TÜBİTAK

Turkish Journal of Chemistry

http://journals.tubitak.gov.tr/chem/

Research Article

Turk J Chem
(2018) 42: 808 – 825

doi:10.3906/kim-1709-34

The first synthesis and antioxidant and anticholinergic activities of 1-(4,5-dihydroxybenzyl)pyrrolidin-2-one derivative bromophenols including natural products

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Received: 20.09.2017 • **Accepted/Published Online:** 02.04.2018 • **Final Version:** 01.06.2018

Abstract: We report herein the first synthesis of biologically active natural bromophenols, namely 1-(2,3,6-tribromo-4,5-dihydroxybenzyl)pyrrolidin-2-one (**2**), 2RS-1-(2,3-dibromo-4,5-dihydroxybenzyl)-5-oxopyrrolidine-2-carboxylic acid (**3**), and 2RS-methyl 1-(2,3-dibromo-4,5-dihydroxybenzyl)-5-oxopyrrolidine-2-carboxylate (**4**), along with their different derivatives (**6** and **8**–**18**). Among the synthesized compounds, **3**, **4**, and their derivatives (**8** and **9**) are yielded in racemic form. Radical scavenging assays of the synthesized molecules were investigated using various bioanalytical antioxidant methods including 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) ($ABTS^{\bullet+}$) and 2,2-diphenyl-1-picrylhydrazyl ($DPPH^{\bullet}$). In addition, the reducing power of the novel bromophenols was investigated by Cu^{2+}–Cu^{+} reducing, Fe^{3+}–Fe^{2+} reducing, and $[Fe^{3+}\text{–}(TPTZ)_2]^{3+}$–$[Fe^{2+}\text{–}(TPTZ)_2]^{2+}$ reducing capacity and ferrous ions (Fe^{2+}) chelating abilities. The molecules demonstrated powerful antioxidant activities when compared to standard antioxidant compounds of α-tocopherol, trolox, butylated hydroxyanisole (BHA), and butylated hydroxytoluene (BHT). Moreover, novel bromophenols were tested against cholinergic enzymes including acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) enzymes in the last part of this study. The presented novel bromophenols showed Ki values in the range of 2.60 ± 0.75–16.36 ± 2.67 nM against AChE and 13.10 ± 3.33–54.47 ± 13.53 nM against BChE.

Key words: Bromination, bromophenol, pyrrolidin-2-one, natural product, antioxidant activity, acetylcholinesterase, butyrylcholinesterase

1. Introduction

Natural or synthetic bromophenols exhibit important biological activities such as feeding deterrent[1] antioxidant,[2–4] antimicrobial,[5,6] and inhibition effects on carbonic anhydrase enzyme.[7–12] Naturally occurring bromophenols are abundantly found in marine life and frequently isolated from red algae of the family Rhodomelaceae.[13–15]

Natural bromophenols **1**–**4** were isolated from marine red algae[2,16–19] and exhibited antioxidant activities (Figure).[2,16–19] It was also reported that compound **2** inhibition had ability on protein tyrosine phosphatase 1B.[17,18] The synthesis of compound **1** and its derivatives like racemate as well as their antioxidant activities were firstly reported by our group.[20]

Reactive oxygen species (ROS), which contain free radicals such as hydroxyl radicals ($OH^{\bullet}$), superoxide anion radicals ($O_2^{\bullet-}$), or nonfree radical species such as singlet oxygen ($^1O_2$) and hydrogen peroxide (H_2O_2),

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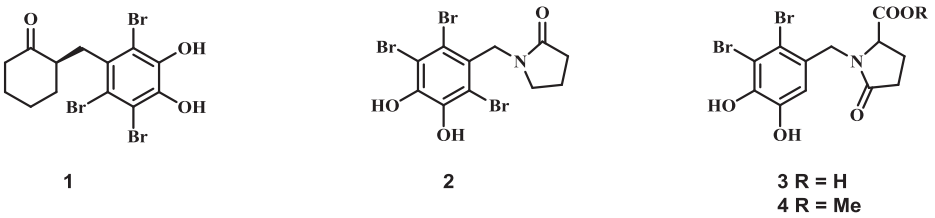


Figure. Some biologically active natural bromophenols.

are diverse types of activated oxygen.[21–23] There is an equilibrium between the formation of ROS molecules and antioxidant defense systems in living organisms.[21] The oxidative stress process occurs when the generation of ROS is higher than the protective ability of the antioxidant defense systems.[24] Under pathological situations, ROS molecules are overproduced and this results in oxidative stress. Additionally, ROS are created when inner antioxidant defenses are incomplete.[25] The imbalance between the antioxidant defense systems and ROS leads to oxidative correction in the intracellular molecules or cellular membrane.[26–28] The oxidative stress has been defined as a key research concern due to its significant role in human diseases such as atherosclerosis, brain dysfunction, and cancer.[29] Antioxidant compounds are bioactive molecules generally used for the protection of cellular compounds in living metabolisms. They maintain food quality and prevent oxidative rancidity.[30–32] Antioxidant molecules eliminate free radicals and protect the human body from cancers, chronic cardiovascular diseases, and ageing by removing or neutralizing ROS.[33]

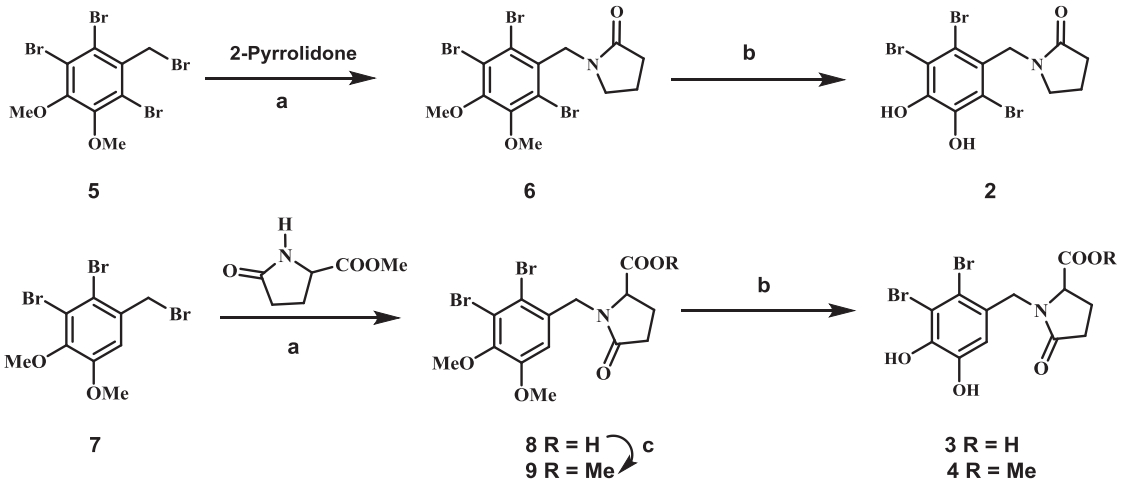
The most commonly used processes to treat Alzheimer disease (AD) are centralized to impede the hydrolysis of acetylcholine (ACh) in cholinergic neurons and hinder synaptic depression. This process is called the cholinergic hypothesis and was recently used in the extension of drugs capable of elevating chemical balance in the slope of the concentration of ACh in patients with AD.[34] AChE performs a main role in ACh hydrolysis in the neural device.[35] The hydrolysis of ACh could be stopped by inhibiting AChE activity. Therefore, different AChE inhibitors (AChEIs) are used in AD therapy. Among them the compound tacrine was the first drug recorded by the Food and Drug Administration (FDA), followed by rivastigmine, galantamine, and donepezil. These compounds as drugs are defined for treating moderate and mild phases of AD, when the patient still enjoys autonomous cognitive acts.[36] AChEIs can be classified as weak or strong inhibitors. Strong inhibitors contain carbamates and organic phosphates, which are frequently used as pesticides and neural toxins, while weak inhibitors have been used in the treatment of Parkinson disease, AD, dementia with Lewy bodies, glaucoma, myasthenia gravis, autism, postural tachycardia syndrome, cognitive disturbances, and insomnia.[37] Cholinergic neurotransmission in the mammalian brain could stop by the hydrolysis of ACh, which is catalyzed by two cholinesterases (ChEs): AChE and BChE. BChE is also used as drug for the therapy of AD at advanced stage. Selective BChE inhibition is significantly beneficial, because it does not have classical cholinergic toxicity, which is a prevalent side effect of AChE inhibitors.[38] It has been recorded that some phenolic molecules had a large potential of biological activity including AChE and BChE inhibition effects and antioxidant properties.[39]

To the best of our knowledge, the synthesis of natural bromophenols **2**–**4** was not reported in the literature previously. The synthesis of them will make a significant contribution to the field because they are natural products and might exhibit potential biological activity such as antioxidant. Therefore, in this study, the synthesis of them and their derivatives was realized and their antioxidant and anticholinergic activities were investigated.

2. Results and discussion

2.1. Chemistry

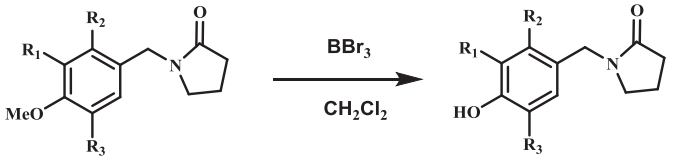
Biologically active natural bromophenols **2**–**4** are N-benzylpyrrolidin-2-one derivatives (Figure). In addition to OH groups in positions 3 and 4 in their benzene rings, three Br in positions 2, 3, and 6 in **2** and two Br in positions 2 and 3 in the others (**3** and **4**) are found. Additionally, COOH and COOMe groups are found in the pyrrolidone rings of **3** and **4**, respectively. For the synthesis of these natural bromophenols **2**–**4**, our method is based on the preparation of benzylic rings with OMe and Br followed by their connection to the corresponding pyrrolidinone rings. Hydroxy (OH) groups are converted to OMe moieties because of the better identification and solubility of the products. It was reported that N-benzyl-2-pyrrolidone could be synthesized from the reaction of benzyl bromide and 2-pyrrolidinone in the presence of KOH and TBAB (tetrabutylammonium bromide).[40] After the synthesis of tetrabromide **5**,[41,42] the reaction of **5** and 2-pyrrolidinone with KOH and TBAB in THF (tetrahydrofurane) did not yield compound **6**. Compound **6** could be synthesized from the reaction of **5** and 2-pyrrolidone with NaH in THF (Scheme 1).



Scheme 1. a) THF-NaH, 0-25 °C, 24 h; b) BBr_3-CH_2Cl_2, 0–25 °C; c) $SOCl_2$ and then MeOH.

For the synthesis of natural bromophenols **3** and **4**, tribromide **7** was obtained from the corresponding reactions using vanillin as starting material.[20,41,42] The reaction of synthesized methyl 5-oxopyrrolidine-2-carboxylate in the basic condition only gave **8**, precursor compounds of natural products **3** and **4**. To use compound **9** in the demethylation reaction of natural products **3** and **4**, compound **8** was also converted into compound **9**. Reactions of each compound **6**, **8**, and **9** with BBr_3 in CH_2Cl_2 gave the natural bromophenols **2**, **3**, and **4**, respectively.

Derivatives with different numbers of Br, without Br and OMe of biologically active natural bromophenol **2** will be important because they might show biological activities. For the synthesis of derivatives **10**–**14**, corresponding bromides **5** and **7** were synthesized by known methods.[20,41–44] The derivatives **10**–**14** were obtained from the reactions of these bromides and 2-pyrrolidinone with NaH in THF (Scheme 2). Similar to the synthesis of bromophenol **2** from the reaction of **6** with BBr_3, phenol derivatives **15**–**18** were obtained by demethylations of derivatives **10**–**13** (Scheme 2). The corresponding phenol derivative could not be obtained from the demethylation reaction of **14** with BBr_3.



10 R_1, R_2 = Br, R_3 = OMe
11 R_1 = Br, R_2 = H, R_3 = OMe
12 R_2 = Br, R_1 = H, R_3 = OMe
13 R_1, R_2 R_3 = H
14 R_1, R_2 = H, R_3 = Br

15 R_1, R_2 = Br, R_3 = OH
16 R_1 = Br, R_2 = H, R_3 = OH
17 R_2 = Br, R_1 = H, R_3 = OH
18 R_1, R_2 R_3 = H

Scheme 2. Some derivatives of natural bromphenol **2** and their synthesis.

2.2. Biological activities

2.2.1. Antioxidant results

Antioxidant activity, which reflected the ability of pure compounds, can inhibit the oxidation process in living systems. In this study, several different antioxidant assays based on different reaction mechanisms were used to detect the potent antioxidant activity of novel 1-(4,5-dihydroxybenzyl)pyrrolidin-2-one derivative bromophenols. The following results should be noted regarding the inhibition of CA, AChE, and BChE enzymes and antioxidant activities of novel bromophenols:

i. The first antioxidant assay performed is based on the Fe^{3+} reduction method. Generally, reducing properties of a compound depend on its reducing power, which have been shown to exert antioxidant activity and radical scavenging ability by donating a hydrogen atom.[45] The $Fe^{3+}(CN^-)_6$ reduction procedure detects the antioxidant effect of any molecule as measuring reducing ability in the reaction. Some 1-(4,5-dihydroxybenzyl)pyrrolidin-2-one derivative bromophenols including natural products of **2**–**4** and some of the other compounds (**15**–**18**) had potent reducing activity using $Fe^{3+}(CN^-)_6$ when compared with the standards (α-tocopherol, trolox, BHT, and BHA). It can be seen in Table 1 that the Fe^{3+} reducing capacity of the newly synthesized compounds of **2**–**4**, **15**–**18**, and the standard compounds was in the following order: BHA (2.322 ± 0.024, r^2: 0.9685) > **4** (1.606 ± 0.006, r^2: 0.9881) > **15** (1.573 ± 0.008, r^2: 0.9638) > **17** (1.520 ± 0.014, r^2: 0.9391) > **2** (1.436 ± 0.049, r^2: 0.9424) > BHT (1.425 ± 0.007, r^2: 0.9770) > **16** (1.266 ± 0.012, r^2: 0.9037) > **3** (1.177 ± 0.011, r^2: 0.9740) > trolox (1.123 ± 0.009, r^2: 0.9889) > α-tocopherol (1.005 ± 0.005, r^2: 0.9990) > **18** (0.716 ± 0.004, r^2: 0.9975) at the same concentration (20 μg/mL). The reducing power of bioactive compounds reflects their electron-donating capacity and is associated with antioxidant activity.

ii. Cupric ion (Cu^{2+}) reducing power of 20 μg/mL concentration of novel 1-(4,5-dihydroxybenzyl)pyrrolidin-2-one derivative bromophenols and standard reducing agents is shown in Table 1. The results of cupric ion (Cu^{2+}) reducing power were similar to the results obtained from the $Fe^{3+}(CN^-)_6$ reduction method. Cu^{2+} reducing capacity of compounds of **2**–**4** and some of the other compounds (**15**–**18**) and standards decreased in the following order: BHA (2.323 ± 0.006, r^2: 0.9846) > BHT (2.204 ± 0.027, r^2: 0.9455) > **3** (2.116 ± 0.013, r^2: 0.9775) > **17** (2.115 ± 0.014, r^2: 0.9563) > **4** (2.064 ± 0.008, r^2: 0.9794) > **15** (1.857 ± 0.004, r^2: 0.9550) > **2** (1.806±0.005, r^2: 0.9744) ≈ **16** (1.782 ± 0.006, r^2: 0.9684) > trolox (1.208±0.005, r^2: 0.9845) > α-tocopherol (1.123 ± 0.006, r^2: 0.9787) > **18** (0.640 ± 0.003,

Table 1. Determination of reducing power of the same concentration (20 μg/mL) of novel bromophenols and their methylated precursors by FRAP methods, ferric ion (Fe^{3+}) reducing and cupric ion (Cu^{2+}) reducing capacity by CUPRAC method.

Antioxidants	Fe^{3+}-Fe^{2+} reducing		Cu^{2+}-Cu^{+} reducing		Fe^{3+}-TPTZ reducing	
	λ_{700}	R^2	λ_{450}	R^2	λ_{593}	R^2
BHA	2.322 ± 0.024	0.9685	2.323 ± 0.006	0.9846	2.637 ± 0.018	0.9575
BHT	1.425 ± 0.007	0.9770	2.204 ± 0.027	0.9455	1.763 ± 0.008	0.9788
$\alpha-$Tocopherol	1.005 ± 0.005	0.9990	1.123 ± 0.006	0.9787	1.678 ± 0.018	0.9262
Trolox	1.123 ± 0.009	0.9889	1.208 ± 0.005	0.9845	2.786 ± 0.018	0.9528
2	1.436 ± 0.049	0.9424	1.806 ± 0.005	0.9744	2.003 ± 0.004	0.9978
3	1.177 ± 0.011	0.9740	2.116 ± 0.013	0.9775	1.990 ± 0.009	0.9788
4	1.606 ± 0.006	0.9881	2.064 ± 0.008	0.9794	2.582 ± 0.010	0.9207
6	0.314 ± 0.012	0.9615	0.480 ± 0.008	0.9969	0.418 ± 0.006	0.9585
8	0.207 ± 0.005	0.9775	0.280 ± 0.002	0.9889	0.656 ± 0.010	0.9627
9	0.297 ± 0.008	0.9832	0.296 ± 0.003	0.9909	0.485 ± 0.007	0.9774
10	0.287 ± 0.008	0.9979	0.253 ± 0.030	0.9108	0.693 ± 0.019	0.9538
11	0.225 ± 0.007	0.9919	0.298 ± 0.005	0.9757	0.393 ± 0.006	0.9538
12	0.242 ± 0.008	0.9440	0.280 ± 0.003	0.9976	0.345 ± 0.011	0.9901
13	0.223 ± 0.005	0.9885	0.305 ± 0.005	0.9844	0.345 ± 0.007	0.9770
14	0.203 ± 0.006	0.9920	0.217 ± 0.007	0.9909	0.376 ± 0.011	0.9507
15	1.573 ± 0.008	0.9638	1.857 ± 0.004	0.9550	1.834 ± 0.006	0.9696
16	1.266 ± 0.012	0.9037	1.782 ± 0.006	0.9684	1.747 ± 0.009	0.9708
17	1.520 ± 0.014	0.9391	2.115 ± 0.014	0.9563	2.204 ± 0.006	0.9879
18	0.716 ± 0.004	0.9975	0.640 ± 0.003	0.9794	0.708 ± 0.023	0.9251

r^2: 0.9794) at the same concentration (20 μg/mL). The CUPRAC assay is a simple, rapid, cost-effective, selective, steady, and versatile antioxidant assay useful for a wide variety of polyphenols, as well as for thiols and synthetic antioxidants.[46] The *ortho*-substitution of HO^- groups with electron-donating groups like methoxy groups (–OCH_3) can also increase antioxidant activity. For example the –CH=CH–COOH groups in cinnamic acid can produce greater H-donating ability and subsequent antioxidant activity than the –COOH groups in benzoic acids through stabilizing the radical by resonance of –C=C–.

iii. The FRAP method measures the capability of antioxidants to reduce the ferric 2,4,6-tripyridyl-s-triazine complex $[Fe(TPTZ)_2]^{3+}$ to the intensely blue colored ferrous complex $[Fe(TPTZ)_2]^{2+}$ in acidic medium.[47] According to the results obtained from the FRAP assay (Table 1) the reducing power of novel 1-(4,5-dihydroxybenzyl)pyrrolidin-2-one derivative bromophenols **2**–**4** and **15**–**18** and standards decreased in the following order: trolox (2.786 ± 0.018, r^2: 0.9528) > BHA (2.637 ± 0.018, r^2: 0.9575) > **4** (2.582 ± 0.010, r^2: 0.9207) > **17** (2.204 ± 0.006, r^2: 0.9879) > **2** (2.003 ± 0.004, r^2: 0.9978) > **3** (1.990 ± 0.009, r^2: 0.9788) > **15** (1.834 ± 0.006, r^2: 0.9696) > BHT (1.763 ± 0.008, r^2: 0.9788) > **16** (1.747 ± 0.009, r^2: 0.9708) > α-tocopherol (1.678 ± 0.018, r^2: 0.9262) > **18** (0.708 ± 0.023, r^2: 0.9251). In this procedure, higher absorbance indicates higher reducing capability. Furthermore, the highest FRAP

reduction capability was recorded for the novel 1-(4,5-dihydroxybenzyl)pyrrolidin-2-one derivative bromophenols **2**–**4**, **17**. It was reported that the FRAP method offers a well-known index of antioxidant, or reducing, potential of samples or pure molecules.[48−51]

iv. The DPPH radical is one of the few stable organic nitrogen radicals and is deep purple. This method is based on the measurement of the neutralizing capability of antioxidants toward DPPH radicals.[52] Table 2 shows half maximal radical scavenging concentrations (IC_{50}) of novel 1-(4,5-dihydroxybenzyl)pyrrolidin-2-one derivative bromophenols and the reference radical scavenger types including BHA, BHT, trolox, and α-tocopherol. IC_{50} amounts for **2**–**4** and **15**–**18** were in the following order: **17** (4.71 μg/mL, r^2: 0.9903) < **3** (5.08 μg/mL, r^2: 0.9204) < **15** (5.68 μg/mL, r^2: 0.9493) < **4** (6.18 μg/mL, r^2: 0.9790) < **2** (6.41 μg/mL, r^2: 0.9993) < **16** (7.01 μg/mL, r^2: 0.9791) < trolox (7.37 μg/mL, r^2: 0.9737) < BHA (8.66 μg/mL, r^2: 0.9594) < BHT (13.07 μg/mL, r^2: 0.9202) < α-tocopherol (16.50 μg/mL, r^2: 0.9490) < **18** (53.30 μg/mL, r^2: 0.9770). It was determined that phenolic moieties in synthesized molecules increased their radical scavenging activities. These activities can vary depending on the position and number of hydroxyl groups attached to the aromatic ring.[53]

Table 2. Determination of half maximal concentrations (IC_{50}, μg/mL) of novel bromophenols, their methylated precursors, and standards for DPPH, ABTS radical scavenging, and metal chelating activities.

Compounds	DPPH· scavenging	R^2	$ABTS^{\bullet+}$ scavenging	R^2	Fe^{2+} Chelating	R^2
BHA	8.66	0.9594	5.21	0.9934	27.72	0.9728
BHT	13.07	0.9202	3.93	0.9737	26.65	0.9922
$\alpha-$Tocopherol	16.50	0.9490	11.17	0.9550	33.01	0.9592
Trolox	7.37	0.9737	7.21	0.9749	38.50	0.9102
EDTA	-	-	-	-	1.88	0.9773
2	6.41	0.9993	31.50	0.9588	19.86	0.9883
3	5.08	0.9204	3.30	0.9905	26.65	0.9393
4	6.18	0.9790	3.41	0.9748	23.10	0.9520
6	85.55	0.9833	41.76	0.9506	135.01	0.9494
8	138.61	0.9939	40.28	0.9518	30.13	0.9190
9	86.62	0.9838	43.31	0.9338	135.88	0.9303
10	99.01	0.9039	69.31	0.9277	135.58	0.9852
11	84.51	0.9945	38.51	0.9424	113.66	0.9022
12	113.60	0.9844	33.02	0.9748	99.08	0.9392
13	76.15	0.9033	46.21	0.9946	131.46	0.9708
14	83.48	0.9334	57.75	0.9686	113.13	0.9912
15	5.68	0.9493	3.28	0.9980	21.01	0.9691
16	7.01	0.9791	3.33	0.9248	16.11	0.9419
17	4.71	0.9903	3.36	0.9910	9.48	0.9889
18	53.30	0.9770	15.75	0.9736	132.96	0.9281

v. Another improved technique for the determination of radical scavenging is $ABTS^{\cdot+}$ scavenging activity. ABTS radicals were generated in an $ABTS/K_2S_2O_8$ system.[54] IC_{50} values for novel 1-(4,5-

dihydroxybenzyl)pyrrolidin-2-one derivative bromophenols **2**–**4**, **15**–**18**, and the reference radical scavenger compounds such as trolox, α-tocopherol, BHT, and BHA were in the following order: **15** (3.28 μg/mL, r^2: 0.9980) < **3** (3.30 μg/mL, r^2: 0.9905) < **16** (3.33 μg/mL, r^2: 0.9248) < **17** (3.36 μg/mL, r^2: 0.9910) < **4** (3.41 μg/mL, r^2: 0.9748) < BHT (3.93 μg/mL, r^2: 0.9737) < BHA (5.21 μg/mL, r^2: 0.9934) < trolox (7.21 μg/mL, r^2: 0.9749) < α-tocopherol (11.17 μg/mL, r^2: 0.9550) < **18** (15.75 μg/mL, r^2: 0.9736) < **2** (31.50 μg/mL, r^2: 0.9588) (Table 2). As in DPPH free radical scavenging activity, a lower IC_{50} amount indicates higher $ABTS^{\cdot+}$ scavenging activity.

Ferrous ion chelating activities of the new 1-(4,5-dihydroxybenzyl)pyrrolidin-2-one derivative bromophenols, BHA, BHT, α-tocopherol, and trolox are shown in Table 2. IC_{50} values of the ferrous ion (Fe^{2+}) chelating effect of the new 1-(4,5-dihydroxybenzyl)pyrrolidin-2-one derivative bromophenols and standards decreased as in the following order: EDTA (1.88 μg/mL, r^2: 0.9773) < **17** (9.48 μg/mL, r^2: 0.9889) < **16** (16.11 μg/mL, r^2: 0.9419) < **2** (19.86 μg/mL, r^2: 0.9883) < **15** (21.01 μg/mL, r^2: 0.9691) < **4** (23.10 μg/mL, r^2: 0.9520) < BHT (26.65 μg/mL, r^2: 0.9922) $\approx$ **3** (26.65 μg/mL, r^2: 0.9393) < BHA (27.72 μg/mL, r^2: 0.9728) < **8** (30.13 μg/mL, r^2: 0.9190) < α-tocopherol (33.01 μg/mL, r^2: 0.9592) < trolox (38.50 μg/mL, r^2: 0.9102) (Table 2). Chelation of ferrous ions (Fe^{2+}) may protect the cells against molecular oxidative damage by inhibiting production of ROS.

2.2.2. Results of anticholinesterase activity

I. AD is a life-threatening illness that accounts for 60%–80% of cases of dementia and that is the sixth leading cause of death in the world.[55] Generally, the final assessment comes at a late step of the disease because clinical signs are difficult to know and AD-relevant biomarkers are not largely accepted. Therapeutic drugs used to treat AD are ChEIs, which are considered to raise availability of ACh, thereby improving cognitive processes, i.e. attention and memory.[56] Indeed, three separate parts in the active site comprise selectivity of BChE inhibitors: the first part is acyl binding pocket, second part is determined near the lip of active site gorge, and the third part is recorded as the choline binding site. Molecules, which can affect these BChE parts via metal interactions or hydrophobic, hydrogen bonding, and salt bridges can create their inhibitory action.[57] In this work, AChE was also extremely inhibited by novel antioxidant bromophenols at the low nanomolar concentrations with K_i values in the range of 2.60 $\pm$ 0.75–16.36 $\pm$ 2.67 nM (Table 3). These results clearly determined that the new synthesized bromophenols had effective AChE inhibition properties. However, the most powerful AChE inhibition was recorded by novel bromophenol **9** with a K_i value of 2.60 $\pm$ 0.75 nM. Moreover, all the remaining newly synthesized bromophenols reported here had highly efficient inhibition constants against AChE. Additionally, tacrine (1, 2, 3, 4-tetrahydroacridin-9-amine), which was the first centrally acting cholinesterase inhibitor recorded for the therapy of AD, showed a K_i value of 86.37 $\pm$ 14.10 nM against AChE.

II. Finally, new antioxidant bromophenols inhibited BChE with K_i values in the range of 13.10 $\pm$ 3.33–54.47 $\pm$ 13.53 nM (Table 3). The K_i values of the new antioxidant bromophenols for BChE and AChE were obtained from Lineweaver–Burk plots. On the other hand, tacrine, which was the first cholinesterase inhibitor to be discovered for the management of AD symptoms in 1993, had a K_i value of 100.40 $\pm$ 15.62 nM. Furthermore, it was considered that donepezil hydrochloride, which is used for the therapy of mild-to-moderate AD and other diverse memory impairments, was shown to lower AChE inhibition activity

(IC_{50}: 55.0 nM).[58] As seen in Table 3, IC_{50} values are in the range of 8.60–29.88 nM and 37.84–83.22 nM towards AChE and BChE, respectively.

Table 3. Acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) enzymes inhibition effects of novel antioxidant bromophenols.

Inhibitors	IC_{50} (nM)				K_i (nM)		
	AChE	r^2	BChE	r^2	AChE	BChE	AChE/BChE
2	13.85	0.9722	38.22	0.9832	7.78 ± 1.07	29.66 ± 7.67	0.25
3	17.10	0.9685	40.57	0.9585	9.13 ± 1.31	31.30 ± 6.65	0.29
4	29.88	0.9965	46.51	0.9848	9.80 ± 1.28	19.53 ± 3.24	0.42
6	13.70	0.9590	39.22	0.9433	6.96 ± 1.77	26.35 ± 5.78	0.26
8	14.87	0.9628	44.58	0.9955	4.20 ± 1.24	22.97 ± 3.45	0.18
9	8.60	0.9593	37.84	0.9985	2.60 ± 0.75	14.97 ± 4.28	0.17
10	19.75	0.9694	42.43	0.9794	12.05 ± 2.95	39.58 ± 7.34	0.30
11	25.94	0.9959	56.20	0.9851	11.38 ± 3.17	54.47 ± 13.53	0.20
12	25.87	0.9749	48.38	0.9643	10.83 ± 0.98	18.75 ± 3.45	0.57
13	28.44	0.9877	70.07	0.9681	16.36 ± 2.67	13.10 ± 3.33	1.24
14	22.18	0.9484	48.26	0.9845	9.01 ± 2.94	23.06 ± 2.18	0.39
15	21.98	0.9945	53.72	0.9750	14.78 ± 2.56	50.18 ± 11.78	0.28
16	21.12	0.9529	67.15	0.9723	6.38 ± 2.88	36.30 ± 5.65	0.16
17	21.77	0.9727	44.73	0.9018	7.84 ± 0.63	52.05 ± 6.12	0.15
18	28.88	0.9318	83.22	0.9288	14.25 ± 6.01	36.26 ± 6.73	0.38
TAC*	136.20	0.9796	174.16	0.9711	86.37 ± 14.10	100.40 ± 15.62	0.86

*Tacrine (TAC) was used as positive standard for acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) enzymes

2.3. Conclusion

Biologically active natural bromophenols **2**–**4**, tetrabromide **5**,[41,42] and tribromide **7**[20,41,42] were synthesized using the procedures described in the literature. Demethylation of compound **6**, obtained from the reaction of tetrabromide **5**, yielded natural bromophenol **2**. In a similar manner, other natural bromophenols **3** and **4** were also synthesized from **8** and **9**. Besides the first synthesis of bromophenols **2**–**4**, the derivatives **10**–**18** of **2** were synthesized as well. Among these compounds, **3**, **4**, **8**, and **9** are racemic compounds. Additionally, the synthesized compounds were evaluated for antioxidant and anticholinergic activities.

3. Experimental

3.1. General

Solvents were purified and dried by known methods. For all compounds, values as well as mp, IR spectra, ^{1}H and ^{13}C NMR spectra, chemical shift, elemental analyses, and antioxidant activities of samples were performed as explained previously.[12,59] Preparative thick-layer chromatography was used with 1 mm of silica gel

60 PF (Merck, Darmstadt, Germany) on glass plates. HRMS data were obtained by LC-MS-TOF electrospray ionization technique (1200/6210, Agilent).

3.2. Synthesis

3.2.1. 1-(2,3,6-Tribromo-4,5-dimethoxybenzyl)pyrrolidin-2-one (6): standard procedure for the substitution reaction with 2-pyrrolidinone (8, 10–13)

To NaH (56.43 mg, 2.35 mmol) in a balloon (50 mL), washed with hexane, was added cold THF solution (0 °C) of 2-pirrolidone (181.94 mg, 2.14 mmol) in THF (20 mL). The mixture was stirred for 30 min and then tetrabromide **5** (500 mg, 1.07 mmol) was added to the mixture. After the mixture was stirred at the same temperature for 30 min more, the cold bath was removed and the mixture was additionally stirred for 24 h. After the removal of solvent from the reaction mixture under vacuum, cold solution (1%, 0 °C, 15 mL) of NaOH was slowly added to the resultant mixture. The new mixture was extracted with ethyl acetate (EtOAc) (2 × 20 mL). After the combined extracts were washed with water (20 mL) and dried over Na_2SO_4, the solvent was removed under vacuum and compound **6** (0.4 g, 80%, white amorphous solid) was yielded. Mp: 126–127 °C; IR (CH_2Cl_2, cm^{-1}): 2942, 1691,1395, 1280, 1121, 1010; R_f = 0.65: MeOH/CH_2Cl_2 (2:8); ^{1}H NMR (400 MHz, $CDCl_3$): 4.94 (s, CH_2, 2H), 3.91 (s, OCH_3, 3H), 3.89 (s, OCH_3, 3H), 3.12 (t, J = 8.1 Hz, CH_2, 2H), 2.40 (t, J = 8.1 Hz, CH_2, 2H), 1.94 (p, J = 8.1 Hz, CH_2, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): 174.65 (CO), 151.95 (C), 150.90 (C), 132.23 (C), 123.83 (C), 122.36 (C), 121.91 (C), 61.12 (NCH_2), 61.10, NCH_2), 49.23 (OCH_3), 45.81 (OCH_3), 30.92 (CH_2), 18.16 (CH_2); HRMS (APCI-TOF) (m/z +H) calcd for $C_{13}H_{14}Br^{79}Br^{79}Br^{81}NO_3$: 471.8582; found: 471.8543.

3.2.2. 1-(2,3-Dibromo-4,5-dihydroxybenzyl)pyrrolidin-2-one (2): standard procedure for the demethylation reaction with BBr_3 (3, 4, 15–18)

A solution of BBr_3 (0.53 g, 2.12 mmol) in CH_2Cl_2 (7 mL) was added dropwise into cold (0 °C) solution of compound **6** (0.50 g, 1.1 mmol) stirred in CH_2Cl_2 (15 mL) under N_2 (g) over 7 min, and the mixture was stirred at the same temperature for 20 min. After the cold bath was removed, the mixture was stirred at RT and under N_2 for 16 h. Then methanol (3 mL) was slowly added to the mixture over 15 min at 0 °C. Next, the solvent was evaporated and then water (25 mL) was added. After the mixture was extracted with EtOAc (2 × 30 mL), the combined organic phases were dried over Na_2SO_4 and the solvent was evaporated. Natural product **2** (310 mg, 65%) was obtained as white amorphous solid. Mp: 166–168 °C (Lit.[2] 166–168 °C); R_f = 0.26: MeOH/CH_2Cl_2 (2:8); ^{1}H NMR (400 MHz, Acetone-d_6) : δ 4.88 (bs, CH_2, 2H), 3.14 (t, J = 7.0 Hz, CH_2, 2H), 2.39 (t, J = 8.0 Hz, CH_2, 2H), 2.06–1.84 (m, CH_2, 2H). ^{13}C NMR (100 MHz, Acetone-d_6) : δ 175.78 (CO), 145.26 (C), 143.84 (C), 125.67 (C), 117.60 (C), 113.40 (C), 113.23 (C), 48.70 (CH_2), 45.81 (CH_2), 30.67 (CH_2), 17.63 (CH_2); HRMS (APCI-TOF) (m/z +H) calcd for $C_{11}H_{10}Br^{79}Br^{81}Br^{81}NO_3$: 441.8289; found: 441.8268.

3.2.3. 2,3-Dibromo-1-(bromomethyl)-4,5-dimethoxybenzene (7)

To a stirred cold (0 °C) mixture of 2,3-dibromo-4,5-dimethoxybenzaldehyde (1.0 g, 3.09 mmol), methanol (10 mL), and diethyl ether (5 mL) $NaBH_4$ (114 mg, 3.09 mmol) was carefully added in portions over 15 min. After the mixture was stirred at the same temperature for half an hour, the cold bath (ice-water) was removed and

the reaction progress was monitored by thin-layer chromatography while the mixture was stirred at RT for 4 h. After the solvent was evaporated, water (20 mL) was added and the resulting mixture was extracted with EtOAc (2 × 15 mL), the combined organic phases were dried over Na_2SO_4, and the solvent was evaporated. To cold (0 °C) solution of the residue including reduction product in CH_2Cl_2 (20 mL) was added Et_3N (0.374 g, 3.70 mmol). After the mixture was stirred at the same temperature for 0.5 h, PBr_3 (1.00 g, 3.70 mmol) was added dropwise and the mixture was additionally stirred for 24 h. As stated above, the remainder of this substitution reaction was realized according to that of the reduction reaction of the aldehyde. Known[20,41,42] tribromide **7** (1.01 g, total 84%) was obtained as a yellow amorphous solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.00 (s, 1H), 4.63 (s, CH_2Br, 2H), 3.89 (s, OMe, 3H), 3.86 (s, OMe, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 152.68 (C), 147.96 (C), 134.16 (C), 122.55 (C), 117.95 (C), 113.69 (CH), 60.59 (OMe), 56.29 (OMe), 35.11 (CH_2Br).

3.2.4. (2RS)-1-(2,3-dibromo-4,5-dimethoxybenzyl)-5-oxopyrrolidine-2-carboxylic acid (8)

This reaction was realized according to the standard procedure described in the synthesis of **6**. Methyl 5-oxopyrrolidine-2-carboxylate (221 mg, 1.54 mmol), THF (20 mL), tribromide **7** (600 mg, 1.54 mmol), and NaH (55.54 mg, 2.31 mmol) were used in the reaction. Methyl 5-oxopyrrolidine-2-carboxylate, synthesized from its acid, was used instead of 2-pirrolidone. The crude compound **8** was purified from $CHCl_3$/hexane as a white amorphous solid (280 mg, 40%). Mp: 157–158 °C; IR (CH_2Cl_2, cm^{-1}): 3081, 2992, 1683, 1402, 1318, 1101 cm^{-1}: R_f = 0.34: MeOH/CH_2Cl_2 (5:95) ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.95 (s, aromatic, 1H), 5.05 (d, A part of AB system, J = 14.9 Hz, CH_2, 1H), 4.35 (d, B part of AB system, J = 14.9 Hz, methylenic, 1H), 4.13 (dd, J = 9.2, 2.6 Hz, methylenic, 1H), 3.84 (s, 2 OCH_3 6H), 2.64–2.53 (m, methylenic, 1H), 2.50–2.28 (m, methylenic, 2H), 2.23–2.14 (m, methylenic, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 176.36 (CO), 175.34 (CO), 152.97 (C), 147.75 (C), 132.68 (C), 122.31 (C), 117.52 (C), 114.19 (CH), 60.76, 59.37, 56.54, 47.68, 29.47 (CH_2), 23.38 (CH_2); HRMS (APCI-TOF) (m/z +H) calcd for $C_{14}H_{15}Br^{79}Br^{81}NO_5$: 437.9375; found: 437.9341.

3.2.5. (2RS)-Methyl 1-(2,3-dibromo-4,5-dimethoxybenzyl)-5-oxopyrrolidine-2-carboxylate (9)

A solution of the compound **8** (100 mg, 0.229 mmol) and $SOCl_2$ (544 mg, 4.58 mmol) in CH_2Cl_2 (8 mL) was stirred at RT for 12 h. After the solvent was removed under vacuum and methanol was added, the formed solution was stirred for 1 h. Then the solvent was removed under vacuum and compound **9** (90 mg, 87%) was obtained as brown liquid; IR (CH_2Cl_2, cm^{-1}): 3081, 2934, 1643, 1463, 1421, 1101, 1066, 1002; R_f = 0.65: MeOH/CH_2Cl_2 (5:95); ^{1}H NMR (400 MHz, $CDCl_3$): 6.92 (s, aromatic, 1H), 5.00 (d, A part of AB system, J = 15.0 Hz, NCH_2, 1H), 4.32 (d, B part of AB system, J = 15.0 Hz, NCH_2, 1H), 4.08 (dd, A part of AB system, J = 9.2, 3.0 Hz, NCH, 1H), 3.83 (s, 2 × OCH_3), 3.70 (s, OCH_3), 2.58–2.49 (m, methylenic, 1H), 2.45–2.33 (m, methylenic, 2H), 2.12–2.03 (m, methylenic, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): 175.64 (CO), 172.42 (CO), 153.00 (C), 147.65 (C), 133.00 (C), 122.22 (C), 117.42 (C), 113.96 (CH), 60.73 (CH), 59.35 (NCH_2), 56.50 (OCH_3), 52.71 (OCH_3), 47.53 (OCH_3), 29.46 (CH_2), 23.27 (CH_2); HRMS (APCI-TOF) (m/z +H) calcd for $C_{15}H_{14}Br^{79}Br^{81}NO_5$: 449.9531; found: 449.9552.

3.2.6. (2RS)-1-(2,3-dibromo-4,5-dihydroxybenzyl)-5-oxopyrrolidine-2-carboxylic acid (3)

This reaction was realized according to the standard procedure described in the synthesis of **2**. Compound **8** (0.5 g, 1.14 mmol), BBr_3 (601.83 mg, 2.40 mmol), CH_2Cl_2 (15 mL), and H_2O (5 mL) were used in the reaction. H_2O was used instead of methanol. The natural product **3** (420 mg, 90%, white amorphous solid) was obtained. Mp: 160–162 °C, (lit.[19] 172–174 °C); R_f = 0.25: MeOH/CH_2Cl_2 (2:8); ^{1}H NMR (400 MHz, Acetone-d_6) : δ 9.13 (bs, OH, 1H), 8.45 (bs, OH, 1H), 6.88 (s, aromatic, 1H), 4.93 (d, A part of AB system, J = 15.2 Hz, methylenic NCH_2, 1H), 4.18 (d, B part of AB system, J = 15.1 Hz, methylenic NCH_2, 1H), 2.51–2.29 (m, methylenic, 3H), 2.21–1.97 (m, methylenic, 2H). ^{13}C NMR (100 MHz, Acetone-d_6) : δ 175.12 (CO), 172.86 (CO), 145.14 (C), 144.31 (C), 128.47 (CH), 115.72 (C), 115.68 (C), 115.15 (C), 58.77 (CHN), 46.53 (CH_2N), 46.47 (CH_2), 23.04 (CH_2); HRMS (APCI-TOF) (m/z +H) calcd for $C_{12}H_{11}Br^{79}Br^{81}NO_5$: 409.9062; found: 409.9038.

3.2.7. (2RS)-Methyl 1-(2,3-dibromo-4,5-dihydroxybenzyl)-5-oxopyrrolidine-2-carboxylate (4)

This reaction was realized according to the standard procedure described in the synthesis of **2**. Compound **9** (400 mg, 0.89 mmol), BBr_3 (444 mg, 1.79 mmol), CH_2Cl_2 (15 mL), and methanol (10 mL) were used in the reaction. The natural product **4** (330 mg, 88%, pale yellow solid) was obtained. Mp: 190–192 °C (lit.[19] 183–185 °C); R_f = 0.34: MeOH/CH_2Cl_2 (2:8); ^{1}H NMR (400 MHz, Acetone-d_6): 10.07 (bs, OH, 1H), 9.63 (bs, OH, 1H), 6.70 (s, aromatic, 1H), 4.69 (d, A part of AB system, J = 15.1 Hz, methylenic CHN, 1H), 4.02 (m, methylenic, 2H), 3.62 (s, OMe, 3H), 2.49–2.45 (m, methylenic, 1H), 2.36–2.24 (m, methylenic, 2H), 2.00–1.92 (m, methylenic, 1H). ^{13}C NMR (100 MHz, Acetone-d_6): 175.19 (CO), 172.88 (CO), 146.07 (C), 144.87 (C), 127.50 (C), 115.97 (CH), 114.75 (C), 113.97 (C), 59.07 (CH_2N), 52.92 (CH_2N), 46.76 (OMe), 29.47 (CH_2), 23.00 (CH_2); HRMS (APCI-TOF) (m/z +H) calcd for $C_{13}H_{13}Br^{81}Br^{81}NO_5$: 425.9198; found: 425.9180.

3.2.8. 1-(2,3-Dibromo-4,5-dimethoxybenzyl)pyrrolidin-2-one (10)

This reaction was realized according to the standard procedure described in the synthesis of **6**. 2-Pyrrolidinone (218.85 mg, 2.57 mmol), THF (20 mL), tribromide **7** (0.5 g, 1.29 mmol), and NaH (67.88 mg, 2.83 mmol) were used in the reaction. Compound **10** (450 mg, 89%) was obtained as a brown liquid; IR (CH_2Cl_2, cm^{-1}): 3316, 2936, 1685, 1467, 1421, 1265, 1160, 1122, 1005; R_f = 0.62: MeOH/CH_2Cl_2 (5:95); ^{1}H NMR (400 MHz, $CDCl_3$): 6.82 (s, aromatic, 1H), 4.52 (bs, methylenic CH_2N, 2H), 3.77 (s, OMe, 3H), 3.76 (s, OMe, 3H), 3.27 (t, methylenic CH_2, J = 6.9 Hz, 2H), 2.36 (t, J = 6.9 Hz, methylenic 2H), 2.00–1.91 (m, methylenic 2H); ^{13}C NMR (100 MHz, $CDCl_3$): 175.46 (CO), 153.19 (C), 147.31 (C), 133.65 (C), 122.21 (C), 116.99 (C), 112.74 (CH), 60.68 (CH_2N), 56.44 (CH_2N), 48.10 (OMe), 47.32 (OMe), 30.87 (CH_2), 18.15 (CH_2); HRMS (APCI-TOF) (m/z +H) calcd for $C_{13}H_{15}Br^{79}Br^{81}NO_3$: 393.9476; found: 393.9446.

3.2.9. 1-(3-Bromo-4,5-dimethoxybenzyl)pyrrolidin-2-one (11)

This reaction was realized according to the standard procedure described in the synthesis of **6**. 2-Pyrrolidinone (274.77 mg, 3.23 mmol), THF (20 mL), 1-bromo-5-(bromomethyl)-2,3-dimethoxybenzene (0.5 g, 1.77 mmol), and NaH (81.29 mg, 3.39 mmol) were used in the reaction. Compound **11** (450 mg, 89%) was obtained as colorless liquid; IR (CH_2Cl_2, cm^{-1}): 2934, 1684, 1568, 1413, 1274, 1138, 1043; R_f = 0.68: MeOH/CH_2Cl_2

(5:95); ^{1}H NMR (400 MHz, $CDCl_3$): 6.80 (s, aromatic, 1H), 6.65 (s, aromatic, 1H), 4.32 (s, methylenic CH_2N, 2H), 3.66 (s, OMe, 3H), 3.64 (s, OMe, 3H), 3.11 (t, methylenic, J = 7.0 Hz, 2H), 2.21 (t, methylenic, J = 8.1 Hz, 2H), 1.85–1.71 (m, methylenic, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): 175.14 (CO), 149.16 (C), 148.86 (C), 127.89 (C), 115.30 (CH), 114.03 (C), 112.92 (CH), 56.24 (OMe), 56.18 (OMe), 46.87 (CH_2N), 46.03 (CH_2N), 30.87 (CH_2), 17.93 (CH_2); HRMS (APCI-TOF) (m/z +H) calcd for $C_{13}H_{16}Br^{79}NO_3$: 314.0392; found: 314.0374.

3.2.10. 1-(2-Bromo-4,5-dimethoxybenzyl)pyrrolidin-2-one (12)

This reaction was realized according to the standard procedure described in the synthesis of **6**. 2-Pyrrolidinone (220 mg, 2.58 mmol), THF (20 mL), 1-bromo-2-(bromomethyl)-4,5-dimethoxybenzene (0.4 g, 1.29 mmol), and NaH (81.29 mg, 3.39 mmol) were used in the reaction. Compound **12** (360 mg, 89%) was obtained as yellow liquid; IR (CH_2Cl_2, cm^{-1}): 1674, 1423, 1290, 1120; R_f = 0.53: MeOH/CH_2Cl_2 (5:95); ^{1}H NMR (400 MHz, $CDCl_3$): 6.91 (s, aromatic, 1H), 6.68 (s, aromatic, 1H), 4.27 (s, methylenic CH_2N, 2H), 3.76 (s, OMe, 3H), 3.74 (s, OMe, 3H), 3.21 (t, J = 7.1 Hz, methylenic, 2 H), 2.35 (t, J = 7.1 Hz, methylenic 2H), 1.93 (p, J = 7.1 Hz, methylenic, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): 175.27 (CO), 154.06 (C), 145.98 (C), 134.05 (C), 124.24 (CH), 117.75 (C), 111.69 (CH), 60.70 (OMe), 56.31 (OMe), 46.86 (CH_2N), 46.19 (CH_2N), 31.02 (CH_2), 17.89 (CH_2); HRMS (APCI-TOF) (m/z +H) calcd for $C_{13}H_{16}Br^{79}NO_3$: 314.0392; found: 314.0349.

3.2.11. 1-(4-Methoxybenzyl)pyrrolidin-2-one (13)

This reaction was realized according to the standard procedure described in the synthesis of **6**. 2-Pyrrolidinone (423.28 mg, 4.97 mmol), THF (20 mL), 1-(bromomethyl)-4-methoxybenzene (0.5 g, 2.49 mmol), and NaH (125.32 mg, 5.22 mmol) were used in the reaction. Compound **13** (450 mg, 89%) was obtained as a brown liquid; IR (CH_2Cl_2, cm^{-1}): 3445, 2086, 1644, 1422, 1120; R_f = 0.5: MeOH/CH_2Cl_2 (5:95); ^{1}H NMR (400 MHz, $CDCl_3$): 7.15 (d, A part of AB system, J = 8.6 Hz, aromatic, 2H), 6.83 (d, B part of AB system, J = 8.6 Hz, aromatic, 2H), 4.36 (s, methylenic CH_2N, 2H), 3.77 (s, OMe, 3H), 3.21 (t, J = 8.1 Hz, methylenic, 2H), 2.40 (t, J = 8.1 Hz, methylenic, 2H), 1.95 (p, J = 8.1 Hz, methylenic, 2H); ^{13}C NMR (100 MHz, $CDCl_3$); 174.98 (CO), 159.21 (C), 129.64 (2 CH), 128.84 (C), 114.18 (2 CH), 55.44 (OMe), 46.66 (CH_2N), 46.12 (CH_2N), 31.22 (CH_2), 17.87(CH_2); HRMS (APCI-TOF) (m/z +H) calcd for $C_{11}H_{12}Br^{79}NO_3$: 206.1181; found: 206.1162.

3.2.12. 1-(3-Bromo-4-methoxybenzyl)pyrrolidin-2-one (14)

To a solution of compound **13** (200 mg, 0.97 mmol) in CH_2Cl_2 (15 mL), molecular bromine (622 mg, 3.90 mmol) was added. After the solution was stirred in the dark at RT for 16 h, the solvent was removed under vacuum and compound **14** (275 mg, 100%) was obtained alone. Mp: 127–129 °C; IR (CH_2Cl_2, cm^{-1}): 2087, 1644, 1422, 1121; R_f = 0.5: MeOH/CH_2Cl_2 (5:95); ^{1}H NMR (400 MHz, $CDCl_3$): 7.47 (d, J = 2.0 Hz, 1H), 7.29 (dd, 1H, J = 8.4, 2.0 Hz, 1H), 6.95 (d, J = 8.4 Hz, 1H), 4.60 (bs, benzylic, 2H), 3.90 (s, OCH_3), 3.71–3.50 (m, methylenic, 2H), 3.11 (t, 8.1 Hz, 2H), 2.39–2.19 (m, methylenic, 2H); ^{1}H NMR (400 MHz, $CDCl_3$): 178.92 (CO), 156.49 (C), 133.76 (CH), 129.72 (CH), 126.41 (CH), 112.80 (CH), 112.31 (C), 56.74 (OCH_3), 50.29 (CH_2N), 48.30 (CH_2N), 31.76 (CH_2), 17.90 (CH_2); HRMS (APCI-TOF) (m/z +H) calcd for $C_{12}H_{14}Br^{79}NO_2$: 284.0286; found: 284.0215.

3.2.13. 1-(2,3-Dibromo-4,5-dihydroxybenzyl)pyrrolidin-2-one (15)

This reaction was realized according to the standard procedure described in the synthesis of **2**. BBr_3 (402 mg, 1.60 mmol), CH_2Cl_2 (20 mL), and **10** (300 mg, 0.763 mmol) were used in the reaction. Compound **15** (230 mg, 82%) were obtained as pale yellow solid. Mp: 210–212 °C; IR (acetone, cm^{-1}): 3435, 1643, 1406, 1280, 1121; R_f = 0.26: MeOH/CH_2Cl_2 (2:8); ^{1}H NMR (400 MHz, CD_3OD): 6.73 (s, aromatic, 1H), 4.49 (s, methylenic CH_2N, 2H), 3.46–3.21 (m, methylenic 2H), 2.42 (t, J = 8.1 Hz, methylenic 2H), 2.11–1.97 (m, methylenic 2H); ^{13}C NMR (100 MHz, CD_3OD): δ 176.46 (CO), 145.51 (C), 144.40 (C), 127.49 (C), 114.60 (C), 114.57 (CH), 113.43 (C), 47.43 (CH_2N), 47.13 (CH_2N), 30.62, 17.58; HRMS (APCI-TOF) (m/z +H) calcd for $C_{11}H_{11}Br^{79}Br^{81}NO_3$: 365.9163; found: 365.9133.

3.2.14. 1-(3-Bromo-4,5-dihydroxybenzyl)pyrrolidin-2-one (16)

This reaction was realized according to the standard procedure described in the synthesis of **2**. BBr_3 (670 mg, 2.67 mmol), CH_2Cl_2 (15 mL), and **11** (0.4 g, 1.27 mmol) were used in the reaction. Compound **16** (300 mg, 80%) was obtained as a brown solid. Mp: 118–120 °C; IR (acetone, cm^{-1}): 3435, 2087, 1643, 1406, 1288, 1121; R_f = 0.37: MeOH/CH_2Cl_2 (2:8); ^{1}H NMR (400 MHz, Acetone-d_6): 6.92 (s, aromatic, 1H), 6.79 (s, aromatic, 1H), 4.28 (s, methylenic CH_2N, 2H), 3.33 (t, J = 8.1 Hz, methylenic, 2H), 2.33 (t, J = 8.1 Hz, methylenic, 2H), 2.05–1.94 (p, J = 8.1 Hz, methylenic, 2H); ^{13}C NMR (100 MHz, Acetone-d_6); 174.78 (CO), 146.39 (C), 142.63 (C), 130.01 (C), 123.15 (CH), 114.21 (CH), 109.04 (C), 46.51 (CH_2N), 45.39 (CH_2N), 30.58 (CH_2), 17.73 (CH_2); HRMS (APCI-TOF) (m/z +H) calcd for $C_{11}H_{12}Br^{79}NO_3$: 286.0034; found: 286.0076.

3.2.15. 1-(2-Bromo-4,5-dihydroxybenzyl)pyrrolidin-2-one (17)

This reaction was realized according to the standard procedure described in the synthesis of **2**. BBr_3 (1.0 g, 4.20 mmol), CH_2Cl_2 (15 mL), and **12** (0.6 g 1.91, mmol) were used in the reaction. Compound **17** (460 mg, 84%) was obtained as a yellow solid. Mp: 170–172 °C; IR (acetone, cm^{-1}): 3445, 1644, 1510, 1423, 1282, 1121; R_f = 0.25: MeOH/CH_2Cl_2 (2:8); ^{1}H NMR (400 MHz, CD_3OD): 9.37 (s, OH, 1H), 9.24 (s, OH, 1H), 6.89 (s, aromatic, 1H), 6.62 (s, aromatic, 1H), 4.23 (s, methylenic CH_2N, 2H), 3.19 (t, J = 7.6 Hz, methylenic CH_2, 2H), 2.25 (t, J = 7.6 Hz, methylenic CH_2, 2H), 1.90 (p, J = 7.6 Hz, methylenic CH_2, 2H); ^{13}C NMR (100 MHz, CD_3OD): 174.60 (CO), 146.52 (C) 145.99 (CH), 126.44 (C), 119.61 (CH), 116.93 (C), 111.12 (C), 46.89 (CH_2N) 45.77 (CH_2N), 30.90 (CH_2) 18.10 (CH_2); HRMS (APCI-TOF) (m/z +H) calcd for $C_{11}H_{12}Br^{79}NO_3$: 286.0079; found: 286.0054.

3.2.16. 1-(4-Hydroxybenzyl)pyrrolidin-2-one (18)

The synthesis of compound **18** was realized according to the standard procedure described in the synthesis of **2**. BBr_3 (1.07 g, 4.29 mmol), CH_2Cl_2 (15 mL), and **13** (0.4 g, 1.95 mmol) were used in the reaction. Compound **18** (310 mg, 83%) was obtained as a brown solid. Mp: 118–120 °C; IR (acetone, cm^{-1}) 3414, 2943, 1641, 1497, 1259, 1110; R_f = 0.31: MeOH/CH_2Cl_2 (2:8); ^{1}H NMR (400 MHz, Acetone-d_6): 7.09 (d, A part of AB system, J = 8.6 Hz, aromatic, 2H), 6.79 (d, B part of AB system, J = 8.6 Hz, aromatic, 2H), 4.31 (s, methylenic CH_2N, 2H), 3.25 (t, J = 8.1 Hz, methylenic CH_2, 2H), 2.28 (t, J = 8.1 Hz, methylenic CH_2, 2H), 2.05 (p, J = 8.1 Hz, methylenic CH_2, 2H); ^{13}C NMR (100 MHz, APT, Acetone-d_6): 173.97 (CO), 156.84

(C), 130.04 (2 CH) 128.42 (C) 115.52 (2 CH) 46.18 (CH_2N) 45.52 (CH_2N) 30.58 (CH_2) 17.74 (CH_2); HRMS (APCI-TOF) (m/z +H) calcd for $C_{11}H_{13}NO_2$: 192.1025; found: 192.1004.

3.3. Biological assays

3.3.1. Antioxidant activity assays

3.3.1.1. Fe^{3+} reducing power assay

For designation of the Fe^{3+} reducing capability of novel 1-(4,5-dihydroxybenzyl)pyrrolidin-2-one derivative bromophenols, the $[Fe(CN)_6]^{3-}$ to $[Fe(CN)_6]^{4-}$ reduction procedure was used.[60] Briefly, varied concentrations of novel 1-(4,5-dihydroxybenzyl)pyrrolidin-2-one derivative bromophenols (10–30 μg/mL) in 0.75 mL of deionized water were added to 1.25 mL of phosphate buffer (0.2 M, pH 6.6) and 1.25 mL of potassium ferricyanide $[K_3Fe(CN)_6]$ (1%). Then the solution was incubated at 40 °C for 15 min. After the incubation period, trichloroacetic acid (TCA) was added (1.25 mL, 10%). Lastly, a portion of $FeCl_3$ (0.5 mL, 0.1%) was transferred to this mixture and the absorbance amount was obtained at 700 nm in a spectrophotometer. According to the results, when reduction capability increases, absorbance shows greater values.[61]

3.3.1.2. Cupric ion (Cu^{2+}) reducing-CUPRAC assay

Cupric ion (Cu^{2+}) reducing power was used as a second reducing capability procedure for novel 1-(4,5-dihydroxybenzyl)pyrrolidin-2-one derivative bromophenols. Cu^{2+} reducing ability was performed conforming to the procedure explained by Apak et al.[62] with slight modification.[63] Briefly, aliquots of $CuCl_2$ solution (0.25 mL, 0.01 M), ethanolic neocuproine solution (0.25 mL, 7.5×10^{-3} M), and NH_4Ac buffer solution (0.25 mL, 1.0 M, pH 6.5) were transferred to a test tube containing novel 1-(4,5-dihydroxybenzyl)pyrrolidin-2-one derivative bromophenols at various concentrations (10–30 μg/mL). Total volume was completed with distilled water to 2 mL followed by vigorous shaking. Absorbance of samples was recorded at 450 nm after 30 min.

3.3.1.3. FRAP Assay

The FRAP method is based upon reduction of Fe^{3+}-TPTZ complex under acidic medium conditions. Enhanced absorbance of blue ferrous form (Fe^{2+}-TPTZ complex) is recorded at 593 nm. TPTZ solution (2.25 mL, 10 mM TPTZ in 40 mM HCl) was freshly prepared and then transferred to 25 mL of acetate buffer (0.3 M, pH 3.6), and $FeCl_3$ solution (2.25 mL, 20 mM) in water. Then various concentrations of novel 1-(4,5-dihydroxybenzyl)pyrrolidin-2-one derivative bromophenols (10–30 μg/mL) were dissolved in 5 mL of appropriate buffer solvent, stirred, and incubated at 37 °C for 30 min. Finally the absorbance of the mixture was measured at 593 nm.[64]

3.3.1.4. $DPPH^{\bullet}$ scavenging activity

The $DPPH^{\bullet}$ scavenging activity of the novel 1-(4,5-dihydroxybenzyl)pyrrolidin-2-one derivative bromophenols was designated as previously explained.[65] Briefly, fresh solution of DPPH radicals (0.1 mM) was prepared in ethanol. Then 1.5 mL of each novel 1-(4,5-dihydroxybenzyl)pyrrolidin-2-one derivative bromophenol in ethanol was added to an aliquot of this solution (0.5 mL, 10–30 μg/mL). These mixtures were mixed vigorously and incubated in the dark for 30 min. Finally the absorbance value was recorded at 517 nm in a spectrophotometer.[66]

3.3.1.5. $ABTS^{\bullet+}$ scavenging activity

The ABTS radical scavenging capacity of the novel 1-(4,5-dihydroxybenzyl)pyrrolidin-2-one derivative bromophenols was characterized using the spectroscopic procedure explained by Re et al.[67] The ABTS radical cation ($ABTS^{\bullet+}$) was acquired by reacting 7 mM solution of ABTS with 2.45 mM $K_2S_2O_8$. Prior to the assay, the $ABTS^{\bullet}$ radical cation solution was diluted with ethyl alcohol to an absorbance of 0.750 $\pm$ 0.05 at 734 nm. Then 1 mL of $ABTS^{\bullet+}$ solution was added to 3 mL of each novel 1-(4,5-dihydroxybenzyl)pyrrolidin-2-one derivative bromophenol (20–60 μg/mL) and control solution. The extent of decolorization was obtained as percentage reduction of absorbance. Percentage of radicals scavenging effects (RSEs) of each novel 1-(4,5-dihydroxybenzyl)pyrrolidin-2-one derivative bromophenol was computed using the following equation: RSEs (%) = [1– (As/Ac)] $\times$ 100, where Ac is the absorbance value of control and As is absorbance value of sample.

3.3.1.6. Metal chelating assay

Ferrous ion (Fe^{2+}) chelating activities of the novel 1-(4,5-dihydroxybenzyl)pyrrolidin-2-one derivative bromophenols were evaluated conforming to the procedure explained previously.[68] Diverse concentrations (10–30 μg/mL) of the novel 1-(4,5-dihydroxybenzyl)pyrrolidin-2-one derivative bromophenols in 0.25 mL of ethanol, 0.25 mL of $FeSO_4$ solution (2 mM), 1 mL of ferrozine solution (0.2% in 0.2 M HCl), 1 mL of Tris-HCl buffer solution (pH 7.4), and 2.5 mL of ethanol solution were placed into a test tube, in that order. The absorbance was measured at 562 nm.

3.3.2. AChE/BChE activity determination and inhibition studies

The inhibitory efficacy of the novel 1-(4,5-dihydroxybenzyl)pyrrolidin-2-one derivative bromophenols on BChE/AChE activities was obtained conforming to the spectrophotometric procedure of Ellman et al.[69] as described previously.[70] Butrylcholine iodide (BChI) and also acetylthiocholine iodide (AChI) compounds were used as substrates of both reactions. In this work, 5,5′-dithio-bis(2-nitro-benzoic)acid (DTNB) was used for the estimation of the BChE/AChE activities. Briefly, 100 μL of buffer solution (pH 8.0, Tris-HCl, 1.0 M) and diverse concentration of sample solutions (1.1–48 nM for AChE and 11–115 nM for BChE) dissolved in deionized water were added to 50 μL of BChE/AChE solutions (5.32 $\times$ 10^{-3} EU). Then the mixture was incubated for 10 min at 20 °C. Finally, 50 μL of DTNB (0.5 mM and 25 mL) of BChI/AChI was added to the incubated mixture. Furthermore, the reaction was initiated by the addition of 50 μL of BChI/AChI. Activities of these enzymes were evaluated spectrophotometrically at a wavelength of 412 nm.

Acknowledgments

This study was financed by the Scientific and Technological Research Council of Turkey (TÜBİTAK) (Project No: 113Z702) and realized at Atatürk University, Faculty of Science, Department of Chemistry. We are grateful to both institutes for their support.

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The first synthesis and antioxidant and anticholinergic activities of 1-(4,5-dihydroxybenzyl)pyrrolidin-2-one derivative bromophenols including natural products

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Abstract: We report herein the first synthesis of biologically active natural bromophenols, namely 1-(2,3,6-tribromo-4,5-dihydroxybenzyl)pyrrolidin-2-one (**2**), 2RS-1-(2,3-dibromo-4,5-dihydroxybenzyl)-5-oxopyrrolidine-2-carboxylic acid (**3**), and 2RS-methyl 1-(2,3-dibromo-4,5-dihydroxybenzyl)-5-oxopyrrolidine-2-carboxylate (**4**), along with their different derivatives (**6** and **8**–**18**). Among the synthesized compounds, **3**, **4**, and their derivatives (**8** and **9**) are yielded in racemic form. Radical scavenging assays of the synthesized molecules were investigated using various bioanalytical antioxidant methods including 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) ($ABTS^{\bullet+}$) and 2,2-diphenyl-1-picrylhydrazyl ($DPPH^{\bullet}$). In addition, the reducing power of the novel bromophenols was investigated by Cu^{2+}–Cu^{+} reducing, Fe^{3+}–Fe^{2+} reducing, and $[Fe^{3+}\text{–}(TPTZ)_2]^{3+}$–$[Fe^{2+}\text{–}(TPTZ)_2]^{2+}$ reducing capacity and ferrous ions (Fe^{2+}) chelating abilities. The molecules demonstrated powerful antioxidant activities when compared to standard antioxidant compounds of α-tocopherol, trolox, butylated hydroxyanisole (BHA), and butylated hydroxytoluene (BHT). Moreover, novel bromophenols were tested against cholinergic enzymes including acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) enzymes in the last part of this study. The presented novel bromophenols showed Ki values in the range of 2.60 ± 0.75–16.36 ± 2.67 nM against AChE and 13.10 ± 3.33–54.47 ± 13.53 nM against BChE.

Key words: Bromination, bromophenol, pyrrolidin-2-one, natural product, antioxidant activity, acetylcholinesterase, butyrylcholinesterase

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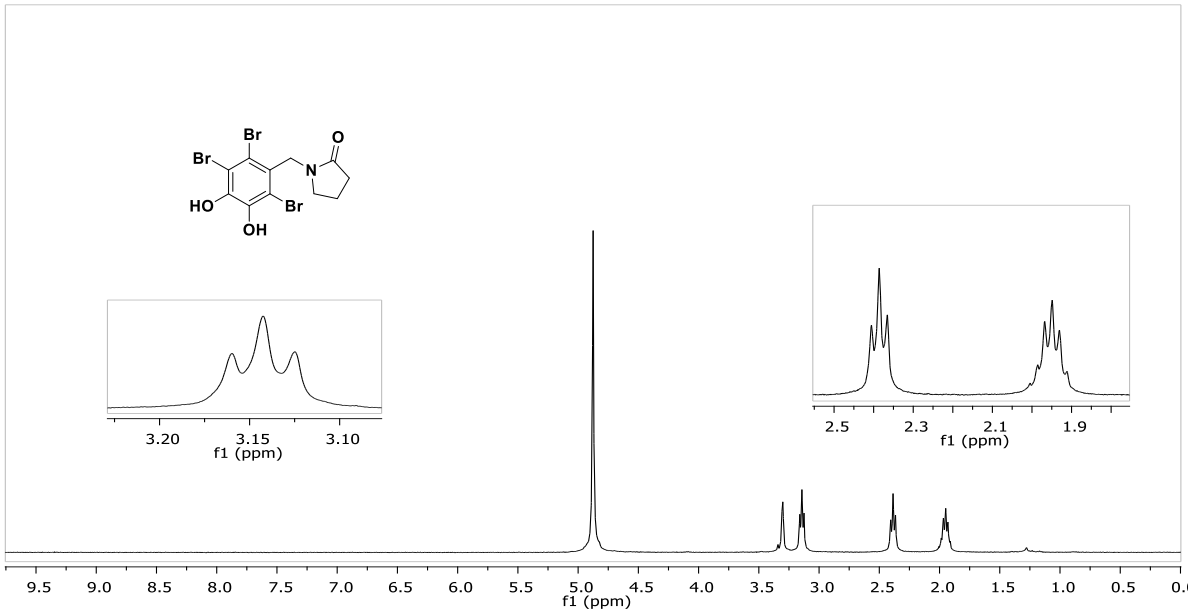
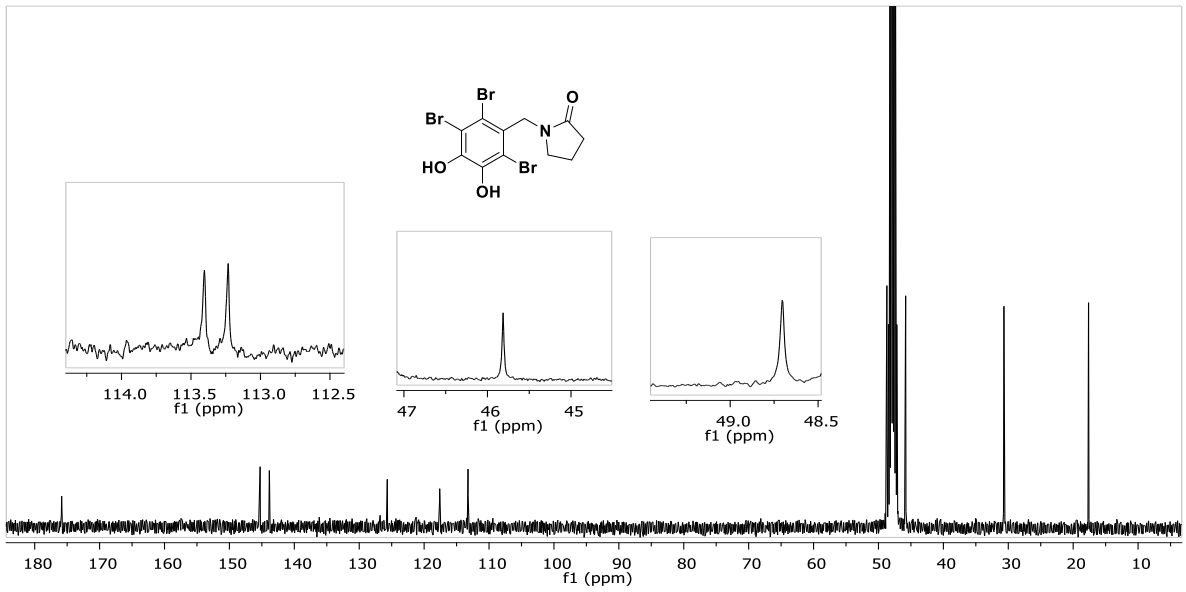
Supplementary material contains NMR spectra of synthesized compounds.



^{1}H NMR spectrum of compound **2** (400 MHz, Acetone-d_6)



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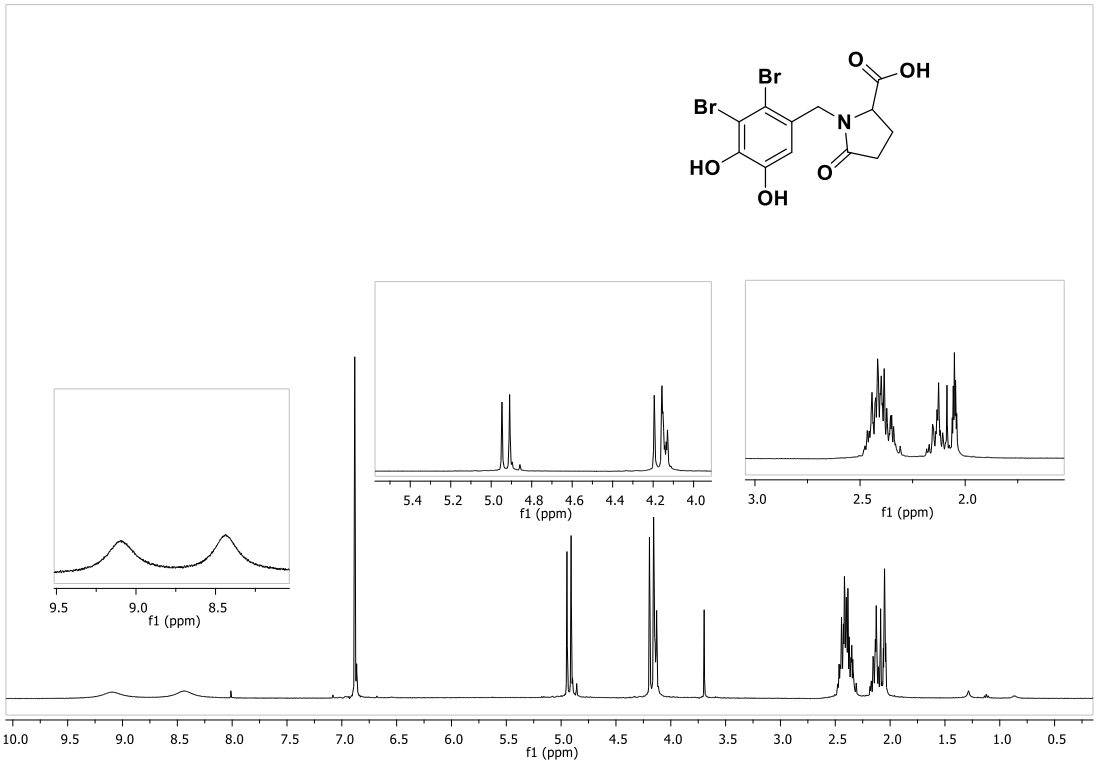

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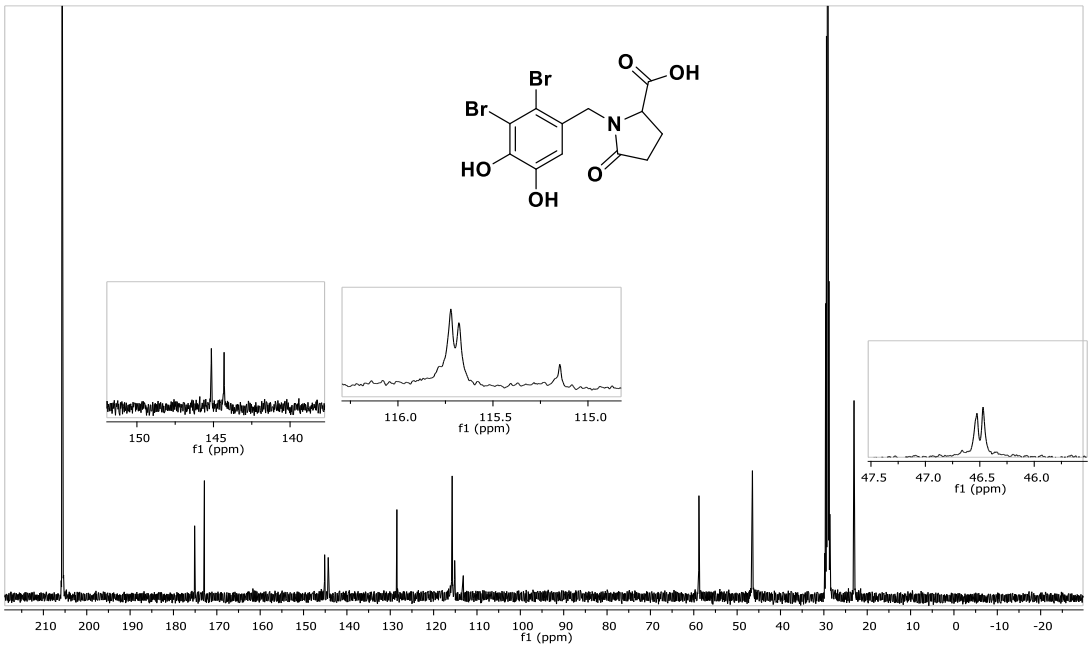
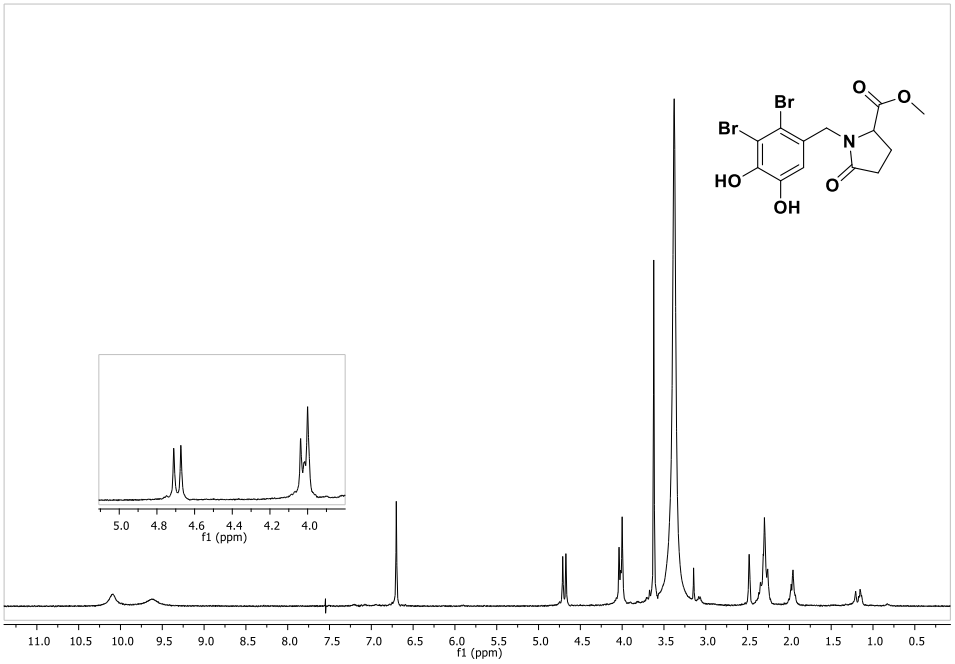
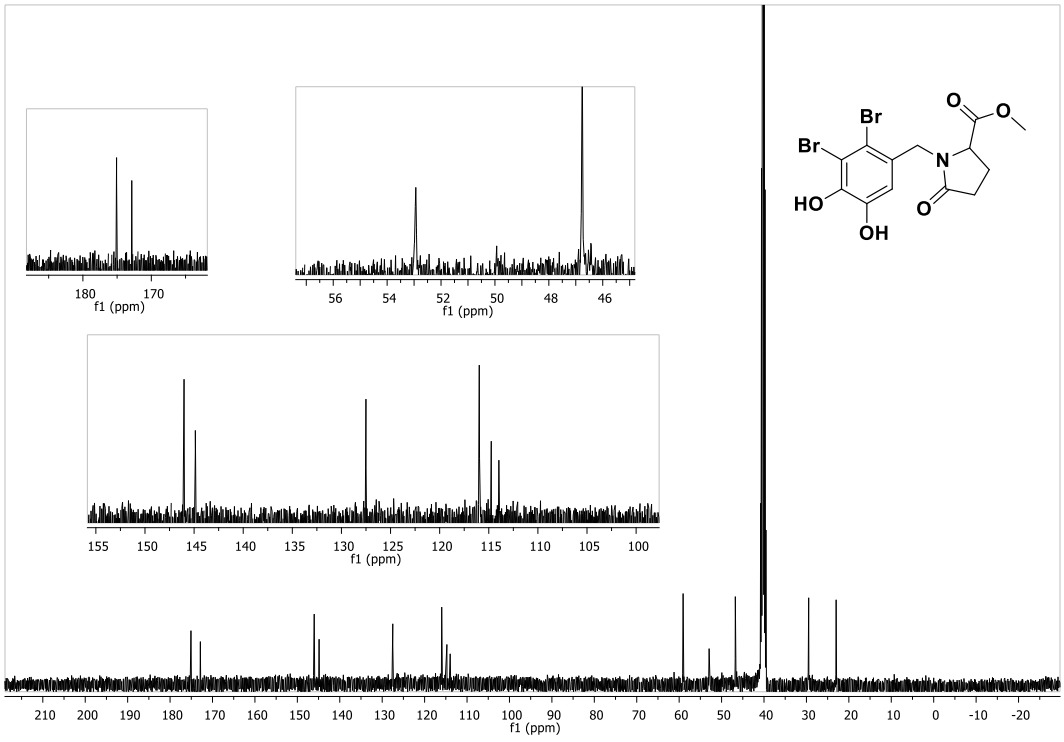


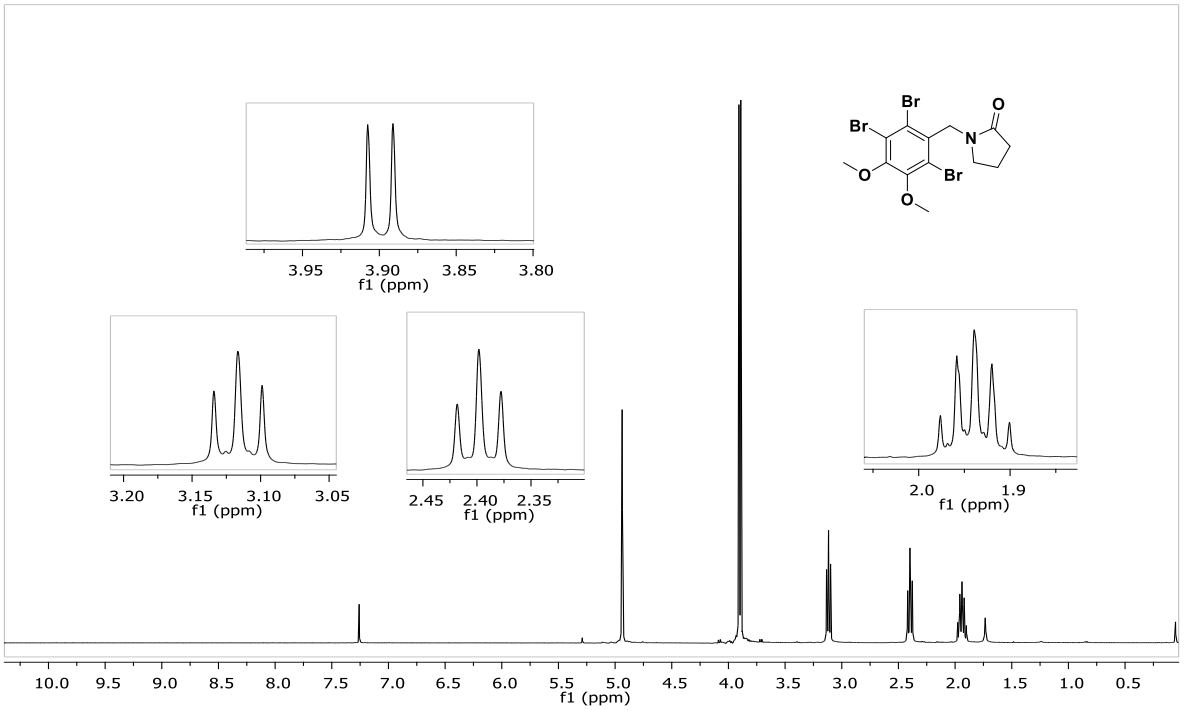
^{13}C NMR spectrum of compound **3** (100 MHz, Acetone-d_6)



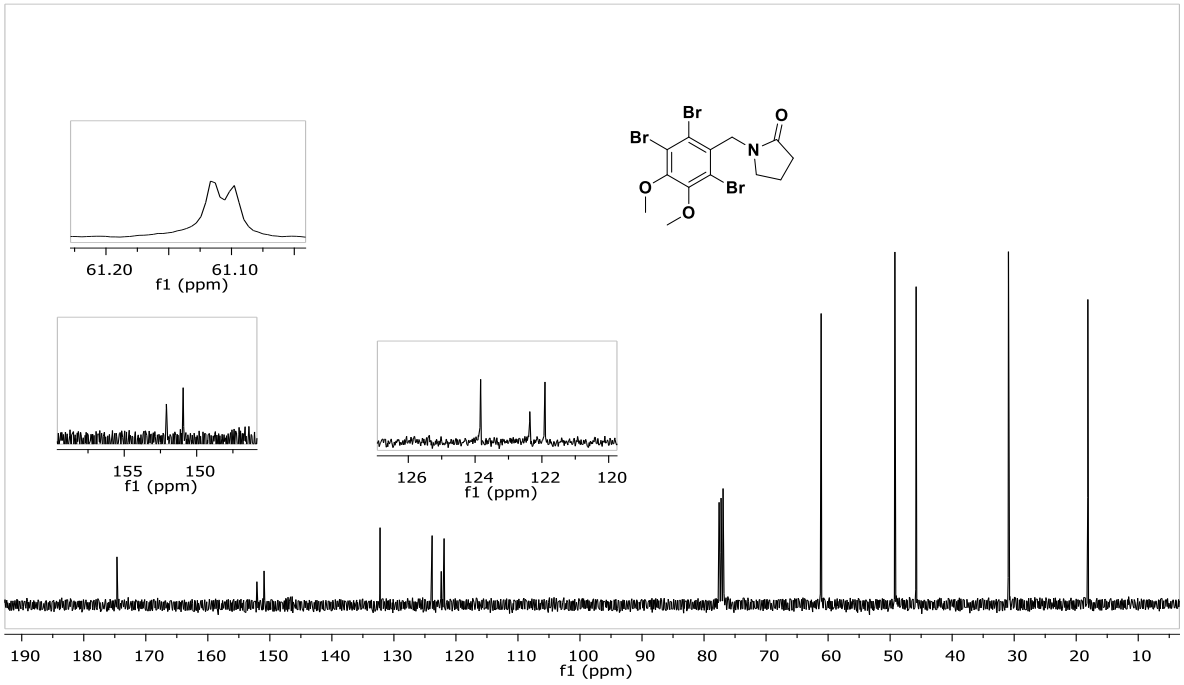
^{1}H NMR spectrum of compound **4** (400 MHz, Acetone-d6)



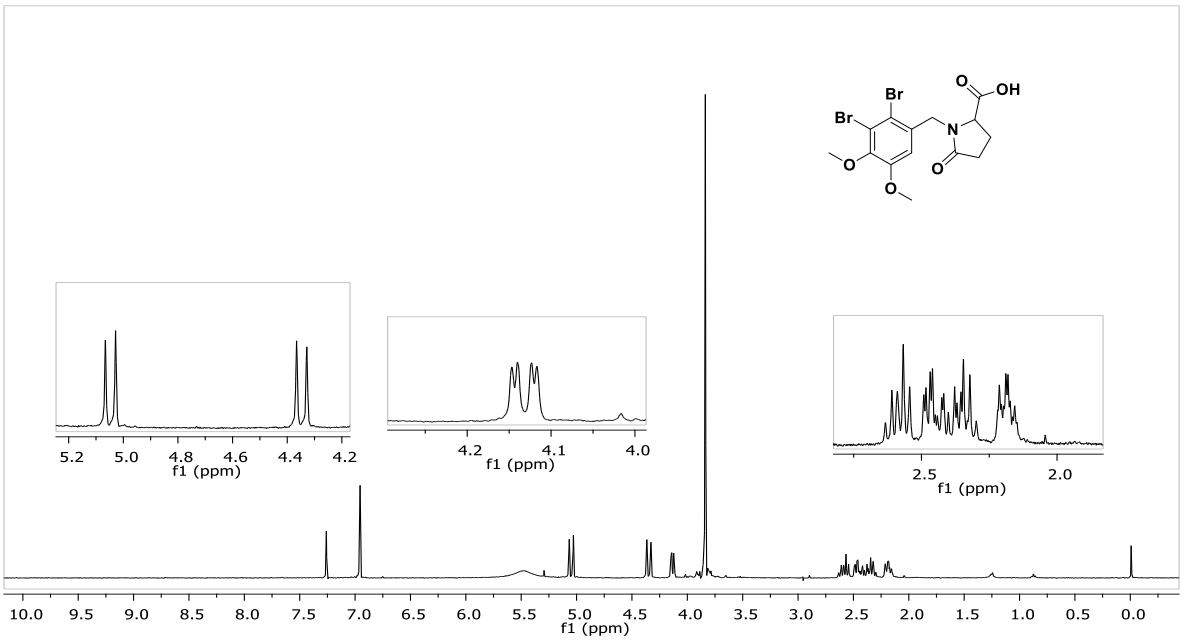
^{13}C NMR spectrum of compound **4** (100 MHz, Acetone-d6)



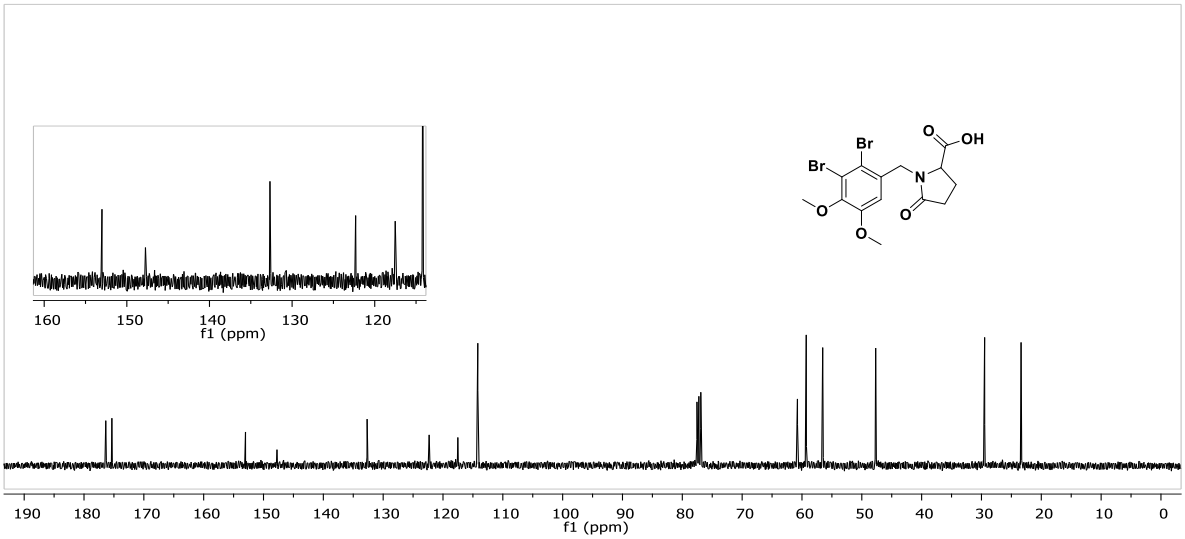
^{1}H NMR spectrum of compound **6** (400 MHz, $CDCl_3$)



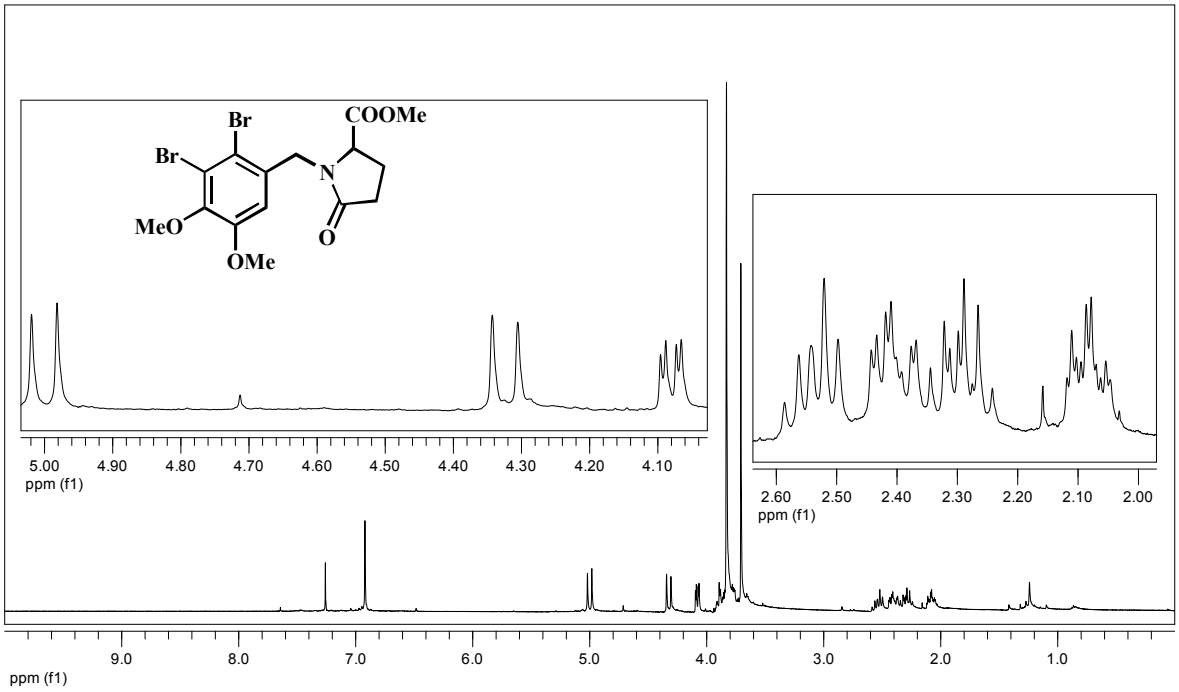
^{13}C NMR spectrum of compound **6** (100 MHz, $CDCl_3$)



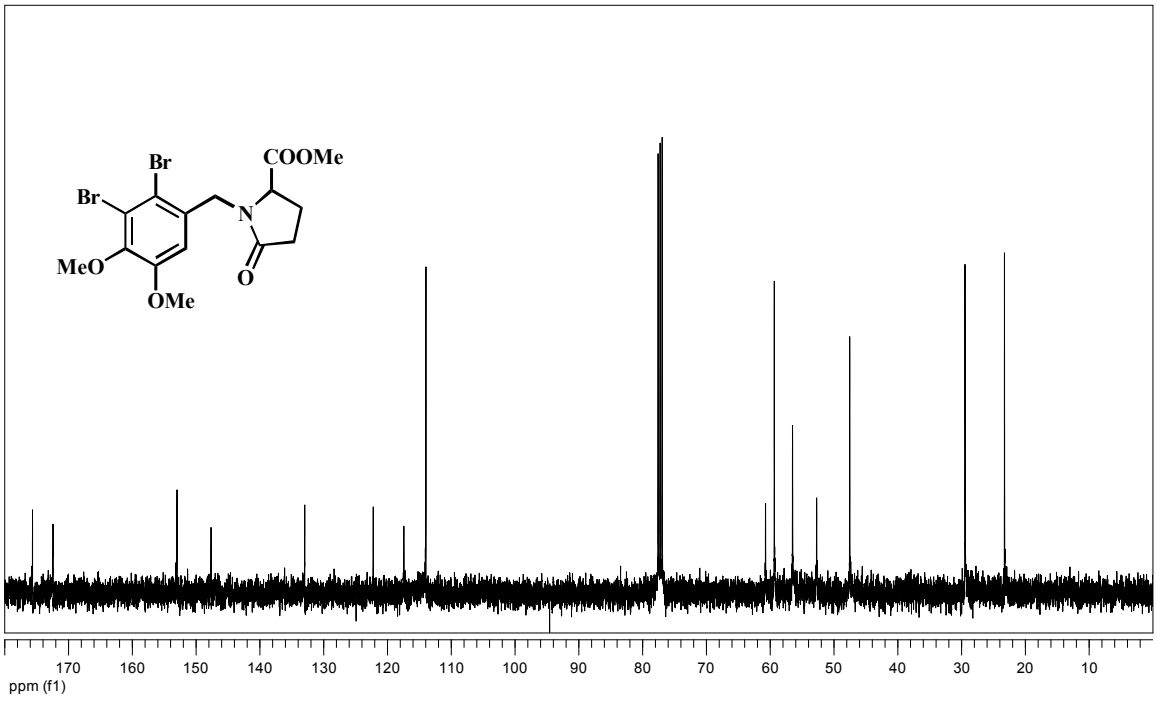
^{1}H NMR spectrum of compound **8** (400 MHz, $CDCl_3$)



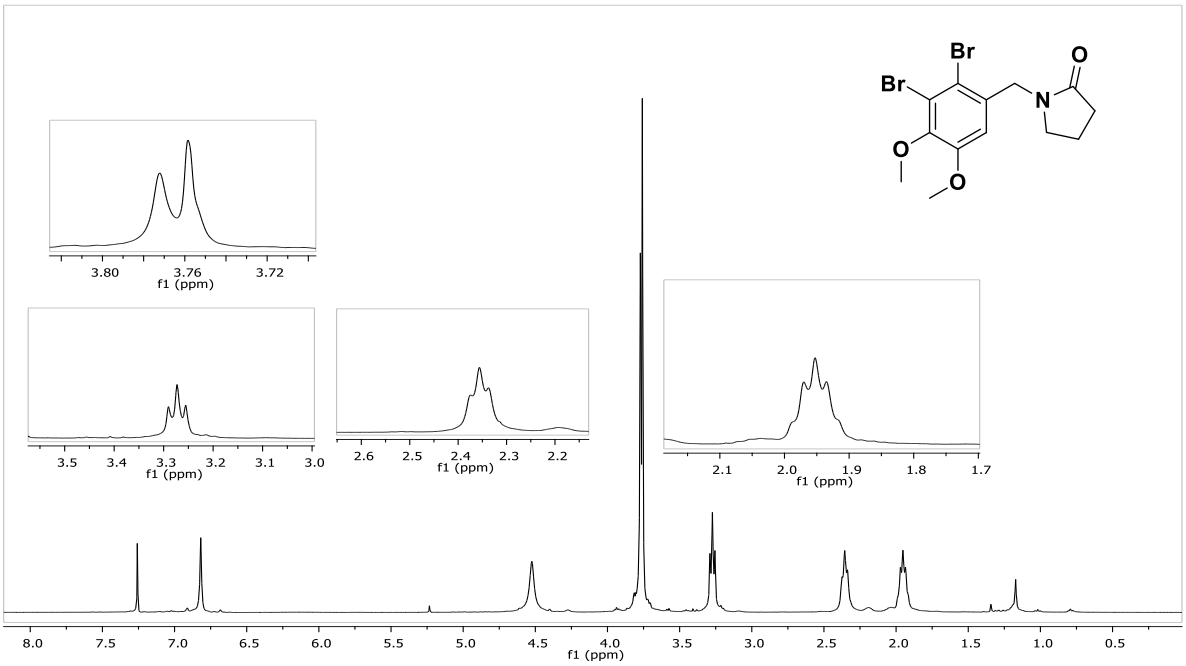
^{13}C NMR spectrum of compound **8** (100 MHz, $CDCl_3$)



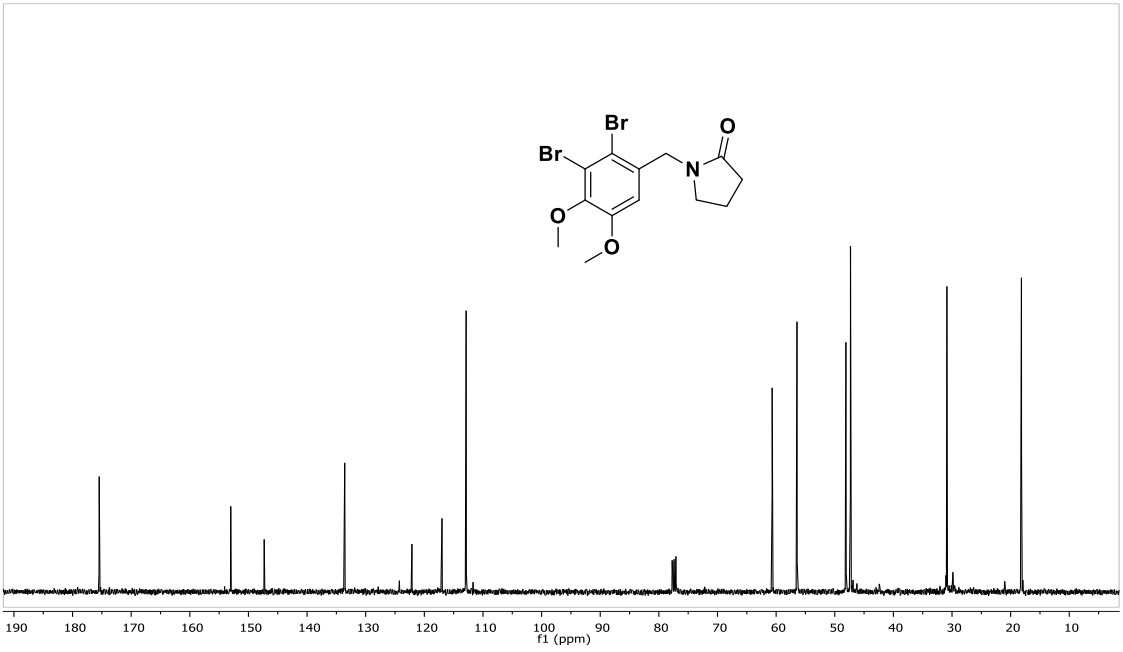
^{1}H-NMR Spectrum of the compound **9** (400 MHz, $CDCl_3$)



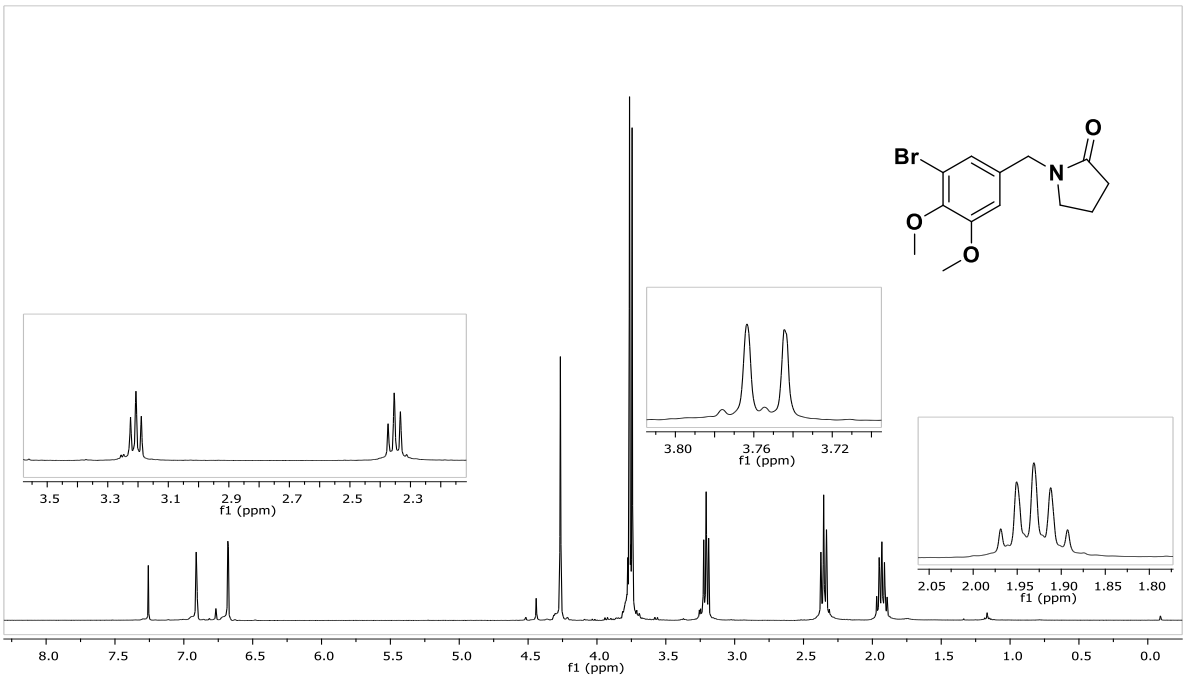
^{13}C NMR spectrum of compound **9** (100 MHz, $CDCl_3$)



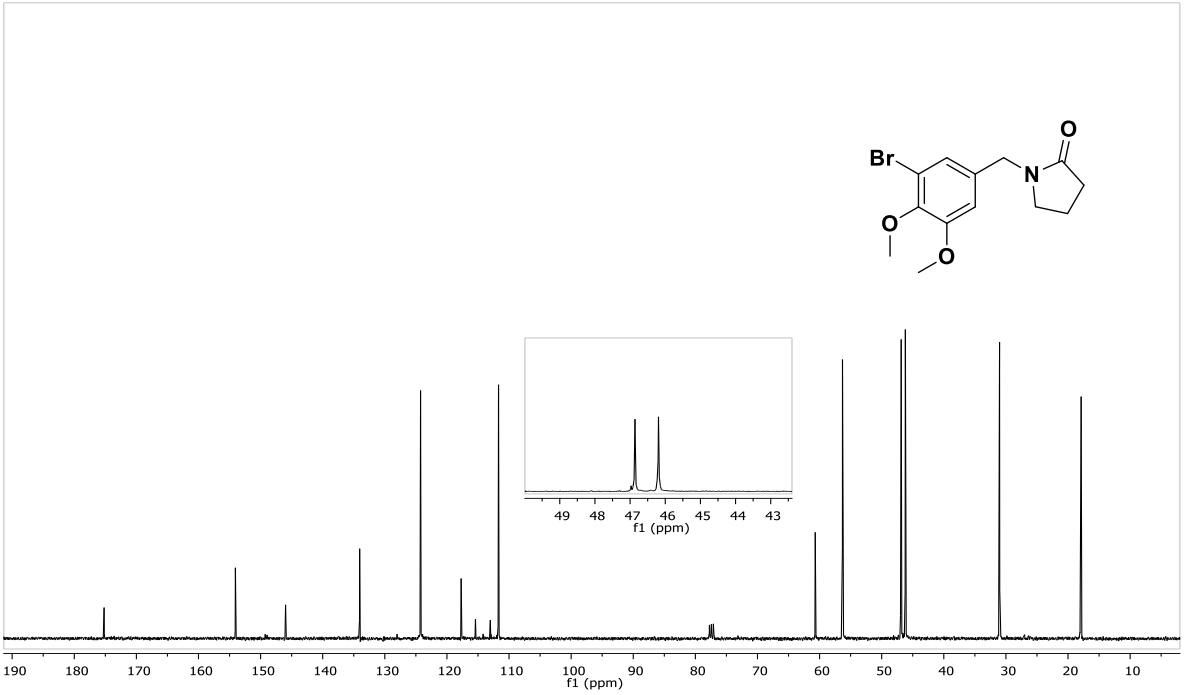
^{13}C NMR spectrum of compound **10** (100 MHz, $CDCl_3$)



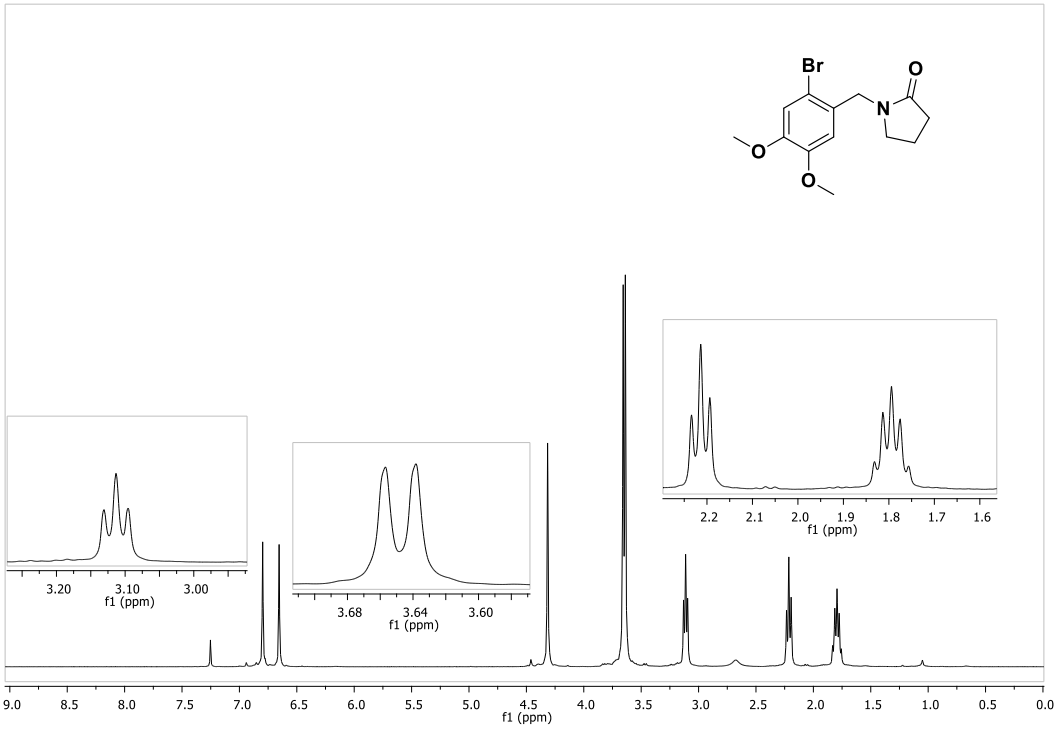
^{13}C-NMR spectrum of compound **10** (100 MHz, $CDCl_3$)



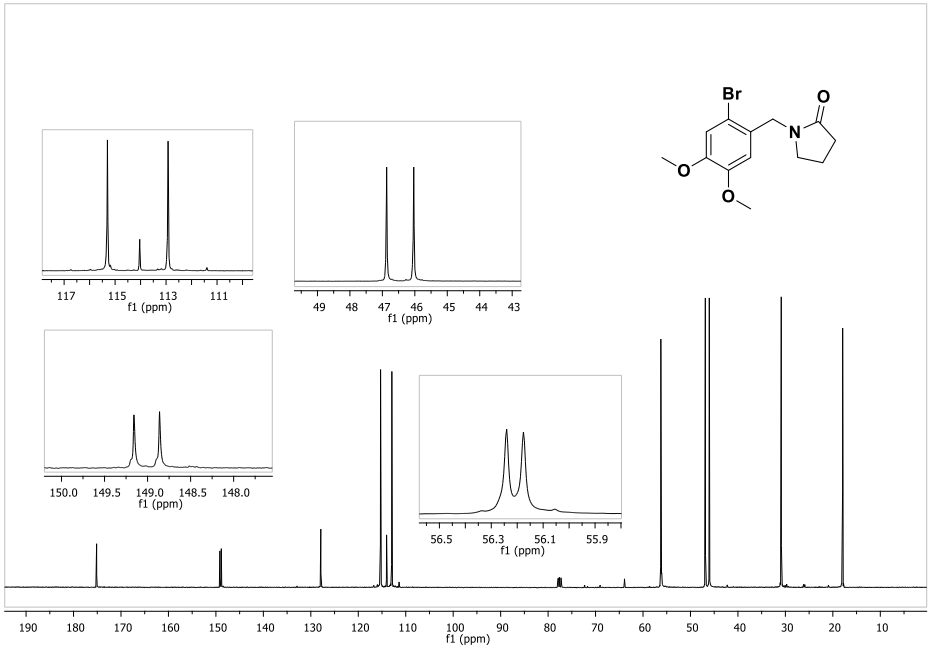
^{13}C NMR spectrum of compound **11** (100 MHz, $CDCl_3$)



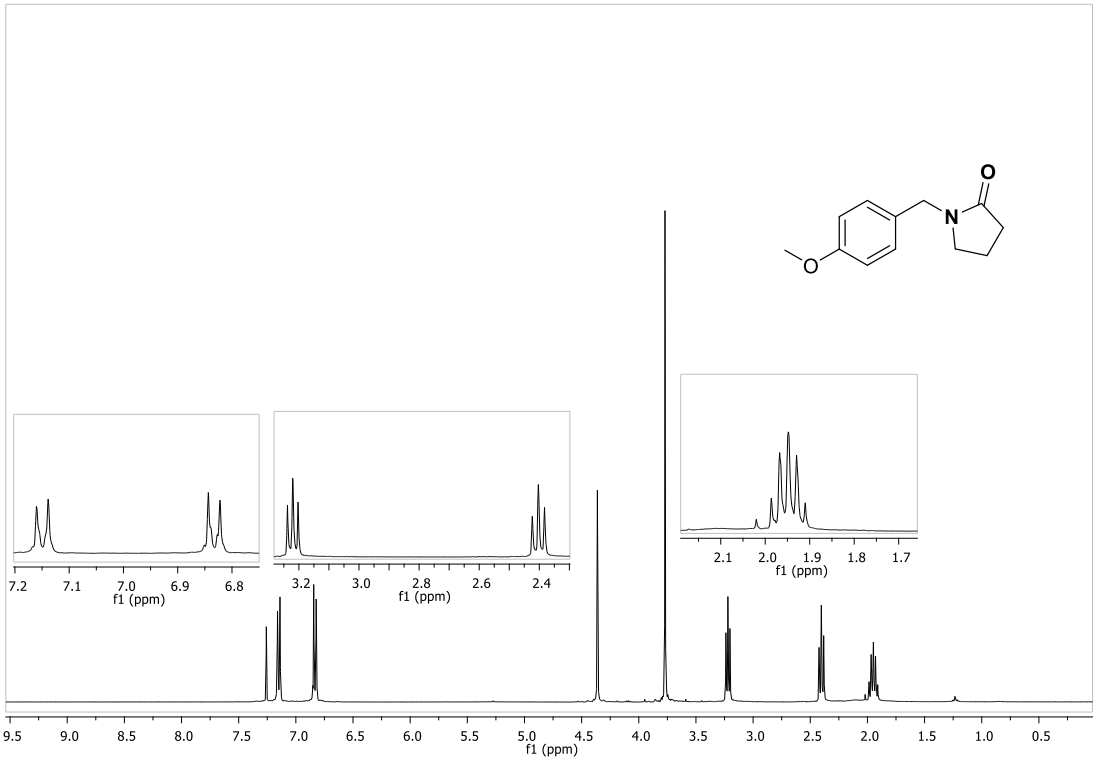
^{13}C NMR spectrum of compound **11** (100 MHz, $CDCl_3$)



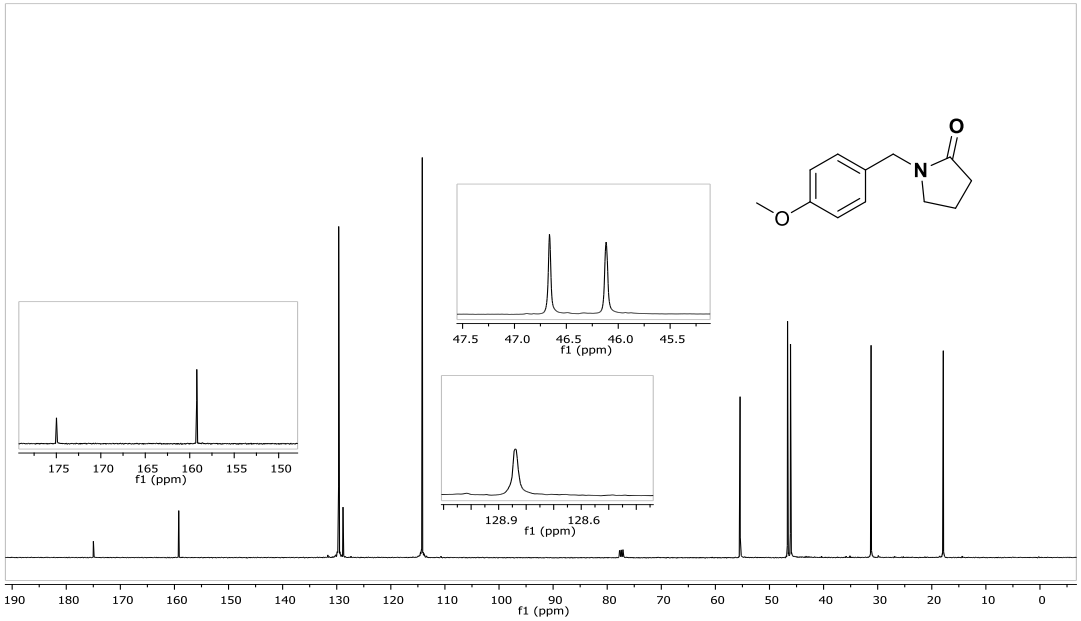
^{13}C NMR spectrum of compound **12** (100 MHz, $CDCl_3$)



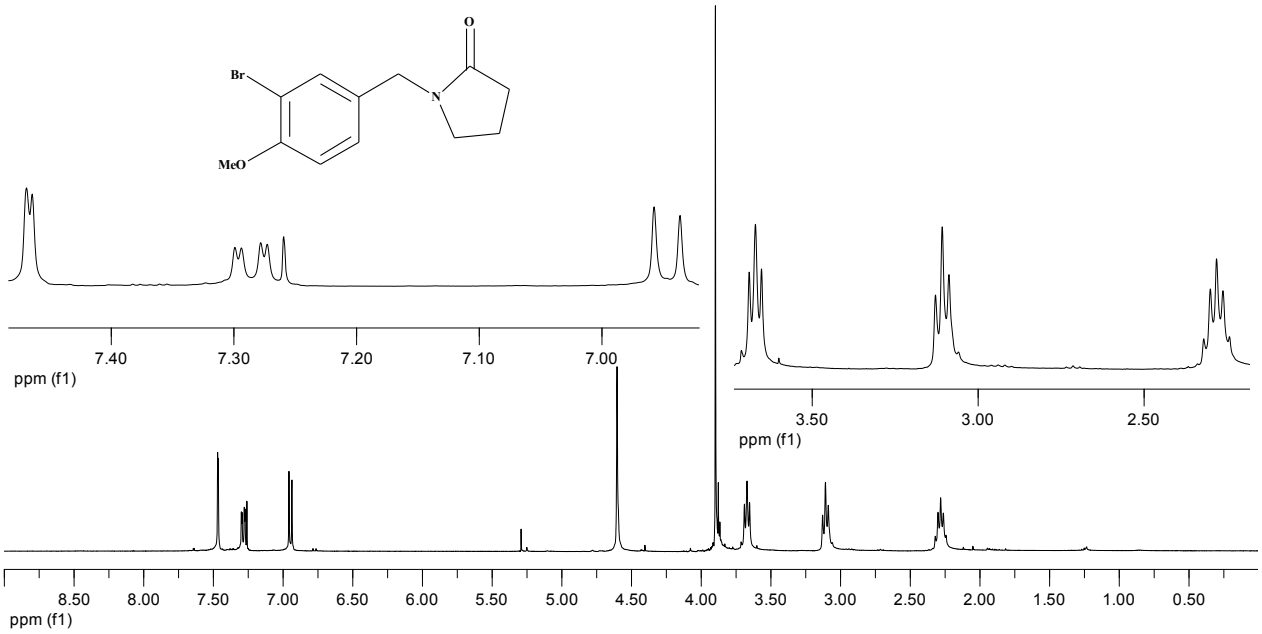
^{13}C NMR spectrum of compound **12** (100 MHz, $CDCl_3$)



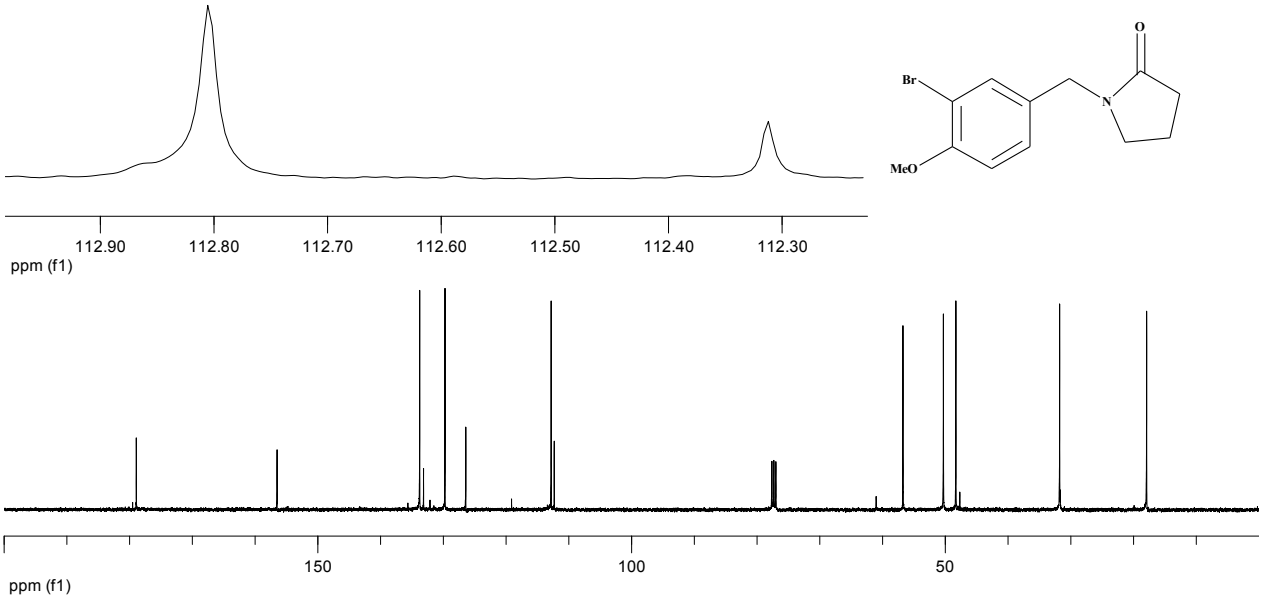
^{1}H NMR spectrum of compound **13** (100 MHz, $CDCl_3$)



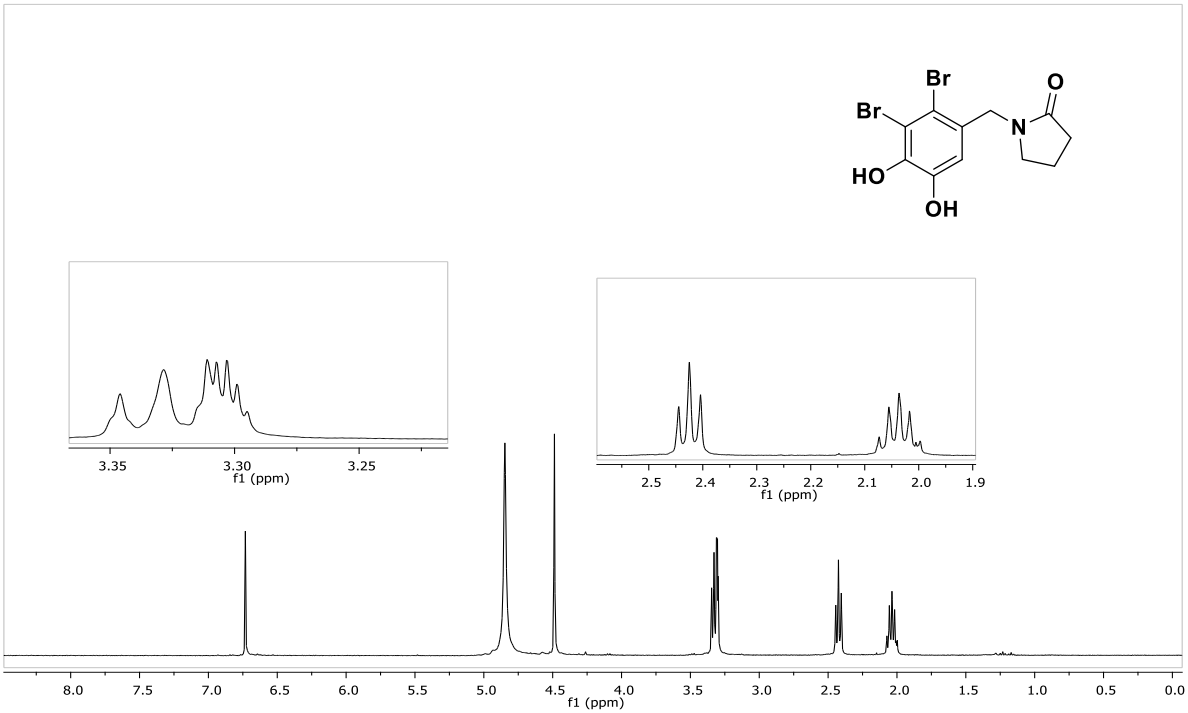
^{13}C NMR spectrum of compound **13** (100 MHz, $CDCl_3$)



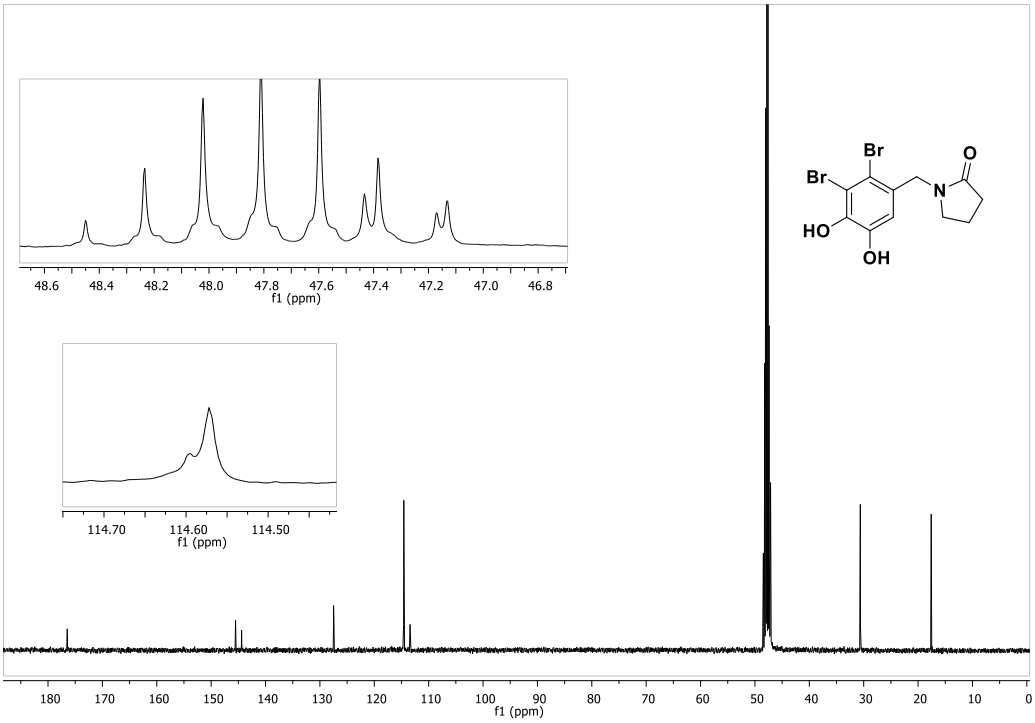
^{1}H NMR spectrum of compound **14** (100 MHz, $CDCl_3$)



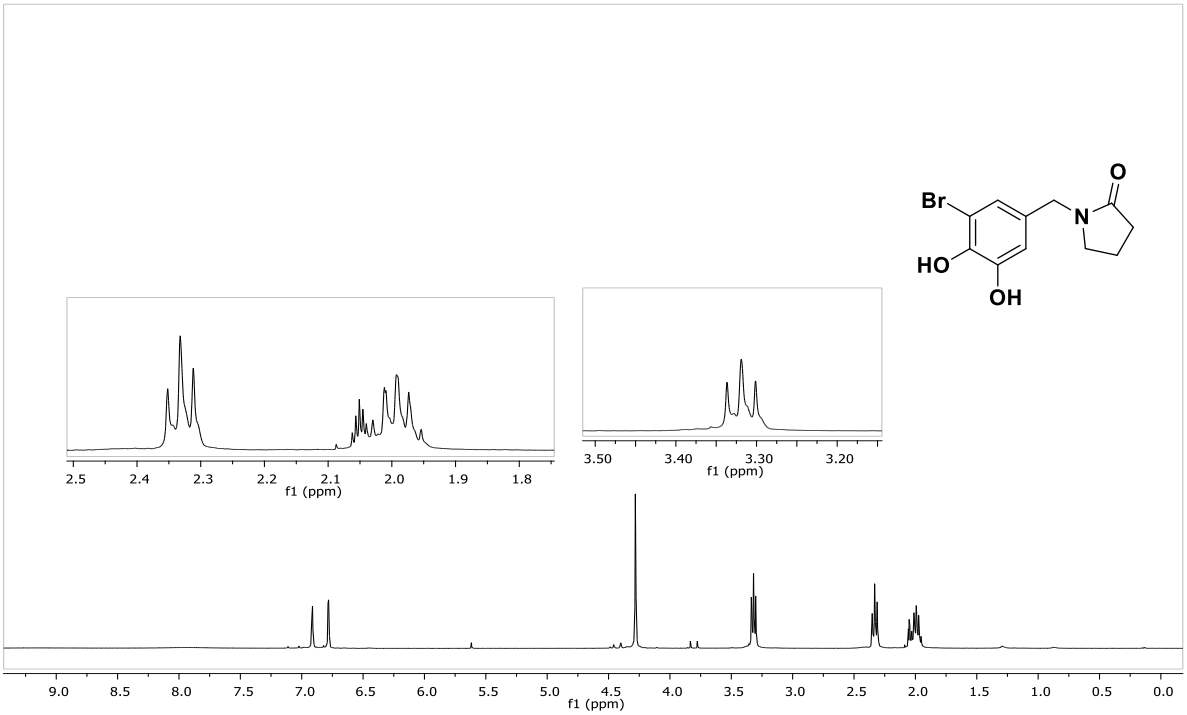
^{13}C NMR spectrum of compound **14** (100 MHz, $CDCl_3$)



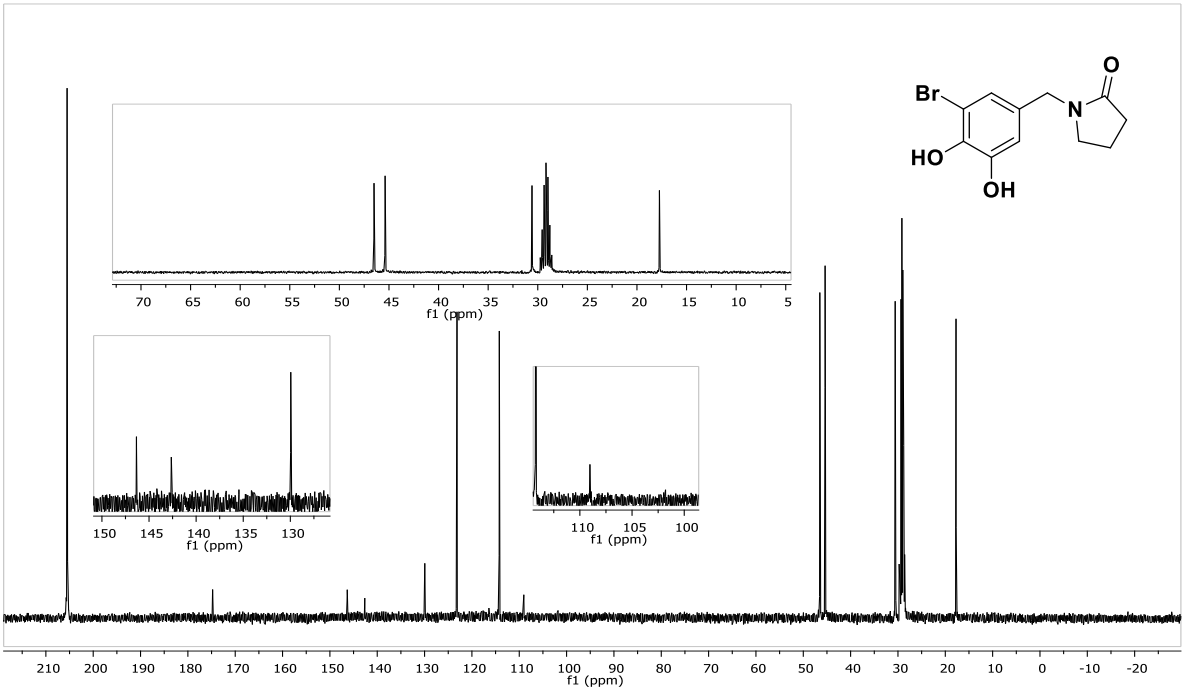
^{1}H NMR spectrum of compound **15** (400 MHz, CD_3OD)



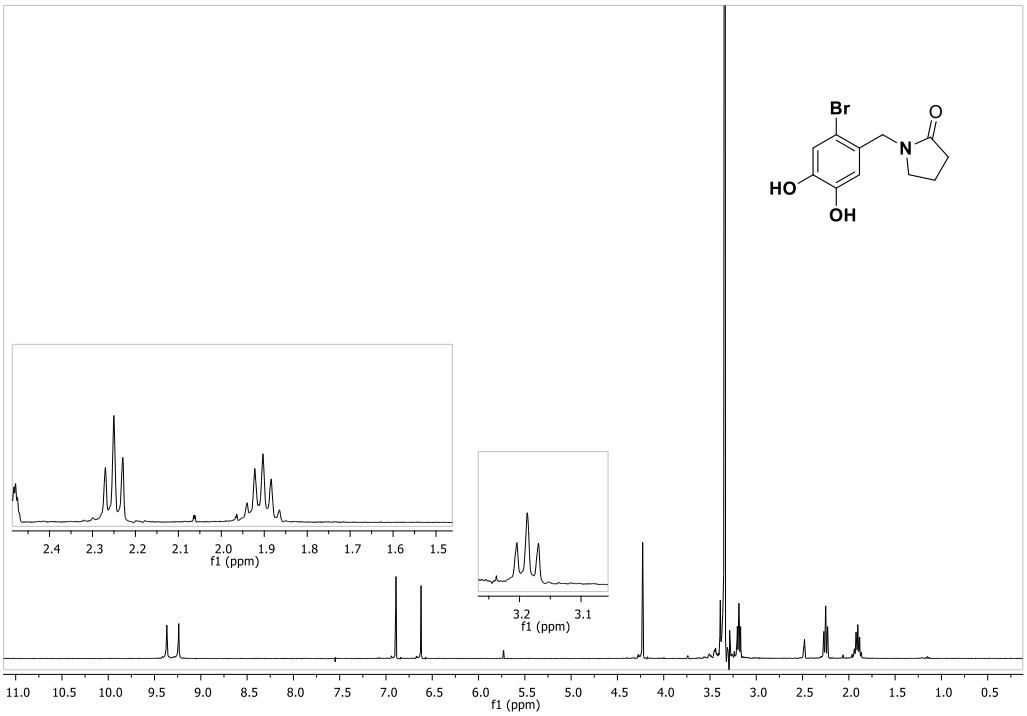
^{13}C NMR spectrum of compound **15** (100 MHz, CD_3OD)



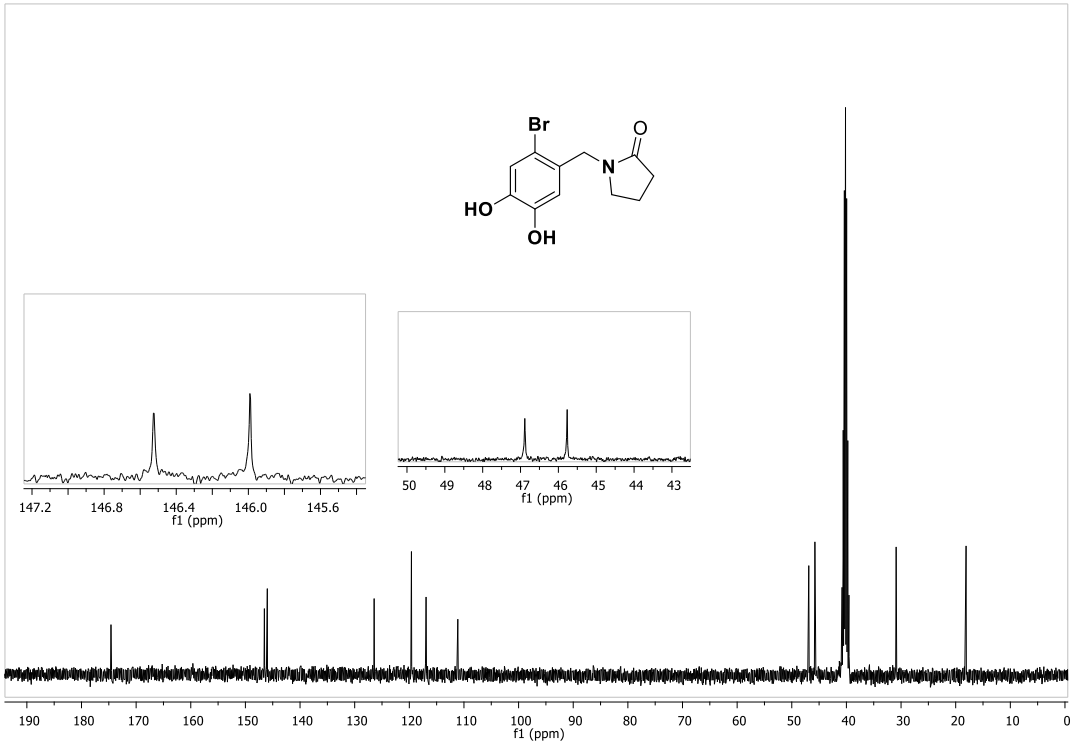
^{1}H NMR spectrum of compound **16** (400 MHz, Acetone-d6)



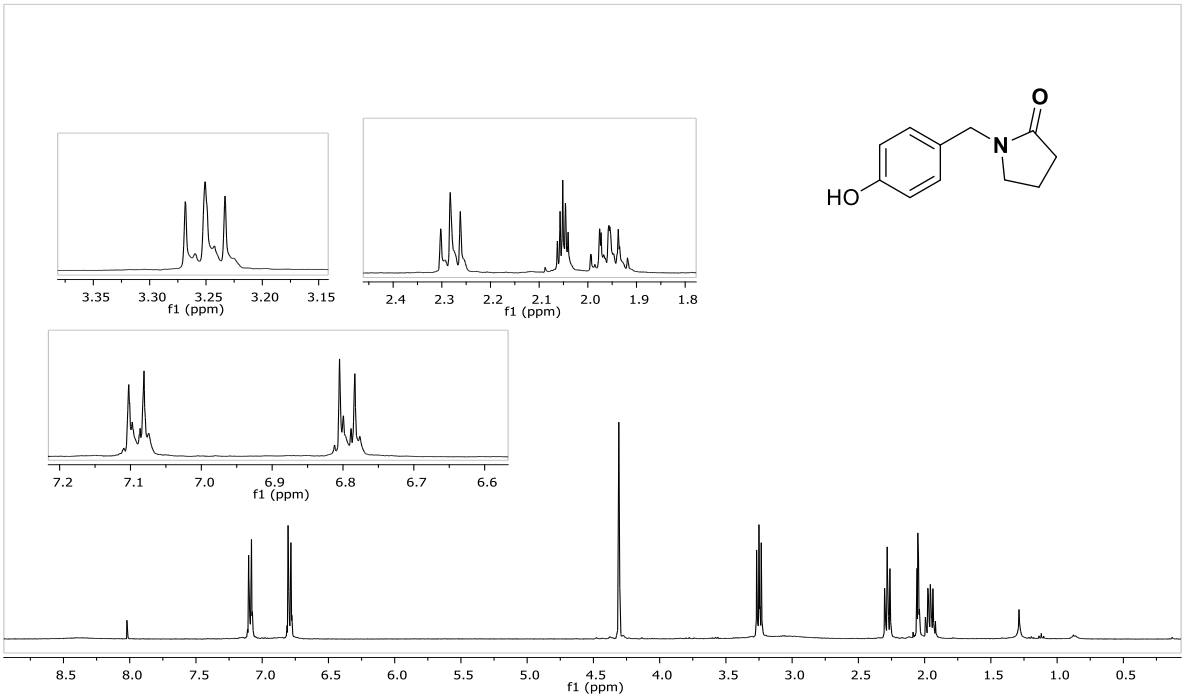
^{13}C NMR spectrum of compound **16** (100 MHz, Acetone-d6)



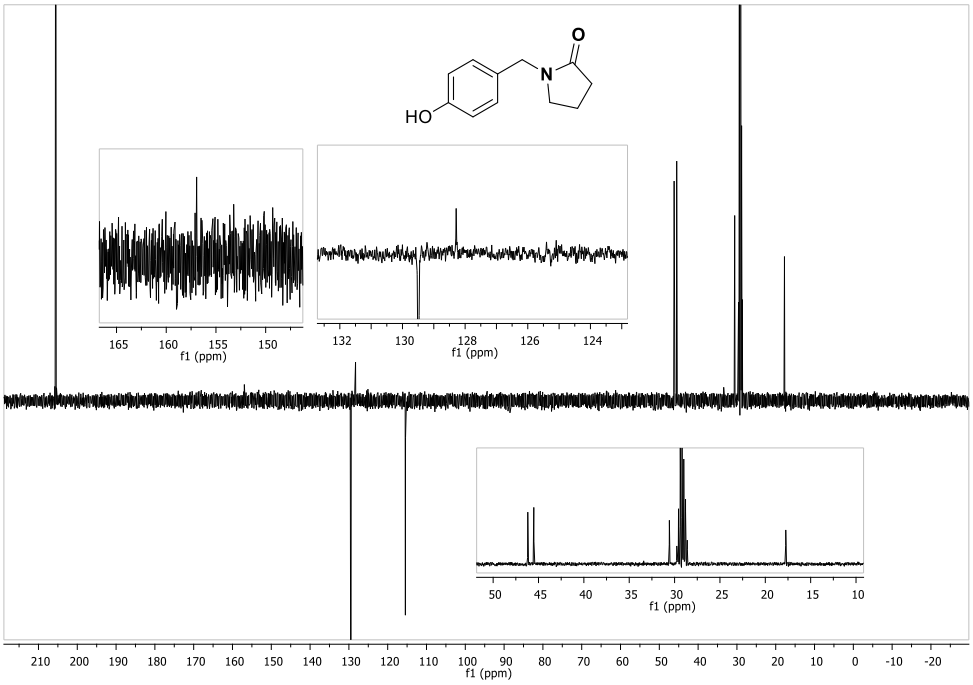
^{1}H NMR spectrum of compound **17** (400 MHz, CD_3OD)



^{13}C NMR spectrum of compound **17** (100 MHz, CD_3OD)



^{1}H NMR spectrum of compound **18** (400 MHz, Acetone-d6)



^{13}C APT spectrum of compound **18** (100 MHz, Acetone-d_6)