SUPPLEMENTARY INFORMATION

Chemical and biological evaluation of unusual sugars, α-aculosides, as novel Michael acceptors

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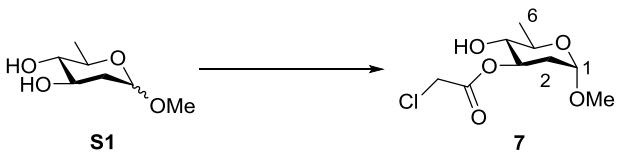
General methods for chemical synthesis.

NMR spectra were recorded on a JEOL ECA-500 (500 MHz for ^{1}H, 125 MHz for ^{13}C) and Varian MVX-300 (300 MHz for ^{1}H) spectrometer. ^{1}H-NMR data are reported as follows; chemical shift in parts par million (ppm) downfield or upfield from tetramethylsilane (δ 0.00), $CDCl_3$ (δ 7.26), integration, multiplicity (br = broad, s = singlet, d = doublet, t = triplet, q = quartet, quin = quintet, and m = multiplet) and coupling constants (Hz). ^{13}C-NMR chemical shifts are reported in ppm downfield or upfield from $CDCl_3$ (δ 77.0). ESI-TOF Mass spectra were measured on a Waters LCT premier XE. MALDI TOF MS spectra were measured on a Bruker Ultra flex. Melting points were determined on a micro hot-stage (Yanako MP-S3). Optical rotations were measured on a JASCO P-2200 polarimeter. Silica gel TLC and column chromatography were performed using Merck TLC 60F-254 (0.25 mm) and Silica Gel 60 N (spherical, neutral, 63-210 μm) (Kanto Chemical Co., Inc.), respectively. Air- and/or moisture-sensitive reactions were carried out under an argon atmosphere using oven-dried glassware. In general, organic solvents were purified and dried using appropriate procedures, and evaporation and concentration were carried out under reduced pressure below 30 °C, unless otherwise noted.

Synthesis of vineomycin B_2 trisaccharide analogues 1-5.

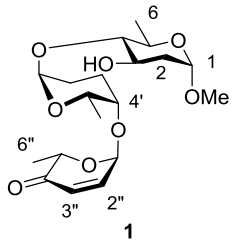
Compound 7



A suspension of **S1**[1)] (474 mg, 2.92 mmol) and Bu_2SnO (2.18 g, 8.76 mmol) in MeOH (9.48 mL) was refluxed for 2 h, and then the reaction mixture was concentrated in *vacuo*. To the residue in CH_2Cl_2 (9.48 mL) were added pyridine (946 μL, 11.7 mmol) and ClAcCl (351 μL, 4.38 mmol) at 0 ºC. After being stirred at 0 ºC for 1 h, the reaction mixture was quenched with H_2O (5 mL). The resulting mixture was extracted with EtOAc (5 mL × 3). The extracts were washed with brine, dried over Na_2SO_4, filtered, and concentrated in *vacuo*. Purification of the residue by flash silica-gel column chromatography (3/1 *n*-hexane/EtOAc) gave **7** (236 mg, 0.991 mmol, 34% yield). Colorless syrup; R_f 0.47 (3/1 *n*-hexane/EtOAc); $[\alpha]^{26}_{D}$ +14.2º (*c* 0.8, $CHCl_3$); ^{1}H-NMR (300 MHz, $CDCl_3$, TMS) δ 5.18 (1H, ddd, $J_{2ax,3}$ = 11.1 Hz, $J_{3,4}$ = 9.3 Hz, $J_{2eq,3}$ = 5.7 Hz, H-3), 4.75 (1H, m, H-1), 4.14 & 4.09 (2H, ABq, J = 15.0 Hz, ClAc), 3.75 (1H, dq, $J_{4,5}$

= 9.3 Hz, $J_{5,6}$ = 6.3 Hz, H-5), 3.34 (s, 3H, OMe), 3.30 (1H, dd, $J_{3,4}$ = $J_{4,5}$ = 9.3 Hz, H-4), 2.23 (1H, ddd, $J_{2ax,2eq}$ = 12.3 Hz, $J_{2eq,3}$ = 5.7 Hz, $J_{1,2eq}$ = 1.5 Hz, H-2eq), 1.78 (1H, ddd, $J_{2ax,2eq}$ = 12.3 Hz, $J_{2ax,3}$ = 11.1 Hz, $J_{1,2ax}$ = 3.9 Hz, H-2ax), 1.34 (3H, d, $J_{5,6}$ = 6.3 Hz, H-6); ^{13}C-NMR (125 MHz, $CDCl_3$); 167.7, 97.8, 75.4, 64.6, 67.7, 54.7, 40.9, 35.0, 17.7; HRMS (ESI-TOF) *m/z* 261.0512 (261.0506 calcd for $C_9H_{15}ClO_5Na$, $[M+Na]^+$).

Compound 1



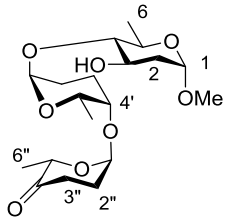
1

A suspension of **6**[2] (13.3 mg, 39.8 μmol), **7** (6.3 mg, 26.5 μmol) and MS 4A (13.3 mg) in dry CH_2Cl_2 (532 μL) was stirred at room temperature for 30 min. And then, the suspension was cooled to −78 ºC and stirred for 30 min at the same temperature. To the mixture were added NIS (17.9 mg, 79.6 μmol) and a solution of TfOH in CH_2Cl_2 (1.0 μL, 79.6 μmol) at −78 ºC. The reaction temperature was gradually warmed to −40 ºC during 2 h. After being stirred at −40 ºC for 12 h, the reaction mixture was quenched with a solution of sat. $NaHCO_3$ aq. / sat. $Na_2S_2O_3$ aq. (1/1, 1 mL). The resulting mixture was extracted with EtOAc (2 mL × 3). The extracts were washed with brine, dried over Na_2SO_4, filtered, and concentrated in *vacuo*. The residue was subjected to flash silica-gel column chromatography (1/1 *n*-hexane/EtOAc) to give the crude product **8**.

To a solution of the above crude product **8** and 2,6-lutidine (11.1 μL, 95.2 μmol) in DMF (770 μL) was added thiourea (7.3 mg, 95.2 μmol). After being stirred at 60 ºC for 3 h, the reaction mixture was quenched with H_2O (2 mL). The resulting mixture was extracted with EtOAc (2 mL × 3). The extracts were washed with brine, dried over Na_2SO_4, filtered, and concentrated in *vacuo*. Purification of the residue by preparative TLC (1/1 *n*-hexane/EtOAc) gave **1** (6.7 mg, 17.4 μmol, 66% yield in 2 steps). Colorless syrup; R_f 0.42 (1/1 *n*-hexane/EtOAc)); $[\alpha]^{21}_D$ +43.5º (*c* 0.5, $CHCl_3$); ^{1}H-NMR (500 MHz, $CDCl_3$, TMS) δ 6.88 (1H, dd, $J_{2'',3''}$ = 9.9 Hz, $J_{1'',2''}$ = 3.5 Hz, H-2''), 6.11 (1H, m, H-3''), 5.24 (1H, d, $J_{1'',2''}$ = 3.5 Hz, H-1''), 4.96 (1H, m, H-1'), 4.76 (1H, br s, H-1), 4.57 (1H, q, $J_{5'',6''}$ = 6.5 Hz, H-5''), 4.21 (1H, dq, $J_{5',6'}$ = 6.5 Hz, $J_{4',5'}$ = 1.5 Hz, H-5'), 3.85 (1H, m, H-3), 3.69-3.63 (2H, m, H-4' & H-5), 3.33 (s, 3H, OMe), 2.98 (1H, m, H-4),

2.17 (1H, m, H-2eq), 2.15-1.50 (5H, m, H-2ax & H-2' & H-3'), 1.38 (3H, d, $J_{5'',6''}$ = 6.5 Hz, H-6''), 1.28-1.20 (6H, m, H-6 & H-6'); ^{13}C-NMR (125 MHz, $CDCl_3$); 196.6, 142.8, 127.5, 120.1, 99.2, 98.3, 95.4, 89.1, 76.3, 70.7, 67.6, 65.5, 54.7, 36.7, 25.2, 24.4, 18.1, 17.0, 15.2; HRMS (ESI-TOF) *m/z* 387.2016 (387.2019 calcd for $C_{19}H_{31}O_8$, $[M+H]^+$).

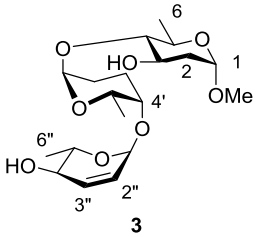
Compound 2



2

A suspension of **1** (5.5 mg, 14.2 μmol) and 10% Pd/C (2.8 mg) in EtOH (275 μL) was stirred under H_2 atmosphere (balloon) at room temperature for 8 h. The mixture was filtered through celite pad, and the filtrate was concentrated in *vacuo*. Purification of the residue by flash silica-gel column chromatography (1/1 *n*-hexane/EtOAc) gave **2** (4.2 mg, 10.8 μmol, 76% yield). Colorless syrup; R_f 0.25 (1/1 *n*-hexane/EtOAc); $[\alpha]^{24}_D$ −98.2° (*c* 1.0, $CHCl_3$); ^{1}H-NMR (300 MHz, $CDCl_3$, TMS) δ 5.22 (1H, br d, *J* = 3.6 Hz, H-1''), 4.93 (1H, m, H-1'), 4.73 (1H, br d, *J* = 3.0 Hz, H-1), 4.35 (1H, m, H-5''), 4.22-4.10 (1H, m, H-5'), 3.84 (1H, ddd, $J_{2ax,3}$ = 11.5 Hz, $J_{3,4}$ = 9.2 Hz, $J_{2eq,3}$ = 5.4 Hz, H-3), 3.73-3.60 (2H, m, H-4' & H-5), 3.32 (s, 3H, OMe), 2.98 (1H, m, H-4), 2.57-1.50 (10H, m, H-2 & H-2' & H-2'' & H-3' & H-3''), 1.33-1.08 (9H, m, H-6 & H-6' & H-6''); ^{13}C-NMR (125 MHz, $CDCl_3$); 210.9, 99.3, 99.1, 98.3, 89.0, 74.8, 71.2, 67.9, 67.6, 65.5, 54.7, 36.6, 33.6, 28.4, 25.3, 24.5, 18.1, 17.0, 14.9; HRMS (ESI-TOF) *m/z* 411.2097 (411.2100 calcd for $C_{19}H_{32}O_8Na$, $[M+Na]^+$).

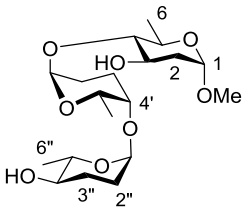
Compound 3



3

A suspension of **1** (14.7 mg, 38.1 μmol) in dry CH_2Cl_2 (588 μL) was cooled to −78 ºC and stirred for 30 min at the same temperature. To the mixture were added $NaBH_4$ (4.3 mg, 114 μmol) and 0.4 M $CeCl_3$ in MeOH (57.3 μL, 229 μmol) at −78 ºC. The reaction temperature was gradually warmed to −60 ºC during 15 min. After being stirred at −60 ºC for 2 h, the reaction mixture was quenched with H_2O (2 mL). The resulting mixture was extracted with EtOAc (2 mL × 3). The extracts were washed with brine, dried over Na_2SO_4, filtered, and concentrated in *vacuo*. Purification of the residue by flash silica-gel column chromatography (1/1 *n*-hexane/EtOAc) gave **3** (12.7 mg, 32.7 μmol, 86% yield). Colorless syrup; R_f 0.67 (1/1 *n*-hexane/EtOAc); $[\alpha]^{25}_D$ −1.9º (*c* 0.6, $CHCl_3$); ^{1}H-NMR (300 MHz, $CDCl_3$, TMS) δ 5.96-5.77 (2H, m, H-2'' & H-3''), 4.98-4.71 (3H, m, H-1 & H-1' & H-1''), 4.22-3.42 (6H, m, H-3 & H-4' & H-4'' & H-5 & H-5' & H-5''), 3.32 (s, 3H, OMe), 3.29 (1H, m, H-4), 2.21 (1H, m, H-2eq), 2.07-1.50 (5H, m, H-2ax & H-2' & H-3') , 1.40-1.03 (9H, m, H-6 & H-6' & H-6''); ^{13}C-NMR (125 MHz, $CDCl_3$); 133.4, 126.6, 99.3, 98.3, 96.6, 89.0, 75.7, 69.7, 68.3, 68.0, 67.6, 65.6, 54.8, 36.7, 25.2, 24.8, 18.1, 18.0, 17.1; HRMS (ESI-TOF) *m/z* 411.1988 (411.1995 calcd for $C_{19}H_{32}O_8Na$, $[M+Na]^+$).

Compound 4

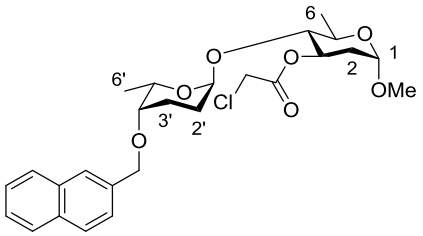


4

To a solution of **3** (8.3 mg, 21.4 μmol) in CH_2Cl_2 (332 μL) were added *o*-nitrobenzene sulfonylhydrazide (28.0 mg, 129 μmol) and Et_3N (23.8 μl, 171 μmol). After being stirred at room temperature for 12 h, the reaction mixture was quenched with H_2O (2 mL). The resulting mixture was extracted with EtOAc (2 mL × 3). The extracts were washed with brine, dried over Na_2SO_4, filtered, and concentrated in *vacuo*. Purification of the residue by preparative TLC (1/1 *n*-hexane/EtOAc) gave **4** (6.3 mg, 16.1 μmol, 75% yield). Colorless syrup; R_f 0.25 (1/1 *n*-hexane/EtOAc); $[\alpha]^{21}_D$ −36.2º (*c* 0.7, $CHCl_3$); ^{1}H-NMR (300 MHz, $CDCl_3$, TMS) δ 4.98 (1H, br d, *J* = 3.6 Hz, H-1''), 4.92 (1H, m, H-1'), 4.73 (1H, br d, *J* = 3.0 Hz, H-1), 4.16 (1H, m, H-5'), 3.83 (1H, m, H-3), 3.90-3.51 (3H, m, H-4'' & H-5 & H-5''), 3.32 (s, 3H, OMe), 3.29 (1H, m, H-4'), 2.97 (1H, m, H-4), 2.23-1.51 (10H, m, H-2 & H-2' & H-2'' & H-3' & H-3''),

1.38-1.08 (9H, m, H-6 & H-6' & H-6''); ^{13}C-NMR (125 MHz, $CDCl_3$); 99.3, 98.7, 98.3, 89.0, 74.5, 72.2, 70.3, 68.2, 67.6, 65.5, 54.7, 36.7, 29.8, 27.9, 25.4, 24.3, 18.1, 17.8, 17.0; HRMS (ESI-TOF) *m/z* 413.2133 (413.2155 calcd for $C_{19}H_{34}O_8Na$, $[M+Na]^+$).

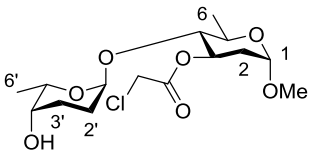
Compound 10



10

A suspension of **9**[2] (17.7 mg, 48.5 μmol), **7** (7.7 mg, 32.3 μmol) and MS 4A (17.6 mg) in dry CH_2Cl_2 (708 μL) was stirred at room temperature for 30 min. And then, the suspension was cooled to −78 ºC and stirred for 30 min at the same temperature. To the mixture were added NIS (21.8 mg, 97.0 mmol) and a solution of TfOH in CH_2Cl_2 (1.0 μL, 0.97 mM) at −78 ºC. The reaction mixture was gradually warmed to −40 ºC during 2 h. After being stirred at −40 ºC for 12 h, the reaction mixture was quenched with a solution of sat. $NaHCO_3$ aq./sat. $Na_2S_2O_3$ aq. (1/1, 2 mL). The resulting mixture was extracted with EtOAc (2 mL × 3). The extracts were washed with brine, dried over Na_2SO_4, filtered, and concentrated in *vacuo*. Purification of the residue by flash silica-gel column chromatography (3/1 *n*-hexane-EtOAc) gave **10** (13.1 mg, 26.6 μmol, 83% yield). Colorless syrup; R_f 0.67 (3/1 *n*-hexane-EtOAc); $[\alpha]^{21}_D$ +28.3º (*c* 0.9, $CHCl_3$); ^{1}H-NMR (500 MHz, $CDCl_3$, TMS) δ 7.86-7.70 (4H, m, ArH), 7.52-7.44 (3H, m, ArH), 5.26 (1H, ddd, $J_{2ax,3}$ = 11.4 Hz, $J_{3,4}$ = 9.3 Hz, $J_{2eq,3}$ = 5.4 Hz, H-3), 4.94 (1H, br s, H-1'), 4.72 (1H, br d, J = 3.3 Hz, H-1), 4.14 & 4.09 (2H, ABq, J = 12.3 Hz, $ArCH_2$), 4.14 & 4.04 (2H, ABq, J = 14.9 Hz, ClAc), 3.97-3.92 (1H, m, H-5'), 3.75 (1H, dq, $J_{4,5}$ = 9.2 Hz, $J_{5,6}$ = 6.5 Hz, H-5), 3.31 (3H, s, OMe), 3.30 (2H, m, H-4 & H-4'), 2.23 (1H, m, H-2eq), 2.10-1.46 (5H, m, H-2ax & H-2' & H-3'), 1.29 (3H, d, $J_{5,6}$ = 6.5 Hz, H-6), 1.18 (3H, d, $J_{5',6'}$ = 6.5 Hz, H-6'); ^{13}C-NMR (125 MHz, $CDCl_3$); 167.6, 136.8, 134.0, 133.8, 129.0, 128.7, 128.5, 127.0, 126.9×2, 126.7, 99.8, 98.5, 82.4, 74.3, 73.6, 72.0, 68.4, 67.5, 55.5, 42.1, 36.1, 25.6, 21.8, 19.2, 18.1; HRMS (ESI-TOF) *m/z* 515.1915 (515.1923 calcd for $C_{26}H_{33}ClO_7Na$, $[M+Na]^+$).

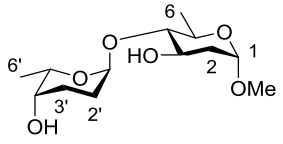
Compound 11



11

To a two-phase mixture of **10** (20.0 mg, 40.6 μmol) in CH_2Cl_2 (4.06 mL) and 0.1 M phosphate buffer (pH 7.2, 5.08 mL) was added DDQ (18.4 mg, 81.2 μmol). After being stirred at room temperature for 12 h, the reaction mixture was quenched with sat. $NaHCO_3$ aq. (5 mL). The resulting mixture was extracted with $CHCl_3$ (5 mL × 3). The extracts were washed with brine, dried over Na_2SO_4, filtered, and concentrated in *vacuo*. Purification of the residue by flash column chromatography (1/1 *n*-hexane-EtOAc) gave **11** (8.7 mg, 24.8 μmol, 61 % yield). Colorless syrup; R_f 0.10 (1/1 *n*-hexane/EtOAc); $[\alpha]^{23}_D$ +21.4º (*c* 0.5, $CHCl_3$); ^{1}H-NMR (500 MHz, $CDCl_3$, TMS) δ 5.27 (1H, ddd, $J_{2ax,3}$ = 11.5 Hz, $J_{3,4}$ = 9.2 Hz, $J_{2eq,3}$ = 5.5 Hz, H-3), 4.92 (1H, br s, H-1'), 4.72 (1H, br d, J = 2.5 Hz, H-1), 4.09 & 4.06 (2H, ABq, J = 15.0 Hz, ClAc), 4.02-3.97 (1H, m, H-5'), 3.75 (1H, dq, $J_{4,5}$ = 9.2 Hz, $J_{5,6}$ = 6.5 Hz, H-5), 3.55 (1H, m, H-4'), 3.32 (1H, m, H-4), 3.30 (s, 3H, OMe), 2.23 (1H, ddd, $J_{2ax,2eq}$ = 13.0 Hz, $J_{2eq,3}$ = 5.5 Hz, $J_{1,2eq}$ = 1.0 Hz, H-2eq), 2.02-1.48 (5H, m, H-2ax & H-2' & H-3'), 1.29 (3H, d, $J_{5,6}$ = 6.5 Hz, H-6), 1.13 (3H, d, $J_{5',6'}$ = 6.5 Hz, H-6'); ^{13}C-NMR (125 MHz, $CDCl_3$); 166.7, 98.8, 97.7, 81.6, 72.9, 67.4, 67.2, 66.6, 54.7, 41.2, 35.2, 25.4, 24.2, 18.4, 17.0; HRMS (ESI-TOF) *m/z* 375.1289 (375.1291 calcd for $C_{15}H_{25}ClO_7Na$, $[M+Na]^+$).

Compound 5



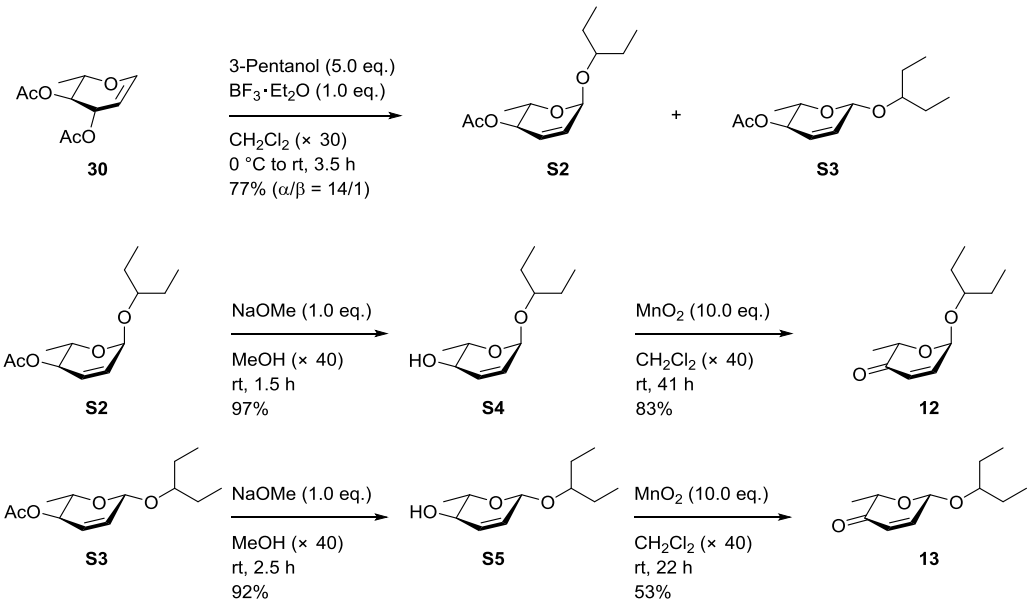
5

To a solution of **11** (14.3 mg, 40.6 μmol) and 2,6-lutidine (18.8 μL, 162 μmol) in DMF (204 μL) was added thiourea (12.3 mg, 162 μmol). After being stirred at 60 ºC for 3 h, the reaction mixture was quenched with H_2O (1 mL). The resulting mixture was extracted with EtOAc (2 mL × 3). The extracts were washed with brine, dried over Na_2SO_4, filtered, and concentrated in *vacuo*. Purification of the residue by preparative TLC (10/1 $CHCl_3$/MeOH) gave **5** (7.2 mg, 26.0 μmol, 64% yield). Colorless syrup; R_f 0.67 (10/1 $CHCl_3$/MeOH); $[\alpha]^{23}_D$ +25.6º (*c* 0.5, $CHCl_3$);

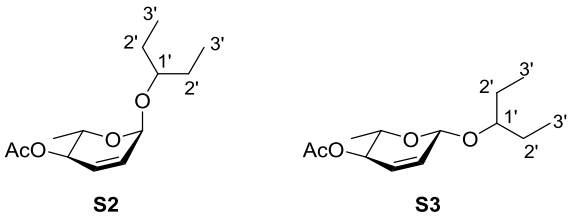
^{1}H-NMR (300 MHz, $CDCl_3$, TMS) δ 4.94 (1H, br s, H-1'), 4.74 (1H, br d, J = 3.3 Hz, H-1), 4.21 (1H, m, H-5'), 3.85 (1H, ddd, $J_{2ax,3}$ = 11.5 Hz, $J_{3,4}$ = 9.2 Hz, $J_{2eq,3}$ = 5.4 Hz, H-3), 3.75 (2H, m, H-5 & H-4'), 3.31 (s, 3H, OMe), 2.98 (1H, m, H-4), 2.18 (1H, m, H-2eq), 2.06-1.50 (5H, m, H-2ax & H-2' & H-3'), 1.35-1.20 (6H, m, H-6 & H-6'); ^{13}C-NMR (125 MHz, $CDCl_3$); 99.4, 98.3, 89.2, 68.0, 67.6, 67.4, 65.5, 54.7, 36.7, 25.5, 24.3, 18.1, 17.0; HRMS (ESI-TOF) *m/z* 299.1573 (299.1570 calcd for $C_{13}H_{25}O_6Na$, $[M+Na]^+$).

Synthesis of aculosides 12 and 13.

Scheme S1. Synthesis of aculosides **12** and **13**.



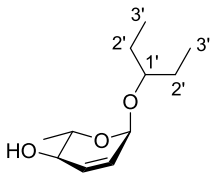
Compounds S2 and S3



To a solution of **30**[3)] (11.6 g, 54.0 mmol) and 3-pentanol (29.1 mL, 270 mmol) in dry CH_2Cl_2 (350 mL) was added $BF_3 \cdot Et_2O$ (6.84 mL, 54.0 mmol) at 0 ºC. After being stirred at room

temperature for 3.5 h, the reaction mixture was quenched with sat. $NaHCO_3$ aq. (100 mL). The resulting mixture was extracted with $CHCl_3$ (300 mL × 3). The extracts were washed with brine, dried over Na_2SO_4, filtered, and concentrated in *vacuo*. Purification of the residue by flash silica-gel column chromatography (80/1 $CHCl_3$/EtOAc) gave **S2** (9.34 g, 38.6 mmol, 72% yield) and **S3** (653 mg, 2.70 mmol, 5% yield). **S2**: Colorless syrup; R_f 0.61 (3/1 *n*-hexane/EtOAc); $[\alpha]^{27}_D$ −144° (c 0.88, $CHCl_3$); ^{1}H-NMR (300 MHz, $CDCl_3$, TMS) δ 5.80 (2H, m, H-2 & H-3), 5.04 (2H, m, H-1 & H-4), 4.03 (1H, dq, $J_{4,5}$ = 9.0 Hz, $J_{5,6}$ = 6.3 Hz, H-5), 3.54 (1H, quin, $J_{1',2'}$ = 6.0 Hz, H-1'), 2.08 (3H, s, Ac), 1.59-1.48 (4H, m, H-2'), 1.21 (3H, d, $J_{5,6}$ = 6.3 Hz, H-6), δ0.95 & 0.90 (each 3H, t, $J_{2',3'}$ = 7.5 Hz, H-3'); ^{13}C-NMR (125 MHz, $CDCl_3$); δ 170.5, 129.4, 128.1, 93.4, 81.0, 70.9, 64.7, 27.3, 26.4, 21.1, 17.8, 9.9, 9.5; HRMS (ESI-TOF) *m/z* 265.1410 (265.1416 calcd. for $C_{13}H_{22}O_4Na$, $[M+Na]^+$). **S3**: Colorless syrup; R_f 0.61 (3/1 *n*-hexane/EtOAc); $[\alpha]^{28}_D$ −85.4° (*c* 0.72, $CHCl_3$); ^{1}H-NMR (300 MHz, $CDCl_3$, TMS) δ 5.89 (2H, m, H-2 & H-3), 5.17 (1H, d, $J_{1,2}$ = 1.5 Hz, H-1), 5.06 (1H, dd, $J_{4,5}$ = 6.3 Hz, $J_{3,4}$ = 1.8 Hz, H-4), 3.83 (1H, dq, $J_{4,5}$ = $J_{5,6}$ = 6.3 Hz, H-5), 3.59 (1H, quin, $J_{1',2'}$ = 6.0 Hz, H-1'), 2.08 (3H, s, Ac), 1.65-1.48 (4H, m, H-2'), 1.30 (3H, d, $J_{5,6}$ = 6.3 Hz, H-6), 0.91 & 0.90 (each 3H, t, $J_{2',3'}$ = 7.8 Hz, H-3'); ^{13}C-NMR (125 MHz, $CDCl_3$); δ 170.5, 131.3, 127.2, 95.4, 81.1, 71.1, 69.8, 27.1, 25.8, 21.1, 18.7, 9.8, 9.3; HRMS (ESI-TOF) *m/z* 265.1427 (265.1416 calcd. for $C_{13}H_{22}O_4Na$, $[M+Na]^+$).

Compound S4

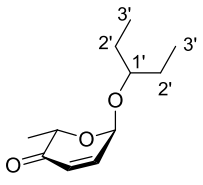


S4

To a solution of **S2** (2.00 g, 8.26 mmol) in MeOH (38.7 mL) was added 0.2 M NaOMe in MeOH (41.3 mL, 8.26 mmol) at room temperature. After being stirred at room temperature for 1.5 h, the reaction mixture was quenched with Amberlite® IR 120 H^+ form. The resulting suspension was filtered, and then the filtrate was concentrated in *vacuo*. Purification of the residue by silica-gel column chromatography (2/1 *n*-hexane/EtOAc) gave **S4** (1.68 g, 8.01 mmol, 97% yield). Colorless syrup; R_f 0.51 (2/1 *n*-hexane/EtOAc); $[\alpha]^{26}_D$ −69.5° (*c* 0.60, $CHCl_3$); ^{1}H-NMR (300 MHz, $CDCl_3$, TMS) δ 5.96-5.88 (1H, m, H-2), 5.78-5.72 (1H, m, H-3), 5.01 (1H, m, H-1), 3.86-3.71 (2H, m, H-4 & H-1'), 3.54 (1H, dq, $J_{4,5}$ = 6.9 Hz, $J_{5,6}$ = 5.7 Hz, H-5), 1.58-1.47 (4H, m, H-2'), 1.31 (3H, d, $J_{5,6}$ = 5.7 Hz, H-6), 0.95 & 0.90 (each 3H, t, $J_{2',3'}$ = 7.5 Hz,

H-3'); ^{13}C-NMR (125 MHz, $CDCl_3$); δ 133.3, 127.0, 93.4, 81.0, 69.7, 68.0, 27.3, 26.5, 17.8, 10.0, 9.5; HRMS (ESI-TOF) *m/z* 223.1307 (223.1310 calcd. for $C_{11}H_{20}O_3Na$, $[M+Na]^+$).

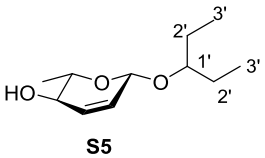
Compound 12



12

To a solution of **S4** (1.63 g, 8.14 mmol) in dry CH_2Cl_2 (65.0 mL) was added MnO_2 (7.08 g, 81.4 mmol) at room temperature. After being stirred at room temperature for 41 h, the mixture was filtered through a pad of Celite. The filtrate was concentrated in *vacuo*. Purification of the residue by silica-gel column chromatography (8/1 *n*-hexane/EtOAc) gave **12** (1.34 g, 6.76 mmol, 83% yield). Colorless syrup; R_f 0.84 (2/1 *n*-hexane/EtOAc); $[\alpha]^{24}_D$ −16.3° (*c* 0.55, $CHCl_3$); ^{1}H-NMR (300 MHz, $CDCl_3$, TMS) δ 6.82 (1H, dd, $J_{2,3}$ = 10.2 Hz, $J_{1,2}$ = 3.3 Hz, H-2), 6.06 (1H, d, $J_{2,3}$ = 10.2 Hz, H-3), 5.26 (1H, d, $J_{1,2}$ = 3.3 Hz, H-1), 4.62 (1H, q, $J_{5,6}$ = 6.9 Hz, H-5), 3.63 (1H, quin, $J_{1',2'}$ = 5.7 Hz, H-1'), 1.63-1.54 (4H, m, H-2'), 1.37 (3H, d, $J_{5,6}$ = 6.9 Hz, H-6), 0.95 & 0.92 (each 3H, t, $J_{2',3'}$ = 7.5 Hz, H-3'); ^{13}C-NMR (125 MHz, $CDCl_3$); δ 197.3, 143.9, 127.0, 92.3, 81.6, 70.3, 64.7, 27.0, 26.0, 15.1, 9.8, 9.3; HRMS (ESI-TOF) *m/z* 199.1344 (199.1334 calcd. for $C_{11}H_{19}O_3$, $[M+H]^+$).

Compound S5

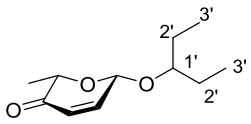


S5

To a solution of **S3** (620 mg, 2.56 mmol) in MeOH (12.0 mL) was added 0.2 M NaOMe in MeOH (12.8 mL, 2.56 mmol) at room temperature. After being stirred at room temperature for 2.5 h, the reaction mixture was quenched with Amberlite® IR 120 H^+ form. The resulting suspension was filtered, and then the filtrate was concentrated in *vacuo*. Purification of the

residue by silica-gel column chromatography (2/1 *n*-hexane/EtOAc) gave **S5** (472 mg, 2.36 mmol, 92% yield). Colorless syrup; R_f 0.51 (2/1 *n*-hexane/EtOAc); $[\alpha]^{26}_{D}$ −13.1° (*c* 1.0, $CHCl_3$); ^{1}H-NMR (300 MHz, $CDCl_3$, TMS) δ 5.95-5.89 (1H, m, H-2), 5.79-5.75 (1H, m, H-3), 5.14 (1H, m, H-1), 3.98-3.88 (1H, m, H-4), 3.58 (2H, m, H-5 & H-1'), 1.67-1.50 (4H, m, H-2'), 1.35 (3H, d, $J_{5,6}$ = 6.3 Hz, H-6), 0.91 & 0.90 (each 3H, t, $J_{2',3'}$ = 7.2 Hz, H-3'); ^{13}C-NMR (125 MHz, $CDCl_3$); δ 131.7, 129.7, 96.2, 81.3, 74.3, 68.7, 27.2, 26.0, 18.4, 9.8, 9.3; HRMS (ESI-TOF) *m/z* 223.1314 (223.1310 calcd. for $C_{11}H_{20}O_3Na$, $[M+Na]^+$).

Compound 13

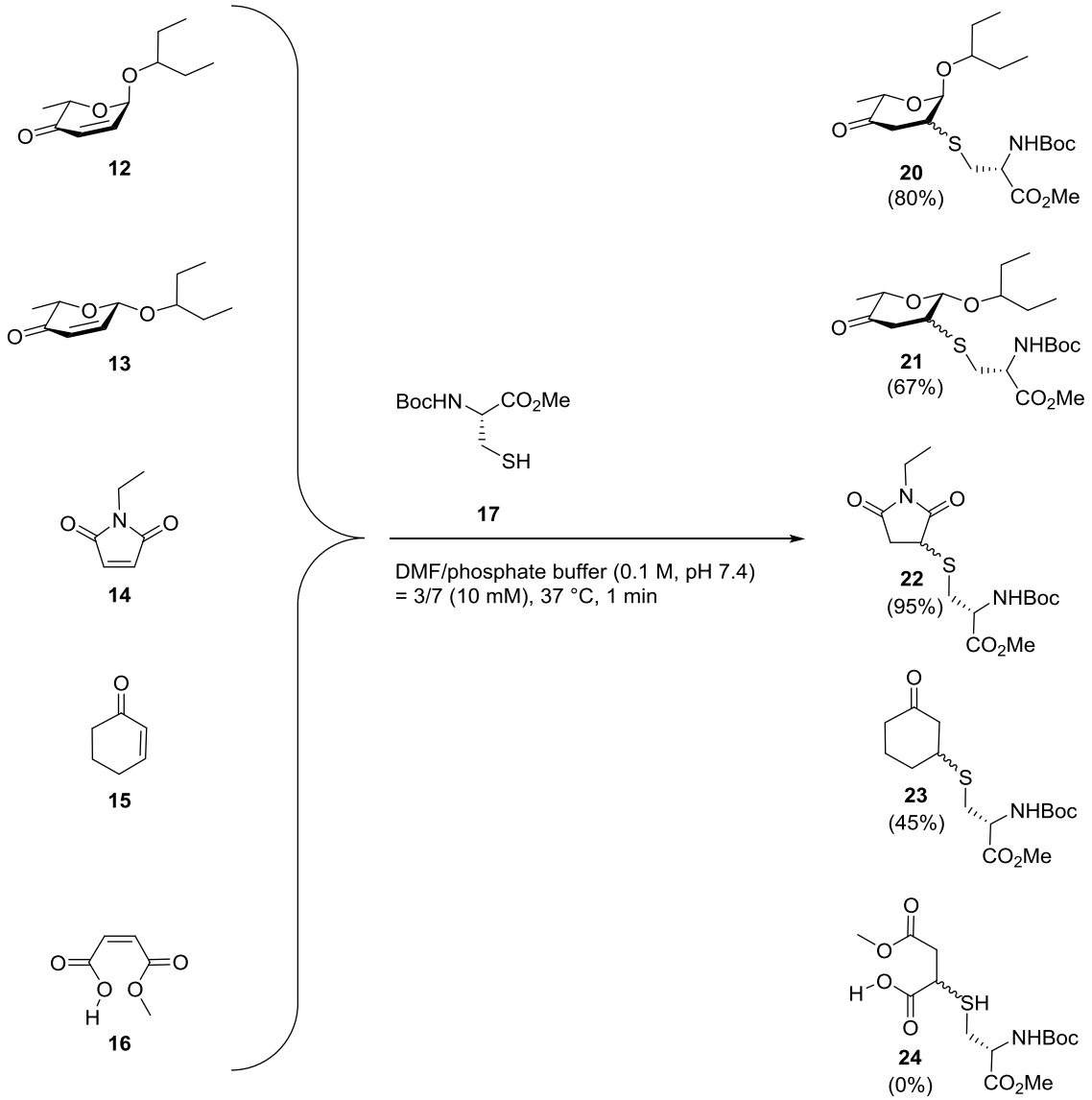


13

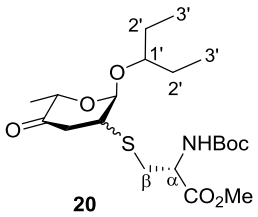
To a solution of **S5** (472 mg, 2.36 mmol) in dry CH_2Cl_2 (18.0 mL) was added MnO_2 (2.05 g, 23.6 mmol) at room temperature. After being stirred at room temperature for 22 h, the mixture was filtered through a pad of Celite. The filtrate was concentrated in *vacuo*. Purification of the residue by silica-gel column chromatography (8/1 *n*-hexane/EtOAc) gave **13** (248 mg, 12.5 mmol, 53% yield). Colorless syrup; R_f 0.84 (2/1 *n*-hexane/EtOAc); $[\alpha]^{28}_{D}$ −2.2° (*c* 0.28, $CHCl_3$); ^{1}H-NMR (300 MHz, $CDCl_3$, TMS) δ 6.89 (1H, dd, $J_{2,3}$ = 10.5 Hz, $J_{1,2}$ = 1.8 Hz, H-2), 6.11 (1H, dd, $J_{2,3}$ = 10.5 Hz, $J_{1,3}$ = 1.5 Hz, H-3), 5.40-5.38 (1H, m, H-1), 4.16 (1H, q, $J_{5,6}$ = 6.6 Hz, H-5), 3.67 (1H, quin, $J_{1',2'}$ = 6.0 Hz, H-1'), 1.68-1.53 (4H, m, H-2'), 1.46 (3H, d, $J_{5,6}$ = 6.6 Hz, H-6), 0.95 & 0.92 (each 3H, t, $J_{2',3'}$ = 7.5 Hz, H-3'); ^{13}C-NMR (125 MHz, $CDCl_3$); δ 197.1, 147.9, 128.1, 95.2, 82.0, 75.1, 27.0, 25.7, 16.8, 9.6, 9.2; HRMS (ESI-TOF) *m/z* 199.1333 (199.1334 calcd. for $C_{11}H_{19}O_3$, $[M+H]^+$).

Michael reactions of 12-16 with 17-19.

Scheme S2. Michael reactions of **12-16** with **17.**

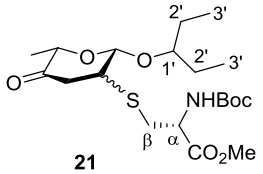


Compound 20



To a solution of **17** (24.5 mg, 104 μmol) in DMF (2.12 mL)/phosphate buffer (0.1 M, pH 7.4, 7.28 mL) was added **12** in DMF (1.00 mL, 104 μmol) at 37 °C. After being stirred at 37 ºC for 1 min, the reaction mixture was quenched with 2-iodoacetamide[4] (96.2 mg, 520 μmol). The resulting mixture was stirred at 37 °C for 10 min, and then poured into sat. $Na_2S_2O_3$ aq. (25 mL). The resulting mixture was extracted with EtOAc (25 mL × 3). The extracts were washed with brine, dried over Na_2SO_4, filtered, and concentrated in *vacuo*. Purification of the residue by silica-gel column chromatography (3/1 *n*-hexane/EtOAc) gave **20** as a mixture of inseparable diastereomers (36.0 mg, 83.2 μmol, 80% yield, 13/1 dr). White solid; R_f 0.47 (3/1 *n*-hexane/EtOAc); ^{1}H-NMR (500 MHz, $CDCl_3$, TMS) δ 5.46 (13/14H, d, J = 7.7 Hz, NH), 5.38-5.30 (1/14H, d, J = 6.6 Hz, NH), 5.02 (1/14H, d, $J_{1,2}$ = 2.6 Hz, H-1), 4.99 (13/14H, d, $J_{1,2}$ = 5.2 Hz, H-1), 4.61-4.48 (1H, m, H-α), 4.30 (1/14H, m, H-5), 4.24 (13/14H, q, $J_{5,6}$ = 6.9 Hz, H-5), 3.76 (3H, s, CO_2Me), 3.65-3.58 (1H, m, H-1'), 3.20-2.95 (3H, m, H-β & H-2), 2.87-2.69 (1/7H, m, H-3), 2.74 (13/14H, dd, $J_{3a,3b}$ = 16.1 Hz, $J_{2,3a}$ = 4.6 Hz, H-3a), 2.58 (13/14H, dd, $J_{3a,3b}$ = 16.1 Hz, $J_{2,3b}$ = 10.3 Hz, H-3b), 1.65-1.53 (4H, m, H-2'), 1.45 (9H, s, Boc), 1.28 (39/14H, d, $J_{5,6}$ = 6.9 Hz, H-6), 1.27 (3/14H, m, H-6), 0.99-0.82 (3/7H, m, H-3'), 0.93 (39/7H, t, $J_{2',3'}$ = 7.5 Hz, H-3'); ^{13}C-NMR (125 MHz, $CDCl_3$), major isomer; δ 208.9, 171.1, 155.1, 99.9, 80.2, 79.9, 71.1, 53.6, 52.6, 44.6, 41.0, 33.7, 28.3, 26.7, 25.5, 14.8, 9.9, 9.3; HRMS (ESI-TOF) *m/z* 456.2020 (456.2032 calcd. for $C_{20}H_{35}NO_7NaS$, $[M+Na]^+$).

Compound 21

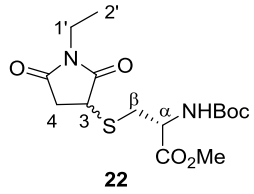


21

To a solution of **17** (24.5 mg, 104 μmol) in DMF (2.12 mL)/phosphate buffer (0.1 M, pH 7.4, 7.28 mL) was added **13** in DMF (1.00 mL, 104 μmol) at 37 °C. After being stirred at 37 ºC for 1 min, the reaction mixture was quenched with 2-iodoacetamide (96.2 mg, 520 μmol). The resulting mixture was stirred at 37 °C for 10 min, and then poured into sat. $Na_2S_2O_3$ aq. (25 mL). The resulting mixture was extracted with EtOAc (25 mL × 3). The extracts were washed with brine, dried over Na_2SO_4, filtered, and concentrated in *vacuo*. Purification of the residue by silica-gel column chromatography (3/1 *n*-hexane/EtOAc) gave **21** as a mixture of diastereomers (30.2 mg, 69.7 μmol, 67% yield, 4/1 dr). Colorless syrup; R_f 0.47 (major isomer), 0.41 (minor isomer) (3/1 *n*-hexane/EtOAc); ^{1}H-NMR (500 MHz, $CDCl_3$, TMS) δ 5.43-5.37 (1/5H, m, NH),

5.30 (4/5H, d, J = 7.5 Hz, NH), 5.10 (1/5H, d, $J_{1,2}$ = 2.1 Hz, H-1), 4.75 (4/5H, d, $J_{1,2}$ = 7.7 Hz, H-1), 4.59-4.50 (1H, m, H-α), 4.09 (1/5H, q, $J_{5,6}$ = 6.9 Hz, H-5), 4.00 (4/5H, q, $J_{5,6}$ = 6.9 Hz, H-5), 3.76 (3/5H, s, CO_2Me), 3.75 (12/5H, s, CO_2Me), 3.65-3.59 (1H, m, H-1'), 3.39-3.33 (1/5H, m, H-2), 3.39-3.27 (4/5H, dd, $J_{\beta a,\beta b}$ = 13.8 Hz, $J_{\alpha,\beta a}$ = 4.3 Hz, H-βa), 3.26-3.18 (4/5H, m, H-2), 3.07 (2/5H, d, $J_{a,\beta}$ = 5.1 Hz, H-β), 2.95 (4/5H, dd, $J_{\beta a,\beta b}$ = 13.8 Hz, $J_{\alpha,\beta b}$ = 6.3 Hz, H-βb), 2.88 (4/5H, dd, $J_{3a,3b}$ = 16.6 Hz, $J_{2,3a}$ = 6.3 Hz, H-3a), 2.75 (2/5H, d, $J_{2,3}$ = 7.8 Hz, H-3), 2.29 (4/5H, dd, $J_{3a,3b}$ = 16.6 Hz, $J_{2,3b}$ = 11.5 Hz, H-3b), 1.68-1.51 (4H, m, H-2'), 1.45 (9H, s, Boc), 1.33 (3/5H, d, $J_{5,6}$ = 6.9 Hz, H-6), 1.31 (12/5H, d, $J_{5,6}$ = 6.9 Hz, H-6), 0.95 & 0.91 (each 12/5H, t, $J_{2',3'}$ = 7.4 Hz, H-3'), 0.95-0.85 (6/5H, m, H-3'); ^{13}C-NMR (125 MHz, $CDCl_3$), major isomer; δ 205.3, 171.4, 155.1, 103.4, 80.9, 80.2, 76.3, 53.0, 52.5, 45.1, 42.0, 34.7, 28.3, 26.7, 25.4, 15.4, 9.5, 9.3; HRMS (ESI-TOF) *m/z* 456.2034 (456.2032 calcd. for $C_{20}H_{35}NO_7NaS$, $[M+Na]^+$).

Compound 22

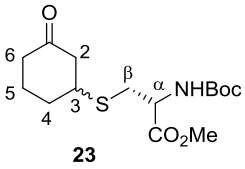


22

To a solution of **17** (24.5 mg, 104 μmol) in DMF (2.12 mL)/phosphate buffer (0.1 M, pH 7.4, 7.28 mL) was added **14** in DMF (1.00 mL, 104 μmol) at 37 °C. After being stirred at 37 ºC for 1 min, the reaction mixture was quenched with 2-iodoacetamide (96.2 mg, 520 μmol). The resulting mixture was stirred at 37 °C for 10 min, and then poured into sat. $Na_2S_2O_3$ aq. (25 mL). The resulting mixture was extracted with EtOAc (25 mL × 3). The extracts were washed with brine, dried over Na_2SO_4, filtered, and concentrated in *vacuo*. Purification of the residue by silica-gel column chromatography (1/1 *n*-hexane/EtOAc) gave **22** as a mixture of inseparable diastereomers (35.6 mg, 98.8 μmol, 95% yield, 7/5 dr). White solid; R_f 0.49 (1/1 *n*-hexane/EtOAc) ; ^{1}H-NMR (500 MHz, $CDCl_3$, TMS) δ 5.56 (5/12H, d, J = 7.8 Hz, NH), 5.35 (7/12H, d, J = 7.8 Hz, NH), 4.64 (1H, m, H-α), 3.91 (7/12H, m, H-3), 3.82 (5/12H, m, H-3), 3.79 (3H, s, CO_2Me), 3.57 (2H, q, $J_{1',2'}$ = 7.2 Hz, H-1'), 3.57 (7/12H, m, H-βa), 3.42 (5/12H, dd, $J_{\beta a,\beta b}$ = 14.1 Hz, $J_{\alpha,\beta a}$ = 5.4 Hz, H-βa), 3.17 (5/12H, dd, $J_{\beta a,\beta b}$ = 13.8 Hz, $J_{\alpha,\beta b}$ = 5.1 Hz, H-βb), 3.11 (7/12H, dd, $J_{4a,4b}$ = 18.9 Hz, $J_{3,4a}$ = 9.3 Hz, H-4a), 3.10 (5/12H, dd, $J_{4a,4b}$ = 18.9 Hz, $J_{3,4a}$ = 9.0 Hz, H-4a), 2.98 (7/12H, dd, $J_{\beta a,\beta b}$ = 13.8 Hz, $J_{\alpha,\beta a}$ = 7.8 Hz, H-βb), 2.46 (5/12H, dd, $J_{4a,4b}$ = 18.9 Hz, $J_{3,4b}$ = 3.9 Hz, H-4b), 2.43 (7/12H, dd, $J_{4a,4b}$ = 18.9 Hz, $J_{3,4b}$ = 3.9 Hz, H-4b), 1.46 (9H,

s, Boc), 1.18 (3H, t, $J_{1',2'}$ = 7.2 Hz, H-2'); ^{13}C-NMR (125 MHz, $CDCl_3$); δ 176.4, 176.2, 174.2, 174.1, 171.3, 155.3, 155.1, 80.3, 53.5, 52.8, 52.7, 52.5, 39.1, 38.4, 36.0, 35.6, 34.5, 34.1, 34.0, 28.2, 12.8; HRMS (ESI-TOF) *m/z* 383.1250 (383.1253 calcd. for $C_{15}H_{24}N_2O_6NaS$, $[M+Na]^+$).

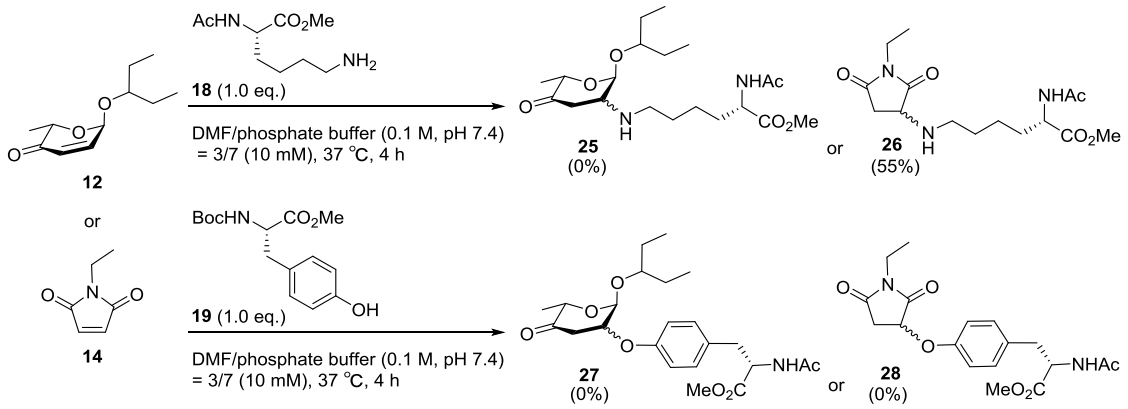
Compound 23



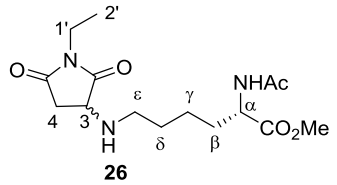
23

To a solution of **17** (24.5 mg, 104 μmol) in DMF (2.12 mL)/phosphate buffer (0.1 M, pH 7.4, 7.28 mL) was added **15** in DMF (1.00 mL, 104 μmol) at 37 ℃. After being stirred at 37 ºC for 1 min, the reaction mixture was quenched with 2-iodoacetamide (96.2 mg, 520 μmol). The resulting mixture was stirred at 37 °C for 10 min, and then poured into sat. $Na_2S_2O_3$ aq. (25 mL). The resulting mixture was extracted with EtOAc (25 mL × 3). The extracts were washed with brine, dried over Na_2SO_4, filtered, and concentrated in *vacuo*. Purification of the residue by silica-gel column chromatography (3/2 *n*-hexane/EtOAc) gave **23** as a mixture of inseparable diastereomers (15.5 mg, 46.8 μmol, 45% yield, 1/1 dr). White solid; R_f 0.47 (3/2 *n*-hexane/EtOAc); ^{1}H-NMR (500 MHz, $CDCl_3$, TMS) δ 5.39-5.28 (1H, m, NH), 4.60-4.51 (1H, m, H-α), 3.77 (3H, s, CO_2Me), 3.15-3.06 (1H, m, H-3), 3.06-2.94 (2H, m, H-β), 2.73-2.66 (1H, m, H-2a), 2.38-2.26 (3H, m, H-2b & H-6), 2.17-2.08 (2H, m, H-5), 1.75-1.67 (2H, m, H-4), 1.26 (9H, s, Boc); ^{13}C-NMR (125 MHz, $CDCl_3$); δ 208.2, 171.2, 155.0, 80.2, 53.3, 53.2, 52.6×2, 47.8, 43.1×2, 40.8×2, 32.8, 32.6, 31.4×2, 28.2, 23.9; HRMS (ESI-TOF) *m/z* 354.1357 (354.1351 calcd. for $C_{15}H_{25}NO_5NaS$, $[M+Na]^+$).

Scheme S3. Michael reactions of **12** and **14** with **18** or **19.**



Compound 26

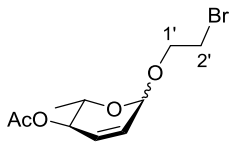


26

To a solution of **18** (24.5 mg, 104 μmol) in DMF (2.12 mL)/phosphate buffer (0.1 M, pH 7.4, 7.28 mL) was added **14** in DMF (1.00 mL, 104 μmol) at 37 ℃. After being stirred at 37 ºC for 4 h, the reaction mixture was quenched with H_2O (25 mL). The resulting mixture was extracted with EtOAc (25 mL × 3). The extracts were washed with brine, dried over Na_2SO_4, filtered, and concentrated in *vacuo*. Purification of the residue by silica-gel column chromatography (6/1 CH_3Cl/MeOH) gave **26** as a mixture of inseparable diastereomers (18.7 mg, 57.2 μmol, 55% yield, 1/1 dr). Colorless syrup; R_f 0.47 (6/1 CH_3Cl/MeOH); ^{1}H-NMR (500 MHz, $CDCl_3$, TMS) δ 6.09 (1H, d, J = 6.6 Hz, NH), 4.62 (1H, m, H-α), 3.75 (3H, s, CO_2Me), 3.75-3.72 (1H, m, H-3), 3.56 (2H, q, $J_{1',2'}$ = 7.2 Hz, H-1'), 2.94-2.88 (1H, dd, $J_{4a,4b}$ = 17.8 Hz, $J_{3,4a}$ = 8.0 Hz, H-4a), 2.73-2.55 (2H, m, H-ε), 2.53-2.46 (1H, dd, $J_{4a,4b}$ = 17.8 Hz, $J_{3,4b}$ = 3.8 Hz, H-4b), 2.03 (3H, s, Ac), 1.90-1.64 (2H, m, H-β), 1.57-1.48 (2H, m, H-δ), 1.44-1.32 (2H, m, H-γ), 1.17 (3H, t, $J_{1',2'}$ = 7.2 Hz, H-2'); ^{13}C-NMR (125 MHz, $CDCl_3$); δ 177.7, 175.1, 173.0, 169.8, 56.3×2, 52.4 2, 51.9, 47.3, 47.2, 36.2×2, 33.8, 32.3×2, 29.4×2, 23.2, 22.7×2, 12.9; HRMS (ESI-TOF) *m/z* 328.1861 (328.1872 calcd. for $C_{15}H_{26}N_3O_5$, $[M+H]^+$).

Synthesis of coumarin-α-aculoside hybrid 29.

Compound 31

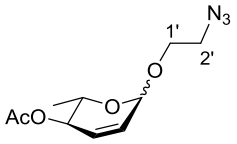


31

To a solution of **30** (1.50 g, 7.00 mmol) and 2-bromoethanol (1.00 mL, 14.0 mmol) in dry CH_2Cl_2 (60.0 mL) was added (±)-10-CSA (325 mg, 1.40 mmol) at 0 ºC. After being stirred at room temperature for 2.5 h, the reaction mixture was quenched with sat. $NaHCO_3$ aq. (60 mL). The resulting mixture was extracted with $CHCl_3$ (60 mL × 3). The extracts were washed with brine, dried over Na_2SO_4, filtered, and concentrated in *vacuo*. Purification of the residue by

silica-gel column chromatography (10/1 toluene/EtOAc) gave **31** as a mixture of inseparable diastereomers (1.11 g, 3.85 mmol, 57% yield, α/β = 8/1). Pale brown syrup; R_f 0.49 (10/1 toluene/EtOAc); ^{1}H-NMR (500 MHz, $CDCl_3$, TMS) δ 5.97-5.80 (2H, m, H-2 & H-3), 5.07 (8/9H, m, H-4), 5.03-5.01 (8/9H, m, H-1), 5.07-5.01 (2/9H, m, H-1 & H-4), 4.10-4.00 (8/9H, m, H-5), 4.10-3.83 (2H, m, H-1'), 3.90-3.83 (1/9H, m, H-5), 3.52 (2H, m, H-2'), 2.09 (3H, s, Ac), 1.32 (1/3H, d, $J_{5,6}$ = 6.3 Hz, H-6), 1.23 (24/9H, d, $J_{5,6}$ = 6.3 Hz, H-6); ^{13}C-NMR (125 MHz, $CDCl_3$), α isomer; δ 170.5, 130.1, 127.2, 94.7, 70.6, 68.5, 65.1, 30.8, 21.0, 17.9; HRMS (ESI-TOF) *m/z* 301.0052 (301.0051 calcd. for $C_{10}H_{!5}O_4NaBr$, $[M+Na]^+$).

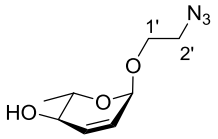
Compound 32



32

To a solution of **31** (1.36 g, 4.87 mmol) in dry DMF (33.0 mL) was added NaN_3 (1.58 g, 24.4 mmol) at room temperature. After being stirred at room temperature for 24 h, the reaction mixture was quenched with H_2O (30 mL). The resulting mixture was extracted with EtOAc (30 mL × 3). The extracts were washed with brine, dried over Na_2SO_4, filtered, and concentrated in *vacuo*. Purification of the residue by silica-gel column chromatography (4/1 *n*-hexane/EtOAc) gave **32** as a mixture of inseparable diastereomers (1.06 g, 4.38 mmol, 90% yield, α/β = 6/1). Colorless syrup; R_f 0.46 (4/1 *n*-hexane/EtOAc); ^{1}H-NMR (300 MHz, $CDCl_3$, TMS) δ 5.98-5.78 (2H, m, H-2 & H-3), 5.10-5.03 (6/7H, m, H-4), 5.02-5.00 (6/7H, m, H-1), 5.10-5.02 (2/7H, m, H-1 & H-4), 4.03-3.90 (6/7H, m, H-5), 4.00-3.85 (1/7H, m, H-5), 4.03-3.65 (2H, m, H-1'), 3.53-3.35 (2H, m, H-2'), 2.10 (18/7H, s, Ac), 2.09 (3/7H, s, Ac), 1.33 (3/7H, d, $J_{5,6}$ = 6.6 Hz, H-6), 1.24 (18/7H, d, $J_{5,6}$ = 6.6 Hz, H-6); ^{13}C-NMR (125 MHz, $CDCl_3$), α isomer; δ 170.5, 130.0, 127.2, 94.7, 70.7, 67.1, 65.0, 50.8, 21.0, 17.9; HRMS (ESI-TOF) *m/z* 242.1132 (242.1141 calcd. for $C_{10}H_{16}N_3O_4$, $[M+H]^+$).

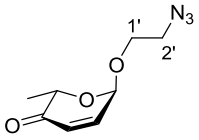
Compound 33



33

To a solution of **32** (44.0 mg, 182 μmol) in MeOH (850 μL) was added 0.2 M NaOMe in MeOH (910 μL, 182 μmol) at room temperature. After being stirred at room temperature for 2.5 h, the reaction mixture was quenched with Amberlite® IR 120 H^+ form. The resulting suspension was filtered, and then the filtrate was concentrated in *vacuo*. Purification of the residue by silica-gel column chromatography (1/1 *n*-hexane/EtOAc) gave **33** (26.9 mg, 135 μmol, 74% yield). Colorless syrup; R_f 0.53 (1/1 *n*-hexane/EtOAc); $[\alpha]^{27}_D$ −14.5° (*c* 1.0, $CHCl_3$); ^{1}H-NMR (500 MHz, $CDCl_3$, TMS) δ 5.98-5.93 (1H, m, H-2), 5.80-5.76 (1H, m, H-3), 5.00-4.97 (1H, m, H-1), 3.90-3.82 (1H, m, H-4), 3.77-3.70 (1H, dq, $J_{4,5}$ = 8.6 Hz, $J_{5,6}$ = 6.3 Hz, H-5), 3.98-3.65 (2H, m, H-1'), 3.49-3.37 (2H, m, H-2'), 1.45 (1H, d, J = 8.3 Hz, OH), 1.34 (3H, d, $J_{5,6}$ = 6.3 Hz, H-6); ^{13}C-NMR (125 MHz, $CDCl_3$); δ 133.7, 126.1, 94.6, 69.5, 68.1, 67.0, 50.8, 17.9; HRMS (ESI-TOF) *m/z* 200.1038 (200.1035 calcd. for $C_8H_{14}N_3O_3$, $[M+H]^+$).

Compound 34

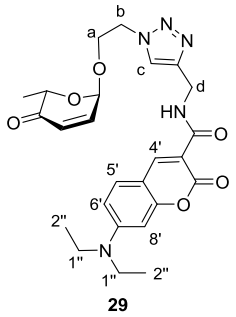


34

To a solution of **33** (48.0 mg, 241 μmol) in dry CH_2Cl_2 (1.44 mL) was added MnO_2 (628 mg, 7.23 mmol) at room temperature. After being stirred at room temperature for 19 h, the mixture was filtered through a pad of Celite. The filtrate was concentrated in *vacuo*. Purification of the residue by silica-gel column chromatography (2/1 *n*-hexane/EtOAc) gave **34** (26.2 mg, 133 μmol, 55% yield). Colorless syrup; R_f 0.61 (2/1 *n*-hexane/EtOAc); $[\alpha]^{23}_D$ +14.5° (*c* 1.0, $CHCl_3$); ^{1}H-NMR (300 MHz, $CDCl_3$, TMS) δ 6.85 (1H, dd, $J_{2,3}$ = 10.2 Hz, $J_{1,2}$ = 3.6 Hz, H-2), 6.11 (1H, d, $J_{2,3}$ = 10.2 Hz, H-3), 5.24 (1H, d, $J_{1,2}$ = 3.6 Hz, H-1), 4.59 (1H, q, $J_{5,6}$ = 6.6 Hz, H-5), 4.18-3.72 (2H, m, H-1'), 3.58-3.40 (2H, m, H-2'), 1.41 (3H, d, $J_{5,6}$ = 6.6 Hz, H-6); ^{13}C-NMR (125 MHz, $CDCl_3$); δ 196.6, 142.7, 127.5, 93.4, 70.5, 67.9, 50.7, 15.2; HRMS (ESI-TOF) *m/z*

198.0889 (198.0879 calcd. for $C_8H_{12}N_3O_3$, $[M+H]^+$).

Compound 29

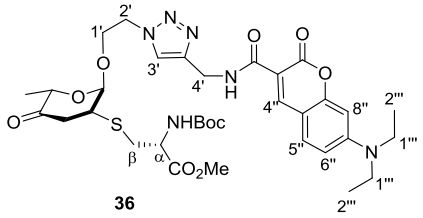


29

To a solution of **34** (9.0 mg, 45.6 μmol) and **35** (11.3 mg, 38.0 μmol) in *t*-BuOH/H_2O/DMF (5/4/3, 1.36 mL) were added sodium ascorbate (15.9 mg, 7.98 μmol), $CuSO_4 \cdot 5H_2O$ (1.98 mg, 7.98 μmol) and triazole ligand (1.97 mg, 3.80 μmol) at room temperature. After being stirred at room temperature for 6 h, the reaction mixture was quenched with H_2O (5 mL). The resulting mixture was extracted with $CHCl_3$ (10 mL × 3). The extracts were washed with brine, dried over Na_2SO_4, filtered, and concentrated in *vacuo*. Purification of the residue by silica-gel column chromatography (9/1 $CHCl_3$/MeOH) gave **29** (10.3 mg, 20.9 μmol, 55% yield). Yellow solid; R_f 0.60 (9/1 $CHCl_3$/MeOH); $[\alpha]^{27}_D$ +58.1° (*c* 0.25, $CHCl_3$); mp 148.0-149.0 °C; ^{1}H-NMR (300 MHz, $CDCl_3$, TMS) δ 9.23 (1H, m, NH), 8.68 (1H, s, H-8'), 7.67 (1H, s, H-c), 7.43 (1H, d, $J_{5',6'}$ = 9.0 Hz, H-6'), 6.76 (1H, dd, $J_{2,3}$ = 10.2 Hz, $J_{1,2}$ = 3.3, H-2), 6.65 (1H, dd, $J_{5',6'}$ = 9.0 Hz, $J_{4',5'}$ = 2.4, H-5'), 6.50 (1H, d, $J_{4',5'}$ = 2.4 Hz, H-4'), 6.05 (1H, d, $J_{2,3}$ = 10.2 Hz, H-3), 5.11 (1H, d, $J_{1,2}$ = 3.3 Hz, H-1), 4.72 (2H. d, J = 5.7 Hz, H-d), 4.65-4.49 (2H, m, H-b), 4.35 (1H, q, $J_{5,6}$ = 6.9 Hz, H-5), 4.28-3.93 (2H, m, H-a), 3.46 (4H, q, $J_{1'',2''}$ = 7.2 Hz, H-1''), 1.33 (3H, d, $J_{5,6}$ = 6.9 Hz, H-6), 1.24 (6H, t, $J_{1'',2''}$ = 7.2 Hz, H-2''); ^{13}C-NMR (125 MHz, $CDCl_3$); δ 196.4, 163.4, 162.6, 157.7, 152.7, 148.2, 145.4, 142.5, 131.2, 127.6, 123.3, 110.0, 109.8, 108.3, 96.6, 93.4, 70.5, 67.3, 50.1, 45.1, 35.3, 15.2, 12.4; HRMS (ESI-TOF) *m/z* 496.2209 (496.2196 calcd. for $C_{25}H_{30}N_5O_6$, $[M+H]^+$).

Michael reaction of 29 with 17.

Compound 36



36

To a solution of **17** (11.4 mg, 23.4 μmol) in DMF (1.54 mL)/phosphate buffer (0.1 M, pH 7.4, 700 μL) was added **29** in DMF (100 μL, 23.4 μmol) at 37 °C. After being stirred at 37 ºC for 1 min, the reaction mixture was quenched with 2-iodoacetamide (21.6 mg, 117 μmol). The resulting mixture was stirred at 37 °C for 10 min, and then poured into sat. $Na_2S_2O_3$ aq. (5 mL). The resulting mixture was extracted with $CHCl_3$ (10 mL × 3). The extracts were washed with brine, dried over Na_2SO_4, filtered, and concentrated in *vacuo*. Purification of the residue by silica-gel column chromatography (4/3 toluene/acetone) gave **36** as a single diastereomer (11.9 mg, 16.4 μmol, 70% yield). Yellow solid; R_f 0.42 (4/3 toluene/acetone); $[\alpha]^{27}_{D}$ −63.6° (*c* 0.58, $CHCl_3$); mp 59.0-60.5 °C; ^{1}H-NMR (500 MHz, $CDCl_3$, TMS) δ 9.27-9.23 (1H, m, NH), 8.68H, s, H-8''), 7.77 (1H, s, H-3'), 7.45 (1H, d, $J_{5'',6''}$ = 8.9 Hz, H-6''), 6.65 (1H, dd, $J_{5'',6''}$ = 8.9 Hz, $J_{4'',5''}$ = 2.1 Hz, H-5''), 6.49 (1H, d, $J_{4'',5''}$ = 2.1 Hz, H-4''), 5.71 (1H, d, J = 8.6 Hz, NHBoc), 4.90 (1H, d, $J_{1,2}$ = 4.6 Hz, H-1), 4.72 (2H, d, J = 5.8 Hz, H-4'), 4.64-4.50 (3H, m, H-α & H-2'), 4.22-3.89 (3H, m, H-1' & H-5), 3.75 (3H, s, CO_2Me), 3.45 (4H, q, $J_{1''',2'''}$ = 7.2 Hz, H-1'''), 3.18-3.10 (1H, m, H-2), 3.06-2.92 (2H, m, H-β), 2.67 (1H, dd, $J_{3a,3b}$ = 16.3 Hz, $J_{2,3a}$ = 4.3 Hz, H-3a), 2.51 (1H, dd, $J_{3a,3b}$ = 16.3 Hz, $J_{2,3b}$ = 10.9 Hz, H-3b), 1.42 (9H, s, Boc), 1.27-1.18 (9H, m, H-2''' & H-6); ^{13}C-NMR (125 MHz, $CDCl_3$); δ 207.7, 171.3, 163.4, 162.6, 157.7, 155.3, 152.7, 148.3, 145.4, 131.2, 123.5, 110.0, 108.3, 102.0, 96.6, 80.2, 71.1, 66.2, 53.3, 52.7, 49.9, 45.1, 43.5, 40.4, 35.5, 33.8, 29.7, 28.3, 14.8, 12.4; HRMS (ESI-TOF) *m/z* 731.3073 (731.3074 calcd. for $C_{34}H_{47}N_6O_{10}S$, $[M+H]^+$).

Cell culture.

MCF-7 human breast cancer cells were grown at 37 °C in 5% CO_2 in air in DMEM medium supplemented with phenol red, L-glutamine (2 mM), penicillin (100 Units/mL), kanamycin (100 μg/mL) and 10% Fetal bovine serum (FBS). Sarcoma 180 solid tumor cells were grown at 37 °C in 5% CO_2 in air in RPMI medium 1640 supplemented with phenol red, L-glutamine (2 mM), penicillin (100 Units/mL), kanamycin (100 μg/mL) and 5% FBS.

MCF-7 and Sarcoma 180 were provided by the RIKEN BRC through the National Bio-Resource Project of the MEXT, Japan. FBS was purchased from MP Biomedicals.

Cell cytotoxicity assay.

MCF-7 or Sarcoma 180 cells were seeded at 5.0×10^3 cells/well in 96-well in 10% FBS DMEM or 5% FBS RPMI, respectively. After 24 h, samples were incubated with the variable concentrations of compounds. Compounds were diluted with DMSO at 400-fold the desired final test concentrations. Cells were then kept for 24 h at 37 °C and in 5% CO_2 in air, and then MTT or WST reagent was added to each well and cells were incubated for up to 3 additional hours at 37 °C. The absorbance at a single wavelength of 540 or 450 nm was read on a plate reader SAFIRE (TECAN).

MALDI-TOF MS analysis.

To a solution of **29** (150 μM) in phosphate buffer (0.1 M, pH 7.4, 667 μL) containing 8.6% DMF was added BSA (30 μM) in phosphate buffer (0.1 M, pH 7.4, 333 μL) containing 8.6% DMF at 37 °C and the reaction proceeded for 1 hour at 37 °C. The reaction mixture was purified using Amicon® Ultra 30K device and the resulting solution was lyophilized. The residue was diluted with H_2O (5.0 μL), and the resulting solution was mixed with a matrix solution (5.0 μL) of 3,5-dimethoxy 4-hydroxycinnamic acid (in 50:50 MeCN/H_2O containing 0.1% TFA). Analyses by MALDI TOF MS were performed in the positive ion mode on a Ultra flex (Bruker).

Fluorescence microscopic analysis.

MCF-7 cells were cultured on ϕ18 mm micro cover glass (Matsunami Glass Industrial, Ltd.) in 6-well plate (1.5×10^5 cells/well) in 10% FBS DMEM. After 24 h, the cells were incubated with the media containing compound **29** or **35** (final concentration of 33 μM) for 30 min at 37 °C and in 5% CO_2 in air. Compounds were diluted with DMSO at 200-fold the desired final test concentration. For the 2-iodoacetamide treated sample, the cells were incubated with the media containing 2-iodoacetamide (final concentration of 10 mM) for 1 h at 37 °C before compound **35** was incubated. Cells were then washed five times with phosphate buffered saline (PBS) and fixed with 4% paraformaldehyde phosphate buffer solution for 30 min at room temperature. After removing paraformaldehyde, cells were washed three times with PBS. The cells were observed by inverted fluorescence microscope (EVOS FL Cell Imaging System; Life

Technologies).

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2) (a) K. Sasaki, S. Matsumura and K. Toshima, *Tetrahedron Lett.*, 2007, **48**, 6982. (b) S. Kusumi, S. Tomono, S. Okuzawa, E. Kaneko, T. Ueda, K. Sasaki, D. Takahashi and K. Toshima, *J. Am. Chem. Soc*., 2013, **135**, 15909.

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4) R. F. Luduena and M. C. Roach, *Biochemistry*, 1981, **20**, 4437.

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

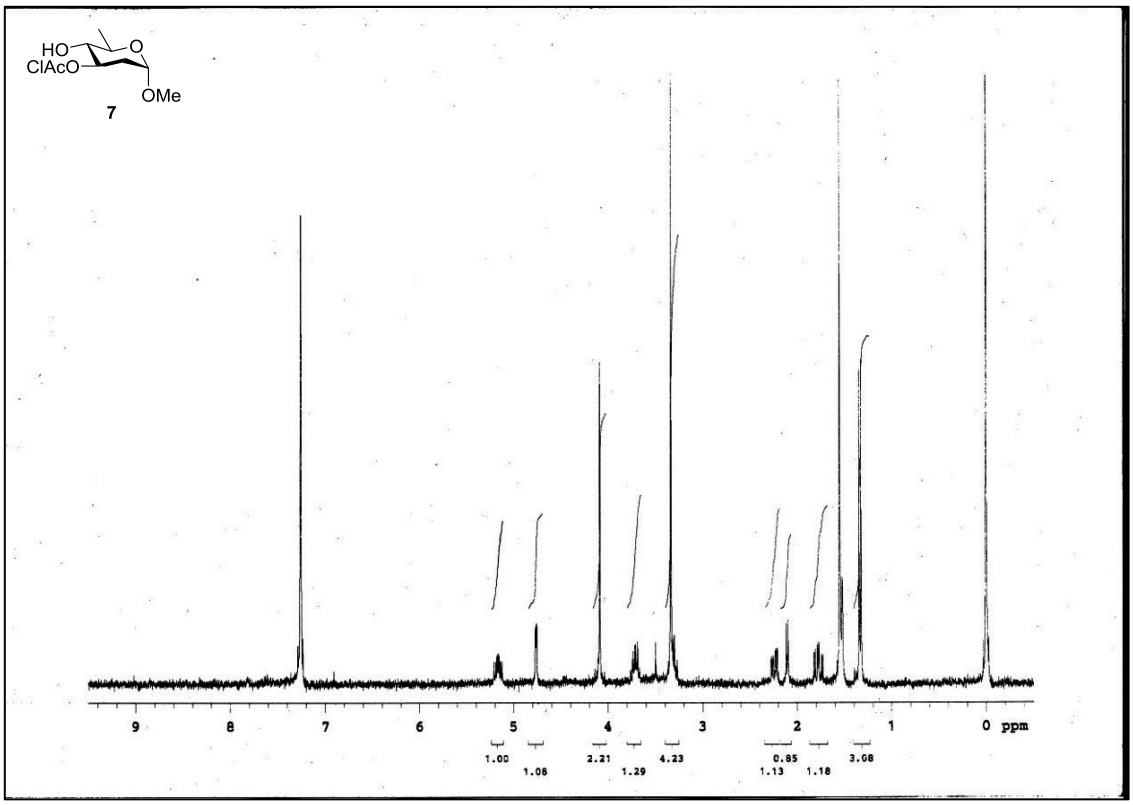

Figure S1 ^{1}H-NMR spectrum of **7**

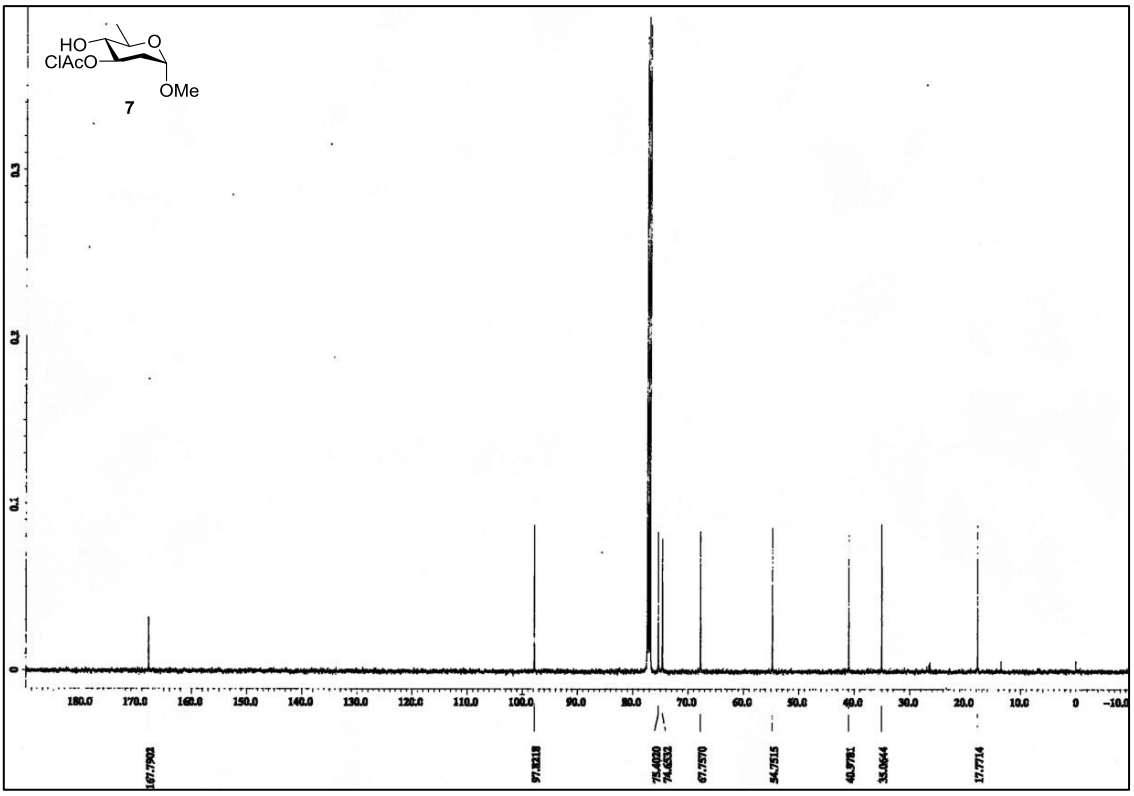

Figure S2 ^{13}C-NMR spectrum of **7**

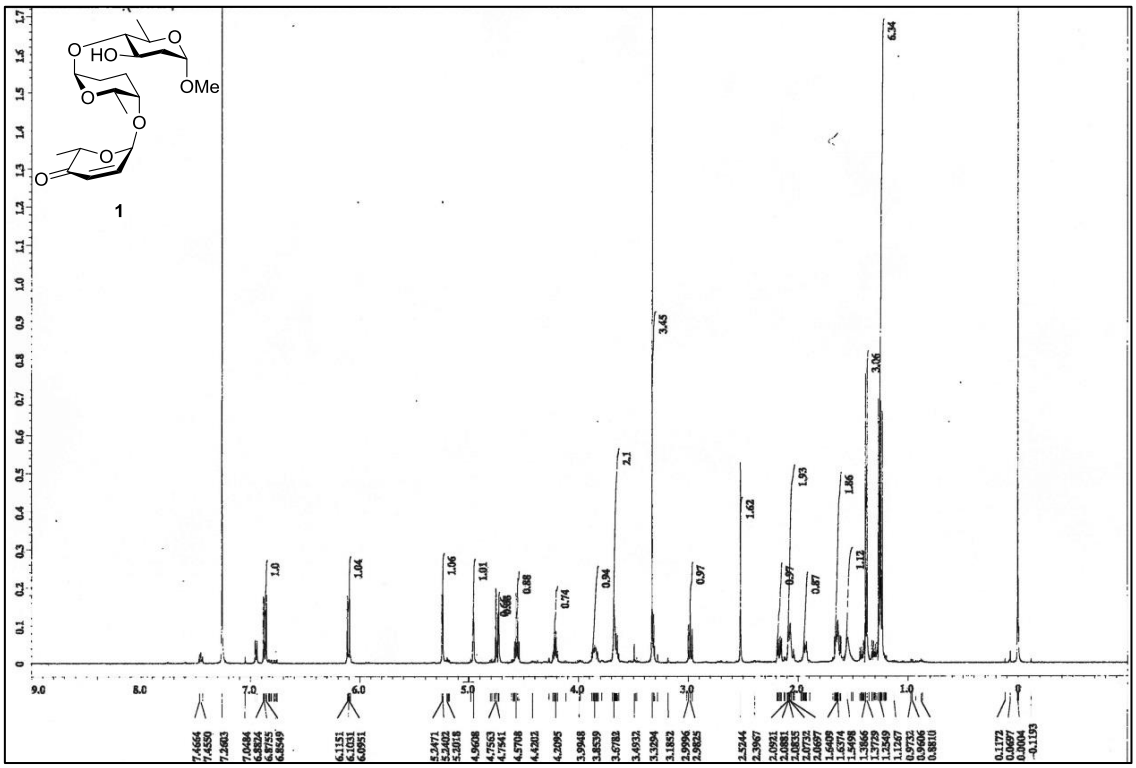

Figure S3 ^{1}H-NMR spectrum of **1**

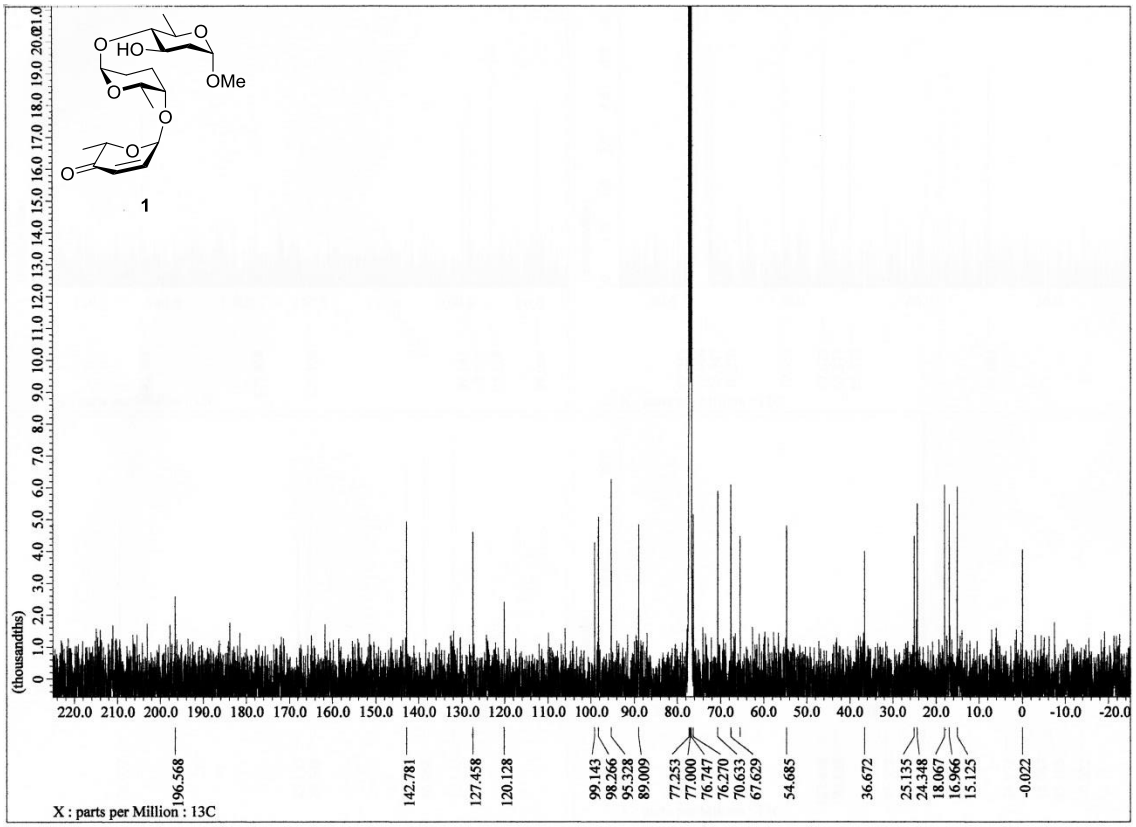

Figure S4 ^{13}C-NMR spectrum of **1**

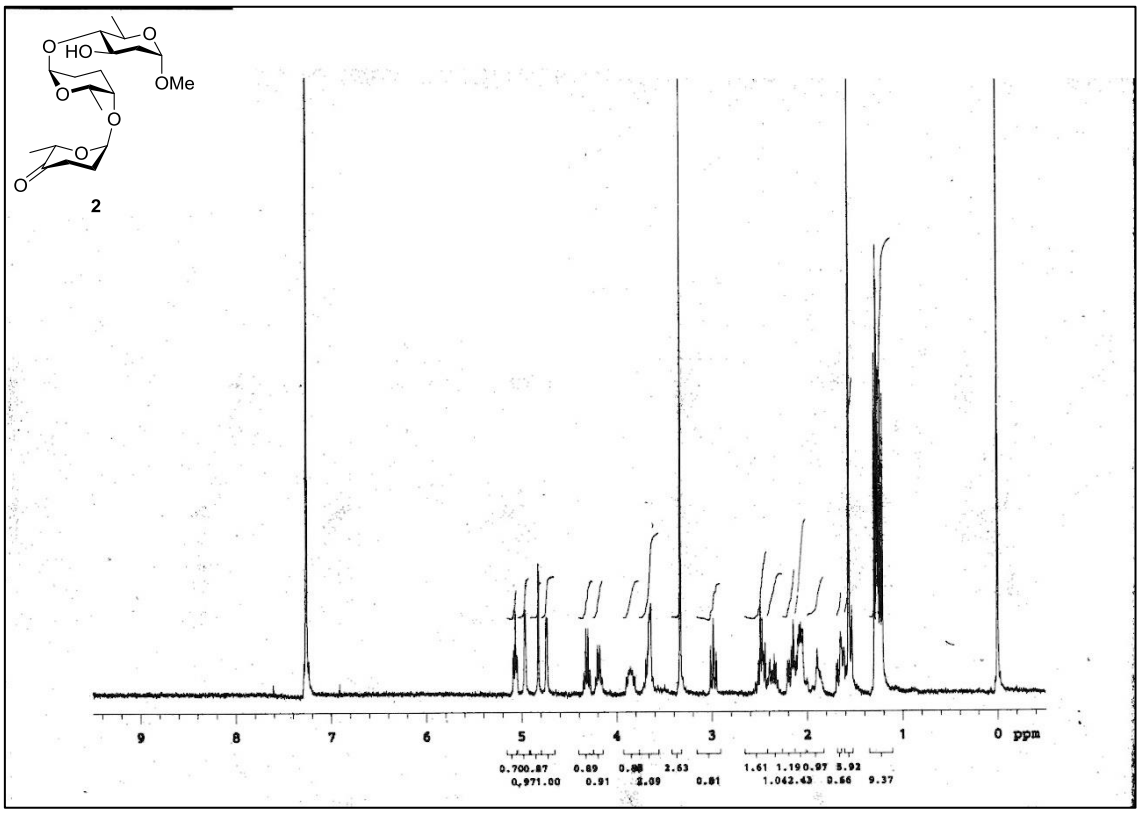

Figure S9 ^{1}H-NMR spectrum of **2**

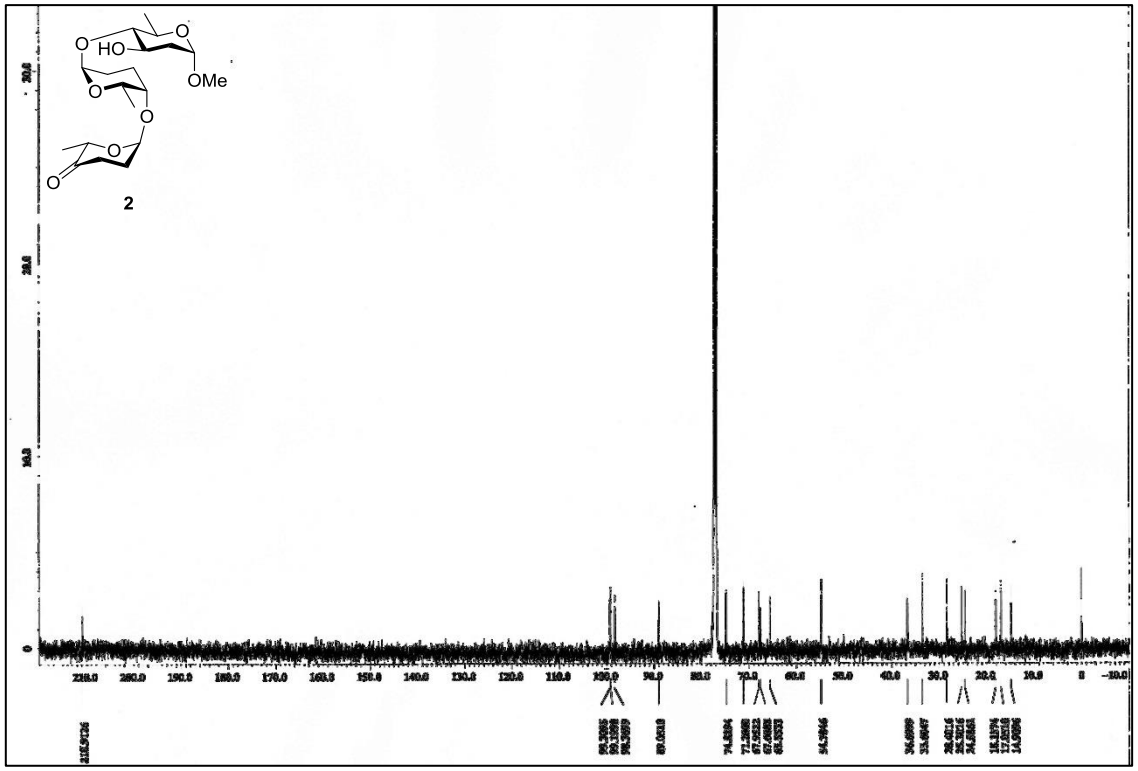

Figure S10 ^{13}C-NMR spectrum of **2**

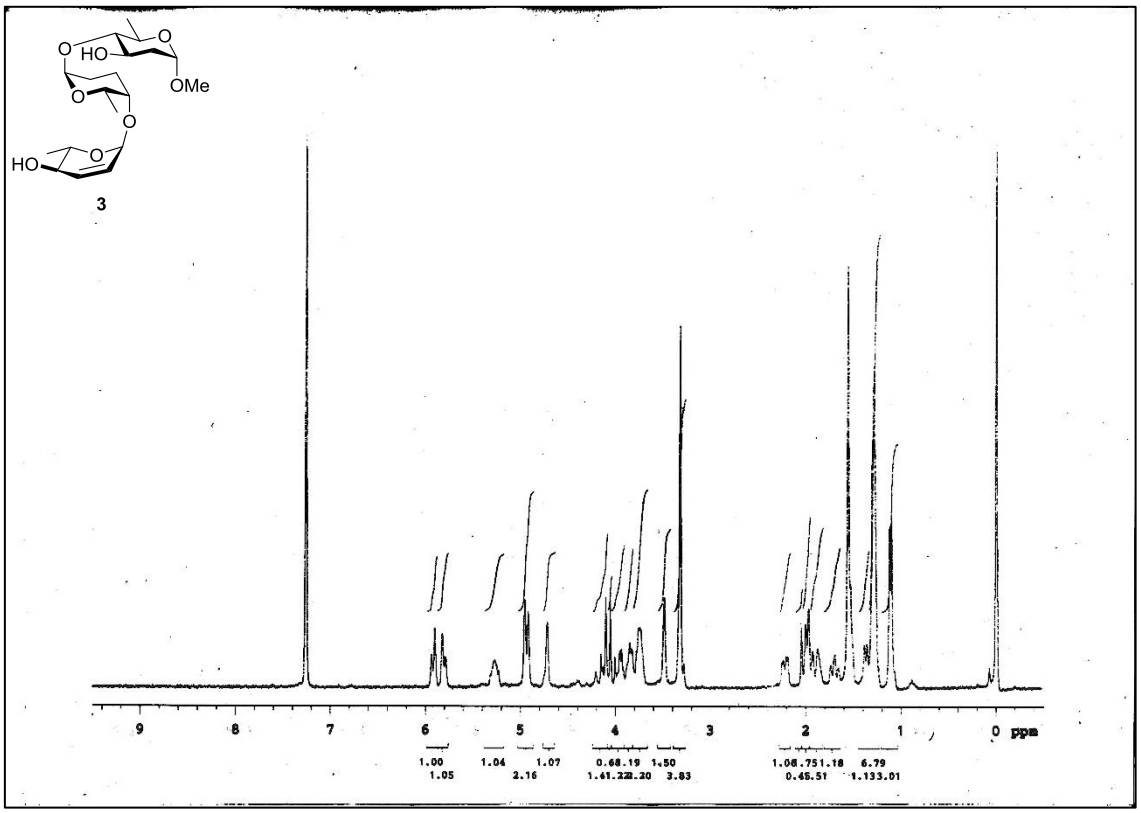

Figure S5 ^{1}H-NMR spectrum of **3**

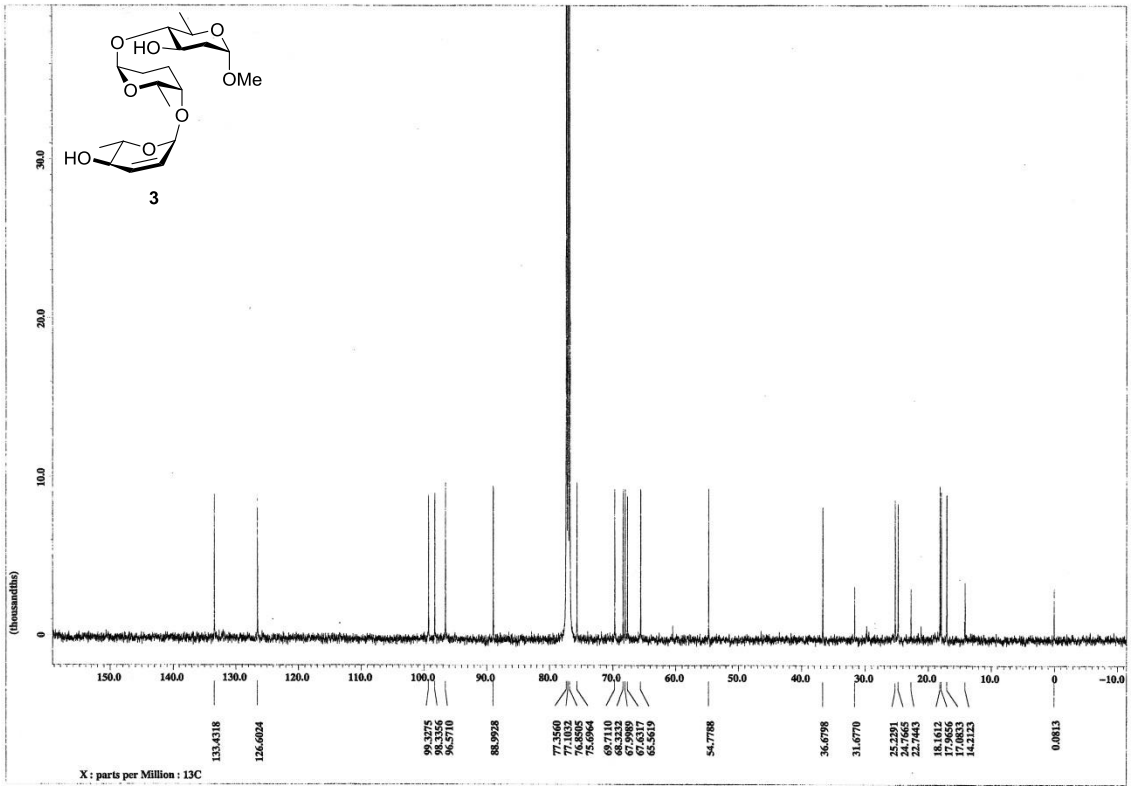

Figure S6 ^{13}C-NMR spectrum of **3**

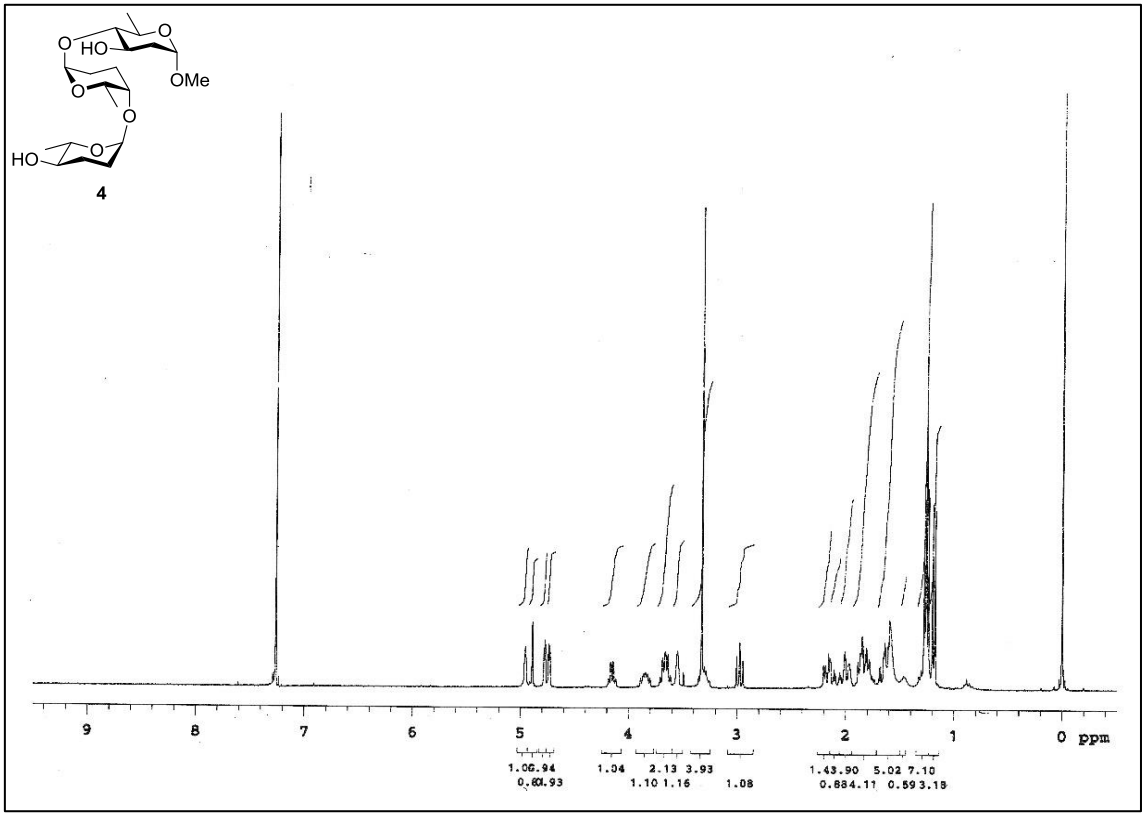

Figure S7 ^{1}H-NMR spectrum of **4**

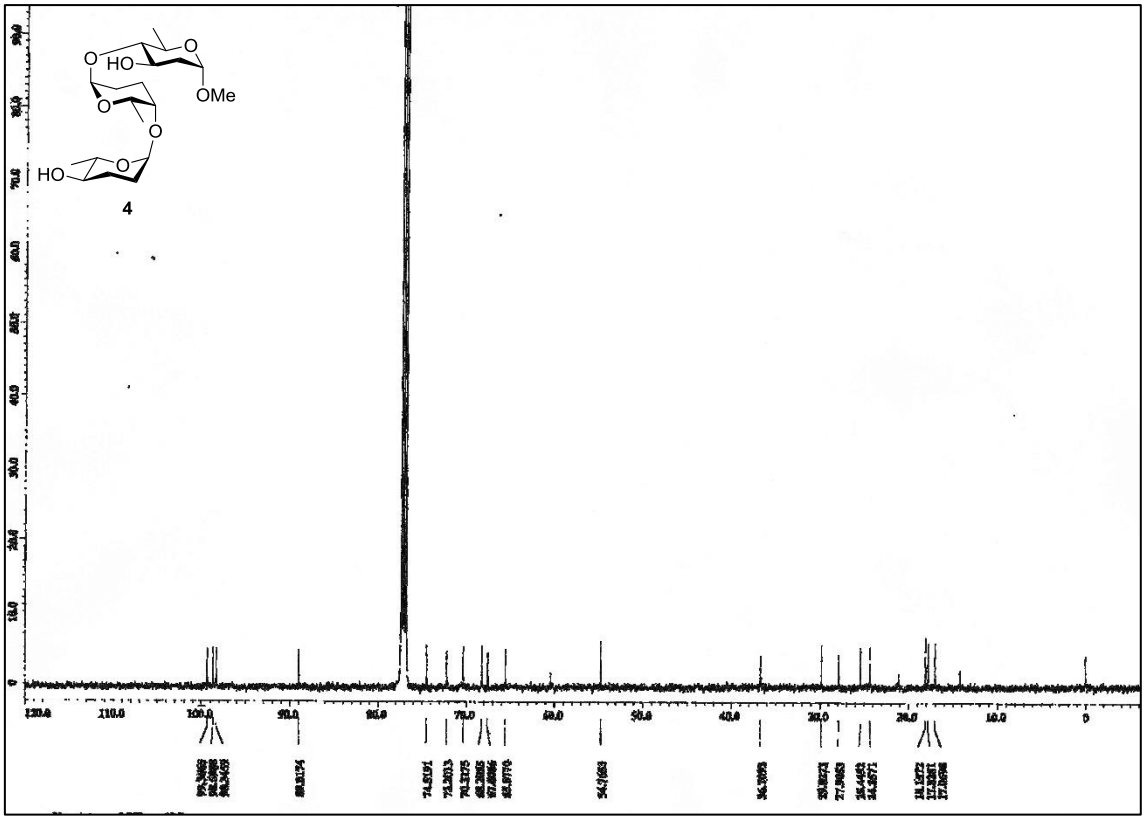

Figure S8 ^{13}C-NMR spectrum of **4**

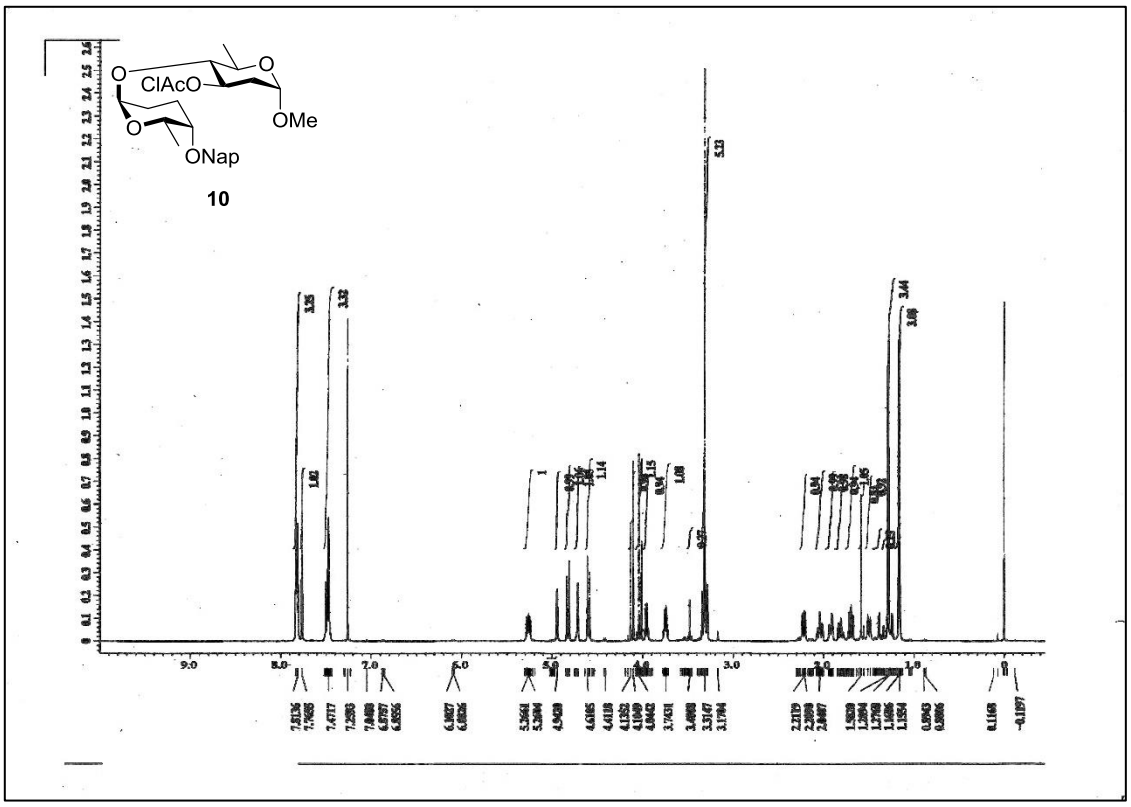

Figure S11 ^{1}H-NMR spectrum of **10**

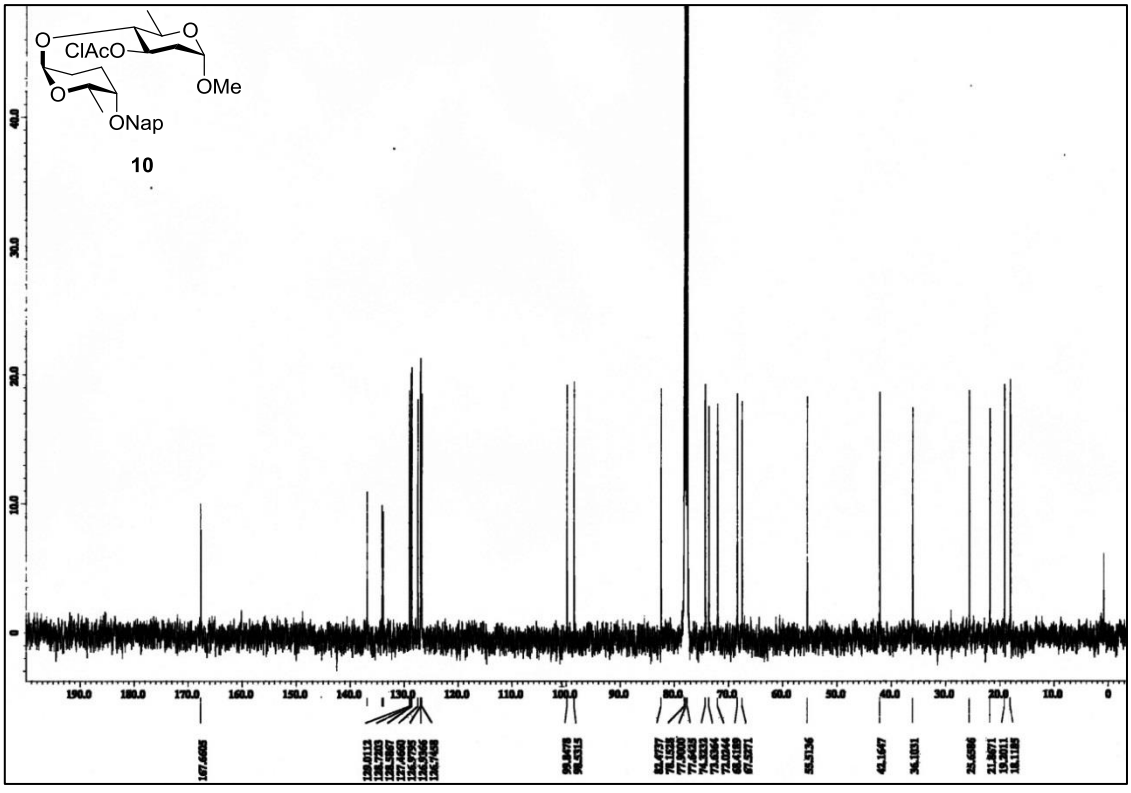

Figure S12 ^{13}C-NMR spectrum of **10**

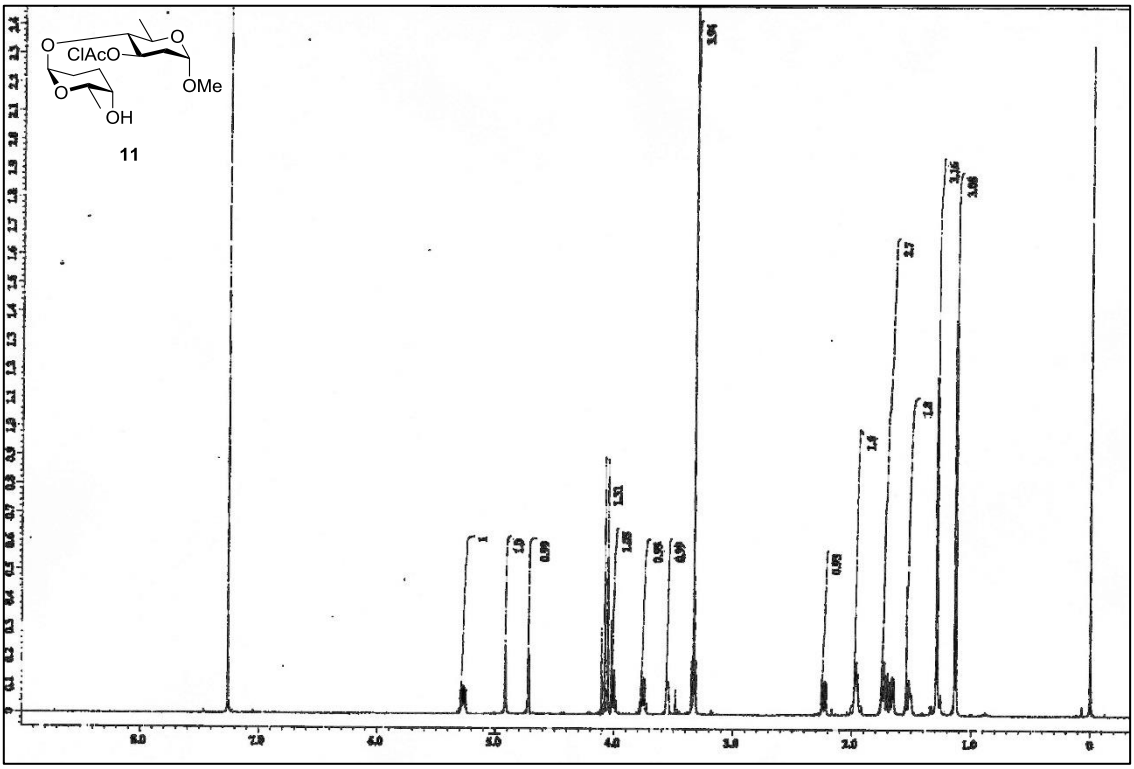

Figure S13 ^{1}H-NMR spectrum of **11**

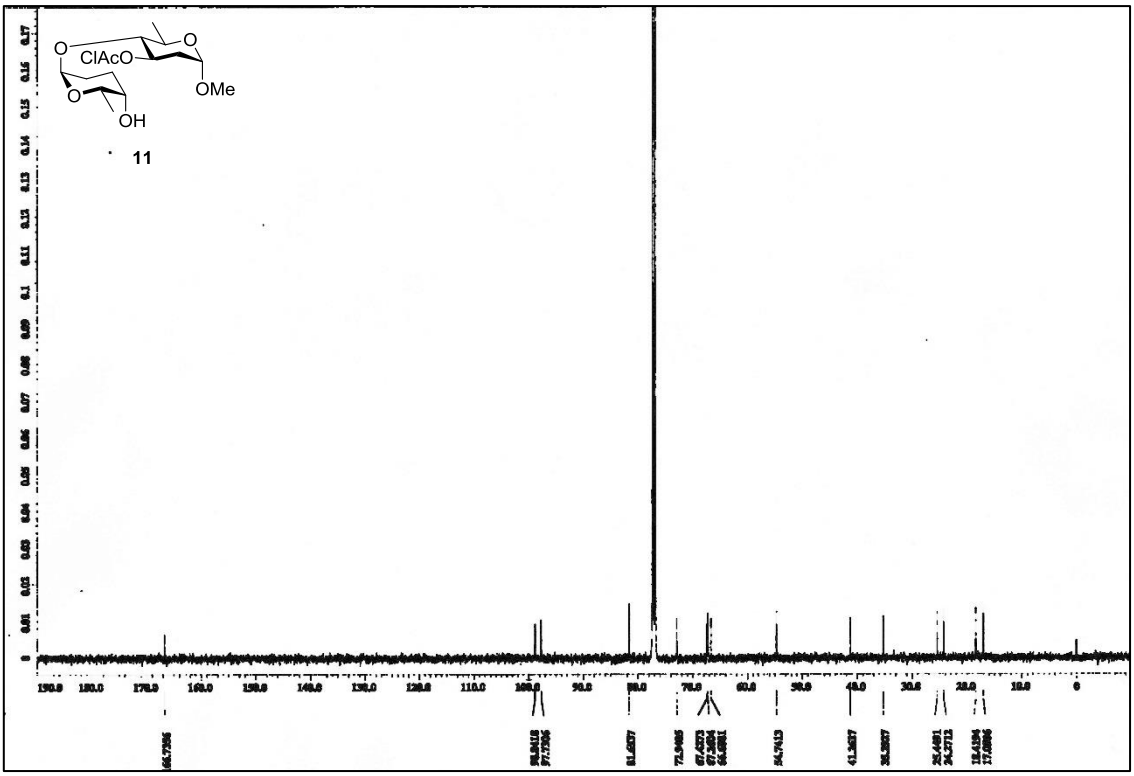

Figure S14 ^{13}C-NMR spectrum of **11**

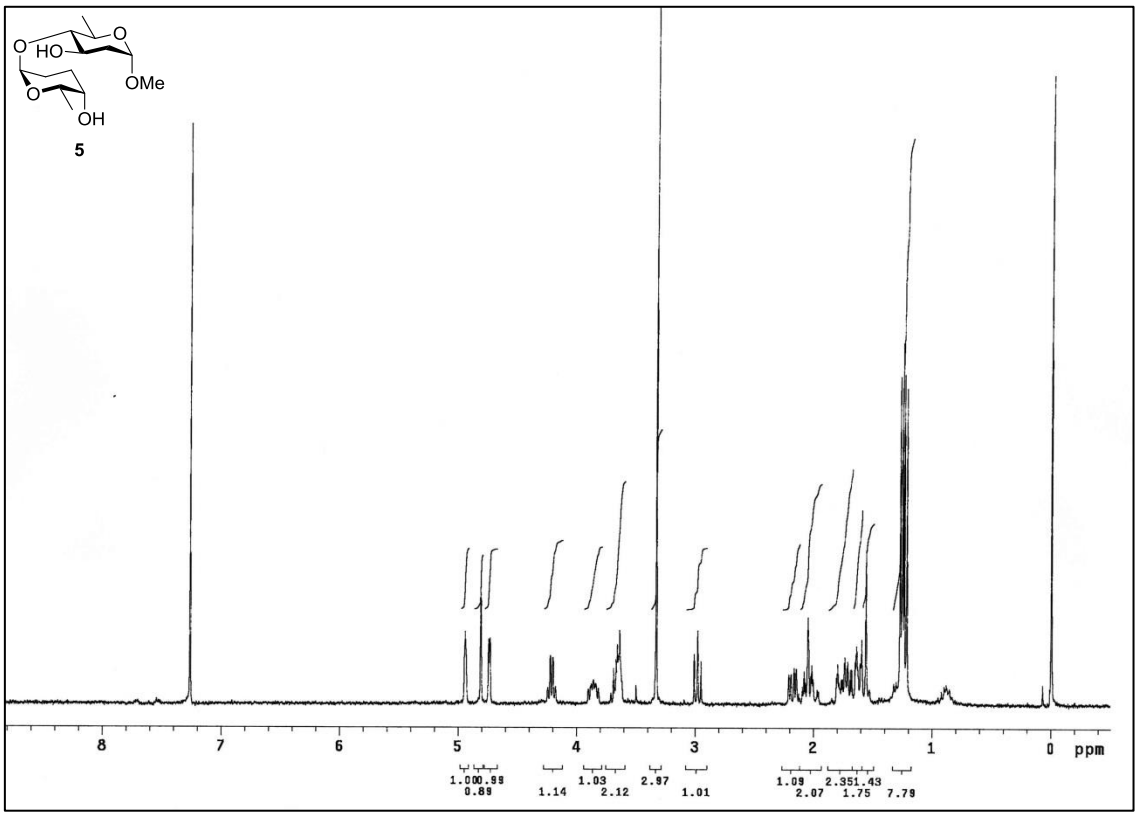

Figure S15 ^{1}H-NMR spectrum of **5**

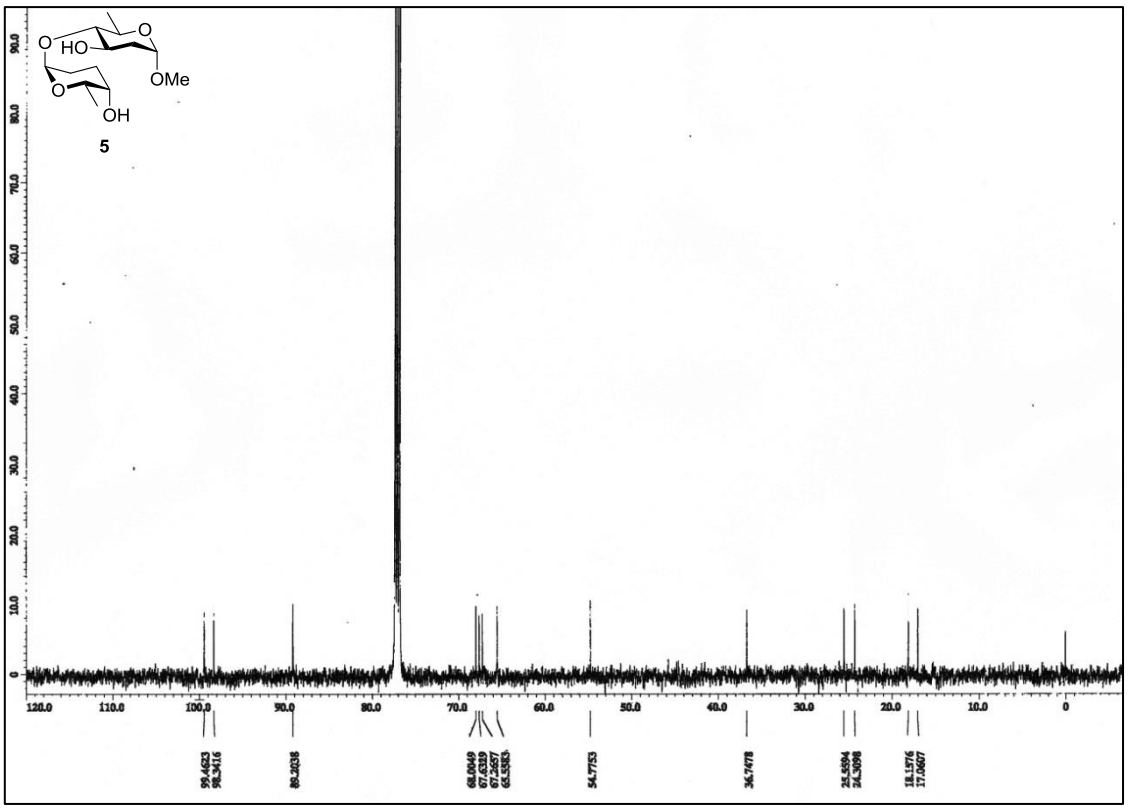

Figure S16 ^{13}C-NMR spectrum of **5**

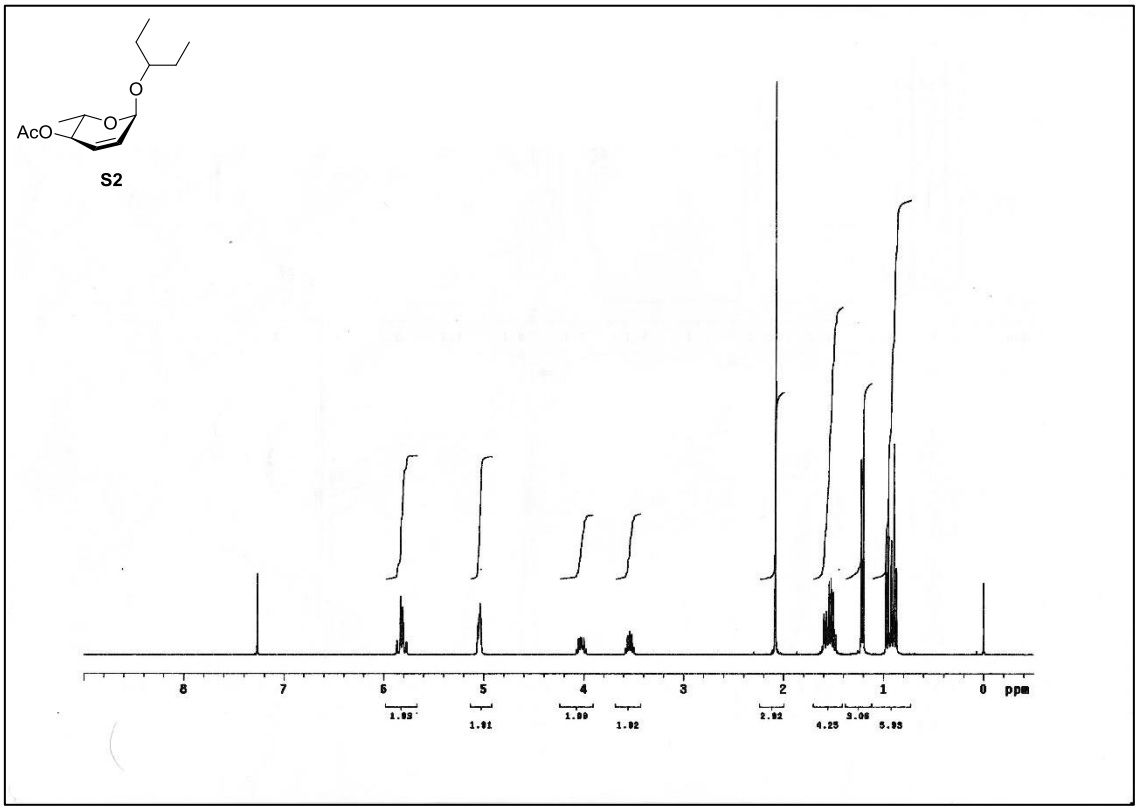

Figure S17 ^{1}H-NMR spectrum of **S2**

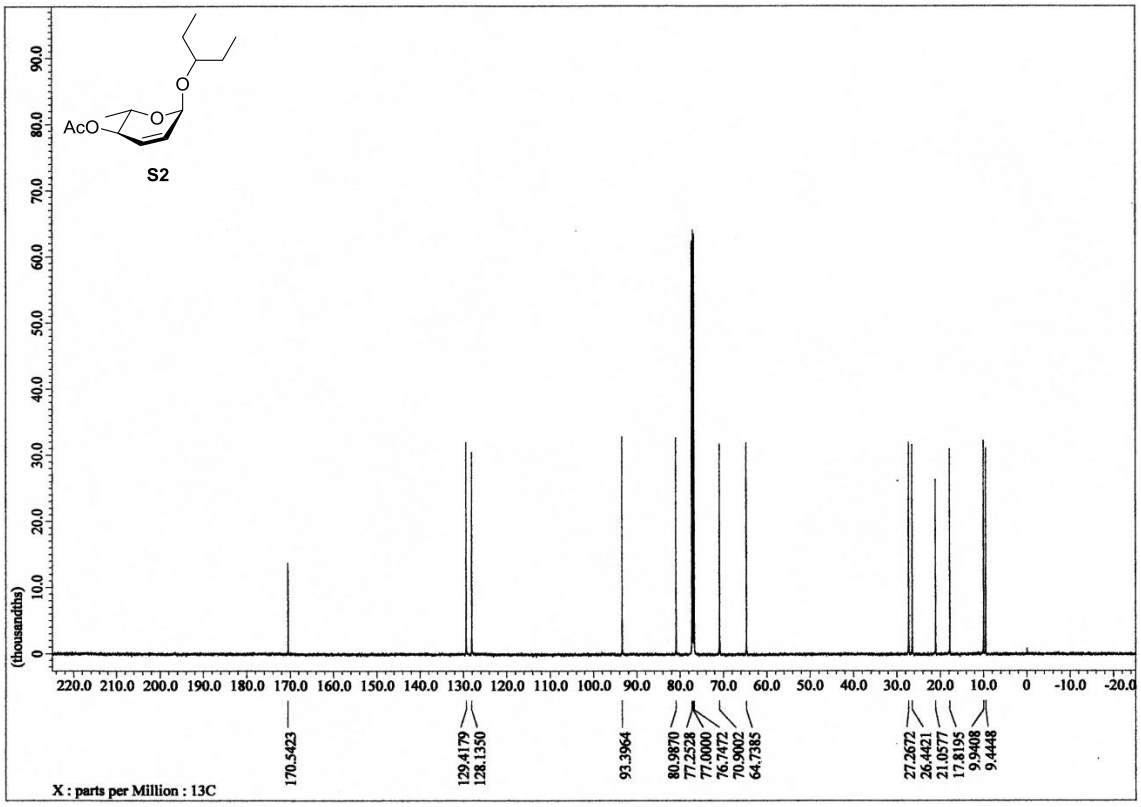

Figure S18 ^{13}C -NMR spectrum of **S2**

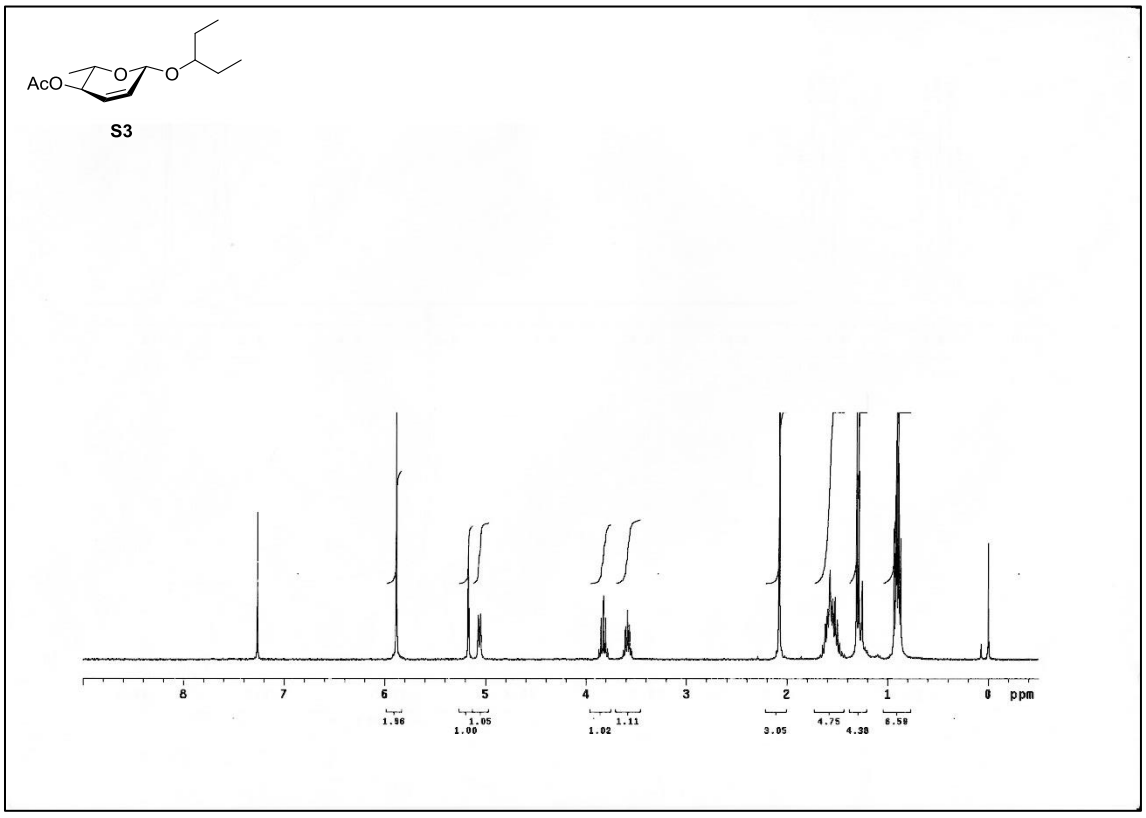

Figure S19 ^{1}H-NMR spectrum of **S3**

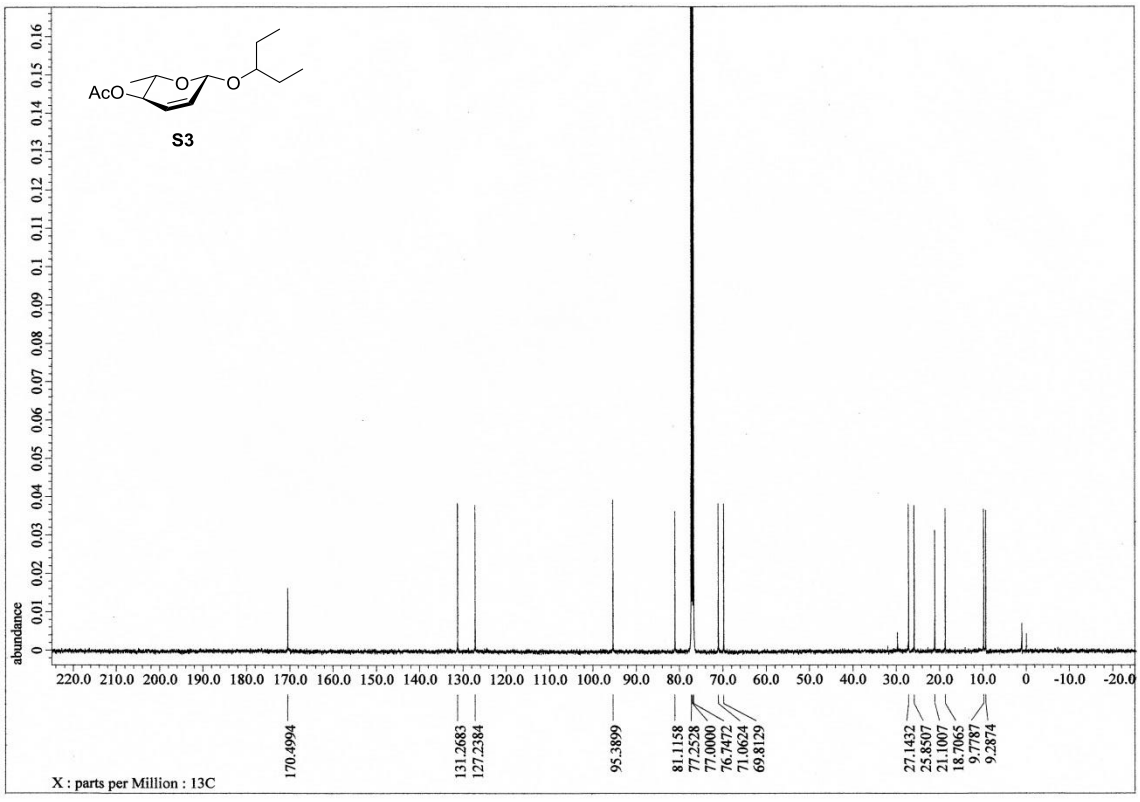

Figure S20 ^{13}C -NMR spectrum of **S3**

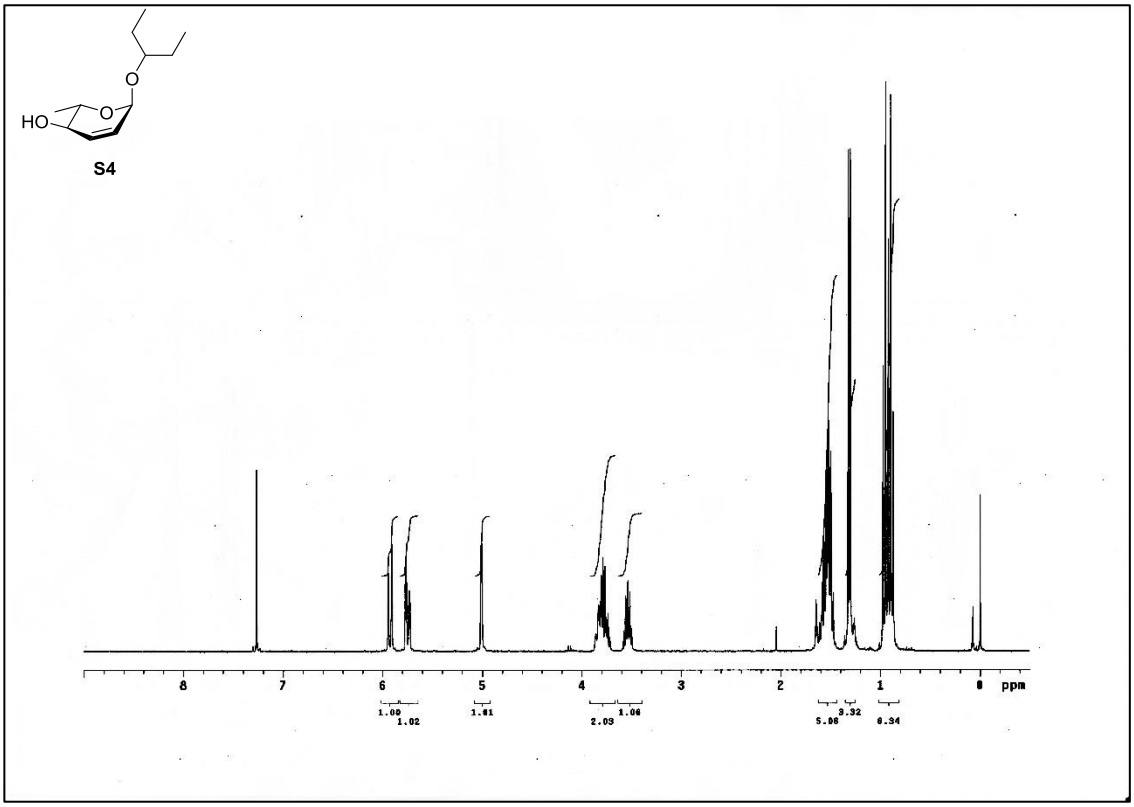

Figure S21 ^{1}H-NMR spectrum of **S4**

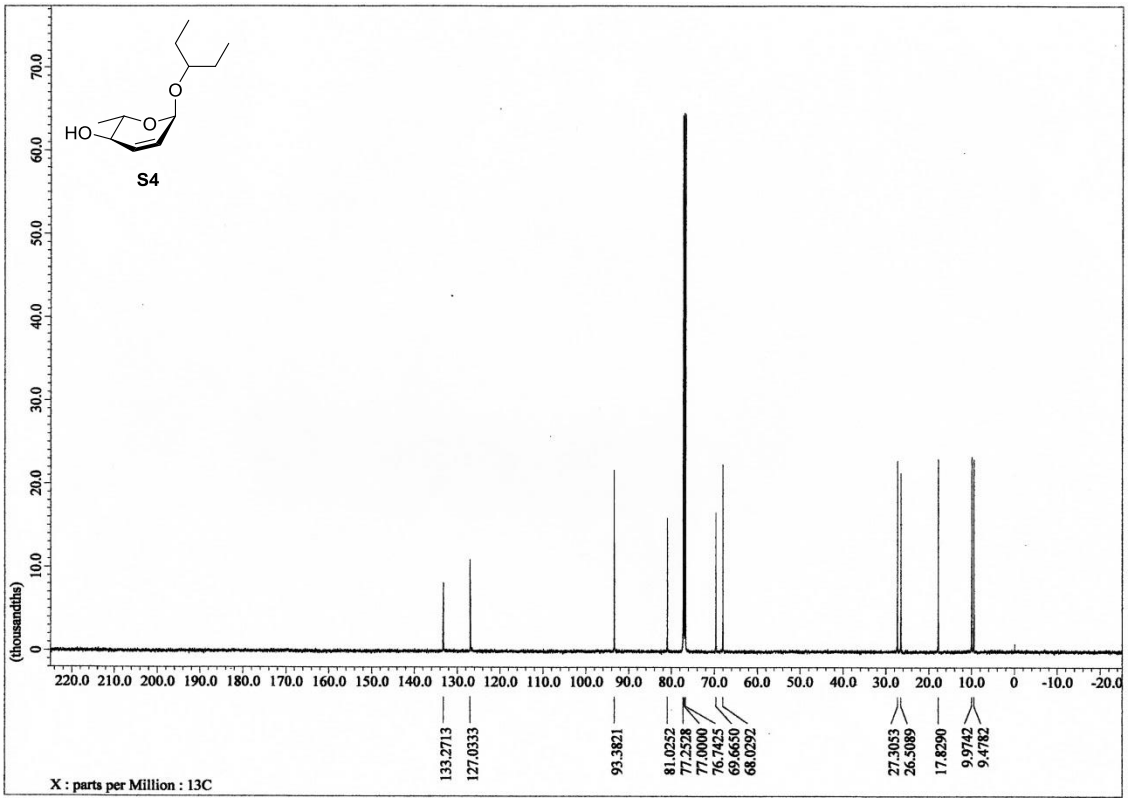

Figure S22 ^{13}C -NMR spectrum of **S4**

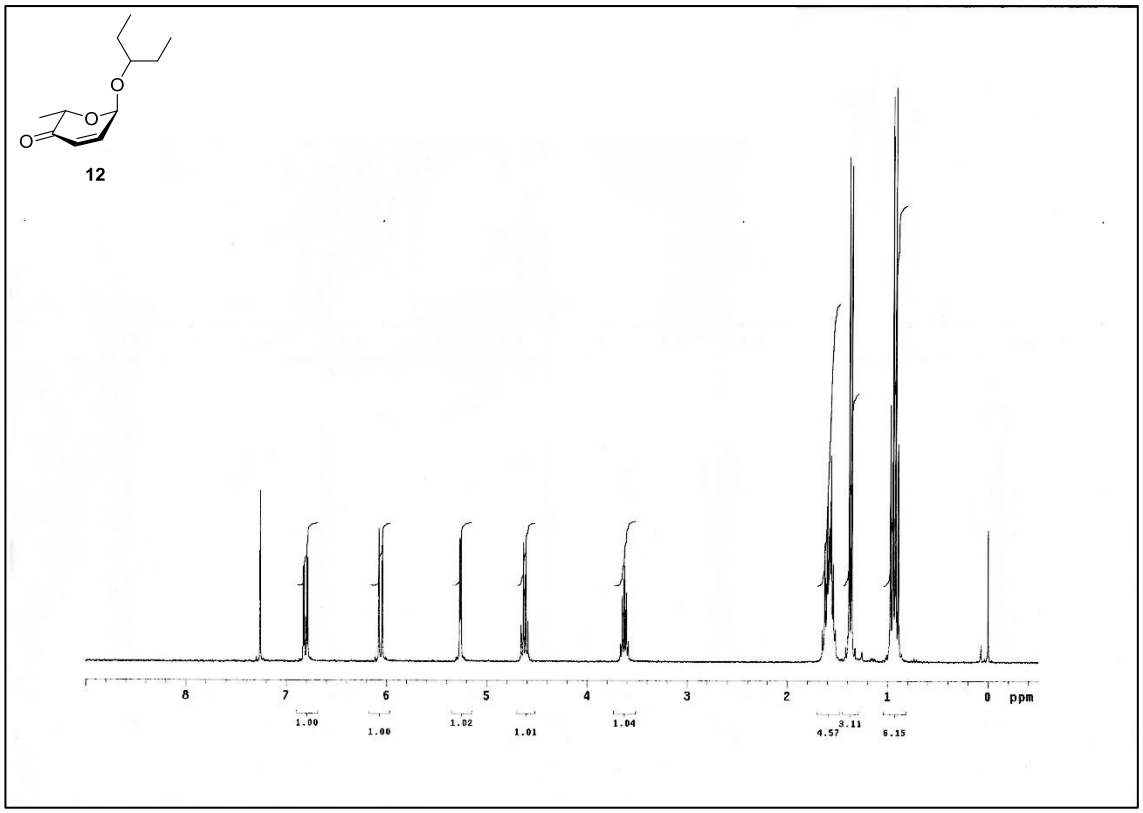

Figure S23 ^{1}H-NMR spectrum of **12**

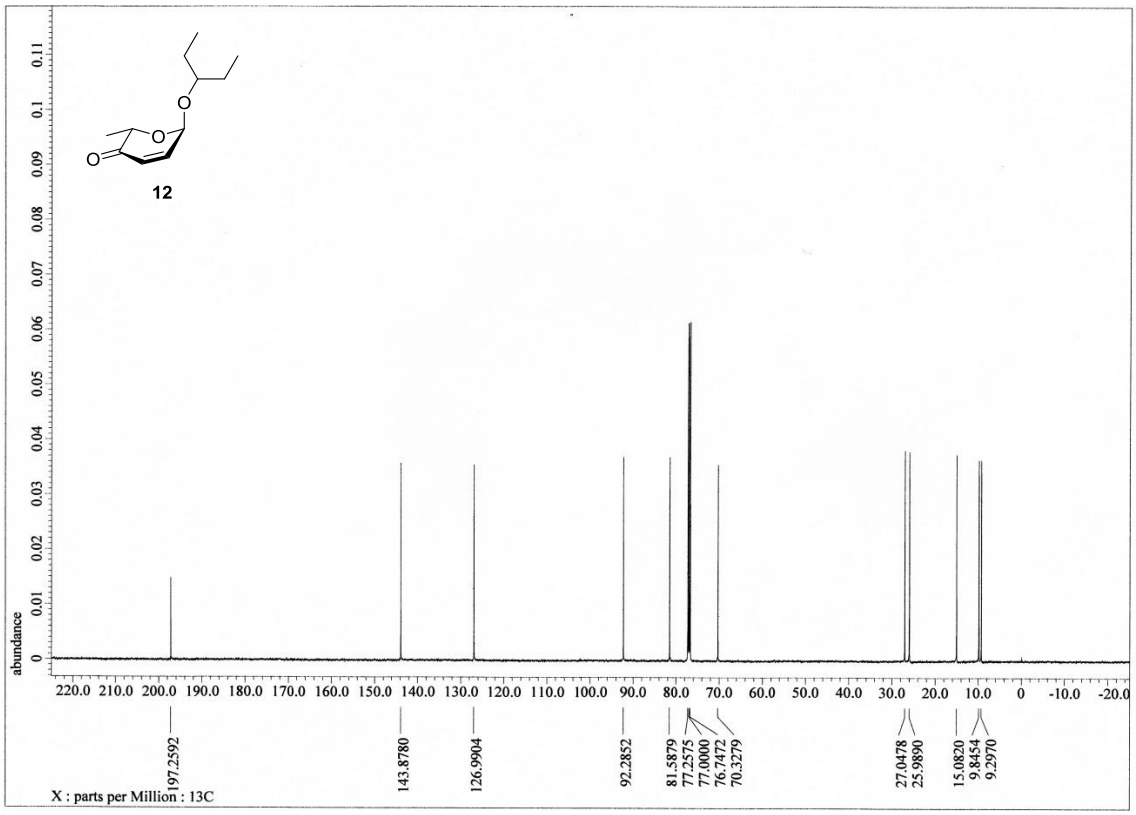

Figure S24 ^{13}C -NMR spectrum of **12**

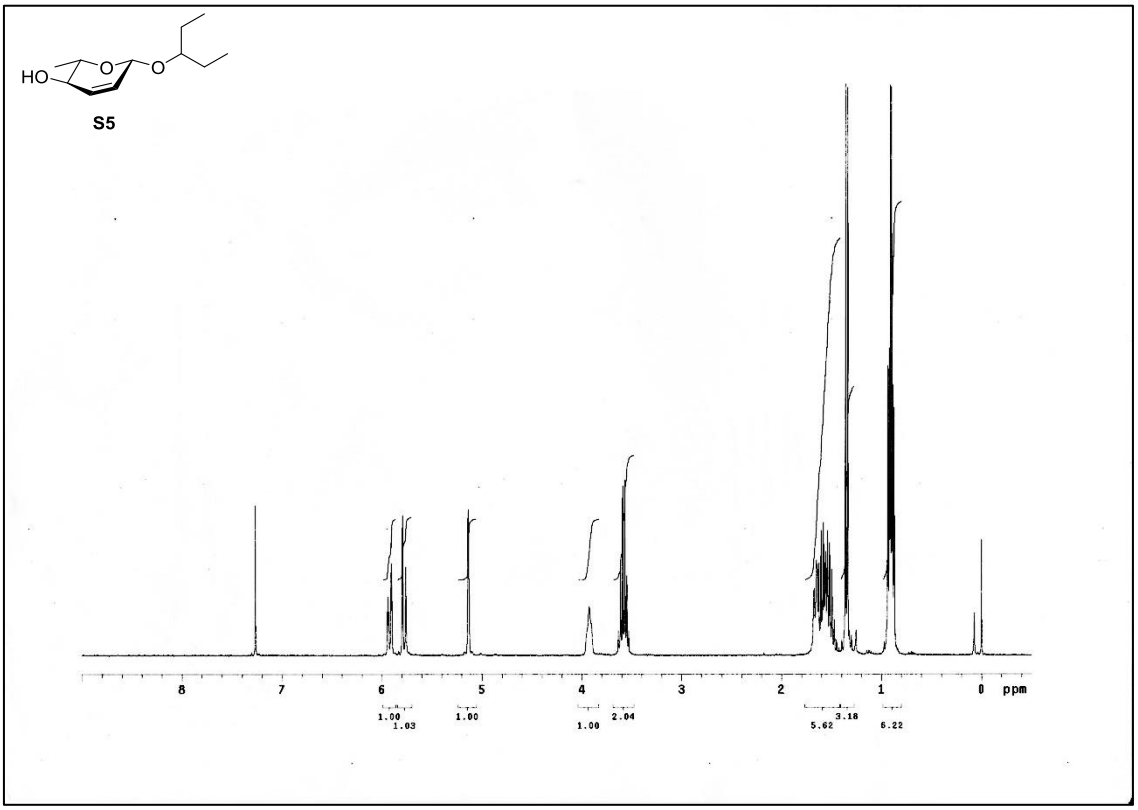

Figure S25 ^{1}H-NMR spectrum of **S5**

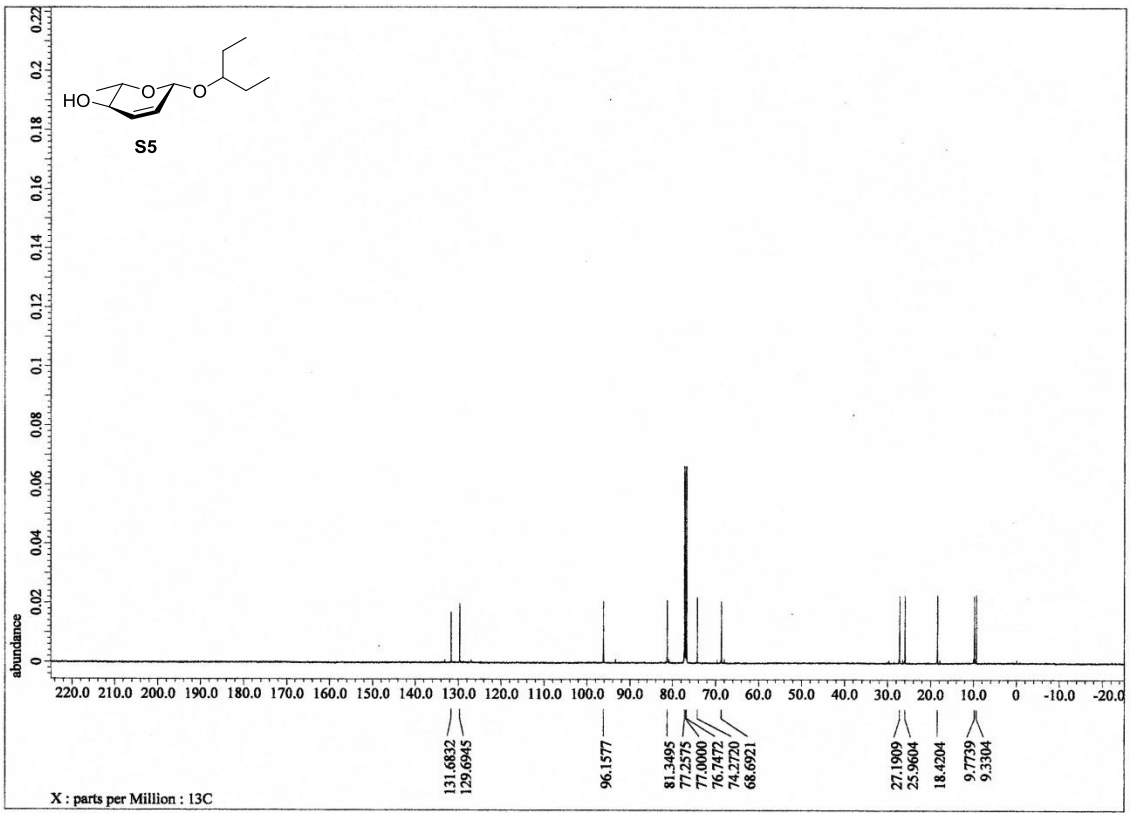

Figure S26 ^{13}C -NMR spectrum of **S5**

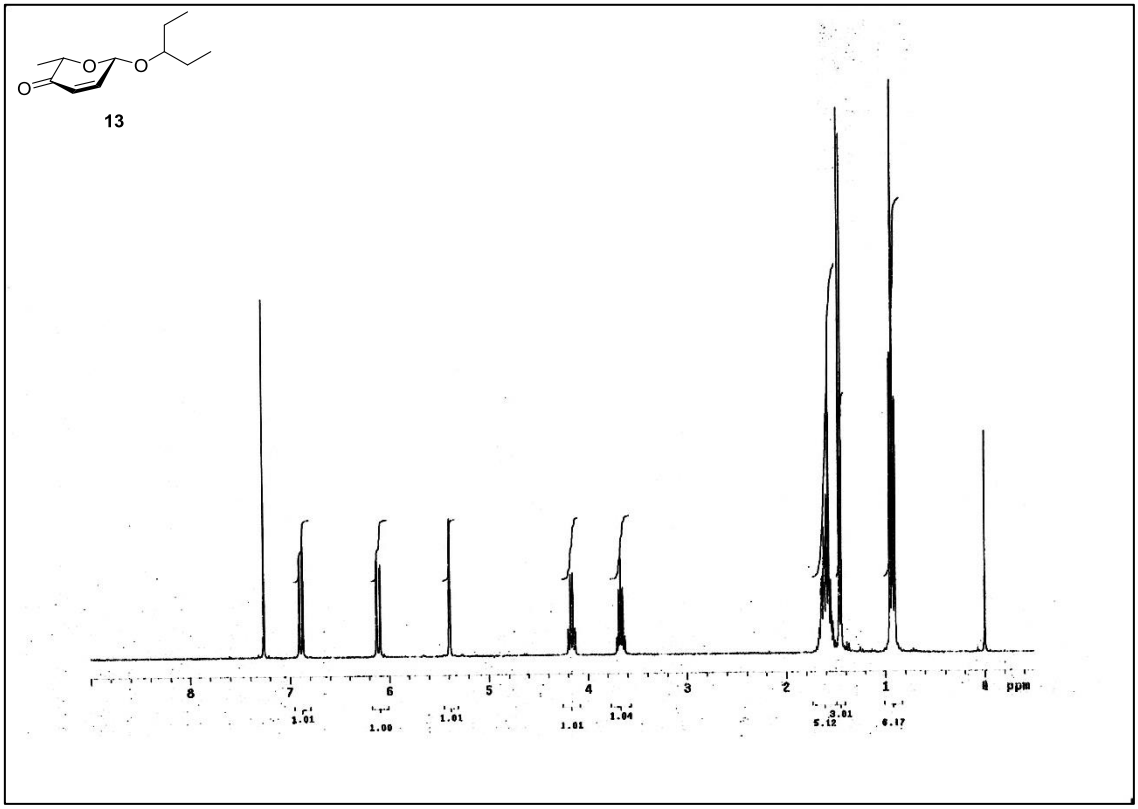

Figure S27 ^{1}H-NMR spectrum of **13**

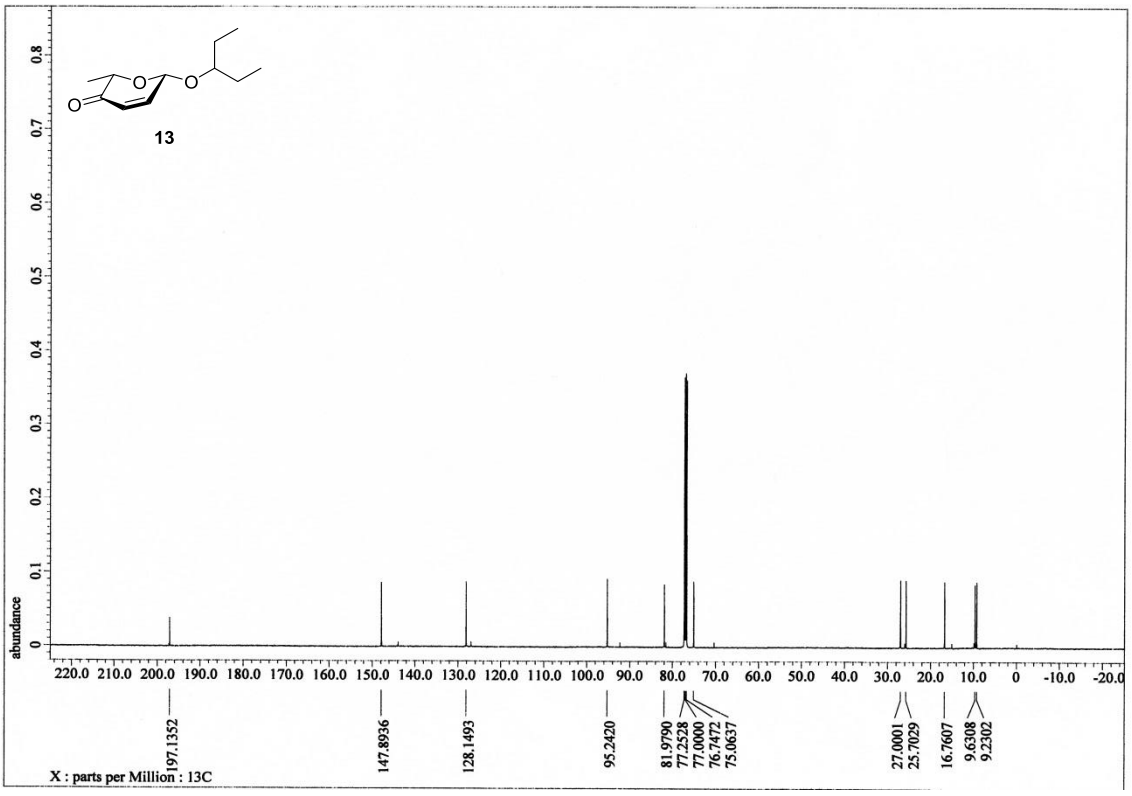

Figure S28 ^{13}C -NMR spectrum of **13**

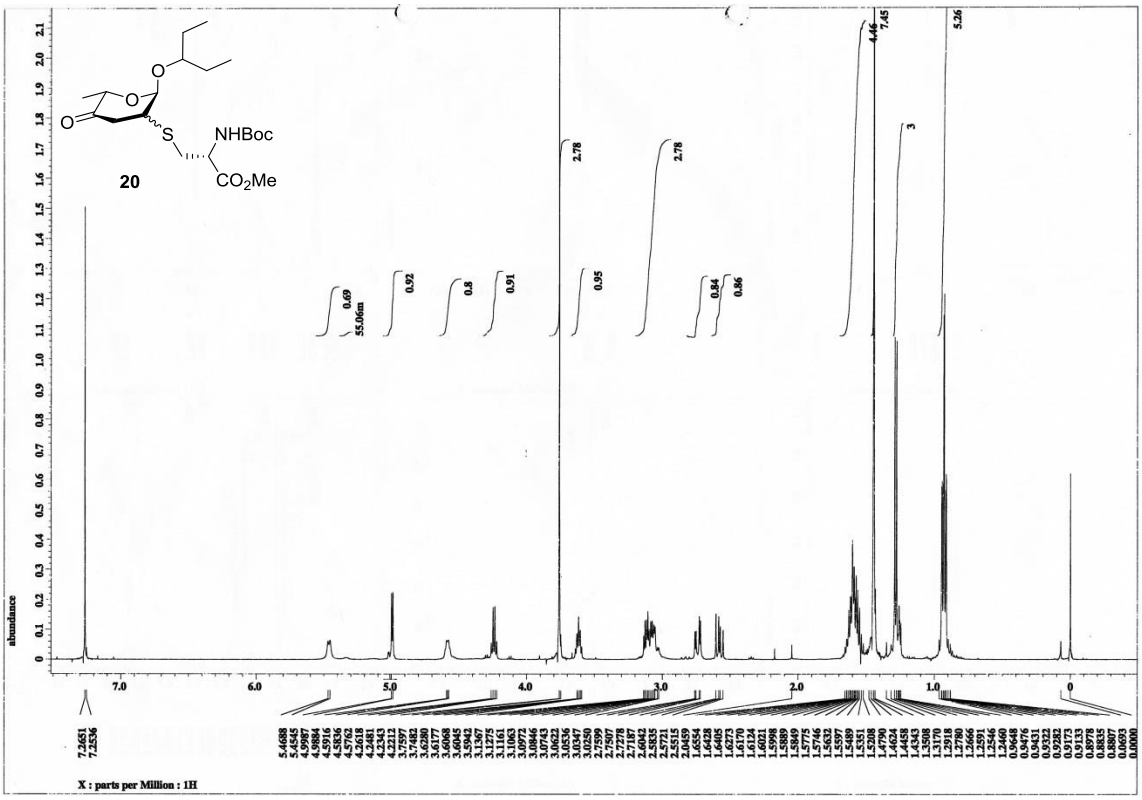

Figure S29 ^{1}H-NMR spectrum of **20**

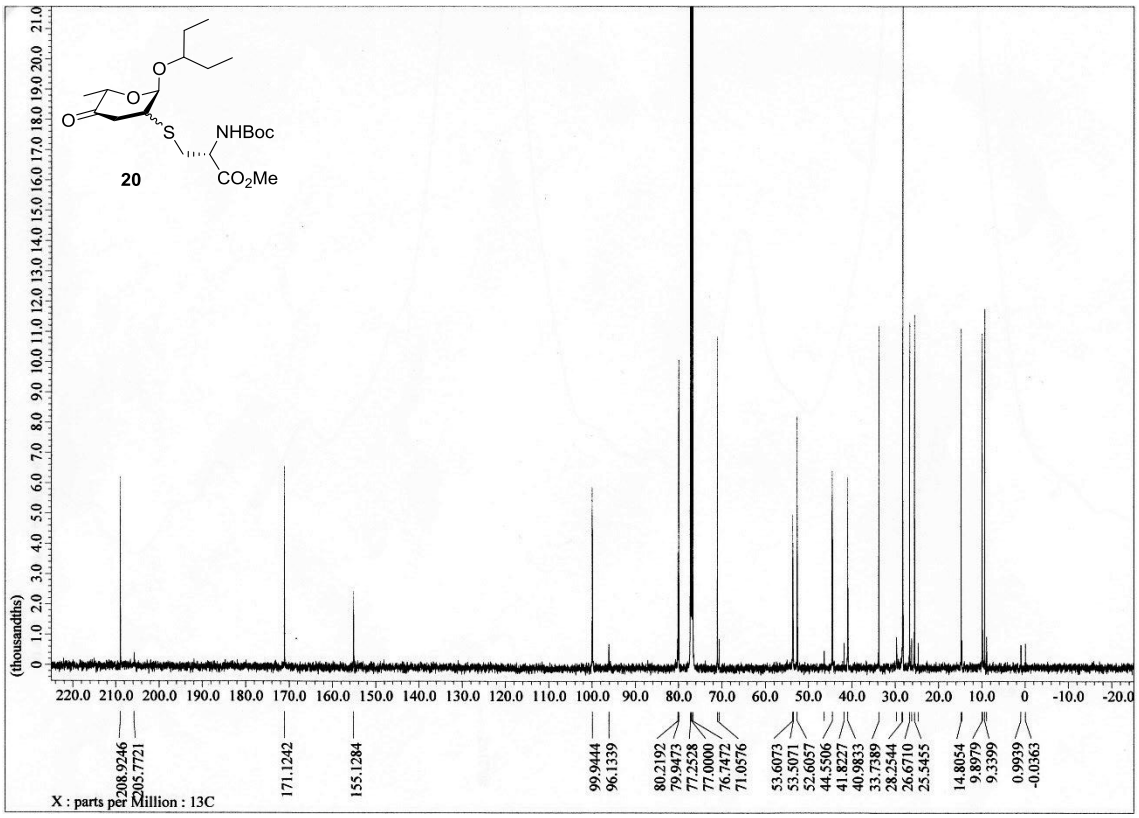

Figure S30 ^{13}C -NMR spectrum of **20**

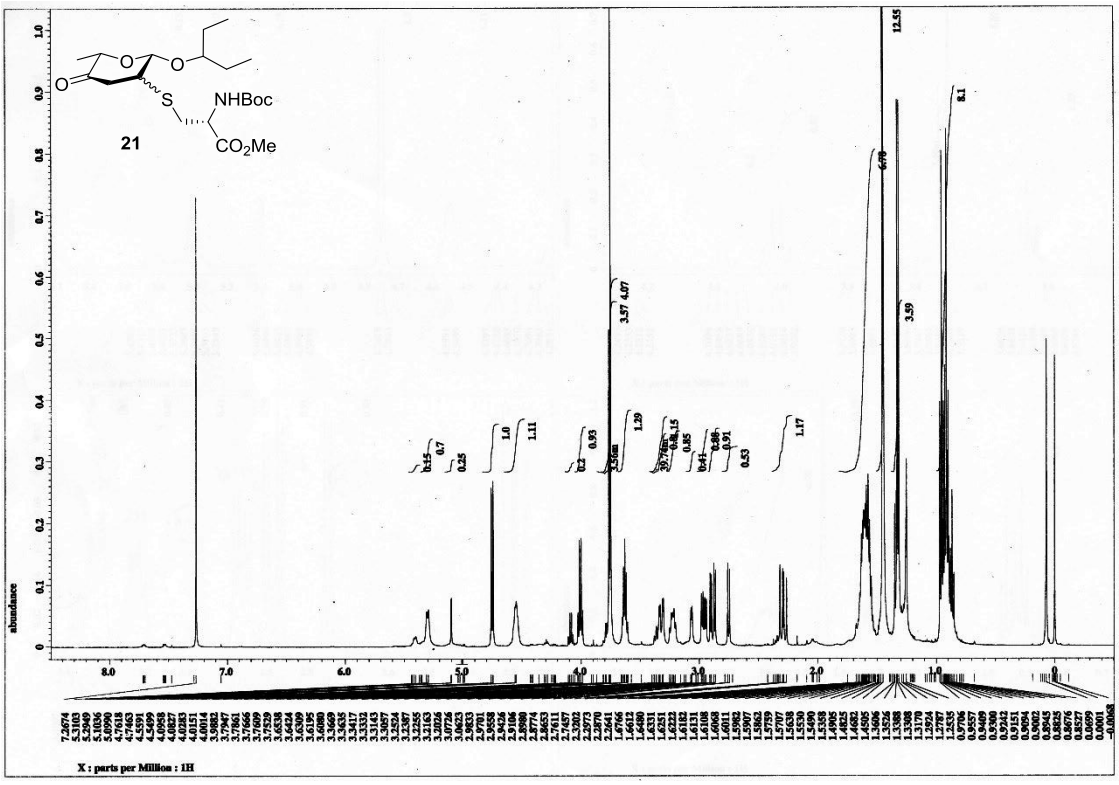

Figure S31 ^{1}H-NMR spectrum of **21**

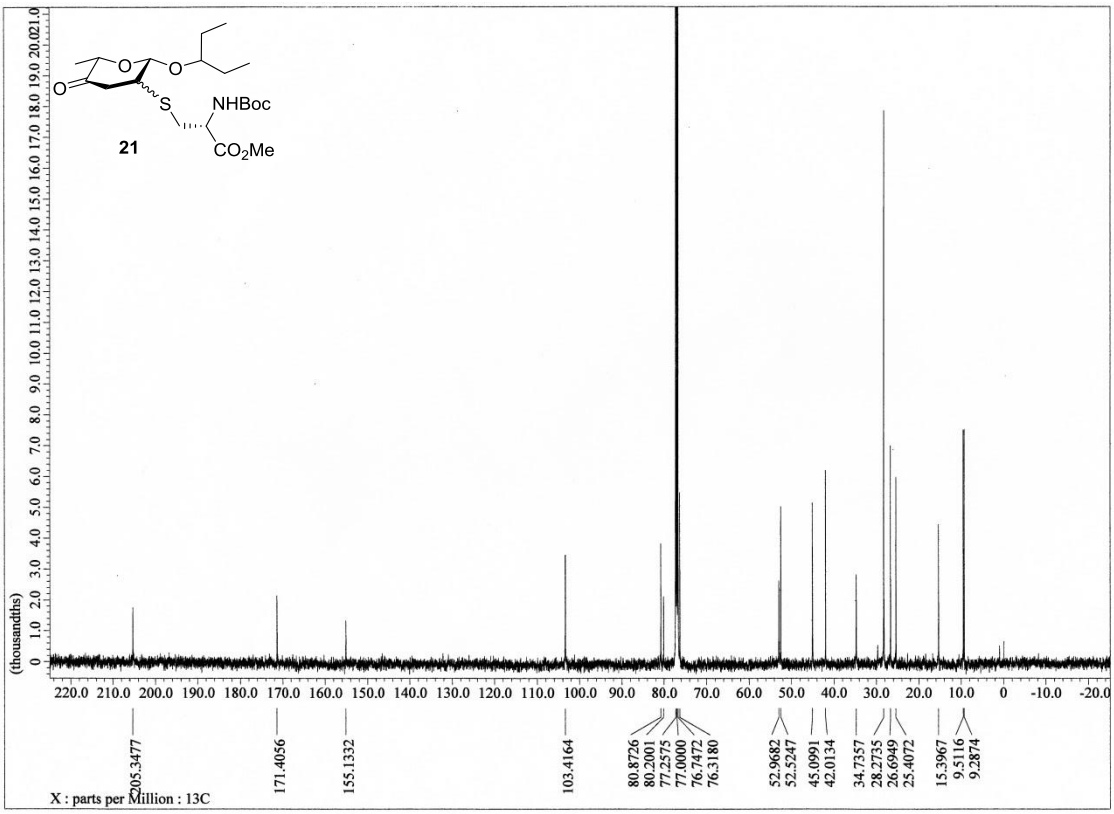

Figure S32 ^{13}C -NMR spectrum of **21**

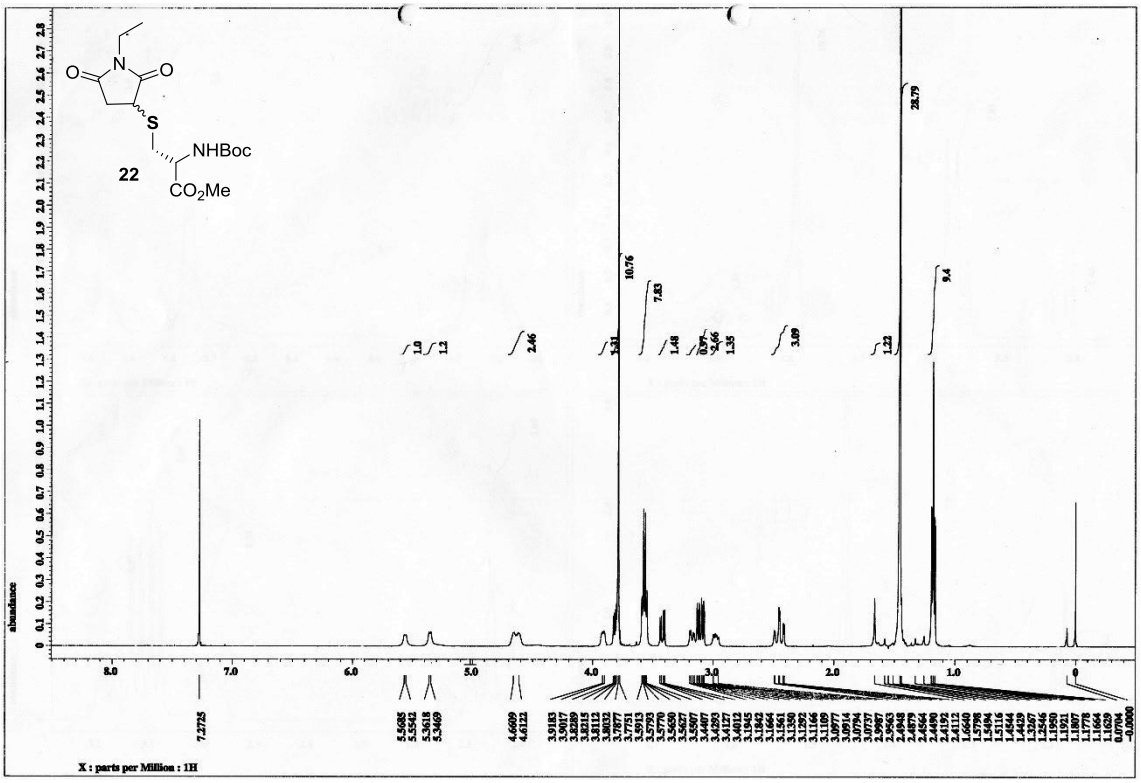

Figure S33 ^{1}H-NMR spectrum of **22**

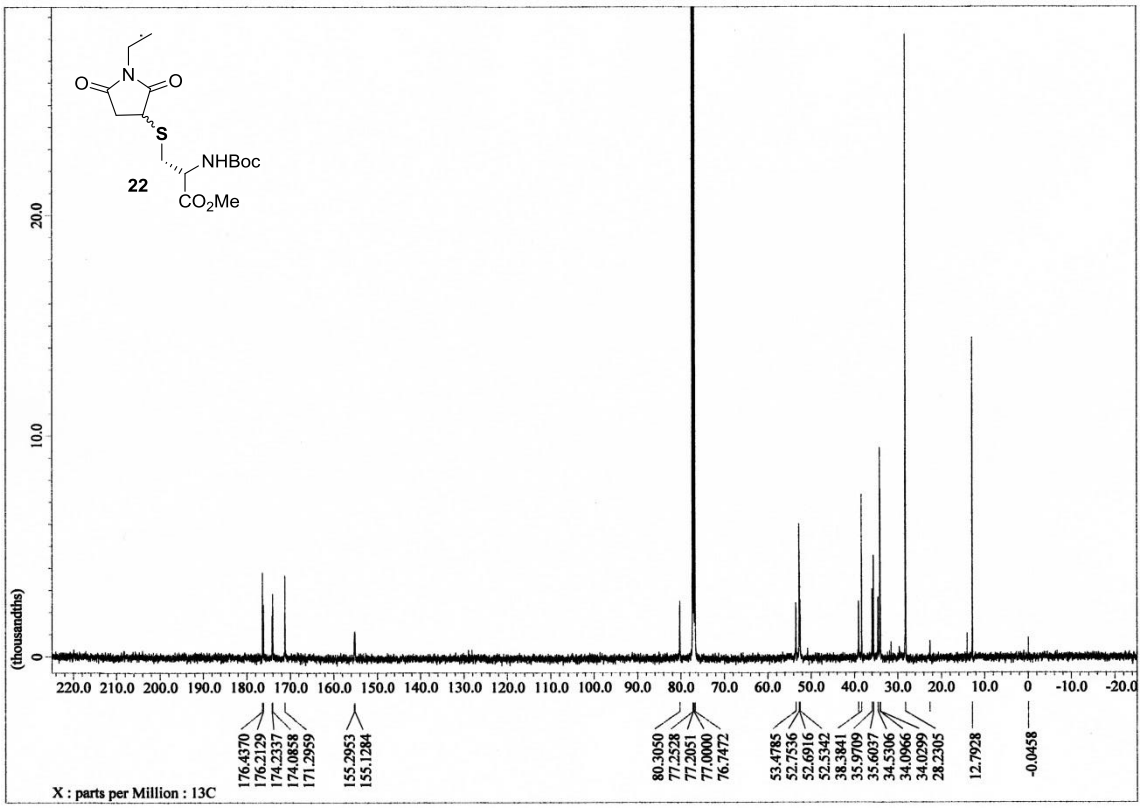

Figure S34 ^{13}C -NMR spectrum of **22**

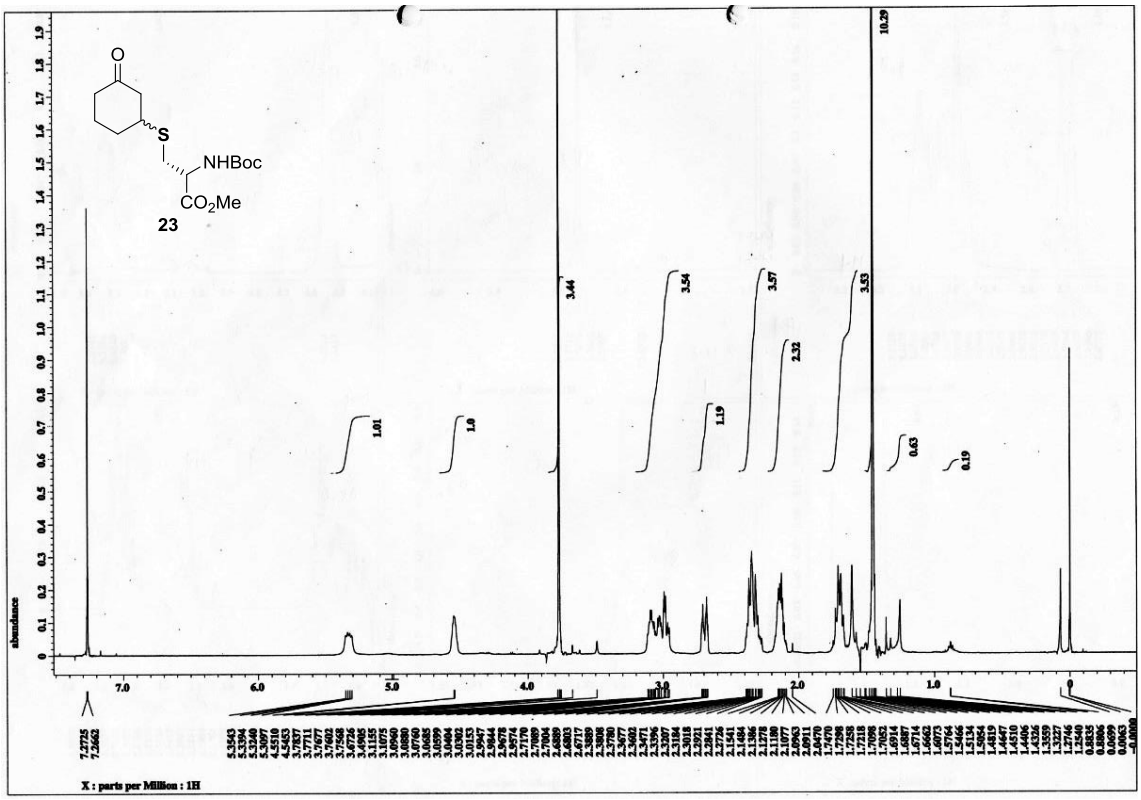

Figure S35 ^{1}H-NMR spectrum of **23**

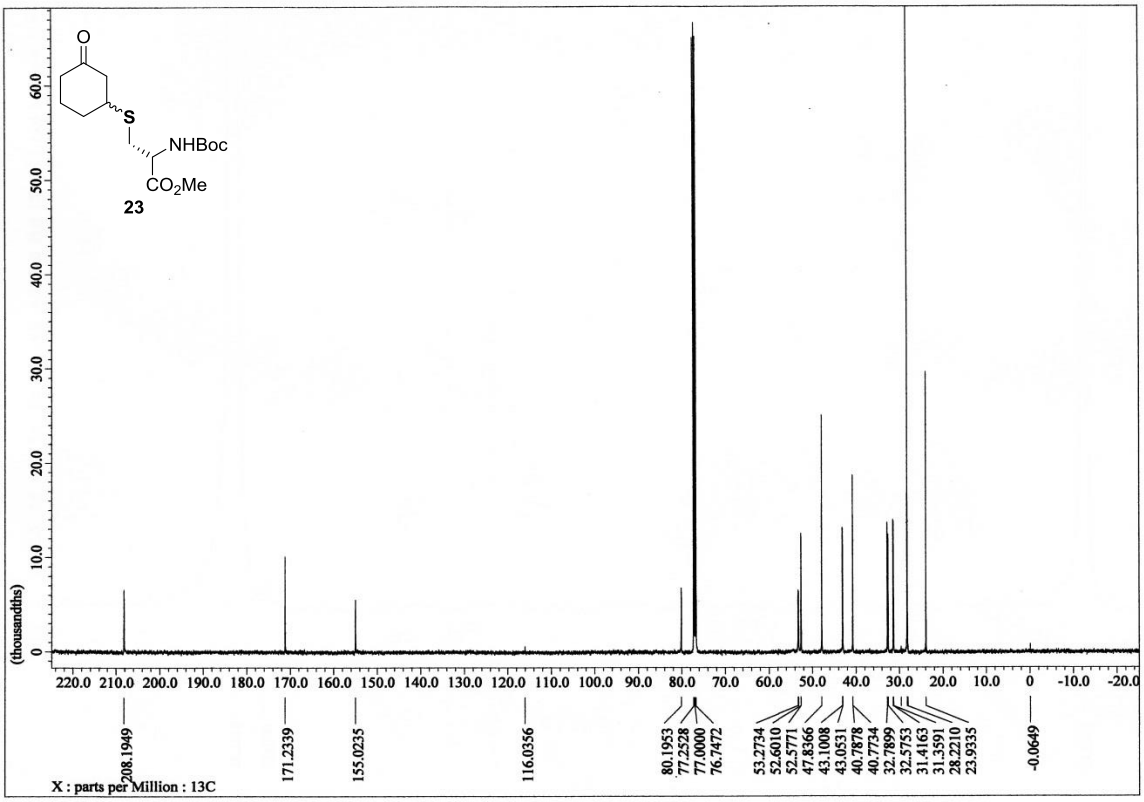

Figure S36 ^{13}C -NMR spectrum of **23**

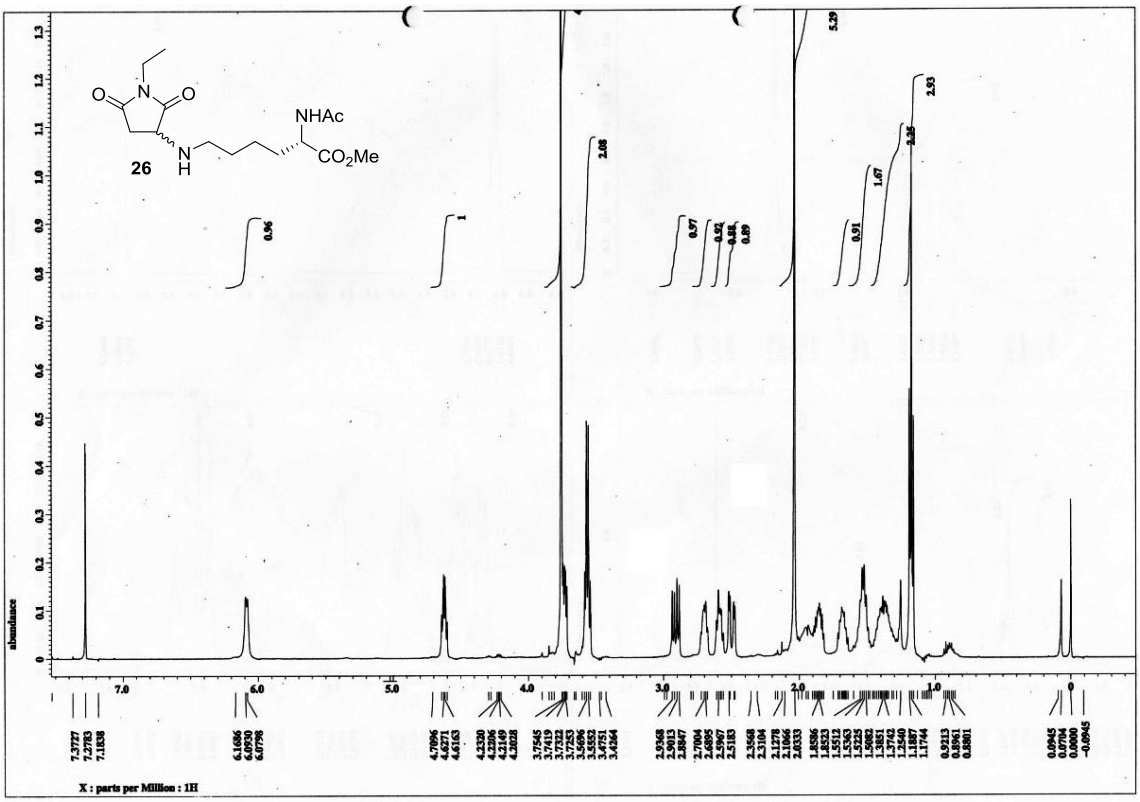

Figure S37 ^{1}H-NMR spectrum of **26**

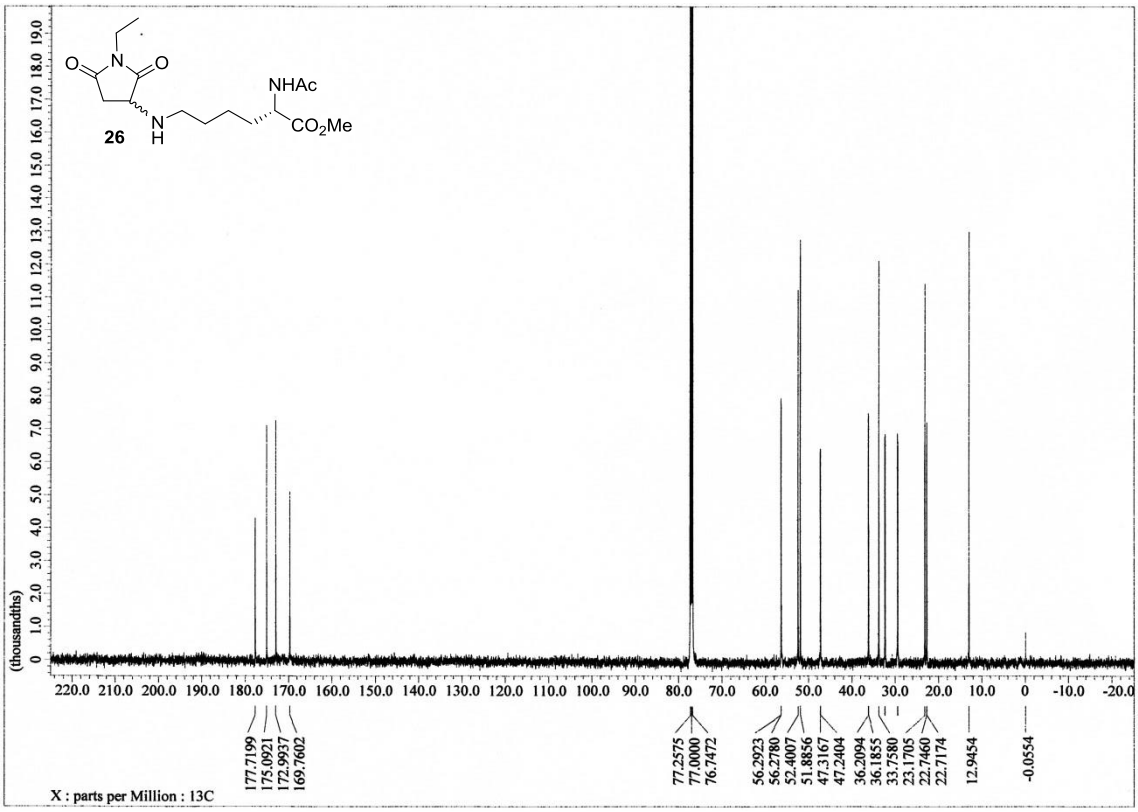

Figure S38 ^{13}C -NMR spectrum of **26**

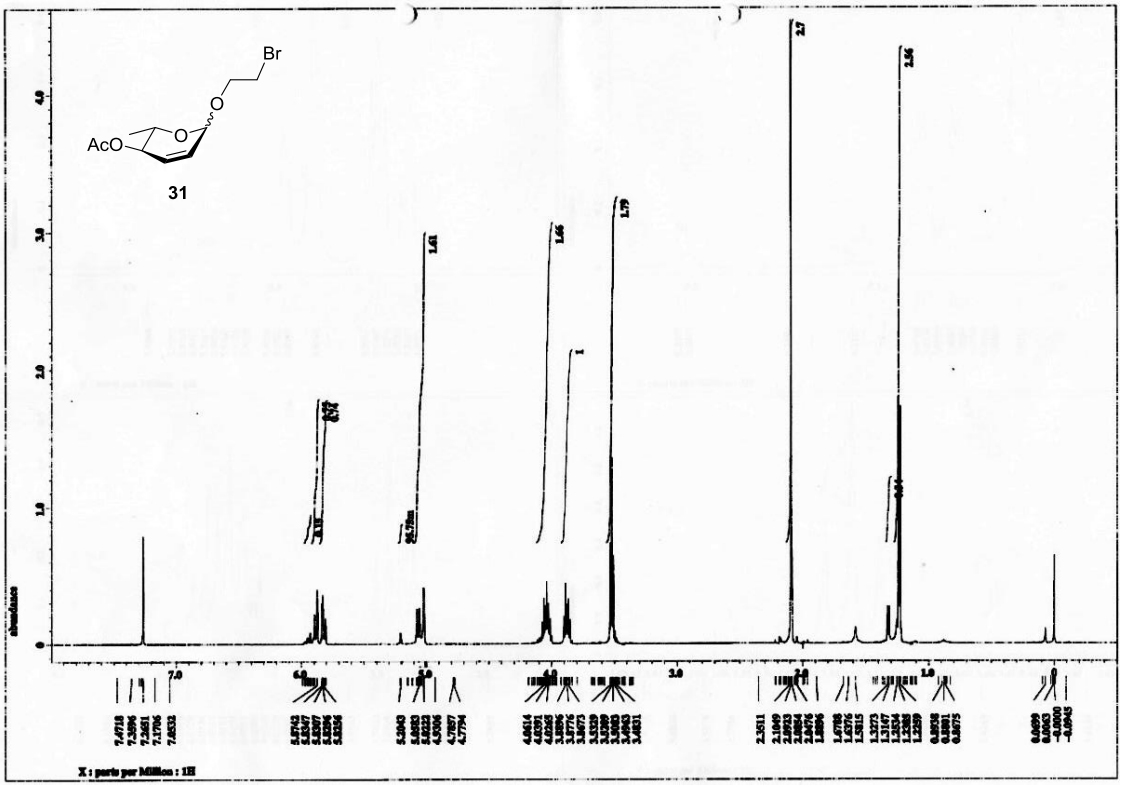

Figure S39 ^{1}H-NMR spectrum of **31**

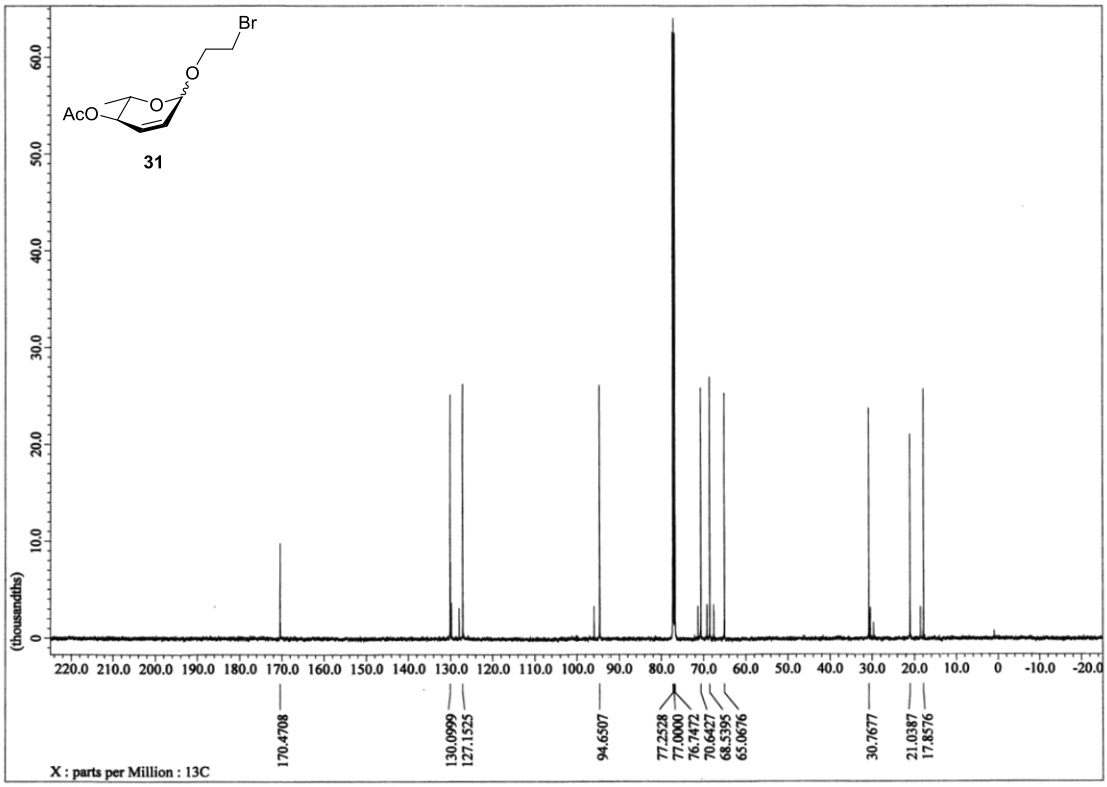

Figure S40 ^{13}C -NMR spectrum of **31**

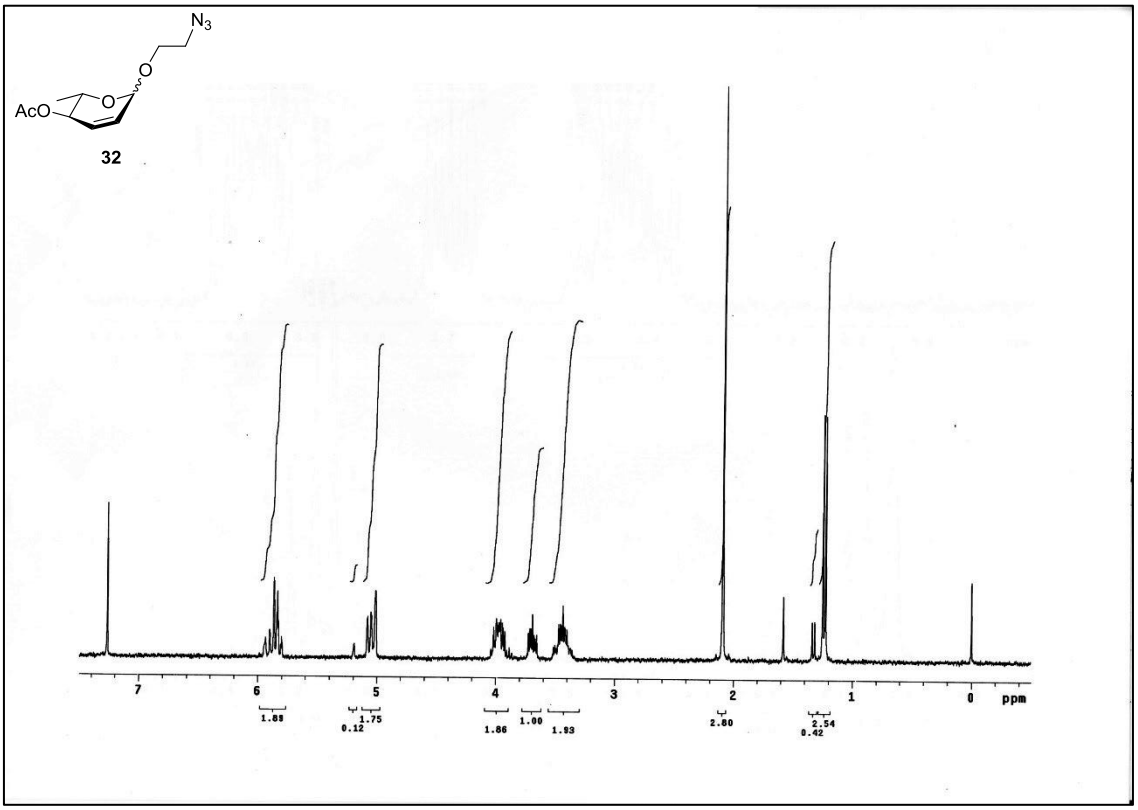

Figure S41 ^{1}H-NMR spectrum of **32**

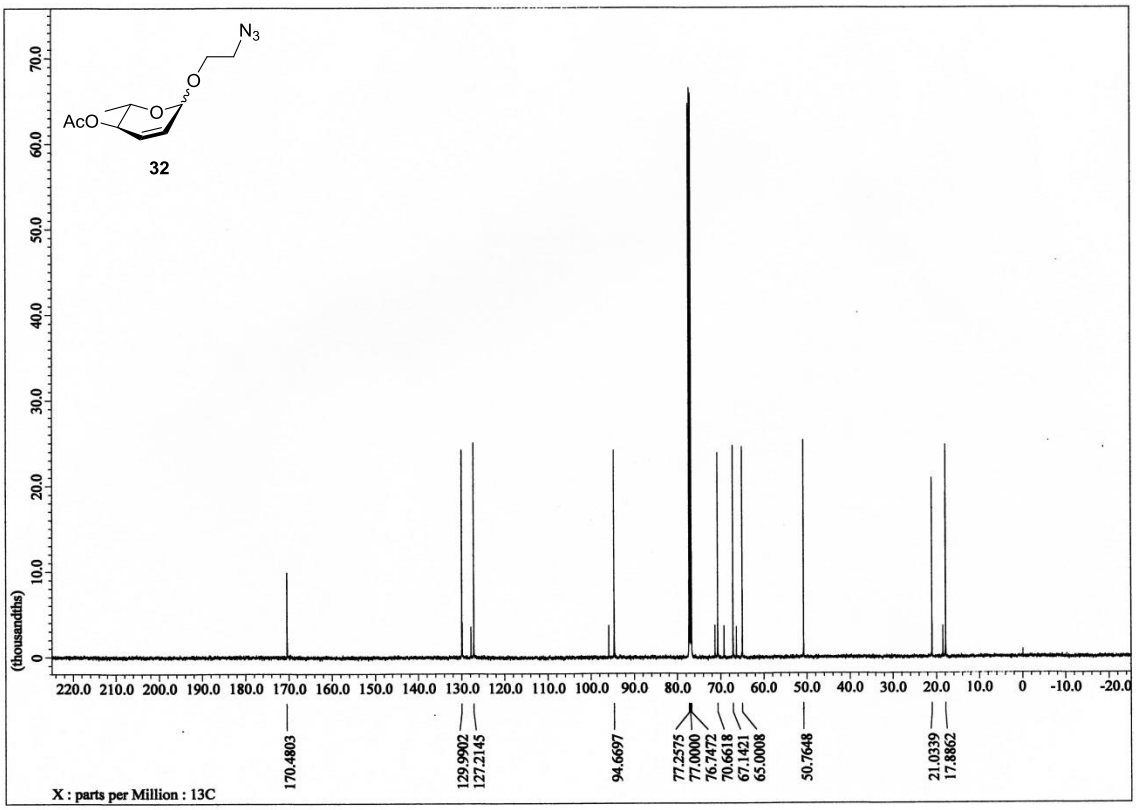

Figure S42 ^{13}C -NMR spectrum of **32**

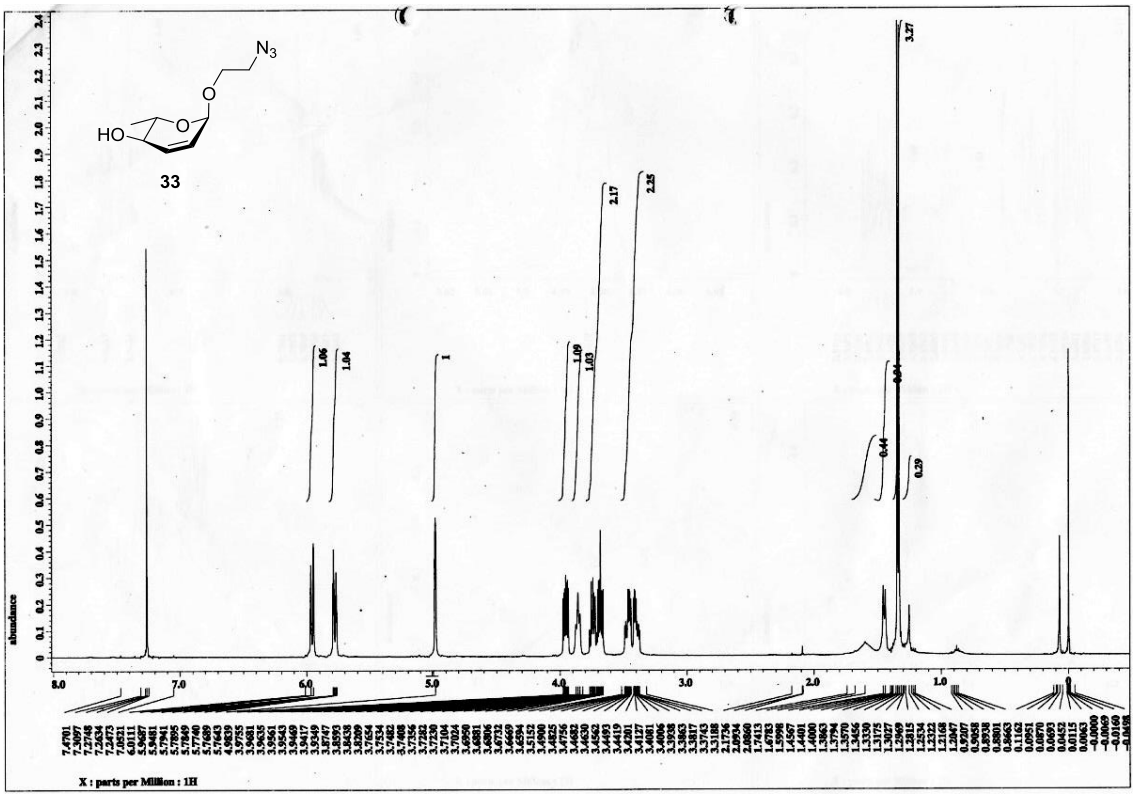

Figure S43 ^{1}H-NMR spectrum of **33**

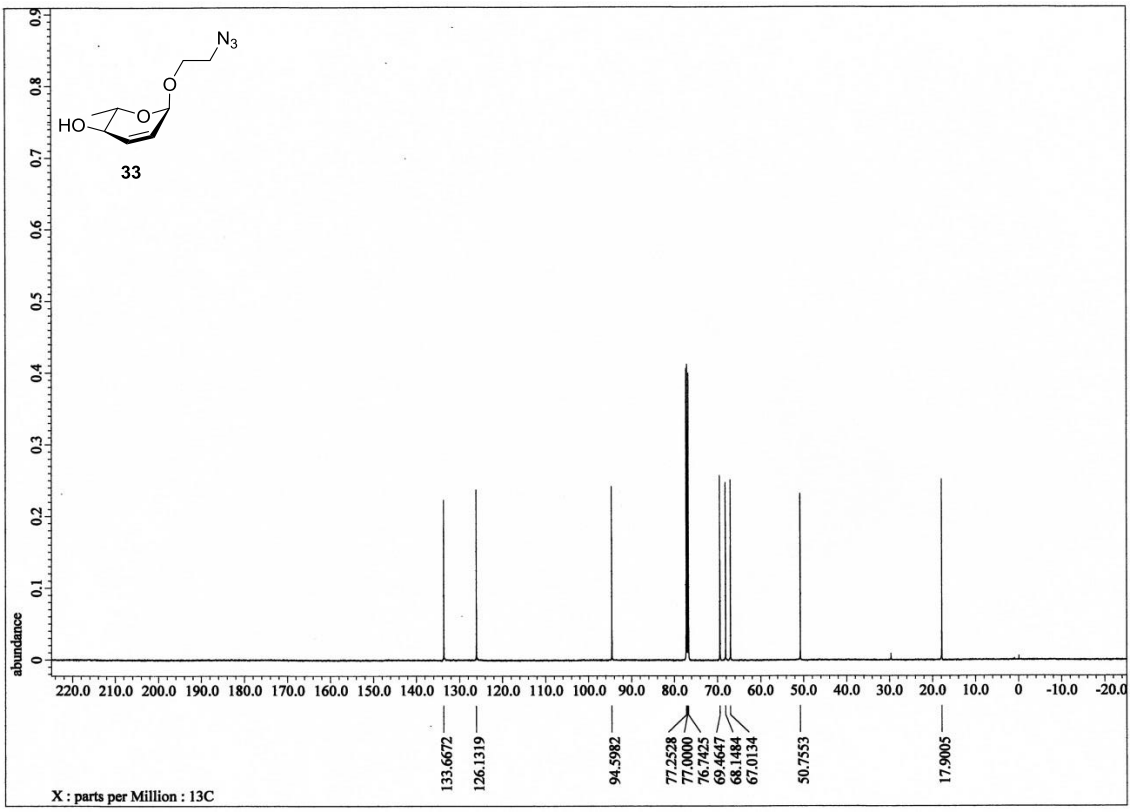

Figure S44 ^{13}C -NMR spectrum of **33**

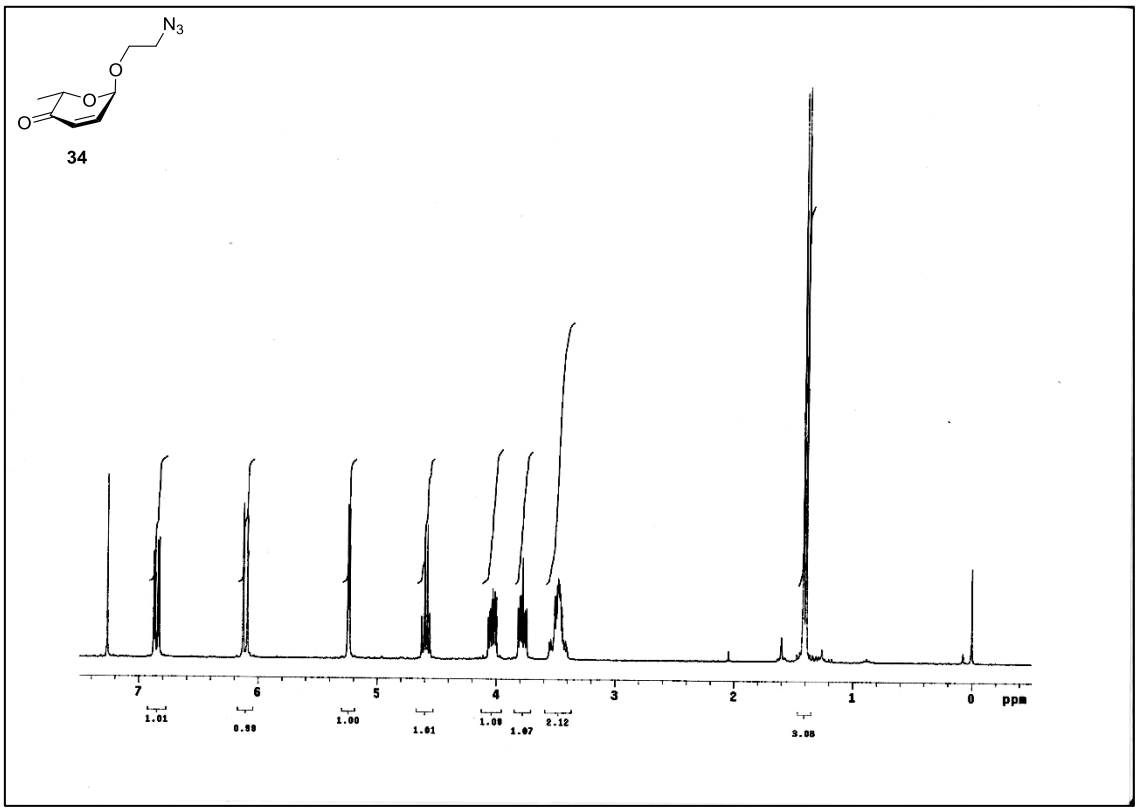

Figure S45 ^{1}H-NMR spectrum of **34**

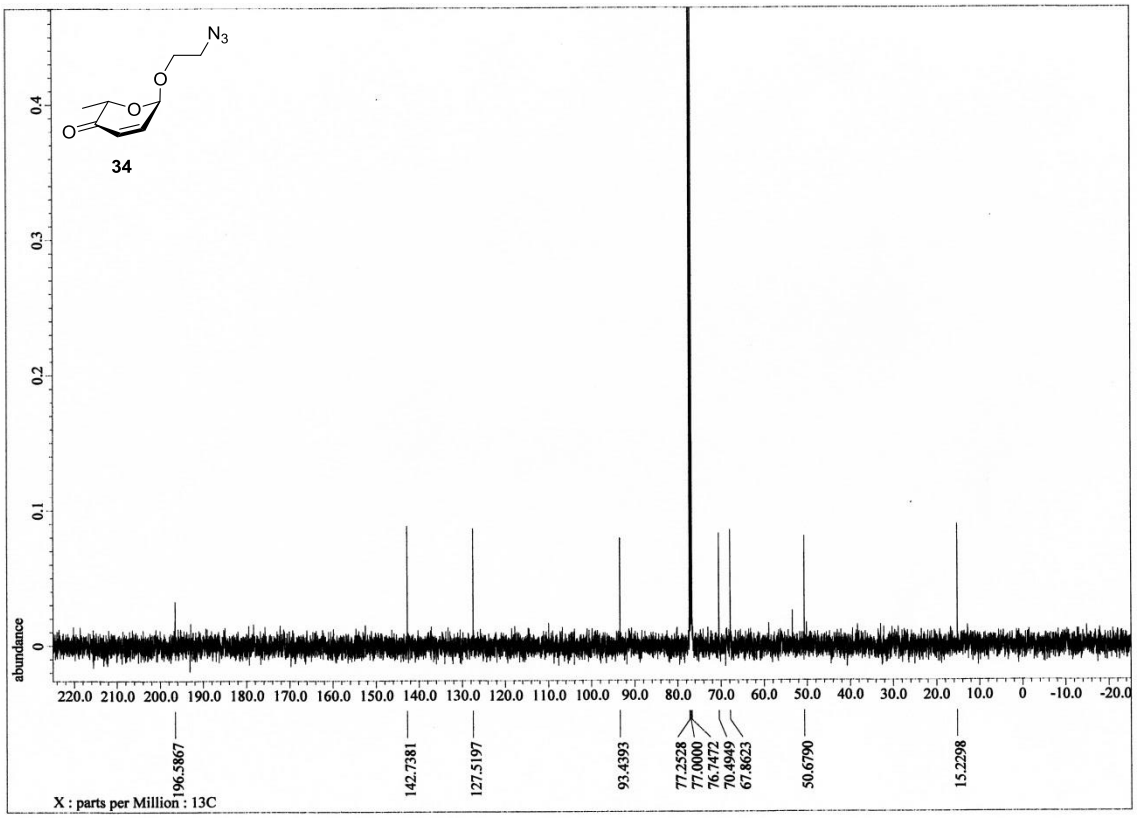

Figure S46 ^{13}C -NMR spectrum of **34**

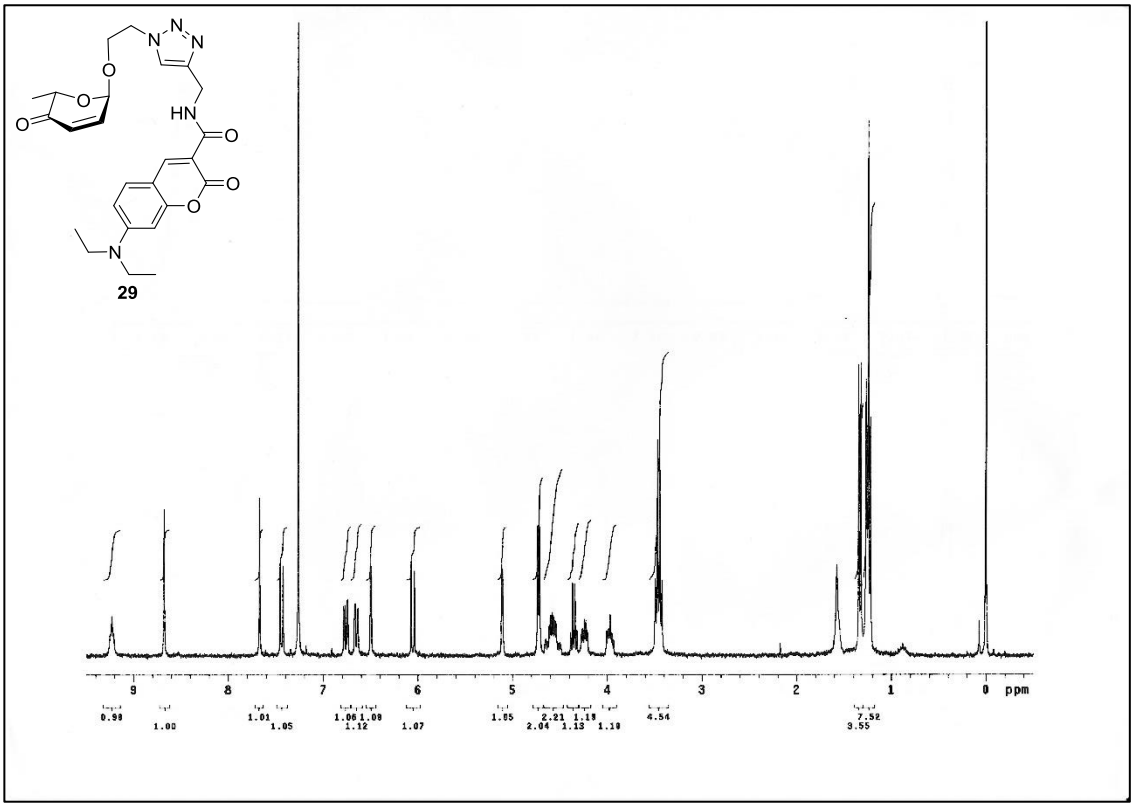

Figure S47 ^{1}H-NMR spectrum of **29**

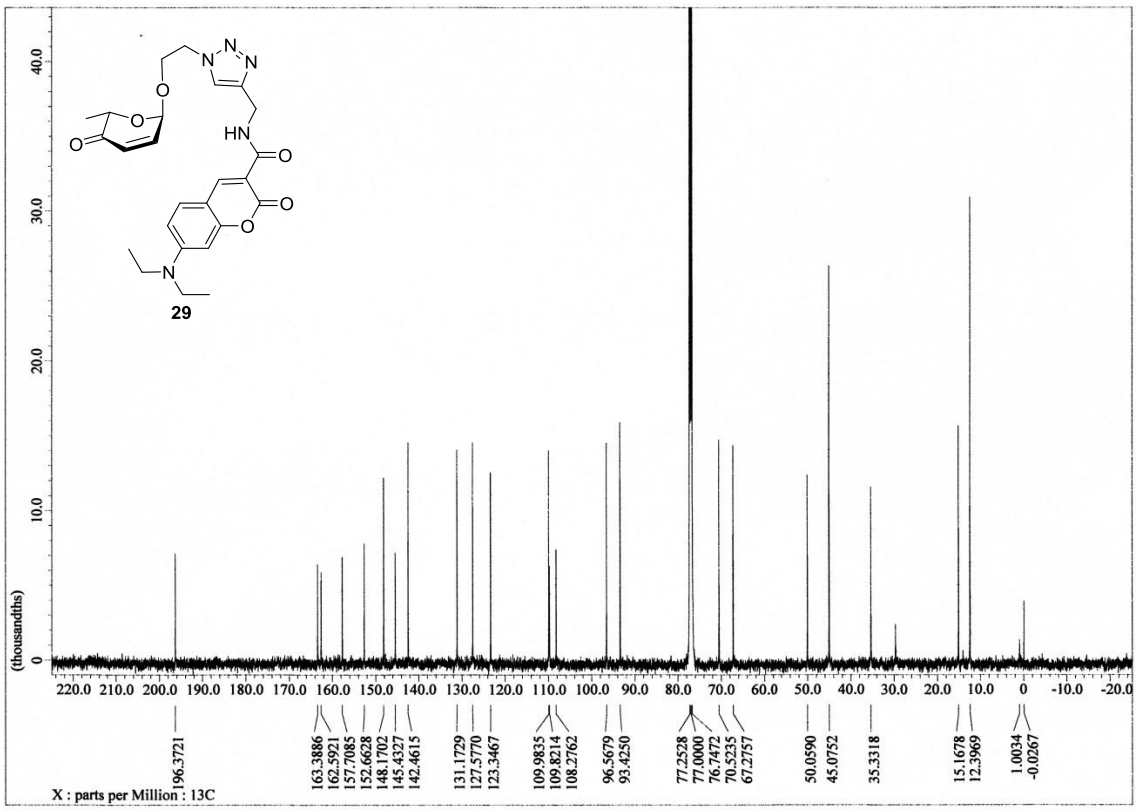

Figure S48 ^{13}C -NMR spectrum of **29**

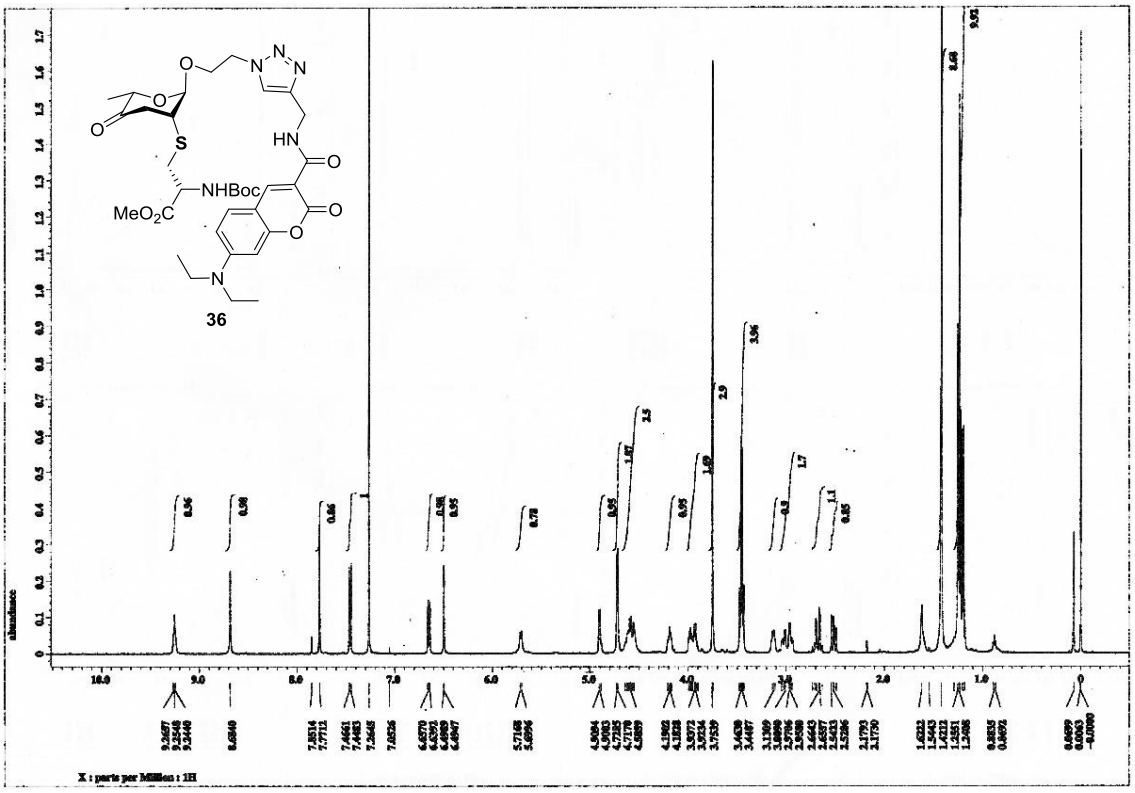

Figure S49 ^{1}H-NMR spectrum of **36**

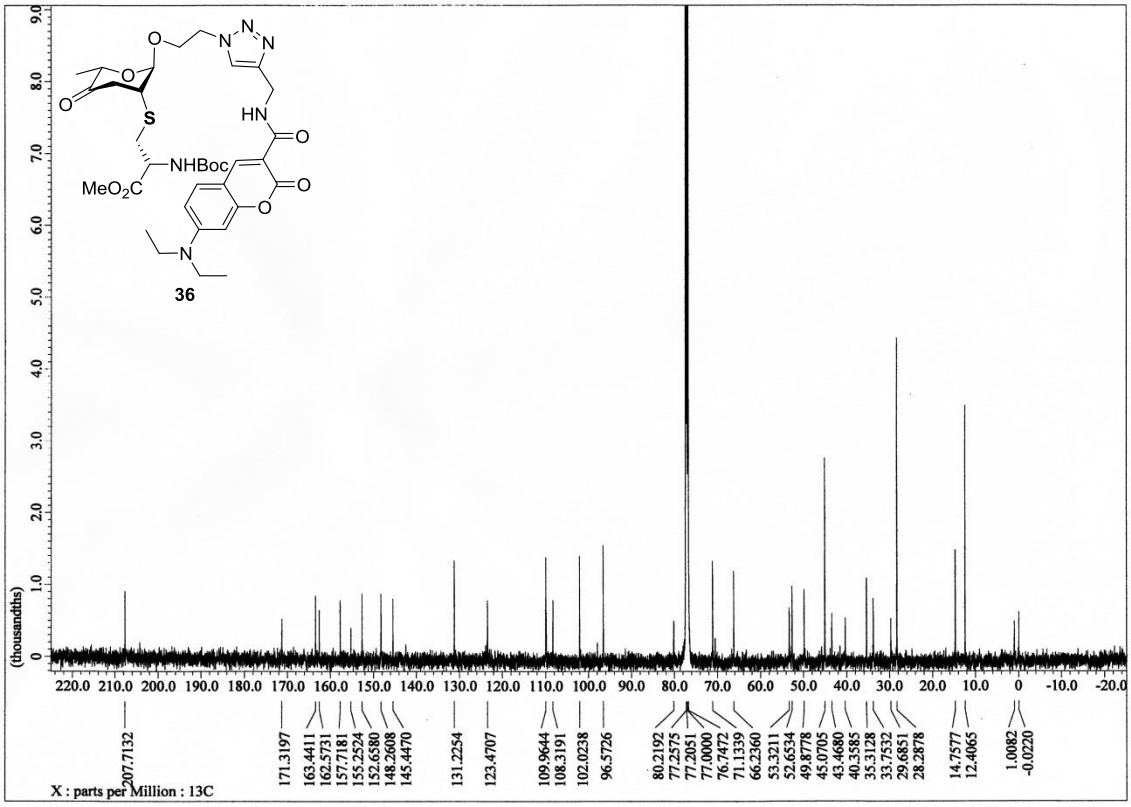

Figure S50 ^{13}C -NMR spectrum of **36**