Chapter 5

Article 3

Inhibition of monoamine oxidase by phthalide analogues

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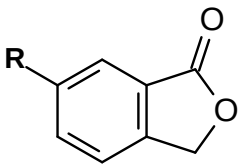
Graphical abstract:



		MAO-A	MAO-B
	R	IC_{50} (μM)	IC_{50} (μM)
6b	4-$ClC_6H_4(CH_2)O$–	0.172	0.0028
6m	$C_6H_5(CH_2)_3O$–	0.096	0.0062
6o	4-$ClC_6H_4O(CH_2)_2O$–	0.137	0.011
6r	$C_6H_{11}CH_2O$–	0.185	0.012

Phthalides as highly potent MAO-A/B inhibitors

Inhibition of monoamine oxidase by phthalide analogues

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Abstract: Based on recent reports that the small molecules, isatin and phthalimide, are suitable scaffolds for the design of high potency monoamine oxidase (MAO) inhibitors, the present study examines the MAO inhibitory properties of a series of phthalide [2-benzofuran-1(*3H*)-one] analogues. Phthalide is structurally related to isatin and phthalimide and it is demonstrated here that substitution at C6 of the phthalide moiety yields compounds endowed with high binding affinities to both human MAO isoforms. Among the nineteen homologues evaluated, the lowest IC_{50} values recorded for the inhibition of MAO-A and –B were 0.096 µM and 0.0014 µM, respectively. In most instances, C6-substituted phthalides exhibit MAO-B specific inhibition. The results also show that the binding modes of representative phthalides are reversible and competitive at both MAO isoforms. Based on these data, C6-substituted phthalides may serve as a lead for the development of therapies for neurodegenerative disorders such as Parkinson's disease.

Keywords: phthalide; 2-benzofuran-1(*3H*)-one; monoamine oxidase; MAO-B; reversible inhibition; structure-activity relationship.

The monoamine oxidases (MAOs) are flavin adenine dinucleotide (FAD) containing enzymes which catalyze the α-carbon oxidation of biogenic and xenobiotic amines.[1] Since the principal function of the MAOs in the central nervous system is to terminate the actions of neurotransmitter amines, they are considered drug targets for the treatment of psychiatric and neurological disorders.[2] MAO-A metabolizes serotonin and MAO-A inhibitors have been used in the treatment of anxiety disorders and depressive illness.[3] MAO-B is considered to be a major dopamine metabolizing enzyme and MAO-B inhibitors are employed in the symptomatic therapy of Parkinson's disease.[2,4,5] In Parkinson's disease, MAO-B inhibitors conserve depleted dopamine stores and prolong the physiological action of dopamine. MAO-B inhibitors also may enhance dopamine levels derived from levodopa, the metabolic precursor of dopamine.[6,7] In addition to a symptomatic benefit, MAO-B inhibitors may also exert a neuroprotective effect by blocking the formation of H_2O_2 and aldehydic species, metabolic by-products of MAO-B-catalyzed substrate metabolism.[1] Considering that central MAO-B activity increases with age,[8–10] inhibition of the MAO-B-catalyzed formation of these potentially harmful species in the aged parkinsonian brain is of particular significance.

It is noteworthy that dopamine is also metabolized by MAO-A in the human brain, and MAO-A inhibitors have been reported to enhance central dopamine levels in primates.[6,7] Non-selective MAO inhibition may therefore represent an attractive strategy to enhance central dopamine levels in Parkinson's disease. In addition, the inhibition of MAO-A may also alleviate depression which is frequently associated with Parkinson's disease.[1,11] MAO-A inhibitors may, however, lead to serious adverse effects when combined with dietary tyramine. Inhibition of the MAO-A-catalyzed metabolism of tyramine in the intestinal endothelial cells results in excessive amounts of tyramine entering the systemic circulation. Tyramine, an indirectly acting sympathomimetic amine, initiates the release of noradrenaline from peripheral adrenergic neurons and as a consequence a severe hypertensive response, which may be fatal, may occur.[1,12] Because of this, the clinical use of MAO-A inhibitors has been limited. Recently developed reversible inhibitors of MAO-A, such as moclobemide, however, are considered safer than irreversible MAO-A inhibitors. For example, moclobemide is essentially free from the tyramine reaction while retaining

its antidepressant efficacy.[13] This suggests that non-selective MAO inhibitors for Parkinson's disease therapy should preferably act reversibly.

Based on these considerations, the present study examines the possibility that a series of phthalide [2-benzofuran-1(*3H*)-one] analogues may act as reversible inhibitors of MAO. Phthalide (**1**) is structurally related to isatin (**2**) and phthalimide (**3**), small molecules which have been shown to be suitable scaffolds for the design of high potency MAO inhibitors (Fig. 1).[14–16] Substitution of isatin at both the C5 and C6 positions with the benzyloxy moiety has yielded potent MAO inhibitors.[15] In this regard, substitution on the C5 position is more favourable for potent MAO-A and –B inhibition compared to the C6 position. Similarly, substitution of phthalimide at the C5 position with the benzyloxy moiety also results in structures endowed with potent MAO inhibitory activities.[16] It is noteworthy that both substituted isatin and phthalimide analogues interact reversibly with the MAO enzymes. The benzyloxy moiety appears to be a particularly suitable side chain for functionalizing MAO inhibitors, and several potent MAO inhibitors possess this moiety. Among these is the non-selective MAO inhibitor, 8-benzyloxycaffeine (**4**).[17] The ability of 8-benzyloxycaffeine to bind to both MAO-A and –B may depend, at least in part, on the rotational freedom of the benzyloxy CH_2–O ether bond. More rigid structures, such as (*E*)-8-(3-chlorostyryl)caffeine (**5**), are MAO-B specific inhibitors and do not bind to MAO-A.

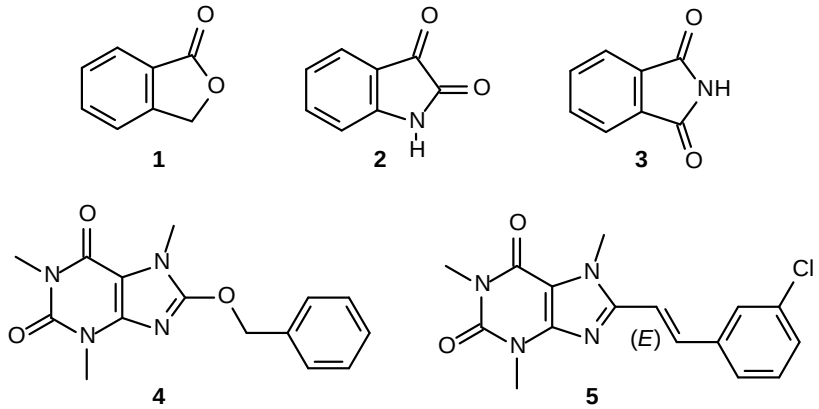
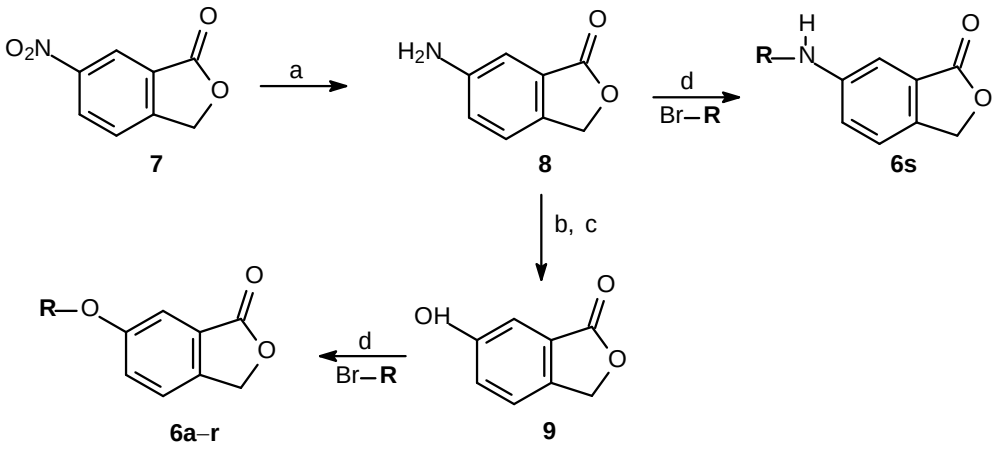


Figure 1. The structures of phthalide (**1**), isatin (**2**), phthalimide (**3**), benzyloxycaffeine (**4**) and (*E*)-8-(3-chlorostyryl)caffeine (**5**).

In the present study we thus substituted phthalide on the C6 position with the benzyloxy moiety and investigated the interactions of the resulting structures with human MAO-A and –B. The C6 position of phthalide is analogous to the C5 position of isatin. As mentioned above, substitution on the C5 position of isatin is more favourable for MAO inhibition than substitution on the C6 position. The proposal that 6-benzyloxyphthalide may act as a human MAO inhibitor is supported by a report, demonstrating that this compound inhibits rat MAO-A and –B with IC_{50} values of 3.6 µM and 0.23 µM, respectively.[18] To establish structure-activity relationships (SARs) a variety of substituents (Cl, Br, F, CF_3, I, CH_3) on the benzyloxy ring were considered (compounds **6a**–**h**). To investigate the importance of the benzyloxy moiety for inhibitory activity, the effects of phenylethoxy (**6i**–**l**), phenylpropoxy (**6m**), phenoxyethoxy (**6n**–**q**), cyclohexylmethoxy (**6r**) and benzylamino (**6s**) substitution on the C6 position of the phthalide ring were also examined.

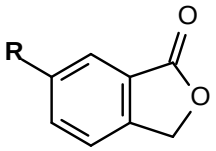
The C6 substituted phthalide analogues were synthesized according to previously established procedures (Scheme 1). For this purpose, commercially available 6-nitrophthalide (**7**) served as key starting material. 6-Nitrophthalide was hydrogenated in the presence of Pd/C (10%) to yield 6-aminophthalide (**8**) in moderate yields (typically 54–73%).[19] Treatment of **8** with $NaNO_2$ in H_2SO_4 (50%) gave the corresponding diazonium salt, which was subsequently hydrolyzed in H_2SO_4 (50%) to yield 6-hydroxyphthalide (**9**) in low yields (typically 10–20%).[20,21] The target phthalide analogues (**6a**–**r**) were obtained by reacting **9** with the appropriately substituted alkyl bromide in the presence of K_2CO_3 (6–83%).[22] 6-Benzylaminophthalide (**6s**) was synthesized according to the same procedure from 6-aminophthalide (**8**) and benzyl bromide (39%). In each instance, the structures and purities of the target compounds were verified by ^{1}H NMR, ^{13}C NMR, mass spectrometry and HPLC analysis as cited in the supplementary material.



Scheme 1. Synthetic route to the C6-substituted phthalide analogues **6a**–**s**. Reagents and conditions: (a) H_2, 10% Pd/C, 18h; (b) 50% H_2SO_4, $NaNO_2$, 2 ºC; (c) 50% H_2SO_4, 125 ºC; (d) DMF, K_2CO_3, 50–100 ºC, 12h.

To evaluate the MAO inhibitory properties of the phthalide analogues, the recombinant human MAO-A and –B enzymes were employed.[23] Kynuramine, a MAO-A/B mixed substrate served as enzyme substrate. Kynuramine undergoes MAO-catalyzed oxidation to yield 6-hydroxyquinoline, a compound which fluoresces (λ_{ex} = 310 nm; λ_{em} = 400 nm) in alkaline media.[17] Concentration measurements of 6-hydroxyquinoline can conveniently be made via fluorescence spectrophotometry since both kynuramine and the phthalide inhibitors are non-fluorescent under these assay conditions. The inhibition potencies of the test compounds were calculated from the sigmoidal dose–response curves and are expressed as the corresponding IC_{50} values. The IC_{50} values for the inhibition of MAO by phthalide analogues **6a**–**s** are given in table 1.

Table 1. The IC_{50} values for the inhibition of recombinant human MAO-A and –B by phthalide (**1**) and phthalide analogues **6a**–**s**.



		IC_{50} (μM)[a]		
	R	MAO-A	MAO-B	SI[b]
1	H–	44.9 ± 2.26	28.6 ± 2.03	1.6
6a	$C_6H_5(CH_2)O$–	1.19 ± 0.075	0.024 ± 0.003	50
6b	4-$ClC_6H_4(CH_2)O$–	0.172 ± 0.010	0.0028 ± 0.0002	61
6c	4-$BrC_6H_4(CH_2)O$–	0.227 ± 0.045	0.0024 ± 0.0002	96
6d	4-$FC_6H_4(CH_2)O$–	0.542 ± 0.025	0.0064 ± 0.0008	84
6e	4-$CF_3C_6H_4(CH_2)_2O$–	0.304 ± 0.037	0.0014 ± 0.0001	214
6f	4-$IC_6H_4(CH_2)O$–	0.344 ± 0.105	0.0018 ± 0.0001	189
6g	4-$CH_3C_6H_4(CH_2)O$–	1.06 ± 0.042	0.0090 ± 0.0004	118
6h	3-$BrC_6H_4CH_2O$–	0.629 ± 0.016	0.0035 ± 0.0003	180
6i	$C_6H_5(CH_2)_2O$–	3.21 ± 0.559	0.047 ± 0.002	68
6j	4-$ClC_6H_4(CH_2)_2O$–	0.498 ± 0.039	0.048 ± 0.009	10
6k	4-$FC_6H_4(CH_2)_2O$–	1.07 ± 0.290	0.068 ± 0.005	16
6l	4-$CH_3C_6H_4(CH_2)_2O$–	7.33 ± 0.234	0.081 ± 0.006	90
6m	$C_6H_5(CH_2)_3O$–	0.096 ± 0.006	0.0062 ± 0.0003	16
6n	$C_6H_5O(CH_2)_2O$–	1.91 ± 0.172	0.018 ± 0.0003	106
6o	4-$ClC_6H_4O(CH_2)_2O$–	0.137 ± 0.009	0.011 ± 0.002	13
6p	4-$BrC_6H_4O(CH_2)_2O$–	0.240 ± 0.010	0.012 ± 0.0009	20
6q	4-$FC_6H_4O(CH_2)_2O$–	0.323 ± 0.017	0.0074 ± 0.0006	43
6r	$C_6H_{11}CH_2O$–	0.185 ± 0.009	0.012 ± 0.001	16
6s	$C_6H_5CH_2NH$–	59.9 ± 22.8	No inhibition[c]	-

[a] All values are expressed as the mean ± standard deviation (SD) of triplicate determinations.

[b] The selectivity index is the selectivity for the MAO-B isoform and is given as the ratio of IC_{50}(MAO-A)/IC_{50}(MAO-B).

[c] No inhibition at a maximum tested concentration of 100 μM.

The results show that the phthalide analogues are highly potent inhibitors of MAO-B, with all homologues, except **6s**, exhibiting IC_{50} values in the low nanomolar range (IC_{50} < 0.081 µM). For example, the most potent MAO-B inhibitor among the test compounds is **6e**, the CF_3 substituted benzyloxyphthalide homologue, with an IC_{50} value of 0.0014 µM. For comparison, the reversible MAO-B selective inhibitor, lazabemide, exhibits an IC_{50} value of 0.091 µM under the same conditions (unpublished data from our laboratory). Of significance is the finding that a wide variety of substituents at C6 of the phthalide moiety yield compounds with potent MAO-B inhibition, with the different substituent groups yielding similar potencies. The IC_{50} values recorded for the differently substituted homologues were as follows: 6-benzyloxyphthalides (**6a**–**h**), IC_{50} = 0.0014–0.024 µM; 6-(phenylethoxy)phthalides (**6i**–**l**), IC_{50} = 0.047–0.081 µM; 6-(phenylpropoxy)phthalide (**6m**), IC_{50} = 0.0062 µM; 6-(phenoxyethoxy)phthalides (**6n**–**q**), IC_{50} = 0.0074–0.018 µM; 6-(cyclohexylmethoxy)phthalide (**6r**), IC_{50} = 0.012 µM. This relatively large degree of tolerance for different substituents makes C6-substituted phthalides good candidates for the design of MAO-B inhibitors since structural modifications to improve drug properties may be made without the risk of loss of inhibition activity. While the 6-benzyloxyphthalides (**6a**–**h**) were all found to be potent MAO-B inhibitors, it is noteworthy that those homologues containing substituents (Cl, Br, F, CF_3, I, CH_3) on the benzyloxy ring were more potent than the unsubstituted homologue **6a**. For example, the substituted homologues **6b**–**h** displayed IC_{50} values ranging from 0.0014–0.009 µM, while the unsubstituted homologue **6a** exhibited an IC_{50} value of 0.024 µM. As mentioned above, 6-benzylaminophthalide (**6s**) did not inhibit MAO-B which suggests that, in contrast to C6 oxy substituents, amino substituents at C6 of phthalide are not suitable for MAO-B inhibition. Compared to the C6 oxy-substituted phthalides **6a**–**q**, phthalide (**1**) was found to be a weak MAO-B inhibitor with an IC_{50} value of 28.6 µM. This is approximately 1000-fold weaker than the MAO-B inhibition potency recorded for **6a** (IC_{50} = 0.024 µM). This finding demonstrates the importance of the C6 substituent for MAO-B inhibition.

The results document that the phthalide analogues **6a**–**s** also are inhibitors of MAO-A. In fact twelve of the nineteen analogues exhibited IC_{50} values in the submicromolar range (0.096–0.629 µM). The most potent MAO-A inhibitor was 6-

(phenylpropoxy)phthalide (**6m**) with an IC_{50} value of 0.096 µM. This compound is also a highly potent MAO-B inhibitor (IC_{50} = 0.0062 µM). Based on the selectivity index (SI) value, **6m** is 16-fold more selective for MAO-B than MAO-A. With the exception of 6-benzylaminophthalide (**6s**), selective inhibition of MAO-B was also observed for the other phthalide analogues examined (SI = 10–214). Although the phthalides are MAO-B selective inhibitors, based on the potent MAO-A inhibitory activities of most homologues, they may still be deemed as suitable drug candidates where both MAO-A and –B inhibition are required. Although many of the phthalide analogues examined here act as potent inhibitors of MAO-A and –B, those phthalides which may be considered as particularly potent dual MAO-A/B inhibitors are: **6b**, **6m**, **6o** and **6r**. These compounds possess IC_{50} values for the inhibition of both MAO isoforms smaller than 0.2 µM. Compound **6j**, also a potent dual inhibitor, was found to be the least selective among the phthalides evaluated (SI = 10). Since potent MAO-A inhibitors were found among all classes of C6 oxy phthalides examined (benzyloxy-, phenylethoxy-, phenylpropoxy-, phenoxyethoxy- and cyclohexylmethoxy-substituted phthalides), it may be concluded that a wide variety of C6 substituents are capable for MAO-A inhibition. As observed for the inhibition of MAO-B, 6-benzylaminophthalide (**6s**) was found, however, to be a weak inhibitor of MAO-A (IC_{50} = 59.9 µM). This suggests that C6 amino substituents are also not suitable for MAO-A inhibition. It is interesting to note that 6-benzyloxyphthalide (**6a**) inhibited MAO-A and –B with IC_{50} values of 1.19 µM and 0.024 µM, respectively. These IC_{50} values are threefold and tenfold more potent than the previously reported values of 3.6 µM and 0.23 µM, respectively, for the inhibition of rat brain MAO-A and –B.[18] This result suggests that, while the rat enzymes may be useful for initial screening, caution should be exercised since relatively large differences may exist between the inhibition potencies obtained with the rat enzymes and those obtained with the human isoforms. As observed for the MAO-B isoform, phthalide (**1**) also was a weak MAO-A inhibitor compared to the C6 oxy-substituted phthalides **6a**–**q**. Compared to **6a**–**q**, phthalide (IC_{50} = 44.9 µM) is 6–467-fold weaker as a MAO-A inhibitor. These data show that the C6 substituent is also an essential structural feature for potent MAO-A inhibition.

To examine the reversibility of MAO inhibition by the phthalide analogues, two representative compounds, **6m** and **6e**, were selected for the evaluation of the reversibility of MAO-A and –B inhibition, respectively. To evaluate the reversibility of inhibition, the recovery of enzyme activities after the dilution of the enzyme-inhibitor complexes was examined.[24] MAO-A and –B were preincubated for 30 minutes with the respective inhibitors at concentrations equal to 10 x IC_{50} and 100 x $IC_{50.}$ The reactions were subsequently diluted 100-fold to yield inhibitor concentrations of 0.1 x IC_{50} and 1 x IC_{50}, and the residual enzyme activities were measured. The results show that after dilution of **6m** and **6e** to 0.1 x IC_{50}, the activities of MAO-A and –B are recovered to levels of 86% and 68% of the control levels, respectively (Fig. 2). After dilution of **6m** and **6e** to 1 x IC_{50}, the activities of MAO-A and –B are recovered to levels of 45% and 14% of the control levels, respectively. This behaviour is consistent with reversible interactions of **6m** and **6e** with MAO-A and –B, respectively. After similar treatment of MAO-A with the irreversible inhibitor, pargyline, and MAO-B with the irreversible inhibitor (R)-deprenyl at concentrations equal to 10 × IC_{50}, and dilution of the resulting complexes to 0.1 x IC_{50}, the MAO activities are not recovered (1% and 2% of control, respectively). Interestingly, after dilution of the **6e**–MAO-B complex to 0.1 × IC_{50} and 1 × IC_{50}, the enzyme activities are not recovered to 90% and 50%, respectively, as would be expected. This result suggests that, for the inhibition of MAO-B, **6e** may possess a quasi-reversible or tight-binding component.

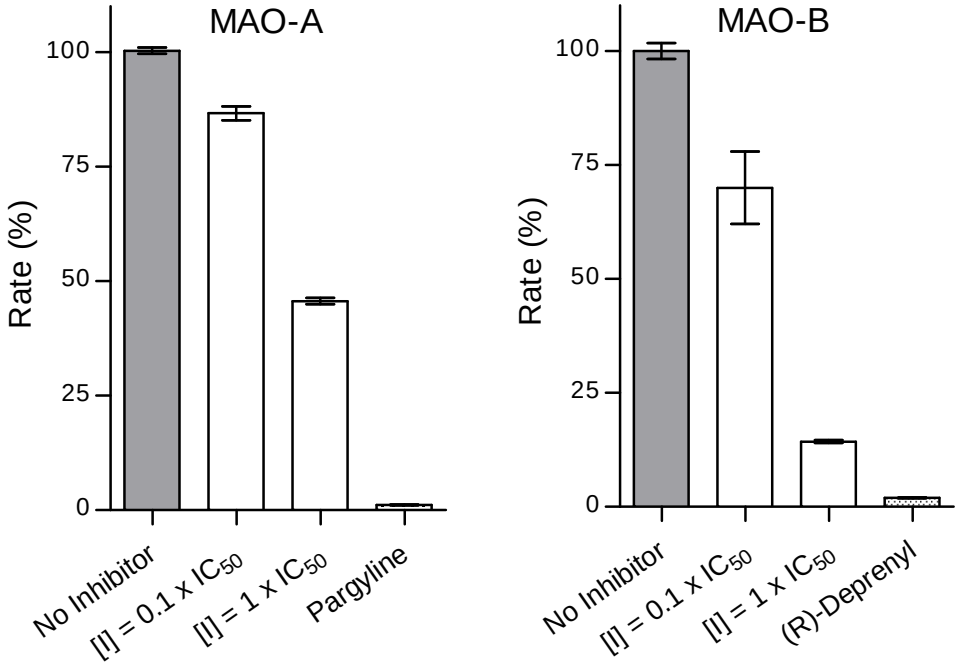


Figure 2. Reversibility of inhibition of MAO-A by **6m** and MAO-B by **6e**. MAO-A and –B were preincubated with the test inhibitors at 10 × IC_{50} and 100 × IC_{50} for 30 min and then diluted to 0.1 × IC_{50} and 1 × IC_{50}, respectively. For comparison, pargyline and (R)-deprenyl, at 10 × IC_{50} were similarly incubated with MAO-A and –B, respectively, and diluted to 0.1 × IC_{50}. The residual enzyme activities were subsequently measured.

The reversibility of inhibition was further examined by constructing sets of Lineweaver-Burk plots for the inhibition of MAO-A and –B by **6m** and **6e**, respectively. The initial MAO catalytic rates were recorded at 4 different substrate concentrations in the absence of inhibitor, and presence of three different inhibitor concentrations. The Lineweaver-Burk plots constructed from these data are given in Fig. 3. The graphs show that for the inhibition of MAO-A by **6m**, and the inhibition of MAO-B by **6e**, the Lineweaver-Burk plots are linear and intersect on the y-axis. This indicates that the modes of inhibition of both MAO-A and –B are competitive and therefore reversible.

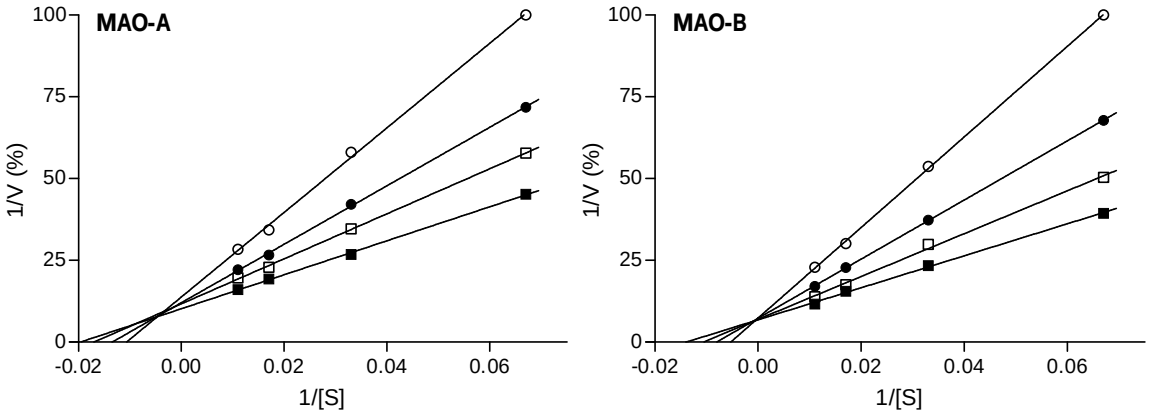


Figure 3. Lineweaver-Burk plots of the oxidation of kynuramine by recombinant human MAO-A (left) and MAO-B (right) in the absence (filled squares) and presence of various concentrations of **6m** and **6e**. For the studies with MAO-A the concentrations of **6m** were: 0.024 µM (open squares), 0.048 µM (filled circles) and 0.096 µM (open circles). For the studies with MAO-B the concentrations of **6e** were: 0.00035 µM (open squares), 0.0007 µM (filled circles) and 0.0014 µM (open circles).

In conclusion, the present study shows that C6-substituted phthalide analogues are inhibitors of both MAO-A and –B. While the phthalides display, for the most part, selective inhibition of MAO-B, the potent inhibition of both MAO isoforms by many of the analogues demonstrates that these compounds are dual MAO-A/B inhibitors. The results further document that phthalides interact reversibly with MAO-A and –B. Both reversibility and dual MAO-A/B inhibition are attributes which are desirable when designing antiparkinsonian therapies. It is noteworthy that a relatively large variety of C6 substituents yield phthalides with potent MAO-A and –B inhibitory activities. This is advantageous when optimizing the properties of these structures since modifications made to these structures, particularly to the C6 substituent, are less likely to abolish MAO inhibition. It should, however, be noted that, in contrast to oxy substituents, C6 amino substituents are not suitable for MAO inhibition. This finding is in accordance to literature which reports that 8-aminocaffeines are weak MAO inhibitors while 8-oxycaffeines are highly potent MAO inhibitors.[17,25] Based on this analysis it may be concluded that phthalides are suitable lead compounds for the development of novel therapies for Parkinson's disease.

Acknowledgements

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Supplementary Material

5.1. Experimental procedures

5.1.1. Chemicals and instrumentation

All reagents were obtained from Sigma-Aldrich and were used without further purification. Proton (^{1}H) and carbon (^{13}C) NMR spectra were recorded on a Bruker Avance III 600 spectrometer in $CDCl_3$. The chemical shifts are reported in parts per million (δ) relative to the signal of $(CH_3)_4Si$. Spin multiplicities are abbreviated as follow: s (singlet), d (doublet), dd (doublet of doublets), t (triplet), q (quartet) or m (multiplet). High resolution mass spectra (HRMS) were recorded with a Bruker micrOTOF-Q II mass spectrometer in atmospheric-pressure chemical ionization (APCI) mode. Melting points (mp) were measured with a Buchi M-545 melting point apparatus and are uncorrected. Fluorescence spectrophotometry was carried out with a Varian Cary Eclipse fluorescence spectrophotometer. Kynuramine.2HBr, phthalide (**1**) and insect cell microsomes containing recombinant human MAO-A and –B (5 mg/mL) were obtained from Sigma-Aldrich.

5.1.2. Synthesis of the C6-substituted phthalide analogues 6a–s

6-Hydroxyphthalide (**9**) (0.33 mmol) was dissolved in N,N-dimethylformamide (DMF; 3 mL) and stirred over K_2CO_3 (1.14 mmol) for 5 min. The appropriately substituted alkyl bromide (0.37 mmol) was added and the reaction mixture was stirred for 12 h at 50–100 ºC. The K_2CO_3 was removed by filtration and the reaction mixture was dried in a stream of air. The resulting residue was recrystallized from ethanol to yield the C6-substituted phthalide analogues **6a**–**r** (Wyrick *et al.*, 1987). Benzylaminophthalide (**6s**) was synthesized according to the same procedure from 6-aminophthalide (**8**) and benzyl bromide

5.1.2.1. 6-Benzyloxyphthalide (6a)

The title compound (cream crystals) was prepared from 6-hydroxyphthalide (**9**) and benzyl bromide. Yield 55%; mp 144 °C, lit mp 143–144 °C (Gnerre et al., 2000); ^{1}H NMR (Bruker Avance III 600, $CDCl_3$) δ 5.10 (s, 2H), 5.24 (s, 2H), 7.30–7.42 (m, 8H); ^{13}C NMR (Bruker Avance III 600, $CDCl_3$) δ 69.5, 70.46, 108.7, 123.0, 123.7, 127.0, 127.5, 128.3, 128.7, 136.0, 139.1, 159.6, 171.1; APCI-HRMS *m*/*z*: calcd for $C_{15}H_{13}O_3$ (MH^+), 241.0865, found 241.0860; Purity (HPLC): 100%.

5.1.2.2. 6-(4-Chlorobenzyloxy)phthalide (6b)

The title compound (white crystals) was prepared from 6-hydroxyphthalide (**9**) and 4-chlorobenzyl bromide. Yield 57%; mp 133 °C; ^{1}H NMR (Bruker Avance III 600, $CDCl_3$) δ 5.07 (s, 2H), 5.25 (s, 2H), 7.29 (dd, 1H, J = 8.3, 2.3 Hz), 7.36 (m, 6H); ^{13}C NMR (Bruker Avance III 600, $CDCl_3$) δ 69.5, 69.7, 108.7, 123.1, 123.7, 127.1, 128.8, 128.9, 134.1, 134.5, 139.3, 159.4, 171.0; APCI-HRMS *m*/*z*: calcd for $C_{15}H_{12}ClO_3$ (MH^+), 275.0475, found 275.0458; Purity (HPLC): 98%.

5.1.2.3. 6-(4-Bromobenzyloxy)phthalide (6c)

The title compound (cream crystals) was prepared from 6-hydroxyphthalide (**9**) and 4-bromobenzyl bromide. Yield 70%; mp 146.2 °C; ^{1}H NMR (Bruker Avance III 600, $CDCl_3$) δ 5.05 (s, 2H), 5.25 (s, 2H), 7.29 (m, 3H), 7.38 (m, 2H), 7.51 (d, 2H, J = 8.3 Hz); ^{13}C NMR (Bruker Avance III 600, $CDCl_3$) δ 69.5, 69.7, 108.7, 122.2, 123.1, 123.7, 127.1, 129.1, 131.9, 135.0, 139.3, 159.4, 171.0; APCI-HRMS *m*/*z*: calcd for $C_{15}H_{12}BrO_3$ (MH^+), 318.9970, found 318.9944; Purity (HPLC): 100%.

5.1.2.4. 6-(4-Fluorobenzyloxy)phthalide (6d)

The title compound (white crystals) was prepared from 6-hydroxyphthalide (**9**) and 4-fluorobenzyl bromide. Yield 48%; mp 131.3 °C; ^{1}H NMR (Bruker Avance III 600, $CDCl_3$) δ 5.06 (s, 2H), 5.25 (s, 2H), 7.07 (t, 2H, J = 8.7 Hz), 7.29 (dd, 1H, J = 2.3, 8.3 Hz), 7.39 (m, 4H); ^{13}C NMR (Bruker Avance III 600, $CDCl_3$) δ 69.5, 69.8, 108.6, 115.6, 115.7, 123.1, 123.7, 127.1, 129.4, 131.8, 139.2, 159.5, 171.1; APCI-HRMS *m*/*z*: calcd for $C_{15}H_{12}FO_3$ (MH^+), 259.0770, found 259.0756; Purity (HPLC): 100%.

5.1.2.5. 6-[4-(Trifluoromethyl)benzyloxy]phthalide (6e)

The title compound (white crystals) was prepared from 6-hydroxyphthalide (**9**) and 4-(trifluoromethyl)benzyl bromide. Yield 43%; mp 126 °C; ^{1}H NMR (Bruker Avance III 600, $CDCl_3$) δ 5.17 (s, 2H), 5.25 (s, 2H), 7.31 (dd, 1H, J = 2.3, 8.3 Hz), 7.38 (m, 2H), 7.55 (d, 2H, J = 7.9 Hz), 7.63 (d, 2H, J = 7.9 Hz); ^{13}C NMR (Bruker Avance III 600, $CDCl_3$) δ 69.5, 69.5, 108.6, 123.2, 123.6, 124.9, 125.7, 127.2, 127.4, 130.3, 139.4, 140.0, 159.3, 171.0; APCI-HRMS *m*/*z*: calcd for $C_{16}H_{12}F_3O_3$ (MH^+), 309.0739, found 309.0731; Purity (HPLC): 100%.

5.1.2.6. 6-(4-Iodobenzyloxy)phthalide (6f)

The title compound (white crystals) was prepared from 6-hydroxyphthalide (**9**) and 4-iodobenzyl bromide. Yield 49%; mp 146.6 °C; ^{1}H NMR (Bruker Avance III 600, $CDCl_3$) δ 5.04 (s, 2H), 5.24 (s, 2H), 7.16 (d, 2H, J = 8.3 Hz), 7.29 (dd, 1H, J = 8.3, 2.3 Hz), 7.36 (m, 2H), 7.71 (d, 2H, J = 8.3 Hz); ^{13}C NMR (Bruker Avance III 600, $CDCl_3$) δ 69.5, 69.8, 93.9, 108.7, 123.1, 123.6, 127.1, 129.3, 135.7, 137.8, 139.3, 159.4, 171.0; APCI-HRMS *m*/*z*: calcd for $C_{15}H_{12}IO_3$ (MH^+), 366.9831, found 366.9807; Purity (HPLC): 100%.

5.1.2.7. 6-(4-Methylbenzyloxy)phthalide (6g)

The title compound (cream crystals) was prepared from 6-hydroxyphthalide (**9**) and 4-methylbenzyl bromide. Yield 43%; mp 126 °C; ^{1}H NMR (Bruker Avance III 600, $CDCl_3$) δ 2.35 (s, 3H), 5.06 (s, 2H), 5.24 (s, 2H), 7.19 (d, 2H, J = 7.9 Hz), 7.30 (m, 3H), 7.34 (d, 1H, J = 8.3 Hz), 7.40 (d, 1H, J = 2.3 Hz); ^{13}C NMR (Bruker Avance III 600, $CDCl_3$) δ 21.2, 69.5, 70.4, 108.7, 122.9, 123.7, 127.0, 127.7, 129.4, 132.9, 138.1, 139.0, 159.7, 171.1; APCI-HRMS *m*/*z*: calcd for $C_{16}H_{15}O_3$ (MH^+), 255.1021, found 255.1003; Purity (HPLC): 99%.

5.1.2.8. 6-(3-Bromobenzyloxy)phthalide (6h)

The title compound (white powder) was prepared from 6-hydroxyphthalide (**9**) and 3-bromobenzyl bromide. Yield 83%; mp 111.1 °C; ^{1}H NMR (Bruker Avance III 600, $CDCl_3$) δ 5.07 (s, 2H), 5.25 (s, 2H), 7.25 (m, 2H), 7.31 (dd, 1H, J = 8.3, 2.3 Hz), 7.34 (d, 1H, J = 7.9 Hz), 7.38 (m, 2H), 7.45 (d, 1H, J = 7.9 Hz); ^{13}C NMR (Bruker Avance III 600, $CDCl_3$) δ 69.5, 108.6, 122.8, 123.1, 123.7, 125.9, 127.1, 130.3, 130.3, 131.4, 138.3, 139.4, 159.3, 171.1; APCI-HRMS *m*/*z*: calcd for $C_{15}H_{12}BrO_3$ (MH^+), 318.9970, found 318.9944; Purity (HPLC): 95%.

5.1.2.9. 6-(2-Phenylethoxy)phthalide (6i)

The title compound (white powder) was prepared from 6-hydroxyphthalide (**9**) and (2-bromoethyl)benzene. Yield 18%; mp 88.6 °C; ^{1}H NMR (Bruker Avance III 600, $CDCl_3$) δ 3.11 (t, 2H, J = 7.2 Hz), 4.22 (t, 2H, J = 7.2 Hz), 5.23 (s, 2H), 7.21–7.34 (m, 8H); ^{13}C NMR (Bruker Avance III 600, $CDCl_3$) δ 35.5, 69.2, 69.5, 108.3, 122.9, 123.4, 126.6, 127.0, 128.5, 128.9, 137.8, 138.8, 159.8, 171.2; APCI-HRMS *m*/*z*: calcd for $C_{16}H_{15}O_3$ (MH^+), 255.1021, found 255.1012; Purity (HPLC): 97%.

5.1.2.10. 6-[2-(4-Chlorophenyl)ethoxy]phthalide (6j)

The title compound (cream crystals) was prepared from 6-hydroxyphthalide (**9**) and 1-(2-bromoethyl)-4-chlorobenzene. Yield 6%; mp 88.3 °C; ^{1}H NMR (Bruker Avance III 600, $CDCl_3$) δ 3.07 (t, 2H, J = 6.8 Hz), 4.19 (t, 2H, J = 6.8 Hz), 5.23 (s, 2H), 7.19–7.34 (m, 7H); ^{13}C NMR (Bruker Avance III 600, $CDCl_3$) δ 34.9, 68.8, 69.5, 108.3, 123.0, 123.4, 127.1, 128.6, 130.3, 132.5, 136.4, 139.0, 159.6, 171.1; APCI-HRMS *m*/*z*: calcd for $C_{16}H_{14}ClO_3$ (MH^+), 289.0631, found 289.0618; Purity (HPLC): 98%.

5.1.2.11. 6-[2-(4-Fluororophenyl)ethoxy]phthalide (6k)

The title compound (yellow crystals) was prepared from 6-hydroxyphthalide (**9**) and 1-(2-bromoethyl)-4-fluorobenzene. Yield 14%; mp 112 °C; ^{1}H NMR (Bruker Avance III 600, $CDCl_3$) δ 3.07 (t, 2H, J = 6.8 Hz), 4.19 (t, 2H, J = 6.8 Hz), 5.23 (s, 2H), 6.99 (t, 2H, J = 8.7), 7.21 (m, 3H), 7.33 (m, 2H); ^{13}C NMR (Bruker Avance III 600, $CDCl_3$) δ 34.7, 69.1, 69.5, 108.27, 115.4, 122.9, 123.4, 127.0, 130.4, 133.5, 138.9, 159.7, 162.5, 171.1; APCI-HRMS *m*/*z*: calcd for $C_{16}H_{14}FO_3$ (MH^+), 273.0927, found 273.0913; Purity (HPLC): 97%.

5.1.2.12. 6-[2-(4-Methylphenyl)ethoxy]phthalide (6l)

The title compound (white powder) was prepared from 6-hydroxyphthalide (**9**) and 1-(2-bromoethyl)-4-methylbenzene. Yield 13%; mp 87.4 °C; ^{1}H NMR (Bruker Avance III 600, $CDCl_3$) δ 2.32 (s, 3H), 3.07 (t, 2H, J = 6.8 Hz), 4.19 (t, 2H, J = 6.8 Hz), 5.23 (s, 2H), 7.12 (d, 2H, J = 7.9 Hz), 7.16 (d, 2H, J = 7.9 Hz), 7.21 (dd, 1H, J = 8.7, 2.3 Hz), 7.32 (m, 2H); ^{13}C NMR (Bruker Avance III 600, $CDCl_3$) δ 21.0, 35.1, 69.3, 69.5, 108.4, 122.9, 123.4 127.0, 128.8, 129.2, 134.7, 136.2, 138.8, 159.8, 171.2; APCI-HRMS *m*/*z*: calcd for $C_{17}H_{17}O_3$ (MH^+), 269.1178, found 269.1156; Purity (HPLC): 98%.

5.1.2.13. 6-(3-Phenylpropoxy)phthalide (6m)

The title compound (cream powder) was prepared from 6-hydroxyphthalide (**9**) and (3-bromopropyl)benzene. Yield 54%; mp 107.6 °C; ^{1}H NMR (Bruker Avance III 600, $CDCl_3$) δ 2.12 (q, 2H, J = 6.4 Hz), 2.80 (t, 2H, J = 7.5 Hz), 3.99 (t, 2H, J = 6.4 Hz), 5.24 (s, 2H), 7.19 (m, 3H), 7.23 (dd, 1H, J = 8.3, 2.3 Hz), 7.26–7.30 (m, 3H), 7.35 (d, 1H, J = 8.3 Hz); ^{13}C NMR (Bruker Avance III 600, $CDCl_3$) δ 30.6, 32.0, 67.5, 69.5,

108.2, 122.9, 123.4, 126.1, 127.0, 128.5, 128.5, 138.7, 141.1, 160.0, 171.3; HRMS *m*/*z*: calcd for $C_{17}H_{17}O_3$ (MH^+), 269.1178, found 269.1170; Purity (HPLC): 100%.

5.1.2.14. 6-(2-Phenoxyethoxy)phthalide (6n)

The title compound (peach crystals) was prepared from 6-hydroxyphthalide (**9**) and (2-bromoethoxy)benzene. Yield 48%; mp 161.8 °C; ^{1}H NMR (Bruker Avance III 600, $CDCl_3$) δ 4.36 (m, 4H), 5.25 (s, 2H), 6.95 (m, 3H), 7.29 (m, 3H), 7.38 (m, 2H); ^{13}C NMR (Bruker Avance III 600, $CDCl_3$) δ 66.2, 67.1, 69.5, 108.3, 114.6, 121.2, 123.0, 123.7, 127.0, 129.5, 139.2, 158.4, 159.6, 171.1; APCI-HRMS *m*/*z*: calcd for $C_{16}H_{15}O_4$ (MH^+), 271.0970, found 271.0965; Purity (HPLC): 100%.

5.1.2.15. 6-[2-(4-Chlorophenoxy)ethoxy]phthalide (6o)

The title compound (brown crystals) was prepared from 6-hydroxyphthalide (**9**) and 1-(2-bromoethoxy)-4-chlorobenzene. Yield 41%; mp 135.3 °C; ^{1}H NMR (Bruker Avance III 600, $CDCl_3$) δ 4.33 (m, 4H), 5.26 (s, 2H), 6.87 (d, 2H, J = 9.0 Hz), 7.23 (d, 2H, J = 8.3 Hz), 7.29 (dd, 1H, J = 8.3, 2.3 Hz), 7.38 (m, 2H); ^{13}C NMR (Bruker Avance III 600, $CDCl_3$) δ 66.6, 67.0, 69.5, 108.2, 115.9, 123.1, 123.7, 126.2, 127.1, 129.4, 139.3, 157.1, 159.5, 171.1; APCI-HRMS *m*/*z*: calcd for $C_{16}H_{14}ClO_4$ (MH^+), 305.0581, found 305.0566; Purity (HPLC): 99%.

5.1.2.16. 6-[2-(4-Bromophenoxy)ethoxy]phthalide (6p)

The title compound (brown crystals) was prepared from 6-hydroxyphthalide (**9**) and 1-(2-bromoethoxy)-4-bromobenzene. Yield 38%; mp 152 °C; ^{1}H NMR (Bruker Avance III 600, $CDCl_3$) δ 4.33 (m, 4H), 5.25 (s, 2H), 6.82 (d, 2H, J = 9.0 Hz), 7.28 (dd, 1H, J = 8.3, 2.3 Hz), 7.38 (m, 4H); ^{13}C NMR (Bruker Avance III 600, $CDCl_3$) δ 66.5, 67.0, 69.5, 108.3, 113.5, 116.5, 123.1, 123.7, 127.1, 132.3, 139.3, 157.6, 159.5, 171.1; APCI-HRMS *m*/*z*: calcd for $C_{16}H_{14}BrO_4$ (MH^+), 351.0055, found 351.0029; Purity (HPLC): 97%.

5.1.2.17. 6-[2-(4-Fluorophenoxy)ethoxy]phthalide (6q)

The title compound (cream crystals) was prepared from 6-hydroxyphthalide (**9**) and 1-(2-bromoethoxy)-4-fluorobenzene. Yield 38%; mp 132.8 °C; ^{1}H NMR (Bruker Avance III 600, $CDCl_3$) δ 4.33 (m, 4H), 5.26 (s, 2H), 6.88 (m, 2H), 6.97 (t, 2H, J = 8.3 Hz), 7.29 (dd, 1H, J = 8.3, 2.3 Hz), 7.37 (d, 2H, J = 8.7 Hz); ^{13}C NMR (Bruker Avance

III 600, $CDCl_3$) δ 67.0, 67.1, 69.5, 108.3, 115.8, 123.1, 123.7, 127.1, 139.3, 154.6, 156.7, 158.3, 159.6, 171.1; APCI-HRMS *m*/*z*: calcd for $C_{16}H_{14}FO_4$ (MH^+), 289.0876, found 289.0869; Purity (HPLC): 99%.

5.1.2.18. 6-(Cyclohexylmethoxy)phthalide (6r)

The title compound (white powder) was prepared from 6-hydroxyphthalide (**9**) and (bromomethyl)cycolohexane. Yield 60%; mp 90 °C; ^1H NMR (Bruker Avance III 600, $CDCl_3$) δ 1.05 (m, 2H), 1.15–1.32 (m, 3H), 1.67–1.85 (m, 6H), 3.78 (d, 2H, J = 6.4 Hz), 5.23 (s, 2H), 7.21 (dd, 1H, J = 8.3, 2.3 Hz), 7.30 (d, 1H, J = 2.3 Hz), 7.33 (d, 1H, J = 8.3 Hz); ^{13}C NMR (Bruker Avance III 600, $CDCl_3$) δ 25.7, 26.4, 29.8, 37.4, 69.5, 74.1, 108.1, 122.8, 123.5, 127.0, 138.5, 160.3, 171.3; APCI-HRMS *m*/*z*: calcd for $C_{15}H_{19}O_3$ (MH^+), 247.1334, found 247.1338; Purity (HPLC): 100%.

5.1.2.19. 6-(Benzylamino)phthalide (1s)

The title compound (white powder) was prepared from 6-aminophthalide (**8**) and benzyl bromide. Yield 39%; mp 145.6 °C; ^1H NMR (Bruker Avance III 600, $CDCl_3$) δ 4.36 (s, 2H), 5.18 (s, 2H), 6.92 (dd, 1H, J = 8.3, 2.3 Hz), 7.04 (d, 1H, J = 2.3 Hz), 7.20–7.34 (m, 7H); ^{13}C NMR (Bruker Avance III 600, $CDCl_3$) δ 48.2, 69.6, 106.6, 120.4, 122.5, 126.9, 127.4, 127.5, 128.8, 135.4, 138.3, 149.0, 171.8; APCI-HRMS *m*/*z*: calcd for $C_{15}H_{14}NO_2$ (MH^+), 240.1025, found 240.1003; Purity (HPLC): 98%.

5.1.3. Synthesis of 6-hydroxyphthalide (9)

6-Hydroxyphthalide was synthesized by reacting a cold suspension of 6-aminophthalide (6.7 mmol) in 10 mL H_2SO_4 (50%) with a cold solution of $NaNO_2$ (7.3 mmol in 3 mL H_2O) to yield the diazonium salt. The resulting solution was added to boiling (125 ºC) H_2SO_4 (50%; 40 mL) and the reaction mixture was boiled for 2 min. The reaction was rapidly cooled in an ice bath, and subsequently extracted to diethyl ether (3 × 50 mL). The ether portions were combined, washed with a saturated solution of $NaHCO_3$ (50 mL) and dried over anhydrous $MgSO_4$. The ether was removed under reduced pressure, leaving the brown 6-hydroxyphthalide residue (Vaughan *et al*., 1946; Yu *et al*., 2007).

5.1.4. Synthesis of 6-aminophthalide (8)

6-Nitrophthalide (20 mmol) was dissolved in ethyl acetate (200 mL) and methanol (50 mL) and hydrogenated at atmospheric pressure in the presence of 10% Pd/C (0.3 g). After 18 h the reaction mixture was filtered through a bed of celite and the filtrate was removed under reduced pressure. The residue was washed with cold ethyl acetate (20 mL) to yield 6-aminophthalide (Mori *et al.*, 2003).

5.1.5. IC_{50} value determination

IC_{50} values for the inhibition of MAO-A and –B were determined using the recombinant human enzymes as described previously (Legoabe et al., 2012). The enzymatic reactions were carried out at pH 7.4 (K_2HPO_4/KH_2PO_4 100 mM, made isotonic with KCl) to a final volume of in 500 µL. The reactions contained the different inhibitor concentrations spanning at least 3 orders of magnitude and the MAO-A/B mixed substrate kynuramine (45 µM for MAO-A and 30 µM for MAO-B). DMSO, as co-solvent (4%) was added to each reaction. The enzyme reactions were initiated with the addition of MAO-A or MAO-B (0.0075 mg protein/mL) and incubated for 20 min at 37 ºC in a waterbath. After termination with the addition of 400 µL NaOH (2 N) and 1000 µL water, the concentrations of the MAO generated 4-hydroxyquinoline were measured by fluorescence spectrophotometry (λ_{em} = 310; λ_{ex} = 400 nm) (Novaroli et al., 2005). For this purpose, a linear calibration curve containing authentic 4-hydroxyquinoline (0.047–1.56 µM) was constructed. The enzyme catalytic rates were calculated and fitted to the one site competition model incorporated into the Prism 5 software package (GraphPad). The IC_{50} values were determined in triplicate and are expressed as mean ± standard deviation (SD).

5.1.6. Recovery of enzyme activity after dilution

The test inhibitors employed were **6m** [IC_{50}(MAO-A) = 0.096 µM] for the evaluation of MAO-A inhibition and **6e** [IC_{50}(MAO-B) = 0.0014 µM] for the evaluation of MAO-B inhibition. The inhibitors at concentrations equal to 10 × IC_{50} and 100 × IC_{50}, were preincubated with recombinant human MAO-A or –B (0.75 mg/ml) for 30 min at 37 ºC (K_2HPO_4/KH_2PO_4 pH 7.4, 100 mM, made isotonic with KCl). All preincubations contained DMSO (4%) as co-solvent. The reactions were subsequently diluted 100-fold with the addition of a solution of kynuramine (in K_2HPO_4/KH_2PO_4 pH 7.4, 100 mM) to yield final concentrations of the test inhibitors equal to 1 × IC_{50} and 0.1 × IC_{50}.

After dilution, the final concentrations of kynuramine were 45 µM and 30 µM for MAO-A and –B, respectively, and the final concentrations of MAO-A and –B were 0.0075 mg/mL. The reactions were incubated for a further 20 min at 37 °C, terminated and the residual rates of 4-hydroxyquinoline formation were measured as described above (Petzer et al., 2012). For comparison, pargyline (IC_{50} = 12.97 µM) and (R)-deprenyl (IC_{50} = 0.079 µM), at a concentrations of 10 × IC_{50} were similarly preincubated with MAO-A and –B, respectively, diluted to 0.1 × IC_{50} and the residual MAO catalytic activities were measured as above (Petzer et al., 2012). Similar reactions, which served as controls, were also conducted in the absence on inhibitor.

5.1.7. The construction of Lineweaver-Burk plots

The modes of MAO-A and –B inhibition were examined by constructing sets of four Lineweaver–Burk plots for each enzyme evaluated. The first plot was constructed in the absence of inhibitor while the remaining three plots were constructed in the presence of different concentrations of the inhibitor. In the present study, compound **6m** was selected as representative inhibitor to evaluate the mode of MAO-A inhibition at the following concentrations: 0.024 µM, 0.048 µM and 0.096 µM. Compound **6e** was selected as representative inhibitor to evaluate the mode of MAO-B inhibition at the following concentrations: 0.00035 µM, 0.0007 µM and 0.0014 µM. Kynuramine at concentrations of 15–90 µM served as substrate and recombinant human MAO-A and –B were used at a concentration of 0.015 mg/mL. The rates of formation of the MAO generated 4-hydroxyquinoline were measured by fluorescence spectrophotometry as described above. Linear regression analysis was performed using Prism 5 (Manley-King et al., 2011).

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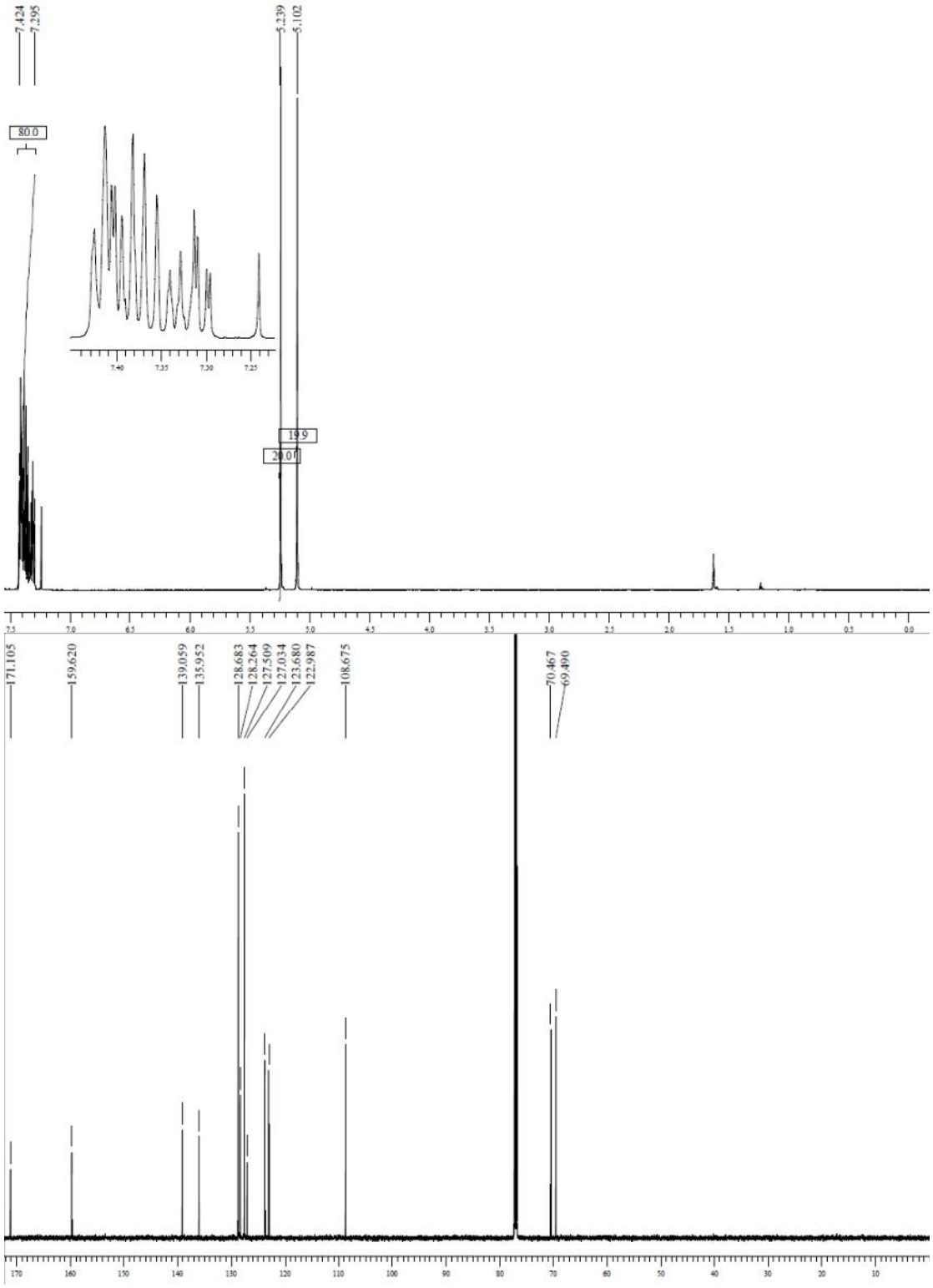
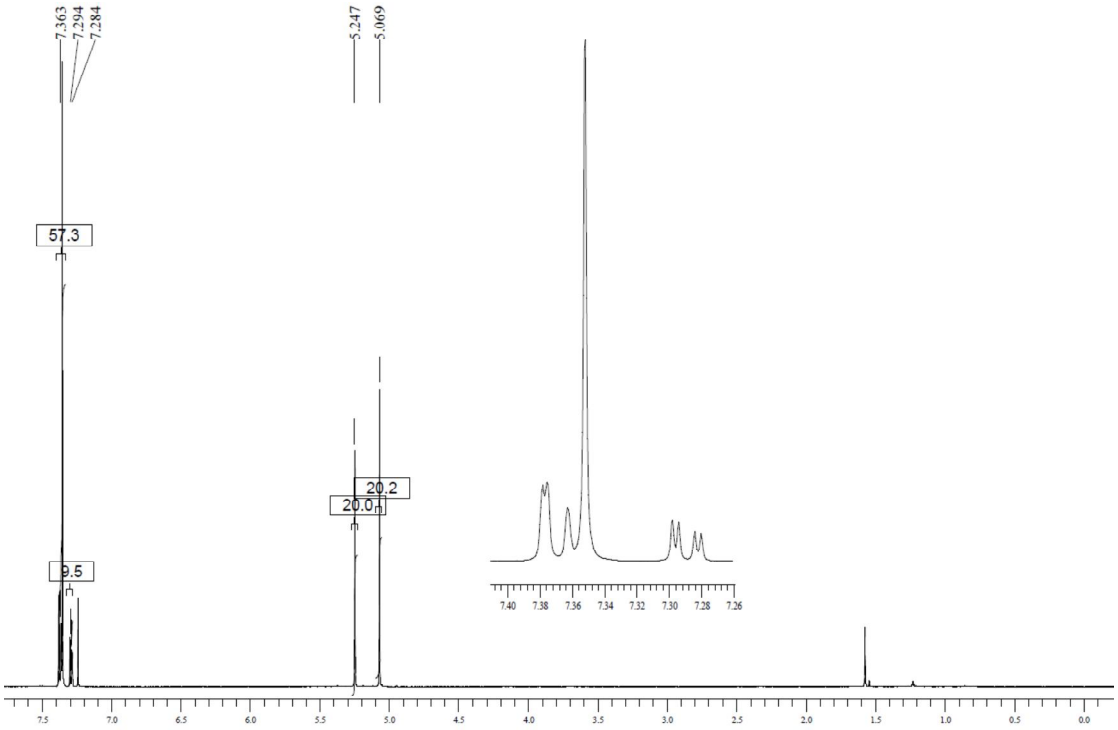
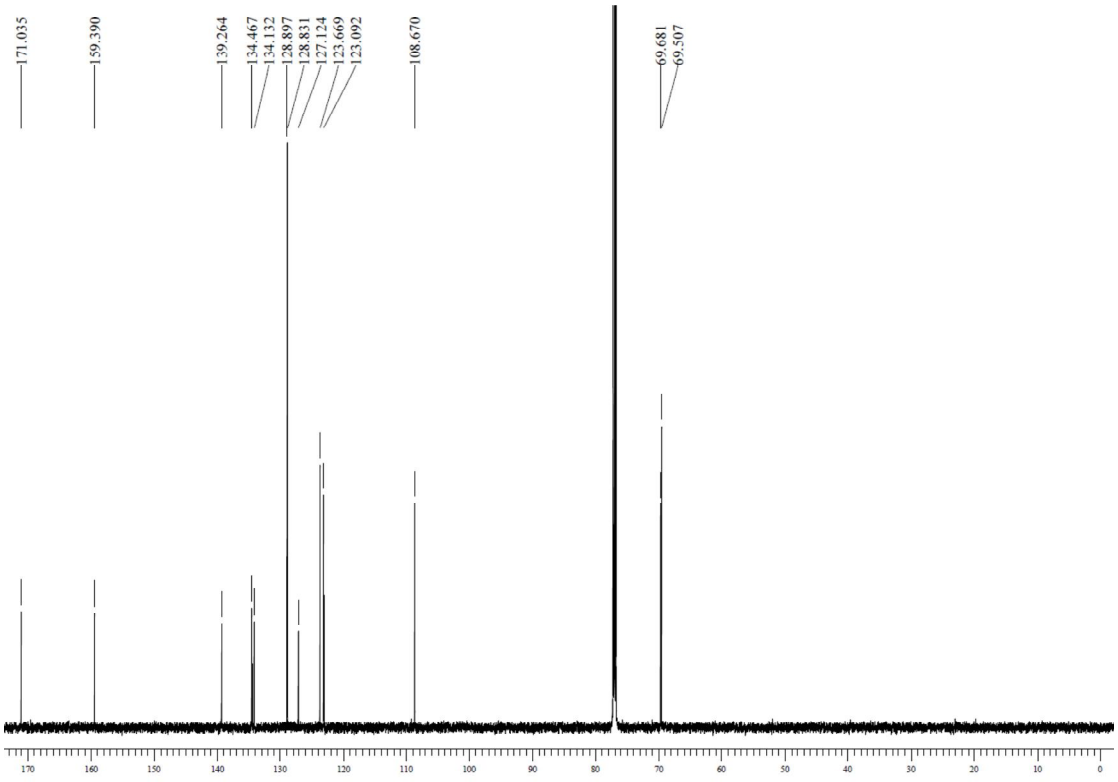
5.2.NMR spectra: 6-Benzyloxyphthalide (6a)



6-(4-Chlorobenzyloxy)phthalide (6b)





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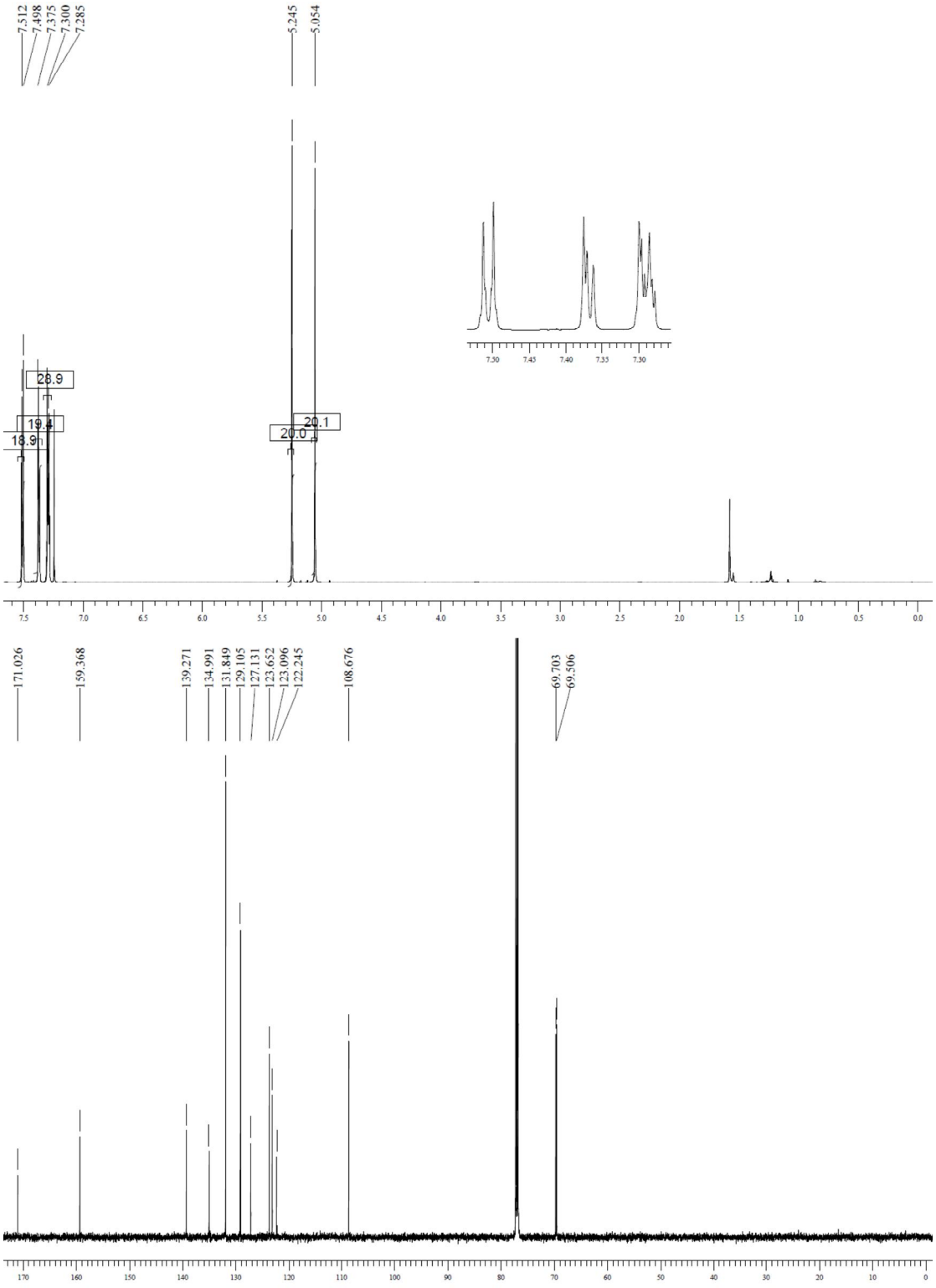

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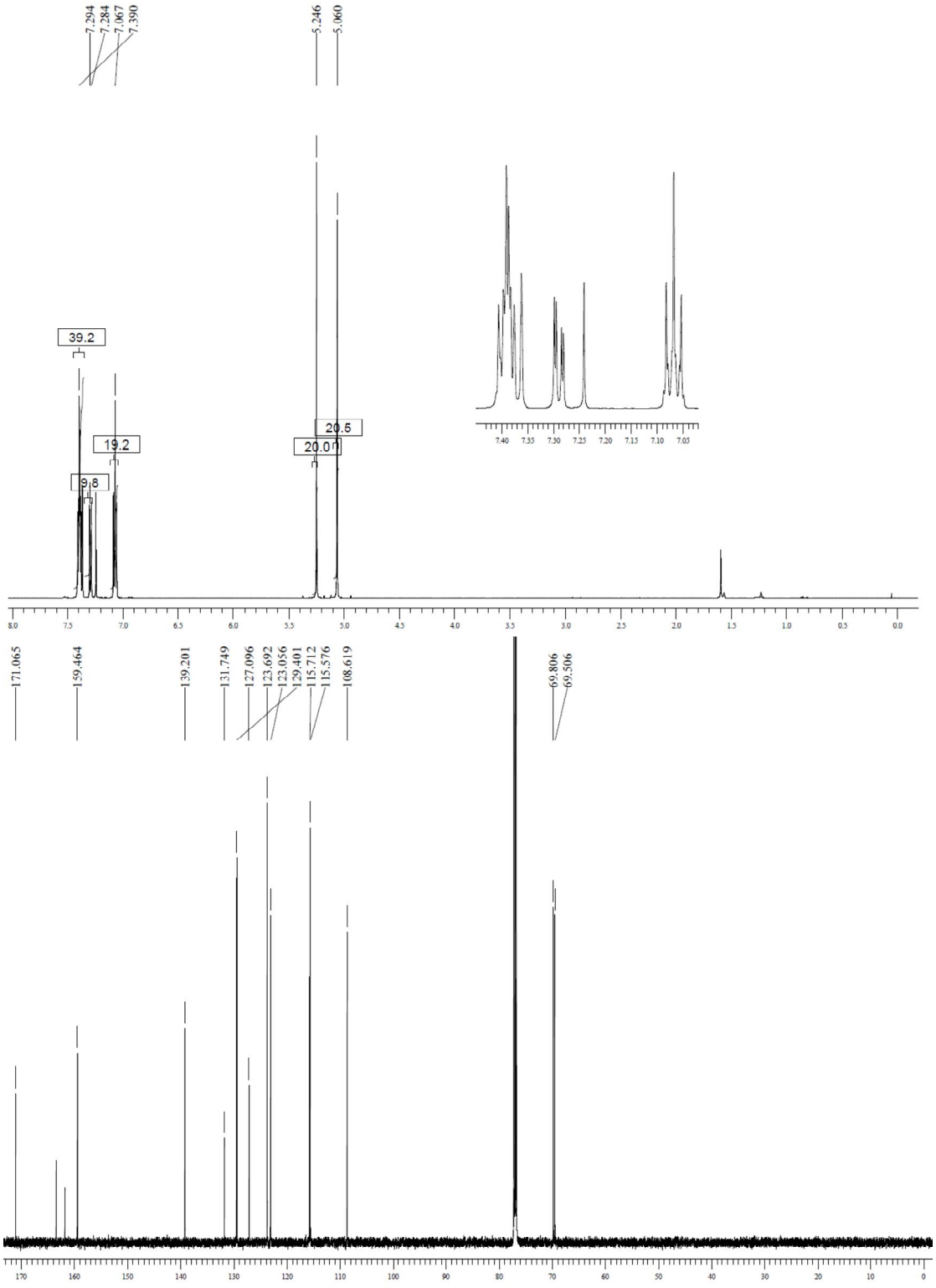
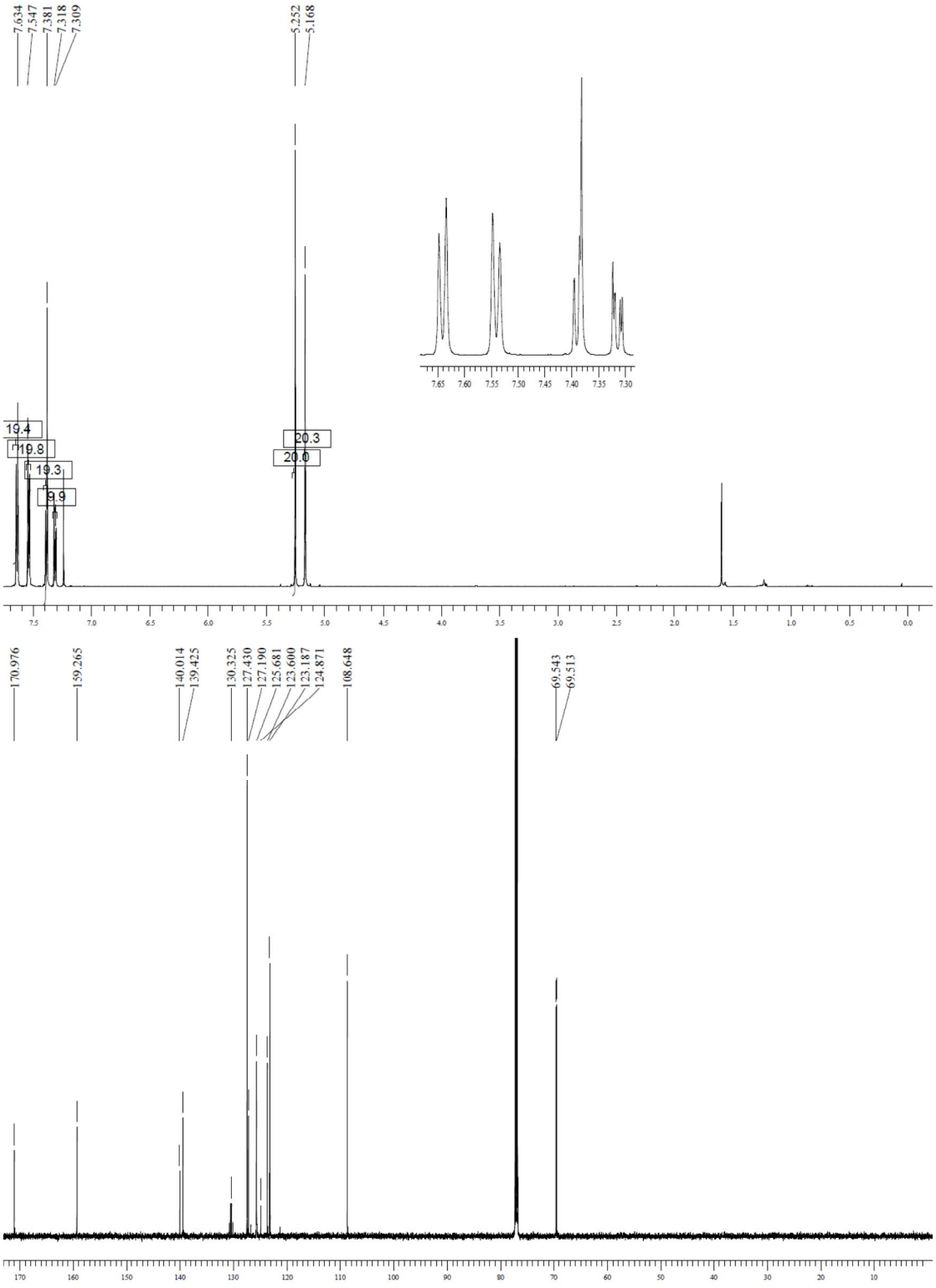
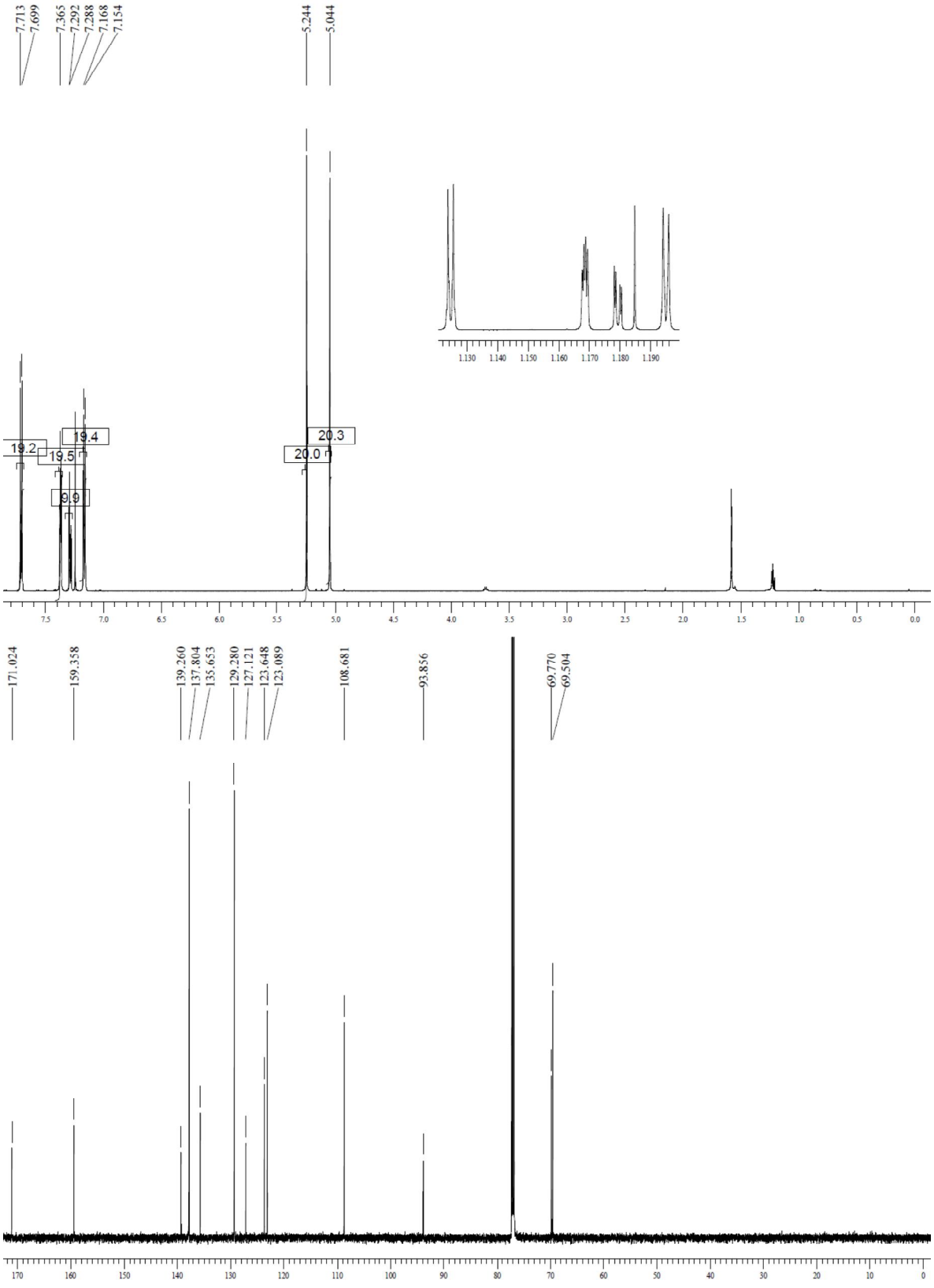


6-[4-(Trifluoromethyl)benzyloxy]phthalide (6e)



6-(4-Iodobenzyloxy)phthalide (6f)



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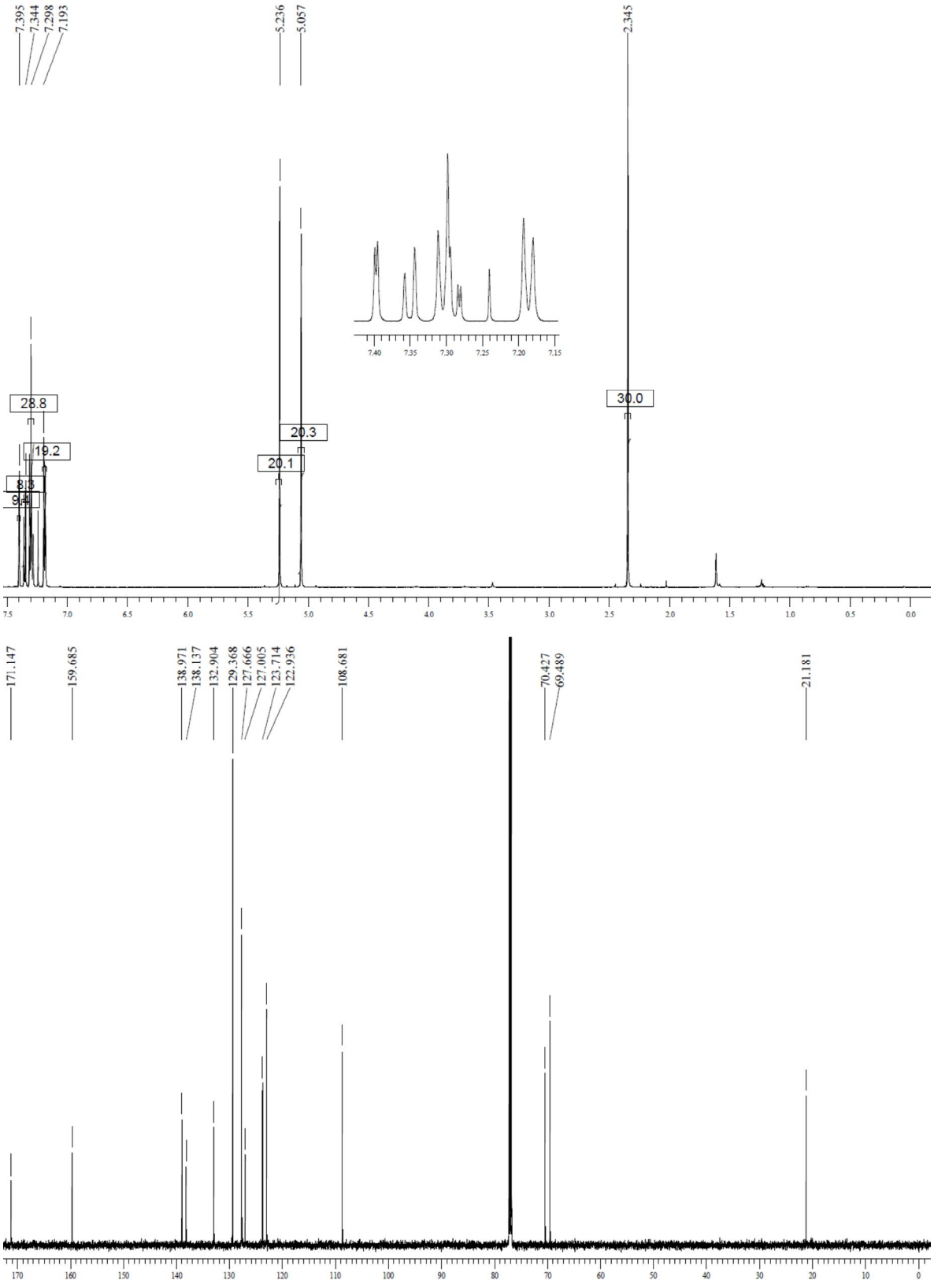

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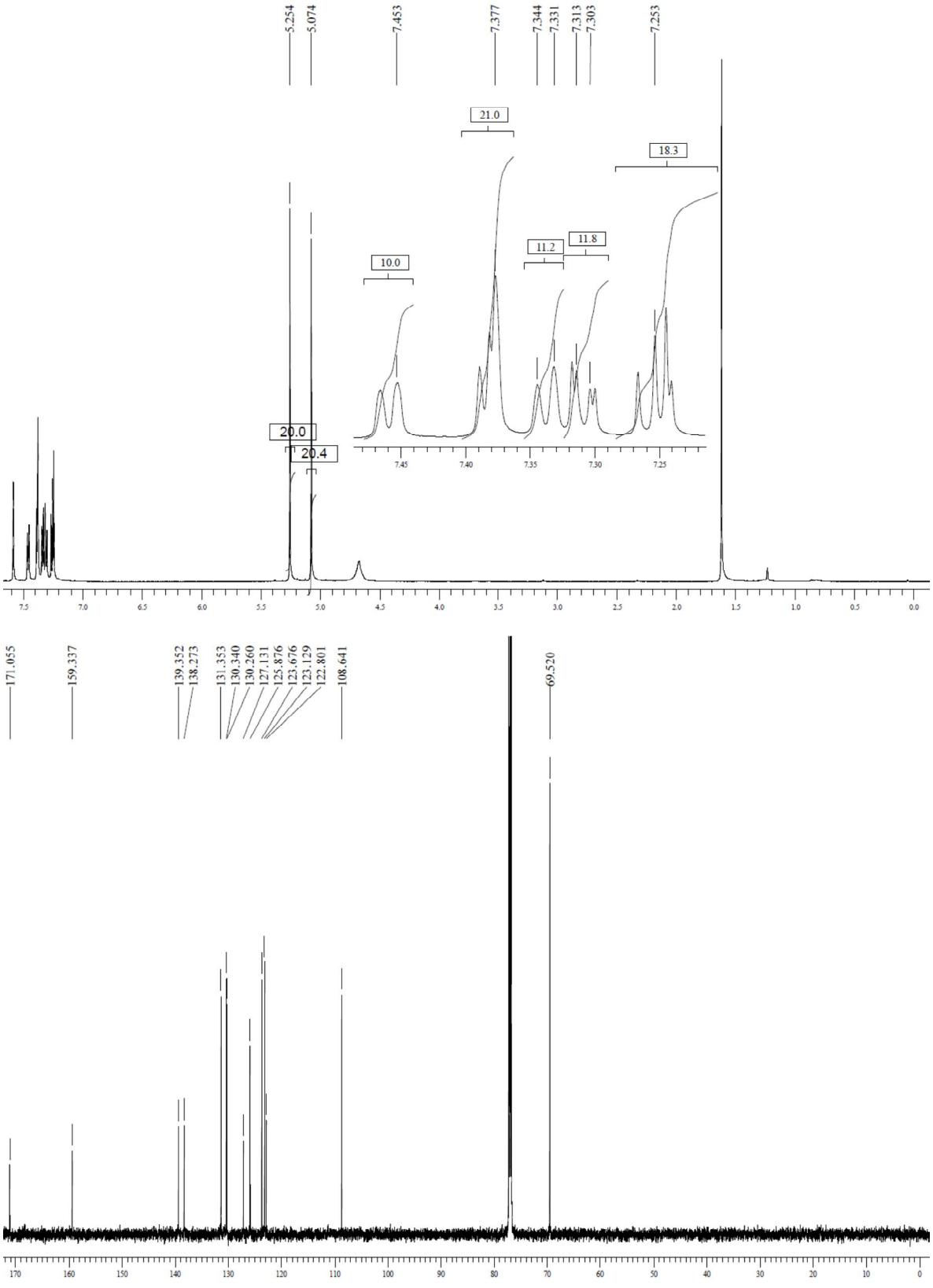

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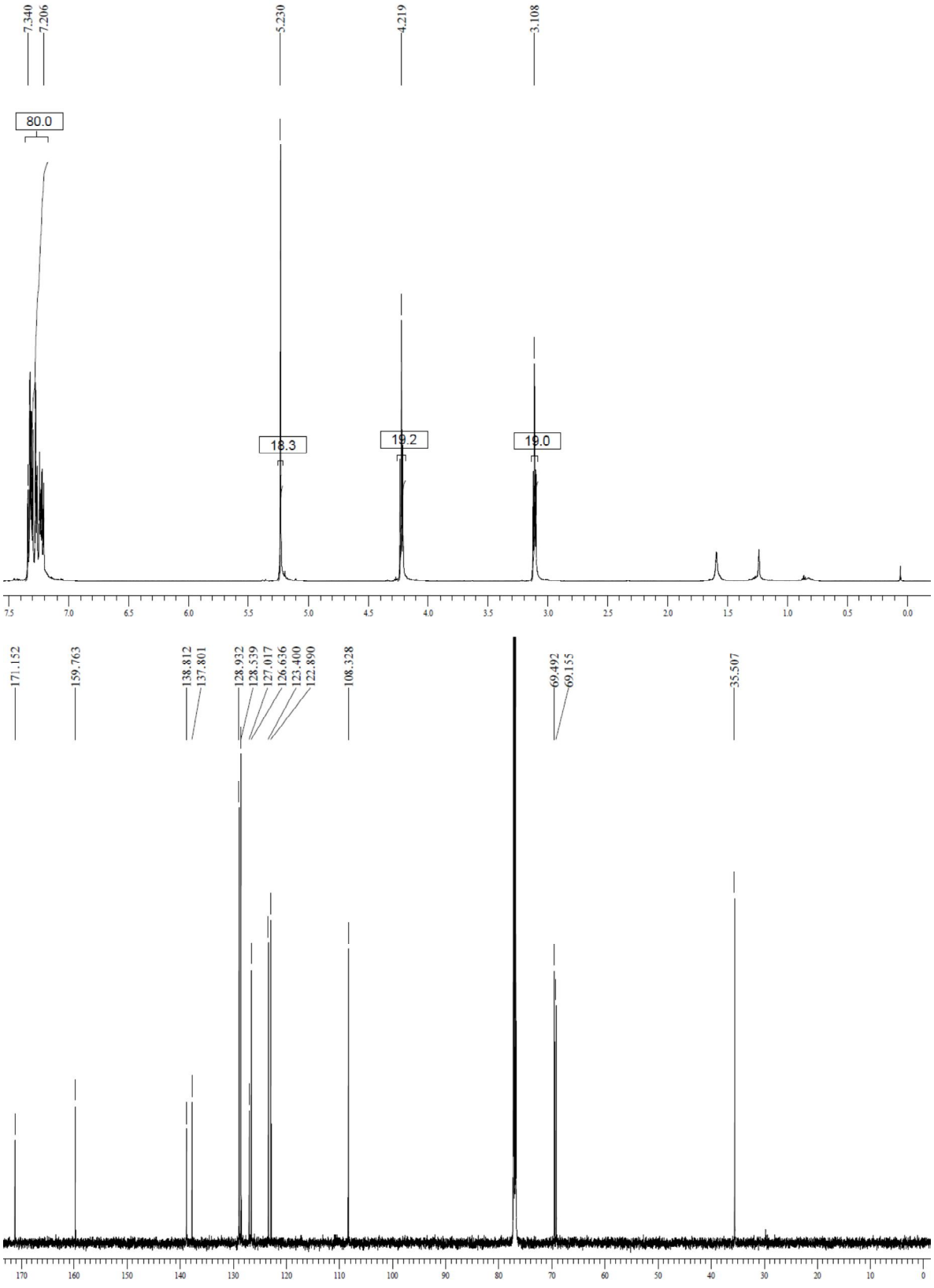

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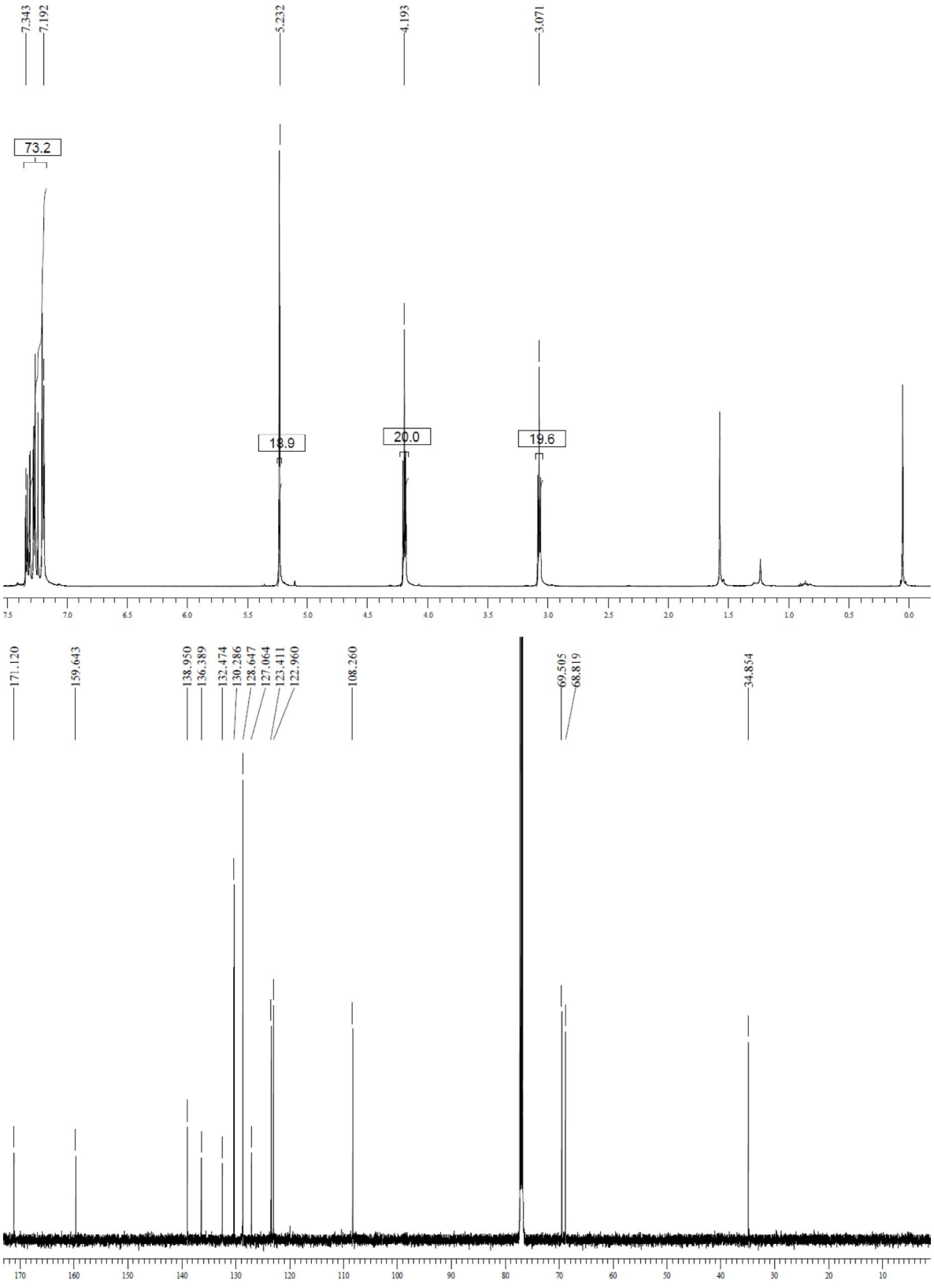
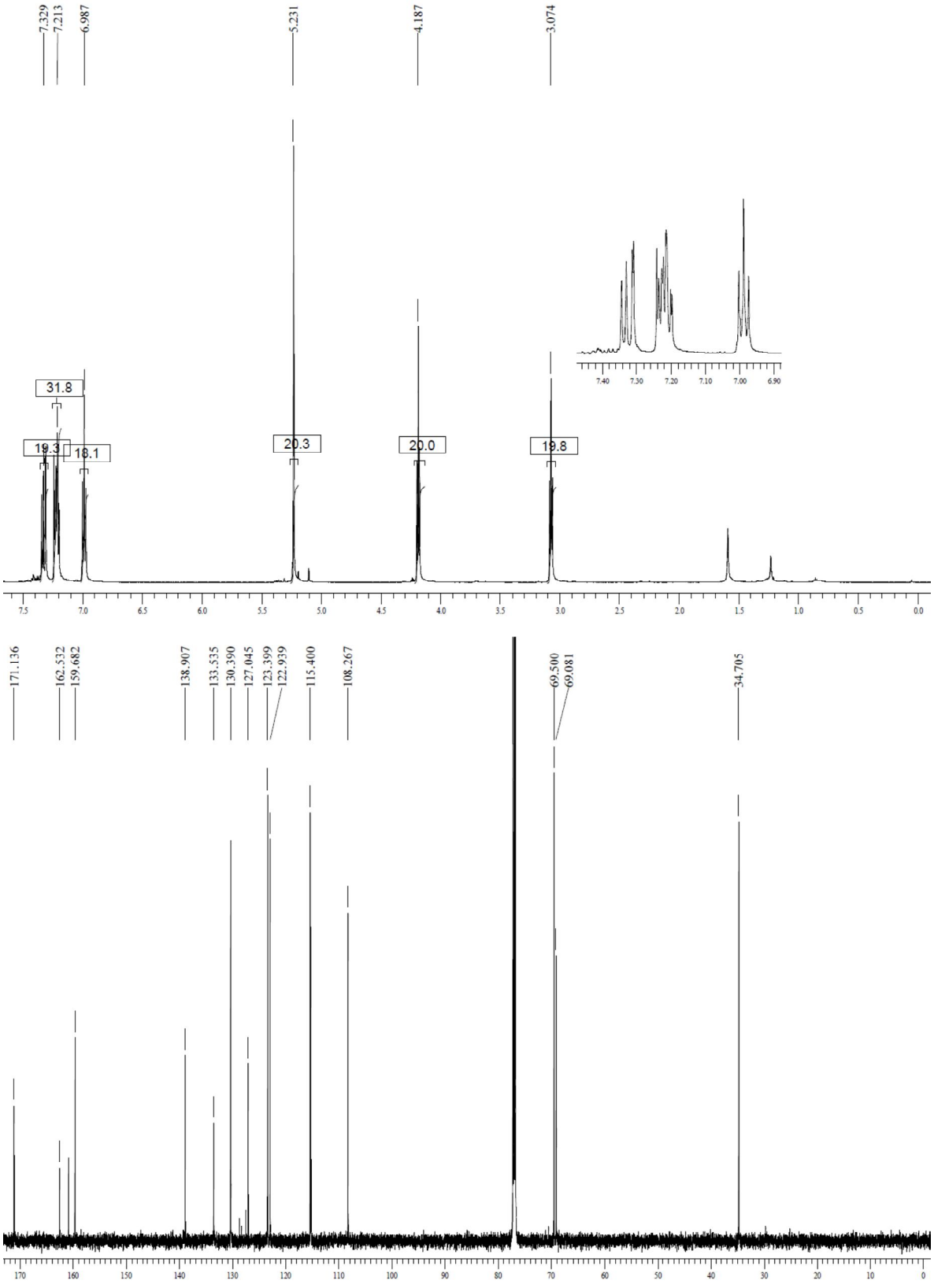


6-[2-(4-Fluorophenyl)ethoxy]phthalide (6k)



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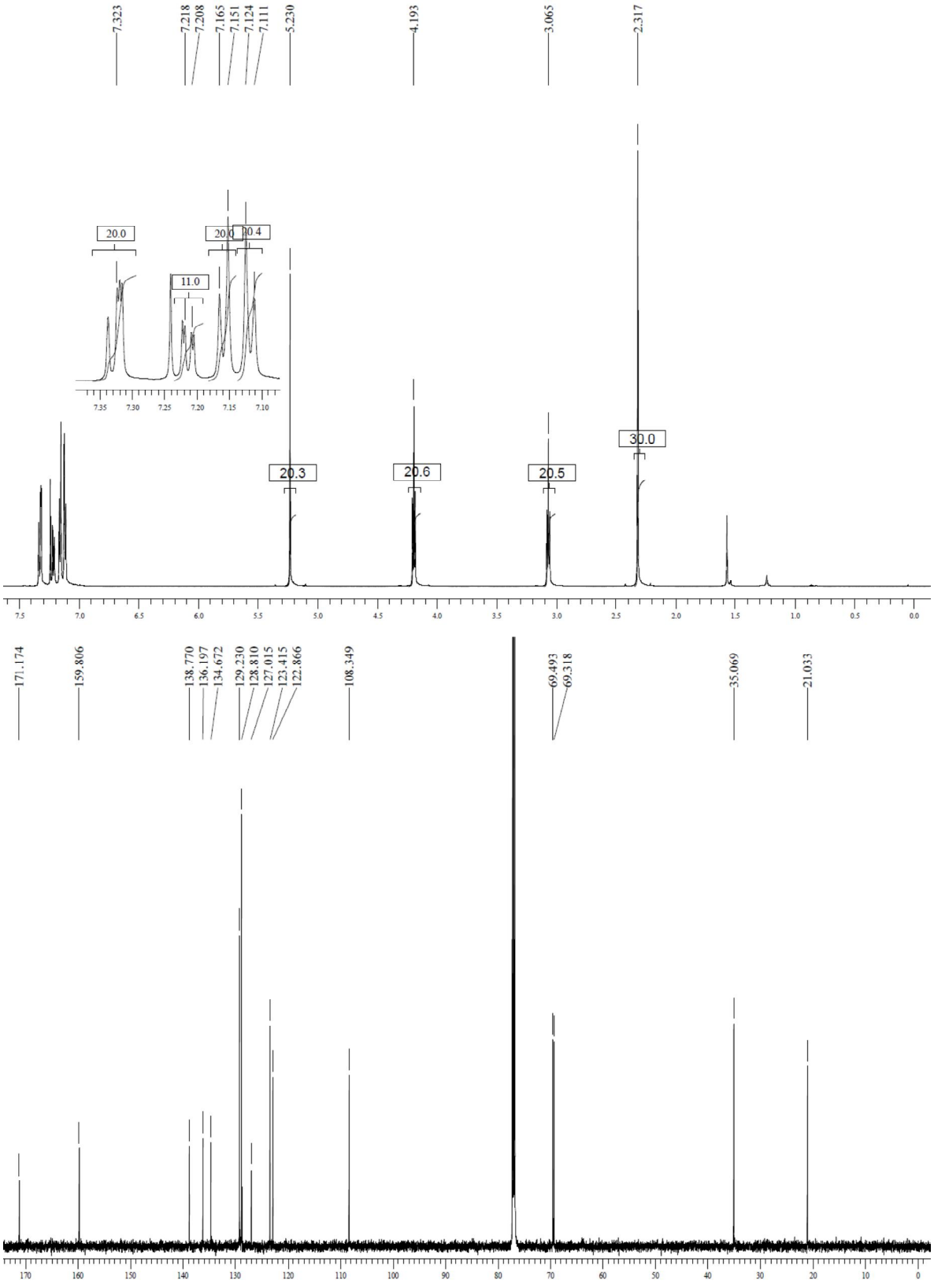

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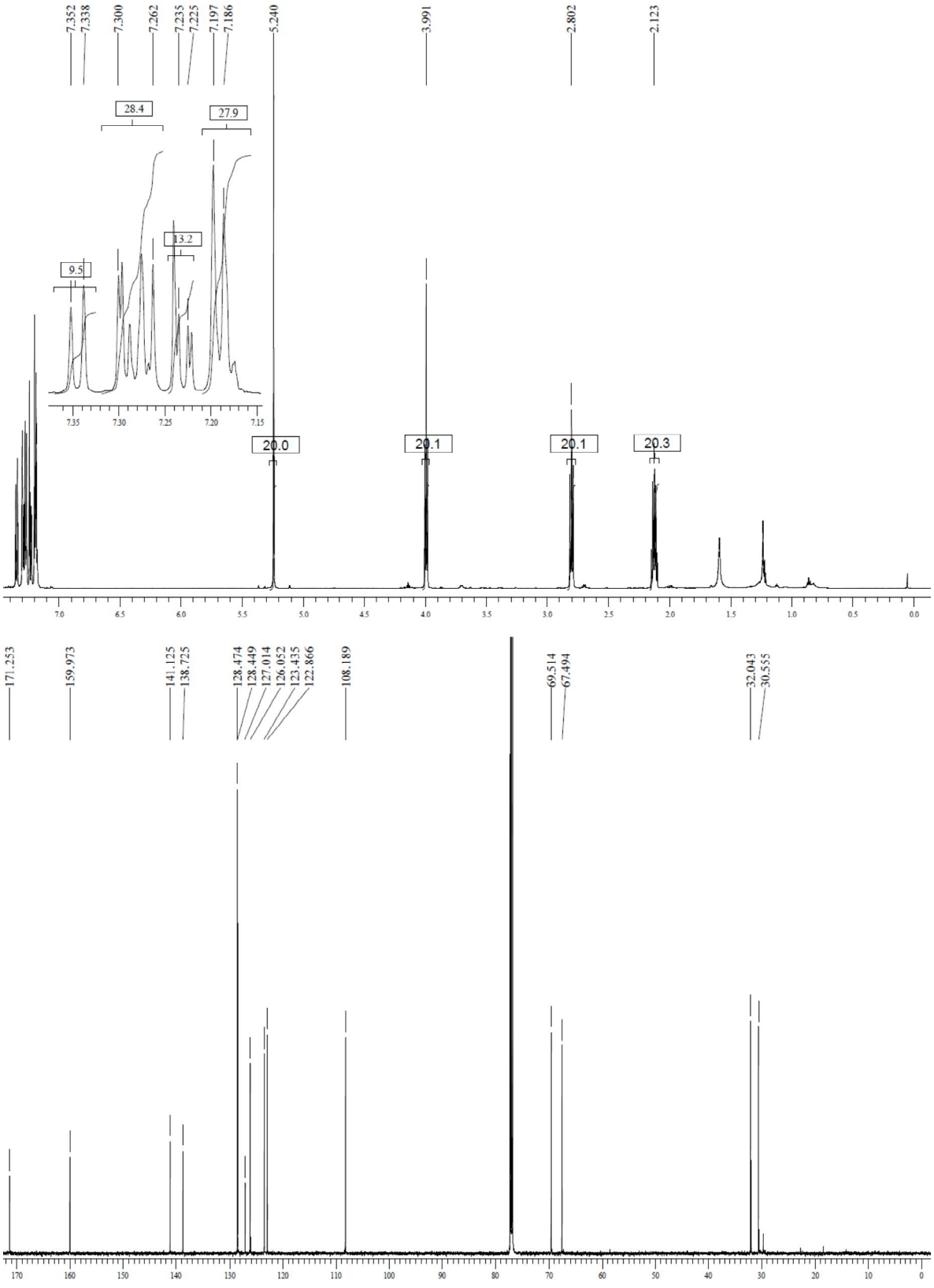

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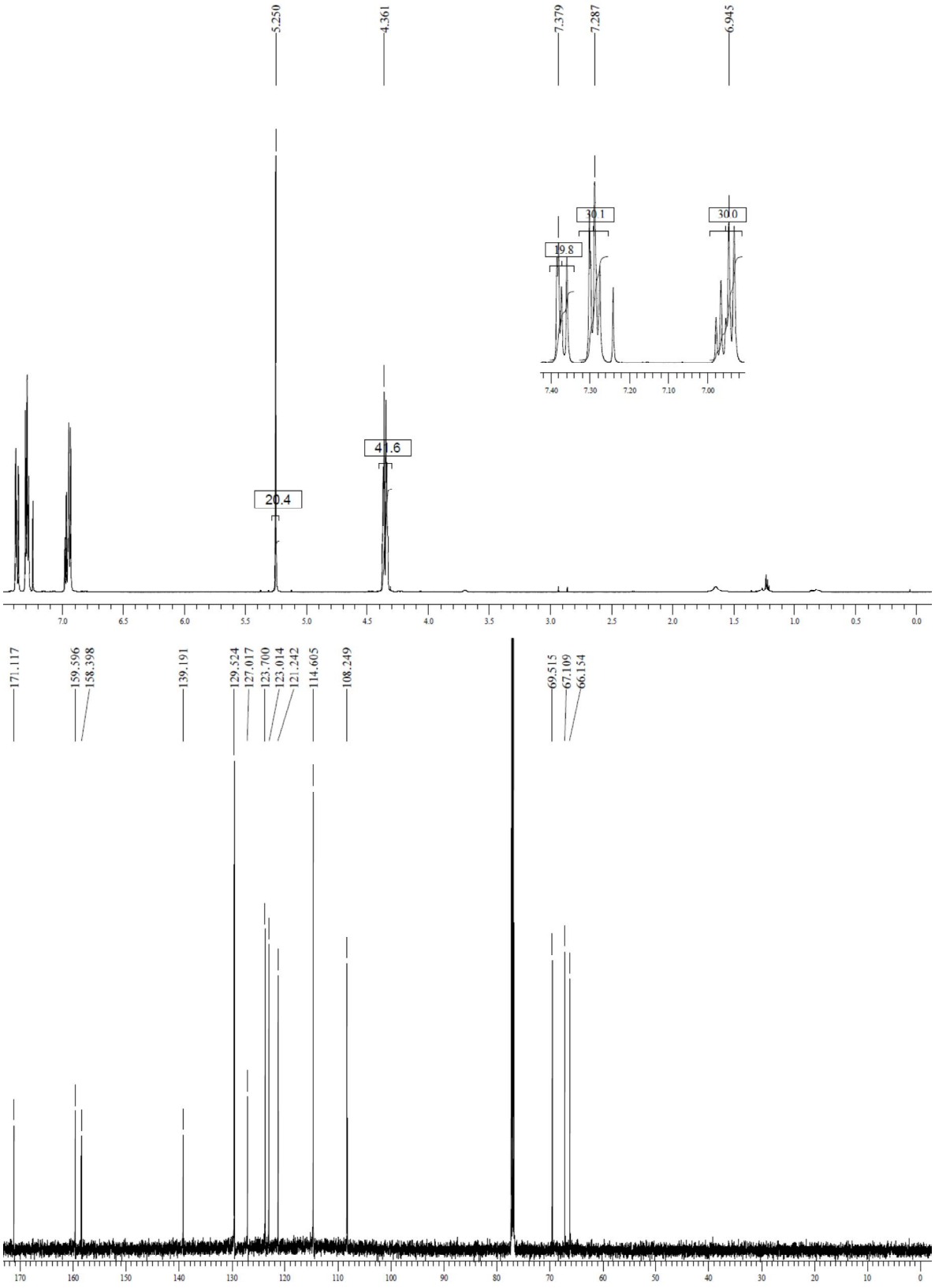

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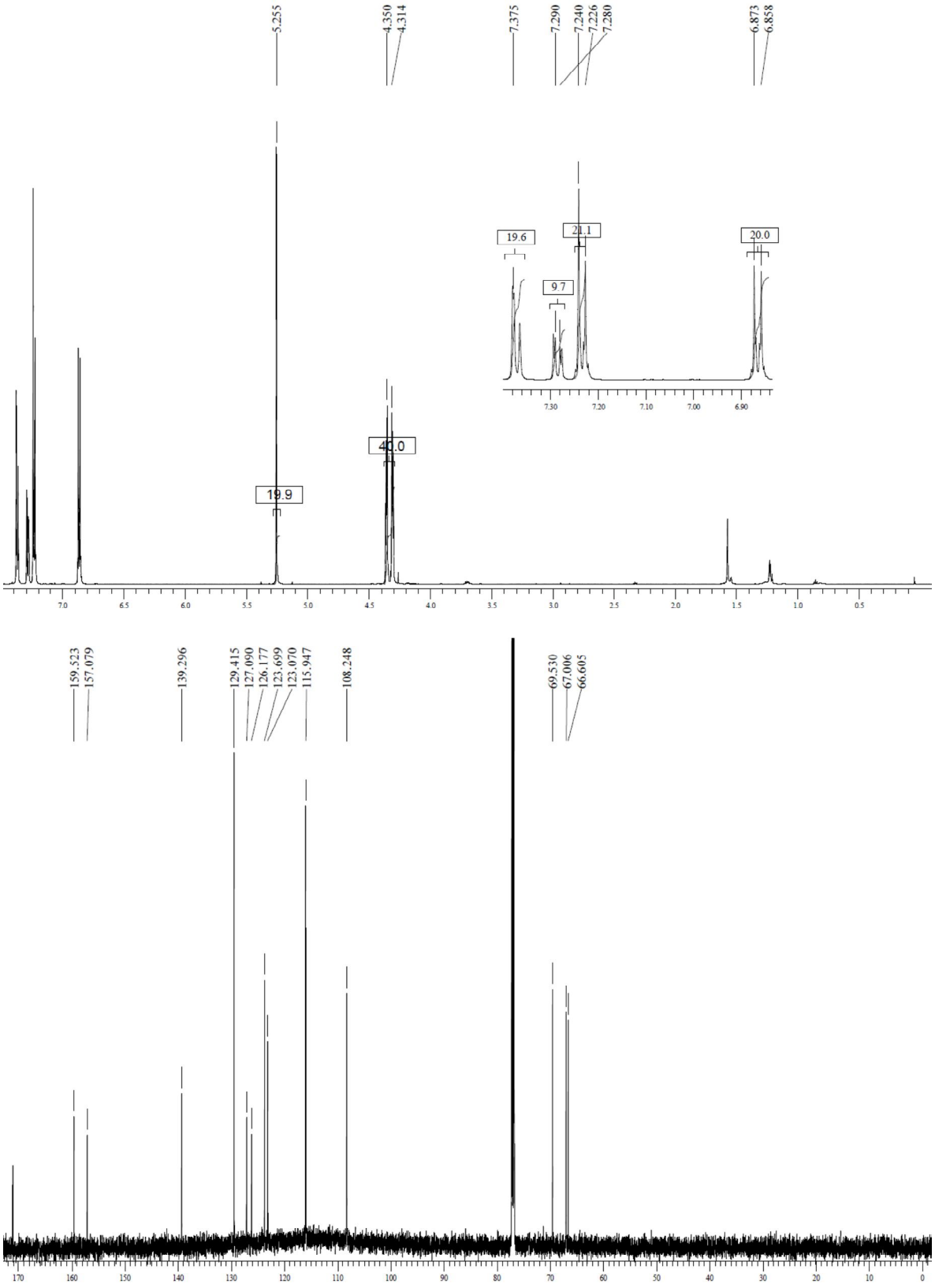
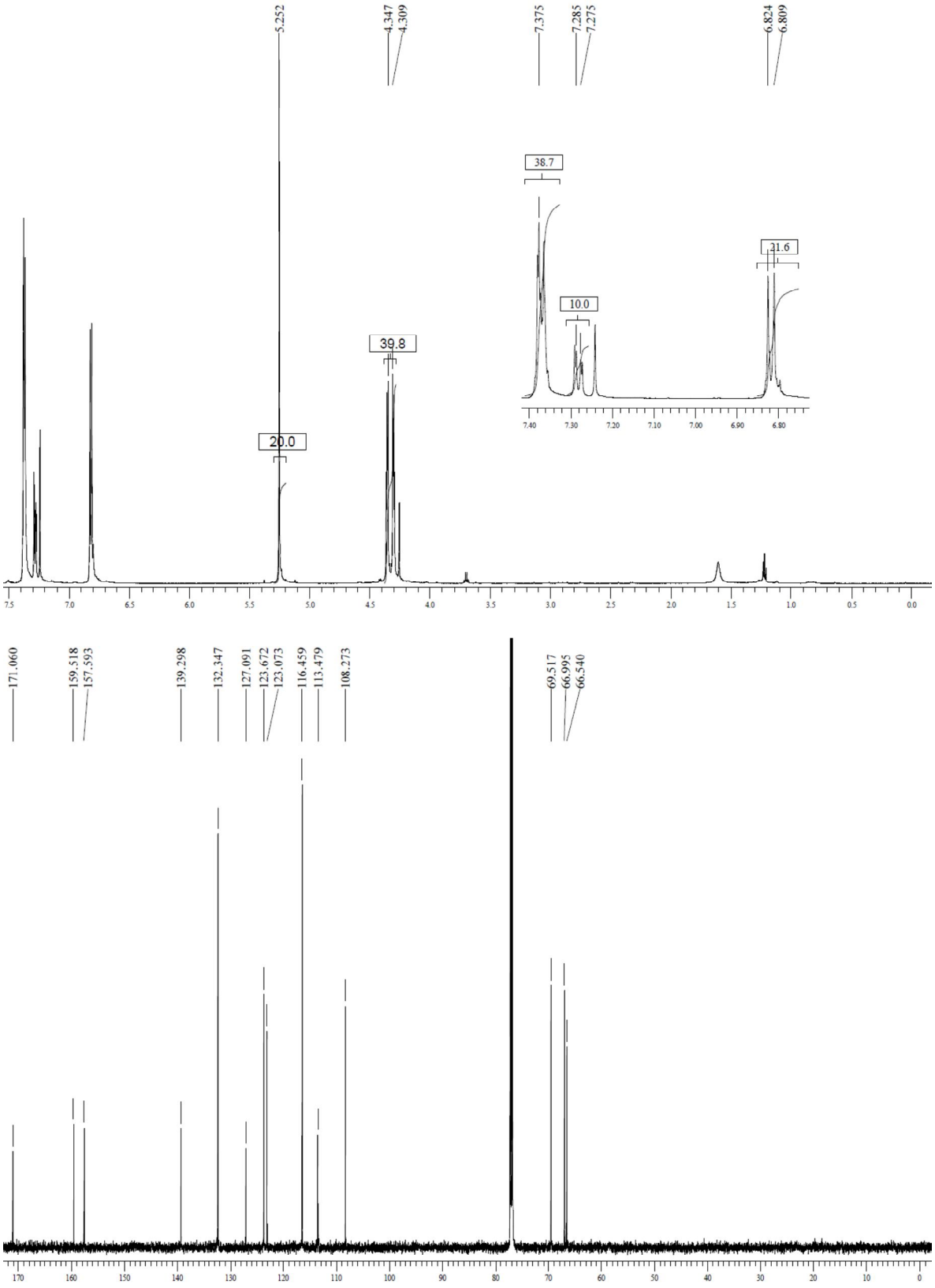
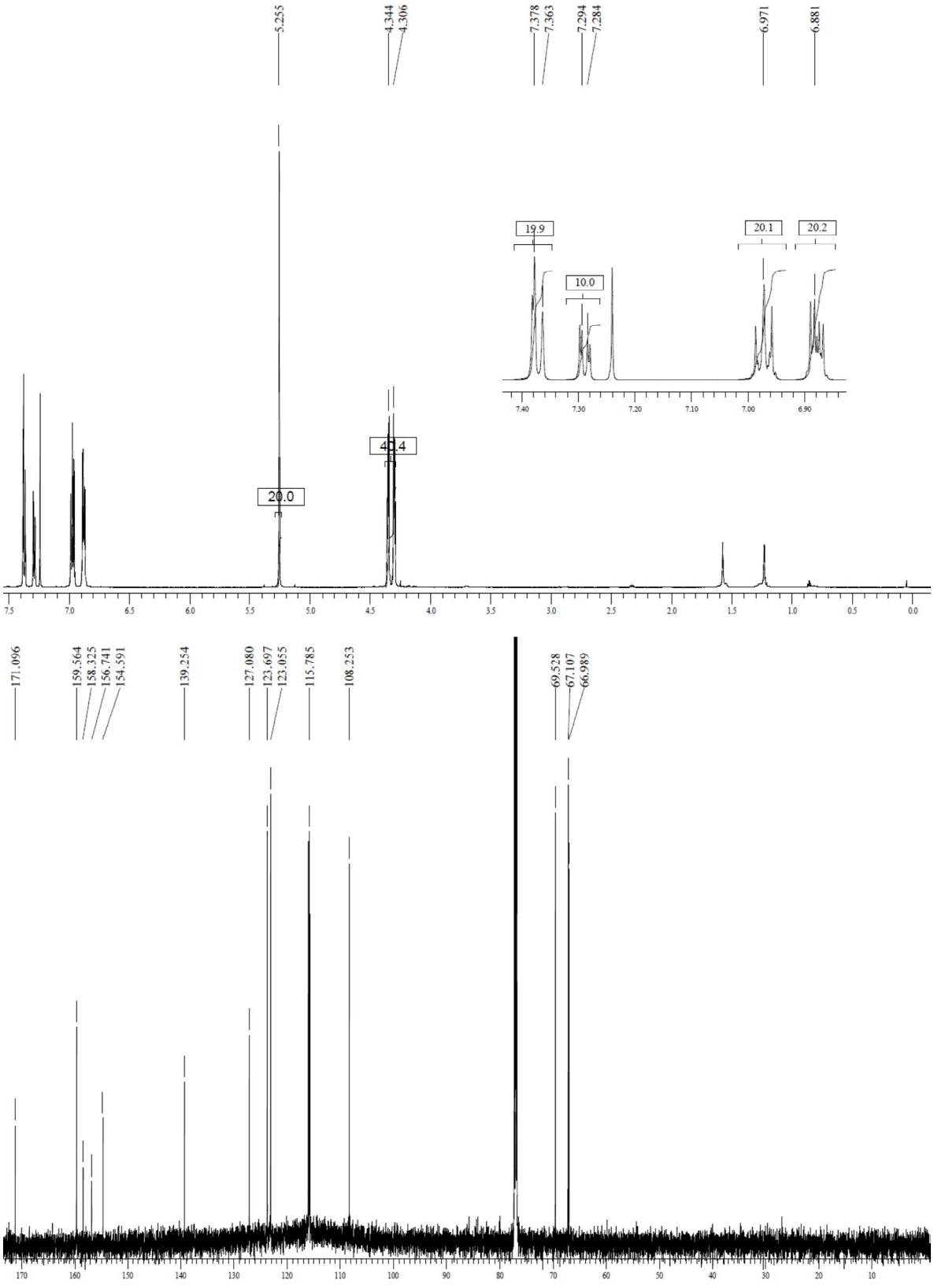


6-[2-(4-Bromophenoxy)ethoxy]phthalide (6p)



6-[2-(4-Fluorophenoxy)ethoxy]phthalide (6q)



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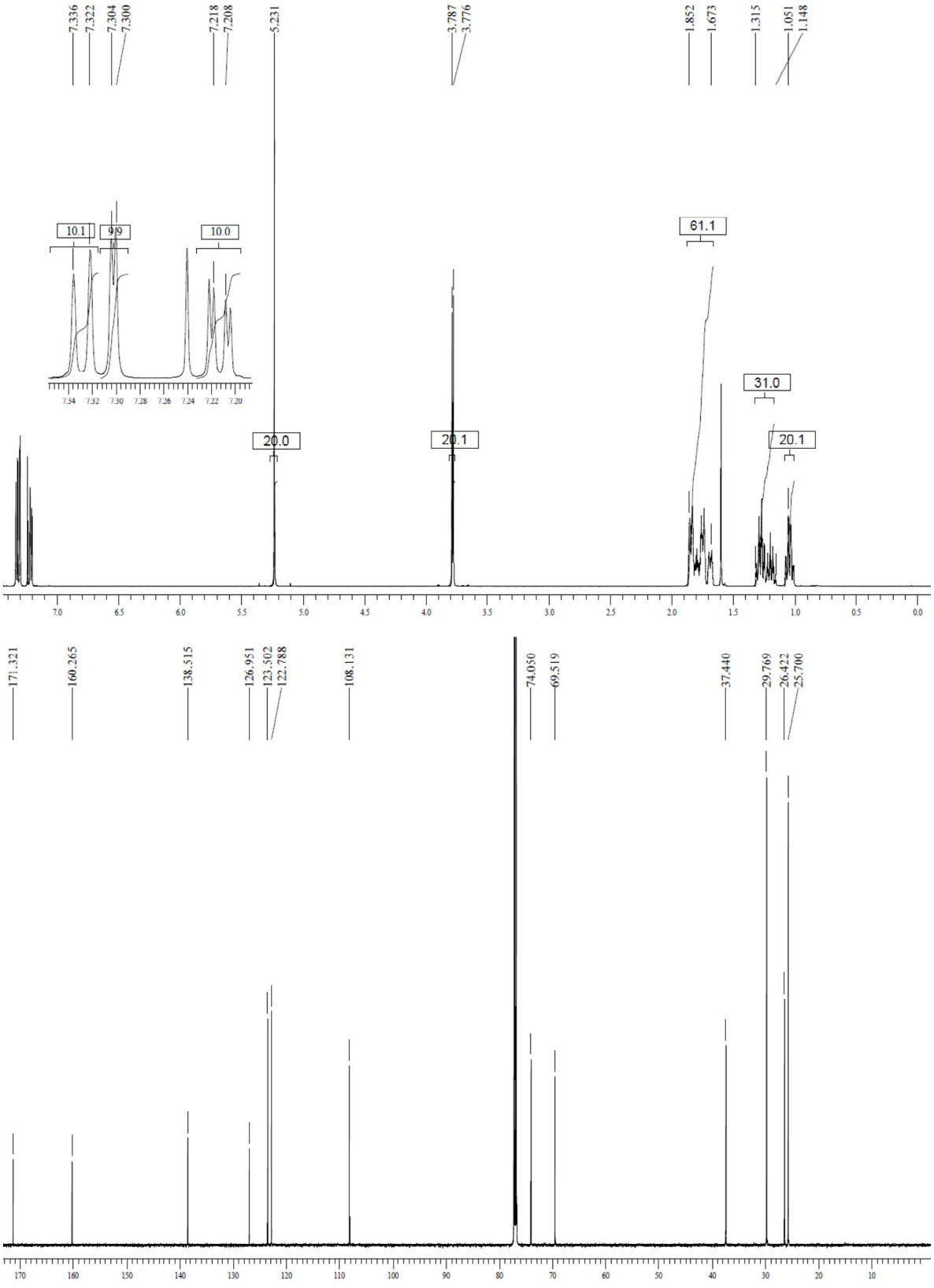
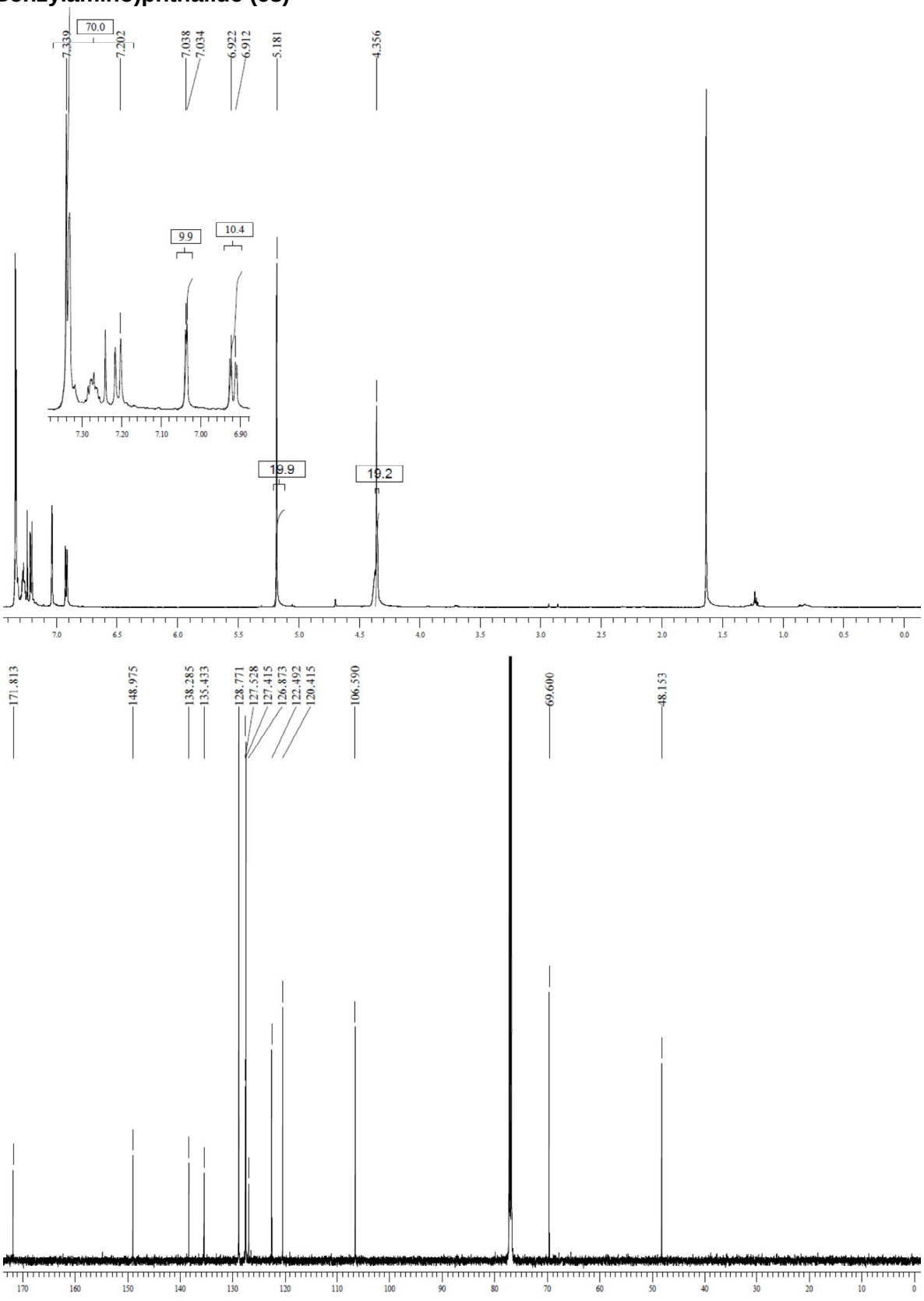


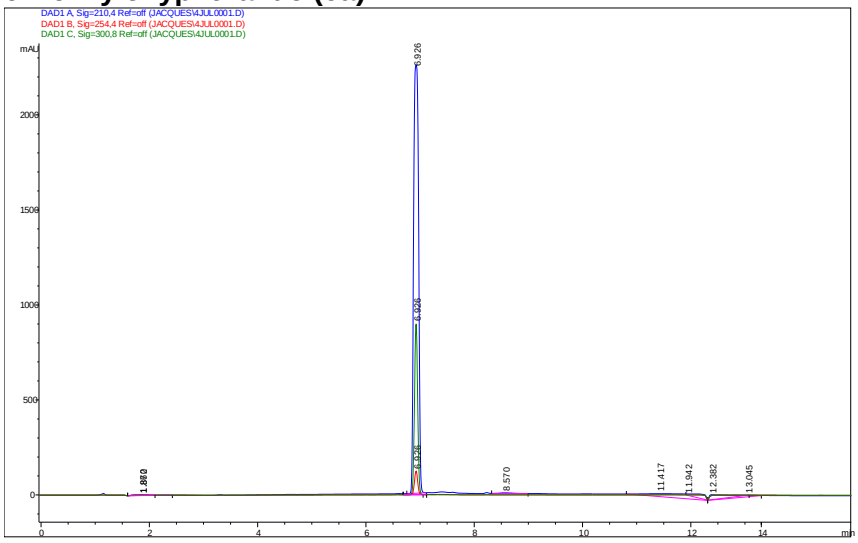
6-(Benzylamino)phthalide (6s)



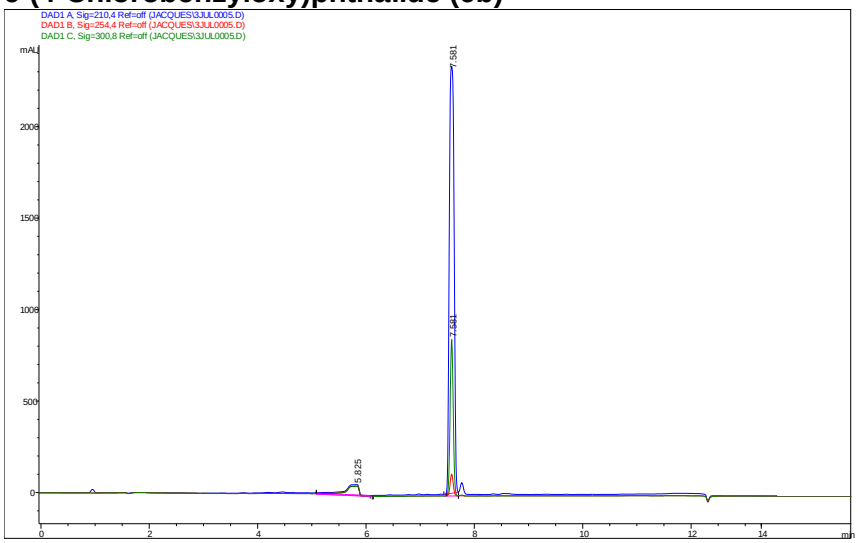
5.3. HPLC traces

The purities of the synthesized compounds were estimated with HPLC analyses. These were carried out with an Agilent 1100 HPLC system equipped with a quaternary pump and an Agilent 1100 series diode array detector. Milli-Q water (Millipore) and HPLC grade acetonitrile (Merck) were used for the chromatography. A Venusil XBP C18 column (4.60 × 150 mm, 5 µm) was used and the mobile phase consisted initially of 30% acetonitrile and 70% MilliQ water at a flow rate of 1 mL/min. At the start of each HPLC run a solvent gradient program was initiated by linearly increasing the composition of the acetonitrile in the mobile phase to 85% acetonitrile over a period of 5 min. Each HPLC run lasted 15 min and a time period of 5 min was allowed for equilibration between runs. A volume of 20 µL of solutions of the test compounds in acetonitrile (1 mM) was injected into the HPLC system and the eluent was monitored at wavelengths of 210, 254 and 300 nm.

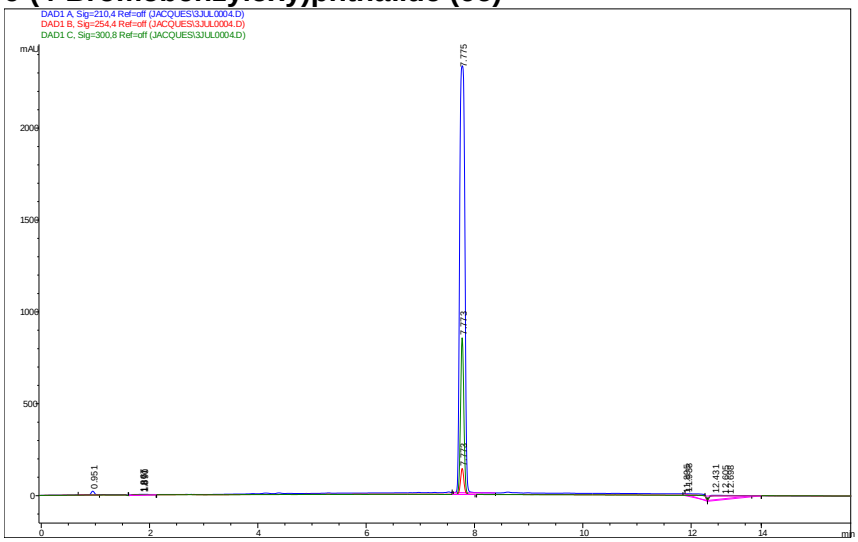
6-Benzyloxyphthalide (6a)



6-(4-Chlorobenzyloxy)phthalide (6b)



6-(4-Bromobenzyloxy)phthalide (6c)



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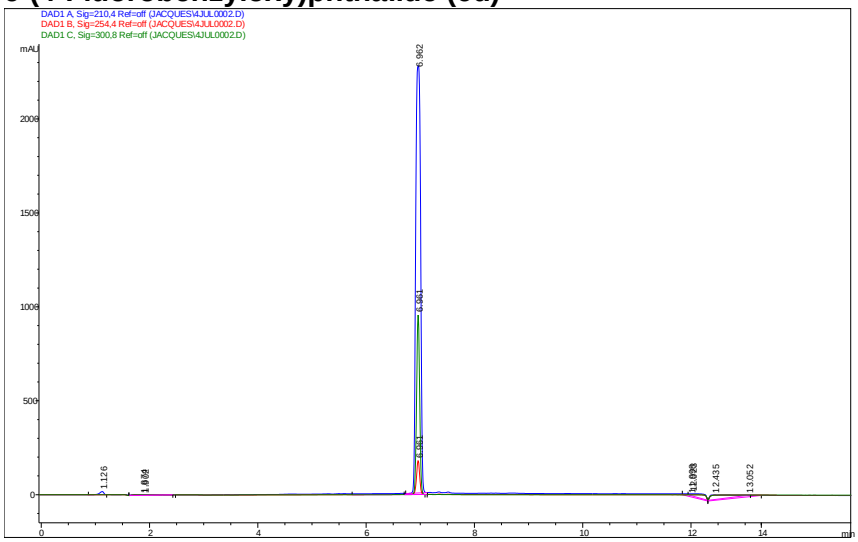
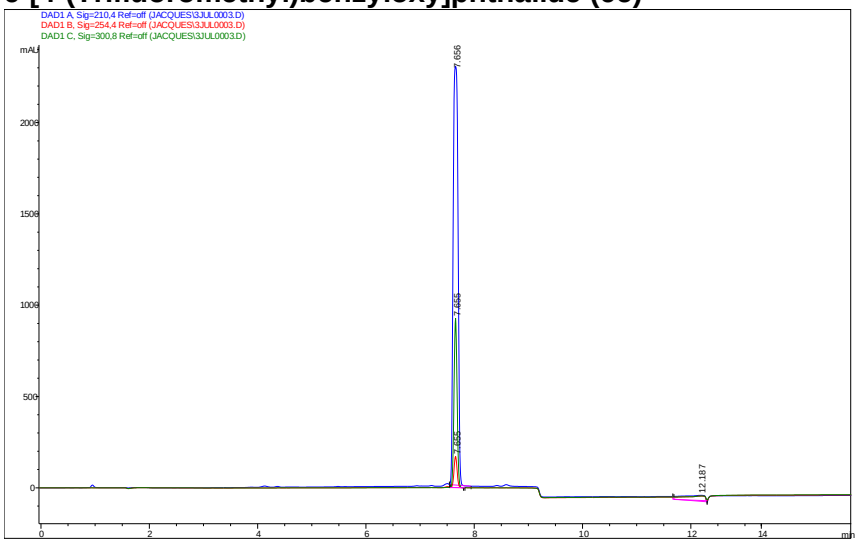
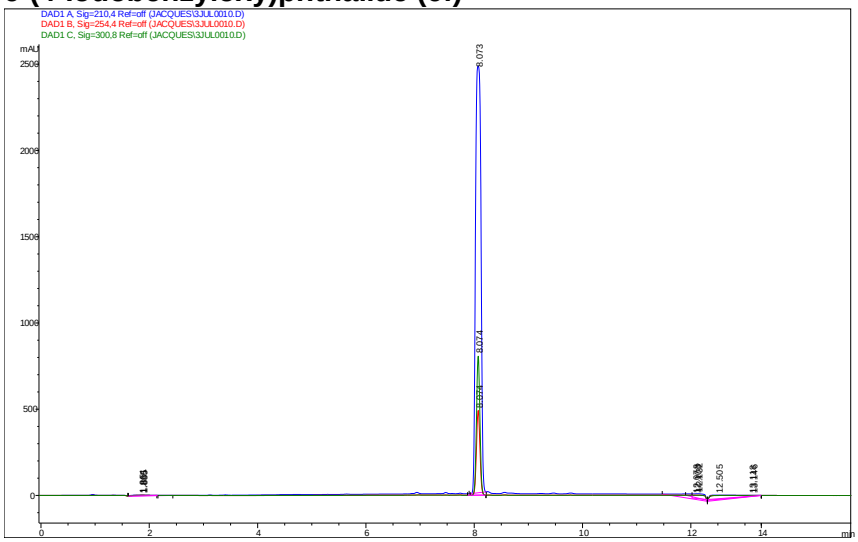


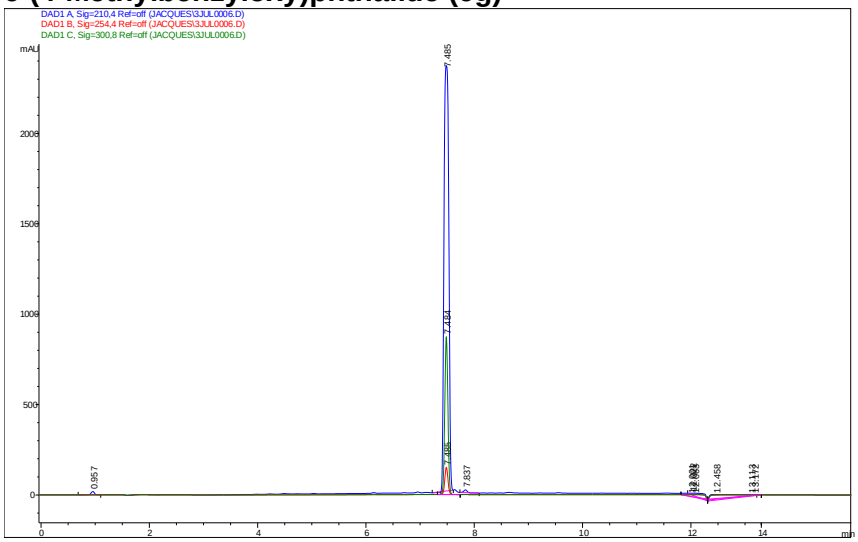
6-[4-(Trifluoromethyl)benzyloxy]phthalide (6e)



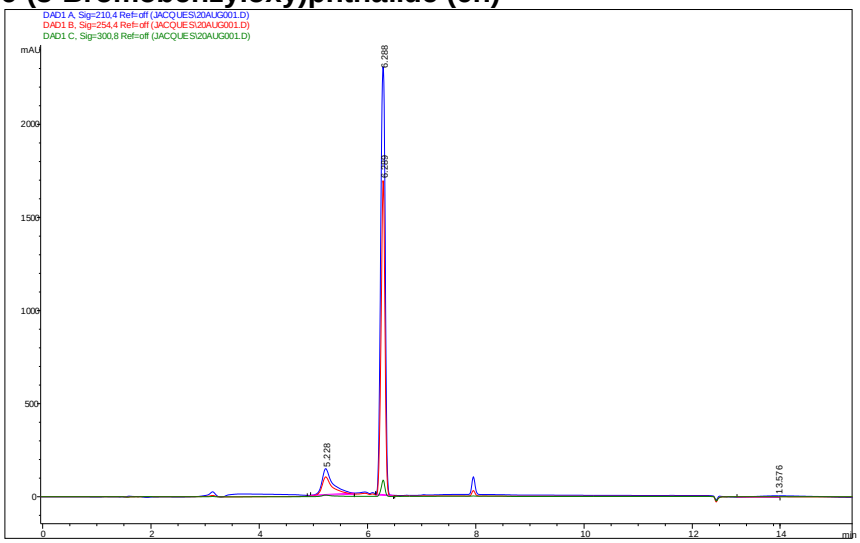
6-(4-Iodobenzyloxy)phthalide (6f)



6-(4-Methylbenzyloxy)phthalide (6g)



6-(3-Bromobenzyloxy)phthalide (6h)



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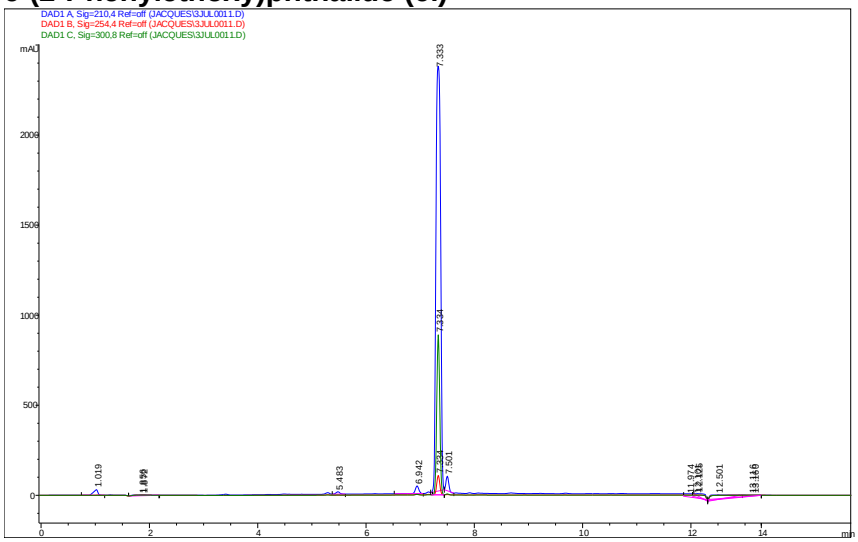
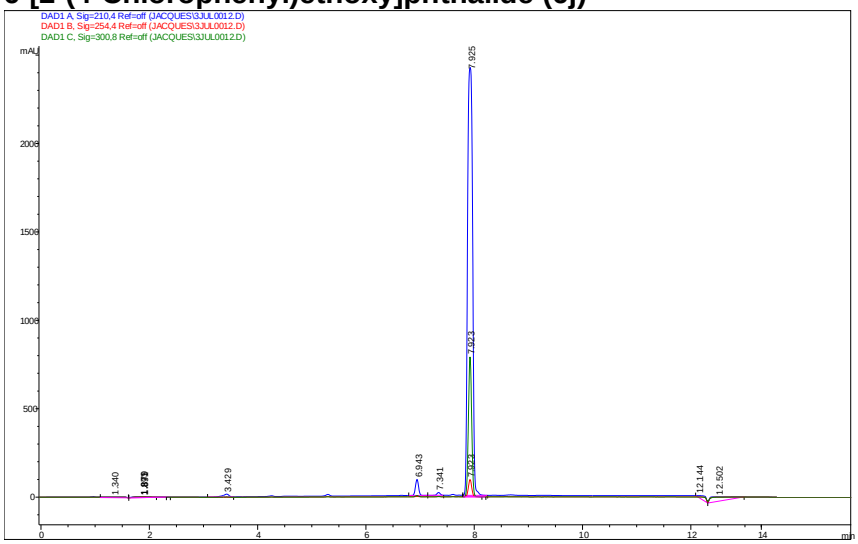


6-[2-(4-Chlorophenyl)ethoxy]phthalide (6j)



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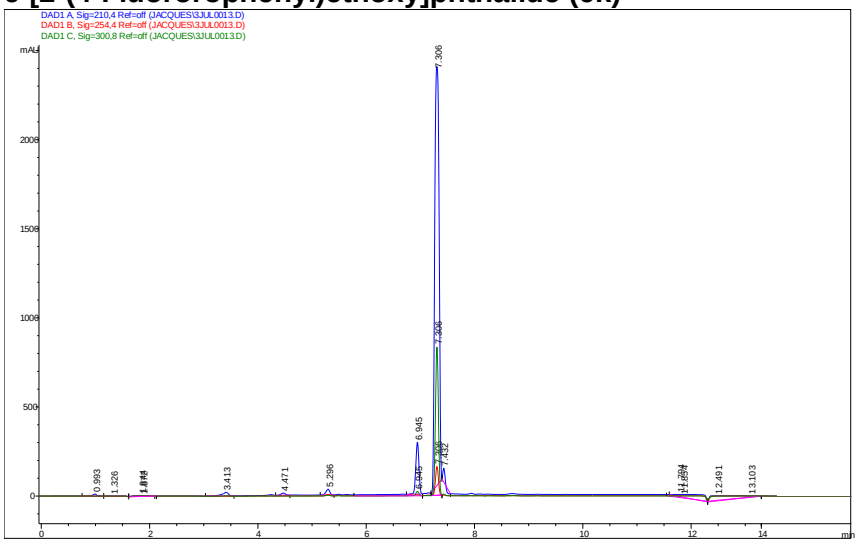

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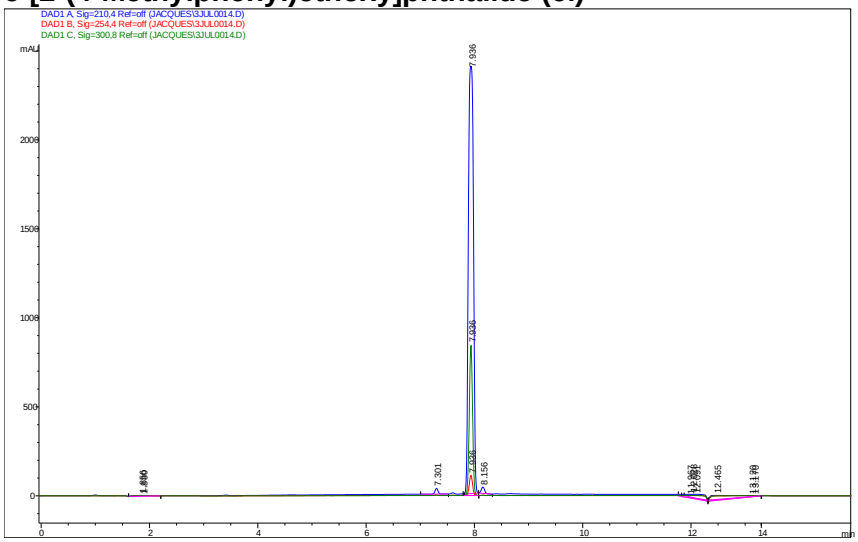

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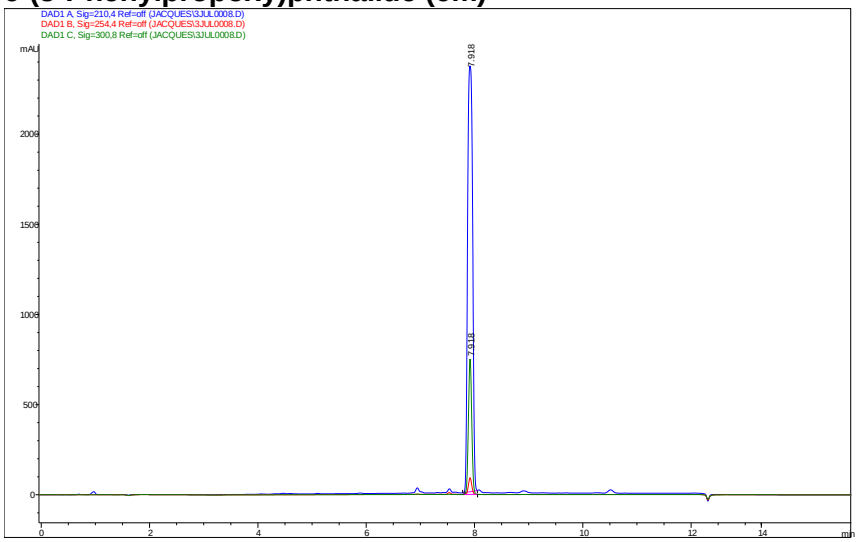

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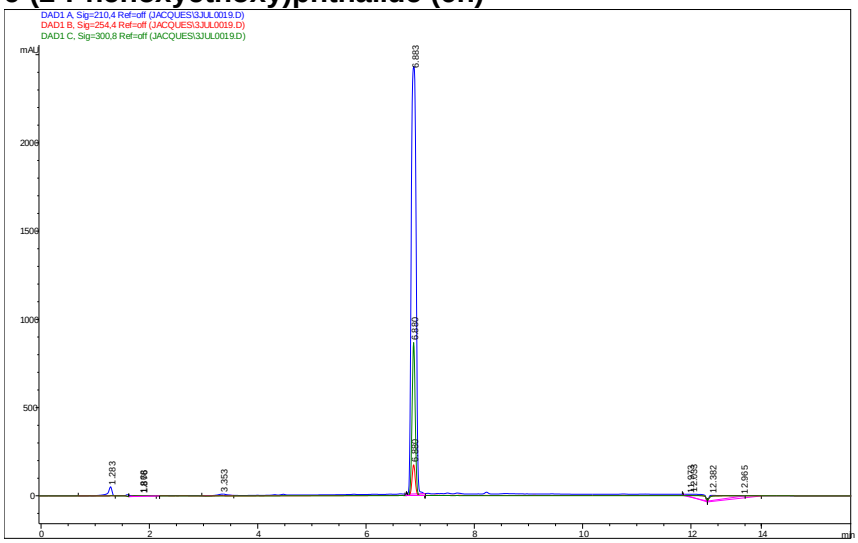

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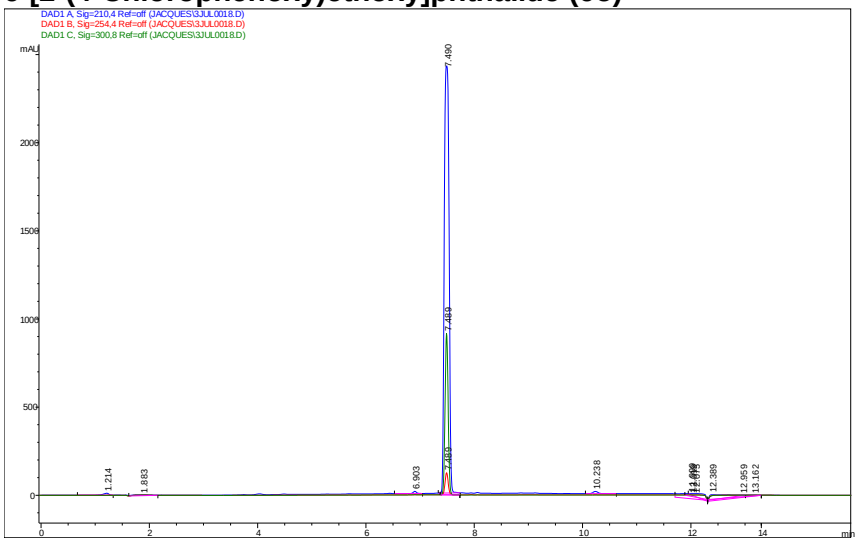

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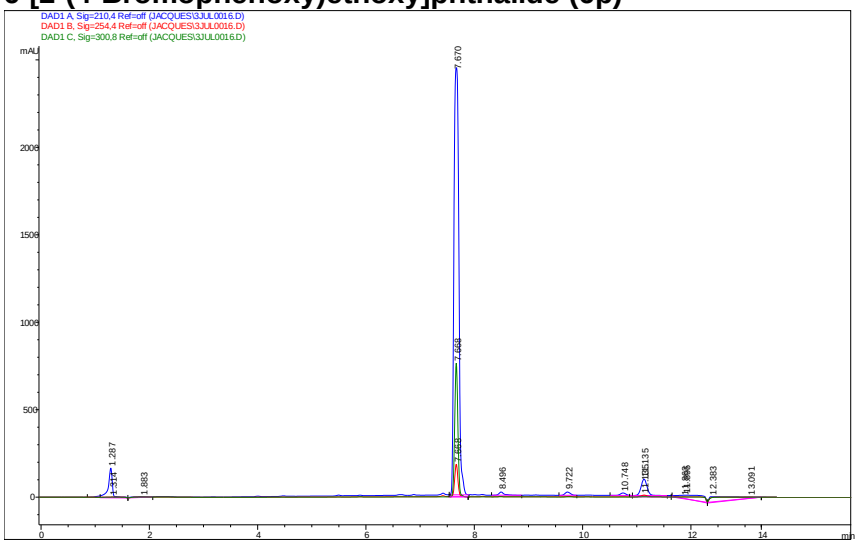

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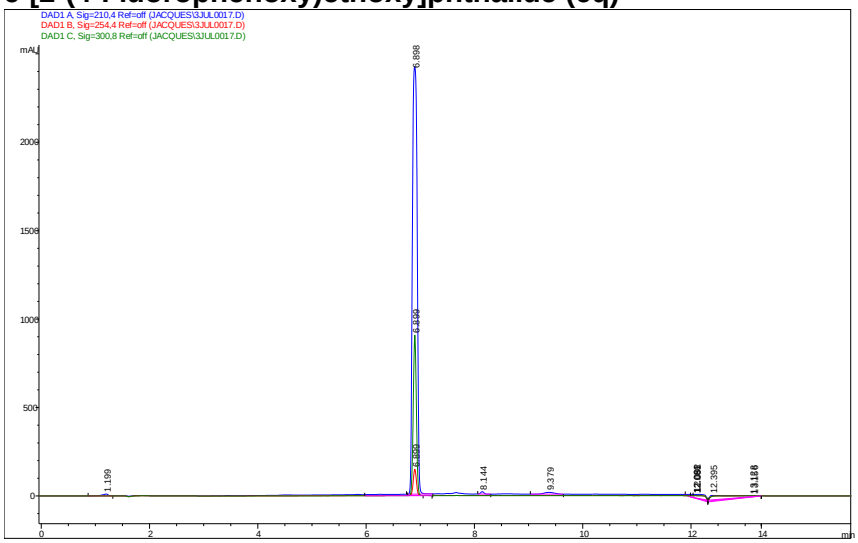

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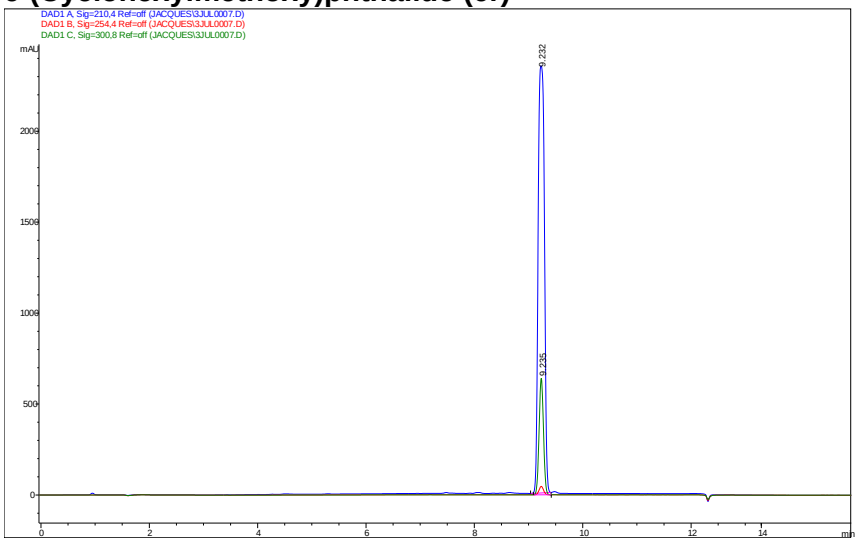

6-(Benzylamino)phthalide (6s)