Electronic Supporting Information

Conditional generation of free radicals by selective activation of alkoxyamines: towards more effective and less toxic targeting of brain tumors

1. Preparation and characterization of alkoxyamines

1.1. General information

All corresponding glassware was oven-dried (100 °C) and/or carefully dried in line with a flameless heat gun. Solvents were used as received if not otherwise stated. THF was distilled from a blue solution of sodium-benzophenone ketyl radical prior to use. Routine monitoring of reactions was performed using Silica gel 60 F_{254}, aluminum supported TLC plates; spots were visualized using UV light, ethanolic acidic *para*-anisaldehyde solution, aqueous basic $KMnO_4$ solution, aqueous ninhydrin solution or ethanolic phosphomolybdic solution, followed by heating. Purifications by means of column chromatography were performed with Silica gel 60 (230-400 mesh) or with automatic flash chromatography system, and using gradients of Et_2O/petroleum ether, AcOEt/petroleum ether, acetone/petroleum ether or MeOH/CH_2Cl_2. ^{1}H, ^{13}C and ^{31}P NMR spectra were recorded in $CDCl_3$ solutions on 300 or 400 MHz spectrometers. Chemical shifts (δ) in ppm are reported using residual non-deuterated solvent peaks as internal reference for ^{1}H and ^{13}C NMR spectra, or by using internal reference of 85% H_3PO_4 for ^{31}P NMR spectra. High-resolution mass spectra (HRMS) have been performed using a mass spectrometer equipped with pneumatically-assisted atmospheric pressure ionization. The sample was ionized in positive mode electrospray in the following conditions: electrospray voltage (ISV): 5500 V; orifice voltage (OR): 80 V; nebulizing gas flow pressure (air): 20 psi. The mass spectrum was obtained using a time-of-flight analyzer (ToF). The measure was realized in triplicate, with double internal standardization. The sample was dissolved in CH_2Cl_2 (400 µL) then diluted (dilution factor $1/10^4$) in a methanolic solution of ammonium acetate (3 mM). The sample solution was infused in the ionization source at a 10 µL/min flow rate. HPLC have been performed in isocratic mode on a 4,6x50 mm (2,7 µM) column using CH_3CN/H_2O (+0,1%

TFA) at 1.0 mL/min, followed by low-resolution mass spectroscopy (LRMS) in ESI+ mode (electrospray voltage: 4000 V; orifice voltage 70V; nebulizing gas flow pressure (air): 20 psi.

1.2. Synthetic procedures

ALK1-3, **ALK7-16** and **ALK20-26** were synthesized following procedures published elsewhere.

ALK4-1 and ALK17-1 – (*RS/SR*)- and (*RR/SS*)-diethyl 1-(*tert*-butyl(1-phenyl-2-(2,2,2-trifluoroacetamido)ethoxy)amino)-2,2-dimethylpropylphosphonate

To a suspension of CuBr (2.37 g, 16.5 mmol, 0.55 equiv.) and Cu powder (2.10 g, 33.0 mmol, 1.10 equiv.) in degassed benzene (35 mL) under argon was added *N,N,N′,N′,N″*-pentamethyldiethylenetriamine (3.45 mL, 16.5 mmol, 0.55 equiv.). After stirring for 10 min, a solution of SG1 nitroxide (8.83 g, 30.0 mmol) and *N*-(bromo(phenyl)methyl)-2,2,2-trifluoroacetamide (11.55 g, 39.0 mmol, 1.30 equiv.) in degassed benzene (35 mL) was cannulated into the first solution. The mixture was allowed to stir for 24 h. The solution was quenched and washed with aq. sat. NH_4Cl solution, water and dried over $MgSO_4$. The solvents were evaporated under reduced pressure. The crude product (3:2 mixture of diastereoisomers by ^1H NMR) was purified by flash-chromatography with a gradient of ethyl acetate in petroleum ether to give white crystals of (*RS/SR*)-**ALK4-1** (5.5 g, 36%) and (*RR/SS*)-**ALK17-1** (8.7 g, 57%). (*RS/SR*)-**ALK4-1**: M.p. = 127-130 °C; ^1H NMR (400 MHz, $CDCl_3$): δ = 10.01 (bs, 1H), 7.27 (m, 5H), 5.04 (t, *J* = 3.5 Hz, 1H), 4.19 (m, 2H), 4.07 (m, 1H), 3.85 (m, 2H), 3.42 (d, *J* = 27.9 Hz, 1H), 1.4 (t, *J* = 7.1 Hz, 3H), 1.39 (t, *J* = 7.1 Hz, 3H), 1.25 (s, 9H), 0.96 (s, 9H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ = 157.32 (q, *J* = 36.8 Hz), 139.73, 128.14, 127.68, 126.78, 116.22 (q, *J* = 863.3 Hz), 86.92, 68.18 (d, *J* = 139.8 Hz), 62.35, 61.65 (d, *J* = 7.1 Hz), 60.28 (d, *J* = 7.6 Hz), 44.30, 35.60 (d, *J* = 5.0 Hz), 30.81 (d, *J* = 5.9 Hz), 28.06, 16.44 (d, *J* = 5.8 Hz), 16.12 (d, *J* = 7.0 Hz) ppm; ^{19}F NMR (377 MHz, $CDCl_3$): δ = -75.54 ppm; ^{31}P NMR (162 MHz, $CDCl_3$): δ = 26.17 ppm. HRMS (ESI) *m/z* calcd for $C_{23}H_{38}F_3N_2O_5P$ 511.2543 ([M+H]), found 511.2545 ([M+H]). (*RR/SS*)-**ALK17-1**: M.p. = 90-93 °C; ^1H NMR (400 MHz, $CDCl_3$): δ = 7.25 (m, 5H), 5.27 (t, *J* = 6.6 Hz, 1H), 4.25 (m, 1H), 3.96 (m, 1H), 3.88 (m, 1H), 3.64 (m, 1H), 3.44 (d, *J* = 26.3 Hz, 1H), 3.34 (m, 2H), 1.62 (bs, 1H), 1.26 (t, *J* = 7.1 Hz, 3 H), 1.24 (s, 9H), 1. 23 (s, 9H), 0.94 (t, *J* = 7.1 Hz, 3 H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ = 156.74 (q, *J* = 36.8 Hz), 138.38, 128.44, 128.24, 128.00, 115.71 (q, *J* = 287.9 Hz), 80.10, 69.98 (d, *J* = 142.1 Hz), 62.13, 61.52 (d, *J* = 6.7 Hz), 59.11 (d, *J* = 7.4 Hz), 43.89, 35.12 (d, *J* = 4.6 Hz), 30.69 (d, *J* = 6.1 Hz), 27.92, 16.13 (d, *J* = 5.8 Hz), 15.97 (d, *J* = 6.9 Hz) ppm; ^{19}F NMR (377 MHz, $CDCl_3$): δ = -75.72 ppm; ^{31}P NMR (400 MHz, $CDCl_3$): δ = 23.96 ppm; HRMS (ESI) *m/z* calcd for $C_{23}H_{38}F_3N_2O_5P$ 511.2543 ([M+H]), found 511.2543 ([M+H]).

ALK4 – (*RS/SR*)-diethyl 1-((2-amino-1-phenylethoxy)(tert-butyl)amino)-2,2-dimethylpropylphosphonate

Alkoxyamine (*RS/SR*)-**ALK4-1** (6.8 g, 13.4 mmol) was dissolved in methanol (100 mL) and then $LiOH•H_2O$ (4.5 g, 107 mmol, 8.0 equiv.) was added. The solution was allowed to stir

for under argon atmosphere. After 24 h the reaction was quenched with aq. sat. $NaHCO_3$ solution, extracted with CH_2Cl_2 and dried over $MgSO_4$. The solvents were evaporated *in vacuo*. The crude product was purified by column chromatography to give (*RS/SR*)-**ALK4** (5.5 g, 99%) as an oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.26 (m, 5H), 4.83 (dd, *J* = 7.6, 4.1 Hz, 1H), 4.36 (m, 1H), 4.13 (m, 2H), 4.0 (m, 1H), 3.44 (dd, *J* = 13.1, 4.2 Hz, 1H), 3.36 (d, *J* = 26.6 Hz, 1H), 3.10 (dd, *J* = 13.0, 7.8 Hz, 1H), 1.52 (bs, 2H), 1.36 (t, *J* = 7.1 Hz, 3H), 1.33 (t, *J* = 7.1 Hz, 3H), 1.24 (s, 9H), 0.89 (s, 9H) ppm; ^{13}C NMR (75 Hz, $CDCl_3$): δ = 141.73, 128.04, 127.77, 127.30, 90.64, 69.24 (d, *J* = 139.0 Hz), 61.45 (d, *J* = 6.3 Hz), 59.09 (d, *J* = 7.4Hz), 46.87, 35.68, 35.60, 30.16 (d, *J* = 5.7 Hz), 28.48, 16.65 (d, *J* = 5.7 Hz), 16.19 (d, J = 6.7 Hz) ppm; ^{31}P NMR (162 MHz, $CDCl_3$): δ = 25.85 ppm ; HRMS (ESI) *m/z* calcd for $C_{21}H_{39}N_2O_4P$ 415.2720 ([M+H]), found 415.2714 ([M+H]).

ALK17 – (*RR/SS*)-diethyl 1-((2-amino-1-phenylethoxy)(tert-butyl)amino)-2,2-dimethylpropylphosphonate

Alkoxyamine (*RR/SS*)-**ALK17-1** (4.6 g, 8.9 mmol) was dissolved in methanol (100 mL) and then LiOH•H_2O (3.0 g, 72 mmol, 8.0 equiv.) was added. The solution was allowed to stir for under argon atmosphere. After 24 h the reaction was quenched with aq. sat. $NaHCO_3$ solution, extracted with CH_2Cl_2 and dried over $MgSO_4$. The solvents were evaporated *in vacuo*. The crude product was purified by column chromatography to give (*RS/SR*)-**ALK17** (3.7 g, 99%) as an oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.26 (m, 5H), 4.83 (dd, *J* = 7.6, 4.1 Hz, 1H), 4.36 (m, 1H), 4.13 (m, 2H), 4.0 (m, 1H), 3.44 (dd, *J* = 13.1, 4.2 Hz, 1H), 3.36 (d, *J* = 26.6 Hz, 1H), 3.10 (dd, *J* = 13.0, 7.8 Hz, 1H), 1.52 (bs, 2H), 1.36 (t, *J* = 7.1 Hz, 3H), 1.33 (t, *J* = 7.1 Hz, 3H), 1.24 (s, 9H), 0.89 (s, 9H) ppm; ^{13}C NMR (75 Hz, $CDCl_3$): δ = 139.81, 128.81, 127.87, 127.79, 84.50, 69.82 (d, *J* = 139.4 Hz), 61.33 (d, *J* = 7.0 Hz), 54.40 (d, *J* = 7.45 Hz), 44.52, 35.16, 35.09, 30.56 (d, *J* = 5.7 Hz), 27.87, 16.15 (d, *J* = 5.7 Hz) 15.99 (d, *J* = 6.9 Hz) ppm; ^{31}P NMR (162 MHz, $CDCl_3$): δ = 24.40 ppm; HRMS (ESI) *m/z* calcd for $C_{21}H_{39}N_2O_4P$ 415.2720 ([M+H]), found 415.2715 ([M+H]).

ALK5-1 and ALK18-1 – Diethyl 1-(*tert*-butyl(1-phenyl-2-(2,2,2-trifluoro-*N*-methylacetamido)ethoxy)amino)-2,2-dimethylpropylphosphonate

To a suspension of CuBr (790 mg, 5.51 mmol, 0,55 equiv.) and Cu (701 mg, 11.0 mmol, 1.1 equiv.) in degassed benzene (12 mL) under argon was added *N,N,N′,N′,N″*-pentamethyldiethylenetriamine (1.2 mL, 5.75 mmol, 0.55 equiv.). After stirring for 10 min a solution of SG1 nitroxide (2.95 g, 10.0 mmol) and *N*-(2-bromo-2-phenylethyl)-2,2,2-trifluoro-*N*-methylacetamide (3.11 g, 10.0 mmol, 1 equiv.) in degassed benzene (12 mL) was cannulated into the first solution. The mixture was allowed to stir for 12 h. The solution was quenched with aq. sat. NH_4Cl solution, washed with water and brine and dried over $MgSO_4$. The solvents were evaporated under reduced pressure. The crude product with a 3:2 (*RR/SS*):(*RS/RS*) diastereomeric ratio (^{1}H NMR ratio) was purified by automatic flash-chromatography with a gradient of ethyl acetate in petroleum ether in order to separate the diastereomers to give (*RS*/*SR*)-**ALK5-1** (460 mg) and (*RR*/*SS*)-**ALK18-1** (712 mg) corresponding to a total yield of 1.17 g (22 %). (*RS/SR*)-**ALK5-1**: M.p. = 116-120 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.27 (m ,5H), 5.19 (dd, *J* = 11.07, 5.04 Hz, 1H), 4.59 (dd, *J* = 13.0, 5.3 Hz, 1H), 4.14 (m, 2H),

3.99 (m, 1H), 3.53 (t, *J* = 12.6 Hz, 1H), 3.35 (d, *J* = 26.6 Hz, 1H), 2.55 (s, 3H), 1.36 (t, *J* = 7.4 Hz, 3H), 1.32 (t, *J* = 6.9 Hz, 3H), 1.27 (s, 9H), 0.90 (s, 9H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ = 154.83 (q, *J* = 34.2 Hz), 139.92, 128.04, 127.66, 114.88 (q, *J* = 290.5 Hz), 84.66, 69.06 (d, *J* = 139.7 Hz), 61.65, 61.37 (d, *J* = 6.7 Hz), 59.18 (d, *J* = 7.5 Hz), 54.21, 35.84 (q, *J* = 10.8 Hz), 35.56 (d, *J* = 5.2 Hz), 30.18 (d, *J* = 5.9 Hz), 28.32, 16.65 (d, *J* = 5.6 Hz), 16.19 (d, *J* = 6.8 Hz) ppm.; ^{31}P NMR (162 MHz, $CDCl_3$): δ = 25.46 ppm; HRMS (ESI) *m/z* calcd for $C_{24}H_4OF_3N_2O_5P$ 525.2700 ([M+H]), found 525.2698 ([M+H]). (*RR/SS*)-**ALK18-1**: M.p. = 74-77 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.38 (m, 2H), 7.26 (m, 3H), 5.28 (dd, *J* = 11.2, 4.4 Hz, 1H), 4.50 (dd, *J* = 12.4, 4.4 Hz, 1H), 3.91 (m, 2H), 3.68 (dd, *J* = 12.4, 11.4 Hz, 1H), 3.42 (d, *J* = 27.0 Hz,1H), 3.35 (m, 2H), 2.66 (m, 3H), 1.22 (m, 21H), 0.93 (t, *J* = 7.1 Hz, 3H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ = 156.97 (q, *J* = 35.6 Hz), 138.95, 128.55, 128.44, 128.08, 116.42 (q, *J* = 289.7 Hz), 79.00, 69.96 (d, *J* = 40.3 Hz), 62.01, 61.64 (d, *J* = 6.5 Hz), 59.07 (d, *J* = 7.6 Hz), 53.08, 35.90 (q, *J* = 3.9 Hz), 35.40 (d, *J* = 4.3 Hz), 30.85 (d, *J* = 6.0 Hz), 28.25, 16.44 (d, *J* = 5.5 Hz), 16.28 (d, *J* = 6.8 Hz) ppm; ^{31}P NMR (162 MHz, $CDCl_3$): δ = 24.30 ppm; HRMS (ESI) *m/z* calcd for $C_{24}H_4OF_3N_2O_5P$ 525.2700 ([M+H]), found 525.2700 ([M+H]).

ALK5 and ALK18 – Diethyl 1-(*tert*-butyl(2-(methylamino)-1-phenylethoxy)amino)-2,2-dimethylpropylphosphonate 2

Diastereomerically pure alkoxyamine (*RS*/*SR*)-**ALK5-1** (355 mg, 0.677 mmol) was dissolved in MeOH (10 mL) and $LiOH{\cdot}H_2O$ (426 mg, 10.2 mmol, 15.1 equiv.) was added. The mixture was allowed to stir for 12 h under argon atmosphere. Then the reaction was quenched with water. The aqueous phase was extracted with CH_2Cl_2, the combined organic layers were dried over $MgSO_4$ and concentrated *in vacuo*. Compound **ALK5** (284 mg, 97 %) was obtained as colorless oil, which crystallized in the refrigerator. The reaction was likewise performed for (*RR*/*SS*)-**ALK18-1** (395 mg, 0.753 mmol) and $LiOH{\cdot}H_2O$ (284 mg, 6.77 mmol, 8.99 equiv.), yielding 297 mg (92 %) of (*RR*/*SS*)-**ALK18** as a colourless oil which also crystallized in the refrigerator. (*RS/SR*)-**ALK5**: M.p. = 79-80 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.32 (m, 5H), 5.00 (dd, *J* = 7.0, 5.1 Hz, 1 H), 4.36 (m, 1H), 4.13 (m, 2H), 3.98 (m, 1H), 3.55 (dd, *J* = 12.1, 5.1 Hz, 1 H), 3.36 (d, *J* = 26.5 Hz, 1H), 2.85 (dd, *J* = 11.8, 8.7 Hz, 1 H), 2.35 (s, 3H), 1.36 (t, *J* = 7.1 Hz, 3H), 1.32 (t, *J* = 7.1 Hz, 3H), 1.25 (s, 9H), 0.84 (s, 9H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ = 142.09, 127.90, 127.70, 127.31, 88.00, 69.42 (d, *J* = 138.1 Hz), 61.28 (d, *J* = 6.4 Hz), 61.11, 58.63 (d, *J* = 7.5 Hz), 56.16, 36.18, 35.47 (d, *J* = 5.9 Hz), 29.85 (d, *J* = 5.7 Hz), 28.40, 16.50 (d, *J* = 5.5 Hz), 16.00 (d, *J* = 6.8 Hz) ppm; ^{31}P NMR (126 MHz, $CDCl_3$): δ = 26.17 ppm; HRMS (ESI) *m/z* calcd for $C_{22}H_{42}N_2O_4P$ 429.2877 ([M+H]), found 429.2879 ([M+H]). (*RR/SS*)-**ALK18**: M.p. = 75-76 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.48 (m, 2H), 7.27 (m, 3H), 5.20 (dd, *J* = 10.2, 4.0 Hz, 1H), 3.96 (m, 1H), 3.84 (m, 1H), 3.54 (dd, *J* = 11.1, 3.9 Hz, 1H), 3.39 (d, *J* = 26.3 Hz, 1H), 3.32 (m, 1H), 3.21 (m, 1H), 2.88 (t, *J* = 10.8 Hz, 1H), 2.38 (s, 3H), 1.23 (t, *J* = 7.1 Hz, 3H), 1.22 (s, 9H), 1.21 (s, 9H), 0.88 (t, *J* = 7.1 Hz, 3H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ = 140.12, 128.42, 127.73, 127.62, 81.45, 69.73 (d, *J* = 139.6 Hz), 61.16, 61.15 (d, *J* = 6.2 Hz), 58.23 (d, *J* = 7.4 Hz), 54.51, 36.56, 35.05 (d, *J* = 5.0 Hz), 27.79, 16.04 (d, *J* = 5.7 Hz), 15.87 (d, *J* = 6.9 Hz) ppm.; ^{31}P NMR (162 MHz,

$CDCl_3$): δ = 24.49 ppm; HRMS (ESI) *m/z* calcd for $C_{22}H_{42}N_2O_4P$ 429.2877 ([M+H]), found 429.2878 ([M+H]).

ALK6 – (*RS/SR*)-diethyl (1-(*tert*-butyl(2-(dimethylamino)-1-phenylethoxy)amino)-2,2-dimethylpropyl)phosphonate

Alkoxyamine (*RS/SR*)-**ALK4** (415 mg, 1.0 mmol) was dissolved in CH_2Cl_2 (10 mL) and then K_2CO_3 (415 mg. 3.0 mmol) was added. To this solution, MeI (125 µL, 2.0 mmol) was added under argon atmosphere at 0°C. After 1 h, the mixture was filtrated and the solvent was evaporated *in vacuo*. The crude product was purified by flash-chromatography with a gradient of MeOH in CH_2Cl_2 to give (*RS/SR*)-**ALK6** (22 mg, 5%) as pale yellow solid. M.p. = 80-82 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.22-7.36 (m, 5H), 4.91 (dd, *J* = 11.3, 3.6 Hz, 1H), 4.27-4.41 (m, 1H), 4.04-4.19 (m, 2H), 3.98 (dquin, *J* = 10.1, 7.1, 7.1, 7.1, 7.1 Hz, 1H), 3.46 (dd, *J* = 12.2, 3.8 Hz, 1H), 3.34 (d, *J* = 27.1 Hz, 1H), 2.65 (t, *J* = 11.9 Hz, 1H), 2.10 (s, 6H), 1.35 (t, *J* = 7.0 Hz, 3H), 1.32 (t, *J* = 7.5 Hz, 3H), 1.27 (s, 9H), 0.84 (s, 9H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ = 142.86, 128.15, 127.75, 127.27, 87.70, 69.62 (d, *J* = 139.2 Hz), 63.40, 61.37 (d, *J* = 6.1 Hz), 61.23, 58.69 (d, *J* = 7.7 Hz), 46.11, 35.52 (d, *J* = 5.5 Hz), 30.06 (d, *J* = 6.1 Hz), 28.45, 16.56 (d, *J* = 5.5 Hz), 16.11 (d, *J* = 6.6 Hz) ppm; ^{31}P NMR (162 MHz, $CDCl_3$) δ 25.51 ppm; HRMS (ESI) *m/z* calcd for $C_{23}H_{44}N_2O_4P$ 443.3033 ([M+H]), found 443.3037 ([M+H]).

ALK19 – (*RR/SS*)-diethyl (1-(*tert*-butyl(2-(dimethylamino)-1-phenylethoxy)amino)-2,2-dimethylpropyl)phosphonate

(*RR/SS*)-**ALK17** (344 mg,0.83 mmol) was dissolved in CH_2Cl_2 (10 mL) and then 1,8-bis(dimethylamino)naphthalene (533 mg, 2.49 mmol) was added. To this solution, trimethyloxonium tetrafluoroborate (614 mg, 4.15 mmol) was added at 0°C. After stirring for 1 h under argon atmosphere, the mixture was quenched with 1 M HCl. The aqueous phase was separated and basified with sat. $NaHCO_3$ and extracted with CH_2Cl_2. The combined organic phase were dried with $MgSO_4$ and the solvent was evaporated *in vacuo*. The crude product was purified by flash-chromatography with a gradient of MeOH in CH_2Cl_2 to give (*RR/SS*)-**ALK19** (15 mg, 4%) as pale yellow solid. M.p. = 85-86 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.49 (m, 2H), 7.25 (m, 3H), 5.23 (dd, *J* = 11.2, 3.0 Hz, 1H), 3.98 (m, 1H), 3.84 (m, 1H), 3.44 (d, *J* = 11.9 Hz, 1H), 3.38 (d, *J* = 26.3 Hz, 1H), 3.26 (m, 1H), 3.10 (m, 1H), 2.72 (t, *J* = 11.5 Hz, 1H), 2.13 (s, 6H), 1.24 (m, 6H), 1.22 (s, 9H), 1.19 (s, 9H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ = 141.29, 129.04, 127.86, 127.76, 81.21, 70.03 (d, *J* = 139.6 Hz), 61.98, 61.51, 61.46 (d, *J* = 7.0 Hz), 58.53 (d, *J* = 7.3 Hz), 46.88, 35.39 (d, *J* = 5.0 Hz), 30.82 (d, *J* = 6.0 Hz), 28.12, 16.35 (d, *J* = 5.8 Hz), 16.19 (d, *J* = 6.9 Hz) ppm; ^{31}P NMR (121 MHz, $CDCl_3$) δ 24.50 ppm; HRMS (ESI) *m/z* calcd for $C_{23}H_{44}N_2O_4P$ 443.3033 ([M+H]), found 443.3035 ([M+H]).

ALK4-MMPp (Ac-PLG-ALK4)

To a stirred solution of **ALK4** (0.60 g, 1.45 mmol) and Fmoc-Pro-Leu-Gly-OH (0.74 g, 1.45 mmol) in CH_2Cl_2 (10 mL) was added HOBt (0.24 g, 1.74 mmol) and DIC (272 µL, 1.74

mmol). The reaction was stirred overnight at rt under Ar, then quenched with water and extracted with CH_2Cl_2. The combined organic phase was washed with brine and dried with $MgSO_4$, and solvents were removed by evaporation. The resulting crude was purified by flash column chromatography (CH_2Cl_2: MeOH = 97: 3) to afford **Fmoc-PLG-ALK4** as white foam (1.22 g, 92%). To a stirred solution of **Fmoc-PLG-ALK4** (1.16 g, 1.28 mmol) in CH_2Cl_2 (15 mL) cooled at 0 ºC was added DBU (230 µL, 1.54 mmol). After stirring overnight under Ar, the reaction mixture was quenched with water, basified with 1 M NaOH and extracted with CH_2Cl_2. The combined organic phase was dried with $MgSO_4$, and solvent was removed by evaporation. The resulting crude material was purified by flash column chromatography (CH_2Cl_2/MeOH = 19: 1) to afford **PLG-ALK4** as a white foam (0.61 g, 70%). To a stirred solution of **PLG-ALK4** (0.53 g, 0.78 mmol) in pyridine (6 mL), acetic anhydride (111 µL, 1.17 mmol) was added. The solution was stirred at rt for 4 h under Ar, the reaction mixture was diluted with CH_2Cl_2 and quenched with water. The mixture was extracted with CH_2Cl_2 and washed with 1 M HCl. The organic phase was dried with $MgSO_4$ and the solvent was removed by evaporation. The crude material was purified with flash column chromatography (CH_2Cl_2/MeOH = 93: 7) to afford **ALK4-MMPp (Ac-PLG-ALK4)** as white foam (0.52 g, 92%). ^{1}H NMR ($CDCl_3$, 400 MHz) and ^{13}C NMR ($CDCl_3$, 75 MHz): complex spectra, as **ALK4-MMPp** exists as a mixture of diastereoisomers and rotamers *(see copies of spectra)* ; ^{31}P NMR ($CDCl_3$, 162 MHz): δ 26.96, 26,89 ppm; HRMS (ESI-TOF) m/z: $[M+H]^+$ Calcd for $C_{26}H_{63}N_5O_8P_1$ 724.4409; Found 724.4413

ALK4-CHYMOp (Suc-AAPF-ALK4)

To a stirred solution of **ALK4** (0.50 g, 1.21 mmol) and Fmoc-Ala-Ala-Pro-Phe-OH (0.91 g, 1.45 mmol) in CH_2Cl_2 (10 mL) was added HOBt (0.20 g, 1.45 mmol) and *N*,*N*-diisopropylcarbodiimide (DIC) (227 µL, 1.45 mmol). The reaction was stirred for 3 h at rt under Ar, then quenched with water and extracted with CH_2Cl_2. The combined organic phase was washed with brine and dried with $MgSO_4$, and solvents were removed by evaporation. The resulting crude was purified by flash column chromatography (CH_2Cl_2: MeOH = 49: 1) to afford **Fmoc-AAPF-ALK4** as white solid (1.19 g, 90%). To a stirred solution of **Fmoc-AAPF-ALK4** (1.05 g, 1.02 mmol) in CH_2Cl_2 (15 mL) cooled at 0 ºC was added DBU (183 µL, 1.23 mmol). After stirring overnight under Ar, the reaction mixture was quenched with water and extracted with CH_2Cl_2. The combined organic phase was washed with water and dried with $MgSO_4$, and solvent was removed by evaporation. The resulting crude material was purified by flash column chromatography (CH_2Cl_2/MeOH = 9: 1) to afford **AAPF-ALK4** as a white powder (0.51 g, 62%). To a stirred solution of **AAPF-ALK4** (0.40 g, 0.50 mmol) and $NaHCO_3$ (0.17 g, 2.0 mmol) in CH_2Cl_2 (10 mL), succinic anhydride (75 mg, 0.75 mmol) was added. The solution was stirred at rt for 3 h under Ar, the reaction mixture was quenched with water and acidified with 1 M HCl and extracted with CH_2Cl_2/MeOH (5:1). The combined organic phase was dried with $MgSO_4$ and the solvent was removed by evaporation. The crude material was purified with flash column chromatography (CH_2Cl_2/MeOH = 4: 1) to afford **Suc-AAPF-ALK4** as white foam (0.43 g, 97%). ^{1}H NMR (CD_3OD, 400 MHz) and ^{13}C NMR (CD_3OD, 75 MHz): complex spectra, as **ALK4-CHYMOp** exists as a mixture of diastereoisomers and rotamers *(see copies of spectra)* ;

^{31}P NMR (CD_3OD, 162 MHz): δ 26.86, 26.79 ppm; HRMS (ESI-TOF) m/z: $[M+H]^+$ Calcd for $C_{45}H_{70}N_6O_{11}P_1$ 901.4835; Found 901.4842

ALK4-FITC

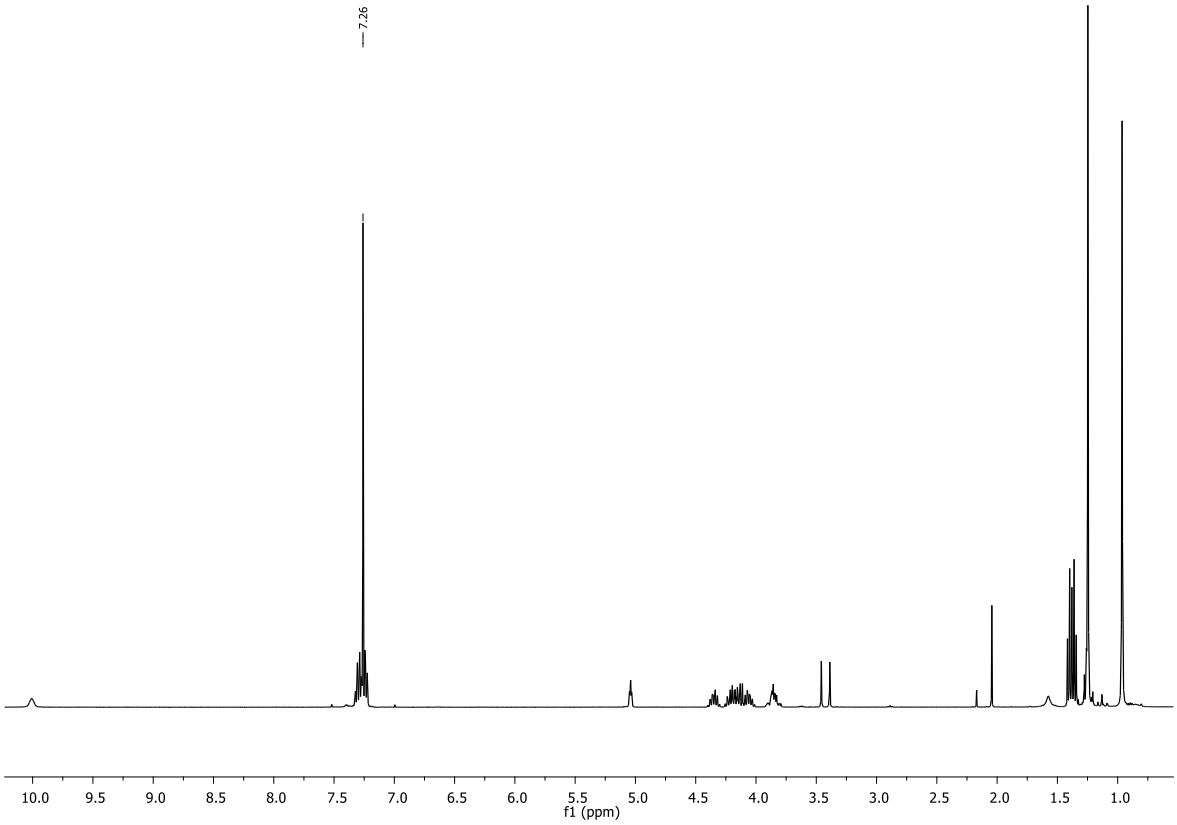
To a stirred solution of **ALK4** (100 mg, 0.24 mmol) in THF (5 mL), FITC (103 mg, 0.26 mmol) was added. The solution was stirred at rt for 12 h under Ar, and the solvent was removed by evaporation. The crude material was purified with flash column chromatography to afford **ALK4-FITC** as red foam (149 mg, 77%). ^{1}H NMR (CD_3OD, 400 MHz): δ 7.91 (br. s, 1H), 7.52-7.26 (m, 7H), 7.04 (d, *J* = 8.3 Hz, 1H), 6.70-6.63 (m, 4H), 6.56-6.53 (m, 2H), 5.35-5.20 (m, 1H), 4.41-3.90 (m, 4H), 3.51 (d, *J* = 27.0 Hz, 1H), 1.42-1.24 (m, 15H), 0.92 (br.s, 9H) ppm; ^{13}C NMR (CD_3OD, 75 MHz): δ 182.6, 171.0, 162.1, 154.3, 148.2, 142.3, 142.0, 139.4, 131.3, 130.3, 129.8, 129.4, 129.1, 128.7, 125.6, 120.1, 114.0, 111.7, 103.6, 87.7, 85.0, 70.5 (d, *J* = 138.1 Hz), 69.5 (d, *J* = 138.7 Hz), 63.6-63.4 (m), 63.0, 62.3 (d, *J* = 7.9 Hz), 61.5 (d, *J* = 7.5 Hz), 50.1, 45.0, 40.4, 36.6 (d, *J* = 5.5 Hz), 36.5, 31.2 (d, *J* = 5.9 Hz), 31.1 (d, *J* = 5.4 Hz), 29.2, 28.8, 25.3, 16.9 (d, *J* = 6.0 Hz), 16.8 (d, J = 6.3 Hz), 16.6 (d, *J* = 6.7 Hz), 16.5 ppm ; ^{31}P NMR (CD_3OD, 162 MHz): δ 27.2, 27.1 ppm; HRMS (ESI-TOF) m/z: $[M+H]^+$ Calcd for $C_{42}H_{51}N_3O_9P_1S_1$ 804.3078; Found 804.3082.

ALK17-FITC

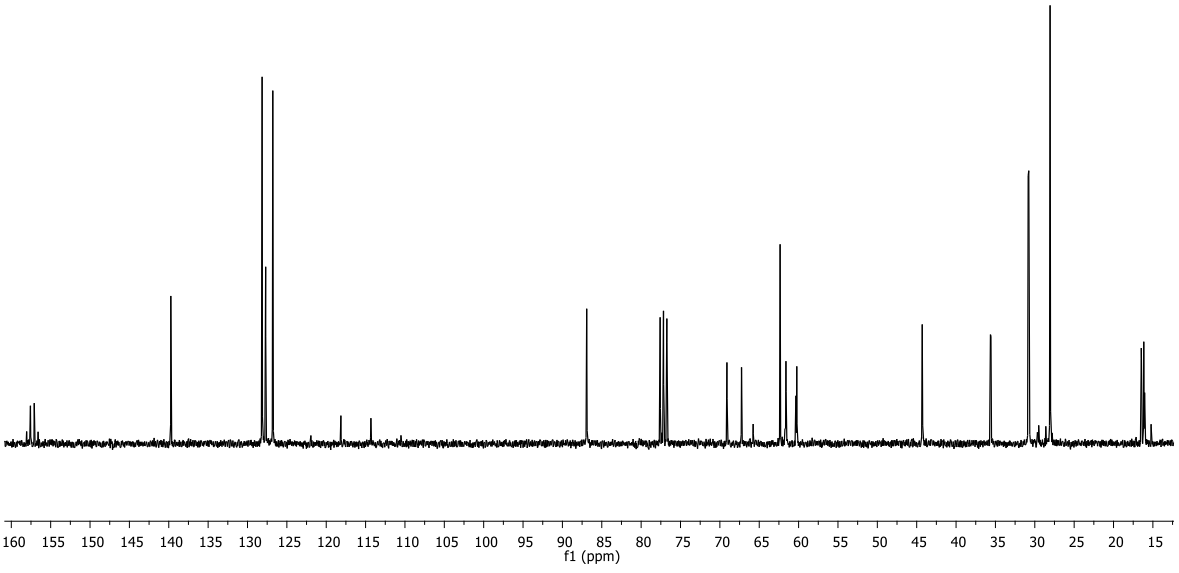
To a stirred solution of **ALK17** (100 mg, 0.24 mmol) in THF (5 mL), FITC (103 mg, 0.26 mmol) was added. The solution was stirred at rt for 12 h under Ar, and the solvent was removed by evaporation. The crude material was purified with flash column chromatography to afford **ALK17-FITC** as red foam (145 mg, 75%). ^{1}H NMR (CD_3OD, 400 MHz): δ 8.04 (br. s, 1H), 7.58-7.53 (m, 3H), 7.39-7.30 (m, 3H), 7.05 (d, *J* = 8.2 Hz, 1H), 6.67 (d, *J* = 2.3 Hz, 2H), 6.63 (d, *J* = 8.8 Hz, 2H), 6.54 (dd, *J* = 8.8 and 2.3 Hz, 2H), 5.30 (dd, *J* = 10.1 and 4.2 Hz, 1H), 3.98-3.86 (m, 4H), 3.54 (d, *J* = 26.6 Hz, 1H), 1.30 (s, 9H), 1.26 (s, 9H), 1.25 (t, *J* = 7.1 Hz, 3H), 0.93 (t, *J* = 7.1 Hz, 3H) ppm ; ^{13}C NMR (CD_3OD, 75 MHz): δ 183.0, 171.0, 161.3, 154.1, 149.7, 142.3, 140.5, 131.6, 130.2, 130.1, 129.7, 129.5, 128.8, 125.5, 119.5, 113.5, 11.4, 103.5, 82.2, 71.3 (d, *J* = 139.6 Hz), 63.3 (d, *J* = 6.7 Hz), 63.1, 60.8 (d, J = 7.5 Hz), 36.4 (d, *J* = 4.8 Hz), 31.4 (d, *J* = 5.9 Hz), 28.7, 16.6 (d, *J* = 5.3 Hz), 16.5 (d, *J* = 6.5 Hz) ppm; ^{31}P NMR (CD_3OD, 162 MHz): δ 23.86 ppm; HRMS (ESI-TOF) m/z: $[M+H]^+$ $C_{42}H_{51}N_3O_9P_1S_1$ 804.3078; Found 804.3085.

1.3. ^{1}H and ^{13}C NMR spectra

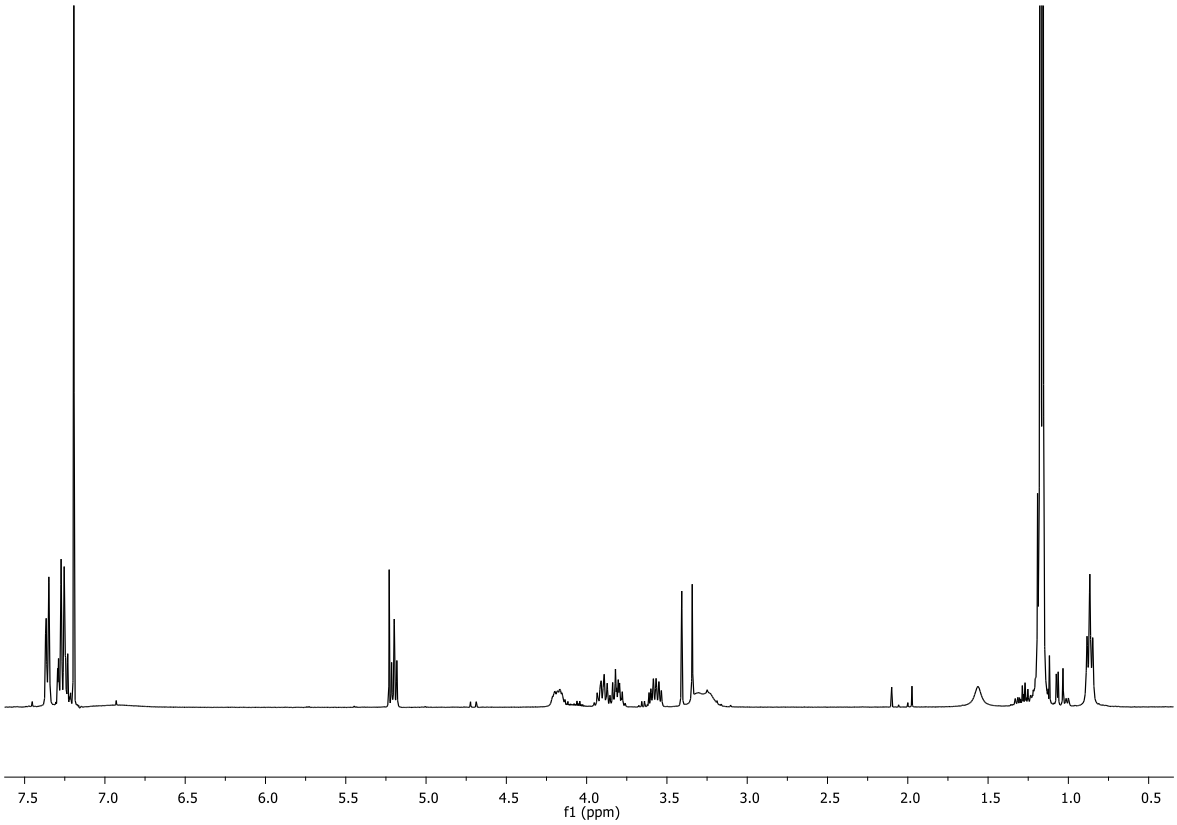
ALK4-1 (400 MHz, $CDCl_3$):



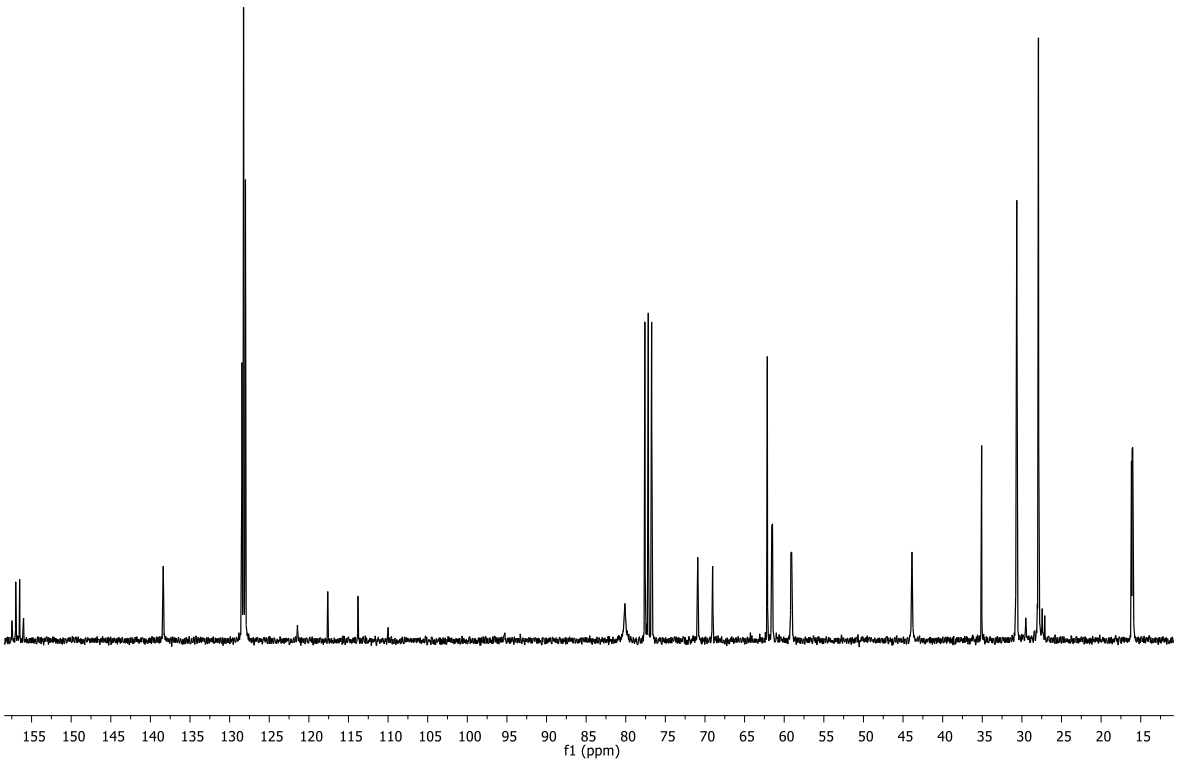
ALK4-1 (75 MHz, $CDCl_3$):



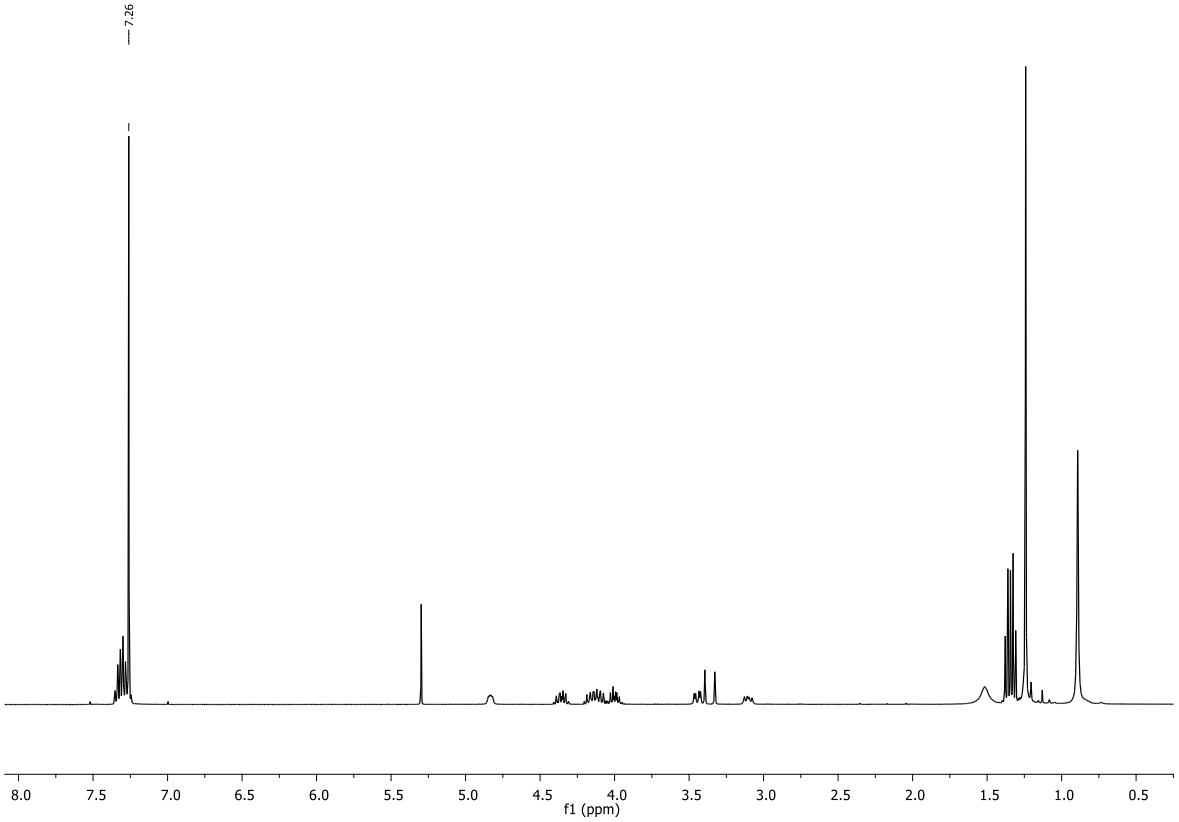
ALK17-1 (400 MHz, $CDCl_3$):



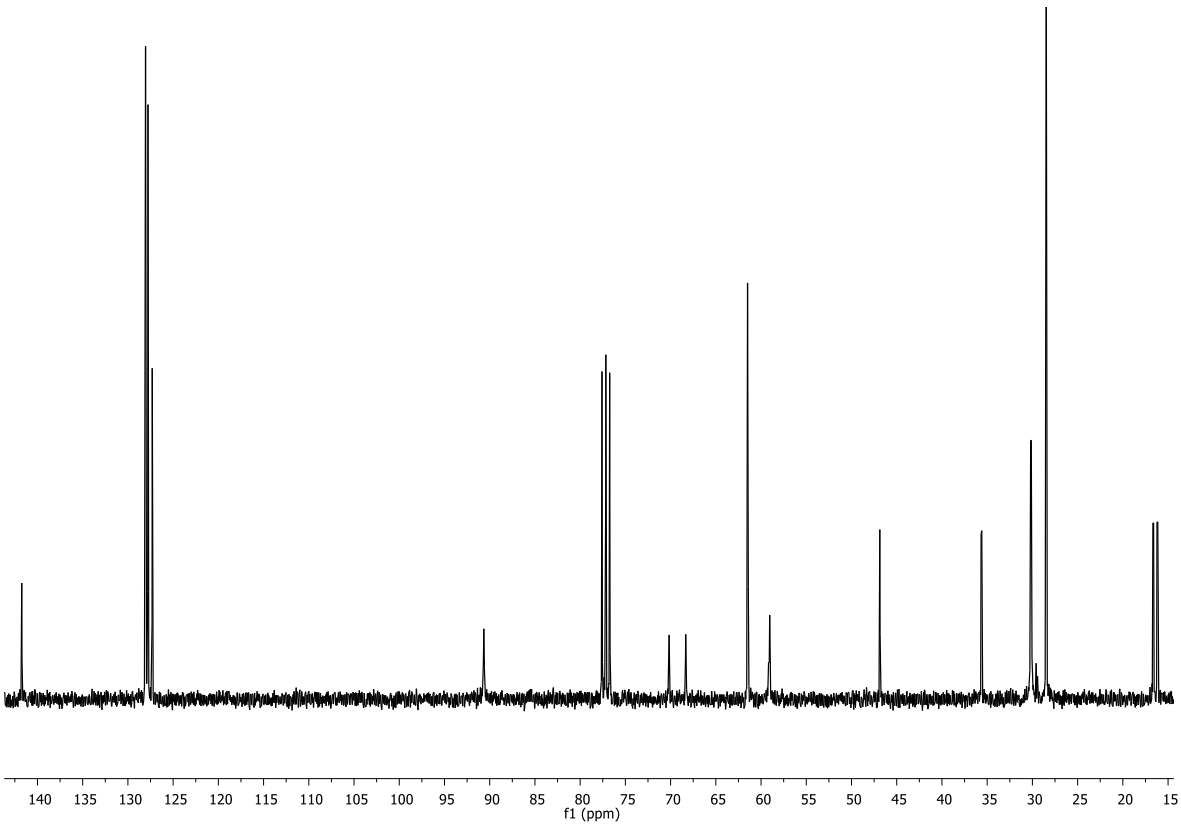
ALK17-1 (75 MHz, $CDCl_3$):



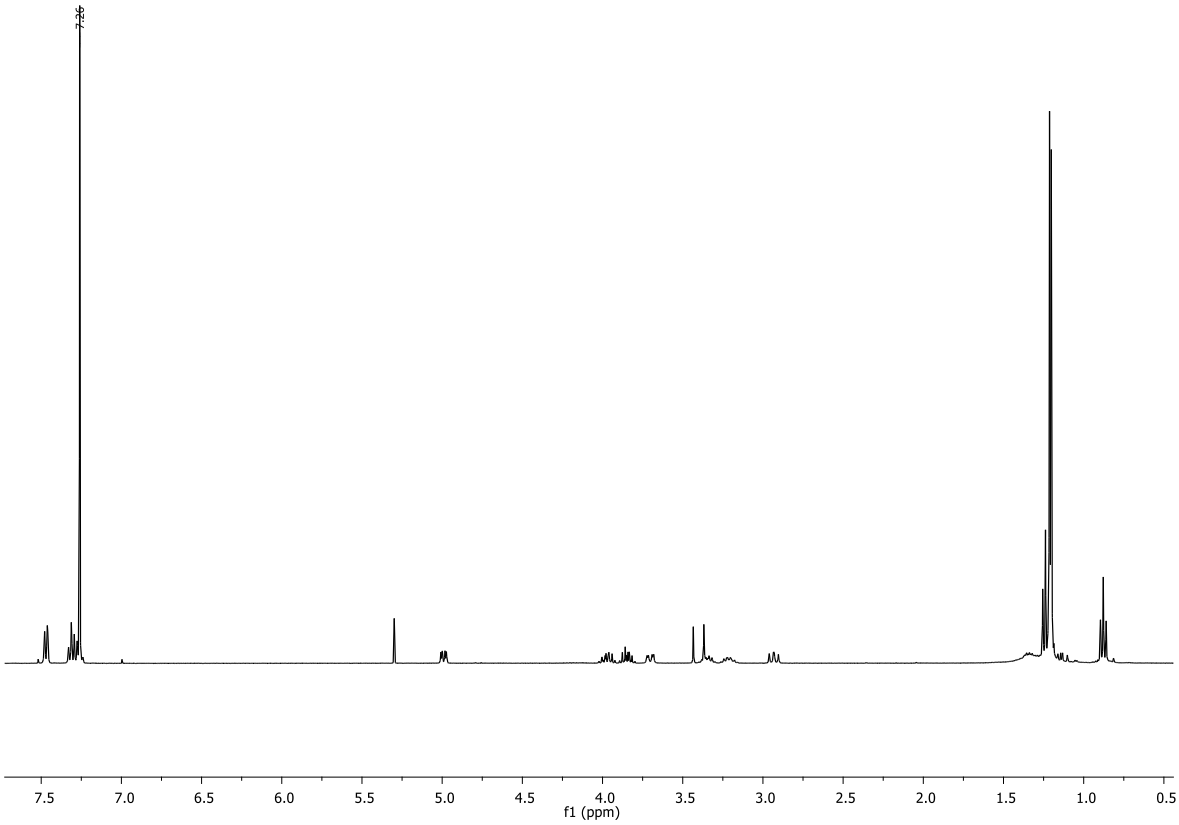
ALK4 (400 MHz, $CDCl_3$):



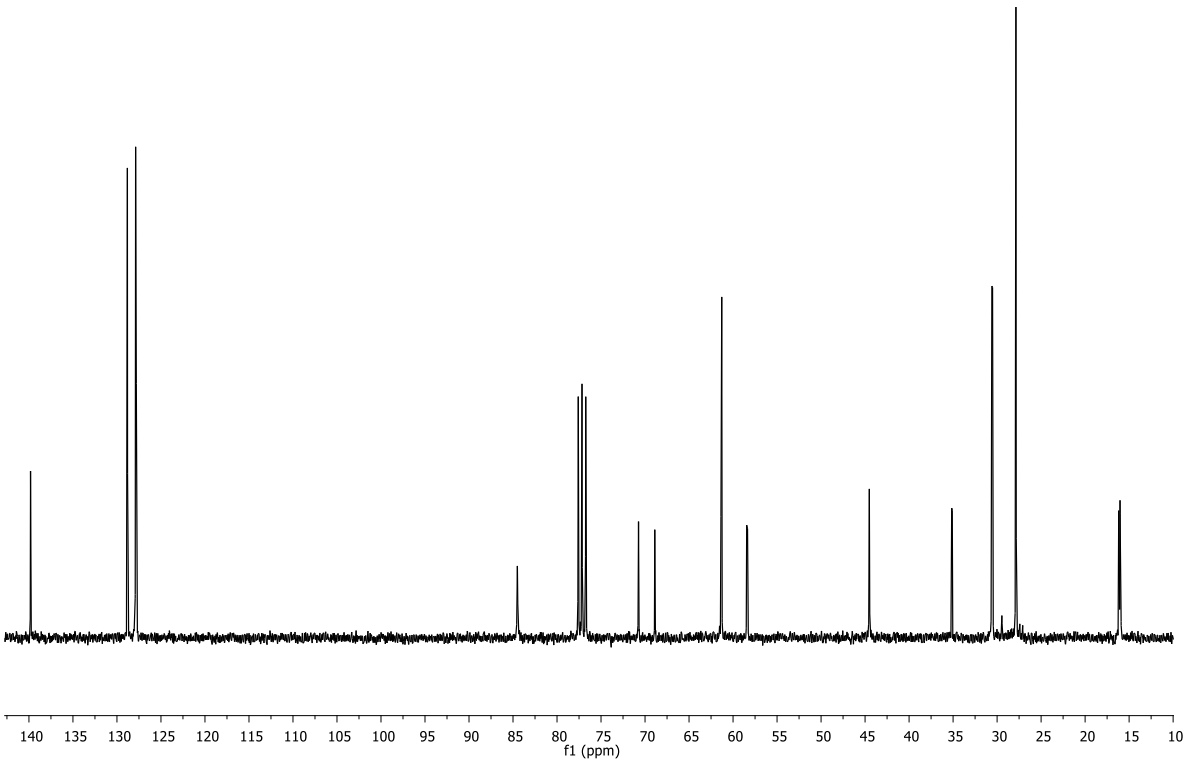
ALK4 (75 MHz, $CDCl_3$):



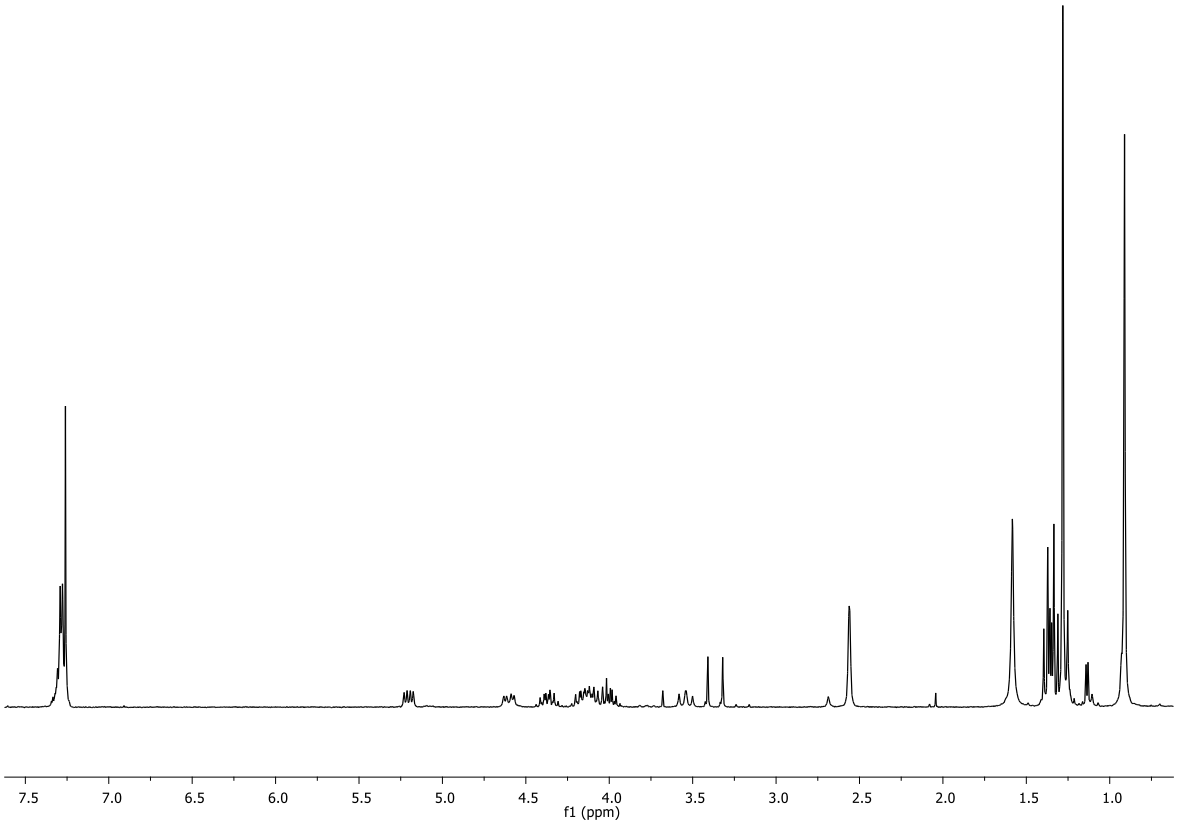
ALK17 (400 MHz, $CDCl_3$):



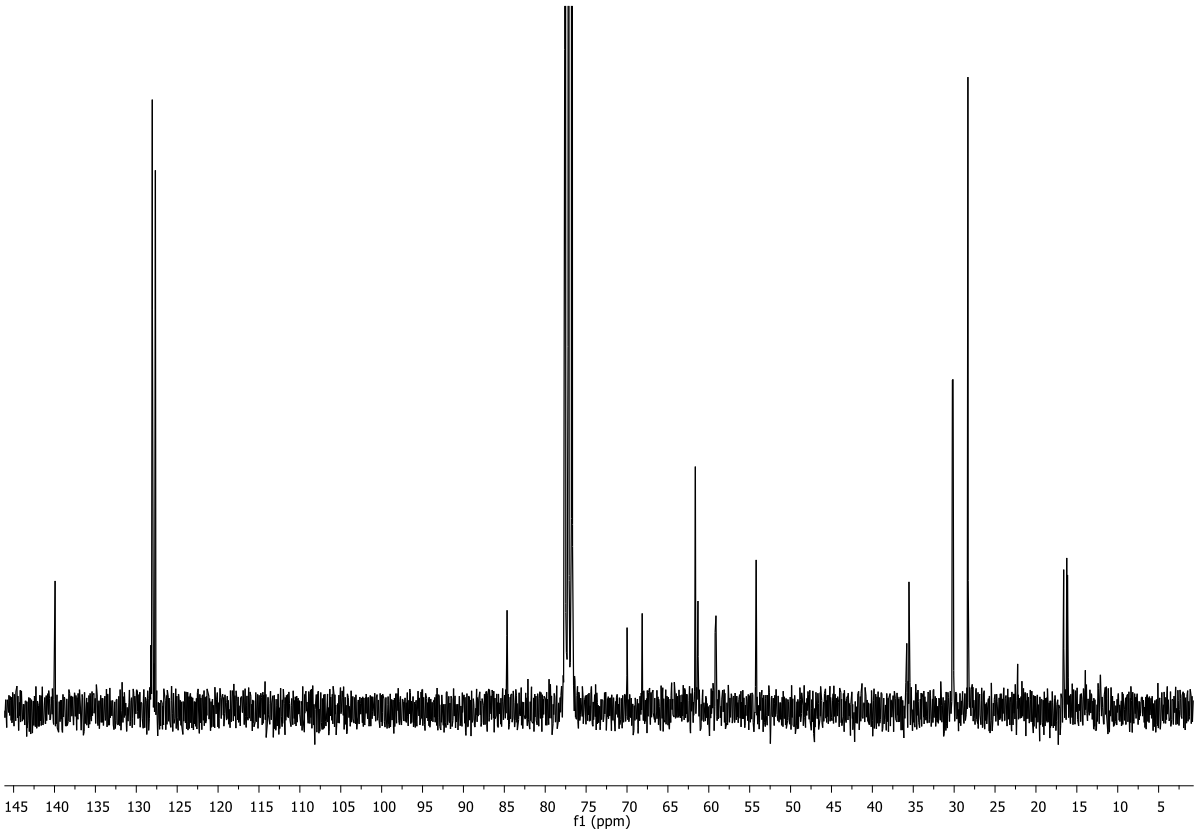
ALK17 (75 MHz, $CDCl_3$):



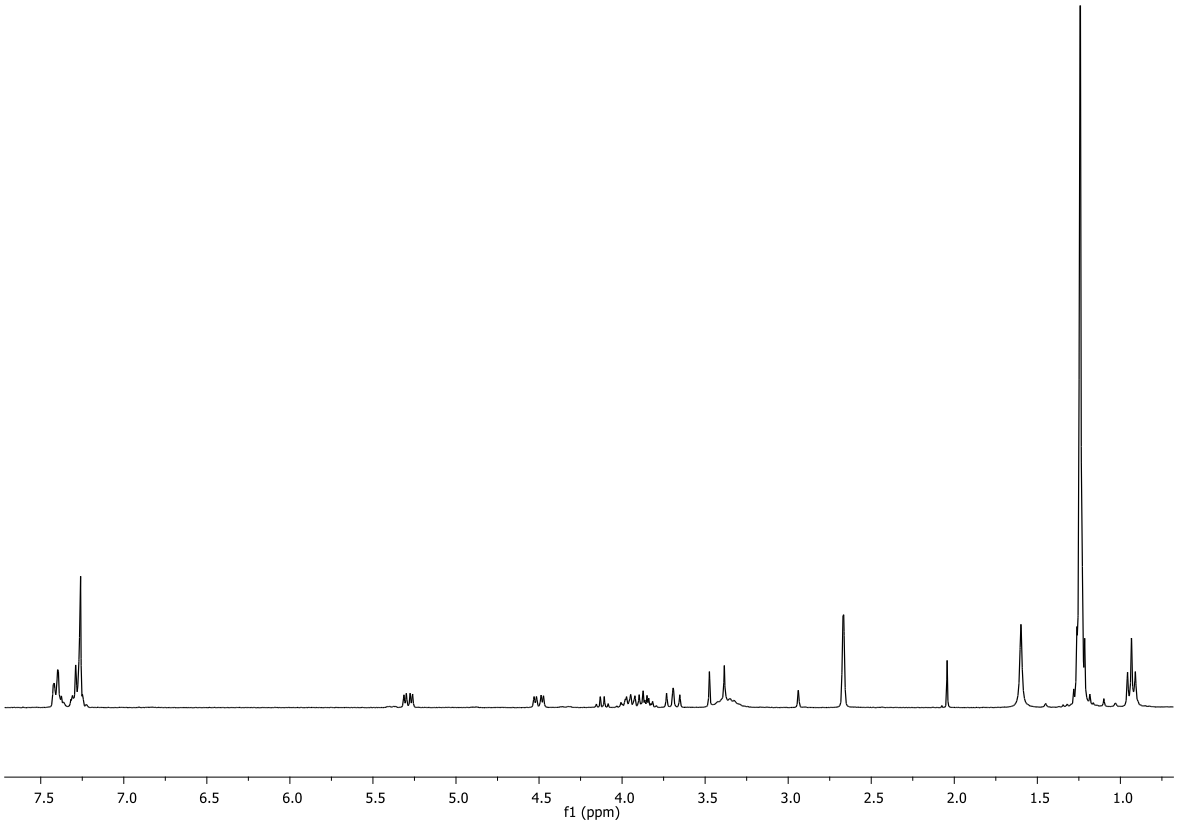
ALK5-1 (300 MHz, $CDCl_3$):



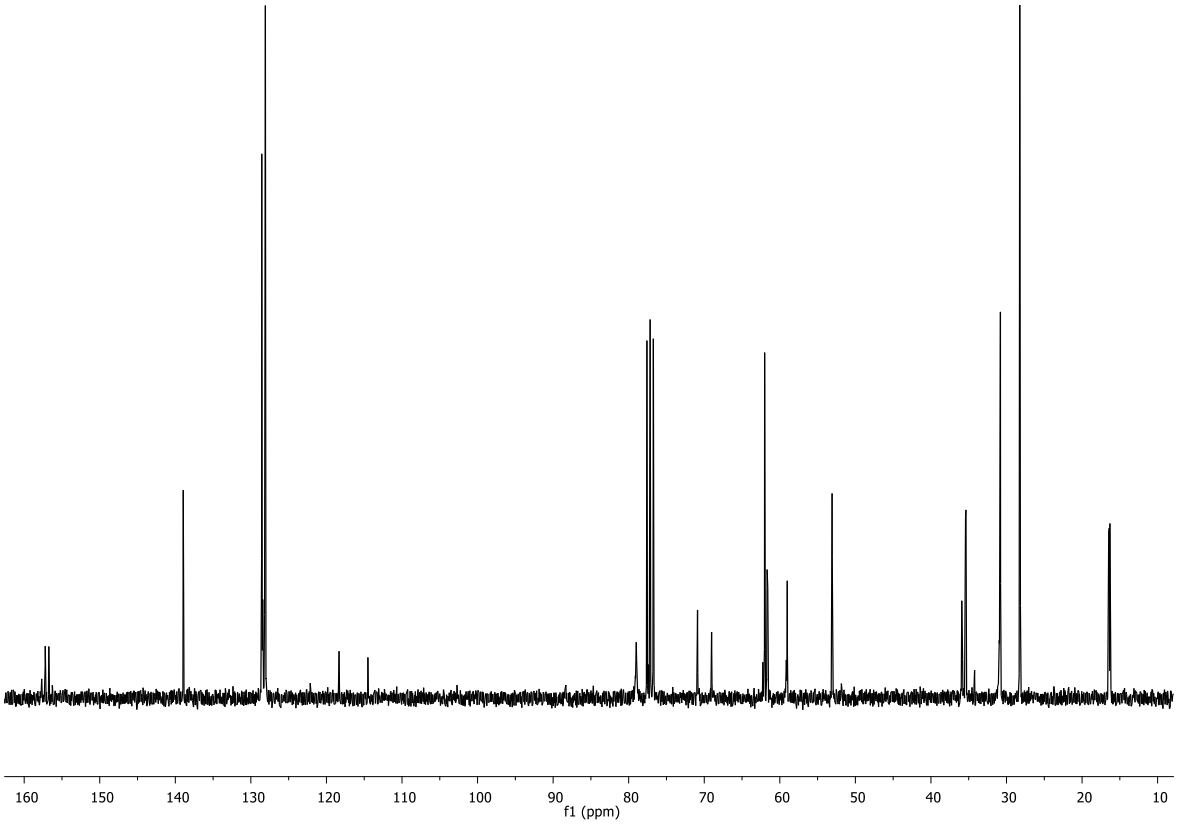
ALK5-1 (75 MHz, $CDCl_3$):



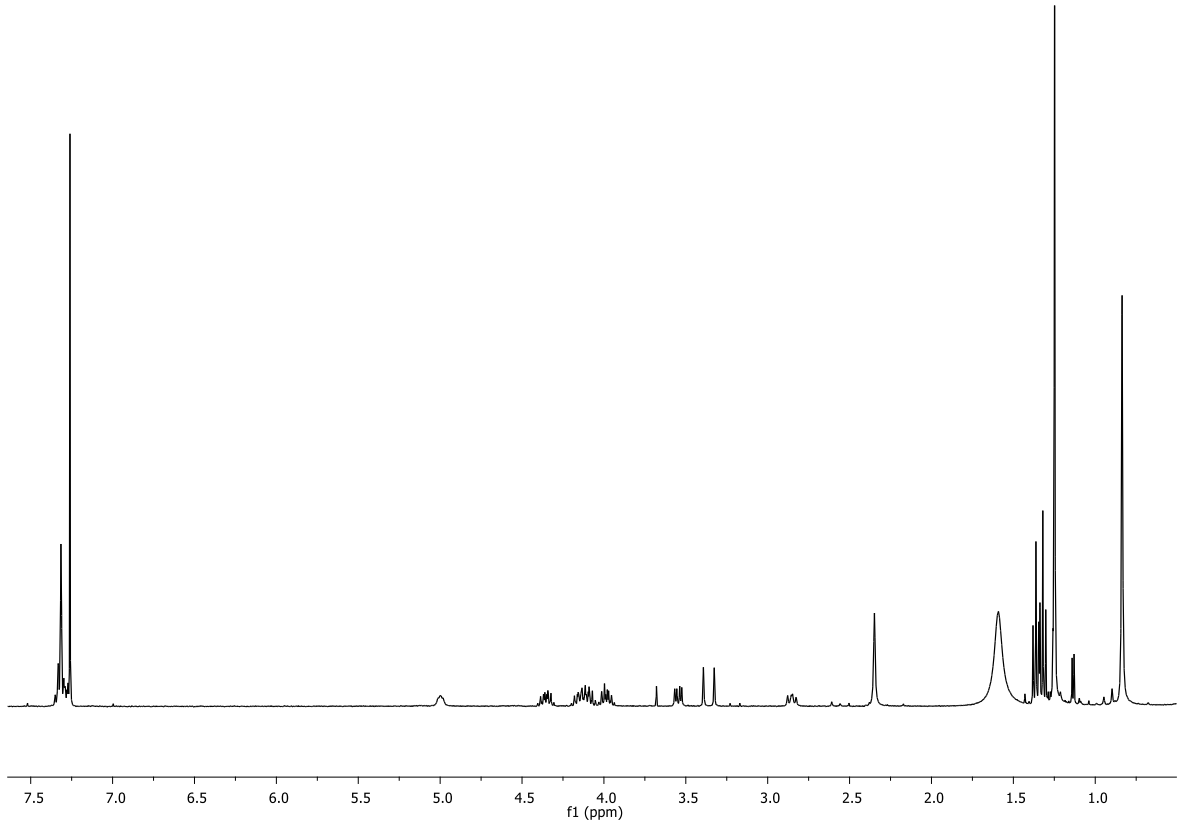
ALK18-1 (300 MHz, $CDCl_3$):



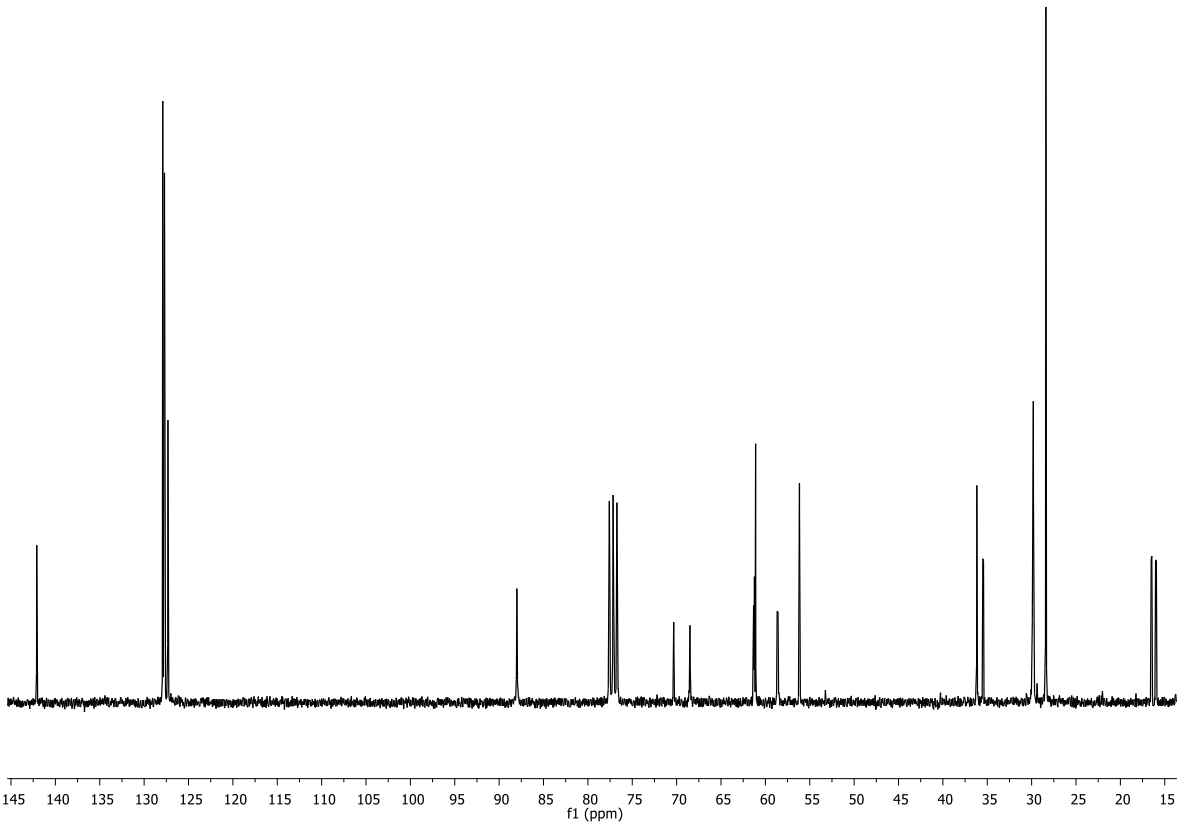
ALK18-1 (75 MHz, $CDCl_3$):



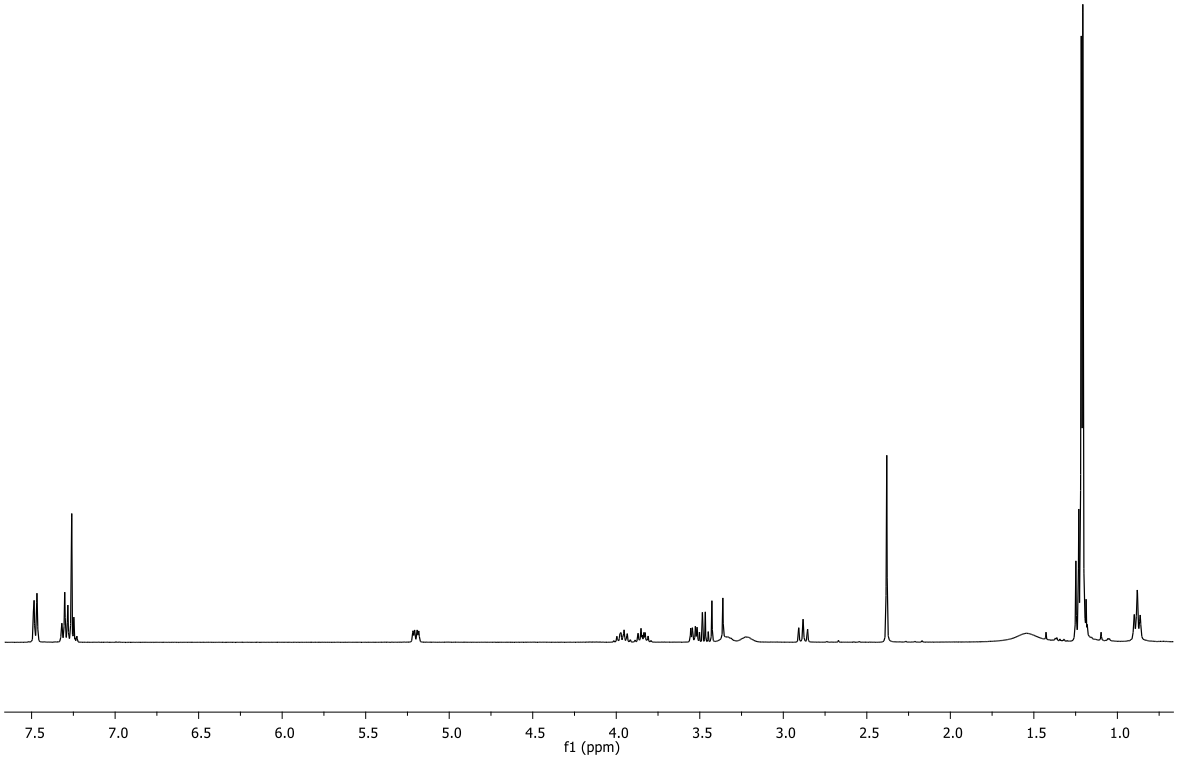
ALK5 (400 MHz, $CDCl_3$):



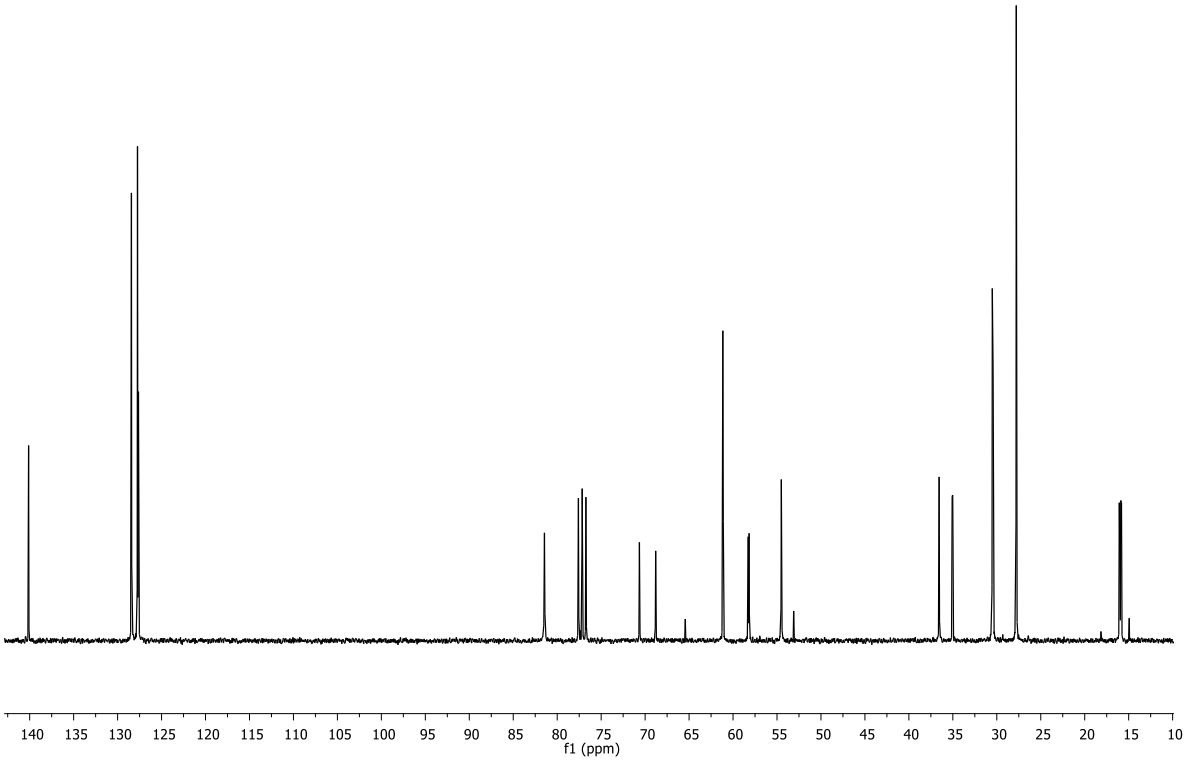
ALK5 (75 MHz, $CDCl_3$):



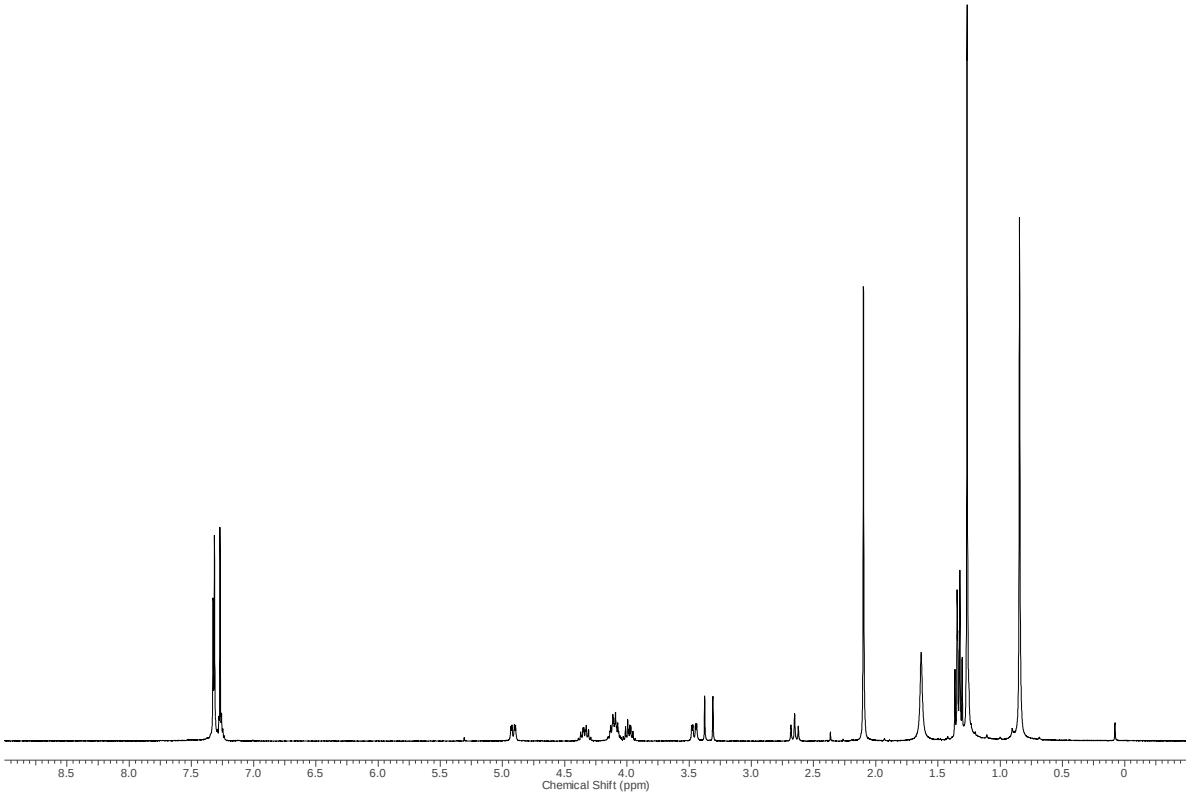
ALK18 (400 MHz, $CDCl_3$):



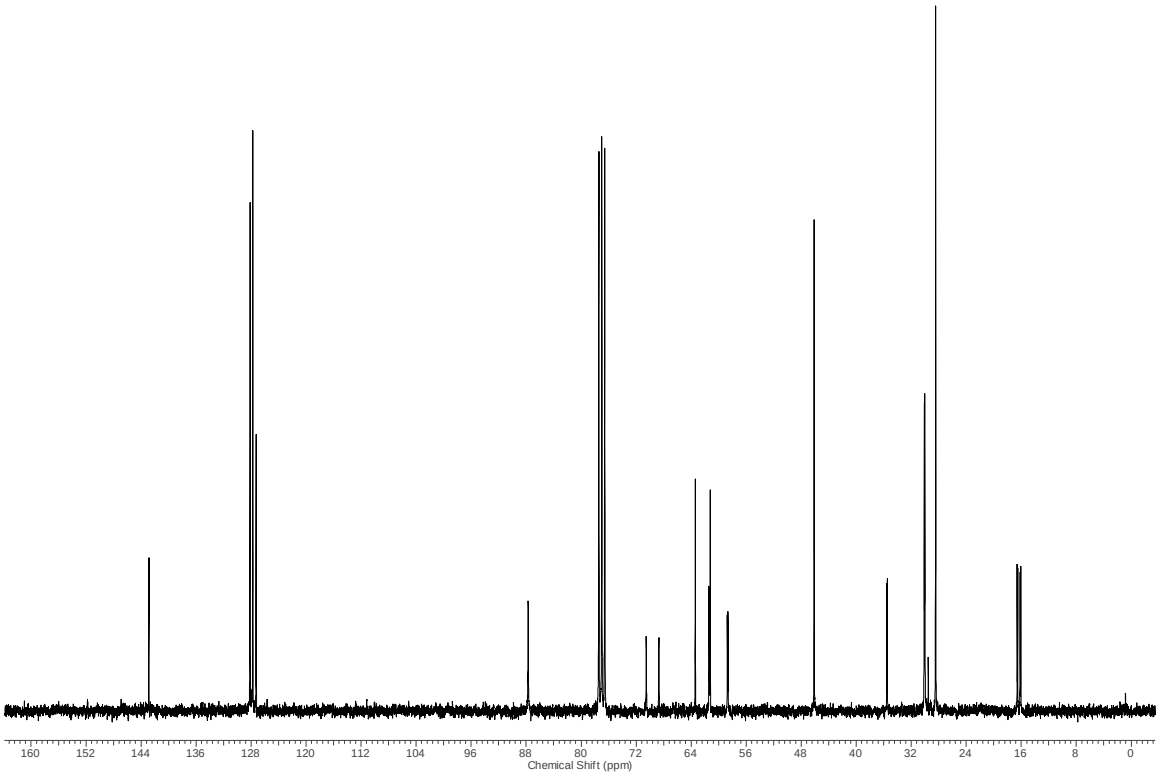
ALK18 (75 MHz, $CDCl_3$):



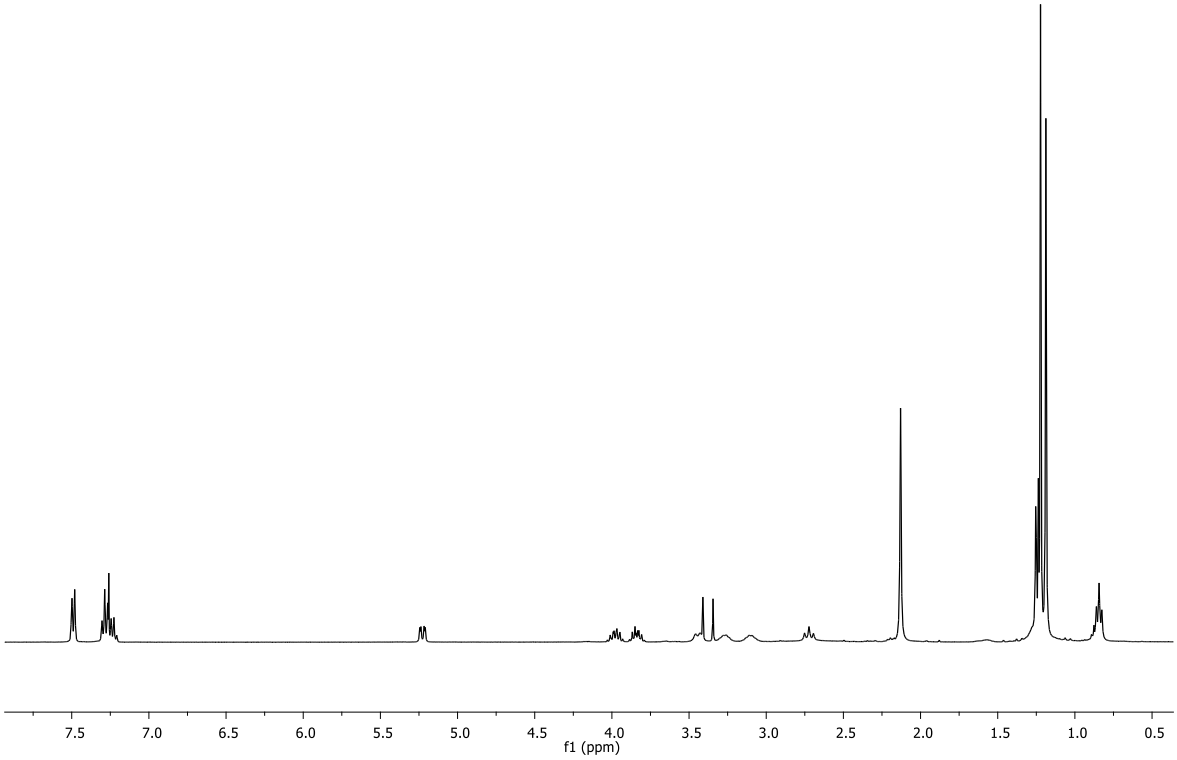
ALK6 (400 MHz, $CDCl_3$):



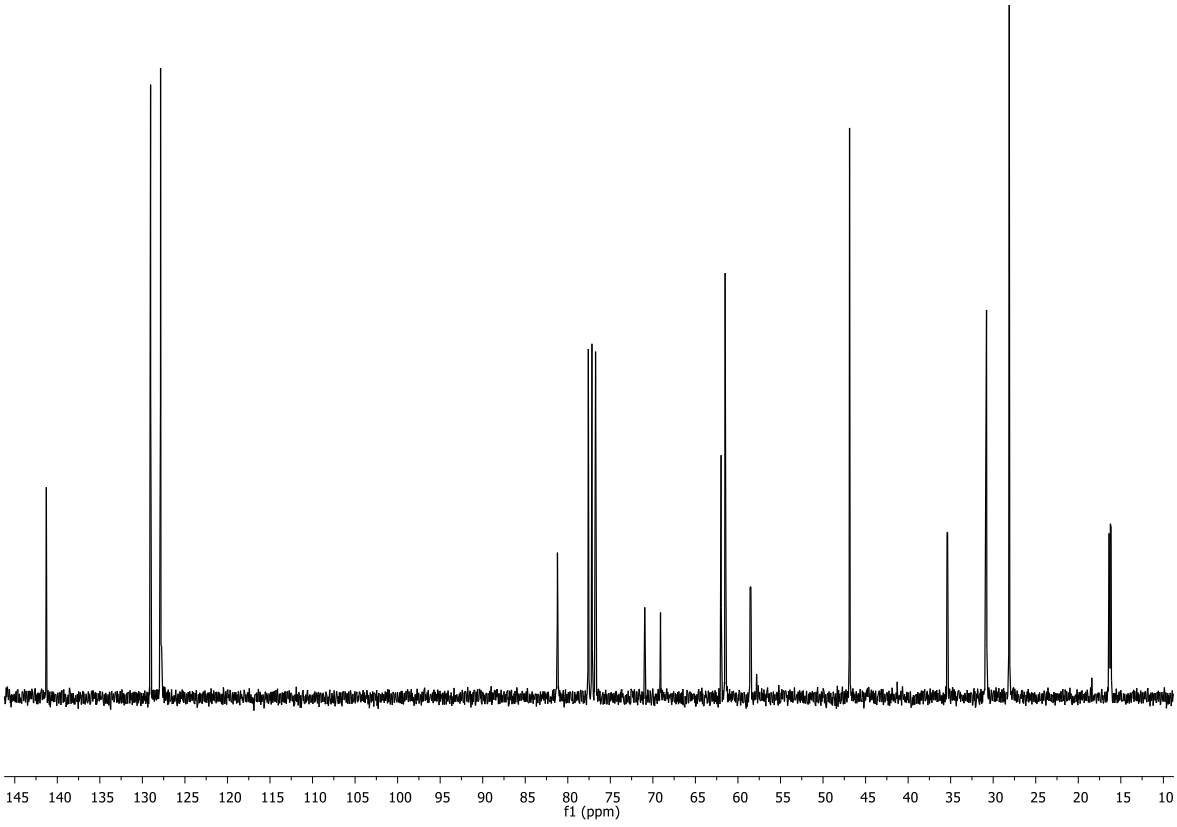
ALK6 (75 MHz, $CDCl_3$):



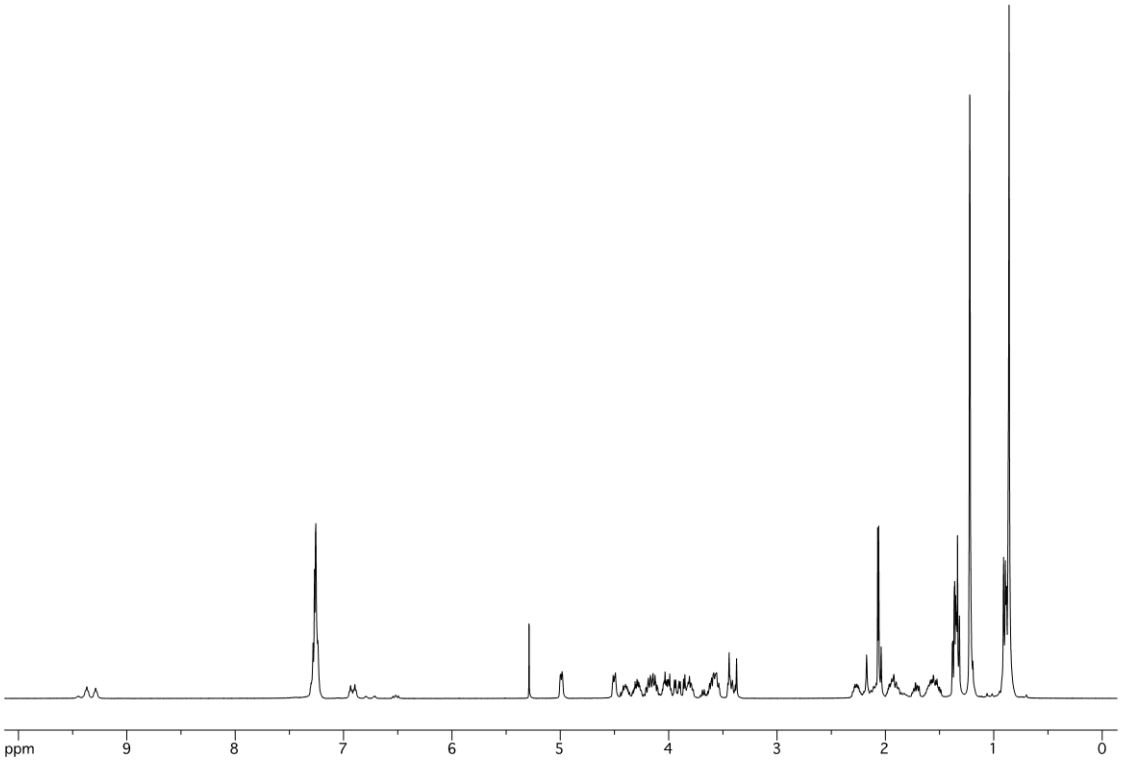
ALK19 (400 MHz, $CDCl_3$):



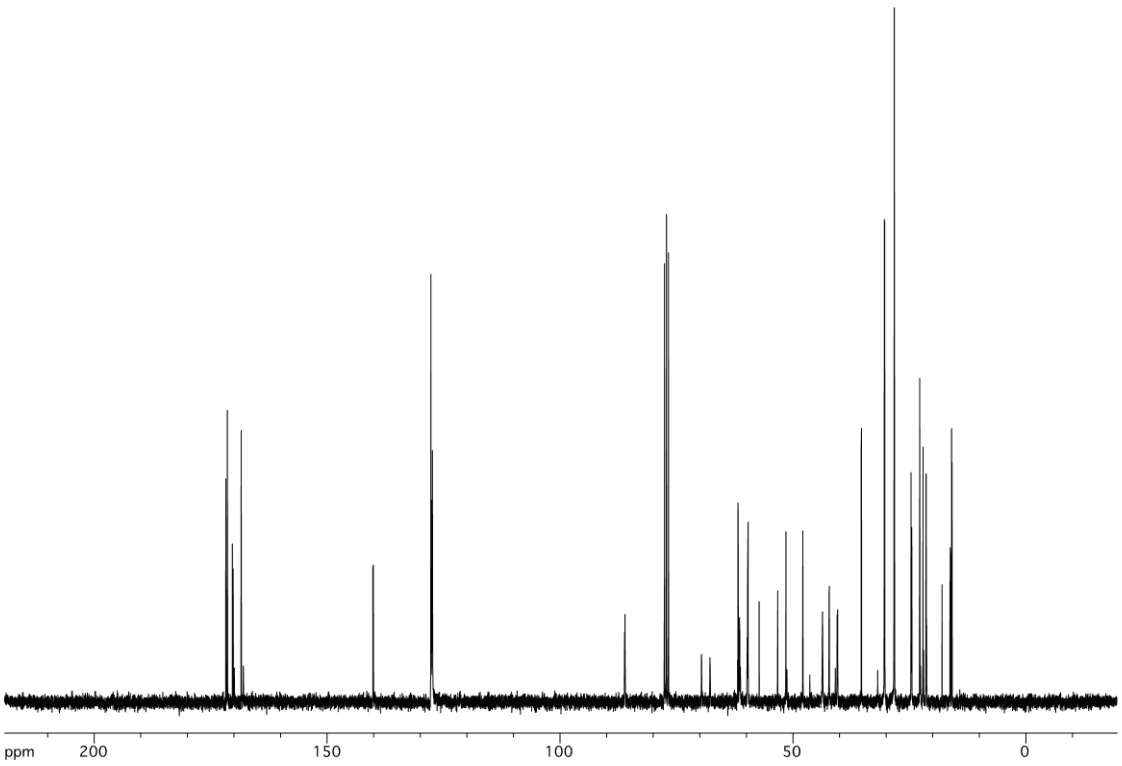
ALK19 (75 MHz, $CDCl_3$):



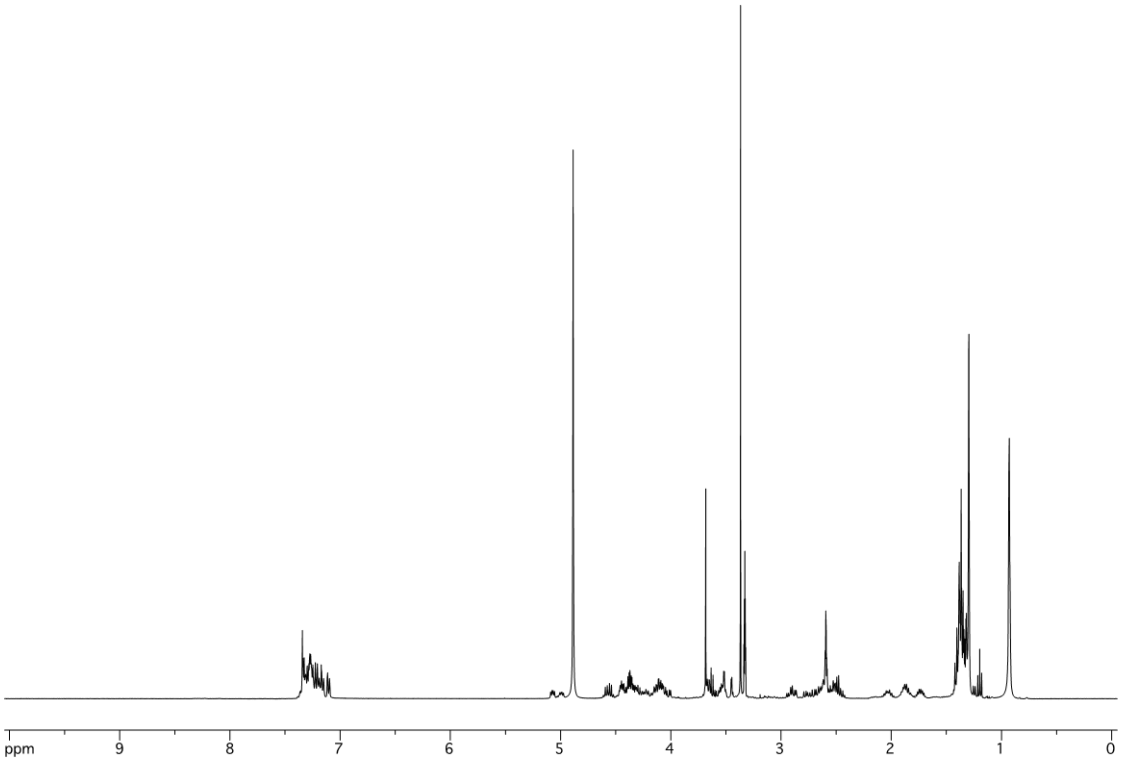
ALK4-MMPp (400 MHz, $CDCl_3$):



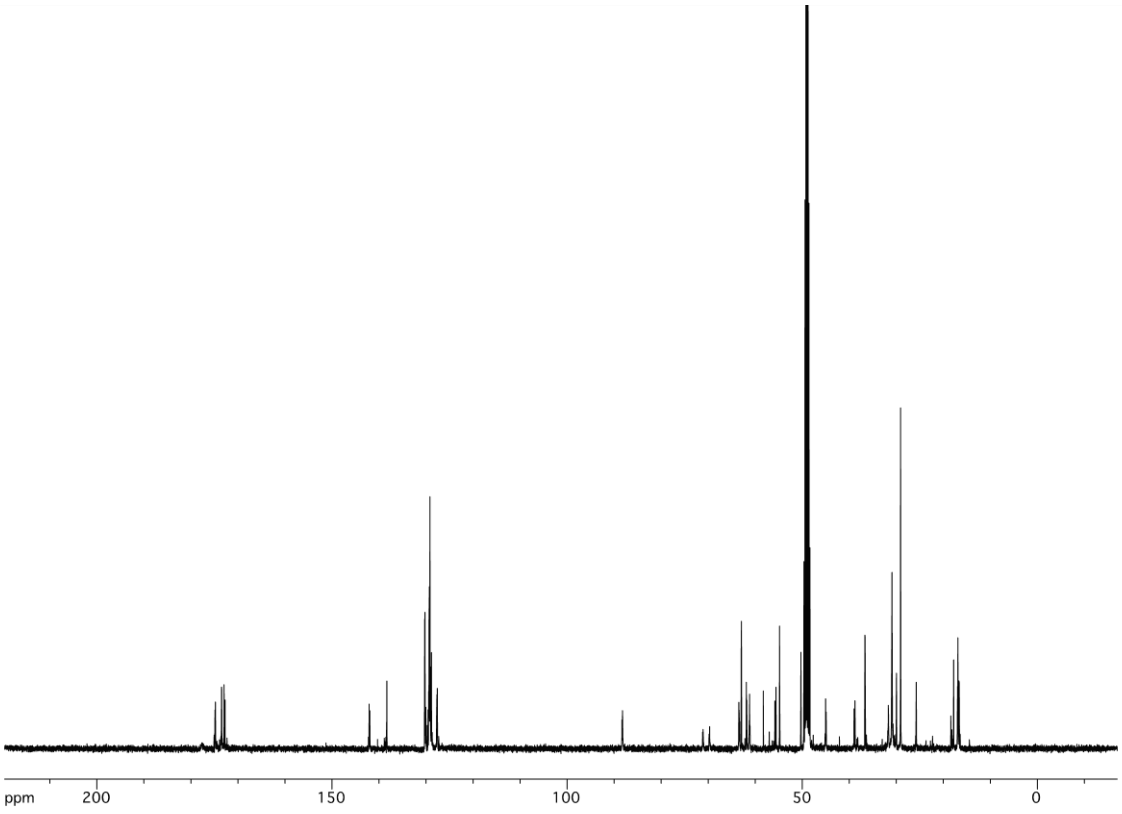
ALK4-MMPp (75 MHz, $CDCl_3$):



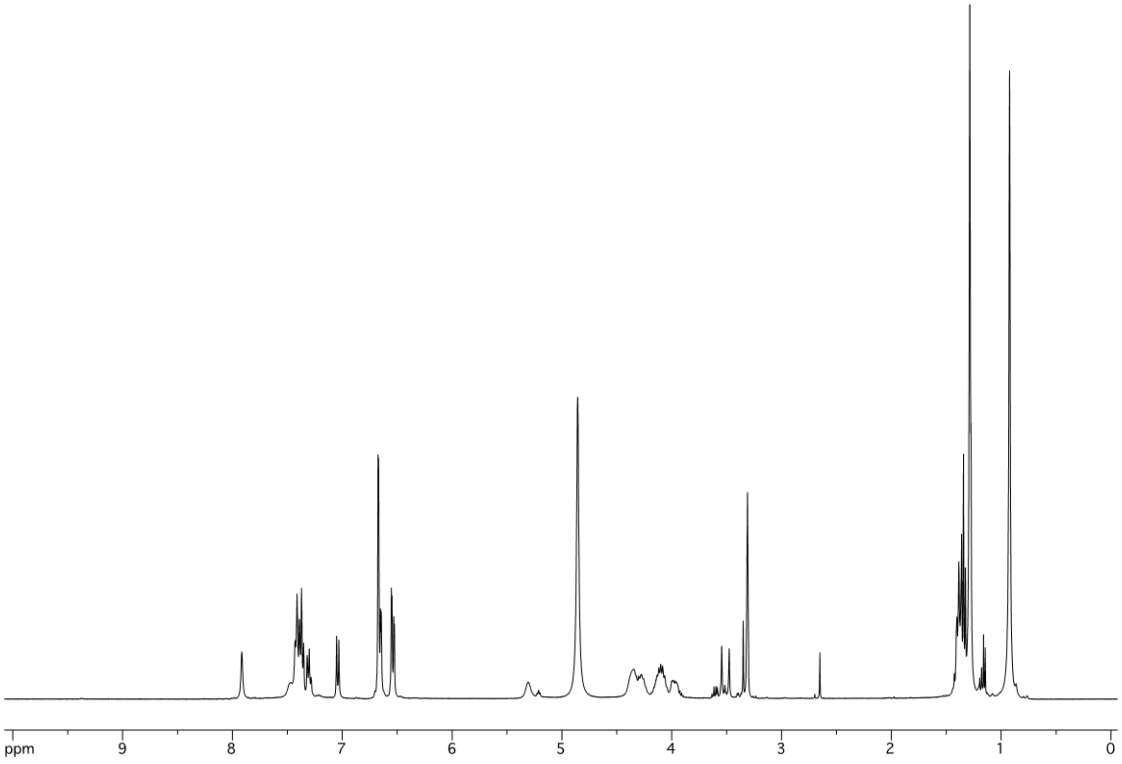
ALK4-CHYMOp (400 MHz, CD_3OD):



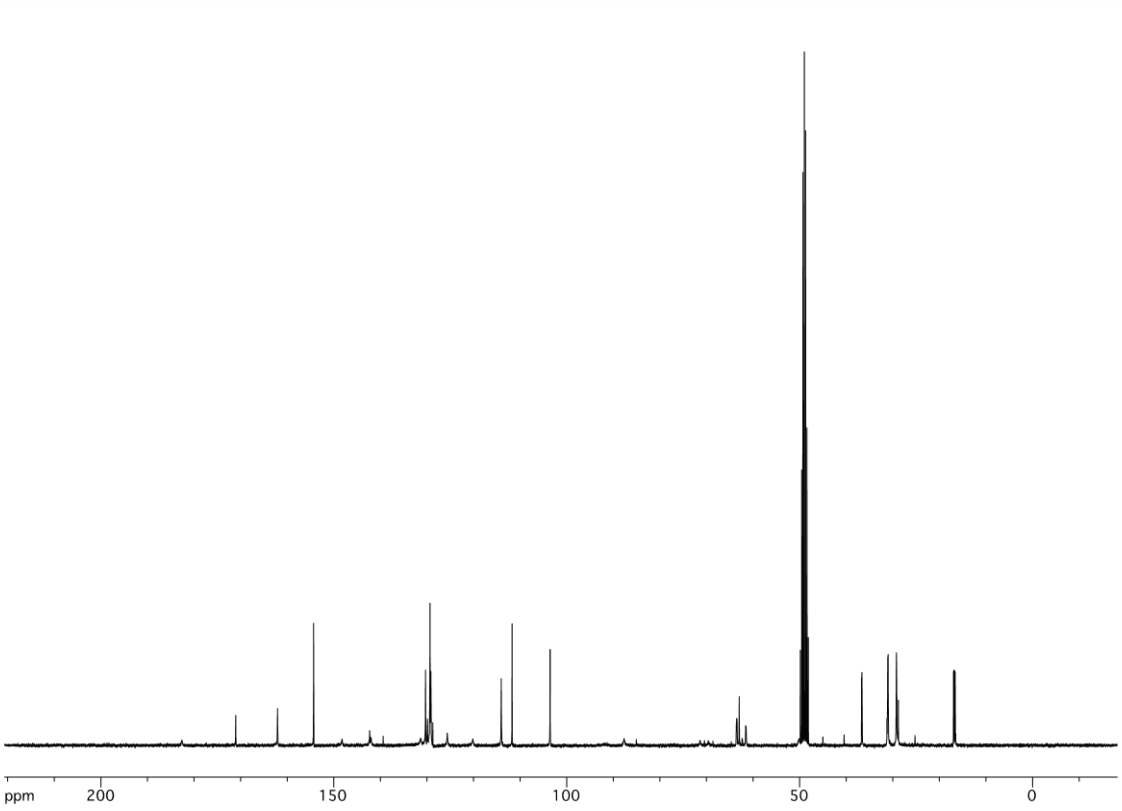
ALK4-CHYMOp (75 MHz, CD_3OD):



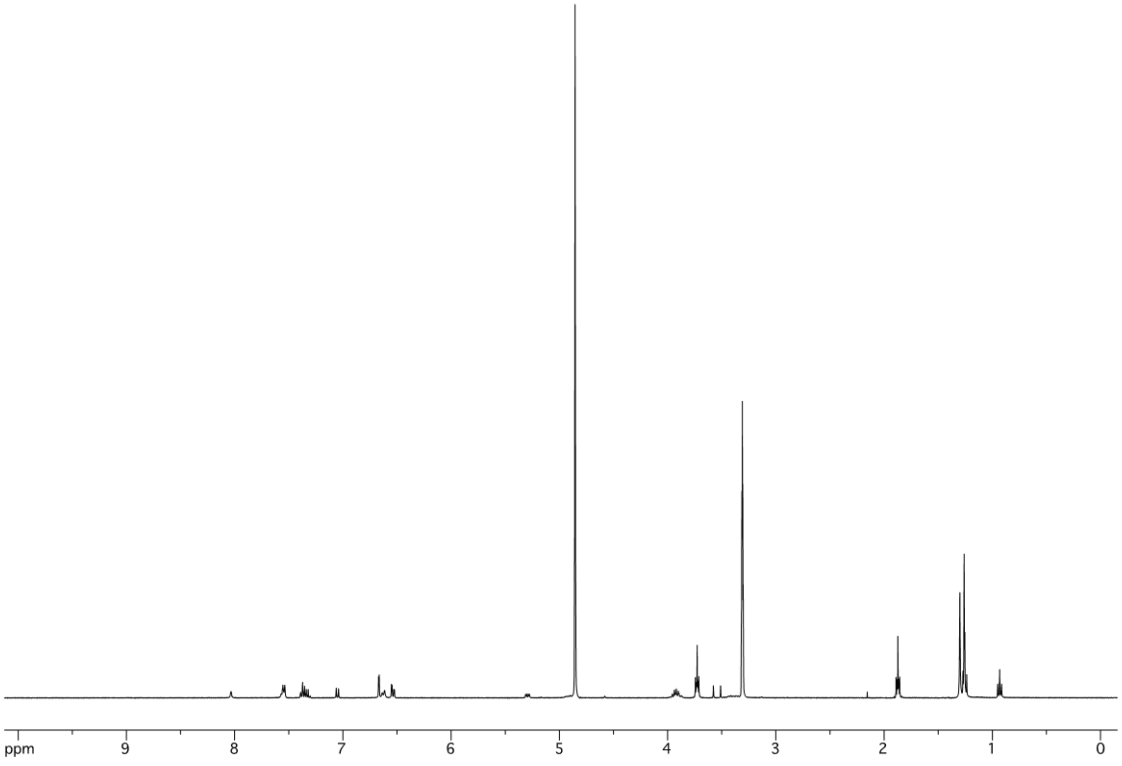
ALK4-FITC (400 MHz, CD_3OD):



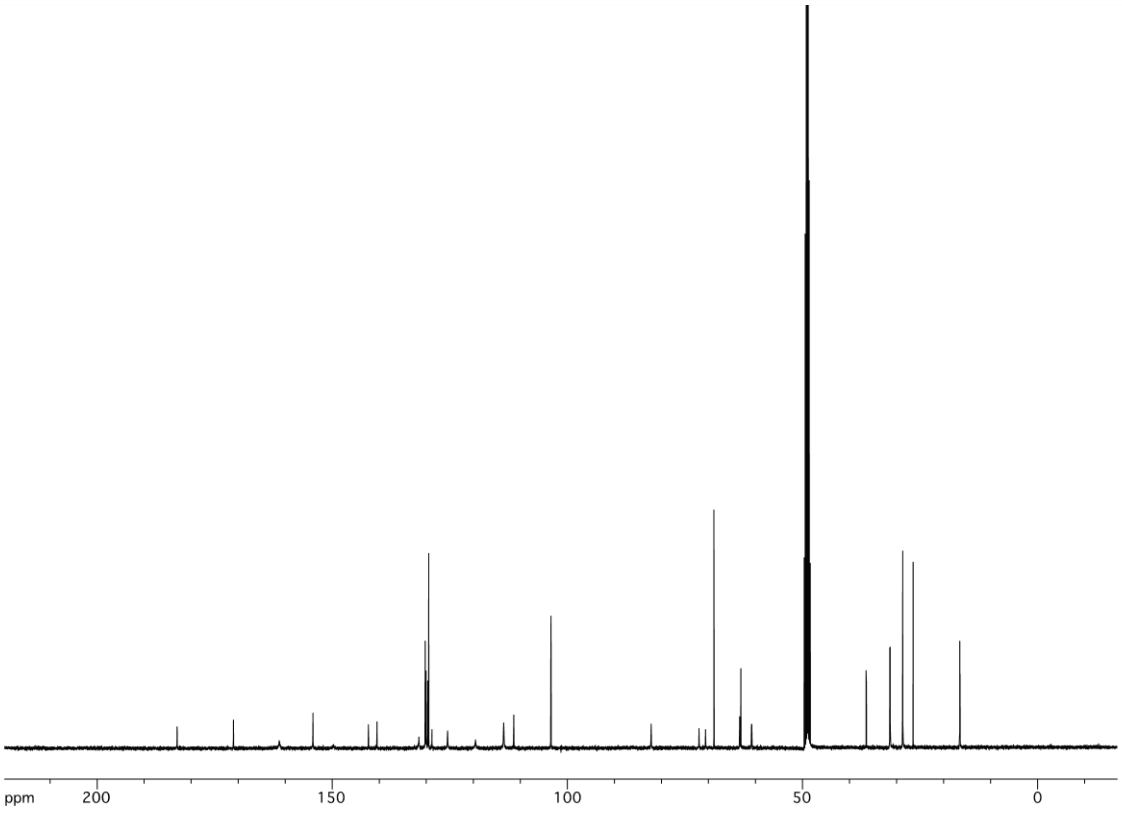
ALK4-FITC (75 MHz, CD_3OD):



ALK17-FITC (400 MHz, CD_3OD):

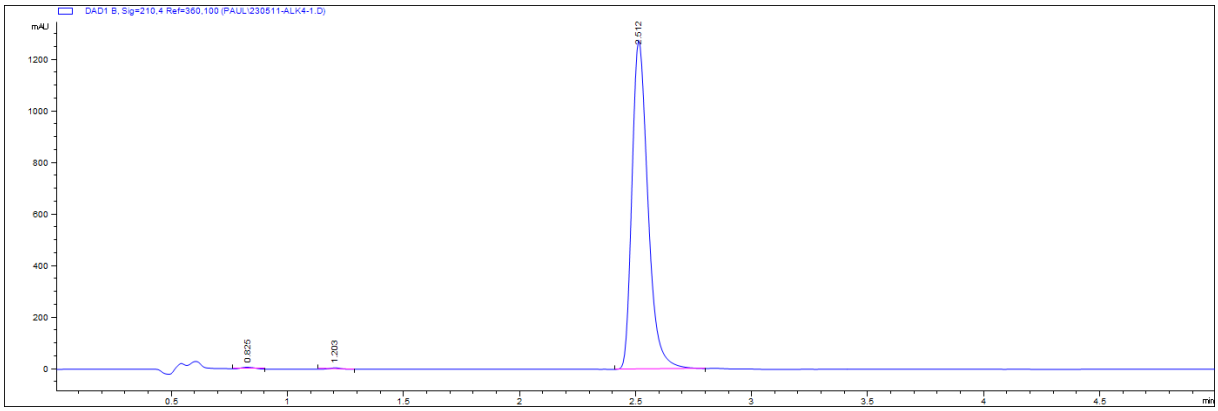


ALK17-FITC (75 MHz, CD_3OD):



1.4. HPLC traces

ALK4-1:



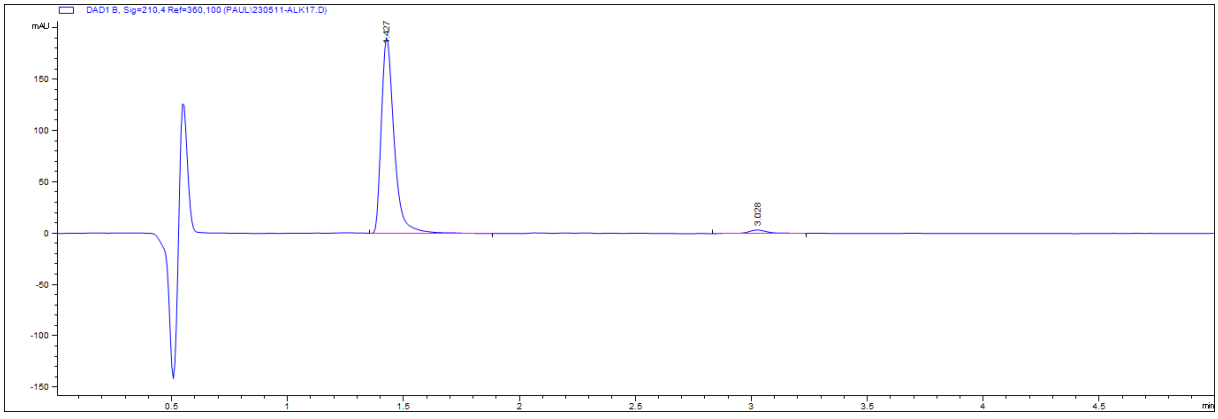
#	Time	Area	Height	Width	Area%	Symmetry
1	0.825	26	6.6	0.0682	0.423	0.815
2	1.203	16.7	5.5	0.0475	0.272	0.939
3	2.512	6102.4	1281.8	0.0735	99.305	0.692

Mobile phase: 80% CH_3CN (+0,1% TFA) / 20% H_2O (+0,1% TFA)

LR-MS (ESI): 533.2 [M+Na]

Purity: 99.3%

ALK17-1:



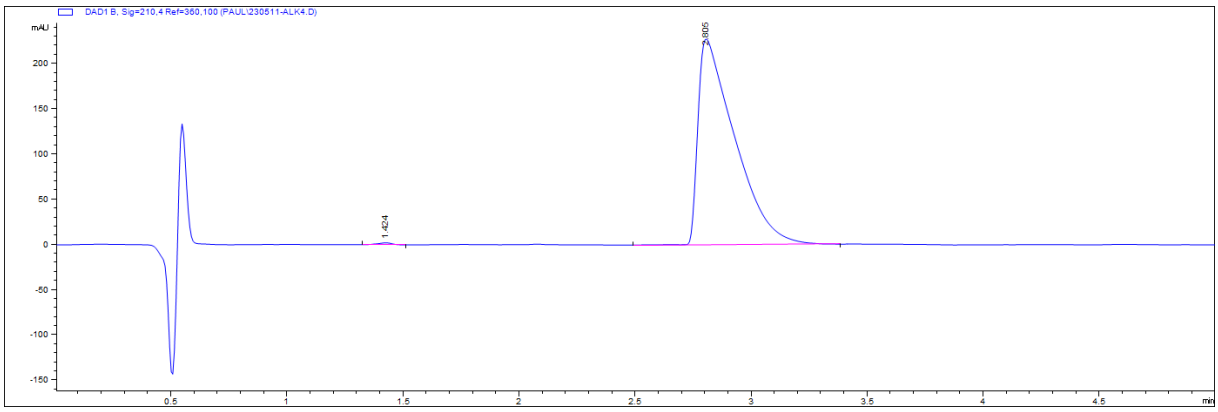
#	Time	Area	Height	Width	Area%	Symmetry
1	1.427	728.4	190.9	0.0585	97.224	0.65
2	3.028	20.8	3.4	0.0889	2.776	0.873

Mobile phase: 70% CH_3CN (+0,1% TFA) / 30% H_2O (+0,1% TFA)

LR-MS (ESI): 533.2 [M+Na]

Purity: 97.2%

ALK4:



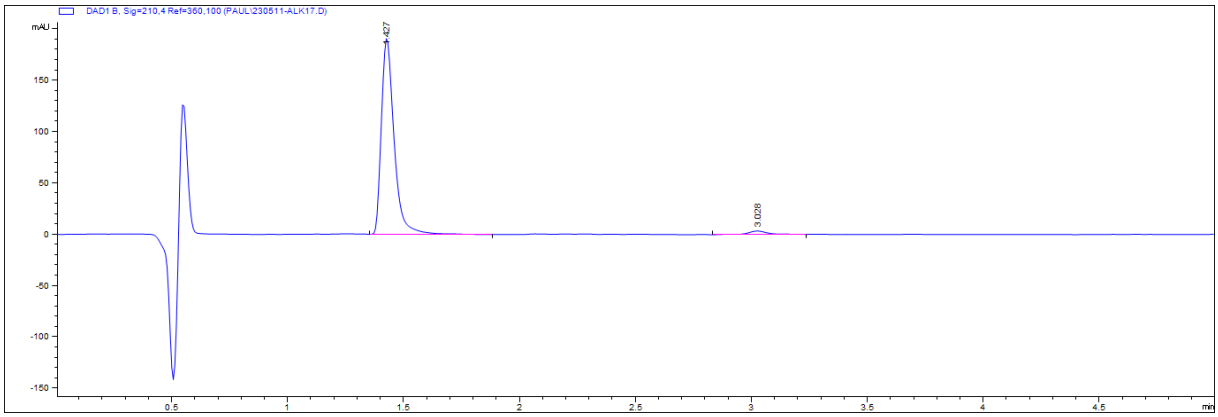
#	Time	Area	Height	Width	Area%	Symmetry
1	1.424	9.3	2.2	0.0634	0.370	1.321
2	2.805	2497.4	227.1	0.1543	99.630	0.267

Mobile phase: 50% CH_3CN (+0,1% TFA) / 50% H_2O (+0,1% TFA)

LR-MS (ESI): 415.3 [M+H]

Purity: 99.6%

ALK17:



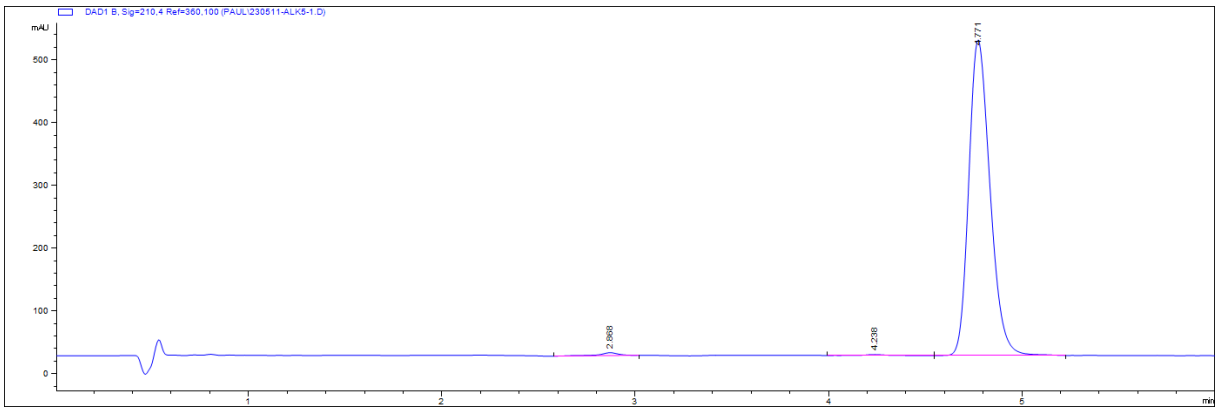
#	Time	Area	Height	Width	Area%	Symmetry
1	1.427	728.4	190.9	0.0585	97.224	0.65
2	3.028	20.8	3.4	0.0889	2.776	0.873

Mobile phase: 50% CH_3CN (+0,1% TFA) / 50% H_2O (+0,1% TFA)

LR-MS (ESI): 415.3 [M+H]

Purity: 97.2%

ALK5-1



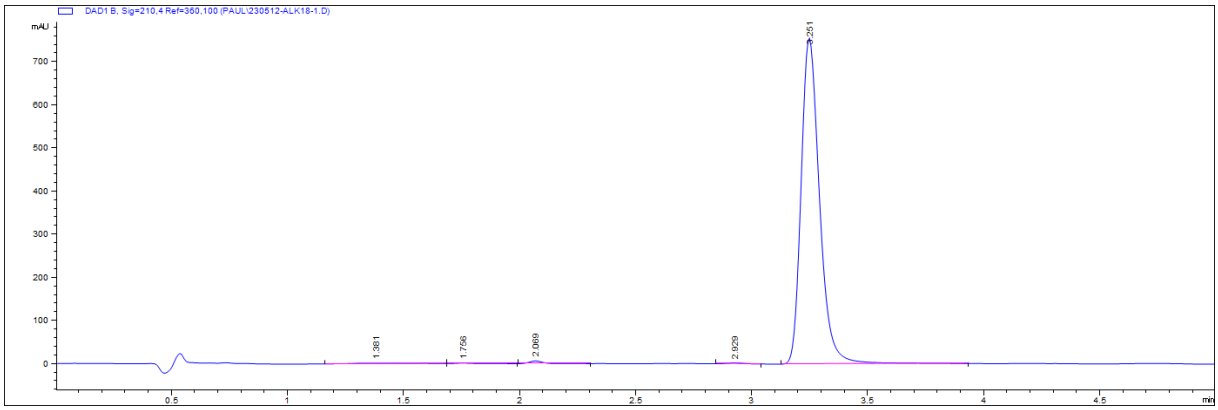
#	Time	Area	Height	Width	Area%	Symmetry
1	2.868	34.3	4.7	0.1056	0.882	1.458
2	4.238	15.2	1.6	0.1357	0.390	0.949
3	4.771	3839.7	503.1	0.119	98.728	0.732

Mobile phase: 70% CH_3CN (+0,1% TFA) / 30% H_2O (+0,1% TFA)

LR-MS (ESI): 547.2 [M+Na]

Purity: 98.7%

ALK18-1



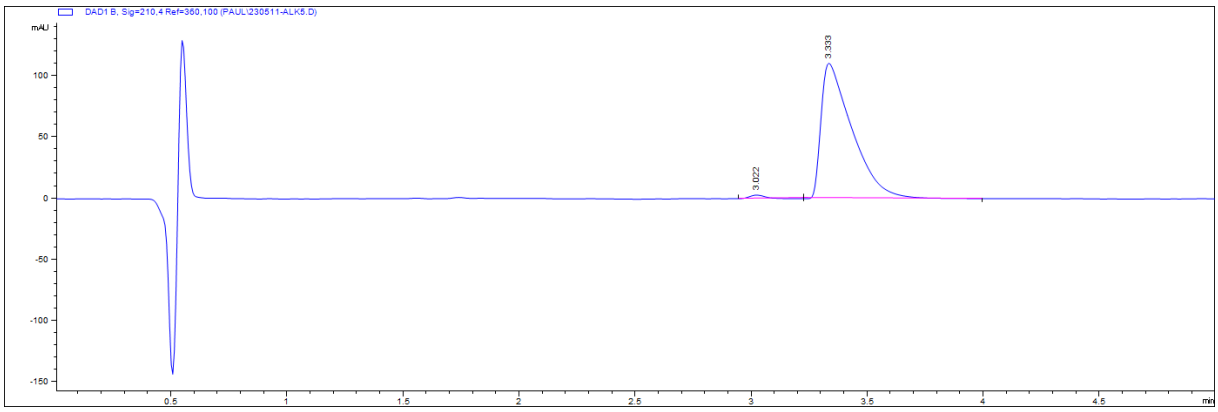
#	Time	Area	Height	Width	Area%	Symmetry
1	1.381	24.6	1.3	0.2281	0.580	0.747
2	1.756	6.4	1.2	0.0787	0.151	0.508
3	2.069	25.2	6.2	0.0634	0.594	0.767
4	2.929	10	2.1	0.0704	0.236	0.785
5	3.251	4173.2	754.9	0.0845	98.439	0.712

Mobile phase: 75% CH_3CN (+0,1% TFA) / 25% H_2O (+0,1% TFA)

LR-MS (ESI): 547.2 [M+Na]

Purity: 98.4%

ALK5



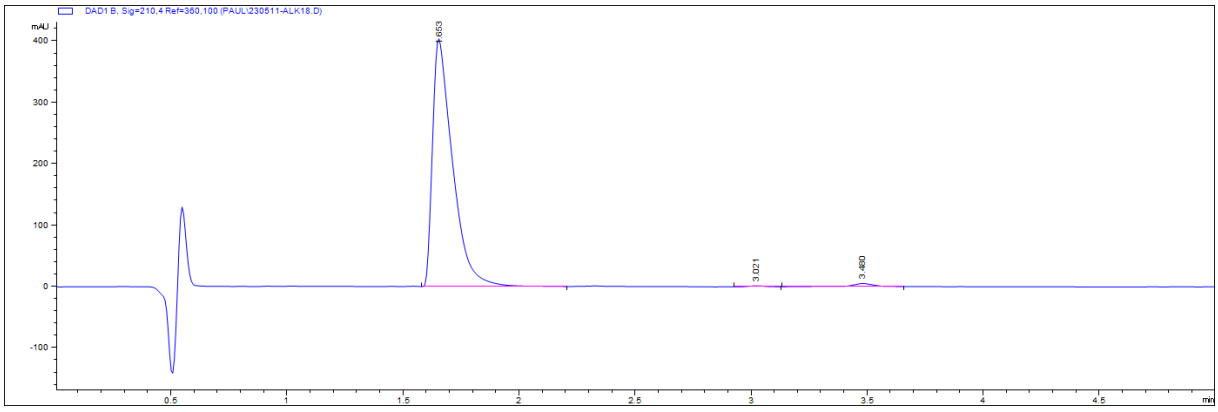
#	Time	Area	Height	Width	Area%	Symmetry
1	3.022	14.6	3	0.075	1.387	0.739
2	3.333	1035.3	111	0.1328	98.613	0.315

Mobile phase: 50% CH_3CN (+0,1% TFA) / 50% H_2O (+0,1% TFA)

LR-MS (ESI): 429.3 [M+H]

Purity: 98.6%

ALK18:



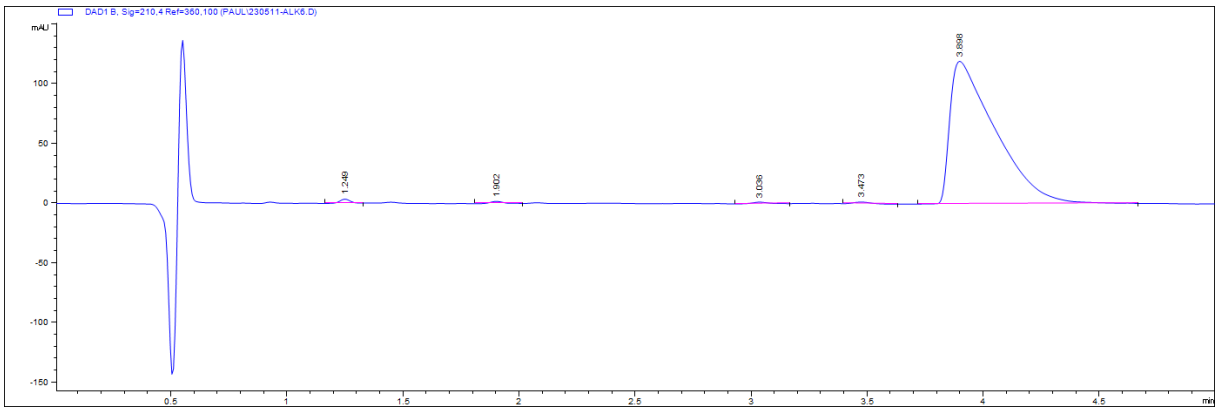
#	Time	Area	Height	Width	Area%	Symmetry
1	1.653	2419.4	403.8	0.0879	98.198	0.414
2	3.021	7.8	1.6	0.0785	0.317	0.86
3	3.48	36.6	5.1	0.1059	1.484	1.228

Mobile phase: 50% CH_3CN (+0,1% TFA) / 50% H_2O (+0,1% TFA)

LR-MS (ESI): 429.3 [M+H]

Purity: 98.2%

ALK6:



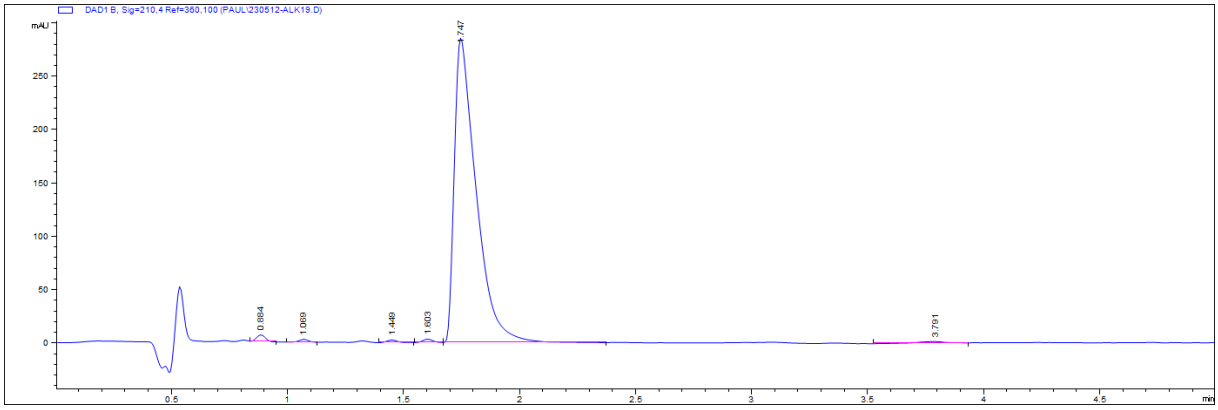
#	Time	Area	Height	Width	Area%	Symmetry
1	1.249	10.4	3.4	0.0478	0.626	1.007
2	1.902	6.8	1.8	0.0604	0.413	0.909
3	3.036	6.2	1.3	0.0684	0.376	0.819
4	3.473	7.2	1.4	0.0777	0.433	0.746
5	3.898	1624.3	119.7	0.1904	98.152	0.254

Mobile phase: 50% CH_3CN (+0,1% TFA) / 50% H_2O (+0,1% TFA)

LR-MS (ESI): 443.3 [M+H]

Purity: 98.1%

ALK19:



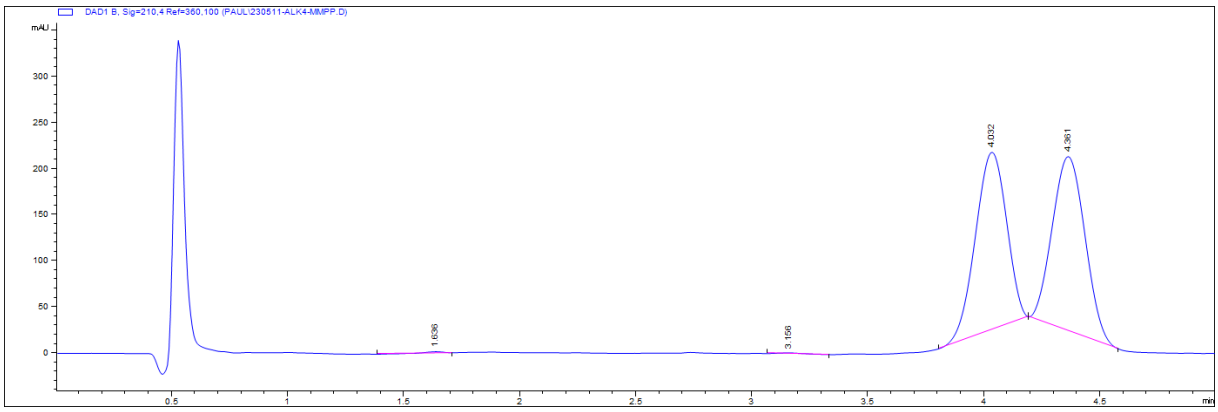
#	Time	Area	Height	Width	Area%	Symmetry
1	0.884	16.2	6.1	0.0428	0.848	0.899
2	1.069	7	2.5	0.0443	0.367	1.051
3	1.449	7.5	2.4	0.0481	0.394	0.866
4	1.603	9	3.1	0.0462	0.472	0.948
5	1.747	1859.5	285.5	0.094	97.229	0.389
6	3.791	13.2	1.5	0.1195	0.689	1.588

Mobile phase: 60% CH_3CN (+0,1% TFA) / 40% H_2O (+0,1% TFA)

LR-MS (ESI): 443.3 [M+H]

Purity: 97.7%

ALK4-MMPp *(mixture of two diastereoisomers)*:



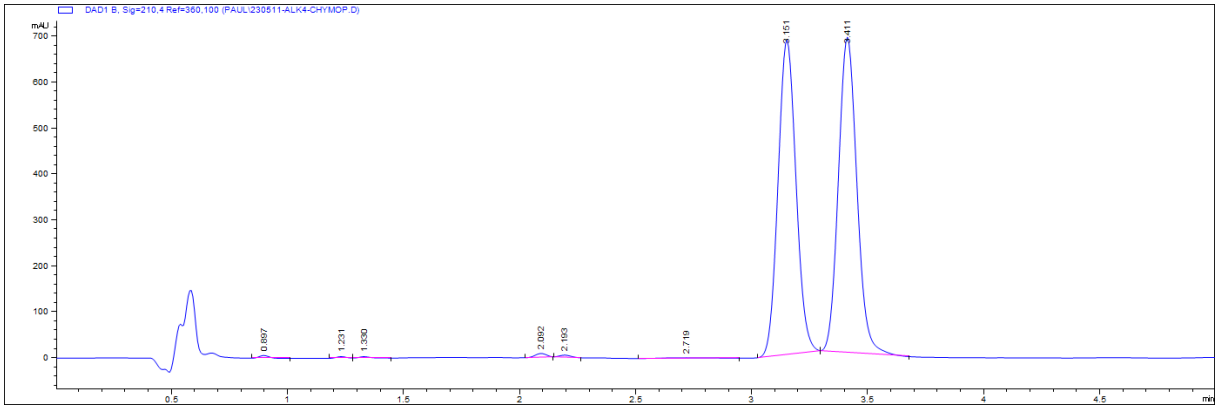
#	Time	Area	Height	Width	Area%	Symmetry
1	1.636	9.3	1.5	0.0952	0.253	1.801
2	3.156	7.6	1.1	0.0951	0.208	0.635
3	4.032	1802.9	193.1	0.1489	49.060	1.126
4	4.361	1855.1	189.4	0.1542	50.479	0.905

Mobile phase: 60% CH_3CN (+0,1% TFA) / 40% H_2O (+0,1% TFA)

LR-MS (ESI): 724.4 [M+H]

Purity: 99.5%

ALK4-CHYMOp *(mixture of two diastereoisomers)*:



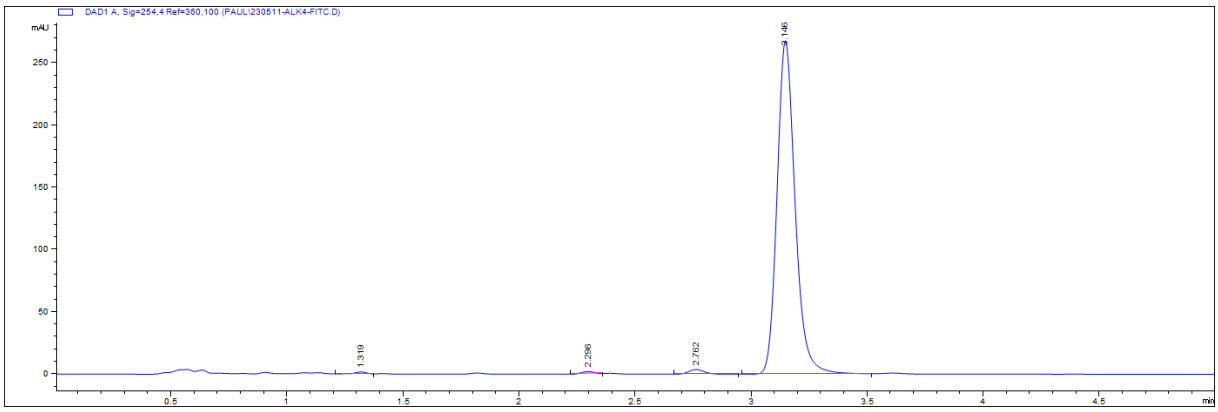
#	Time	Area	Height	Width	Area%	Symmetry
1	0.897	17.5	5.9	0.0484	0.231	0.895
2	1.231	9	3.2	0.0447	0.119	1.031
3	1.33	8.7	2.9	0.0474	0.115	0.77
4	2.092	26.9	8.1	0.0526	0.357	1.127
5	2.193	14.6	4.4	0.0543	0.193	0.75
6	2.719	17.5	1.2	0.1808	0.232	1.012
7	3.151	3667.7	683.4	0.0846	48.553	0.91
8	3.411	3792.1	684.7	0.0866	50.200	0.865

Mobile phase: 60% CH_3CN (+0,1% TFA) / 40% H_2O (+0,1% TFA)

LR-MS (ESI): 901.3 [M+H]

Purity: 98.7%

ALK4-FITC:



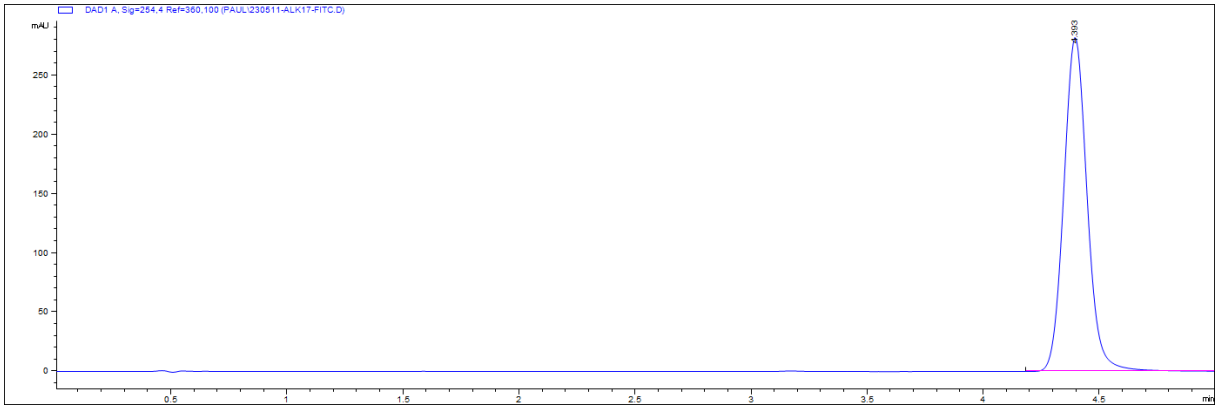
#	Time	Area	Height	Width	Area%	Symmetry
1	1.319	5.5	1.6	0.0539	0.373	1.278
2	2.296	6.6	1.9	0.0573	0.443	1.071
3	2.762	17.5	3.8	0.0725	1.184	0.864
4	3.146	1450.8	268.5	0.083	98.000	0.799

Mobile phase: 70% CH_3CN (+0,1% TFA) / 30% H_2O (+0,1% TFA)

LR-MS (ESI): 804.2 [M+H]

Purity: 98.0%

ALK17-FITC:



#	Time	Area	Height	Width	Area%	Symmetry
1	4.393	1971.2	281.8	0.1076	100.000	0.883

Mobile phase: 60% CH_3CN (+0,1% TFA) / 40% H_2O (+0,1% TFA)

LR-MS (ESI): 804.2 [M+H]

Purity: >99.9%

2. Kinetic measurements

	Conditions	*T* (°C)	k_d (s^{-1})	E_a (kJ/mol)	$t_{1/2}$ (37 ºC)
ALK4	pH = 11	80	$1.54\ 10^{-4}$	123.0	*17 d*
	pH = 7.4	80	$1.46\ 10^{-3}$	116.4	*32 h*
	pH = 6.0	80	$2.11\ 10^{-3}$	115.3	*21 h*
ALK5	pH = 11	80	$8.83\ 10^{-4}$	117.9	*2 d*
	pH = 7.4	70	$4.82\ 10^{-3}$	109.7	*2 h*
	pH = 6.0	70	$5.06\ 10^{-3}$	109.5	*2 h*
ALK6	pH = 7.4	50	$4.68\ 10^{-3}$	103.4	*13 min*
ALK17	pH = 11	80	$1.22\ 10^{-4}$	123.7	*23 d*
	pH = 7.4	80	$7.61\ 10^{-4}$	118.3	*3 d*
	pH = 6.0	80	$1.00\ 10^{-3}$	117.5	*2 d*
ALK18	pH = 11	90	$1.48\ 10^{-3}$	119.6	*5 d*
	pH = 7.4	70	$1.94\ 10^{-3}$	112.3	*7 h*
	pH = 6.0	70	$1.51\ 10^{-3}$	113.0	*8 h*
ALK19	pH = 7.4	50	$3.04\ 10^{-3}$	104.5	*19 min*

3. pK_a measurements

The dependence of the chemical shifts of alkoxyamines upon protonation was investigated by means of ^{1}H NMR spectroscopy. 0.02 M samples in a D_2O/CD_3OD (*v/v* 1:1) mixture of alkoxyamines were used. The values of pH were adjusted with DCl or NaOD and controlled by a pH-meter with a micro-pH electrode (o.d. 3 mm glass, 180 mm stem length). ^{1}H NMR spectra were recorded on a 400 MHz spectrometer.

All pK_a values were determined using the Henderson-Hasselbach relationship:

$$d_{\mathrm{pH}} = d_1 + \frac{d_{1\mathrm{H}^+} - d_1}{1+10^{\mathrm{p}K_a - \mathrm{pH}}}$$

4. Supplementary figures

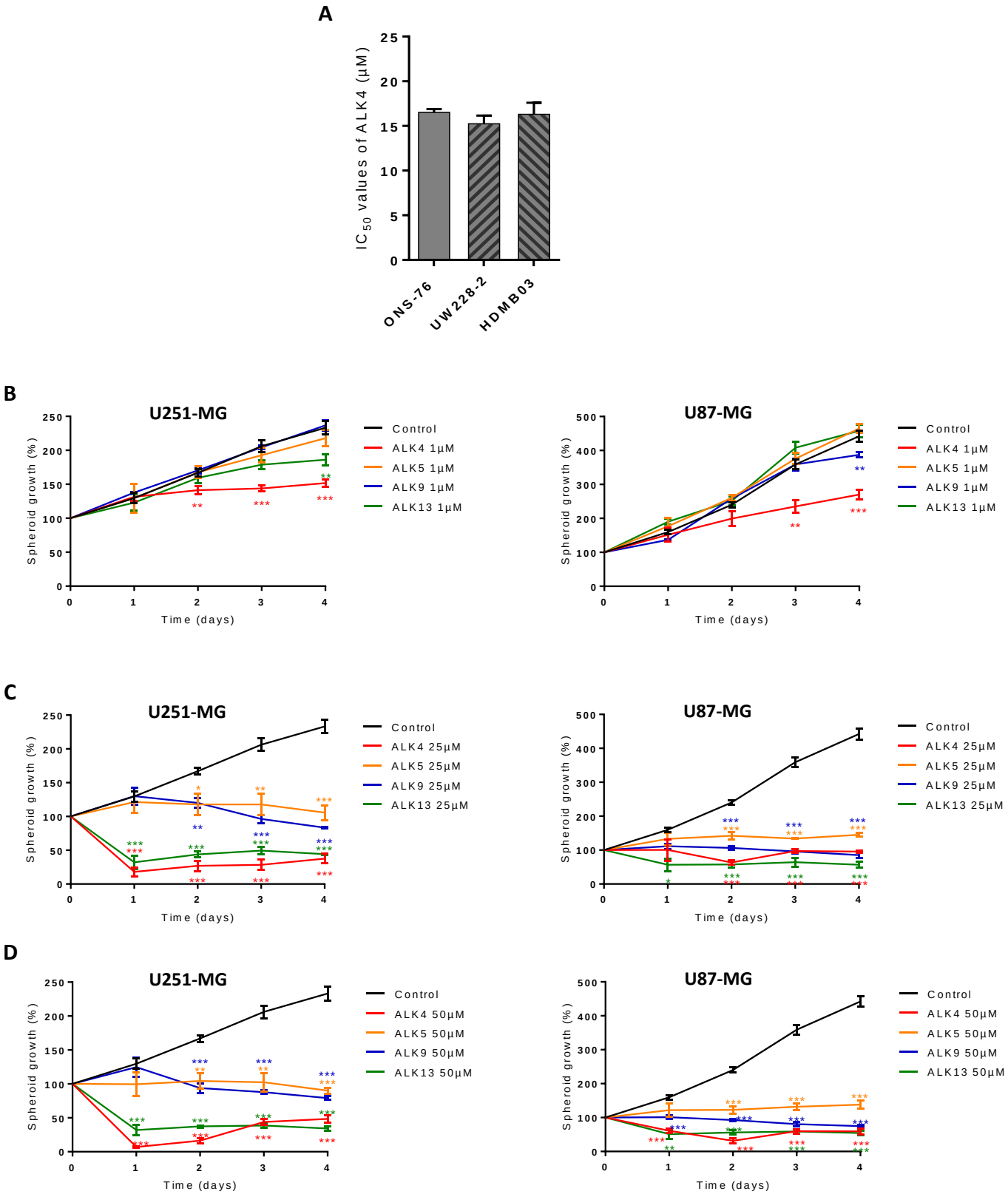


Fig. S1 Evaluation of the cytotoxicity of ALK4, ALK5, ALK9 and ALK13 in U251-MG and U87-MG human glioblastoma spheroids. (A) Evaluation of the cytotoxicity of ALK4 in ONS-76, UW228-2 and HDMB-03 MB cell lines. Monitoring the growth of U251-MG and U87-MG spheroids by fluorescence quantification after treatment with ALK4, ALK5, ALK9 and ALK13 at (B) 1 µM, (C) 25 µM and (D) 50 µM. Values are the average of at least three independent experiments ± SEM. * p<0,05 ; ** p<0,01 ; *** p<0,001

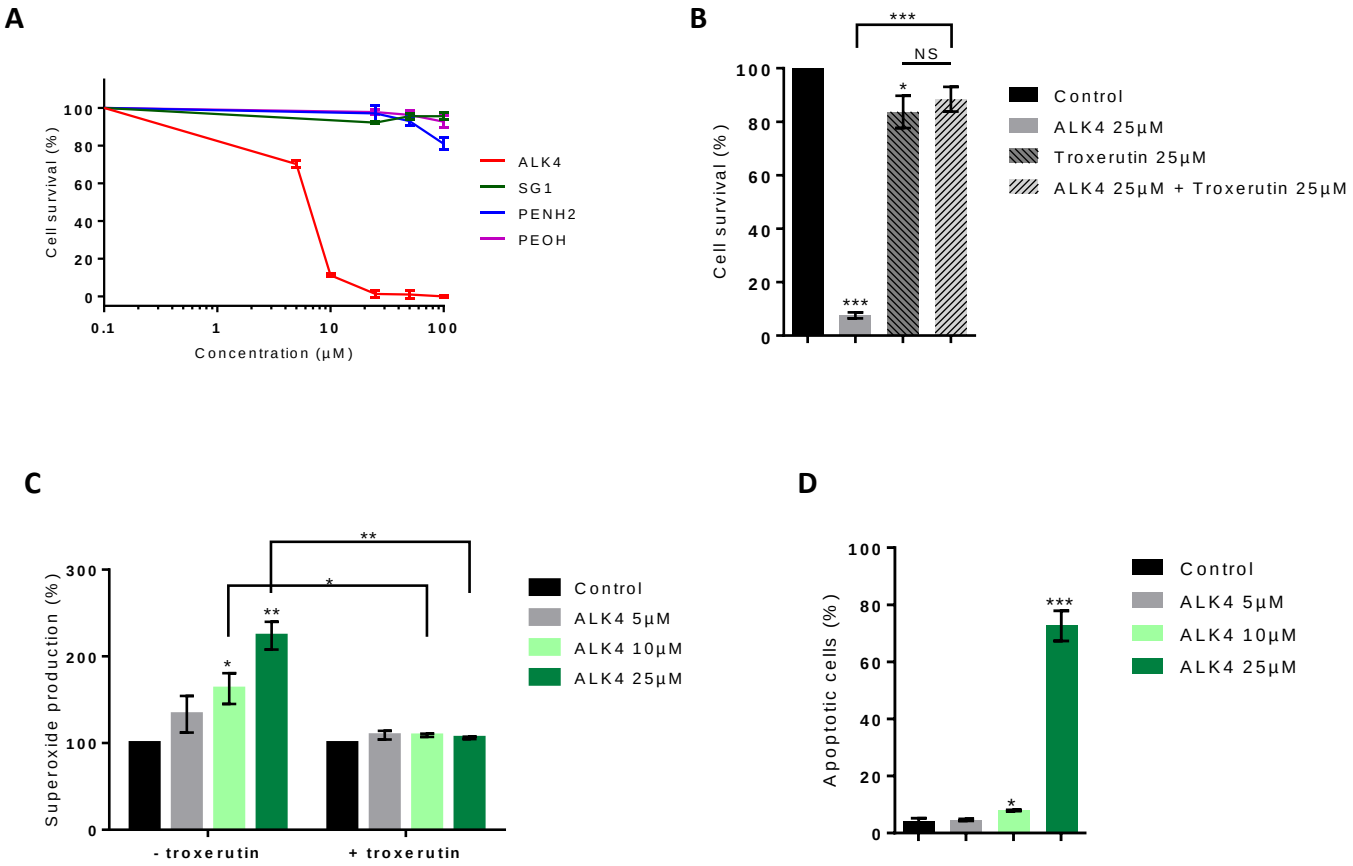


Fig. S2 Study of the mechanism of action of ALK4 in U87-MG cells. (A) Cell survival measured by MTT-assay after 72 hours of treatment with ALK4, its nitroxide SG1 and its alkyl radical on inactive forms PEA and PEA-OH, in 2D-culture of U87-MG cells. (B) Cell survival measured by MTT-assay of ALK4 in presence of alkyl scavenger Troxerutin at 25 µM in 2D-culture of U87-MG cells after 72 hours of treatment; (C) Superoxide production in 2D culture of U87-MG cells upon treatment with ALK4 only and in combination with alkyl scavenger troxerutin for 6 hours. (D) Measurement of apoptotic induction in 2D-culture of U87-MG cells upon treatment with ALK4 for 48 hours. * p<0,05 ; ** p<0,01 ; *** p<0,001.

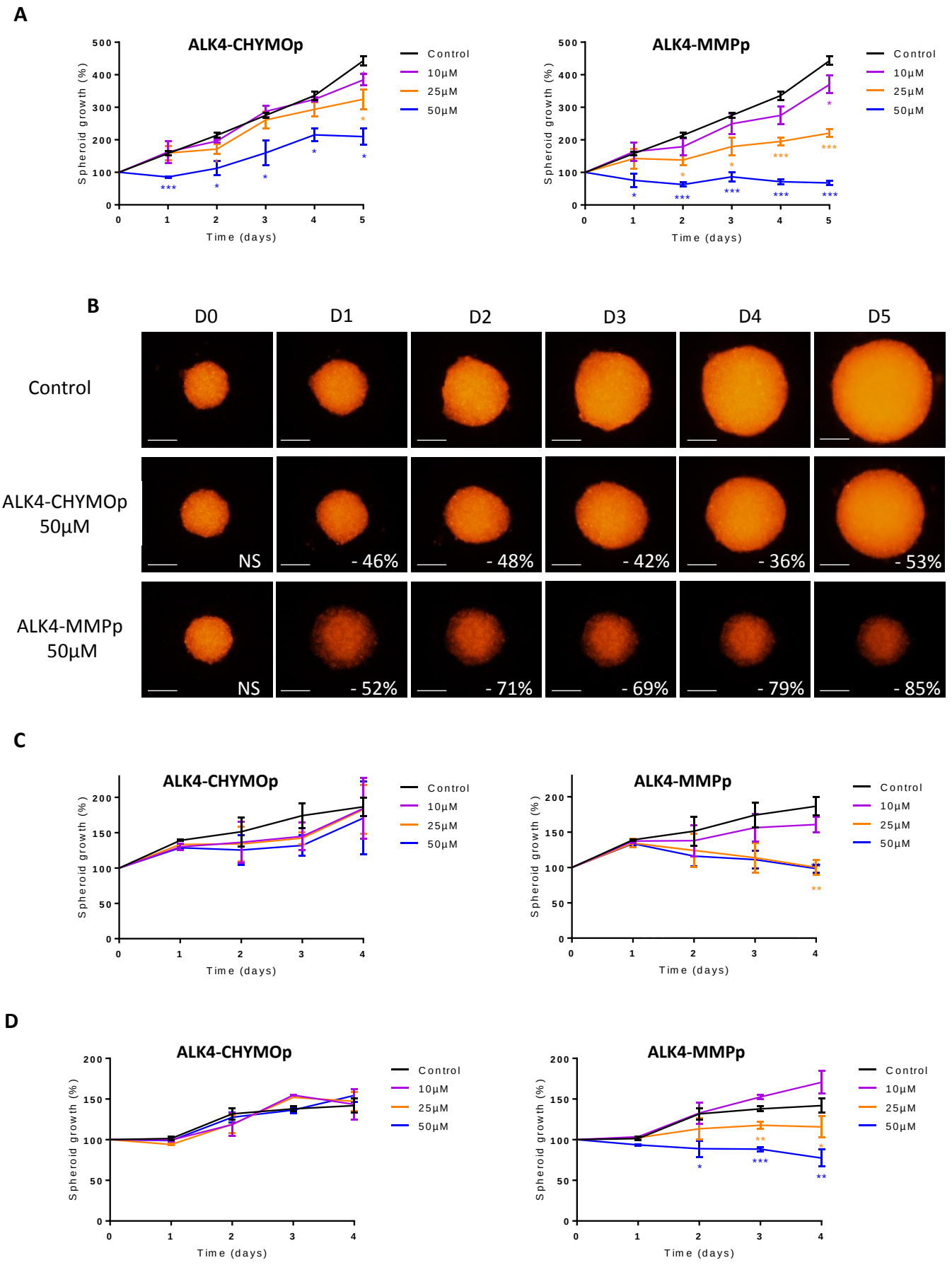


Fig. S3 Evaluation of the cytotoxicity of the bioconjugates ALK4-CHYMOp and ALK4-MMPp in U87-MG GBM spheroids and HD-MB03 and ONS-76 MB spheroids. Monitoring of the growth of (A) U87-MG, (C) HD-MB03 and (D) ONS-76 spheroids by fluorescence quantification for 4 days after treatment with ALK4-CHYMOp and with ALK4-MMPp. (B) Representative pictures of U87-MG spheroids before treatment and for 4 days after treatment with 50 µM of ALK4-CHYMOp and ALK4-MMPp. Scale bar = 250 µm. Values are the average of at least three independent experiments ± SEM.* p<0,05 ; ** p<0,01 ; *** p<0,001.

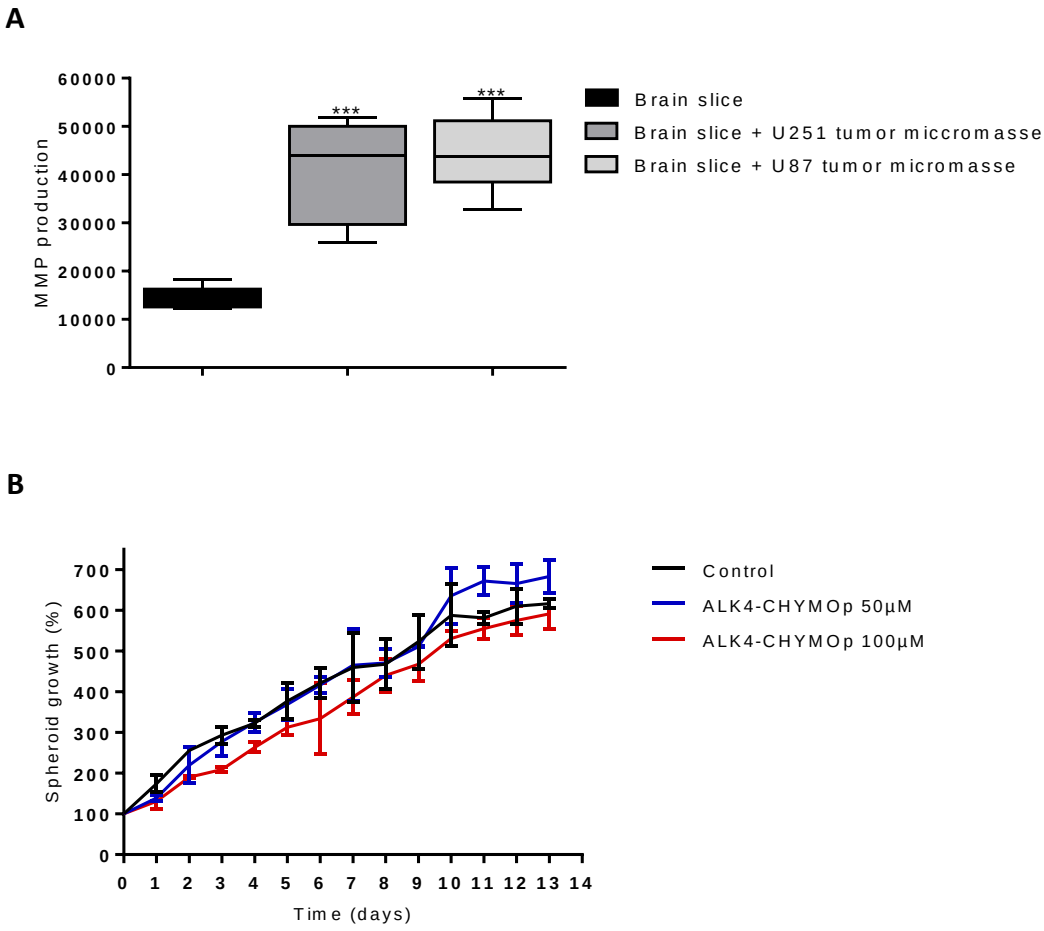


Fig. S4 (A) Production of MMP enzymes on organotypic brain slices with and without glioblastoma tumor graft. (B) Monitoring of the growth of U87-MG spheroids in brain slices by fluorescence quantification for 14 days after treatment with the bioconjugates ALK4-CHYMOp. Values are the average of at least three independent experiments ± SEM. * p<0,05 ; ** p<0,01 ; *** p<0,001.