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Electronic Supplementary Information

Evaluation of a ^{125}I-Labelled Benzazepinone derived Voltage-Gated Sodium Channel Blocker for Imaging with SPECT

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Appendix 1. Synthetic procedures

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Appendix 1. Synthetic procedures

Compounds **2**[1], **3**[2], **4**-**7**[3] and **11**[4] have previously been described and our analytical data are in agreement with these published results.

3-Azido-4,5-dihydro-1*H*-benzo[*b*]azepin-2(3*H*)-one (2). A solution of 3-bromo-4,5-dihydro-1*H*-benzo[*b*]azepin-2(3*H*)-one (**1**, 10 g, 42 mmol), sodium azide (4.0 g, 63 mmol) and sodium iodide (6.0 g, 42 mmol) in DMF (20 mL) was stirred for 24 hours at room temperature. Upon addition of water, a solid was formed which was filtered and washed with water. After drying, pure product was obtained as a white solid (7.84 g, 92 %), mp 140-142°C (lit.[1] mp 142-145°C); IR (neat): 3189, 1665, 1584, 1489, 1270 cm^{-1}; ^{1}H-NMR (500 MHz, $CDCl_3$) δ in ppm: 2.31 (m, 1H); 2.52 (m, 1H); 2.73 (m, 1H); 2.97 (m, 1H); 3.89 (dd, ^{3}J=11.3, ^{3}J=8.0 Hz, 1H); 7.08 (d, ^{3}J=7.8 Hz, 1H); 7.17 (dd, ^{3}J=7.4, ^{4}J=1.0 Hz, 1H); 7.25 (m, 2H); 8.77 (s, 1H, NH). ^{13}C-NMR (125 MHz, $CDCl_3$) δ in ppm: 28.4 (CH_2); 35.0 (CH_2); 59.2 (CH); 122.5 (CH); 126.5 (CH); 128.1 (CH); 129.8 (CH); 133.5; 136.3; 171.7. HRMS-CI [$(M+H)^+$]: 203.0938 m/z (calculated for $C_{10}H_{11}N_4O$ 203.0933).

(*R*)-3-amino-4,5-dihydro-1*H*-benzo[*b*]azepin-2(3*H*)-one (*R*-3). 3-azido-4,5-dihydro-1*H*-benzo[*b*]azepin-2(3*H*)-one (**2**, 7.84 g, 38 mmol) and triphenylphosphine (9.9 g, 76 mmol) were placed in a flask which was sealed and flushed with argon, and 135 mL of THF and 15 mL of water were added. The mixture was stirred for 18 hours at room temperature and then at 60 °C for another two hours. The solution was allowed to cool down to room temperature and concentrated to almost dryness under reduced pressure to afford a whitish residue which was redissolved in 50 mL of ethyl acetate. Upon addition of 4 M HCl (20 mL) the formation of a white crystalline solid was observed. The mixture was left standing for one hour and the solid collected by filtration and washed with ethyl acetate. The solid was then treated with 2 M NaOH (25 mL) and the resulting mixture extracted with ethyl acetate. The organic fractions are combined, dried over magnesium sulphate and concentrated to yield pure product as a white solid (5.4 g, 81 %).

A solution of **3** (5.2 g, 29 mmol), D-pyroglutamic acid (3.84 g, 29 mmol) and 5-nitrosalicylaldehyde (0.15 g, 3 mol %) in a mixture of isopropanol (114 mL) and water (6 mL) was heated under argon at 70 °C for 48 hours (a precipitate formed after a short time). The precipitate was collected by filtration and washed with isopropanol. The isolated salt was then transferred to a flask where 100 mL of acetonitrile/water 9:1 were added. The slurry was heated at 73 °C for 40 minutes and then the resulting solid was filtered and washed with acetonitrile/water 9:1. Ethyl acetate (150 mL) and 2M NaOH (25 mL) were added to the isolated salt for extraction. The organic layer was dried over magnesium sulphate and, after removal of ethyl acetate evaporated under vacuum, pure *R*-**3** was obtained as a white solid (3.56 g, 68 %, >96 % ee), mp 148-150°C (lit.[2] mp 147-149°C); IR (neat): 1665, 1590, 1488, 1405, 1261 cm^{-1}; ^{1}H-NMR (500 MHz, $CDCl_3$) δ in ppm: 1.7 (bs, 2H, NH_2); 1.92 (m, 1H); 2.49 (m, 1H); 2.63 (dd, ^{2}J=13.7 Hz, ^{3}J=6.9 Hz, 1H); 2.89 (m, 1H); 3.42 (dd, ^{3}J=11.4 Hz, ^{3}J=8.0 Hz, 1H); 6.99 (d, ^{2}J=7.7 Hz, 1H); 7.13 (m, 1H); 7.23 (m, 2H); 8.27 (s, 1H, NH). ^{13}C-NMR (125 MHz, $CDCl_3$) δ in ppm: 29.1 (CH_2); 39.3(CH_2); 51.6 (CH); 122.1 (CH); 126.1 (CH); 127.6 (CH); 129.7 (CH); 134.6; 136.7; 177.3. HRMS-EI [$(M+H)^+$]: 176.0941 m/z (calculated for $C_{10}H_{12}N_2O$ 176.0944).

(*R*)-3-Tritylamino-4,5-dihydro-1*H*-benzo[*b*]azepin-2(3*H*)-one (4). Over a solution of *R*-**3** (4.2 g, 24 mmol) in anhydrous DMF (12 mL) was added triethylamine (7.0 mL) and then trityl

chloride (7.4 g, 27.mmol) dissolved in chloroform (45 mL). The mixture was stirred for 20 hours under argon after which time water (25 mL) was added. The organic layer was collected and washed with water (2x), dried over magnesium sulphate and the solvent removed under reduced pressure. The resulting syrupy residue was treated with methanol/water 1:1 (100 mL) in an ultrasound bath for an hour. Filtration of the mixture afforded the desired product as a white solid (8.95 g, 89 %), mp 96-98°C; IR (neat): 3055, 2858, 1670, 1489, 1376 cm^{-1}; ^{1}H-NMR (500 MHz, $CDCl_3$) δ in ppm: 2.20 (m, 1H); 2.54 (m, 1H); 2.71 (m, 1H); 3.23 (m, 1H); 3.32 (bs, 1H); 6.64 (d, 7.6, 1H), 6.80 (s, 1H); 7.05-7.23 (m, 12H); 7.44 (d, ^{3}J=7.5 Hz, 6H). ^{13}C-NMR (125 MHz, $CDCl_3$) δ in ppm: 28.9 (CH_2); 38.7 (CH_2); 52.9 (CH); 71.6; 122.1 (CH); 126.1 (CH); 126.3 (CH); 127.5 (CH); 127.7 (CH); 128.9 (CH); 129.7 (CH); 134.9; 136.5; 146.3; 175.3. HRMS-ES [$(M+Na)^+$]: m/z 441.1929 (calculated for $C_{29}H_{26}N_2ONa$ 441.1943).

(*R*)-3-Tritylamino-1-isopropyl-4,5-dihydro-1*H*-benzo[*b*]azepin-2(3*H*)-one (5). A sealed flask containing sodium hydride (180 mg, 7.2 mmol) was placed in an ice/water bath and a solution of **4** (3.0 g, 7.2 mmol) in anhydrous DMF (20 mL) was added dropwise under argon. The mixture was stirred for 40 minutes. Keeping the flask in the bath, 2-iodopropane (2.7 mL, 29 mmol) was added and the resulting solution stirred for 17 hours at room temperature. Water was then added (15 mL) and the flask placed in an ultrasound bath for 20 minutes. The resulting solid was collected by filtration and chromatographed over silica gel using petroleum ether/ethyl acetate 10:1 as eluent. Pure **5** was thus obtained as a white solid (1.78 g, 54 %), mp 168-170°C ; IR (neat): 3030, 3058, 2951, 1655, 1489, 1447 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ in ppm: 0.77 (d, ^{3}J=7.0 Hz, 3H); 1.05 (d, ^{3}J=7.0 Hz, 3H); 2.15 (m, 1H); 2.38 (m, 1H); 2.45 (m, 1H); 2.62 (m, 1H); 2.98 (m, 1H); 3.47 (d, ^{3}J=7.8 Hz, 1H); 4.43 (sept, ^{3}J=7.0 Hz, 1H); 6.79 (d, ^{3}J=7.6 Hz, 1H); 7.08-7.20 (m, 12H); 7.39 (d ^{3}J=7.3 Hz, 6H). ^{13}C-NMR (125 MHz, $CDCl_3$) δ in ppm: 20.0 (CH_3); 22.8 (CH_3); 28.8 (CH_2); 38.3 (CH_2); 48.8 (CH); 53.8 (CH); 71.4; 125.1 (CH); 126.1 (CH); 126.9 (CH); 127.3 (CH); 127.6 (CH); 128.7 (CH); 128.9 (CH); 137.3; 139.5; 147.0; 173.5. HRMS-CI [$(M+H)^+$]: m/z 461.2603 (calculated for $C_{32}H_{33}N_2O$ 461.2593).

(*R*)-3-Amino-1-isopropyl-1,3,4,5-tetrahydro-benzo[*b*]azepin-2-one (6). Over a suspension of **5** (1.0 g, 2.2 mmol) in methanol (15 mL), *p*-toluenesulfonic acid (1.24 g, 6.6 mmol) was added. The resulting clear solution was stirred at 75 °C for 45 minutes and then allowed to cool down to room temperature. Methanol was removed under reduced pressure and the thick residue obtained was treated with petroleum ether (30 mL) and water (15 mL). Phases were separated and the aqueous phase extracted again with petroleum ether (2x). The aqueous layer was finally made basic by adding solid sodium carbonate and then extracted with ethyl acetate (3x). The organic fractions were combined, dried over magnesium sulphate and concentrated to yield the pure product as a colourless oil which solidified upon standing (0.39 g, 83 %). ^{1}H-NMR ($CDCl_3$) δ in ppm: 1.02 (d, ^{3}J=7.0 Hz, 3H); 1.38 (d, ^{3}J=7.0 Hz, 3H); 1.60 (s, 2H); 1.67 (m, 1H); 2.18 (m, 1H); 2.45 (dd, ^{2}J=13.4, ^{3}J=6.8 Hz, 1H); 2.68 (m, 1H); 3.14 (dd, ^{3}J=11.3, ^{3}J=7.7, 1H); 4.73 (sept, ^{3}J=7.0 Hz, 1H); 7.11-7.21 (m, 4H). ^{13}C-NMR (125 MHz, $CDCl_3$) δ in ppm: 20.2 (CH_3); 22.8 (CH_3); 29.0 (CH_2); 39.0 (CH_2); 49.8 (CH); 51.9 (CH); 124.9 (CH); 127.2 (CH); 127.2 (CH); 129.0 (CH); 137.2; 139.8; 175.0. HRMS-CI [$(M+H)^+$]: m/z 219.1502 (calculated for $C_{13}H_{19}N_2O$ 219.1497).

***Tert*-butyl-3-(3-fluorophenyl)-1-((*R*)1-isopropyl-2-oxo-2,3,4,5-tetrahydro-1*H*-benzo[*b*] azepin-3-ylamino)-1-oxopropan-2-ylcarbamate (7).** Over a solution of **6** (0.20 g, 0.9 mmol), *N*-Boc-DL-3-fluorophenyalanine (0.9 mmol) and BOP (0.45 g, 1 mmol) in dry dichloromethane (4 mL) was added diisopropylethylamine (0.33 mL, 1.8 mmol). The solution was stirred at room temperature for 4 hours and then the solvent was removed. The residue was redissolved in ethyl

acetate (30 mL) and the resulting solution was sequentially washed with 5 % potassium hydrogensulfate (3x), brine, 5 % sodium bicarbonate (3x) and finally brine. The organic phase was then dried over magnesium sulphate and, after removal of solvents, crude product (0.41 g) was obtained as a white foam which was purified by column chromatography over silica gel (petrol/ethyl acetate 1:1). A colourless oil that solidified upon standing was obtained (0.38 g, 1:1 mixture of diastereoisomers, 88 %). Purity was assessed by HPLC on an Agilent C18 Eclipse Plus column using water (0.1 % formic acid; solvent A) and methanol (0.1 % formic acid; solvent B) as eluents and going from 20 % to 90 % B over 12 min, then held at 90 % B for another two minutes, at a flow rate of 1 mL/min. The retention time was 12.1 min and the purity > 98 %. ^{1}H-NMR (500 MHz, $CDCl_3$) δ in ppm: 1.10 (d, 3H, CH_3); 1.39, 1,40 (s, 9H, $(CH_3)_3$); 1.44 (d, 3H, CH_3); 1.61, 1.76, 2.45, 2.55 (m, 2H, H4); 2.55, 2.77 (m, 2H, H5); 2.99 (m, 2H, CH_2 (Phe)); 4.20, 4.26 (m, 1H, H3); 4.30 (m, 1H, CHN (Phe)); 4.70 (m, 1H, $CHMe_2$); 5.11 (d, 1H, NHBoc); 6.8 (s, 1H, NHCO); 6.9 (m, 3H, aromatic, FPh); 7.2-7.3 (m, 5H, aromatic); ^{13}C-NMR (125 MHz, $CDCl_3$) δ in ppm: 20.2 (CH_3); 22.6 (CH_3); 28.3 (CH_3); 28.4, 28.5 (CH_2); 35.6, 35.8 (CH_2); 38.4, 38.8 (CH_2); 49.9, 50.1 (CH); 50.3, 50.4 (CH); 55.4, 55.7 (CH); 80.2; 113.9 (CH); 116.3 (CH); 125.0 (CH); 125.1 (CH); 127.6 (CH); 127.8, 127.8 (CH); 129.4, 129.4 (CH); 130.1 (CH); 136.2; 139.0, 139.1; 139.3, 139.4; 155.2; 162.9 (CF); 169.6, 169.7; 169.9, 170.1. HRMS-FAB [$(M+Na)^+$]: m/z 506.2434 (calculated for $C_{27}H_{34}N_3O_4FNa$ 506.2431).

2-amino-3-(2-fluorophenyl)-*N*-((*R*)-1-isopropyl-2-oxo-2,3,4,5-tetrahydro-1*H*-benzo[*b*] azepin-3-yl)propanamide (8). A mixture of trifluoroacetic acid (1.5 mL) and **7** (0.32 g, 0.66 mmol) in dichloromethane (1.5 mL) was stirred at ambient temperature for 5 hours. Dichloromethane (20 mL) was then added, and the resulting solution was poured into a mixture of ice/solid sodium bicarbonate. The organic phase was washed with 2 M sodium hydroxide (5 mL) and collected, dried over magnesium sulfate and concentrated under vacuum to afford pure product as a yellowish oil (0.22 g, 1:1 mixture of diastereomers, 87%). Purity was assessed by HPLC on an Agilent C18 Eclipse Plus column using water (0.1 % formic acid; solvent A) and methanol (0.1 % formic acid; solvent B) as eluents going from 40 % to 70 % B over 20 mins, at a flow rate of 1 mL/min. The retention time for the two diastereomers was 15.2 and 15.9 mins and the purity > 95%. IR (DCM): 2932, 1650, 1489 cm^{-1}, ^{1}H-NMR (500 MHz, $CDCl_3$) δ in ppm: 1.10 (d, 3H, CH_3); 1.46 (d, 3H, CH_3); 1.8 (bs, 2H, NH_2); 1.75, 2.48 (m, 2H, H4); 2.48, 2.75 (m, 2H, H5); 2.61, 2.72, 3.02 (m, 2H, CH_2 (Phe)); 3.51 (bs, 1H, CHN (Phe)); 4.26 (m, 1H, H3); 4.67 (m, 1H, $CHMe_2$); 6.7-6.9 (m, 3H, Ar); 7.1-7.3 (m, 5H, Ar); 7.93/8.02 (d, 1H, NH). ^{13}C-NMR (125 MHz, $CDCl_3$) δ in ppm: 20.0, 22.6 (CH_3); 28.5 (CH_2); 35.8, 36.0 (CH_2); 40.8, 40.9 (CH_2); 49.6, 49.7 (CH); 50.3, 50.3 (CH); 56.1, 56.6 (CH); 113.6 (CH); 116.2 (CH); 125.0 (CH); 125.1; 125.2; 127.5; 127.6; 127.7; 129.3; 130.0; 136.3; 139.2; 140.3; 140.8; 162.9; 170.5; 172.9. HRMS-ES [$(M+H)^+$]: m/z 384.2086 (calculated for $C_{22}H_{27}N_3O_2F$ 384.2087).

***N*-(3-(3-fluorophenyl)-1-((*R*)-1-isopropyl-2-oxo-2,3,4,5-tetrahydro-1*H*-benzo[*b*]azepin-3-ylamino)-1-oxopropan-2-yl)-2-iodobenzamide (9).** To a solution of **8** (0.22 g 0.57 mmol), 2-iodobenzoic acid (0.14 g, 0.57 mmol) and BOP (0.26 g, 0.63 mmol) in dichloromethane (4 mL) was added diisopropylethylamine (0.2 mL, 1.2 mmol). The solution was stirred for 4 hours at room temperature after which time dichloromethane was removed and the resulting yellowish residue redissolved in ethyl acetate (30 mL). This solution was sequentially washed with 5 % potassium hydrogensulfate (3x), brine, 5 % sodium bicarbonate (3x) and finally brine. The organic phase was then dried over magnesium sulphate and, after removal of solvents, a solid mixture containing the product was obtained. This mixture was chromatographed over silica gel (petroleum ether/ethyl acetate 9:1 to 10:3) to afford the desired product as a white solid (0.29 g,

1:1 mixture of diastereomers, 83 %), mp 131-135 ºC. Further purification by column chromatography over silica gel (petroleum ether/ethyl acetate 10:3, very slow elution) yielded enriched samples of both diastereomers (~5:1). Diastereomerically pure *h*-**9** and *l*-**9** were obtained as white solids by semipreparative HPLC chromatography using a C18 Chromolith semipreparative column (100 x 10 mm) and gradient elution from 60 to 90 % solvent B over 200 minutes at a flow rate of 3 mL/min, (retention time: *h*-**9**: 14 minutes, *l*-**9**: 16 minutes). The purity for both diastereomers was > 99%.

^{1}H-NMR ($CDCl_3$) *h*-**9**, δ in ppm,: 1.11 (d, ^{3}J=7.0 Hz, 3H, CH_3); 1.47 (d, ^{3}J=7.0 Hz, 3H, CH_3); 1.82 (m, 1H, CH, H4); 2.55 (m, 1H, CH, H5); 2.59 (m, 1H, CH, H4); 2.79 (m, 1H, CH, H5); 3.2 (m, 2H, CH_2 (Phe)); 4.23 (m, 1H, H3); 4.73 (m, ^{3}J=7.0 Hz, 1H, $CHMe_2$); 4.84 (m, 1H, CHNCO (Phe)); 6.39 (d, ^{3}J=7.8 Hz, 1H, NH); 6.83 (d, ^{3}J=7.3 Hz, 1H, NH); 6.92 (m, 2H, Ar); 7.04 (d, ^{3}J=7.6 Hz, 1H, Ar); 7.15 (m, 1H, Ar); 7.20-7.35 (m, 7H, Ar); 7.81 (dd, ^{3}J=8.0 Hz, ^{4}J=0.7 Hz, 1H, Ar). ^{13}C-NMR (125 MHz, $CDCl_3$) δ in ppm: 20.2 (CH_3); 22.6 (CH_3); 28.5 (CH_2); 35.9 (CH_3); 38.3 (CH_3); 50.3 (CH); 50.4 (CH); 54.6 (CH); 92.4; 114.1 (CH); 116.5 (CH); 125.1 (CH); 125.1 (CH); 127.7 (CH); 127.8 (CH); 128.3 (CH); 128.4 (CH); 129.4 (CH); 130.2 (CH); 131.4 (CH); 136.1; 138.7; 139.0; 140.0 (CH); 141.4; 162.9 (CF); 168.8; 168.8; 169.7.

^{1}H-NMR ($CDCl_3$) *l*-**9**, δ in ppm: 1.08 (d, ^{3}J=7.0 Hz, 3H, CH_3); 1.44 (d, ^{3}J=7.0 Hz, 3H, CH_3); 1.61 (m, 1H, CH, H4); 2.47 (m, 1H, CH, H4); 2.55 (m, 1H, CH, H5); 2.77 (m, 1H, CH, H5); 3.2 (m, 2H, CH_2 (Phe)); 4.28 (m, 1H, H3); 4.73 (m, ^{3}J=7.0 Hz, 1H, $CHMe_2$); 4.84 (m, 1H, CHNCO (Phe)); 6.45 (d, ^{3}J=7.7 Hz, 1H, NH); 6.74 (d, ^{3}J=6.8 Hz, 1H, NH); 6.95 (m, 2H, Ar); 7.08 (m, 2H, Ar); 7.17 (d, ^{3}J=7.7 Hz, 1H, Ar); 7.22-7.33 (m, 6H, Ar); 7.83 (d, ^{3}J=7.9 Hz, 1H, Ar). ^{13}C-NMR (125 MHz, $CDCl_3$) δ in ppm: 20.2 (CH_3); 22.7 (CH_3); 28.4 (CH_2); 35.6 (CH_3); 38.7 (CH_3); 50.1 (CH); 50.2 (CH); 54.9 (CH); 92.4; 114.1 (CH); 116.6 (CH); 125.1 (CH); 125.2 (CH); 127.6 (CH); 127.8 (CH); 128.2 (CH); 128.3 (CH); 129.4 (CH); 130.3 (CH); 131.4 (CH); 136.1; 138.8; 140.0 (CH); 141.4; 162.9 (CF); 168.7; 168.7; 169.9.

HRMS-FAB, **9** [$(M+Na)^+$]: m/z 636.1125 (calculated for $C_{29}H_{29}N_3O_3FINa$ 636.1135).

2-Iodo-benzoic acid 2,5-dioxo-pyrrolidin-1-yl ester (11). To a solution of 2-iodobenzoic acid (**10**, 0.30 g, 1.2 mmol) and TSTU (0.41 g, 1.3 mmol) in DMF (4 mL) was added triethylamine (0.8 mL). The resulting mixture was stirred at ambient temperature for three hours. After this time, water (10 mL) was added and the flask placed in an ice/water bath. The white precipitate thus formed was filtered and washed with water to yield the desired pure product (0.17 g, 41 %), mp 160-163°C; ^{1}H-NMR (300 MHz, $CDCl_3$) δ in ppm: 2.91 (s, 4H); 7.28 (ddd, ^{3}J=^{3}J=7.8 Hz, ^{4}J=1.7 Hz, 1H); 7.49 (ddd, ^{3}J=^{3}J=7.8 Hz, ^{4}J=1.1 Hz, 1H); 8.11 (m, 2H). ^{13}C-NMR (125 MHz, $CDCl_3$) δ in ppm: 25.8 (CH_2); 95.8: 128.3 (CH); 129.4; 132.3 (CH); 134.7 (CH); 142.2 (CH); 161.2; 169.1. HRMS-EI [M^+]: m/z 344.9496 (calculated for $C_{11}H_8NO_4I$ 344.9493).

2-Trimethylstannanyl-benzoic acid 2,5-dioxo-pyrrolidin-1-yl ester (12). Over a solution of 2-iodo-benzoic acid 2,5-dioxo-pyrrolidin-1-yl ester (**11**, 0.10 g, 0.28 mmol) in degassed toluene (4 mL) were added sequentially hexamethylditin (0.08 mL, 0.35 mmol) and tetrakis(triphenylphosphine)palladium (0) (30 mg, 0.03 mmol) dissolved in degassed toluene (2 mL). The solution was heated at 115 ºC for five hours under argon after which time it was allowed to cool to room temperature and decanted. Toluene was removed under reduced pressure and the resulting light brown residue was chromatographed on silica gel using petroleum ether/ethyl acetate 4:1 as eluent to get the pure product as an off-white solid (75 mg, 70 %), mp 126-128°C; IR (DCM): 3052, 2994, 1757, 1730, 1233, 1205 cm^{-1}; ^{1}H-NMR (300

MHz, $CDCl_3$) δ in ppm: 0.27 (s, 9H); 2.90 (s, 4H); 7.46 (dd, ^3J=^3J=7.5 Hz, 1H); 7.60 (dd, ^3J=^3J=7.5 Hz, 1H); 7.72 (d, ^3J=7.5 Hz, 1H); 8.28 (d, ^3J=7.5 Hz, 1H). ^{13}C-NMR (125 MHz, $CDCl_3$) δ in ppm: -7.8 (CH_3); 25.7 (CH_2); 128.5 (CH); 130.5; 130.9 (CH); 133.5 (CH); 137.0 (CH); 149.1; 164.0; 169.1. HRMS-CI [$(M+H)^+$]: m/z 384.0269 (calculated for $C_{14}H18NO_4Sn$ 384.0258).

***N*-(3-(3-fluorophenyl)-1-((*R*)-1-isopropyl-2-oxo-2,3,4,5-tetrahydro-1*H*-benzo[*b*]azepin-3-ylamino)-1-oxopropan-2-yl)-2-trimethylstannanylbenzamide (13).** Triethylamine (0.04 mL) was added to a solution of 2-trimethylstannanyl-benzoic acid 2,5-dioxo-pyrrolidin-1-yl ester (**12**, 45 mg, 0.12 mmol) and **8** (46 mg, 0.12 mmol) in DMF (0.9 mL) and the mixture was stirred for four days at room temperature. The flask was then placed in an ice/water bath and water was (3mL) added. The resulting suspension was stirred for five minutes and then filtered. The solid was washed with water and dried in vacuo, pure product was obtained as a white solid (35 mg, 1:1 mixture of diastereomers, 46 %), mp 103-105°C; IR (neat): 3285, 2960, 2931, 1635, 1488, 1250 cm^{-1}; ^{1}H-NMR (500 MHz, $CDCl_3$) δ in ppm: 0.22 (s, 9H); 1.09 (d, ^3J=7.0 Hz, 3H); 1.45 (d, ^3J=7.0 Hz, 3H); 1.61, 1.83 (m, 1H); 2.6 (m, 2H); 2.79 (m, 1H); 3.14 (m, 2H); 4.24 (m, 1H); 4.72 (m, 1H); 4.83 (m, 1H); 6.75 (d, 1H, NH); 6.9 (d, 1H, NH); 6. 9 (m, 3H); 7.17-7.32 (m, 6H); 7.43 (m, 1H); 7.51 (m, 1H); 7.67 (d, ^3J=7.1 Hz, 1H). ^{13}C-NMR (125 MHz, $CDCl_3$) δ in ppm: -6.9 (CH_3); 20.2 (CH_3); 22.7 (CH_3); 28.4 (CH_2); 35.5, 35.9 (CH_2); 38.6, 38.8 (CH_3); 50.0, 50.2 (CH); 50.3, 50.4 (CH); 54.6, 54.9 (CH); 114.1, 114.1 (CH); 116.4, 116.5 (CH); 125.1, 125.2 (CH); 125.2 (CH); 125.5 (CH); 127.6, 127.7 (CH); 127.8, 127.9 (CH); 128.2, 128.3 (CH); 129.3, 129.4 (CH); 130.1, 130.2 (CH); 130.9 (CH); 136.1, 136.2; 137.0, 137.0 (CH); 138.2, 138.3; 138.8; 139.0, 139.0; 146.0; 162.9, 162.9 (CF); 168.6, 168.6; 169.2, 169.4; 169.8, 169.9. MS (FAB) Mass HRMS-FAB [$(M+Na)^+$]: m/z 674.1817 (calculated for $C_{32}H_{38}N_3O_3FSnNa$ 674.1828).

Labelling of [^{125}I]9. Precursor **13** (50 μg) was placed in a glass vial and dissolved in methanol (100 μL). Hydrochloric acid (60 μL of a 0.4 M solution), hydrogen peroxide (50 μL of a 6 % v/v solution) and finally [^{125}I]NaI (5-10 μL) were then sequentially added. The vial was sealed, shaken and placed in an oil bath at 60 ºC for 30 minutes. The mixture was then allowed to cool down, diluted with MeOH/water 1:1 (500 μL) and injected into the HPLC system using a glass syringe. The two diastereomers were isolated using a C18 Agilent Eclipse Plus (4.6 x 150 mm, 5 μm) with the following eluent: water (0.1 % TFA) as solvent A and methanol (0.1 % TFA) as solvent B, going from 60 % B to 90 % B over 32 min. Retention times of compounds [^{125}I]*h*-**9** and [^{125}I]*l*-**9** were 21 and 22 min, respectively. Integration of the UV absorption peak of each diastereoisomer allowed the determination of the specific activity. The fractions containing the radioligands were diluted with water (10 mL) and passed through a Sep-Pak C18 light cartridge (Waters) using a 20 mL glass syringe. The loaded cartridge was washed with water (10 mL) and the radioligand was finally released with ethanol (0.5 mL, > 95 % recovery).

Appendix 2. Selected ^{1}H and ^{13}C NMR spectra

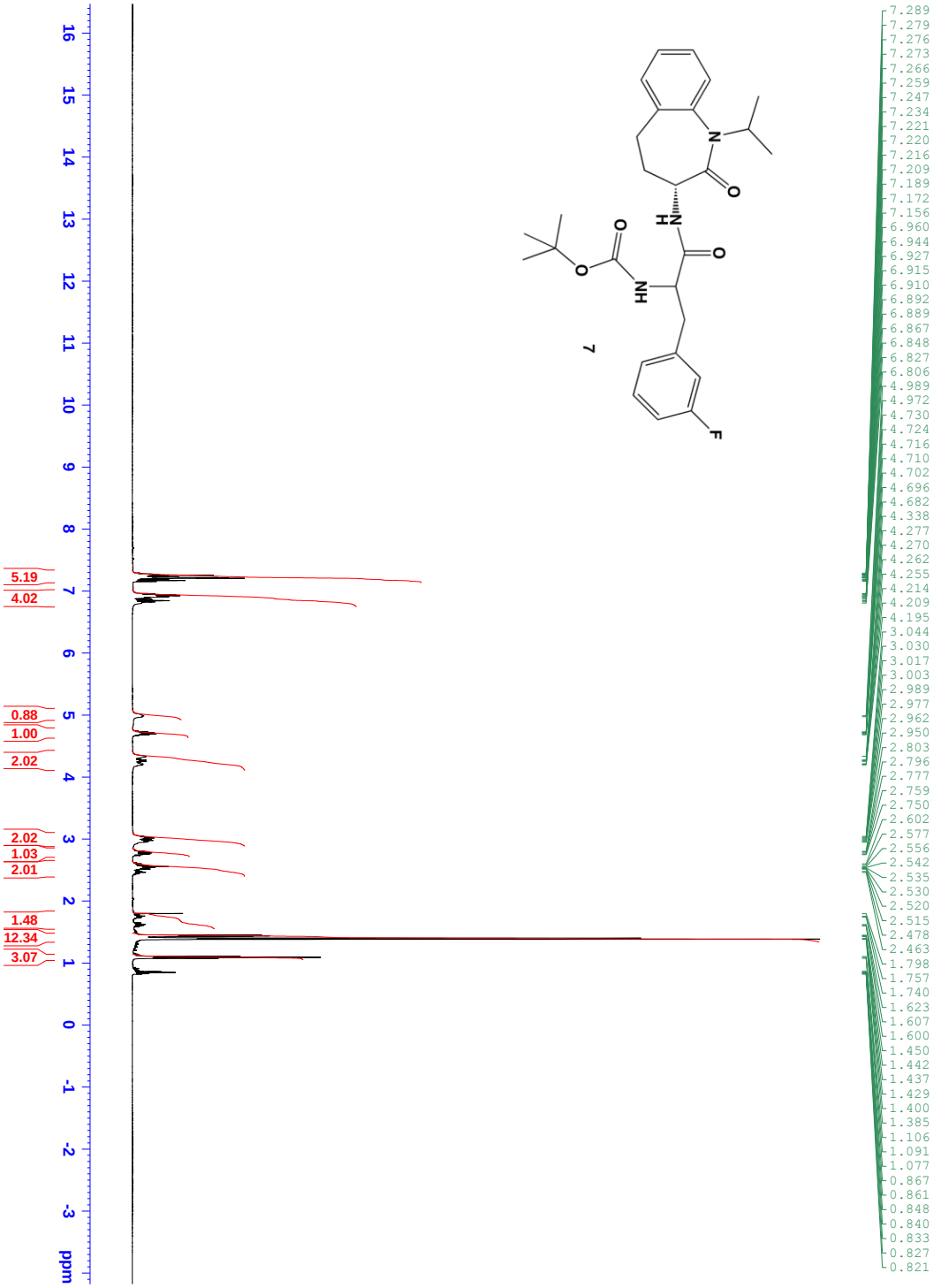

Figure 1. ^{1}H-NMR spectrum of compound **7** (mixture of diastereomers).

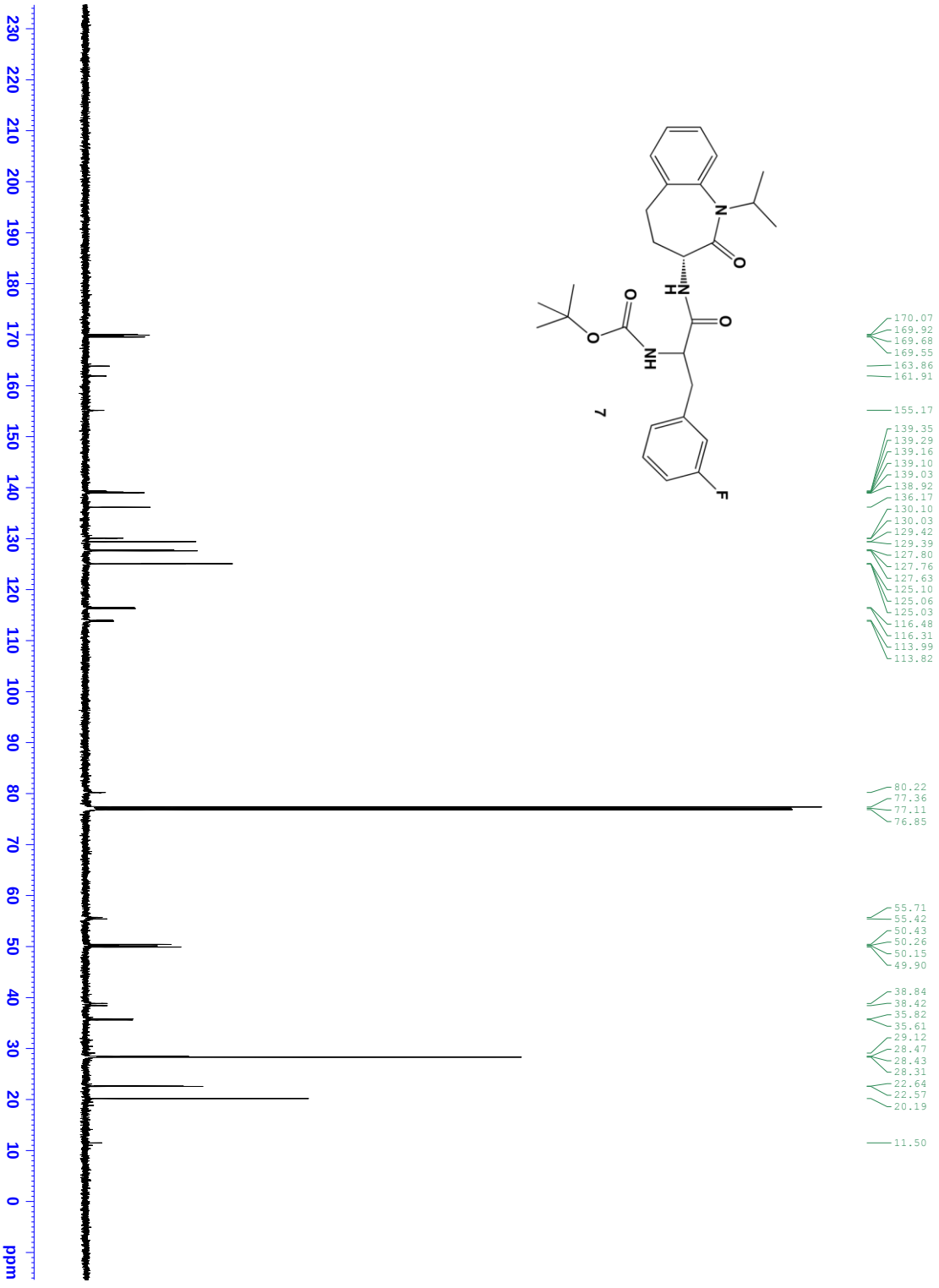

Figure 2. ^{13}C-NMR spectrum of compound **7** (mixture of diastereomers).

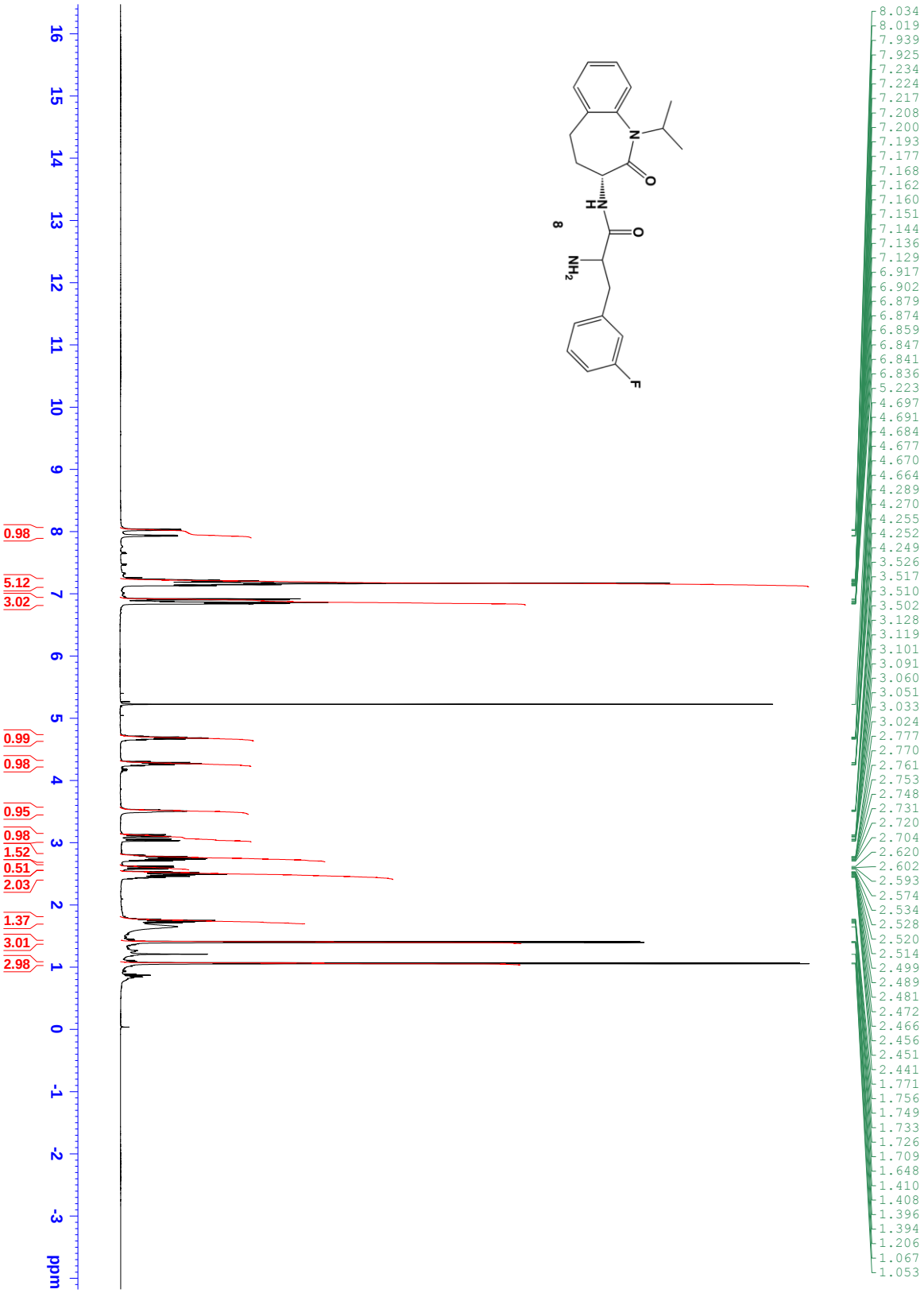

Figure 3. ^{1}H-NMR spectrum of compound **8** (mixture of diastereoisomers).

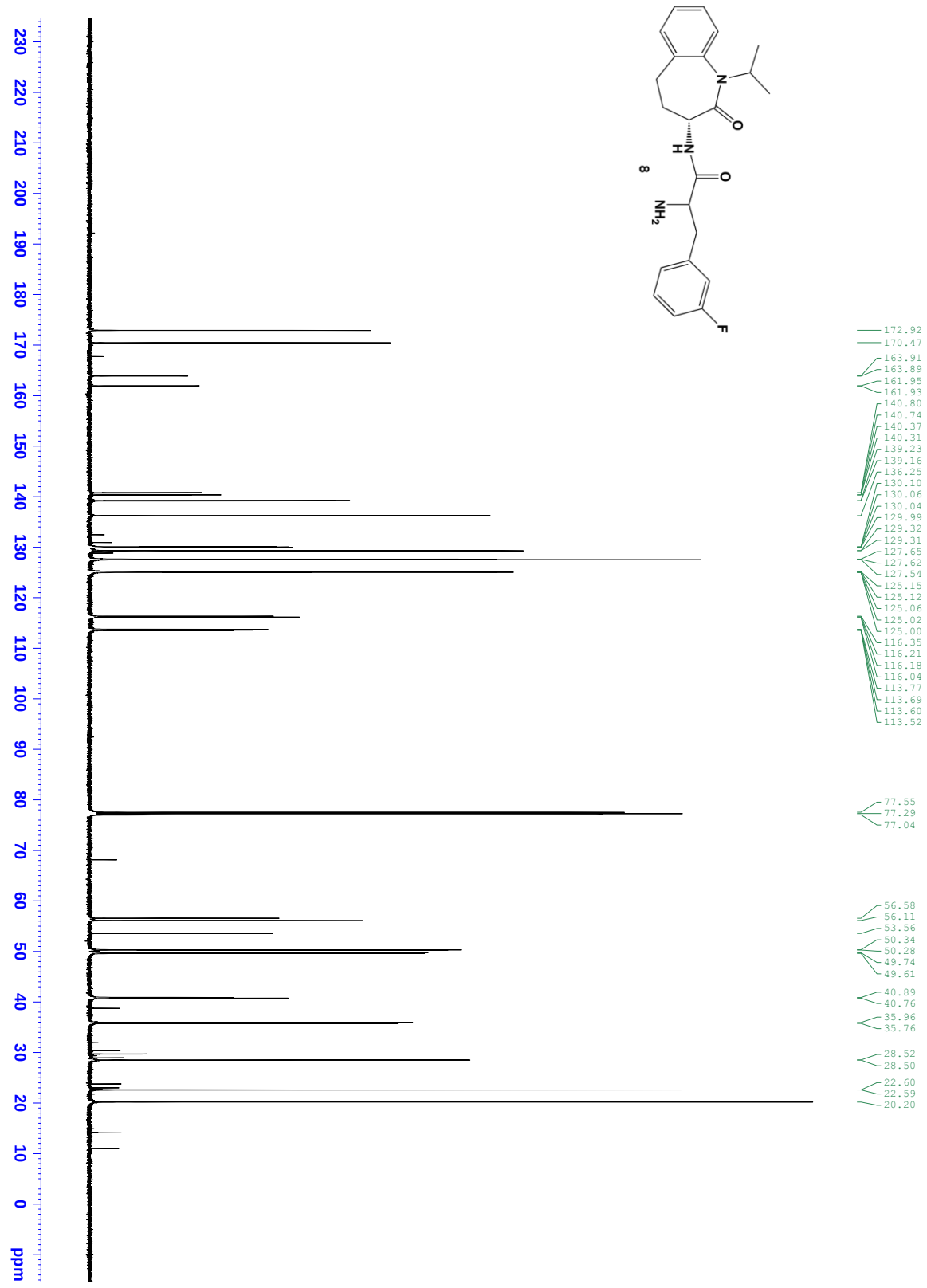

Figure 4. ^{13}C-NMR spectrum of compound **8** (mixture of diastereoisomers).

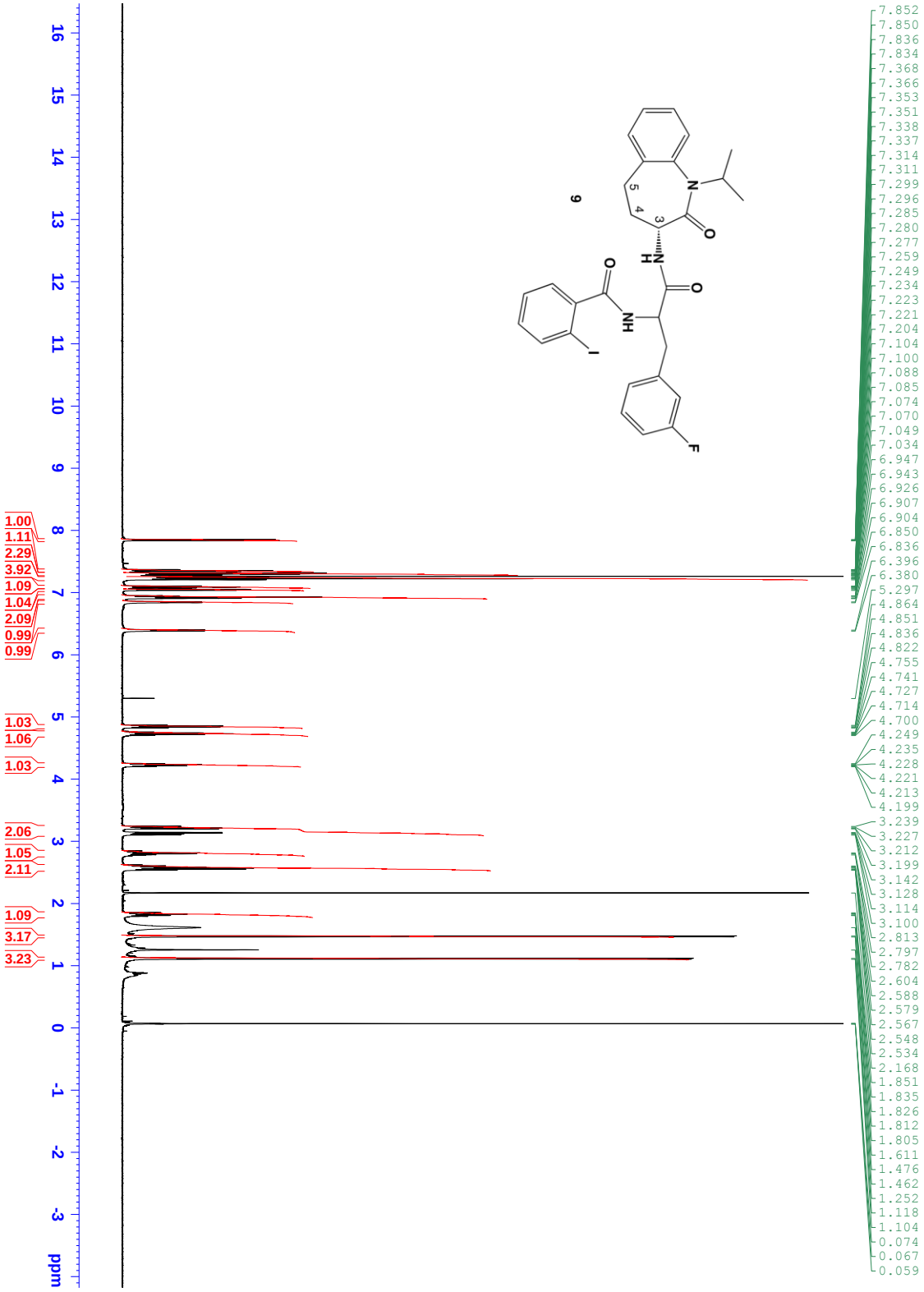

Figure 5. ^{1}H-NMR spectrum of compound *h*-**9**.

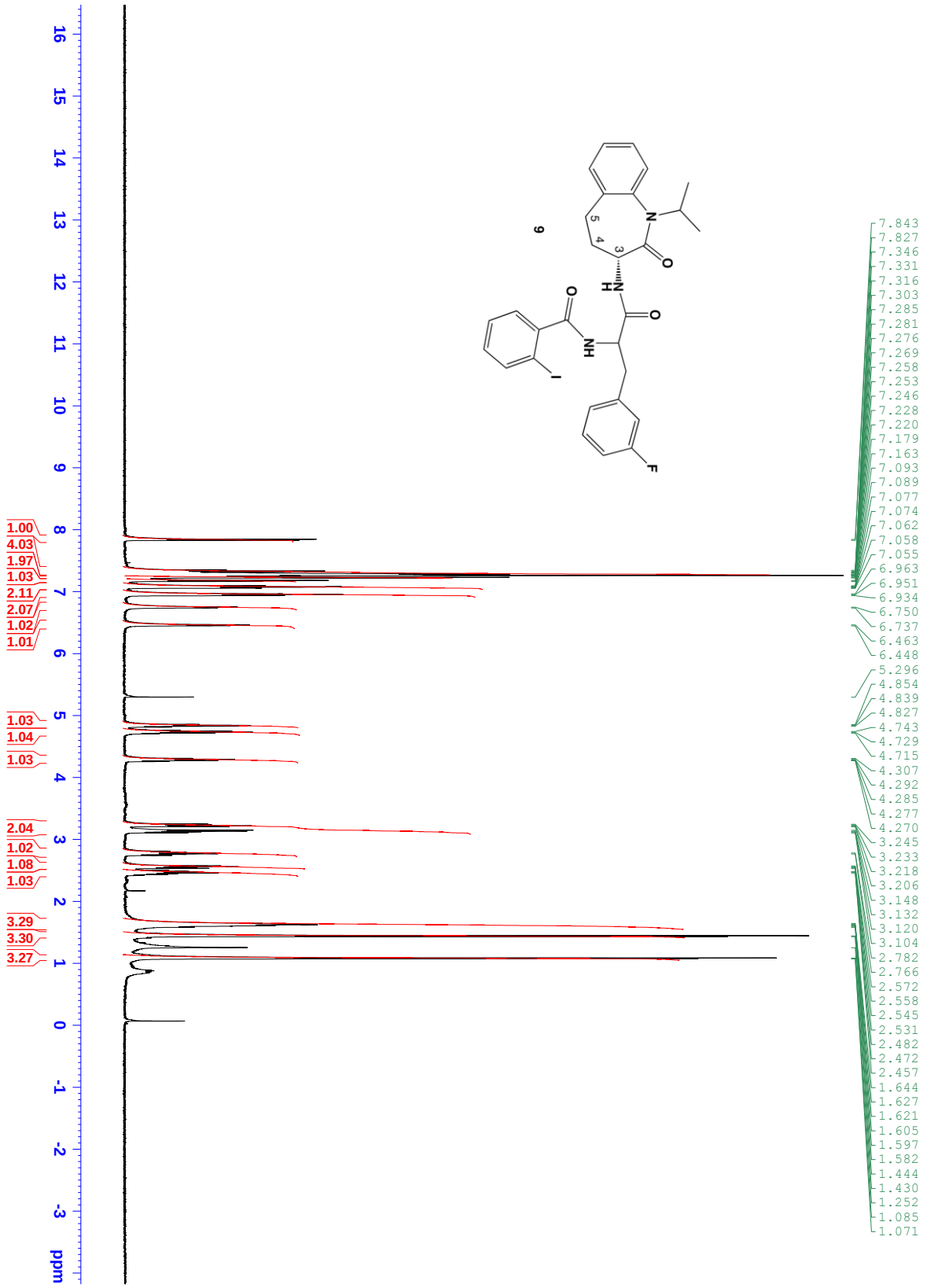

Figure 6. ^{1}H-NMR spectrum of compound *l*-**9**.

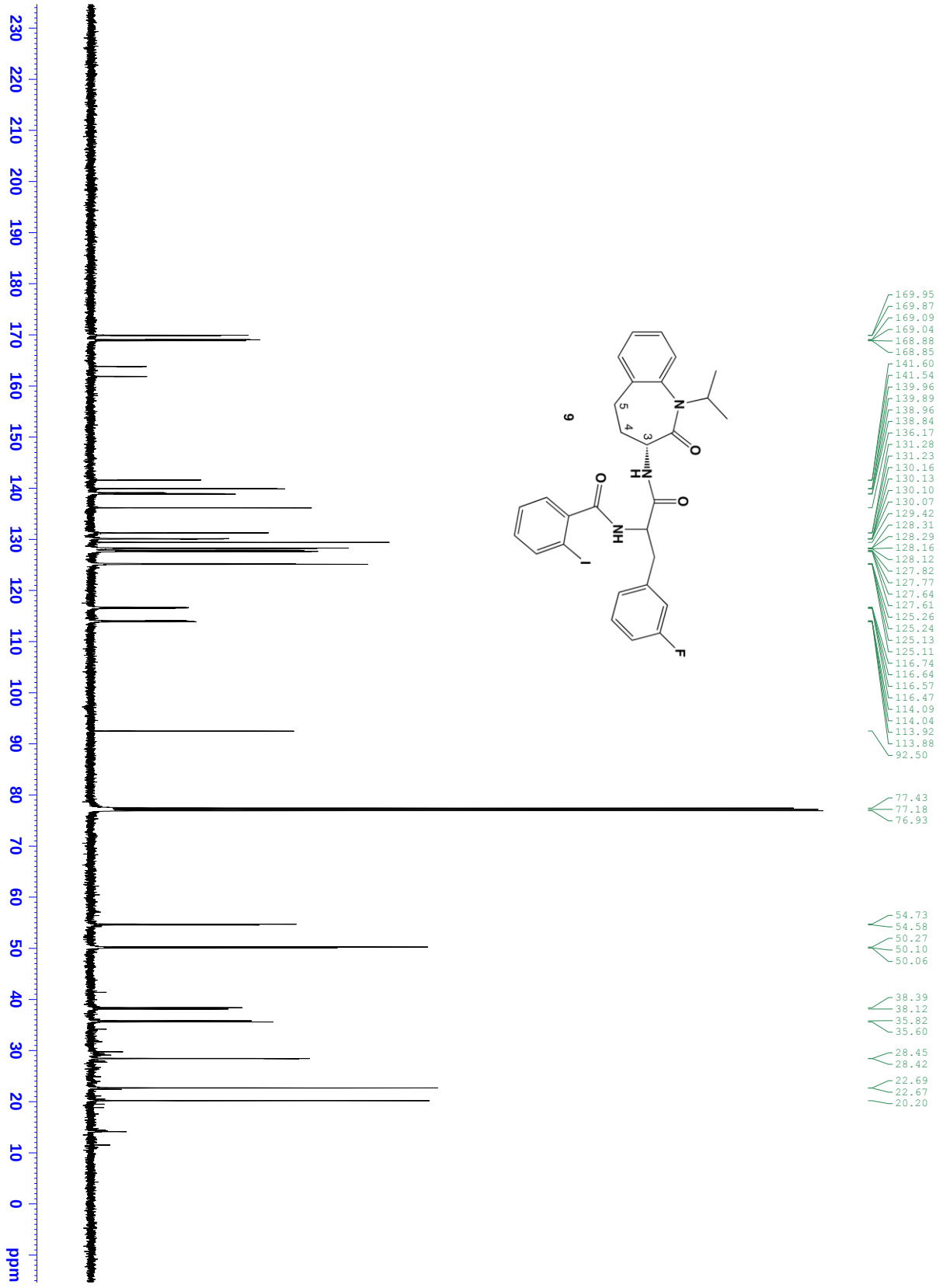

Figure 7. ^{13}C-NMR spectrum of compound **9** (mixture of diastereomers).

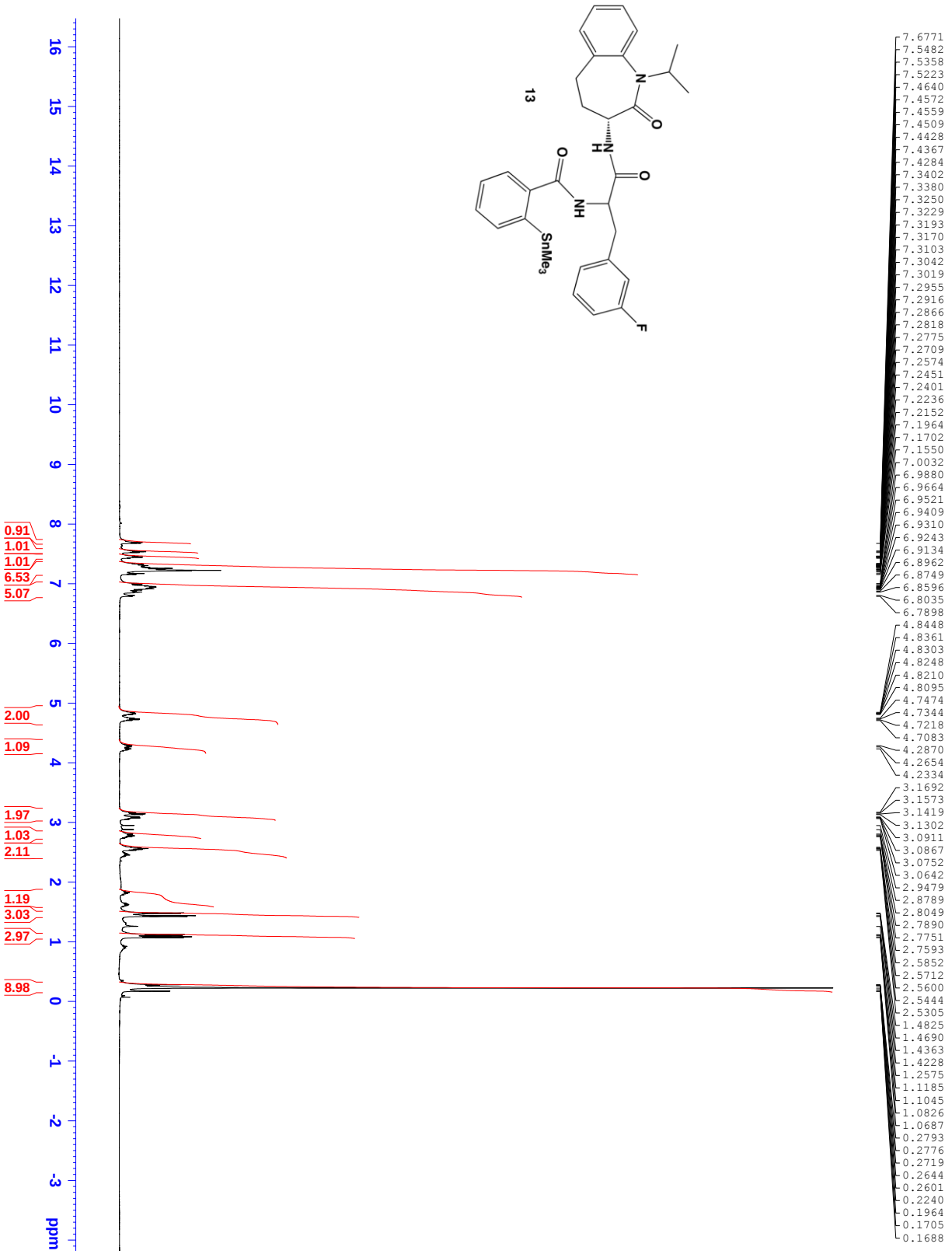

Figure 8. ^{1}H-NMR spectrum of compound **13** (mixture of diastereoisomers).

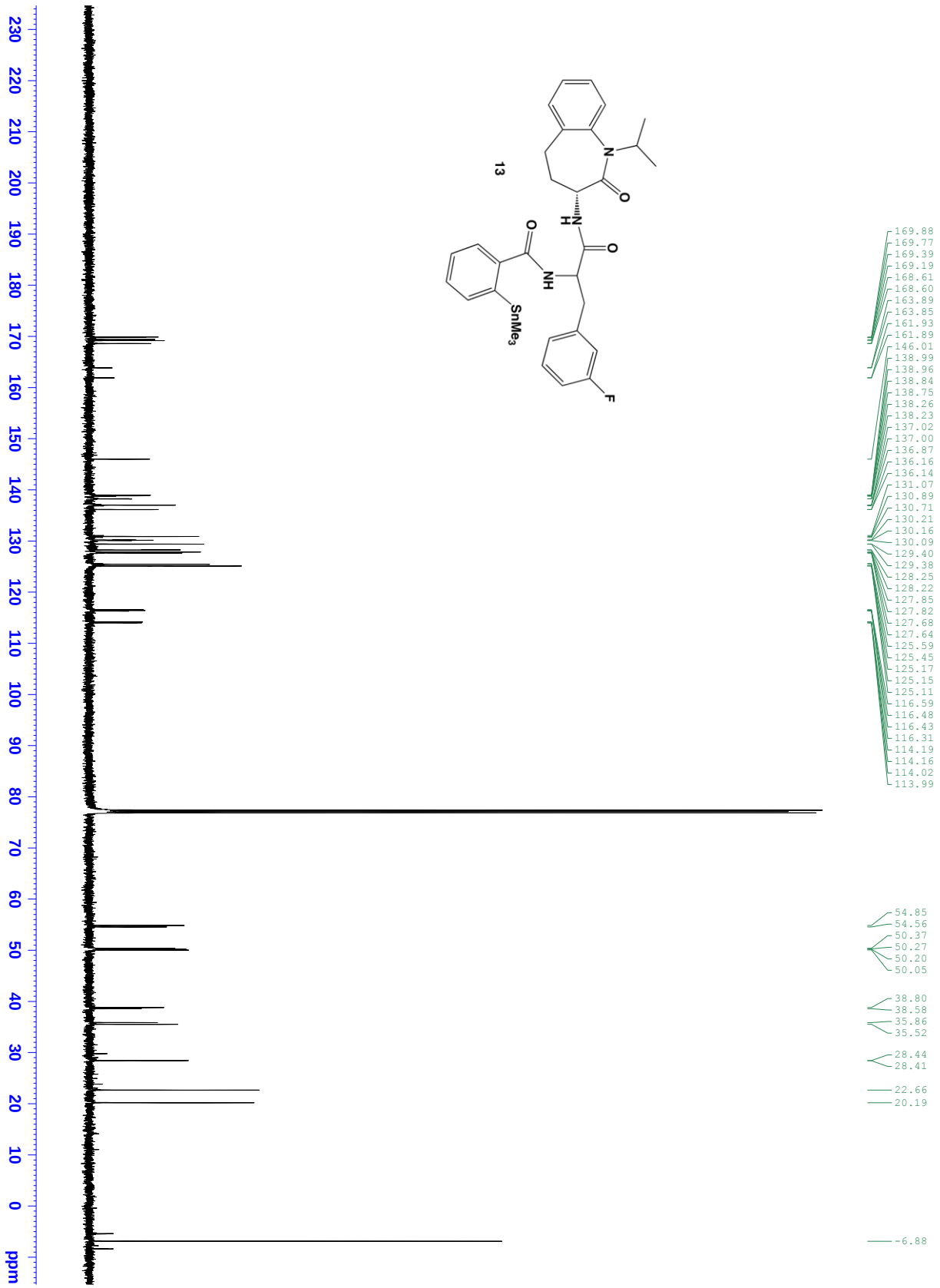

Figure 9. ^{13}C-NMR spectrum of compound **13** (mixture of diastereoisomers).

Appendix 3. Selected radio-HPLC chromatograms

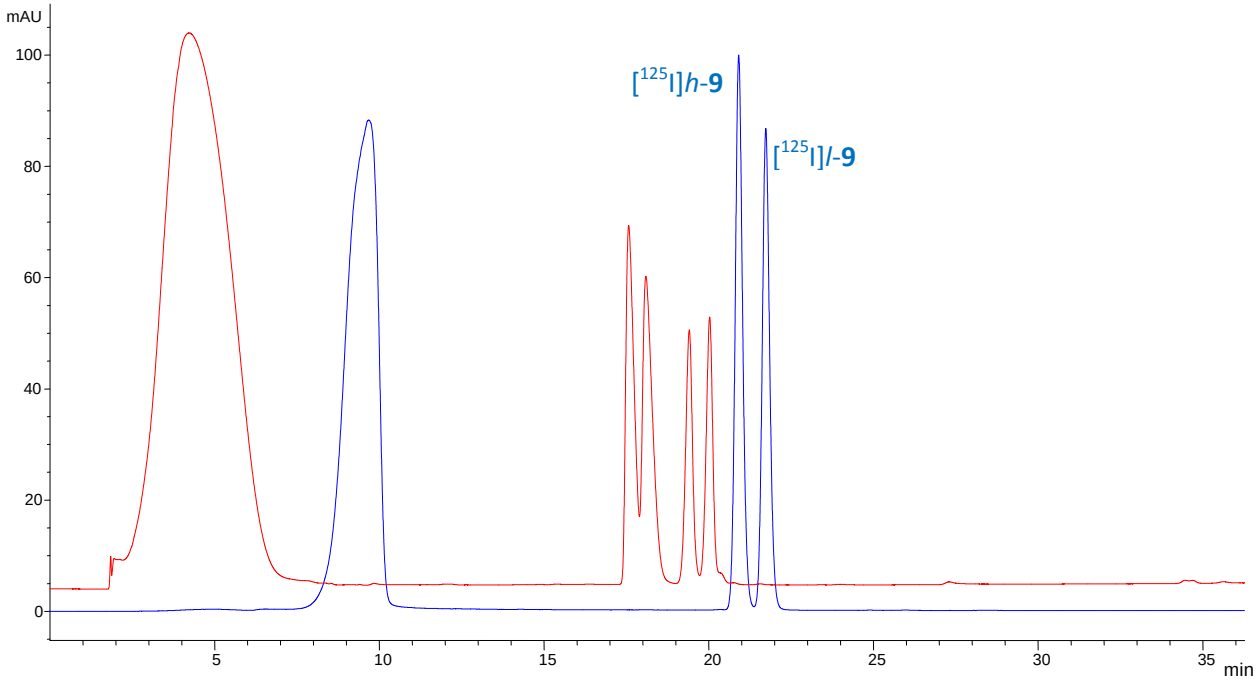

Figure 10. HPLC chromatogram of the crude reaction mixture resulting from the radiolabelling of [^{125}I]**9**, showing radioactivity (blue trace) and UV absorption at 254 nm (red trace).

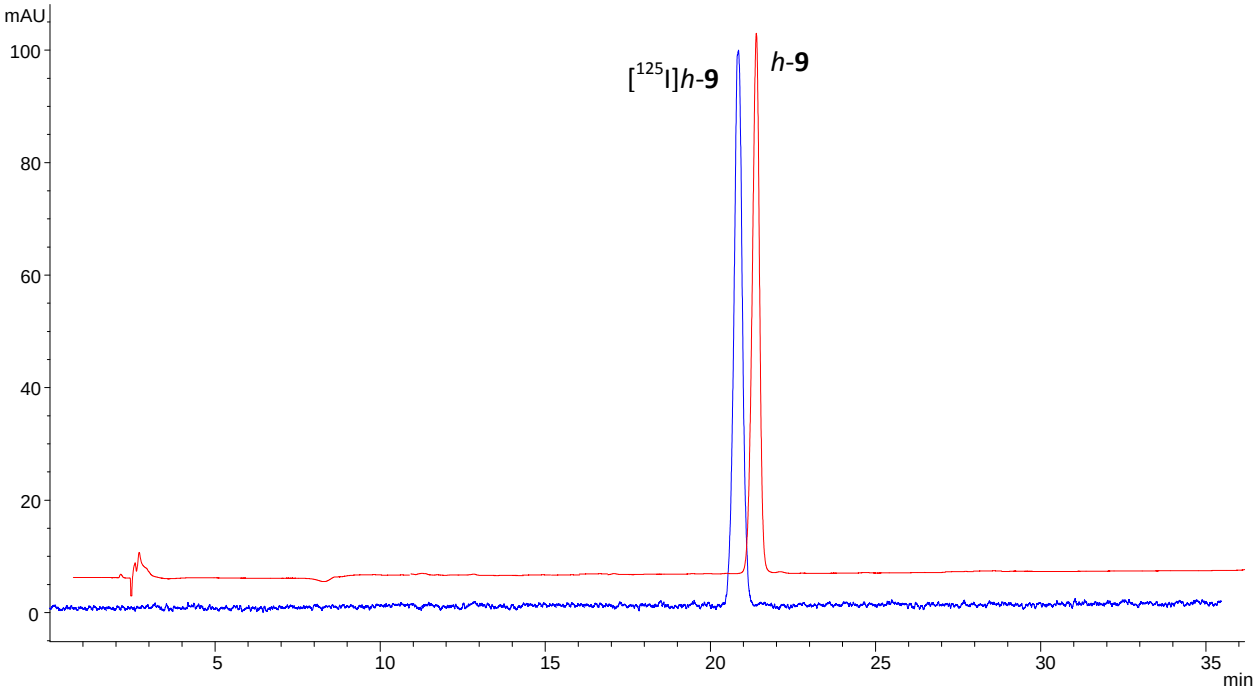

Figure 11. HPLC chromatogram of a mixture of *h*-**9** and [^{125}I]*h*-**9**, showing radioactivity (blue trace) and UV absorption at 254 nm (red trace)(2 % offset between signals).

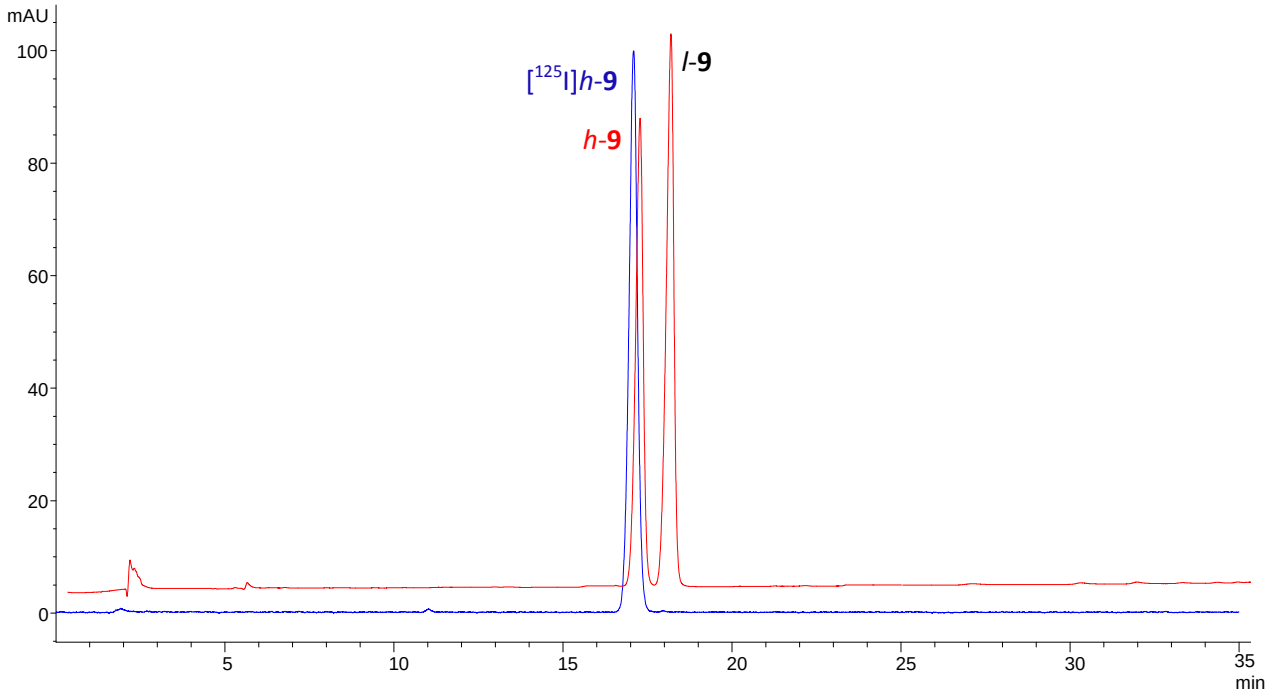

Figure 12. HPLC chromatogram of a mixture of *h*-**9** and *l*-**9** and [^{125}I]*h*-**9**, showing radioactivity (blue trace) and UV absorption at 254 nm (red trace)(1 % offset between signals).

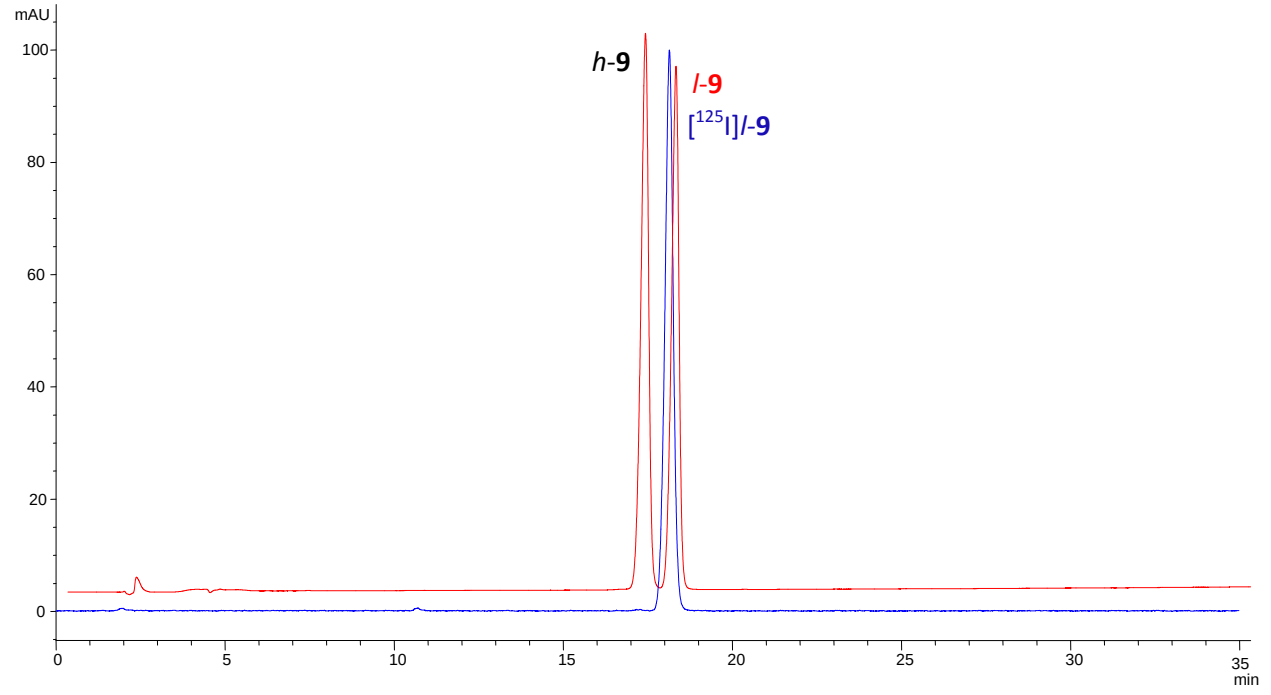

Figure 13. HPLC chromatogram of a mixture of *h*-**9** and *l*-**9** and [^{125}I]*l*-**9**, showing radioactivity (blue trace) and UV absorption at 254 nm (red trace)(1 % offset between signals).

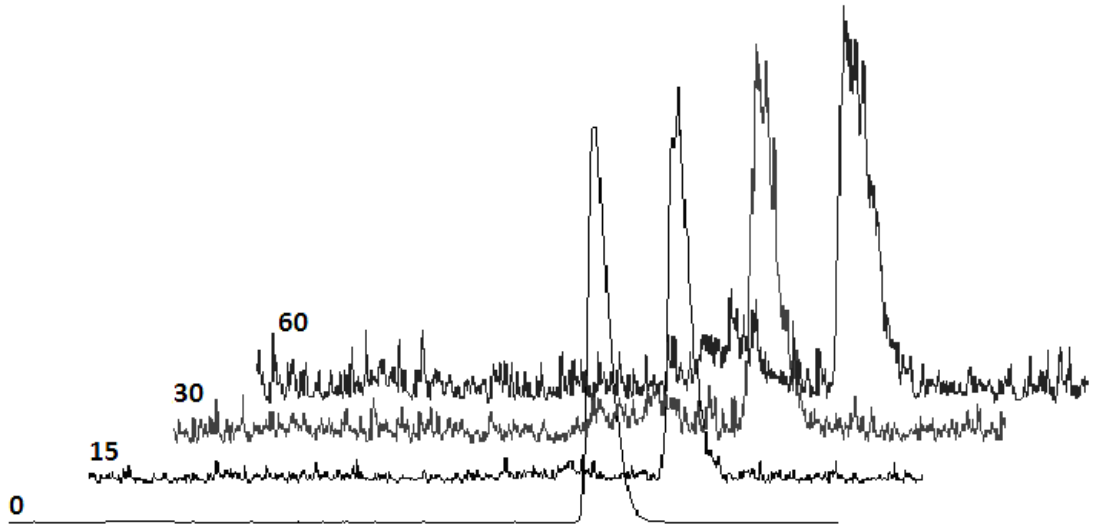

Figure 14. HPLC radioactivity profiles of brain samples at 0, 15, 30 and 60 min after injection of [^{125}I]*h*-**9**.

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