Supporting Information

Multifunctional Cholinesterase and Amyloid Beta Fibrillization Modulators. Synthesis and Biological Investigation

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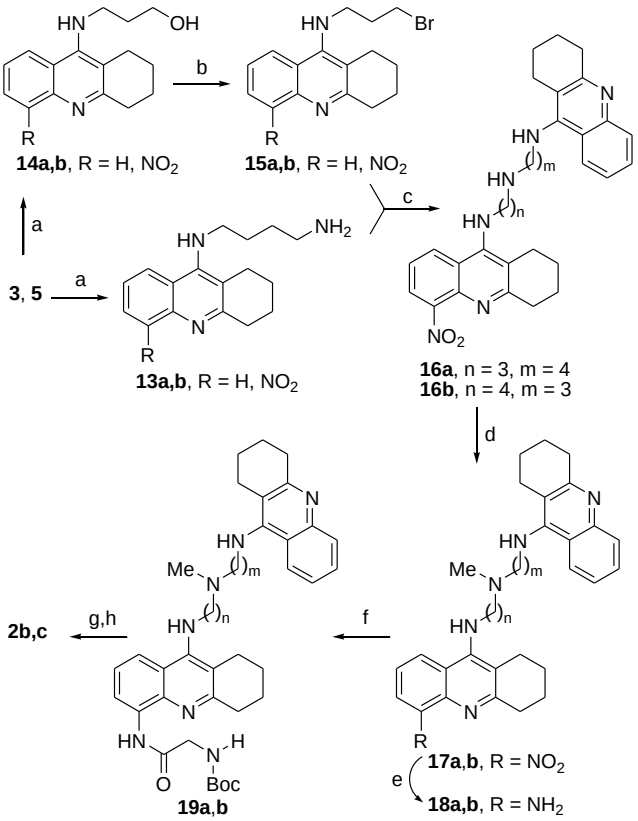
Further CE notes, supplementary figures and table, details for synthesis, analytical data, and biological studies**.**

Chemistry

Scheme 1 of the Main Text highlights the convergent synthetic strategy to **2a**. Compound **3** was converted into its nitro derivative **5** which was coupled with the amine **4**, in turn obtained by combining **3** with 1,8-diaminooctane, to afford the nitro bis-tacrine **6**. After reduction of the nitro group (**7**) and coupling with *N*-Boc glycine, compound **8** was obtained. Boc deprotection and immediate coupling of the free amine with the peptide **12** gave compound **2a**. The synthesis of the peptide moiety **12** needed for the convergent synthetic strategy to **2a** and **2b,c** (see Scheme 2) starts from **Fmoc-L-glutamic acid 5-*tert*-butyl ester** (**9**) which was coupled with L-phenylalanine benzyl ester by application of a standard protocol for in solution peptide chemistry to give the dipeptide **10**. After removal of the Fmoc protecting group and coupling with glycine benzyl ester the appropriate orthogonally protected tripeptide **11** was obtained. Hydrolysis of the *tert*-butyl ester (**12**) followed by coupling reaction with the appropriate amine gave **2a**.

The synthesis of the bis-tacrine derivatives **2b,c** is described in Scheme 1SI**.** Compounds **3** and **5** were coupled with 1,4-diaminobutane or 3-aminopropanol in a palladium catalyzed reaction and furnished compounds **13a,b** and **14a,b** respectively. The alcohol derivatives **14a,b** were brominated with CBr_4 and PPh_3, giving **15a,b,** that were used for the alkylation of the amines **13a,b** to give the nitro bis-tacrine compounds **16a,b**. These latter compounds were carefully methylated in the presence of iodomethane, and the tertiary amines **17a,b** were obtained in moderate yield. The reduction of the nitro group with $SnCl_2$ furnished **18a,b.** The anilines were coupled with *N*-Boc-glycine previously activated with ethylchloroformate and derivatives **19a,b** were obtained in good yield. The removal of the Boc functionality with a methanolic solution of HCl, furnished the amines **20a,b** as hydrochloride salts that were immediately coupled with peptide **12** to give the final compounds **2b,c**.

Scheme 1SI. Synthesis of compounds 2b,c.[a]



[a]Reagents and conditions: (a) 1,4-diaminobutane (for **13a,b**), 3-aminopropan-1-ol (for **14a,b**) K_2CO_3, (±)-BINAP, $Pd(OAc)_2$, 1,4-dioxane, reflux, 12 h, 55-77%; (b) CBr_4, PPh_3, DCM, rt, 30 min, 75-98%; (c) K_2CO_3, 18-crown-6, MeCN, 60 °C, 12 h, 40-63%; (d) MeI, K_2CO_3, MeCN, rt, 30 min, 35-45%; (e) $SnCl_2 \cdot 2H_2O$ EtOH, rt, 12 h, 92-95%; (f) N-Boc-Gly-OH, ethylchloroformate, THF/DCM, from -10 °C to rt, 3 h, 51-55%; (g) MeCOCl, MeOH; rt, 10 min, 99% (h) Z-Gly-L-Glu-L-Phe-OBn (**12**), EDCI, HOBt, TEA, from 0 °C to rt, 12 h, 45-50%.

Table 1SI. XP glide scores of selected compounds concerning the best poses derived from the 1st and 2nd cluster of docked solutions obtained by means of IFD protocol.

Compound	XP glide score[a] of the best pose derived from 1st cluster	XP glide score[a] of the best pose derived from 2nd cluster
2a	-19.603 Kcal/mol	-13.467 Kcal/mol
2b	-20.561 Kcal/mol	-9.321 Kcal/mol
2c	-21.143 Kcal/mol	-10.543 Kcal/mol

[a]XP glide scores were calculated as reported in the Induced Fit Docking (IFD) paragraph in the Computational Details section

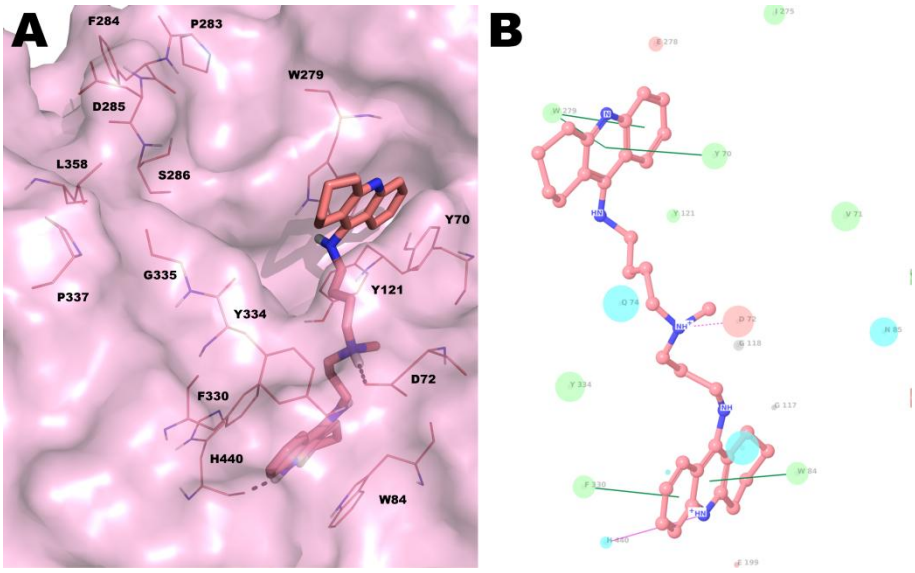


Figure 1SI. (A) Docked pose of **1c** (pink sticks) into the *Tc*AChE binding site (*glide XP score: -19.120 Kcal/mol*) obtained by means of Induced Fit Docking (IFD) protocol (pink surface for protein; key residues are represented by lines). H-bonds were reported as dotted lines. The picture was generated by PyMOL (PyMOL is an OPEN SOURCE program distributed under the "Python" license. The PyMOL Molecular Graphics System, v1.6-alpha; Schrödinger LLC: New York, 2013). Nonpolar hydrogens were omitted for clarity; (B) Representation of key interactions of **1c** (pink ball and stick model) into the *Tc*AChE binding site The picture was generated by Maestro (Maestro, Version 9.2, Schrödinger, LLC, New York, NY, 2011) using ligand-interaction diagram 3D script (green lines represent π-π stacking, purple lines represent polar contacts).

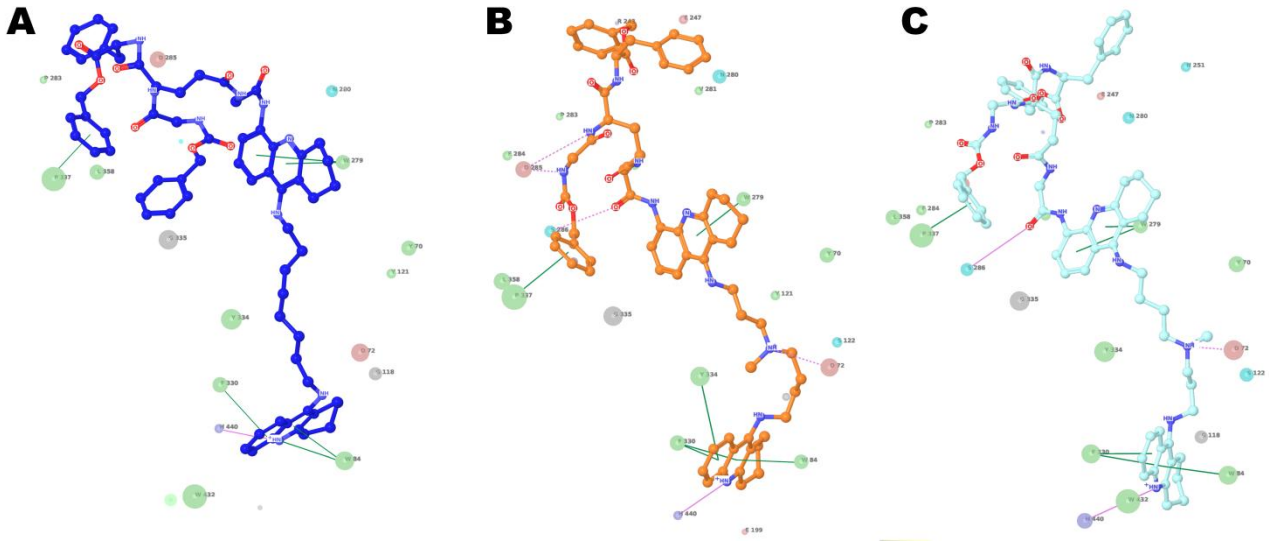


Figure 2SI. (A) Representation of key interactions of **2a** (blue balls and sticks model) into the *Tc*AChE binding site (*glide XP score: -19.603 Kcal/mol*). (B) Representation of key interactions of **2b** (orange balls and sticks model) into the *Tc*AChE binding site (*glide XP score: -20.561 Kcal/mol*). (C) Representation of key interactions of **2c** (cyan balls and sticks model) into the *Tc*AChE binding site (*glide XP score: -21.143 Kcal/mol*). The picture was generated by Maestro (Maestro, Version 9.2, Schrödinger, LLC, New York, NY, 2011) using ligand-interaction diagram 3D script (green lines represent π-π stacking, purple lines represent polar contacts).

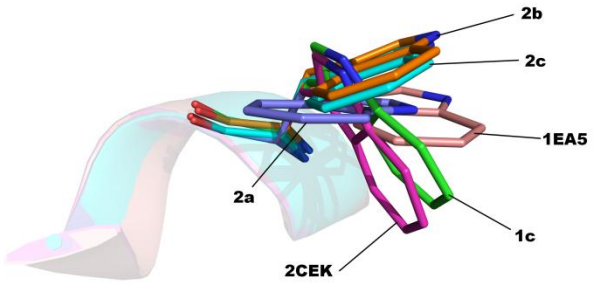


Figure 3SI. Different orientation of W279 when diverse ligands are bound to *Tc*AChE. (2CEK crystallographic structure of **1b** in complex with *Tc*AChE; 1EA5 native state of *Tc*AChE)

Capillary electrophoresis

It is well known that the in vitro aggregation kinetics of Aβ peptide may differ depending on many variables such as, among others, the peptide supplier.[1] As in this work the peptide supplier differs from that previously used by us,[2] all the features therein described have been here confirmed: i) the dynamic equilibrium between the two electrophoretic peaks detected over time (Figure 4SI for the peptide profile at the beginning of solubilisation); ii) fibril presence at the end of the analysis (Figure 5SI); iii) the molecular weight range (Figure 6SI); iv) the toxicity of separated species (Figure 7SI).

In Figure 4SI two peaks A and B are present. The two electrophoretic peaks are at equilibrium: over time peak B increases at the expenses of the peak A (see Figure 4SI inset). Compared to our previous report[2] peak A is a single and sharp peak instead of a group of unresolved peaks, but two distinct protein populations are anyway observed. For experimental conditions see experimental section.

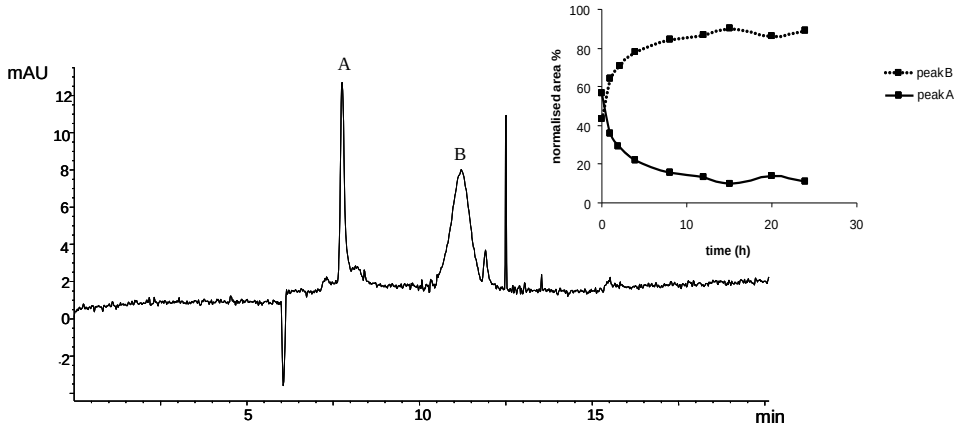


Figure 4SI. $A\beta_{1-42}$ (Anaspec, San Jose, CA, USA). CE profile immediately after supernatant withdrawal (t_0). Inset: Plot of the normalized area% [(peak area/electrophoretic migration time) x 100]/ total area of peaks)] of peaks A and B vs. the different elapsed times from t_0.

In Figure 5SI, a TEM image of $A\beta_{1-42}$ after five days from sample solubilization (i.e. when precipitation occurs) exhibits classical amyloid fibrils with a diameter of 10 nm (see arrow), as previously reported.[2a]

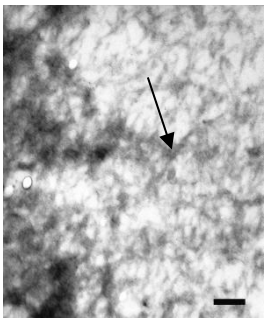


Figure 5SI. Electron micrograph of negatively stained sample exhibits non-branched fibrils of $A\beta_{1-42}$. Scale bar: 100 nm.

Ultrafiltration studies were carried out to assign a molecular mass range to the separated $A\beta_{1-42}$ peaks. ($A\beta_{1-42}$ monomer: 4514.15). All the peaks correspond to species characterized by molecular masses above 10000 Da (data not shown). The filtered and the retained solutions were injected into the CE and the electropherograms obtained were compared with that of Figure 6SI panel (a). As previously reported,[2b] peak A corresponds to species with molecular mass range of 10000–50000 Da (trimers-undecamers), see Figure 6SI panel (c); species with a molecular mass higher than 100000 Da (>22 mers) migrate under peak B, see Figure 6SI panel (b).

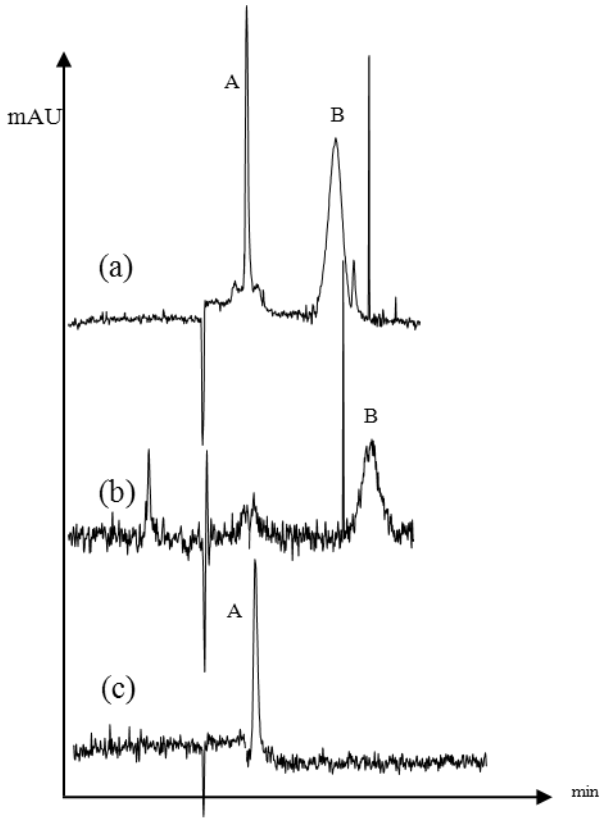


Figure 6SI. Ultrafiltration experiments and injection of $A\beta_{1-42}$ filtered and retained solutions in CE. Electrophoretic profiles of a) control peptide not filtered; b) $A\beta_{1-42}$ retained solution (cut-off of 100000 Da); c) $A\beta_{1-42}$ filtered solution (cut-off of 50000 Da). Experimental conditions as previously reported.[2b]

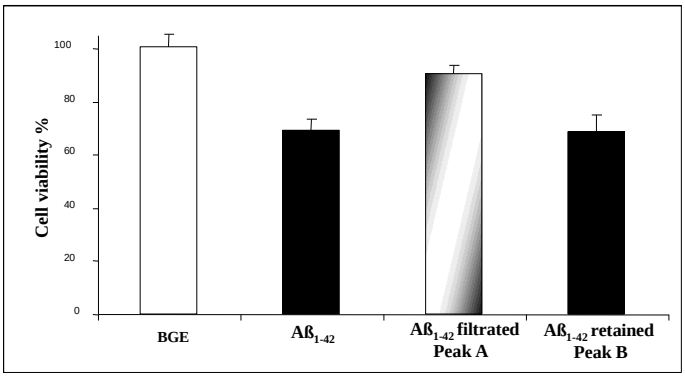


Figure 7SI. MTT colorimetric assay: high molecular mass oligomers (peak B) are responsible for the cytotoxicity of $A\beta_{1-42}$. Experimental conditions as previously reported.[2a]

Figure 7SI reports the cell toxicity played by $A\beta_{1-42}$ in toto (cell viability 69.24 % ± 4.38) and by species filtrated and retained by a filter (cut-off of 100000 Da). Filtrated $A\beta_{1-42}$ (trimers-undecamers, peak A), exhibits a cell viability of 90.41 % ± 3.43. Retained $A\beta_{1-42}$ multimeric species (>22 mers, peak B), shows cell viability of 68.73 % ± 6.42. Cell viability is expressed as % of untreated cells and determined by the MTT reduction assay. Results are consistent with previous data.[2a]

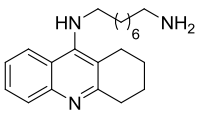
Experimental Section

Chemistry

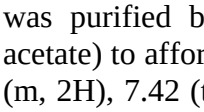
General procedures.

Unless otherwise specified, materials were purchased from commercial suppliers and used without further purification. Reaction progress was monitored by TLC using silica gel 60 F254 (0.040-0.063 mm) with detection by UV. Silica gel 60 (0.040-0.063 mm) or aluminum oxide 90 (0.063.0.200 mm) were used for column chromatography. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Varian 300 MHz spectrometer by using the residual signal of the deuterated solvent as internal standard. Splitting patterns are described as singlet (s), doublet (d), triplet (t), quartet (q), quintet (p), and broad (br); the value of chemical shifts (δ) are given in ppm and coupling constants (*J*) in Hertz (Hz). Number of overlapping carbon signals are reported in brackets (equivalent carbon atoms are always counted once). ESI-MS spectra were performed by an Agilent 1100 Series LC/MSD spectrometer. Melting points were determined in Pyrex capillary tubes using an Electrothermal 8103 apparatus and are uncorrected. Optical rotation values were measured at room temperature using a Perkin-Elmer model 343 polarimeter operating at λ = 589 nm, corresponding to the sodium D line. Yields refer to purified products and are not optimized. All moisture-sensitive reactions were performed under argon atmosphere using oven-dried glassware and anhydrous solvents. ESI-MS spectra for exact mass determination were performed on a LTQ Orbitrap Thermo Fischer Scientific instrument.

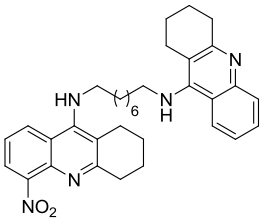
***N*^{1}-(1,2,3,4-Tetrahydroacridin-9-yl)octane-1,8-diamine (4).** To a solution of **3** (350 mg, 1.61 mmol) in freshly distilled 1,4-dioxane (15.0 mL), 1,8-diaminooctane (464 mg, 3.22 mmol), anhydrous K_2CO_3 (4.4 g, 32.2 mmol), (±)-BINAP (53 mg, 0.08 mmol), and $Pd(OAc)_2$ (18 mg, 0.08 mmol) were added. The resulting mixture was refluxed for 12 h under argon atmosphere. Then the solvent was removed under reduced pressure and water was added. The aqueous phase was extracted with ethyl acetate (3 x 30 mL), the combined organic layers were dried over Na_2SO_4, filtered and concentrated. The residue was purified by means of flash chromatography (0.5:1:10 ammonium hydroxide/MeOH/ethyl acetate) to afford title compound (62% yield) as a yellow oil; ^{1}H NMR (300 MHz, $CDCl_3$) δ = 7.82 (m, 2H), 7.42 (t, *J* = 7.6 Hz, 1H), 7.21 (t, *J* = 7.6 Hz, 1H), 3.85 (br s, 1H), 3.34 (m, 2H), 2.94 (m, 2H), 2.56 (m, 4H), 1.85 (m, 4H), 1.51 (m, 2H), 1.27-1.11 (m, 12H); ^{13}C NMR (75 MHz, $CDCl_3$) δ = 158.5, 150.8, 147.7, 128.9, 128.2, 123.5, 123.0, 120.4, 115.9, 49.5, 42.3, 34.3, 33.9, 31.8, 29.5, 29.4, 27.0, 26.9, 24.9, 23.2, 23.0; ESI-MS *m/z*: 326 $[M+H]^+$.



9-Chloro-5-nitro-1,2,3,4-tetrahydroacridine (5). Compound **3** (6.0 g, 27.56 mmol) was dissolved in concentrated sulfuric acid (90.0 mL), then nitric acid (6.0 mL) was added dropwise to the solution previously cooled at 0 °C. The reaction mixture was stirred at rt for 30 min and successively poured into ice and neutralized with a 33% ammonium hydroxide solution. The aqueous phase was extracted with DCM (3 x 150 mL) and the combined organic layers were dried over Na_2SO_4, filtered and concentrated. The crude was purified by means of flash chromatography (1:8 ethyl acetate/*n*-hexane) to afford title compound (50% yield) as a pale yellow solid, mp: 134-136 °C (hexane); ^{1}H NMR (300 MHz, $CDCl_3$) δ = 8.36 (m, 1H), 7.92 (d, *J* = 7.4 Hz, 1H), 7.57 (t, *J* = 8.0 Hz, 1H), 3.14 (m, 2H), 3.03 (m , 2H), 1.95 (m, 4H); ^{13}C NMR (75 MHz, $CDCl_3$) δ = 162.8, 148.2, 141.4, 138.1, 131.2, 127.7, 126.4, 125.1, 123.1, 34.7, 27.8, 22.5 (2C); **ESI-MS** *m/z*: 285 $[M+Na]^+$, 263 $[M+H]^+$ (100).

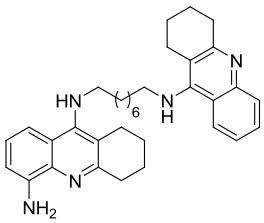


***N*^{1}-(5-Nitro-1,2,3,4-tetrahydroacridin-9-yl)-*N*^{8}-(1,2,3,4-tetrahydroacridin-9-yl)octane-1,8-diamine (6).** Starting from **5** and amine **4**, the title compound was obtained following the procedure described for **4**. The crude was purified by means of flash chromatography (1:10 MeOH/chloroform) to afford title compound (65% yield) as a yellow oil; ^{1}H NMR (300 MHz, $CDCl_3$) δ = 8.08 (d, *J* = 8.5 Hz, 1H), 7.92 (m, 2H), 7.69 (d, *J* = 7.4 Hz, 1H), 7.49 (t, *J* = 7.5 Hz, 1H), 7.25

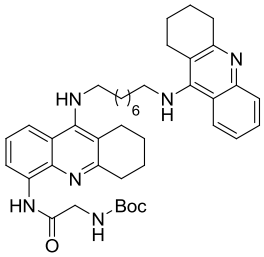


(m, 2H), 4.19 (br s, 1H), 4.06 (br s, 1H), 3.55-3.45 (m, 4H), 3.08-2.94 (m, 4H), 2.61 (m, 4H), 1.83 (m, 8H), 1.70-1.50 (m, 4H), 1.40-1.27 (m, 8H); ^{13}C NMR (75 MHz, $CDCl_3$) δ = 161.2, 158.0, 151.2, 150.7, 148.5, 147.1, 139.4, 128.6, 128.3, 127.5, 123.7, 123.2, 122.0, 121.5, 121.3, 120.1, 117.0, 115.6, 49.8, 49.5, 34.6, 33.9, 31.8 (2C), 29.4 (2C), 27.0 (2C), 24.9, 24.7, 23.2, 22.9 (2C), 22.6; ESI-MS *m/z*: 552 $[M+H]^+$, 276 $[M+2H]^{2+}/2$ (100). Anal ($C_{34}H_{41}N_5O_2$), C, H, N.

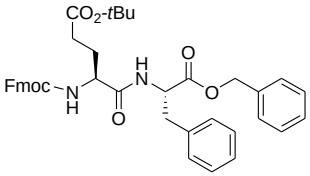
***N*^{1}-(5-Amino-1,2,3,4-tetrahydroacridin-9-yl)-*N*^{8}-(1,2,3,4-tetrahydroacridin-9-yl)octane-1,8-diamine (7).** To a solution of **6** (920 mg, 1.67 mmol) in EtOH (30.0 mL), $SnCl_2{\cdot}2H_2O$ (1.9 g, 8.35 mmol) was added. The mixture was stirred at rt for 12 h, then it was poured into ice and neutralized with a 5% aqueous solution of $NaHCO_3$. The suspension was filtered through a small plug of Celite, and washed with MeOH. The organic solvent was removed in vacuo and the aqueous phase was extracted with chloroform (3 x 25 mL). The combined organic layers were dried over Na_2SO_4, filtered and concentrated. Pure title compound was obtained without any further purification (60% yield) as brown oil; ^{1}H NMR (300 MHz, $CDCl_3$) δ = 7.93, (m, 2H), 7.54 (t, *J* = 7.5 Hz, 1H), 7.33 (t, *J* = 7.6 Hz, 1H), 7.24 (m, 1H), 7.12 (t, *J* = 7.9 Hz, 1H), 6.78 (d, *J* = 7. 2 Hz, 1H), 4.93 (br s, 2H), 3.89 (br s, 2H), 3.43 (m, 4H), 3.04 (m, 4H), 2.70 (m, 4H), 1.90 (m, 8H), 1.60 (m, 4H), 1.31 (m, 8H); ^{13}C NMR (75 MHz, $CDCl_3$) δ = 158.5, 156.2, 151.1, 150.8, 147.5, 143.8, 137.9, 128.8, 128.6, 124.5, 123.8, 123.1, 120.9, 120.4, 116.8, 116.0, 111.2, 109.0, 49.7, 49.4, 34.3, 34.2, 32.0, 31.9, 29.5 (2C), 27.1 (2C), 25.3, 25.0, 23.5, 23.3, 23.2, 23.0; ESI-MS *m/z*: 522 $[M+H]^+$, 261 $[M+2H]^{2+}/2$ (100).). Anal ($C_{34}H_{43}N_5$), C, H, N.



***N*^{1}-(5-(2-*tert*-Butoxycarbonylaminoacetamido)-1,2,3,4-tetrahydroacridin-9-yl)-*N*^{8}-(1,2,3,4-tetrahydroacridin-9-yl)octane-1,8-diamine (8).** To a cold solution (-10 °C) of *N*-Boc-Gly-OH (168 mg, 0.96 mmol) in dry THF (8.0 mL), TEA (148 μL, 1.06 mmol) and ethylchloroformate (91 μL, 0.96 mmol) were added. The mixture was stirred at -10 °C for 20 min before adding a solution of **7** (500 mg, 0.96 mmol) in 12.0 mL of dry DCM. The reaction mixture was stirred at rt for 3 h, then the solvents were evaporated under reduced pressure, and the residue was purified by means of flash chromatography (1:5 MeOH/DCM) to afford pure title compound (55% yield) as an amorphous yellow solid; ^{1}H NMR (300 MHz, $CDCl_3$) δ = 10.45 (br s, 1H), 8.45 (d, *J* = 7.3 Hz, 1H), 8.00 (m, 2H), 7.50 (m, 2H), 7.21 (m, 2H), 5.66 (br s, 1H), 4.95 (br s, 1H), 4.03 (m, 3H), 3.55 (m, 2H), 3.40 (m, 2H), 3.03 (m, 2H), 2.92 (m, 2H), 2.59 (m, 4H), 1.80 (m, 8H), 1.63-1.53 (m, 4H), 1.43 (s, 9H), 1.25 (m, 8H); ^{13}C NMR (75 MHz, $CDCl_3$) δ = 167.9, 156.8, 156.2, 155.7, 152.7, 151.0, 144.4, 137.9, 133.4, 129.8, 125.8, 124.2, 123.7, 123.6, 119.6, 118.8, 117.4, 116.2, 115.1, 114.2, 80.1, 49.4, 49.2, 45.5, 34.3, 32.1, 31.8, 31.6, 29.4 (2C), 28.6, 26.9, 25.0, 24.7, 23.3, 22.8 (2C), 22.2; ESI-MS *m/z*: 679 $[M+H]^+$, 340 $[M+2H]^{2+}/2$ (100).



Fmoc-L-Glu(O*t*Bu)-L-Phe-OBn (10). To a cold (0 °C) solution of Fmoc-L-Glu(O*t*Bu)-OH (**9**) (730 mg, 1.72 mmol) in dry DCM (10.0 mL), HOBt (278 mg, 2.06 mmol) and EDCI (395 mg, 2.06 mmol) were added. The solution was stirred at 0 °C for 1 h before adding L-Phe-OBn (500 mg, 1.72 mmol). The reaction mixture was stirred for 12 h at rt then the solvent was removed under reduced pressure and water was added. The aqueous phase was extracted with DCM (3 x 10 mL) and the combined organic layers were dried over Na_2SO_4, filtered and evaporated. The crude was purified by means of flash chromatography (1:2 ethyl acetate/*n*-hexane) to afford title compound (93% yield) as a white solid, mp: 123-125 °C (hexane); $[\alpha]^{20}_D$= –22.3 cm^3 g^{-1} dm^{-1} (*c* = 0.01 g cm^{-3} in MeOH); ^{1}H NMR (300 MHz, $CDCl_3$) δ = 7.77 (d, *J* = 7.5 Hz, 2H), 7.61 (d, *J* = 7.2 Hz, 2H), 7.44-7.26 (m, 9H), 7.19 (m, 3H) 7.06 (m, 2H), 6.96 (br d, 1H), 5.77 (br d, 1H), 5.14 (m, 2H), 4.91 (dd, J_1 = 13.7 Hz, J_2 = 6.3 Hz, 1H), 4.41-4.12 (m, 4H), 3.12 (m, 2H), 2.38 (m, 2H), 2.20-1.85 (m, 2H), 1.50 (s, 9H). ^{13}C NMR (75 MHz, $CDCl_3$) δ = 173.1, 171.2 (2C), 156.4, 144.1, 144.0, 141.5 (2C), 135.8, 135.3, 129.5, 128.8 (2C), 128.7, 128.0, 127.3, 125.4, 120.2, 81.2,



67.5, 67.4, 54.3, 53.7, 47.4, 38.1, 31.9, 28.5, 28.3; ESI-MS *m/z*: 701[M+K]$^+$, 685 [M+Na]$^+$ (100), 663 [M+H]$^+$.

Z-Gly-L-Glu(O*t*Bu)-L-Phe-OBn (11). To a solution of **10** (1.2 g, 1.80 mmol) in dry DCM (6.0 mL), distilled Et_2NH (4.0 mL) was added. The reaction mixture was stirred at rt for 1 h. The solution was concentrated and added to a solution of *Z*-Gly-OH (376 mg, 1.64 mmol) in the same solvent, previously activated for 1 h at 0 °C with EDCI (345 mg, 1.80 mmol), HOBt (243 mg, 1.80 mmol) and TEA (250 µL, 1.80 mmol). The mixture was stirred for 12 h at rt, then the solvent was removed under reduced pressure and water was added. The aqueous phase was extracted with DCM (3 x 10 mL) and the combined organic layers were dried over Na_2SO_4, filtered and evaporated. The crude was purified by means of flash chromatography (1:20 MeOH/chloroform) to afford title compound (65% yield) as a white solid after crystallization from chloroform/*n*-hexane, mp: 183-185 °C (hexane, dec); $[\alpha]^{20}_D$ = –22.7 cm^3 g^{-1} dm^{-1} (*c* = 0.01 g cm^{-3} in MeOH); ^{1}H NMR (300 MHz, $CDCl_3$) δ = 7.35-7.21 (m, 13H), 7.07-6.99 (m, 3H), 6.92 (br d, 1H), 5.36 (br s, 1H), 5.14 (m, 4H), 4.85 (dd, J_1 = 13.6 Hz, J_2 = 6.7 Hz, 1H), 4.41 (dd, J_1 = 13.3 Hz, J_2 = 7.0 Hz, 1H), 3.83 (d, *J* = 5.3 Hz, 2H), 3.10 (m, 2H), 2.32 (m, 2H), 2.02-1.80 (m, 2H), 1.41 (m, 9H); ^{13}C NMR (75 MHz, $CDCl_3$) δ = 173.4, 171.2, 170.8, 169.1, 156.7, 136.4, 135.9, 135.3, 129.5, 128.8 (2C), 128.4, 128.3, 127.3, 124.6, 120.5, 81.4, 67.5, 67.4, 53.7, 52.6, 44.6, 37.9, 31.9, 28.3, 28.0; ESI-MS *m/z*: 654 [M+Na]$^+$.

Z-Gly-L-Glu-L-Phe-OBn (12). A solution of **11** (100 mg, 0.15 mmol) in 5.0 mL of formic acid was stirred at rt for 3 h. The formic acid was evaporated under reduced pressure, and an ice-cold 6% aqueous solution of $NaHCO_3$ was added to the residue and extracted with ethyl acetate (3 x 15 mL). The combined organic layers were dried over Na_2SO_4, filtered and concentrated. Pure title compound was obtained without any further purification (99% yield) as a white solid, mp: 150°C (hexane, dec); $[\alpha]^{20}_D$ = -6.5 cm^3 g^{-1} dm^{-1} (*c* = 0.01 g cm^{-3} in DMF);^{1}H NMR (300 MHz, DMSO-d_6) δ = 8.90 (br s, 1H); 8.26 (br s, 1H), 7.43-7.18 (m, 16H), 5.03 (m, 4H), 4.46 (m, 1H), 4.29 (m, 1H), 3.60 (d, *J* = 5.7 Hz, 2H), 3.05-2.91 (m, 2H), 2.19-2.07 (m, 2H), 1.70 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ = 181.3, 172.3 (2C), 170.8, 157.3, 136.6, 136.4, 136.2, 135.3, 134.7, 129.5, 128.9, 128.6 (2C), 128.5, 128.2, 127.0, 67.3, 67.1, 54.2, 53.0, 44.6, 37.8, 33.9, 29.5; ESI-MS *m/z*: 574 [M–H]$^-$.

Z-Gly-L-Gln-[N^1-(5-(2-acetamide)-1,2,3,4-tetrahydroacridin-9-yl)-N^8-(1,2,3,4-tetrahydroacridin-9-yl)octane-1,8-diamine]-L-Phe-OBn (2a).

N^1-(5-(2-Aminoacetamido)-1,2,3,4-tetrahydroacridin-9-yl)-N^8-(1,2,3,4-tetrahydroacridin-9-yl)octane-1,8-diamine trihydrochloride. A 0.1 N solution of acetyl chloride in MeOH was carefully added to a solution of **8** in MeOH, and the resulting mixture was evaporated. The treatment was repeated until complete deprotection (ESI-MS monitoring). Pure amine was obtained as an amorphous white solid, and was immediately used for the following reaction without any further purification. ESI-MS *m/z*: 579 [M+H]$^+$. To a solution of **12** (55 mg, 0.096 mmol) in dry DCM (5.0 mL), EDCI (18 mg, 0.096 mmol), HOBt (13 mg, 0.096 mmol) and TEA (15 µL, 0.096 mmol) were added at 0 °C. The reaction mixture was maintained for 1 h at 0 °C then it was allowed to reach rt. The above amine (0.087 mmol) dissolved in 4.0 mL of dry DCM and TEA (12 µL, 0.087 mmol) was added to the reaction mixture, which was stirred for 12 h at rt. The solution was washed with a $NaHCO_3$ saturated solution and the organic layer was dried over Na_2SO_4, filtered and concentrated. The crude was purified by means of chromatography on aluminum oxide (1:100 MeOH/chloroform) to afford title compound (42% yield) as an amorphous white solid, $[\alpha]^{20}_D$ = –1.9 cm^3 g^{-1} dm^{-1} (*c* = 0.01 g cm^{-3} in chloroform); ^{1}H NMR (300 MHz, $CDCl_3$) δ = 10.22 (br s, 1H), 8.52 (d, *J* = 7.4 Hz, 1H), 8.09 (br d 1H), 7.93 (m,

2H), 7.58 (m, 2H), 7.40-7.09 (m, 18H), 5.58 (br s, 1H), 5.13 (m, 5H), 4.89 (m, 1H), 4.66 (m, 1H), 4.34 (br d, 1H), 4.16-3.63 (m, 5H), 3.48 (m, 4H), 3.26-2.93 (m, 6H), 2.70 (m, 4H), 2.54-1.98 (m, 4H), 1.92 (m, 8H), 1.64 (m, 4H), 1.35 (m, 8H); ^{13}C NMR (75 MHz, $CDCl_3$) δ = 173.5, 172.4, 171.3, 169.0, 167.6, 158.5, 157.1, 156.7, 151.1, 151.0, 147.5, 137.8, 136.5, 136.4, 135.2, 133.2, 129.5 (2C), 129.3, 128.8 (2C), 128.7 (2C), 128.6 (2C), 128.5, 128.3, 128.2, 127.1, 123.8, 123.0, 119.6, 117.6, 116.3, 116.0, 115.7, 67.6, 67.3, 53.9, 52.4, 49.7, 49.5, 44.6, 43.9, 37.8, 34.3, 34.2, 32.4, 31.9 (2C), 29.4 (2C), 29.2, 27.0 (2C), 25.0 (2C), 23.3 (2C), 23.0, 22.9; ESI-MS *m/z*: 1137 $[M+H]^+$, 569 $[M+2H]^{2+}/2$ (100); HRMS (ESI) *m/z* $[M+H]^+$ calcd for $C_{67}H_{77}N_9O_8$ 1136.5968, found 1136.5966, $[M+2H]^+/2$ calcd for $C_{67}H_{77}N_9O_8$ 568.80205, found 568.8014 (100).

***N*^{1}-(1,2,3,4-Tetrahydroacridin-9-yl)butane-1,4-diamine (13a).** Starting from **3** and 1,4-diaminobutane, the title compound was obtained following the same procedure described for **4**. The crude was purified by means of flash chromatography (0.5:1:10 ammonium hydroxide/MeOH/ethyl acetate) to afford title compound (55% yield) as a pale yellow oil; ^{1}H NMR (300 MHz, $CDCl_3$) δ = 7.79 (m, 2H), 7.39 (m, 1H), 7.20 (m, 1H), 4.03 (br s, 1H), 3.34 (m, 2H), 2.89 (m, 2H), 2.54 (m, 4H), 2.12 (br s, 2H), 1.90-1.64 (m, 4H), 1.54 (m, 2H), 1.39 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ = 158.3, 151.1, 147.2, 128.6, 128.5, 123.9, 123.1, 120.2, 115.9, 49.5, 41.9, 33.9, 31.0, 29.3, 25.0, 23.2, 22.9; ESI-MS *m/z*: 270 $[M+H]^+$.

***N*^{1}-(5-Nitro-1,2,3,4-tetrahydroacridin-9-yl)butane-1,4-diamine (13b).** Starting from **5** and 1,4-diamonibutane, the title compound was obtained following the same procedure described for **4**. The crude was purified by means of flash chromatography (0.5:1:10 ammonium hydroxide/MeOH/ethyl acetate) to afford title compound (77% yield) as a yellow solid, mp**:** 134-136 °C (hexane); ^{1}H NMR (300 MHz, $CDCl_3$) δ = 8.08 (d, *J* = 8.6 Hz, 1H), 7.69 (d, *J* = 7.4 Hz, 1H), 7.23 (m, 1H), 4.21 (br s, 1H), 3.45 (t, *J* = 7.0 Hz, 2H), 2.94 (m, 2H), 2.68 (t, *J* = 6.8 Hz, 2H), 2.59 (m, 2H), 1.80 (m, 4H), 1.65 (m, 2H), 1.47 (m, 2H), 1.27 (br s, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ = 161.5, 150.8, 148.6, 139.4, 127.4, 122.2, 121.6, 121.5, 117.4, 49.8, 41.7, 34.6, 30.5, 29.2, 24.9, 23.0, 22.7; ESI-MS *m/z*: 315 $[M+H]^+$.

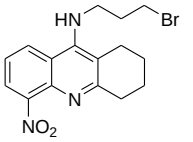
3-((1,2,3,4-Tetrahydroacridin-9-yl)amino)propan-1-ol (14a). Starting from **3** and 3-aminopropan-1-ol, the title compound was obtained following the same procedure described for **4**. The crude was purified by means of flash chromatography (1:1:15 TEA/MeOH/ethyl acetate) to afford title compound (63% yield) as a white solid, mp 132-135 °C (hexane); ^{1}H NMR (300 MHz, $CDCl_3$) δ = 7.98 (d, *J* = 8.5 Hz, 1H), 7.88 (d, *J* = 8.5 Hz, 1H), 7.50 (m, 1H), 7.28 (m, 1H), 4.71 (br s, 1H), 3.88 (m, 3H), 3.66 (m, 2H), 2.99 (m, 2H), 2.68 (m, 2H), 1.95-1.80 (m, 6H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ = 158.5, 151.1, 147.4, 130.9, 128.6, 123.8, 123.1, 120.3, 116.2, 61.6, 47.9, 34.0, 33.6, 25.1, 23.2, 23.0; **ESI-MS** *m/z*: 257 $[M+H]^+$.

3-(5-Nitro-1,2,3,4-tetrahydroacridin-9-ylamino)propan-1-ol (14b). Starting from **5** and 3-aminopropan-1-ol, the title compound was obtained following the same procedure reported for **4**. The crude was purified by means of flash chromatography (ethyl acetate) to afford title compound (65% yield) as an amorphous white solid; ^{1}H NMR (300 MHz, $CDCl_3$) δ = 8.16 (d, *J* = 8.5 Hz, 1H), 7.72 (d, *J* = 7.3 Hz, 1H), 7.20 (t, *J* = 8.0 Hz, 1H), 4.95 (br s, 1H), 3.82 (m, 2H), 3.64 (m, 2H), 3.15 (br s, 1H), 2.94 (m, 2H), 2.60 (m, 2H), 1.89-1.79 (m, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ = 161.3, 151.0, 148.3, 139.4, 127.7, 122.4, 121.5, 121.3, 117.2, 61.8, 48.4, 34.5, 33.3, 24.9, 23.0, 22.7; ESI-MS *m/z* 302 $[M+H]^+$.

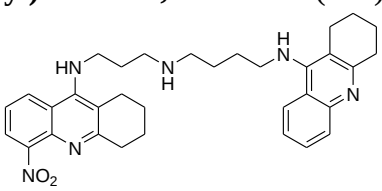
***N*-(3-Bromopropyl)-1,2,3,4-tetrahydroacridin-9-amine (15a).** To a solution of compound **14a** (1.2 g, 3.99 mmol) in dry DCM (50.0 mL), CBr_4 (1.5 g, 4.39 mmol) and PPh_3 (1.2 g, 4.39 mmol) were added. The solution was stirred at rt for 30 min, then a 15% aqueous NaOH solution was added. The aqueous phase was extracted with DCM (3 x 50 mL) and the combined organic layers were dried over Na_2SO_4, filtered and evaporated. The crude was purified by means of flash chromatography (3:7 *n*-hexane/ethyl acetate)

to afford title compound (98% yield) as a brown solid, mp 117-120 °C (hexane); ^{1}H NMR (300 MHz, $CDCl_3$) δ = 7.86 (d, J = 8.6 Hz, 2H), 7.49 (t, J = 7.6 Hz, 1H), 7.29 (m, 1H), 3.96 (br t, 1H), 3.50 (m, 2H), 3.38 (t, J = 6.4 Hz, 2H), 3.00 (m, 2H), 2.64 (m, 2H), 2.07 (p, J = 6.6 Hz, 2H), 1.83 (m, 4H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ = 161.5, 150.8, 148.6, 139.5, 127.4, 122.2, 121.6, 121.5, 117.4, 41.9, 34.7, 30.9, 29.4, 24.9, 23.0, 22.7; ESI-MS *m/z* 319 $[M+H]^+$.

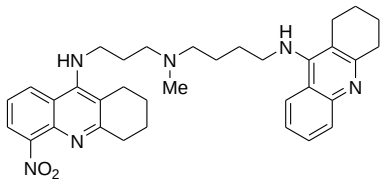
***N*-(3-Bromopropyl)-5-nitro-1,2,3,4-tetrahydroacridin-9-amine (15b).** Starting from **14b**, the title compound was obtained following the same procedure reported for **15a**. The crude was purified by means of flash chromatography (ethyl acetate) to afford title compound (75% yield) as an orange oil; ^{1}H NMR (300 MHz, $CDCl_3$) δ = 8.09 (d, J = 8.5 Hz, 1H), 7.73 (d, J = 7.3 Hz, 1H), 7.29 (t, J = 8.0 Hz, 1H), 4.12 (br t, 1H), 3.58 (m, 2H), 3.46 (t, J = 6.3 Hz, 2H), 2.95 (m, 2H), 2.65 (m, 2H), 2.16 (p, J = 6.5 Hz, 2H), 1.81 (m, 4H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ = 161.5, 150.3, 148.4, 139.1, 127.2, 122.2, 122.0, 121.8, 118.5, 47.7, 34.6, 33.9, 30.9, 24.9, 22.9, 22.6; ESI-MS *m/z*: 364 $[M+H]^+$.



***N*^{1}-(3-(5-Nitro-1,2,3,4-tetrahydroacridin-9-ylamino)propyl)-*N*^{4}-(1,2,3,4-tetrahydroacridin-9-yl)butane-1,4-diamine** (**16a**). To a solution of compound **13a** (314 mg, 1.17 mmol) in dry MeCN (15.0 mL), K_2CO_3 (234 mg, 1.76 mmol) and 18-crown-6 (catalytic amount) were added. The mixture was stirred for 1 h at rt, then compound **15b** (510 mg, 1.40 mmol) was added. The reaction was stirred at 60 °C for 12 h. The mixture was cooled to rt and the solvent was removed under reduced pressure. The residue was taken up with water and extracted with DCM (3 x 20 mL). The combined organic layers were dried over Na_2SO_4, filtered and concentrated. The crude was purified by means of flash chromatography (1:1:10 TEA/MeOH/ethyl acetate) to afford title compound (40% yield) as a yellow oil; ^{1}H NMR (300 MHz, $CDCl_3$) δ = 8.08 (d, J = 8.5 Hz, 1H), 7.84 (t, J = 9.6 Hz, 2H), 7.61 (d, J = 7.4 Hz, 1H), 7.44 (t, J = 7.6 Hz, 1H), 7.22 (t, J = 7.6 Hz, 1H), 7.11 (t, J = 7.9 Hz, 1H), 5.52 (br s, 1H), 3.52 (m, 2H), 3.41 (m, 2H), 2.96 (m, 2H), 2.88 (m, 2H), 2.68 (m, 2H), 2.57 (m, 6H), 2.10-1.46 (m, 16H); ^{13}C NMR (75 MHz, $CDCl_3$) δ = 161.4, 158.8, 151.0, 150.7, 148.7, 147.7, 139.6, 129.1, 128.5, 127.5, 123.9, 122.9, 122.0, 121.5, 121.1, 120.5, 116.9, 116.4, 50.1, 49.5 (2C), 48.9, 34.6, 34.3, 31.1, 29.8, 27.7, 25.4, 25.1, 23.3, 23.1, 23.0, 22.7; ESI-MS *m/z* 553 $[M+H]^+$, 277 $[M+2H]^{2+}$/2 (100).



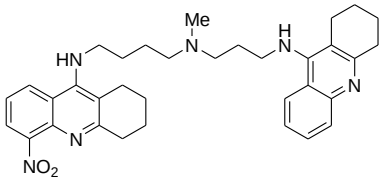
***N*^{1}-(5-Nitro-1,2,3,4-tetrahydroacridin-9-yl)-*N*^{4}-(3-(1,2,3,4-tetrahydroacridin-9-ylamino)propyl)butane-1,4-diamine (16b).** Starting from **15a** and **13b**, the title compound was obtained following the same procedure described for **16a**. The crude was purified by means of flash chromatography (1:1:10 TEA/MeOH/ethyl acetate) to afford title compound (63% yield) as a yellow oil; ^{1}H NMR (300 MHz, $CDCl_3$) δ = 8.04 (d, J = 8.5 Hz, 1H), 7.93 (d, J = 8.5 Hz, 1H), 7.85 (d, J = 8.4 Hz, 1H), 7.65 (d, J = 7.3 Hz, 1H), 7.45 (t, J = 7.5 Hz, 1H), 7.21 (m, 2H), 5.09 (br s, 1H), 4.12 (br s, 2H), 3.53 (t, J = 6.3 Hz, 2H), 3.42 (m, 2H), 2.98 (m, 2H), 2.91 (m, 2H), 2.71 (t, J = 6.2 Hz, 2H), 2.50 (m, 6H), 1.93-1.69 (m, 10H), 1.63 (m, 2H), 1.52 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ = 161.5, 158.3, 151.4, 150.6, 148.7, 147.3, 139.4, 128.6 (2C), 127.3, 123.7, 123.1, 122.1, 121.6, 121.5, 120.2, 117.5, 115.9, 50.0, 49.9, 48.9, 48.8, 34.6, 34.0, 31.2, 29.8, 27.6, 25.5, 24.8, 23.3, 23.0 (2C), 22.7; ESI-MS *m/z*: 553 $[M+H]^+$, 277 $[M+2H]^{2+}$/2 (100).



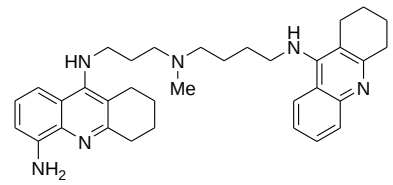
***N*^{1}-Methyl-*N*^{1}-(3-(5-nitro-1,2,3,4-tetrahydroacridin-9-ylamino)-propyl)-*N*^{4}-(1,2,3,4-tetrahydroacridin-9-yl)butane-1,4-diamine (17a).** Compound **16a** (290 mg, 0.53 mmol) was dissolved in 15.0 mL of dry MeCN, then K_2CO_3 (146 mg, 1.06 mmol) and 18-crown-6 (catalytic amount) were added. The mixture was stirred for 30 min at rt before adding iodomethane (40.0 μL, 0.48 mmol). The reaction was stirred at rt for 1 h. The solvent was removed and aqueous NaOH 1N solution was added. The aqueous phase was extracted with DCM (3 x 20 mL) and the combined organic layers were dried over Na_2SO_4, filtered and concentrated. The crude was purified by means of flash chromatography

(1:1:15 TEA/MeOH/ethyl acetate) to afford pure title compound (35% yield) as a yellow oil; ^{1}H NMR (300 MHz, $CDCl_3$) δ = 8.13 (d, J = 8.5 Hz, 1H), 7.90 (d, J = 8.5 Hz, 2H), 7.72 (d, J = 7.2 Hz, 1H), 7.52 (t, J = 7.7 Hz, 1H), 7.25 (m, 2H), 5.54 (br s, 1H), 3.87 (br s, 1H), 3.59 (m, 2H), 3.47 (t, J = 6.6 Hz, 2H), 3.01 (m, 4H), 2.34 (m, 4H), 2.49 (t, J = 6.0 Hz, 2H), 2.39 (t, J = 7.0 Hz, 2H), 2.25 (s, 3H), 1.97-1.72 (m, 10H), 1.72-1.48 (m, 4H); ^{13}C NMR (75 MHz, $CDCl_3$) δ = 161.3, 158.8, 151.0, 150.7, 148.6, 147.8, 139.6, 129.1, 128.4, 127.5, 123.9, 122.8, 122.0, 121.4, 121.0, 120.6, 116.6, 116.5, 58.2, 56.9, 49.7, 49.5, 42.6, 34.6, 34.4, 30.0, 28.2, 25.2, 25.1, 24.8, 23.3, 23.1, 23.0, 22.7; ESI-MS *m/z* 567 $[M+H]^+$, 295 $[M+H+Na]^{2+}/2$ (100).

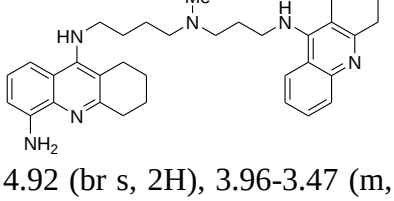
N^1-Methyl-N^4-(5-nitro-1,2,3,4-tetrahydroacridin-9-yl)-N^1-(3-(1,2,3,4-tetrahydroacridin-9-ylamino)propyl)butane-1,4-diamine (17b). Starting from **16b**, the title compound was obtained following the same procedure reported for **17a**. The crude was purified by means of flash chromatography (1:1:10 TEA/MeOH/ethyl acetate) to afford pure title compound (45% yield) as a yellow oil; ^{1}H NMR (300 MHz, $CDCl_3$) δ = 8.07 (d, J = 8.6, 1H), 7.96 (d, J = 8.3 Hz, 1H), 7.90 (d, J = 8.3 Hz, 1H), 7.76 (d, J = 6.3 Hz, 1H), 7.52 (m, 1H), 7.28 (m, 2H), 5.15 (br s, 1H), 3.99 (t, J = 5.9 Hz, 1H), 3.58 (m, 2H), 3.46 (m, 2H), 3.03 (m, 4H), 2.70 (t, J = 5.9 Hz, 2H), 2.58 (m, 2H), 2.49 (t, J = 6.3 Hz, 2H), 2.38 (t, J = 7.0 Hz, 2H), 2.25 (s, 3H), 1.97-1.72 (m, 10H), 1.72-1.46 (m, 4H); ^{13}C NMR (75 MHz, $CDCl_3$) δ = 161.5, 158.6, 151.2, 150.6, 148.7, 147.6, 139.4, 128.9, 128.4, 127.2, 123.6, 123.1, 122.1, 121.6, 121.5, 120.3, 117.5, 115.7, 57.9, 56.7, 49.9, 49.1, 42.6, 34.6, 34.2, 29.9, 28.4, 25.5, 24.9, 24.8, 23.4, 23.0, 22.9, 22.7; ESI-MS *m/z*: 567 $[M+H]^+$, 295 $[M+H+Na]^{2+}/2$ (100).



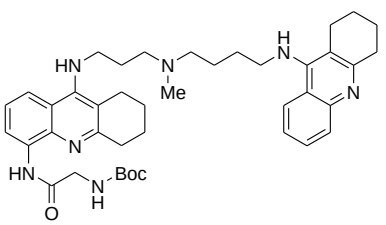
N^1-Methyl-N^1-(3-(5-amino-1,2,3,4-tetrahydroacridin-9-ylamino)propyl)-N^4-(1,2,3,4-tetrahydroacridin-9-yl)butane-1,4-diamine (18a). Starting from **17a**, the title compound was obtained following the same procedure described for **7**. Pure title compound (92% yield) was obtained without any further purification; ^{1}H NMR (300 MHz, $CDCl_3$) δ = 7.93 (t, J = 8.1 Hz, 2H), 7.55 (t, J = 7.7 Hz, 1H), 7.29 (m, 2H), 7.08 (t, J = 7.9 Hz, 1H), 6.75 (d, J = 7.6 Hz, 1H), 4.95 (br s, 2H), 3.50 (m, 4H), 3.04 (m, 4H), 2.69 (m, 2H), 2.61 (m, 2H), 2.47 (t, J = 5.8 Hz, 2H), 2.37 (t, J = 6.6 Hz, 2H), 2.24 (s, 3H), 1.93-1.44 (m, 16H); ^{13}C NMR (75 MHz, $CDCl_3$) δ = 158.7, 156.2, 150.9, 150.7, 147.8, 143.8, 137.9, 129.0, 128.4, 124.2, 123.8, 122.9, 120.8, 120.5, 116.5, 116.3, 111.2, 108.9, 57.9, 56.6, 49.5, 48.5, 42.5, 34.3 (2C), 29.9, 28.6, 25.6, 25.0 (2C), 23.5, 23.2, (2C), 23.0; ESI-MS *m/z* 288 $[M+H+K]^{2+}/2$ (100).



N^1-Methyl-N^4-(5-amino-1,2,3,4-tetrahydroacridin-9-yl)-N^1-(3-(1,2,3,4-tetrahydroacridin-9-ylamino)propyl)butane-1,4-diamine (18b). Starting from **18a**, the title compound was obtained following the same procedure reported for **7**. Pure title compound (95% yield) was obtained without any further purification; ^{1}H NMR (300 MHz, $CDCl_3$) δ = 7.97 (d, J = 8.5 Hz, 1H), 7.90 (d, J = 8.5 Hz, 1H), 7.52 (t, J = 7.6 Hz, 1H), 7.28 (m, 1H), 7.20 (d, J = 8.5 Hz, 1H), 7.09 (t, J = 7.8 Hz, 1H), 6.76 (d, J = 7.3 Hz, 1H), 5.21 (br s, 1H), 4.92 (br s, 2H), 3.96-3.47 (m, 3H), 3.42 (m, 2H), 3.03 (m, 4H), 2.66 (m, 4H), 2.48 (t, J = 6.3 Hz, 2H), 2.37 (m, 2H), 2.24 (s, 3H), 1.99-1.71 (m, 10H), 1.71-1.34 (m, 4H); ^{13}C NMR (75 MHz, $CDCl_3$) δ = 158.5, 156.2, 151.3, 150.6, 147.6, 143.9, 137.9, 128.8, 128.5, 124.6, 123.6, 123.2, 121.0, 120.3, 117.0, 115.6, 111.1, 109.0, 58.1, 56.8, 49.2, 49.1, 42.6, 34.3, 34.1, 29.9, 28.4, 25.4, 25.3, 24.9, 23.4, 23.3, 23.1, 23.0; ESI-MS *m/z*: 269 $[M+2H]^{2+}/2$.

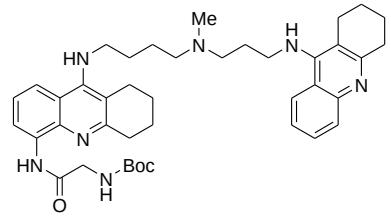


N^1-Methyl-N^1-((5-(2-*tert*-butoxycarbonylaminoacetamido)-1,2,3,4-tetrahydroacridin-9-ylamino)propyl)-N^4-(1,2,3,4-tetrahydroacridin-9-yl)butane-1,4-diamine (19a). Starting from **18a**, the title compound was obtained following the same procedure described for **8**. The crude was purified by means of chromatography on aluminum oxide (ethyl

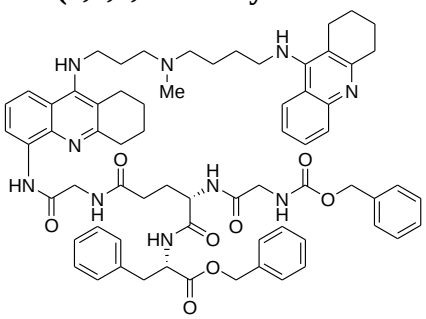


acetate) to afford pure title compound (51% yield) as a yellow oil; ^{1}H NMR (300 MHz, $CDCl_3$) δ = 10.44 (br s, 1H), 8.47 (d, *J* = 7.5 Hz, 1H), 7.89 (dd, *J* = 16.9, 8.3 Hz, 2H), 7.59 (d, *J* = 8.5 Hz, 1H), 7.48 (m, 1H), 7.22 (m, 2H), 5.61 (br s, 1H), 5.24 (br s, 1H), 4.55-3.75 (m, 3H), 3.56 (t, *J* = 6.2 Hz, 2H), 3.45 (t, *J* = 6.9 Hz, 2H), 3.01 (m, 2H), 2.94 (m, 2H), 2.62 (m, 2H), 2.55 (m, 2H), 2.45 (t, *J* = 6.2 Hz, 2H), 2.36 (t, *J* = 6.9 Hz, 2H), 2.22 (s, 3H), 1.98-1.71 (m, 10H), 1.71-1.50 (m, 4H), 1.46 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$) δ = 167.5, 158.8, 156.9, 156.1, 151.3, 150.7, 147.8, 138.0, 133.3, 129.1, 128.4, 123.8, 123.6, 122.9, 120.5, 119.3, 117.5, 116.3, 115.7, 115.2, 80.2, 58.1, 56.8, 49.5, 49.1, 45.4, 42.6, 34.3 (2C), 30.0, 28.6, 28.3, 25.4, 25.0, 24.9, 23.4, 23.2, 23.0, 22.9; ESI-MS *m/z* 694 $[M+H]^+$, 347 $[M+2H]^{2+}/2$ (100).

N^1-Methyl-N^4-(5-(2-tert-butoxycarbonylaminoacetamido)-1,2,3,4-tetrahydroacridin-9-yl)-N^1-(3-(1,2,3,4-tetrahydroacridin-9-yl)propyl)butane-1,4-diamine (19b). Starting from **18b**, the title compound was obtained following the same procedure described for **8**. The crude was purified by means of chromatography on aluminum oxide (ethyl acetate) to afford pure title compound (55% yield) as a yellow oil; ^{1}H NMR (300 MHz, $CDCl_3$) δ = 10.42 (br s, 1H), 8.52 (d, *J* = 7.5 Hz, 1H), 7.99-7.83 (m, 2H), 7.56 (d, *J* = 8.4 Hz, 1H), 7.49 (t, *J* = 7.2 Hz, 1H), 7.23 (m, 2H), 5.51 (br s, 1H), 5.09 (br s, 1H), 4.17-3.91 (m, 3H), 3.60-3.39 (m, 4H), 3.11-2.84 (m, 4H), 2.73-2.50 (m, 4H), 2.46 (t, *J* = 6.3 Hz, 2H), 2.36 (t, *J* = 6.9 Hz, 2H), 2.22 (s, 3H), 1.98-1.70 (m, 10H), 1.70-1.52 (m, 4H), 1.48 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$) δ = 167.7, 158.7, 156.9, 156.1, 151.2, 150.9, 147.7, 137.9, 133.5, 128.9, 128.3, 123.9, 123.5, 123.1, 120.3, 119.7, 117.2, 116.4, 115.7, 115.3, 80.2, 57.9, 56.7, 49.3, 49.0, 45.4, 42.6, 34.3, 34.2, 29.9, 28.6, 28.4, 25.4, 25.0, 24.9, 23.4, 23.2, 23.1, 22.9; ESI-MS *m/z*: 694 $[M+H]^+$, 347 $[M+2H]^{2+}/2$ (100).



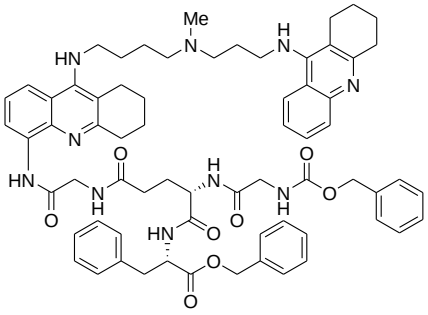
Z-Gly-L-Gln-[N^1-methyl-N^1-((5-(2-acetamide)-1,2,3,4-tetrahydroacridin-9-ylamino)propyl)-N^4-(1,2,3,4-tetrahydroacridin-9-yl)butane-1,4-diamine]-L-Phe-OBn (2b).



N^1-Methyl-N^1-((5-(2-aminoacetamido)-1,2,3,4-tetrahydroacridin-9-ylamino)propyl)-N^4-(1,2,3,4-tetrahydroacridin-9-yl)butane-1,4-diamine tetrahydrochloride. Starting from **19a**, the free amine was obtained following the same procedure described for the preparation of **2a** and was immediately used for the following reaction without any further purification; ESI-MS *m/z* 594 $[M+H]^+$, 297 $[M+2H]^{2+}/2$ (100).

Starting from **12** and the above amine, the title compound was obtained following the procedure described for **2a**. The crude was purified by means of chromatography on aluminum oxide (1:100 MeOH/chloroform) to afford pure title compound (50% yield) as an amorphous white solid, $[\alpha]^{20}_D$: -14.4 (c = 0.01, chloroform); ^{1}H NMR ($CDCl_3$, 300 MHz) δ = 10.22 (br s, 1H), 8.47 (d, *J* = 7.5 Hz, 1H), 8.10 (br d, 1H), 7.89 (dd, *J* = 3.4, 8.4 Hz, 2H), 7.64 (m, 1H), 7.56–7.42 (m, 2H), 7.38–7.00 (m, 18H), 5.61 (br t, 1H), 5.36 (br t, 1H), 5.10 (m, 4H), 4.87 (m, 1H), 4.64 (m, 1H), 4.32 (dd, *J* = 6.2, 16.8 Hz, 1H), 4.18–3.93 (m, 2H), 3.81 (d, *J* = 5.3 Hz, 2H), 3.61 (m, 2H), 3.46 (m, 2H), 3.28–2.80 (m, 6H), 2.69-2.53 (m, 4H), 2.49 (t, *J* = 6.1 Hz, 2H), 2.44–2.28 (m, 4H), 2.25 (s, 3H), 2.15-1.95 (m, 2H), 1.95–1.73 (m, 10H), 1.72-1.49 (m, 4H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ = 173.6, 172.3, 171.4, 169.2, 167.6, 158.4, 157.0, 156.7, 151.3, 151.0, 147.6, 137.9, 136.5, 136.4, 135.3, 133.2, 129.5 (2C), 128.8, 128.7 (2C), 128.6 (2C), 128.3, 128.2, 127.1, 123.9, 123.4, 123.0, 120.3, 120.2, 119.4, 117.7, 116.1, 115.7, 115.5, 67.6, 67.3, 58.1, 56.8, 54.0, 52.5, 49.4, 49.1, 44.6, 44.0, 42.6, 37.8, 34.2, 33.9, 32.4, 29.9, 29.0, 28.3, 25.5, 24.9 (2C), 23.3, 23.1, 22.9 (2C); ESI-MS *m/z*: 576 $[M+2H]^{2+}/2$; HRMS (ESI) *m/z* $[M+H]^+$ calcd for $C_{67}H_{78}N_{10}O_8$, 1151,6077, found 1151,6076, $[M+2H]^+/2$ calcd for $C_{67}H_{78}N_{10}O_8$ 576.3075, found 576,3070 (100).

Z-Gly-L-Gln-[N^1-methyl-N^4-(5-(2-acetamide)-1,2,3,4-tetrahydroacridin-9-yl)-N^1-(3-(1,2,3,4-tetrahydroacridin-9-yl)propyl)butane-1,4-diamine]-L-Phe-OBn (2c).



N^1-Methyl-N^4-(5-(2-aminoacetamido)-1,2,3,4-tetrahydroacridin-9-yl)-N^1-(3-(1,2,3,4-tetrahydroacridin-9-yl)propyl)butane-1,4-diamine tetrahydrohydrochloride. Starting from **19b**, the free amine was obtained following the same procedure described for the preparation of **2a** and was immediately used for the following reaction without any further purification; ESI-MS *m/z*: 297 $[M+2H]^{2+}/2$ (100), 594 $[M+H]^+$.

Starting from **12** and the above amine, the title compound was obtained following the procedure described for **2a**. The crude was purified by means of chromatography on aluminum oxide (1:100 MeOH/chloroform) to afford title compound (45% yield) as an amorphous white solid, $[\alpha]^{20}_D$: -14.7 (c = 0.01, chloroform); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 10.20 (br s, 1H), 8.46 (d, *J* = 7.4 Hz, 1H), 8.07 (br d, 1H), 7.91 (m, 2H), 7.52 (m, 3H), 7.38-7.12 (m, 15H), 7.04 (m, 1H), 6.94 (br d, 1H), 6.59 (br s, 1H),5.68 (br s, 1H), 5.36 (br s, 1H), 5.32-5.13 (m, 4H), 4.92 (m, 1H), 4.65 (m, 1H), 4.32 (m, 1H), 4.01 (m, 2H), 3.82 (m, 2H), 3.55 (m, 4H), 3.19-2.90 (m, 6H), 2.69-2.32 (m 10H), 2.32-1.93 (m, 5H), 1.92-1.72 (m, 10H), 1.72-1.50 (m, 4H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 173.6, 172.3, 171.4, 169.2, 167.7, 158.1, 157.1, 156.7, 151.5, 150.9, 149.0, 137.8, 136.5, 136.3, 136.2, 135.3, 133.3, 129.5, 128.9, 128.8, 128.7 (2C), 128.6, 128.3, 128.2, 127.1, 123.8, 123.6, 123.2, 121.8, 119.9, 119.6, 117.4, 116.5 (2C), 115.6, 67.6, 67.3, 58.0, 56.9, 54.0, 52.5, 49.3, 49.2, 44.6, 44.0, 42.6, 37.8, 34.2, 33.7, 32.5, 30.0, 29.0, 28.2, 25.3, 25.0, 24.9, 23.2 (2C), 22.9 (2C); ESI-MS *m/z*: 576 $[M+2H]^{2+}/2$; HRMS (ESI) *m/z* $[M+H]^+$ calcd for $C_{67}H_{78}N_{10}O_8$, 1151,6077, found 1151,6072, $[M+2H]^+/2$ calcd for $C_{67}H_{78}N_{10}O_8$ 576.3075, found 576,3067 (100).

Determination of the Inhibitory Potency on Aβ$_{1-40}$ Aggregation Induced by *hr*AChE.[3]

Aliquots of 2 µL of Aβ$_{1-40}$ (Bachem AG, Switzerland), lyophilized from 2 mg mL^{-1} HFIP (1,1,1,3,3,3-hexafluoro-2-propanol) and dissolved in dimethylsulfoxide (DMSO) at a final concentration of 230 µM, were incubated for 24 h at rt in 0.215 M sodium phosphate buffer (pH 8.0). For co-incubation experiments aliquots of *hr*AChE (2.30 µM, ratio 100:1) and *hr*AChE in the presence of the tested compound were added. **1a**, **1c** and **2a-b** were screened at a single concentration (100 µM) while for the determination of the IC_{50} value compound **2c** concentrations ranging from 25 µM to 250 µM were used. Blanks containing Aβ alone, *hr*AChE alone, or Aβ plus the tested compound, and *hr*AChE plus the tested compound in 0.215 M sodium phosphate buffer (pH 8.0) were prepared. The final volume of each vial was 20 µL.

To quantify amyloid fibril formation, the thioflavine T (ThT) fluorescence method was used.[4] ThT binds to amyloid fibrils, giving rise to an intense specific emission band at 490 nm in its fluorescent emission spectrum. Therefore, after incubation, the samples were diluted to a final volume of 2 mL with 50 mM glycine-NaOH buffer (pH 8.5) containing 1.5 µM ThT. A 300 s time scan of fluorescence intensity was carried out (λ_{exc} = 446 nm, λ_{em} = 490 nm), and values at the plateau were averaged after subtraction of the background fluorescence of the 1.5 µM ThT solution.

Determination of the Inhibitory Potency on *A*β$_{1-42}$ Self-aggregation (protocol 1).

In order to investigate the Aβ$_{1-42}$ self-aggregation, a ThT based fluorometric assay was performed. HFIP pre-treated Aβ$_{1-42}$ samples (Bachem AG, Switzerland) were resolubilized with a MeCN/Na_2CO_3/NaOH (48.4/48.4/3.2) mixture to have a stable stock solution ([Aβ$_{1-42}$] = 500 μM).[5]Experiments were performed by incubating the peptide in 10 mM phosphate buffer (pH = 8.0) containing 10 mM NaCl, at 30 °C for 24 h (final Aβ concentration = 50 μM) with and without inhibitor. The inhibitor was dissolved in methanol and diluted in the assay buffer. Blanks containing inhibitor and ThT were also prepared and evaluated to account for quenching and fluorescence properties. In order to determine the IC_{50} values at least five different inhibitor concentrations were tested in duplicate. To quantify amyloid fibril formation, the ThT fluorescence method was used.[4] After incubation, samples were diluted to a final volume of 2.0 mL with 50 mM glycine-NaOH buffer (pH 8.5) containing 1.5 μM ThT. A 300-seconds-time scan of fluorescence intensity was carried out (λ_{exc} = 446 nm; λ_{em} = 490 nm), and values at plateau were averaged after subtracting the background fluorescence of 1.5 μM ThT solution. The fluorescence intensities were compared and the % inhibition was calculated.

Determination of the Inhibitory Potency on *A*β$_{1-42}$ Self-aggregation (protocol 2).

A stock solution of Aβ$_{1-42}$ (2 mg/mL dissolved in DMSO) was prepared. 100 mg (50 mL) of Aβ$_{1-42}$ was dried and reconstituted in 1 mL of PBS. Each well contained 20 mg of Aβ$_{1-42}$ and 5 μM concentration of compound **2a**. Total volume of the reaction mixture was 200 mL.

Aβ$_{1-42}$ fibrillization was carried out by stirring the samples with 150 rpm on a stirring plate at room temperature. Aβ$_{1-42}$ fibrillization reading (ThT fluorescence) after fibrillization was 528 nm and that of un-fibrillized Aβ$_{1-42}$ was 75 nm (standard control). The fluorescence intensities were compared and the % inhibition was calculated.

Determination of the Inhibitory Effect on Aβ$_{1-42}$ oligomerization process.

0.5 mg of Aβ$_{1-42}$ (Anaspec, San Jose, CA, USA) were dissolved in 40 μL of 1% NH_4OH to obtain a clear solution and then in 20 mM phosphate buffer (pH 7.4). The solution was divided in aliquots, freeze-dried and stored at −20 °C. In vitro aggregation kinetics of Aβ peptides and in general of amyloidogenic proteins may be influenced by the presence of organic solvents, including EtOH.[6] Therefore, before the analysis, each aliquot was redissolved in 20 mM phosphate buffer, pH 7.4 (Figure 4SI) or in buffer containing 15% EtOH (control Aβ$_{1-42}$ at t_0, Figure 4A of the main text), to obtain a 100 μM concentration. Stock solutions of **2a** (500 μM) were prepared in 15% aqueous EtOH and diluted in the same solvent to obtain 200 and 50 μM concentrations. All solutions were kept in the dark and stored at −20 °C. When co-incubation studies were carried out, the lyophilized peptide was resuspended in an appropriately diluted **2a** solution, so as to keep the peptide concentration at 100 μM and obtain different peptide/compound ratios. All the samples were sonicated with an ultrasonic bath for 3 min and then centrifuged at 3326 g for 20 min (4 °C). The supernatant was immediately injected to obtain the electropherogram at $t=t_0$. It was then kept at rt and injected at different elapsed times (Figures 4 A-C of the main text). The CE method was described previously.[2a] The electrophoretic migration of **2a** does not interfere in the time window where the peptide oligomeric species are detected (data not shown). After sample precipitation, TEM experiments were carried out (Figures 4D-F of the main text), according to previous report.[2b]

Computational Details

All calculations performed in this work were carried out on Cooler Master Centurion 5 (Intel Core i5–2500 CPU @ 3.30 GHz Quad) with Ubuntu 10.04 LTS (long-term support) operating system running Maestro 9.2 (Schrödinger, LLC, New York, NY, 2011).

a) Ligand preparation

Three-dimensional structure building for all compounds in this study was carried out by means of Maestro.[7] Molecular energy minimizations were performed in MacroModel using the Optimized Potentials for Liquid Simulations-all atom (OPLS-AA) force field 2005.[8] The solvent effects are simulated using the analytical Generalized-Born/Surface-Area (GB/SA) model,[9] and no cutoff for

nonbonded interactions was selected. Polak-Ribiere conjugate gradient (PRCG)[10] method with 1000 maximum iterations and 0.001 gradient convergence threshold was employed. All compounds reported in this paper were treated by LigPrep application,[11] implemented in Maestro suite 2011, generating the most probable ionization state of any possible enantiomers and tautomers at cellular pH value (7 ± 2).

b) Protein preparation

The three-dimensional structure of the *Tc*AChE enzyme was taken from PDB entry 2CEK.[12] The protein was imported into Schrödinger Maestro molecular modeling environment[7] and the molecules of water were removed and the resulting structure was submitted to protein preparation wizard implemented in Maestro suite 2011 (Protein Preparation Wizard workflow 2011; http://www.schrodinger.com/supportdocs/18/16). This protocol allowed us to obtain a reasonable starting structure of protein for molecular docking calculations by a series of computational steps. In particular, we performed three steps to (1) add hydrogens, (2) optimize the orientation of hydroxyl groups, Asn, and Gln, and the protonation state of His, and (3) perform a constrained refinement with the impref utility, setting the max RMSD of 0.30. The impref utility consists of a cycles of energy minimization based on the impact molecular mechanics engine and on the OPLS_2005 force field.[8b,8c]

c) Induced Fit Docking (IFD)

Molecular docking was carried out using the Schrödinger suite 2011[13] by applying the IFD protocol.[14] This procedure induces conformational changes in the binding site to accommodate the ligand and exhaustively identify possible binding modes and associated conformational changes by side-chain sampling and backbone minimization. The protein and the ligands used were prepared as reported in the previous paragraphs. The box for docking calculation was built taking into account the centroid of the co-crystallized ligand with default setting. IFD includes protein side-chain flexibility in a radius of 5.0 Å around the poses found during the initial docking stage of the IFD protocol. Complexes within 30.0 kcal/mol of minimum energy structure were taken forward for redocking. The Glide redocking stage was performed by XP (Extra Precision) methods. The calculations were performed using default IFD protocol parameters. No hydrogen bonding or other constraints were used.

d) *Tc*AChE/Aβ docking

Protein-protein docking calculation was performed by means of HADDOCK.[15] The developed protocol makes use of biochemical and/or biophysical interaction data such as chemical shift perturbation data, mutagenesis data, or bioinformatic predictions and thus, incorporates structural knowledge of the target to drive the docking procedure.[15] Moreover, HADDOCK introduces the possibility to drive the selection step by identified data about the interacting regions of the proteins. This information is introduced as ambiguous interaction restraints (AIRs). The docking is then driven by a force that pulls the selected regions together. The calculation was performed with default parameters using the web server version of HADDOCK.[16] For *Tc*AChE 2CEK without ligand and water molecules was used after protein preparation wizard treatment (as reported in protein preparation paragraph). While for Aβ the information was extracted from pdb file 1IYT (also the peptide was prepared as reported in protein preparation paragraph). The potential residues of *Tc*AChE and Aβ domains involved in the protein-protein interaction were defined according to the studies performed by De Ferrari and colleagues.[17] In particular, for *Tc*AChE the active residues for docking calculation are: P232, V236, R289, P283, S307, N310. Concerning Aβ interacting residues, the active residues involved in the docking calculation are: H6, Y10, V24, G25, I32. After specification of active regions of the proteins, passive residues were automatically defined around the active sites. Docking protocol consists of three steps: 1) rigid-body energy minimization, 2) a semi-flexible refinement by simulated annealing in torsional angle space, 3) a finishing refinement of each complex in explicit solvent (water) in order to improve the reliability of the model. After execution of these steps, the docked conformations are scored and ranked by the scoring function to facilitate the selection of the best conformations. HADDOCK score takes into account the weighted

sum of van der Waals, electrostatic, desolvation and restraint violation energies together with buried surface area. In this study the complex was chosen considering higher score derived from HADDOCK coupled to a visual inspection to find the right orientation taking into account the model obtained by De Ferrari and colleagues.[17] Finally, the complex was minimized using OPLS_2005 as force field,[8b,8c] GB/SA model[9] for simulating the solvent effects and no cutoff for non-bonded interactions. PRCG method with 100,000 maximum iterations and 0.001 gradient convergence thresholds was employed. The output of this computational step is reported in Figure 3 of the main text.

Elemental Analysis Data

Compound	Formula		Calcd			Found	
		C	H	N	C	H	N
6	$C_{34}H_{41}N_5O_2$	74.02	7.49	12.69	74.28	7.76	12.53
7	$C_{34}H_{43}N_5$	78.27	8.31	13.42	78.44	8.15	13.61

References

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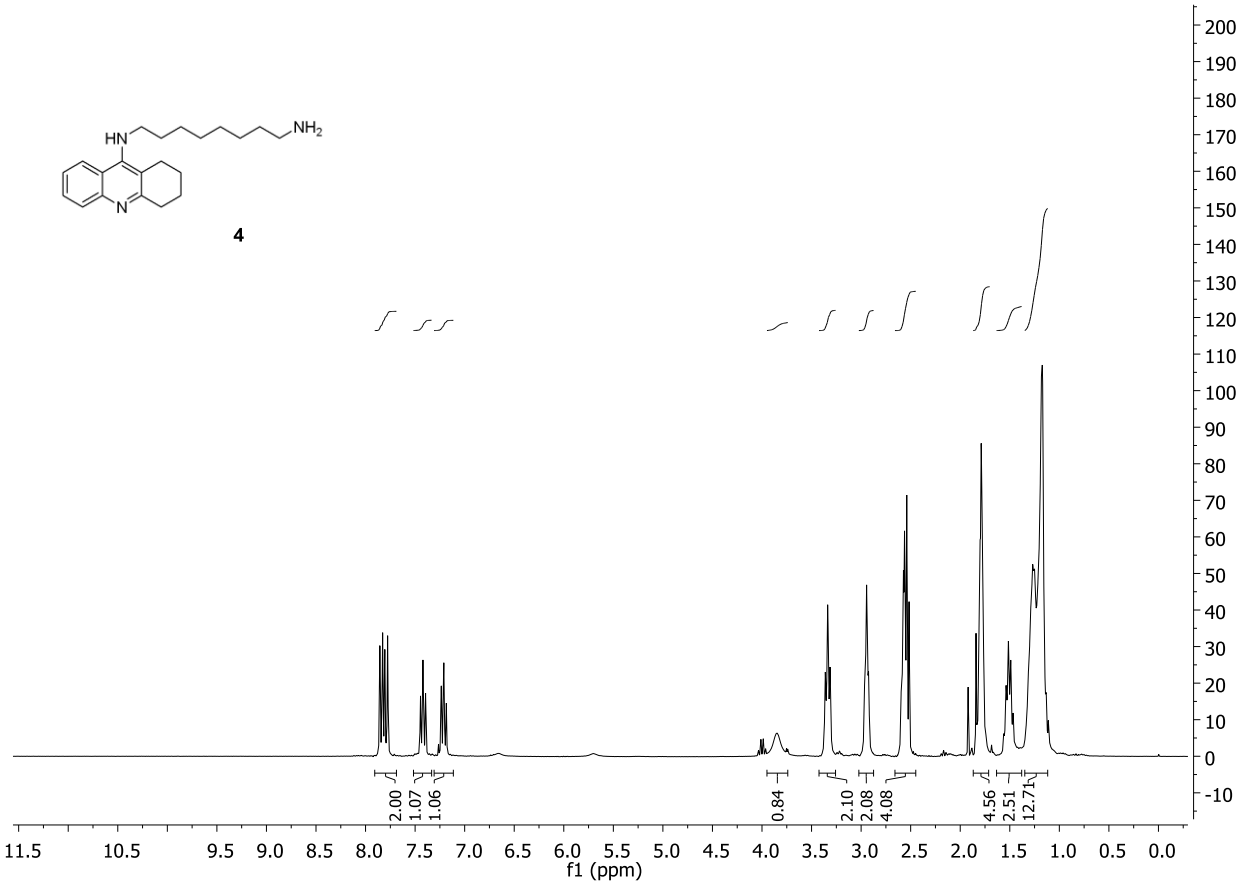
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2.10
2.08
4.08
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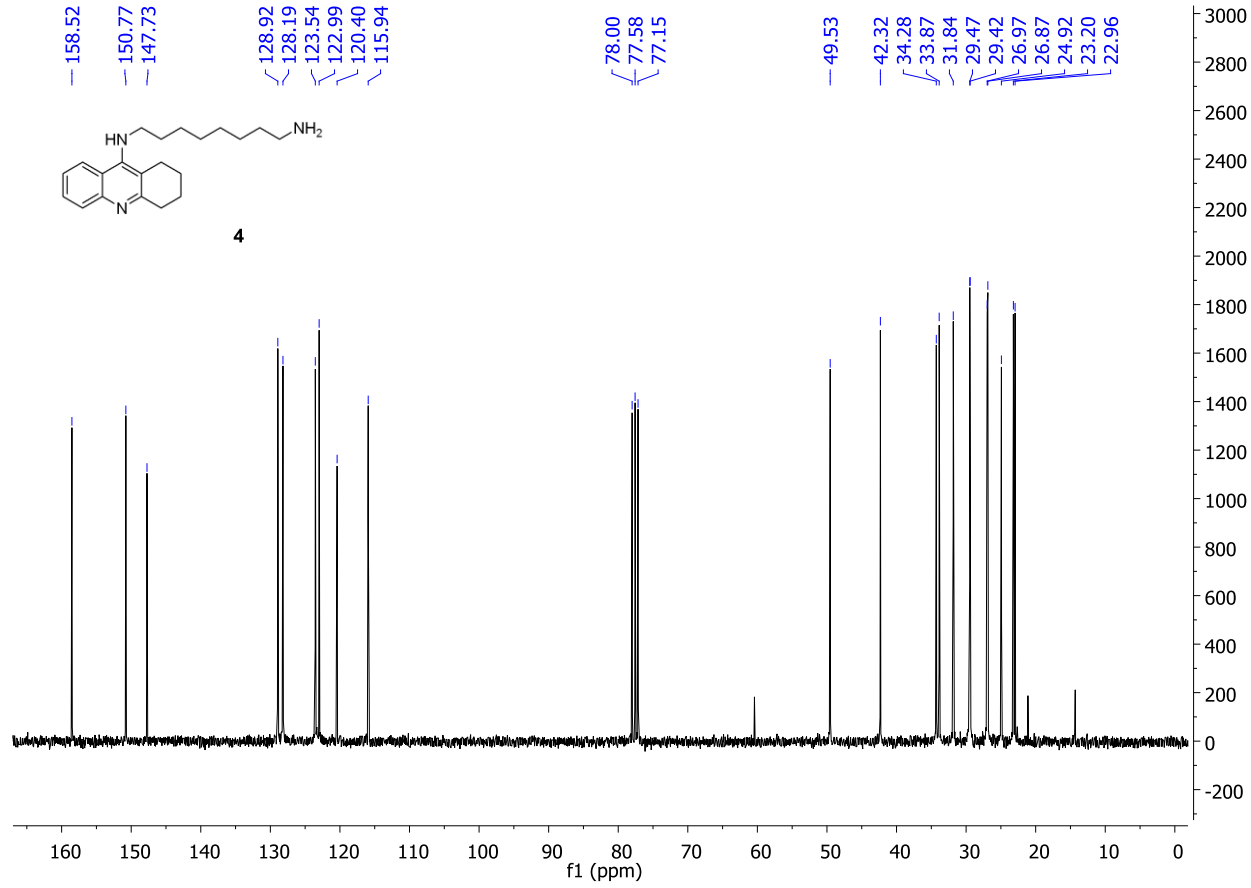
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77.58
77.15
49.53
42.32
34.28
33.87
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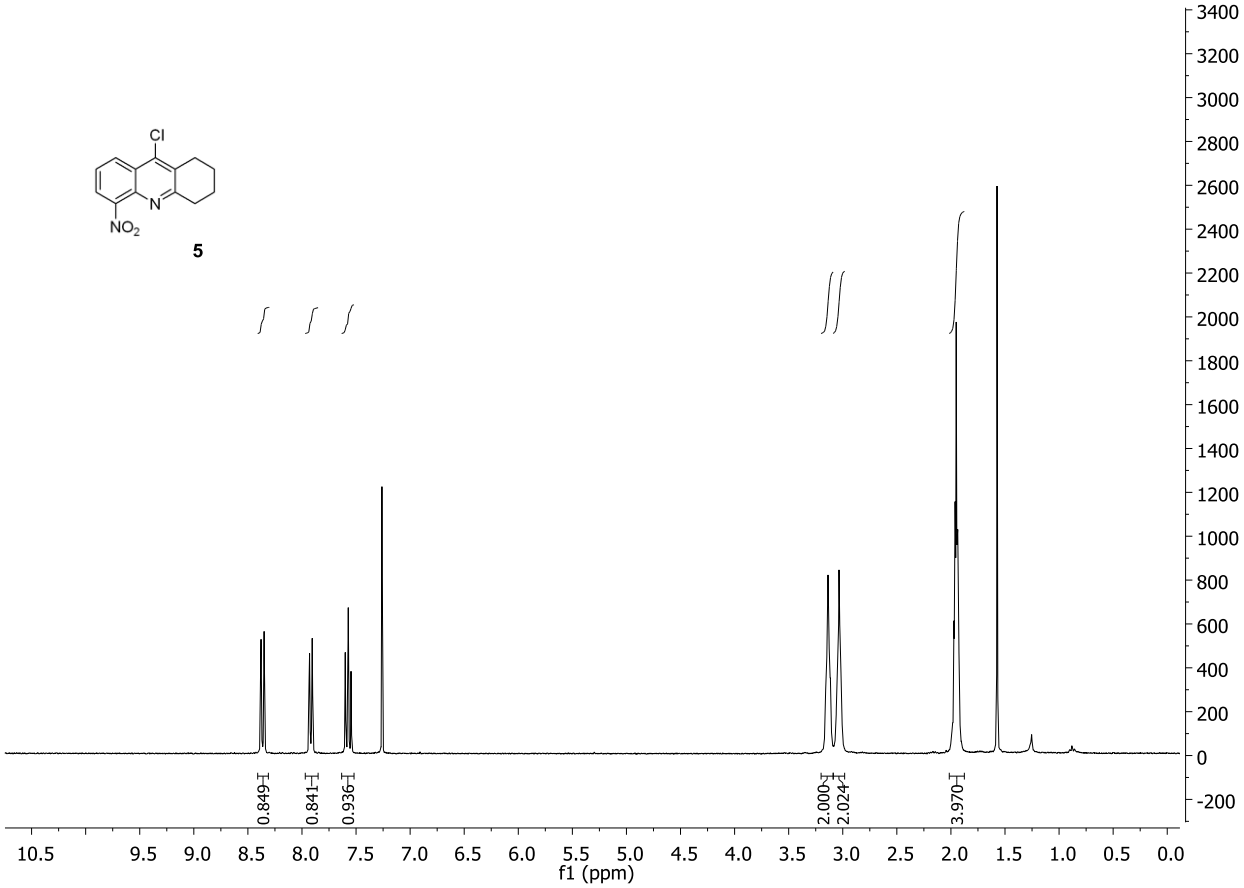
4
f1 (ppm)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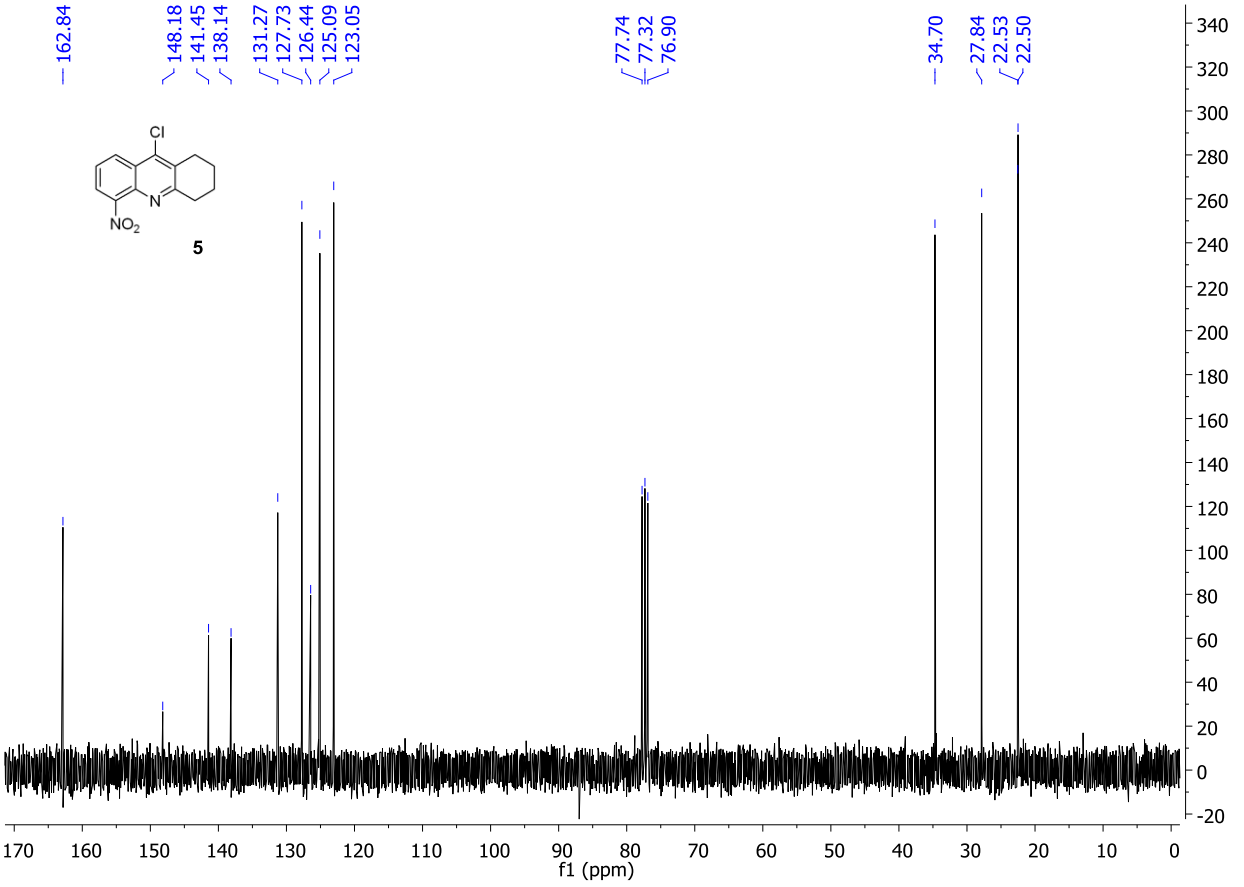
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22.53
22.50
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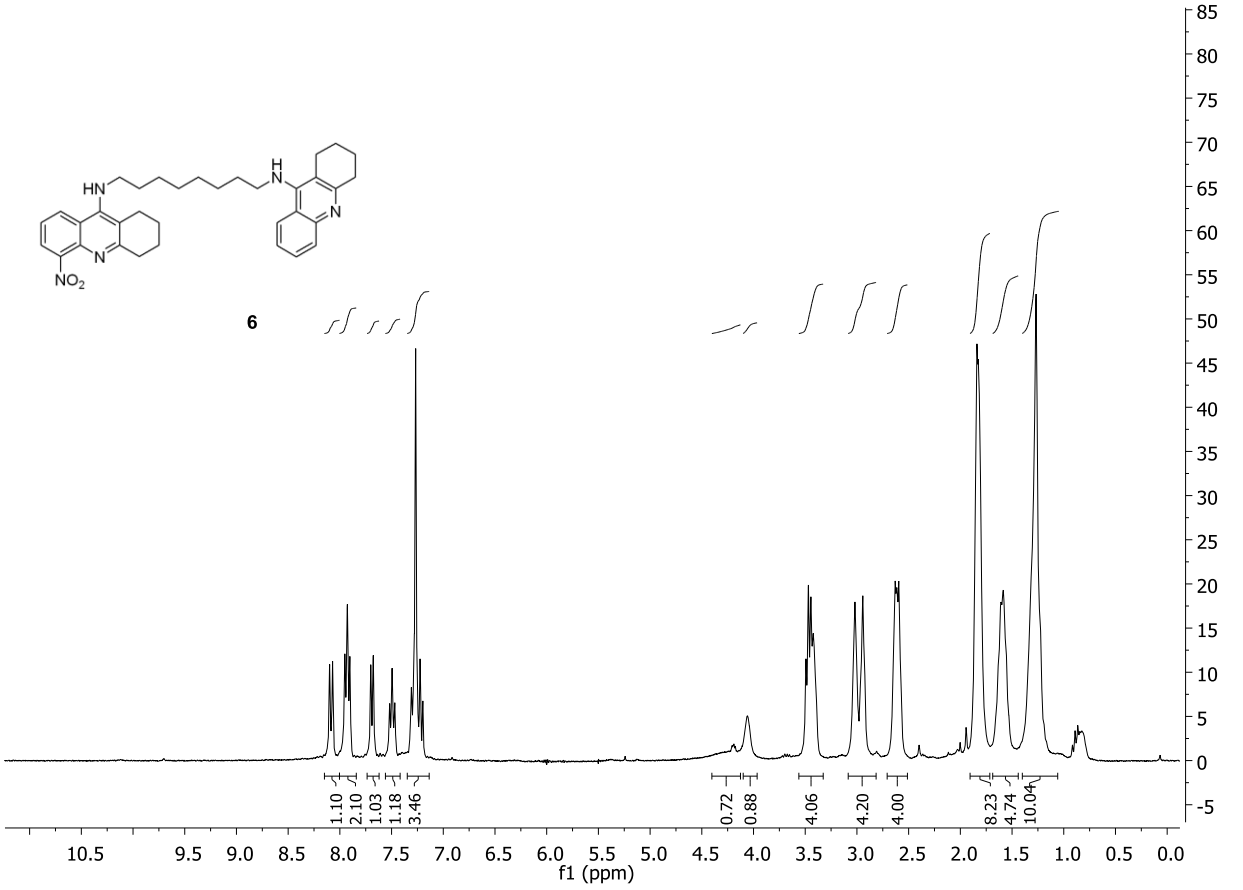
NO2
5
f1 (ppm)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H
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N
NO2
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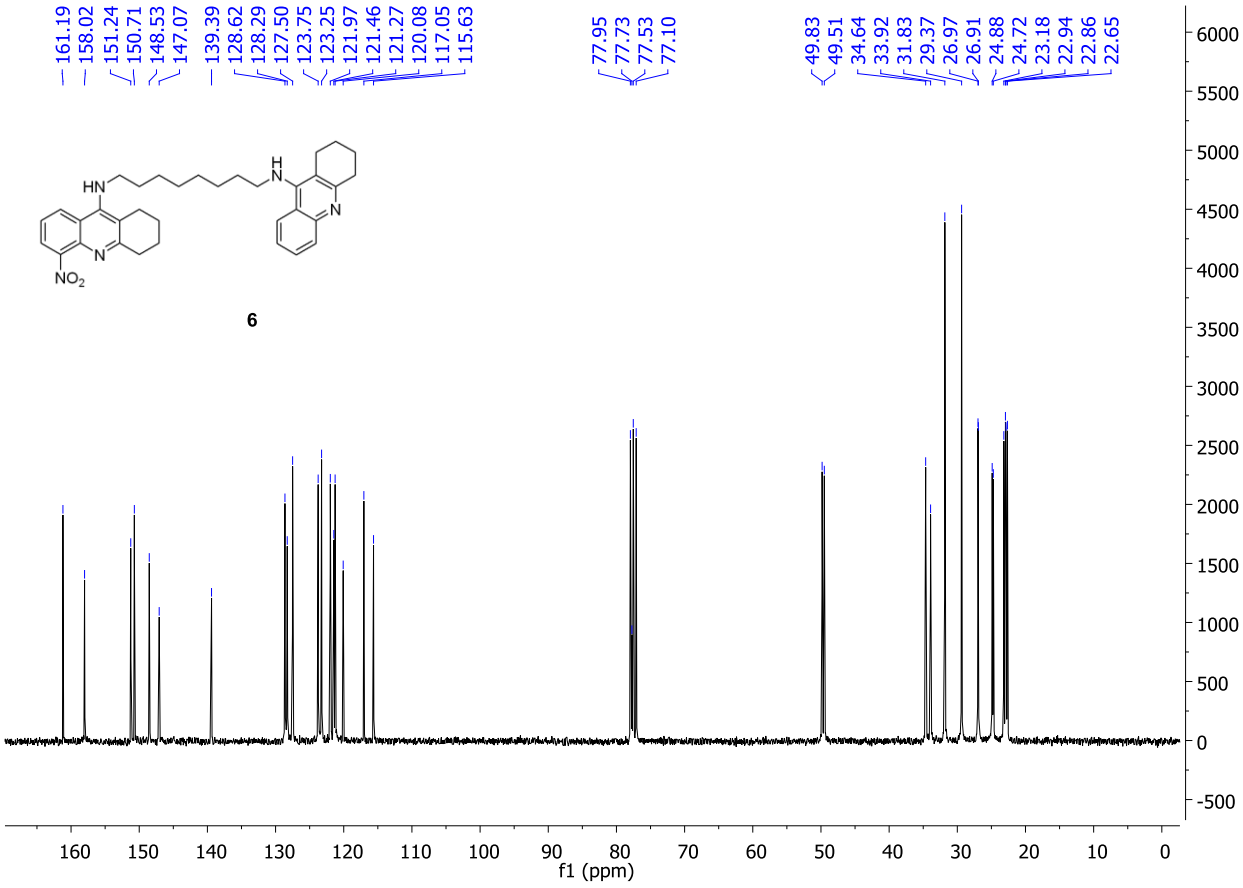
f1 (ppm)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121.27
120.08
117.05
115.63
77.95
77.73
77.53
77.10
49.83
49.51
34.64
33.92
31.83
29.37
26.97
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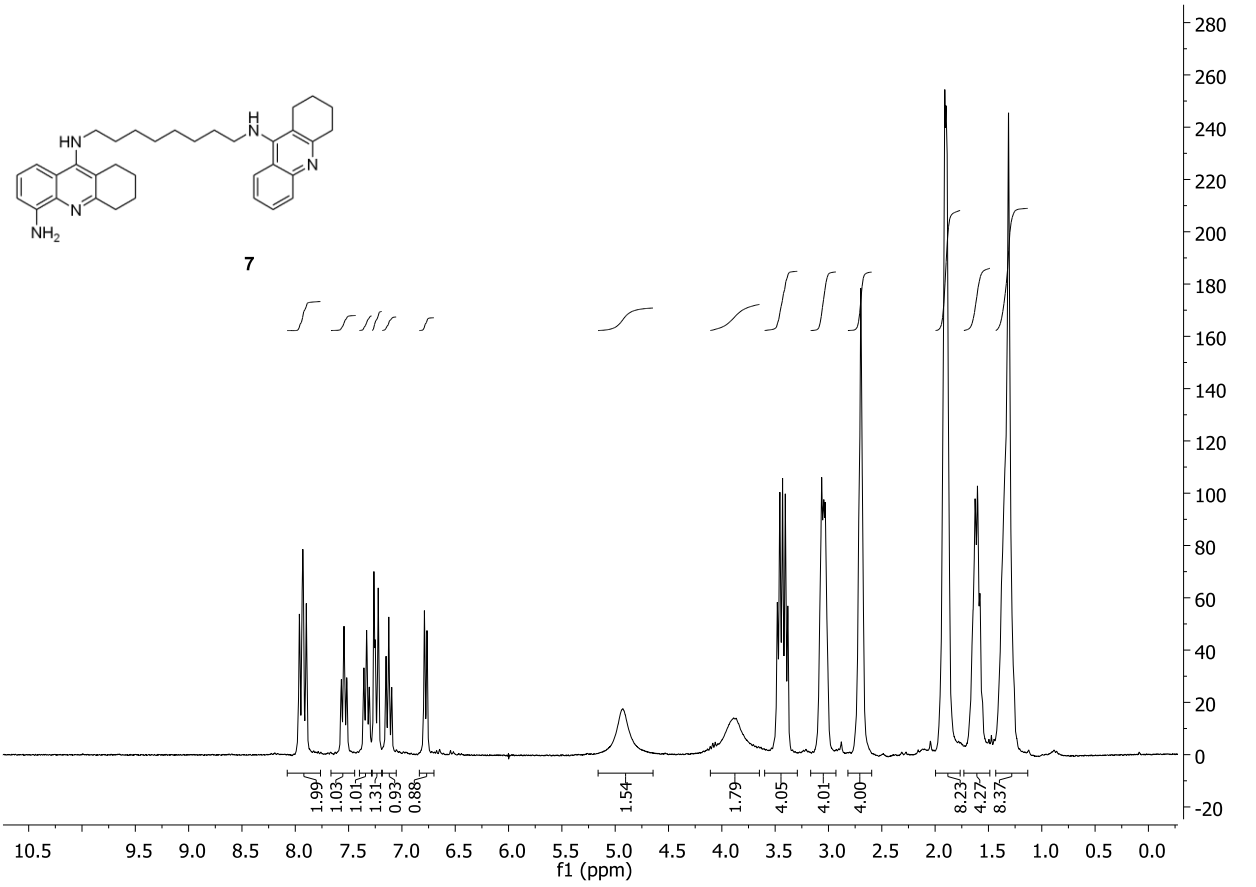
H
N
N
NO2
6
f1 (ppm)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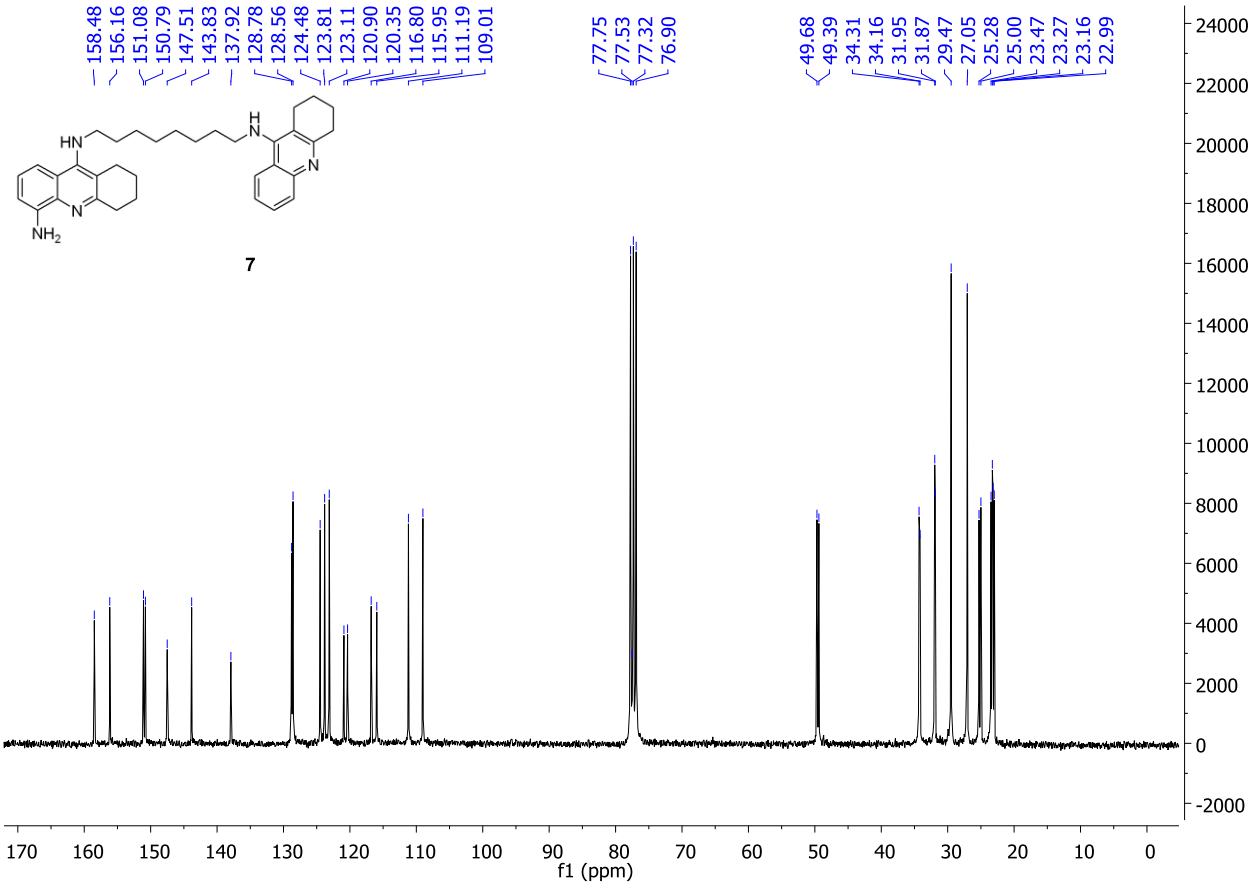
f1 (ppm)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120.35
116.80
115.95
111.19
109.01
77.75
77.53
77.32
76.90
49.68
49.39
34.31
34.16
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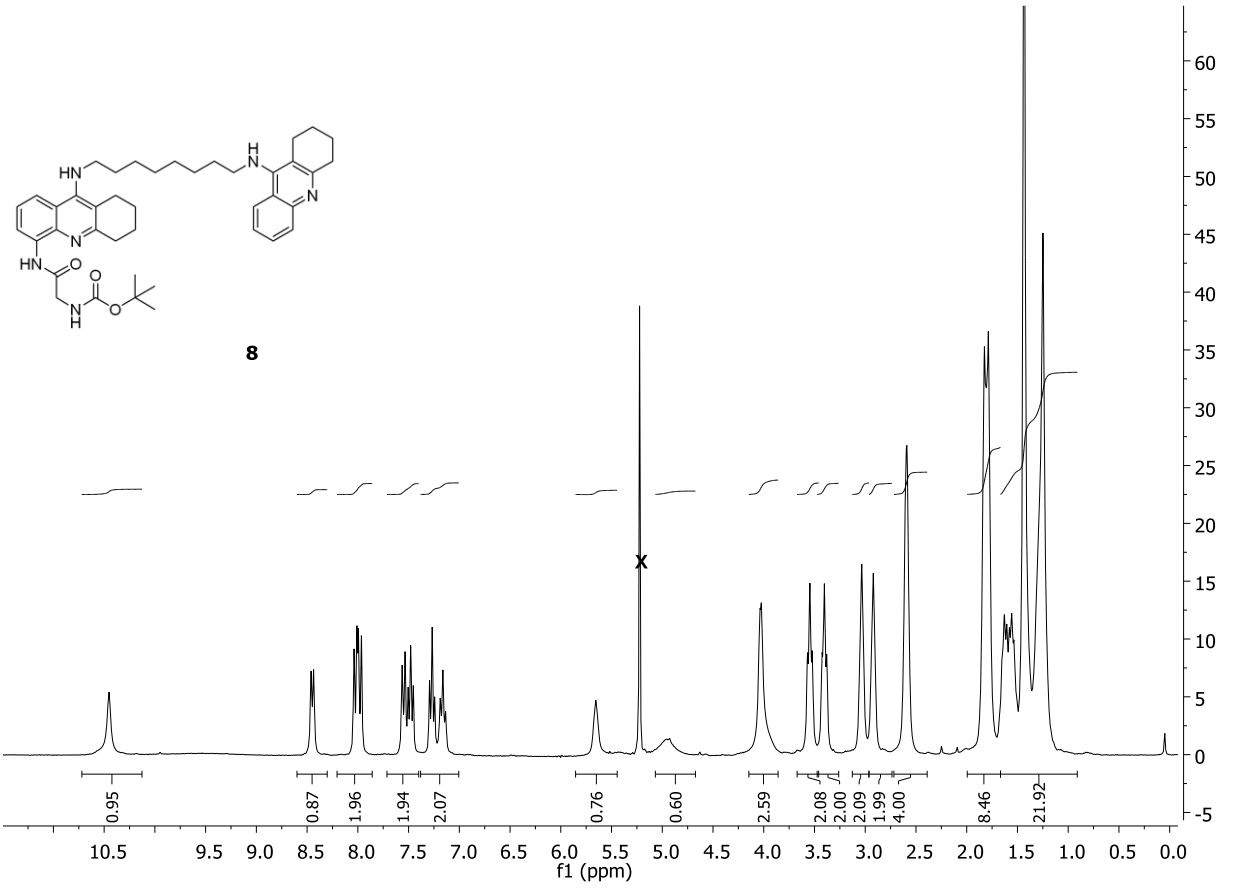
23.47
23.27
23.16
22.99
7
f1 (ppm)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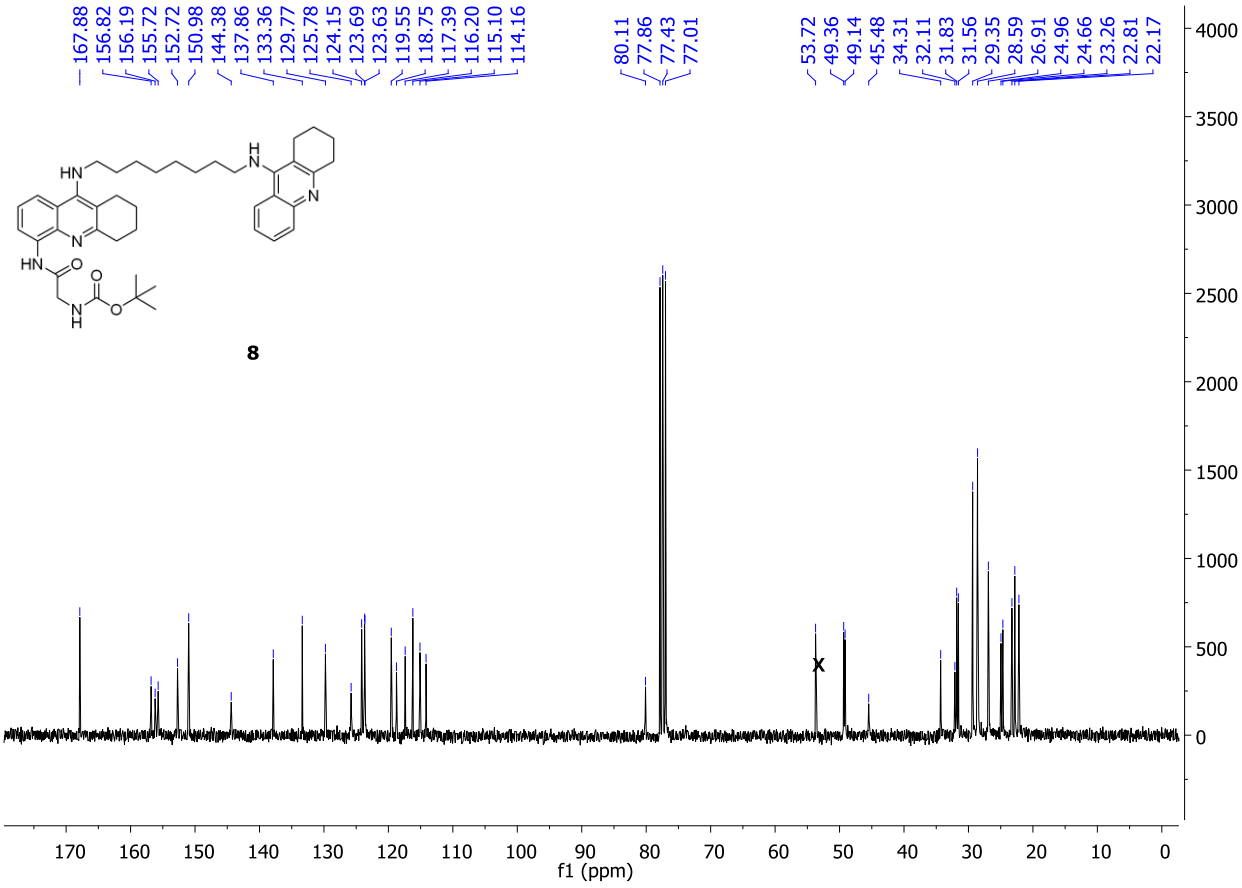
2.08
2.00
2.09
1.99
4.00
8.46
21.92
f1 (ppm)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123.69
123.63
119.55
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117.39
116.20
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77.43
77.01
53.72
49.36
49.14
45.48
34.31
32.11
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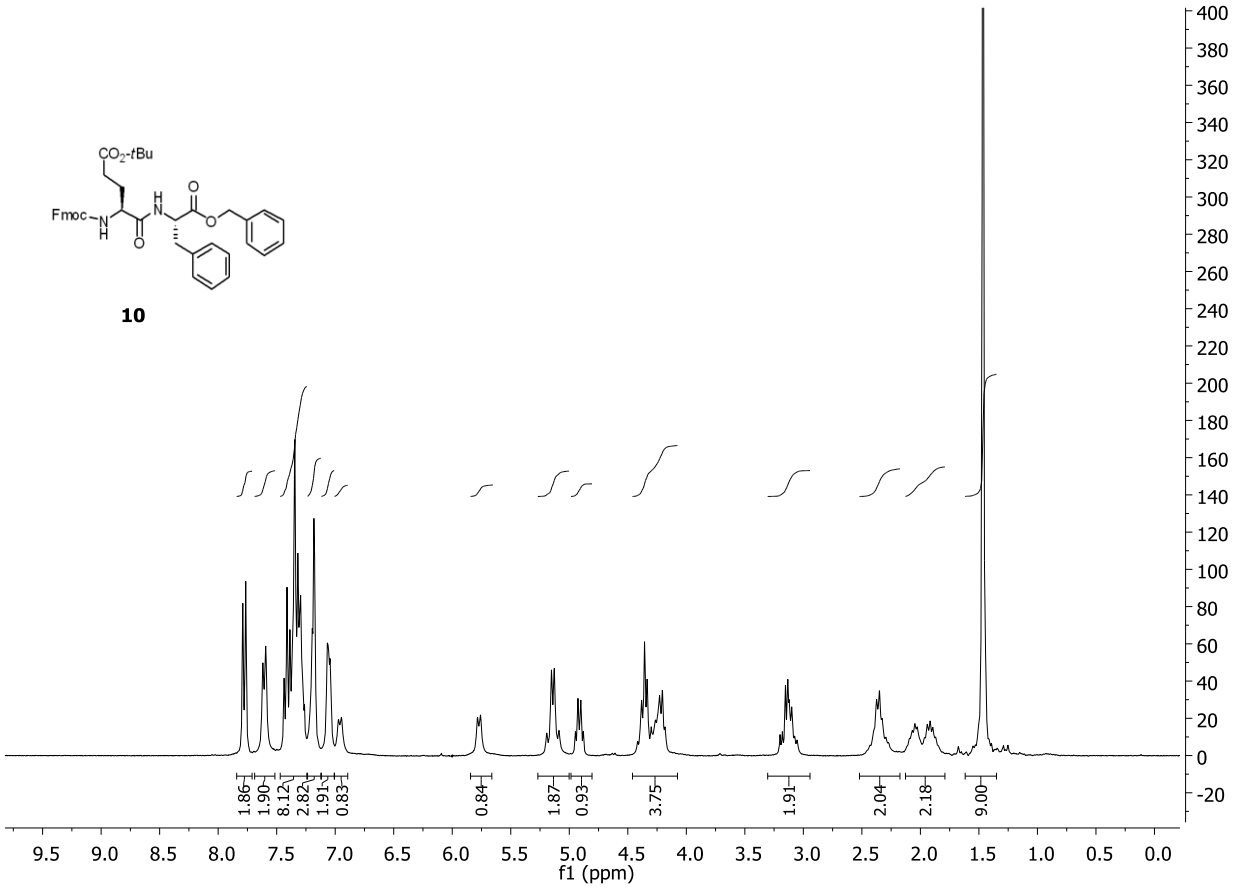
8
f1 (ppm)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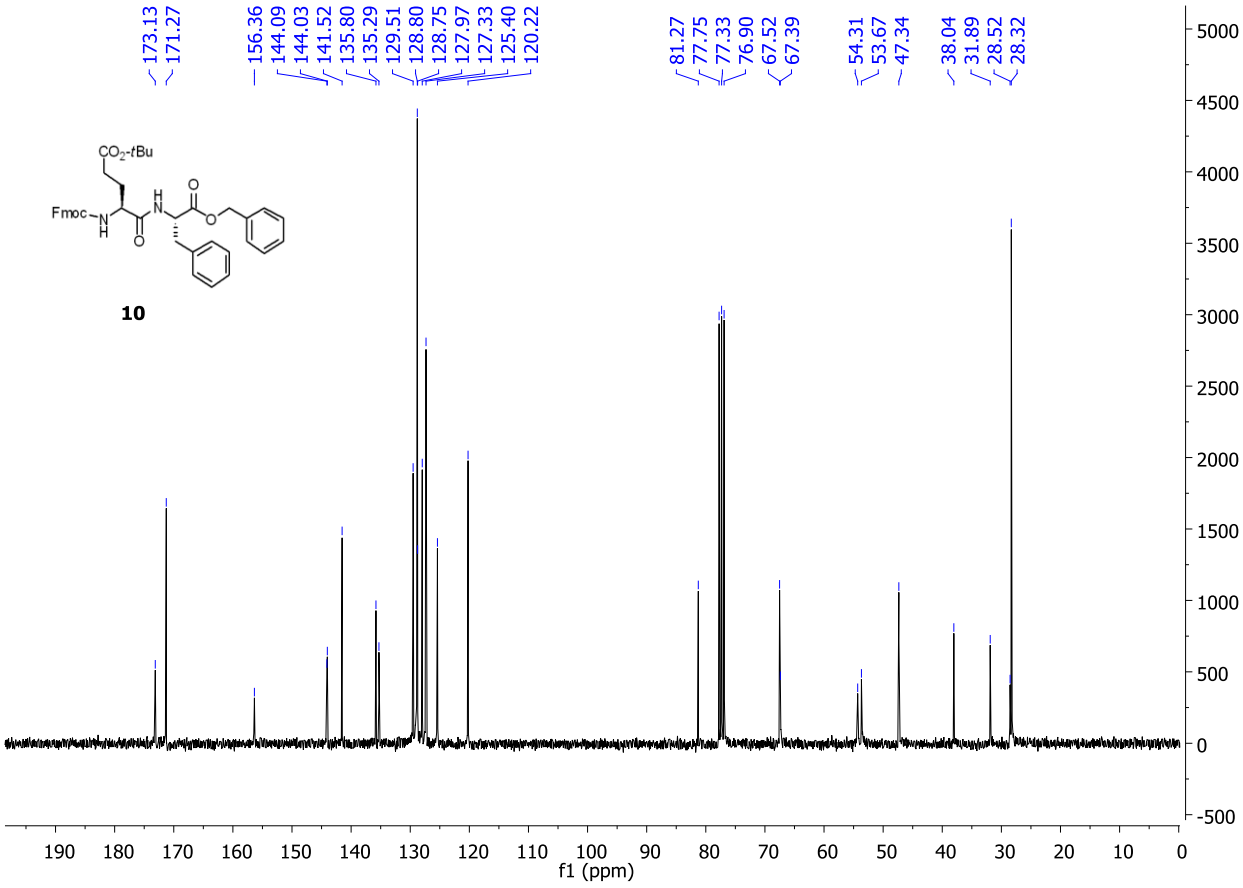
f1 (ppm)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127.33
125.40
120.22
81.27
77.75
77.33
76.90
67.52
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38.04
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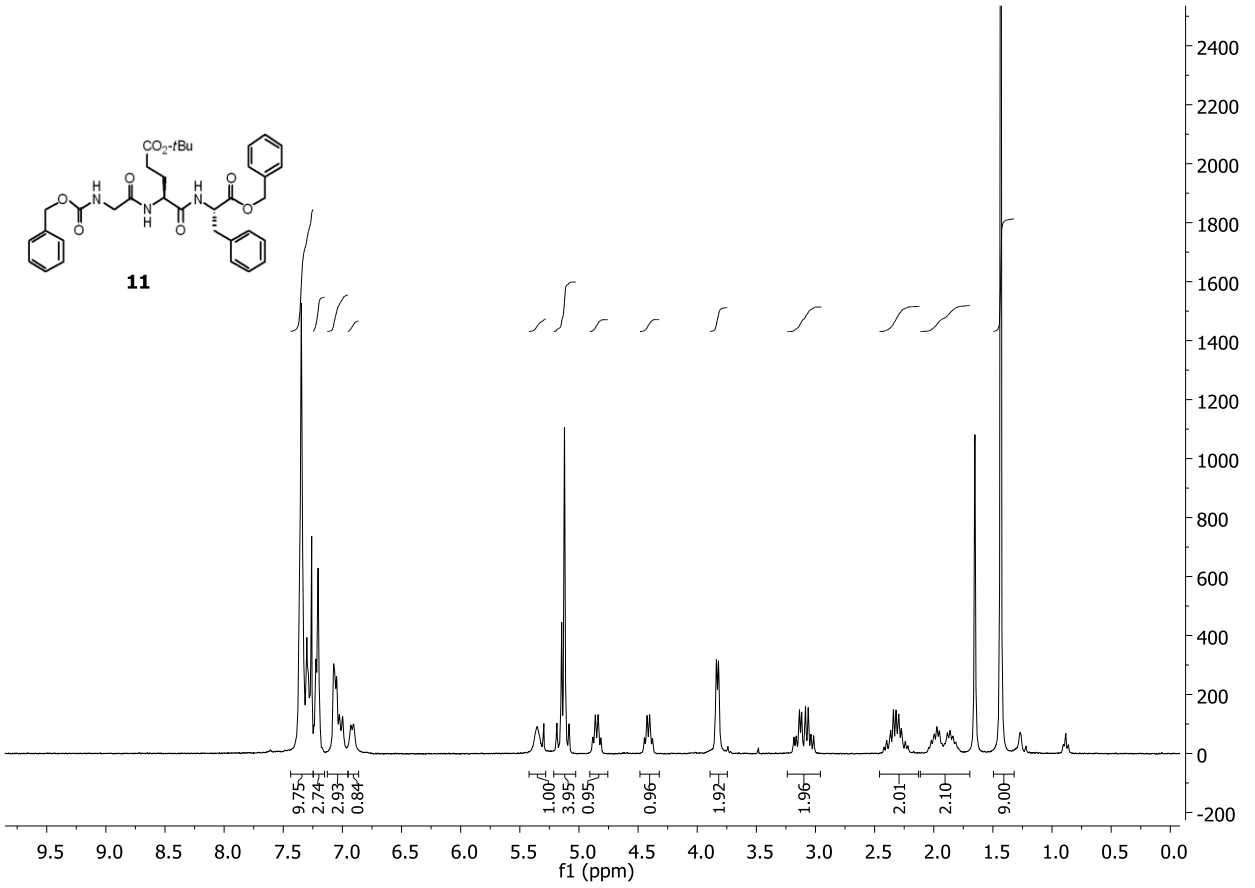
28.32
CO2-tBu
Fmoc
10
f1 (ppm)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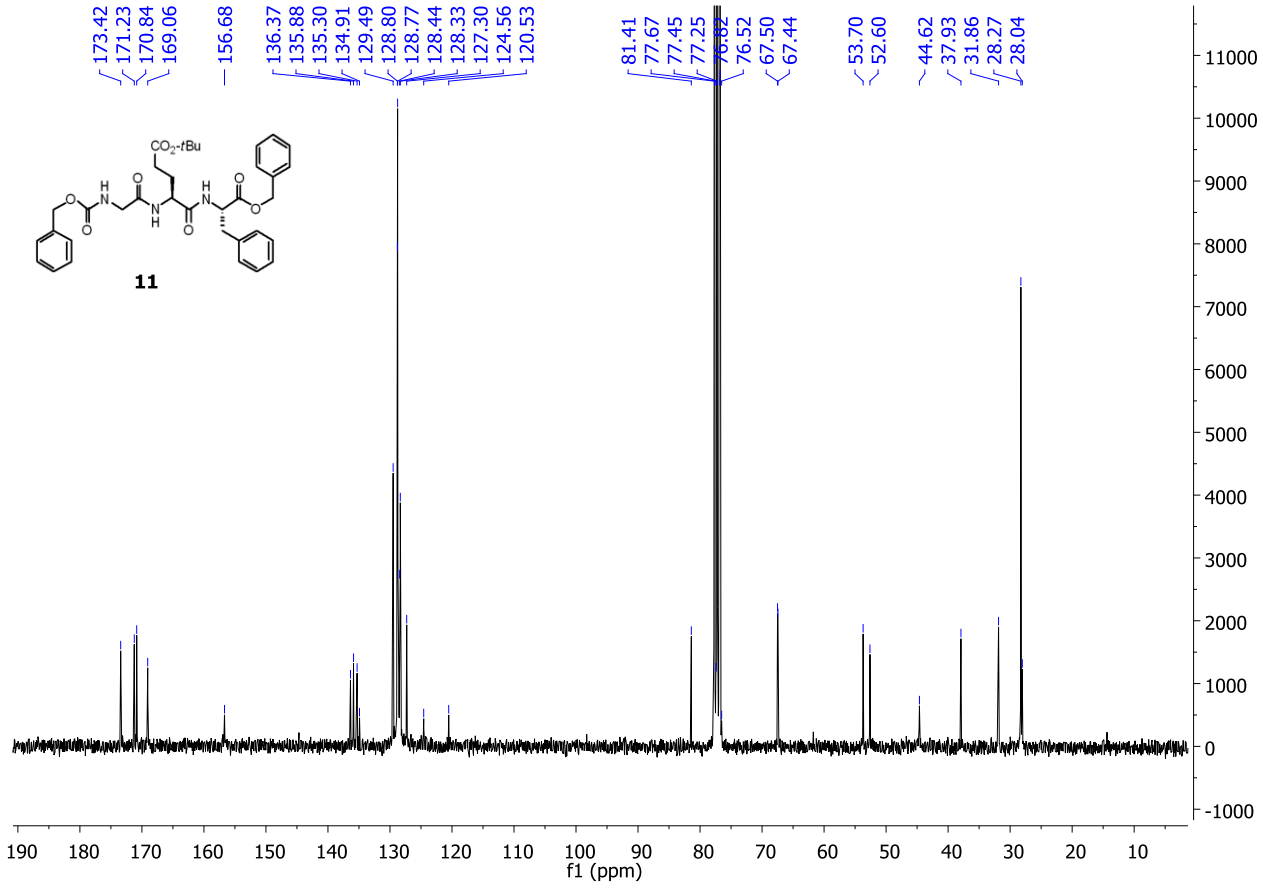
9.00
f1 (ppm)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128.33
127.30
124.56
120.53
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77.45
77.25
76.82
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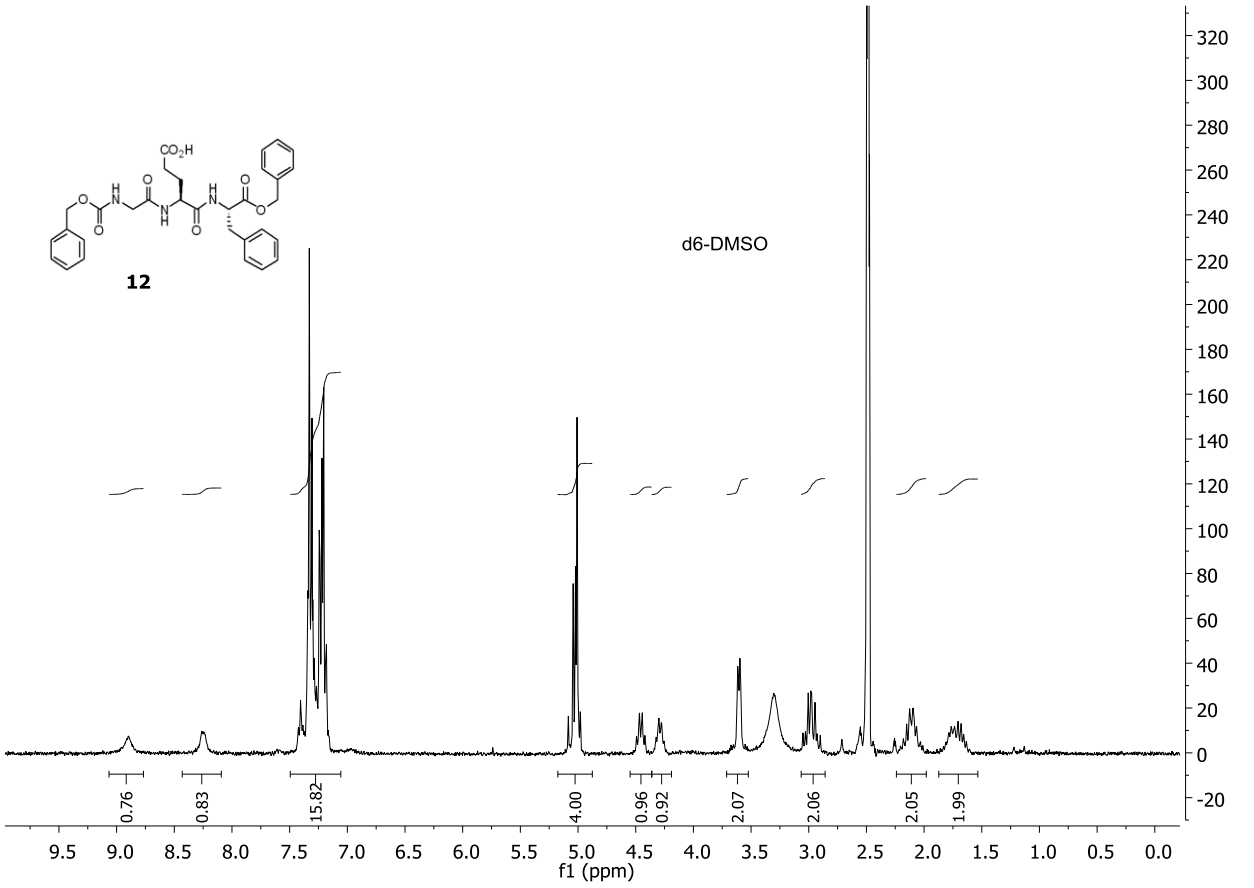
28.04
11
f1 (ppm)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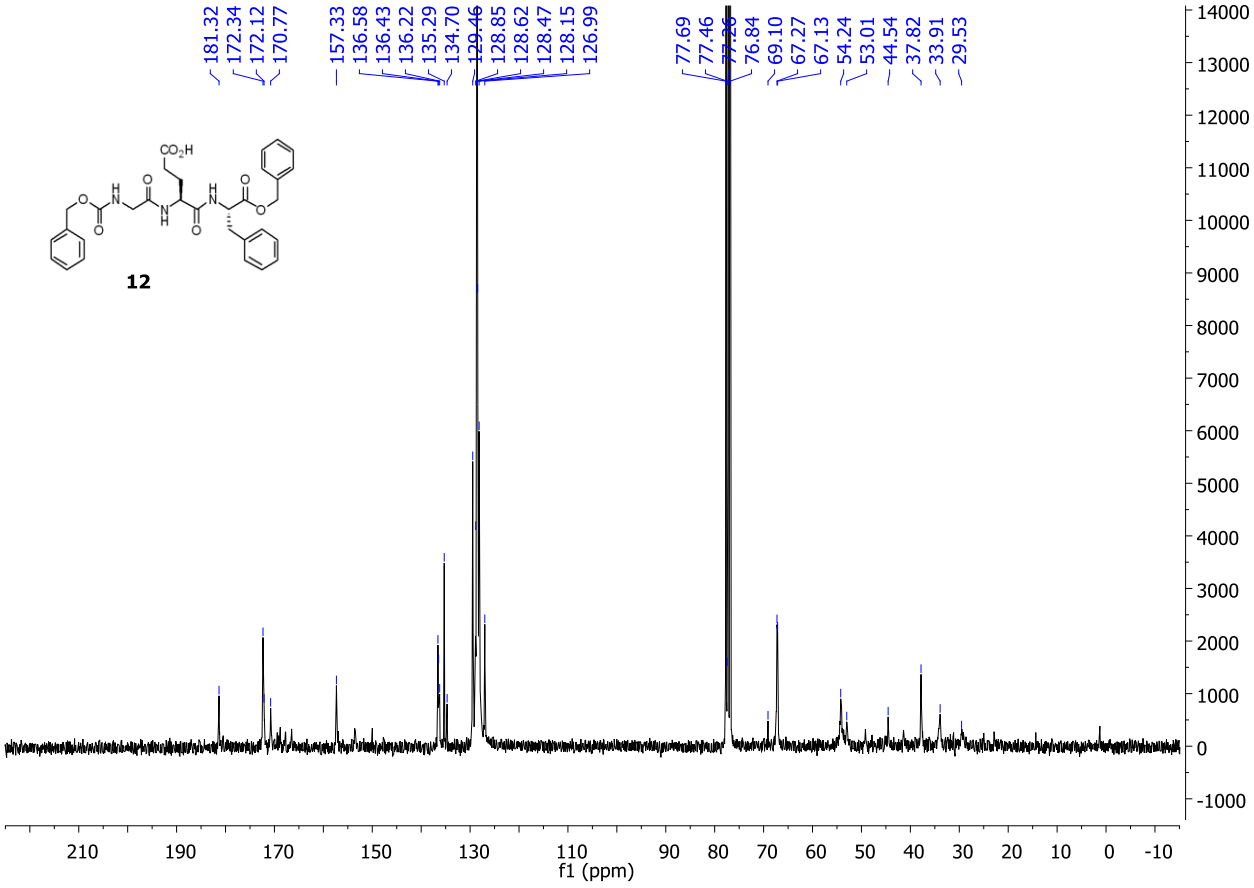
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128.15
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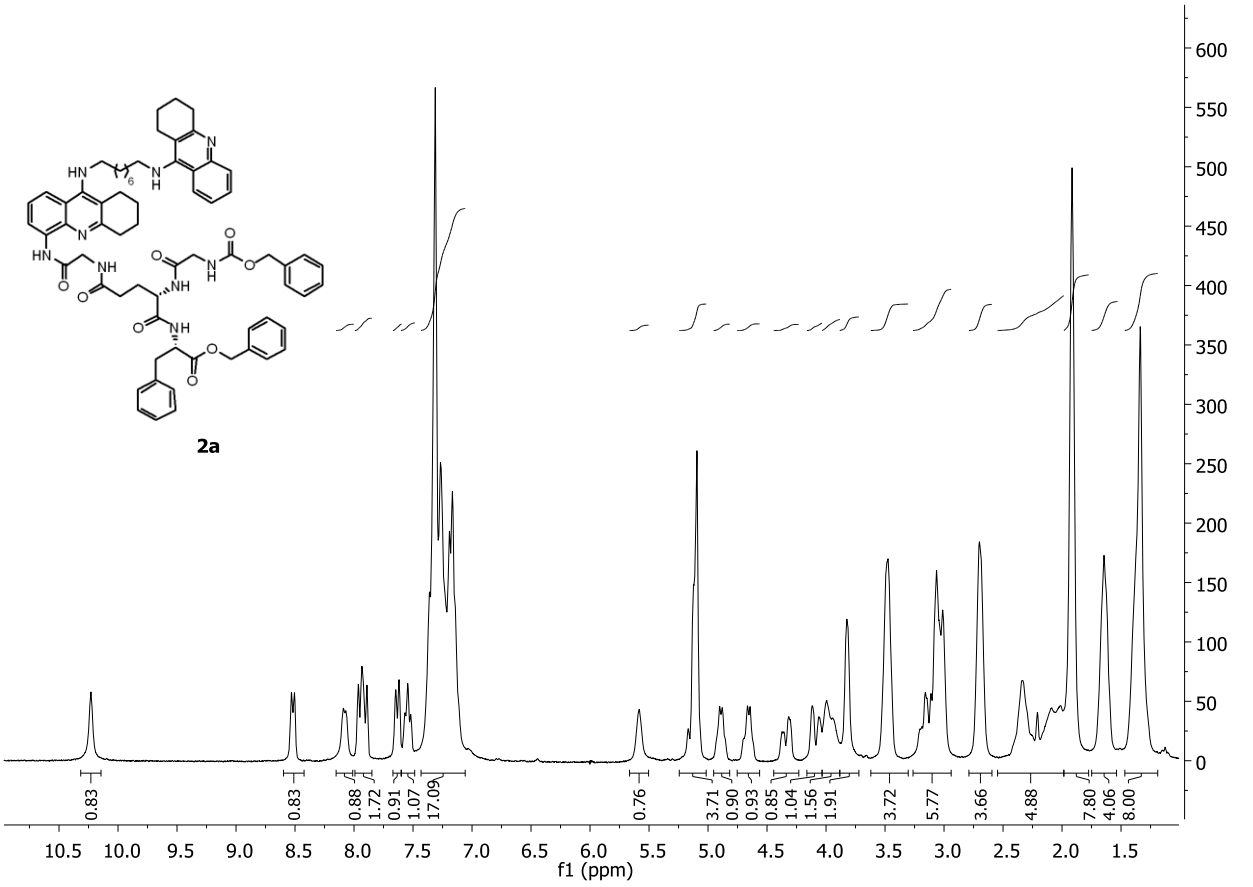
f1 (ppm)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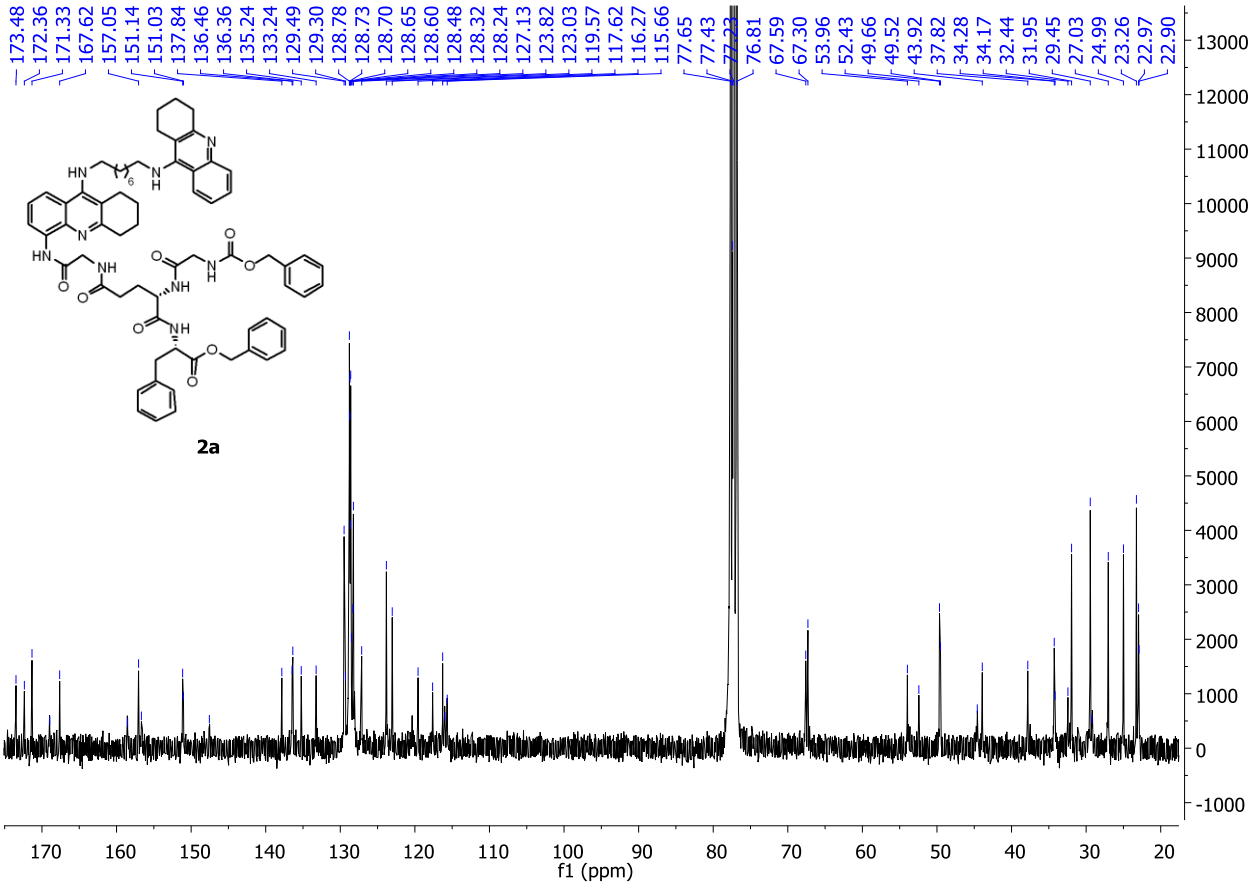
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128.60
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128.24
127.13
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77.23
76.81
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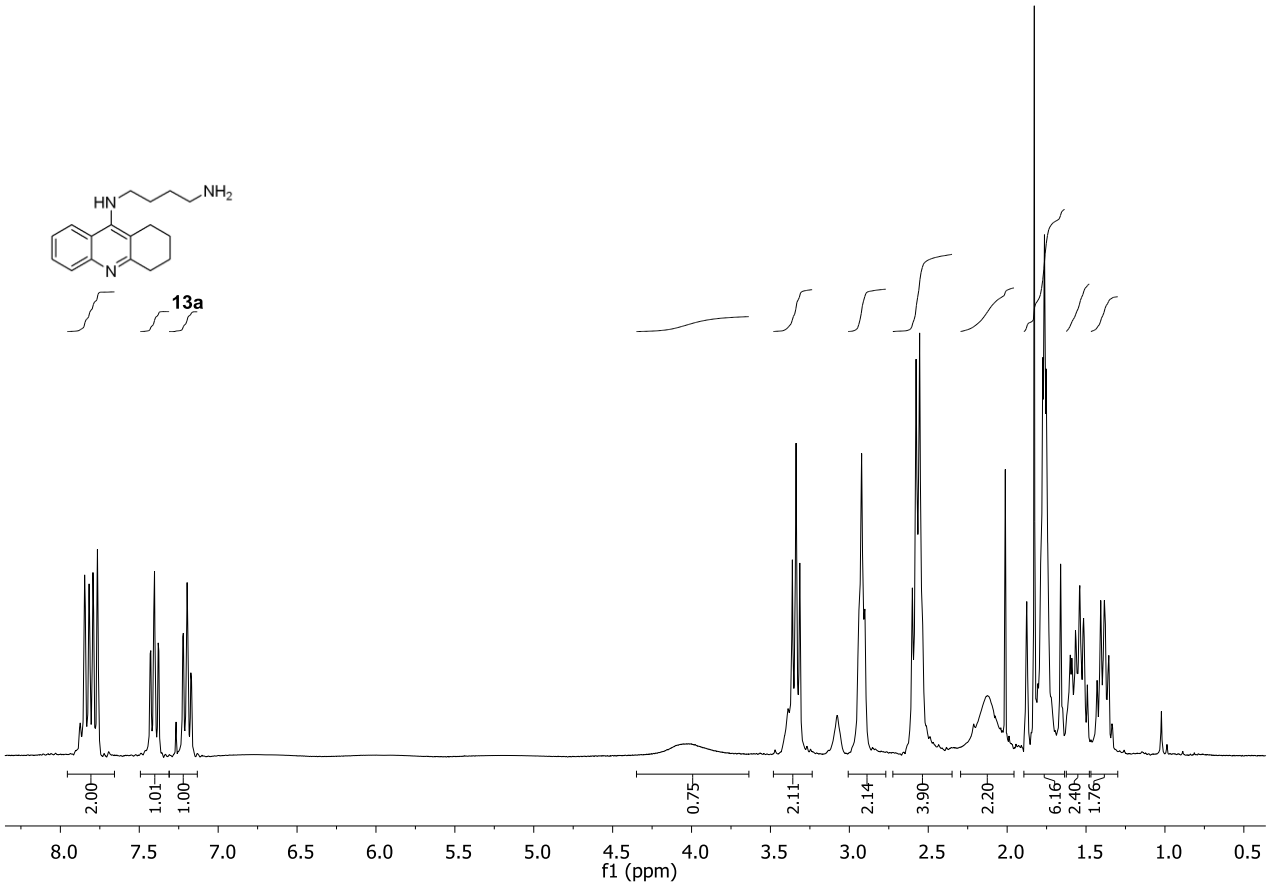
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7.0
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6.0
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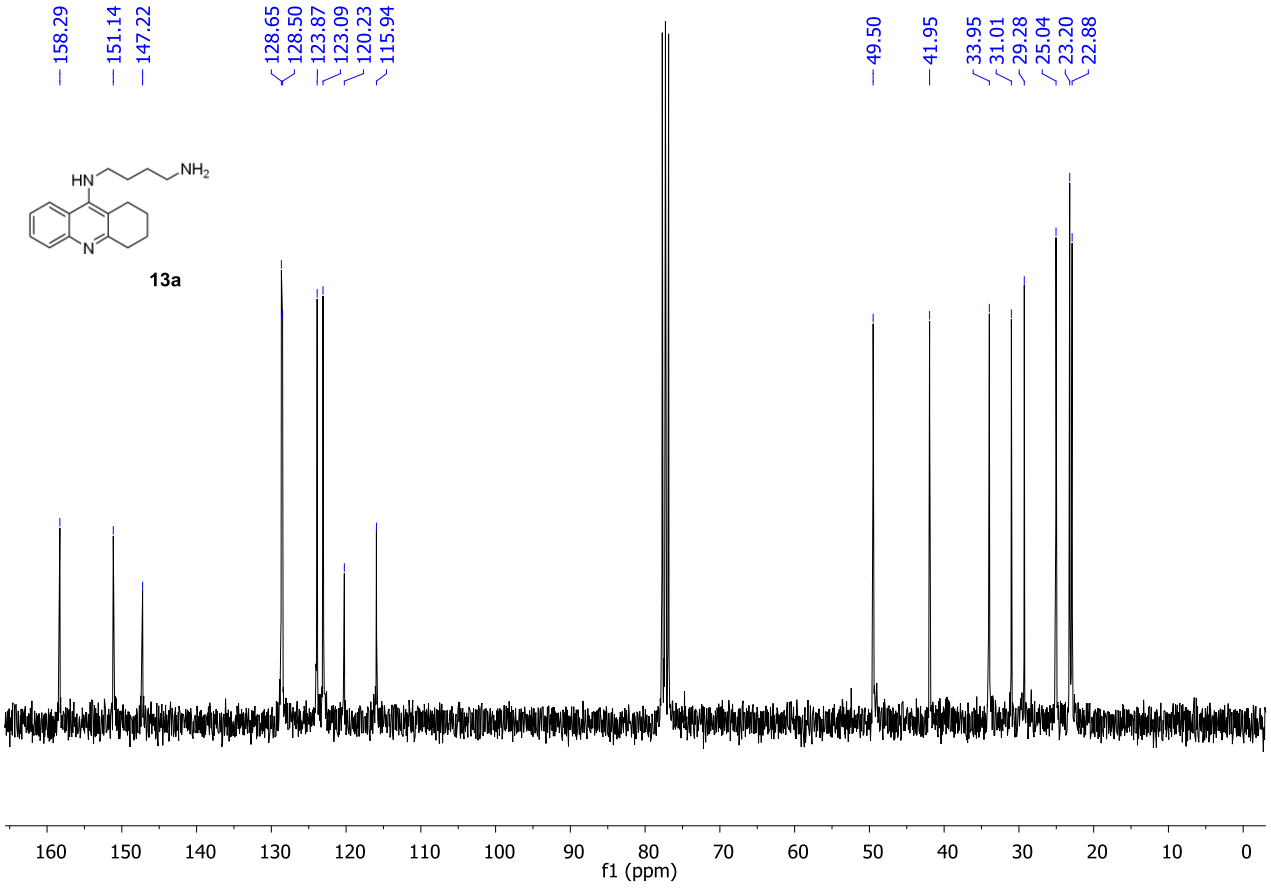
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29.28
25.04
23.20
22.88
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NH2
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50
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20
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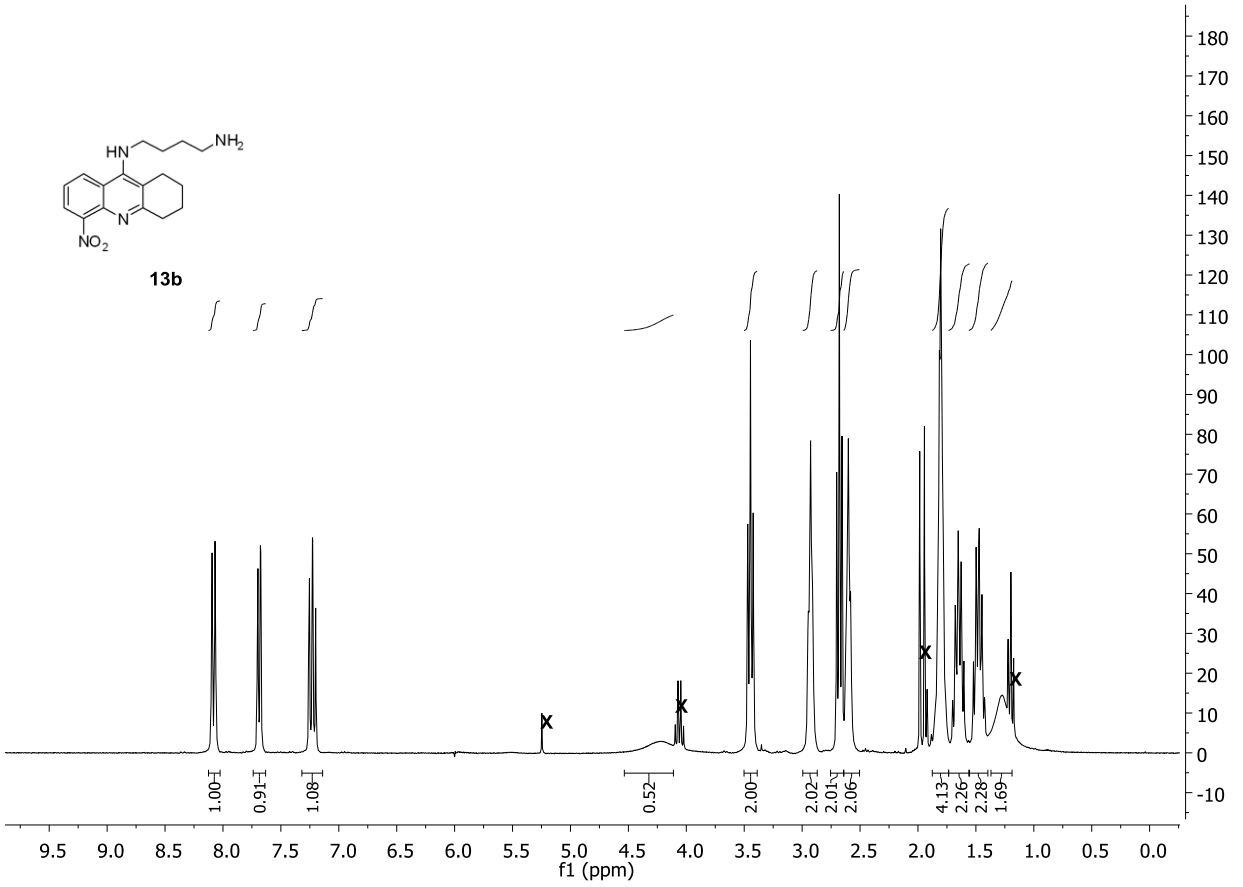
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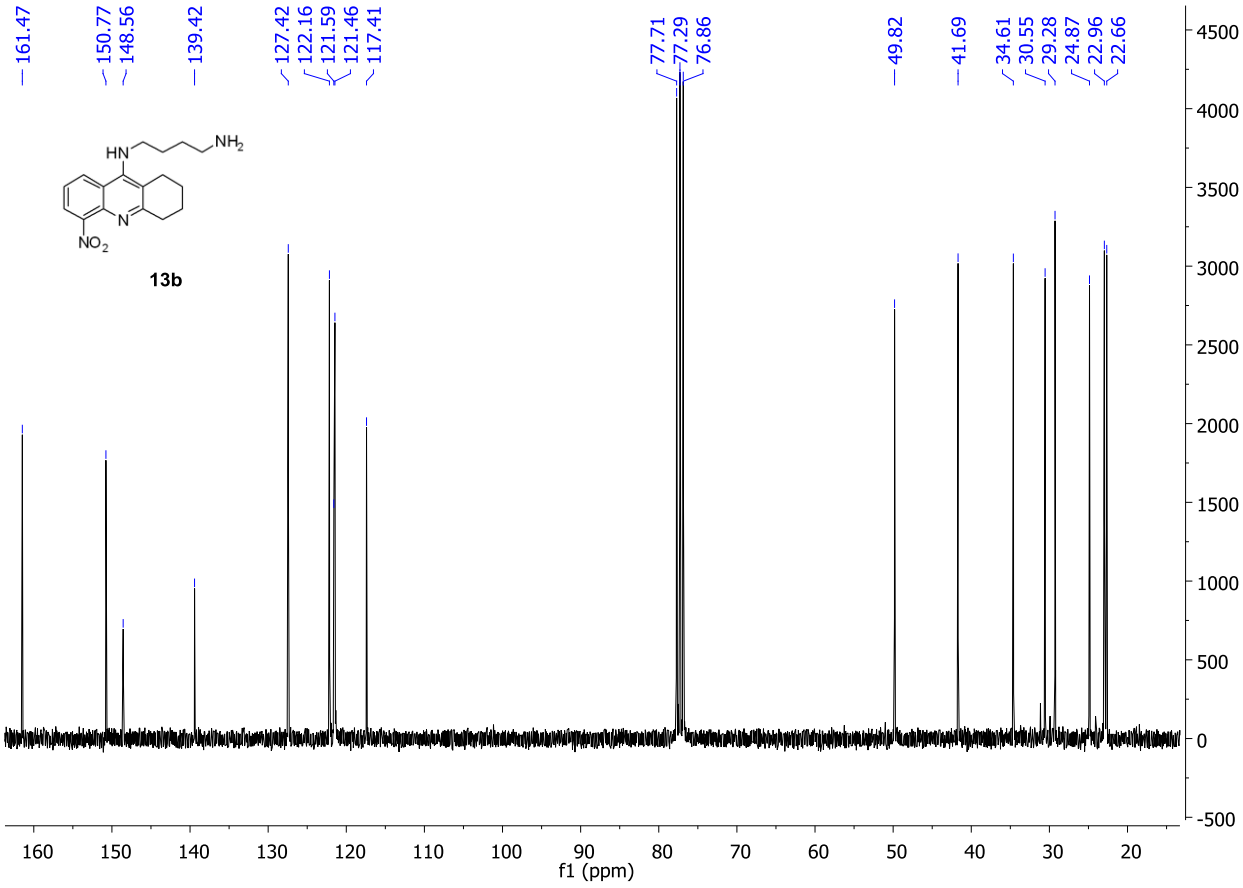
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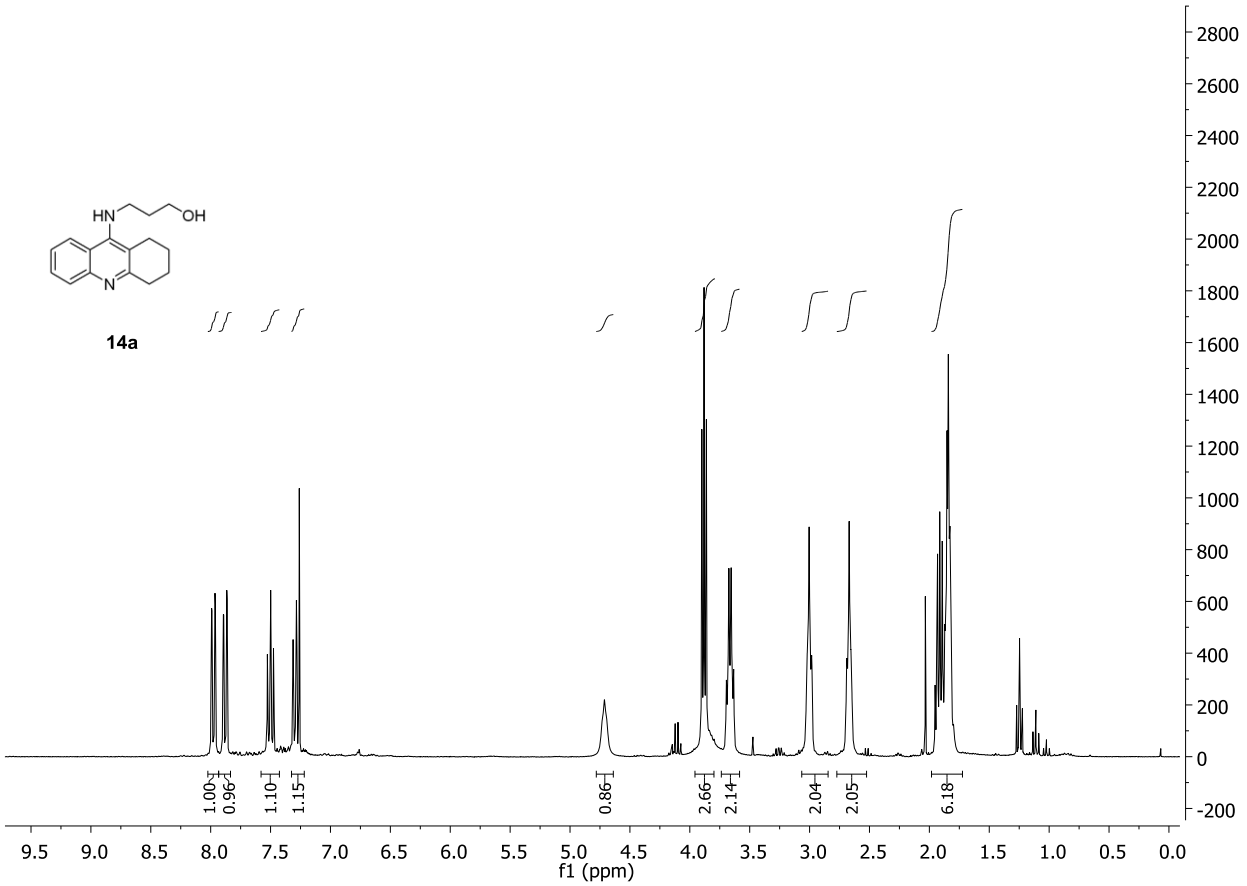

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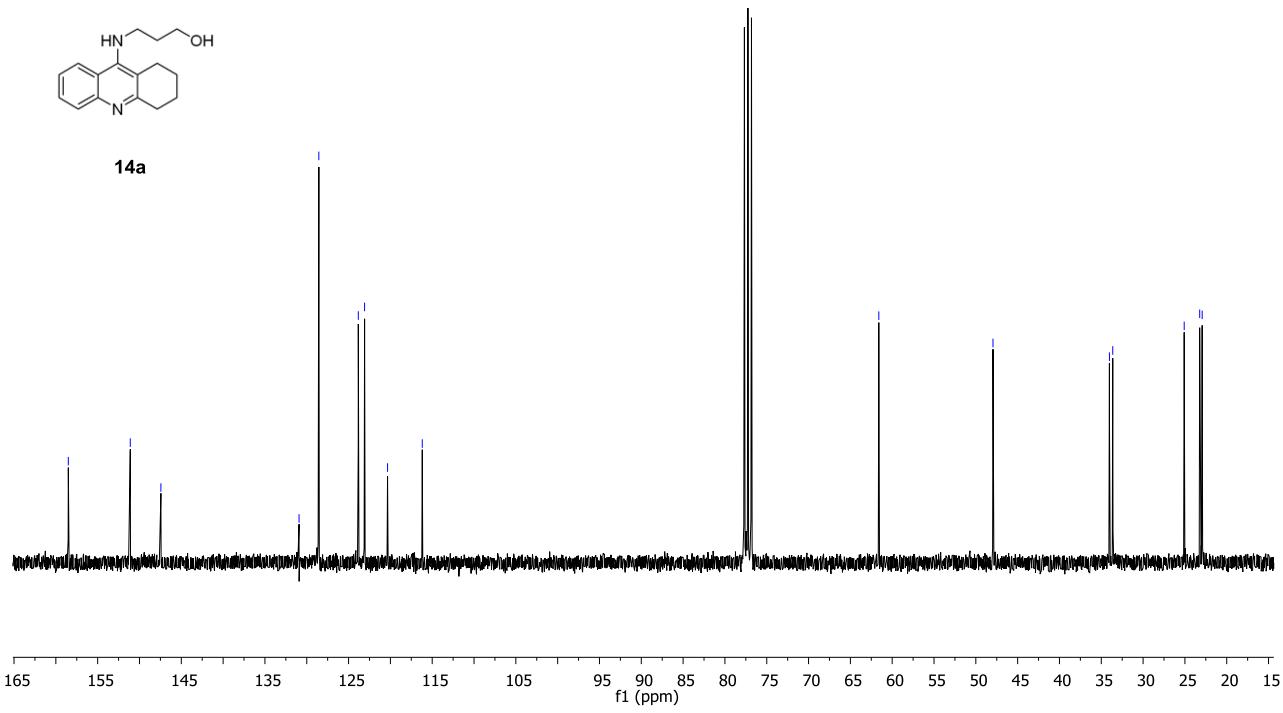

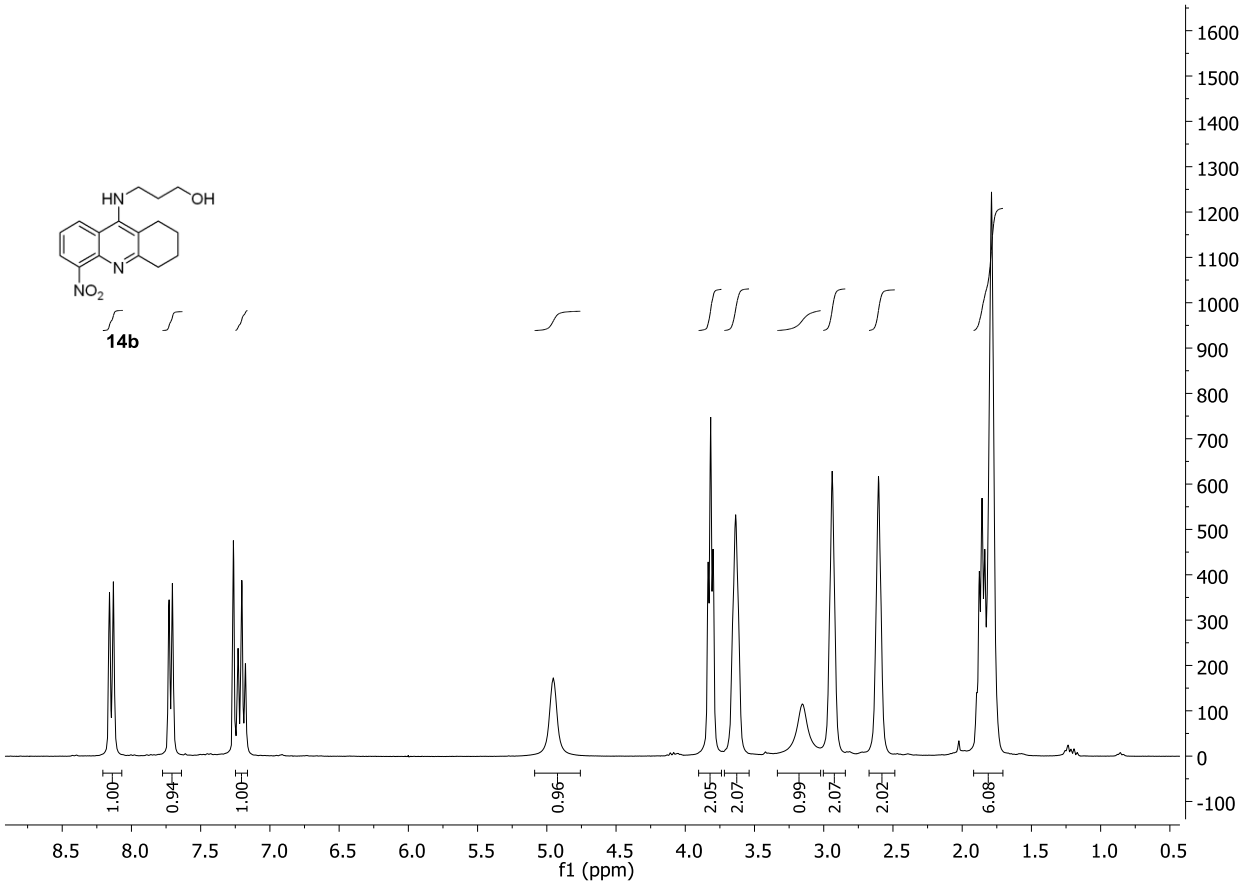
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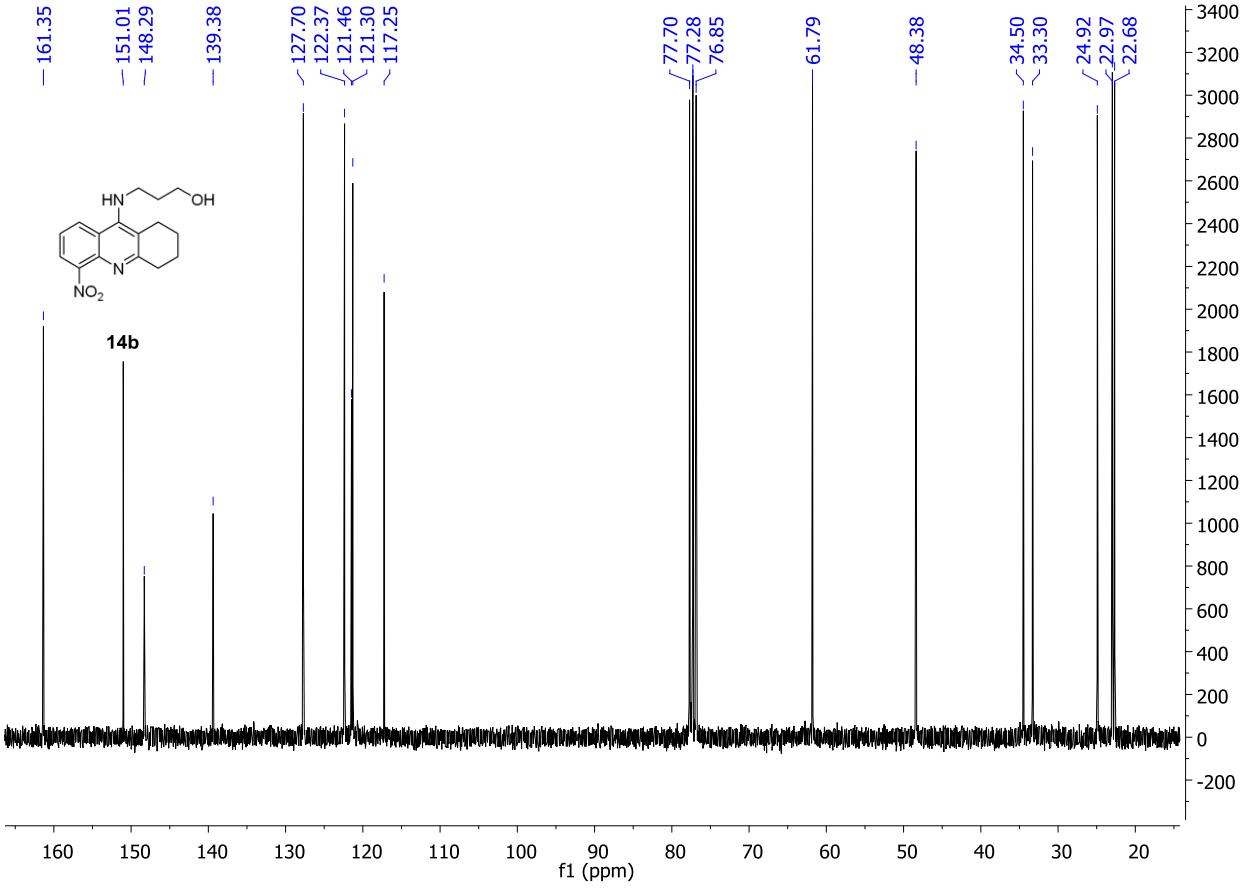
6.08
f1 (ppm)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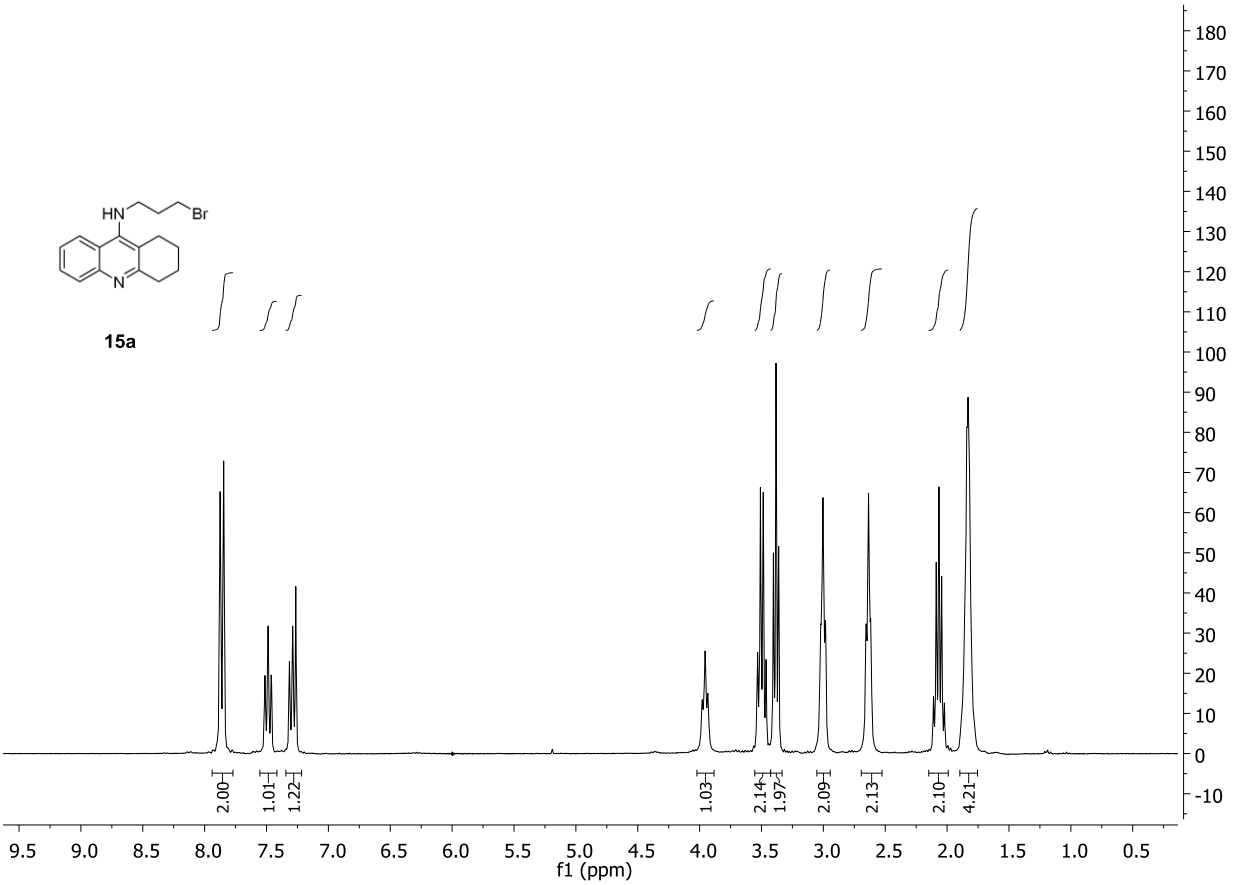
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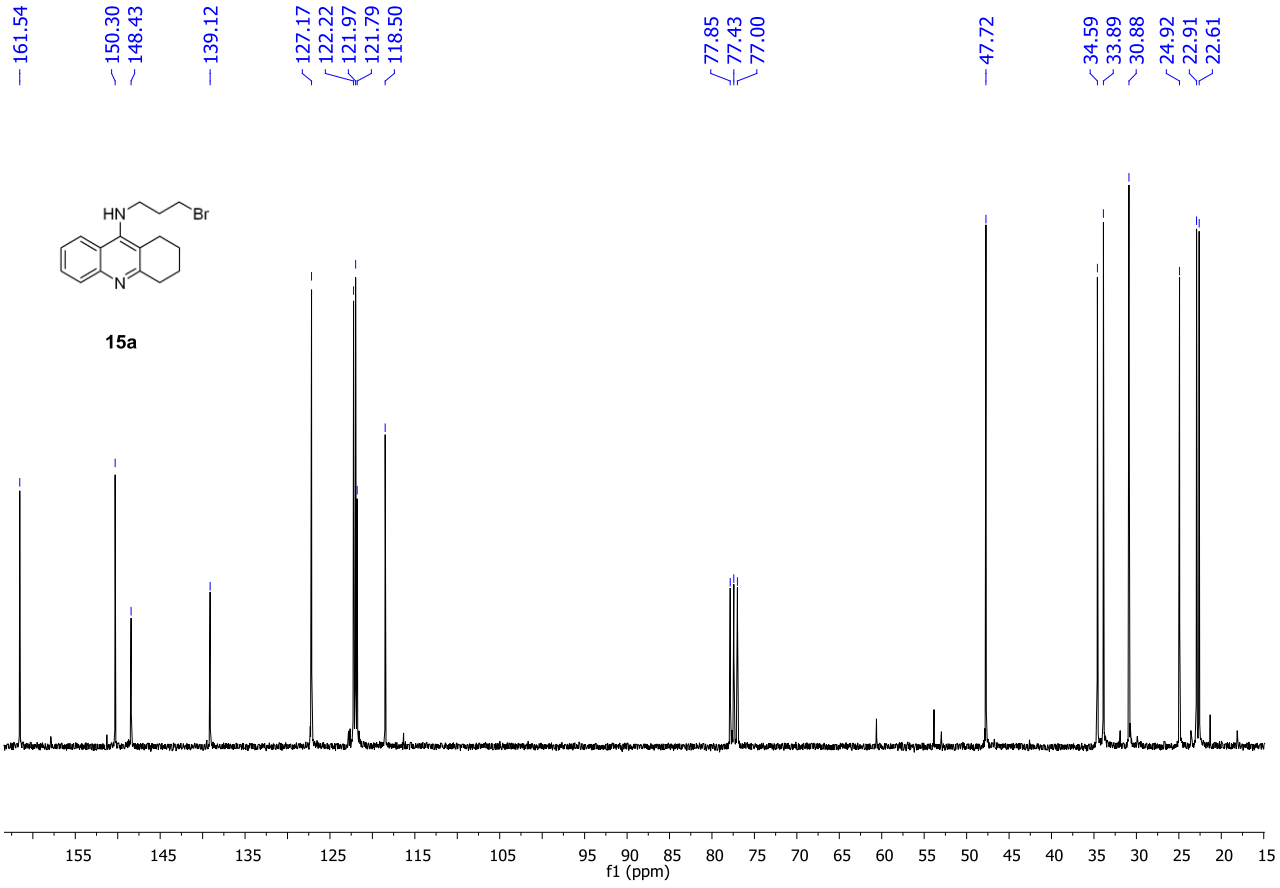
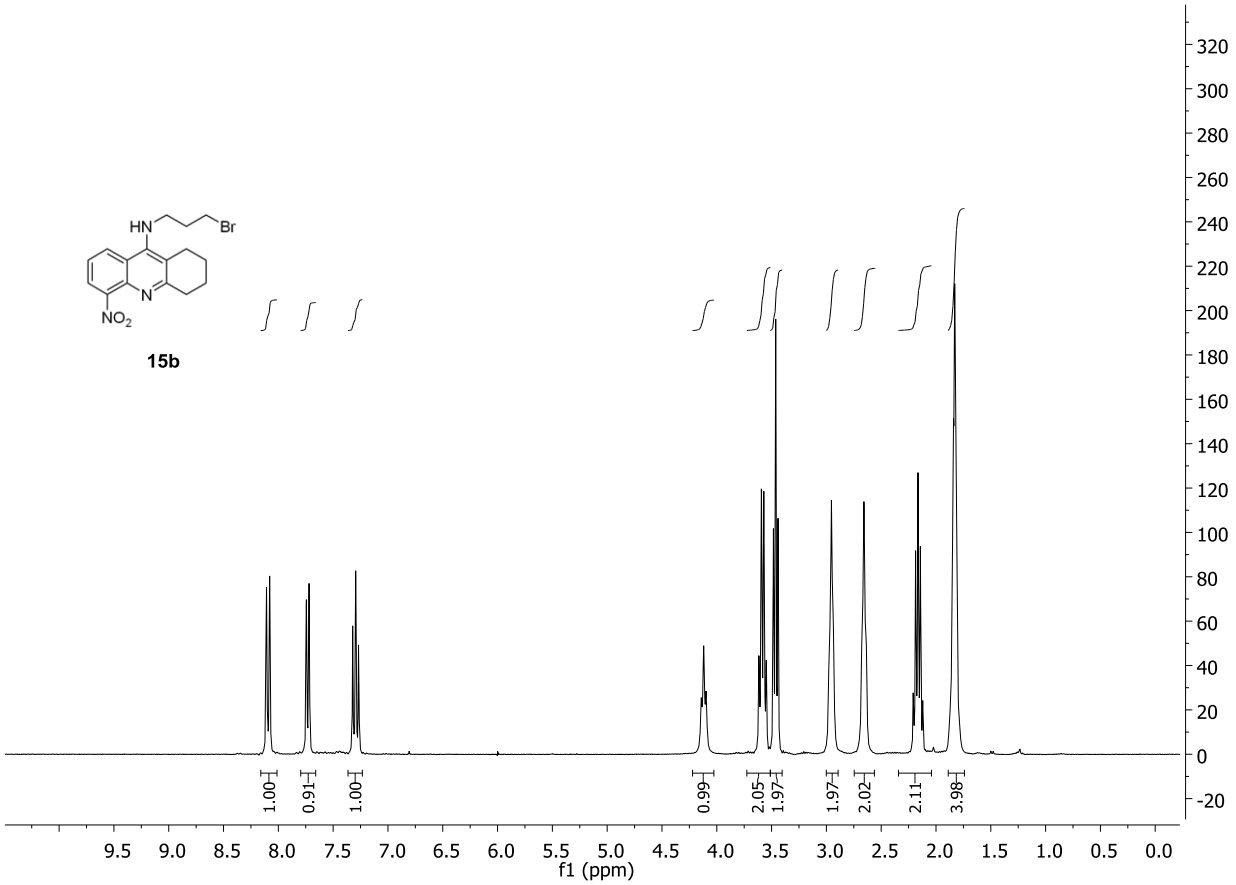
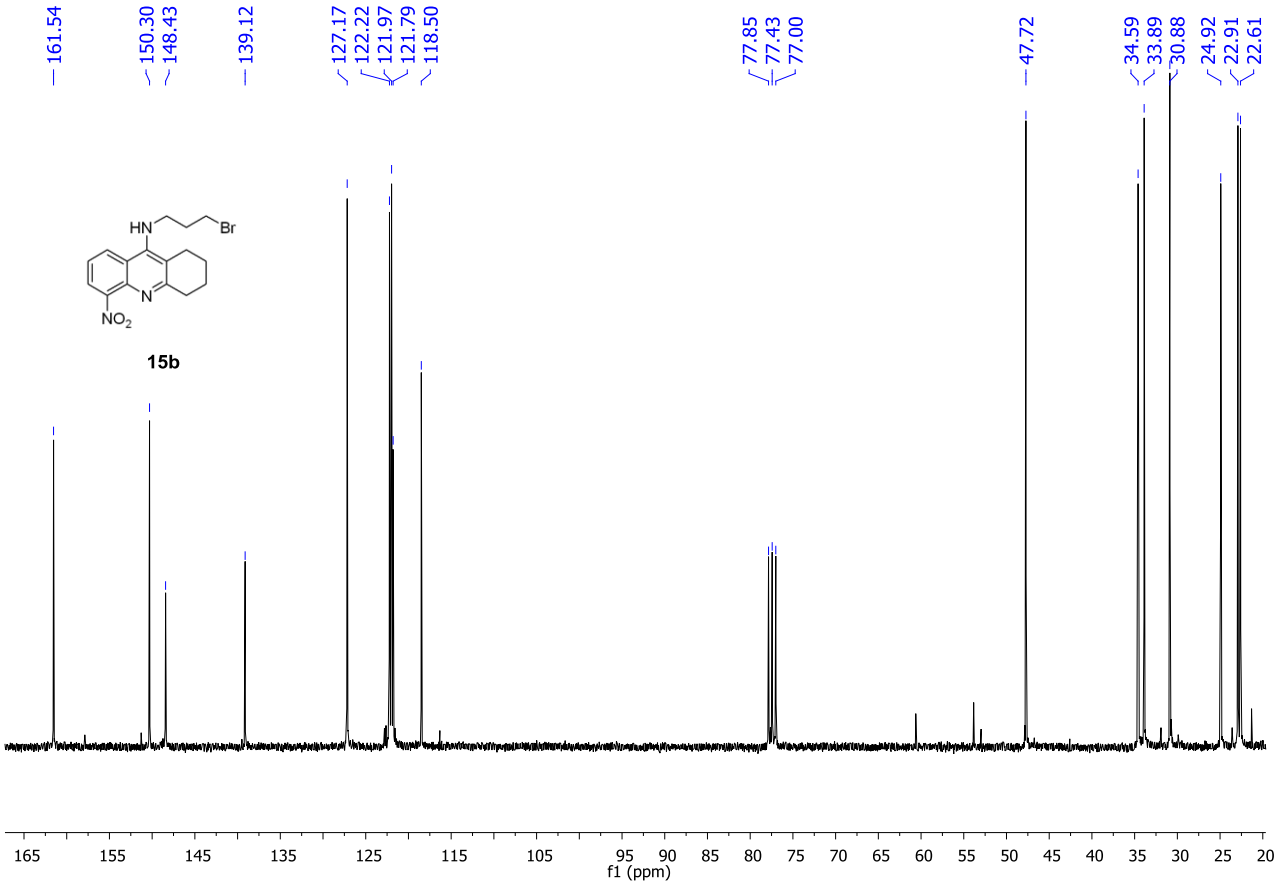
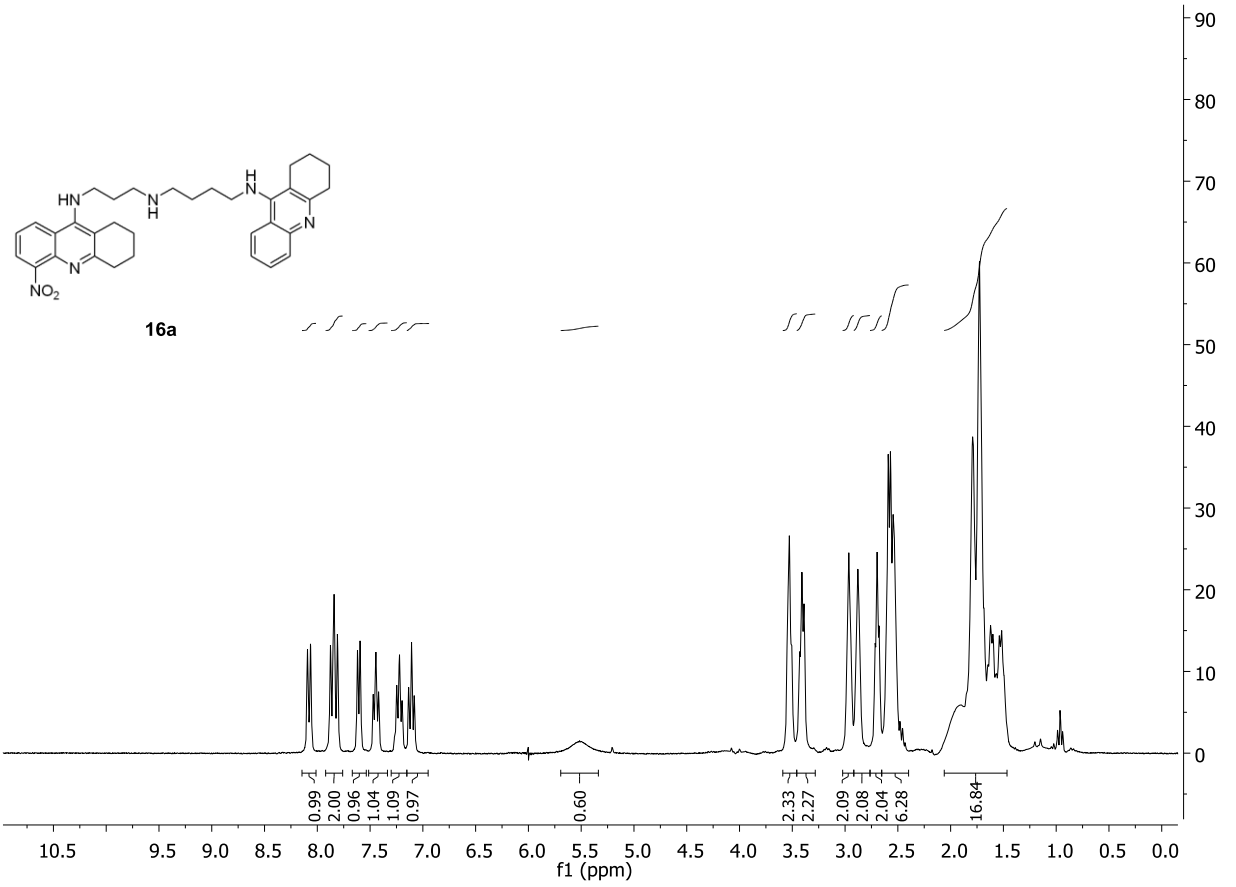
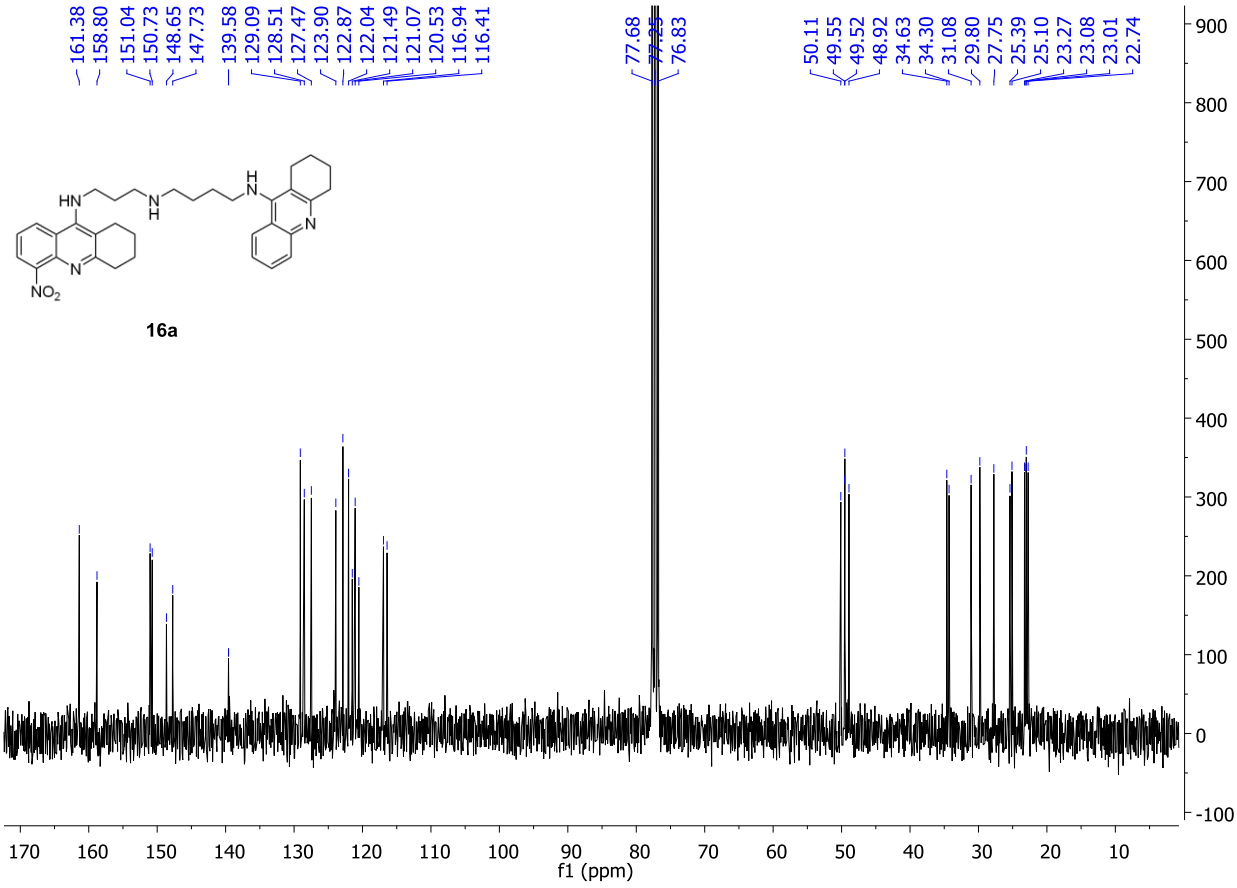
4.21
f1 (ppm)

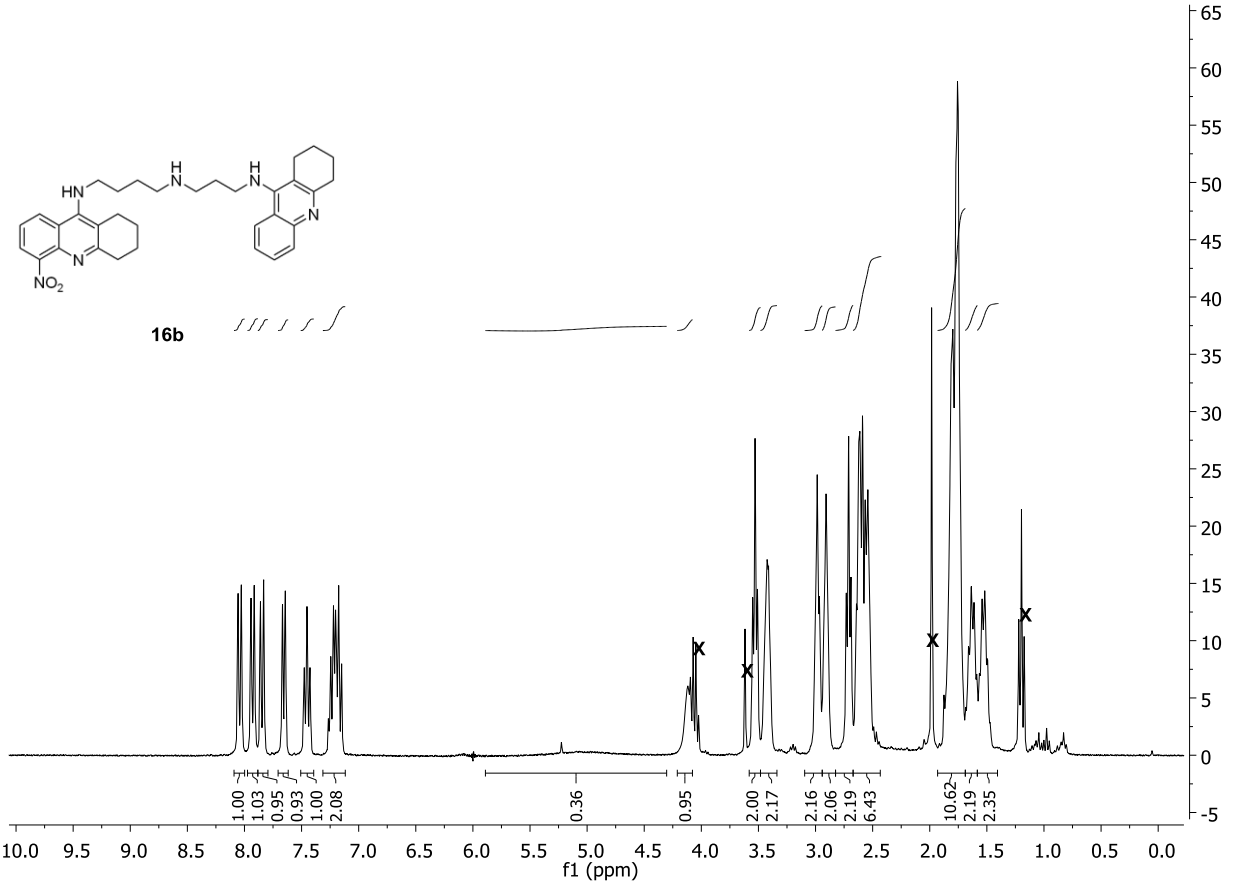

161.54
150.30
148.43
139.12
127.17
122.22
121.97
121.79
118.50
77.85
77.43
77.00
47.72
34.59
33.89
30.88
24.92
22.91
22.61
HN
Br
N
15a
f1 (ppm)

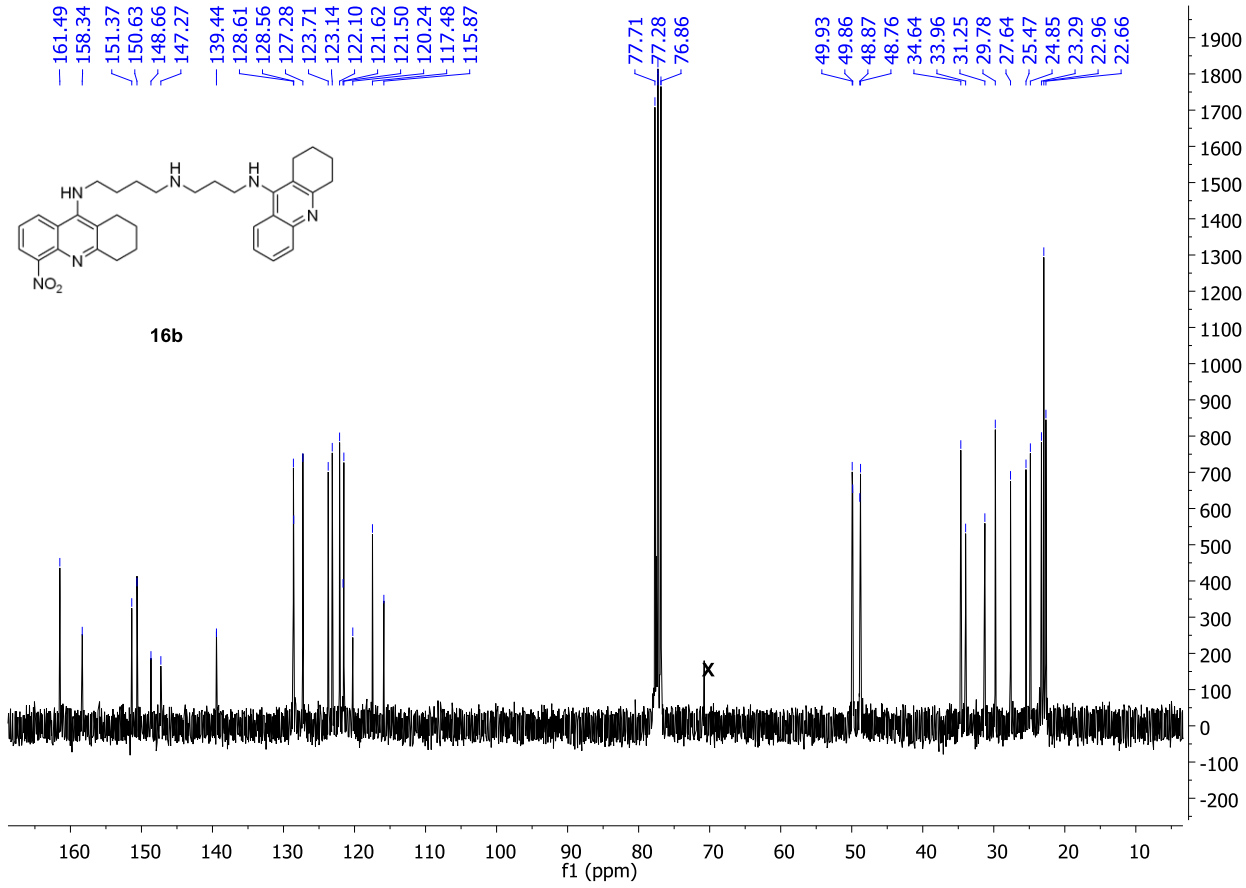

HN
Br
N
NO2
15b
1.00
0.91
1.00
0.99
2.05
1.97
1.97
2.02
2.11
3.98
f1 (ppm)

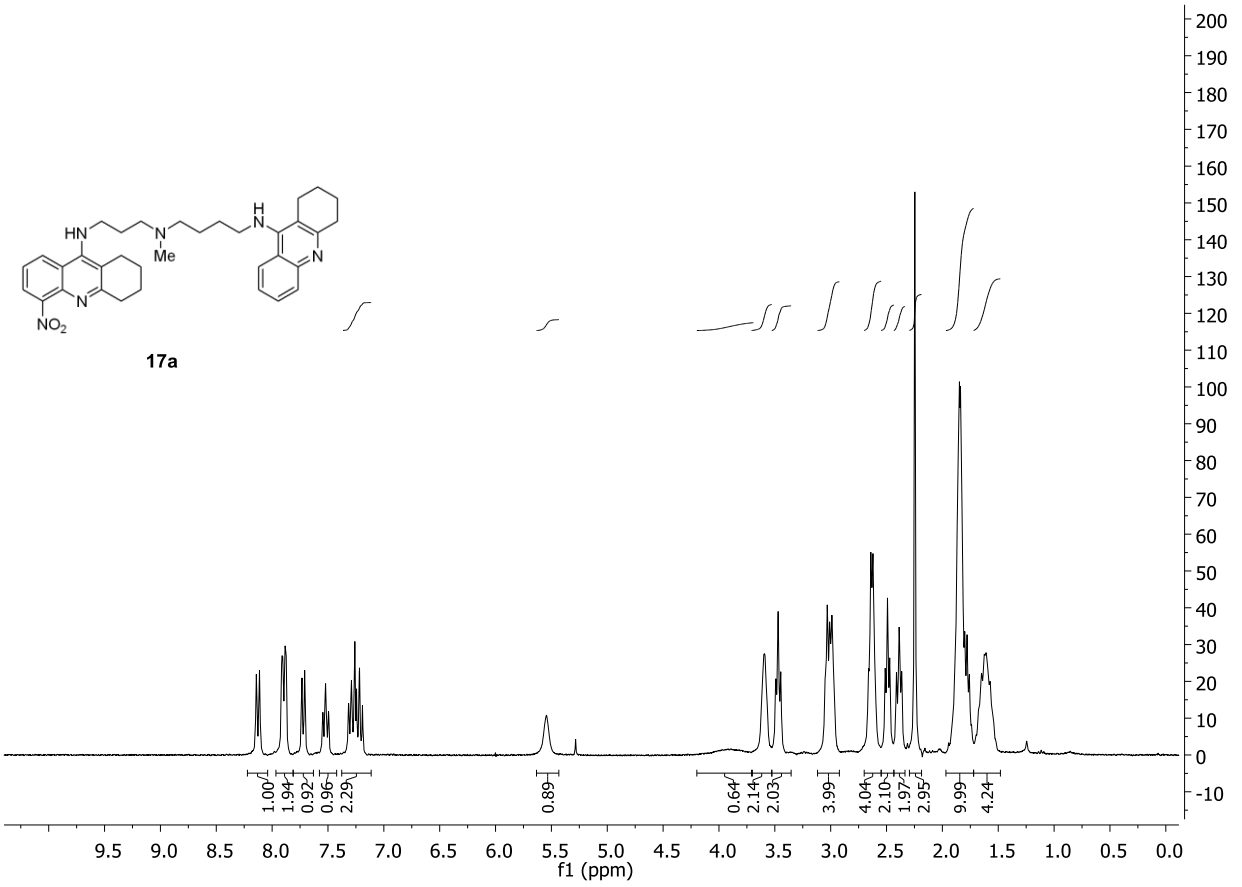

161.54
150.30
148.43
139.12
127.17
122.22
121.97
121.79
118.50
77.85
77.43
77.00
47.72
34.59
33.89
30.88
24.92
22.91
22.61
HN
Br
N
NO2
15b
f1 (ppm)

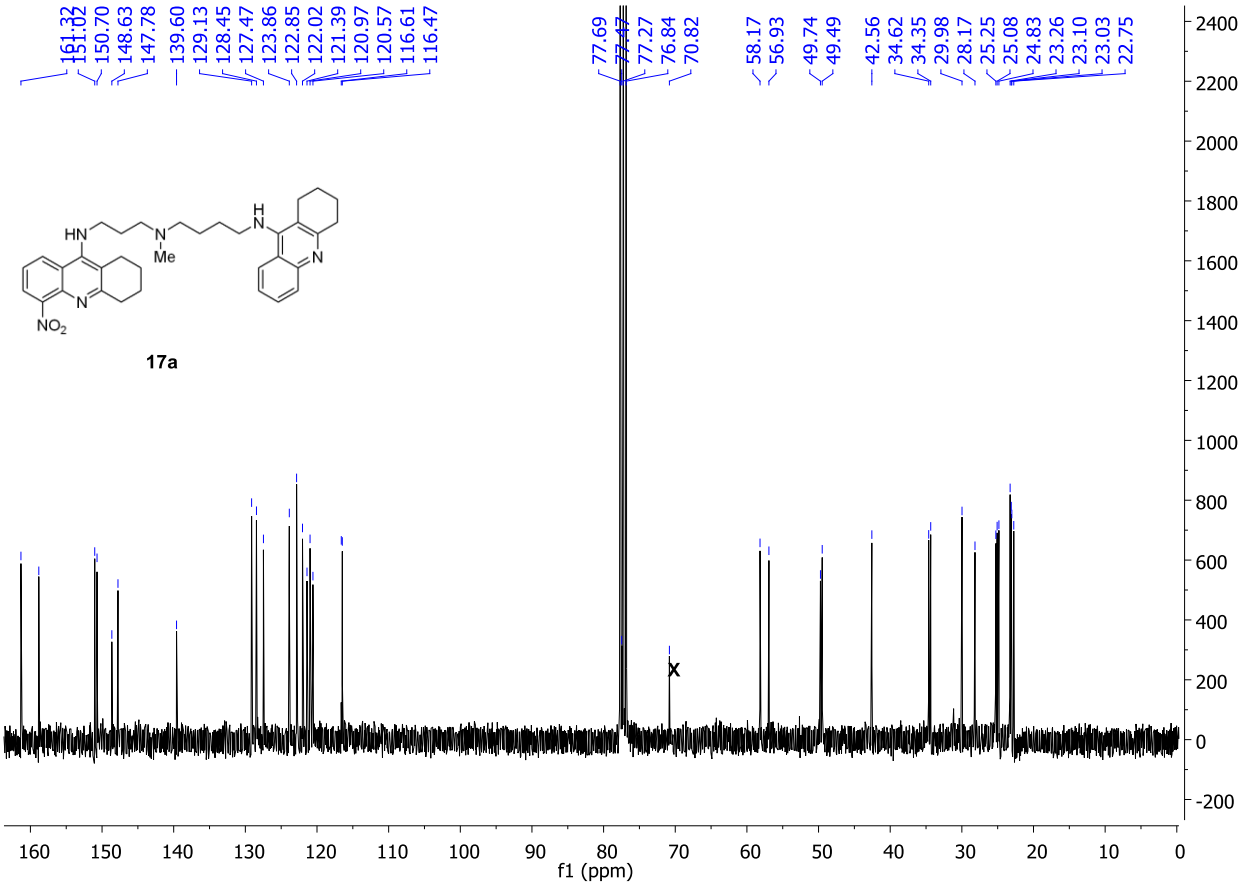

16a
0.99
2.00
0.96
1.04
1.09
0.97
0.60
2.33
2.27
2.09
2.08
2.04
6.28
16.84
f1 (ppm)

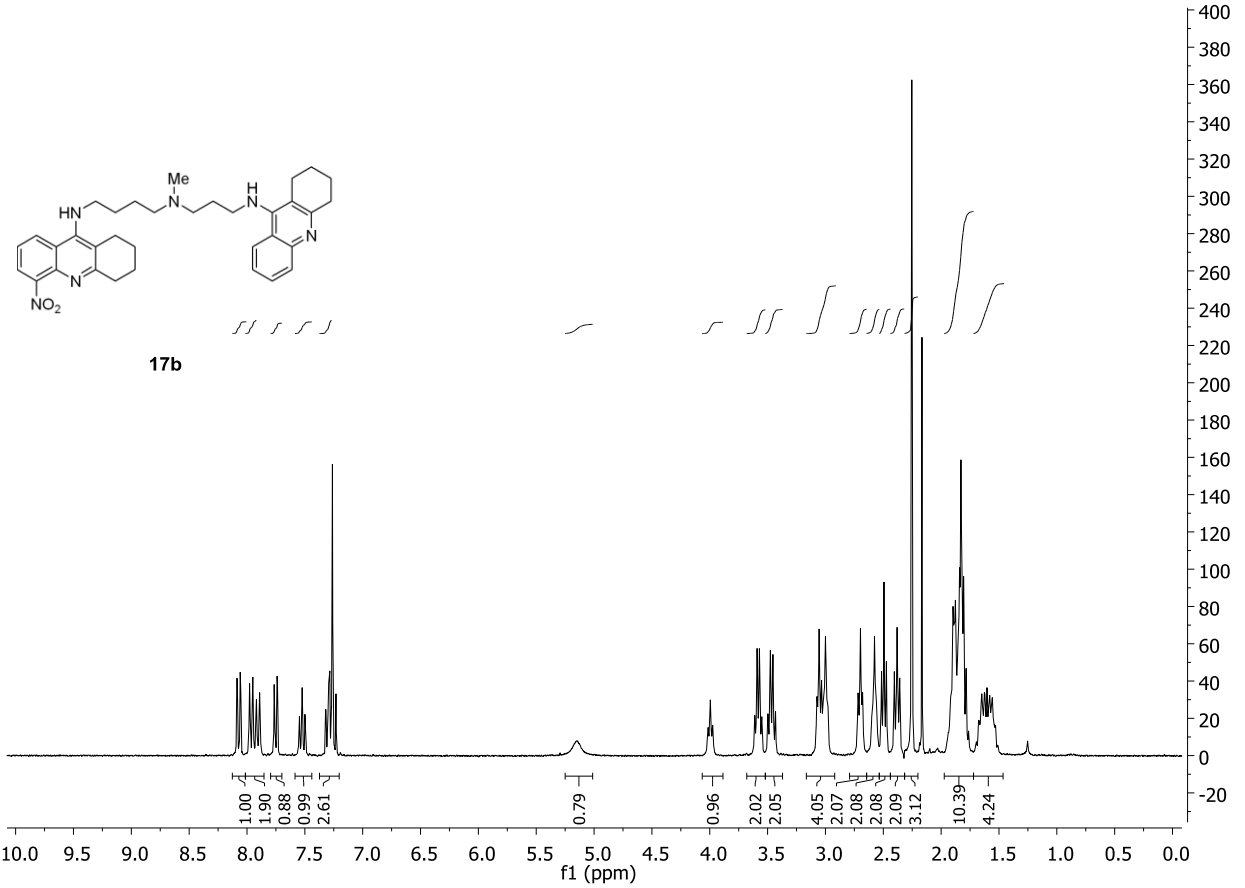

161.38
158.80
151.04
150.73
148.65
147.73
139.58
129.09
128.51
127.47
123.90
122.87
122.04
121.49
121.07
120.53
116.94
116.41
77.68
77.25
76.83
50.11
49.55
49.52
48.92
34.63
34.30
31.08
29.80
27.75
25.39
25.10
23.27
23.08
23.01
22.74
16a
f1 (ppm)

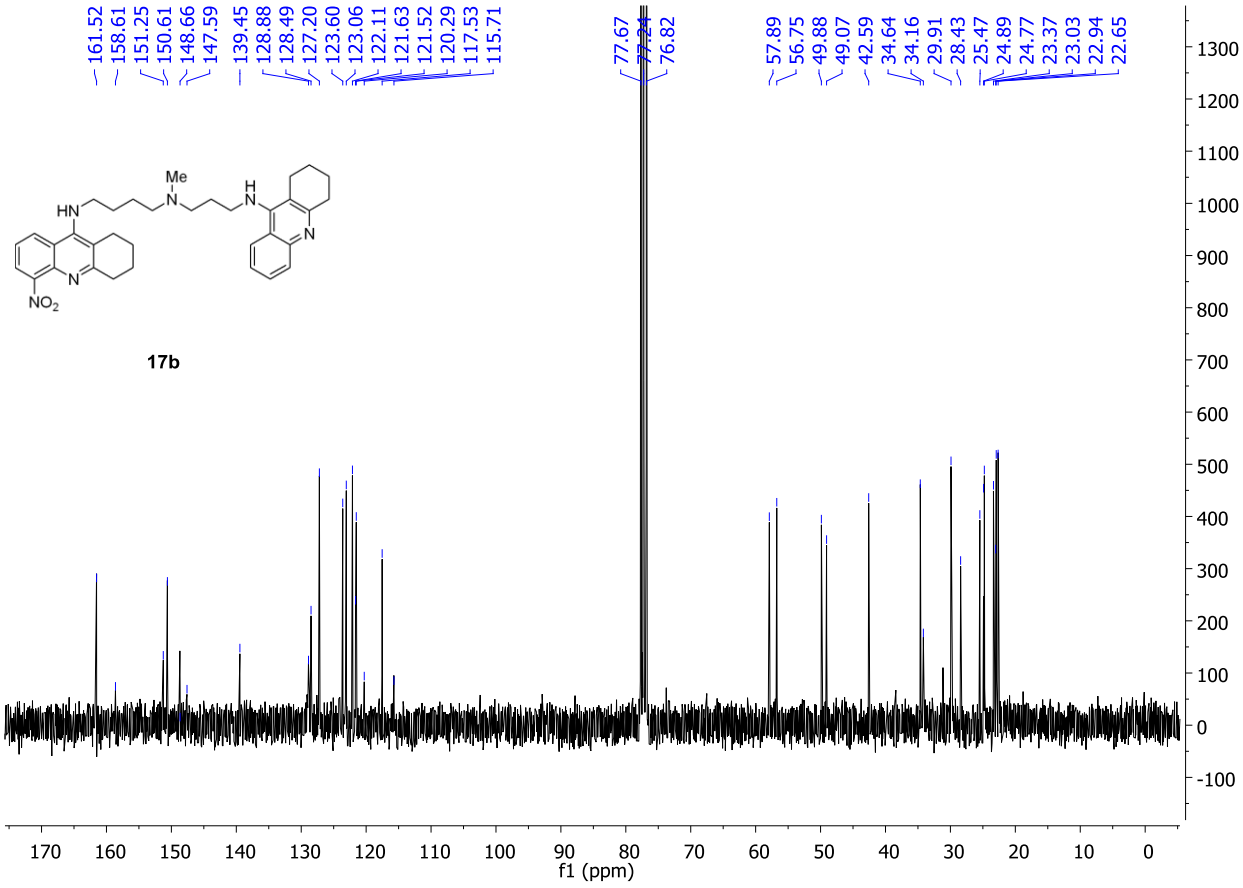

16b
1.00
1.03
0.95
0.93
1.00
2.08
0.36
0.95
2.00
2.17
2.16
2.06
2.19
6.43
10.62
2.19
2.35
f1 (ppm)

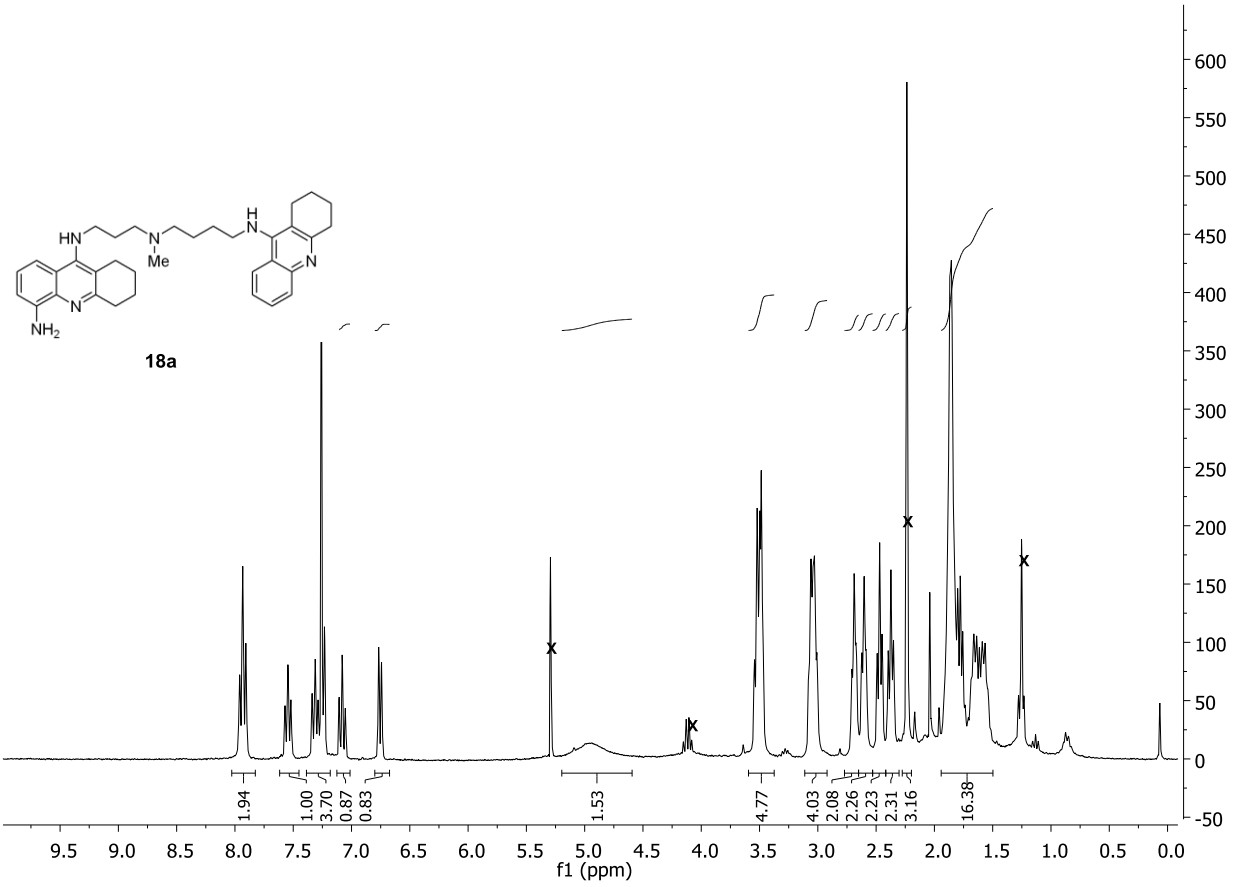

161.49
158.34
151.37
150.63
148.66
147.27
139.44
128.61
128.56
127.28
123.71
123.14
122.10
121.62
121.50
120.24
117.48
115.87
77.71
77.28
76.86
49.93
49.86
48.87
48.76
34.64
33.96
31.25
29.78
27.64
25.47
24.85
23.29
22.96
22.66
16b
f1 (ppm)

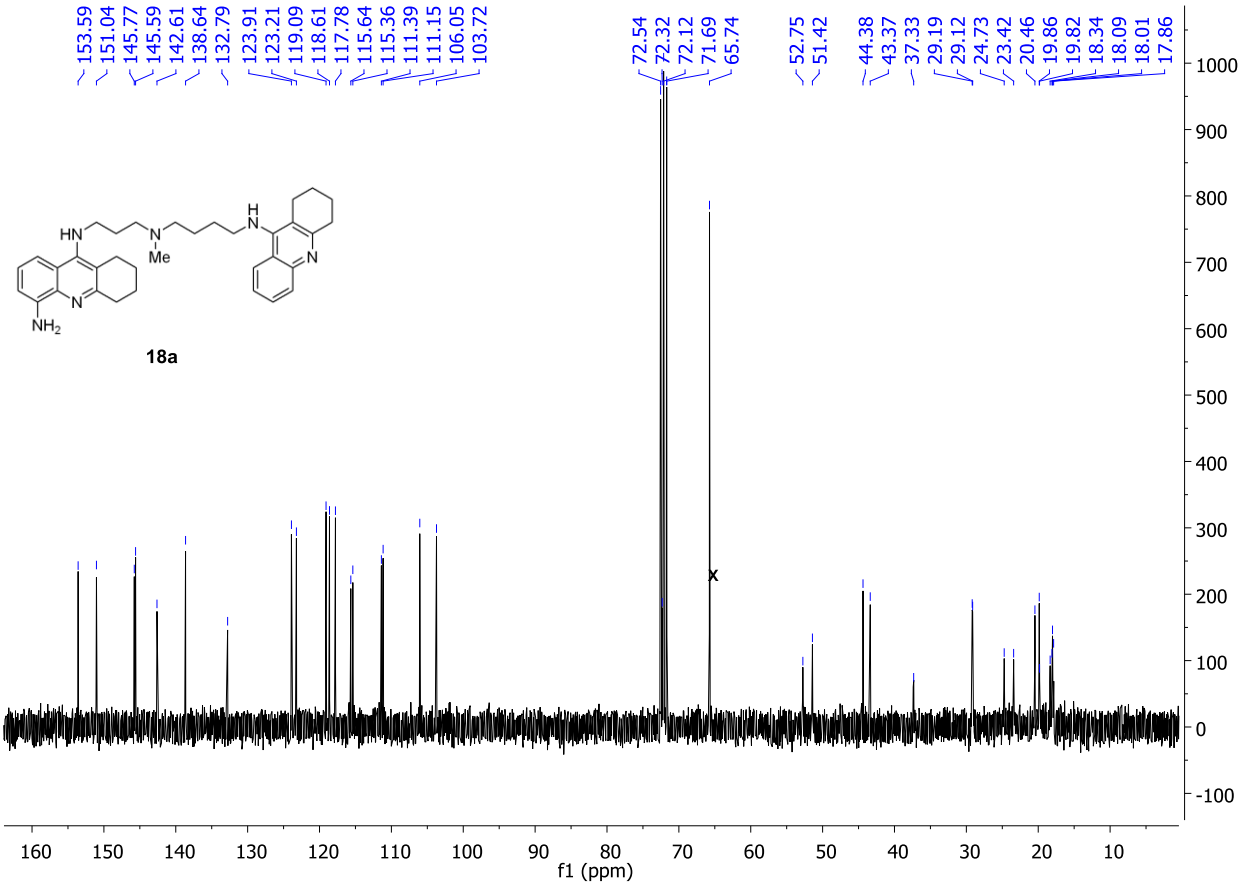

HN
N
Me
H
N
N
NO2
17a
1.00
1.94
0.92
0.96
2.29
0.89
0.64
2.14
2.03
3.99
4.04
2.10
1.97
2.95
9.99
4.24
f1 (ppm)

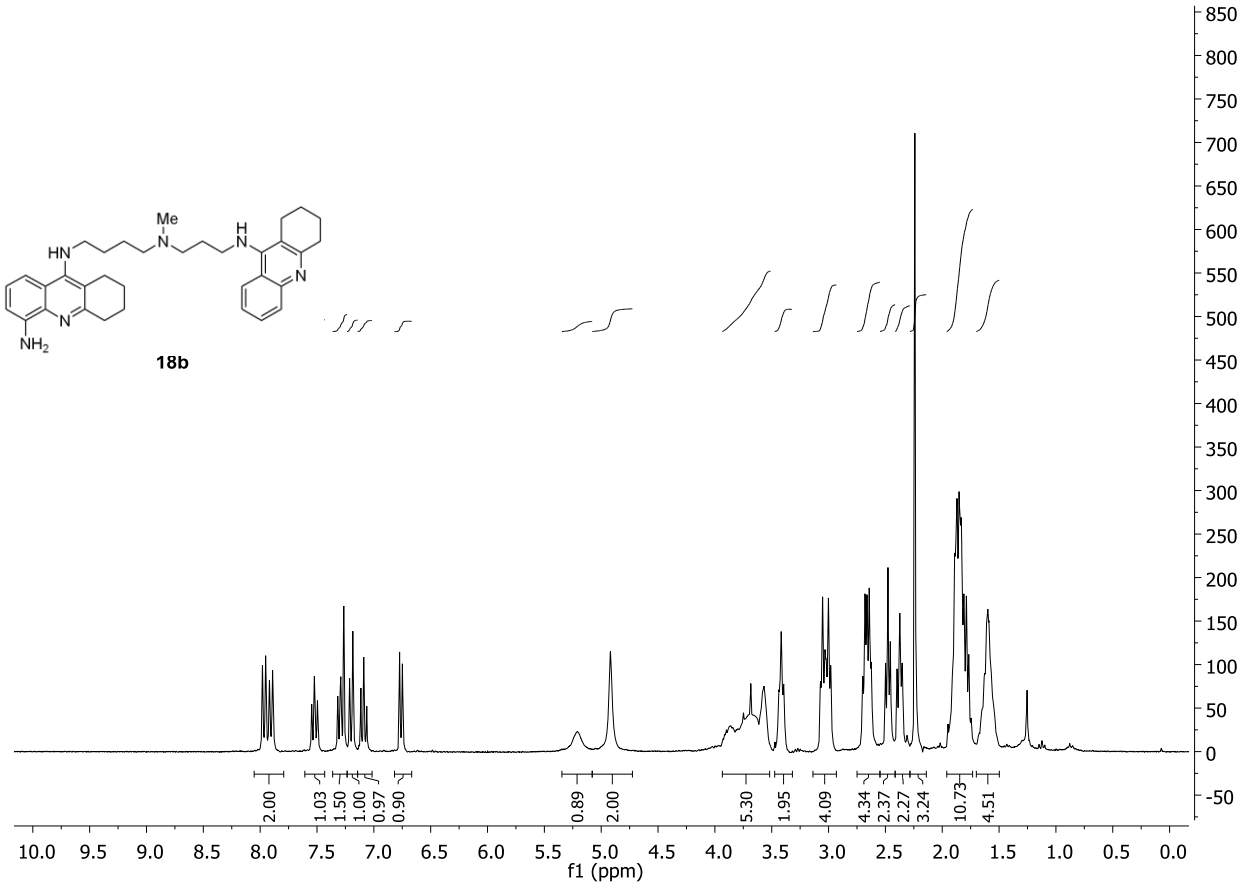

161.32
151.02
150.70
148.63
147.78
139.60
129.13
128.45
127.47
123.86
122.85
122.02
121.39
120.97
120.57
116.61
116.47
77.69
77.47
77.27
76.84
70.82
58.17
56.93
49.74
49.49
42.56
34.62
34.35
29.98
28.17
25.25
25.08
24.83
23.26
23.10
23.03
22.75
HN
N
Me
H
N
N
NO2
17a
f1 (ppm)

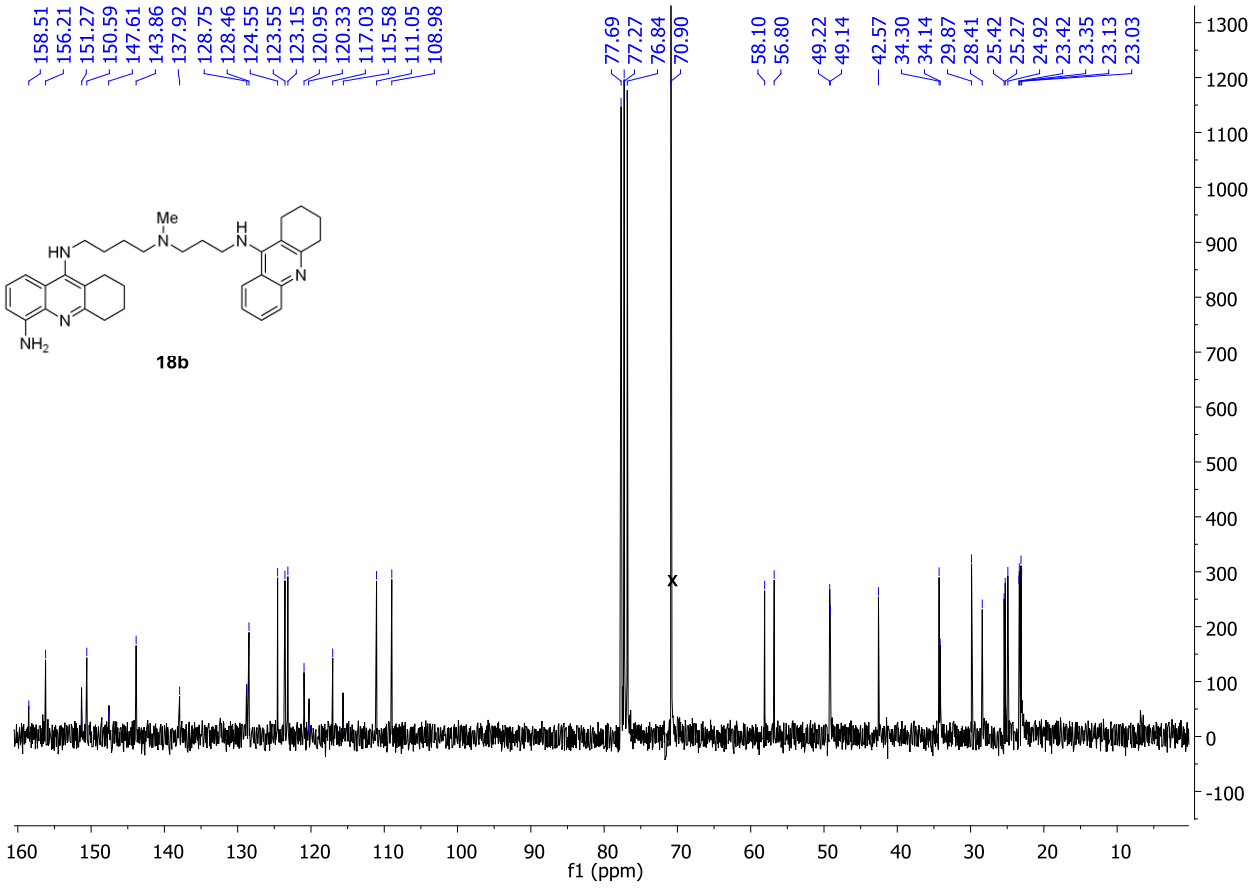

Me
HN
N
H
N
N
NO2
17b
1.00
1.90
0.88
0.99
2.61
0.79
0.96
2.02
2.05
4.05
2.07
2.08
2.08
2.09
3.12
10.39
4.24
f1 (ppm)

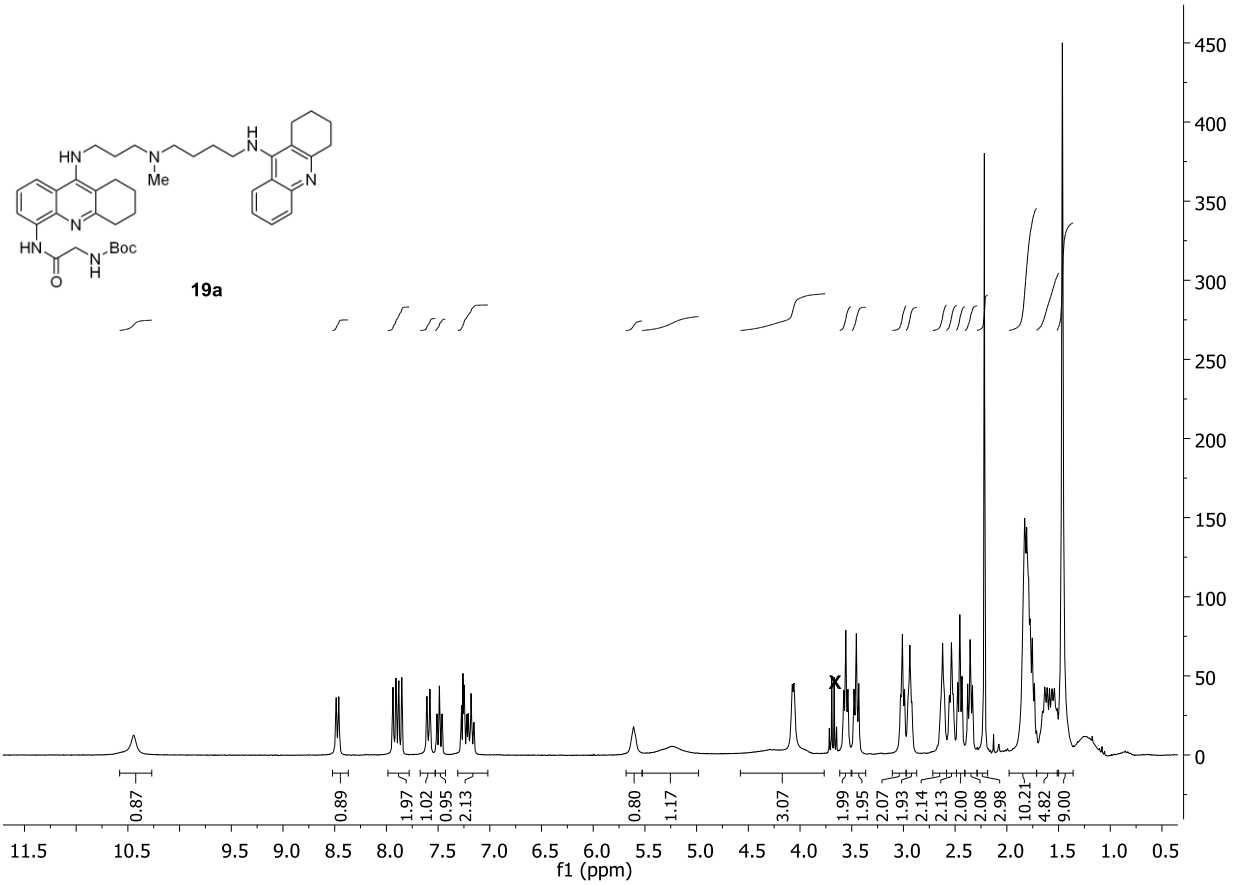

161.52
158.61
151.25
150.61
148.66
147.59
139.45
128.88
128.49
127.20
123.60
123.06
122.11
121.63
121.52
120.29
117.53
115.71
77.67
77.24
76.82
57.89
56.75
49.88
49.07
42.59
34.64
34.16
29.91
28.43
25.47
24.89
24.77
23.37
23.03
22.94
22.65
Me
HN
N
H
N
N
NO2
17b
f1 (ppm)

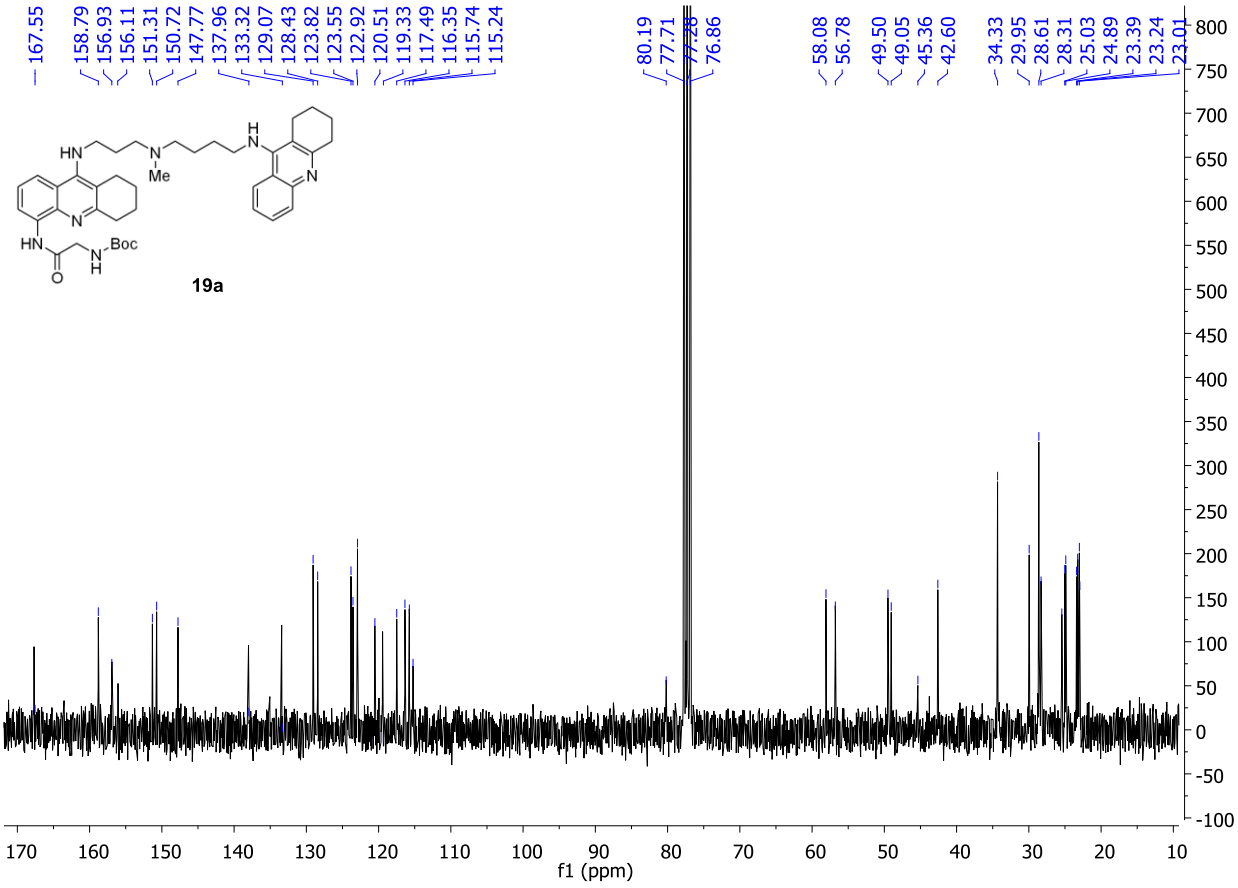

18a
1.94
1.00
3.70
0.87
0.83
1.53
4.77
4.03
2.08
2.26
2.23
2.31
3.16
16.38
f1 (ppm)

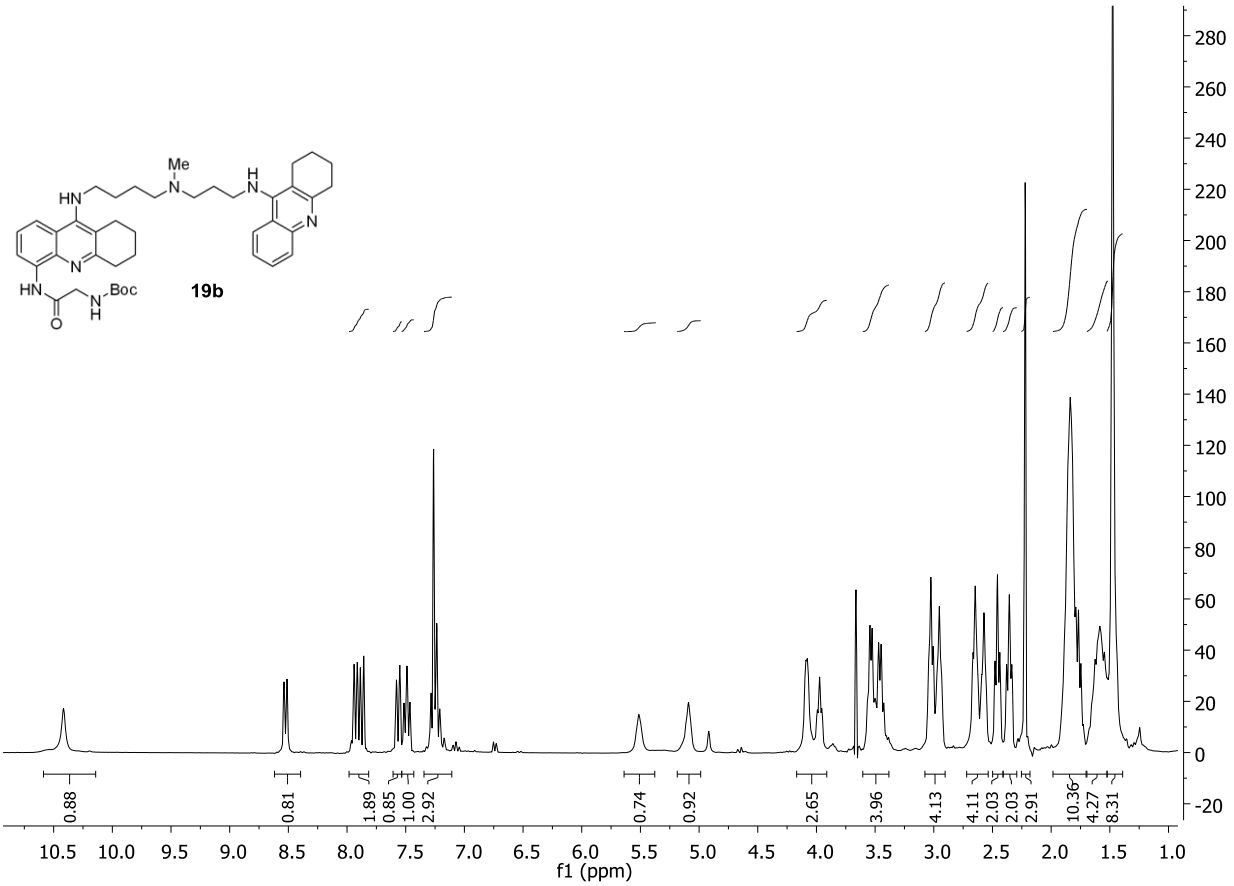

153.59
151.04
145.77
145.59
142.61
138.64
132.79
123.91
123.21
119.09
118.61
117.78
115.64
115.36
111.39
111.15
106.05
103.72
72.54
72.32
72.12
71.69
65.74
52.75
51.42
44.38
43.37
37.33
29.19
29.12
24.73
23.42
20.46
19.86
19.82
18.34
18.09
18.01
17.86
18a
f1 (ppm)

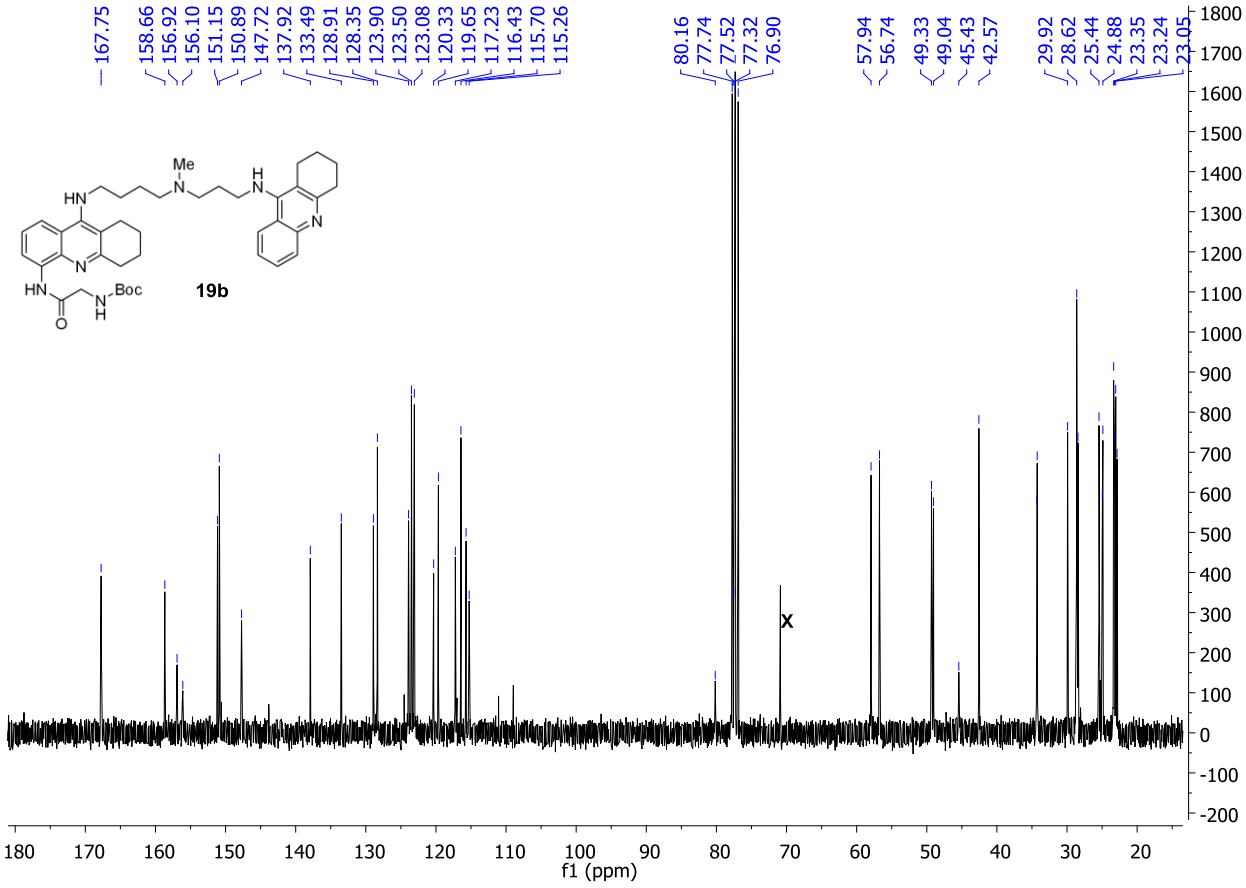

Me
HN
N
H
N
N
NH2
18b
2.00
1.03
1.50
1.00
0.97
0.90
0.89
2.00
5.30
1.95
4.09
4.34
2.37
2.27
3.24
10.73
4.51
f1 (ppm)

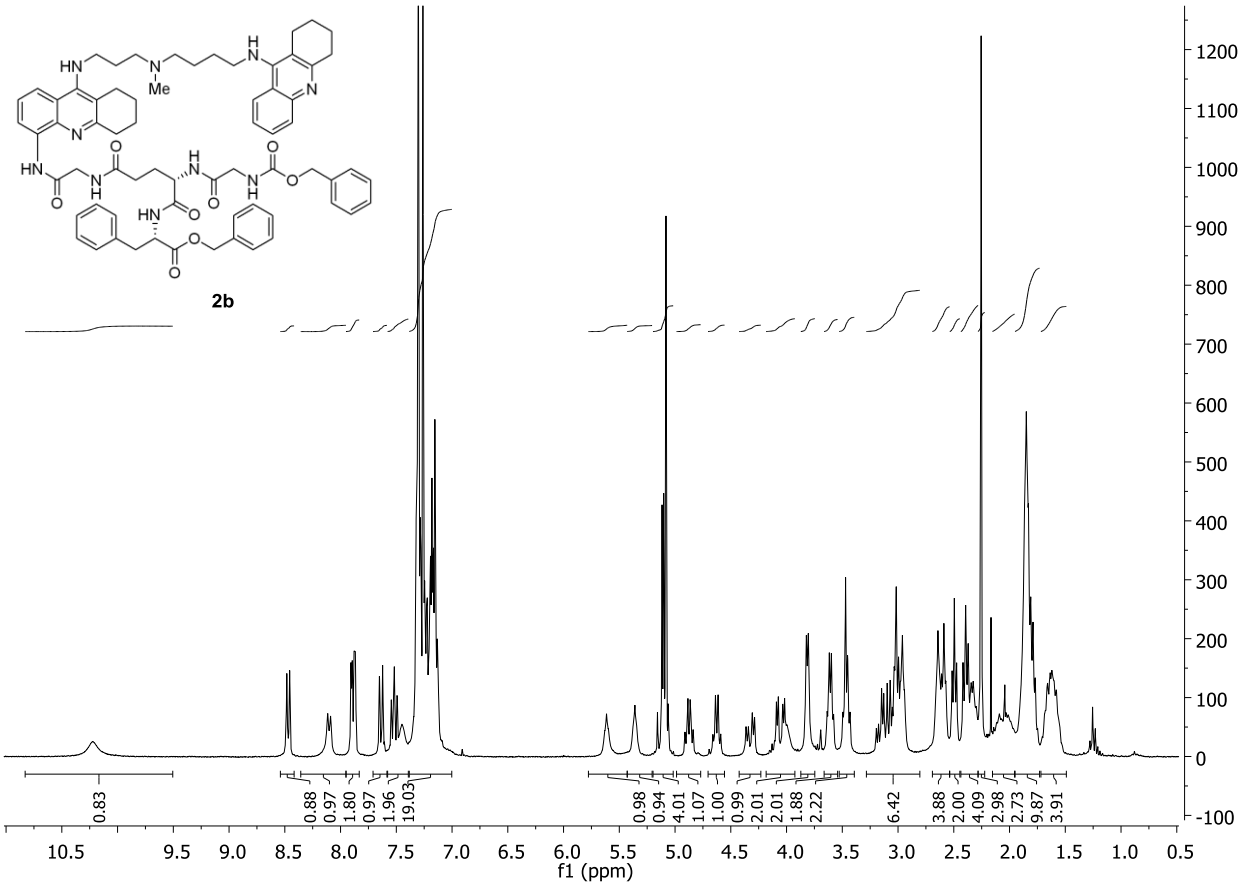

158.51
156.21
151.27
150.59
147.61
143.86
137.92
128.75
128.46
124.55
123.55
123.15
120.95
120.33
117.03
115.58
111.05
108.98
77.69
77.27
76.84
70.90
58.10
56.80
49.22
49.14
42.57
34.30
34.14
29.87
28.41
25.42
25.27
24.92
23.42
23.35
23.13
23.03
Me
HN
N
H
N
N
NH2
18b
f1 (ppm)

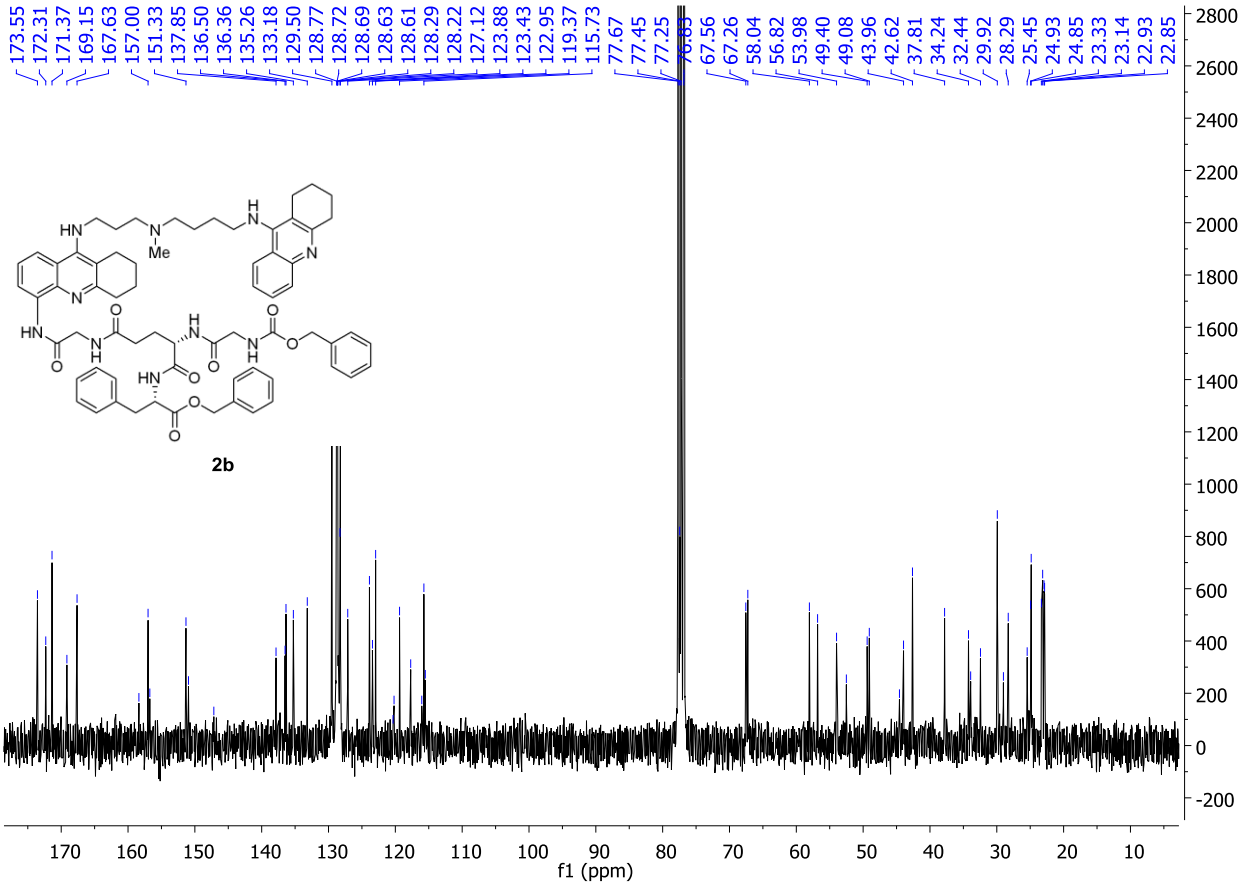

19a
f1 (ppm)

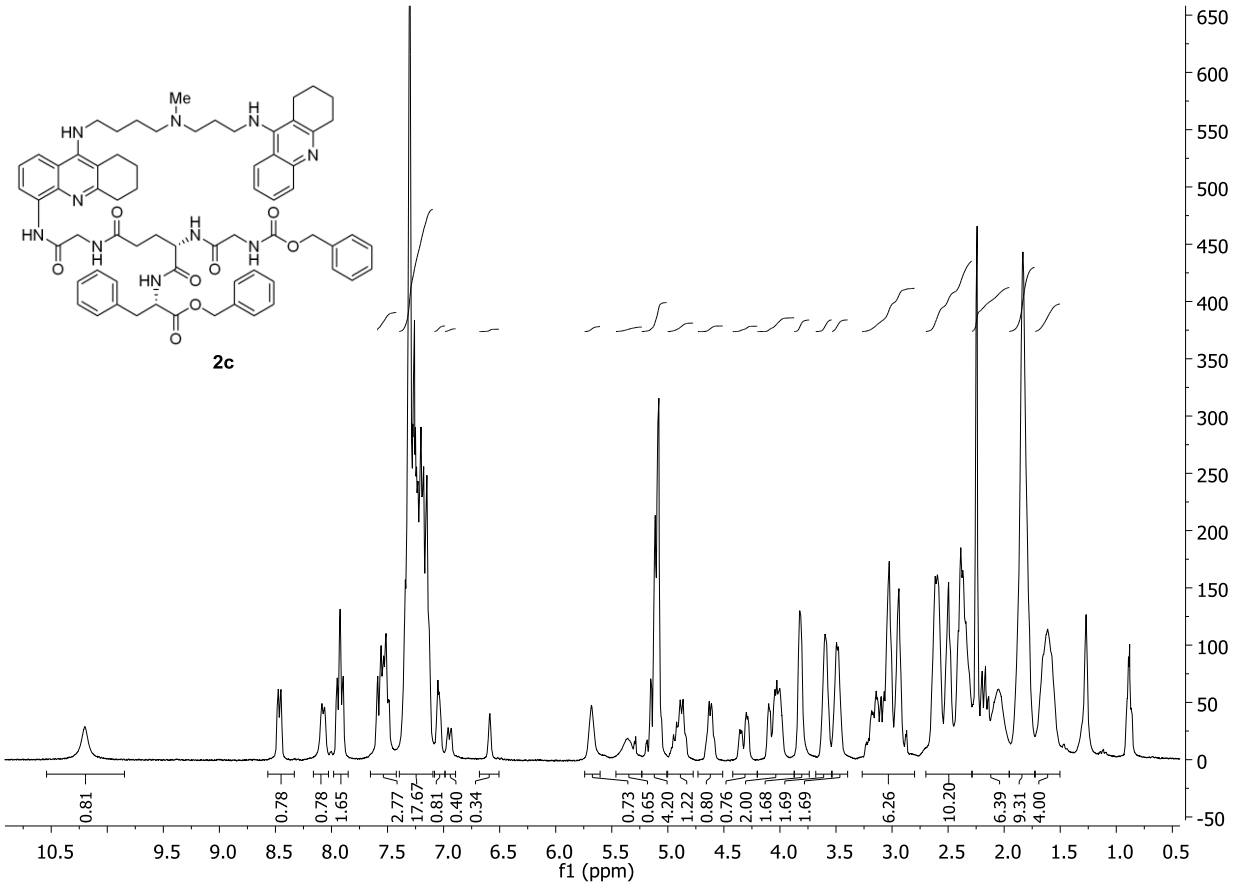

19a
f1 (ppm)

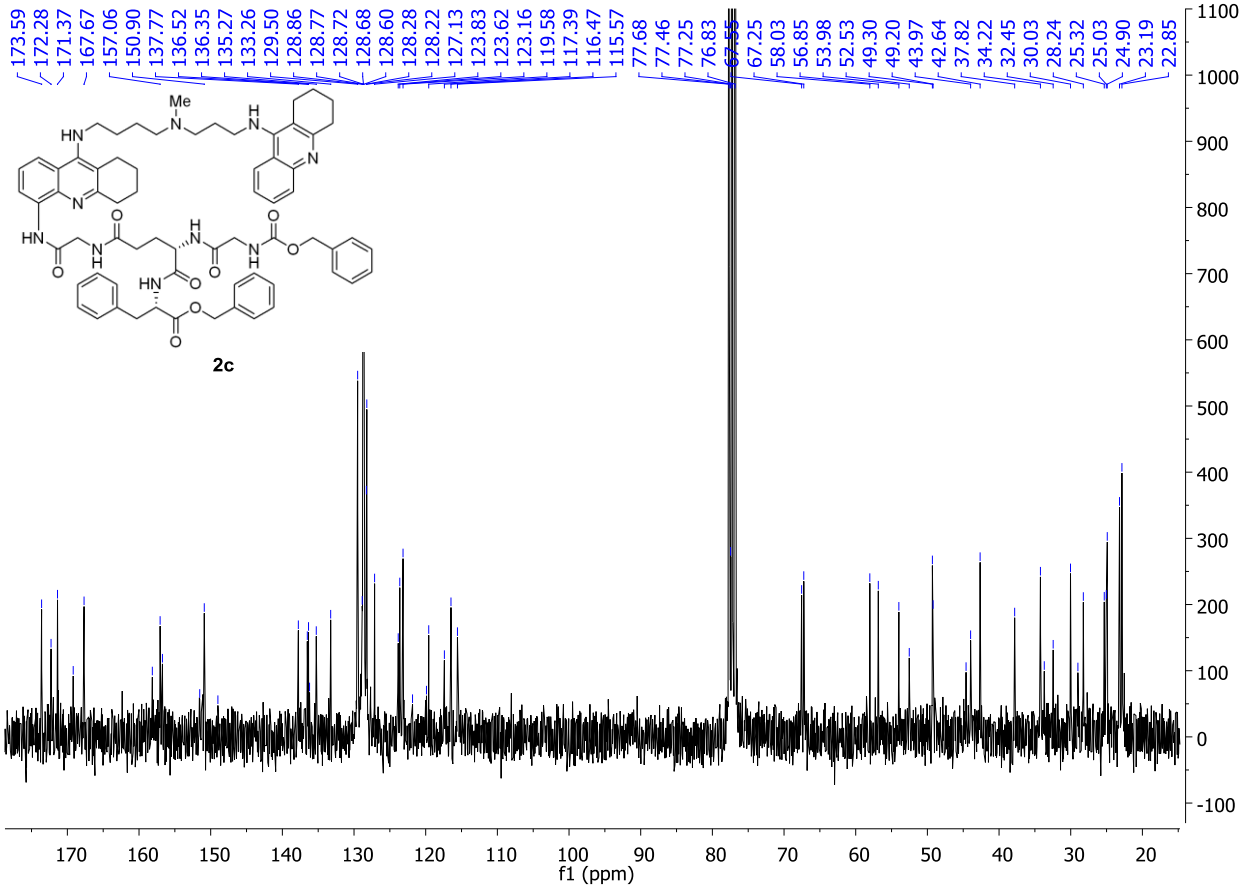

Me
HN
N
H
N
HN
N
Boc
N
H
O
19b
0.88
0.81
1.89
0.85
1.00
2.92
0.74
0.92
2.65
3.96
4.13
4.11
2.03
2.03
2.91
10.36
4.27
8.31
f1 (ppm)


167.75
158.66
156.92
156.10
151.15
150.89
147.72
137.92
133.49
128.91
128.35
123.90
123.50
123.08
120.33
119.65
117.23
116.43
115.70
115.26
80.16
77.74
77.52
77.32
76.90
57.94
56.74
49.33
49.04
45.43
42.57
29.92
28.62
25.44
24.88
23.35
23.24
23.05
Me
HN
N
H
N
HN
N
Boc
N
H
O
19b
X
f1 (ppm)


2b
0.83
0.88
0.97
1.80
0.97
1.96
19.03
0.98
0.94
4.01
1.07
1.00
0.99
2.01
2.01
1.88
2.22
6.42
3.88
2.00
4.09
2.98
2.73
9.87
3.91
f1 (ppm)


173.55
172.31
171.37
169.15
167.63
157.00
151.33
137.85
136.50
136.36
135.26
133.18
129.50
128.77
128.72
128.69
128.63
128.61
128.29
128.22
127.12
123.88
123.43
122.95
119.37
115.73
77.67
77.45
77.25
76.83
67.56
67.26
58.04
56.82
53.98
49.40
49.08
43.96
42.62
37.81
34.24
32.44
29.92
28.29
25.45
24.93
24.85
23.33
23.14
22.93
22.85
2b
f1 (ppm)


2c
0.81
0.78
0.78
1.65
2.77
17.67
0.81
0.40
0.34
0.73
0.65
4.20
1.22
0.80
0.76
2.00
1.68
1.69
1.69
6.26
10.20
6.39
9.31
4.00
f1 (ppm)


173.59
172.28
171.37
167.67
157.06
150.90
137.77
136.52
136.35
135.27
133.26
129.50
128.86
128.77
128.72
128.68
128.60
128.28
128.22
127.13
123.83
123.62
123.16
119.58
117.39
116.47
115.57
77.68
77.46
77.25
76.83
67.25
58.03
56.85
53.98
52.53
49.30
49.20
43.97
42.64
37.82
34.22
32.45
30.03
28.24
25.32
25.03
24.90
23.19
22.85
2c
f1 (ppm)