10.1071/CH15569_AC

Australian Journal of Chemistry 69(7), 746-752

Supplementary Material

Synthesis and binding affinity of fluorine containing N^G-acyl and -sulfonyl BIBP3226 derivatives: Ligands for the NPY Y_1 receptor

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S1. Experimental details and analytical data for compounds 2, 4, 6, 7 and **8**

S1.1 Succinimidyl 2,2-diphenylacetate (2).[1, 2]

The synthesis of **(2)** was carried out as described in the published literature in 88% yield. The ^{1}H NMR data obtained was consistent with the published literature.

S1.2. 4-*tert*-Butoxybenzylamine (4).[3]

The synthesis of **(4)** was carried out as described in the published literature in 72% yield. The ^{1}H NMR data obtained was consistent with the published literature.

S1.3. (*R*)-*N*-(4-*tert*-Butoxybenzyl-N^{α}-(2,2-diphenylacetyl)ornithinamide (6).[4]

The synthesis of **(6)** was carried out as described in the published literature in 92% yield. The ^{1}H NMR data obtained was consistent with the published literature.

S1.4. *N*-Benzyloxycarbonyl-*N'*-*tert*-butoxycarbonyl-1*H*-pyrazole-1-carboxamidine (7).[5, 6]

The synthesis of **(7)** was carried out as described in the published literature (via the *N*-benzyloxycarbonyl-1*H*-pyrazole-1-carboxamidine intermediate, [4, 7]) in 41% yield. The ^{1}H NMR data obtained was consistent with the published literature.

S1.5. (R)-*N*-(4-*tert*-Butoxybenzyl)-N^{ω}-*tert*-butoxycarbonyl-N^{α}-(2,2-diphenylacetyl)argininamide (8).[8]

The synthesis of **(8)** was carried out as described in the published literature in 81% yield. The ^{1}H NMR data obtained was consistent with the published literature.

S2. Attempted preparation of N^{ω}-(2-fluoroethylsulfonyl)BIBP3226

Under an atmosphere of N_2, **8** (50.0 mg, 0.079 mmol) was dissolved in dry CH_3CN (4 mL). After cooling in a salt-ice bath Et_3N (50 μL, 0.36 mmol) followed by slow addition of 2-fluoroethylsulfonyl chloride (10 μl, 0.11 mmol) was added to the mixture. The mixture was stirred for 20 h and allowed to warm to r.t. The mixture was taken to dryness under high vacuum to give a white solid that was dissolved in a mixture of dry CH_3CN (3 mL) and TFA (0.5 mL) and heated at reflux for 1 - 3 hrs. The mixture was taken to dryness to give a yellow oil. The crude material was initially separated using a silica gel plug with n-heptane/EtOAc/CH_3OH, the products eluted with a 0/90/10 (n-heptane/EtOAc/CH_3OH) mixture of solvents to give a pale yellow oil containing 2 peaks in the HPLC trace. This material was further purified by preparative HPLC employing an isocratic solvent system (28/62/10, CH_3CN/H_2O/1% TFA in H_2O; n.b. at higher percentages of H_2O filter blockages were encountered presumably caused by precipitation of the compounds) on a Grace Alltima C_{18} semi-prep column (10 μm, 250 mm × 10 mm ID). Four fractions were collected F1 (14.93 - 16.45 min, 0.8 mg), F2

(16.45 - 18.18 min, 8.0 mg), F3 (20.07 - 22.21 min, 17.4 mg) and F4 (29.77 - 35.11 min, 3.4 mg, mixture of compounds).

S2.1. F1, Product #1 (mixture of 2 compounds)

LR-MS (ES^-): m/z 384.75, 520.72, 656.73, 676.04 $[C_{31}H_{33}F_3N_5O_7S]^-$ = $[C_{29}H_{33}N_5O_5S]$ + $CF_3CO_2^-$, 792.68; ^1H NMR (CD_3CN, 400.13 MHz): (2 compounds) 1.39 - 1.65 (m), 1.69 - 1.79 (m), 2.04 - 2.13 (m), 2.99 (m), 3.06 - 3.31 (m), 3.55 (m), 4.21 (m), 4.34 (m), 5.02 (s), 5.3 (s), 5.36 (br s), 6.70 - 6.77 (m), 8.84 (m), 7.00 - 7.09 (m), 7.16 (m) 7.22 - 7.37(m).

S2.2. F2, Product #2

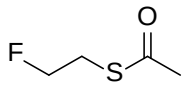
^1H NMR (CD_3CN, 400.13 MHz): 1.41 (m, 2H), 1.51 - 1.79 (m, 2H), 2.95 (m, 2H), 3.08 (m, 4H), 4.02 (m, 2H), 4.19 (m, 2H), 4.37 (m, 1H), 5.04 (s, 1H), 6.05 (br s, 1H), 6.73 (AB doublet, 2H), 6.83 (m, 1H), 7.03 (AB doublet, 2H), 7.18 - 7.37 (m, 10H); $^{13}C\{^1H\}$NMR (CD_3CN, 100.61 MHz): δ 14.39, 22.66, 26.45, 29.93, 30.35, 39.29, 40.17, 43.00, 44.74, 54.01, 58.34, 116.13, 116.59, 127.91, 127.96, 129.41, 129.47, 129.70, 129.71, 129.74, 131.12, 131.88, 141.05, 141.17, 156.38, 157.08, 158.81, 172.88, 173.59; LR-MS (ES^-): m/z 676.04 $[C_{31}H_{33}F_3N_5O_7S]^-$ = $[C_{29}H_{33}N_5O_5S]$ + $CF_3CO_2^-$; LR-MS (ES^+): m/z 585.98 $[C_{29}H_{33}N_5O_5SNa]^+$.

S2.3. F3: Product #3

^1H NMR (CD_3CN, 400.13 MHz): 1.48 - 1.83 (m, 2H), 2.04 - 2.15 (m, 2H), 3.62 - 3.73 (m, 4H), 3.84 (m, 2H), 4.18 (m, 2H), 5.00 (s, 1H), 6.71 (AB d, 2H), 7.02 (AB d, 2H), 7.18 - 7.35 (m, 10H); LR-MS (ES^-): m/z 676.00 $[C_{31}H_{33}F_3N_5O_7S]^-$ = $[C_{29}H_{33}N_5O_5S]$ + $CF_3CO_2^-$; LR-MS (ES^+): m/z 563.97 $[C_{29}H_{34}N_5O_5S]^+$, 585.96 $[C_{29}H_{33}N_5O_5SNa]^+$.

S3. Fluoroalkylsulfonyl compounds

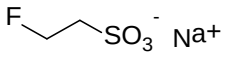
S3.1. Thioactetic acid S-(2-fluoroethyl) ester



2-Fluoroethyl methanesulfonate[9] (7.03 g, 49.45 mmol) and potassium thioacetate (11.63g, 100.8 mmol) were dissolved in CH_3CN (200 mL) and stirred at r.t. for 3 days, the mixture became bright orange over this time. The mixture was poured into DeI H_2O (600 mL) and extracted with Et_2O (3 × 150 mL). The combined organic fractions were dried over $MgSO_4$, filtered and taken to dryness. The viscous brown oil was purified under reduced pressure employing a Kugelrohr apparatus, 70 - 80 °C @ 50 mmHg, giving 2.86 g of a yellow oil. ^1H NMR ($CDCl_3$, 400.13 MHz): δ 3.18 (dt, J_1 = 21.45 Hz, J_2 = 6.2 Hz, 2H), 4.48

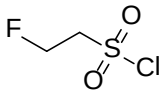
(dt J_1 = 46.93 Hz, J_2 = 6.2 Hz, 2H); $^{13}C\{^1H\}$ NMR ($CDCl_3$, 100.62 MHz): δ 29.56 (d, J_1 = 21.97 Hz, CH_2), 30.60 (CH_3) 81.83 (d, J_1 = 170.83 Hz, CH_2), 194.95 (CO).

S3.2. Sodium 2-fluoroethylsulfonate



Thioacetic acid S-(2-fluoroethyl) ester (2.86 g, 23.41 mmol) was dissolved in glacial AR acetic acid (40 mL). After warming the solution to 60 °C, 30% aqueous H_2O_2 (12 mL) was added dropwise, rapidly discharging the yellow color from solution, the mixture was heated for a further 3 h after addition. After cooling to r.t. the mixture was reduced to dryness to give a thick oil, before being dried under high vacuum overnight. The residue was dissolved in DeI H_2O (50 mL) before being carefully neutralized with 1M aqueous Na_2CO_3. The solution was then taken to dryness to give a fine white powder that was dried under high vacuum overnight. The crude material was used without further purification. ^1H NMR (D_2O - internal Me_2CO reference, 400.13 MHz): δ 3.31 (dt, J_1 = 25.3 Hz, J_2 = 5.4 Hz, 2H), 4.82 (dt, J_1 = 46.7 Hz, J_2 = 5.4 Hz, 2H); $^{13}C\{^1H\}$ NMR (D_2O - internal Me_2CO reference, 100.62 MHz): δ 51.92 (d, J = 20.1 Hz) 80.11 (d, J = 164.5 Hz).

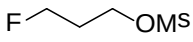
S3.3. 2-Fluoroethylsulfonyl chloride



Crude sodium 2-fluoroethylsulfonate (3.32 g) was suspended in dry CH_2Cl_2 (100 mL) to which was carefully added $SOCl_2$ (6 mL) and DMF (0.1 mL). The mixture was heated to reflux under a nitrogen atmosphere for 5 h. The solution was filtered through a pad of oven-dried celite and the eluate taken to dryness under high vacuum to give a yellow liquid. The crude product was purified by distillation under reduced pressure using a Kugelrohr apparatus, 120 - 140 °C at 10 - 15 mmHg to give a yellow liquid, 1.98 g. ^1H NMR ($CDCl_3$, 400.13 MHz): δ 4.06 (dt, J_1 = 21.2 Hz, J_2 = 5.2 Hz, 2H), 4.98 (dt, J_1 = 46.4 Hz, J_2 = 5.2 Hz, 2H); $^{13}C\{^1H\}$ NMR ($CDCl_3$, 100.61 MHz): δ 64.88 (d, J = 22.1 Hz, CH_2), 76.68 (d, J = 177.9 Hz, CH_2); $^{19}F\{^1H\}$ NMR (CD_3CN, 376.50 MHz): δ -222.58 (s, -CH_2F).

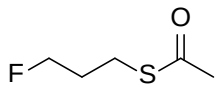
S3.4. 3-Fluoropropyl methanesulfonate

Prepared in a similar manner to that described for 2-fluoroethyl methanesulfonate.[9]



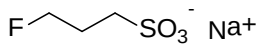
A 2-neck fitted with a dropping funnel and nitrogen inlet was charged with 3-fluoropropanol (5.94 g, 76 mmol) and Et_3N (11.57 g, 114 mmol) and anhydrous Et_2O (150 mL). Methanesulfonyl chloride (13.11 g, 114 mmol) dissolved in Et_2O (50 mL) was added to the dropping funnel. The RB flask was cooled in a salt-ice bath for 30 min before drop-wise addition of the $MeSO_2Cl/Et_2O$ mixture was commenced. A thick white precipitate rapidly began to form rapidly. After complete addition the mixture was allowed to warm to r.t. The white precipitate (Et_3NHCl) was removed by filtration and was washed with Et_2O (2 × 150 mL). The volume of the filtrate was reduced to ~100 mL before being washed quickly with H_2O (3 × 100 mL), dried over anhydrous $MgSO_4$, filtered and taken to dryness to give a place brown liquid. The crude material was subjected to fractional distillation to remove any residual $MeSO_2Cl$ followed by distillation, pressure/ temperature, of the desired product as a colorless liquid, 4.30 g. ^1H NMR ($CDCl_3$, 400.13 MHz): δ 2.13 (m, 2H), 3.02 (s, 3H), 4.36 (t, J =6.11 Hz, 2H), 4.57 (dt, J1 = 46.5 Hz, J2 = 5.7 Hz, 2H).

S3.5. Thioactetic acid S-(3-fluoropropyl) ester



3-Fluoropropyl methanesulfonate (4.30 g, 27.53 mmol) and potassium thioacetate (6.33 g, 55.42 mmol) were dissolved in CH_3CN (150 mL) and stirred at r.t. for 3 days, the mixture became red-brown over this time. The mixture was poured into DeI H_2O (600 mL) and extracted with Et_2O (3 × 150 mL). The combined organic fractions were dried over anhydrous $MgSO_4$, filtered and taken to dryness. The residue was purified under reduced pressure employing a Kugelrohr apparatus, 85 - 100 °C @ 35 mmHg, giving 2.04g of a pale yellow oil. ^1H NMR (d_6-DMSO, 400.13 MHz): δ 1.89 (ttd, J_1 = 5.90 Hz, J_1 = 7.28 Hz, J_3 = 1.4 Hz, 2H), 2.33 (s, 3H), 2.91 (t, J1 = 7.28 Hz, 2H), 4.46 (dt, J_1 = 47.4 Hz, J_2 = 5.90 Hz); $^{13}C\{^1H\}$ NMR (d_6-DMSO, 100.62 MHz): δ 24.41 (d, J = 5.85 Hz, CH_2), 30.00 (d, J = 91.81 Hz, CH_2), 30.49 (CH_3), 82.38 (d, J = 162.74 Hz, CH_2), 195.09 (CO).

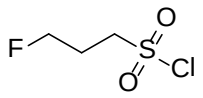
S3.6. Sodium 3-fluoropropylsulfonate



Thioacetic acid S-(3-fluoropropyl) ester (2.04 g, 14.98 mmol) was dissolved in glacial AR acetic acid (40 mL). After warming the solution to 60 °C, 30% aqueous H_2O_2 (7.5 mL) was added dropwise, rapidly discharging the yellow color from solution, the mixture was heated for a further 3 h after addition. After cooling to r.t. the mixture was reduced to dryness to give a thick oil, before being dried under high vacuum overnight. The residue was dissolved in DeI H_2O (50 mL) before being carefully neutralized

with 1M aqueous Na_2CO_3. The solution was then taken to dryness to give a fine white powder that was dried under high vacuum overnight. The crude material was used without further purification. ^{1}H NMR (D_2O - internal Me_2CO reference, 400.13 MHz): δ 2.13 (m, 2H), 3.03 (m, 2H), 4.61 (dt, J_1 = 46.9 Hz, J_2 = 5.95 Hz); $^{13}C\{^1H\}$ NMR (D_2O - internal Me_2CO reference, 100.62 MHz): δ 26.11 (d, J = 20.1 Hz, CH_2), 47.76 (d, J = 5.92 Hz, CH_2), 83.85 (d, J = 160.2 Hz, CH_2).

S3.7. 3-Fluoropropylsulfonyl chloride



Crude sodium 3-fluoropropylsulfonate, from the previous reaction, (2.6 g) was suspended in dry CH_2Cl_2 (100 mL) to which was carefully added $SOCl_2$ (6 mL) and DMF (0.1 mL). The mixture was heated to reflux under a nitrogen atmosphere for 5 h. The solution was filtered through a pad of oven-dried celite and the eluate taken to dryness under high vacuum to give a yellow liquid. The crude product was purified by distillation under reduced pressure using a Kugelrohr apparatus, 120 - 130 °C at 10 - 12 mmHg to give a pale orange oil, 2.00 g. ^{1}H NMR ($CDCl_3$, 400.13 MHz): δ 2.44 (m, 2H), 3.83 (m, 2H), 4.62 (dt, J_1 = 46.7 Hz, J_2 = 5.5Hz, 2H); $^{13}C\{^1H\}$ NMR ($CDCl_3$, 100.62 MHz): δ 25.91 (d, J = 20.50), 61.63 (d, J = 3.91), 80.55 (d, J = 169.16); $^{19}F\{^1H\}$ NMR (CD_3CN, 376.50 MHz): δ -222.28 (s, $-CH_2F$).

S4. Preparation of membranes from mouse brain

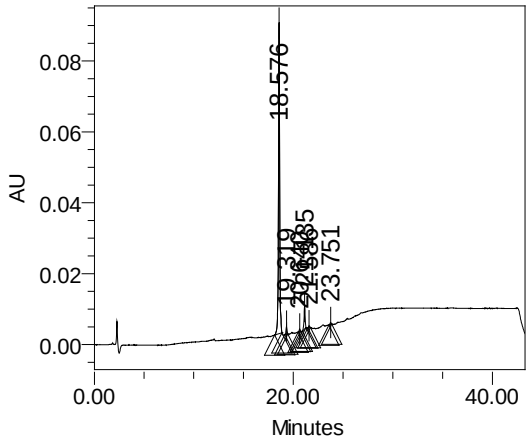
To test the Y_1R affinity of the synthesized ligands, receptor binding assays (described below) were performed on crude membranes prepared from the brains of Y_2R- and Y_4R-deficient mice ($Y2^{-/-}Y4^{-/-}$), where Y_1R accounts for the majority of remaining Y receptors. Membranes were prepared following modified membrane extraction protocol published elsewhere (McCrea KE, Herzog H. Radioligand binding studies. Pharmacological profiles of cloned Y-receptor subtypes. Methods Mol Biol. 2000; 153:231-9). In brief, fresh frozen $Y2^{-/-}Y4^{-/-}$ mouse brains were cut into small cubes and homogenized in ice-cold homogenization buffer (50 mM Tris-HCl, 10 mM NaCl, 5 mM $MgCl_2$, 2.5 mM $CaCl_2$, pH7.4, supplemented with 1 mg/mL bacitracin (250,000U; Calbiochem-Novabiochem., La Jolla, CA, USA) prior to use) on ice with a glass homogenizer (Wheaton, USA) using 30 strokes. Subsequently, the homogenates were centrifuged at 32,000g for 15 minutes at 4ºC. The resulting pellet was re-suspended in ice-cold homogenization buffer and re-homogenized using 30 strokes on ice, followed by centrifugation at 32,000g for 15 minutes at 4ºC to obtain the final pellet. The final pellet was re-suspended in ice-cold homogenization buffer and flash frozen in liquid nitrogen. The protein concentration of the suspension was determined using Bradford protein assay (Quick Start™ Bradford Protein Assay, Bio-Rad Laboratories Pty., Ltd., Hercules, CA, USA).

S4.1. Receptor binding assays

Competition assays were performed on $Y2^{-/-}Y4^{-/-}$ mouse brain membrane preparations following procedures published previously (McCrea KE, Herzog H. Radioligand binding studies. Pharmacological profiles of cloned Y-receptor subtypes. Methods Mol Biol. 2000; 153:231-9). Briefly, equal volumes (25 µL) of non-radioactive ligands and ^{125}I-human polypeptide YY (^{125}I-hPYY, 2200 Ci/mmol; PerkinElmer Life Science Products, Boston, MA, USA) were added into each assay. The final concentration of ^{125}I-hPYY in the assay was 25^{-12} M. The binding of ^{125}I-hPYY was competed by Y_1R ligands of interest at increasing concentrations ranging from 10^{-12} M to 10^{-6} M. Non-radioactive human PYY (Auspep, Parkville, VIC, Australia) at 10^{-6} M was used as the non-specific binding control. The reaction was initiated by the addition of 50 µL of membrane suspension containing 30 µg of protein into the assay mixture and incubated for 2 hours at r.t. After the incubation, each sample was layered with 200 µL of pre-cooled (4ºC) horse serum and centrifuged at 13,000g for 4 minutes to separate of bound from free ^{125}I-PYY. The supernatant solution was removed and resultant pellet was harvested and counted for radioactivity using a γ-counter (Wallac 1470 WIZARD® Gamma Counter; PerkinElmer Life Sciences, Turku, Finland). Finally, the data were analyzed with GraphPad Prism Software to determine the IC_{50} value.

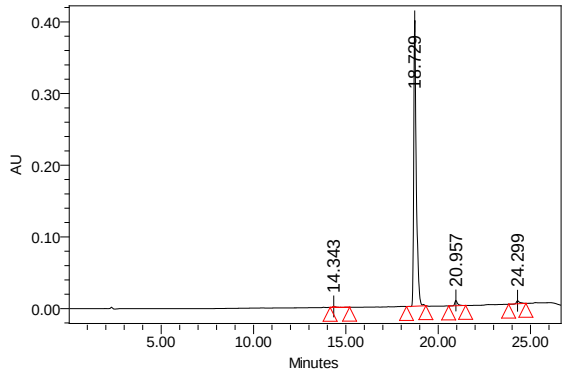
S5. HPLC purity analysis

S5.1. 9b 90.03% purity, t_R = 18.57 min.



	t_R	Area	% Area	Height
1	18.576	726430	90.03	88273
2	19.319	16194	2.01	2280
3	20.640	5282	0.65	779
4	21.135	49683	6.16	6313
5	21.586	4660	0.58	572
6	23.751	4648	0.58	570

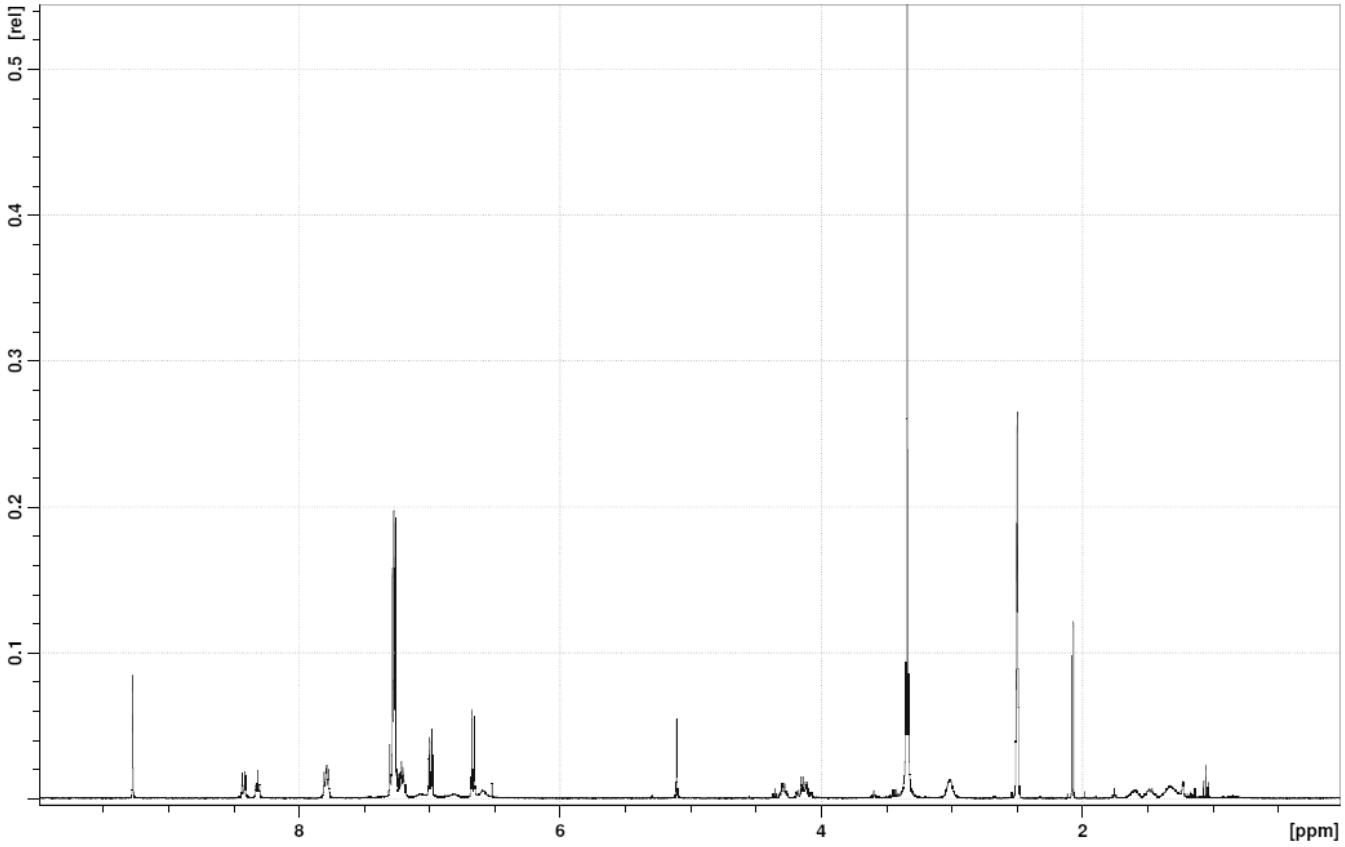
S5.2. 9c 96.24% purity, t_R = 18.73 min.



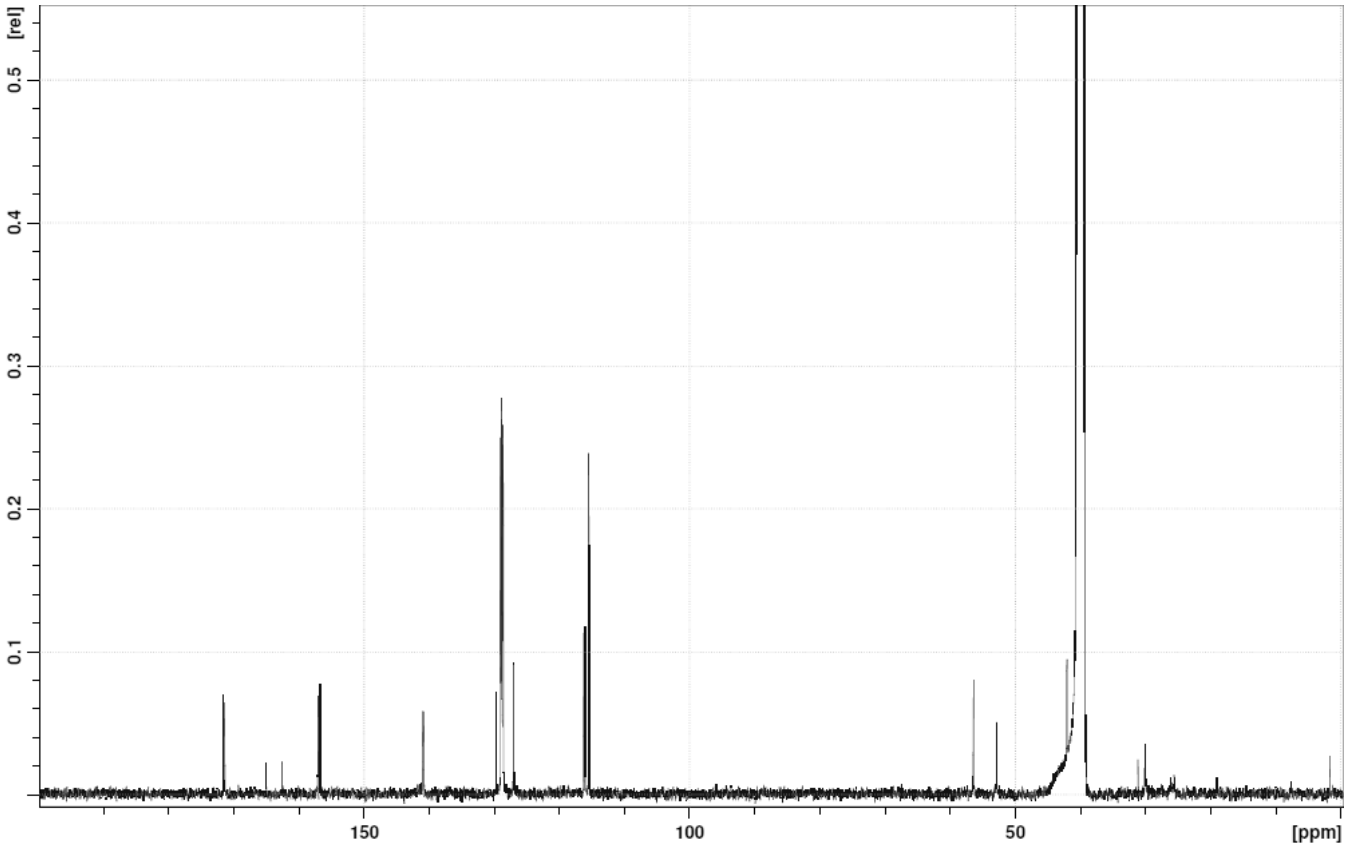
	t_R	Area	% Area	Height
1	14.343	15134	0.38	1477
2	18.729	3822738	96.24	398935
3	20.957	76998	1.94	7392
4	24.299	57195	1.44	4191

S6. NMR spectra

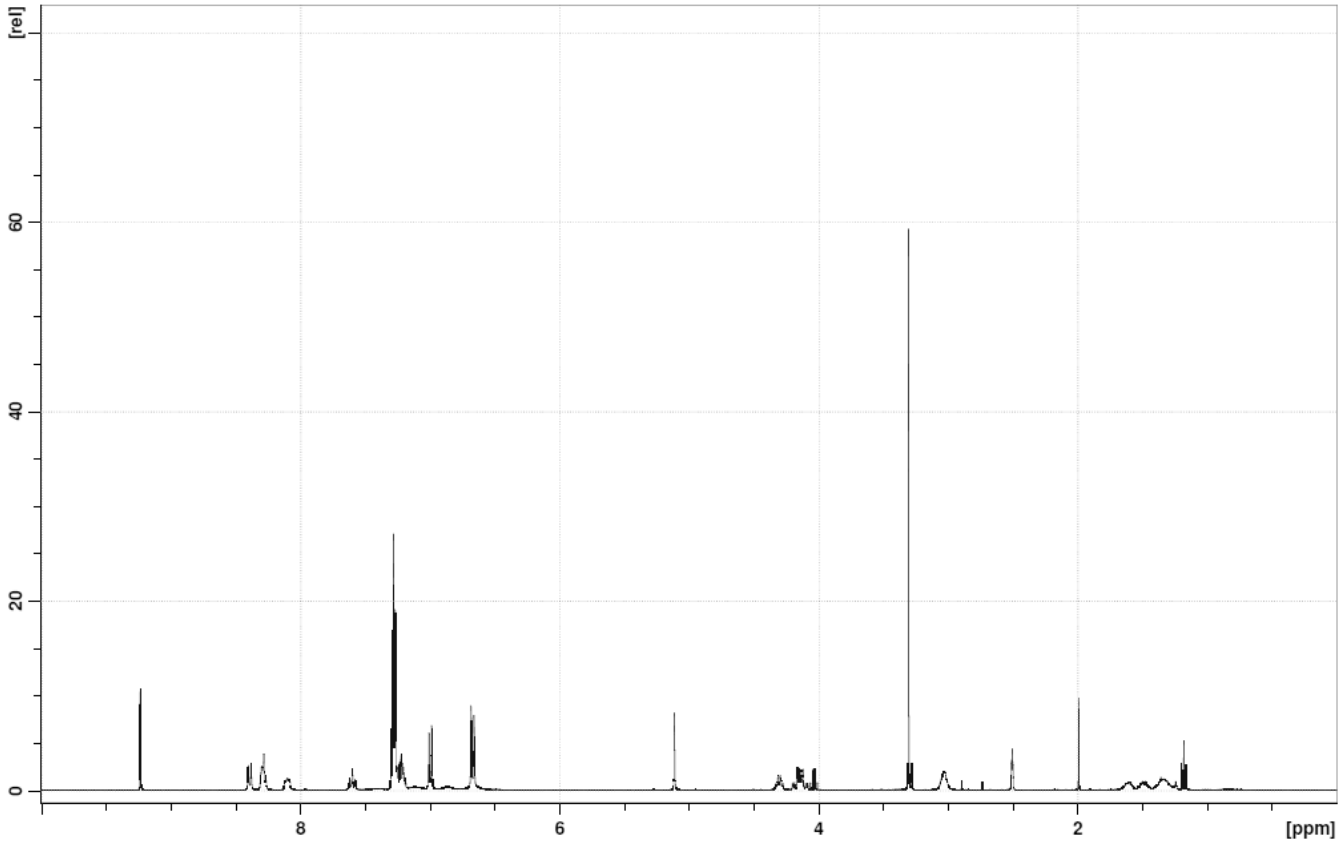
S6.1. 9b ^{1}H NMR



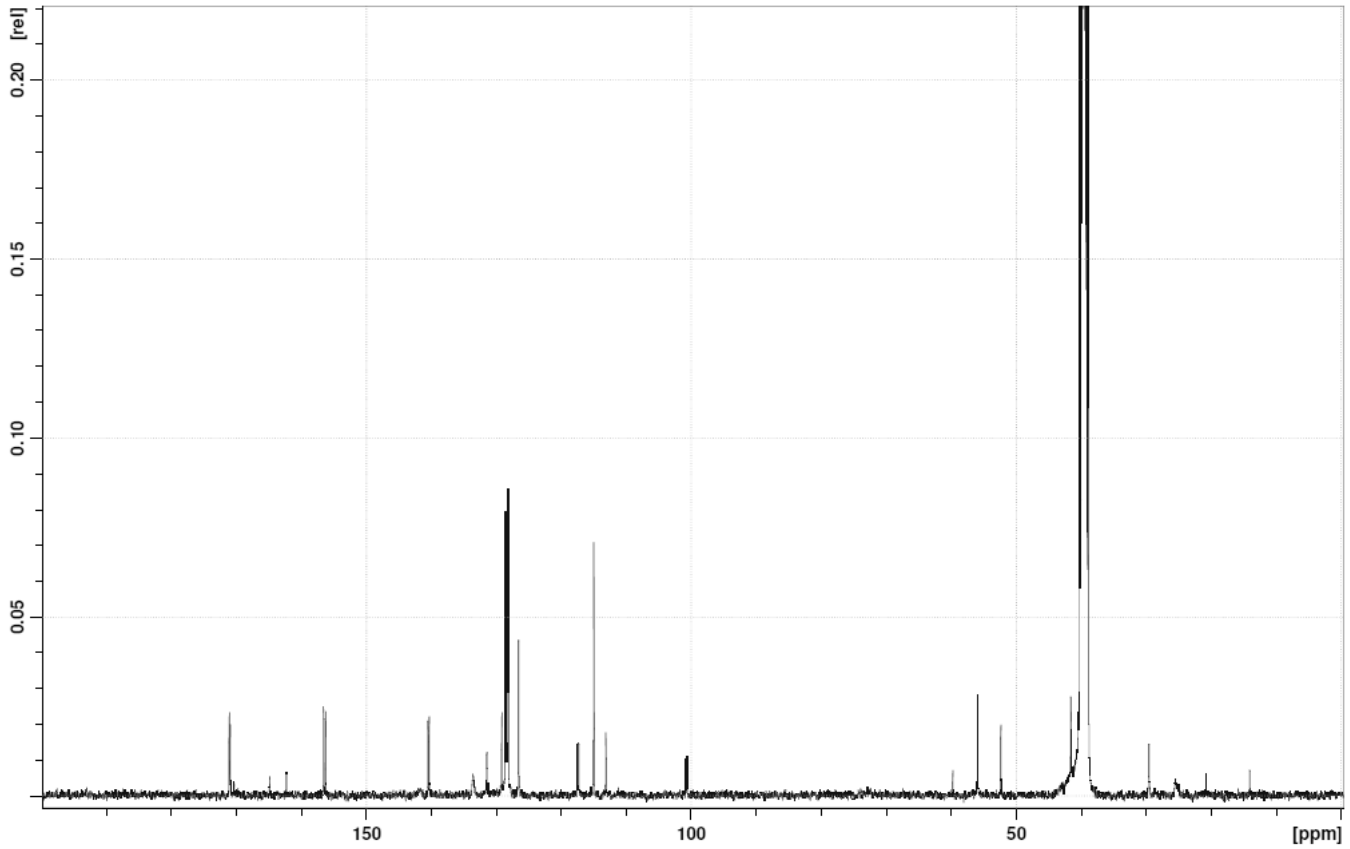
S6.2. 9b ^{13}C NMR



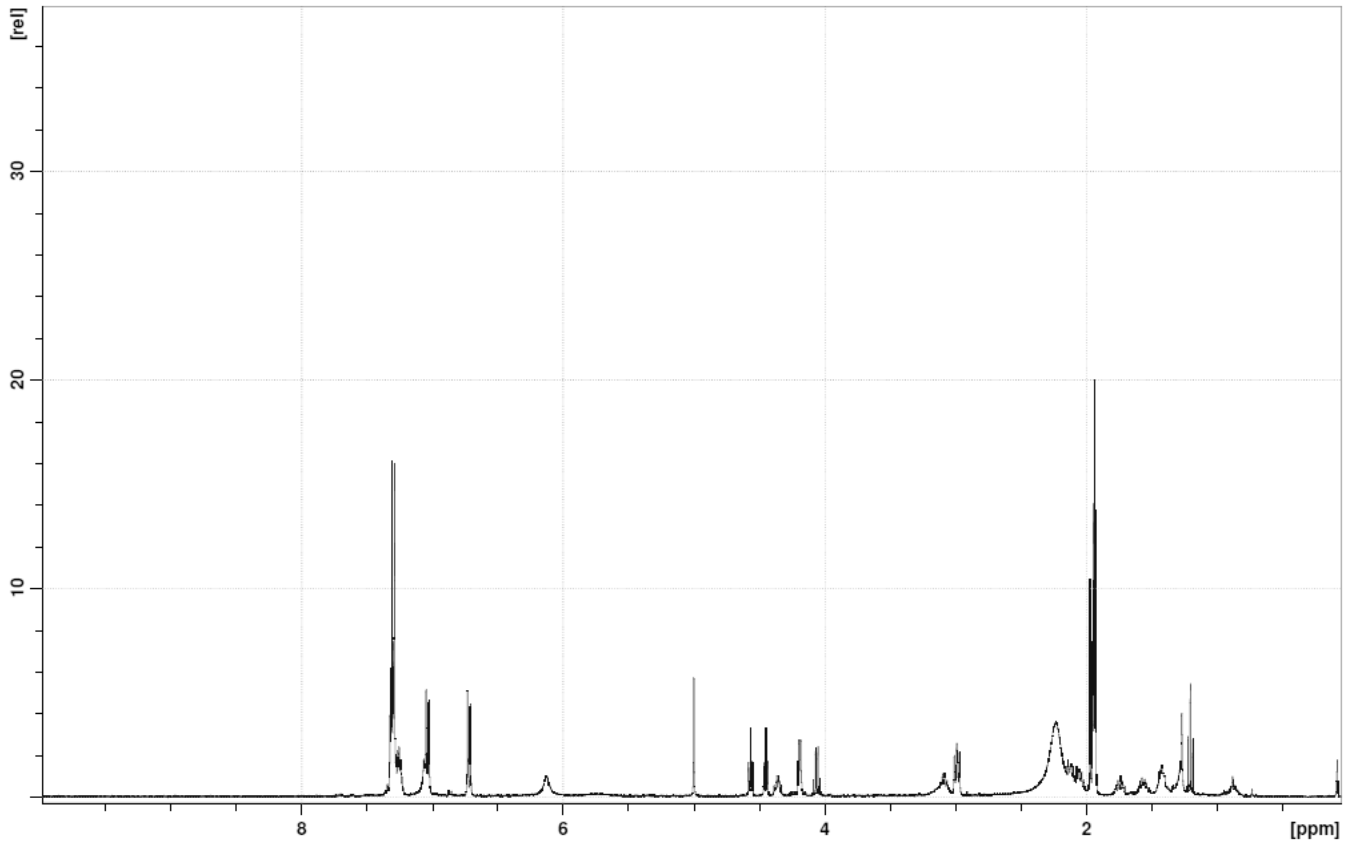
S6.3. 9c ^{1}H NMR



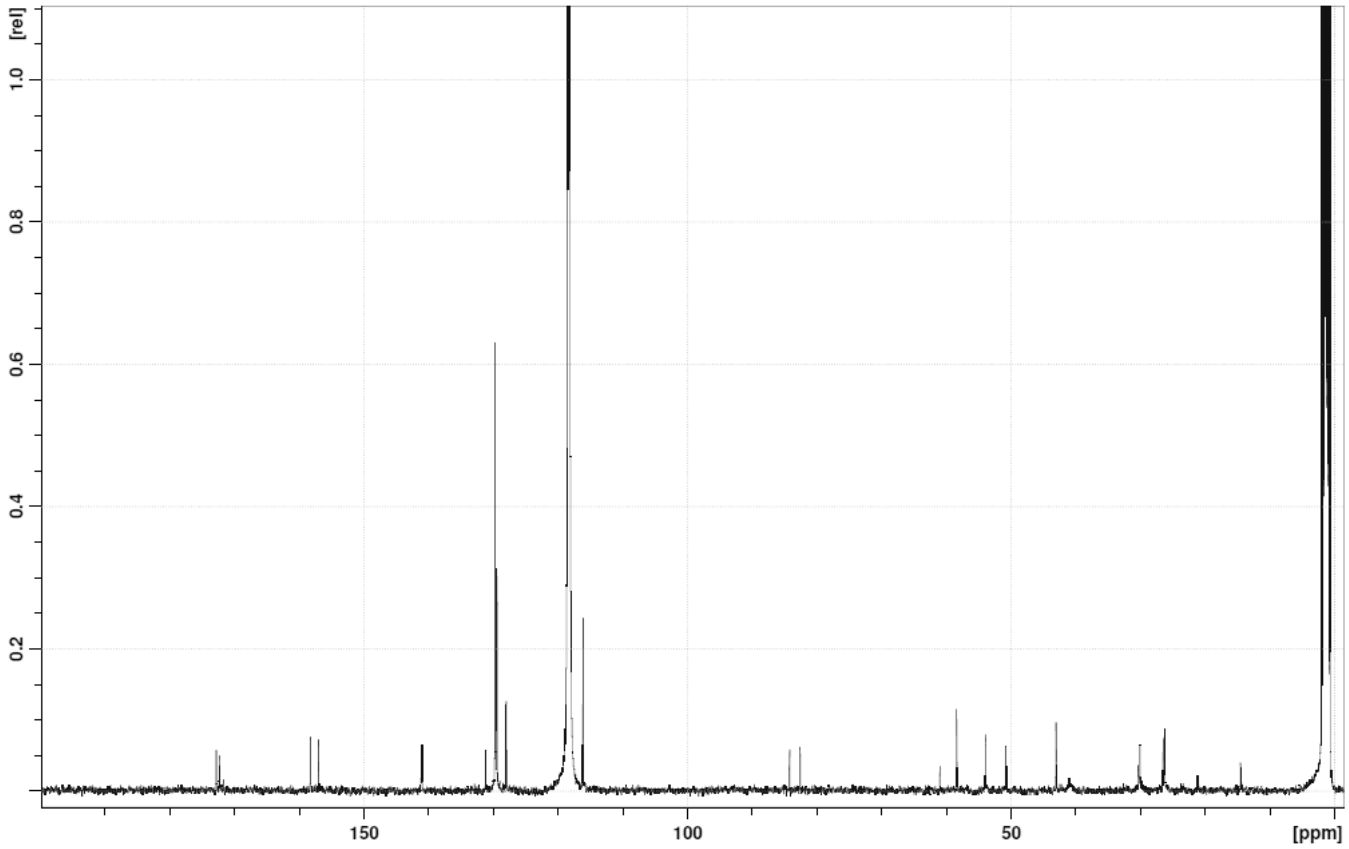
S6.4. 9c ^{13}C NMR



S6.5. 9d ^{1}H NMR



S6.6. 9d ^{13}C NMR



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