Experimental

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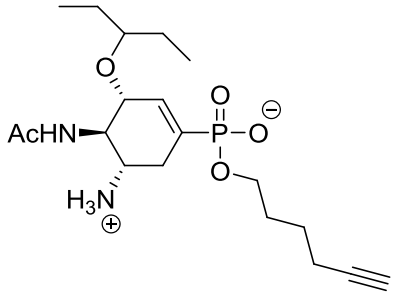
General

Reaction solvents were purchased anhydrous and used as received. Solvents for chromatography were distilled before use. Reactions were monitored by TLC using precoated silica gel 60 F_{254} plates. Compounds were detected by UV absorption and/or by staining with a molybdenum phosphate reagent (20 g ammonium molybdate and 0.4 g cerium(IV) sulfate in 400 mL of 10% aq. sulfuric acid) or a basic $KMnO_4$-solution and subsequent heating at 120 °C for 5 min. Silica gel 60 A 'Davisil' (particle size 35-70 μm) from Fisher Scientific, UK and silica gel 100 C18 reversed phase (particle size 40-63 μm) from Fluka Analytical, UK were used for flash chromatography. ^{1}H NMR, ^{13}C NMR, ^{31}P NMR and all multidimensional NMR spectra were recorded on Varian VNMRS spectrometers (600 MHz, 500 MHz or 400 MHz, see compound characterisation for individual experiments). Chemical shifts in ^{1}H NMR and ^{13}C NMR spectra were referenced to the residual proton resonance of the respective deuterated solvent, $CDCl_3$ (7.26 ppm), D_2O (4.80 ppm), CD_3OD (3.31 ppm). For ^{31}P NMR spectra H_3PO_4 was used as external standard (0 ppm). In some cases, ^{13}C chemical shifts were deduced from heteronuclear multiple spin correlation (HSQC) spectra. The H-6_{ax} and H-6_{eq} assignments refer to the pseudoaxial and pseudoequatorial protons in the cyclohexene systems, respectively, obtained by ROESY spectroscopy. HR-ESI MS spectra were recorded on a Bruker Daltonics Apex III in positive mode with MeOH/H_2O as solvent. Fluorescence was measured in a JASCO FP-6300 fluorimeter. Silica-based MPLC chromatography was carried out on the Büchi Sepacore system equipped with glass columns packed with LiChroprep Si 60 (15-25 μm) from Merck, Darmstadt, Germany. Gel permeation chromatography was carried out in the 1-10 mg scale on a XK 16/70 column (bed volume

130 mL), from Amersham packed with Sephadex G-10 (particle size 40-120 μm) and 0.1 M NH_4HCO_3 as buffer. Detection was achieved with a differential refractometer from Knauer, Berlin, Germany. Fine chemicals were purchased from Aldrich-, Sigma- or Acros-Chemicals and were of the highest purity available. Abbreviations: THF (tetrahydrofuran), TIPS (trisiopropylsilyl), EA (ethyl acetate), Tol (toluene), TFA (trifluoroacetic acid), TBAF (tetrabutylammonium fluoride), DMAP (*p*-dimethylaminopyridine), DMF (dimethyl formamide), MUNANA (2'-(4-methylumbelliferyl)-α-D-*N*-acetylneuraminic acid) DE (diethyl ether), DCM (dichloromethane), AcOH (acetic acid), MeOH (methanol), TLC (thin layer chromatography).

Syntheses

(Hex-5-yn-1-yl) (3*R*,4*R*,5*S*)-4-acetamido-5-amino-3-(1-ethylpropoxy)-1-cyclohexene-1-phosphonic acid (3a)



Hexyne-conjugate **12** (15 mg, 0.0291 mmol) and sodium iodide (44 mg, 0.291 mmol) were dried *in vacuo* in the absence of light for 20 minutes and then placed under an N_2 (g) atmosphere. Dry acetone (1 mL) was then added to the reaction flask followed by ultrasonication. The solution was then refluxed over 24 hours by which time TLC had indicated the absence of starting material and the formation of a new baseline spot. The resulting crude product was purified over a short silica plug (EA → EA/MeOH; 1:1) to give the demethylated product (12.5mg, 86%). This demethylated product (6 mg, 0.0120 mmol) was then stirred in a solution of TFA/H_2O (1:1) (1 mL) and 1,4-dioxane (0.2 mL) overnight. The solvent was then evaporated *in vacuo* and the residue was purified by gel permeation chromatography to afford compound target compound **3a** (4.1 mg, 85%).
^{1}H NMR (600 MHz, MeOH-D_4) δ_H: 0.85 – 0.95 (6 H, m, -OCH($CH_2C\underline{H}_3$)$_2$), 1.47 – 1.57 (4 H, m, -OCH($C\underline{H}_2CH_3$)$_2$), 1.58 – 1.65 (2 H, m, -$C\underline{H}_2CH_2C{\equiv}CH$), 1.70 – 1.77 (1 H, m, -$OCH_2C\underline{H}_2$-), 2.03 (3 H, s, -NHCOC$\underline{H}_3$), 2.15 – 2.27 (3 H, m, -$CH_2C{\equiv}C\underline{H}$, -$C\underline{H}_2C{\equiv}CH$), 2.39 (1 H, m, H_{6ax}), 2.78 (1 H, m, H_{6eq}), 3.36 – 3.45 (2 H, m, -OC$\underline{H}$(CH_2CH_3)$_2$, H_5), 3.81 (2 H, dt,

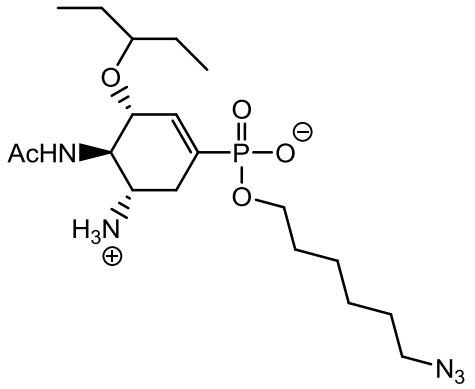
J = 6.2, 6.4 Hz, -OC$\underline{H_2}$CH$_2$-), 3.95 (1 H, dd, J = 8.6, 8.6 Hz, H$_4$), 4.10 (1 H, d, J = 7.3 Hz, H$_3$), 6.40 (1 H, d, J = 18.7 Hz, H$_2$);

^{13}C NMR (150.8 MHz, MeOH-D$_4$) δ_C: 9.58, 9.89 (-OCH(CH$_2\underline{C}$H$_3$)$_2$), 18.75 (-$\underline{C}$H$_2$C≡CH), 23.12 (-NHCO$\underline{C}$H$_3$), 26.20 (-$\underline{C}$H$_2$CH$_2$≡CH), 26.64, 27.31 (-OCH($\underline{C}$H$_2$CH$_3$)$_2$), 30.62 (d, J = 10.5 Hz, C6), 31.03 (d, J = 7.1 Hz, -OCH$_2\underline{C}$H$_2$-), 51.49 (d, J = 13.2 Hz, C5), 54.63 (C4), 65.03 (d, J = 5.3 Hz, -O$\underline{C}$H$_2$-), 69.73 (-C≡$\underline{C}$H), 76.39 (d, J = 18.8 Hz, C3), 83.28 (-O$\underline{C}$H(CH$_2$CH$_3$)$_2$), 84.78 (-$\underline{C}$≡CH), ~132.4 (d, J_{P-1} = 174 Hz, C1), 136.34 (d, J_{P-2} = 6.4 Hz, C2), 174.66 (-NH$\underline{C}$OCH$_3$);

^{31}P NMR (242.7 MHz, MeOH-D$_4$) δP: 8.05 (s).

HR-ESI-MS (m/z) calculated for $C_{19}H_{33}N_2O_5P$ $(M+Na)^+$ 423.2019, found 423.2038.

(6-Azido hexyl) (3*R*,4*R*,5*S*)-4-acetylamino-5-amino-3-(1-ethylpropoxy)-1-cyclohexene-1-phosphonic acid (3b)



Azido-conjugate **11** (18 mg, 0.0322 mmol) and sodium iodide (50 mg, 0.333 mmol) were dried *in vacuo* in the absence of light for 20 minutes and then placed under an N_2 (g) atmosphere. Dry acetone (1 mL) was then added to the reaction flask followed by ultrasonication. The solution was then refluxed over 24 hours by which time TLC had indicated the absence of starting material and the formation of a new baseline spot. The resulting crude product was purified over a short silica plug (EA → EA/MeOH; 1:1) to give the demethylated product (10 mg, 58%). This demethylated product (10 mg, 0.0183 mmol) was then stirred in a solution of TFA/H_2O (1:1) (1.2 mL) and 1,4-dioxane (0.8 mL) overnight. The solvent was then evaporated *in vacuo* and the residue was purified by gel permeation chromatography to afford target compound **3b** (7.4 mg, 91%).

^{1}H NMR (600 MHz, D_2O) δ_H: 0.87 (3 H, t, J = 7.4 Hz, -OCH(CH$_2$C$\underline{H_3}$)$_2$), 0.92 (3 H, t, J = 7.4 Hz, -OCH(CH$_2$C$\underline{H_3}$)$_2$), 1.40 – 1.45 (4 H, bm, -C$\underline{H_2}$C$\underline{H_2}$CH$_2$CH$_2$N$_3$), 1.46 – 1.52, 1.53 – 1.61 (4 H, m, -OCH(C$\underline{H_2}$CH$_3$)$_2$), 1.54 – 1.61 (4 H, m, -OCH$_2$C$\underline{H_2}$-, N$_3$CH$_2$C$\underline{H_2}$-), 2.10 (3 H, s, -

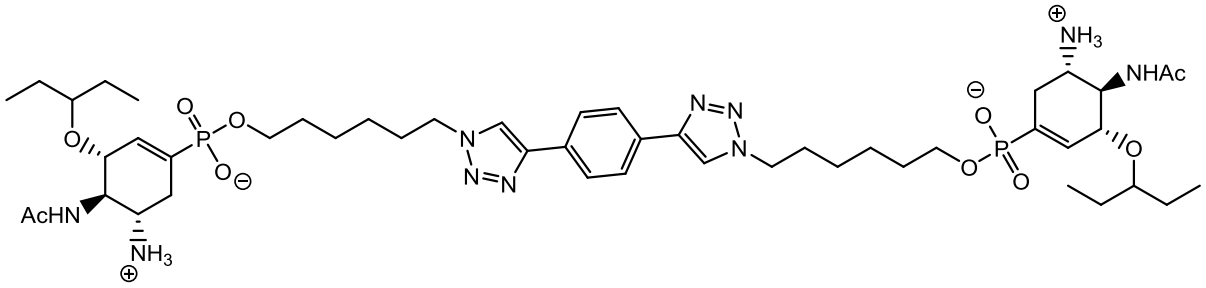
NHCOC$\underline{H}_3$), 2.40 (1 H, m, H_{6ax}), 2.75 (1 H, m, H_{6eq}), 3.35 (2 H, t, J = 6.9 Hz, $N_3C\underline{H}_2CH_2$-), 3.40 – 3.50 (1 H, m, H_5), 3.55 (1 H, dd, J = 5.6, 11.4 Hz, -OC$\underline{H}$(CH_2CH_3)$_2$), 3.83 (2 H, m, -O$C\underline{H}_2$-), 4.00 (1 H, m, H_4), 4.26 (1 H, m, H_3), 6.35 (1 H, d, J_{P-2} = 19.3 Hz, H_2);

^{13}C NMR (150.8 MHz, D_2O) δ_C: 8.47, 8.54 (-OCH($CH_2\underline{C}H_3$)$_2$), 22.26 (-NHCO$\underline{C}H_3$), 24.55, 25.14, 25.47, 25.56, 27.82, 29.70, 29.75 (-O$CH_2CH_2\underline{C}H_2\underline{C}H_2CH_2CH_2N_3$, -OCH($\underline{C}H_2CH_3$)$_2$, $N_3CH_2\underline{C}H_2$-, -O$CH_2\underline{C}H_2$-), ~30.81 (m, C6), ~49.6 (d, J = 13.7 Hz, C5), 51.07 ($N_3\underline{C}H_2$-), 54.61 (C4), 65.17 (m, -O$\underline{C}H_2$-), ~76.2 (m, C3), ~84.0 (m, -O$\underline{C}$H(CH_2CH_3)$_2$), ~130.6 (d, J_{P-1} = 171.6 Hz, C1), 136.25 (m, C2), 160.15, 174.94 (-NH$\underline{C}$OCH_3);

^{31}P NMR (242.7 MHz, D_2O) δP: 12.77 (s).

HR-ESI-MS (m/z) calculated for $C_{19}H_{36}N_2O_5P$ $(M+Na)^+$ 468.2346, found 468.2355.

1,4-Bis [1-(hex-1'-yl [(*3R,4R,5S*)-4-acetamido-5-amino-3-(1-ethylpropoxy)-1-cyclohexene-1-phosphonic acid])-1*H*-1,2,3-triazol-4-yl]-benzene (3c)



Compound **13** (20 mg, 0.016mmol) and NaI (24 mg, 0.016mmol) were refluxed in dry acetone (2 mL) under an atmosphere of dry nitrogen. After approximately 72 hours stirring under reflux, the solvent was evaporated and the crude product purified (silica plug; EA→MeOH) to give the demethylated intermediate (17 mg, 87%). R_f: 0.15 (EA:MeOH; 1:1). The deprotected intermediate (12 mg, 0.010mmol) was then dissolved in a 50% TFA/H_20 solution (1 mL) with dioxane (0.1 mL). The solution was left to stir for 48 hours and was observed to turn a bright orange colour before the solvents were evaporated *in vacuo*. The product was then purified by gel permeation chromatography, and was lyophilised to give compound **3c** (7 mg, 67 %). R_f: 0.25 (MeOH:EA; 1:1).

^{1}H NMR (500 MHz, MeOH-D_4) δ_H: 0.83 - 0.91 (12 H, m, -OCH($CH_2C\underline{H}_3$)$_2$), 1.35 - 1.43 (4 H, m, -$CH_2\underline{C}H_2CH_2CH_2$N-), 1.43 - 1.55 (12 H, m, -OCH($C\underline{H}_2CH_3$)$_2$, -$OCH_2CH_2\underline{C}H_2$-), 1.59 - 1.67 (4 H, m, -$OCH_2C\underline{H}_2$-), 1.94 - 2.01 (-$C\underline{H}_2CH_2$N-), 2.03 (6 H, s, -COC$\underline{H}_3$), 2.39 (2 H, m, H_{6ax}), 2.78 (2 H, m, H_{6eq}), 3.36 - 3.43 (4 H, m, H_5, H_7), 3.80 (4 H, dt, J = 6.1, 6.2 Hz, -O$C\underline{H}_2CH_2$-), 3.95 (2 H, dd, J = 8.3, 8.4 Hz, H_4), 4.09 (2 H, d, J = 7.4 Hz, H_3), 4.47 (4 H, t, J =

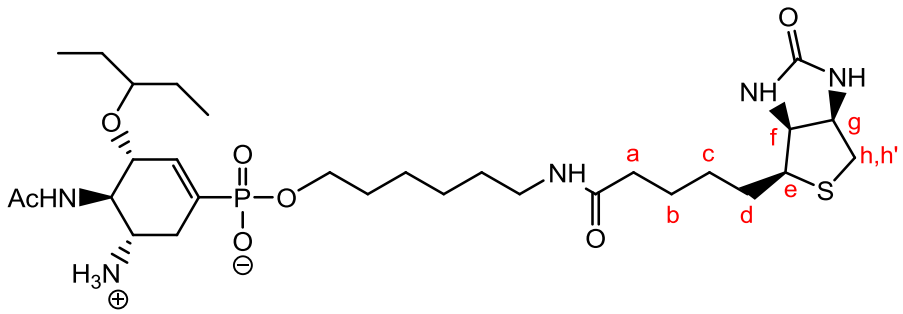
6.9 Hz, -CH_2CH_2N-), 6.38 (2 H, d, J_{P-2} = 18.6 Hz), 7.91 (4 H, s, H-C_{aryl}), 8.40 (2 H, s, H-C=C- triazole);

^{13}C NMR (100.5 MHz, MeOH-D_4) δ_C: 9.58, 9.89 (-OCH(CH_2CH_3)$_2$), 23.15 (-NHCOCH_3), 26.36, 26.65, 27.18, 27.30 (-OCH(CH_2CH_3)$_2$, -$CH_2CH_2CH_2CH_2$N-), ~30.71 (m, C6), 31.19 (-CH_2CH_2N-), ~31.77 (d, J = 6.7 Hz, -OCH_2CH_2), 51.45 (-CH_2N-), 51.58 (C5), 54.69 (C4), ~65.40 (d, J = 5.1 Hz, -OCH_2-), 76.36, 76.55 (C3), 83.29 (-OCH(CH_2CH_3)$_2$), 122.48 (H-C=C- triazole), 127.20 (H-C_{aryl}), 131.68 (C_{aryl}), ~132.41 (d, J_{P-1} = 176 Hz, C1), ~136.19 (d, J_{P-2} = 6.6 Hz, C2), 148.35 (H-C=C- triazole), 174.62 (-NHCOCH$_3$);

^{31}P NMR (161.7 MHz, $CDCl_3$) δ_P: 12.28 (s).

HR-ESI-MS (m/z) calculated for $C_{48}H_{79}N_{10}O_{10}P_2$ $(M+H)^+$ 1017.5450, found 1017.5491.

(6-*d*-Biotinamidohex-1-yl) (3*R*,4*R*,5*S*)-4-acetamido-5-amino-3-(1-ethylpropoxy)-1-cyclohexene-1-phosphonic acid (3d)



Protected biotin-conjugate **14** (8.4 mg, 0.0110 mmol) and sodium iodide (30 mg, 0.200 mmol) were dried *in vacuo* in the absence of light and then placed under an N_2 (g) atmosphere. Dry acetone (1 mL) was then added to the reaction flask followed by ultrasonication. The solution was then refluxed over 36 hours (maintaining the volume of acetone in the reaction flask) by which time TLC had indicated the absence of starting material. The resulting crude product was purified over a short silica plug (DCM/MeOH; 10:1 + 3% AcOH) to give the demethylated product (7 mg, 87%). The demethylated product (7 mg, 0.00938 mmol) was then stirred in a solution of TFA/H_2O (1:1) (1 mL) overnight. The solvent was then evaporated *in vacuo* and the residue was purified by gel permeation chromatography to afford target compound compound **3d** (5 mg, 74%).

^{1}H NMR (600 MHz, MeOH-D_4) δ_H: 0.87, 0.92 (6 H, 2t, J = 7.4 Hz, -OCH(CH_2CH_3)$_2$), 1.34 – 1.79 (18 H, m, -OCH(CH_2CH_3)$_2$, Hb, Hc, Hd, -$OCH_2CH_2CH_2CH_2CH_2CH_2$NH-), 2.11 (3 H, s, -NHCOCH_3), 2.27, (2 H, t, J = 7.1 Hz, Ha), 2.48 (1 H, m, H_{6ax}), 2.77 – 2.84 (1 H, m, H_{6eq}),

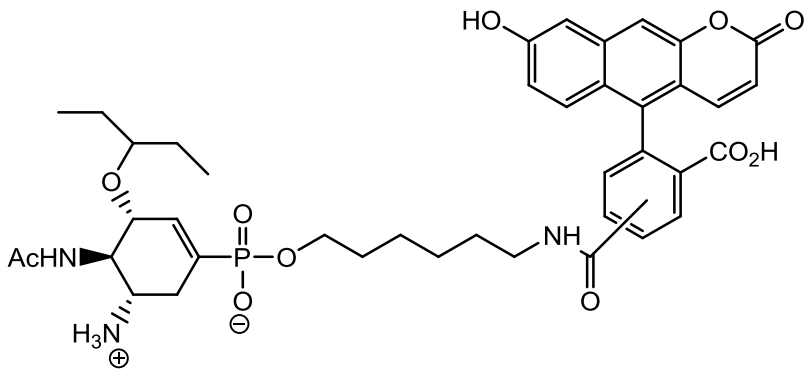
2.81 (1 H, d, J = 13.1 Hz, Hh'), 3.02 (1 H, dd, J = 5.0, 13.2 Hz, Hh), 3.21 (2 H, t, J = 6.8 Hz, -CH_2CH_2NH-), 3.36 (1 H, dd, J = 4.7, 4.8 Hz, He), 3.54 – 3.61 (2 H, m, H_5, -$OCH(CH_2CH_3)_2$), 3.83 (2 H, m, -OCH_2CH_2-), 4.07 (1 H, dd, J = 9.0, 9.2 Hz, H_4), 4.28 (1 H, d, J = 8.5 Hz, H_3), 4.45 (1 H, dd, J = 4.4, 4.5 Hz, Hf), 4.64 (1 H, dd, J = 4.9, 5.0 Hz, Hg), 6.35 (1 H, d, J_{P-2} = 19.3 Hz, H_2);

^{13}C NMR (150.8 MHz, MeOH-D_4) δ_C: 8.46, 8.60 (-$OCH(CH_2CH_3)_2$), 22.28 (-$NHCOCH_3$), 24.76, 25.12, 25.18, 25.50, 25.77, 27.66, 27.84, 28.22 (Cb, Cc, Cd, -$OCH_2CH_2CH_2CH_2$-CH_2CH_2NH-, -$OCH(CH_2CH_3)_2$), 29.25 (m, C6), 29.84 (-OCH_2CH_2-), 35.48 (Ca), 39.20 (-CH_2CH_2NH-), 39.63 (Ch,h'), ~49.6 (d, J = 14.2 Hz, C5), 52.93 (C4), 55.39 (Ce), 60.17 (Cg), 62.06 (Cf), 65.35 (m, -OCH_2CH_2-), ~75.8 (m, C3), 84.05 (-$OCH(CH_2CH_3)_2$), ~130.0 (d, J_{P-1} = 174 Hz, C1), 135.80 (C2), 165.25 (-NH-C(O)-NH-), 175.12 (-$NHCOCH_3$), 176.48 (-CH_2-C(O)NH-CH_2-);

^{31}P NMR (242.7 MHz, MeOH-D_4) δP: 12.44 (s).

HR-ESI-MS (m/z) calculated for $C_{29}H_{52}N_5O_7PS$ $(M+Na)^+$ 668.3217, found 668.3244.

(6-(Fluoresceinyl-5,6-carbonylamino)-hex-1-yl) (3*R*,4*R*,5*S*)-4-acetylamino-5-amino-3-(1-ethylpropoxy)-1-cyclohexene-1-phosphonic acid (3e)



Protected fluorescein-conjugate **15** (9 mg, 0.0101 mmol) and sodium iodide (15 mg, 0.0101 mmol) were dried *in vacuo* in the absence of light and then placed under an N_2 (g) atmosphere. Dry acetone (1 mL) was then added to the reaction flask followed by ultrasonication. The solution was then refluxed overnight by which time TLC had indicated the absence of starting material. The resulting crude product was purified over a short silica plug (EA/MeOH; 10:1 + 3% AcOH → 1:1 + 3% AcOH) to give the demethylated product (7 mg, 80%). This demethylated product (6 mg, 0.00673 mmol) was then stirred in a solution of TFA/H_2O (1:1) (1 mL) overnight. The solvent was then evaporated *in vacuo* and the residue

was purified by reversed-phase silica chromatography (short-plug) (MeOH/H_2O; 1:1 → 3:1) to afford target compound **3e** (5 mg, 94%).

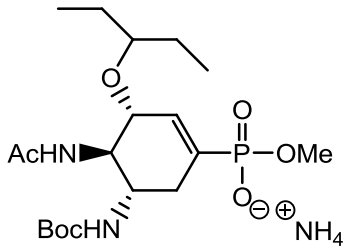
^{1}H NMR (600 MHz, MeOH-D_4) δ_H: 0.87 (6 H, m, -OCH($CH_2C\underline{H}_3$)$_2$), 1.40-1.60 (8 H, m, -OCH($C\underline{H}_2CH_3$)$_2$, -$OCH_2CH_2C\underline{H}_2C\underline{H}_2CH_2CH_2NH$-), 1.61 – 1.70 (4 H, m, -$OCH_2C\underline{H}_2$-$CH_2CH_2C\underline{H}_2CH_2NH$-), 1.97, 2.00 (3 H, 2s, -$NHCOC\underline{H}_3$), 2.38 (1 H, m, H_{6ax}), 2.76 (1 H, m, H_{6eq}), 3.26 – 3.45 (2 H, bm, -$CH_2C\underline{H}_2NH$- (partially obscured by MeOH)), 3.35 - 3.45 (2 H, m, H_7, H_5), 3.73 – 3.83 (2 H, m, -$OC\underline{H}_2CH_2$-), 3.94 (1 H, m, H_4), 4.08 (1 H, m, H_3), 6.38 (1 H, m, H_2), 6.50 – 8.42 (9 H, m, $\underline{H}$-Fluoroscein);

^{13}C NMR (150.8 MHz, MeOH-D_4) δ_C: 9.58, 9.53 (-OCH($CH_2\underline{C}H_3$)$_2$), 23.14 (-$NHCO\underline{C}H_3$), 26.25, 27.32, 27.69, 30.32, 30.35, 31.86 (-$OCH_2\underline{C}H_2\underline{C}H_2\underline{C}H_2\underline{C}H_2CH_2NH$-, -OCH($\underline{C}H_2CH_3$)$_2$), 30.73 (m, C6), 41.13 (-$CH_2\underline{C}H_2NH$-), ~51.5 (d, J = 12.0 Hz, C5), 54.67 (C4), 65.46 (-$O\underline{C}H_2CH_2$-), ~76.5 (m, C3), 83.26 (-$O\underline{C}H(CH_2CH_3)_2$), 103.66 ($\underline{C}$H(Fluorescein)), 111.40, 114.51 ($\underline{C}$H(Fluorescein)), 124.49, 125.26, 126.21, 126.67 ($\underline{C}$H(Fluorescein)), 130.37, 131.91, 133.06, 134.85 (C1, $\underline{C}$H(Fluorescein)), ~136.3 (m, C2), 137.97, 154.54, 168.42 (-$\underline{C}$(O)NH-CH_2-), 170.81, 174.65 (-$NH\underline{C}OCH_3$);

^{31}P NMR (242.7 MHz, MeOH-D_4) δP: 8.14 (s).

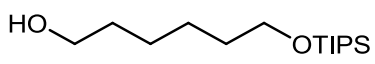
HR-ESI-MS (m/z) calculated for $C_{40}H_{48}N_3O_{11}P$ $(M+Na)^+$ 800.2919, found 800.2919; calculated for $C_{40}H_{48}N_3O_{11}P$ $[M+(2Na-H)]^+$ 822.2738, found 822.2767.

Ammonium [methyl (3*R*,4*R*,5*S*)-4-acetamido-5-(1,1-dimethylethyloxycarbonylamino)-3-(1-ethylpropoxy)-1-cyclohexene-1-phosphonate] (4)



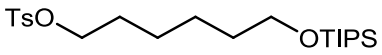
Monoester **4** was synthesised from the 'acetamido-azide' precursor as published previously.[1]

***O*-Triisopropylsilylhexane-1,6-diol (5)**



1,6-Hexanediol (2 g, 0.0169 mmol) and imidazole (3.46 g, 0.0507 mmol) were dried *in vacuo* in a round-bottomed flask for 1 hour, then placed under an N_2 (g) atmosphere. Dry DCM (7 mL) was added and the resulting suspension was briefly sonicated. The suspension was cooled to 0°C (ice-bath) and TIPSCl (4.4 mL, 0.0253 mmol) was then added dropwise. Once TLC had indicated that the reaction was no longer progressing, the solvent was evaporated to dryness and the crude product was purified by column chromatography (Tol/EA; 1:0 → 10:1) to give **5** as a clear oil (1.82 g, 40%). R_f : 0.23 (Tol/EA; 10:1).
^{1}H NMR (500 MHz, $CDCl_3$) δ_H: 1.02 – 1.11 (21 H, m, -Si(C<u>H</u>$(CH_3)_2)_3$, -Si(CH(C<u>H</u>$_3)_2)_3$), 1.38 (4 H, m, -C<u>H</u>$_2$C<u>H</u>$_2$CH$_2$CH$_2$OSi-), 1.57 (4 H, m, HOCH$_2$C<u>H</u>$_2$CH$_2$CH$_2$C<u>H</u>$_2$CH$_2$O-), 3.62 – 3.70 (4 H, m, HOC<u>H</u>$_2$-, -C<u>H</u>$_2$OSi-);
^{13}C NMR (125.7 MHz, $CDCl_3$) δ_C: 12.21 (-Si(<u>C</u>H$(CH_3)_2)_3$), 18.19 (-Si(CH(<u>C</u>H$_3)_2)_3$), 25.74, 25.82 (HOCH$_2$CH$_2$<u>C</u>H$_2$<u>C</u>H$_2$CH$_2$CH$_2$O-), 32.99, 33.13 (HOCH$_2$<u>C</u>H$_2$CH$_2$CH$_2$<u>C</u>H$_2$CH$_2$CH$_2$O-), 63.18, 63.51 (HO<u>C</u>H$_2$- , -<u>C</u>H$_2$OSi-).
HR-ESI-MS (m/z) calculated for $C_{15}H_{34}O_2Si$ $(M+Na)^+$ 297.2220, found 297.2218.

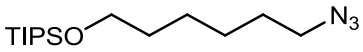
_O_-Toluenesulfonyl-_O_-triisopropylsilylhexane-1,6-diol (6)



Under an atmosphere of dry nitrogen, alcohol **5** (3.86 g, 14.06 mmol) was dissolved in dry pyridine (12 mL). DMAP (172 mg, 1.41 mmol) and *p*-toluenesulfonyl chloride (4.02 g, 21.09 mmol) were added to the solution and the mixture stirred a 0°C for 4 hours. The reaction was quenched by addition of saturated aqueous NH_4Cl (5 mL). After evaporation to dryness, the residue was dissolved in DCM (15 mL) which was then extracted with NH_4Cl (5 mL), washed with brine (2x5 mL), the organic phase was dried over $MgSO_4$ and the solvent evaporated. Purification by flash chromatography (cyclohexane/DE; 10:1) gave compound **6** (4.79 g, 80%) as a clear oil. R_f : 0.27 (cyclohexane/DE; 10:1).
^{1}H NMR (500 MHz, $CDCl_3$) δ_H: 1.00-1.10 (21 H, m, -Si(C<u>H</u>$(CH_3)_2)_3$, -Si(CH(C<u>H</u>$_3)_2)_3$), 1.27 – 1.35 (4 H, m, HOCH$_2$CH$_2$C<u>H</u>$_2$C<u>H</u>$_2$-), 1.48 (2 H, m, -C<u>H</u>$_2$CH$_2$OSi-), 1.64 (2 H, m, -C<u>H</u>$_2$CH$_2$OTs), 2.44 (3 H, s, <u>H</u>$_3$C-Ph-), 3.63 (2 H, t, J = 6.4 Hz, -CH$_2$OSi-), 4.02 (2 H, t, J = 6.4, 6.5 Hz, Ts-OCH$_2$-), 7.33 (2 H, d, J = 8.0 Hz, H_{Aryl}), 7.78 (2 H, d, J = 8.1 Hz, H_{Aryl});

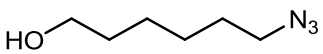
^{13}C NMR (125.7 MHz, $CDCl_3$) δ_C: 12.15 (-Si(CH(CH_3)$_2$)$_3$), 18.16 (-Si(CH(CH_3)$_2$)$_3$), 21.73 (CH_3-Ph-), 25.36, 25.40 (TsOCH$_2$CH$_2$CH$_2$CH$_2$CH$_2$CH$_2$O-), 29.01, 32.86 (TsOCH$_2$CH$_2$CH$_2$CH$_2$CH$_2$CH$_2$O-), 63.31 (-CH$_2$OSi-),70.75 (TsOCH$_2$-), 128.00, 129.91 (CH$_{Aryl}$), 133.50, 144.71 (C$_{Aryl}$);
HR-ESI-MS (m/z) calculated for $C_{22}H_{40}O_4SSi$ $(M+Na)^+$ 451.2296, found 451.2309.

6-Azido-*O*-triisopropylsilylhexane-1-ol (7)



Compound **6** (59 mg, 0.138 mmol) was placed under an N_2 (g) atmosphere and dissolved in DMF (1.0mL). The solution was cooled to 0°C and NaN_3 (27 mg, 0.414 mmol) was then quickly added. The solution was stirred for 4 hours at which time the reaction was complete. The DMF was evaporated *in vacuo* and the resulting residue was suspended in DCM and washed with cold H_2O, semi-saturated NH_4Cl (aq.) and brine. The organic phase was dried over $MgSO_4$ and purified by flash chromatography to give azide **7** as an oily liquid (35 mg, 84%). R_f : 0.84 (DE:cyclohexane; 1:10).
^{1}H NMR (500 MHz, $CDCl_3$) δ_H: 1.03, 1.04 (21 H, 2s, -Si(CH(CH_3)$_2$)$_3$, 1.38 (4 H, m, -OCH$_2$CH$_2$CH$_2$CH$_2$CH$_2$CH$_2$-N$_3$), 1.53 (2 H, m, -OCH$_2$CH$_2$-), 1.60 (2 H, m, N$_3$CH$_2$CH$_2$-), 3.24 (2 H, t, J = 7.0 Hz, N$_3$CH$_2$-), 3.66 (2 H, t, 6.4 Hz, -OCH$_2$-);
^{13}C NMR (125.7 MHz, $CDCl_3$) δ_C: 12.27, 18.25 -Si(CH(CH_3)$_2$)$_3$, 25.67 (-OCH$_2$CH$_2$-CH$_2$CH$_2$CH$_2$CH$_2$N$_3$), 26.80 (-Si(CH(CH_3)$_2$)$_3$), 29.09 (N$_3$CH$_2$CH$_2$-), 33.05 (-OCH$_2$CH$_2$-), 51.69 (N$_3$CH$_2$-), 63.46 (-OCH$_2$-);
HR-ESI-MS (m/z) calculated for $C_{15}H_{33}N_3OSi$ $(M+Na)^+$ 322.2285, found 322.2286.

6-Azido-1-hexanol (8)



Compound **7** (489 mg, 1.63 mmol) was dissolved in THF (11.0 mL), then AcOH (0.1 mL) and TBAF.3H_2O (1.54 g, 4.89 mmol) were added to the solution and stirred overnight. On completion of the reaction, the solvent was evaporated *in vacuo* and the residue was suspended in DCM, washed with $NaHCO_3$ (aq.) and brine. The organic phase was dried over

$MgSO_4$, the solvent was removed and the residue was purified by flash chromatography to give **8** as a yellowish oil (212 mg, 90%). R_f : 0.17 (Tol:EA; 10:1).

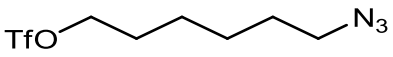
The physical data correspond to the literature.[2]

^{1}H NMR (500 MHz, $CDCl_3$) δ_H: 1.35 (bm, 4H, -$OCH_2CH_2C\underline{H_2}C\underline{H_2}CH_2CH_2N_3$), 1.53 (m, 2H, -$OCH_2C\underline{H_2}$-), 1.57 (m, 2H, $N_3CH_2C\underline{H_2}$-), 1.88 (bs, 1H, -O<u>H</u>), 3.22 (t, 2H, J = 6.8, 6.9 Hz, $N_3C\underline{H_2}$-), 3.58 (t, 2H, J = 6.6 Hz, -$OC\underline{H_2}$-).

^{13}C NMR (125.7 MHz, $CDCl_3$) δ_C: 25.47, 26.66 (-$OCH_2CH_2\underline{C}H_2\underline{C}H_2CH_2CH_2N_3$), 28.94 ($N_3CH_2\underline{C}H_2$-), 32.66 (-$OCH_2\underline{C}H_2$-), 51.52 ($N_3\underline{C}H_2$-), 62.78 (-$O\underline{C}H_2$-).

HR-ESI-MS (m/z) calculated for $C_6H_{13}N_3O$ $(M+Na)^+$ 166.0951, found 166.0952.

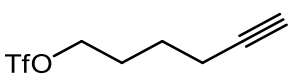
6-Azido-*O*-trifluoromethanesulfonyl-hexan-1-ol (9)



Alcohol **8** (55.9 mg, 0.390 mmol) was placed under an N_2 (g) atmosphere and suspended in dry DCM (1 mL). Lutidine (90 µL, 0.780 mmol) was then added and the solution was cooled to -40°C. Triflic anhydride (86 µL, 0.507 mmol) was added to dry DCM (1 mL), cooled to -40°C for a few minutes and then added dropwise to the stirring solution of **8**. The reaction was stirred at this temperature for 30 minutes by which time the reaction was complete. The DCM solution was then poured into a separating funnel containing cold saturated KH_2PO_4 (aq.) and washed. The organic layer was separated, dried over $MgSO_4$ and the solvent evaporated to give the desired triflate **9** as brown oil (94 mg, 87%) which was used without further purification.

^{1}H NMR (500 MHz, $CDCl_3$) δ_H: 1.46 (4 H, m, -$C\underline{H_2}C\underline{H_2}CH_2CH_2N_3$), 1.62 (2 H, tt, J = 6.8, 7.3 Hz, -$C\underline{H_2}CH_2N_3$), 1.85 (2 H, tt, J = 6.5, 6.9 Hz, -$C\underline{H_2}CH_2$OTf), 3.29 (2 H, t, J = 6.6 Hz, -$C\underline{H_2}N_3$), 4.54 (2 H, t, J = 6.5 Hz, -$C\underline{H_2}$OTf).

***O*-Trifluoromethanesulfonylhex-5-yn-1-ol (10)**

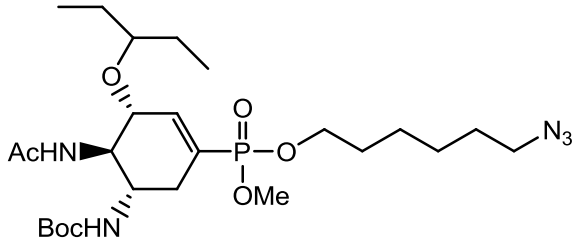


5-Hexyn-1-ol (55.2 µL, 0.509 mmol) was placed under an N_2 (g) atmosphere and suspended in dry DCM (1 mL). Lutidine (118 µL, 1.02 mmol) was then added and the solution was

cooled to -60°C. Triflic anhydride (103µL, 0.611 mmol) was added to dry DCM (1 mL), cooled to -60°C for a few minutes and then added dropwise to the stirring solution of the alcohol. The reaction was stirred at this temperature for 1 hour by which time the reaction was complete. The DCM solution was then poured into a separating funnel containing cold saturated KH_2PO_4 (aq.) and washed. The organic layer was separated, dried over $MgSO_4$ and the solvent evaporated to give the desired triflate **10** as a slightly coloured oil (113 mg, 96%) which was used without further purification.

^{1}H NMR (500 MHz, $CDCl_3$) δ_H: 1.64 – 1.73 (2 H, m, -C$\underline{H_2}$CH$_2$C≡CH), 1.94 – 2.02 (3 H, m, -OCH$_2$C$\underline{H_2}$CH$_2$CH$_2$C≡C$\underline{H}$), 2.29 (2 H, m, -CH$_2$C$\underline{H_2}$C≡CH), 4.48 (2 H, t, J = 6.2 Hz, -OC$\underline{H_2}$CH$_2$-), 5.30 (s, residual CH_2Cl_2).

Methyl (6-azidohex-1-yl) [(3*R*,4*R*,5*S*)-4-acetamido-5-(1,1-dimethylethyloxycarbonylamino)-3-(1-ethylpropoxy)-1-cyclohexene-1-phosphonate] (11)



Monoester **4** was converted into the triethylammonium salt by treatment with Amberlite IR-120 (H^+) ion exchange resin in water, filtration, addition of triethylamine and lyophilisation. The triethylammonium salt of **4** (35 mg, 0.0653 mmol) was then placed under an N_2 (g) atmosphere and dry acetonitrile (0.4 mL) was added. Azido triflate **9** (21 mg, 0.763 mmol) was dissolved in dry acetonitrile (0.3 mL) under an N_2 (g) atmosphere and then quickly added to the stirring solution of the triethylammonium salt of **4**. After an hour, TLC analysis indicated the reaction had not progressed any further and that the azide triflate was degrading in solution. The solvent was evaporated *in vacuo* and the residue was directly purified by flash chromatography (Tol:EA; 1:1 → EA → MeOH) to give **11** (17.0 mg, 72% based on recovered starting material). R_f: 0.54 (EA).

^{1}H NMR (500 MHz, $CDCl_3$) δ_H: 0.85 (6 H, dd, J = 7.5, 14.9 Hz, -OCH(CH$_2$C$\underline{H_3}$)$_2$), 1.37 - 1.42 (13 H, s/bm, -NHCOC(C$\underline{H_3}$)$_3$, -OC$\underline{H_2}$C$\underline{H_2}$CH$_2$CH$_2$N$_3$), 1.47 (4 H, m, -OCH(C$\underline{H_2}$CH$_3$)$_2$), 1.58 (2 H, dd, J = 6.3, 12.9 Hz, N$_3$CH$_2$C$\underline{H_2}$-), 1.66 (2 H, m, -OCH$_2$C$\underline{H_2}$-), 1.96 (3 H, s, -NHCOC$\underline{H_3}$), 2.17 (1 H, m, H_{6ax}), 2.58 (1 H, ddd, J = 5.9, 12.3, 17.3 Hz, H_{6eq}), 3.25 (2 H, dd,

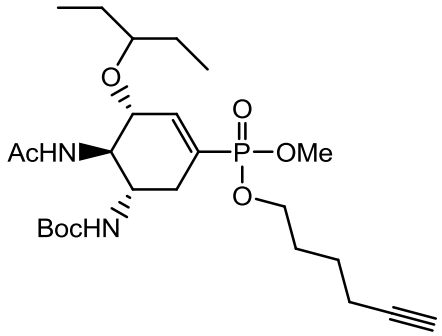
J = 6.8, 6.8 Hz, -C$\underline{H}_2$N$_3$), 3.31 (1 H, dd, J = 5.5, 11.1 Hz, -OC$\underline{H}$(CH$_2$CH$_3$)$_2$), 3.68 (3 H, dd, J = 1.8, 11.0 Hz, P-OC$\underline{H}_3$), 3.77 (1 H, ddd, J = 5.3, 9.8, 15.0 Hz, H$_5$), 3.90 (1 H, m, H$_3$), 3.94 - 4.08 (3 H, m, H$_4$, -OC$\underline{H}_2$-), 5.17 (1 H, d, J = 8.4 Hz, -N$\underline{H}$COC(CH$_3$)$_3$), 5.94, 6.00 (1 H, 2d, J = 9.0 Hz, -N$\underline{H}$COCH$_3$), 6.58 (1 H, d, $J_{P\text{-}2}$ = 21.7 Hz, H$_2$).

^{13}C NMR (100.5 MHz, CDCl$_3$) δ_C: 9.26, 9.68 (-OCH(CH$_2\underline{C}$H$_3$)$_2$), 23.48 (-NHCO$\underline{C}$H$_3$), 25.26, 25.71, 26.24, 26.42 (-OCH$_2$CH$_2\underline{C}$H$_2\underline{C}$H$_2$CH$_2$CH$_2$N$_3$, -OCH($\underline{C}$H$_2$CH$_3$)$_2$), 28.46 (-NHCOC(CH$_3$)$_3$), 28.86 (N$_3$CH$_2\underline{C}$H$_2$-), 30.46 (d, J = 6.0 Hz, -OCH$_2\underline{C}$H$_2$-), 31.44 (d, J = 9.3 Hz, C6), 49.35 (d, J = 14.6 Hz, C5), 51.46 (-$\underline{C}$H$_2$N$_3$), 52.48-52.77 (m, P-O$\underline{C}$H$_3$), 54.60 (m, C4), 66.02 (d, J = 6.0 Hz, -O$\underline{C}$H$_2$-), 76.28, 76.50 (C3), 79.87 (-NHCO$\underline{C}$(CH$_3$)$_2$), 82.31 (-O$\underline{C}$H(CH$_2$CH$_3$)$_2$), ~126.9 (d, $J_{P\text{-}1}$= 182 Hz, C1), 142.20 (d, $J_{P\text{-}2}$ = 6.8 Hz, C2), 156.40 (-NH$\underline{C}$OC(CH$_3$)$_3$), 170.97 (-NH$\underline{C}$OCH$_3$);

^{31}P NMR (161.7 MHz, CDCl$_3$) δP: 18.28 (s), 18.32 (s).

HR-ESI-MS (m/z) calculated for C$_{25}$H$_{46}$N$_5$O$_7$P (M+Na)$^+$ 582.3027, found 582.3058.

Methyl (hex-5-yn-1-yl) [(3*R*,4*R*,5*S*)-4-acetamido-5-(1,1-dimethylethyloxycarbonylamino)-3-(1-ethylpropoxy)-1-cyclohexene-1-phosphonate] (12)



Hexyne-triflate **10** (51 mg, 0.221 mmol) was placed under an N$_2$ (g) atmosphere and dry acetonitrile (0.7 mL) was added. The triethylammonium salt of **4** (80 mg, 0.184 mmol, repared as described for **11**) was then quickly added to the stirring solution. After an hour, TLC analysis indicated the reaction had not progressed any further and that the hexyne-triflate was degrading in solution. The solvent was evaporated *in vacuo* and the residue was directly purified by flash chromatography (Tol:EA; 1:1 → EA → MeOH) to give **12** (30.5 mg, 65% based on recovered starting material). R_f : 0.40 (EA).

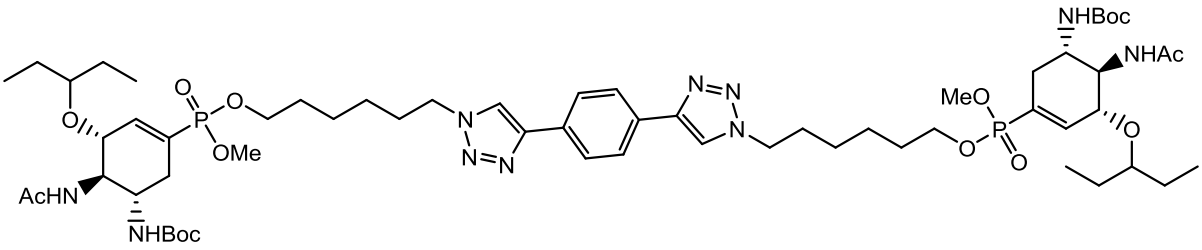
^{1}H NMR (500 MHz, $CDCl_3$) δ_H: 0.87 (6 H, m, -OCH(CH_2CH_3)$_2$), 1.40 (9 H, s, -NHCOC(CH_3)$_3$), 1.49 (4 H, m, -OCH(CH_2CH_3)$_2$), 1.61 (2 H, m, -CH_2CH_2C≡CH), 1.79 (2 H, m, -OCH_2CH_2-), 1.92 (1 H, bs, -CH_2C≡CH), 1.97 (3 H, s, -NHCOCH_3), 2.16-2.25 (3 H, m, -CH_2C≡CH, H_{6ax}), 2.59 (1 H, m, H_{6eq}), 3.33 (1 H, d, J = 5.0 Hz, -OCH(CH_2CH_3)$_2$), 3.69 (3 H, 2d, J = 10.9 Hz, P-OCH_3), 3.79 (1 H, m, H_5), 3.91 (1 H, m, H_3), 3.98 – 4.09 (3 H, m, -OCH_2CH_2-, H_4), 5.11 (1 H, d, J = 7.1 Hz, -NHCOCH_3), 5.85 (1 H, m, -NHCOC(CH_3)$_3$), 6.59 (1 H, d, J_{P-2} = 21.8 Hz, H_2);

^{13}C NMR (100.5 MHz, $CDCl_3$) δ_C: 9.27, 9.67 (-OCH(CH_2CH_3)$_2$), 18.07 (-CH_2CH_2C≡CH), 23.46 (-NHCOCH_3), 24.60 (-CH_2CH_2C≡CH), 25.71, 26.23 (-OCH(CH_2CH_3)$_2$), 28.46 (-NHCOC(CH_3)$_2$), ~29.5 (m, -OCH_2CH_2-), ~31.3 (d, J = 8.7 Hz, C6), ~49.3 (m, C5), ~52.6 (m, -P(OCH_3)$_2$), ~54.5 (m, C4), ~65.6 (m, -OCH_2-), 69.01, 69.07 (-C≡CH), ~76.3 (m, C3), 79.85 (-NHCOC(CH_3)$_2$), 82.30 (-OCH(CH_2CH_3)$_2$), 83.83 (-C≡CH), ~126.8 (2d, J_{P-1} = 182 Hz, C1), ~142.2 (d, J_{P-2} = 7.9 Hz, C2), 156.39 (-NHCOC(CH_3)$_2$), 170.97 (-NHCOCH$_3$);

^{31}P NMR (161.7 MHz, $CDCl_3$) δP: 18.30 (s).

HR-ESI-MS (m/z) calculated for $C_{25}H_{43}N_2O_7P$ $(M+Na)^+$ 537.2700, found 537.2709.

1,4-Bis [1-(hex-1'-yl methyl [(*3R,4R,5S*)-4-acetamido-5-(1,1 dimethylethyloxycarbonylamino)-3-(1-ethylpropoxy)-1-cyclohexene-1-phosphonate])-1*H*-1,2,3-triazol-4-yl]-benzene (13)



1,4-Diethynylbenzene (10.8 mg, 0.086 mmol) and CuI (12.2 mg, 0.064 mmol) were added to a round bottomed flask. Separately, compound **11** (120 mg, 0.214 mmol) was dissolved in toluene (2 mL). Under an atmosphere of dry nitrogen the solution of compound **11** was added to the flask. DIPEA (0.336mL, 1.923mmol) was then added, followed by sonication. The subsequent pale yellow solution was heated at 80^{o}C for 48 hours (and monitored by TLC). The solvent was then evaporated *in vacuo* and the crude mixture was purified by flash chromatography (EA:MeOH; 20:1 → 1:1) to give the desired product, compound **13** (80 mg, 77%). R_f: 0.42 (EA:MeOH; 10:1).

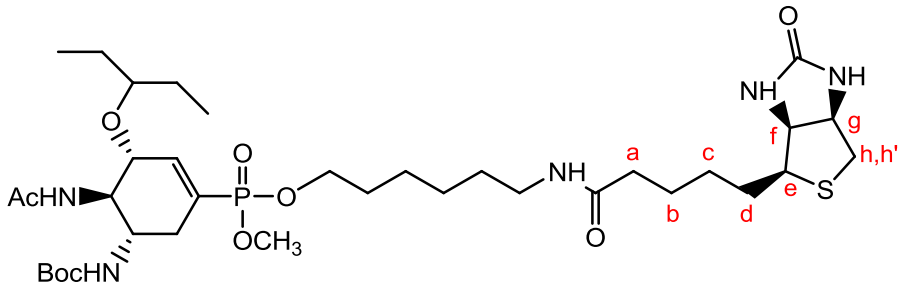
^{1}H NMR (500 MHz, $CDCl_3$) δ_H: 0.83 - 0.89 (12 H, m, -OCH(CH_2CH_3)$_2$), 1.38 -1.42 (18 H, m, -NHCOC(CH_3)), 1.37 - 1.54 (16 H, m, -CH(CH_2CH_3)$_2$, -$OCH_2CH_2CH_2CH_2CH_2CH_2$N-), 1.64 - 1.71 (4 H, m, -OCH_2CH_2-), 1.95 - 2.02 (10 H, m, -$COCH_3$, -CH_2CH_2N-), 2.21 (2 H, m, H_{6ax}), 2.62 (2 H, m, H_{6eq}), 3.33 (4 H, p, J = 4.9 Hz, -OCH(CH_2CH_3)$_2$), ~3.69 (2d, J = 11.0 Hz, -$POCH_3$), 3.81 (2 H, m, H_5), 3.95 (2 H, bm, H_3), 3.98 - 4.10 (6 H, m, -OCH_2CH_2-, H_4), 4.42 (4 H, t, J = 7.0 Hz, -CH_2CH_2N-), 5.18, 5.23 (2 H, 2d, J = 8.8 Hz, -NHCOC(CH_3)), 5.94, 6.06 (2 H, 2d, J = 8.9 Hz, -$NHCOCH_3$), 6.60 (2 H, d, J_{P-2} = 21.9 Hz, H_2), 7.85 (2 H, d, J = 8.7 Hz, H-C=C- triazole), 7.88 - 7.92 (4 H, m, H-C_{aryl});

^{13}C NMR (100.5 MHz, $CDCl_3$) δ_C: 9.09, 9.13, 9.50, 9.54 (-OCH(CH_2CH_3)$_2$), 23.30, 23.33 (-NHCOCH_3), 24.94, 24.99, 25.54, 25.57, 25.93, 25.96, 26.08 (-OCH(CH_2CH_3)$_2$, -$CH_2CH_2CH_2CH_2$N-), 28.31 (-NHCOC(CH_3)$_3$), 30.04, 30.09, 30.14, 30.18 (-OCH_2CH_2-, -CH_2CH_2N-), ~31.25 (m, C6), ~49.25 (m, C5), 50.21 (-CH_2N-), ~52.47 (m, -$POCH_3$), ~54.30, ~54.55 (2m, C4), ~65.79 (m, -OCH_2-), 75.96 - 76.35 (m, C3), 79.59, 79.68 (-NHCOC(CH_3)$_3$), 82.15, 82.18 (-OCH(CH_2CH_3)$_2$), 119.67, 119.69 (H-C=C- triazole), 126.10, 126.11 (H-C_{aryl}), ~126.70 (d, J_{P-1} = 182 Hz), 130.32, 130.37, 130.41 (C_{aryl}), ~142.17 (d, J_{P-2} = 7.8 Hz), 147.35, 147.38 (H-C=C- triazole), 156.18, 156.27 (-NHCOC(CH_3)$_3$), 170.91, 171.01 (-$NHCOCH_3$);

^{31}P NMR (161.7 MHz, $CDCl_3$) δ_P: 18.40 (bs).

HR-ESI-MS (m/z) calculated for $C_{60}H_{98}N_{10}O_{14}P_2$ $(M+Na)^+$ 1267.6706, found 1267.6631.

Methyl (6-*d*-biotinamidohex-1-yl) [(3*R*,4*R*,5*S*)-4-acetamido-5-(1,1-dimethylethyloxycarbonylamino)-3-(1-ethylpropoxy)-1-cyclohexene-1-phosphonate] (14)



Azido-conjugate **11** (26 mg, 0.0473 mmol) was placed under an N_2 (g) atmosphere and dry THF (1 mL) was added. PMe_3 (1M stock solution in THF, 61.5 µL) was then added dropwise to the stirring azido-conjugate solution. The reaction was monitored by TLC over the course

of 4 hours after which time the reaction was complete. Deionised water (0.5 mL) was then added and the solution was stirred for an additional 30 minutes. The solvent was then removed directly *in vacuo* and the crude product was placed on a short silica plug (DCM/MeOH; 10:1 +3% NEt_3) to give the isolated amino-conjugate without additional purification. d-Biotin (15.5 mg, 0.0633 mmol) and PyBOP (33 mg, 0.0633 mmol) were dried *in vacuo* and then placed under an N_2 (g) atmosphere. Dry DMF (1 mL) was then added followed by DIPEA (16 µL, 0.0974 mmol). The reaction flask was then sonicated briefly and cooled to 0°C (ice-bath). After a few minutes the isolated amino-conjugate (25 mg, 0.0487 mmol) was dissolved in dry DMF (1 mL) and was added dropwise to the stirring d-Biotin solution. The solution was allowed to warm slowly to room temperature over 5 hours after which time TLC indicated the reaction was complete. The solvent was evaporated directly *in vacuo* and then purified by chromatography (EA/MeOH; 10:1 + 2% AcOH → 2:1 + 2% AcOH) to give protected target compound **14** (23 mg, 90%). R_f: 0.36 (DCM/MeOH + 3% AcOH).

^{1}H NMR (500 MHz, MeOH-D_4) δ_H: 0.88, 0.92 (6 H, 2t, J = 7.1 Hz, -OCH($CH_2C\underline{H}_3$)$_2$), 1.43 (9 H, s, -NHCOC($C\underline{H}_3$)$_3$), 1.34 – 1.78 (18 H, m, -OCH($C\underline{H}_2CH_3$)$_2$, Hb, Hc, Hd, -$OCH_2C\underline{H}_2$-$C\underline{H}_2C\underline{H}_2$-$C\underline{H}_2CH_2$NH-), 1.96 (3 H, s, -NHCOC$\underline{H}_3$), 2.20 (2 H, dd, J = 6.9, 7.0 Hz, Ha), 2.18 – 2.27 (1 H, m, H_{6ax}), 2.51 (1 H, m, H_{6eq}), 2.71 (1 H, d, J = 12.7 Hz, Hh'), 2.93 (1 H, dd, J = 4.7, 12.6 Hz, Hh), 3.15-3.24 (3 H, m, -$CH_2C\underline{H}_2$NH-, He), 3.41 (1 H, m, -OC$\underline{H}$(CH_2CH_3)$_2$), 3.70 – 3.78 (1 H, m, H_5), 3.74 (3 H, d, J = 11.0 Hz, P-OC$\underline{H}_3$), 3.86 (1 H, dd, J = 9.5, 9.7 Hz, H_4), 4.01 – 4.08 (2 H, m, -$OC\underline{H}_2CH_2$-), 4.11 (1 H, m, H_3), 4.30, (1 H, dd, J = 4.3, 7.4 Hz, Hf), 4.49 (1 H, dd, J = 5.8, 6.0 Hz, Hg), 6.57 (1 H, d, $J_{P\text{-}2}$ = 21.8 Hz, H_2);

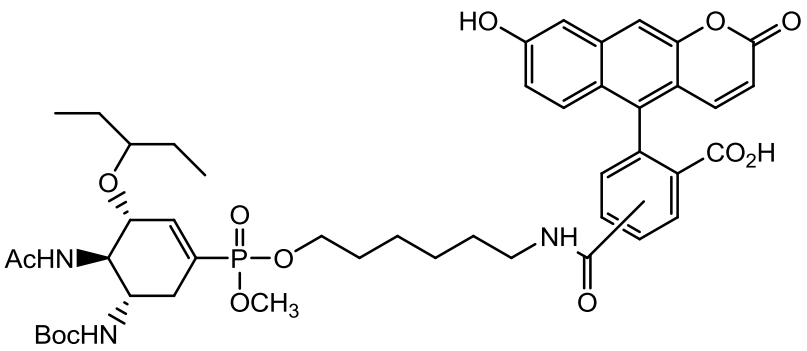
^{13}C NMR (100.5 MHz, MeOH-D_4) δ_C: 9.63, 9.93 (-OCH($CH_2\underline{C}H_3$)$_2$), 22.98 (-NHCO$\underline{C}H_3$), 26.34, 26.81, 26.94, 29.55, 29.80, 30.32 (Cb, Cc, Cd, -OCH_2CH_2-$\underline{C}H_2\underline{C}H_2$-$\underline{C}H_2CH_2$NH-), 27.28, 27.46 (-OCH($\underline{C}H_2CH_3$)$_2$), 28.73 (-NHCOC($\underline{C}H_3$)$_2$), ~31.4 (d, J = 5.9 Hz, -$OCH_2\underline{C}H_2$-), ~32.1 (m, C6), 36.86 (Ca), 40.23 (-$CH_2\underline{C}H_2$NH-), 41.07 (Ch,h'), ~50.4 (m, C5), ~53.4 (d, J = 5.8 Hz, -P(OC$\underline{H}_3$)$_2$), 56.10 (m, C4), 57.05 (Ce), 61.62 (Cg), 63.42 (Cf), 67.69 (m, -O$\underline{C}H_2CH_2$-), ~77.4 (m, C3), 80.35 (-NHCO$\underline{C}$(CH_3)$_2$), 83.82 (-O$\underline{C}$H(CH_2CH_3)$_2$), 124.29, ~127.4 (d, $J_{P\text{-}1}$ = 183 Hz, C1), 144.17 (m, C2), 157.99 (-NH$\underline{C}$OC(CH_3)$_2$), 166.07 (-NH-$\underline{C}$(O)-NH-), 173.75 (-NH$\underline{C}$OCH_3), 175.90 (-CH_2-$\underline{C}$(O)NH-CH_2-);

^{31}P NMR (161.7 MHz, MeOH-D_4) δP: 22.77 (s).

HR-ESI-MS (m/z) calculated for $C_{35}H_{62}N_5O_9PS$ $(M+Na)^+$ 782.3898, found 782.3900.

Methyl (6-(fluoresceinyl-5,6-carbonylamino)-hex-1-yl) [(3*R*,4*R*,5*S*)-4-acetamido-5-(1,1-

dimethylethyloxycarbonylamino)-3-(1-ethylpropoxy)-1-cyclohexene-1-phosphonate] (15)



Azido-conjugate **11** (16 mg, 0.0293 mmol) was placed under an N_2 (g) atmosphere and dry THF (1 mL) was added. PMe_3 (1M stock solution in THF, 40 µL) was then added dropwise to the stirring azido-conjugate solution. The reaction was monitored by TLC over the course of 4 hours after which time the reaction was complete. Deionised water (0.5 mL) was then added and the solution was stirred for an additional 30 minutes. The solvent was then removed directly *in vacuo* and the crude product was placed on a short silica plug (DCM/MeOH; 10:1 +3% NEt_3) to give the isolated amino-conjugate without additional purification. The amino-conjugate (10 mg, 0.0187 mmol) and NHS-Fluorescein (9 mg, 0.0206 mmol) were dried *in vacuo* in the absence of light then placed under an N_2 (g) atmosphere. Dry DMF (0.6 mL) was added to the flask (strong yellow coloured solution) to which was added dry triethylamine (~10 µL, 0.0748 mmol) (forming an orange coloured solution of pH~9). The solution was allowed to stir in the absence of light over 4 hours after which time the solvent was removed directly *in vacuo* (no heating). Chromatography of the crude product (EA + 3% AcOH → EA/MeOH; 10:1 + 3% AcOH) gave the desired protected target compound **15** as a yellow solid (15 mg, 83%). R_f : 0.58 (EA/MeOH; 10:1 + 3% AcOH).

^{1}H NMR (500 MHz, MeOH-D_4) δ_H: 0.82 – 0.93 (6 H, m, -OCH($CH_2C\underline{H}_3$)$_2$), 1.41 (9 H, s, -NHCOC($C\underline{H}_3$)$_3$), 1.30 – 1.55 (8 H, bm, -OCH($C\underline{H}_2CH_3$)$_2$, -$C\underline{H}_2C\underline{H}_2CH_2CH_2NH$-), 1.55 – 1.78 (4 H, m, -$OCH_2C\underline{H}_2CH_2CH_2C\underline{H}_2CH_2NH$-), 1.93 – 1.95 (3 H, 2s, -NHCOC$\underline{H}_3$), 2.23 (1 H, m, H_{6ax}), 2.52 (1 H, m, H_{6eq}), 3.23 – 3.36, 3.42 – 3.48 (2 H, bm, -$CH_2C\underline{H}_2$NH-(partially under MeOH resonance), 3.36 – 3.42 (1 H, m, -OC$\underline{H}$(CH_2CH_3)$_2$), 3.66 – 3.80 (3 H, 2d, J = 11.3 Hz, P-OC$\underline{H}_3$), 3.66 – 3.80 (1 H, m, H_5), 3.86 (1 H, dd, J = 9.5, 10.4 Hz, H_4), 3.98 – 4.16

(3 H, m, -OC$\underline{H_2}$CH$_2$-, H$_3$), 6.35 – 6.76 (7 H, m, H$_2$, $\underline{H}$-Fluoroscein), 7.24 – 8.48 (3 H, m, $\underline{H}$-Fluoroscein);

^{13}C NMR (100.5 MHz, MeOH-D$_4$) δ_C: 9.61, 9.92 (-OCH(CH$_2\underline{C}$H$_3$)$_2$), 22.98 (NHCO$\underline{C}$H$_3$), 26.33, 26.39, 26.79, 27.27, 27.51 (-OCH$_2$CH$_2\underline{C}$H$_2\underline{C}$H$_2$-, -OCH($\underline{C}$H$_2$CH$_3$)$_2$), 28.72 (-NHCOC($\underline{C}$H$_3$)$_2$), 30.19, 30.32, 31.28, 31.34, 31.40 (-OCH$_2\underline{C}$H$_2$CH$_2$CH$_2\underline{C}$H$_2$CH$_2$NH-), 32.11 (d, J = 9.2 Hz, C6), 41.06, 41.10 (-CH$_2\underline{C}$H$_2$NH-), 50.37 (m, C5), 53.36 (m, -P(O$\underline{C}$H$_3$)$_2$), 56.08 (m, C4), 67.69, 67.70, 67.74 (m, -O$\underline{C}$H$_2$CH$_2$-), 77.29, 77.51 (C3), 80.34 (-NHCO$\underline{C}$(CH$_3$)$_2$), 83.76, 83.79 (-O$\underline{C}$H(CH$_2$CH$_3$)$_2$), 103.70 ($\underline{C}$-H (Fluorescein)), ~111.6 (m), ~114.9 (m), ~124.9 ~125.5, ~126.5, ~126.9, ~128.4 (C1, $\underline{C}$-H (Fluorescein)), 130.12, 130.18, 130.45, 130.65 ($\underline{C}$-H (Fluorescein)), ~134.6 (m), 137.93, ~144.1 (m, C2), ~154.8 (m), 157.96 (-NH$\underline{C}$OC(CH$_3$)$_2$), 168.16, 168.41, 170.95, 173.76 (-NH$\underline{C}$OCH$_3$, -CH$_2$-$\underline{C}$(O)NH-CH$_2$-);

^{31}P NMR (242.7 MHz, MeOH-D$_4$) δP: 18.91, 18.96, 18.98 (s).

HR-ESI-MS (m/z) calculated for C$_{46}$H$_{58}$N$_3$O$_{13}$P (M+Na)$^+$ 914.3599, found 914.3603.

Inhibition of neuraminidase activity

Neuraminidase (NA) enzymatic activity (purified NA from influenza virus X31 (H3N2)) was studied using the fluorescent substrate (2'-4-methylumbelliferyl)-α-D-*N*-acetylneuraminic acid (MUNANA). Measurements were made at 37 °C in 32.5 mM MES (pH 6.5) + 100 mM NaCl using a JASCO FP-6300 fluorimeter with excitation at 365 nm and emission at 450 nm. Michaelis-Menten constants for the enzyme were determined as described before.[13] Inhibition constants (KI) were determined by measuring the extent to which different concentrations of inhibitor (8, 16, 24 nM) reduced the steady-state rate of MUNANA hydrolysis (25 uM MUNANA, 0.16 nM NA). The data were interpreted as described.[13,22]

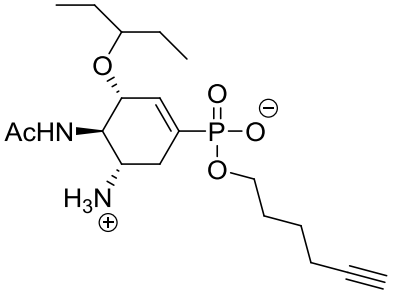
Fluorescence experiments

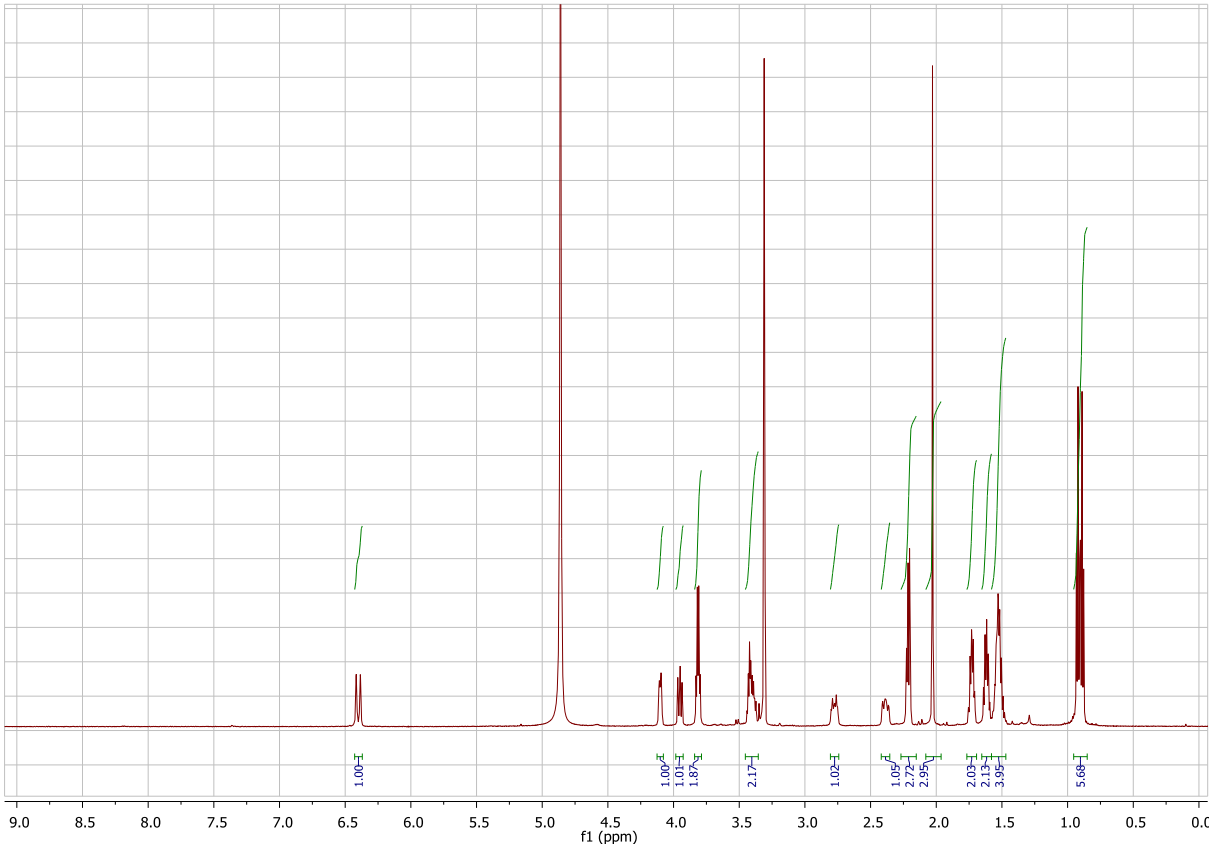
The binding experiment was carried out at 5 °C, using a JASCO FP-6300 fluorimeter and concentrations of **3e** and NA were comparable (9 nM and 12 nM, respectively).

The fluorescence titration experiment was carried out at 37 °C, using a JASCO FP-6300 fluorimeter by titrating **3e** (2 nM) with X31 N2 in 32.5 mM MES (pH 6.5) + 100 mM NaCl. The dissociation constant determined using standard non-linear least-squares methods was 0.23 ± 0.05 nM, in reasonable agreement with the value determined by inhibition of MUNANA hydrolysis.

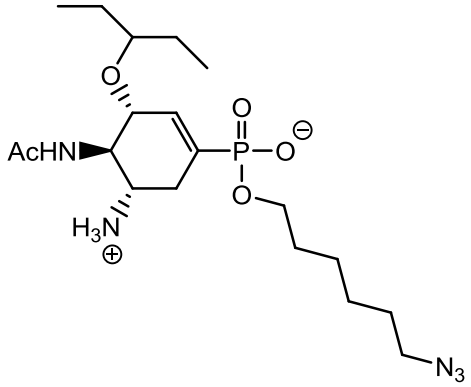
^{1}H-NMR-spectra

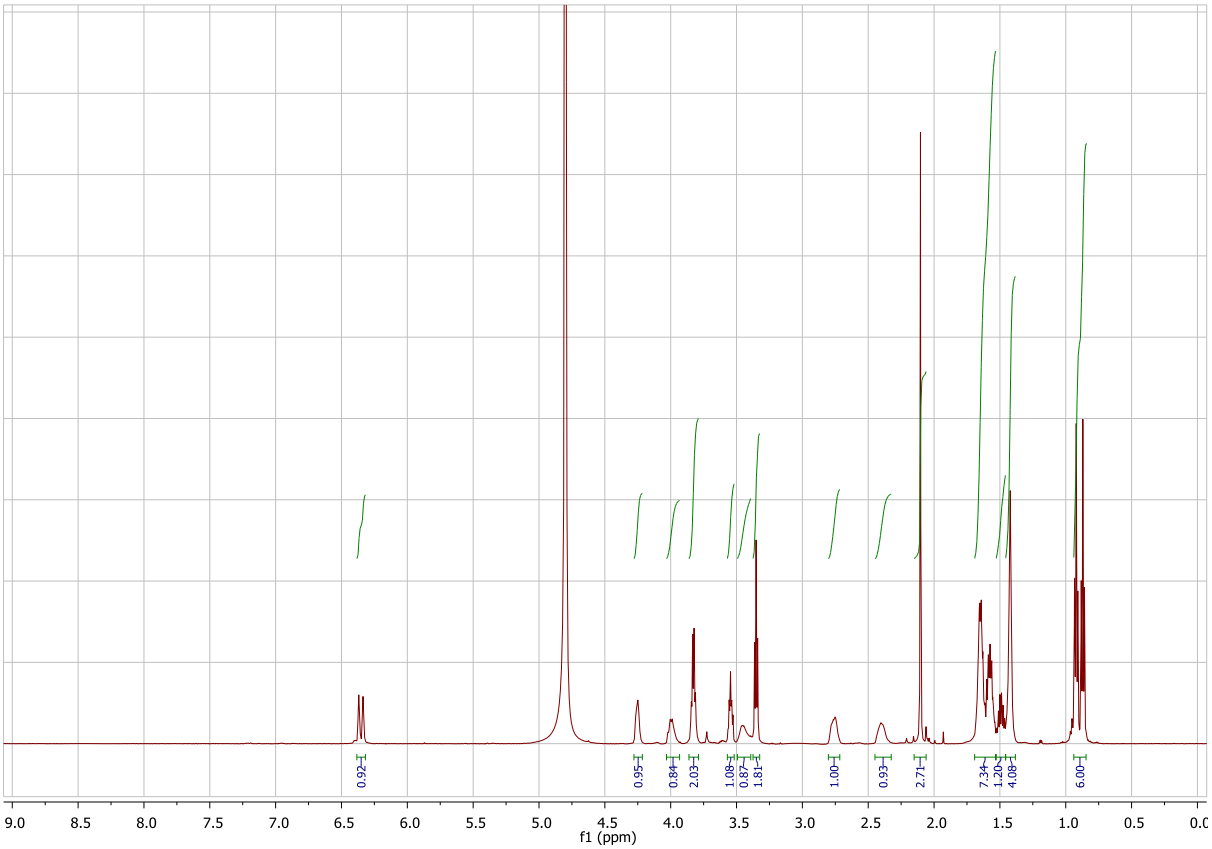
(Hex-5-yn-1-yl) (3*R*,4*R*,5*S*)-4-acetamido-5-amino-3-(1-ethylpropoxy)-1-cyclohexene-1-phosphonic acid (3a)



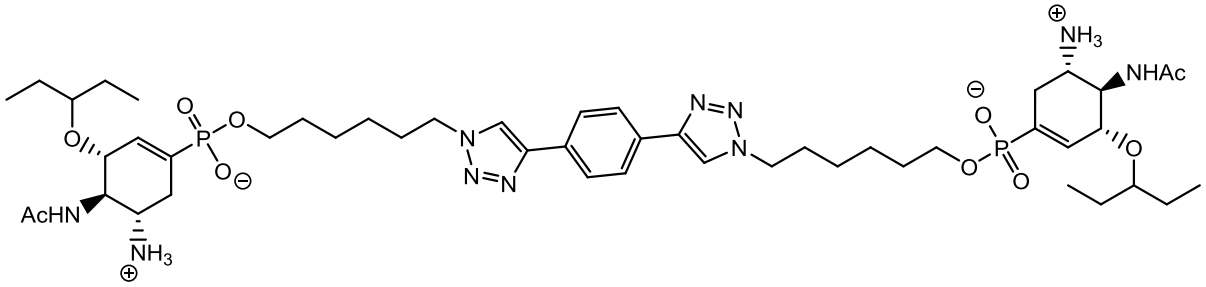


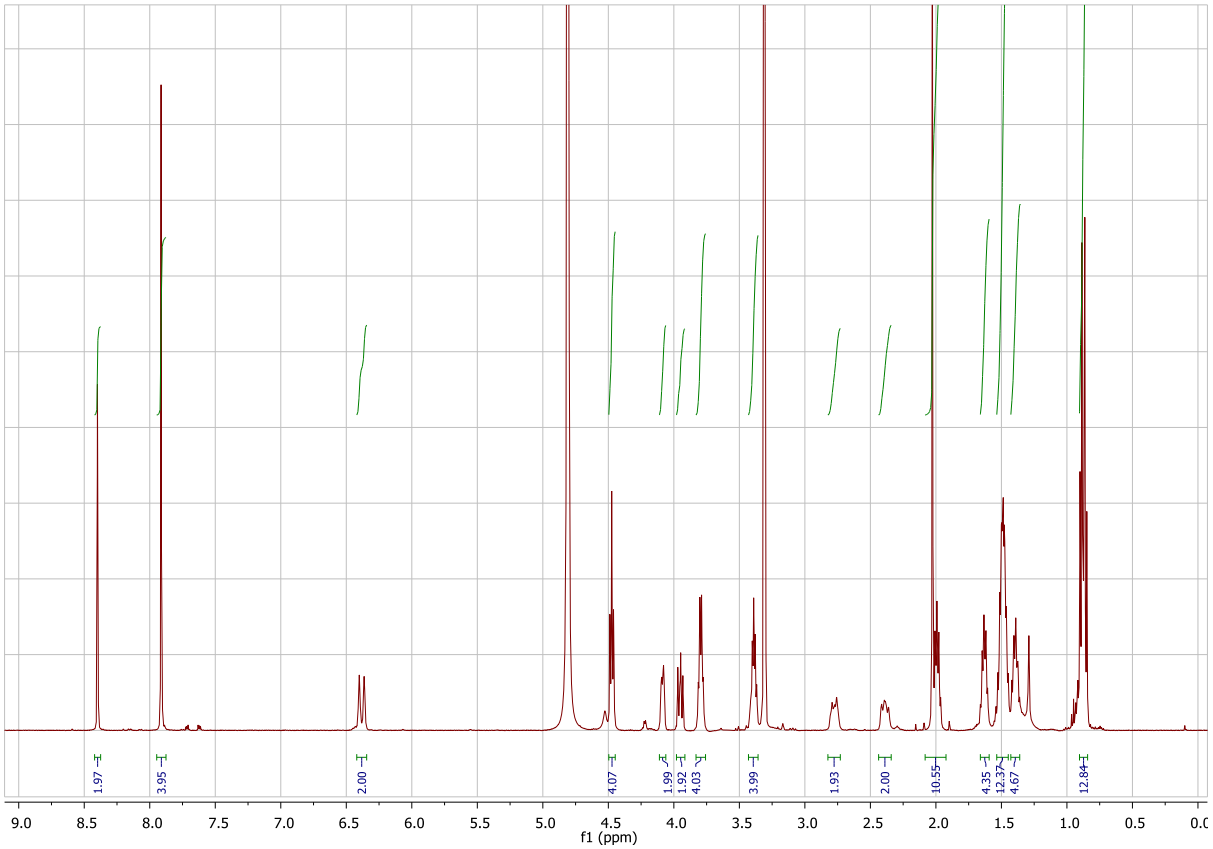
(6-Azido hexyl) (3*R*,4*R*,5*S*)-4-acetylamino-5-amino-3-(1-ethylpropoxy)-1-cyclohexene-1-phosphonic acid (3b)



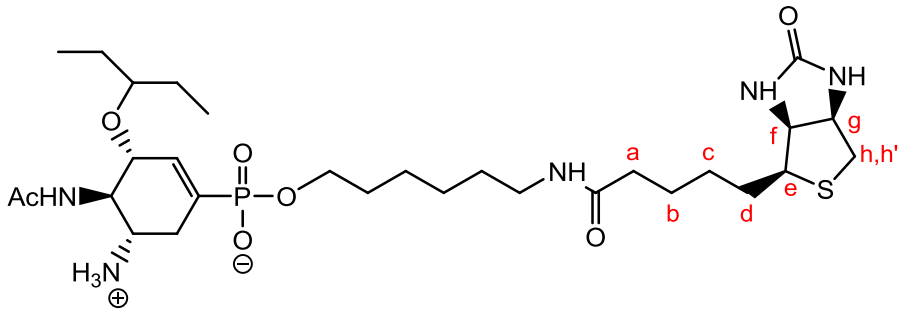


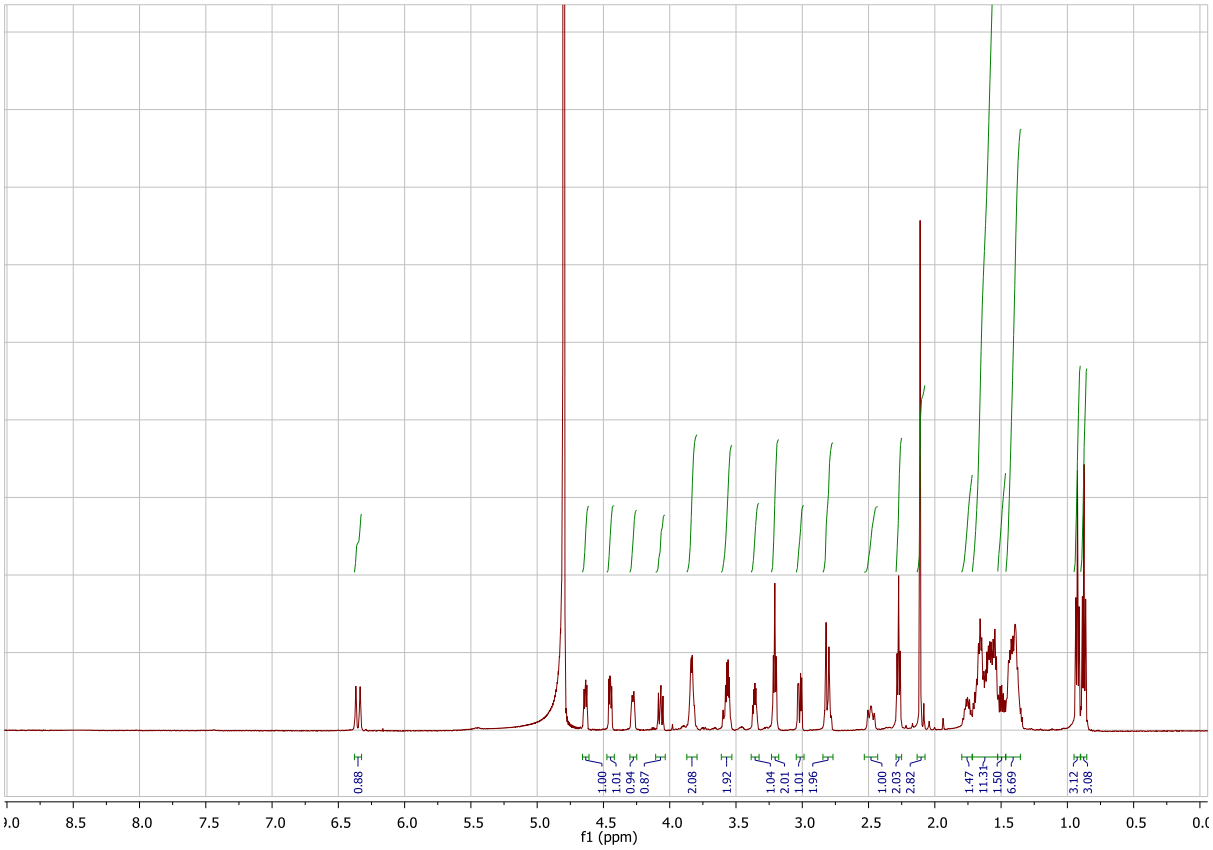
1,4-Bis [1-(hex-1'-yl [(*3R,4R,5S*)-4-acetamido-5-amino-3-(1-ethylpropoxy)-1-cyclohexene-1-phosphonic acid])-1*H*-1,2,3-triazol-4-yl]-benzene (3c)



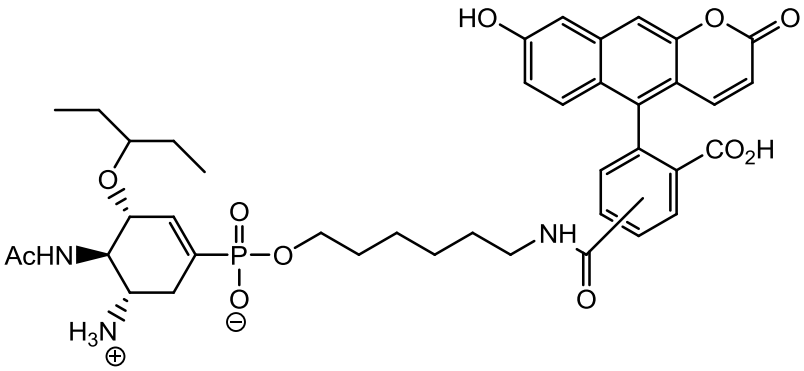


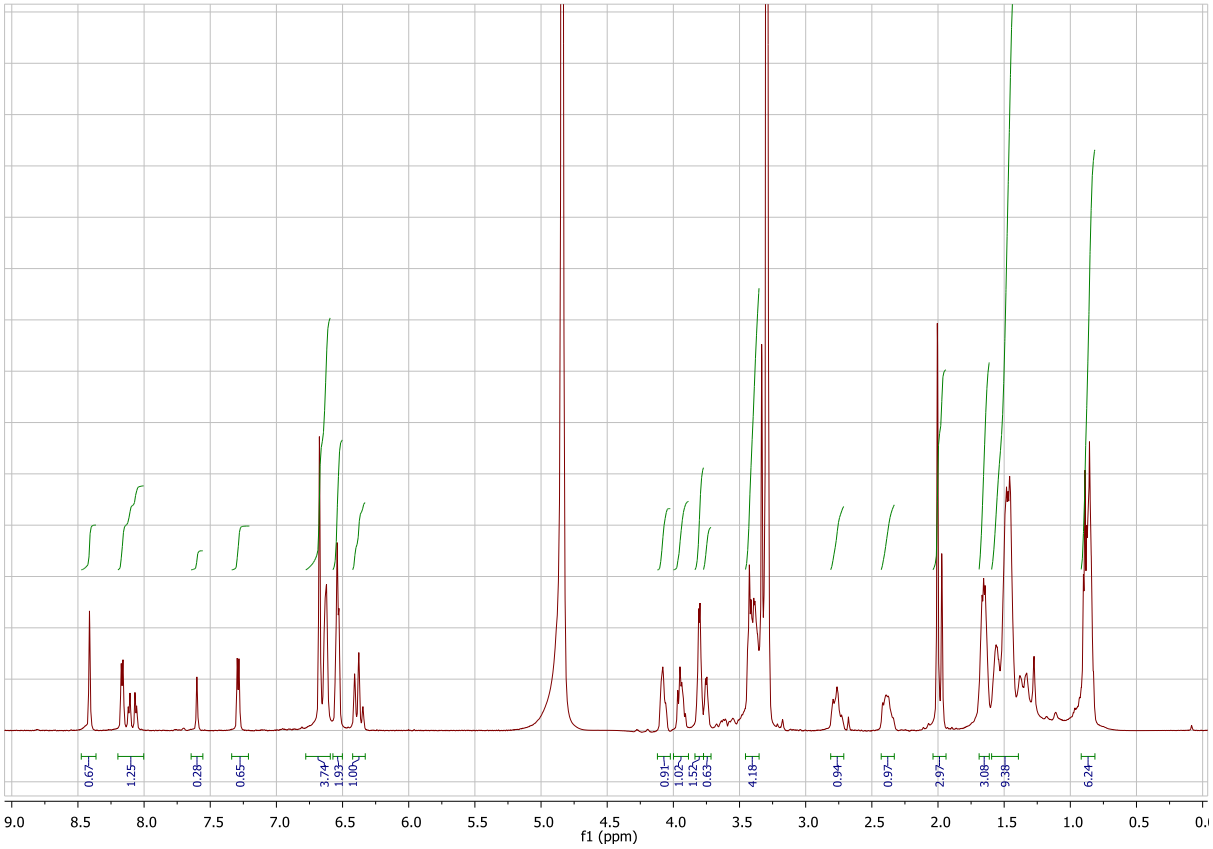
(6-*d*-Biotinamidohex-1-yl) (3*R*,4*R*,5*S*)-4-acetamido-5-amino-3-(1-ethylpropoxy)-1-cyclohexene-1-phosphonic acid (3d)



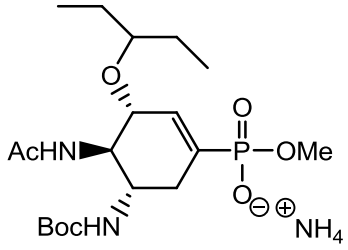


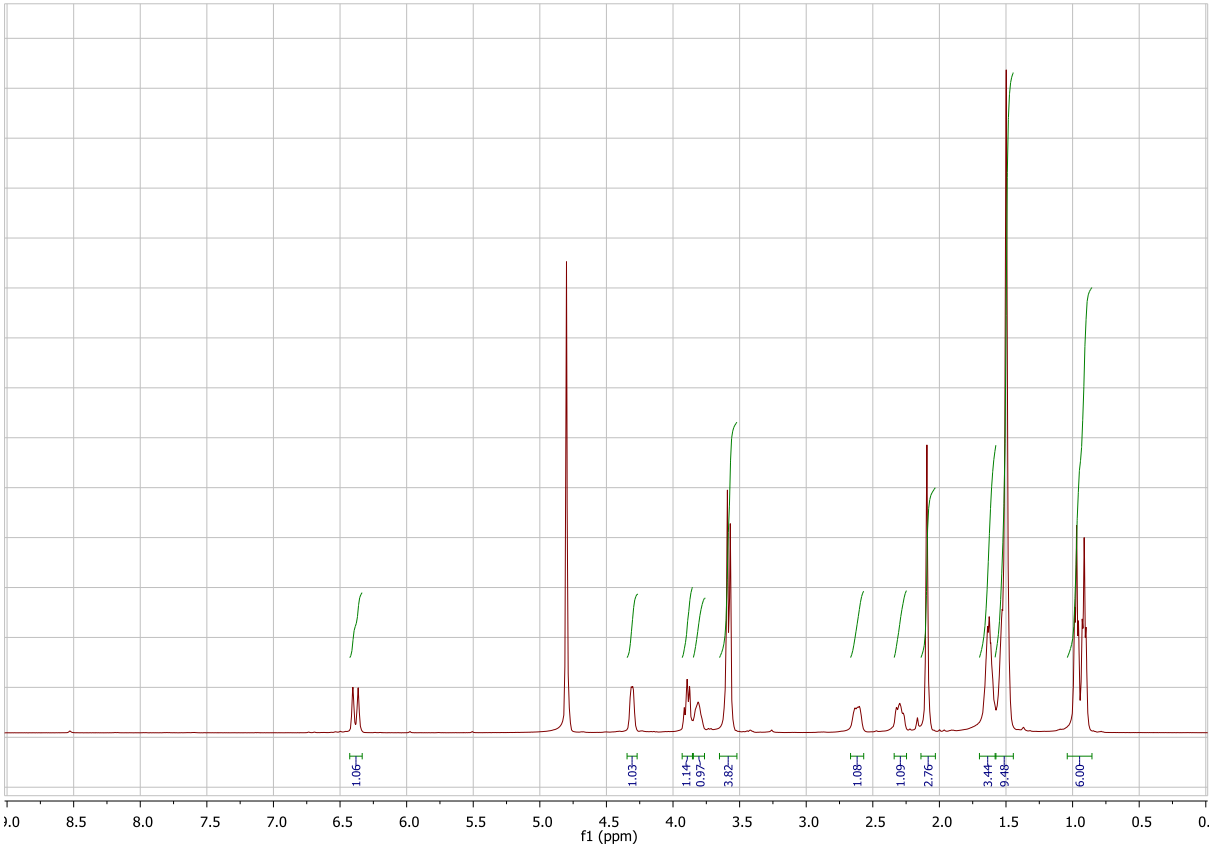
(6-(Fluoresceinyl-5,6-carbonylamino)-hex-1-yl) (3*R*,4*R*,5*S*)-4-acetylamino-5-amino-3-(1-ethylpropoxy)-1-cyclohexene-1-phosphonic acid (3e)



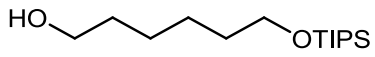


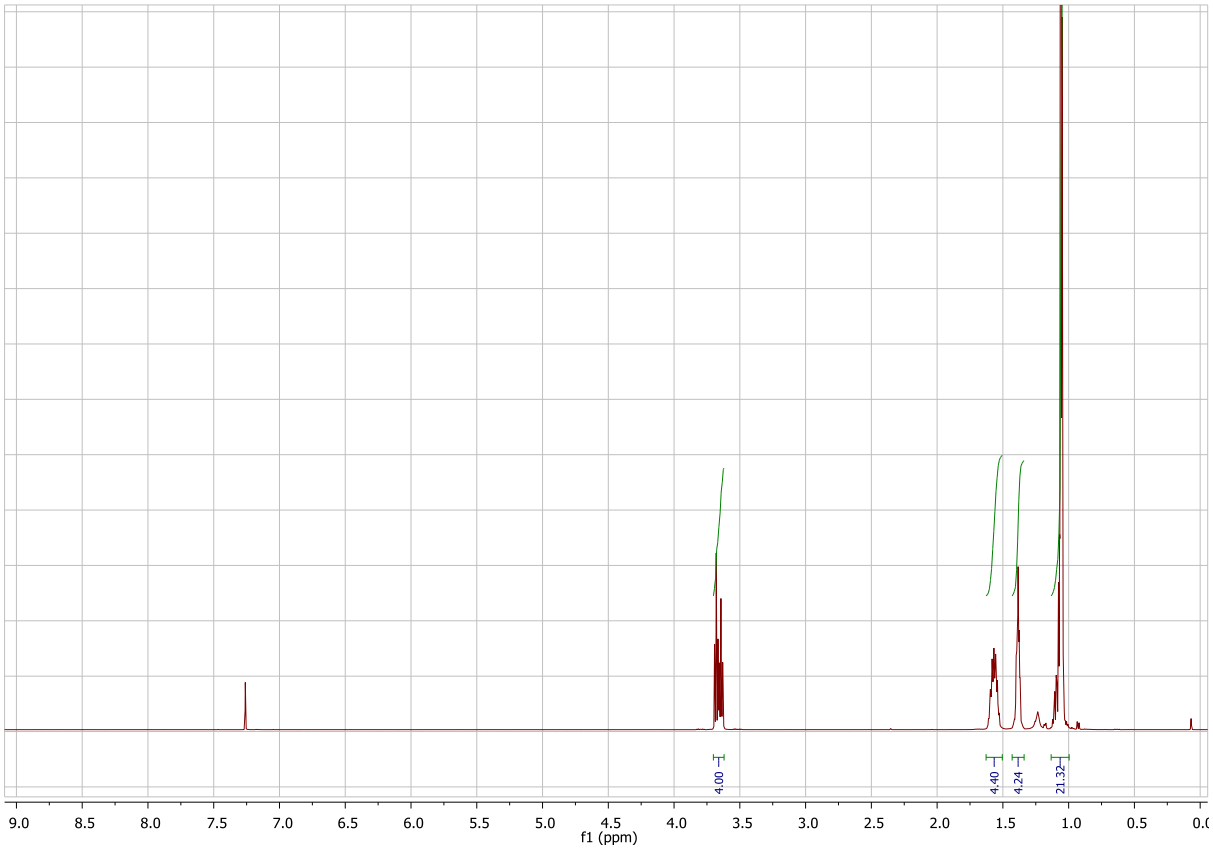
Ammonium [methyl (3*R*,4*R*,5*S*)-4-acetamido-5-(1,1-dimethylethyloxycarbonylamino)-3-(1-ethylpropoxy)-1-cyclohexene-1-phosphonate] (4)



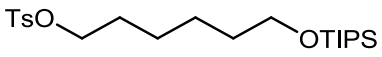


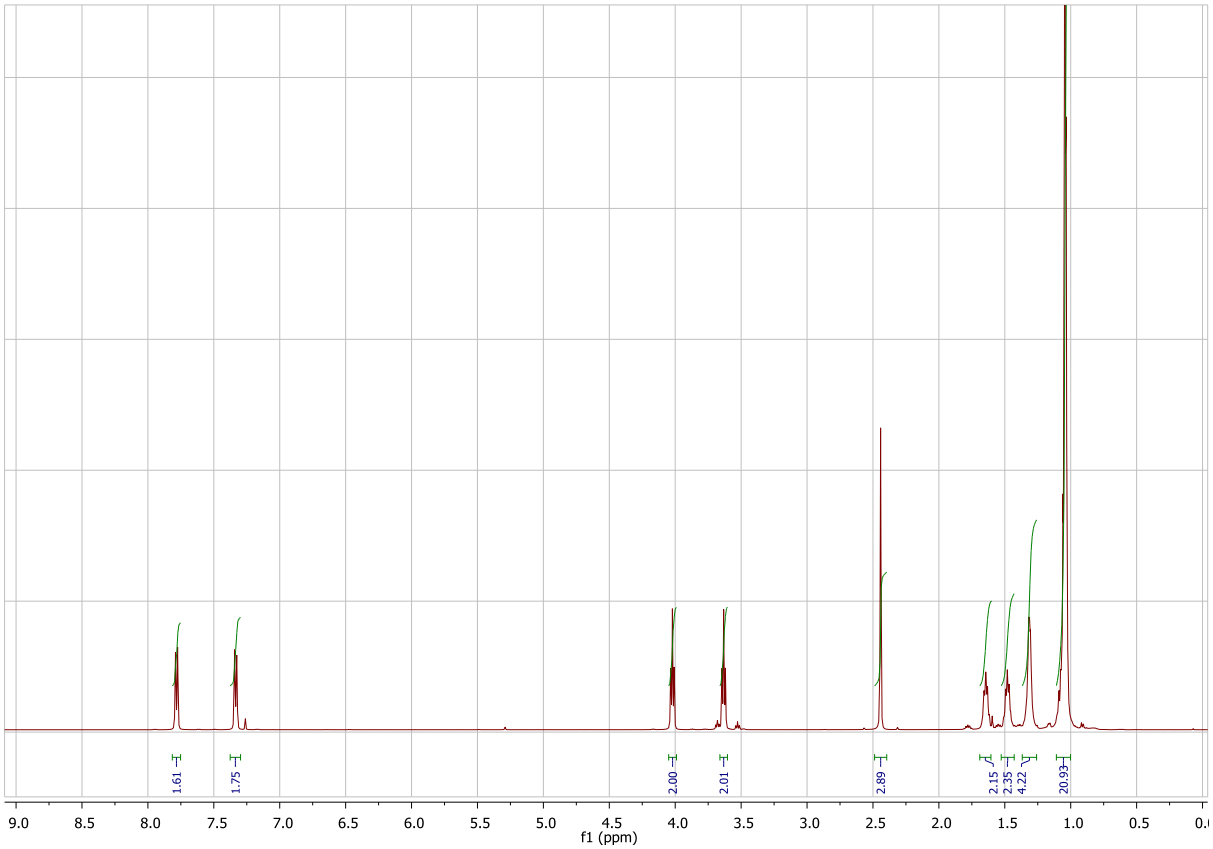
O-Triisopropylsilylhexane-1,6-diol (5)



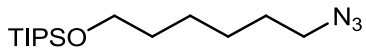


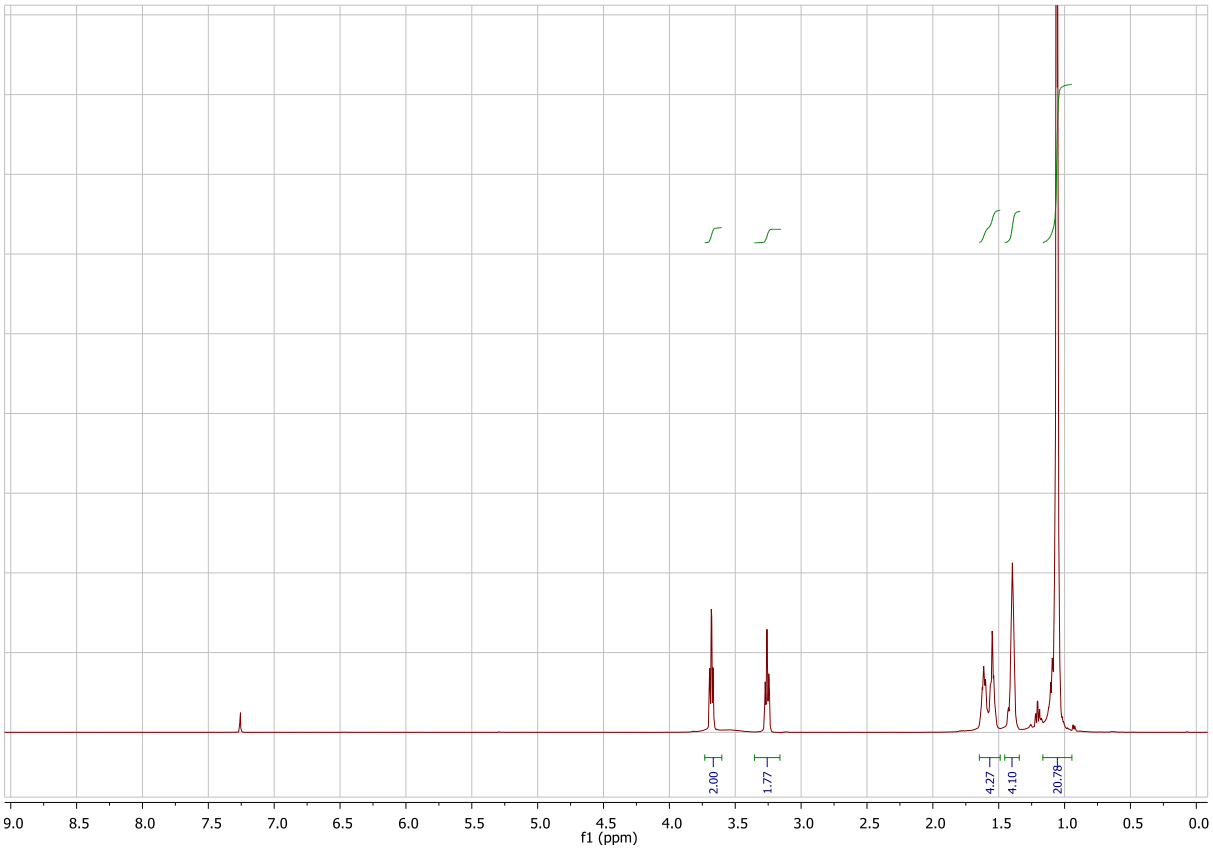
O-Toluenesulfonyl-*O*-triisopropylsilylhexane-1,6-diol (6)



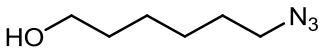


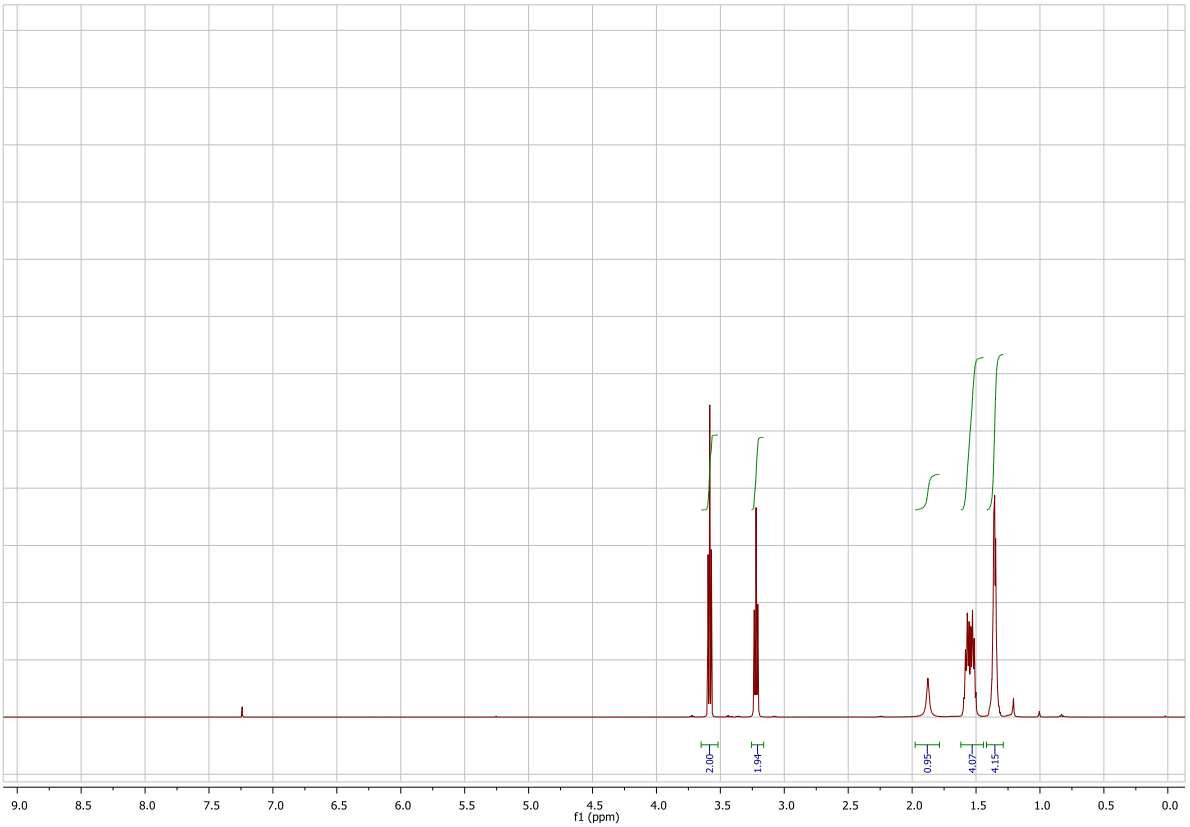
6-Azido-*O*-triisopropylsilylhexane-1-ol (7)



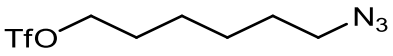


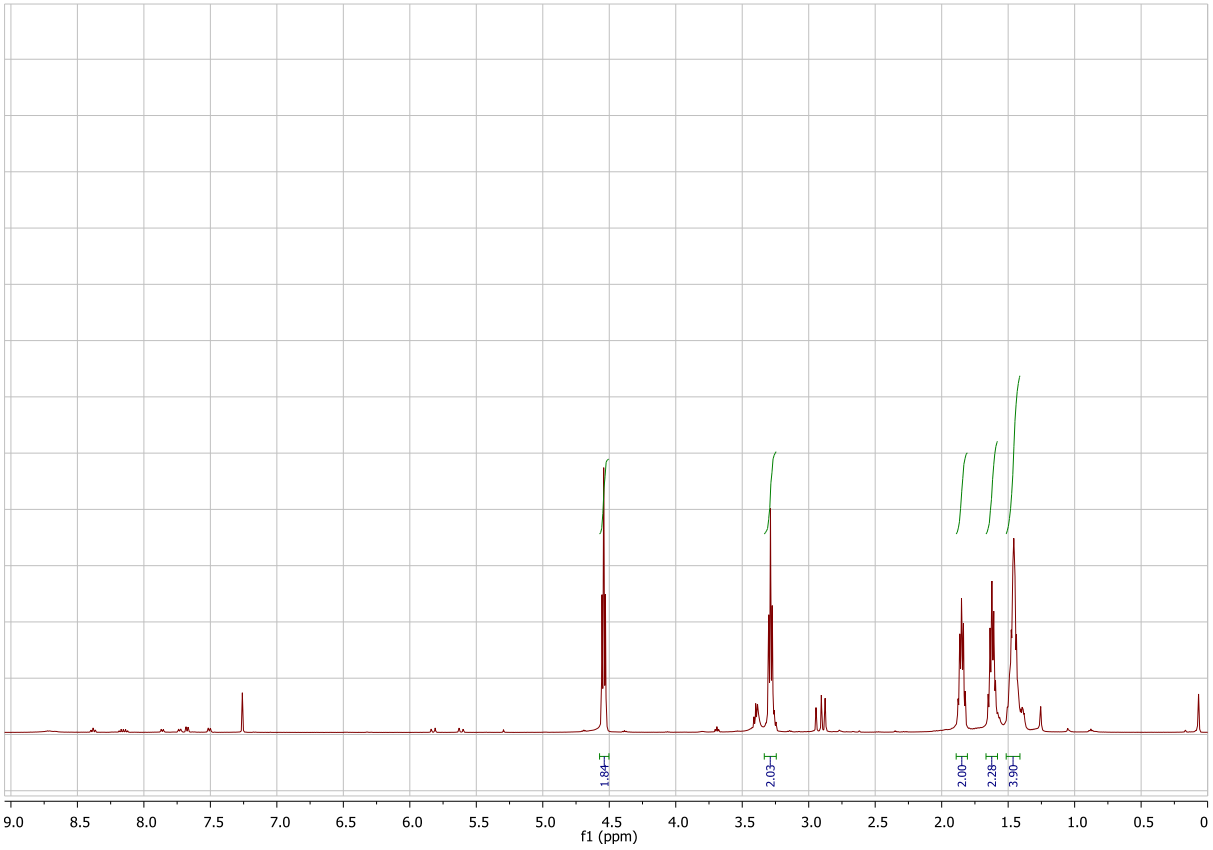
6-Azido-1-hexanol (8)



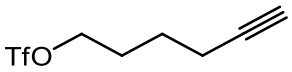


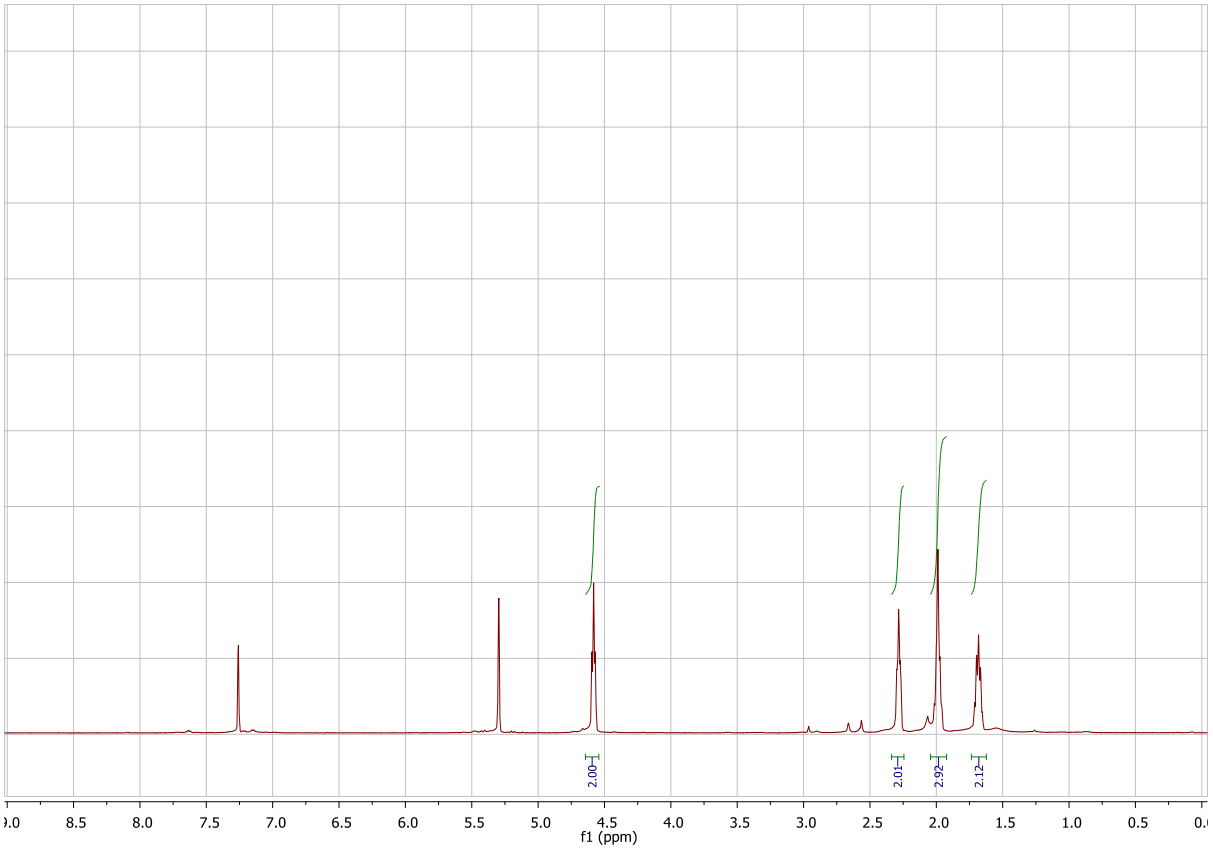
6-Azido-*O*-trifluoromethanesulfonyl-hexan-1-ol (9)



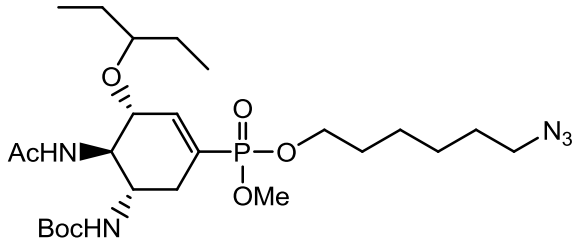


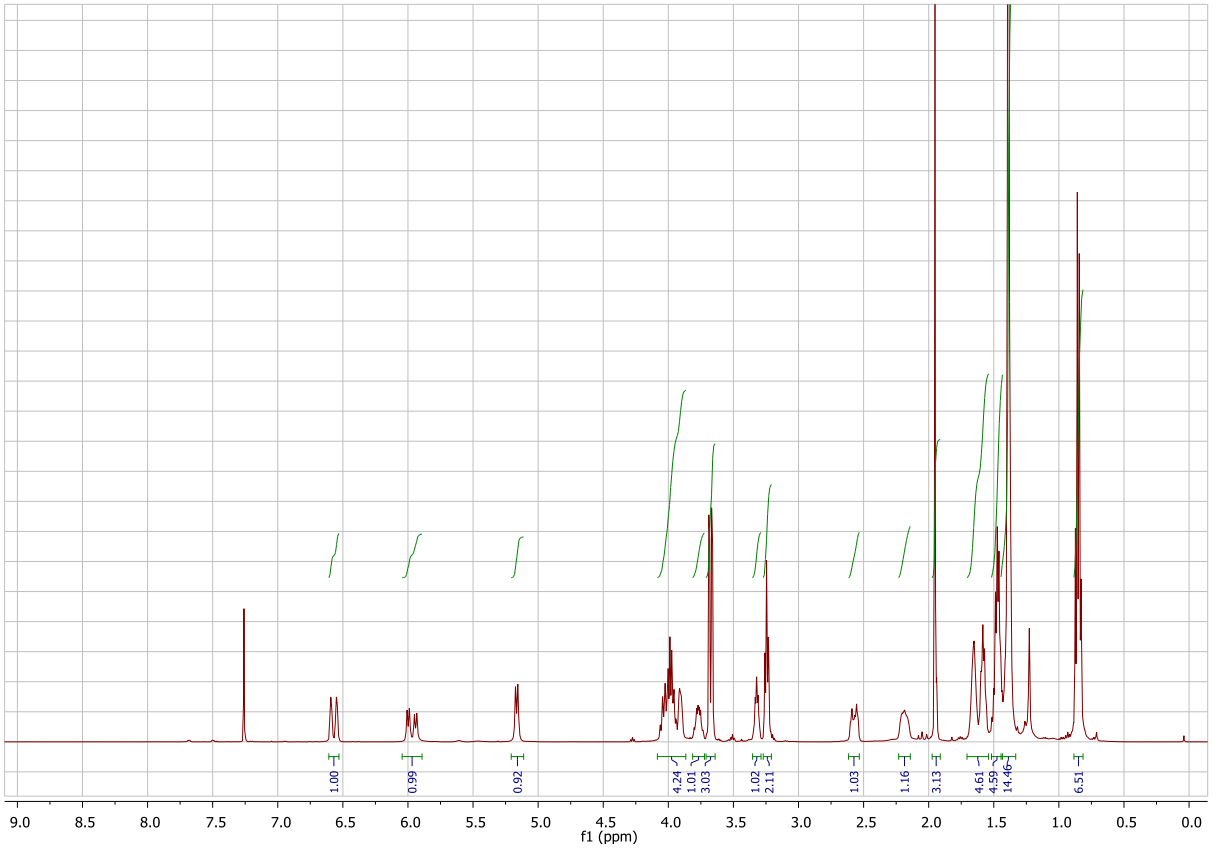
_O_-Trifluoromethanesulfonylhex-5-yn-1-ol (10)



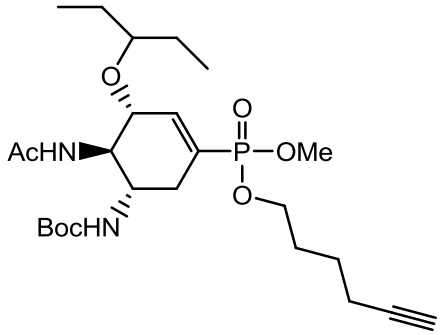


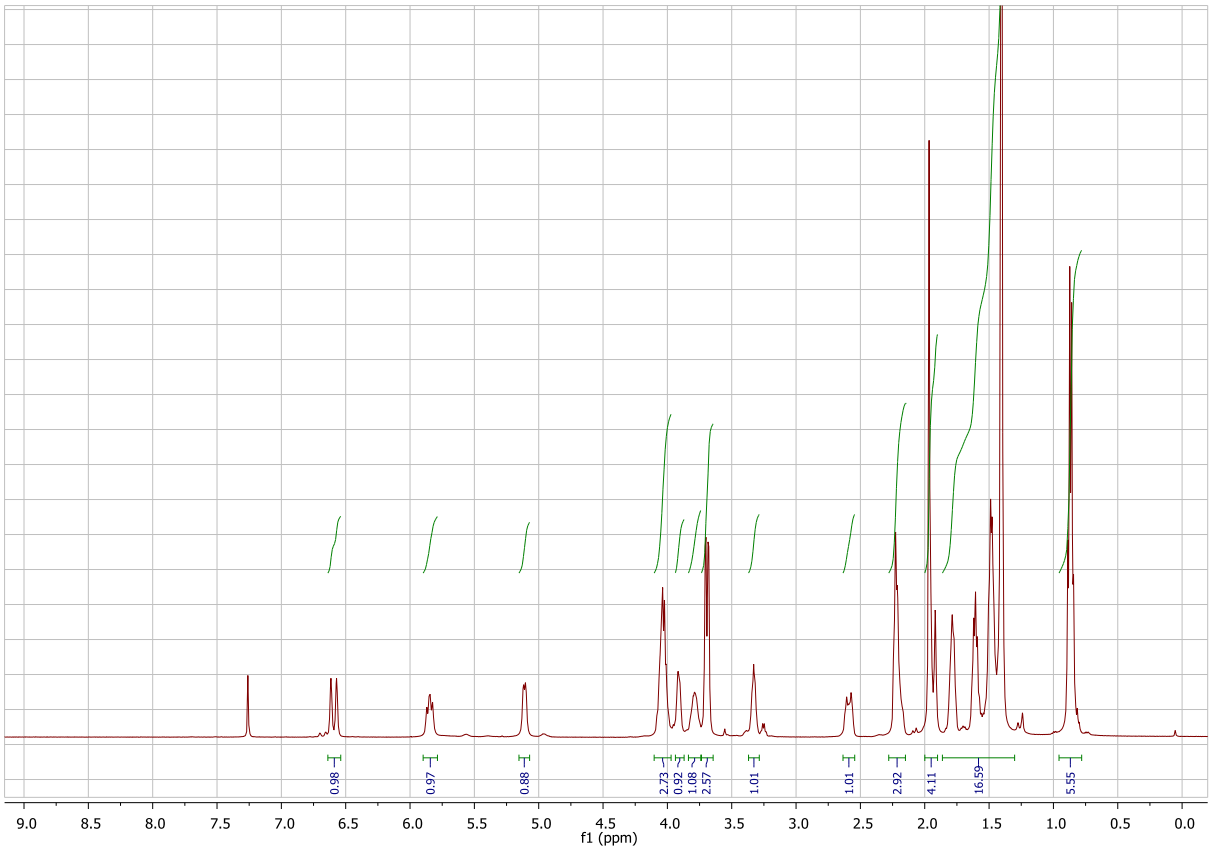
Methyl (6-azidohex-1-yl) [(3*R*,4*R*,5*S*)-4-acetamido-5-(1,1-dimethylethyloxycarbonylamino)-3-(1-ethylpropoxy)-1-cyclohexene-1-phosphonate] (11)



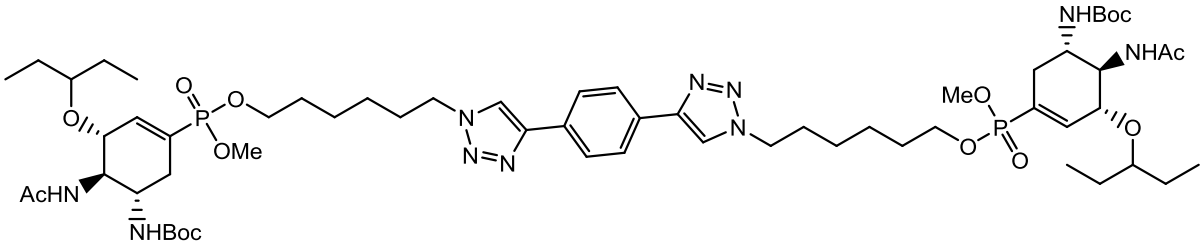


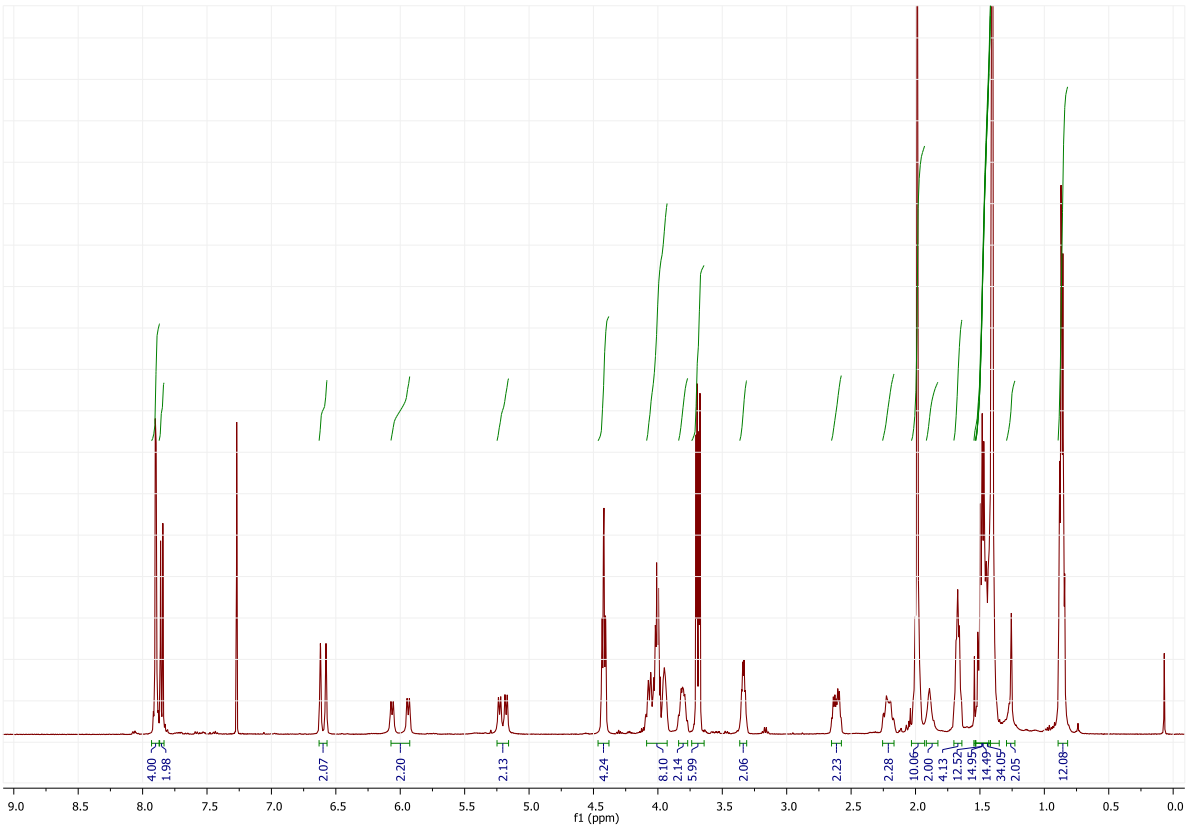
Methyl (hex-5-yn-1-yl) [(3*R*,4*R*,5*S*)-4-acetamido-5-(1,1-dimethylethyloxycarbonylamino)-3-(1-ethylpropoxy)-1-cyclohexene-1-phosphonate] (12)



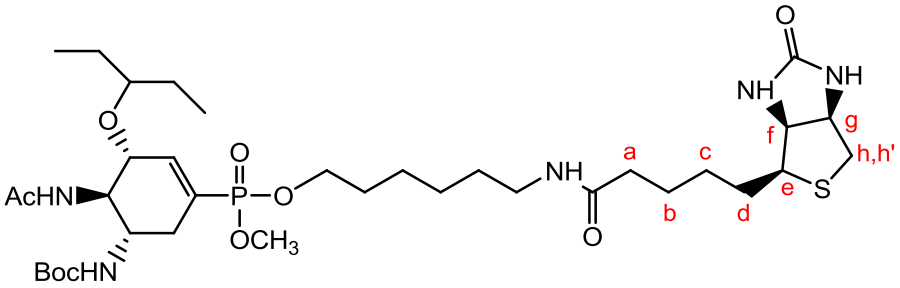


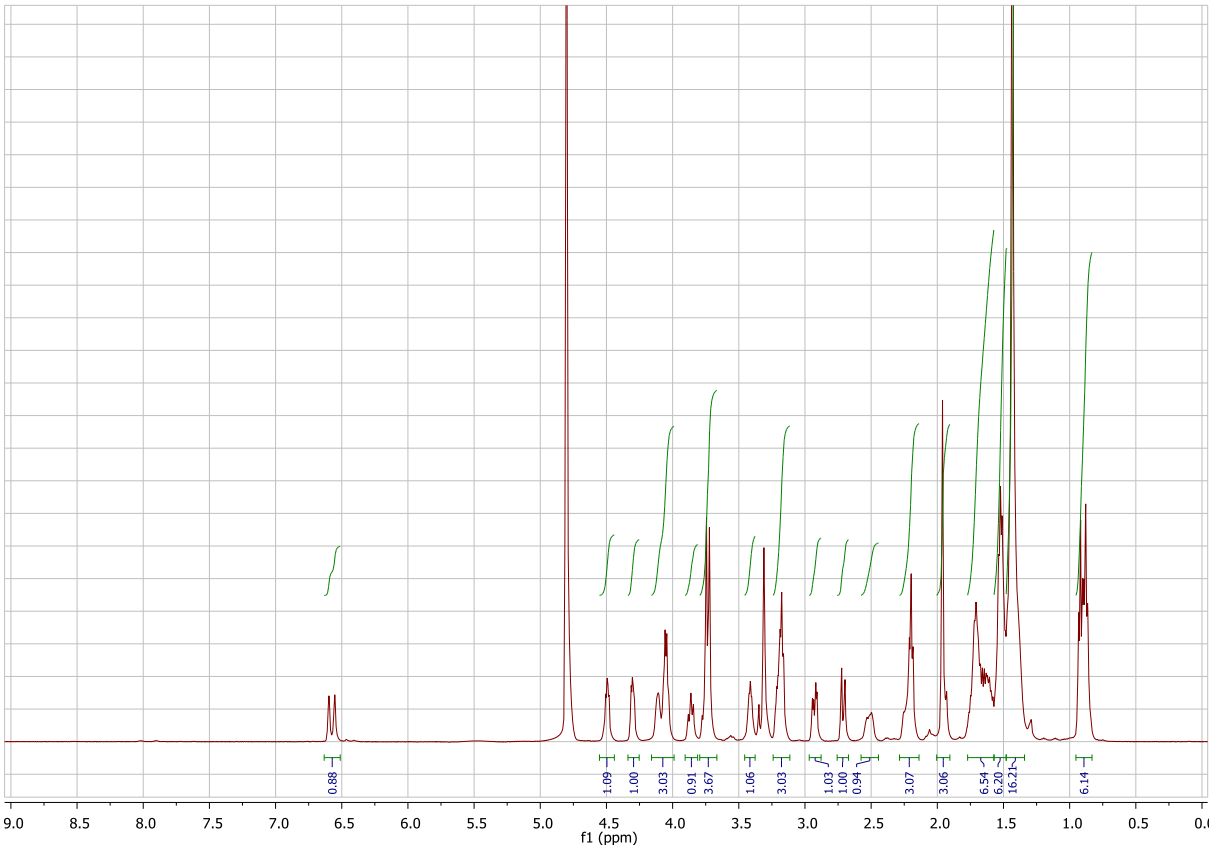
1,4-Bis [1-(hex-1'-yl methyl [(*3R,4R,5S*)-4-acetamido-5-(1,1 dimethylethyloxycarbonylamino)-3-(1-ethylpropoxy)-1-cyclohexene-1-phosphonate])-1*H*-1,2,3-triazol-4-yl]-benzene (13)



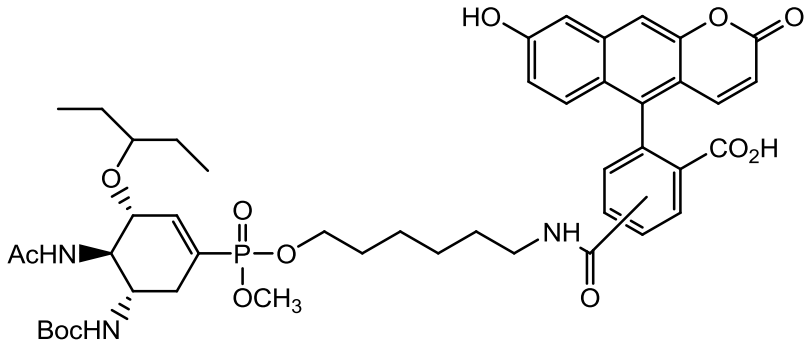


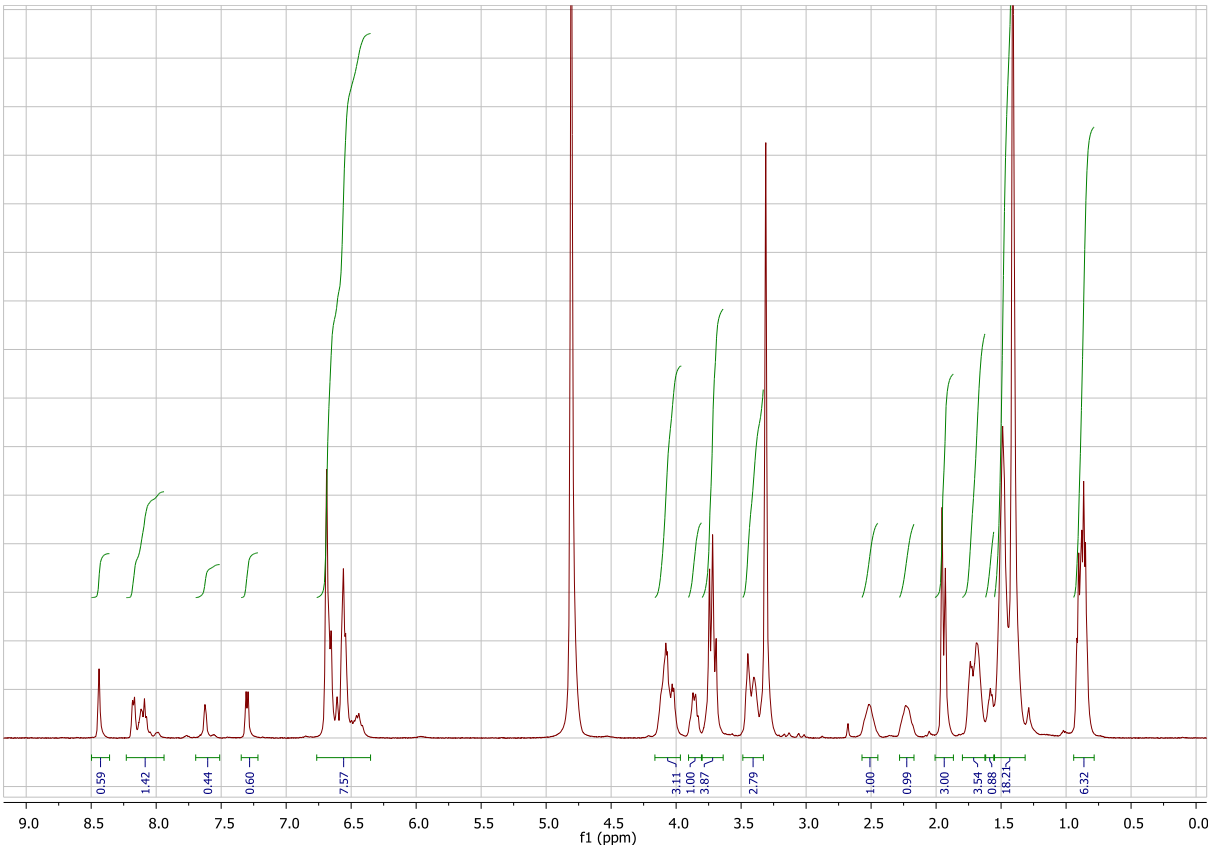
Methyl (6-*d*-biotinamidohex-1-yl) [(3*R*,4*R*,5*S*)-4-acetamido-5-(1,1-dimethylethyloxycarbonylamino)-3-(1-ethylpropoxy)-1-cyclohexene-1-phosphonate] (14)





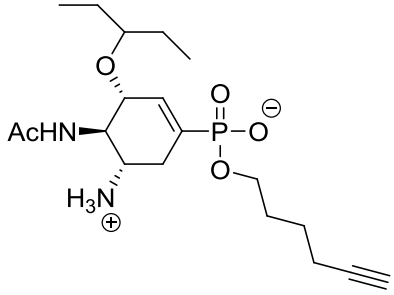
Methyl (6-(fluoresceinyl-5,6-carbonylamino)-hex-1-yl) [(3*R*,4*R*,5*S*)-4-acetamido-5-(1,1-dimethylethyloxycarbonylamino)-3-(1-ethylpropoxy)-1-cyclohexene-1-phosphonate] (15)

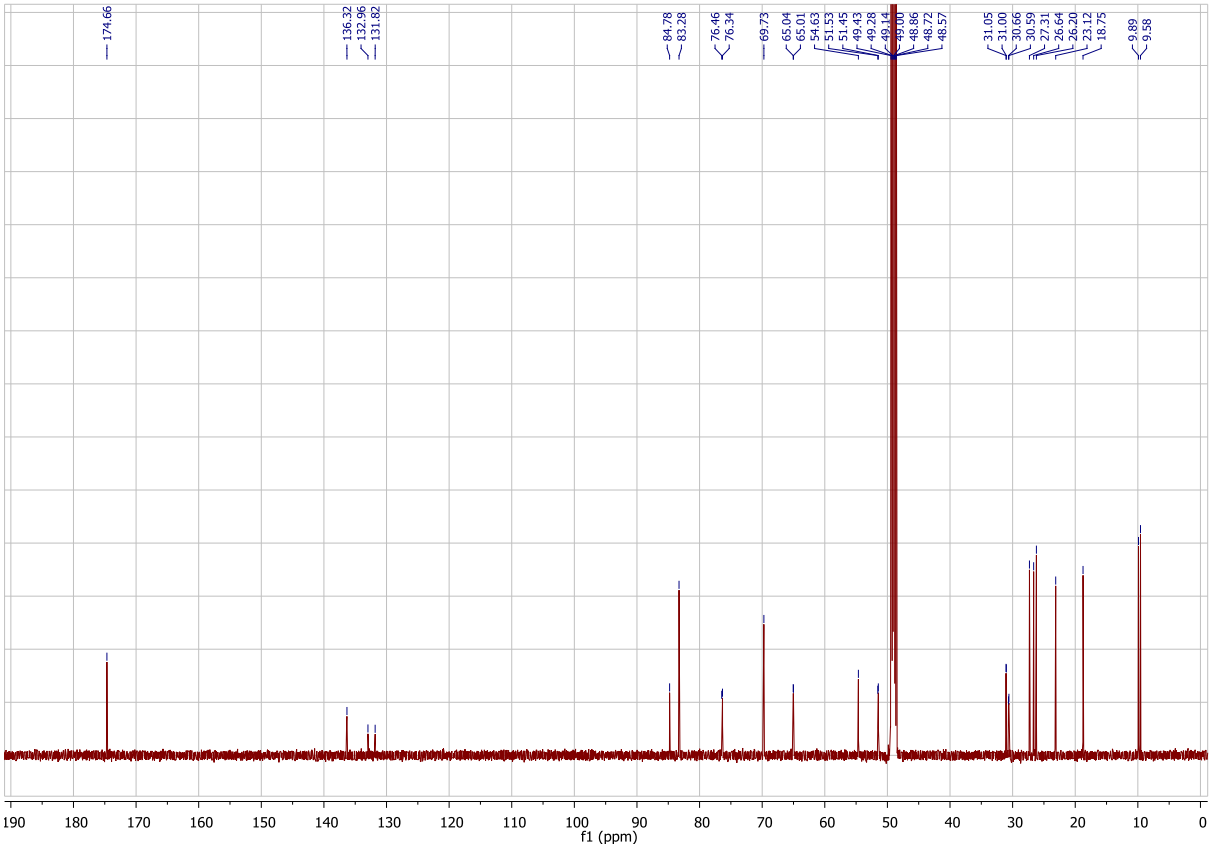




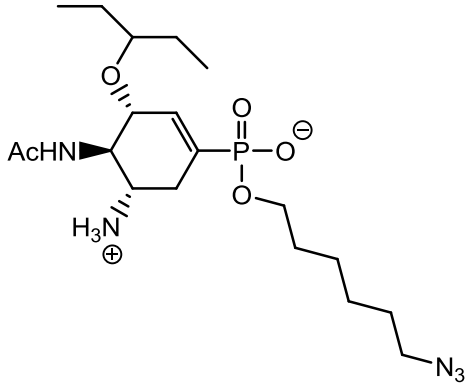
^{13}C-NMR-spectra

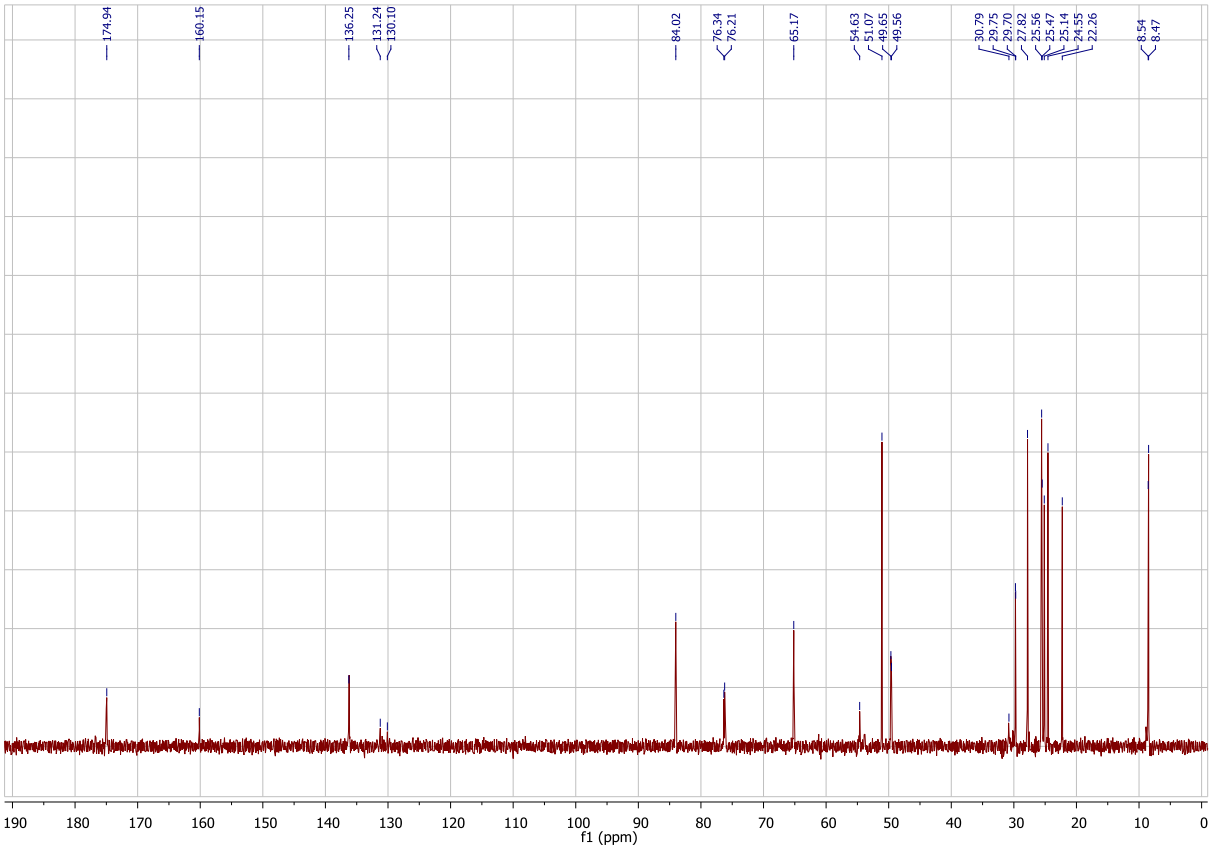
(Hex-5-yn-1-yl) (3*R*,4*R*,5*S*)-4-acetamido-5-amino-3-(1-ethylpropoxy)-1-cyclohexene-1-phosphonic acid (3a)



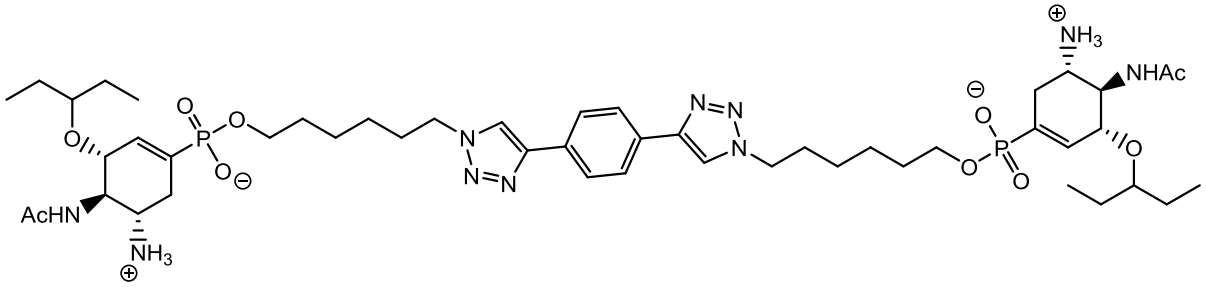


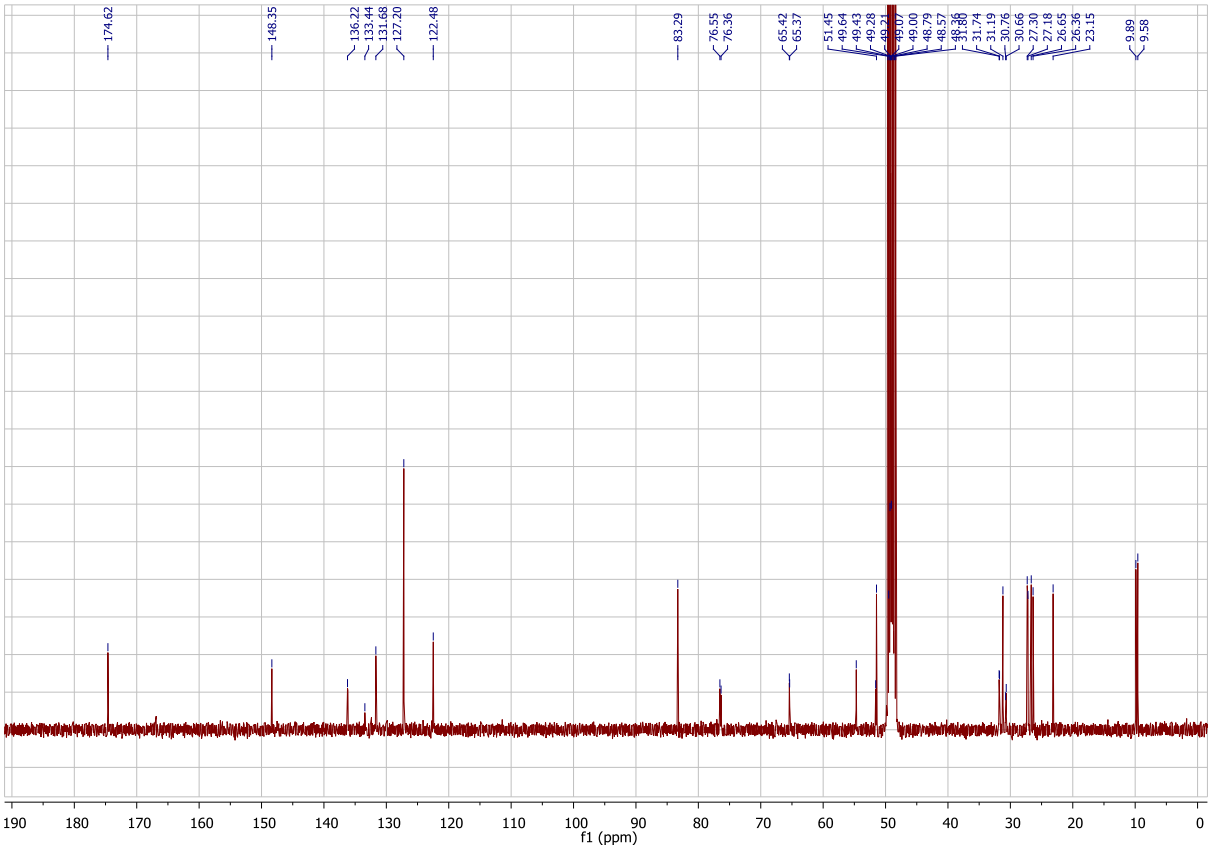
(6-Azido hexyl) (3*R*,4*R*,5*S*)-4-acetylamino-5-amino-3-(1-ethylpropoxy)-1-cyclohexene-1-phosphonic acid (3b)



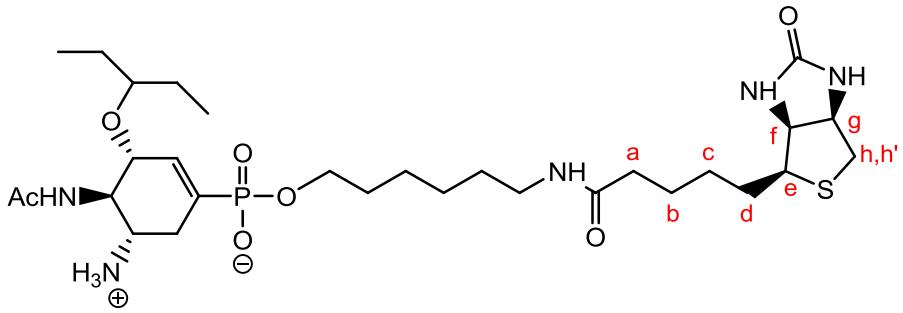


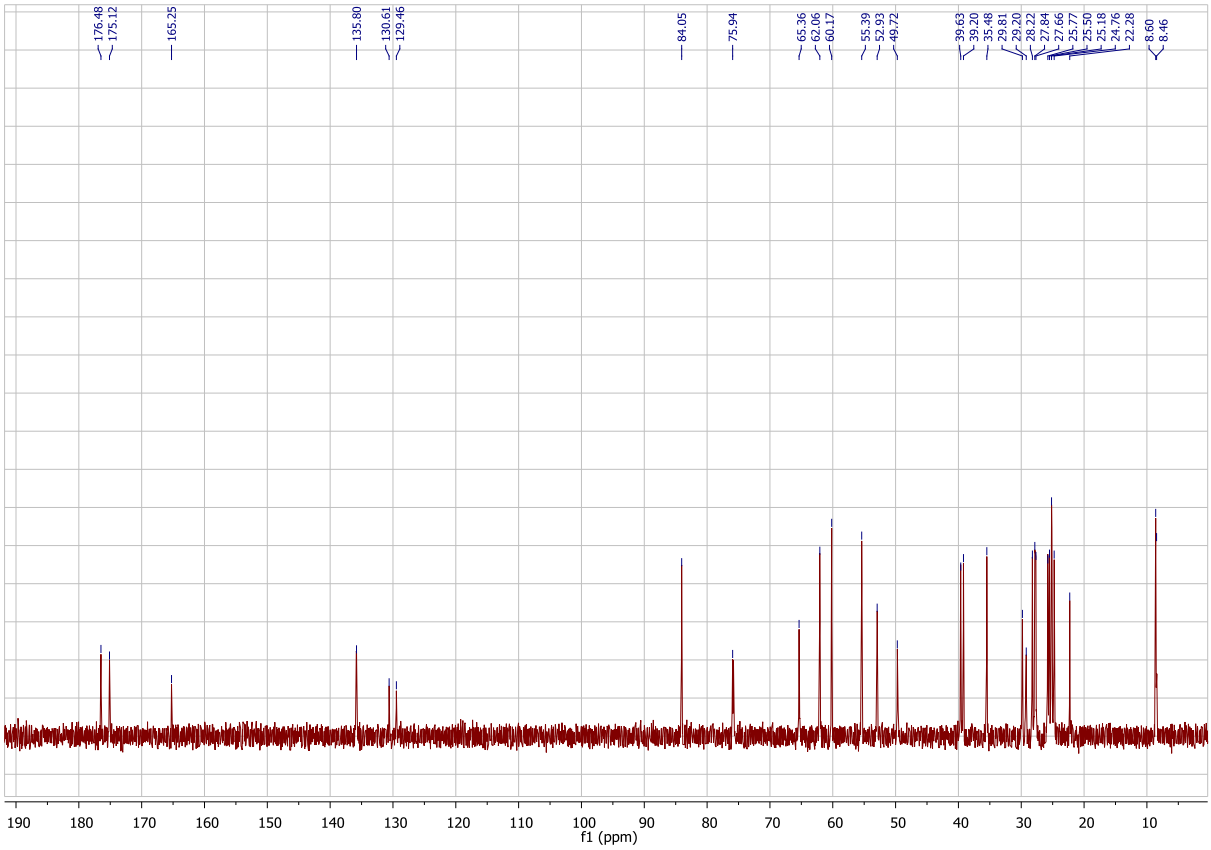
1,4-Bis [1-(hex-1'-yl [(*3R,4R,5S*)-4-acetamido-5-amino-3-(1-ethylpropoxy)-1-cyclohexene-1-phosphonic acid])-1*H*-1,2,3-triazol-4-yl]-benzene (3c)



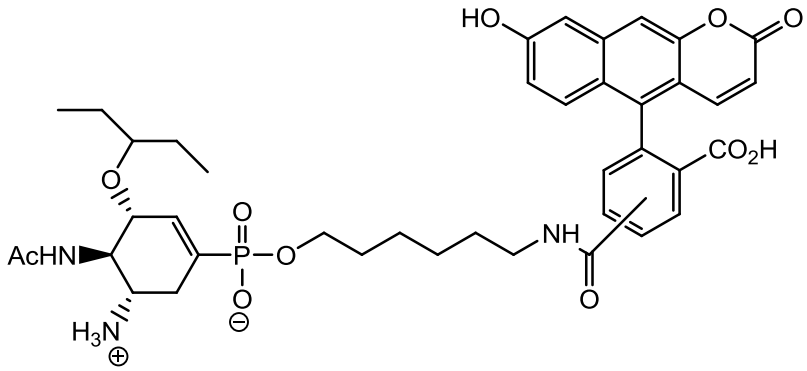


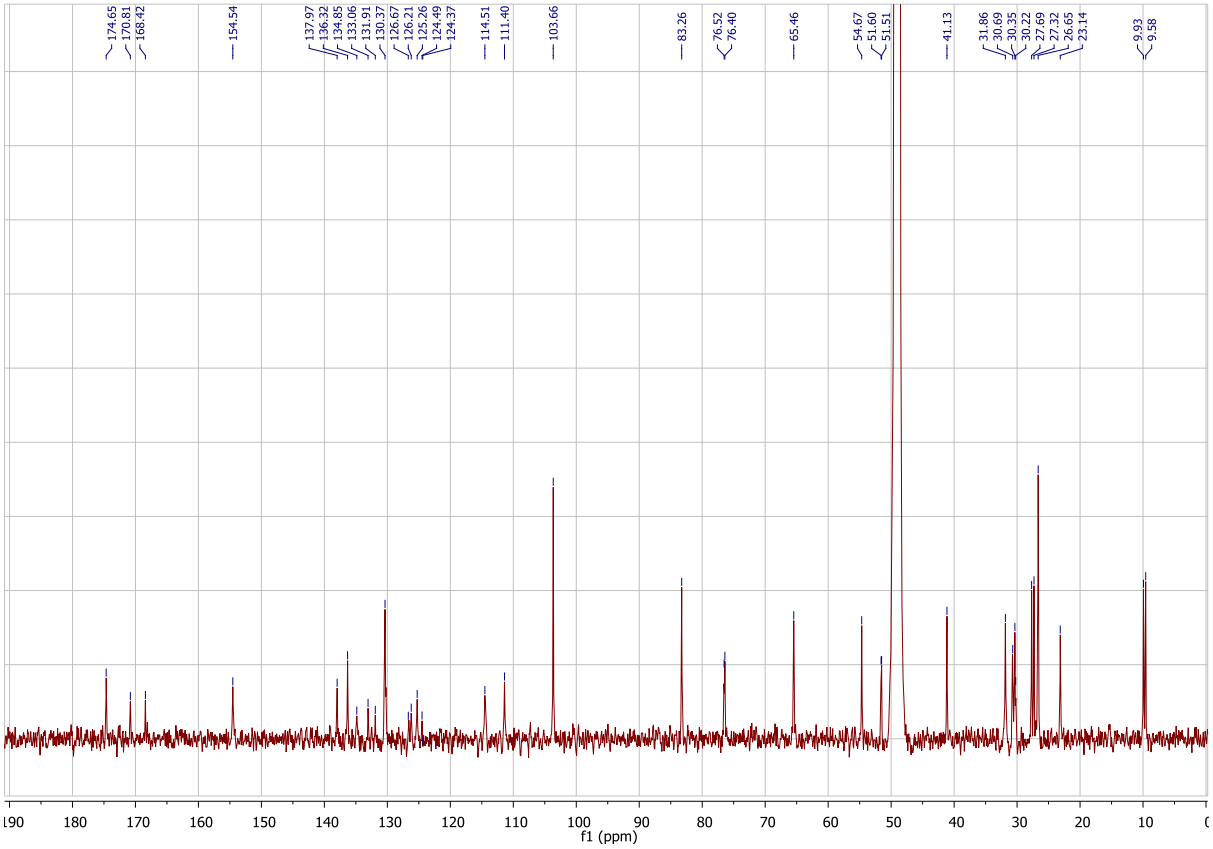
(6-*d*-Biotinamidohex-1-yl) (3*R*,4*R*,5*S*)-4-acetamido-5-amino-3-(1-ethylpropoxy)-1-cyclohexene-1-phosphonic acid (3d)



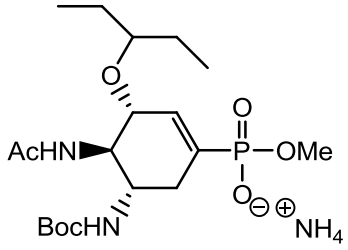


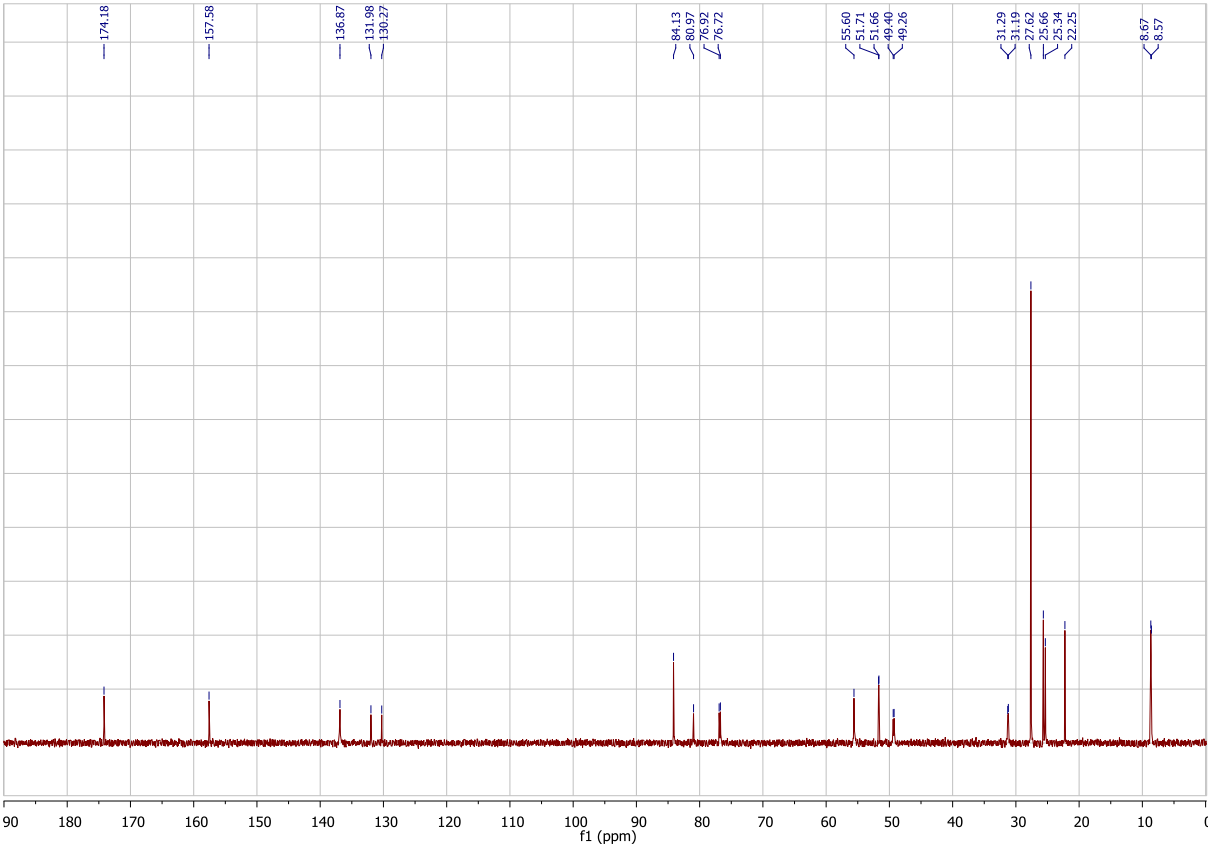
(6-(Fluoresceinyl-5,6-carbonylamino)-hex-1-yl) (3*R*,4*R*,5*S*)-4-acetylamino-5-amino-3-(1-ethylpropoxy)-1-cyclohexene-1-phosphonic acid (3e)



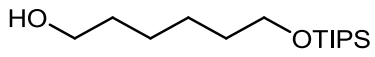


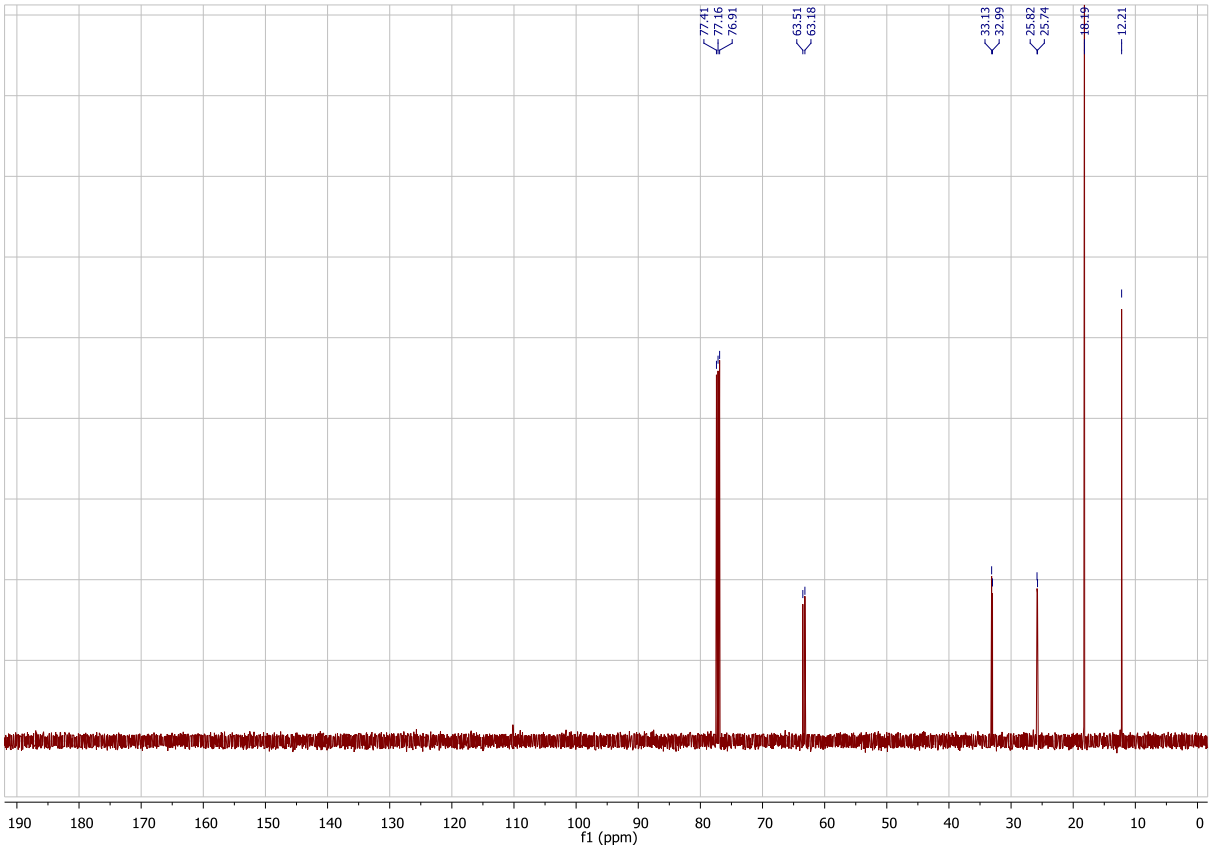
Ammonium [methyl (3*R*,4*R*,5*S*)-4-acetamido-5-(1,1-dimethylethyloxycarbonylamino)-3-(1-ethylpropoxy)-1-cyclohexene-1-phosphonate] (4)



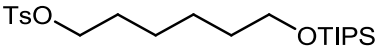


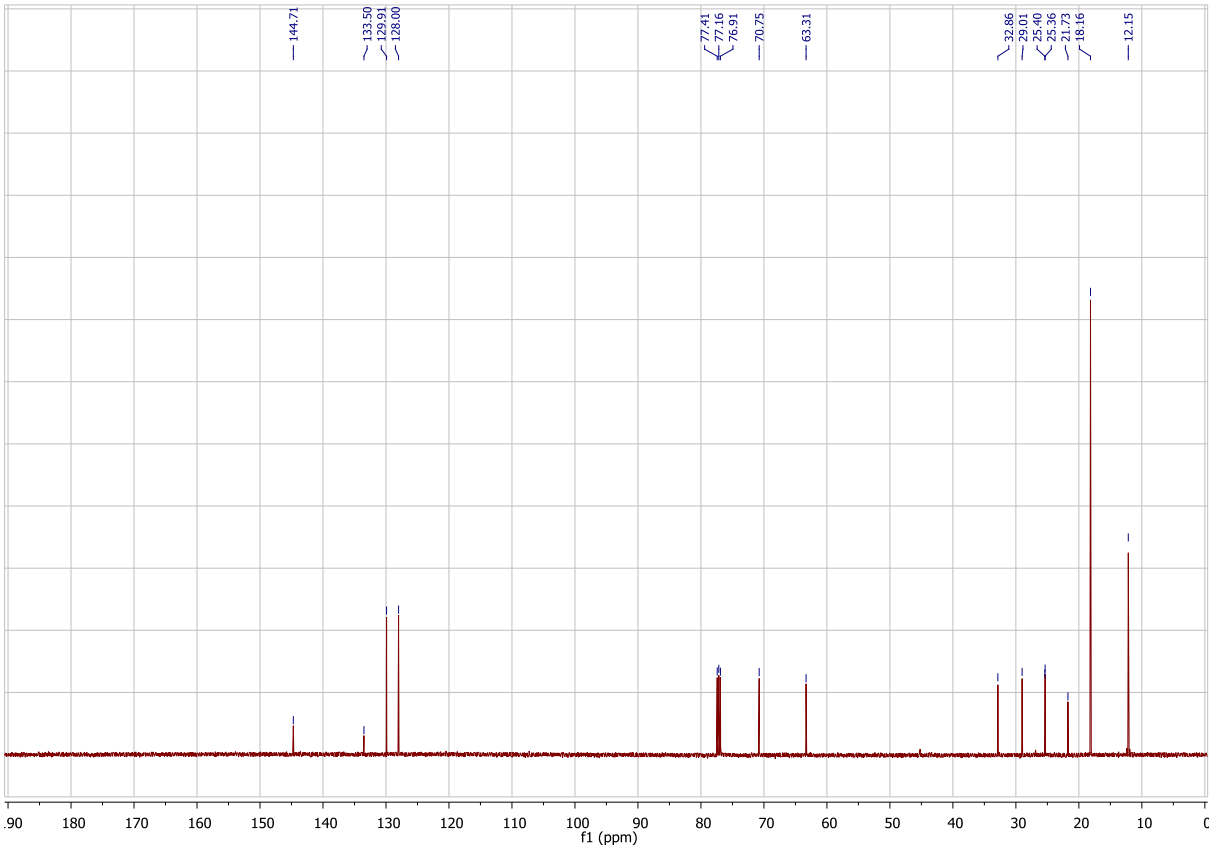
O-Triisopropylsilylhexane-1,6-diol (5)



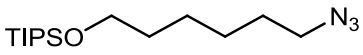


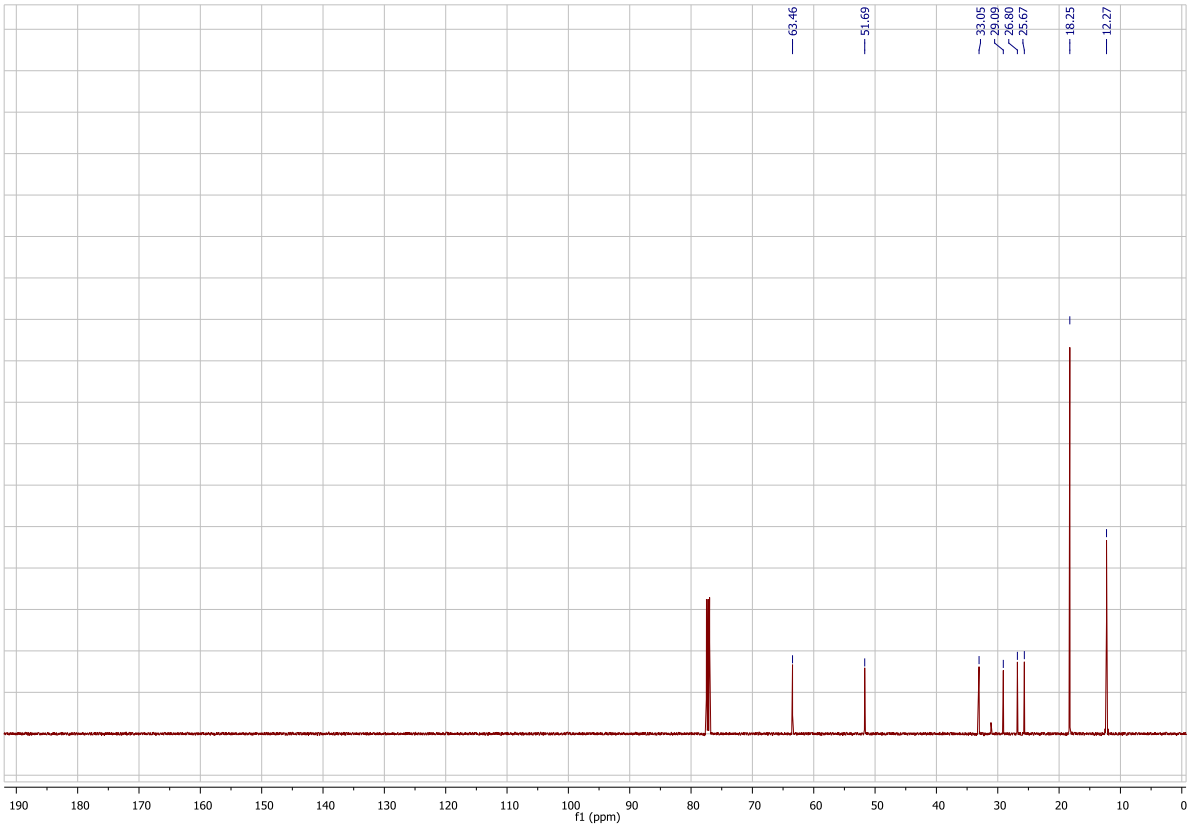
O-Toluenesulfonyl-*O*-triisopropylsilylhexane-1,6-diol (6)



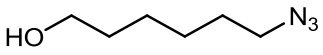


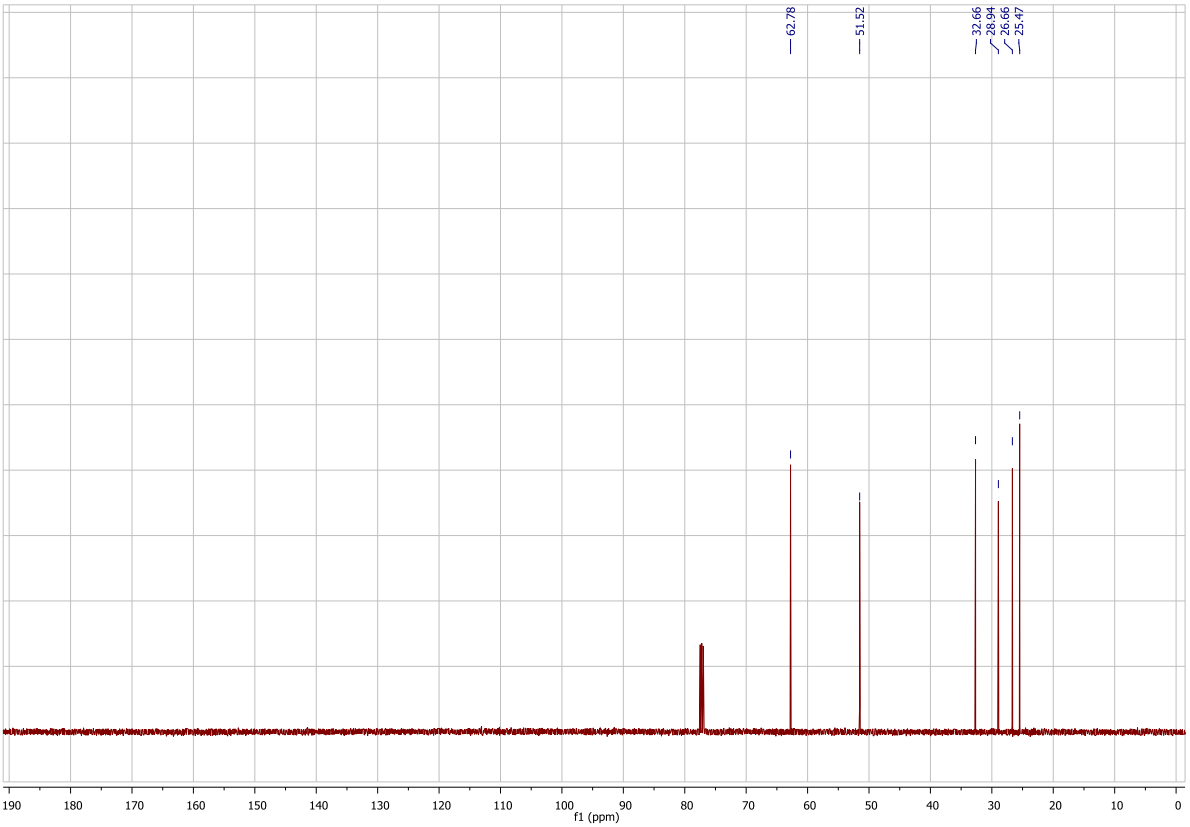
6-Azido-*O*-triisopropylsilylhexane-1-ol (7)



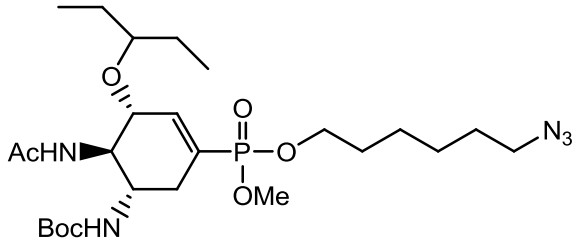


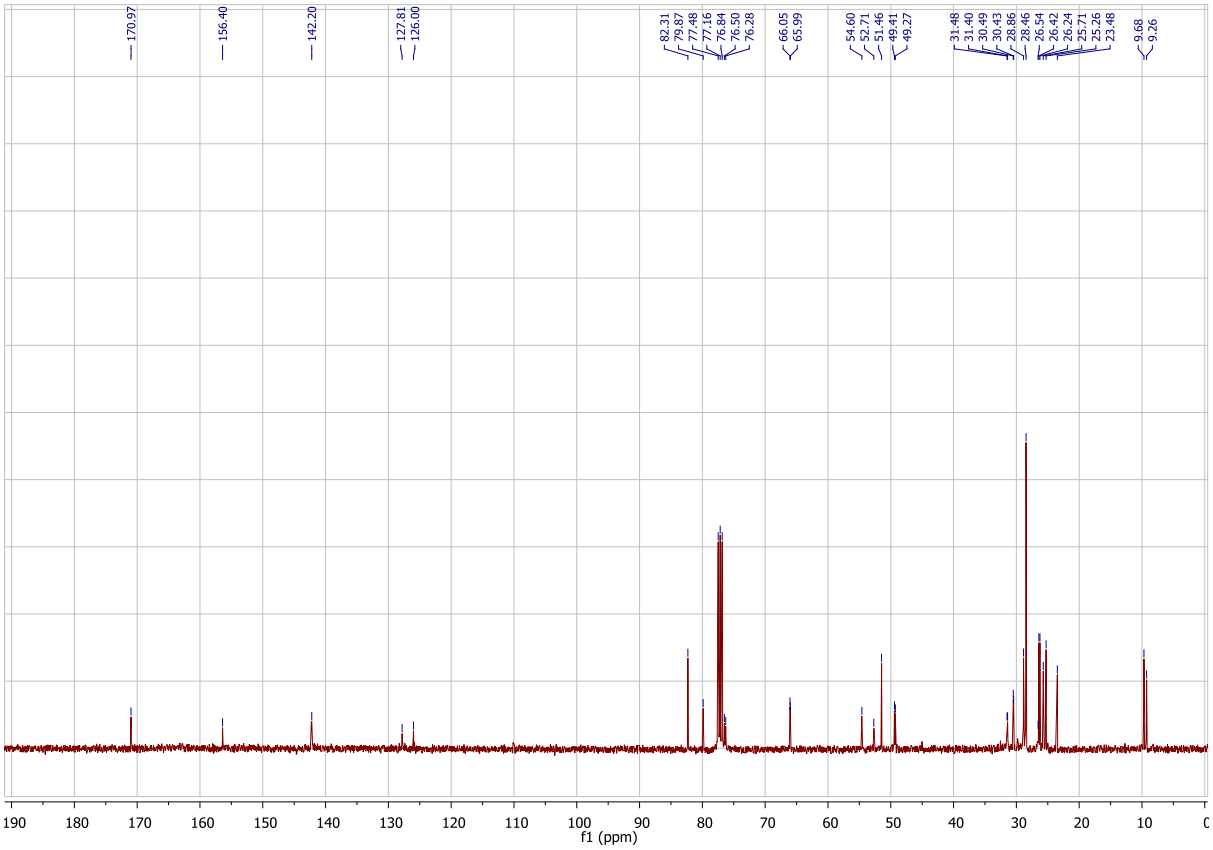
6-Azido-1-hexanol (8)



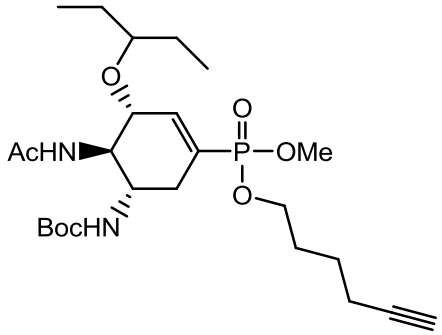


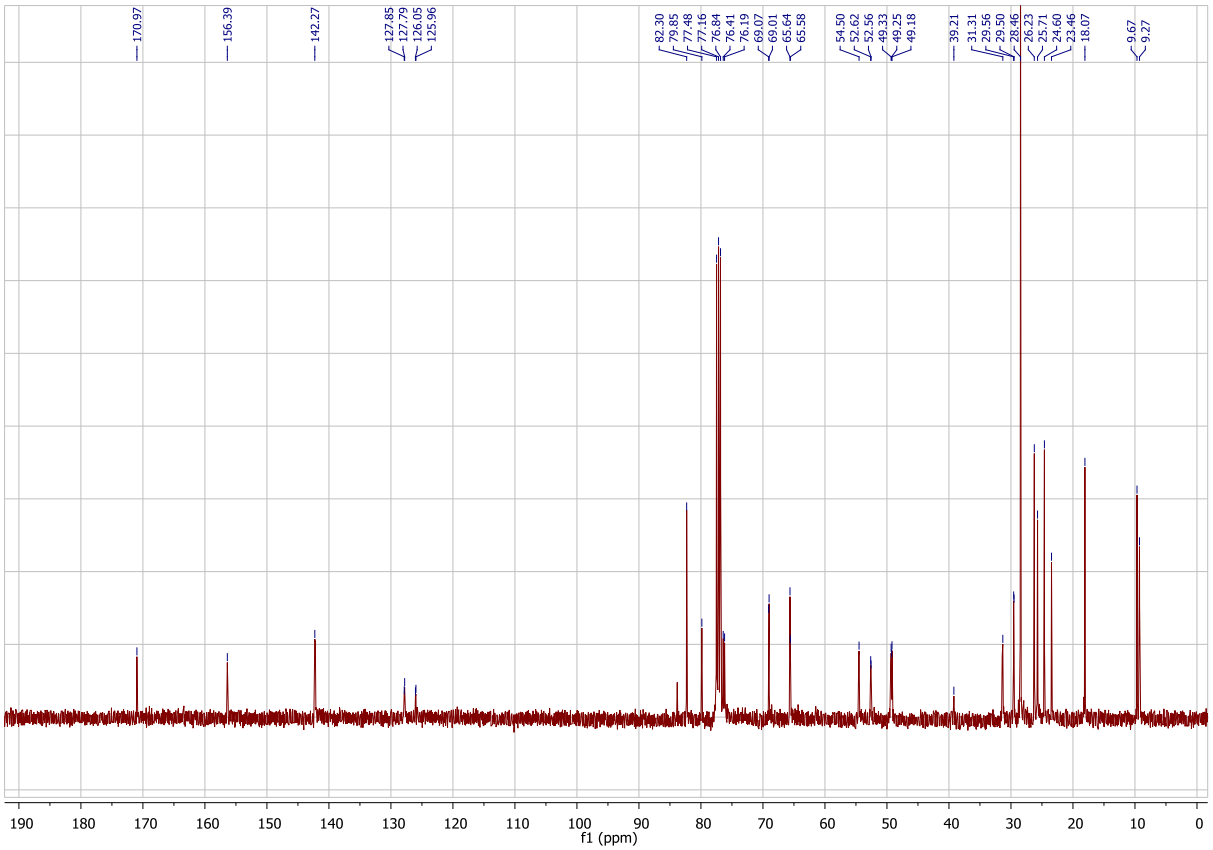
Methyl (6-azidohex-1-yl) [(3*R*,4*R*,5*S*)-4-acetamido-5-(1,1-dimethylethyloxycarbonylamino)-3-(1-ethylpropoxy)-1-cyclohexene-1-phosphonate] (11)



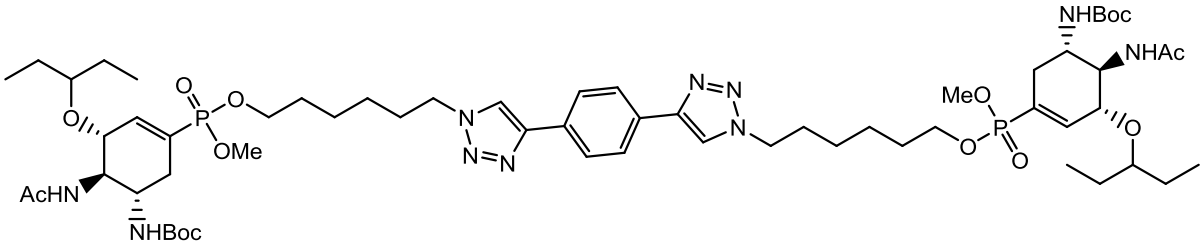


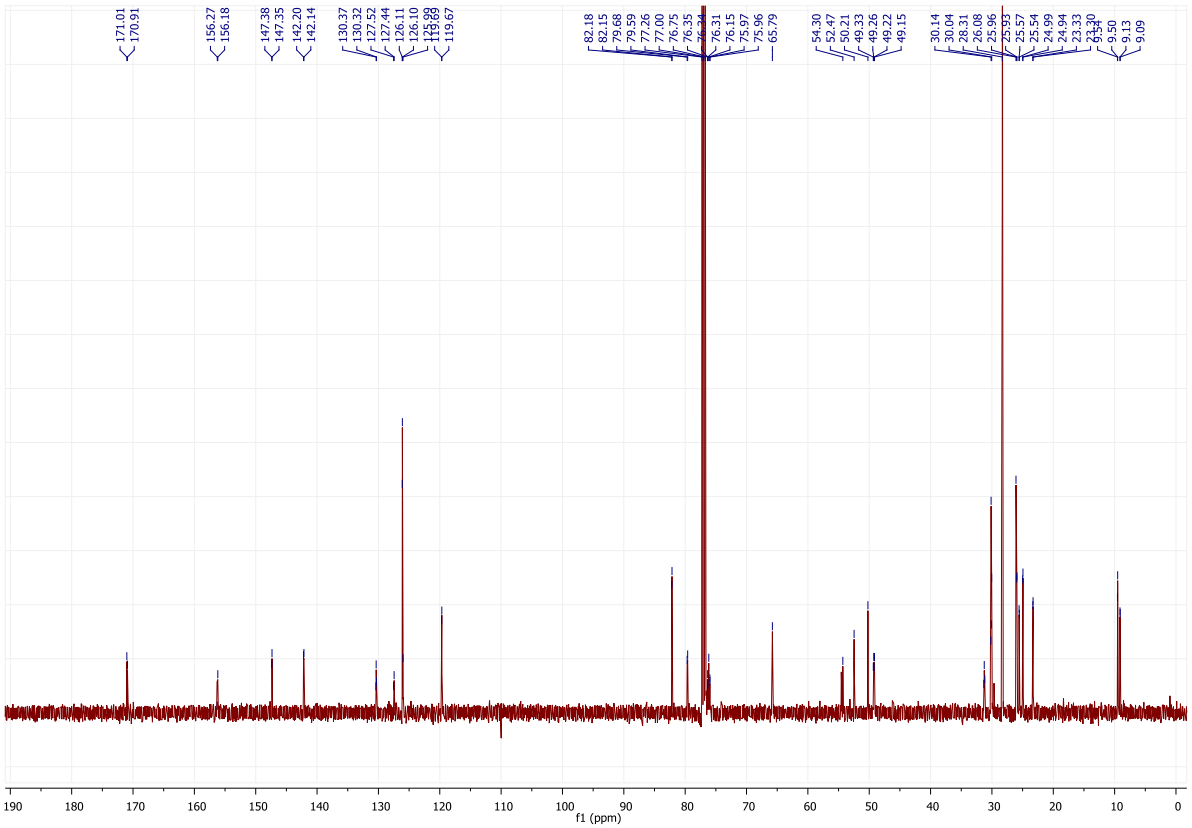
Methyl (hex-5-yn-1-yl) [(3*R*,4*R*,5*S*)-4-acetamido-5-(1,1-dimethylethyloxycarbonylamino)-3-(1-ethylpropoxy)-1-cyclohexene-1-phosphonate] (12)



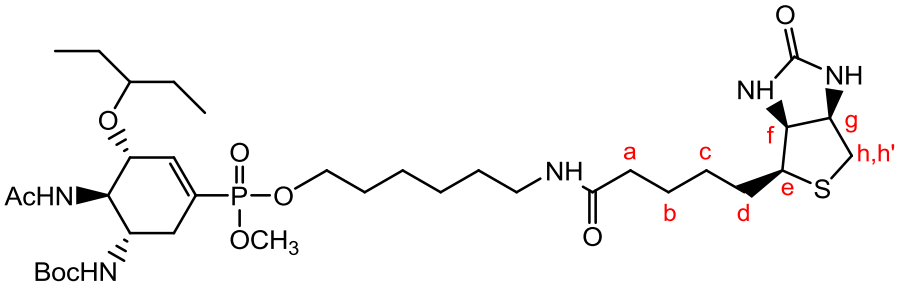


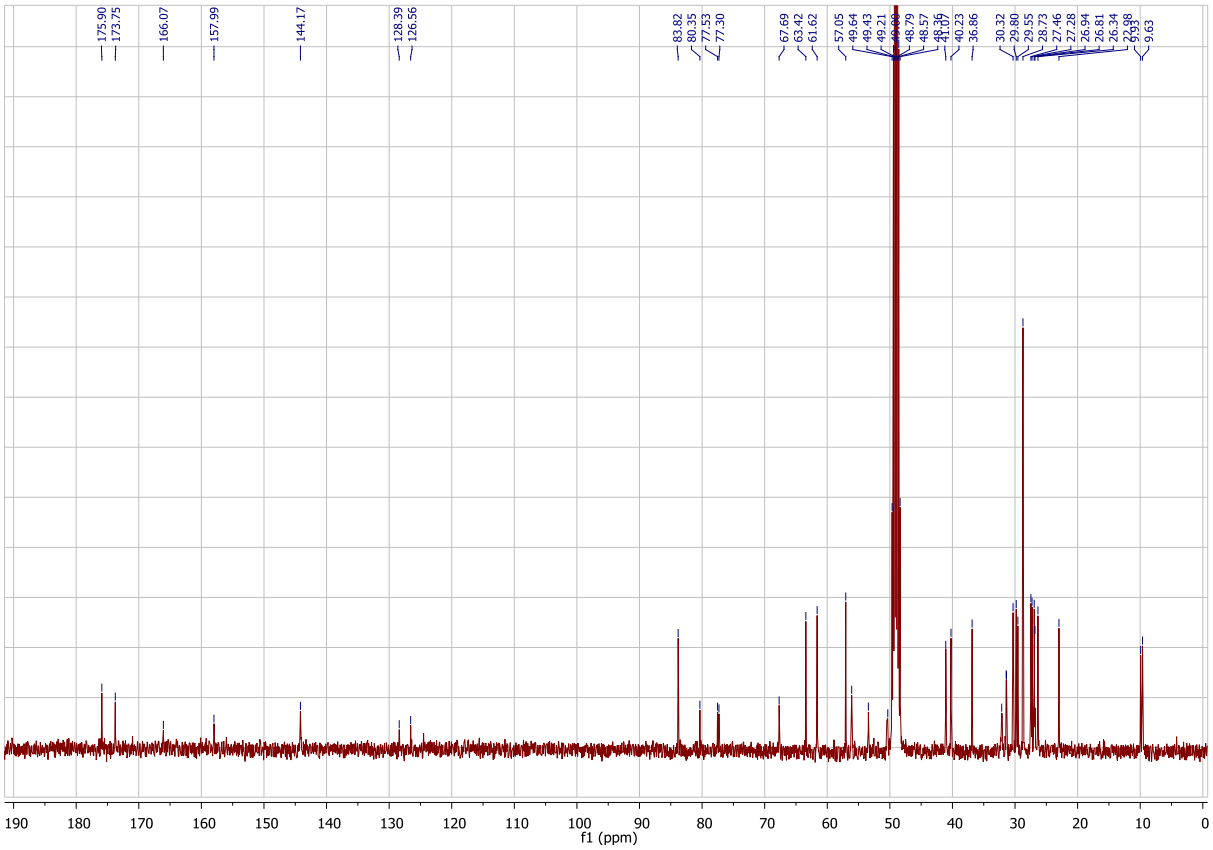
1,4-Bis [1-(hex-1'-yl methyl [(*3R*,*4R*,*5S*)-4-acetamido-5-(1,1 dimethylethyloxycarbonylamino)-3-(1-ethylpropoxy)-1-cyclohexene-1-phosphonate])-1*H*-1,2,3-triazol-4-yl]-benzene (13)



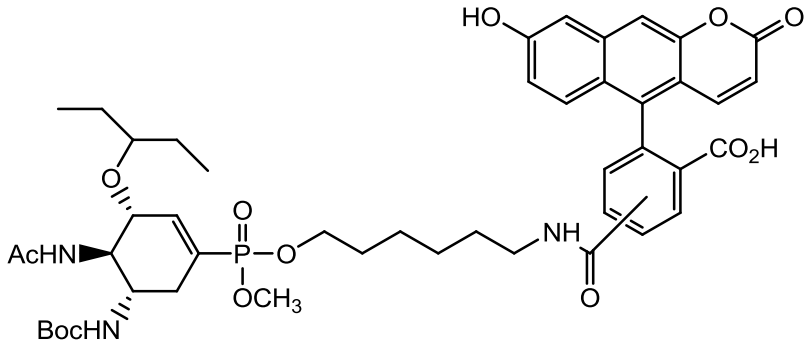


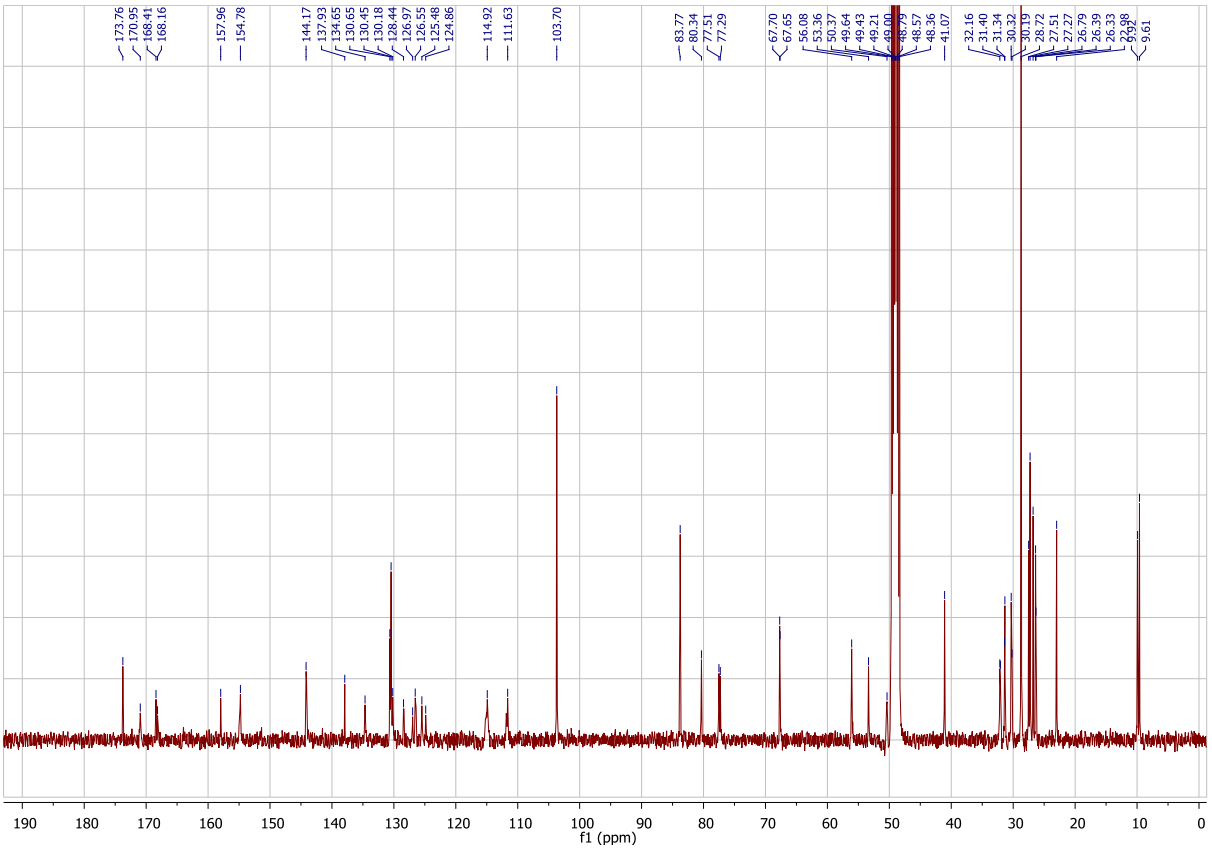
Methyl (6-*d*-biotinamidohex-1-yl) [(3*R*,4*R*,5*S*)-4-acetamido-5-(1,1-dimethylethyloxycarbonylamino)-3-(1-ethylpropoxy)-1-cyclohexene-1-phosphonate] (14)





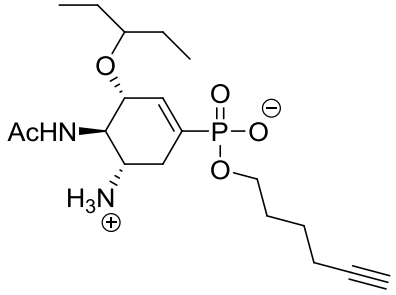
Methyl (6-(fluoresceinyl-5,6-carbonylamino)-hex-1-yl) [(3*R*,4*R*,5*S*)-4-acetamido-5-(1,1-dimethylethyloxycarbonylamino)-3-(1-ethylpropoxy)-1-cyclohexene-1-phosphonate] (15)

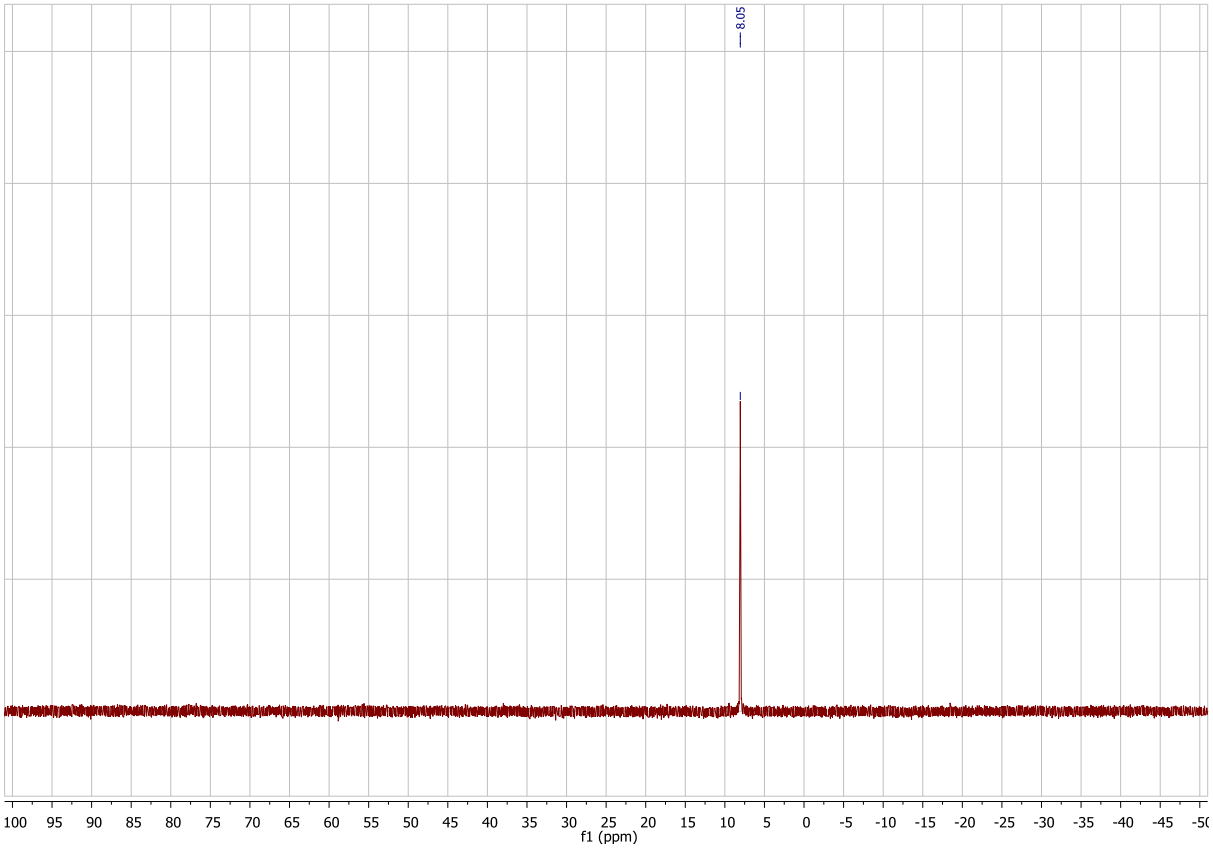




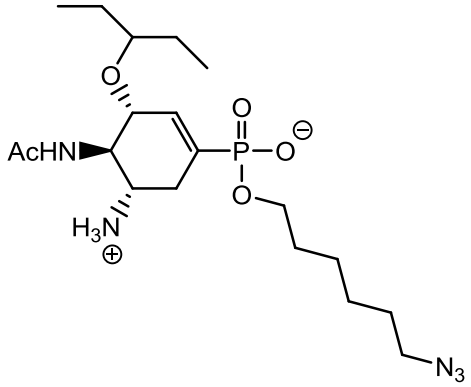
^{31}P-NMR-spectra

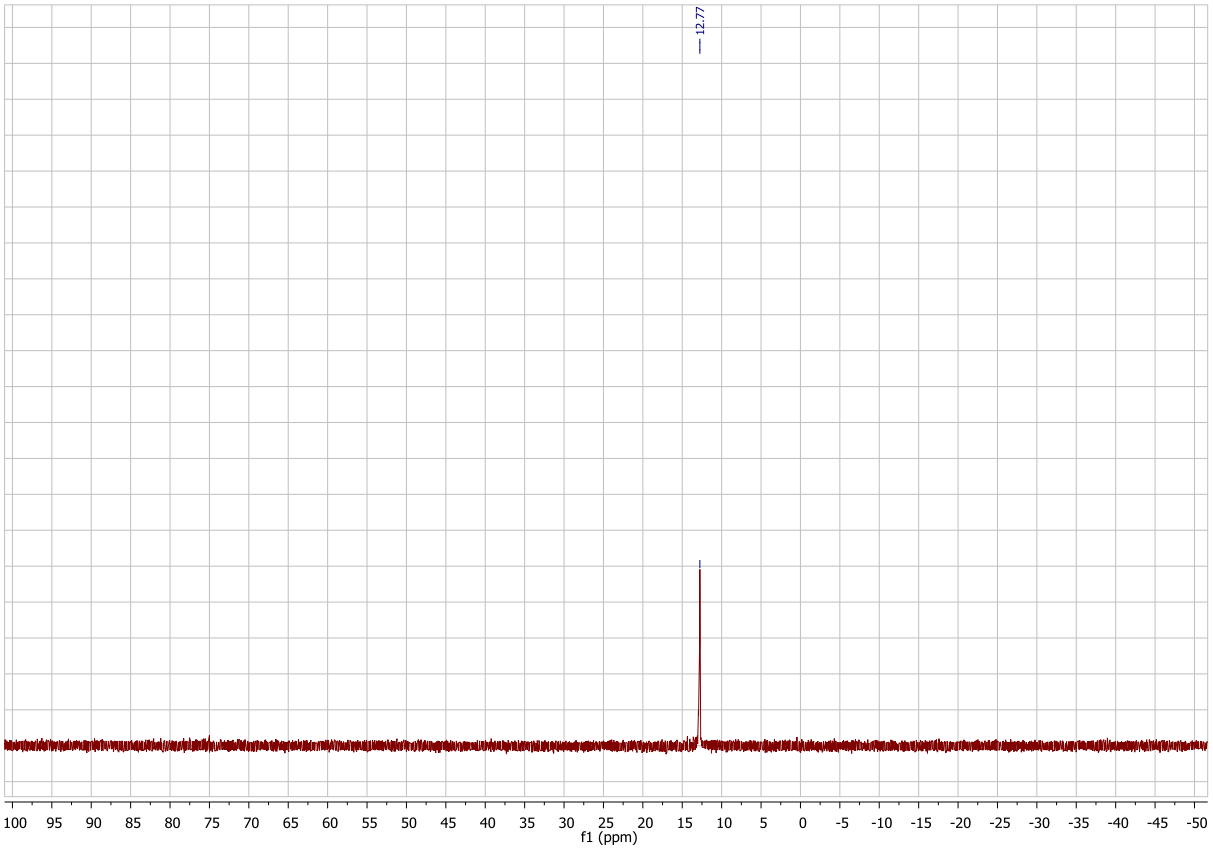
(Hex-5-yn-1-yl) (3*R*,4*R*,5*S*)-4-acetamido-5-amino-3-(1-ethylpropoxy)-1-cyclohexene-1-phosphonic acid (3a)



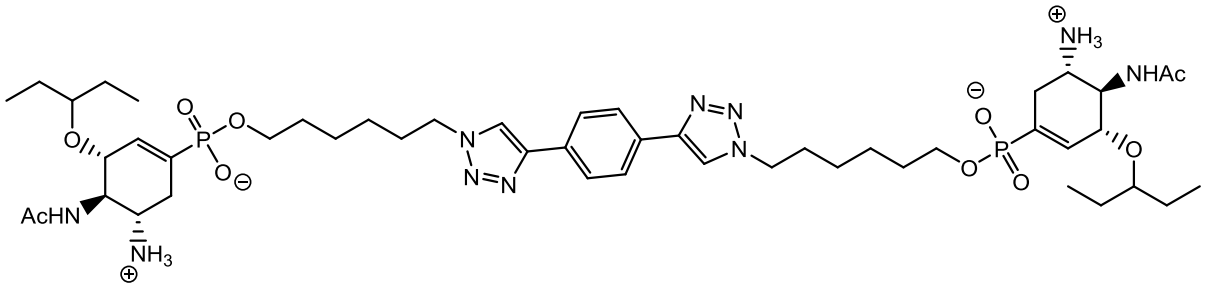


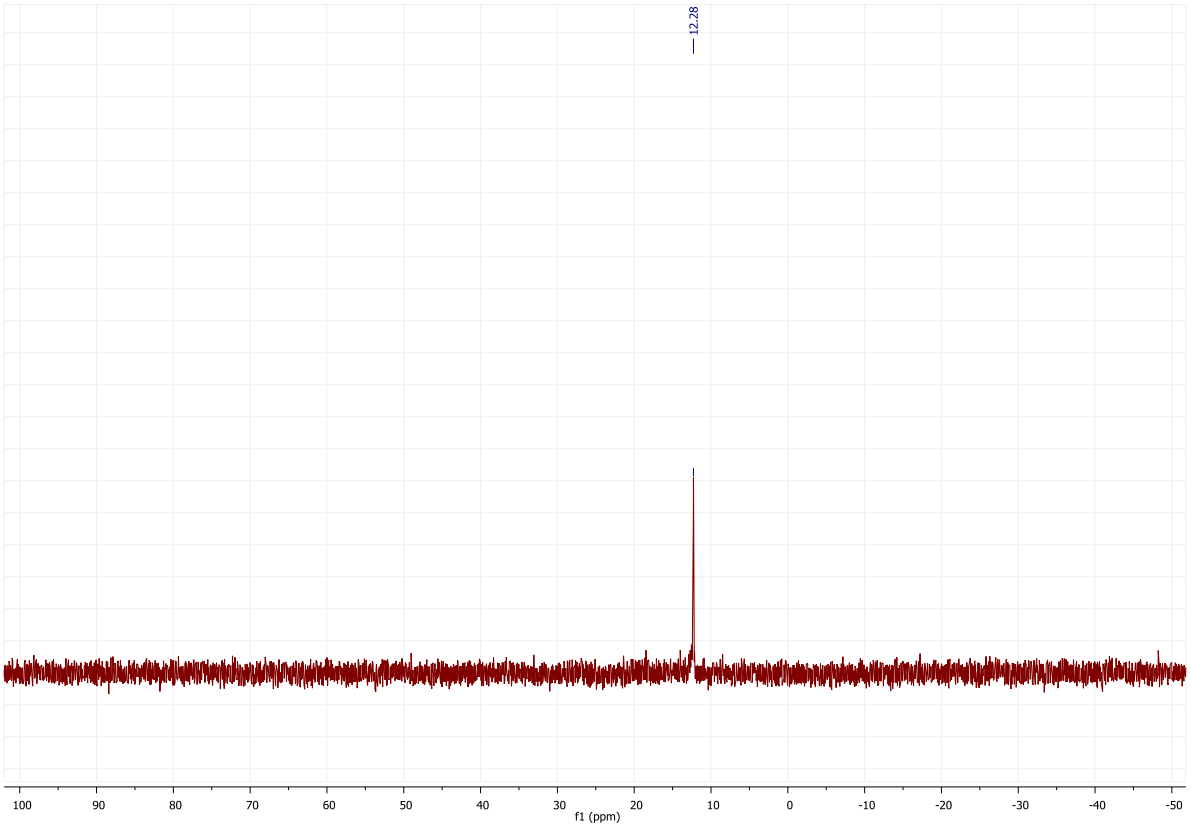
(6-Azido hexyl) (3*R*,4*R*,5*S*)-4-acetylamino-5-amino-3-(1-ethylpropoxy)-1-cyclohexene-1-phosphonic acid (3b)



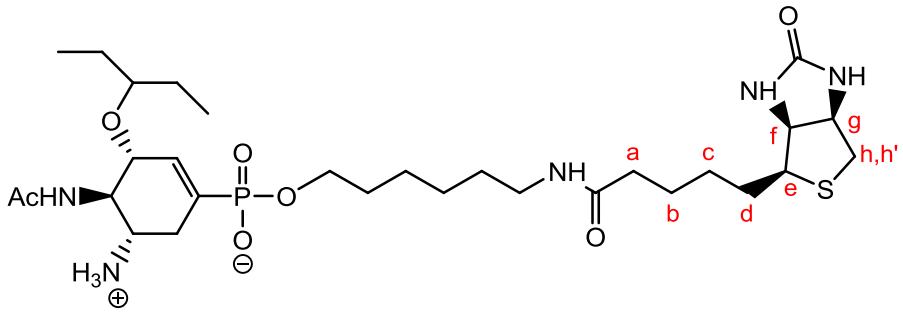


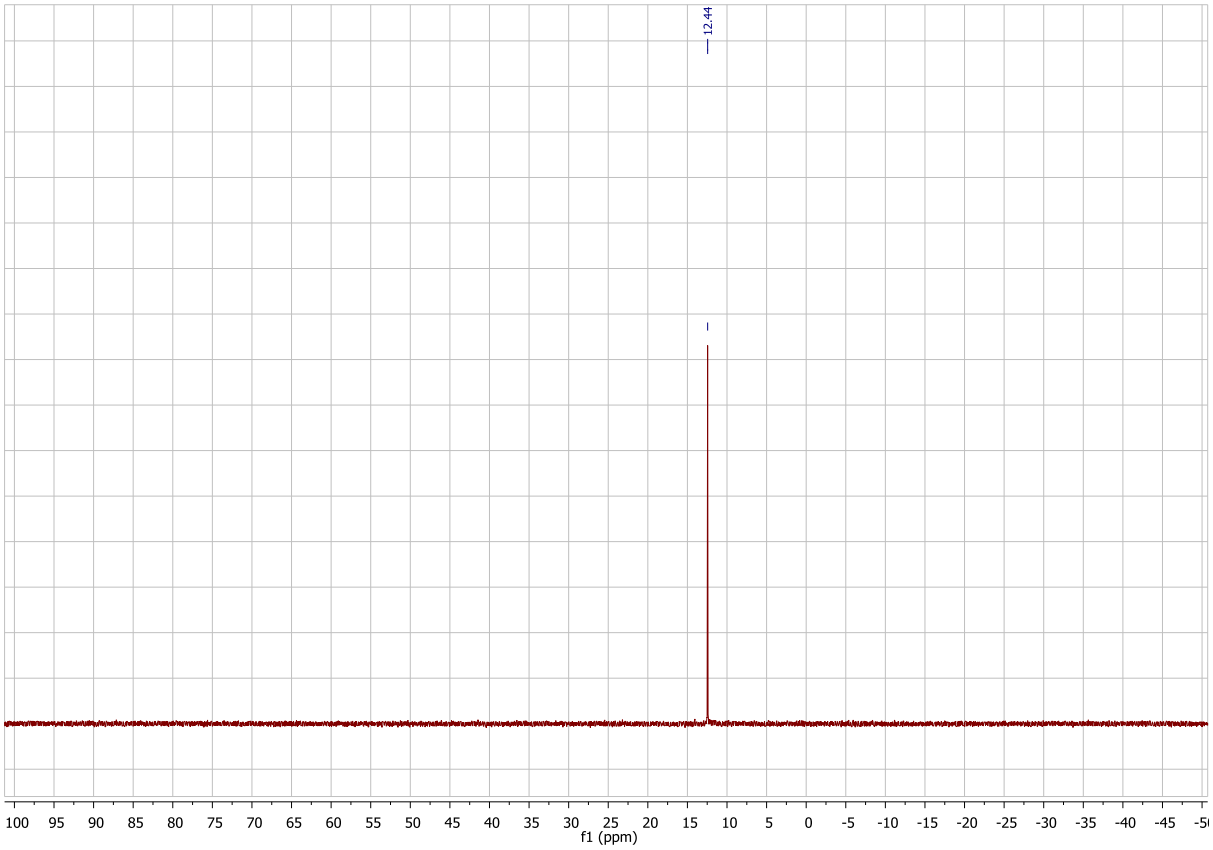
1,4-Bis [1-(hex-1'-yl [(*3R,4R,5S*)-4-acetamido-5-amino-3-(1-ethylpropoxy)-1-cyclohexene-1-phosphonic acid])-1*H*-1,2,3-triazol-4-yl]-benzene (3c)



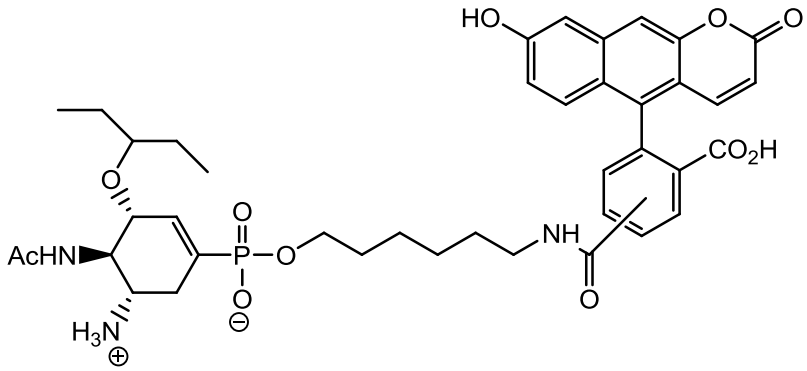


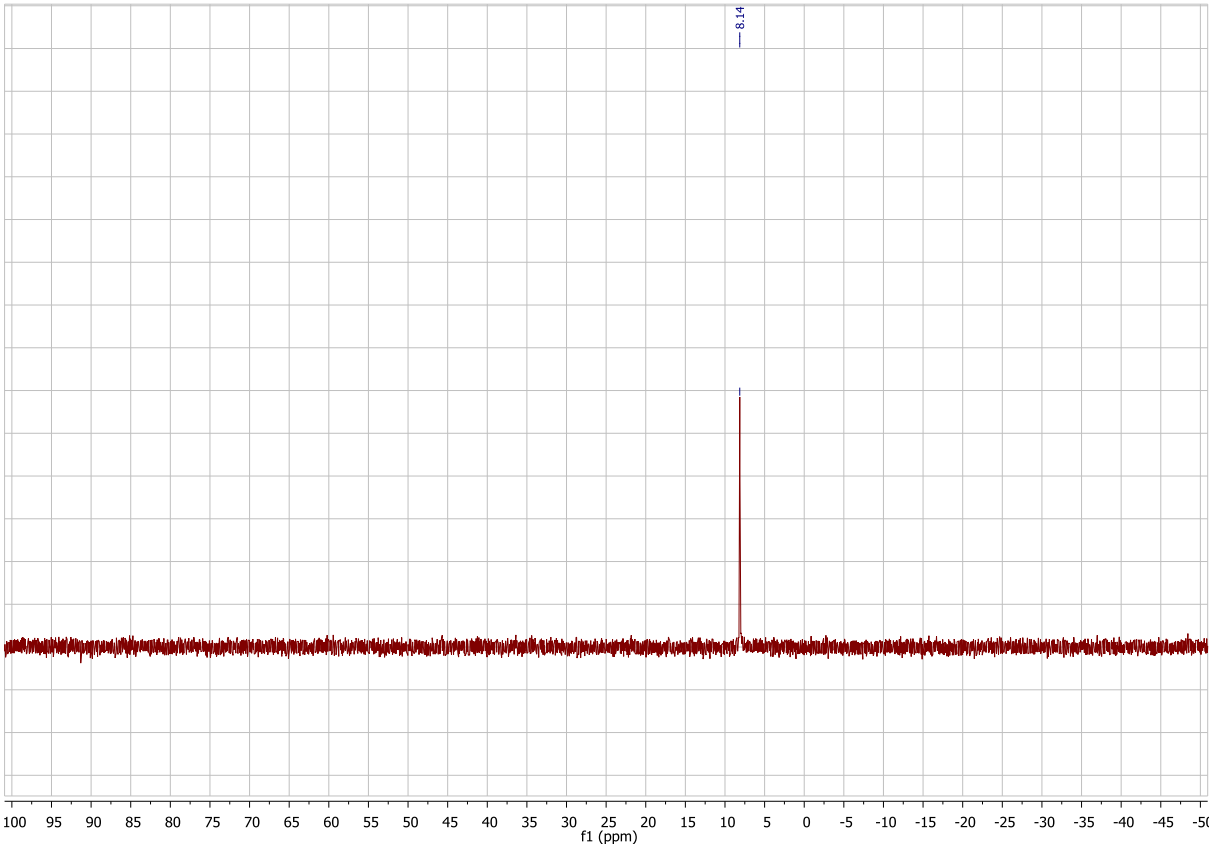
(6-*d*-Biotinamidohex-1-yl) (3*R*,4*R*,5*S*)-4-acetamido-5-amino-3-(1-ethylpropoxy)-1-cyclohexene-1-phosphonic acid (3d)



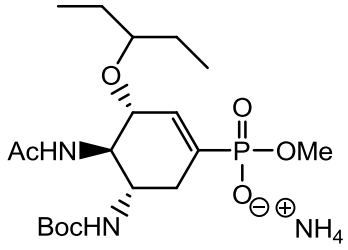


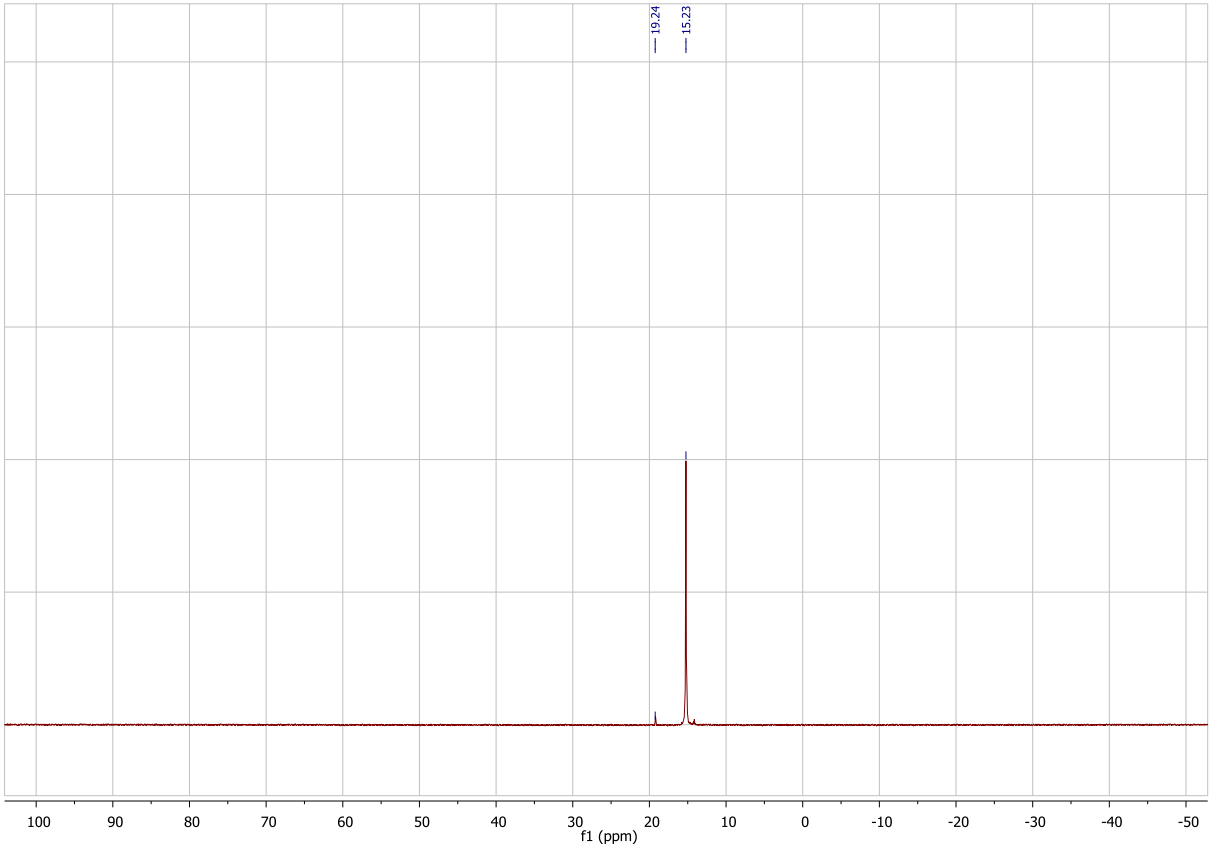
(6-(Fluoresceinyl-5,6-carbonylamino)-hex-1-yl) (3*R*,4*R*,5*S*)-4-acetylamino-5-amino-3-(1-ethylpropoxy)-1-cyclohexene-1-phosphonic acid (3e)



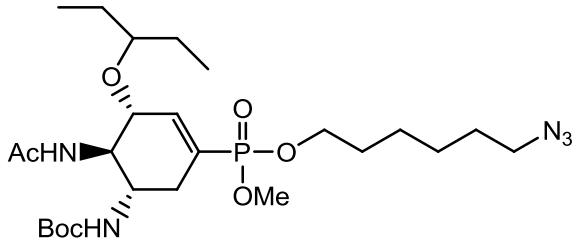


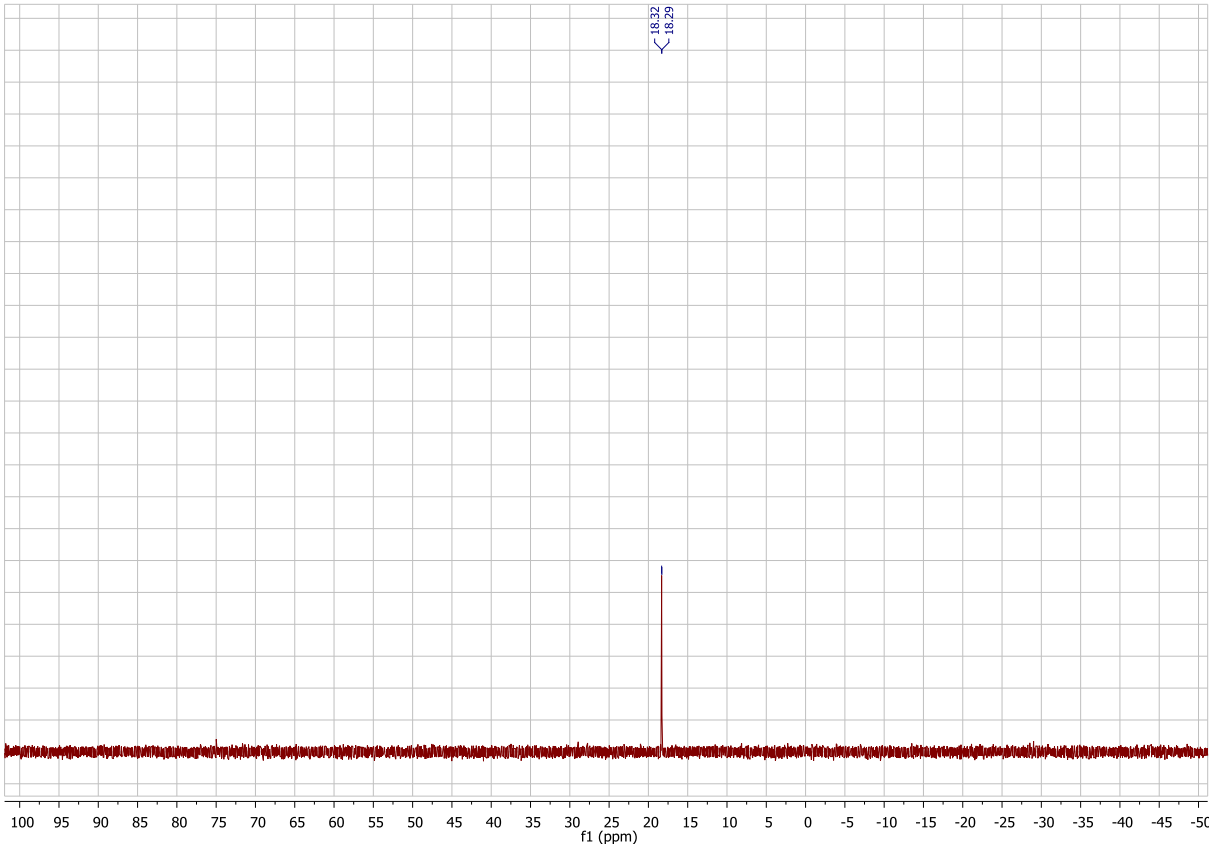
Ammonium [methyl (3*R*,4*R*,5*S*)-4-acetamido-5-(1,1-dimethylethyloxycarbonylamino)-3-(1-ethylpropoxy)-1-cyclohexene-1-phosphonate] (4)



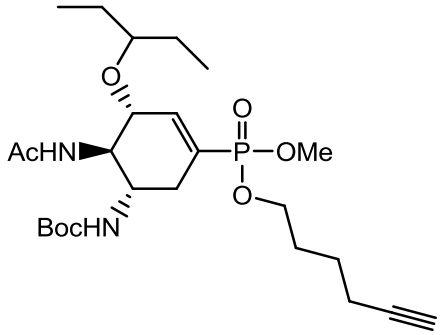


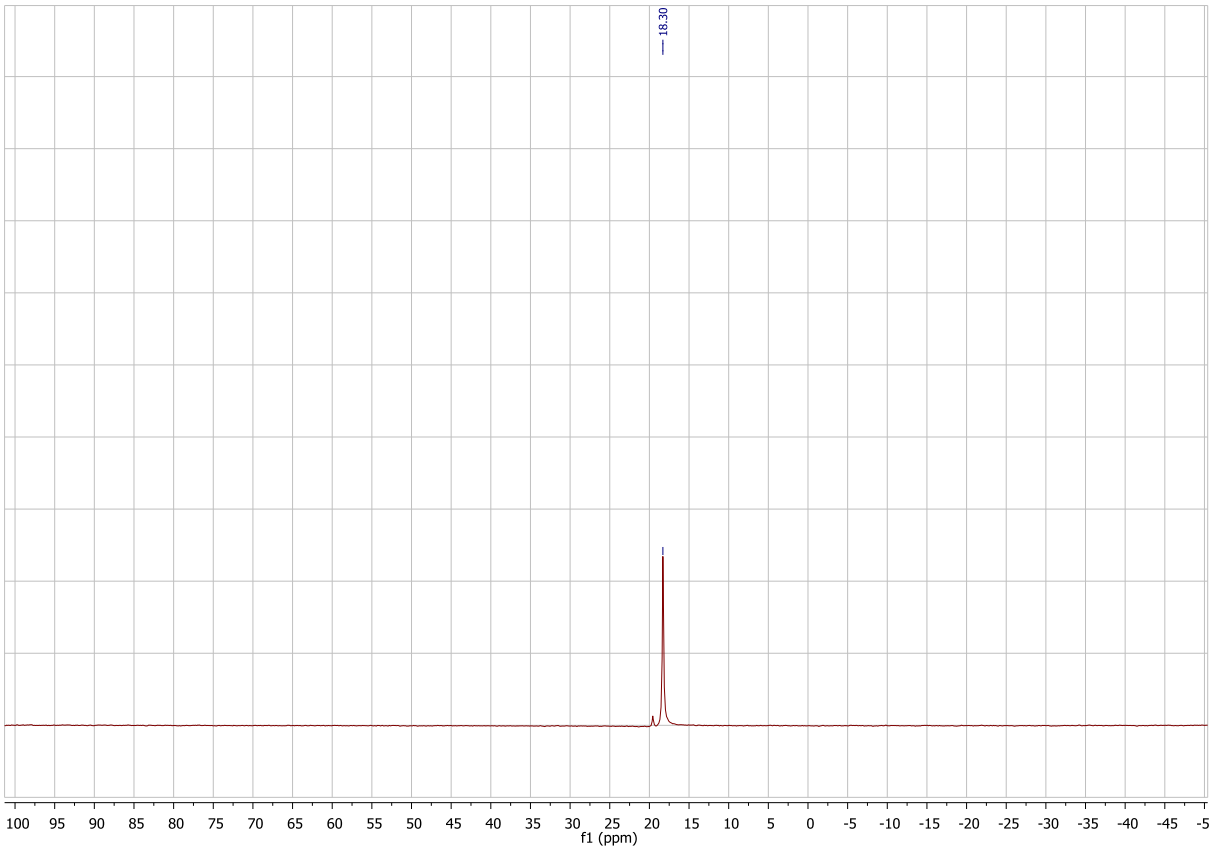
Methyl (6-azidohex-1-yl) [(3*R*,4*R*,5*S*)-4-acetamido-5-(1,1-dimethylethyloxycarbonylamino)-3-(1-ethylpropoxy)-1-cyclohexene-1-phosphonate] (11)



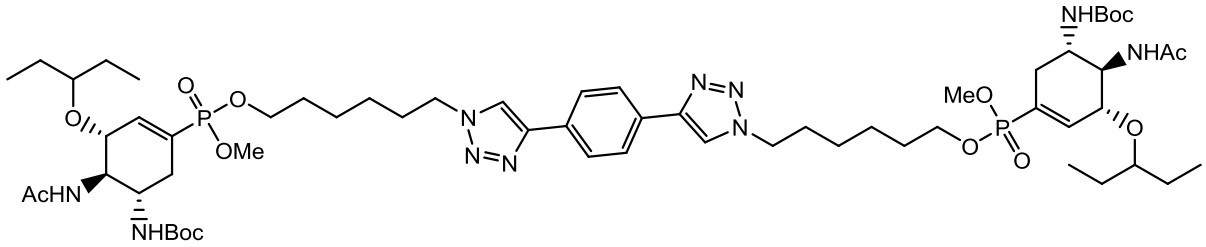


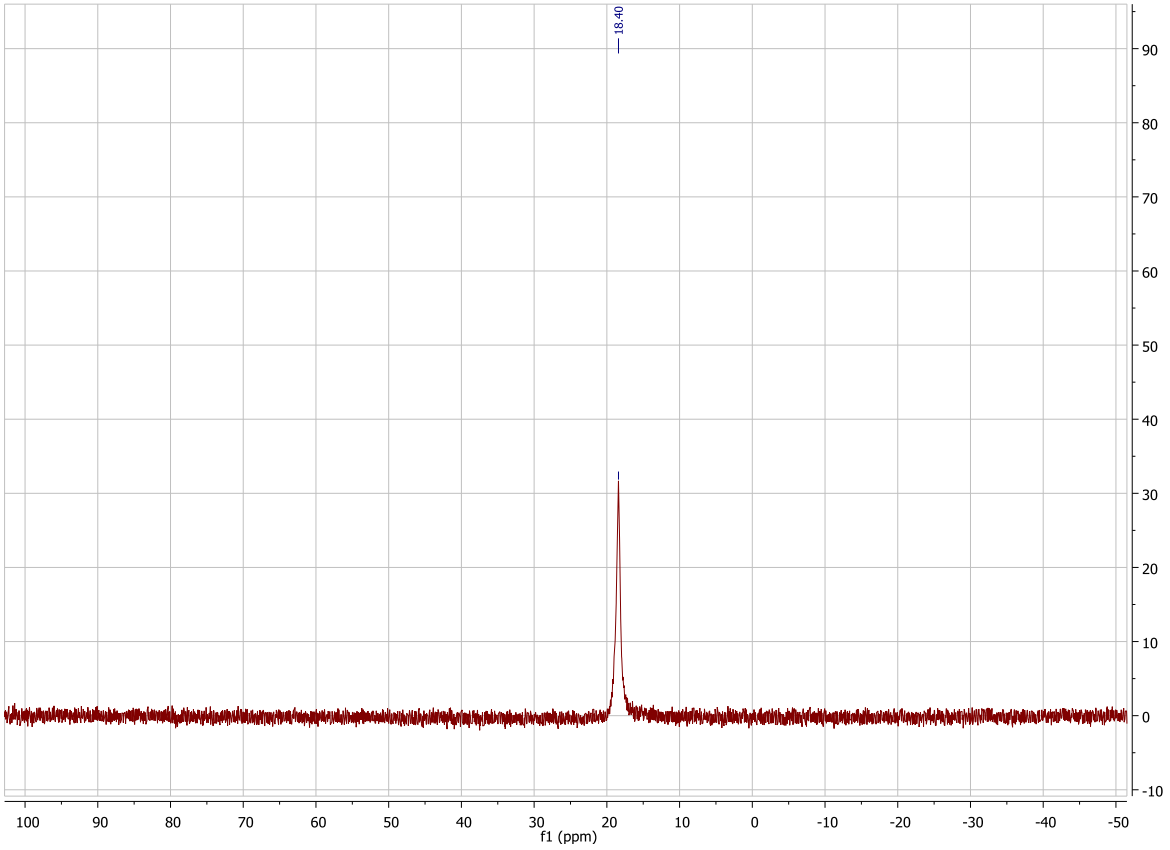
Methyl (hex-5-yn-1-yl) [(3*R*,4*R*,5*S*)-4-acetamido-5-(1,1-dimethylethyloxycarbonylamino)-3-(1-ethylpropoxy)-1-cyclohexene-1-phosphonate] (12)



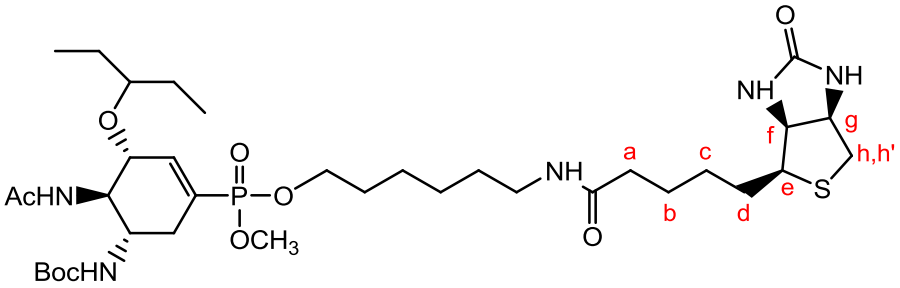


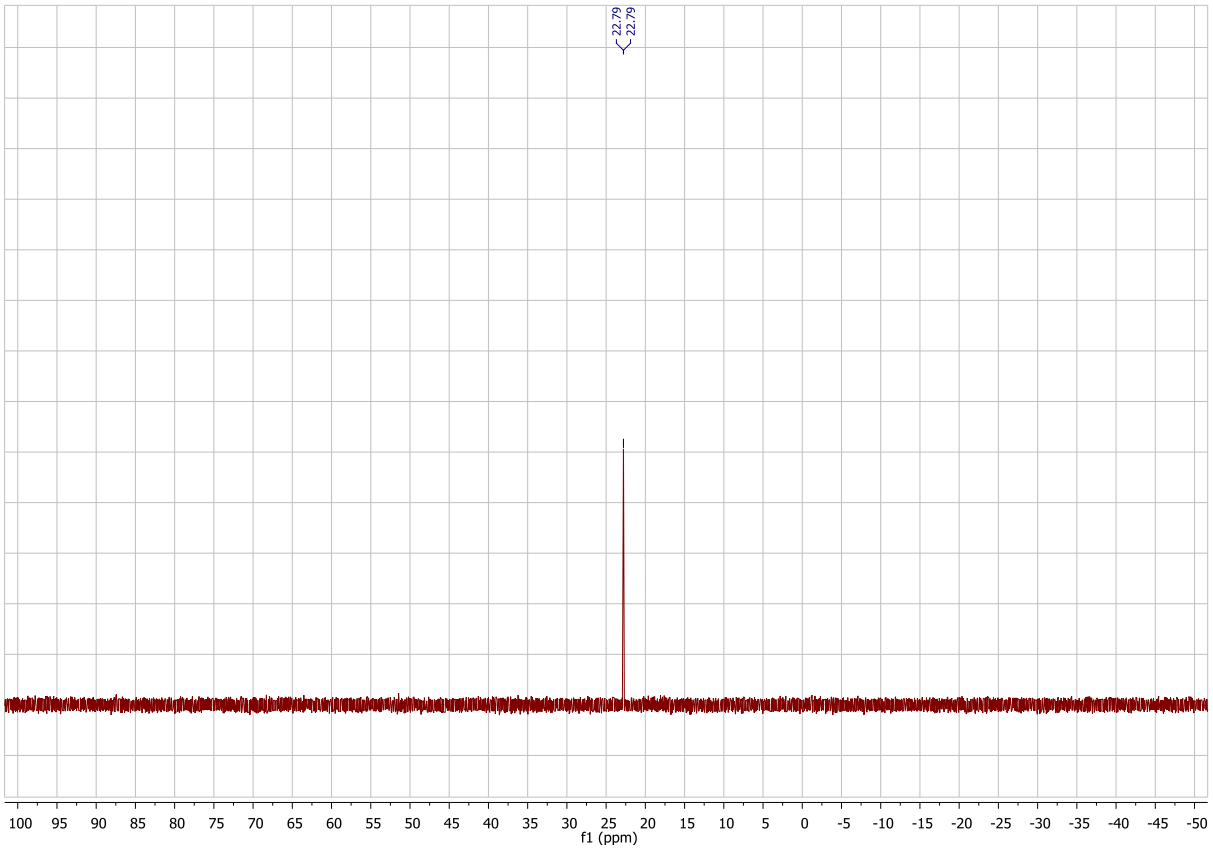
1,4-Bis [1-(hex-1'-yl methyl [(*3R,4R,5S*)-4-acetamido-5-(1,1 dimethylethyloxycarbonylamino)-3-(1-ethylpropoxy)-1-cyclohexene-1-phosphonate])-1*H*-1,2,3-triazol-4-yl]-benzene (13)



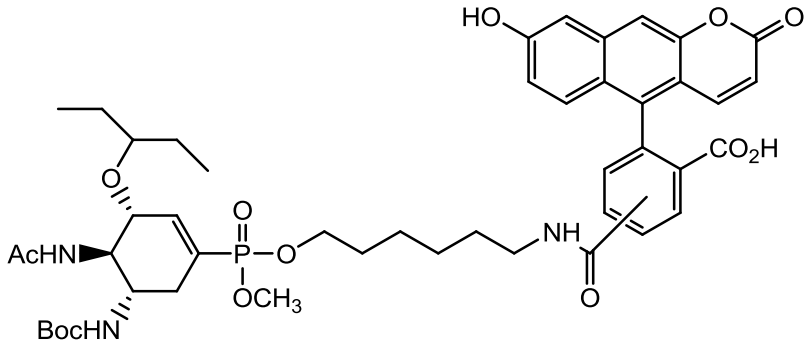


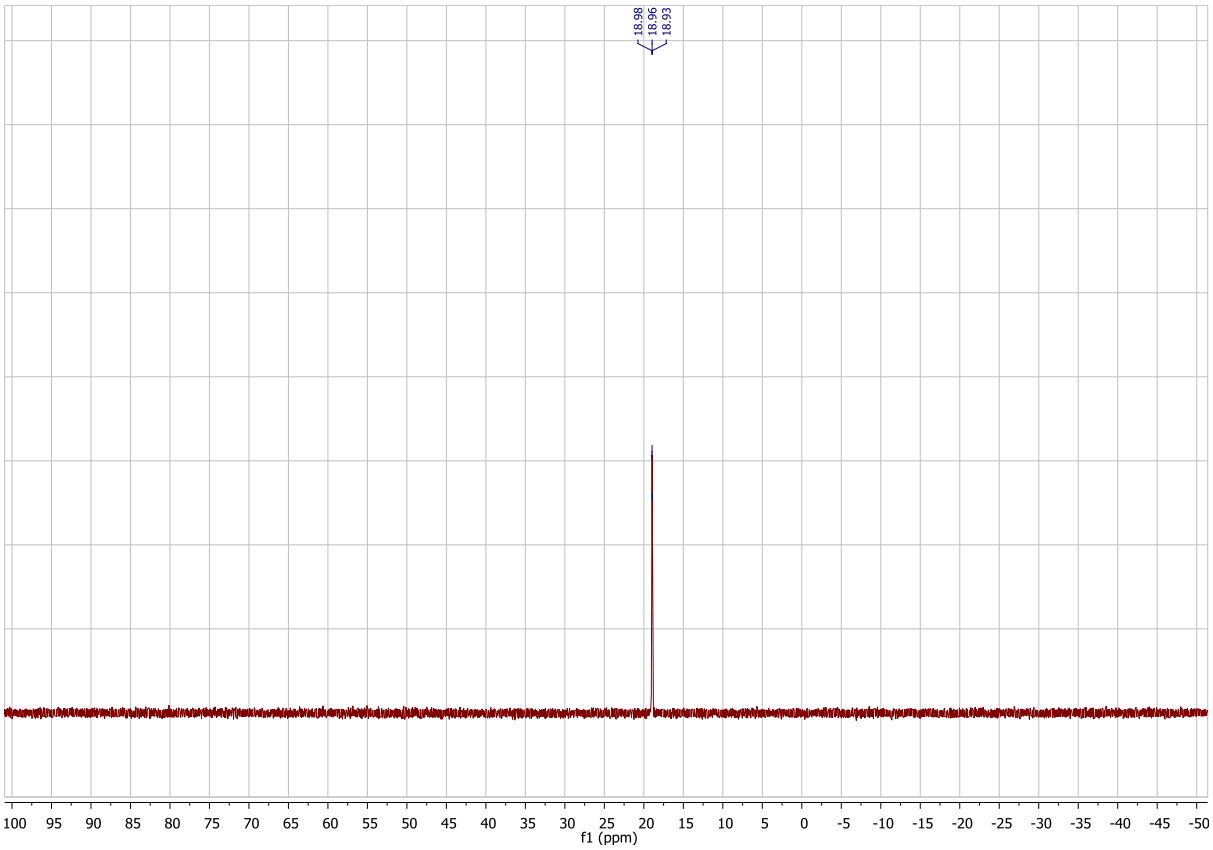
Methyl (6-*d*-biotinamidohex-1-yl) [(3*R*,4*R*,5*S*)-4-acetamido-5-(1,1-dimethylethyloxycarbonylamino)-3-(1-ethylpropoxy)-1-cyclohexene-1-phosphonate] (14)





Methyl (6-(fluoresceinyl-5,6-carbonylamino)-hex-1-yl) [(3*R*,4*R*,5*S*)-4-acetamido-5-(1,1-dimethylethyloxycarbonylamino)-3-(1-ethylpropoxy)-1-cyclohexene-1-phosphonate] (15)





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