Carbohydrate SAMs Characterized by Sum-Frequency Generation Spectroscopy

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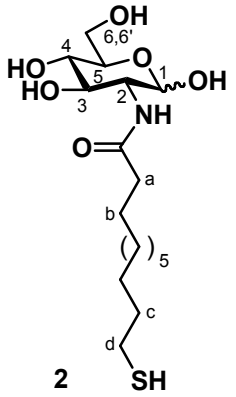
Supporting Information

Materials and Methods: All reagents were purchased from Aldrich unless otherwise mentioned. HOBT and HBTU were purchased from Alfa Aesar. Except as otherwise indicated, reactions were carried out under argon atmosphere. All reactions were monitored using thin layer chromatography on 0.25 mm Dynamic Adsorbents, L.L.C. precoated silica gel (particle size 0.03-0.07 mm, catalog no. 84111, lot #LA2006). Column chromatography was performed using Whatman Purasil 60 Å (230-400 mesh ASTM) silica gel. Yields refer to chromatographically and spectroscopically pure compounds except where noted. A Thermolyne, Maxi-Mix III type 65800 was used for shaking gold slides with the appropriate linker. BD Falcon Petri dishes (50 x 9 mm) from BD Biosciences purchased through VWR was used to house the gold slides and media for SAM. ^{1}H and ^{13}C NMR spectra were recorded on Varian Unity 300, Varian Mercury 400, Varian Unity 500 and Varian 500 Direct Drive System spectrometers. The residual singlet at δ 7.26 ppm and δ 77 ppm for $CDCl_3$, δ 2.50 ppm and δ 39.5 ppm for $(CD_3)_2SO$, δ 3.31 ppm and δ 49.0 ppm for CD_3OD were used as the standard for ^{1}H NMR and ^{13}C NMR spectra respectively. Mass spectra were recorded on a Micromass GCT at 70 eV.

11-mercapto-*N*-((*2R*,*3S*,*4S*,*5S*)-2,4,5-trihydroxy-6-(hydroxymethyl)tetrahydro-2H-pyran-3-yl)undecanamide (2)

1 mL of trifluoroacetic acid was added to 0.15 g (0.241 mmol) of tritylated compound at 0 ˚C (turned a yellow color) and 100 µL of triisopropylsilane was then added drop-wise until the yellow color disappeared. The reaction mixture was stirred for 10 min at 0 ˚C. TFA was removed under reduced pressure and the residue was sequentially washed with ice-cold diethyl ether (3 x 4 mL) and methylenechloride (2 x 5 mL) to afford 0.084 g of *11-mercapto-N-((2R,3S,4S,5S)-2,4,5-trihydroxy-6-(hydroxymethyl)tetrahydro-2H-pyran-3-yl)undecanamide* (**2**) as a white solid in 92% yield. Rf value 0.4 in CH_2Cl_2:MeOH (88:12), mp. = 108 ˚C.



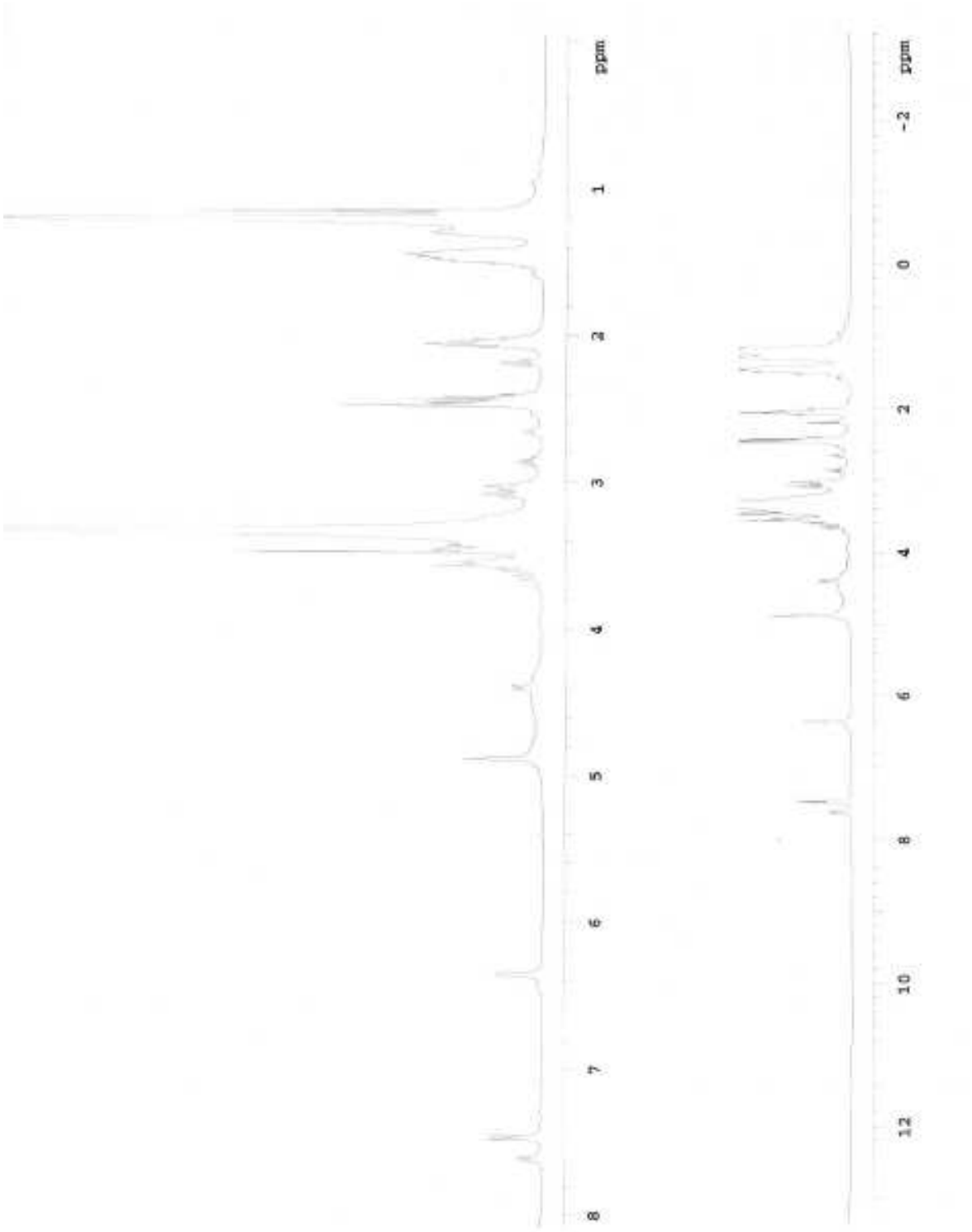
I.R: ν_{max} cm^{-1} 720, 800, 1020, 1060, 1080, 1140, 1380, 1460, 1555, 1620, 1645, 2855, 2905, 2925, 2960, 3100, 3300, 3400.

^{1}H NMR (400 MHz, $(CD_3)_2SO$, (α and β mixture)): δ 1.12-1.36 (m, 12 H, 6 CH_2), 1.38-1.62 (m, 4H, H_b, H_c), 2.00-2.10 (m, 2H, H_a, β), 2.19 (t, 1H, *J* = 8.0 Hz, H_a, α), 2.38-2.52 (m, 3H, H_d, (2 β, 1 α)), 2.66 (t, 1H, *J* = 6.4 Hz, -OH), 2.82-2.90 (m, 1H, H_d, (1 α)), 2.98-3.74 (m, 9H, H_2, H_3, H_4, H_5, H_6, $H_{6'}$, 3-OH, α and β), 4.40 (d, 1H, *J* = 8.0 Hz, -OH), 4.89 (bs, 2H, H_6, (α, β)), 6.36 (bd, 2H, *J* = 6.4 Hz, H_1, (α and β)), 7.47 (d, 1H, *J* = 8.0 Hz, N-H, (β-isomer)), 7.61 (d, 1H, *J* = 8.0 Hz, N-H, (α-isomer)).

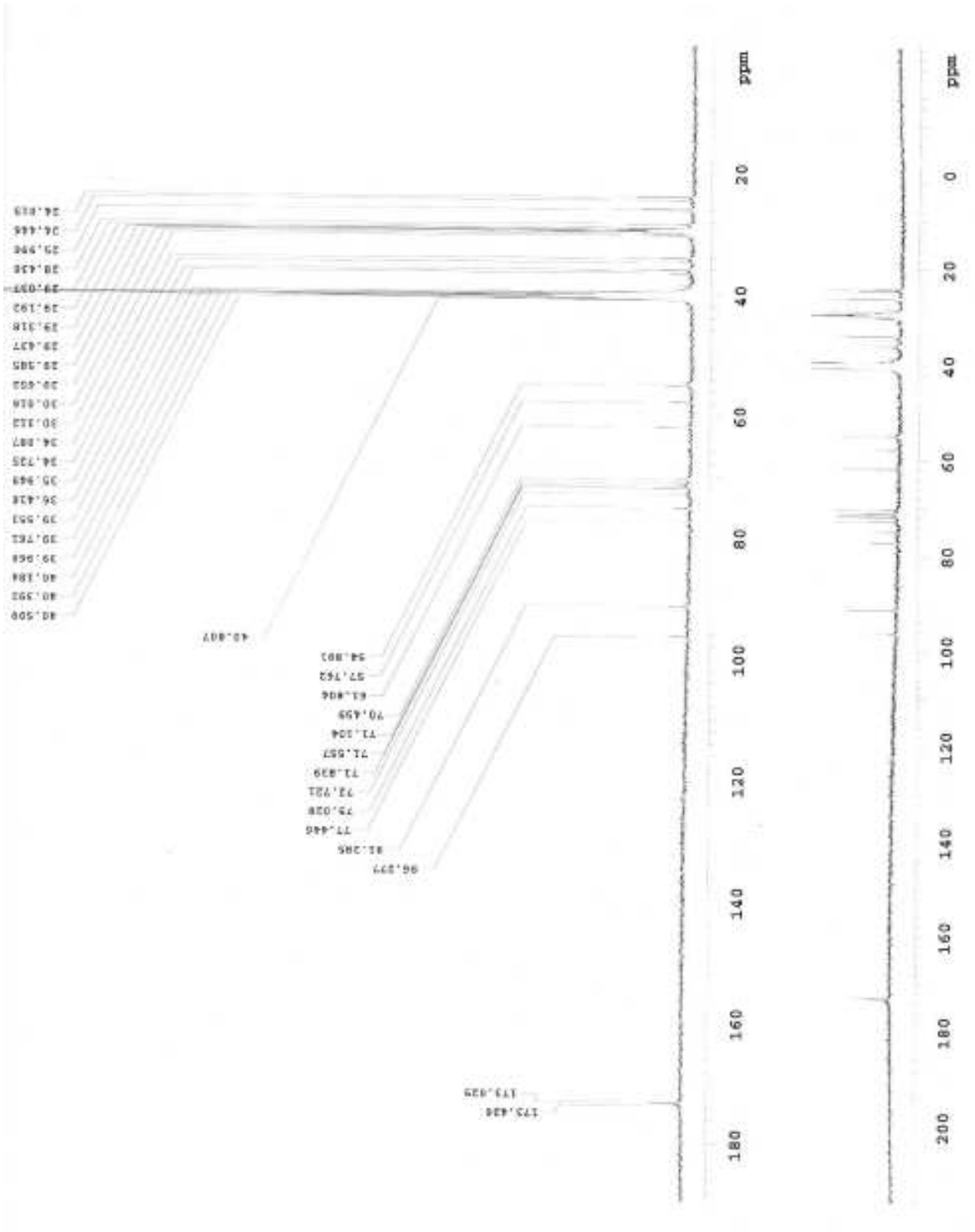
^{13}C NMR (100 MHz, $(CD_3)_2SO$): δ 24.01 (C_b, β), 24.45 C_b, α), 25.99, 28.43, 29.03, 29.19, 29.32, 29.44, 29.58, 29.65, 30.01 (C_c, β), 30.31 (C_c, α), 34.09 (C_d, β), 34.72 (C_d, α), 35.95 (C_a, β), 36.42 (C_a, α), 54.89 (C_2, β), 57.76 (C_2, α), 61.80 (C_5, β), 70.46 (C_6, β), 71.10 (C_6, α), 71.56 (C_5, α), 71.84 (C_4, β), 72.72 (C_3, β), 75.03 (C_4, α), 77.44 (C_3, α), 91.28 (C_1, β), 96.27 (C_1, α), 173.02 (CO, β-isomer), 173.42 (CO, α-isomer).

ESI-MS for $C_{17}H_{33}NO_6S$: calcd, 379.38; found, 402.2 $(M + Na)^+$, 418.1 $(M + K)^+$ and 781.3 $(2M + Na)^+$.

^{1}H NMR of compound- **2** (400 MHz, $(CD_3)_2SO$):

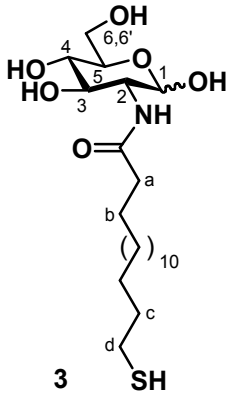


^{13}C NMR of compound- **2** (100 MHz, $(CD_3)_2SO$):



16-mercapto-*N*-((*2R*,*3S*,*4S*,*5S*)-2,4,5-trihydroxy-6-(hydroxymethyl)tetrahydro-2H-pyran-3-yl)hexadecanamide (3)

1 mL of trifluoroacetic acid was added to 0.15 g (0.216 mmol) of tritylated compound at 0 ˚C (turned a yellow color) and 100 µL of triisopropylsilane was then added drop-wise until the yellow color disappeared. The reaction mixture was stirred for 10 min at 0 ˚C. TFA was removed under reduced pressure and the residue was sequentially washed with ice-cold diethyl ether (3 x 4 mL) and methylenechoride (2 x 5 mL) to afford 0.087 g of *16-mercapto-N-((2R,3S,4S,5S)-2,4,5-trihydroxy-6-(hydroxymethyl)tetrahydro-2H-pyran-3-yl)hexadecanamide* (**3**) as a white solid in 90% yield. Rf value 0.4 in CH_2Cl_2:MeOH (88:12), mp. = 106 ˚C.



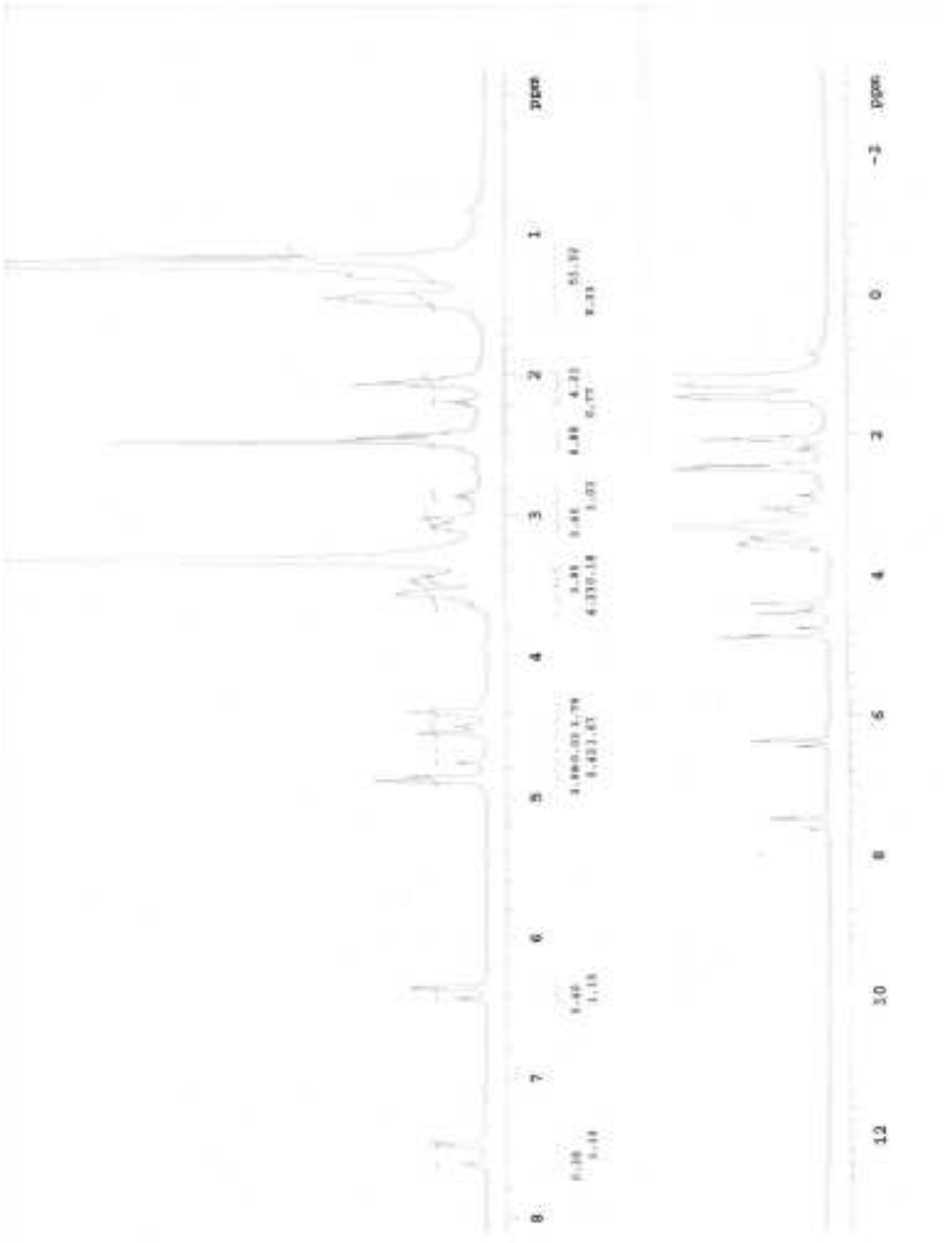
I.R: ν_{max} cm^{-1} 720, 800, 1020, 1065, 1080, 1100, 1115, 1130, 1380, 1460, 1555, 1640, 2850, 2905, 2922, 2960, 3100, 3300, 3400.

^{1}H NMR (400 MHz, $(CD_3)_2SO$, (α and β mixture)): δ 1.11-1.34 (m, 22 H, 11 CH_2), 1.39-1.53 (m, 4H, H_b, H_c), 1.98-2.11 (m, 3H, $2H_a$, (β), $1H_a$, (α)), 2.19 (t, 1H, J = 7.6 Hz, H_a, α), 2.38-2.56 (m, 3H, $2H_d$, (β), $1H_d$, (α)), 2.82-2.90 (m, 1H, $1H_d$, (α)), 2.98-3.68 (m, 9H, H_2, H_3, H_4, H_5, H_6, $H_{6'}$, 3-OH, α and β), 4.36-4.44 (m, 2H), 4.49 (t, 1H, J = 10.8 Hz, -OH), 4.54 (d, 1H, J = 5.2 Hz, H_6), 4.76 (d, 1H, J = 4.8 Hz, -OH), 4.83-4.92 (m, 2H), 6.35 (d, 1H, J = 4.0 Hz, H_1, (α)), 6.43 (d, 1H, J = 6.0 Hz, H_1, (β)), 7.47 (d, 1H, J = 8.0 Hz, N-H, (β-isomer)), 7.61 (d, 1H, J = 8.0 Hz, N-H, (α-isomer)).

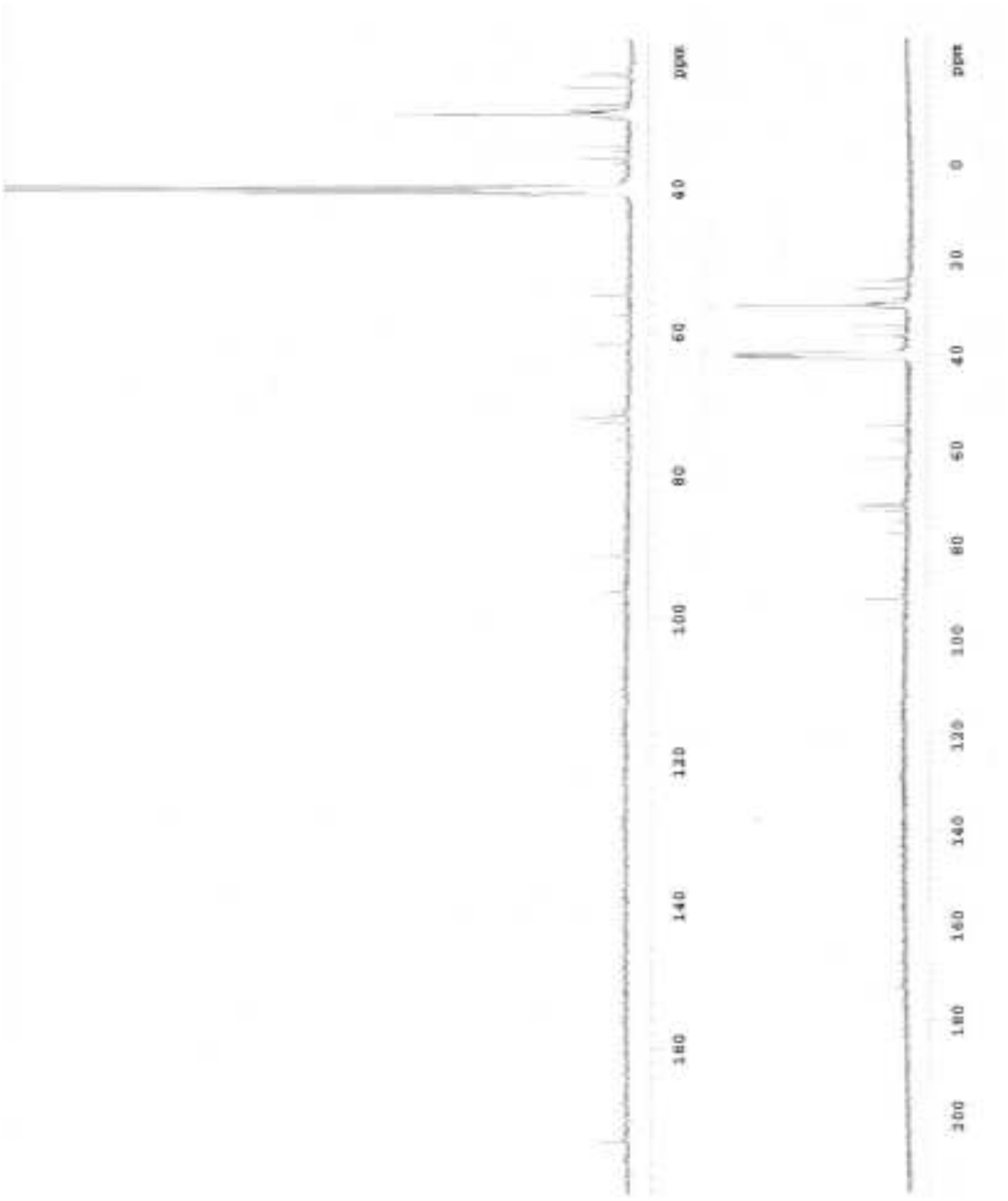
^{13}C NMR (100 MHz, $(CD_3)_2SO$): δ 24.02 (C_b, β), 24.44 (C_b, α), 26.00, 28.42, 29.00, 29.19, 29.28, 29.36, 29.45, 29.66, 29.75, 29.98 (C_c, β), 30.30 (C_c, α), 34.07 (C_d, β), 34.72 (C_d, α), 35.96 (C_a, β), 36.42 (C_a, α), 54.89 (C_2, β), 57.76 (C_2, α), 61.80 (C_5, β), 71.10 (C_6, β), 71.55 (C_6, α), 71.56 (C_5, α), 71.84 (C_4, β), 72.72 (C_3, β), 75.02 (C_4, α), 77.45 (C_3, α), 91.28 (C_1, β), 96.28 (C_1, α), 173.02 (CO, β-isomer), 173.42 (CO, α-isomer).

ESI-MS for $C_{22}H_{43}NO_6S$: calcd, 449.28; found, 448.3 (-ve, M – 1), 472.2 $(M + Na)^+$, 488.2 $(M + K)^+$ and 937.4 $(2M + K)^+$.

^{1}H NMR of compound- **3** (400 MHz, $(CD_3)_2SO$):

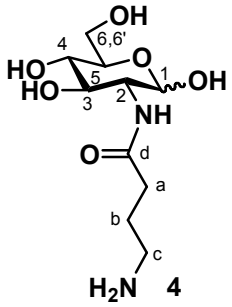


^{13}C NMR of compound- **3** (100 MHz, $(CD_3)_2SO$):



4-amino-*N*-((*2R*,*3S*,*4S*,*5S*)-2,4,5-trihydroxy-6-(hydroxymethyl)tetrahydro-2H-pyran-3-yl)butanamide (4)

2 mL of trifluoroacetic acid was added to 0.2 g (0.54 mmol) of compound **7** at 0 ˚C. After 2 min., 200 μL of anisole was added to the reaction mixture and stirred for 1 h at room temperature. TFA was removed under reduesed pressure and the residue was washed sequentially with ice-cold diethyl ether (3 x 5 mL) and dichloromethane (2 x 5 mL). Solvent was removed under reduced pressure to afford 0.142 g of *4-amino-N-((2R,3S,4S,5S)-2,4,5-trihydroxy-6-(hydroxymethyl)tetrahydro-2H-pyran-3-yl)butanamide* **(4**) as yellow syrup in 98% yield. Rf value 0.1 in CH_2Cl_2:MeOH (85:15).



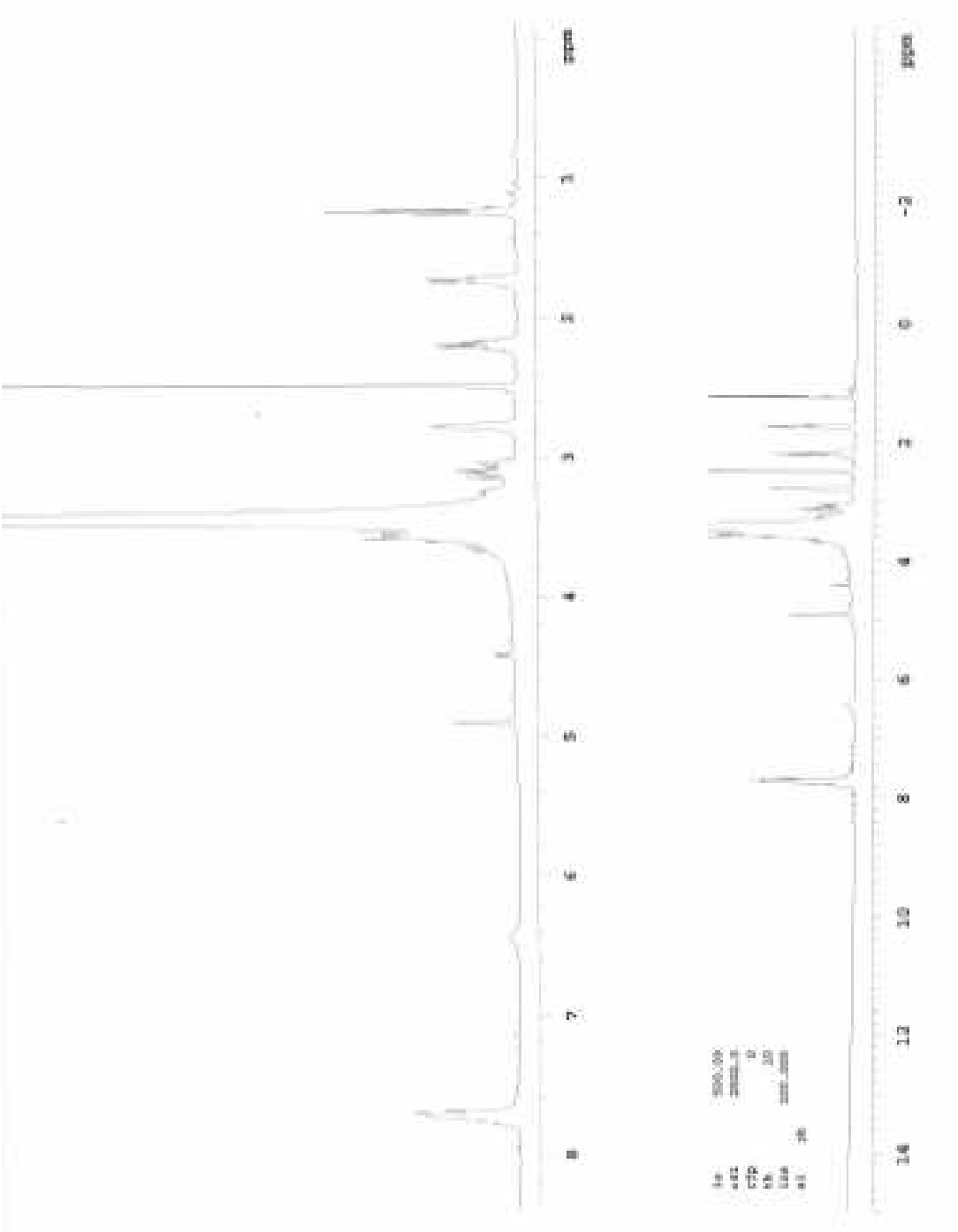
I.R: ν_{max} cm^{-1} 720, 805, 1020, 1080, 1130, 1210, 1420, 1460, 1550, 2855, 2905, 2925, 2960, 3100, 3330, 3400.

^{1}H NMR (500 MHz, $(CD_3)_2SO$, (α and β mixture)): δ 1.20-1.27 (m, 2 H), 1.69-1.78 (m, 2H, H_b), 2.13-2.24 (m, 2H, H_a), 2.83 (m, 2H, H_c), 3.01-3.13 (m, 2H, H_3), 3.14 (bd, J = 1.0 Hz, -OH), 3.22-3.68 (m, 8H), 4.41 (d, 1H, J = 7.5 Hz, H_1, (β)), 4.89 (d, 1H, J = 2.5 Hz, H_1, (α)), 6.44 (bs, 1H, -N<u>H</u>), 7.72 (bs, 1H, -N<u>H</u>$_2$).

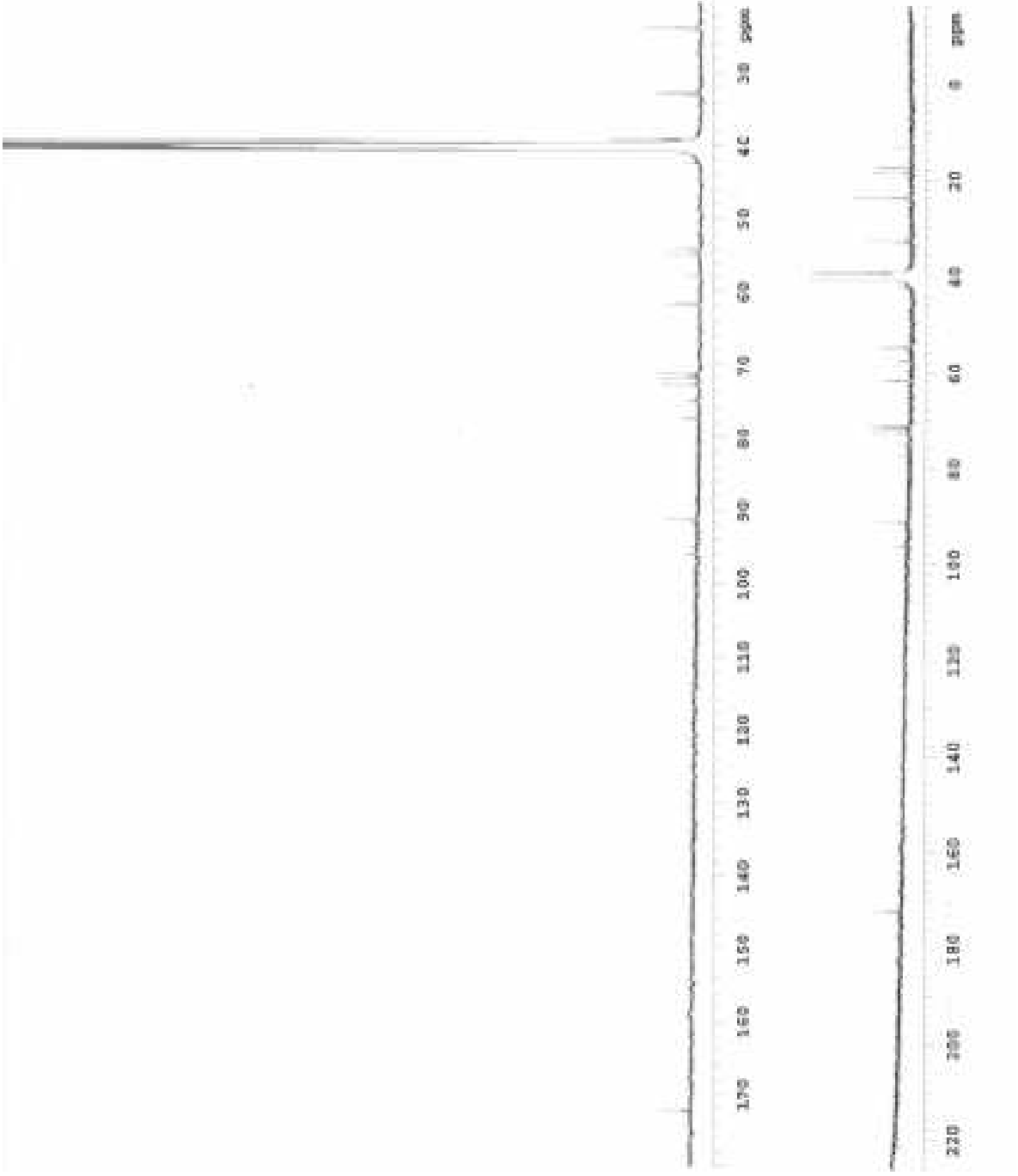
^{13}C NMR (125 MHz, $(CD_3)_2SO$): δ 23.93 (C_b, α and β), 32.67 (C_a, β), 33.18 (C_a, α), 39.26 (C_c, β) , 42.49 (C_c, α), 54.96 (C_2, β), 57.71 (C_2, α), 61.76 (C_6, α and β), 71.14 (C_5, β), 71.48 (C_5, α), 71.79 (C_4, β), 72.76 (C_3, β), 74.95 (C_4, α), 77.49 (C_3, α), 91.27 (C_1, β), 96.06 (C_1, α), 172.12 (CO, C_d of β-isomer), 172.22 (CO, C_d of α-isomer).

HRMS: EIMS (M^+) calcd for $C_{10}H_{20}N_2O_6$ 264.1321, found 265.3 (M + H).

^{1}H NMR of compound- **4** (500 MHz, $(CD_3)_2SO$):

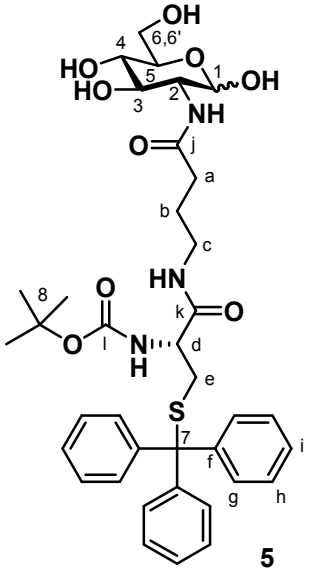


^{13}C NMR of compound- **4** (125 MHz, $(CD_3)_2SO$):



tert-butyl-(*2R*)-1-oxo-1-(4-oxo-4-((*2R*,*3S*,*4S*,*5S*)-2,4,5-trihydroxy-6-(hydroxymethyl)tetrahydro-2H-pyran-3-ylamino)butylamino)-3-(tritylthio)propan-2-ylcarbamate (5)

0.240 g (0.905 mmol) of compound **4** was dissolved in 1.0 mL of dry DMSO and then added drop-wise to a mixture of 0.3 g (0.647 mmol) of (*R*)-2-(tert-butoxycarbonylamino)-3-(tritylthio)propanoic acid (NH-Boc, S-Tr, *L*-cysteine), 0.245 g (0.647 mmol) of HBTU, 0.087 g (0.647 mmol) of HOBT and 0.225 mL (1.284 mmol) of DIPEA in 3 mL of dry DMF and stirred for 12 h at room temperature. After completion of the reaction, as denoted by TLC, the reaction mixture was added in a drop-wise fashion to ice-cold diethylether (50 mL) and stirred at 0 ˚C for 15 min. The precipitate was collected on filter paper and washed twice with ice-cold ether (2 x 25 mL). The residue solid was purified by silica gel column chromatography using dichloromethane and methanol (92:8) to afford 0.312 g of *tert-butyl (2R)-1-oxo-1-(4-oxo-4-((2R,3S,4S,5S)-2,4,5-trihydroxy-6-(hydroxymethyl)tetrahydro-2H-pyran-3-ylamino)butylamino)-3-(tritylthio)propan-2-ylcarbamate* **(5)** as a light yellow solid in 68% yield. Rf value 0.55 in CH_2Cl_2:MeOH (91:9), mp. = 126 ˚C.



5

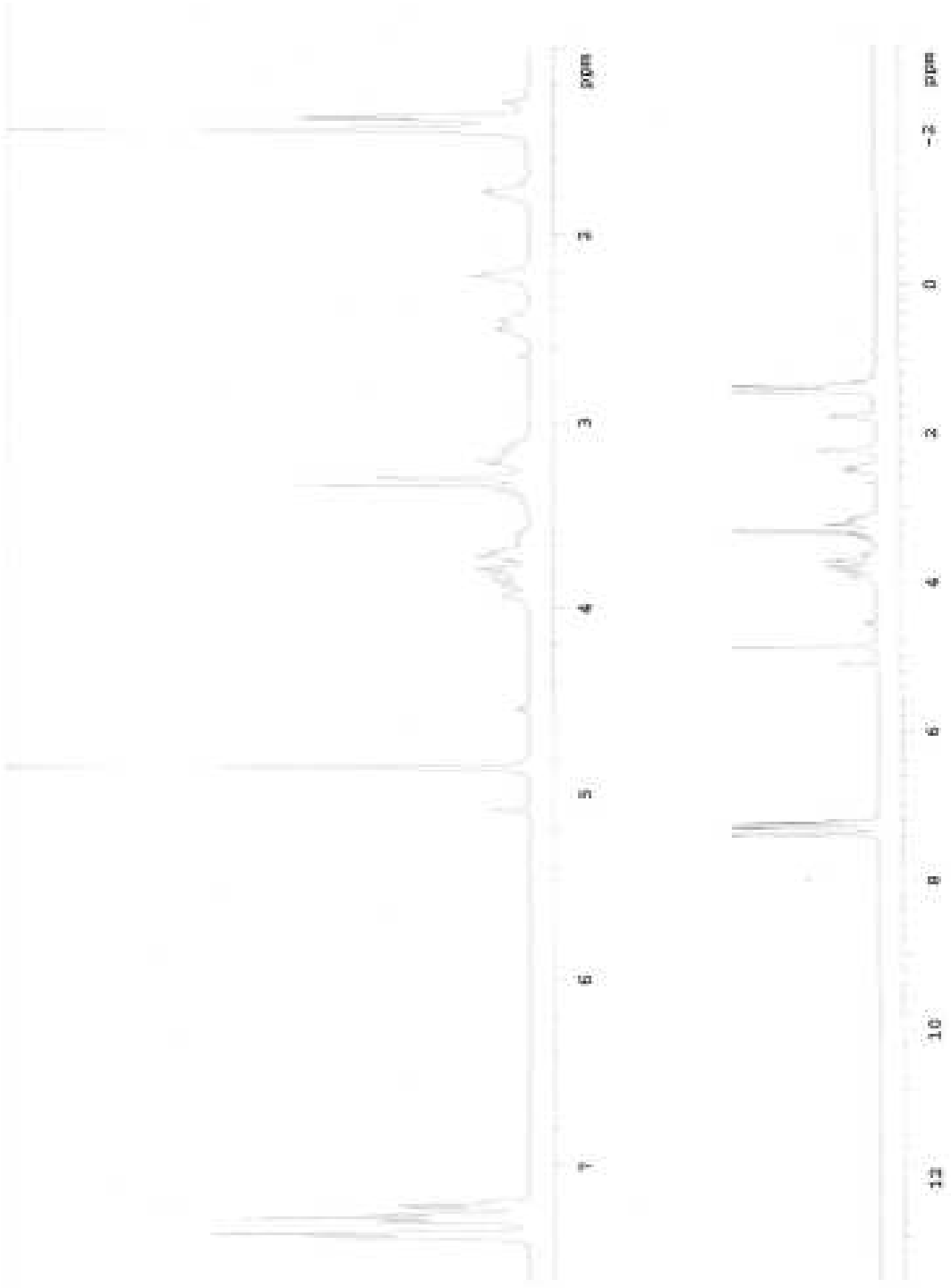
I.R: ν_{max} cm^{-1} 698, 743, 843, 1022, 1165, 1368, 1443, 1540, 1652, 2849, 2919, 3409

^{1}H NMR (400 MHz, CD_3OD, (α and β mixture)): δ 1.34-1.47 (s and d 9 H, J = 8.0 Hz, Boc- C$\underline{H}_3$, this may be due to long range coupling with Ph-H), 1.68-1.84 (m, 2H, H_b), 2.21 (t, 2H, J = 6.4 Hz, H_a), 2.40-2.56 (m, 2H, H_c), 3.08-3.26 (m, 3H, $2H_e$, -OH), 3.32-3.47 (m, 3H, H_2, H_4, H_5), 3.58-4.00 (m, 6H), 4.54 (d, 1H, J = 9.2 Hz, H_1, β), 5.08 (d, 1H, J = 2.4 Hz, H_1, α), 7.21 (t, 3H, J = 7.6 Hz, Ar-$\underline{H}$, H_i), 7.28 (t 6H, J = 7.2 Hz, Ar-$\underline{H}$, H_h), 7.37 (d, 6H, J = 8.0 Hz, Ar-$\underline{H}$, H_g).

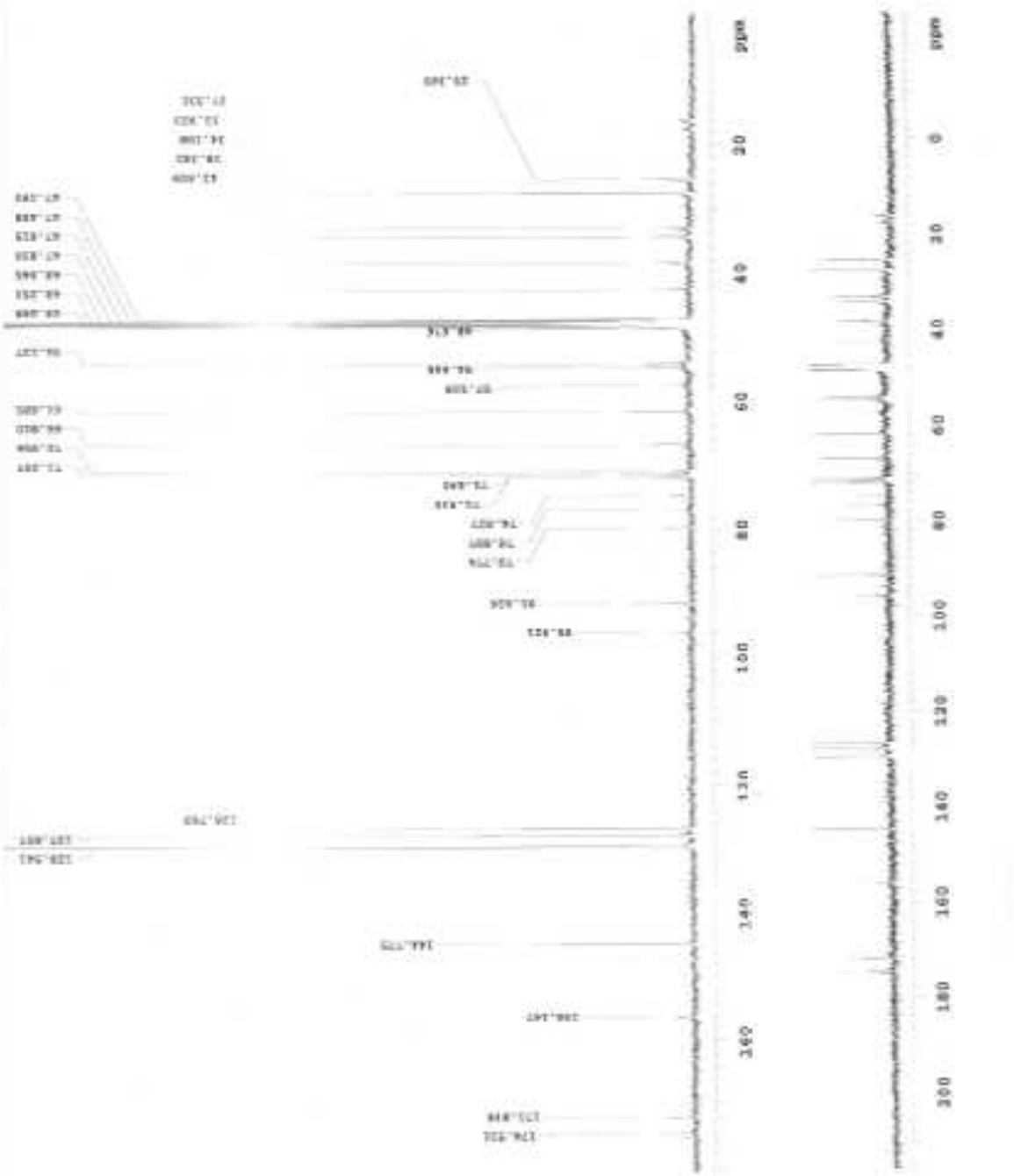
^{13}C NMR (100 MHz, CD_3OD): δ 25.36 (C_b), 27.53 (Boc $\underline{C}H_3$), 32.92 (C_a, α and β), 34.19 (C_e, α and β), 38.38 (C_c, β), 42.61 (C_c, α) , 48.67 (C_d, β), 54.12 (C_d, α) 54.64 (C_2, β), 57.54 (C_2, α), 61.68 (C_6, α and β), 70.95 (C_7, α and β), 71.29 (C_5, β), 71.49 (C_4, β),

71.93 (C_3, β), 74.83 (C_5, α), 76.89 (C_4, α), 79.77 (C_3, α), 91.42 (C_1, β), 93.67 (C_8, α and β), 95.92 (C_1, α), 126.76 (C_i), 127.86 (C_g), 129.54 (C_h), 144.77 (C_f), 156.16 (CO, C_l), 171.89 (CO, C_k), 174.53 (CO, C_j).
ESI-MS for $C_{37}H_{47}N_3O_8S$: calcd, 709.3033; found, 708.3 (M – H, -ve), 732.2 $(M + Na)^+$, 748.3 $(M + K)^+$ and 1441.4 $(2M + Na)^+$.

^{1}H NMR of compound- **5** (400 MHz, (CD_3OD):



^{13}CNMR of compound- **5** (100 MHz, (CD_3OD):



4-((*R*)-2-amino-3-mercaptopropanamido)-*N*-((*2R*,*3S*,*4S*,*5S*)-2,4,5-trihydroxy-6-(hydroxymethyl)tetrahydro-2H-pyran-3-yl)butanamide (**6**)

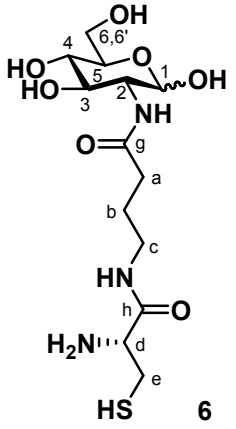
Method 1: 1 mL of trifluoroacetic acid was added to 0.080 g (0.112 mmol) of compound **5** at 0 ˚C and 50 µL of triisopropylsilane was added in a drop-wise fashion until the yellow color disappeared. The reaction mixture was subsequently stirred for 10 min at 0 ˚C. TFA was removed under reduced pressure and the residue was sequentially washed with ice-cold diethyl ether (3 x 4 mL) and dichloromethane (2 x 5 mL) to afford 0.033 g of *4-((R)-2-amino-3-mercaptopropanamido)-N-((2R,3S,4S,5S)-2,4,5-trihydroxy-6-(hydroxymethyl)tetrahydro-2H-pyran-3-yl)butanamide* (**6**) as a white solid in 81% yield. Rf value 0.05 in CH_2Cl_2:MeOH (85:15). mp. = 96 ˚C.

Fmoc deprotection strategy to get compound **6**.

Method 2: 0.050 g (0.084 mmol) of compound **11** was taken in 1 mL of dry DMF and added 100 µL of TBAF (1 mol solution in THF) at 0 ˚C and stirred at rt for 10 min and immediately quenched with 3- 4 drops of MeOH and removed the solvent under reduced pressure then sequentially washed with ice-cold diethyl ether (3 x 4 mL) and dichloromethane (2 x 5 mL) to get 0.024 g of (**6**) as a white solid in 78% yield.

Method 3: 0.050 g (0.084 mmol) of compound **11** was taken up in 1 mL of dry DMF and then 100 µL of 20% piperidine in DMF was added to the mixture at 0 ˚C. It was stirred at room temperature for 10 min and immediately quenched with 2-3 drops of MeOH. The solvents and piperidine were removed under reduced pressure then sequentially washed with ice-cold diethyl ether (3 x 4 mL) and dichloromethane (2 x 5 mL) to afford 0.026 g of (**6**) as a white solid in 84% yield.

(**Method 1** yields more pure compound of **6** than either **Method 2** or **3**. **Methods 2** and **3** require recrystallization with MeOH and Et_2O for obtaining pure compound.)



6

I.R: ν_{max} cm^{-1} 720, 805, 1020, 1080, 1130, 1210, 1420, 1460, 1550, 1640, 1680, 2855, 2905, 2925, 2960, 3100, 3300, 3400.

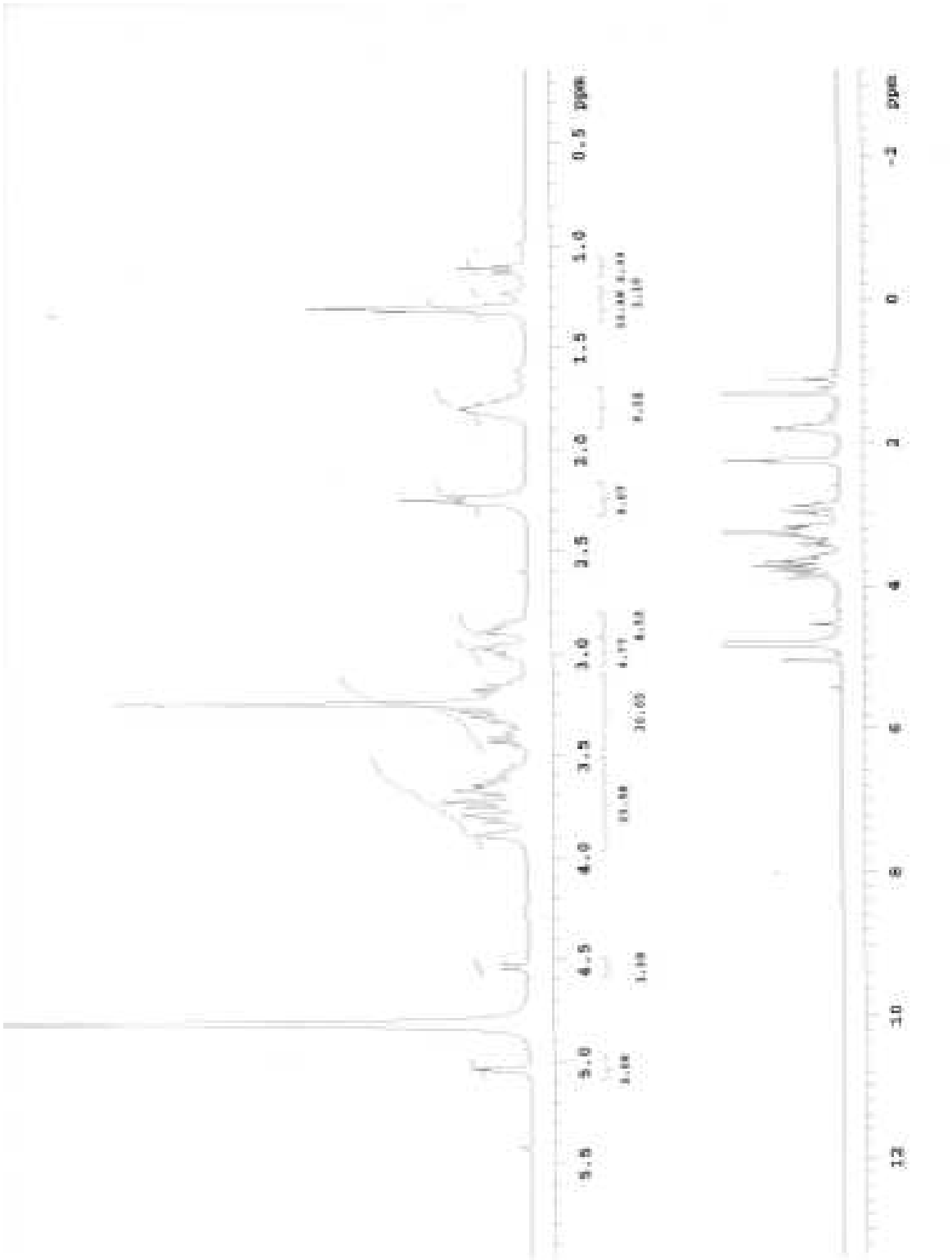
^{1}H NMR (400 MHz, CD_3OD, (α and β mixture): δ 1.64 (d, 1H, *J* = 18.8 Hz, -SH), 1.72-1.87 (m, 2H, H_b), 2.24 (t, 2H, *J* = 6.4 Hz, H_a), 2.83-2.92 (m, 1H, H_e), 2.94-3.40 (m, 1H,

H_c), 3.13-3.47 (m, 3H), 3.54- 3.84 (m, 4H), 3.88 (t, 1H, J = 5.6 Hz, H_d), 4.53 (d, 1H, J = 9.2 Hz, H_1, (β)), 5.02 (d, 1H, J = 4.0 Hz, H_1, (α)).

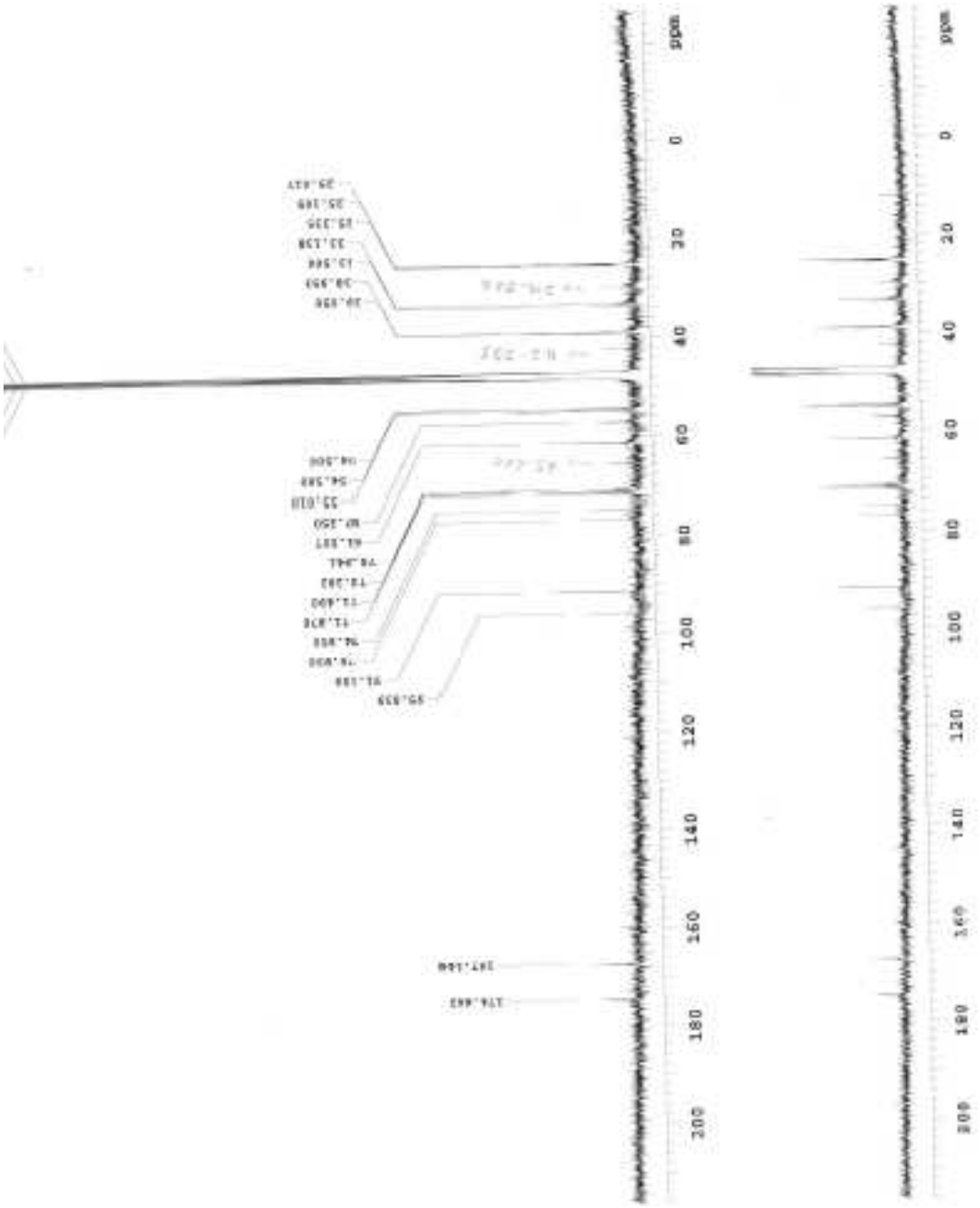
^{13}C NMR (100 MHz, CD_3OD, (α and β mixture): δ 25.01 (C_b, β), 25.16 (C_b, α), 25.33 (C_e, β), 29.82 (C_e, α), 33.14 (C_a, β), 33.57 (C_a, α), 38.95 (C_c, β), 39.05 (C_c, α), 54.50 (C_2, β), 55.01 (C_d, α and β), 57.25 (C_2, α), 61.50 (C_6, α and β), 70.94 (C_5, α), 71.28 (C_5, β), 71.49 (C_4, β), 71.87 (C_3, β), 74.85 (C_4, α), 76.83 (C_3, α), 91.36 (C_1, β), 95.84 (C_1, α), 167.14 (CO, C_h, α and β-isomer), 174.44 (CO, C_g, β), 174.96 (CO, C_g, α).

ESI-MS for $C_{13}H_{25}N_3O_7S$: calcd, 367.1413; found, 368.2 (M + H), 374.3 $(M + Li)^+$, 390.2 $(M + Na)^+$ and 741.3 $(2M + Li)^+$, 757.3 $(2M + Na)^+$.

^{1}H NMR of compound- **6** (400 MHz, (CD_3OD):



^{13}C NMR of compound- **6** (100 MHz, (CD_3OD):



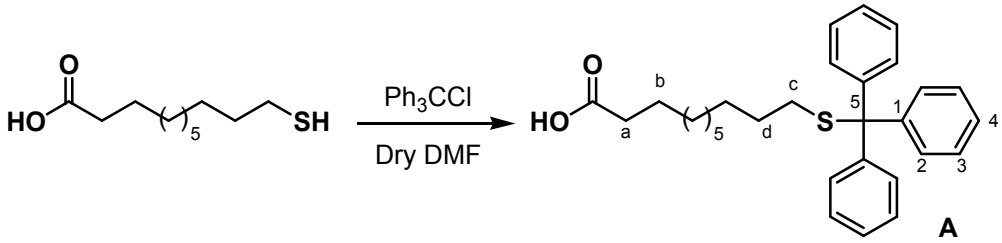
SAM attachment of maleimide and thiol-linked carbohydrates.

To a Petri-dish containing thin layered gold slides (120 mm x 90 mm x 0.8 mm) was added 5 mL of vigorously dried MeOH and thiol-maleimide linker (a final concentration of 2M solution). The Petri-dish was then covered and placed on a rotary shaker for 48 hr stirring at 220 rpm at room temperature. After the elapsed period of time, the gold slides were washed successfully (5 x 5 mL) with MeOH. Each adlayer went through this exact protocol.

Other Synthetic Supplementary Information (All compounds that were synthesized)

11-(tritylthio)undecanoic acid (A)

1.0 g (4.58 mmol) of 11-mercaptoundecanoic acid and 1.276 g (4.58 mmol) of chlorotriphenylmethane were taken up in 7 mL of dry DMF and stirred at room temperature for 6 h under an atmosphere of argon. The reaction was monitored by TLC. After completion of the reaction, as denoted by TLC, 50 mL of H_2O was added to the reaction mixture and then it was extracted with ethyl acetate (2 x 50 mL). The organic layer was washed with water (50 mL), brine (40 mL) and dried over Na_2SO_4. The ethyl acetate was evaporated under reduced pressure and the remaining residue was purified by silica column chromatography using ethyl acetate and hexane (40:60) to afford 1.980 g of *11-(tritylthio)undecanoic acid* (**A**) as a white solid in 94% yield. R_f value 0.5 in EtOAc:Hexane (40:60). mp. = 56 ˚C.



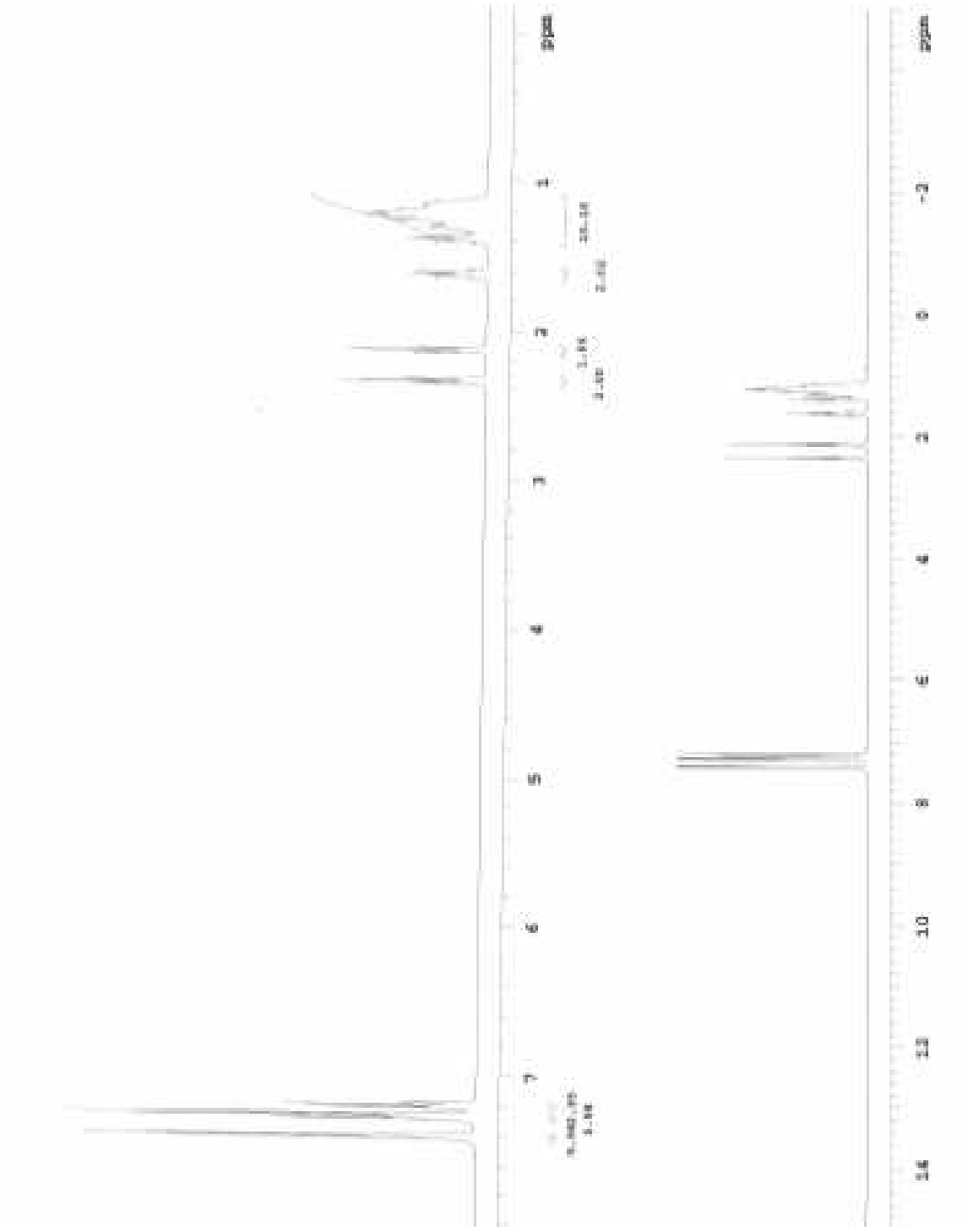
I.R: ν_{max} cm^{-1} 700, 740, 758, 1440, 1446, 1468, 1486, 1592, 2852, 2926, 3016, 3028, 3055.

^1H NMR (500 MHz, $CDCl_3$): δ 1.10-1.32 (m, 12 H, 6 CH_2), 1.38 (quintet, 2H, J = 7.5 Hz, H_b), 1.62 (quintet, 2H, J = 7.5 Hz, H_d), 2.13 (t, 2H, J = 7.5 Hz, H_a), 2.34 (t, 2H, J = 7.5 Hz, H_c), 7.20 (t, 3H, J = 7.0 Hz, H_4), 7.27 (t, 6H, J = 7.5 Hz, H_2), 7.42 (d, 6H, J = 8.0 Hz, H_3).

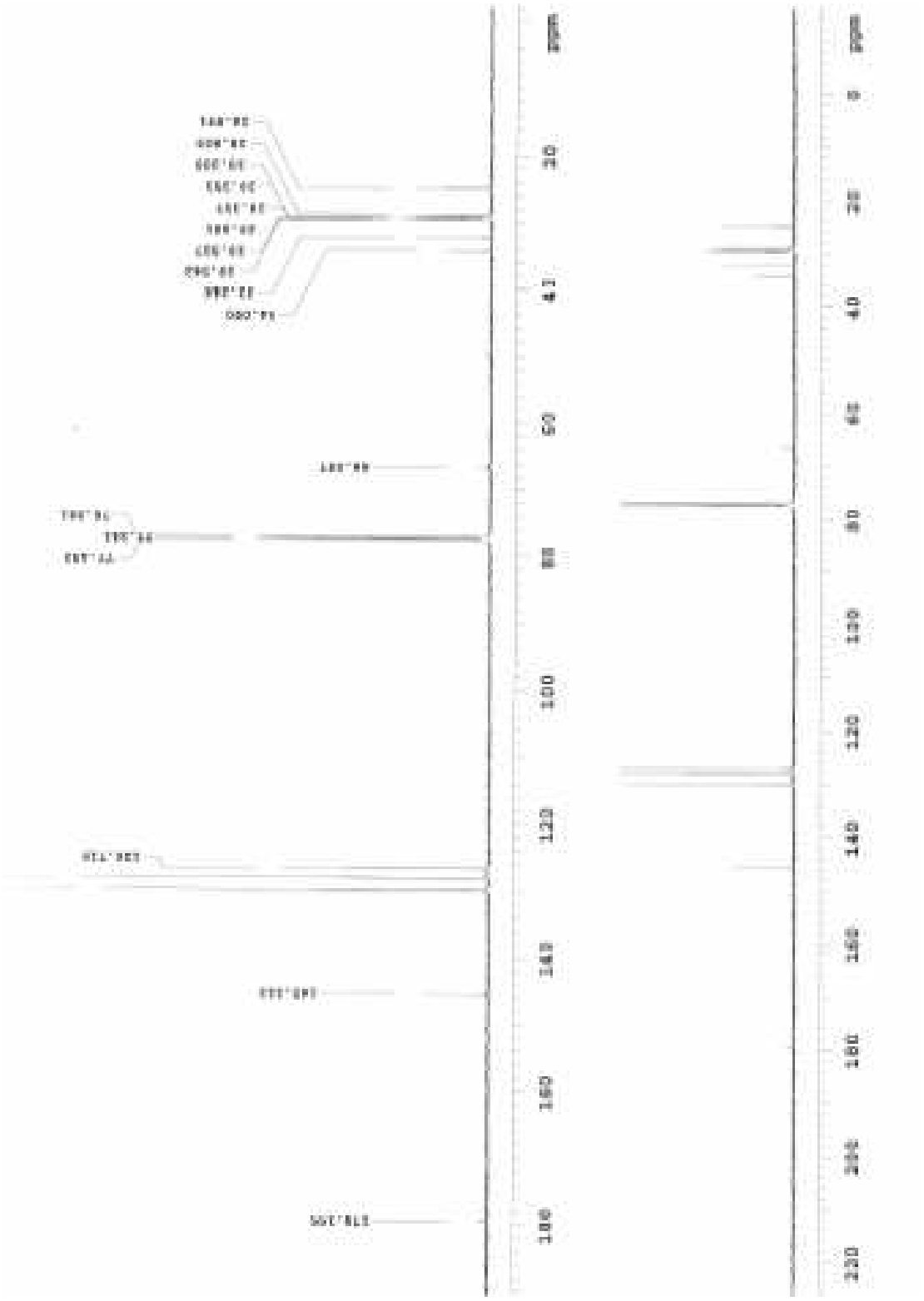
^{13}C NMR (125 MHz, $CDCl_3$): δ 24.89 (C_b), 28.80, 29.21, 29.25, 29.35, 29.40, 29.52, 32.24 (C_c), 34.09 (C_a), 66.59 (C_5), 126.71 (C_4), 128.01 (C_3), 129.84 (C_2), 145.32 (C_1), 179.35 (CO).

ESI-MS for $C_{30}H_{36}O_2S$: calcd, 460.2436; found, 459.2 (-ve, M - H)$^+$, 460.2 (M)$^+$ and 919.4 (2M - H)$^+$.

^{1}H NMR of compound- **A** (500 MHz, $CDCl_3$):

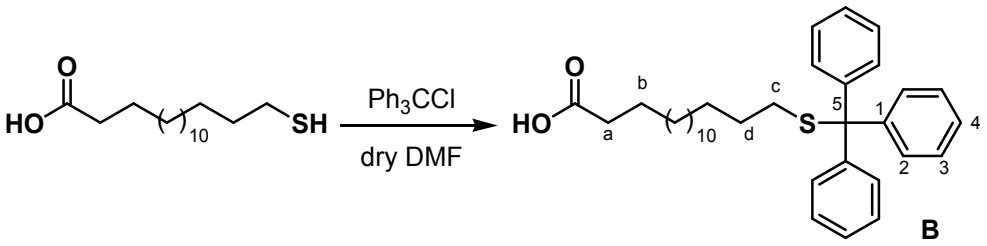


^{13}C NMR of compound- **A** (500 MHz, $CDCl_3$):



16-(tritylthio)hexadecanoic acid (B)

0.290 g (1.00 mmol) of 16-mercaptohexadecanoic acid and 0.280 g (1.00 mmol) of chlorotriphenylmethane were taken up in 4 mL dry DMF and the same procedure as described for compound **1** was followed. The solvent was evaporated under reduced pressure and purified by silica gel column chromatography using ethyl acetate and hexane (40:60) to afford 0.490 g of *16-(tritylthio)hexadecanoic acid* (**B**) as a white solid in 92% yield. Rf value 0.55 in EtOAc:Hexane (50:50). mp. = 58 ˚C.



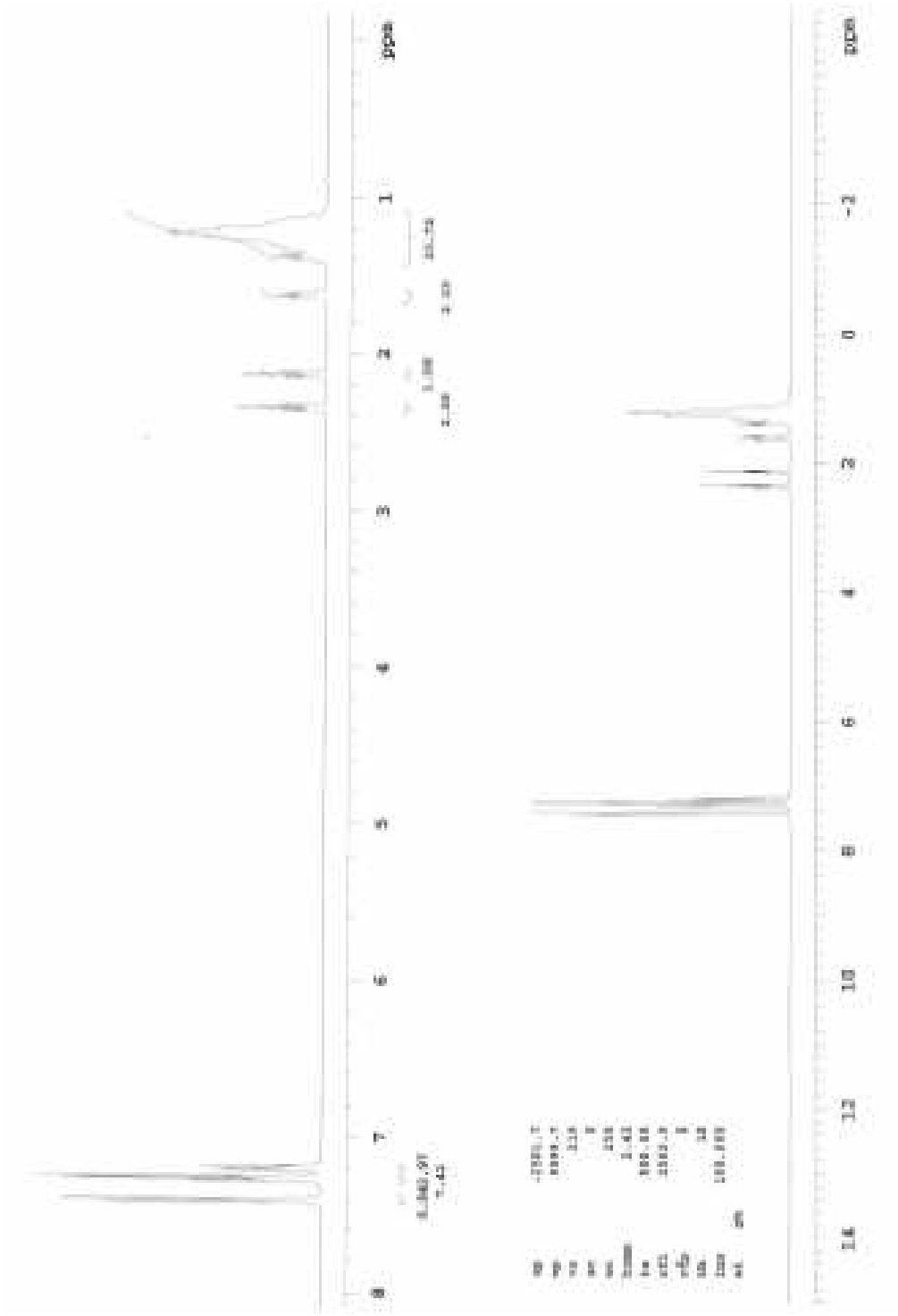
I.R: ν_{max} cm^{-1} 699, 741, 758, 1035, 1467, 1486, 1594, 1707, 2850, 2922, 3016, 3028, 3055.

^{1}H NMR (500 MHz, $CDCl_3$): δ 1.10-1.36 (m, 22 H, 11 CH_2), 1.38 (quintet, 2H, J = 8.0 Hz, H_b), 1.63 (quintet, 2H, J = 7.0 Hz, H_d), 2.13 (t, 2H, J = 7.5 Hz, H_a), 2.34 (t, 2H, J = 7.5 Hz, H_c), 7.20 (t, 3H, J = 7.5 Hz, H_4), 7.27 (t, 6H, J = 7.0 Hz, H_2), 7.40 (d, 6H, J = 7.5 Hz, H_3).

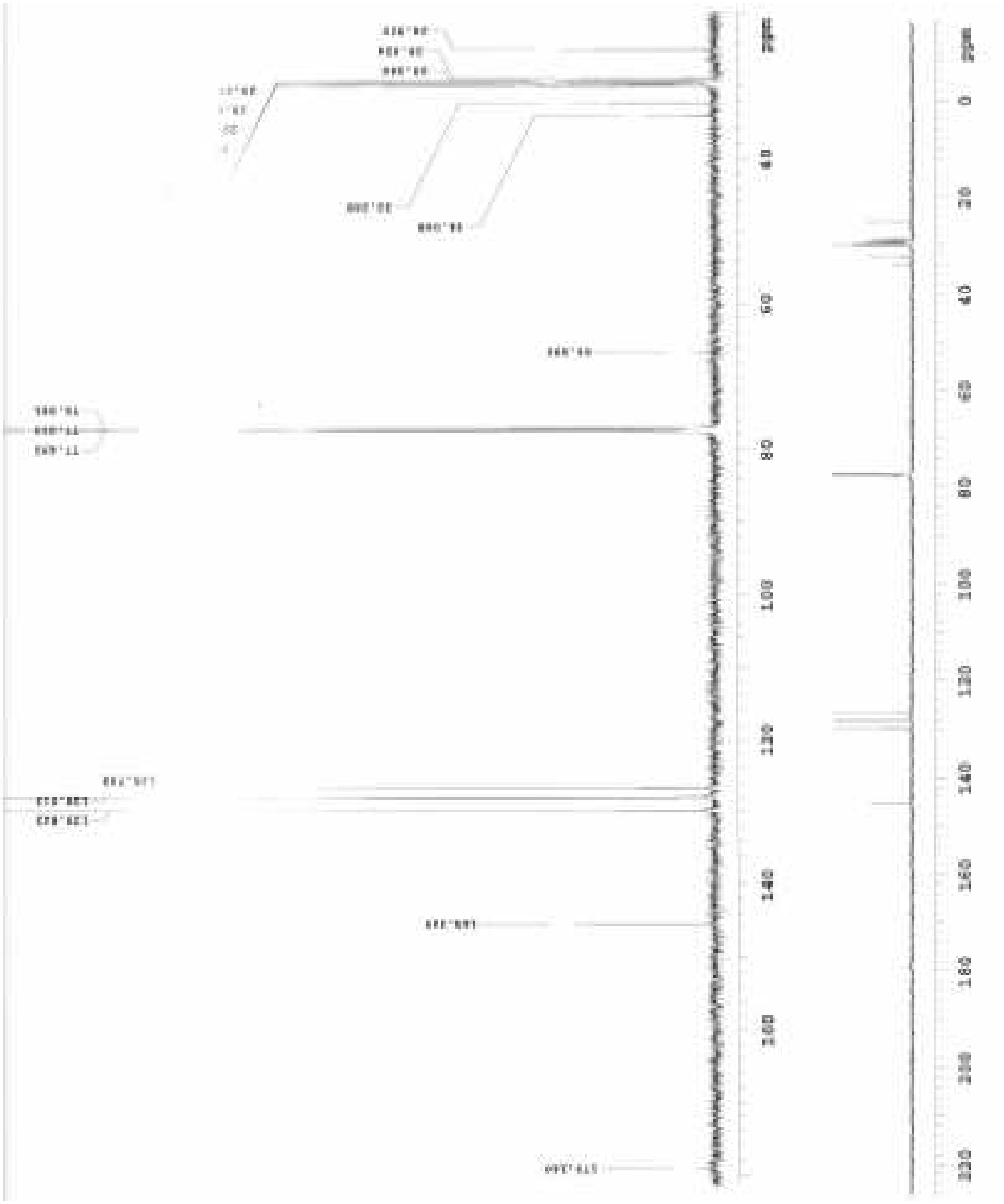
^{13}C NMR (100 MHz, $CDCl_3$): δ 24.92 (C_b), 28.82, 29.24, 29.24, 29.29, 29.40, 29.69, 29.70, 29.83, 29.87, 29.90, 32.26 (C_c), 34.06 (C_a), 66.59 (C_5), 126.70 (C_4), 128.01 (C_3), 129.84 (C_2), 145.32 (C_1), 179.14 (CO).

ESI-MS for $C_{35}H_{46}O_2S$: calcd, 530.8035; found, 529.3 (-ve, M - H)$^+$, 530.3 (M)$^+$,and 1059.6 (2M - H)$^+$ and 1060.6 (2M)$^+$.

^{1}H NMR of compound- **B** (500 MHz, $CDCl_3$):

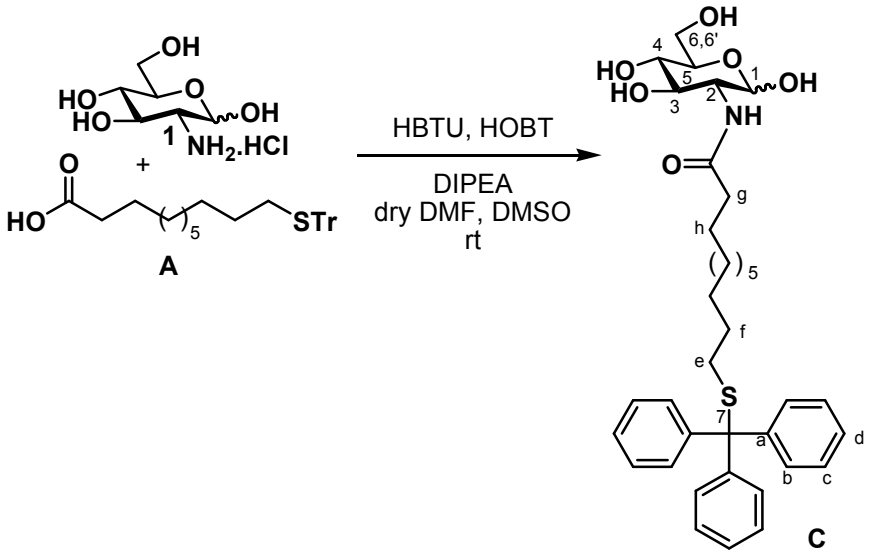


^{13}C NMR of compound- **B** (500 MHz, $CDCl_3$):



***N*-((*2R,3S,4S,5S*)-2,4,5-trihydroxy-6-(hydroxymethyl)tetrahydro-2H-pyran-3-yl)-11-(tritylthio)undecanamide (C)**

0.224 g (1.038 mmol) of glucosamine hydrochloride (**1**) was dissolved in 1 mL of dry DMSO and to it was added 0.225 mL (1.30 mmol) of DIPEA at room temperature. The mixture was then stirred for 15 min. To this solution was added (drop-wise) 0.3 g (0.65 mmol) of acid **A**, 0.18 g (0.65 mmol) of HBTU, 0.088 g (0.65 mmol) of HOBT and 0.225 mL (1.30 mmol) of DIPEA in 3 mL of dry DMF. The resulting mixture was stirred for 12 h at room temperature. After completion of the reaction, as denoted by TLC, the reaction mixture was added to ice-cold diethylether (50 mL) in a drop-wise fashion and stirred at 0 ˚C for 15 min. The precipitate was filtered and washed twice with ice-cold ether (2 x 25 mL). The residue was purified by silica gel column chromatography using dichloromethane and methanol (92:08) to afford 0.270 g of *N-((2R,3S,4S,5S)-2,4,5-trihydroxy-6-(hydroxymethyl)tetrahydro-2H-pyran-3-yl)-11-(tritylthio)undecanamide* (**C**) as an inseparable mixture of α and β anomers in 1.5:1 ratio as noted by ^{1}H NMR. **C** was a white solid obtained in 68% yield. Rf value 0.4 in CH_2Cl_2:MeOH (88:12). mp. = 98 ˚C.



I.R: ν_{max} cm^{-1} 1025, 1128, 1384, 1469, 1554, 1643, 2851, 2922, 3396, 3409.

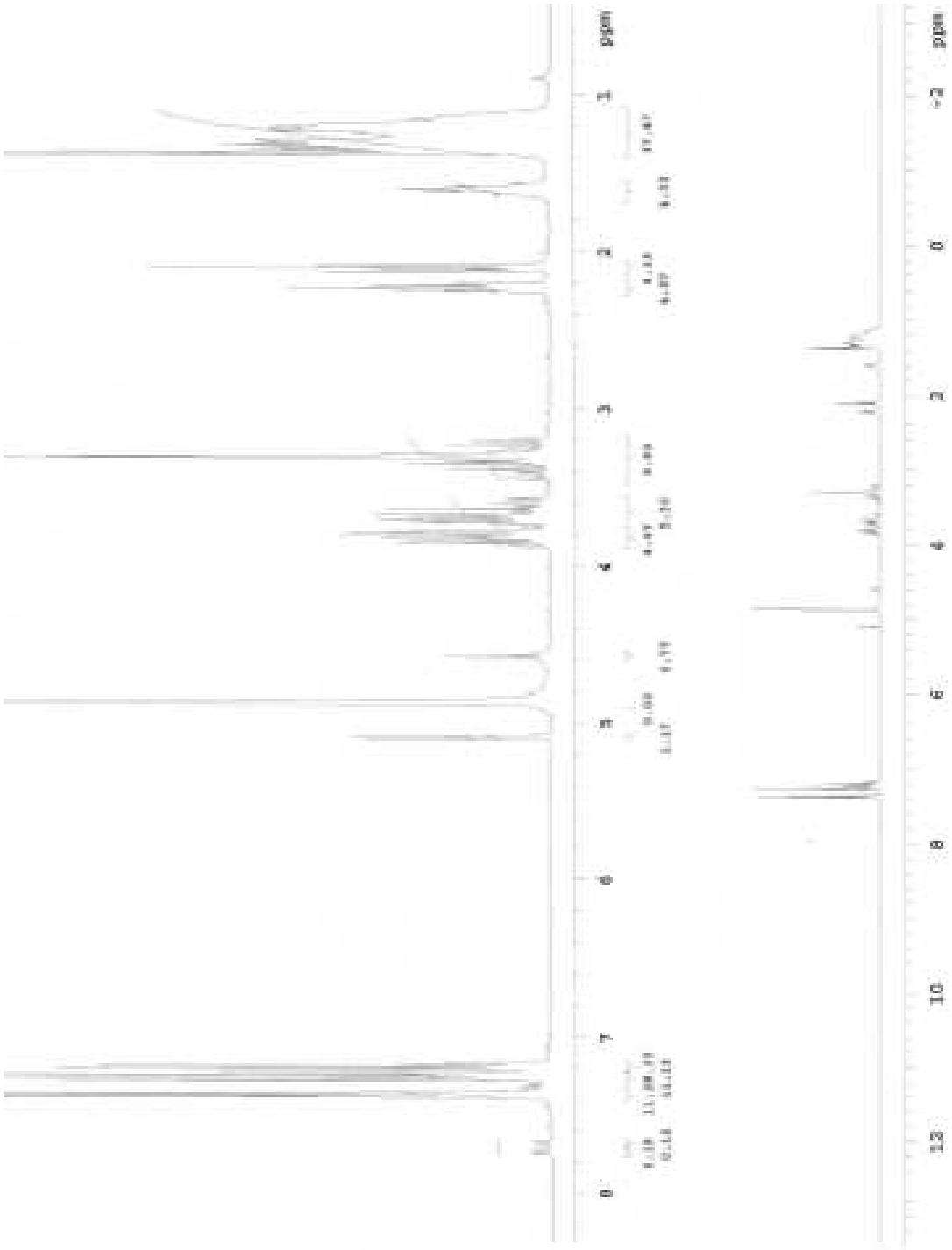
^{1}H NMR (400 MHz, CD_3OD): δ 1.08-1.38 (m, 16 H, $2H_f$, 7 CH_2), 1.59 (quintet, 2H, J = 7.6 Hz, H_h), 2.10 (t, 2H, J = 7.2 Hz, H_g), 2.19-2.26 (m, 2H, H_e), 3.17-3.45 (m, 5H, H_6, H_5, H_4, 2-OH), 3.55-3.88 (m, 5H, $H_{6'}$, H_3, H_2, 2-OH), 4.57 (d, 1H, J = 8.0 Hz, H_1, (β-isomer)), 5.09 (d, 1H, J = 3.2 Hz, H_1, (α-isomer)), 7.17-7.22 (m, 3H, H_d), 7.23-7.29 (m, 6H, H_b), 7.34 (m, 6H, H_c), 7.68 (d, 1H, J = 6.4 Hz, N-H, (β-isomer)), 7.74 (d, 1H, J = 8.0 Hz, N-H, (α-isomer)).

^{13}C NMR (75 MHz, $(CD_3)_2SO$): δ 25.99 (C_h, β and α), 28.66, 28.96, 29.10, 29.34, 29.44, 29.53, 31.90 (C_f, β and α), 35.95 (C_g, β), 36.39 (C_g, α), 42.18 (C_e, β and α), 53.90 (C_2, β),

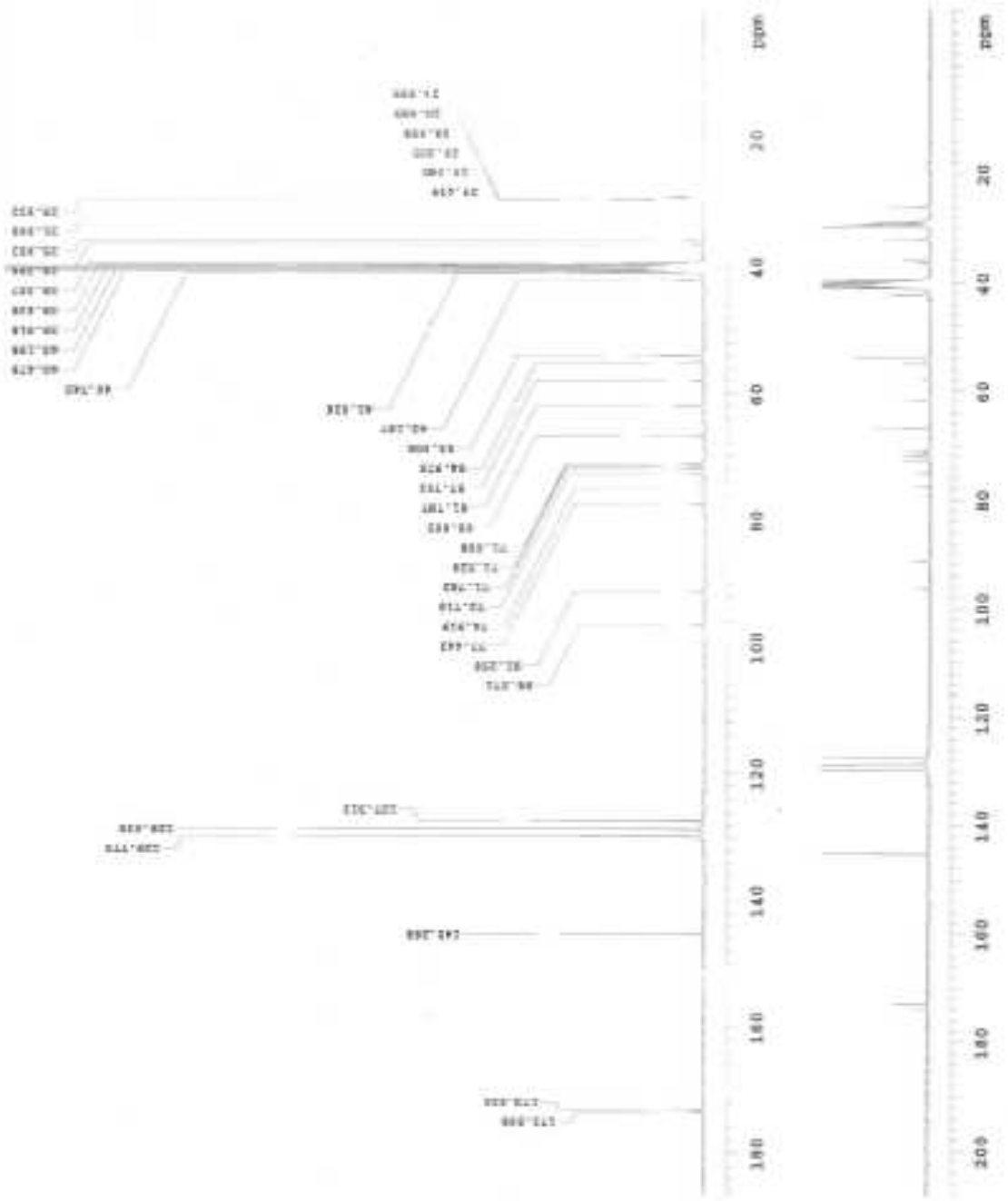
54.97 (C_2, α), 57.79 (C_7 β), 61.79 (C_5, β), 66.67 (C_6, α and β), 71.00 (C_7, α), 71.53 (C_5, α), 71.78 (C_4, β), 72.71 (C_3, β), 74.92 (C_4, α), 77.44 (C_3, α), 91.26 (C_1, β), 96.37 (C_1, α), 127.31 (C_d), 128.63 (C_b), 129.77 (C_c), 145.27 (C_a) 173.02 (CO, β-isomer), 173.57 (CO, α-isomer).

ESI-MS for $C_{36}H_{47}NO_6S$: calcd, 621.39; found, 644.3 $(M + Na)^+$, 660.2 $(M + K)^+$, and 1265.5 $(2M + Na)^+$.

^{1}H NMR of compound- **C** (400 MHz, CD_3OD):

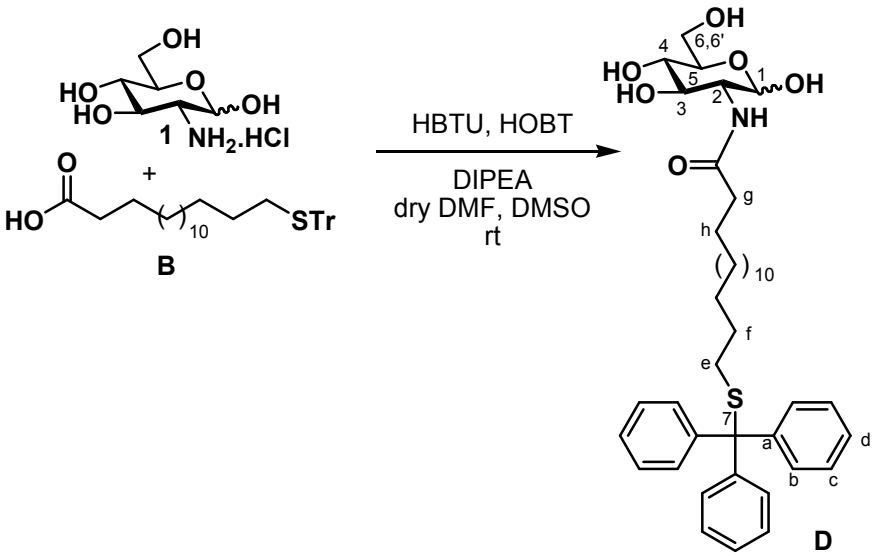


^{13}C NMR of compound- **C** (75 MHz, $(CD_3)_2SO$):



***N*-((*2R,3S,4S,5S*)-2,4,5-trihydroxy-6-(hydroxymethyl)tetrahydro-2H-pyran-3-yl)-16-(tritylthio)hexadecanamide (D)**

0.194 g (0.904 mmol) of glucosamine hydrochloride (**1**) was dissolved in 1 mL of dry DMSO and to it was added 0.2 mL (1.13 mmol) of DIPEA at room temperature. It was subsequently stirred for 15 min. To this solution was added in a drop-wise fashion a mixture of 0.3 g (0.56 mmol) of acid **2**, 0.157 g (0.56 mmol) of HBTU, 0.076 g (0.56 mmol) of HOBT and 0.2 mL (1.13 mmol) of DIPEA in 3 mL of dry DMF. The same procedure for **C** was then followed. The residue was purified by silica gel column chromatography using dichloromethane and methanol (92:08) to afford 0.275 g of *N-((2R,3S,4S,5S)-2,4,5-trihydroxy-6-(hydroxymethyl)tetrahydro-2H-pyran-3-yl)-16-(tritylthio)hexadecanamide* (**D**) as an unseparable α and β mixture in a 1.5:1 ratio (as denoted by ^{1}H NMR). It was a white solid obtained in 70% yield. Rf value 0.45 in CH_2Cl_2: MeOH (88:12). mp. = 97 ˚C.



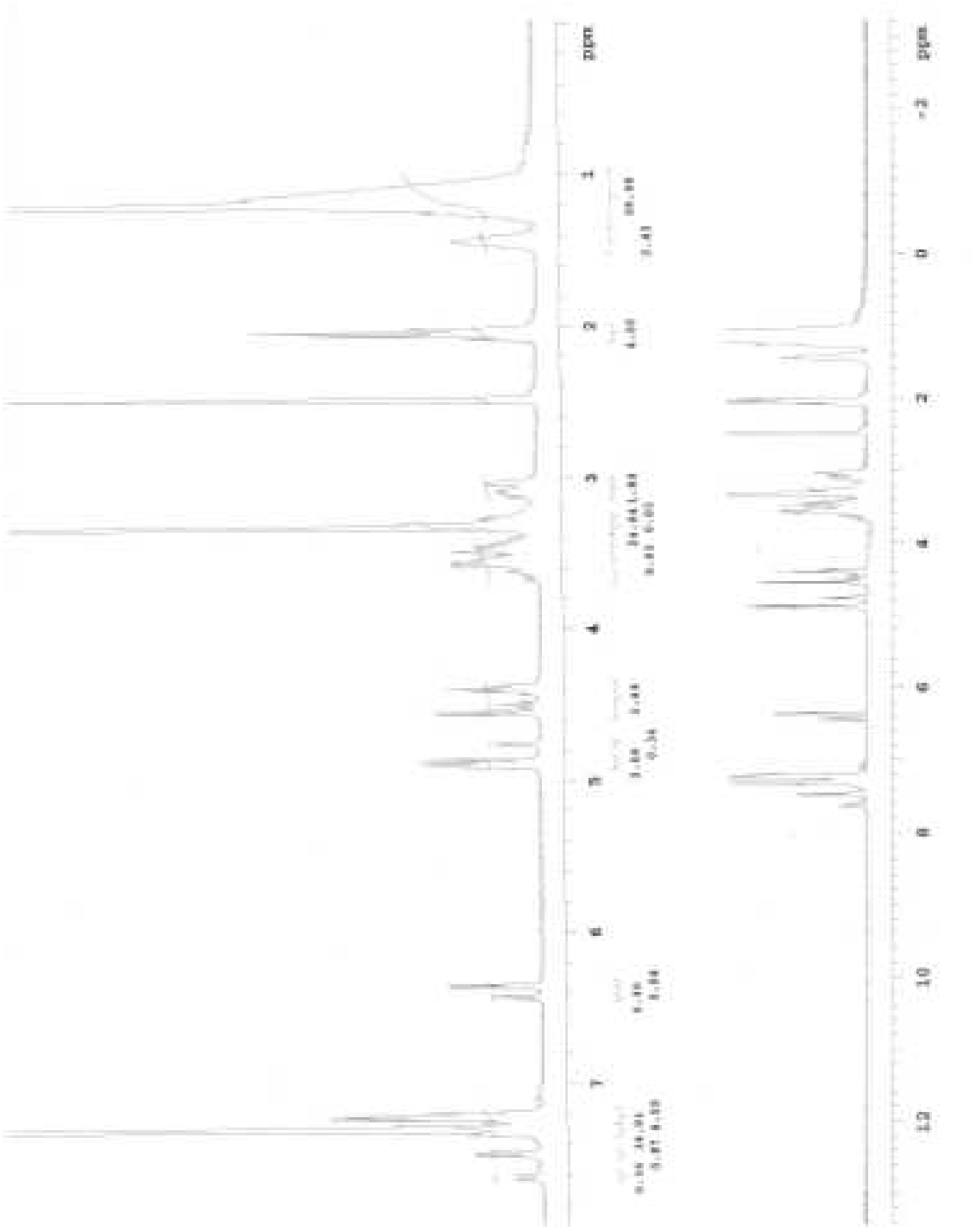
I.R: ν_{max} cm^{-1} 1023, 1065, 1081, 1445, 1487, 1554, 1626, 1641, 2850, 2919, 3396, 3409.

^{1}H NMR (300 MHz, $(CD_3)_2SO$, (α and β mixture)): δ 0.98-1.32 (m, 24 H, 2H_f, 11 CH_2), 1.37-1.51 (m, 2H, H_h), 1.99-2.11 (m, 4H, H_g, H_e), 3.00 (m, 2H, H_3, (β), -OH), 3.22-3.69 (m, 11H, H_6, $H_{6'}$, H_4 (2), H_3 (α), H_2, H_5, 4-OH), 4.36-4.44 (m, 1H, H_5), 4.49 (t, 1H, *J* = 8.0 Hz, -OH), 4.54 (d, 1H, *J* = 6.8 Hz, H_6), 4.75 (d, 1H, *J* = 6.4 Hz, H_6, α), 4.86 (d, 1H, *J* = 7.2 Hz, $H_{6'}$, β), 4.89 (t, 1H, d, 1H, *J* = 4.8 Hz, $H_{6'}$, α), 6.35 (d, 1H, *J* = 4.8 Hz, H_1-α-isomer), 6.43 (d, 1H, *J* = 8.8 Hz, H_1- β-isomer), 7.17-7.27 (m, 3H, Ph-H, H_d), 7.27-7.35 (m, 12H, Ph-H, H_b and H_c), 7.46 (d, 1H, *J* = 10.0 Hz, N-H, (β-isomer)), 7.62 (d, 1H, *J* = 10.0 Hz, N-H, (α-isomer)).

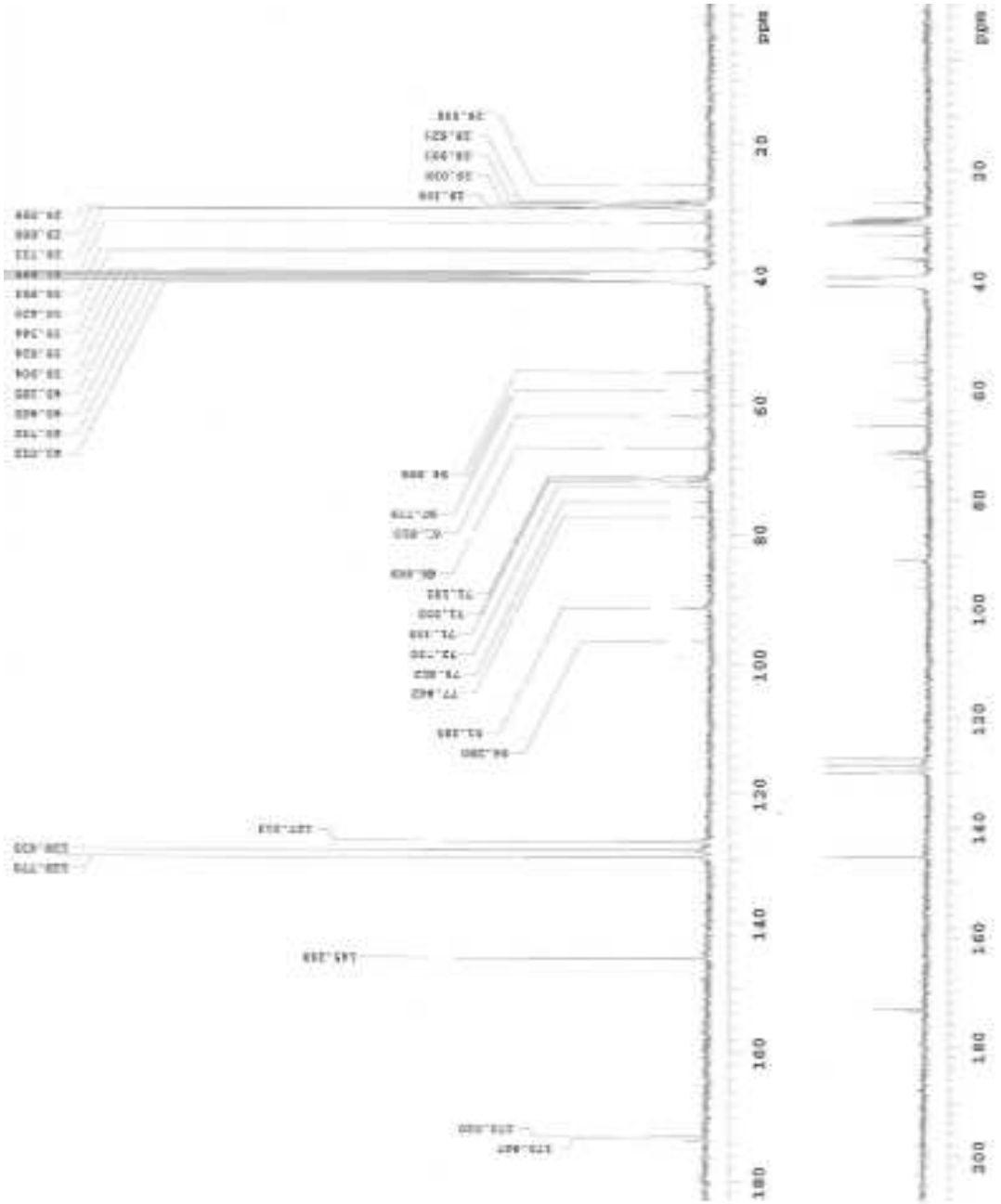
^{13}C NMR (75 MHz, $(CD_3)_2SO$): δ 26.00 (C_h, β and α), 28.62, 28.90, 29.04, 29.39, 29.45, 29.56, 29.66, 29.73, 31.89 (C_e, β and α), 35.95 (C_g, β), 36.42 (C_g, α), 54.89 (C_2, β), 57.77 (C_2, α), 61.81 (C_5, β), 66.67 (C_6, α and β), 71.10 (C_7, β and α), 71.55 (C_5, α), 71.84 (C_4, β), 72.73 (C_3, β), 75.01 (C_4, α), 77.44 (C_3, α), 91.29 (C_1, β), 96.29 (C_1, α), 127.31 (C_d), 128.63 (C_b), 129.77 (C_c), 145.26 (C_a) 173.02 (CO, β-isomer), 173.44 (CO, α-isomer).

ESI-MS for $C_{41}H_{57}NO_6S$: calcd, 691.39; found, 714.3 $(M + Na)^+$, 730.3 $(M + K)^+$, 1405.5 $(2M + Na)^+$, and 1421.6 $(2M + K)^+$.

^{1}H NMR of compound- **D** (300 MHz, $(CD_3)_2SO$):

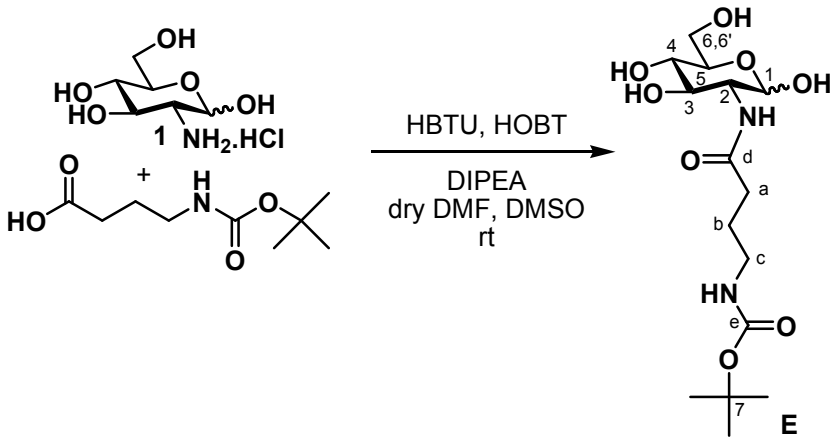


^{13}C NMR of compound- **D** (75 MHz, $(CD_3)_2SO$):



tert-butyl-4-oxo-4-((*2R*,*3S*,*4S*,*5S*)-2,4,5-trihydroxy-6-(hydroxymethyl)tetrahydro-2H-pyran-3-ylamino)butylcarbamate (E)

0.346 g (1.606 mmol) of glucosamine hydrochloride (**1**) was dissolved in 1 mL of dry DMSO and to it was added 0.35 mL (2.007 mmol) of DIPEA at room temperature. The mixture was then stirred for 15 min. To this solution was added drop-wise a mixture of 0.204 g (1.02 mmol) of 4-*N*-(*tert*-butoxycarbonyl)-aminobutanoic acid, 0.38 g (1.02 mmol) of HBTU, 0.134 g (1.02 mmol) of HOBT and 0.35 mL (2.007 mmol) of DIPEA in 3 mL of dry DMF. The same procedure as described for compound **C** was then followed. The residue was purified by silica gel column chromatography using dichloromethane and methanol (90:10) to afford 0.248 g of *tert-butyl 4-oxo-4-((2R,3S,4S,5S)-2,4,5-trihydroxy-6-(hydroxymethyl)tetrahydro-2H-pyran-3-ylamino)butylcarbamate* (**E**) as a light yellow colored solid in 68% yield. Rf value 0.5 in CH_2Cl_2:MeOH (88:12). mp. = 108 ˚C.



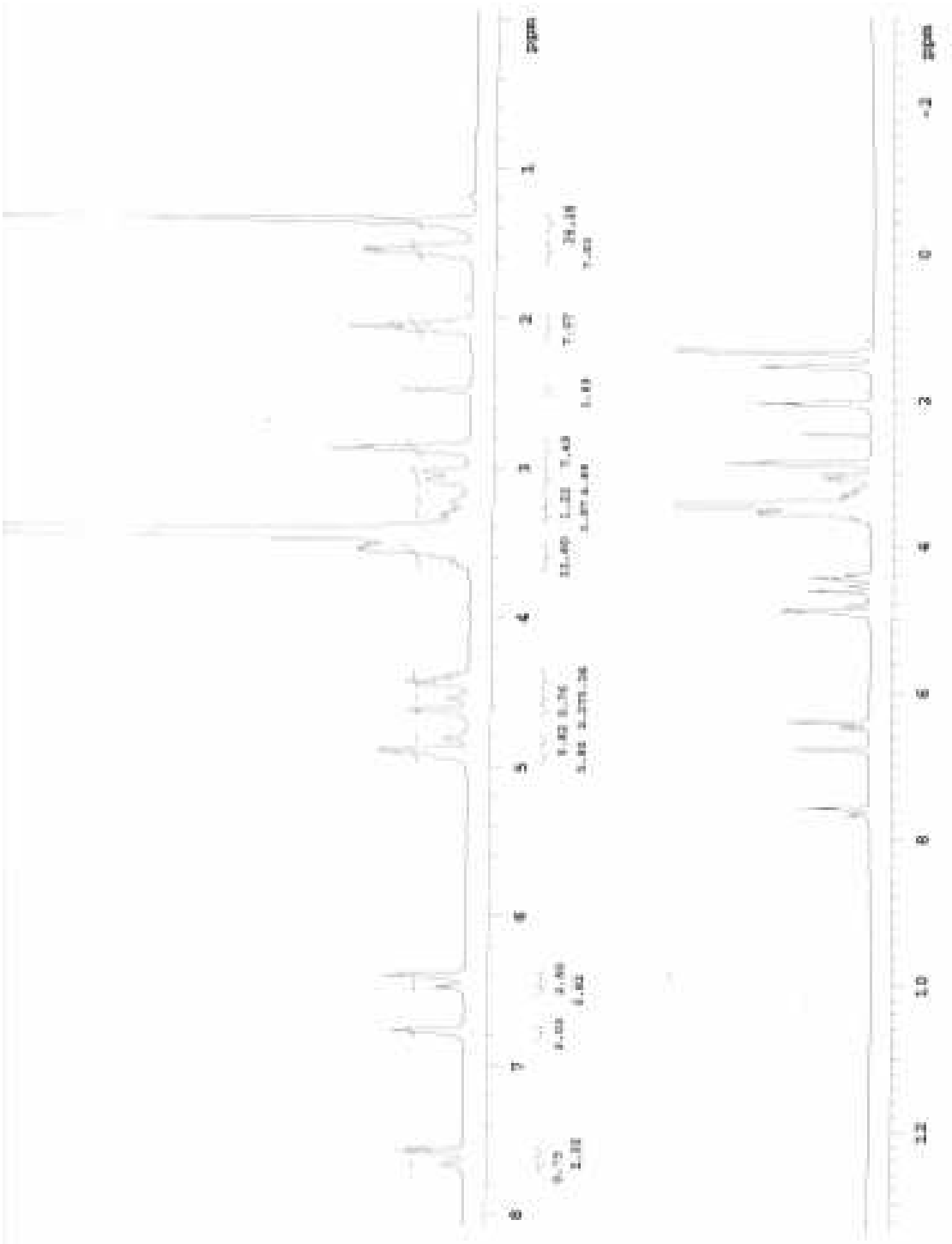
I.R: ν_{max} cm^{-1} 807, 1020, 1080, 1130, 1210, 1420, 1460, 1550, 1640, 1750, 2855, 2905, 2960, 3100, 3300, 3400.

^{1}H NMR (400 MHz, $(CD_3)_2SO$, (α and β mixture)): δ 1.35 (s, 9 H, Boc- C$\underline{H}_3$), 1.54 (quintet, 2H, J = 7.2 Hz, H_b), 2.01-2.11 (m, 2H, H_a), 2.84-2.93 (m, 2H, H_c), 3.00-3.12 (m, 2H, H_3, -OH), 3.20-3.30 (bm, 1H), 3.38-3.50 (m, 2H), 3.51-3.68 (m, 3H), 4.39 (t, 1H, J = 6.0 Hz, H_5), 4.50 (t, 1H, J = 6.4 Hz, H_5), 4.58 (d, 1H, J = 5.6 Hz, H_1, β), 4.79 (d, 1H, J = 5.6 Hz, -OH), 4.86-4.93 (m, 2H), 6.38 (d, 1H, J = 4.0 Hz, H_1, (α)), 6.45 (d, 1H, J = 6.4 Hz, N-$\underline{H}$), 6.79 (t 1H, J = 5.6 Hz, N-$\underline{H}$), 7.56 (d, 1H, J = 8.0 Hz, N-$\underline{H}$), 7.65 d, 1H, J = 8.0 Hz, N-$\underline{H}$).

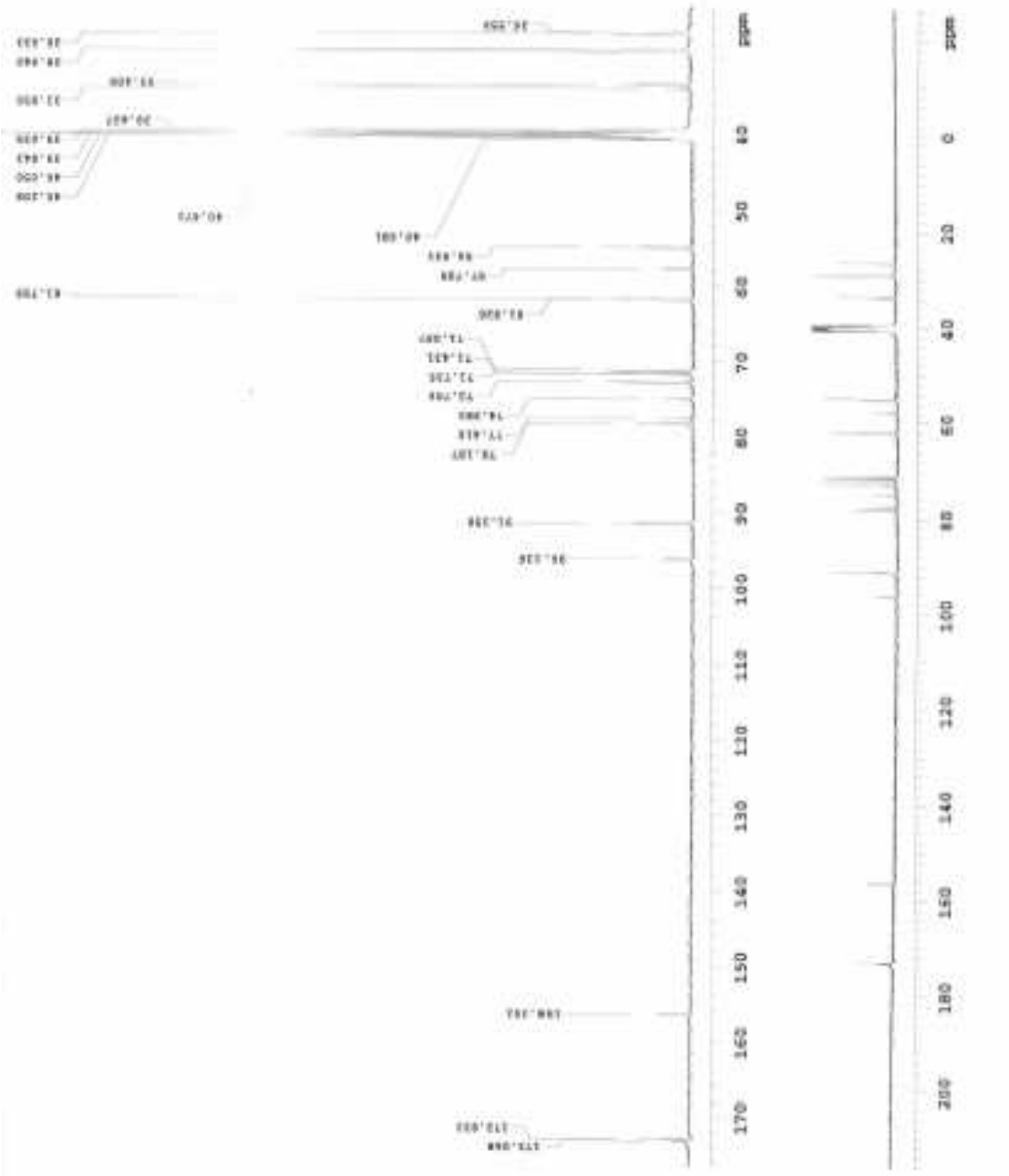
^{13}C NMR (100 MHz, $(CD_3)_2SO$): δ 26.65 (C_b), 28.96 (Boc $\underline{C}H_3$), 33.41 (C_a, β), 39.54 (C_a, α), 40.06 (C_c, α) , 40.79 (C_c, β), 54.93 (C_2, β), 57.77 (C_2, α), 61.79 (C_6, α and β), 71.13 (C_5, β), 71.47 (C_5, α), 71.56 (C_5, α), 71.80 (C_4, β), 72.74 (C_3, β), 75.00 (C_4, α), 77.48 (C_3, α),78.09 (C_7, α and β), 91.27 (C_1, β), 96.19 (C_1, α), 156.25 (CO, C_e), 172.67 (CO, C_d).

ESI-MS for $C_{15}H_{28}N_2O_8$: calcd, 364.1846; found, 371.3 (M + Li), 387.2 $(M + Na)^+$, 403.2 $(M + K)^+$ and 735.4 $(2M + Li)^+$.

^{1}H NMR of compound- **E** (400 MHz, $(CD_3)_2SO$):

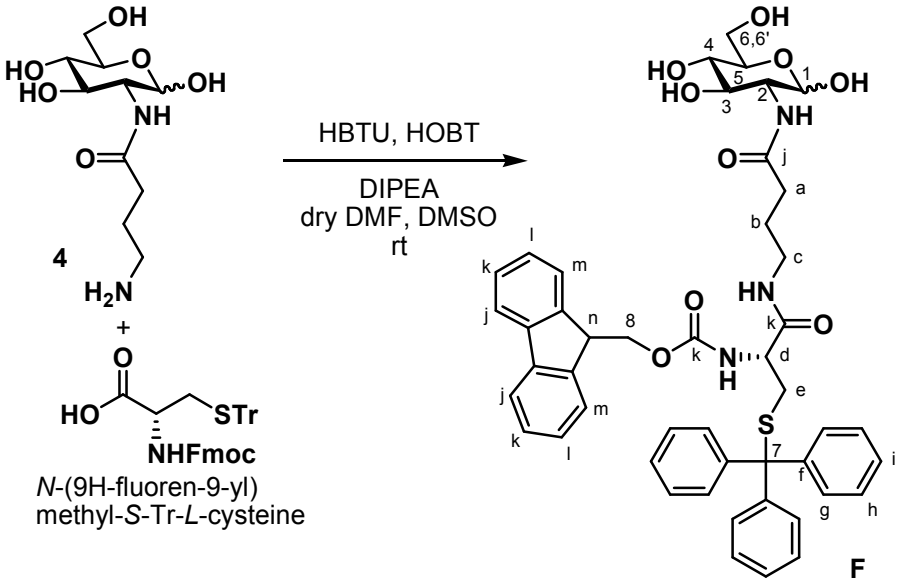


^{13}C NMR of compound- **E** (100 MHz, $(CD_3)_2SO$):



(9H-fluoren-9-yl)methyl (2R)-1-oxo-1-(4-oxo-4-((2R,3S,4S,5S)-2,4,5-trihydroxy-6-(hydroxymethyl)tetrahydro-2H-pyran-3-ylamino)butylamino)-3-(tritylthio)propan-2-ylcarbamate (F)

0.125 g (0.473 mmol) of compound **4** was dissolved in 0.75 mL dry DMSO and then added in a drop-wise fashion to a mixture of 0.276 g (0.473 mmol) of *N*-(9H-fluoren-9-yl)methyl-S-Tr-*L*-cysteine, 0.179 g (0.473 mmol) HBTU, 0.063 g (0.473 mmol) of HOBT and 0.33 mL (1.892 mmol) of DIPEA in 3 mL of dry DMF. The same procedure as described for compound **C** was then followed. The residue was purified by silica gel column chromatography using dichloromethane and methanol (91:9) as the eluent to afford 0.250 g of *(9H-fluoren-9-yl)methyl (2R)-1-oxo-1-(4-oxo-4-((2R,3S,4S,5S)-2,4,5-trihydroxy-6-(hydroxymethyl)tetrahydro-2H-pyran-3-ylamino)butylamino)-3-(tritylthio)propan-2-ylcarbamate* (**F**) as a light yellow colored solid in 64% yield. Rf value 0.55 in CH_2Cl_2:MeOH (91:9). mp. = 138 ˚C.



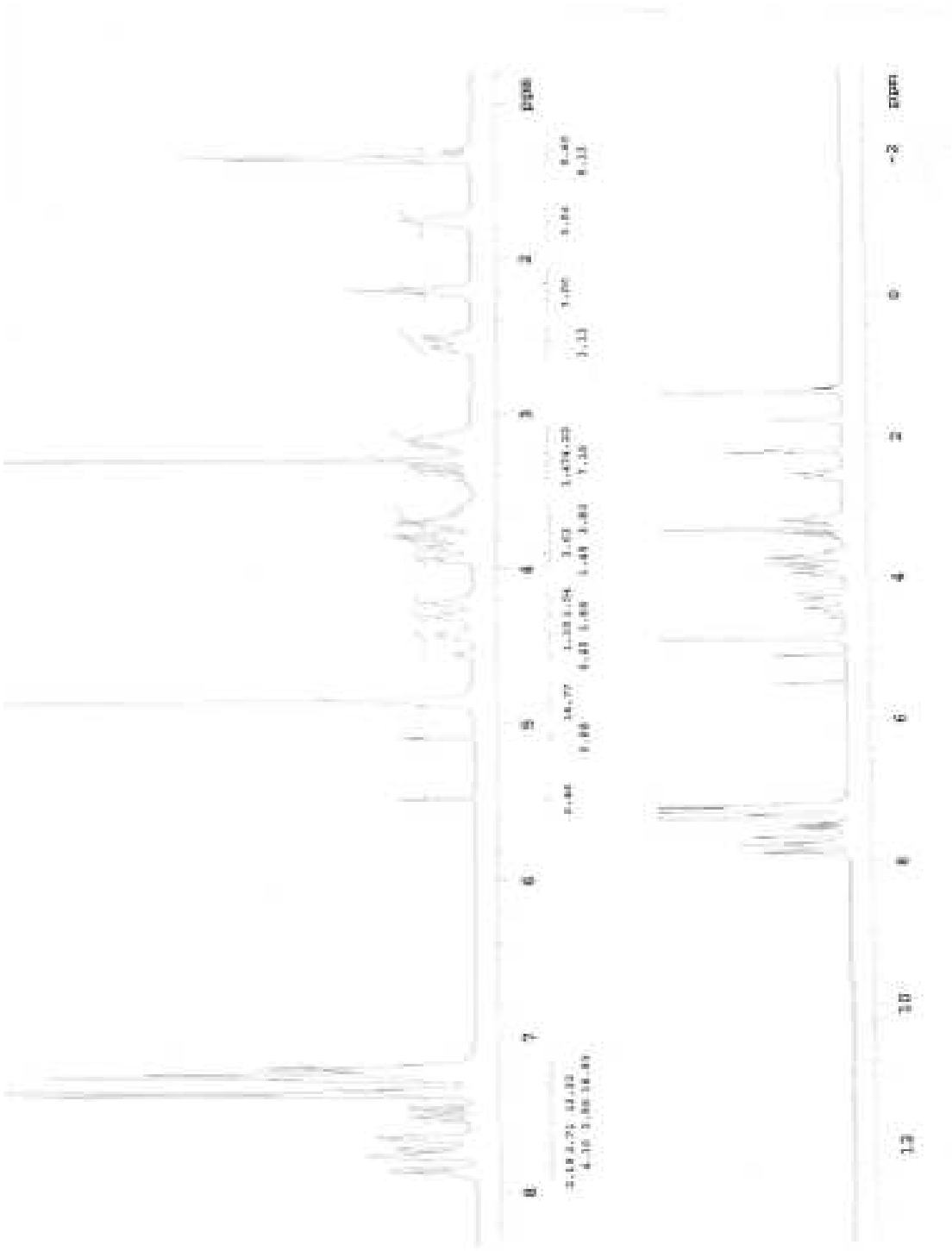
I.R: ν_{max} cm^{-1} 721, 802, 1025, 1206, 1261, 1468, 1558, 1627, 1642, 2852, 2925, 3409.

^{1}H NMR (400 MHz, CD_3OD, (α and β mixture)): δ 1.70-1.82 (m, 2H, H_b), 2.21 (t, 2H, *J* = 6.4 Hz, H_a), 2.46-2.62 (m, 2H, H_c), 3.10-3.25 (m, 3H, $2H_e$, -OH), 3.30-3.45 (m, 2H, H_2, H_5), 3.58-3.75 (m, 3H, H_2, H_4, -OH), 3.76-3.89 (m, 3H, H_6, $H_{6'}$, -OH), 3.93 (t, 1H, *J* = 7.6 Hz, H_n), 4.21 (bt, 1H, H_8), 4.54 (d, 1H, *J* = 8.4 Hz, H_1, β), 5.08 (d, 1H, *J* = 3.2 Hz, H_1, α), 7.17-7.30 (m, 9H, Tr-H), 7.32-7.40 (m, 6H, Tr-H), 7.46 (t, 1H, *J* = 7.2 Hz, Ar-H, H_l), 7.52 (t, 1H, *J* = 7.6 Hz, Ar-H, H_l), 7.65 (d, 2H, *J* = 6.8 Hz, Ar-H, H_m), 7.71-7.80 (m, 2H, H_k), 7.85 (d, 2H, *J* = 8.0 Hz, Ar-H, H_j).

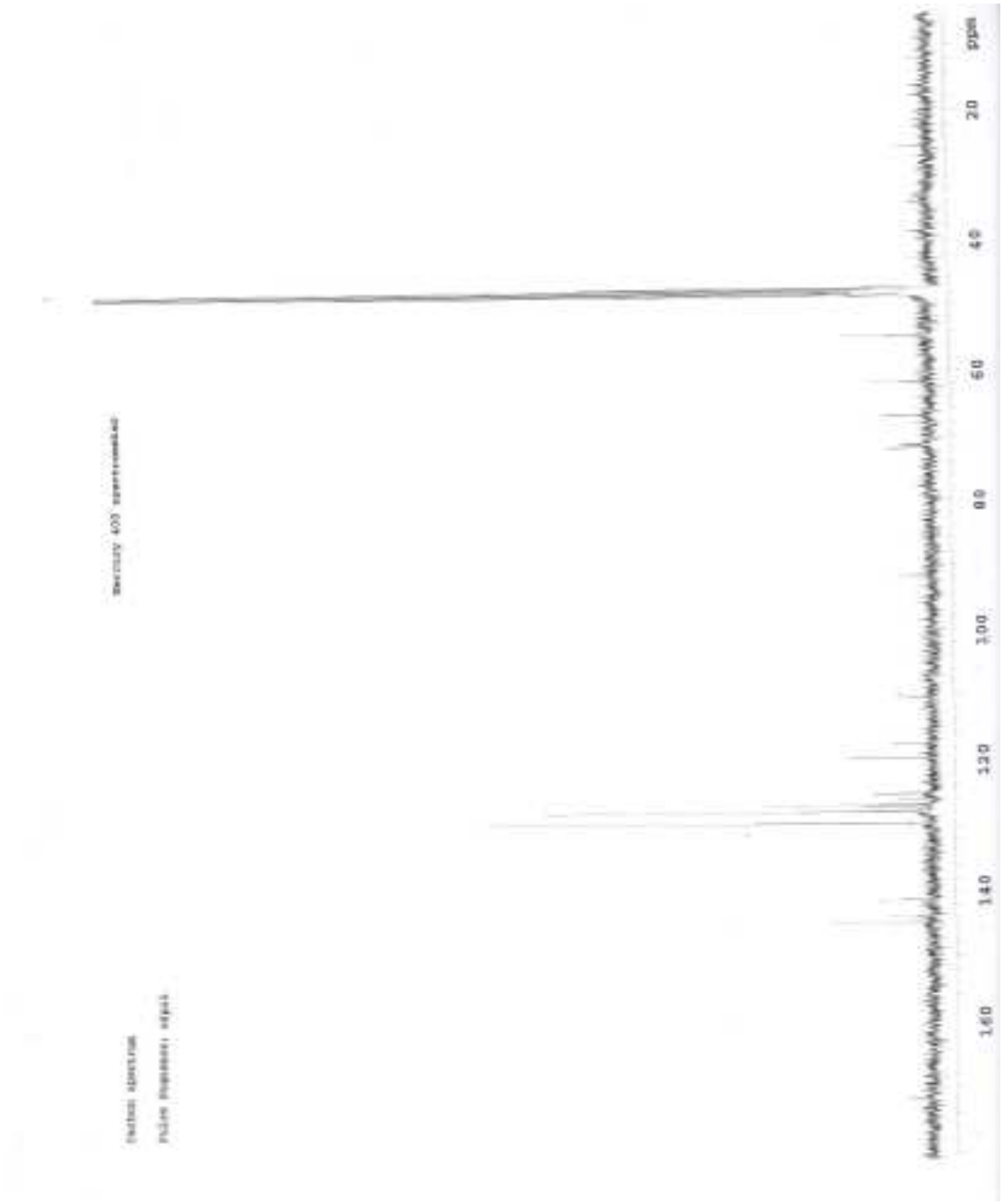
^{13}C NMR (100 MHz, CD_3OD): δ 25.32 (C_b), 28.91 (C_e), 34.02 (C_a, α and β), 38.47 (C_c, α and β), 42.60 (Cn), 54.66 (C_2), 61.68 (C_6), 66.92 (C_5), 68.18 (C_d), 68.21 (C_8), 71.37 (C_7), 71.54 (C_4), 71.93 (C_3), 91.42 (C_1, β), 95.86 (C_1, α), 110.31, 117.48, 119.74, 125.06, 125.90, 127.04, 127.62, 127.85, 129.56, 141.40, 143.91, 144.76, 171.62, 173.12.

ESI-MS for $C_{47}H_{49}N_3O_9S$: calcd, 831.3190; found, 838.3 (M + Li), 854.3 $(M + Na)^+$, 870.2 $(M + K)^+$ and 1670.5 $(2M + Li)^+$.

^{1}H NMR of compound- **F** (400 MHz, (CD_3OD):

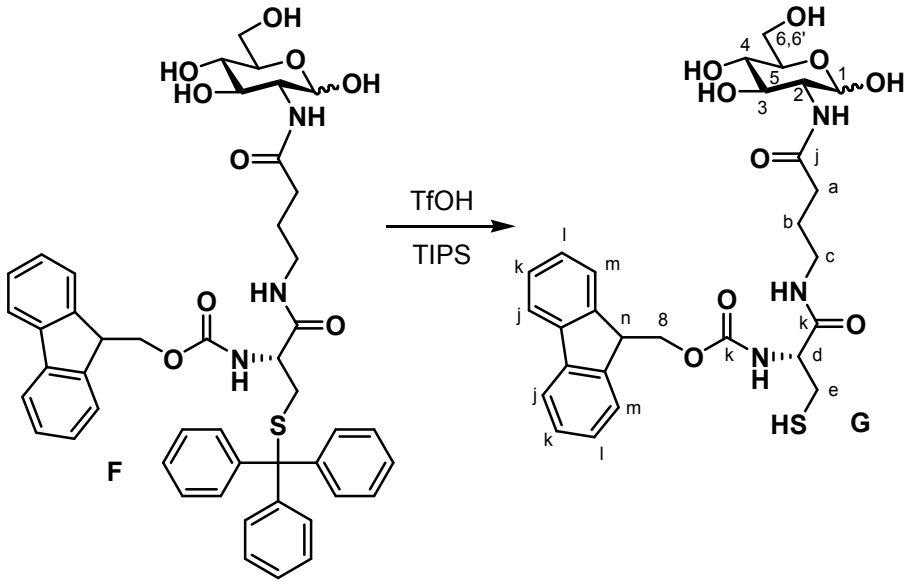


^{13}C NMR of compound- **F** (100 MHz, (CD_3OD):



(9H-fluoren-9-yl)methyl (2R)-3-mercapto-1-oxo-1-(4-oxo-4-((2R,3S,4S,5S)-2,4,5-trihydroxy-6-(hydroxymethyl)tetrahydro-2H-pyran-3-ylamino)butylamino)propan-2-ylcarbamate (G)

1 mL of trifluoroacetic acid was added to 0.2 g (0.24 mmol) of compound **F** at 0 ˚C. Subsequent drop-wise addition of 100 µL triisopropylsilane rid the reaction mixture of a yellow color and the mixture was stirred for 10 min at 0 ˚C. TFA was removed under reduced pressure and the residue was sequentially washed with ice-cold diethyl ether (3 x 4 mL) and dichloromethane (2 x 5 mL) to afford 0.127 g of *(9H-fluoren-9-yl)methyl (2R)-3-mercapto-1-oxo-1-(4-oxo-4-((2R,3S,4S,5S)-2,4,5-trihydroxy-6-(hydroxymethyl)tetrahydro-2H-pyran-3-ylamino)butylamino)propan-2-ylcarbamate* (**G**) as a white solid in 90% yield. Rf value 0.4 in CH_2Cl_2:MeOH (88:12).

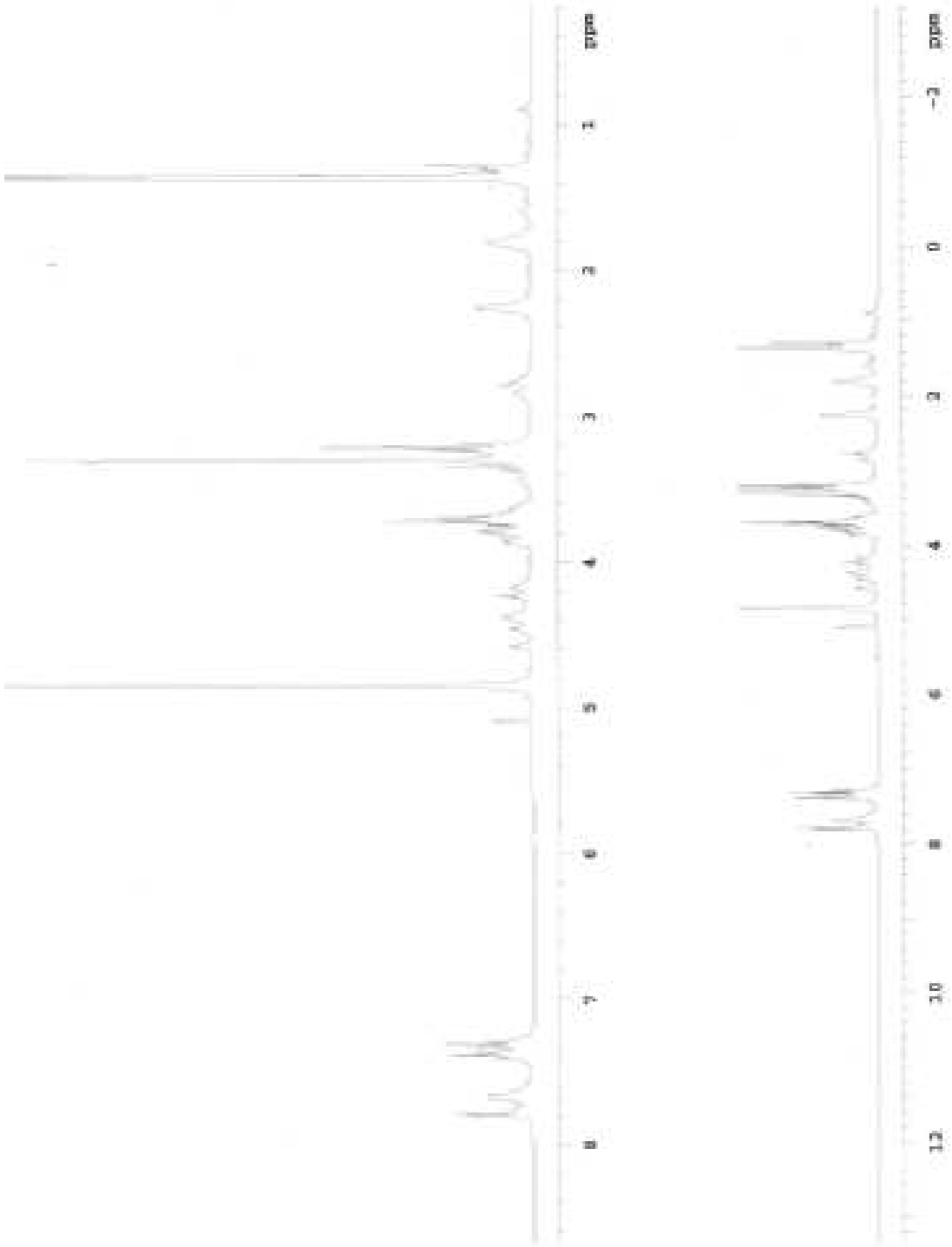


I.R: ν_{max} cm^{-1} 690, 720, 802, 1026, 1206, 1260, 1385, 1468, 1558, 1627, 1642, 2480, 2852, 2925, 3409.

^{1}H NMR (400 MHz, CD_3OD, (α and β mixture): δ 1.60 (bs, 1H, -SH), 1.72-1.86 (m, 2H, H_b), 2.20-2.30 (m, 2H, H_a), 2.72-2.90 (m, 3H, H_c and OH), 3.16-3.40 (m, 6H,), 3.60-3.90 (m, 8H), 4.12- 4.50 (m, 6H), 4.58 (d, 1H, J = 8.0 Hz, H_1, (β)), 5.08 (d, 1H, J = 3.2 Hz, H_1, (α)), 7.30 (t, 2H, J = 7.2 Hz, H_l), 7.38 (t, 2H, J = 8.0 Hz, H_m), 7.62-7.82 (m, 4H, H_k and H_j)

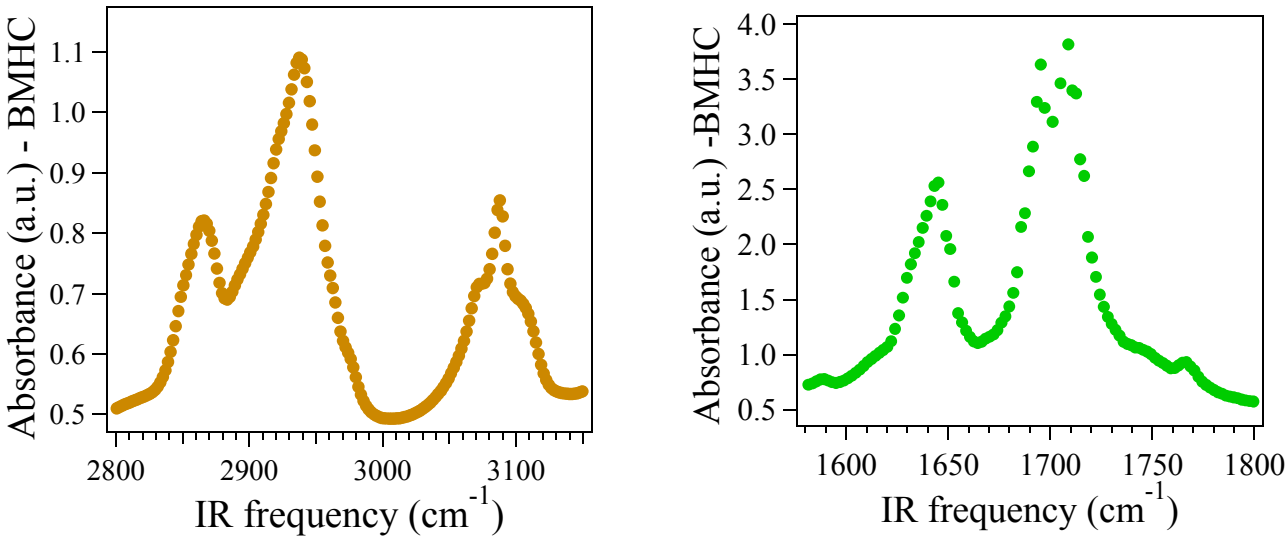
ESI-MS for $C_{28}H_{35}N_3O_9S$: calcd, 589.2094; found, 612.2 $(M + Na)^+$, 628.2 $(M + K)^+$ and 1201.4 $(2M + Na)^+$.

^{1}H NMR of compound- **G** (400 MHz, (CD_3OD):



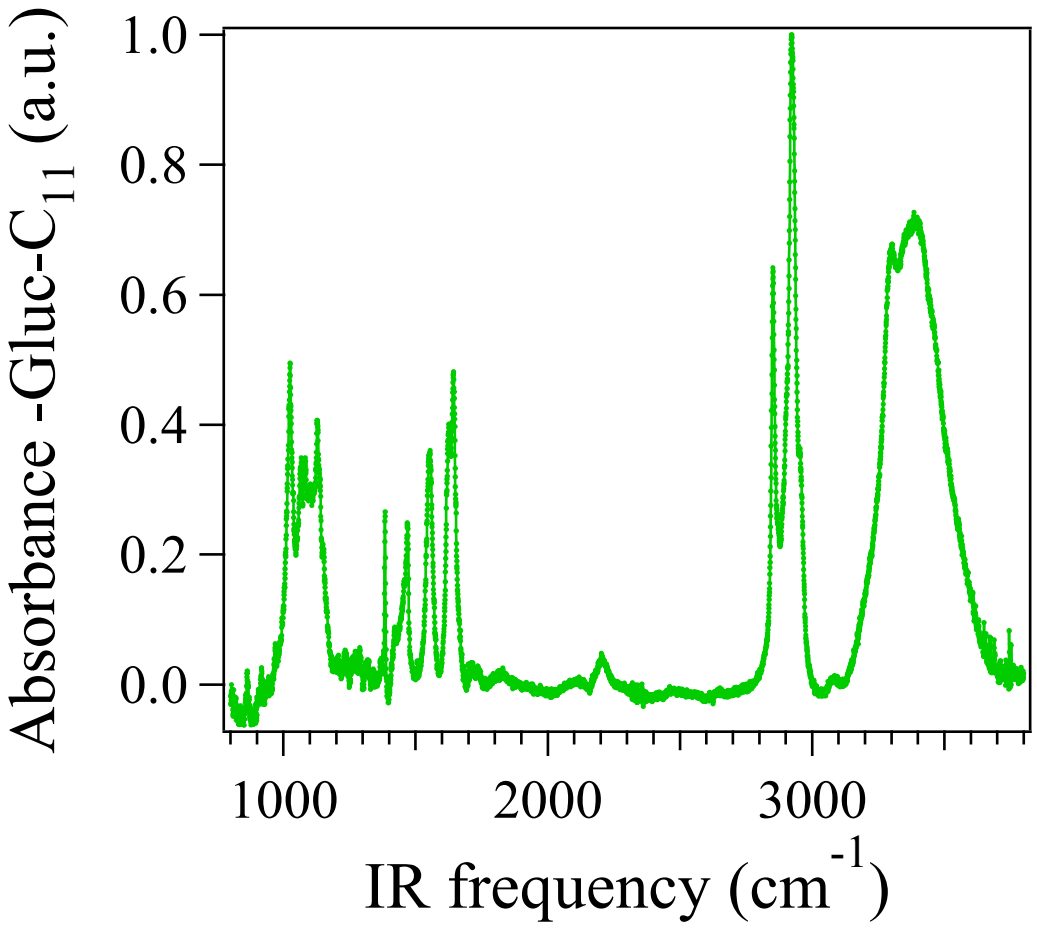
FTIR spectra

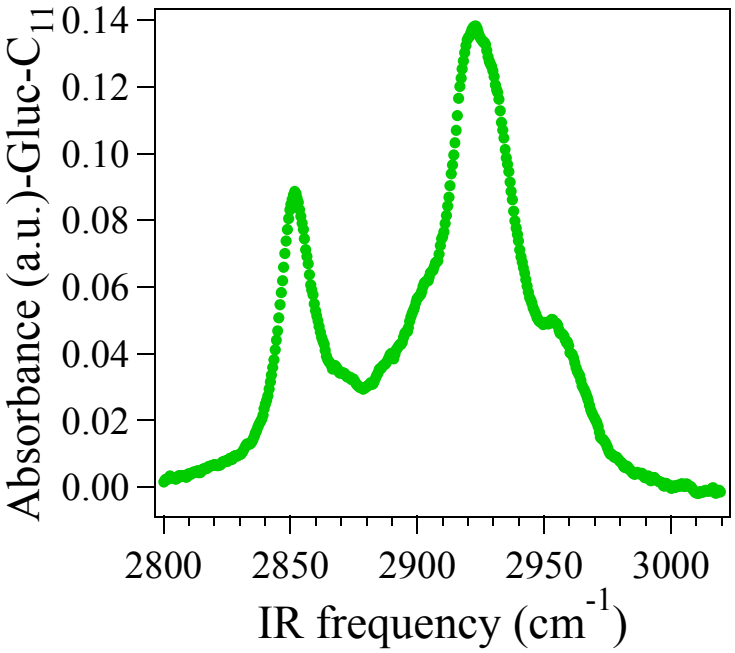
(a) N,N'-bis(maleimidylhexanoyl)cystamine [BMHC]



Frequency/cm^{-1}	Transition
3105	Maleimide ring C-H asymmetric stretch
3087	Maleimide ring C-H symmetric stretch
3072	Maleimide ring C-H symmetric stretch
2938	CH_2 asymmetric stretch
2865	CH_2 symmetric stretch
1710	Maleimide C=O
1645	Amide C=O

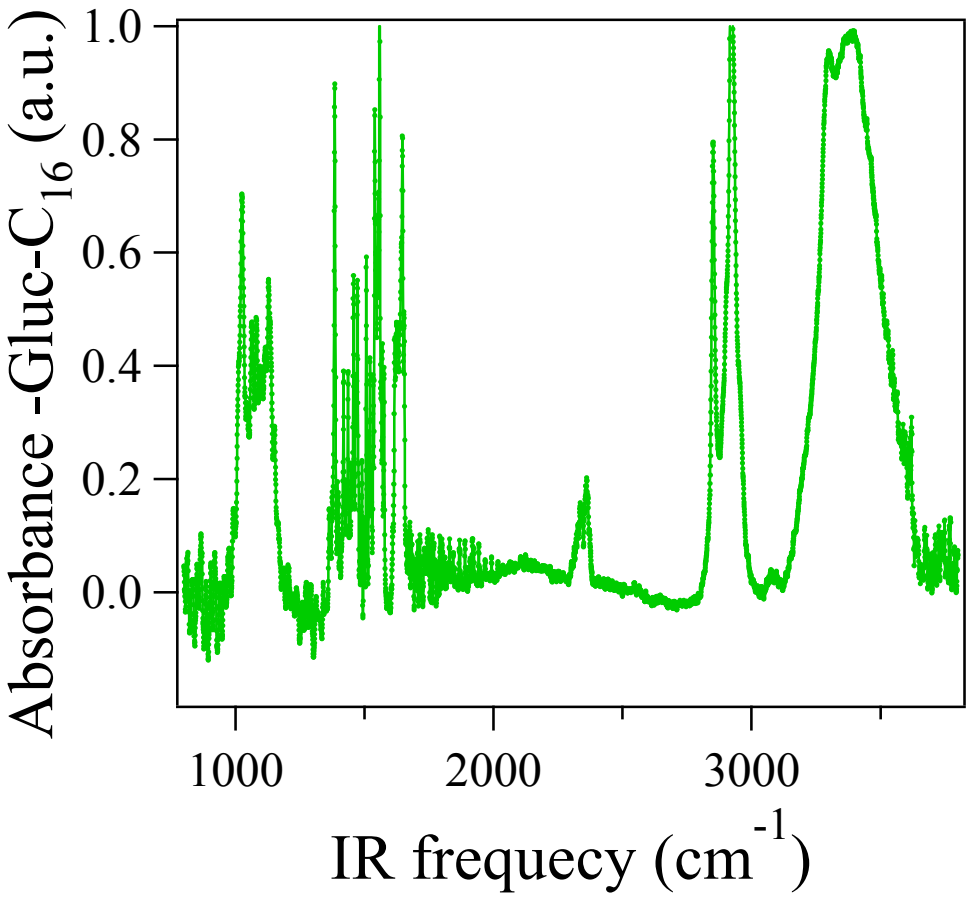
(b) 11-mercapto-N-((2R,3S,4S,5S)-2,4,5-trihydroxy-6-(hydroxymethyl)tetrahydro-2H-pyran-3-yl)undecanamide [Gluc-C_{11} thiol], Compound 2.

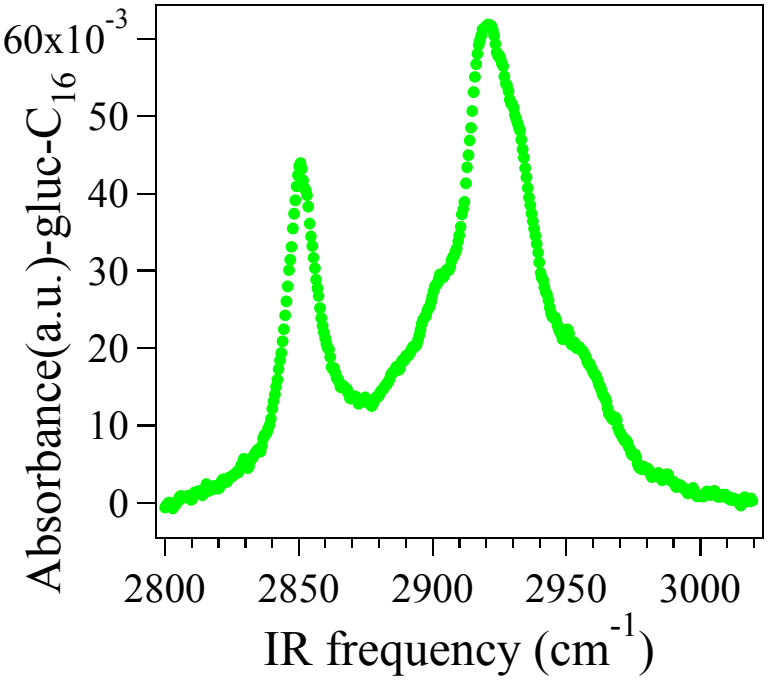




Frequency/cm^{-1}	**Transition**
2852	CH_2 symmetric stretch
2890	Glucose C-H stretch
2905	Glucose C-H stretch
2925	CH_2 asymmetric stretch
2965	Glucose C-H stretch

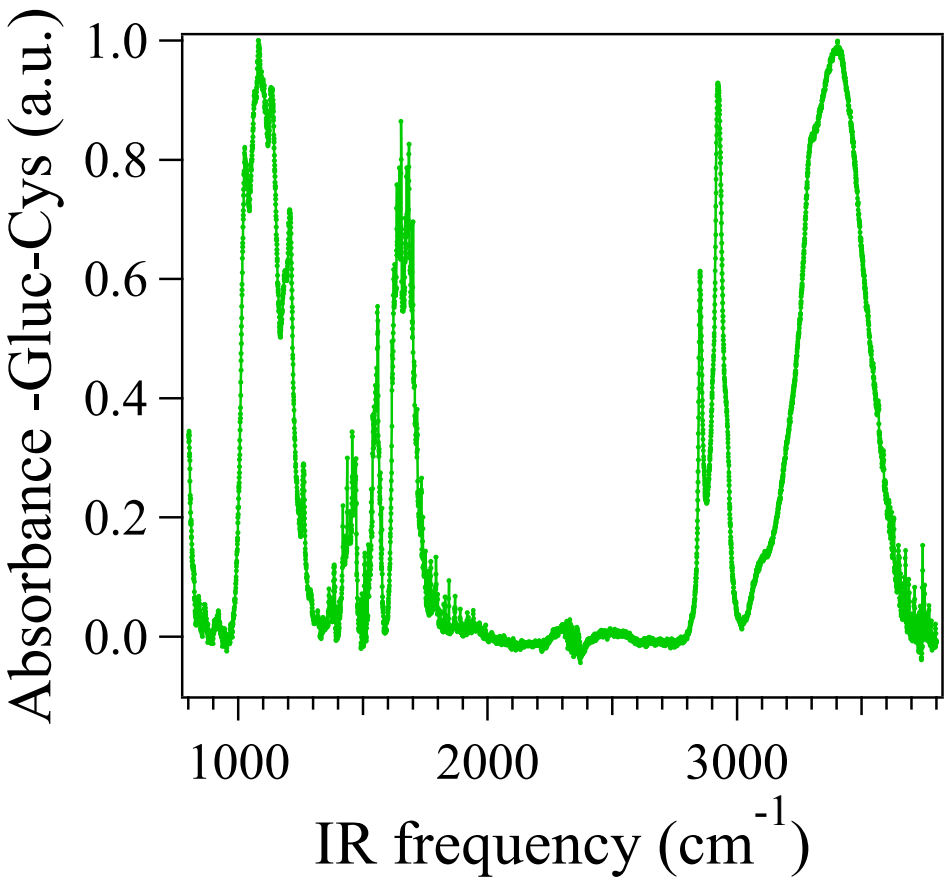
(c) 16-mercapto-N-((2R,3S,4S,5S)-2,4,5-trihydroxy-6-(hydroxymethyl)tetrahydro-2H-pyran-3-yl)hexadecanamide [Gluc-C_{16} thiol], Compound 3.

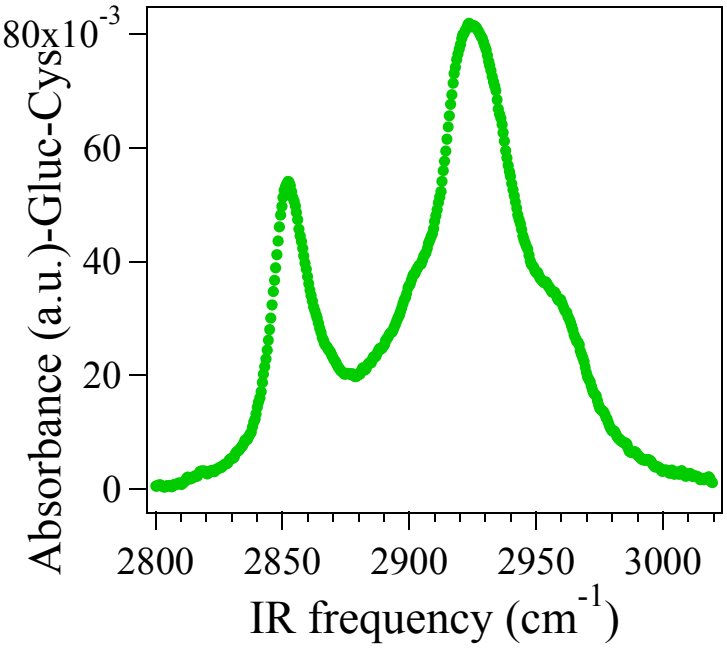




Frequency/cm^{-1}	Transition
2852	CH_2 symmetric stretch
2890	Glucose C-H stretch
2905	Glucose C-H stretch
2925	CH_2 asymmetric stretch
2965	Glucose C-H stretch

(d) 4-((R)-2-amino-3-mercaptopropanamido)-N-((2R,3R,4S,5S)-2,4,5-trihydroxy-6-(hydroxymethyl)tetrahydro-2H-pyran-3-yl)butanamid [Gluc-Cys], Compound 6.





Frequency/cm^{-1}	Transition
2850	CH_2 symmetric stretch
2905	Glucose C-H stretch
2925	CH_2 asymmetric stretch
2965	Glucose C-H stretch

VSFG spectroscopy set-up

The broad-band vibrational sum frequency generation (VSFG) spectroscopy set-up, which follows the pioneering development by Richter, Petralli-Mallow, and Stephenson [*Opt. Lett.* **1998**, *23*, 1594-1596], is based on a high power amplified femtosecond Ti-Sapphire laser system (Spectra Physics Spitfire sub-50 fs HP). 50% of the 2 mJ fundamental output pulse (800 nm, FWHM 35 fs) is used to pump an Optical Parametric Amplifier (OPA) followed by the signal–idler difference frequency mixing in a 0.5 mm thick $AgGaS_2$ crystal producing 75 fs IR pulses (300 cm^{-1} spectral FWHM). Spectra of the IR pulses were measured using an IR grating (blazed at 5 μm) in the monochromator and liquid nitrogen cooled MCT detector (IR Associates). The broad-band VSFG scheme was employed that uses spectrally broad (FWHM~250 cm^{-1}) IR and narrow-band visible pulses to obtain the spectrum by frequency-dispersing the SFG signal. A zero-chirp 4-f design pulse stretcher consisting of a grating, collimating lens and a mirror equipped with a tunable slit was used to narrow the spectrum of the visible (800 nm) pulse from 430 cm^{-1} of the Spitfire output down to the desired spectral width. Since the full width at half maximum (FWHM) of the spectral features of the SAM samples was determined to be greater than 7 cm^{-1}, we selected the visible pulse spectral width to be $\leq$ 5 cm^{-1} Gaussian FWHM. The IR and visible beams were spatially and temporally overlapped at the sample surface with the incidence angle for both beams 65^{o} from surface normal. The beam diameter at the sample is ~150 μm for both visible and IR beams. The laser power at the sample is 4 μJ/pulse for IR and up to 17-18 μJ/pulse for the visible at 1 kHz repetition rate. The SFG signal reflected from the sample surface was recollimated, spatially and frequency filtered, passed through a 300 mm monochromator (Acton Spectra-Pro 300i), and detected using a Liquid N_2-cooled CCD (Princeton Instruments Spec-10:100B, 100x1340 pixels). Five pixel binning along the horizontal axis was performed to reduce noise. With this binning, the spectrometer resolution is 2.7 cm^{-1}. The SFG spectrum was obtained by vertical integration of the CCD image. The signals were averaged over many laser shots by using the CCD as the integrator, with acquisition time typically 1-3 min per spectrum. The background spectrum for each spectrum measurement was recorded by blocking IR beam on to the sample for the same acquisition time. The background correction was performed by subtracting from the signal region of interest (e.g., horizontal strips 40-45), a non-illuminated region of background spectrum of the same size. The IR frequencies are calculated by subtracting the central frequency of the narrow visible pulse (measured using the same monochromator) from the SFG frequency. In addition, we calibrate the IR frequency scale using a known SFG surface spectrum of dimethyl sulfoxide (DMSO). We estimate our IR frequency calibration accuracy to be ±2 cm^{-1}. Polarization of the visible beam is controlled by using a zero-order half-wave plate, while the IR beam polarization can be made either horizontal or vertical by using a periscope before the sample. The polarization combination used in these experiments is PPP (SFG-vis-IR).

VSFG Data Fitting

In order to obtain correct vibrational transition frequencies we used multi-Lorentzian approximation to fit our data.

$$I_{SFG}\left(\omega_{IR}\right) \propto \left| a_{NR} e^{i\phi} + \sum_{v} \frac{b_v \Gamma_v}{\left(\omega_{IR} - \omega_v\right) + i\Gamma_v} \right|^2$$

Each vibrational mode v described by amplitude b_v , line width Γ, and transition frequency ω_v . First term express the non resonant contribution of the response with amplitude a_{NR} and phase φ with respect to the vibrationally resonant contribution.

Table 1: Fitting parameters for SFG spectra (Figure 1E) of maleimide-terminated precursor SAM formed from BMHC precursor on gold substrate and the product of the attachment reaction: unsaturated C-H stretch region (spectra in PPP polarization[a]

	Before		**After modifications**
Vibrational mode	Unsaturated C-H AS	Unsaturated C-H SS	No peaks
ω_v (cm^{-1}) [b]	3130	3066	
b	-0.60	-0.92	
Γ (cm^{-1})	16.03	25.56	
a_{NR}	1.3		
φ	-1.4		

[a] After modifications the two transitions disappeared.
[b] The uncertainty determining the transition frequency is ± 2 cm^{-1}.

Table 2: Fitting parameters for SFG spectra (Figure 1C) of maleimide-terminated precursor SAM formed from BMHC precursor on gold substrate and the product of the attachment reaction: C=O stretch region, PPP polarization

	Before	**After modification**
Vibrational mode	α-β unsaturated ring C=O stretch	Saturated ring C=O stretch
ω_v (cm^{-1}) [b]	1728	1746
b	-0.80	-0.37
Γ (cm^{-1})	9.0	9.0
a_{NR}	0.95	0.83
φ	-0.87	-1.09

Table 3: Fitting parameters for SFG spectra of maleimide-terminated precursor SAM formed from BMHC precursor on gold substrate (Figure 1D) and the product of the attachment reaction of glucose on C_{11} alkanethiol linker **2** (Figure 2A): saturated C-H stretch region, PPP polarization.

	Before		**After** Glucose-C_{11} modification					
Vibrational mode	d^+ CH_2 SS[b]	d^- CH_2 AS[b]	d^+ CH_2 SS[b]	Glucose C-H stretch	Glucose C-H stretch	Glucose C-H stretch	d^- CH_2 AS[b]	Glucose C-H stretch
ω_v (cm^{-1}) [b]	2862	2937	2865	2887	2910	2919	2940	2974
b	-0.55	-2.42	-1.33	-1.16	-0.15	-0.43	-2.20	-1.13
Γ (cm^{-1})	8.96	22.95	8.45	8.20	3.50	7.46	12.82	7.95
a_{NR}	1.03		0.69					
φ	-1.04		-1.77					

[a] The uncertainty determining the transition frequency is ± 2 cm^{-1}
[b] The transition frequencies obtained according to FTIR measurements

Table 4: Fitting parameters for SFG spectra of maleimide-terminated precursor SAM formed from BMHC precursor on gold substrate (Figure 1D) and the product of the attachment reaction of glucose on C_{16} alkanethiol linker **3** (Figure 2B): saturated C-H stretch region, PPP polarization.

	Before		**After** Glucose-C_{16} modification					
Vibrational mode	d^+ CH_2 SS[b]	d^- CH_2 AS[b]	d^+ CH_2 SS[b]	Glucose C-H stretch	Glucose C-H stretch	Glucose C-H stretch	d^- CH_2 AS[b]	Glucose C-H stretch
ω_v (cm^{-1}) [b]	2862	2937	2865	2887	2910	2919	2938	2974
b	-0.55	-2.42	-1.34	-0.97	-0.11	-0.34	-2.23	-1.20
Γ (cm^{-1})	8.96	22.95	9.86	9.22	3.50	7.46	14.20	9.55
a_{NR}	1.03		0.99					
φ	-1.04		-1.85					

[a] The uncertainty determining the transition frequency is ± 2 cm^{-1}
[b] The transition frequencies obtained according to FTIR measurements

Table 6: Fitting parameters for SFG spectra of maleimide-terminated precursor SAM formed from BMHC precursor on gold substrate (Figure 1D) and the product of the attachment reaction of glucose on cysteine-containing linker **6** (Figure 2C): saturated C-H stretch region, PPP polarization.

	Before		**After** Glucose-cystein modification					
Vibrational mode	d^+ CH_2 SS[b]	d^- CH_2 AS[b]	d^+ CH_2 SS[b]	Glucose C-H stretch	Glucose C-H stretch	Glucose C-H stretch	d^- CH_2 AS[b]	Glucose C-H stretch
ω_v (cm^{-1}) [b]	2862	2937	2865	2887	2910	2920	2944	2974
b	-0.55	-2.42	-1.32	-0.99	-0.16	-1.19	-1.68	-1.71
Γ (cm^{-1})	8.96	22.95	14.60	12.26	6.00	9.10	13.91	11.07
a_{NR}	1.03		1.04					
φ	-1.04		-1.68					

[a] The uncertainty determining the transition frequency is ± 2 cm^{-1}
[b] The transition frequencies obtained according to FTIR measurements