Supplemental Material for:

Design, synthesis and initial characterisation of a radiolabelled [^{18}F]pyrimidoindolone probe for detecting activated caspase-3/7

A. Udemba, G. Smith, Q-D. Nguyen, M. Kaliszczak, L. Carroll, R. Fortt, M. J. Fuchter and E. O. Aboagye*

email: l.carroll@imperial.ac.uk

Table of Contents

1. **General experimental procedures, materials and instrumentation**

All reactions were performed under anhydrous conditions and an atmosphere of nitrogen in flame-dried glassware unless otherwise stated. Yields refer to chromatographically and spectroscopically (^{1}H-NMR) homogenous materials.

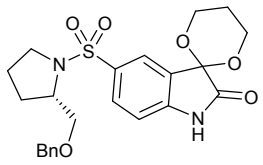
Solvents and reagents: All solvents were purified and dried according to standard methods prior to use. All chemicals were handled in accordance with COSHH regulations. All reagents were used as commercially supplied.

Flash chromatography (FC) was always performed on silica gel (Merck Kieselgel 60 F_{254} 320-400 mesh, Sigma-Aldrich, Gillingham, UK) according to the method of W. C. Still, unless otherwise stated.[1] Thin Layer Chromatography (TLC) was performed on Merck aluminium-backed plates pre-coated with silica (0.2 mm, 60 F_{254}) which were visualised either by quenching of ultraviolet fluorescence (λ = 254 and 366 nm) or by charring with 10% $KMnO_4$ in 1M H_2SO_4. ^{1}H NMR spectra: These were recorded at 400 MHz on a Bruker AV-400 or on a Bruker AV-500 instrument (Coventry, UK). Chemical shifts (δ_H) are quoted in parts per million (ppm), referenced to the appropriate residual solvent peak. Coupling constants (*J*) are reported to the nearest 0.5 Hz. ^{13}C NMR spectra: These were recorded at 100 MHz on a Bruker AV-400 instrument. Chemical shifts (δ_C) are quoted in ppm, referenced to the appropriate residual solvent peak. Mass spectra: Low resolution mass spectra (*m/z*) were recorded on either a VG platform II or VG AutoSpec spectrometers (Waters, Elstree, UK), with only molecular ions (M^+, MH^+, MNa^+, MK^+, MNH_4^+) and major peaks being reported with intensities quoted as percentages of the base peak.

[^{18}F]Fluoride was produced by a cyclotron (GE PETrace, GE Healthcare, Amersham, UK) using the $^{18}O(p,n)^{18}F$ nuclear reaction with 16.4 MeV proton irradiation of an enriched [^{18}O]H_2O target.

2. **Synthesis of non-labelled compounds**

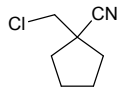
(*S*)-5'-(2-(Benzyloxymethyl)pyrrolidin-1-ylsulfonyl)spiro[[1,3]dioxane-2,3'-indolin]-2'-one **1**



1

To a solution of isatin sulfonamide (2.42 g, 6.00 mmol) and 1,3-propanediol (4.3 mL, 60.00 mmol) in toluene (100 mL), was added toluenesulfonic acid (0.29 g, 1.50 mmol) and the mixture was refluxed for 12 hours, collecting water with a Dean Stark trap. The mixture was subsequently allowed to cool to room temperature, washed with saturated aqueous Na_2CO_3 solution (70 mL), water (100 mL) and brine (100 mL), before drying over $MgSO_4$. After concentration *in vacuo*, the crude product was purified by flash column chromatography, eluting with 1:4 – 1:1 ethyl acetate/petroleum ether to give the ketal protected sulfonamide **1** as a colourless foam (2.39 g, 87 % yield): ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.64 (m, 3H), 1.92 (m, 2H), 2.39 (m, 1H), 3.08 (m, 1H), 3.48 (m, 2H), 3.82 (m, 2H), 4.00 (m, 2H), 4.57 (m, 2H), 4.94 (m, 2H), 6.88 (d, *J* = 8.3 Hz, 1H), 7.35 (m, 5H), 7.80 (m, 2H), 7.91 (d, *J* = 2.0 Hz, 1H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 24.0, 25.1, 28.8, 49.3, 59.2, 61.2, 72.8, 73.5, 93.0, 110.0, 124.4, 127.7, 127.7, 128.4, 128.6, 131.1, 132.3, 138.2, 143.9, 173.0; νmax/cm-1 3280 (N-H), 1620 (C=O), 1473, 1335 (-SO2-N), 1201 (O-C-O); HRMS (ES) *m/z* = 459.1583 [M+H]+ found, $C_{23}H_{27}N_2O_6S$ needs 459.1584; R_f = 0.32 (1:1 ethyl acetate/ petroleum ether).

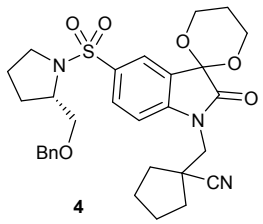
1-(Chloromethyl)cyclopentanecarbonitrile **3**



3

A mixture of cyclopentanecarbonitrile (6.6 mL, 63.00 mmol) and bromochloromethane (14.4 mL, 221.00 mmol) in THF (100 mL) was cooled to -78 ^{O}C. A solution of lithium diisopropylamide (LDA), prepared by the addition of *n*BuLi (1.6 M in hexane, 59 mL, 94.50 mmol) to diisopropylamine (13.26 mL, 94.50 mmol) in THF (50 mL) at 0 ^{O}C, was cooled to -78 ^{O}C in a separate flask. The two solutions were quickly mixed and stirred at -78 ^{O}C for 3 hours, before allowing to stand at room temperature overnight. This was followed by quenching with water (150 mL) and washing with brine (50 mL). The organic extract was dried over $MgSO_4$ and concentrated *in vacuo*. Purification by flash column chromatography, eluting with 1:19 ethyl acetate/petroleum ether gave the 1-chloromethyl cyclopentanecarbonitrile **3** as a pale yellow oil (2.16 g, 24 % yield): ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.85 (m, 6H), 2.24 (m, 2H), 3.61 (s, 2H)); ^{13}C NMR (101 MHz, $CDCl_3$) δ 24.7, 36.7, 45.4, 48.5, 123.1; HRMS (CI) *m/z* = 161.0846 [M+NH_4]+ found, $C_7H_{14}ClN_2$ needs 161.0846; R_f = 0.30 (1:5 ethyl acetate/ petroleum ether).

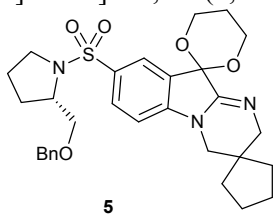
(*S*)-1-((5'-(2-(Benzyloxymethyl)pyrrolidin-1-ylsulfonyl)-2'-oxospiro[[1,3]dioxane-2,3'-indoline]-1'-yl)methyl)cyclopentanecarbonitrile **4**



4

A mixture of ketal-protected isatin sulfonamide **1** (1.60 g, 3.50 mmol) and *t*BuOK (0.98 g, 8.75 mmol) in DMF (10 mL) was stirred at room temperature for 1 hour. The alkylating agent, 1-chloromethyl cyclopentanecarbonitrile **3** (1.50 g, 10.50 mmol) was added and stirring was continued at 140 ^{O}C for 2 days. The reaction mixture was subsequently concentrated *in vacuo* and purified by flash column chromatography, eluting with 1:4 – 1:1 ethyl acetate/petroleum ether to give the nitrile **4** as a colourless foam (1.40 g, 71 % yield): ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.91 (m, 8H), 2.17 (m, 3H), 2.42 (m, 1H), 3.09 (m, 1H), 3.49 (m, 3H), 3.66 (m, 1H), 3.77 (m, 1H), 3.86 (m, 3H), 4.01 (m, 2H), 4.57 (m, 2H), 4.92 (m, 2H), 7.20 (d, *J* = 8.3 Hz, 1H), 7.36 (m, 5H), 7.86 (dd, *J* = 2.0 Hz, 8.3 Hz, 1H), 7.93 (d, *J* = 2.0 Hz, 1H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 23.8, 24.0, 25.1, 28.8, 36.5, 43.9, 45.5, 49.2, 59.2, 61.3, 72.8, 73.4, 92.7, 109.7, 124.0, 127.7, 127.7, 128.0, 128.4, 131.1, 132.8, 132.8, 138.2, 145.9, 172.7; νmax/cm-1 2235 (C≡N), 1721 (C=O), 1661 (C=N), 1342 (-SO2-N), 1055 (O-C-O); HRMS (ES) *m/z* = 566.2325 [M+H]+ found, $C_{30}H_{36}N_3O_6S$ needs 566.2325; R_f = 0.52 (1:1 ethyl acetate/petroleum ether).

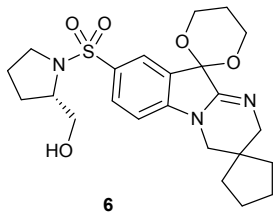
(*S*)-8'-(2-(Benzyloxymethyl)pyrrolidin-1-ylsulfonyl)-2'*H*-spiro[cyclopentane-1,3'-pyrimido[1,2-a]indole]-10',10'-(1,3-dioxolane) **5**



5

A mixture of the nitrile **4** (0.113 g, 0.20 mmol) and a spatula of wet Raney Ni in 2M methanolic ammonia (5 mL) was hydrogenated in an autoclave at a hydrogen pressure of ~70 psi for 7 hours. The suspension was filtered through Celite® and the filtrate was refluxed for a further 5 hours. After cooling to room temperature and concentration *in vacuo*, the crude product was purified by flash column chromatography, eluting with 1:4 – 1:1 ethyl acetate/petroleum ether to give the amidine **5** as a colourless foam (0.091 g, 83 % yield): ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.48 (m, 2H), 1.59 (m, 4H), 1.75 (m, 5H), 1.90 (m, 2H), 2.27 (m, 1H), 3.07 (m, 1H), 3.30 (s, 2H), 3.45 (m, 4H), 3.79 (m, 2H), 3.97 (m, 2H), 4.57 (m, 2H), 5.07 (m, 2H), 6.62 (d, *J* = 8.4 Hz, 1H), 7.36 (m, 5H), 7.80 (dd, *J* = 1.8 Hz, 8.4 Hz, 1H), 7.92 (d, *J* = 1.8 Hz, 1H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 24.0, 25.0, 25.5, 28.8, 35.9, 38.5, 49.2, 50.2, 55.9, 59.0, 60.2, 73.0, 73.4, 96.0, 105.5, 124.0, 127.6, 127.7, 128.4, 128.5, 129.1, 131.4, 138.3, 147.6, 156.2; νmax/cm-1 2954, 1611 (C=N), 1494, 1466, 1335, 1264, 1153; HRMS (ES) *m/z* = 552.2532 [M+H]+ found, $C_{30}H_{38}N_3O_5S$ needs 552.2532; R_f = 0.34 (1:1 ethyl acetate/ petroleum ether).

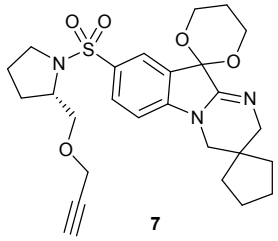
(*S*)-8'-(2-(Hydroxymethyl)pyrrolidin-1-ylsulfonyl)-2'*H*-spiro[cyclopentane-1,3'-pyrimido[1,2-a]indole]-10',10'(1,3)-dioxolane **6**



6

A mixture of ketal/benzyl protected pyrimidoindolone **5** (0.50 g, 0.91 mmol) and 10 % Pd/C (0.05 g) in EtOH (2 mL) was degassed for 20 minutes. 1,4-Cyclohexadiene (15 mL) was added and the mixture was refluxed for 2 days. After cooling to room temperature, the suspension was filtered through Celite® and the resulting filtrate was concentrated *in vacuo*. Purification by flash column chromatography, eluting with 3:7 acetone/ petroleum ether gave the benzyl deprotected ketal **6** as a colourless foam (0.27 g, 64 % yield); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.56 (m, 5H), 1.75 (m, 8H), 2.31 (m, 1H), 2.84 (m, 1H), 3.28 (m, 3H), 3.46 (m, 3H), 3.69 (m, 3H), 3.97 (m, 2H), 5.09 (br td, *J* = 11.2 Hz, 2.7 Hz, 2H), 6.65 (d, *J* = 8.3 Hz, 1H), 7.81 (dd, *J* = 8.3 Hz, 1.8 Hz, 1H), 7.92 (d, *J* = 1.8 Hz, 1H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 24.3, 25.0, 25.4, 28.9, 29.2, 35.9, 38.6, 50.2, 55.9, 60.2, 61.8, 66.0, 97.0, 105.6, 124.1, 128.4, 128.7, 131.5, 147.9, 155.9; νmax/cm-1 3427 (O-H), 1671, 1611 (C=N), 1266, 1152 (O-C-O); HRMS (ES) *m/z* = 462.2063 [M+H]+ found, $C_{23}H_{32}N_3O_5S$ needs 462.2063; R_f = 0.26 (ethyl acetate).

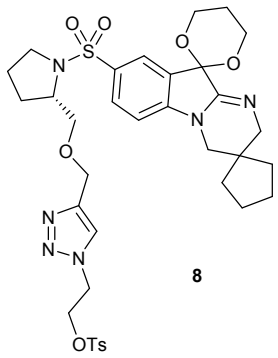
(*S*)-10',10'-(1,3)Dioxolane-8'-(2-((prop-2-ynyloxy)methyl)pyrrolidin-1-ylsulfonyl)-4',10'-dihydro-2'*H*-spiro[cyclopentane-1,3'-pyrimido[1,2-a]indole] **7**



7

To a solution of alcohol **6** (0.016 g, 0.03 mmol) in THF (0.5 mL) was added NaH (60 % in mineral oil, 1.00 mg, 0.07 mmol) and the mixture was stirred at room temperature for 10 minutes. Propargyl bromide (80 % in toluene, 0.01 mL, 0.14 mmol) was added and stirring was continued at room temperature for 12 hours. Then, the reaction mixture was poured into 1M NaOH solution (3 mL) and partitioned between water (5 mL) and DCM (5 mL). The organic layer was dried over $MgSO_4$ and concentrated *in vacuo*. Purification of the crude material by flash column chromatography, eluting with 1:4 – 1:0 ethyl acetate/ petroleum ether gave the alkyne **7** as a colourless foam (0.009 g, 60 % yield): ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.50 (m, 2H), 1.61 (m, 4H), 1.74 (m, 5H), 1.90 (m, 2H), 2.31 (m, 1H), 2.48 (t, *J* = 2.3 Hz, 1H), 3.09 (m, 1H), 3.30 (s, 2H), 3.47 (m, 4H), 3.81 (m, 2H), 3.98 (m, 2H), 4.21 (d, *J* = 2.3 Hz, 2H), 5.07 (m, 2H), 6.64 (d, *J* = 8.3 Hz, 1H), 7.81 (dd, *J* = 8.3 Hz, 1.8 Hz, 1H), 7.93 (d, *J* = 1.8 Hz, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 24.0, 25.1, 25.5, 28.8, 35.9, 38.6, 49.3, 50.3, 56.0, 58.6, 58.8, 60.2, 72.7, 74.6, 79.7, 96.0, 105.6, 124.0, 128.6, 129.0, 131.5, 147.7, 156.2; νmax/cm-1 3279 (C≡C), 1670, 1612 (C=N), 1464, 1335 (-SO2-N), 1263 (C-O-C), 1155, 1018; HRMS (ES) *m/z* = 500.2219 [M+H]+ found, $C_{26}H_{34}N_3O_5S$ needs 500.2219; [αD]20 = -65.6 (c 0.0025, $CDCl_3$); R_f = 0.40 (ethyl acetate).

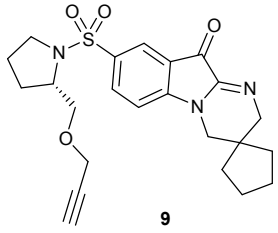
(*S*)-2-(4-(((1-(10',10'-(1,3)Dioxolane-4',10'-dihydro-2'*H*-spiro[cyclopentane-1,3'-pyrimido[1,2-a]indole]-8'-ylsulfonyl)pyrrolidin-2-yl)methoxy)methyl)-1*H*-1,2,3-triazol-1-yl)ethyl 4-methylbenzenesulfonate **8**



8

A mixture of $CuSO_4.5H_2O$ (0.011 g, 0.045 mmol), L-ascorbic acid (0.016 g, 0.09 mmol) and the alkyne **7** (0.045 g, 0.09 mmol) in DMF (1.0 mL) was stirred at room temperature for 10 minutes to give a pale yellow solution. Tosylethylazide (0.027 g, 0.11 mmol) in DMF (0.5 mL) was added and stirring was continued at room temperature for 1 hour. This was followed by quenching with 10 % NH_4Cl aqueous solution (10 mL) and extraction with ethyl acetate (2 x 10 mL). The combined organic extracts were washed with brine (2 x 15 mL) and water (2 x 15 mL), dried over $MgSO_4$ and concentrated *in vacuo*. The crude mixture was purified by flash column chromatography, eluting with 1:1 – 1:0 ethyl acetate/ petroleum ether to give the tosylate **8** as a colourless foam (0.036 g, 54 % yield); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.50 (m, 2H), 1.61 (m, 4H), 1.75 (m, 5H), 1.89 (m, 2H), 2.31 (m, 1H), 2.47 (s, 3H), 3.09 (m, 1H), 3.31 (s, 2H), 3.44 (br s, 3H), 3.54 (m, 1H), 3.79 (m, 2H), 3.96 (m, 2H), 4.44 (t, *J* = 5.1 Hz, 2H), 4.68 (m, 4H), 5.08 (m, 2H), 6.65 (d, *J* = 8.3 Hz, 1H), 7.36 (d, *J* = 8.3 Hz, 2H), 7.66 (s, 1H), 7.72 (d, *J* = 8.3 Hz, 2H), 7.80 (dd, *J* = 8.3 Hz, 1.7 Hz, 1H), 7.91 (d, *J* = 1.7 Hz, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 21.7, 24.0, 25.1, 25.5, 28.8, 35.9, 38.6, 49.0, 49.3, 50.3, 56.0, 59.0, 60.2, 64.6, 67.6, 73.1, 96.0, 105.6, 123.7, 124.0, 127.9, 128.6, 129.0, 130.1, 131.4, 132.1, 145.3, 145.5, 147.7, 156.2; ν max/cm-1 1674, 1612 (C=N), 1465, 1335 (-SO2-N), 1152 (C-O-C), 1117, 1032 (O-C-O); HRMS (ES) *m/z* = 741.2740 [M+H]+ found, $C_{35}H_{45}N_6O_8S_2$ needs 741.2740; [αD]20 = -50.4 (c 0.004, $CDCl_3$); R_f = 0.16 (ethyl acetate).

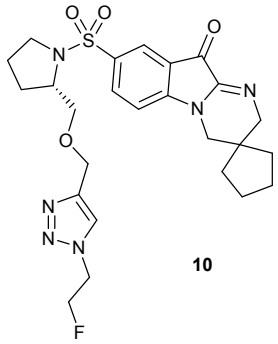
(*S*)-8'-(2-((Prop-2-ynyloxy)methyl)pyrrolidin-1-ylsulfonyl)-2'H-spiro[cyclopentane-1,3'-pyrimido[1,2- a]indol]-10'(4'*H*)-one, **9**



9

A solution of the ketal-protected alkyne **7** (0.18 g, 0.36 mmol) in methanesulfonic acid (2 mL) was stirred at room temperature for 12 hours. The mixture was slowly quenched with saturated aqueous Na2CO3 solution until a pH of ~8 was reached, before extracting with DCM (3 x 15 mL). This was followed by drying over $MgSO_4$ and concentration *in vacuo*. Purification by flash column chromatography, eluting with 1:1 – 1:0 ethyl acetate/ petroleum ether, gave deprotected alkyne **9** as a yellow foam (0.091 g, 58 % yield); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.65 (m, 10H), 1.93 (m, 2H), 2.49 (t, *J* = 2.4 Hz, 1H), 3.13 (m, 1H), 3.44 (m, 3H), 3.54 (m, 1H), 3.71 (s, 2H), 3.77 (m, 2H), 4.19 (d, *J* = 2.4 Hz, 2H), 6.89 (d, *J* = 8.4 Hz, 1H), 8.04 (dd, *J* = 8.4 Hz, 1.8 Hz, 1H), 8.11 (d, *J* = 1.8 Hz, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 24.0, 25.1, 28.9, 36.1, 39.9, 49.4, 50.0, 57.5, 58.6, 59.0, 72.4, 74.7, 79.6, 108.2, 119.2, 124.9, 130.5, 137.0, 147.9, 154.3, 183.3; ν max/cm-1 2924 (C≡C), 1727 (C=O), 1605 (C=N), 1464, 1334, 1151, 1093; HRMS (ES) *m/z* = 442.1801 [M+H]+ found, $C_{23}H_{28}N_3O_4S$ needs 442.1801; [αD]20 = -60.3(c 0.003, $CDCl_3$); R_f = 0.37 (ethyl acetate).

(*S*)-8'-(2-(((1-(2-Fluoroethyl)-1*H*-1,2,3-triazol-4-yl)methoxy)methyl)pyrrolidin-1-ylsulfonyl)-2'*H*-spiro[cyclopentane-1,3'-pyrimido[1,2-a]indol]-10'(4'*H*)-one, **10**



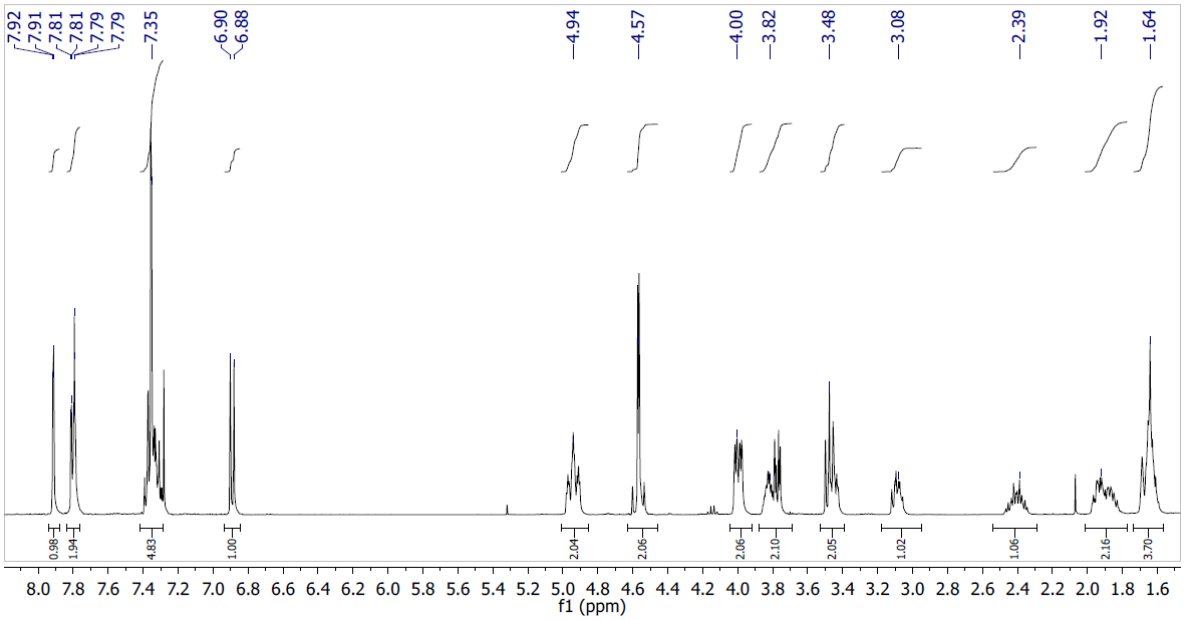
10

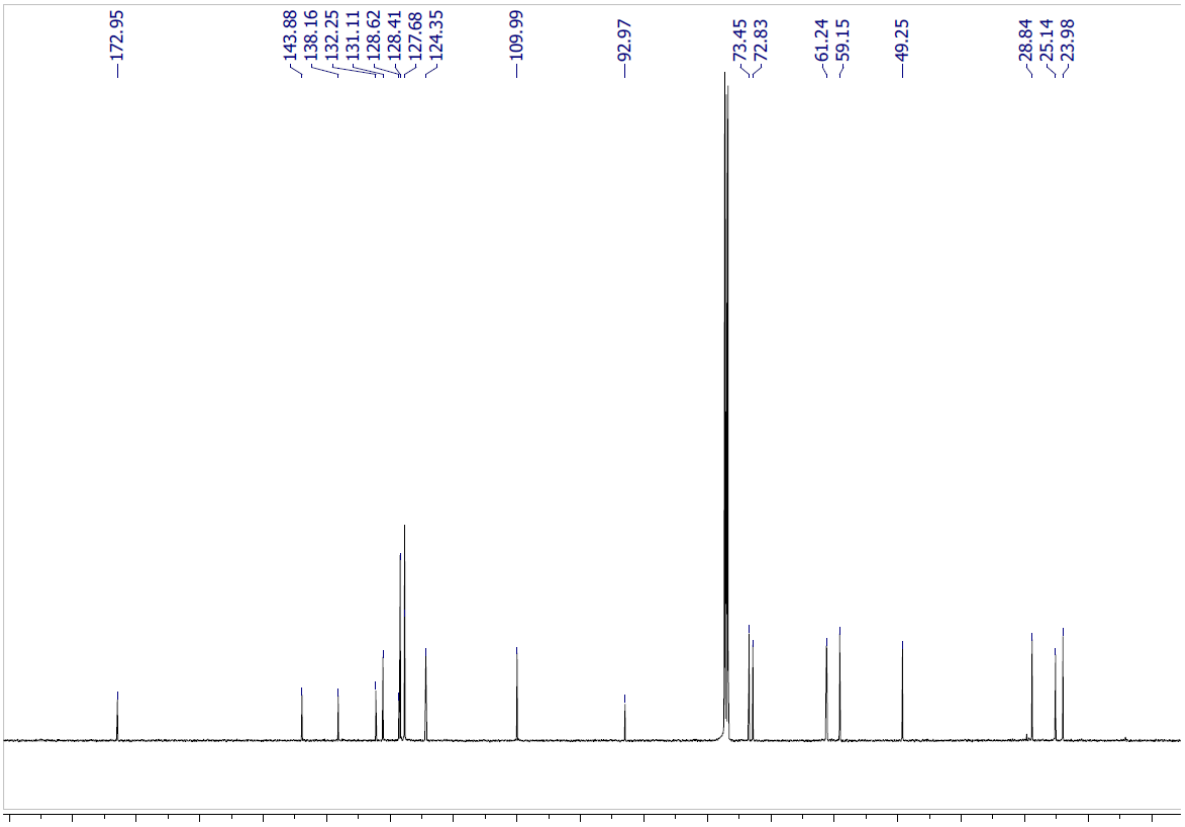
Part 1: To a solution of 1-fluoroethyltosylate (0.10 g, 0.46 mmol) in DMF (2 mL) was added NaN_3 (0.09 g, 1.40 mmol) and the mixture was stirred at room temperature for 15 hours. A TLC showed the full disappearance of the 1-fluoroethyltosylate and a new polar product, indicating conversion to 1-fluoroethylazide.

Part 2: $CuSO_4.5H_2O$ (0.006 g, 0.025 mmol) and L-ascorbic acid (0.009 g, 0.05 mmol) were added to a separate reaction flask, followed by the addition of the alkyne precursor **9** (0.022 g, 0.05 mmol) in DMF (1.5 mL). The mixture was stirred to give a dark brown solution. Then, fluoroethylazide (0.26 mL, 0.06 mmol of the filtered solution from *part 1*) was added and stirring was continued at room temperature for 1 hour. This was followed by quenching with 10 % aqueous NH_4Cl solution (10 mL) and extraction with ethyl acetate (2 x 10 mL). The combined organic extracts were washed with brine (2 x 15 mL) and water (2 x 5 mL), dried over MgSO4 and concentrated *in vacuo*. Purification by flash column chromatography, eluting with 1:1 – 1:0 ethyl acetate/ petroleum ether gave the pyrimidoindolone target **10** as a yellow foam (0.013 g, 50 % yield); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.55 (m, 12H), 3.15 (m, 1H), 3.42 (m, 3H), 3.54 (m, 1H), 3.76 (m, 4H), 4.67 (m, 3H), 4.75 (m, 2H), 4.89 (m, 1H), 6.88 (d, J = 8.5 Hz, 1H), 7.73 (s, 1H), 8.02 (dd, J = 8.5 Hz, 1.8 Hz, 1H), 8.04 (d, J = 1.8 Hz, 1H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 24.1, 25.1, 28.9, 36.1, 39.9, 49.3, 50.0, 50.5 (d, J = 20.7 Hz), 57.5, 59.1, 64.6, 72.7, 81.0 (d, J = 173.3 Hz), 108.1, 119.1, 123.6, 124.7, 130.8, 137.0, 145.2, 147.9, 154.2, 183.3; ν max/cm-1 1728 (C=O), 1660, 1603 (C=N), 1484, 1461, 1334 (-SO2-N), 1276, 1151, 1127 (C-F), 1075 (C-O-C); HRMS (ES) *m/z* = 531.2183 [M+H]+ found, $C_{25}H_{32}FN_6O_4S$ needs 531.2184; [αD]20 = -50.4 (c 0.004, CHCl3); R_f = 0.11 (ethyl acetate).

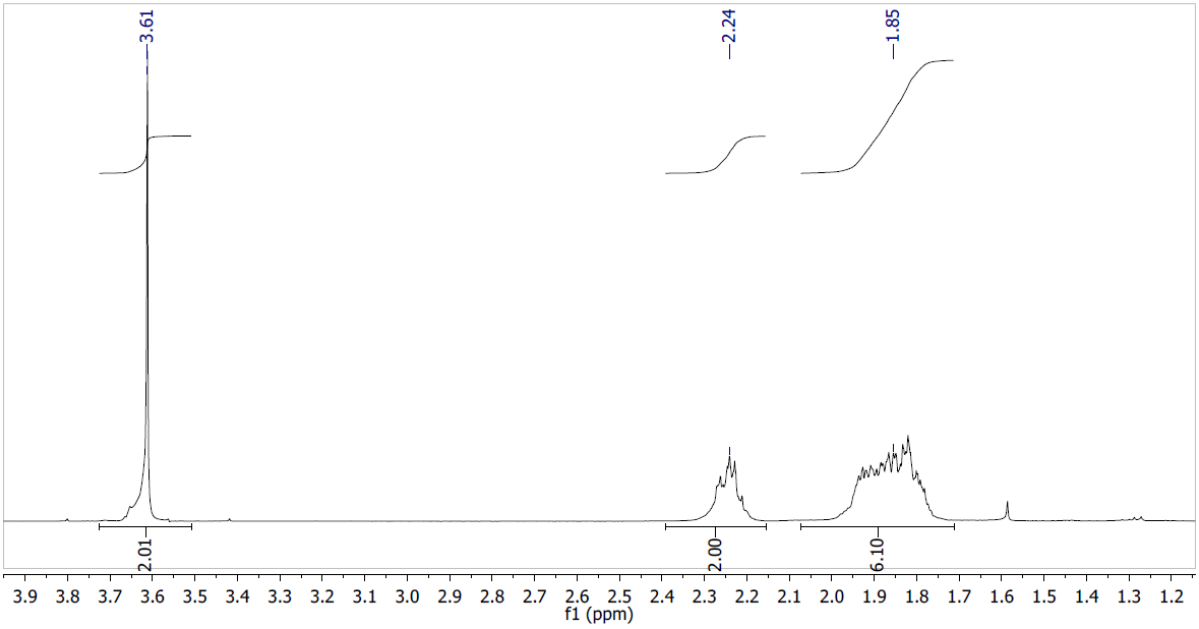
3. NMR Spectra of relevant compounds

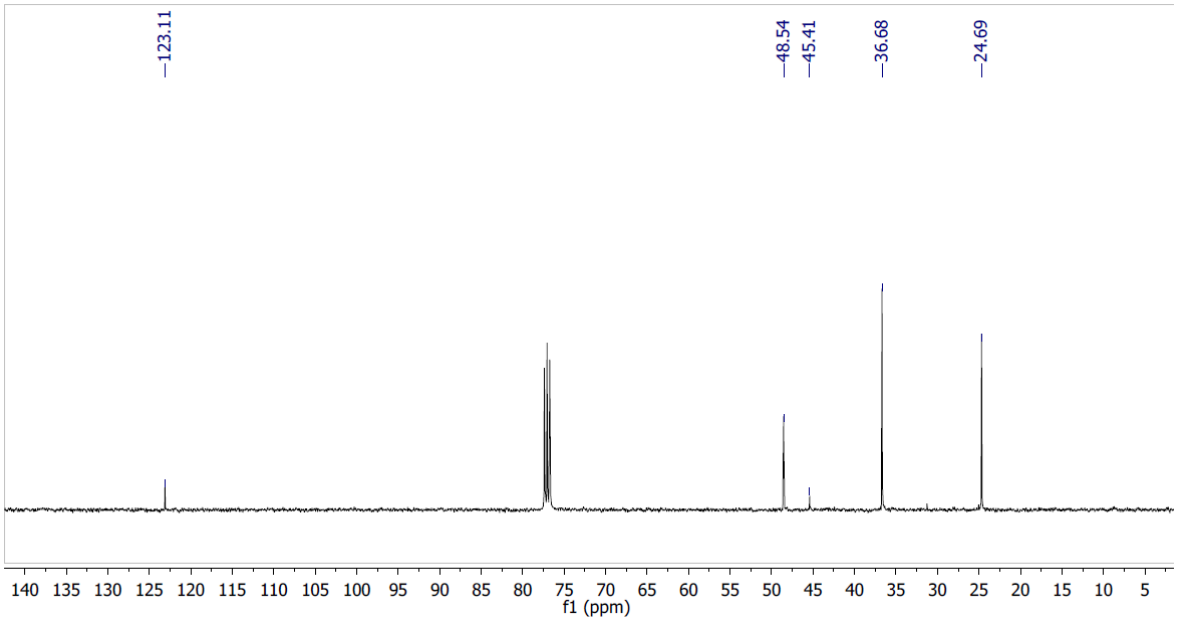
Compound 1



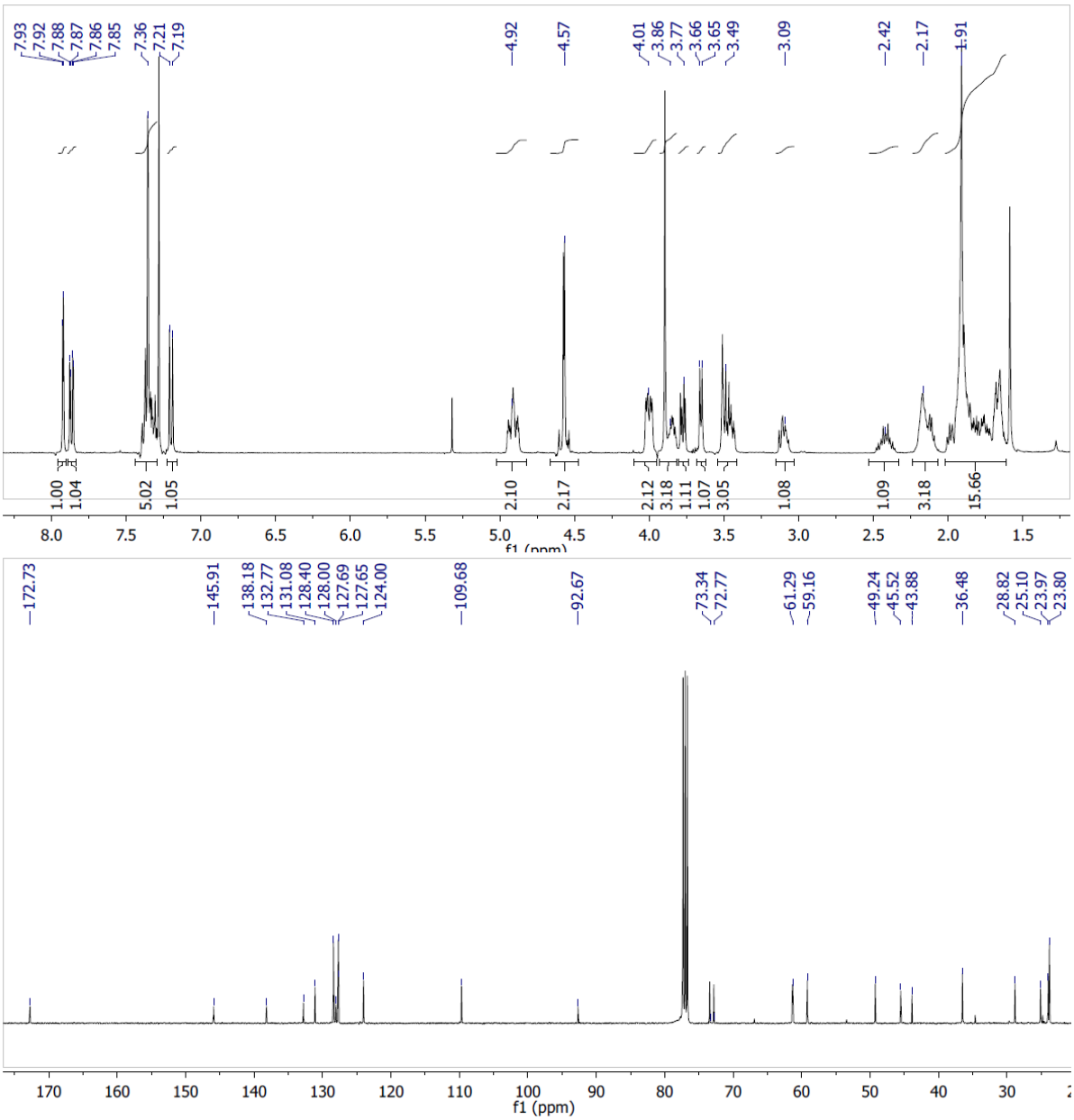


Compound 3

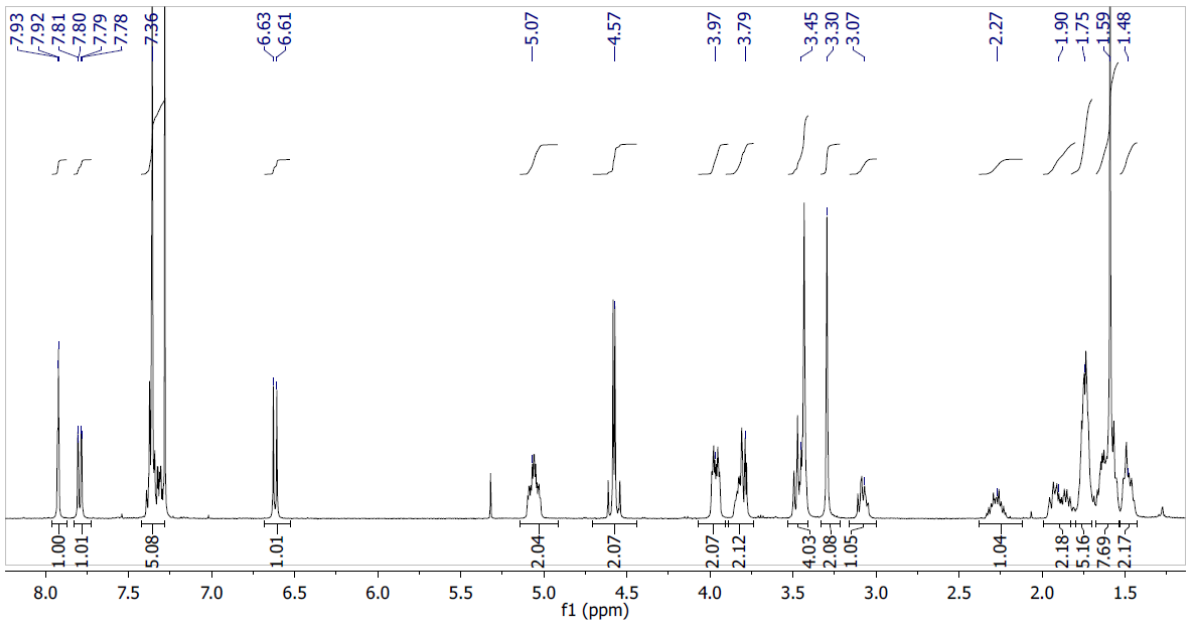

3.61
2.24
1.85
2.01
2.00
6.10
3.9 3.8 3.7 3.6 3.5 3.4 3.3 3.2 3.1 3.0 2.9 2.8 2.7 2.6 2.5 2.4 2.3 2.2 2.1 2.0 1.9 1.8 1.7 1.6 1.5 1.4 1.3 1.2
f1 (ppm)

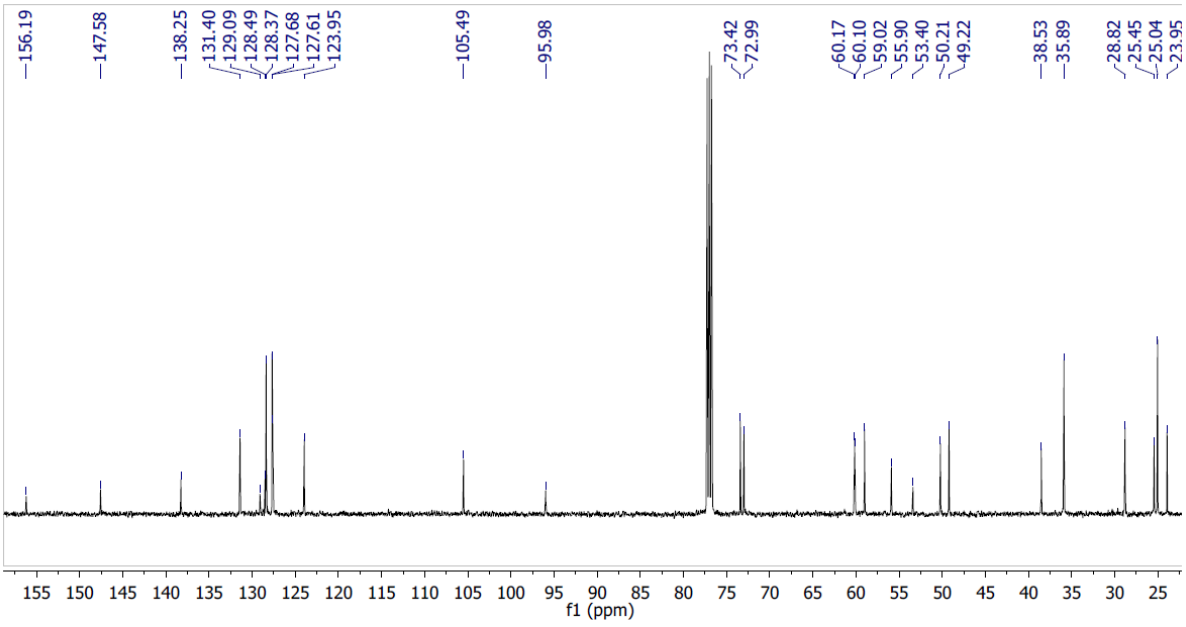

123.11
48.54
45.41
36.68
24.69
140 135 130 125 120 115 110 105 100 95 90 85 80 75 70 65 60 55 50 45 40 35 30 25 20 15 10 5
f1 (ppm)

Compound 4

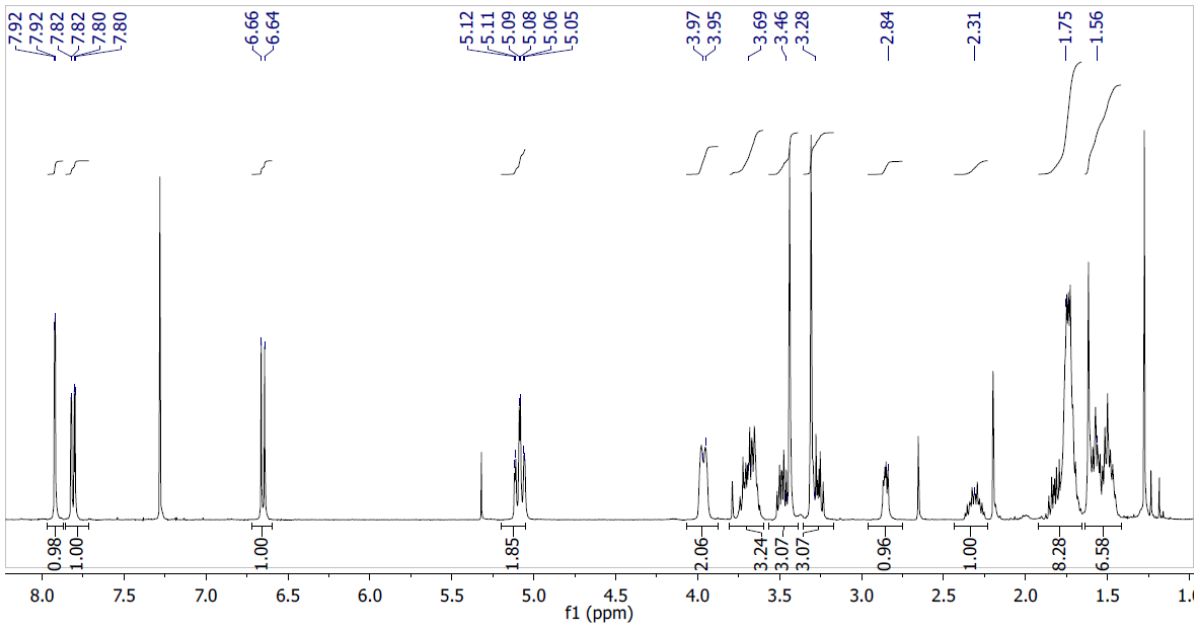

7.93
7.92
7.88
7.87
7.86
7.85
7.36
7.21
7.19
4.92
4.57
4.01
3.86
3.77
3.66
3.65
3.49
3.09
2.42
2.17
1.91
1.00
1.04
5.02
1.05
2.10
2.17
2.12
3.18
1.11
1.07
3.05
1.08
1.09
3.18
15.66
f1 (ppm)
172.73
145.91
138.18
132.77
131.08
128.40
128.00
127.69
127.65
124.00
109.68
92.67
73.34
72.77
61.29
59.16
49.24
45.52
43.88
36.48
28.82
25.10
23.97
23.80
f1 (ppm)

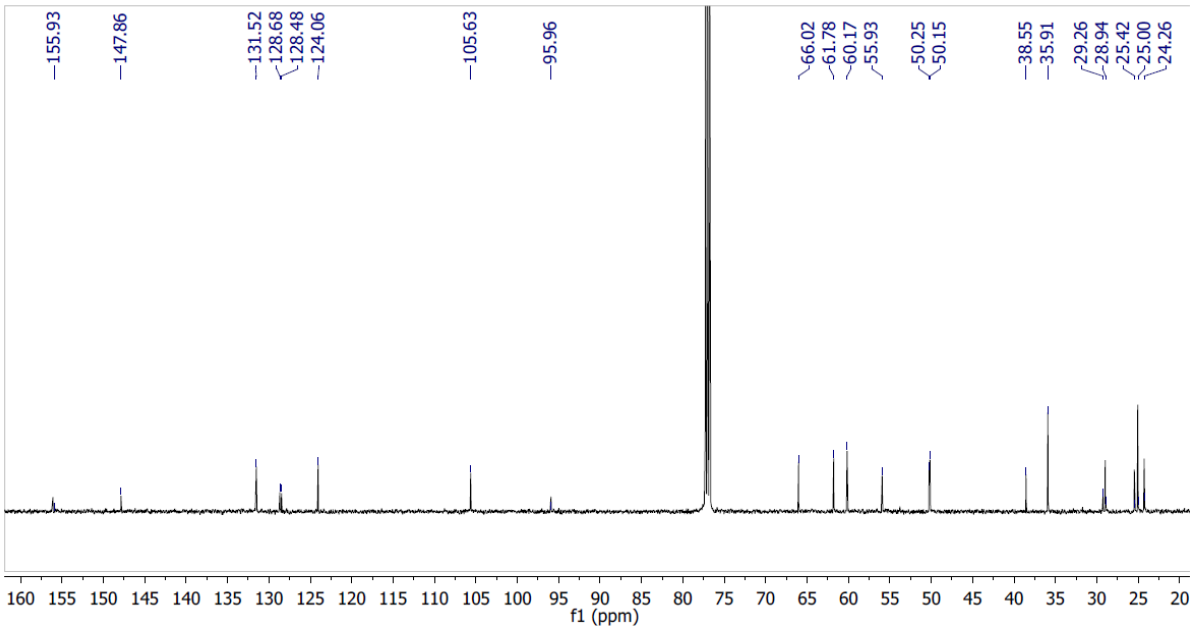
Compound 5


7.93
7.92
7.81
7.80
7.79
7.78
7.36
6.63
6.61
5.07
4.57
3.97
3.79
3.45
3.30
3.07
2.27
1.90
1.75
1.59
1.48
1.00
1.01
5.08
1.01
2.04
2.07
2.07
2.12
4.03
2.08
1.05
1.04
2.18
5.16
7.69
2.17
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
f1 (ppm)

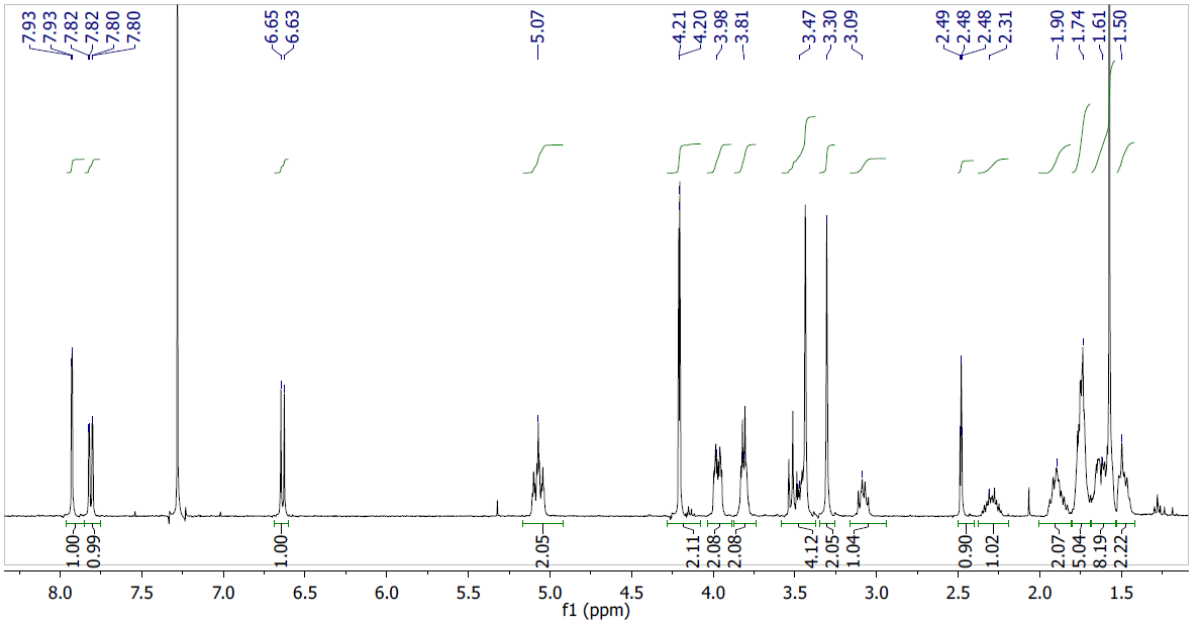

156.19
147.58
138.25
131.40
129.09
128.49
128.37
127.68
127.61
123.95
105.49
95.98
73.42
72.99
60.17
60.10
59.02
55.90
53.40
50.21
49.22
38.53
35.89
28.82
25.45
25.04
23.95
155
150
145
140
135
130
125
120
115
110
105
100
95
90
85
80
75
70
65
60
55
50
45
40
35
30
25
f1 (ppm)

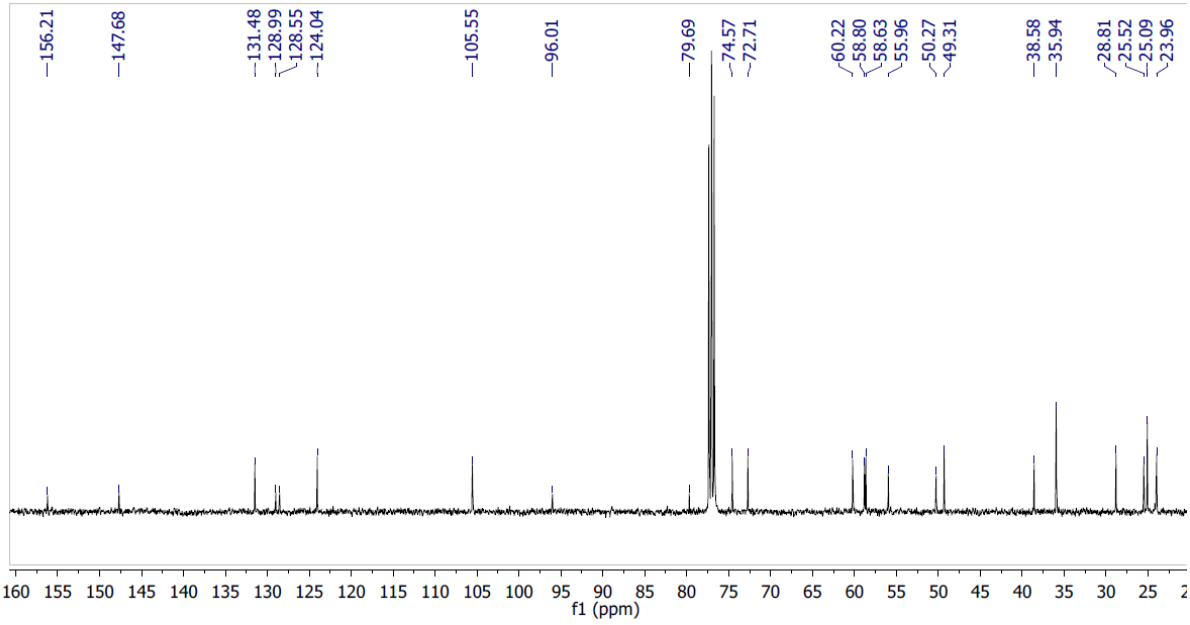
Compound 6


7.92
7.92
7.82
7.82
7.80
7.80
6.66
6.64
5.12
5.11
5.09
5.08
5.06
5.05
3.97
3.95
3.69
3.46
3.28
2.84
2.31
1.75
1.56
0.98
1.00
1.00
1.85
2.06
3.24
3.07
3.07
0.96
1.00
8.28
6.58
f1 (ppm)

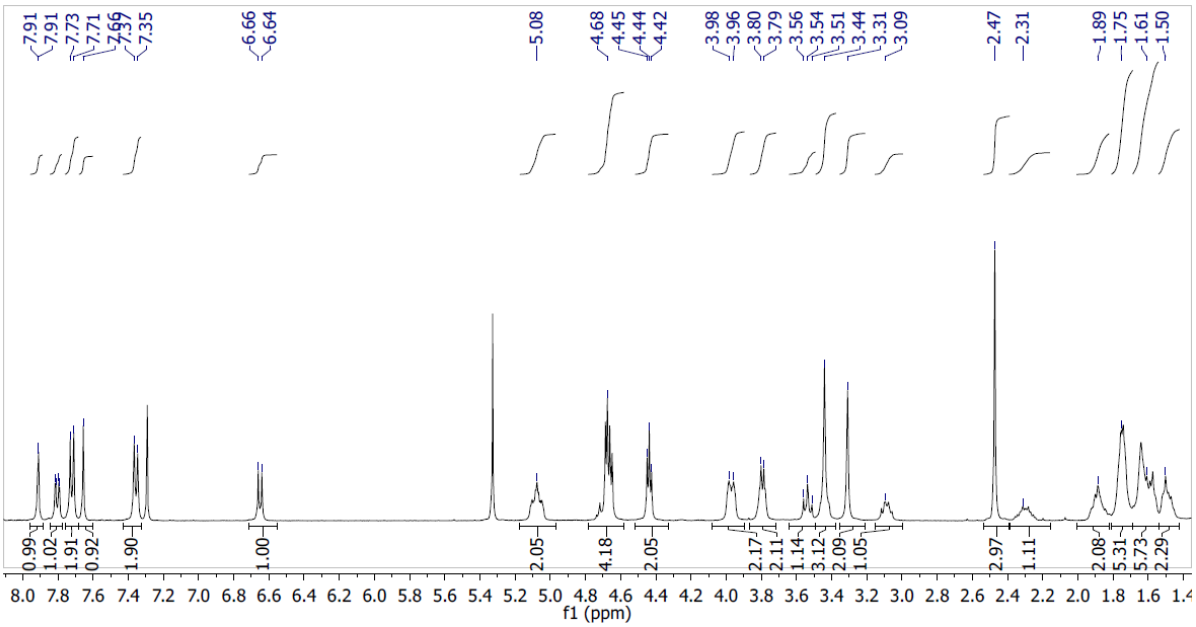

155.93
147.86
131.52
128.68
128.48
124.06
105.63
95.96
66.02
61.78
60.17
55.93
50.25
50.15
38.55
35.91
29.26
28.94
25.42
25.00
24.26
f1 (ppm)

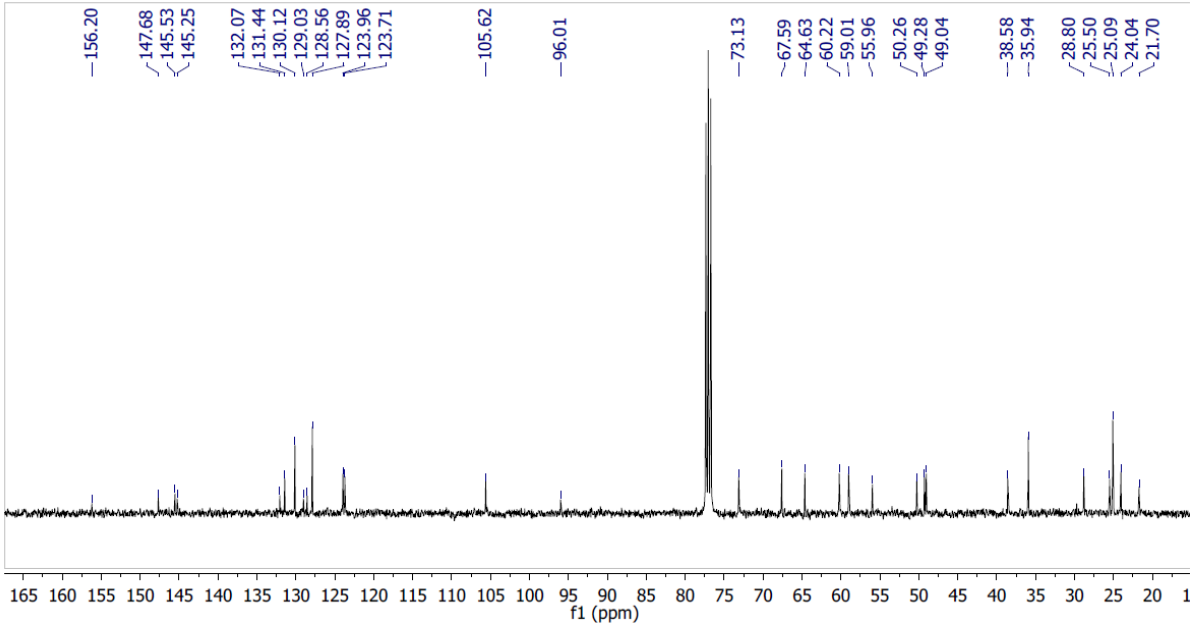
Compound 7


7.93
7.93
7.82
7.82
7.80
7.80
6.65
6.63
5.07
4.21
4.20
3.98
3.81
3.47
3.30
3.09
2.49
2.48
2.48
2.31
1.90
1.74
1.61
1.50
1.00
0.99
1.00
2.05
2.11
2.08
2.08
4.12
2.05
1.04
0.90
1.02
2.07
5.04
8.19
2.22
f1 (ppm)

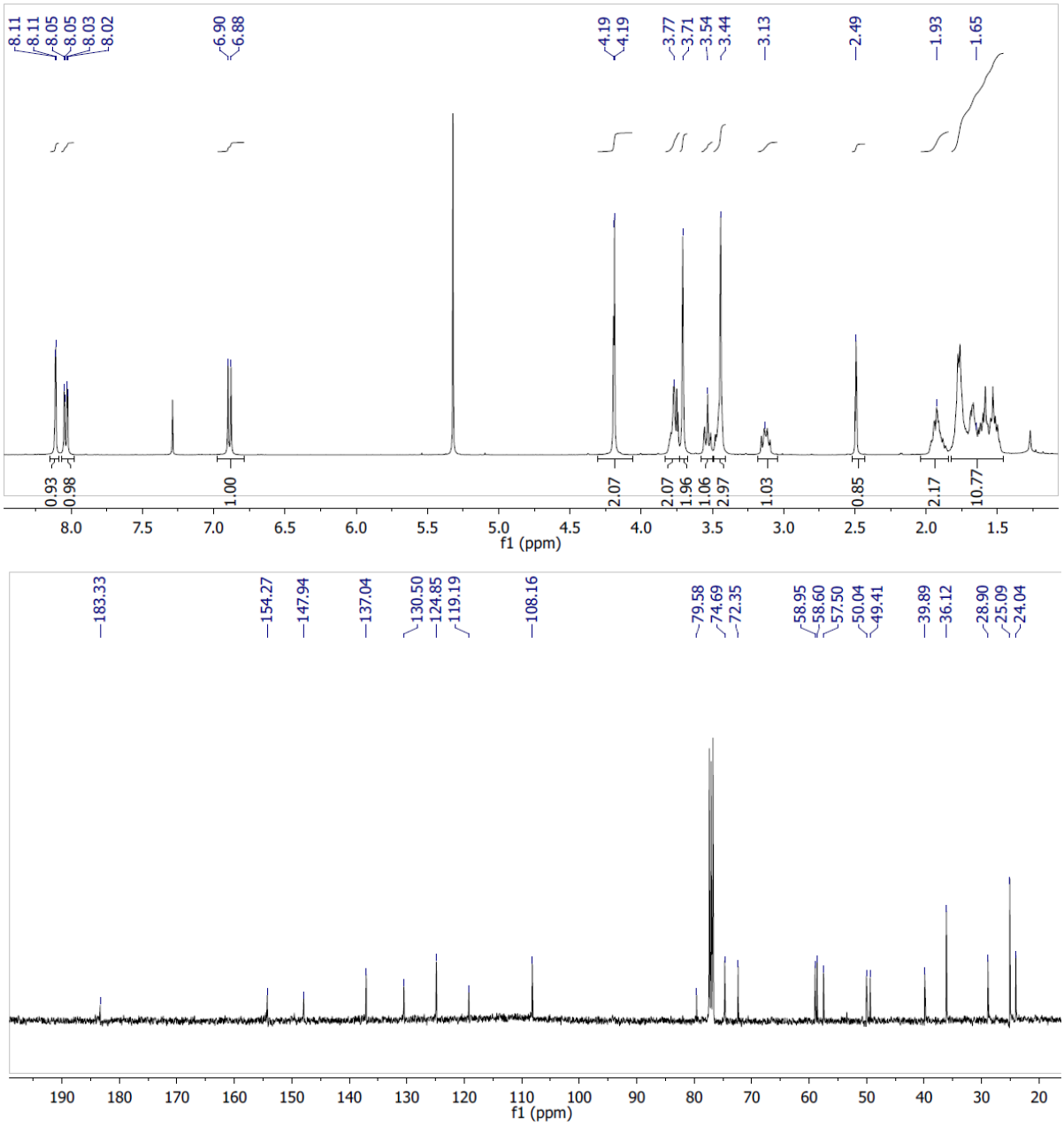

156.21
147.68
131.48
128.99
128.55
124.04
105.55
96.01
79.69
74.57
72.71
60.22
58.80
58.63
55.96
50.27
49.31
38.58
35.94
28.81
25.52
25.09
23.96
f1 (ppm)

Compound 8

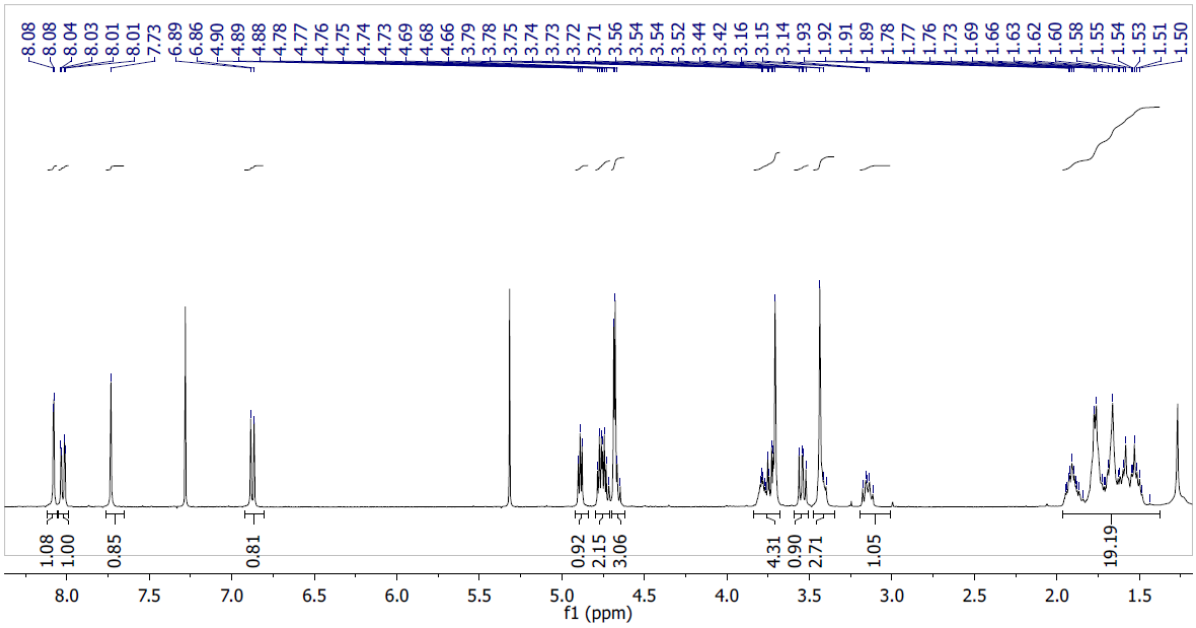

7.91
7.91
7.73
7.71
7.35
6.66
6.64
5.08
4.68
4.45
4.44
4.42
3.98
3.96
3.80
3.79
3.56
3.54
3.51
3.44
3.31
3.09
2.47
2.31
1.89
1.75
1.61
1.50
0.99
1.02
1.91
0.92
1.90
1.00
2.05
4.18
2.05
2.17
2.11
1.14
3.12
2.09
1.05
2.97
1.11
2.08
5.31
5.73
2.29
f1 (ppm)

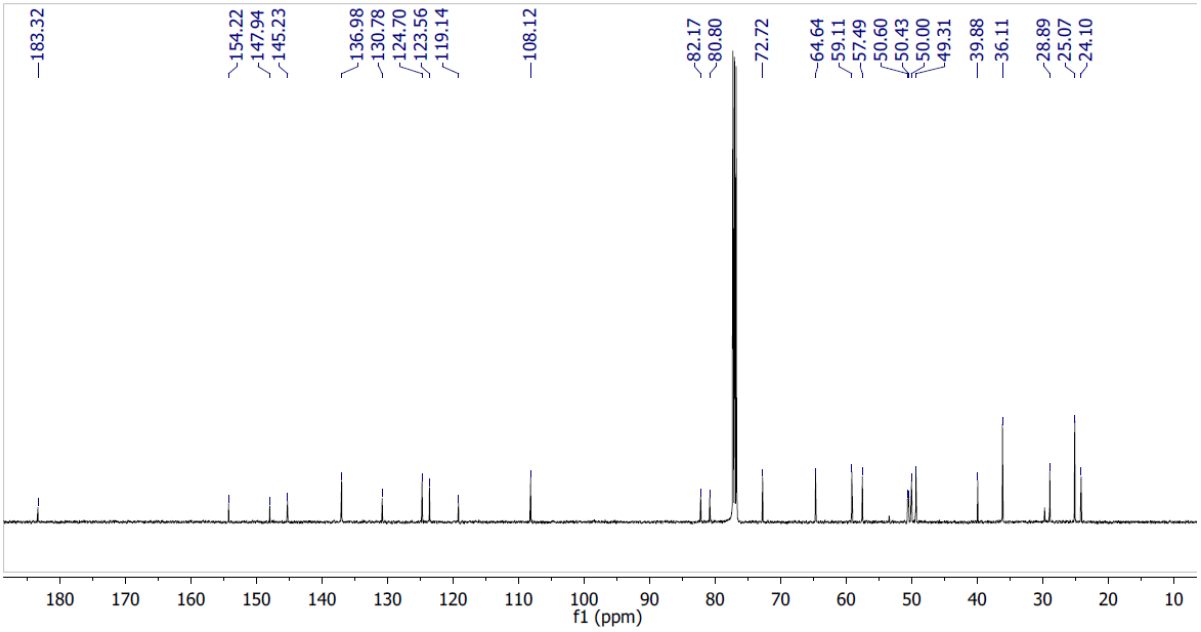

156.20
147.68
145.53
145.25
132.07
131.44
130.12
129.03
128.56
127.89
123.96
123.71
105.62
96.01
73.13
67.59
64.63
60.22
59.01
55.96
50.26
49.28
49.04
38.58
35.94
28.80
25.50
25.09
24.04
21.70
f1 (ppm)

Compound 9


8.11
8.11
8.05
8.05
8.03
8.02
6.90
6.88
4.19
4.19
3.77
3.71
3.54
3.44
3.13
2.49
1.93
1.65
0.93
0.98
1.00
2.07
2.07
1.96
1.06
2.97
1.03
0.85
2.17
10.77
f1 (ppm)
183.33
154.27
147.94
137.04
130.50
124.85
119.19
108.16
79.58
74.69
72.35
58.95
58.60
57.50
50.04
49.41
39.89
36.12
28.90
25.09
24.04
f1 (ppm)

Compound 10


8.08
8.08
8.04
8.03
8.01
8.01
7.73
6.89
6.86
4.90
4.89
4.88
4.78
4.77
4.76
4.75
4.74
4.73
4.69
4.68
4.66
3.79
3.78
3.75
3.74
3.73
3.72
3.71
3.56
3.54
3.54
3.52
3.44
3.42
3.16
3.15
3.14
1.93
1.92
1.91
1.89
1.78
1.77
1.76
1.73
1.69
1.66
1.63
1.62
1.60
1.58
1.55
1.54
1.53
1.51
1.50
1.08
1.00
0.85
0.81
0.92
2.15
3.06
4.31
0.90
2.71
1.05
19.19
f1 (ppm)


183.32
154.22
147.94
145.23
136.98
130.78
124.70
123.56
119.14
108.12
82.17
80.80
72.72
64.64
59.11
57.49
50.60
50.43
50.00
49.31
39.88
36.11
28.89
25.07
24.10
f1 (ppm)

4. ^{18}F Radiosynthesis of compound 10

Click Radiolabelling Procedure

Part 1: To a Wheaton vial containing an aqueous solution of K_2CO_3 (50 μL, 1.00 mg), K222 (5.00 mg) and acetonitrile (600 μL), was added an aqueous solution of [^{18}F]fluoride (50 -100 μL, < 740 MBq). The mixture was dried by azeotropic distillation using acetonitrile (3 x 600 μL). Then, a solution of tosylethylazide (1.5 μL) in acetonitrile (250 μL) was added to the vial and the resulting mixture was stirred at 80 °C for 15 minutes. The product [^{18}F]fluoroethylazide was then purified by distillation using a thermospray.

Part 2: A buffered solution (sodium phosphate buffer, pH 6.0, 250 mM) of sodium L-ascorbate (25 μL, pH 6.0, 2.53 mg, 12.79 μmol) was added to a Wheaton vial containing an aqueous solution of $CuSO_4.5H_2O$ (25 μL, 0.51 mg, 2.06 μmol). Then, a solution of the relevant alkyne precursor **9** or **7** (1.00 – 1.30 mg) in DMF or DMSO (50 μL) was added, followed by the addition of the distilled [^{18}F]-2-fluoroethylazide (< 740 MBq) in acetonitrile (30 – 100 μL). The mixture was stirred at room temperature or heated as appropriate. In reactions using bathophenanthrolinedisulfonate (BPDS) ligand, a solution of BPDS (25 μL, 2.22 mg, 4.13 μmol) in acetate buffer (250 mM, pH 5.0) was added to the Wheaton vial along with $CuSO_4$ and sodium L-ascorbate solution.

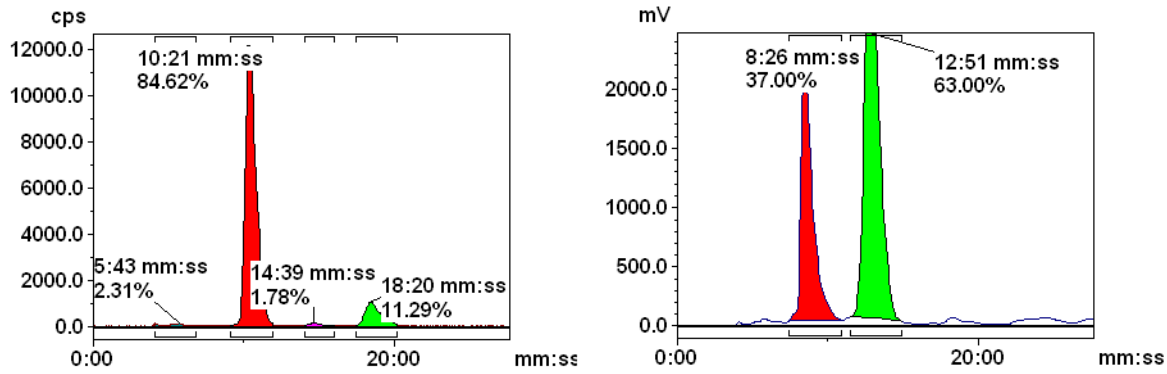
Reaction optimisation studies by analytical radio-HPLC: 10 μL samples of the reaction mixture were taken at time intervals, diluted with acetonitrile/water (1:1, 300 μL) and analysed by HPLC (1100 Agilent HPLC, using a gradient of: 0 minutes, 95% H_20:5% MeCN; 5 minutes, 95% H_2O:5% MeCN; 20 minutes, 5% H_2O:95% MeCN; 25 minutes, 5% H_2O:95% MeCN; 30 mintues, 95% H_20:5% MeCN; Flow-rate: 1ml/min; C18 Gemini 150 mm x 3.6 mm).

Radio-product isolation and formulation by Solid Phase Extraction (SPE): The reaction mixture was diluted with acetonitrile/water (1:1, 1.5 mL) and the resulting mixture was purified by prep radio-HPLC. The isolated HPLC fraction was diluted with water (5 mL) and loaded onto a SepPak C-18 cartridge (Waters, Elstree, UK), pre-conditioned with ethanol (5 mL) and water (10 mL). The loaded cartridge was flushed with water (5 mL) and the product was eluted with ethanol (4 x 100 μL fractions).

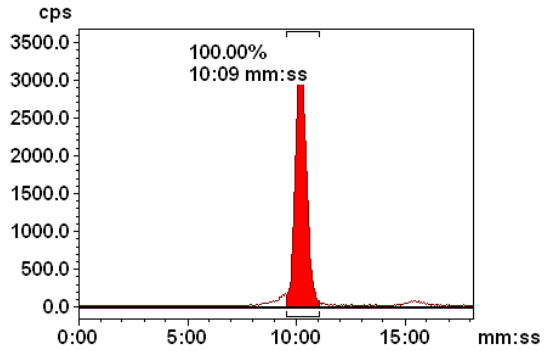
Direct radiolabelling with [^{18}F]fluoride

To a Wheaton vial containing K222 (~ 7.0 mg), K_2CO_3 (0.05 mL aqueous solution, 1.0 mg) and acetonitrile (0.6 mL), was added an aqueous solution of [^{18}F]fluoride (50 – 100 μL, < 740 MBq). The mixture was dried by azeotropic distillation using acetonitrile (3 x 0.6 mL). Subsequently, a solution of tosylate **8** (1.0 - 1.3 mg) in acetonitrile (0.2 mL) was added to the vial and the resulting mixture was heated at 110 - 120 °C for 15 minutes to give the intermediate. Acetonitrile was then removed by distillation, followed by the addition of hydrochloric acid (5M, 0.3 mL) and heating at 110 - 120 °C for a further 20 minutes. The reaction mixture was then transferred into a vial containing aqueous PBS buffer (0.4 mL) and diluted with 3M aqueous NaOAc solution (0.6 mL). Then, the mixture was further diluted with water (10 mL) and loaded onto a SepPak C18 cartridge conditioned with ethanol (5 mL) and water (10 mL). The cartridge was washed with water (20 mL) and eluted with ethanol (0.6 mL). The crude mixture was subsequently diluted with water (0.6 mL) prior to HPLC prep purification to give the radiolabelled product [^{18}F]**10**.

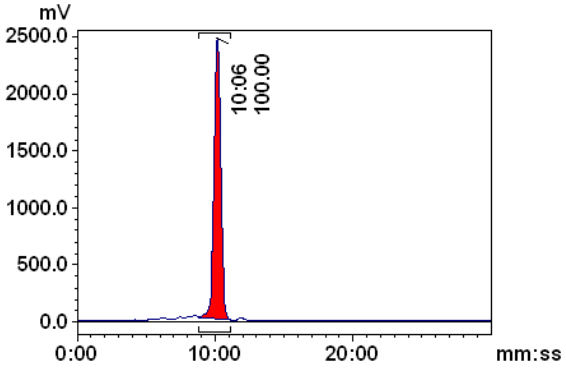
5. ^{18}F Radiosynthesis HPLC traces



Semi-preparative radio- and UV-HPLC of [^{18}F]**10**



Analytical radio-HPLC of [^{18}F]**10** following semi-prep HPLC purification



Analytical HPLC of [^{19}F]**10** as reference standard

6. Biological Procedures

Caspase-3 and 8 fluorogenic enzyme competition assay

Recombinant human caspases-3 and -8, and their peptide-specific substrates (Ac-DEVD-AMC and Ac-IETD-AMC, respectively) were purchased from Biomol International, Exeter, UK. Substrate competition with Pyrimido **10** and ICMT-11 was assessed using a fluorometric assay that measures the accumulation of the fluorogenic product, 7-amino-4-methylcoumarin (7-AMC). All assays were performed in 96-well plates in a 200 µl reaction volume per well (in triplicate). The reactions were performed at 37 ºC in a buffer comprised of 0.1% 3-[(3-Cholamidopropyl)dimethylammonio]-1-propanesulfonate hydrate buffer (CHAPS), 100 mM NaCl, 20 mM HEPES (pH 7.4), 2 mM EDTA, and 10% sucrose. Serial dilutions of Pyrimido **10** and ICMT-11 were prepared in DMSO and added to the reaction mixture at a final concentration of 500, 50, 5 µM; 500, 50, 5 nM; 500, 50, 5 pM; the final concentration of DMSO in all wells was 5% of the total volume. Recombinant caspases were used at 0.5 units per assay (~500 pmol substrate converted per h). All reagents except the peptide substrate were pre-incubated for 10 min. The peptide substrate (final concentration 10 µM) was then added and the plate was incubated for 15, 30, 60 or 90 min. Respective control wells contained all reaction components without enzyme. The amount of 7-AMC produced was measured on a fluorescence microplate reader (Victor2; Perkin-Elmer Life sciences, Coventry, UK) at excitation and emission wavelengths of 355 nm and 460 nm, respectively. The concentration of Pyrimido **10** and ICMT-11 that inhibits the caspase activity by 50% (IC_{50}) was estimated by non-linear regression analysis using GraphPad Prism (v5.0 for Mac, GraphPad Software, San Diego California USA).

In vitro [^{18}F]**10** cell uptake assay

38C13 cells, derived from a B-cell lymphoma that arose in a C3H mouse treated with 7,12-dimethybenz[a]anthracene, were kindly provided by R. Levy (Division of Oncology, Stanford School of Medicine, CA). The cells were plated in triplicate in 12-well plates 2 or 3 days before the experiments, and treated with 4-hydroperoxycyclophosphamide (4-HC, 3 µg/mL, 24 h). On the day of the experiment, ~0.37 MBq per well of [^{18}F]**10** was added and allowed to accumulate into cells for 60 min at 37 °C. Cells were collected, washed, and resuspended in PBS. Samples were transferred into counting tubes and fluorine-18 radioactivity was immediately determined using a gamma counter (Cobra II Auto-Gamma counter, Packard Biosciences, Pangbourne, UK). To enable normalization of data to total cellular protein content, the Bicinchoninic acid (BCA) protein assay was performed for all samples according to the manufacturer's instructions. Data were expressed as counts per mg of protein.

In vivo [^{18}F]**10** tissue biodistribution

All animal experiments were done by licensed investigators in accordance with the UK Home Office Guidance on the Operation of the Animal (Scientific Procedures) Act 1986 (HMSO, London, UK, 1990) and within guidelines set out by the UK National Cancer Research Institute Committee on Welfare of Animals in Cancer Research.[2] Male C3H mice (Harlan, Bicester, United Kingdom) were injected intravenously *via* the lateral tail vein with [^{18}F]**10** (~3.7 MBq), and at 60 min post-injection mice were sacrificed by exsanguination *via* cardiac puncture under general anesthesia (isoflurane inhalation). Tissues were then excised and placed in tubes for counting. Tissue radioactivity was determined on a gamma counter and decay corrected. Data were expressed as percentage injected

dose per gram.

Statistical analysis

Data were expressed as mean ± SEM and the significance of comparison between 2 datasets was determined using Student t test (Prism v5.0 for Mac, GraphPad Software, San Diego California USA) and defined as significant (*, $0.01 < P < 0.05$), very significant (**, $0.001 < P < 0.01$), and extremely significant (***, $P < 0.001$).

Transwell Assay

Caco-2 cells were seeded on to cell culture chambers (24 well plate – 0.4 μm pore size from Fisher, Loughborough UK) for 21 days, and the media changed every other day. The transepithelial electric resistance was measured using an epithelial voltohmmeter equipped with an STX2 electrode (World Precision Instruments, Sarasota, Florida, USA), and it was verified to be >300 Ω/cm^2 prior to all experiments. The assay was started by replacing the culture medium with HBSS containing 20 μM of drug. The plates were placed on an orbital shaker (60 rpm) during the experiment. After 2 hr, a volume of 50 μL of each chamber was collected for analysis by LC/MS. The ability of a test compound to pass through caco-2 bilayers was defined by the following equation (Eq. 1):[3] Papp $(dQ=dt) / AC_0$

Where A is the surface area of the transwell membrane, C_0 is the molar drug concentration in the donor chamber at time and dQ/dt is the rate of transfer of the compound to the receiver chamber, determined from the slope of the graph concentration (dQ) versus time (dt). It has been stated that if a compound exerts a ratio between the secretion (permeability from the basal to the apical side (Papp B→A)) and the absorption (permeability from the apical to the basal side (Papp A→B)) > 3, it can be considered as being actively effluxed.[4] At the end of the assay, the integrity of the monolayer was verified by measuring the amount of lucifer yellow, a fluorophore, which has passed through the membrane using a plate reader (Wallac EnVision reader, PerkinElmer, Boston, Massachusetts, USA) and verified to be < 2% of the concentration in the donor chamber.[5, 6] The integrity of the monolayer was assessed post-drug transport as lucifer yellow may interfere with LC/MS analysis.

7. References

(1) W. C. Still, M. Kahn, A. Mitra, *J. Org. Chem.* **1978**, *43*, 2923.
(2) P. Workman, E. O. Aboagye, F. Balkwill, A. Balmain, G. Bruder, D. J. Chaplin, *et al*. *Br. J. Cancer* **2010**,*102,*1555–77.
(3) P. Artursson, J. Karlsson. *Biochem. Biophys Res. Commun*. **1991**, *175,* 880–5.
(4) G. Szakacs, J. K. Paterson, J. A. Ludwig, C. Booth-Genthe, M. M. Gottesman *Nature Rev.* **2006**, *5*, 219–34.
(5) I. J. Hidalgo, T. J. Raub, R. T. Borchardt. *Gastroent.* **1989**, *96*, 736–49.
(6) D. S. Samiulla, V. V. Vaidyanatha, P. C. Arun, G. Balan, M. Blaze, S. Bondre, *et al*. *Mol. Divers.* **2005**, *9*, 131–9.