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Appendix A

Supplementary data

for

Design, synthesis, in vitro and in vivo characterization of 1-{4-[4-(substituted)-piperazin-1-yl]butyl}guanidines and their piperidine analogues, as histamine H_3 receptor antagonists

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Author Contributions

K. Walczyński was responsible for the supervision and development of the whole project.
M. Staszewski performed the chemical syntheses of the newly synthesized compounds and performed preliminary pharmacological studies *in vitro*, both at H_3 and H_1 receptor, elaborated and described the results.
A. Stasiak performed the extended pharmacological studies *in vivo*, elaborated and described the results.
T. Karcz performed the *h*H_3 and *h*H_4 binding affinity tests, elaborated and described the results.

D. McNaught Flores performed the *r*H_3 binding affinity test, elaborated and described the results.
W. A. Fogel coordinated the advanced pharmacological studies *in vivo* and interpreted the obtained results.
K. Kieć-Kononowicz coordinated the *h*H_3 and *h*H_4 binding affinity tests and interpreted the obtained results
R. Leurs coordinated the *r*H_3 binding affinity test and interpreted the obtained results.

1. Chemical synthesis and analysis data

1.1 Compounds 2a.1-2a.11

2a.1. *Preparation of* 7-(pyridin-4-yl)heptan-1-ol hydrochloride

2.5M solution BuLi in hexane (1.2 eq) was added dropwise to 4-picoline (1eq.) in anhydrous THF cooled to -60°C under argon atmosphere. The mixture temperature was increased to room temperature and stirred 1 hour. Then the temperature was decreased to -60°C again and 2-(6-Bromohexyloxy)tetrahydro-2*H*-pyran (1.1eq) was slowly dropped in. The reaction was stirred overnight at room temperature then the mixture was poured into cold water and extracted with EtOAc. The organic layer was concentrated and dissolved in 5% HCl in EtOH solution. The reaction was stirred overnight at room temperature then the solvent was removed under vacuum and the crude product was purified through crystallization (isopropyl alcohol/EtOH/isopropyl acetate – 10/1/20) to yield pure product.

2a.1 - 7-(pyridin-4-yl)heptan-1-olhydrochloride: $C_{12}H_{19}NOxHCl$. M=229.75.White solid.45.3% yield.mp: 167.0-168.2°C.^{1}H NMR (300 MHz, D_2O) δ ppm 8.60-8.57 (m, 2H$^{Py.}$), 7.89-7.87 (m, 2H$^{Py.}$), 3.55-3.51 (t, 2H, OC$\underline{H}_2$, *J*=6.65Hz). 2.93-2.88 (t, 2H, Py.-C$\underline{H}_2$; *J*=7.64Hz), 1.75-1.66 (m, 2H, $CH_2C\underline{H}_2CH_2$), 1.52-1.43 (m, 2H, $CH_2C\underline{H}_2CH_2$), 1.29-1.24 (m, 6H, $CH_2C\underline{H}_2CH_2$).

2a.2. Preparation of 7-(piperidin-4-yl)heptan-1-ol

To a solution of 7-(pyridin-4-yl)heptan-1-of hydrochloride **(2a.1)** (1eq.) in anhydrous ethanol was added PtO_2 (0.1eq.). The reaction was stirred 48 hours at room temperature under 19atm H_2. The precipitate was discarded and the solvent was removed under vacuum. The crude product was dissolved in water and alkalized by 5% sol. of sodium hydroxide, then extracted with EtOAc and dried over $MgSO_4$.The solvent was removed under vacuum and

the crude product was purified by column chromatography (DCM/MeOH/25% NH_3 aq.19:10:1) to yield pure product.

2a.2 - 7-(piperidin-4-yl)heptan-1-ol: $C_{12}H_{25}NO$. M=199.34.White solid.91.9% yield. R_f=0.23 (DCM/MeOH/25% NH_3aq. 19:10:1). mp: 74.1-75.8°C. ^{1}H NMR (300 MHz, *CDCl$_3$*) δ ppm 3.65-3.60 (t,2H,OC$\underline{H}_2$,*J*=6.65Hz), 3.07-3.03 (m,2H^{Piperidine}), 2.61-2.52 (m, 2H^{Piperidine}), 2.06 (s,2H:N$\underline{H}$,O$\underline{H}$,*), 1.69-1.64 (m,2H^{Piperidine}), 1.61-1.51 (qt,2H,$CH_2C\underline{H}_2CH_2$), 1.40-1.01 (m, 13H:3H^{Piperidine} , $CH_2C\underline{H}_2CH_2$).

2a.3. *Preparation of* 7-[1-(*tert*-butoxycarbonyl)piperidin-4-yl]heptan-1-ol

To a solution of 7-(piperidin-4-yl)heptan-1-ol (**2a.2**) (1eq.) and trimethylamine (6eq.) in methanol di-*tert*-butyl dicarbonate (2eq.) in methanol were added dropwise. The reaction was stirred 45 minutes at 60°C. The solvent was removed under vacuum and the crude product was purified by column chromatography (hexane/EtOAc 3:1) to yield pure product.

2a.3 - 7-[1-(*tert*-butoxycarbonyl)piperidin-4-yl]heptan-1-ol: $C_{17}H_{33}NO_3$. M=299.46. Colorless sticky oil. 68.5% yield. R_f=0.26 (hexane/EtOAc 3:1).^{1}H NMR (300 MHz, *CDCl$_3$*) δ ppm4.05 (m, 2H^{Piperidine}), 3.66-3.62 (t, 2H, OC$\underline{H}_2$,*J*=6.65Hz), 2.70-2.62 (m, 2H^{Piperidine}), 1.66-1.23 (m, 25H: 3H^{Piperidine}, $CH_2C\underline{H}_2CH_2$, O$\underline{H}$, C$\underline{H}_3$), 1.12-0.99 (m, 2H^{Piperidine}).

2a.4. *Preparation of* 1-(*tert*-butoxycarbonyl)-4-(7-bromoheptyl)piperidine

To a solution of 7-[1-(*tert*-butoxycarbonyl)piperidin-4-yl]heptan-1-ol **(2a.3)** (1eq.) and triphenylphosphine (2eq.) in DCM under argon atmosphere was added dropwise tetrabromomethane (6eq.) in DCM. The reaction was stirred 20 hours at room temperature. The solvent was removed under vacuum and the crude product was extracted twice by diethyl ether. The solvent was distilled off and the residue was purified by column chromatography (hexane/EtOAc 9:1) to yield pure product

2a.4 - 1-(*tert*-butoxycarbonyl)-4-(7-bromoheptyl)piperidine: $C_{17}H_{32}BrNO_2$. M=362.35. Colorless oil. 29.9% yield. R_f=0.45 (hexane/EtOAc 9:1). ^{1}H NMR (300 MHz, *CDCl$_3$*) δ ppm 4.05 (m,2H^{Piperidine}), 3.43-3.39 (t,2H,BrC$\underline{H}_2$,*J*=6.84Hz), 2.70-2.62 (m,2H^{Piperidine}), 1.90-1.80 (qt, 2H,$CH_2C\underline{H}_2CH_2$), 1.66-1.52 (m,2H^{Piperidine}), 1.45-0.99 (m,22H: 3H^{Piperidine}, $CH_2C\underline{H}_2CH_2$,C$\underline{H}_3$).

2a.5. *Preparation of* 1-(*tert*-butoxycarbonyl)-4-(7-phenoxyheptyl)piperidine

To a dissolved in anhydrous ethanol sodium (1.1eq) was added phenol (1.1eq.) and stirred 30 minutes at room temperature. To a solution of 1-(*tert*-butoxycarbonyl)-4-(7-bromoheptyl)piperidine **(2a.4)** (1eq.) in anhydrous ethanol freshly prepared sodium phenoxide solution was added dropwise. The reaction was stirred overnight at 80°C. The solvent was removed under vacuum and the crude product was alkalized by 5% NaOH aq. The solution then extracted with DCM and dried over $MgSO_4$. The solvent was removed under vacuum and purified by column chromatography (hexane/EtOAc 9:1) to yield pure product.

2a.5 - 1-(*tert*-butoxycarbonyl)-4-(7-phenoxyheptyl)piperidine: $C_{23}H_{37}NO_3$. M=375.56. Colorless oil. 65.5% yield; .R_f=0.64 (hexane/EtOAc 9:1). ^{1}H NMR (300 MHz, *CDCl$_3$*) δ ppm 7.31-7.24 (m, 2H$^{phenoxy.}$, C(CHC<u>H</u>)$_2$CH), 6.95-6.87 (m, 3H$^{phenoxy.}$, C(C<u>H</u>CH)$_2$C<u>H</u>), 4.08-4.04 (m, 2H^{Piperidine}), 3.97-3.93 (t, 2H, OC<u>H$_2$</u>,*J*=6.55Hz), 2.70-6.62 (m, 2H^{Piperidine}), 1.82-1.73 (qt, 2H, CH$_2$C<u>H$_2$</u>CH$_2$), 1.66-1.21 (m, 22H: 3H^{Piperidine}, CH$_2$C<u>H$_2$</u>CH$_2$, C<u>H$_3$</u>), 1.12-0.99 (m, 2H^{Piperidine}).

2a.6. *Preparation of* 4-(7-phenoxyheptyl)piperidine

To a solution of the 1-(*tert*-butoxycarbonyl)-4-(7-phenoxyheptyl)piperidine (**2a.5**) (1eq.) in chloroform was added dropwise 4M solution hydrogen chloride in dioxane (20 eq.). The reaction was stirred overnight at room temperature then the solvent was removed under vacuum. The crude product was evaporated twice with chloroform, dissolved in water and alkalized by 5% NaOH aq. The solution was then extracted with DCM and dried over Na_2SO_4. The solvent was removed under vacuum to yield pure product.

2a.6 - 4-(7-phenoxyheptyl)piperidine: $C_{18}H_{29}NO$.M=275.44. White solid.89.0% yield. R_f=0.26 (DCM/MeOH/25% NH_3 aq.139:10:1). mp: 39.4-40.9°C. ^{1}H NMR (300 MHz, *CDCl$_3$*) δ ppm 7.31-7.24 (m, 2H$^{phenoxy.}$, C(CHC<u>H</u>)$_2$CH), 6.95-6.87 (m, 3H$^{phenoxy.}$, C(C<u>H</u>CH)$_2$C<u>H</u>), 3.97-3.93 (t, 2H, OC<u>H$_2$</u>,*J*=6.55Hz), 3.07-3.03 (m, 2H^{Piperidine}), 2.61-2.52 (m, 2H^{Piperidine}), 1.82-1.73 (m, 3H: CH$_2$C<u>H$_2$</u>CH$_2$, N<u>H</u>), 1.68-1.64 (m, 2H^{Piperidine}), 1.48-1.21 (m, 11H: 1H^{Piperidine}, CH$_2$C<u>H$_2$</u>CH$_2$), 1.15-1.01 (m, 2H^{Piperidine}).

2a.7. *Preparation of* 4-[4-(7-phenoxyheptyl)piperidin-1-yl]butanenitrile

4-Bromobutyronitrile (1.1eq.) was added to a mixture of 4-(7-phenoxyheptyl)piperidine (**2a.6**) (1eq.) and potassium carbonate (4eq.) in acetonitrile. The reaction was stirred overnight at room temperature. The mixture was filtered then the solvent was removed under vacuum

and the crude product was purified by column chromatography (DCM/MeOH - 49:1) to yield pure product.

2a.7 - 4-(4-(7-phenoxyheptyl)piperidin-1-yl)butanenitrile: $C_{22}H_{34}N_2O$. M=342.53.Colorless oil. 80.4% yield. R_f=0.11 (DCM/MeOH49:1). ^{1}H NMR (300 MHz, *CDCl$_3$*) δ ppm 7.31-7.24 24 (m, 2H$^{phenoxy.}$, C(CHC<u>H</u>)$_2$CH),6.95-6.87 (m, 3H$^{phenoxy.}$, C(C<u>H</u>CH)$_2$C<u>H</u>), 3.97-3.92 (t, 2H, OC<u>H$_2$</u>,*J*=6.55Hz), 2.87-2.84 (m, 2H^{Piperidine}), 2.45-2.39 (m, 4H: C<u>H$_2$</u>CN, C<u>H$_2$</u>N), 1.97-1.21 (m, 21H: 7H^{Piperidine}, CH$_2$C<u>H$_2$</u>CH$_2$).

2a.8. *Preparation of* 4-[4-(7-phenoxyheptyl)piperidin-1-yl]butan-1-amine

To a solution of 4-[4-(7-phenoxyheptyl)piperidin-1-yl]butanenitrile (**2a.7**) (1eq.) in diethyl ether was added $LiAlH_4$ (4eq.). The reaction was stirred overnight at room temperature then the mixture was quenched by dropwise addition of water (16eq.) and stirred for 30 minutes then filtered. The precipitate was discarded. The organic layer was dried over Na_2SO_4 then the solvent was removed under vacuum and the crude product was purified with column chromatography (DCM/MeOH/25% NH_3 aq. 49:10:1) to yield pure product (**2a.8**).

2a.8 - 4-[4-(7-phenoxyheptyl)piperidin-1-yl]butan-1-amine: $C_{22}H_{38}N_2O$. M=346.56. White solid. 89.9% yield. R_f=0.64 (DCM/MeOH/25% NH_3 aq.49:10:1). mp: 66.2-68.2°C. ^{1}H NMR (300 MHz, *CDCl$_3$*) δ ppm 7.29-7.24 (m, 2H$^{phenoxy.}$, C(CHC<u>H</u>)$_2$CH), 6.95-6.88 (m, 3H$^{phenoxy.}$, C(C<u>H</u>CH)$_2$C<u>H</u>), 3.97-3.92 (t, 2H, OC<u>H$_2$</u>,*J*=6.55Hz), 2.94-2.90 (m, 2H^{Piperidine}), 2.73-2.69 (m, 2H, C<u>H$_2$</u>NH$_2$), 2.34-2.29 (m, 2H, C<u>H$_2$</u>-Piperidine), 2.04 (br, 2H, N<u>H$_2$</u>, *), 1.89-1.73 (m, 4H: 2H^{Piperidine}, CH$_2$C<u>H$_2$</u>CH$_2$), 1.66-1.65 (m, 2H^{Piperidine}), 1.56-1.20 (m, 17H: 3H^{Piperidine}, CH$_2$C<u>H$_2$</u>CH$_2$).

2a.9. *Preparation of N*-{4-[4-(7-phenoxyheptyl)piperidin-1-yl]butyl}-4-(trifluoromethyl)benzamide

4-(Trifluoromethyl)benzoyl chloride (1.1eq.) was added dropwise to mixture of 4-[4-(7-phenoxyheptyl]piperidin-1-yl)butan-1-amine (**2a.8)** (1eq.) and triethylamine (2eq.) in DCM. The reaction was stirred 2 hours at room temperature. The mixture was washed three times with water and dried over anhydrous Na_2SO_4. Then the solvent was removed under vacuum and the crude product was purified by column chromatography (EtOAc/MeOH/Triethylamine 89:10:1) to yield pure product.

2a.9 - *N*-(4-(4-(7-phenoxyheptyl)piperidin-1-yl)butyl)-4-(trifluoromethyl)benzamide: $C_{30}H_{41}F_3N_2O_2$. M=518.67. White solid. 68.4% yield. R_f=0.32 (EtOAc/MeOH/Triethylamine 89:10:1). mp: 118.5-119.4°C. ^{1}H NMR (300 MHz, *CDCl$_3$*) δ ppm 7.94-7.91 (m, 2H$^{arom.}$), 7.70-7.69 (m, 2H$^{arom.}$), 7.54 (br, 1H, NH), 7.30-7.24 (m, 2H$^{phenoxy.}$, C(CHCH)$_2$CH), 6.95-6.87 (m, 3H$^{phenoxy.}$, C(CHCH)$_2$CH), 3.97-3.93 (t, 2H, OCH$_2$,*J*=6.55Hz), 3.48-3.46 (m, 2H, CH$_2$NH), 3.02-2.98 (m, 2H^{Piperidine}), 2.48-2.47 (m, 2H, CH$_2$-Piperidine), 2.05-1.17 (m, 23H: 7H^{Piperidine}, CH$_2$CH$_2$CH$_2$).

2a.10. *Preparation of* 4-[4-(7-phenoxyheptyl)piperidin-1-yl]-*N*-[4-(trifluoromethyl)benzyl]butan-1-amine

$LiAlH_4$ (4eq.) was added to a mixture of *N*-{4-[4-(7-phenoxyheptyl)piperidin-1-yl]butyl}-4-(trifluoromethyl)benzamide **(2a.9)** (1eq.) in diethyl ether. The reaction was stirred overnight at room temperature then the mixture was quenched by dropwise addition of water (16eq.) and stirred for 30 minutes then filtered. The precipitate was discarded. The organic phase was dried over Na_2SO_4 then the solvent was removed under vacuum and the crude product was purified with column chromatography (EtOAc/MeOH/Triethylamine 39:10:1) to yield pure product.

2a.10 - 4-[4-(7-phenoxyheptyl)piperidin-1-yl]-*N*-[4-(trifluoromethyl)benzyl]butan-1-amine: $C_{30}H_{43}F_3N_2O$. M=504.69. White solid. 80.7% yield. R_f=0.41 (EtOAc/MeOH/Triethylamine 39:10:1). mp: 59.6-61.3°C. ^{1}H NMR (300 MHz, *CDCl$_3$*) δ ppm 7.59-7.56 (m, 2H$^{arom.}$), 7.47-7.44 (m, 2H$^{arom.}$), 7.31-7.24 (m, 2H$^{phenoxy.}$, C(CHCH)$_2$CH), 6.96-6.87 (m, 3H$^{phenoxy.}$, C(CHCH)$_2$CH), 3.97-3.93 (t, 2H, OCH$_2$,*J*=6.65Hz), 3.85 (s, 2H, CH$_2$Ph), 2.94-2.91 (m, 2H^{Piperidine}), 2.67-2.62 (m, 2H, CH$_2$CH$_2$NH), 2.36-2.31 (m, 2H, CH$_2$N^{piperidine}), 2.08 (br, 1H, NH, *), 1.98-1.20 (m, 23H: 7H^{Piperidine}, CH$_2$CH$_2$CH$_2$).

2a.11. *Preparation of* 2,3-di(*tert*-butoxycarbonyl)-1-{4-[4-(7-phenoxyheptyl)piperidin-1-yl]butyl}-1-[4-(trifluoromethyl)benzyl]guanidine

1,3-bis(*tert*-butoxycarbonyl)-2-methyl-2-thiopseudourea (1.1eq.) and mercury II chloride (1.1eq) were sequentially added to an ice-cooled mixture of 4-[4-(7-phenoxyheptyl)piperidin-1-yl]-*N*-[4-(trifluoromethyl)benzyl]butan-1-amine **(2a.10)** (1eq.) and trimethylamine (5eq.) in DCM. The ice bath was removed and the reaction was stirred 18 hours at room temperature then filtered. The precipitate was discarded. The filtrate was washed sequentially twice with H_2O and twice with brine. The organic phase was dried over Na_2SO_4 then the solvent was

removed under vacuum and the crude product was purified with column chromatography (EtOAc/MeOH/Triethylamine 189:10:1) to yield pure product.

2a.11 - 2,3-di(*tert*-butoxycarbonyl)-1-{4-[4-(7-phenoxyheptyl)piperidin-1-yl]butyl}-1-(4-(trifluoromethyl)benzyl)guanidine: $C_{41}H_{61}F_3N_4O_5$. M=746.96. White solid. 81.9% yield. R_f=0.21 (EtOAc/MeOH/Triethylamine 189:10:1). mp: 112.7-113.9°C. ^{1}H NMR (300 MHz, *CDCl*$_3$) δ ppm 9.91 (br, 1H, NH), 7.60-7.58 (m, 2H$^{arom.}$), 7.43-7.40 (m, 2H$^{arom.}$), 7.30-7.23 (m, 2H$^{phenoxy.}$, C(CHCH)$_2$CH), 6.95-6.86 (m, 3H$^{phenoxy.}$, C(CHCH)$_2$CH), 4.16-4.08 (s, 2H, CH$_2$Ph), 3.97-3.92(t, 2H, OCH$_2$,*J*=6.55Hz), 3.34-3.29 (m, 2H, CH$_2$CH$_2$NC(N)), 2.87-2.83 (m, 2H^{Piperidine}), 2.28-2.26 (m, 2H, CH$_2$N^{piperidine}), 1.96-1.19 (m, 41H: 7H^{Piperidine}, CH$_2$CH$_2$CH$_2$, CH$_3$).

***1.2 Compounds* 2b.1 - 2b.7**

2b.1 *Preparation of* methyl 4-(piperidin-4-yl)butanoate hydrochloride

4-Piperidine butyric acid hydrochloride (1eq.) was added dropwise to 8.7% solution hydrogen chloride-methanol (1.6eq.). The reaction was stirred 20 hours at 70°C then the solvent was removed under vacuum and the crude product was directly used in the next step without further purification.

2b.1 - Methyl 4-(piperidin-4-yl)butanoate hydrochloride: $C_{10}H_{19}NO_2$·HCl. M=221.73. ^{1}H NMR (300 MHz, *DMSO*) δ ppm 8.95 (br, 1H, NH), 8.72 (br, 1H, HCl), 3.58 (s, 3H, OCH$_3$), 3.22-3.18 (m, 2H^{piperidine}), 2.84-2.73 (m, 2H^{piperidine}), 2.32-2.18 (dt, 2H, CH$_2$C(O), *J*=7.34Hz), 1.78-1.73 (m, 2H^{piperidine}), 1.58-1.45 (m, 3H: 1H^{piperidine}, CH$_2$CH$_2$CH$_2$), 1.35-1.19 (m, 4H: 2H^{piperidine}, 2H$^{alif.}$,CHCH$_2$).

2b.2. *Preparation of* 4-(piperidin-4-yl)butanamide

To a crude methyl 4-(piperidin-4-yl)butanoate hydrochloride (**2b.1**) (1eq.) was added 25% ammonium hydroxide water solution (6eq.). The reaction was stirred 4 hours at room temperature under gaseous ammonia atmosphere then the solvent was removed under vacuum and the crude product was directly used in the next step without further purification.

2b.2 - 4-(piperidin-4-yl)butanamide: $C_9H_{18}N_2O$.M=170.26. ^{1}H NMR (300 MHz, *D$_2$O*) δ ppm 3.39-3.34 (m, 2H^{piperidine}), 2.98-2.88 (m, 2H^{piperidine}), 2.32-2.21 (dt, 2H, CH$_2$C(O), *J*=7.44Hz), 1.94-1.89 (m, 2H^{piperidine}), 1.64-1.52 (m, 3H: 1H^{piperidine}, CH$_2$CH$_2$CH$_2$), 1.39-1.25 (m, 4H: 2H^{piperidine}, 2H$^{alif.}$,CHCH$_2$).

2b.3. *Preparation of* 4-[1-(7-phenoxyheptyl)piperidin-4-yl]butanamide

To a crude 4-(piperidin-4-yl)butanamide **(2b.2)** (1eq.) in methanol was added 7-phenoxyheptyl bromide (1eq.) and potassium carbonate (4eq.). The reaction was stirred 20 hours at 70°C, then filtered. The precipitate was discarded then the solvent was removed under vacuum. and the crude product (**2b.3**) was directly used in the next step without further purification.

2b.3 - 4-[1-(7-phenoxyheptyl)piperidin-4-yl]butanamide: $C_{22}H_{36}N_2O_2$. M=360.54. White solid. R_f=0.32 (DCM/MeOH/25% NH_3 aq. 139:10:1). mp: 102.8-104.8°C. ^{1}H NMR (300 MHz, *CDCl$_3$*) δ ppm 7.31-7.24 (m, 2H$^{phenoxy.}$, C(CHC<u>H</u>)$_2$CH), 6.95-6.86 (m, 3H$^{phenoxy.}$, C(C<u>H</u>CH)$_2$C<u>H</u>), 5.40-5.29 (br, 2H, N<u>H$_2$</u>), 3.97-3.92 (t, 2H, OC<u>H$_2$</u>, *J*=6.55Hz), 3.02-2.98 (m, 2H^{piperidine}), 2.40-2.35 (m, 2H, C<u>H$_2$</u>C(O)), 2.23-2.18 (m, 2H, C<u>H$_2$</u>N^{piperidine}), 1.98-1.27 (m, 21H: 7H^{piperidine}, 14H$^{alif.}$,CHC<u>H$_2$</u>, CH$_2$C<u>H$_2$</u>CH$_2$).

2b.4. *Preparation of* 4-[1-(7-phenoxyheptyl)piperidin-4-yl]butan-1-amine

To a crude 4-[1-(7-phenoxyheptyl)piperidin-4-yl]butanamide **(2b.3)** (1eq.)in diethyl ether was added $LiAlH_4$ (4eq.). The reaction was stirred overnight at room temperature then the mixture was quenched by dropwise addition of water (16eq.) and stirred for 30 minutes then filtered. The precipitate was discarded. The organic phase was dried over Na_2SO_4, then the solvent was distilled off and the crude product was purified with column chromatography (DCM/MeOH/25% NH_3 aq. 89:10:1) to yield pure product (**2b.4**).

2b.4 - 4-(1-(7-phenoxyheptyl)piperidin-4-yl)butan-1-amine: $C_{22}H_{38}N_2O$. M=346.56.White solid. 3.70% yield (counted from **2b.1**) .R_f=0.25 (DCM/MeOH/25% NH_3 aq. 89:10:1).mp: 78.5-80.5°C.^{1}H NMR (300 MHz, *CDCl$_3$*) δ ppm 7.30-7.23 (m, 2H$^{phenoxy.}$, C(CHC<u>H</u>)$_2$CH), 6.95-6.86 (m, 3H$^{phenoxy.}$, C(C<u>H</u>CH)$_2$C<u>H</u>), 3.96-3.92 (t, 2H, OC<u>H$_2$</u>,*J*=6.55Hz), 2.93-2.89 (m, 2H^{piperidine}), 2.69-2.65 (t, 2H, C<u>H$_2$</u>NH$_2$,*J*=6.85Hz), 2.31-2.28 (t, 2H, C<u>H$_2$</u>N^{piperidine},*J*=6.76Hz), 1.98-1.75 (m, 4H: 2H^{piperidine}, CH$_2$C<u>H$_2$</u>CH$_2$), 1.68-1.23 (m, 21H: 5H^{piperidine},CH$_2$C<u>H$_2$</u>CH$_2$, N<u>H$_2$</u>, *).

2b.5 *Preparation of N*-{4-[1-(7-phenoxyheptyl)piperidin-4-yl]butyl}-4-(trifluoromethyl)benzamide

4-(Trifluoromethyl)benzoyl chloride (1.1eq.) was added dropwise to mixture of 4-[1-(7-phenoxyheptyl)piperidin-4-yl]butan-1-amine (**2b.4**) (1eq.) and triethylamine (2eq.) in DCM. The reaction was stirred 2 hours at room temperature. The mixture was washed 3-times with water and dried over Na_2SO_4. The solvent was removed under vacuum and the crude

product was purified by column chromatography (DCM/MeOH/25% NH_3 aq. 189:10:1) to yield pure product.

2b.5 - *N*-{4-[1-(7-phenoxyheptyl)piperidin-4-yl]butyl}-4-(trifluoromethyl)benzamide: $C_{30}H_{41}F_3N_2O_2$. M=518.67.White solid. 52.63% yield. R_f=0.42 (DCM/MeOH/25% NH_3 aq. 189:10:1). mp: 124.8-125.8°C. ^{1}H NMR (300 MHz, *CDCl$_3$*) δ ppm 7.87-7.85 (m, 2H$^{arom.}$), 7.71-7.68 (m, 2H$^{arom.}$), 7.29-7.24 (m, 2H$^{phenoxy.}$, C(CHC<u>H</u>)$_2$CH), 6.95-6.87 (m, 3H$^{phenoxy.}$, C(C<u>H</u>CH)$_2$C<u>H</u>),6.10 (m, 1H, N<u>H</u>), 3.96-3.92 (t, 2H, OC<u>H</u>$_2$,*J*=6.55Hz), 3.50-3.44 (m, 2H, C<u>H</u>$_2$NH), 2.93-2.90 (m, 2H^{Piperidine}), 2.32-2.27 (m, 2H, C<u>H</u>$_2$N^{piperidine}), 1.86-1.25 (m, 23H: 7H^{piperidine}, CH$_2$C<u>H</u>$_2$CH$_2$).

2b.6 *Preparation of* 4-[1-(7-phenoxyheptyl)piperidin-4-yl]-*N*-[4-(trifluoromethyl)benzyl]butan-1-amine

$LiAlH_4$ (4eq.) was added to a mixture of *N*-{4-[1-(7-phenoxyheptyl)piperidin-4-yl]butyl}-4-(trifluoromethyl)benzamide (**2b.5**) (1eq.) in diethyl ether. The reaction was stirred overnight at room temperature then the mixture was quenched by dropwise addition of water (16eq.) and stirred for 30 minutes then filtered. The precipitate was discarded. The organic phase was dried over Na_2SO_4, then the solvent was removed under vacuum and the crude product was purified through column chromatography (DCM/MeOH/25% NH_3 aq. 189:10:1) to yield pure product.

2b.6 - 4-[1-(7-phenoxyheptyl)piperidin-4-yl]-*N*-[4-(trifluoromethyl)benzyl]butan-1-amine: $C_{30}H_{43}F_3N_2O$. M=506.69. White solid. 99.8% yield. R_f=0.24 (DCM/MeOH/25% NH_3 aq. 189:10:1). mp: 57.4-59.4°C. ^{1}H NMR (300 MHz, *CDCl$_3$*) δ ppm 7.61-7.56 (m, 2H$^{arom.}$), 7.45-7.42 (m, 2H$^{arom.}$), 7.29-7.24 (m, 2H$^{phenoxy.}$, C(CHC<u>H</u>)$_2$CH), 6.95-6.87(m, 3H$^{phenoxy.}$, C(C<u>H</u>CH)$_2$C<u>H</u>), 3.96-3.92 (t, 2H, OC<u>H</u>$_2$; *J*=6.55Hz), 3.83 (s, 2H, C<u>H</u>$_2$Ph), 2.92-2.87 (m, 2H^{Piperidine}), 2.62-2.58 (t, 2H, CH$_2$C<u>H</u>$_2$NH; *J*=7.04Hz), 2.29-2.25 (m, 2H, C<u>H</u>$_2$N^{piperidine}), 1.83-1.21 (m, 24H: 7H^{Piperidine}, CH$_2$C<u>H</u>$_2$CH$_2$,N<u>H</u>, *).

2b.7 *Preparation of* 2,3-di(*tert*-butoxycarbonyl)-1-{4-[1-(7-phenoxyheptyl)piperidin-4-yl]butyl}-1-[4-(trifluoromethyl)benzyl]guanidine

1,3-bis(*tert*-butoxycarbonyl)-2-methyl-2-thiopseudourea (1.1eq.) and mercury II chloride (1.1eq) were sequentially added to an ice-cooled mixture of 4-[1-(7-phenoxyheptyl)piperidin-4-yl]-*N*-[4-(trifluoromethyl)benzyl]butan-1-amine **(2b.6)** (1eq.) and trimethylamine (5eq.) in DCM. The ice bath was removed and the reaction was stirred 18 hours at room temperature

then filtered. The precipitate was discarded. The filtrate was washed sequentially twice with H_2O and twice with brine. The organic phase was dried over Na_2SO_4 then the solvent was removed under vacuum and the crude product was purified with column chromatography (EtOAc/MeOH/Triethylamine 189:10:1) to yield pure product.

2b.7 - 2,3-di(*tert*-butoxycarbonyl)-1-{4-[1-(7-phenoxyheptyl)piperidin-4-yl]butyl}-1-[4-(trifluoromethyl)benzyl]guanidine: $C_{41}H_{61}F_3N_4O_5$. M=746.96. White solid. 47.0% yield. R_f=0.30 (EtOAc/MeOH/Triethylamine 189:10:1). mp: 111.7-112.3°C. ^{1}H NMR (600 MHz, *CDCl$_3$)* δ ppm 9.95 (br, 1H, N<u>H</u>), 7.59-7.58 (m, 2H$^{arom.}$), 7.43-7.41 (m, 2H$^{arom.}$), 7.28-7.25 (m, 2H$^{phenoxy.}$, C(CHC<u>H</u>)$_2$CH), 6.93-6.90 (m, 1H$^{phenoxy.}$, C(CHCH)$_2$C<u>H</u>), 6.89-6.87 (m, 2H$^{phenoxy.}$, C(CHCH)$_2$C<u>H</u>), 4.77 (s, 2H, C<u>H</u>$_2$Ph), 3.95-3.93 (t, 2H, OC<u>H</u>$_2$, *J*=6.55Hz), 3.29 (m, 2H, CH$_2$C<u>H</u>$_2$NC(N)), 2.88-2.86 (m, 2H^{Piperidine}), 2.28-2.26 (m, 2H, C<u>H</u>$_2$N^{piperidine}), 1.84-1.81 (m, 2H^{Piperidine}), 1.79-1.74 (qt, 2H, CH$_2$C<u>H</u>$_2$CH$_2$), 1.61-1.59 (m, 4H: 2H^{Piperidine}, CH$_2$C<u>H</u>$_2$CH$_2$), 1.53-1.43 (m, 23H: 1H^{Piperidine}, CH$_3$, CH$_2$C<u>H</u>$_2$CH$_2$), 1.39-1.34 (m, 2H, CH$_2$C<u>H</u>$_2$CH$_2$), 1.33-1.29 (m, 2H, CH$_2$C<u>H</u>$_2$CH$_2$), 1.27-1.16 (m, 6H: 2H^{Piperidine}, CH$_2$C<u>H</u>$_2$CH$_2$).

1.3 Compounds 2c.2 *and* 2d.2

2c.1/2d.1 *Preparation of* 1,3-di(*tert*-butoxycarbonyl)-1-benzyl-2-methyl-2-thiopseudourea **2c.1** and 1,3-di(*tert*-butoxycarbonyl)-1-[4-(trifluoromethyl)benzyl]-2-methyl-2thiopseudourea **2d.1**

First, 60% sodium hydride oil dispersion (10eq.) was added to an ice-cooled mixture of 1,3-bis(*tert*-butoxycarbonyl)-2-methyl-2-thiopseudourea (1eq.) in DMF. The reaction was stirred 1 hour at room temperature then cooled to 0°C and the appropriate benzyl bromide **10** and **11** (1.15eq.) was added. The reaction was stirred overnight, then the water was added and the mixture was extracted with EtOAc. The organic phase was washed twice with brine and was dried over $MgSO_4$. The solvent was removed under vacuum and the crude product was purified by column chromatography (hexane/EtOAc/Triethylamine 160:10:1) to yield pure product.

2c.1 **-** 1,3-di(*tert*-butoxycarbonyl)-1-benzyl-2-methyl-2-thiopseudourea: $C_{17}H_{28}N_2O_4S$. M=380.51. Sticky colorless oil. 96.9% yield. R_f=0.20 (hexane/EtOAc/Triethylamine 160:10:1). ^{1}H NMR (300 MHz, *CDCl$_3$*) δ ppm 7.36-7.25 (m, 5H$^{arom.}$), 4.78 (s, 2H, C<u>H</u>$_2$), 2.29 (s, 3H, SC<u>H</u>$_3$), 1.52 (s, 9H, CC<u>H</u>$_3$), 1.40 (m, 9H, CC<u>H</u>$_3$).

2d.1 - 1,3-di(*tert*-butoxycarbonyl)-1-[4-(trifluoromethyl)benzyl]-2-methyl-2thiopseudourea: $C_{20}H_{27}F_3N_2O_4S$. M=448.51.White solid. 92.2% yield. R_f=0.13 (hexane/EtOAc/Triethylamine 160:10:1). mp: 48.8-50.5°C. ^{1}H NMR (300 MHz, *CDCl$_3$*) δ ppm 7.60-7.58 (m, 2H$^{arom.}$), 7.48-7.45 (m, 2H$^{arom.}$), 4.82 (s, 2H, C$\underline{H}_2$), 2.33 (s, 3H, SC$\underline{H}_3$), 1.53 (s, 9H, CC$\underline{H}_3$), 1.41 (s, 9H, CC$\underline{H}_3$).

2c.2/2d.2 *Preparation of* 2,3-di(*tert*-butyloxycarbonyl)-3-benzyl-1-{4-[4-(7-phenoxyheptyl)piperazin-1-yl]butyl}guanidine **2c.2** *and* 2,3-di(*tert*-butyloxycarbonyl)-1-{4-[4-(7-phenoxyheptyl)piperazin-1-yl]butyl}-3-[4-(trifluoromethyl)benzyl]guanidine **2d.2**,

To a solution of 4-(4-(7-phenoxyheptyl)piperazin-1-yl)butyl-1-amine (2eq.) in THF-water (10:1) mixture the appropriate 1,3-di(*tert*-butoxycarbonyl)-1-[4-(substituted)benzyl]-2-methyl-2thiopseudourea **2c.1** and **2d.1** (1eq.) was added. The reaction was stirred 2 hours at 70°C then overnight at room temperature. The mixture was diluted in EtOAc and washed twice with water and twice by brine. The organic phase was dried over anhydrous $MgSO_4$ then the solvent was removed under vacuum and the crude product **2c.2** was purified by column chromatography (DCM/MeOH 48:2) to yield pure product.

2c.2 - 2,3-di(*tert*-butyloxycarbonyl)-3-benzyl-1-{4-[4-(7-phenoxyheptyl)piperazin-1-yl]butyl}guanidine: $C_{39}H_{61}N_5O_5$. M=679.95. Sticky yellowish oil. 76.0% yield. R_f=0.38 (DCM/MeOH 48:2). ^{1}H NMR (300 MHz, *CDCl$_3$*) δ ppm 9.55 (s, 1H, N$\underline{H}$), 7.30-7.24 (m, 7H: 5H$^{arom.}$, 2H$^{phenoxy.}$), 6.95-6.86 (m, 3H$^{phenoxy.}$, C(C$\underline{H}$CH)$_2$C$\underline{H}$), 4.85 (s, 2H, C$\underline{H}_2$Ph), 3.97-3.92 (t, 2H, OC$\underline{H}_2$; *J*=6.45Hz), 3.02 (m, 2H, CH$_2$C$\underline{H}_2$NC(N)), 2.43-2.29 (m, 10H: 8H^{pip}, C$\underline{H}_2$N^{piperazine}), 2.20 (br, 2H, C$\underline{H}_2$N^{piperazine}), 1.82-1.73 (m, 2H, CH$_2$C$\underline{H}_2$CH$_2$), 1.51-1.32 (m, 30H: CH$_2$C$\underline{H}_2$CH$_2$, C$\underline{H}_3$).

2d.2 - 2,3-di(*tert*-butyloxycarbonyl)-1-{4-[4-(7-phenoxyheptyl)piperazin-1-yl]butyl}-3-[4-(trifluoromethyl)benzyl]guanidine: $C_{40}H_{60}F_3N_5O_5$. M=747.95. Sticky colorless oil. 100% yield. R_f=0.40 (DCM/MeOH 48:2).^{1}H NMR (300 MHz, *CDCl$_3$*) δ ppm 9.51 (s, 1H, N$\underline{H}$), 7.58-7.55 (m, 2H$^{arom.}$), 7.44-7.42 (m, 2H$^{arom.}$), 7.29-7.23 (m, 2H$^{phenoxy.}$, C(CHC$\underline{H}$)$_2$CH), 6.95-6.86 (m, 3H$^{phenoxy.}$, C(C$\underline{H}$CH)$_2$C$\underline{H}$), 4.92 (s, 2H, C$\underline{H}_2$Ph), 3.97-3.92 (t, 2H, OC$\underline{H}_2$,*J*=6.55Hz), 3.09 (m, 2H, CH$_2$C$\underline{H}_2$NC(N)), 2.45-2.31 (m, 10H: 8H^{pip}, C$\underline{H}_2$N^{piperazine}), 2.25 (m, 2H, C$\underline{H}_2$N^{piperazine}), 1.80-1.73 (m, 2H, CH$_2$C$\underline{H}_2$CH$_2$), 1.49-1.27 (m, 30H: CH$_2$C$\underline{H}_2$CH$_2$, C$\underline{H}_3$).

1.4 Compound 2e.2

Preparation of phenyl 1-cyano-2-phenylisourea **2e.1**

To a solution of the *N*-cyano-diphenylimidocarbonate **13** (1eq.) in DCM, a 10% ammonia solution in methanol (10eq.) was added dropwise. The reaction was stirred overnight at room temperature then the solvent was removed under vacuum and the crude product was purified by column chromatography (DCM/EtOAc 8:2 and EtOAc) to yield pure product.

2e.1 - 1-cyano-2-phenylisourea: $C_8H_7N_3O$. M=161.16. White solid. 81.4% yield. R_f=0.38 (DCM/EtOAc 8:2). mp: 154.0-155.1°C. ^{1}HNMR (600MHz,*CDCl*$_3$) δppm 7.42-7.39 (m, 2H$^{phenoxy.}$, C(CHC<u>H</u>)$_2$CH), 7.31-7.28 (m,1H$^{phenoxy.}$,C(CHCH)$_2$C<u>H</u>), 7.12-7.10 (m,2H$^{phenoxy.}$,C(C<u>H</u>CH)$_2$CH), 6.10 (br,2H,N<u>H</u>$_2$).

Preparation of 1-benzyl-3-cyano-1-{4-[4-(7-phenoxyheptyl)piperazin-1-yl]but-1-yl}- guanidine **2e.2**

To a solution of the 1-cyano-2-phenylisourea **(2e.1)** (1eq.) in acetonitrile, the *N*-benzyl-4-[4-(7-phenoxyheptyl)piperazin-1-yl]butan-1-amine (1.1eq.) was added. The reaction was stirred 48 hours at 85°C, then the solvent was removed under vacuum and the crude product was purified with column chromatography (EtOAc/MeOH/Triethylamine 89:10:1) to yield pure product.

2e.2 - 1-benzyl-3-cyano-1-{4-[4-(7-phenoxyheptyl)piperazin-1-yl]but-1-yl}guanidine: $C_{30}H_{44}N_6O$. M=504.72. Light yellow solid. 30.9% yield. R_f=0.29 (EtOAc/MeOH/Triethylamine 89:10:1). mp: 84.1-85.5°C.^{1}H NMR (600 MHz, *CDCl*$_3$) δ ppm 7.37-7.22 (m, 7H: 5H$^{arom.}$, 2H$^{phenoxy.}$), 6.94-6.87 (m, 3H$^{phenoxy.}$, C(C<u>H</u>CH)$_2$C<u>H</u>), 6.37 (br, 2H, N<u>H</u>$_2$), 4.60 (s, 2H, C<u>H</u>$_2$Ph), 3.96-3.94 (t, 2H, OC<u>H</u>$_2$, *J*=6.51Hz), 3.16 (m, 2H, CH$_2$C<u>H</u>$_2$NC(N)), 2.60-2.31 (m, 12H: 8H^{pip}, C<u>H</u>$_2$N^{piperazine}), 1.81-1.75 (m, 2H, CH$_2$C<u>H</u>$_2$CH$_2$), 1.60-1.53 (m, 2H, CH$_2$C<u>H</u>$_2$CH$_2$), 1.52-1.43 (m, 6H, CH$_2$C<u>H</u>$_2$CH$_2$), 1.40-1.33 (m, 4H, CH$_2$C<u>H</u>$_2$CH$_2$).

1.5 Compounds 2f.1 – 2f.5

2f.1 *Preparation of* 1,4-bis(4-nitrilobutyl)piperazine

Piperazine (1eq.) in acetonitrile was added dropwise to a mixture of bromobutyronitrile (2.1eq.) and potassium carbonate (8eq.) in acetonitrile. The reaction was stirred overnight at room temperature, then filtered. The precipitate was discarded. The solvent was removed under vacuum and the crude product was purified by column chromatography (EtOAc/MeOH 48:2) to yield pure product.

2f.1 - 1,4-Bis(4-nitrilobutyl)piperazine :$C_{12}H_{20}N_4$. M=220.32.White solid. 82.26% yield. R_f=0.18 (EtOAc/MeOH48:2). mp: 105.3-106.4°C. ^{1}H NMR (300 MHz, *CDCl$_3$*) δ ppm 2.47-2.40 (m, 16H: 8H^{piperazine}, C$\underline{H_2}$N^{piperazine}, C$\underline{H_2}$CN), 1.87-1.77 (qt, 4H, $CH_2C\underline{H_2}CH_2$).

2f.2 *Preparation of* 1,4-bis(4-aminobutyl)piperazine

To a solution of 1,4-bis(4-nitrilobutyl)piperazine **(2f.1)** (1eq.) in diethyl ether $LiAlH_4$ (4 eq.) was added. The reaction was stirred 2.5 hours at room temperature, then the mixture was quenched by dropwise addition of water (16eq.) and stirred for 30 minutes and filtered. The precipitate was discarded. The organic layer was dried over Na_2SO_4 then the solvent was removed under vacuum and the crude product was directly used in the next step without further purification.

2f.2 - 1,4-Bis(4-aminobutyl)piperazine :$C_{12}H_{28}N_4$. M=228.38.b.p. 175°C/14 mm Hg[32] ^{1}H NMR (300 MHz, *CDCl$_3$*) δ ppm 2.73-2.68 (t, 4H, C$\underline{H_2}$NH$_2$, *J*=6.74Hz), 2.66-2.39 (m, 8H^{piperazine}), 2.37-2.32 (t, 4H, C$\underline{H_2}$N^{piperazine}), 1.59-1.21 (m, 12H: $CH_2C\underline{H_2}CH_2$, N$\underline{H_2}$, *).

2f.3 *Preparation of* 1,4-Bis[*N*-(4-trifluoromethylbenzoil)aminobut-4-yl]piperazine

To a mixture of 1,4-Bis(4-aminobutyl)piperazine **(2f.2)** (1eq.) and triethylamine (4eq.) in DCM 4-(trifluoromethyl)benzoyl chloride (2.2eq.) was added dropwise. The reaction was stirred 24 hours at room temperature. The mixture was washed 3-times by water and dried over Na_2SO_4. Then the solvent was removed under vacuum and the crude product was purified by column chromatography (EtOAc/MeOH/Triethylamine 49:10:1) to yield pure product.

2f.3 - 1,4-Bis(*N*-(4-trifluoromethylbenzoil)aminobut-4-yl)piperazine: $C_{28}H_{34}N_4O_2F_6$. M=572.60. White solid. 27.80% yield. R_f=0.15 (EtOAc/MeOH/Triethylamine 49:10:1). mp: 208.4-210.4°C.^{1}H NMR (300 MHz, *CDCl$_3$*) δ ppm7.81-7.71 (m, 4H$^{arom.}$), 7.68-7.27 (m, 4H$^{arom.}$), 6.86 (br, 2H, N$\underline{H}$), 3.51-3.45 (q, 4H, HNC$\underline{H_2}$), 2.36-2.31 (m, 12H: 8H^{piperazine}, C$\underline{H_2}$N^{piperazine}), 1.70-1.61 (m, 8H, $CH_2C\underline{H_2}CH_2$).

2f.4 *Preparation of* 1,4-Bis[*N*-(4-trifluoromethylbenzyl)aminobut-4-yl]piperazine

To a mixture of 1,4-Bis(*N*-(4-trifluoromethylbenzoil)aminobut-4-yl)piperazine **2f.3** (1eq.) in diethyl ether $LiAlH_4$ (6 eq.) was added. The reaction was stirred overnight at room temperature and, then the mixture was quenched by dropwise addition of water (24 eq.) and stirred for 30 minutes and filtered. The precipitate was discarded. The organic phase was dried over Na_2SO_4 then the solvent was removed under vacuum and the crude product was purified with column chromatography (DCM/MeOH/25% NH_3 aq. 139:10:1) to yield pure product

2f.4 - 1,4-Bis[*N*-(4-trifluoromethylbenzyl)aminobut-4-yl]piperazine: $C_{28}H_{38}N_4F_6$. M=544.63. White solid. 30.23% yield. R_f=0.28 (DCM/MeOH/25% NH_3 aq. 139:10:1). mp: 76.9-78.9°C.^{1}H NMR (300 MHz, *CDCl$_3$*) δ ppm 7.58-7.51 (m, 4H$^{arom.}$), 7.46-7.39 (m, 4H$^{arom.}$), 3.84 (s, 4H, C$\underline{H}_2$Ph), 2.63-2.28 (m, 16H: 8H^{piperazine}, C$\underline{H}_2$N^{piperazine}, CH$_2$C$\underline{H}_2$NH), 1.98 (br, 2H, N$\underline{H}$, *), 1.56-1.54 (m, 8H, CH$_2$C$\underline{H}_2$CH$_2$).

2f.5 *Preparation of* 1,4-Bis{4-[1-(4-trifluoromethylbenzyl)-2,3-di(*tert*-butyloxycarbonyl)guandin-1-yl]but-1-yl}piperazine

To an ice-cooled mixture of 1,4-Bis[*N*-(4-trifluoromethylbenzyl)aminobut-4-yl]piperazine **2f.4** (1eq.) and trimethylamine (5eq.) in DCM 1,3-bis(tert-butoxycarbonyl)-2-methyl-2-thiopseudourea (1.1eq.) and mercury II chloride (1.1eq).were sequentially added The ice bath was removed and the reaction was stirred 18 hours at room temperature then filtered. The precipitate was discarded. The filtrate was washed sequentially twice with H_2O and twice with brine. The organic phase was dried over Na_2SO_4 then the solvent was removed under vacuum and the crude product was purified through column chromatography (EtOAc/MeOH/Triethylamine 139:10:1) to yield pure product.

2f.5 - 1,4-Bis{4-[1-(4-trifluoromethylbenzyl)-2,3-di(*tert*-butyloxycarbonyl)guandin-1-yl]but-1-yl}piperazine: $C_{50}H_{74}N_8O_8F_6$. M=1029.16. White solid. 69.38% yield. R_f=0.17 (EtOAc/MeOH/Triethylamine139:10:1). mp: 168.7-170.2°C.^{1}H NMR (300 MHz, *CDCl$_3$*) δ ppm 9.98 (br, 2H, N$\underline{H}$), 7.61-7.58 (m, 4H$^{arom.}$), 7.43-7.40 (m, 4H$^{arom.}$), 4.76 (br, 4H, C$\underline{H}_2$Ph), 3.32 (m, 4H, CH$_2$C$\underline{H}_2$NC(N)), 2.46-2.28 (m, 12H: 8H^{piperazine}, C$\underline{H}_2$N^{piperazine}), 1.62-1.43 (m, 44H: CH$_2$C$\underline{H}_2$CH$_2$, CC$\underline{H}_3$).

1.6 Compound 2g.1

2g.1 *Preparation of* 1,4-bis(7-phenoxyheptyl)piperazine

To a mixture of 7-phenoxyheptylbromide (1eq.) and potassium carbonate (4eq.) in acetonitrile piperazine (0.5eq.) was added. The reaction was stirred overnight at room temperature then filtered. The precipitate was discarded. The solvent was removed under vacuum and the crude product was purified by column chromatography (DCM/MeOH 9:1) to yield pure product.

2g.1 - 1,4-bis(7-phenoxyheptyl)piperazine: $C_{30}H_{46}N_2$. M=466.70. White solid. 17.50% yield. R_f=0.88 (DCM/MeOH 9:1). mp: 96.4-98.4°C. ^{1}H NMR (300 MHz, *CDCl$_3$*) δ ppm 7.28-7.25 (m, 4H$^{phenoxy.}$, C(CHC$\underline{H}$)$_2$CH), 6.93-6.88 (m, 6H$^{phenoxy.}$, C(C$\underline{H}$CH)$_2$C$\underline{H}$), 3.96-3.93 (m, 4H, OC$\underline{H}_2$, *J* = 6.54Hz), 2.50-2.32 (12H, 8H^{piperazine}, C$\underline{H}_2$N^{piperazine}), 1.79-1.75 (qt, 4H, CH$_2$C$\underline{H}_2$CH$_2$), 1.53-1.44 (m, 8H, CH$_2$C$\underline{H}_2$CH$_2$), 1.40-1.30 (m, 8H, CH$_2$C$\underline{H}_2$CH$_2$)

1.7 Compounds 2h.1 – 2h.5

2h.1 *Preparation of* 7-phenoxyheptanenitrile

To a dissolved in anhydrous ethanol sodium (1.1eq) phenol (1.1eq.) was added and the solution was stirred 30 minutes at room temperature. To a solution of 7-bromoheptanenitrile **16** (1eq.) in anhydrous ethanol freshly prepared sodium phenoxide solution was added dropwise. The reaction was stirred overnight at 80°C. The solvent was removed under vacuum and the mixture was purified by column chromatography (hexane/DCM 1:1) to yield pure product.

2h.1 7-phenoxyheptanenitrile: $C_{13}H_{17}NO$. M=203.28. Colorless liquid. 76.2% yield. R_f=0.39 (hexane/DCM 1:1). ^{1}H NMR (600 MHz, *CDCl$_3$*) δ ppm 7.28-7.26 (m, 2H$^{phenoxy.}$, C(CHC$\underline{H}$)$_2$CH), 6.94-6.92 (m, 1H$^{phenoxy.}$, C(CHCH)$_2$C$\underline{H}$), 6.89-6.88 (2H$^{phenoxy.}$, C(C$\underline{H}$CH)$_2$CH), 3.97-3.95 (t, 2H, OC$\underline{H}_2$, *J* = 6.33Hz), 2.35-2.33 (t, 2H, C$\underline{H}_2$CN, *J* = 7.12Hz), 1.82-1.77 (qt, 2H, CH$_2$C$\underline{H}_2$CH$_2$), 1.71-1.66 (qt, 2H, CH$_2$C$\underline{H}_2$CH$_2$), 1.53-1.51 (m, 4H, CH$_2$C$\underline{H}_2$CH$_2$)

2h.2 *Preparation of* 7-phenoxyheptan-1-amine

To a solution of 7-phenoxyheptanenitrile **2h.1** (1eq.) in diethyl ether $LiAlH_4$ (4eq.) was added. The reaction was stirred 2.5 hours at room temperature, then the mixture was quenched by dropwise addition of water (16eq.) and stirred for 30 minutes, and then filtered. The precipitate was discarded. The organic layer was dried over Na_2SO_4, then the solvent was removed under vacuum and the crude product was purified with column chromatography (DCM/MeOH/25% NH_3 aq. 39:10:1) to yield pure product.

2h.2 7-phenoxyheptan-1-amine: $C_{13}H_{21}NO$. M=207.31. White solid. 92.7% yield. R_f=0.64 (DCM/MeOH/25% NH_3 aq. 39:10:1). mp: 92.5-94.5°C. ^{1}H NMR (600 MHz, *CDCl$_3$*) δ ppm 7.28-7.25 (m, 2H$^{phenoxy.}$, C(CHC<u>H</u>)$_2$CH), 6.93-6.88 (m, 3H$^{phenoxy.}$, C(C<u>H</u>CH)$_2$C<u>H</u>), 3.96-3.94 (t, 2H, OC<u>H$_2$</u>, *J* = 6.55Hz), 2.69-2.67 (t, 2H, C<u>H$_2$</u>NH$_2$, *J* = 7.01Hz), 1.80-1.76 (qt, 2H, CH$_2$C<u>H$_2$</u>CH$_2$), 1.49-1.43 (m, 4H, CH$_2$C<u>H$_2$</u>CH$_2$), 1.39-1.32 (m, 4H, CH$_2$C<u>H$_2$</u>CH$_2$), 1.19 (br, 2H, *, N<u>H$_2$</u>).

2h.3 *Preparation of N*-(7-phenoxyheptyl)benzamide

To a mixture of 7-phenoxyheptan-1-amine **2h.2** (1eq.) and triethylamine (4eq.) in DCM benzoyl chloride, **17** (1.1eq.) was added dropwise. The reaction was stirred 2 hours at room temperature. The mixture was washed 3-times with water and dried over Na_2SO_4. Then the solvent was removed under vacuum and the crude product was purified by column chromatography (DCM/MeOH 100:1) to yield pure product.

2h.3 - *N*-(7-phenoxyheptyl)benzamide: $C_{20}H_{25}NO_2$. M=311.42. White solid. 73.1% yield. R_f=0.72 (DCM/MeOH 100:1). mp: 89.8-91.0°C. ^{1}H NMR (600 MHz, *CDCl$_3$*) δ ppm 7.76-7.74 (m, 2H$^{arom.}$, C(C<u>H</u>CH)$_2$CH), 7.49-7.47 (m, 1H$^{arom.}$, C(CHCH)$_2$C<u>H</u>), 7.43-4.41 (m, 2H$^{arom.}$, C(CHC<u>H</u>)$_2$CH), 7.28-7.25 (m, 2H$^{phenoxy.}$, C(CHC<u>H</u>)$_2$CH), 6.93-6.88 (m, 3H$^{phenoxy.}$, C(C<u>H</u>CH)$_2$C<u>H</u>), 6.12 (br, 1H, N<u>H</u>), 3.96-3.94 (t, 2H, OC<u>H$_2$</u>, *J* = 6.49Hz), 3.47-3.44 (q, 2H, C<u>H$_2$</u>NH), 1.81-1.76 (qt, 2H, CH$_2$C<u>H$_2$</u>CH$_2$), 1.66-1.61 (m, 2H, CH$_2$C<u>H$_2$</u>CH$_2$), 1.51-1.46 (qt, 2H, CH$_2$C<u>H$_2$</u>CH$_2$), 1.43-1.41 (m, 4H, CH$_2$C<u>H$_2$</u>CH$_2$)

2h.4 *Preparation of N*-benzyl-7-phenoxyheptan-1-amine

To a mixture of *N*-(7-phenoxyheptyl)benzamide **2h.3** (1eq.) in diethyl ether, $LiAlH_4$ (4eq.) was added. The reaction was stirred overnight at room temperature, then the mixture was quenched by dropwise addition of water (16 eq.) and stirred for 30 minutes then filtered. The precipitate was discarded. The organic phase was dried over Na_2SO_4, then the solvent was removed and the crude product was purified by column chromatography (EtOAc/MeOH/Triethylamine 139:10:1) to yield pure product.

2h.4 - *N*-benzyl-7-phenoxyheptan-1-amine: $C_{20}H_{27}NO$. M=297.43. White solid. 75.3% yield. R_f=0.28 (EtOAc/MeOH/Triethylamine 139:10:1). mp: 37.2-38.8°C. ^{1}H NMR (600 MHz, *CDCl$_3$*) δ ppm 7.32-7.31 (m, 4H$^{arom.}$, C(C<u>H</u>C<u>H</u>)$_2$CH), 7.28-7.24 (m, 3H: 2H$^{phenoxy.}$, C(CHC<u>H</u>)$_2$CH; 1H$^{arom.}$, C(CHCH)$_2$C<u>H</u>), 6.93-6.88 (m, 3H$^{phenoxy.}$, C(C<u>H</u>CH)$_2$C<u>H</u>), 3.95-3.93 (t, 2H, OC<u>H$_2$</u>, *J* =

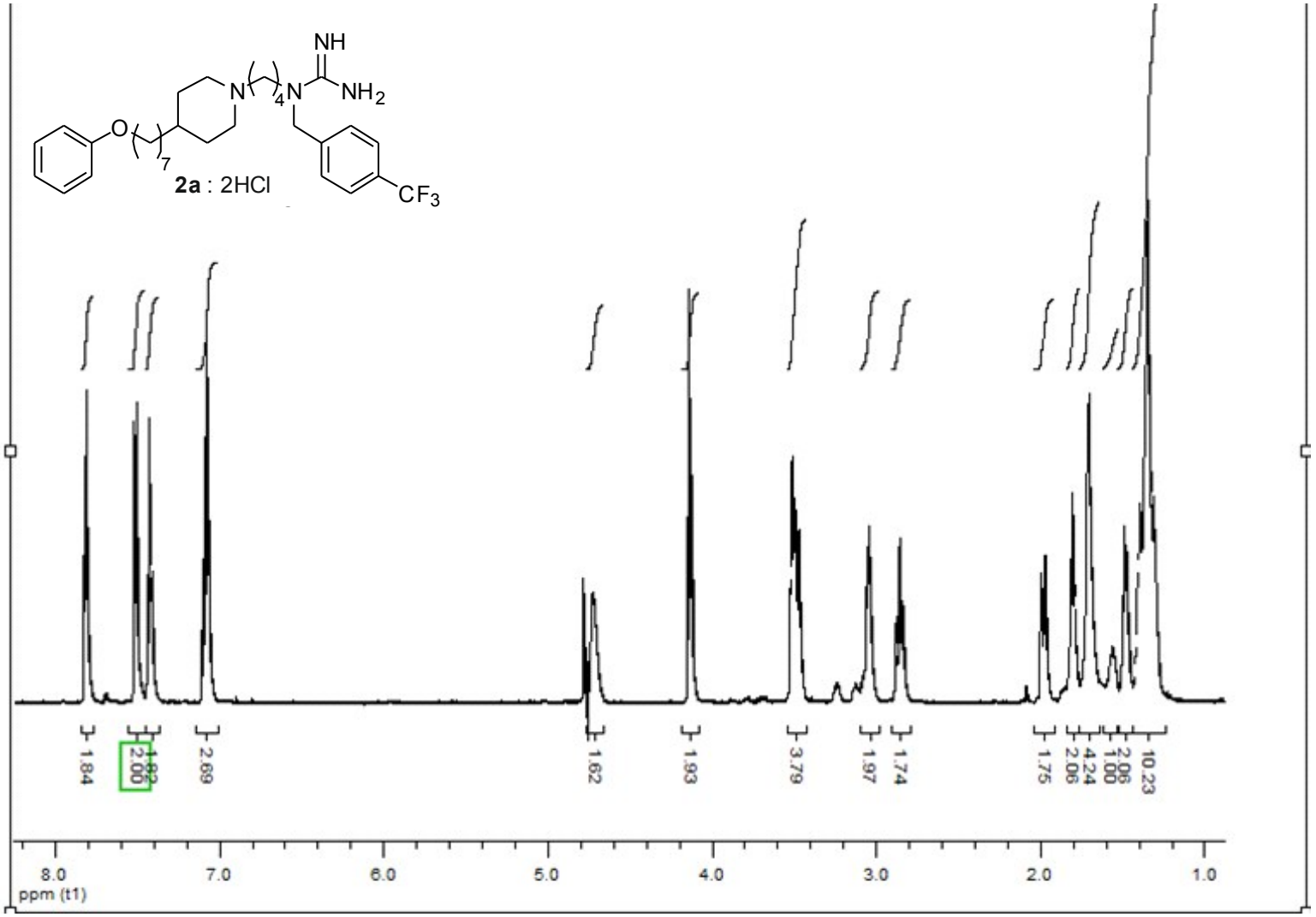
6.55Hz), 3.78 (s, 2H, $C\underline{H}_2$-arom.), 2.64-2.62 (t, 2H, $CH_2C\underline{H}_2NH$), 1.79-1.74 (qt, 2H, $CH_2C\underline{H}_2CH_2$), 1.55-1.49 (qt, 2H, $CH_2C\underline{H}_2CH_2$), 1.48-1.43 (qt, 2H, $CH_2C\underline{H}_2CH_2$),1.37-1.35 (m, 5H, $CH_2C\underline{H}_2CH_2$, N$\underline{H}$).

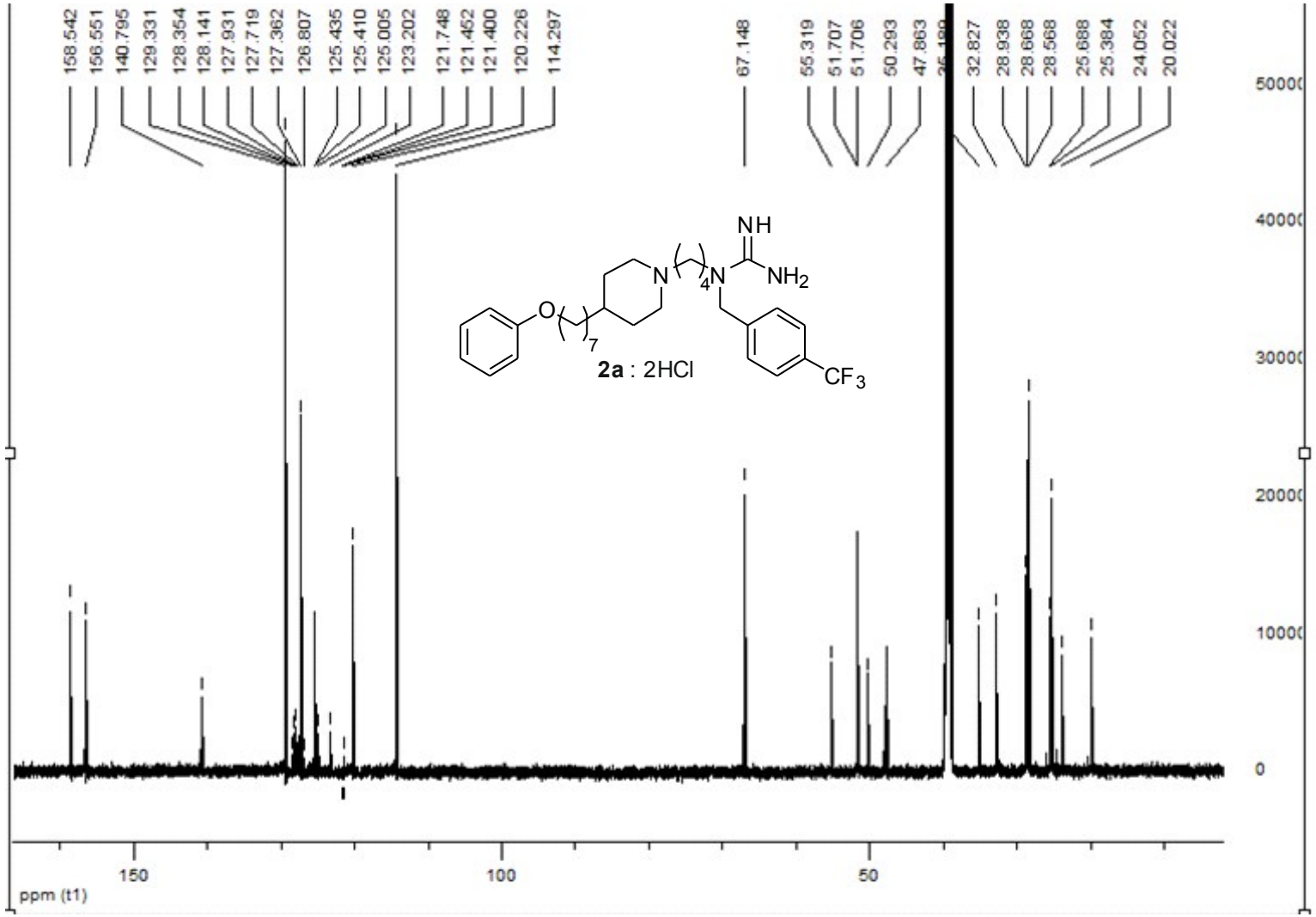
2h.5 *Preparation of* 1-benzyl-1-(7-phenoxyhept-1-yl)-2,3-di(*tert*-butyloxycarbonyl)guanidine

To an ice-cooled mixture of *N*-benzyl-7-phenoxyheptan-1-amine **2h.4** (1eq.) and trimethylamine (5eq.) in DCM 1,3-bis(tert-butoxycarbonyl)-2-methyl-2-thiopseudourea (1.1eq.) and mercury II chloride (1.1eq) were sequentially added. The ice bath was removed and the reaction was stirred 18 hours at room temperature, then filtered. The precipitate was discarded. The filtrate was washed sequentially twice with H_2O and twice with brine. The organic phase was dried over Na_2SO_4, then the solvent was removed under vacuum and the crude product was purified with column chromatography (hexane/EtOAc 9:1) to yield pure product.

2h.5 - 1-benzyl-1-(7-phenoxyhept-1-yl)-2,3-di(*tert*-butyloxycarbonyl)guanidine: $C_{31}H_{45}N_3O_5$. M=539.71. Colorless sticky oil. 89.6% yield. R_f=0.38 (hexane/EtOAc 9:1). ^{1}H NMR (600 MHz, *CDCl$_3$*) δ ppm 9.91 (1H, N$\underline{H}$), 7.33-7.31 (m, 2H$^{arom.}$, C(CHC$\underline{H}$)$_2$CH), 7.27-7.25 (m, 5H: 2H$^{phenoxy.}$, C(CHC$\underline{H}$)$_2$CH, 3H$^{arom.}$, C(C$\underline{H}$CH)$_2$C$\underline{H}$), 6.93-6.90 (m, 1H$^{phenoxy.}$, C(CHCH)$_2$C$\underline{H}$), 6.89-6.87 (m, 2H$^{phenoxy.}$, C(CHCH)$_2$C$\underline{H}$), 4.67 (br, 2H, PhC$\underline{H}_2$), 3.93-3.91 (t, 2H, OC$\underline{H}_2$, *J* = 6.54Hz), 3.23 (br, 2H, $CH_2C\underline{H}_2C(N)N$), 1.76-1.71 (qt, 2H, $CH_2C\underline{H}_2CH_2$), 1.58-1.53 (qt, 2H, $CH_2C\underline{H}_2CH_2$), 1.49 (m, 18H: C$\underline{H}_3$), 1.43-1.38 (qt, 2H, $CH_2C\underline{H}_2CH_2$), 1.33-1.24 (m, 4H, $CH_2C\underline{H}_2CH_2$)

1.8 ^{1}H and ^{13}C NMR spectral data of **compounds 2a-h**

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156.551
140.795
129.331
128.354
128.141
127.931
127.719
127.362
126.807
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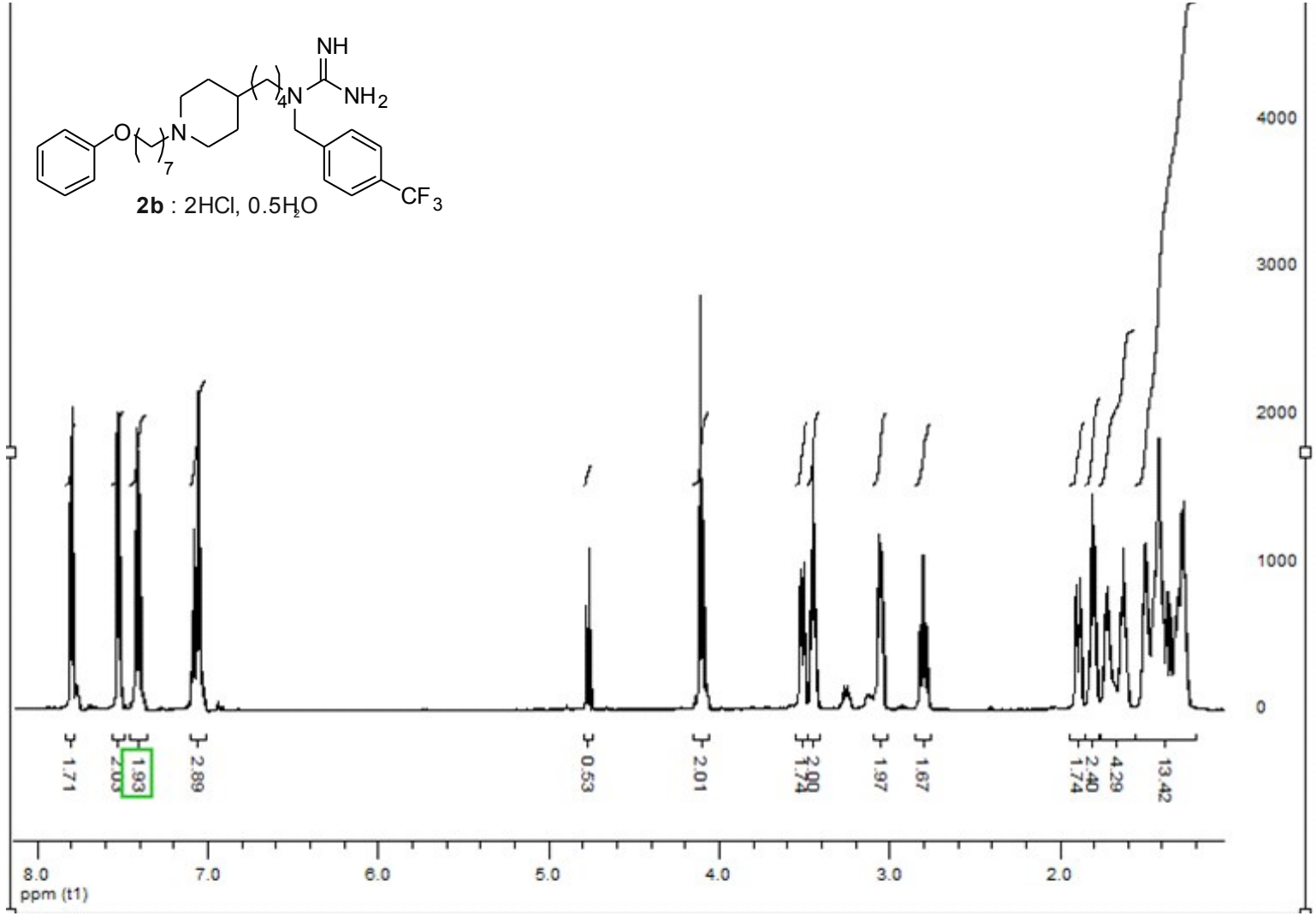
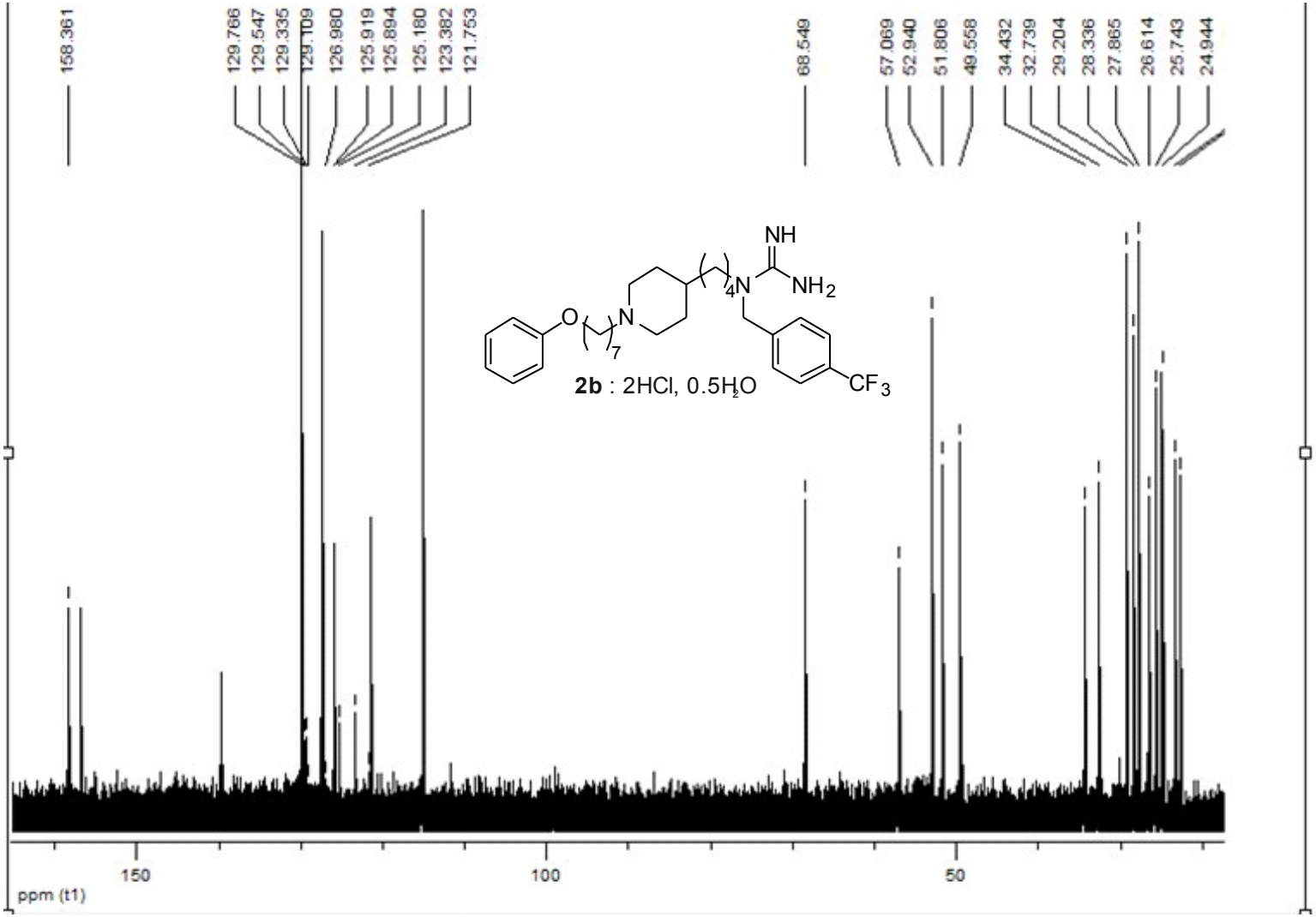
125.410
125.005
123.202
121.748
121.452
121.400
120.226
114.297
67.148
55.319
51.707
51.706
50.293
47.863
32.827
28.938
28.668
28.568
25.688
25.384
24.052
20.022
NH
NH2
N
N
O
7
4
CF3
2a : 2HCl
50000
40000
30000
20000
10000
0
150
100
50
ppm (t1)


NH
NH2
N
4
O
N
7
CF3
2b : 2HCl, 0.5H2O
4000
3000
2000
1000
0
1.71
2.03
1.93
2.89
0.53
2.01
1.74
2.00
1.97
1.67
1.74
2.40
4.29
13.42
8.0
7.0
6.0
5.0
4.0
3.0
2.0
ppm (t1)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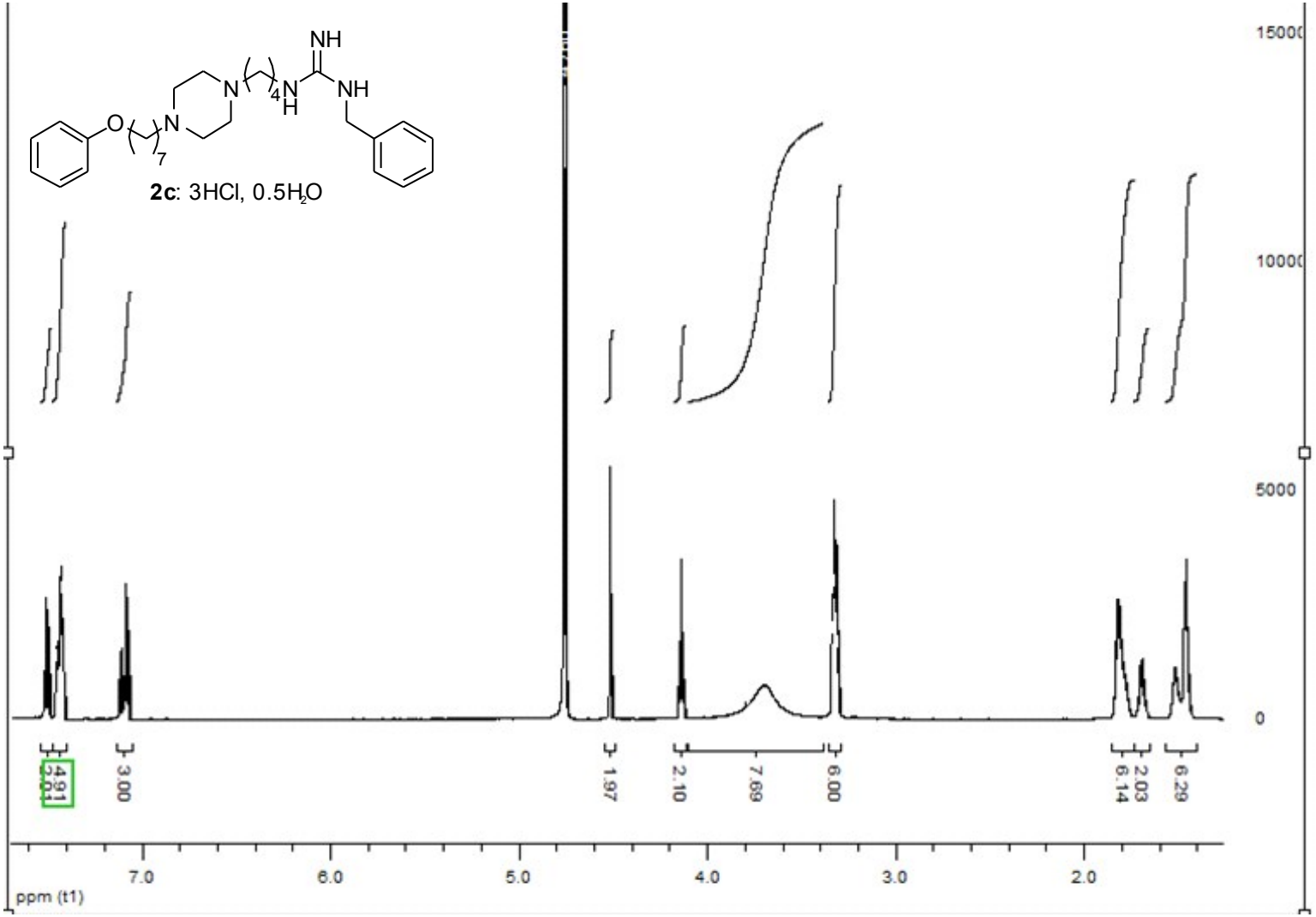
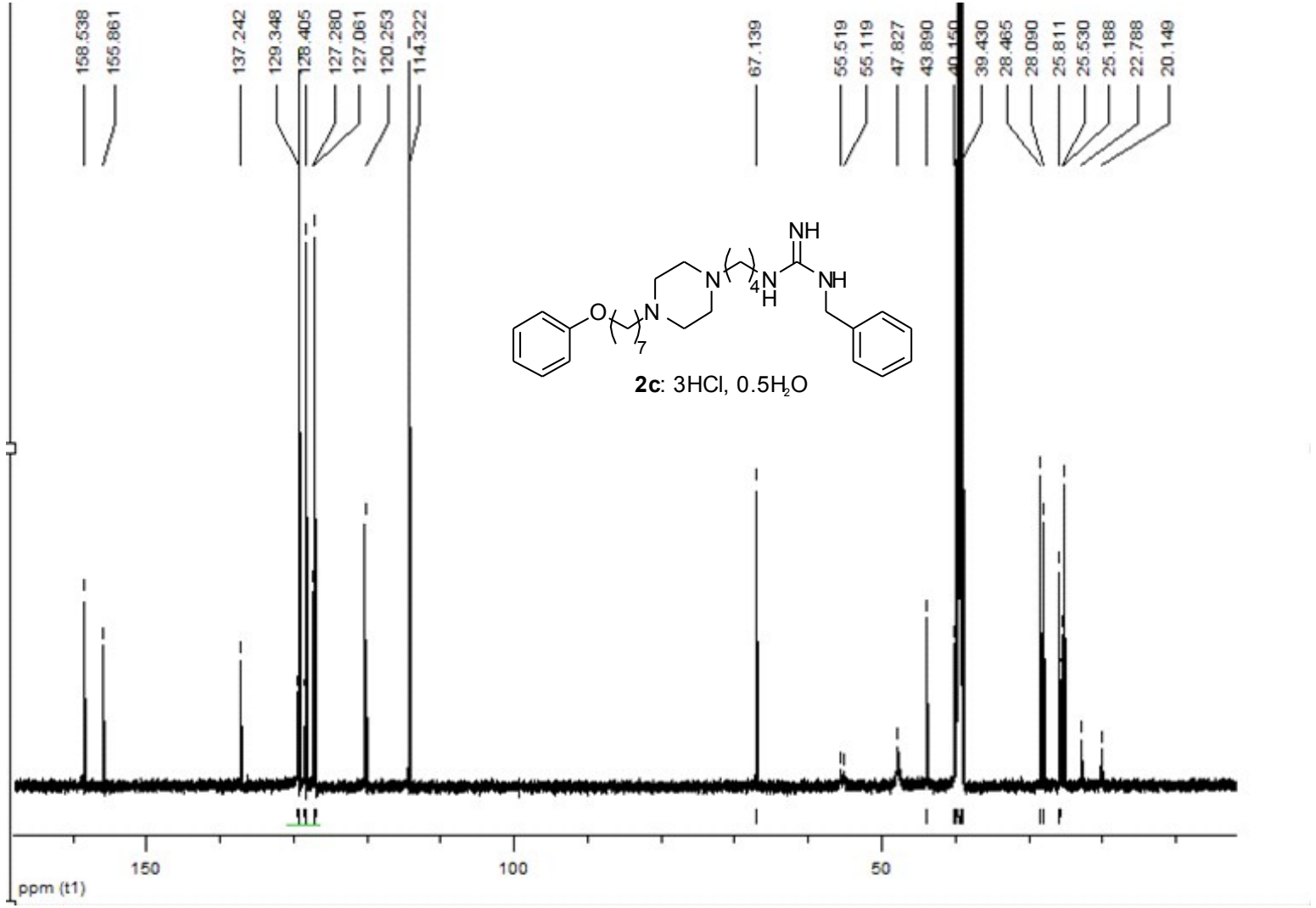
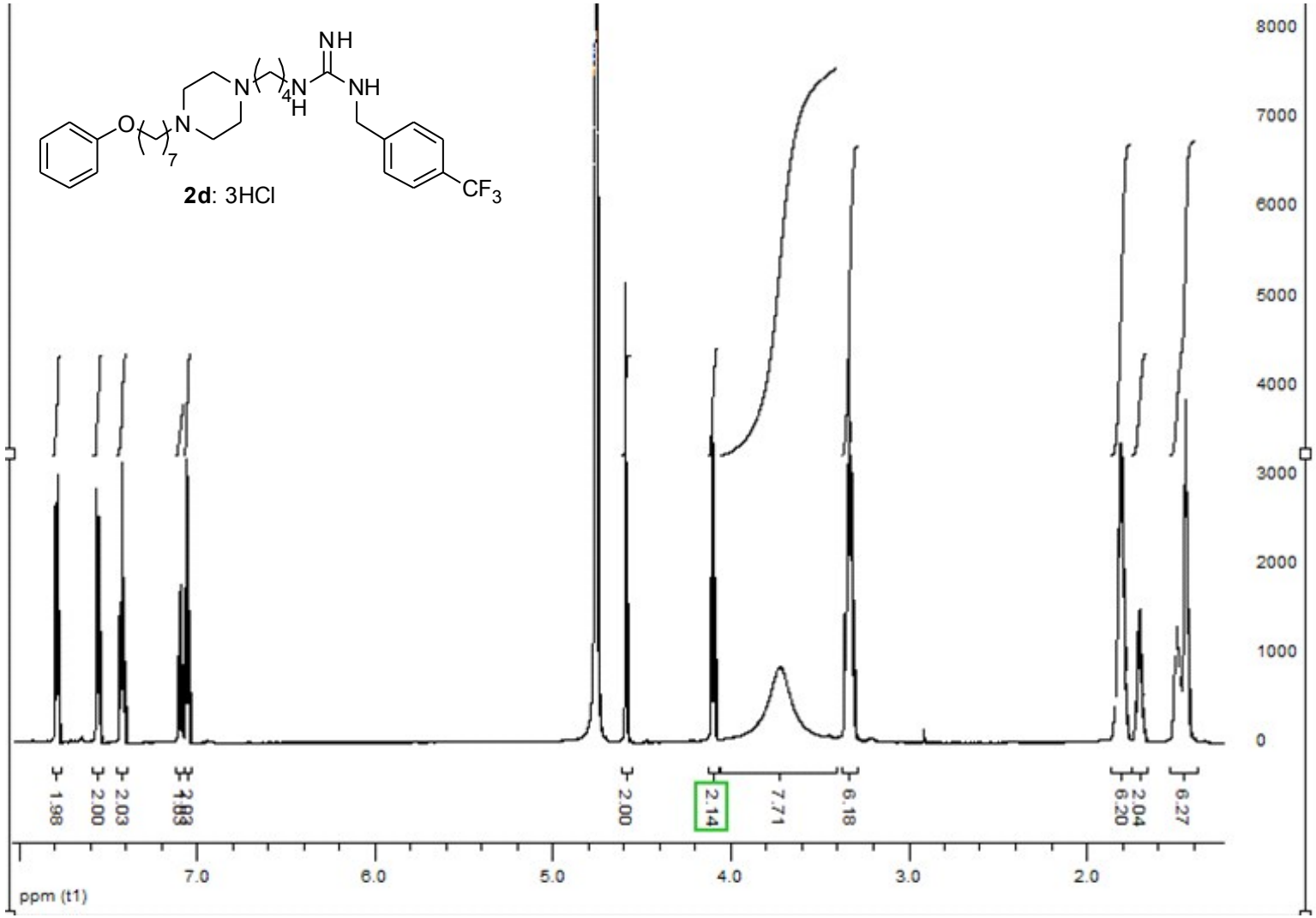
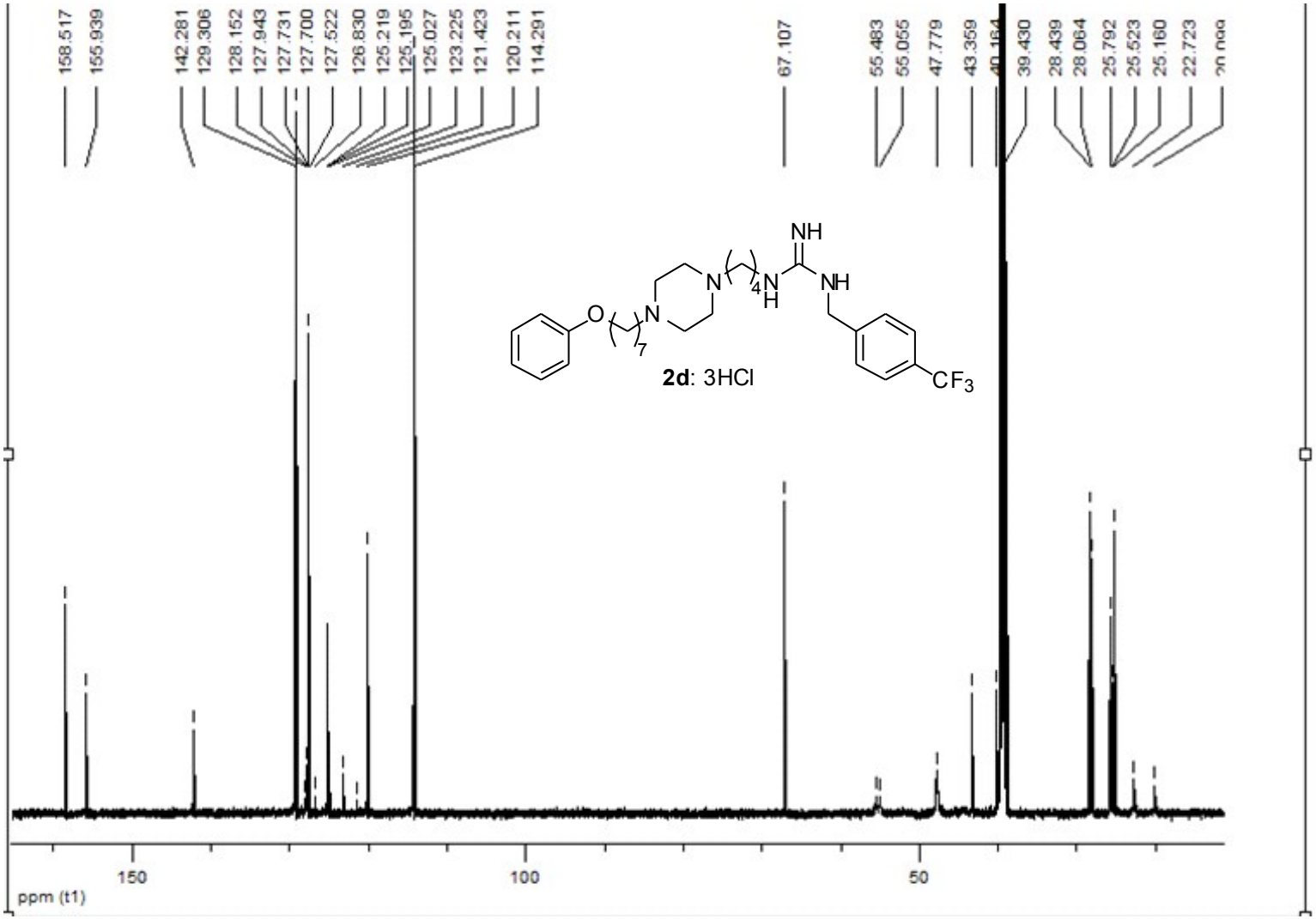
129.766
129.547
129.335
129.109
126.980
125.919
125.894
125.180
123.382
121.753
68.549
57.069
52.940
51.806
49.558
34.432
32.739
29.204
28.336
27.865
26.614
25.743
24.944
NH
NH2
N
4
O
N
7
CF3
2b : 2HCl, 0.5H2O
150
100
50
ppm (t1)


NH
N
4
N
H
NH
O
N
7
2c: 3HCl, 0.5H2O
15000
10000
5000
0
4.91
0.01
3.00
1.97
2.10
7.69
6.00
6.14
2.03
6.29
7.0
6.0
5.0
4.0
3.0
2.0
ppm (t1)



2c: 3HCl, 0.5H_2O


NH
NH
NH
O
N
N
4
7
H
CF3
2d: 3HCl
8000
7000
6000
5000
4000
3000
2000
1000
0
1.98
2.00
2.03
2.00
2.14
7.71
6.18
2.04
6.20
6.27
7.0
6.0
5.0
4.0
3.0
2.0
ppm (t1)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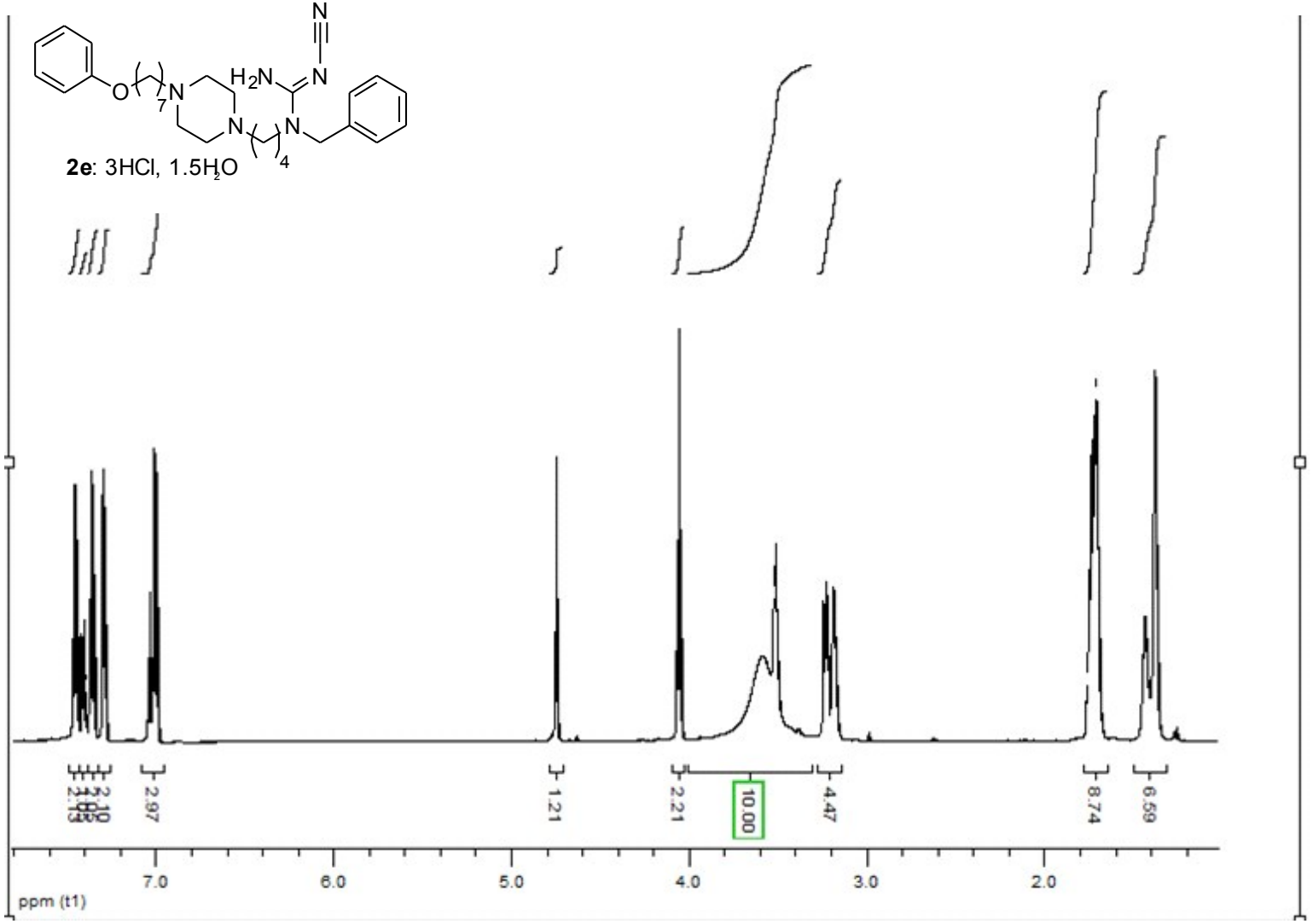
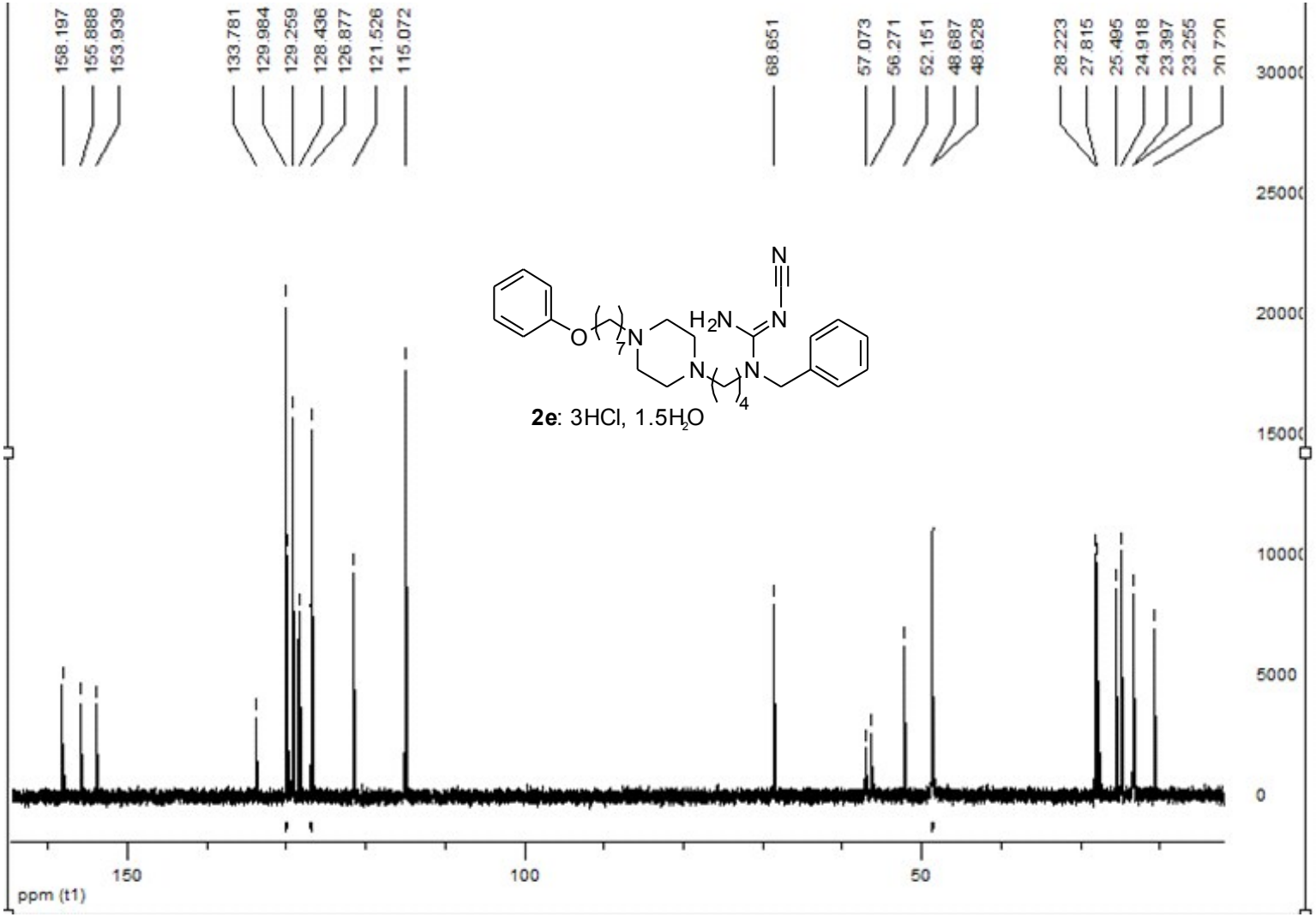
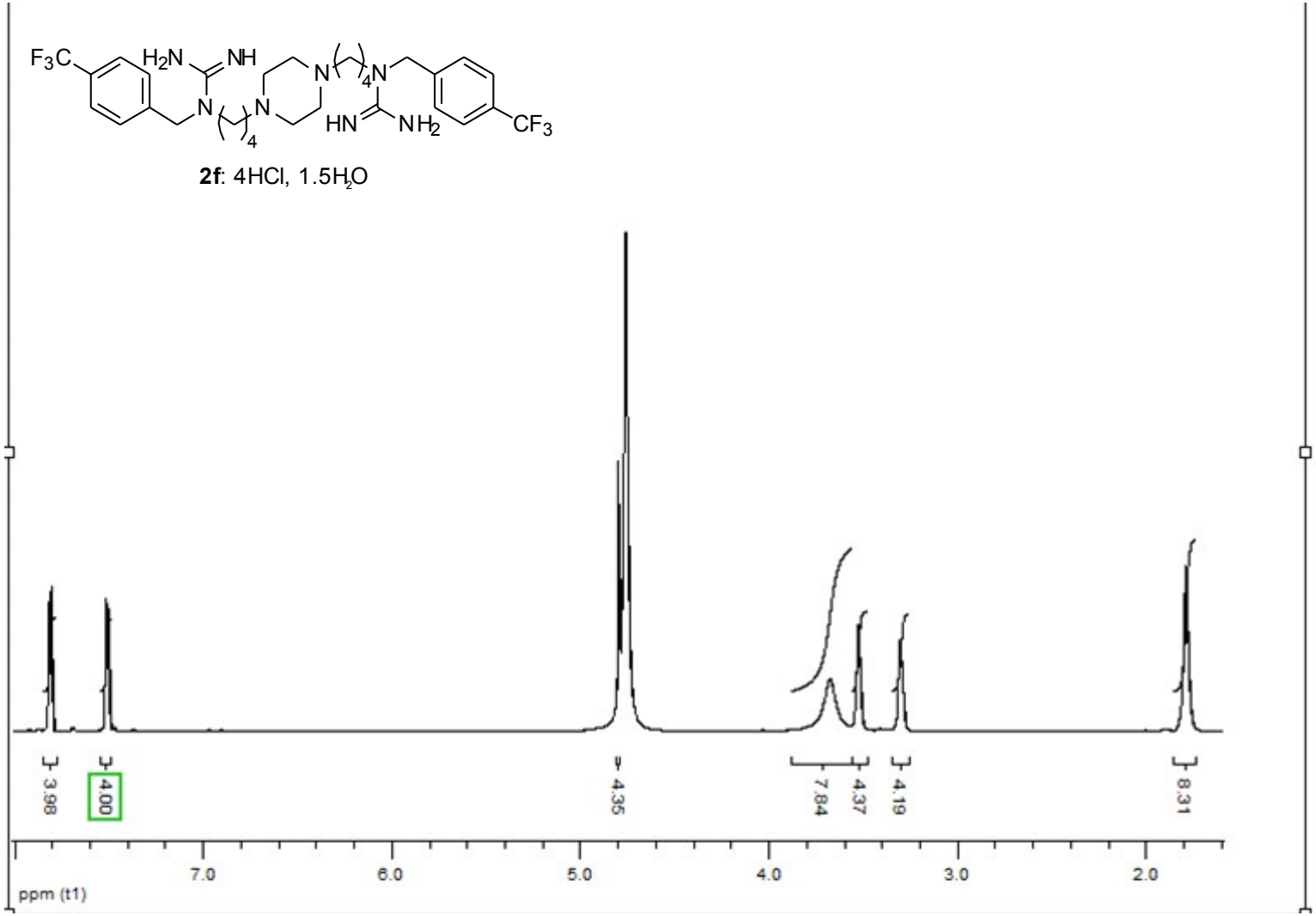
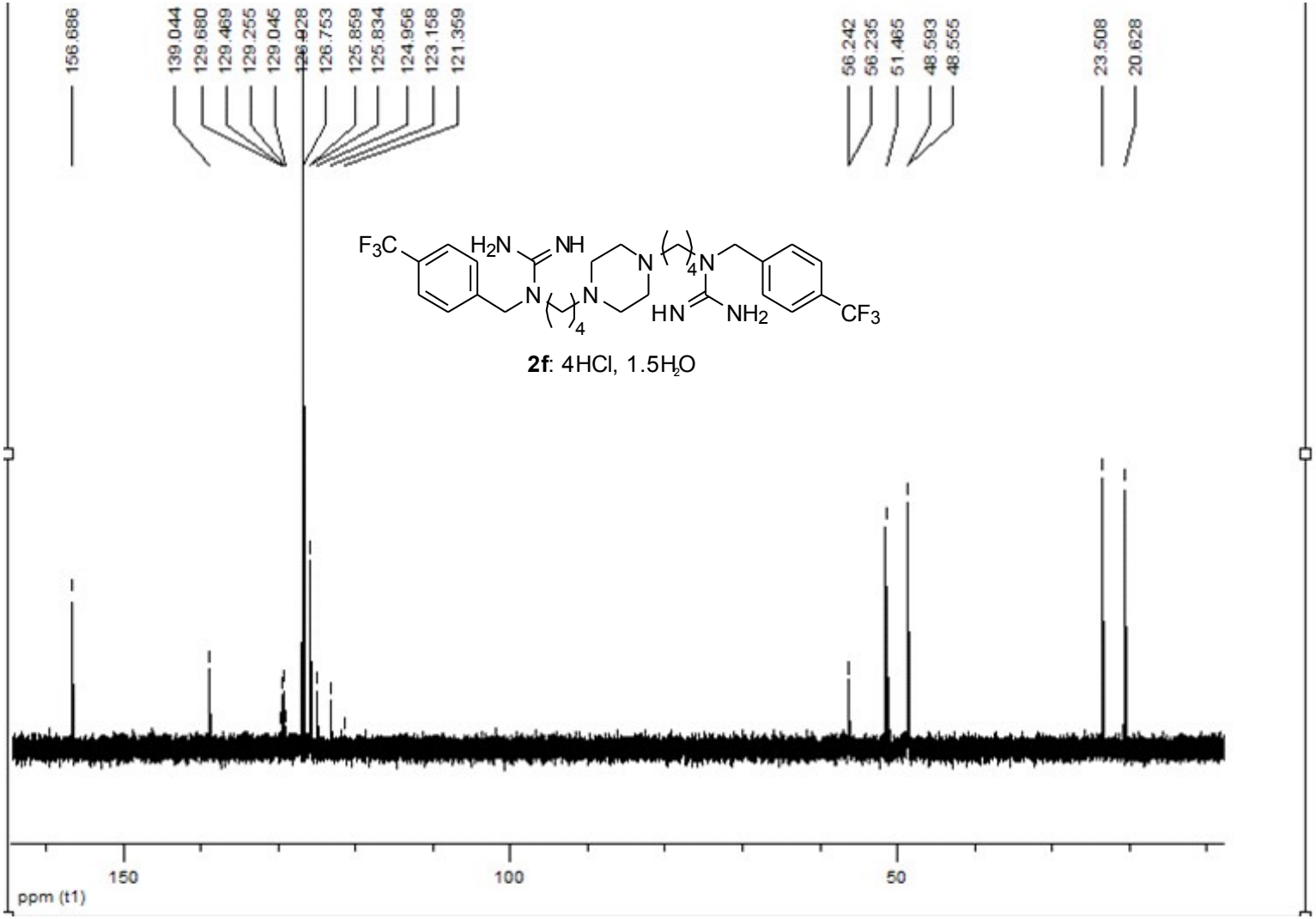
155.939
142.281
129.306
128.152
127.943
127.731
127.700
127.522
126.830
125.219
125.195
125.027
123.225
121.423
120.211
114.291
67.107
55.483
55.055
47.779
43.359
39.430
28.439
28.064
25.792
25.523
25.160
22.723
NH
NH
NH
N
N
O
4
7
CF3
2d: 3HCl
150
100
50
ppm (t1)


N
H2N
N
O
7
N
N
N
4
2e: 3HCl, 1.5H2O
2.97
1.21
2.21
10.00
4.47
8.74
6.59
7.0
6.0
5.0
4.0
3.0
2.0
ppm (t1)

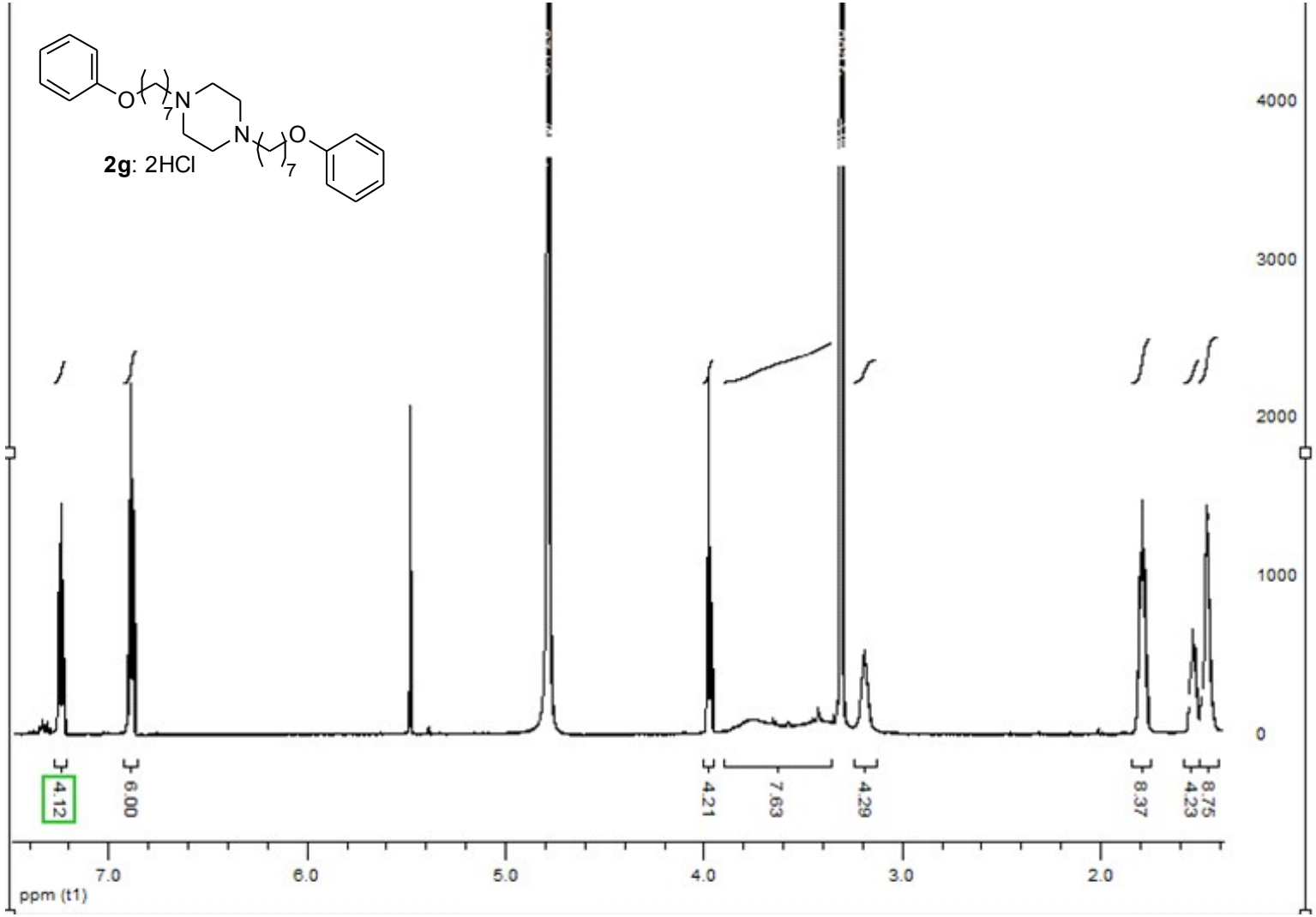


2e: 3HCl, 1.5H_2O


F3C
H2N
NH
N
N
4
N
HN
NH2
CF3
4
2f: 4HCl, 1.5H2O
3.98
4.00
4.35
7.84
4.37
4.19
8.31
7.0
6.0
5.0
4.0
3.0
2.0
ppm (t1)



2f: 4HCl, 1.5H_2O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4000
3000
2000
1000
0
4.12
6.00
4.21
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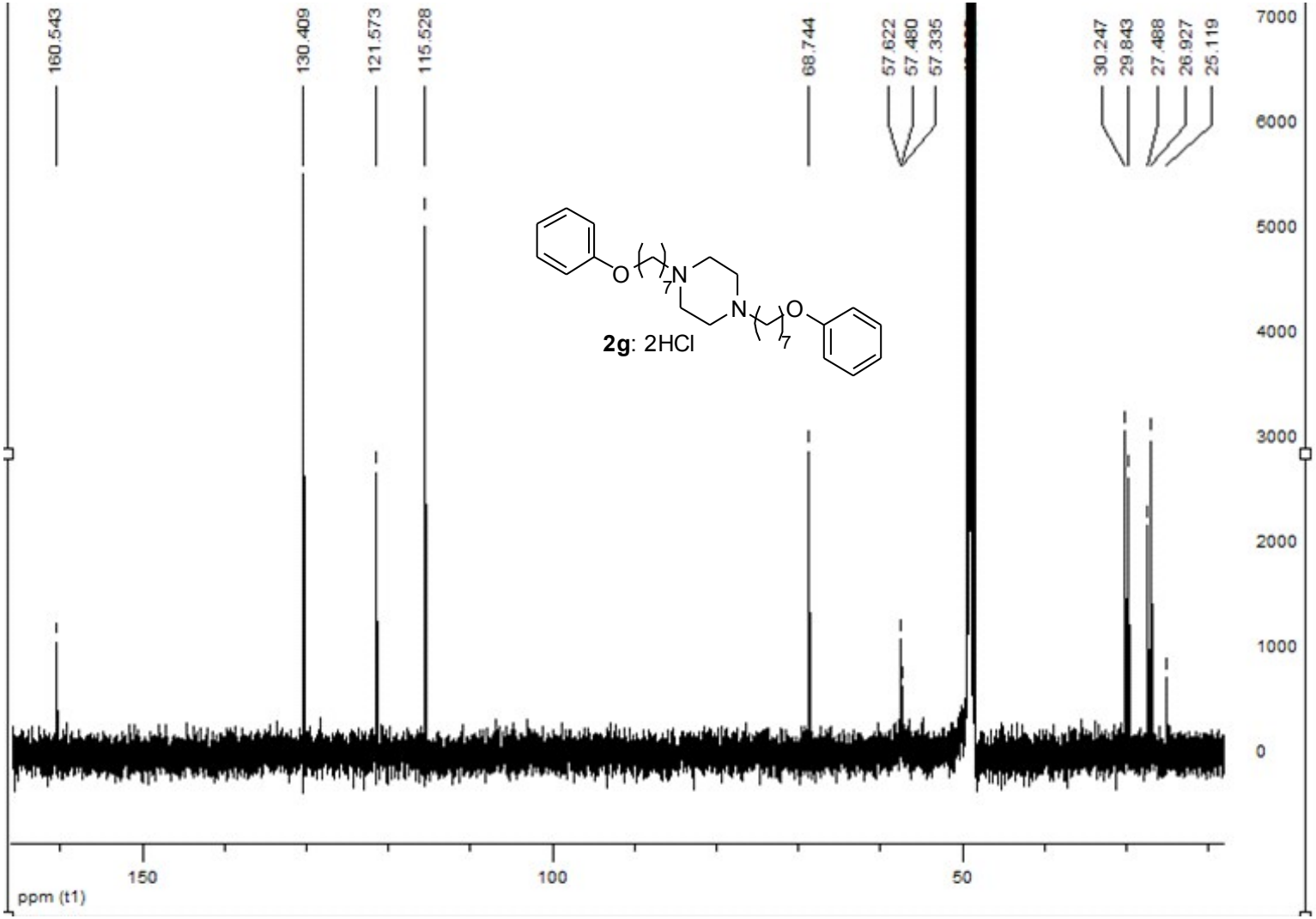
4.23
8.75
7.0
6.0
5.0
4.0
3.0
2.0
ppm (t1)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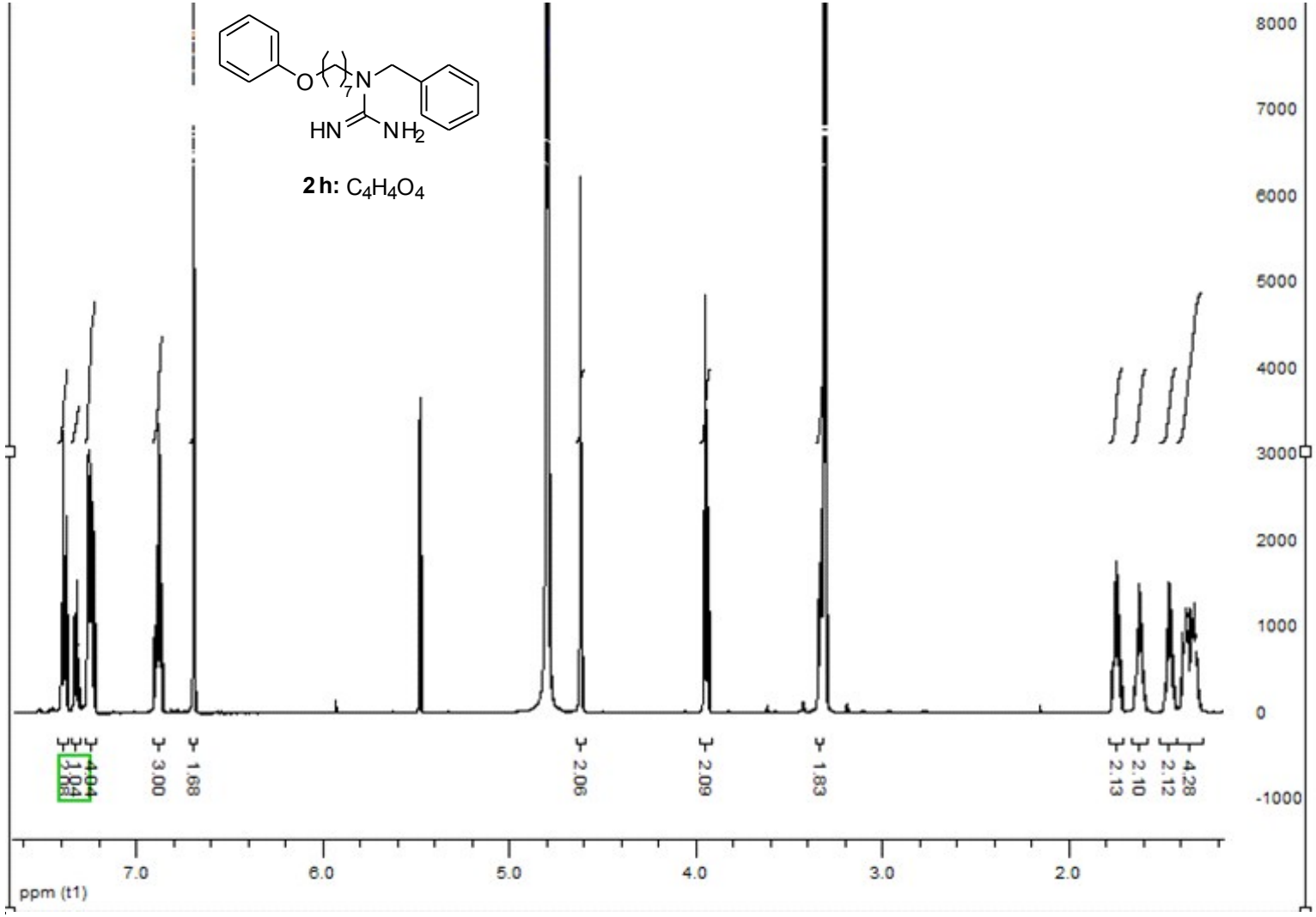
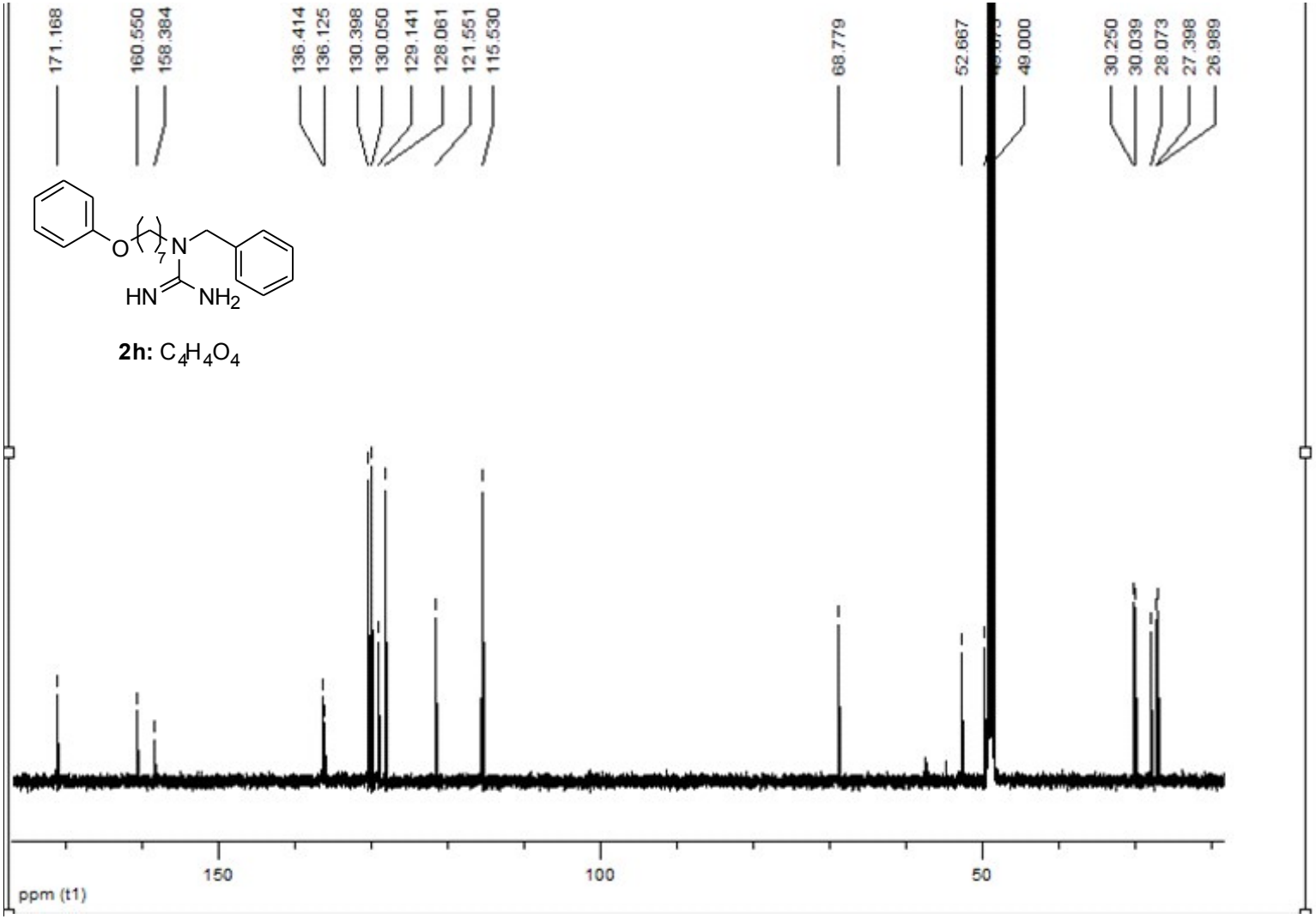
130.409
121.573
115.528
68.744
57.622
57.480
57.335
30.247
29.843
27.488
26.927
25.119
7000
6000
5000
4000
3000
2000
1000
0
150
100
50
ppm (t1)
O
7
N
N
7
O
2g: 2HCl


2 h: C4H4O4
HN
NH2
8000
7000
6000
5000
4000
3000
2000
1000
0
-1000
4.04
1.04
1.00
3.00
1.68
2.06
2.09
1.83
2.13
2.10
2.12
4.28
7.0
6.0
5.0
4.0
3.0
2.0
ppm (t1)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136.125
130.398
130.050
129.141
128.061
121.551
115.530
68.779
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49.000
30.250
30.039
28.073
27.398
26.989
O
7
N
HN
NH2
150
100
50
ppm (t1)

2h: $C_4H_4O_4$

Appendix B

Supplementary data
for

Design, and synthesis of active - *in vitro* and *in vivo* – guanidine derivatives, as histamine H_3 receptor antagonists

Marek Staszewski[1*] Anna Stasiak[2], Tadeusz Karcz[3] Daniel McNaught Flores[4], Wiesława, Agnieszka Fogel[2], Katarzyna Kieć-Kononowicz[3], Rob Leurs[4], and Krzysztof Walczyński[1**]

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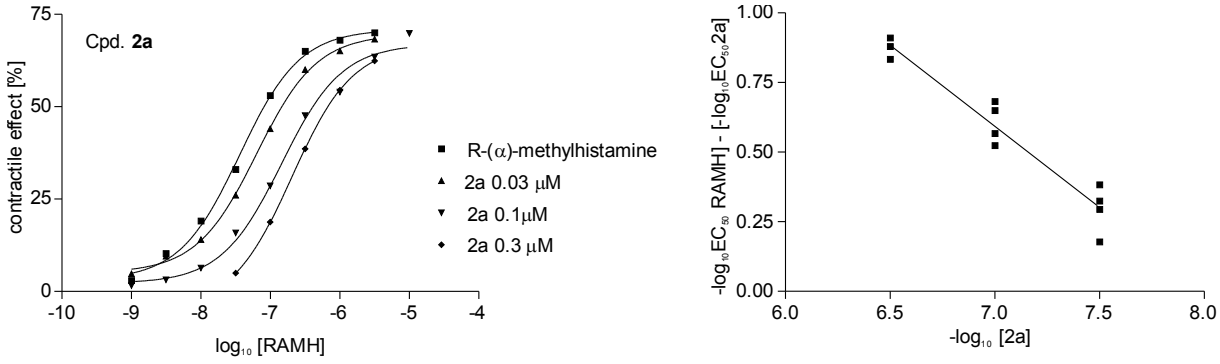
[2] *Department of Hormone Biochemistry, Medical University of Lodz, ul. Żeligowskiego 7/9, 90-752 Łódź, Poland*
[3] *Department of Technology and Biotechnology of Drugs, Faculty of Pharmacy, Jagiellonian UniversityMedical College, ul. Medyczna 9, 30-688 Kraków, Poland*
[4] *Amsterdam Institute of Molecules, Medicines & Systems, Division of Medicinal Chemistry, Vrije Universiteit Amsterdam, De Boelelaan 1108, 1081 HZ Amsterdam, The Netherlands*

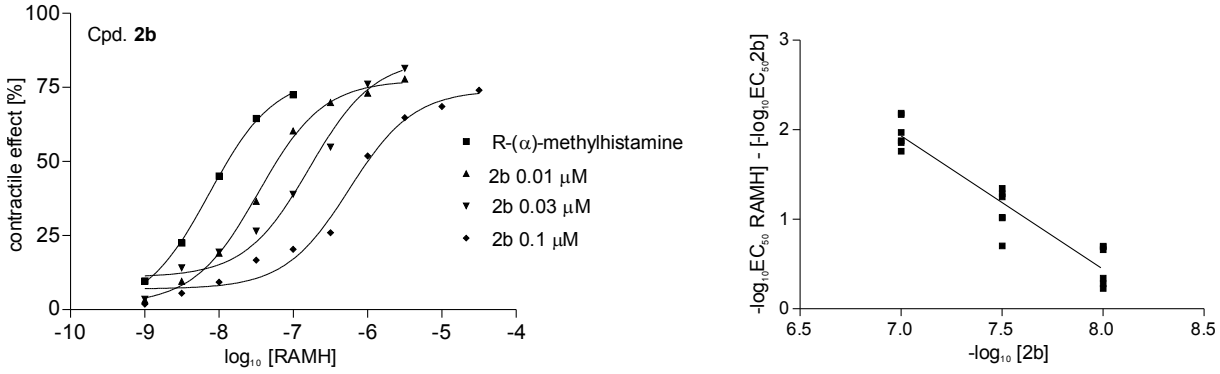
marek.staszewski@umed.lodz.pl, anna.stasiak@umed.lodz.pl, t.karcz@uj.edu.pl d.mcnaughtflores@vu.nl, wieslawa.agnieszka.fogel@umed.lodz.pl, mfkonono@cyf-kr.edu.pl, r.leurs@vu.nl, krzysztof.walczynski@umed.lodz.pl

2.2. Pharmacology

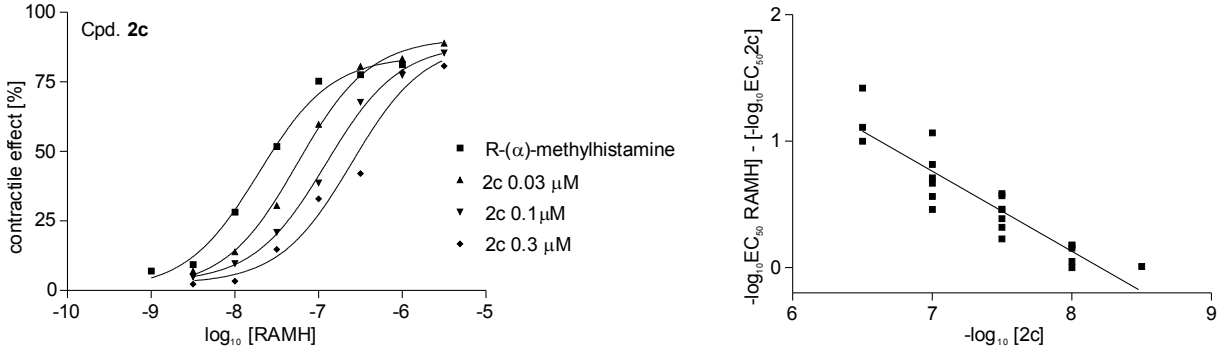
2.2.1.1 Antagonism by **2a-h** (ADS-1025 – 1032) and thioperamide of the inhibitory effect of R-(-)- α-methylhistamine (RAMH) on the electrically induced contraction of guinea-pig ileum strips



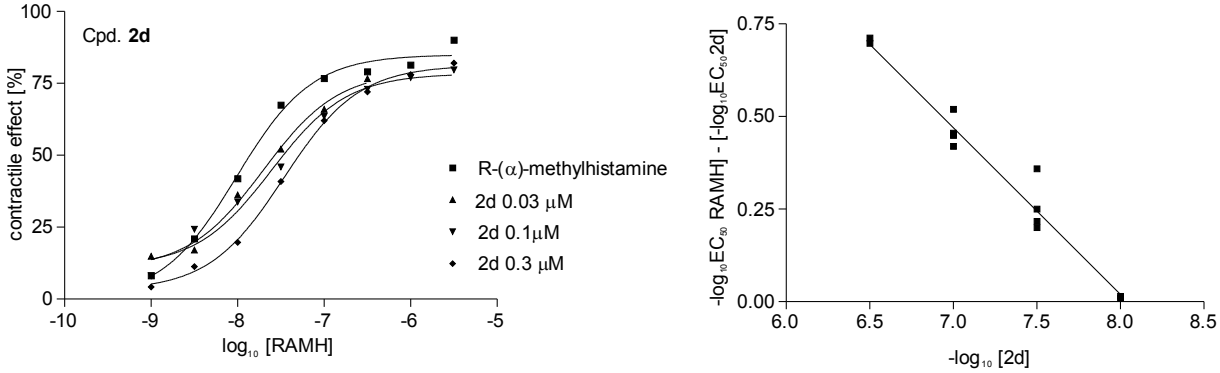
Contraction of electrically evoked guinea-pig ileum by R-(α)-methylhistamine in the absence (■) and presence (▲,▼, ♦) of compound **2a**.



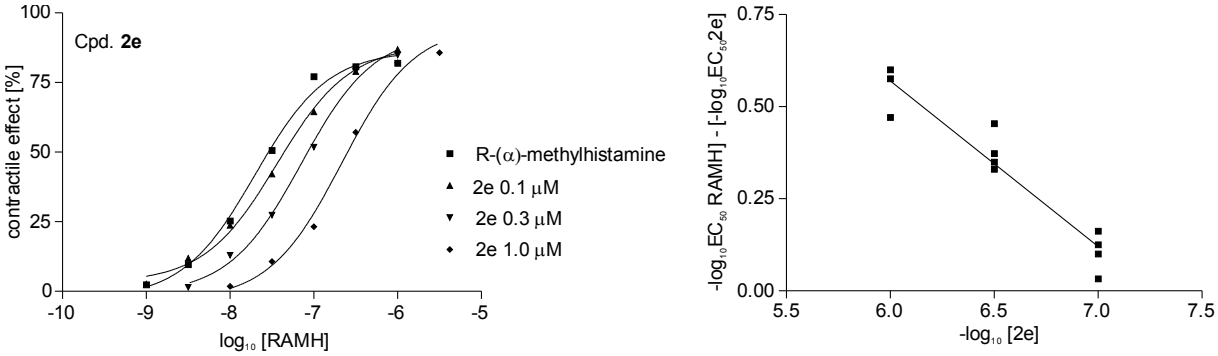
Contraction of electrically evoked guinea-pig ileum by R-(α)-methylhistamine in the absence (■) and presence (▲,▼, ♦) of compound **2b**.



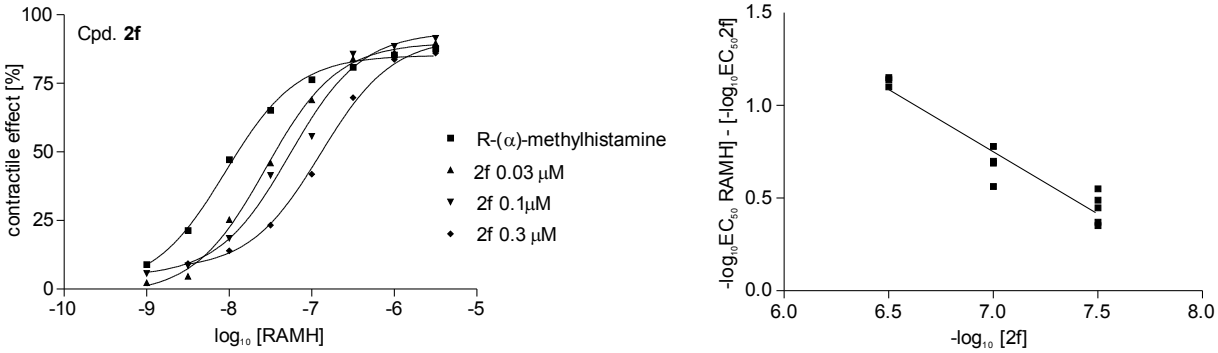
Contraction of electrically evoked guinea-pig ileum by R-(α)-methylhistamine in the absence (■) and presence (▲,▼, ♦) of compound **2c**.



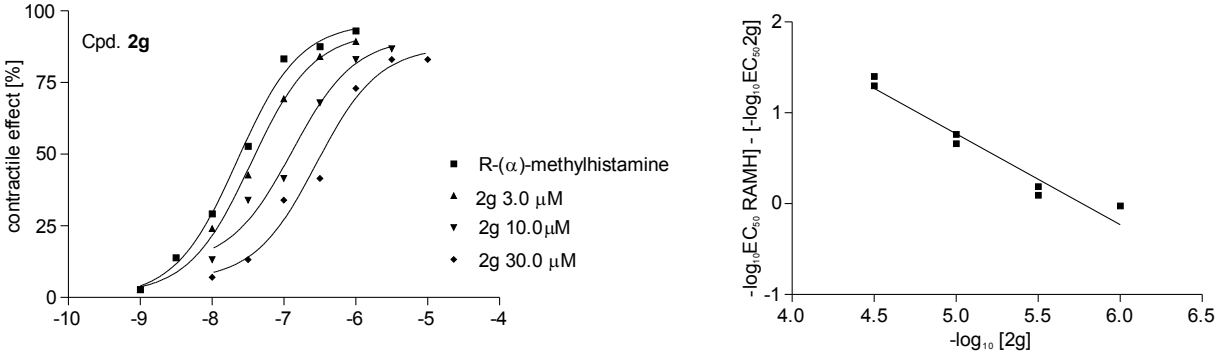
Contraction of electrically evoked guinea-pig ileum by R-(α)-methylhistamine in the absence (■) and presence (▲,▼, ♦) of compound **2d.**



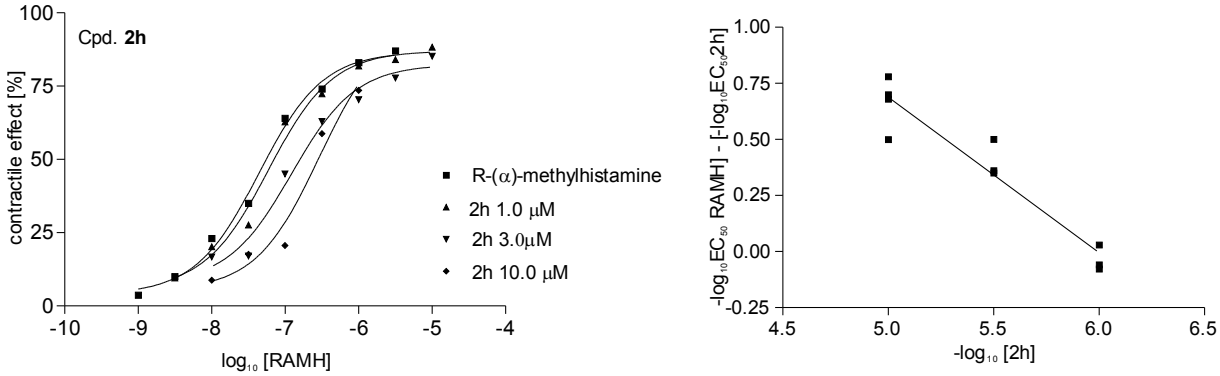
Contraction of electrically evoked guinea-pig ileum by R-(α)-methylhistamine in the absence (■) and presence (▲,▼, ♦) of compound **2e.**



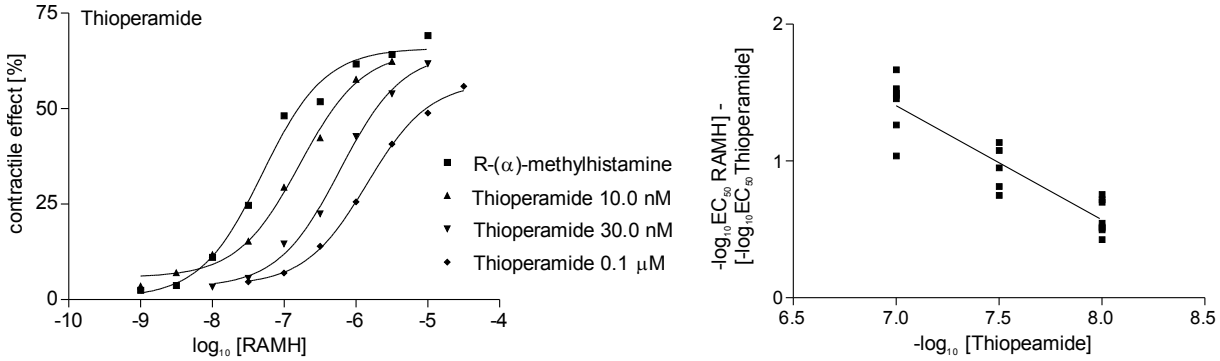
Contraction of electrically evoked guinea-pig ileum by R-(α)-methylhistamine in the absence (■) and presence (▲,▼, ♦) of compound **2f.**



Contraction of electrically evoked guinea-pig ileum by R-(α)-methylhistamine in the absence (■) and presence (▲,▼, ♦) of compound **2g.**



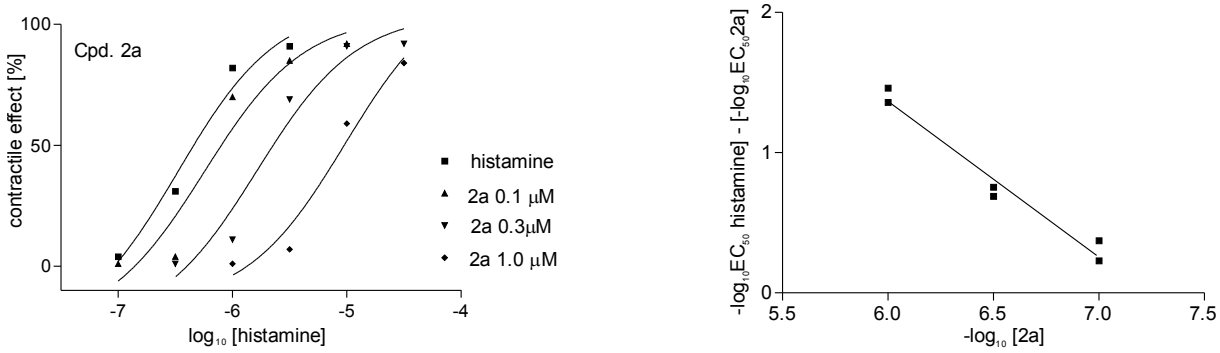
Contraction of electrically evoked guinea-pig ileum by R-(α)-methylhistamine in the absence (■) and presence (▲,▼, ♦) of compound **2h.**



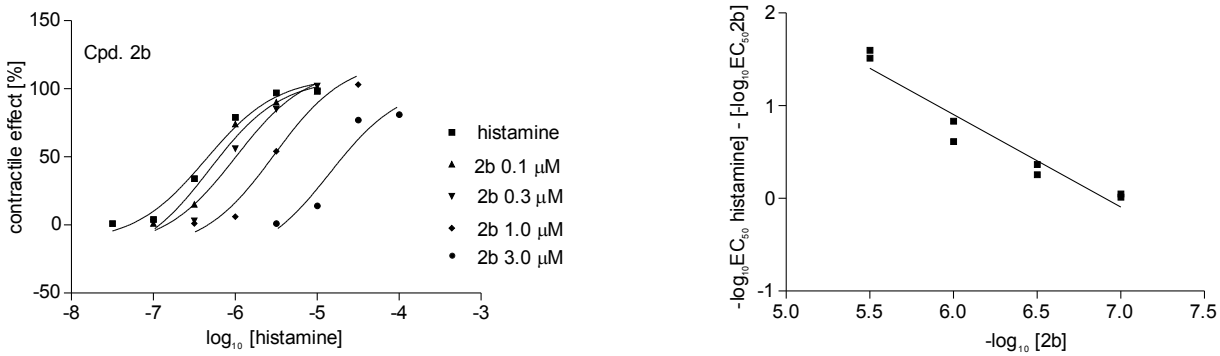
Contraction of electrically evoked guinea-pig ileum by R-(α)-methylhistamine in the absence (■) and presence (▲,▼, ♦) of the reference compound – thioperamide

Figure 2 Antagonism by **2a-h** (ADS-1025 – 1032) and thioperamide of the inhibitory effect of R-(-)- α-methylhistamine (RAMH) on the electrically induced contraction of guinea-pig ileum strips

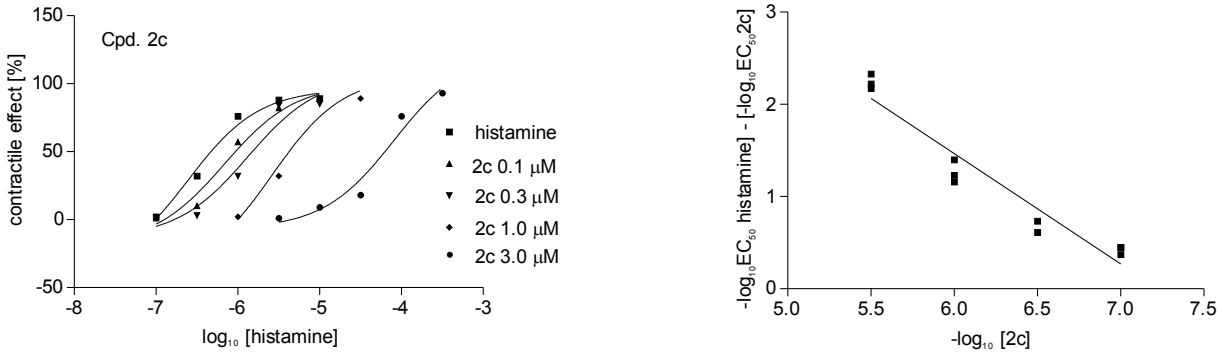
2.2.1.2 Antagonism by **2a-h** (ADS-1025 – ADS-1032) and pyrilamine of the inhibitory effect of histamine on the contraction of guinea-pig ileum strips



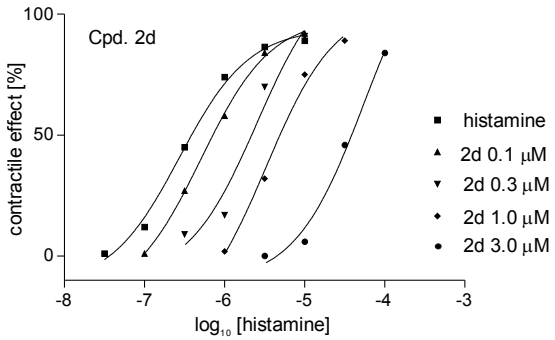
Contraction of guinea-pig ileum by histamine in the absence (■) and presence (▲,▼, ♦) of compound **2a**

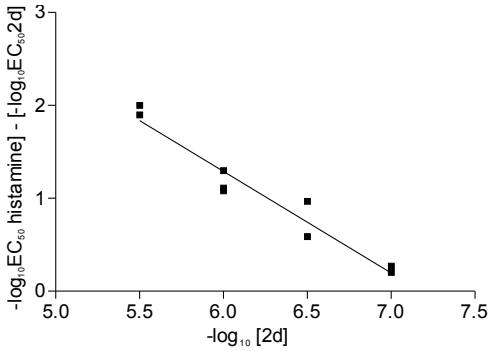


Contraction of guinea-pig ileum by histamine in the absence (■) and presence (▲,▼, ♦, ●) of compound **2b**

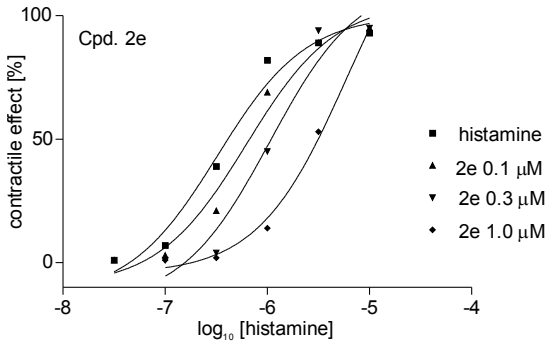


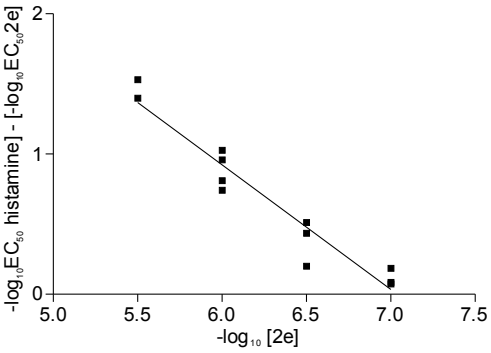
Contraction of guinea-pig ileum by histamine in the absence (■) and presence (▲,▼, ♦, ●) of compound **2c**



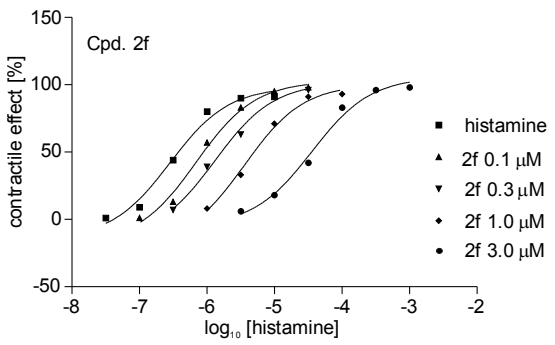


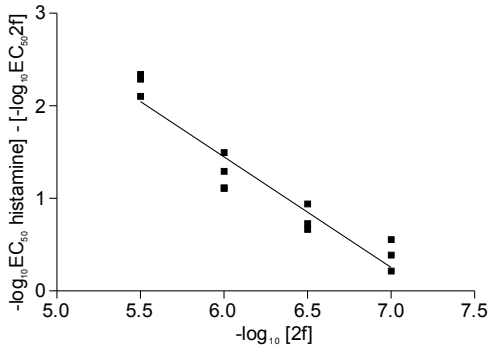
Contraction of guinea-pig ileum by histamine in the absence (■) and presence (▲,▼, ♦, ●) of compound **2d**



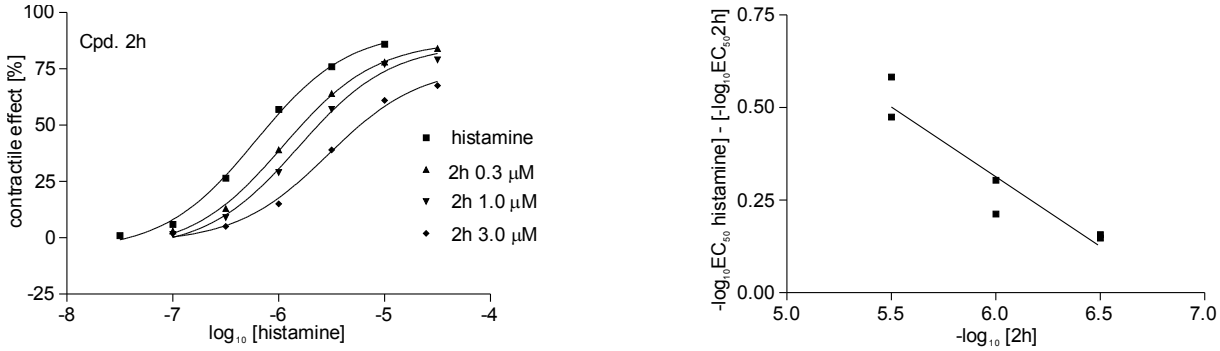


Contraction of guinea-pig ileum by histamine in the absence (■) and presence (▲,▼, ♦) of compound **2e**.

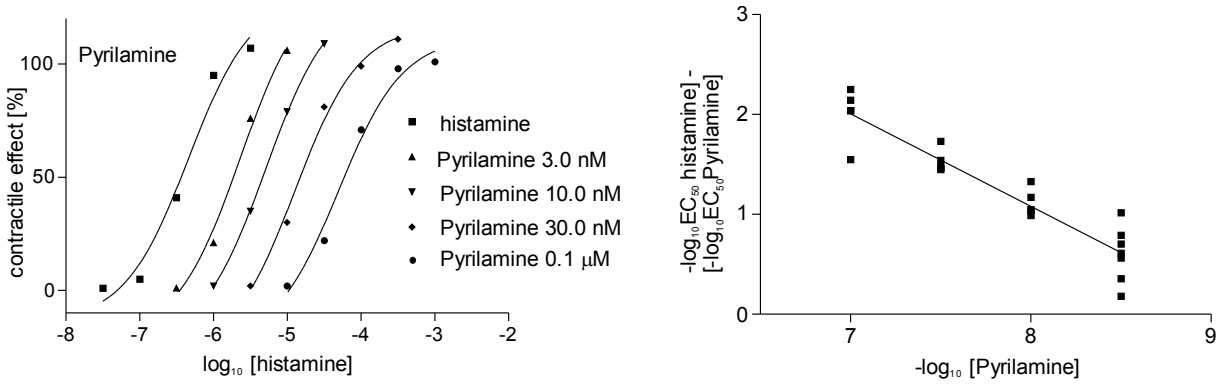




Contraction of guinea-pig ileum by histamine in the absence (■) and presence (▲,▼, ♦, ●) of compound **2f.**



Contraction of guinea-pig ileum by histamine in the absence (■) and presence (▲,▼, ♦) of compound **2h**.



Contraction of guinea-pig ileum by histamine in the absence (■) and presence (▲,▼, ♦, ●) of **Pyrilamine**

Figure 3. Antagonism by **2a-h** (ADS-1025 – ADS-1032) and pyrilamine of the inhibitory effect of histamine on the contraction of guinea-pig ileum strips

2.2.2.1 Histamine H_3 receptor affinity

Saturation of the rat H_3 receptors

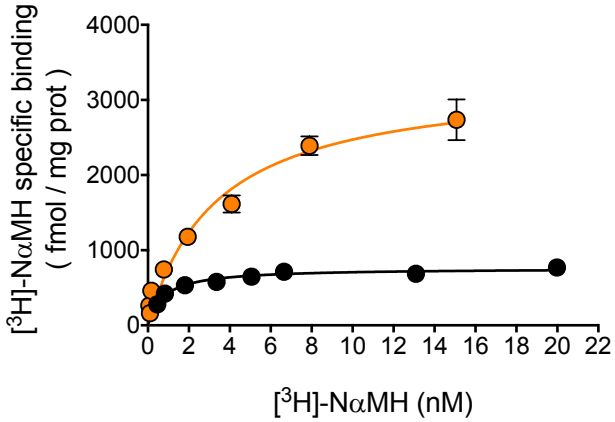


Figure 4. Saturation of the rat H_3R.

Competition binding of H_3 receptor ligands

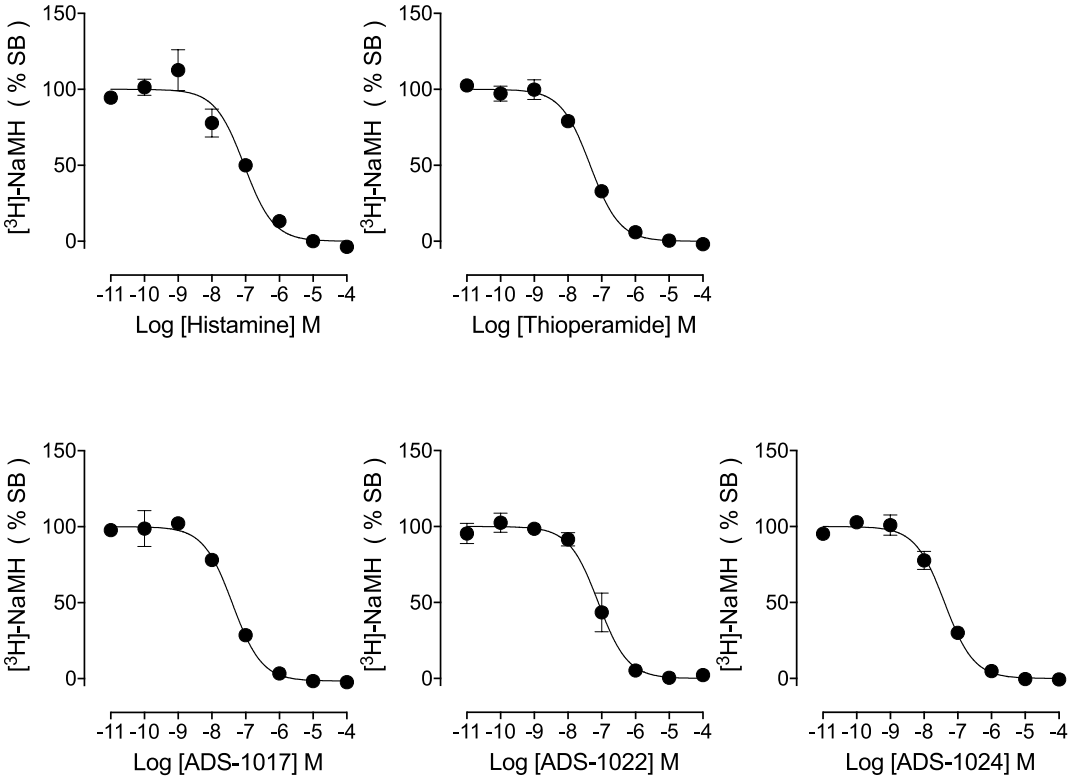


Figure 5 Competition binding of H_3R ligands on rat H_3 receptor.

*2.2.3.1 Post-mortem biochemical analysis of the brain tissues of **1a**, **1c**, and **1d** treated rats*

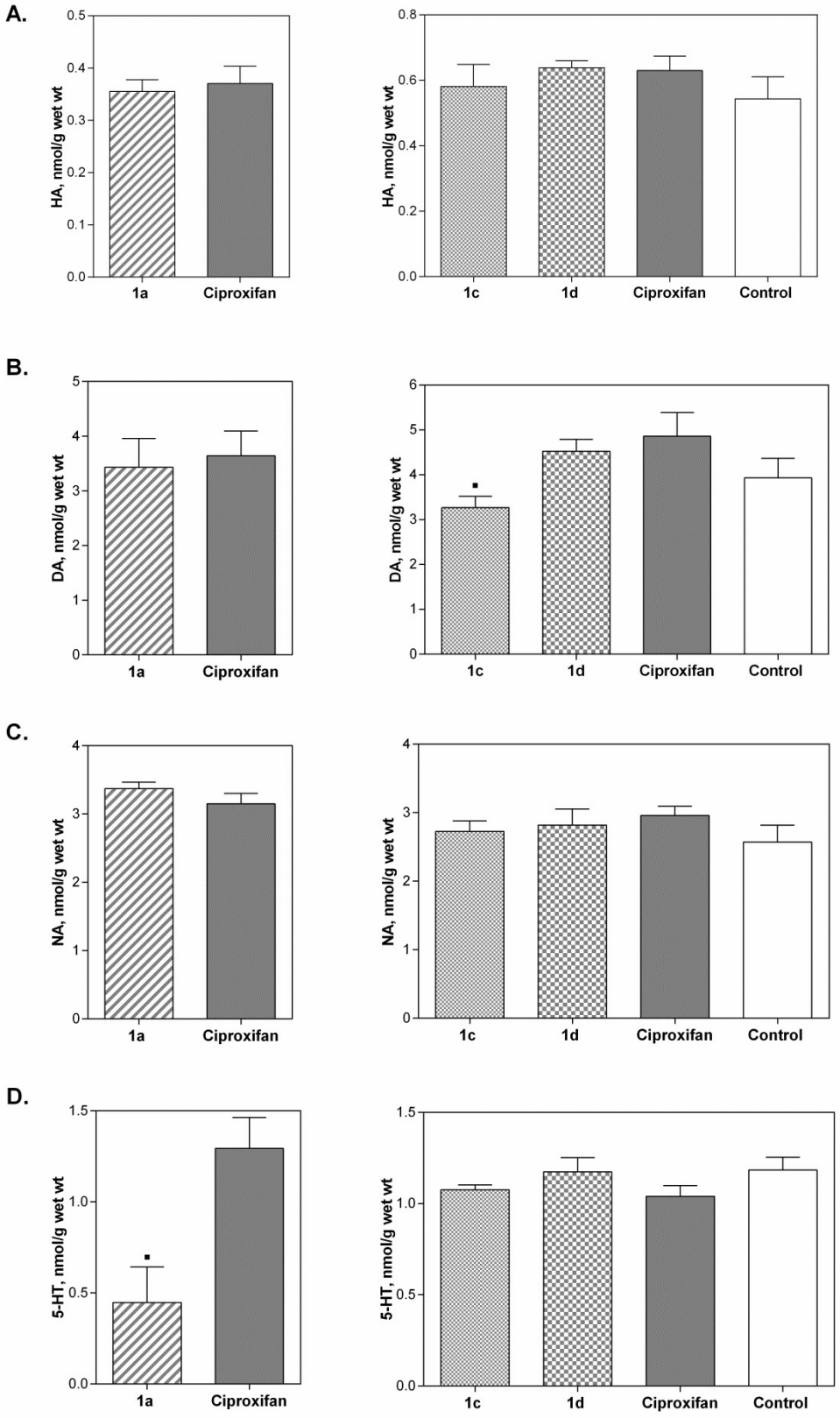


Figure 6 Cerebral amine neurotransmitters concentration in rats subchronically treated with newly synthesized **1a**, **1c** and **1d** histamine H_3 receptor antagonists and reference ciproxifan. **1a** examined with Wistar, 1c and 1d with Lewis rats.

One-way ANOVA and Tukey's multiple comparisons test: vs. Ciproxifan, $P < 0.05$.
The drugs were administered subcutaneously at a dose of 3 mg/kg of body mass for five consecutive days and the rats were sacrificed 24 h following the last dose, i.e., after its effects on food consumption have been recorded.
HA – histamine, DA – dopamine, NA – noradrenaline, 5-HT – serotonin.

Table 2. The effect of subchronic administration of **1a**, **1c** and **1d** histamine H_3 receptor antagonists (s.c. 3 mg/kg/daily for 5 days) and Ciproxifan (s.c. 3 mg/kg/daily for 5 days) on cerebral MAOs and HNMT activities

	Group	HMNT	MAO-A	MAO-B
		pmol/min/mg protein	*pmol/min/mg protein*	
Wistar rats	**1a**	46.74 ± 2.19	1654 ± 35.19	1037 ± 45.98
	Ciproxifan	46.90 ± 3.59	1654 ± 31.42	1049 ± 56.16
	Control	45.97 ± 1.28	1563 ± 14.89	1054 ± 19.31
Lewis rats	**1c**	57.41 ± 1.47	1047 ± 30.49	1220 ± 54.73
	1d	59.69 ± 1.24	1103 ± 76.10	1232 ± 55.26
	Ciproxifan	56.35 ± 0.84	1005 ± 43.98	1122 ± 38.36
	Control	60.58 ± 1.00	1116 ± 108.10	1098 ± 50.29

The values are means ± sem for 4-8 rats.
One-way ANOVA and Tukey's multiple comparisons test showed no statistically significant differences.
The drugs were administered subcutaneously at a dose of 3 mg/kg of body mass for 5 consecutive days and rats sacrificed 24 h following the last dose after its effects on food consumption have been recorded.
MAO – monoamine oxidase, HNMT – histamine *N*-methyltransferase.