Supporting Information

Synthesis and antiproliferative effect of the proposed stereoisomer of the marine sponge metabolite halisphingosine A

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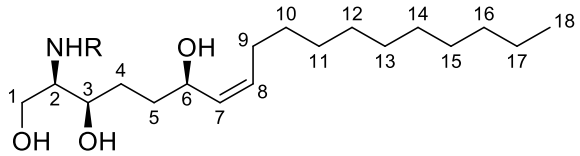
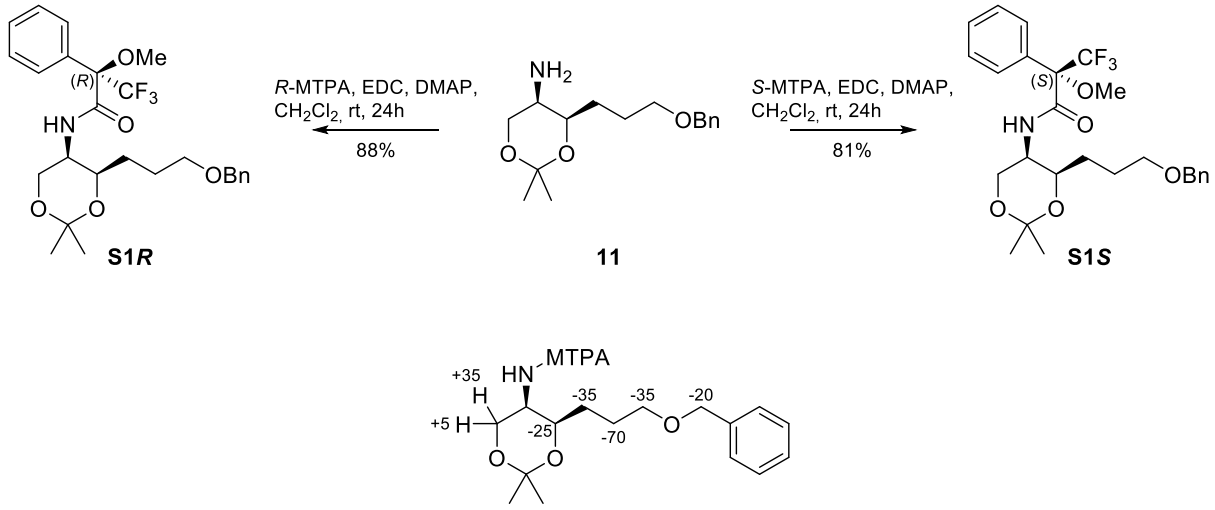
Table 1. Comparison of ^{13}C and ^{1}H NMR shifts of isolated and synthetic Halisphingosine A (**1**) and *N*-Boc-Halisphingosine A (**15**)



1: R = H
15: R = Boc

	isolated **1** (lit.[1]) in CD_3OD		synthetic **1** in CD_3OD		**15** prepared from isolated **1** (lit.[2]) in CD_3OD		our fully synthetic **15** in CD_3OD	
Pos.	δ_C	δ_H (mult., *J* [Hz])	δ_C	δ_H (mult., *J* [Hz])	δ_C	δ_H (mult., *J* [Hz])	δ_C	δ_H (mult., *J* [Hz])
1	60.9	3.74 (dd, 11.5, 4.5)	63.4	3.65 (dd, 11.0, 4.6)	62.8	3.60 (m)	63.2	3.60 (m)
		3.62 (dd, 11.5, 7.0)		3.52 (dd, 11.0, 6.7)		3.55(m)		3.56 (m)
2	59.0	2.99 (dt, 7.0, 4.5)	58.4	2.77 (dt, 6.7, 5.2)	56.5	3.54 (m)	56.8	3.53 (m)
3	69.3	3.64 (m)	71.4	3.58 (m)	70.7	3.75 (m)	71.2	3.76 (m)
4	35.0	1.52 (m)	33.08	1.58 (m)	34.7	1.45 (m)	33.1	1.48 (m)
		1.42 (m)		1.52 (m)				
5	38.7	1.55 (m)	34.8	1.72 (m)	38.5	1.56 (m)	35.1	1.69 (m)
		1.35 (m)		1.48 (m)		1.38 (m)		1.53 (m)
6	68.3	4.35 (m)	68.1	4.41 (td, 7.8, 5.8)	67.9	4.37 (dt, 9.0, 5.7)	68.4	4.37 (dt, 8.5, 6.7)
7	134.0	5.31 (dd, 11.0, 9.0)	133.8	5.36 (dd, 11.0, 9.0)	133.7	5.31 (ddd, 9.3, 7.8, 1.5)	133.9	5.33 (dd, 11.0, 8.5)
8	132.2	5.44 (dt, 11.0, 7.5)	132.4	5.45 (dt, 11.0, 7.3)	131.8	5.45 (dt, 9.3, 6.4)	132.4	5.44 (dt, 11.0, 7.6)
9	28.7	2.08 (m)	28.7	2.08 (m)	28.4	2.09 (m)	28.7	2.09 (m)
10-17	23.7 - 32.9	1.28 - 1.37 (m)	23.7 - 30.9	1.24 – 1.40 (m)	23.5 – 32.5	1.29-1.41 (m)	23.8 – 31.2	1.28 – 1.40 (m)
18	14.1	0.90 (t, 7.0)	14.5	0.90 (t, 7.0)	14.1	0.91 (t, 6.7)	14.5	0.90 (t, 7.0)
Boc-CH_3	-	-	-	-	28.5	1.45 (s)	28.8	1.45 (s)
Boc-C=O	-	-	-	-	156.4	-	158.4	-
Boc-*C*CH_3	-	-	-	-	79.6	-	80.1	-

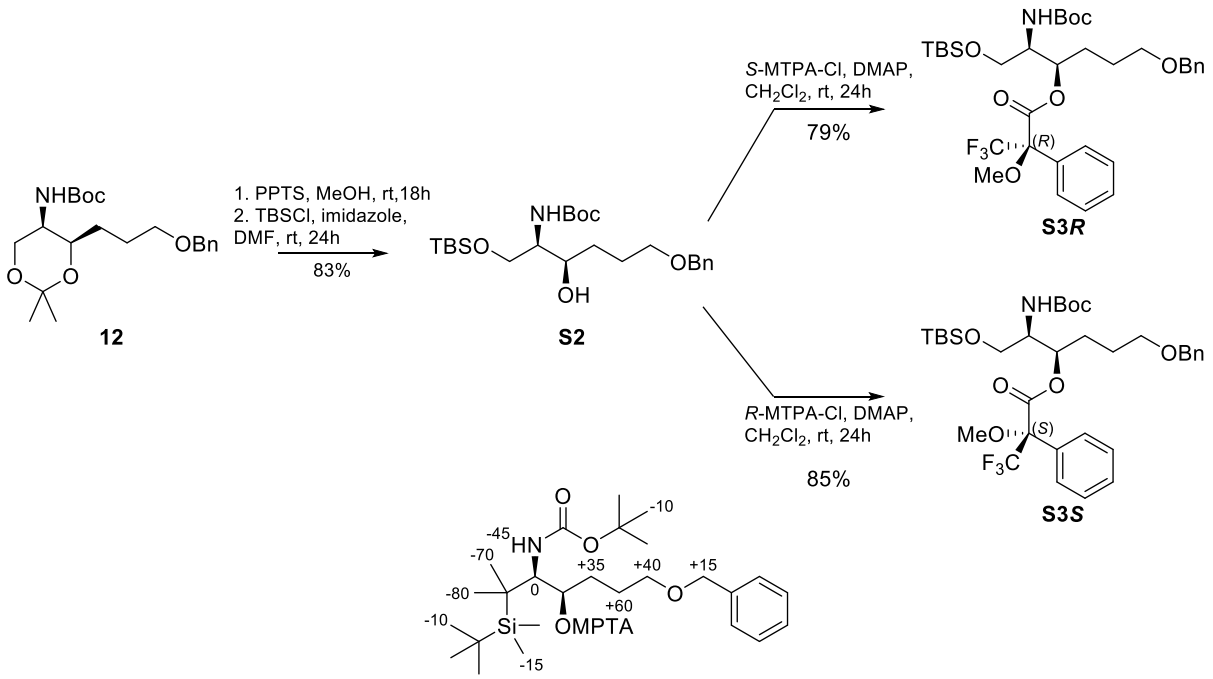
Synthesis and analysis of Mosher amides and Mosher esters S1-S4[3,4]



Scheme S1. Synthesis and $\Delta\delta_{SR} = \delta_S - \delta_R$ values in Hz of the ^{1}H NMR spectra of Mosher-amides **S1*R*** and **S1*S***.

(*R*)- and (*S*)-N-((4R,5R)-4-(3-(benzyloxy)propyl)-2,2-dimethyl-1,3-dioxan-5-yl)-3,3,3-trifluoro-2-methoxy-2-phenylpropanamide (S1*R*, S1*S*)

A solution of amine **11** (25 mg, 89 µmol, 1.0 equiv.) in dry CH_2Cl_2 (1 mL) was treated with NEt_3 (25 µL, 179 µmol, 2.0 equiv.), EDC·HCl (21 mg, 107 µmol, 1.2 equiv.) and (*S*)- or (*R*)-MTPA (25 mg, 107 µmol, 1.2 equiv.). The mixture was stirred at ambient temperature for 18 h and volatiles were removed *in vacuo*. The crude products were purified by flash chromatography (*n*-hexane/ethyl acetate 2:1) to give **S1*R*** and **S1*S*** as colorless oils; R_f = 0.57 (*n*-hexane/ethyl acetate 2:1); IR (ν_{max}/cm^{-1}) 3427, 2951, 1698, 1508, 1271, 1166, 1103, 714, 698; HRMS (ESI) *m/z* $[M + H]^+$ calcd for $C_{26}H_{33}O_5NF_3^+$ 496.2305, found 496.2288, $[M + Na]^+$ calcd for $C_{26}H_{32}O_5NF_3Na^+$ 518.2125, found 518.2105; **S1*R*** (43 mg, 72 µmol, 81%): $[\alpha]^{23}_D$ = -33.8 (*c* 1.0 in $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.37-1.41 (3 H, s, CH_3), 1.42-1.46 (3 H, s, CH_3), 1.48-1.57 (1 H, m, C*H*HCCOBn), 1.58-1.77 (3 H, m, CH*H*C*H*$_2$COBn), 3.39 (3 H, s, OCH_3), 3.43-3.52 (2 H, m, CH_2OBn), 3.70, (1 H, dd, *J* = 12.1, 2.0 Hz, 6-H^a), 3.90 (1 H, ddd, *J* = 9.5, 1.7, 1.7 Hz, C*H*NH), 4.00-4.04 (1 H, m, 4-H), 1.06 (1 H, dd, *J* = 12.1, 2.0 Hz, 6-H^b), 4.44-4.54 (2 H, m, CH_2Ph), 7.26-7.37 (4 H, m, Ph), 7.39-7.45 (3 H, m, Ph), 7.53-7.63 (3 H, m, Ph); ^{13}C NMR (126 MHz, $CDCl_3$) δ 18.5 (CH_3), 25.0 (*C*COBn), 28.6 (*C*CCOBn), 29.7 (CH_3), 45.7 (C-5), 54.9 (OCH_3), 64.6 (C-6), 69.8 (*C*H$_2$OBn), 70.8 (C-4), 72.9 (*C*H$_2$Ph), 84.0 ($^2J_{FC}$ = 26.3 Hz, *C*CF$_3$), 99.1 (C-2), 123.9 ($^1J_{FC}$ = 290.6 Hz, CF_3), 123.9, 127.5, 127.6, 127.7, 128.3, 128.6, 129.4, 132.2, 138.5, 166.0 (NCO); **S1*S*** (47 mg, 79 µmol, 88%): $[\alpha]^{23}_D$ = -5.8 (*c* 1.0 in $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.22-1.37 (2 H, m, CH_2CCOBn), 1.39 (3 H, s, CH_3), 1.42 (3 H, s, CH_3), 1.48-1.65 (2 H, m, CH_2COBn), 3.28-3.40 (2 H, m, CH_2OBn), 3.50 (3 H, s, OCH_3), 3.77 (1 H, dd, *J* = 11,9, 1.8 Hz, 6-H^a), 3.90 (1 H, ddd, *J* = 9.7, 1.8, 1.8 Hz, C*H*NH), 3.98 (1 H, td, *J* = 6.9, 1.8 Hz, 4-H), 4.07 (1 H, dd, *J* = 11.9, 1.8 Hz, 6-H^b), 4.45 (2 H, q, *J* = 12.1 Hz, CH_2Ph), 7.26-7.40 (8 H, m, Ph), 7.52-7.62 (2 H, m, Ph); ^{13}C NMR (126 MHz, $CDCl_3$) δ 18.5 (CH_3), 24.9 (*C*COBn), 28.6 (*C*CCOBn), 29.7 (CH_3), 45.5 (C-5), 55.1 (OCH_3), 64.8 (C-6), 69.7 (*C*H$_2$OBn), 70.8 (C-4), 72.9 (*C*H$_2$Ph), 84.0 ($^2J_{FC}$ = 25.4 Hz, *C*CF$_3$), 99.2 (C-2), ($^1J_{FC}$ = 289.7 Hz, CF_3) 127.4, 127.6, 127.7, 128.3, 128.4, 129.4, 132.9, 128.4, 166.1 (NCO); The absolute configuration of amine **S1** was confirmed to be *R*.



Scheme S2. Synthesis of alcohol **S2**; synthesis and $\Delta\delta_{SR} = \delta_S - \delta_R$ values in Hz of the ^{1}H NMR spectra of Mosher esters **S3*R*** and **S3*S***.

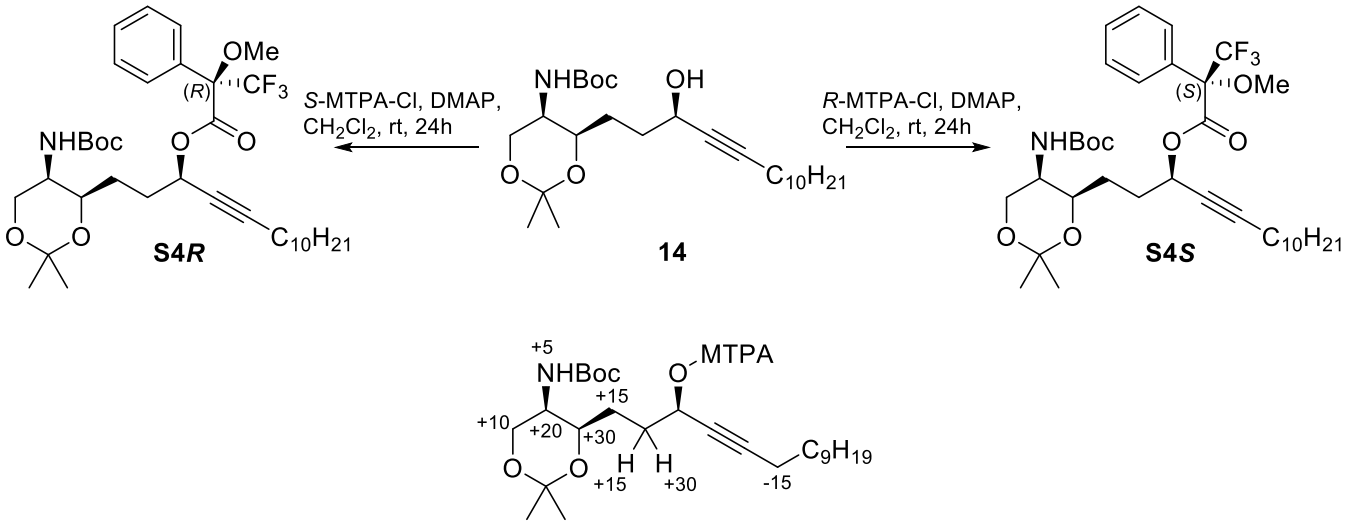
tert-Butyl ((2*R*,3*R*)-6-(benzyloxy)-1-((tert-butyldimethylsilyl)oxy)-3-hydroxyhexan-2-yl)carbamate (S2)

A solution of carbamate **12** (100 mg, 264 µmol, 1.0 equiv.) in MeOH (2 mL), was treated with PPTS (28 mg, 111 µmol, 0.5 equiv.) and stirred at ambient temperature for 18 h. Volatiles were removed *in vacuo*. The residue was taken up in EtOAc (10 mL) and filtered through a plug of silica. The filtrate was concentrated to dryness. The crude diol was dissolved in DMF (2 mL) and imidazole (30 mg, 441 µmol, 2.0 equiv.) and TBSCl (33 mg, 218 µmol, 1.0 equiv.) were added. The mixture was stirred at ambient temperature for 18 h. Saturated aqueous NH_4Cl (10 mL) was added and the aqueous phase was extracted with Et_2O (3 x 10 mL). The combined organic phases were dried over $MgSO_4$ and evaporated to dryness. The crude product was purified by flash chromatography (*n*-hexane/ethyl acetate 6:1) to give alcohol **S2** as a colorless oil (70 mg, 135 µmol, 51%). R_f = 0.35 (*n*-hexane/ethyl acetate 6:1); $[\alpha]^{23}_D$ = -16.7 (*c* 1.0 in $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.02-0.09 (6 H, m, $Si(CH_3)_2$), 0.84-0.92 (9 H, m, $SiC(CH_3)_3$), 1.44 (9 H, s, $C(CH_3)_3$),1.51-1.82 (4 H, m, 4-H, 5-H), 3.47-3.55 (3 H, m, CH_2OBn, C*H*NH), 3.77 (1 H, dd, *J* = 10.3, 2.9 Hz, 1-H^a), 3.87 (1 H, dd, *J* = 10.3, 4.0 Hz, 1-H^b), 3.93-3.99 (1 H, m, 3-H), 4.46 (2 H, m, CH_2Ph), 5.16 (1 H, d, *J* = 8.9 Hz, NH), 7.26-7.30 (1 H, m, Ph), 7.31-7.36 (4 H, m, Ph); ^{13}C NMR (126 MHz, $CDCl_3$) δ -5.5 ($Si(CH_3)_2$), 18.1 ($SiC(CH_3)_3$); 25.8 ($SiC(CH_3)_3$), 26.0 (C-5), 28.4 ($C(CH_3)_3$), 31.0 (C-4), 53.8 (C-2), 66.2 (C-6), 70.2 (C-1), 72.8 (C-3), 72.9 (CH_2Ph), 79.2 ($C(CH_3)_3$), 127.5, 127.6, 128.3, 138.4, 156.0 (NCO); IR (ν_{max}/cm^{-1}) 3449, 2928, 2857, 1715, 1692, 1496, 1365, 1252, 1168, 1096, 835, 776, 734, 696; HRMS (ESI) *m/z* $[M + H]^+$ calcd for $C_{24}H_{44}O_5NSi^+$ 454.2983, found 454.2970, $[M+Na]^+$ calcd for $C_{24}H_{43}O_5NNaSi^+$ 476.2803, found 476.2790.

(2*R*,3*R*)-6-(benzyloxy)-2-((tert-butoxycarbonyl)amino)-1-((tert-butyldimethylsilyl)oxy)hexan-3-yl-3,3,3-trifluoro-2-methoxy-2-phenylpropanoate (S3*R*, S3*S*)

A solution of alcohol **S2** (25 mg, 55 µmol, 1.0 equiv.) in dry CH_2Cl_2 (1 mL) was treated with DMAP (14 mg, 110 µmol, 2.0 equiv.), and (*S*)- or (*R*)-MTPA-Cl (12 µL, 66 µmol, 1.2 equiv.). The mixture was stirred at ambient temperature for 18 h and volatiles were removed *in vacuo*. The crude products were purified by flash chromatography (*n*-hexane/ethyl acetate 8:1) to give the esters **S3*R*** and **S3*S*** as colorless oils. R_f = 0.43 (*n*-hexane/ethyl acetate 8:1); IR (ν_{max}/cm^{-1}) 2953, 2930, 2857, 1746, 1717, 1497,

1253, 1166, 1103, 1016, 835, 778, 697; HRMS (ESI) *m/z* [M + H]$^+$ calcd for $C_{34}H_{51}O_7NF_3Si^+$ 670.3381, found 670.3360, [M + Na]$^+$ calcd for $C_{34}H_{50}O_7NF_3NaSi^+$ 692.3201, found 692.3178; **S3*R*** (28 mg, 42 µmol, 77%): $[\alpha]^{23}_D$ = +7.8 (*c* 1.0 in $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.05 (6 H, s, $Si(CH_3)_2$), 0.90 (9 H, s, $SiC(CH_3)_3$), 1.39-1.46 (9 H, m, $C(CH_3)_3$), 1.49-1.61 (2 H, m, 4-H), 1.76 (2 H, q, *J* = 7.4 Hz, 5-H), 3.40 (2 H, t, *J* = 6.1 Hz, CH_2OBn), 3.47 (1 H, dd, *J* = 10.2, 6.6 Hz, 1-H^a), 3.53 (3 H, s, OCH_3), 3.62 (1 H, dd, *J* = 10.2, 4.4 Hz, 1-H^b), 3.84-3.93 (1 H, m, C*H*NH), 4.45 (2 H, s, CH_2Ph), 4.66 (1 H, d, *J* = 9.8 Hz, NH), 5.39 (1 H, q, *J* = 6.1 Hz, 3-H), 7.26-7.36 (5 H, m, Ph), 7.36-7.43 (3 H, m, Ph), 7.49-7.58 (2 H, m, Ph); ^{13}C NMR (126 MHz, $CDCl_3$) δ -5.5 ($Si(CH_3)_2$), 18.1 ($SiC(CH_3)_3$), 25.1 (C-5), 25.7 ($SiC(CH_3)_3$), 27.5 (C-4), 28.3 ($C(CH_3)_3$), 53.7 (C-2), 55.3 (OCH_3), 62.0 (C-1), 69.4 (CH_2OBn), 72.7 (CH_2Ph), 74.9 (C-3), 79.6 ($C(CH_3)_3$), 84.6 ($^2J_{FC}$ = 27.7 Hz, CCF_3), 123.3 ($^1J_{FC}$ = 288.8 Hz; CF_3), 127.4, 128.4, 129.6, 132.0, 138.4, 155.4 (NCO), 166.1 (COO); **S3*S*** (31 mg, 46 µmol, 85%): $[\alpha]^{23}_D$ = -16.6 (*c* 1.0 in $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.02 (6 H, s, $Si(CH_3)_2$), 0.88 (9 H, s, $SiC(CH_3)_3$), 1.38-1.47 (9 H, m, $C(CH_3)_3$), 1.57-1.76 (2 H, m, 4-H), 1.78-1.68 (2 H, m, 5-H), 3.31 (1 H, dd, *J* = 9.9, 7.5 Hz, 1-H^a), 3.45-3.51 (3 H, m, 1-H^b, CH_2OBn), 3.53 (3 H, s, OCH_3), 3.85-3.93 (1 H, m, C*H*NH), 4.48 (2 H, s, CH_2Ph), 4.52 (1 H, d, *J* = 9.8 Hz, NH), 5.42 (1 H, td, *J* = 6.4, 3.7 Hz, 3-H), 7.27-7.36 (5 H, m, Ph), 7.37-7.44 (3 H, m, Ph), 7.53-7.55 (2 H, m, Ph); ^{13}C NMR (126 MHz, $CDCl_3$) δ -5.5 ($Si(CH_3)_2$), 18.1 ($SiC(CH_3)_3$), 25.3 (C-5), 25.7 ($SiC(CH_3)_3$), 27.7 (C-4), 28.3 ($C(CH_3)_3$), 55.3 (C-2), 55.4 (OCH_3), 61.7 (C-1), 69.4 (CH_2OBn), 72.7 (CH_2Ph), 74.8 (C-3), 79.6 ($C(CH_3)_3$), 123.4 ($^1J_{FC}$ = 288.7 Hz; CF_3), 127.5, 127.6, 128.3, 128.4, 129.7, 132.1, 138.4, 155.4 (NCO), 166.1 (COO); The absolute configuration of alcohol **S2** was confirmed to be *R.*



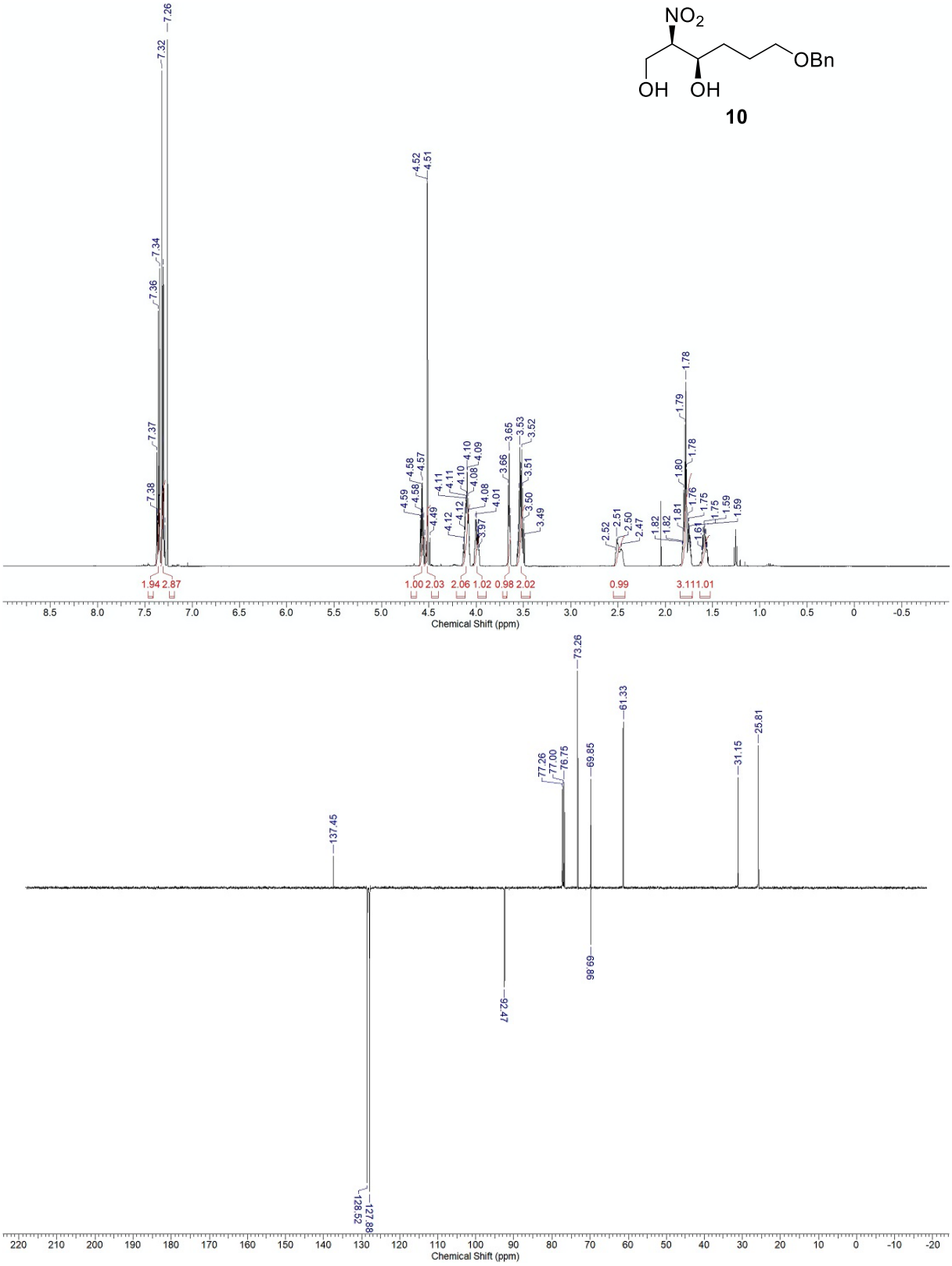
Scheme S3. Synthesis and $\Delta\delta_{SR} = \delta_S - \delta_R$ values in Hz of the ^{1}H NMR spectra of Mosher-esters **S4*R*** and **S4*S***

(*R*)-1-((4''*R*,5''*R*)-5''-((tert-butoxycarbonyl)amino)-2'',2''-dimethyl-1'',3''-dioxan-4''-yl)pentadec-4'-yn-3'-yl-3,3,3-trifluoro-2-methoxy-2-phenylpropanoate (S4*R*, S4*S*)

A solution of alcohol **14** (25 mg, 55 µmol, 1.0 equiv.) in dry CH_2Cl_2 (1 mL) was treated with DMAP (14 mg, 110 µmol, 2.0 equiv.), and (*S*)- or (*R*)-MTPA-CL (12 µL, 66 µmol, 1.2 equiv.). The mixture was stirred at ambient temperature for 18 h and volatiles were removed *in vacuo*. The crude products were purified by flash chromatography (*n*-hexane/ethyl acetate 9:1) to give **S4*R*** and **S4*S*** as colorless oils. R_f = 0.35 (*n*-hexane/ethyl acetate 9:1); IR (ν_{max}/cm^{-1}) 2962, 2856, 1750, 1713, 1496, 1244, 1164, 1119, 1081, 986, 718; HRMS (ESI) *m/z* [M + H]$^+$ calcd for $C_{36}H_{55}O_7NF_3^+$ 670.3925, found 670.3904, [M + Na]$^+$ calcd for $C_{36}H_{54}O_7NF_3Na^+$ 692.3745, found 692.3721; **S4*R*** (32 mg, 50 µmol, 90%): $[\alpha]^{23}_D$ = +17.1 (*c* 1.0 in $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.78-0.83 (3 H, m, CH_3), 1.15-1.24 (12 H, m, $(CH_2)_6$), 1.28-1.31 (3 H, m, CH_3), 1.32-1.35 (3 H, m, CH_3), 1.35-1.40 (9 H, m, $C(CH_3)_3$), 1.40-1.55 (4 H, m, 4-H, CH_2, 1'-H), 1.67-

1.84 (2 H, m, 2'-H), 2.13 (2 H, td, *J* = 7.2, 2.1 Hz, 6'-H), 3.37 (1 H, dd, *J* = 9.8, 1.8 Hz, C*H*NH), 3.51 (3 H, s, OCH_3), 3.62-3.69 (1 H, m, 6''-H[a]), 3.79 (1 H, ddd, *J* = 8.5, 5.1, 1.8 Hz, 4''-H), 3.94 (1 H, dd, *J* = 12.1, 2.0 Hz, 6''-H[b]), 5.17 (1 H, d, *J* = 9.8 Hz, NH), 5.45 (1 H, tt, *J* = 6.7, 1.9 Hz, 3'-H), 7.30-7.38 (3 H, m, Ph), 7.46-7.52 (2 H, m, Ph); ^{13}C NMR (126 MHz, $CDCl_3$) δ 14.1 (CH_3), 18.5 (CH_3), 18.6 (CH_2), 22.7 (CH_2), 27.1 (CH_2), 28.3 (CH_2), 28.4 (C(*C*H_3)$_3$), 28.8 (CH_2), 29.1 (CH_2), 29.3 (CH_2), 29.5 (CH_2), 29.6 (CH_3), 30.4 (CH_2), 31.9 (CH_2), 46.9 (C-5''), 55.4 (OCH_3), 65.2 (C-6''), 66.4 (C-3'), 70.6 (C-4''), 76.2 (C-5'), 79.4 (*C*(CH$_3$)$_3$), 87.9 (C-4'), 99.1 (C-2''), 123.3 ($^1J_{FC}$ = 288.8 Hz, CF_3), 126.6, 128.3, 129.6, 129.9, 132.4, 155.7 (NCO), 165.7 (COO); ^{19}F NMR (282 MHz, $CDCl_3$) δ -71.6 (major), -71.9 (minor); **S4*S*** (30 mg, 47 µmol, 86%): $[\alpha]^{23}_D$ = -14.1 (*c* 1.0 in $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): 0.85-0.90 (3 H, m, CH_3), 1.20-1.35 (12 H, m, $(CH_2)_6$), 1.37-1.40 (3 H, m, CH_3), 1.41-1.49 (12 H, m, C*H*$_3$, C(C*H*$_3$)$_3$), 1.52-1.66 (2 H, m, 1'-H), 1.76-1.97 (2 H, m, 2'-H), 2.16 (2 H, td, *J* = 7.2, 1.8 Hz, 6'-H), 3.47 (1 H, dd, *J* = 9.9, 1.9 Hz, C*H*NH), 3.55 (3 H, s, OCH_3), 3.73 (1 H, dd, *J* = 12.0, 1.8 Hz, 6''-H[a]), 3.92 (1 H, ddd, *J* = 8.2, 5.2, 1.8 Hz, 4''-H), 4.02 (1 H, dd, *J* = 12.0, 2.0 Hz, 6''-H[b]), 5.25 (1 H, d, *J* = 10.1 Hz, NH), 5.45 (1 H, tt, *J* = 6.7, 1.8 Hz, 3'-H), 7.38-7.42 (3 H, m, Ph), 7.52-7.55 (2 H, m, Ph); ^{13}C NMR (126 MHz, $CDCl_3$) δ 14.1 (CH_3), 18.5 (CH_3), 18.6 (CH_2), 22.7 (CH_2), 27.4 (CH_2), 28.4 (C(*C*H_3)$_3$), 28.8 (CH_2), 29.1 (CH_2), 29.3 (CH_2), 29.5 (CH_2), 29.6 (CH_3), 30.3 (CH_2), 31.9 (CH_2), 46.9 (C-5''), 55.5 (OCH_3), 65.2 (C-6''), 66.8 (C-3'), 70.8 (C-4''), 76.0 (C-5'), 79.5 (*C*(CH$_3$)$_3$), 87.7 (C-4'), 99.1 (C-2''), 123.2 ($^1J_{FC}$ = 287.9 Hz, CF_3), 127.5, 128.3, 129.6, 132.0, 155.7 (NCO), 165.6 (COO); ^{19}F NMR (282 MHz, $CDCl_3$) δ -71.6 (minor), -71.9 (major); The absolute configuration of alcohol **14** was confirmed to be *R.*

NMR spectra and chromatograms



Custom Report

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Injection Volume: 20 µL
Concentration: 0.9 mg/mL in 10% *i*-PrOH in *n*-Hexane
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Analyzed: 4/12/2018 12:58:38 PM
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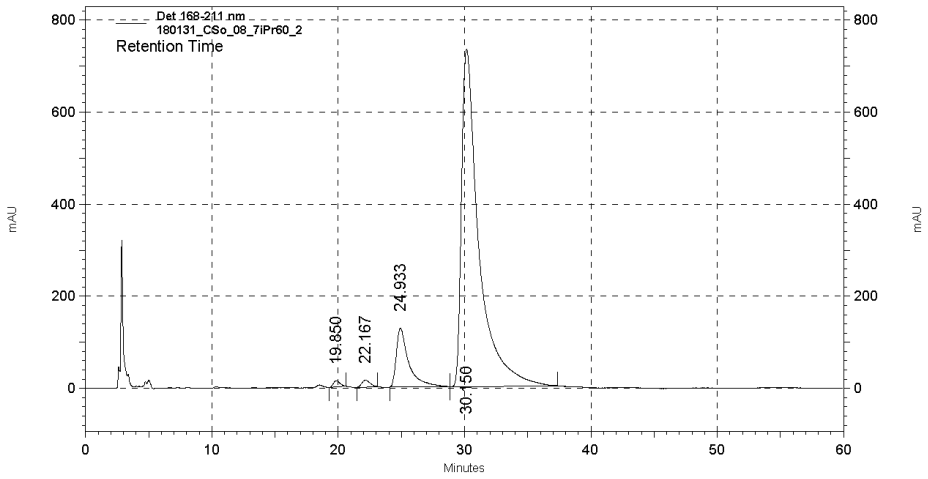
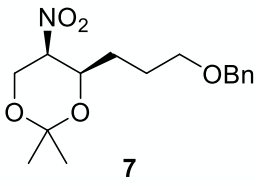


Det 168-211 nm
Results

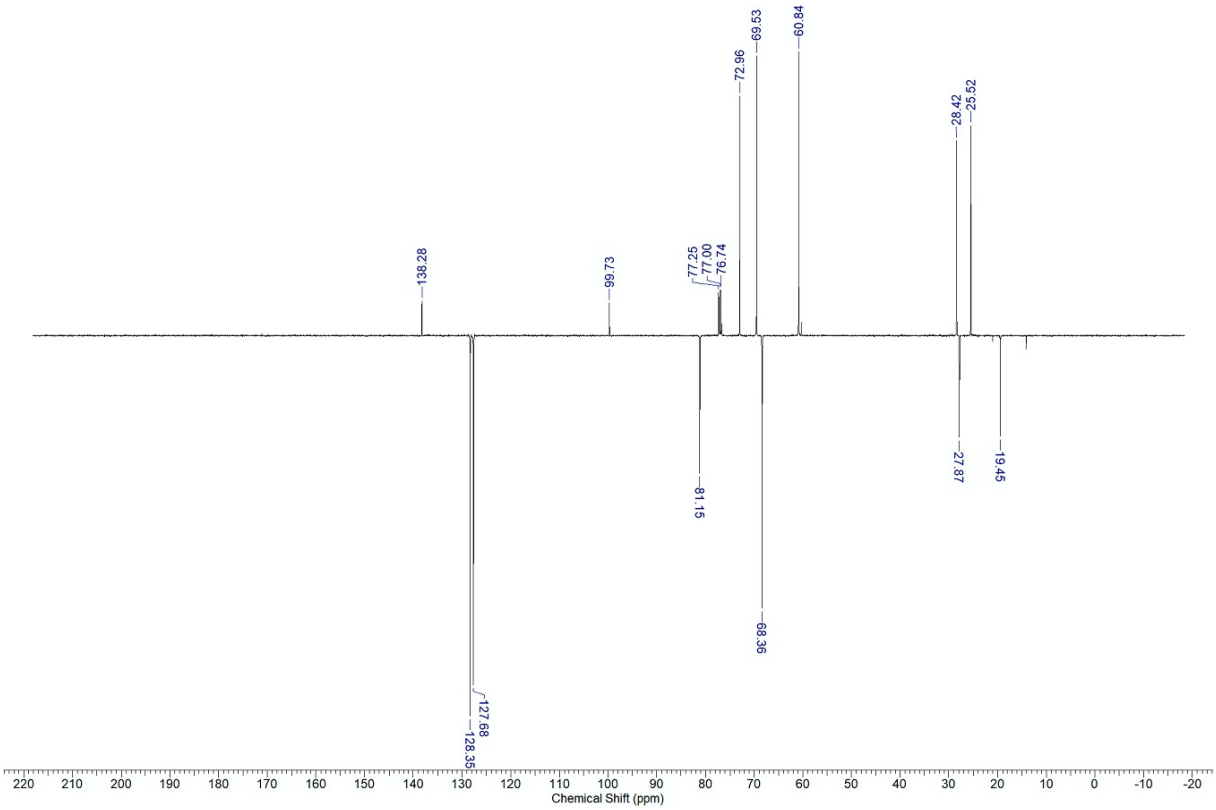
Pk #	Time	Area	Height	Area Percent
1	19.850	489773	13413	0.614
2	22.167	661137	15138	0.829
3	24.933	8758190	127522	10.981
4	30.150	69848641	732468	87.576
Totals		79757741	888541	100.000

HPLC trace of **10**; Phenomenex Lux® Amylose-1; 95:5 Hex/IPA; 1.0 mL/min; *anti* isomer: *tr* (major) = 24.93 min, *tr* (minor) = 19.85 min, 89% *ee*; *syn* isomer: *tr* (major) = 30.15 min, *tr* (minor) = 22.17 min, 98% *ee*; *dr* (*syn/anti*) = 88:12.


NO2
OBn
O
O

7

1.46
1.39
7.33
7.32
7.35
7.31
7.29
7.36
4.47
4.49
4.52
4.34
4.32
4.31
4.14
4.13
4.11
4.11
4.10
4.09
4.09
4.08
3.49
3.48
3.50
3.47
3.48
3.47
1.82
1.80
1.76
1.68
1.67
1.66
1.64
1.65
4.72
2.96
1.00
2.26
1.98
4.11
2.95
2.96
Chemical Shift (ppm)

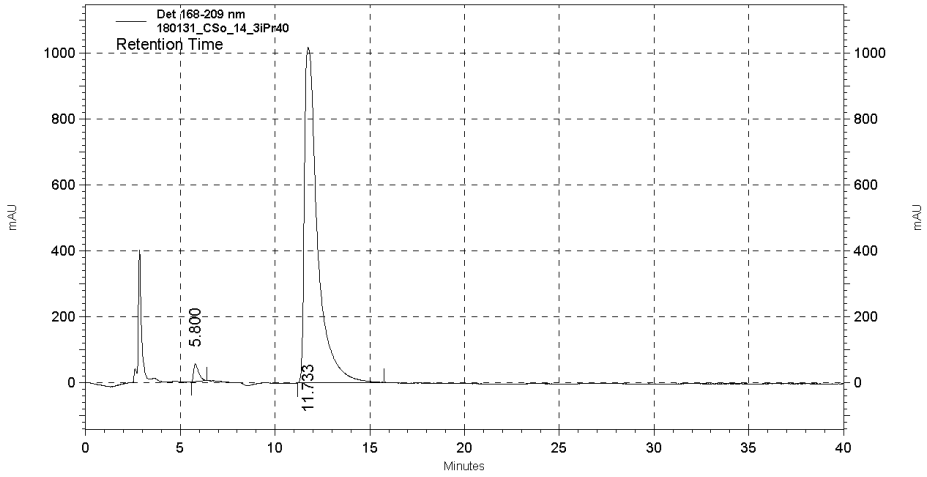

69.53
60.84
72.96
28.42
25.52
138.28
99.73
77.25
77.00
76.74
81.15
68.36
27.87
19.45
127.68
128.35
Chemical Shift (ppm)

Custom Report

Data File: C:\32Karat\Projects\ABa\Data\180131_CSo_14_3iPr40

Method: C:\32Karat\Projects\ABa\Methods\180123_CSo_08_7iPr60m.met

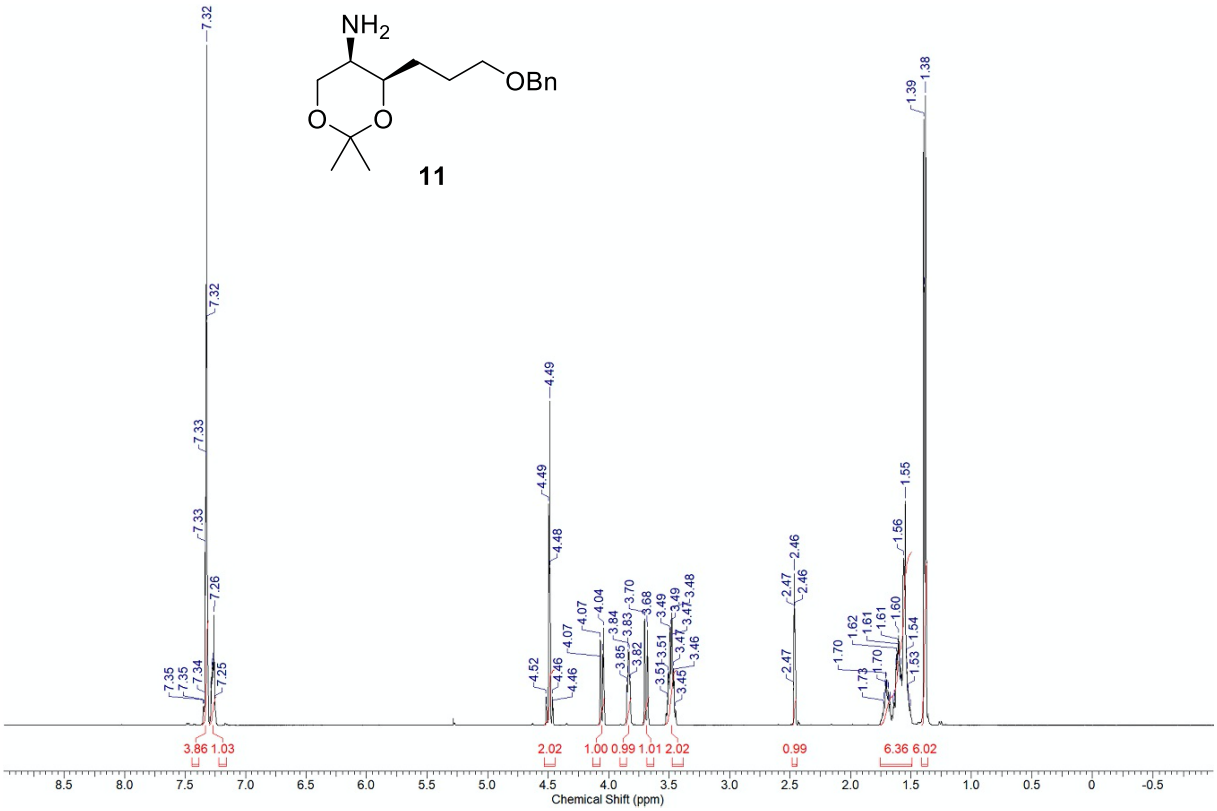
Instrument Name: HPLC2 (Offline)
Injection Volume: 20 µL
Concentration: 0.5 mg/mL
Analyst: Admin
Acquired: 1/31/2018 10:45:00 AM
Analyzed: 1/31/2018 11:59:16 AM
Printed: 1/31/2018 12:00:32 PM

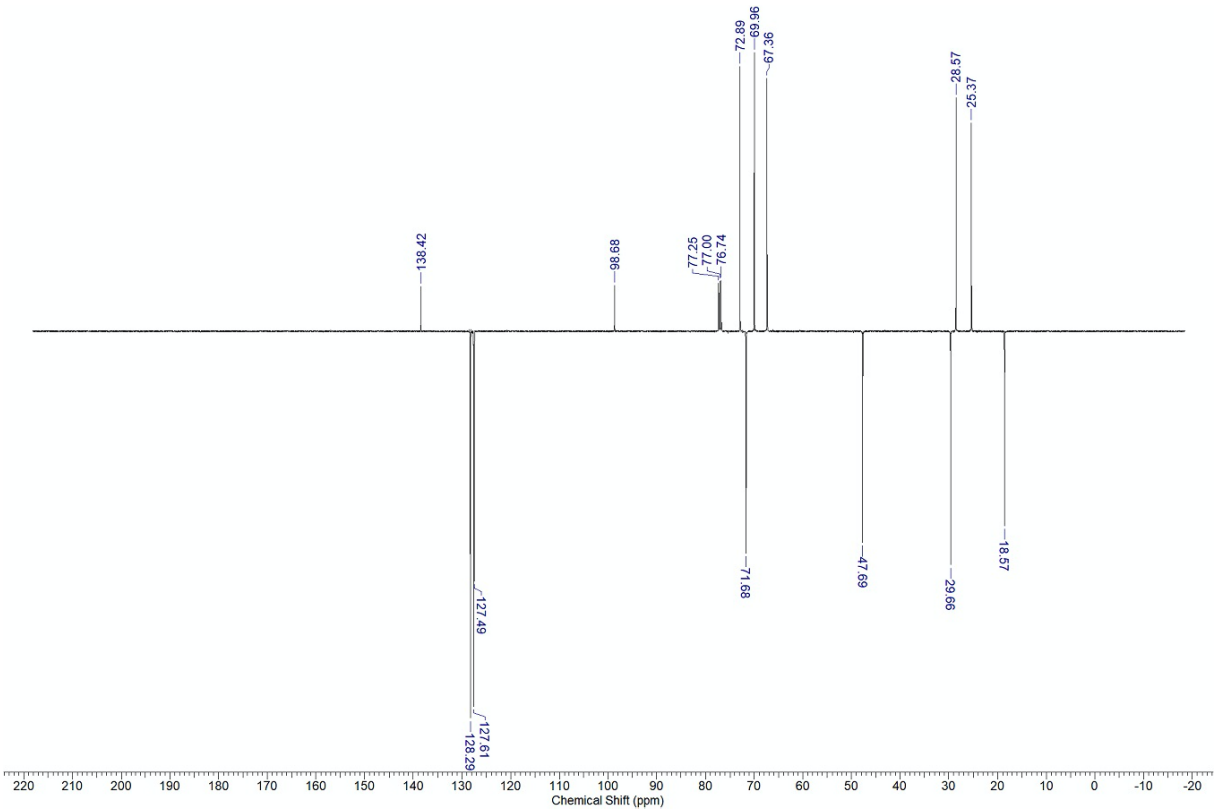


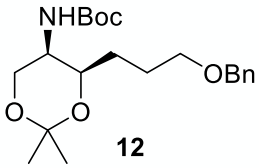
Det 168-209 nm Results

Pk #	Time	Area	Height
1	5.800	956137	54111
2	11.733	48883578	1017759
Totals		49839715	1071870

HPLC trace of **7**; Phenomenex Lux® Amylose-1; 95:5 Hex/IPA; 1.0 mL/min; *syn* isomer: *tr* (major) = 11.73 min, *tr* (minor) = 5.80 min

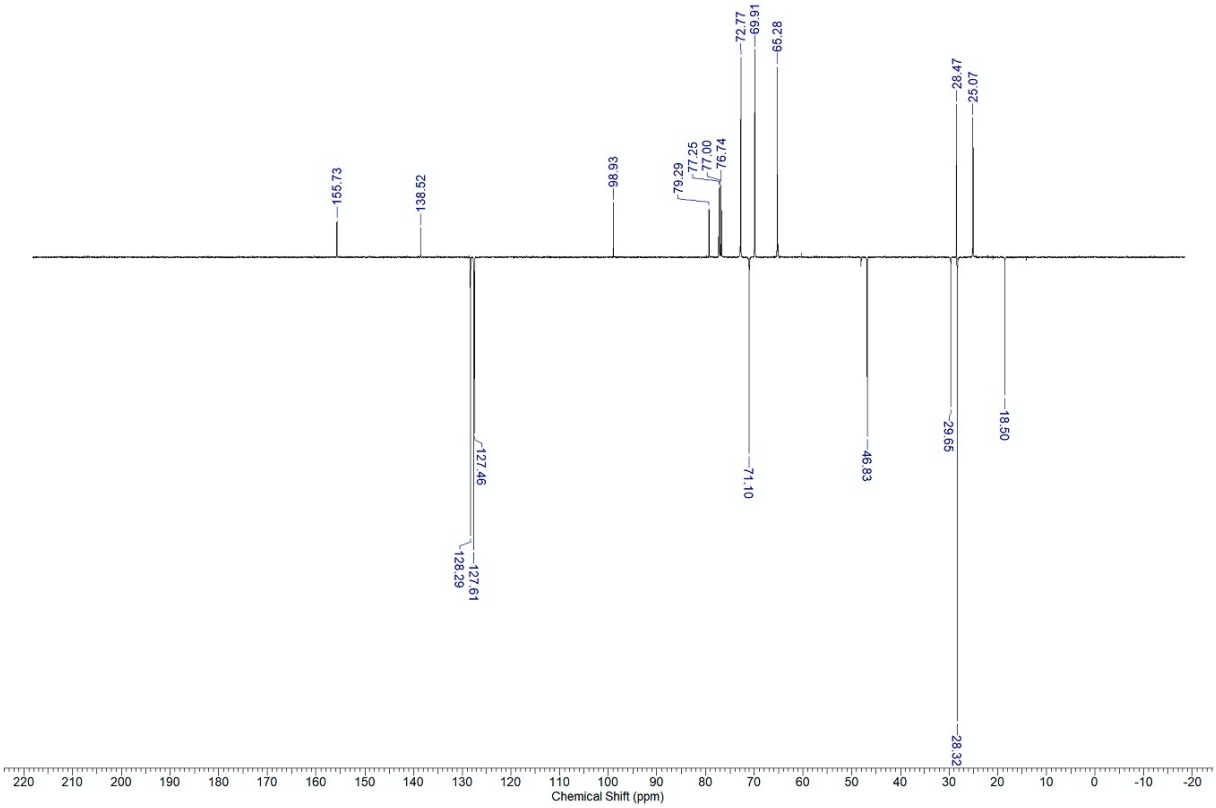

NH2
OBn
O
O
11
Chemical Shift (ppm)

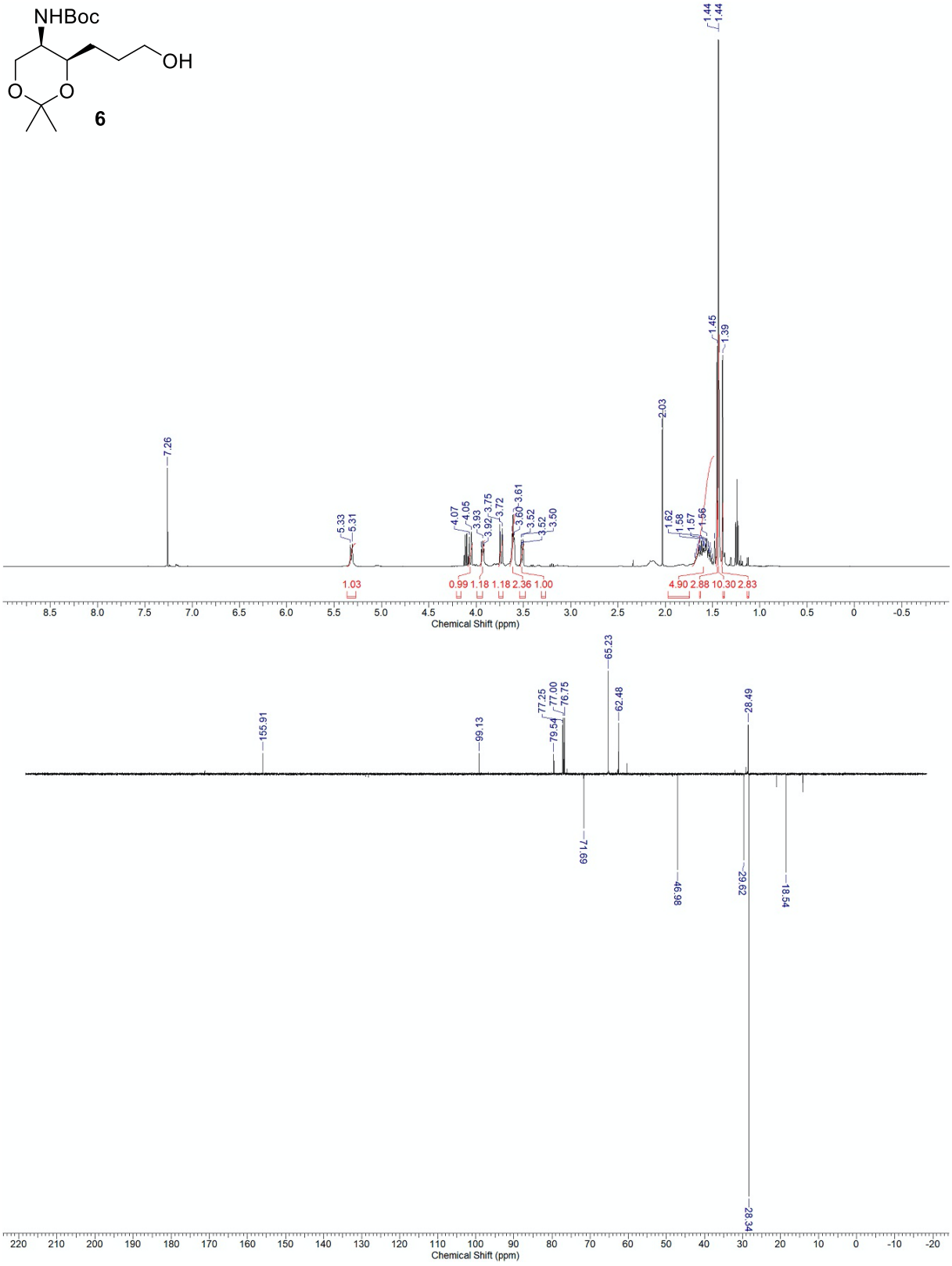

Chemical Shift (ppm)

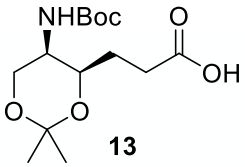

NHBoc
OBn
O
O

12

1.44
7.32
7.33
7.33
7.28
7.27
7.26
1.40
1.38
4.49
4.47
4.51
4.45
4.02
4.00
3.92
3.74
3.72
3.50
3.48
3.47
3.46
3.45
3.44
5.30
5.28
1.68
1.67
1.67
1.65
1.57
1.56
4.96
0.86
2.01
0.92
0.90
1.00
2.93
2.11
2.25
8.98
2.75
3.01
Chemical Shift (ppm)

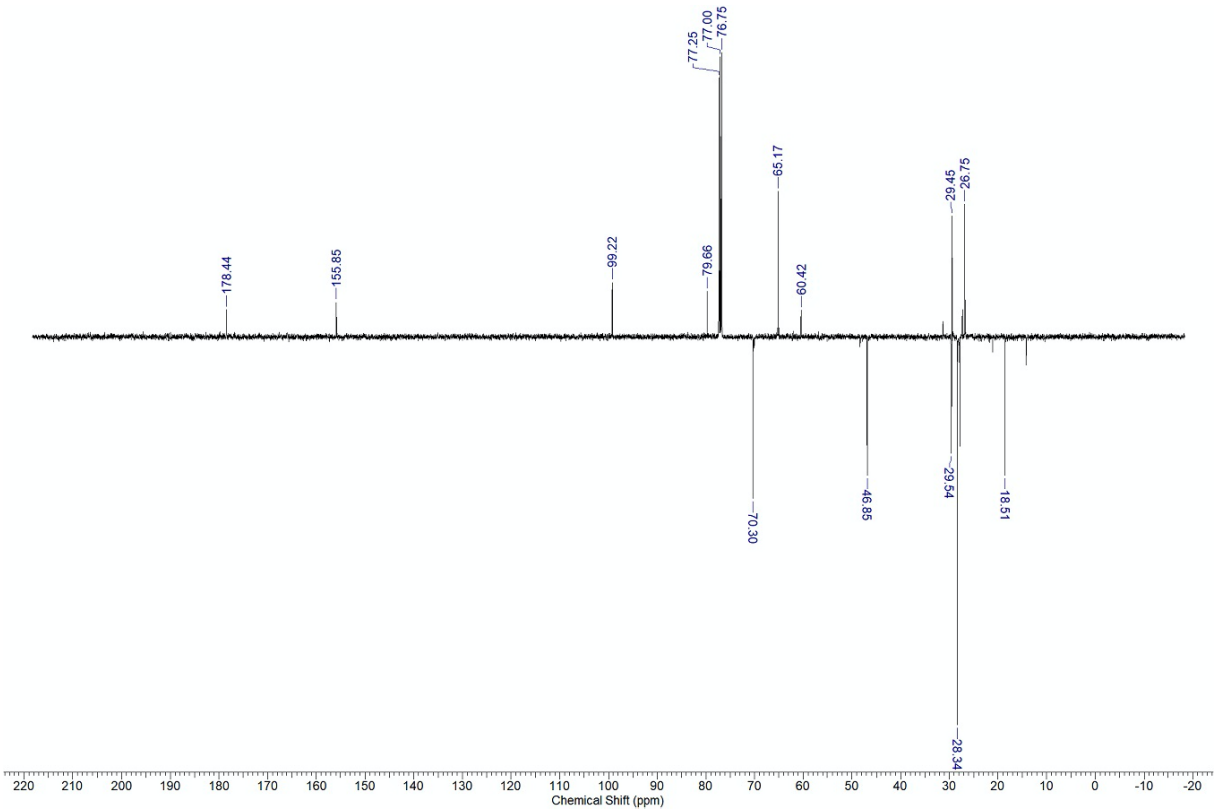

72.77
69.91
65.28
28.47
25.07
155.73
138.52
98.93
79.29
77.25
77.00
76.74
127.46
128.29
127.61
71.10
46.83
29.65
18.50
28.32
Chemical Shift (ppm)

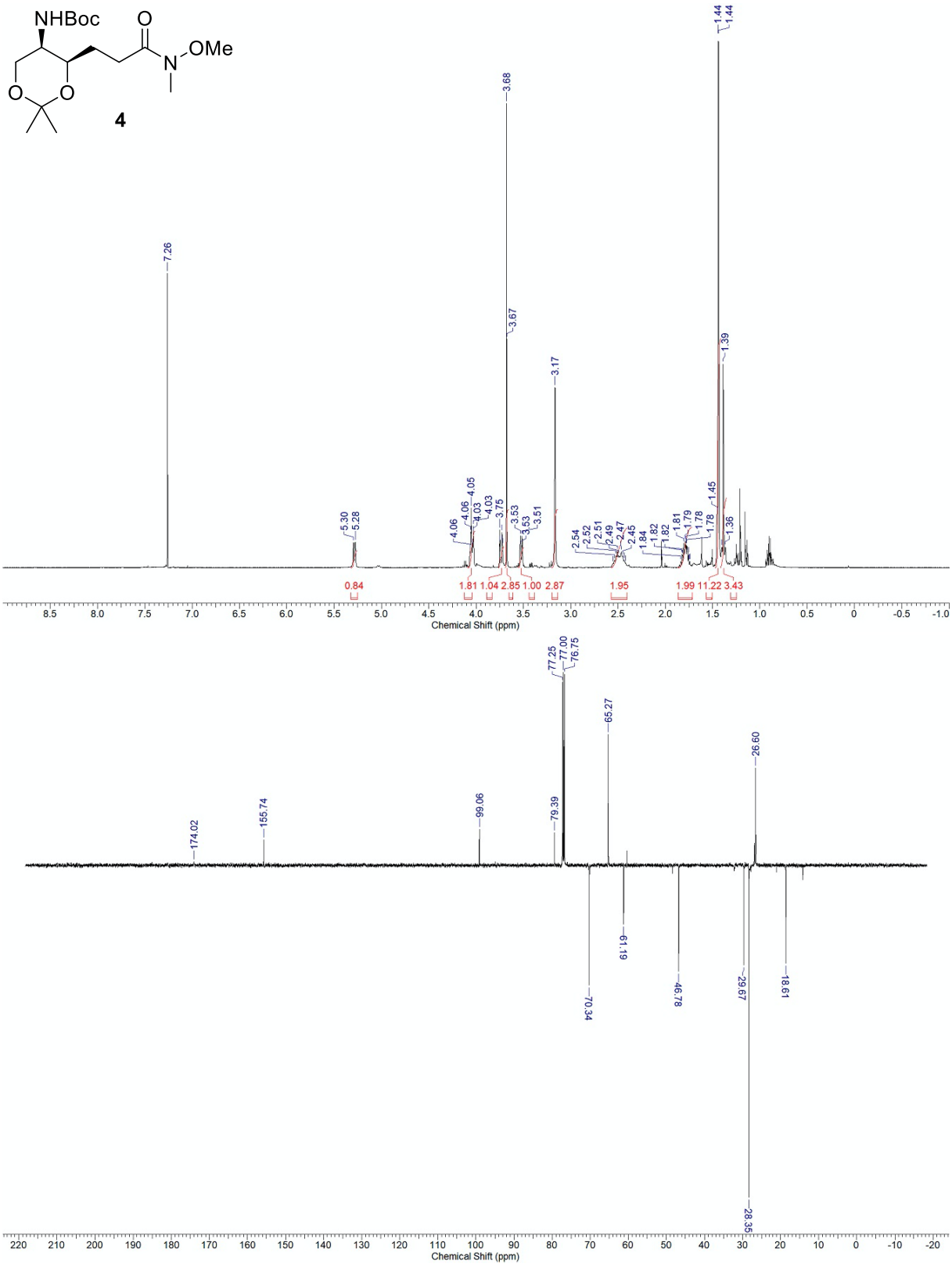

NHBoc
OH
O
O
6
7.26
5.33
5.31
4.07
4.05
3.93
3.92
3.75
3.72
3.60-3.61
3.52
3.50
2.03
1.62
1.58
1.57
1.56
1.45
1.44
1.44
1.39
1.03
0.99 1.18 1.18 2.36 1.00
4.90 2.88 10.30 2.83
Chemical Shift (ppm)
155.91
99.13
79.54
77.25
77.00
76.75
65.23
62.48
28.49
71.69
46.98
29.62
18.54
28.34
Chemical Shift (ppm)

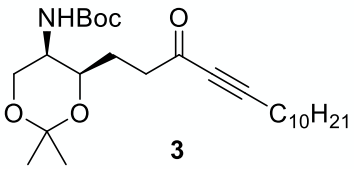

NHBoc
O
OH
O
O

13

7.26
1.45
1.44
1.39
5.32
5.30
4.08
4.07
4.05
4.05
4.01
4.00
3.75
3.73
3.54
3.54
3.52
3.52
2.46
2.45
2.44
2.42
1.83
1.82
1.80
1.79
1.78
1.77
1.77
1.46
0.82
0.91
0.93
0.99
0.90
1.96
2.02
8.80
2.72
2.95
Chemical Shift (ppm)

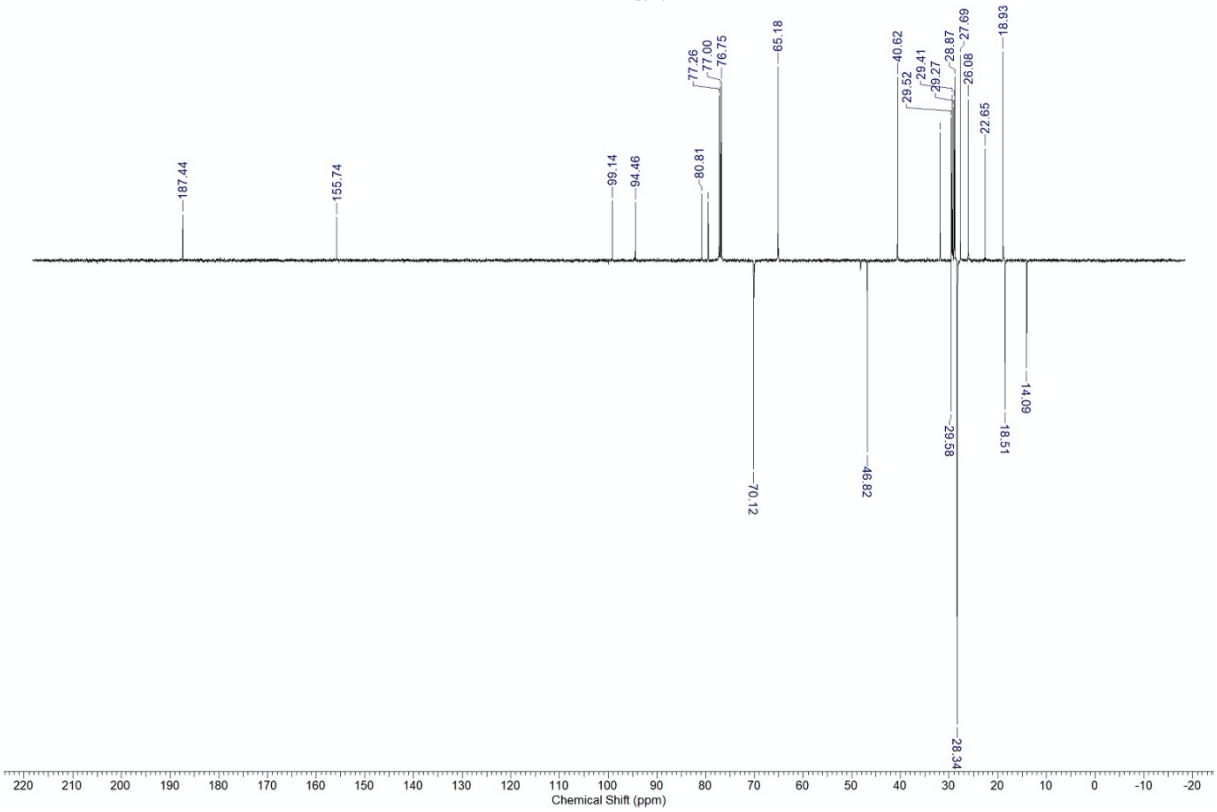

77.25
77.00
76.75
178.44
155.85
99.22
79.66
65.17
60.42
29.45
26.75
70.30
46.85
29.54
18.51
28.34
Chemical Shift (ppm)

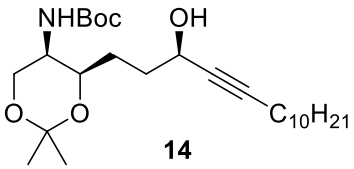

NHBoc
O
N
OMe
O
O
4
7.26
5.30
5.28
4.06
4.06
4.05
4.03
4.03
3.75
3.68
3.67
3.53
3.53
3.51
3.17
2.54
2.52
2.51
2.49
2.47
2.45
1.84
1.82
1.82
1.81
1.79
1.78
1.78
1.45
1.44
1.44
1.39
1.36
0.84
1.81
1.04
2.85
1.00
2.87
1.95
1.99
11.22
3.43
Chemical Shift (ppm)
77.25
77.00
76.75
65.27
26.69
174.02
155.74
99.06
79.39
70.34
61.16
46.78
29.67
18.61
28.35
Chemical Shift (ppm)


NHBoc
O
O
O
$C_{10}H_{21}$

3

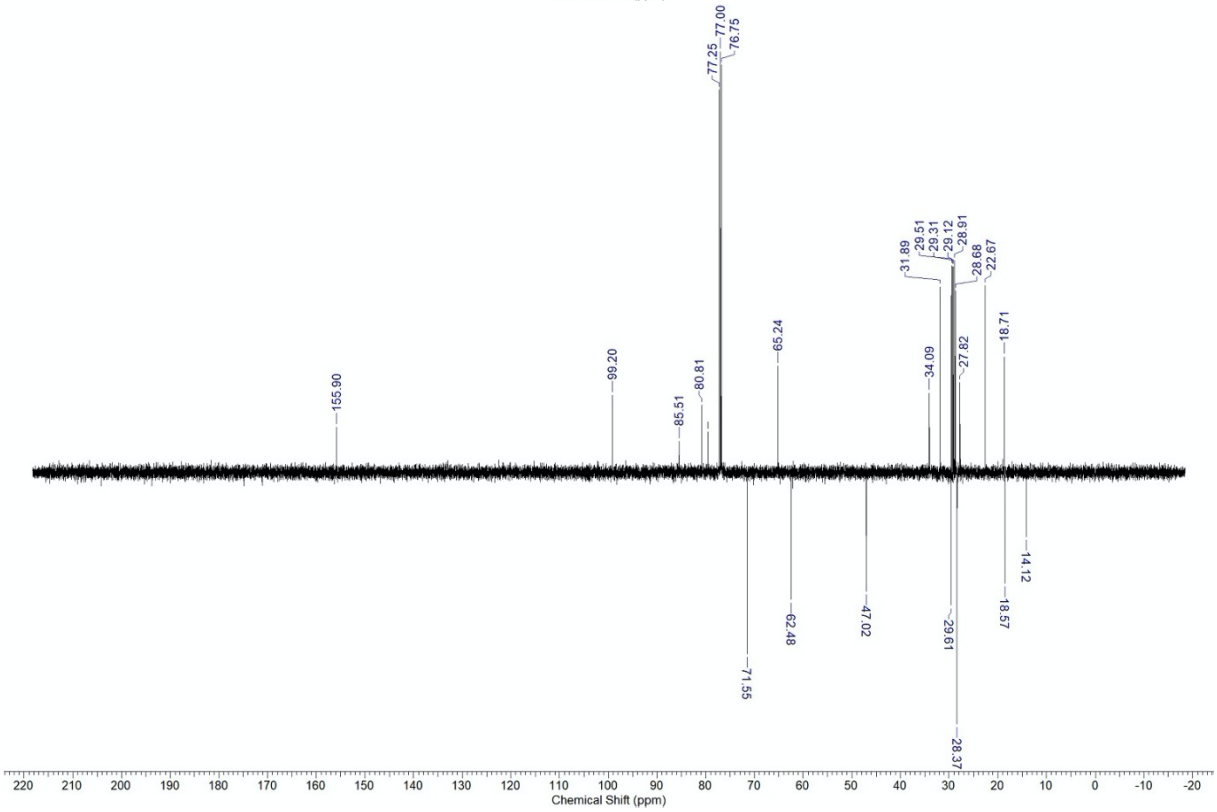
7.26
5.28
5.26
4.05
4.04
4.02
4.02
3.94
3.74
3.74
3.72
3.72
3.51
3.51
3.49
2.62
2.61
2.59
2.35
2.34
2.32
1.81
1.79
1.79
1.58
1.56
1.44
1.38
1.25
0.88
0.87
0.86
0.98
1.02 1.00 1.11 1.00
2.18 2.26 2.15 2.41 9.26 3.29 6.08 14.09 3.44
Chemical Shift (ppm)

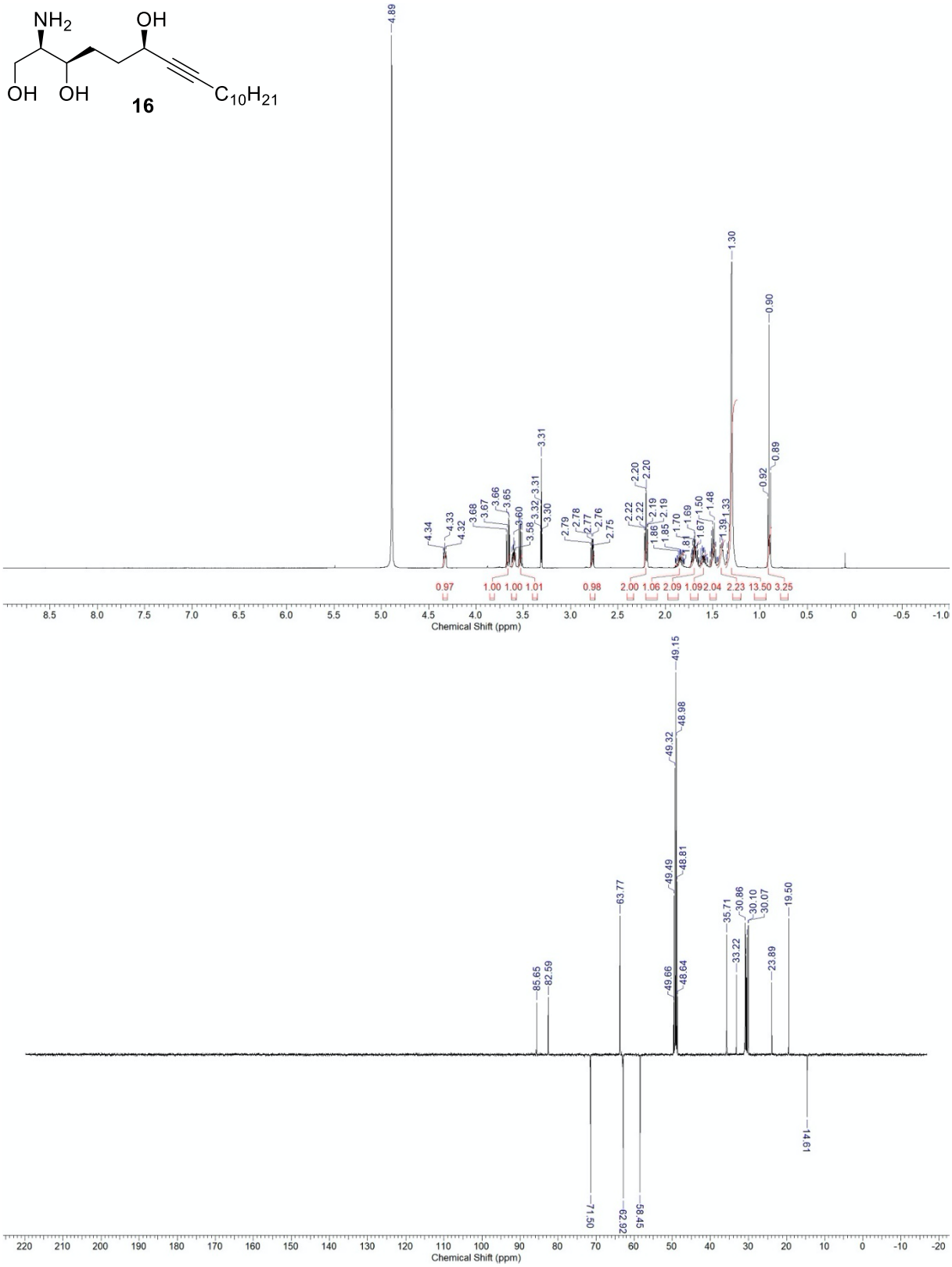

187.44
155.74
99.14
94.46
80.81
77.26
77.00
76.75
65.18
70.12
46.82
40.62
29.52
29.41
29.27
28.87
28.34
27.69
26.08
22.65
18.93
18.51
14.09
Chemical Shift (ppm)

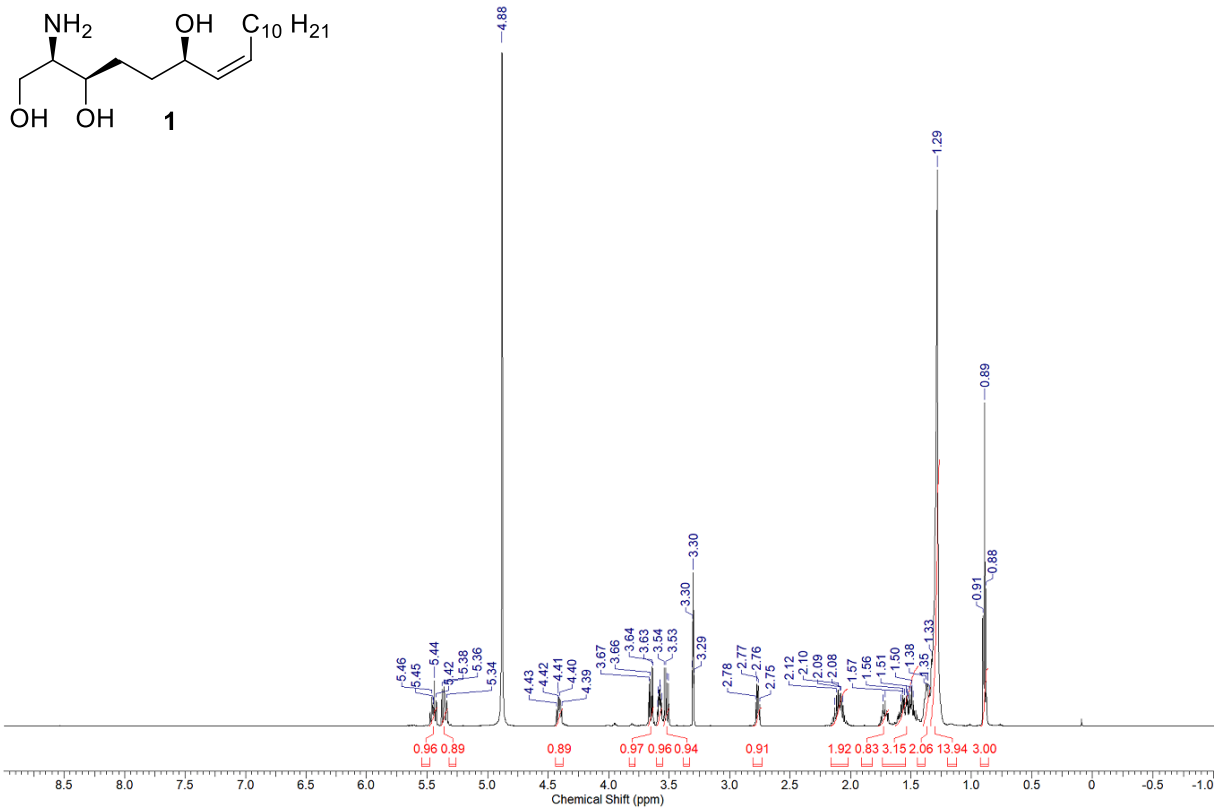

NHBoc
OH
O
O
$C_{10}H_{21}$

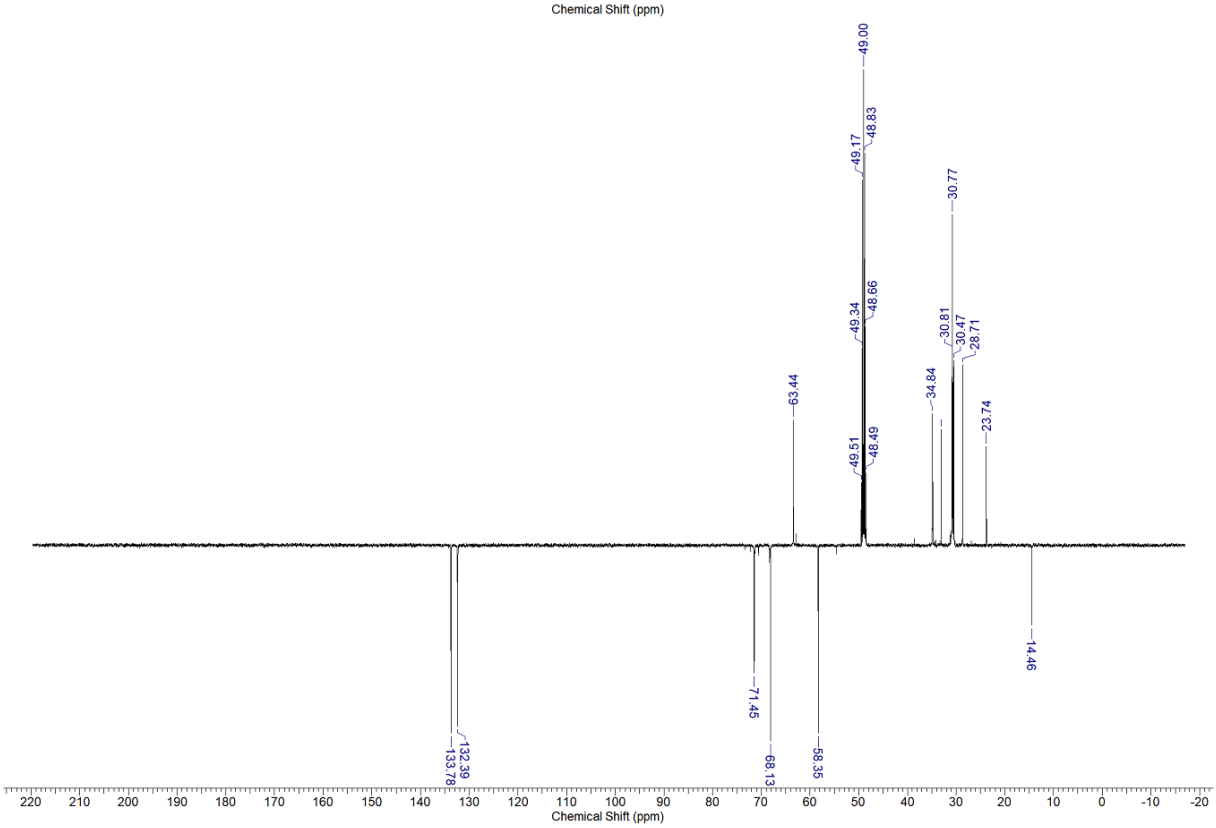
14

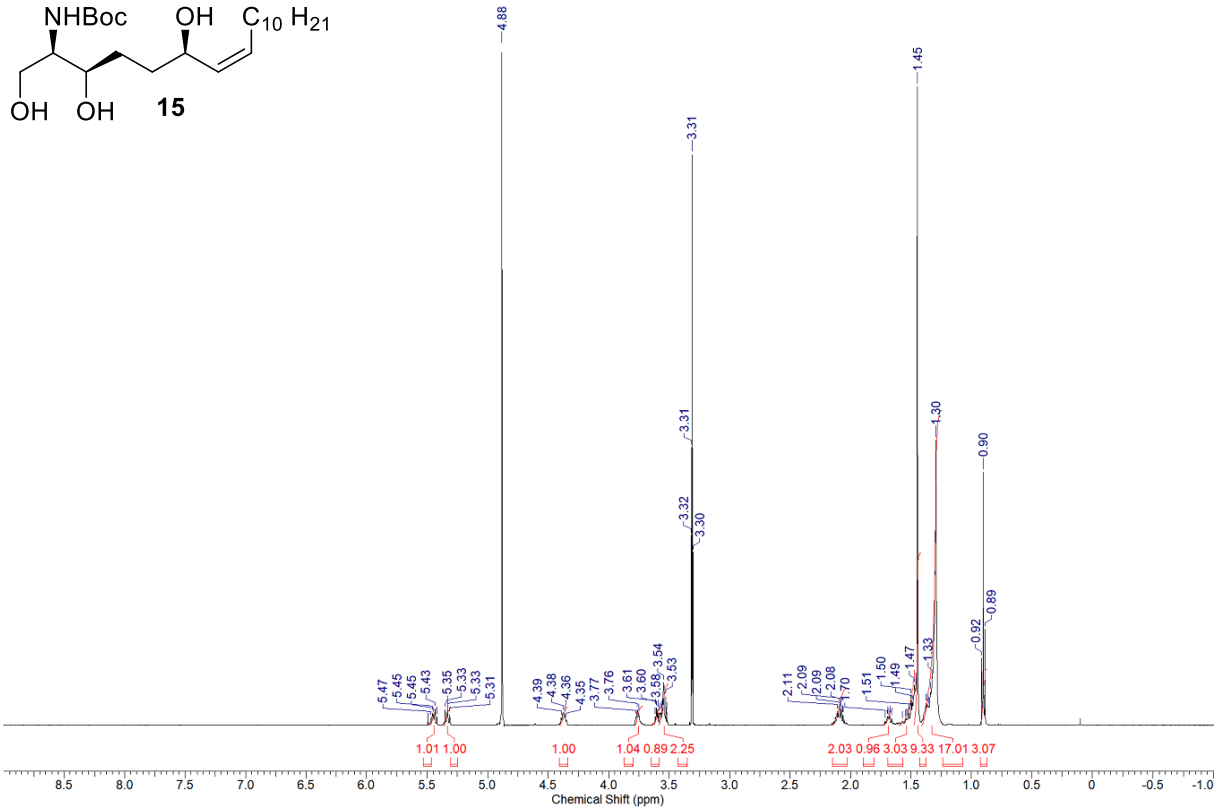
7.26
5.31
5.29
4.36
4.36
4.36
4.07
4.07
4.05
4.04
3.96
3.76
3.76
3.74
3.74
3.53
3.51
3.51
2.34
2.34
2.33
2.20
2.18
2.18
2.17
2.16
1.79
1.77
1.76
1.66
1.58
1.46
1.45
1.40
1.28
1.26
0.90
0.89
0.88
0.86
0.91
1.00
1.01
0.96
1.01
0.98
0.93
1.93
1.83
1.89
14.03
2.63
2.69
12.64
3.17
Chemical Shift (ppm)

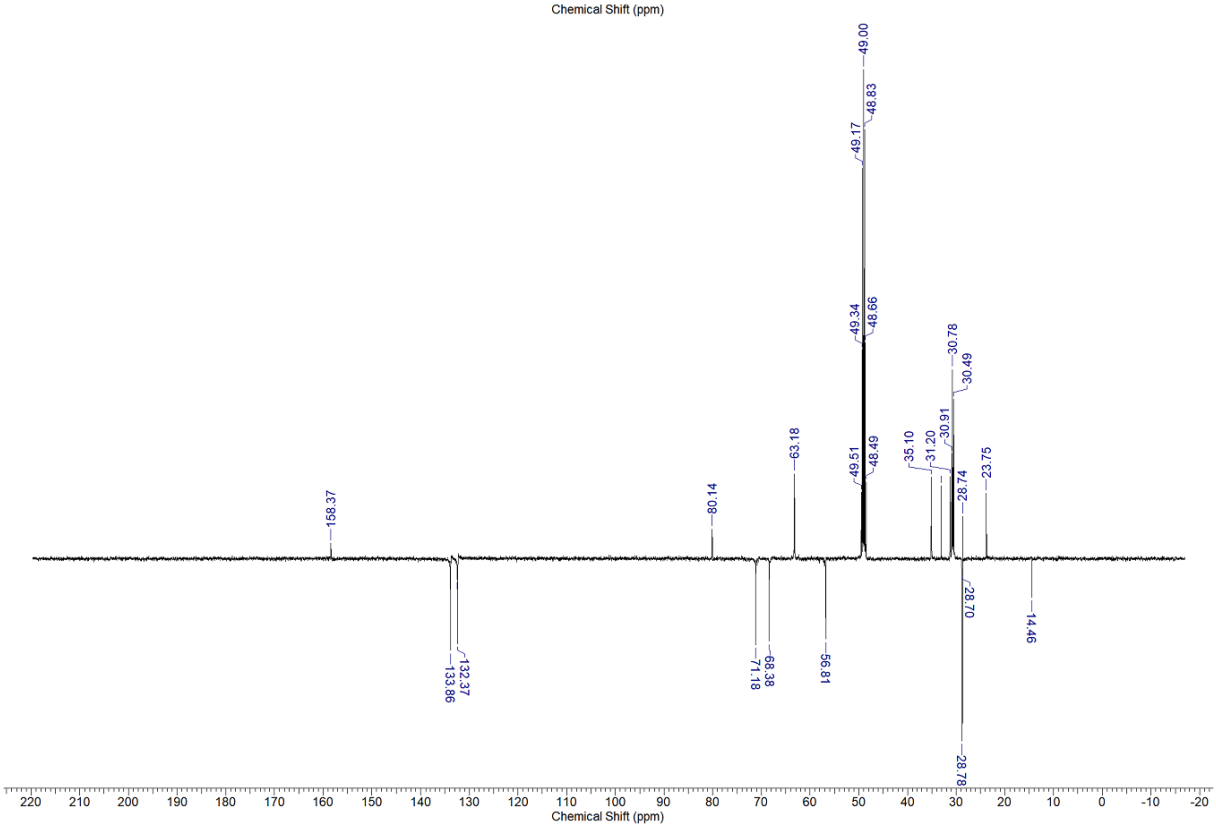

77.25
77.00
76.75
155.90
99.20
85.51
80.81
65.24
34.09
31.89
29.51
29.31
29.12
28.91
28.68
27.82
22.67
18.71
14.12
18.57
29.48
47.02
29.61
71.55
28.37
Chemical Shift (ppm)

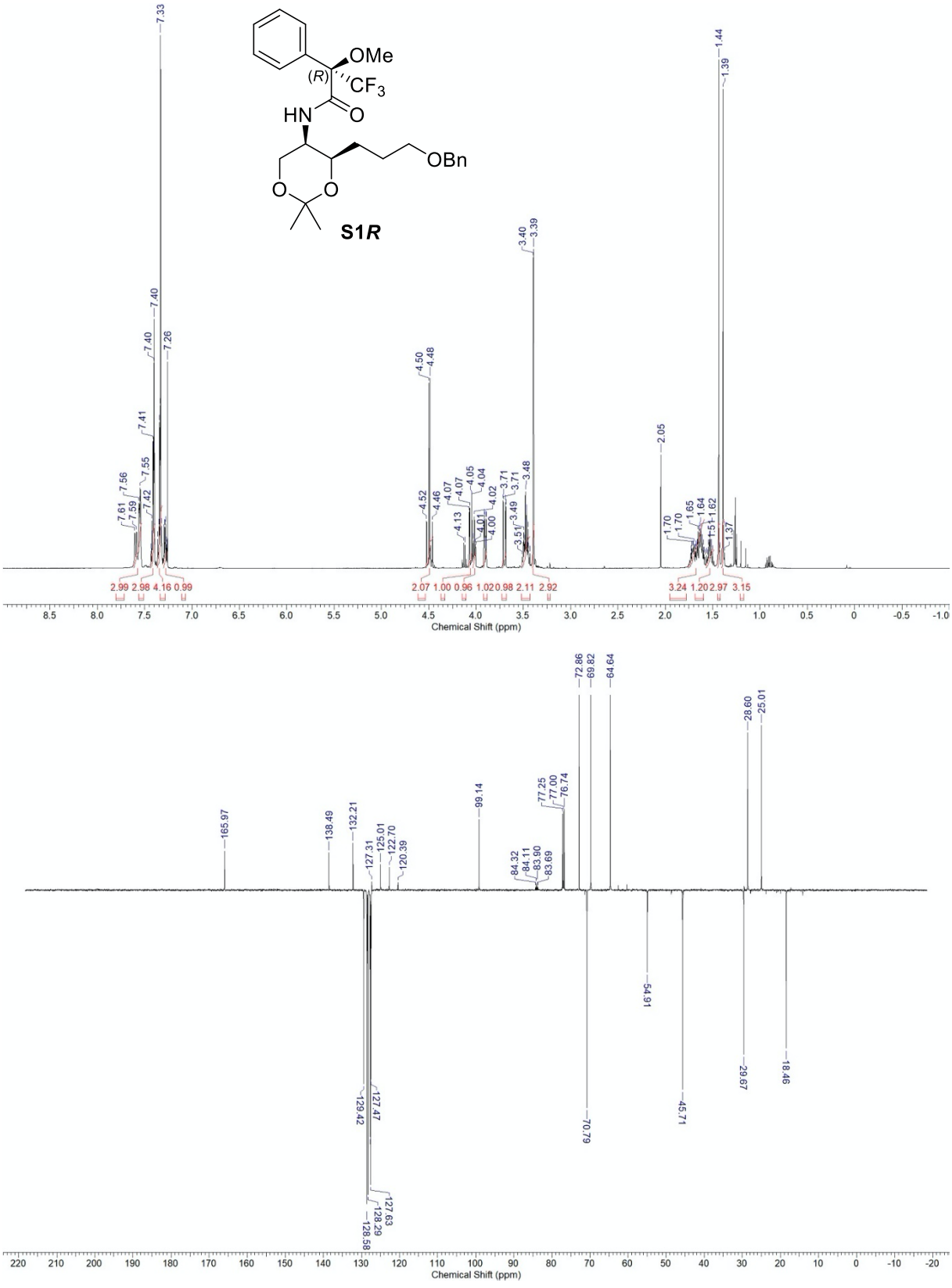

NH2
OH
OH
OH
C10H21
16
Chemical Shift (ppm)
Chemical Shift (ppm)

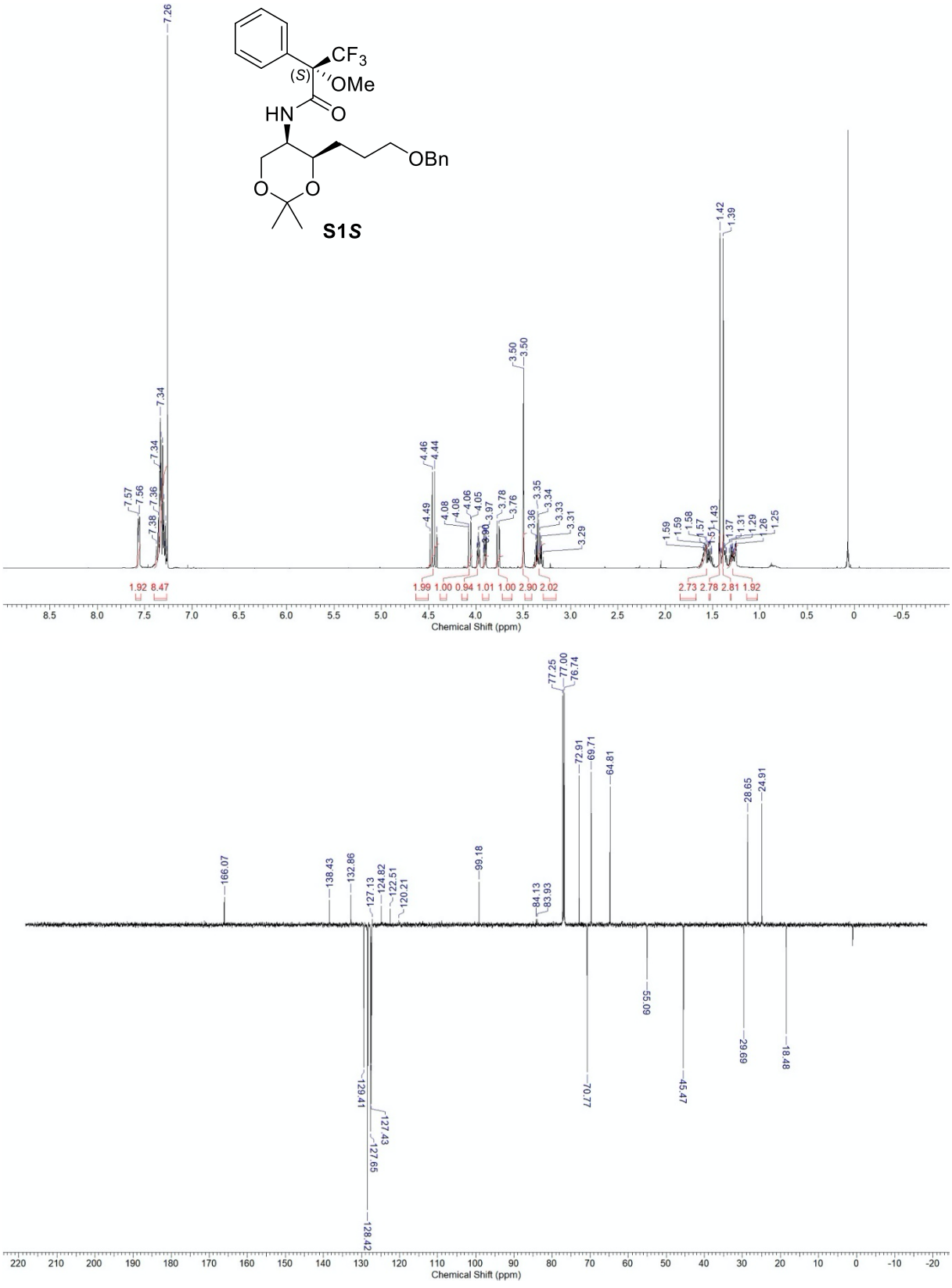

NH2
OH
C10 H21
OH
OH
1
4.88
1.29
0.89
0.88
0.91
3.30
3.30
3.29
1.33
5.46
6.45
5.44
5.42
5.38
5.36
5.34
4.43
4.42
4.41
4.40
4.39
3.67
3.66
3.64
3.63
3.54
3.53
2.78
2.77
2.76
2.75
2.12
2.10
2.09
2.08
1.57
1.56
1.51
1.50
1.38
1.35
0.96 0.89
0.89
0.97 0.96 0.94
0.91
1.92 0.83 3.15 2.06 13.94 3.00
Chemical Shift (ppm)

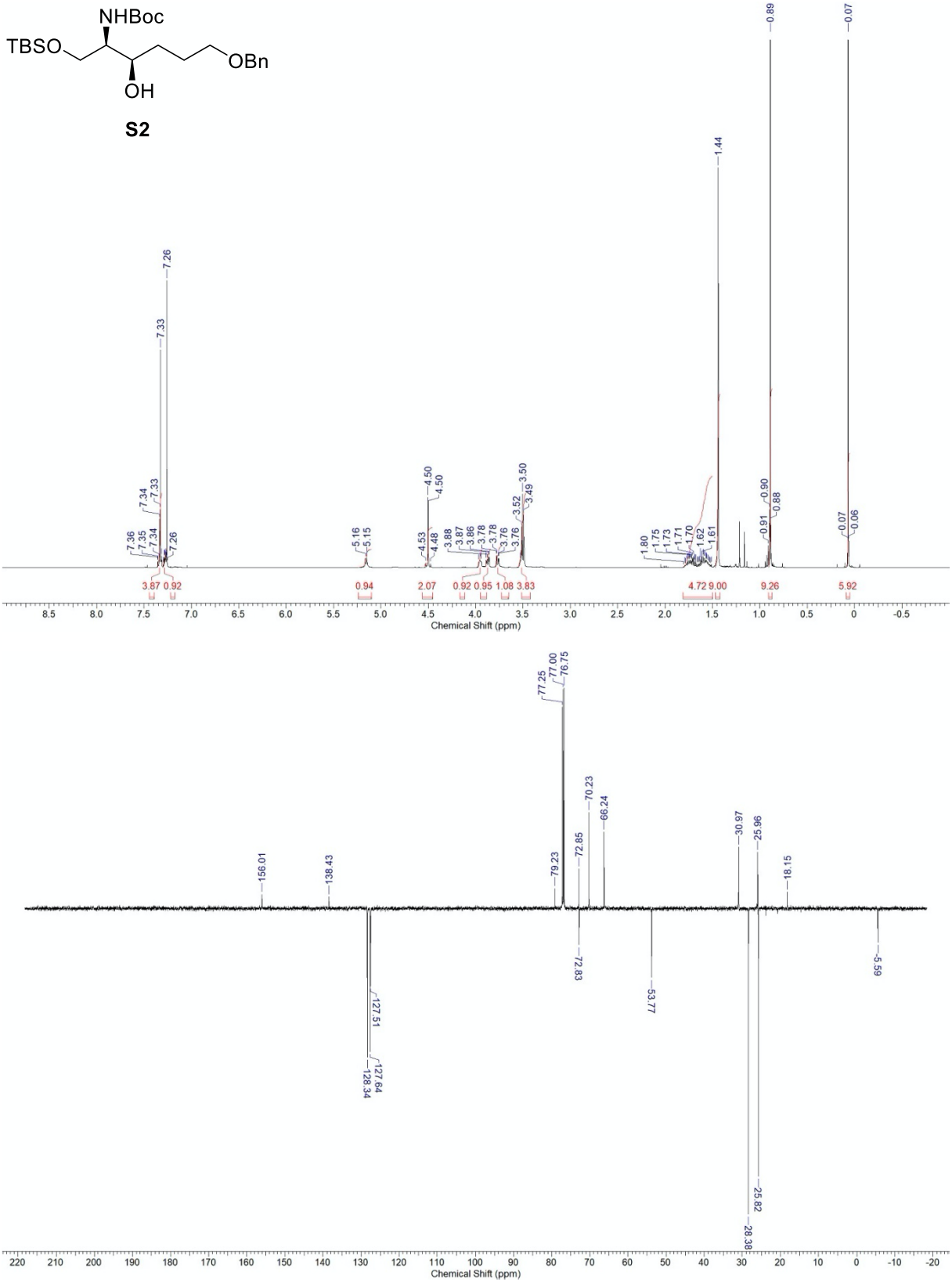

49.00
48.83
49.17
30.77
48.66
49.34
30.81
30.47
28.71
34.84
63.44
23.74
48.49
49.51
14.46
71.45
68.13
58.35
133.78
132.39
Chemical Shift (ppm)

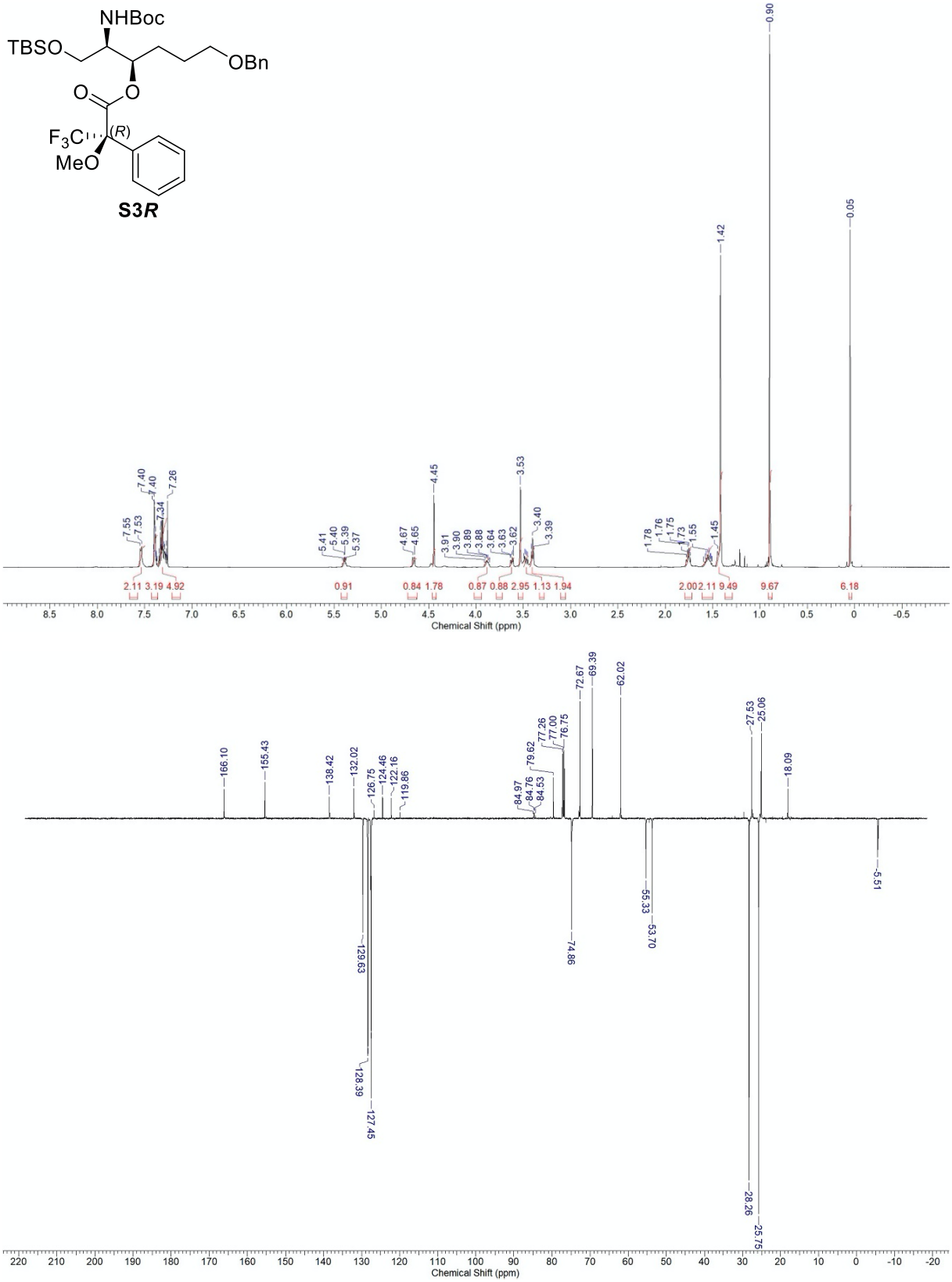

NHBoc
OH
$C_{10}H_{21}$
OH
OH
15
4.88
3.31
1.45
3.31
1.30
0.90
3.32
3.30
0.89
0.92
1.33
1.47
1.49
1.50
1.51
1.70
2.08
2.09
2.11
3.53
3.54
3.58
3.60
3.61
3.76
3.77
4.35
4.36
4.38
4.39
5.31
5.33
5.33
5.35
5.43
5.45
5.47
1.01 1.00
1.00
1.04 0.89 2.25
2.03 0.96 3.03 9.33 17.01 3.07
Chemical Shift (ppm)

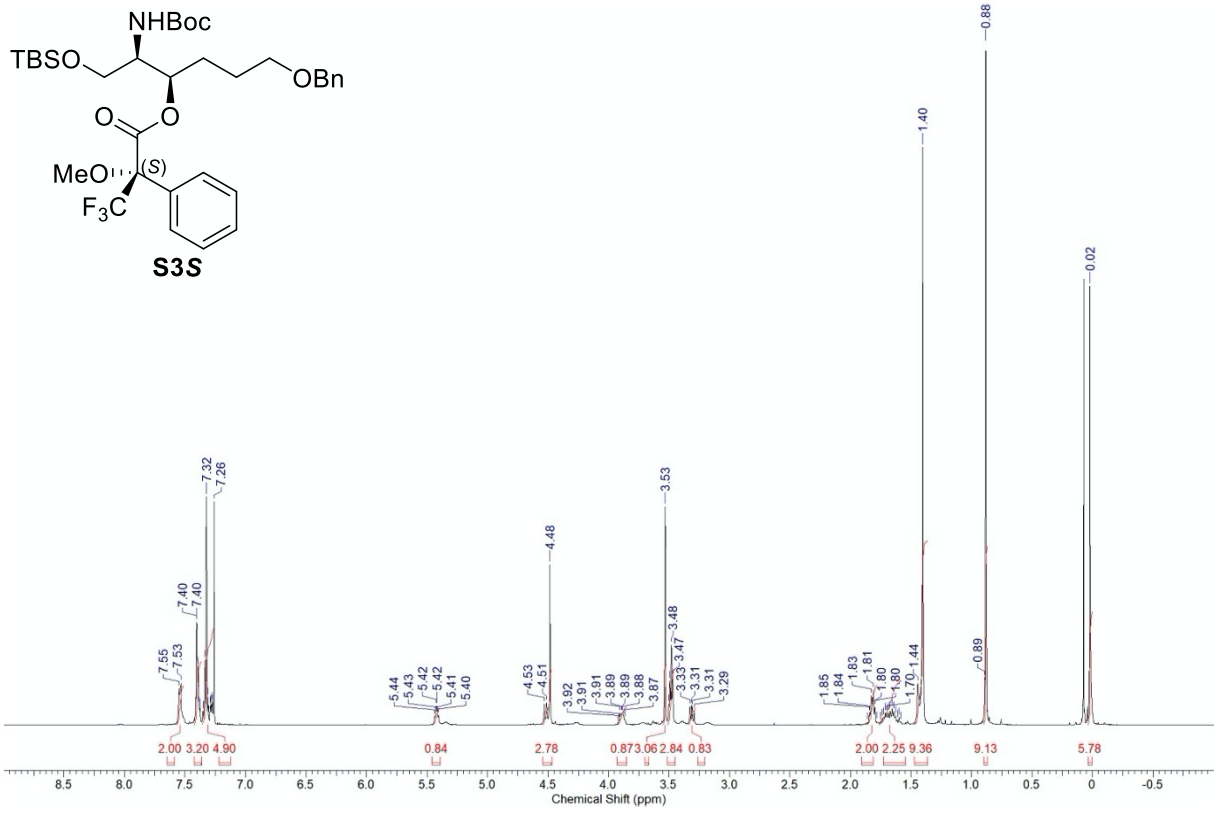

49.00
48.83
49.17
49.34
48.66
30.78
30.49
63.18
49.51
48.49
35.10
31.20
30.91
23.75
28.74
158.37
80.14
28.70
14.49
132.37
133.86
71.18
68.38
56.81
28.78
Chemical Shift (ppm)


OMe
(R)
CF3
HN
O
OBn
O
O
S1R
Chemical Shift (ppm)
Chemical Shift (ppm)


CF3
(S)
OMe
HN
O
OBn
O
O
S1S
Chemical Shift (ppm)
Chemical Shift (ppm)

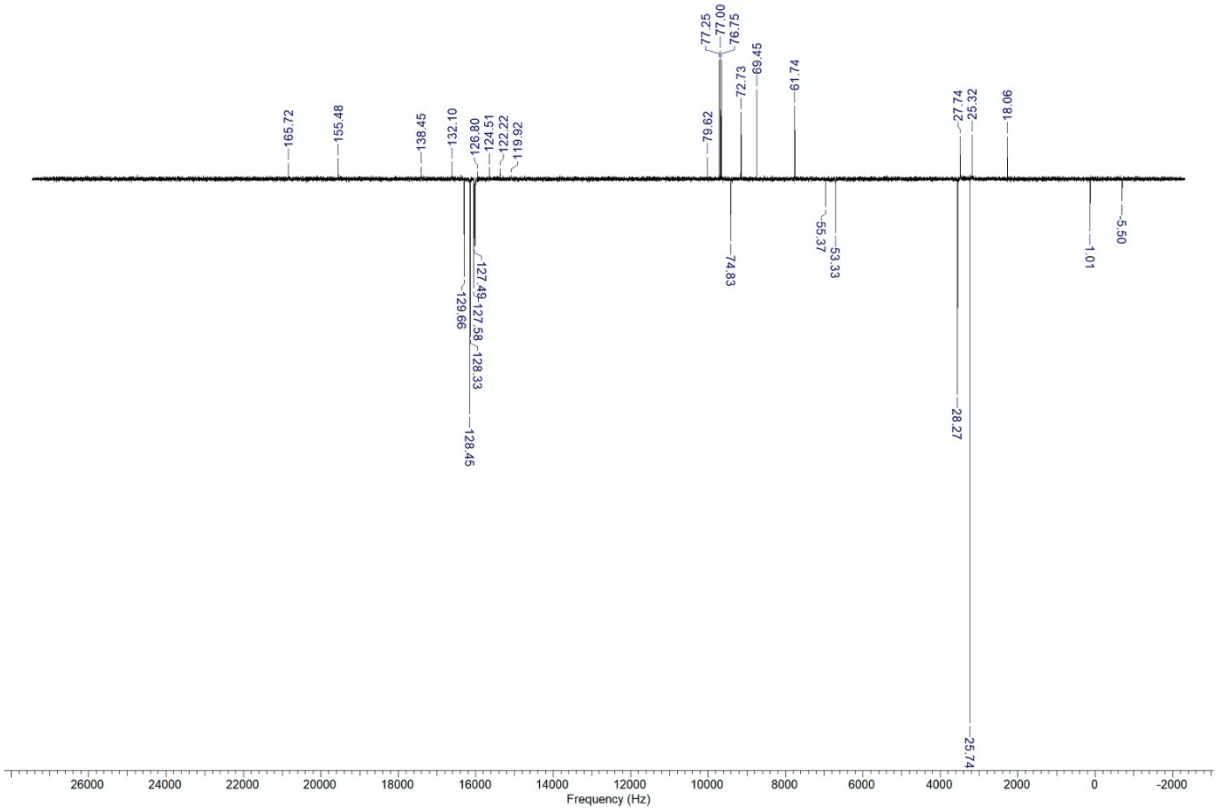

NHBoc
TBSO
OBn
OH
S2
Chemical Shift (ppm)
Chemical Shift (ppm)

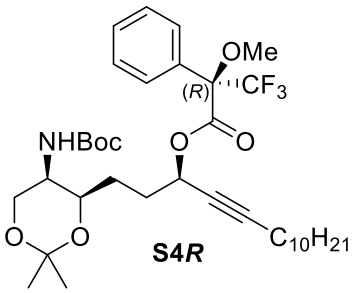

NHBoc
TBSO
OBn
O
O
F3C
(R)
MeO
S3R
Chemical Shift (ppm)
Chemical Shift (ppm)


NHBoc
TBSO
OBn
O
O
MeO
(S)
F3C

S3*S*

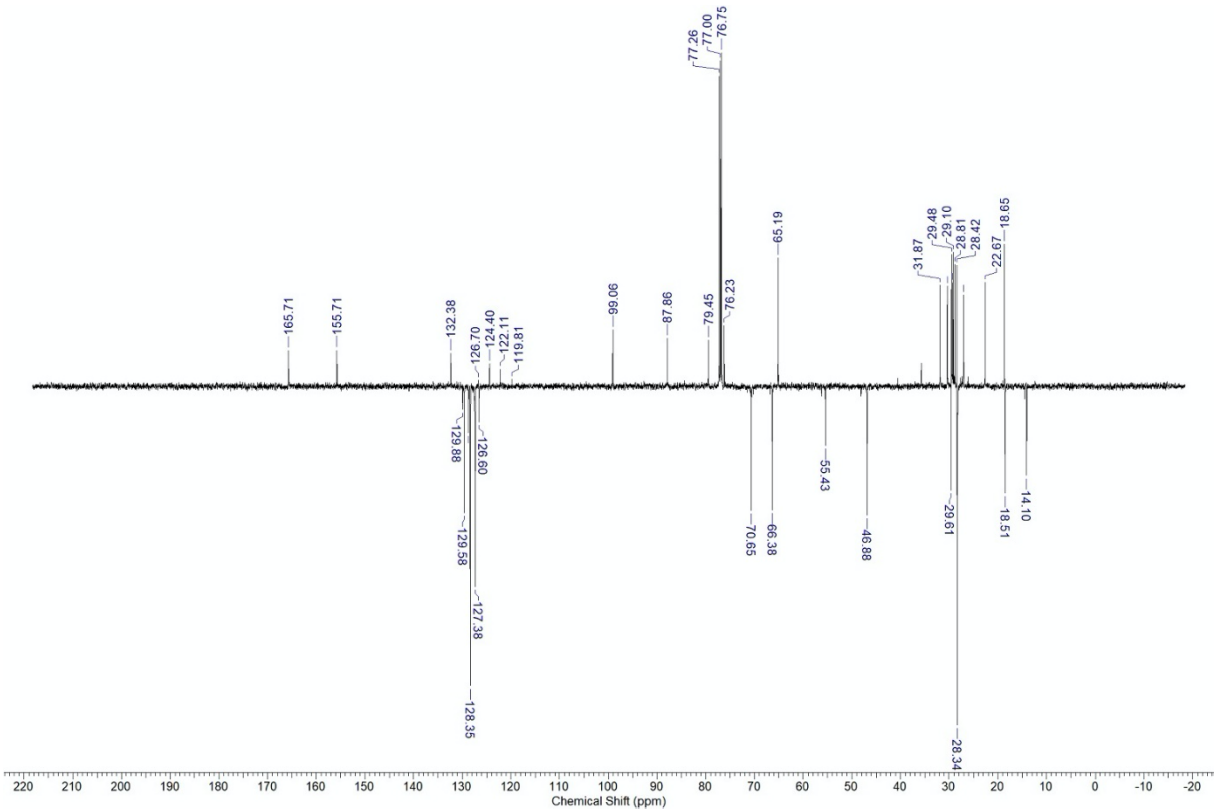
7.55
7.53
7.40
7.40
7.32
7.26
5.44
5.43
5.42
5.42
5.41
5.40
4.53
4.51
4.48
3.92
3.91
3.91
3.89
3.89
3.88
3.87
3.53
3.48
3.47
3.33
3.31
3.31
3.29
1.85
1.84
1.83
1.81
1.80
1.80
1.70
1.44
1.40
0.89
0.88
0.02
2.00 3.20 4.90
0.84
2.78
0.87 3.06 2.84 0.83
2.00 2.25 9.36
9.13
5.78
Chemical Shift (ppm)

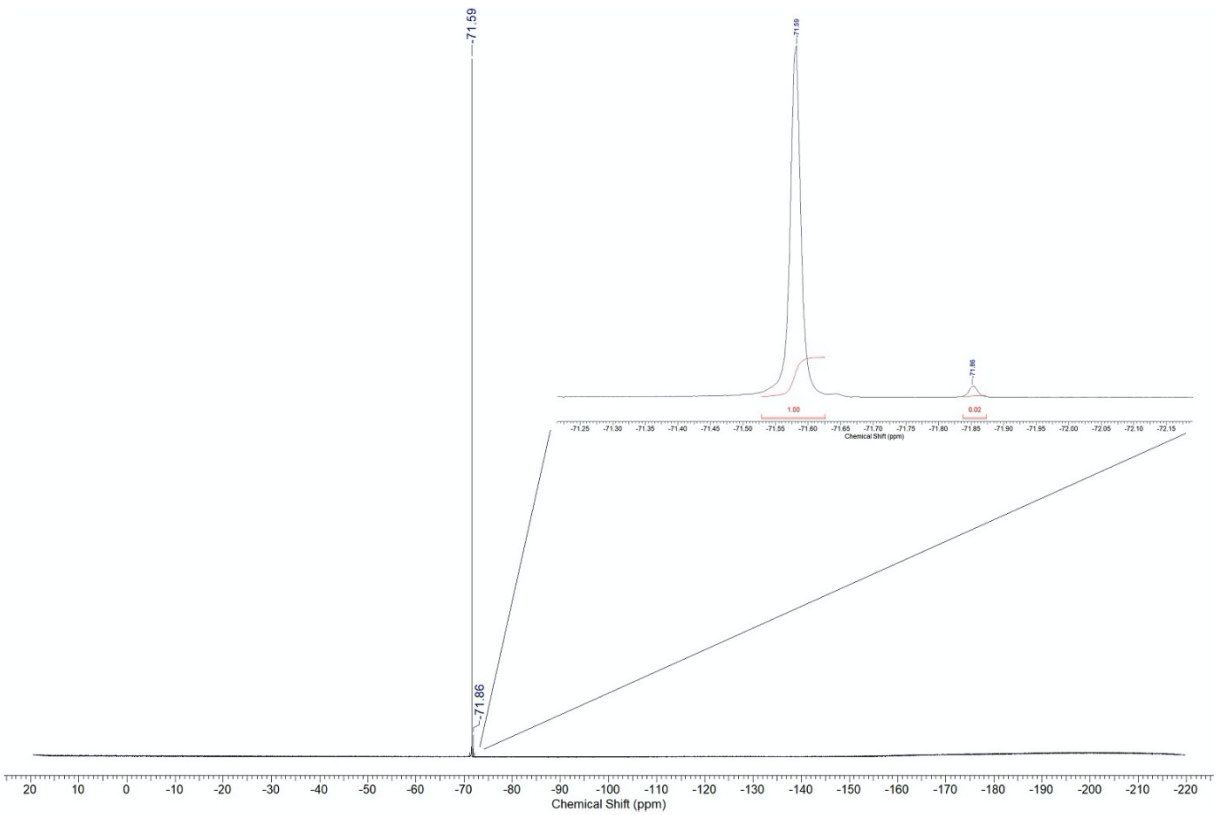

165.72
155.48
138.45
132.10
130.80
124.51
122.22
119.92
129.66
127.49
127.58
128.33
128.45
77.25
77.00
76.75
79.62
74.83
72.73
69.45
61.74
55.37
53.33
27.74
25.32
18.06
28.27
1.01
-5.50
25.74
Frequency (Hz)

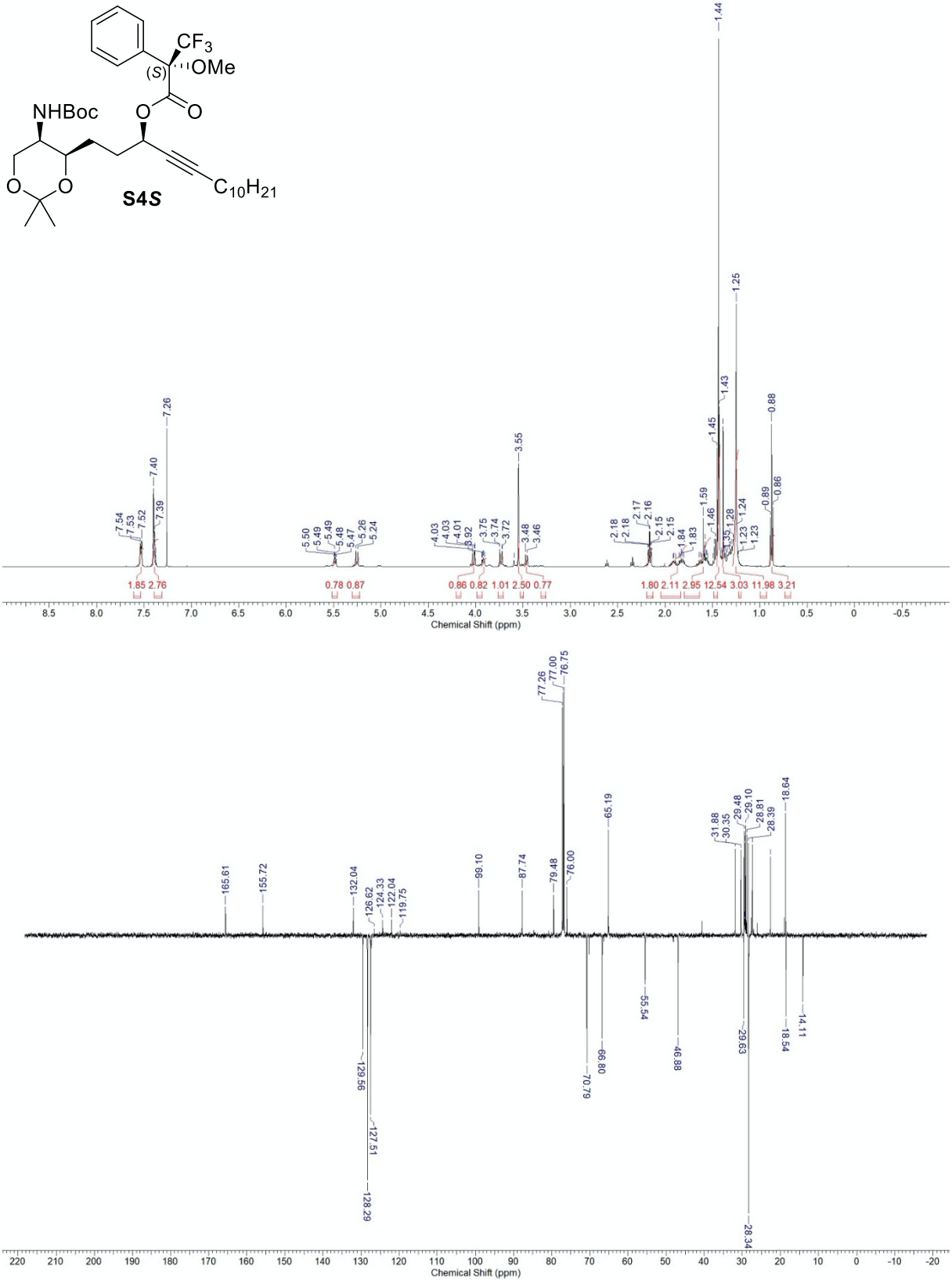

OMe
(R)
CF3
NHBoc
O
O
O
O
C10H21

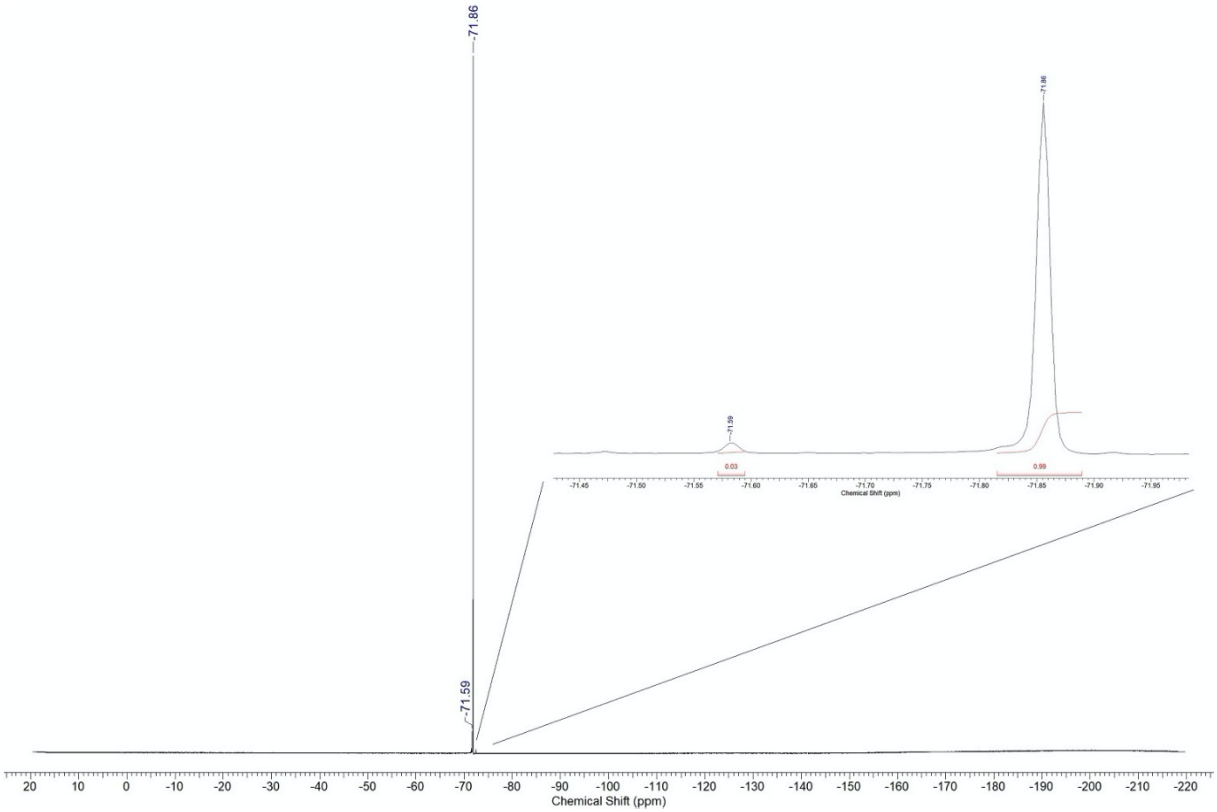
S4*R*

1.96 3.08
0.73 0.71
0.83 0.72 1.62 2.50 0.74
1.68 2.36 3.88 9.01 2.97 3.07 11.97 2.99
Chemical Shift (ppm)


Chemical Shift (ppm)


-71.59
-71.86
1.00
0.02
Chemical Shift (ppm)


CF3
(S)
OMe
NHBoc
O
O
O
O
O
C10H21
S4S
Chemical Shift (ppm)
Chemical Shift (ppm)


-71.86
-71.59
0.03
0.99
Chemical Shift (ppm)
20 10 0 -10 -20 -30 -40 -50 -60 -70 -80 -90 -100 -110 -120 -130 -140 -150 -160 -170 -180 -190 -200 -210 -220
Chemical Shift (ppm)

Cell culture conditions and MTT-assay

Cell culture conditions and stock solutions

The HCT-116^{wt} (DSMZ ACC-581) as well as its knockout mutant HCT-116$^{p53-/-}$ colon carcinoma cells, the 518A2 (Department of Radiotherapy & Radiobiology, University Hospital Vienna, Austria) melanoma cells, the U87 glioblastoma cells, the HeLa (DSMZ ACC-57) cervix carcinoma cells and the Ea.Hy926 (ATCC CRL-2922) endothelial cells were cultivated in Dulbeccos Modified Eagle Medium (Gibco, ThermoFisher), supplemented with 10% fetal bovine serum (Biochrom) and 1% Antibiotic-Antimycotic (Gibco, ThermoFisher) at 37 °C, 95% humidity and 5% CO_2. If not indicated otherwise all incubation steps of the assay were conducted under these cell culture conditions. Halisphingosine A (**1**) was dissolved in DMSO (10 mM) and freshly diluted appropriately with sterile Milli-Q water.

Anti-proliferative activity (MTT-assay)[5]

1 was investigated for its anti-proliferative effect on various human carcinoma cell lines via the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, Glentham life sciences) based proliferation assay. Cells were seeded at 0.05×10^6 cells per mL (cpm) into the wells of 96-well microtiter plates (100 µL/well) and incubated for 24 h. Appropriate dilutions in H_2O of halisphingosine A (**1**) or equal amounts of the solvent were added into the wells (final concentrations 100 µM–5 nM) and the cells were further incubated for 72 h. Before staining of the viable cells the plates were centrifuged (300 g, 5 min, 4 °C) and the back medium was discarded. 50 µL of a 0.05% MTT solution (PBS) were added to each well. After another 2 h of incubation the plates were centrifuged as before and the MTT solution was discarded again. To dissolve the cells and the formed violet water-insoluble formazan, 25 µL of an SDS/DMSO solution (10%, 0.6% AcOH) were added to each well and the plates were further incubated for at least 1 h. The absorbances at 570 nm (formazan) and at 630 nm (background) were measured. The absorbance of formazan is directly linked to the amount of metabolically active (viable) cells in the wells. The absorbance of the wells treated with the solvent was set to 100% viable cells, and the percentage of viable cells in the wells treated with halisphingosine A was calculated accordingly. IC_{50} values were determined using Graphpad Prism, means and SD were calculated from four independent experiments.

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