

**Design, synthesis and biological evaluation of
selected 7-azaindole derivatives
and structurally related compounds as
potential inhibitors of disease-related protein
kinases**

L Pieterse



orcid.org/0000-0001-8895-9897

Thesis accepted in fulfillment of the requirements for the
degree Doctor of Philosophy in Pharmacy with
Pharmaceutical chemistry at the North-West University

Promoter: Prof LJ Legoabe

Co-promotor: Dr RM Beteck

Assistant promotor: Dr OJ Jesumoroti

Graduation: June 2021

Student number: 23347368

The financial assistance of National Research Foundation (NRF) (Grant specific unique reference number (UID) 112348), South Africa towards this study is hereby acknowledged. Opinions expressed and conclusions arrived at, are those of the author and are not necessarily to be attributed to the NRF.

“It always seems impossible until it is done”

Nelson Mandela

ACKNOWLEDGEMENTS

I am wholeheartedly thankful that this seemingly never ending “Doctoral Chapter” has drawn to a close and I offer my sincere thanks to everyone who played a part in making this journey easier.

A special note of thanks to the following:

To my promoter, Prof Lesetja Legoabe, co-promoter Dr Richard Beteck and assistant promoter Dr Bola Jesumoroti thank you for your collective guidance, it was greatly appreciated.

To all my co-authors, thank you for your contributions; without it I would not have been able to publish.

To Dr Johan Jordaan, Dr Daniël Otto and Prof Frank van Der Kooy, I am grateful for the MS, NMR and HPLC data, respectively.

To my dear friends Heleen, Rialette and Stéfan thank you for all the laughter, joy and coffee you supplied. I am immensely grateful for your friendship; without it I would not have stayed sane.

To my parents, I am grateful that you are always on my side and for equipping me to undertake this mammoth task.

To my husband, Thomas. You are by far my favourite person in the world. Thank you for all the encouragement and support. You were and always will be a ray of sunshine when skies are dark.

Above all, I thank my heavenly Father, in whose hands we are safely carried and steered toward greater purpose.

PREFACE

This thesis is the pinnacle of an eventful journey into the world of protein kinases. It examines a space where chemistry meets biology, more specifically 7-azaindole and 7-deazapurine derivatives meet a panel of protein kinases relevant to disease. The thesis is completed in article format and includes two (2) articles. The first article is published in the Medical Chemistry Research journal and the permission of reproduction for this article can be found under Annexure B, while a sign letter from the co-authors follows the preface. The last article has been submitted for publication to Chemical Biology and Drug Design and the email confirmation of the submission can be found under Annexure E. All the synthesis, data analysis and preparation of scholarly documents pertaining to this thesis were performed by Ms. L. Pieterse.

LETTER OF PERMISSION FROM CO-AUTHORS

LETTER OF PERMISSION

November 2020

To whom it may concern,

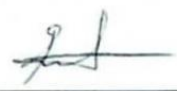
CO-AUTHORSHIP ON ORIGINAL ARTICLES

The undersigned are co-authors of the original articles featured in this thesis, and hereby, give permission to Ms. L. Pieterse to use these articles in the thesis: Design, synthesis and biological evaluation of selected 7-azaindole derivatives and structurally related compounds as potential inhibitors of disease-related kinases, submitted for the degree Doctor of Philosophy in Pharmacy with Pharmaceutical Chemistry at the North-West University (NWU).

Sincerely,



Lesetja J. Legoabe



Richard M. Beteck



Blandine Baratte



Stéphane Bach



Thomas Robert



Sandrine Ruchaud



Omobolanle J. Jesumoroti

ABSTRACT

The bulk of the myriad cellular processes pertaining to normal cellular function in humans are governed by a superfamily of proteins known as protein kinases. Protein kinases account for 1.7% of all human genes and are vastly explored due to their involvement in various diseases. This study focused on a panel of disease-related protein kinases, namely: *HsCDK2/Cyclin A*, *HsCDK5/p25*, *HsCDK9/Cyclin T*, *HsHaspin*, *HsPIM1*, *SscCK1δ/ε*, *SscGSK3α/β* and *LmCK1*. The selected scaffold of interest, 7-azaindole, has been known to have a propensity for producing derivatives that inhibit protein kinases. Therefore, a total of thirty-five 7-azaindole derivatives were synthesised for *in vitro* assessment for protein kinase inhibition against the designated panel of kinases. The C4-substituted 7-azaindole derivatives exhibited the best inhibition activity against the Haspin and CDK9/Cyclin T kinases, in general. 4-(4-Methoxyphenyl)-1*H*-pyrrolo[2,3-*b*]pyridine exhibited best Haspin activity of the 7-azaindole derivatives; IC₅₀ value of 0.118 μM. 4-(4-Fluorophenyl)-1*H*-pyrrolo[2,3-*b*]pyridine exhibited the best CDK9/Cyclin T activity; IC₅₀ value of 0.063 μM. Prompted by the success of the 7-azaindole scaffold, 19 structurally related C4-substituted 7*H*-pyrrolo[2,3-*d*]pyrimidine (7-deazapurine) derivatives were synthesised and also evaluated against the designated kinase panel. The most potent 7-deazapurine derivatives were 4-(7*H*-pyrrolo[2,3-*d*]pyrimidin-4-yl)phenol and 4-(4-methoxyphenyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine with IC₅₀ values of 0.38 μM and 0.11 μM, against CDK9/CyclinT and Haspin, respectively. Both CDK9/Cyclin T and Haspin have been implicated in cancer, therefore, it may be concluded that the 7-azaindole and 7-deazapurine scaffolds are ideal for producing protein kinase inhibitors that may have potential benefit in cancer therapy in the future.

Keywords: 7-azaindole; 7*H*-pyrrolo[2,3-*d*]pyrimidine; protein kinase inhibitors; Haspin; CDK9/Cyclin T.

OPSOMMING

Die meerderheid van die talle sellulêre prosesse, wat vir normale selffunksie in mense nodig is, is grootliks afhanklik van 'n "superfamilie" van proteïene wat bekend staan as proteïenkinases. Proteïenkinases maak 1.7% van alle menslike gene uit en weens hulle betrokkenheid in verskeie siektetoestande word hulle breedvoerig ondersoek. In hierdie studie is daar van 'n paneel van proteïenkinases gebruik gemaak. Die paneel bestaan uit die volgende proteïenkinases: *HsCDK2/Cyclin A*, *HsCDK5/p25*, *HsCDK9/Cyclin T*, *HsHaspin*, *HsPIM1*, *SscCK1δ/ε*, *SscGSK3α/β* and *LmCK1*. 7-Asaïndool is as die uitverkore skelet verkies omdat die analoë van hierdie verbinding geneig is, om as belowende proteïenkinase-inhibeerders op te tree. Na aanleiding hiervan, is daar vyf-en-dertig 7-asaïndoolanaloeë, met die oog op die *in vitro* analise teenoor die paneel van die proteïenkinases, gesintetiseer. In die geheel, is die beste resultate teenoor die Haspin en CDK9/Cyclin T kinases, met die C4-gesubstitueerde-7-asaïndoolanaloeë, verkry. 4-(4-Metoksifeniel)-1*H*-pirollo[2,3-*b*]piridien is die verbinding wat die beste aktiwiteit teenoor Haspin vertoon het (IK_{50} waarde van 0.118 μ M), terwyl 4-(4-fluorofeniel)-1*H*-pirollo[2,3-*b*]piridien die verbinding is, wat die beste aktiwiteit teenoor CDK9/Cyclin T vertoon het (IK_{50} waarde van 0.063 μ M). Die sukses wat met die 7-asaïndoolreeks behaal is, het die sintese van 19 struktureel-verwante C4-gesubstitueerde-7*H*-pirollo[2,3-*d*]pirimidienanaloeë aangespoor. In hierdie reeks het die 4-(7*H*-pirollo[2,3-*d*]pirimidin-4-iel)fenol en die 4-(4-metoksifeniel)-7*H*-pirollo[2,3-*d*]pirimidien die beste gevaar, met 'n IK_{50} waarde van 0.38 μ M en 0.11 μ M teenoor die CDK9/Cyclin T en Haspin kinase, onderskeidelik. Beide die Haspin en CDK9/Cyclin T kinases word tans vir hulle waarde in kankerterapie ondersoek. Dus na aanleiding van hierdie studie, kan die gevolgtrekking gemaak word, dat die 7-asaïndool- en 7*H*-pirollo[2,3-*d*]pirimidienskelette moontlik van waarde in die stryd teen kanker mag wees.

Sleuteltermes: 7-asaïndool; 7*H*-pirollo[2,3-*d*]pirimidien; proteïenkinase-inhibeerders; Haspin; CDK9/Cyclin T.

TABLE OF CONTENTS

ACKNOWLEDGEMENTS	III
PREFACE.....	IV
LETTER OF PERMISSION FROM CO-AUTHORS.....	V
ABSTRACT	VI
OPSOMMING	VII
LIST OF TABLES	XIII
LIST OF FIGURES.....	XIV
LIST OF ABBREVIATIONS	XV
CHAPTER 1: INTRODUCTION AND BACKGROUND	1
1.1 General Introduction	1
1.2 The function of protein kinases - an overview	1
1.2.1 The cell-division cycle and the role that protein kinases play	2
1.3 The 7-azaindole scaffold and its relation to protein kinase inhibition	4
1.3.1 The proposed protein kinase panel.....	7
1.4 Problem statement and research hypothesis.....	11
1.5 Aim of the study	12
1.6 Objectives of the study	12
1.7 Structure of the study	12
1.7.1 Synthesis of proposed compounds.....	14
1.8 Conclusion.....	14

CHAPTER 2: DISEASES RELATED TO THE PROPOSED PROTEIN KINASE PANEL	15
2.1	Introduction 15
2.1.1	What is cancer? 15
2.1.2	How is it caused? 16
2.1.3	Current treatment options 16
2.1.3.1	Chemotherapy 16
2.1.3.2	Surgery 18
2.1.3.2.1	Diagnostic 18
2.1.3.2.2	Staging 19
2.1.3.2.3	Curative surgery or tumour removal 19
2.1.3.2.4	Debulking surgery 19
2.1.3.2.5	Palliative surgery 19
2.1.3.2.6	Reconstructive surgery 19
2.1.3.2.7	Preventative surgery 19
2.1.3.3	Radiation therapy 20
2.1.4	The shortcomings of current cancer treatment 21
2.1.4.1	Chemotherapy 21
2.1.4.2	Surgery 21
2.1.4.3	Radiation therapy 22
2.1.4.4	Conclusion 22
2.2	Neurodegenerative diseases 22
2.2.1	Alzheimer's disease 23
2.2.2	Parkinson's disease 23

2.2.3	Huntington's disease	26
2.2.4	Amyotrophic lateral sclerosis	27
2.3	Type 2 diabetes mellitus	27
2.3.1	Current therapy for type 2 diabetes mellitus.....	27
2.3.2	Conclusion.....	30
2.4	Leishmaniasis.....	30
2.4.1	Treatment of Leishmaniasis.....	31
2.4.1.1	Treatment of visceral leishmaniasis	31
2.4.1.2	Treatment of cutaneous and mucocutaneous leishmaniasis	31
2.4.2	Conclusion.....	31
CHAPTER 3: PUBLISHED ARTICLE		32
CHAPTER 4: MANUSCRIPT SUBMITTED TO CHEMICAL BIOLOGY AND DRUG DESIGN		46
4.1	Author Guidelines for submitting a manuscript to Chemical Biology and Drug Design.....	46
4.2	Synthesis and biological evaluation of selected 7<i>H</i>-pyrrolo[2,3- <i>d</i>]pyrimidine derivatives as novel CDK9/CyclinT and Haspin inhibitors. ...	46
4.2.1	Acknowledgements	46
4.2.2	Conflict of interest.....	46
4.2.3	Abstract	47
4.2.4	Keywords:.....	47
4.2.5	Introduction.....	47
4.2.6	Material and methods	49

4.2.7	Experimental	50
4.2.7.1	Chemistry	50
4.2.7.2	General synthetic procedure for the C6-substituted 7-azaindole derivatives (5a–m)	51
4.2.7.3	General synthetic procedure for the selected C4-substituted-7 <i>H</i> -pyrrolo[2,3- <i>d</i>]pyrimidine derivatives (7a–p)	51
4.2.7.4	General synthetic procedure for the selected 4,7-bis-substituted-7 <i>H</i> -pyrrolo[2,3- <i>d</i>]pyrimidine derivatives (8a–c)	51
4.2.7.5	Protein kinase assays	61
4.2.7.6	Cytotoxicity prediction	62
4.2.7.7	SwissADME prediction	62
4.2.8	Results and discussion	63
4.2.9	Conclusion	64
4.2.10	Tables	71
4.2.11	Figures:	80
4.2.12	Graphical Table of Contents for Chemical Biology and Drug Design Manuscript	82
CHAPTER 5: CONCLUSION		83
BIBLIOGRAPHY		89
ANNEXURE A: ETHICAL APPROVAL- NO RISK STUDY		110
ANNEXURE B: PERMISSION FOR THE USE OF PUBLISHED ARTICLE		111
ANNEXURE C: PERMISSION FOR USE OF FIGURE 1.3		116
ANNEXURE D: SUPPLEMENTARY INFORMATION FOR PUBLISHED ARTICLE		120

ANNEXURE E: EMAIL CONFIRMATION OF SUBMISSION OF MANUSCRIPT TO CHEMICAL BIOLOGY AND DRUG DESIGN BY THE CORRESPONDING AUTHOR	219
ANNEXURE F: CHEMICAL BIOLOGY AND DRUG DESIGN AUTHOR GUIDELINES.....	220
ANNEXURE G: SUPPLEMENTARY INFORMATION FOR SUBMITTED MANUSCRIPT	235

LIST OF TABLES

Table 1.1: The protein kinase panel and the possible disease application	10
Table 2.1: Current chemotherapeutic agents and their drug classes	17
Table 2.2: The alternative surgical techniques in cancer treatment	20
Table 2.3: The current available treatment options in Alzheimer's disease	23
Table 2.4: The current treatment options in Parkinson's disease and the challenges associated with each.....	24
Table 2.5: The current pharmacological treatment in type 2 diabetes mellitus	28
Table 4.1: A table depicting the synthesis of C6-substituted 7-azaindole derivatives, target compounds and yields achieved	71
Table 4.2: A table depicting the synthesis of the C4-substituted 7 <i>H</i> -pyrrolo[2,3- <i>d</i>]pyrimidine derivatives, target compounds and yields achieved.....	72
Table 4.3: A table depicting the synthesis of the C4, 7-bissubstituted 7 <i>H</i> -pyrrolo[2,3- <i>d</i>]pyrimidine derivatives, target compounds and yields achieved.....	74
Table 4.4: Percentage of residual activity for compounds 5a–k, 7a–p and 8a–c at 10 μ M and 1 μ M using a representative kinase panel	75
Table 4.5: Secondary screening of compounds reported as active against CDK9/CyclinT and Haspin (compounds inhibiting >70% of the kinase activity at 1 μ M, see table 4.4). Activities were assessed in duplicate using 10 μ M ATP	77
Table 4.6: Predicted cytotoxicity of the most promising compounds (compounds inhibiting >70% of kinase activity at 1 μ M).....	78

LIST OF FIGURES

Figure 1.1: A visual representation of the known eukaryotic protein kinase groups and examples of their members (Duong-Ly & Peterson, 2013).	2
Figure 1.2: A basic illustration of the eukaryotic cell-division cycle	3
Figure 1.3: The ATP binding site; adapted from (Le Corre <i>et al.</i> , 2010)	4
Figure 1.4: A depiction of various 7-azaindole derivatives known to inhibit different PKs.	6
Figure 1.5: An illustration of the existing 7-deazapurine and related structures that inhibit different PKs. References: (13) (Lawhorn <i>et al.</i> , 2015), (14) (Nishioka <i>et al.</i> , 1991) and (Traxler <i>et al.</i> , 1996).	7
Figure 1.6: The structures of the 7-azaindole and 7-deazapurine scaffolds	12
Figure 1.7: An illustration of the proposed structure for the study	13
Figure 4.1: A depiction of the 7-azaindole and the 7-deazapurine scaffolds	80
Figure 4.2: A BOILED-egg representation of SwissADME predictions for the most promising compounds	81
Figure 4.3: Graphical Table of Content for Submission to Chemical Biology and Drug Design.....	82
Figure 5.1: The best compound against CDK9/CyclinT in relation to its corresponding counterparts in each series	85
Figure 5.2: The compound with the best Haspin activity in relation to its corresponding counterpart in each series	86
Figure 5.3: The effect of the additional N on the pyrimidine ring of the 7-deazapurine scaffold on CDK9/CyclinT activity.....	87

LIST OF ABBREVIATIONS

A

AD	Alzheimer's disease
AGC	A eukaryotic protein kinase group that includes protein kinases A , G and C
ALK	Anaplastic lymphoma kinase
ALS	Amyotrophic lateral sclerosis
ATP	Adenosine triphosphate

C

CAMK	Calcium/calmodulin-dependent kinase
CAMKI	Calcium/calmodulin-dependent kinase I
CAMKII	Calcium/calmodulin-dependent kinase II
CAMKIII	Calcium/calmodulin-dependent kinase III
CAMKIV	Calcium/calmodulin-dependent kinase IV
CAMKV	Calcium/calmodulin-dependent kinase V
Cdc7	Cell division cycle 7 related protein kinase
CDK	Cyclin-dependent kinase
CK1	Casein kinase 1
CK1δ	Casein kinase 1 delta
CK1ε	Casein kinase 1 epsilon
CMGC	A eukaryotic protein kinase group that includes c yclin-dependent, m itogen-activated, g lycogen synthase and c ell-division cycle like kinases
COMT	Catechol-O-methyltransferase
CuOAc ₂	Cupric acetate

D

DDC	Dopa-decarboxylase
DNA	Deoxyribonucleic acid
DRT	Dopamine replacement therapy

E

EFG-R PTK	Epidermal growth factor receptor protein tyrosine kinase
-----------	--

F

FAK	Focal adhesion kinase
-----	-----------------------

G

G ₁	First gap
G ₂	Second gap
GSK3	Glycogen synthase kinase 3

H

Haspin	Haploid germ cell-specific nuclear kinase
HD	Huntington's disease
HGH	Human growth hormone
HPLC	High performance liquid chromatography
M	
M phase	Mitosis
m-TOR	Mammalian target of rapamycin kinase
MAO	Monoamine oxidase
MS	Mass spectra
N	
N	Nitrogen
NH	Amine
NMDA	N-methyl-D-aspartate
NMR	Nuclear magnetic resonance
P	
p38 α MAP	p38 α Mitogen activated kinase
Pim-1	Proto-oncogene serine/threonine kinase 1
PD	Parkinson's disease
Pd(PPh ₃) ₄	Tetrakis triphenylphosphine
PK	Protein kinase
PKA	Protein kinase A
PKC	Protein kinase C
PKG	Protein kinase G
PPI4K	Phosphatidylinositol-4-kinase
S	
S phase	Synthesis
SAR	Structure activity relationship
STE	A eukaryotic protein kinase group that includes "sterile" kinase
STE7	Sterile kinase 7
STE11	Sterile kinase 11
STE20	Sterile kinase 20
T	
T2DM	Type 2 diabetes mellitus
TK	Tyrosine kinase
TKL	Tyrosine-like kinase
TNNI3K	Troponin I-interacting kinase
TrKA	Tropomyosin-related kinase A

R

RGC

Receptor guanyl cyclase

CHAPTER 1: INTRODUCTION AND BACKGROUND

1.1 General Introduction

This chapter describes a journey into the realm of protein kinases (PKs), some of the diseases they are related to (like cancer, neurodegenerative diseases, diabetes (type 2) and leishmaniasis) and the scaffolds that will be assessed for PK inhibition in this study.

1.2 The function of protein kinases - an overview

The quintessential eukaryote's viability is dependent on a vast number of cellular processes, the majority of which are sustained by a process known as protein phosphorylation (Hanks & Hunter, 1995; Hunter, 1995). Protein phosphorylation is an integral component of eukaryotic signal transduction pathways and it is mediated by a superfamily of homologous proteins known as PKs (Hanks & Hunter, 1995; Hunter, 1995). Recently, the nearly completed human genome sequencing project revealed the extent to which PKs permeate the human body. A total of 518 PKs have been recorded to date, this accounts for 1.7% of all known human genes (Manning *et al.*, 2002). 478 of these PKs belong to one superfamily, which can be further divided into groups, families and sub-families based on sequence similarity and biochemical function (Manning, 2019) (see **Figure 1.1** that depicts the known eukaryotic protein kinase groups). They promote the transfer of a phosphoryl group from a phosphoryl donor, usually adenosine triphosphate (ATP), to receptor substrates, usually small molecule, lipid or protein substrates (Lawson *et al.*, 2016). Furthermore, these PKs not only regulate signal transduction but also mediate other cellular processes like metabolism, cell cycle progression, cytoskeletal rearrangement and cell movement, apoptosis and differentiation (Manning *et al.*, 2002). Regarding the magnitude of PK involvement in normal cellular processes, it is thus prudent to consider the possible implications of phosphorylation disruption.

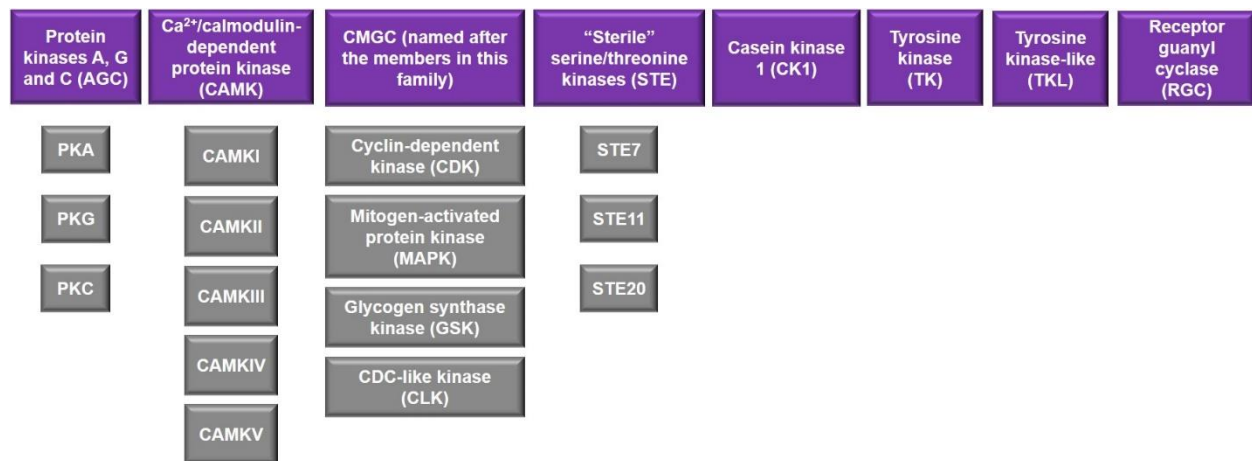


Figure 1.1: A visual representation of the known eukaryotic protein kinase groups and examples of their members (Duong-Ly & Peterson, 2013).

1.2.1 The cell-division cycle and the role that protein kinases play

The cell-division cycle (cell cycle) is critical to normal cellular function. It typically comprises of four systematic processes: cell growth, deoxyribonucleic acid (DNA) replication, distribution of the duplicated chromosomes to daughter cells and cell division (Cooper *et al.*, 2000). These processes occur during two fundamental stages of cell cycle: mitosis and interphase. Mitosis (nuclear division or M phase) is considered the more distinctive stage of the two, it is equivalent to the parting of daughter chromosomes and is customarily divided into five stages: prophase, prometaphase, metaphase, anaphase and telophase that ordinarily culminates in cell-division (cytokinesis). Nevertheless, mitosis accounts for only approximately 10% of the cell cycle, while 90% is spent in interphase between mitoses (Campbell *et al.*, 2008). Interphase consists of three distinct phases: G₁ phase (first gap), S phase (synthesis), G₂ phase (second gap) (Underwood & Cross, 2009). G₁ phase, is the gap that follows mitosis but precedes the initiation of DNA replication (Cooper *et al.*, 2000). During G₁ the cell remains metabolically active and cell growth is continued but DNA replication is presently halted. S follows the G₁ phase, where DNA replication commences and upon completion of DNA synthesis, G₂ phase is initiated. During G₂ cell growth is resumed and the appropriate proteins are synthesised in preparation for mitosis. Accordingly, the M phase ensues, thereby bringing the cell cycle to a close (Cooper *et al.*, 2000). See **Figure 1.2**.

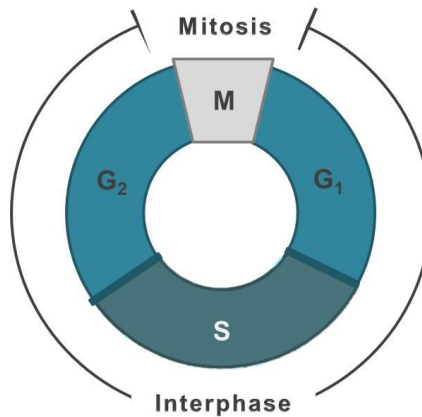


Figure 1.2: A basic illustration of the eukaryotic cell-division cycle

During the cell cycle, DNA replication needs to be achieved with absolute integrity from one generation to the next. To ensure this integrity, the cell cycle is bound to regulatory events at specific times during the cell cycle (Underwood & Cross, 2009). Regulation of the eukaryotic cell cycle is achieved by a combination of external events and internal checkpoints. External events dictate whether a cell set for replication will experience inhibition or activation. These events range from uncomplicated events (apoptosis in close vicinity to the cell) to extensive events (release of growth promoting hormones, such as human growth hormone (HGH)) (Open Stax, 2012). Occurrences like the crowding of cells can inhibit division, whereas cell growth, to the point where the cell becomes less efficient, can trigger division. Once an external progression signal is received, the cell proceeds into interphase, if not, cell cycle progression is halted (Open Stax, 2012). These parameters thus ensure that daughter cells are unequivocal duplicates of the parent cells because inaccurate duplication or distribution of the chromosomes can bring about mutations that could be passed on to every new cell produced. Hence, internal control mechanisms curtail the continuous transfer of possible mutations to new daughter cells. These mechanisms function at three main cell cycle checkpoints to halt any unfavourable division. They are located near the end of G_1 , at the G_2 to M transition and during the metaphase of mitosis (Open Stax, 2012).

PKs are prominent enzymes in the regulation of the cell cycle (Blagden & Bono, 2005). Examples of PKs involved in cell cycle regulation are the cyclin-dependent kinases (CDKs); they regulate the punctual production and activation of several different proteins involved in DNA replication and mitotic spindle formation, among other things (Underwood & Cross, 2009). Upon execution of their function(s), PKs are expected to undergo prompt termination or inactivation.

This forms part of the regulation of cell cycle progression through the different phases and is necessary for normal cell function (Blagden & Bono, 2005). Therefore, erroneous PK activity can exert devastating consequences on processes like cell growth, differentiation, cell development, normal cell function and cell death. As such, abnormal PK activity is often associated with cell mutation, cancer, neurodegenerative diseases like Alzheimer's disease, inflammation and diabetes (Flight, 2013; Huse & Kuriyan, 2002; Noh & King, 2007).

Therefore, PKs have garnered much attention as likely drug targets; with nearly 30 kinase inhibitors reaching Phase I clinical trials, the majority of which are being investigated for cancer. Furthermore, considering that PKs are involved in almost every signal transduction process in the human body, it is understood that inhibition of these kinases might exert valuable physiological responses in certain diseases (Zhang *et al.*, 2009).

1.3 The 7-azaindole scaffold and its relation to protein kinase inhibition

PK inhibitors are ordinarily designed with the goal of binding to the ATP binding site in mind. The ATP binding site is usually positioned in the deep cleft situated between the N- and C-terminal lobes of the catalytic binding domain (Liao, 2007). The 1*H*-pyrrolo[2,3-*b*]pyridine (7-azaindole scaffold) exhibits a proclivity for PK inhibition, because the nitrogen (N) on the pyridine ring act as a hydrogen bond acceptor, whilst the secondary amine (-NH) on the pyrrole ring serves as a hydrogen bond donor, rendering bidentate hydrogen bonds with the hinge region of the ATP binding site, see **Figure 1.3** (Irie & Sawa, 2018; Mérour *et al.*, 2014; Zuccotto *et al.*, 2009).

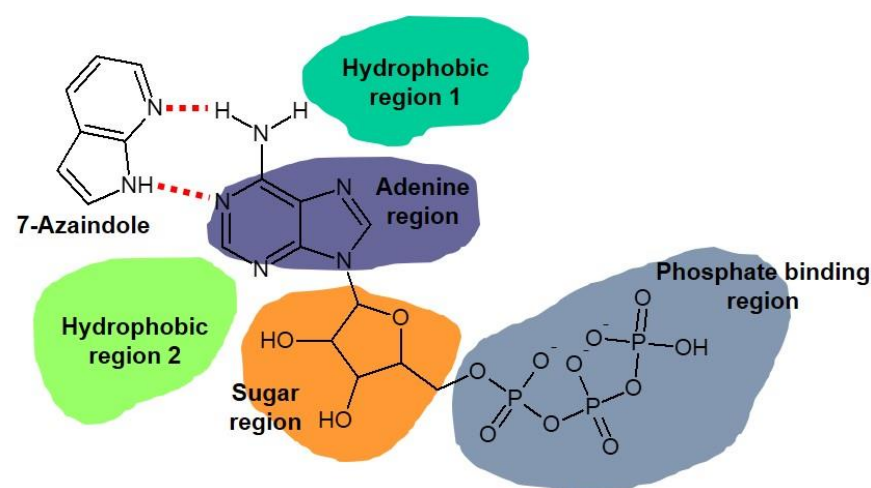


Figure 1.3: The ATP binding site; adapted from (Le Corre *et al.*, 2010)

7-azaindole based compounds have been reported to inhibit several kinase, including anaplastic lymphoma kinase (ALK) (Gummadi *et al.*, 2013), the aurora kinases (Bavetsias *et al.*, 2013), cell

division kinase 7 (Cdc7) (Harrington *et al.*, 2013), C-met kinase (Kim *et al.*, 2008), DYRK1A (Gourdain *et al.*, 2013), focal adhesion kinase (FAK) (Heinrich *et al.*, 2013), I κ B kinase (IKK) (Liddle *et al.*, 2009), KIT/FMS dual kinase (Zhang *et al.*, 2013), p38 α mitogen activated protein kinase (p38 α MAP) (Mavunkel *et al.*, 2010), proto-oncogene serine/threonine kinase 1 (Pim-1) (Nakano *et al.*, 2012), mammalian target of rapamycin kinase (m-TOR) (Tsou *et al.*, 2010) and tropomyosin-related kinase A (TrKA) (Hong *et al.*, 2012). See **figure 1.4** for examples of the structures and their corresponding IC₅₀ values.

The 7H-pyrrolo[2,3-*d*]pyrimidine scaffold (7-deazapurine) is structurally related to 7-azaindole and has recently been deemed as a privileged scaffold in the design of antitumor and antiviral nucleosides (Perlíková & Hocek, 2017). It bears an additional nitrogen in the form of a pyrimidine ring instead of the pyridine ring on the 7-azaindole scaffold. In terms of PK inhibition, studies on 7-deazapurines are limited. Nevertheless, the following are some examples of PK inhibition by the 7-deazapurine scaffold and closely related compounds: troponin I-interacting kinase (TNNI3K) (Lawhorn *et al.*, 2015), phosphatidylinositol 4-kinase (PPI4K) (Nishioka *et al.*, 1991) and epidermal growth factor receptor protein tyrosine kinase (EGF-R PTK) (Traxler *et al.*, 1996). See **figure 1.5** for examples of structures and their corresponding IC₅₀. These examples suggest that compounds incorporating 7-azaindole and/or 7-deazapurine scaffolds could act as novel PK inhibitors. Consequently, a study focused on screening selected known and novel 7-azaindole derivatives and 7-deazapurine derivatives against a selected panel of PKs (discussed below), might identify novel and potent PK inhibitors, whilst also highlighting important SARs. This could lead to new treatment options in diseases like cancer, neurodegenerative diseases, diabetes and leishmaniasis and will also enable future studies to use the knowledge generated by this study to streamline new PK studies.

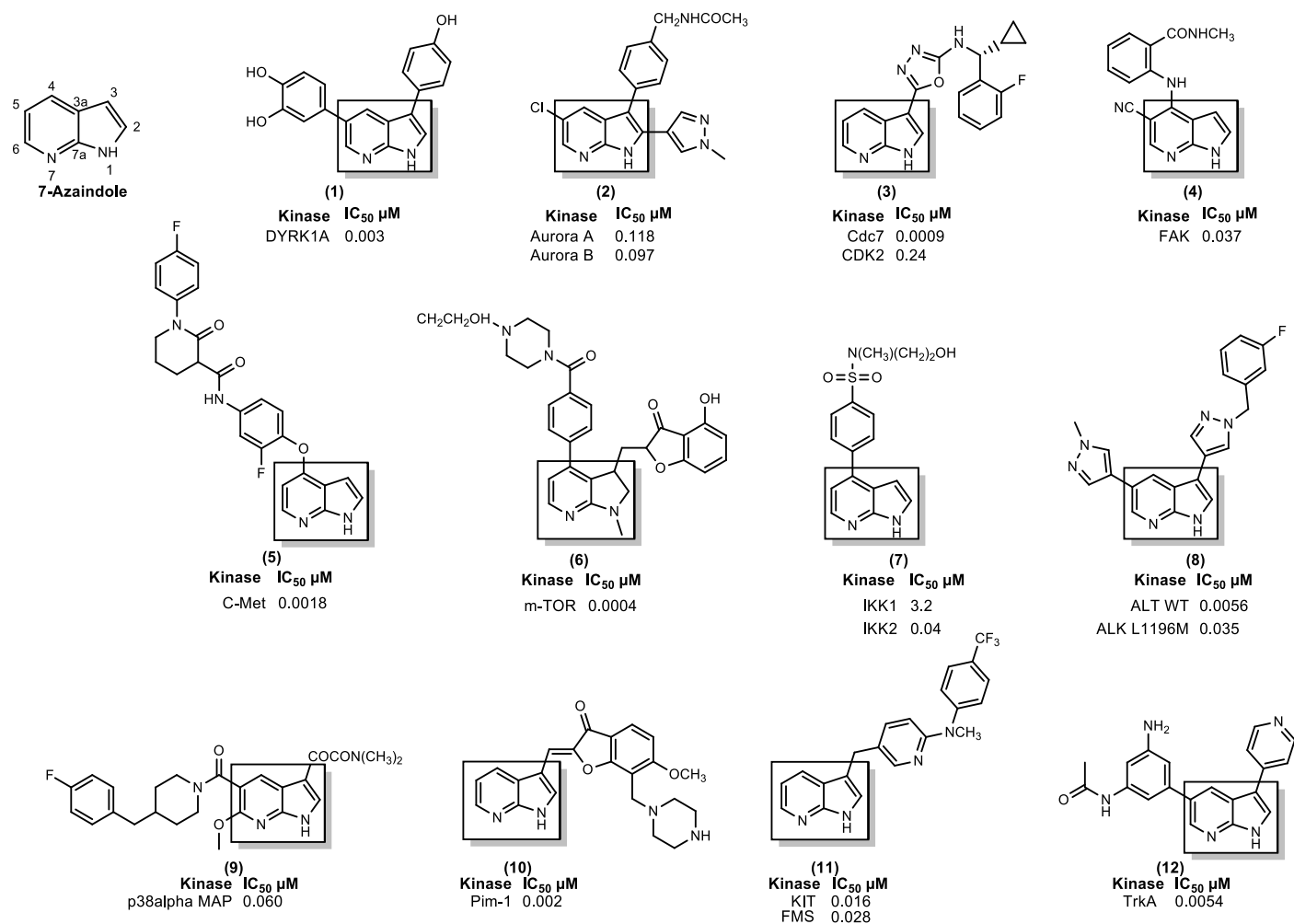


Figure 1.4: A depiction of various 7-azaindole derivatives known to inhibit different PKs.

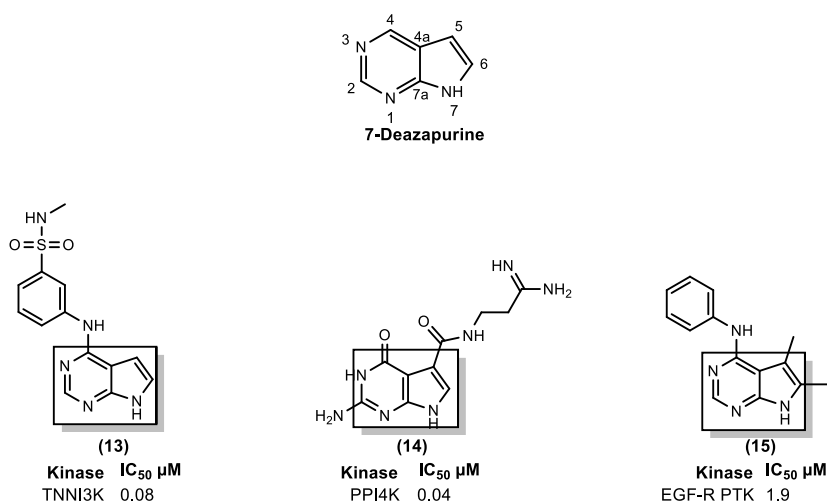


Figure 1.5: An illustration of the existing 7-deazapurine and related structures that inhibit different PKs. References: (13) (Lawhorn et al., 2015), (14) (Nishioka et al., 1991) and (Traxler et al., 1996).

1.3.1 The proposed protein kinase panel

The PKs listed below have been implicated in several diseases of humans and are regarded as potential drug targets

CDK2/Cyclin A, CDK5/p25 and CDK9/Cyclin T

The CDKs form a unique family of serine-threonine kinases within the larger superfamily of PKs. After careful consideration (of seeming contradictory evidence), there was decided upon a unifying notion that explains how CDKs control virtually all key transitions in the cell cycle (Nurse, 1990). The G₁ to S phase transition is believed to be reliant on activation of specific proteins essential for DNA replication. Activation, in turn, occurs by means of phosphorylation which is CDK dependent (Blow & Nurse, 1990; D'urso et al., 1990; Fang & Newport, 1991; Furukawa et al., 1990; Pagano et al., 1993; Tsai et al., 1993). Similarly, the G₂ to M phase transition is dependent on CDK phosphorylation and activation of proteins but differs from G₁ to S phase transition in that a different set of proteins are activated. In this case, the activated proteins enable mitosis and cytokinesis (Arion et al., 1988; Dunphy et al., 1988; Fang & Newport, 1991; Gautier et al., 1988; Labbe et al., 1988; Reed & Wittenberg, 1990; Riabowol et al., 1989). Thus, CDK activation is deemed as one of the principal points of control for normal cell proliferation. In contrast, anomalous proliferation of cells, mediated by the disruption of the normal cell cycle mechanisms, is regarded as the tell-tale sign of nearly all cancer cells.

Therefore, compounds that inhibit the CDK-cyclin complexes, such as CDK2/Cyclin A, CDK5/ p25 and CDK9/Cyclin T are auspicious anti-tumour agents or adjuvant therapy to existing therapies (Harper & Elledge, 1996; Pevarello *et al.*, 2004). Furthermore, the CDK5/p25 complex has also been implicated in diseases like Alzheimer's disease (AD), amyotrophic lateral sclerosis (ALS), Parkinson's disease (PD) and Niemann-Pick type C disease (Bu *et al.*, 2002; Lau & Ahljanian, 2003; Nguyen & Julien, 2003; Smith *et al.*, 2003), whereas the CDK9/Cyclin T complex has been identified as a possible target against which to develop antiviral agents (Yamamoto *et al.*, 2014).

Haspin

The protein kinase, Haploid germ cell-specific nuclear kinase or Haspin, is one of the recent additions to the kinome and belongs to the serine-threonine kinase family. It is specifically attuned for phosphorylation of histone 3 at threonine 3 (H3T3ph) and its function is of cardinal importance in mitosis, prompting chromosome cohesion, metaphase alignment and cell cycle progression (Dai *et al.*, 2009; Dai *et al.*, 2006; Dai *et al.*, 2005). Haspin like other mitotic kinases is involved in cell proliferation, therefore, non-proliferating cells will not be affected by Haspin inhibition (Huertas *et al.*, 2012b). In addition, Haspin inhibition might induce fewer adverse effects than other mitotic kinases as it is classified as an atypical kinase (belonging to a family that diverges from other human kinases) (Eswaran *et al.*, 2009; Higgins, 2001a; Villa *et al.*, 2009). Furthermore, it has only one known target in the human body (H3T3). Considering the above, it's deducible that inhibitors of Haspin may be safe and effective anti-mitotic agents (Huertas *et al.*, 2012b).

Pim-1

Pim-1 is a serine-threonine kinase was originally cloned to serve as a proviral insertion in murine T-cell lymphomas, hence known as Pim. (Selten *et al.*, 1985). Later on, the Pim kinases were connected to the development of prostate cancer when DNA-microarrays brought to light that Pim-1 is overexpressed in human prostate cancer (Dhanasekaran *et al.*, 2001). Now is it believed that the grade of overexpression is directly related to the grade of prostate cancer (Xu *et al.*, 2005), especially since moderate-to-strong cytoplasmic staining of Pim-1 illustrates the presence of Pim-1 kinases in 68% of the tumours of patients with a Gleason score of 7 or higher (Valdman *et al.*, 2004). Nevertheless, there is still a limited amount of known Pim-inhibitors (Xia *et al.*, 2008), but with the recent clarification of the Pim-1 crystal structure the possibility of discovering novel Pim-selective inhibitors exists (Jacobs *et al.*, 2005; Kumar *et al.*, 2005; Qian *et al.*, 2005).

CK1δ/ε

The two isoforms, casein kinase 1 delta (CK1δ) and casein kinase 1 epsilon (CK1ε) form part of a well-conserved family of serine-threonine protein kinases. CK1δ and CK1ε are expressed in multiple isoforms (α, β, γ1, γ2, γ3, δ, ε) in eukaryotes (Cozza *et al.*, 2008). Despite the fact that all CK1 isoforms have highly conserved kinase domains, the N- and C- terminals differ in length and primary structure. (Knippschild *et al.*, 2005). Moreover, the CK1 family is a constitutively active serine-threonine kinases which rely on prephosphorylation of the substrate by another kinase, such as glycogen synthase kinase 3β (GSK3β), in order to achieve basal activity (Flotow *et al.*, 1990). Notwithstanding CK1's constitutive activation, certain mechanisms that are controlled by CK1 activity have been acknowledged, such as inhibitory autophosphorylation, proteolytic cleavage of the C-terminal domain and subcellular localisation and compartmentalisation (Knippschild *et al.*, 2005). The CK1 family are also parallel to the regulation of particular cellular events, namely transduction of the Wnt signalling pathway, circadian rhythm, DNA damage response and late cell cycle progression (Beyaert *et al.*, 1995; Eide *et al.*, 2002; McKay *et al.*, 2001; Meek & Knippschild, 2003; Murakami *et al.*, 1999; Sillibourne *et al.*, 2002; Vielhaber *et al.*, 2000). Accordingly, disturbance in CK1 homeostasis could cause disease such as neurodegenerative diseases, including Alzheimer's and Parkinson's disease (CK1δ), the familial advanced sleep phase syndrome (CK1δ and ε) and cancer (CK1δ and ε) (Meek & Knippschild, 2003; Singh *et al.*, 1995; Takano *et al.*, 2004) Consequently, the two isoforms, CK1δ and CK1ε, have generated interest as potential drug targets in treatment of neurodegenerative diseases, abnormal circadian behaviour, cancer and drug use disorders (Long *et al.*, 2012).

LmCK1

The CK1 family is not restricted to humans but it is also found amongst the protozoan parasites of the genus *Leishmania*. This study will, however, focus on the *Leishmania major* (LmCK1) strain. In eukaryotes, protein phosphorylation plays a vital role in the regulation of cellular responses to environmental signal, including cell-cell interactions. The function of intracellular PKs in complex regulatory cascades, controlling processes like differentiation, metabolism, growth, gene expression and other cellular processes, has been well defined (Hunter, 1995). The functions concerning the externally located cell surface PKs (or ecto-PKs) of protozoan parasites, have not been clearly defined as of yet (Sacerdoti-Sierra & Jaffe, 1997). Regarding *Leishmania*, there are two constitutively shed ecto-PKs that have been biochemically characterised and recognised to have CK1-like activities (Sacerdoti-Sierra & Jaffe, 1997; Vieira *et al.*, 2002).

It has been propounded that the aforementioned PKs impact parasite-host interaction by phosphorylating the host proteins in order to aid the infiltration of the parasite into host cells (Allocco *et al.*, 2006). Thus, members of the leishmanial CK1 family (especially LmCK1) denote attractive therapeutic targets in combating leishmaniasis (Rachidi *et al.*, 2014). Especially since the known CDK inhibitor purvalanol B, proved to be a more potent inhibitor of parasitic CK1 than its mammalian counterpart (Gray *et al.*, 1998). This accentuated the possibility of selectively in targeting leishmanial CK1 by exploiting the parasite-host differences, thereby diminishing the probability of adverse effects in humans (Allocco *et al.*, 2006).

GSK3 α/β

Glycogen synthase kinase 3 (GSK3) was discovered in 1980 and its function in the human body was originally trivialised to mere glycogen metabolism. It was later determined that GSK3 is indispensable to numerous cell functions, including signalling by insulin, growth factors and nutrients, and the specification of cell fate during embryonic development (Cohen & Frame, 2001; Embi *et al.*, 1980; Martinez *et al.*, 2002). Furthermore, control of cell division, apoptosis and microtubule function have also been linked to GSK3 (Cohen & Frame, 2001). There are two known forms of the enzyme, GSK3 α and GSK3 β , and inhibitors of GSK3 are deemed potentially valuable in the treatment of neurodegenerative diseases like Alzheimer's disease, Parkinson's disease, and Huntington's disease (Parr *et al.*, 2012). Furthermore, GSK-3 has also been associated with diabetes type II, bipolar disorder, schizophrenia, stroke, cancer and chronic inflammatory disease (Martinez *et al.*, 2002).

Table 1.1: The protein kinase panel and the possible disease application

Protein kinase	Possible disease applications
CDK2/Cyclin A	Antitumor agents (Davies <i>et al.</i> , 2001) .
CDK5/p25	Alzheimer's disease (Lau & Ahljanian, 2003). Amyotrophic lateral sclerosis (Nguyen & Julien, 2003). Parkinson's disease (Smith <i>et al.</i> , 2003). Niemann-Pick type C disease (Bu <i>et al.</i> , 2002). Prostate cancer (Lin <i>et al.</i> , 2004).
CDK9/Cyclin T	Antitumor agents (Walsby <i>et al.</i> , 2014). Antiviral agents (Yamamoto <i>et al.</i> , 2014).
Haspin	Antitumor agents (Huertas <i>et al.</i> , 2012b).
Pim-1	Prostate cancer (Xu <i>et al.</i> , 2005) Diverse leukaemias (Amson <i>et al.</i> , 1989)

Protein kinase	Possible disease applications
CK1 δ/ϵ	Disrupted circadian behaviour (CK1 δ)(Meng <i>et al.</i> , 2010) Alzheimer's disease (Singh <i>et al.</i> , 1995) Cancer (Brockschmidt <i>et al.</i> , 2008; Yang & Stockwell, 2008)
GSK3 α/β	Alzheimer's' disease (Parr <i>et al.</i> , 2012) Parkinson's disease (Parr <i>et al.</i> , 2012) Huntington's disease (Parr <i>et al.</i> , 2012) Type 2 diabtetes (Martinez <i>et al.</i> , 2002) Cancer (Martinez <i>et al.</i> , 2002)
LmCK1	Leishmaniasis (Rachidi <i>et al.</i> , 2014)

1.4 Problem statement and research hypothesis

PKs are ubiquitous throughout the human body and have been implicated in various important cellular functions. As such, disruption of normal PK activity elicits ill effects on processes like cell growth, differentiation, cell development and cell death, thus, disrupting normal cell function. Haphazard cell function is associated with disease development and consequently connects PK dysregulation with diseases such as cancer, neurodegenerative diseases (like Alzheimer's and Parkinson's disease), sleep disorders, inflammation and diabetes, to name a few (Flight, 2013; Huse & Kuriyan, 2002; Noh & King, 2007).

Although research on PK inhibition exists (ample 7-azaindole and sparse 7-deazapurine in PK inhibition research), the current research has only begun to scratch the surface of how PK inhibitors could change the world of medicine and disease. Therefore, considering the burden diseases such as cancer, the neurodegenerative diseases, diabetes and leishmaniasis impose on their victims and families combined with the need for more knowledge on PK inhibition, a study aimed at finding novel solutions to old burdens, in the form of PK inhibitors, may prove to be of value. Not only will the knowledge contribute to the scientific community, but it could also improve the lives of the countless people affected by these diseases in future. Thus, screening a set of both selected known and novel 7-azaindole derivatives and structurally related compounds (7-deazapurine derivatives) for inhibition against a panel of protein kinases, potent novel PK inhibitors can be discovered. The PK inhibitors may have future value as therapeutic options in at least one of the diseases applicable to this study.

1.5 Aim of the study

The aim of this study is to synthesise and explore different 7-azaindole and structurally related compounds to find potent inhibitors of selected disease related PKs and to evaluate structure activity relationships (SARs) to determine which structural features of the derivatives are associated with potency against the selected PKs.

1.6 Objectives of the study

To achieve the aims of the study, the following objectives have been set:

- The design, synthesis and characterisation (NMR, MS, melting points) of the proposed 7-azaindole and 7-deazapurine derivatives, as well as the determination of the purities of the proposed 7-azaindole and 7-deazapurine derivatives HPLC (Pieterse *et al.*, 2020)
- The *in vitro* evaluation of the proposed 7-azaindole and 7-deazapurine derivatives against a panel of protein kinases.
- Structure activity relationship analysis.

1.7 Structure of the study

In light of the previous successes with the 7-azaindole scaffold, this study will aim to deliver selected known and novel 7-azaindole derivatives, as well as structurally related 7-deazapurine derivatives, to allow for structure activity relationship comparison. These compounds are to be screened against the panel of PKs (*HsCDK2/CyclinA*, *HsCDK5/p25*, *HsCDK9/CyclinT*, *HsHaspin*, *HsPim-1*, *SscCKδ/ε*, *SscGSK3α/β* and *LmCK1*, discussed above) in an attempt to bring forth new compounds active in at least one of the diseases identified as applicable to this study (cancer, neurodegenerative diseases, diabetes and leishmaniasis, see **Chapter 2**).

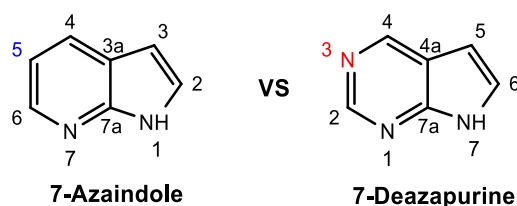
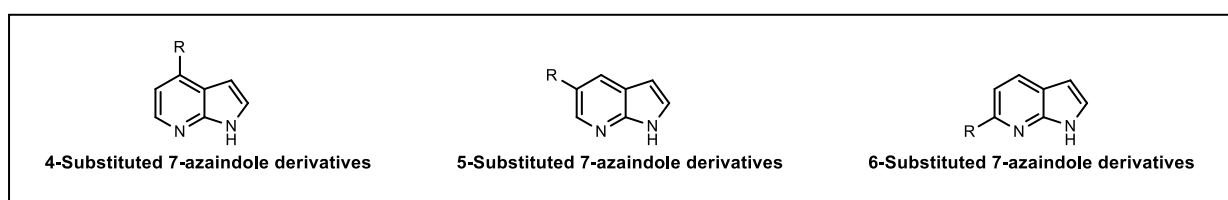


Figure 1.6: The structures of the 7-azaindole and 7-deazapurine scaffolds

The proposed compounds will be prepared in two phases. The first phase will entail the synthesis of 3 series of 7-azaindole derivatives: 1) C4-substituted 7-azaindole derivatives 2) C5-substituted 7-azaindole derivatives and 3) C6-substituted 7-azaindole derivatives. Substitution on the different positions will lend insight as to how the position of substitution may hinder or improve binding to the relevant binding site. All the 7-azaindole derivatives will then undergo screening for biological activity against the panel of kinases and the series with the best performance will determine the position of substitution for second phase. Subsequently, the second phase will entail a series of selected 7-deazapurine derivatives using the optimal position for substitution, as determined in phase 1 (position 2 or 4 in the case of 7-deazapurine). In addition, a select few 7-deazapurine derivatives, bearing identical substitution (bis-substitution) on the optimal position and the N of the pyrrole ring, will be synthesised. These compounds are included for their novelty as well as interesting SAR information that may be obtained.

PHASE ONE



PHASE TWO

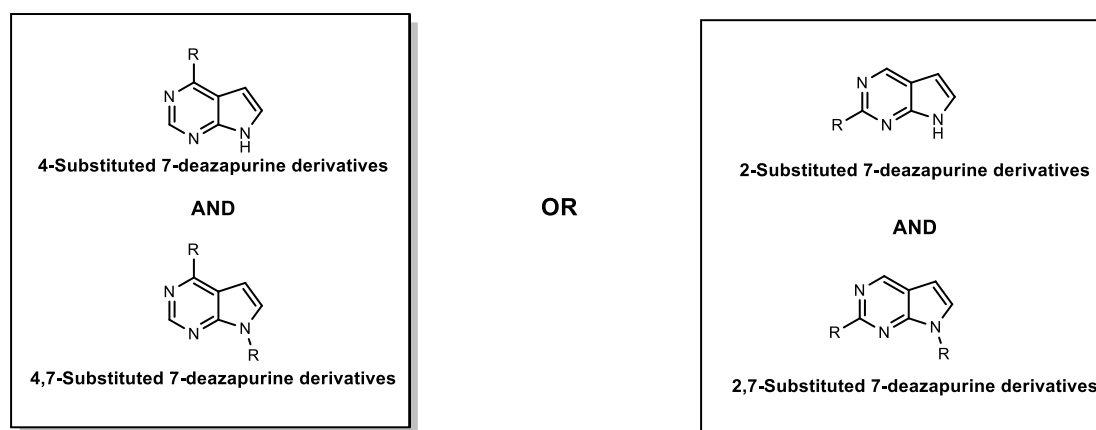


Figure 1.7: An illustration of the proposed structure for the study

1.7.1 Synthesis of proposed compounds

The synthesis of the final mono-substituted products is based on synthesis by means of Suzuki coupling, while the bis-substituted compounds will be synthesised by a combination of Suzuki coupling and Chan-Lam coupling. Suzuki-coupling refers to a carbon-carbon cross coupling reaction between organoboron compounds and organic halides or triflates (Kim *et al.*, 2002; Wolfe & Buchwald, 1999). In this case, the organic halide is the 7-azaindole and 7-deazapurine scaffolds, while a variety of boronic acids will act as organoboron compounds in the presence of tetrakis(triphenylphosphine)palladium(0) ($\text{Pd}(\text{PPh}_3)_4$) as catalyst. The Chan-Lam coupling represents the reaction between an aryl boronic acid and an alcohol or amine with a copper complex as catalyst (Qiao & Lam, 2011). Here the 7-deazapurine scaffold will play the role of amine in the presence of cupric acetate ($\text{Cu}(\text{OAc})_2$) and a collection of aryl boronic acids.

1.8 Conclusion

In conclusion, this study aims to synthesise 3 series of 7-azaindole derivatives (C4-, C5- and C6-substituted compounds) and 1 series 7-deazapurine derivatives (substituted on the optimal position determined in the first phase of the study). These compounds will be screened against the proposed panel of PKs in order to determine their inhibitory activity against said PKs. Potent novel PK inhibitors could lead to novel therapies in various diseases. Cancer, neurodegenerative diseases, diabetes and leishmaniasis have been selected as diseases applicable to this study and will be briefly discussed in **chapter 2**, which will aim to highlight the necessity for new treatment options. The articles that form **chapters 3** and **4** will give more insight into the synthesis and biological evaluation of each series.

CHAPTER 2: DISEASES RELATED TO THE PROPOSED PROTEIN KINASE PANEL

2.1 Introduction

Chapter 1 defined a proposed PK panel which consists of the following kinases: *HsCDK2/Cyclin A*, *HsCDK5/p25*, *HsCDK9/Cyclin T*, *HsHaspin*, *HsPim-1*, *SscCKδ/ε*, *SscGSK3α/β* and *LmCK1*. This chapter will take a glance at some of the diseases related to the activation of these PKs. Diseases such as cancer, neurodegenerative diseases (Alzheimer's disease (AD), Parkinson's disease (PD), Amyotrophic lateral sclerosis (ALS) and Huntington's disease (HD)), type 2 diabetes and leishmaniasis (*Leishmania major*).

Globally, cancer is the second leading cause of death, claiming an estimated 9.6 million lives in 2018 alone; thus, the shadow of cancer pervades modern life. The most significant cancer-induced deaths were: lung cancer (1.76 million), colorectal cancer (862 000), stomach cancer (783 000), liver cancer (782 000) and breast cancer (627 000) (WHO, 2018). Not discounting the social impact, cancer also inflicts an enormous economic burden on its victims and the caretakers of these victims. The World Cancer Report of 2020 states that the premature death and loss of productivity associated with cancer costs approximately US\$ 1.16 trillion per year (Wild *et al.*, 2020). Consequently, cancer is a growing socio-economic burden that needs to be addressed.

2.1.1 What is cancer?

Cancer is a broad term that encompasses a group of more than 100 different disease states. It customarily refers to malignancies that originate due to the transformation of genetic material, followed by the continuous mutations that inevitably result in a state of uncontrolled proliferation of progeny cells (Balmain *et al.*, 1993; Hanahan & Weinberg, 2000). The abovementioned process is known as carcinogenesis and is governed by six crucial alterations in cell physiology, namely: self-sufficiency in growth signals, insensitivity to growth-inhibitory (antigrowth) signals, avoidance of programmed cell-death (apoptosis), limitless replicative potential, sustained angiogenesis, as well as tissue invasion and metastasis (Hanahan & Weinberg, 2000).

2.1.2 How is it caused?

Carcinogenesis is thought to occur as the result of an interaction between certain genetic factors and external carcinogens. These external carcinogens are divided into three categories: physical carcinogens (such as ultraviolet and ionising radiation), chemical carcinogens (such as asbestos, components of tobacco smoke, aflatoxin in food and arsenic in drinking water) and biological carcinogens (such as infection with certain viruses, bacteria or parasites). Furthermore, the natural process of ageing is also regarded as a key factor in the development of cancer (WHO, 2018).

2.1.3 Current treatment options

Cancer therapy, at present, is mostly constrained to a series of invasive procedures. These procedures can include the insertion of catheters to allow the administration of chemotherapy to curtail existing cancer cells. It could also entail surgery to remove the tumour(s) where possible, followed by additional chemotherapy and radiation therapy (Brannon-Peppas & Blanchette, 2012). Some other strategies beside chemotherapy, surgery and radiation therapy exist, such as targeted therapies, hormone therapy and immunotherapy (WebMD Medical Reference, 2018).

2.1.3.1 Chemotherapy

Upon confirmation of a cancer diagnosis, chemotherapy is the option most likely to form part of a treatment regime. It is generally used as an adjuvant to localised options, like surgery and radiation. It has been the breath of life for a multitude of cancer survivors diagnosed with a variety of malignancies, including breast and colorectal cancers (Hutchinson *et al.*, 2012; Vardy & Tannock, 2007). The principle of chemotherapy rests on the administration of cytotoxic chemicals to kill the tumour cells, exploiting the cancer cells' susceptibility to chemotherapeutics. This susceptibility is deemed as a side effect of their accelerated growth rate (Brannon-Peppas & Blanchette, 2012; Nygren, 2001).

Regarding available chemotherapeutic agents, there are a myriad of options available for prescription. Naturally, not all chemotherapeutic agents fight cancer cells in the same manner, therefore, every chemotherapeutic drug is assigned to a class of drugs according to certain properties, such as their mechanism of action and chemical structure (Chabner *et al.*, 2006). Current chemotherapeutic drug classes include alkylating agents, antimetabolites, anti-tumour antibiotics, topoisomerase inhibitors and mitotic inhibitors (Chabner *et al.*, 2006; The American Cancer Society medical and editorial content team, 2016b). See **Table 2.1** for more information on the chemotherapeutic agents.

Table 2.1: Current chemotherapeutic agents and their drug classes

Chemotherapeutic Agents	
Drug class	Subclass and examples
Alkylating agents: Alkylation agents interfere with cell reproduction by damaging a cell's DNA. They are active in all phases of the cell cycle and are employed in the treatment of various cancers (like lung, breast, ovarian, leukaemia, lymphoma, Hodgkin's disease, multiple myeloma and sarcoma). It is important to note that the use of alkylation agents is accompanied by a dose-dependent risk of developing leukaemia in later years (highest risk 5 to 10 years after treatment) (The American Cancer Society medical and editorial content team, 2016a).	Nitrogen mustards: • mechlorethamine • cyclophosphamide • ifosfamide • chlorambucil Nitrosoureas: • chloroethyl diazohydroxide • isocyanate Platinum agents: • cisplatin • carboplatin • oxaliplatin
Mitotic inhibitors: Mitotic inhibitors have two main mechanisms of action. They function by preventing proliferation of cancer cells and also have the ability to halt production of the proteins needed by cancer cells to survive (The American Cancer Society medical and editorial content team, 2016a)	Mitotic inhibitors: • Docetaxel • Estramustine • Ixabepilone • Paclitaxel • Vinblastine • Vincristine • Vinorelbine
Antimetabolites: This class of drugs represents molecules that mimic natural substances required for healthy DNA function. When consumed, they alter the function of enzymes requisite in cell metabolism and protein synthesis. In effect antimetabolites starve cancer cells to death. Antimetabolites are deemed as cell cycle specific and are most valuable during the S-phase of cell division because they essentially "target" cells undergoing synthesis of DNA for the formation of new cells (SEER Training Modules, 2019)	Folic acid antagonist: • Methotrexate Pyrimidine antagonist: • 5-Fluorouracil • Foxuridine • Cytarabine • Capecitabine • Gemcitabine. Purine antagonist: • 6-Mercaptopurine • 6-Thioguanine. Adenosine deaminase inhibitor: • Cladribine • Fludarabine • Nelarabine • Pentostatin

Chemotherapeutic Agents (continued)	
Drug class	Subclass and examples
Antitumor antibiotics: Antitumor antibiotics are not to be confused with the antibiotics used in treating bacterial infections. This drug class' mechanism of action is cell cycle non-specific. Antitumor antibiotics negatively impact cell survival by binding with DNA and thereby impede RNA synthesis. RNA synthesis is critical to protein synthesis and the loss thereof equates cell death (SEER Training Modules, 2019).	Anthracyclines: • Doxorubicin • Daunorubicin • Epirubicin • Mitoxantrone • Idarubicin Chromomycins: • Dactinomycin • Plicamycin Miscellaneous: • Mitomycin • Bleomycin
Topoisomerase inhibitors: Topoisomerase inhibitors are part of a therapeutic class that act by disrupting the function of the topoisomerase enzymes I and II. Topoisomerase enzymes are typically responsible for controlling the manipulation of the DNA structure necessary for replication (Chemocare, 2019)	Topoisomerase I inhibitors: • Irinotecan • Topotecan Topoisomerase II inhibitors: • Amsacrine • Etoposide • Etoposide phosphate • Teniposide

2.1.3.2 Surgery

Surgery is the oldest type of cancer treatment. Surgical excision is regarded as the lynchpin in treatment of solid tumours; however, various additional types of surgery also play a role in the treatment of cancer (American Society of Clinical Oncology, 2019; Coffey *et al.*, 2003). A surgeon may opt to implement one of several alternative surgical techniques, see **Table 2.2**.

2.1.3.2.1 Diagnostic

On the whole, most types of cancer are diagnosed by means of biopsy, where a surgeon removes some or all of the questionable tissue by making an incision into the skin. The sample is evaluated by a pathologist, reported to the oncologist and diagnosed accordingly (American Society of Clinical Oncology, 2019).

2.1.3.2.2 Staging

Generally, staging surgery is employed in cases where it is necessary to determine the extent of a tumour and if or where the cancer has spread. This is achieved by removing lymph nodes in close proximity to the known cancer cells in order to establish if the cancer has spread (American Society of Clinical Oncology, 2019).

2.1.3.2.3 Curative surgery or tumour removal

In the case of curative surgery, the tumour and some of the adjacent healthy tissue that constitute the margins are removed. It is usually performed by large incisions through skin, muscle and sometimes bone, although, in some cases the surgery could be performed with less invasive techniques. Curative surgery may be performed as an exclusive treatment option or as adjuvant to chemotherapy and radiation therapy (American Society of Clinical Oncology, 2019).

2.1.3.2.4 Debulking surgery

Certain tumours cannot be removed in their entirety or the removal would cause too much damage to the surrounding tissues. These cases are dealt with by combining adjuvant therapies like radiation and chemotherapy with partial resection of the tumour in order to remove the greatest possible portion of the tumour (American Society of Clinical Oncology, 2019).

2.1.3.2.5 Palliative surgery

Palliative surgery aims to relieve some of the adverse effects caused by cancer; thereby improving the quality of life for patients in the advance stages of the disease. Reasons for palliative surgery include the relieve of pain, to stop bleeding, insert a feeding tube or prevent broken bones (American Society of Clinical Oncology, 2019)

2.1.3.2.6 Reconstructive surgery

In some cases, a patient may opt to have reconstructive surgery to restore the body to its pre-cancer appearance and function. A typical example of reconstructive surgery is breast reconstruction after a mastectomy (American Society of Clinical Oncology, 2019).

2.1.3.2.7 Preventative surgery

This type of surgery is done in order to reduce the risk of developing cancer. Examples of preventative surgery includes removal of polyps in the colon to prevent colon cancer and mastectomy or oophorectomy in the case of high risk for breast or ovarian cancer, respectively (American Society of Clinical Oncology, 2019).

Table 2.2: The alternative surgical techniques in cancer treatment

Alternative surgical techniques employed in the treatment of cancer	
Technique	Application
Cryosurgery: Cryosurgery is a procedure where extreme cold, generated by liquid nitrogen or argon gas, is employed to terminate abnormal tissue cells (National Cancer Institute, 2015).	• Early-stage skin cancer • Retinoblastoma • Precancerous growths of the skin and cervix
Laser therapy: Laser therapy refers to a treatment where cell tissue is destroyed by means of powerful beams of light. Laser beams allow for extreme accuracy and precision, making this treatment effective in tiny areas (National Cancer Institute, 2015)	• Basal cell carcinoma • Cervical cancer • Vaginal cancer • Oesophageal cancer • Non-small cell lung cancer
Hyperthermia: Hyperthermia is a treatment that implements high temperatures to small areas of the body. The high heat damages or kills the cancer cells or make them more sensitive to radiation and chemotherapy (National Cancer Institute, 2015)	• At present hyperthermia is not widely available and is currently being studied in clinical trials
Photodynamic therapy: Photodynamic therapy is based on using drugs that react to specific types of light. Once the drugs are administered and the cancer cells are exposed to light, the drugs become active and kill the nearest cells (National Cancer Institute, 2015)	• To ameliorate the symptoms of skin cancer, mycosis fungoides and non-small cell lung cancer

2.1.3.3 Radiation therapy

Radiation therapy (radiotherapy) is one of the principal therapies in the treatment of cancer and as the name suggests, it employs radiation (in high doses) to eliminate cancer cells and shrink tumours. Radiotherapy is a lengthy process; initially the onset of cell death is slow, ranging from days to weeks. Nonetheless, the cancer cells will continue to experience cell death for weeks to months after radiation was administered. There are two key types of radiation therapy, external beam therapy and brachytherapy. External beam therapy refers to the radiation therapy where high-energy beams are directed at the precise locations of the cancer from the outside, whereas brachytherapy relies on the implantation of a radiation source (seeds, ribbons or capsules) inside the body (Peschel & Colberg, 2003). The high doses of radiation impede the cellular growth of cancerous cells by damaging their DNA. Once the DNA is damaged beyond repair, the cells lose

their ability to proliferate and cell death follows. Subsequently, the cells are broken down and expelled from the body (Ling *et al.*, 2010). The aim of radiotherapy is to harm as few healthy cells as possible but some damage to healthy cells can be expected. Fortunately, healthy cells tend to exhibit more resilience toward radiation and are able to repair themselves (Mayo Clinic Staff, 2018)

2.1.4 The shortcomings of current cancer treatment

2.1.4.1 Chemotherapy

Unfortunately, chemotherapy does not come without challenges, resistance to cytotoxic drugs and several adverse effects plague the use of chemotherapy. Adverse effects, such as myelosuppression, higher risk of infection, bleeding, fatigue as well as nausea and vomiting often accompany chemotherapy. It is also not uncommon for patients to suffer from changes in cognitive functions (as reported by 10-50% of cancer patients) (Hutchinson *et al.*, 2012; Vardy & Tannock, 2007); but more worrying than the frequent adverse effects, is the fact that chemotherapy often exhibits an inadequate antitumour effect in the treatment of malignant tumours. Numerous tumour types (cancer of the lung, gastrointestinal tract and kidney) are resistant to most cytotoxic agents from the moment treatment commenced, while others react initially (cancer of the breast, ovaries and haematological malignancies) only to become resistant later on, even to drugs not part of the initial regime (Nygren, 2001; Yang & Warner, 1993).

2.1.4.2 Surgery

There is no discounting the fact that surgery is of paramount importance in the management of solid tumours. Especially as absolute resection can be wholly curative. Unfortunately, the metastatic recurrence of cancer cells after surgery is a great encumbrance, as it remains one of the main causes of morbidity and mortality. (Tavare *et al.*, 2012). Furthermore, reports on surgery in lung cancer indicate that a substantial number of patients die within the first six months after the surgery and the surviving patients experience continued pain and impairment in functional health (Handy Jr *et al.*, 2002). In breast cancer, surgery is accompanied by long-term persistent pain and sensory disturbances (Gärtner *et al.*, 2009), while another study found that patients that undergo surgery for rectal cancer, both male and female, are prone to developing sexual dysfunction (Hendren *et al.*, 2005). Regrettably, these findings eclipse some of the value of surgery in the treatment of cancer.

2.1.4.3 Radiation therapy

Although radiation therapy is regarded as one of the model therapies in cancer, the rationale of radiotherapy is precariously balanced on the ledge of delivering a high dose to the tumour cells while preserving healthy tissue. Thus, normal tissue tolerance and susceptible organs limit the amount of radiation that can be safely administered, hampering the maximum efficacy (Njeh, 2008). Additionally, failure of radiation therapy to eradicate tumour cells is a reality, as depicted by the following study: in patients receiving radiation therapy for unresectable non-oat cell carcinoma of the lung, the failure rate was determined to be 48 % with 4000 cGy continuously, 38% with 4000 cGy split course or 5000 cGy continuously and 27% for patients receiving 6000 cGy continuously. Among these patients the incidence of distant metastases was 70–80% (Perez *et al.*, 1987). Another study reported an unexpected high rate of complications in patients undergoing radiation therapy for meningiomas (Mathiesen *et al.*, 2003). Interestingly, radiation therapy can also temporarily or permanently damage patients' pacemakers while undergoing the therapy (Souliman & Christie, 1994).

2.1.4.4 Conclusion

Although current treatment methods are generally effective in the treatment of different types of cancer, it is clear to see from their shortcomings that to remain complacent with the available treatment is not an option. Therefore, it is imperative to engage in the study of novel therapeutic options. The discovery of novel drugs, like CDK2/CyclinA, CDK5/p25, CDK9/CyclinT, Haspin, Pim-1, CK1 δ/ϵ and GSK3 α/β inhibitors, could in the future allow for a complete change of treatment, one that is safer and more effective.

2.2 Neurodegenerative diseases

Neurodegenerative diseases are disorders that, by large, give rise to progressive and incapacitating cognitive, behavioural and motor dysfunctions. At present, neurological disorders are collectively deemed as the dominant cause of disability globally (Feigin *et al.*, 2019). During the initial stages of neurodegenerative diseases, misfolded proteins are inclined to selectively aggregate within small, vulnerable neuron populations of specific brain regions (Graveland *et al.*, 1985; Hyman *et al.*, 1984; Seeley *et al.*, 2006). Consequently, synapses cease to function as they should and the extent of damage spreads to envelope new regions; thus, initiating a progressive cycle of destruction followed by the worsening of clinical deficits (Selkoe, 2002).

2.2.1 Alzheimer's disease

AD is the embodiment of a progressive decline in cognitive function, more specifically, a progressive decline in memory, thinking and capacity for learning (Khachaturian, 1985). It is more prevalent among individuals aged 65 and above. It should be distinguished from the normal and expected age-induced decline in cognitive function, which occurs at a more gradual pace and is less debilitating. Customarily, AD originates with mild symptoms and ends in severe brain damage (Carr *et al.*, 1997).

This disease affects more than 30 million people worldwide and projections estimate that by 2050 the current prevalence will have tripled (Alzheimer's Association, 2010; Hebert *et al.*, 2003; Trojanowski, 2004; Weiner *et al.*, 2010). The problem of AD is exacerbated by the fact that there is no available cure and existing treatment is at best, inadequate (Alzheimer's Association, 2019). Thus, new advances in the field of AD are urgently needed and novel PK inhibitors such as GSK3 α/β , CDK5/p25 and CK1 δ/ϵ inhibitors, could prove to be beneficial in the treatment of AD. See **Table 2.3** for current available medicinal treatment in AD (Lau & Ahljanian, 2003; Parr *et al.*, 2012; Singh *et al.*, 1995).

Table 2.3: The current available treatment options in Alzheimer's disease

Drugs in Alzheimer's disease	
Drug class	Examples
Cholinesterase inhibitors Cholinesterase inhibitors bring about symptomatic relief of the disease by enhancing the levels of cell-to-cell communication by preserving acetylcholinesterase in the brain (NIH, 2018).	• Donepezil • Galantamine • Rivastigmine
Memantine Memantine is an N-methyl-D-aspartate (NMDA) antagonist that combats the progression of symptoms in moderate to severe AD. Its effects are elicited by blocking the toxic adverse effects associated with excess glutamate and assists with glutamate regulation (NIH, 2018).	• Memantine

2.2.2 Parkinson's disease

Parkinson's disease (PD) is a neurodegenerative disease that hardly needs any introduction. The clinical syndrome of PD is primarily characterised by the degeneration of the *substantia nigra pars compacta*, resulting in dopamine depletion (Ehringer & Hornykiewicz, 1960; Jankovic, 2008; Parkinson, 2002). Roughly 200 years from being first addressed, PD is the second most prevalent neurodegenerative disease and is now considered the neurological disease with fastest growth rate regarding prevalence, disability and mortality (Feigin *et al.*, 2017). In 2016, the incidence rate

of PD among people aged above 64 years was approximately 1% (a predicted 6.2 million people worldwide); this number is expected to double by 2040 (Dorsey & Bloem, 2018; Dorsey *et al.*, 2018).

Physically, PD is associated with diminished quality of life. The disease encumbers its victims with the characteristic muscle rigidity, resting tremor, bradykinesia and postural instability (Jankovic, 2008). In addition to the physical symptoms, PD also exacts a toll on normal cognitive function (Lees & Smith, 1983). Treatment of the disease has remained more or less unchanged in the last few decades. Levodopa and other dopamine replacement therapies (DRTs) forms the foundation of PD treatment since the 1960s (Kalia & Lang, 2016). Even though a great deal of the motor dysfunction associated with PD can be partially reversed by current treatment therapies, these treatments are fraught with challenges, see **Table 2.4** (Titova *et al.*, 2017). Therefore, the discovery of novel CDK5/p25 and/or GSK3 α / β inhibitors could serve to broaden the scope of current PD treatment (Parr *et al.*, 2012; Smith *et al.*, 2003).

Table 2.4: The current treatment options in Parkinson's disease and the challenges associated with each

Drugs in Parkinson's disease		
Drug class	Challenges	Examples
Levodopa: Levodopa is a dopamine precursor and provides symptomatic relief of some PD symptoms such as bradykinesia and rigidity (Fahn, 2006)	Levodopa use (long-term) has been linked to the development of dyskinesia and the likelihood of expediting neurodegeneration (Cotzias <i>et al.</i> , 1969; Parkinson Study Group, 2004).	Levodopa
Dopamine agonists: The dopamine agonists stimulate the dopamine receptors to bring about positive motor effects (Stocchi, 1998).	The use of these agents as monotherapy in PD is associated with a higher risk of inducing nausea, vomiting, hallucination, gastralgia and postural hypotension, especially in geriatric patients (Stocchi, 1998).	Ergot derivatives: - Bromocriptine - Pergolide - Lisuride - Cabergoline Non-ergot derivatives: - Apomorphine - Pramipexole - Ropinirole

Drugs in Parkinson's disease (continued)		
Drug class	Challenges	Examples
Dopa-decarboxylase (DDC) inhibitors: DDC inhibitors are administered in combination with levodopa to minimise the amount of levodopa that is peripherally metabolised by decarboxylase. This allows a higher concentration of levodopa to cross the blood-brain barrier and reduces the amount of adverse effects (Kaakkola, 2000; Tarsy, 2006).	This class of drug is not suitable for monotherapy as it does not cross the blood-brain barrier and its use is restricted to combination therapy with levodopa (Tarsy, 2006).	• Carbidopa • Benserazide
Catechol-O-methyltransferase (COMT) inhibitors: These agents are similar to the DDC inhibitors in the sense that they are also co-administered with levodopa to reduce the peripheral metabolism thereof. The only exception is that they block the COMT enzyme instead of the decarboxylase enzyme (Kaakkola, 2000).	The original derivatives in this class were non-selective, not very potent and worse of all, toxic. Hence, great care needs to be implemented when designing novel COMT-inhibitors to ensure selectivity and safe therapeutic use (Kaakkola, 2000).	• Tolcapone • Entacapone
Monoamine oxidase (MAO) inhibitors: The MAO inhibitors function by hindering the degradation of biogenic amine neurotransmitters like dopamine, thereby increasing the concentration of dopamine available in the synaptic cleft (Riederer & Laux, 2011).	Non-selective MAO inhibitors can cause severe hypertensive crisis and limits the drug class use to selective MAO-B inhibitors (Bernheimer <i>et al.</i> , 1962; Victor & Waters, 2003).	• Selegiline • Rasagiline
Muscarinic receptor antagonists: The mechanism of action of the anticholinergic agents in PD treatment is not completely understood. It is suggested that the drugs of this class affect the receptors that ordinarily mediate intrinsic cholinergic innervation in the neostriatum	The drugs of this class are only somewhat active against PD and cause uncomfortable side effects such as, urinary retention, constipation, confusion, sedation and cycloplegia (Standaert & Roberson, 2011).	• Trihexyphenidyl • Benztropine • Diphenhydramine

to have a positive effect in PD (Standaert & Roberson, 2011).		
---	--	--

Drugs in Parkinson's disease (continued)		
Drug class	Challenges	Examples
Amantadine: This drug was originally designed as an anti-viral drug used in treatment of Influenza A virus and was discovered to have a moderate effect in PD. Amantadine has an effect on dopamine release in the striatum, possess anticholinergic properties and also blocks NMDA glutamate receptors. It has not been defined which of these play a role in ameliorating PD (Standaert & Roberson, 2011).	Effects of this drug are only modest and adverse effects include sleep disturbance, lethargy, dizziness and anticholinergic effects.	Amantadine

2.2.3 Huntington's disease

Huntington's disease (HD) is a fatal neurodegenerative disorder. It is characterised as an autosomal-dominant, progressive disorder with a striking phenotype that presents as dystonia, incoordination, behavioural complications, cognitive deficits and chorea (Ross & Tabrizi, 2011; Walker, 2007). The disease is thought to be the result of mutations of the HTT gene and is estimated to affect 3 – 7 individuals per 100 000 people of European descent. Individuals from Chinese, Japanese and African ancestry are less often affected (Genetics Home Reference, 2020; Rawlins *et al.*, 2016). At present, the disease is incurable and treatment focusses on symptomatic relief. Treatment includes tetrabenazine (depletes dopamine), antipsychotic drugs such as risperidone and haloperidol (to improve chorea, control hallucinations and delusions) and antidepressants like citalopram, sertraline and fluoxetine (Cunha, 2019). Regarding the severity of the disease and the fact that current treatment is limited, the discovery of novel PK inhibitors (GSK3 α/β in this instance) might be of therapeutic value in HD (Parr *et al.*, 2012).

2.2.4 Amyotrophic lateral sclerosis

Amyotrophic lateral sclerosis (ALS), like PD, is an idiopathic neurodegenerative disorder and can also be classified as a fatal neurodegenerative disorder of the human motor system (Kiernan *et al.*, 2011). ALS presents with clinical features that involve both the upper and lower motor neuron pathways (Mitchell & Borasio, 2007). Symptoms include fatigue and reduced exercise capability, dysphagia, weight loss, malnutrition, dyspnoea, orthopnoea and hypoventilation. Once the patient develops dyspnoea at rest, death is imminent (Kiernan *et al.*, 2011; Leigh *et al.*, 2003; Ramirez *et al.*, 2008; Stambler *et al.*, 1998). Incidence of ALS has been reported as an estimated 5.2 cases per 100 000 individuals in the United States with total cases amounting to 16583 in 2015 (Mehta *et al.*, 2018). Like HD, ALS is incurable and the current treatment is only able to stall the inevitable disease progression. Drugs available for the treatment of ALS include edaravone and riluzole (Bhandari & Kuhad, 2018; Dharmadasa & Kiernan, 2018). Consequently, new PK inhibitors, specifically CDK5/p25 inhibitors, may broaden the number of option available in the treatment of ALS (Nguyen & Julien, 2003).

2.3 Type 2 diabetes mellitus

Type 2 diabetes mellitus (T2DM) is a metabolic disorder that is marked by a chronic increase in blood sugar and concomitant metabolic dysfunctions attributable to a lack of insulin or insulin resistance (Korsgren, 2020). T2DM is an encumbrance to more than 450 million people across the globe; a number that is expected to reach 650 million by 2040 (Khan *et al.*, 2020). Poor control of the condition could result in a prognosis that includes complications such as nephropathy, retinopathy, neuropathy and cardiovascular disease (Zheng *et al.*, 2018). The PK GSK α/β has been linked to T2DM and novel inhibitors thereof could augment the existing treatment options (Martinez *et al.*, 2002).

2.3.1 Current therapy for type 2 diabetes mellitus

Treatment of T2DM is centred around mitigating the symptoms associated with hyperglycaemia and preventing or reducing the acute and chronic complications of T2DM (Powers & D'Alessio, 2011). There are various pharmacological interventions that may be used in treatment of T2DM and will be briefly explored in **Table 2.5**. Notwithstanding the number of pharmacological options at hand, certain non-pharmacological interventions should always be considered in an attempt to modify the pathophysiological mechanisms of the disease. The combination of regular physical exercise and appropriate dietary interventions play a key role in the management of T2DM. These lifestyle changes have the power to not only augment glycaemic outcomes but also improve

outcomes of associated diseases such as dyslipidaemia, hypertension and other cardiovascular diseases (Raveendran *et al.*, 2018).

Table 2.5: The current pharmacological treatment in type 2 diabetes mellitus

Treatment of Type 2 diabetes mellitus		
Drug class	Shortcomings	Examples
Insulin therapy: Insulin is an anchor in the treatment of T2DM and aids glycaemic control by activation of the insulin receptors (Inzucchi <i>et al.</i> , 2015; Powers & D'Alessio, 2011).	Initiation of insulin therapy may be met with resistance by both patient and doctor. This is likely caused by the psychological impact of injection, the need for training, the patients perception that the disease has gotten worse and the possibility of hypoglycaemia (Collins, 2002; Inzucchi <i>et al.</i> , 2015).	Short-acting: - Aspart - Glulisine - Lispro - Regular insulin Long-acting: - Detemir - Glargine - NPH Combination-preparations: 75/25 protamine lispro: lispro 70/30 protamine aspart: aspart 50/50 protamine lispro: lispro 70/30 NPH: regular insulin
Sulfonylureas The sulfonylureas work by binding to a specific site on the β cell of the K_{ATP} channel complex, resulting in depolarisation of the membrane. Subsequently, a cascade of events causes insulin secretion (Powers & D'Alessio, 2011).	The agents of this class can cause significant weight gain. In addition, these agents (especially the longer-acting agents chlorpropamide and glyburide) have the ability to result in clinical hypoglycaemia that limits their use in T2DM (Collins, 2002).	First generation: - Chlorpropamide - Tolazamide - Tolbutamide Second-generation: - Glimepiride - Glipizide - Glyburide
Meglitinides The meglitinides are structurally unrelated to the sulfonylureas but like the sulfonylureas, exact insulin secretion by blocking K_{ATP} channels in the pancreatic β cells (Inzucchi <i>et al.</i> , 2015; Powers & D'Alessio, 2011).	The meglitinides have also been associated with weight gain and hypoglycaemia but less pronounced than with sulfonylurea administration (Collins, 2002).	- Repaglinide - Nateglinide

Treatment of Type 2 diabetes mellitus (continued)		
Drug class	Shortcomings	Examples
Biguanides The one drug in this class, metformin, acts as an AMP-dependant protein kinase (AMPK) activator. The activation of AMPK promotes fatty acid oxidation, nonoxidative metabolism and glucose uptake, whilst reducing lipogenesis and gluconeogenesis. This ultimately means increased glucose storage in skeletal muscle, diminished hepatic glucose production, increased insulin sensitivity and finally, lower blood glucose levels (Powers & D'Alessio, 2011).	Metformin is chiefly dependent on renal excretion and is therefore not advised in patients that suffer from renal impairment. It is also contra-indicated in metabolic acidosis, hepatic impairment, dehydration, sepsis, diabetic ketoacidosis, cardiogenic shock, acute congestive heart failure and myocardial infarction. In rare cases, metformin can cause lactic acidosis, a potentially fatal adverse effect. Lastly, metformin is to be used with caution in patients over the age of 75 (Collins, 2002).	Metformin
Thiazolidinediones: These compounds work by activating the peroxisome proliferating activating receptor γ (PPAR γ) to increase insulin sensitivity (Inzucchi <i>et al.</i> , 2015).	Thiazolidinediones have been linked to weight gain and fluid retention. Special care needs to be taken if the patient has heart failure; constant monitoring of the patient for signs and symptoms of worsening cardiac function (Collins, 2002).	- Rosiglitazone - Pioglitazone
GLP-1-Receptor agonists: Activates the incretin, GLP-1 receptor and stimulates insulin secretion (Inzucchi <i>et al.</i> , 2015; Powers & D'Alessio, 2011).	The GLP-1 receptor agonists have been associated with gastrointestinal upset such as nausea and vomiting and also an increase in heart rate. These agents are injectable and thus the patient needs training in administering (Inzucchi <i>et al.</i> , 2015).	- Exenatide - Liraglutide
Dipeptidyl Peptidase-4 (DDP-4) Inhibitors: DPP-4 is a serine protease that is essential in the inhibition of GLP-1. Thus, inhibition of DPP-4 allows for activation of GLP-1 (Powers & D'Alessio, 2011).	These agents may be associated with angioedema, urticaria and other dermatological side effects (Inzucchi <i>et al.</i> , 2015)	- Saxagliptin - Sitagliptin - Vildagliptin
Alpha Glucosidase Inhibitors: These agents work by decreasing the amount of starch, dextrin and disaccharides that in absorbed from the intestine by inhibiting alpha-glucosidase in the intestinal brush border (Powers & D'Alessio, 2011).	The use of alpha glucosidase inhibitors may result in gastrointestinal adverse effects such as abdominal discomfort, gas formation and loose stools (DeFronzo, 2000). These agents are rarely used as monotherapy in T2DM because their modest ability to reduce overall glucose levels (Collins, 2002).	Acarbose

2.3.2 Conclusion

Although there is no shortage of drugs in the treatment of T2DM, the discovery of novel compounds active against T2DM could result in therapy that could rival insulin's efficacy without having to be injected. It could also result in drugs that have little or no adverse effects. Therefore, exploring compounds to ascertain their value in GSK3 α/β inhibition could lead to novel treatment options in T2DM.

2.4 Leishmaniasis

Leishmaniasis refers to a collection of diseases originating from protozoan parasites from more than 20 *Leishmania* species (the PK relevant to *Leishmania* in this study, *LmCK1*, was extracted from the *Leishmania major* species). The parasites are transferred from vector to human by the bites of the infected female phlebotomine sandfly and can typically present in 3 different forms: visceral leishmaniasis (VL), cutaneous leishmaniasis (CL) and mucocutaneous leishmaniasis (MCL) (Khatami *et al.*, 2020; WHO, 2020).

VL is generally caused by the *L. donovani* and *L. infantum* species and is the most perilous of the three forms. In VL, the parasite infiltrates the internal organs (more so the liver, spleen and bone marrow) and when left untreated is fatal in more than 95% of cases (CDC, 2020; WHO, 2020). Individuals suffering from VL can present with sporadic spells of fever, weight loss (cachexia), enlargement of the liver and spleen (hepatosplenomegaly), pancytopenia (anaemia, leukaemia and thrombocytopaenia) and total high protein levels with low albumin and hypergammaglobulinemia (CDC, 2020; Chappuis *et al.*, 2007; WHO, 2020). The World Health Organization (WHO) considers VL as a parasitic disease with a high risk of outbreak and mortality; annual new cases are estimated at 50 000 – 90 000 worldwide (with only 25 – 45% reported to the WHO each year) (WHO, 2020). CL, as the term suggests, is a disease of the skin and typically exhibits as skin lesions (primarily ulcers) on the unsheltered parts of the body. These lesions tend to leave permanent scars and disability, leaving the victim open to stigma and discrimination (Bailey & Lockwood, 2007; Reithinger *et al.*, 2007; WHO, 2020). CL is predominantly associated with *L. major*, *L. aethiopica*, *L. tropica*, *L. donovani* and *L. infantum* in the Eastern hemisphere (Old World), whereas the *L. mexicana* species complex (*L. amazonensis*, *L. L. mexicana* and *L. venezuelensis*) and the subgenus *Viannia* (*L.[V.] braziliensis*, *L. [V.] guyanensis*, *L. [V.] peruviana* and *L. [V.] panamensis*) mainly exist in the Western hemisphere (New World) (CDC, 2020; Reithinger *et al.*, 2007). The WHO reports that between 600 000 and a million new CL cases arise annually (WHO, 2020). The last leishmaniasis form, ML, refers to leishmaniasis of the face, where partial or complete destruction of the mucous membranes of the

nose can be observed and is mainly limited to Brazil, Bolivia, Peru and Ethiopia (Minodier & Parola, 2007; WHO, 2020).

2.4.1 Treatment of Leishmaniasis

Despite great advances in medicine, there is still no single effective therapy against all *Leishmania* species and syndromes. Treatment is greatly dependent on the unique host and parasitic factors of each case; VL and ML should always receive treatment, while the necessity for treatment of CL should be evaluated per case (CDC, 2020; Copeland & Aronson, 2015).

2.4.1.1 Treatment of visceral leishmaniasis

Treatment of VL rests on a combination of systemic therapy and supportive care. Systemic therapy consist of liposomal amphotericin B (drug of choice), conventional amphotericin B deoxycholate, miltefosine and pentavalent antimonial (Sb^V) therapy (CDC, 2020; Phillips & Stanley, 2006). Supportive care complements systemic therapy by addressing anaemia, malnutrition and intercurrent infections. Unfortunately, the available systemic treatment has some drawbacks. These drawbacks are limited treatment options, toxicity (conventional amphotericin B deoxycholate), contra-indicated in pregnancy and breastfeeding (miltefosine) and drug resistance (liposomal amphotericin B and the pentavalent antimonial (Sb^V) therapy) (CDC, 2020; Copeland & Aronson, 2015; Hamill, 2013). Furthermore, because of the diverse nature of the disease, it is near impossible to standardise clinical guidelines (Copeland & Aronson, 2015).

2.4.1.2 Treatment of cutaneous and mucocutaneous leishmaniasis

Treatment of CL and ML often overlaps with treatment of VL. Parenteral preparations of conventional amphotericin B (rescue therapy), lipid amphotericin B, pentamidine isethionate and pentavalent antimonial (Sb^V) are utilised in CL and ML therapy. Treatment may also include oral agents such as miltefosine, ketoconazole, itraconazole and fluconazole. In some cases, local therapy such as thermotherapy, cryotherapy, intralesional administration of Sb^V and topical paromomycin, may be indicated (CDC, 2020; Phillips & Stanley, 2006).

2.4.2 Conclusion

Treatment of VL is essential for a victim's survival, while lack of CL treatment can cause severe disability. Even though treatment is considered as vital, limited treatment options and shortcomings hamper effective treatment of the disease. Considering that leishmaniasis generally affects some of the poorest people in the world, the development of novel antileishmanial therapy could be of great value to these communities.

CHAPTER 3: PUBLISHED ARTICLE

Medicinal Chemistry Research (2020) 29:1449–1462
<https://doi.org/10.1007/s00044-020-02560-1>

MEDICINAL
CHEMISTRY
RESEARCH

ORIGINAL RESEARCH



Synthesis and biological evaluation of selected 7-azaindole derivatives as CDK9/Cyclin T and Haspin inhibitors

Lianie Pieterse¹ · Lesetja J. Legoabe¹ · Richard M. Beteck¹ · Béatrice Josselin^{2,3} · Stéphane Bach^{2,3} · Sandrine Ruchaud²

Received: 28 March 2020 / Accepted: 7 May 2020 / Published online: 22 May 2020
© Springer Science+Business Media, LLC, part of Springer Nature 2020

Abstract

The 7-azaindole scaffold attracts attention due to its value in the design of inhibitors of diseases related protein kinases. However, this scaffold has not been evaluated against Haploid germ cell-specific nuclear protein kinase (Haspin). Herein, we report the synthesis of a select set of 7-azaindole derivatives and their evaluation against Haspin. The compounds were also evaluated against CDK9/Cyclin T kinase. The synthesis of 7-azaindole derivatives was achieved through Suzuki coupling using appropriate halogenated 7-azaindole and boronic acids. Seven of the screened compounds exhibited activity as CDK9/Cyclin T and/or Haspin inhibitors in a nanomolar to low micromolar range. The most promising dual inhibiting compound **18c**, exhibited an inhibitory potential of 0.206 μ M against CDK9/Cyclin T and 0.118 μ M against Haspin. The dual inhibition of CDK9/Cyclin T and Haspin could afford a potentially potent antimitotic agent of value in further anticancer studies.

Keywords Protein kinases · 7-Azaindole · anticancer · CDK9/ Cyclin T · Haspin

Introduction

The majority of the manifold cellular processes in the human body are vastly dependent on a superfamily of homologous proteins, known as protein kinases (PKs) (Hanks and Hunter 1995; Hunter 1995). The PKs that make up the human kinome tally up to a total of 518 PKs, thus accounting for 1.7% of human genes (Manning et al. 2002) and are integrally linked to the process of protein phosphorylation which regulates signal transduction, as these proteins facilitate the transfer of phosphoryl groups from

phosphate donors such as ATP, to receptor substrates that range from small molecules to lipids and proteins (Lawson et al. 2016). Signal transduction, in turn is linked to cell growth, metabolism, differentiation and apoptosis (Liao 2007). Therefore, any dysregulation of PKs (such as over-expression) could result in serious ramifications, the likes of which include, cell transformation and cancer, inflammation, diabetes and neurodegenerative diseases like Alzheimer's disease (Flight 2013; Huse and Kuriyan 2002; Noh and King 2007). Hence, PKs have become appealing targets for drug discovery, even being touted as the second most important drug targets after G-protein-coupled receptors (Cohen 2002).

This forms the backdrop against which the discovery of novel PK inhibitors plays out and indeed, a number of kinase inhibitors are in clinical use or undergoing clinical trials, like imatinib, for the treatment of chronic myelogenous leukaemia (CML) and trastuzumab in breast cancer (Fedorov et al. 2010). Nevertheless, these inhibitors target only a slight portion of the kinome and numerous clinically relevant kinases still remain relatively underexplored (Duong-Ly and Peterson 2013).

One such PK is the Haploid germ cell-specific nuclear protein kinase (Haspin). Haspin has only recently been added to the ranks of known PKs and is thought to phosphorylate histone H3 during mitosis (Higgins 2010).

Supplementary information The online version of this article (<https://doi.org/10.1007/s00044-020-02560-1>) contains supplementary material, which is available to authorized users.

✉ Lesetja J. Legoabe
lesetja.legoabe@nwu.ac.za

¹ Centre of Excellence for Pharmaceutical Sciences, North-West University, Private Bag X6001, Potchefstroom 2520, South Africa

² Sorbonne Université, CNRS, UMR 8227, Integrative Biology of Marine Models Laboratory (LBIM), Station Biologique de Roscoff, CS 90074, 29680 Roscoff Cedex, France

³ Sorbonne Université, CNRS, FR2424, Plateforme de criblage KISSf (Kinase Inhibitor Specialized Screening facility), Station Biologique de Roscoff, CS90074, 29680 Roscoff Cedex, France

It is believed that identification of Haspin inhibitors might supply valuable candidates for biological studies and cancer treatment. This belief stems from the following factors: (i) Haspin is deemed important in the regulation of mitosis and Haspin RNAi in tumour cell lines thwarts chromosome alignment and completion of the normal mitotic process (Dai et al. 2005; Dai et al. 2006). This suggests that Haspin inhibitors have the potential to act as novel antimetabolic agents with an aptitude for halting the proliferation of cancer cells (de Cárcer et al. 2007; Schmidt and Bastians 2007). (ii) Haspin mRNA is predominantly expressed in proliferative cells and not in non-dividing cells, making it another attractive target for cancer treatment (Higgins 2001a, b). (iii) Not only is the primary sequence of Haspin structurally different to that of other known kinases, but it also does not fit into any of the known eukaryotic kinase families and humans appear to have only one Haspin homologue, indicating that the design of highly selective Haspin inhibitors is probable (Higgins 2003; Higgins 2001a, b; Kannan et al. 2007). Thus, designing a study that aims to explore the Haspin target, may yield valuable novel knowledge in the field of PK inhibition and treatment of disease.

In general, PK inhibitors are largely designed to bind to the ATP binding site situated within the deep cleft between the N- and C-terminal lobes of the kinase catalytic domain. Binding is generally the product of hydrogen bond interactions with the hinge region that connects the two lobes (Liao 2007), and as such, the 7-azaindole scaffold (1) has been highlighted as a prime candidate for targeting PKs because of the scaffolds enhanced fit within the ATP binding site:

the pyridine N serves as a hydrogen bond acceptor, while the NH of the pyrrole ring acts as a hydrogen bond donor, together they form bidentate hydrogen bonds with the hinge region of the kinase (Irie and Sawa 2018; Mérour et al. 2014; Zuccotto et al. 2009). Therefore, it is hardly surprising that in recent years, the 7-azaindole scaffold has been widely explored in terms of PK inhibition and a few examples of PK successfully inhibited using the 7-azaindole scaffold include: anaplastic lymphoma kinase (ALK) (Gummadi et al. 2013), the aurora kinases (Bavetsias et al. 2013), cell division cycle 7 kinase (Cdc 7) (Harrington et al. 2013), C-Met kinase (Kim et al. 2008), DYRK1A (Gourdain et al. 2013), focal adhesion kinase (FAK) (Heinrich et al. 2013), I κ B kinase (IKK) (Liddle et al. 2009), KIT/FMS dual kinase (Zhang et al. 2013), p38 α mitogen-activated protein kinase (p38 α MAP) (Mavunkel et al. 2010), proto-oncogene serine/threonine-protein kinase 1 (PIM1) (Nakano et al. 2012), mammalian target of rapamycin kinase (m-TOR) (Tsou et al. 2010), tropomyosin-related kinase A (TrkA) (Hong et al. 2012) (see Fig. 1).

It is important to note that the 7-azaindole scaffold has never been explored for Haspin inhibition. Prompted by the relative novelty of the Haspin target and the general success of the 7-azaindole scaffold in PK inhibition, twenty-four 7-azaindole derivatives (divided into two series: 4-substituted 7-azaindoles and 5-substituted 7-azaindoles, see Tables 1 and 2), were synthesised and evaluated in vitro as potential Haspin inhibitors. The compounds were selected for their simplicity and comparatively inexpensive method of synthesis, while also being novel to the Haspin target. A SciFinder search of each compound was conducted to determine the novelty of each compound. Compounds **18c**, **18f**, **18h**, **18i**, **19e**, **19l** and **19o** had no known references in SciFinder and are deemed novel. In turn, compounds **18a** (Metz et al. 2011), **18b** (Arnold et al. 2007), **18d** (Ledeboer et al. 2006), **18e** (Allegretti et al. 2001) and **18g** (Ibrahim et al. 2007), **19a** (Ibrahim et al. 2007), **19b** (Huang et al. 2019), **19c** (Ibrahim et al. 2007), **19d** (Huang et al. 2019), **19f** (Ibrahim et al. 2007), **19g** (Huang et al. 2019), **19h** (Huang et al. 2019), **19i** (Huang et al. 2019), **19j** (Liu et al. 2019), **19k** (Huang et al. 2019), **19m** (Ibrahim et al. 2007) and **19n** (Ibrahim et al. 2007) have previously featured in literature. Alongside Haspin, the derivatives were screened against other PKs: (i) *HsCDK2/Cyclin A*, (ii) *HsCDK2/p25*, (iii) *HsCDK9/Cyclin T*, (iv) *HsPim-1*, (v) *SscCK1 δ/ϵ* , (vi) *SscGSK3 α/β* and (vii) *LmCK1*.

Materials and methods

All compounds were synthesised according to the general methods discussed in the experimental section. Starting materials were procured from AK Scientific, CHR-6494 and flavopiridol were purchased from Sigma Aldrich and were used without further purification, unless otherwise specified. Proton (¹H) and carbon (¹³C) nuclear magnetic resonance (NMR) spectra were recorded by means of a Bruker Avance III 600 spectrometer at frequencies of 600 MHz and 151 MHz, respectively, in deuterated dimethylsulfoxide (DMSO-d₆). The chemical shifts (δ) are reported in parts per million (ppm) and internally referenced to the signal of tetramethylsilane (TMS). Spin multiplicities are indicated as follow: s (singlet), d (doublet), dd (doublet of doublets), td (triplet of doublets), t (triplet), q (quartet) and m (multiplet). Coupling constants (*J*) are reported in Hz. High resolution mass spectra (HRMS) were recorded by means of a Bruker micrOTOF-Q II mass spectrometer in atmospheric pressure chemical ionisation (APCI) mode (positive ion polarity). High performance liquid chromatography (HPLC) analyses were performed by means of an Agilent 1100 HPLC system, using a Venusil XBP C18(2) (4.6 mm $\times$ 150 mm) 5 μ m column with mobile phase A (water + 0.1% formic acid) and mobile phase B (acetonitrile + 0.1% formic acid).

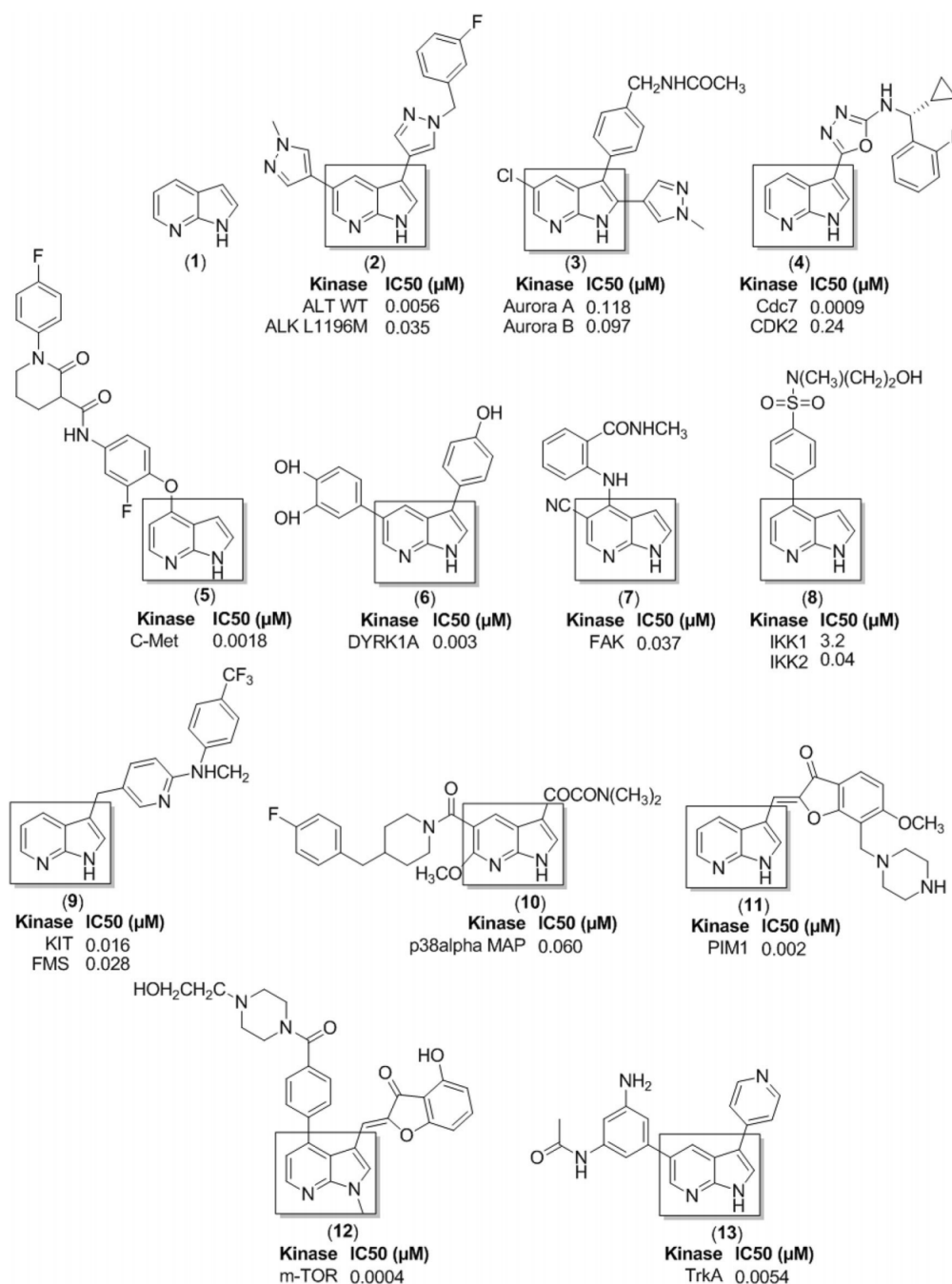


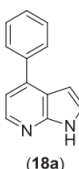
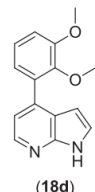
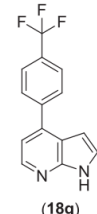
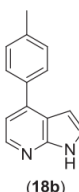
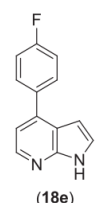
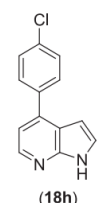
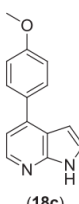
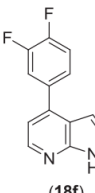
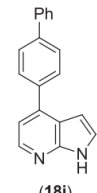
Fig. 1 A depiction of several compounds bearing the 7-azaindole structure known to inhibit various PKs. References: (2)(Gummadi et al. 2013), (3) (Bavetsias et al. 2013), (4) (Harrington et al. 2013), (5) (Kim et al. 2008), (6) (Gourdain et al. 2013), (7) (Heinrich et al. 2013),

(8) (Liddle et al. 2009), (9) (Zhang et al. 2013), (10) (Mavunkel et al. 2010), (11) (Nakano et al. 2012), (12) (Tsou et al. 2010), (13) (Hong et al. 2012)

against a gradient of 50% B (0–2 min), 100% B (2–8 min), 100% B (8–11 min), 50% B (11–11.1 min) and 50% B (11.1–13 min) at 22 °C. Melting points (mp) were

determined by a Buchi B545 melting point apparatus and are uncorrected. Thin layer chromatography (TLC) was accomplished using silica gel 60 TLC plates (Merck) and a

Table 1 Table depicting synthesis of 4-substituted 7-azaindole derivative, target compounds, and yields achieved

$ \begin{array}{c} \text{R}-\text{B}(\text{OH})_2 + \text{4-chloro-7-azaindole} \xrightarrow[\text{toluene:ethanol (3:1)}]{\text{K}_2\text{CO}_3, \text{Pd(PPh}_3)_4, \text{reflux overnight at } 120^\circ\text{C}} \text{4-substituted-7-azaindole} \\ \text{(15)} \qquad \qquad \qquad \text{(16)} \qquad \qquad \qquad \qquad \qquad \qquad \qquad \qquad \qquad \qquad \qquad \qquad \qquad \qquad \text{(18)} \end{array} $					
Compound	Yield (%)	Compound	Yield (%)	Compound	Yield (%)
 (18a)	42	 (18d)	38	 (18g)	75
 (18b)	39	 (18e)	34	 (18h)	39
 (18c)	29	 (18f)	63	 (18i)	59

UV254 fluorescent indicator to determine retention factor (R_f) using petroleum ether: ethyl acetate (1:1) as eluent.

Experimental

Chemistry

Numerous 4-, and 5-substituted 7-azaindole derivatives were prepared by means of Suzuki-Miyaura cross coupling between either 4-chloro-1*H*-pyrrolo[2,3-*b*]pyridine or 5-bromo-1*H*-pyrrolo[2,3-*b*]pyridine and an assortment of commercially available boronic acids in a single step reaction. Suzuki coupling affords an excellent platform for carbon-carbon bond formation and entails a palladium-catalysed cross-coupling reaction between organoboron compounds and organic halides or triflates (Kim et al. 2002; Wolfe and Buchwald 1999). Tetrakis(triphenylphosphine)palladium(0) (Pd(PPh₃)₄) served as the catalyst and the two series (4-substituted and 5-substituted 7-

azaindole derivatives) were obtained in varying yields of 11–82%. (see Table 1 and Table 3). Synthesis of the 5-substituted 7-azaindole derivatives (20a – 20o) proceeded smoothly as per standard literature methods (Littke and Fu 2002). Noteworthy, although the reaction for the 4-substituted 7-azaindole derivatives was performed with the same basic reagents, the reaction of 4-chloro-7-azaindole and appropriate boronic acid required a greater ratio of boronic acid (at least 4 equivalences of azaindole) for successful coupling. Thus, a total of 24 compounds were produced according to conditions discussed above. The synthesised compounds were confirmed by NMR and HRMS analysis.

General synthetic procedure for the selected 7-azaindole derivatives Compounds 18a–19o were synthesised by means of Suzuki coupling (also known as Suzuki-Miyaura coupling) under nitrogen atmosphere. To a stirred solution of either 4-chloro-7-azaindole for series 1 or 5-bromo-7-

Table 2 Percentage of residual activity for compounds **18a–b** and **19a–m** at 1 and 10 μ M using a representative kinase panel

Cpd	Hs CDK2/ CyclinA	Hs CDK5/ p25	Hs CDK9/ CyclinT	Hs Haspin	Hs PIM1	Ssc CK1 δ/ϵ	Ssc GSK3 α/β	Lm CK1
18a	64 (99)	66 (91)	5 (10)	19 (95)	50 (81)	54 (83)	70 (89)	72 (80)
18b	81 (97)	62 (≥ 100)	9 (26)	14 (29)	66 (99)	71 (86)	70 (91)	91 (96)
18c	73 (73)	53 (82)	14 (30)	10 (21)	10 (35)	79 (89)	78 (91)	79 (≥ 100)
18d	59 (99)	83 (≥ 100)	15 (44)	58 (63)	90 (98)	87 (86)	75 (91)	46 (62)
18e	53 (77)	6 (78)	6 (11)	15 (30)	60 (≥ 100)	67 (95)	87 (93)	63 (85)
18f	59 (≥ 100)	54 (75)	10 (26)	20 (47)	20 (39)	79 (95)	73 (≥ 100)	94 (99)
18g	≥ 100 (91)	71 (≥ 100)	24 (74)	18 (73)	29 (99)	82 (≥ 100)	73 (≥ 100)	70 (79)
18h	63 (71)	79 (96)	3 (15)	8 (28)	77 (87)	76 (86)	78 (86)	41 (56)
18i	≥ 100 (89)	98 (≥ 100)	70 (82)	73 (83)	≥ 100 (≥ 100)	93 (95)	≥ 100 (89)	19 (44)
19a	24 (77)	45 (61)	31 (94)	41 (73)	80 (≥ 100)	27 (57)	51 (86)	37 (≥ 100)
19b	63 (88)	75 (89)	54 (81)	50 (71)	≥ 100 (≥ 100)	55 (90)	68 (94)	66 (82)
19c	61 (≥ 100)	68 (≥ 100)	44 (62)	32 (64)	59 (≥ 100)	47 (84)	89 (91)	99 (81)
19d	≥ 100 (94)	78 (≥ 100)	78 (63)	50 (59)	46 (≥ 100)	68 (78)	96 (95)	56 (69)
19e	90 (≥ 100)	89 (94)	47 (69)	85 (71)	≥ 100 (77)	66 (79)	86 (94)	75 (66)
19f	78 (88)	50 (86)	60 (74)	71 (76)	31 (≥ 100)	59 (71)	88 (97)	52 (82)
19g	37 (79)	63 (93)	74 (63)	49 (56)	≥ 100 (≥ 100)	48 (67)	78 (71)	63 (≥ 100)
19h	79 (≥ 100)	75 (≥ 100)	54 (88)	43 (53)	≥ 100 (≥ 100)	61 (77)	66 (98)	61 (≥ 100)
19i	93 (≥ 100)	≥ 100 (≥ 100)	92 (≥ 100)	79 (81)	50 (78)	92 (99)	94 (≥ 100)	≥ 100 (≥ 100)
19j	65 (87)	64 (≥ 100)	71 (89)	≥ 100 (97)	83 (87)	72 (88)	71 (97)	≥ 100 (≥ 100)
19k	68 (93)	≥ 100 (88)	89 (73)	77 (62)	≥ 100 (98)	71 (63)	93 (78)	≥ 100 (61)
19l	≥ 100 (91)	84 (≥ 100)	72 (73)	73 (90)	≥ 100 (≥ 100)	69 (78)	83 (95)	36 (88)
19m	99 (≥ 100)	77 (93)	49 (81)	40 (64)	37 (58)	58 (89)	86 (92)	71 (83)
19n	61 (87)	20 (≥ 100)^a	21 (56)	7 (29)	39 (55)	49 (73)	60 (92)	62 (90)
19o	79 (≥ 100)	98 (≥ 100)	74 (81)	47 (52)	56 (≥ 100)	64 (81)	77 (93)	≥ 100 (≥ 100) ^a

Values represent the percentage of residual kinase activity for compounds used at 10 μ M and 1 μ M concentrations. The values obtained with compounds at 1 μ M are in bracket. The most significant results are presented in bold-italic. Activities were assessed in duplicate using 10 μ M ATP.

azaindole for series 2 (1.523 mmol), in 25 mL of a 3:1 toluene: ethanol mixture, Pd(PPh₃)₄ (0.023 mmol) and 1.6 mL of a 2 M K₂CO₃ solution, the appropriate boronic acid (1.523 mmol for series 1 and 6.092 mmol for series 2) was added. Followed by additional 0.5 mmol additions if deemed necessary during monitoring of TLC plates. The reaction mixture was stirred continuously under reflux (120°C) for at least 24 h and then monitored via TLC until complete. Subsequently, the reaction mixture was allowed to cool to room temperature and the solvent was removed under reduced pressure. The resulting residue was extracted with distilled water and dichloromethane (3 $\times$ 25 mL). The organic layers were combined and dried with MgSO₄. The solvent was once again removed under reduced pressure and the resulting product was recrystallized from a suitable solvent.

Physical properties and characterization The NMR spectra of 4/5-Halo-7-azaindole—starting material distinctly bears 7 carbons and 5 hydrogens peaks. The proton NMR

spectra of 4/5-Phenyl-7-azaindole derivatives: the target compounds bear no hydrogen on C4 in the case of 4-substituted compounds, or C5 in the case of 5-substituted compounds and the presence of additional aromatic signals suggest that aromatic moieties were successfully coupled. Furthermore, the singlet at approximately 12 ppm in ¹H-NMR is assignable to N-H, this signifies that no reaction took place at NH-position (position 1). The NMR spectra and other characterisation data can be found in the Supporting information.

4-Phenyl-1H-pyrrolo[2,3-b]pyridine (18a): compound was synthesised from phenylboronic acid and 4-chloro-7-azaindole in a yield of 42.37%, recrystallized from acetonitrile, dark beige crystals, mp 208 – 210 °C, R_f = 0.48 (1 petroleum ether: 1 ethyl acetate). ¹H NMR (DMSO-*d*₆, 600 MHz) δ 11.86 (1H, s, NH), 8.33 (1H, d, *J* = 4.8 Hz, H-6), 7.81 (2H, d, *J* = 7.6 Hz, H-2', H-6'), 7.60 (3H, m, H-2, H-3', H-5'), 7.50 (1H, t, *J* = 7.3 Hz, H-4'), 7.23 (1H, d, *J* = 4.8 Hz, H-5), 6.68–6.63 (1H, m, H-3). ¹³C NMR (DMSO-*d*₆, 150 MHz) δ 149.6 (C-7a), 143.4 (C-6), 140.7 (C-1'),

Table 3 A table depicting 5-substituted 7-azaindole derivative synthesis, compounds synthesised and yields achieved

$ \begin{array}{c} \text{R}-\text{B}(\text{OH})_2 + \text{Br}-\text{7-azaindole} \xrightarrow[\text{toluene:ethanol (3:1)}]{\text{K}_2\text{CO}_3, (\text{Pd}(\text{PPh}_3)_4), \text{reflux overnight at } 120^\circ\text{C}} \text{R}-\text{7-azaindole} \\ (15) \qquad (17) \qquad \qquad \qquad (19) \end{array} $					
Compound	Yield (%)	Compound	Yield (%)	Compound	Yield (%)
	63		46		34
	60		53		20
	63		43		45
	83		53		45
	11		42		61

139.0 (C-4), 129.5 (C-3', C-5'), 128.8 (C-3a), 128.7 (C-2', C-6'), 127.1 (C-4'), 117.7 (C-2), 114.7 (C-5), 99.4 (C-3). APCI-HRMS m/z : calc. for $\text{C}_{13}\text{H}_{11}\text{N}_2$ (MH^+), 195.0916, found 195.0920; Purity (HPLC): 98% (column: Venusil XBP C18(2) 4.6 mm $\times$ 150 mm; retention time: 15 min).

4-(4-Methylphenyl)-1H-pyrrolo[2,3-b]pyridine (18b): compound was synthesised from 4-methylphenylboronic acid and 4-chloro-7-azaindole in a yield of 38.80%, recrystallized from acetonitrile, yellow powder, mp 169–171 °C, Rf = 0.38 (4 petroleum ether: 1 ethyl acetate). ^1H NMR ($\text{DMSO}-d_6$, 600 MHz) δ 11.78 (1H, s, NH), 8.27 (1H, d, J = 4.7 Hz, H-6), 7.67 (2H, d, J = 7.9 Hz, H-2', H-6'), 7.53 (1H, d, J = 2.9 Hz, H-2), 7.36 (2H, d, J = 7.7 Hz, H-3'-H-5'), 7.16 (1H, d, J = 4.7 Hz, H-5), 6.60 (1H, d, J = 2.9 Hz, H-3), 2.39 (3H, s, Me-4'). ^{13}C NMR ($\text{DMSO}-d_6$, 150 MHz) δ 149.6 (C-7a), 143.4 (C-6), 140.7 (C-4'), 138.3 (C-1'), 136.1 (C-4), 130.0 (C-3', C-5'), 128.6 (C-2', C-6'), 126.9 (C-3a), 117.7 (C-2), 114.5 (C-5), 99.5 (C-3), 21.3

(Me-4'). APCI-HRMS m/z : calc. for $\text{C}_{14}\text{H}_{13}\text{N}_2$ (MH^+), 209.1073, found 209.1067; Purity (HPLC): 97% (column: Venusil XBP C18(2) 4.6 mm $\times$ 150 mm; retention time: 9 min).

0.4-(4-Methoxyphenyl)-1H-pyrrolo[2,3-b]pyridine (18c): compound was synthesised from 4-methoxyphenylboronic acid and 4-chloro-7-azaindole in a yield of 29.33%, recrystallized from acetonitrile, pale yellow powder, mp 212–212 °C, Rf = 0.18 (2 petroleum ether: 1 ethyl acetate). ^1H NMR ($\text{DMSO}-d_6$, 600 MHz) δ 11.75 (1H, s, NH), 8.25 (1H, d, J = 4.8 Hz, H-6), 7.73 (2H, d, J = 8.5 Hz, H-2', H-6'), 7.52 (1H, t, J = 2.8 Hz, H-2), 7.14 (1H, d, J = 4.4 Hz, H-5), 7.12 (2H, d, J = 8.6 Hz, H-3', H-5'), 6.61 (1H, d, J = 2.9 Hz, H-3), 3.84 (3H, s, MeO-4'). ^{13}C NMR ($\text{DMSO}-d_6$, 150 MHz) δ 159.9 (C-4'), 149.6 (C-7a), 143.4 (C-6), 140.5 (C-1'), 131.2 (C-4), 129.9 (C-2', C-6'), 126.8 (C-3a), 117.5 (C-2), 114.9 (C-3', C-5'), 114.3 (C-5), 99.5 (C-3), 55.7 (MeO-4'). APCI-HRMS m/z : calc. for $\text{C}_{14}\text{H}_{13}\text{N}_2\text{O}$ (MH^+),

225.1022, found 225.1017; Purity (HPLC): 99% (column: Venusil XBP C18(2) 4.6 mm × 150 mm; retention time: 7 min).

4-(2,3-Dimethoxyphenyl)-1H-pyrrolo[2,3-b]pyridine (18d): compound was synthesised from 2,3-dimethoxyphenylboronic acid and 4-chloro-7-azaindole in a yield of 37.73%, recrystallized from acetonitrile, sand coloured powder, mp 191–192 °C, Rf = 0.16 (4 petroleum ether: 1 ethyl acetate). ¹H NMR (DMSO-*d*₆, 600 MHz) δ 11.69 (1H, s, NH), 8.24 (1H, d, *J* = 4.8 Hz, H-6), 7.47–7.44 (1H, m, H-2), 7.18 (1H, t, *J* = 7.8 Hz, H-5'), 7.14 (1H, dd, *J* = 8.6, 1.2 Hz, H-4'), 7.05 (1H, d, *J* = 5.1 Hz, H-5), 6.99 (1H, dd, *J* = 7.4, 1.0 Hz, H-6'), 6.25 (1H, s, H-3), 3.87 (3H, s, MeO-2'), 3.48 (3H, s, MeO-3'). ¹³C NMR (DMSO-*d*₆, 150 MHz) δ 152.9 (C-3'), 148.8 (C-7a), 146.2 (C-6), 142.1 (C-2'), 137.9 (C-4), 132.4 (C-3a), 126.0 (C-5'), 124.0 (C-6'), 122.2 (C-1'), 118.7 (C-2), 116.0 (C-5), 112.9 (C-4'), 99.4 (C-3), 60.4 (C-11'), 55.8 (C-13'). APCI-HRMS *m/z*: calc. for C₁₅H₁₅N₂O₂ (MH⁺), 255.1128, found 255.1129; Purity (HPLC): 100% (column: Venusil XBP C18(2) 4.6 mm × 150 mm; retention time: 5 min).

4-(4-Fluorophenyl)-1H-pyrrolo[2,3-b]pyridine (18e): compound was synthesised from 4-fluorophenylboronic acid and 4-chloro-7-azaindole in a yield of 34.10%, recrystallized from acetonitrile, pale yellow powder, mp 219.3–219.7 °C, Rf = 0.44 (2 petroleum ether: 1 ethyl acetate). ¹H NMR (DMSO-*d*₆, 600 MHz) δ 11.84 (1H, s, NH), 8.28 (1H, d, *J* = 4.9 Hz, H-6), 7.82 (2H, dd, *J* = 8.5, 5.6 Hz, H-2', H-6'), 7.56 (1H, t, *J* = 3.0 Hz, H-2), 7.42–7.36 (2H, m, H-3', H-5'), 7.18 (1H, d, *J* = 4.9 Hz, H-5), 6.60 (1H, d, *J* = 3.3 Hz, H-3). ¹³C NMR (DMSO-*d*₆, 150 MHz) δ 162.7 (d, *J* = 245.5 Hz, C-4'), 149.6 (s, C-7a), 143.4 (s, C-6), 139.6 (s, C-5), 135.4 (d, *J* = 3.0 Hz, C-1'), 130.8 (d, *J* = 8.3 Hz, C-2', C-6'), 127.2 (s, C-3a), 117.6 (s, C-2), 116.4 (d, *J* = 21.4 Hz, C-3', C-5'), 114.7 (s, C-5), 99.3 (s, C-3). APCI-HRMS *m/z*: calc. for C₁₃H₁₀FN₂ (MH⁺), 213.082253, found 213.0826; Purity (HPLC): 99% (column: Venusil XBP C18(2) 4.6 mm × 150 mm; retention time: 5 min).

4-(3,4-Difluorophenyl)-1H-pyrrolo[2,3-b]pyridine (18f): compound was synthesised from 3,4-difluorophenylboronic acid and 4-chloro-7-azaindole in a yield of 62.68%, recrystallized from acetonitrile, pale orange crystals, mp 172–174 °C, Rf = 0.20 (4 petroleum ether: 1 ethyl acetate). (DMSO-*d*₆, 600 MHz) δ 11.93 (1H, s, NH), 8.35 (1H, d, *J* = 4.8 Hz, H-6), 7.90–7.84 (1H, m, H-5'), 7.71–7.65 (2H, m, H-2', H-4'), 7.64 (1H, t, *J* = 2.8 Hz, H-2), 7.26 (1H, d, *J* = 4.8 Hz, H-5), 6.68 (1H, m, H-3). ¹³C NMR (DMSO-*d*₆, 150 MHz) δ 150.9 (dd, *J* = 33.0, 12.7 Hz, C-4'), 149.6 (s, C-7a), 149.3 (dd, *J* = 34.6, 12.5 Hz, C-3'), 143.4 (s, C-6), 138.4 (s, C-4), 136.5 (dd, *J* = 5.0, 4.3 Hz, C-1'), 127.6 (s, C-3a), 125.7 (dd, *J* = 6.5, 3.2 Hz, C-2'), 118.6 (d, *J* = 17.2 Hz, C-6'), 117.7 (d, *J* = 17.6 Hz, C-5'), 117.5 (s, C-2),

114.8 (s, C-5), 99.2 (s, C-3). APCI-HRMS *m/z*: calc. for C₁₃H₉F₂N₂ (MH⁺), 231.0728, found 231.0736; Purity (HPLC): 98% (column: Venusil XBP C18(2) 4.6 mm × 150 mm; retention time: 4 min).

4-[4-(Trifluoromethyl)phenyl]-1H-pyrrolo[2,3-b]pyridine (18g): compound was synthesised from 4-trifluorophenylboronic acid and 4-chloro-7-azaindole in a yield of 75.44%, recrystallized from acetonitrile, sand coloured crystals, mp 204–205 °C, Rf = 0.13 (4 petroleum ether: 1 ethyl acetate). ¹H NMR (DMSO-*d*₆, 600 MHz) δ 11.90 (1H, s, NH), 8.33 (1H, d, *J* = 4.8 Hz, H-6), 7.99 (2H, d, *J* = 7.9 Hz, H-3', H-5'), 7.91 (2H, d, *J* = 7.9 Hz, H-2', H-6'), 7.62–7.58 (1H, m, H-2), 7.26 (1H, d, *J* = 4.8 Hz, H-5), 6.65–6.62 (1H, m, H-3). ¹³C NMR (DMSO-*d*₆, 150 MHz) δ 149.6 (s, C-7a), 143.5 (s, C-6), 143.0 (s, C-1'), 139.0 (s, C-4), 129.5 (s, C-3a), 129.1 (q, *J* = 31.7 Hz, C-4'), 127.7 (s, C-2', C-6'), 126.3 (q, *J* = 3.6 Hz, C-3', C-5'), 124.7 (d, *J* = 272.1 Hz, CF₃), 117.7 (s, C-2), 114.9 (s, C-5), 99.2 (s, C-3). APCI-HRMS *m/z*: calc. for C₁₄H₁₀F₃N₂ (MH⁺), 263.0790, found 263.0798; Purity (HPLC): 99% (column: Venusil XBP C18(2) 4.6 mm × 150 mm; retention time: 6 min).

4-(4-Chlorophenyl)-1H-pyrrolo[2,3-b]pyridine (18h): compound was synthesised from 4-chlorophenylboronic acid and 4-chloro-7-azaindole in a yield of 38.51%, recrystallized from acetonitrile, yellow powder, mp 222–223 °C, Rf = 0.24 (2 petroleum ether: 1 ethyl acetate). ¹H NMR (DMSO-*d*₆, 600 MHz) δ 11.81 (1H, s, NH), 8.29 (1H, d, *J* = 4.8 Hz, H-6), 7.79 (2H, d, *J* = 7.7 Hz, H-3', H-5'), 7.60 (2H, d, *J* = 7.6 Hz, H-2', H-6'), 7.57–7.55 (1H, m, H-2), 7.18 (1H, d, *J* = 4.8 Hz, H-2), 6.61–6.58 (1H, m, H-3). ¹³C NMR (DMSO-*d*₆, 150 MHz) δ 149.6 (C-7a), 143.4 (C-6), 139.3 (C-1'), 137.7 (C-4), 133.6 (C-4'), 130.4 (C-3', C-5'), 129.4 (C-2', C-6'), 127.3 (C-3a), 117.5 (C-2), 114.6 (C-5), 99.3 (C-3). APCI-HRMS *m/z*: calc. for C₁₃H₁₀ClN₂ (MH⁺), 229.0527, found 229.0537; Purity (HPLC): 83% (column: Venusil XBP C18(2) 4.6 mm × 150 mm; retention time: 6 min).

4-(Biphenyl-4-yl)-1H-pyrrolo[2,3-b]pyridine (18i): compound was synthesised from 4-biphenylboronic acid and 4-chloro-7-azaindole in a yield of 58.74%, recrystallized from acetonitrile, olive green powder, mp 266–267 °C, Rf = 0.43 (2 petroleum ether: 1 ethyl acetate). ¹H NMR (DMSO-*d*₆, 600 MHz) δ 11.81 (1H, s, NH), 8.30 (1H, d, *J* = 4.6 Hz, H-6), 7.88 (2H, d, *J* = 8.1 Hz, C-3', C11'), 7.85 (2H, d, *J* = 7.6 Hz, C-2', C12'), 7.76 (2H, d, *J* = 7.5 Hz, H-6', H-10'), 7.56 (1H, d, *J* = 2.8 Hz, H-2), 7.51 (2H, t, *J* = 7.4 Hz, H-7', H-9'), 7.41 (1H, t, *J* = 7.4 Hz, H-8'), 7.24 (1H, d, *J* = 4.6 Hz, H-5), 6.67 (1H, d, *J* = 2.2 Hz, H-3). ¹³C NMR (DMSO-*d*₆, 150 MHz) δ 149.7 (C-7a), 143.4 (C-6), 140.5 (C-4'), 140.2 (C-5'), 140.0 (C-1'), 138.0 (C-4), 129.5 (C-7', C-9'), 129.3 (C-3', C-11'), 128.2 (C-3a), 127.7 (C2', C12'), 127.3 (C8'), 127.2 (C-6', C-10'), 117.7 (C-2), 114.6 (C-5), 99.5 (C-3). APCI-HRMS *m/z*: calc. for C₁₉H₁₅N₂ (MH⁺),

271.1229, found 271.1224; Purity (HPLC): 89% (column: Venusil XBP C18(2) 4.6 mm × 150 mm; retention time: 5 min).

5-Phenyl-1H-pyrrolo[2,3-b]pyridine (19a): compound was synthesised from phenylboronic acid and 5-bromo-7-azaindole in a yield of 62.93%, recrystallized from ethanol, pale orange crystals, mp 160–162 °C, Rf = 0.57 (2 petroleum ether: 1 ethyl acetate). ¹H NMR (DMSO-*d*₆, 600 MHz) δ 11.71 (1H, s, NH), 8.52 (1H, d, *J* = 2.1 Hz, H-6), 8.21 (1H, d, *J* = 2.0 Hz, H-4), 7.71 (2H, d, *J* = 7.3 Hz, H-2', H-6'), 7.53–7.51 (1H, m, H-2), 7.48 (2H, t, *J* = 7.6 Hz, H-3', H-5'), 7.36 (1H, t, *J* = 7.3 Hz, H-4'), 6.52–6.50 (1H, m, H-3). ¹³C NMR (DMSO-*d*₆, 150 MHz) δ 148.6 (C-7a), 142.0 (C-6), 139.6 (C-1'), 129.5 (C-5), 128.7 (C-3', C-5'), 127.4 (C-4'), 127.4 (C-4), 127.3 (C-2', C-6'), 126.6 (C-2), 120.2 (C-3a), 100.7 (C-3). APCI-HRMS *m/z*: calc. for C₁₃H₁₁N₂ (MH⁺), 195.0916, found 195.0918; Purity (HPLC): 99% (column: Venusil XBP C18(2) 4.6 mm × 150 mm; retention time: 14 min).

5-(4-Methylphenyl)-1H-pyrrolo[2,3-b]pyridine (19b): compound was synthesised from *p*-tolylboronic acid and 5-bromo-7-azaindole in a yield of 59.94%, recrystallized from acetonitrile, brownish orange crystals, mp 182–184 °C, Rf = 0.71 (2 petroleum ether: 1 ethyl acetate). ¹H NMR (DMSO-*d*₆, 600 MHz) δ 11.71 (1H, s, NH), 8.49 (1H, d, *J* = 1.7 Hz, H-6), 8.16 (1H, d, *J* = 1.7 Hz, H-4), 7.59 (2H, d, *J* = 7.9 Hz, H-2', H-6'), 7.51 (1H, t, *J* = 2.8 Hz, H-2), 7.28 (2H, d, *J* = 7.9 Hz, H-3', H-5'), 6.52–6.47 (1H, m, H-3), 2.4 (3H, s, Me-4'). ¹³C NMR (DMSO-*d*₆, 150 MHz) δ 148.4 (C-7a), 141.8 (C-6), 136.7 (C-4'), 136.5 (C-1'), 130.1 (C-5), 128.6 (C-3', C-5'), 127.4 (C-4), 127.2 (C-2', C-6'), 126.3 (C-2), 120.2 (C-3a), 100.6 (C-3), 21.1 (Me-4'). APCI-HRMS *m/z*: calc. for C₁₄H₁₃N₂ (MH⁺), 209.1073, found 209.1071; Purity (HPLC): 87% (column: Venusil XBP C18(2) 4.6 mm × 150 mm; retention time: 11 min).

5-(3-Methoxyphenyl)-1H-pyrrolo[2,3-b]pyridine (19c): compound was synthesised from 3-methoxyphenylboronic acid and 5-bromo-7-azaindole in a yield of 63.05%, recrystallized from acetonitrile, pale yellow crystals, mp 140–141 °C, Rf = 0.63 (2 petroleum ether: 1 ethyl acetate). ¹H NMR (DMSO-*d*₆, 600 MHz) δ 11.73 (1H, s, NH), 8.52 (1H, d, *J* = 2.1 Hz, H-6), 8.22 (1H, d, *J* = 2.1 Hz, H-4), 7.52 (1H, t, *J* = 2.9 Hz, H-2'), 7.39 (1H, td, *J* = 7.7, 0.8 Hz, H-5'), 7.28 (1H, dd, *J* = 5.8, 2.0 Hz, H-4'), 7.25–7.23 (1H, m, H-2), 6.93 (1H, dd, *J* = 8.2, 2.4 Hz, H-6'), 6.52–6.50 (1H, m, H-3), 3.8 (3H, s, Me-3'). ¹³C NMR (DMSO-*d*₆, 150 MHz) δ 160.3 (C-3'), 148.6 (C-7a), 142.0 (C-6), 141.1 (C-1'), 130.5 (C-5), 128.5 (C-5'), 127.5 (C-6'), 126.7 (C-4), 120.1 (C-2), 119.7 (C-3a), 113.0 (C-4'), 112.7 (C-2'), 100.7 (C-3), 55.6 (Me-3'). APCI-HRMS *m/z*: calc. for C₁₄H₁₃N₂O (MH⁺), 225.1022, found 225.1030; Purity (HPLC): 69% (column: Venusil XBP C18(2) 4.6 mm × 150 mm; retention time: 8 min).

5-(4-Methoxyphenyl)-1H-pyrrolo[2,3-b]pyridine (19d): compound was synthesised from 4-methoxyphenylboronic acid and 5-bromo-7-azaindole in a yield of 82.70%, recrystallized from acetonitrile, mustard coloured crystals, mp 227–228 °C, Rf = 0.23 (2 petroleum ether: 1 ethyl acetate). ¹H NMR (DMSO-*d*₆, 600 MHz) δ 11.68 (1H, s, NH), 8.47 (1H, d, *J* = 1.9 Hz, H-6), 8.13 (1H, d, *J* = 1.8 Hz, H-4), 7.63 (2H, d, *J* = 8.1 Hz, H-3', H-5'), 7.50 (1H, t, *J* = 3.1 Hz, H-2), 7.04 (2H, d, *J* = 8.1 Hz, H-2', H-6'), 6.50–6.46 (1H, m, H-3), 3.80 (3H, s, Me-4'). ¹³C NMR (DMSO-*d*₆, 150 MHz) δ 159.0 (C-4'), 148.2 (C-7a), 141.7 (C-6), 132.0 (C-1'), 128.4 (C-5), 127.7 (C-2', C-6'), 127.3 (C-4), 126.0 (C-2), 120.2 (C-3a), 114.9 (C-3', C-5'), 100.5 (C-3), 55.6 (MeO-4'). APCI-HRMS *m/z*: calc. for C₁₄H₁₃N₂O (MH⁺), 225.1022, found 225.1023; Purity (HPLC): 100% (column: Venusil XBP C18(2) 4.6 mm × 150 mm; retention time: 11 min).

5-(2,3-Dimethoxyphenyl)-1H-pyrrolo[2,3-b]pyridine (19e): compound was synthesised from 2,3-dimethoxyphenylboronic acid and 5-bromo-7-azaindole in a yield of 11.11%, recrystallized from acetonitrile, white crystals, mp 136–138 °C, Rf = 0.36 (2 petroleum ether: 1 ethyl acetate). ¹H NMR (CDCl₃, 600 MHz) δ 10.19 (1H, s, NH), 8.54 (1H, d, *J* = 1.8 Hz, H-6), 8.15 (1H, d, *J* = 1.7 Hz, H-4), 7.39–7.37 (1H, m, H-2), 7.15 (1H, t, *J* = 7.9 Hz, H-5'), 7.02 (1H, dd, *J* = 7.7, 1.3 Hz, H-4'), 6.96 (1H, dd, *J* = 8.2, 1.2 Hz, H-6'), 6.56 (1H, dd, *J* = 3.3, 1.7 Hz, H-3), 3.94 (3H, s, Me-2'), 3.6 (3H, s, Me-3'). ¹³C NMR (CDCl₃, 151 MHz) δ 153.4 (C-3'), 148.0 (C-7a), 146.9 (C-6), 144.0 (C-2'), 134.2 (C-5), 129.7 (C-5'), 126.6 (C-6'), 125.4 (C-4), 124.4 (C-1'), 123.1 (C-2), 120.0 (C-3a), 111.7 (C-4'), 101.4 (C-3), 60.7 (MeO-2'), 56.1 (MeO-3'). APCI-HRMS *m/z*: calc. for C₁₅H₁₅N₂O₂ (MH⁺), 255.1128, found 255.1130; Purity (HPLC): 99% (column: Venusil XBP C18(2) 4.6 mm × 150 mm; retention time: 6 min).

5-(3,4-Dimethoxyphenyl)-1H-pyrrolo[2,3-b]pyridine (19f): compound was synthesised from 3,4-dimethoxyphenylboronic acid and 5-bromo-7-azaindole in a yield of 46.25%, recrystallized from acetonitrile, yellow crystals, mp 210–211 °C, Rf = 0.18 (2 petroleum ether: 1 ethyl acetate). ¹H NMR (DMSO-*d*₆, 600 MHz) δ 11.68 (1H, s, NH), 8.51 (1H, d, *J* = 2.0 Hz, H-6), 8.18 (1H, d, *J* = 1.9 Hz, H-4), 7.50 (1H, t, *J* = 2.8 Hz, H-2), 7.27 (1H, d, *J* = 1.9 Hz, H-2'), 7.22 (1H, dd, *J* = 8.2, 2.0 Hz, H-5'), 7.05 (1H, d, *J* = 8.3 Hz, H-6'), 6.50–6.48 (1H, m, H-3), 3.9 (3H, s, Me-3'), 3.8 (3H, s, Me-4'). ¹³C NMR (DMSO-*d*₆, 150 MHz) δ 149.6 (C-7a), 148.5 (C-4'), 148.3 (C-3'), 141.9 (C-6), 132.4 (C-1'), 128.7 (C-5), 127.3 (C-6'), 126.2 (C-4), 120.1 (C-2), 119.3 (C-3a), 112.8 (C-5'), 111.2 (C-2'), 100.5 (C-3), 56.1 (MeO-3'), 56.0 (MeO-4'). APCI-HRMS *m/z*: calc. for C₁₅H₁₅N₂O₂ (MH⁺), 255.1128, found 255.1124; Purity (HPLC): 100% (column: Venusil XBP C18(2) 4.6 mm × 150 mm; retention time: 11 min).

5-(4-Fluorophenyl)-1H-pyrrolo[2,3-b]pyridine (19g): compound was synthesised from 4-fluorophenylboronic acid and 5-bromo-7-azaindole in a yield of 52.63%, recrystallized from acetonitrile, dark yellow crystals, mp 182–184 °C, Rf = 0.48 (2 petroleum ether: 1 ethyl acetate). ¹H NMR (DMSO-*d*₆, 600 MHz) δ 11.74 (1H, s, NH), 8.49 (1H, d, *J* = 2.2 Hz, H-6), 8.19 (1H, d, *J* = 2.1 Hz, H-4), 7.76–7.73 (2H, m, H-2', H-6'), 7.53 (1H, t, *J* = 2.8 Hz, H-2), 7.32–7.29 (2H, m, H-3', H-5'), 6.52–6.49 (1H, m, H-3). ¹³C NMR (DMSO-*d*₆, 150 MHz) δ 162.1 (d, *J* = 243.6 Hz, C-4'), 148.5 (s, C-7a), 141.9 (s, C-6), 136.1 (d, *J* = 2.9 Hz, C-1'), 129.3 (d, *J* = 8.1 Hz, C-2', C-6'), 127.7 (s, C-5), 127.5 (s, C-4), 126.6 (s, C-2), 120.1 (s, C-3a), 116.2 (d, *J* = 21.3 Hz, C-3', C-5'), 100.6 (s, C-3). APCI-HRMS *m/z*: calc. for C₁₃H₁₀FN₂ (MH⁺), 213.0822, found 213.0823; Purity (HPLC): 100% (column: Venusil XBP C18(2) 4.6 mm × 150 mm; retention time: 8 min).

5-(3,4-Difluorophenyl)-1H-pyrrolo[2,3-b]pyridine (19h): compound was synthesised from 3,4-difluorophenylboronic acid and 5-bromo-7-azaindole in a yield of 43.02%, recrystallized from acetonitrile, mustard coloured crystals, mp 194–196 °C, Rf = 0.47 (2 petroleum ether: 1 ethyl acetate). ¹H NMR (DMSO-*d*₆, 600 MHz) δ 11.79 (1H, s, NH), 8.53 (1H, d, *J* = 2.2 Hz, H-6), 8.24 (1H, d, *J* = 2.2 Hz, H-4), 7.83 (1H, ddd, *J* = 12.2, 7.8, 2.2 Hz, H-2'), 7.59–7.56 (1H, m, H-5'), 7.55–7.53 (1H, m, H-2), 7.53–7.49 (1H, m, H-6'), 6.52–6.50 (1H, m, H-3). ¹³C NMR (DMSO-*d*₆, 150 MHz) δ 150.5 (dd, *J* = 157.2, 12.7 Hz, C-4'), 148.9 (dd, *J* = 157.2, 12.7 Hz, C-3'), 148.7 (s, C-7a), 141.9 (s, C-6), 137.3 (dd, *J* = 6.3, 3.6 Hz, C-1'), 127.7 (s, C-5), 126.8 (s, C-4), 126.5 (s, C-2), 123.9 (dd, *J* = 6.2, 3.1 Hz, C-6'), 120.1 (s, C-3a), 118.4 (d, *J* = 17.0 Hz, C-5'), 116.3 (d, *J* = 17.6 Hz, C-2'), 100.7 (s, C-3). APCI-HRMS *m/z*: calc. for C₁₃H₉F₂N₂ (MH⁺), 231.0728, found 231.0724; Purity (HPLC): 100% (column: Venusil XBP C18(2) 4.6 mm × 150 mm; retention time: 9 min).

5-[4-(Trifluoromethyl)phenyl]-1H-pyrrolo[2,3-b]pyridine (19i): compound was synthesised from 4-trifluorophenyl boronic acid and 5-bromo-7-azaindole in a yield of 52.88%, recrystallized from acetonitrile, pale yellow crystals, mp 236–237 °C, Rf = 0.50 (2 petroleum ether: 1 ethyl acetate). ¹H NMR (DMSO-*d*₆, 600 MHz) δ 11.84 (1H, s, NH), 8.59 (1H, d, *J* = 1.8 Hz, H-6), 8.31 (1H, d, *J* = 1.8 Hz, H-4), 7.96 (2H, d, *J* = 8.1 Hz, H-3', H-5'), 7.82 (2H, d, *J* = 8.1 Hz, H-2', H-6'), 7.56 (1H, d, *J* = 3.2 Hz, H-2), 6.54 (1H, d, *J* = 3.3 Hz, H-3). ¹³C NMR (DMSO-*d*₆, 150 MHz) δ 148.9 (s, C-7a), 143.7 (s, C-6), 142.1 (s, C-1'), 128.0 (s, C-5), 127.8 (s, C-4), 127.7 (d, *J* = 31.9 Hz, C-4'), 127.1 (d, *J* = 6.1 Hz, C-2', C-6'), 126.3 (d, *J* = 6.1 Hz, C-3', C-5'), 126.2 (s, C-2), 125.0 (d, *J* = 271.7 Hz, CF₃), 120.2 (s, C-3a), 100.9 (s, C-3). APCI-HRMS *m/z*: calc. for C₁₄H₁₀F₃N₂ (MH⁺), 263.0790, found 263.0787; Purity (HPLC): 100% (column: Venusil XBP C18(2) 4.6 mm × 150 mm; retention time: 10 min).

5-(4-Chlorophenyl)-1H-pyrrolo[2,3-b]pyridine (19j): compound was synthesised from 4-chlorophenylboronic acid and 5-bromo-7-azaindole in a yield of 70.40%, recrystallized from acetonitrile, gold crystals, mp 195–197 °C, Rf = 0.41 (2 petroleum ether: 1 ethyl acetate). ¹H NMR (DMSO-*d*₆, 600 MHz) δ 11.77 (1H, s, NH), 8.52 (1H, d, *J* = 2.2 Hz, H-6), 8.22 (1H, d, *J* = 2.0 Hz, H-4), 7.76–7.73 (2H, m, H-2', H-6'), 7.54–7.51 (3H, m, H-2, H-3', H-5'), 6.52–6.50 (1H, m, H-3). ¹³C NMR (DMSO-*d*₆, 150 MHz) δ 148.6 (C-7a), 141.9 (C-6), 138.5 (C-1'), 132.2 (C-4'), 129.4 (C-5), 129.1 (C-3', C-5'), 127.6 (C-2', C-6'), 127.3 (C-4), 126.6 (C-2), 120.1 (C-3a), 100.7 (C-3). APCI-HRMS *m/z*: calc. for C₁₃H₁₀ClN₂ (MH⁺), 229.0527, found 229.0536; Purity (HPLC): 100% (column: Venusil XBP C18(2) 4.6 mm × 150 mm; retention time: 8 min).

4-(1H-Pyrrolo[2,3-b]pyridin-5-yl)benzonitrile (19k): compound was synthesised from 4-cyanoboronic acid and 5-bromo-7-azaindole in a yield of 33.53%, recrystallized from acetonitrile, pale yellow crystals, mp 287–293 °C, Rf = 0.38 (2 petroleum ether: 1 ethyl acetate). ¹H NMR (DMSO-*d*₆, 600 MHz) δ 11.85 (1H, s, NH), 8.61 (1H, d, *J* = 2.2 Hz, H-6), 8.33 (1H, d, *J* = 2.1 Hz, H-4), 7.95 (2H, d, *J* = 8.5 Hz, H-3', H-5'), 7.93 (2H, d, *J* = 8.5 Hz, H-2', H-6'), 7.56 (1H, t, *J* = 3.3 Hz, H-2), 6.55–6.53 (1H, m, H-3). ¹³C NMR (DMSO-*d*₆, 150 MHz) δ 149.0 (C-7a), 144.3 (C-6), 142.1 (C-1'), 133.3 (C-3', C-5'), 128.0 (C-2', C-6'), 127.9 (C-5), 127.2 (C-4), 126.7 (C-2), 120.2 (C-3a), 119.5 (CN), 109.8 (C-4'), 101.0 (C-3). APCI-HRMS *m/z*: calc. for C₁₄H₁₀N₃ (MH⁺), 220.086924, found 220.0872; Purity (HPLC): 99% (column: Venusil XBP C18(2) 4.6 mm × 150 mm; retention time: 5 min).

5-(Biphenyl-4-yl)-1H-pyrrolo[2,3-b]pyridine (19l): compound was synthesised from 4-biphenylboronic acid and 5-bromo-7-azaindole in a yield of 19.90%, recrystallized from acetonitrile, white powder, mp 264–265 °C, Rf = 0.36 (2 petroleum ether: 1 ethyl acetate). ¹H NMR (DMSO-*d*₆, 600 MHz) δ 11.76 (1H, s, NH), 8.59 (1H, d, *J* = 2.1 Hz, H-6), 8.27 (1H, d, *J* = 2.0 Hz, H-4), 7.82 (2H, d, *J* = 8.3 Hz, H-2, H-12'), 7.78 (2H, d, *J* = 8.4 Hz, H-3', H-11'), 7.74 (2H, d, *J* = 7.3 Hz, H-6', H-10'), 7.55–7.53 (1H, m, H-2), 7.50 (2H, t, *J* = 7.7 Hz, H-7', H-9'), 7.39 (1H, t, *J* = 7.4 Hz, H-8'), 6.53 (1H, m, H-3). ¹³C NMR (DMSO-*d*₆, 150 MHz) δ 148.6 (C-7a), 141.9 (C-6), 140.2 (C-4'), 139.0 (C-5'), 138.6 (C-1'), 129.5 (C-7', C-9'), 128.1 (C-5), 127.9 (C-8'), 127.8 (C-3', C-11'), 127.7 (C-2', C-12'), 127.5 (C-4), 127.0 (C-6', C-10'), 126.5 (C-2), 120.2 (C-3a), 100.7 (C-3). APCI-HRMS *m/z*: calc. for C₁₉H₁₅N₂ (MH⁺), 271.1229, found 271.1222; Purity (HPLC): 73% (column: Venusil XBP C18(2) 4.6 mm × 150 mm; retention time: 7 min).

5-(Pyridin-3-yl)-1H-pyrrolo[2,3-b]pyridine (19m): compound was synthesised from pyridin-3-ylboronic acid and 5-bromo-7-azaindole in a yield of 44.78%, recrystallized from acetonitrile and ethanol, yellow crystals, mp 176–177 °C, Rf = 0.07 (2 petroleum ether: 1 ethyl acetate).

^1H NMR (DMSO- d_6 , 600 MHz) δ 11.81 (1H, s, NH), 8.95 (1H, d, $J = 1.9$ Hz, H-6), 8.58–8.55 (2H, m, C-2', C-4'), 8.30 (1H, d, $J = 1.9$ Hz, H-4), 8.15–8.12 (1H, m, H-6'), 7.55 (1H, t, $J = 2.8$ Hz, H-2), 7.51–7.48 (1H, m, H-5'), 6.55–6.52 (1H, m, H-3). ^{13}C NMR (DMSO- d_6 , 150 MHz) δ 100.8 (C-7a), 120.2 (C-2'), 124.4 (C-4'), 125.5 (C-6), 126.9 (C-6'), 127.7 (C-5), 134.7 (C-1'), 135.1 (C-4), 141.9 (C-2), 148.2 (C-5'), 148.4 (C-3a), 148.8 (C-3). APCI-HRMS m/z : calc. for $\text{C}_{12}\text{H}_{10}\text{N}_3$ (MH^+), 196.0869, found 196.0882; Purity (HPLC): 100% (column: Venusil XBP C18(2) 4.6 mm $\times$ 150 mm; retention time: 7 min).

5-(Pyridin-4-yl)-1H-pyrrolo[2,3-b]pyridine (19n): compound was synthesised from pyridin-4-ylboronic acid and 5-bromo-7-azaindole in a yield of 45.12%, recrystallized from acetonitrile, yellow powder, mp 211.3–214.0 °C, Rf = 0.07 (2 petroleum ether: 1 ethyl acetate). ^1H NMR (DMSO- d_6 , 600 MHz) δ 11.88 (1H, s, NH), 8.67 (1H, d, $J = 2.1$ Hz, H-6), 8.63 (2H, dd, $J = 5.3, 1.3$ Hz, H-3', H-5'), 8.40 (1H, d, $J = 2.1$ Hz, H-4), 7.79 (2H, dd, $J = 5.6, 1.5$ Hz, H-2', H-6'), 7.58–7.56 (1H, m, H-2), 6.57–6.54 (1H, m, H-3). ^{13}C NMR (DMSO- d_6 , 150 MHz) δ 150.6 (C-7a), 149.3 (C-3'-C-5'), 146.6 (C-6), 141.9 (C-1'), 128.0 (C-5), 127.00 (C-4), 125.4 (C-2), 121.7 (C-2', C-6'), 120.2 (C-3a), 101.0 (C-3). APCI-HRMS m/z : calc. for $\text{C}_{12}\text{H}_{10}\text{N}_3$ (MH^+), 196.0869, found 196.0886; Purity (HPLC): 99% (column: Venusil XBP C18(2) 4.6 mm $\times$ 150 mm; retention time: 5 min).

5-(6-Fluoropyridin-3-yl)-1H-pyrrolo[2,3-b]pyridine (19o): compound was synthesised from 2-fluoropyridine-5-boronic acid and 5-bromo-7-azaindole in a yield of 60.62%, recrystallized from acetonitrile, gold powder, mp 204–205 °C, Rf = 0.26 (2 petroleum ether: 1 ethyl acetate). ^1H NMR (DMSO- d_6 , 600 MHz) δ 11.82 (1H, s, NH), 8.59 (1H, d, $J = 2.4$ Hz, H-6), 8.55 (1H, d, $J = 2.2$ Hz, H-4'), 8.36–8.32 (1H, m, H-5'), 8.28 (1H, t, $J = 2.3$ Hz, H-2), 7.56 (1H, s, H-2'), 7.30 (1H, dd, $J = 8.8, 2.9$ Hz, H-6'), 6.52 (1H, m, H-3). ^{13}C NMR (DMSO- d_6 , 150 MHz) δ 162.8 (d, $J = 235.3$ Hz, C-4'), 148.8 (s, C-7a), 145.8 (d, $J = 15.0$ Hz, C-2'), 141.9 (s, C-6), 140.9 (d, $J = 7.9$ Hz, C-1'), 133.7 (d, $J = 4.5$ Hz, C-6'), 127.8 (s, C-5), 126.9 (s, C-4), 124.4 (s, C-2), 120.1 (s, C-), 110.1 (d, $J = 37.7$ Hz, C-5'), 100.7 (s, C-3). APCI-HRMS m/z : calc. for $\text{C}_{12}\text{H}_9\text{FN}_3$ (MH^+), 214.077502, found 214.0791; Purity (HPLC): 99% (column: Venusil XBP C18(2) 4.6 mm $\times$ 150 mm; retention time: 4 min).

Protein kinase assays

Kinase activities were assayed in duplicate and described in Nguyen et al (Nguyen et al. 2019) except for the following enzymes: (i) *LmCK1* (from *Leishmania major*, recombinant, expressed in bacteria) was assayed in buffer A with 170 μM of the following CK1-specific peptide substrate: RRKHAAGSpAYSITA (“Sp” stands for phosphorylated

serine); (ii) *SscCK1 δ/ϵ* (casein kinase 1 δ/ϵ , porcine brain, native, affinity purified) was assayed in buffer A with 170 μM of the CK1-specific peptide substrate; (iii) *SscGSK-3 α/β* (glycogen synthase kinase-3, porcine brain, native, affinity purified) isoforms were assayed in buffer A with 20 μM of GS-1 peptide, a GSK-3-selective substrate (YRRAAVPPSPSLSRHSSPHQSpEDEEE). Composition of buffer A: 10 mM MgCl_2 , 1 mM EGTA, 1 mM DTT, 25 mM Tris-HCl pH 7.5, 50 $\mu\text{g}/\text{mL}$ heparin. The peptides were obtained from Proteogenix (Oberhausbergen, France).

To measure the kinase activities, we used the ADP-GloTM assay kit according to the recommendations of the manufacturer (Promega, Madison, WI). This assay is a luminescent ADP detection assay that provides an homogeneous and high-throughput screening method to measure kinase activity by quantifying the amount of ADP produced during a kinase reaction. Briefly, the reactions were carried out in 384-well plates in a final volume of 6 μL for 30 min at 30 °C in appropriate kinase buffer, with either protein or peptide as substrate in the presence of 10 μM ATP. After that, 6 μL of ADP-GloTM Kinase Reagent was added to stop the kinase reaction. After an incubation time of 50 min at room temperature (RT), 12 μL of Kinase Detection Reagent was added for one hour at RT. The transmitted signal was measured using the Envision (PerkinElmer, Waltham, MA) microplate luminometer and expressed in Relative Light Unit (RLU). The compounds were first diluted at 10 mM in DMSO (stock solution) before to be analyzed as described hereafter. In this study, we used a two-step process to evaluate the effect of the 7-azaindole derivatives on kinase activity: (Step 1) As primary screening, the compounds were tested at 10 and 1 μM against a panel of eight disease-related kinases. Activities were expressed in % of maximal activity, i.e. measured with dimethyl sulfoxide (DMSO) diluted at 1% (v/v) concentration; (Step 2) The more active compounds (dubbed “hits”) were next tested in duplicate over a wide range of concentrations (usually from 0.0003 to 10 μM) against the targets identified during “step 1”, here Haspin and CDK9/CyclinT, and IC_{50} values were determined from the dose response curves using Prism-GraphPad (GraphPad Software, San Diego, CA, USA).

Cytotoxicity prediction

The cytotoxicity for the most promising compounds were predicted using CLC-Pred: in silico prediction of cytotoxicity for tumour and non-tumour cell-lines (Lagunin et al. 2018). CLC-Pred was developed by using the previously developed Prediction of Activity Spectra for Substances (PASS) algorithm to create and validate the classification structure-activity relationship (SAR) models for predicting

the cytotoxicity of structures against various types of human cell lines using ChEMBL experimental data. The experimental data from ChEMBL (IG₅₀, IC₅₀ and % inhibition values) forms the basis for a training set of 59882 structures of compounds and the average accuracy of predicting area-under-the-curve (AUC) is given as 0.930 and 0.927 for 278 cancer cell-lines, when calculated by leave-one-out and 20-fold cross-validation procedure, respectively (Lagunin et al. 2018).

Results and discussion

7-Azaindole derivatives have been investigated against numerous PKs wherein they have exhibited good inhibition potentials (see Fig. 1). The inhibitory potential of this compound class against Haspin, a kinase with less similarity to those mentioned in Fig. 1, has not been investigated (Amoussou et al. 2018). Hence, in the current study a library of twenty-four 7-azaindole derivatives was synthesised and screened against Haspin. The compounds were also screened against a panel of other PKs. The screening panel consisted of kinases: *HsCDK2/CyclinA*, *HsCDK5/p25*, *HsCDK9/CyclinT*, *HsHaspin*, *HsPIM1*, *SscCK1δ/ε*, *SscGSK3α/β* and *LmCK1*. *Haspin* is of particular interest as a novel biological target with regards to these compounds.

Initially, the compounds were screened at concentrations of 10 and 1 μM (diluted in 1% DMSO v/v). Only the compounds exhibiting inhibition greater than 70% at 1 μM (as arbitrary threshold) underwent more thorough testing (at a wide range of concentrations, usually 0.0003 μM–10 μM) and IC₅₀ values were determined by means of dose-response curves (Prism-GraphPad). The results of the in vitro kinase assays can be found in Table 3 and Table 4.

The 7-azaindole derivatives of this study proved to exhibit inhibitory activity against two of the eight PKs in the panel, namely, CDK9/Cyclin T and Haspin. Interestingly, the compounds that were deemed active (**18a**, **18b**, **18c**, **18e**, **18f**, **18h**, & **19n**) all belong to the 4-substituted 7-azaindole derivatives, with the exception of one 5-substituted 7-azaindole derivative, **19n**. Their 5-substituted 7-azaindole counterparts **19a**, **19b**, **19d**, **19g**, **19h** and **19j** lacked significant activity, thus, it can be postulated that substitution on the 4-position may greatly improve inhibition of CDK9/Cyclin T and Haspin. Moreover, active compounds seem to favour inhibition of CDK9/Cyclin T over Haspin with inhibition ranging from 0.063 μM (**18e**) ≤ IC₅₀ ≤ 1.2 μM (**18h**) for CDK9/Cyclin T and 0.118 μM (**18c**) ≤ IC₅₀ ≤ 1.016 μM (**18e**) for Haspin.

Considering solely the inhibitory activity for CDK9/CyclinT, the best results were achieved by substituting the 4-position of the 7-azaindole with a 4'-fluorophenyl

Table 4 Kinase inhibitory activity of compounds selected after primary screening (compounds inhibiting >70% of the kinase activity at 1 μM). Activities were assessed in duplicate using 10 μM ATP

Cpd	<i>HsCDK9/CyclinT</i>	<i>HsHaspin</i>
18a	0.081	NP
18b	0.481	0.221
18c	0.206	0.118
18e	0.063	1.016
18f	0.137	NP
18h	1.200	0.284
19n	NP	0.664
Flavopiridol	0.015	NP
CHR-6494	NP	0.055

Values represent IC₅₀ reported in μM. Kinase activities were assessed in duplicate

NP Not performed

substituent (**18e**), which exhibited inhibitory activity against CDK9/CyclinT of 0.063 μM, followed closely by substitution with an unsubstituted phenyl ring (**18a**), which also achieved sub-micromolar activity (0.081 μM). Comparison of the active 4-substituted 7-azaindoles with the 4-substituted compounds that did not exhibit significant activity, revealed that the molecular weight of the substituent (in addition to position of the substituent) may have an impact of inhibitory activity against CDK9/Cyclin T. The compounds deemed active all bear a substituent of low molecular weight (< 130 g/mol), where 130 g/mol per substituent is surpassed (**18d**, **18g**, **18i**), the compound has lower or insignificant inhibitory CDK9/Cyclin T activity in comparison to the active 4-substituted 7-azaindole derivatives. In conjunction with molecular weight, inhibitory activity for CDK9/Cyclin T may also be governed by the electronegativity of the substituent. Three compounds bearing comparable electronegative *para*-substituted phenyl rings were assessed, it was found that the more electronegative the atom (electronegativity: F > OCH₃ > CH₃), the better the inhibitory activity of the compound (CDK9/CyclinT activity: **18e** > **18c** > **18b**) see Fig. 2. However, the impact exacted by molecular weight of substituents seems to outweigh the impact exacted by the electronegativity of substituents, as both **18g** and **18h** are highly electronegative, but exhibit only a poor activity and relatively low inhibitory activity (e.g. IC₅₀ of 1.2 μM for **18h**). Haspin, like CDK9/Cyclin T, seems to favour the lower molecular weight 4-substituted 7-azaindole derivatives (**18b**, **18c**, **18e**, **18h**) with regards to inhibitory activity, with the exception of **18a** (substituted with an unsubstituted phenyl ring) which exhibits no significant inhibitory activity.

Regarding the cytotoxicity of the active compounds, the CLC-Pred prediction platform was used to predict the

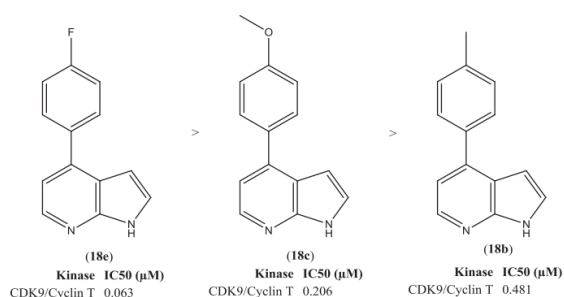


Fig. 2 Examples of 7-azaindole derivatives showing the significance of substitution with a more electronegative substituent in the para-position of the phenyl ring

cytotoxicity of these compounds (Lagunin et al. 2018). All of the active compounds (**18a**, **18b**, **18c**, **18e**, **18f**, **18h**, & **19n**) were predicted to have a cytotoxicity score higher than at least 60% of the compounds in the training set for oligodendrogliomas (Hs 683). Compound **18h**, exhibited the highest predicted cytotoxicity score (0.787), meaning that **18h** can be expected to be more cytotoxic against oligodendrogliomas than 78.7% of the compounds in the training set. Remarkably, the only active compound from the second series **19n**, is also the only active compound that is predicted to have cytotoxic activity against more than one cell-line, oligodendroglioma and malignant melanoma (Hs 683: 0.683 and A-375: 0.545) (see Table 5).

Overall, 7 active inhibitors of CDK9/Cyclin T and/or Haspin have been identified (**18a**, **18b**, **18c**, **18e**, **18f**, **18h**, & **19n**) with a high likelihood of being active against oligodendrogliomas. Thus, these compounds may have value as potential antimitotic agents in cancer therapy.

Conclusions

This article describes the synthesis of selected 4-, and 5-substituted azaindole derivatives, formed by the combination of the 7-azaindole scaffold and appropriate boronic acids. Protein kinase assays unveiled seven promising compounds; two compounds exhibited selective activity against CDK9/Cyclin T and one compound selectively against Haspin, while four compounds were identified as dual-target inhibitors of both CDK9/Cyclin T and Haspin, all with IC₅₀ values in a nanomolar to low micromolar range. Furthermore, compound **18c** not only proved to be the most potent of the target inhibitors, but also, when considered separately, was identified as the compound with the best Haspin inhibitory activity (IC₅₀ value of 0.118 μM against Haspin and 0.206 μM against CDK9/Cyclin T). Regarding CDK9/Cyclin T, compound **18e** reigned supreme with an IC₅₀ value of 0.063 μM. Both Haspin and CDK9/Cyclin T, have been connected to normal cell

Table 5 Predicted cytotoxicity most promising compounds (compounds inhibiting >70% of the kinase activity at 1 μM)

Cpd	Probability to be active (Pa)	Cell-line	Cell-line full name
18a	0.721	Hs 683	Oligodendroglioma
18b	0.636	Hs 683	Oligodendroglioma
18c	0.652	Hs 683	Oligodendroglioma
18e	0.759	Hs 683	Oligodendroglioma
18f	0.734	Hs 683	Oligodendroglioma
18h	0.787	Hs 683	Oligodendroglioma
19n	0.638	Hs 683	Oligodendroglioma
	0.545	A-375	Malignant melanoma
Flavopiridol	0.509	MRC5	Embryonic lung fibroblast (non-tumour cell-line)
CHR-6494	0.385	A-375	Malignant melanoma
	0.328	PA-1	Ovarian carcinoma
	0.319	SJSA-1	Osteosarcoma
	0.317	RKO	Colon carcinoma
	0.338	Kasumi 1	Childhood acute
	0.368	DMS-114	myeloid leukaemia with
	0.357	NCI-H838	maturation
	0.303	SK-MEL-1	Lung carcinoma
	0.307	MDA-MB-453	Non-small cell lung cancer
			Metastatic melanoma
			Breast carcinoma

Pa values represent the percentage of active compounds (from the training set) that is predicted to have a lower cytotoxicity for a specific cell-line than the investigated compound (Lagunin et al. 2018)

proliferation and have been identified as targets in cancer therapy, thus compounds **18a**, **18b**, **18c**, **18e**, **18f**, **18h** and **19n** may potentially be of value as novel anti-proliferative agents (Huertas et al. 2012; Walsby et al. 2014). Furthermore, it is postulated that compound **18c** will make an excellent lead compound for further exploration of 7-azaindole derivatives as Haspin inhibitors.

Acknowledgements The authors are grateful to Dr. D.Otto and Dr. J. Jordaan of the SASOL Centre for Chemistry, North-West University, for recording the MS and NMR spectra, respectively. The authors thank the “Ligue contre le Cancer du Grand-Ouest” comity, (districts: 29, 22, 56, 35, 45, and 79), Cancéropôle Grand Ouest (axis: natural sea products in cancer treatment network), IBISA (French Infrastructures en sciences du vivant: biologie, santé et agronomie) and Biogenouest (Western France life science and environment core facility network) for supporting the KISSf screening facility (FR2424, CNRS and Sorbonne Université), Roscoff, France.

Compliance with ethical standards

Conflict of interest Stéphane Bach is a founder and member of the SAB of SeaBeLife Biotech (Roscoff, France). This company is developing novel therapies for treating liver and kidney acute disorders. This work was conducted in the absence of any commercial or

financial relationships that could be construed as a potential conflict of interest. The authors declare that they have no conflict of interest

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

References

- Allegretti M, Arcadi A, Marinelli F, Nicolini L (2001) Palladium-catalysed functionalisation at 4-and 6-position of the 7-azaindole system. *Synlett* 5:0609–0612
- Amoussou NG, Bigot A, Roussakis C, Robert J-MH (2018) Haspin: a promising target for the design of inhibitors as potent anticancer drugs. *Drug Discov Today* 23:409–415
- Arnold LD, Chen X, Dong H, Garton A., Mulvihill MJ, Smith CPS, Thomas GH, Krulle TM, Wang J. (2007) Fused heterobicyclic kinase inhibitors. US20070208053A1. Accessed from <https://patents.google.com/patent/US20070208053A1/en> on 06/05/2020
- Bavetsias V, Faisal A, Crumpler S, Brown N, Kosmopoulou M, Joshi A, Atrash B, Pérez-Fuertes Y, Schmitt JA, Boxall KJ (2013) Aurora isoform selectivity: design and synthesis of imidazo [4, 5-b] pyridine derivatives as highly selective inhibitors of Aurora-A kinase in cells. *J Med Chem* 56:9122–9135
- Cohen P (2002) Protein kinases—the major drug targets of the twenty-first century? *Nat Rev Drug Discov* 1:309–315
- Dai J, Sultan S, Taylor SS, Higgins JM (2005) The kinase haspin is required for mitotic histone H3 Thr 3 phosphorylation and normal metaphase chromosome alignment. *Genes Dev* 19:472–488
- Dai J, Sullivan BA, Higgins JM (2006) Regulation of mitotic chromosome cohesion by Haspin and Aurora B. *Dev Cell* 11:741–750
- de Cárcer G, Perez de Castro I, Malumbres M (2007) Targeting cell cycle kinases for cancer therapy. *Curr Med Chem* 14:969–985
- Duong-Ly KC, Peterson JR (2013) The human kinome and kinase inhibition. *Curr Protoc Pharmacol* 60:2.9. 1–2.9. 14
- Fedorov O, Müller S, Knapp S (2010) The (un) targeted cancer kinome. *Nat Chem Biol* 6:166–169
- Flight MH (2013) Neurodegenerative diseases: new kinase targets for Alzheimer's disease. *Nat Rev Drug Discov* 12:739
- Gourdain SP, Dairou J, Denhez Cm, Bui LC, Rodrigues-Lima F, Janel N, Delabar JM, Cariou K, Dodd RH (2013) Development of DANDYs, new 3, 5-diaryl-7-azaindoles demonstrating potent DYRK1A kinase inhibitory activity. *J Med Chem* 56:9569–9585
- Gummadi VR, Rajagopalan S, Looi C-Y, Paydar M, Renukappa GA, Ainan BR, Krishnamurthy NR, Panigrahi SK, Mahasweta K, Raghuramachandran S (2013) Discovery of 7-azaindole based anaplastic lymphoma kinase (ALK) inhibitors: wild type and mutant (L1196M) active compounds with unique binding mode. *Bioorg Med Chem Lett* 23:4911–4918
- Hanks SK, Hunter T (1995) Protein kinases 6. The eukaryotic protein kinase superfamily: kinase (catalytic) domain structure and classification. *The FASEB J* 9:576–596
- Harrington PE, Bourbeau MP, Fotsch C, Frohn M, Pickrell AJ, Reichelt A, Sham K, Siegmund AC, Bailis JM, Bush T (2013) The optimization of aminooxadiazoles as orally active inhibitors of Cdc7. *Bioorg Med Chem Lett* 23:6396–6400
- Heinrich T, Seenisamy J, Emmanuvel L, Kulkarni SS, Bomke Jr, Rohdich F, Greiner H, Esdar C, Krier M, Grädler U (2013) Fragment-based discovery of new highly substituted 1 H-pyrrolo [2, 3-b]-and 3 H-imidazo [4, 5-b]-pyridines as focal adhesion kinase inhibitors. *J Med Chem* 56:1160–1170
- Higgins J (2003) Structure, function and evolution of haspin and haspin-related proteins, a distinctive group of eukaryotic protein kinases. *CMLS* 60:446–462
- Higgins JM (2001a) The Haspin gene: location in an intron of the Integrin α E gene, associated transcription of an Integrin α E-derived RNA and expression in diploid as well as haploid cells. *Gene* 267:55–69
- Higgins JM (2001b) Haspin-like proteins: a new family of evolutionarily conserved putative eukaryotic protein kinases. *Protein Sci* 10:1677–1684
- Higgins JM (2010) Haspin: a newly discovered regulator of mitotic chromosome behavior. *Chromosoma* 119:137–147
- Hong S, Kim J, Seo JH, Jung KH, Hong S-S, Hong S (2012) Design, synthesis, and evaluation of 3, 5-disubstituted 7-azaindoles as Trk inhibitors with anticancer and antiangiogenic activities. *J Med Chem* 55:5337–5349
- Huang Z, Tremblay MS, Wu TY-H, Ding Q, Hao X, Baaten J, Hill R, Liu B, Meeusen S, Zou Y (2019) Discovery of 5-(3, 4-Difluorophenyl)-3-(pyrazol-4-yl)-7-azaindole (GNF3809) for β -Cell Survival in Type 1 Diabetes. *ACS Omega* 4:3571–3581
- Huertas D, Soler M, Moreto J, Villanueva A, Martinez A, Vidal A, Charlton M, Moffat D, Patel S, McDermott J (2012) Antitumor activity of a small-molecule inhibitor of the histone kinase Haspin. *Oncogene* 31:1408–1418
- Hunter T (1995) Protein kinases and phosphatases: the yin and yang of protein phosphorylation and signaling. *Cell* 80:225–236
- Huse M, Kuriyan J (2002) The conformational plasticity of protein kinases. *Cell* 109:275–282
- Ibrahim PN, Artis DR, Bremer R, Habets G, Mamo S, Nespi M, Zhang C, Zhang J, Zhu Y, Zuckerman R (2007) Pyrrolo [2, 3-b] pyridine derivatives as protein kinase inhibitors. *Int. Pat. Appl.* Accessed from <https://patents.google.com/patent/EP2395004A2/ko> on 06/05/2020
- Irie T, Sawa M (2018) 7-Azaindole: a versatile scaffold for developing kinase inhibitors. *Chem Pharm Bull* 66:29–36
- Kannan N, Taylor SS, Zhai Y, Venter JC, Manning G (2007) Oceanic metagenomics: structural and functional diversity of the microbial kinome. *PLoS Biol* 5:e17
- Kim KS, Zhang L, Schmidt R, Cai Z-W, Wei D, Williams DK, Lombardo LJ, Trainor GL, Xie D, Zhang Y (2008) Discovery of pyrrolopyridine—pyridone based inhibitors of Met kinase: Synthesis, X-ray crystallographic analysis, and biological activities. *J Med Chem* 51:5330–5341
- Kim S-W, Kim M, Lee WY, Hyeon T (2002) Fabrication of hollow palladium spheres and their successful application to the recyclable heterogeneous catalyst for Suzuki coupling reactions. *J Am Chem Soc* 124:7642–7643
- Lagunin AA, Dubovskaja VI, Rudik AV, Pogodin PV, Druzhilovskiy DS, Glorizova TA, Filimonov DA, Sastry NG, Poroikov VV (2018) CLC-Pred: a freely available web-service for in silico prediction of human cell line cytotoxicity for drug-like compounds. *PLoS One* 13:e019183
- Lawson M, Rodrigo J, Baratte B, Robert T, Delehouzé C, Lozach O, Ruchaud S, Bach S, Brion J-D, Alami M (2016) Synthesis, biological evaluation and molecular modeling studies of imidazo [1, 2-a] pyridines derivatives as protein kinase inhibitors. *Eur J Med Chem* 123:105–114
- Ledeboer MW, Wannamaker M, Farmer L (2006). Pyrrolopyridines useful as inhibitors of protein kinase and their preparation, pharmaceutical compositions, and use in the treatment of various diseases, WO2006127587A1
- Liao JJ-L (2007) Molecular recognition of protein kinase binding pockets for design of potent and selective kinase inhibitors. *J Med Chem* 50:409–424
- Liddle J, Bamborough P, Barker MD, Campos S, Cousins RP, Cutler GJ, Hobbs H, Holmes DS, Ioannou C, Mellor GW (2009) 4-Phenyl-7-azaindoles as potent and selective IKK2 inhibitors. *Bioorg Med Chem Lett* 19:2504–2508

- Littke AF, Fu GC (2002) Palladium-catalyzed coupling reactions of aryl chlorides. *Angew Chem Int Ed* 41:4176–4211
- Liu B, Yuan X, Xu B, Zhang H, Li R, Wang X, Ge Z, Li R (2019) Synthesis of novel 7-azaindole derivatives containing pyridin-3-ylmethyl dithiocarbamate moiety as potent PKM2 activators and PKM2 nucleus translocation inhibitors. *Eur J Med Chem* 170:1–15
- Manning G, Whyte DB, Martinez R, Hunter T, Sudarsanam S (2002) The protein kinase complement of the human genome. *Science* 298:1912–1934
- Mavunkel BJ, Perumattam JJ, Tan X, Luedtke GR, Lu Q, Lim D, Kizer D, Dugar S, Chakravarty S, Xu Y-j (2010) Piperidine-based heterocyclic oxalyl amides as potent p38 α MAP kinase inhibitors. *Bioorg Med Chem Lett* 20:1059–1062
- Mérour J-Y, Buron F, Plé K, Bonnet P, Routier S (2014) The azaindole framework in the design of kinase inhibitors. *Molecules* 19:19935–19979
- Metz JT, Johnson EF, Soni NB, Merta PJ, Kifle L, Hajduk PJ (2011) Navigating the kinome. *Nat Chem Biol* 7:200–202
- Nakano H, Saito N, Parker L, Tada Y, Abe M, Tsuganezawa K, Yokoyama S, Tanaka A, Kojima H, Okabe T (2012) Rational evolution of a novel type of potent and selective proviral integration site in Moloney murine leukemia virus kinase 1 (PIM1) inhibitor from a screening-hit compound. *J Med Chem* 55:5151–5164
- Nguyen T-N-D, Feizbakhsh O, Sfecci E, Baratte B, Delehouzé C, Garcia A, Moulin C, Colas P, Ruchaud S, Mehiri M (2019) Kinase-based screening of marine natural extracts leads to the identification of a cytotoxic high molecular weight metabolite from the mediterranean sponge *crambe tailliezi*. *Mar Drugs* 17:e569
- Noh H, King G (2007) The role of protein kinase C activation in diabetic nephropathy. *Kidney Int Suppl* 72:S49–S53
- Schmidt M, Bastians H (2007) Mitotic drug targets and the development of novel anti-mitotic anticancer drugs. *Drug Resist Updat* 10:162–181
- Tsou H-R, MacEwan G, Birnberg G, Grosu G, Bursavich MG, Bard J, Brooijmans N, Toral-Barza L, Hollander I, Mansour TS (2010) Discovery and optimization of 2-(4-substituted-pyrrolo [2, 3-b] pyridin-3-yl) methylene-4-hydroxybenzofuran-3 (2H)-ones as potent and selective ATP-competitive inhibitors of the mammalian target of rapamycin (mTOR). *Bioorg Med Chem Lett* 20:2321–2325
- Walsby E, Pratt G, Shao H, Abbas AY, Fischer PM, Bradshaw TD, Brennan P, Fegan C, Wang S, Pepper C (2014) A novel Cdk9 inhibitor preferentially targets tumor cells and synergizes with fludarabine. *Oncotarget* 5:375–385
- Wolfe JP, Buchwald SL (1999) A highly active catalyst for the room-temperature amination and Suzuki coupling of aryl chlorides. *Angew Chem Int Ed* 38:2413–2416
- Zhang C, Ibrahim PN, Zhang J, Burton EA, Habets G, Zhang Y, Powell B, West BL, Matusow B, Tsang G (2013) Design and pharmacology of a highly specific dual FMS and KIT kinase inhibitor. *PNAS* 110:5689–5694
- Zuccotto F, Ardini E, Casale E, Angiolini M (2009) Through the “gatekeeper door”: exploiting the active kinase conformation. *J Med Chem* 53:2681–2694

CHAPTER 4: MANUSCRIPT SUBMITTED TO CHEMICAL BIOLOGY AND DRUG DESIGN

4.1 Author Guidelines for submitting a manuscript to Chemical Biology and Drug Design

The manuscript submitted to Chemical Biology and Drug Design was prepared in accordance with this journal's Author Guidelines. These Author Guidelines can be found under Annexure F.

4.2 Synthesis and biological evaluation of selected 7*H*-pyrrolo[2,3-*d*]pyrimidine derivatives as novel CDK9/CyclinT and Haspin inhibitors.

Short running title: 7-Deazapurine derivatives as novel CDK9/CyclinT and Haspin inhibitors

Lianie Pieterse¹, Richard M. Beteck¹, Blandine Baratte^{2,3}, Omobolanle J. Jesumoroti¹, Thomas Robert^{2,3}, Sandrine Ruchaud², Stéphane Bach^{1,2,3}, Lesetja J. Legoabe^{1*}

¹ Centre of Excellence for Pharmaceutical Sciences, North-West University, Private Bag X6001, Potchefstroom 2520, South Africa

² Sorbonne Université, CNRS, UMR8227, Integrative Biology of Marine Models Laboratory (LBI2M), Station Biologique de Roscoff, 29680 Roscoff, France

³ Sorbonne Université, CNRS, FR2424, Plateforme de criblage KISSf (Kinase Inhibitor Specialized Screening facility), Station Biologique de Roscoff, 29680 Roscoff Cedex, France

4.2.1 Acknowledgements

The authors are grateful to Dr. Otto and Dr. Jordaan of the SASOL Centre for Chemistry, North-West University, for recording the NMR and MS spectra, respectively. The authors also thank the Cancéropôle Grand Ouest (3MC network - Marine Molecules, Metabolism and Cancer), GIS IBiSA (Infrastructures en Biologie Santé et Agronomie) and Biogenouest (Western France life science and environment core facility network) for supporting KISSf screening facility.

4.2.2 Conflict of interest

Stéphane Bach is a founder and member of the scientific advisory board of SeaBeLife Biotech, which is developing novel therapies for treating liver and kidney acute disorders. This work was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

4.2.3 Abstract

Protein kinases, including CDK9/CyclinT and Haspin, are regarded as potential drug targets in cancer therapy. Findings from a previous study suggested 7-azaindole as a privileged scaffold for producing inhibitors of CDK9/CyclinT and Haspin. Inspired by these findings, the current study synthesised and evaluated eleven (11) C6-substituted 7-azaindole and nineteen (19) C4-substituted structurally related 7*H*-pyrrolo[2,3-*d*]pyrimidine derivatives against a panel of protein kinases, including CDK9/CyclinT and Haspin. Ten of the 7*H*-pyrrolo[2,3-*d*]pyrimidine derivatives exhibited activity toward CDK9/CyclinT, while 4 of compounds had activity against Haspin. The best CDK9/CyclinT (IC₅₀ of 0.38 µM) and Haspin (IC₅₀ of 0.11 µM) activities were achieved by compounds **7d** and **7f**, respectively. Hence, these compounds may be valuable starting points for development of new anti-cancer drugs.

4.2.4 Keywords:

Protein kinase; 7-deazapurine; CDK9/CylinT; Haspin; anticancer

4.2.5 Introduction

In 1955 Fischer and Krebs reported evidence that the conversion of phosphorylase *b* to *a*, as observed in cell-free muscle extracts, is dependent on nucleotides with high energy phosphates in the presence of high energy divalent metal ions (Fischer & Krebs, 1955). This opened a door into the world of protein kinases (PKs) as phosphorylase became the first known PK to be purified (Krebs *et al.*, 1959). Since then, it has become apparent that the human kinome consists of 518 PKs (equating to 1.7 % of all human genes) and is indispensable to normal cellular function (Manning *et al.*, 2002). PKs play a key part in regulating signal transduction by promoting the transfer of phosphoryl groups from phosphate donors, like adenosine triphosphate (ATP), to receptor substrates (Lawson *et al.*, 2016). The associated signal transduction pathways significantly influence biological systems as they determine cellular responses to environmental stimuli and modify gene expression (Pokholok *et al.*, 2006). Erroneous signal transduction may lead to disruption of normal biological processes and could ultimately result in diseases such as, cancer, inflammation, diabetes and neurodegenerative disease (Flight, 2013; Huse & Kuriyan, 2002; Noh & King, 2007; Sever & Brugge, 2015). Thus, the appeal of PK inhibitors as drug targets is rooted in the expectation that erroneous signal transduction can be halted by inhibition of the appropriate PK. Nowadays, 52 PK inhibitor-based drugs are approved by the US food and drugs administration, where 46 of the 52 is used in the treatment of neoplastic diseases. Examples include abemaciclib, palbociclib and ricociclib (CDK4/6 inhibitors) (Roskoski Jr, 2020).

Cyclin-dependant kinase CDK9/CyclinT complex is an example of such a therapeutic target. CDK9 is a member of CMGC family of kinases: for Cyclin-dependent kinase (CDK), Mitogen-activated protein kinase (MAPK), Glycogen synthase kinase (GSK) and CDC-like kinase (CLK). CMGC kinases are key regulators involved in the regulation of DNA repair and cell fate (cell proliferation, differentiation, apoptosis) (Franco *et al.*, 2018). CDK9 is indispensable in the governance of non-ribosomal transcription, which includes super-enhancers regulated gene expression. Super-enhancers are clusters of DNA regulatory components that guide transcription of genes of significance in cell-identity (Hnisz *et al.*, 2013). As abnormalities in the regulation of CDK9 activity have been described in acute myeloid leukaemia (AML), other haematological malignancies and solid tumours, the chemical inhibition of CDK9 is considered as a promising strategy in cancer therapy (Franco *et al.*, 2018).

Another kinase of interest is the Haploid germ cell-specific nuclear protein kinase (Haspin). Haspin is one of the newer additions to the kinome and remains relatively underexplored as a drug target (Higgins, 2010). It is present across a variety of eukaryotic species (animal, fungi and plants) and was first detected in male germ cells (Amoussou *et al.*, 2018; Tanaka *et al.*, 1994). This Ser/Thr kinase is structurally unique as compared to other members of the PK family (Eswaran *et al.*, 2009). It displays essential function during cell division and is involved in histone code printing (Dai & Higgins, 2005; Dai *et al.*, 2006; Higgins, 2010). Haspin depletion by CRISPR/Cas9 in Breast cancer HeLa cells triggers severe mitotic defects and ultimately cell death by mitotic catastrophe (Huertas *et al.*, 2012)

In humans, Haspin mRNA expression has been marked in all proliferating cell-lines such as, thymus, bone marrow and foetal liver cell-lines. Haspin has also been found overexpressed in several highly proliferative malignant tumours such as melanomas, myelomas, pancreatic and bladder cancer (Chen *et al.*, 2020; Han *et al.*, 2017; Han *et al.*, 2019; Melms *et al.*, 2020). The degree of Haspin expression in tissue correlates with the degree of cellular proliferation and differentiation each tissue experiences and often correlates with the most aggressive stages of the cancers (Dai *et al.*, 2006; de Cárcer *et al.*, 2007; Higgins, 2003; Higgins, 2001; Villa *et al.*, 2009). Furthermore, Haspin inhibition or knockdown has been shown to slow down the development of several cancers in animal models (Chen *et al.*, 2020; Han *et al.*, 2019; Tanaka *et al.*, 2020; Yu *et al.*, 2020). Consequently, Haspin could prove to be a valuable target in mitosis and thus cancer therapy.

In a previous study (Pieterse *et al.*, 2020), 7-azaindole derivatives were identified as dual-inhibitors of CDK9/CyclinT and Haspin (see **1** and **2**, **Fig. 1**). This illustrates the ability of the 7-azaindole scaffold to produce potential novel CDK9/CyclinT and Haspin inhibitors. Prompted by the success of the previous study, a series of selected 6-substituted 7-azaindole derivatives and

a series of structurally related 7*H*-pyrrolo[2,3-*d*]pyrimidine (7-deazapurine) derivatives were synthesised. These compounds were evaluated *in vitro* against CDK9/CyclinT and Haspin kinases (see **Tables 1–3**). Additionally, these compounds were screened against other PKs: (i) CDK2/CyclinA, (ii) CDK5/p25, (iii) Pim-1, (iv) CK1 ϵ , (v) GSK-3 β , (vi) *Lm*CK1 (from the parasite *Leishmania major*) and (vii) ABL1. As per the previous study, these compounds were singled out due to their simplicity, comparatively economical synthesis and concomitant novelty to the CDK9/CyclinT and Haspin kinase (Pieterse *et al.*, 2020). The structures of the selected 7-azaindole and 7-deazapurine derivatives were subjected to a SciFinder search in order to establish the novelty of each compound. The structure search for compounds **5b**, **5c**, **5f**, **5g–k**, **7c–e**, **7g**, **7h**, **7l–n**, and **8a – c** produced no former references and they were considered novel. Compounds **5a** (Allegretti *et al.*, 2001), **5d**, **5e** (Cincinelli *et al.*, 2014), **7a**, **7b**, **7f** (Li *et al.*, 2019), **7i**, **7j**, **7k** (Sabat *et al.*, 2016), **7o** (Kashyap *et al.*, 2014) and **7p** (Bhagirath *et al.*, 2015) had been referenced in previous literature works. Nevertheless, none of the compounds had previously been examined as CDK9/CyclinT or Haspin inhibitors. In this study, we described the inhibitory activity in the low micromolar range of six compounds against CDK9/CyclinT and five compounds against Haspin. Among these molecular hits, four compounds are dual-target inhibitors.

4.2.6 Material and methods

The selected 7-azaindole and 7-deazapurine derivatives were synthesised as described in the experimental section. Materials for synthesis were sourced from AK Scientific (for **5a–k**, **7a**, **7b**, **7e**, **7f**, **7g**, **7l**, **7m**, **7n**, **7o**, **7p** and **8a – c**) and Ambeed (for **7c**, **7d**, **7h**, **7i**, **7j** and **7k**), while CHR 6494 and flavopiridol were purchased from Sigma Aldrich. All materials were used without further purification, unless otherwise specified. Proton (^1H) and carbon (^{13}C) nuclear magnetic resonance (NMR) spectra were recorded utilizing a Bruker Avance III 600 spectrometer at frequencies of 600 MHz and 150 MHz, respectively. Deuterated dimethyl sulfoxide (DMSO-*d*₆) served as the solvent for all NMR analysis. The chemical shifts (δ) are reported in parts per million (ppm) and internally referenced to the signal of tetramethyl saline (TMS). Spin multiplicities are indicated as follow: s (singlet), d (doublet), t (triplet), q (quartet), dd (doublet of doublets) and m (multiplet) and ddd (doublet of doublet of doublets). Coupling constants (*J*) are reported in Hz. High resolution mass spectra (HRMS) were recorded by means of a Bruker micrOTOF-Q II mass spectrometer in atmospheric pressure chemical ionisation (APCI) mode (positive ion polarity). High performance liquid chromatography (HPLC) analyses were performed to determine purity by means of an Agilent 1100 HPLC system, using a Venusil XBP C18(2) (4.6 mm x 150 mm) 5 μm column with mobile phase A (water + 0.1% formic acid) and mobile phase B (acetonitrile + 0.1% formic acid) against a gradient of 50% B (0–2min), 100% B (2–8min), 100% B (8–11min), 50% B (11.1–13min) at 22°C. Melting points (mp) were determined as part of the characterisation of the

synthesised compounds by a Buchi B545 melting point apparatus and are uncorrected. Thin layer chromatography (TLC) was performed using silica gel 60 TLC plates (Merck) and a UV254 fluorescent indicator to determine retention factor (Rf), with petroleum ether: ethyl acetate (1:1) as eluent.

4.2.7 Experimental

4.2.7.1 Chemistry

6-Chloro-7-azaindole and 4-chloro-7*H*-pyrrolo[2,3-*d*]pyrimidine (6-Chloro-7-deazapurine) were reacted with a variety of commercially available boronic acids, by means of Suzuki-Miyaura coupling, to afford a series of C6-substituted 7-azaindole derivatives (**5a–k**) and a series of C4-substituted-7*H*-pyrrolo[2,3-*d*]pyrimidine derivatives (**7a–p**), respectively. Furthermore, the 4,7-bis-substituted-7*H*-pyrrolo[2,3-*d*]pyrimidine derivatives (**8a–c**) were derived from 4-chloro-7*H*-pyrrolo[2,3-*d*]pyrimidine subjected to a combination of Suzuki-Miyaura coupling (at the 4-Cl) and Chan-Lam coupling (at the NH) with the appropriate boronic acids. Suzuki-Miyaura coupling promotes carbon-carbon cross coupling between organoborons and organic halides in the presence of a palladium catalyst (Kim *et al.*, 2002; Wolfe & Buchwald, 1999); whereas Chan-Lam coupling refers to a reaction between aryl boronic acids and an alcohols or amines and is catalysed by copper complexes (Qiao & Lam, 2011). In the instance of compounds **5a–k** and **7a–p**, tetrakis(triphenylphosphine)palladium(0) (Pd(PPh₃)₄), served as catalyst, while compounds **8a–c**, were synthesised by means of cupric acetate Cu(OAc)₂ in addition to Pd(PPh₃)₄.

Verification of the compounds were achieved by utilising NMR and HRMS analyses. In terms of HRMS, the MH⁺ for every compound was determined and compared to the predicted MH⁺. All the determined MH⁺ values corresponded to the predicted values, thus proving that each compound exhibits the correct molecular weight for its structure. Concomitantly, the ¹H and ¹³C NMR spectra revealed the anticipated number of hydrogen and carbon atoms, respectively, for each compound. In general, the ¹H NMR spectra of all the 7-azaindole derivatives depict a peak at approximately 6.40 that can be assigned to H3. In the case of the 7*H*-pyrrolo[2,3-*d*]pyrimidine derivatives, a similar peak is seen at approximately 6.80 and denotes the corresponding hydrogen H5. Furthermore, the 7*H*-pyrrolo[2,3-*d*]pyrimidine derivatives possess a very distinct H2 singlet at approximately 8.80 ppm. Additionally, the 7-azaindole and 7*H*-pyrrolo[2,3-*d*]pyrimidine derivatives produce a broad singlet at approximately 12.20 ppm, in most cases. The absence of this peak in the proton spectra of the 4,7-bissubstituted 7*H*-pyrrolo[2,3-*d*]pyrimidine derivatives, could suggest that the successful Chan-Lam coupling has been successful. All the characterization data can be found in the supporting info.

4.2.7.2 General synthetic procedure for the C6-substituted 7-azaindole derivatives (5a–m)

Compounds **5a–k** were synthesised under nitrogen atmosphere, in accordance with required conditions for Suzuki-Miyaura coupling reaction. For each compound, a mixture of 6-chloro-7-azaindole (1.523 mmol), Pd(PPh₃)₄ (0.0023 mmol), 1.6 mL of a 2M K₂CO₃ aqueous solution and 6.092 mmol of the appropriate boronic acid in 25 mL of a 3:1 toluene: ethanol mixture was prepared. The reaction mixture was stirred continuously under reflux at 120 °C for at least 24 hours. TLC plates were used to monitor the progress of the reaction. Upon completion of the reaction, the reaction mixture was left to cool to room temperature before the solvent was removed *in vacuo*. This yielded a residue that was dissolved in dichloromethane and washed with distilled water (3 x 25 mL). The combined organic layers were dried over a suitable drying agent (MgSO₄), filtered and evaporated *in vacuo*. The resulting product was recrystallised from suitable solvents.

4.2.7.3 General synthetic procedure for the selected C4-substituted-7H-pyrrolo[2,3-d]pyrimidine derivatives (7a–p)

Compounds **7a–p** were synthesised under inert nitrogen atmosphere. The preparation of each compound entailed mixing 4-chloro-7H-pyrrolo[2,3-d]pyrimidine (6-Chloro-7-deazapurine) (1.95 mmol), Pd(PPh₃)₄ (0.05 mmol), 1.6 mL of a 2M K₂CO₃ solution and 5.85 mmol of the appropriate boronic acid in 50 mL of a 3:1 toluene: ethanol mixture. The reaction mixture was stirred under reflux at 120 °C for a minimum period of 24 hours. Progress was monitored with TLC plates, using petroleum ether: ethyl acetate (1:1 v/v) mixture as mobile phase. Upon completion of the reaction, the solvent was removed under reduced pressure. This yielded a residue that was dissolved in dichloromethane and washed with distilled water (3 x 25 mL). The combined organic layers were dried with MgSO₄, filtered and subjected to reduced pressure. The resulting residues were recrystallised from suitable solvents.

4.2.7.4 General synthetic procedure for the selected 4,7-bis-substituted-7H-pyrrolo[2,3-d]pyrimidine derivatives (8a–c)

Compounds **8a–c** were synthesised under inert nitrogen atmosphere. To prepare each of the bis-substituted compounds, a mixture of 4-chloro-7H-pyrrolo[2,3-d]pyrimidine (6-Chloro-7-deazapurine) (3.26 mmol), Pd(PPh₃)₄ (0.10 mmol), Cu(OAc)₂ (3.88 mmol), K₂CO₃ (17.37 mmol) and 16.3 mmol of the appropriate boronic acid in 50 mL of a 1:1 water: ethanol mixture was refluxed at 120 °C for a minimum of 24 h. Progress was monitored with TLC plates using petroleum ether: ethyl acetate (1:1 v/v) as mobile phase. Upon completion of the reaction, the

reaction mixture was filtered through Celite® and the filtrate concentrated under reduced pressure. The resulting residue was dissolved in dichloromethane and washed with distilled water (3 x 25 mL). The combined organic layers were dried with MgSO₄ and evaporated *in vacuo*. The resulting product was recrystallised from a suitable solvent.

6-Phenyl-1H-pyrrolo[2,3-b]pyridine (5a):

Compound was synthesised from phenylboronic acid and 6-chloro-7-azaindole in a yield of 26%, recrystallised from acetonitrile, gold-coloured crystals, mp 189 °C, R_f = 0.71 (dichloromethane: ethyl acetate; 4:1). ¹H NMR (DMSO-*d*₆, 600 MHz) δ 11.73 (1H, s, NH), 8.10 (2H, d, J = 7.8 Hz, Ar-H), 8.03 (1H, d, J = 8.2 Hz, Ar-H), 7.66 (1H, d, J = 8.2 Hz, Ar-H), 7.51 – 7.46 (3H, m, Ar-H), 7.39 (1H, t, J = 7.3 Hz, Ar-H), 6.48 (1H, m, Ar-H). ¹³C NMR (DMSO-*d*₆, 150 MHz) δ 149.96, 149.06, 140.37, 129.40, 129.13, 128.53, 127.24, 126.89, 119.28, 113.08, 100.36. APCI-HRMS *m/z*: calcd for C₁₃H₁₀N₂ (MH⁺), 195.0917, found: 195.0913; Purity (HPLC): 99%.

6-(4-Methylphenyl)-1H-pyrrolo[2,3-b]pyridine (5b):

Compound was synthesised from 4-methylphenylboronic acid and 6-chloro-7-azaindole in a yield of 94%, recrystallised from acetonitrile, maroon coloured crystals, mp 218 °C, R_f = 0.67 (dichloromethane: ethyl acetate; 4:1). ¹H NMR (DMSO-*d*₆, 600 MHz) δ 11.66 (1H, s, NH), 8.01 – 7.95 (Ar-H), 7.61 (1H, d, J = 8.2 Hz, Ar-H), 7.47 – 7.44 (1H, m, Ar-H), 7.28 (2H, d, J = 7.6 Hz, Ar-H), 6.46 – 6.43 (1H, m, Ar-H), 2.35 (3H, s, Me-4'). ¹³C NMR (DMSO-*d*₆, 150 MHz) δ 149.55, 148.54, 137.36, 137.14, 129.23, 128.82, 126.46, 126.28, 118.54, 112.30, 99.83, 20.76. APCI-HRMS *m/z*: calcd. for C₁₄H₁₂N₂ (MH⁺), 209.1073, found 209.1077; Purity (HPLC): 99%.

6-(3-Methoxyphenyl)-1H-pyrrolo[2,3-b]pyridine (5c):

Compound was synthesised from 3-methoxyphenylboronic acid and 6-chloro-7-azaindole in a yield of 54%, recrystallised from acetonitrile, brown coloured crystals, mp 139 °C, R_f = 0.67 (4 dichloromethane: 1 ethyl acetate). ¹H NMR (DMSO-*d*₆, 600 MHz) δ 11.69 (1H, s, NH), 7.97 (1H, d, J = 8.2 Hz, Ar-H), 7.62 – 7.60 (3H, m, Ar-H), 7.45 – 7.43 (1H, m, Ar-H), 7.35 – 7.32 (1H, m, Ar-H), 6.90 (1H, ddd, J = 8.1, 2.5, 0.8 Hz, Ar-H), 6.42 – 6.41 (1H, m, Ar-H), 3.79 (3H, s, MeO-3'). ¹³C NMR (DMSO-*d*₆, 150 MHz) δ 159.67, 149.25, 148.51, 141.43, 129.71, 128.88, 126.46, 126.85, 118.96, 118.84, 113.90, 112.81, 111.55, 99.91, 55.12 (MeO-3'). APCI-HRMS *m/z*: calcd. for C₁₄H₁₂N₂O (MH⁺), 225.1022, found 225.1020; Purity (HPLC): 92%.

6-(4-Methoxyphenyl)-1H-pyrrolo[2,3-b]pyridine (5d):

Compound was synthesised from 4-methoxyphenylboronic acid and 6-chloro-7-azaindole in a yield of 72%, recrystallised from acetonitrile, dark yellow-coloured crystals, mp 185 °C, R_f = 0.62 (dichloromethane: ethyl acetate; 4:1). ¹H NMR (DMSO-*d*₆, 600 MHz) δ 11.61 (1H, s, NH), 8.03 (2H, d, *J* = 8.0 Hz, Ar-H), 7.96 (1H, d, *J* = 8.2 Hz, Ar-H), 7.56 (1H, d, *J* = 8.2 Hz, Ar-H), 7.43 (1H, s, Ar-H), 7.02 (2H, d, *J* = 8.0 Hz, Ar-H), 6.42 (1H, s, Ar-H), 3.80 (3H, s, MeO-4'). ¹³C NMR (DMSO-*d*₆, 150 MHz) δ 159.41, 149.43, 148.54, 132.44, 128.83, 127.62, 126.16, 118.17, 114.02, 111.97, 99.82, 55.16 (MeO-4'). APCI-HRMS *m/z*: calcd. for C₁₄H₁₂N₂O (MH⁺), 225.1022, found 225.1035; Purity (HPLC): 97%.

6-(4-Fluorophenyl)-1H-pyrrolo[2,3-b]pyridine (5e):

Compound was synthesised from 4-fluorophenylboronic acid and 6-chloro-7-azaindole in a yield of 68%, recrystallised from acetonitrile, light brown coloured crystals, mp 217 °C, R_f = 0.89 (dichloromethane: ethyl acetate; 4:1). ¹H NMR (DMSO-*d*₆, 600 MHz) δ 11.69 (1H, s, NH), 8.16 – 8.09 (2H, m, Ar-H), 8.01 (1H, d, *J* = 8.1 Hz, Ar-H), 7.62 (1H, d, *J* = 8.1 Hz, Ar-H), 7.47 (1H, s, Ar-H), 7.28 (2H, d, *J* = 8.3 Hz, Ar-H), 6.45 (1H, s, Ar-H). ¹³C NMR (DMSO-*d*₆, 150 MHz) δ 163.08, 161.46 (s), 148.49 (d, *J* = 0.8 Hz), 136.38 (d, *J* = 3.0 Hz), 128.98 (s), 128.38 (d, *J* = 8.3 Hz), 126.77 (s), 118.73 (s), 115.42 (d, *J* = 21.4 Hz), 112.40 (s), 99.88 (s). APCI-HRMS *m/z*: calcd. for C₁₃H₉FN₂ (MH⁺), 213.0823, found 213.0824; Purity (HPLC): 99%.

6-(3,4-Difluorophenyl)-1H-pyrrolo[2,3-b]pyridine (5f):

Compound was synthesised from 3,4-difluorophenylboronic acid and 6-chloro-7-azaindole in a yield of 13%, recrystallised from acetonitrile, dark green coloured crystals, mp 173 °C, R_f = 0.85 (dichloromethane: ethyl acetate; 4:1). ¹H NMR (DMSO-*d*₆, 600 MHz) δ 11.72 (1H, s, NH), 8.07 (1H, ddd, *J* = 12.5, 8.0, 2.1 Hz, Ar-H), 7.99 (1H, d, *J* = 8.2 Hz, Ar-H), 7.92 (1H, ddd, *J* = 4.2, 3.5, 2.0 Hz, Ar-H), 7.65 (1H, d, *J* = 8.2 Hz, Ar-H), 7.50 – 7.45 (2H, m, Ar-H), 6.44 – 6.42 (1H, m, Ar-H). ¹³C NMR (DMSO-*d*₆, 150 MHz) δ 150.45 (dd, *J* = 45.3, 12.9 Hz), 148.82 (dd, *J* = 47.5, 12.7 Hz), 148.42 (s), 147.08 (s), 137.63 (dd, *J* = 5.7, 3.5 Hz), 130.15 (dd, *J* = 411.0, 10.8 Hz), 129.10 (s), 127.32 (s), 123.00 (dd, *J* = 6.4, 3.1 Hz), 119.27 (s), 116.38 (dd, *J* = 398.9, 17.7 Hz), 112.57 (s), 100.01 (s). APCI-HRMS *m/z*: calcd. C₁₃H₈F₂N₂ (MH⁺), 231.0728, found 231.0736; Purity (HPLC): 96%.

6-[4-(Trifluoromethyl)phenyl]-1H-pyrrolo[2,3-b]pyridine (5g):

Compound was synthesised from 4-trifluoromethylphenylboronic acid and 6-chloro-7-azaindole in a yield of 50%, recrystallised from acetonitrile, gold-coloured crystals, mp 247 °C, R_f = 0.71 (dichloromethane: ethyl acetate; 4:1). ¹H NMR (DMSO-*d*₆, 600 MHz) δ 11.80 (1H, s, NH), 8.31 (2H, d, *J* = 7.9 Hz, Ar-H), 8.07 (1H, d, *J* = 7.2 Hz, Ar-H), 7.82 (2H, d, *J* = 7.8 Hz, Ar-H), 7.74 (1H, d, *J* = 8.0 Hz, Ar-H), 7.55 (1H, s, Ar-H), 6.49 (1H, s, Ar-H). ¹³C NMR (DMSO-*d*₆, 150 MHz) δ 148.56 (s), 147.58 (s), 143.73 (s), 129.07 (s), 128.15 (d, *J* = 31.8 Hz), 127.65 (s), 126.97 (s), 125.54 (q, *J* = 3.6 Hz), 124.43 (q, *J* = 271.8 Hz), 119.63 (s), 113.05 (s), 100.01 (s). APCI-HRMS *m/z*: calcd. for C₁₄H₉F₃N₂ (MH⁺), 263.0791, found 263.0807; Purity (HPLC): 99%.

4-(1H-Pyrrolo[2,3-b]pyridin-6-yl)benzonitrile (5h):

Compound was synthesised from 4-cyanophenylboronic acid and 6-chloro-7-azaindole in a yield of 58%, recrystallised from acetonitrile, gold-coloured crystals, mp 216 °C, R_f = 0.65 (dichloromethane: ethyl acetate; 4:1). ¹H NMR (DMSO-*d*₆, 600 MHz) δ 11.80 (1H, s, NH), 8.27 – 8.24 (2H, m, H-2', H-6'), 8.04 (1H, d, *J* = 8.2 Hz, H-4), 7.90 – 7.87 (2H, m, Ar-H), 7.74 (1H, d, *J* = 8.2 Hz, Ar-H), 7.54 (1H, d, *J* = 3.4 Hz, Ar-H), 6.47 (1H, d, *J* = 3.4 Hz, Ar-H). ¹³C NMR (DMSO-*d*₆, 150 MHz) δ 148.57, 147.16, 144.18, 132.67, 129.12, 128.02, 127.03, 119.89, 119.04, 113.27, 110.31, 100.12. APCI-HRMS *m/z*: calcd. for C₁₄H₉N₃ (MH⁺), 220.0869, found 220.0881; Purity (HPLC): 98%.

6-(Pyridin-3-yl)-1H-pyrrolo[2,3-b]pyridine (5i):

Compound was synthesised from 3-pyridinylboronic acid and 6-chloro-7-azaindole in a yield of 32%, recrystallised from acetonitrile, pale orange coloured powder, mp 177 °C, R_f = 0.11 (dichloromethane: ethyl acetate; 4:1). ¹H NMR (DMSO-*d*₆, 600 MHz) δ 11.76 (1H, s, NH), 9.23 (1H, d, *J* = 1.5 Hz, Ar-H), 8.52 (1H, d, *J* = 5.6 Hz, Ar-H), 8.38 – 8.36 (1H, m, Ar-H), 8.01 (1H, d, *J* = 8.14 Hz, Ar-H), 7.67 (1H, d, *J* = 8.2 Hz, Ar-H), 7.49 – 7.47 (1H, m, Ar-H), 7.44 (1H, dd, *J* = 7.8, 4.7 Hz, Ar-H), 6.45 – 6.43 (1H, m, Ar-H). ¹³C NMR (DMSO-*d*₆, 150 MHz) δ 148.91, 148.65, 147.70, 146.96, 135.24, 133.69, 129.16, 127.32, 123.76, 119.39, 112.83, 100.04. APCI-HRMS *m/z*: calcd. for C₁₂H₉N₃ (MH⁺), 196.0869, found 196.0886; Purity (HPLC): 91%.

6-(Pyridin-4-yl)-1H-pyrrolo[2,3-b]pyridine (5j):

Compound was synthesised from 4-pyridinylboronic acid and 6-chloro-7-azaindole in a yield of 59%, recrystallised from acetonitrile, yellow coloured powder, mp 167 °C, R_f = 0.28 (dichloromethane: ethyl acetate; 4:1). ¹H NMR (DMSO-*d*₆, 600 MHz) δ 11.85 (1H, s, NH), 8.64 (2H, dd, *J* = 4.5, 1.6 Hz, Ar-H), 8.08 (1H, d, *J* = 8.2 Hz, Ar-H), 8.05 (2H, dd, *J* = 4.5, 1.6 Hz, Ar-

H), 7.79 (1H, d, J = 8.2 Hz, Ar-H), 7.57 (1H, d, J = 3.4 Hz, Ar-H), 6.50 (1H, d, J = 3.4 Hz, Ar-H). ^{13}C NMR (DMSO- d_6 , 150 MHz) δ 150.15, 148.55, 146.74, 146.42, 129.05, 128.11, 120.57, 120.31, 113.03, 100.11. APCI-HRMS m/z : calcd. for $\text{C}_{12}\text{H}_9\text{N}_3$ (MH^+), 196.0869, found 196.0875, Purity (HPLC): 91%.

6-(6-Fluoropyridin-3-yl)-1H-pyrrolo[2,3-b]pyridine (5k):

Compound was synthesised from 6-fluoro-3-pyridinylboronic acid and 6-chloro-7-azaindole in a yield of 59%, recrystallised from acetonitrile, yellow coloured powder, mp 194 °C, R_f = 0.28 (dichloromethane: ethyl acetate; 4:1). ^1H NMR (DMSO- d_6 , 600 MHz) δ 11.79 (1H, s, NH), 8.91 (1H, d, J = 2.5 Hz, Ar-H), 8.62 (1H, d, J = 8.3, 2.6 Hz, Ar-H), 8.06 (1H, d, J = 8.1 Hz, H-4), 7.71 (1H, d, J = 8.2 Hz, H-5), 7.52 (1H, d, J = 3.4 Hz, H-2), 7.28 (1H, d, J = 8.6, 2.7 Hz, Ar-H), 6.48 (1H, d, J = 3.4 Hz, Ar-H). ^{13}C NMR (DMSO- d_6 , 150 MHz) δ 162.85 (d, J = 236.3 Hz), 148.51 (s), 145.85 (s), 145.44 (d, J = 15.4 Hz), 139.86 (d, J = 8.1 Hz), 133.99 (d, J = 4.4 Hz), 129.18 (s), 127.34 (s), 119.36 (s), 112.65 (s), 109.47 (d, J = 37.7 Hz), 100.01 (s). APCI-HRMS m/z : calcd. for $\text{C}_{12}\text{H}_8\text{FN}_3$ (MH^+), 214.0775, found 214.0781; Purity (HPLC): 93%.

4-Phenyl-7H-pyrrolo[2,3-d]pyrimidine (7a):

Compound was synthesised from phenylboronic acid and 6-chloro-7-deazapurine in a yield of 53%, recrystallised from methanol, green coloured powder, mp 221 °C, R_f = 0.46 (hexane: ethyl acetate: 1:1). ^1H NMR (DMSO- d_6 , 600 MHz) δ 8.82 (1H, s, Ar-H), 8.18 – 8.15 (2H, m, Ar-H), 7.64 (1H, d, J = 3.5 Hz, Ar-H), 7.57 (2H, m, Ar-H), 7.52 (1H, t, J = 7.2 Hz, Ar-H), 6.86 (1H, d, J = 3.5 Hz, Ar-H). ^{13}C NMR (DMSO- d_6 , 150 MHz) δ 155.47, 152.63, 150.88, 137.94, 129.95, 128.79, 128.52, 127.75, 114.50, 99.90. APCI-HRMS m/z : calcd. for $\text{C}_{12}\text{H}_9\text{N}_3$ (MH^+), 196.0869 found 196.0863; Purity (HPLC): 99%.

4-(4-Methylphenyl)-7H-pyrrolo[2,3-d]pyrimidine (7b):

Compound was synthesised from 4-methylphenylboronic acid and 6-chloro-7-deazapurine in a yield of 24%, recrystallised from methanol, pale yellow crystals, mp 207 °C, R_f = 0.61 (hexane: ethyl acetate: 1:1). ^1H NMR (DMSO- d_6 , 600 MHz) δ 12.21 (1H, s, NH), 8.80 (1H, s, Ar-H), 8.08 (2H, d, J = 8.1 Hz, Ar-H), 7.63 (1H, d, J = 3.5 Hz, Ar-H), 7.38 (2H, d, J = 8.0 Hz, Ar-H), 6.87 (1H, d, J = 3.6 Hz, Ar-H), 6.87 (3H, s, Me-4'). ^{13}C NMR (DMSO- d_6 , 150 MHz) δ 155.49, 152.52, 150.86, 139.73, 135.19, 129.38, 128.45, 127.41, 114.24, 99.99, 20.96 (Me-4'). APCI-HRMS m/z : calcd. for $\text{C}_{13}\text{H}_{11}\text{N}_3$ (MH^+), 210.1026, found 210.1029; Purity (HPLC): 94%.

3-(7H-Pyrrolo[2,3-d]pyrimidin-4-yl)phenol (7c):

Compound was synthesised from 3-hydroxyphenylboronic acid and 6-chloro-7-deazapurine in a yield of 35%, recrystallised from methanol, white crystals, mp 263 °C, Rf = 0.57 (hexane: ethyl acetate: 1:1). ¹H NMR (DMSO-*d*₆, 600 MHz) δ 12.23 (1H, s, NH), 9.66 (1H, s, OH), 8.85 (1H, s, Ar-H), 7.69 – 7.67 (1H, m, Ar-H), 7.67 – 7.64 (2H, m, Ar-H), 7.42 (1H, t, *J* = 7.8 Hz, Ar-H), 6.98 (1H, dd, *J* = 8.1, 1.7 Hz, Ar-H), 6.88 (1H, d, *J* = 3.5 Hz, Ar-H). ¹³C NMR (DMSO-*d*₆, 150 MHz) δ 157.70, 155.62, 152.66, 150.87, 139.28, 129.84, 127.56, 119.39, 117.10, 115.24, 114.51, 100.00. APCI-HRMS *m/z*: calcd. for C₁₂H₉N₃O (MH⁺), 212.0818, found 212.0816; Purity (HPLC): 99%.

4-(7H-Pyrrolo[2,3-d]pyrimidin-4-yl)phenol (7d):

Compound was synthesised from 4-hydroxyphenylboronic acid and 6-chloro-7-deazapurine in a yield of 82%, recrystallised from methanol, gold crystals, mp > 300 °C, Rf = 0.61 (hexane: ethyl acetate: 1:1). ¹H NMR (DMSO-*d*₆, 600 MHz) δ 12.13 (1H, s, NH), 9.98 (1H, s, OH), 8.74 (1H, s, Ar-H), 8.08 (2H, d, *J* = 8.3 Hz, Ar-H), 7.58 (1H, s, Ar-H), 6.95 (2H, d, *J* = 8.2 Hz, Ar-H), 6.87 (1H, s, Ar-H). ¹³C NMR (DMSO-*d*₆, 150 MHz) δ 159.45, 155.54, 152.44, 150.82, 130.20, 128.77, 126.87, 115.61, 113.61, 100.18. APCI-HRMS *m/z*: calcd. for C₁₂H₉N₃O (MH⁺), 212.0818, found 212.0814; Purity (HPLC): 90%.

4-(3-Methoxyphenyl)-7H-pyrrolo[2,3-d]pyrimidine (7e):

Compound was synthesised from 3-methoxyphenylboronic acid and 6-chloro-7-deazapurine in a yield of 62%, recrystallised from methanol, gold crystals, mp 220 °C, Rf = 0.48 (hexane: ethyl acetate: 1:1). ¹H NMR (DMSO-*d*₆, 600 MHz) δ 12.27 (1H, s, NH), 8.84 (1H, s, Ar-H), 7.74 (1H, d, *J* = 7.8 Hz, Ar-H), 7.68 (1H, dd, *J* = 2.4, 1.6 Hz, Ar-H), 7.65 (1H, d, *J* = 3.5 Hz, Ar-H), 7.50 (1H, t, *J* = 7.9 Hz, Ar-H), 7.13 – 7.10 (1H, m, Ar-H), 6.68 (1H, d, *J* = 3.5 Hz, Ar-H), 3.86 (1H, s, MeO-3'). ¹³C NMR (DMSO-*d*₆, 150 MHz) δ 159.51, 155.31, 152.59, 150.84, 139.36, 129.90, 127.72, 120.94, 115.98, 114.53, 113.25, 99.91, 55.18 (MeO-3'). APCI-HRMS *m/z*: calcd. for C₁₃H₁₁N₃O (MH⁺), 226.0975, found 226.0981; Purity (HPLC): 99%.

4-(4-Methoxyphenyl)-7H-pyrrolo[2,3-d]pyrimidine (7f):

Compound was synthesised from 4-methoxyphenylboronic acid and 6-chloro-7-deazapurine in a yield of 39%, recrystallised from methanol, dark green crystals, mp 207 °C, R_f = 0.51 (hexane: ethyl acetate: 1:1). ¹H NMR (DMSO-*d*₆, 600 MHz) δ 8.78 (1H, s, Ar-H), 8.20 – 8.17 (2H, m, Ar-H), 7.61 (1H, d, *J* = 3.5 Hz, Ar-H), 7.15 – 7.11 (2H, m, Ar-H), 6.88 (1H, d, *J* = 3.6 Hz, Ar-H), 3.85 (1H, s, MeO-4'). ¹³C NMR (DMSO-*d*₆, 150 MHz) δ 160.78, 155.11, 152.53, 150.80, 130.37, 130.05, 127.27, 114.20, 113.84, 99.99, 55.30 (MeO-4'). APCI-HRMS *m/z*: calcd. for C₁₃H₁₁N₃O (MH⁺), 226.0975, found 226.0978; Purity (HPLC): 97%.

4-(2,3-Dimethoxyphenyl)-7H-pyrrolo[2,3-d]pyrimidine (7g):

Compound was synthesised from 2,3-dimethoxyphenylboronic acid and 6-chloro-7-deazapurine in a yield of 57%, recrystallised from methanol, yellow crystals, mp 142 °C, R_f = 0.52 (hexane: ethyl acetate: 1:1). ¹H NMR (DMSO-*d*₆, 600 MHz) δ 12.11 (1H, s, NH), 8.81 (1H, s, Ar-H), 7.53 (1H, d, *J* = 3.5 Hz, Ar-H), 7.20 (2H, d, *J* = 4.6 Hz, Ar-H), 7.09 (1H, d, *J* = 4.6 Hz, Ar-H), 6.36 (1H, d, *J* = 3.5 Hz, Ar-H), 3.89 (3H, s, Me-2'), 3.57 (3H, s, Me-3'). ¹³C NMR (DMSO-*d*₆, 150 MHz) δ 155.78, 152.72, 151.60, 150.58, 146.68, 132.35, 126.67, 123.84, 122.18, 116.75, 113.80, 100.25, 60.83 (MeO-2'), 55.83 (MeO-3'). APCI-HRMS *m/z*: calcd. for C₁₄H₁₃N₃O₂(MH⁺), 256.1081, found 256.1089; Purity (HPLC): 99%.

4-(4-Phenoxyphenyl)-7H-pyrrolo[2,3-d]pyrimidine (7h):

Compound was synthesised from 4-phenoxyphenylboronic acid and 6-chloro-7-deazapurine in a yield of 24%, recrystallised from methanol, white powder, mp 210 °C, R_f = 0.76 (hexane: ethyl acetate: 1:1). ¹H NMR (DMSO-*d*₆, 600 MHz) δ 12.23 (1H, s, NH), 8.80 (1H, s, Ar-H), 8.23 (2H, d, *J* = 7.9 Hz, Ar-H), 7.64 (1H, s, Ar-H), 7.46 (2H, t, *J* = 7.1 Hz, Ar-H), 7.22 (1H, t, *J* = 6.9 Hz, Ar-H), 7.15 (4H, t, *J* = 7.5 Hz, Ar-H), 6.90 (1H, s, Ar-H). ¹³C NMR (DMSO-*d*₆, 150 MHz) δ 158.67, 155.75, 154.73, 152.58, 150.85, 132.73, 130.45, 130.25, 127.52, 124.23, 119.57, 118.04, 114.08, 99.98. APCI-HRMS *m/z*: calcd. for C₁₈H₁₃N₃O (MH⁺), 288.1131, found 288.1129; Purity (HPLC): 97%.

4-(Furan-2-yl)-7H-pyrrolo[2,3-d]pyrimidine (7i):

Compound was synthesised from 2-furanylboric acid and 6-chloro-7-deazapurine in a yield of 32%, recrystallised from methanol, green crystals, mp 205 °C, Rf = 0.61 (hexane: ethyl acetate: 1:1). ¹H NMR (DMSO-*d*₆, 600 MHz) δ 12.19 (1H, s, NH), 8.71 (1H, s, Ar-H), 8.04 – 8.03 (1H, m, Ar-H), 7.62 (1H, d, *J* = 3.6 Hz, Ar-H), 7.43 (1H, d, *J* = 3.4 Hz, Ar-H), 6.95 (1H, d, *J* = 3.5 Hz, Ar-H), 6.77 – 6.76 (1H, m, Ar-H). ¹³C NMR (DMSO-*d*₆, 150 MHz) δ 152.81, 152.66, 150.85, 146.00, 145.92, 127.64, 112.64, 112.51, 111.77, 100.22. APCI-HRMS *m/z*: calcd. for C₁₀H₇N₃O (MH⁺), 186.0662, found 186.0655; Purity (HPLC): 93%.

4-(1-Benzofuran-2-yl)-7H-pyrrolo[2,3-d]pyrimidine (7j):

Compound was synthesised from 2-benzofuranylboric acid and 6-chloro-7-deazapurine in a yield of 23%, recrystallised from methanol, white powder, mp 272 °C, Rf = 0.42 (hexane: ethyl acetate: 1:1). ¹H NMR (DMSO-*d*₆, 600 MHz) δ 12.31 (1H, s, NH), 8.86 (1H, s, Ar-H), 7.91 (1H, s, Ar-H), 7.83 (2H, m, Ar-H), 7.75 (1H, d, *J* = 3.5 Hz, Ar-H), 7.49 (1H, d, *J* = 7.8 Hz, Ar-H), 7.39 (1H, t, *J* = 7.5 Hz, Ar-H), 7.20 (1H, d, *J* = 3.5 Hz, Ar-H). ¹³C NMR (DMSO-*d*₆, 150 MHz) δ 155.42, 154.69, 153.18, 151.10, 146.01, 128.58, 128.03, 126.40, 123.84, 122.46, 113.30, 111.96, 108.53, 100.72. APCI-HRMS *m/z*: calcd. for C₁₄H₉N₃O (MH⁺), 236.0818, found 236.0792; Purity (HPLC): 97%.

4-(Thiophen-2-yl)-7H-pyrrolo[2,3-d]pyrimidine (7k):

Compound was synthesised from 2-thienylboronic acid and 6-chloro-7-deazapurine in a yield of 44%, recrystallised from methanol, pale green powder, mp 266 °C, Rf = 0.64 (hexane: ethyl acetate: 1:1). ¹H NMR (DMSO-*d*₆, 600 MHz) δ 12.25 (1H, s, NH), 8.74 (1H, s, Ar-H), 8.17 (1H, dd, *J* = 4.1, 1.3 Hz, Ar-H), 7.86 (1H, dd, *J* = 5.0, 0.6 Hz, Ar-H), 7.69 (1H, d, *J* = 3.6 Hz, Ar-H), 7.35 – 7.32 (1H, m, Ar-H), 7.07 (1H, d, *J* = 3.6 Hz, Ar-H). ¹³C NMR (DMSO-*d*₆, 150 MHz) δ 152.72, 150.69, 149.67, 142.96, 130.23, 129.14, 128.92, 127.90, 112.17, 99.86. APCI-HRMS *m/z*: calcd. for C₁₀H₇N₃S (MH⁺), 202.0433, found 202.0442; Purity (HPLC): 97%.

4-(1-Benzothiophen-2-yl)-7H-pyrrolo[2,3-d]pyrimidine (7l):

Compound was synthesised from benzo[*b*]thien-2-ylboronic acid and 6-chloro-7-deazapurine in a yield of 44%, recrystallised from methanol, gold crystals, mp 287 – 289 °C, R_f = 0.31 (hexane: ethyl acetate: 1:1). ¹H NMR (DMSO-*d*₆, 600 MHz) δ 12.33 (1H, s, NH), 8.79 (1H, s, Ar-H), 8.54 (1H, s, Ar-H), 8.05 – 8.03 (1H, m, Ar-H), 8.03 – 8.01 (1H, m, Ar-H), 7.75 (1H, d, *J* = 3.6 Hz, Ar-H), 7.46 – 7.44 (2H, m, Ar-H), 7.21 (1H, d, *J* = 3.6 Hz, Ar-H). ¹³C NMR (DMSO-*d*₆, 150 MHz) δ 152.50, 150.39, 149.29, 142.86, 140.21, 139.86, 128.30, 125.88, 125.75, 124.71, 124.54, 122.33, 112.74, 99.68. APCI-HRMS *m/z*: calcd. C₁₄H₉N₃S (MH⁺), 252.0590, found 252.0577; Purity (HPLC): 98%.

4-(4-Fluorophenyl)-7H-pyrrolo[2,3-d]pyrimidine (7m):

Compound was synthesised from 4-fluorophenylboronic acid and 6-chloro-7-deazapurine in a yield of 23%, recrystallised from methanol, dark green coloured crystals, mp 243 °C, R_f = 0.36 (hexane: ethyl acetate: 1:1). ¹H NMR (DMSO-*d*₆, 600 MHz) δ 8.82 (1H, s, Ar-H), 8.25 (2H, dd, *J* = 8.6, 5.7 Hz, H-2', H-6'), 7.66 (1H, d, *J* = 3.5 Hz, H-6), 7.40 (2H, m, H-3', H-5'), 6.88 (1H, d, *J* = 3.5 Hz, Ar-H). ¹³C NMR (DMSO-*d*₆, 150 MHz) δ 163.21 (d, *J* = 247.8 Hz), 154.30 (s), 152.67 (s), 150.82 (s), 134.41 (d, *J* = 2.9 Hz), 130.81 (d, *J* = 8.7 Hz), 127.94 (s), 115.78 (d, *J* = 21.6 Hz), 114.29 (s), 99.81 (s). APCI-HRMS *m/z*: calcd. for C₁₂H₈FN₃ (MH⁺), 214.0775, found 214.0776; Purity (HPLC): 99%

4-(3,4-Difluorophenyl)-7H-pyrrolo[2,3-d]pyrimidine (7n):

Compound was synthesised from 3,4-difluorophenylboronic acid and 6-chloro-7-deazapurine in a yield of 23%, recrystallised from methanol, pale green crystals, mp 246 °C, R_f = 0.56 (hexane: ethyl acetate: 1:1). ¹H NMR (DMSO-*d*₆, 600 MHz) δ 8.84 (1H, s, Ar-H), 8.19 – 8.13 (1H, m, H-Ar-H), 8.10 – 8.05 (1H, m, H-2'), 7.70 (1H, d, *J* = 3.5 Hz, Ar-H), 7.64 (1H, dd, *J* = 18.9, 8.6 Hz, Ar-H), 6.94 (1H, d, *J* = 3.6 Hz, Ar-H). ¹³C NMR (DMSO-*d*₆, 150 MHz) δ 152.91 (d, *J* = 4.6 Hz), 152.78 (s), 152.01 (dd, *J* = 197.7, 25.8 Hz), 150.74 (s), 149.35 (d, *J* = 220.0, 80.9 Hz), 135.45 (dd, *J* = 10.5, 7.0 Hz), 128.37 (s), 125.69 (dd, *J* = 6.8, 3.2 Hz), 118.00 (dd, *J* = 18.1, 4.9 Hz), 117.26 (dd, *J* = 18.9, 5.7 Hz), 114.34 (s), 99.72 (s). APCI-HRMS *m/z*: calcd. for C₁₂H₇F₂N₃ (MH⁺), 232.0681, found 232.0669; Purity (HPLC): 96%.

4-[4-(Trifluoromethyl)phenyl]-7H-pyrrolo[2,3-d]pyrimidine (7o):

Compound was synthesised from 4-(trifluoromethyl)phenylboronic acid and 6-chloro-7-deazapurine in a yield of 53%, recrystallised from methanol, yellow crystals, mp 218 °C, Rf = 0.64 (hexane: ethyl acetate: 1:1). ¹H NMR (DMSO-*d*₆, 600 MHz) δ 8.89 (1H, s, Ar-H), 8.38 (2H, d, *J* = 8.1 Hz, Ar-H), 7.93 (2H, d, *J* = 8.3 Hz, Ar-H), 7.73 (1H, d, *J* = 3.6 Hz, Ar-H), 6.93 (1H, d, *J* = 3.6 Hz, Ar-H). ¹³C NMR (DMSO-*d*₆, 150 MHz) δ 153.68 (s), 152.81 (s), 150.88 (s), 141.73 (s), 129.88 (q, *J* = 31.9 Hz), 129.29 (s), 128.53 (s), 125.68 (q, *J* = 3.6 Hz), 124.15 (q, *J* = 272.3 Hz), 114.84 (s), 99.65 (s). APCI-HRMS *m/z*: calcd. for C₁₃H₈F₃N₃ (MH⁺), 264.0743, found 264.0759; Purity (HPLC): 97%.

4-(4-Chlorophenyl)-7H-pyrrolo[2,3-d]pyrimidine (7p):

Compound was synthesised from 4-chlorophenylboronic acid and 6-chloro-7-deazapurine in a yield of 23%, recrystallised from methanol, white powder, mp 255 °C, Rf = 0.42 (hexane: ethyl acetate: 1:1). ¹H NMR (DMSO-*d*₆, 600 MHz) δ 12.27 (1H, s, NH), 8.84 (1H, s, Ar-H), 8.21 (2H, d, *J* = 8.5 Hz, Ar-H), 7.67 (1H, d, *J* = 3.5 Hz, Ar-H), 7.64 (2H, d, *J* = 8.5 Hz, Ar-H), 6.89 (1H, d, *J* = 3.6 Hz, Ar-H). ¹³C NMR (DMSO-*d*₆, 150 MHz) δ 154.04, 152.61, 150.77, 136.64, 134.72, 130.18, 128.79, 127.89, 114.32, 99.71. APCI-HRMS *m/z*: calcd. for C₁₂H₈ClN₃ (MH⁺), 230.0480, found 230.0467; Purity (HPLC): 96%.

4,7-Bis(4-methylphenyl)-7H-pyrrolo[2,3-d]pyrimidine (8a):

Compound was synthesised from 4-methylphenylboronic acid and 6-chloro-7-deazapurine in a yield of 23%, recrystallised from methanol, white powder, mp 158 °C, Rf = 0.42 (hexane: ethyl acetate: 1:1). ¹H NMR (DMSO-*d*₆, 600 MHz) δ 8.90 (1H, s, H-2), 8.11 (2H, d, *J* = 8.0 Hz, Ar-H), 8.05 (1H, d, *J* = 3.7 Hz, Ar-H), 7.76 (2H, d, *J* = 8.2 Hz, Ar-H), 7.43 (2H, d, *J* = 7.9 Hz, Ar-H'), 7.39 (2H, d, *J* = 8.1 Hz, Ar-H), 7.12 (1H, d, *J* = 3.7 Hz, Ar-H), 2.43 (3H, s, Me-4'), 2.40 (3H, s, Me-10'). ¹³C NMR (DMSO-*d*₆, 150 MHz) δ 156.54, 151.50, 151.02, 140.12, 136.29, 134.74, 134.66, 129.95, 129.69, 129.50, 128.61, 123.71, 115.45, 101.46, 20.99 (Me-4'), 20.58 (Me-10'). APCI-HRMS *m/z*: calcd. for C₂₀H₁₈N₃ (MH⁺), 300.1495, found 300.1504; Purity (HPLC): 96%.

4,7-Bis(4-methoxyphenyl)-7H-pyrrolo[2,3-d]pyrimidine (8b):

Compound was synthesised from 4-methoxyphenylboronic acid and 6-chloro-7-deazapurine in a yield of 23%, recrystallised from methanol, white powder, mp 175 °C, Rf = 0.42 (hexane: ethyl acetate: 1:1). ¹H NMR (DMSO-*d*₆, 600 MHz) δ 8.85 (1H, s, Ar-H), 8.21 (2H, d, *J* = 8.7 Hz, H-2', H-6'), 7.98 (1H, d, *J* = 3.7 Hz, H-6), 7.74 (2H, d, *J* = 8.8 Hz, H-8', H-12'), 7.16 (2H, d, *J* = 8.7 Hz, Ar-H), 7.14 (2H, d, *J* = 8.8 Hz, Ar-H), 7.11 (1H, d, *J* = 3.7 Hz, Ar-H), 3.88 (3H, s, MeO-4'), 3.84 (3H, s, MeO-10'). ¹³C NMR (DMSO-*d*₆, 150 MHz) δ 161.13, 158.13, 156.20, 151.50, 151.12, 138.72, 130.31, 130.24, 130.05, 125.52, 114.91, 114.53, 114.41, 101.28, 55.57 (MeO-4'), 55.44 (MeO-10'). APCI-HRMS *m/z*: calcd. for C₂₀H₁₈N₃O₂ (MH⁺), 332.1394, found 332.1403; Purity (HPLC): 93%.

4,7-Bis(4-fluorophenyl)-7H-pyrrolo[2,3-d]pyrimidine (8c):

Compound was synthesised from 4-fluorophenylboronic acid and 6-chloro-7-deazapurine in a yield of 23%, recrystallised from methanol, white powder, mp 163 °C, Rf = 0.42 (hexane: ethyl acetate: 1:1). ¹H NMR (DMSO-*d*₆, 600 MHz) δ 8.94 (1H, s, Ar-H), 8.28 (2H, m, H-2', Ar-H), 8.12 (1H, d, *J* = 3.8 Hz, Ar-H), 7.92 (2H, m, Ar-H), 7.45 (4H, m, Ar-H), 7.17 (1H, d, *J* = 3.8 Hz, Ar-H). ¹³C NMR (DMSO-*d*₆, 150 MHz) δ 163.41 (d, *J* = 248.3), 160.58 (d, *J* = 244.0 Hz), 155.48 (s), 151.57 (s), 151.14 (s), 133.61 (dd, *J* = 72.2, 2.7 Hz), 131.03 (d, *J* = 8.8 Hz), 130.37 (s), 130.09 (dd, *J* = 411.9, 10.8 Hz), 126.06 (d, *J* = 8.6 Hz), 116.90 (d, *J* = 22.9 Hz), 115.93 (d, *J* = 21.7 Hz), 115.43 (s), 101.51 (s). APCI-HRMS *m/z*: calcd. for C₁₈H₁₂F₂N₃ (MH⁺), 308.0994, found 308.0976; Purity (HPLC): 92%.

4.2.7.5 Protein kinase assays

All the PK assays were executed following methods described by Nguyen and co-workers, in duplicate (Nguyen *et al.*, 2019), apart from: (i) *Lm*CK1 (from *Leishmania major*, recombinant, expressed in bacteria) was assayed in buffer A with 170 μM of CK1-specific peptide substrate, RRKHAAIGSpAYSITA (where "Sp" represents phosphorylated serine); (ii) *Hs*CK1ε (casein kinase 1ε, human, recombinant, expressed by baculovirus in Sf9 insect cells) was assayed in buffer A with 170 μM of the CK1-specific peptide substrate; (iii) *Hs*GSK-3β (glycogen synthase kinase-3, human, recombinant, expressed by baculovirus in Sf9 insect cells) isoforms were assayed in buffer A with 20 μM of GS-1 peptide, YRRAAVPPSPSLSRHSSPHQSpEDEEE, a GSK-3-selective substrate. Buffer A is composed of 10 mM MgCl₂, 1mM EGTA, 1 mM DTT, 25 mM Tris-HCl pH 7.5, 50 μg/mL heparin. The peptides were sourced from Proteogenix (Oberhausbergen, France). The kinase activities were measured by means of the ADP-GLO™ assay kit according to the manufacturer recommendations (Promega, Madison, WI). The assay

is a luminescent ADP detection assay that provides a homogeneous and high-throughput screening method to measure kinase activity by quantifying the amount of ADP produced during a kinase reaction. The kinase reactions were performed in 384-well plates in a final volume of 6 μ L (initially, the compounds were diluted in DMSO to obtain 10 mM stock solutions) for 30 min at 30 °C in an appropriate kinase buffer, with either protein or peptide as substrate in the presence of 10 μ M ATP. Subsequently, 6 μ L of ADP-Glo™ kinase reagent was added to quench the kinase reaction, followed by a 50 min incubation time at room temperature (RT). Hereafter, 12 μ L of Kinase Detection Reagent was added and the mixture was left for an hour at RT. The resulting signal was measured by the Envision microplate luminometer (PerkinElmer, Waltham, MA) and expressed in Relative Light Unit (RLU).

The effect of the 7-azaindole and 7-deazapurine derivatives on kinase activity were evaluated as a two-step process: Step 1- served as the primary screening, where all the compounds were tested at 1 and 10 μ M against a panel of eight disease-related kinases and the activities were expressed in % of maximal activity, i.e. measured with DMSO diluted at 1% v/v concentration; Step 2- The more active compounds (deemed “hits”) were then tested in duplicate over a wide range of concentrations (usually between 0.0003 and 10 μ M) against the appropriate targets identified in step 1 (here Haspin and CDK9/Cyclin T).

4.2.7.6 Cytotoxicity prediction

The cytotoxicity of the most promising compounds were predicted by using a prediction tool known as, CLC-Pred: *in silico* prediction of cytotoxicity for tumour and non-tumour cell-lines (Lagunin *et al.*, 2018). The tool was developed by making use of the existing Prediction of Activity Spectra for Substances (PASS) algorithm to create and validate the classification structure-activity relationship (SAR) models for predicting cytotoxicity of structures against numerous types of human cell-lines using ChEMBL experimental data. The experimental data from ChEMBL (IG₅₀, IC₅₀ and % inhibition values) forms the foundation for a training set of 59882 structures of compounds and the average accuracy of predicting area-under-the-curve (AUC) is given as 0.930 and 0.927 for 278 cancer cell-lines, when calculated by leave-one-out and 20-fold cross-validation procedure, respectively (Lagunin *et al.*, 2018).

4.2.7.7 SwissADME prediction

In addition to the cytotoxicity, the most promising compounds were also evaluated using the SwissADME prediction tool (Daina *et al.*, 2017). SwissADME is a free web tool that utilises a set of fast, but robust predictive models. These models enable the user to generate physiochemical properties, lipophilicity, water solubility, pharmacokinetic properties, druglikeness and qualities

useful in medicinal chemistry for the desired compounds. The complete results for compounds **7a**, **7b**, **7c**, **7d**, **7e**, **7f**, **7k**, **7m**, **7n** and **7p** can be found in the supplementary information.

4.2.8 Results and discussion

Previously, it has been determined that derivatives of the 7-azaindole scaffold is likely to be PK inhibitors (Pieterse *et al.*, 2020). In this study, eleven (11) 7-azaindole and nineteen (19) 7-deazapurine derivatives were synthesised and screened against the CDK9/CyclinT and Haspin kinases. In addition, the compounds were also screened against **HsCDK2/CyclinA**, **HsCDK5/p25**, **HsPim-1**, **HsCK1 ϵ** , **HsGSK3 β** , **LmCK1** and **HsABL1**. Screenings were performed at 10 and 1 μ M concentrations (diluted in 1% DMSO v/v). The most promising compound were subjected to further testing at a wider range of concentrations. The IC₅₀ values were determined from dose-response curves (Prism-GraphPad). The results of the screening and additional testing can be found in Table 4 and Table 5, respectively.

In general, the results of this study echo those of a previous study. It was observed that the position of substituents on the pyridine ring of the 7-azaindole scaffold, greatly affects a compounds ability to exhibit PK inhibition. The 4-position was deemed optimal for CDK9/CyclinT and Haspin inhibition (Pieterse *et al.*, 2020). Like the 5-substituted 7-azaindole derivatives in the previous study, the 6-substituted 7-azaindole derivatives display a lack of inhibitory activity towards PKs deployed in this study (at the concentrations tested). Additionally, the structurally related 4-substituted 7-deazapurines derivatives (substituted on the equivalent to the 4-position on the 7-azaindole scaffold) displayed a greater inclination toward PK inhibition.

The screening against CDK9/CyclinT revealed 10 active compounds (**7a–f**, **7m**, **7n**, and **7p**) and 9 relatively inactive compounds (**7g–7j**, **7l**, **7o** and **8a–c**). IC₅₀ values for the active compounds ranged from 0.38 μ M to 1.44 μ M. The most active compound proved to be the C4-hydroxyphenyl substituted derivative (**7d**) exhibiting an IC₅₀ value of 0.38 μ M, followed by the C3-hydroxyphenyl substituted derivative (**7c**) with an IC₅₀ value of 0.59 μ M against CDK9/CyclinT. Interestingly, the C3-methoxyphenyl (**7e**) and the C4-methoxyphenyl substituted derivative (**7f**) performed equivalently, with 1.24 μ M and 1.26 μ M IC₅₀ values, respectively. Additionally, the results show that the C4-hydroxyphenyl is preferred over the C4-methoxyphenyl and C4-methylphenyl substituted derivatives (IC₅₀ values: 0.38 μ M (**7d**) > 1.24 μ M (**7f**) > 1.39 μ M (**7b**). Considering the halogen-substituted compounds, compound **7m** (4-fluorophenyl substituted derivative) was 2.32 times more active than compound **7p** (4-chlorophenyl substituted derivative), with a 1.44 μ M IC₅₀ value. Furthermore, the C3,4-difluorophenyl substituted derivative (**7n**: IC₅₀ value of 0.72) exhibits a slight decrease in activity when compared to the C4-fluorophenyl monosubstituted derivative (**7m**: IC₅₀ value of 0.62), while the 4-trifluoromethylphenyl substituted derivative (**7o**) was deemed

relatively inactive. Noteworthy, compound **7k**, which bears a 2-thiophene ring, is the only one of the compounds with an alternative ring structure to exhibit activity for CDK9/CyclinT, while the bis-substituted compounds (**8a–c**) were all deemed to be relatively inactive.

Haspin screening data revealed four promising compounds (**7c**, **7d**, **7f** and **7k**). Here the IC₅₀ values ranged from 0.11 µM to 0.42 µM. In contrast to the CDK9/CyclinT results, the 4-methoxy substituted 7-deazapurine derivative (**7f**) proved to be the most potent Haspin inhibitor (IC₅₀ value of 0.11 µM), while the C4-hydroxy substituted 7-deazapurine derivative was 4-fold less potent (IC₅₀ value of 0.42 µM). Additionally, the C3-hydroxy substituted 7-deazapurine derivative seems to be favoured over the C4-hydroxy substituted 7-deazapurine derivative (IC₅₀ values of 0.19 and 0.42, respectively). Once again, compound **7k** is the only compound bearing an alternative ring that possessed activity toward Haspin, at the concentrations tested.

Regarding the cytotoxicity, the prediction was achieved by using the CLC-Pred prediction platform (see the “Cytotoxicity prediction” of the material and methods section) and illustrated that most of the 7-deazapurine derivatives are expected to have a cytotoxicity score higher than at least 0.400 for the given cell-lines (see Table 6). The score indicates the percentage of compounds in the training data set that will likely be less cytotoxic than the tested compound for the given cell-line. Based on the results, compound **7m** exhibited the highest cytotoxicity score of all the derivatives for any given cell-line (cytotoxicity score of 0.672 against Hs 683 (Oligodendroglioma). Translated this means that compound **7m** is 67.2 % more likely than the known compounds in the training data set to be cytotoxic against the Hs 683 cell line. In addition to the cytotoxicity prediction, the SwissADME tool revealed that these compounds are likely to cross the blood-brain barrier, thus highlighting their potential use in brain tumours (see Figure 2). The most promising compounds are also expected to have high gastrointestinal absorption. Unfortunately, the predicted bioavailability for these compounds is moderate (achieving a bioavailability score of 0.55) and could benefit from investigating options for increasing drug-likeness. The full set of properties and values predicted by the SwissADME tool can be found in the supplementary information.

4.2.9 Conclusion

This study evaluated the inhibitory potential of two structurally related chemical series against selected disease-related protein kinases. The first series consisted of C6-substituted 7-azaindole derivatives while second series is constituted by C4-substituted 7H-pyrrolo[2,3-d]pyrimidine derivatives. The kinase-based screening assays performed against several PKs, revealed that compounds belonging to the 7-deazapurine scaffold and substituted on the C4-position, exhibited activity toward the CDK9/CyclinT and Haspin kinases. On the other hand, the C6-substituted 7-azaindole derivatives lacked activity at the concentrations tested. In conjunction with previous

results, it is postulated that the substituents at C4-position play a key role in activity against CDK9/CyclinT and Haspin. The most active compound against CDK9/CyclinT proved to be the C4-hydroxyphenyl substituted 7H-pyrrolo[2,3-d]pyrimidine derivative (**7d**) with an IC_{50} value of 0.38 μ M. On the other hand, the C4-methoxyphenyl substituted 7H-pyrrolo[2,3-d]pyrimidine derivative (**7f**) exhibited the best activity against Haspin (with IC_{50} value of 0.11 μ M), making it the most potent hit compound of this study.

References

- Allegretti, M., Arcadi, A., Marinelli, F., & Nicolini, L. (2001). Palladium-catalysed functionalisation at 4-and 6-position of the 7-azaindole system. *Synlett*, 2001(05), 0609-0612. doi:10.1055/s-2001-13366
- Amoussou, N. G., Bigot, A., Roussakis, C., & Robert, J.-M. H. (2018). Haspin: a promising target for the design of inhibitors as potent anticancer drugs. *Drug Discovery Today*, 23(2), 409-415. doi:10.1016/j.drudis.2017.10.005
- Bhagirath, N., Dominique, R., Kennedy-Smith, J., Lopez-Tapia, F. J., Mertz, E., Qiao, Q., & So, S.-S. (2015). Inhibitors of bruton's tyrosine kinase. In: Google Patents.
- Chen, Y., Fu, D., Zhao, H., Cheng, W., & Xu, F. (2020). GSG2 (Haspin) promotes development and progression of bladder cancer through targeting KIF15 (Kinase-12). *Aging*, 12(10).
- Cincinelli, R., Musso, L., Merlini, L., Giannini, G., Vesci, L., Milazzo, F. M., . . . Artali, R. (2014). 7-Azaindole-1-carboxamides as a new class of PARP-1 inhibitors. *Bioorganic & Medicinal Chemistry*, 22(3), 1089-1103. doi:10.1016/j.bmc.2013.12.031
- Dai, J., & Higgins, J. M. (2005). Haspin: a mitotic histone kinase required for metaphase chromosome alignment. *Cell cycle*, 4(5), 665-668.
- Dai, J., Sullivan, B. A., & Higgins, J. M. (2006). Regulation of mitotic chromosome cohesion by Haspin and Aurora B. *Developmental cell*, 11(5), 741-750.
- Daina, A., Michielin, O., & Zoete, V. (2017). SwissADME: a free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. *Scientific reports*, 7, 42717. doi:10.1038/srep42717
- de Cárcer, G., Perez de Castro, I., & Malumbres, M. (2007). Targeting cell cycle kinases for cancer therapy. *Current medicinal chemistry*, 14(9), 969-985. doi:10.2174/092986707780362925
- Eswaran, J., Patnaik, D., Filippakopoulos, P., Wang, F., Stein, R. L., Murray, J. W., . . . Knapp, S. (2009). Structure and functional characterization of the atypical human kinase haspin. *Proceedings of the National Academy of Sciences*, 106(48), 20198-20203.
- Fischer, E. H., & Krebs, E. G. (1955). Conversion of phosphorylase b to phosphorylase a in muscle extracts. *Journal of Biological Chemistry*, 216(1), 121-132.

Flight, M. H. (2013). Neurodegenerative diseases: New kinase targets for Alzheimer's disease. *Nature Reviews Drug Discovery*, 12(10), 739.

Franco, L. C., Morales, F., Boffo, S., & Giordano, A. (2018). CDK9: A key player in cancer and other diseases. *Journal of Cellular Biochemistry*, 119(2), 1273-1284. doi:10.1002/jcb.26293

Han, L., Wang, P., Sun, Y., Liu, S., & Dai, J. (2017). Anti-melanoma activities of haspin inhibitor CHR-6494 deployed as a single agent or in a synergistic combination with MEK inhibitor. *Journal of Cancer*, 8(15), 2933.

Han, X., Kuang, T., Ren, Y., Lu, Z., Liao, Q., & Chen, W. (2019). Haspin knockdown can inhibit progression and development of pancreatic cancer in vitro and vivo. *Experimental cell research*, 385(1), 111605.

Higgins, J. (2003). Structure, function and evolution of haspin and haspinrelated proteins, a distinctive group of eukaryotic protein kinases. *Cellular and Molecular Life Sciences CMLS*, 60(3), 446-462. doi:10.1007/s000180300038

Higgins, J. M. (2001). The Haspin gene: location in an intron of the Integrin α E gene, associated transcription of an Integrin α E-derived RNA and expression in diploid as well as haploid cells. *Gene*, 267(1), 55-69. doi: 10.1016/S0378-1119(01)00387-0

Higgins, J. M. (2010). Haspin: a newly discovered regulator of mitotic chromosome behavior. *Chromosoma*, 119(2), 137-147.

Hnisz, D., Abraham, B. J., Lee, T. I., Lau, A., Saint-André, V., Sigova, A. A., . . . Young, R. A. (2013). Super-enhancers in the control of cell identity and disease. *Cell*, 155(4), 934-947. doi:10.1016/j.cell.2013.09.053

Huertas, D., Soler, M., Moreto, J., Villanueva, A., Martinez, A., Vidal, A., . . . McDermott, J. (2012). Antitumor activity of a small-molecule inhibitor of the histone kinase Haspin. *Oncogene*, 31(11), 1408-1418.

Huse, M., & Kuriyan, J. (2002). The conformational plasticity of protein kinases. *Cell*, 109(3), 275-282.

Kashyap, S., Sandler, J., Peters, U., Martinez, E. J., & Kapoor, T. M. (2014). Using 'biased-privileged'scaffolds to identify lysine methyltransferase inhibitors. *Bioorganic & Medicinal Chemistry*, 22(7), 2253-2260. doi:10.1016/j.bmc.2014.02.024

Kim, S.-W., Kim, M., Lee, W. Y., & Hyeon, T. (2002). Fabrication of hollow palladium spheres and their successful application to the recyclable heterogeneous catalyst for Suzuki coupling reactions. *Journal of the American Chemical Society*, 124(26), 7642-7643.

Krebs, E. G., Graves, D. J., & Fischer, E. H. (1959). Factors affecting the activity of muscle phosphorylase b kinase. *Journal of Biological Chemistry*, 234(11), 2867-2873.

Lagunin, A. A., Dubovskaja, V. I., Rudik, A. V., Pogodin, P. V., Druzhilovskiy, D. S., Glorizova, T. A., . . . Poroikov, V. V. (2018). CLC-Pred: a freely available web-service for in silico prediction of human cell line cytotoxicity for drug-like compounds. *PloS one*, 13(1), e0191838. doi:10.1371/journal.pone.0191838

Lawson, M., Rodrigo, J., Baratte, B., Robert, T., Delehouzé, C., Lozach, O., . . . Alami, M. (2016). Synthesis, biological evaluation and molecular modeling studies of imidazo [1, 2-a] pyridines derivatives as protein kinase inhibitors. *European journal of medicinal chemistry*, 123, 105-114.

Li, Q., Persoons, L., Daelemans, D., & Herdewijn, P. (2019). Iron/Copper Co-Catalyzed Cross-Coupling Reaction for the Synthesis of 6-Substituted 7-Deazapurines and the Corresponding Nucleosides. *The Journal of Organic Chemistry*, 85(2), 403-418. doi:10.1021/acs.joc.9b02414

Manning, G., Whyte, D. B., Martinez, R., Hunter, T., & Sudarsanam, S. (2002). The protein kinase complement of the human genome. *Science*, 298(5600), 1912-1934.

Melms, J. C., Vallabhaneni, S., Mills, C. E., Yapp, C., Chen, J.-Y., Morelli, E., . . . Moret, N. (2020). Inhibition of haspin kinase promotes cell-intrinsic and extrinsic antitumor activity. *Cancer Research*, 80(4), 798-810.

Nguyen, T.-N.-D., Feizbakhsh, O., Sfecchi, E., Baratte, B., Delehouzé, C., Garcia, A., . . . Mehiri, M. (2019). Kinase-based screening of marine natural extracts leads to the identification of a cytotoxic high molecular weight metabolite from the mediterranean sponge *crambe tailliezi*. *Marine drugs*, 17(10), 569. doi:10.3390/md17100569

Noh, H., & King, G. (2007). The role of protein kinase C activation in diabetic nephropathy. *Kidney International*, 72, S49-S53.

Pieterse, L., Legoabe, L. J., Beteck, R. M., Josselin, B., Bach, S., & Ruchaud, S. (2020). Synthesis and biological evaluation of selected 7-azaindole derivatives as CDK9/Cyclin T and Haspin inhibitors. *MEDICINAL CHEMISTRY RESEARCH*. doi:10.1007/s00044-020-02560-1

Pokholok, D. K., Zeitlinger, J., Hannett, N. M., Reynolds, D. B., & Young, R. A. (2006). Activated signal transduction kinases frequently occupy target genes. *Science*, 313(5786), 533-536. doi:10.1126/science.1127677

Qiao, J. X., & Lam, P. Y. (2011). Recent Advances in Chan–Lam Coupling Reaction: Copper-Promoted C–Heteroatom Bond Cross-Coupling Reactions with Boronic Acids and Derivatives. *Boronic Acids: Preparation and Applications in Organic Synthesis, Medicine and Materials*, 1, 315-361.

Roskoski Jr, R. (2020). Properties of FDA-approved small molecule protein kinase inhibitors: A 2020 update. *Pharmacological Research*, 152, 104609.

Sabat, N., Nauš, P., Matyašovský, J., Dziuba, D., Slavětínská, L. P., & Hocek, M. (2016). Synthesis of Fluorescent 2-Substituted 6-(Het) aryl-7-deazapurine Bases {4-(Het) aryl-pyrrolo [2, 3-d] pyrimidines} by Aqueous Suzuki–Miyaura Cross-Coupling Reactions. *Synthesis*, 48(07), 1029-1045. doi:10.1002/chin.201633165

Sever, R., & Brugge, J. S. (2015). Signal transduction in cancer. *Cold Spring Harbor perspectives in medicine*, 5(4), a006098.

Tanaka, H., Wada, M., & Park, J. (2020). HASPIN kinase inhibitor CHR-6494 suppresses intestinal polyp development, cachexia, and hypogonadism in Apcmin/+ mice. *European Journal of Cancer Prevention*, 29(6), 481.

Tanaka, H., Yoshimura, Y., Nishina, Y., Nozaki, M., Nojima, H., & Nishimune, Y. (1994). Isolation and characterization of cDNA clones specifically expressed in testicular germ cells. *FEBS letters*, 355(1), 4-10. doi:10.1016/0014-5793(94)01155-9

Taylor, E. C., Macor, J. E., & Pont, J. L. (1987). Intramolecular diels-alder reactions of 1, 2, 4-triazines.: A general synthesis of furo [2, 3-b] pyridines, 2, 3-dihydropyrano [2, 3-b] pyridines, and pyrrolo [2, 3-b] pyridines. *Tetrahedron*, 43(21), 5145-5158. doi:10.1016/S0040-4020(01)87690-0

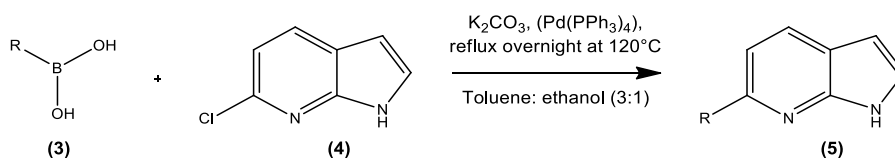
Villa, F., Capasso, P., Tortorici, M., Forneris, F., De Marco, A., Mattevi, A., & Musacchio, A. (2009). Crystal structure of the catalytic domain of Haspin, an atypical kinase implicated in chromatin organization. *Proceedings of the National Academy of Sciences*, 106(48), 20204-20209.

Wolfe, J. P., & Buchwald, S. L. (1999). A highly active catalyst for the room-temperature amination and Suzuki coupling of aryl chlorides. *Angewandte Chemie International Edition*, 38(16), 2413-2416.

Yu, F., Lin, Y., Xu, X., Liu, W., Tang, D., Zhou, X., . . . Xie, A. (2020). Knockdown of GSG2 inhibits prostate cancer progression in vitro and in vivo. *International journal of oncology*, 57(1), 139-150.

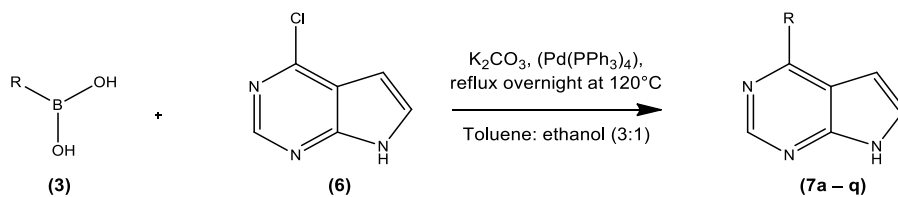
4.2.10 Tables

Table 4.1: A table depicting the synthesis of C6-substituted 7-azaindole derivatives, target compounds and yields achieved



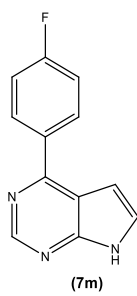
Cpd	Yield (%)	Cpd	Yield (%)	Cpd	Yield (%)
	26		94		54
	72		68		13
	50		30		32
	59		83		

Table 4.2: A table depicting the synthesis of the C4-substituted 7H-pyrrolo[2,3-*d*]pyrimidine derivatives, target compounds and yields achieved

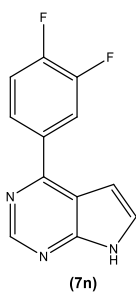


Cpd	Yield (%)	Cpd	Yield (%)	Cpd	Yield (%)	Cpd	Yield (%)
	53		24		35		82
	62		39		57		24
	32		31		44		40

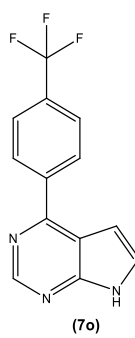
Table 4.2 Continued



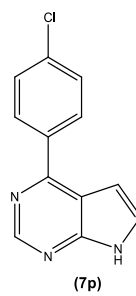
23



23



53



23

Table 4.3: A table depicting the synthesis of the C4, 7-bissubstituted 7H-pyrrolo[2,3-d]pyrimidine derivatives, target compounds and yields achieved

Cpd	Yield (%)	Cpd	Yield (%)	Cpd	Yield (%)
 (8a)	11	 (8b)	17	 (8c)	7

Table 4.4: Percentage of residual activity for compounds 5a–m, 7a–q and 8a–c at 10 μ M and 1 μ M using a representative kinase panel

Cpd	Hs CDK2/ CyclinA	Hs CDK5/ p25	Hs CDK9/ CyclinT	Hs Haspin	Hs PIM 1	Ssc CK1 δ/ϵ	Ssc GSK3 α/β	Lm CK1	Hs ABL1
5a	73 (88)	≥ 100 (95)	68 (67)	60 (88)	81 (95)	84 (80)	92 (71)	59 (84)	NP
5b	81 (92)	97 (82)	82 (≥ 100)	84 (81)	≥ 100 (≥ 100)	92 (90)	76 (84)	51 (62)	NP
5c	90 (≥ 100)	≥ 100 (≥ 100)	97 (98)	67 (72)	83 (93)	76 (80)	77 (82)	≥ 100 (88)	61 (61)
5d	94 (94)	89 (≥ 100)	90 (≥ 100)	65 (69)	92 (93)	95 (79)	≥ 100 (87)	≥ 100 (99)	65 (64)
5e	97 (≥ 100)	≥ 100 (≥ 100)	92 (84)	100 (93)	100 (93)	100 (83)	96 (93)	≥ 100 (86)	100 (88)
5f	≥ 100 (96)	≥ 100 (≥ 100)	83 (96)	73 (78)	99 (93)	93 (88)	86 (96)	99 (96)	87 (73)
5g	98 (94)	≥ 100 (≥ 100)	≥ 100 (≥ 100)	94 (87)	89 (≥ 100)	≥ 100 (≥ 100)	≥ 100 (≥ 100)	97 (85)	62 (78)
5h	≥ 100 (90)	≥ 100 (98)	87 (≥ 100)	87 (79)	92 (93)	≥ 100 (99)	87 (≥ 100)	96 (95)	74 (80)
5i	93 (96)	≥ 100 (≥ 100)	94 (≥ 100)	70 (68)	92 (92)	≥ 100 (91)	82 (≥ 100)	≥ 100 (84)	66 (65)
5j	≥ 100 (81)	≥ 100 (≥ 100)	72 (≥ 100)	46 (70)	76 (≥ 100)	82 (≥ 100)	74 (≥ 100)	≥ 100 (94)	74 (62)
5k	98 (≥ 100)	≥ 100 (≥ 100)	≥ 100 (≥ 100)	72 (68)	83 (96)	80 (79)	80 (81)	86 (97)	56 (63)
7a	90 (≥ 100)	≥ 100 (≥ 100)	11 (40)	65 (92)	89 (≥ 100)	73 (92)	≥ 100 (≥ 100)	74 (92)	72 (70)
7b	92 (≥ 100)	≥ 100 (≥ 100)	28 (73)	36 (74)	56 (98)	68 (94)	85 (89)	86 (94)	66 (74)
7c	55 (≥ 100)	63 (≥ 100)	5 (40)	16 (60)	48 (≥ 100)	32 (93)	55 (77)	84 (93)	32 (72)
7d	50 (95)	76 (≥ 100)	0 (22)	14 (38)	41 (91)	51 (77)	54 (86)	67 (89)	36 (74)
7e	98 (99)	≥ 100 (≥ 100)	17 (55)	29 (44)	93 (≥ 100)	47 (76)	66 (76)	77 (≥ 100)	47 (56)
7f	88 (87)	≥ 100 (≥ 100)	19 (60)	10 (24)	60 (≥ 100)	65 (76)	66 (90)	82 (69)	40 (56)

Table 4.4 Continued									
Cpd	Hs CDK2/ CyclinA	Hs CDK5/ p25	Hs CDK9/ CyclinT	Hs Haspin	Hs PIM 1	Ssc CK1δ/ε	Ssc GSK3α/β	Lm CK1	Hs ABL1
7g	≥100 (99)	≥100 (88)	50 (83)	83 (≥100)	100 (89)	88 (97)	100 (93)	93 (71)	86 (67)
7h	≥100 (93)	86 (85)	93 (86)	99 (98)	91 (89)	98 (95)	100 (81)	97 (88)	96 (93)
7i	75 (96)	≥100 (≥100)	37 (82)	11 (62)	90 (94)	38 (97)	96 (89)	71 (82)	56 (67)
7j	93 (92)	≥100 (≥100)	53 (86)	14 (21)	36 (≥100)	60 (76)	73 (87)	87 (89)	61 (59)
7k	57 (≥100)	66 (≥100)	3 (53)	10 (57)	50 (≥100)	22 (88)	49 (76)	55 (85)	34 (57)
7l	94 (≥100)	≥100 (≥100)	72 (76)	19 (56)	22 (≥100)	65 (86)	71 (79)	84 (86)	62 (70)
7m	100 (≥100)	≥100 (≥100)	6 (33)	58 (58)	57 (94)	84 (84)	100 (85)	95 (90)	76 (76)
7n	≥100 (≥100)	≥100 (≥100)	7 (40)	58 (67)	64 (≥100)	79 (79)	81 (80)	87 (94)	70 (56)
7o	71 (≥100)	≥100 (≥100)	61 (72)	19 (74)	73 (≥100)	64 (88)	70 (≥100)	77 (74)	55 (55)
7p	99 (≥100)	97 (≥100)	24 (72)	28 (67)	30 (≥100)	72 (78)	74 (76)	90 (81)	61 (76)
8a	97 (99)	≥100 (≥100)	≥100 (100)	88 (83)	≥100 (92)	90 (99)	(82 (87)	98 (85)	76 (82)
8b	98 (77)	80 (≥100)	≥100 (100)	85 (69)	49 (49)	81 (88)	≥100 (89)	59 (81)	80 (≥100)
8c	99 (97)	88 (≥100)	≥100 (100)	83 (87)	≥100 (≥100)	80 (94)	89 (82)	87 (98)	65 (74)

Values represent the percentage of residual kinase activity for compounds used at 10μM and 1μM concentrations, respectively. The values in brackets signify the results obtained with 1μM. The most significant results are presented in bold italics. Activities were assessed in duplicate using 10μM ATP. NP not performed.

Table 4.5: Secondary screening of compounds reported as active against CDK9/CyclinT and Haspin (compounds inhibiting >70% of the kinase activity at 1 μ M, see table 4.4). Activities were assessed in duplicate using 10 μ M ATP

Cpd	<i>Hs</i> CDK9/ Cyclin T (μM)	<i>Hs</i> Haspin (μM)
7a	0.94	NP
7b	1.39	NP
7c	0.59	0.19
7d	0.38	0.42
7e	1.26	NP
7f	1.24	0.11
7k	0.67	0.21
7m	0.62	NP
7n	0.72	NP
7p	1.44	NP
Flavopiridol	0.015	NP
CHR-6494	NP	0.055

IC₅₀ values (in μ M) were determined from dose-response curves by using Prism-GraphPad (GraphPad software, San Diego, CA, USA). NP Not Performed. Results obtained for dual-target inhibitors are presented in bold.

Table 4.6: Predicted cytotoxicity of the most promising compounds (compounds inhibiting >70% of kinase activity at 1 μ M)

Compound	Probability of activity (Pa)	Cell-line code	Cell-line full name
7a	0.581	Hs 683	Oligodendroglioma
	0.480	NCI-H295R	Adrenal cortex carcinoma
	0.468	A-375	Malignant melanoma
7b	0.485	Hs 683	Oligodendroglioma
	0.450	NCI-H295R	Adrenal cortex carcinoma
	0.430	A-375	Malignant melanoma
7c	0.577	Hs 683	Oligodendroglioma
	0.452	NCI-H295R	Adrenal cortex carcinoma
	0.410	NCI-H23	Non-small cell lung carcinoma
7d	0.614	Hs 683	Oligodendroglioma
	0.455	NCI-H295R	Adrenal cortex carcinoma
	0.418	NCI-H23	Non-small cell lung carcinoma
7e	0.464	Hs 683	Oligodendroglioma
	0.433	NCI-H295R	Adrenal cortex carcinoma
	0.406	DU-145	Prostate carcinoma
7f	0.503	Hs 683	Oligodendroglioma
	0.435	NCI-H295R	Adrenal cortex carcinoma
	0.415	A-375	Malignant melanoma
7g	0.430	Hs 683	Oligodendroglioma
	0.426	DMS-114	Lung carcinoma
	0.408	DU-145	Prostate carcinoma
7h	0.669	Hs 683	Oligodendroglioma
	0.463	A-375	Malignant melanoma
	0.426	NCI-H295R	Adrenal cortex carcinoma

7i	0.499	NCI-H23	Non-small cell lung carcinoma
	0.498	HeLa	Cervical adenocarcinoma
	0.485	ACHN	Papillary renal carcinoma
7j	0.460	HeLa	Cervical adenocarcinoma
	0.457	MOLT-4	Acute T-lymphoblastic leukaemia
	0.433	ACHN	Papillary renal carcinoma
7k	0.523	NCI-H23	Non-small cell lung carcinoma
	0.491	NCI-H295R	Adrenal cortex carcinoma
	0.438	HeLa	Cervical adenocarcinoma
7m	0.672	Hs 683	Oligodendroglioma
	0.462	A-375	Malignant melanoma
	0.438	Kasumi 1	Childhood acute myeloid leukaemia with maturation
7n	0.596	Hs 683	Oligodendroglioma
	0.485	Kasumi 1	Childhood acute myeloid leukaemia with maturation
	0.451	A-375	Malignant melanoma
7o	0.437	Hs 683	Oligodendroglioma
	0.390	NCI-H295R	Adrenal cortex carcinoma
	0.359	NALM-6	Adult B acute lymphoblastic leukaemia
7p	0.663	Hs 683	Oligodendroglioma
	0.447	NCI-H23	Non-small cell lung carcinoma
	0.414	A-375	Malignant melanoma
8a	0.464	NCI-H295R	Adrenal cortex carcinoma
	0.421	DMS-114	Lung carcinoma
	0.418	YAPC	Pancreatic carcinoma
8b	0.449	NCI-H23	Non-small cell lung carcinoma
	0.427	PC-9	Lung carcinoma
	0.390	HCT-15	Colon carcinoma
8c	0.518	Hs 683	Oligodendroglioma

	0.447	NCI-H23	Non-small cell lung carcinoma
	0.420	Kasumi 1	Childhood acute myeloid leukaemia with maturation

4.2.11 Figures:

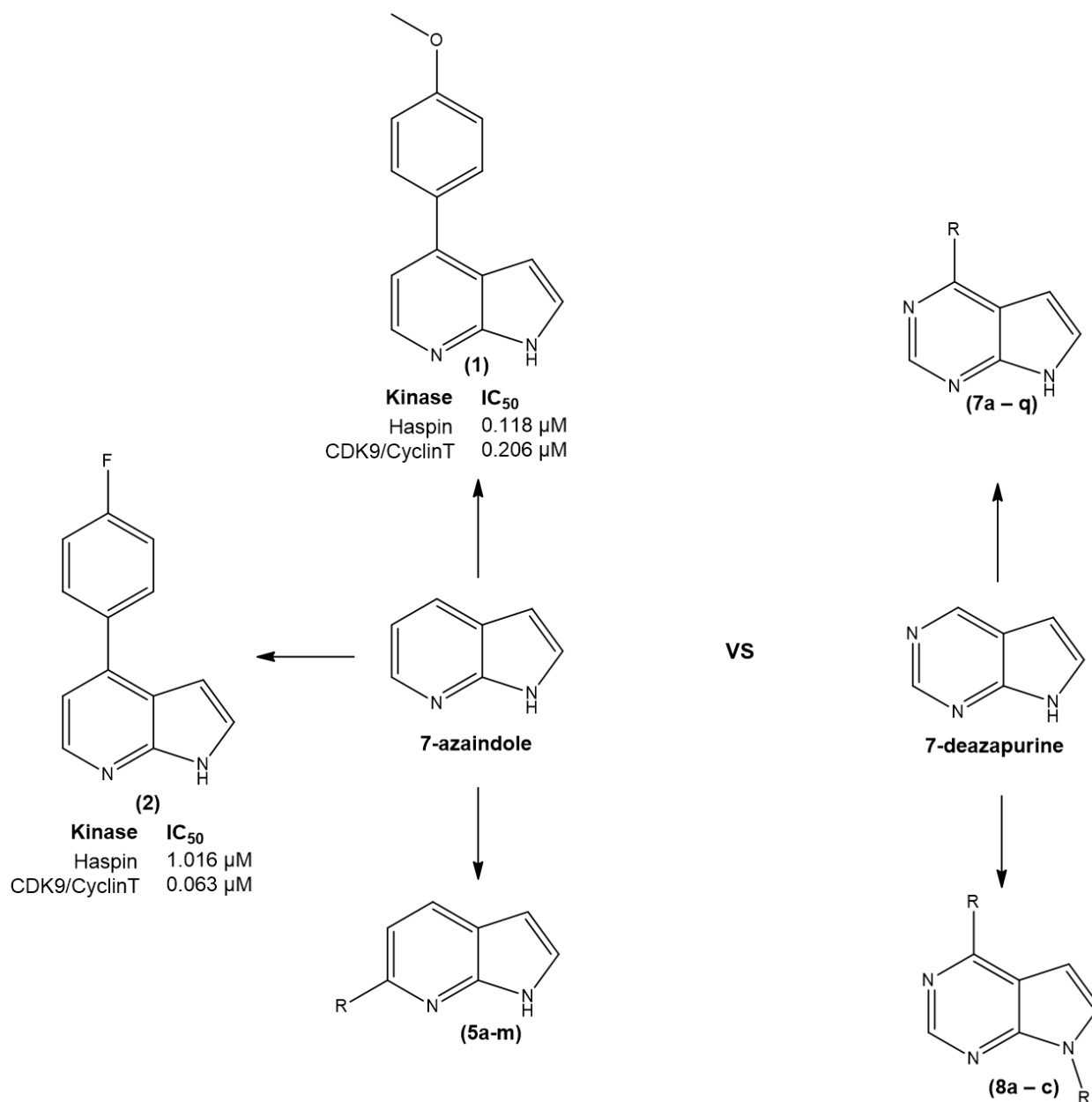
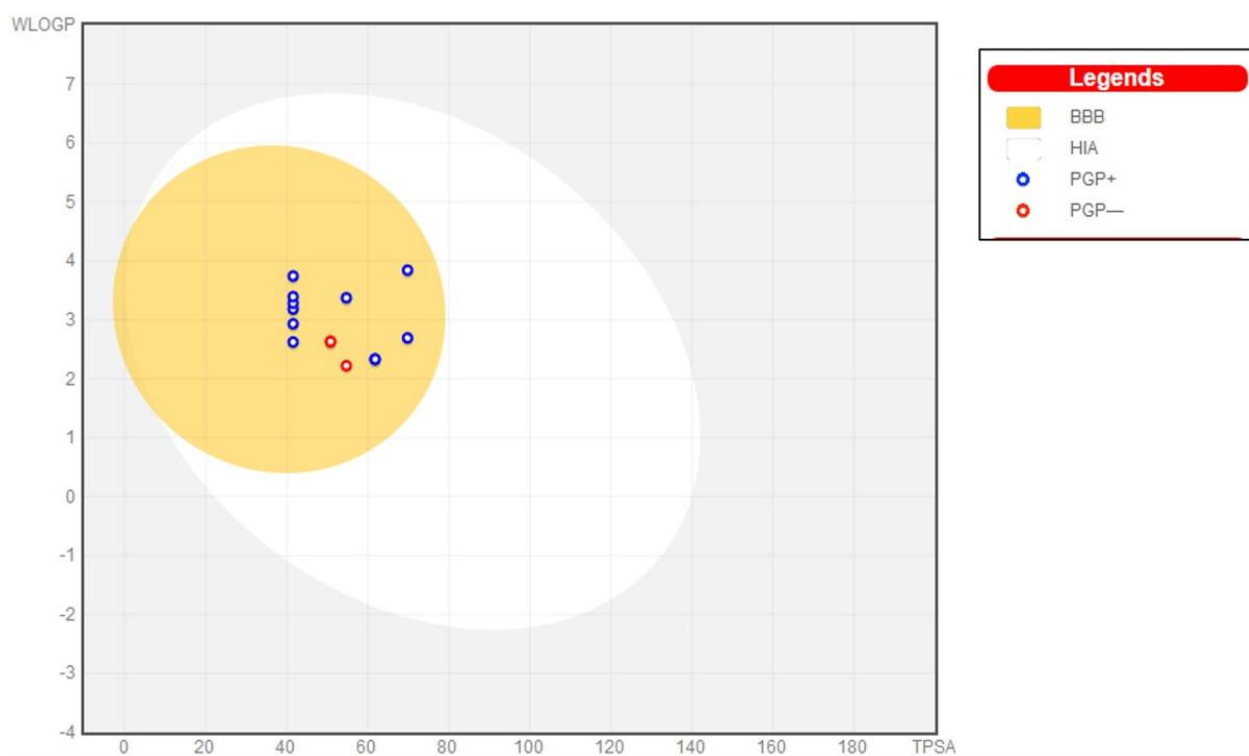


Figure 4.1: A depiction of the 7-azaindole and the 7-deazapurine scaffolds



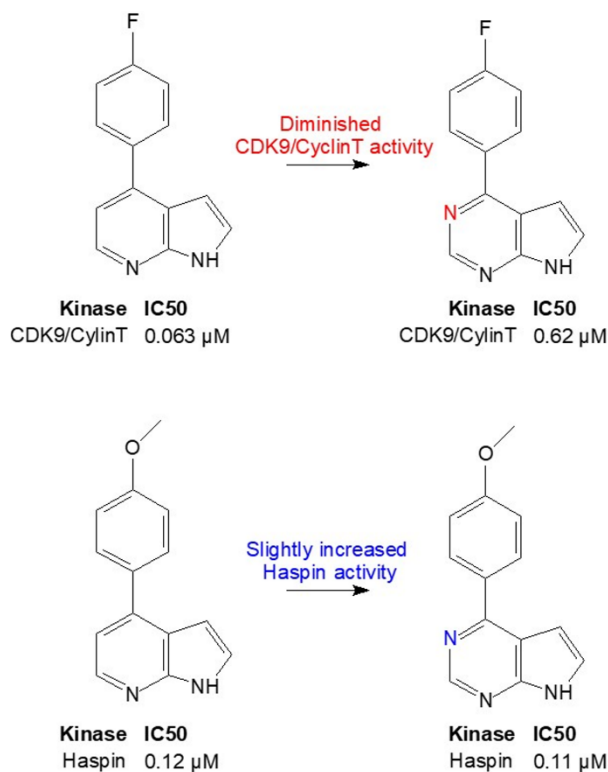
BBB: points located in the yellow represent compounds that are expected to passively cross the blood-brain barrier; HIA: points located in the white are expected to be passively absorbed by the gastrointestinal tract; PGP+: compounds that are expected to experience efflux via PGP; PGP-: compounds that are not expected to experience efflux via PGP.

Figure 4.2: A BOILED-egg representation of SwissADME predictions for the most promising compounds

4.2.12 Graphical Table of Contents for Chemical Biology and Drug Design Manuscript

Synthesis and biological evaluation of selected 7H-pyrrolo[2,3-d]pyrimidine derivatives as novel CDK9/CyclinT and Haspin inhibitors in the low micromolar range

Lianie Pieterse¹, Richard M. Beteck¹, Blandine Baratte^{2,3}, Omobolanle J. Jesumoroti¹, Thomas Robert^{2,3}, Sandrine Ruchaud², Stéphane Bach^{1,2,3}, Lesetja J. Legoabe^{1*}



7H-Pyrrolo[2,3-d]pyrimidine derivatives were evaluated against a panel of protein kinases based on success with the related 7-azaindo scaffold. In relation to their 7-azaindo counterparts, the 7H-pyrrolo[2,3-d]pyrimidine derivatives exhibited diminished CDK9/CyclinT activity. Haspin activity was retained or improved, making them useful starting points in cancer drug discovery endeavours.

Figure 4.3: Graphical Table of Content for Submission to Chemical Biology and Drug Design

CHAPTER 5: CONCLUSION

Controlled protein kinase (PK) function is paramount to normal cellular function; when PK activity falters, processes like cell growth, differentiation, cell development and apoptosis go awry. Dysregulation of PK activity has been implicated in diseases such as diabetes, cancer and neurodegenerative disorders. In the instance of disease, inhibition of the relevant PK may be of therapeutic value (see **Chapter 2**).

To execute the aims of this study (see **Chapter 1**), fifty-four compounds, thirty-five 7-azaindole derivatives and nineteen 7*H*-pyrrolo[2,3-*d*]pyrimidine (7-deazapurine) derivatives, were synthesised. Subsequently, these compounds were evaluated against a proposed protein kinase panel comprised of *HsCDK2/CyclinA*, *HsCDK5/p25*, *HsCDK9/CyclinT*, *HsHaspin*, *HsPim-1*, *SscCKδ/ε*, *SscGSK3α/β* and *LmCK1* (see **Chapters 3 and 4**). This evaluation revealed sixteen CDK9/CyclinT and nine Haspin inhibitors in the high nanomolar to low micromolar range. Thus, the hypothesis that novel, potent PK inhibitors could be discovered by utilising the 7-azaindole and 7-deazapurine scaffolds, was fulfilled.

The content of the study was divided into two original works, one a published article (see **Chapter 3**) and the other a manuscript submitted for publication (see **Chapter 4**). The published article entailed the C4- and C5-substituted 7-azaindole series. The results of the article revealed that the 7-azaindole scaffold is selective toward the CDK9/CyclinT and Haspin kinases. Comparison of the C4- and C5-substituted derivatives unveiled that only **one** C5-substituted 7-azaindole derivative exhibited activity (toward Haspin) at the concentrations tested. In turn, four of the C4-substituted 7-azaindole derivatives exhibited activity toward both Haspin and CDK9/CyclinT. Additionally, two of the C4-substituted 7-azaindole derivatives displayed activity solely towards CDK9/CyclinT.

The second work contains the results of the C6-substituted 7-azaindole and the C4-substituted 7-deazapurine series. In this manuscript, a similar pattern was observed. The 7-deazapurine scaffold displayed selectivity toward the CDK9/CyclinT and Haspin kinases, while the C6-substituted 7-azaindole derivatives, like the C5-substituted 7-azaindole derivatives in the published article, were void of activity at the concentrations tested. The C4-substituted 7-deazapurine scaffold produced ten CDK9/CyclinT and four Haspin inhibitors in the high nanomolar to low micromolar range. Thus, it is likely that substitution of the C4-position is favourable for CDK9/CyclinT and Haspin activity on both the 7-azaindole and 7-deazapurine scaffolds.

The best 7-azaindole compounds were 4-(4-fluorophenyl)-1*H*-pyrrolo[2,3-*b*]pyridine and 4-(4-methoxyphenyl)-1*H*-pyrrolo[2,3-*b*]pyridine with IC₅₀ values of 0.063 μM and 0.118 μM for CDK9/CyclinT and Haspin, respectively. In turn, the best 7-deazapurine compounds resulted in IC₅₀ values of 0.38 μM (4-(4-hydroxyphenyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine) for CDK9/CyclinT and 0.11 μM (4-(4-methoxyphenyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine) for Haspin. Unfortunately, neither the C5-substituted or C6-substituted 7-azaindole derivatives, nor the 4,7-bis-substituted-7*H*-pyrrolo[2,3-*d*]pyrimidine derivatives exhibited any PK inhibition activity at the concentrations tested, this is likely caused by impeded binding due to the position of the substituents. See **Figures 5.1** and **5.2** for the compounds with best activity against CDK9/CyclinT and Haspin in relation to their corresponding counterparts across the other series.

Considering the results as a whole, the C4-substituted 7-azaindole derivatives produced the best CDK9/CyclinT inhibitors, while the 7-deazapurine scaffold produced the best Haspin inhibitors. 4-(4-Fluorophenyl)-1*H*-pyrrolo[2,3-*b*]pyridine achieved the best activity against CDK9/CyclinT with an IC₅₀ of 0.063 μM, while 4-(4-methoxyphenyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine exhibited an IC₅₀ of 0.11 μM against Haspin. Interestingly, the additional N on the pyrimidine ring of the 7-deazapurine scaffold resulted in diminished CDK9/CyclinT when compared to their 7-azaindole counterparts (see **Figures 5.3**).

Both CDK9/CyclinT and Haspin have been linked to cancer. Therefore, the most promising 7-azaindole and 7-deazapurine derivatives were also subjected to cytotoxicity prediction via an *in-silico* cytotoxicity prediction tool (CLC-Pred: prediction of cytotoxicity for tumour and non-tumour cell-lines). According to the prediction data, the active C4-substituted 7-azaindole derivatives are likely to be active against oligodendroglioma cells (see **Chapter 3**), while the active 7-deazapurine derivatives have a more modest likelihood of activity against oligodendroglioma, non-small cell lung carcinoma, cervical adenocarcinoma, acute T-lymphoblastic leukaemia, breast carcinoma and adrenal cortex carcinoma cells (see **Chapter 4**). In addition to the cytotoxicity prediction, the most promising compounds were screened using the SwissADME tool. This illustrated that the compounds used in this study are likely to cross the blood-brain barrier and have moderate bioavailability.

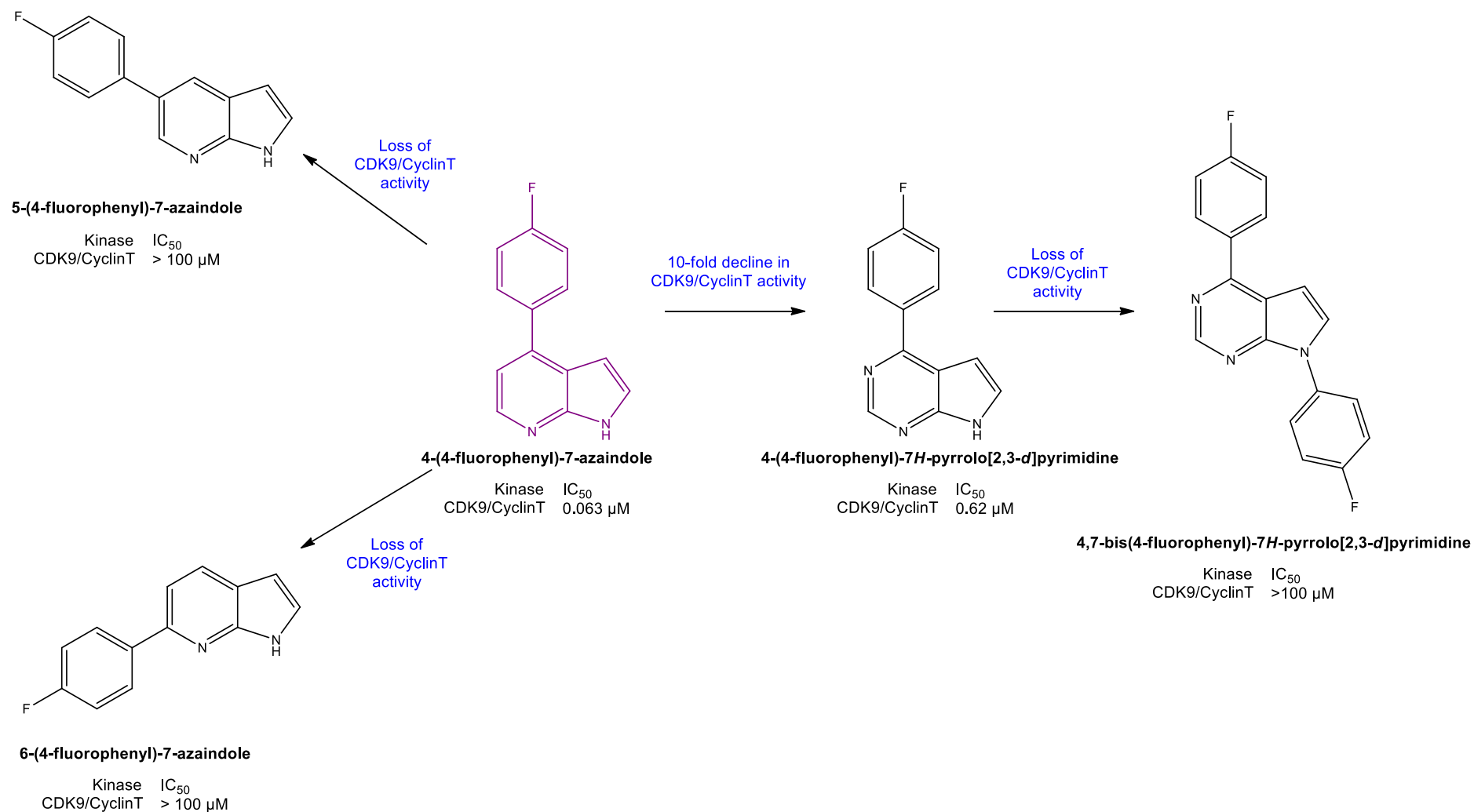


Figure 5.1: The best compound against CDK9/CyclinT in relation to its corresponding counterparts in each series

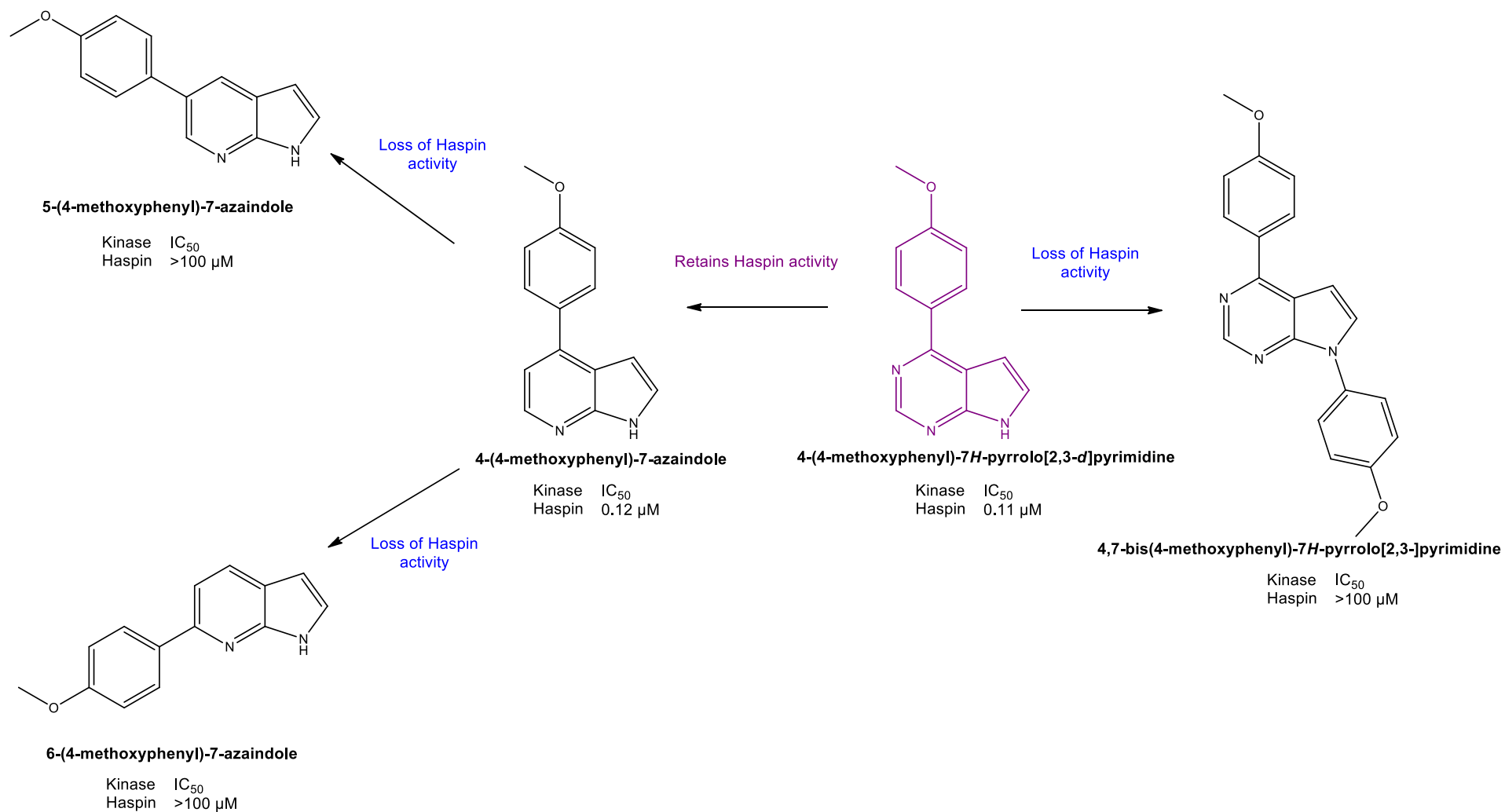


Figure 5.2: The compound with the best Haspin activity in relation to its corresponding counterpart in each series

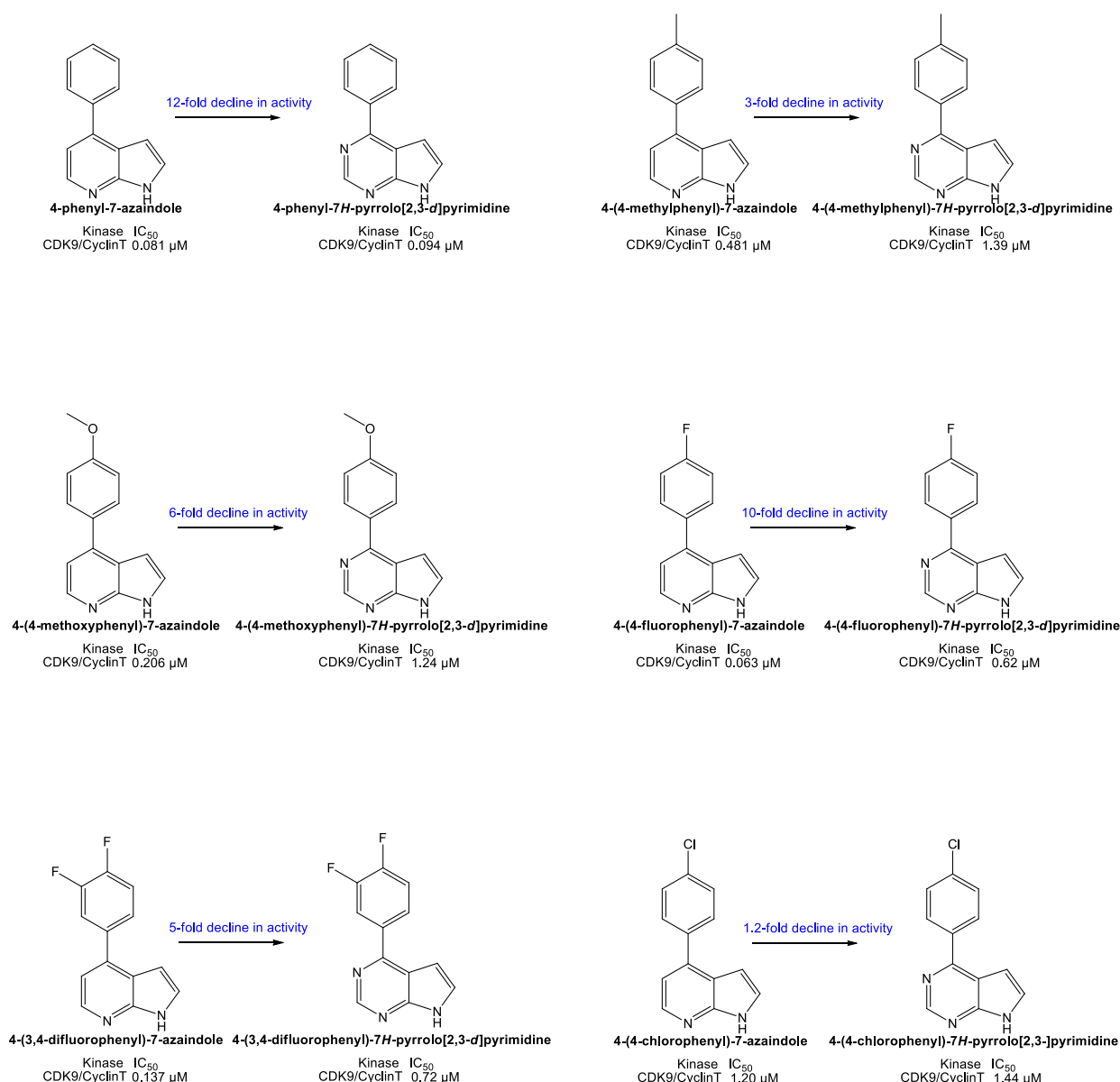


Figure 5.3: The effect of the additional N on the pyrimidine ring of the 7-deazapurine scaffold on CDK9/CyclinT activity

Thus, this study culminates in the conclusion that i) both the 7-azaindole and 7-deazapurine scaffolds are valuable in the search for PK inhibitors, especially for CDK9/CyclinT and Haspin inhibitors, ii) the substitution of the C4-position of the 7-azaindole scaffold may greatly affect the potency toward CDK9/CyclinT and Haspin; in future a greater library of C4-substituted 7-azaindoles could deliver remarkable CDK9/CyclinT and Haspin inhibitors, iii) the 7-azaindole scaffold is likely to produce more potent CDK9/CyclinT inhibitors, while the 7-deazapurine scaffold may be better equipped for producing potent Haspin inhibitors, however, considering the relatively small difference in IC₅₀ values between corresponding counterparts, a more in depth screening

of various equivalent 7-azaindole and 7-deazapurine derivatives may have to be performed to confirm and iv) the novel CDK9/CyclinT and Haspin inhibitors generated in this study may be beneficial in the fight against cancer and it is recommended that these compounds be examined against the appropriate cancer cell-lines.

BIBLIOGRAPHY

- Allegretti, M., Arcadi, A., Marinelli, F. & Nicolini, L. 2001. Palladium-catalysed functionalisation at 4-and 6-position of the 7-azaindole system. *Synlett*, 2001(05):0609-0612.
- Allocco, J.J., Donald, R., Zhong, T., Lee, A., Tang, Y.S., Hendrickson, R.C., Liberator, P. & Nare, B. 2006. Inhibitors of casein kinase 1 block the growth of *Leishmania major* promastigotes in vitro. *International journal for parasitology*, 36(12):1249-1259.
- Alzheimer's Association. 2010. 2010 Alzheimer's disease facts and figures. *Alzheimer's & dementia*, 6(2):158-194.
- Alzheimer's Association. 2019. What Is Alzheimer's? <https://www.alz.org/alzheimers-dementia/what-is-alzheimers> Date of access: 26 July, 2019.
- American Society of Clinical Oncology. 2019. What is Cancer Surgery? <https://www.cancer.net/navigating-cancer-care/how-cancer-treated/surgery/what-cancer-surgery> Date of access: 23 July, 2019.
- Amoussou, N.G., Bigot, A., Roussakis, C. & Robert, J.-M.H. 2018. Haspin: a promising target for the design of inhibitors as potent anticancer drugs. *Drug Discovery Today*, 23(2):409-415.
- Amson, R., Sigaux, F., Przedborski, S., Flandrin, G., Givol, D. & Telerman, A. 1989. The human protooncogene product p33pim is expressed during fetal hematopoiesis and in diverse leukemias. *Proceedings of the National Academy of Sciences*, 86(22):8857-8861.
- Arion, D., Meijer, L., Brizuela, L. & Beach, D. 1988. cdc2 is a component of the M phase-specific histone H1 kinase: evidence for identity with MPF. *Cell*, 55(2):371-378.
- Bailey, M.S. & Lockwood, D.N. 2007. Cutaneous leishmaniasis. *Clinics in dermatology*, 25(2):203-211.
- Balmain, A., Barrett, J.C., Moses, H. & Renan, M.J. 1993. How many mutations are required for tumorigenesis? Implications from human cancer data. *Molecular carcinogenesis*, 7(3):139-146.
- Bavetsias, V., Faisal, A., Crumpler, S., Brown, N., Kosmopoulou, M., Joshi, A., Atrash, B., Pérez-Fuertes, Y., Schmitt, J.A. & Boxall, K.J. 2013. Aurora isoform selectivity: design and synthesis of imidazo [4, 5-b] pyridine derivatives as highly selective inhibitors of Aurora-A kinase in cells. *Journal of medicinal chemistry*, 56(22):9122-9135.

- Bernheimer, H., Birkmayer, W. & Hornykiewicz, O. 1962. Behavior of monoamine oxidase in the brain of man after therapy with monoamine oxidase inhibitors. *Wiener klinische Wochenschrift*, 74:558.
- Beyaert, R., Vanhaesebroeck, B., Declercq, W., Van Lint, J., Vandenabeele, P., Agostinis, P., Vandenheede, J.R. & Fiers, W. 1995. Casein kinase-1 phosphorylates the p75 tumor necrosis factor receptor and negatively regulates tumor necrosis factor signaling for apoptosis. *Journal of Biological Chemistry*, 270(40):23293-23299.
- Bhagirath, N., Dominique, R., Kennedy-Smith, J., Lopez-Tapia, F.J., Mertz, E., Qiao, Q. & So, S.-S. 2015. Inhibitors of bruton's tyrosine kinase: Google Patents.
- Bhandari, R. & Kuhad, A. 2018. Edaravone: a new hope for deadly amyotrophic lateral sclerosis. *Drugs of today (Barcelona, Spain: 1998)*, 54(6):349-360.
- Blagden, S. & Bono, J.D. 2005. Drugging cell cycle kinases in cancer therapy. *Current drug targets*, 6(3):325-335.
- Blow, J.J. & Nurse, P. 1990. A cdc2-like protein is involved in the initiation of DNA replication in *Xenopus* egg extracts. *Cell*, 62(5):855-862.
- Brannon-Peppas, L. & Blanchette, J.O. 2012. Nanoparticle and targeted systems for cancer therapy. *Advanced drug delivery reviews*, 64:206-212.
- Brockschmidt, C., Hirner, H., Huber, N., Eismann, T., Hillenbrand, A., Giamas, G., Radunsky, B., Ammerpohl, O., Bohm, B. & Henne-Bruns, D. 2008. Anti-apoptotic and growth-stimulatory functions of CK1 delta and epsilon in ductal adenocarcinoma of the pancreas are inhibited by IC261 in vitro and in vivo. *Gut*, 57(6):799-806.
- Bu, B., Li, J., Davies, P. & Vincent, I. 2002. Deregulation of cdk5, hyperphosphorylation, and cytoskeletal pathology in the Niemann–Pick type C murine model. *Journal of Neuroscience*, 22(15):6515-6525.
- Campbell, N.A., Reece, J.B., Urry, L.A., Cain, M.L., Wasserman, S.A., Minorsky, P.V. & Jackson, R.B. 2008. Biology eight edition. *San Francisco*:231.
- Carr, D., Goate, A., Phil, D. & Morris, J. 1997. Current concepts in the pathogenesis of Alzheimer's disease. *The American journal of medicine*, 103(3):3S-10S.

CDC. (Centers for Disease Control and Prevention). 2020. Parasites-Leishmaniasis. https://www.cdc.gov/parasites/leishmaniasis/health_professionals/index.html Date of access: 21 October 2020.

Chabner, B.A., Amrein, P.C., Druker, B.J., Michaelson, M.D., Mitsaides, C.S., Goss, P.E., Ryan, D.P., Ramachandra, S., Richardson, P.G., Supko, J.G. & Wyndham, W.H. 2006. Chemotherapy of Neoplastic Diseases. (In Laurence, L.B., ed. Goodman & Gilman's The Pharmacological Basis of THERAPEUTICS. New York: McGraw-Hill.

Chappuis, F., Sundar, S., Hailu, A., Ghalib, H., Rijal, S., Peeling, R.W., Alvar, J. & Boelaert, M. 2007. Visceral leishmaniasis: what are the needs for diagnosis, treatment and control? *Nature reviews microbiology*, 5(11):S7-S16.

Chemocare. 2019. Types of Chemotherapy <http://chemocare.com/chemotherapy/what-is-chemotherapy/types-of-chemotherapy.aspx> Date of access: 22 July 2019.

Chen, Y., Fu, D., Zhao, H., Cheng, W. & Xu, F. 2020. GSG2 (Haspin) promotes development and progression of bladder cancer through targeting KIF15 (Kinase-12). *Aging*, 12(10).

Cincinelli, R., Musso, L., Merlini, L., Giannini, G., Vesci, L., Milazzo, F.M., Carenini, N., Perego, P., Penco, S. & Artali, R. 2014. 7-Azaindole-1-carboxamides as a new class of PARP-1 inhibitors. *Bioorganic & Medicinal Chemistry*, 22(3):1089-1103.

Coffey, J.C., Wang, J., Smith, M., Bouchier-Hayes, D., Cotter, T. & Redmond, H. 2003. Excisional surgery for cancer cure: therapy at a cost. *The Lancet oncology*, 4(12):760-768.

Cohen, P. & Frame, S. 2001. The renaissance of GSK3. *Nature reviews Molecular cell biology*, 2(10):769.

Collins, F.M. 2002. Current treatment approaches to type 2 diabetes mellitus: successes and shortcomings. *American Journal of Managed Care*, 8:S460-S471.

Cooper, G.M., Hausman, R.E. & Hausman, R.E. 2000. The cell: a molecular approach. Vol. 10: ASM press Washington, DC.

Copeland, N.K. & Aronson, N.E. 2015. Leishmaniasis: treatment updates and clinical practice guidelines review. *Current opinion in infectious diseases*, 28(5):426-437.

Cotzias, G.C., Papavasiliou, P.S. & Gellene, R. 1969. Modification of Parkinsonism—chronic treatment with L-dopa. *New England Journal of Medicine*, 280(7):337-345.

Cozza, G., Gianoncelli, A., Montopoli, M., Caparrotta, L., Venerando, A., Meggio, F., Pinna, L.A., Zagotto, G. & Moro, S. 2008. Identification of novel protein kinase CK1 delta (CK1 δ) inhibitors through structure-based virtual screening. *Bioorganic & medicinal chemistry letters*, 18(20):5672-5675.

Cunha, J.P. 2019. Huntington's disease facts.

https://www.medicinenet.com/huntington_disease/article.htm Date of access: 15 October 2020.

D'urso, G., Marraccino, R.L., Marshak, D.R. & Roberts, J.M. 1990. Cell cycle control of DNA replication by a homologue from human cells of the p34cdc2 protein kinase. *Science*, 250(4982):786-791.

Dai, J. & Higgins, J.M. 2005. Haspin: a mitotic histone kinase required for metaphase chromosome alignment. *Cell cycle*, 4(5):665-668.

Dai, J., Kateneva, A.V. & Higgins, J.M. 2009. Studies of haspin-depleted cells reveal that spindle-pole integrity in mitosis requires chromosome cohesion. *Journal of Cell Science*, 122(22):4168-4176.

Dai, J., Sullivan, B.A. & Higgins, J.M. 2006. Regulation of mitotic chromosome cohesion by Haspin and Aurora B. *Developmental cell*, 11(5):741-750.

Dai, J., Sultan, S., Taylor, S.S. & Higgins, J.M. 2005. The kinase haspin is required for mitotic histone H3 Thr 3 phosphorylation and normal metaphase chromosome alignment. *Genes & development*, 19(4):472-488.

Daina, A., Michielin, O. & Zoete, V. 2017. SwissADME: a free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. *Scientific reports*, 7:42717.

Davies, T.G., Tunnah, P., Meijer, L., Marko, D., Eisenbrand, G., Endicott, J.A. & Noble, M.E. 2001. Inhibitor binding to active and inactive CDK2: the crystal structure of CDK2-cyclin A/indirubin-5-sulphonate. *Structure*, 9(5):389-397.

de Cárcer, G., Perez de Castro, I. & Malumbres, M. 2007. Targeting cell cycle kinases for cancer therapy. *Current medicinal chemistry*, 14(9):969-985.

DeFronzo, R.A. 2000. Pharmacologic therapy for type 2 diabetes mellitus. *Annals of internal medicine*, 133(1):73-74.

Dhanasekaran, S.M., Barrette, T.R., Ghosh, D., Shah, R., Varambally, S., Kurachi, K., Pienta, K.J., Rubin, M.A. & Chinnaiyan, A.M. 2001. Delineation of prognostic biomarkers in prostate cancer. *Nature*, 412(6849):822.

Dharmadasa, T. & Kiernan, M.C. 2018. Riluzole, disease stage and survival in ALS. *The Lancet Neurology*, 17(5):385-386.

Dorsey, E.R. & Bloem, B.R. 2018. The Parkinson pandemic—a call to action. *JAMA neurology*, 75(1):9-10.

Dorsey, E.R., Elbaz, A., Nichols, E., Abd-Allah, F., Abdelalim, A., Adsuar, J.C., Ansha, M.G., Brayne, C., Choi, J.-Y.J. & Collado-Mateo, D. 2018. Global, regional, and national burden of Parkinson's disease, 1990–2016: a systematic analysis for the Global Burden of Disease Study 2016. *The Lancet Neurology*, 17(11):939-953.

Dunphy, W.G., Brizuela, L., Beach, D. & Newport, J. 1988. The Xenopus cdc2 protein is a component of MPF, a cytoplasmic regulator of mitosis. *Cell*, 54(3):423-431.

Duong-Ly, K.C. & Peterson, J.R. 2013. The human kinome and kinase inhibition. *Current protocols in pharmacology*, 60(1):2.9. 1-2.9. 14.

Ehringer, H. & Hornykiewicz, O. 1960. Verteilung von Noradrenalin und Dopamin (3-Hydroxytyramin) im Gehirn des Menschen und ihr Verhalten bei Erkrankungen des extrapyramidalen Systems. *Klinische Wochenschrift*, 38(24):1236-1239.

Eide, E.J., Vielhaber, E.L., Hinz, W.A. & Virshup, D.M. 2002. The circadian regulatory proteins BMAL1 and cryptochromes are substrates of casein kinase I ϵ . *Journal of Biological Chemistry*, 277(19):17248-17254.

Embi, N., Rylatt, D.B. & Cohen, P. 1980. Glycogen Synthase Kinase-3 from Rabbit Skeletal Muscle: Separation from Cyclic-AMP-Dependent Protein Kinase and Phosphorylase Kinase. *European Journal of biochemistry*, 107(2):519-527.

Eswaran, J., Patnaik, D., Filippakopoulos, P., Wang, F., Stein, R.L., Murray, J.W., Higgins, J.M. & Knapp, S. 2009. Structure and functional characterization of the atypical human kinase haspin. *Proceedings of the National Academy of Sciences*, 106(48):20198-20203.

Fahn, S. 2006. Levodopa in the treatment of Parkinson's disease. *Oxidative Stress and Neuroprotection*. Springer. p. 1-15).

Fang, F. & Newport, J.W. 1991. Evidence that the G1-S and G2-M transitions are controlled by different cdc2 proteins in higher eukaryotes. *Cell*, 66(4):731-742.

Feigin, V.L., Abajobir, A.A., Abate, K.H., Abd-Allah, F., Abdulle, A.M., Abera, S.F., Abyu, G.Y., Ahmed, M.B., Aichour, A.N. & Aichour, I. 2017. Global, regional, and national burden of neurological disorders during 1990–2015: a systematic analysis for the Global Burden of Disease Study 2015. *The Lancet Neurology*, 16(11):877-897.

Feigin, V.L., Nichols, E., Alam, T., Bannick, M.S., Beghi, E., Blake, N., Culpepper, W.J., Dorsey, E.R., Elbaz, A. & Ellenbogen, R.G. 2019. Global, regional, and national burden of neurological disorders, 1990–2016: a systematic analysis for the Global Burden of Disease Study 2016. *The Lancet Neurology*, 18(5):459-480.

Fischer, E.H. & Krebs, E.G. 1955. Conversion of phosphorylase b to phosphorylase a in muscle extracts. *Journal of Biological Chemistry*, 216(1):121-132.

Flight, M.H. 2013. Neurodegenerative diseases: New kinase targets for Alzheimer's disease. *Nature Reviews Drug Discovery*, 12(10):739.

Flotow, H., Graves, P.R., Wang, A., Fiol, C.J., Roeske, R.W. & Roach, P. 1990. Phosphate groups as substrate determinants for casein kinase I action. *Journal of Biological Chemistry*, 265(24):14264-14269.

Franco, L.C., Morales, F., Boffo, S. & Giordano, A. 2018. CDK9: A key player in cancer and other diseases. *Journal of Cellular Biochemistry*, 119(2):1273-1284.

Furukawa, Y., Piwnica-Worms, H., Ernst, T.J., Kanakura, Y. & Griffin, J.D. 1990. cdc2 gene expression at the G1 to S transition in human T lymphocytes. *Science*, 250(4982):805-808.

Gärtner, R., Jensen, M.-B., Nielsen, J., Ewertz, M., Kroman, N. & Kehlet, H. 2009. Prevalence of and factors associated with persistent pain following breast cancer surgery. *Jama*, 302(18):1985-1992.

Gautier, J., Norbury, C., Lohka, M., Nurse, P. & Maller, J. 1988. Purified maturation-promoting factor contains the product of a *Xenopus* homolog of the fission yeast cell cycle control gene *cdc2+*. *Cell*, 54(3):433-439.

Genetics Home Reference. 2020. Huntington disease.

<https://medlineplus.gov/genetics/condition/huntington-disease/#frequency> Date of access: 15 October 2020.

- Gourdain, S.P., Dairou, J., Denhez, C.M., Bui, L.C., Rodrigues-Lima, F., Janel, N., Delabar, J.M., Cariou, K. & Dodd, R.H. 2013. Development of DANDYs, new 3, 5-diaryl-7-azaindoles demonstrating potent DYRK1A kinase inhibitory activity. *Journal of medicinal chemistry*, 56:9569-9585.
- Graveland, G., Williams, R. & DiFiglia, M. 1985. Evidence for degenerative and regenerative changes in neostriatal spiny neurons in Huntington's disease. *Science*, 227(4688):770-773.
- Gray, N.S., Wodicka, L., Thunnissen, A.-M.W., Norman, T.C., Kwon, S., Espinoza, F.H., Morgan, D.O., Barnes, G., LeClerc, S. & Meijer, L. 1998. Exploiting chemical libraries, structure, and genomics in the search for kinase inhibitors. *Science*, 281(5376):533-538.
- Gummadi, V.R., Rajagopalan, S., Looi, C.-Y., Paydar, M., Renukappa, G.A., Ainan, B.R., Krishnamurthy, N.R., Panigrahi, S.K., Mahasweta, K. & Raghuramachandran, S. 2013. Discovery of 7-azaindole based anaplastic lymphoma kinase (ALK) inhibitors: wild type and mutant (L1196M) active compounds with unique binding mode. *Bioorganic & medicinal chemistry letters*, 23:4911-4918.
- Hamill, R.J. 2013. Amphotericin B formulations: a comparative review of efficacy and toxicity. *Drugs*, 73(9):919-934.
- Han, L., Wang, P., Sun, Y., Liu, S. & Dai, J. 2017. Anti-melanoma activities of haspin inhibitor CHR-6494 deployed as a single agent or in a synergistic combination with MEK inhibitor. *Journal of Cancer*, 8(15):2933.
- Han, X., Kuang, T., Ren, Y., Lu, Z., Liao, Q. & Chen, W. 2019. Haspin knockdown can inhibit progression and development of pancreatic cancer in vitro and vivo. *Experimental cell research*, 385(1):111605.
- Hanahan, D. & Weinberg, R.A. 2000. The hallmarks of cancer. *cell*, 100(1):57-70.
- Handy Jr, J.R., Asaph, J.W., Skokan, L., Reed, C.E., Koh, S., Brooks, G., Douville, E.C., Tsen, A.C., Ott, G.Y. & Silvestri, G.A. 2002. What happens to patients undergoing lung cancer surgery?: Outcomes and quality of life before and after surgery. *Chest*, 122(1):21-30.
- Hanks, S.K. & Hunter, T. 1995. Protein kinases 6. The eukaryotic protein kinase superfamily: kinase (catalytic) domain structure and classification. *The FASEB journal*, 9(8):576-596.
- Harper, J.W. & Elledge, S.J. 1996. Cdk inhibitors in development and cancer. *Current opinion in genetics & development*, 6(1):56-64.

- Harrington, P.E., Bourbeau, M.P., Fotsch, C., Frohn, M., Pickrell, A.J., Reichelt, A., Sham, K., Siegmund, A.C., Bailis, J.M. & Bush, T. 2013. The optimization of aminooxadiazoles as orally active inhibitors of Cdc7. *Bioorganic & medicinal chemistry letters*, 23:6396-6400.
- Hebert, L.E., Scherr, P.A., Bienias, J.L., Bennett, D.A. & Evans, D.A. 2003. Alzheimer disease in the US population: prevalence estimates using the 2000 census. *Archives of neurology*, 60(8):1119-1122.
- Heinrich, T., Seenisamy, J., Emmanuvel, L., Kulkarni, S.S., Bomke, J.r., Rohdich, F., Greiner, H., Esdar, C., Krier, M. & Grädler, U. 2013. Fragment-based discovery of new highly substituted 1 H-pyrrolo [2, 3-b]-and 3 H-imidazolo [4, 5-b]-pyridines as focal adhesion kinase inhibitors. *Journal of Medicinal Chemistry*, 56:1160-1170.
- Hendren, S.K., O'Connor, B.I., Liu, M., Asano, T., Cohen, Z., Swallow, C.J., MacRae, H.M., Gryfe, R. & McLeod, R.S. 2005. Prevalence of male and female sexual dysfunction is high following surgery for rectal cancer. *Annals of surgery*, 242(2):212.
- Higgins, J. 2003. Structure, function and evolution of haspin and haspinrelated proteins, a distinctive group of eukaryotic protein kinases. *Cellular and Molecular Life Sciences CMLS*, 60(3):446-462.
- Higgins, J.M. 2001a. Haspin-like proteins: A new family of evolutionarily conserved putative eukaryotic protein kinases. *Protein Science*, 10(8):1677-1684.
- Higgins, J.M. 2001b. The Haspin gene: location in an intron of the Integrin α E gene, associated transcription of an Integrin α E-derived RNA and expression in diploid as well as haploid cells. *Gene*, 267(1):55-69.
- Higgins, J.M. 2010. Haspin: a newly discovered regulator of mitotic chromosome behavior. *Chromosoma*, 119(2):137-147.
- Hnisz, D., Abraham, B.J., Lee, T.I., Lau, A., Saint-André, V., Sigova, A.A., Hoke, H.A. & Young, R.A. 2013. Super-enhancers in the control of cell identity and disease. *Cell*, 155(4):934-947.
- Hong, S., Kim, J., Seo, J.H., Jung, K.H., Hong, S.-S. & Hong, S. 2012. Design, synthesis, and evaluation of 3, 5-disubstituted 7-azaindoles as Trk inhibitors with anticancer and antiangiogenic activities. *Journal of medicinal chemistry*, 55:5337-5349.

Huertas, D., Soler, M., Moreto, J., Villanueva, A., Martinez, A., Vidal, A., Charlton, M., Moffat, D., Patel, S. & McDermott, J. 2012a. Antitumor activity of a small-molecule inhibitor of the histone kinase Haspin. *Oncogene*, 31(11):1408-1418.

Huertas, D., Soler, M., Moreto, J., Villanueva, A., Martinez, A., Vidal, A., Charlton, M., Moffat, D., Patel, S. & McDermott, J. 2012b. Antitumor activity of a small-molecule inhibitor of the histone kinase Haspin. *Oncogene*, 31(11):1408.

Hunter, T. 1995. Protein kinases and phosphatases: the yin and yang of protein phosphorylation and signaling. *Cell*, 80(2):225-236.

Huse, M. & Kuriyan, J. 2002. The conformational plasticity of protein kinases. *Cell*, 109(3):275-282.

Hutchinson, A.D., Hosking, J.R., Kichenadasse, G., Mattiske, J.K. & Wilson, C. 2012. Objective and subjective cognitive impairment following chemotherapy for cancer: a systematic review. *Cancer treatment reviews*, 38(7):926-934.

Hyman, B.T., Van Hoesen, G.W., Damasio, A.R. & Barnes, C.L. 1984. Alzheimer's disease: cell-specific pathology isolates the hippocampal formation. *Science*, 225(4667):1168-1170.

Inzucchi, S.E., Bergenstal, R.M., Buse, J.B., Diamant, M., Ferrannini, E., Nauck, M., Peters, A.L., Tsapas, A., Wender, R. & Matthews, D.R. 2015. Management of hyperglycemia in type 2 diabetes, 2015: a patient-centered approach: update to a position statement of the American Diabetes Association and the European Association for the Study of Diabetes. *Diabetes care*, 38(1):140-149.

Irie, T. & Sawa, M. 2018. 7-Azaindole: A versatile scaffold for developing kinase inhibitors. *Chemical and Pharmaceutical Bulletin*, 66(1):29-36.

Jacobs, M.D., Black, J., Futer, O., Swenson, L., Hare, B., Fleming, M. & Saxena, K. 2005. Pim-1 ligand-bound structures reveal the mechanism of serine/threonine kinase inhibition by LY294002. *Journal of Biological Chemistry*, 280(14):13728-13734.

Jankovic, J. 2008. Parkinson's disease: clinical features and diagnosis. *Journal of neurology, neurosurgery & psychiatry*, 79(4):368-376.

Kaakkola, S. 2000. Clinical pharmacology, therapeutic use and potential of COMT inhibitors in Parkinson's disease. *Drugs*, 59(6):1233-1250.

- Kalia, L.V. & Lang, A.E. 2016. Parkinson disease in 2015: evolving basic, pathological and clinical concepts in PD. *Nature reviews Neurology*, 12(2):65.
- Kashyap, S., Sandler, J., Peters, U., Martinez, E.J. & Kapoor, T.M. 2014. Using 'biased-privileged'scaffolds to identify lysine methyltransferase inhibitors. *Bioorganic & medicinal chemistry*, 22(7):2253-2260.
- Khachaturian, Z.S. 1985. Diagnosis of Alzheimer's disease. *Archives of neurology*, 42(11):1097-1105.
- Khan, M.A.B., Hashim, M.J., King, J.K., Govender, R.D., Mustafa, H. & Al Kaabi, J. 2020. Epidemiology of Type 2 Diabetes—Global Burden of Disease and Forecasted Trends. *Journal of epidemiology and global health*, 10(1):107.
- Khatami, M., Alijani, H.Q., Mousazadeh, F., Hashemi, N., Mahmoudi, Z., Darijani, S., Bamorovat, M., Keyhani, A., Abdollahpour-Alitappeh, M. & Borhani, F. 2020. Calcium carbonate nanowires: greener biosynthesis and their leishmanicidal activity. *RSC Advances*, 10(62):38063-38068.
- Kiernan, M.C., Vucic, S., Cheah, B.C., Turner, M.R., Eisen, A., Hardiman, O., Burrell, J.R. & Zoing, M.C. 2011. Amyotrophic lateral sclerosis. *The Lancet*, 377(9769):942-955.
- Kim, K.S., Zhang, L., Schmidt, R., Cai, Z.-W., Wei, D., Williams, D.K., Lombardo, L.J., Trainor, G.L., Xie, D. & Zhang, Y. 2008. Discovery of pyrrolopyridine– pyridone based inhibitors of Met kinase: Synthesis, X-ray crystallographic analysis, and biological activities. *Journal of medicinal chemistry*, 51:5330-5341.
- Kim, S.-W., Kim, M., Lee, W.Y. & Hyeon, T. 2002. Fabrication of hollow palladium spheres and their successful application to the recyclable heterogeneous catalyst for Suzuki coupling reactions. *Journal of the American Chemical Society*, 124(26):7642-7643.
- Knippschild, U., Gocht, A., Wolff, S., Huber, N., Löhler, J. & Stöter, M. 2005. The casein kinase 1 family: participation in multiple cellular processes in eukaryotes. *Cellular signalling*, 17(6):675-689.
- Korsgren, O. 2020. The role of vitamin D in the aetiology of type 1 diabetes. *Diabetologia*.
- Krebs, E.G., Graves, D.J. & Fischer, E.H. 1959. Factors affecting the activity of muscle phosphorylase b kinase. *Journal of Biological Chemistry*, 234(11):2867-2873.

- Kumar, A., Mandiyan, V., Suzuki, Y., Zhang, C., Rice, J., Tsai, J., Artis, D.R., Ibrahim, P. & Bremer, R. 2005. Crystal structures of proto-oncogene kinase Pim1: a target of aberrant somatic hypermutations in diffuse large cell lymphoma. *Journal of molecular biology*, 348(1):183-193.
- Labbe, J., Lee, M., Nurse, P., Picard, A. & Doree, M. 1988. Activation at M-phase of a protein kinase encoded by a starfish homologue of the cell cycle control gene *cdc2+*. *Nature*, 335(6187):251.
- Lagunin, A.A., Dubovskaja, V.I., Rudik, A.V., Pogodin, P.V., Druzhilovskiy, D.S., Glorizova, T.A., Filimonov, D.A., Sastry, N.G. & Poroikov, V.V. 2018. CLC-Pred: a freely available web-service for in silico prediction of human cell line cytotoxicity for drug-like compounds. *PloS one*, 13(1):e0191838.
- Lau, L.-F. & Ahljanian, M.K. 2003. Role of cdk5 in the pathogenesis of Alzheimer's disease. *Neurosignals*, 12(4-5):209-214.
- Lawhorn, B.G., Philp, J., Zhao, Y., Louer, C., Hammond, M., Cheung, M., Fries, H., Graves, A.P., Shewchuk, L. & Wang, L. 2015. Identification of purines and 7-deazapurines as potent and selective type I inhibitors of troponin I-interacting kinase (TNNI3K). *Journal of medicinal chemistry*, 58(18):7431-7448.
- Lawson, M., Rodrigo, J., Baratte, B., Robert, T., Delehouzé, C., Lozach, O., Ruchaud, S., Bach, S., Brion, J.-D. & Alami, M. 2016. Synthesis, biological evaluation and molecular modeling studies of imidazo [1, 2-a] pyridines derivatives as protein kinase inhibitors. *European journal of medicinal chemistry*, 123:105-114.
- Le Corre, L., Girard, A.-L., Aubertin, J., Radvanyi, F., Benoist-Lasselin, C., Jonquoy, A., Mugniery, E., Legeai-Mallet, L. & Le Merrer, Y. 2010. Synthesis and biological evaluation of a triazole-based library of pyrido [2, 3-d] pyrimidines as FGFR3 tyrosine kinase inhibitors. *Organic & biomolecular chemistry*, 8(9):2164-2173.
- Lees, A. & Smith, E. 1983. Cognitive deficits in the early stages of Parkinson's disease. *Brain*, 106(2):257-270.
- Leigh, P., Abrahams, S., Al-Chalabi, A., Ampong, M., Goldstein, L., Johnson, J., Lyall, R., Moxham, J., Mustfa, N. & Rio, A. 2003. The management of motor neurone disease. *Journal of neurology, neurosurgery & psychiatry*, 74(suppl 4):iv32-iv47.

- Li, Q., Persoons, L., Daelemans, D. & Herdewijn, P. 2019. Iron/Copper Co-Catalyzed Cross-Coupling Reaction for the Synthesis of 6-Substituted 7-Deazapurines and the Corresponding Nucleosides. *The journal of organic chemistry*, 85(2):403-418.
- Liao, J.J.-L. 2007. Molecular recognition of protein kinase binding pockets for design of potent and selective kinase inhibitors. *Journal of medicinal chemistry*, 50(3):409-424.
- Liddle, J., Bamborough, P., Barker, M.D., Campos, S., Cousins, R.P., Cutler, G.J., Hobbs, H., Holmes, D.S., Ioannou, C. & Mellor, G.W. 2009. 4-Phenyl-7-azaindoles as potent and selective IKK2 inhibitors. *Bioorganic & medicinal chemistry letters*, 19:2504-2508.
- Lin, H., Juang, J.-L. & Wang, P.S. 2004. Involvement of Cdk5/p25 in digoxin-triggered prostate cancer cell apoptosis. *Journal of biological chemistry*, 279(28):29302-29307.
- Ling, C.C., Gerweck, L.E., Zaider, M. & Yorke, E. 2010. Dose-rate effects in external beam radiotherapy redux. *Radiotherapy and oncology*, 95(3):261-268.
- Long, A., Zhao, H. & Huang, X. 2012. Structural basis for the interaction between casein kinase 1 delta and a potent and selective inhibitor. *Journal of medicinal chemistry*, 55(2):956-960.
- Manning, G. 2019. Protein Kinases: Introduction.
<https://www.cellsignal.com/contents/science/protein-kinases-introduction/kinases> Date of access: 10 June 2019.
- Manning, G., Whyte, D.B., Martinez, R., Hunter, T. & Sudarsanam, S. 2002. The protein kinase complement of the human genome. *Science*, 298(5600):1912-1934.
- Martinez, A., Castro, A., Dorronsoro, I. & Alonso, M. 2002. Glycogen synthase kinase 3 (GSK-3) inhibitors as new promising drugs for diabetes, neurodegeneration, cancer, and inflammation. *Medicinal research reviews*, 22(4):373-384.
- Mathiesen, T., Kihlström, L., Karlsson, B. & Lindquist, C. 2003. Potential complications following radiotherapy for meningiomas. *Surgical neurology*, 60(3):193-198.
- Mavunkel, B.J., Perumattam, J.J., Tan, X., Luedtke, G.R., Lu, Q., Lim, D., Kizer, D., Dugar, S., Chakravarty, S. & Xu, Y.-j. 2010. Piperidine-based heterocyclic oxalyl amides as potent p38 α MAP kinase inhibitors. *Bioorganic & medicinal chemistry letters*, 20:1059-1062.
- Mayo Clinic Staff. 2018. Radiation therapy. <https://www.mayoclinic.org/tests-procedures/radiation-therapy/about/pac-20385162> Date of access: 25 July, 2019.

- McKay, R.M., Peters, J.M. & Graff, J.M. 2001. The casein kinase I family in Wnt signaling. *Developmental biology*, 235(2):388-396.
- Meek, D.W. & Knippschild, U. 2003. Posttranslational modification of MDM2. *Molecular cancer research*, 1(14):1017-1026.
- Mehta, P., Kaye, W., Raymond, J., Punjani, R., Larson, T., Cohen, J., Muravov, O. & Horton, K. 2018. Prevalence of amyotrophic lateral sclerosis—United States, 2015. *Morbidity and mortality weekly report*, 67(46):1285.
- Melms, J.C., Vallabhaneni, S., Mills, C.E., Yapp, C., Chen, J.-Y., Morelli, E., Waszyk, P., Kumar, S., Deming, D. & Moret, N. 2020. Inhibition of haspin kinase promotes cell-intrinsic and extrinsic antitumor activity. *Cancer research*, 80(4):798-810.
- Meng, Q.-J., Maywood, E.S., Bechtold, D.A., Lu, W.-Q., Li, J., Gibbs, J.E., Dupré, S.M., Chesham, J.E., Rajamohan, F. & Knafels, J. 2010. Entrainment of disrupted circadian behavior through inhibition of casein kinase 1 (CK1) enzymes. *Proceedings of the National Academy of Sciences*, 107(34):15240-15245.
- Mérour, J.-Y., Buron, F., Plé, K., Bonnet, P. & Routier, S. 2014. The azaindole framework in the design of kinase inhibitors. *Molecules*, 19:19935-19979.
- Minodier, P. & Parola, P. 2007. Cutaneous leishmaniasis treatment. *Travel medicine and infectious disease*, 5(3):150-158.
- Mitchell, J.D. & Borasio, G.D. 2007. Amyotrophic lateral sclerosis. *The Lancet*, 369(9578):2031-2041.
- Murakami, A., Kimura, K. & Nakano, A. 1999. The Inactive Form of a Yeast Casein Kinase I Suppresses the Secretory Defect of the sec12 Mutant implication of negative regulation by the Hrr25 kinase in the vesicle budding from the endoplasmic reticulum. *Journal of Biological Chemistry*, 274(6):3804-3810.
- Nakano, H., Saito, N., Parker, L., Tada, Y., Abe, M., Tsuganezawa, K., Yokoyama, S., Tanaka, A., Kojima, H. & Okabe, T. 2012. Rational evolution of a novel type of potent and selective proviral integration site in Moloney murine leukemia virus kinase 1 (PIM1) inhibitor from a screening-hit compound. *Journal of medicinal chemistry*, 55:5151-5164.
- National Cancer Institute. 2015. <https://www.cancer.gov/about-cancer/treatment/types/surgery>
Date of access: 23 July, 2019.

Nguyen, M.D. & Julien, J.-P. 2003. Cyclin-dependent kinase 5 in amyotrophic lateral sclerosis. *Neurosignals*, 12(4-5):215-220.

Nguyen, T.-N.-D., Feizbakhsh, O., Sfecci, E., Baratte, B., Delehouzé, C., Garcia, A., Moulin, C., Colas, P., Ruchaud, S. & Mehiri, M. 2019. Kinase-based screening of marine natural extracts leads to the identification of a cytotoxic high molecular weight metabolite from the mediterranean sponge *crambe tailliezi*. *Marine drugs*, 17(10):569.

NIH (National Institutes of Health). 2018. Treatment of Alzheimer's disease. <https://www.nia.nih.gov/health/how-alzheimers-disease-treated> Date of access: 11 September, 2019.

Nishioka, H., Sawa, T., Nakamura, H., Iinuma, H., Ikeda, D., Sawa, R., Naganawa, H., Hayashi, C., Hamada, M. & Takeuchi, T. 1991. Isolation and structure determination of novel phosphatidylinositol kinase inhibitors, echiguanines A and B, from *Streptomyces* sp. *Journal of natural products*, 54(5):1321-1325.

Njeh, C. 2008. Tumor delineation: The weakest link in the search for accuracy in radiotherapy. *Journal of medical physics/Association of Medical Physicists of India*, 33(4):136.

Noh, H. & King, G. 2007. The role of protein kinase C activation in diabetic nephropathy. *Kidney international*, 72:S49-S53.

Nurse, P. 1990. Universal control mechanism regulating onset of M-phase. *Nature*, 344(6266):503.

Nygren, P. 2001. What is cancer chemotherapy? *Acta oncologica*, 40(2-3):166-174.

Open Stax. 2012. Control of the Cell Cycle. <https://courses.lumenlearning.com/biology1/chapter/control-of-the-cell-cycle/> Date of access: 13 June 2019.

Pagano, M., Pepperkok, R., Lukas, J., Baldin, V., Ansorge, W., Bartek, J. & Draetta, G. 1993. Regulation of the cell cycle by the cdk2 protein kinase in cultured human fibroblasts. *The Journal of cell biology*, 121(1):101-111.

Parkinson, J., Member of the Royal College of Surgeons. 2002. An Essay on the Shaking Palsy. *The Journal of neuropsychiatry and clinical neurosciences*, 14(2):223-236.

Parkinson Study Group. 2004. Levodopa and the progression of Parkinson's disease. *New England journal of medicine*, 351(24):2498-2508.

- Parr, C., Carzaniga, R., Gentleman, S.M., Van Leuven, F., Walter, J. & Sastre, M. 2012. Glycogen synthase kinase 3 inhibition promotes lysosomal biogenesis and autophagic degradation of the amyloid- β precursor protein. *Molecular and cellular biology*, 32(21):4410-4418.
- Perez, C.A., Pajak, T.F., Rubin, P., Simpson, J.R., Mohiuddin, M., Brady, L.W., Perez-Tamayo, R. & Rotman, M. 1987. Long-term observations of the patterns of failure in patients with unresectable non-oat cell carcinoma of the lung treated with definitive radiotherapy report by the radiation therapy oncology group. *Cancer*, 59(11):1874-1881.
- Perlíková, P. & Hocek, M. 2017. Pyrrolo [2, 3-d] pyrimidine (7-deazapurine) as a privileged scaffold in design of antitumor and antiviral nucleosides. *Medicinal research reviews*, 37(6):1429-1460.
- Peschel, R.E. & Colberg, J.W. 2003. Surgery, brachytherapy, and external-beam radiotherapy for early prostate cancer. *The Lancet oncology*, 4(4):233-241.
- Pevarello, P., Brasca, M.G., Amici, R., Orsini, P., Traquandi, G., Corti, L., Piutti, C., Sansonna, P., Villa, M. & Pierce, B.S. 2004. 3-Aminopyrazole inhibitors of CDK2/cyclin A as antitumor agents. 1. Lead finding. *Journal of medicinal chemistry*, 47(13):3367-3380.
- Phillips, M.A. & Stanley, S.L. 2006. Chemotherapy of Protozoal Infections. (In Brunton, L.L., ed. Goodman & Gilman's The Pharmacological Basis of Therapeutics New York: McGraw-Hill.
- Pieterse, L., Legoabe, L.J., Beteck, R.M., Josselin, B., Bach, S. & Ruchaud, S. 2020. Synthesis and biological evaluation of selected 7-azaindole derivatives as CDK9/Cyclin T and Haspin inhibitors. *Medicinal chemistry research*, 29:1449-1462.
- Pokholok, D.K., Zeitlinger, J., Hannett, N.M., Reynolds, D.B. & Young, R.A. 2006. Activated signal transduction kinases frequently occupy target genes. *Science*, 313(5786):533-536.
- Powers, A.C. & D'Alessio, D. 2011. Endocrine Pancreas and Pharmacotherapy of Diabetes Mellitus and Hypoglycemia. (In L., B.L., ed. Goodman & Gilman's The Pharmacological Basis of Therapeutics. 12th ed. New York: McGraw-Hill.
- Qian, K.C., Wang, L., Hickey, E.R., Studts, J., Barringer, K., Peng, C., Kronkaitis, A., Li, J., White, A. & Mische, S. 2005. Structural basis of constitutive activity and a unique nucleotide binding mode of human Pim-1 kinase. *Journal of biological chemistry*, 280(7):6130-6137.

- Qiao, J.X. & Lam, P.Y. 2011. Recent Advances in Chan–Lam Coupling Reaction: Copper-Promoted C–Heteroatom Bond Cross-Coupling Reactions with Boronic Acids and Derivatives. *Boronic acids: preparation and applications in organic synthesis, medicine and materials*, 1:315-361.
- Rachidi, N., Taly, J.F., Durieu, E., Leclercq, O., Aulner, N., Prina, E., Pescher, P., Notredame, C., Meijer, L. & Späth, G.F. 2014. Pharmacological assessment defines *Leishmania donovani* casein kinase 1 as a drug target and reveals important functions in parasite viability and intracellular infection. *Antimicrobial agents and chemotherapy*, 58(3):1501-1515.
- Ramirez, C., Pimentel Piemonte, M.E., Callegaro, D. & Almeida Da Silva, H.C. 2008. Fatigue in amyotrophic lateral sclerosis: frequency and associated factors. *Amyotrophic lateral sclerosis*, 9(2):75-80.
- Raveendran, A.V., Chacko, E.C. & Pappachan, J.M. 2018. Non-pharmacological treatment options in the management of diabetes mellitus. *European endocrinology*, 14(2):31.
- Rawlins, M.D., Wexler, N.S., Wexler, A.R., Tabrizi, S.J., Douglas, I., Evans, S.J. & Smeeth, L. 2016. The prevalence of Huntington's disease. *Neuroepidemiology*, 46(2):144-153.
- Reed, S.I. & Wittenberg, C. 1990. Mitotic role for the Cdc28 protein kinase of *Saccharomyces cerevisiae*. *Proceedings of the National Academy of Sciences*, 87(15):5697-5701.
- Reithinger, R., Dujardin, J.-C., Louzir, H., Pirmez, C., Alexander, B. & Brooker, S. 2007. Cutaneous leishmaniasis. *The Lancet infectious diseases*, 7(9):581-596.
- Riabowol, K., Draetta, G., Brizuela, L., Vandre, D. & Beach, D. 1989. The cdc2 kinase is a nuclear protein that is essential for mitosis in mammalian cells. *Cell*, 57(3):393-401.
- Riederer, P. & Laux, G. 2011. MAO-inhibitors in Parkinson's Disease. *Experimental neurobiology*, 20(1):1-17.
- Roskoski Jr, R. 2020. Properties of FDA-approved small molecule protein kinase inhibitors: A 2020 update. *Pharmacological research*, 152:104609.
- Ross, C.A. & Tabrizi, S.J. 2011. Huntington's disease: from molecular pathogenesis to clinical treatment. *The Lancet neurology*, 10(1):83-98.
- Sabat, N., Nauš, P., Matyašovský, J., Dziuba, D., Slavětínská, L.P. & Hocek, M. 2016. Synthesis of Fluorescent 2-Substituted 6-(Het) aryl-7-deazapurine Bases {4-(Het) aryl-pyrrolo

[2, 3-d] pyrimidines} by Aqueous Suzuki–Miyaura Cross-Coupling Reactions. *Synthesis*, 48(07):1029-1045.

Sacerdoti-Sierra, N. & Jaffe, C.L. 1997. Release of Ecto-protein Kinases by the Protozoan Parasite *Leishmania major*. *Journal of biological chemistry*, 272(49):30760-30765.

Seeley, W.W., Carlin, D.A., Allman, J.M., Macedo, M.N., Bush, C., Miller, B.L. & DeArmond, S.J. 2006. Early frontotemporal dementia targets neurons unique to apes and humans. *Annals of Neurology: Official journal of the American neurological association and the child neurology society*, 60(6):660-667.

SEER Training Modules. 2019. Types of Chemotherapy Drugs. <https://training.seer.cancer.gov/treatment/chemotherapy/types.html> Date of access: 22 July 2019.

Selkoe, D.J. 2002. Alzheimer's disease is a synaptic failure. *Science*, 298(5594):789-791.

Selten, G., Cuypers, H.T. & Berns, A. 1985. Proviral activation of the putative oncogene Pim-1 in MuLV induced T-cell lymphomas. *The EMBO journal*, 4(7):1793-1798.

Sever, R. & Brugge, J.S. 2015. Signal transduction in cancer. *Cold Spring Harbor perspectives in medicine*, 5(4):a006098.

Sillibourne, J.E., Milne, D.M., Takahashi, M., Ono, Y. & Meek, D.W. 2002. Centrosomal anchoring of the protein kinase CK1 δ mediated by attachment to the large, coiled-coil scaffolding protein CG-NAP/AKAP450. *Journal of molecular biology*, 322(4):785-797.

Singh, T.J., Grundke-Iqbal, I. & Iqbal, K. 1995. Phosphorylation of τ Protein by Casein Kinase-1 Converts It to an Abnormal Alzheimer-Like State. *Journal of neurochemistry*, 64(3):1420-1423.

Smith, P.D., Crocker, S.J., Jackson-Lewis, V., Jordan-Sciutto, K.L., Hayley, S., Mount, M.P., O'Hare, M.J., Callaghan, S., Slack, R.S. & Przedborski, S. 2003. Cyclin-dependent kinase 5 is a mediator of dopaminergic neuron loss in a mouse model of Parkinson's disease. *Proceedings of the National Academy of Sciences*, 100(23):13650-13655.

Souliman, S. & Christie, J. 1994. Pacemaker failure induced by radiotherapy. *Pacing and clinical electrophysiology*, 17(3):270-273.

Stambler, N., Charatan, M. & Cedarbaum, J.M. 1998. Prognostic indicators of survival in ALS. *Neurology*, 50(1):66-72.

- Standaert, D.G. & Roberson, E.D. 2011. Treatment of Central Nervous System Degenerative Disorders. (*In* Brunton, L.L., ed. Goodman & Gilman's The Pharmacological Basis of THERAPEUTICS. 12th ed. New York: McGraw-Hill. p. 609).
- Stocchi, F. 1998. Dopamine Agonists in Parkinson's Disease. *CNS drugs*, 10(3):159-170.
- Takano, A., Isojima, Y. & Nagai, K. 2004. Identification of mPer1 phosphorylation sites responsible for the nuclear entry. *Journal of biological chemistry*, 279(31):32578-32585.
- Tanaka, H., Wada, M. & Park, J. 2020. HASPIN kinase inhibitor CHR-6494 suppresses intestinal polyp development, cachexia, and hypogonadism in Apcmin/+ mice. *European journal of cancer prevention*, 29(6):481.
- Tanaka, H., Yoshimura, Y., Nishina, Y., Nozaki, M., Nojima, H. & Nishimune, Y. 1994. Isolation and characterization of cDNA clones specifically expressed in testicular germ cells. *FEBS letters*, 355(1):4-10.
- Tarsy, D. 2006. Initial treatment of Parkinson's disease. *Current treatment options in neurology*, 8(3):224-235.
- Tavare, A.N., Perry, N.J., Benzonana, L.L., Takata, M. & Ma, D. 2012. Cancer recurrence after surgery: direct and indirect effects of anesthetic agents. *International journal of cancer*, 130(6):1237-1250.
- Taylor, E.C., Macor, J.E. & Pont, J.L. 1987. Intramolecular diels-alder reactions of 1, 2, 4-triazines.: A general synthesis of furo [2, 3-b] pyridines, 2, 3-dihydropyrano [2, 3-b] pyridines, and pyrrolo [2, 3-b] pyridines. *Tetrahedron*, 43(21):5145-5158.
- The American Cancer Society medical and editorial content team. 2016a. How Chemotherapy Drugs Work. <https://www.cancer.org/treatment/treatments-and-side-effects/treatment-types/chemotherapy/how-chemotherapy-drugs-work.html> Date of access: 22 July, 2019.
- The American Cancer Society medical and editorial content team. 2016b. How Chemotherapy Drugs Work. https://www.cancer.org/treatment/treatments-and-side-effects/treatment-types/chemotherapy/how-chemotherapy-drugs-work.html#written_by Date of access: 16 May 2019.
- Titova, N., Jenner, P. & Chaudhuri, K.R. 2017. The future of Parkinson's treatment—personalised and precision medicine. *European neurological review*, 12(1):15-16.

- Traxler, P.M., Furet, P., Mett, H., Buchdunger, E., Meyer, T. & Lydon, N. 1996. 4-(Phenylamino) pyrrolopyrimidines: potent and selective, ATP site directed inhibitors of the EGF-receptor protein tyrosine kinase. *Journal of medicinal chemistry*, 39(12):2285-2292.
- Trojanowski, J. 2004. Searching for the biomarkers of Alzheimer's. *Practical neurology*, 3:30-34.
- Tsai, L.-H., Lees, E., Faha, B., Harlow, E. & Riabowol, K. 1993. The cdk2 kinase is required for the G1-to-S transition in mammalian cells. *Oncogene*, 8(6):1593-1602.
- Tsou, H.-R., MacEwan, G., Birnberg, G., Grosu, G., Bursavich, M.G., Bard, J., Brooijmans, N., Toral-Barza, L., Hollander, I. & Mansour, T.S. 2010. Discovery and optimization of 2-(4-substituted-pyrrolo [2, 3-b] pyridin-3-yl) methylene-4-hydroxybenzofuran-3 (2H)-ones as potent and selective ATP-competitive inhibitors of the mammalian target of rapamycin (mTOR). *Bioorganic & medicinal chemistry letters*, 20:2321-2325.
- Underwood, J.C. & Cross, S.S. 2009. General and Systematic Pathology Elsevier Health Sciences.
- Valdman, A., Fang, X., Pang, S.T., Ekman, P. & Egevad, L. 2004. Pim-1 expression in prostatic intraepithelial neoplasia and human prostate cancer. *The prostate*, 60(4):367-371.
- Vardy, J. & Tannock, I. 2007. Cognitive function after chemotherapy in adults with solid tumours. *Critical reviews in oncology/hematology*, 63(3):183-202.
- Victor, D. & Waters, C. 2003. Monoamine oxidase inhibitors in parkinson's disease. *Neurological disease and therapy*, 59:425-436.
- Vieira, L.L., Sacerdoti-Sierra, N. & Jaffe, C.L. 2002. Effect of pH and temperature on protein kinase release by *Leishmania donovani*. *International journal for parasitology*, 32(9):1085-1093.
- Vielhaber, E., Eide, E., Rivers, A., Gao, Z.-H. & Virshup, D.M. 2000. Nuclear entry of the circadian regulator mPER1 is controlled by mammalian casein kinase I ϵ . *Molecular and cellular biology*, 20(13):4888-4899.
- Villa, F., Capasso, P., Tortorici, M., Forneris, F., De Marco, A., Mattevi, A. & Musacchio, A. 2009. Crystal structure of the catalytic domain of Haspin, an atypical kinase implicated in chromatin organization. *Proceedings of the National Academy of Sciences*, 106(48):20204-20209.
- Walker, F.O. 2007. Huntington's disease. *The Lancet*, 369(9557):218-228.

Walsby, E., Pratt, G., Shao, H., Abbas, A.Y., Fischer, P.M., Bradshaw, T.D., Brennan, P., Fegan, C., Wang, S. & Pepper, C. 2014. A novel Cdk9 inhibitor preferentially targets tumor cells and synergizes with fludarabine. *Oncotarget*, 5(2):375.

WebMD Medical Reference. 2018. How Chemotherapy Works Continued. <https://www.webmd.com/cancer/how-chemo-works#2> Date of access: 16 May 2019.

Weiner, M.W., Aisen, P.S., Jack Jr, C.R., Jagust, W.J., Trojanowski, J.Q., Shaw, L., Saykin, A.J., Morris, J.C., Cairns, N. & Beckett, L.A. 2010. The Alzheimer's disease neuroimaging initiative: progress report and future plans. *Alzheimer's & Dementia*, 6(3):202-211. e207.

WHO (World Health Organization). 2018. Cancer. <https://www.who.int/en/news-room/fact-sheets/detail/cancer> Date of access: 30 April 2019.

WHO (World Health Organization). 2020. Leishmaniasis. <https://www.who.int/news-room/fact-sheets/detail/leishmaniasis> Date of access: 20 October 2020.

Wild, C.P., Weiderpass, E. & Stewart, B.W. 2020. World Cancer Report.

Wolfe, J.P. & Buchwald, S.L. 1999. A highly active catalyst for the room-temperature amination and Suzuki coupling of aryl chlorides. *Angewandte chemie international edition*, 38(16):2413-2416.

Xia, Z., Knaak, C., Ma, J., Beharry, Z.M., McInnes, C., Wang, W., Kraft, A.S. & Smith, C.D. 2008. Synthesis and evaluation of novel inhibitors of Pim-1 and Pim-2 protein kinases. *Journal of medicinal chemistry*, 52(1):74-86.

Xu, Y., Zhang, T., Tang, H., Zhang, S., Liu, M., Ren, D. & Niu, Y. 2005. Overexpression of PIM-1 is a potential biomarker in prostate carcinoma. *Journal of surgical oncology*, 92(4):326-330.

Yamamoto, M., Onogi, H., Kii, I., Yoshida, S., Iida, K., Sakai, H., Abe, M., Tsubota, T., Ito, N. & Hosoya, T. 2014. CDK9 inhibitor FIT-039 prevents replication of multiple DNA viruses. *The journal of clinical investigation*, 124(8):3479-3488.

Yang, S.S. & Warner, H.R. 1993. The Underlying molecular, cellular, and immunological factors in cancer and aging: Springer.

Yang, W.S. & Stockwell, B.R. 2008. Inhibition of casein kinase 1-epsilon induces cancer-cell-selective, PERIOD2-dependent growth arrest. *Genome biology*, 9(6):R92.

Yu, F., Lin, Y., Xu, X., Liu, W., Tang, D., Zhou, X., Wang, G., Zheng, Y. & Xie, A. 2020. Knockdown of GSG2 inhibits prostate cancer progression in vitro and in vivo. *International journal of oncology*, 57(1):139-150.

Zhang, C., Ibrahim, P.N., Zhang, J., Burton, E.A., Habets, G., Zhang, Y., Powell, B., West, B.L., Matusow, B. & Tsang, G. 2013. Design and pharmacology of a highly specific dual FMS and KIT kinase inhibitor. *Proceedings of the National Academy of Sciences*, 110(14):5689-5694.

Zhang, J., Yang, P.L. & Gray, N.S. 2009. Targeting cancer with small molecule kinase inhibitors. *Nature reviews cancer*, 9(1):28.

Zheng, Y., Ley, S.H. & Hu, F.B. 2018. Global aetiology and epidemiology of type 2 diabetes mellitus and its complications. *Nature reviews endocrinology*, 14(2):88.

Zuccotto, F., Ardini, E., Casale, E. & Angiolini, M. 2009. Through the “gatekeeper door”: exploiting the active kinase conformation. *Journal of medicinal chemistry*, 53:2681-2694.

ANNEXURE A: ETHICAL APPROVAL- NO RISK STUDY

Dear Mrs Joubert

Please find attached an extract from the minutes of the NWU-HREC meeting at which Ms Pieterse's proposal was determined to be a no-risk study. This was before we were required by SCRE to provide ethics numbers for no-risk studies. Please let me know if you have any further queries. I wish you a good day.

Yours sincerely

Wayne



Prof Wayne Towers

Associate professor / Medeprofessor

Head: Health Sciences Ethics Office for Research, Training and Support / Hoof: Gesondheidswetenskappe Etiekkantoor vir Navorsing, Opleiding en Steun

Faculty of Health Sciences / Fakulteit Gesondheidswetenskappe

North-West University (Potchefstroom Campus) / Noordwes-Universiteit (Potchefstroom Kampus)

T: +27 (0)18 299 2092

Wayne.Towers@nwu.ac.za

www.nwu.ac.za



Health Science Ethics Office for Research, Training and Support

MINUTES

Meeting:	NWU-HREC (North-West University Health Research Ethics Committee) REC-130913-037
Date of meeting:	14 August 2019
Time of meeting	08:00-09:00 HREC members only 09:00-17:00 Applicants included
Meeting room:	Building G16 Room 233 (Dean's Seminar Room)

1.1 Pharmacoen Drug Discovery

Prof Towers confirmed that two notifications of no-risk studies were received from chairperson of the aforementioned Scientific Committee.

19.11.1 LJ Legoabe-L Pieterse: Design, Synthesis and Biological evaluation of selected 7-azaindole derivatives and structurally related compounds as potential inhibitors of disease-related protein kinases

19.11.2 JP Petzer-A Petzer: The use of commercially available human and animal proteins in medicinal chemistry research

The two notifications were ratified by the NWU-HREC members present.

ANNEXURE B: PERMISSION FOR THE USE OF PUBLISHED ARTICLE

SPRINGER NATURE LICENSE TERMS AND CONDITIONS

Oct 12, 2020

This Agreement between Ms. Lianie Pieterse ("You") and Springer Nature ("Springer Nature") consists of your license details and the terms and conditions provided by Springer Nature and Copyright Clearance Center.

License Number 4926381037105

License date Oct 12, 2020

Licensed Content Publisher Springer Nature

Licensed Content Publication Medicinal Chemistry Research

Licensed Content Title Synthesis and biological evaluation of selected 7-azaindole derivatives as CDK9/Cyclin T and Haspin inhibitors

Licensed Content Author Lianie Pieterse et al

Licensed Content Date May 22, 2020

Type of Use Thesis/Dissertation

Requestor type academic/university or research institute

Format electronic

Portion full article/chapter

Will you be translating? no

Circulation/distribution	1 - 29
Author of this Springer Nature content	yes
Title	Design, synthesis and biological evaluation of selected 7-azaindole derivatives and structurally related compounds as potential inhibitors of disease-related protein kinases
Institution name	NWU-Potchefstroom
Expected presentation date	Nov 2020
Order reference number	LP12
Requestor Location	Ms. Lianie Pieterse 7 Kiaat str Dalpark Brakpan, Gauteng 1541 South Africa Attn: Ms. Lianie Pieterse
Total	0.00 USD
Terms and Conditions	

Springer Nature Customer Service Centre GmbH
Terms and Conditions

This agreement sets out the terms and conditions of the licence (the **Licence**) between you and **Springer Nature Customer Service Centre GmbH** (the **Licensor**). By clicking 'accept' and completing the transaction for the material (**Licensed Material**), you also confirm your acceptance of these terms and conditions.

1. Grant of License

1.1. The Licensor grants you a personal, non-exclusive, non-transferable, world-wide licence to reproduce the Licensed Material for the purpose specified in your order only. Licences are granted for the specific use requested in the order and for no other use, subject to the conditions below.

1. 2. The Licensor warrants that it has, to the best of its knowledge, the rights to license reuse of the Licensed Material. However, you should ensure that the material you are requesting is original to the Licensor and does not carry the copyright of another entity (as credited in the published version).

1. 3. If the credit line on any part of the material you have requested indicates that it was reprinted or adapted with permission from another source, then you should also seek permission from that source to reuse the material.

2. Scope of Licence

2. 1. You may only use the Licensed Content in the manner and to the extent permitted by these Ts&Cs and any applicable laws.

2. 2. A separate licence may be required for any additional use of the Licensed Material, e.g. where a licence has been purchased for print only use, separate permission must be obtained for electronic re-use. Similarly, a licence is only valid in the language selected and does not apply for editions in other languages unless additional translation rights have been granted separately in the licence. Any content owned by third parties are expressly excluded from the licence.

2. 3. Similarly, rights for additional components such as custom editions and derivatives require additional permission and may be subject to an additional fee.

Please apply to

Journalpermissions@springernature.com/bookpermissions@springernature.com for these rights.

2. 4. Where permission has been granted **free of charge** for material in print, permission may also be granted for any electronic version of that work, provided that the material is incidental to your work as a whole and that the electronic version is essentially equivalent to, or substitutes for, the print version.

2. 5. An alternative scope of licence may apply to signatories of the [STM Permissions Guidelines](#), as amended from time to time.

3. Duration of Licence

3. 1. A licence for is valid from the date of purchase ('Licence Date') at the end of the relevant period in the below table:

Scope of Licence	Duration of Licence
Post on a website	12 months
Presentations	12 months
Books and journals	Lifetime of the edition in the language purchased

4. Acknowledgement

4. 1. The Licensor's permission must be acknowledged next to the Licenced Material in print. In electronic form, this acknowledgement must be visible at the same time as the figures/tables/illustrations or abstract, and must be hyperlinked to the journal/book's homepage. Our required acknowledgement format is in the Appendix below.

5. Restrictions on use

5. 1. Use of the Licensed Material may be permitted for incidental promotional use and minor editing privileges e.g. minor adaptations of single figures, changes of format, colour and/or style where the adaptation is credited as set out in Appendix 1 below. Any other changes including but not limited to, cropping, adapting, omitting material that affect the meaning, intention or moral rights of the author are strictly prohibited.

5. 2. You must not use any Licensed Material as part of any design or trademark.

5. 3. Licensed Material may be used in Open Access Publications (OAP) before publication by Springer Nature, but any Licensed Material must be removed from OAP sites prior to final publication.

6. Ownership of Rights

6. 1. Licensed Material remains the property of either Licensor or the relevant third party and any rights not explicitly granted herein are expressly reserved.

7. Warranty

IN NO EVENT SHALL LICENSOR BE LIABLE TO YOU OR ANY OTHER PARTY OR ANY OTHER PERSON OR FOR ANY SPECIAL, CONSEQUENTIAL, INCIDENTAL OR INDIRECT DAMAGES, HOWEVER CAUSED, ARISING OUT OF OR IN CONNECTION WITH THE DOWNLOADING, VIEWING OR USE OF THE MATERIALS REGARDLESS OF THE FORM OF ACTION, WHETHER FOR BREACH OF CONTRACT, BREACH OF WARRANTY, TORT, NEGLIGENCE, INFRINGEMENT OR OTHERWISE (INCLUDING, WITHOUT LIMITATION, DAMAGES BASED ON LOSS OF PROFITS, DATA, FILES, USE, BUSINESS OPPORTUNITY OR CLAIMS OF THIRD PARTIES), AND WHETHER OR NOT THE PARTY HAS BEEN ADVISED OF THE POSSIBILITY OF SUCH DAMAGES. THIS LIMITATION SHALL APPLY NOTWITHSTANDING ANY FAILURE OF ESSENTIAL PURPOSE OF ANY LIMITED REMEDY PROVIDED HEREIN.

8. Limitations

8. 1. **BOOKS ONLY:** Where 'reuse in a dissertation/thesis' has been selected the following terms apply: Print rights of the final author's accepted manuscript (for clarity,

NOT the published version) for up to 100 copies, electronic rights for use only on a personal website or institutional repository as defined by the Sherpa guideline (www.sherpa.ac.uk/romeo/).

9. Termination and Cancellation

9. 1. Licences will expire after the period shown in Clause 3 (above).

9. 2. Licensee reserves the right to terminate the Licence in the event that payment is not received in full or if there has been a breach of this agreement by you.

Appendix 1 — Acknowledgements:

For Journal Content:

Reprinted by permission from [the Licensor]: [Journal Publisher (e.g. Nature/Springer/Palgrave)] [JOURNAL NAME] [REFERENCE CITATION (Article name, Author(s) Name), [COPYRIGHT] (year of publication)]

For Advance Online Publication papers:

Reprinted by permission from [the Licensor]: [Journal Publisher (e.g. Nature/Springer/Palgrave)] [JOURNAL NAME] [REFERENCE CITATION (Article name, Author(s) Name), [COPYRIGHT] (year of publication), advance online publication, day month year (doi: 10.1038/sj.[JOURNAL ACRONYM].)]

For Adaptations/Translations:

Adapted/Translated by permission from [the Licensor]: [Journal Publisher (e.g. Nature/Springer/Palgrave)] [JOURNAL NAME] [REFERENCE CITATION (Article name, Author(s) Name), [COPYRIGHT] (year of publication)]

Note: For any republication from the British Journal of Cancer, the following credit line style applies:

Reprinted/adapted/translated by permission from [the Licensor]: on behalf of Cancer Research UK: : [Journal Publisher (e.g. Nature/Springer/Palgrave)] [JOURNAL NAME] [REFERENCE CITATION (Article name, Author(s) Name), [COPYRIGHT] (year of publication)]

For Advance Online Publication papers:

Reprinted by permission from The [the Licensor]: on behalf of Cancer Research UK: [Journal Publisher (e.g. Nature/Springer/Palgrave)] [JOURNAL NAME] [REFERENCE CITATION (Article name, Author(s) Name), [COPYRIGHT] (year of publication), advance online publication, day month year (doi: 10.1038/sj.[JOURNAL ACRONYM].)]

For Book content:

Reprinted/adapted by permission from [the Licensor]: [Book Publisher (e.g. Palgrave Macmillan, Springer etc)] [Book Title] by [Book author(s)] [COPYRIGHT] (year of publication)]

ANNEXURE C: PERMISSION FOR USE OF FIGURE 1.3



Marketplace™

Royal Society of Chemistry - License Terms and Conditions

This is a License Agreement between Lianie Pieterse ("You") and Royal Society of Chemistry ("Publisher") provided by Copyright Clearance Center ("CCC"). The license consists of your order details, the terms and conditions provided by Royal Society of Chemistry, and the CCC terms and conditions.

All payments must be made in full to CCC.

Order Date	08-Oct-2020	Type of Use	Republish in a thesis/dissertation
Order license ID	1068687-1	Publisher	ROYAL SOCIETY OF CHEMISTRY
ISSN	1477-0539	Portion	Chart/graph/table/figure

LICENSED CONTENT

Publication Title	Organic & biomolecular chemistry	Country	United Kingdom of Great Britain and Northern Ireland
Author/Editor	Royal Society of Chemistry (Great Britain)	Rightholder	Royal Society of Chemistry
Date	01/01/2003	Publication Type	e-Journal
Language	English	URL	http://www.rsc.org/is/journals/current/obc/obcpub.htm

REQUEST DETAILS

Portion Type	Chart/graph/table/figure	Distribution	Worldwide
Number of charts / graphs / tables / figures requested	1	Translation	Original language of publication
Format (select all that apply)	Electronic	Copies for the disabled?	No
Who will republish the content?	Academic institution	Minor editing privileges?	No
Duration of Use	Life of current edition	Incidental promotional use?	No
Lifetime Unit Quantity	Up to 499	Currency	USD
Rights Requested	Main product		

NEW WORK DETAILS

Title	Design, synthesis and biological evaluation of selected 7-azaindole derivatives and structurally related compounds as potential inhibitors of disease-related protein kinases	Institution name	NWU-Potchefstroom
		Expected presentation date	2020-11-25

Instructor name LJ Legoabe

ADDITIONAL DETAILS

Order reference number	N/A	The requesting person / organization to appear on the license	Lianie Pieterse
------------------------	-----	---	-----------------

REUSE CONTENT DETAILS

Title, description or numeric reference of the portion(s)	Pharmacophore model of ATP binding site	Title of the article/chapter the portion is from	Synthesis and biological evaluation of a triazole-based library of pyrido[2,3-d]pyrimidines as FGFR3 tyrosine kinase inhibitors
Editor of portion(s)	N/A	Author of portion(s)	Royal Society of Chemistry (Great Britain)
Volume of serial or monograph	N/A	Issue, if republishing an article from a serial	N/A
Page or page range of portion	2164	Publication date of portion	2010-02-17

CCC Republication Terms and Conditions

1. Description of Service; Defined Terms. This Republication License enables the User to obtain licenses for republication of one or more copyrighted works as described in detail on the relevant Order Confirmation (the "Work(s)"). Copyright Clearance Center, Inc. ("CCC") grants licenses through the Service on behalf of the rightsholder identified on the Order Confirmation (the "Rightsholder"). "Republication", as used herein, generally means the inclusion of a Work, in whole or in part, in a new work or works, also as described on the Order Confirmation. "User", as used herein, means the person or entity making such republication.
2. The terms set forth in the relevant Order Confirmation, and any terms set by the Rightsholder with respect to a particular Work, govern the terms of use of Works in connection with the Service. By using the Service, the person transacting for a republication license on behalf of the User represents and warrants that he/she/it (a) has been duly authorized by the User to accept, and hereby does accept, all such terms and conditions on behalf of User, and (b) shall inform User of all such terms and conditions. In the event such person is a "freelancer" or other third party independent of User and CCC, such party shall be deemed jointly a "User" for purposes of these terms and conditions. In any event, User shall be deemed to have accepted and agreed to all such terms and conditions if User republishes the Work in any fashion.
3. Scope of License; Limitations and Obligations.
 - 3.1. All Works and all rights therein, including copyright rights, remain the sole and exclusive property of the Rightsholder. The license created by the exchange of an Order Confirmation (and/or any invoice) and payment by User of the full amount set forth on that document includes only those rights expressly set forth in the Order Confirmation and in these terms and conditions, and conveys no other rights in the Work(s) to User. All rights not expressly granted are hereby reserved.
 - 3.2. General Payment Terms: You may pay by credit card or through an account with us payable at the end of the month. If you and we agree that you may establish a standing account with CCC, then the following terms apply: Remit Payment to: Copyright Clearance Center, 29118 Network Place, Chicago, IL 60673-1291. Payments Due: Invoices are payable upon their delivery to you (or upon our notice to you that they are available to you for downloading). After 30 days, outstanding amounts will be subject to a service charge of 1-1/2% per month or, if less, the maximum rate allowed by applicable law. Unless otherwise specifically set forth in the Order Confirmation or in a separate written agreement signed by CCC, invoices are due and

payable on "net 30" terms. While User may exercise the rights licensed immediately upon issuance of the Order Confirmation, the license is automatically revoked and is null and void, as if it had never been issued, if complete payment for the license is not received on a timely basis either from User directly or through a payment agent, such as a credit card company.

- 3.3. Unless otherwise provided in the Order Confirmation, any grant of rights to User (i) is "one-time" (including the editions and product family specified in the license), (ii) is non-exclusive and non-transferable and (iii) is subject to any and all limitations and restrictions (such as, but not limited to, limitations on duration of use or circulation) included in the Order Confirmation or invoice and/or in these terms and conditions. Upon completion of the licensed use, User shall either secure a new permission for further use of the Work(s) or immediately cease any new use of the Work(s) and shall render inaccessible (such as by deleting or by removing or severing links or other locators) any further copies of the Work (except for copies printed on paper in accordance with this license and still in User's stock at the end of such period).
- 3.4. In the event that the material for which a republication license is sought includes third party materials (such as photographs, illustrations, graphs, inserts and similar materials) which are identified in such material as having been used by permission, User is responsible for identifying, and seeking separate licenses (under this Service or otherwise) for, any of such third party materials; without a separate license, such third party materials may not be used.
- 3.5. Use of proper copyright notice for a Work is required as a condition of any license granted under the Service. Unless otherwise provided in the Order Confirmation, a proper copyright notice will read substantially as follows: "Republished with permission of [Rightsholder's name], from [Work's title, author, volume, edition number and year of copyright]; permission conveyed through Copyright Clearance Center, Inc. " Such notice must be provided in a reasonably legible font size and must be placed either immediately adjacent to the Work as used (for example, as part of a by-line or footnote but not as a separate electronic link) or in the place where substantially all other credits or notices for the new work containing the republished Work are located. Failure to include the required notice results in loss to the Rightsholder and CCC, and the User shall be liable to pay liquidated damages for each such failure equal to twice the use fee specified in the Order Confirmation, in addition to the use fee itself and any other fees and charges specified.
- 3.6. User may only make alterations to the Work if and as expressly set forth in the Order Confirmation. No Work may be used in any way that is defamatory, violates the rights of third parties (including such third parties' rights of copyright, privacy, publicity, or other tangible or intangible property), or is otherwise illegal, sexually explicit or obscene. In addition, User may not conjoin a Work with any other material that may result in damage to the reputation of the Rightsholder. User agrees to inform CCC if it becomes aware of any infringement of any rights in a Work and to cooperate with any reasonable request of CCC or the Rightsholder in connection therewith.
4. Indemnity. User hereby indemnifies and agrees to defend the Rightsholder and CCC, and their respective employees and directors, against all claims, liability, damages, costs and expenses, including legal fees and expenses, arising out of any use of a Work beyond the scope of the rights granted herein, or any use of a Work which has been altered in any unauthorized way by User, including claims of defamation or infringement of rights of copyright, publicity, privacy or other tangible or intangible property.
5. Limitation of Liability. UNDER NO CIRCUMSTANCES WILL CCC OR THE RIGHTSHOLDER BE LIABLE FOR ANY DIRECT, INDIRECT, CONSEQUENTIAL OR INCIDENTAL DAMAGES (INCLUDING WITHOUT LIMITATION DAMAGES FOR LOSS OF BUSINESS PROFITS OR INFORMATION, OR FOR BUSINESS INTERRUPTION) ARISING OUT OF THE USE OR INABILITY TO USE A WORK, EVEN IF ONE OF THEM HAS BEEN ADVISED OF THE POSSIBILITY OF SUCH DAMAGES. In any event, the total liability of the Rightsholder and CCC (including their respective employees and directors) shall not exceed the total amount actually paid by User for this license. User assumes full liability for the actions and omissions of its principals, employees, agents, affiliates, successors and assigns.
6. Limited Warranties. THE WORK(S) AND RIGHT(S) ARE PROVIDED "AS IS". CCC HAS THE RIGHT TO GRANT TO USER THE RIGHTS GRANTED IN THE ORDER CONFIRMATION DOCUMENT. CCC AND THE RIGHTSHOLDER DISCLAIM ALL

OTHER WARRANTIES RELATING TO THE WORK(S) AND RIGHT(S), EITHER EXPRESS OR IMPLIED, INCLUDING WITHOUT LIMITATION IMPLIED WARRANTIES OF MERCHANTABILITY OR FITNESS FOR A PARTICULAR PURPOSE. ADDITIONAL RIGHTS MAY BE REQUIRED TO USE ILLUSTRATIONS, GRAPHS, PHOTOGRAPHS, ABSTRACTS, INSERTS OR OTHER PORTIONS OF THE WORK (AS OPPOSED TO THE ENTIRE WORK) IN A MANNER CONTEMPLATED BY USER; USER UNDERSTANDS AND AGREES THAT NEITHER CCC NOR THE RIGHTSHOLDER MAY HAVE SUCH ADDITIONAL RIGHTS TO GRANT.

7. Effect of Breach. Any failure by User to pay any amount when due, or any use by User of a Work beyond the scope of the license set forth in the Order Confirmation and/or these terms and conditions, shall be a material breach of the license created by the Order Confirmation and these terms and conditions. Any breach not cured within 30 days of written notice thereof shall result in immediate termination of such license without further notice. Any unauthorized (but licensable) use of a Work that is terminated immediately upon notice thereof may be liquidated by payment of the Rightsholder's ordinary license price therefor; any unauthorized (and unlicensable) use that is not terminated immediately for any reason (including, for example, because materials containing the Work cannot reasonably be recalled) will be subject to all remedies available at law or in equity, but in no event to a payment of less than three times the Rightsholder's ordinary license price for the most closely analogous licensable use plus Rightsholder's and/or CCC's costs and expenses incurred in collecting such payment.

8. Miscellaneous.

8.1. User acknowledges that CCC may, from time to time, make changes or additions to the Service or to these terms and conditions, and CCC reserves the right to send notice to the User by electronic mail or otherwise for the purposes of notifying User of such changes or additions; provided that any such changes or additions shall not apply to permissions already secured and paid for.

8.2. Use of User-related information collected through the Service is governed by CCC's privacy policy, available online here: <https://marketplace.copyright.com/rs-ui-web/mp/privacy-policy>

8.3. The licensing transaction described in the Order Confirmation is personal to User. Therefore, User may not assign or transfer to any other person (whether a natural person or an organization of any kind) the license created by the Order Confirmation and these terms and conditions or any rights granted hereunder; provided, however, that User may assign such license in its entirety on written notice to CCC in the event of a transfer of all or substantially all of User's rights in the new material which includes the Work(s) licensed under this Service.

8.4. No amendment or waiver of any terms is binding unless set forth in writing and signed by the parties. The Rightsholder and CCC hereby object to any terms contained in any writing prepared by the User or its principals, employees, agents or affiliates and purporting to govern or otherwise relate to the licensing transaction described in the Order Confirmation, which terms are in any way inconsistent with any terms set forth in the Order Confirmation and/or in these terms and conditions or CCC's standard operating procedures, whether such writing is prepared prior to, simultaneously with or subsequent to the Order Confirmation, and whether such writing appears on a copy of the Order Confirmation or in a separate instrument.

8.5. The licensing transaction described in the Order Confirmation document shall be governed by and construed under the law of the State of New York, USA, without regard to the principles thereof of conflicts of law. Any case, controversy, suit, action, or proceeding arising out of, in connection with, or related to such licensing transaction shall be brought, at CCC's sole discretion, in any federal or state court located in the County of New York, State of New York, USA, or in any federal or state court whose geographical jurisdiction covers the location of the Rightsholder set forth in the Order Confirmation. The parties expressly submit to the personal jurisdiction and venue of each such federal or state court. If you have any comments or questions about the Service or Copyright Clearance Center, please contact us at 978-750-8400 or send an e-mail to support@copyright.com.

ANNEXURE D: SUPPLEMENTARY INFORMATION FOR PUBLISHED ARTICLE

Supplementary Material:

Synthesis and Biological Evaluation of Selected 7-Azaindole Derivatives as dual CDK9/Cyclin T and Haspin Inhibitors

Lianie Pieterse¹; Lesetja J. Legoabe¹, Richard M. Beteck¹, Béatrice Josselin^{2,3}, Stéphane Bach^{2,3}, Sandrine Ruchaud²

✉ Lesetja J. Legoabe
lesetja.legoabe@nwu.ac.za

¹ Centre of Excellence for Pharmaceutical Sciences, North-West University, Private Bag X6001, Potchefstroom 2520, South Africa,

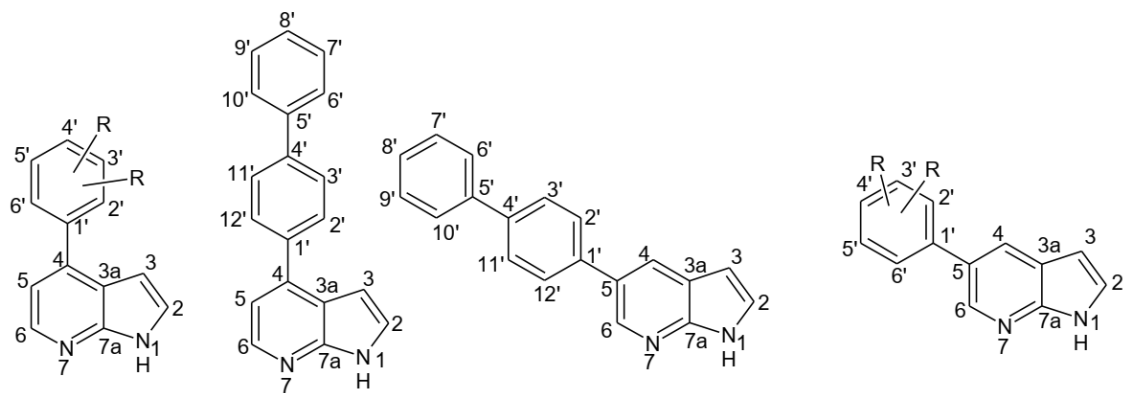
² Sorbonne Université, CNRS, UMR 8227, Integrative Biology of Marine Models Laboratory (LBI2M), Station Biologique de Roscoff, CS 90074, 29688 Roscoff Cedex, France

³ Sorbonne Université, CNRS, FR2424, Plateforme de criblage KISSf (Kinase Inhibitor Specialized Screening facility), Station Biologique de Roscoff, CS90074, 29688 Roscoff Cedex, France

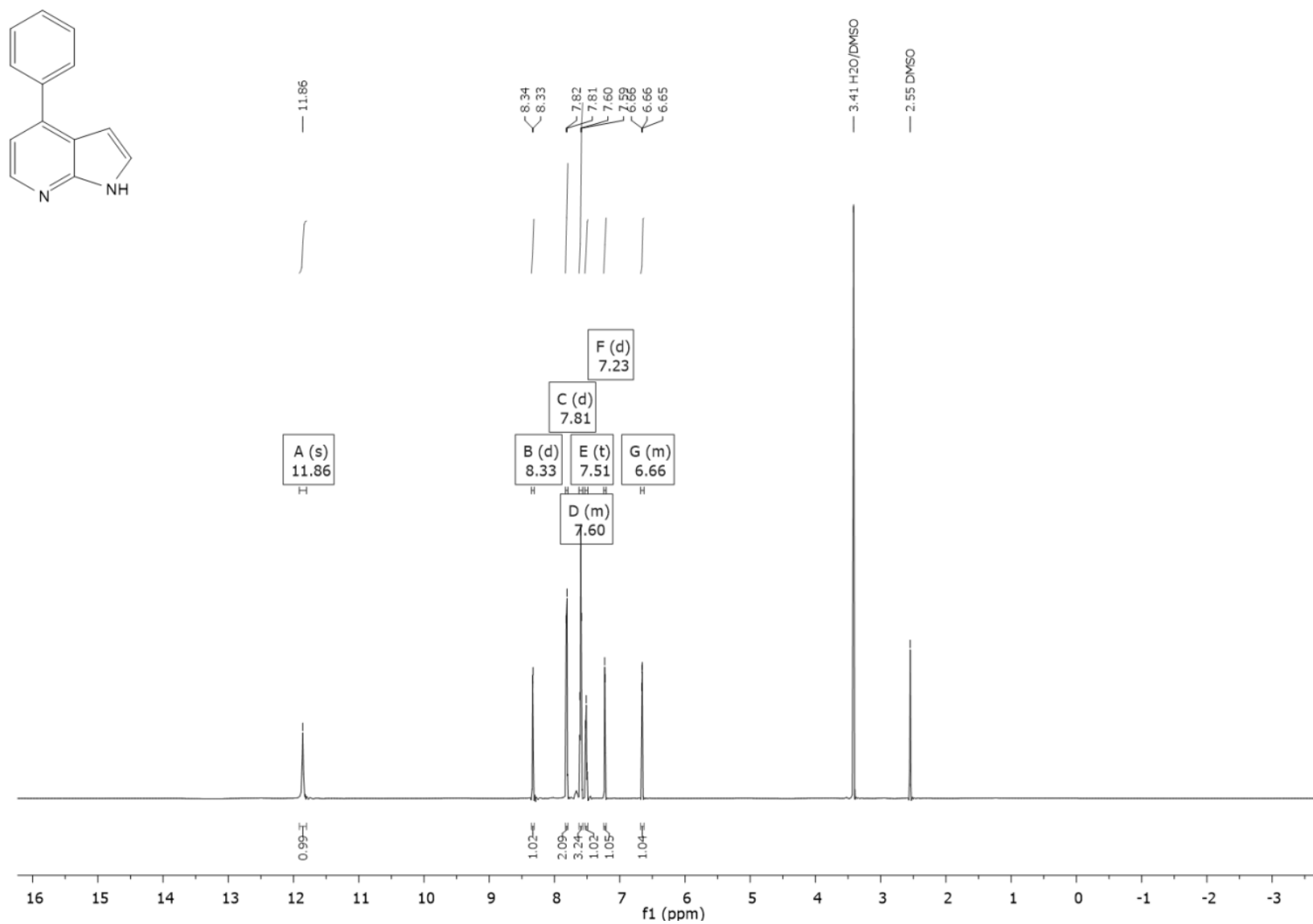
Index:

1. ^1H and ^{13}C NMR spectra
2. HRMS data
3. HPLC data

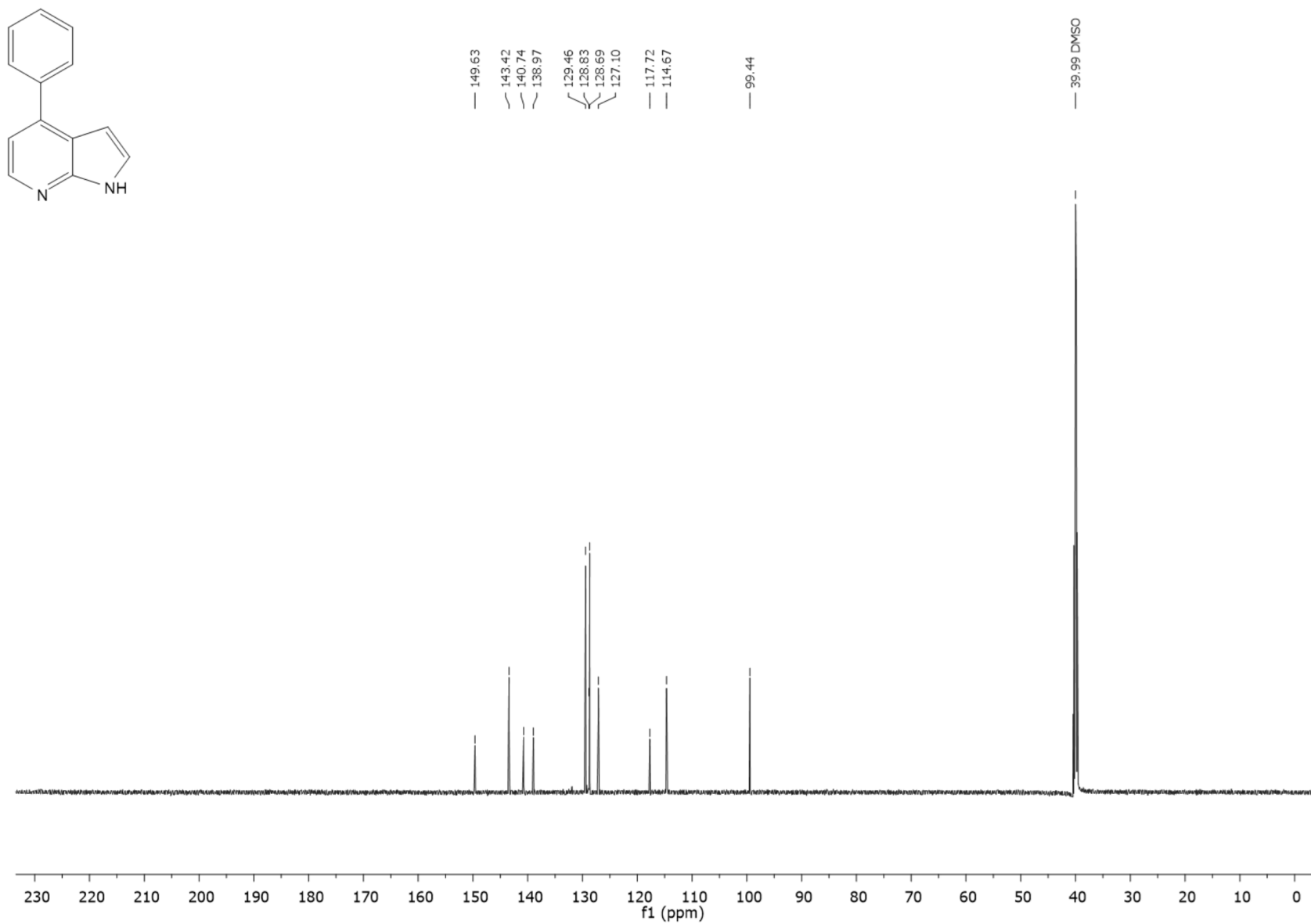
Numbering of compounds relevant to NMR spectra:



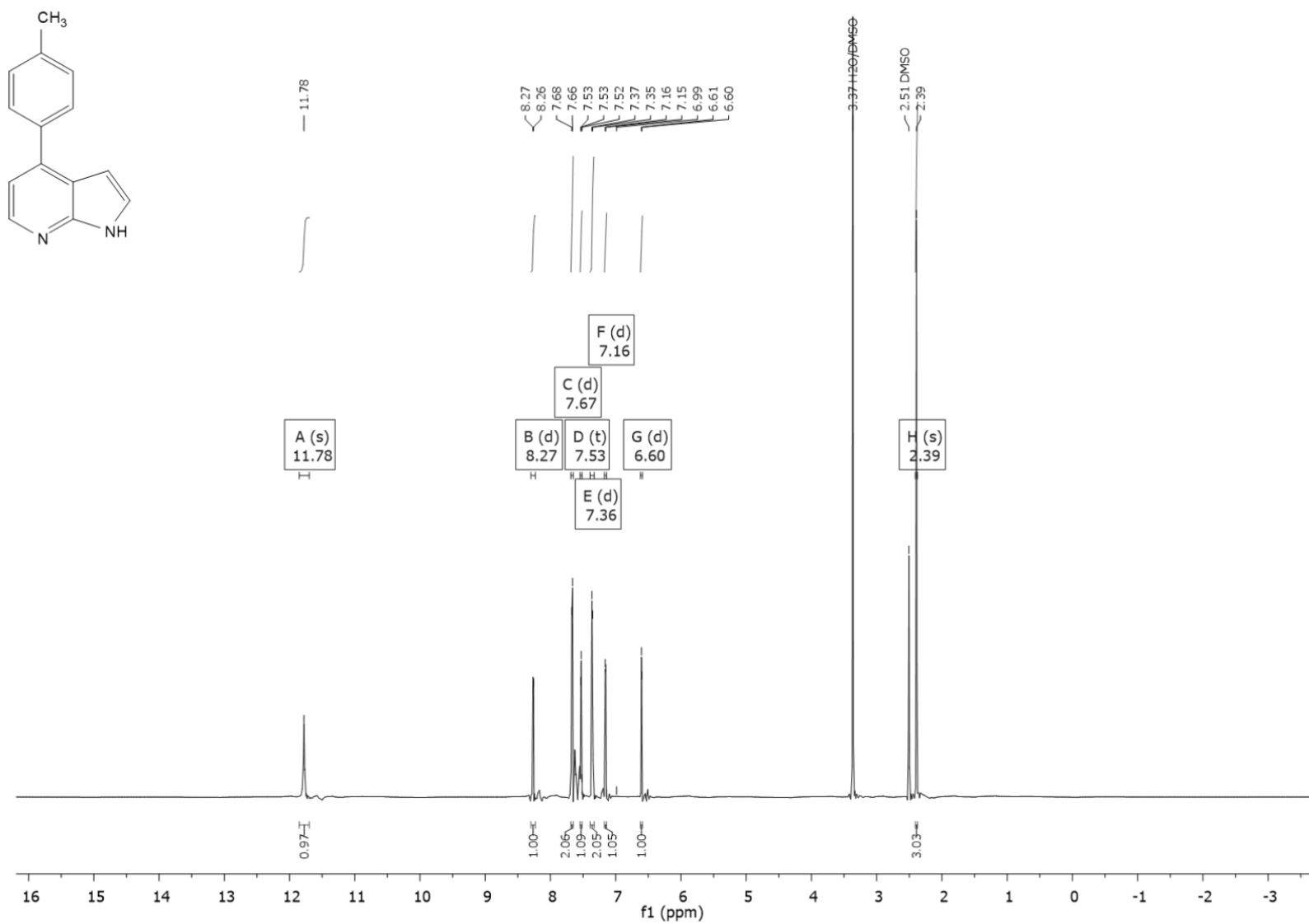
4-phenyl-1*H*-pyrrolo[2,3-*b*]pyridine (18a) (¹H):



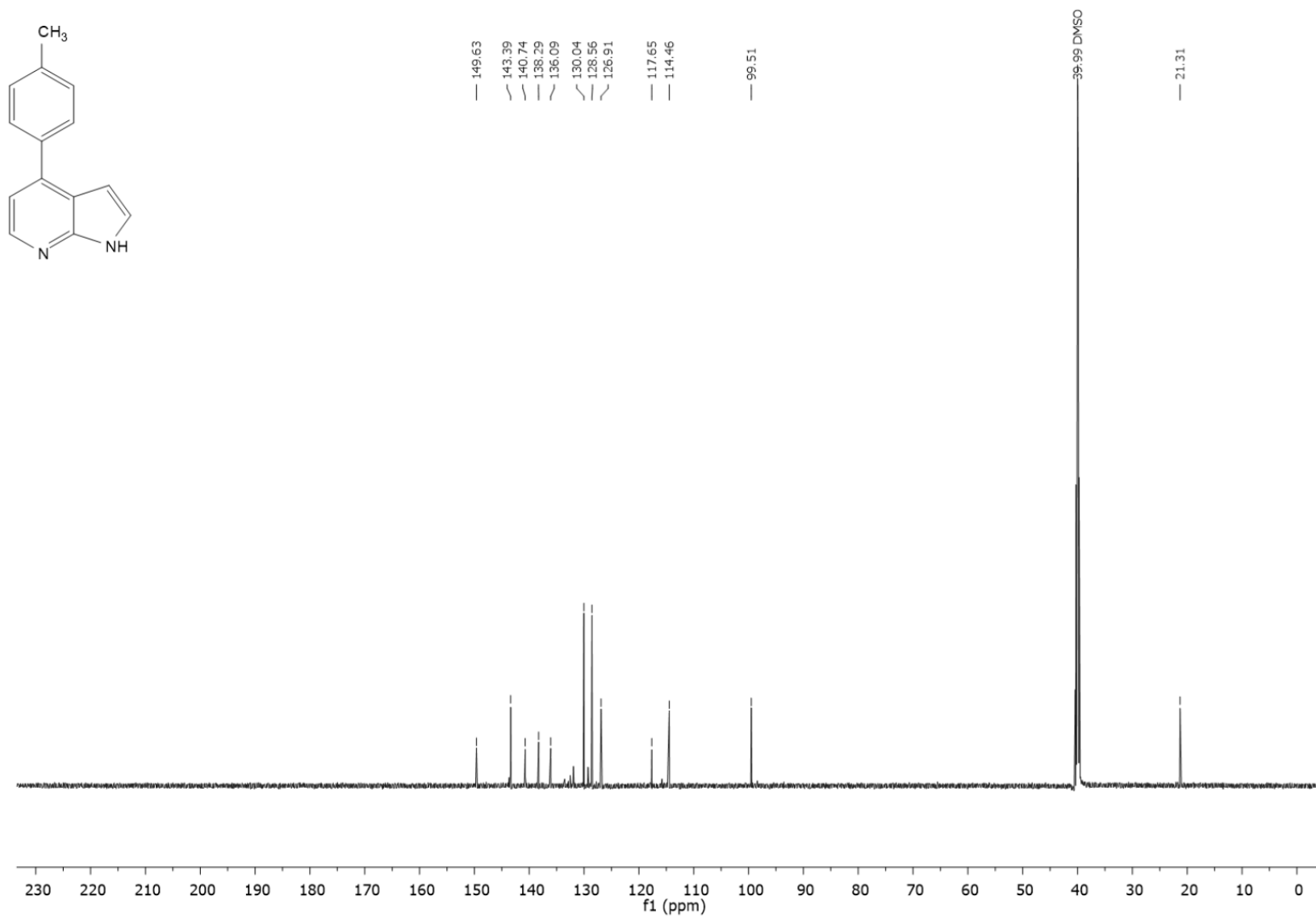
4-phenyl-1*H*-pyrrolo[2,3-*b*]pyridine (18a) (¹³C):



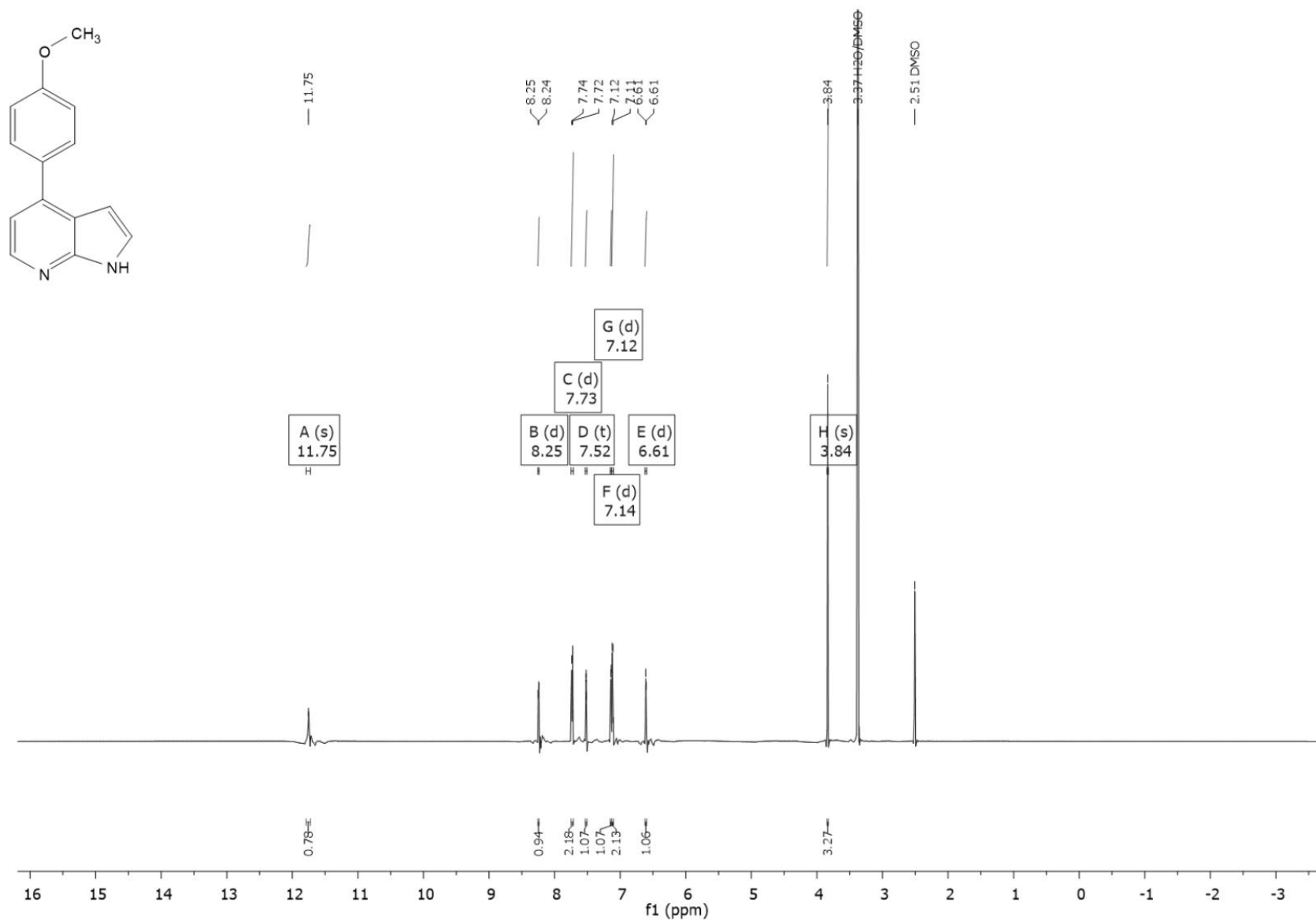
4-(4-methylphenyl)-1*H*-pyrrolo[2,3-*b*]pyridine (18b) (¹H):



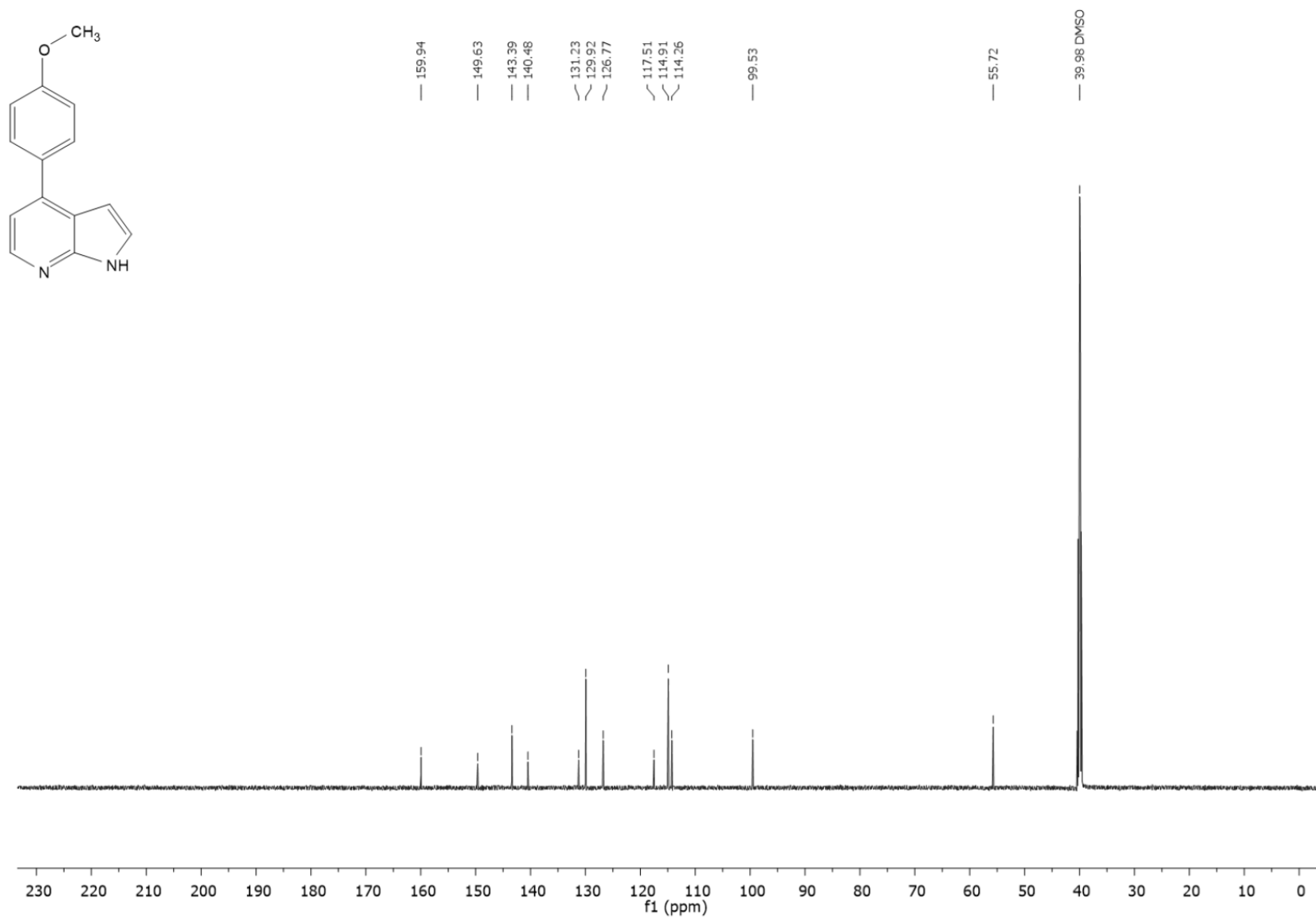
4-(4-methylphenyl)-1*H*-pyrrolo[2,3-*b*]pyridine (18b) (¹³C):



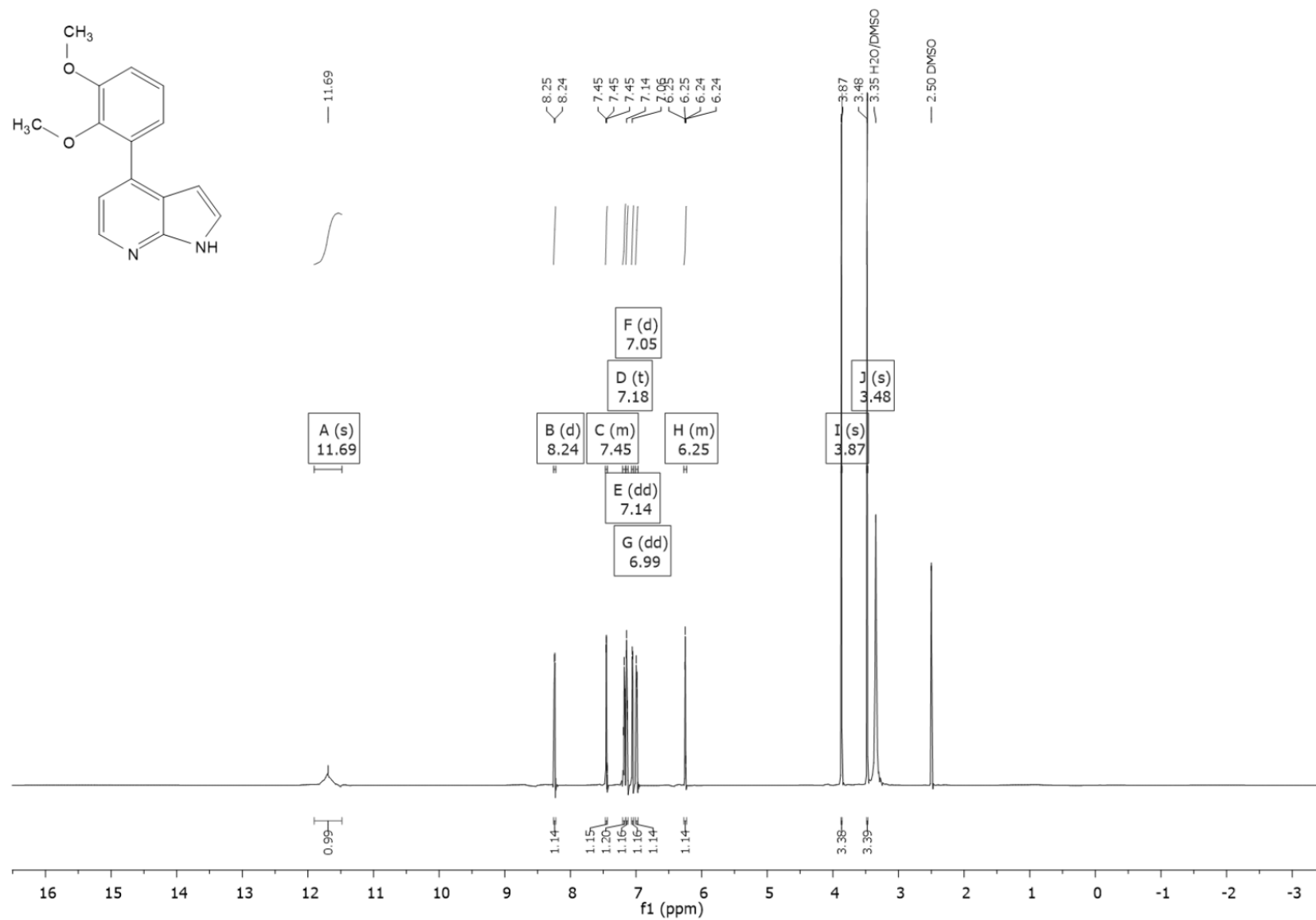
4-(4-methoxyphenyl)-1*H*-pyrrolo[2,3-*b*]pyridine (18c) (¹H):



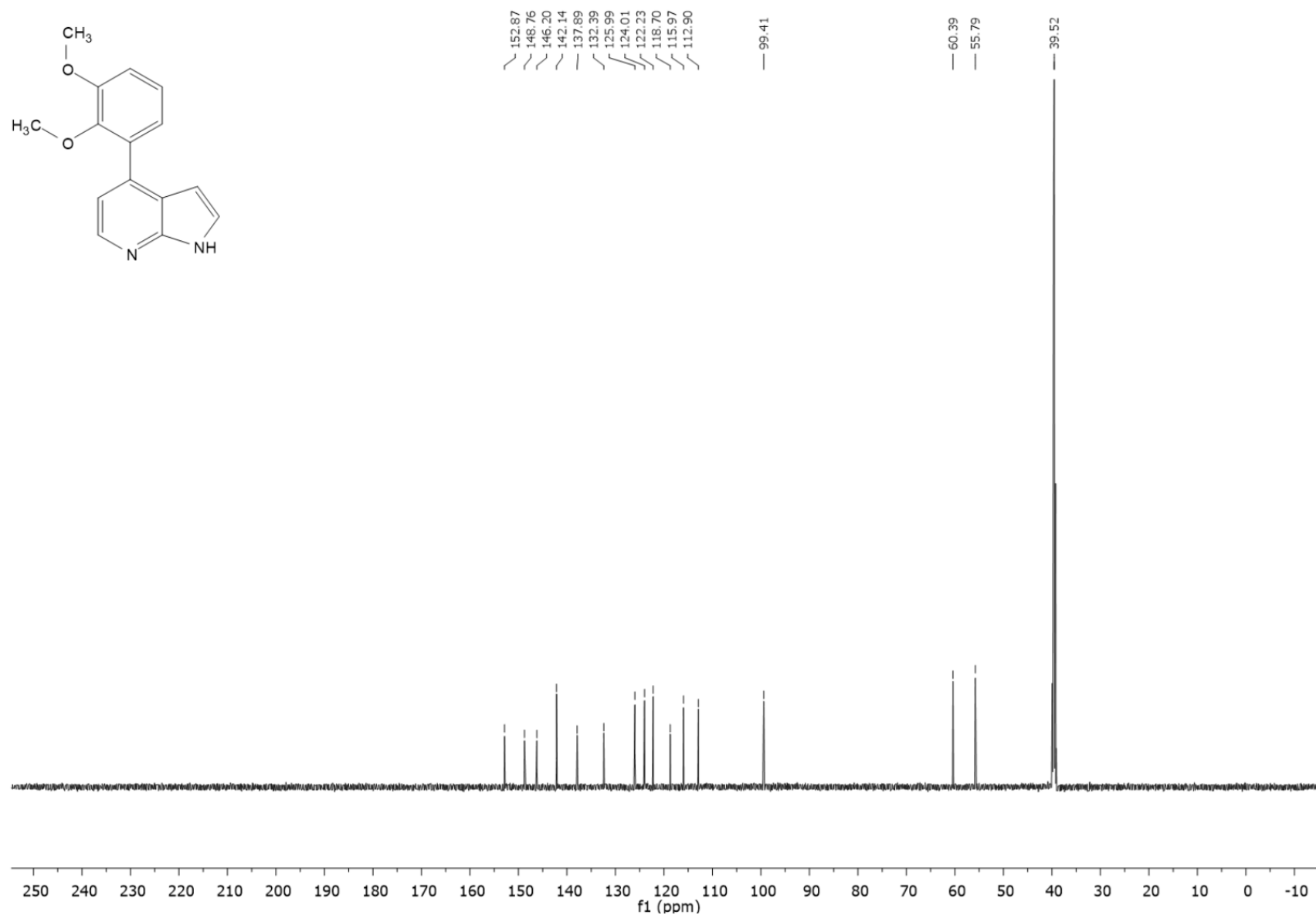
4-(4-methoxyphenyl)-1*H*-pyrrolo[2,3-*b*]pyridine (18c) (¹³C):



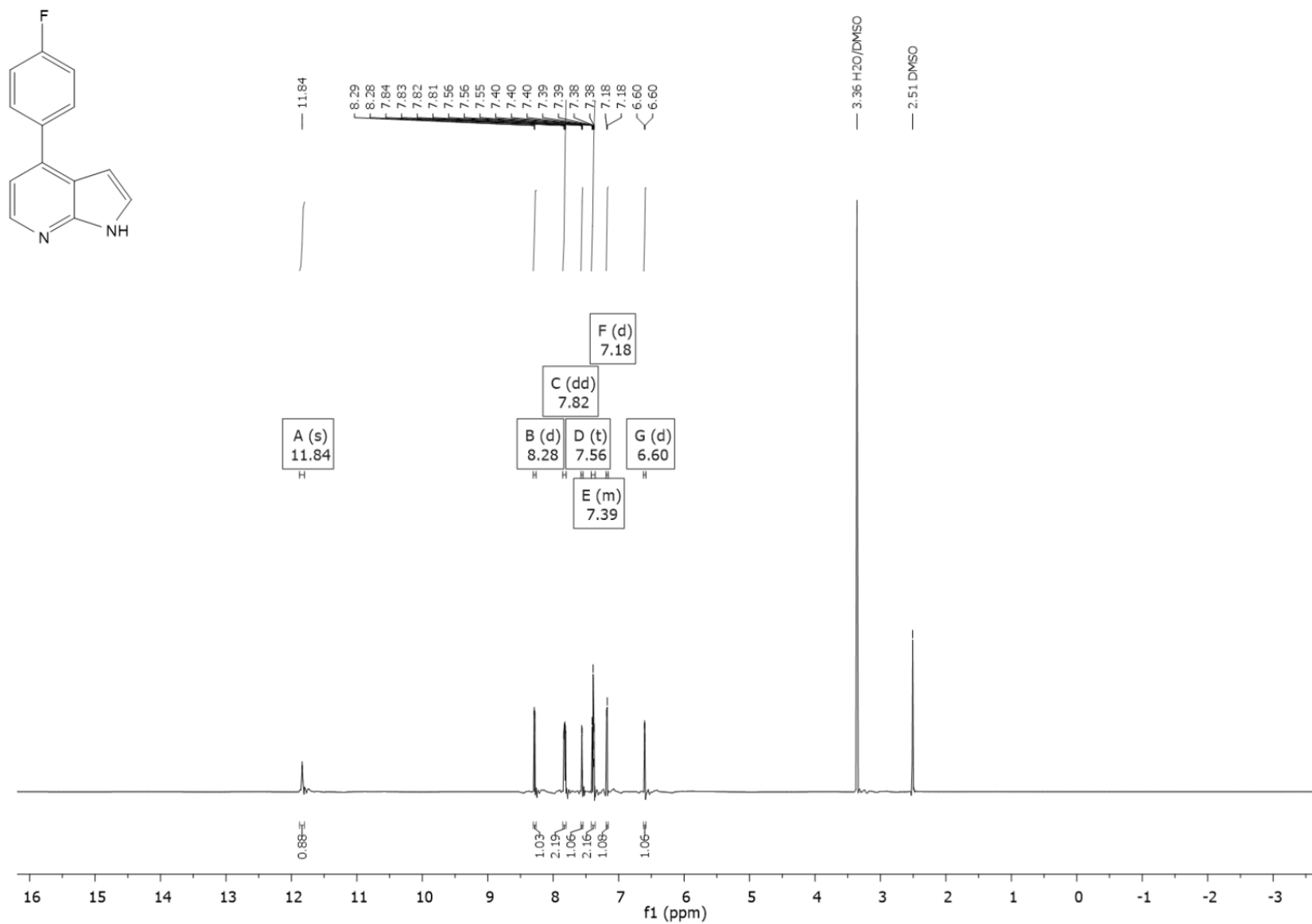
4-(2,3-dimethoxyphenyl)-1*H*-pyrrolo[2,3-*b*]pyridine (18d) (¹H):



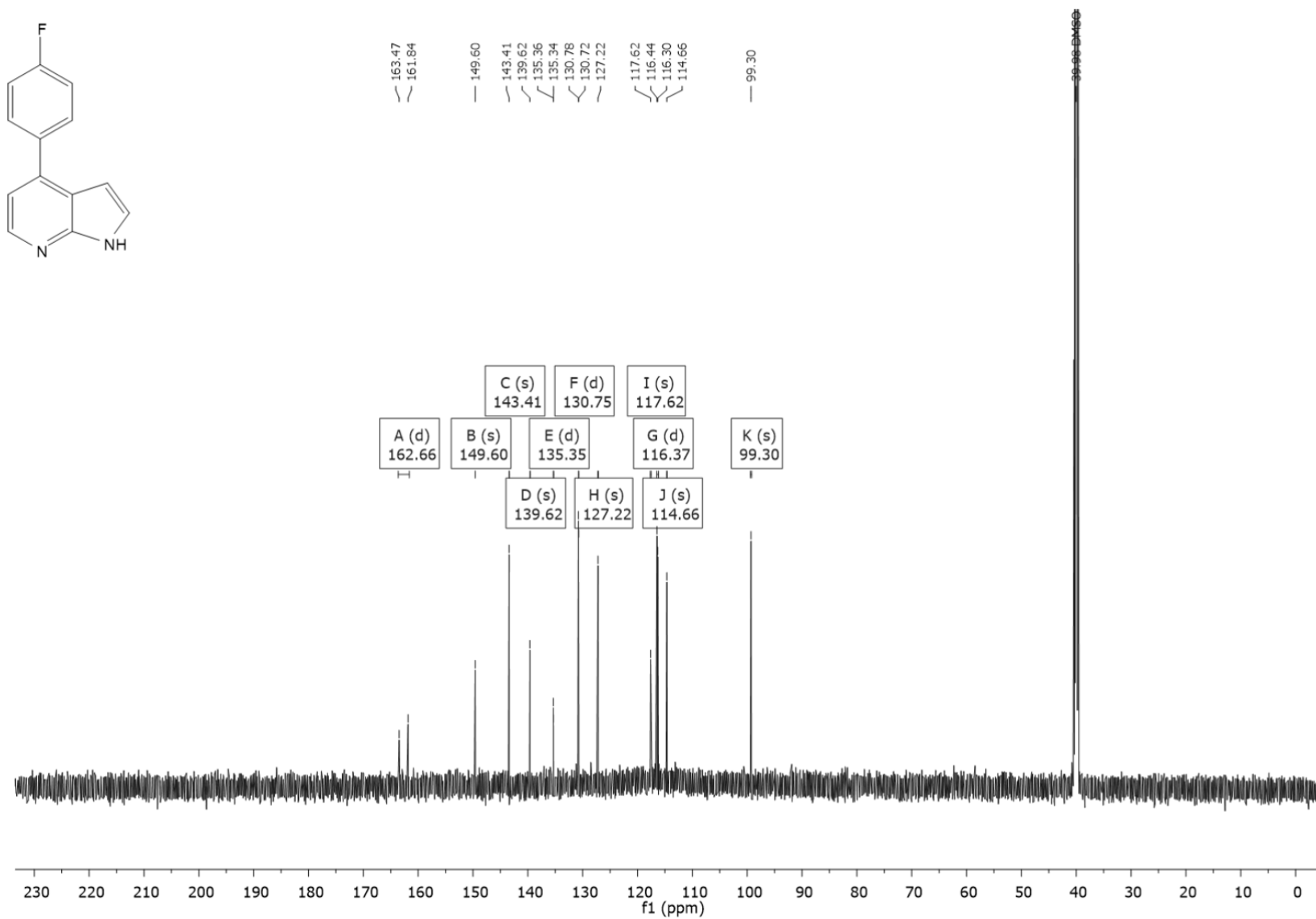
4-(2,3-dimethoxyphenyl)-1*H*-pyrrolo[2,3-*b*]pyridine (18d) (¹³C):



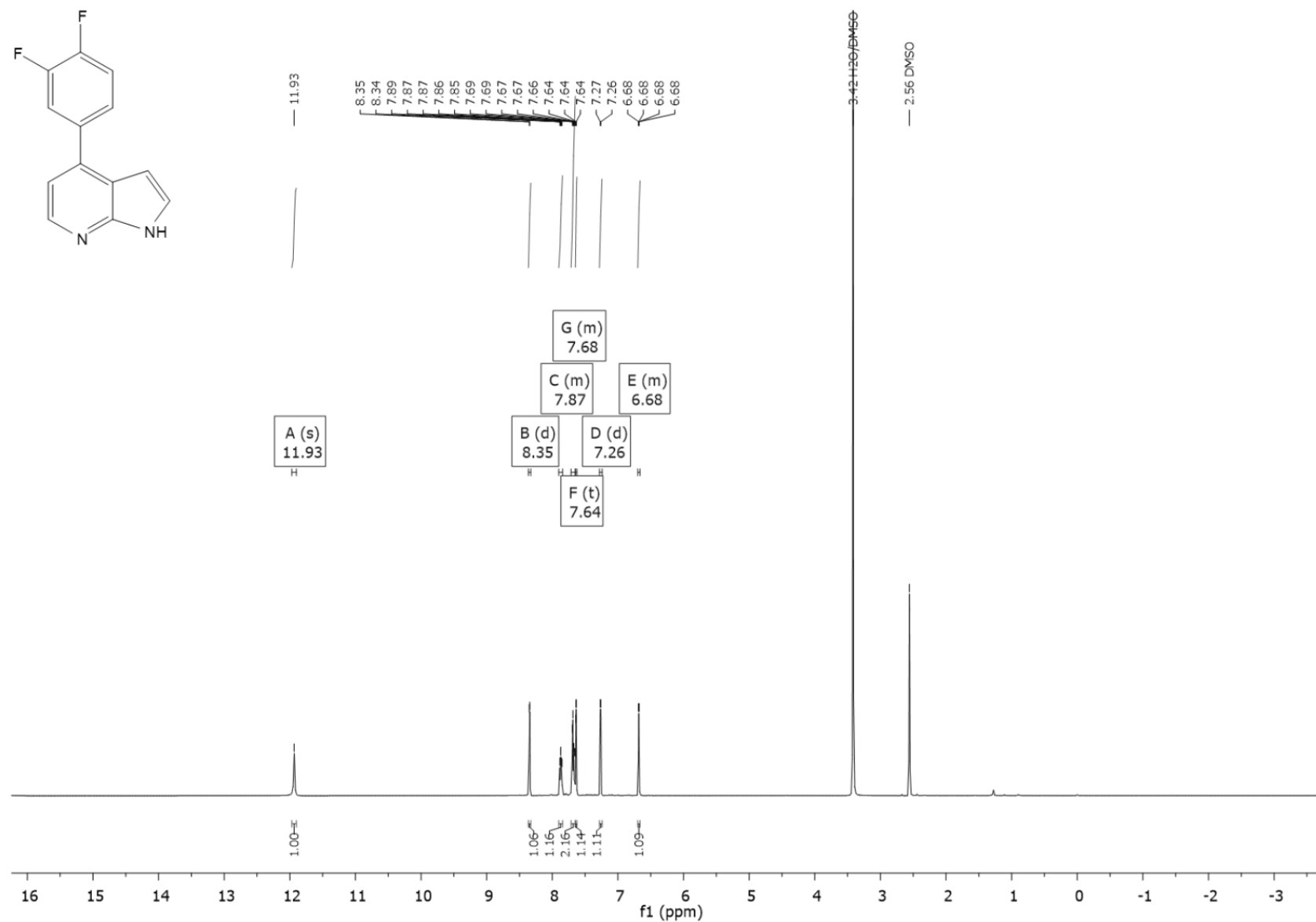
4-(4-fluorophenyl)-1*H*-pyrrolo[2,3-*b*]pyridine (18e) (¹H):



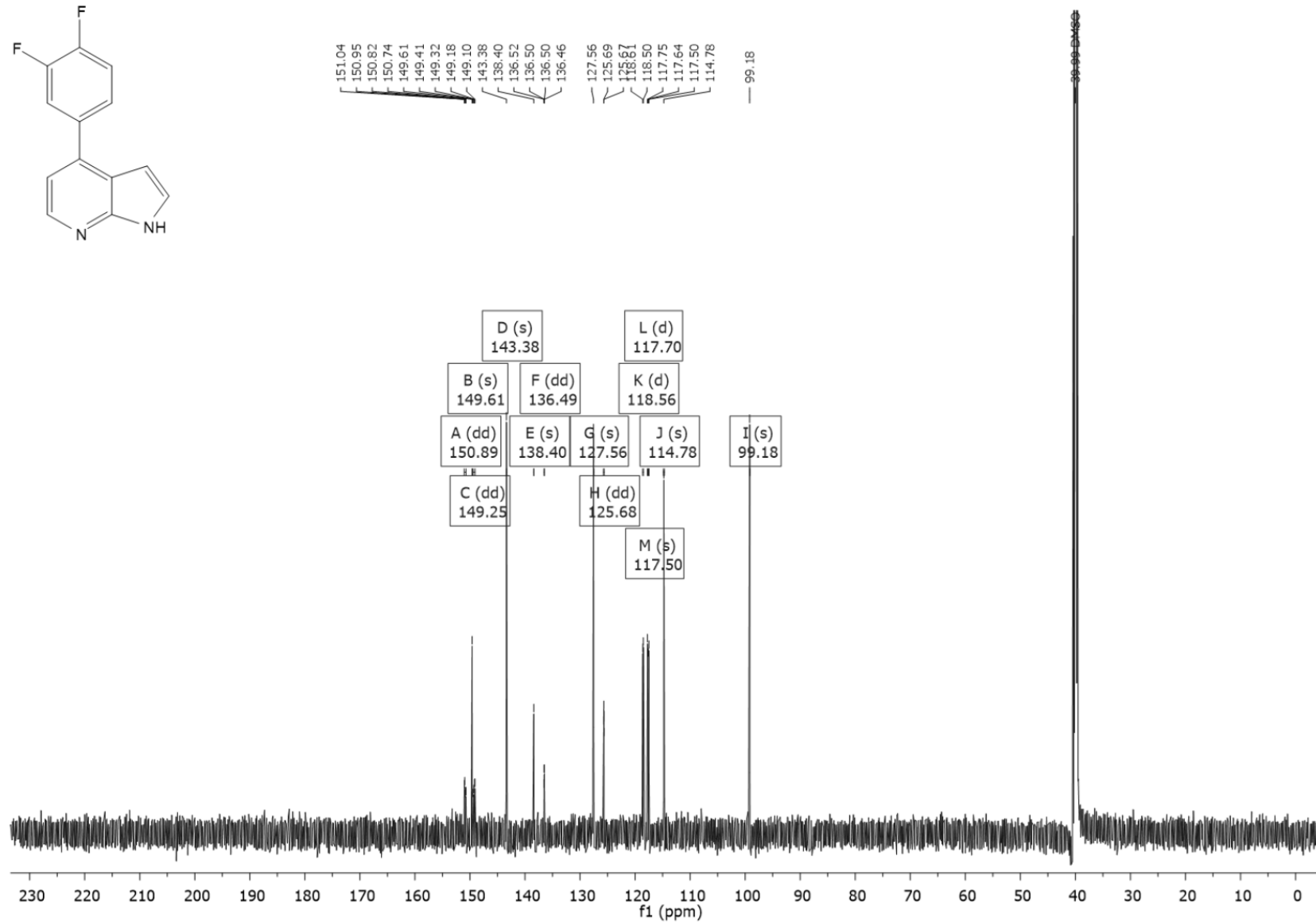
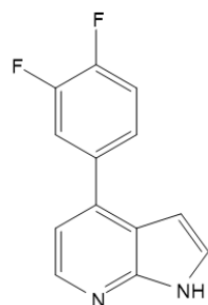
4-(4-fluorophenyl)-1*H*-pyrrolo[2,3-*b*]pyridine (18e) (¹³C):



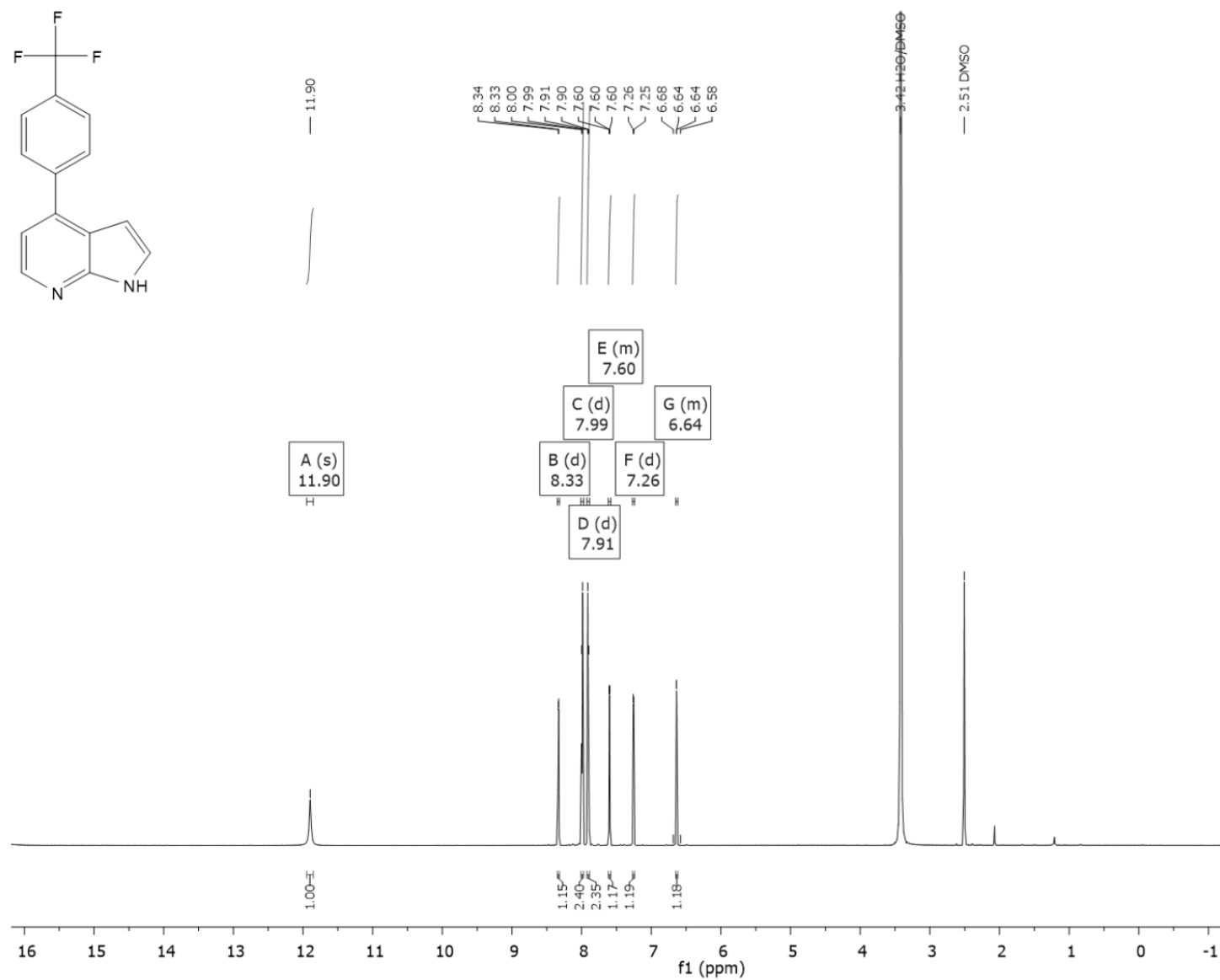
4-(3,4-difluorophenyl)-1*H*-pyrrolo[2,3-*b*]pyridine (18f) (¹H):



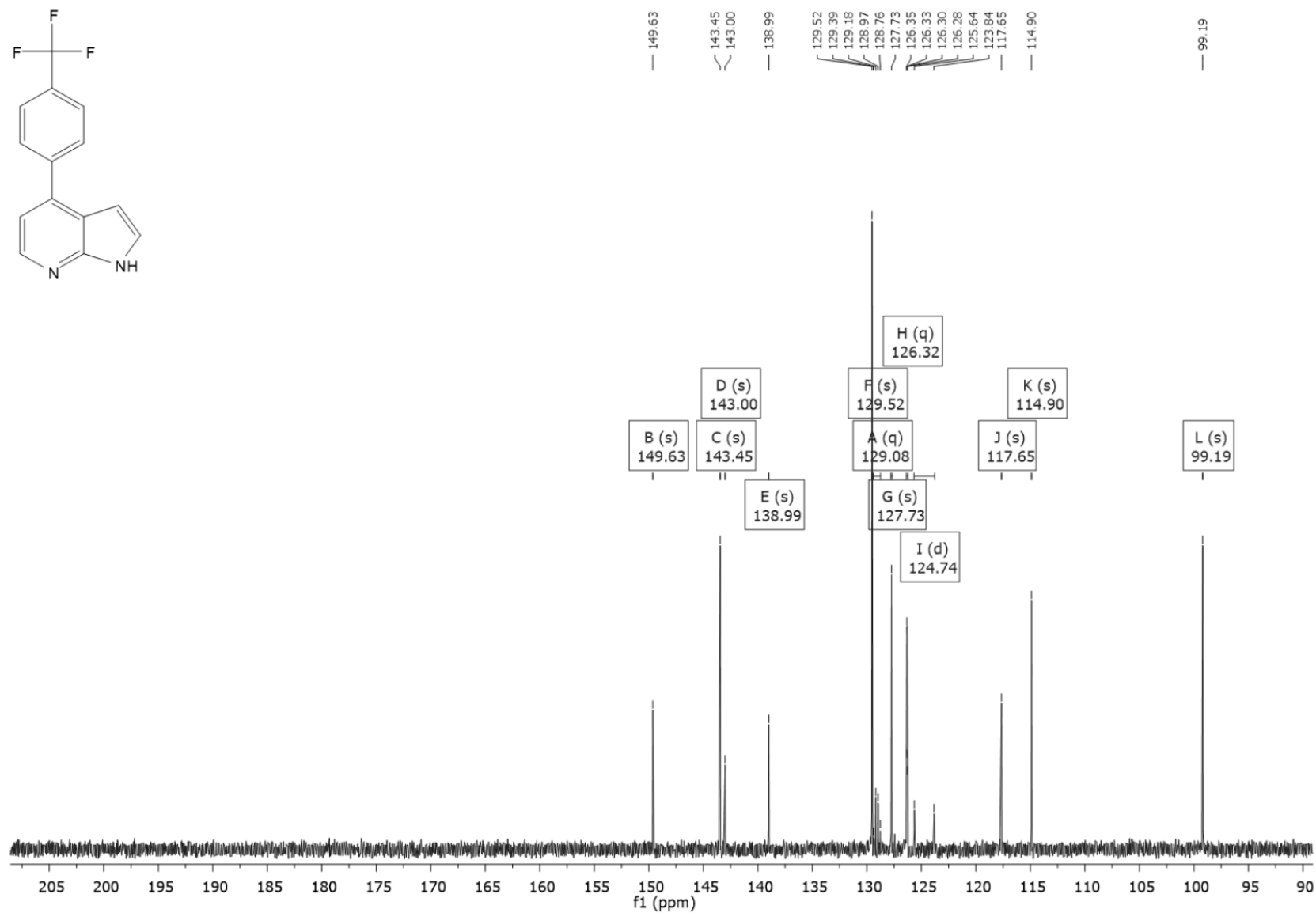
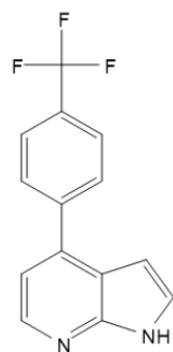
4-(3,4-difluorophenyl)-1*H*-pyrrolo[2,3-*b*]pyridine (18f) (¹³C):



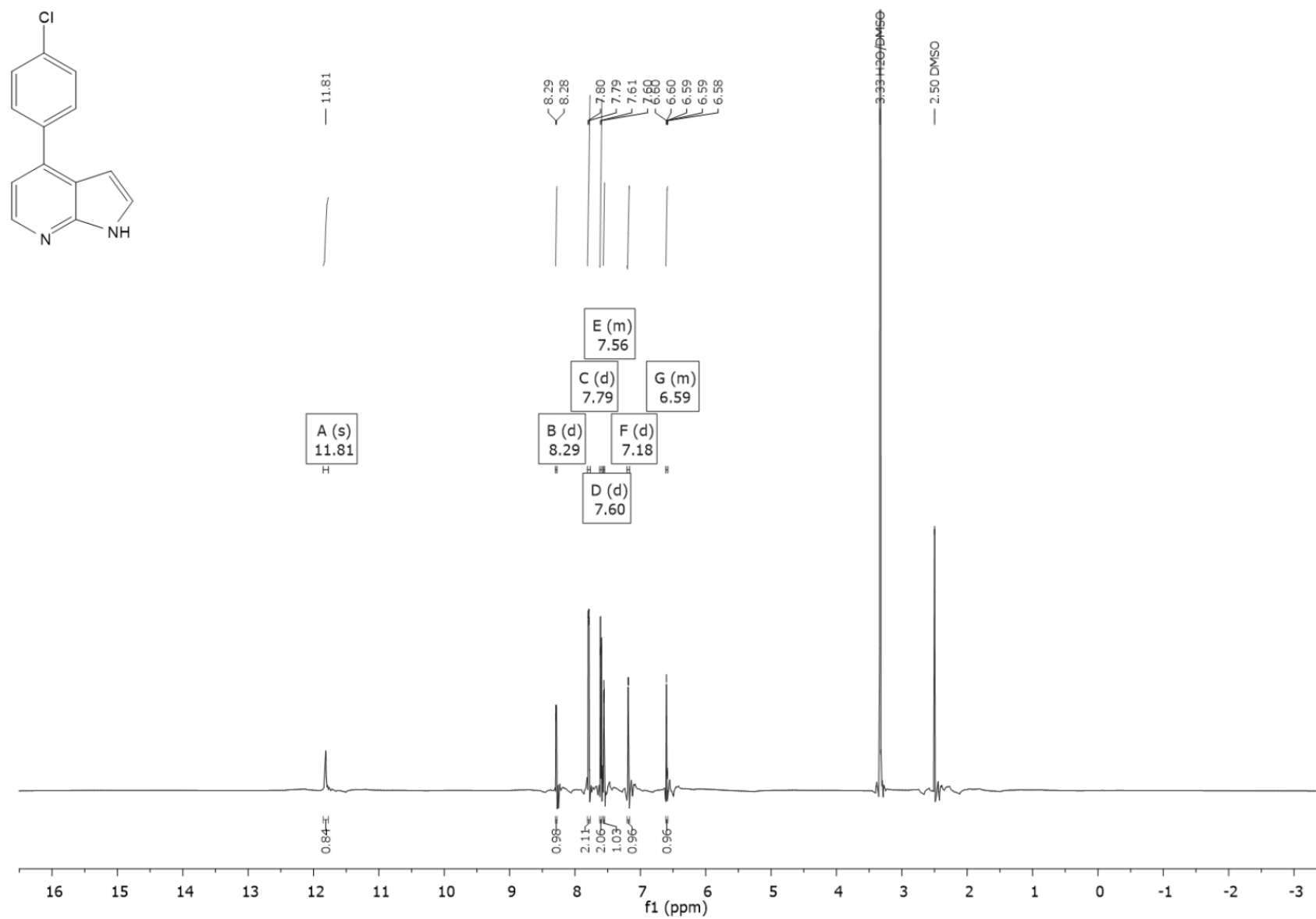
4-[4-(trifluoromethyl)phenyl]-1*H*-pyrrolo[2,3-*b*]pyridine (18g) (¹H):



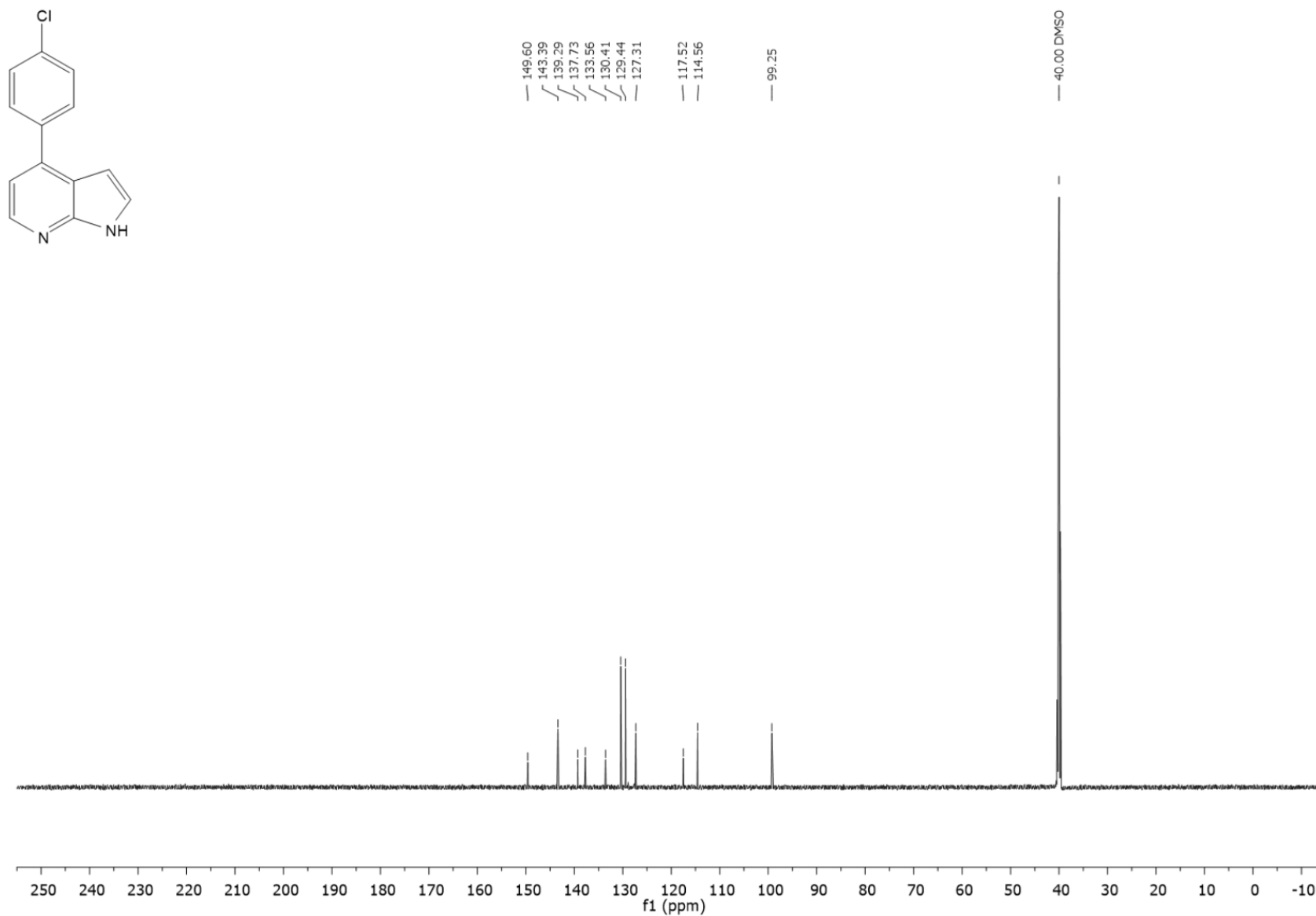
4-[4-(trifluoromethyl)phenyl]-1*H*-pyrrolo[2,3-*b*]pyridine (18g) (¹³C):



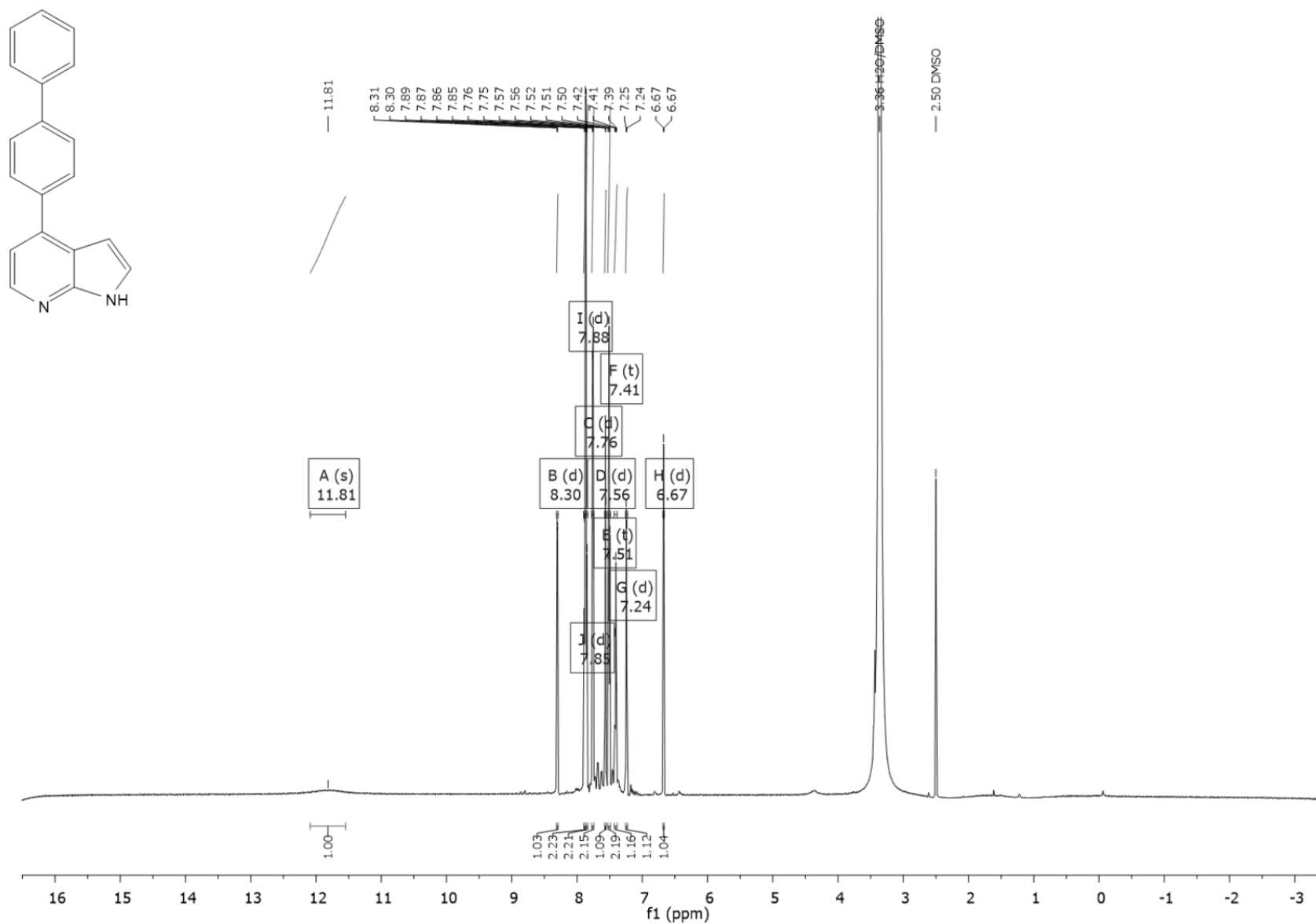
4-(4-chlorophenyl)-1*H*-pyrrolo[2,3-*b*]pyridine (18h) (¹H):



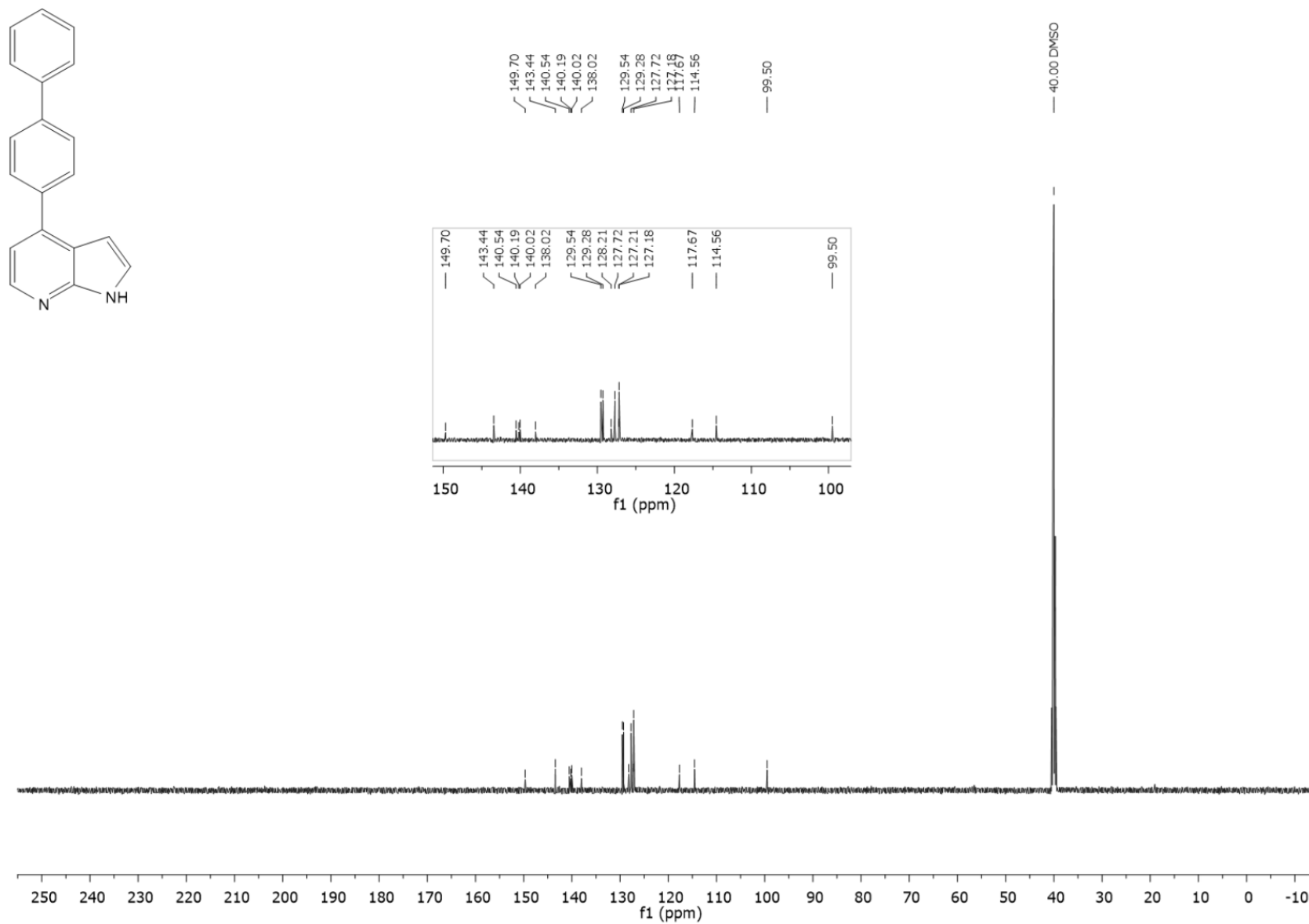
4-(4-chlorophenyl)-1*H*-pyrrolo[2,3-*b*]pyridine (18h) (^{13}C):



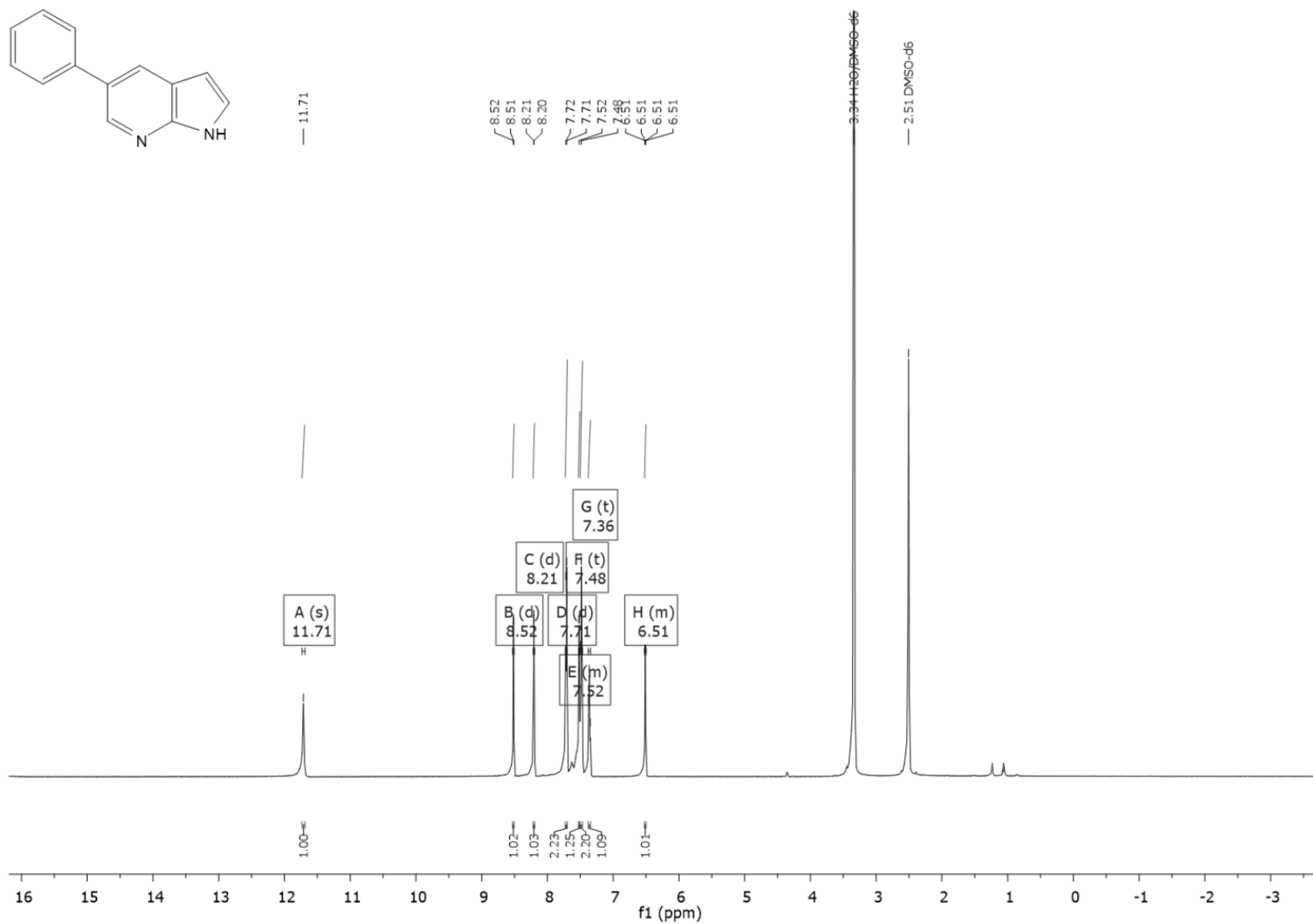
4-(biphenyl-4-yl)-1*H*-pyrrolo[2,3-*b*]pyridine (18i) (¹H):



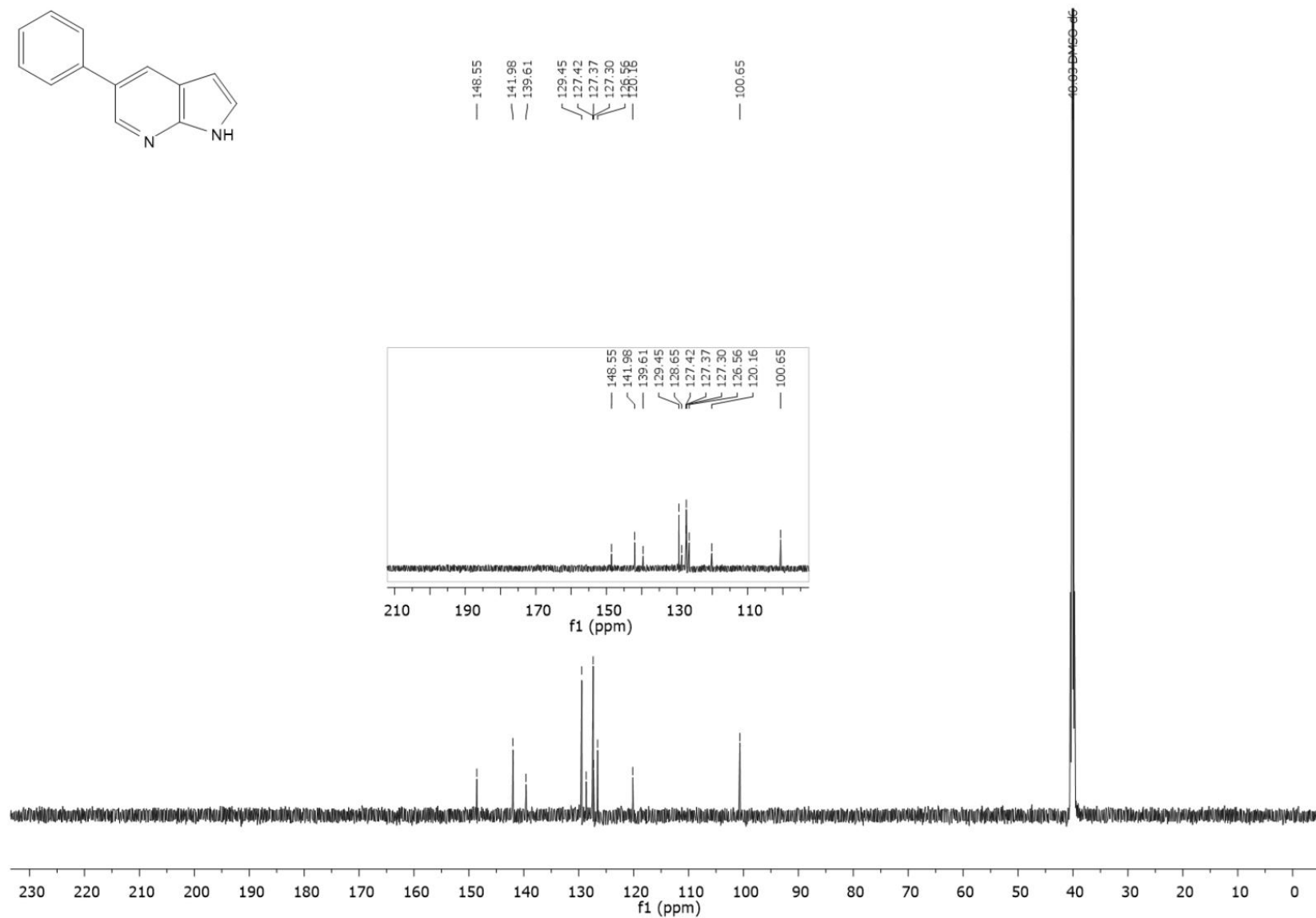
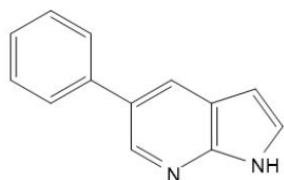
4-(biphenyl-4-yl)-1*H*-pyrrolo[2,3-*b*]pyridine (18i) (^{13}C):



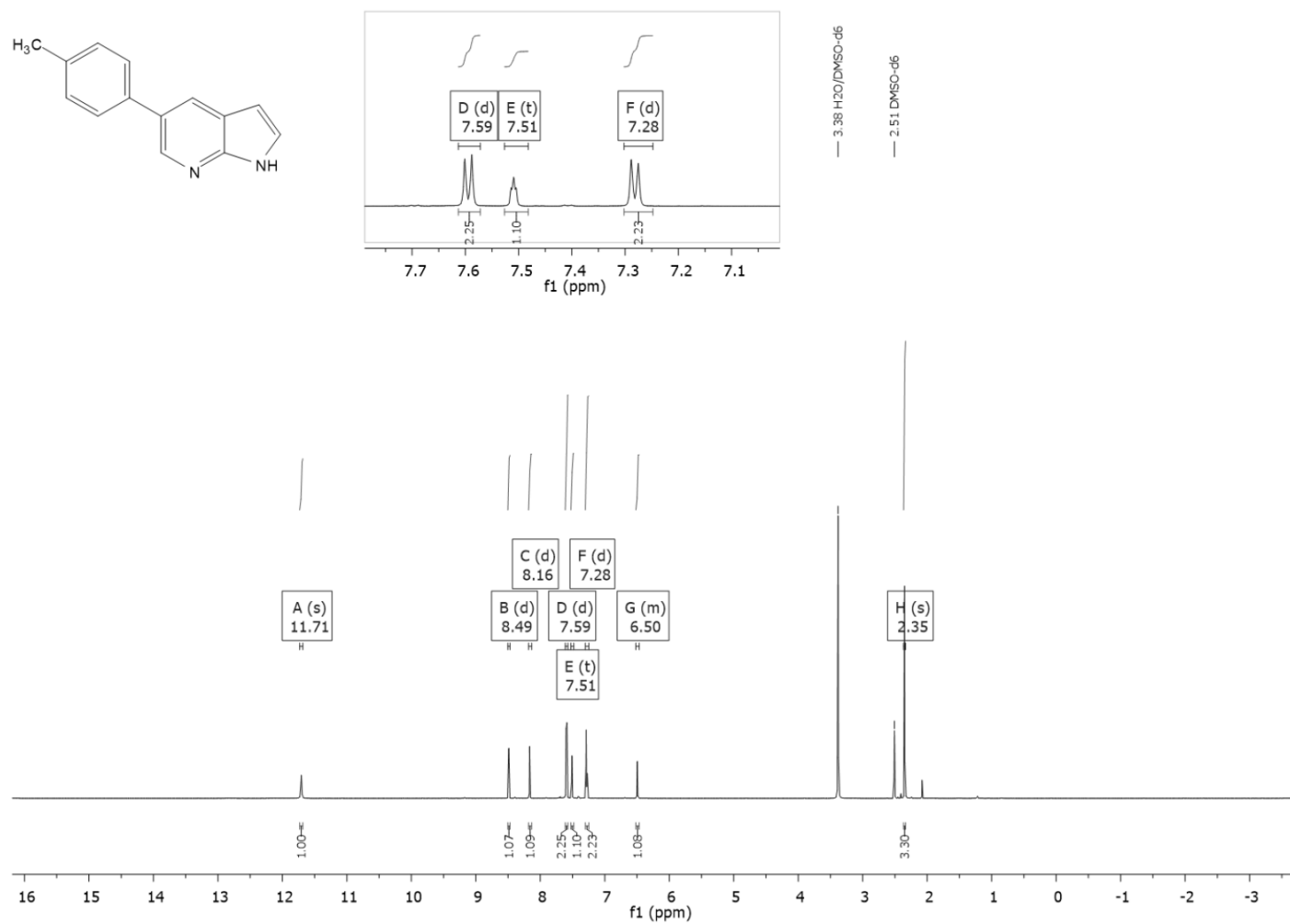
5-phenyl-1*H*-pyrrolo[2,3-*b*]pyridine (19a) (¹H):



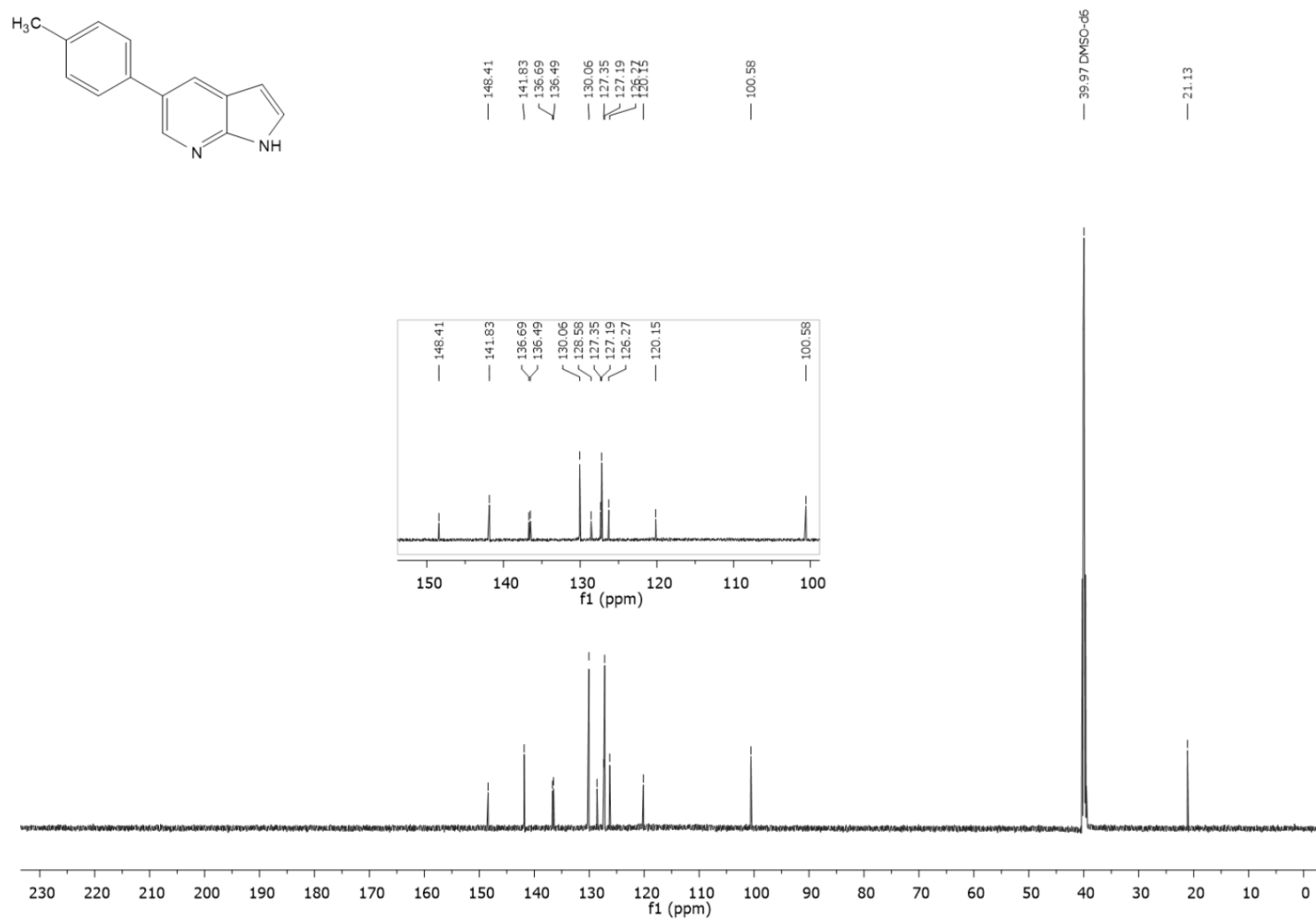
5-phenyl-1*H*-pyrrolo[2,3-*b*]pyridine (19a) (¹³C):



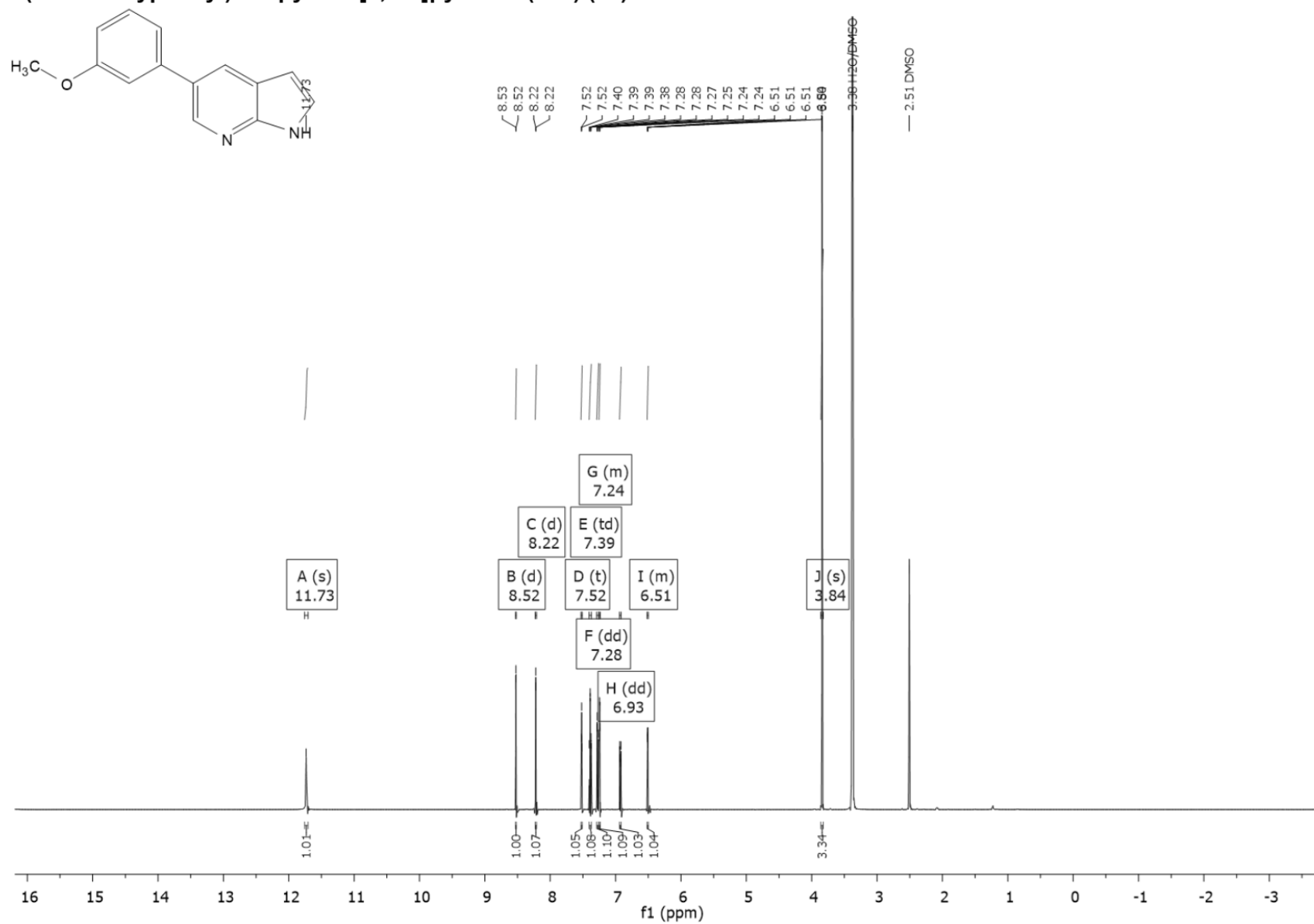
5-(4-methylphenyl)-1*H*-pyrrolo[2,3-*b*]pyridine (19b) (¹H): :



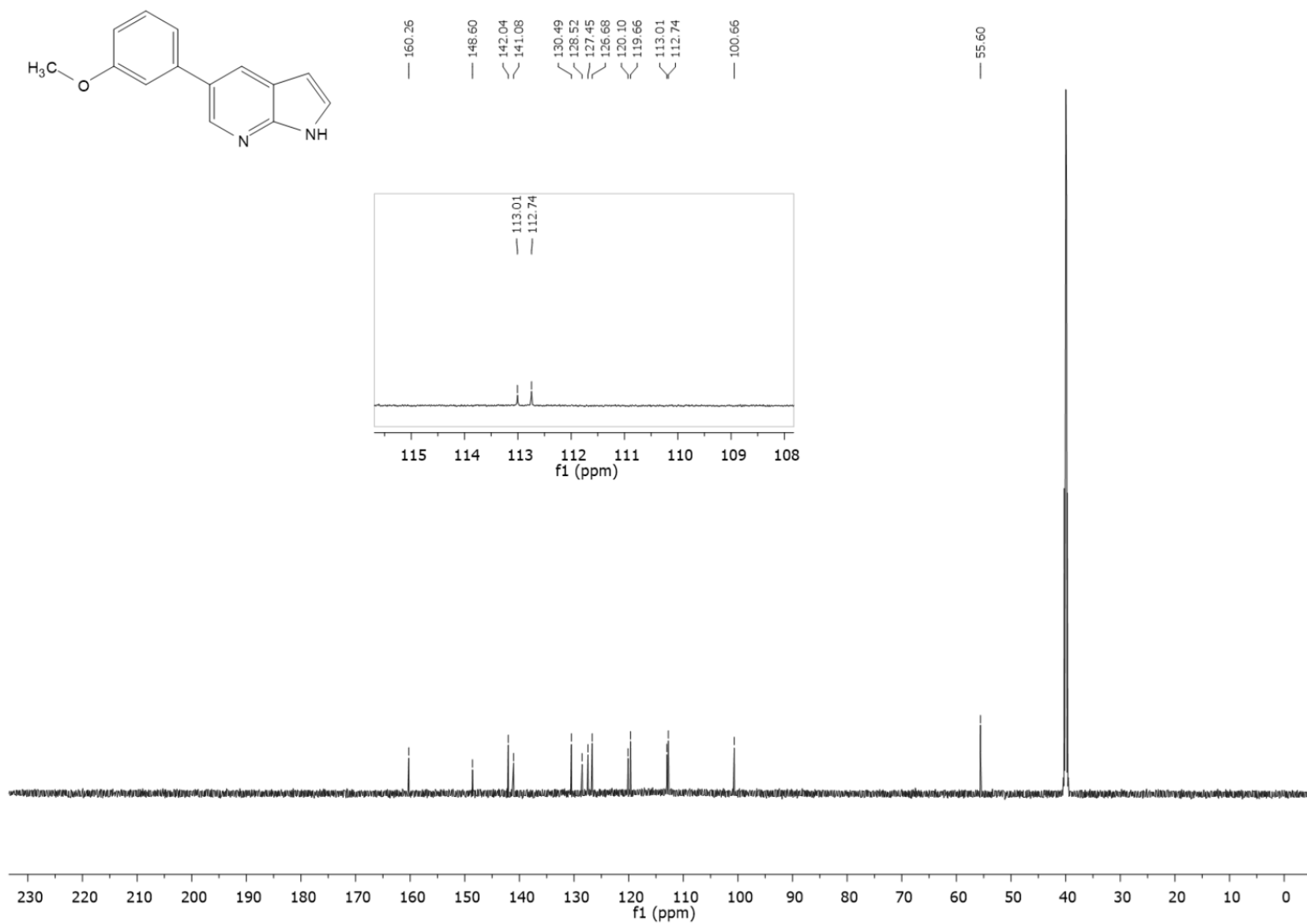
5-(4-methylphenyl)-1*H*-pyrrolo[2,3-*b*]pyridine (19b) (^{13}C):



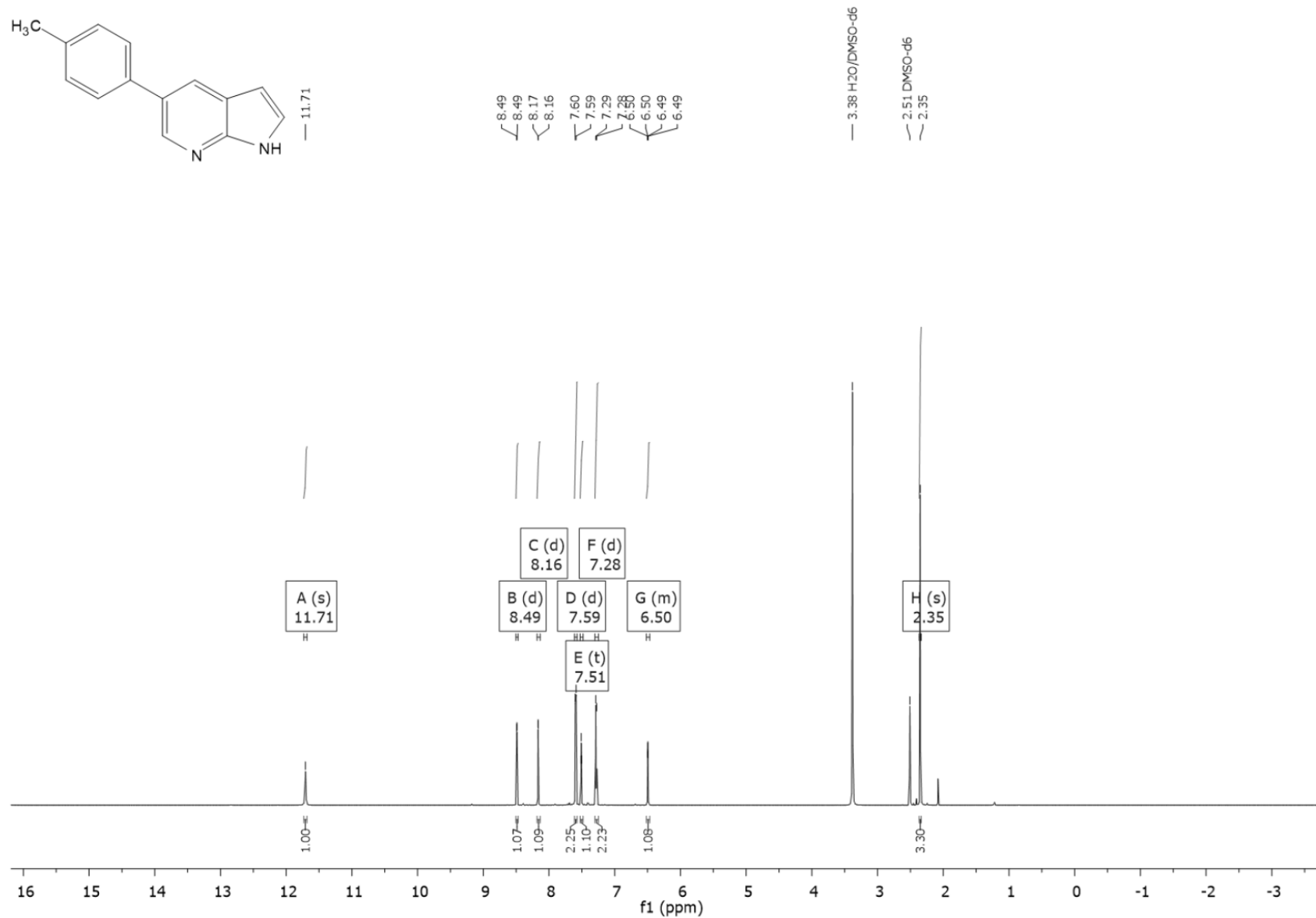
5-(3-methoxyphenyl)-1*H*-pyrrolo[2,3-*b*]pyridine (19c) (¹H):



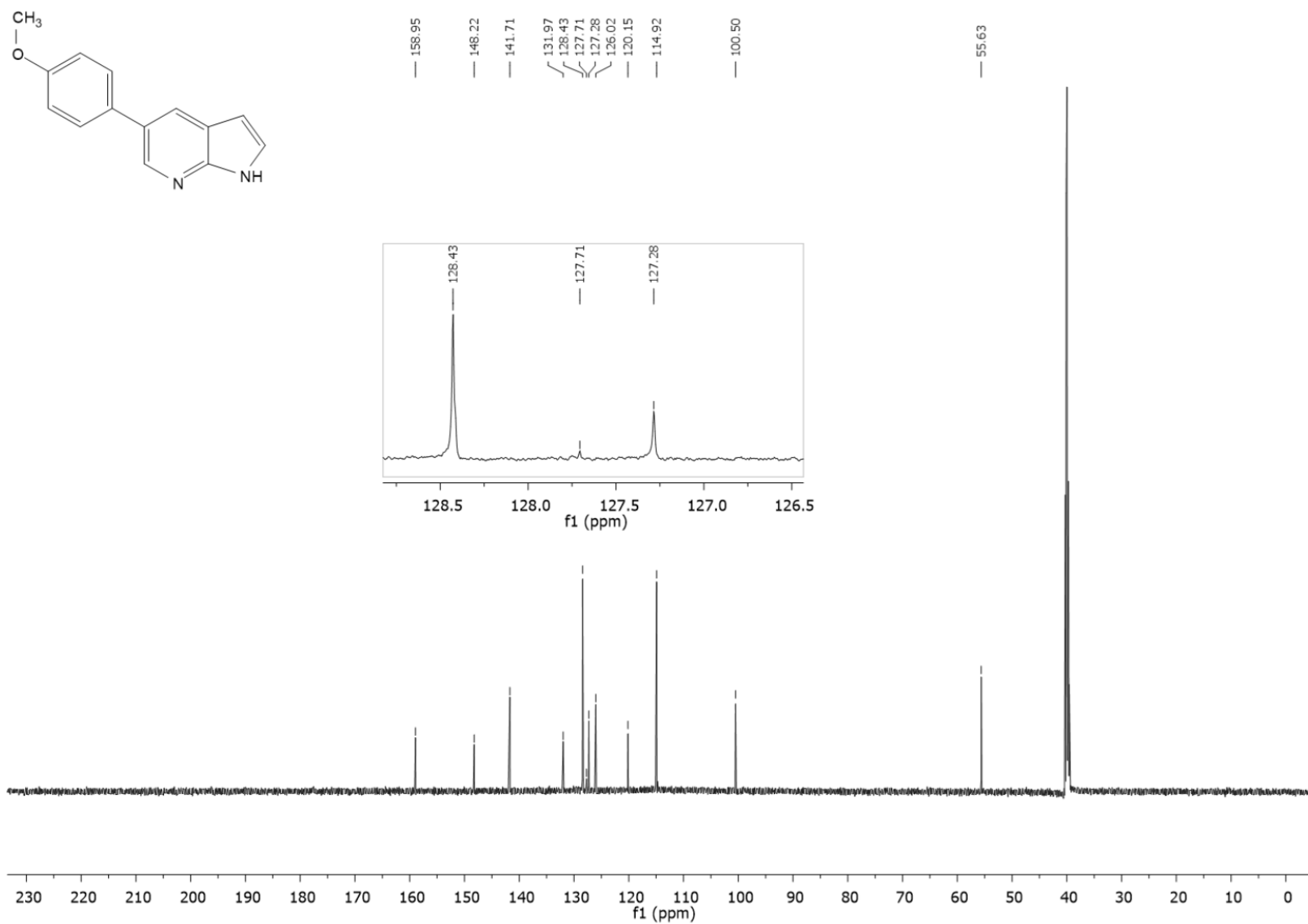
5-(3-methoxyphenyl)-1*H*-pyrrolo[2,3-*b*]pyridine (19c) (¹³C):



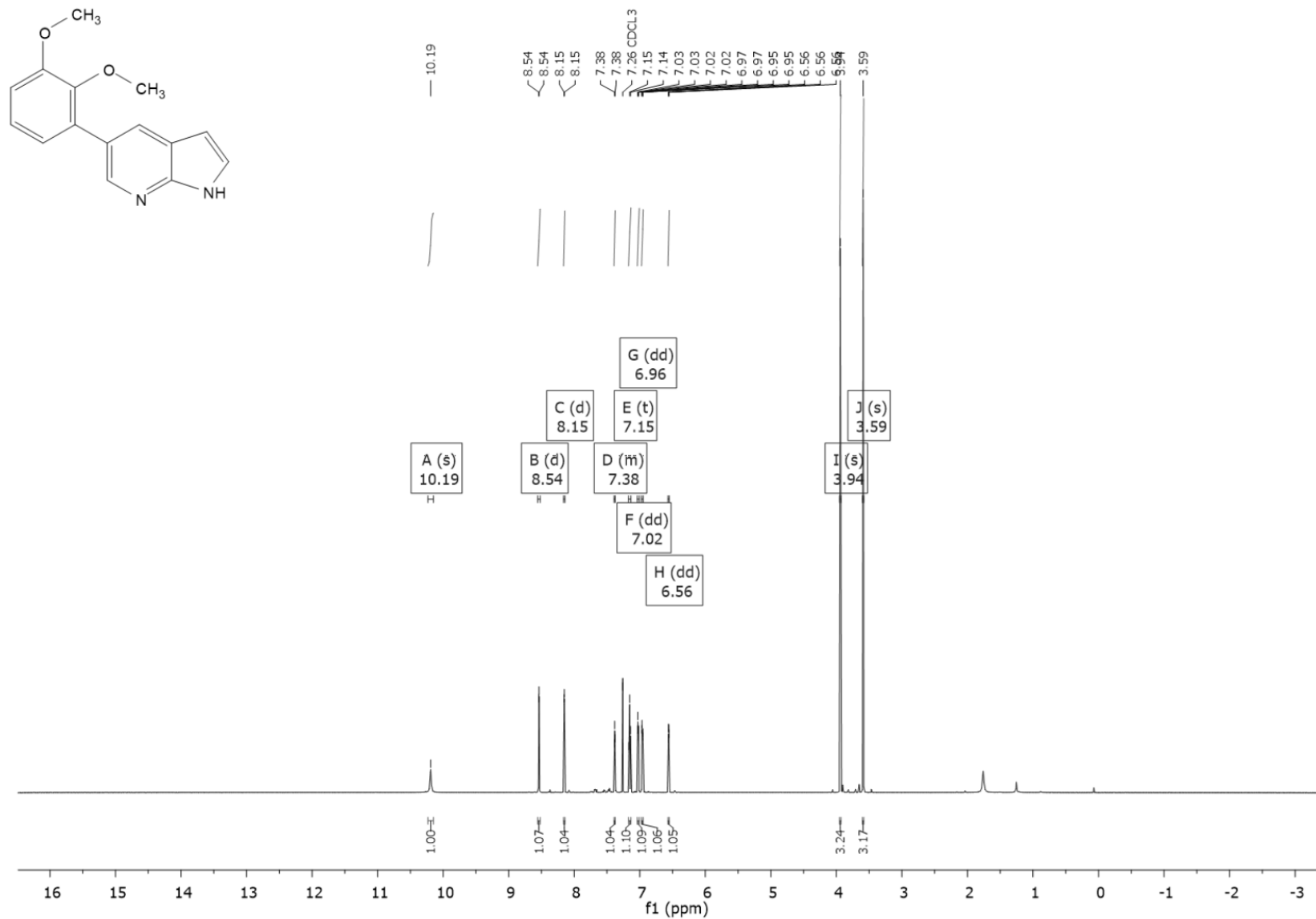
5-(4-methoxyphenyl)-1*H*-pyrrolo[2,3-*b*]pyridine (19d) (¹H):



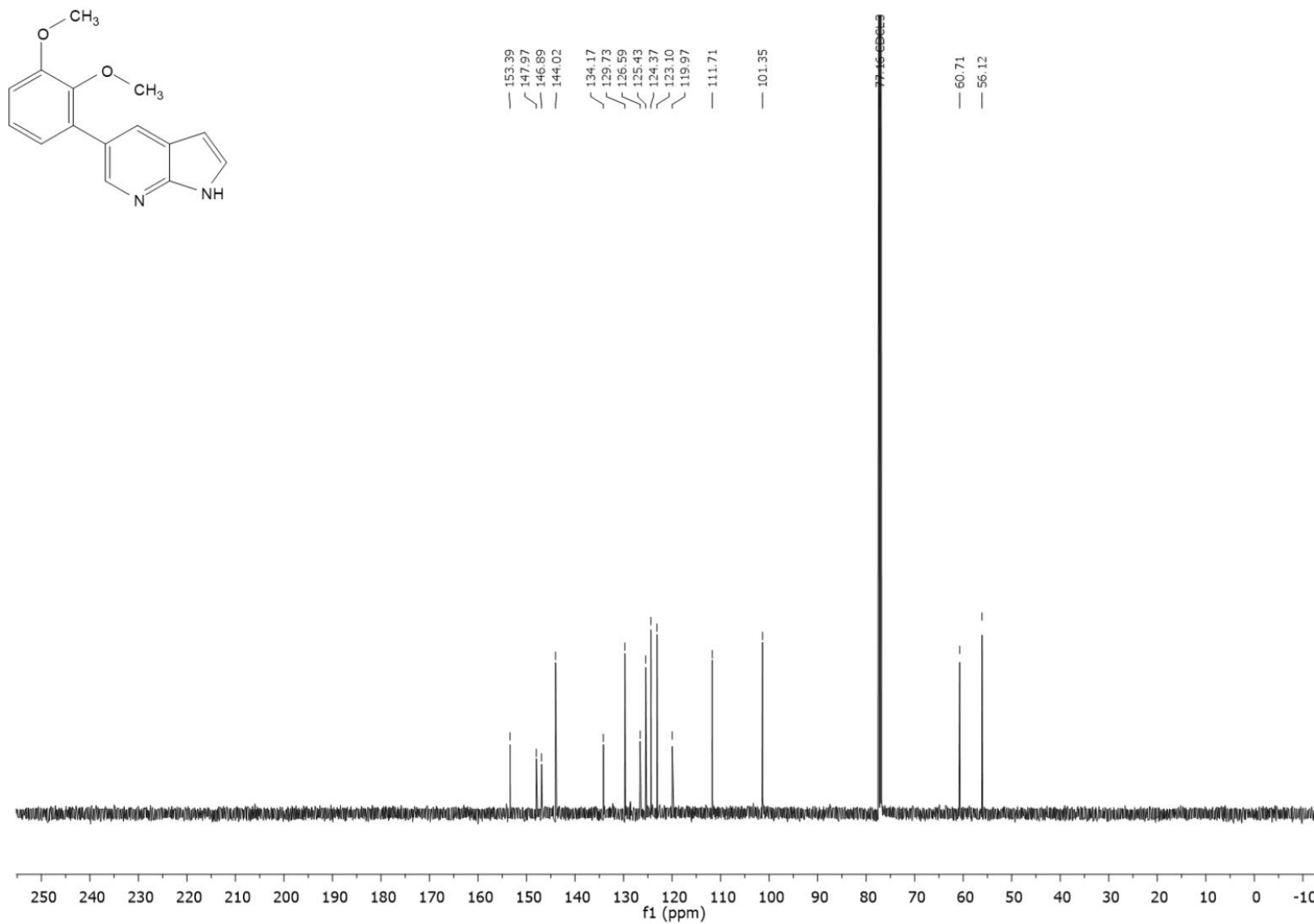
5-(4-methoxyphenyl)-1*H*-pyrrolo[2,3-*b*]pyridine (19d) (¹³C):



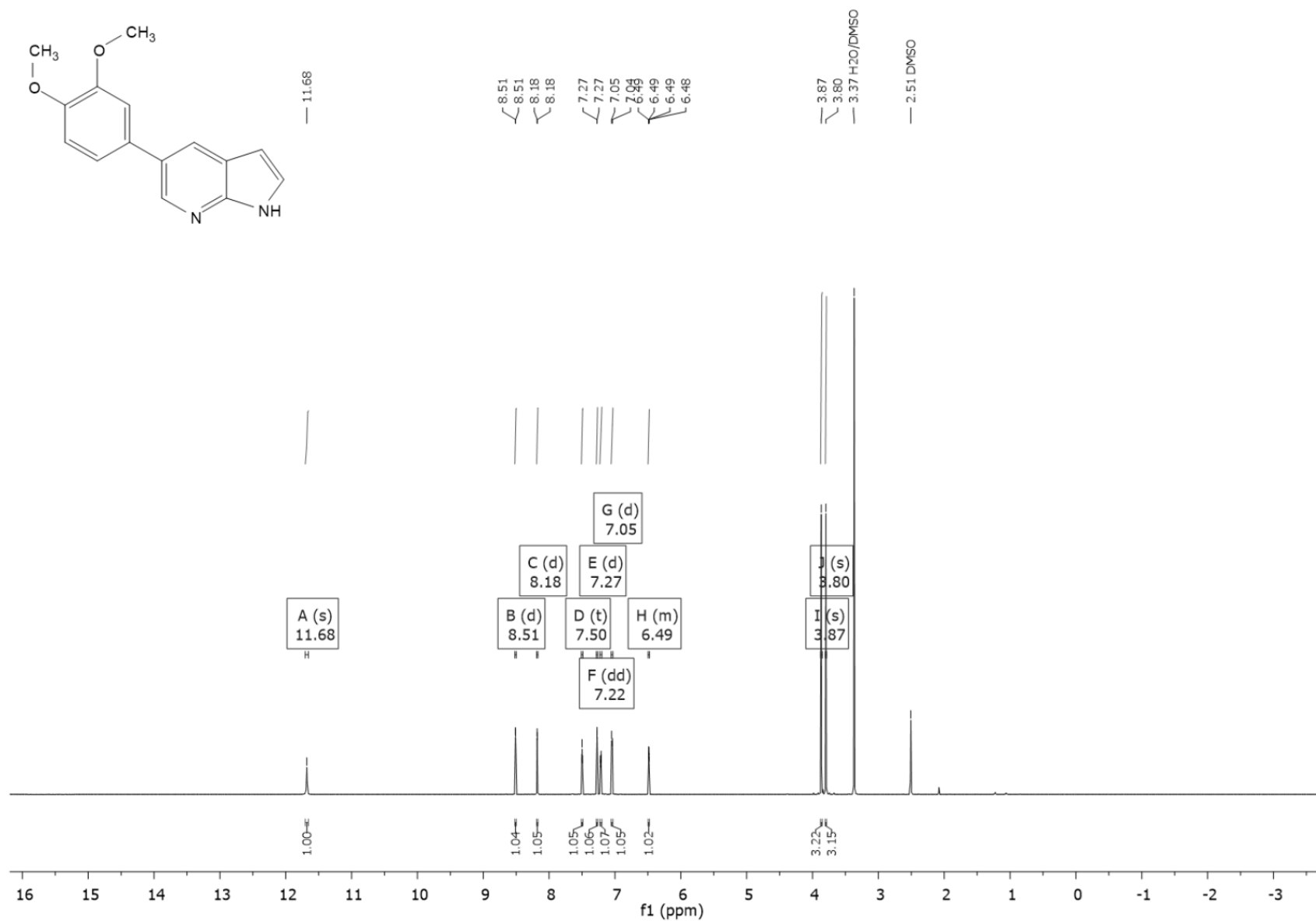
5-(2,3-dimethoxyphenyl)-1*H*-pyrrolo[2,3-*b*]pyridine (19e) (¹H):



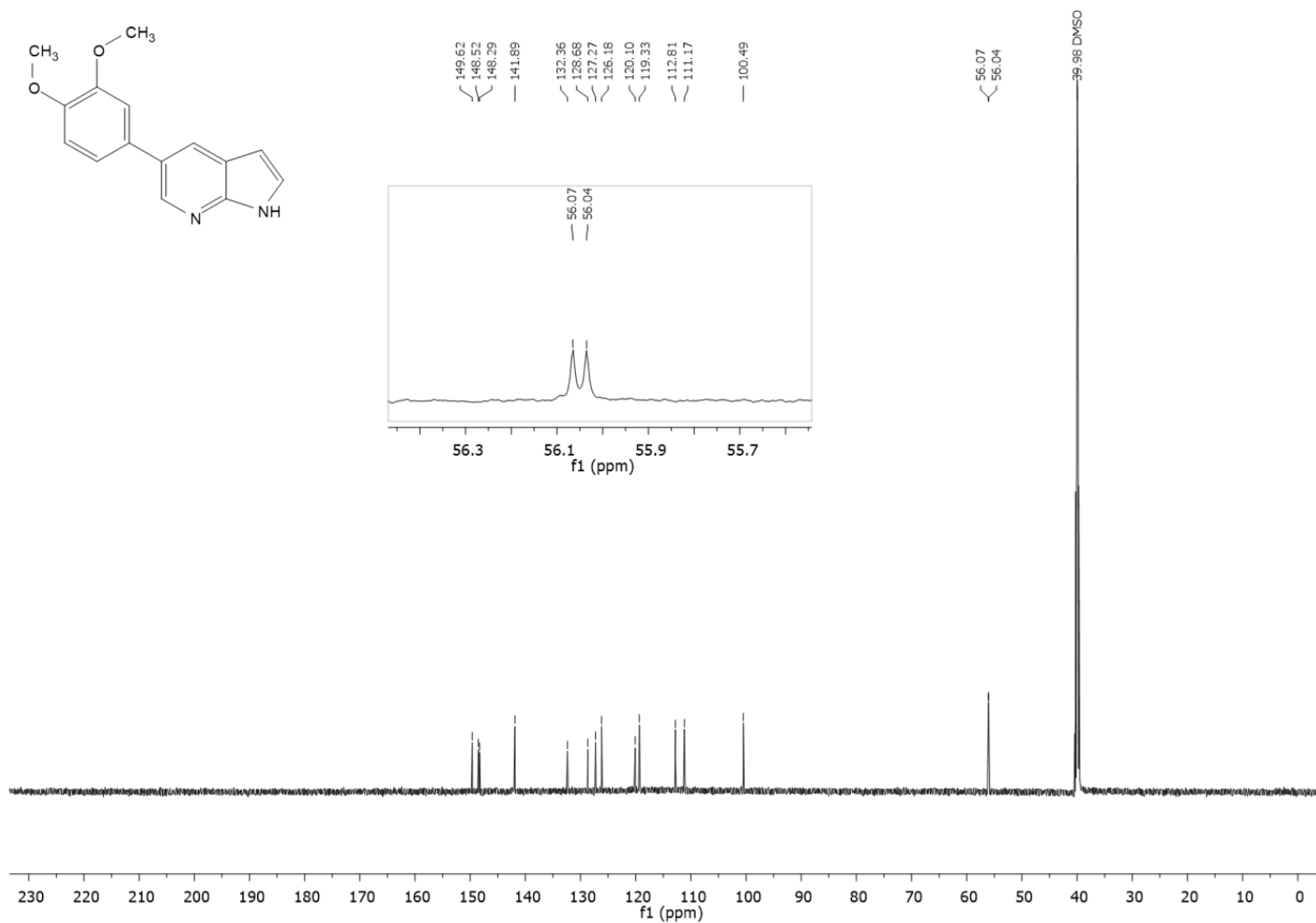
5-(2,3-dimethoxyphenyl)-1*H*-pyrrolo[2,3-*b*]pyridine (19e) (¹³C):



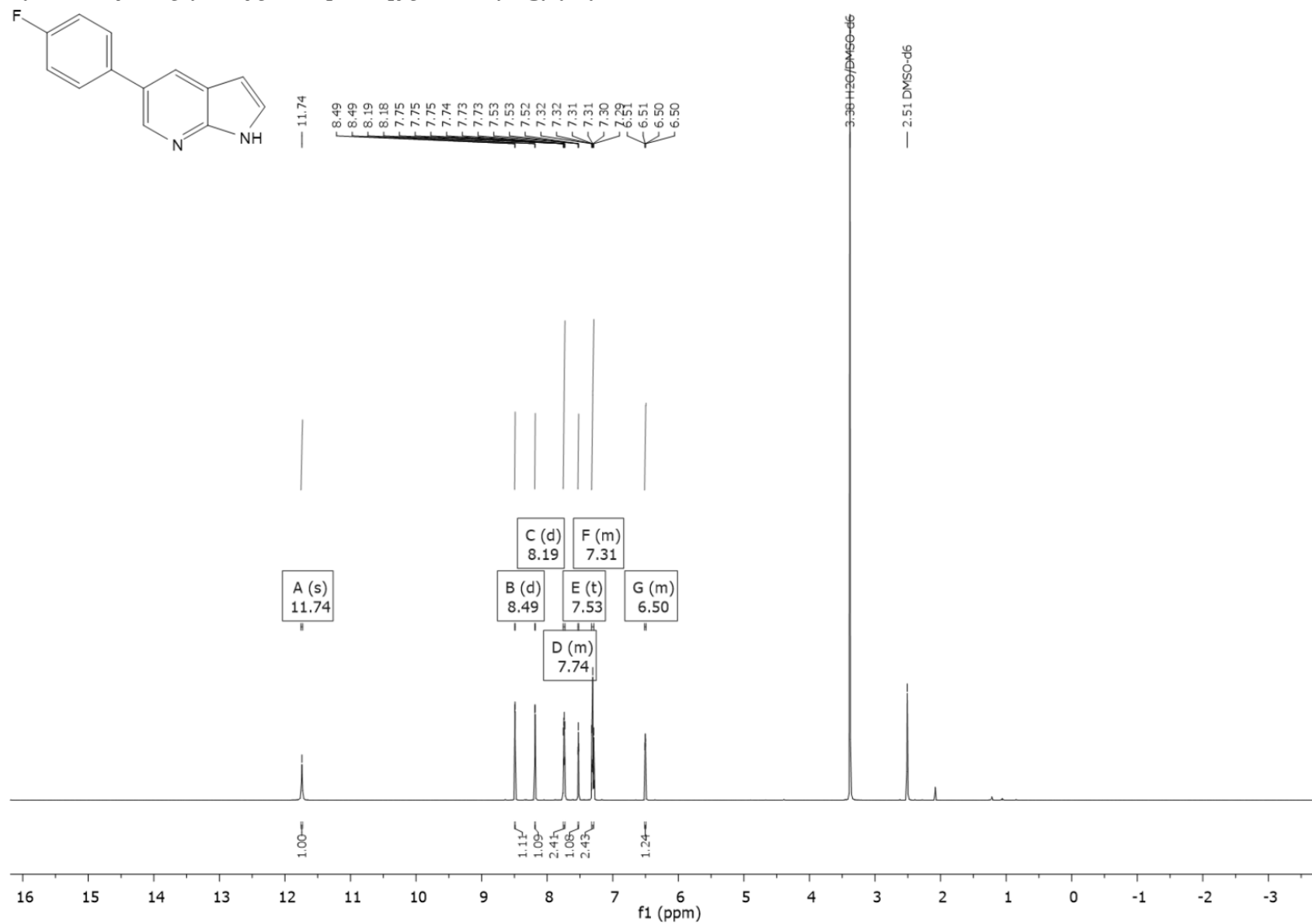
5-(3,4-dimethoxyphenyl)-1*H*-pyrrolo[2,3-*b*]pyridine (19f) (¹H):



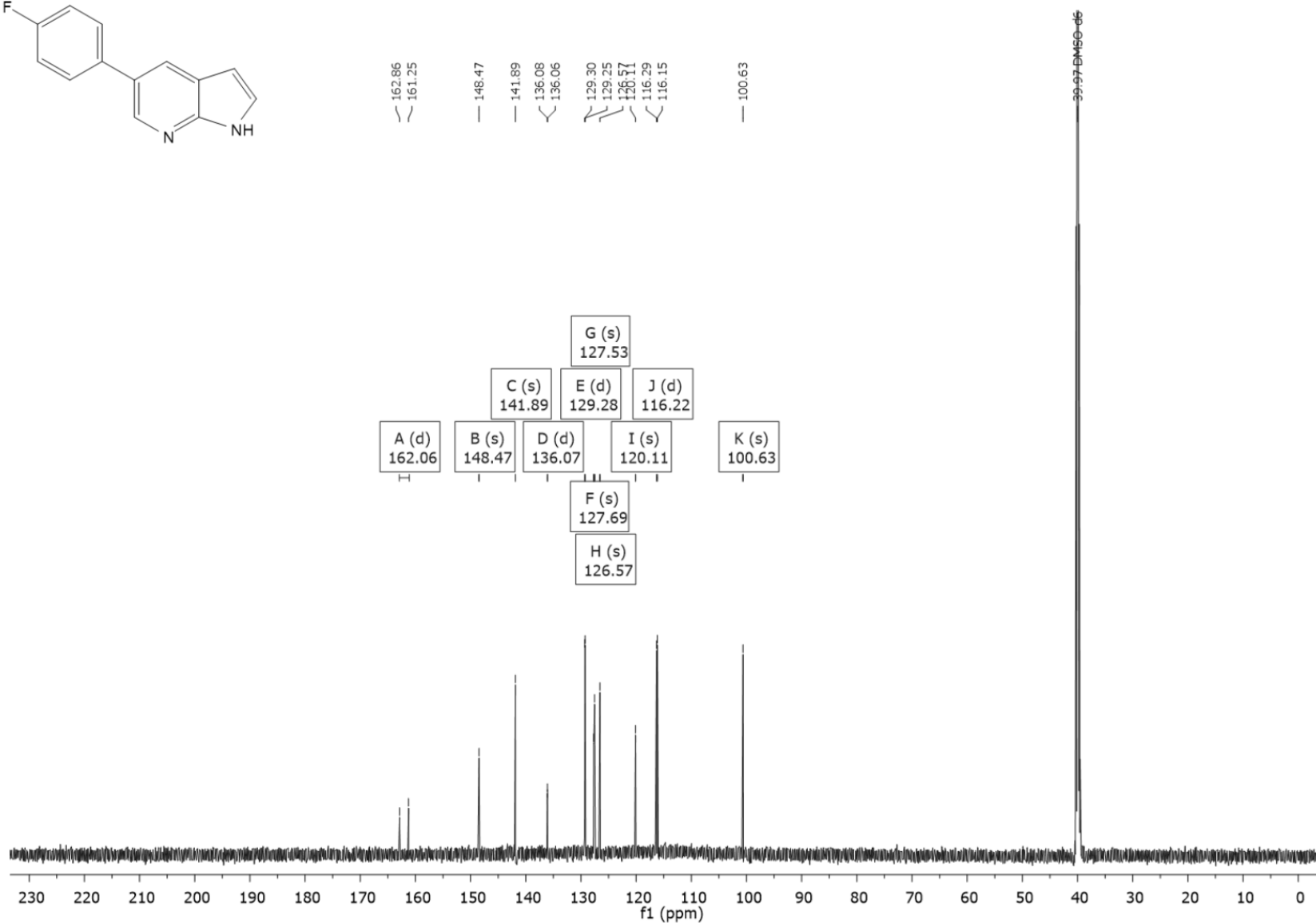
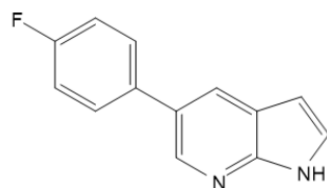
5-(3,4-dimethoxyphenyl)-1*H*-pyrrolo[2,3-*b*]pyridine (19f) (¹³C):



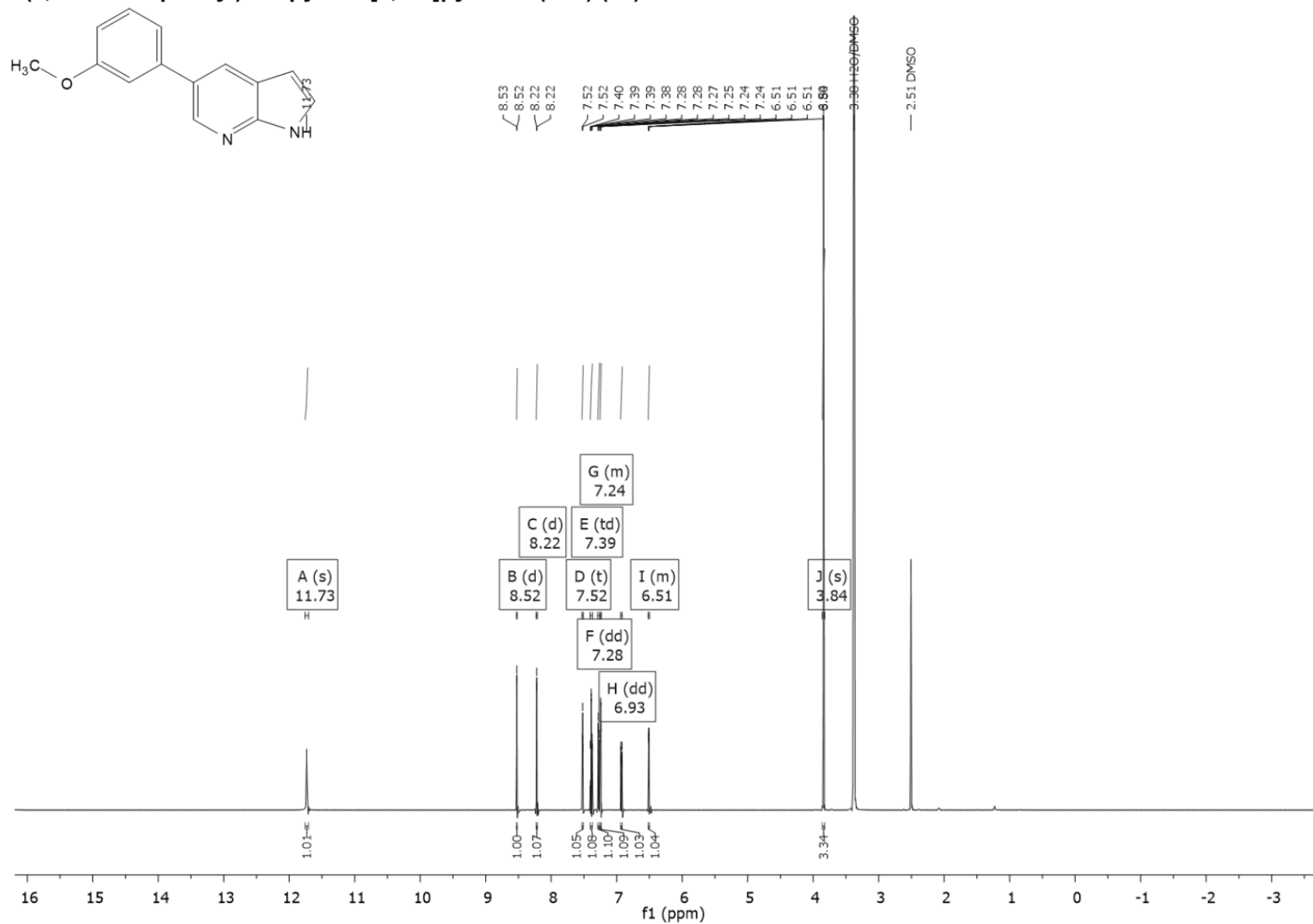
5-(4-fluorophenyl)-1*H*-pyrrolo[2,3-*b*]pyridine (19g) (¹H):



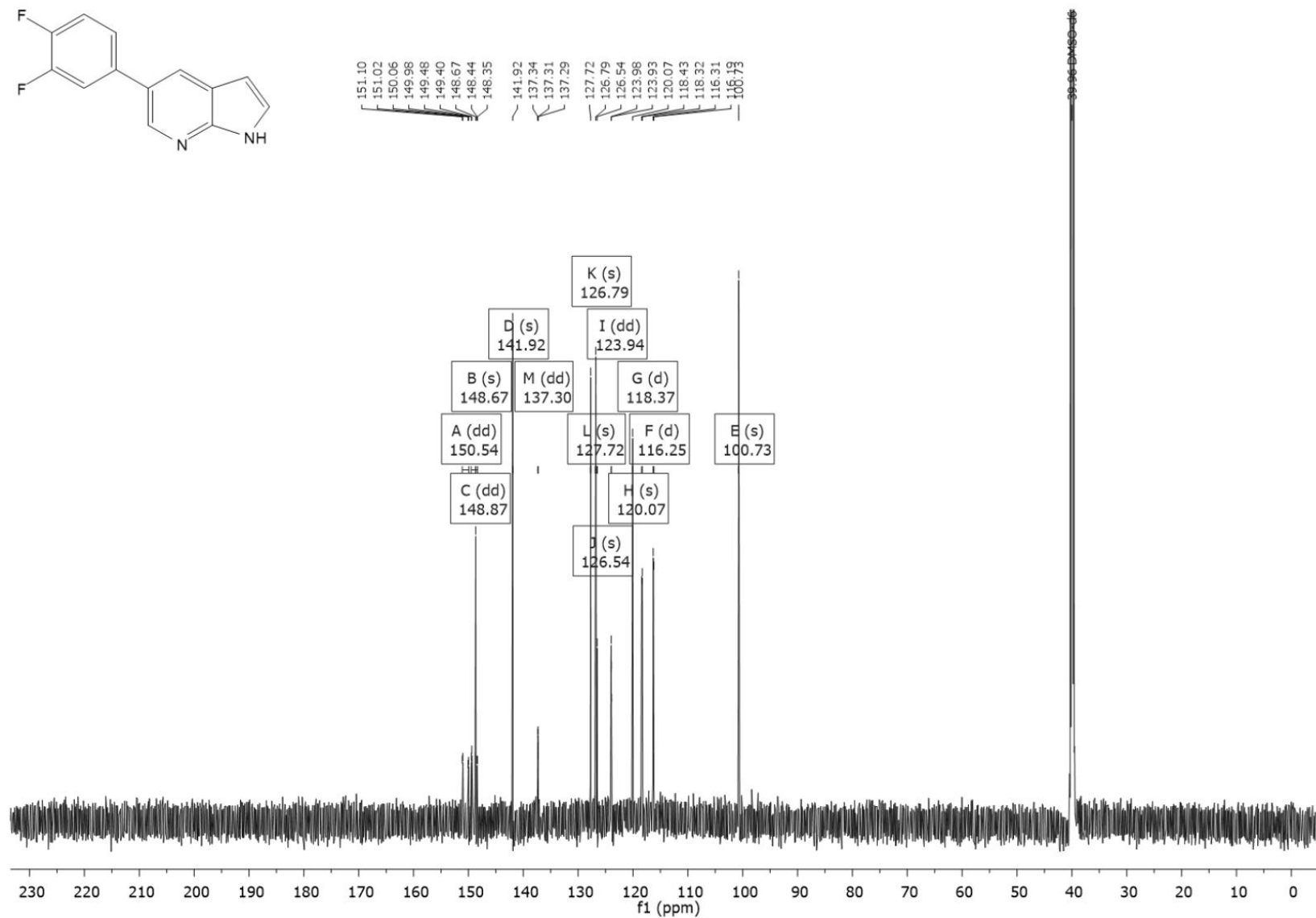
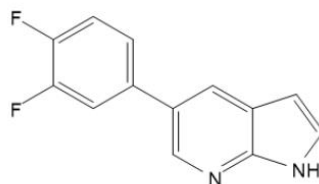
5-(4-fluorophenyl)-1*H*-pyrrolo[2,3-*b*]pyridine (19g) (¹³C):



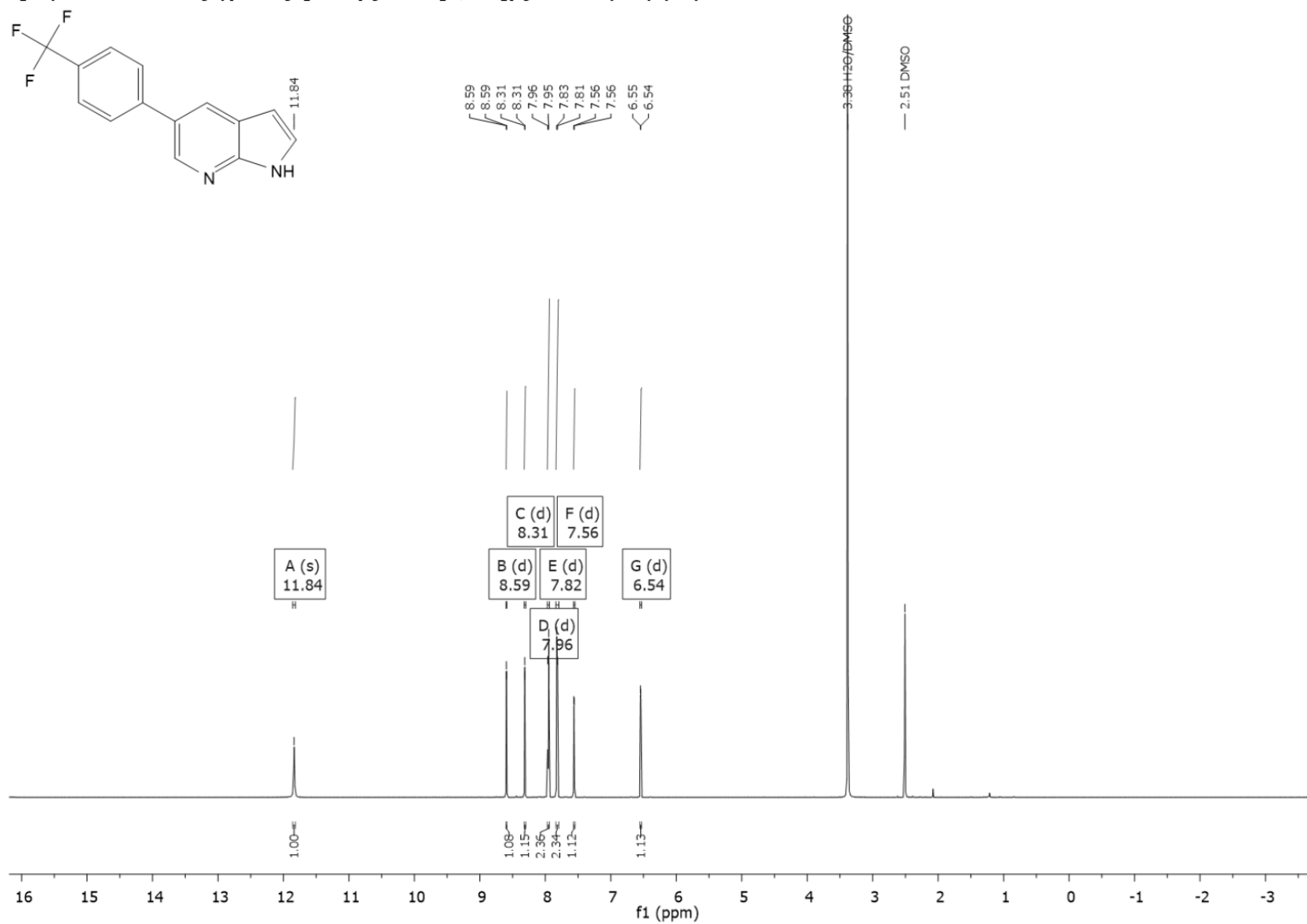
5-(3,4-difluorophenyl)-1*H*-pyrrolo[2,3-*b*]pyridine (19h) (¹H):



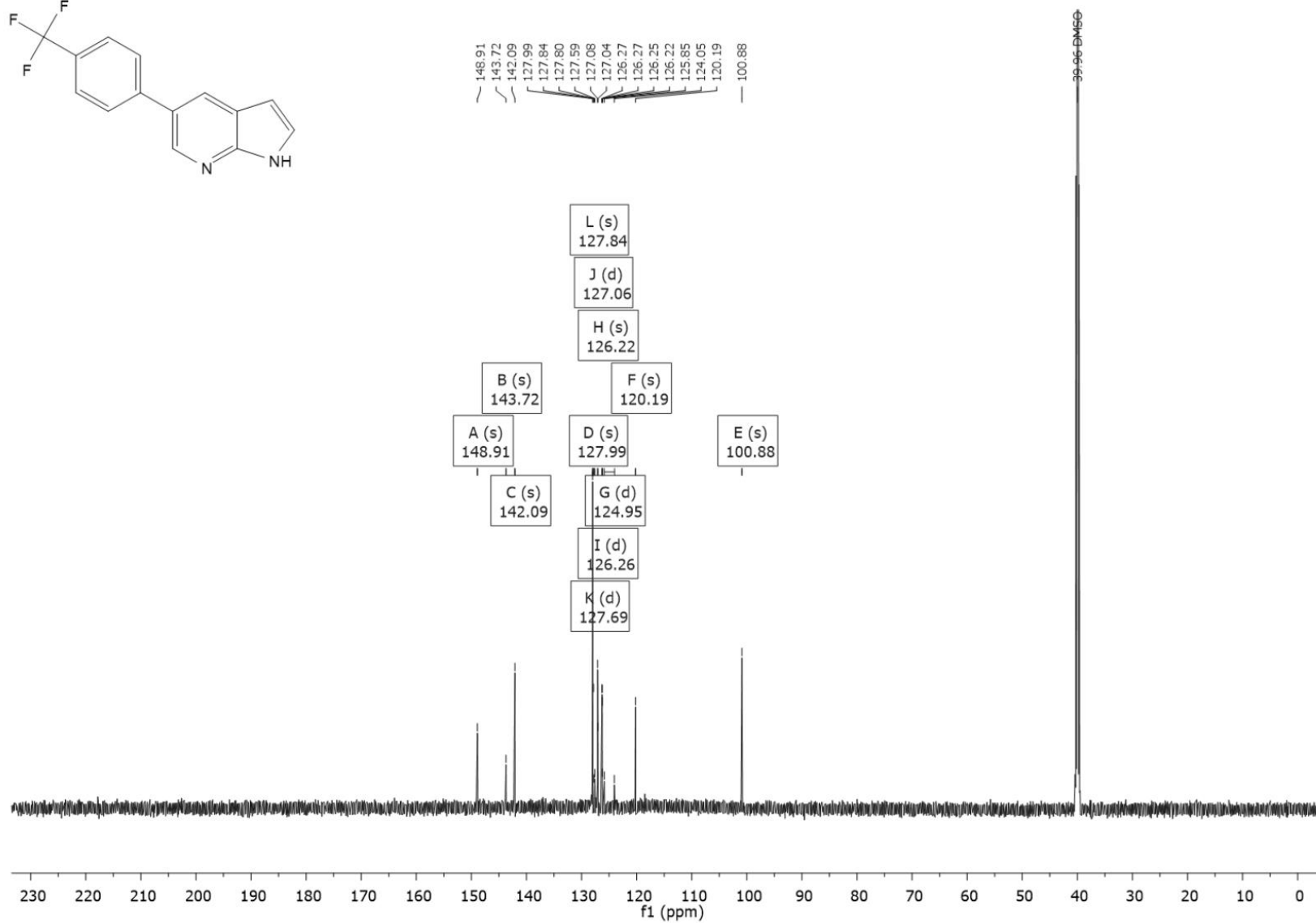
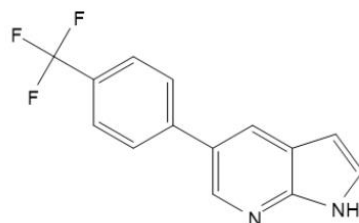
5-(3,4-difluorophenyl)-1*H*-pyrrolo[2,3-*b*]pyridine (19h) (¹³C):



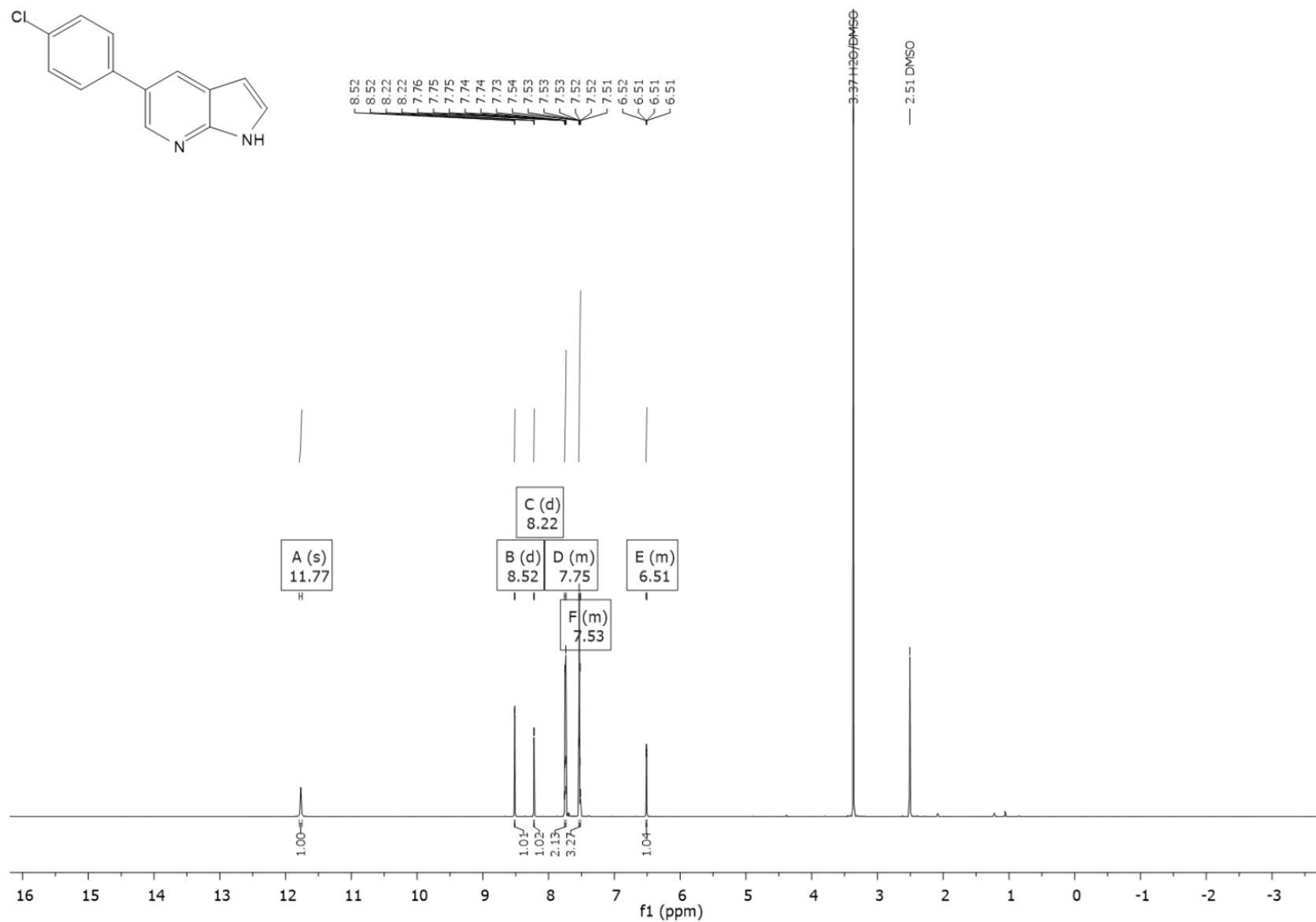
5-[4-(trifluoromethyl)phenyl]-1*H*-pyrrolo[2,3-*b*]pyridine (19i) (¹H):



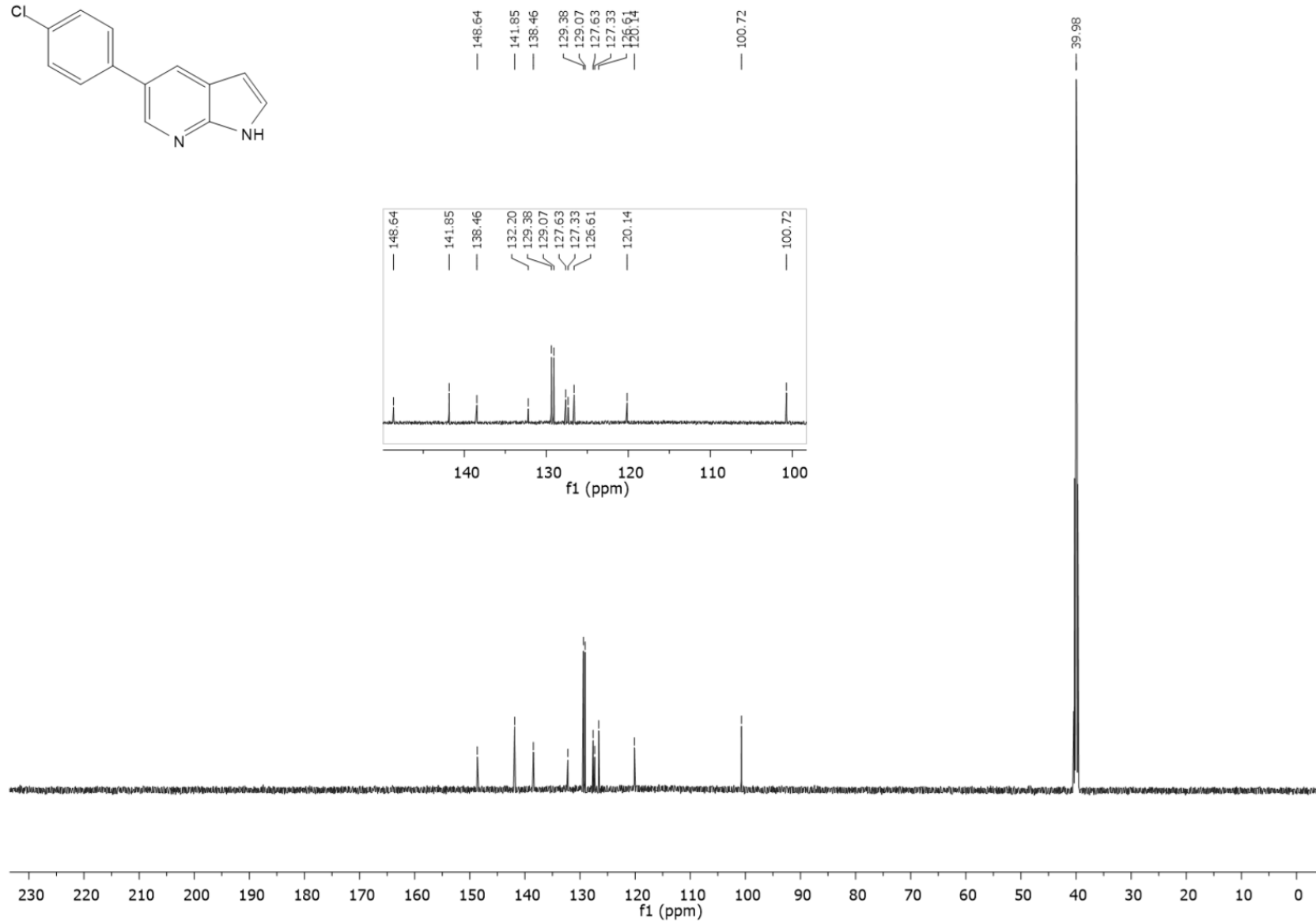
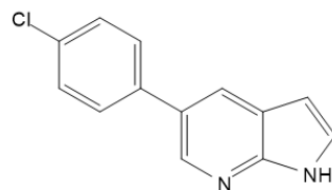
5-[4-(trifluoromethyl)phenyl]-1*H*-pyrrolo[2,3-*b*]pyridine (19i) (¹³C):



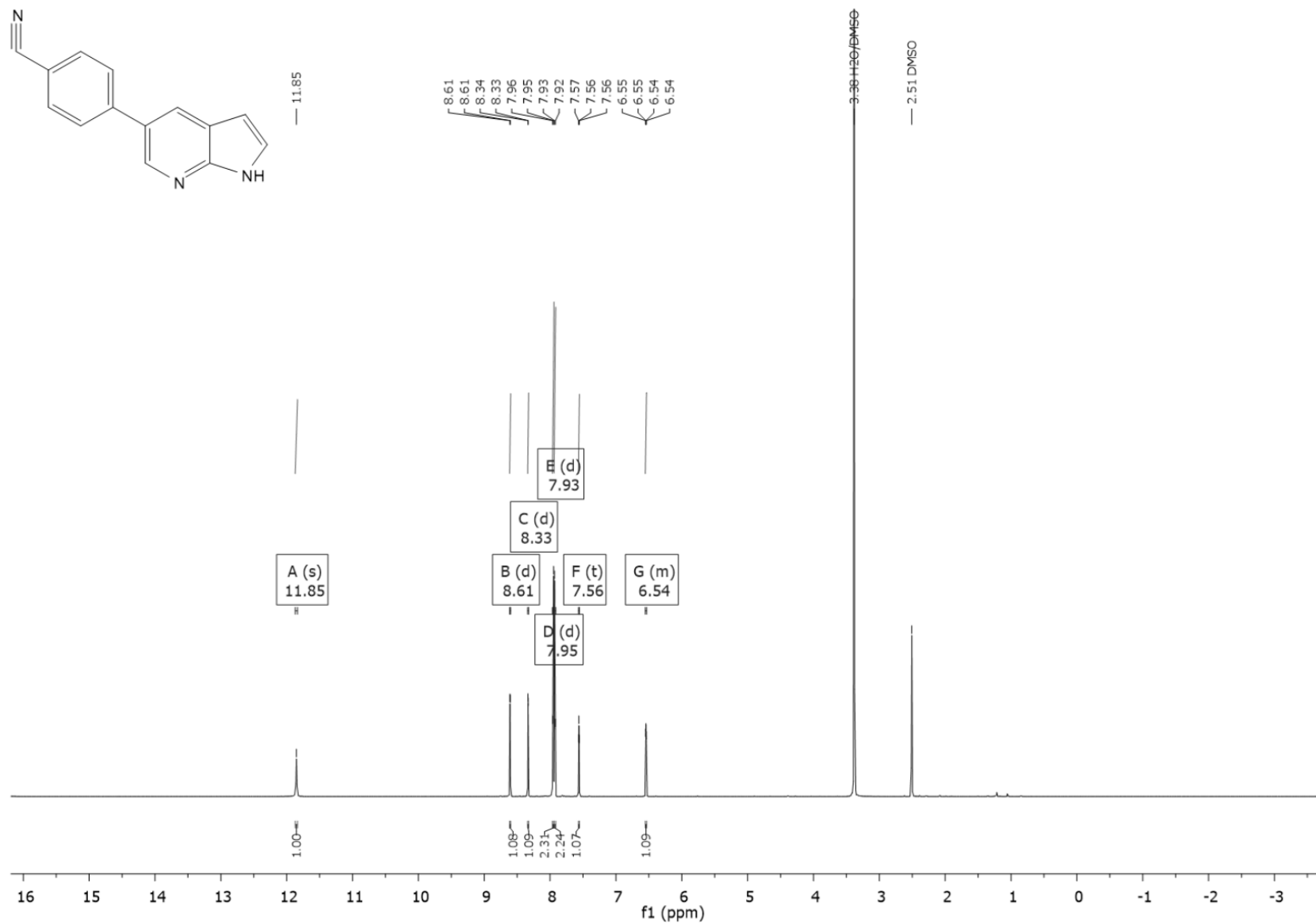
5-(4-chlorophenyl)-1*H*-pyrrolo[2,3-*b*]pyridine (19j) (¹H):



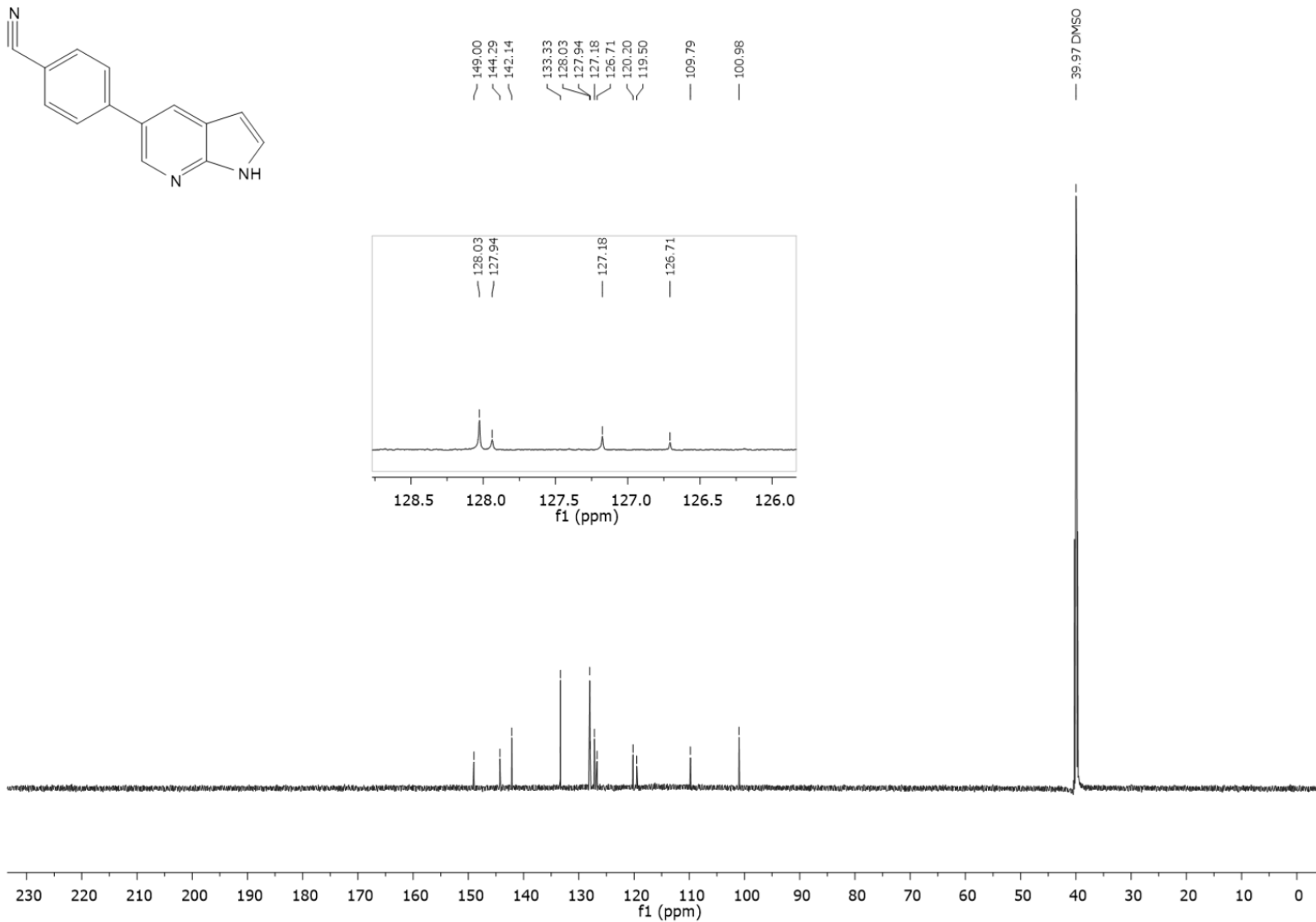
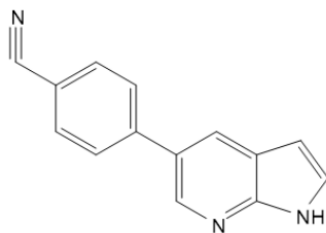
5-(4-chlorophenyl)-1*H*-pyrrolo[2,3-*b*]pyridine (19j) (^{13}C):



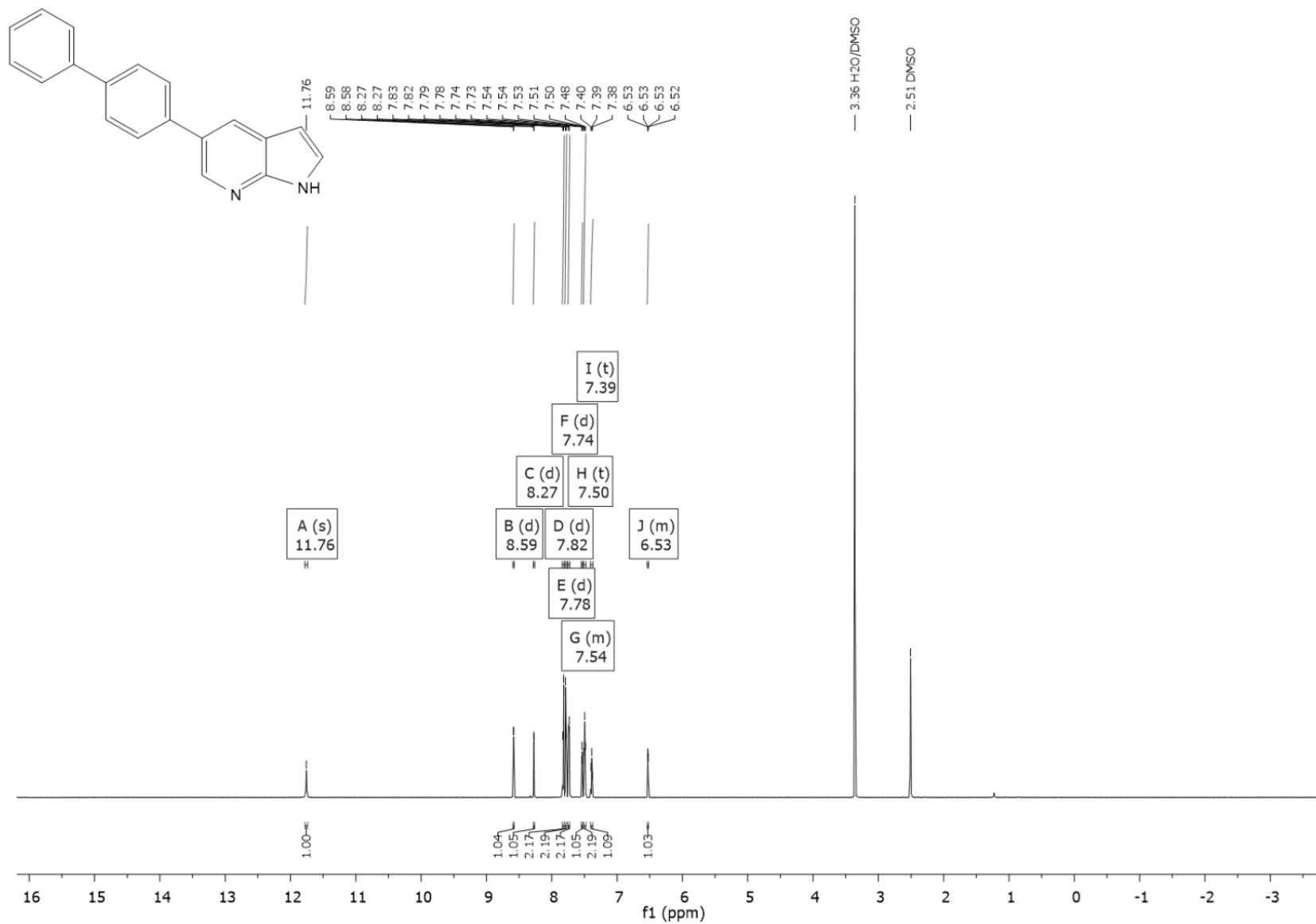
4-(1*H*-pyrrolo[2,3-*b*]pyridin-5-yl)benzonitrile (19k) (¹H):



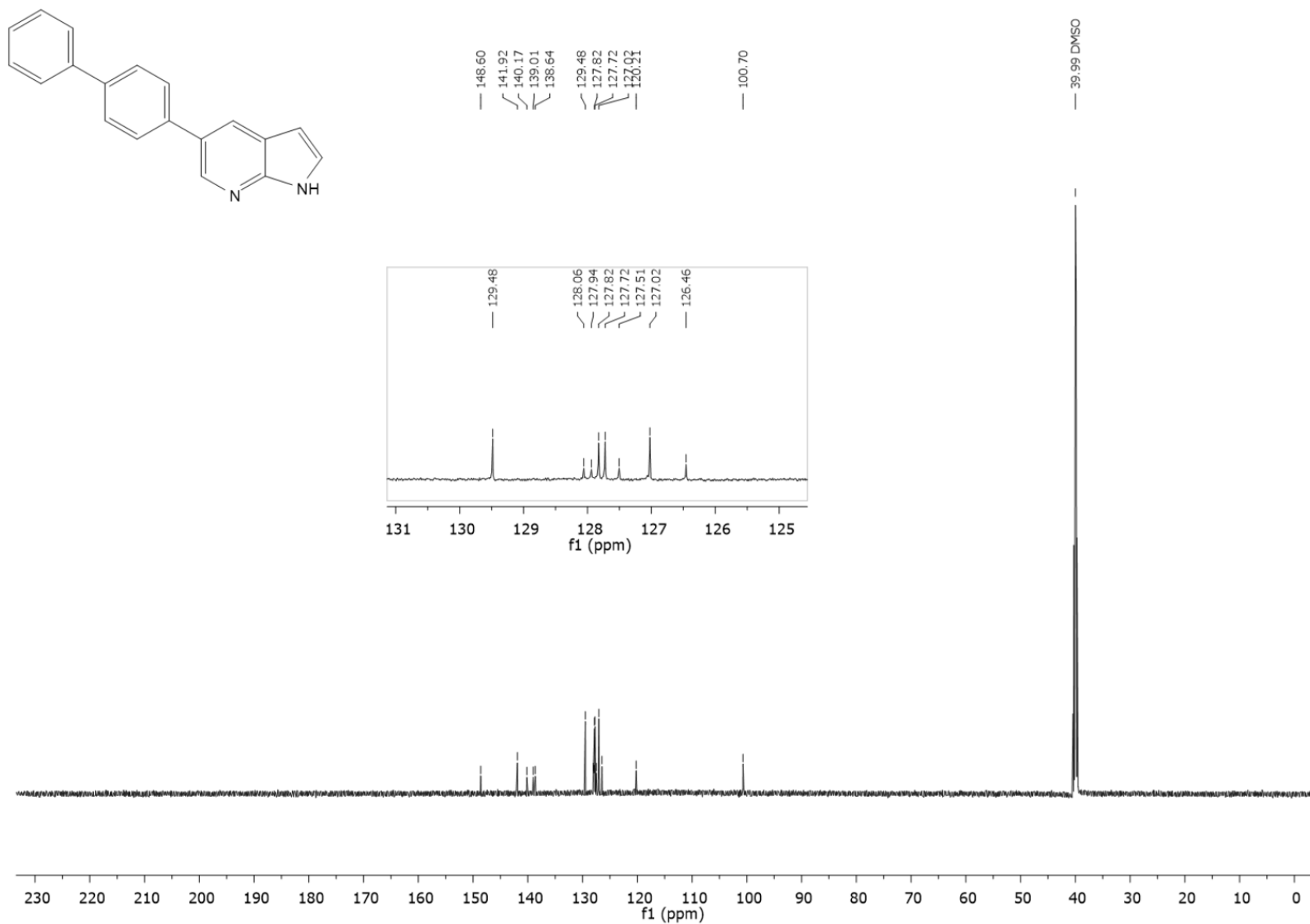
4-(1*H*-pyrrolo[2,3-*b*]pyridin-5-yl)benzonitrile (19k) (^{13}C):



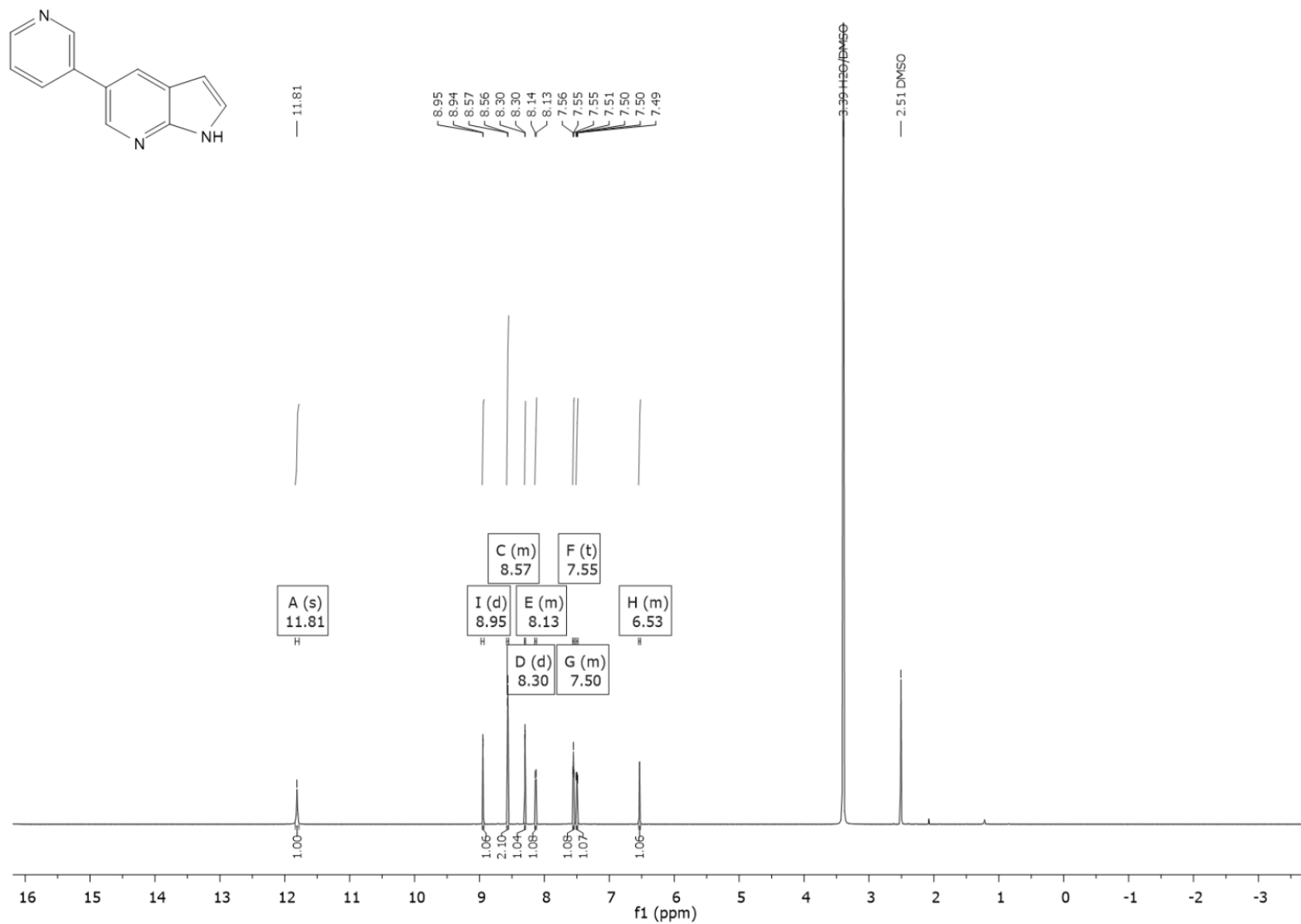
5-(biphenyl-4-yl)-1*H*-pyrrolo[2,3-*b*]pyridine (19l) (¹H):



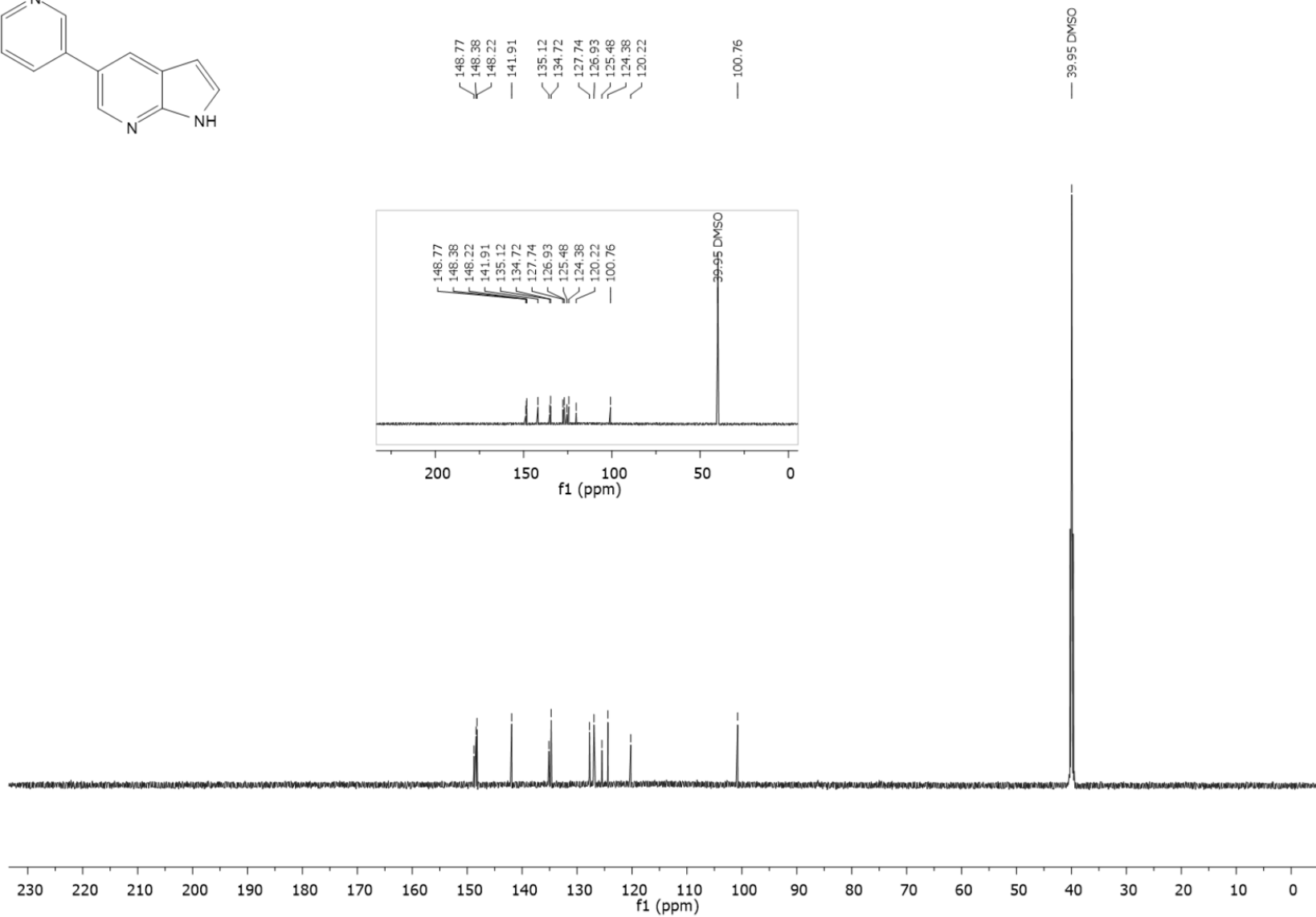
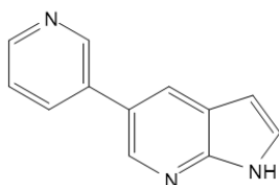
5-(biphenyl-4-yl)-1*H*-pyrrolo[2,3-*b*]pyridine (19l) (¹³C):



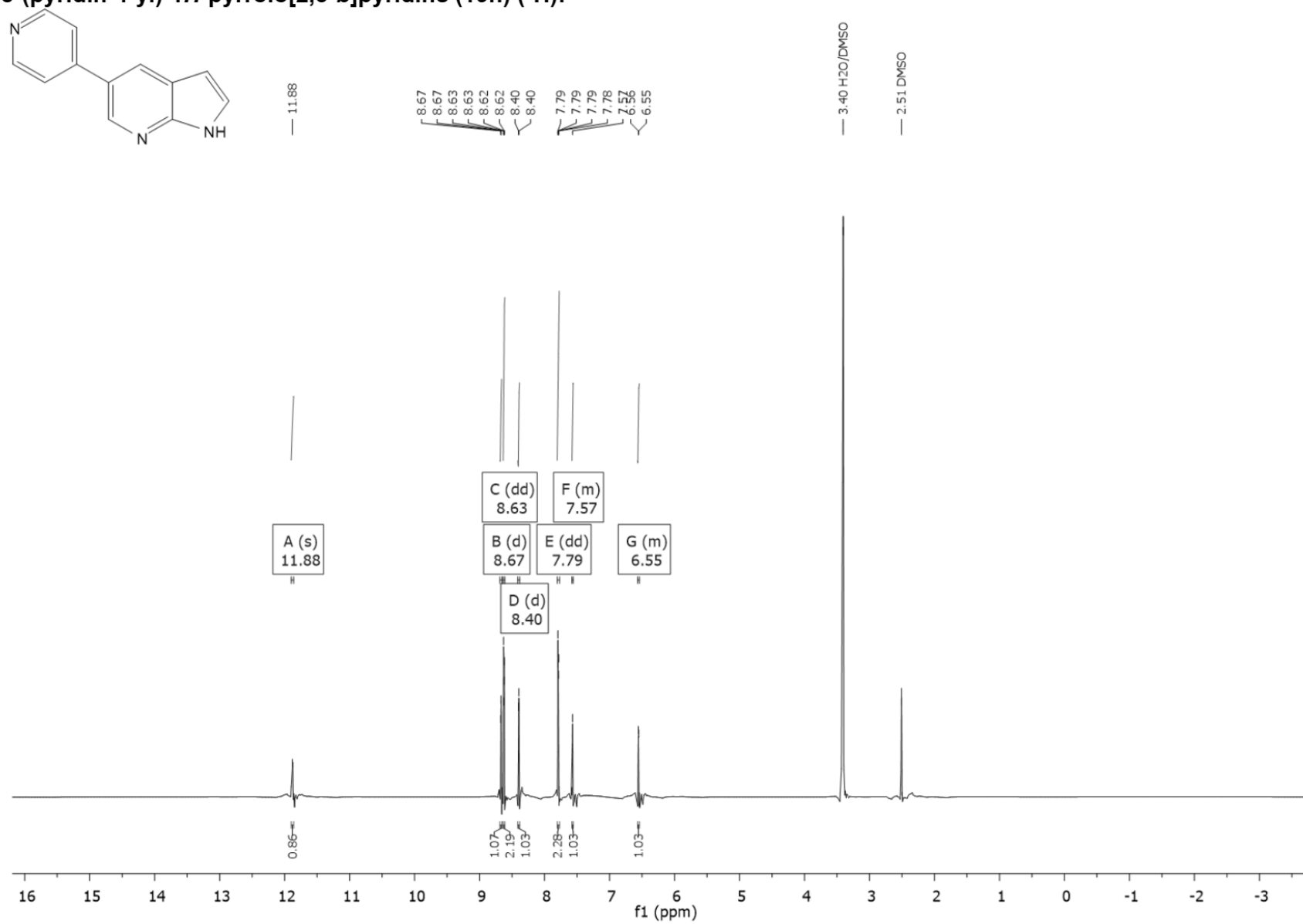
5-(pyridin-3-yl)-1*H*-pyrrolo[2,3-*b*]pyridine (19m) (¹H):



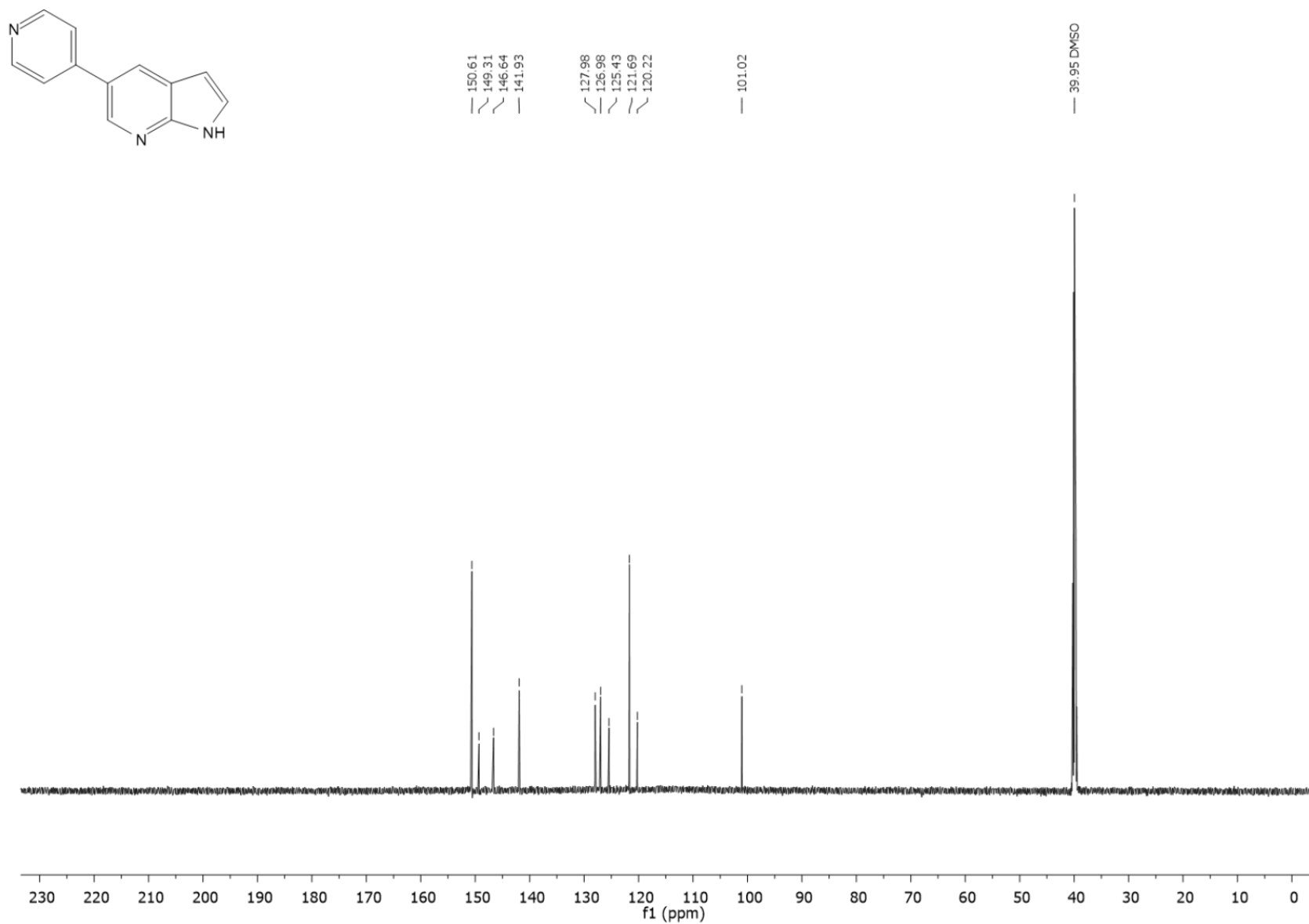
5-(pyridin-3-yl)-1*H*-pyrrolo[2,3-*b*]pyridine (19m) (¹³C):



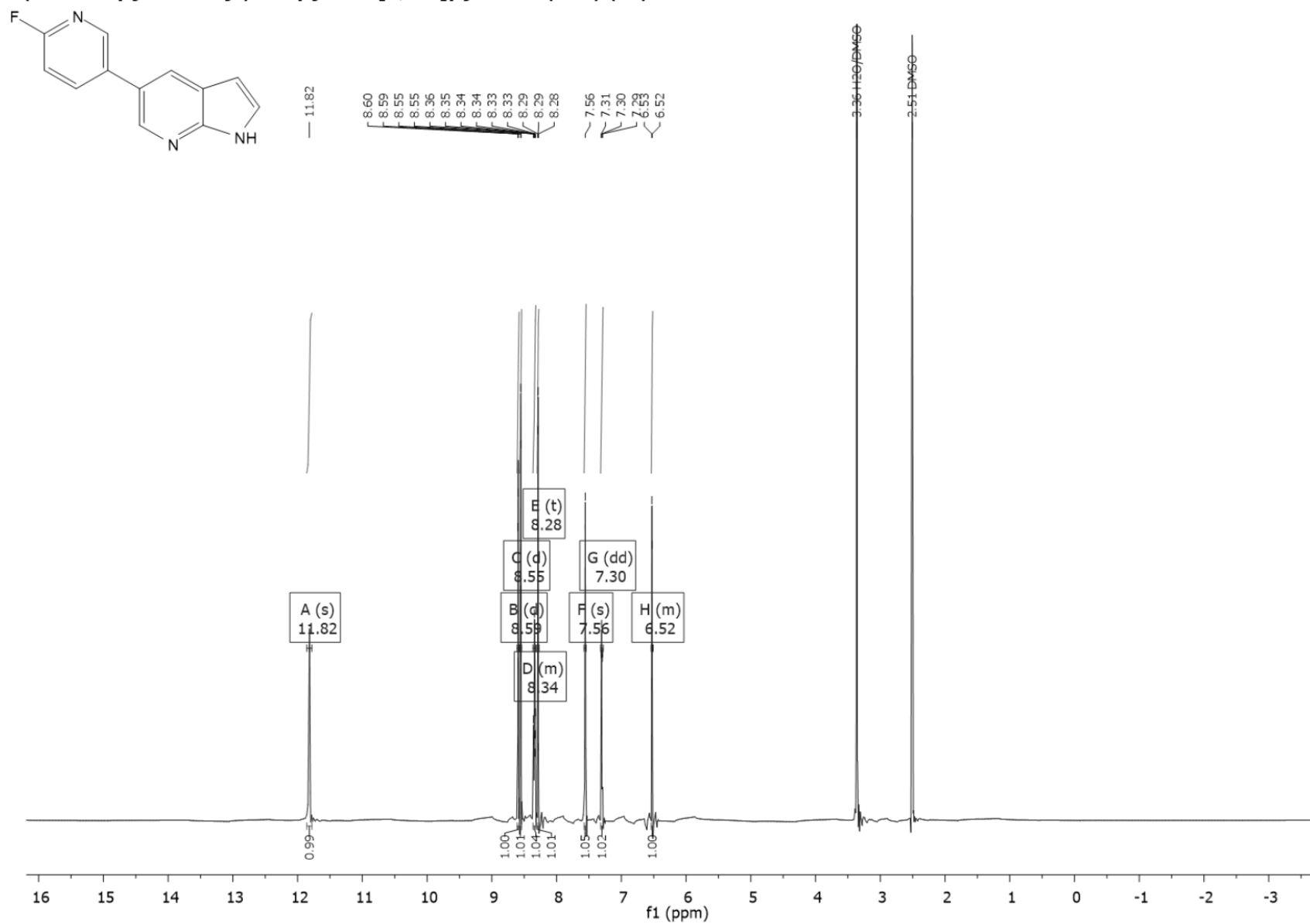
5-(pyridin-4-yl)-1*H*-pyrrolo[2,3-*b*]pyridine (19n) (¹H):



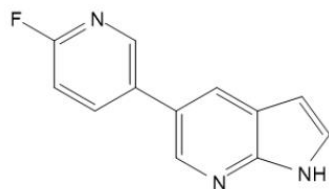
5-(pyridin-4-yl)-1*H*-pyrrolo[2,3-*b*]pyridine (19n) (¹³C):



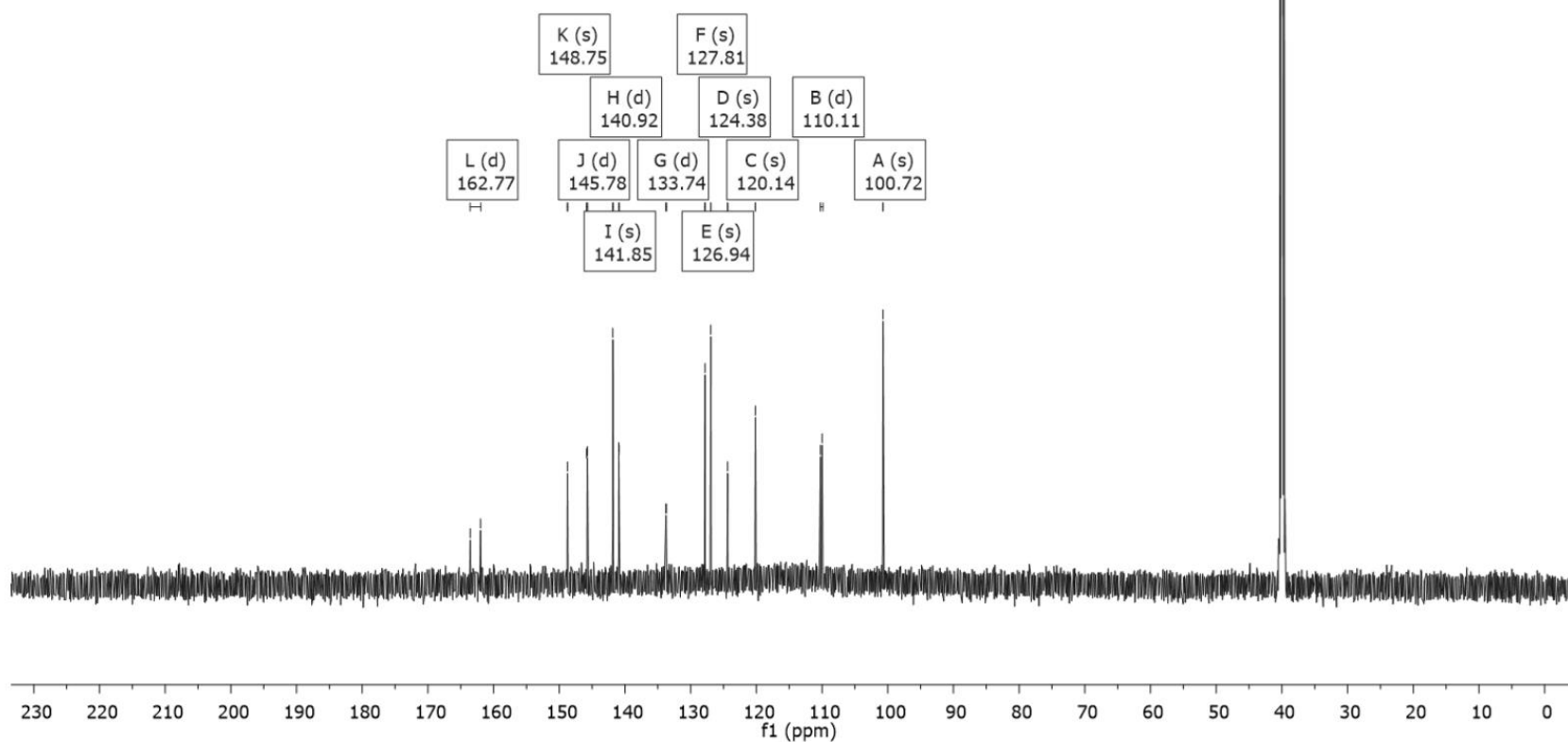
5-(6-fluoropyridin-3-yl)-1H-pyrrolo[2,3-b]pyridine (19o) (¹H):



5-(6-fluoropyridin-3-yl)-1*H*-pyrrolo[2,3-*b*]pyridine (19o) (¹³C):



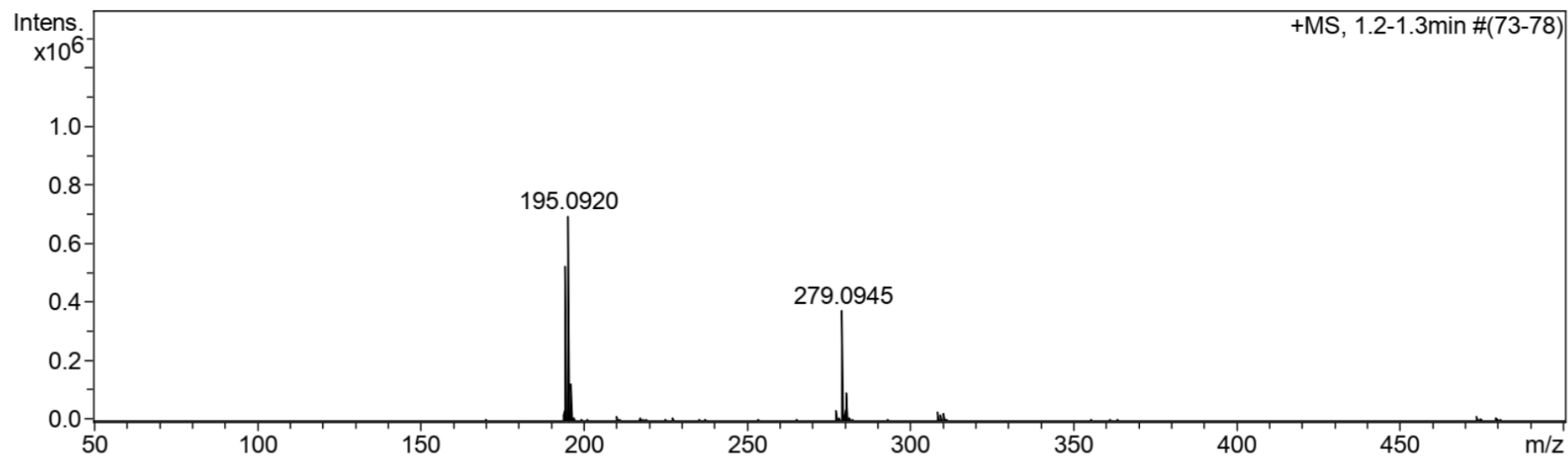
¹³C NMR peaks (ppm):
 163.55, 161.99, 148.75, 145.83, 145.73, 141.85, 140.95, 140.90, 133.76, 133.73, 127.81, 126.94, 124.38, 120.14, 110.23, 109.98, 100.72



2. MS data

4-phenyl-1*H*-pyrrolo[2,3-*b*]pyridine (18a):

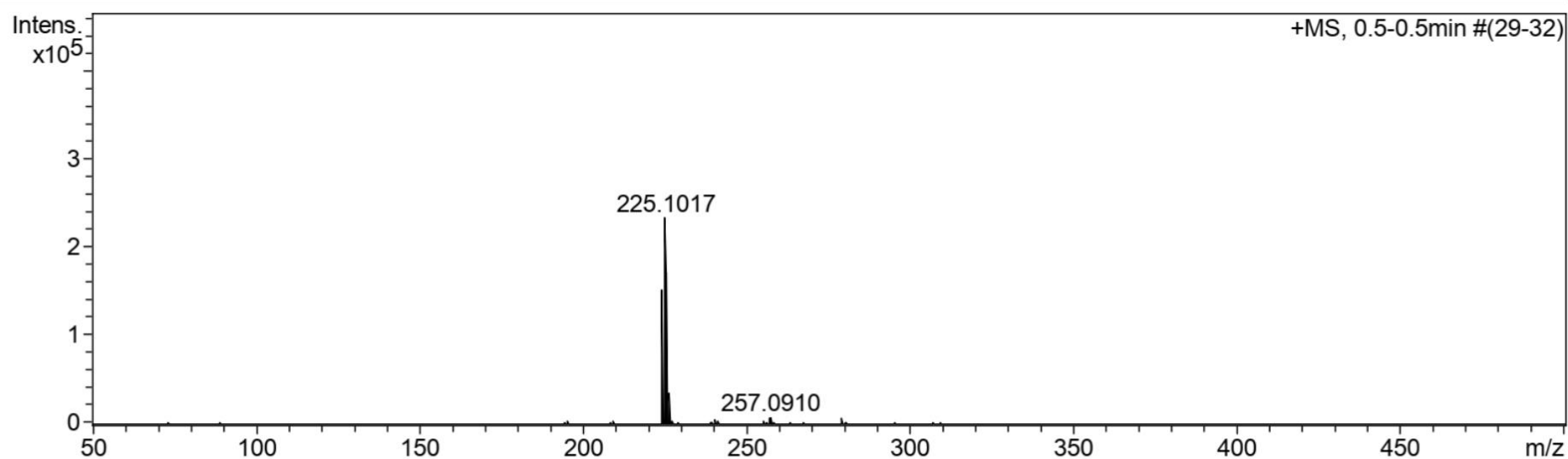
APCI-HRMS *m/z*: calc. for C₁₃H₁₀N₂ (MH⁺), 195.091675, found 195.0920:



Meas. <i>m/z</i>	#	Formula	Score	<i>m/z</i>	err [mDa]	err [ppm]	mSigma	rdb	e ⁻ Conf	N-Rule
195.0920	1	C 13 H 11 N 2	100.00	195.0917	-0.3	-1.8	18.9	9.5	even	ok
	2	C 10 H 13 N O 3	9.15	195.0890	-3.0	-15.5	39.7	5.0	odd	ok
279.0945	1	C 18 H 15 O 3	7.29	279.1016	7.1	25.4	30.8	11.5	even	ok
	2	C 17 H 13 N O 3	100.00	279.0890	-5.5	-19.7	35.0	12.0	odd	ok
	3	C 16 H 13 N 3 O 2	63.94	279.1002	5.7	20.6	37.2	12.0	odd	ok

4-(4-methoxyphenyl)-1*H*-pyrrolo[2,3-*b*]pyridine (18c):

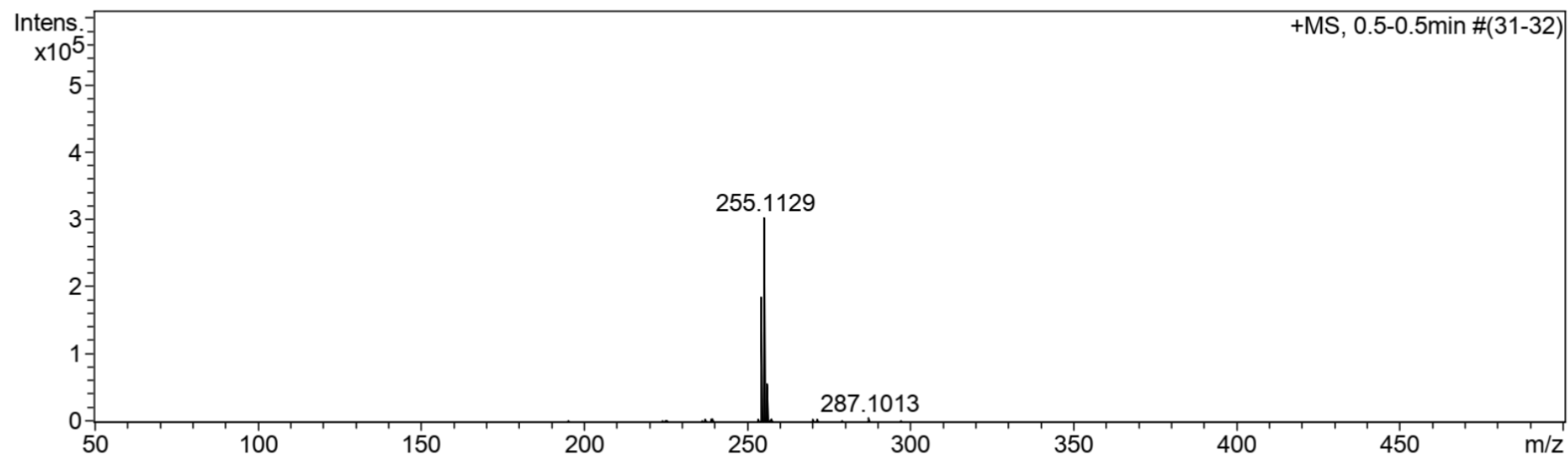
APCI-HRMS *m/z*: calc. for C₁₄H₁₂N₂O (MH⁺), 225.102239, found 225.1017:



Meas. <i>m/z</i>	#	Formula	Score	<i>m/z</i>	err [mDa]	err [ppm]	mSigma	rdb	e ⁻	Conf	N-Rule
225.1017	1	C 14 H 13 N 2 O	100.00	225.1022	0.5	2.4	5.3	9.5	even		ok

4-(2,3-dimethoxyphenyl)-1*H*-pyrrolo[2,3-*b*]pyridine (18d):

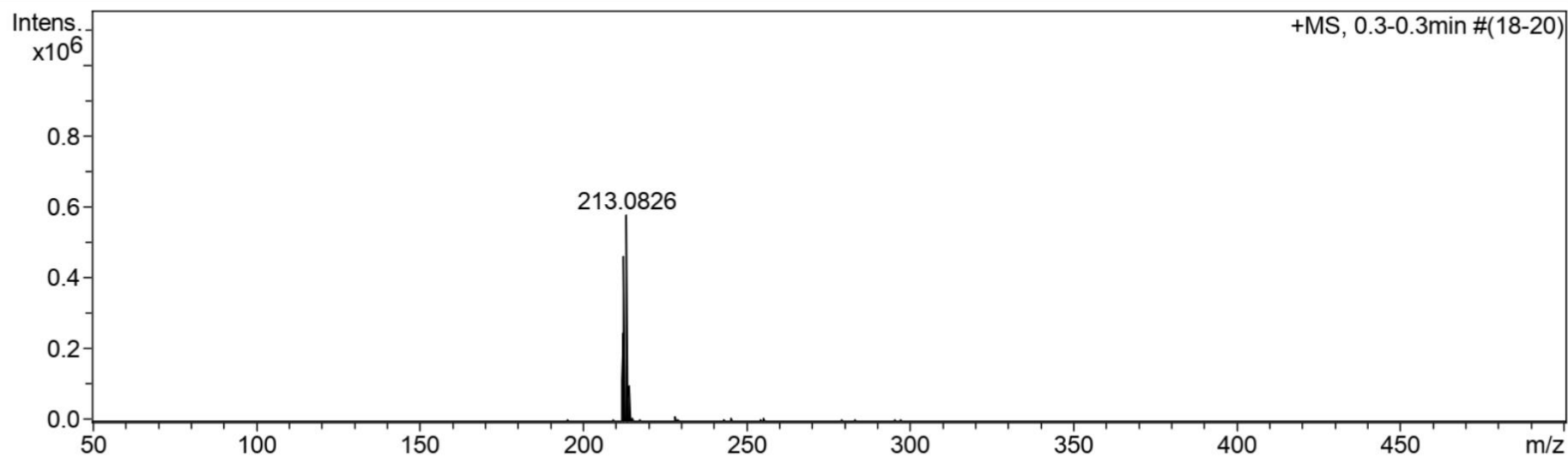
APCI-HRMS *m/z*: calc. for C₁₅H₁₄N₂O₂ (MH⁺), 255.112804, found 255.1129:



Meas. <i>m/z</i>	#	Formula	Score	<i>m/z</i>	err [mDa]	err [ppm]	mSigma	rdb	e ⁻ Conf	N-Rule
255.1129	1	C 15 H 15 N 2 O 2	100.00	255.1128	-0.1	-0.5	10.7	9.5	even	ok

4-(4-fluorophenyl)-1*H*-pyrrolo[2,3-*b*]pyridine (18e):

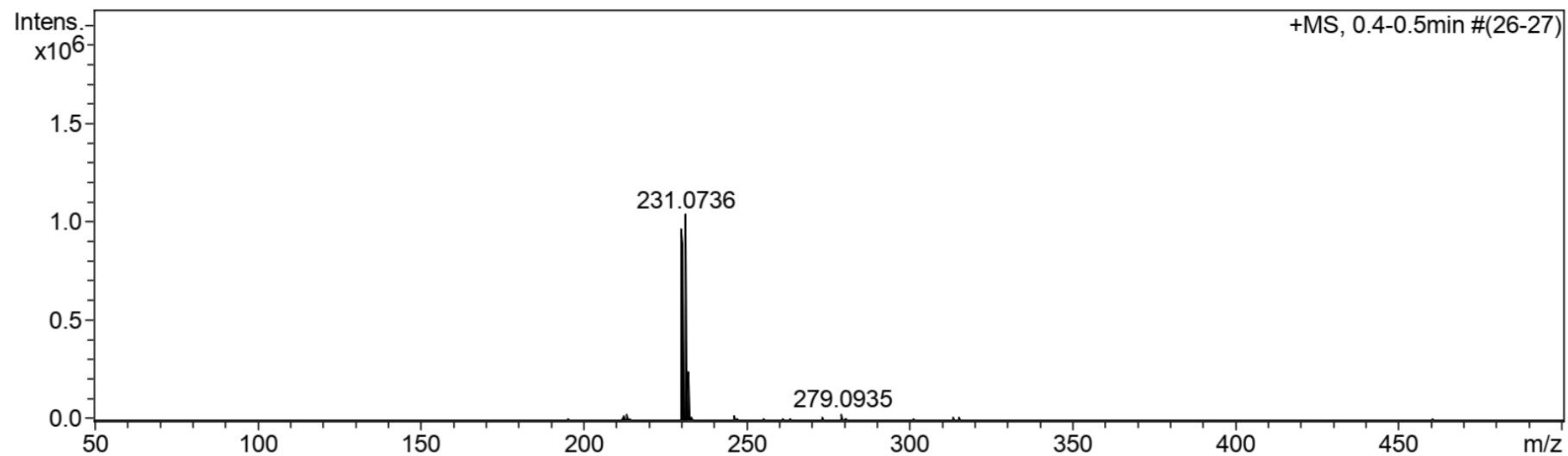
APCI-HRMS *m/z*: calc. for C₁₃H₉FN₂ (MH⁺), 213.082253, found 213.0826:



Meas. m/z	#	Formula	Score	m/z	err [mDa]	err [ppm]	mSigma	rdb	e ⁻ Conf	N-Rule
213.0826	1	C 13 H 10 F N 2	100.00	213.0823	-0.4	-1.7	12.1	9.5	even	ok

4-(3,4-difluorophenyl)-1*H*-pyrrolo[2,3-*b*]pyridine (18f):

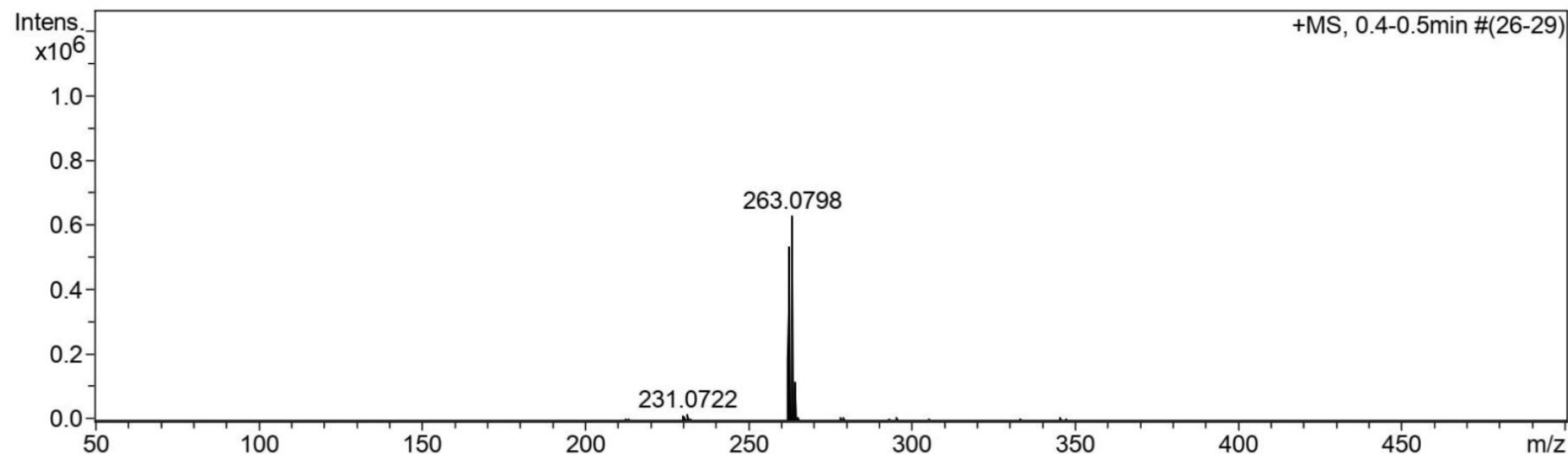
APCI-HRMS *m/z*: calc. for C₁₃H₈F₂N₂ (MH⁺), 231.072831, found 231.0736



Meas. m/z	#	Formula	Score	m/z	err [mDa]	err [ppm]	mSigma	rdb	e ⁻ Conf	N-Rule
231.0736	1	C 13 H 9 F 2 N 2	100.00	231.0728	-0.8	-3.3	51.0	9.5	even	ok

4-[4-(trifluoromethyl)phenyl]-1*H*-pyrrolo[2,3-*b*]pyridine (18g):

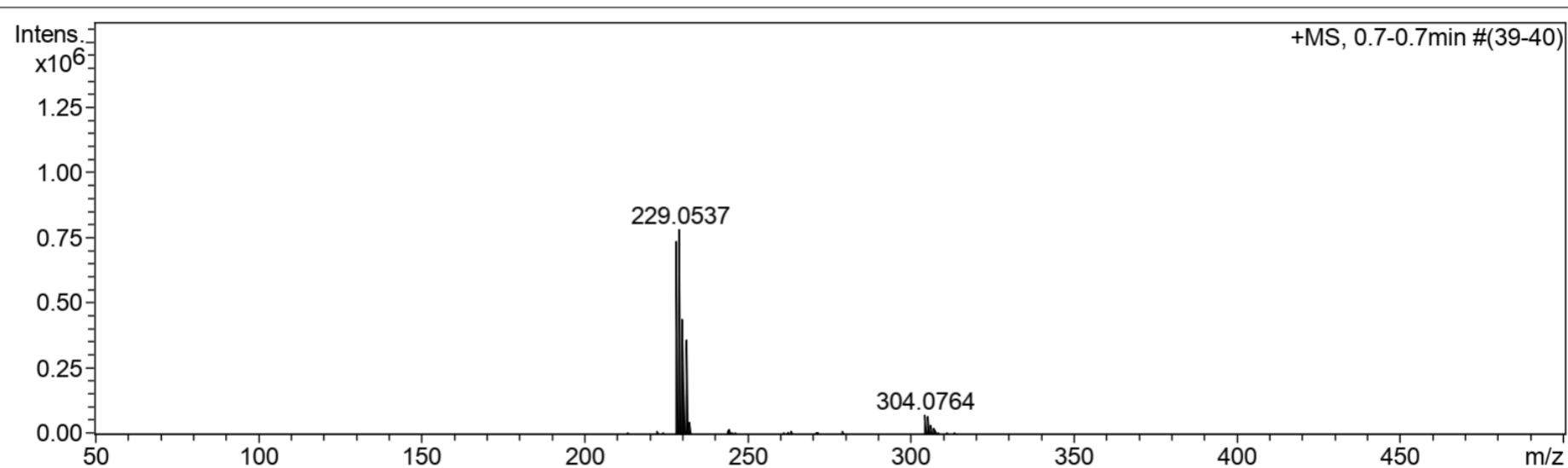
APCI-HRMS *m/z*: calc. for C₁₄H₉F₃N₂ (MH⁺), 263.079059, found 263.0798:



Meas. <i>m/z</i>	#	Formula	Score	<i>m/z</i>	err [mDa]	err [ppm]	mSigma	rdb	e ⁻ Conf	N-Rule
263.0798	1	C 14 H 10 F 3 N 2	100.00	263.0791	-0.8	-3.0	15.0	9.5	even	ok

4-(4-chlorophenyl)-1*H*-pyrrolo[2,3-*b*]pyridine (18h):

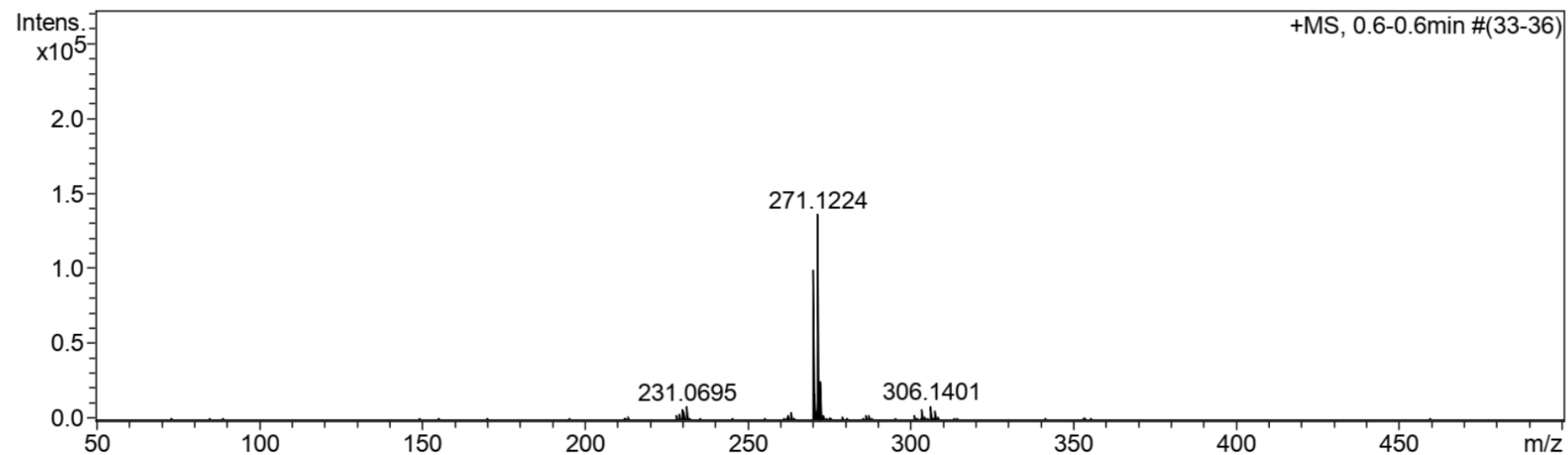
APCI-HRMS *m/z*: calc. for C₁₃H₉ClN₂ (MH⁺), 229.052702, found 229.0537:



Meas. m/z	#	Formula	Score	m/z	err [mDa]	err [ppm]	mSigma	rdb	e ⁻ Conf	N-Rule
228.0464	1	C 13 H 9 Cl N 2	100.00	228.0449	-1.6	-6.9	488.1	10.0	odd	ok
229.0537	1	C 13 H 10 Cl N 2	100.00	229.0527	-1.0	-4.6	216.8	9.5	even	ok

4-(biphenyl-4-yl)-1*H*-pyrrolo[2,3-*b*]pyridine (18i):

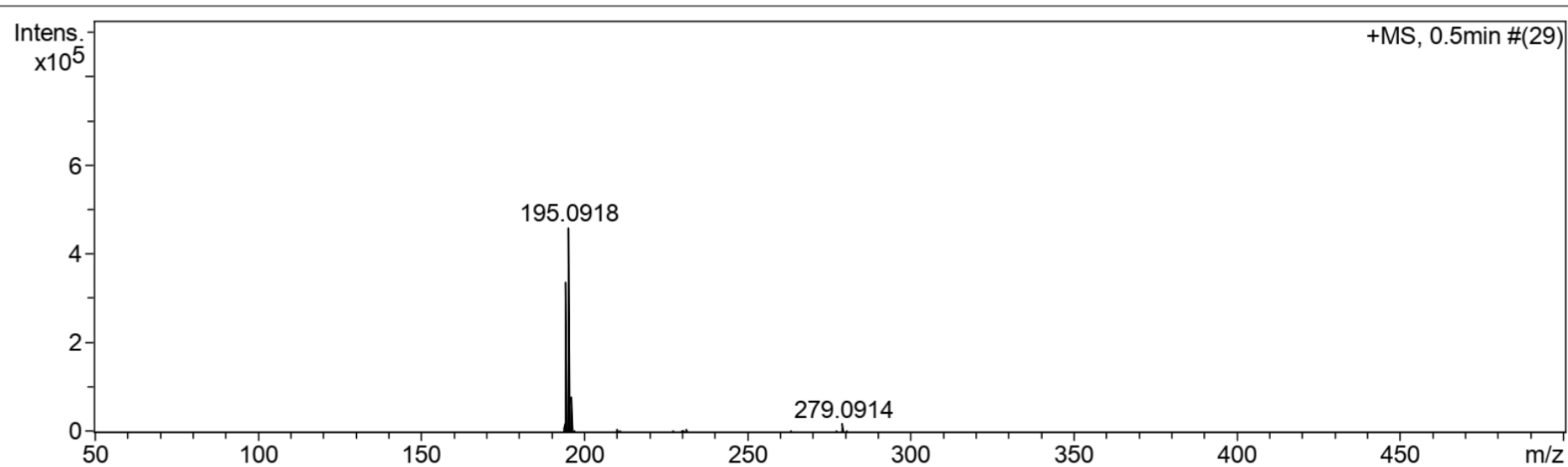
HRMS m/z : calc. for $C_{19}H_{14}N_2$ (MH^+), 271.122975, found 271.1224:



Meas. m/z	#	Formula	Score	m/z	err [mDa]	err [ppm]	mSigma	rdb	e ⁻ Conf	N-Rule
271.1224	1	C ₁₉ H ₁₅ N ₂	100.00	271.1230	0.6	2.2	14.3	13.5	even	ok

5-phenyl-1*H*-pyrrolo[2,3-*b*]pyridine (19a):

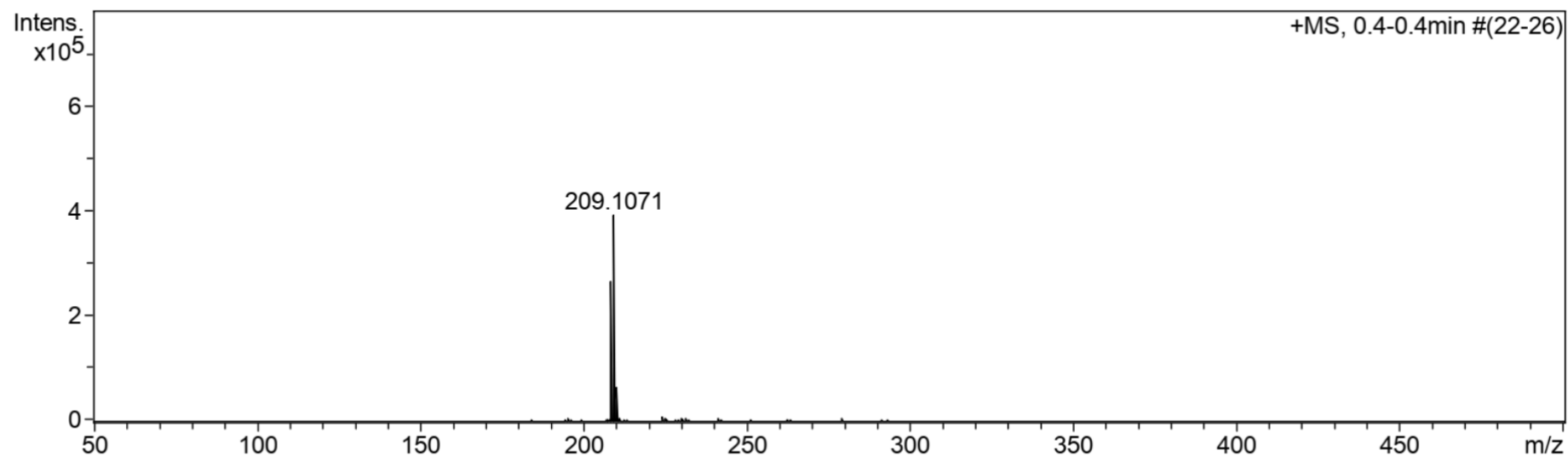
APCI-HRMS *m/z*: calc. for C₁₃H₁₀N₂ (MH⁺), 195.091675, found 195.0918



Meas. m/z	#	Formula	Score	m/z	err [mDa]	err [ppm]	mSigma	rdb	e ⁻ Conf	N-Rule
195.0918	1	C 13 H 11 N 2	100.00	195.0917	-0.1	-0.5	13.6	9.5	even	ok

5-(4-methylphenyl)-1*H*-pyrrolo[2,3-*b*]pyridine (19b):

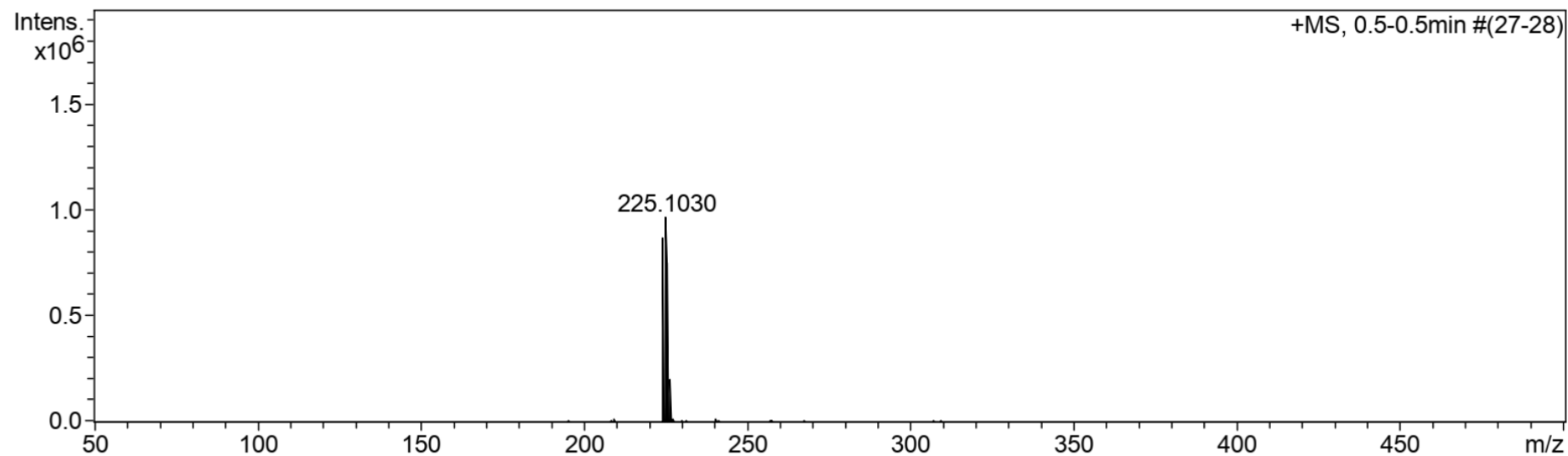
APCI-HRMS *m/z*: calc. for C₁₄H₁₂N₂ (MH⁺), 209.107325, found 209.1071



Meas. m/z	#	Formula	Score	m/z	err [mDa]	err [ppm]	mSigma	rdb	e ⁻ Conf	N-Rule
209.1071	1	C 14 H 13 N 2	100.00	209.1073	0.2	0.9	2.1	9.5	even	ok

5-(3-methoxyphenyl)-1*H*-pyrrolo[2,3-*b*]pyridine (19c):

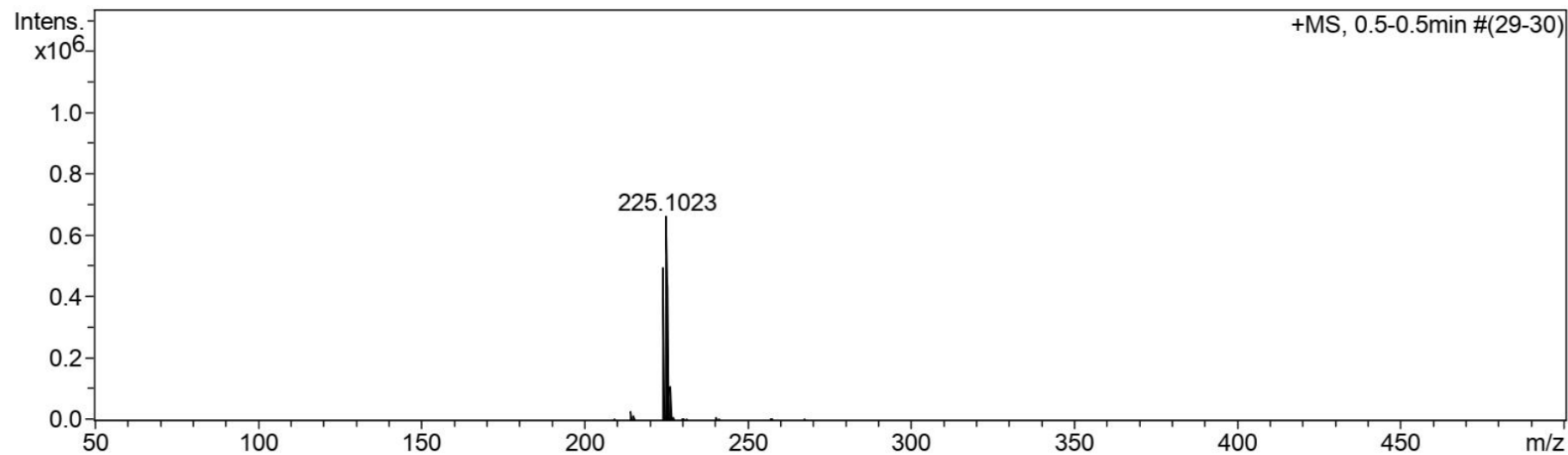
APCI-HRMS *m/z*: calc. for C₁₄H₁₂N₂O (MH⁺), 225.102239, found 225.1030



Meas. m/z	#	Formula	Score	m/z	err [mDa]	err [ppm]	mSigma	rdb	e ⁻ Conf	N-Rule
225.1030	1	C 14 H 13 N 2 O	100.00	225.1022	-0.7	-3.3	29.7	9.5	even	ok

5-(4-methoxyphenyl)-1*H*-pyrrolo[2,3-*b*]pyridine (19d):

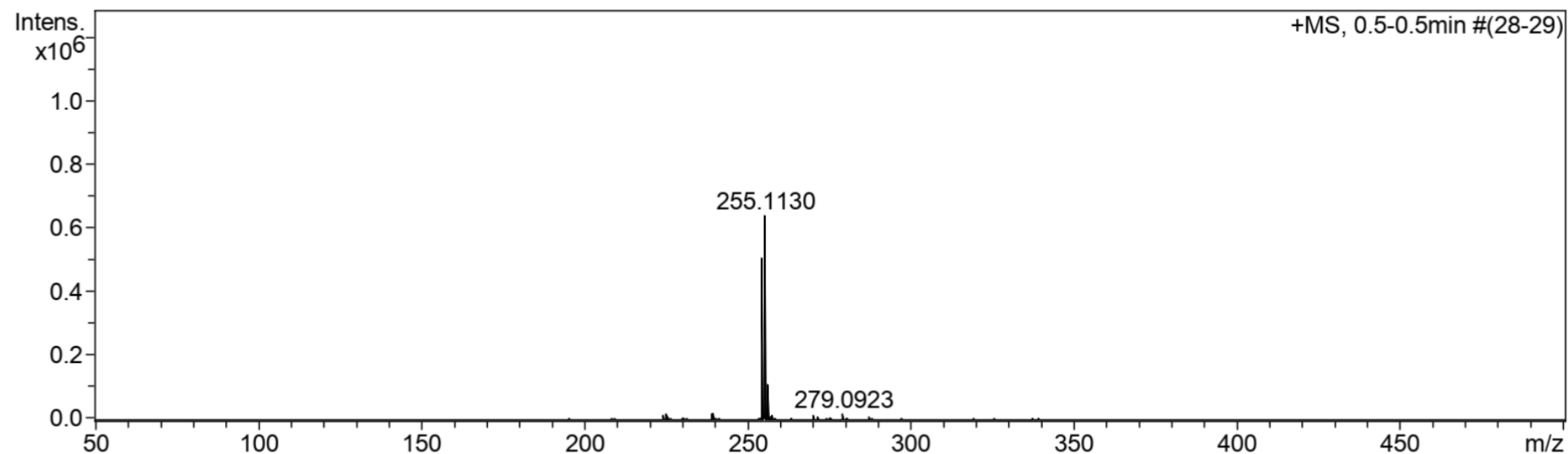
APCI-HRMS *m/z*: calc. for C₁₄H₁₂N₂O (MH⁺), 225.102239, found 225.1023



Meas. <i>m/z</i>	#	Formula	Score	<i>m/z</i>	err [mDa]	err [ppm]	mSigma	rdb	e ⁻ Conf	N-Rule
225.1023	1	C 14 H 13 N 2 O	100.00	225.1022	-0.1	-0.4	6.3	9.5	even	ok

5-(2,3-dimethoxyphenyl)-1*H*-pyrrolo[2,3-*b*]pyridine (19e):

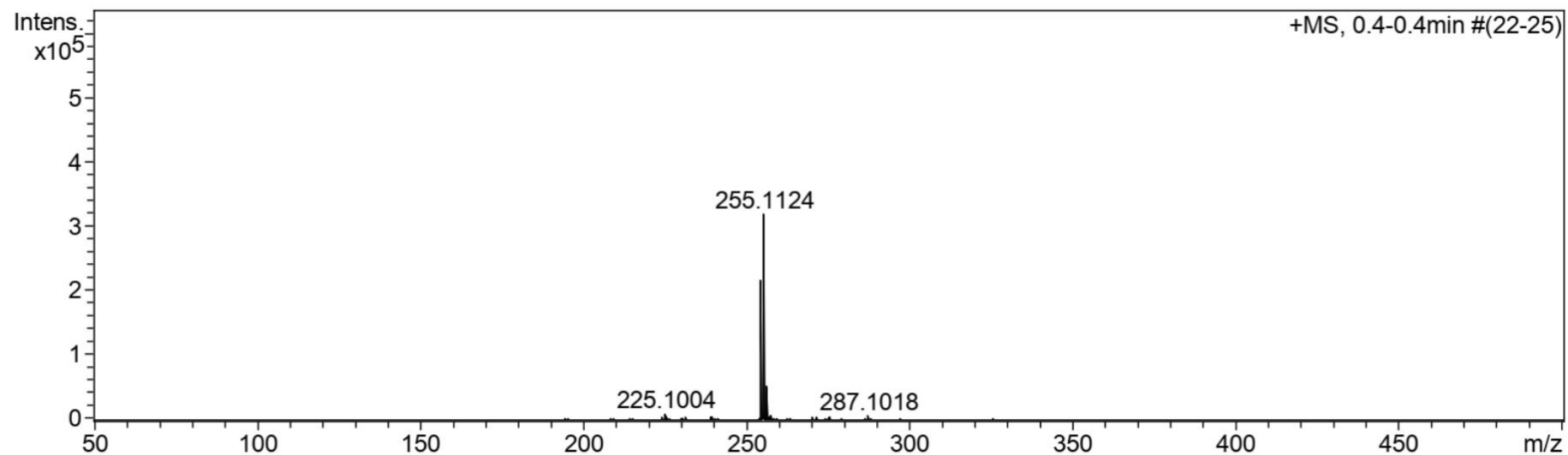
APCI-HRMS *m/z*: calc. for C₁₅H₁₄N₂O₂ (MH⁺), 255.112804, found 255.1130



Meas. <i>m/z</i>	#	Formula	Score	<i>m/z</i>	err [mDa]	err [ppm]	mSigma	rdb	e ⁻ Conf	N-Rule
255.1130	1	C 15 H 15 N 2 O 2	100.00	255.1128	-0.1	-0.6	3.0	9.5	even	ok

5-(3,4-dimethoxyphenyl)-1*H*-pyrrolo[2,3-*b*]pyridine (19f):

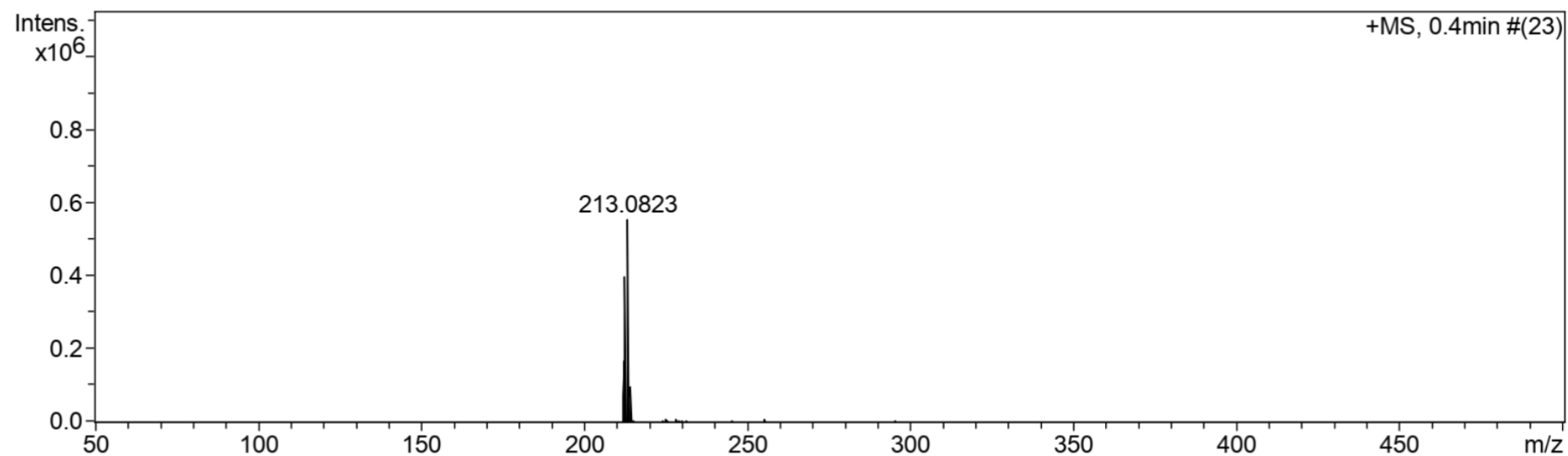
APCI-HRMS *m/z*: calc. for C₁₅H₁₄N₂O₂ (MH⁺), 255.112804, found 255.1124:



Meas. <i>m/z</i>	#	Formula	Score	<i>m/z</i>	err [mDa]	err [ppm]	mSigma	rdb	e ⁻ Conf	N-Rule
255.1124	1	C 15 H 15 N 2 O 2	100.00	255.1128	0.5	1.8	4.1	9.5	even	ok

5-(4-fluorophenyl)-1*H*-pyrrolo[2,3-*b*]pyridine (19g):

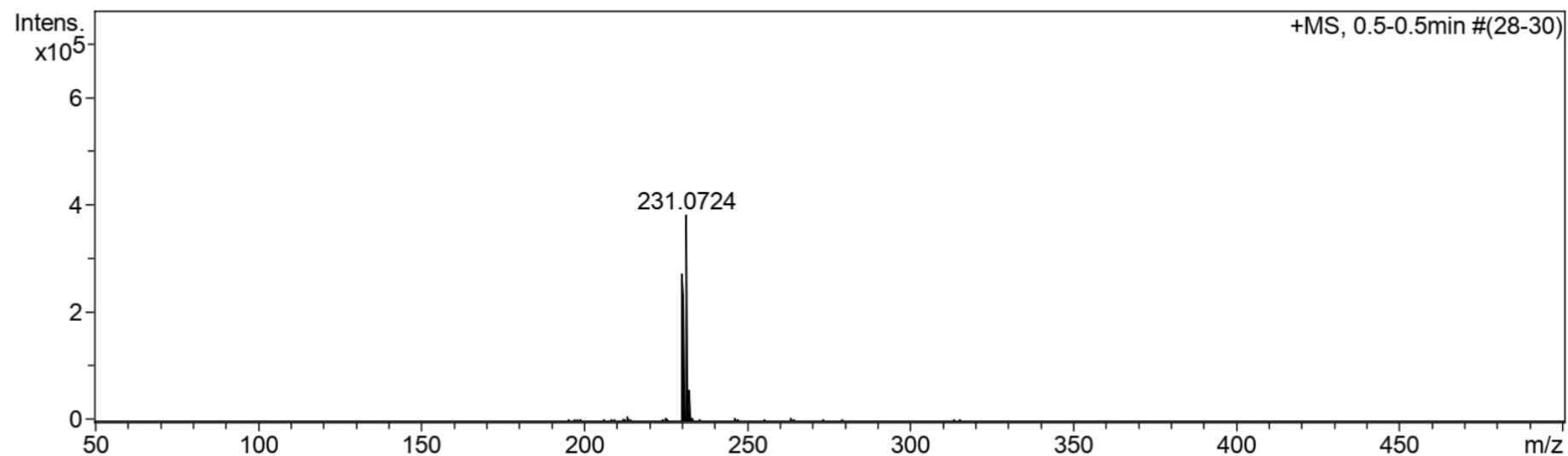
APCI-HRMS *m/z*: calc. for C₁₃H₉FN₂ (MH⁺), 213.082253, found 213.0823



Meas. m/z	#	Formula	Score	m/z	err [mDa]	err [ppm]	mSigma	rdb	e ⁻ Conf	N-Rule
213.0823	1	C 13 H 10 F N 2	100.00	213.0823	-0.0	-0.1	17.1	9.5	even	ok

5-(3,4-difluorophenyl)-1*H*-pyrrolo[2,3-*b*]pyridine (19h):

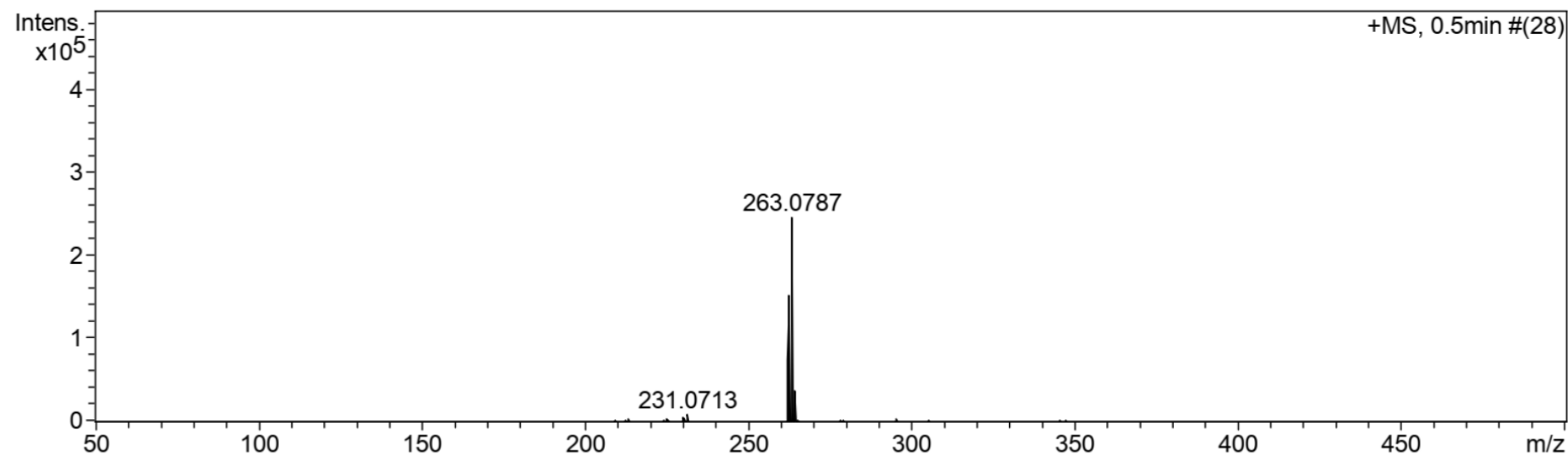
APCI-HRMS *m/z*: calc. for C₁₃H₈F₂N₂ (MH⁺), 231.072831, found 231.0724



Meas. m/z	#	Formula	Score	m/z	err [mDa]	err [ppm]	mSigma	rdb	e ⁻ Conf	N-Rule
231.0724	1	C 13 H 9 F 2 N 2	100.00	231.0728	0.5	2.0	0.5	9.5	even	ok

5-[4-(trifluoromethyl)phenyl]-1*H*-pyrrolo[2,3-*b*]pyridine (19i):

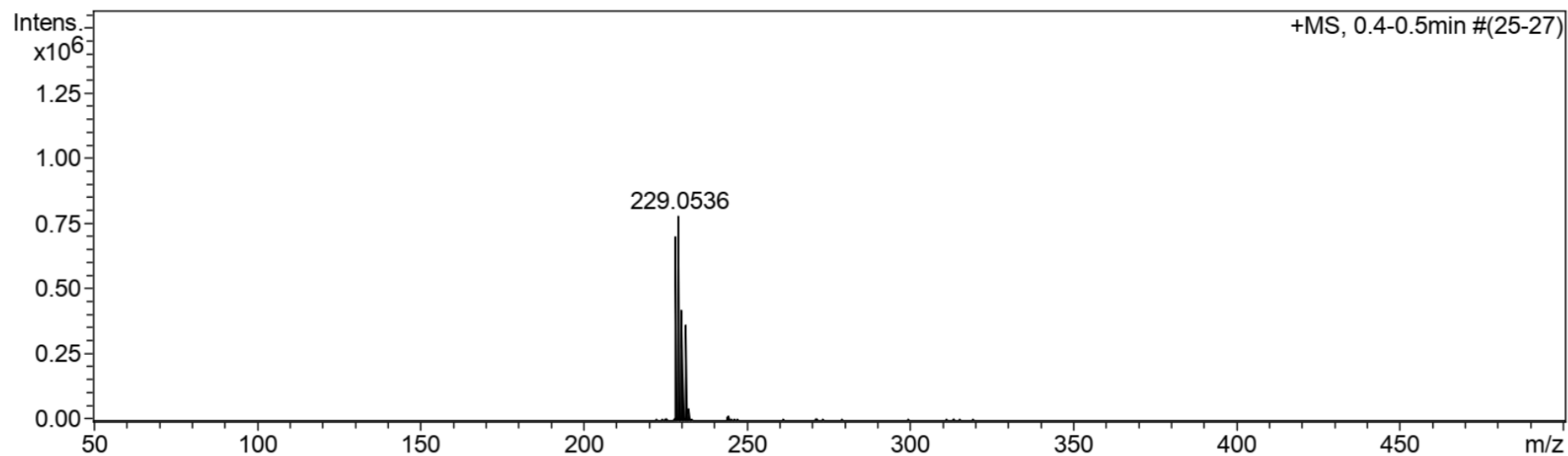
APCI-HRMS *m/z*: calc. for C₁₄H₉F₃N₂ (MH⁺), 263.079059, found 263.0787:



Meas. <i>m/z</i>	#	Formula	Score	<i>m/z</i>	err [mDa]	err [ppm]	mSigma	rdb	e ⁻ Conf	N-Rule
263.0787	1	C 14 H 10 F 3 N 2	100.00	263.0791	0.4	1.5	1.9	9.5	even	ok

5-(4-chlorophenyl)-1*H*-pyrrolo[2,3-*b*]pyridine (19j):

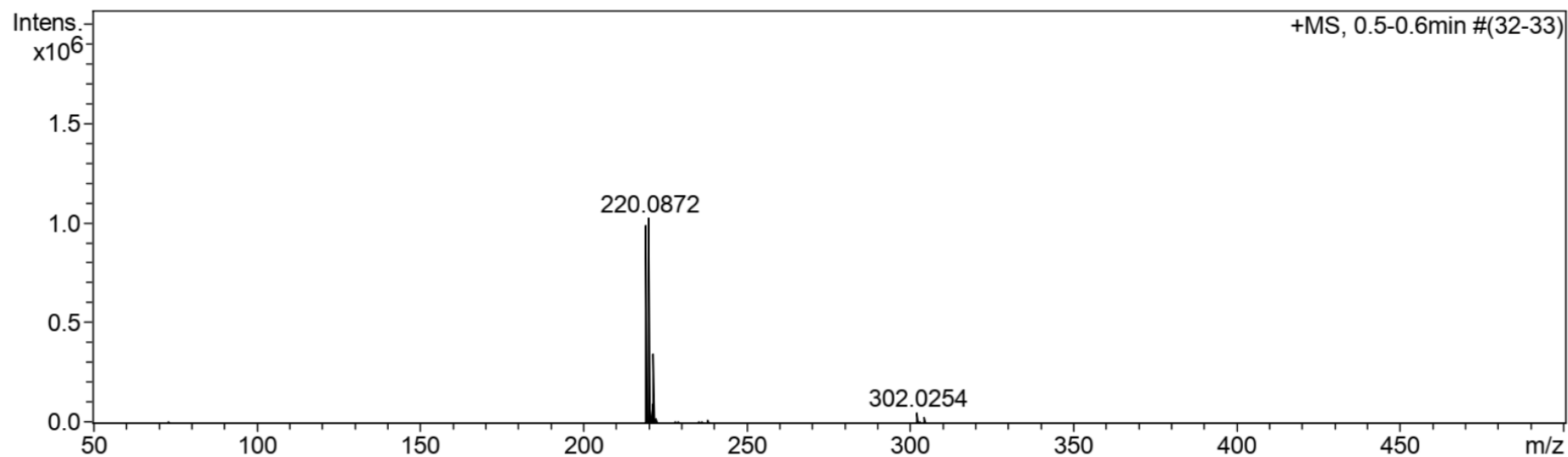
APCI-HRMS *m/z*: calc. for C₁₃H₉ClN₂ (MH⁺), 229.052702, found 229.0536:



Meas. <i>m/z</i>	#	Formula	Score	<i>m/z</i>	err [mDa]	err [ppm]	mSigma	rdb	e ⁻ Conf	N-Rule
229.0536	1	C 13 H 10 Cl N 2	100.00	229.0527	-0.9	-3.8	205.8	9.5	even	ok

4-(1*H*-pyrrolo[2,3-*b*]pyridin-5-yl)benzonitrile (19k):

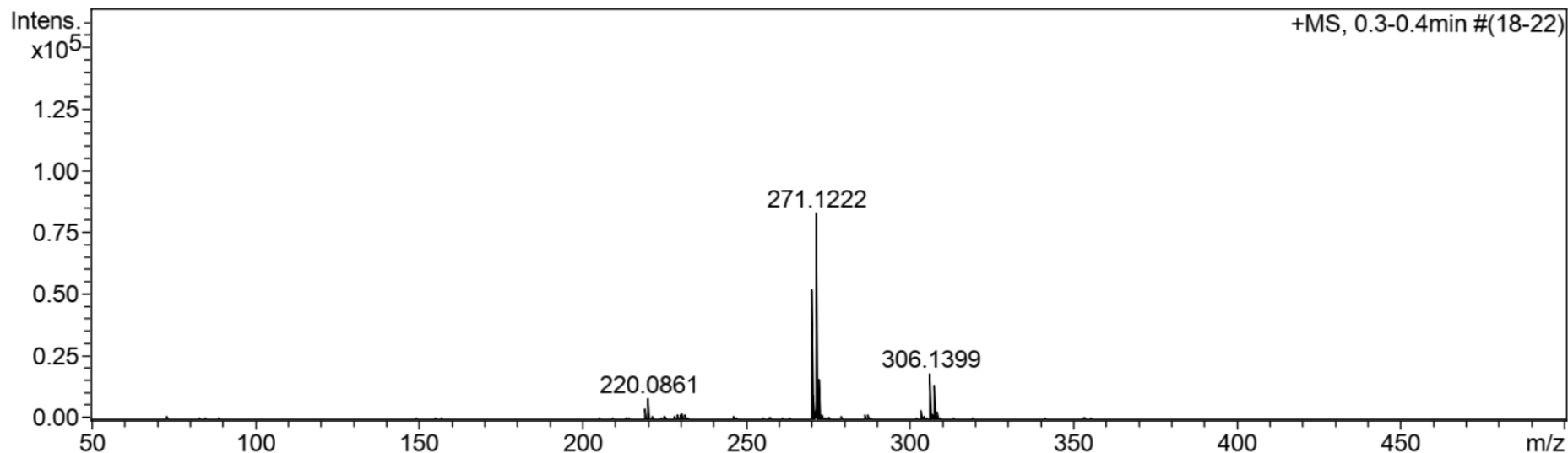
APCI-HRMS *m/z*: calc. for C₁₄H₉N₃ (MH⁺), 220.086924, found 220.0872:



Meas. m/z	#	Formula	Score	m/z	err [mDa]	err [ppm]	mSigma	rdb	e ⁻ Conf	N-Rule
220.0872	1	C 14 H 10 N 3	100.00	220.0869	-0.2	-1.0	102.9	11.5	even	ok

5-(biphenyl-4-yl)-1*H*-pyrrolo[2,3-*b*]pyridine (19l):

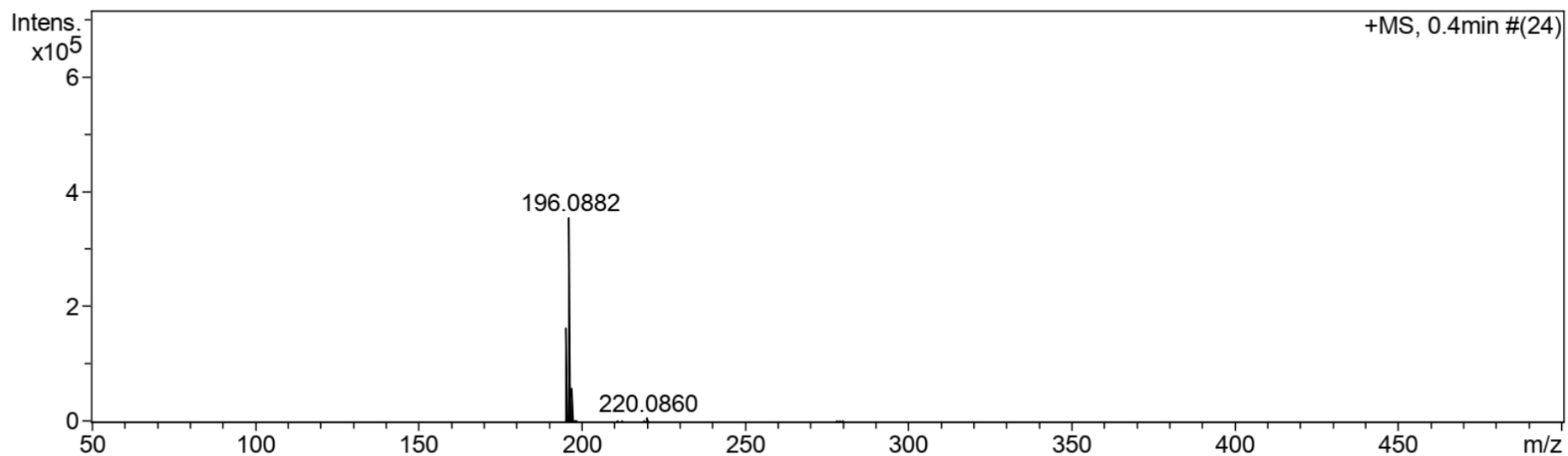
APCI-HRMS *m/z*: calc. for C₁₉H₁₄N₂ (MH⁺), 271.122975, found 271.1222:



Meas. <i>m/z</i>	#	Formula	Score	<i>m/z</i>	err [mDa]	err [ppm]	mSigma	rdb	e ⁻ Conf	N-Rule
271.1222	1	C 19 H 15 N 2	100.00	271.1230	0.8	2.8	12.2	13.5	even	ok
306.1399	1	C 24 H 18	100.00	306.1403	0.4	1.4	294.2	16.0	odd	ok
	2	C 19 H 18 N 2 O 2	0.29	306.1363	-3.6	-11.7	321.3	12.0	odd	ok
	3	C 12 H 22 N 2 O 7	0.00	306.1422	2.3	7.5	364.3	3.0	odd	ok

5-(pyridin-3-yl)-1*H*-pyrrolo[2,3-*b*]pyridine (19m):

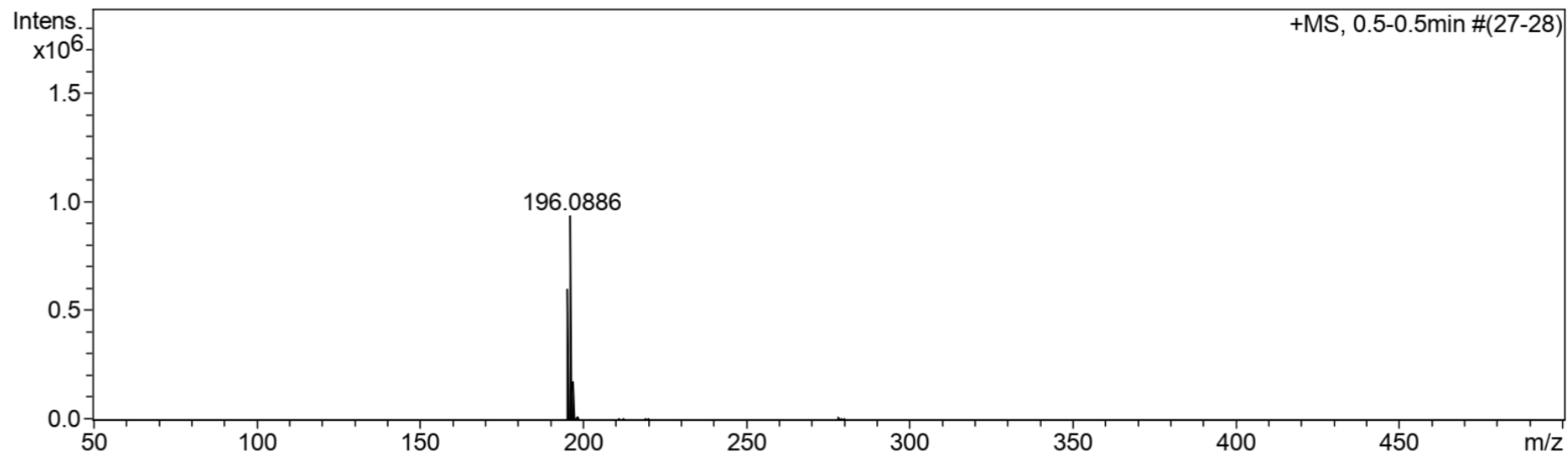
APCI-HRMS *m/z*: calc. for C₁₂H₉N₃ (MH⁺), 196.086924, found 196.0882:



Meas. m/z	#	Formula	Score	m/z	err [mDa]	err [ppm]	mSigma	rdb	e ⁻ Conf	N-Rule
196.0882	1	C 12 H 10 N 3	100.00	196.0869	-1.3	-6.6	16.4	9.5	even	ok

5-(pyridin-4-yl)-1*H*-pyrrolo[2,3-*b*]pyridine (19n):

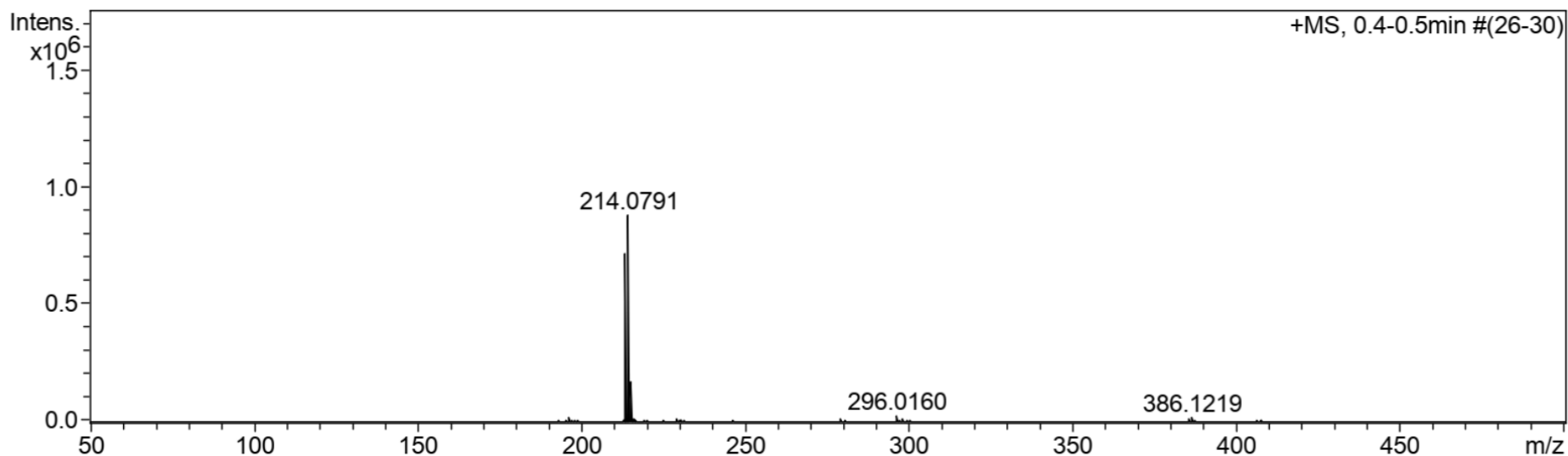
APCI-HRMS *m/z*: calc. for C₁₂H₉N₃ (MH⁺), 196.086924, found 196.0886:



Meas. m/z	#	Formula	Score	m/z	err [mDa]	err [ppm]	mSigma	rdb	e ⁻ Conf	N-Rule
196.0886	1	C 12 H 10 N 3	100.00	196.0869	-1.6	-8.3	29.6	9.5	even	ok

5-(6-fluoropyridin-3-yl)-1*H*-pyrrolo[2,3-*b*]pyridine (19o):

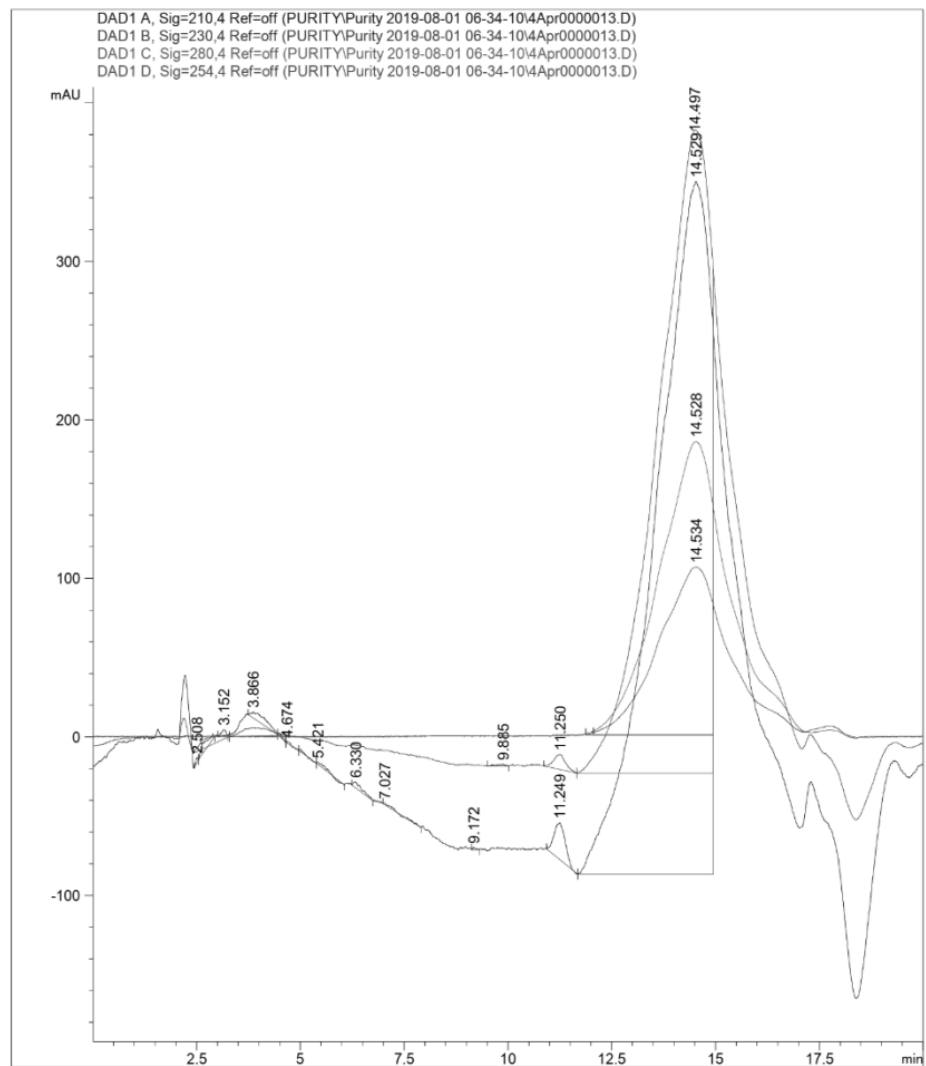
APCI-HRMS *m/z*: calc. for C₁₂H₈FN₃ (MH⁺), 214.077502, found 214.0791:



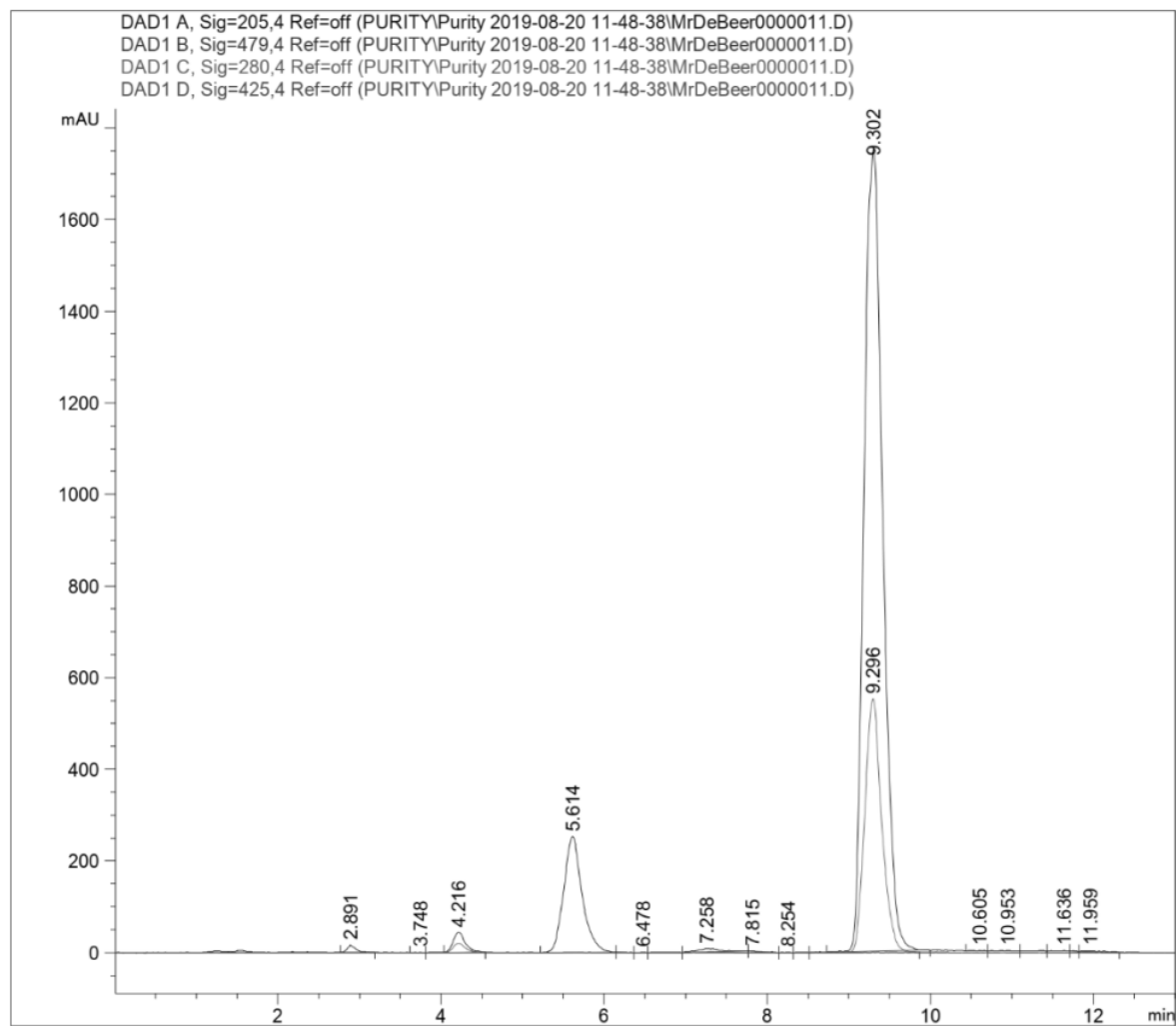
Meas. m/z	#	Formula	Score	m/z	err [mDa]	err [ppm]	mSigma	rdb	e ⁻ Conf	N-Rule
214.0791	1	C 17 H 10	100.00	214.0777	-1.4	-6.4	6.3	13.0	odd	ok
	2	C 12 H 9 F N 3	52.48	214.0775	-1.6	-7.3	30.8	9.5	even	ok

3. HPLC data

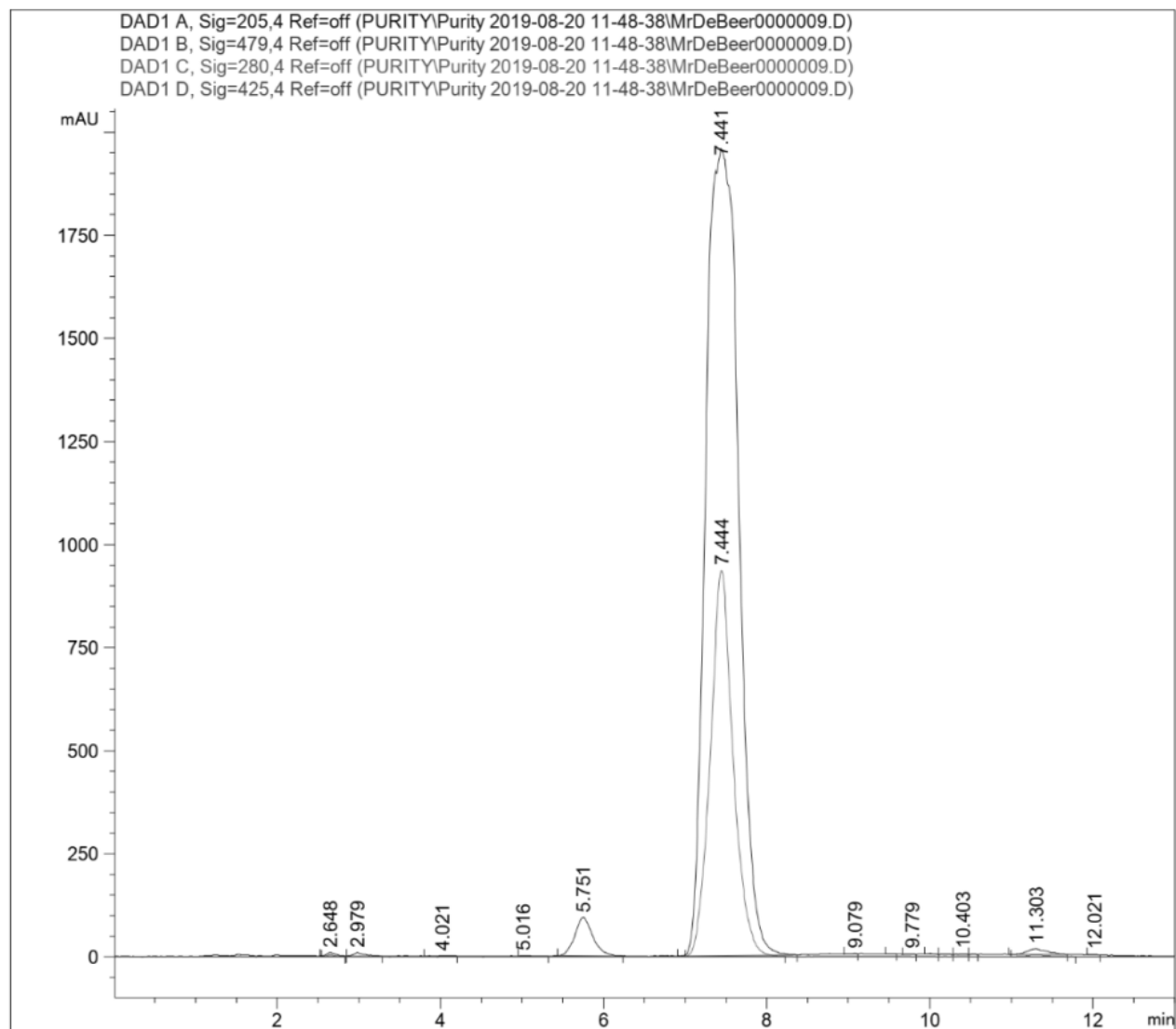
4-phenyl-1*H*-pyrrolo[2,3-*b*]pyridine (18a):



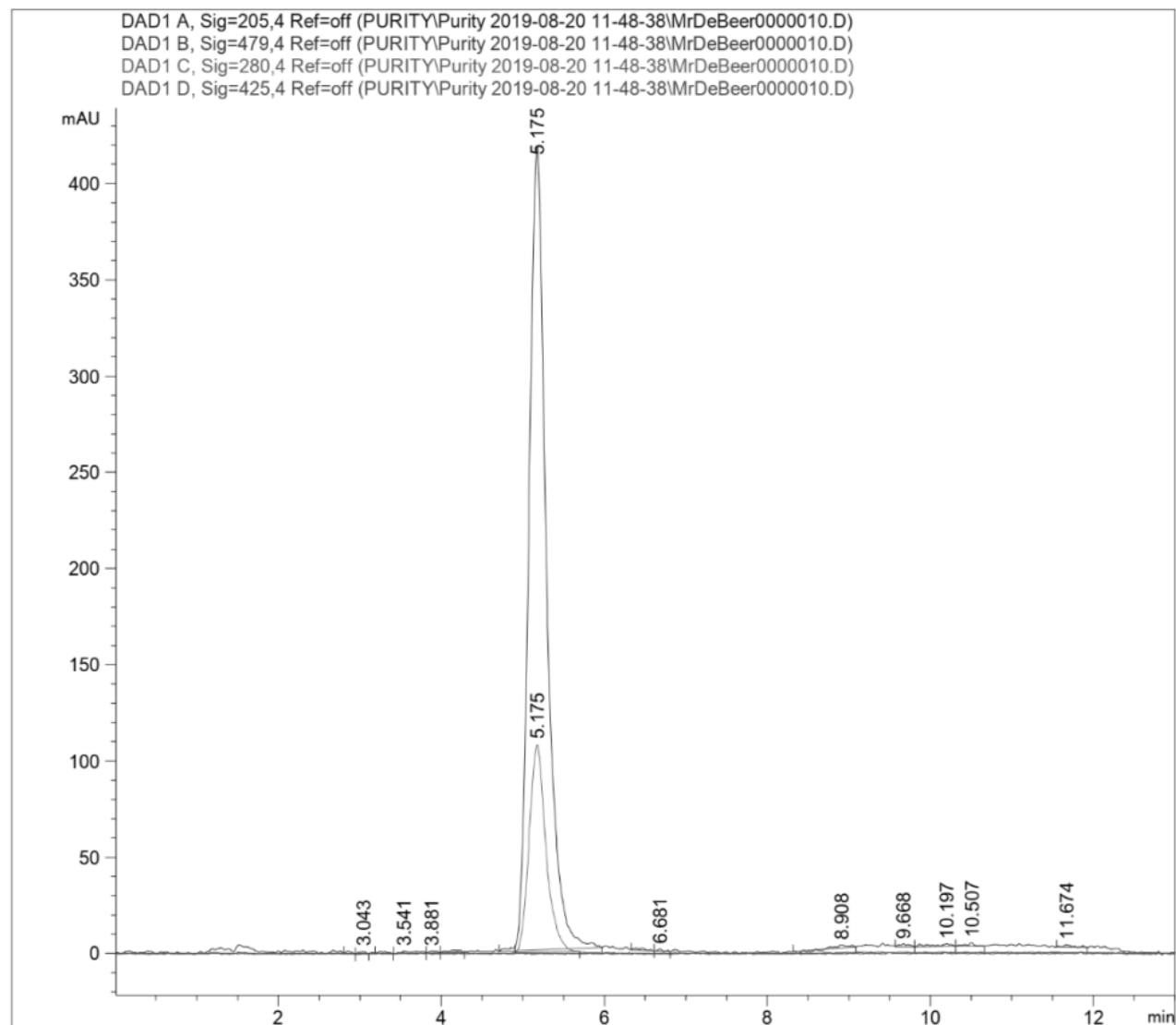
4-(4-methylphenyl)-1*H*-pyrrolo[2,3-*b*]pyridine (18b):



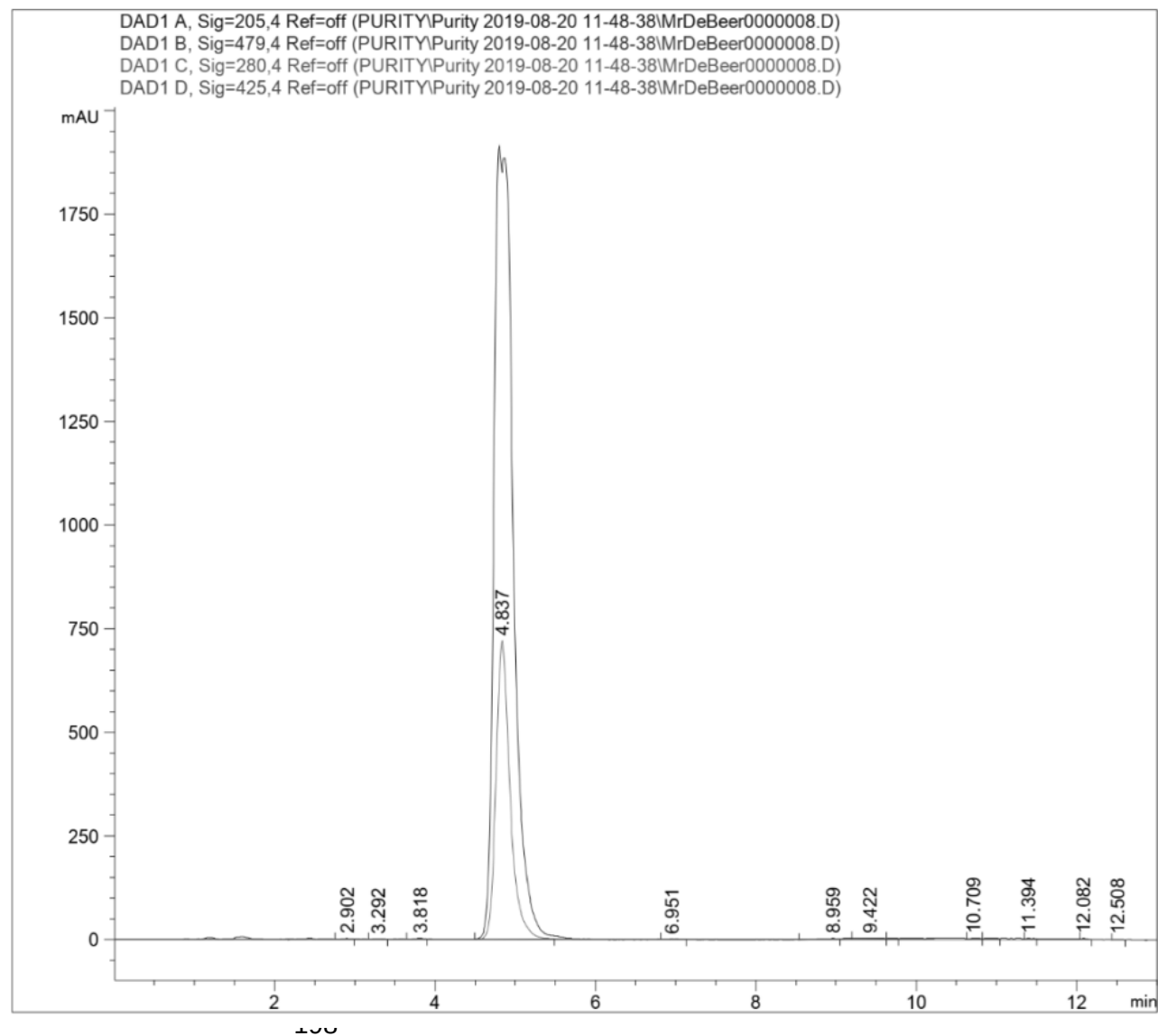
4-(4-methoxyphenyl)-1*H*-pyrrolo[2,3-*b*]pyridine (18c):



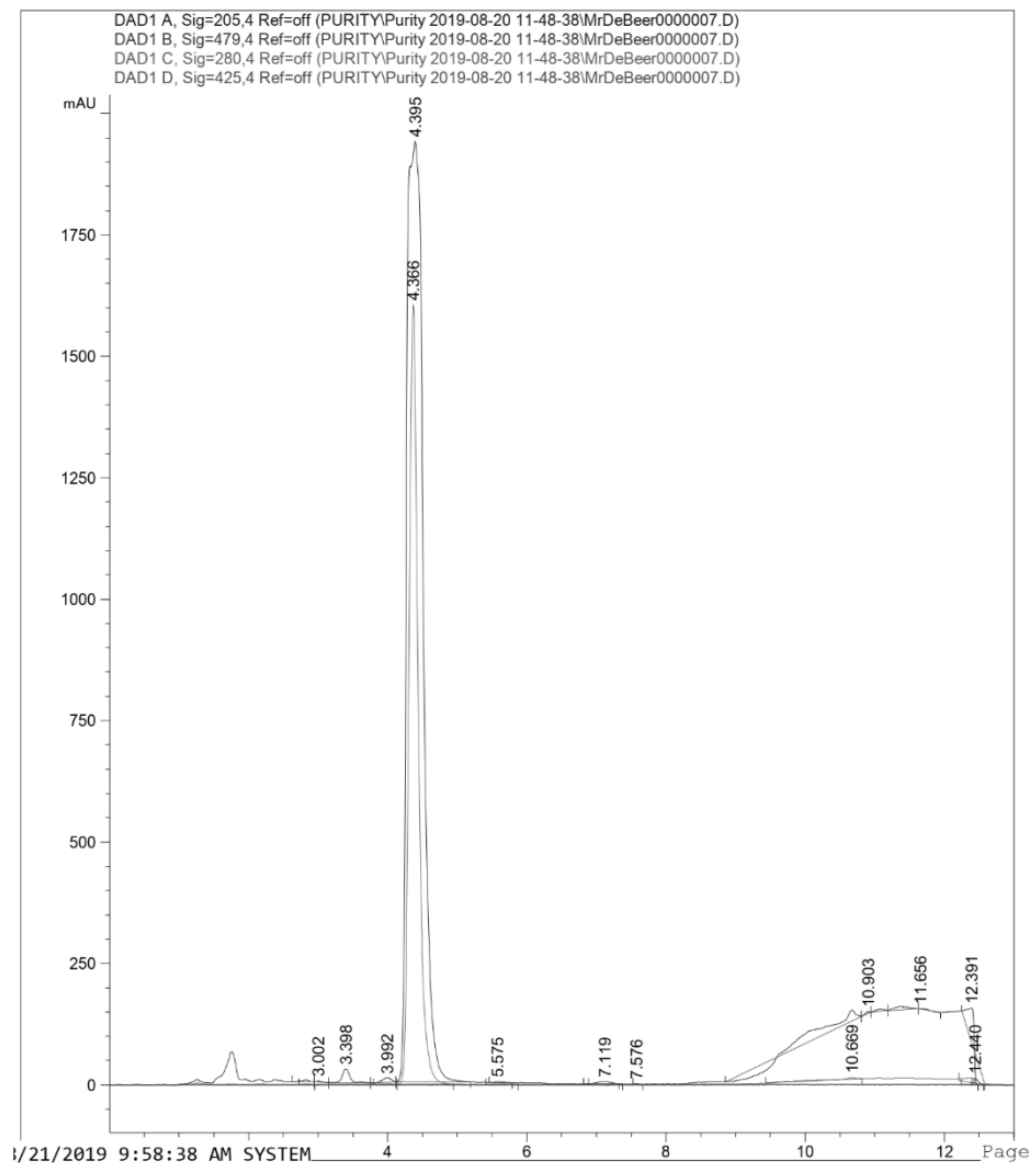
4-(2,3-dimethoxyphenyl)-1*H*-pyrrolo[2,3-*b*]pyridine (18d):



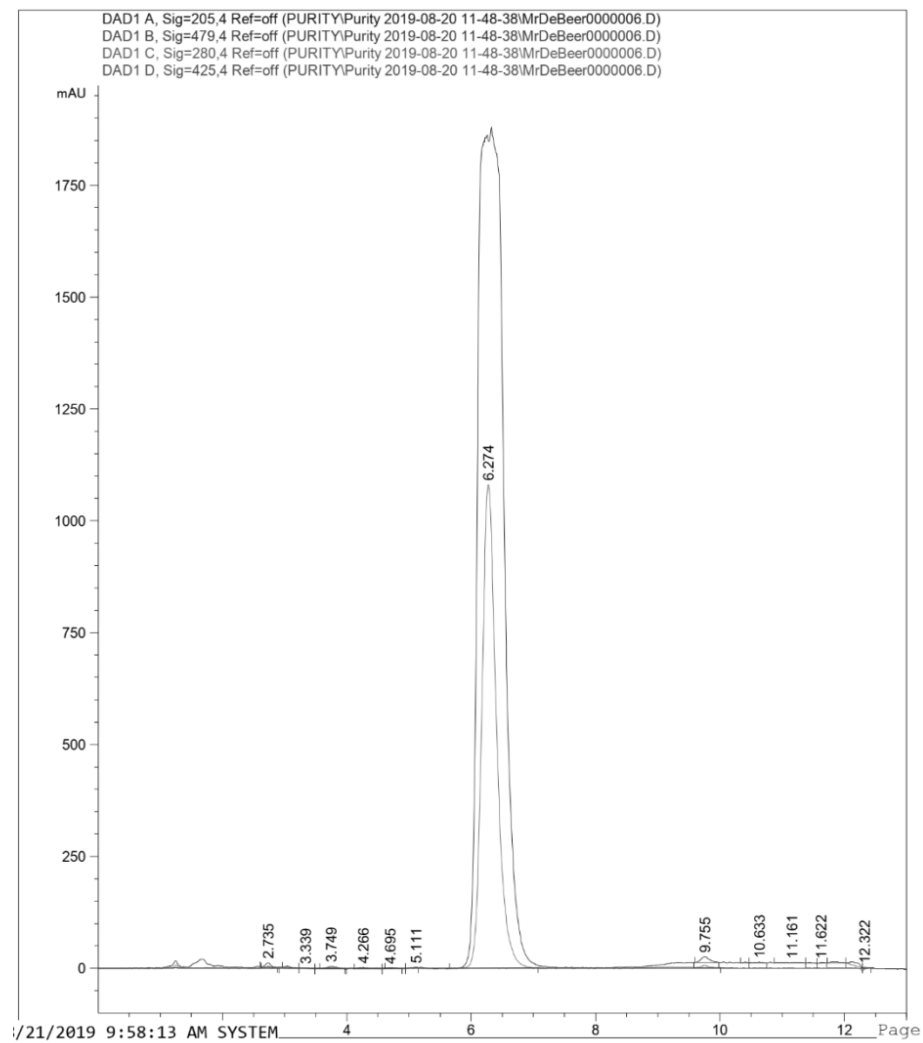
4-(4-fluorophenyl)-1*H*-pyrrolo[2,3-*b*]pyridine (18e):



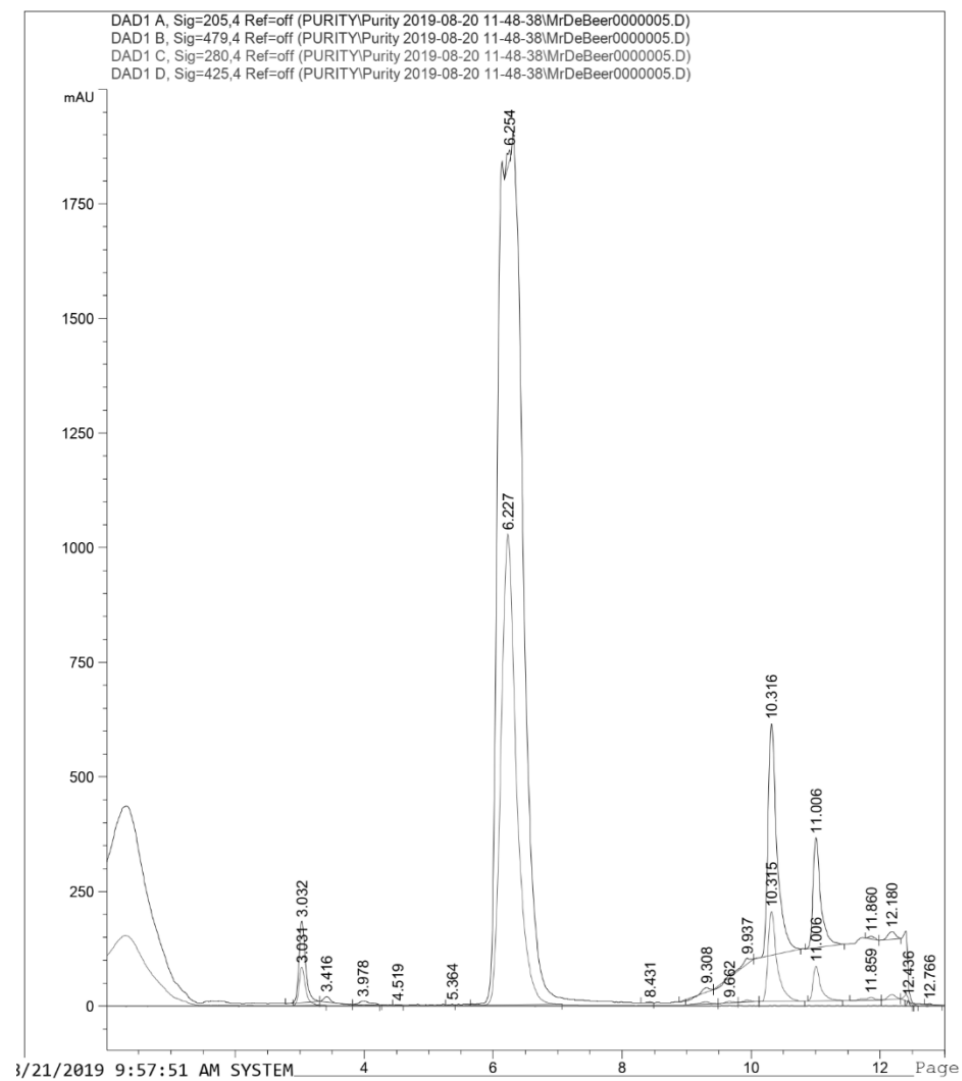
4-(3,4-difluorophenyl)-1*H*-pyrrolo[2,3-*b*]pyridine (18f):



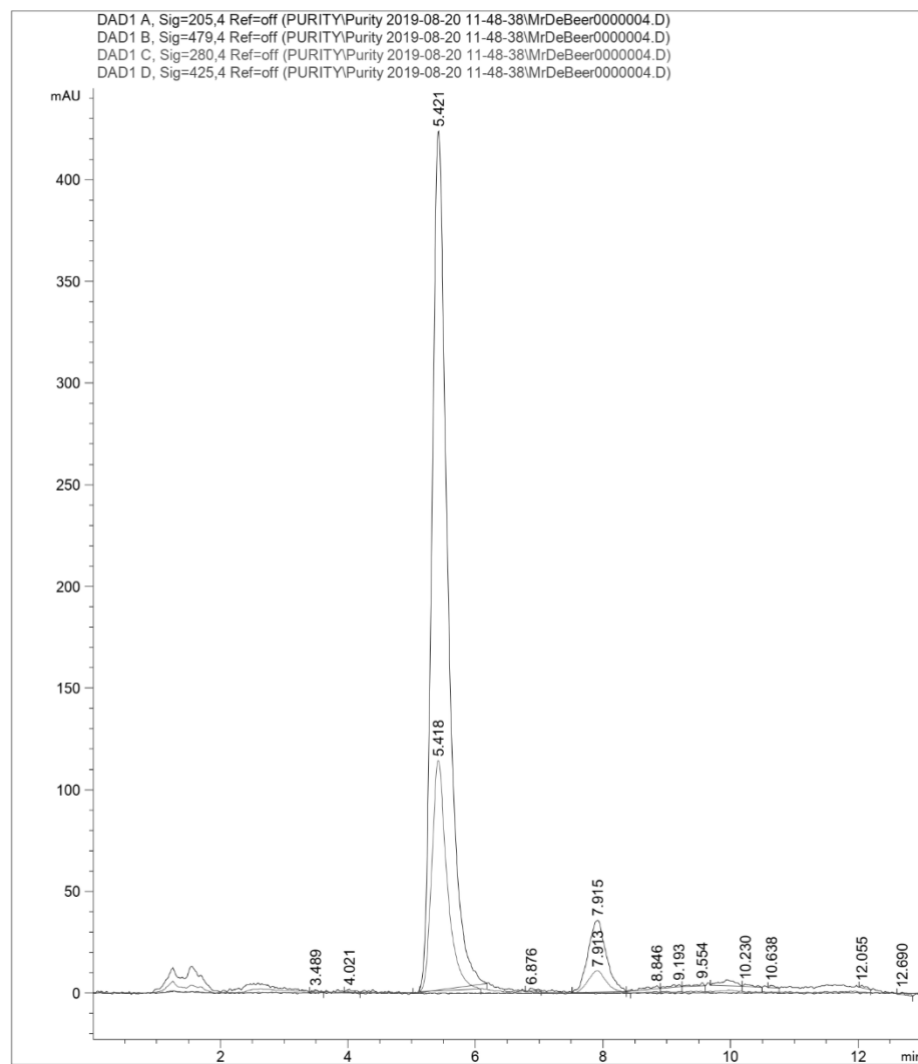
4-[4-(trifluoromethyl)phenyl]-1*H*-pyrrolo[2,3-*b*]pyridine (18g):



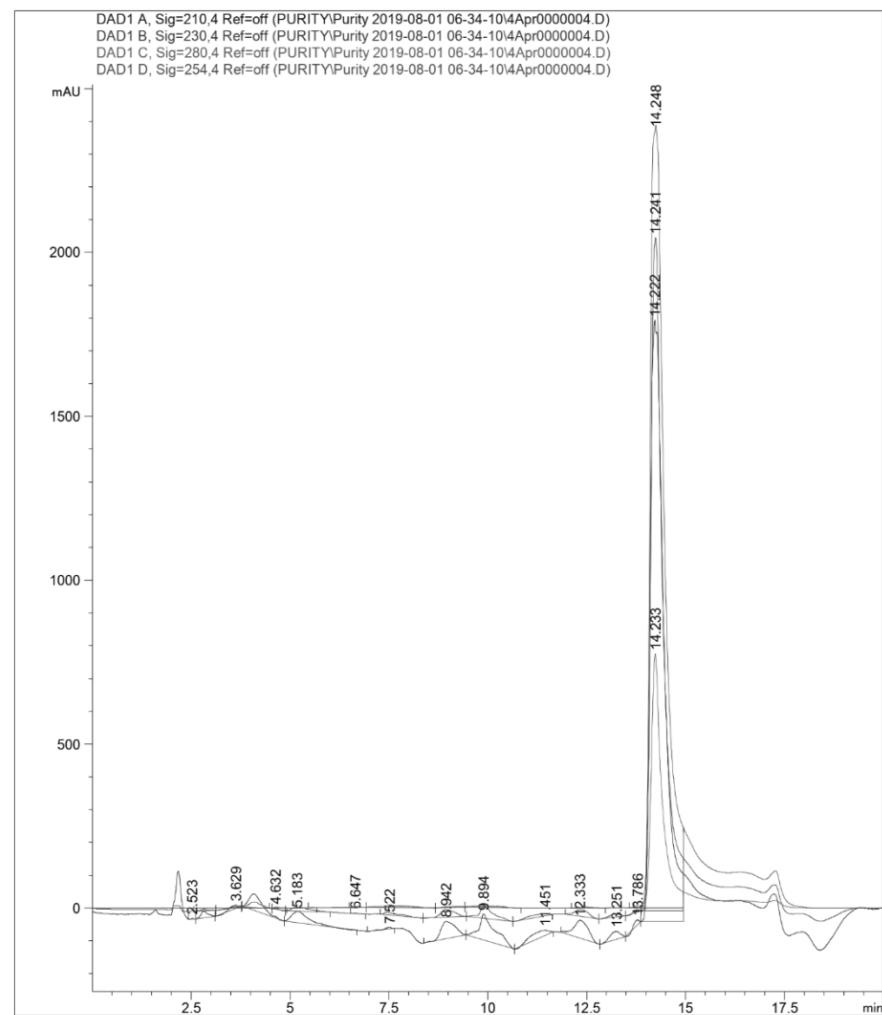
4-(4-chlorophenyl)-1*H*-pyrrolo[2,3-*b*]pyridine (18h):



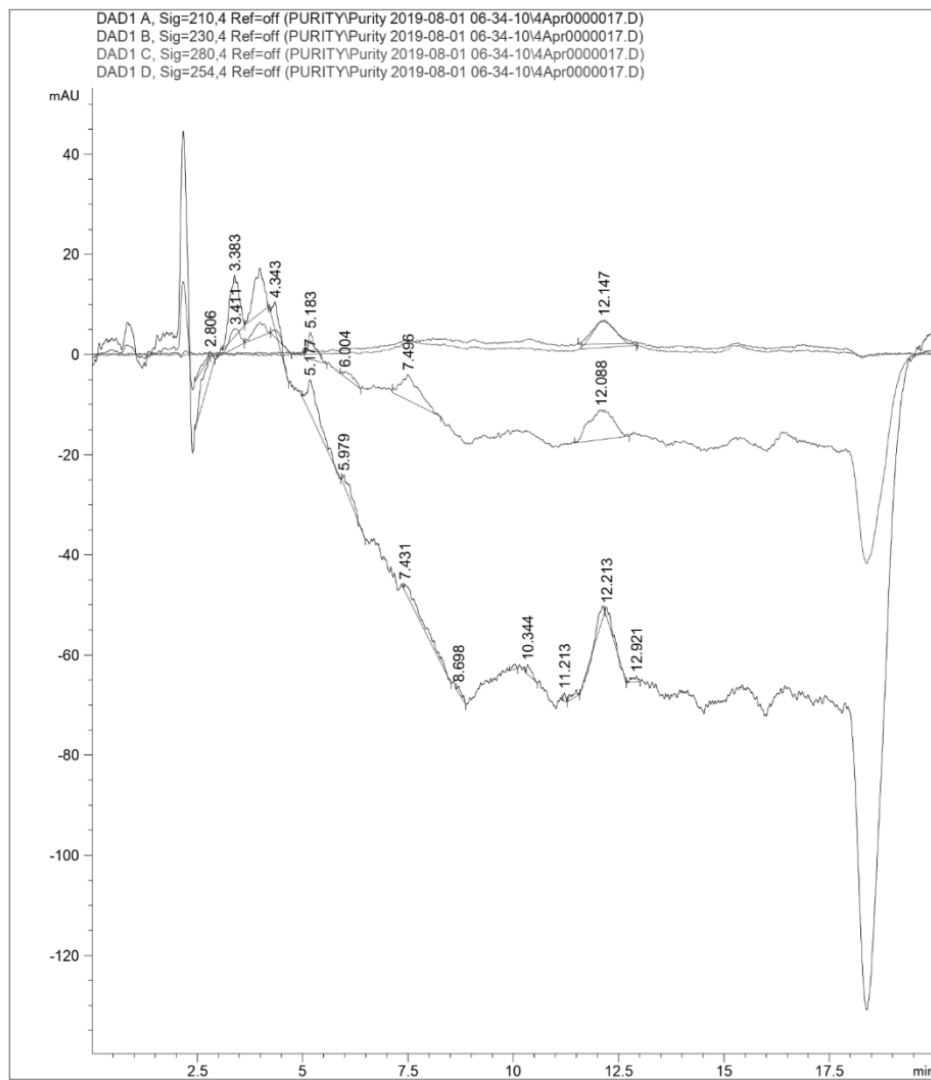
4-(biphenyl-4-yl)-1*H*-pyrrolo[2,3-*b*]pyridine (18i):



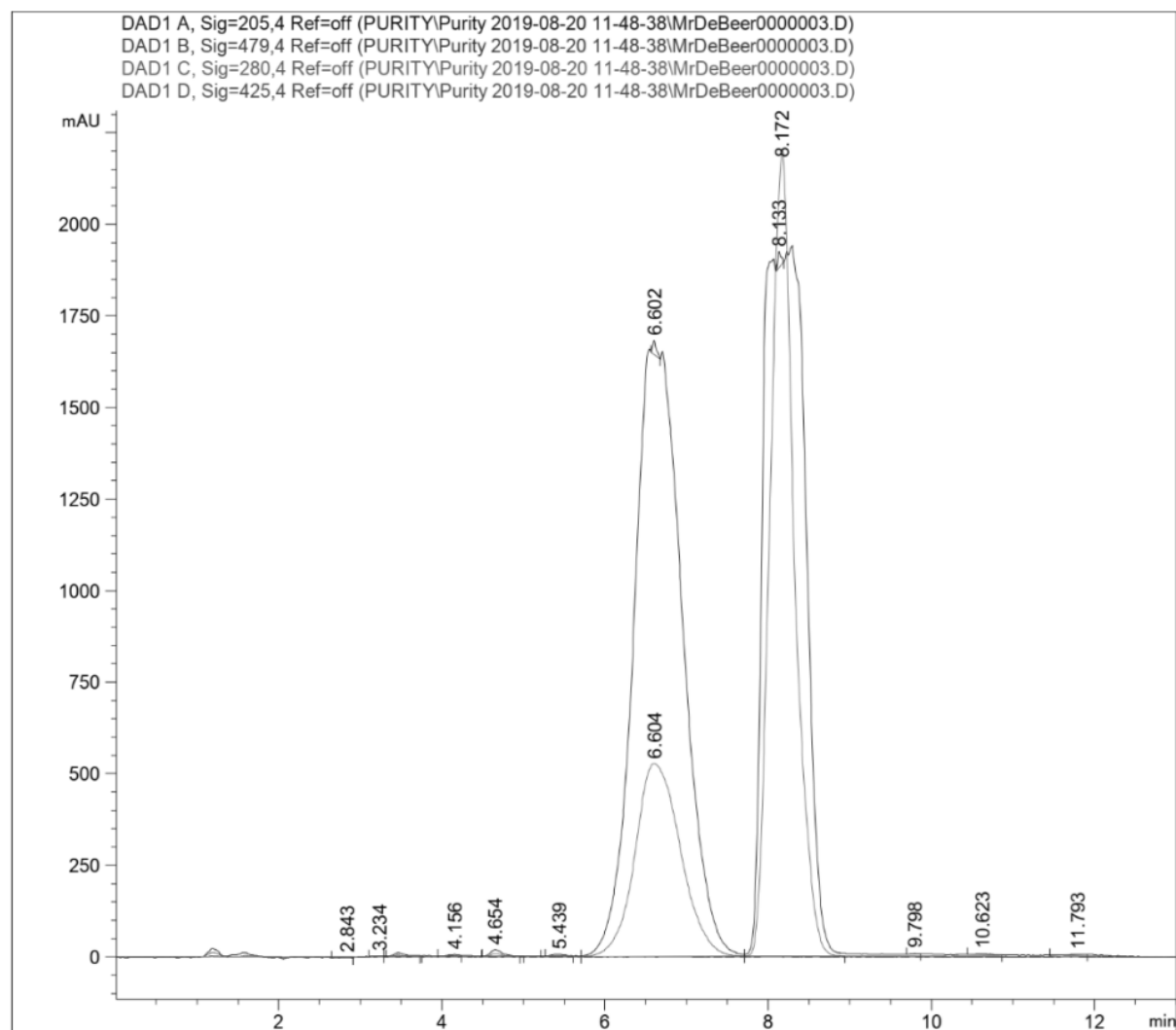
5-phenyl-1*H*-pyrrolo[2,3-*b*]pyridine (19a):



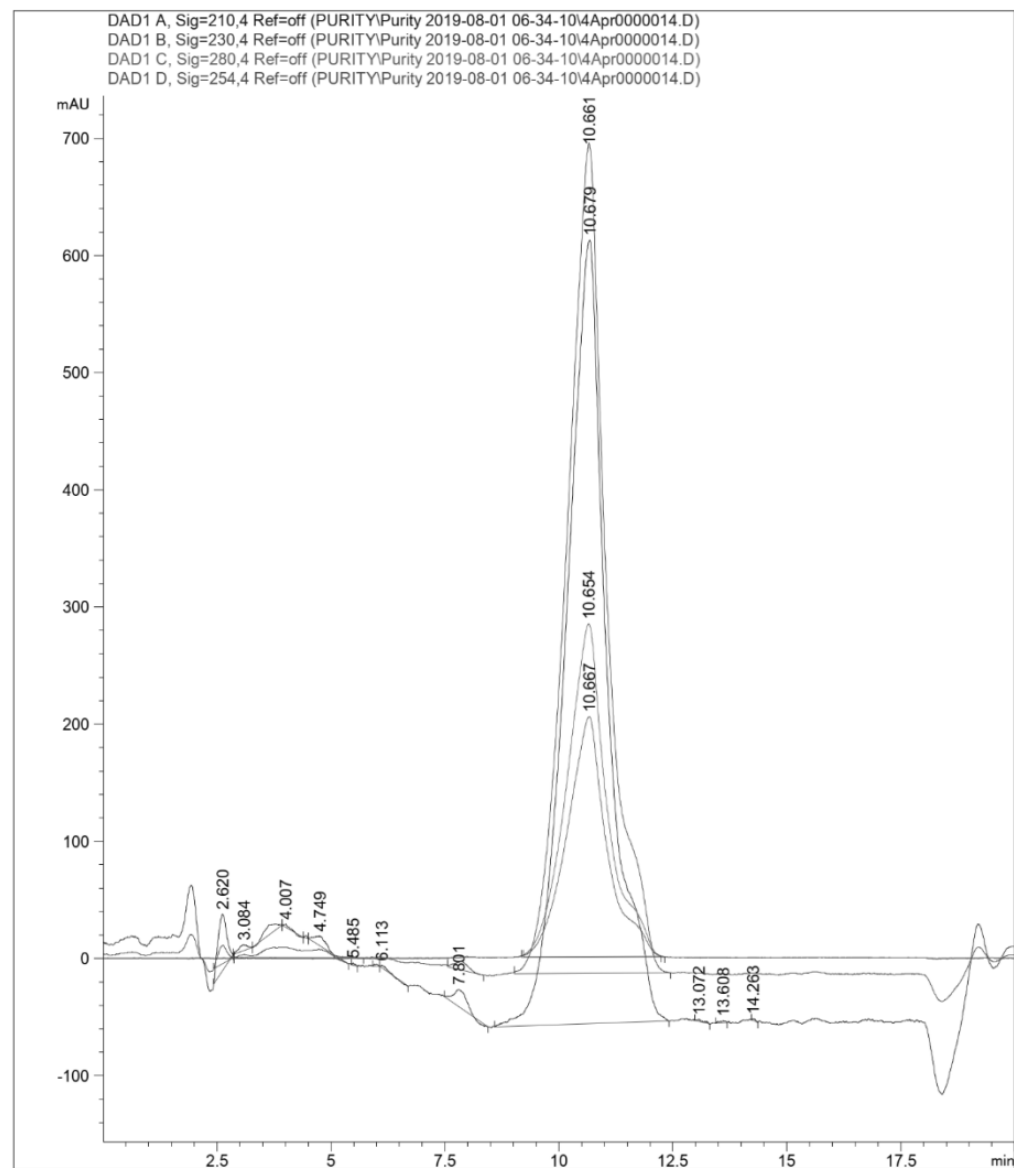
5-(4-methylphenyl)-1*H*-pyrrolo[2,3-*b*]pyridine (19b):



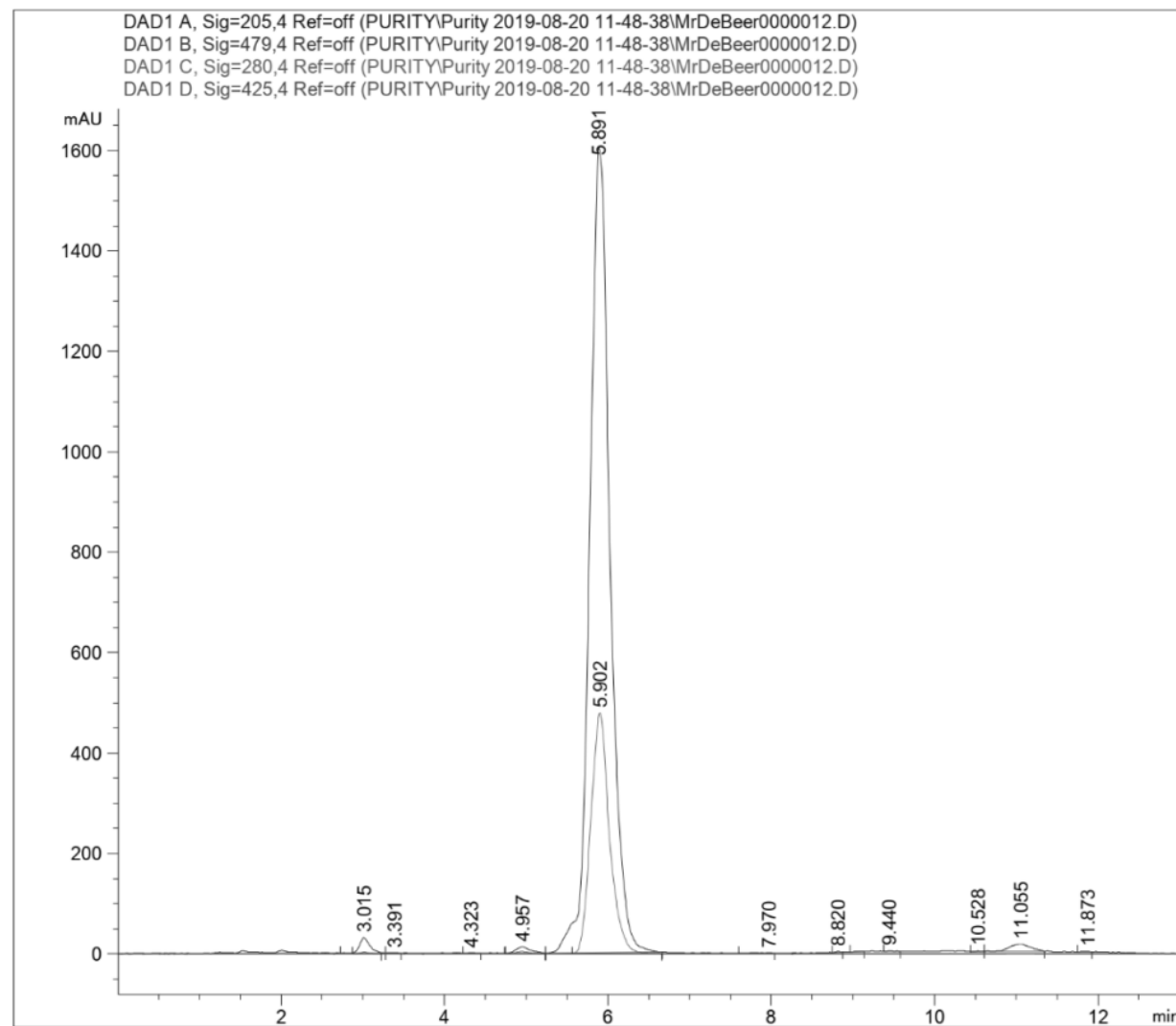
5-(3-methoxyphenyl)-1*H*-pyrrolo[2,3-*b*]pyridine (19c):



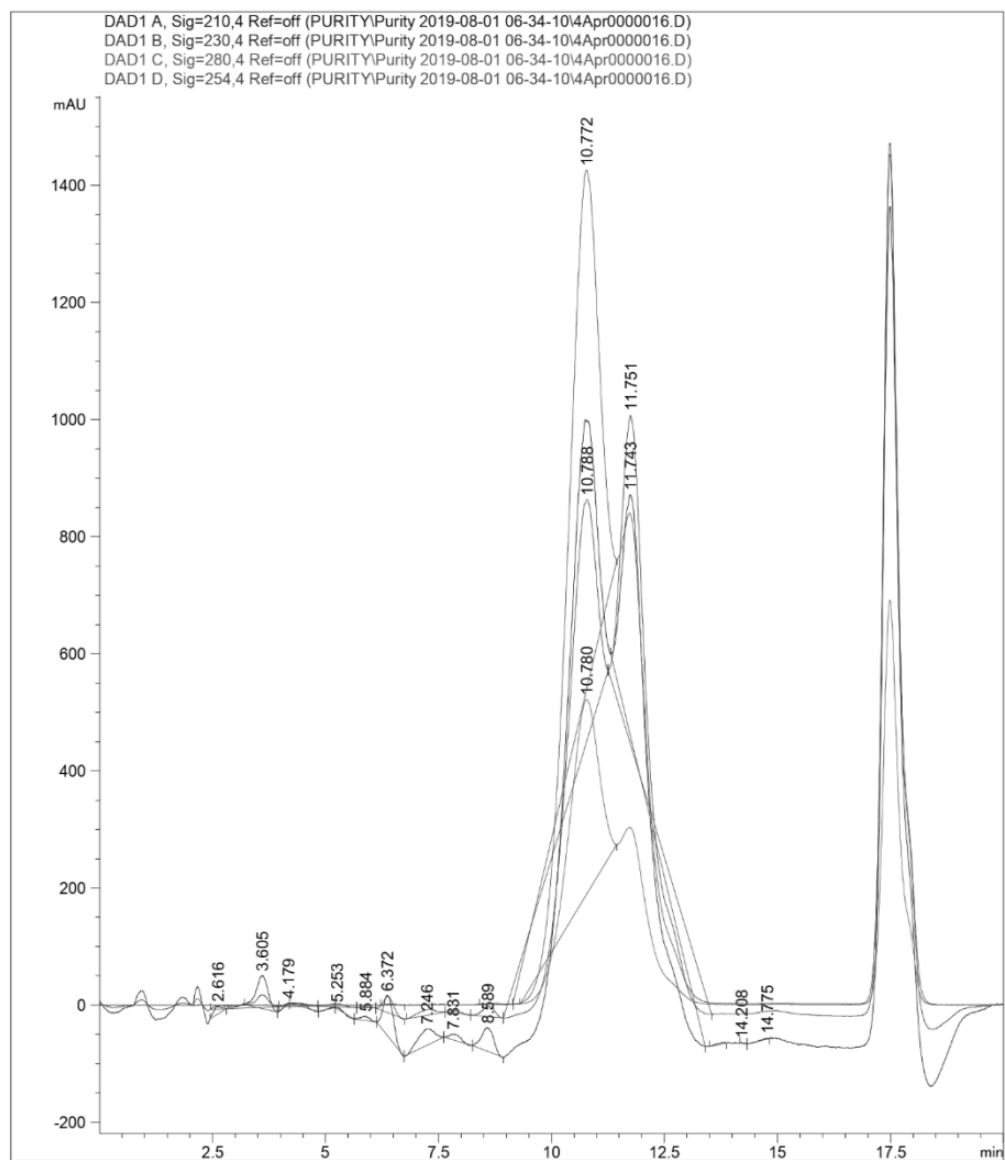
5-(4-methoxyphenyl)-1*H*-pyrrolo[2,3-*b*]pyridine (19d):



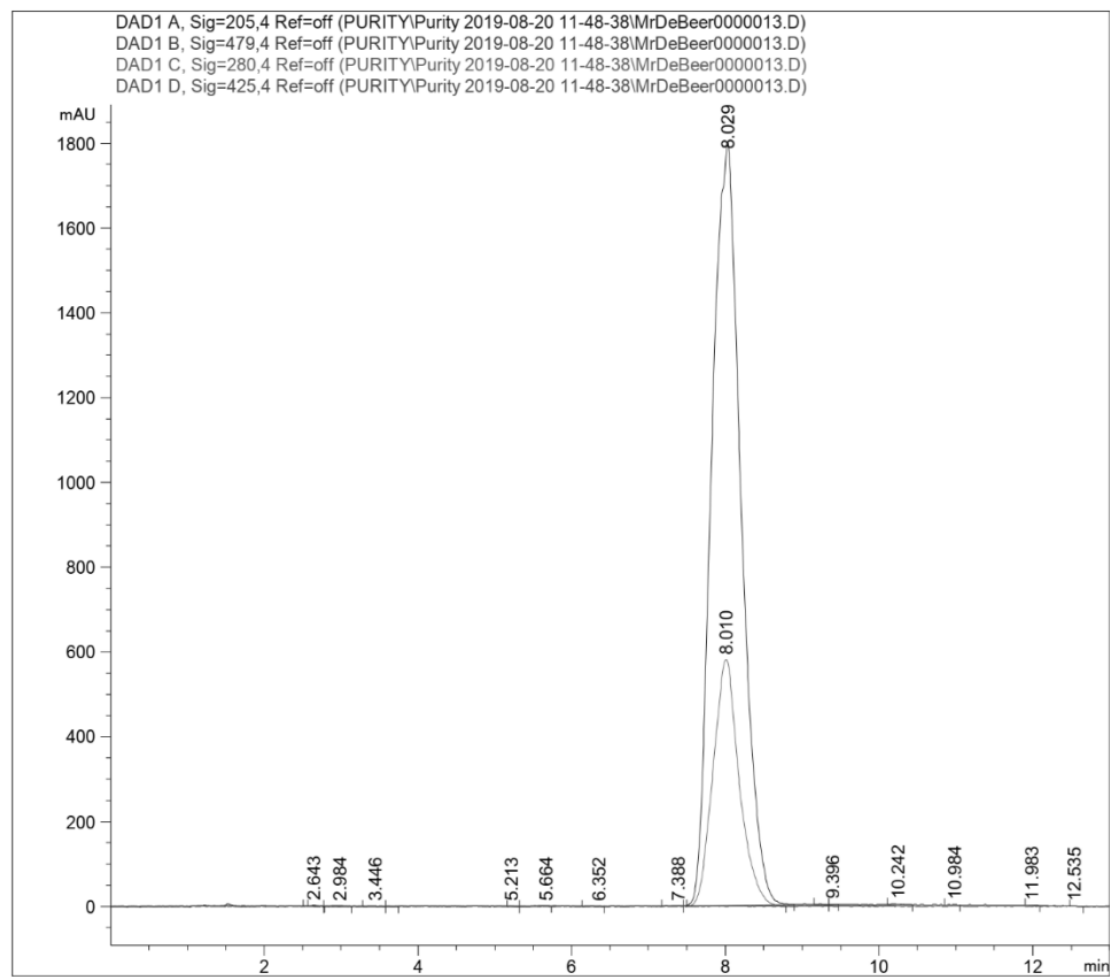
5-(2,3-dimethoxyphenyl)-1*H*-pyrrolo[2,3-*b*]pyridine (19e):



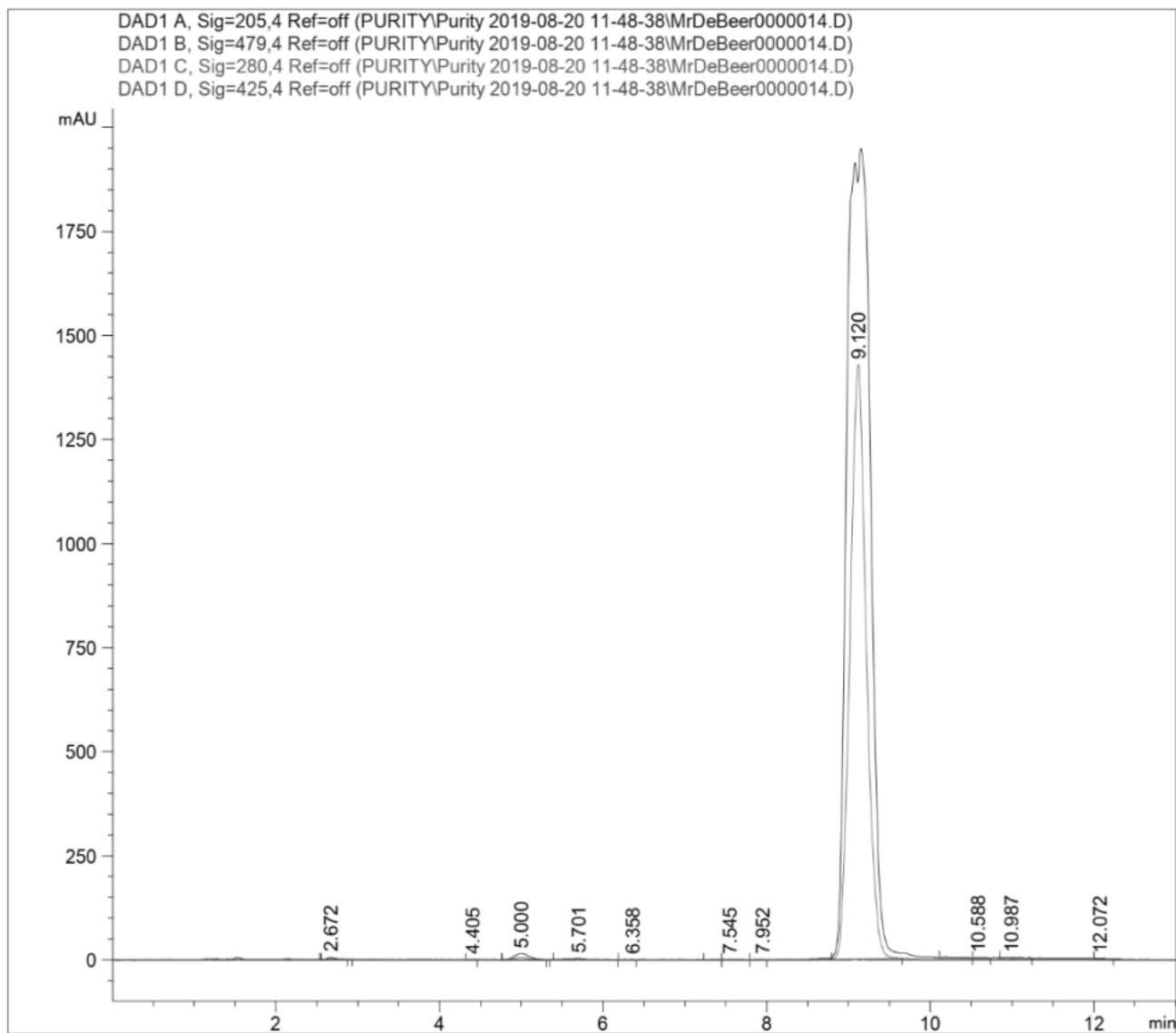
5-(3,4-dimethoxyphenyl)-1*H*-pyrrolo[2,3-*b*]pyridine (19f):



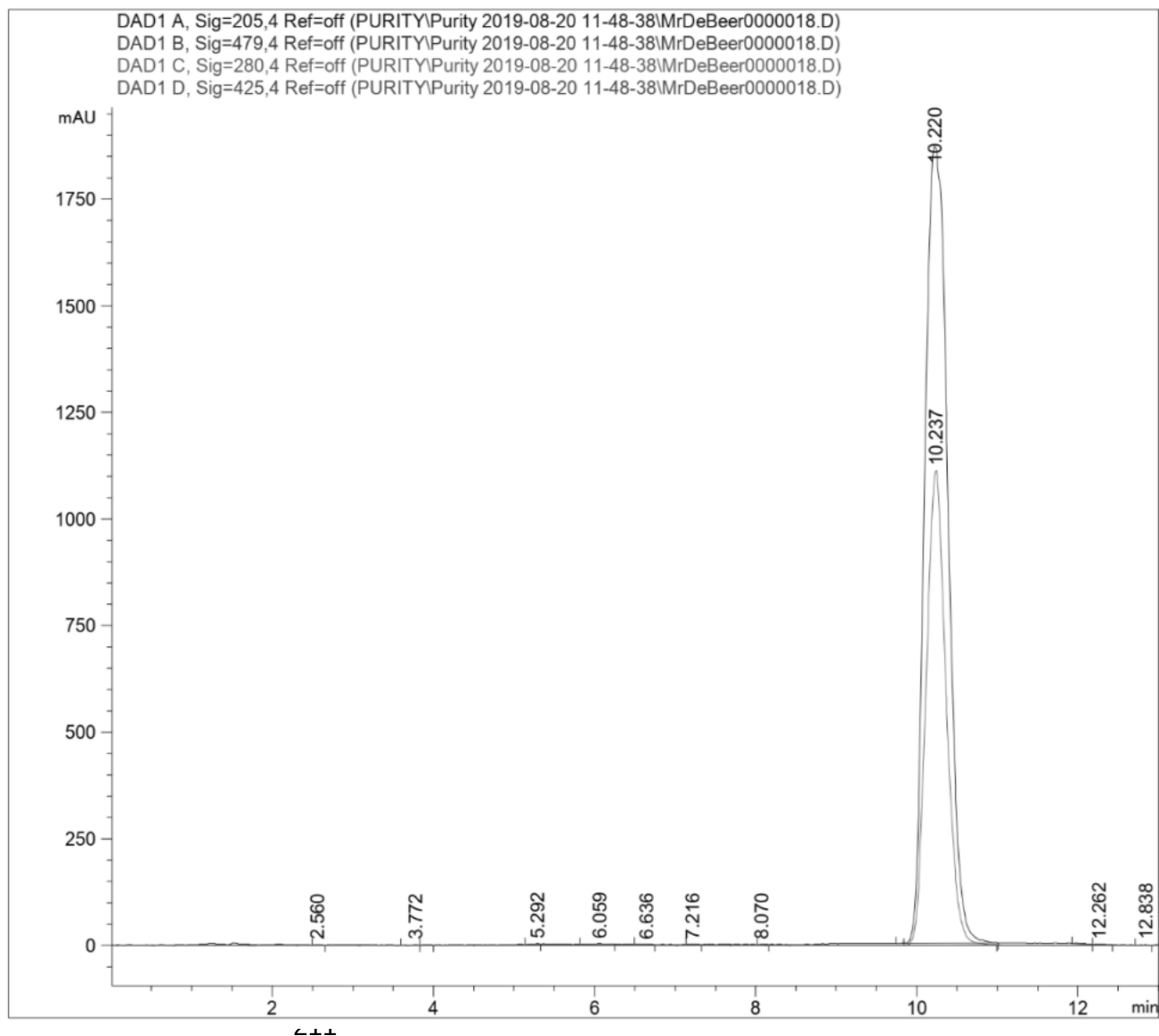
5-(4-fluorophenyl)-1*H*-pyrrolo[2,3-*b*]pyridine (19g):



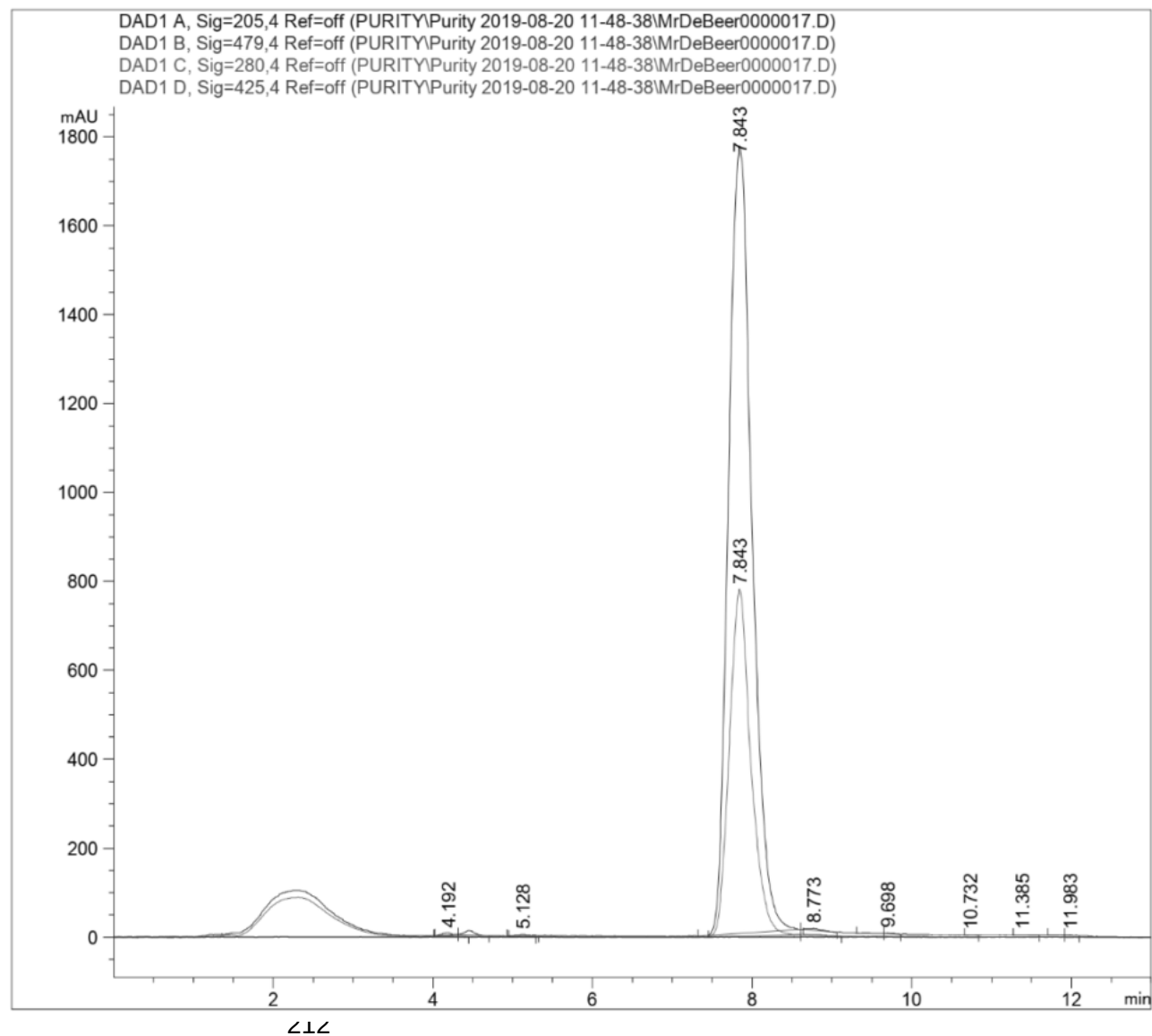
5-(3,4-difluorophenyl)-1*H*-pyrrolo[2,3-*b*]pyridine (19h):



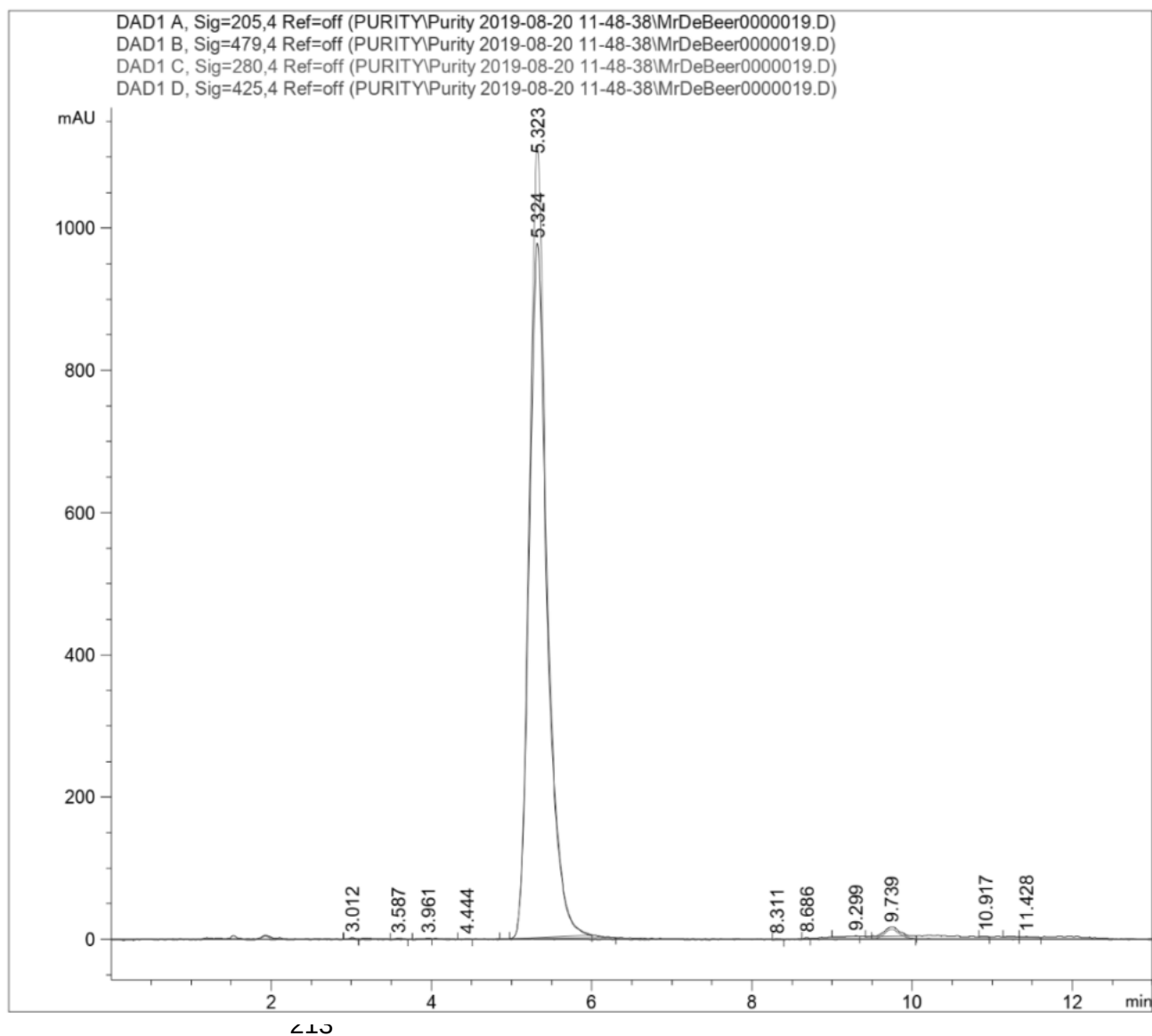
5-[4-(trifluoromethyl)phenyl]-1*H*-pyrrolo[2,3-*b*]pyridine (19i):



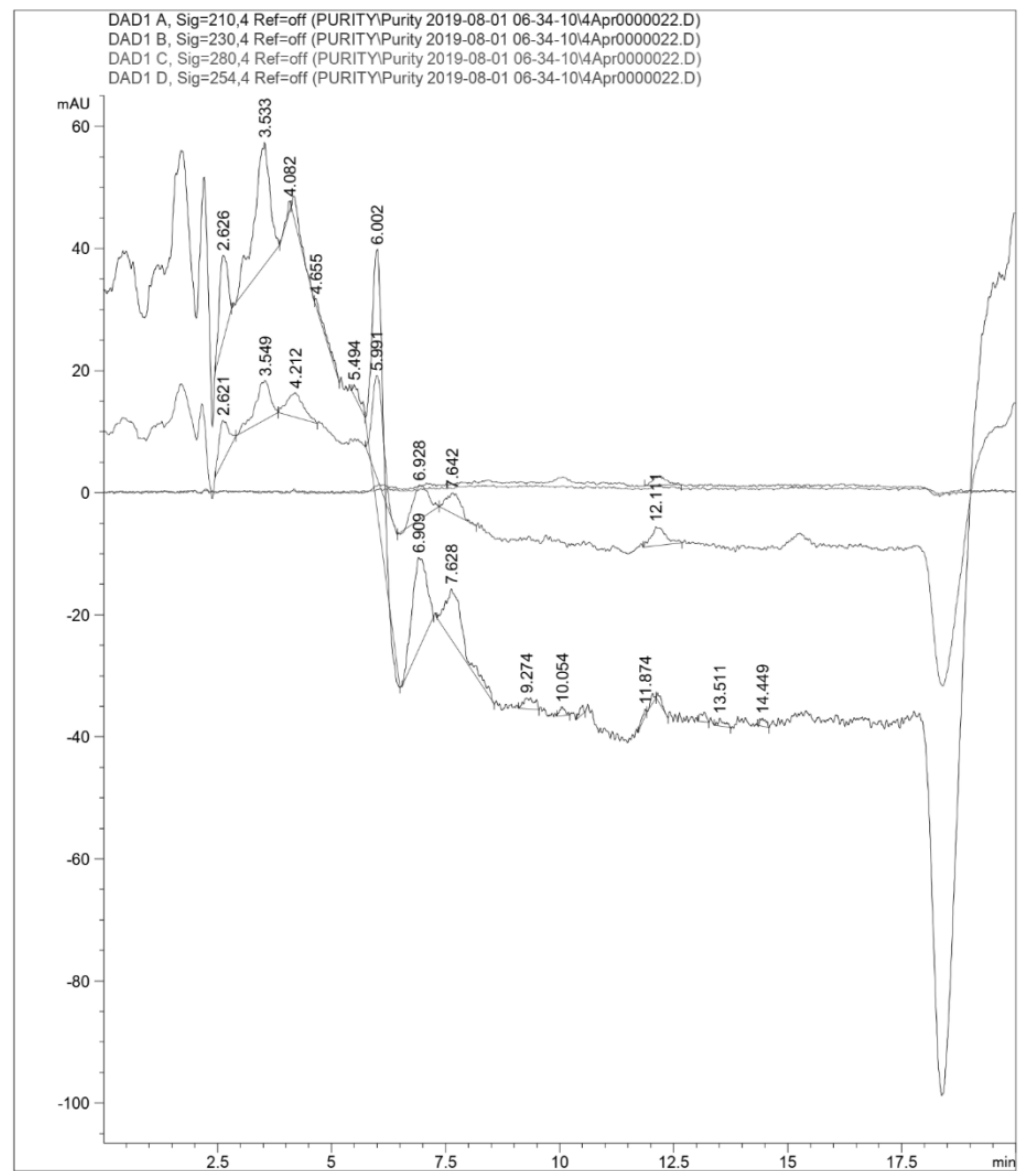
5-(4-chlorophenyl)-1*H*-pyrrolo[2,3-*b*]pyridine (19j):



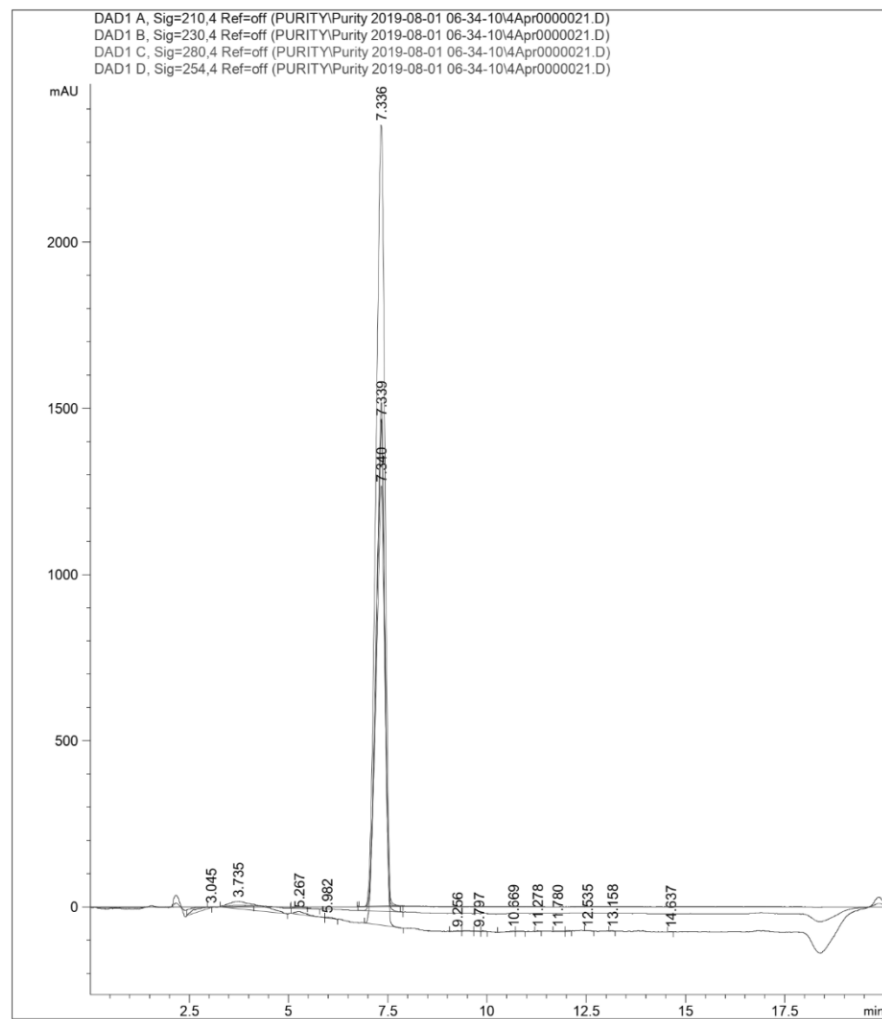
4-(1*H*-pyrrolo[2,3-*b*]pyridin-5-yl)benzonitrile (19k):



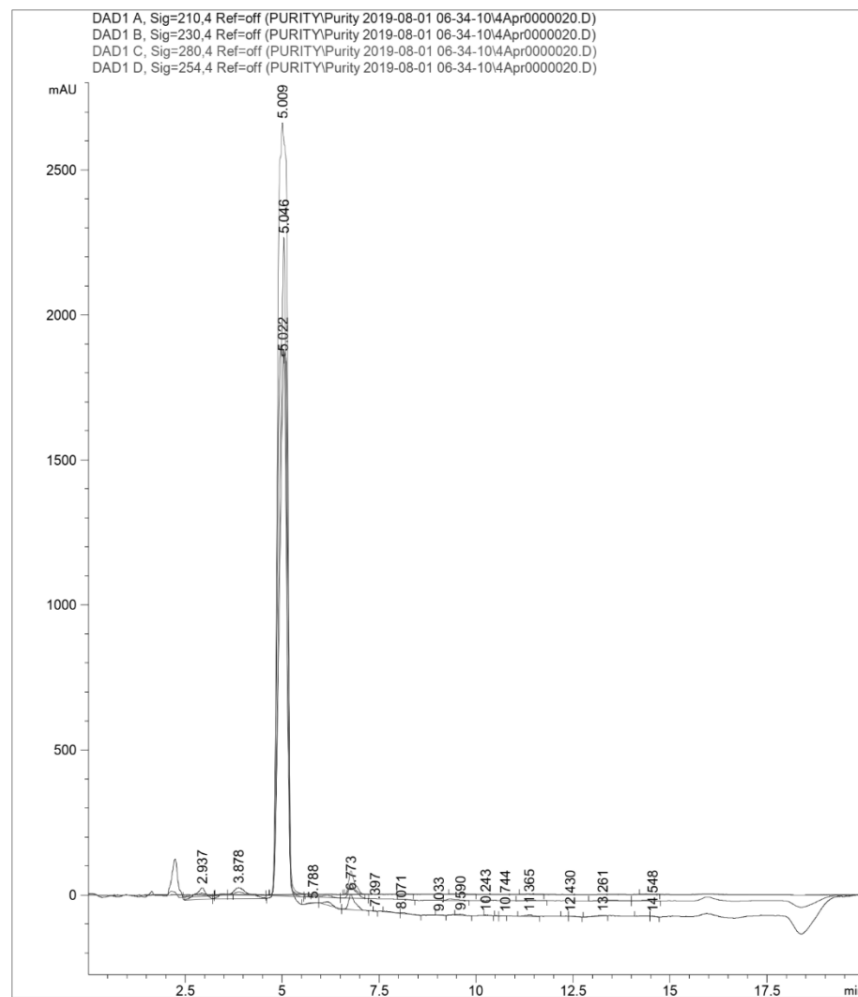
5-(biphenyl-4-yl)-1*H*-pyrrolo[2,3-*b*]pyridine (19l):



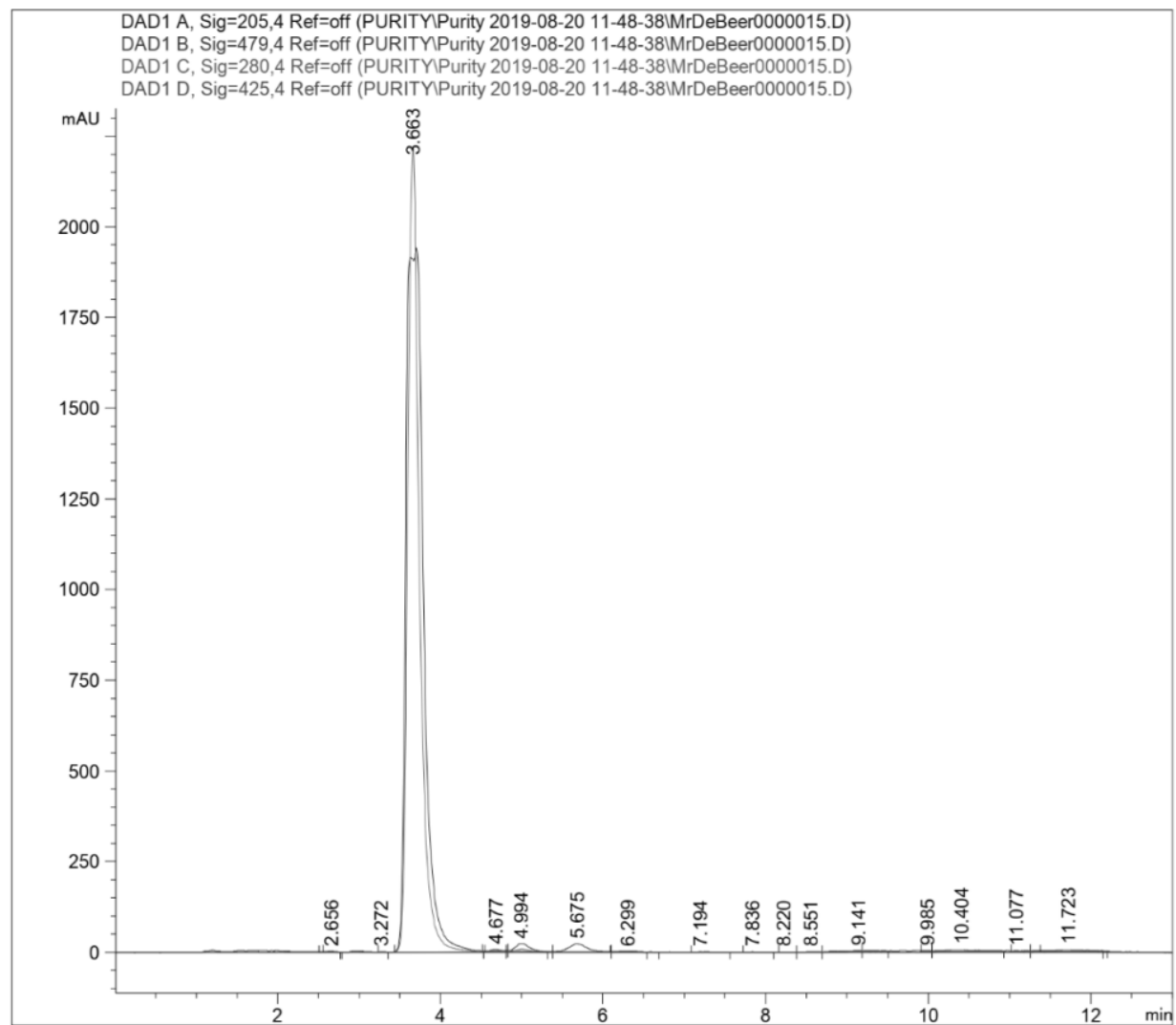
5-(pyridin-3-yl)-1*H*-pyrrolo[2,3-*b*]pyridine (19m):



5-(pyridin-4-yl)-1*H*-pyrrolo[2,3-*b*]pyridine (19n):



5-(6-fluoropyridin-3-yl)-1*H*-pyrrolo[2,3-*b*]pyridine (19o):



References:

Nguyen, T.-N.-D., Feizbakhsh, O., Sfecci, E., Baratte, B., Delehouzé, C., Garcia, A., . . . Mehiri, M. (2019). Kinase-Based Screening of Marine Natural Extracts Leads to the Identification of a Cytotoxic High Molecular Weight Metabolite from the Mediterranean Sponge *Crambe tailliezi*. *Mar. Drugs*, *17*, 569. doi:10.3390/md17100569

ANNEXURE E: EMAIL CONFIRMATION OF SUBMISSION OF MANUSCRIPT TO CHEMICAL BIOLOGY AND DRUG DESIGN BY THE CORRESPONDING AUTHOR



Lesetja Legoabe

to me ▾

9:57 PM (4 minutes ago)



FYI

NWU CORONA VIRUS: <http://www.nwu.ac.za/coronavirus/>

Vrywaringsklousule / Disclaimer: <http://www.nwu.ac.za/it/gov-man/disclaimer.html>

>>> Chemical Biology & Drug Design <no-reply@wiley.com> 2020/12/17 21:46 >>>

Dear Lesetja Legoabe,

Your manuscript entitled "Synthesis and biological evaluation of selected 7 H -pyrrolo[2,3- d]pyrimidine derivatives as novel CDK9/CyclinT and Haspin inhibitors." has been successfully submitted online and is being delivered to the Editorial Office of *Chemical Biology & Drug Design* for consideration.

You will receive a follow-up email with further instructions from our electronic editorial office platform, ScholarOne, typically within one business day. That message will confirm that the Editorial Office has received your submission and will provide your Manuscript ID.

Thank you for submitting your manuscript to *Chemical Biology & Drug Design*

Sincerely,

The Editorial Staff at Chemical Biology & Drug Design

By submitting a manuscript to or reviewing for this publication, your name, email address, and affiliation, and other contact details the publication might require, will be used for the regular operations of the publication, including, when necessary, sharing with the publisher (Wiley) and partners for production and publication. The publication and the publisher recognize the importance of protecting the personal information collected from users in the operation of these services and have practices in place to ensure that steps are taken to maintain the security, integrity, and privacy of the personal data collected and processed. You can learn more by reading our [data protection policy](#). In case you don't want to be contacted by this publication again, please send an email to cbddeditorial@wiley.com.

ANNEXURE F: CHEMICAL BIOLOGY AND DRUG DESIGN AUTHOR GUIDELINES

Sections

1. Submission
2. Aims and Scope
3. Manuscript Categories and Requirements
4. Preparing the Submission
5. Editorial Policies and Ethical Considerations
6. Author Licensing
7. Publication Process After Acceptance
8. Offprints
9. Post Publication
10. Editorial Office Contact Details

1. SUBMISSION

Authors should kindly note that submission implies that the content has not been published or submitted for publication elsewhere except as a brief abstract in the proceedings of a scientific meeting or symposium.

Once the submission materials have been prepared in accordance with the Author Guidelines, manuscripts should be submitted online.

New submissions should be made via the Research Exchange submission portal at <https://submission.wiley.com/journal/CBDD>.

For technical help with the submission system, please review our [FAQs](#) or contact submissionhelp@wiley.com.

Free Format Submission

Chemical Biology & Drug Design now offers free format submission for a simplified and streamlined submission process.

Before you submit, you will need:

- Your manuscript: this can be a single file including text, figures, and tables, or separate files – whichever you prefer. All required sections should be contained in your manuscript, including abstract, introduction, methods, results, and conclusions. Figures and tables should have legends. References may be submitted in any style or format, as long as it is consistent throughout the manuscript. If the manuscript, figures or tables are difficult for you to read, they will also be difficult for the editors and reviewers. If your manuscript is difficult to read, the editorial office may send it back to you for revision.

- the title page of the manuscript, including statements relating to our ethics and integrity policies:
 - data availability statement
 - funding statement
 - conflict of interest disclosure
 - ethics approval statement
 - patient consent statement
 - permission to reproduce material from other sources
 - clinical trial registration
- Your co-author details, including affiliation and email address. *(Why is this important? We need to keep all co-authors informed of the outcome of the peer review process.)*
 - An ORCID ID, freely available at <https://orcid.org>. *(Why is this important? Your article, if accepted and published, will be attached to your ORCID profile. Institutions and funders are increasingly requiring authors to have ORCID IDs.)*

Data Protection

By submitting a manuscript to or reviewing for this publication, your name, email address, and affiliation, and other contact details the publication might require, will be used for the regular operations of the publication, including, when necessary, sharing with the publisher (Wiley) and partners for production and publication. The publication and the publisher recognize the importance of protecting the personal information collected from users in the operation of these services, and have practices in place to ensure that steps are taken to maintain the security, integrity, and privacy of the personal data collected and processed. You can learn more at <https://authorservices.wiley.com/statements/data-protection-policy.html>.

Open Access

This journal is a subscription journal that offers OnlineOpen, an open access option. You'll have the option to choose to make your article open access after acceptance, which will be subject to an APC. You can [read more about APCs](#) and whether you may be eligible for waivers or discounts, through your institution, funder, or a country waiver.

Preprint Policy

Wiley believes that journals publishing for communities with established pre-print servers should allow authors to submit manuscripts which have already been made available on a non-commercial preprint server. Allowing submission does not, of course, guarantee that an article will be sent out for review. It simply reflects our belief that journals should not rule out reviewing a paper simply because it has already been available on a non-commercial server. Please see below for the specific policy language.

However, Wiley also knows that the use of preprint servers is not universally accepted and that individual journals and/or societies may approach submission of preprints differently:

This journal will consider for review articles previously available as preprints on non-commercial servers such as ArXiv, bioRxiv, psyArXiv, SocArXiv, engrXiv, etc. Authors may also post the submitted version of a manuscript to non-commercial servers at any time. Authors are requested to update any pre-publication versions with a link to the final published article.

Article Preparation Support

[Wiley Editing Services](#) offers expert help with English Language Editing, as well as translation, manuscript formatting, figure illustration, figure formatting, and graphical abstract design – so you can submit your manuscript with confidence. Also, check out our resources for [Preparing Your Article](#) for general guidance about writing and preparing your manuscript.

For help with submissions, please contact: cbddeditorial@wiley.com

2. AIMS AND SCOPE

Chemical Biology & Drug Design is a peer-reviewed scientific journal that is dedicated to the advancement of innovative science, technology and medicine with a focus on the multidisciplinary fields of chemical biology and drug design. It is the aim of *Chemical Biology & Drug Design* to capture significant research and drug discovery that highlights new concepts, insight and new findings within the scope of chemical biology and drug design.

The following scientific topics will exemplify the scope of the journal:

- Protein, peptide, peptidomimetic, nucleic acid, lipids, carbohydrate and natural product chemical biology and drug design
- Receptor agonist/antagonist, protease substrate/inhibitor, and signal transduction modulator chemical biology and drug design
- Small-molecule diversity and synthetic methods important to investigate chemical space relationships to drug discovery
- Structure-based drug design, molecular modelling, virtual screening and related in silico chemoinformatics technologies
- NMR, X-ray crystallography, calorimetry and related biophysical technologies
- Therapeutic target proteomics, chemical genomics and molecular screening technologies
- Emerging chemical, biological and drug design concepts of significance to pharmacology, ADMET and drug delivery
- Novel proof-of-concept ligands and breakthrough medicines (CB&DD thematic issues)

3. MANUSCRIPT CATEGORIES AND REQUIREMENTS

Chemical Biology & Drug Design welcomes correspondence in the following categories:

- **Reviews and Perspectives** will be focused on a significantly important theme to chemical biology and drug design. Reviews should incorporate judicious use of tables, figures, and schemes. Perspectives are short reviews on a narrow topic.
- **Research Articles** will provide detailed description of scientific findings within the scope of the journal and be formatted according to the guidance highlighted below with respect to subheadings, inclusion of tables and/or figures, reference style, etc.
- **Research Letters** will be a short version of a Research Article, but should be continuous text (subheadings for 'Results and Discussion' and 'Conclusions and Future Directions' are optional).
- **Commentaries** will be invited by the Editors to provide focused viewpoints of specific topics of interest, and written as continuous text with no subheadings.
- **Tools and protocols in chemical biology** - The central idea of this new journal section is to assemble a definitive collection of protocols and methods that can be a useful and reliable resource for the scientific community going forward. These could be completely new ideas or optimised methods for existing tools. The methodology must take centre stage - this should pay special attention to the key transformations, methods or computational aspects. The focus should be on reproducibility. The data does not lie, it's our interpretation of it that is flawed. The structure should follow:

- Introduction (authors should include a description of how the method/protocol supplied is optimised, or more reproducible than previous report).
- Protocols (includes full details of the key transformation or method).
- Results (to illustrate the success of the method).
- Discussion (final conclusions and relevance to the scientific field).

4. PREPARING THE SUBMISSION

New submissions should be made via the Research Exchange submission portal at <https://submission.wiley.com/journal/CBDD>.

For technical help with the submission system, please review our [FAQs](#) or contact submissionhelp@wiley.com.

Parts of the Manuscript

Chemical Biology & Drug Design now offers [free format submission](#) for a simplified and streamlined submission process.

For the initial submission, the manuscript can be a single file including text, figures, and tables, or separate files – whichever you prefer. All required sections, including abstract, introduction, methods, results, and conclusions, should be contained in your manuscript. Figures and tables should have legends. References may be submitted in any style or format, as long as it is consistent throughout the manuscript.

For revised submissions, the manuscript should be submitted as a single pdf file containing the main text, tables and figures as, outlined in the following sections. On acceptance, separate files and high quality image files of the figures will be required.

Main Text File

The text file should be presented in the following order:

- i. A short informative title containing the major key words. The title should not contain abbreviations (see Wiley's [best practice SEO tips](#));
- ii. A short running title of less than 50 characters;
- iii. The full names of the authors;
- iv. The author's institutional affiliations where the work was conducted, with a footnote for the author's present address if different from where the work was conducted;
- v. Acknowledgments;
- vi. Abstract and keywords;
- vii. Main text;
- viii. References;
- ix. Tables (each table complete with title and footnotes);
- x. Figure legends;
- xi. Appendices (if relevant).

Figures and supporting information should be supplied as separate files.

Authorship

Please refer to the journal's Authorship policy in the [Editorial Policies and Ethical Considerations](#) section for details on author listing eligibility.

Acknowledgments

Contributions from anyone who does not meet the criteria for authorship should be listed, with permission from the contributor, in an Acknowledgments section. Financial and material support should also be mentioned. Thanks to anonymous reviewers are not appropriate.

Conflict of Interest Statement

Authors will be asked to provide a conflict of interest statement during the submission process. For details on what to include in this section, see the 'Conflict of Interest' section in the [Editorial Policies and Ethical Considerations](#) section below. Submitting authors should ensure they liaise with all co-authors to confirm agreement with the final statement.

Abstract

Please provide an abstract of no more than 200 words containing the major keywords and a sentence that clarifies the novelty of the manuscript and its place in the field.

Keywords

Please provide between 5 and 10 keywords.

Main Text

- The journal uses British spelling; however, authors may submit using either option, as spelling of accepted papers is converted during the production process.
- Footnotes to the text are not allowed and any such material should be incorporated into the text as parenthetical matter.

References

References should be prepared according to the *Publication Manual of the American Psychological Association* (6th edition). This means in text citations should follow the author-date method whereby the author's last name and the year of publication for the source should appear in the text, for example, (Jones, 1998). The complete reference list should appear alphabetically by name at the end of the paper. Please note that for journal articles, issue numbers are not included unless each issue in the volume begins with page 1, and a DOI should be provided for all references where available.

Reference examples follow:

Journal article

Beers, S. R. , & De Bellis, M. D. (2002). Neuropsychological function in children with maltreatment-related posttraumatic stress disorder. *The American Journal of Psychiatry*, 159, 483–486.

doi:[10.1176/appi.ajp.159.3.483](https://doi.org/10.1176/appi.ajp.159.3.483)

Book

Bradley-Johnson, S. (1994). *Psychoeducational assessment of students who are visually impaired or blind: Infancy through high school* (2nd ed.). Austin, TX: Pro-ed.

Internet Document

Norton, R. (2006, November 4). How to train a cat to operate a light switch [Video file]. Retrieved from <http://www.youtube.com/watch?v=Vja83KLQXZs>

Tables

Tables should be self-contained and complement, not duplicate, information contained in the text. They should be supplied as editable files, not pasted as images. Legends should be concise but comprehensive – the table, legend, and footnotes must be understandable without reference to the text. All abbreviations must be defined in footnotes. Footnote symbols: †, ‡, §, ¶, should be used (in that order) and *, **, *** should be reserved for P-values. Statistical measures such as SD or SEM should be identified in the headings.

Figure Legends

Legends should be concise but comprehensive – the figure and its legend must be understandable without reference to the text. Include definitions of any symbols used and define/explain all abbreviations and units of measurement.

Figures

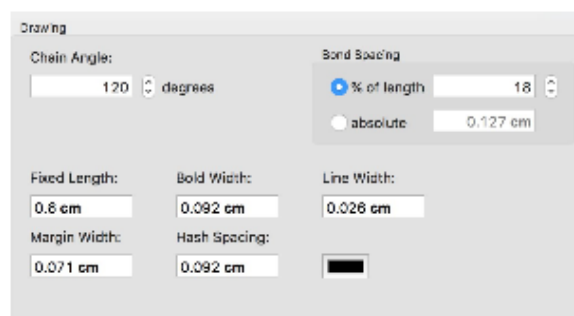
Although authors are encouraged to send the highest-quality figures possible, for peer-review purposes, a wide variety of formats, sizes, and resolutions are accepted.

[Click here](#) for the basic figure requirements for figures submitted with manuscripts for initial peer review, as well as the more detailed post-acceptance figure requirements.

Colour figures. Figures submitted in colour may be reproduced in colour online free of charge. Please note, however, that it is preferable that line figures (e.g. graphs and charts) are supplied in black and white so that they are legible if printed by a reader in black and white. If an author would prefer to have figures printed in colour in hard copies of the journal, a fee will be charged by the Publisher.

Figures: These should be at a resolution of at least 300 dpi at final size.

Chemical structures: should be prepared using a chemical drawing program (e.g., ChemDraw) as standard practice and saved as TIFF or EPS files in submitting along with Tables and manuscript (text and references). In Chemdraw select object, apply object settings from, and chose Wiley document. Settings are as shown in the screenshot below



Data Citation

In recognition of the significance of data as an output of research effort, Wiley has endorsed [the FORCE11 Data Citation Principles](#) and is implementing a mandatory data citation policy. Wiley journals require data to be cited in the same way as article, book, and web citations and authors are required to include data citations as part of their reference list.

Data citation is appropriate for data held within institutional, subject focused, or more general data repositories. It is not intended to take the place of community standards such as in-line citation of GenBank accession codes.

When citing or making claims based on data, authors must refer to the data at the relevant place in the manuscript text and in addition provide a formal citation in the reference list. We recommend the format proposed by the [Joint Declaration of Data Citation Principles](#):

[dataset] Authors; Year; Dataset title; Data repository or archive; Version (if any); Persistent identifier (e.g. DOI)

Appendices

Appendices will be published after the references. For submission they should be supplied as separate files but referred to in the text.

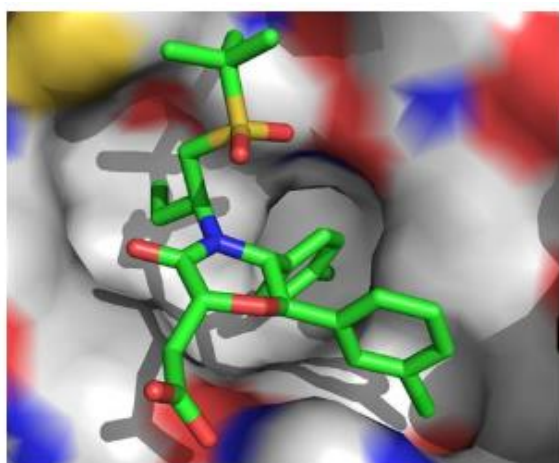
Graphical Table of Contents

The journal's table of contents will be presented in graphical form with a brief abstract.

The table of contents entry must include the article title, the authors' names (with the corresponding author indicated by an asterisk), no more than 80 words or 3 sentences of text summarising the key findings presented in the paper and a figure that best represents the scope of the paper (see the section on abstract writing for more guidance).

Table of contents entries should be submitted to Research Exchange in one of the generic file formats and uploaded as 'Supplementary material for review' during the initial manuscript submission process.

The image supplied should fit within the dimensions of 50mm x 60mm, and be fully legible at this size. See below for a suitable example from [Chen et al. 2018](#):



Supporting Information

Supporting information is information that is not essential to the article, but provides greater depth and background. It is hosted online and appears without editing or typesetting. It may include tables, figures, videos, datasets, etc.

[Click here](#) for Wiley's FAQs on supporting information.

Note: if data, scripts, or other artefacts used to generate the analyses presented in the paper are available via a publicly available data repository, authors should include a reference to the location of the material within their paper.

General Style Points

The following points provide general advice on formatting and style.

- **Abbreviations:** In general, terms should not be abbreviated unless they are used repeatedly and the abbreviation is helpful to the reader. Initially, use the word in full, followed by the abbreviation in parentheses. Thereafter use the abbreviation only.
- **Units of measurement:** Measurements should be given in SI or SI-derived units. Visit the [Bureau International des Poids et Mesures \(BIPM\) website](#) for more information about SI units.
- **Numbers:** numbers under 10 are spelt out, except for: measurements with a unit (8mmol/l); age (6 weeks old), or lists with other numbers (11 dogs, 9 cats, 4 gerbils).
- **Trade Names:** Chemical substances should be referred to by the generic name only. Trade names should not be used. Drugs should be referred to by their generic names. If proprietary drugs have been used in the study, refer to these by their generic name, mentioning the proprietary name and the name and location of the manufacturer in parentheses.

Wiley Author Resources

Manuscript Preparation Tips: Wiley has a range of resources for authors preparing manuscripts for submission available [here](#). In particular, we encourage authors to consult Wiley's best practice tips on [Writing for Search Engine Optimization](#).

Editing, Translation, and Formatting Support: [Wiley Editing Services](#) can greatly improve the chances of a manuscript being accepted. Offering expert help in English language editing, translation, manuscript formatting, and figure preparation, Wiley Editing Services ensures that the manuscript is ready for submission.

5. EDITORIAL POLICIES AND ETHICAL CONSIDERATIONS

Peer Review and Acceptance

The acceptance criteria for all papers are the quality and originality of the research and its significance to journal readership. Papers will only be sent to review if the Editor-in-Chief determines that the paper meets the appropriate quality and relevance requirements.

Wiley's policy on the confidentiality of the review process is [available here](#).

Human Studies and Subjects

For manuscripts reporting medical studies that involve human participants, a statement identifying the ethics committee that approved the study and confirmation that the study conforms to recognized standards is required, for example: [Declaration of Helsinki](#); [US Federal Policy for the Protection of Human Subjects](#); or [European Medicines Agency Guidelines for Good Clinical Practice](#). It should also state clearly in the text that all persons gave their informed consent prior to their inclusion in the study.

Patient anonymity should be preserved. When detailed descriptions, photographs, or videos of faces or identifiable body parts are used that may allow identification, authors should obtain the individual's free prior informed consent. Authors do not need to provide a copy of the consent form to the publisher; however, in signing the author license to publish, authors are required to confirm that consent has been obtained. Wiley has a [standard patient consent form available](#) for use. Where photographs are used they need to be cropped sufficiently to prevent human subjects being recognized; black eye bars should not be used as they do not sufficiently protect an individual's identity).

Animal Studies

A statement indicating that the protocol and procedures employed were ethically reviewed and approved, as well as the name of the body giving approval, must be included in the Methods section of the manuscript. Authors are encouraged to adhere to animal research reporting standards, for example the [ARRIVE guidelines](#) for reporting study design and statistical analysis; experimental procedures; experimental animals and housing and husbandry. Authors should also state whether experiments were performed in accordance with relevant institutional and national guidelines for the care and use of laboratory animals:

- US authors should cite compliance with the US National Research Council's [Guide for the Care and Use of Laboratory Animals](#), the US Public Health Service's [Policy on Humane Care and Use of Laboratory Animals](#), and [Guide for the Care and Use of Laboratory Animals](#).
- UK authors should conform to UK legislation under the [Animals \(Scientific Procedures\) Act 1986 Amendment Regulations \(SI 2012/3039\)](#).
- European authors outside the UK should conform to [Directive 2010/63/EU](#).

Clinical Trial Registration

The journal requires that clinical trials are prospectively registered in a publicly accessible database and clinical trial registration numbers should be included in all papers that report their results. Authors are asked to include the name of the trial register and the clinical trial registration number at the end of the abstract. If the trial is not registered, or was registered retrospectively, the reasons for this should be explained.

Research Reporting Guidelines

Accurate and complete reporting enables readers to fully appraise research, replicate it, and use it. Authors are encouraged to adhere to recognised research reporting standards. The EQUATOR Network collects more than 370 reporting guidelines for many study types, including for:

- Randomised trials: CONSORT
- Observational studies: STROBE
- Systematic reviews: PRISMA
- Case reports: CARE
- Qualitative research: SRQR
- Diagnostic / prognostic studies: STARD
- Quality improvement studies: SQUIRE
- Economic evaluations: CHEERS
- Animal pre-clinical studies: ARRIVE
- Study protocols: SPIRIT
- Clinical practice guidelines: AGREE

We also encourage authors to refer to and follow guidelines from:

- Future of Research Communications and e-Scholarship (FORCE11)
- National Research Council's Institute for Laboratory Animal Research guidelines
- The Gold Standard Publication Checklist from Hooijmans and colleagues
- Minimum Information Guidelines from Diverse Bioscience Communities (MIBBI) website
- FAIRsharing website

Species Names

Upon its first use in the title, abstract, and text, the common name of a species should be followed by the scientific name (genus, species, and authority) in parentheses. For well-known species, however, scientific names may be omitted from article titles. If no common name exists in English, only the scientific name should be used.

Genetic Nomenclature

Sequence variants should be described in the text and tables using both DNA and protein designations whenever appropriate. Sequence variant nomenclature must follow the current HGVS guidelines; see varnomen.hgvs.org, where examples of acceptable nomenclature are provided.

Nucleotide sequence data can be submitted in electronic form to any of the three major collaborative databases: DDBJ, EMBL, or GenBank. It is only necessary to submit to one database as data are exchanged between DDBJ, EMBL, and GenBank on a daily basis. The suggested wording for referring to accession-number information is: 'These sequence data have been submitted to the DDBJ/EMBL/GenBank databases under accession number U12345'. Addresses are as follows:

- DNA Data Bank of Japan (DDBJ): ddbj.nig.ac.jp
- EMBL Nucleotide Archive: ac.uk/ena
- GenBank: ncbi.nlm.nih.gov/genbank

Proteins sequence data should be submitted to either of the following repositories:

- Protein Information Resource (PIR): georgetown.edu
- SWISS-PROT: ch/sprot/sprot-top

Structural Data

For papers describing structural data, atomic coordinates and the associated experimental data should be deposited in the appropriate databank (see below). **Please note that the data in databanks must be released, at the latest, upon publication of the article.** We trust in the cooperation of our authors to ensure that atomic coordinates and experimental data are released on time.

- **Organic and organometallic compounds:** Crystallographic data should not be sent as Supporting Information, but should be deposited with the *Cambridge Crystallographic Data Centre* (CCDC) at cam.ac.uk/services/structure%5Fdeposit.
- **Inorganic compounds:** *Fachinformationszentrum Karlsruhe* (FIZ; fiz-karlsruhe.de).
- **Proteins and nucleic acids:** *Protein Data Bank* (org/pdb).
- **NMR spectroscopy data:** *BioMagResBank* (wisc.edu).
- **Chemical structures and biological assay data:** ChEMBL, pubchem compound and pubchem bioassay.

Conflict of Interest

The journal requires that all authors disclose any potential sources of conflict of interest. Any interest or relationship, financial or otherwise that might be perceived as influencing an author's objectivity is considered a potential source of conflict of interest. These must be disclosed when directly relevant or directly related to the work that the authors describe in their manuscript. Potential sources of conflict of interest include, but are not limited to: patent or stock ownership, membership of a company board of directors, membership of an advisory board or committee for a company, and consultancy for or receipt of speaker's fees from a company. The existence of a conflict of interest does not preclude publication. If the authors have no conflict of interest to declare, they must also state this at submission. It is the responsibility of the corresponding author to review this policy with all authors and collectively to disclose with the submission ALL pertinent commercial and other relationships.

Funding

Authors should list all funding sources in the Acknowledgments section. Authors are responsible for the accuracy of their funder designation. If in doubt, please check the Open Funder Registry for the correct nomenclature: <https://www.crossref.org/services/funder-registry/>

Authorship

The list of authors should accurately illustrate who contributed to the work and how. All those listed as authors should qualify for authorship according to the following criteria:

1. Have made substantial contributions to conception and design, or acquisition of data, or analysis and interpretation of data; and
2. Been involved in drafting the manuscript or revising it critically for important intellectual content; and
3. Given final approval of the version to be published. Each author should have participated sufficiently in the work to take public responsibility for appropriate portions of the content; and
4. Agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Contributions from anyone who does not meet the criteria for authorship should be listed, with permission from the contributor, in an Acknowledgments section (for example, to recognize contributions from people who provided technical help, collation of data, writing assistance, acquisition of funding, or a department chairperson who provided general support). Prior to submitting the article all authors should agree on the order in which their names will be listed in the manuscript.

Additional Authorship Options. Joint first or senior authorship: In the case of joint first authorship, a footnote should be added to the author listing, e.g. 'X and Y should be considered joint first author' or 'X and Y should be considered joint senior author.'

Data Sharing and Data Accessibility

Chemical Biology & Drug Design recognizes the many benefits of archiving research data. *Chemical Biology & Drug Design* expects you to archive all the data from which your published results are derived in a public repository. The repository that you choose should offer you guaranteed preservation (see the registry of research data repositories at <https://www.re3data.org/>) and should help you make it findable, accessible, interoperable, and re-useable, according to FAIR Data Principles (<https://www.force11.org/group/fairgroup/fairprinciples>).

All accepted manuscripts are required to publish a data availability statement to confirm the presence or absence of shared data. If you have shared data, this statement will describe how the data can be accessed, and include a persistent identifier (e.g., a DOI for the data, or an accession number) from the repository where you shared the data. Authors will be required to confirm adherence to the policy. If you cannot share the data described in your manuscript, for example for legal or ethical reasons, or do not intend to share the data then you must provide the appropriate data availability statement. *Chemical Biology & Drug Design* notes that FAIR data sharing allows for access to shared data under restrictions (e.g., to protect confidential or proprietary information) but notes that the FAIR principles encourage you to share data in ways that are as open as possible (but that can be as closed as necessary). Sample statements are available [here](#). If published, all statements will be placed in the heading of your manuscript.

Human subject information in databases. The journal refers to the [World Health Medical Association Declaration of Taipei on Ethical Considerations Regarding Health Databases and Biobanks](#).

Publication Ethics

This journal is a member of the [Committee on Publication Ethics \(COPE\)](#). Note this journal uses iThenticate's CrossCheck software to detect instances of overlapping and similar text in submitted manuscripts. Read Wiley's Top 10 Publishing Ethics Tips for Authors [here](#). Wiley's Publication Ethics Guidelines can be found [here](#).

ORCID

As part of the journal's commitment to supporting authors at every step of the publishing process, the journal encourages the submitting author (only) to provide an ORCID iD when submitting a manuscript. This takes around 2 minutes to complete. [Find more information here](#).

6. AUTHOR LICENSING

If a paper is accepted for publication, the author identified as the formal corresponding author will receive an email prompting them to log in to Author Services, where via the Wiley Author Licensing Service (WALS) they will be required to complete a copyright license agreement on behalf of all authors of the paper.

Authors may choose to publish under the terms of the journal's standard copyright agreement, or [OnlineOpen](#) under the terms of a Creative Commons License.

General information regarding licensing and copyright is available [here](#). To review the Creative Commons License options offered under OnlineOpen, please [click here](#). (Note that certain funders mandate a particular type of CC license be used; to check this please click [here](#).)

Self-Archiving Definitions and Policies: Note that the journal's standard copyright agreement allows for self-archiving of different versions of the article under specific conditions. Please click [here](#) for more detailed information about self-archiving definitions and policies.

Open Access fees: Authors who choose to publish using OnlineOpen will be charged a fee. A list of Article Publication Charges for Wiley journals is available [here](#).

Funder Open Access: Please click [here](#) for more information on Wiley's compliance with specific Funder Open Access Policies.

7. PUBLICATION PROCESS AFTER ACCEPTANCE

Accepted Article Received in Production

When an accepted article is received by Wiley's production team, the corresponding author will receive an email asking them to login or register with [Wiley Author Services](#). The author will be asked to sign a publication license at this point.

Accepted Articles

The journal offers Wiley's Accepted Articles service for all manuscripts. This service ensures that accepted 'in press' manuscripts are published online shortly after acceptance, prior to copy-editing or typesetting. Accepted Articles are published online a few days after final acceptance and appear in PDF format only. They are given a Digital Object Identifier (DOI), which allows them to be cited and tracked and are indexed by PubMed. After the final version article is published (the article of record), the DOI remains valid and can still be used to cite and access the article.

Accepted Articles will be indexed by PubMed; submitting authors should therefore carefully check the names and affiliations of all authors provided in the cover page of the manuscript so it is accurate for indexing. Subsequently, the final copyedited and proofed articles will appear in an issue on Wiley Online Library; the link to the article in PubMed will update automatically.

Proofs

Authors will receive an e-mail notification with a link and instructions for accessing HTML page proofs online. Page proofs should be carefully proofread for any copyediting or typesetting errors. Online guidelines are provided within the system. No special software is required, most common browsers are supported. Authors should also make sure that any renumbered tables, figures, or references match text citations and that figure legends correspond with text citations and actual figures. Proofs must be returned within 48 hours of receipt of the email. Return of proofs via e-mail is possible in the event that the online system cannot be used or accessed.

Page Charges

Original research papers are supposed to be no longer than **9 full typeset pages**. In the event of a paper being longer, the Publisher will charge 100 USD per extra page to the author. Authors need to approve these costs upon submission of their paper in Research Exchange. Each typeset page can roughly be estimated to contain approximately 750 words, 2 or 3 Figures or Tables, or 50 references. Please note this is only an approximate guide.

Invited reviews have no page charges. Uninvited reviews should be limited to 20 pages. If additional pages are required, there will be a charge to the authors of 100 USD per extra page. **For articles in special issues, page charges may differ on a case by case basis.**

The author will be notified of the cost of page charges when they receive the proofs, along with instructions on how to pay for the charges.

Accepted Articles

This journal offers rapid publication via Wiley's Accepted Articles Service. Accepted Articles are published on Wiley Online Library within 5 days of receipt, without waiting for a copyright agreement (they are protected under a general copyright statement).

Early View

The journal offers rapid publication via Wiley's Early View service. [Early View](#) (Online Version of Record) articles are published on Wiley Online Library before inclusion in an issue. Note there may be a delay after corrections are received before the article appears online, as Editors also need to review proofs. Once the article is published on Early View, no further changes to the article are possible. The Early View article is fully citable and carries an online publication date and DOI for citations.

8. OFFPRINTS

If you wish to purchase additional printed copies of your article, please click on the link and follow the instructions provided: www.sheridan.com/wiley/eoc

9. POST PUBLICATION

Access and Sharing

When the article is published online:

- The author receives an email alert (if requested).
- The link to the published article can be shared through social media.
- The author will have free access to the paper (after accepting the Terms & Conditions of use, they can view the article).
- For non-open access articles, the corresponding author and co-authors can nominate up to ten colleagues to receive a publication alert and free online access to the article.

Promoting the Article

To find out how to best promote an article, click [here](#).

Article Promotion Support

[Wiley Editing Services](#) offers professional video, design, and writing services to create shareable video abstracts, infographics, conference posters, lay summaries, and research news stories for your research – so you can help your research get the attention it deserves.

Measuring the Impact of an Article

Wiley also helps authors measure the impact of their research through specialist partnerships with [Kudos](#) and [Altmetric](#).

10. EDITORIAL OFFICE CONTACT DETAILS

cbddeditorial@wiley.com

Author Guidelines updated August 2020

ANNEXURE G: SUPPLEMENTARY INFORMATION FOR SUBMITTED MANUSCRIPT

Supplementary Material:

Synthesis and biological evaluation of selected 7*H*-pyrrolo[2,3-*d*]pyrimidine derivatives as novel CDK9/CyclinT and Haspin inhibitors in the low micromolar range.

Lianie Pieterse¹; Richard M. Beteck¹, Blandine Barratte², Stéphane Bach^{2,3}, Lesetja J. Legoabe¹

✉ Lesetja J. Legoabe

¹ Centre of Excellence for Pharmaceutical Sciences, North-West University, Private Bag X6001, Potchefstroom 2520, South Africa

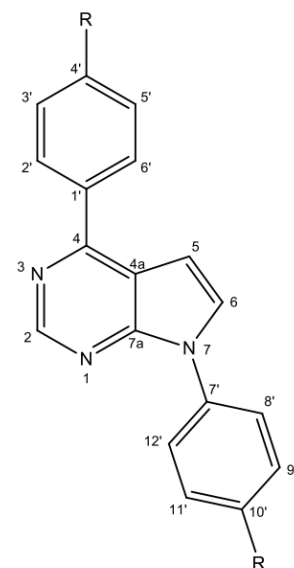
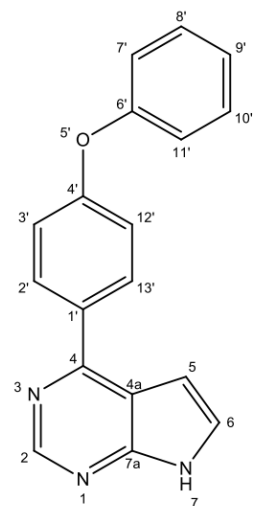
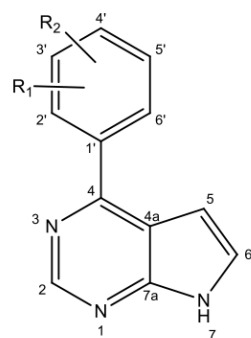
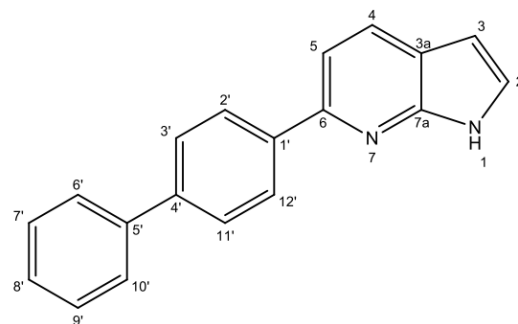
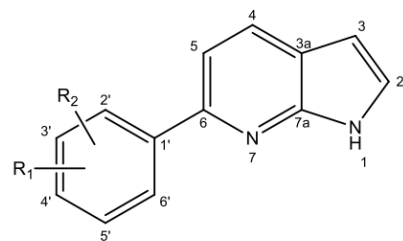
² Sorbonne Université, CNRS, FR2424, Plateforme de criblage KISSf (Kinase Inhibitor Specialized Screening facility), Station Biologique de Roscoff, 29680 Roscoff Cedex, France

³ Sorbonne Université, CNRS, UMR8227, Integrative Biology of Marine Models Laboratory (LBIM), Station Biologique de Roscoff, 29680 Roscoff, France

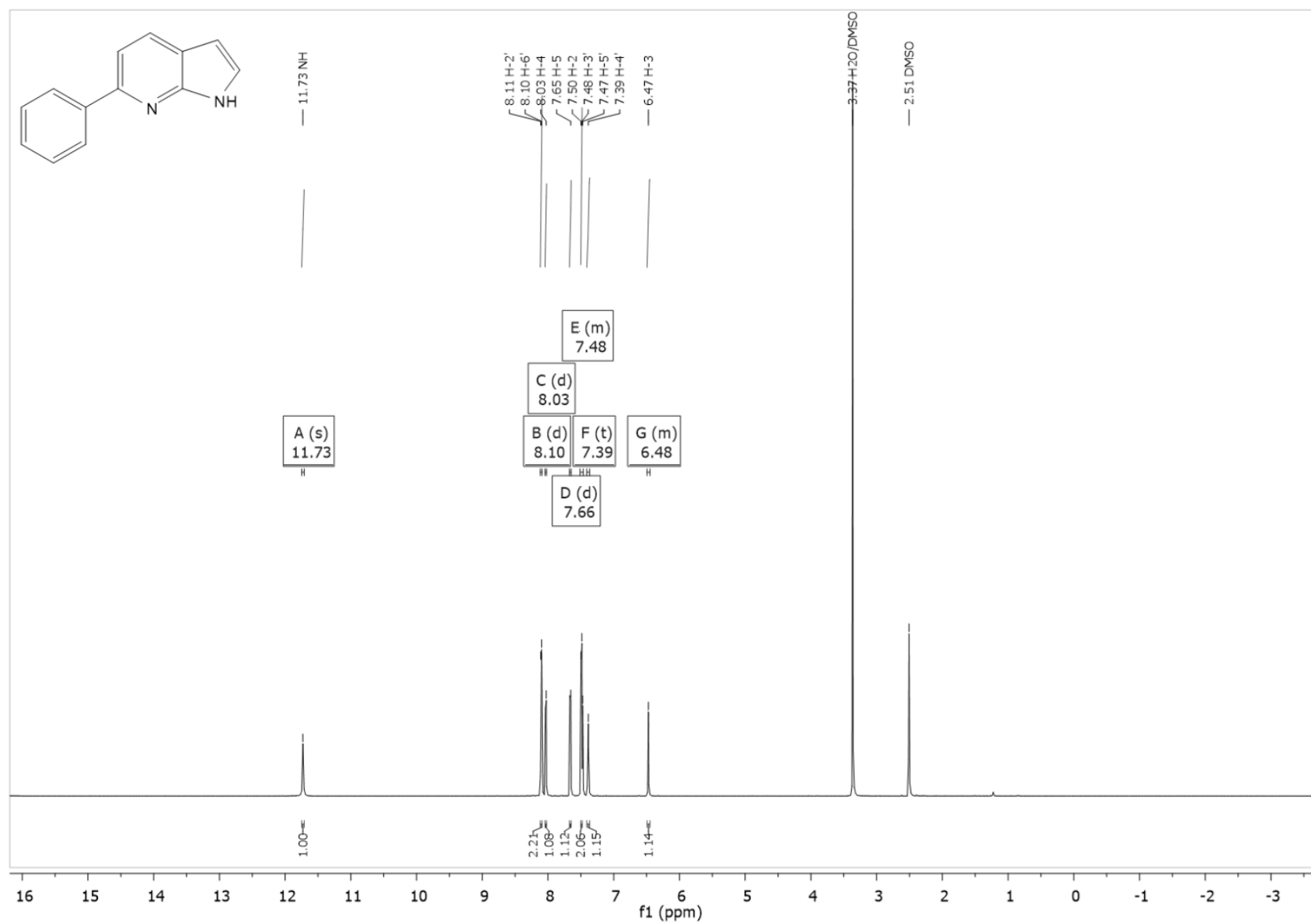
Index:

1. ^1H and ^{13}C NMR spectra
2. HRMS data
3. HPLC data

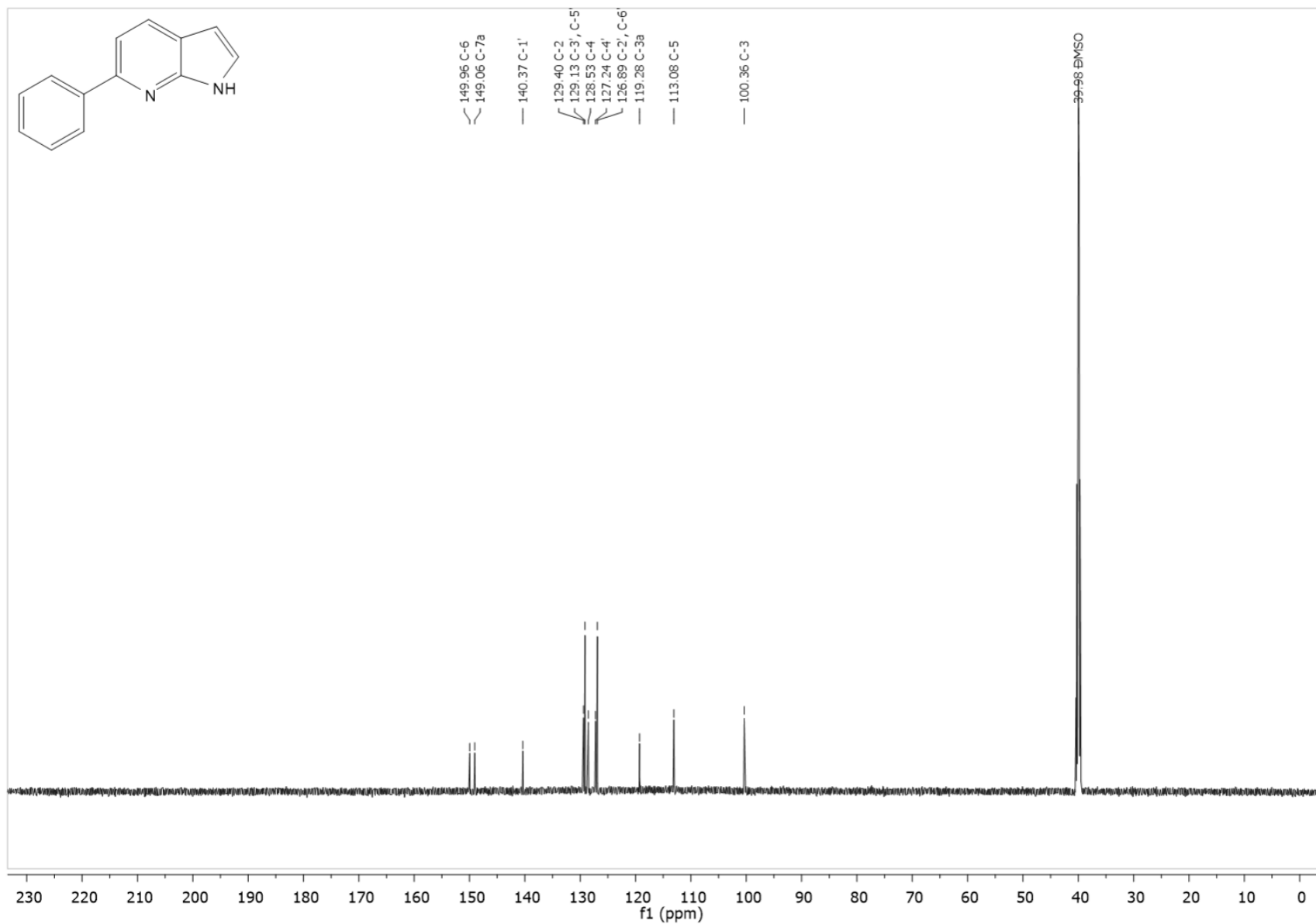
Numbering of compounds relevant to NMR spectra:



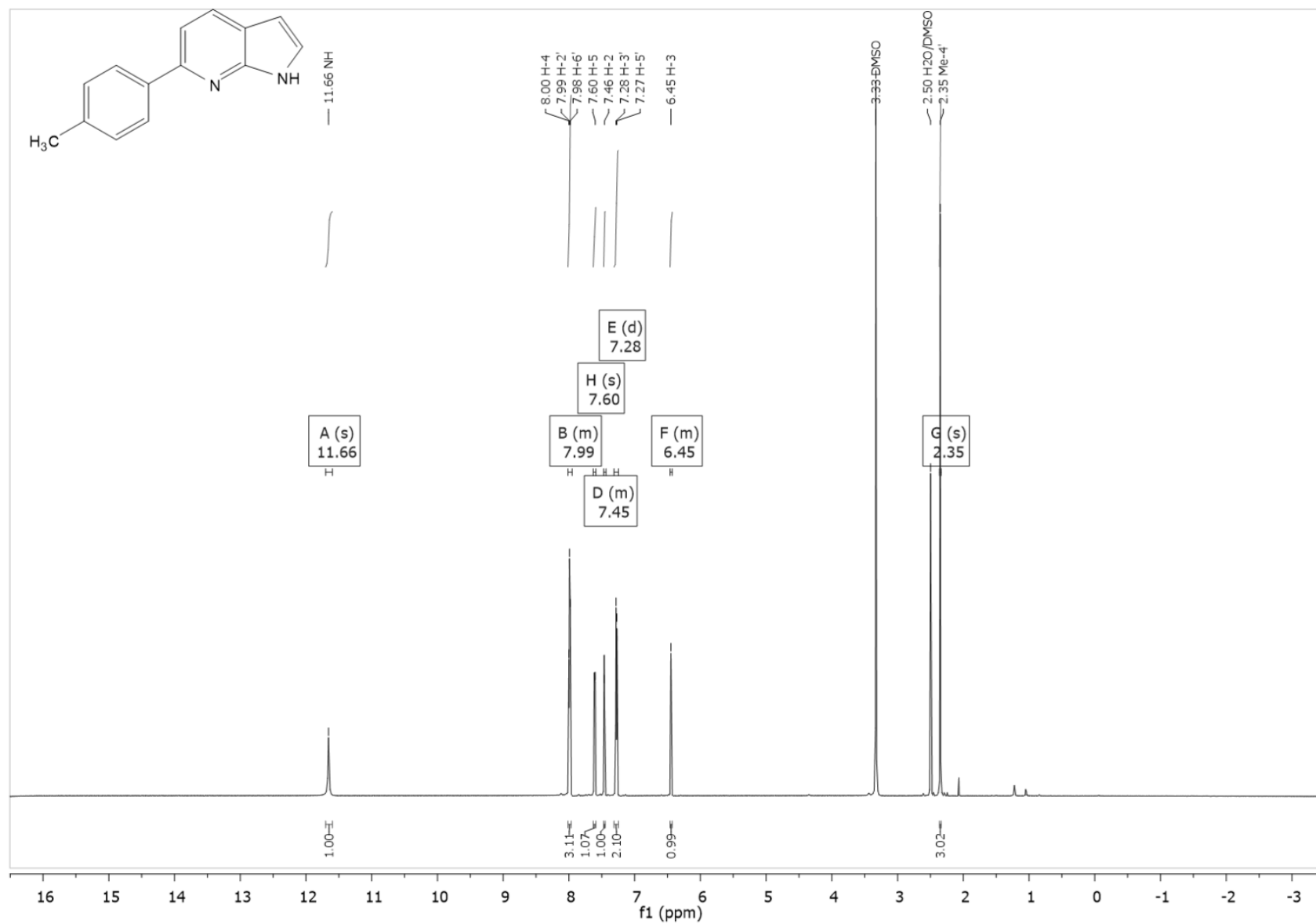
6-phenyl-7*H*-pyrrolo[2,3-*d*]pyrimidine (¹H) **5a**:



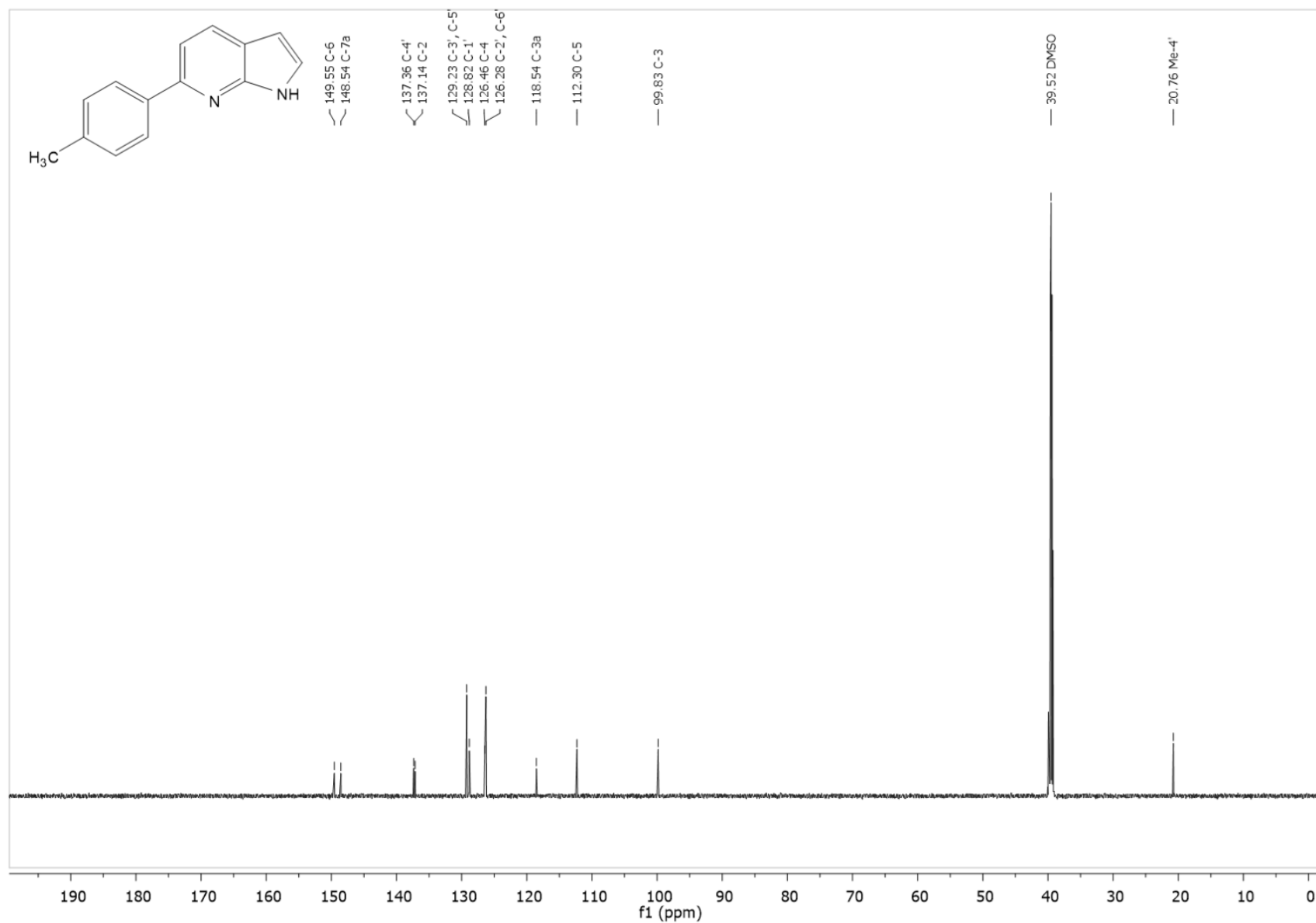
6-phenyl-7*H*-pyrrolo[2,3-*d*]pyrimidine (¹³C) **5a**:



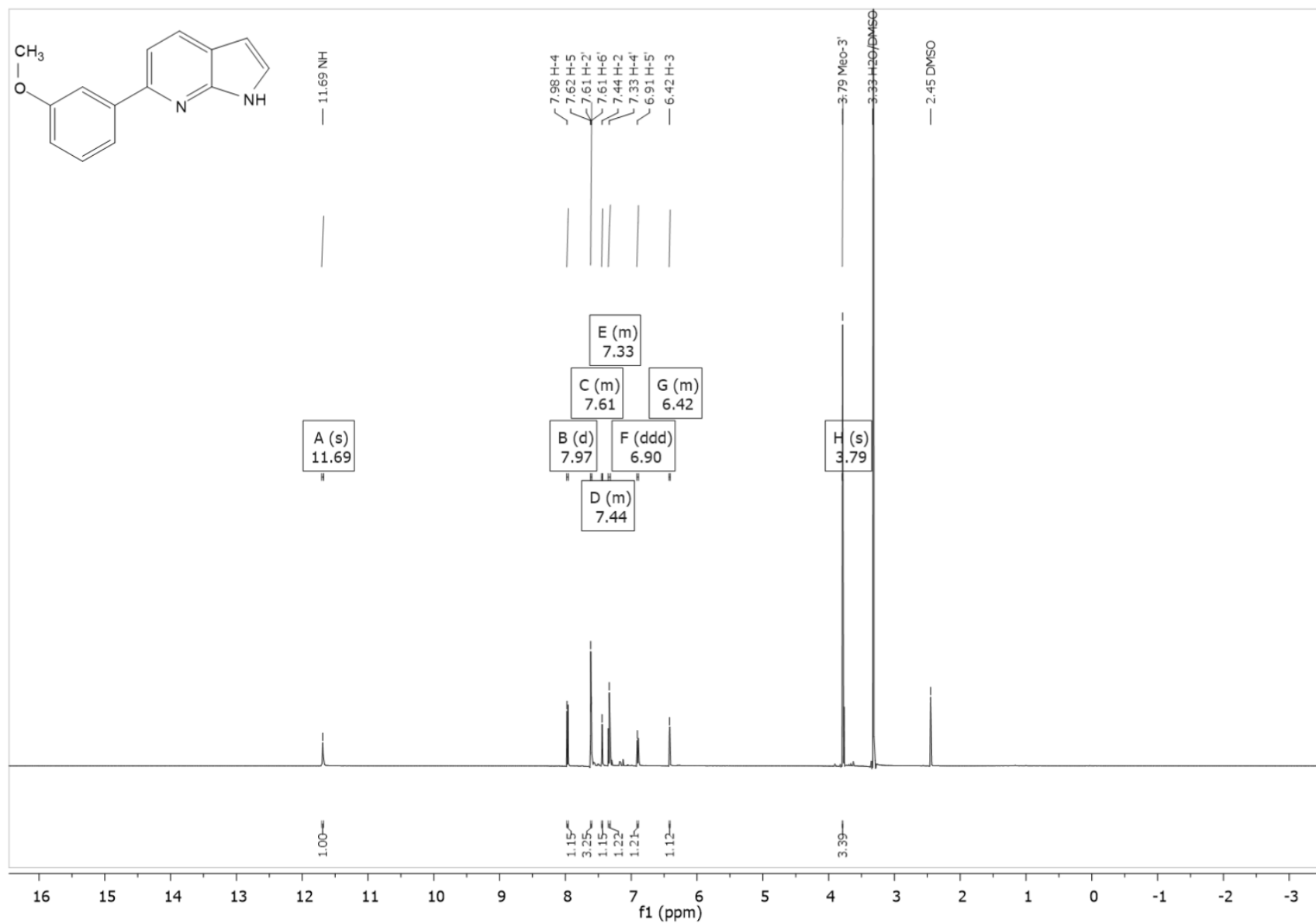
6-(4-methylphenyl)-1*H*-pyrrolo[2,3-*b*]pyridine (¹H) **5b**:



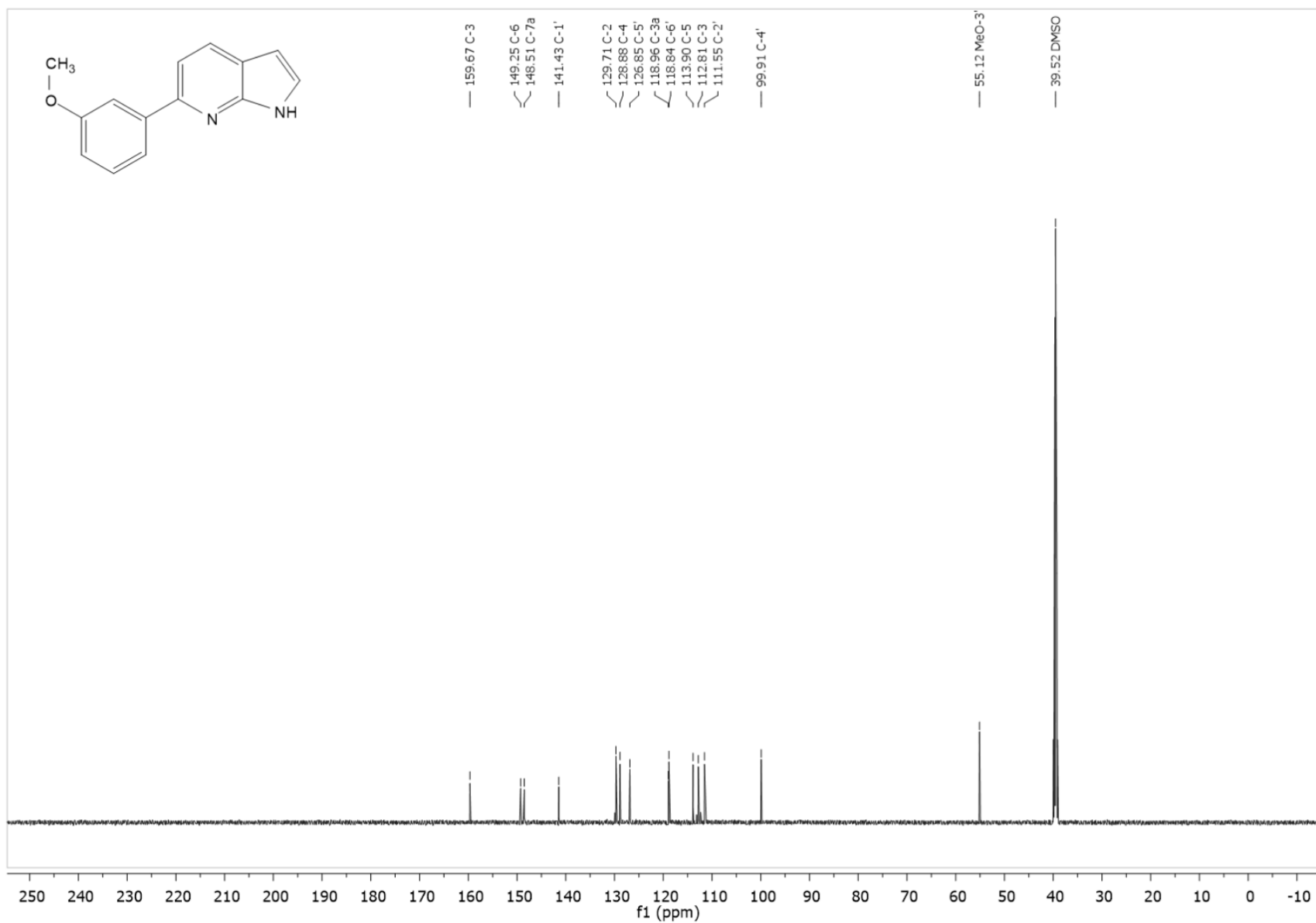
6-(4-methylphenyl)-1*H*-pyrrolo[2,3-*b*]pyridine (^{13}C) **5b**:



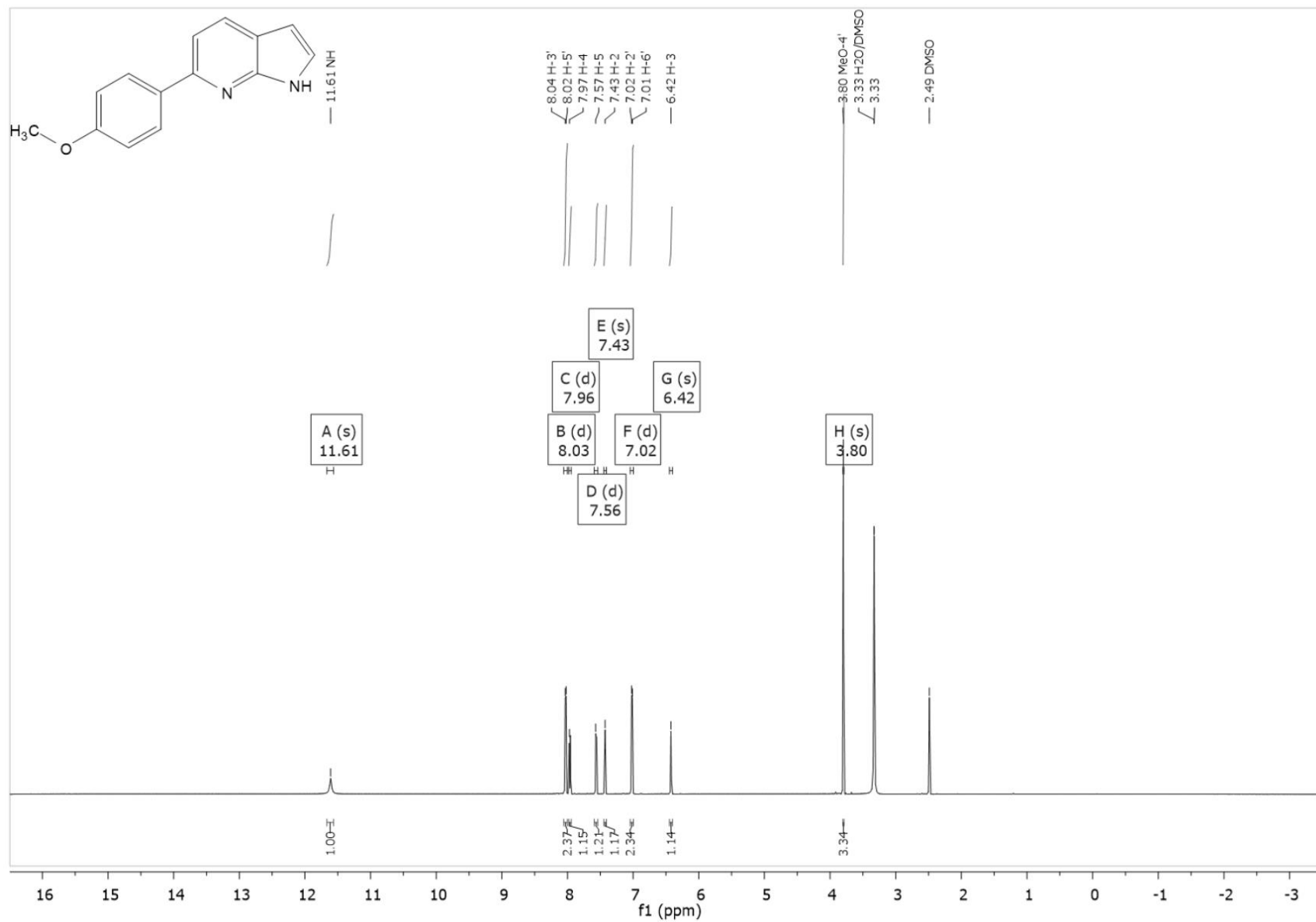
6-(3-methoxyphenyl)-1*H*-pyrrolo[2,3-*b*]pyridine (¹H) **5c**:



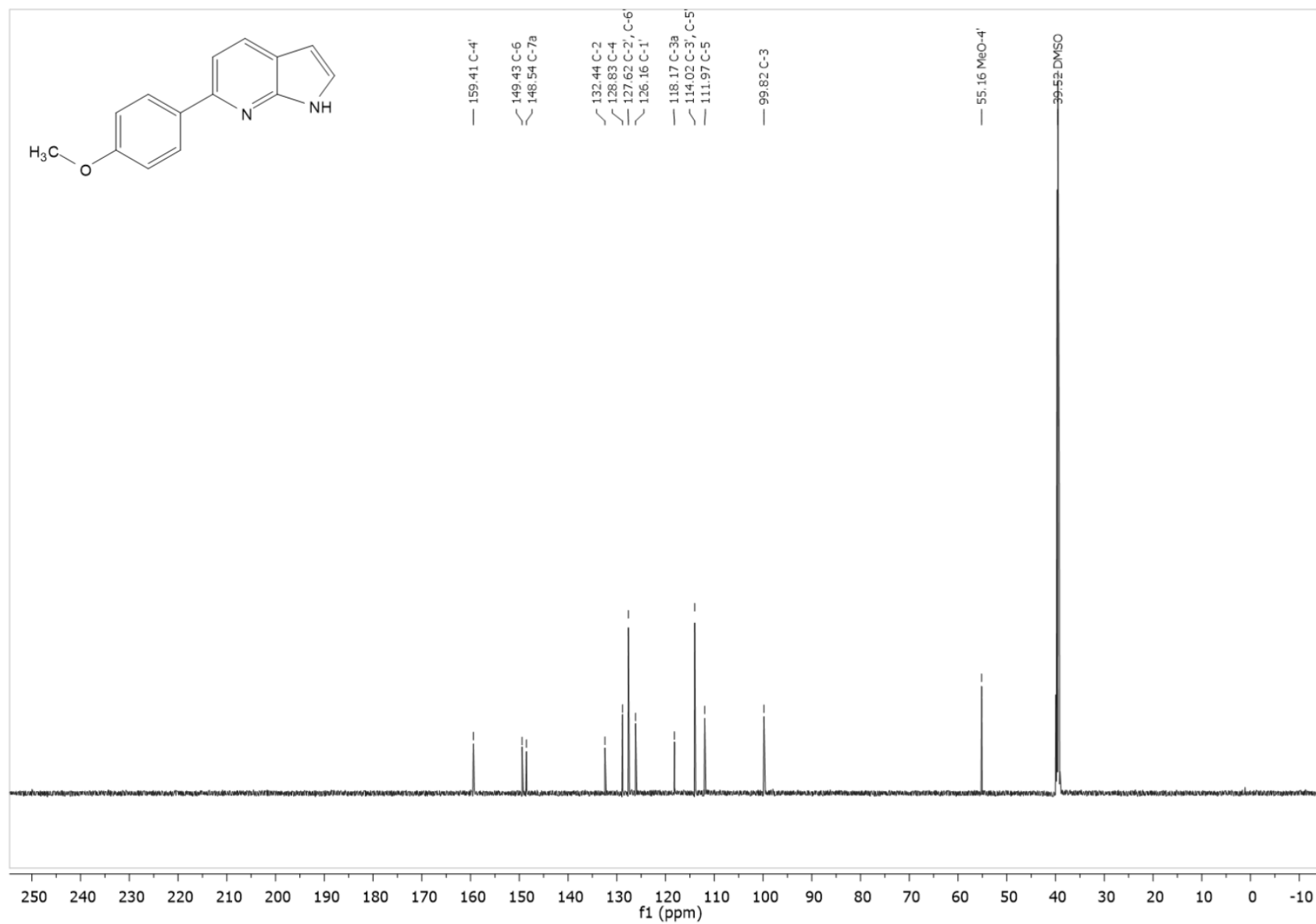
6-(3-methoxyphenyl)-1*H*-pyrrolo[2,3-*b*]pyridine (^{13}C) **5c**:



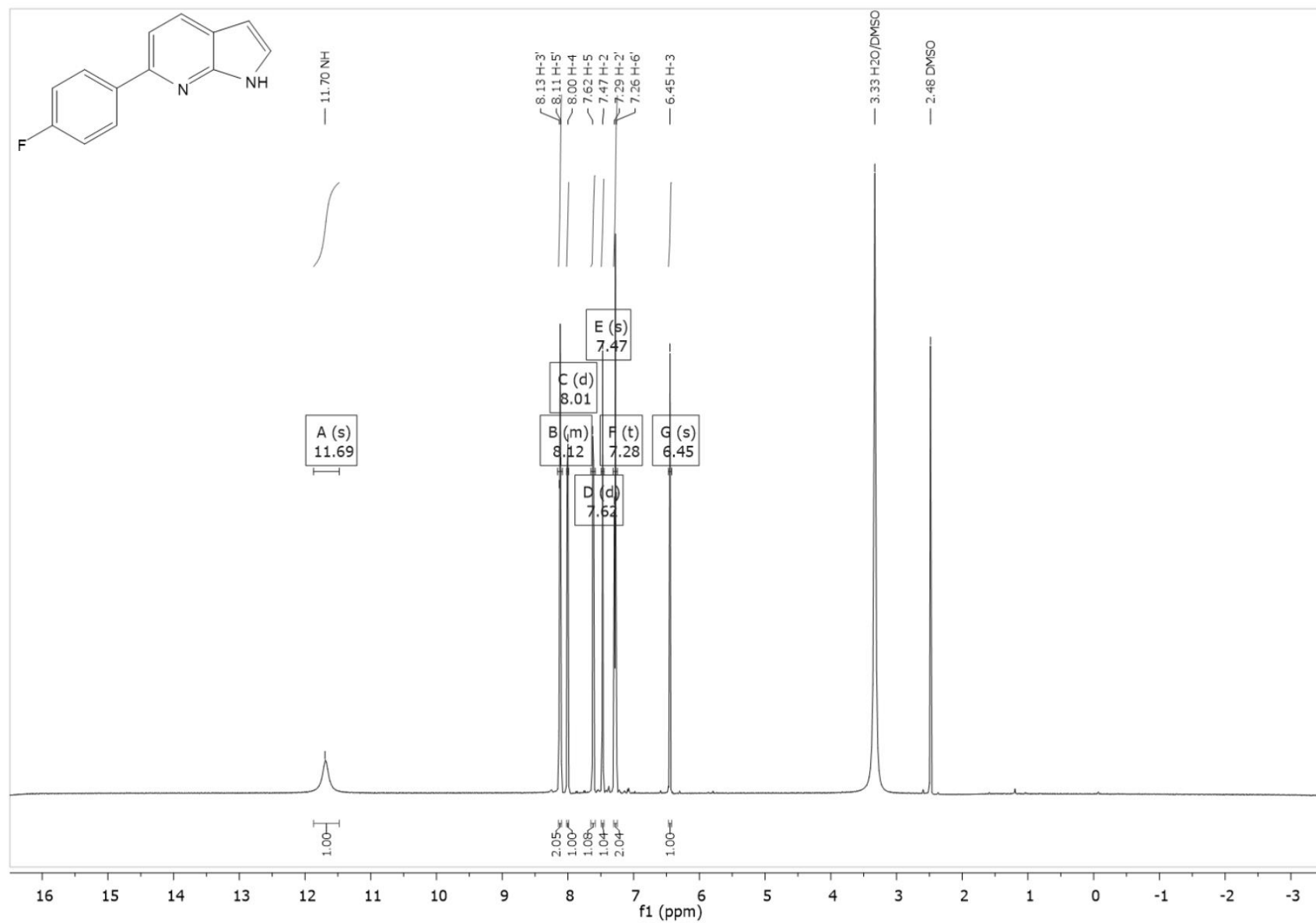
6-(4-methoxyphenyl)-1*H*-pyrrolo[2,3-*b*]pyridine (¹H) **5d**:



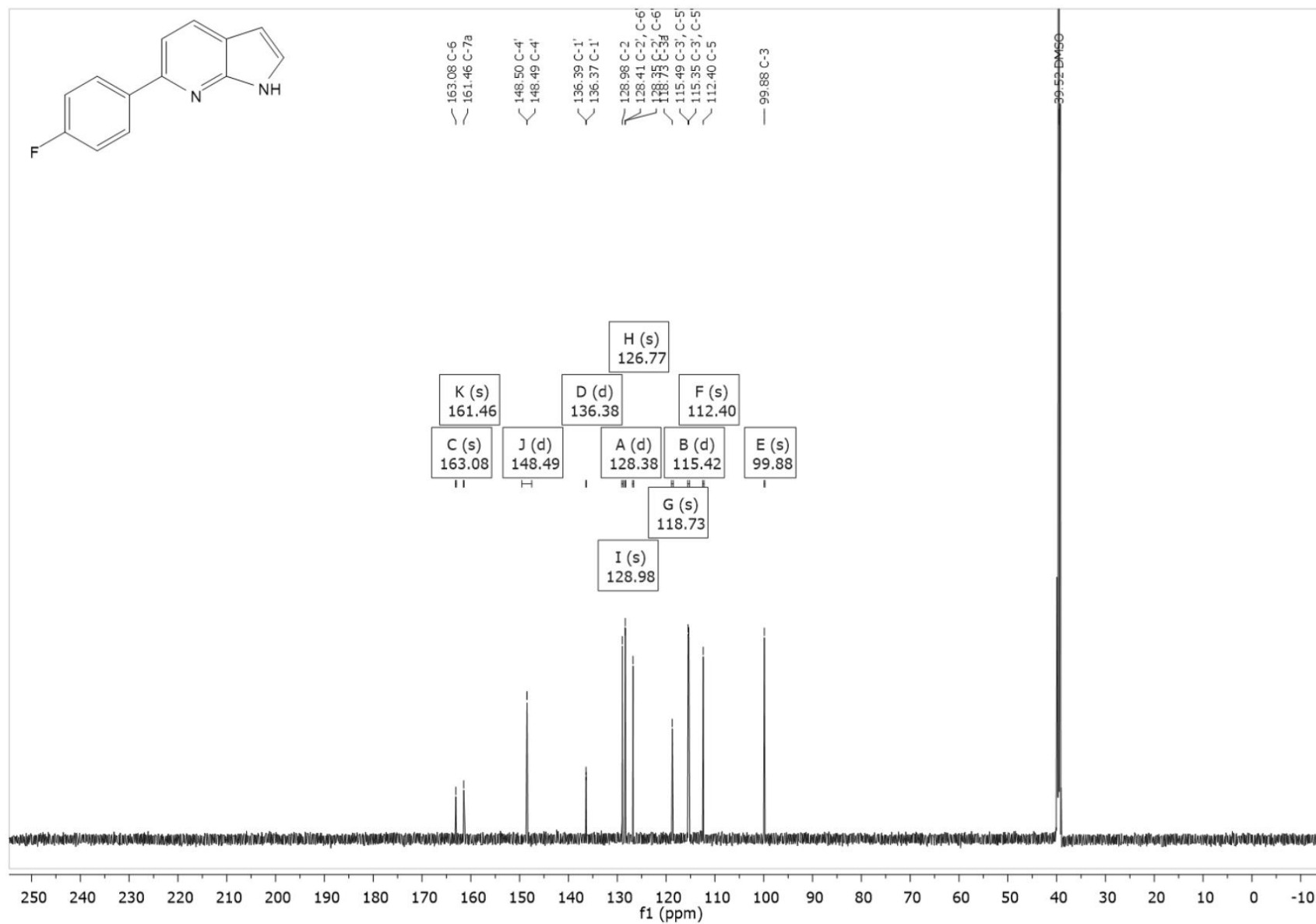
6-(4-methoxyphenyl)-1*H*-pyrrolo[2,3-*b*]pyridine (¹³C) **5d**:



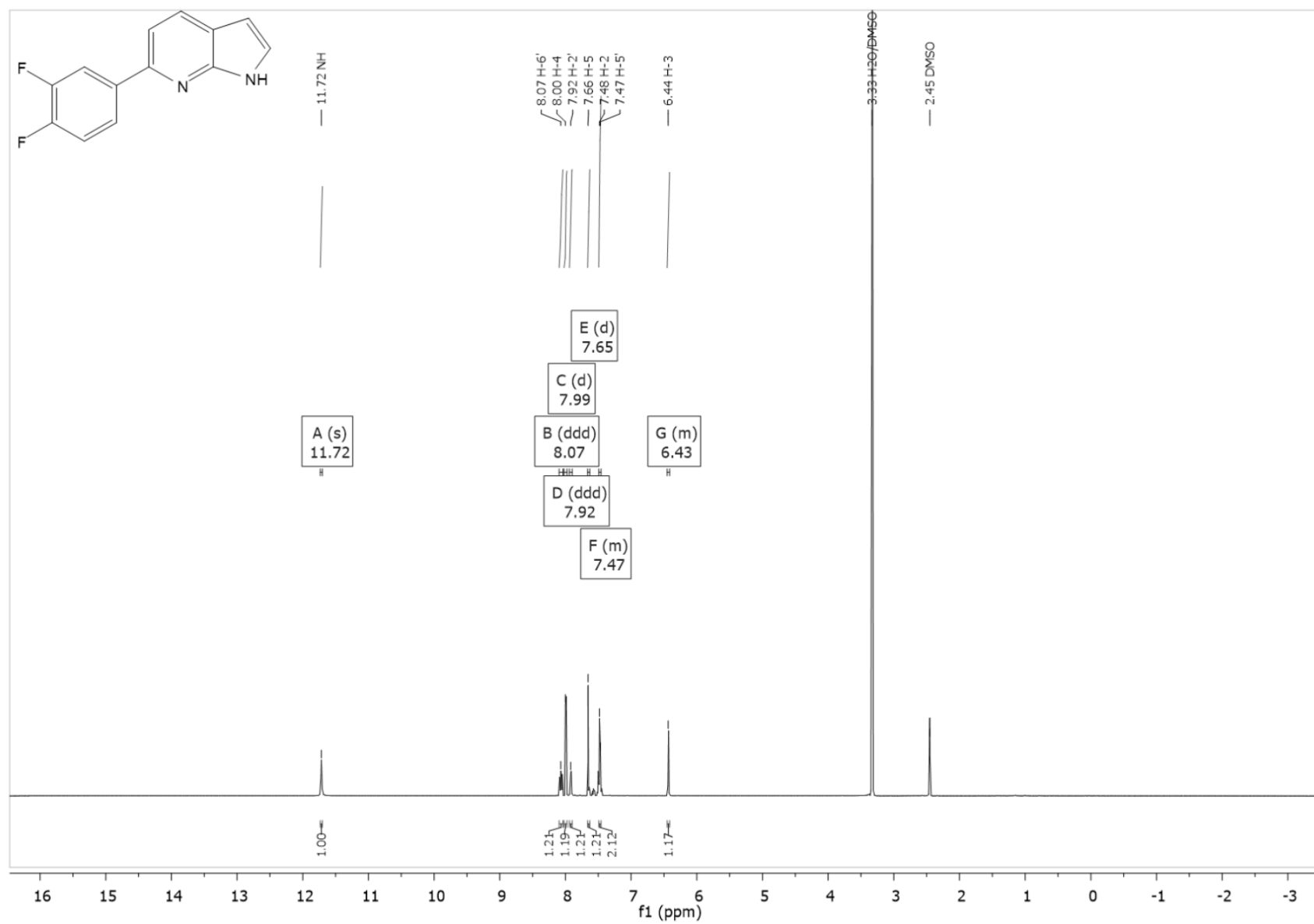
6-(4-fluorophenyl)-1*H*-pyrrolo[2,3-*b*]pyridine (¹H) **5e**:



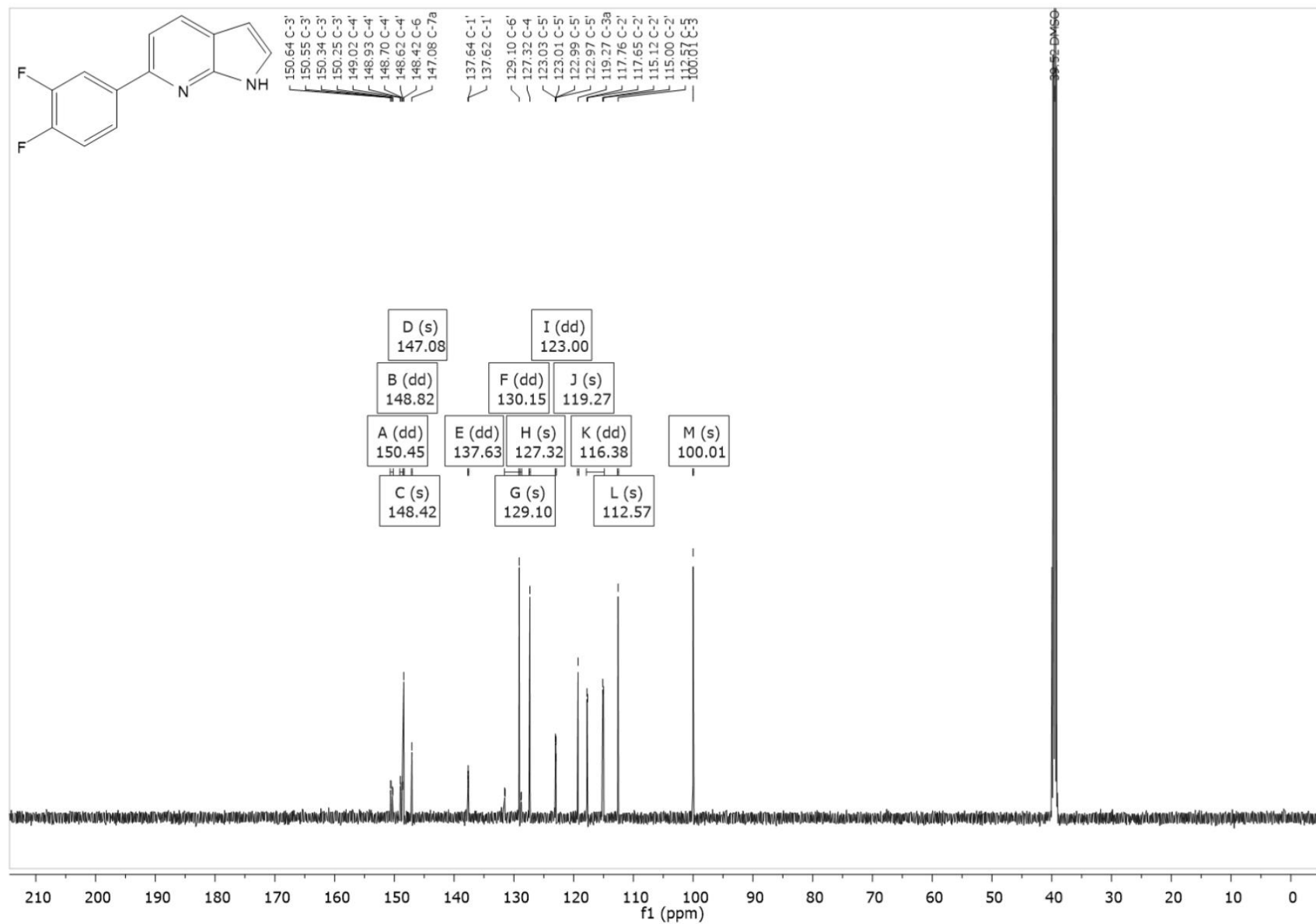
6-(4-fluorophenyl)-1*H*-pyrrolo[2,3-*b*]pyridine (^{13}C) **5e**:



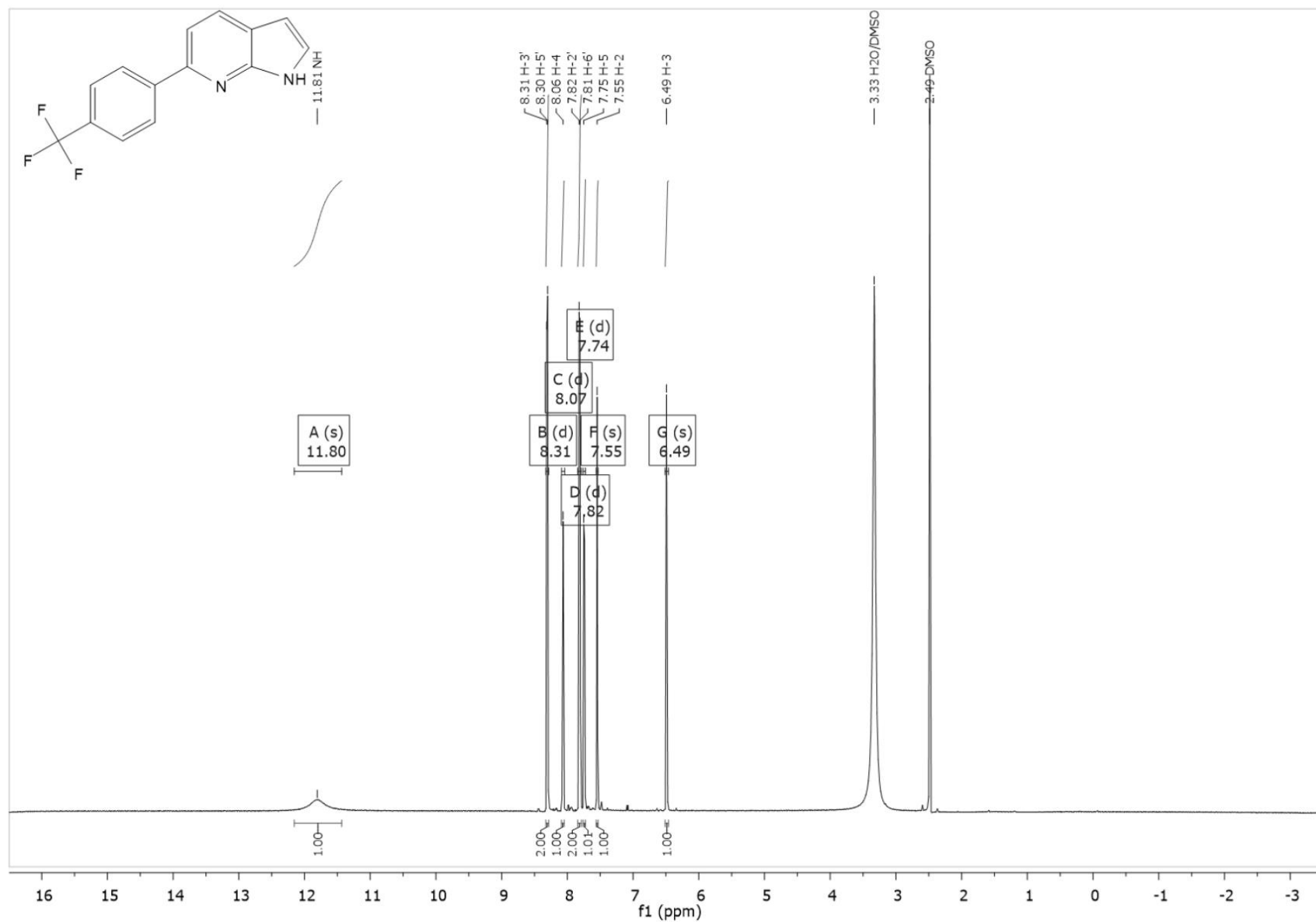
6-(3,4-difluorophenyl)-1*H*-pyrrolo[2,3-*b*]pyridine (¹H) **5f**:



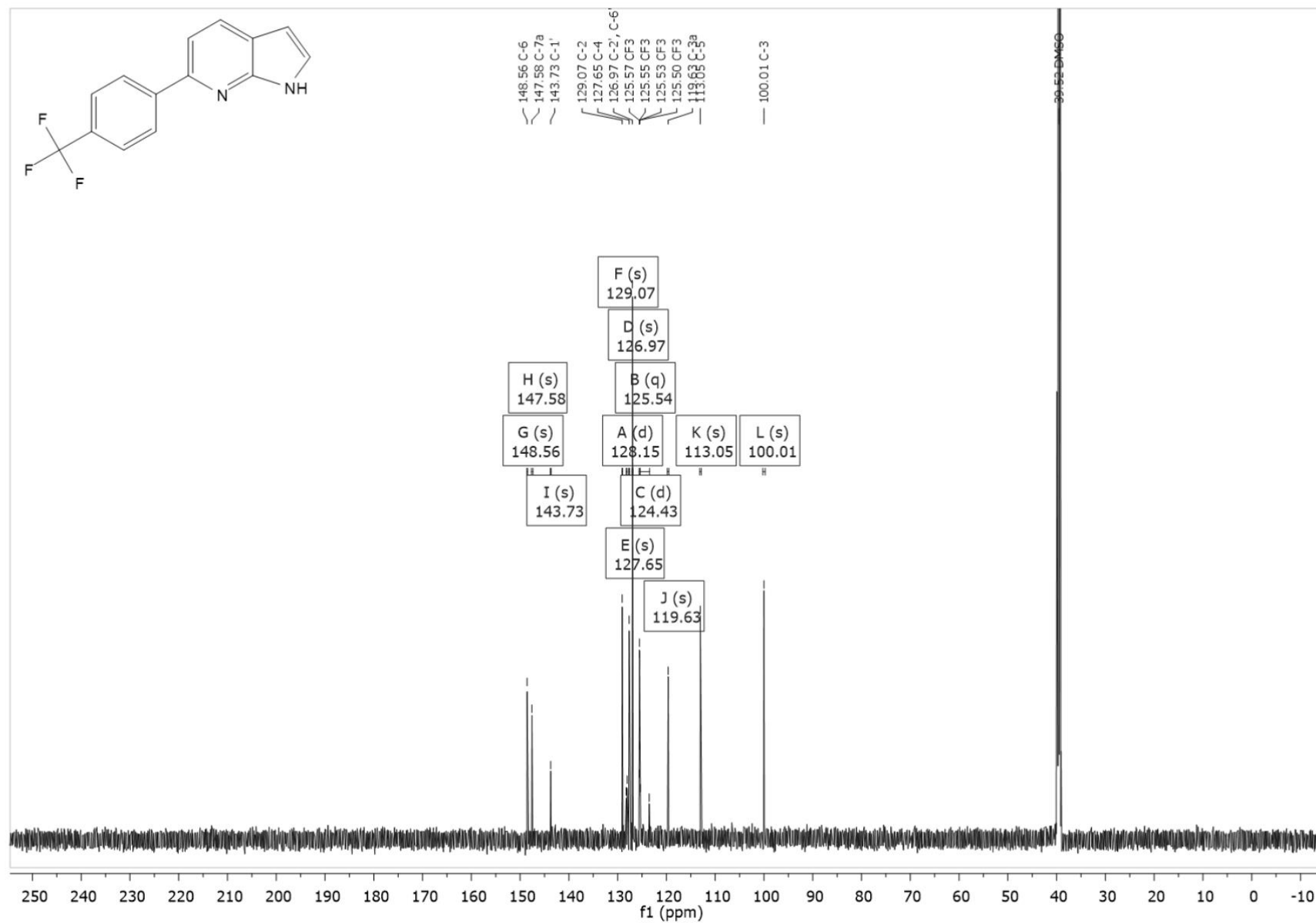
6-(3,4-difluorophenyl)-1*H*-pyrrolo[2,3-*b*]pyridine (¹³C) **5f**:



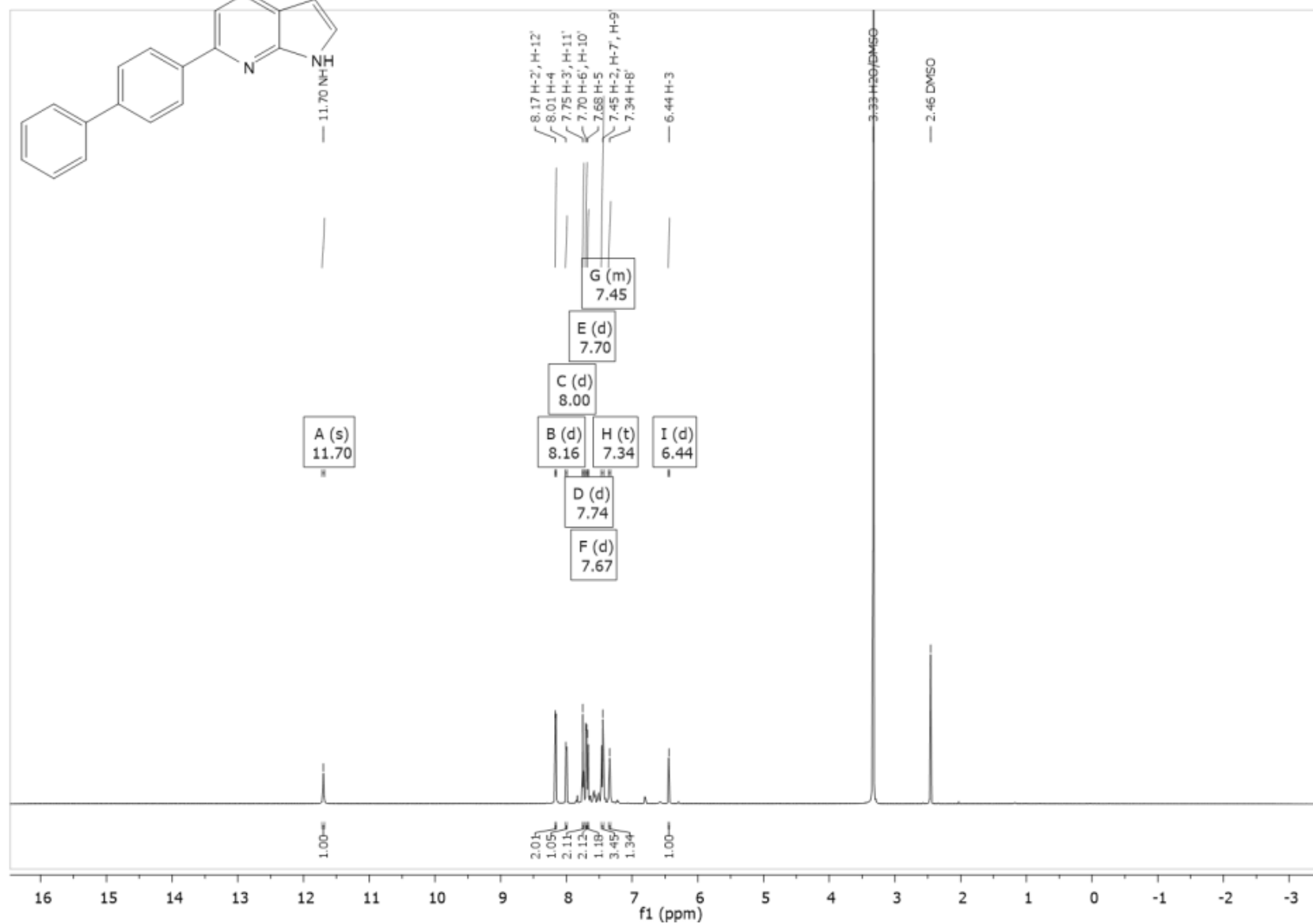
6-[4-(trifluoromethyl)phenyl]-1*H*-pyrrolo[2,3-*b*]pyridine (¹H) **5g**:



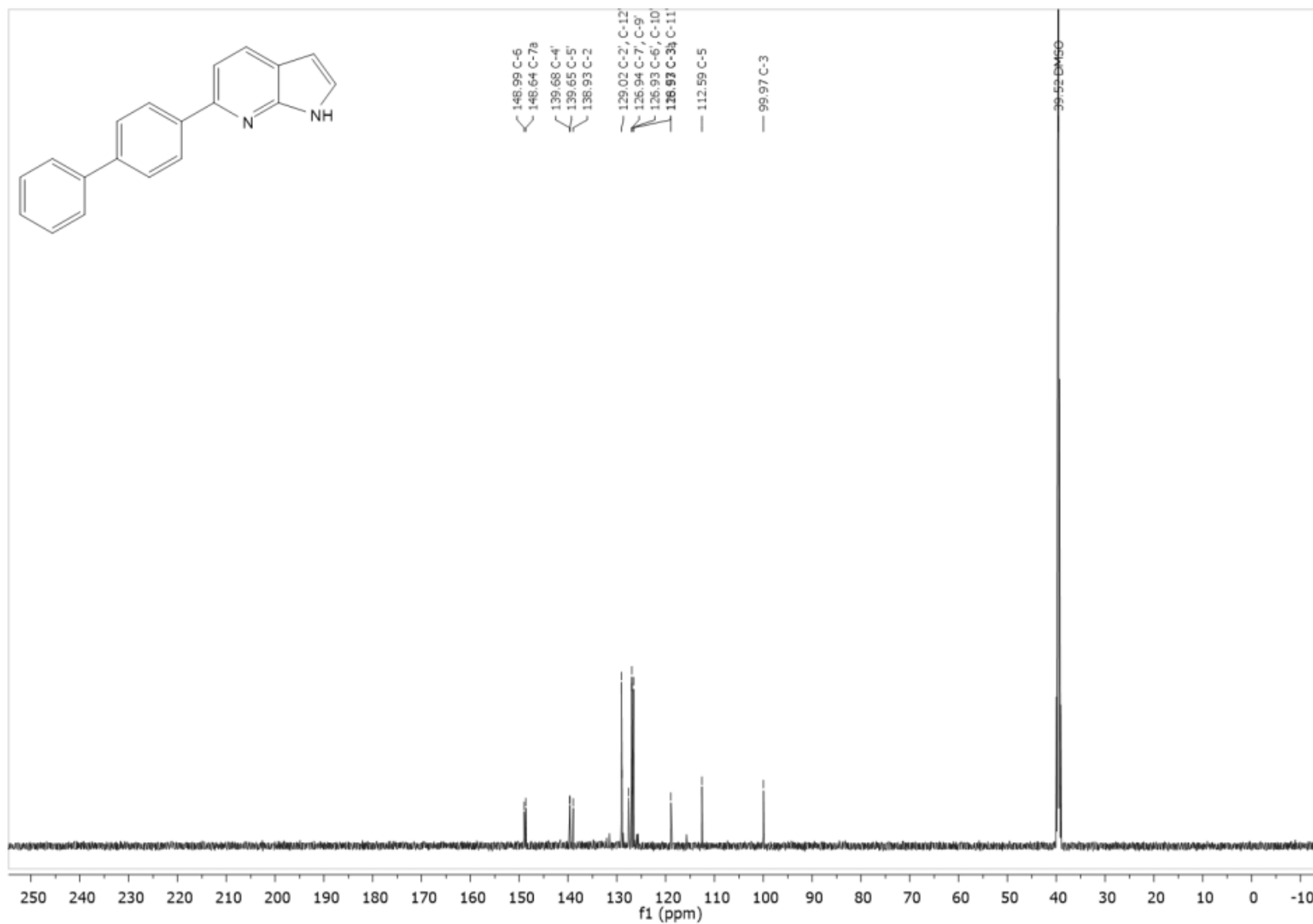
6-[4-(trifluoromethyl)phenyl]-1*H*-pyrrolo[2,3-*b*]pyridine (¹³C) **5g**:



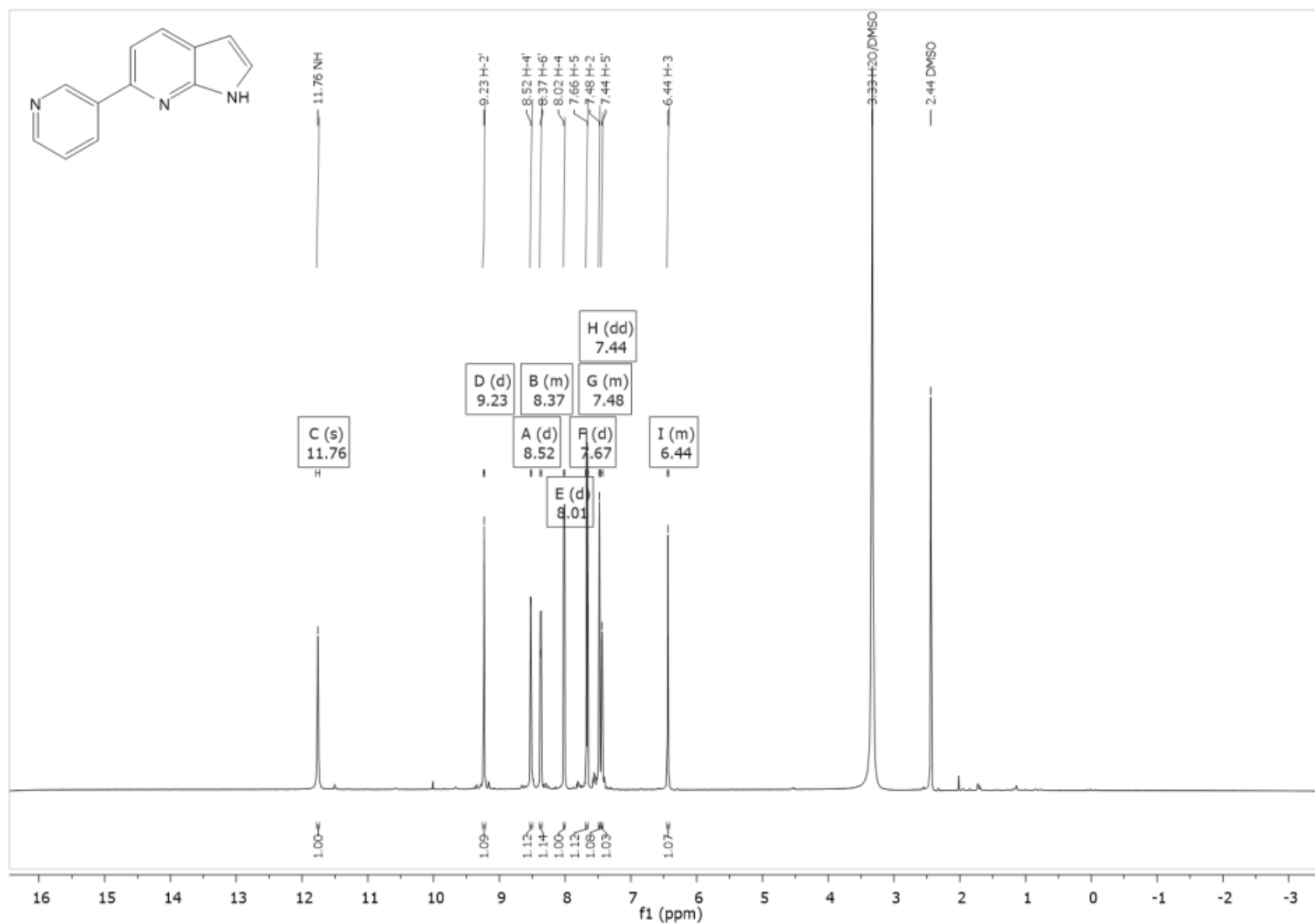
6-([1,1'-biphenyl]-4-yl)-1*H*-pyrrolo[2,3-*b*]pyridine (¹H) **5h**:



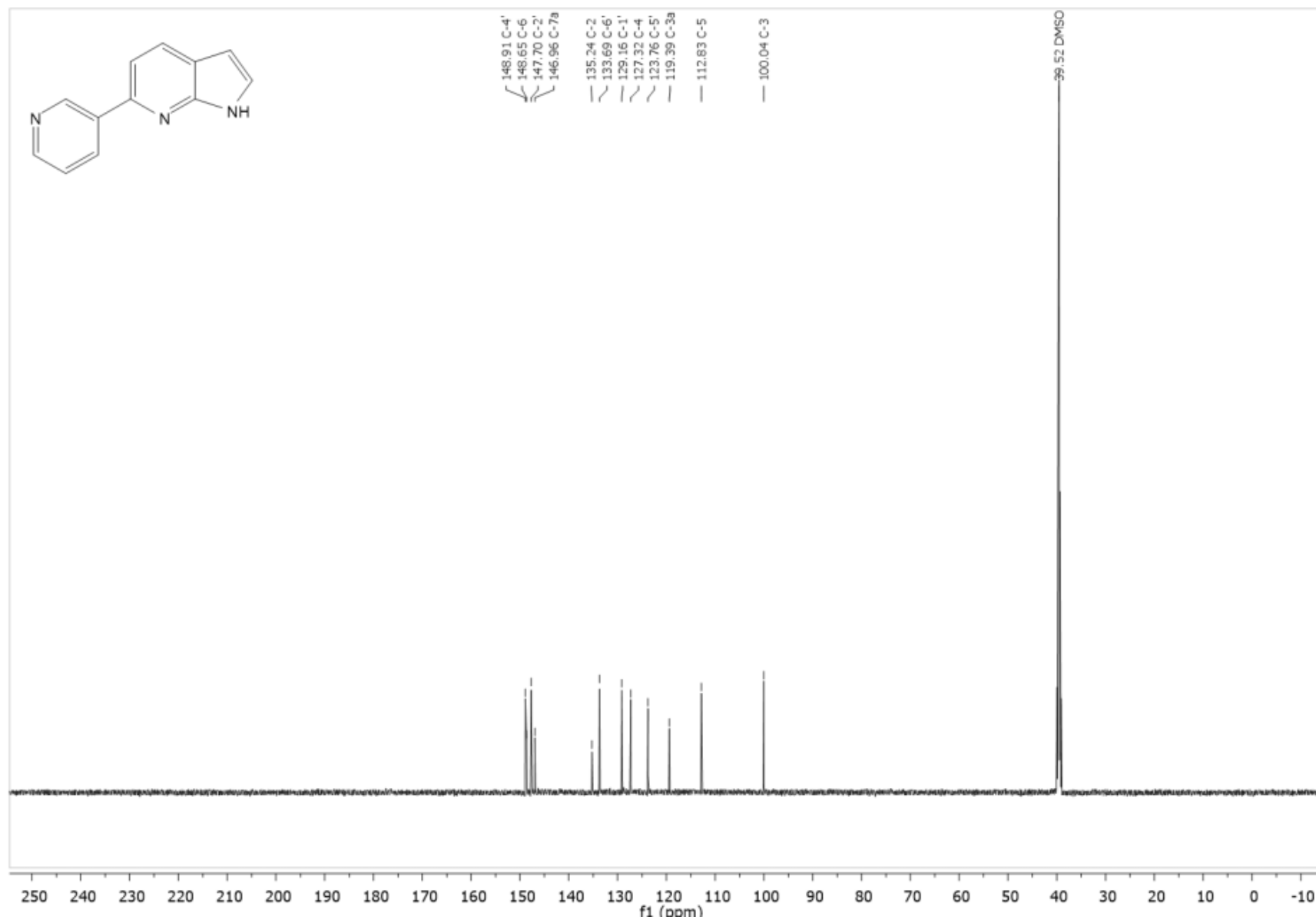
6-([1,1'-biphenyl]-4-yl)-1*H*-pyrrolo[2,3-*b*]pyridine (¹³C) **5h**:



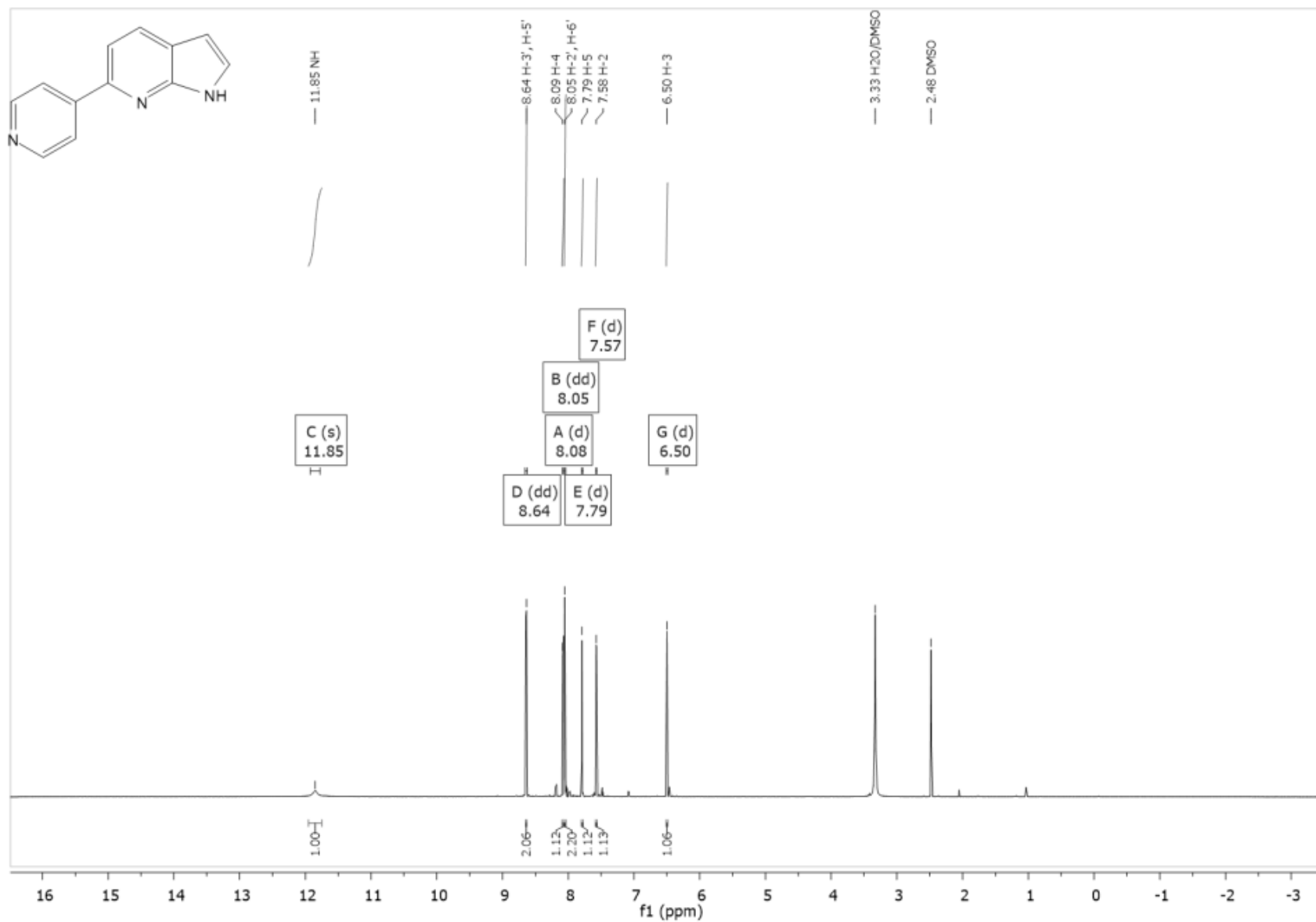
6-(pyridin-3-yl)-1*H*-pyrrolo[2,3-*b*]pyridine (¹H) **5i**:



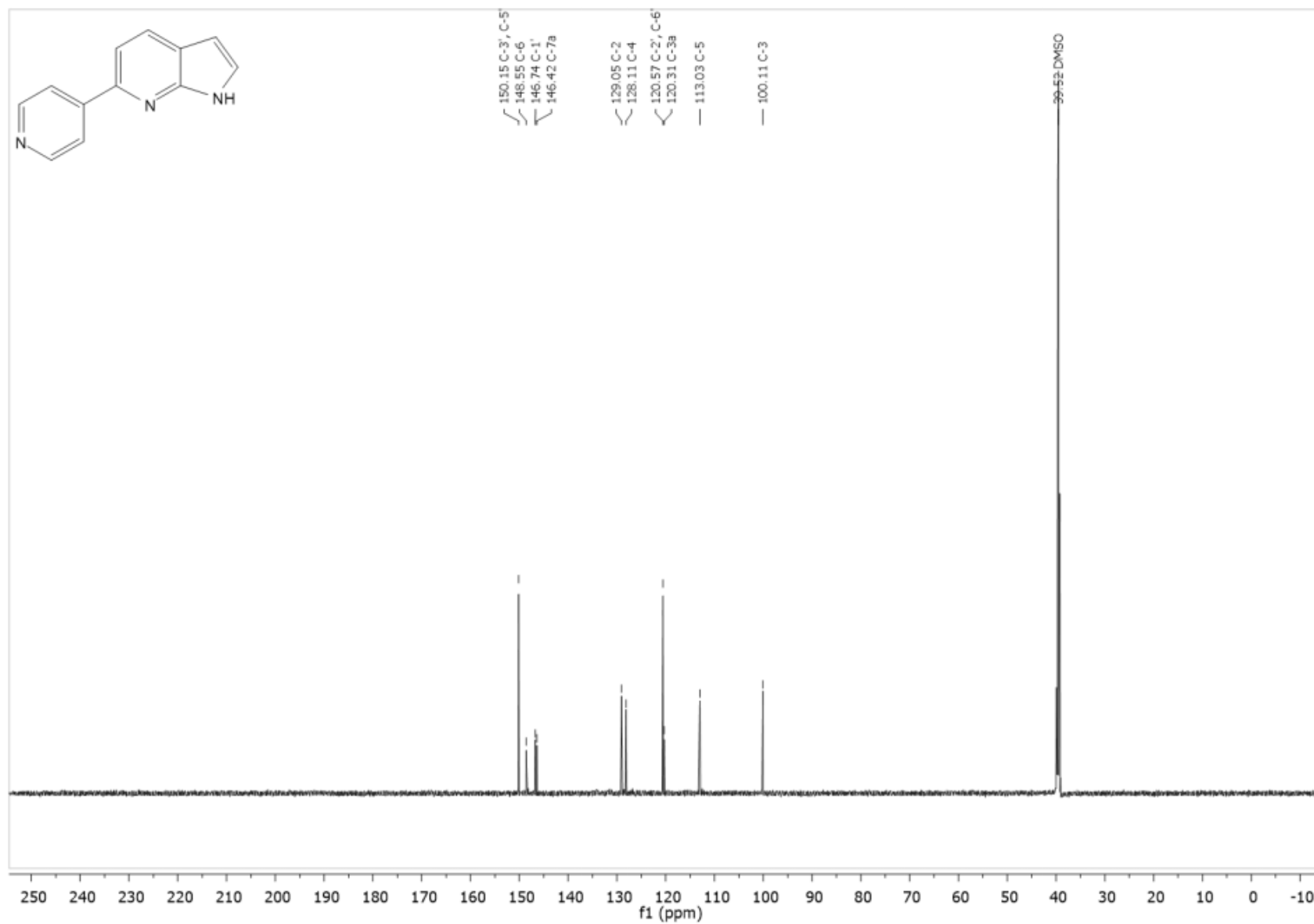
6-(pyridin-3-yl)-1*H*-pyrrolo[2,3-*b*]pyridine (^{13}C) **5i**:



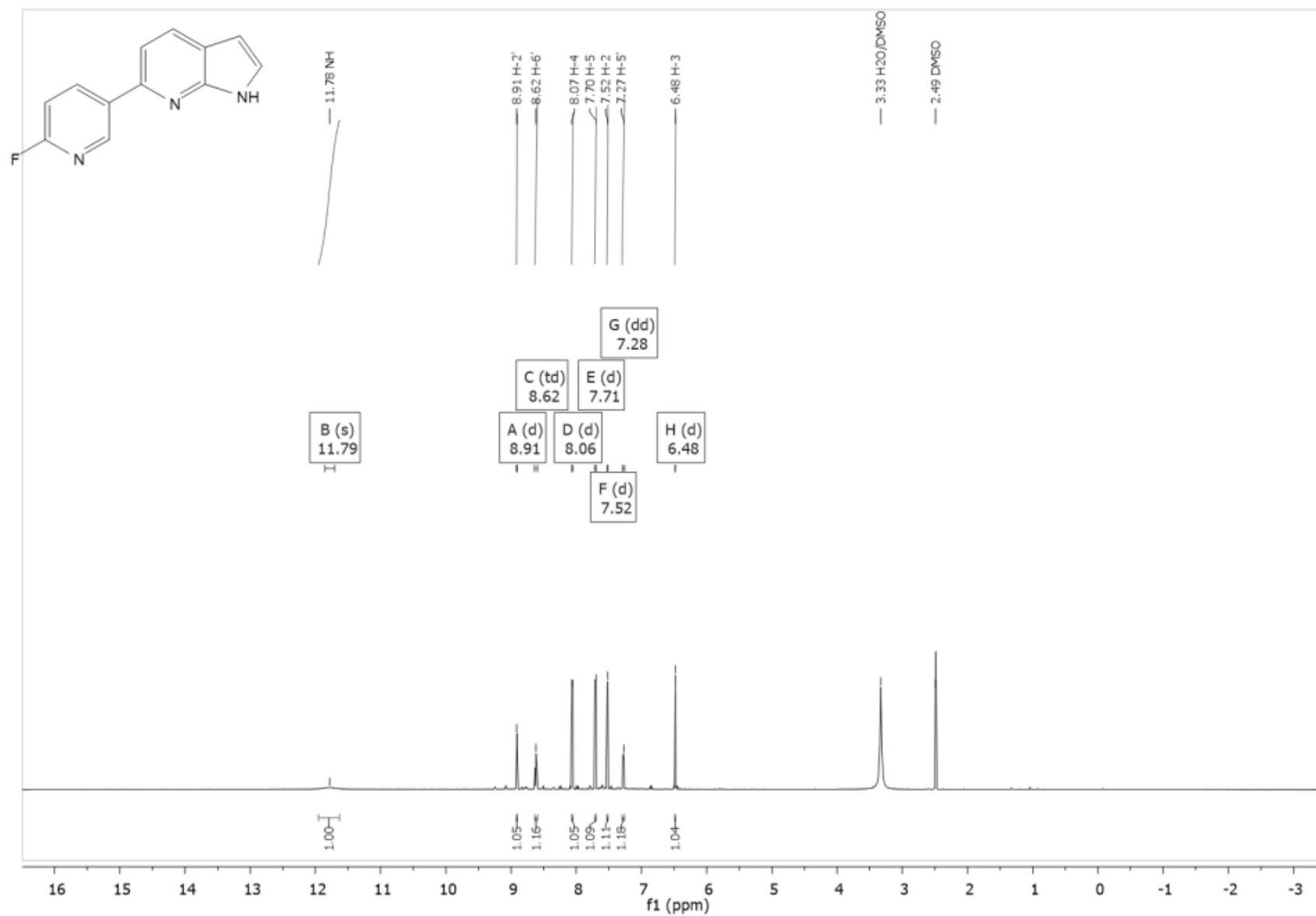
6-(pyridin-4-yl)-1*H*-pyrrolo[2,3-*b*]pyridine (¹H) **5j**:



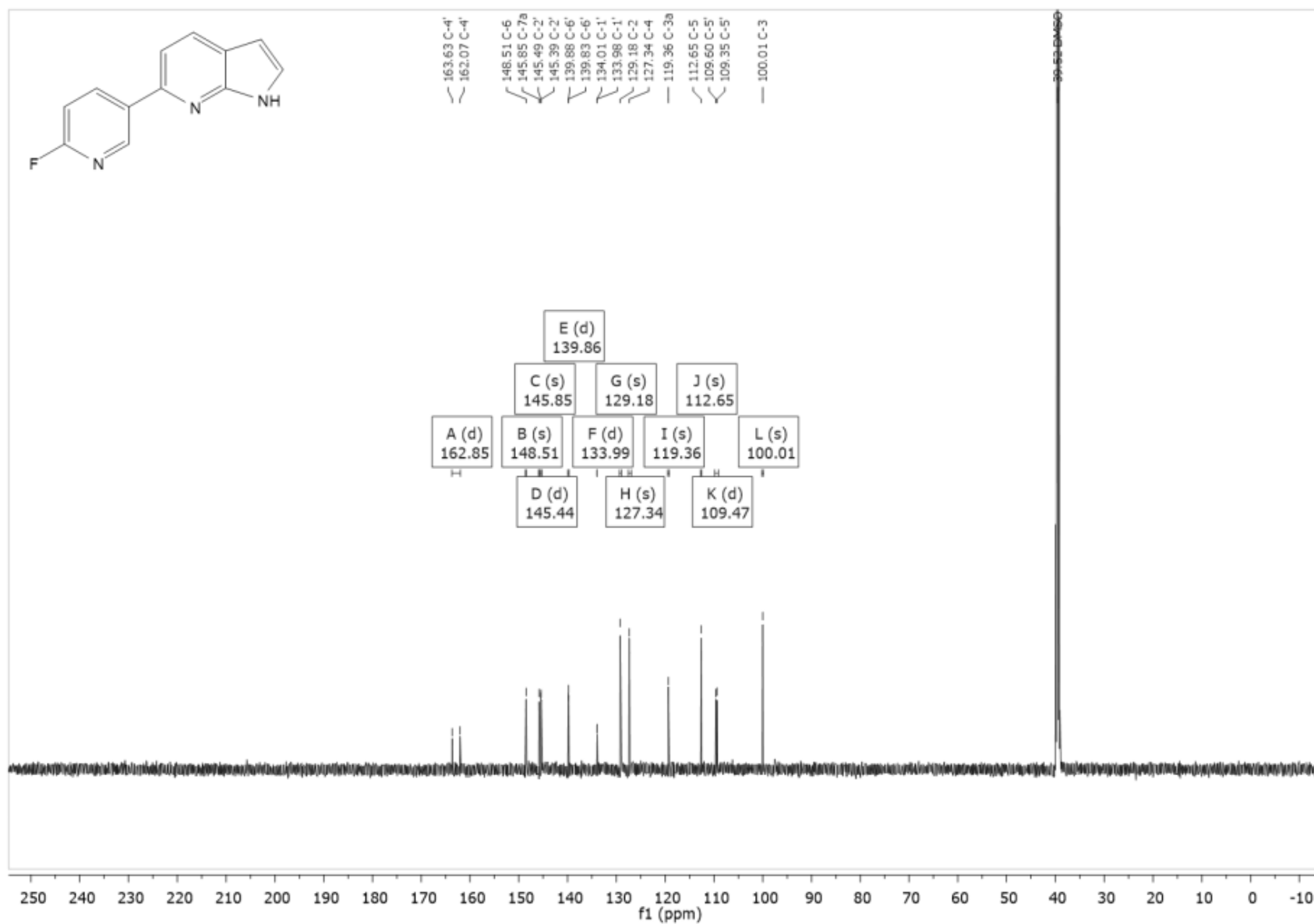
6-(pyridin-4-yl)-1*H*-pyrrolo[2,3-*b*]pyridine (¹³C) **5j**:



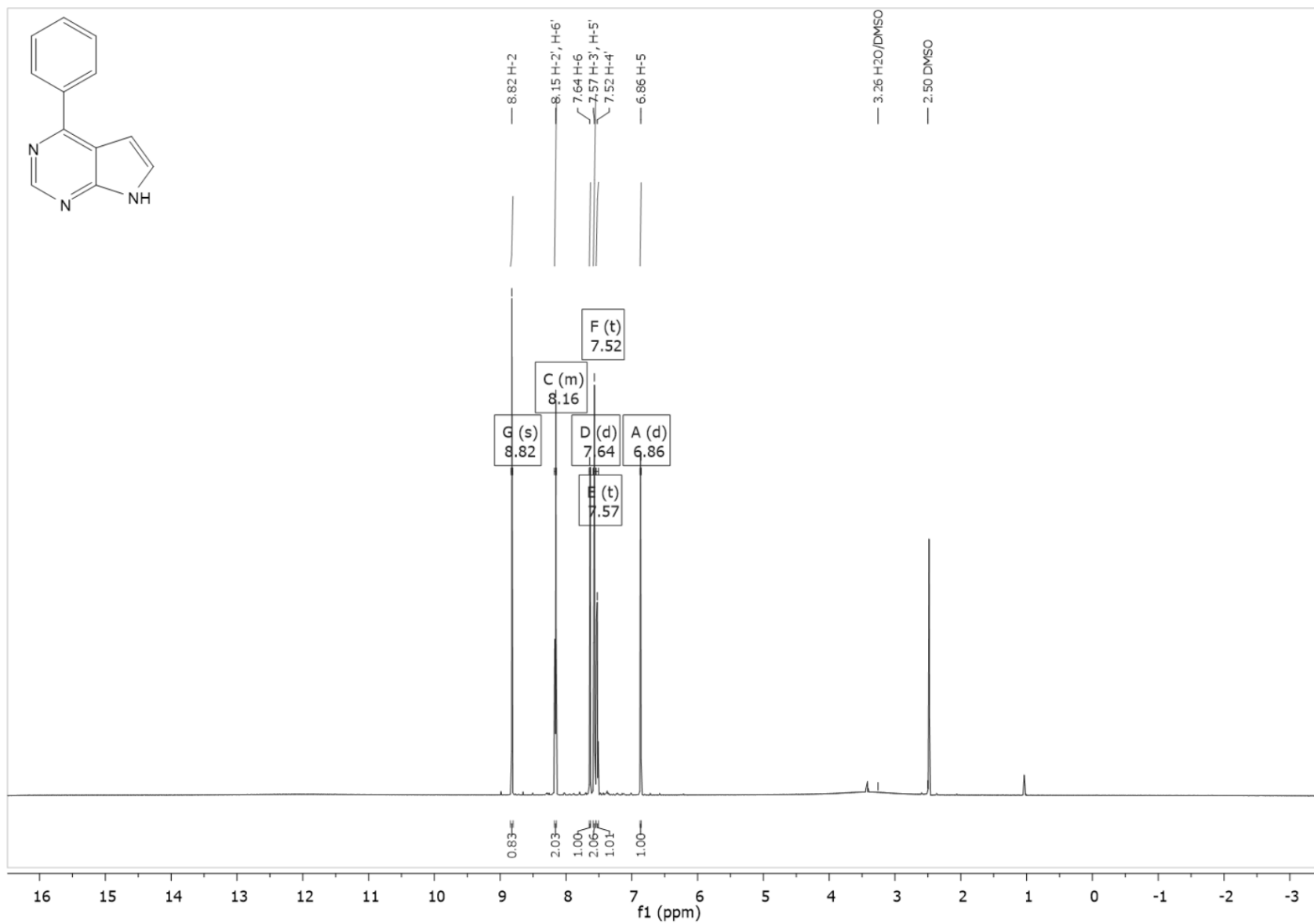
6-(6-fluoropyridin-3-yl)-1*H*-pyrrolo[2,3-*b*]pyridine (¹H) **5k**:



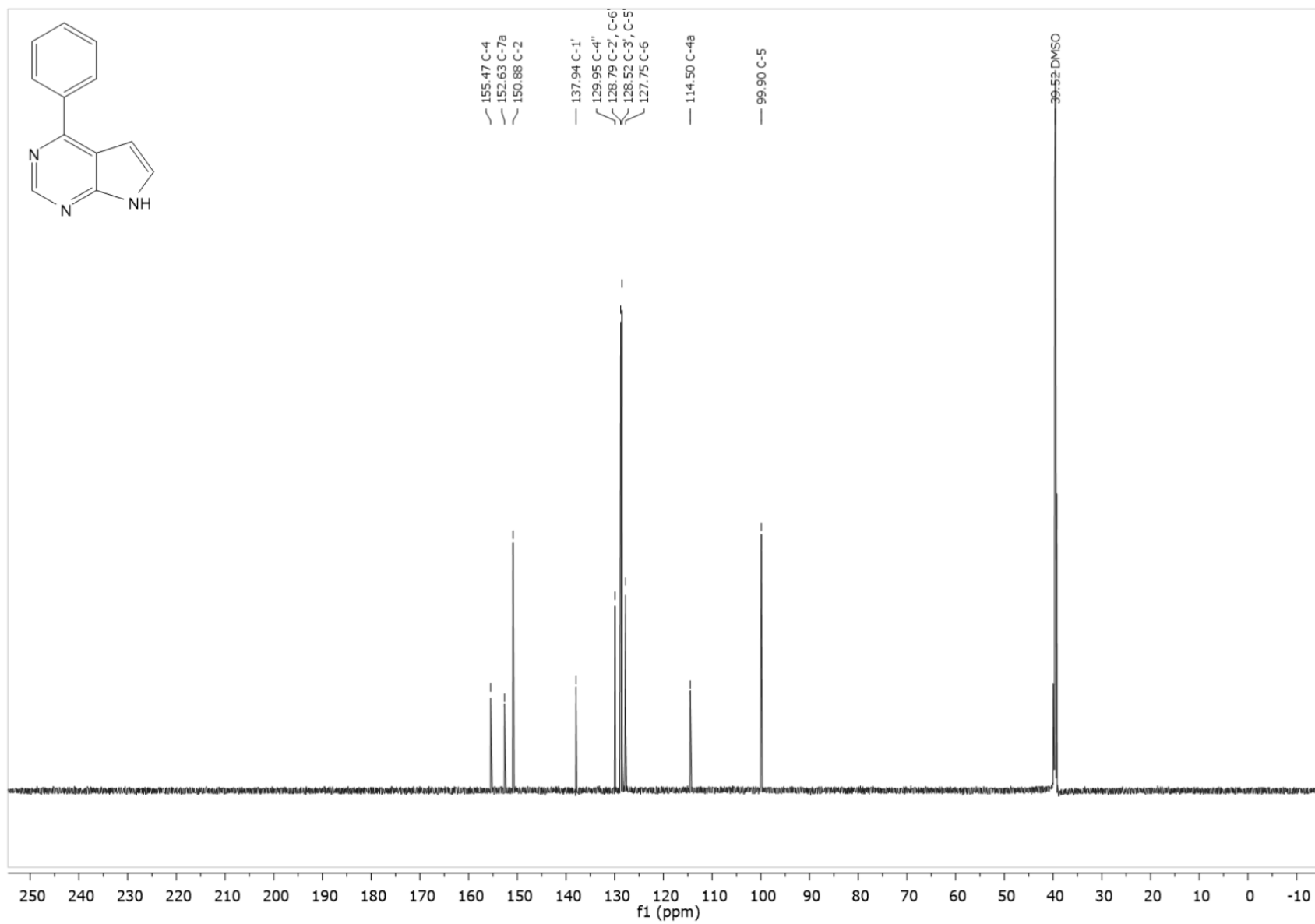
6-(6-fluoropyridin-3-yl)-1*H*-pyrrolo[2,3-*b*]pyridine (^{13}C) **5k**:



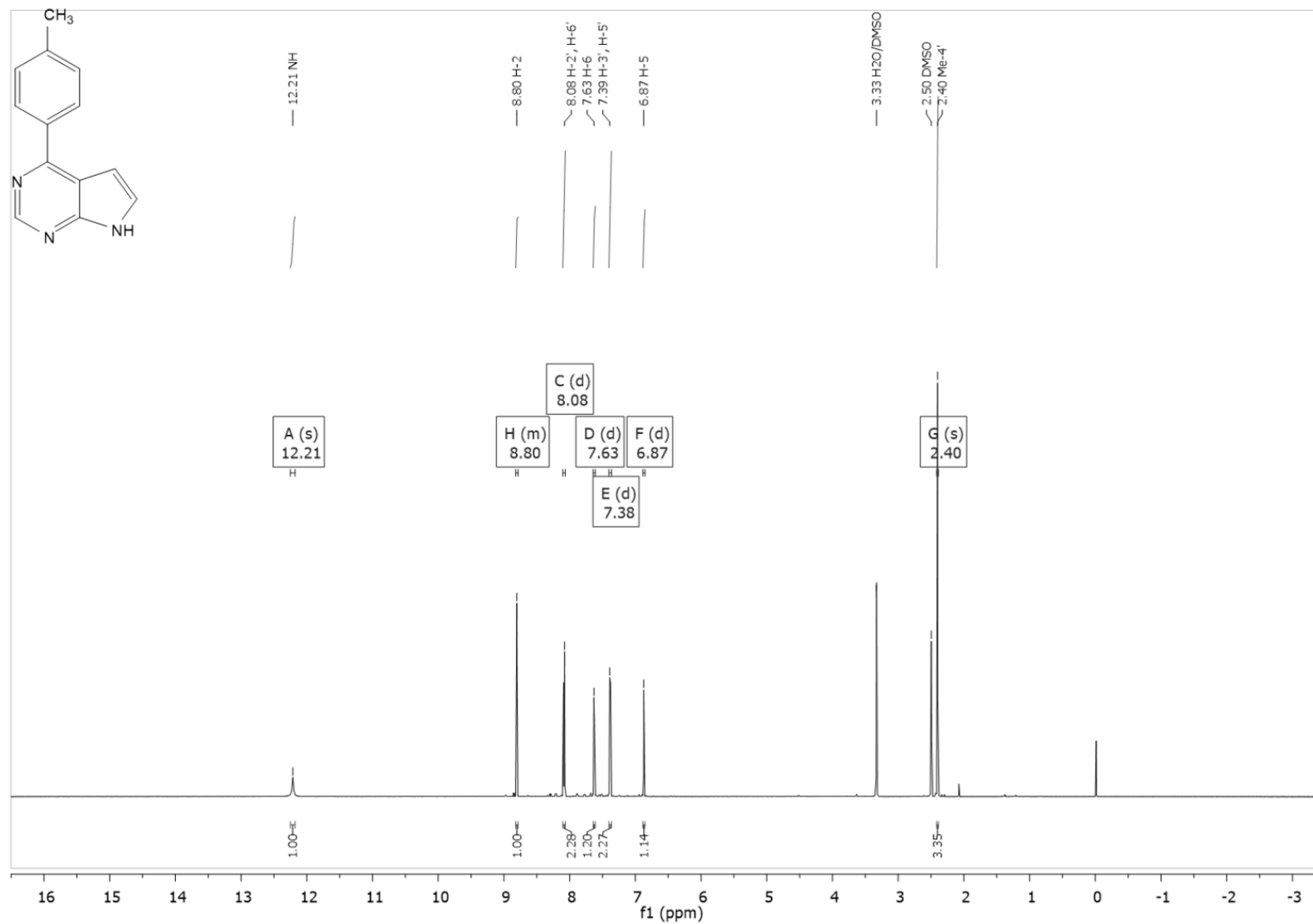
4-Phenyl-7H-pyrrolo[2,3-d]pyrimidine (¹H) **7a**:



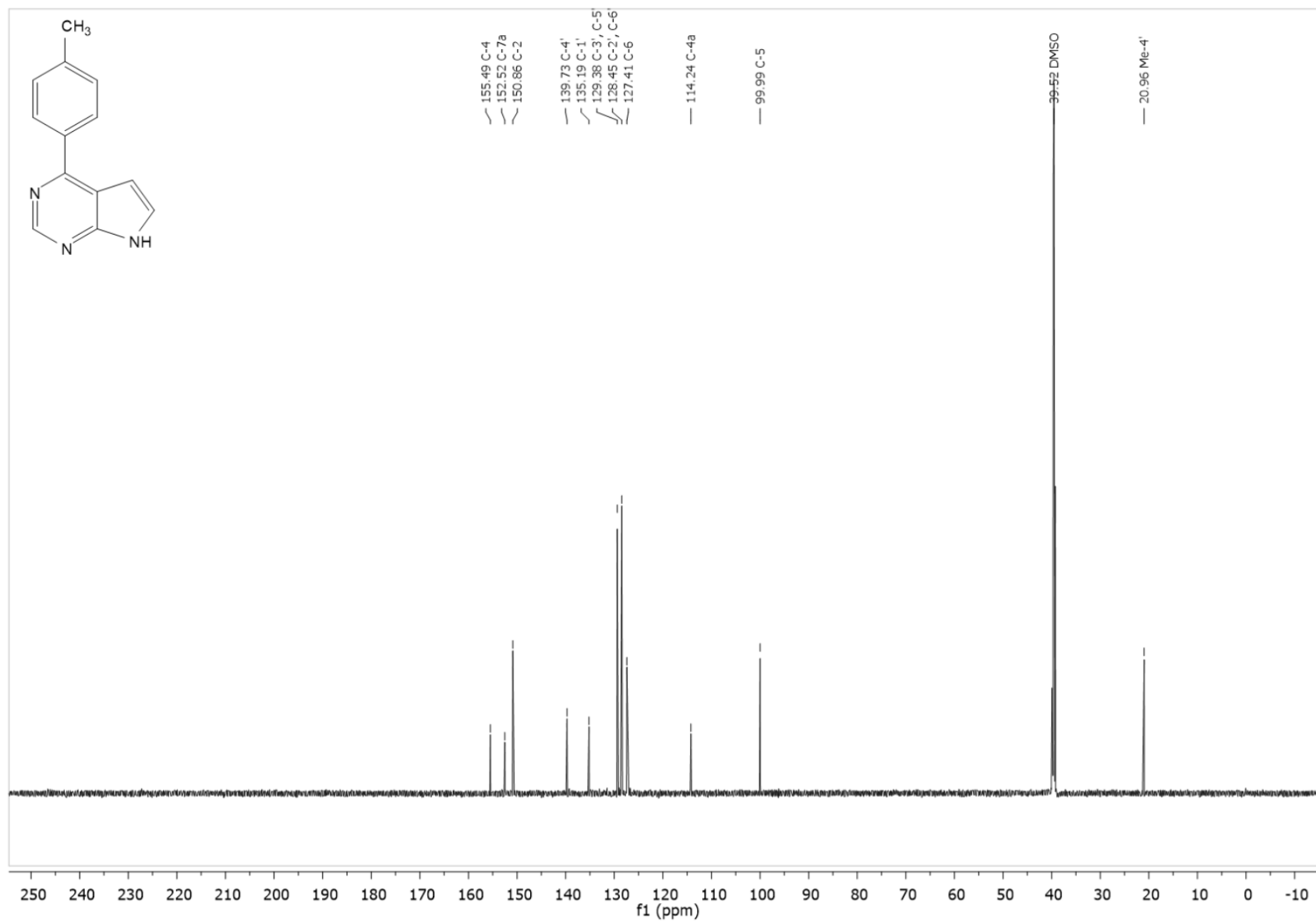
4-Phenyl-7*H*-pyrrolo[2,3-*d*]pyrimidine (¹³C) **7a**:



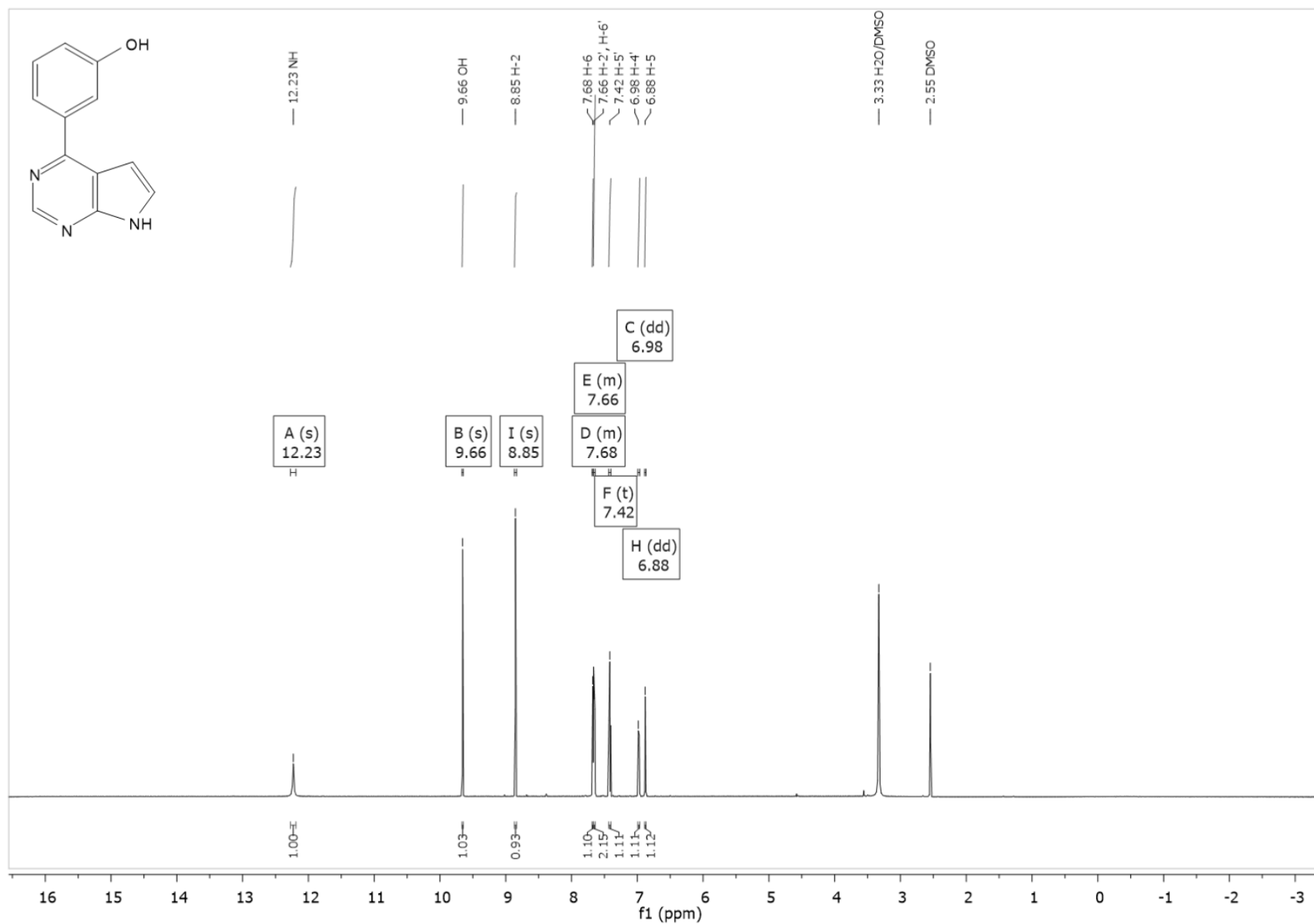
4-(4-Methylphenyl)-7H-pyrrolo[2,3-d]pyrimidine (¹H) **7b**:



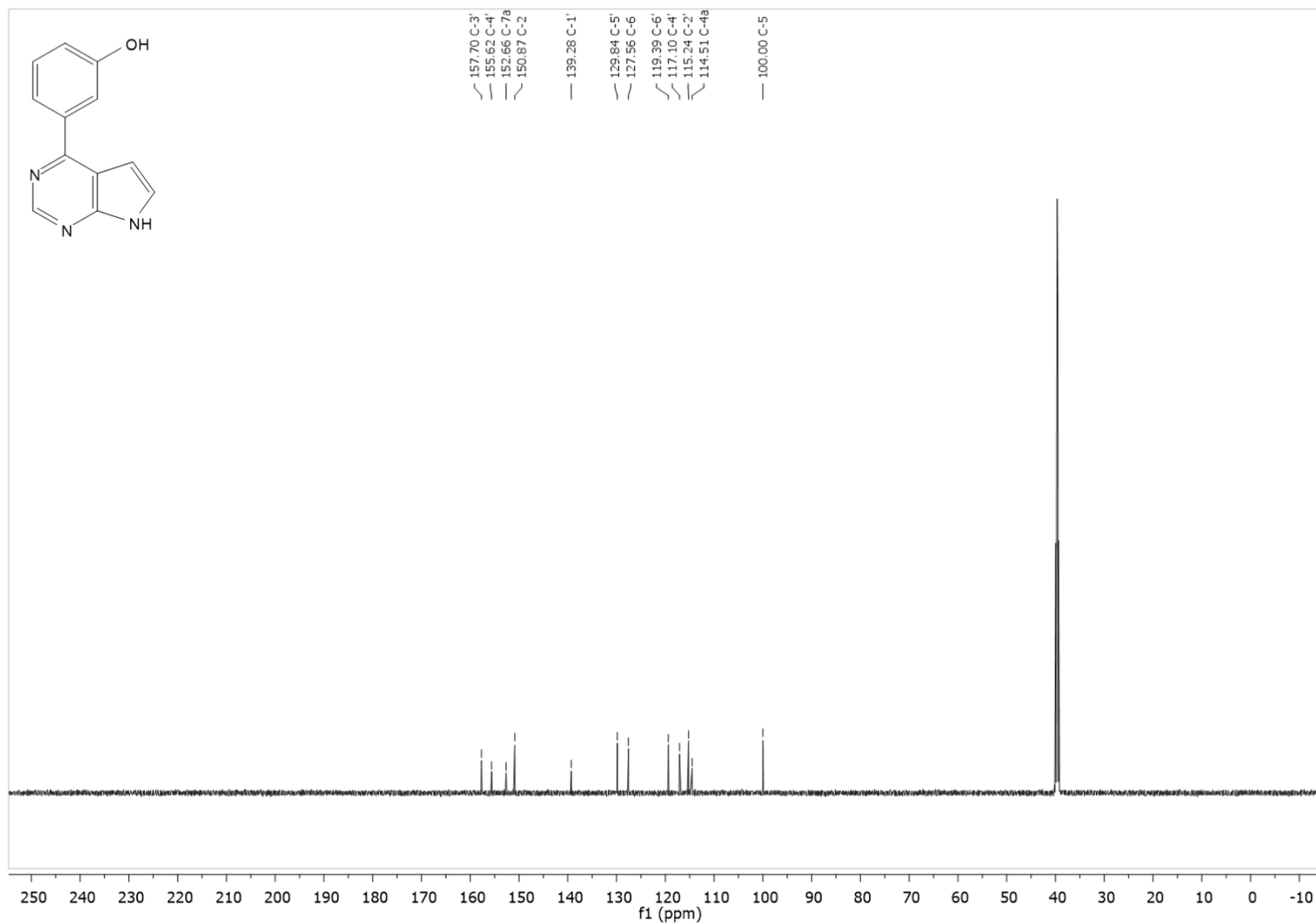
4-(4-Methylphenyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine (¹³C) **7b**:



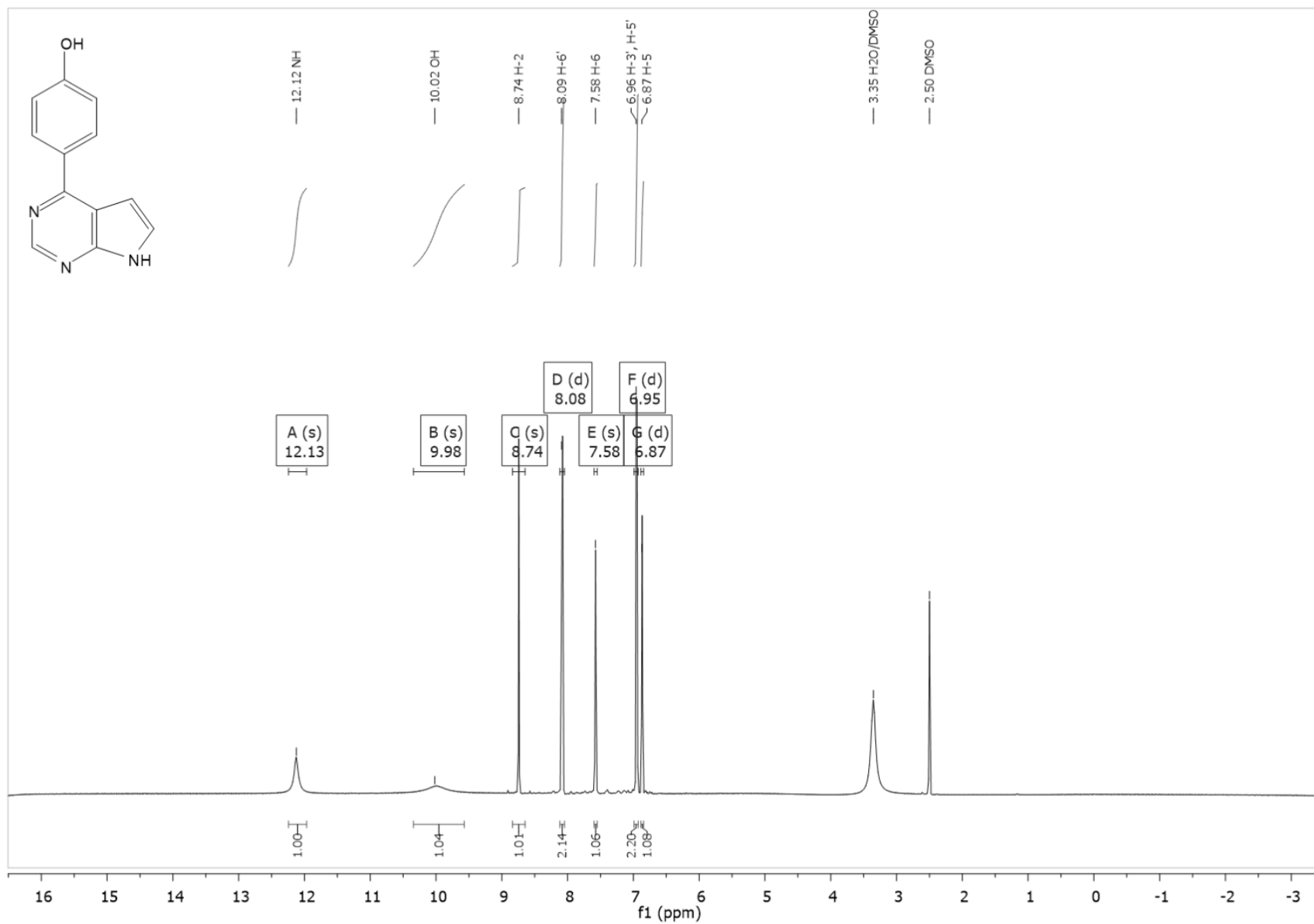
3-(7*H*-Pyrrolo[2,3-*d*]pyrimidin-4-yl)phenol (¹H) **7c**:



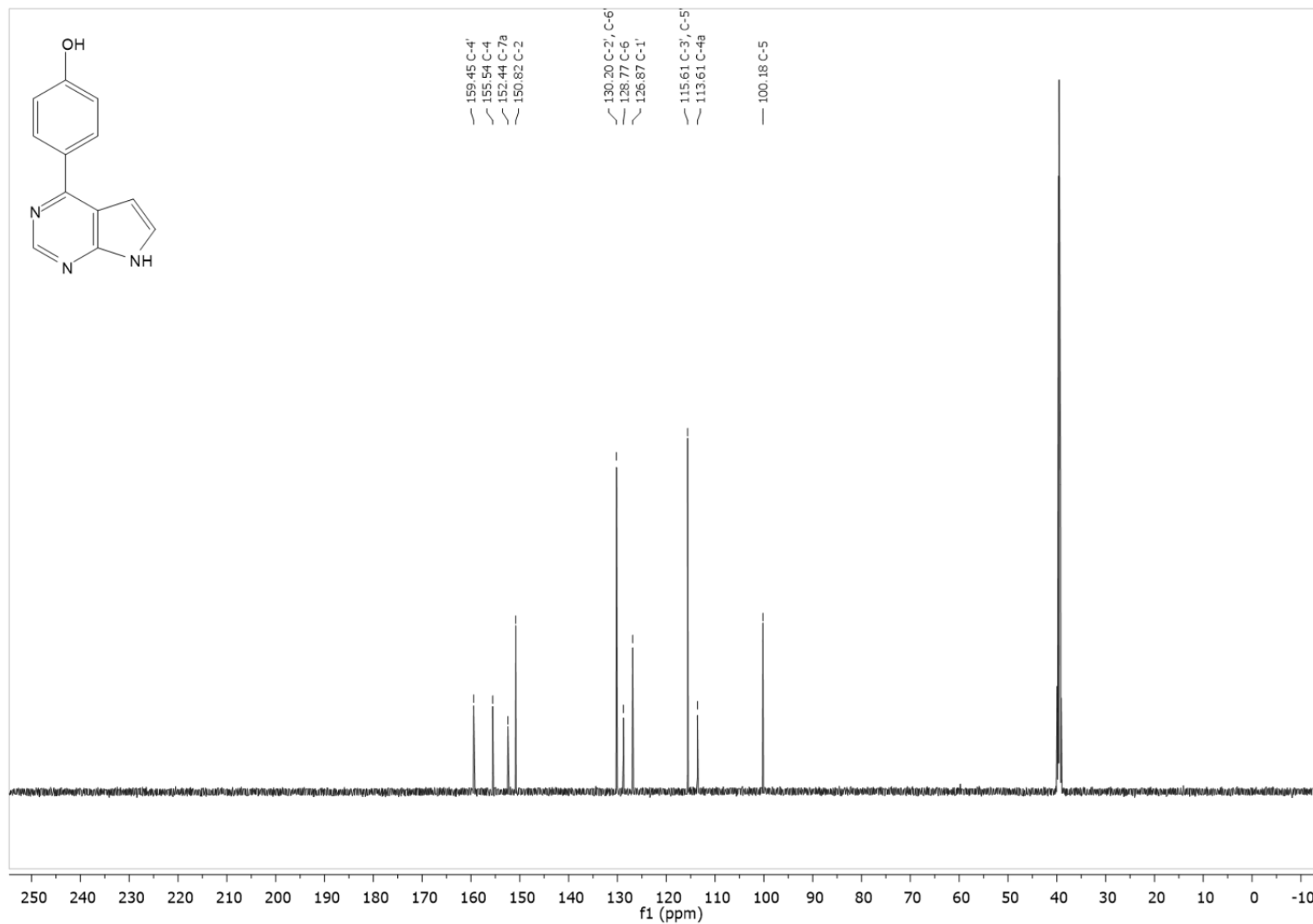
3-(7*H*-Pyrrolo[2,3-*d*]pyrimidin-4-yl)phenol (¹³C) **7c**:



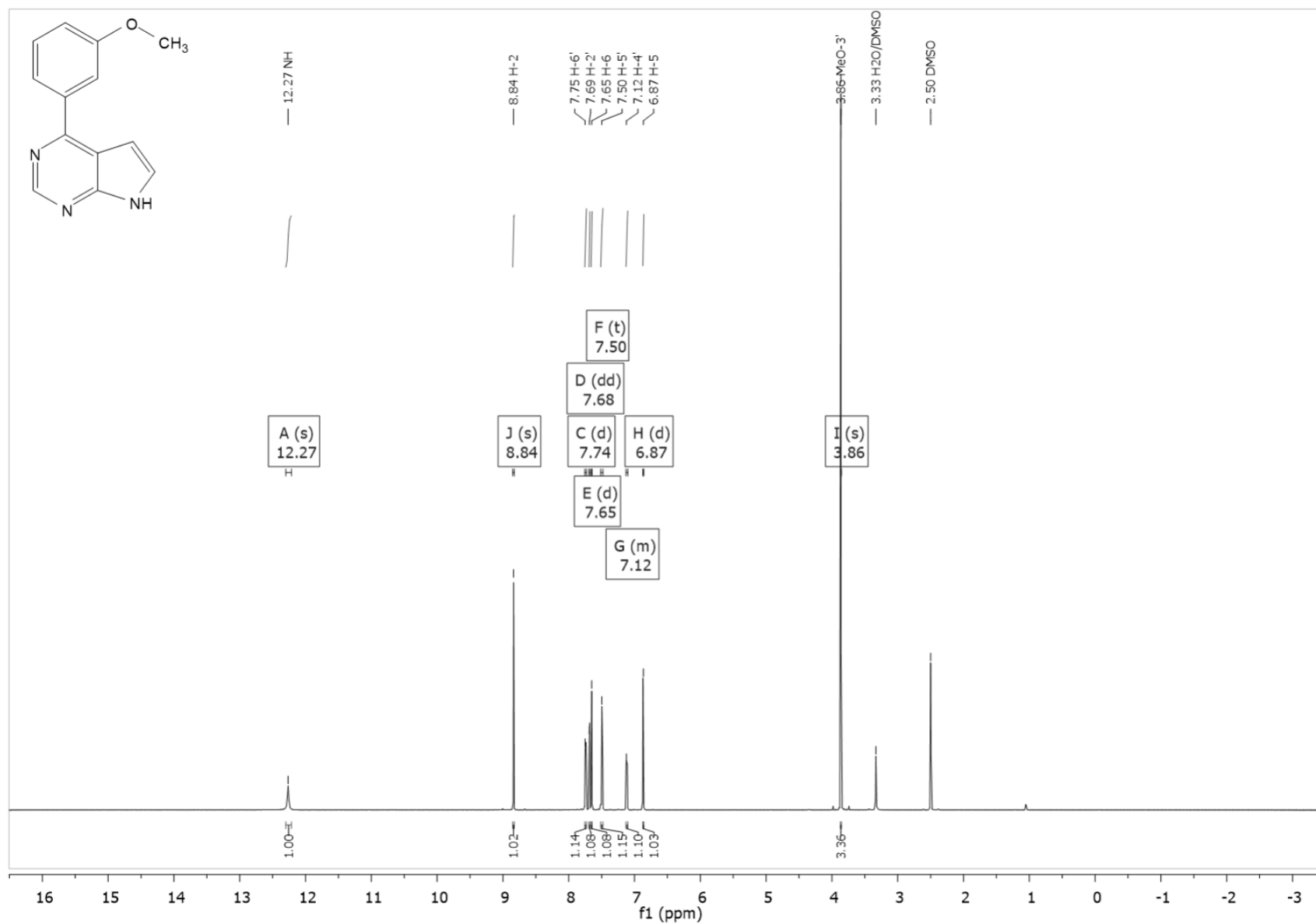
4-(7H-Pyrrolo[2,3-d]pyrimidin-4-yl)phenol (¹H) **7d**:



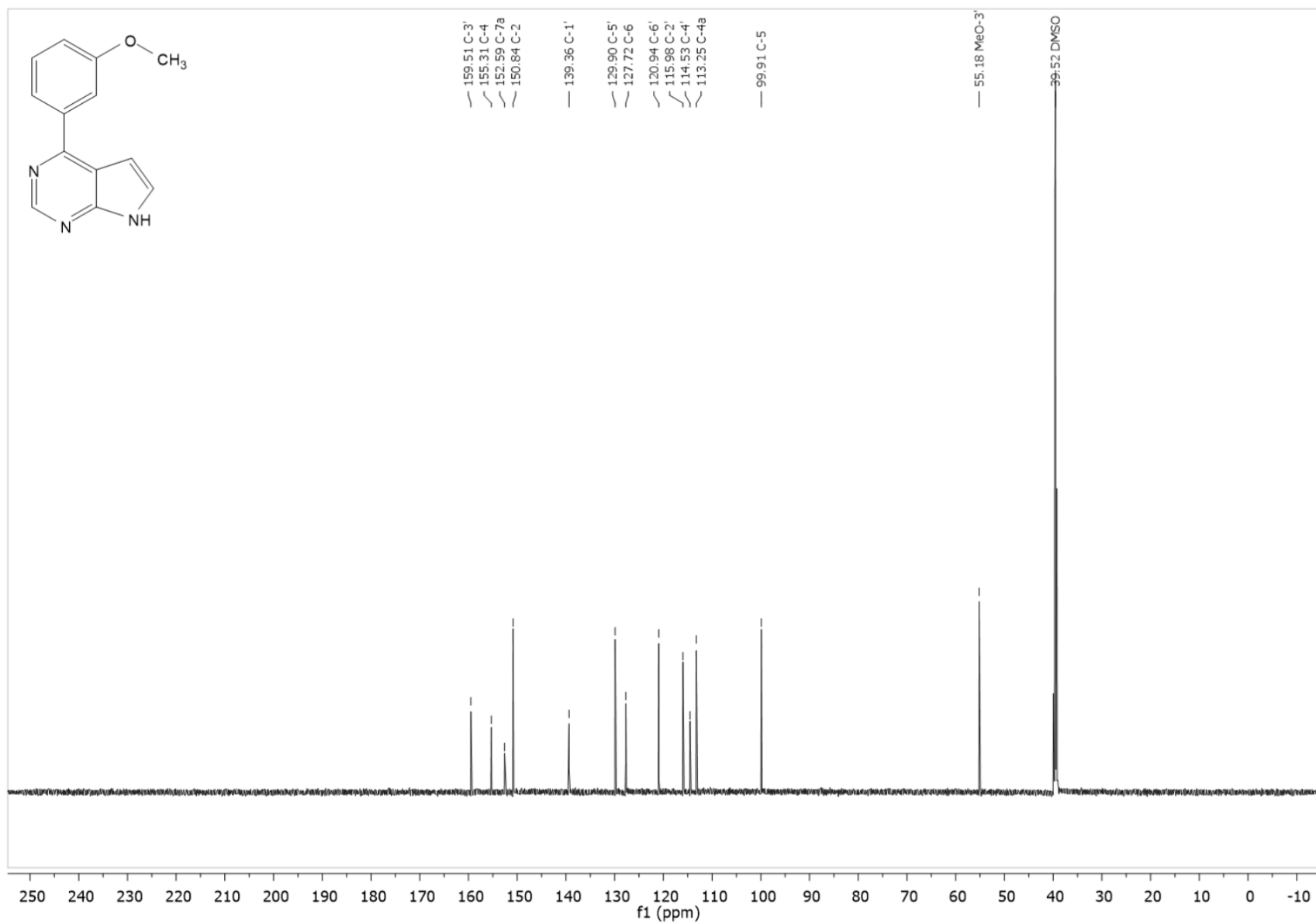
4-(7H-Pyrrolo[2,3-d]pyrimidin-4-yl)phenol (^{13}C) **7d**:



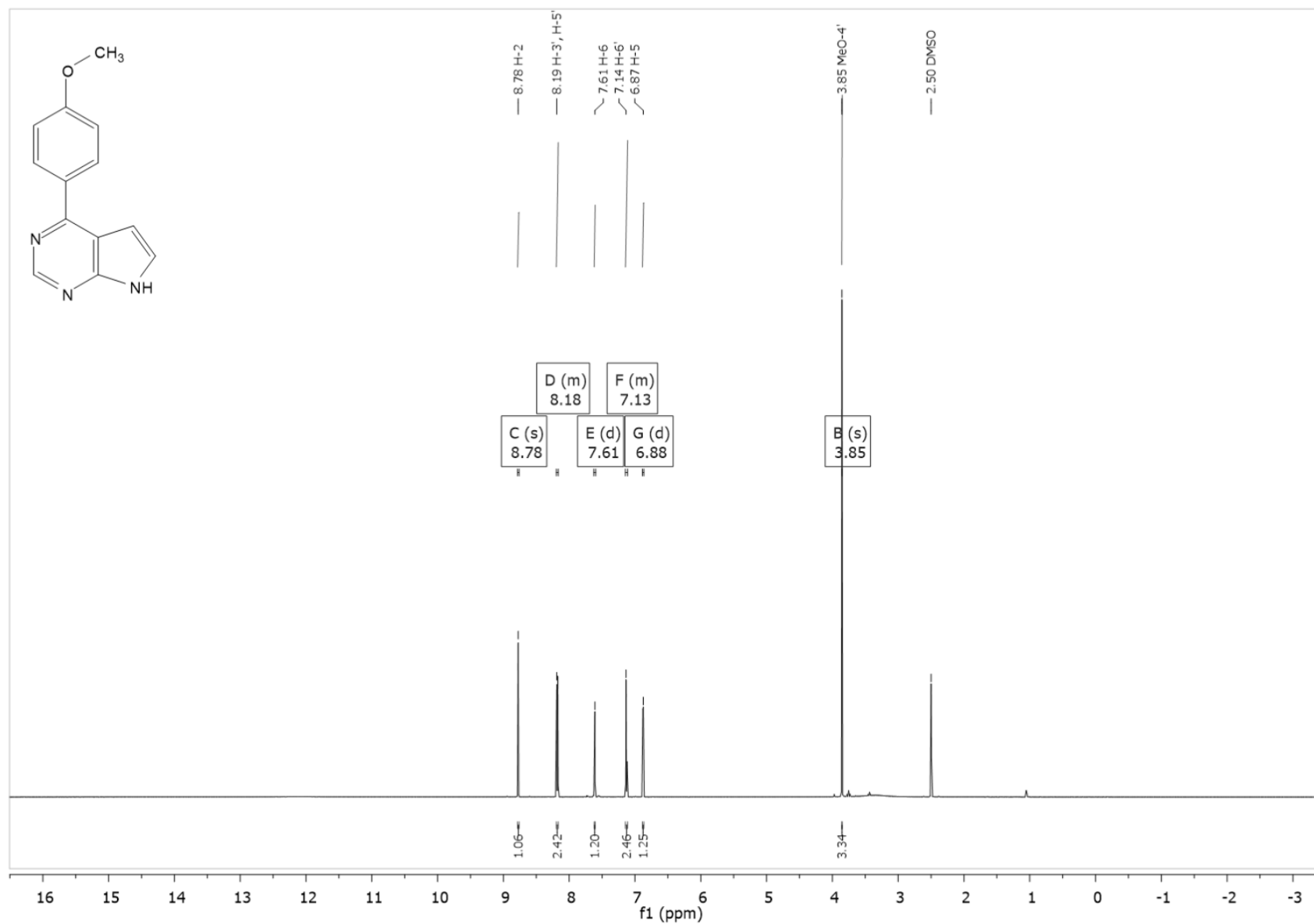
4-(3-Methoxyphenyl)-7H-pyrrolo[2,3-d]pyrimidine (¹H) **7e**:



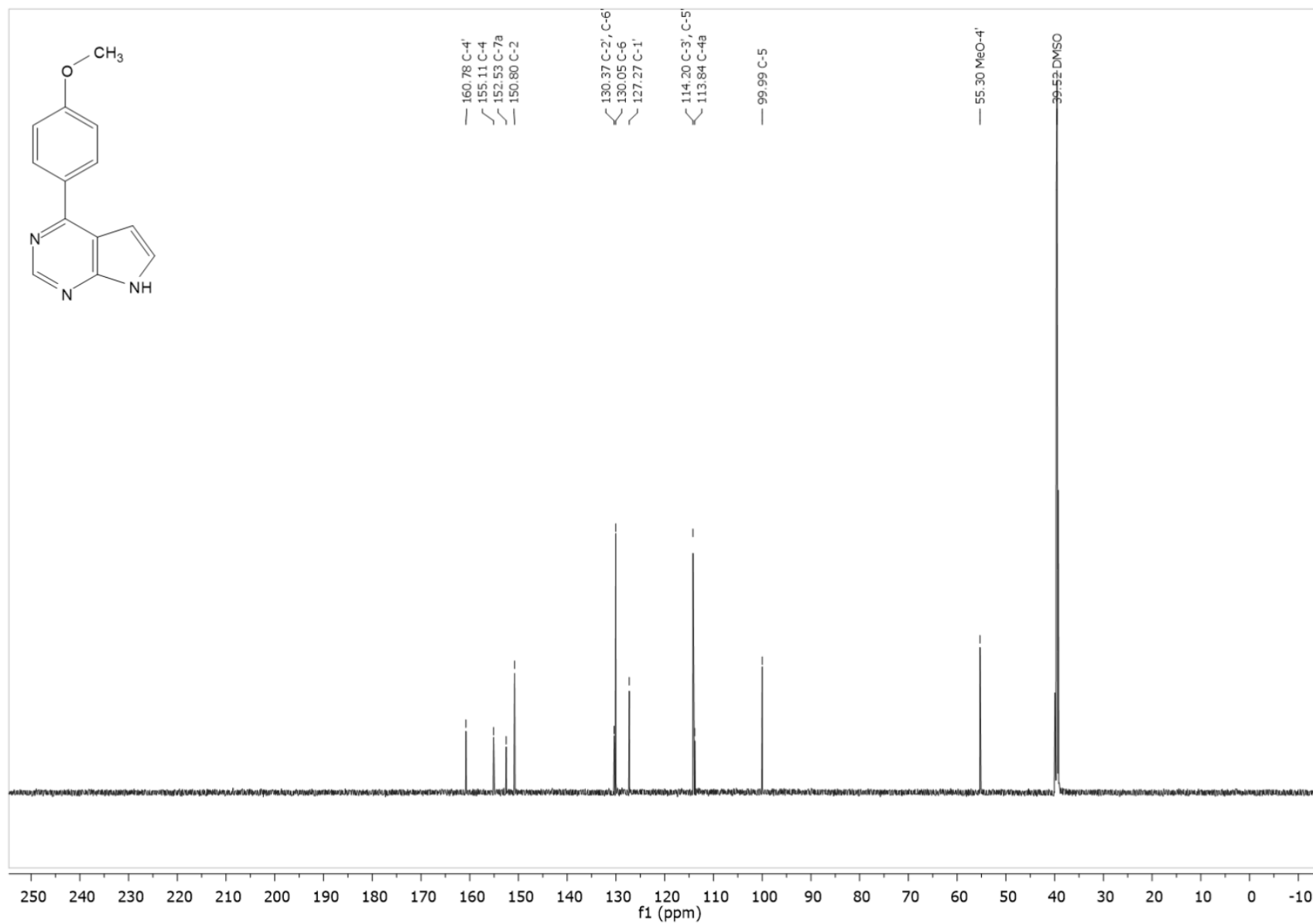
4-(3-Methoxyphenyl)-7H-pyrrolo[2,3-d]pyrimidine (^{13}C) **7e**:



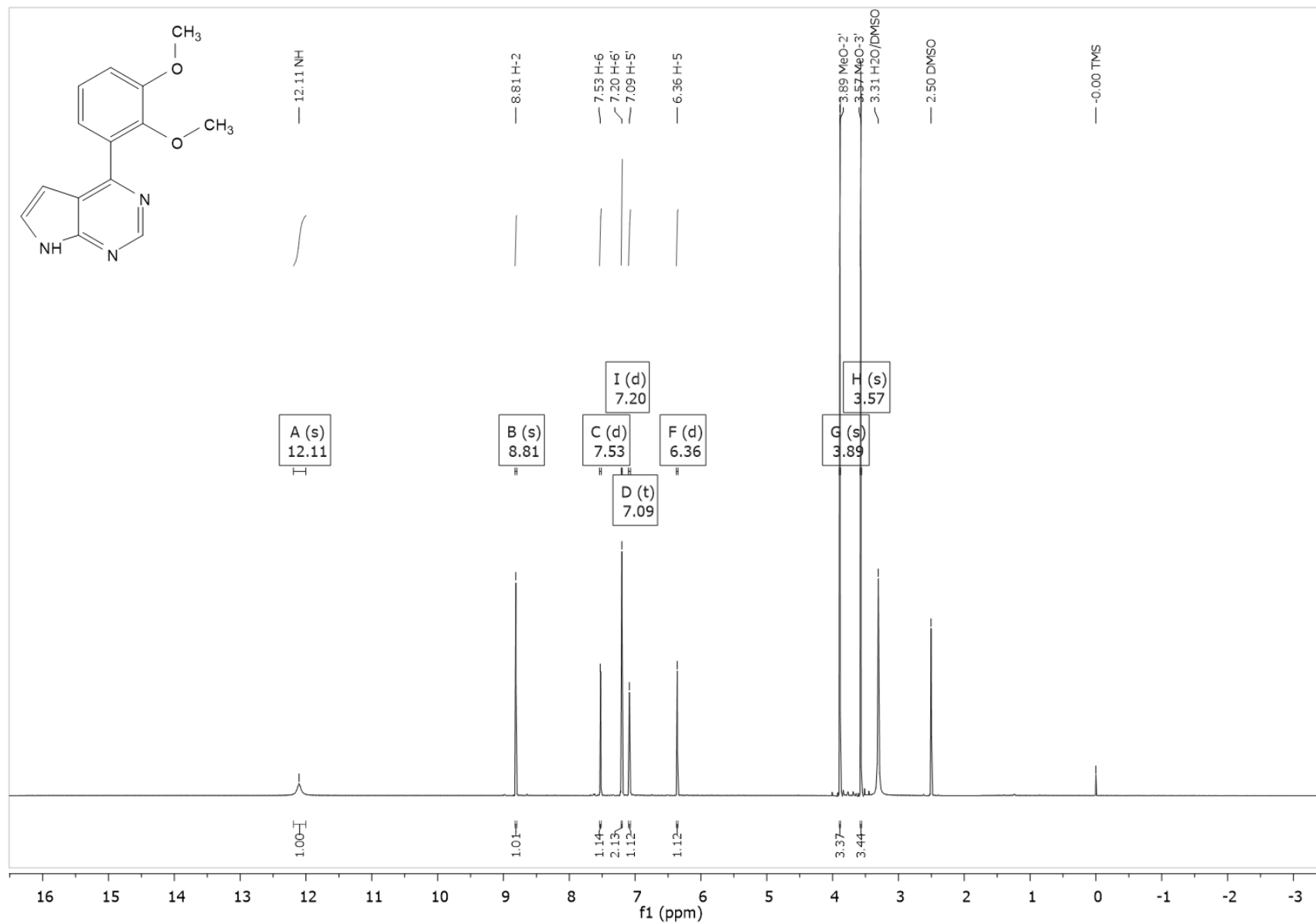
4-(4-Methoxyphenyl)-7H-pyrrolo[2,3-d]pyrimidine (¹H) **7f**:



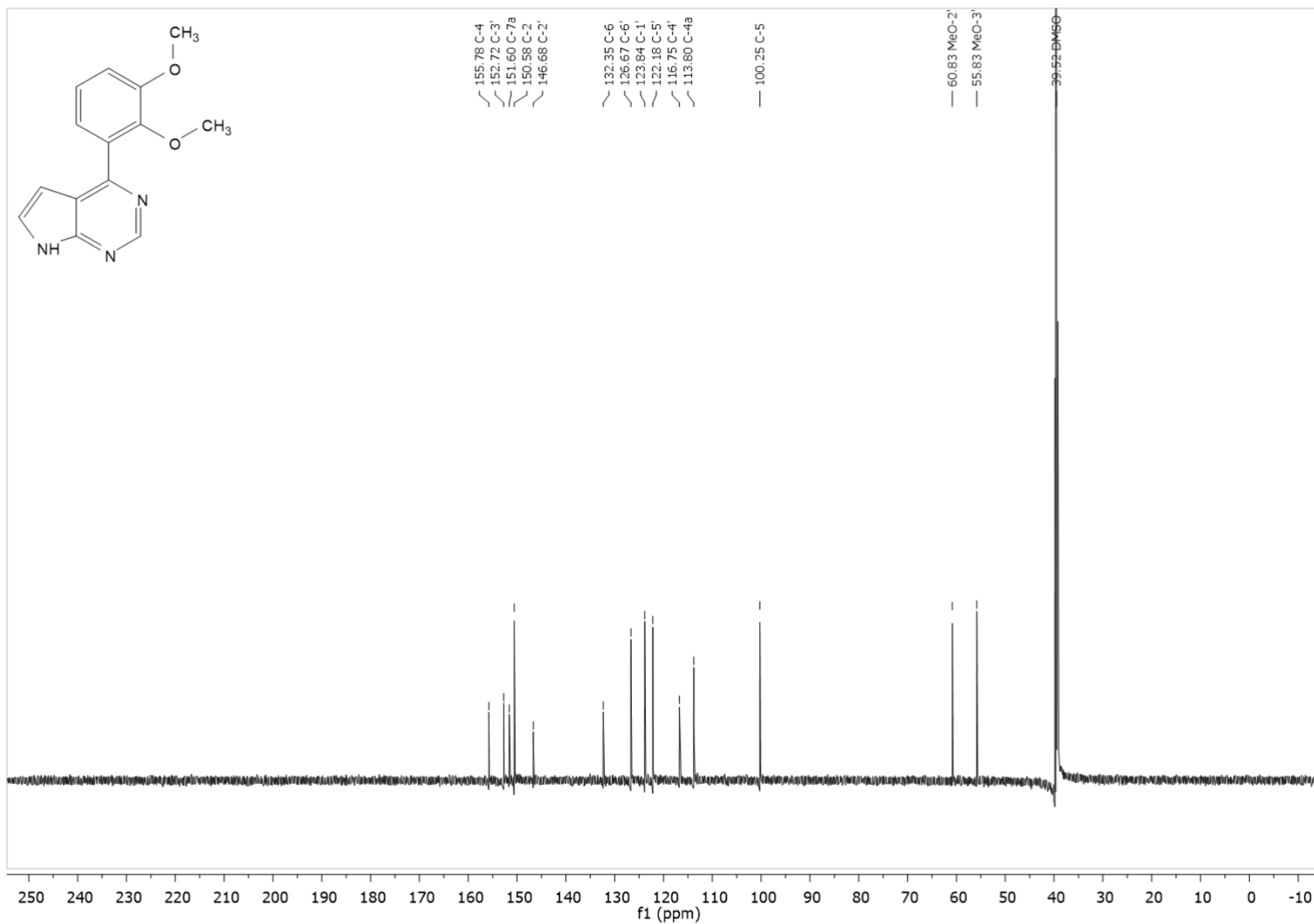
4-(4-Methoxyphenyl)-7H-pyrrolo[2,3-d]pyrimidine (^{13}C) **7f**:



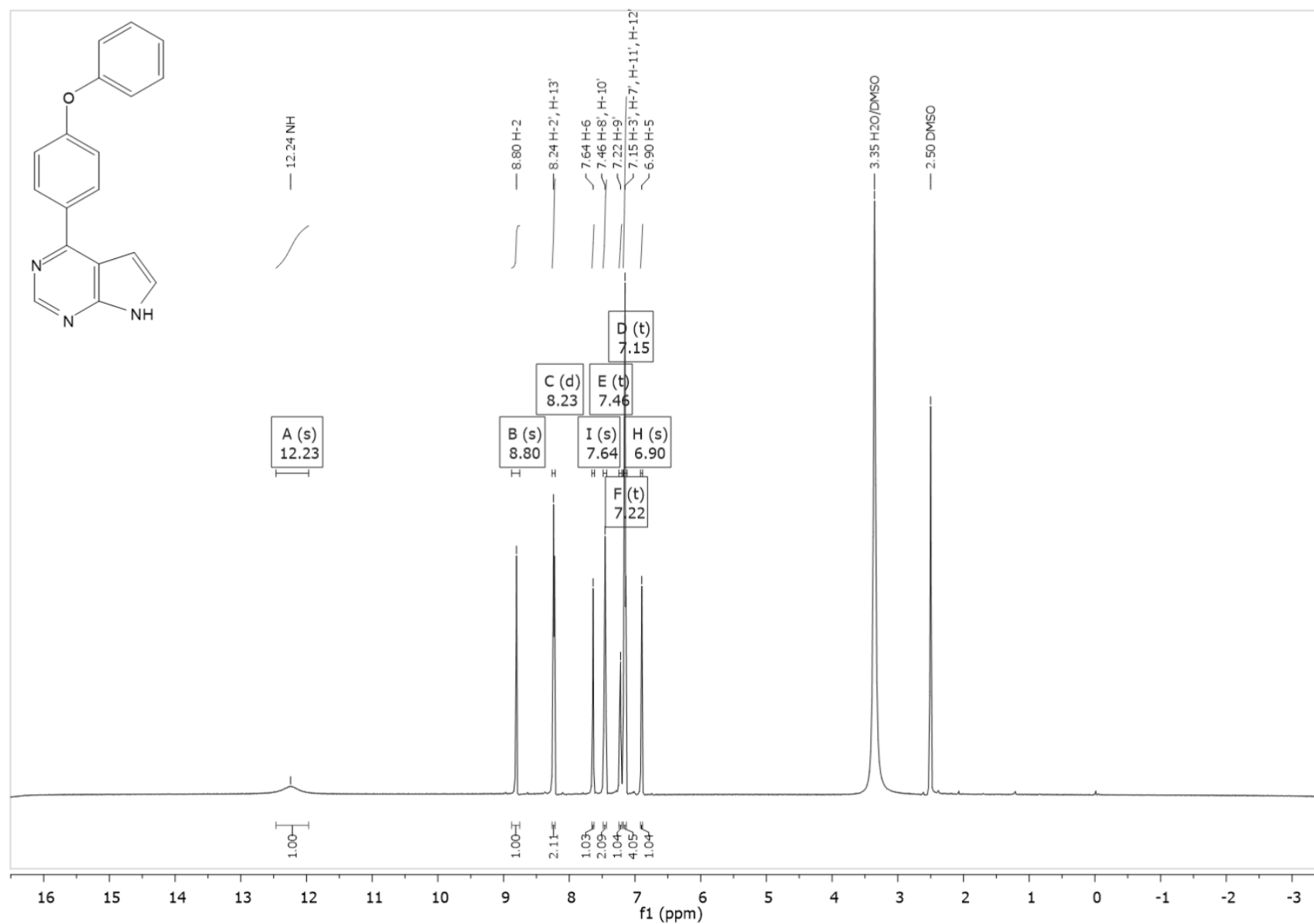
4-(2,3-Dimethoxyphenyl)-7H-pyrrolo[2,3-d]pyrimidine (¹H) **7g**:



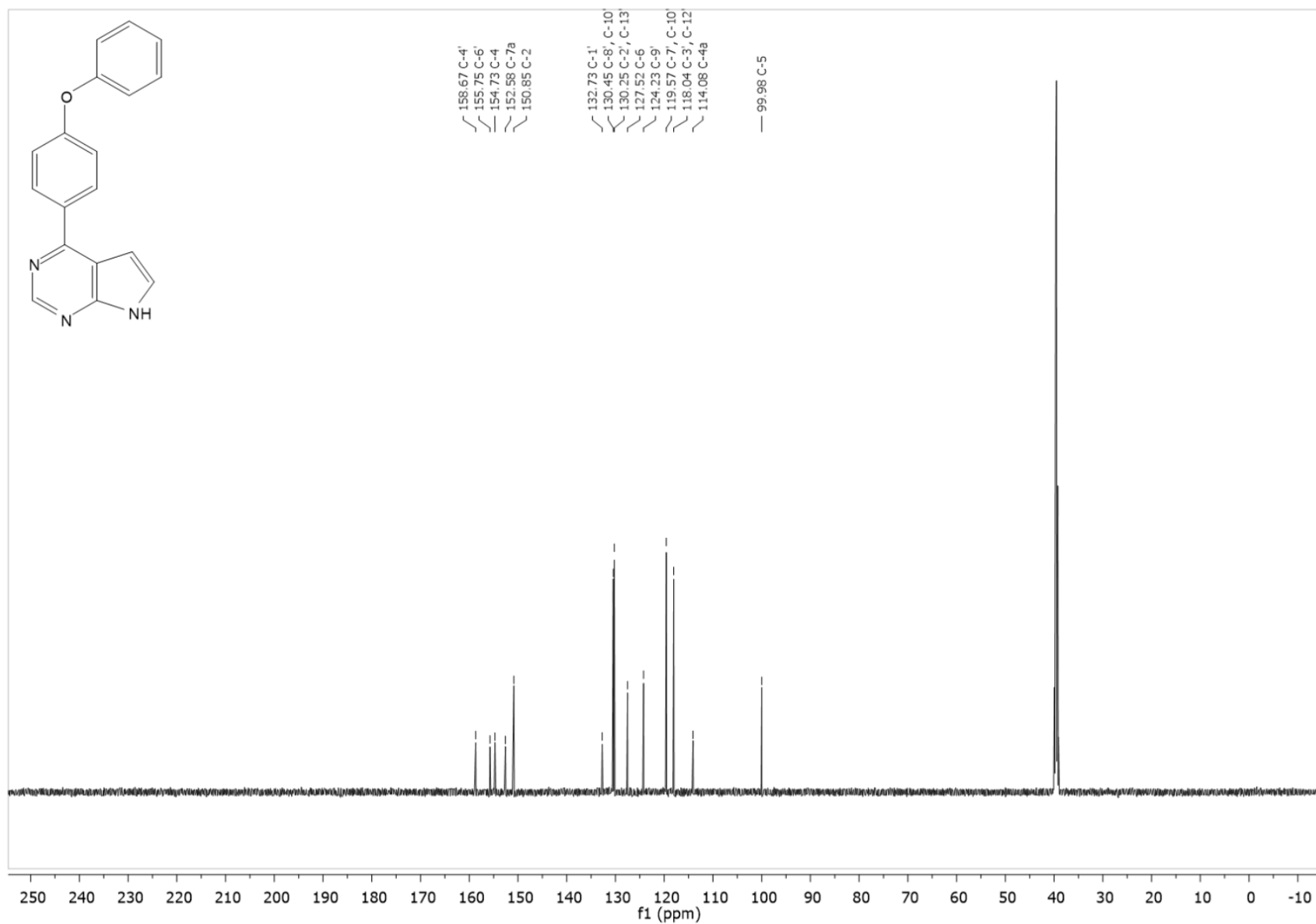
4-(2,3-Dimethoxyphenyl)-7H-pyrrolo[2,3-d]pyrimidine (^{13}C) **7g**:



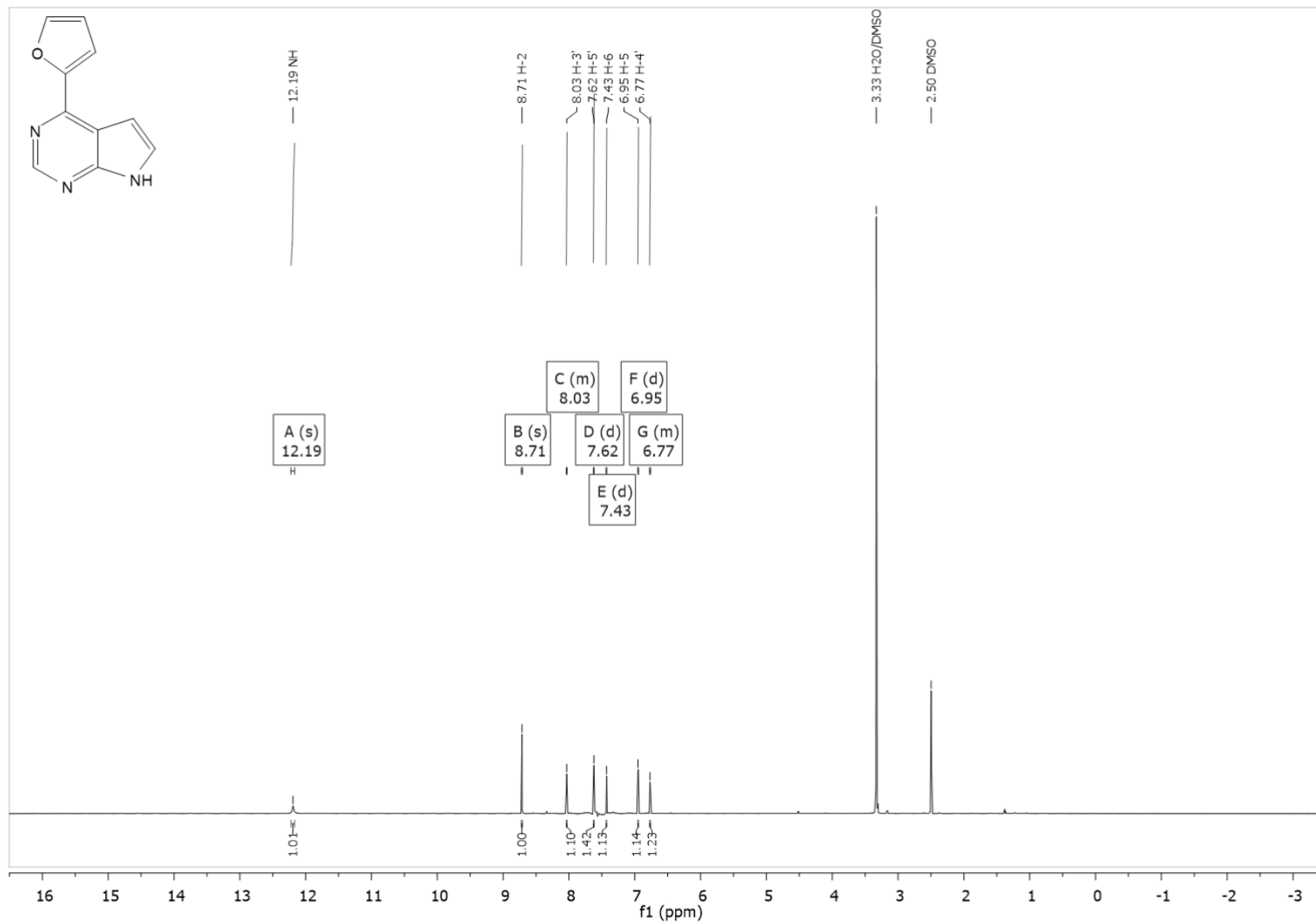
4-(4-Phenoxyphenyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine (¹H) **7h**:



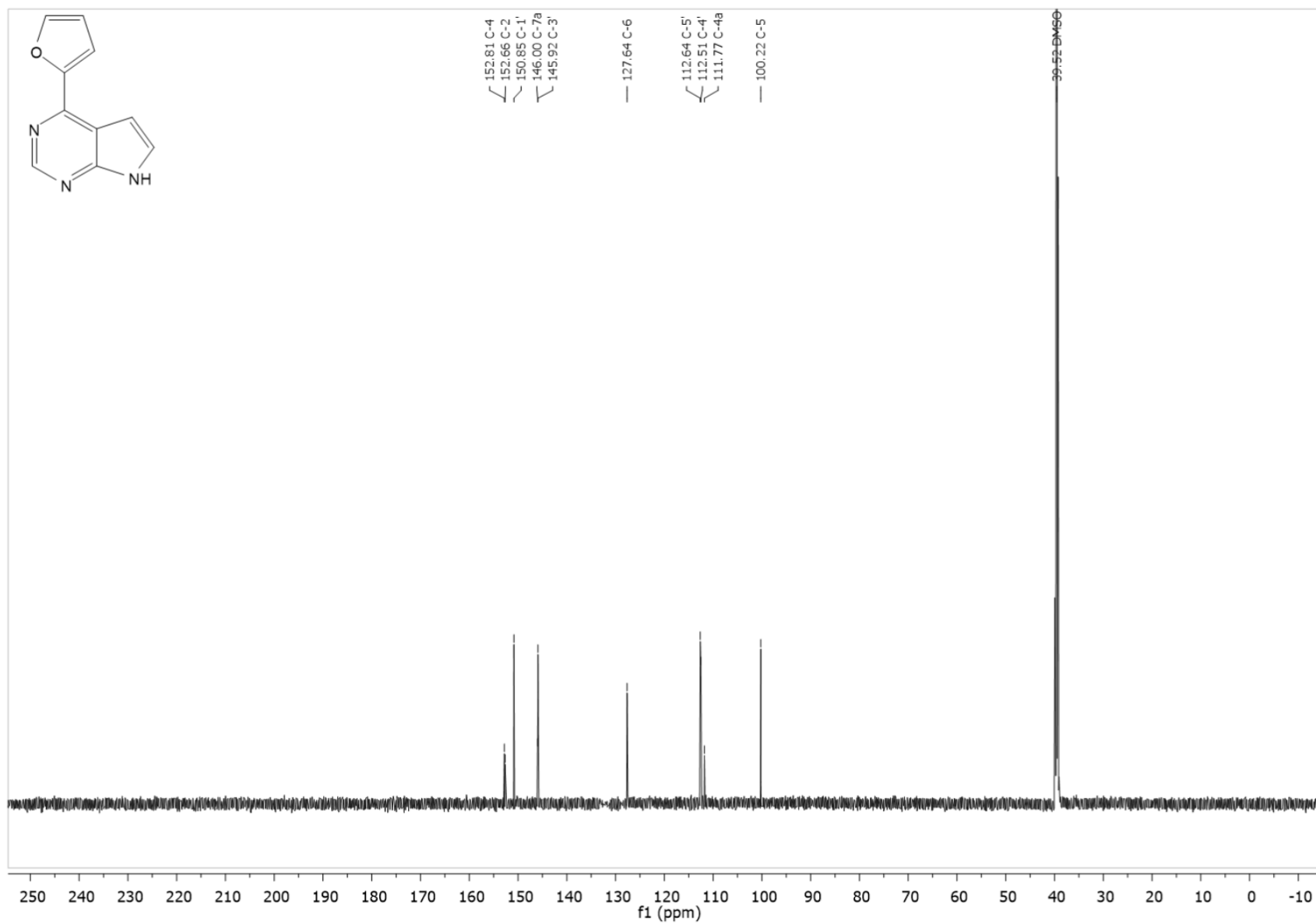
4-(4-Phenoxyphenyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine (¹³C) **7h**:



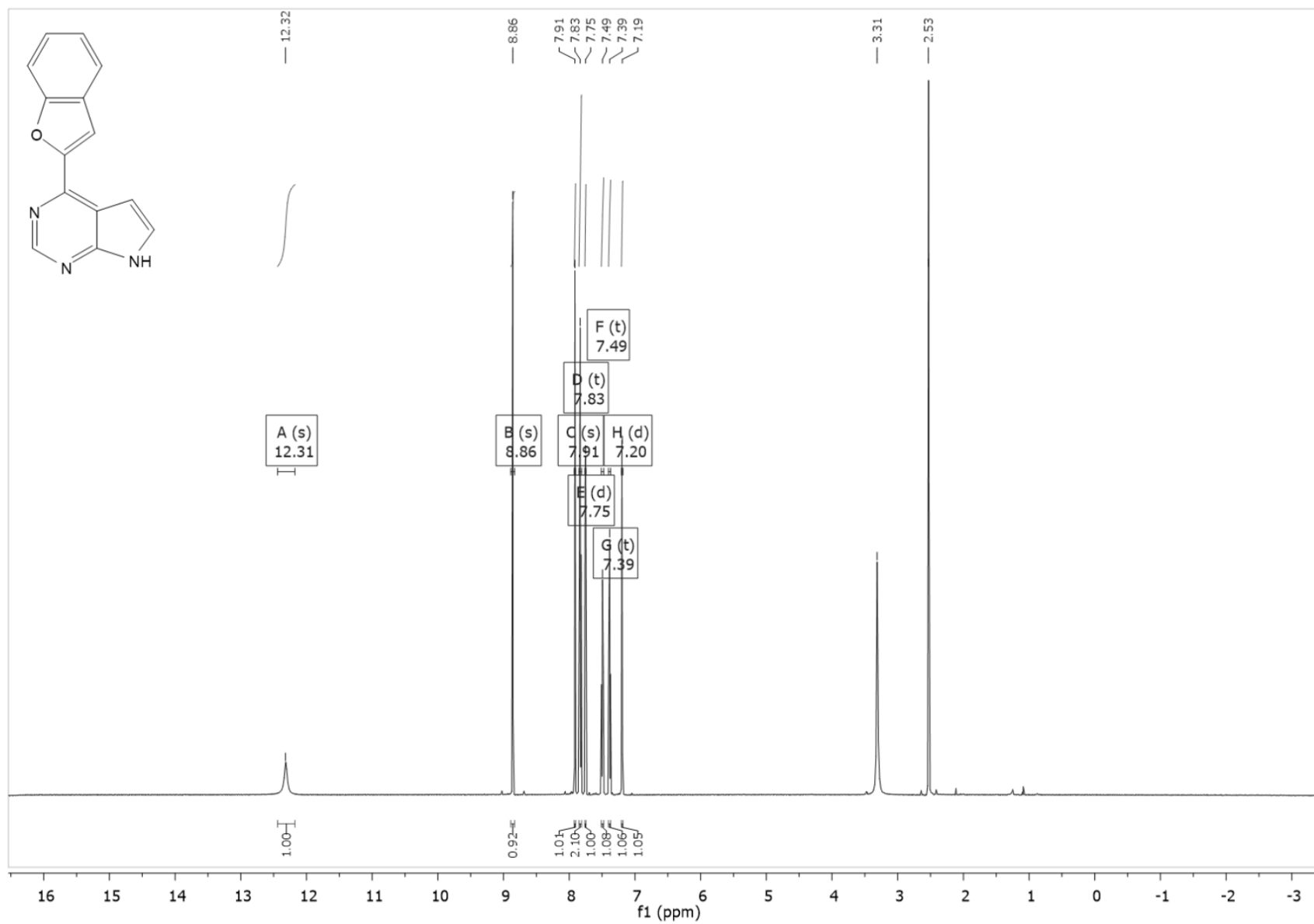
4-(Furan-2-yl)-7*H*-pyrrolo[2,3-*d*]pyrimidine (¹H) **7i**:



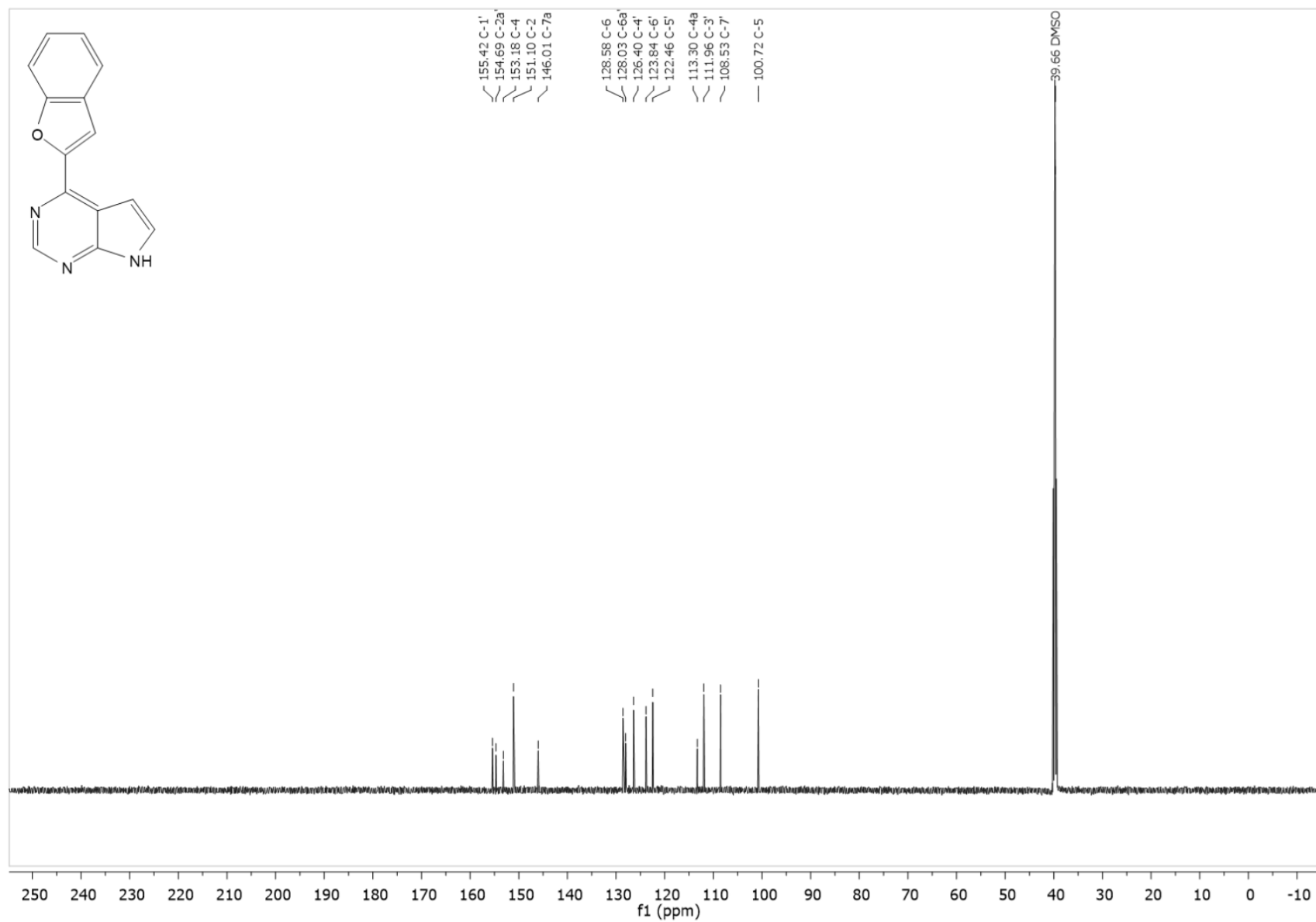
4-(Furan-2-yl)-7*H*-pyrrolo[2,3-*d*]pyrimidine (¹³C) **7i**:



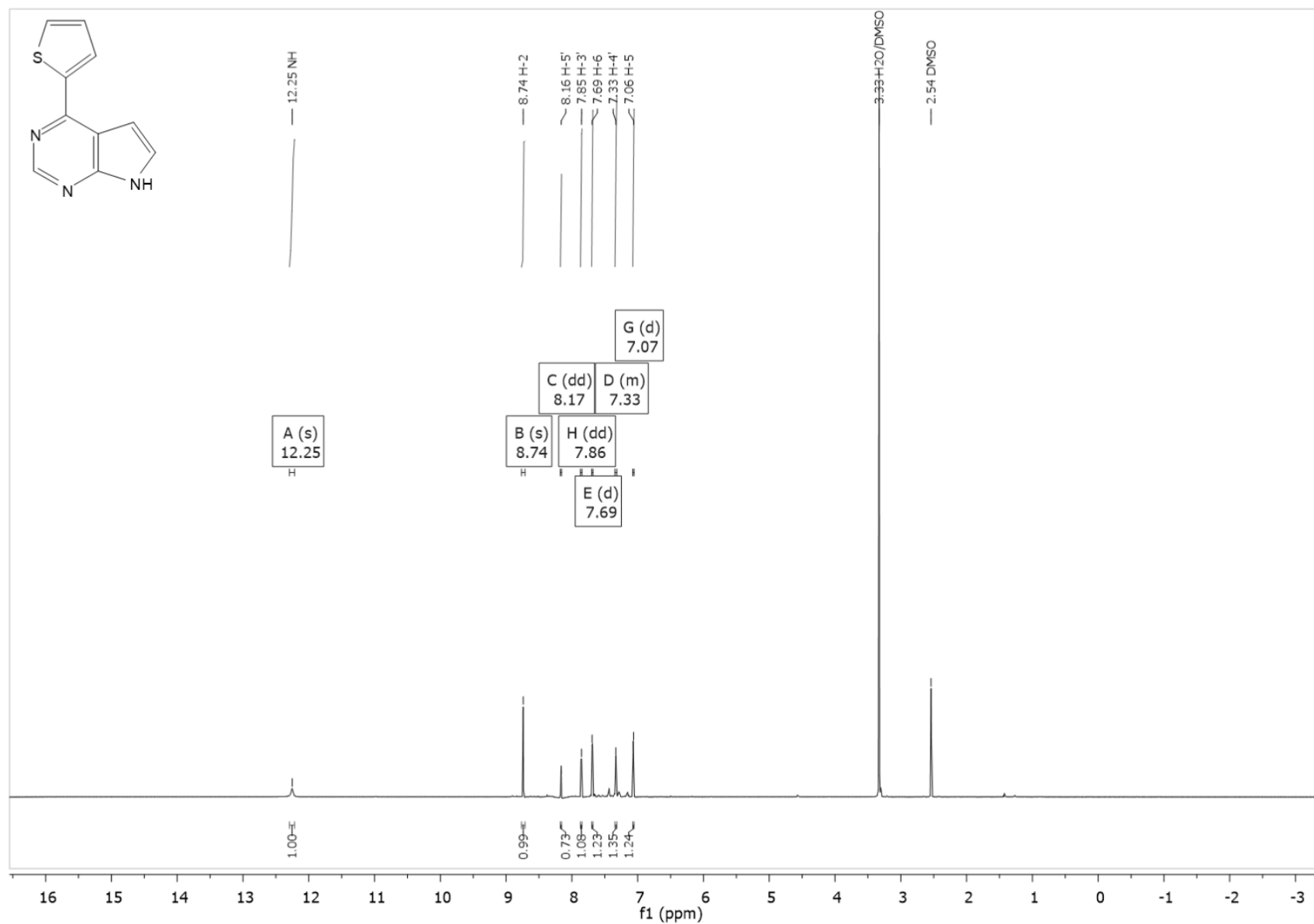
4-(1-Benzofuran-2-yl)-7H-pyrrolo[2,3-d]pyrimidine (¹H) **7j**:



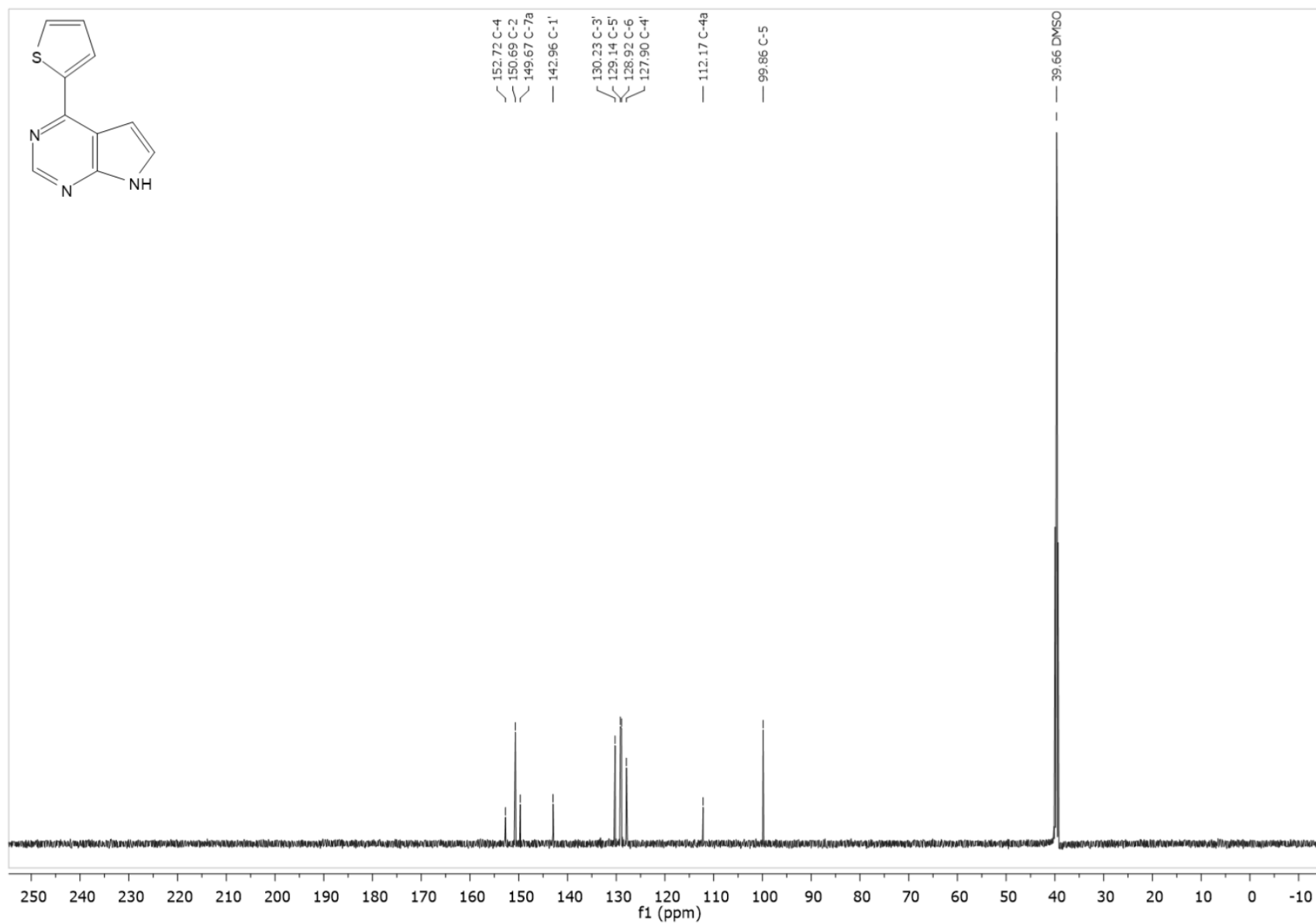
4-(1-Benzofuran-2-yl)-7H-pyrrolo[2,3-d]pyrimidine(¹³C) **7j**:



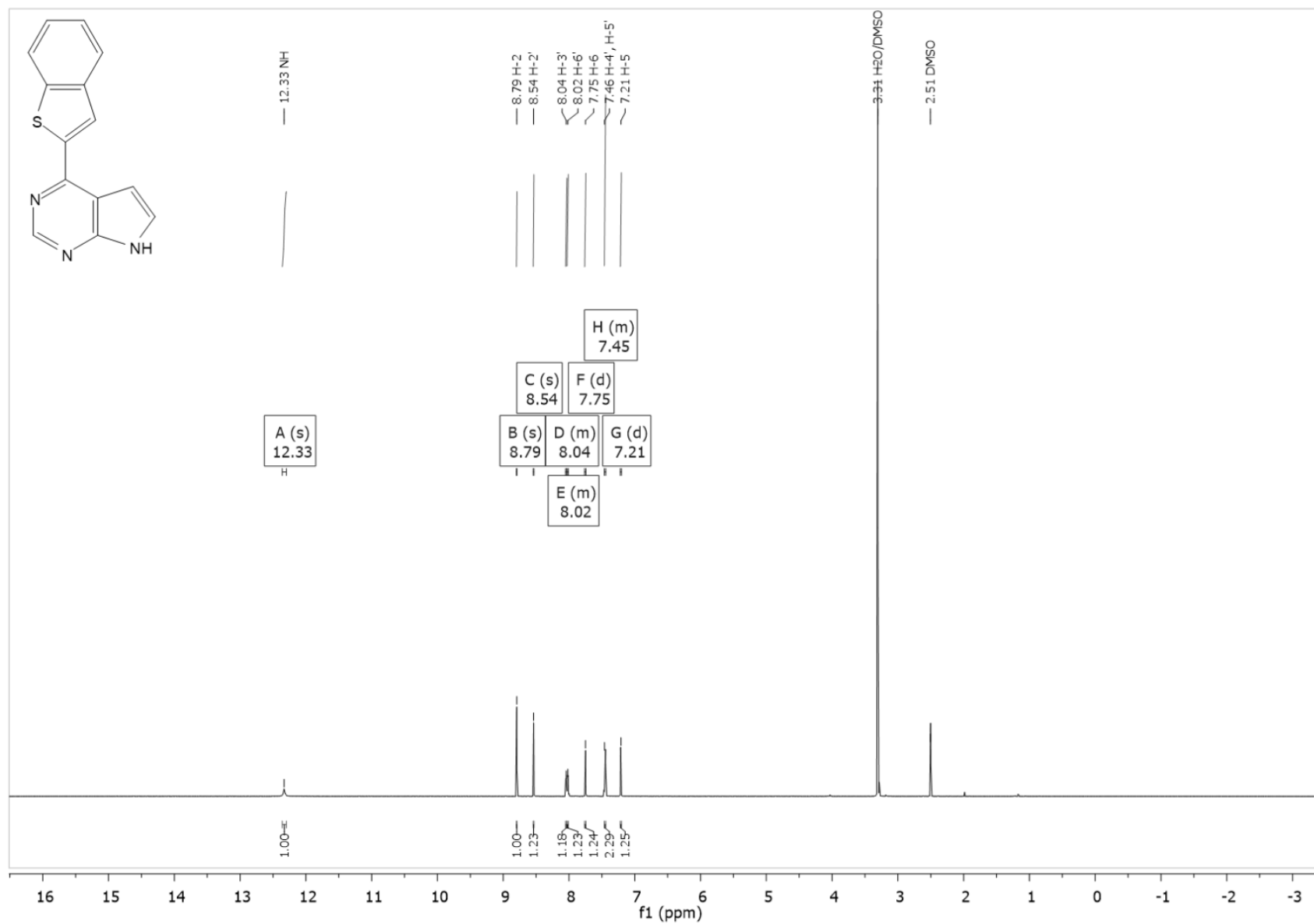
4-(Thiophen-2-yl)-7*H*-pyrrolo[2,3-*d*]pyrimidine (¹H) **7k**:



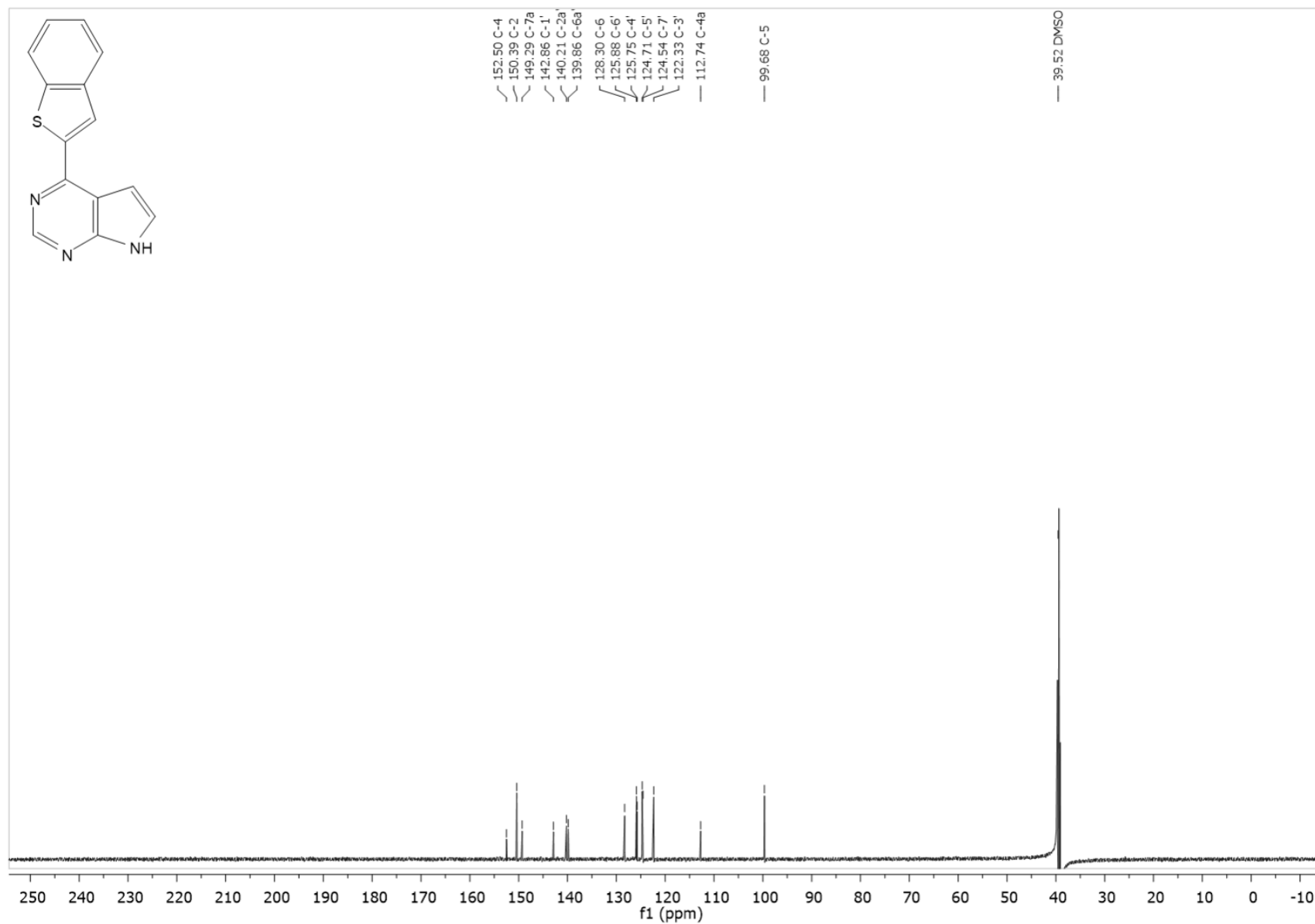
4-(Thiophen-2-yl)-7H-pyrrolo[2,3-d]pyrimidine (^{13}C) **7k**:



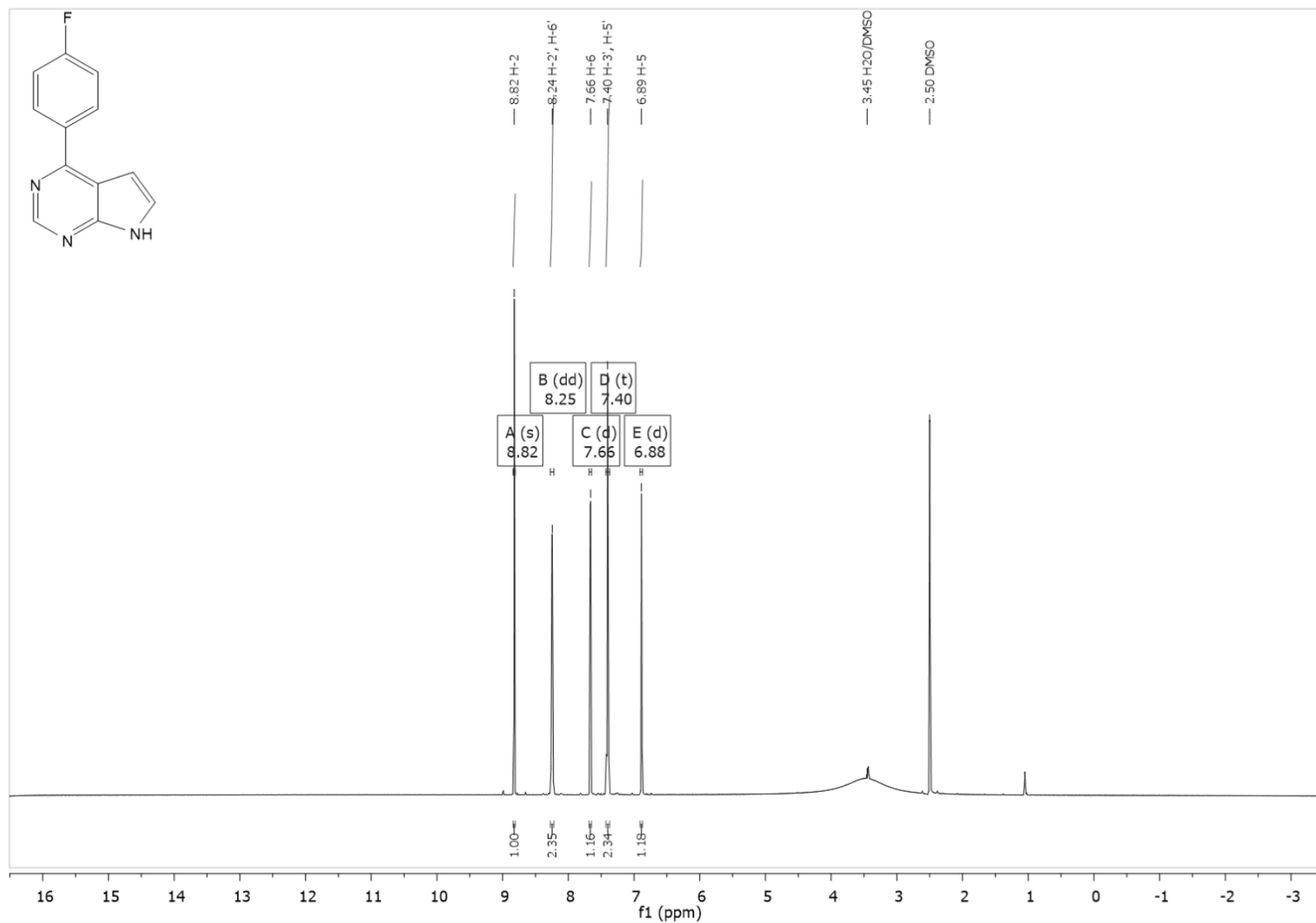
4-(1-Benzothiophen-2-yl)-7H-pyrrolo[2,3-d]pyrimidine (1H) **7I**:



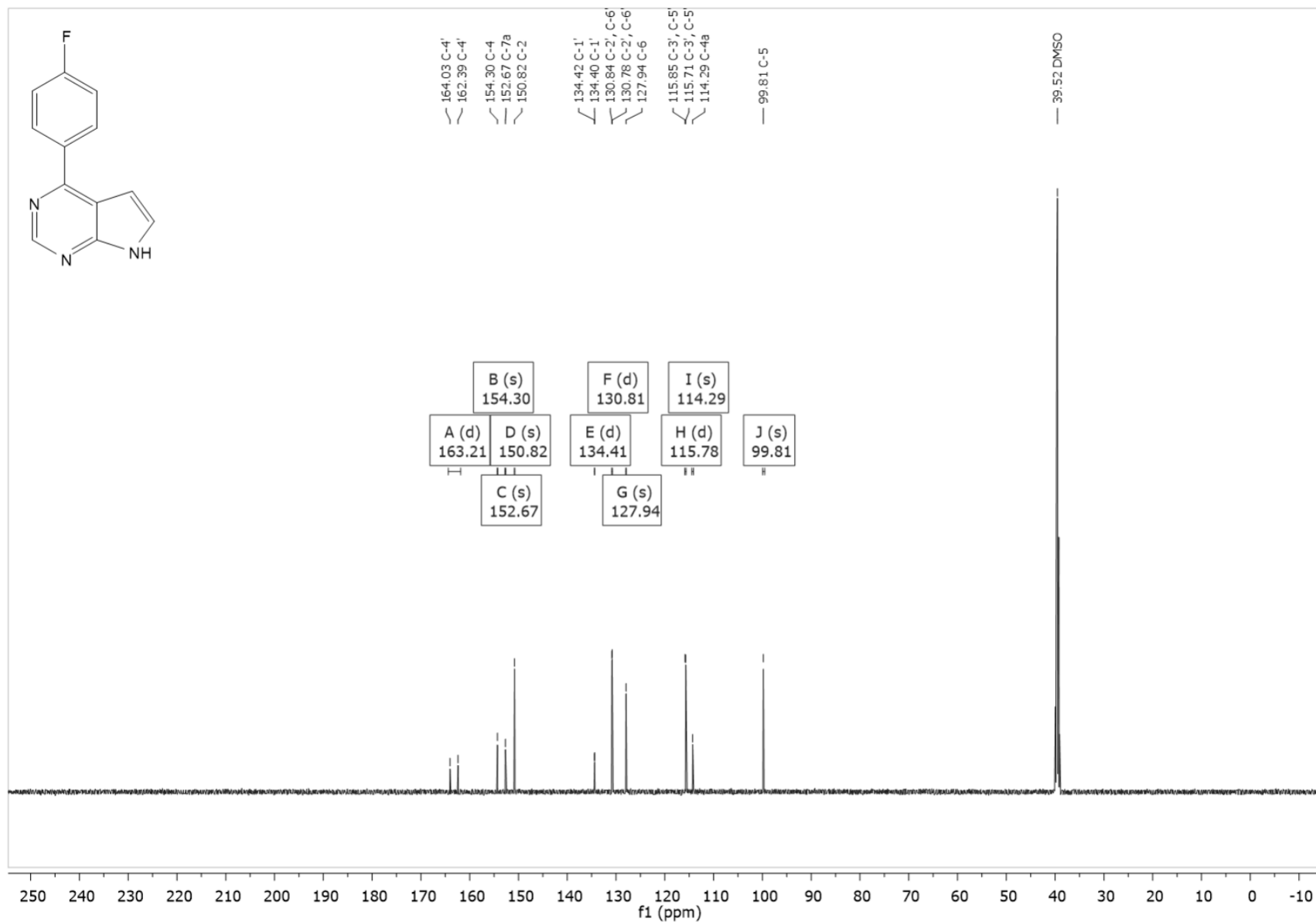
4-(1-Benzothiophen-2-yl)-7H-pyrrolo[2,3-d]pyrimidine (^{13}C) **7l**:



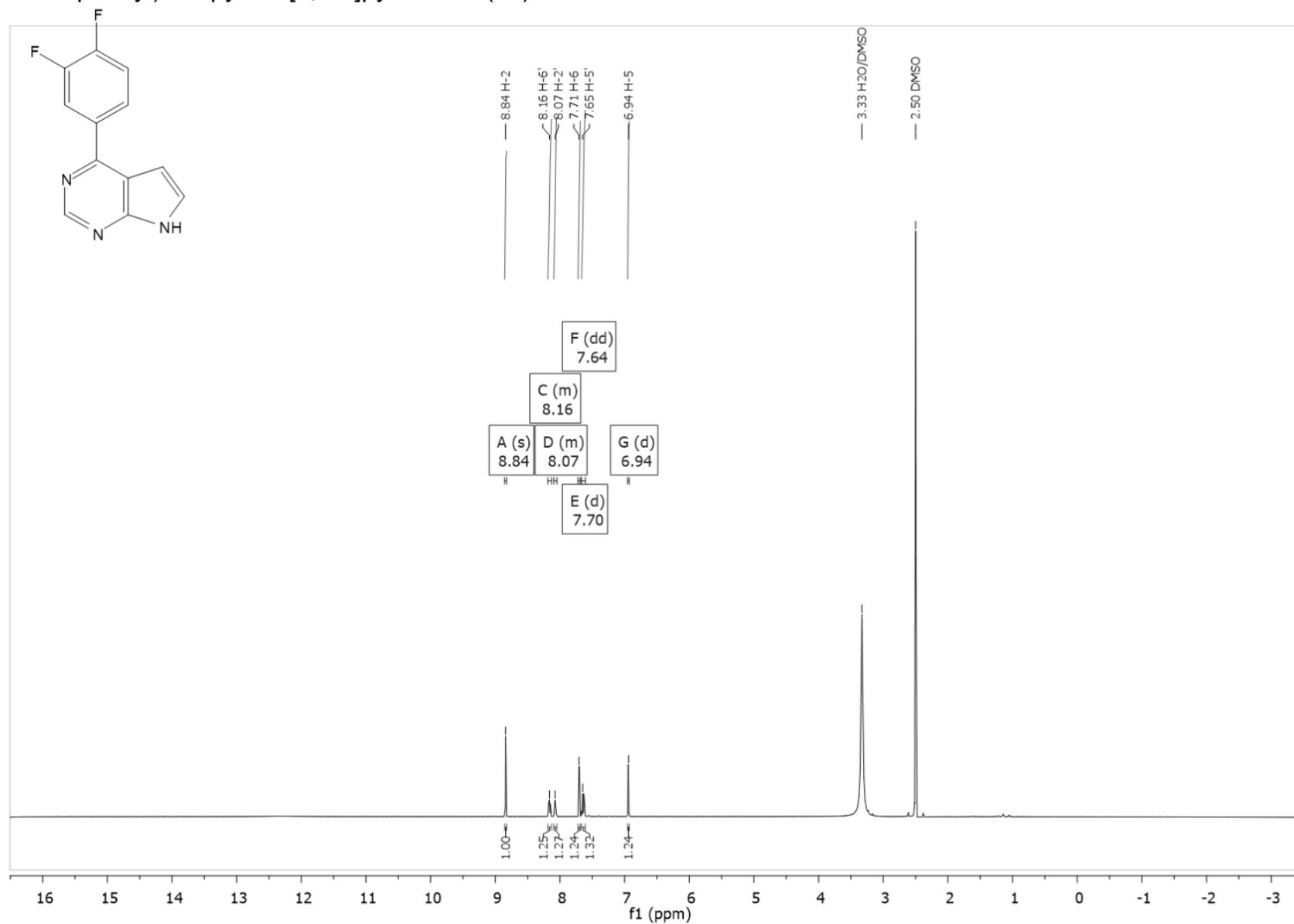
4-(4-Fluorophenyl)-7H-pyrrolo[2,3-d]pyrimidine (¹H) **7m**:



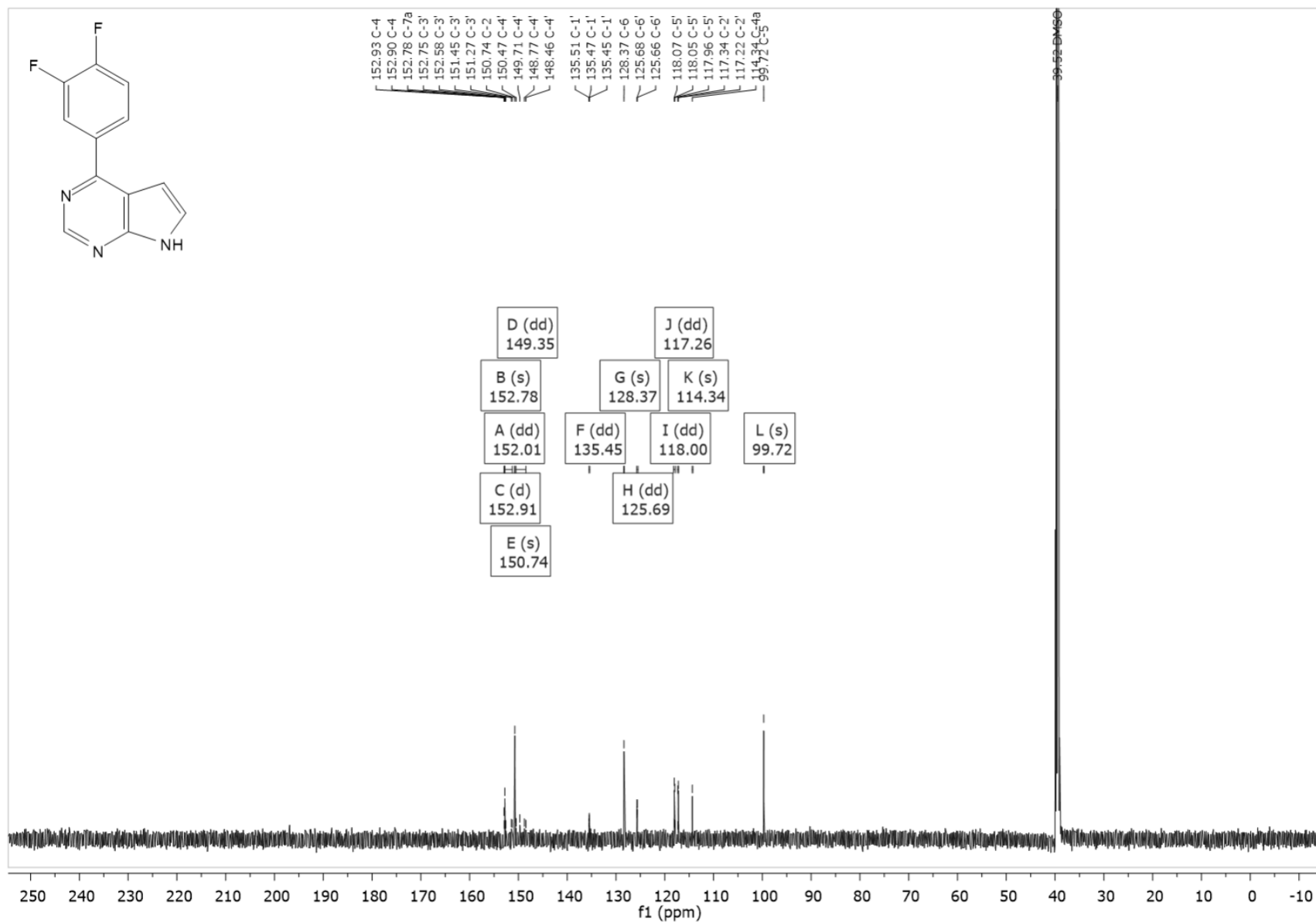
4-(4-Fluorophenyl)-7H-pyrrolo[2,3-d]pyrimidine (^{13}C) **7m**:



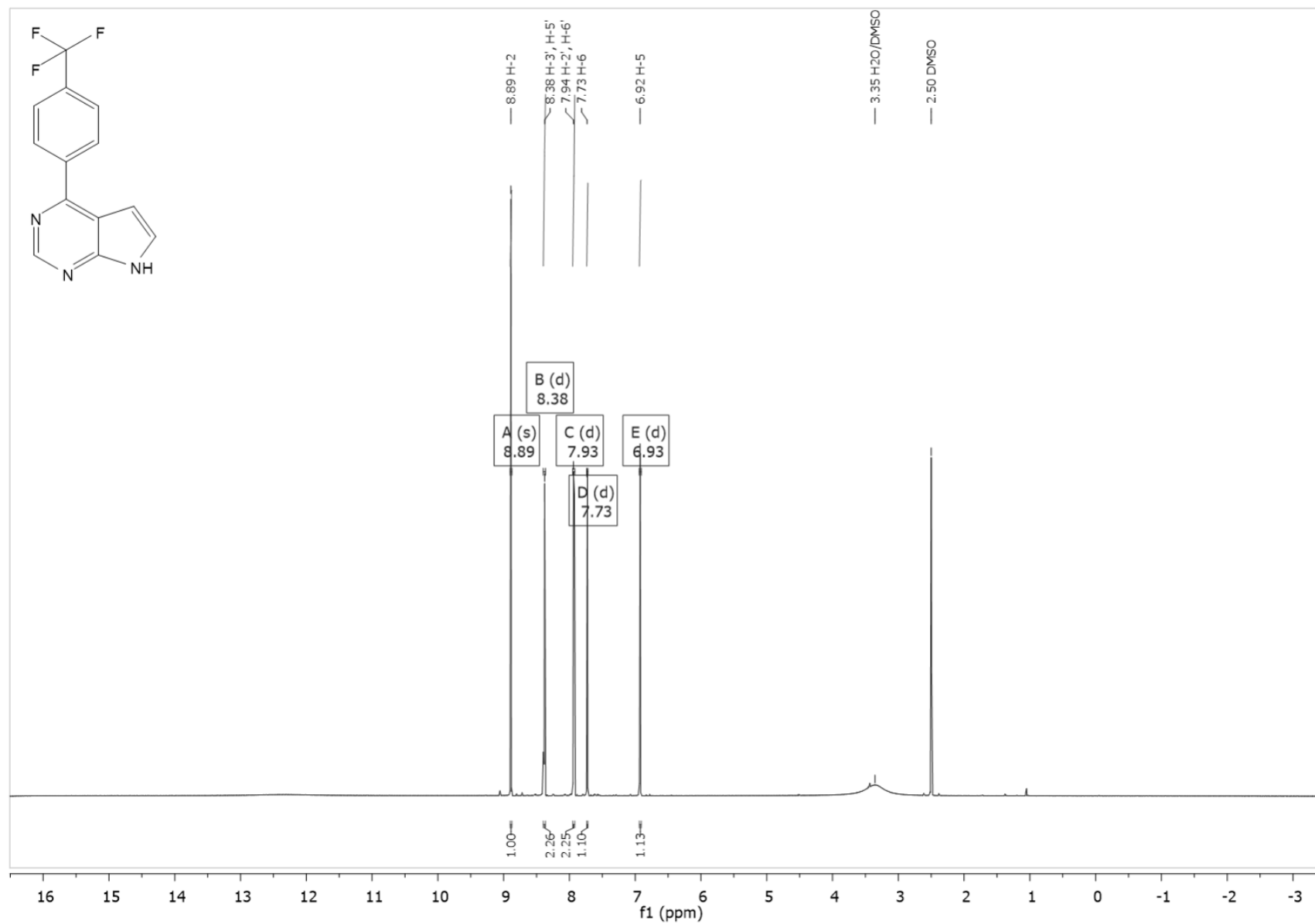
4-(3,4-Difluorophenyl)-7H-pyrrolo[2,3-d]pyrimidine (¹H) **7n**:



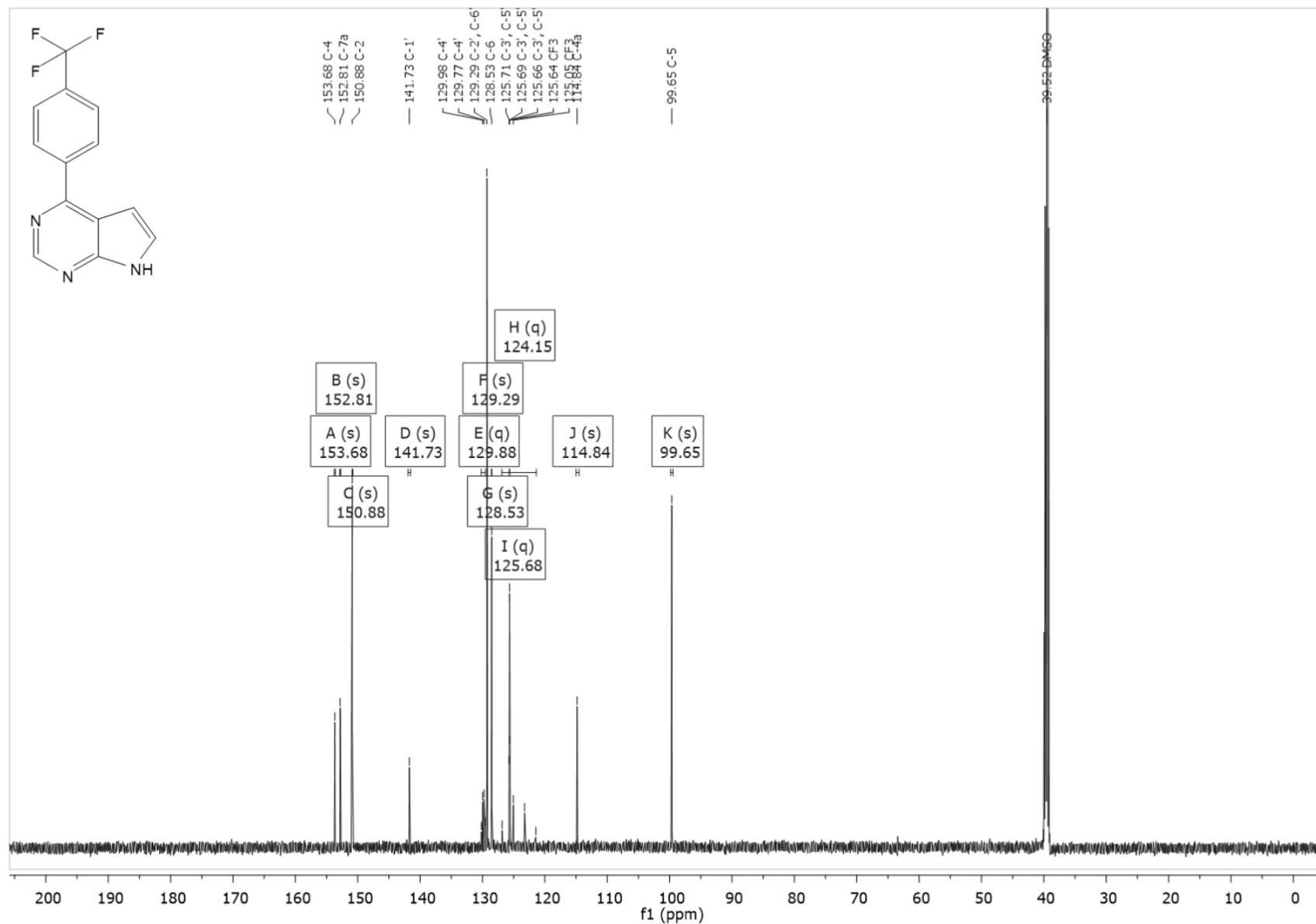
4-(3,4-Difluorophenyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine (¹³C) **7n**:



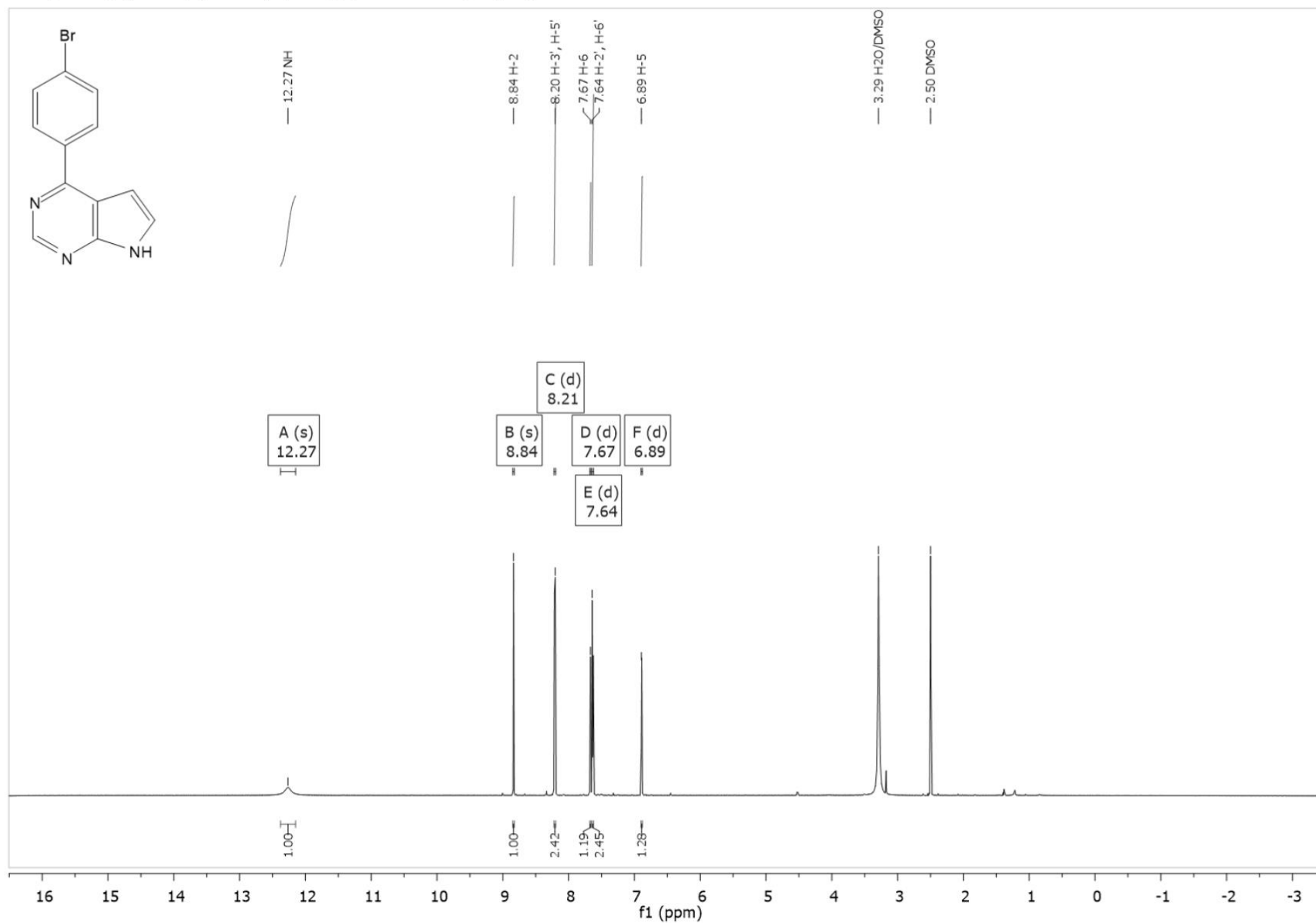
4-[4-(Trifluoromethyl)phenyl]-7H-pyrrolo[2,3-d]pyrimidine (¹H) **7o**:



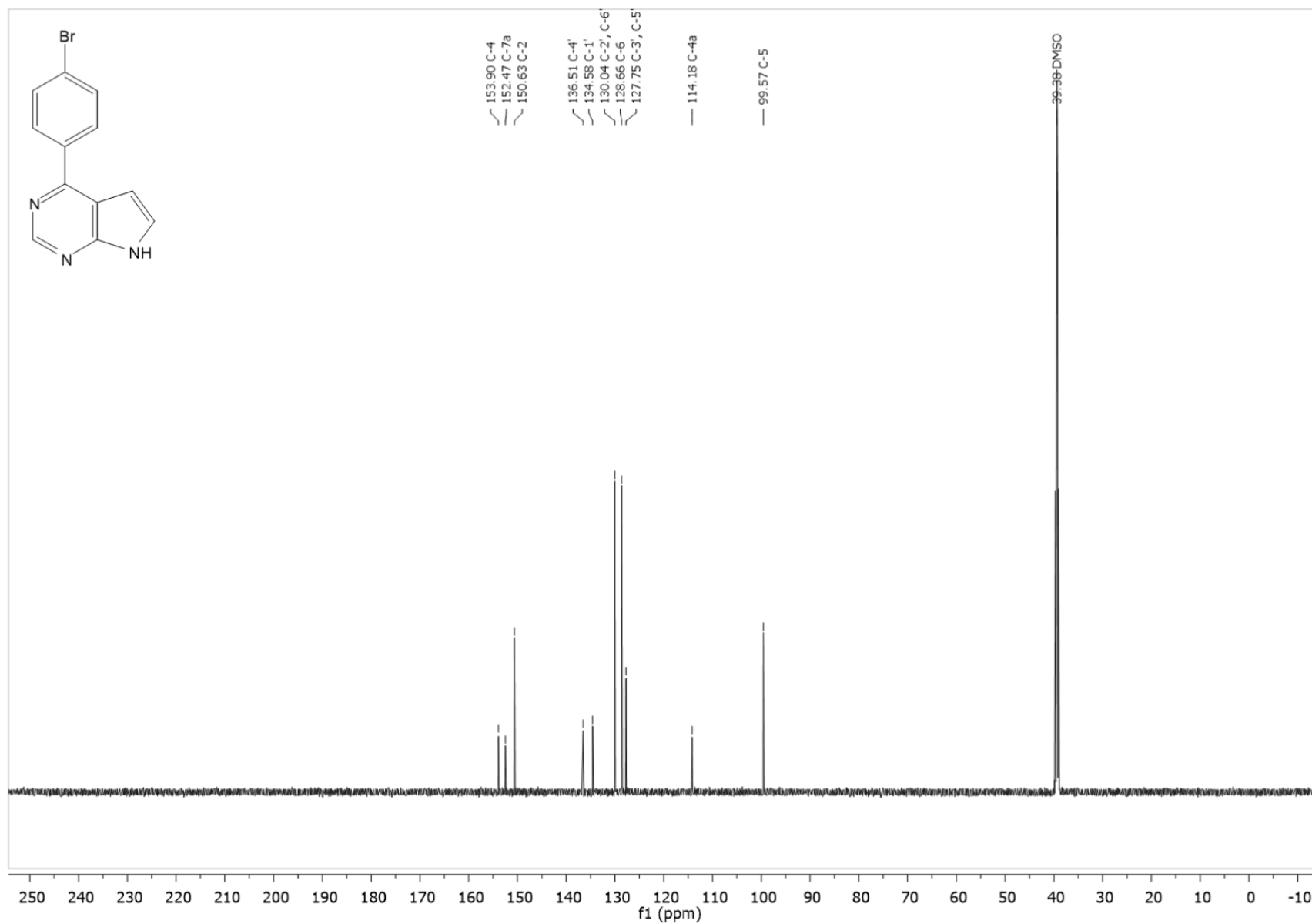
4-[4-(Trifluoromethyl)phenyl]-7*H*-pyrrolo[2,3-*d*]pyrimidine (¹³C) **7o**:



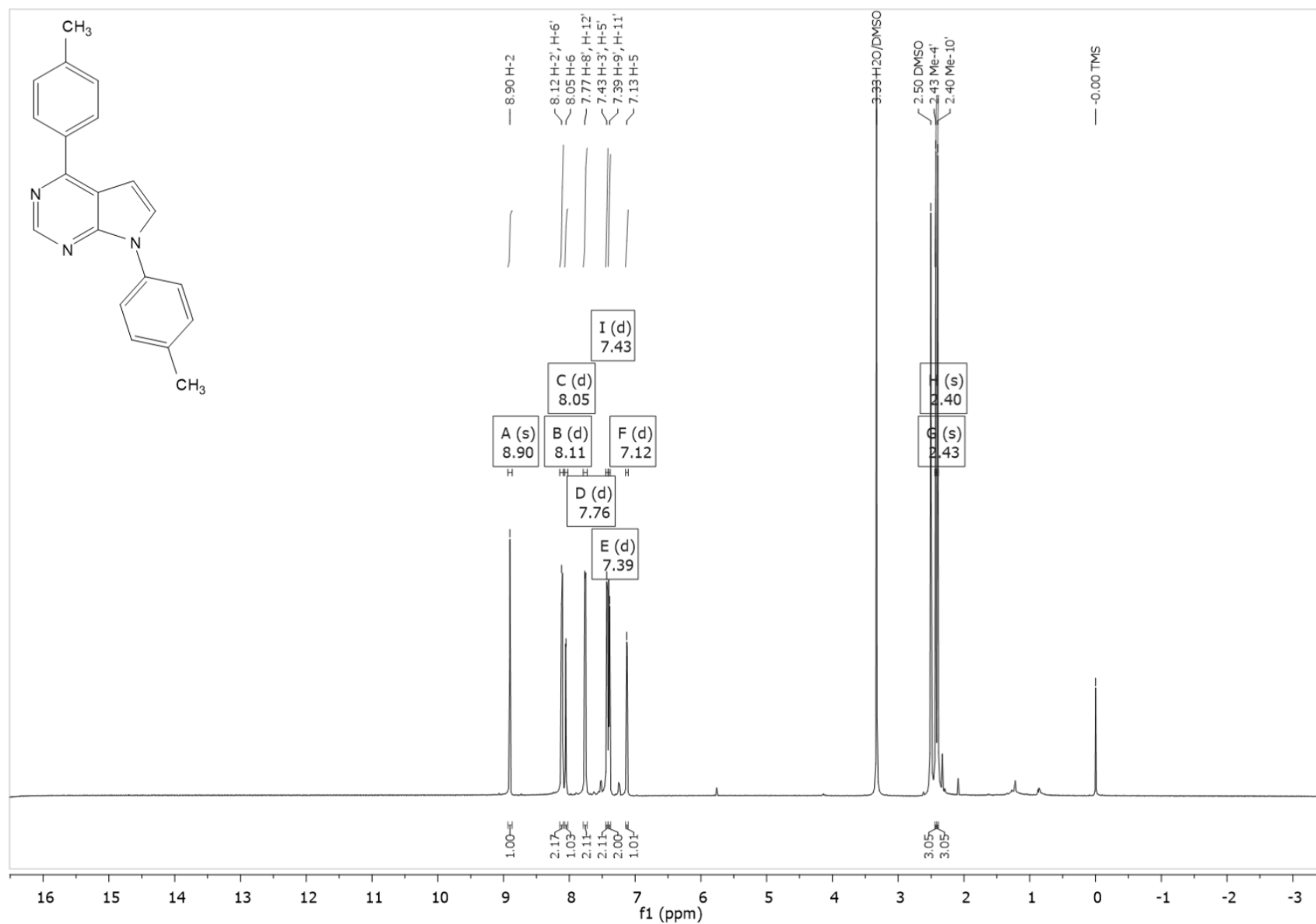
4-(4-Chlorophenyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine (¹H) **7p**:



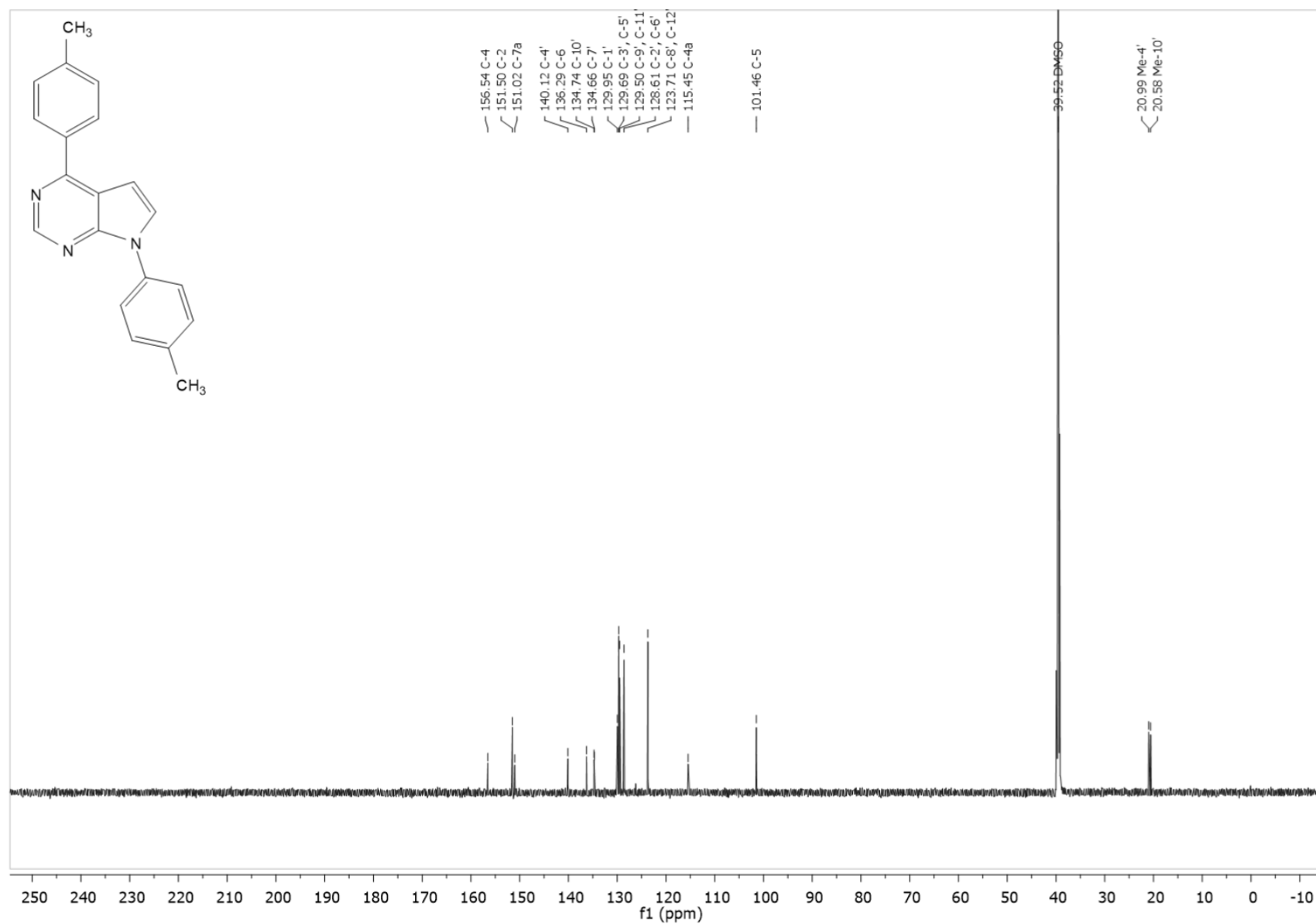
4-(4-Chlorophenyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine (^{13}C) **7p**:



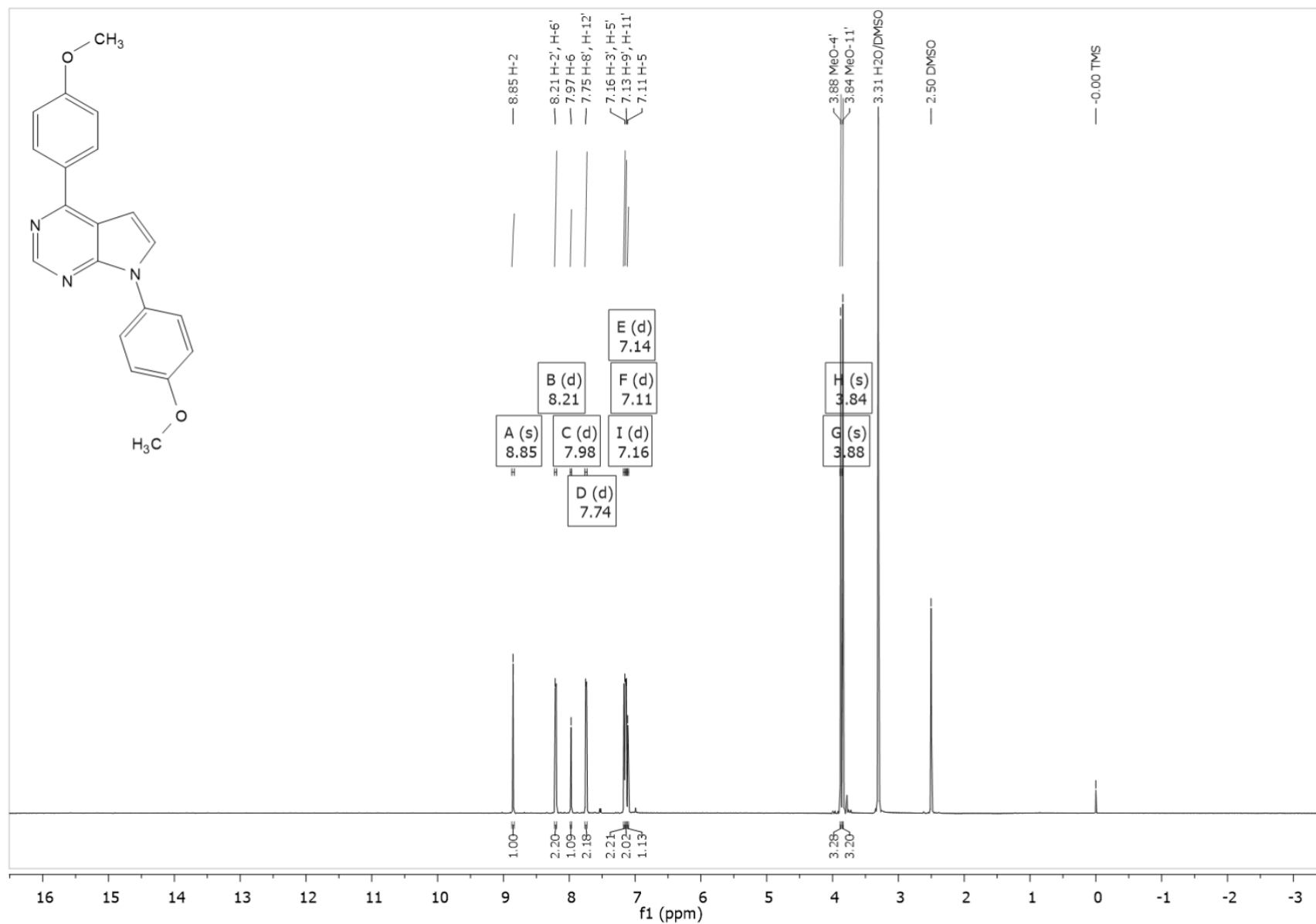
4,7-Bis(4-methylphenyl)-7H-pyrrolo[2,3-d]pyrimidine (¹H) **8a**:



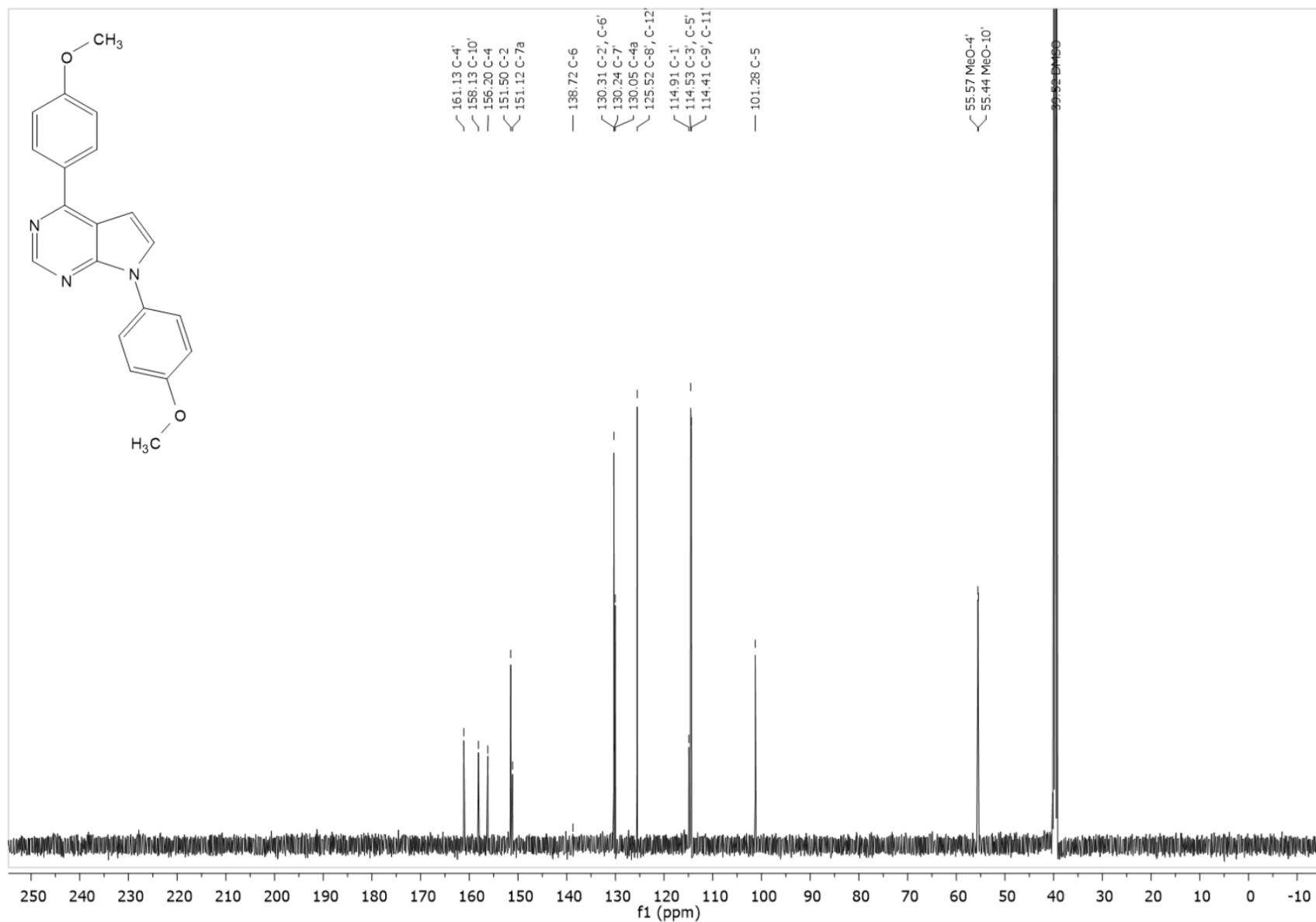
4,7-Bis(4-methylphenyl)-7H-pyrrolo[2,3-d]pyrimidine (^{13}C) **8a**:



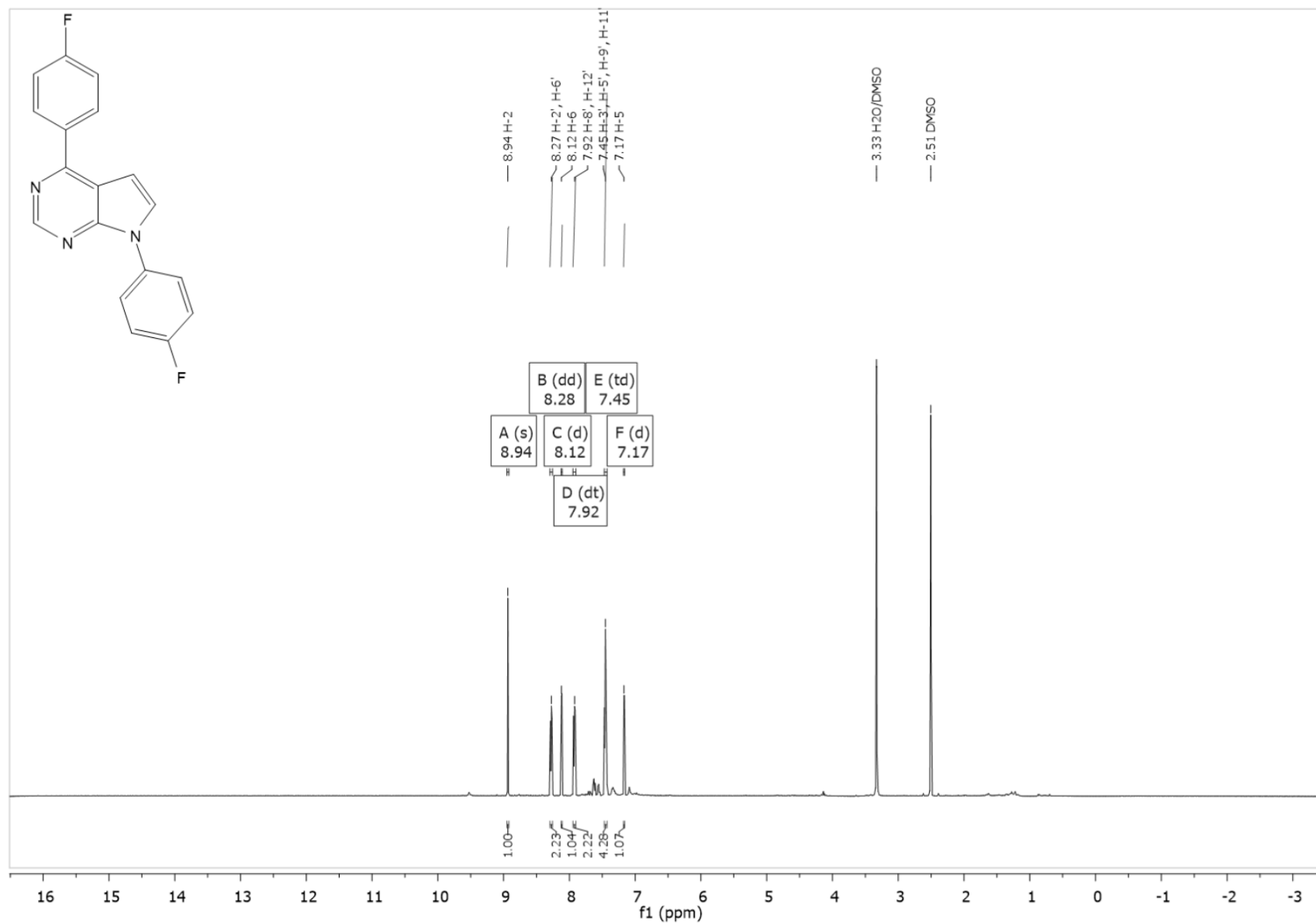
4,7-Bis(4-methoxyphenyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine (¹H) **8b**:



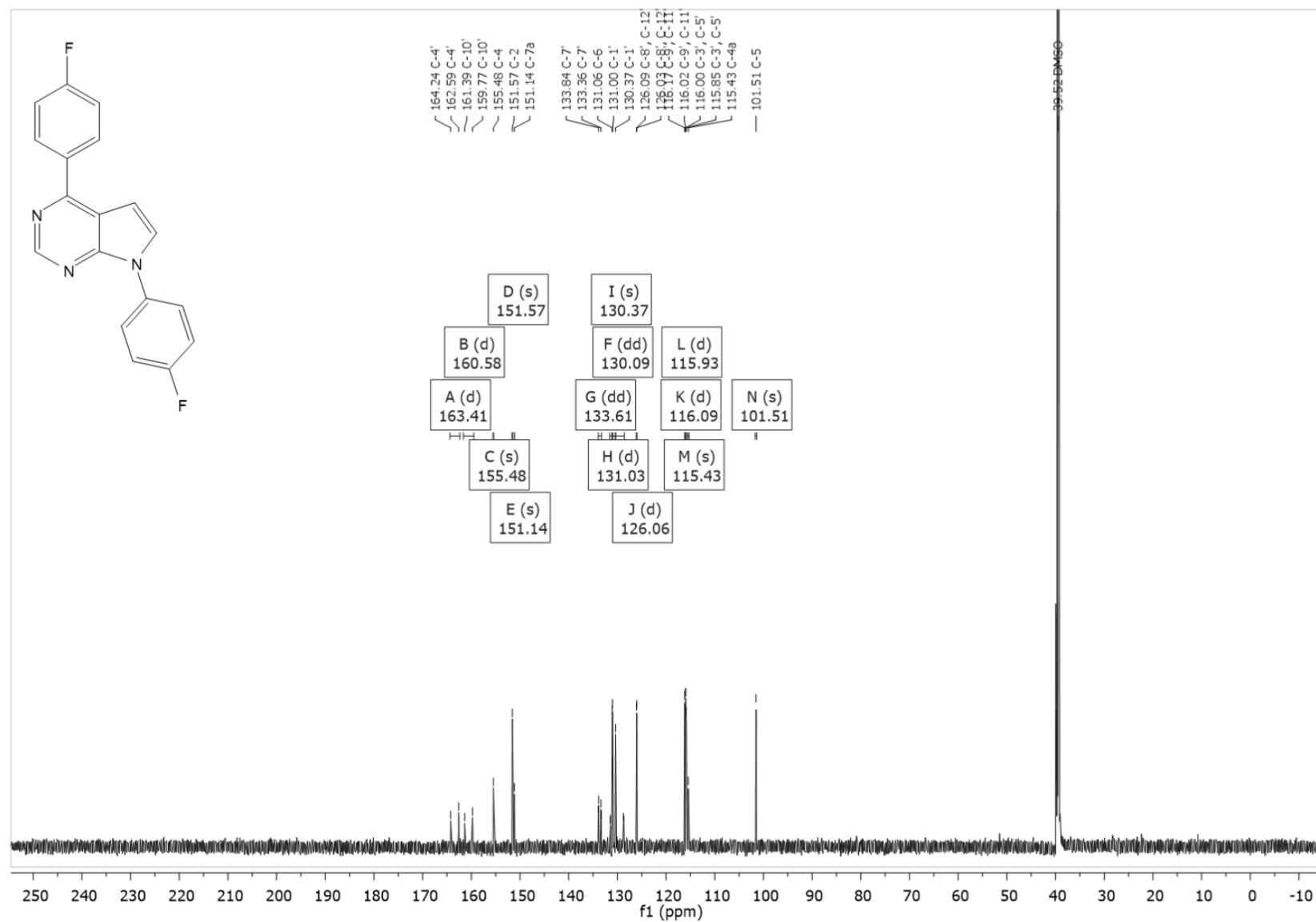
4,7-Bis(4-methoxyphenyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine (¹³C) **8b**:



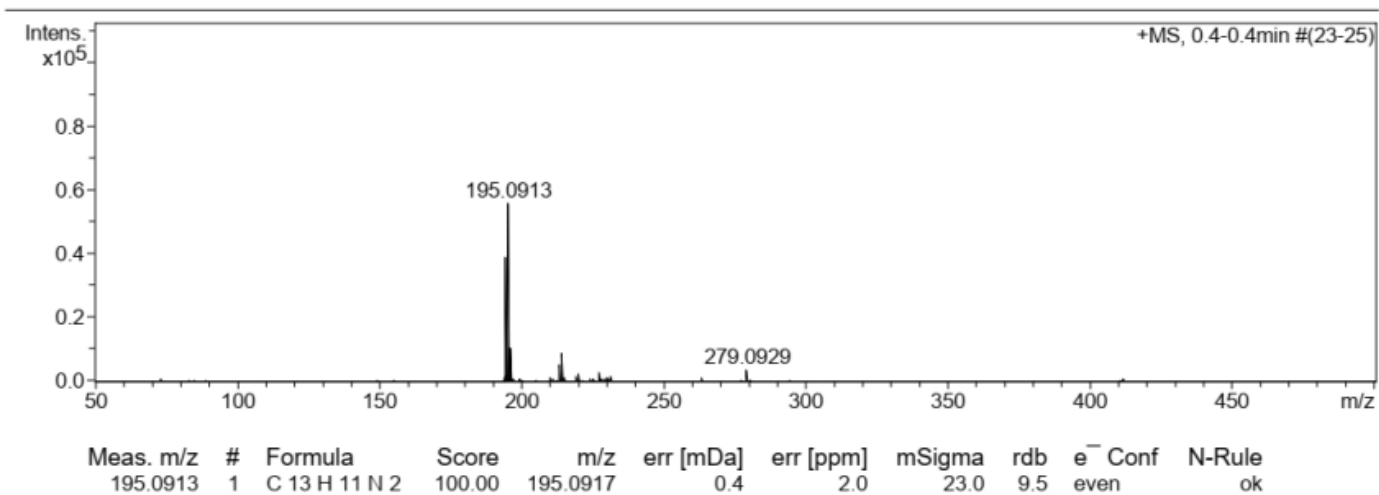
4,7-Bis(4-fluorophenyl)-7H-pyrrolo[2,3-d]pyrimidine (¹H) **8c**:



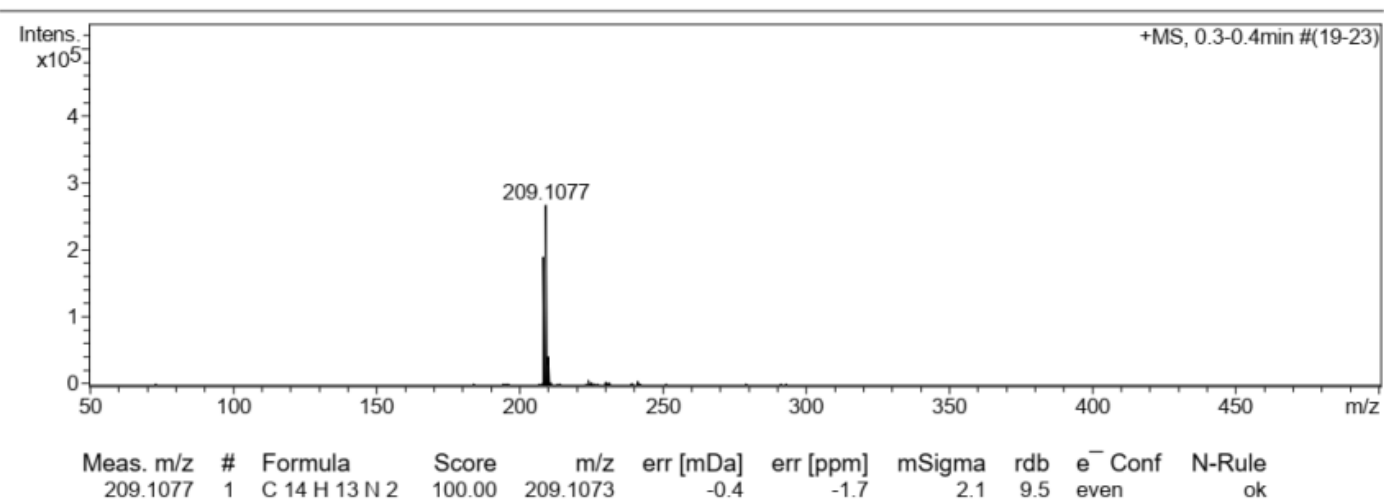
4,7-Bis(4-fluorophenyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine (¹³C) **8c**:



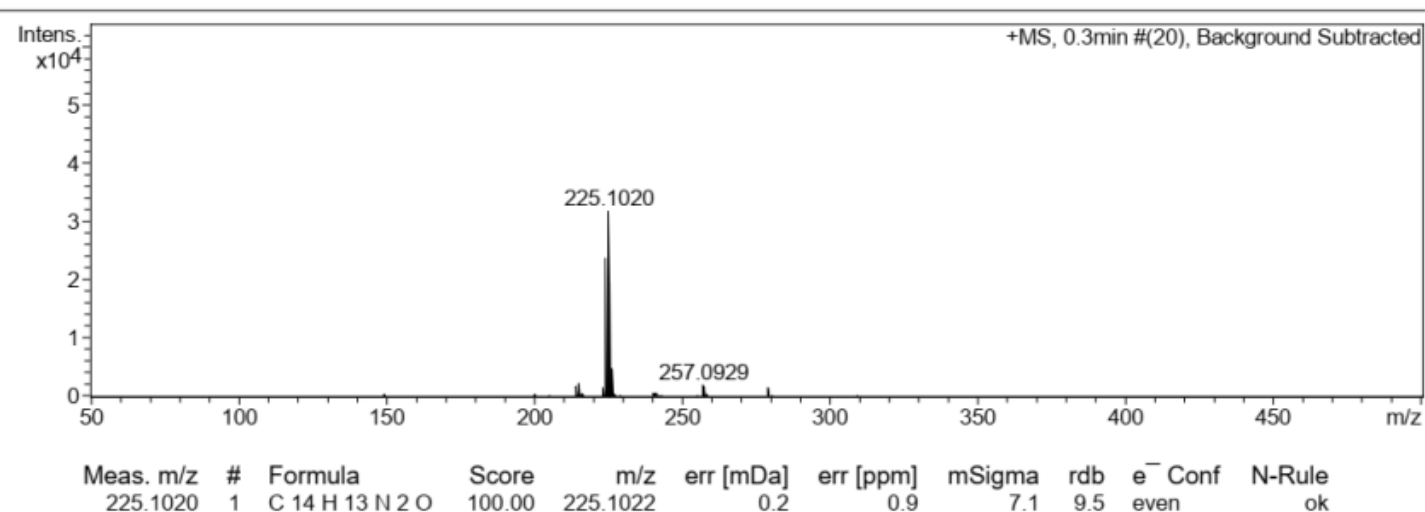
6-phenyl-7*H*-pyrrolo[2,3-*d*]pyrimidine (MS) **5a**:



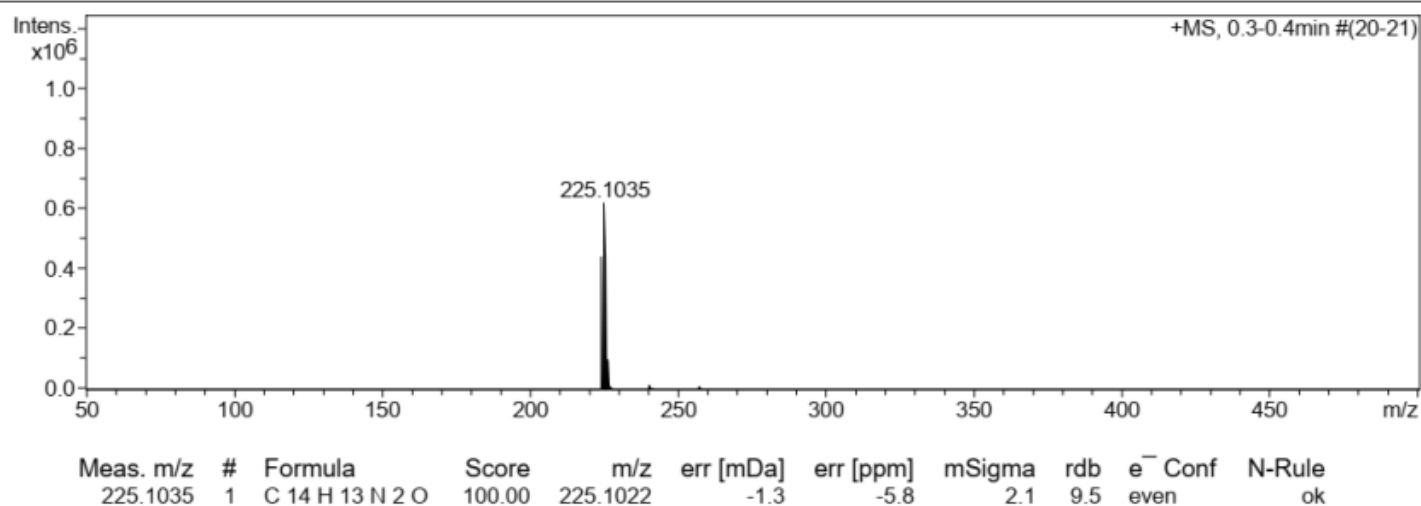
6-(4-Methylphenyl)-1*H*-pyrrolo[2,3-*b*]pyridine (MS) **5b**:



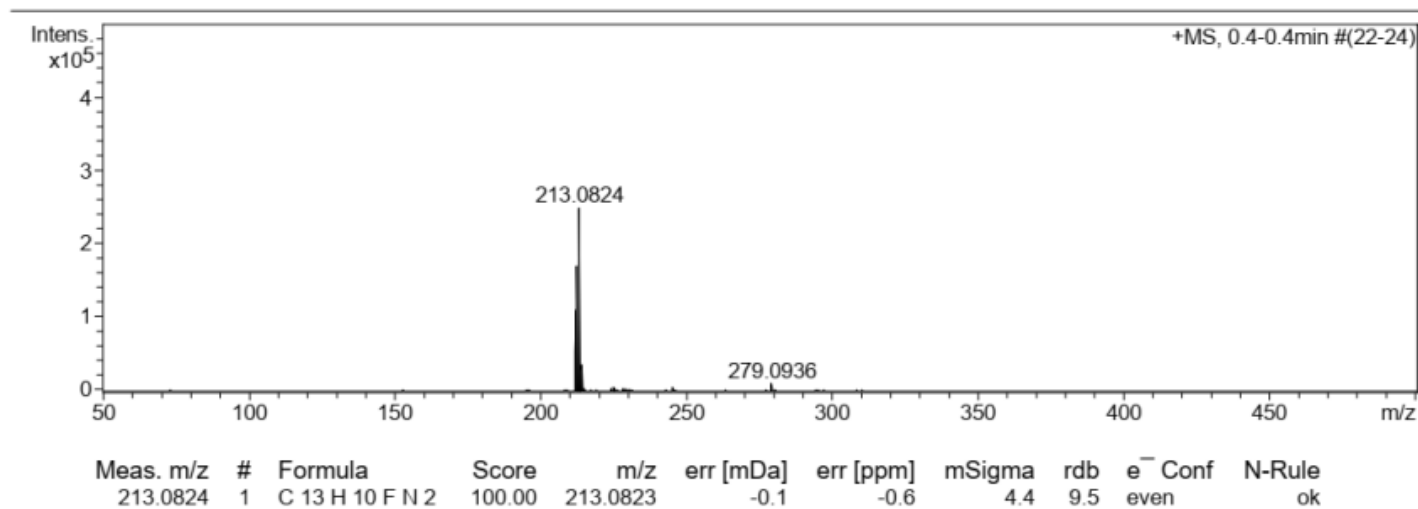
6-(3-Methoxyphenyl)-1*H*-pyrrolo[2,3-*b*]pyridine (MS) **5c**:



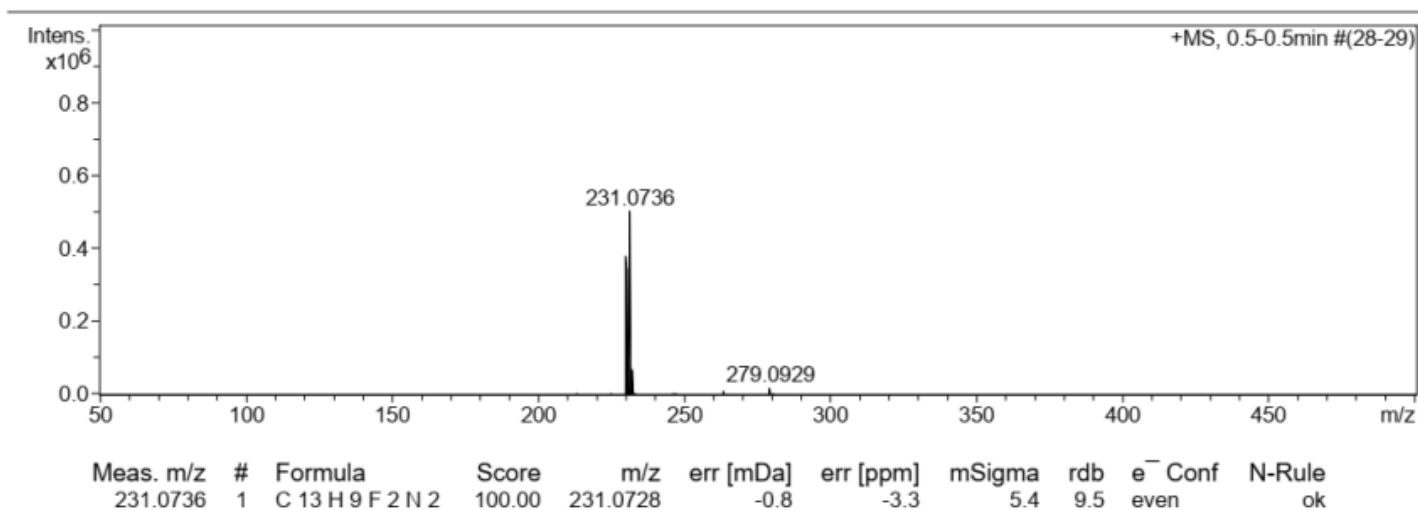
6-(4-Methoxyphenyl)-1*H*-pyrrolo[2,3-*b*]pyridine (MS) **5d**:



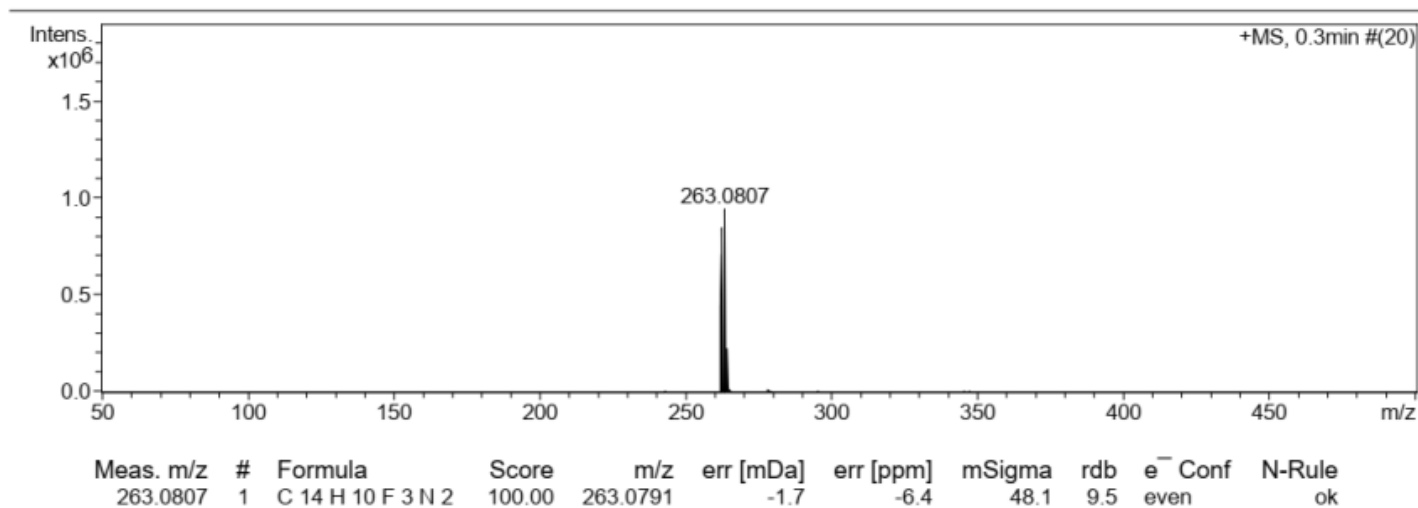
6-(4-Fluorophenyl)-1*H*-pyrrolo[2,3-*b*]pyridine (MS) **5e**:



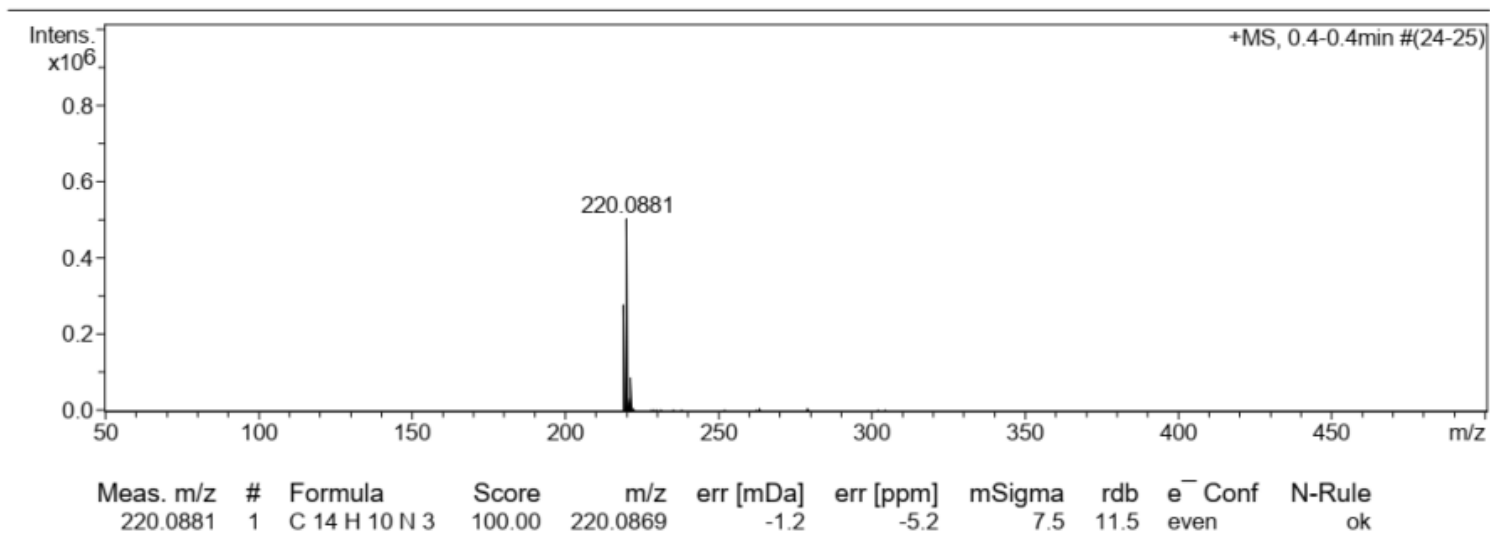
6-(3,4-Difluorophenyl)-1*H*-pyrrolo[2,3-*b*]pyridine (MS) **5f**:



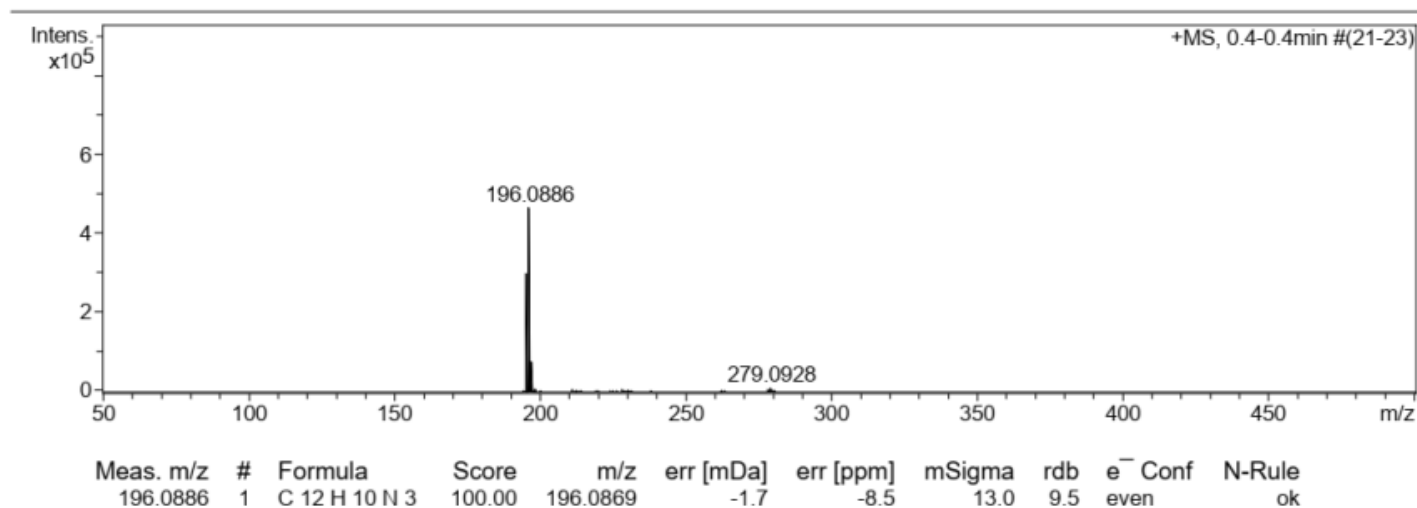
6-[4-(Trifluoromethyl)phenyl]-1*H*-pyrrolo[2,3-*b*]pyridine (MS) **5g**:



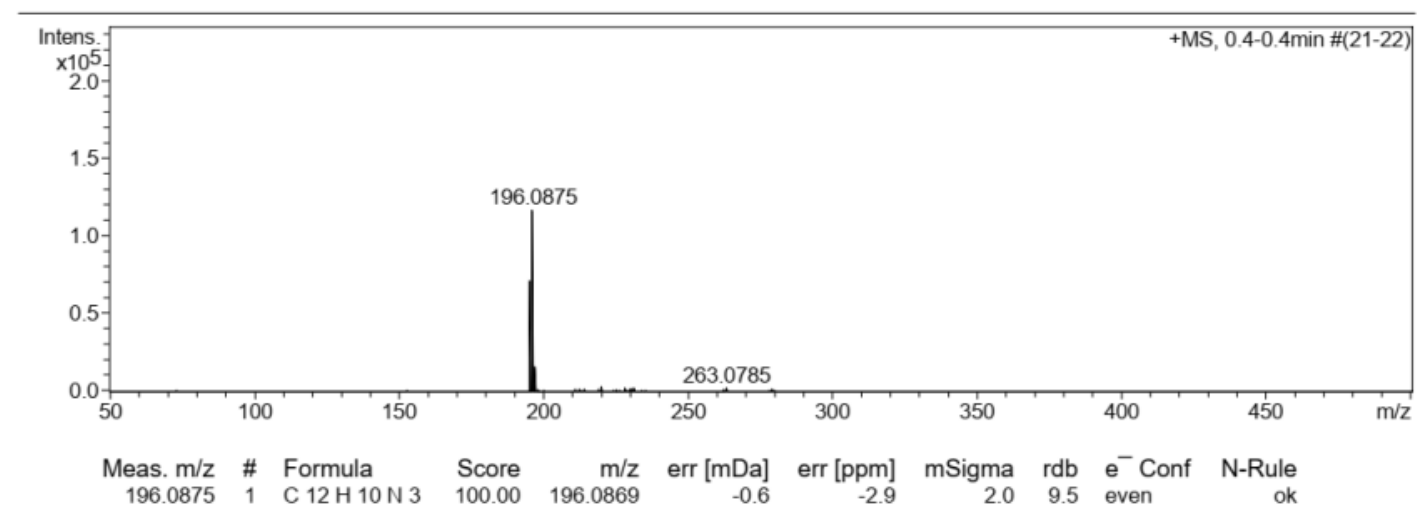
4-(1*H*-Pyrrolo[2,3-*b*]pyridin-6-yl)benzonitrile (MS) **5h**:



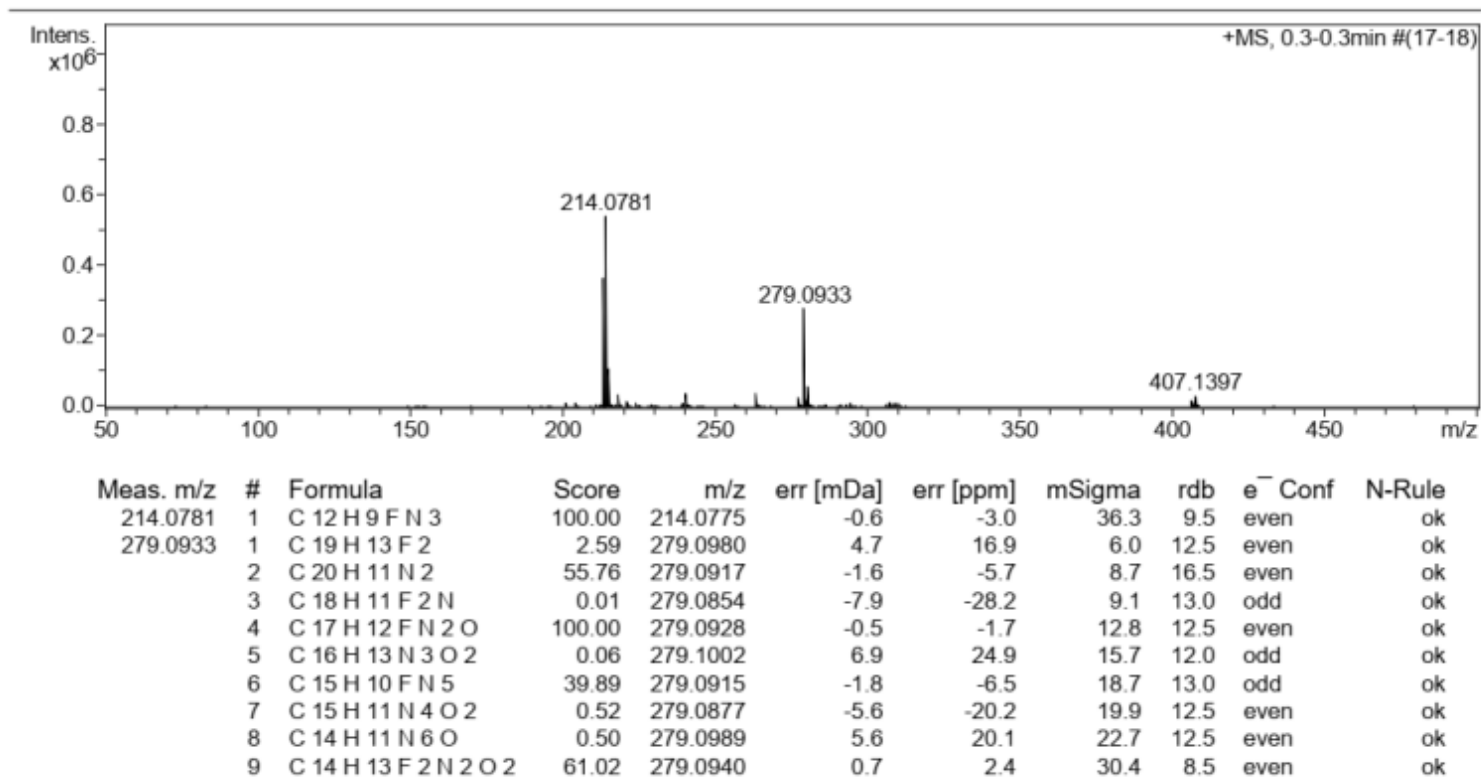
6-(Pyridin-3-yl)-1*H*-pyrrolo[2,3-*b*]pyridine (MS) **5i**:



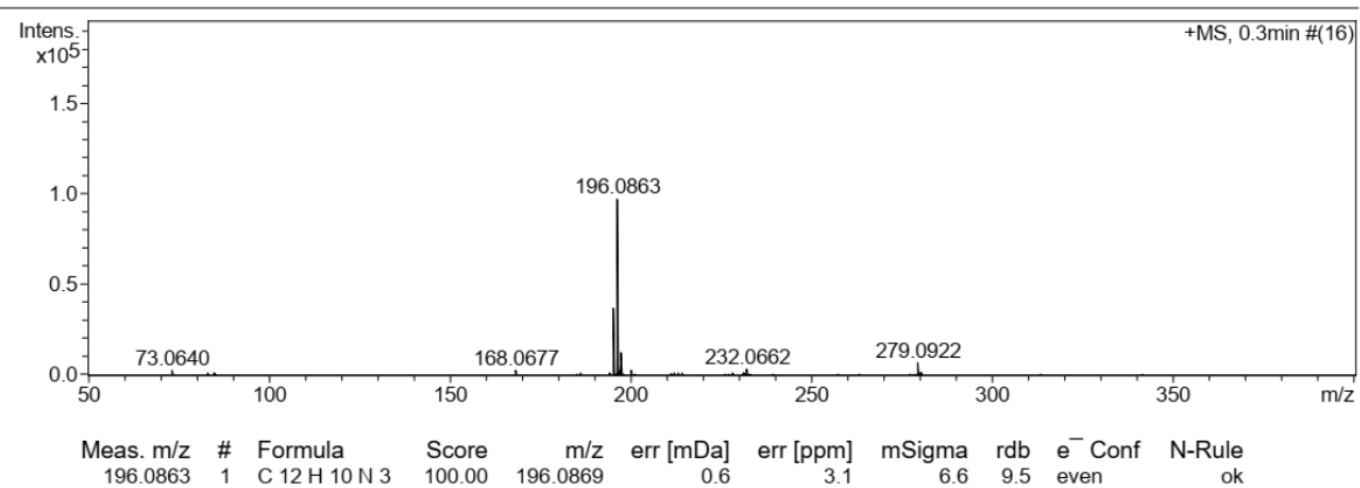
6-(Pyridin-4-yl)-1*H*-pyrrolo[2,3-*b*]pyridine (MS) **5j**:



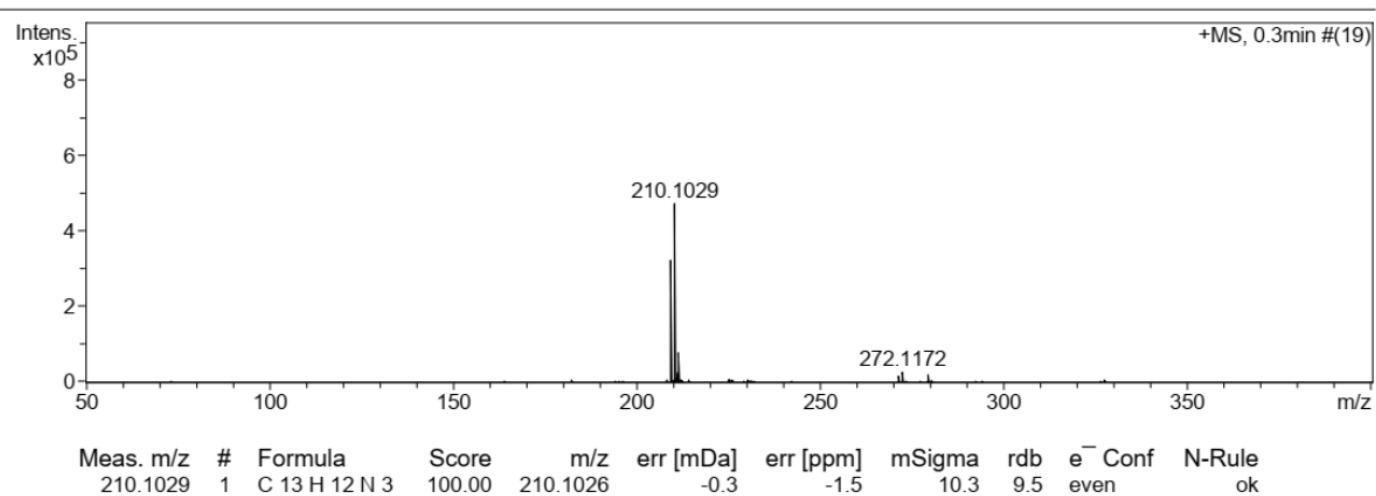
6-(6-Fluoropyridin-3-yl)-1*H*-pyrrolo[2,3-*b*]pyridine (MS) **5k**:



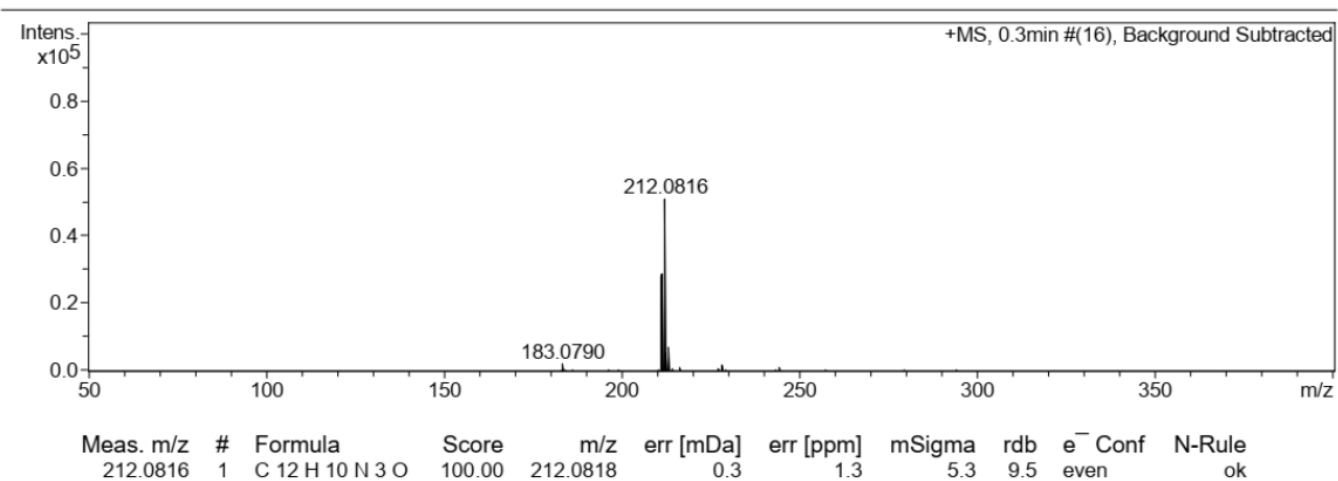
4-Phenyl-7*H*-pyrrolo[2,3-*d*]pyrimidine (MS) **7a**:



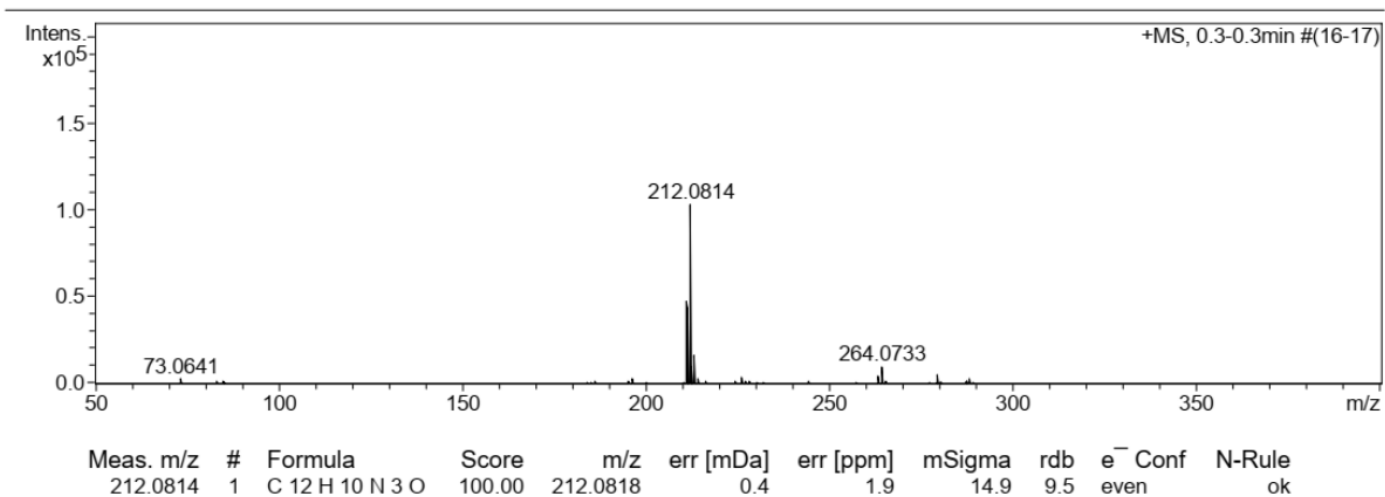
4-(4-Methylphenyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine (MS) **7b**:



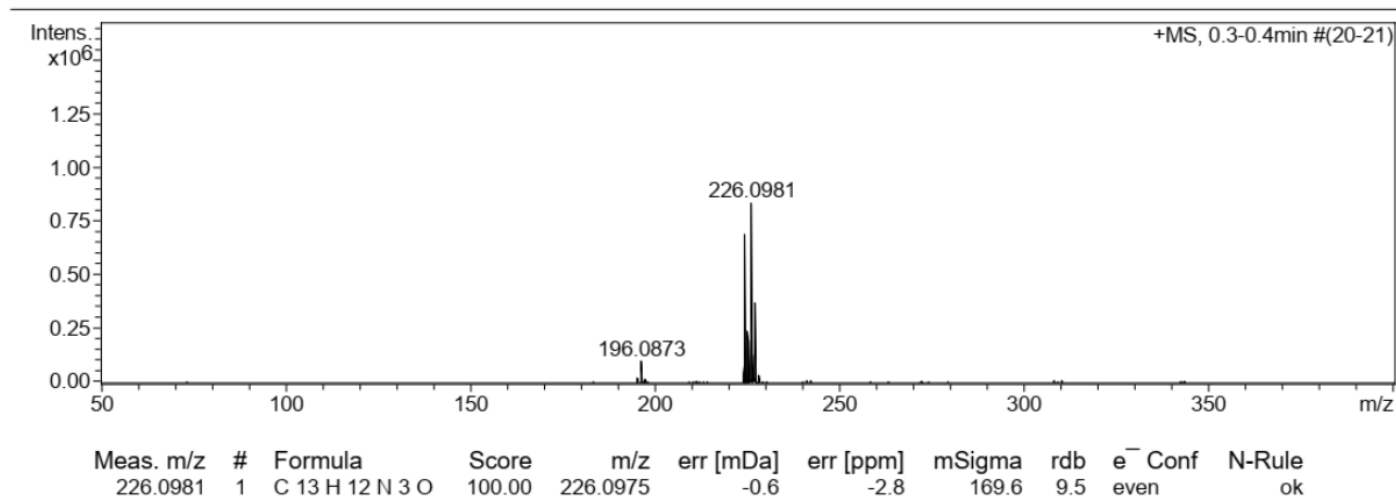
3-(7*H*-Pyrrolo[2,3-*d*]pyrimidin-4-yl)phenol **7c**:



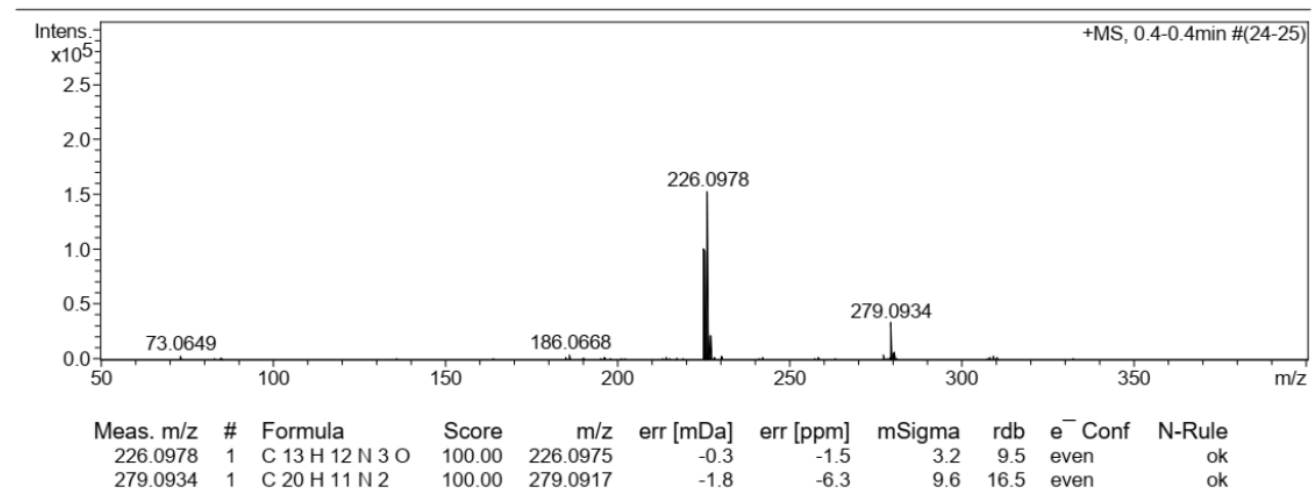
4-(7*H*-Pyrrolo[2,3-*d*]pyrimidin-4-yl)phenol **7d**:



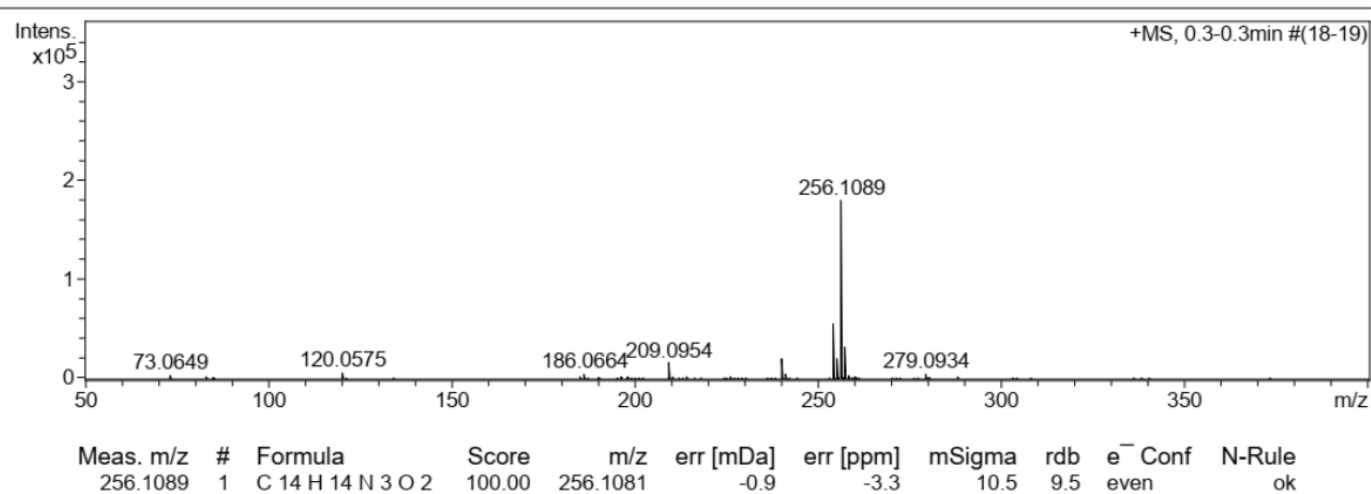
4-(3-Methoxyphenyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine **7e**:



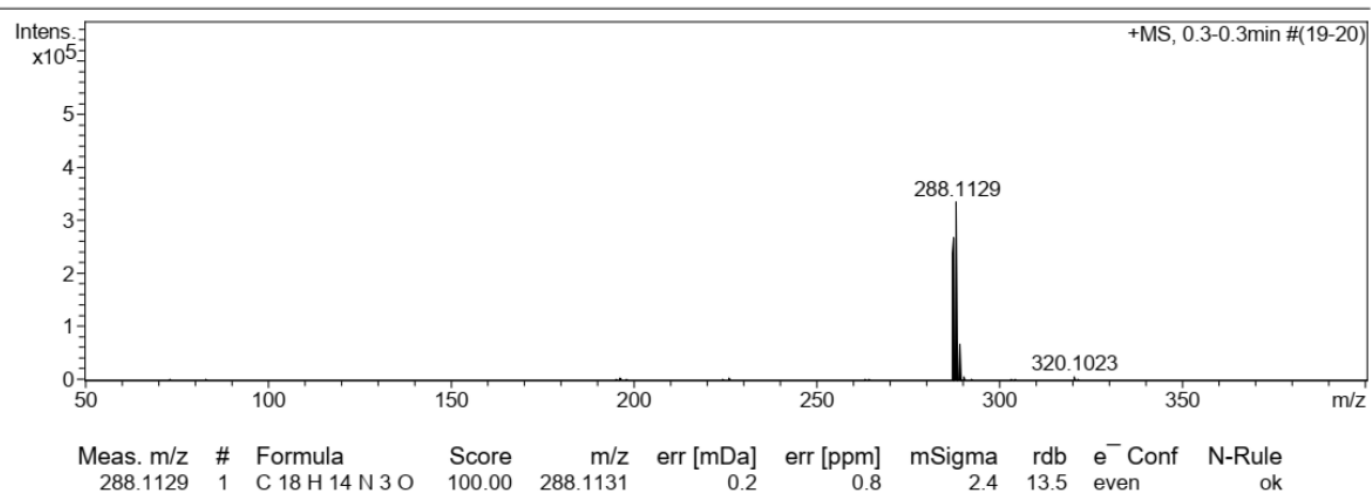
4-(4-Methoxyphenyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine **7f**:



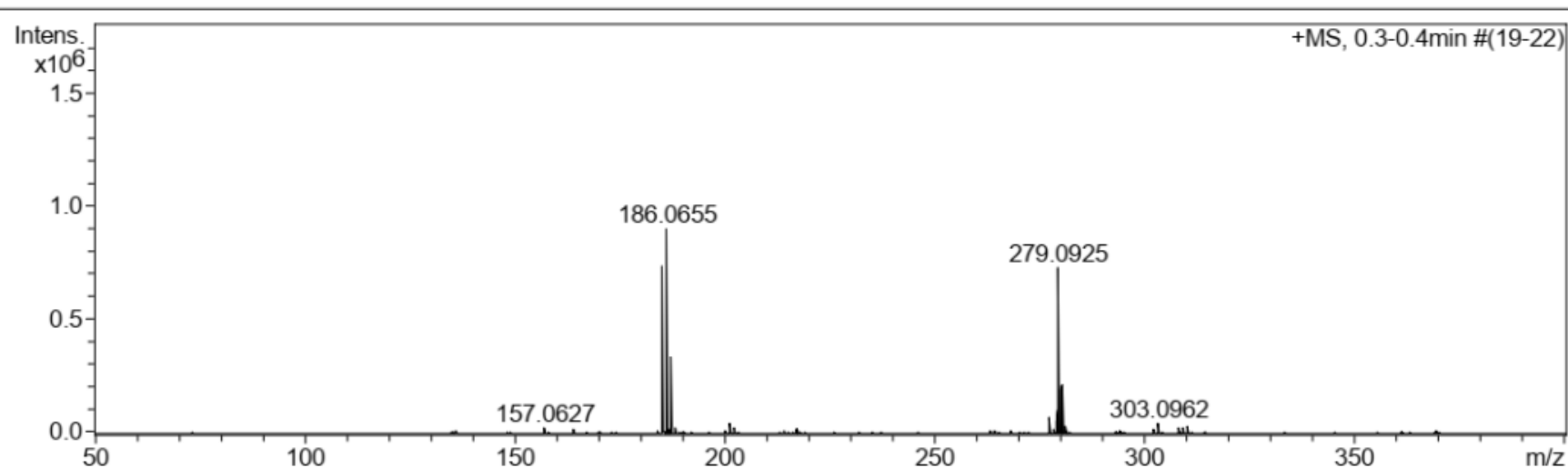
4-(2,3-Dimethoxyphenyl)-7H-pyrrolo[2,3-d]pyrimidine **7g**:



4-(4-Phenoxyphenyl)-7H-pyrrolo[2,3-d]pyrimidine **7h**:

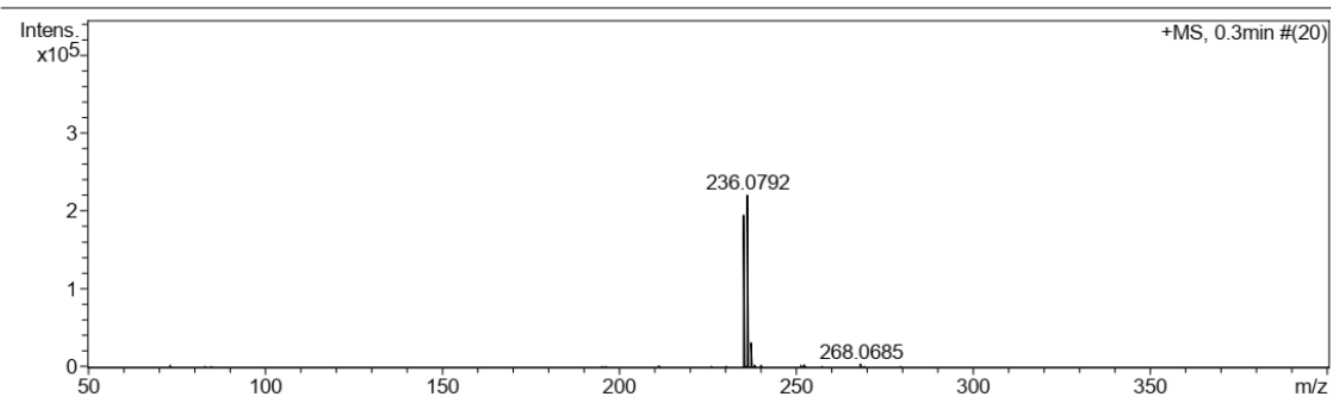


4-(Furan-2-yl)-7*H*-pyrrolo[2,3-*d*]pyrimidine **7i**:



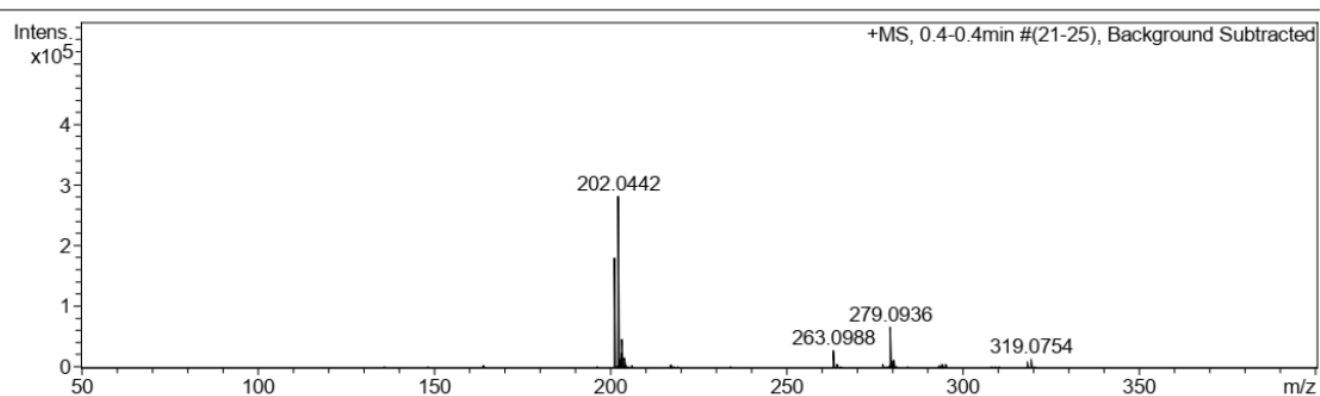
Meas. m/z	#	Formula	Score	m/z	err [mDa]	err [ppm]	mSigma	rdB	e ⁻ Conf	N-Rule
185.0586	1	C ₁₀ H ₇ N ₃ O	100.00	185.0584	-0.2	-1.3	561.2	9.0	odd	ok
186.0655	1	C ₁₀ H ₈ N ₃ O	100.00	186.0662	0.7	3.9	147.6	8.5	even	ok
279.0925	1	C ₂₀ H ₁₁ N ₂	100.00	279.0917	-0.8	-2.9	41.5	16.5	even	ok
	2	C ₁₇ H ₁₃ N ₃ O	6.25	279.0890	-3.5	-12.5	62.1	12.0	odd	ok
	3	C ₁₁ H ₁₃ N ₅ O ₄	1.52	279.0962	3.7	13.4	91.2	8.0	odd	ok
	4	C ₉ H ₁₁ N ₈ O ₃	4.25	279.0949	2.4	8.5	97.5	8.5	even	ok
	5	C ₁₀ H ₁₇ N ₃ O ₈	2.89	279.0949	2.4	8.6	105.5	3.0	odd	ok
	6	C ₈ H ₁₅ N ₄ O ₇	5.39	279.0935	1.0	3.8	111.9	3.5	even	ok
	7	C ₆ H ₁₃ N ₇ O ₆	5.72	279.0922	-0.3	-1.1	118.1	4.0	odd	ok
	8	C ₄ H ₁₁ N ₁₀ O ₅	1.90	279.0908	-1.6	-5.9	124.3	4.5	even	ok

4-(1-Benzofuran-2-yl)-7*H*-pyrrolo[2,3-*d*]pyrimidine **7j**:



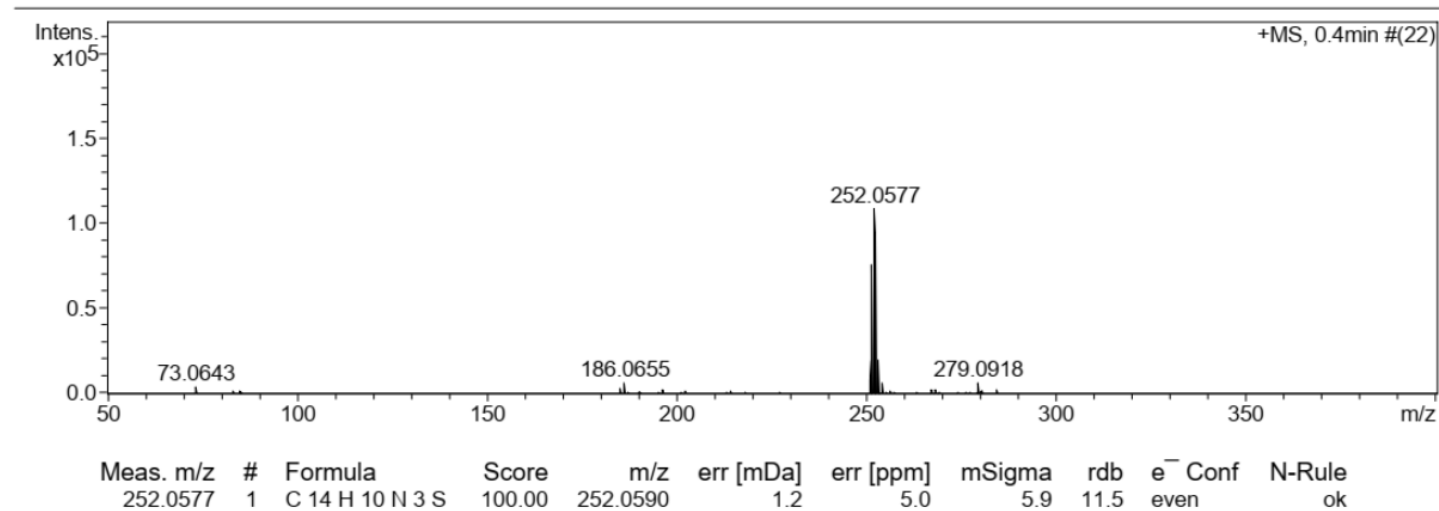
Meas. <i>m/z</i>	#	Formula	Score	<i>m/z</i>	err [mDa]	err [ppm]	<i>mSigma</i>	<i>rdb</i>	<i>e</i> ⁻ Conf	N-Rule
235.0722	1	C ₁₄ H ₉ N ₃ O	100.00	235.0740	1.8	7.9	494.6	12.0	odd	ok
236.0792	1	C ₁₄ H ₁₀ N ₃ O	100.00	236.0818	2.7	11.2	8.3	11.5	even	ok

4-(Thiophen-2-yl)-7*H*-pyrrolo[2,3-*d*]pyrimidine **7k**:

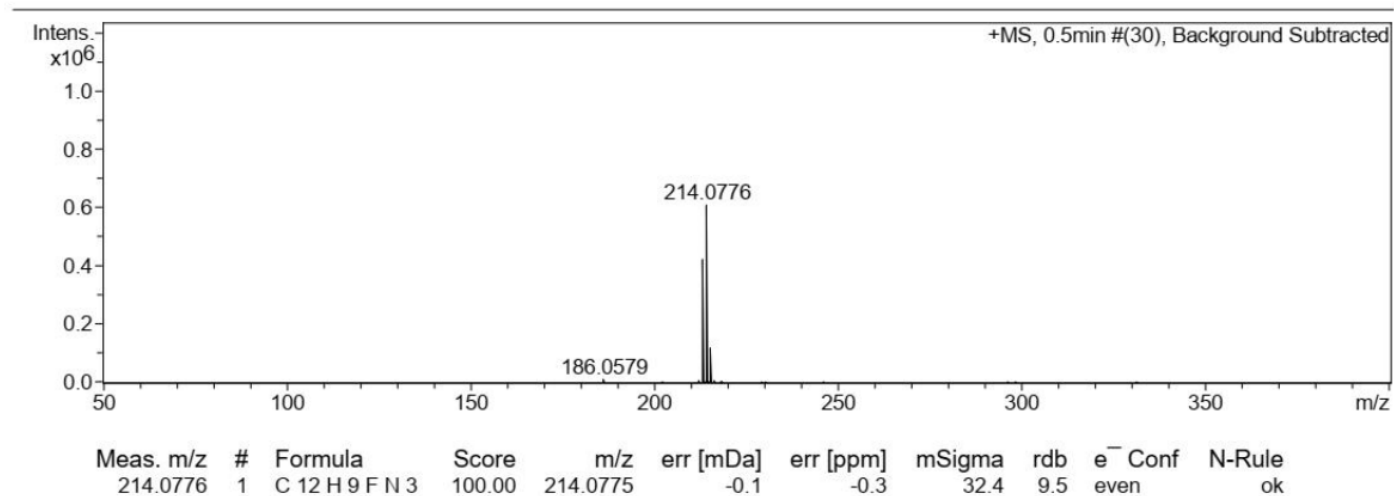


Meas. <i>m/z</i>	#	Formula	Score	<i>m/z</i>	err [mDa]	err [ppm]	<i>mSigma</i>	<i>rdb</i>	<i>e</i> ⁻ Conf	N-Rule
202.0442	1	C ₁₀ H ₈ N ₃ S	100.00	202.0433	-0.9	-4.3	20.9	8.5	even	ok
279.0936	1	C ₁₇ H ₁₅ N ₂ S	100.00	279.0950	1.5	5.4	17.1	11.5	even	ok

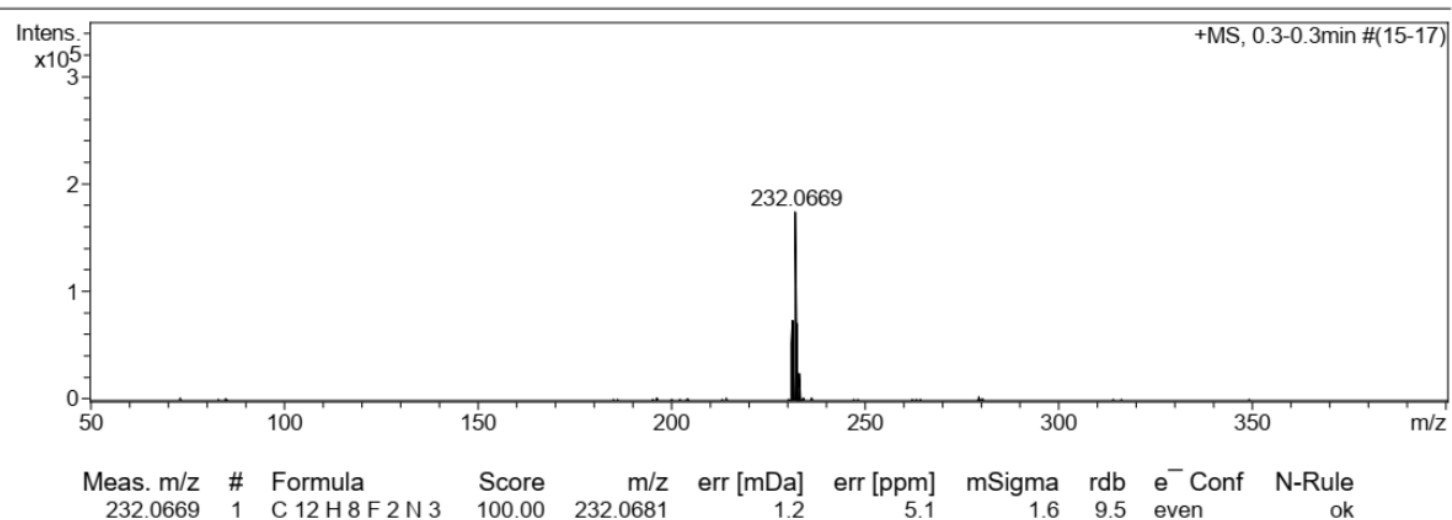
4-(1-Benzothiophen-2-yl)-7*H*-pyrrolo[2,3-*d*]pyrimidine **7l**:



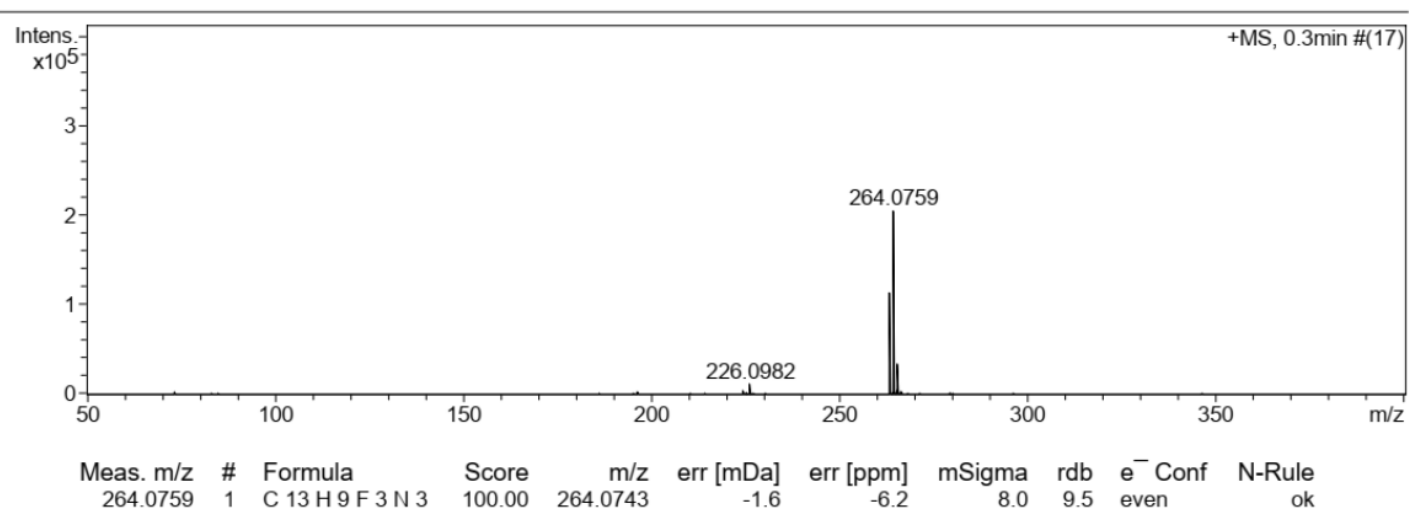
4-(4-Fluorophenyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine **7m**:



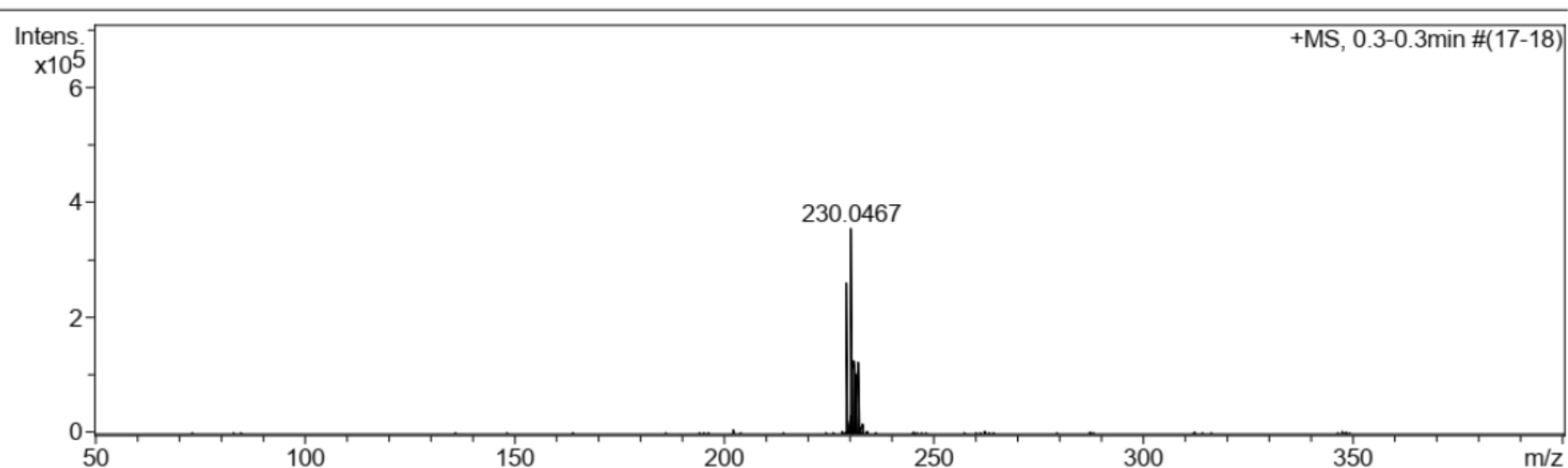
4-(3,4-Difluorophenyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine **7n**:



4-[4-(Trifluoromethyl)phenyl]-7*H*-pyrrolo[2,3-*d*]pyrimidine **7o**:

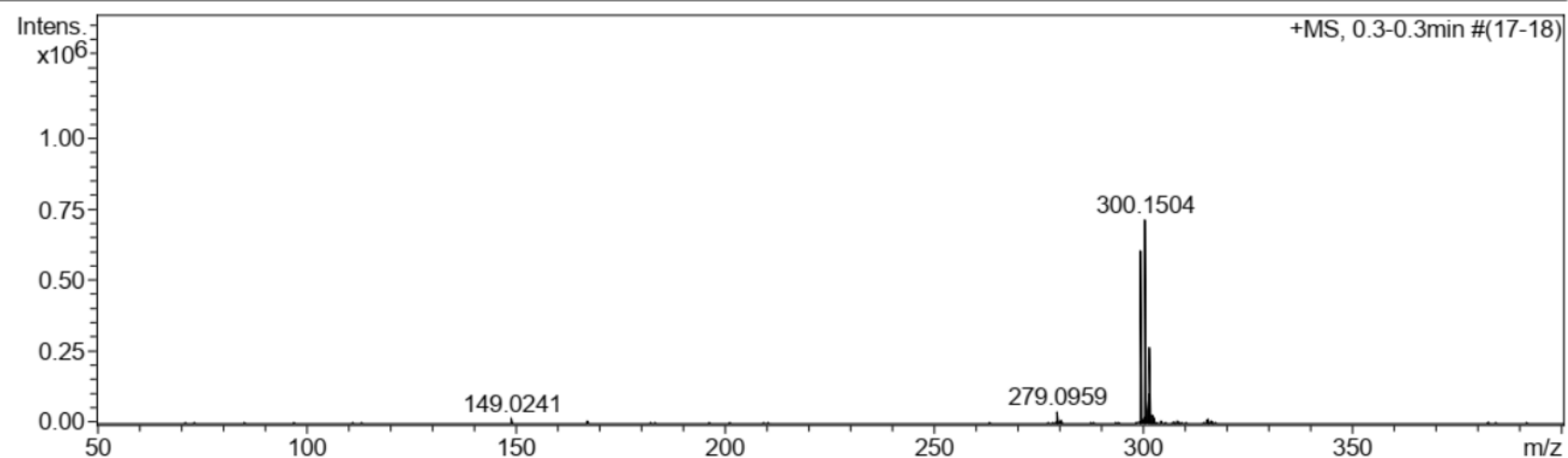


4-(4-Chlorophenyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine **7p**:



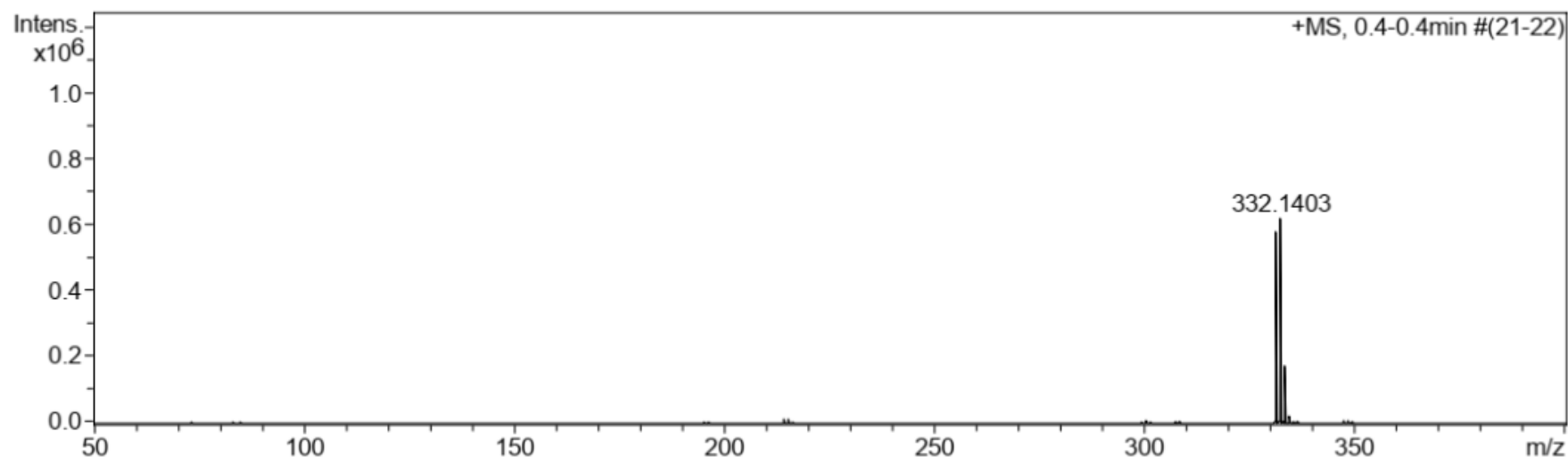
Meas. m/z	#	Formula	Score	m/z	err [mDa]	err [ppm]	mSigma	rdb	e ⁻ Conf	N-Rule
229.0392	1	C 14 H 10 Cl O	100.00	229.0415	2.2	9.7	469.3	9.5	even	ok
	2	C 12 H 8 Cl N 3	89.65	229.0401	0.9	3.8	474.6	10.0	odd	ok
	3	C 15 H 5 N 2 O	0.00	229.0396	0.4	1.7	540.0	14.5	even	ok
230.0467	1	C 14 H 11 Cl O	48.13	230.0493	2.6	11.1	102.1	9.0	odd	ok
	2	C 12 H 9 Cl N 3	100.00	230.0480	1.2	5.2	107.4	9.5	even	ok
	3	C 15 H 6 N 2 O	0.04	230.0475	0.7	3.1	222.0	14.0	odd	ok

4,7-Bis(4-methylphenyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine **8a**:



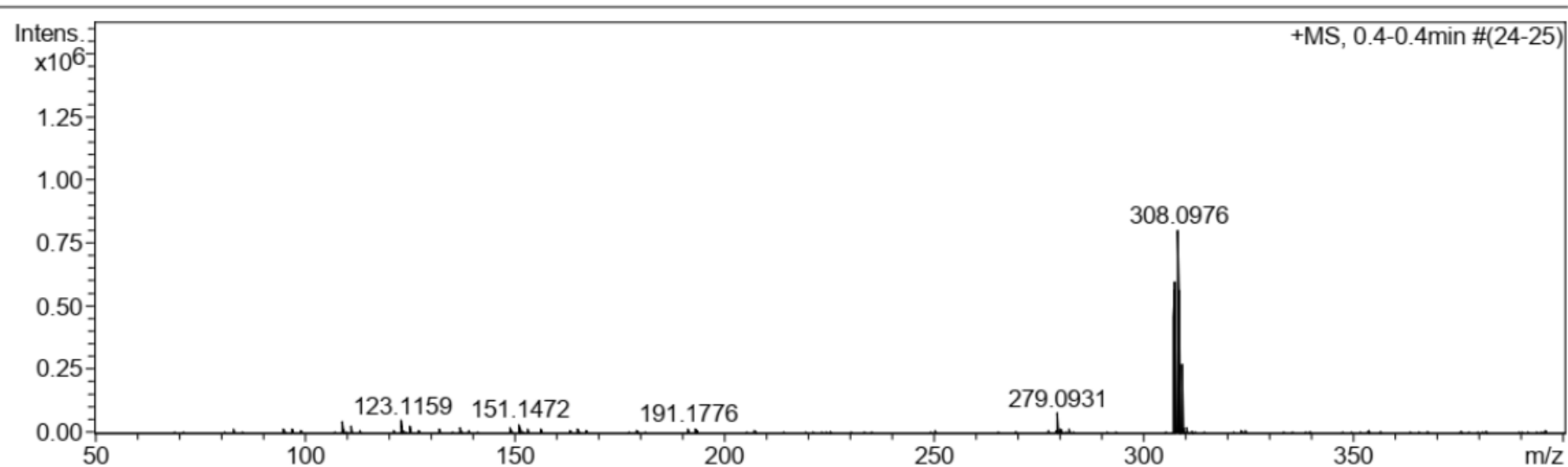
Meas. m/z	#	Formula	Score	m/z	err [mDa]	err [ppm]	mSigma	rdb	e ⁻ Conf	N-Rule
300.1504	1	C ₂₂ H ₂₀ O	100.00	300.1509	0.4	1.5	78.4	13.0	odd	ok
	2	C ₂₀ H ₁₈ N ₃	61.50	300.1495	-0.9	-3.0	84.6	13.5	even	ok
	3	C ₁₇ H ₂₀ N ₂ O ₃	2.67	300.1468	-3.6	-11.9	105.5	9.0	odd	ok
	4	C ₁₀ H ₂₄ N ₂ O ₈	0.75	300.1527	2.3	7.6	149.1	0.0	odd	ok

4,7-Bis(4-methoxyphenyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine **8b**:



Meas. m/z	#	Formula	Score	m/z	err [mDa]	err [ppm]	mSigma	rdb	e ⁻	Conf	N-Rule
332.1403	1	C ₂₅ H ₁₈ N	21.90	332.1434	3.1	9.3	2.3	17.5	even		ok
	2	C ₂₄ H ₁₆ N ₂	0.00	332.1308	-9.5	-28.6	6.4	18.0	odd		ok
	3	C ₂₂ H ₂₀ O ₃	100.00	332.1407	0.4	1.2	23.1	13.0	odd		ok
	4	C ₂₀ H ₁₈ N ₃ O ₂	65.91	332.1394	-0.9	-2.8	29.4	13.5	even		ok
	5	C ₁₈ H ₂₂ N ₂ O ₅	0.00	332.1492	9.0	27.0	46.1	8.5	even		ok
	6	C ₁₇ H ₂₀ N ₂ O ₅	4.12	332.1367	-3.6	-10.9	50.3	9.0	odd		ok
	7	C ₁₅ H ₂₄ O ₈	0.06	332.1466	6.3	18.9	66.9	4.0	odd		ok
	8	C ₁₄ H ₂₂ N ₂ O ₈	0.05	332.1340	-6.3	-19.0	71.1	4.5	even		ok
	9	C ₁₃ H ₂₂ N ₃ O ₇	0.36	332.1452	4.9	14.9	73.1	4.5	even		ok

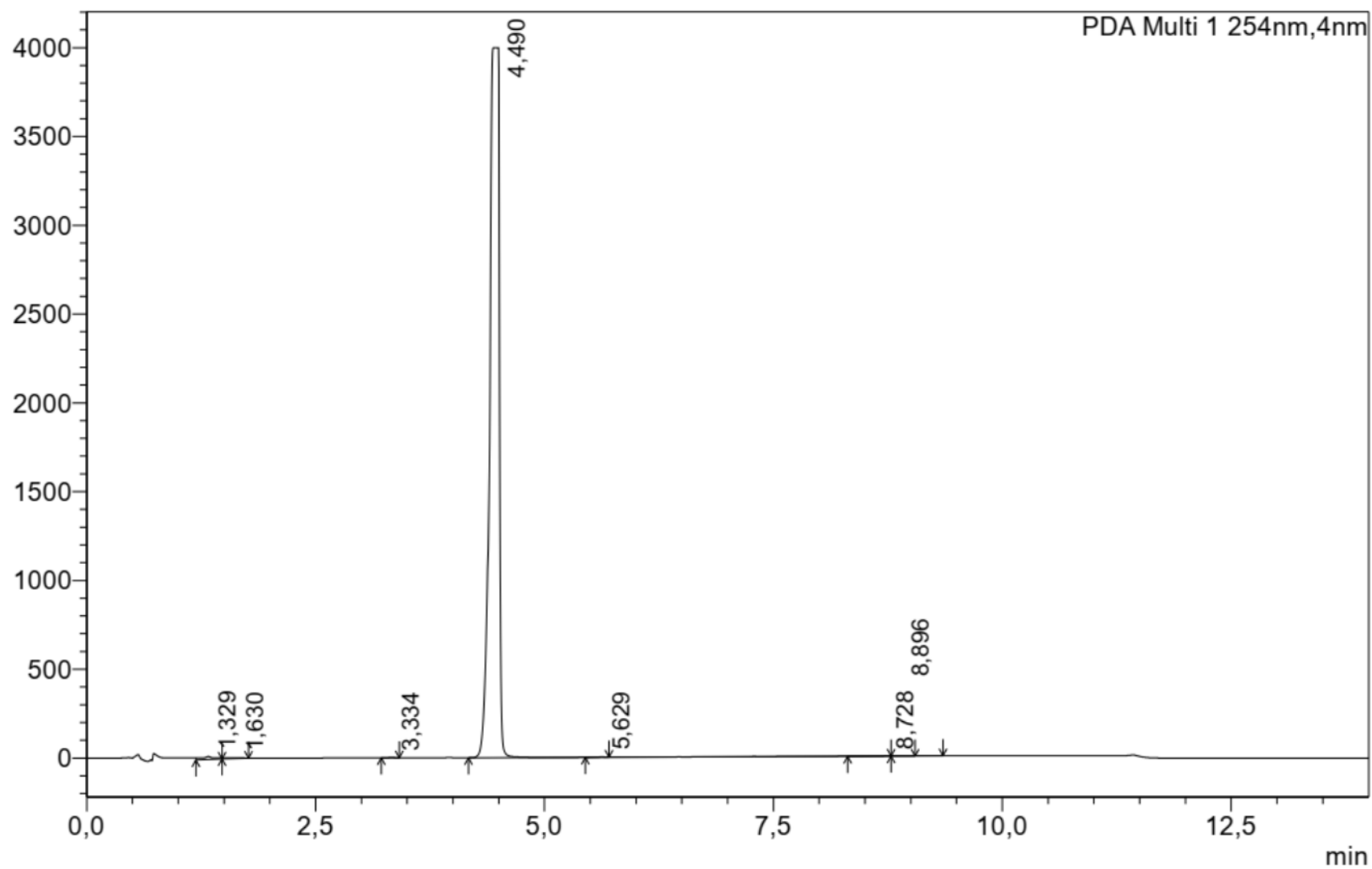
4,7-Bis(4-fluorophenyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine **8c**:



Meas. m/z	#	Formula	Score	m/z	err [mDa]	err [ppm]	mSigma	rdb	e ⁻ Conf	N-Rule
307.0901	1	C ₂₁ H ₁₁ N ₂ O	100.00	307.0866	-3.5	-11.6	500.2	17.5	even	ok
	2	C ₁₄ H ₁₅ N ₂ O ₆	0.08	307.0925	2.3	7.6	539.7	8.5	even	ok
	3	C ₁₁ H ₁₇ N ₂ O ₉	0.00	307.0898	-0.4	-1.2	558.3	4.0	odd	ok
	4	C ₈ H ₁₉ O ₁₂	0.00	307.0871	-3.0	-9.9	574.9	-0.5	even	ok
308.0976	1	C ₂₁ H ₁₂ N ₂ O	100.00	308.0944	-3.2	-10.5	61.0	17.0	odd	ok
	2	C ₁₄ H ₁₆ N ₂ O ₆	28.54	308.1003	2.6	8.6	104.7	8.0	odd	ok
	3	C ₁₁ H ₁₈ N ₂ O ₉	50.27	308.0976	-0.0	-0.1	125.5	3.5	even	ok

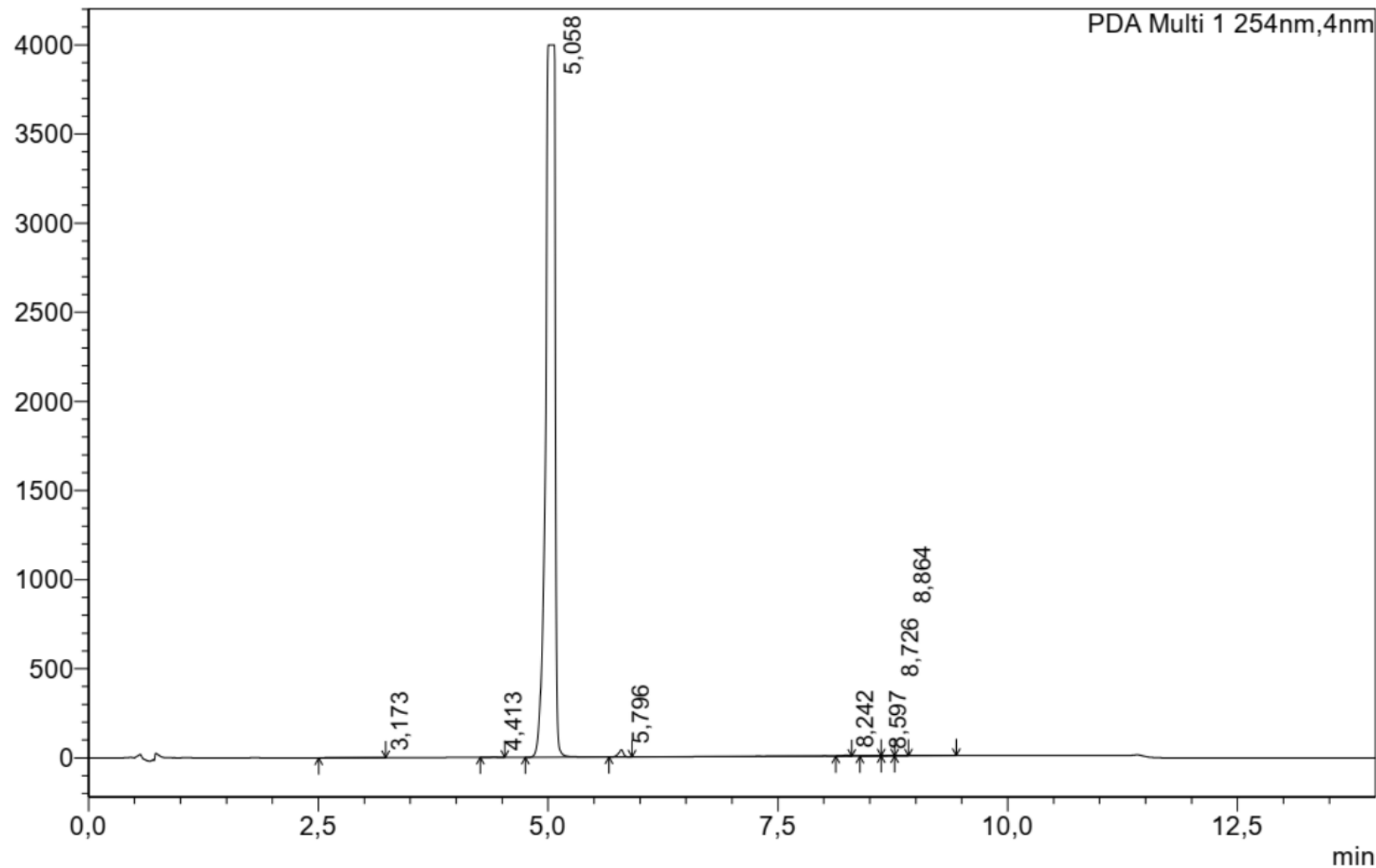
6-phenyl-7*H*-pyrrolo[2,3-*d*]pyrimidine (HPLC) **5a**:

mAU



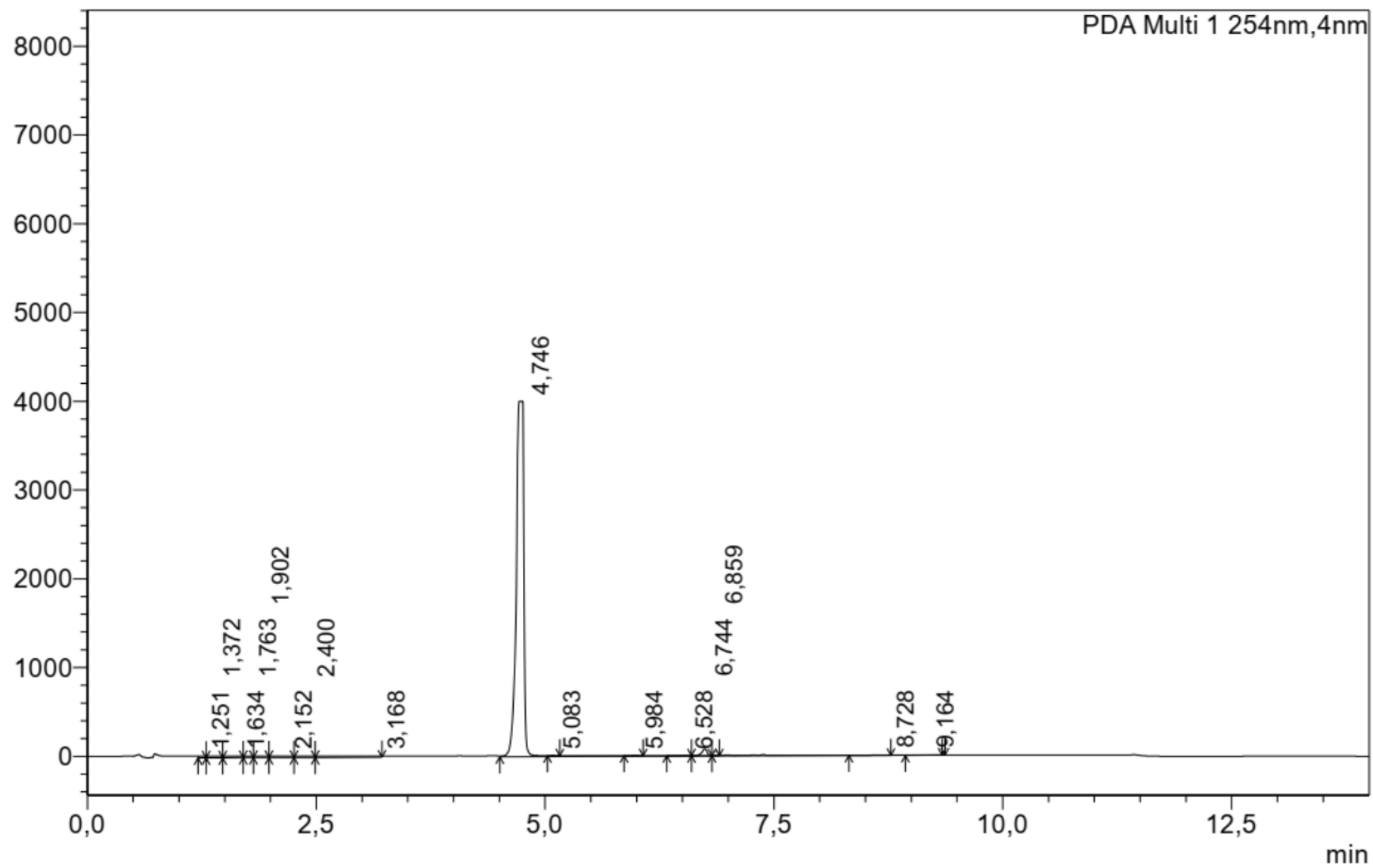
6-(4-Methylphenyl)-1*H*-pyrrolo[2,3-*b*]pyridine (HPLC) **5b**:

mAU



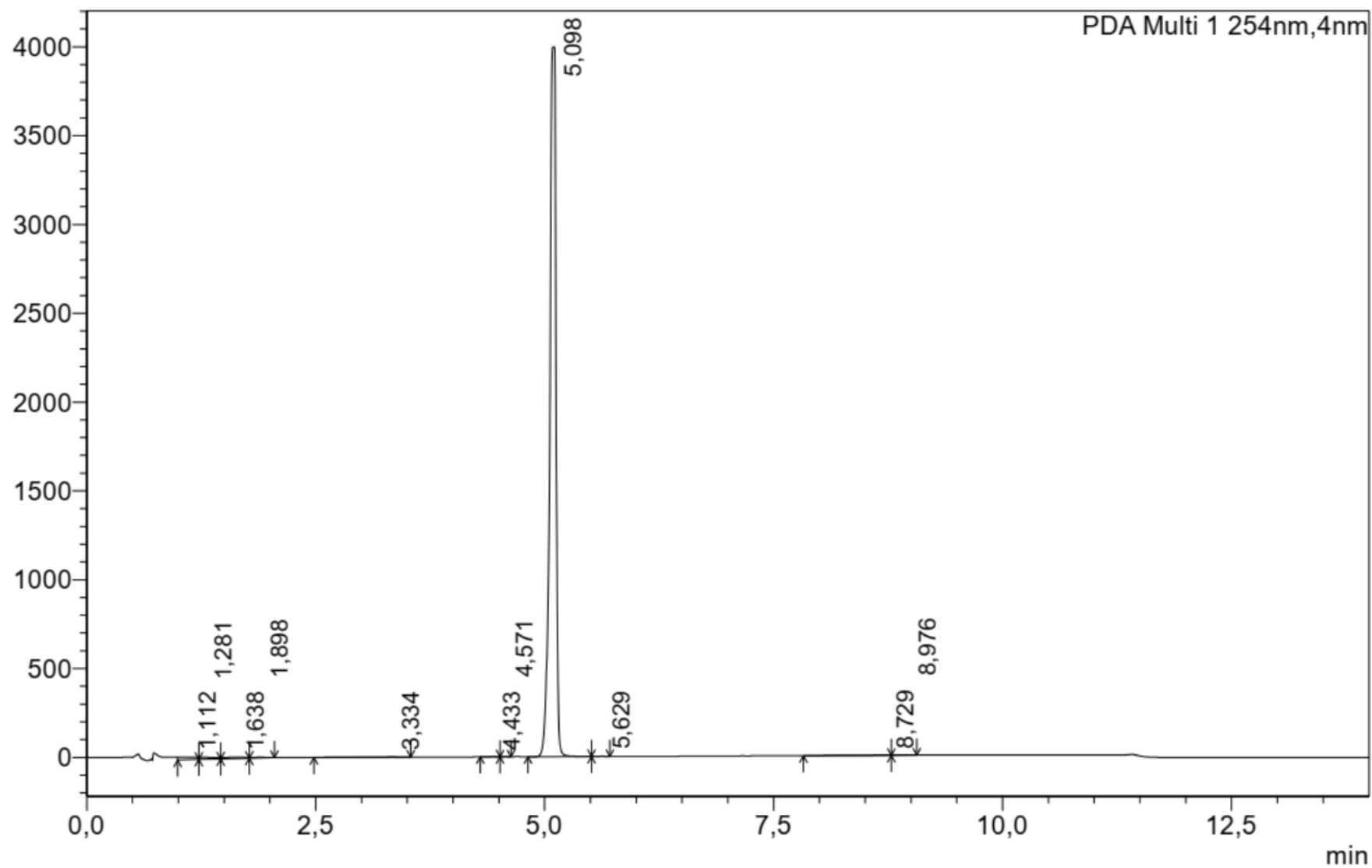
6-(3-Methoxyphenyl)-1*H*-pyrrolo[2,3-*b*]pyridine (HPLC) **5c**:

mAU



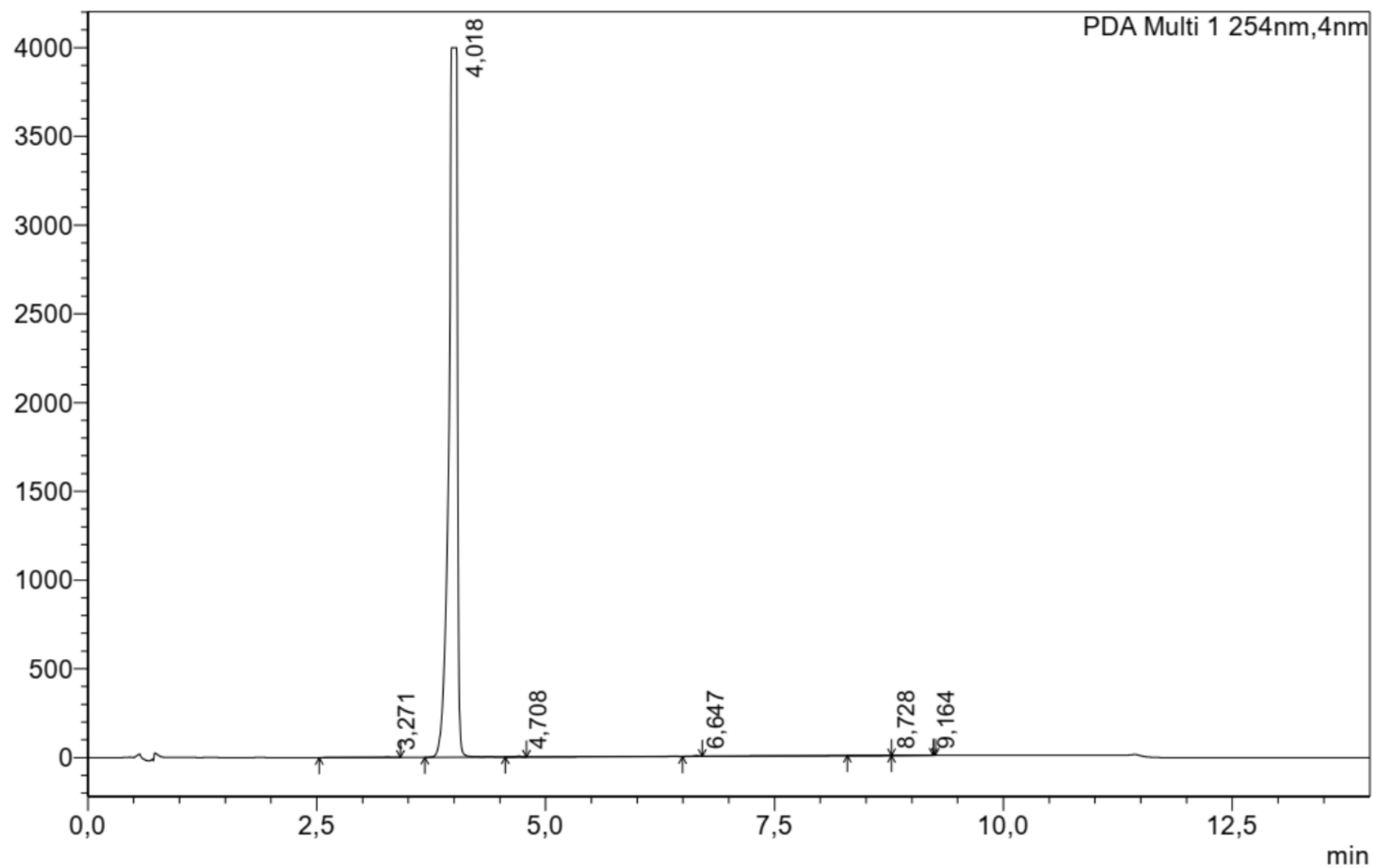
6-(4-Methoxyphenyl)-1*H*-pyrrolo[2,3-*b*]pyridine (HPLC) **5d**:

mAU



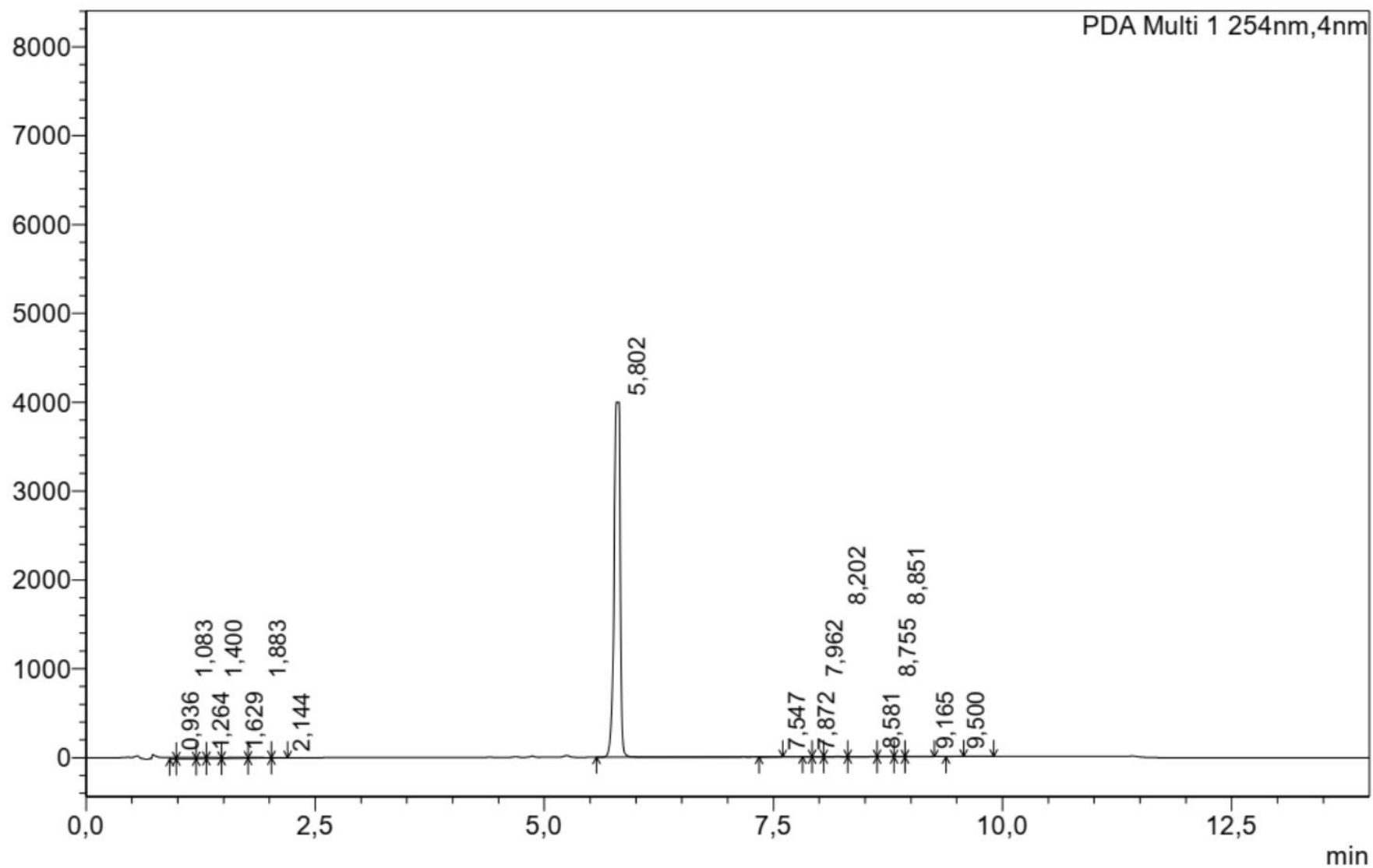
6-(4-Fluorophenyl)-1*H*-pyrrolo[2,3-*b*]pyridine (HPLC) **5e**:

mAU

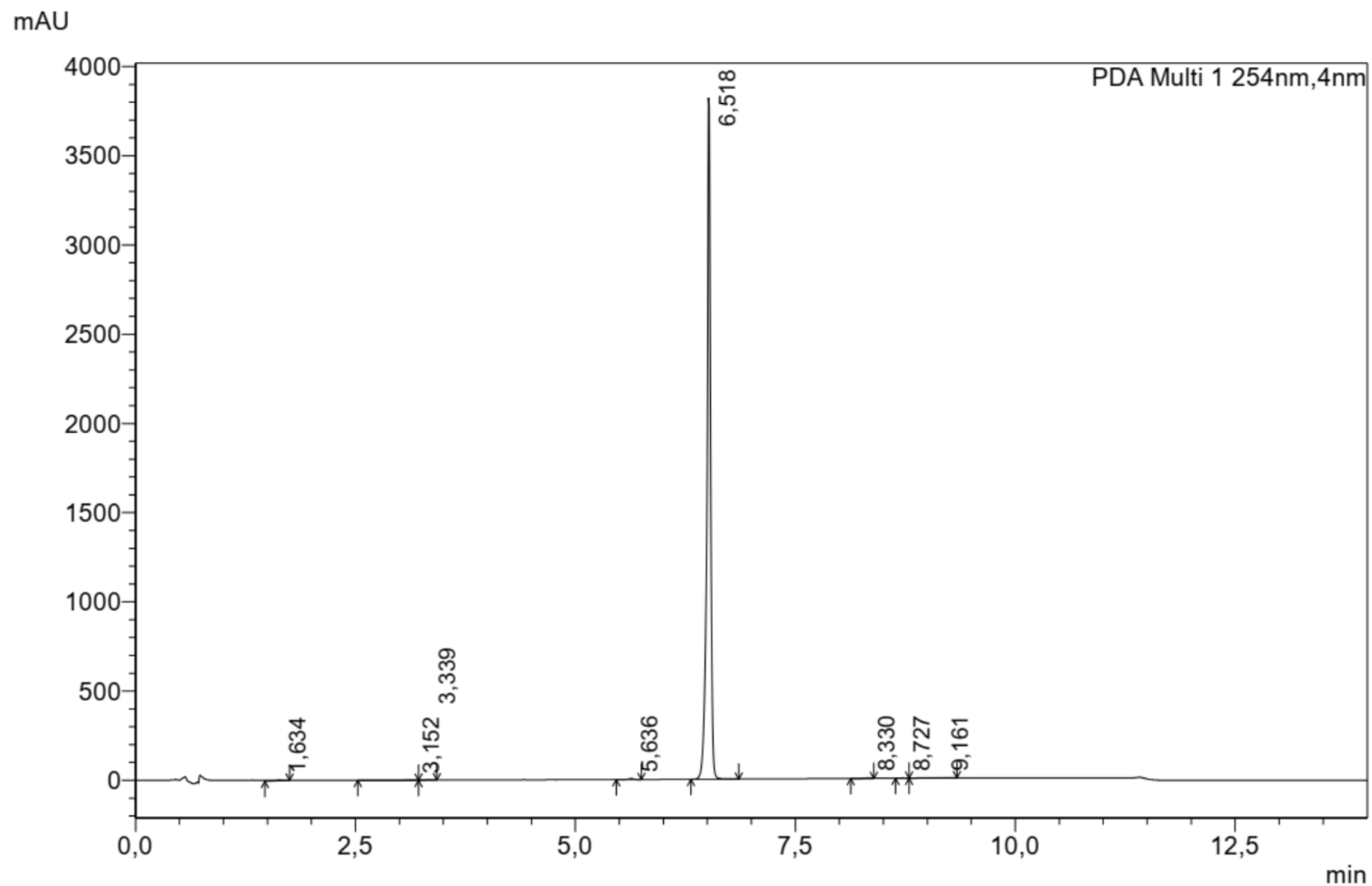


6-(3,4-Difluorophenyl)-1*H*-pyrrolo[2,3-*b*]pyridine (HPLC) **5f**:

mAU

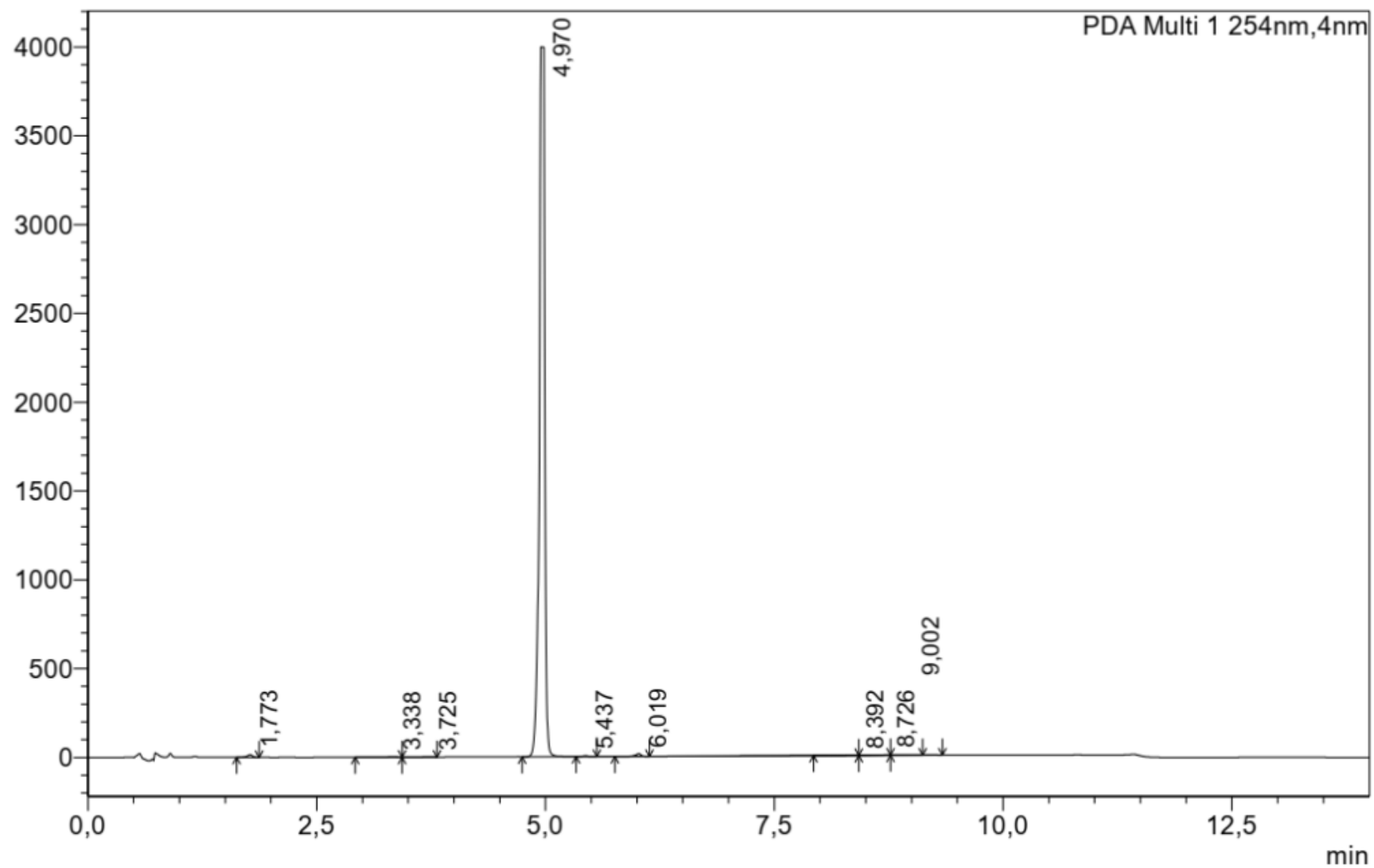


6-[4-(Trifluoromethyl)phenyl]-1*H*-pyrrolo[2,3-*b*]pyridine (HPLC) **5g**:

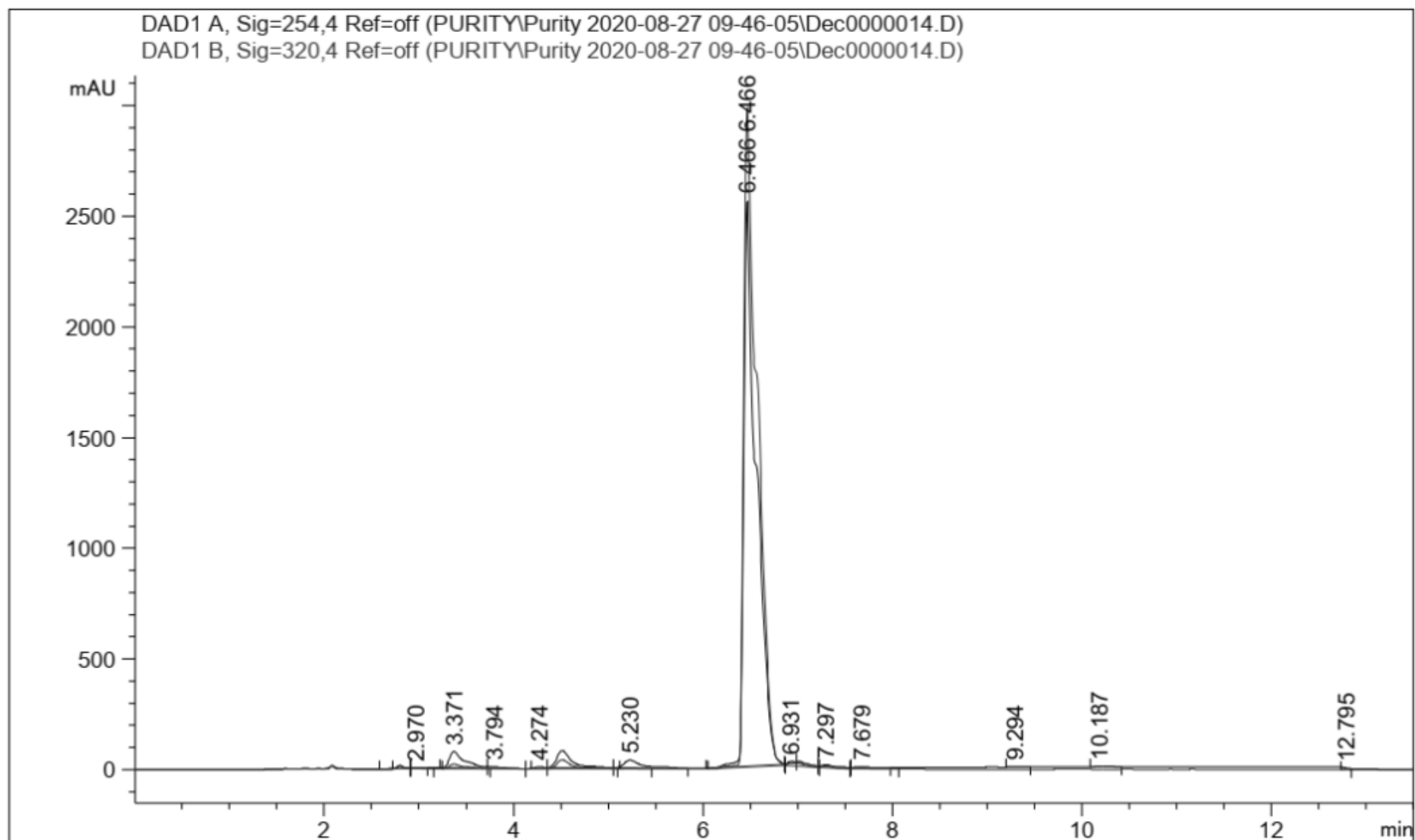


4-(1H-Pyrrolo[2,3-b]pyridin-6-yl)benzonitrile (HPLC) **5h**:

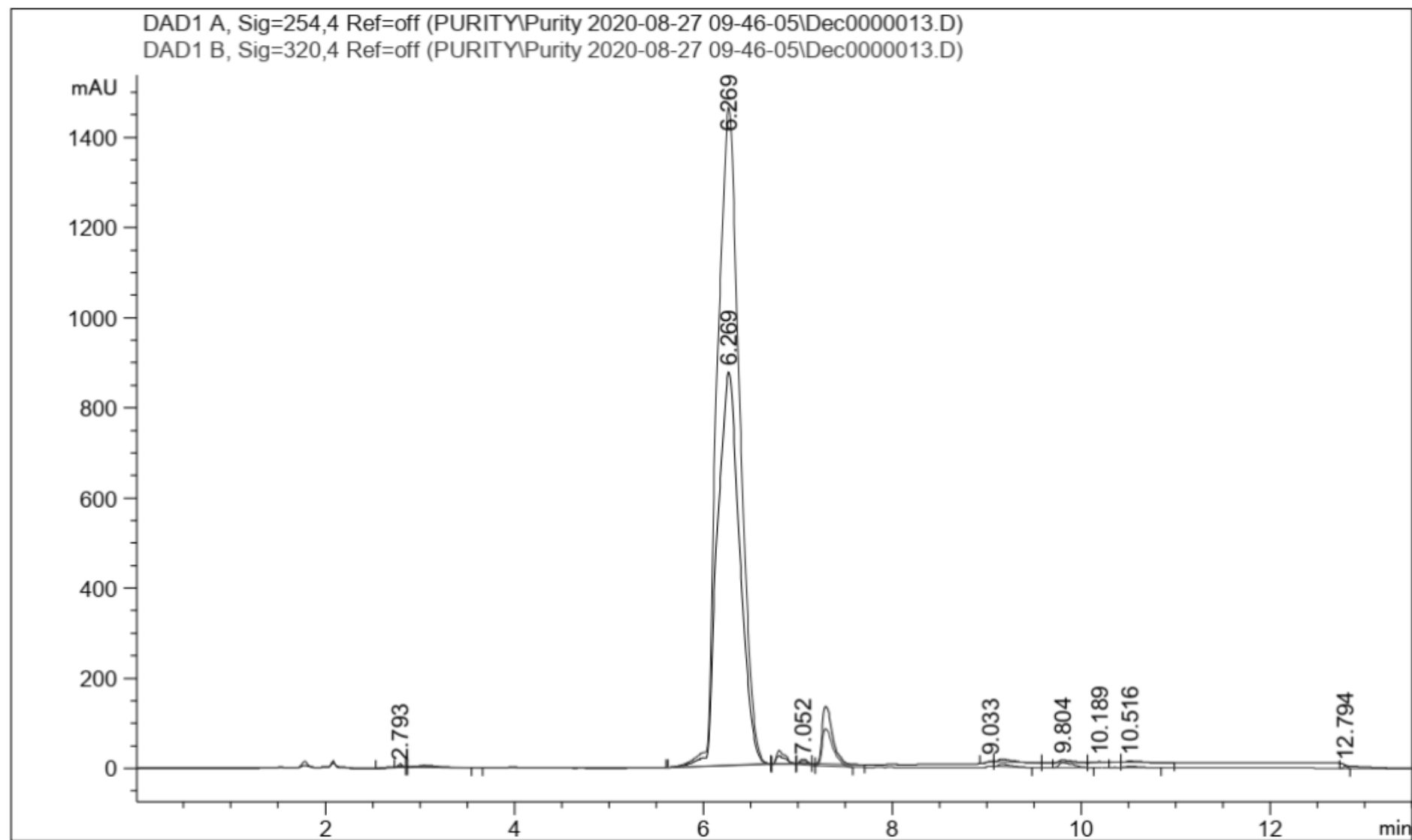
mAU



6-(Pyridin-3-yl)-1*H*-pyrrolo[2,3-*b*]pyridine (HPLC) **5i**:

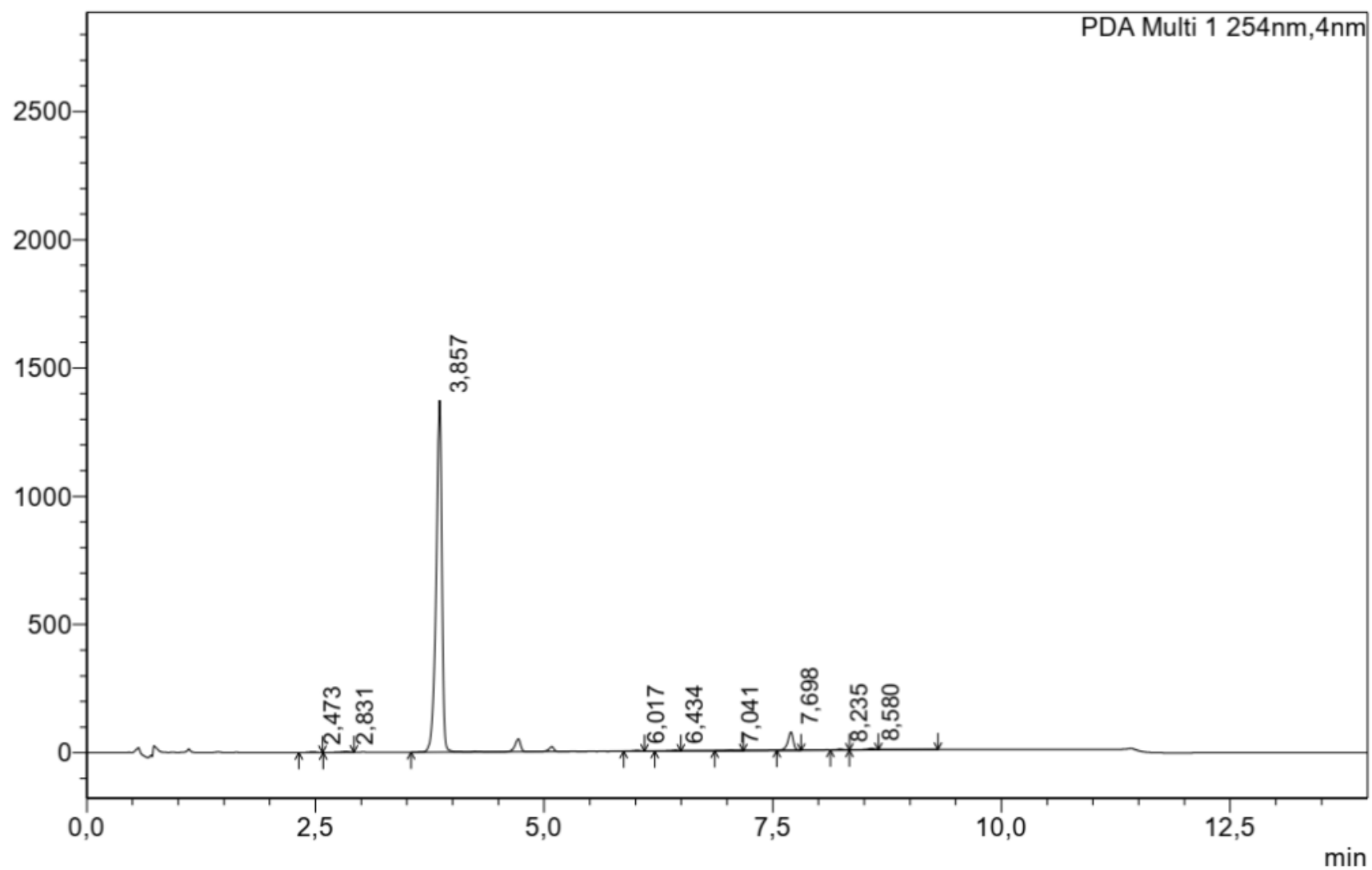


6-(Pyridin-4-yl)-1*H*-pyrrolo[2,3-*b*]pyridine (HPLC) **5j**:

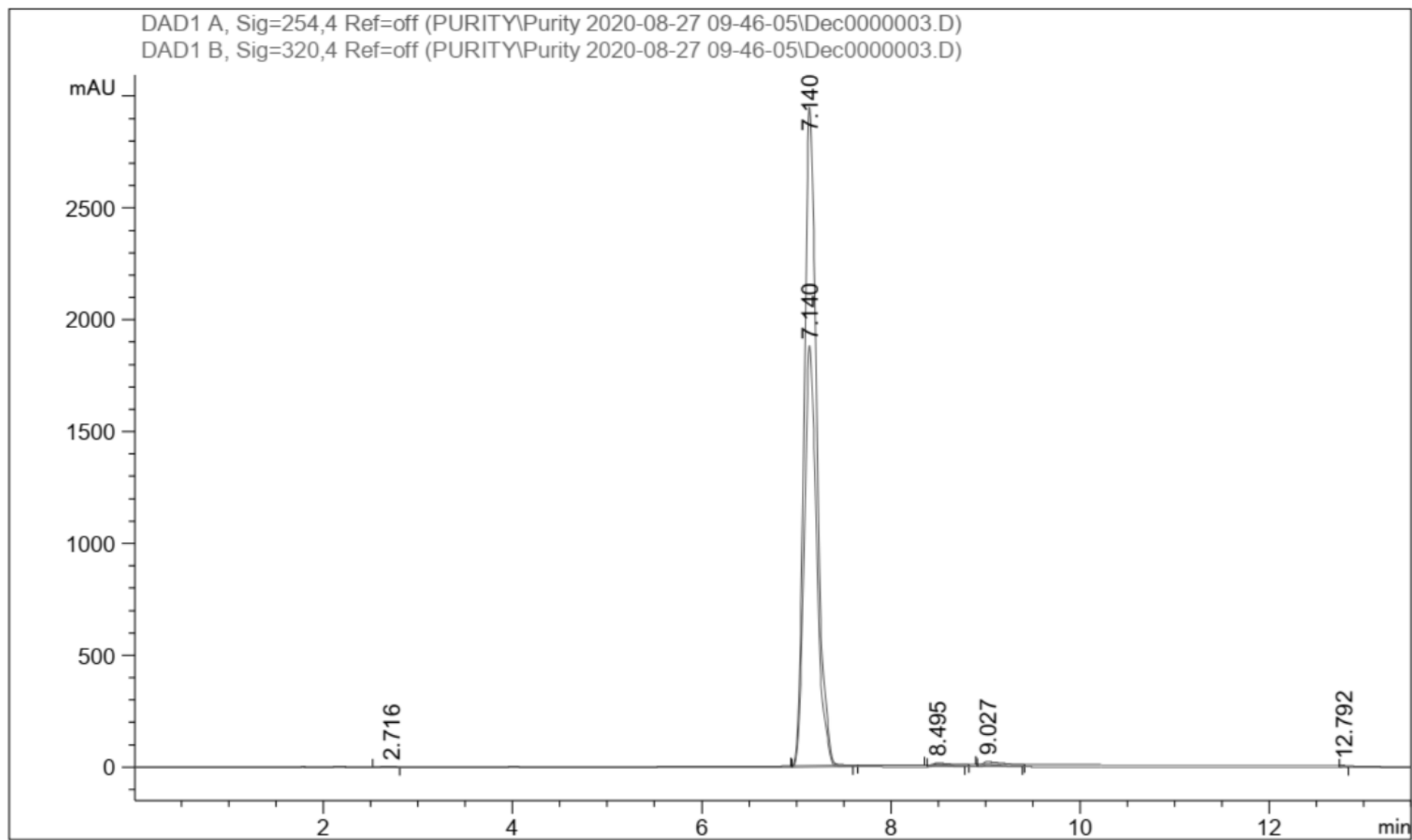


6-(6-Fluoropyridin-3-yl)-1*H*-pyrrolo[2,3-*b*]pyridine (HPLC) **5k**:

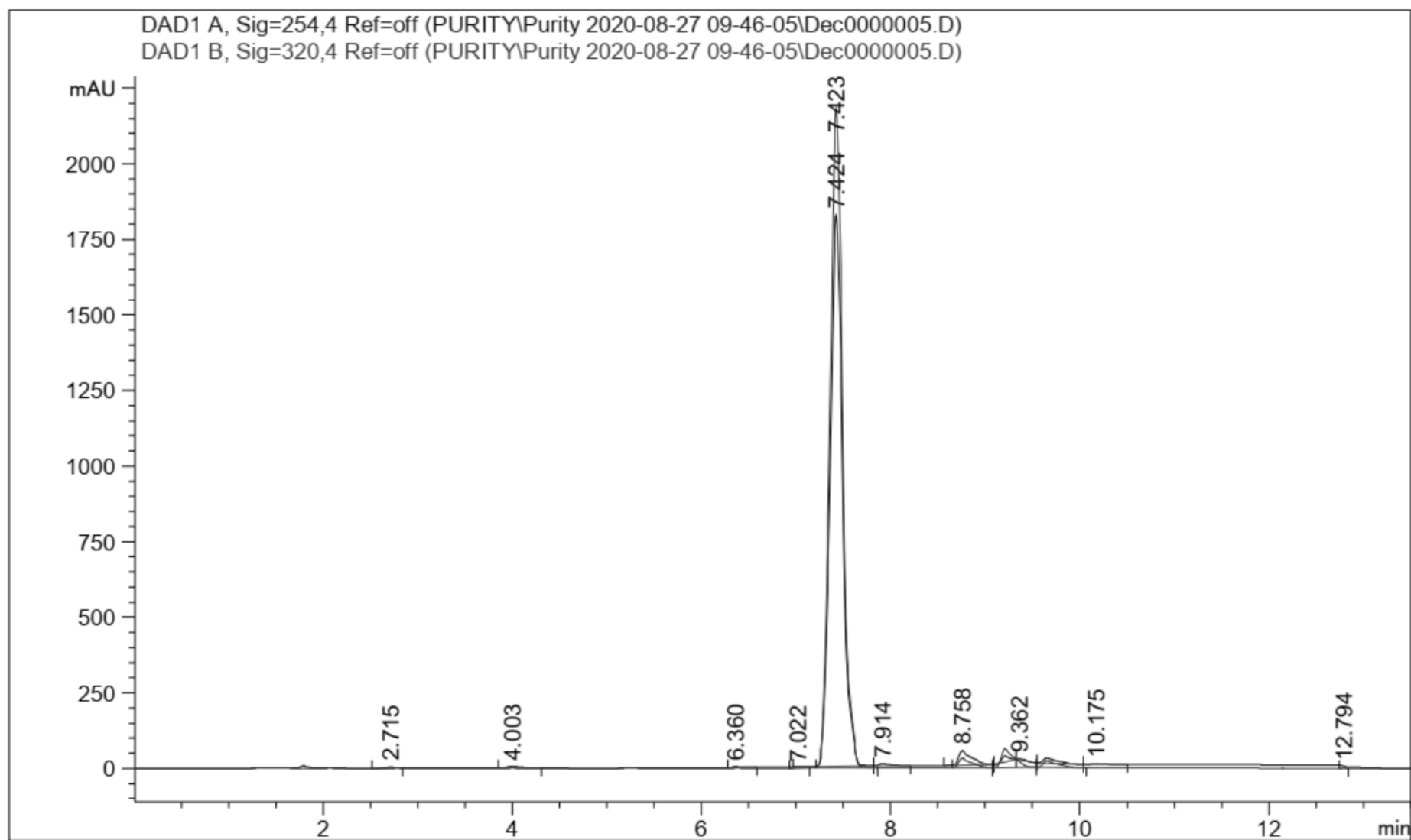
mAU



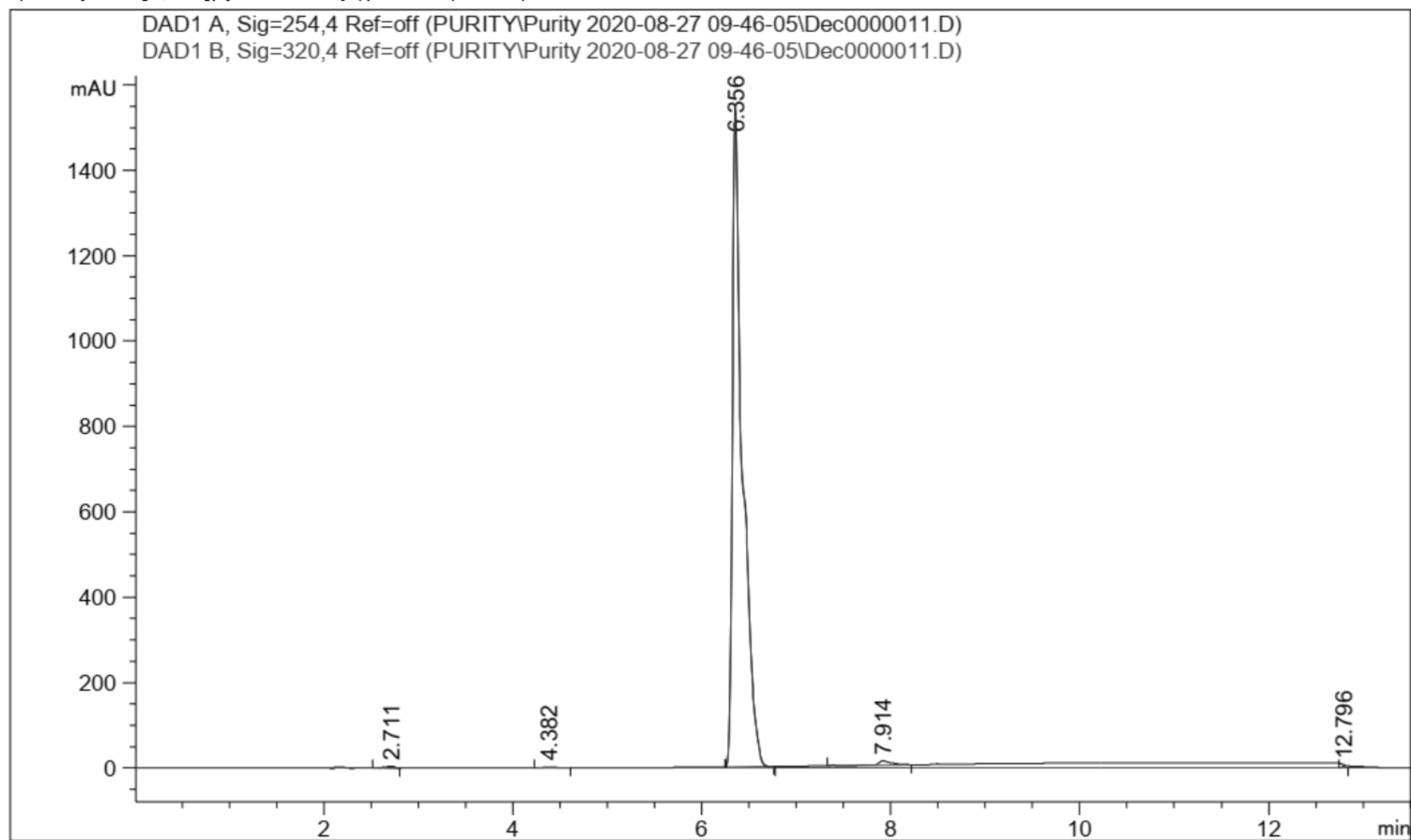
4-Phenyl-7*H*-pyrrolo[2,3-*d*]pyrimidine (HPLC) **7a**:



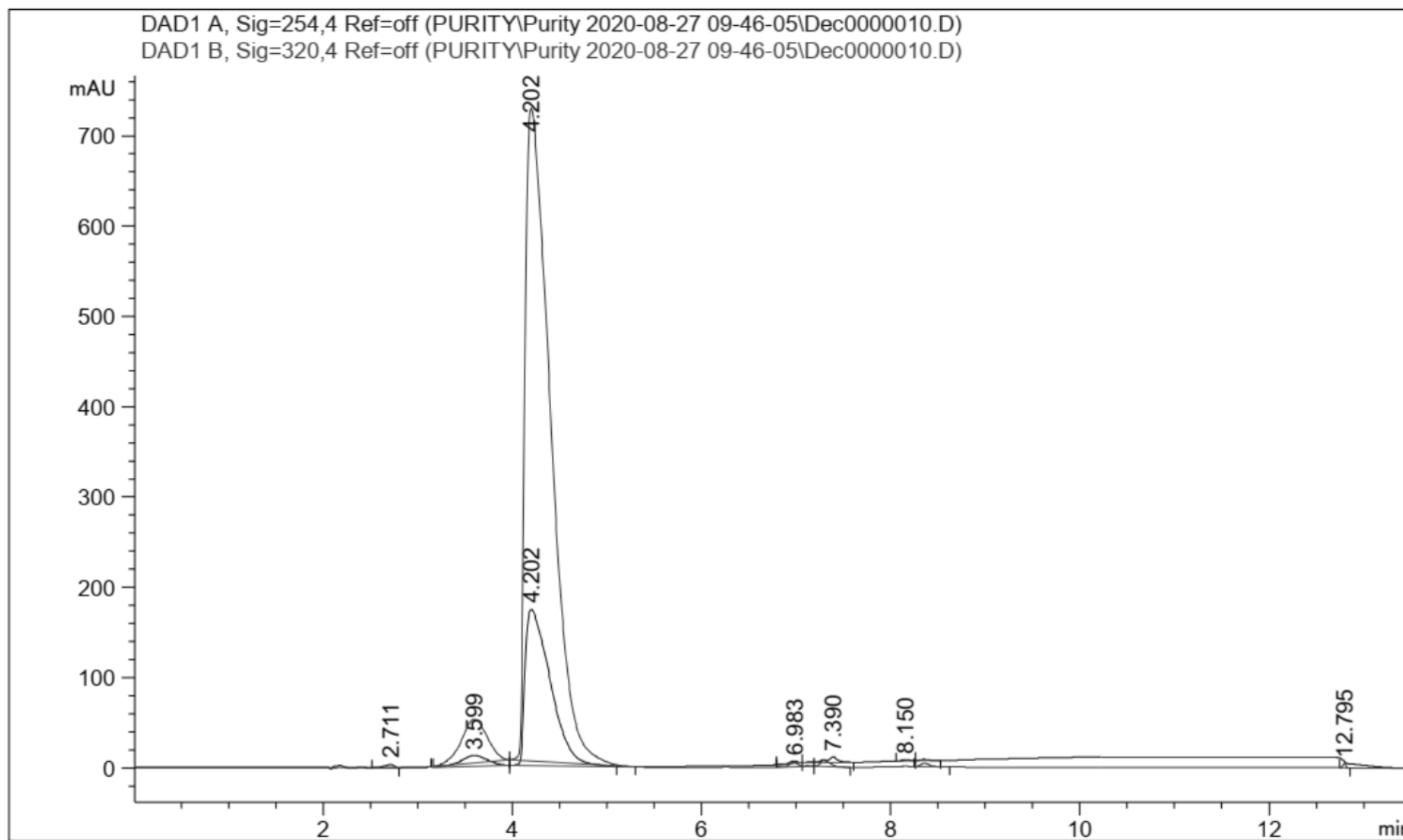
4-(4-Methylphenyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine (HPLC) **7b**:



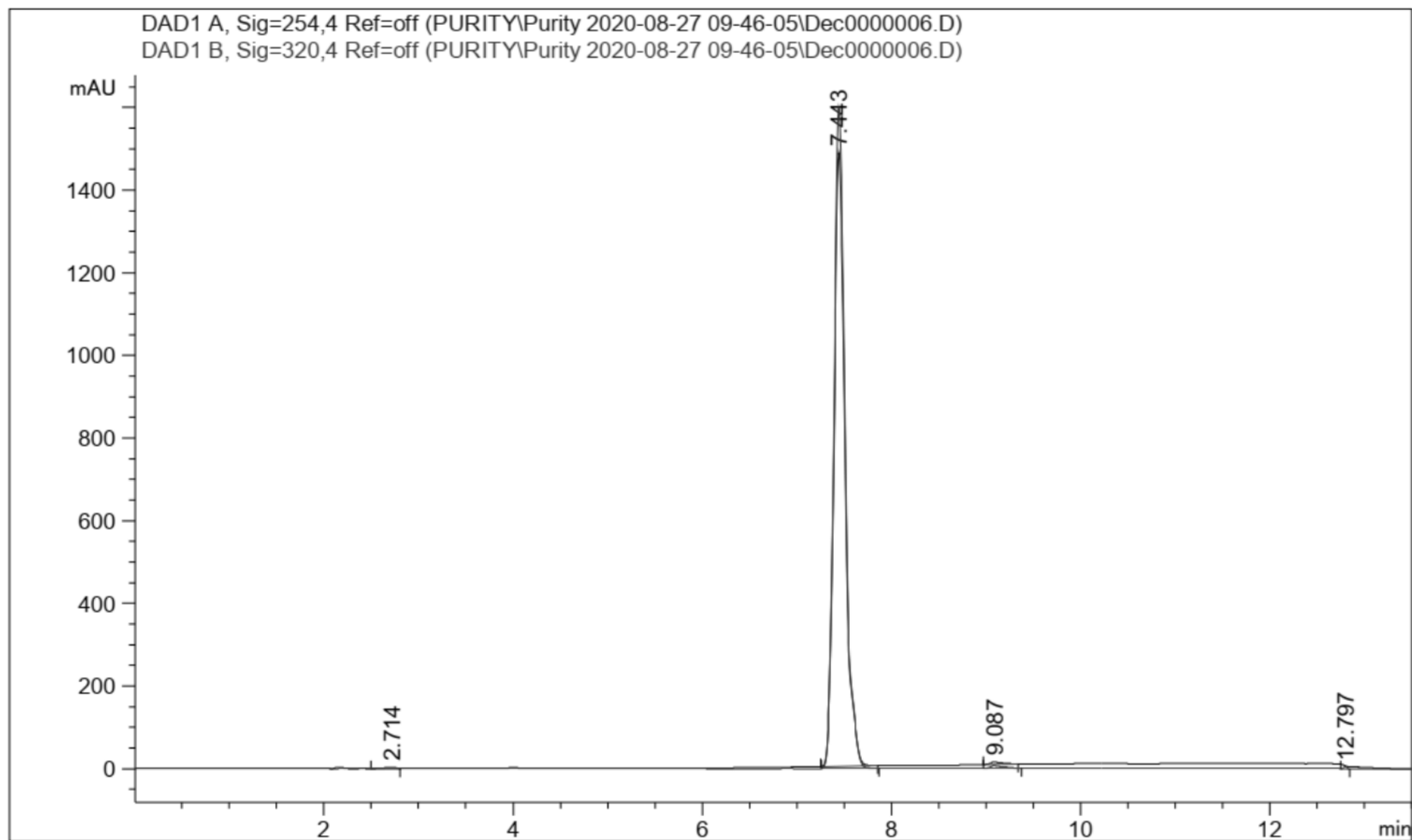
3-(7*H*-Pyrrolo[2,3-*d*]pyrimidin-4-yl)phenol (HPLC) **7c**:



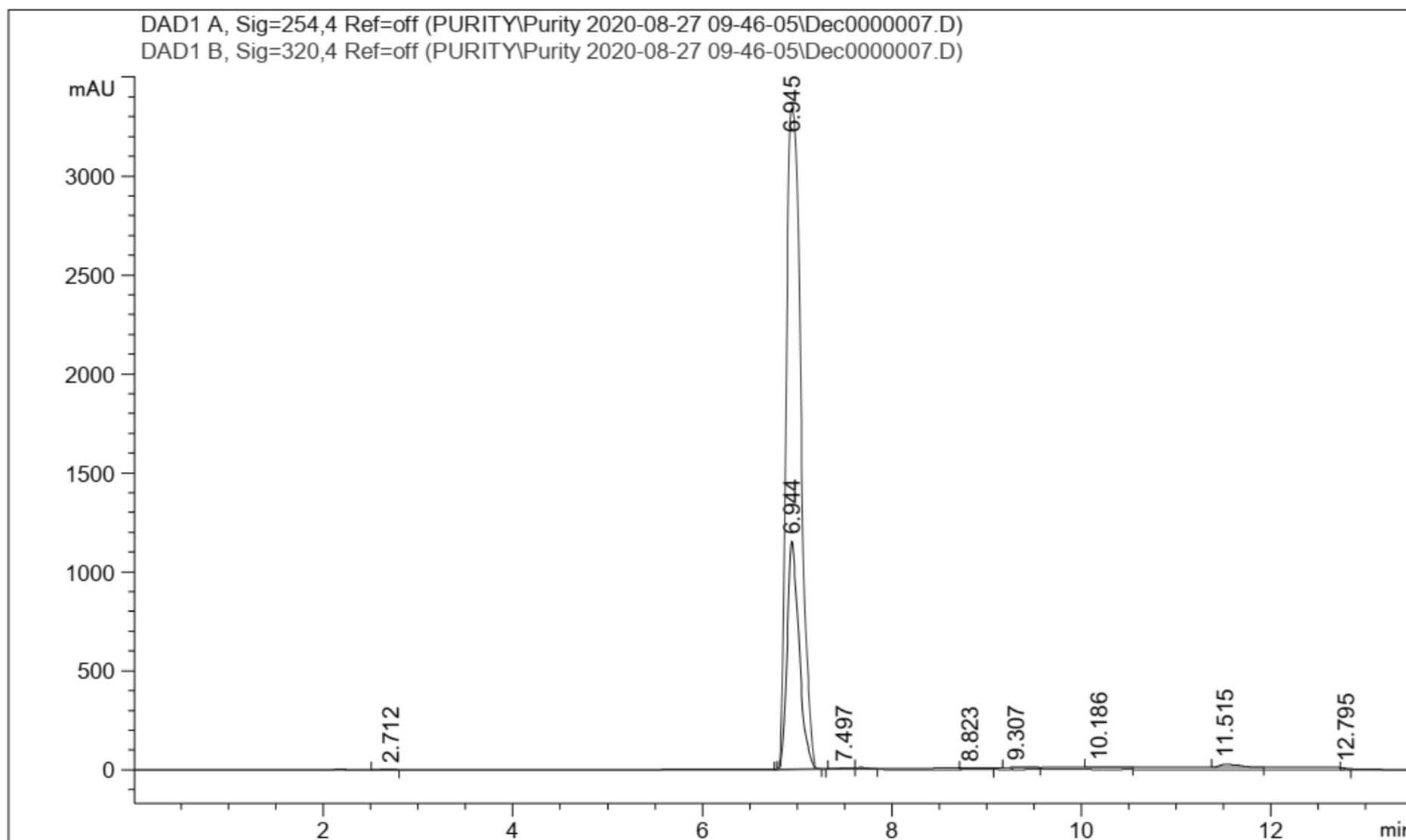
4-(7*H*-Pyrrolo[2,3-*d*]pyrimidin-4-yl)phenol (HPLC) **7d**:



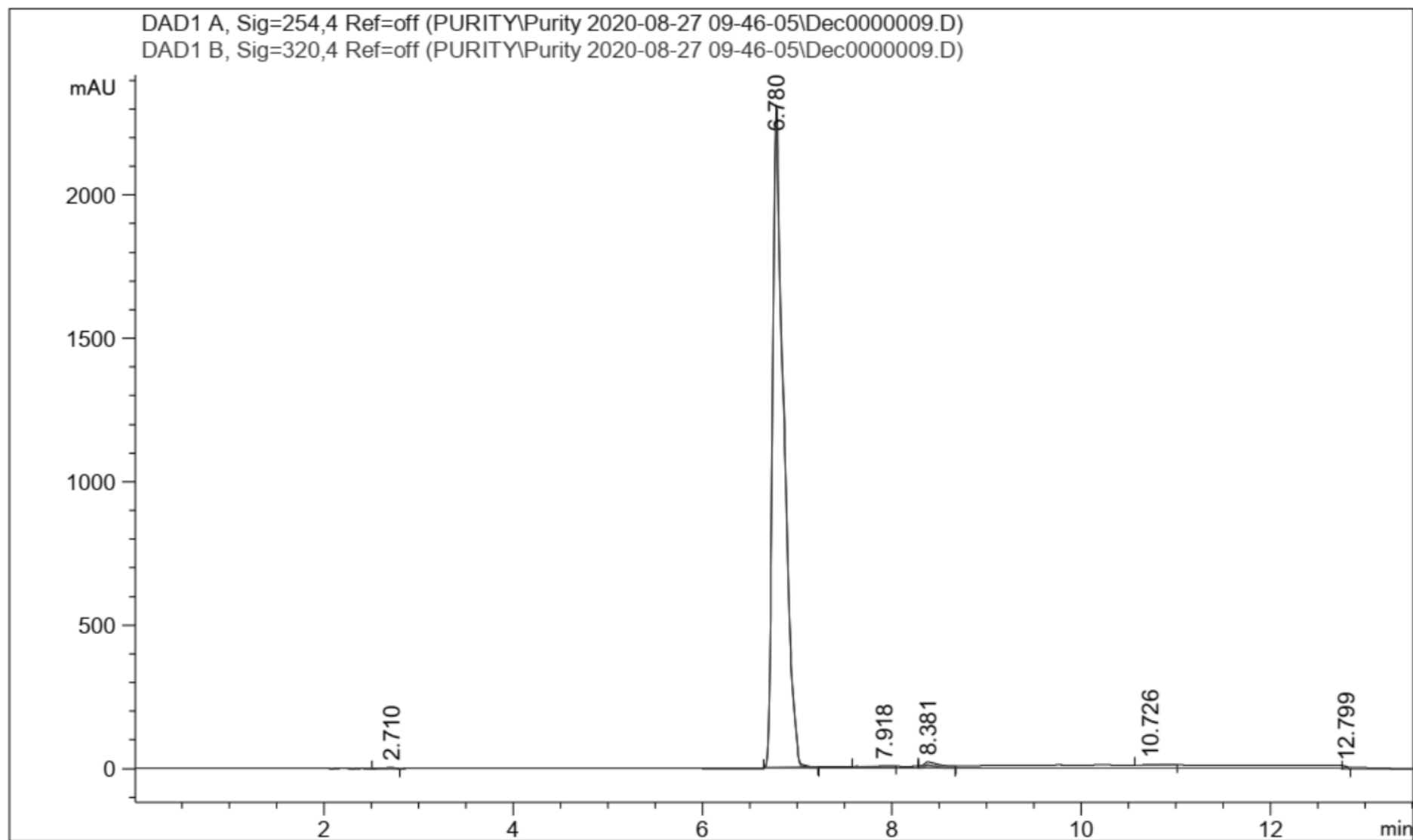
4-(3-Methoxyphenyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine (HPLC) **7e**:



4-(4-Methoxyphenyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine (HPLC) **7f**:

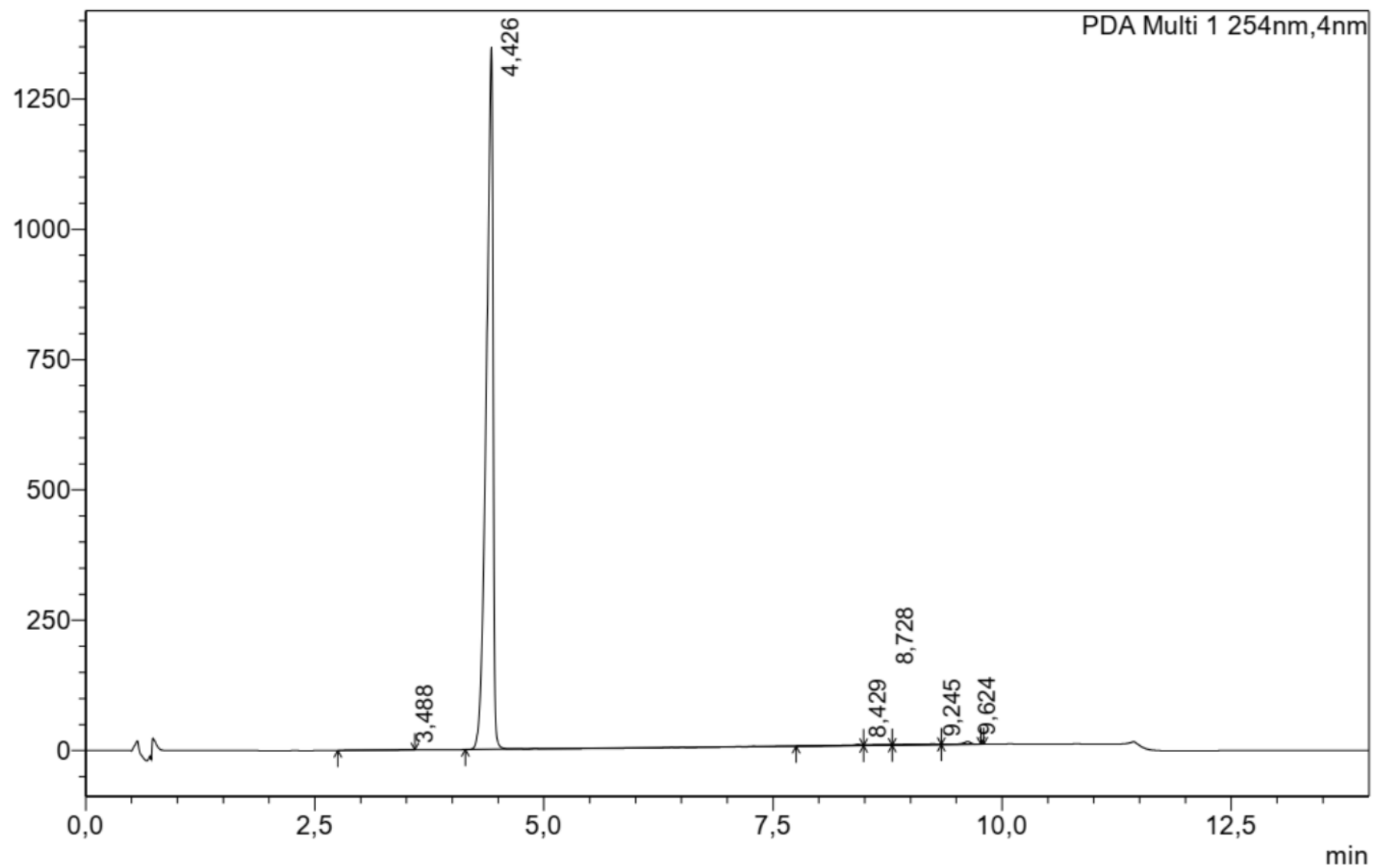


4-(2,3-Dimethoxyphenyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine (HPLC) **7g**:

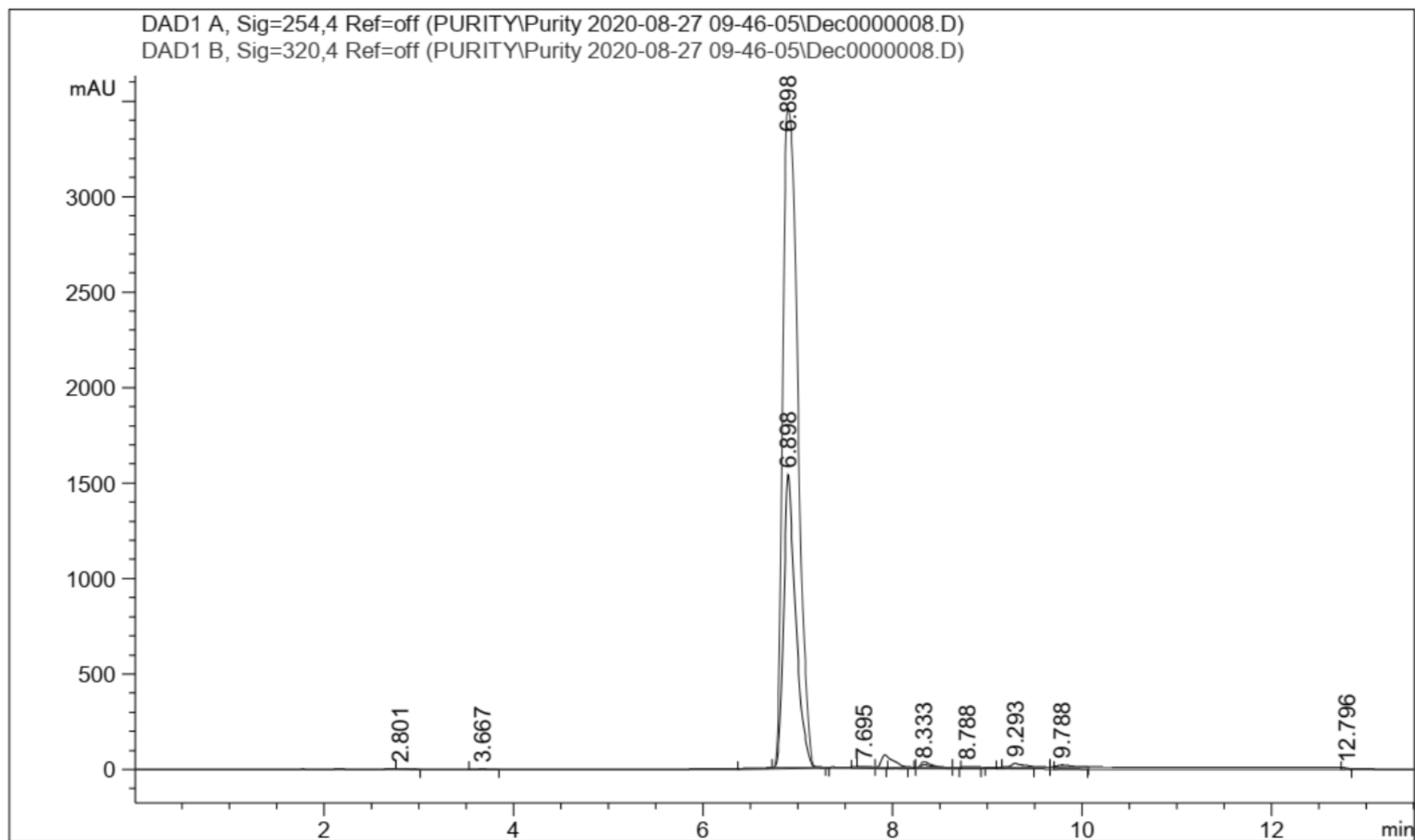


4-(4-Phenoxyphenyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine (HPLC) **7h**:

mAU

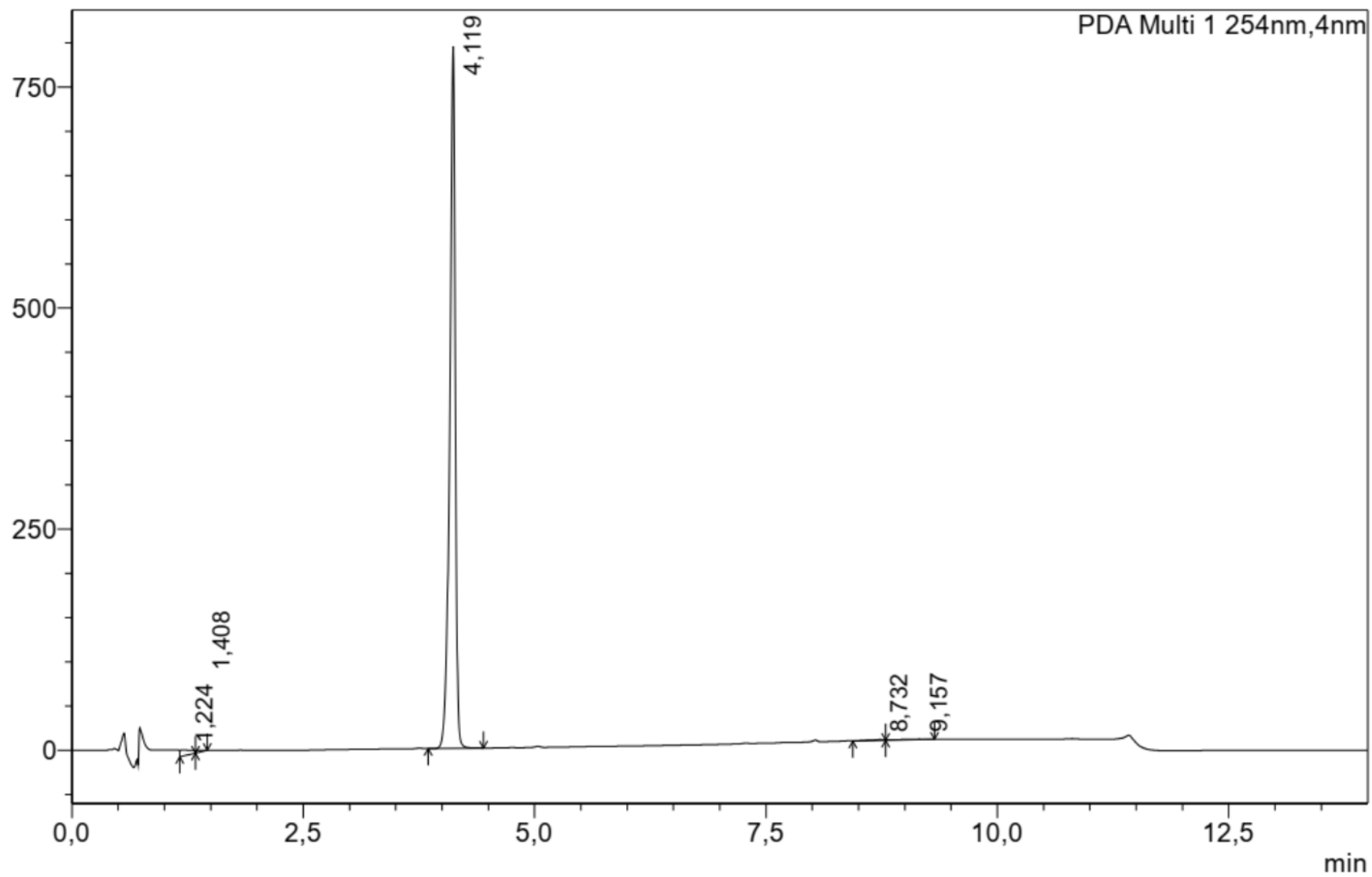


4-(Furan-2-yl)-7*H*-pyrrolo[2,3-*d*]pyrimidine (HPLC) **7i**:

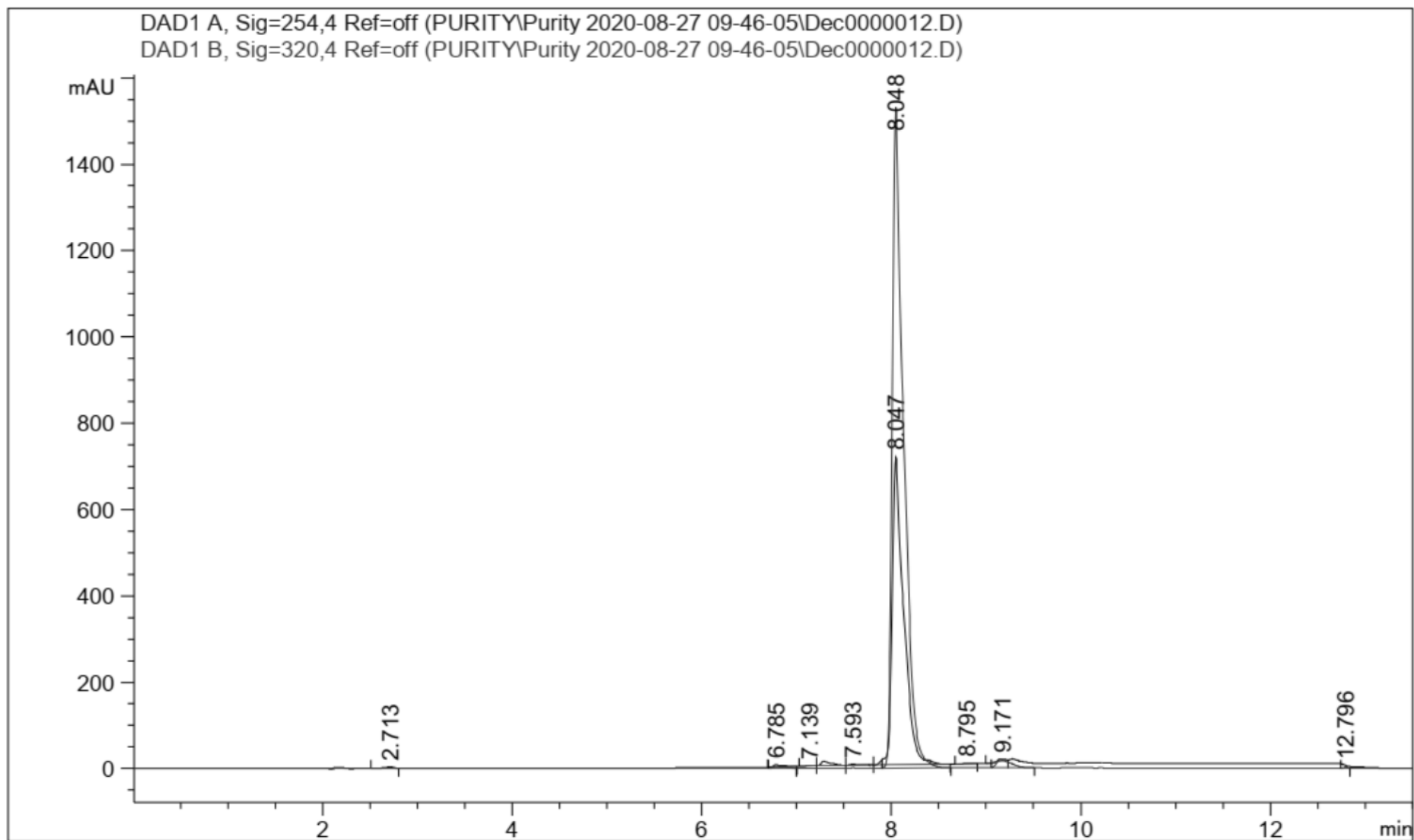


4-(1-Benzofuran-2-yl)-7*H*-pyrrolo[2,3-*d*]pyrimidine (HPLC) **7j**:

mAU

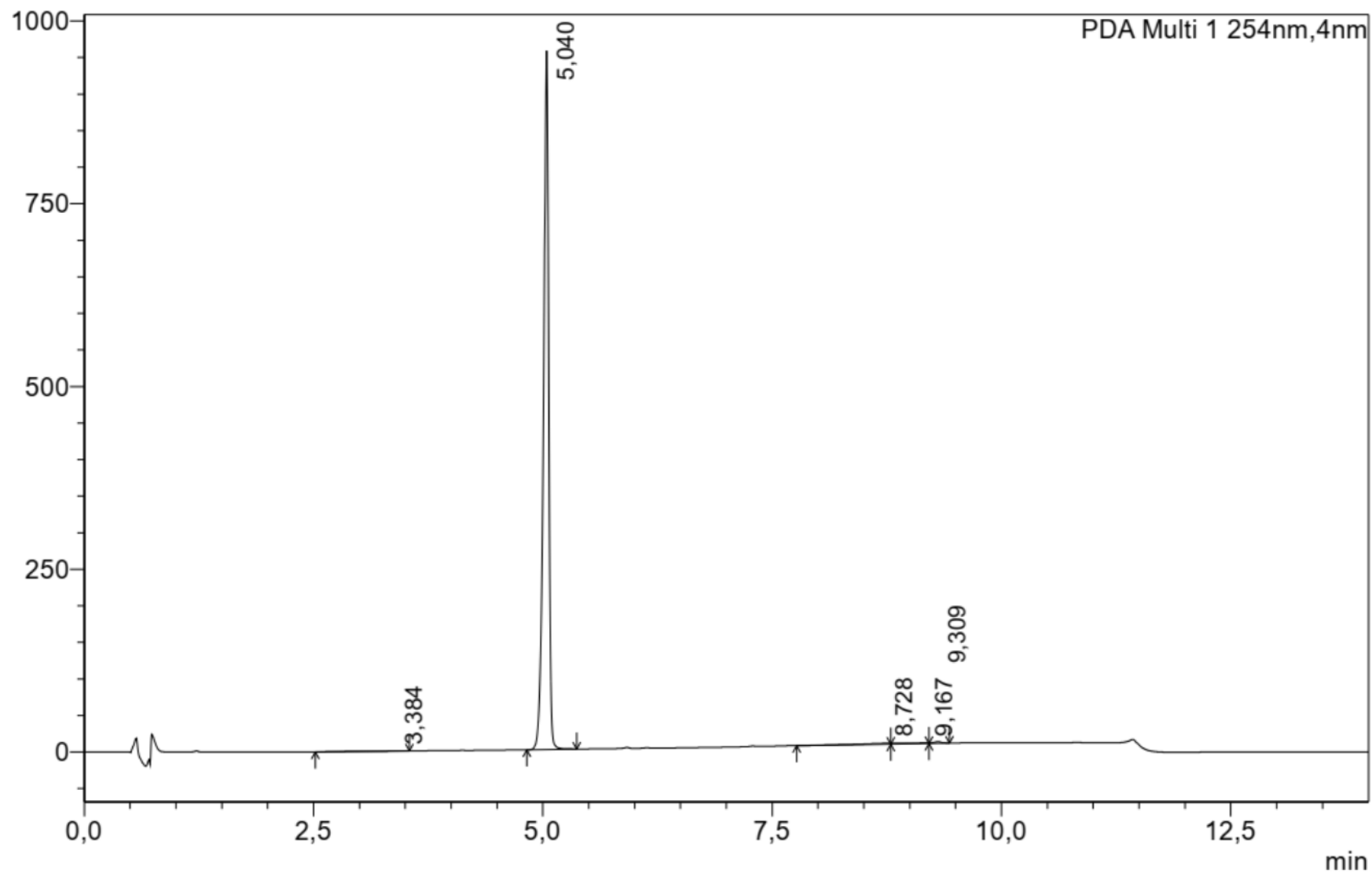


4-(Thiophen-2-yl)-7H-pyrrolo[2,3-d]pyrimidine (HPLC) **7k**:

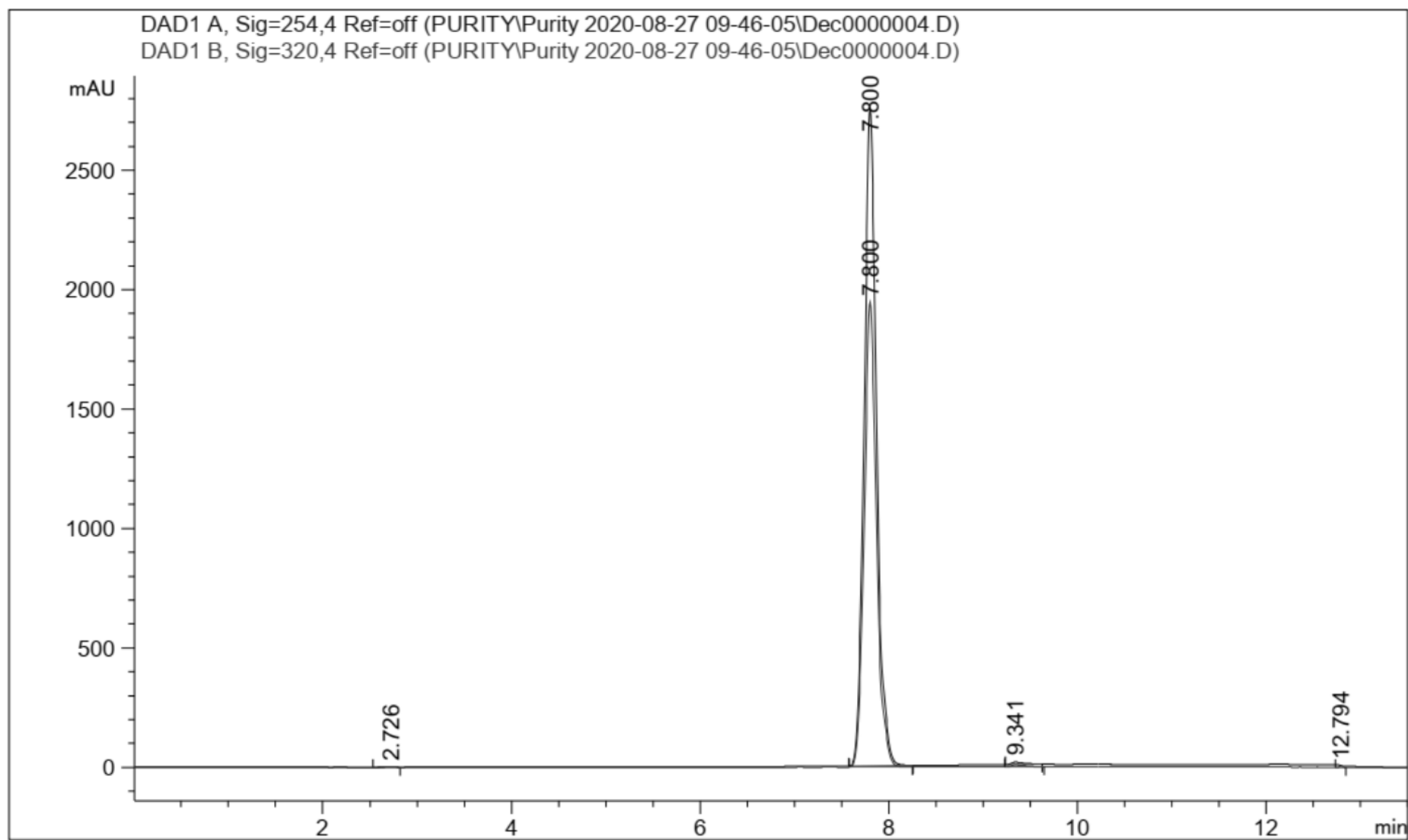


4-(1-Benzothiophen-2-yl)-7*H*-pyrrolo[2,3-*d*]pyrimidine (HPLC) 7I:

mAU

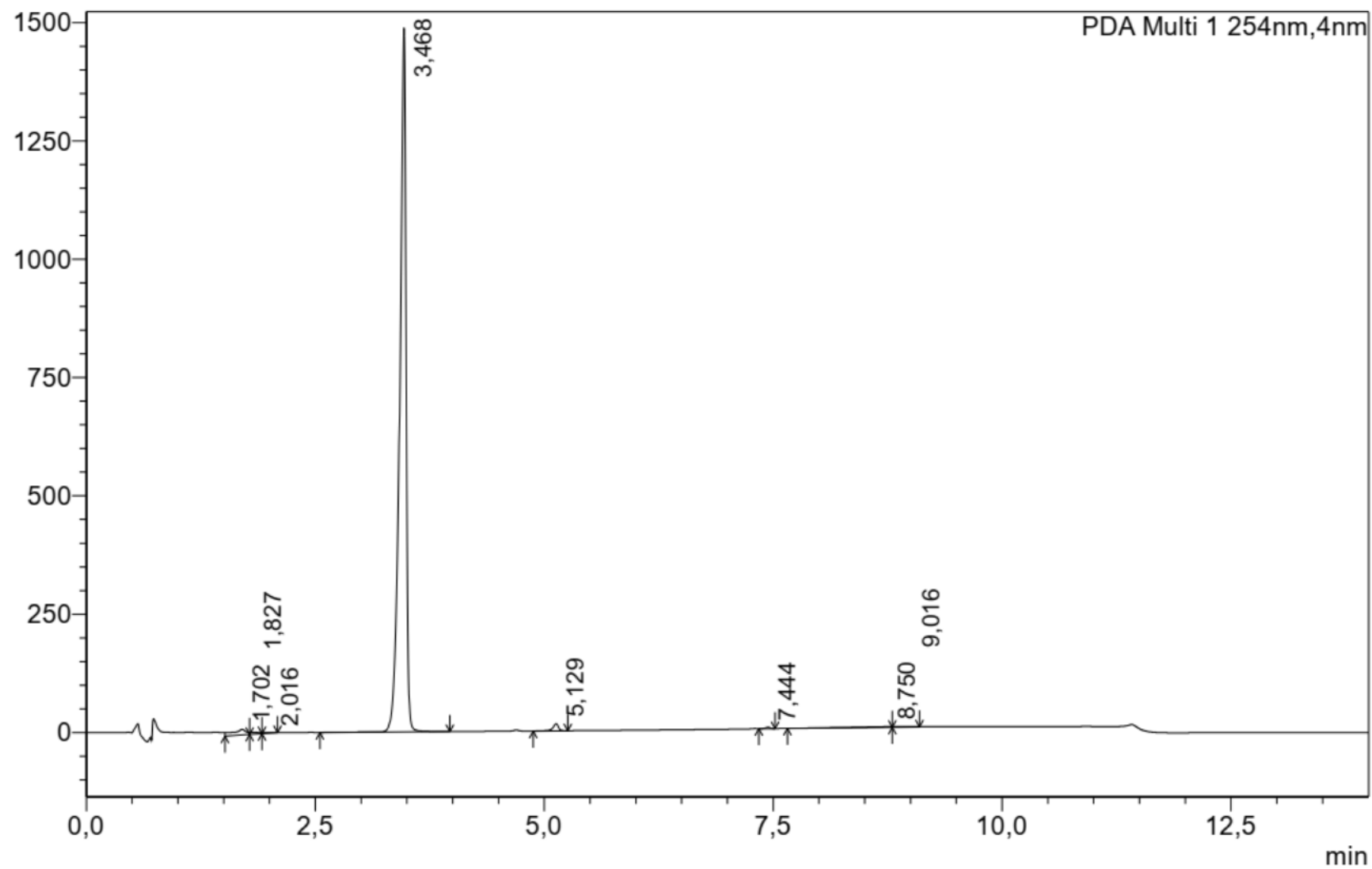


4-(4-Fluorophenyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine (HPLC) **7m**:



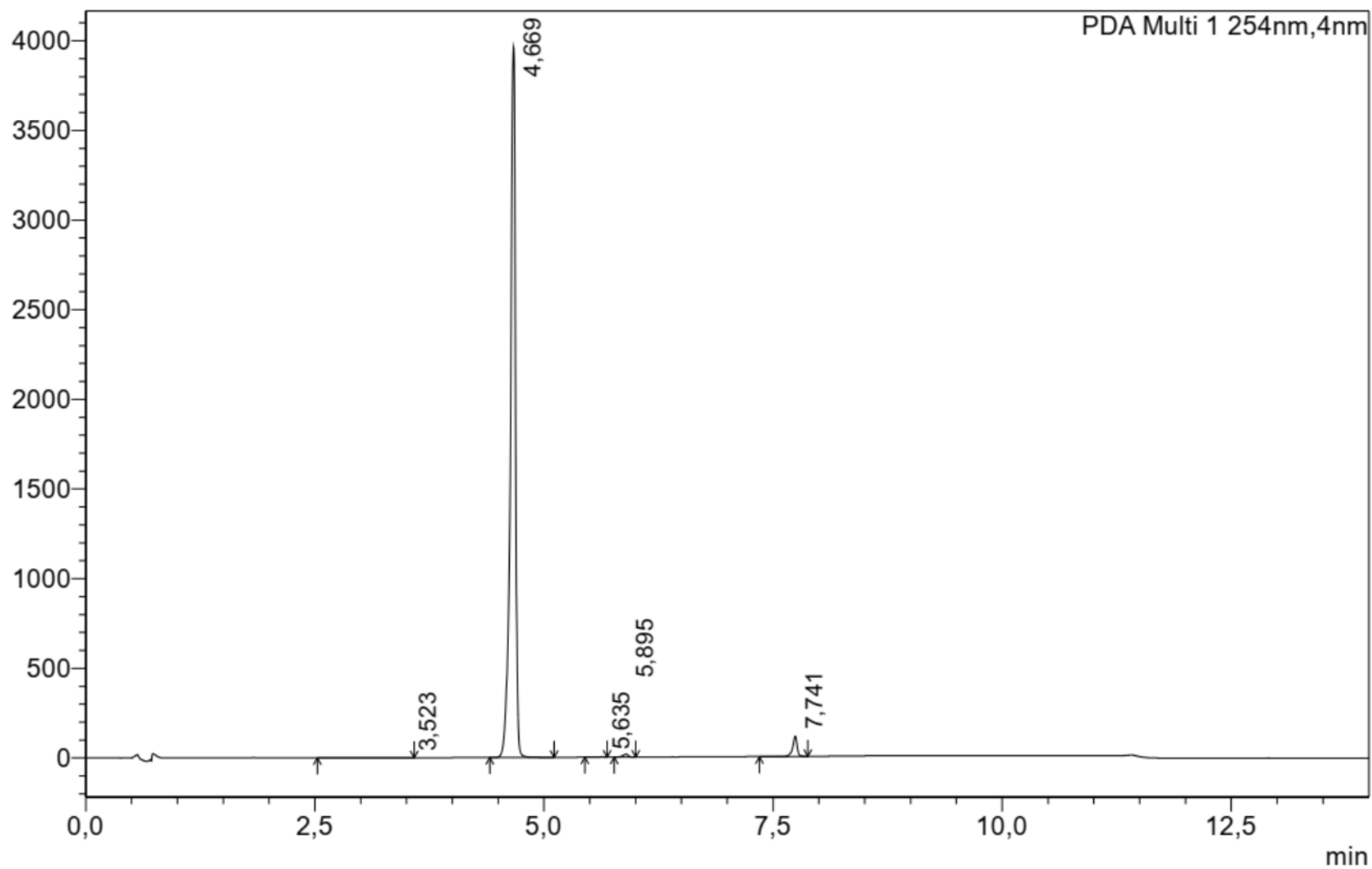
4-(3,4-Difluorophenyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine (HPLC) **7n**:

mAU



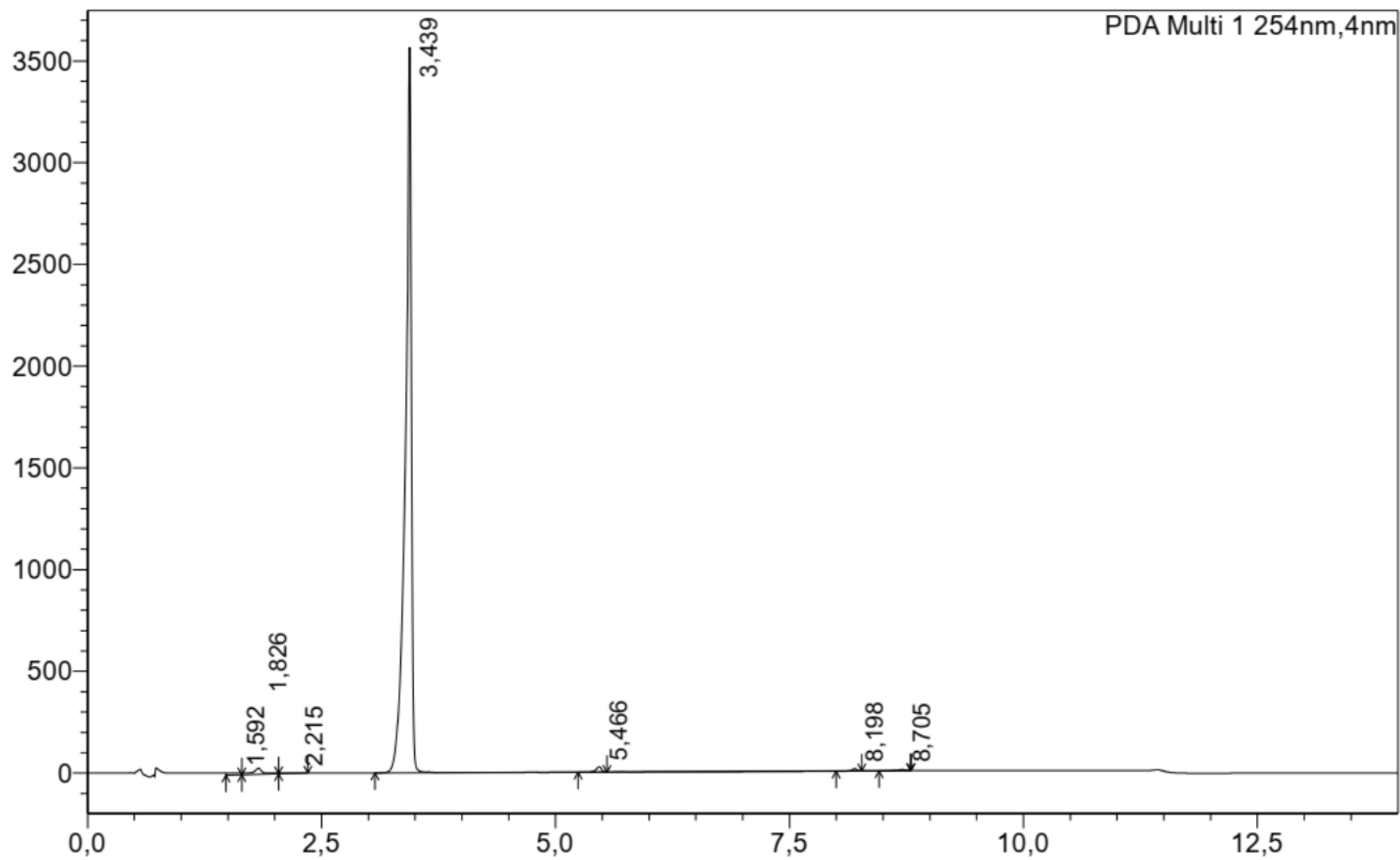
4-[4-(Trifluoromethyl)phenyl]-7*H*-pyrrolo[2,3-*d*]pyrimidine (HPLC) **7o**:

mAU



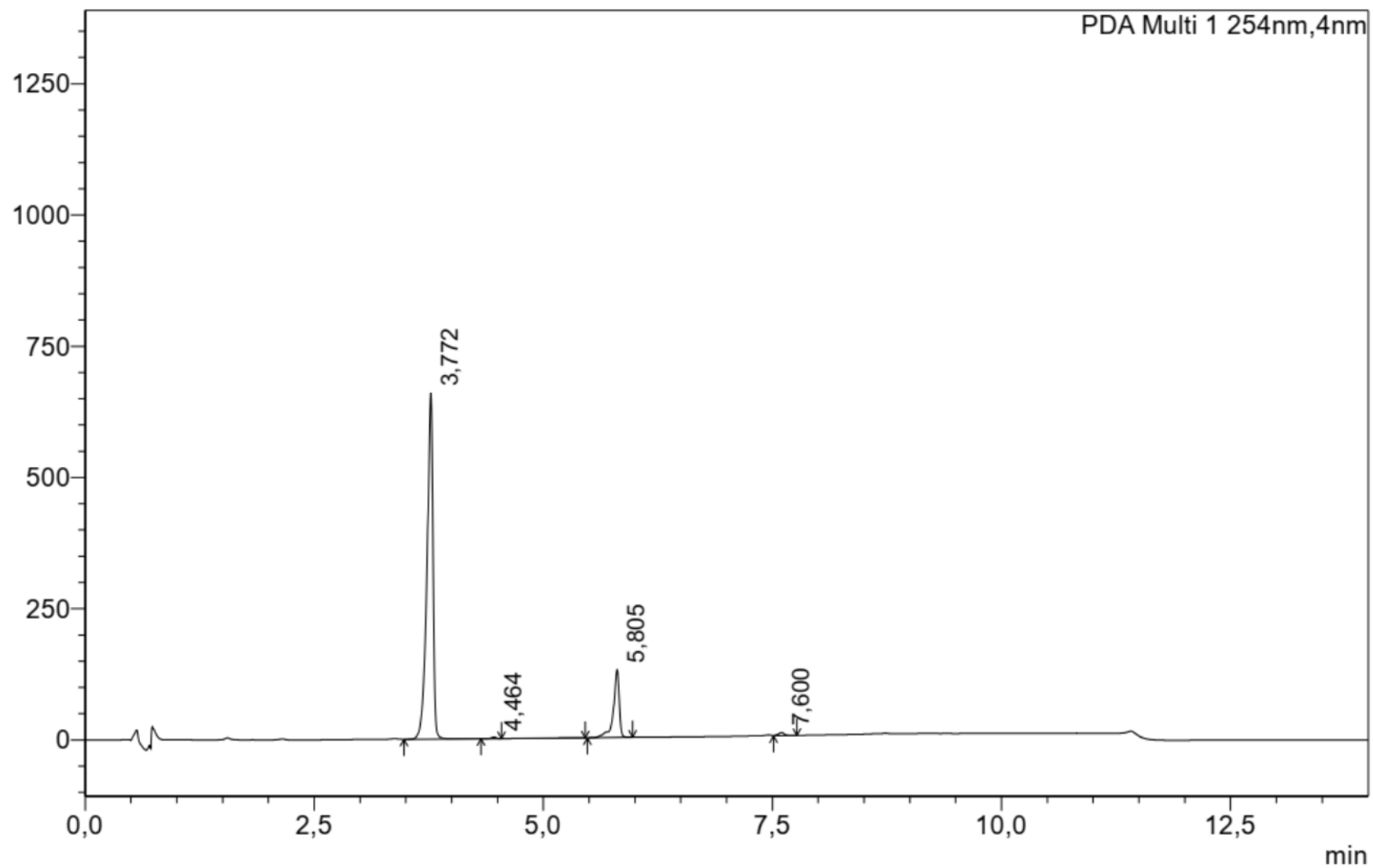
4-(4-Chlorophenyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine (HPLC) **7p**:

mAU



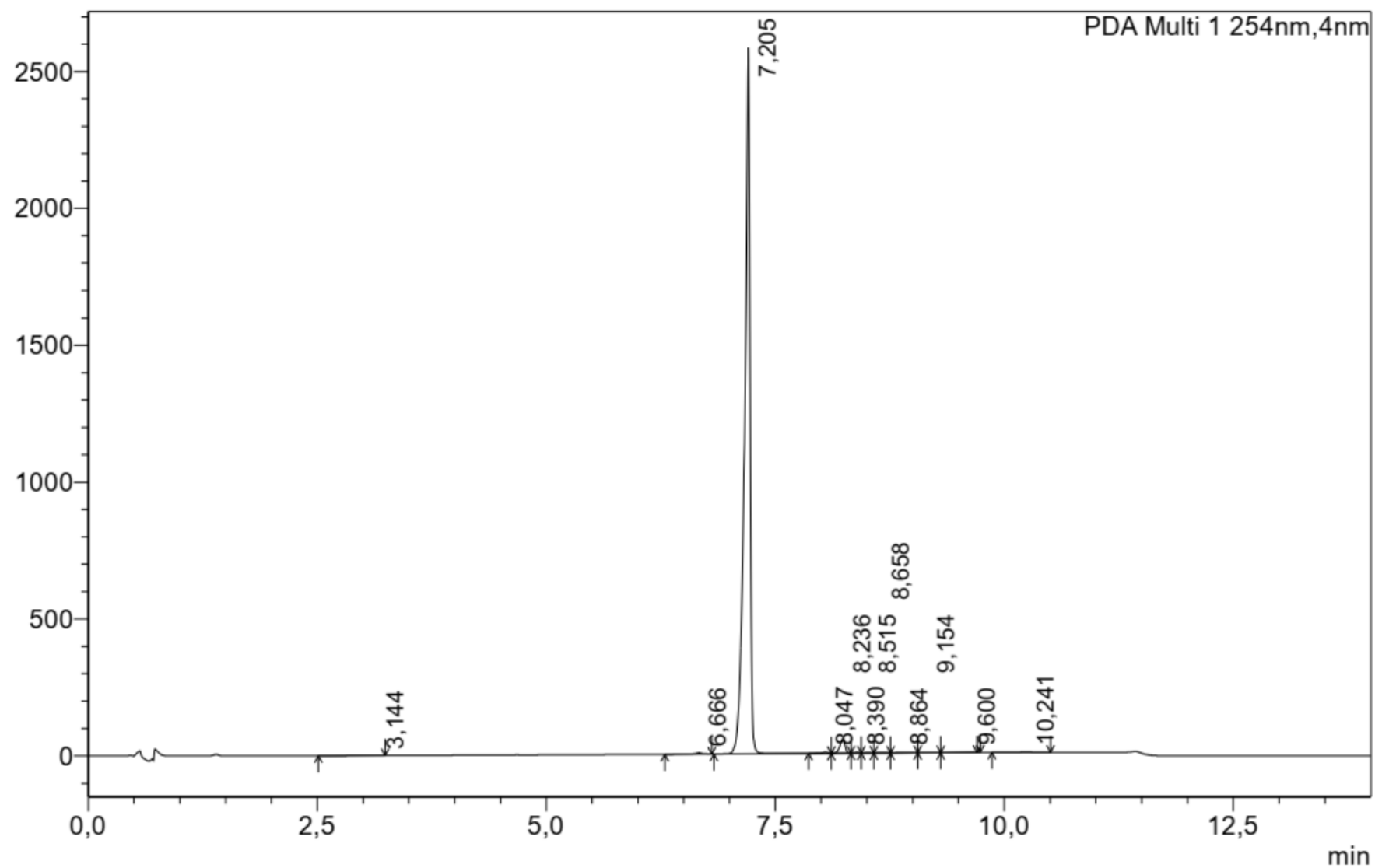
4-(4-Bromophenyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine (HPLC) **7q**:

mAU



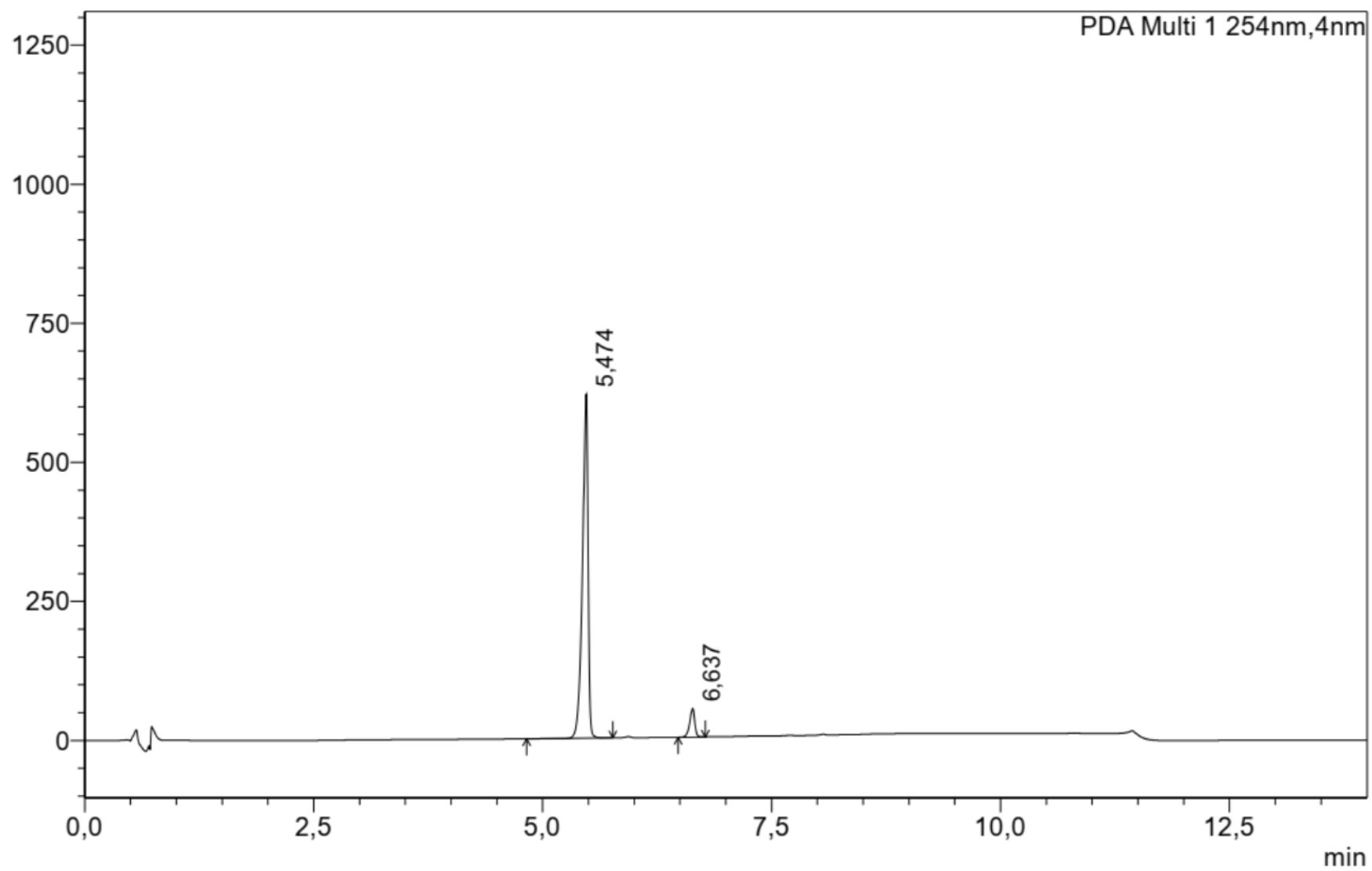
4,7-Bis(4-methylphenyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine (HPLC) **8a**:

mAU



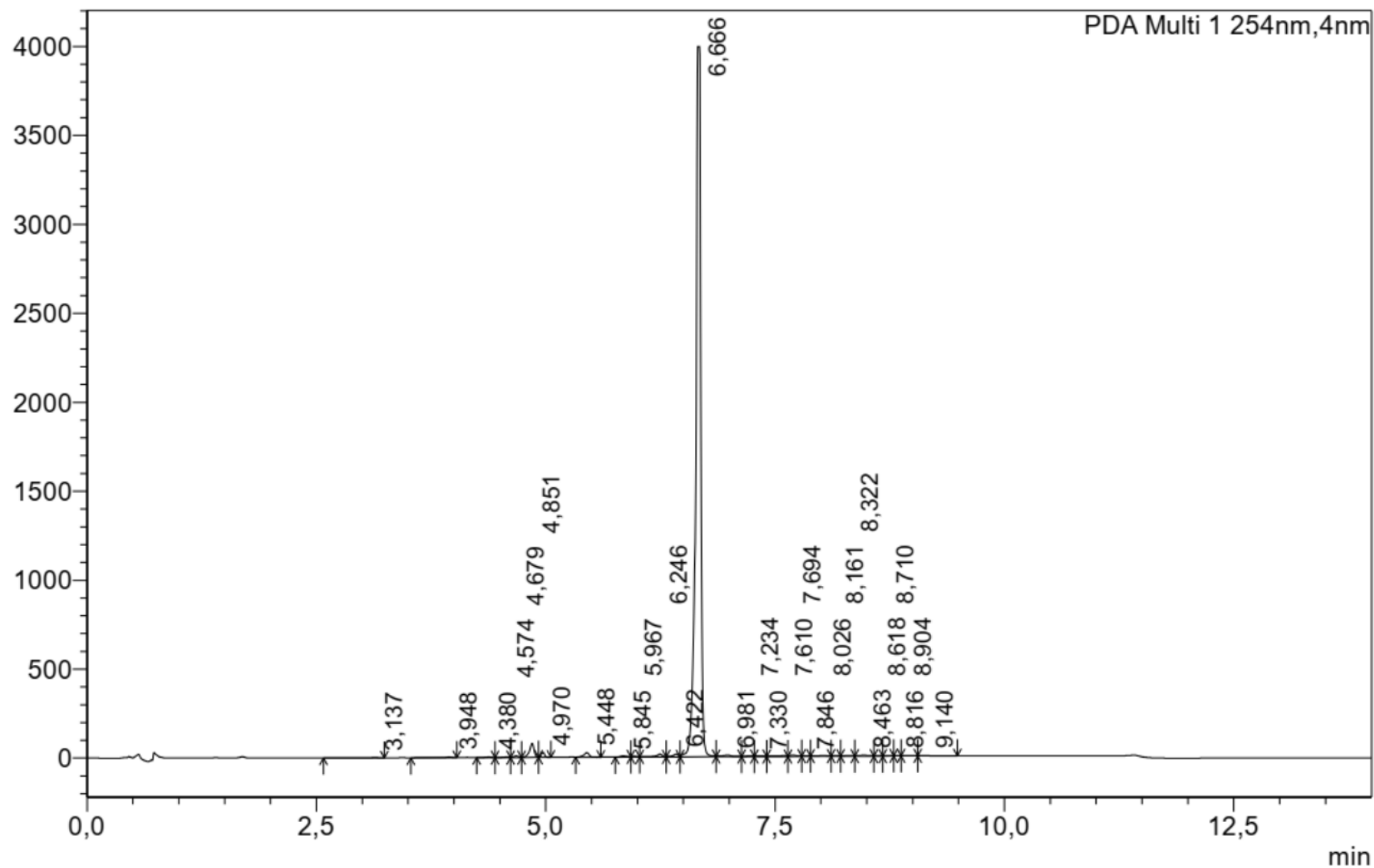
4,7-Bis(4-methoxyphenyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine (HPLC) **8b**:

mAU

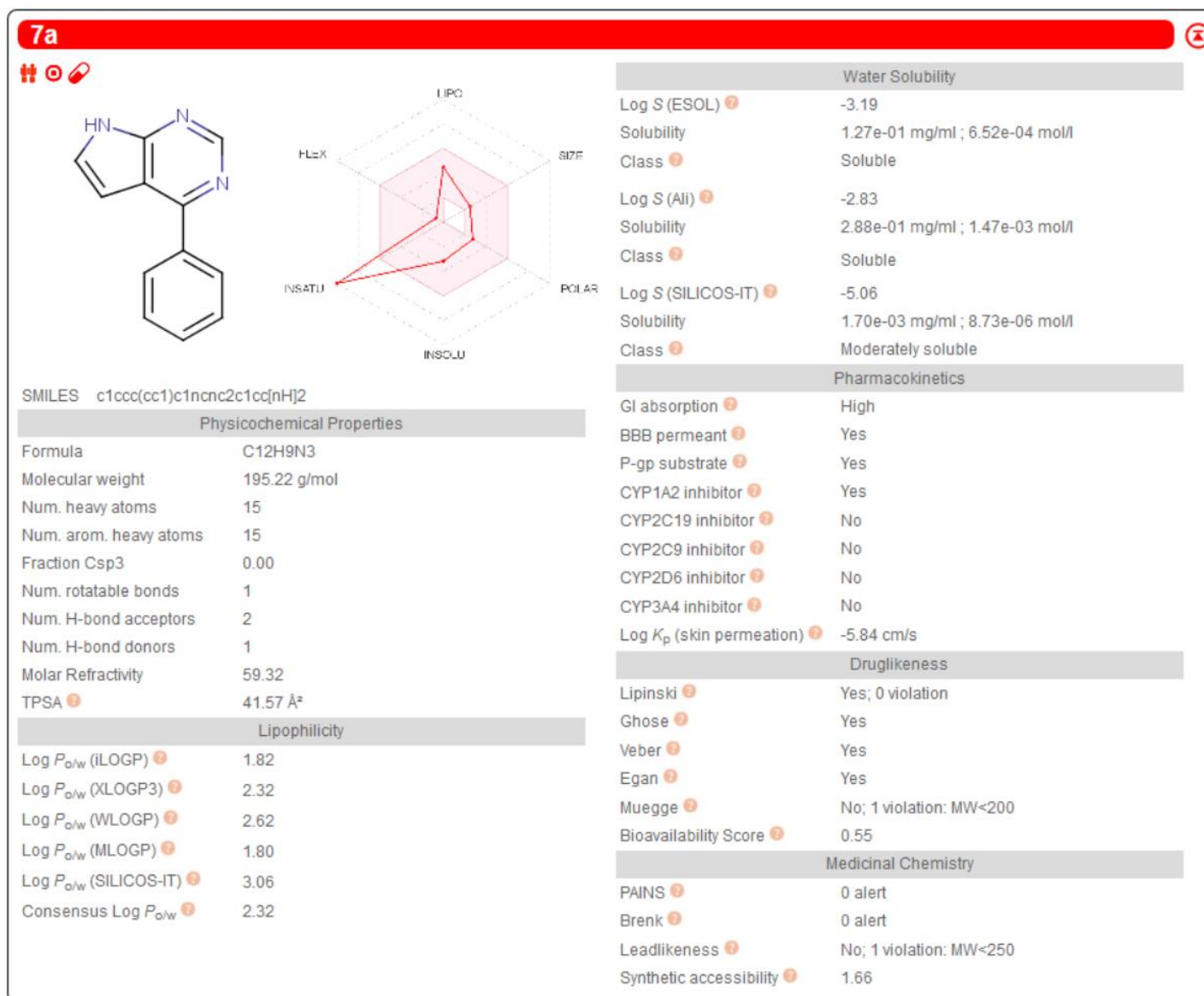


4,7-Bis(4-fluorophenyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine (HPLC) **8c**:

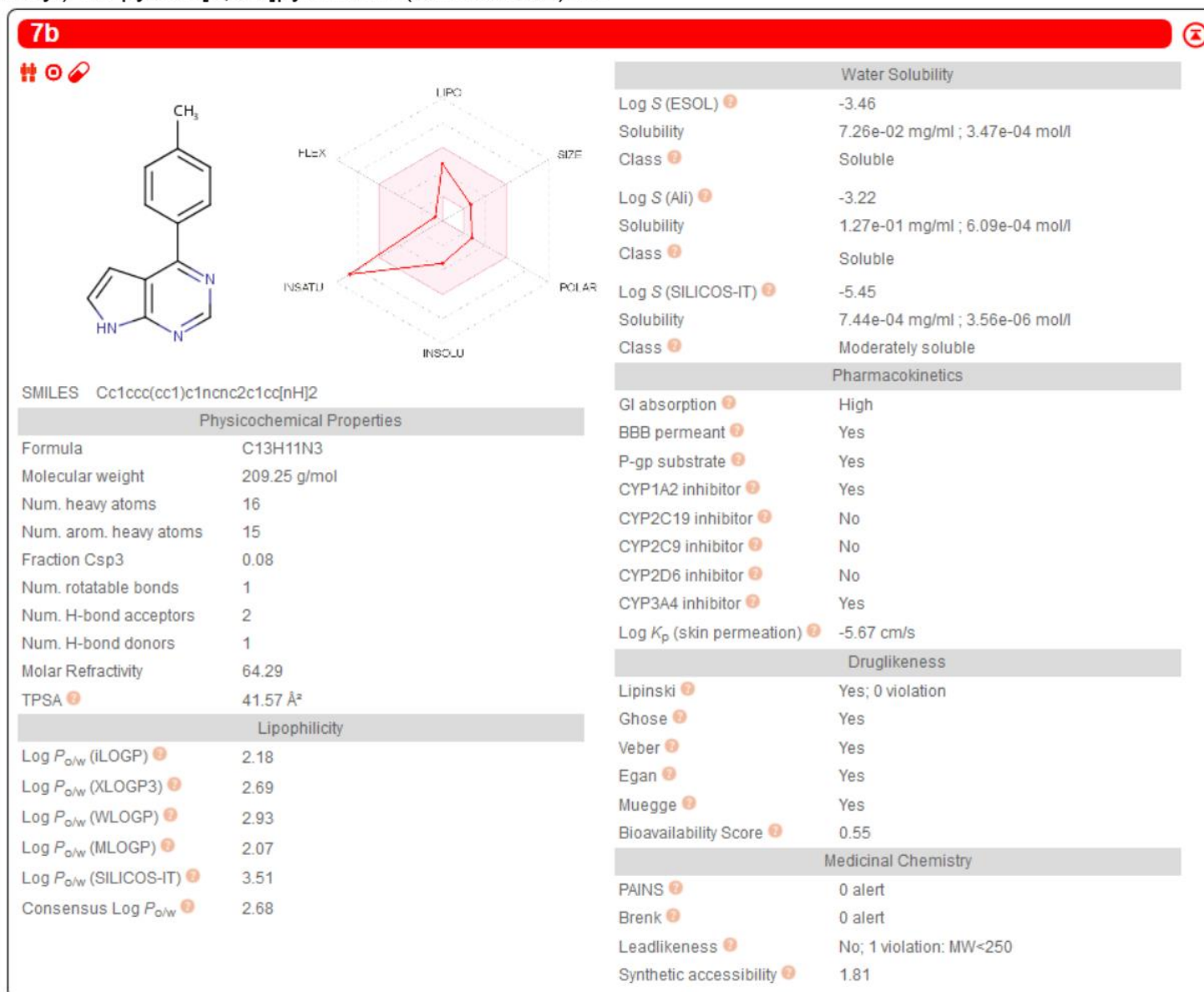
mAU



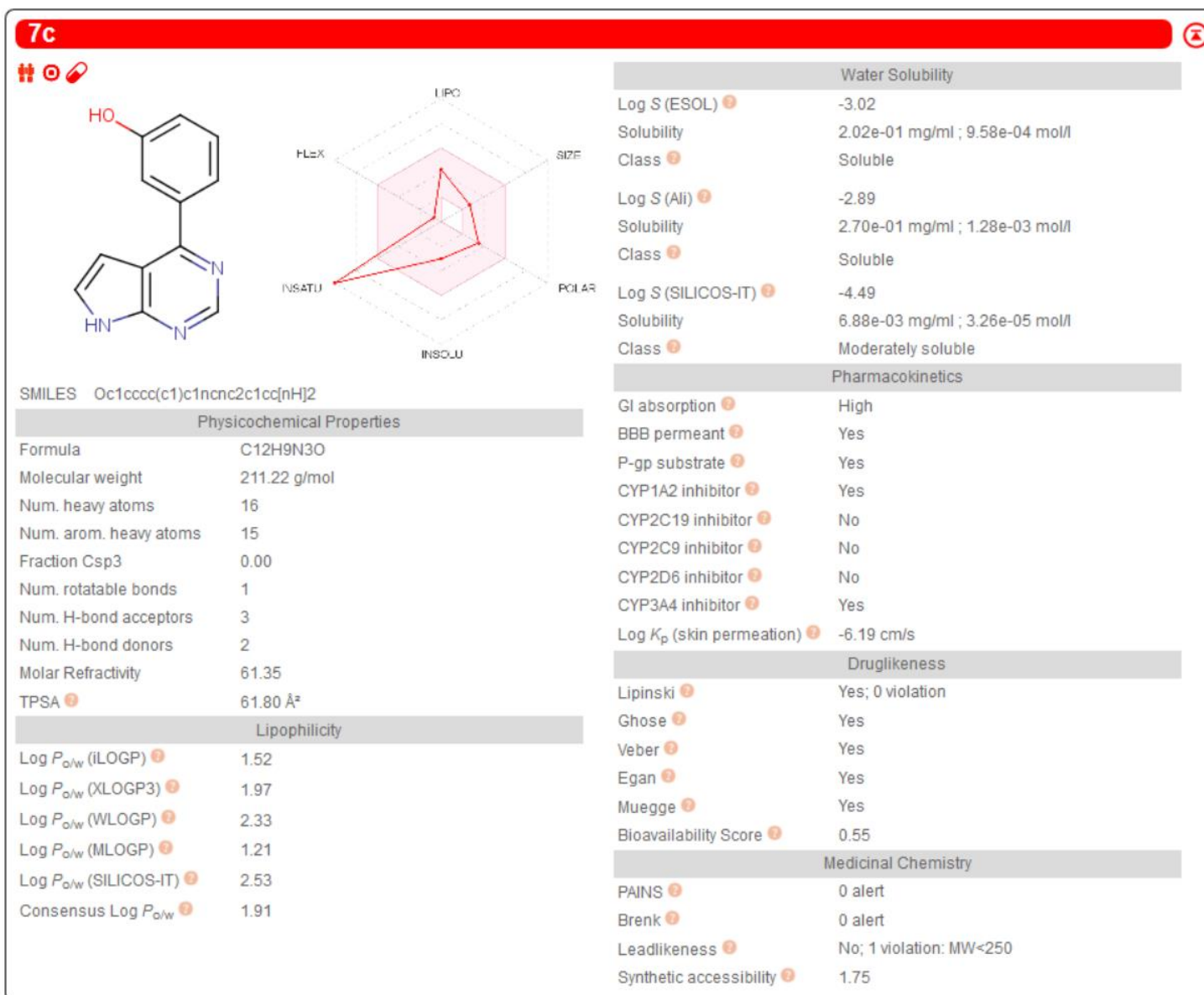
4-Phenyl-7H-pyrrolo[2,3-d]pyrimidine (SwissADME) **7a**:



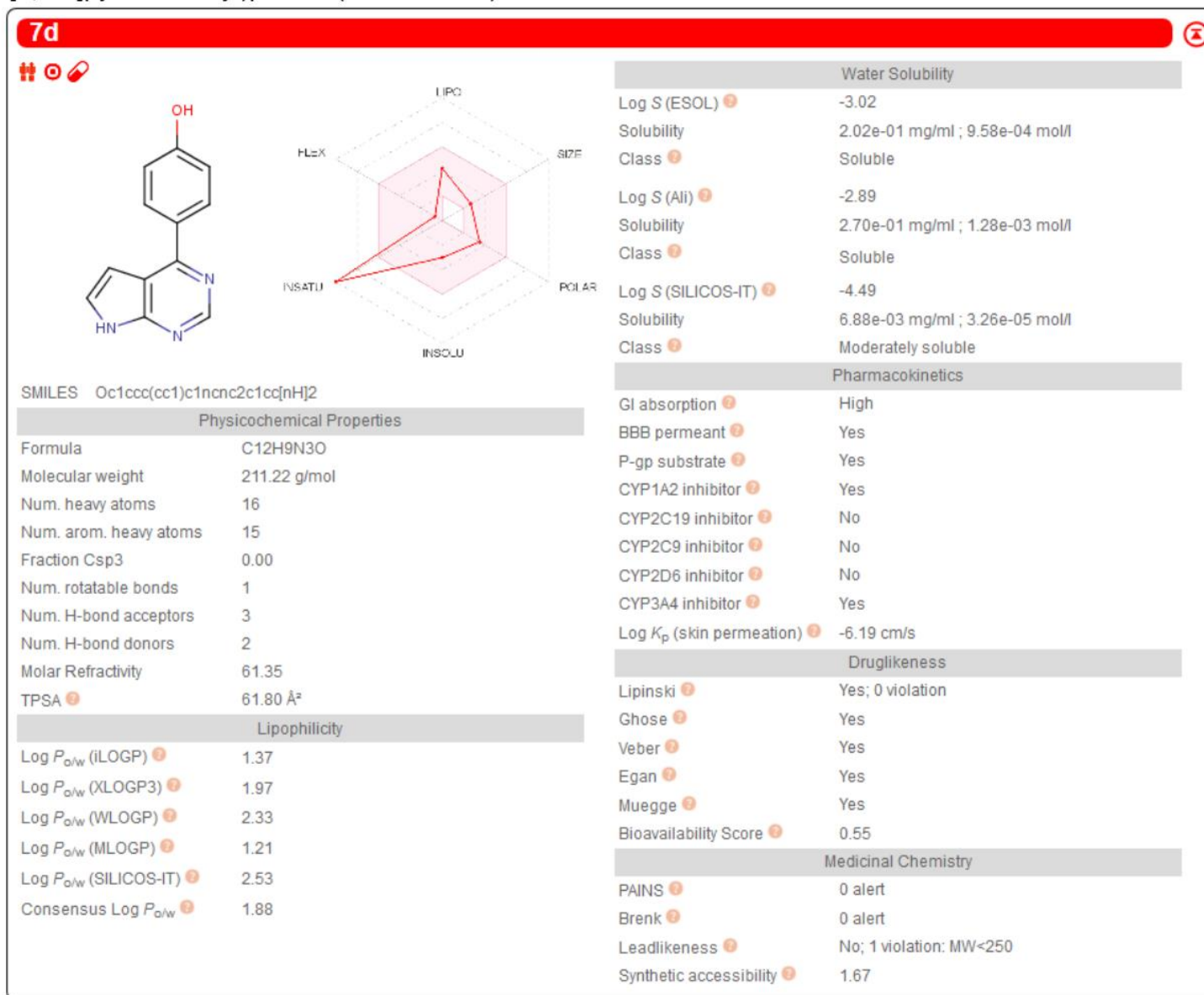
4-(4-Methylphenyl)-7H-pyrrolo[2,3-d]pyrimidine (SwissADME) **7b**:



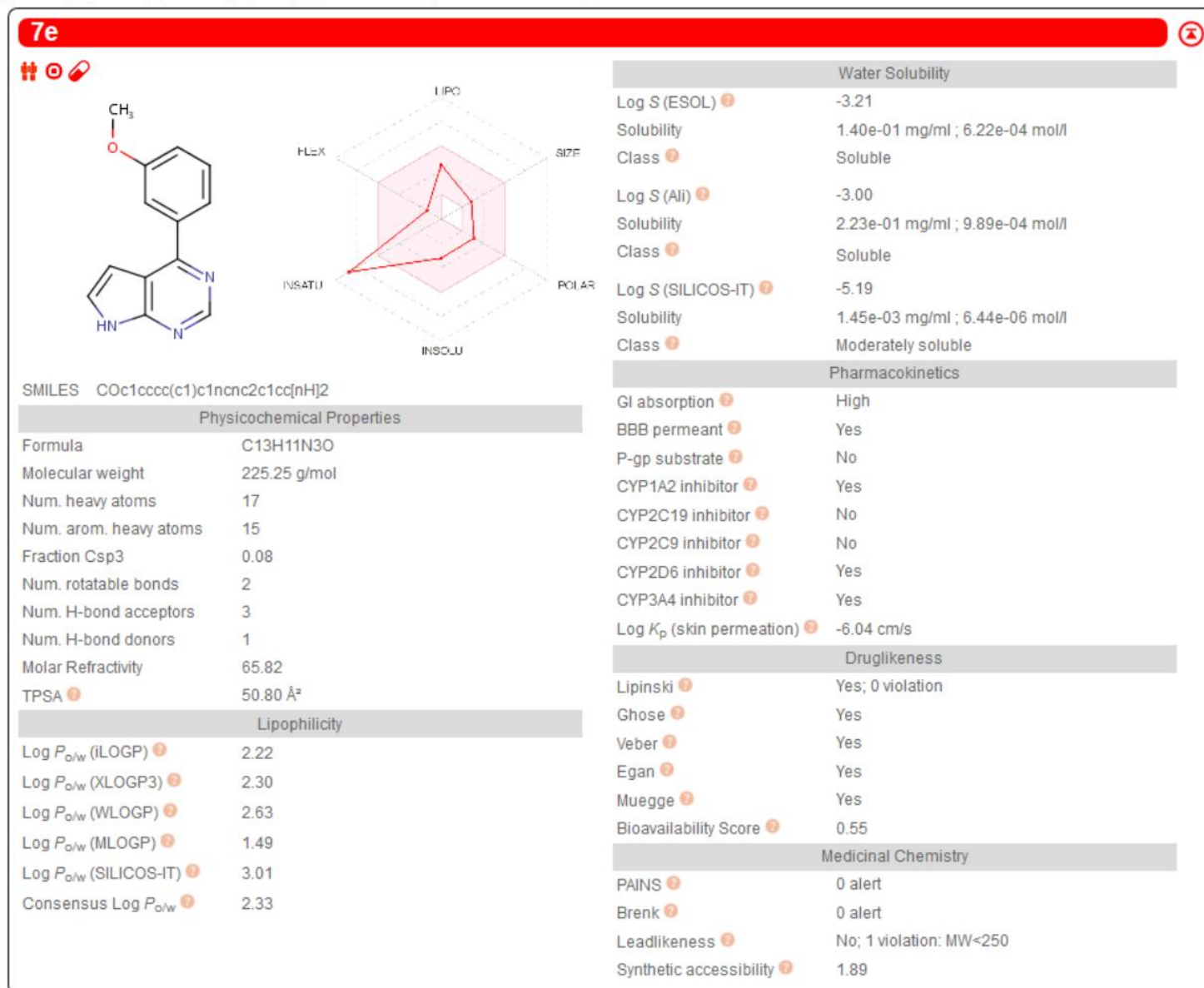
3-(7H-Pyrrolo[2,3-d]pyrimidin-4-yl)phenol (SwissADME) **7c**:



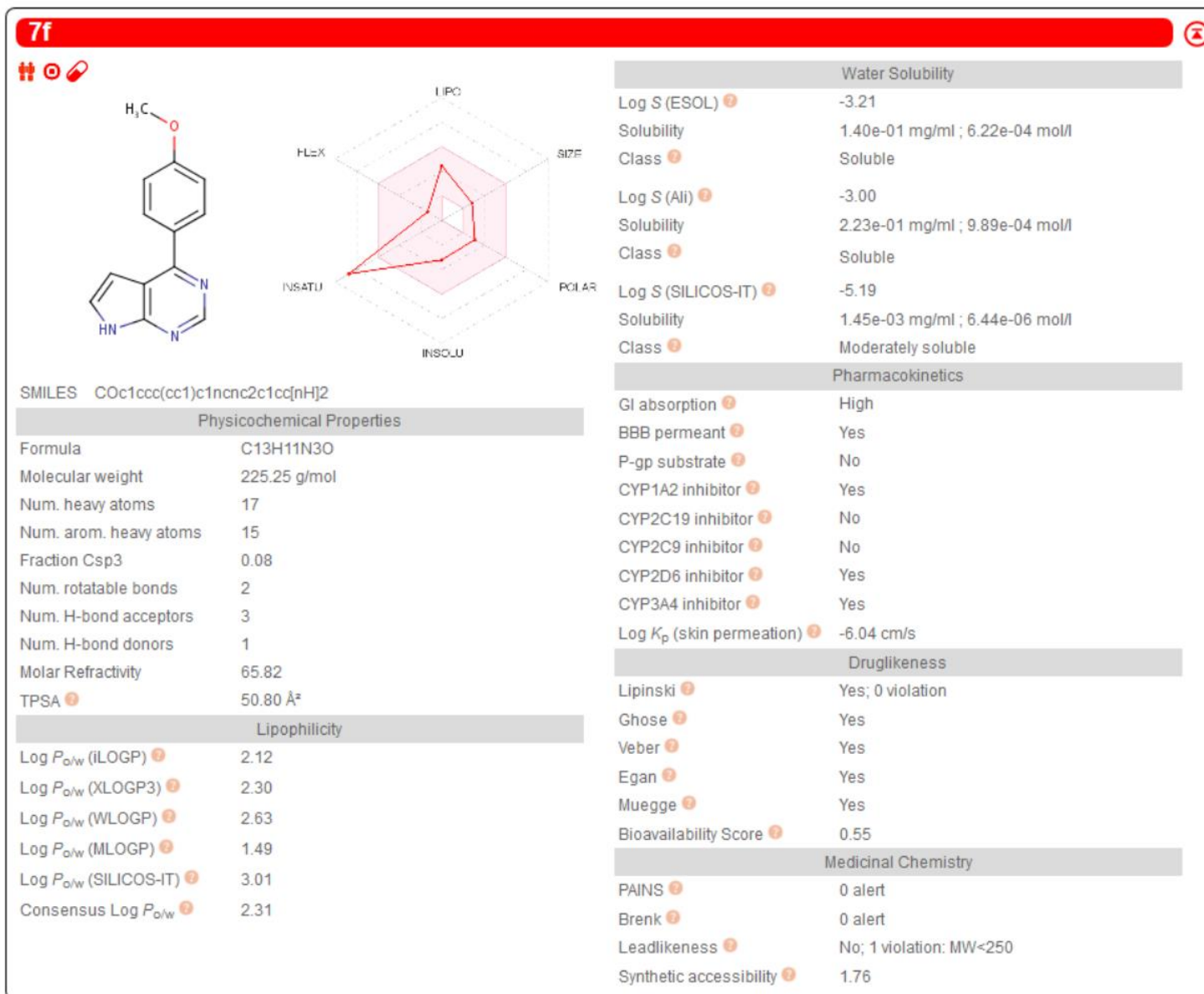
4-(7H-Pyrrolo[2,3-d]pyrimidin-4-yl)phenol (SwissADME) **7d**:



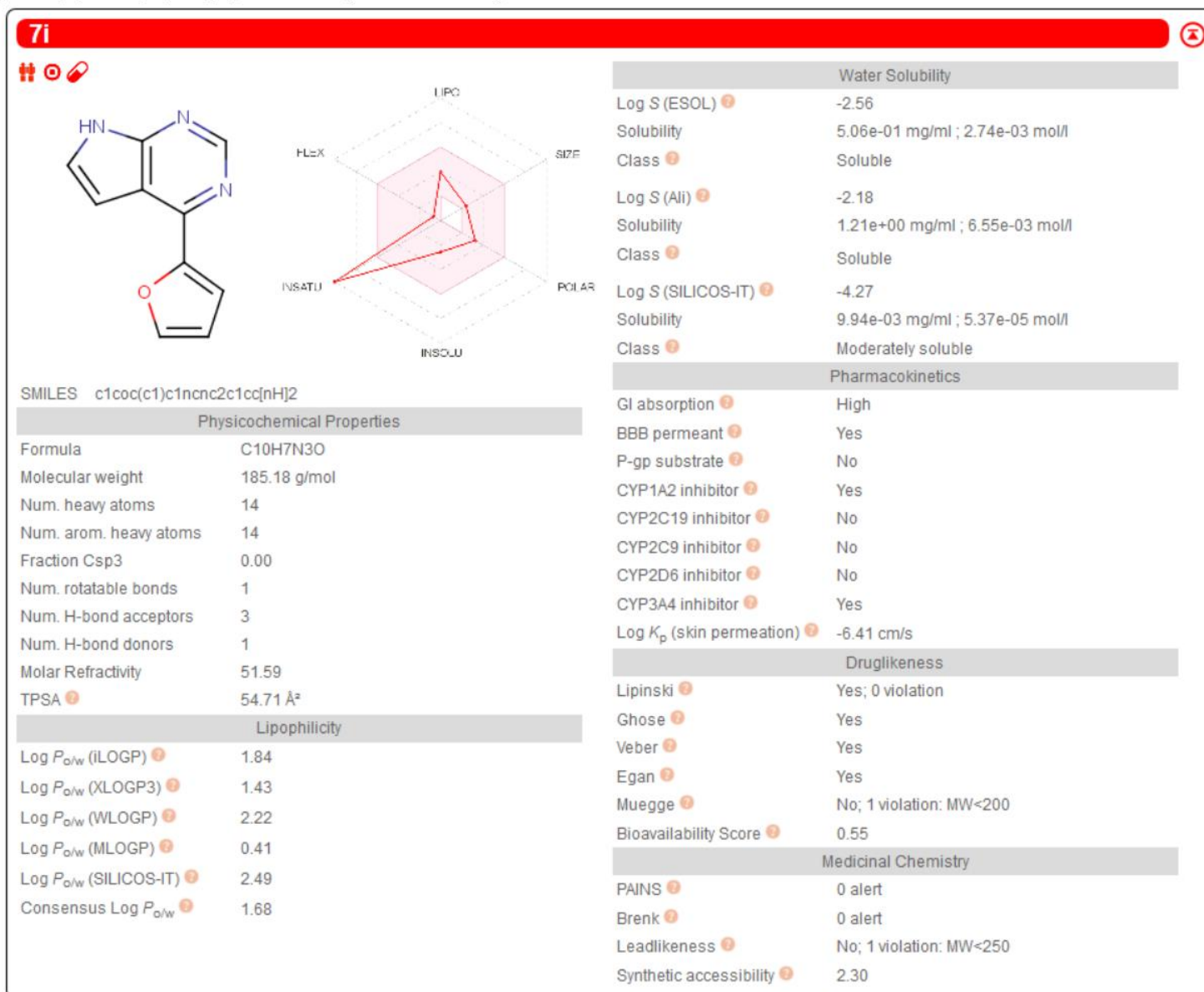
4-(3-Methoxyphenyl)-7H-pyrrolo[2,3-d]pyrimidine (SwissADME) **7e**:



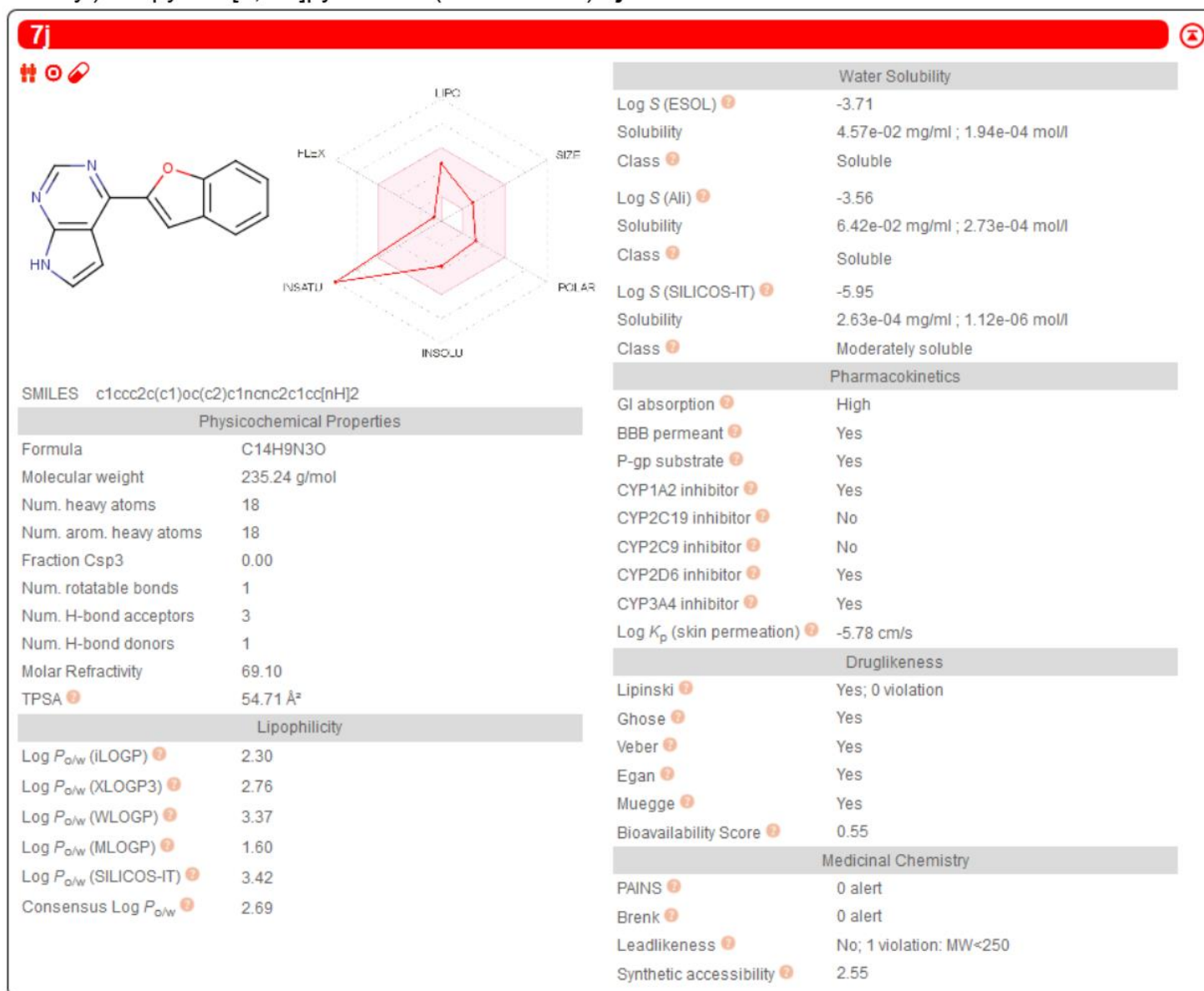
4-(4-Methoxyphenyl)-7H-pyrrolo[2,3-d]pyrimidine (SwissADME) **7f**:



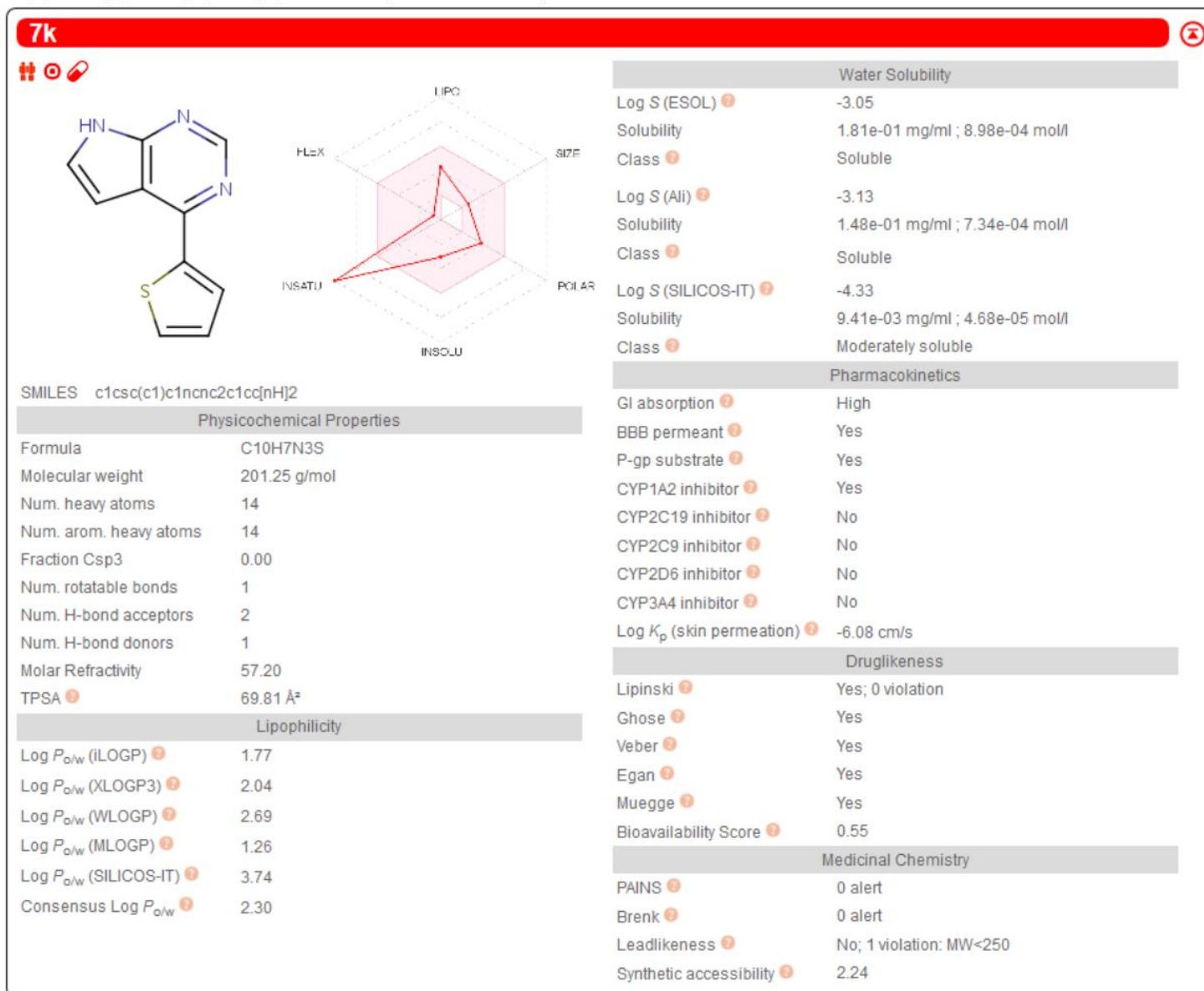
4-(Furan-2-yl)-7H-pyrrolo[2,3-d]pyrimidine (SwissADME) **7i**:



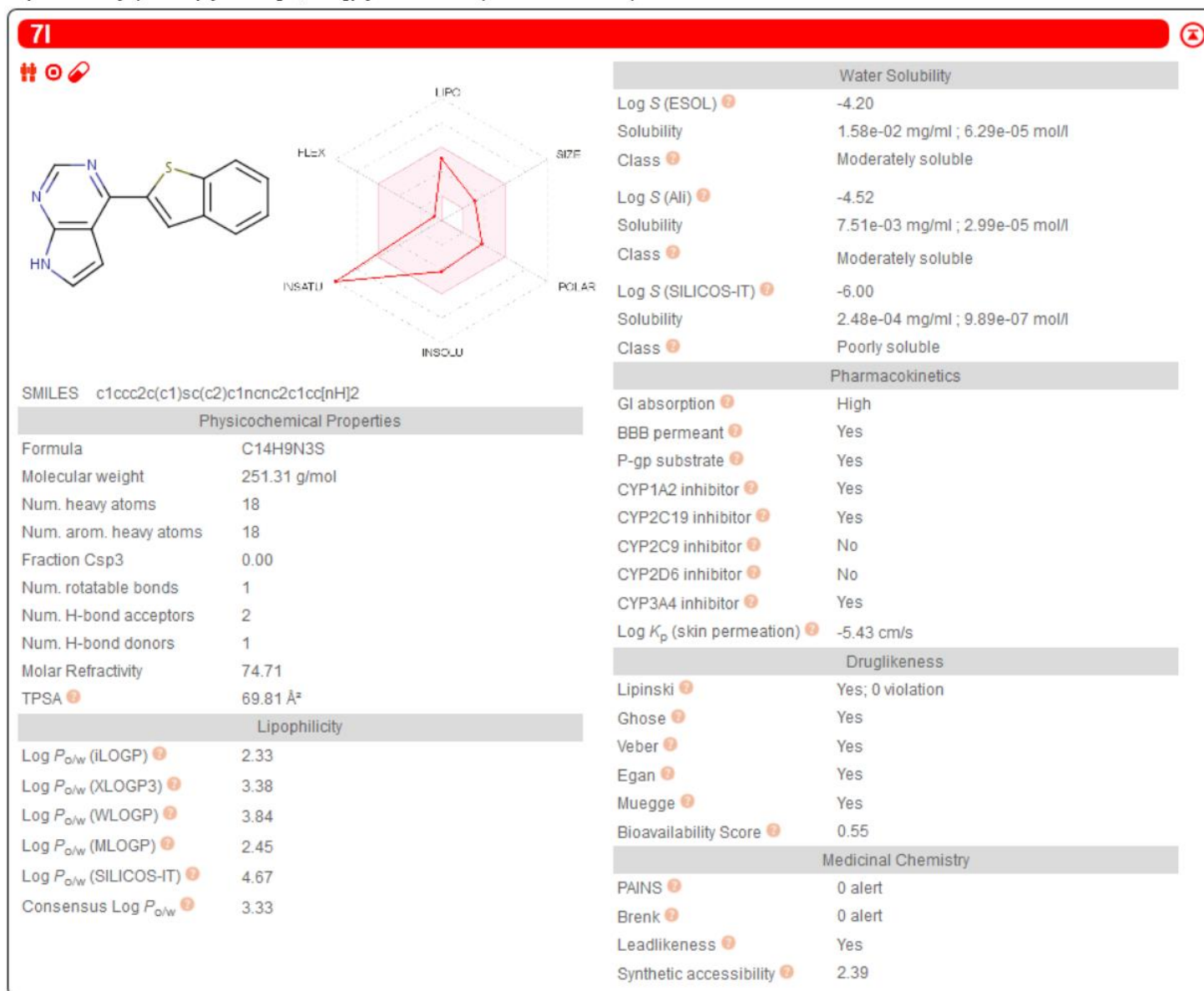
4-(1-Benzofuran-2-yl)-7H-pyrrolo[2,3-d]pyrimidine (SwissADME) 7j:



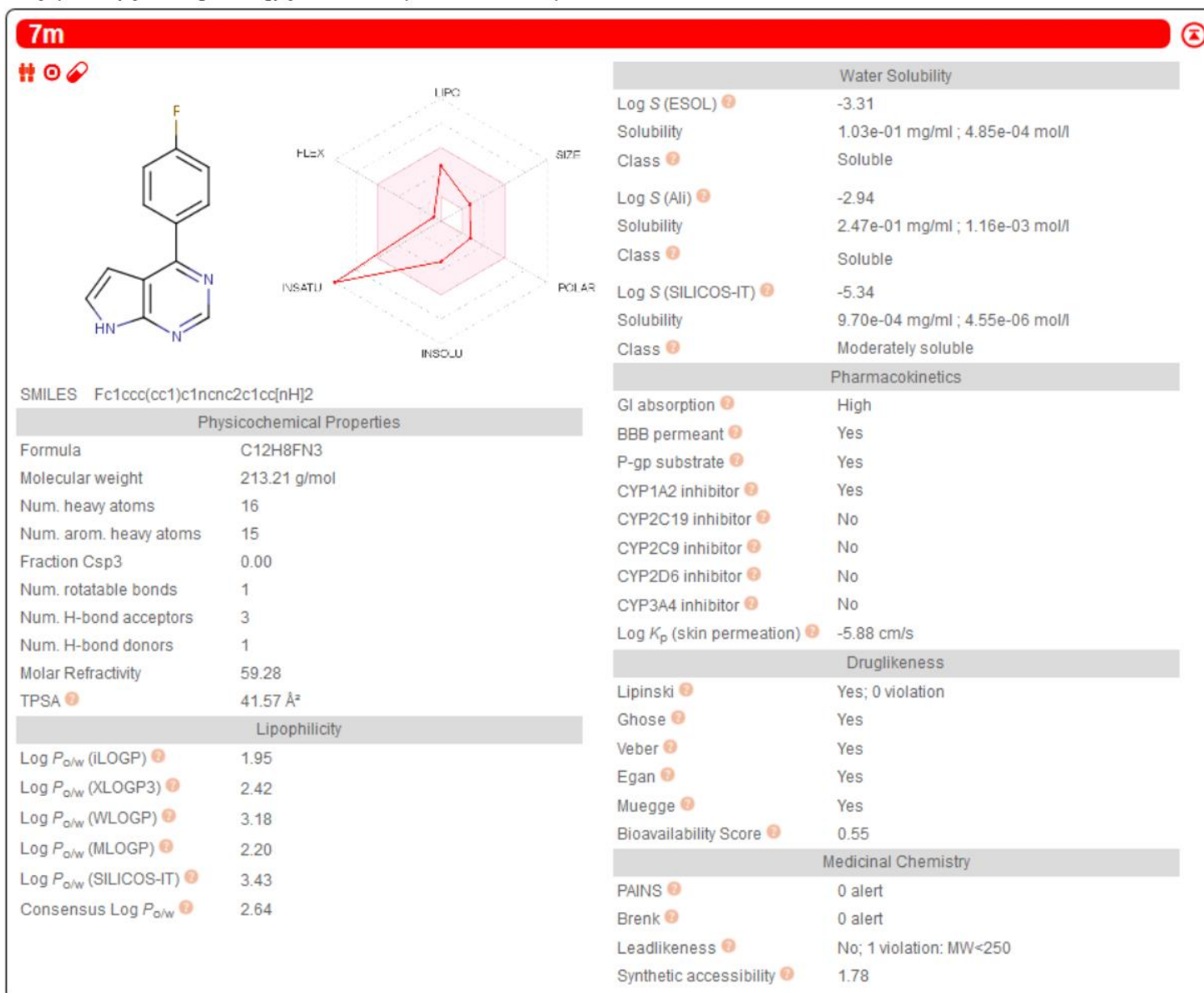
4-(Thiophen-2-yl)-7H-pyrrolo[2,3-d]pyrimidine (SwissADME) **7k**:



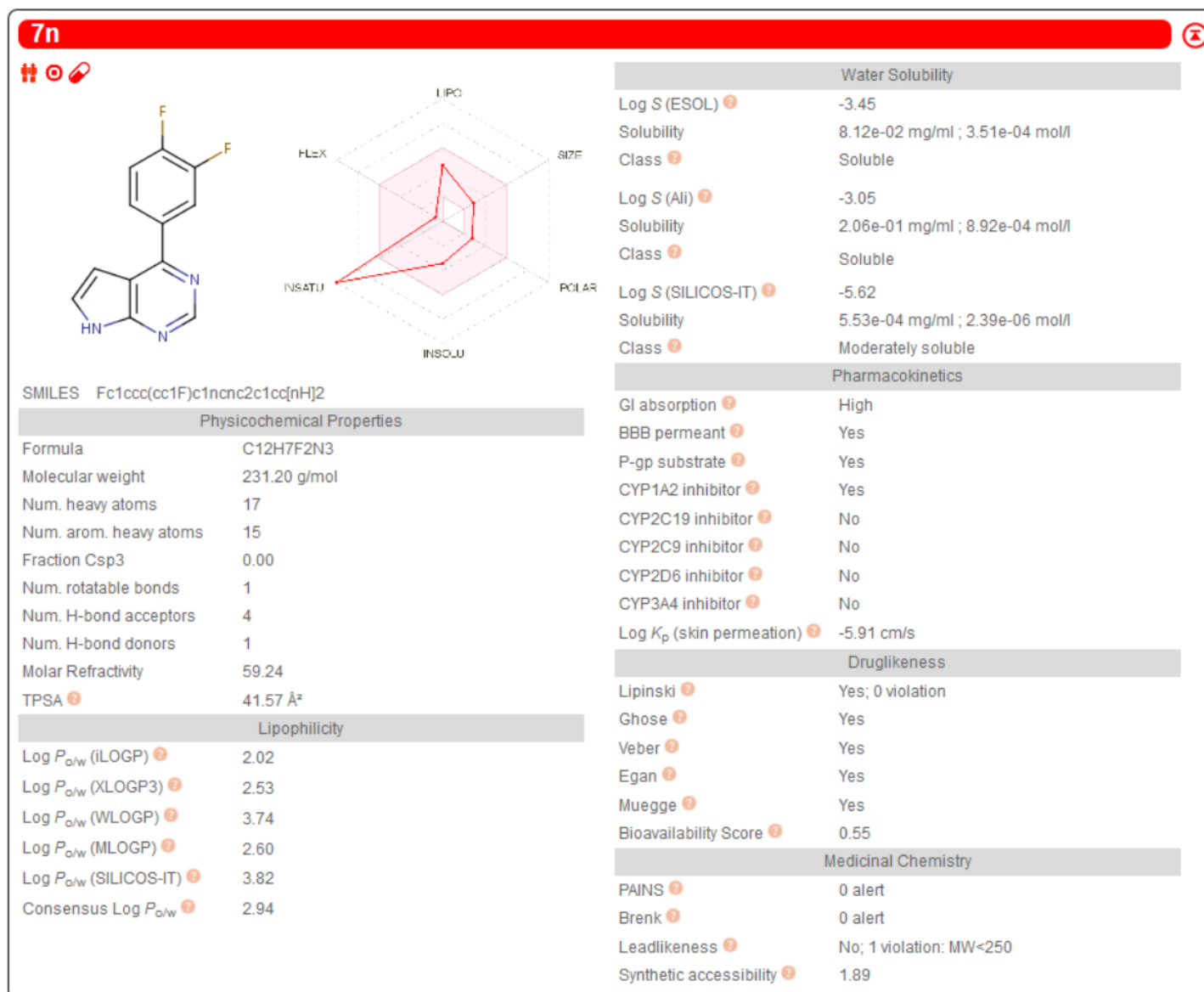
4-(1-Benzothiophen-2-yl)-7H-pyrrolo[2,3-d]pyrimidine (SwissADME) **71**:



4-(4-Fluorophenyl)-7H-pyrrolo[2,3-d]pyrimidine (SwissADME) 7m:



4-(3,4-Difluorophenyl)-7H-pyrrolo[2,3-d]pyrimidine (SwissADME) **7n**:



4-(4-Chlorophenyl)-7H-pyrrolo[2,3-d]pyrimidine (SwissADME) **7p**:

