# Paper II

# Marine AChE inhibitors isolated from *Geodia barretti*: Natural compounds and their synthetic analogs

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**Abstract**
Barettin, 8,9-dihydrobarettin, bromoconicamin and a novel brominated marine indole were isolated from the boreal sponge *Geodia barretti* collected off the Norwegian coast. The compounds were evaluated as inhibitors of electric eel acetylcholinesterase. Barettin and 8,9-dihydrobarettin displayed significant inhibition of the enzyme, with inhibition constants ($K_i$) of 29 and 19 µM respectively via a reversible noncompetitive mechanism. These activities are comparable to several other natural acetylcholine esterase inhibitors. Bromoconicamin was less potent and the novel compound was inactive. Based on the inhibitory activity, a library of 22 simplified synthetic analogs was designed and prepared to probe the role of the brominated indole, common to all the isolated compounds. From the structure activity investigation it was shown that the brominated indole motif is not sufficient to generate a high inhibitory activity, even when combined with natural cationic ligands for the acetylcholinesterase active site. The study illustrates how both barettin and 8,9-dihydrobarettin display additional bioactivities which may help to explain their biological role in the producing organism. The findings also provide new insights into the structure activity relationship of both natural and synthetic AChE inhibitors.

## Introduction

The diverse array of organisms inhabiting the marine world offers access to new and exciting chemical scaffolds which have demonstrated potential for drug development[1-3]. Additionally, marine organisms provide more pharmacologically interesting molecules when compared with those from terrestrial sources[4, 5]. About 500 novel marine natural products are reported each year and almost half of those have been isolated from sponges (*Porifera*), making it the most productive marine taxon[5, 6]. In analogy to many of the drug leads isolated from terrestrial organisms, it is often the symbiontic microorganisms that are the actual producers of the bioactive compounds in marine organisms[2, 7, 8]. Marine microorganisms are generally notoriously challenging to cultivate and, therefore, the collection and analysis of marine macroorganisms remains a highly valuable strategy in the search for novel bioactive compounds[9, 10]. Marine sponges have been shown to be particularly rich sources of both microorganism and bioactive natural products[1]. As up to 35 % of the weight of a sponge can be composed of microorganisms, the phylum *Porifera* continue to attract particular attention from marine scientists[1, 11, 12].

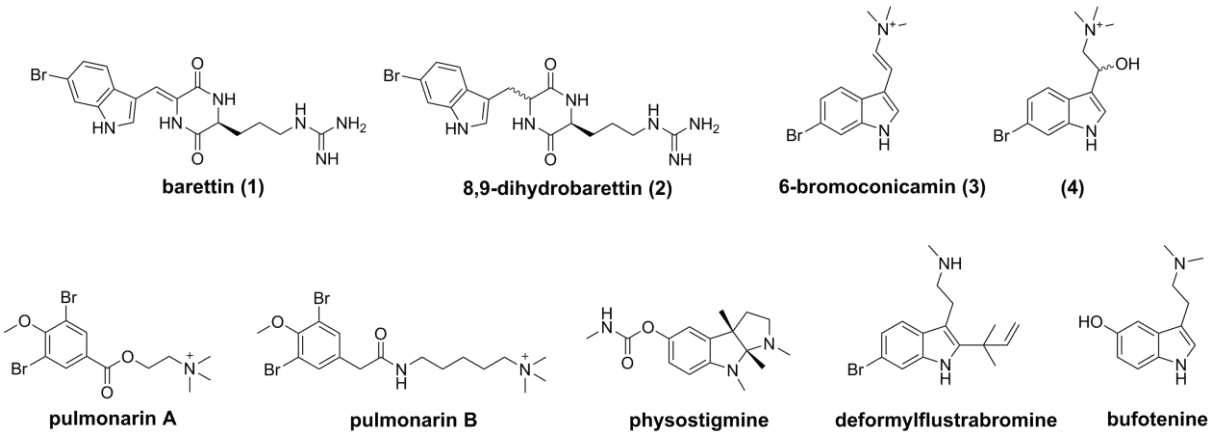
Inhibition of the enzyme acetylcholinesterase (AChE) is a common approach for the management of several disease states[13]. Most notably, AChE inhibitors are used to alleviate the symptoms of dementia and Alzheimer's disease[14, 15], glaucoma[16], and also as muscle relaxants during surgery[17, 18]. Commercial AChE inhibitors are diverse in structure and they also range significantly in affinity for AChE[19]. A common structural feature of natural and synthetic AChE inhibitors is the presence of one or more quaternary ammonium groups. Nature has provided several clinically approved AChE inhibitors that are currently in use[13]. The first approved example being the alkaloid physostigmine ($K_i$ = 30 nM) which was isolated from the Calabar bean (*Physostigma veneosum*)[20]. Other examples are Galanthamine ($K_i$ = 2-10 µM)[21], isolated from the bulbs of the common snowdrop (*Galanthus woronowii*)[22], and huperazine A ($K_i$ = 7-500 nM)[23] which is obtained from the Chinese club moss *Huperzia serrata*[19]. So far, no AChE inhibitors from the marine realm have been developed into commercial products.

Our previous studies of Arctic marine secondary metabolites of the colonial ascidian *Synoicum pulmonary* recently yielded two selective AChE inhibitors, namely pulmonarin A and B[17]. These small, dibrominated compounds displayed AChE inhibition in the pharmaceutically

relevant range ($K_i$ = 90 and 20 µM respectively) and represent interesting marine leads for further studies.

Other marine compounds that have been shown to induce AChE inhibition are the 3-alkylpyridinium polymers isolated from the Mediterranean sponge *Reniera sarai*, that act as strong, irreversible AChE inhibitors in the nanomolar range[24]. Furthermore, the bromotyrosine derivative aplysamine-4, isolated from an unidentified Red sea marine sponge[25], as well pseudozoanthoxanthin from the Adriatic soft coral *Parazoanthus axinellae*[26] were shown to act as reversible AChE inhibitors. Their potencies, $K_i$ = 16 and 4 µM, respectively, are similar to that of pulmonarin B. Onchidal and turbotoxin A from the marine molluscs *Onchidella binney* and *Turbo marmorata* are other examples of marine secondary metabolites with documented affinity for AChE[19]. Deformylflustrabromine was originally isolated from the North sea bryozoan *Flustra foliacea*[27] and is structurally similar to the currently investigated compounds. Deformylflustrabromine has been used in several mechanistic studies of AChE inhibition but only displays a moderate $IC_{50}$ of 150 µM against α4β2 nicotinic acetylcholine receptors[28, 29]. Neuroactive marine natural products was recently reviewed by Sakai and Swanson[3].

*Geodia barretti* (Bowerbank, 1958) is a large white boreal sponge with a generally smooth surface. It can reach 50 cm in diameter and possess a pungent characteristic smell. It is found in dense colonies in the northern Atlantic Ocean and is common to the Norwegian coast and in the areas around Bear Island and Svalbard. Although it has been observed at depths from 30-2000 m, most northeast Atlantic records report it residing at depths between 200 and 500 m[30]. *G. barretti* is a known source for bioactive secondary metabolites[31]. The most studied are the monobrominated 2,5-diketopiperazines barettin (**1**) and 8,9-dihydrobarettin (**2**) as described by Bohlin and others[32]. **1** and **2** have been shown to display diverse biological activities such as antifouling and a high affinity for the human serotonin receptor (5-HT), **1** has additionally been found to have antioxidant and anti-inflammatory activities[33, 34, 35].




The current study presents the results from our continued search for novel AChE inhibitors of marine origin, this time in the sponge *G. barretti.* Four brominated marine natural products (two known, one novel and one not previously reported in *G. barretti* before) were isolated and screened for inhibitory activity against electric eel AChE. Based on the observed bioactivities and structures of the isolated compounds, a library of simplified analogs were designed, synthesised and tested in an attempt to establish the structure activity relationship (SAR). The current study expands the knowledge of marine AChE inhibitors and also provides insight into the role of bromination in marine secondary metabolites.

## Results and discussion

Four monobrominated, low molecular weight compounds were found in sufficient amounts in the organic phase of the *G. barretti* extract and were isolated using mass guided preparative HPLC. The two major compounds were identified as barettin (**1**) and 8,9-dihydrobarettin (**2**), previously described from *G. barretti,* based on spectroscopic analysis[33]. In addition, the known marine indole bromoconicamin (**3**), recently described in the Okinawian sponge *Suberites sp*, was also identified and isolated for the first time from *G. barretti*[36]. The fourth compound (**4**) was novel and isolated as an amorphous solid.

The HRMS spectrum of compound **4** showed a molecular ion at *m/z* 297.0586, corresponding to the molecular formula of $C_{13}H_{18}BrN_2O$, with an isotopic pattern characteristic for a mono-brominated molecule. The $^{1}H$ and $^{13}C$ NMR spectra of **4** (Figures S6 and S7) displayed signals consistent with an indole motif. Furthermore, the $^{1}H$ NMR spectrum of **4** run in methanol-$d_3$ displayed a singlet at 10.83 ppm which was attributed to the indole N-H functionality. Multiplicity and coupling constant analysis of the relevant signals in the $^{1}H$ NMR spectrum of **4** (Table 1) suggested di-substitution of the indole moiety at the 5 or 6, and 2 or 3 positions. ROESY and HMBC correlations (Figure 2, spectra displayed in Figures S10, S11 and S13), and comparison with the NMR spectra reported for **1**[37], supported that **4** was brominated at C-6 and functionalised at C-3. The nature of the C-3 substituent was deduced by considering the remaining three resonances in the $^{1}H$ and $^{13}C$ NMR spectra, which consisted of a methine, a methylene and a methyl carbon. The methyl carbon resonance, a strong singlet at 3.32 ppm, was identified as a *N*,*N*,*N*-trimethylamino group based on an integral of 9, its equivalence resulting in overlapping $^{1}J_{CH}$ and $^{3}J_{CH}$ correlations and its relatively symmetric configuration giving an observable quadrupolar $^{14}N$ splitting for the methyl carbons (Figure S7). HMBC correlations for H-Me to C-2' and H-1' to C-2 indicated that the β-hydroxy-*N*,*N*,*N*-trimethylethanaminium group was connected to C-3 of the indole. The chemical shifts of C-6 of 116.4 ($\Delta\delta_{pred}$=0.2 ppm) and C-1' of 64.2 ppm ($\Delta\delta_{pred}$=5.7 ppm) were consistent with the bromine atom attached to C-6 and the remaining hydroxyl group residing at C-1'. All experimental and predicted chemical shifts were in agreement, the mean error being 2.3 ppm for $^{13}C$ and 0.18 ppm for $^{1}H$ for compound **1** (Figure S5). The absolute configuration of **4** was not established. Key correlations used to elucidate the structure of **4** are presented in Figure 1.

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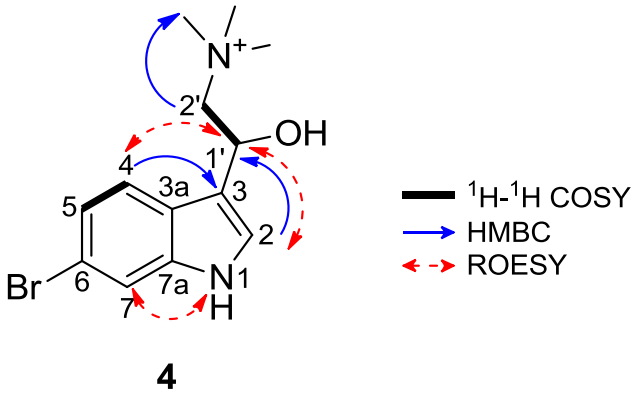



**4**

**Figure 1.** Key gHMBC (H→C), gCOSY and ROESY correlations of **4**.

**Table 1.** NMR spectroscopic data[a] (600 MHz, methanol-*d3*) for compound **4**.

| Position | $\delta_C$, type | $\delta_H$ (*J* in Hz) | $^1$H-$^1$H COSY | HMBC |
|---|---|---|---|---|
| Me | 54.85, $CH_3$ | 3.34, s | | 2’ |
| 1’ | 71.79, CH | 5.59, d (10.1) | 2’ | 2’, 2, 3 |
| 2’ | 64.18, $CH_2$ | 3.88, dd (13.5, 10.5), 3.52, dd (13.6, 2.2) | 1’ | Me |
| 1 | NH | 10.87, s | | |
| 2 | 124.86, CH | 7.35, s | | 1’, 3, 3a, 7a |
| 3 | 116.31, C | | | |
| 3a | 125.43, C | | | |
| 4 | 121.49, CH | 7.68, d (8.5) | 5 | 3, 3a, 7a |
| 5 | 123.53, CH | 7.19, dd (8.5, 1.6) | 4 | 3a |
| 6 | 116.38, C | | | |
| 7 | 115.52, CH | 7.56, d (1.4) | | |
| 7a | 139.16, C | | | |

[a] $^1$H, $^{13}$C, $^1$H-$^1$H COSY and HMBC NMR spectra are included in the supporting info, Figures S6-S9 and S13.

The four natural compounds were evaluated as potential AChE inhibitors. AChE belongs to the serine protease family of enzymes and represents the key enzyme in the nervous system as it enables the transmission of the signals in cholinergic synapses through a degradation of the neurotransmitter acetylcholine[38]. AChE represents a highly conserved enzyme and the sequence identity between species such as mammals, bird, fish and insects is significant with 14 conserved amino acids in the active site[13]. In the current study, electric eel AChE was employed as a model esterase[39]. The colorimetric assay developed by Ellman was employed to study the kinetics of the enzyme inhibition[40] and the data is presented in Table 2. Dixon plots were used to determine the inhibition constants and the type of inhibition as shown in Figure 2.

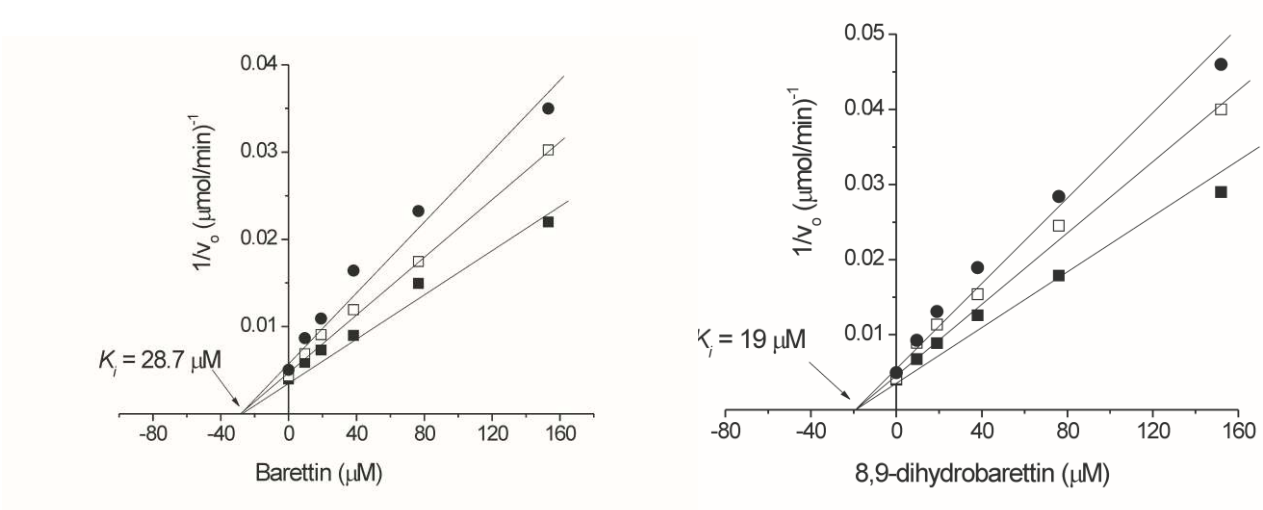



**Figure 2.** Determination of the type of inhibition and the inhibition constant $K_i$ for **1** (barettin, left graph) and **2** (8,9-dihydrobarettin, right graph) by Dixon plot analysis. The concentrations of the substrate acetylthiocholine were 0.125 (●), 0.25 (□), and 0.50 mM (■). $K_i$ was determined to 28.7 µM for **1** and 19 µM for **2**.

**Table 2** Inhibition of electric eel acetylcholinesterase by natural compounds and their synthetic analogs.

| Compound | $IC_{50}$ (µM)[a] | $K_i$ (µM) |
|---|---|---|
| **1** | 36 | 29 |
| **2** | 29 | 19 |
| **3** | 230 | 90 |
| **4** | > 690 | n.d[b] |
| **5a** | 1046 | n.d[b] |
| **6a** | 360 | 323 |
| **7a** | 92 | 42 |
| **8a** | 82 | 49 |
| **9a** | 78 | 57 |
| **5b** | 374 | 307 |
| **6b** | 260 | 230 |
| **7b** | 669 | n.d[b] |
| **8b** | 281 | 255 |
| **9b** | 138 | 92 |
| **5c** | 157 | 116 |
| **6c** | 156 | 130 |
| **7c** | 230 | 176 |
| **8c** | 187 | 124 |
| **9c** | 319 | 223 |
| **5d** | 637 | n.d[b] |
| **6d** | 293 | 271 |
| **7d** | 1046 | n.d[b] |
| **8d** | 142 | 97 |
| **9d** | 319 | 213 |
| **8e** | 478 | 369 |
| **9e** | 492 | 398 |
| Pulmonarin A[c] | 150 | 90 |
| Pulmonarin B[c] | 36 | 20 |

[a] $IC_{50}$ is determined as the concentration of the compound inducing 50% inhibition of the enzyme activity
[b] $K_i$ not determined for compounds displaying an $IC_{50}$ > 500 µM
[c] Data taken from ref 14

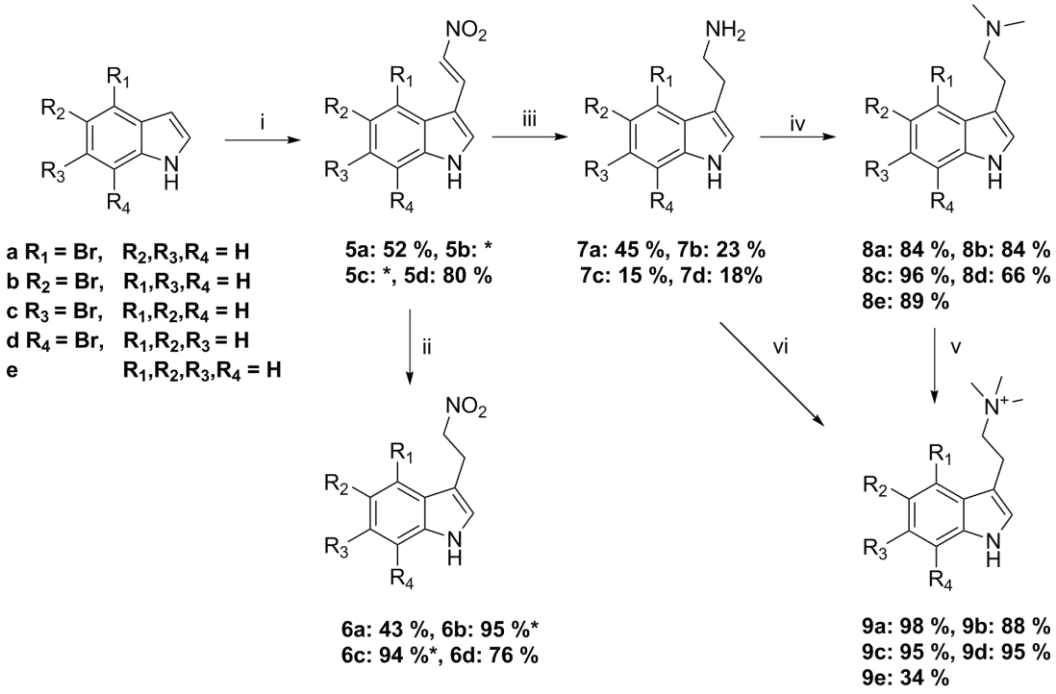
Compounds **1-4** displayed ranging potencies as inhibitors of AChE (Table 2). Both **1** and **2** displayed $IC_{50}$ values (36 and 29 µM respectively) which are similar to the activities of marine AChE inhibitors pulmonarin B (36 µM) and turbotoxin A (28 µM) and places the activity of these compounds in the pharmaceutically interesting realm. Dixon plot analysis revealed that both compounds were non-competitive inhibitors of electric eel AChE as presented in Figure2. Compound **3** was moderately potent while **4** was inactive at the highest concentration employed. The inactivity of **4** was unexpected given its structural resemblance with other known natural AChE inhibitors such as the pulmonarins[17], deformylflustrabromine[27] and bufotenine[41].

Both **1** and **2** have been described as ligands for several subtypes of the human serotonin receptor (for **1** the 5-$HT_{2A}$, 5-$HT_{2C}$, and 5-$HT_4$ subtypes) with binding affinities at low micromolar concentrations, close to that of endogenous serotonin[35]. The inhibition of these types of receptors by **1** has been hypothesized to be the underlying mechanism of the antifouling activity of **1** towards barnacle crustaceans. A 5-$HT_2$ receptor has recently been cloned and functionally characterized from the spiny lobster, *Panulirus interruptus*[42] and DNA sequences homologous to a human 5-$HT_{1A}$ receptor has been found in cyprid larvae of the barnacle, *B. amphitrite*[43]. Serotonin has been shown to display AChE inhibition (63 % at 100 μM) indicating that the indole is accommodated in the active site[44]. It was therefore not unexpected to also see affinity and inhibitory activity towards AChE for **1** and **2** given the structural resemblance. The well-established antifouling potential of these compounds could also be due to their AChE-inhibitory potential. In fact, a functional set of cholinergic molecules is present in *B. amphitrite* cyprids, predominantly in the thoracic appendages and the caudal rami extending from the cyprid's thoracic region. AChE activity was also detected in the setae of the antennules, which play an important role in the process of substratum recognition and subsequent settlement[45]. It is possible that AChE is involved in larval settlement and is therefore a viable target for inhibiting this process.

The active site of AChE, located at the bottom of a 20 Å deep enzyme gorge, is composed of two subsites: the esteratic subsite which contains the catalytic triad responsible for acetylcholine hydrolysis, and the anionic site with is responsible for the accommodation of the positive quaternary amine of acetylcholine[38]. At the rim of the gorge, another choline binding site exists - the peripheral anionic site. Ligand binding at this site destabilizes binding of the substrate, and this site is also responsible for AChE inhibition by excess acetylcholine. In analogy with the binding mode of acetylcholine, both anionic and peripheral anionic sites can bind compounds carrying quaternary nitrogen[46].

Based on the pronounced activity of **1** and **2**, it was decided to probe the individual structural contributions to this activity. Given the challenging syntheses needed for preparation of synthetic **1** and **2**, focus was placed on the contribution from the brominated indole[47, 48]. This epitope has previously been found in compounds with affinities for AChE[49]. The brominated indole is a common structural motif to the four natural compounds of this study and is also found in many other marine natural products[50]. For the analog library, the indole was monobrominated in the 4-7 positions to probe the bromine contribution to activity (Scheme 1). Amines (the corresponding nitro intermediates were also included) at the C-2' position, with varying degrees of alkylation, represented the cationic element required for AChE binding. This generated a focused library of bromotryptamines. resembling both **3** and **4** in terms of size and distribution of charge and lipophilicity. The library also resembles the structure of the marine AChE inhibitors pulmonarins A and B which can be regarded as *N*-trimethylated ammonium derivatives of dibrominated tyrosine[17]. The pulmonarins display a similar degree of AChE-inhibitory activity as **1** and **2**, illustrating that these simpler analogs can be good inhibitors. The library is also closely adhering to the structure of bufotenine a natural AChE inhibitor both isolated from the fungus *Amanita mappa* and from several frog skin excretions[51].

The 22 analogs (**5a**-**9e**) were prepared from indole, monobrominated in the 4-7 positions. TFA-promoted condensation with dimethylaminonitroethylene followed by either $LiAlH_4$ or $NaBH_4$ mediated reduction converted the starting material to either bromotryptamine derivatives or their nitro analogs. The dibromotryptamines were prepared from the bromotryptamines by reaction with $NaBH_3CN$ and formaldehyde. Methylation with MeI provided the trimethylated tryptamines in high yields.




**Scheme 1.** Reagents, solvents, conditions and isolated yield: (i) *N,N*-dimethylamino nitroethylene,TFA, (ii) $H_3BO_3$, $NaBH_4$, THF, isopropanol (iii) $LiAlH_4$, THF, (iv) acetic acid, $NaBH_3CN$, formaldehyde (v) MeI, $CHCl_3$ (vi) MeI, isopropanol. *Yield over two steps reported.

The inhibitory activity of the analogs ranged from that being inactive at concentrations >1000 µM to those moderately potent. None of the simpler synthetic compounds were as active as **1** or **2** and the most active analog (**9a**) displayed an $IC_{50}$ of 78 µM (Table 2). The analog library was designed to probe the role of bromination and also the degree of alkylation of the amine. The analogs were monobrominated at the 4-7 positions and it is clear from the biological data that bromination in the 4-position yields the most potent AChE inhibitors, when combined with a positive charge. Bromination in the 5-position, which creates mimics of serotonin, generates analogs with similar activity as those brominated in the 6-position. The 6-position is the common position for natural marine bromination[50] and it is interesting to see the lack of distinction in inhibition between the 5-and 6-isomers. Bromination in the 7-position generated the poorest synthetic analogs. Acetylcholine and many other strong AChE binders display quaternary ammonium groups as ligands for the AChE anionic peripheral site[52]. For this reason, it was expected that the trimethylated analogs would display significantly higher inhibitory effects but that is not the case for the current library. The degree of amine alkylation appears to be insignificant for the role of binding these compounds to the active site of AChE. The fact that the nitro analogs also display similar inhibitory activities implies that the compounds perhaps are not effectively accessing the binding pockets in the active site. Analogs **8e** and **9e** lack any bromine functionality and were active at 500 µM. This illustrates that the synergistic effects of having both a bromine and a charge in the analogs are present but not particularly pronounced. No link between hydrophobicity and inhibitory activity was seen for the synthetic analogs. These findings are interesting from a structure activity viewpoint. In a recent report by Queiroz *et al*, similar compounds to those prepared synthetically were isolated from the bark of the Brazilian tree *Tetraptery mucronata* and tested for AChE inhibition[41]. The difference between the synthetic molecules of this work and the natural products in their study is the

replacement of the bromine with a hydroxyl or a methoxy group. The 5-hydroxyl analog (bufotenine) of **8b** displays an $IC_{50}$ of 12.5 µM, clearly indicating that the smaller hydroxyl group is a much-preferred substituent when compared to the bromine used in the synthetic analog library. The methoxylated analogs also displayed similar activity[41]. Synthetic **8e**, prepared without any substituent, is not particularly active either, suggesting that the 5-hydroxyl, found in serotonin and several other natural compounds, is beneficial for the inhibition of AChE. The recently reported structure-activity relationship study of desformylflustrabromine by German *et al.* also suggested that the natural bromine in the 6-position could be omitted whilst maintaining activity as positive allosteric modulator of α4β2 neuronal nicotinic acetylcholine receptors[49].

Collectively, these finding suggest that the mechanistic reasons for the higher inhibitory effects seen for **1** and **2** stretches beyond the brominated indole. It is likely that both the 2,5-diketopiperazine core and the cationic arm are involved in additional distal interactions to generate a higher inhibitory effect. The calculated ClogP values for **1** and **2** are -0.19 and 0.20, respectively. This indicates that these two compounds are more hydrophilic in comparison with the other natural and synthetic compounds (ClogP ranging between 1.73 to 2.88). These differences in polarity may also influence the binding to, and inhibition of AChE to some extent. In previous bioactivity studies on 2,5-diketopiperazines from *G. barretti* **2** has consistently displayed decreased activity in comparison with **1**. As a 5-HT ligand, **2** only displayed binding to the 5-$HT_{2C}$ receptor[35] and the antifouling activity is 10-fold lower than that of **1**[33]. The slightly increased AChE inhibitiory activity in the current study is therefore worth noting. The reduction of the exocyclic double bond generates a higher flexibility for the brominated indole sidechain which may reach the desired binding epitope at a lower energy cost.

Several AChE inhibitors of natural origin are not only potent but also deadly, as exemplified by the fasiculin peptides from the green mamba (*Dendroaspis angusticeps*)[53] and several of the marine inhibitors previously discussed[19]. No animal studies were performed in the present investigation but previous studies have indicated a low cellular toxicity (>100 µM) for **1** against human fibroblasts (MRC-5) and hepatocytes (HepG2)[34]. This illustrates the potential therapeutic window for these compounds. The current study highlights the broad activities of **1** and **2**. Furthermore, the AChE inhibition discovered may contribute to the antifouling effect of **1** and **2** observed against marine biota.

**1** and **2** are cyclized dipeptides arising from coupling of tryptophan and arginine. It is not known when the bromoperoxidase catalysed bromination[50] of the indole occurs. Whether the two smaller compounds, also isolated from the *G. barretti* extract, are degradation products or precursors of **1** was not ascertained. The ratio of the compounds in the extract was constant over time and remained stable irrespective of sample handling which illustrates their individual stabilities. The quaternary ammonium group coupled via an ethylene linker of **3** and **4** implies a biosynthetic route involving choline and it therefore appears unlikely that they represent precursors of **1**.

A pronounced activity towards both AChE and several subtypes of the human serotonin receptor subtypes makes the two barettins (**1** and **2**) interesting compounds for further studies directed towards the treatment central nervous system diseases. Additionally, **1** is a powerful antioxidant and also displays anti-inflammatory properties[34]. The particular combination of receptor affinities have recently been highlighted as a promising approach for new therapies against Alzheimer's disease[54]. Multitarget-directed ligands (MTDL) acting as both AChE inhibitors and 5-HT receptor agonists are currently under development[54]. Natural products have

been heralded as particularly promising for MTDL design and the barettin scaffold may add additional insights into the structural requirements for the design of optimized MTDL in the future[55].

## Conclusion

Four marine natural products, three known and one novel, were isolated from an organic extract of *G. barretti*. The compounds were screened for inhibitory activity against electric eel AChE. It was shown that **1** and **2** displayed inhibitory properties comparable to several other marine AChE inhibitors and also to the commercially used AChE inhibitor galanthamine. A library of simplified synthetic analogs were designed and prepared to mainly investigate the role of the brominated indole. None of the synthetic analogs were as active as the two active natural products (**1** and **2)** and illustrate that the brominated indole is not sufficient for high activity. A surprisingly low effect was seen when incorporating the natural ligand for the AChE active site into the synthetic compounds. The study illustrates that **1** and **2** display additional bioactivities which may help to explain their biological role in the organism. The findings also provide new insights into the structure-activity relationship of AChE inhibitors.

## Experimental section

### General Experimental Procedures

High resolution MS spectra were acquired on either a UPLC-ToF system, for the natural products **3** and **4**, or an orbitrap instrument for the synthetic compounds **5a**-**9e**. The Waters UPLC-ToF system (Milford, MA, USA), with MassLynx version 1.1 as software, was used for the accurate mass determination. This system included Waters LCT permier and Waters acquity UPLC. The compounds were separated on a Acquity UPLC® BEH C18 (2.1 × 50 mm, 1.7 µm) column. Gradients of $H_2O$ with 0.1% formic acid (FA) (A) and acetonitrile with 0.1% FA (B) were used at a flow rate of 0.35 mL/min (20-100% B over 3.5 min) An LTQ Orbitrap XL Hybrid Fourier Transform mass spectrometer from Thermo Scientific and the Thermo Scientific Accela HPLC-LTQ Ion Trap-Orbitrap Discovery system was used to determine accurate mass of the synthetic compounds. ChemDraw Pro V 12.0.2 was used to calculate the exact masses of all the compounds. All starting materials were purchased from Sigma-Aldrich. Chemicals for the synthesis were used without further purification. 1D and 2D NMR spectra of natural product **4** were acquired on a Varian Inova spectrometer operating at 599.936 MHz for $^1$H equipped with a 5 mm inverse triple resonance cryogenically enhanced HCN probe. The compounds were dissolved in 100 µl solvent in 3 mm solvent matched shigemi tubes. Data was acquired in methanol-$d_3$ and methanol-*d4* at 25 °C. Carbon resonances were either acquired directly or derived from gHMBC experiments. Chemical shifts were referenced to the residual solvent peaks, for methanol-*d4* : $\delta_H$ 3.310 ppm and $\delta_C$ 49,000 ppm, and for Acetone-$d_6$: $\delta_H$ 2.050 ppm and $\delta_C$ 29.840 ppm. . 1D and 2D NMR spectra of the synthetic compounds were acquired on either a Varian Inova or a Varian Mercury plus spectrometer. Infrared spectra were recorded on an Avatar 320 FT-IR spectrometer from Nicolet. Synthetic compounds **7a**, **b** and **d** were purified using a Waters Xbridge BEH C18 OBD (19 × 250 mm, 5 µm) column.

**Extraction, Isolation and Identification of Natural Products 1-4** Compounds **1-4** were isolated from the sponge *Geodia barretti*, which was collected in Varangerfjorden (Norway 2012) by the marine biobank Marbank. After being freeze dried and ground, extraction with water was followed by an extraction of the pellet (DCM:MeOH, 1:1). The extracts were stored at -24°C, at MabCent, the Research Park, Norway until use. Due to a larger content of the

brominated compounds only the organic extract was used. The organic extract was dissolved in hexane (100 mL) was partitioned twice with 90% MeOH (2 × 50 mL) at room temperature to remove highly lipophilic compounds. Following, the volatiles were removed *in vacuo* at 40°C and SpeedVac™ before dissolved in 1:1 $H_2O$/MeOH and loaded on a preparative HPLC column. A Waters (Milford, MA, USA) purification system, and a fraction collector with MassLynx version 4.1 SCN 714 as software was utilized. A Waters 515 pump, Waters flow splitter (99% of the flow proceeded to the fraction collector and 1% to the MS), Waters 3100 mass spectrometer (in positive mode, with an ESI-electrospray source), Waters 2996 photo diode array detector and Waters 2767 sample manager constituted the purification system. The fraction collection was triggered when the intensity of the target mass exceeded the threshold specified in the method. A Waters X-terra MS-C18 (10 × 250 mm, 5 μm) column was used to isolate the compounds. Gradients of $H_2O$ with 0.1% FA (A) and acetonitrile with 0.1% FA (B) were used at a flow rate of 6 mL/min and optimized for each compound (compounds **1** and **2** 10-40 % B over 10 min, compound **3** 20-35% B over 25 min and compound **4**: 10-30% B over 10 min).

Obtained MS data in combination with Elemental Composition version 4.0 software were used for estimation of possible elemental compositions of the isolated compounds. Compounds **1**-**3** were identified based on their elemental compositions and spectral comparison with published compounds. **1** and **2** have previously been found in *G. barretti*. The structure of **4** was elucidated using combination of HRMS and NMR.

**2-(6-bromo-1H-indol-3-yl)-2-hydroxy-*N,N,N*-trimethylethanaminium (4)**. Yellow oil; UV (MeOH) $\lambda_{max}$ (log $\varepsilon$) 283.8 nm (3.8); $[\alpha]^{22}_D \pm 0°$ (c 0.1, MeOH); IR $\nu_{max}$ 3190, 1586, 1343 and 807 $cm^{-1}$; $^1$H NMR (600 MHz, methanol-*d4* and methanol-*d3*) δ 10.87 (1H, s, H-NH), 7.68 (1H, d, *J* = 8.5 Hz, H-4), 7.56 (1H, d, *J* = 1.8 Hz, H-7), 7.35 (1H, s, H-2), 7.19 (1H, dd, *J* = 8.4, 1.8 Hz, H-5), 5.61 – 5.57 (1H, d, H-1'), 3.88 (1H, dd, *J* = 13.6, 10.5 Hz, H-2'a), 3.52 (1H, dd, *J* = 13.6, 2.5 Hz, H-2'b), 3.34 (9H, s, H-Me); $^{13}$C NMR (151 MHz, methanol-*d4*) $\delta_C$ 139.16 (C-7a), 125.43 (C-3a), 124.86 (C-2), 123.53 (C-5), 121.49 (C-4), 116.38 (C-6), 116.31 (C-3), 115.52 (C-7), 71.79 (C-1'), 64.18 (C-2'), 54.85 (C-Me); HRESIMS *m/z* 297.0594 $[M]^+$ (calcd for $C_{13}H_{18}{}^{79}BrN_2O^+$, 297.0597).

## Synthesis

### General procedure for the preparation of compounds (5a-d).

**4-bromo-3-(2-nitroethenyl)-indole (5a)**[56]. 4-bromoindole (0.5 g, 2.55 mmol) and 1-(dimethylamino)-2-nitroethylene (0.296 g, 2.55 mmol) were treated with trifluoroacetic acid (5 mL) at room temperature. The reaction was stirred for 50 min and then it was quenched by slowly adding saturated aqueous $NaHCO_3$ (25 mL). The resultant slurry was extracted with EtOAc (3 × 25 mL), the combined organic layers washed with water (2 × 100 mL), brine (100 mL) and dried with anhydrous $Na_2SO_4$. The solvents were removed *in vacuo* and the resulting residue purified by column chromatography (heptane:EtOAc 1:1) to afford the title compound as a brown/red amorphous solid (0.353 g, 52%); IR $\nu_{max}$ 3265, 3106 $cm^{-1}$; $^1$H NMR (600 MHz, methanol-*d4*) $\delta_H$ 9.27 (1H, d, *J* = 13.3 Hz, H-1'), 8.17 (1H, s, H-2), 7.82 (1H, d, *J* = 13.3 Hz, H-2'), 7.48 (1H, d, *J* = 8.1 Hz, H-7), 7.39 (1H, d, *J* = 7.6 Hz, H-5), 7.12 (1H, t, *J* = 7.9 Hz, H-6); $^{13}$C NMR (151 MHz, methanol-*d4*) $\delta_C$ 139.92 (C-7a), 134.67 (C-2'), 133.15 (C-5), 130.77 (C-6), 127.22 (C-3a), 125.92 (C-4), 124.89 (C-2), 114.37 (C-1'), 113.16 (C-7), 109.47 (C-3); HRESIMS *m/z* 264.9620 $[M + H]^-$ (calcd for $C_{10}H_6{}^{79}BrN_2O_2^-$, 264.9613).

**5-bromo-3-(2-nitroethenyl)-indole (5b)**[57]. Orange amorphous solid (yield over two steps reported); IR $\nu_{max}$ 3337, 3109 $cm^{-1}$; $^1$H NMR (600 MHz, methanol-*d4*) $\delta_H$ 8.32 (1H, d, *J* = 13.5

Hz, H-1'), 7.94 (1H, d, *J* = 1.7 Hz, H-7), 7.93 (1H, s, H-2), 7.82 (1H, d, *J* = 13.5 Hz, H-2'), 7.43 – 7.35 (2H m, H-4 and H-6); $^{13}$C NMR (151 MHz, methanol-*d4*) $\delta_C$ 137.92 (C-7a), 136.19 (C-2'), 134.47 (C-5), 133.09 (C-6), 127.78 (C-3a), 127.29 (C-4), 123.56 (C-2), 116.12 (C-1'), 115.05 (C-7), 109.24 (C-3); HRESIMS *m/z* 266.9766 $[M + H]^+$ (calcd for $C_{10}H_8{}^{79}BrN_2O_2^+$, 266.9764).

**6-bromo-3-(2-nitroethenyl)-indole** (**5c**)[57]. Brown amorphous solid (yield over two steps reported); IR $\nu_{max}$ 3226, 3109, 3044 cm$^{-1}$; $^1$H NMR (600 MHz, methanol-*d4*) $\delta_H$ 8.35 (1H, d, *J* = 13.5 Hz, H-1'), 7.93 (1H, s, H-2), 7.89 (1H, d, *J* = 13.4 Hz, H-2'), 7.77 (1H, d, *J* = 8.2 Hz, H-4), 7.66 (1H, d, *J* = 1.7 Hz, H-7), 7.38 (1H, dd, *J* = 8.5, 1.8 Hz, H-5); $^{13}$C NMR (151 MHz, methanol-*d4*) $\delta_C$ 140.11 (C-7a), 136.06 (C-2'), 134.55 (C-5), 133.28 (C-6), 125.93 (C-3a), 125.01 (C-4), 122.49 (C-2), 117.70 (C1'), 116.33 (C-7), 109.88 (C-3); HRESIMS *m/z* 266.9768 $[M + H]^+$ (calcd for $C_{10}H_8{}^{79}BrN_2O_2^+$, 266.9764).

**7-bromo-3-(2-nitroethenyl)-indole (5d)**[58]. Orange amorphous solid (1.095 g, 80% yield); IR $\nu_{max}$ 3246, 2792, 2361 cm$^{-1}$; $^1$H NMR (600 MHz, acetone-*d6*) $\delta_H$ 8.38 (1H, d, *J* = 13.6 Hz, H-1'), 8.25 (1H, s, H-2,), 8.03 (1H, d, *J* = 8.0 Hz, H-4), 7.95 (1H, d, *J* = 13.6 Hz, H-2'), 7.53 (1H, d, *J* = 7.6 Hz, H-6), 7.25 (1H, t, *J* = 7.6 Hz H-5); $^{13}$C NMR (151 MHz, methanol-*d4*) $\delta_C$ 137.90 (C-7a) 135.88 (C-2'), 134.68 (C-5), 133.74 (C-6), 127.81 (C-3a), 127.20 (C-4), 124.15 (C-2), 120.54 (C-1') 110.81 (C-7), 106.62 (C-3); HRESIMS *m/z* 266.9768 $[M + H]^+$ (calcd for $C_{10}H_8{}^{79}BrN_2O_2^+$, 266.9764).

**General procedure for the reduction of 5a-d (6a-d)**[59] .

**4-bromo-3-(2-nitroethyl)-indole (6a)**. To **5a** (0.051 g, 0.19 mmol), in a mixed solvent of tetrahydrofuran (3 mL) and isopropanol (9 mL), was added $H_3BO_3$ powder (0.071 g, 1.15 mmol) followed by $NaBH_4$ (0.043 g, 1.15 mmol) in portions. The reaction was stirred at room temperature for 2 hours and then it was quenched by slowly adding 1 M aq. HCl (1.2 mL). The solvents were removed *in vacuo*, the resulting solid added water (10 mL) and the aqueous solution extracted with EtOAc (3 × 10 mL). The combined organic layers were washed with water (30 mL), brine (30 mL) and dried with anhydrous $Na_2SO_4$. The solvents were removed *in vacuo* to afford the title compound as a pale yellow oil (0.022 g, 43%) to be used without further purification. IR $\nu_{max}$ 3414, 3135 cm$^{-1}$; $^1$H NMR (400 MHz, methanol-*d4*) $\delta_H$ 7.33 (1H, d, *J* = 8.2 Hz, H-7), 7.19 (1H, d, *J* = 7.5 Hz, H-5), 7.15 (1H, s, H-2), 6.96 (1H, t, *J* = 7.8 Hz, H-6), 4.76 (2H, t, *J* = 7.1 Hz, H1'), 3.65 (2H, t, *J* = 7.2 Hz, H-2'); $^{13}$C NMR (151 MHz, acetone-*d6*) $\delta_C$ 139.16 (C-7a), 126.85 (C-2), 125.78 (C-3a), 124.27 (C-5), 123.53 (C-6), 113.78 (C-4), 112.26 (C-7), 110.77 (C-3), 77.87 (C-2'), 25.11 (C-1'); HRESIMS *m/z* 266,9778 $[M + H]^-$ (calcd for $C_{10}H_8{}^{79}BrN_2O_2^-$, 266,9769).

**5-bromo-3-(2-nitroethyl)-indole** (**6b**)[60]. Orange oil (0.261 g, 95% over two steps), IR $\nu_{max}$ 3427 cm$^{-1}$; $^1$H NMR (400 MHz, methanol-*d4*) $\delta_H$ 7.70 (1H, d, *J* = 1.7 Hz, H-7), 7.26 (1H, d, *J* = 8.4 Hz, H-4), 7.22 – 7.17 (1H, m, H-6), 7.12 (1H, s, H-2), 4.70 (2H, t, *J* = 7.0 Hz, H-1'), 3.39 (2H, t, *J* = 7.0 Hz, H-2'); $^{13}$C NMR (151 MHz, methanol-*d4*) $\delta_C$ 136.59 (C-7a), 129.90 (C-3a), 125.60 (C-2), 125.35 (C-6), 121.52 (C-5), 114.02 (C-4), 113.16 (C-3), 110.38 (C-7), 76.87 (C-2'), 24.27 (C-1'); HRESIMS *m/z* 266.9773 $[M + H]^-$ (calcd for $C_{10}H_8{}^{79}BrN_2O_2^-$, 266.9769).

**6-bromo-3-(2-nitroethyl)-indole** (**6c**)[60]. Orange oil (0.235 g, 94% over two steps), IR $\nu_{max}$ 3427, 2918 cm$^{-1}$; $^1$H NMR (600 MHz, methanol-*d4*) $\delta_H$ 7.50 (1H, d, *J* = 1.7 Hz, H-4), 7.45 (1H, d, *J* = 8.3 Hz, H-7), 7.16 – 7.11 (1H, m, H-5), 7.09 (1H, s, H-2), 4.70 (2H, t, *J* = 7.0 Hz, H-1'), 3.40 (2H, t, *J* = 7.0 Hz, H-2'); $^{13}$C NMR (151 MHz, methanol-*d4*) $\delta_C$ 138.83 (C-7a), 127.13 (C-

3a), 124.99 (C-2), 123.10 (C-6), 120.41 (C-5), 116.04 (C-4), 115.24 (C-3), 110.99 (C-7), 76.87 (C-2'), 24.36 (C-1'); HRESIMS *m/z* 266.9773 $[M + H]^-$ (calcd for $C_{10}H_8{}^{79}BrN_2O_2^-$, 266.9769).

**7-bromo-3-(2-nitroethyl)-indole** (**6d**). Yellow oil (0.153 g, 76% yield); IR $\nu_{max}$ 3423 $cm^{-1}$; $^1H$ NMR (600 MHz, methanol-*d4*) $\delta_H$ 7.54 (1H, d, *J* = 7.9 Hz, H-4), 7.28 (1H, d, *J* = 7.6 Hz, H-6), 7.17 (1H, s, H-2), 6.95 (1H, t, *J* = 7.8 Hz, H-5), 4.72 (2H, t, *J* = 7.0 Hz, H-1'), 3.42 (3H, t, *J* = 7.0 Hz, H-2'); $^{13}C$ NMR (151 MHz, methanol-*d4*) $\delta_C$ 136.44 (C-7a), 129.71 (C-3a), 125.22 (C-2), 125.21 (C-6), 121.24 (C-5), 118.43 (C-4), 111.97 (C-3), 105.66 (C-7), 76.83 (C-2'), 24.56 (C-1'); HRESIMS *m/z* 266.9775 $[M + H]^-$ (calcd for $C_{10}H_8{}^{79}BrN_2O_2^-$, 266.9769).

**General procedure for the preparation of compounds (7a-d)**. [61] **4-bromotryptamine** (**7a**) [56]. $LiAlH_4$ (0.444 g, 11.7 mmol) was added dry THF (25 mL) and stirred for 5 min at room temperature under an inert atmosphere. The reaction was cooled down to -78 °C for 10 min and then **6a** (0.521 g) dissolved in dry THF (25 mL) was added drop wise while the reaction was stirred. It was allowed to warm to room temperature overnight and after 12 hours the flask was placed in an ice bath and cooled for 5 min. The reaction was quenched by slowly adding 0.52 mL of water, followed by 0.52 mL of 15% aq. NaOH, followed by 1.56 mL of water. The flask was stirred for 30 min, and a small amount of $Na_2SO_4$ was added. The mixture was then filtered, the solid washed with THF, before the solvents were removed *in vacuo*. The product was either purified either using flash chromatography (80:12:0 to 80:12:5 $CHCl_3$/MeOH/$NH_4OH$ or 50:5:1 DCM/MeOH/TFA) or HPLC (A gradient of $H_2O$ with 0.1% TFA (A) and acetonitrile with 0.1% TFA (B), 5-30% B over 35 min followed by 30-95% B over 4 min, was used at a flow rate of 25 mL/min). Only the amount required for the continuing steps of the synthesis was purified. **7a**: Pale brown oil, (0.074 g, 16%); IR $\nu_{max}$ 3412, 3134, 2921, 2851 $cm^{-1}$; $^1H$ NMR (600 MHz, methanol-*d4*) $\delta_H$ 7.37 (1H, d, *J* = 8.1 Hz, H-7), 7.25 (1H, s, H-2), 7.20 (1H, d, *J* = 7.4 Hz, H-5), 6.99 (1H, t, *J* = 7.9 Hz, H-6), 3.35 – 3.27 (4H m, H-1' and H-2'); $^{13}C$ NMR (151 MHz, methanol-*d4*) $\delta_C$ 139.82 (C-7a), 134.00 (C-3a) 126.81 (C-2), 126.12 (C-6), 124.40 (C-5), 123.69 (C-4), 114.16 (C-3), 112.25, 111.02 (C-7), 42.66 (C-2'), 25.45 (C-1'); HRESIMS *m/z* 239.0175 $[M + H]^+$ (calcd for $C_{10}H_{12}{}^{79}BrN_2^+$, 239.0178).

**5-bromotryptamine (7b)**[57, 60]. Colorless oil (0.061 g, 23%); IR $\nu_{max}$ 3146, 3021, 2923, 2852 $cm^{-1}$; $^1H$ NMR (600 MHz, methanol-*d4*) $\delta_H$ 7.74 (1H, d, *J* = 1.9 Hz, H-7), 7.30 (1H, d, *J* = 8.6 Hz, H-4), 7.24 – 7.19 (2H, m, H-2 and H-6), 3.21 (2H, t, *J* = 7.5 Hz, H-1'), 3.08 (2H, t, *J* = 7.4 Hz, H-2'); $^{13}C$ NMR (252 MHz, methanol-*d4*) $\delta_C$ 136.93 (C-7a), 130.01 (C-3a), 125.88 (C-2), 125.49 (C-6), 121.54 (C-5), 114.21 (C-4), 113.22 (C-3), 110.17 (C-7), 41.14 (C-2'), 24.27 (C-1'); HRESIMS *m/z* 239.0181 $[M + H]^+$ (calcd for $C_{10}H_{12}{}^{79}BrN_2^+$, 239.0178).

**6-bromotryptamine (7c)**[57, 60]. Yellow oil (0.041 g, 15%); IR $\nu_{max}$ 3411, 3210 $cm^{-1}$; $^1H$ NMR (600 MHz, methanol-*d4*) $\delta_H$ 7.50 (1H, d, *J* = 2.0 Hz, H-7), 7.45 (1H, d, *J* = 8.4 Hz, H-4), 7.11 (1H, dd, *J* = 8.7, 1.8 Hz, H-5), 7.09 (1H, s, H-2), 3.01 – 2.88 (4H, m, H-1' and H-2'); $^{13}C$ NMR (151 MHz, methanol-*d4*) $\delta_C$ 139.05 (C-7a), 127.64 (C-3a), 124.59 (C-2), 122.77 (C-6), 120.69 (C-5), 115.84 (C-4), 115.15 (C-3), 113.36 (C-7), 42.75 (C-2'), 28.56 (C-1'); HRESIMS *m/z* 239,0178 $[M + H]^+$ (calcd for $C_{10}H_{12}{}^{79}BrN_2^+$, 239.0178).

**6-bromotryptamine (7d)**[62, 63]. Pale brown oil (0.052 g, 18); IR $\nu_{max}$ 3343 $cm^{-1}$; $^1H$ NMR (400 MHz, methanol-*d4*) $\delta_H$ 7.55 (1H, d, *J* = 7.9 Hz, H-4), 7.29 (1H, d, *J* = 7.7 Hz, H-6), 7.26 (1H, s, H-2), 6.96 (1H, t, *J* = 7.7 Hz, H-5), 3.22 (2H, t, *J* = 7.4 Hz, H-1'), 3.10 (2H, t, *J* = 7.4 Hz, H-2'); $^{13}C$ NMR (151 MHz, methanol-*d4*) $\delta_C$ 136.70 (C-7a), 129.74 (C-3a), 125.47 (C-2), 125.32 (C-6), 121.29 (C-5), 118.41 (C-4), 111.74 (C-3), 105.78 (C-7), 41.08 (C-2'), 24.50 (C-1'); HRESIMS *m/z* 239.0178 $[M + H]^+$ (calcd for $C_{10}H_{12}{}^{79}BrN_2^+$, 239.0178).

**General procedure for the preparation of compounds (8a-e)**[64].

**4-Bromo-N,N-dimethyltryptamine 8a**[65]. To a stirred solution of **7a** (0.07 g, 0.29 mmol) in MeOH (6 mL) acetic acid (0.067 mL, 1.17 mmol) was added followed by sodium cyanoborohydride (0.037 g, 0.59 mmol) under $N_2$ at 0 °C. A solution of formaldehyde (37%, 0.053 mL, 0.7 mmol) in MeOH (2 mL) was then added dropwise over 20 min, and the resulting solution was stirred at room temperature for 24 h. The reaction was quenched by slowly adding aqueous $Na_2CO_3$ (2 N) to pH 8–9 and the solvents were removed *in vacuo*. The residue was partitioned between $CHCl_3$ (3 × 7 mL) and water (10 mL). The organic layer was washed with water and brine (10 mL), dried over $Na_2SO_4$, and concentrated *in vacuo* to yield **8a** without purification: yellow oil (0.106 g, 84%); IR $\nu_{max}$ 3412, 3134, 2924, 2851$cm^{-1}$; $^1H$ NMR (600 MHz, methanol-*d4*) $\delta_H$ 7.30 (1H, d, *J* = 8.1 Hz, H-7), 7.14 (1H, d, *J* = 7.7 Hz, H-5), 7.11 (1H, s, H-2), 6.91 (1H, t, *J* = 7.8 Hz, H-6), 3.16 – 3.12 (2H, m, H-1'), 2.66 – 2.62 (2H, m, H-2'), 2.31 (6H, s, H-Me); $^{13}C$ NMR (151 MHz, methanol-*d4*) $\delta_C$ 139.50 (C-7a), 126.45 (C-3a), 125.65 (C-2), 124.05 (C-5), 123.08 (C-4), 114.51 (C-6), 114.33 (C-7), 111.89 (C-3), 63.07 (C-Me), 45.42 (C-2'), 24.88 (C-1'); HRESIMS *m/z* 267,0498 $[M + H]^+$ (calcd for $C_{12}H_{16}{}^{79}BrN_2^+$, 267.0491).

**5-Bromo-*N,N*-dimethyltryptamine (8b)**[65]. Yellow oil (0.015 g, 84%); IR $\nu_{max}$ 2940, 2855, 2822, 2776 $cm^{-1}$; $^1H$ NMR (400 MHz, methanol-*d4*) $\delta_H$ 7.66 (1H, d, *J* = 2.1 Hz, H-4), 7.25 (1H, d, *J* = 8.6 Hz, H-7), 7.17 (1H, dd, *J* = 8.6, 1.9 Hz, H-6), 7.09 (1H, s, H-2), 2.92 – 2.86 (2H, m, H-1'), 2.66 – 2.61 (2H, m, H-2'), 2.34 (6H, s, H-Me); $^{13}C$ NMR (151 MHz, methanol-*d4*) $\delta_C$ 136.73 (C-7a), 130.45 (C-3a), 125.01 (C-2), 124.75 (C-5), 121.73 (C-4), 113.91 (C-6), 113.37 (C-7), 112.74 (C-3), 61.22 (C-Me), 45.33 (C-2'), 23.99 (C-1'); HRESIMS *m/z* 267.0495 $[M + H]^+$ (calcd for $C_{12}H_{16}{}^{79}BrN_2^+$, 267.0491).

**6-Bromo-*N,N*-dimethyltryptamine (8c)**. Pale yellow oil (0.043 g, 96%); IR $\nu_{max}$ 2940, 2858, 2818, 2780 $cm^{-1}$; $^1H$ NMR (600 MHz, methanol-*d4*) $\delta_H$ 7.49 (1H, d, *J* = 1.7 Hz, H-4), 7.45 (1H, d, *J* = 8.5 Hz, , H-7), 7.11 (1H, dd, *J* = 8.7, 1.7 Hz, H-5), 7.08 (1H, s, H-2), 2.97 – 2.91 (2H, m, H-1'), 2.73 – 2.69 (2H, m, H-2'), 2.39 (6H, s, H-Me); $^{13}C$ NMR (151 MHz, methanol-*d4*) $\delta_C$ 138.95 (C-7a), 127.59 (C-3a), 124.16 (C-2), 122.72 (C-5), 120.62 (C-4), 115.77 (C-6), 115.12 (C-7), 113.67 (C-3), 61.09 (C-Me), 45.21 (C-2'), 23.87 (C-1'); HRESIMS *m/z* 267.0491 $[M + H]^+$ (calcd for $C_{12}H_{16}{}^{79}BrN_2^+$, 267.0491).

**7-Bromo-*N,N*-dimethyltryptamine (8d)**[66]. Pale yellow oil (0.037 g, 66%); IR $\nu_{max}$ 2941, 2854, 2819, 2778 $cm^{-1}$; $^1H$ NMR (600 MHz, methanol-*d4*) $\delta_H$ 7.53 (1H, d, *J* = 7.9 Hz, H-4), 7.26 (1H, d, *J* = 7.5 Hz, H-6), 7.15 (1H, s, H-2), 6.93 (1H, t, *J* = 7.8 Hz, H-5), 2.97 – 2.91 (2H, m, H-1'), 2.70 – 2.65 (2H, m, H-2'), 2.36 (6H, s, H-Me); $^{13}C$ NMR (151 MHz, methanol-*d4*) $\delta_C$ 136.50 (C-7a), 130.23 (C-3a), 124.89 (C-2), 124.32 (C-5), 120.82 (C-4), 118.67 (C-6), 114.94 (C-7), 105.57 (C-3), 61.21 (C-Me), 45.34 (C-2'), 24.24 (C-1'); HRESIMS *m/z* 267,0497 $[M + H]^+$ (calcd for $C_{12}H_{16}{}^{79}BrN_2^+$, 267,0491).

***N,N*-dimethyltryptamine (8e)**[64]. White oil (0.104 g, 89%); IR $\nu_{max}$ 2939, 2858, 2822, 2776 $cm^{-1}$; $^1H$ NMR (600 MHz, methanol-*d4*) $\delta_H$ 7.52 (1H, d, *J* = 7.9 Hz, H-4), 7.32 (1H, d, *J* = 8.1 Hz,

H-7), 7.10 – 7.06 (1H, m, H-6), 7.02 – 6.98 (2H, m, H-5 and H-2), 2.93 – 2.87 (2H, m, H-1'), 2.63 – 2.58 (2H, m, H-2'), 2.28 (6H, s, H-Me); $^{13}$C NMR (151 MHz, methanol-*d4*) $\delta_C$ 138.09 (C-7a), 128.60 (C-3a), 123.05 (C-2), 122.28 (C-5), 119.53 (C-4), 119.16 (C-6), 113.45 (C-7), 112.24 (C-3), 61.27 (C-Me), 45.26 (C-2'), 24.14 (C-1'); HRESIMS *m/z* 189,1386 $[M + H]^+$ (calcd for $C_{12}H_{17}N_2^+$, 189,1386).

**General procedure for the preparation of compounds** (**9a**-**d**).
**4-bromo-*N,N,N*-trimethyltryptamine (9a)**. **8** was dissolved in $CHCl_3$ and MeI was added at 0 °C. The reaction was stirred at room temperature and the reaction followed by TLC by the loss of starting material. Further equivalents of MeI were added at intervals of at least 1 h if starting material was still observed on TLC, total reaction time 27 h. The solvent was removed and the precipitated product was concentrated *in vacuo*. MeOH was added, and removed *in vacuo*, 3 times to remove excess MeI. No further purification was performed. **9a**: Colorless oil (0.052 g, 98%); IR $\nu_{max}$ 3209 cm$^{-1}$; $^1$H NMR (600 MHz, methanol-*d4*) $\delta_H$ 7.39 – 7.35 (2H, m, H-7 and H-2), 7.22 (1H, d, *J* = 7.3 Hz, H-5), 7.00 (1H, t, *J* = 7.9 Hz, H-6), 3.65 – 3.58 (2H, m, H-1'), 3.53 (2H, m, H-2'), 3.27 (9H, s, H-Me); $^{13}$C NMR (151 MHz, methanol-*d4*) $\delta_C$ 139.57 (C-7a), 127.41 (C-2), 125.93 (C-3a), 124.48 (C-5), 123.71 (C-6), 122.82 (C-4), 120.14 (C-7), 112.37 (C-3), 69.26 (C-1'), 53.89 (C-Me), 21.04 (C-2'); HRESIMS *m/z* 281.0657 $[M + H]^+$ (calcd for $C_{13}H_{18}{}^{79}BrN_2^+$, 281.0648).

**5-bromo-*N,N,N*-trimethyltryptamine (9b)**. Colorless oil (0.015 g, 88%); IR $\nu_{max}$ 3230, 3001, 2922 cm$^{-1}$; $^1$H NMR (400 MHz, methanol-*d4*) $\delta_H$ 7.80 (1H, d, *J* = 1.8 Hz, H-7), 7.30 (2H, d, *J* = 8.6 Hz, H-4 and H-2), 7.22 (1H, dd, *J* = 8.6, 1.8 Hz, H-6), 3.67 – 3.60 (4H, m, H-1' and H-2'), 3.26 (s, 9H); $^{13}$C NMR (151 MHz, methanol-*d4*) $\delta_C$ 136.72 (C-7a), 129.86 (C-3a), 125.97 (C-2), 125.60 (C-6), 121.59 (C-7), 114.21 (C-4), 113.35 (C-5), 109.22 (C-3), 67.64 (C2'), 53.71 (C-Me), 20.10 (C-1'); HRESIMS *m/z* 281.0652 $[M + H]^+$ (calcd for $C_{13}H_{18}{}^{79}BrN_2^+$, 281.0648).

**6-bromo-*N,N,N*-trimethyltryptamine (9c)**. Pale yellow oil (0.013 g, 95%); IR $\nu_{max}$ 3234, 3009 cm$^{-1}$; $^1$H NMR (400 MHz, acetone-*d6*) $\delta_H$ 7.69 – 7.63 (2H, m, H-4 and H-7), 7.45 (1H, s, H-2), 7.16 (1H, dd, *J* = 8.3, 1.7 Hz, H-5), 3.98 – 3.91 (2H, m, H-1'), 3.54 (9H, s, H-Me), 3.45 (2H, m, H-2'); $^{13}$C NMR (151 MHz, methanol-*d4*) $\delta_C$ 138.91 (C-7a), 127.03 (C-3a), 125.31 (C-2), 123.28 (C-5), 120.57 (C-4), 116.23 (C-6), 115.38 (C-7), 67.65 (C-3), 53.72 (C-2'), 29.54 (C-Me), 20.16 (C-1'); HRESIMS *m/z* 281.0650 $[M + H]^+$ (calcd for $C_{13}H_{18}{}^{79}BrN_2^+$, 281.0648).

**7-bromo-*N,N,N*-trimethyltryptamine (9d)**. Colorless oil (0.022 g, 95%); IR $\nu_{max}$ 3213, 3012, 2957 cm$^{-1}$; $^1$H NMR (600 MHz, methanol-*d4*) $\delta_H$ 7.63 (1H, d, *J* = 7.6 Hz, H-4), 7.32 (2H, m, H-6 and H-2), 7.00 (1H, t, *J* = 7.8 Hz, H5), 3.67 – 3.63 (2H, m, H-1' and H-2' overlapping), 3.26 (9H, s, H-Me); $^{13}$C NMR (151 MHz, methanol-*d4*); $\delta_C$ 136.55 (C-7a), 129.59 (C-3a), 125.50 (C-2), 125.46 (C-6), 121.43 (C-5), 118.50 (C-4), 110.80 (C-3), 105.81 (C-7), 67.63 (C-2'), 53.61 (C-Me), 20.35 (C-1'); HRESIMS *m/z* 281.0652 $[M + H]^+$ (calcd for $C_{13}H_{18}{}^{79}BrN_2^+$, 281.0648).

**Procedure for the preparation of *N,N,N*-trimethyltryptamine (9e)**[67].
MeI (3.42 g, 24 mmol, 1.5 mL) was added to a solution of tryptamine (0.7 g, 4.4 mmol) dissolved in isopropanol (30 mL), and stirred for 2 h at room temperature. The resulting solid product was filtered and washed with isopropanol (4 × 30 mL) before drying. **9e** was obtained as a white powder (0.303 g, 34%) without further purification. IR $\nu_{max}$ 3401, 3010 cm$^{-1}$; $^1$H NMR (600 MHz, methanol-*d4*) $\delta_H$ 7.61 (1H, d, *J* = 7.5 Hz, H-4), 7.37 (1H, d, *J* = 7.9 Hz, H-7), 7.21 (1H, s, H-2), 7.14 (1H, t, *J* = 7.5 Hz, H-6), 7.07 (1H, t, *J* = 7.2 Hz, H-5), 3.66 – 3.62 (3H, m, H-1' and H-2' overlapping), 3.25 (9H, s, H-Me); $^{13}$C NMR (151 MHz, methanol-*d4*) $\delta_C$

141.05 (C-7a), 130.88 (C-3a), 124.22, (C-2), 122.85, (C-6), 120.16, (C-5), 118.85, (C-4), 112.56, (C-7) 67.90 (C-2'), 53.58 (C-Me), 20.36 (C-1'); HRESIMS *m/z* 203.1537 $[M + H]^+$ (calcd for $C_{13}H_{19}N_2^+$, 203.1543). MP 183-184°C

**Acetylcholinesterase Inhibition Assay**

AChE activity was measured by Ellman's method adapted for microtiter plates as described in Defant et al. [68, 69] Stock solutions (2 mg/mL) were prepared in methanol and progressively diluted in 100 mM potassium phosphate buffer (pH 7.4). Then, acetylthiocholine chloride and 5,5′ dithiobis-2-nitrobenzoic acid at final concentrations of 1 mM and 0.5 mM, respectively, dissolved in the same buffer, were added to the wells of the microtiter plate to the final volume of 150 µL. Electric eel AChE as a source of enzyme (50 µL; final concentration in the test 0.0075 U/mL), dissolved in the same buffer, was added to start the reaction, which was followed spectrophotometrically at 405 nm and 25°C for 5 min using a VIS microplate reader (Anthos, UK). All readings were corrected for their appropriate blanks and a run with only acetylthiocholine chloride served as assay positive control. Every measurement was repeated at least three times. For the determination of the inhibitory constants, the kinetics was monitored using three different substrate concentrations (0.125, 0.25, and 0.5 mM, respectively).

ASSOCIATED CONTENT

**Supporting Information**. 1D and 2D NMR and HR-MS data of natural products **4** and $^1H$ and $^{13}C$ NMR for synthetic compounds not previously reported in the literature.

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**Author Contributions**

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

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**Table of content graphic**

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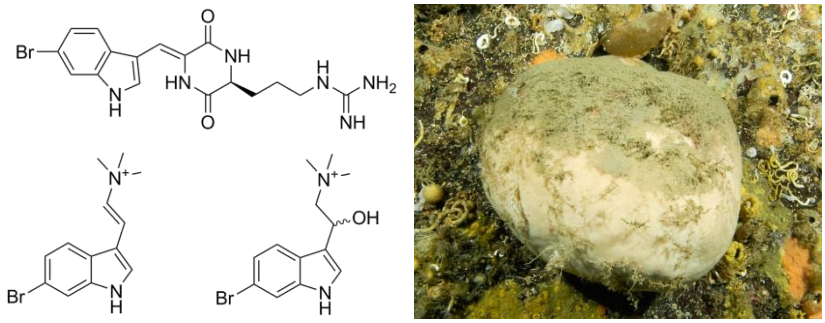

**Supporting Information, Paper II**

# Marine AChE inhibitors isolated from *Geodia baretti*: Natural compounds and their synthetic analogs

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**Contents**

**Figure S16.** $^{1}$H-NMR spectrum of **6d** in methanol-*d4*.

**Figure S17.** $^{13}$C-NMR spectrum of **6d** in methanol-*d4*.

**Figure S18.** $^{1}$H-NMR spectrum of **8c** in methanol-*d4*.

**Figure S19.** $^{13}$C-NMR spectrum of **8c** in methanol-*d4*.

**Figure S20.** $^{1}$H-NMR spectrum of **9a** in methanol-*d4*.

**Figure S21.** $^{13}$C-NMR spectrum of **9a** in methanol-*d4*.

**Figure S22.** $^{1}$H-NMR spectrum of **9b** in methanol-*d4*.

**Figure S23.** $^{13}$C-NMR spectrum of **9b** in methanol-*d4*.

**Figure S24.** $^{1}$H-NMR spectrum of **9c** in acetone-*d6*.

**Figure S25.** $^{13}$C-NMR spectrum of **9c** in methanol-*d4*.

**Figure S26.** $^{1}$H-NMR spectrum of **9d** in methanol-*d4*.

**Figure S27.** $^{13}$C-NMR spectrum of **9d** in methanol-*d4*.

**Figure S1.** High-resolution MS spectrum of **1**

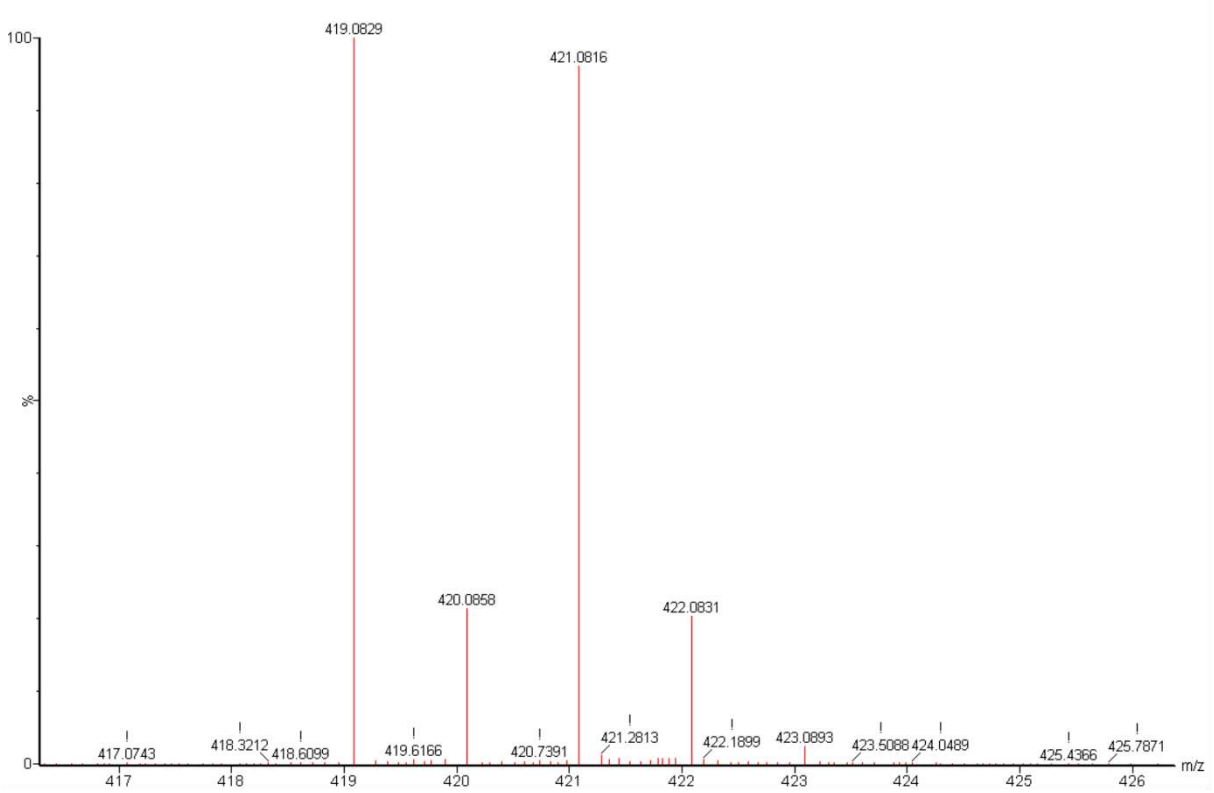


**Figure S2.** High-resolution MS spectrum of **2**.

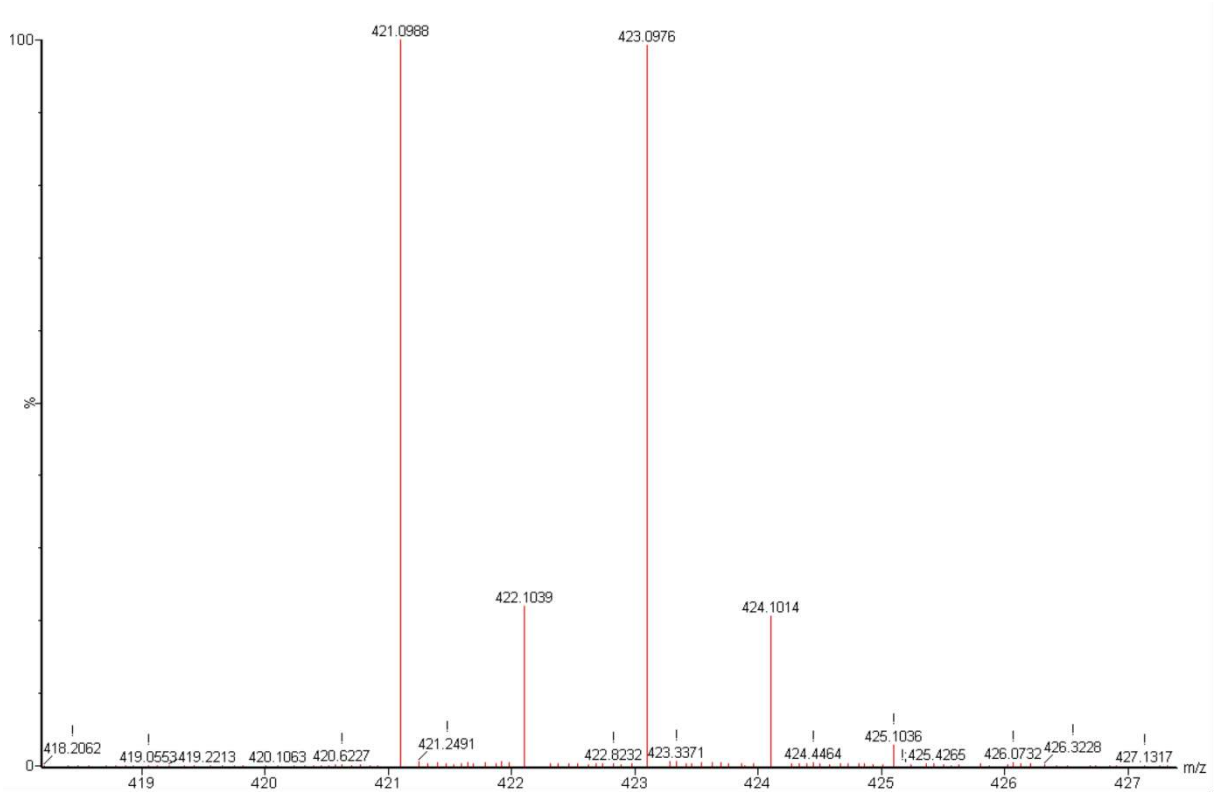


**Figure S3.** High-resolution MS spectrum of **3**.

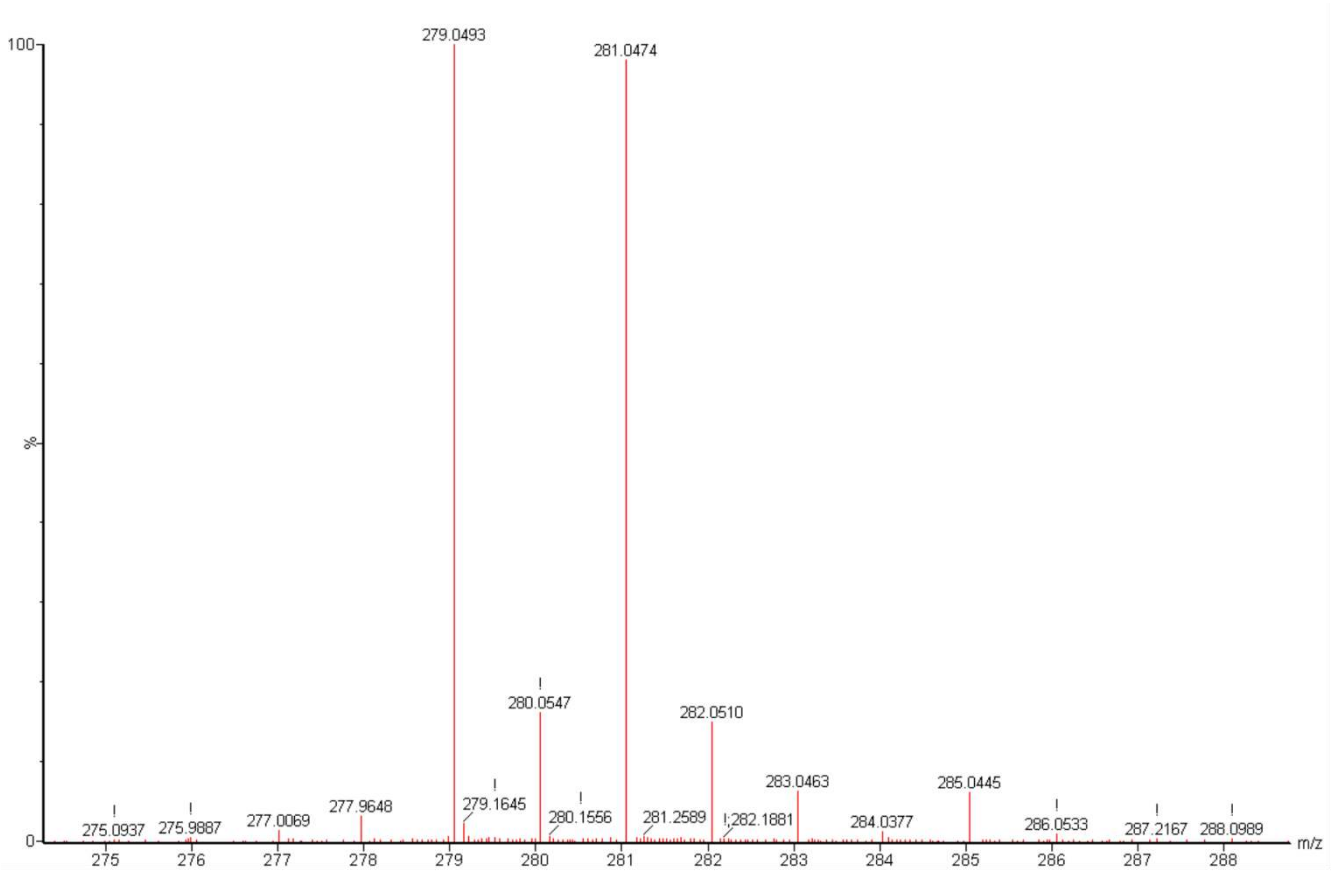


**Figure S4.** High-resolution MS spectrum of **4**.

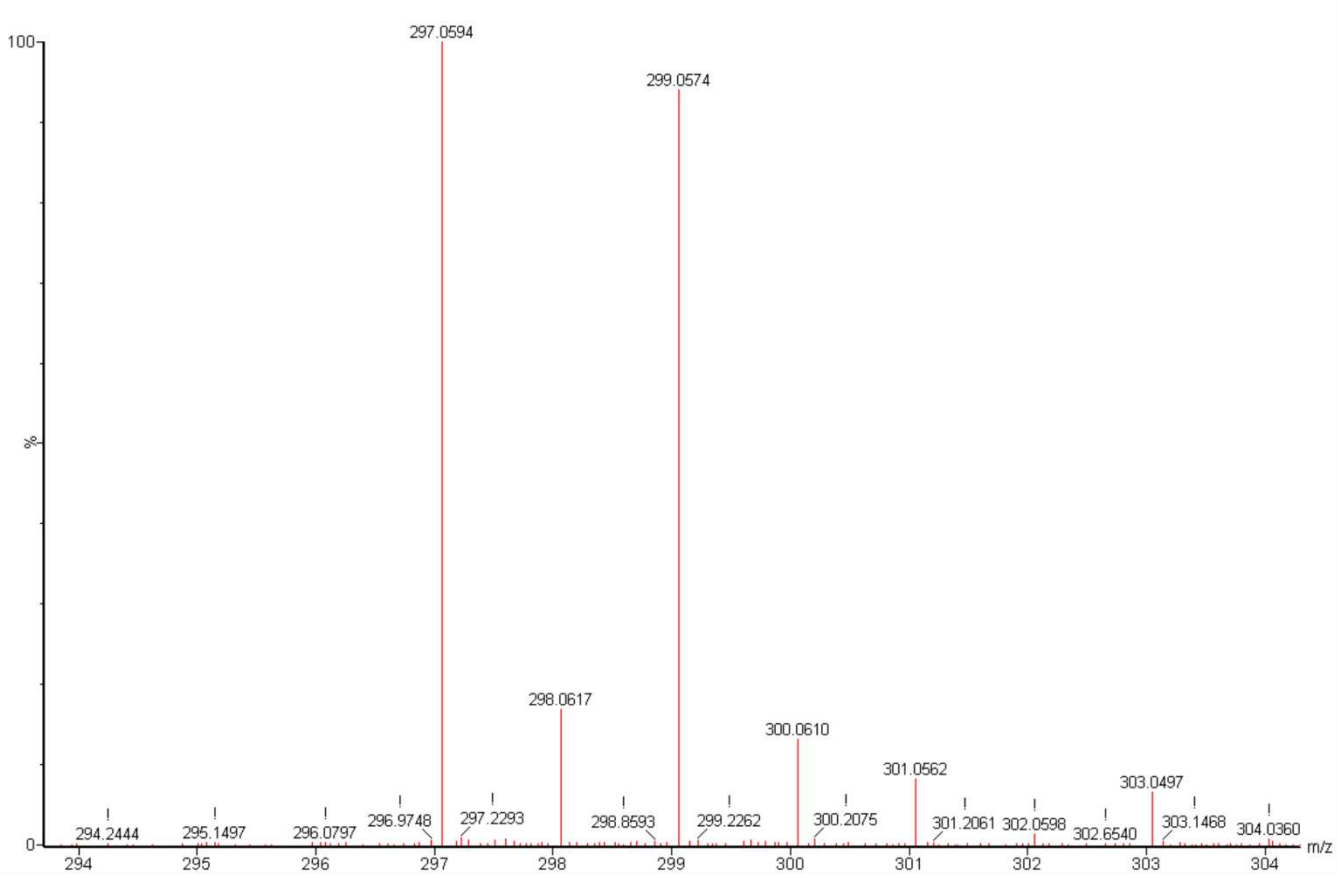
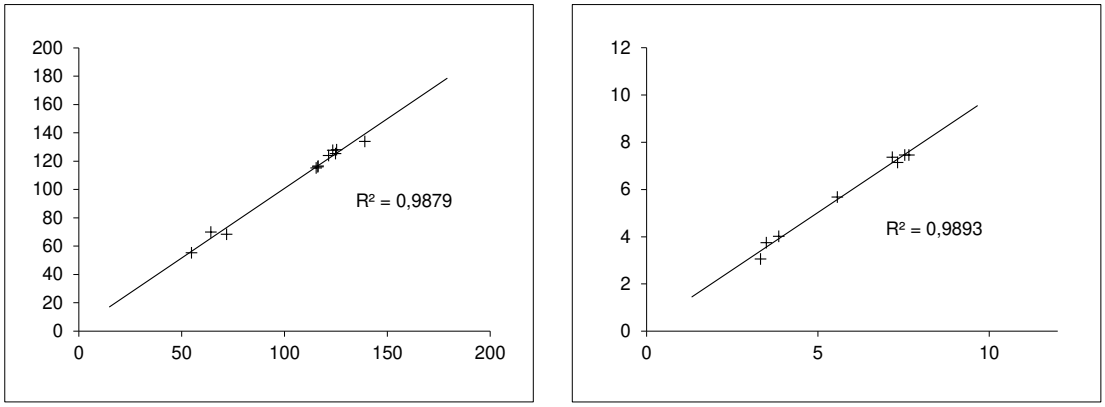


**Table S1** Experimental parameters for acquired NMR spectra in methanol-$d_4$ and methanol-$d_3$ at 25 ºC

| Experiment | Pulse sequence | Parameters |
|---|---|---|
| 1D $^1$H | Proton | sw: 16 ppm, complex points: 24k, nt: 64, d1: 10s |
| | wet1D | sw: 16 ppm, complex points: 24k, nt: 64, d1: 1.5s, wet suppression @ 22ms pulse width (wet) |
| | dpfgsewater | sw: 20 ppm, complex points: 36k, nt: 64, d1: 1s, water flipback |
| $^1$H,$^1$H-DQFCOSY | gDQCOSY | sw: 16 ppm, complex points: 4000x200, nt: 8, d1: 1s, wet, homospoils, gradient selected |
| $^1$H,$^1$H-ROESY | ROESYAD | sw: 14 ppm, complex points: 2000x128, nt: 8, d1: 1s, mix: 300ms @ 8188 Hz, wet, homospoils, adiabatic |
| $^1$H,$^{13}$C-HSQC | gc2hsqcse | sw: 16x220 ppm, complex points: 2000x200, nt: 32, $^1J_{CH}$: 146 Hz, ME, BIP, wet, homospoils, gradient selected |
| $^1$H,$^{13}$C-HMBC | gc2hmbc | sw: 16x240 ppm, complex points: 1440x256, nt: 32, $^nJ_{CH}$: 8 and 3 Hz, dual $^1J_{CH}$ suppression: 165 and 130 Hz, BIP, wet, homospoils, gradient selected |

**Figure S5.** Correlation plots between neural network based predicted chemical shifts and experimental chemical shifts for $^{13}$C, mean error: 2.3 ppm, and $^1$H, mean error: 0.18 ppm.




$^{13}$C $^1$H

**Figure S6.** [1]H-NMR spectrum of **4** in methanol-*d4*.

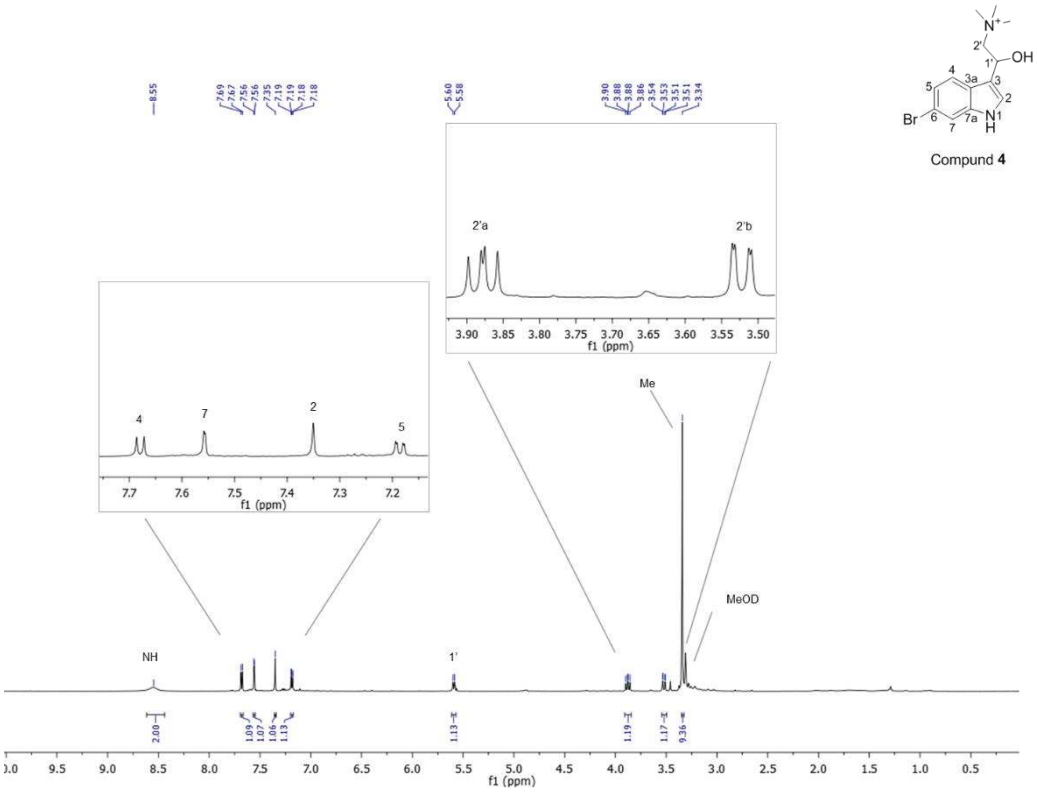



**Figure S7.** [13]C-NMR spectrum of **4** in methanol-*d4*.

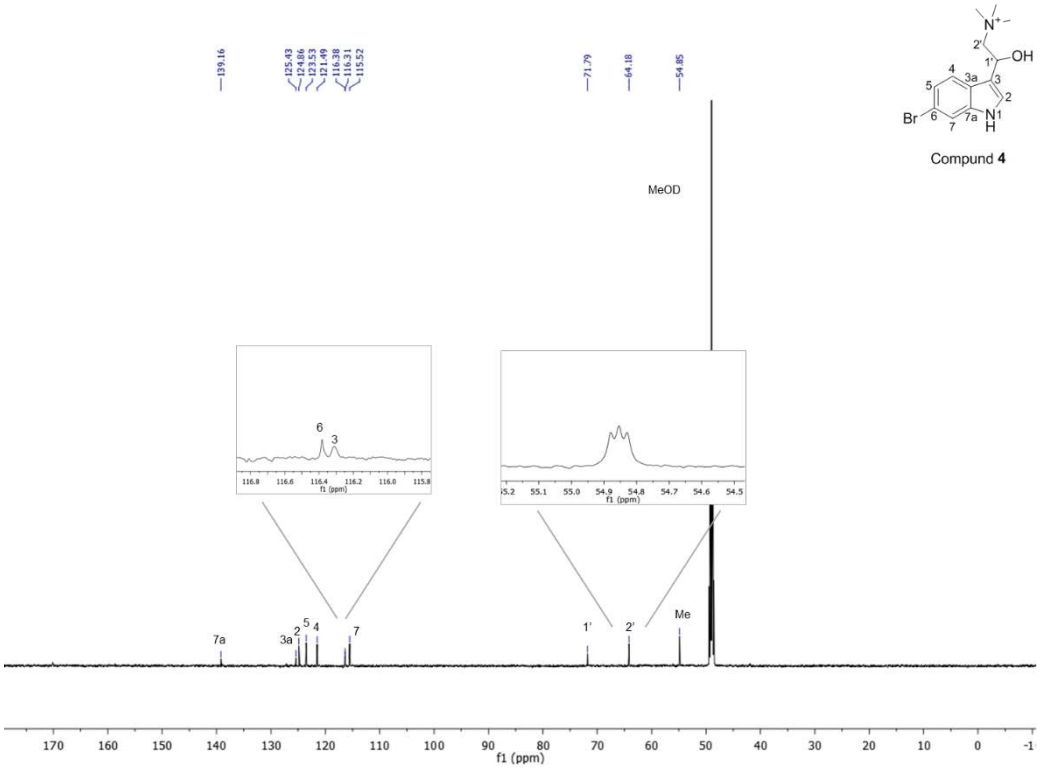


**Figure S8.** $^{1}$H-$^{1}$H COSY spectrum of **4** in methanol-*d4*.

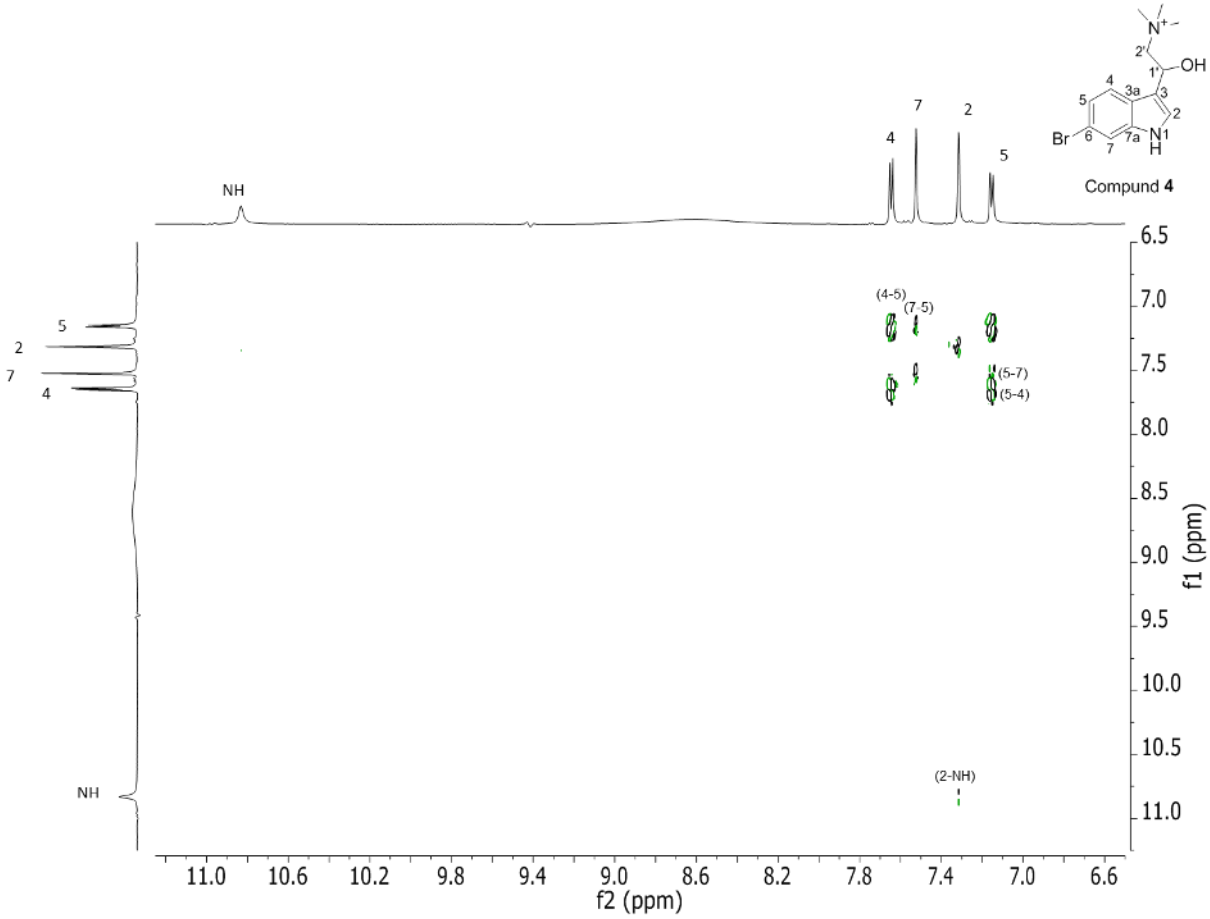


**Figure S9.** $^{1}$H-$^{1}$H COSY spectrum of **4** in methanol-*d4*.

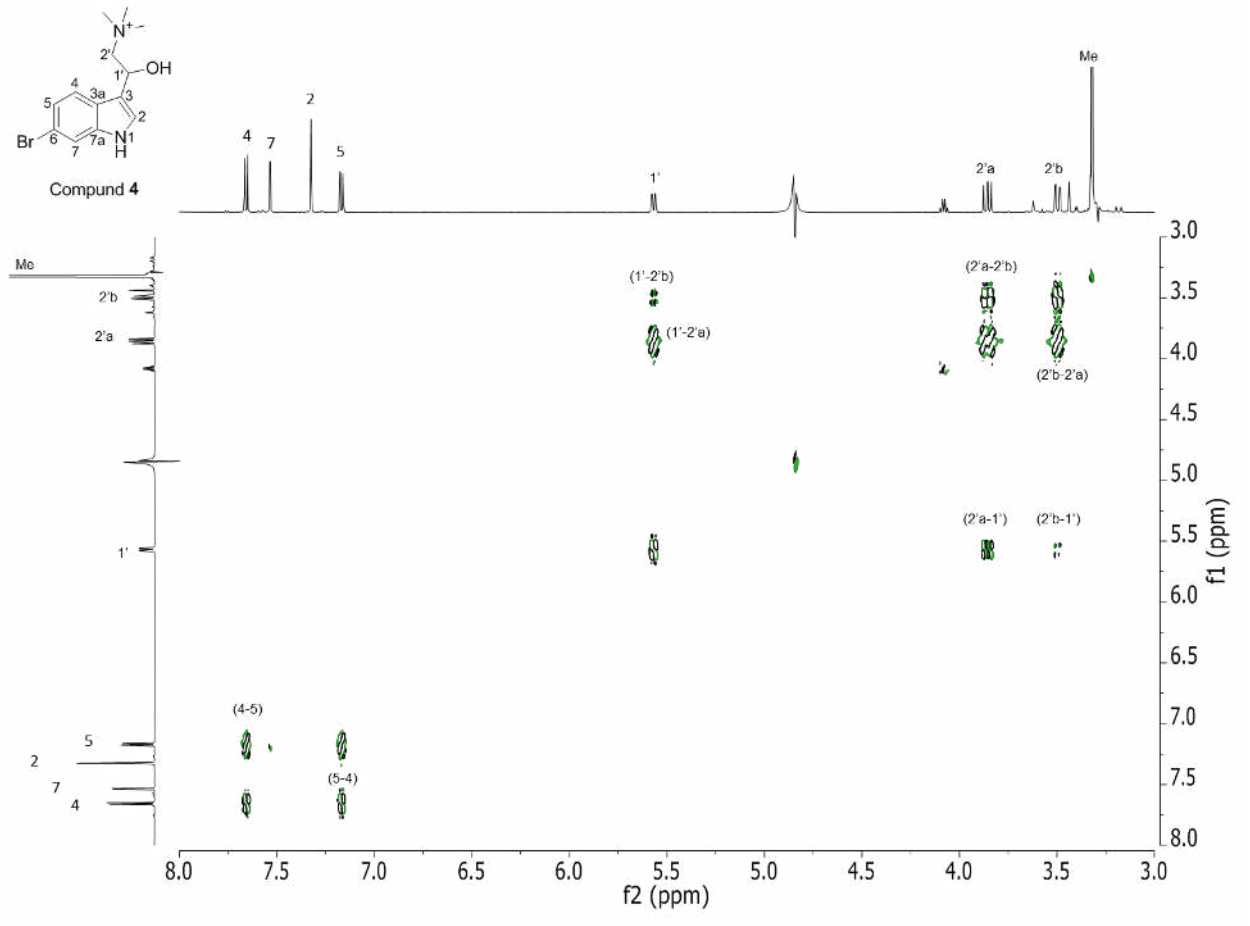


**Figure S10.** ROSEY spectrum of **4** in methanol-*d4*.

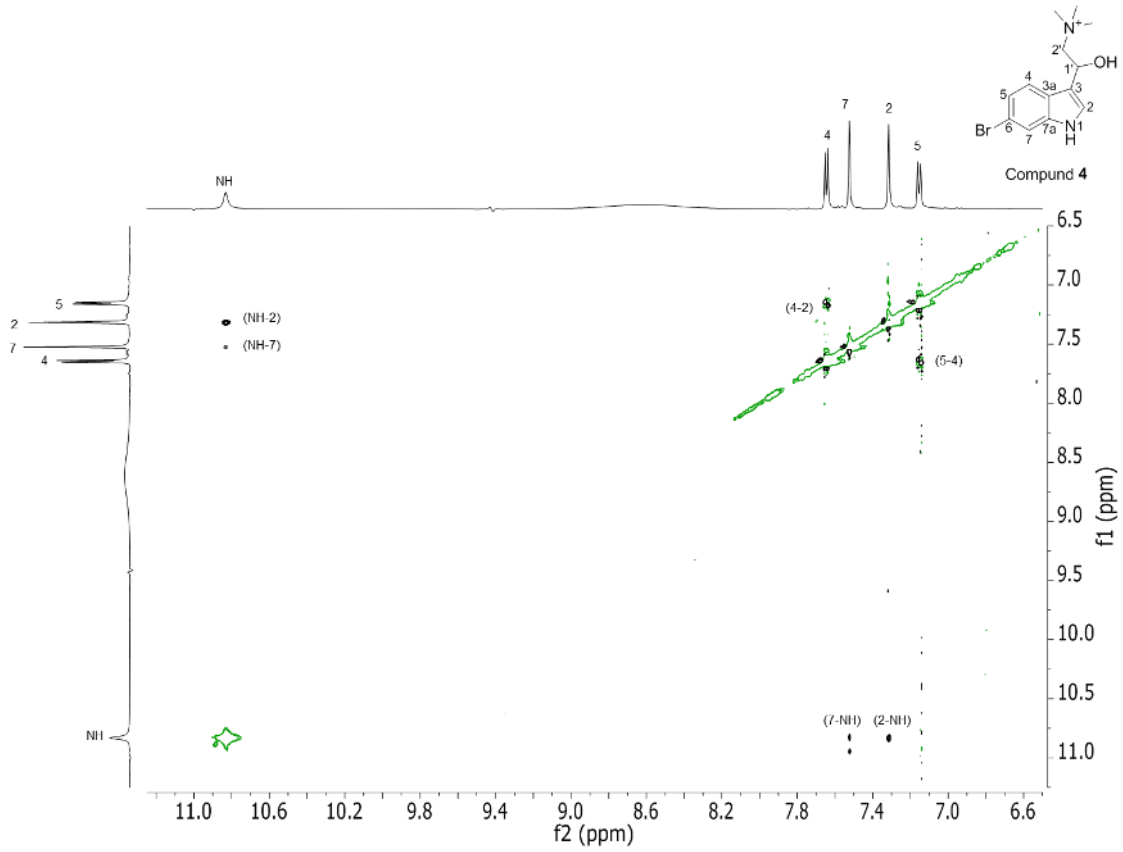


**Figure S11.** ROSEY spectrum of **4** in methanol-*d4*.

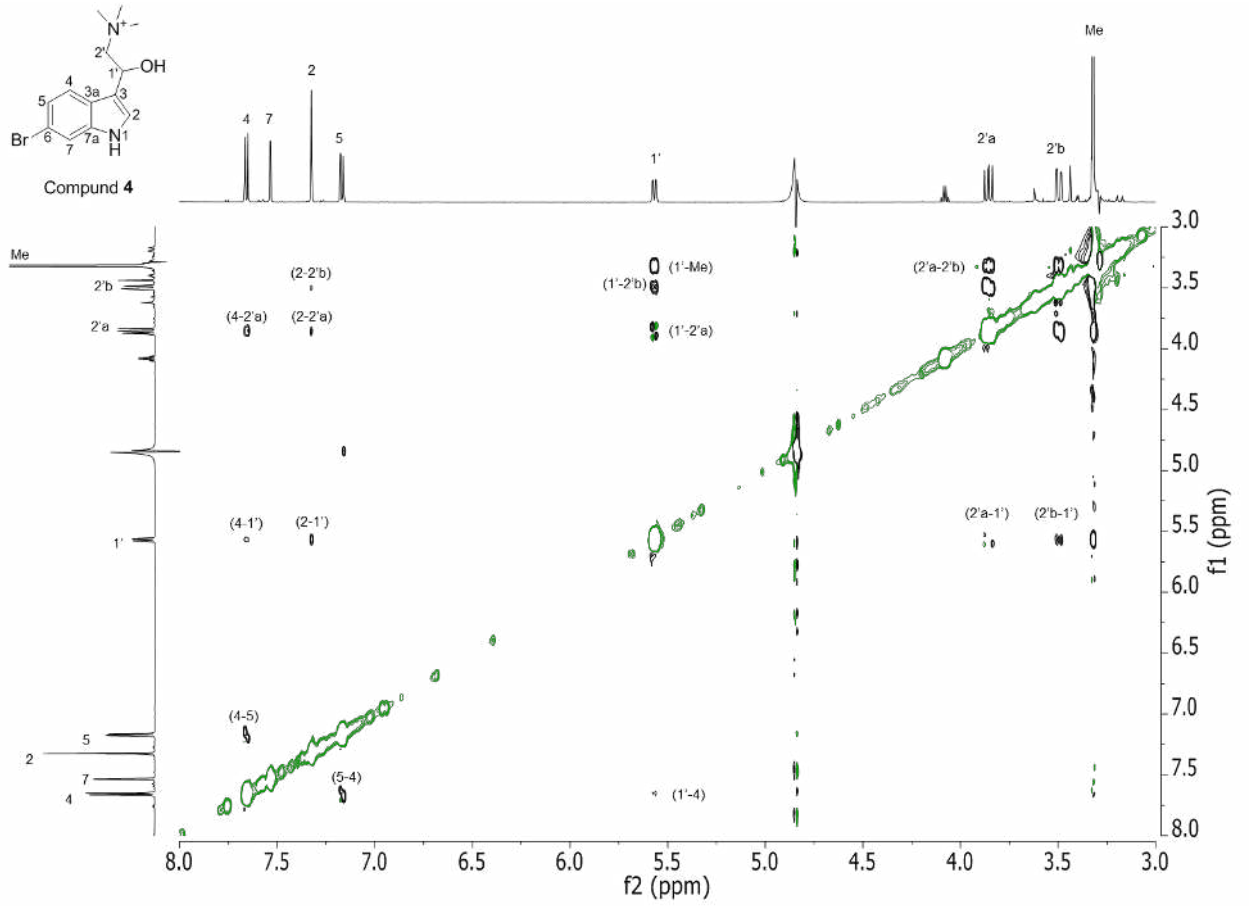


**Figure S12.** gHSQC spectrum of **4** in methanol-*d4*.

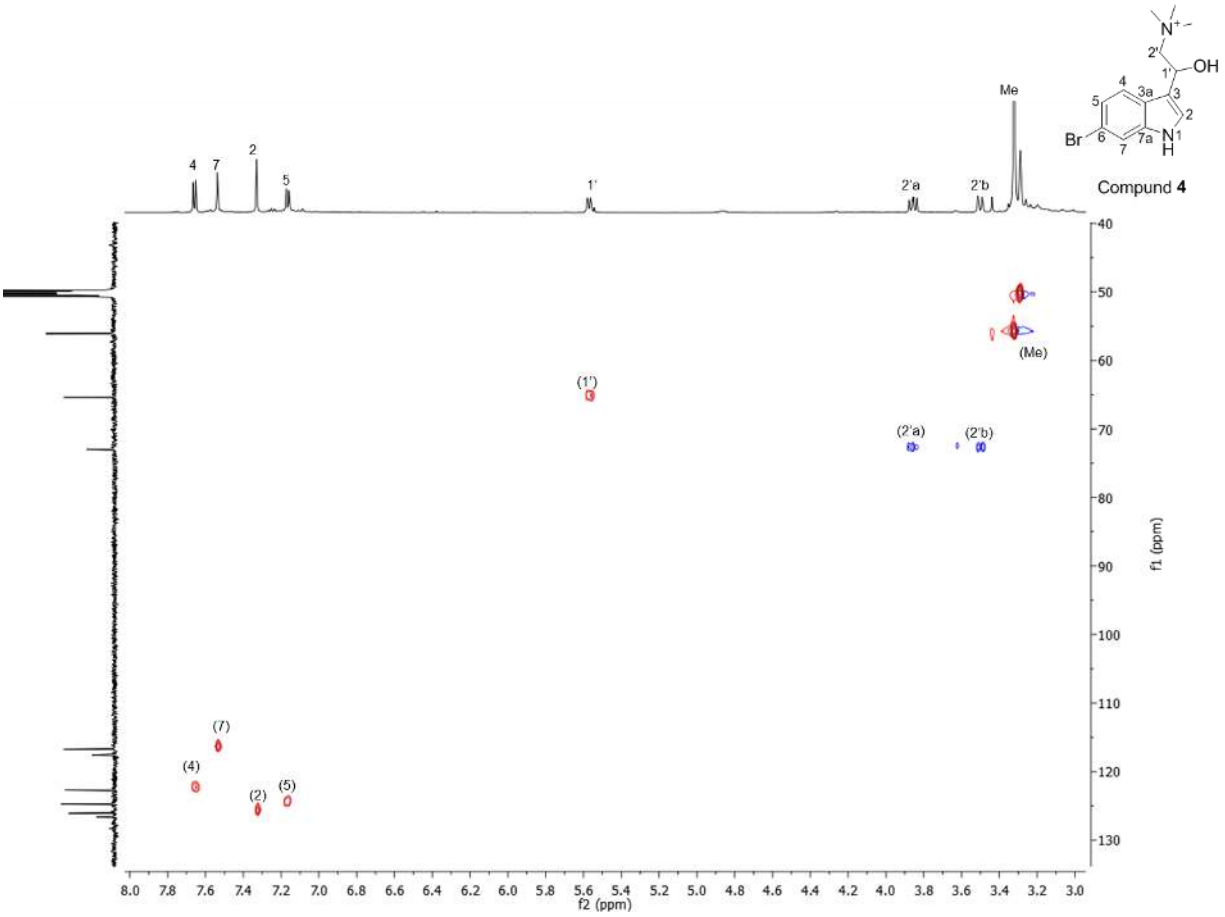


**Figure S13.** gHMBC spectrum of **4** in methanol-*d4*.

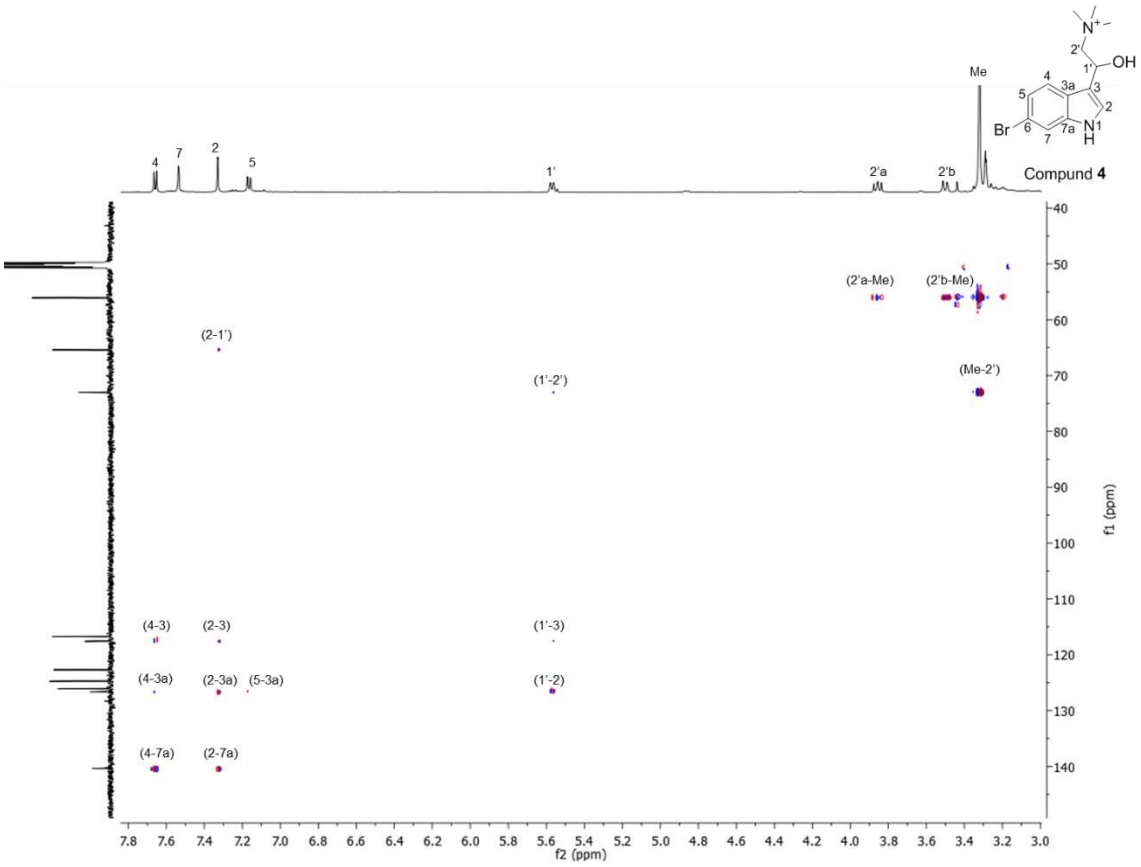


**Figure S14.** [1]H-NMR spectrum of **6a** in methanol-*d4*.

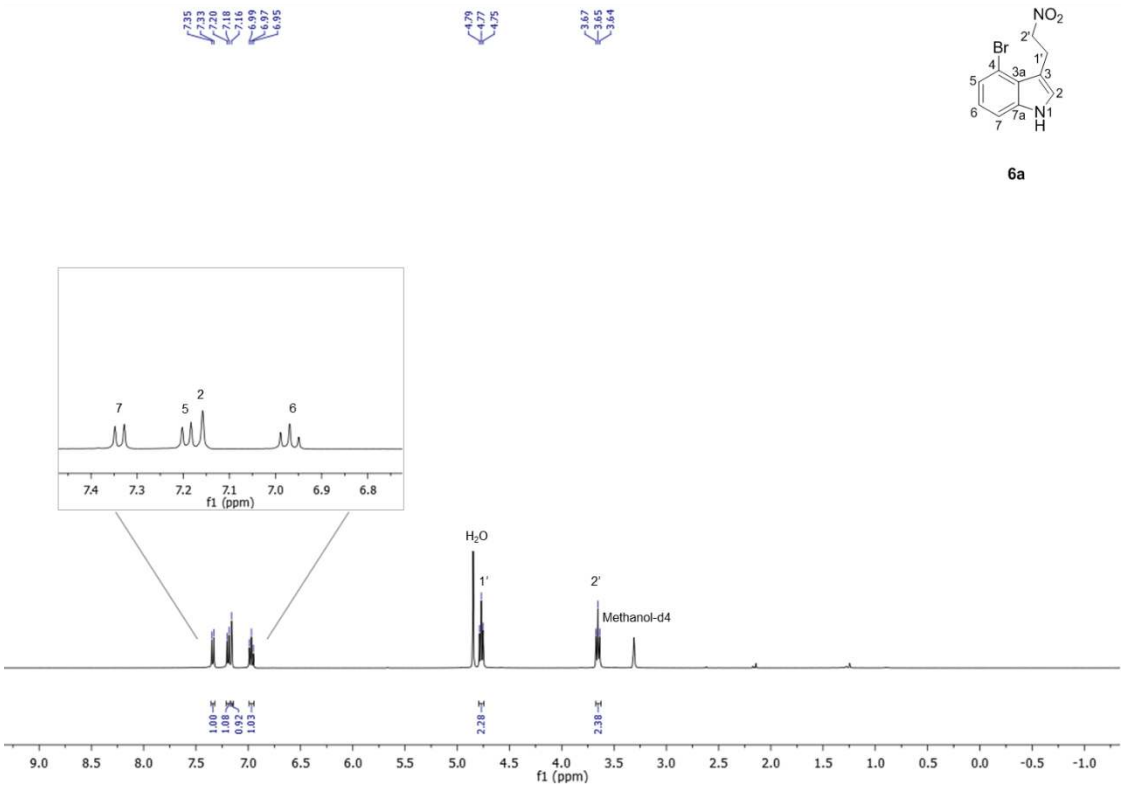



**Figure S15.** [13]C-NMR spectrum of **6a** in acetone-*d6*.

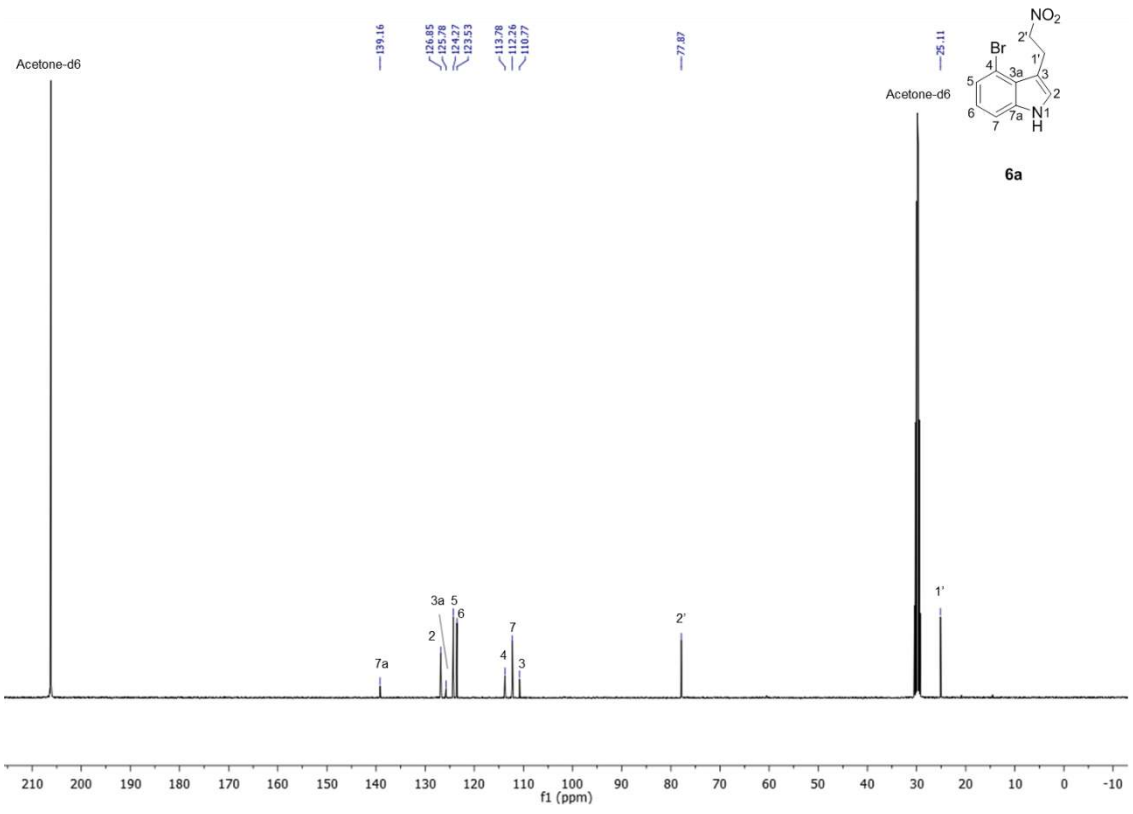


**Figure S16.** [1]H-NMR spectrum of **6d** in methanol-*d4*.

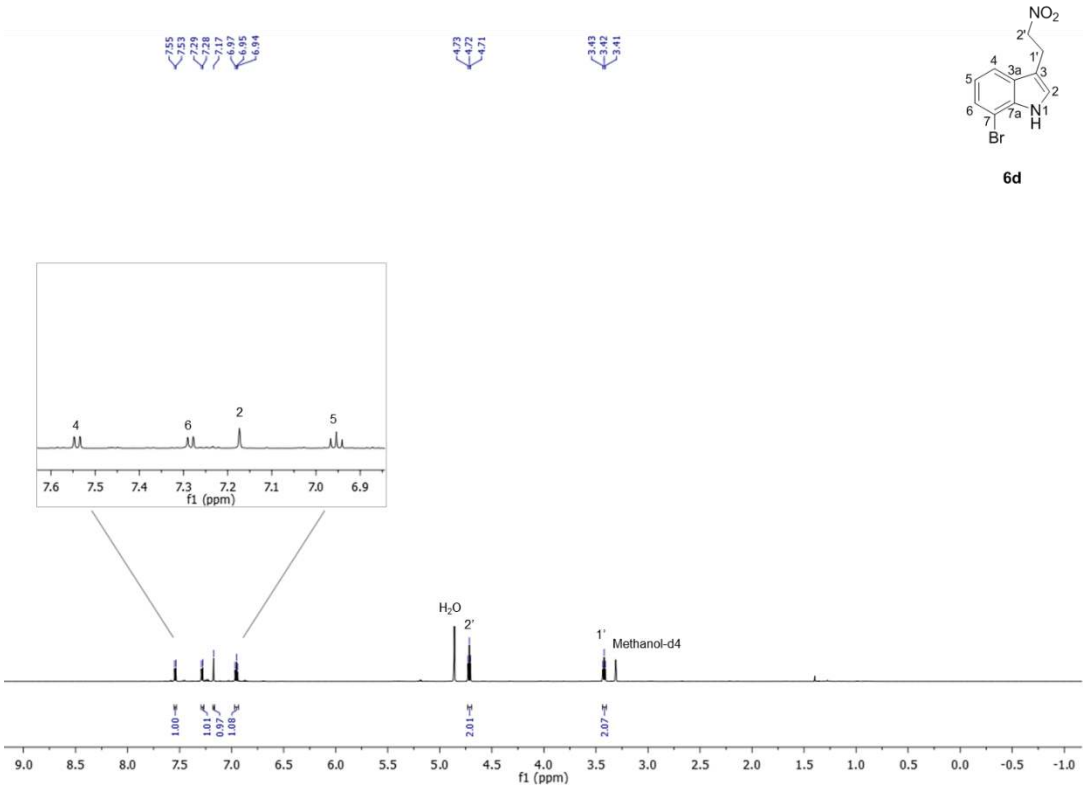



**Figure S17.** [13]C-NMR spectrum of **6d** in methanol-*d4*.

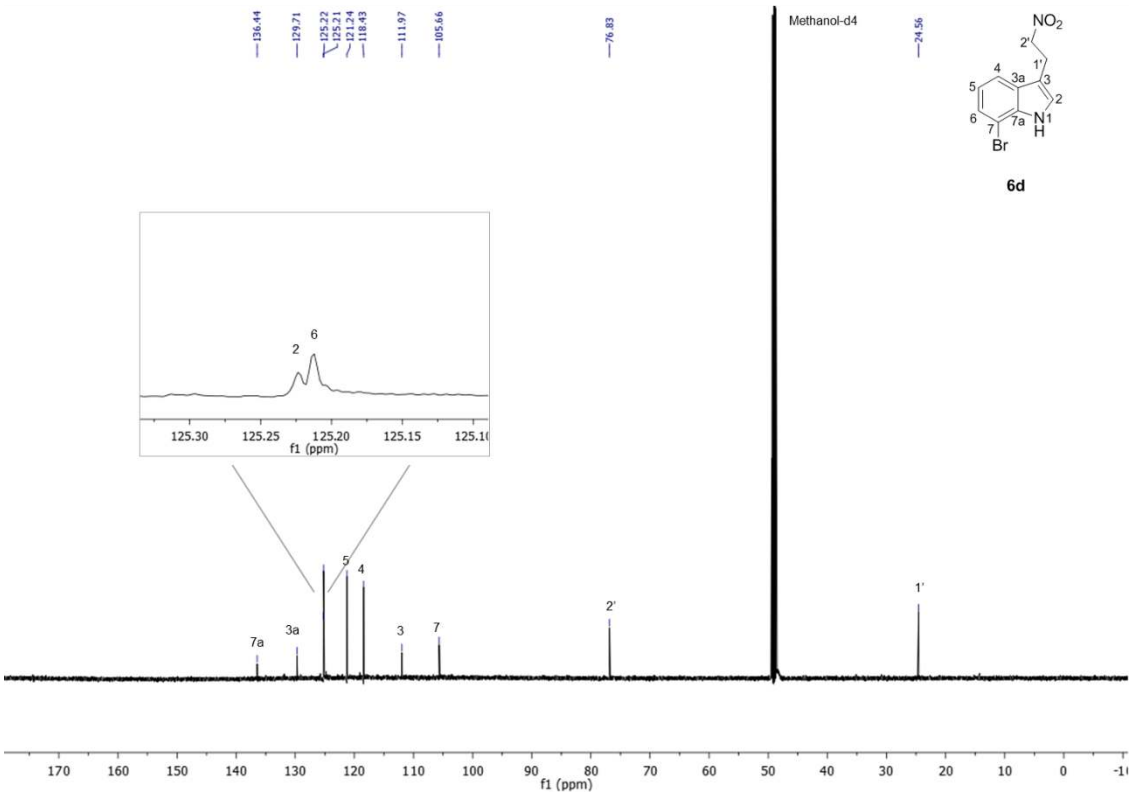


**Figure S18.** [1]H-NMR spectrum of **8c** in methanol-*d4*.

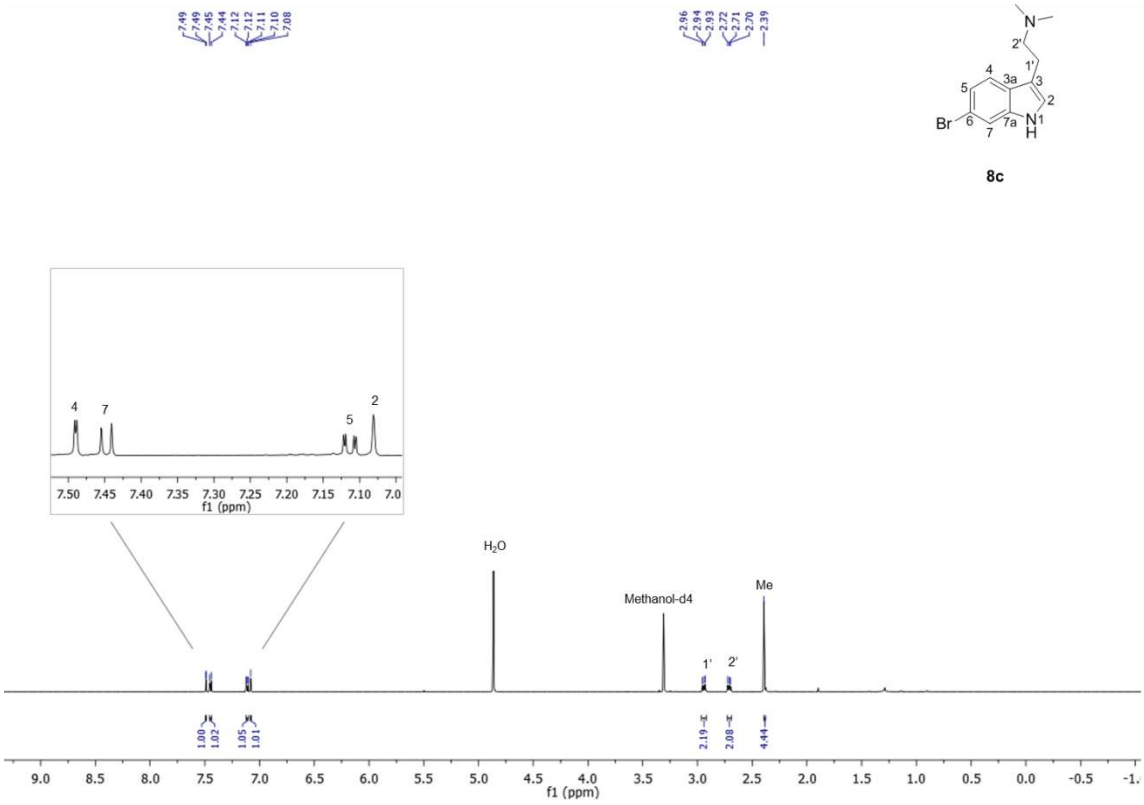



**Figure S19.** [13]C-NMR spectrum of **8c** in methanol-*d4*.

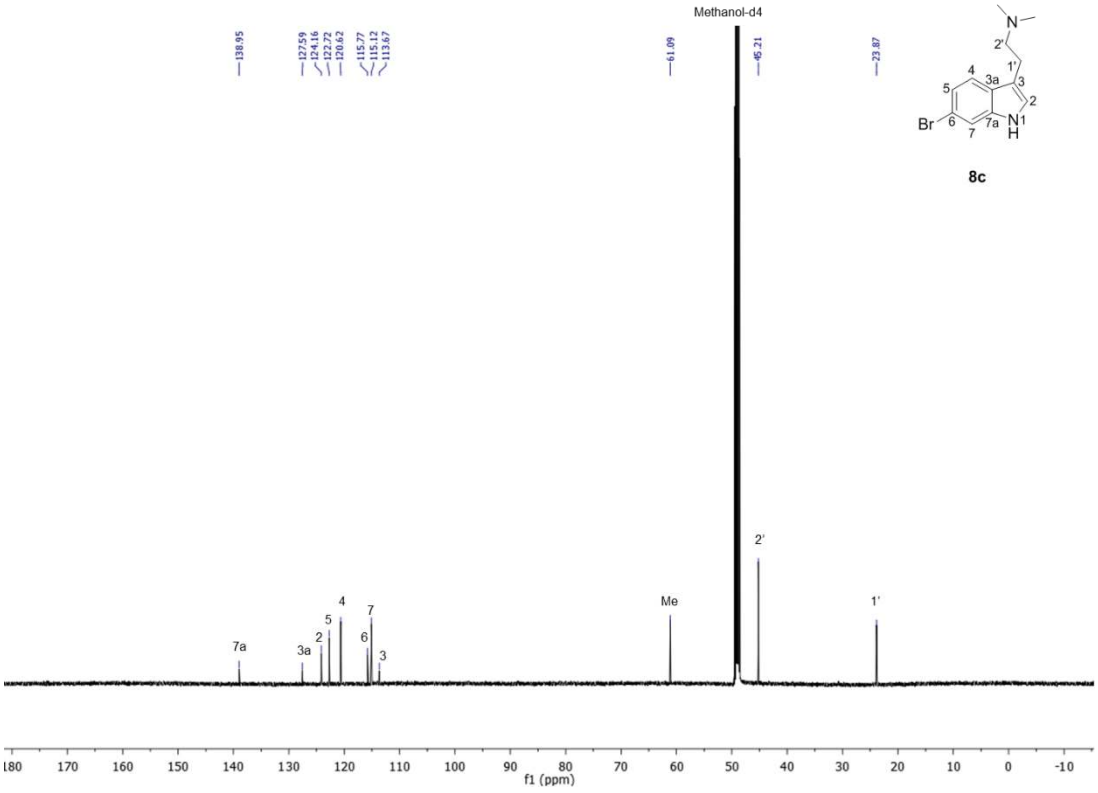


**Figure S20.** [1]H-NMR spectrum of **9a** in methanol-*d4*.

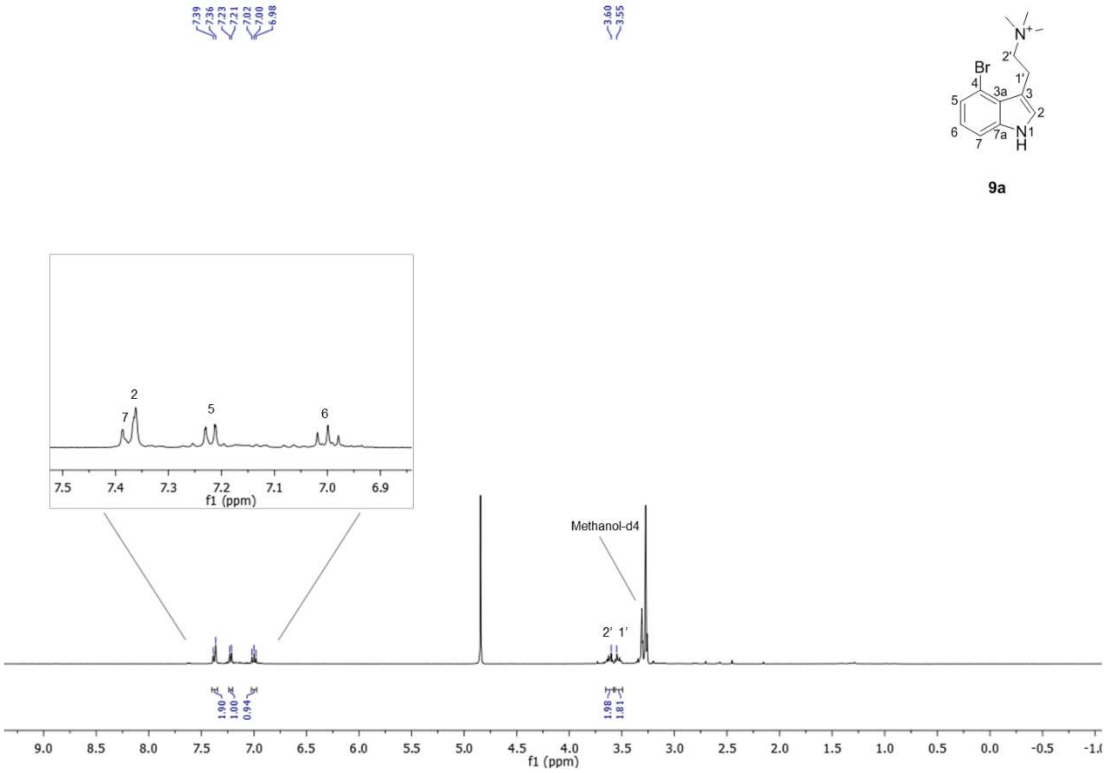



**Figure S21.** [13]C-NMR spectrum of **9a** in methanol-*d4*.

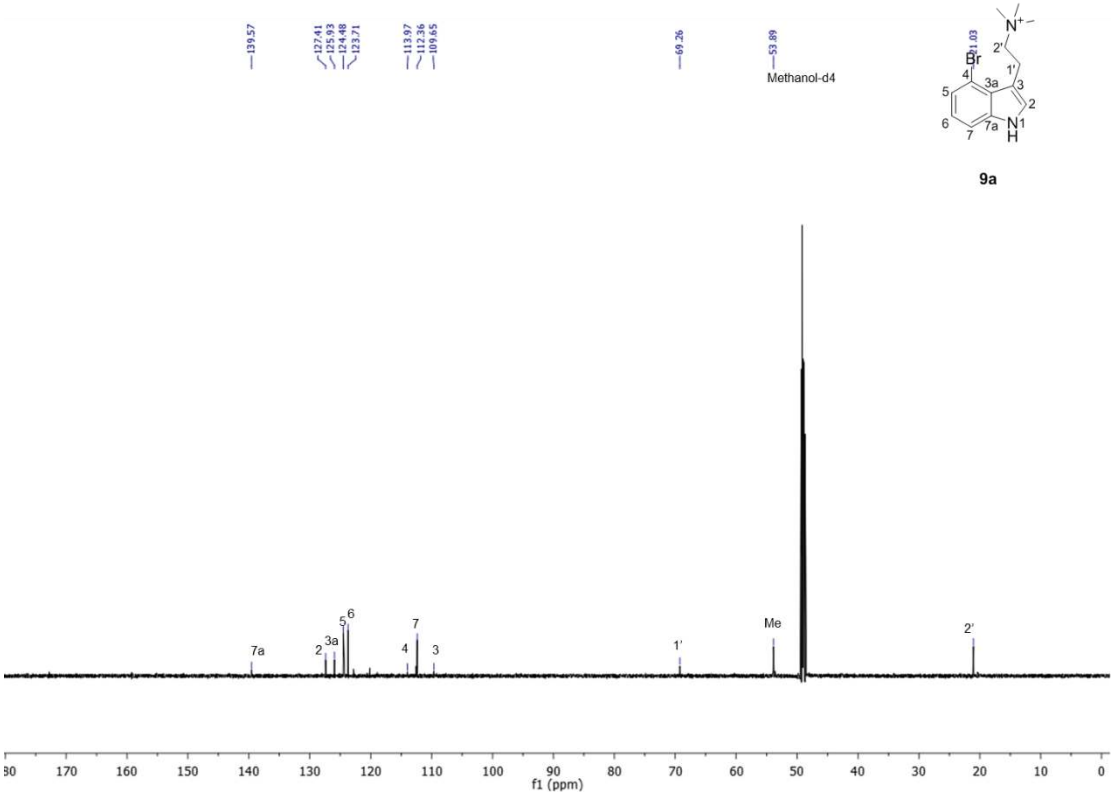


**Figure S22.** [1]H-NMR spectrum of **9b** in methanol-*d4*. The 1’ and Me-peak overlaps.

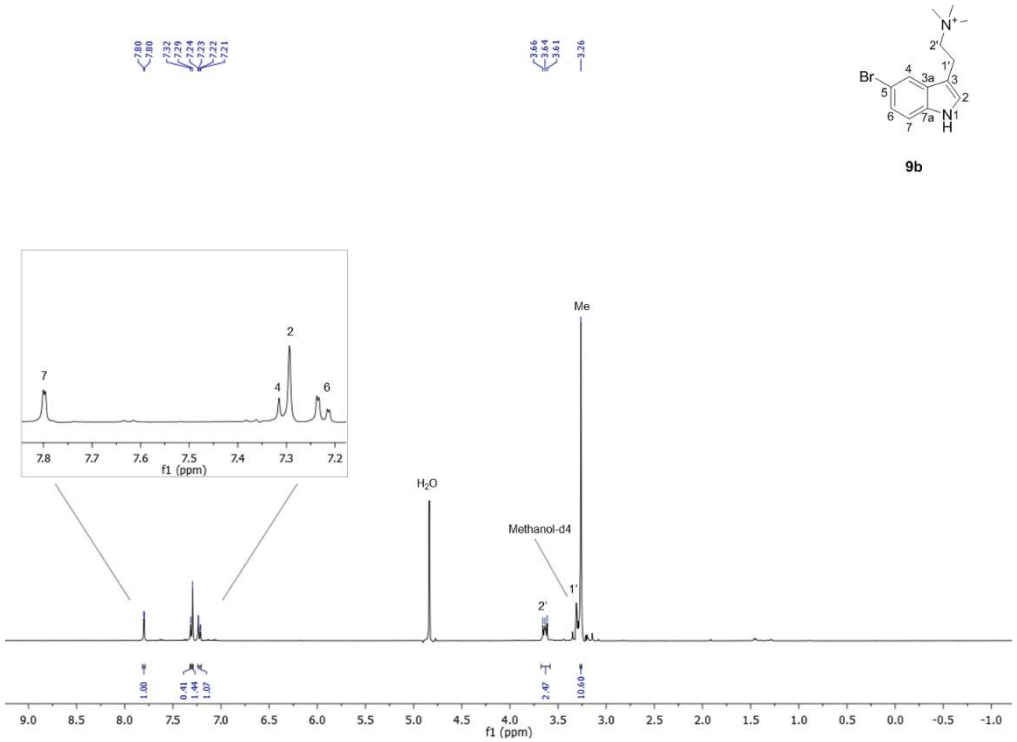



**Figure S23.** [13]C-NMR spectrum of **9b** in methanol-*d4*.

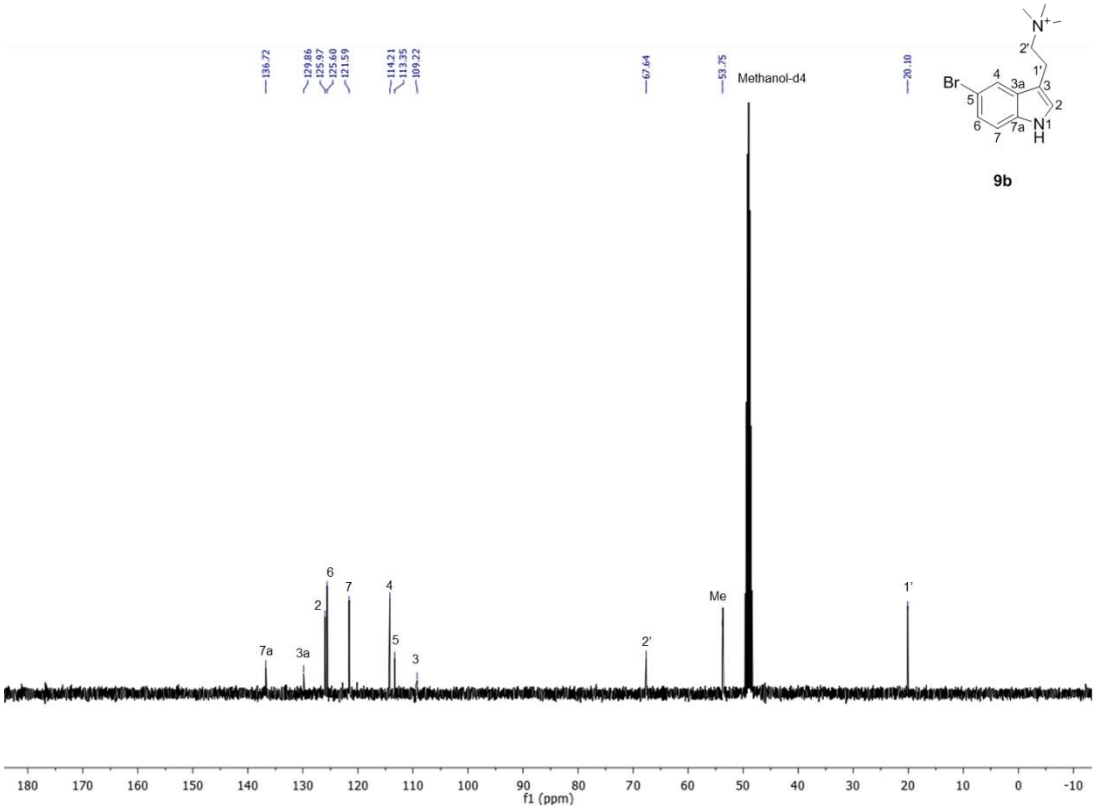


**Figure S24.** [1]H-NMR spectrum of **9c** in acetone-*d6*.

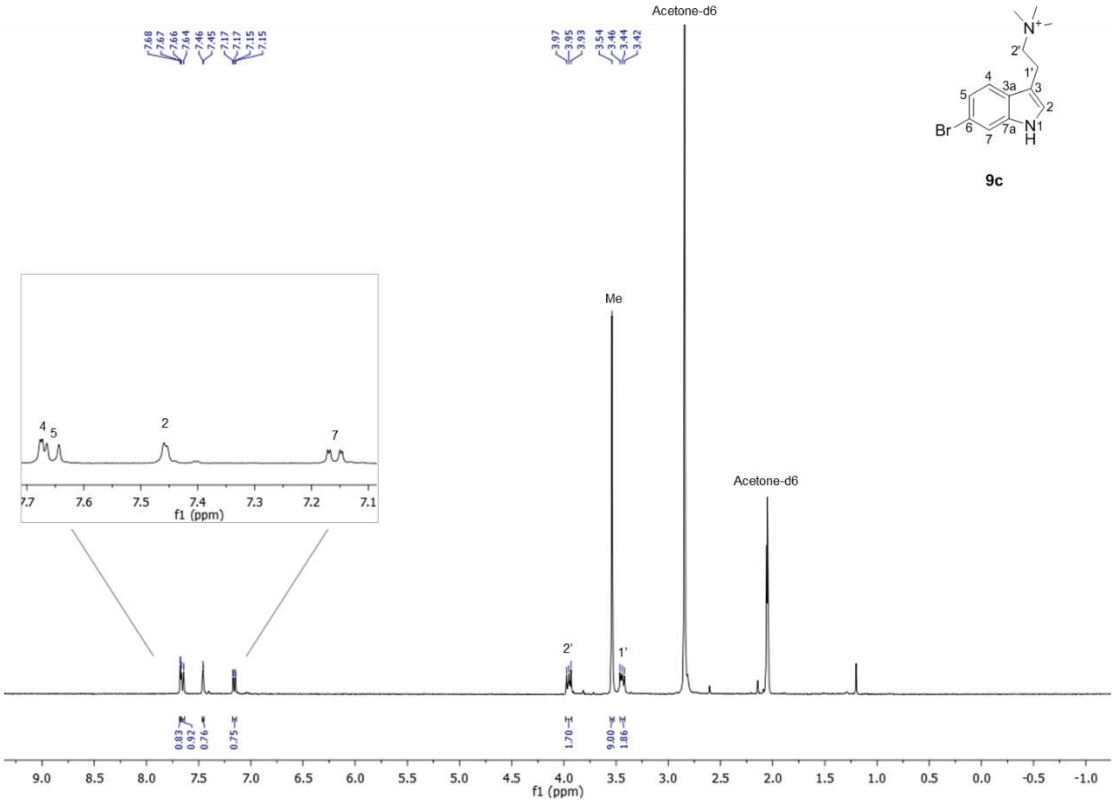



**Figure S25.** [13]C-NMR spectrum of **9c** in methanol-*d4*.

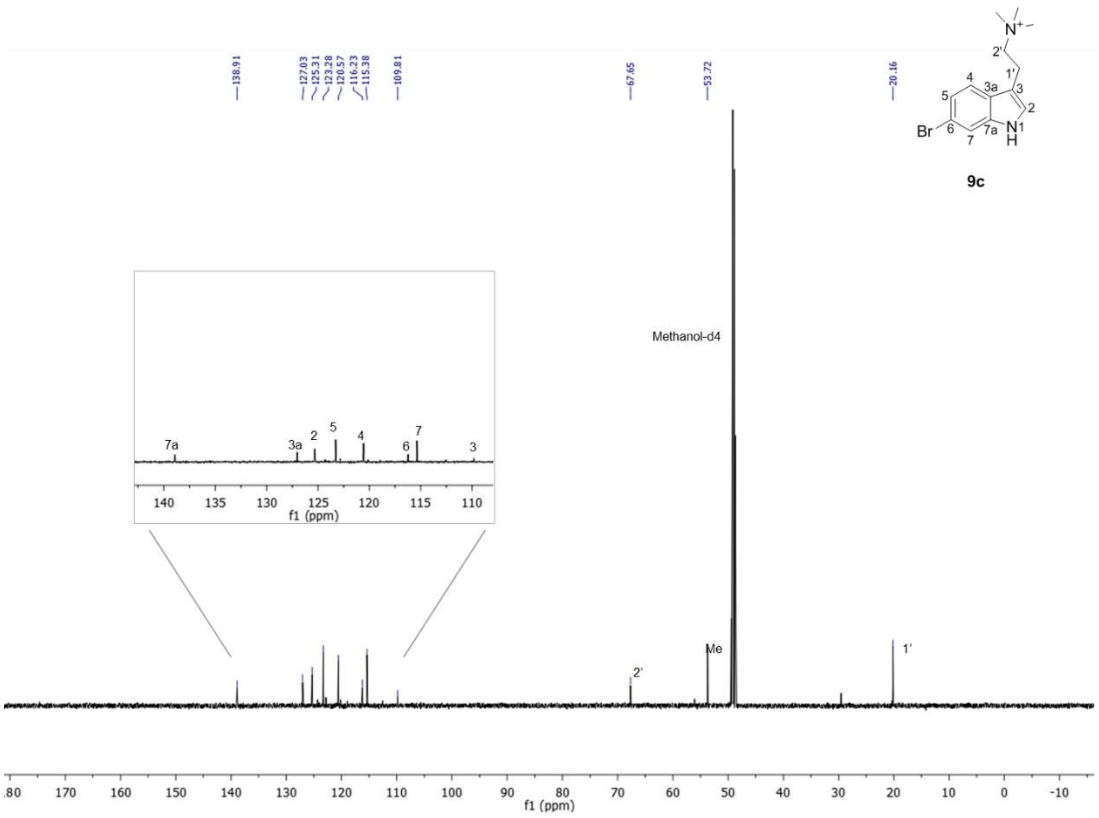


**Figure S26.** [1]H-NMR spectrum of **9d** in methanol-*d4*. The singlet of 2 appears in the middle of the 6 doublet. The peaks of 1' and 2' are overlapping.

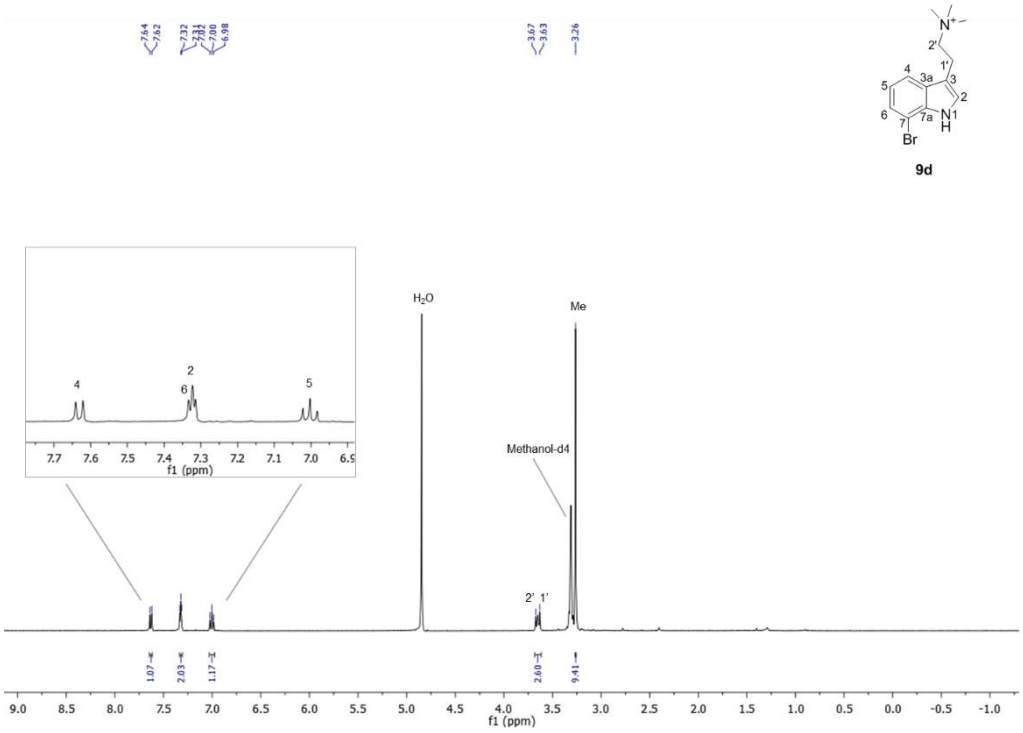



**Figure S27.** [13]C-NMR spectrum of **9d** in methanol-*d4*.