Angewandte Chemie
Eine Zeitschrift der Gesellschaft Deutscher Chemiker

Supporting Information

for

Angew. Chem. Int. Ed. Z51950

69451 Weinheim, Germany

A Practical Solid-Phase Synthesis of Glycosylphosphatidylinositol (GPI) Precursors

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Experimental section

General methods: Dichloromethane and acetonitrile were distilled from calciumhydride. Solid-phase reactions were conducted in custom-designed schlenk-type glass-reactors of 5,10 and 30ml volume, equipped with sintered glass filter and sealed with rubber septum. Reactors were shaken on IKA-Vibramax-VXR shakers. All reactions were carried out under an argon atmosphere in predried glassware unless otherwise stated. Analytical thin layer chromatography (TLC) were performed on silica gel 60 F_{254} precoated on aluminium plates (Merck) and the compounds were detected by staining with phosphomolybdic acid/EtOH or with anisaldehyde solution (anisaldehyde (25 mL) with sulphuric acid (25 mL), ethanol (450 mL) and acetic acid (1mL)) with detection by heating over 200ºC. Column chromatography was carried out on silica gel 60 (0.2-0.5 mm, 0.2-0.063 mm or 0.040-0.015 mm; Merck). Preparative tlc-purifications were carried out on Merck 20x20 cm silica-gel 60 F_{254}-plates. Optical rotations were determined with a Perkin-Elmer 341 polarimeter. ^{1}H- and ^{13}C-NMR spectra were acquired on Bruker DPX-300, DRX-400 and DRX-500 spectrometers and chemical shifts are given in ppm (δ) relative to tetramethylsilane as an internal reference or relative to D_2O. Mass spectra (fast atom bombardment, FAB MS) were carried out by the Mass Spectrometry Service, Facultad Química, Seville, with a Kratos MS-80 RFA spectrometer.

O-(2-O-Acetyl-3-O-t-butyldiphenylsilyl-6-O-(9-fluorenylmethoxycarbonyl)-4-O-levulinoyl-α-D-manno-pyranosyl-(1→4)-O-(2-Azido-3,6-di-O-benzyl-2-deoxy-α–D-glucopyranosyl)-(1→6)-1,2-di-O-acetyl-3,4,5-tri-O-benzyl-D-myo-inositol (1)

Trisaccharide was cleaved from the resin **21** (75mg resin, 1.5 ml 10% TFA in $CHCl_3$, 30mg crude Trisaccharide **1** after 1 cycle) as described in the general cleavage procedure and acetylated with pyridine/acetic anhydride (2ml/1ml), DMAP cat. at 0°C for 2h. After aqueous workup, drying of the organic fraction and evaporation of the solvent the residue was purified by preparative TLC (toluene/ethylacetate 3:1) to afford **1** (30mg, 0.022mmol, 87%) as a colorless film.

Rf=0.55 (toluene/ethylacetate), $[\alpha]_D$=+55.29° (c=0.85 in $CHCl_3$),
^{1}H-NMR (500MHz, $CDCl_3$) δ=7.63 (d, J=7.0Hz, 2H; Ph), 7.58 (d, J=7.0Hz, 2H; Ph), 7.41-7.09 (m, 31H; Ph), 5.73 (s, 1H; H_2), 5.34 (t, J=9.8Hz, 1H; $H_{4''}$), 5.26 (d, J=3.7Hz, 1H; $H_{1'}$), 5.09 (s, 1H; $H_{1''}$), 5.02-4.91 (m, 3H; CH_2Ph, H_1, $H_{2''}$), 4.71-4.67 (m, 2H; CH_2Ph), 4.59 (d, J=11.4Hz, 1H; CH_2Ph), 4.51-4.43 (m, 3H; CH_2Ph), 4.27 (m, 2H; CH_2Ph), 4.19 (dd, J=3.0Hz, J=9.7Hz, 1H; $H_{3''}$), 4.10 (t, J=9.8Hz, 1H; H_6), 3.97 (d, J=4.14, J=12.4Hz, 1H; $H_{6a''}$), 3.95 (t, J=9.5Hz, 1H; H_4), 3.78-3.75 (m, 3H; $H_{4'}$, $H_{5'}$, $H_{6b''}$), 3.66 (dd, J=2.6Hz, J=9.8Hz, 1H; H_3), 3.63-3.58 (m, 1H, $H_{3'}$), 3.51-3.45 (m, 1H, H_5), 3.41-3.39 (m, 1H, $H_{5''}$), 3.17 (d, J=11.0Hz, 1H, $H_{6a'}$), 3.06 (dd, J=3.6Hz, J=10.3Hz, 1H; $H_{2'}$), 3.01 (d, J=10.9Hz, 1H; $H_{6b'}$), 2.72-2.65 (m, 1H; Lev), 2.49-2.39 (m, 2H; Lev), 2.15 (s, 3H; Ac), 2.14 (s, 3H; Ac), 2.04 (s, 3H; Lev), 2.02 (s, 3H; Ac), 1.97 (s, 3H; Ac), 0.93 (s, 9H; t-Bu); FAB-MS *m/z* 1506 (MNa^+)

3,4,6-tri-O-benzyl-2-O-(9-fluorenylmethoxycarbonyl)-α-D-manno-pyranosyl-(1→6)-(O-(2-O-Acetyl-3-O-t-butyldiphenylsilyl-6-O-(9-fluorenylmethoxycarbonyl)-4-O-levulinoyl-α-D-manno-pyranosyl-(1→4)-O-(2-Azido-3,6-di-O-benzyl-2-deoxy-α–D-glucopyranosyl)-(1→6)-1,2-di-O-acetyl-3,4,5-tri-O-benzyl-D-myo-inositol (2)

Tetrasaccharide-loaded resin **22** (155mg) was treated with 10% TFA in $CHCl_3$ (3ml) as described in the general cleavage procedure and the free hydroxylgroups acetylated with pyridine/acetic anhydride and a catalytic amount of DMAP for 1h at rt. Aqueous workup, drying over $MgSO_4$ and coevaporation with toluene, followed by purification via preparative TLC yielded tetrasaccharide **2 (**28mg, 0.015mmol, 31%) as a slightly yellow oil.

Rf=0.54 (toluene/ethylacetate), $[\alpha]_D$=+52.35° (c=0.68 in $CHCl_3$), ^{1}H-NMR (500MHz, $CDCl_3$) δ= 7.62 (d, J=7.0Hz, 2H; Ph), 7.56 (d, J=7.0Hz, 2H; Ph), 7.40-7.09 (m, 46H; Ph), 5.71 (t, J=2.6Hz, 1H; H_{2a}), 5.39-5.35 (m, 2H; H_{4c}, H_{2d}), 5.22 (d, J=3.8Hz, 1H; H_{1b}), 5.10 (m, 1H; H_{1c}), 4.99 (d, J=11.3Hz, 1H; Ph), 4.97 (d, J=2.7Hz, 1H; H_{1a}), 4.95-4.93 (m, 1H; H_{2c}) 4.91 (d, J=10.5Hz, 1H; CH_2Ph), 4.75 (d, J=1.3Hz; H_{1d}), 4.71-4.59 (m, 6H; CH_2Ph), 4.51-4.36 (m, 6H; CH_2Ph), 4.22-4.19 (m, 1H; H_{3c}), 4.09 (t, J=9.7Hz, 1H; H_{6a}), 3.94 (dd, J=3.4Hz, J=9.4Hz, 1H; H_{3d}), 3.90 (t, J=9.5Hz, 1H; H_{4a}), 3.83 (t, J=9.7Hz, 1H; H_{4d}), 3.79-3.75 (m, 2H; H_{4b}, H_{5b}), 3.67-3.62 (m, 2H; H_{5d}, H_{3a}), 3.59-3.57 (m, 1H; H_{3b}), 3.55-3.44 (m, 4H; H_{6d}, $H_{6d'}$, H_{6c}, H_{5a}), 3.26 (d, J=9.8Hz, 1H; $H_{6c'}$), 3.16 (d, J=10.3Hz, 1H; H_{6b}), 3.08-3.03 (m, 2H; H_{2b}, $H_{6b'}$), 2.66-2.61 (m, 1H; Lev), 2.42-2.34 (m, 2H; Lev), 2.12 (s, 3H; Ac), 2.07 (s, 3H; Lev), 2.00 (s, 3H; Ac), 1.99 (s, 3H; Ac), 1.98 (s, 3H; Ac), 1.87-1.83 (m, 1H; Lev), 0.93 (s, 9H; t-Bu)

^{13}C-NMR (125MHz, $CDCl_3$) δ=206.2 (*C*OOR, Lev), 171.5 (*C*O, Lev), 170.1 (2x), 169.8, 169.7 (*C*O, Ac), 138.7, 138.4, 138.2, 137.1, 137.9, 137.8, 137.3, 136.1, 135.9 (quat.C , Ph),132.5, 130.2, 129.8, 128.5, 128.4, 128.3, 128.2, 128.1, 128.0, 127.9, 127.8, 127.7, 127.6, 127.5, 127.4, 127.3 (arom. C, Ph), 98.9 (C_{1b}), 98.4 (C_{1c}), 81.8, 80.6, 79.5, 78.5, 76.9, 76.8, 76.7, 75.9, 75.6, 75.3, 75.2, 75.0, 74.2, 73.9, 73.4, 73.2, 72.6, 72.3, 71.8, 71.4, 70.6, 70.0, 69.4, 69.2, 68.8, 68.4, 68.4, 68.2, 67.5, 66.1, 62.9 (CH_2, sugar core), 37.7 (*C*H_2CO (Lev)), 29.7 (CO*C*H_3), 27.7 (*C*H_2COOR (Lev)), 26.6 (t-Bu) 21.1, 20.9, 20.8, 20.7 (CH_3, Ac), 19.2 ($C_{quat.}$, TBDPS) ; FAB-MS *m/z* 1939 (MNa^+)

2-O-acetyl-3,4,6tri-O-benzyl-α-D-manno-pyranosyl-(1→2)-3,4,6-tri-O-benzyl-2-O-(9-fluorenylmethoxycarbonyl)-α-D-manno-pyranosyl-(1→6)-(O-(2-O-Acetyl-3-O-t-butyldiphenylsilyl-6-O-(9-fluorenylmethoxycarbonyl)-4-O-levulinoyl-α-D-manno-pyranosyl-(1→4)-O-(2-Azido-3,6-di-O-benzyl-2-deoxy-α–D-glucopyranosyl)-(1→6)-1,2-di-O-acetyl-3,4,5-tri-O-benzyl-D-myo-inositol (3)

Pentasaccharide-loaded resin **23** (205mg) was treated with 10%TFA in $CHCl_3$ as described in the general cleavage procedure. After workup the free hydroxylgroups were acetylated with pyridine/acetic anhydride and a catalytic amount of DMAP overnight at rt. Aqueous workup, drying over $MgSO_4$ and coevaporation with toluene, followed by purification via preparative TLC yielded pseudo-pentasaccharide **1** (34mg, 0.014mmol, 23%) as a slightly yellow oil.

Rf=0.67 (toluene/ethylacetate 3:1), $[\alpha]_D$=+30.75° (c= 0.53 in $CHCl_3$), ^{1}H-NMR (500MHz, $CDCl_3$) δ= 7.62 (d, J=6.9Hz, 2H; Ph), 7.57 (d, J=6.9Hz, 2H; Ph), 7.39-7.07 (m, 61H; Ph), 5.71 (t, J=2.6Hz, 1H; H_{2a}),5.50 (s, 1H; H_{2e}), 5.30 (t, J=9.8Hz, 1H; H_{4c}), 5.22 (d, J=3.8Hz, 1H; H_{1b}), 5.08 (s, 1H; H_{1c}), 5.03 (s, 1H; H_{1e}), 4.99-4.94 (m, 3H; CH_2Ph, H_{1a}, H_{2c}), 4.90 (d, J=10.5Hz, 1H; CH_2Ph), 4.80-4.77 (m, 3H; CH_2Ph, H_{2d}), 4.69-4.65 (m, 3H; CH_2Ph), 4.3-4.28 (m, 16H; H_{1d}, CH_2Ph), 4.20-4.18 (m, 1H; H_{3c}), 4.12-4.08 (m, 1H; H_{6a}), 3.96 (bs,1H; H_{3d}), 3.92 (bs, 1H; H_{3e}), 3.90-3.85 (m, 3H; H_{4a}, H_{5b}, H_{4e}), 3.83-3.70 (m, 5H; H_{6d}, $H_{6d'}$, H_{6e}, $H_{6e'}$, H_{5e}), 3.54 (dd, J=2.7Hz, J=9.8Hz, 1H; H_{3a}), 3.60-3.48 (m, 4H; H_{3b}, H_{6c}, H_{5a}, H_{5c}), 3.20 (d, J=10.0Hz, 1H; $H_{6c'}$), 3.15 (d, J=10.0Hz, 1H; H_{6b}), 3.09-3.03 (m, 2H; $H_{6b'}$, H_{2b}), 2.61-2.56 (m, 1H; Lev), 2.38-2.29 (m, 2H; Lev), 2.12 (s, 3H; Ac), 2.05 (s, 3H; Lev), 1.97 (s, 3H; Ac),1.96 (s, 3H; Ac), 1.87 (s, 3H; Ac), 0.91 (s, 9H; t-Bu)
^{13}C-NMR (125MHz, $CDCl_3$) δ=206.3 (*C*OOR, Lev), 171.6 (*C*O, Lev), 170.1, 169.8 (2x), 169.6, (CO, Ac), 138.7, 138.5, 138.3, 137.2, 138.1, 137.9, 137.8, 137.3, 136.1, 135.9 (quat.C , Ph), 129.7, 128.5, 128.4, 128.3, 128.2, 128.1, 128.0, 127.9, 127.8, 127.7, 127.6, 127.5, 127.4, 127.3 (arom. C, Ph), 99.6 (C_{1b}), 99.2 (C_{1c}), 99.0 (C_{1e}), 98.3 (C_{1d}), 81.8, 80.2, 80.0, 79.5, 78.2, 77.9, 75.9, 75.7, 75.5, 75.0, 74.6, 74.5, 74.1, 73.9, 73.3, 73.2, 72.7, 72.3, 72.2, 72.0, 70.5, 70.0, 69.5, 69.4, 69.3, 69.1, 68.7, 68.5, 68.3, 67.5, 62.8 (CH_2, sugar core), 37.6 (*C*H_2CO (Lev)), 29.7 (CO*C*H_3), 26.6 (t-Bu), 27.8 (*C*H_2COOR (Lev)), 21.1, 20.9, 20.8, 20.7 (CH_3, Ac), 19.1 ($C_{quat.}$, TBDPS) ; FAB-MS *m/z* 2373 (MNa^+)

***O-(2-Azido-3-O-benzyl-6-O-benzyl-2-deoxy-4-O-levulinoyl-α–D-glucopyranyl)-(1→6)-3,4,5-tri-O-benzyl-1,2-O-(L-1,7,7-trimethyl[2.2.1]bicyclohept-6-ylidine)-D-myo-inositol* (5)**

(1.2g, 1.26mmol) and DCC (1.3g, 6.3mmol) were dissolved in DCM (13ml) and under stirring levulinic acid (1.3ml, 12.6mmol) added slowly via syringe. After a few seconds precipitation of the insoluble urea occurred. After addition of an catalytical amount of DMAP (50mg) the suspension was stirred at rt for 3h until tlc-analysis showed no advance of the reaction. The reaction was diluted with DCM (50ml) and filtered over a pad of celite, the filtrate concentrated in vacuo and purified over a column of silica gel using hexane/ethylacetate (4:1) as the mobile phase. The product containing fractions were concentrated under reduced pressure and after disolvation in a minimum amount of diethylether foamed under high vacuum to afford **6** as colorless stable foam (1.26g, 1.20mmol, 95%) .**6** was obtained as a mixture (1.3/1) of regioisomers due to the acid-promoted isomerisation of the camphoracetal and was used as such in the following step. FAB-MS *m/z* 1072 (MNa^+)

O-(2-Azido-3,6-di-O-benzyl-2-deoxy-α–D-glucopyranosyl)-(1→6)-3,4,5-tri-O-benzyl-D-myo-inositol (7)

6 (1.16g, 1.10mmol) was dissolved in chloroform (35.2ml) , cooled to 0°C and slowly treated with TFA (8.8ml, 20%v)added by syringe. The icebath was then removed and the solution stirred for 20h at rt . The reaction was diluted with DCM (50ml) and neutralised with saturated Na_2CO_3-solution in a separation funnel. Washing with brine (30ml) , drying over anhydrous $MgSO_4$ and concentration under reduced pressure afforded a crude product, which was further purified over a column of silica gel (hexane/ethylacetate 4:1→2:1). After

concentration of the product containing fractions foaming from a minimum amount of diethylether furnished **7** as a colorless stable foam (784mg, 0.86mmol, 78%).

R_f= 0.16 (hexane/AcOEt 2:1), $[\alpha]_D$=42.2° (c= 0.6 in $CHCl_3$), ^1H NMR (500Mhz, $CDCl_3$, 25°C): δ= 7.32-7.18 (m, 25H; Ph), 5.46 (d, J=3.2Hz, 1H; $H_{1'}$), 5.16 (t, J=19.5Hz, 1H; $H_{4'}$), 4.96 (d, J=11.1Hz, 1H; CH_2), 4.92 (d, J=10.6Hz, 1H; CH_2), 4.76 (d, J=2.9Hz, 1H; CH_2), 4.73 (d, J=2.4Hz, 1H; CH_2), 4.71-4.69 (m, 3H; CH_2), 4.62 (d, J=11.1Hz, 1H; CH_2), 4.29(d, J=11.8Hz, 1H; CH_2), 4.18 (d, J=11.8Hz, 1H; CH_2), 4.14 (m, 2H; H_2), 4.04-3.89 (m, 3H; $H_{5'}$, H_4, $H_{3'}$), 3.64-3.59 (m, 1H; H_1), 3.54-3.50 (m, 2H; $H_{2'}$, H_6), 3.46 (dd, J=2.7Hz, J=9.5Hz, 1H; H_3), 3.39 (t, J=9.4Hz, 1H; H_5), 3.12 (dd, J=10.7Hz, J=2.8Hz, 1H; $H_{6a'}$), 3.01 (dd, J=3.5Hz, J=10.6Hz, 1H; $H_{6b'}$) 2.59-2.45 (m, 3H; CH_2 (Lev), OH-2), 2.22 (t, J=6.7Hz, 2H; CH_2 (Lev)), 2.09 (s, 3H; Lev (CH_3)) ^{13}C NMR (125MHz, $CDCl_3$): δ= 206.1 (*C*OOR, Lev), 171.3 (*C*O, Lev), 138.6, 138.5, 138.0, 137.8, 137.7 (quat. arom. C, Ph), 129.6, 128.6, 128.5, 128.4, 128.3, 128.2, 128.1, 128.0, 127.9, 127.8, 127.6, 127.5, 127.4, 127.1, 1270 (arom. C, Ph), 98.0 ($C_{1'}$), 81.6, 80.9, 80.0, 79.5, 78.2, 75.9, 75.0, 74.6, 73.4, 73.0, 72.7, 70.8, 69.7, 69.2, 68.0, 63.8 (CH_2, sugar core), 37.7 (*C*H_2CO (Lev)), 29.8 (CO*C*H_3), 27.8 (*C*H_2COOR (Lev)) FAB-MS *m/z* 937 (MNa^+)

Formation of stannylene-acetal, attachment of pseudodisaccharide to resin and capping of unreacted linker sites (8)

Formation of stannylene-acetal

7 (766mg, 0.86mmol) and Bn_2SnO (235mg, 095mmol) were suspended in MeOH (20ml) under an Argon-atmosphere and heated to reflux temperature. After approximately 1h the turbid solution became clear. Stirring was continued for 2h, the reaction cooled to rt, concentrated under reduced pressure and dried over night under high vacuum. Due to the high instability of the stannylene-acetal **8** the compound was used as such in the next step and not further characterised.

Attachment to resin

Argo-Gel-Wang-Cl resin (1.3319g, 0.573mmol) with an initial loading of 0.43mmol/g was placed into a tared 50ml custom-made schlenk equipped with a stopcock and a glass filter. After adding solid CsF (262mg, 1.72mmol) and TBAI (318mg, 0.86mmol), the schlenk was flushed with inert gas , sealed with a septum and the stannilydene-acetal **8**, dissolved in acetonitrile (10ml), was transferred via cannula to the flask. After shaking for 10min at rt using a modified IKA-vibrax-vxr shaker to swell the geltype resin, the schlenk, equipped with a balloon containing argongas, was heated to 60°C using a oil bath and shaking was continued for 4h. MeOH (5ml) was added to “quench” unreacted linker and the resin shaken for 20min. After colling to rt the resin was washed with MeOH (3x10ml), DCM(4x10ml), water (2x5ml), MeOH(4x10ml), THF(4x10ml), DCM(4x10ml). Inbetween washings the resin was stirred thoroughly using a IKA MS1 minishaker. The first methanol- and DCM-washings were collected to recover unreacted pseudodisaccharide **7**. The resin **9** was dried over night under high vacuum and the schlenk weighed to determine the amount of attached pseudodisaccharide (377mg, 0.41mmol, 72%, loading 0.31mmol/g).

To selectively cap the unreacted linker, the resin (1513mg, 0.16mmol free linker) was swollen in DCM (10ml), shaken for 10 min under an inert gas atmosphere, treated with trichloroacetonitrile (240µl, 2.4mmol, 15eq.), cooled to 0°C and after addition of DBU (20µl) shaken for 40min at 0°C. After removal of the liquid, the resin was washed subsequently with DCM(4x7ml), DMSO(4x7ml), THF(4x7ml), DCM(5x7ml), dried in vacuo and used directly for the following methylation. For the capping reaction with methanol the resin was swollen in a mixture of Cyclohexane/DCM/methanol (5ml/7ml/0.5ml), and the reaction started by addition of BF_3OEt_2 (40µl, mmol). After 10min shaking, the solvent was removed under pressure, using the incorporated glass filter, and the resin washed with MeOH(4x5ml) and DCM (4x5ml).

^{13}C-gel-phase-NMR (75MHz, $CDCl_3$) δ= 206.54 (*C*O, Lev), 171.56 (*C*OOR, Lev), 138.88, 138.73, 138.44, 138.31, 138.19 (aromat. C, Bn), 130.19, 128.90, 128.79, 128.53, 128.38, 128.21, 127.83, 127.72, 127.50, 127.36 (aromat. C, Bn, resin), 115.04 (aromat. C, Bn), 97.42 (C-1b), 81.76, 81.44, 79.89, 78.22, 77.99, 77.63, 76.99, 76.31, 75.85, 74.87, 74.63, 74.33, 73.74, 73.01, 71.95, 71.73, 70.06, 68.78, 67.85, 66.54, 62.97 (*C*H_2Ph, C sugar core, C resin backbone), 38.07 (*C*H_2CO, Lev), 30.29 (*CO C*H_3, Lev), 28.11 (*C*H_2COOR)

Acetylation of the free inositol-hydroxygroup (9)

To protect the remaining free hydroxylgroup of the inositolmoiety, the resin **9** (1.485mg) was swollen in DCM/pyr (6ml/4ml) treated with acetic anhydride (2ml), DMAP (10mg), dissolved in a small amount of DCM, and shaken at rt for 2.5h. After removal of the solvents, washing with MeOH(4x6ml) and DCM(5x6ml), the procedure was repeated. Cleavage (TFA 10% in $CHCl_3$, 3ml, 2h) of a resin sample (91mg) reproduced the calculated loading (0.3 mmol/g) and NMR analysis of the cleaved product showed the existence of two regioisomers (2:1).

^{13}C-gel-phase-NMR (75MHz, $CDCl_3$) δ= 206.48 (*C*O, Lev), 171.55 (*C*OOR, Lev), 170.81(COOR, Ac), 138.76, 138.60, 138.41, 138.28, 138.19, 137.92 (aromat. C, Bn),131.10, 130.57, 128.93, 128.73, 128.20, 128.05, 127.55 (aromat. C, Bn, resin), 114.92 (aromat. C, Bn), 97.50 (C-1b), 82.05, 81.30, 79.44, 78.47, 77.62, 76.24, 76.11, 75.31, 75.00, 74.72, 74.45, 74.29,73.73, 73.27, 72.58, 71.88, 71.76, 70.09, 68.79, 67.82, 66.23, 62.82 (*C*H_2Ph, C sugar core, C resin backbone), 38.06 (*C*H_2CO, Lev), 30.27 (*CO C*H_3, Lev), 28.10 (*C*H_2COOR, Lev), 21.38 (*C*H_3, Ac)

Deslevulination on solid support (10)

The resin containing pseudodisaccharide **9** (1.467g, 0.356mmol) was swollen in DCM (9ml) shaken for 10min under argon atmosphere and rt, hydrazine-acetate (98mg, 1.068mmol) dissolved in MeOH (1ml) added and after shaking for 20h at rt the excess hydrazine consumed by addition of acetylacetone (2ml) and prolonged shaking (30min). The soluble fraction was removed, the resin washed with MeOH (4x5ml), DCM (4x5ml) and the deprotection repeated for 10h. Repeated washing and drying over night afforded the liberated receptor resin **10** in quantitative yield as suggested by tlc analysis of an cleaved resin sample (hexane/ethylacetate 3:2, Rf= 0.59) ^{13}C-gel-phase analysis showed the disappearance of the lev-group.

^{13}C-gel-phase-NMR (75MHz, $CDCl_3$) δ 170.83 (COOR, Ac), 138.70, 138.60, 138.44, 137.92 (aromat. C, Bn), 132.32, 130.66, 130.51, 128.93, 128.73, 128.20, 128.05, 127.55 (aromat. C, Bn, resin), 115.06, 114.91 (aromat. C, Bn, resin), 97.79 (C-1b), 81.35, 79.53, 78.57, 78.19, 77.63, 76.45, 76.14, 75.16, 73.72, 72.57, 72.34, 71.89, 70.08, 68.02, 67.81, 66.23, 62.89 (*C*H_2Ph, C sugar core, C resin backbone), 21.55 (*C*H_3, Ac)

Typical glycosylation procedure

To the dried acceptor-loaded resin **10** (1.427g, 0.356mmol)) was added 2 eq of e.g. donor **7** (657mg, 0.712mmol) either as a colorless solid, or as a solution in DCM via cannula when donor was an oil, the schlenk flushed with argon, swollen in DCM (12ml) agitated for 10 min at rt, cooled to –20°C and the reaction started by addition of 0.1eq of a freshly prepared 0.5 M TMSOTf solution in DCM. After 2h of vigorous shaking at –20°C tlc-analysis of the liquid phase showed the complete consumption/transformation of the donor. The resin was diluted with 5ml of DCM, the solvents filtered off rapidly at low temperature and the resin washed successively with 75ml DCM and dried overnight under high vacuum. To recover hydrolysed excess donor, the collected washing fractions were washed with saturated $NaHCO_3$-solution, dried over $MgSO_4$ and concentrated under reduced pressure. Cleavage and Tlc-analysis of a small resin sample (4-5mg) indicated, if glycosylation had to be repeated or not.

^{13}C-gel-phase-NMR of pseudotrisaccharide fully protected (75MHz, $CDCl_3$) δ 206.58 (*C*O, Lev), 172.36 (*C*OOR, Lev), 170.79, 170.05 (COOR, Ac), 155.09 (*C*O, Fmoc), 144.02, 143.76, 141.62, 138.79, 138.51, 138.23, 137.90, 136.50, 136.22, 133.97 (aromat. C, Bn), 132.55, 130.63, 130.17, 129.95, 128.87, 128.64, 128.36, 128.25, 128.02, 127.84, 127.56, 127.14, 125.68, 120.40 (aromat. C, Bn, resin), 114.88 (aromat. C, Bn, resin), 100.16 (c-1c), 97.99 (C-1b), 82.20, 80.89, 79.67, 79.42, 78.52, 78.18, 76.31, 75.71, 75.49, 74.16, 73.56, 73.32, 72.66, 71.74, 70.16, 69.75, 69.32, 68.48, 67.78, 66.56, 66.11, 62.89 (*C*H_2Ph, C sugar core, C resin backbone), 47.08 (*C*H_2, Fmoc), 38.20 (*C*H_2CO, Lev), 30.17 (CO*C*H_3, Lev), 28.05 (*C*H_2COOR, Lev), 26.90 (C(*C*H_3), TBDPS), 21.57 (*C*H_3, Ac), 21.30 (*C*H_3, Ac), 19.58 (*C*(CH_3), TBDPS)

Typical deprotection procedure for the Fmoc-group on solid-phase

In a typical experiment 1.5g of resin were swollen in a 20% solution of Et_3N in DCM and shaken for 4h at rt. The solvents were filtered off using the incorporated sintered glass filter and the resin washed continuously with MeOH and DCM until the was tlc-analysis of the washing fractions indicated the absence of the highly uv-active Fmoc-derivate. The deprotection was repeated until no uv-absorbance was observed for a tlc of the liquid phase. In general the deprotection was complete after 2 cycles. In addition the completion of the deprotection was observed by cleaving a sample from the resin and by performing tlc and MALDI-Tof- analysis.

General procedure for cleavage of intermediates from the resin, acetylation and purification

After removal of the Fmoc-group (for procedure see above, Fmoc-group is partially cleaved under acetylation conditions) a resin sample (150mg) was treated with 10% TFA in $CHCl_3$

(2ml) and shaken for 2h at rt. The solvents were carefully filtered off and collected, the resin thoroughly washed with DCM (5x3ml). All washing fractions were combined, neutralised with saturated $NaHCO_3$ solution, dried over $MgSO_4$ and concentrated in vacuo. The deprotection procedure was repeated twice, and the united cleavage products were acetylated with pyridine/acetic anhydride (1ml/0.5ml) and DMAP(cat) at 0° for 2h. After dilution with ethylacetate (10ml) and extraction with water (3x10ml), the organic fraction was dried over $MgSO_4$, concentrated in vacuo and coevaporated twice with toluene (2x2ml) to remove last traces of pyridine. The slightly yellow product was purified by preparative TLC (toluene/ethylacetate 3:1) affording a single compound of high purity.

Phenyl 4,6-O-Benzylidene-3-O-t-butyldiphenylsilyl-1-thio-α-D-manno-pyranoside (15)

A suspension of **14** (4g , 11.1mmol) and Dibutyltinoxide (3.04g , 12.2mmol) in 80 ml of Methanol is refluxed for 1h until an almost clear solution is observed. The solvent is removed under reduced pressure and the resulting foam dried for 2h under high vaccum.

To solution of the stannilidene-acetal in DMF (60ml) at rt , TBDPSCl (7.5ml, 28.8mmol) and TBAI (1g, 0.24mmol) in 2ml of DCM are added by syringe. The stirred solution is left overnight, quenched with 2ml of MeOH and washed twice subsequently with water and brine. The organic phase is dried over $MgSO_4$, filtered and the solvent removed under reduced pressure. Chromatographic purification of the residue over a column of silica gel (hexane/ethylacetate 9:1) and removal of the solvent, afforded compound **15** as a white foam (4.7g, 7.9mmol, 71%) next to 762mg (20%) of unreacted starting material and traces of the regioisomer.

R_f 0.52 (hexane/AcOEt 4:1), $[\alpha]_D^{20}$= +163° (c= 0.87 in $CHCl_3$), ^{1}H NMR (500Mhz, $CDCl_3$, 25°C): δ= 7.68-7.64 (m, 4H; Ph), 7.35-7.21 (m, 16H; Ph), 5.6 (s, 1H, H_1), 5.46 (s, 1H; CHPh), 4.08-4.23 (m, 4H; H_3, H_4, $H_{6a,b}$), 3.85 (d, $^3J_{2,1}$=2.6Hz, 1H; H_2), 3.82-3.73 (m, 1H; H_5), 2.93 (s, 1H; OH-2), 1.08 (s, 9H; t-Bu) ; ^{13}C NMR (125MHz, $CDCl_3$) δ= 137.7, 136.3, 136.2, 133.7, 133.2, 132.1, 130.6, 130.2, 129.5, 129.3, 128.4, 128.3, 128.0, 126.7(Ph), 101.9 (CHPh), 87.4 (C_1), 78.9, 72.6, 10.9, 68.4, 64.4 (sugar core), 26.9 (t-Bu), 19.7 (C_{quat} TBDPS) FAB-MS *m/z* 621 (MNa^+)

Phenyl 2-O-Acetyl- 4,6-O-benzylidene-3-O-t-butyldiphenylsilyl-1-thio-α-D-manno-pyranoside (16)

To a solution of **15** (4.7g, 7.86mmol) in a mixture of pyridine (55ml) and acetic anhydride (22ml) was added a catalytical amount of DMAP (100mg, 0.1eq) dissolved in DCM (1ml) . The solution was stirred for two hours at rt until tlc-analysis indicated the total consumption of the starting material. Great part of the pyridine was removed by extraction of the product with ethylacetate and repeated washing of the organic fraction with water and finally brine. Drying over $MgSO_4$, filtration and removal of the solvent by reduced pressure and coevaportion with toluene afforded crude **16** (4.78g, 7.5mmol, 95%) which was further purified by silica gel chromatography (hexane/ethylacetate 12:1).

R_f 0.42 (hexane/AcOEt 6:1), $[\alpha]_D^{20}$ not measured, ^{1}H NMR (300Mhz, $CDCl_3$, 25°C): δ= 7.68-7.19 (m, 20H; Ph), 5.46 (s, 1H; CHPh), 5.29 (d, $^3J_{1,2}$=1.2Hz, 1H; H_1), 5.04-5.03 (m, 1H;

H_2), 4.28 (dd, J=9.4Hz, J=3.4Hz, 1H; H_3), 4.18-4.10 (m, 3H; H_4, $H_{6a,b}$), 3.78 (t, J=9.7Hz, 1H; H_5), 2.19 (s, 3H; Ac), 1.02 (s, 9H; t-Bu) ; ^{13}C NMR (75MHz, $CDCl_3$, 25°C) from HMQC (selected values) δ = 102.9 (CHPh), 87.2 (C_1), 79.2 (C_4), 74.5 (C_2), 68.4(C_3) , 68.1 (C_5), 68.0 (C_6), 27.1 (t-Bu), 22.2 (CH_3) FAB-MS *m/z* 663 (MNa^+)

Phenyl 2-O-Acetyl-3-O-t-butyldiphenylsilyl-1-thio-α-D-manno-pyranoside (17)

To a solution of **16** (3.95g, 7.15mmol) in DCM (50ml) was added via syringe Ethanthiol (5.3 ml, 3.57mmol) and BF_3OEt_2 (45µl, 0.36mmol). The reaction was allowed to proceed at rt for 30 min and stopped with triethylamine (250µl). Solvent and excess thiol were removed in a rotaevaporotor located in a hood and equipped with a water pump. The crude residue was purified by silica gel chromatography (hexane/ethylacetate 3:1 → 1:2) affording pure **17** (3.63g, 6.58mmol, 92%) as a colorless foam.

R_f 0.26 (hexane/AcOEt 3:1), $[\alpha]_D^{20}$=+60.5° (c=0.78 in $CHCl_3$), ^{1}H NMR (500Mhz, $CDCl_3$, 25°C): δ= 7.69-7.22 (m, 15H, Ph), 5.30 (d, $^3J_{1,2}$=1.4Hz, 1H, H_1), 5.03-5.02 (m, 1H, H_2), 4.04-4.02 (m, 2H, H_3, H_4), 3.99-3.94 (m, 1H, H_5), 3.79-3.75 (m, 2H, $H_{6a,b}$), 2.10 (s, 3H, Ac), 1.95 (d, J=2.9Hz, 1H,OH_4), 1.81 (t, J=6.7Hz, 1H, OH_6), 1.05(s, 9H,t-Bu) ; ^{13}C (75MHz, $CDCl_3$) from HMQC (selected values) δ= 87.2 (C_1), 74.5 (C_2), 72.3 (C_3, C_4), 68.1 (C_5), 63.3 (C_6), 27.5 (t-Bu), 22.3 (CH_3) FAB-MS *m/z* 575 (MNa^+)

Phenyl 2-O-Acetyl-3-O-t-butyldiphenylsilyl-6-O-(9-fluorenylmethoxycarbonyl)-1-thio-α D-manno-pyranoside (18)

To a solution of diol **17** (2g, 3.62mmol) and DMAP (5mg, 0.04mmol) in acetonitrile (60ml) and pyridine (15ml) cooled to –15°C by means of a ice/salt bath FmocCl (1.87g, 7.24 mmol) was added in small amounts over 10 min. The reaction was complete after one hour as disappearence of starting material in tlc indicated. The reaction was stopped through additon of water (2ml) , the reaction mixture poured into a funnel containing DCM/water (150ml/150ml) the organic phase repeatedly washed with water. The collected organic fractions were dried over $MgSO_4$, filtered and the solvent removed under reduced pressure. The crude residue was purified over silica gel (hexane/ ethylacetate 9:1) affording **18** (2.43g, 3.08mmol, 85%) as slightly yellow foam.

R_f 0.62 (hexane/AcOEt 3:1), $[\alpha]_D^{20}$= +33.9° (c=0.59 in $CHCl_3$), ^{1}H NMR (500Mhz, $CDCl_3$, 25°C): δ= 7.75 (d, J=7.56Hz, 2H; Fmoc), 7.70-7.67 (m, 4H; Ph), 7.60-7.58 (m, 2H;Fmoc), 7.47-7.17 (m, 17H; Fmoc, Ph), 5.32 (d, $^3J_{1,2}$=1.1Hz, 1H; H_1), 5.01-5.00 (m, 1H; H_2), 4.43-4.36 (m, 4H; $H_{6a,b}$, CH_2 (Fmoc)), 4.24-4.18 (m, 2H; H_5,H_9(Fmoc)), 4.03 (dd, 1H, $^3J_{3,4}$=9.15Hz, $^3J_{3,2}$=3.24Hz, 1H; H_3) 3.98-3.95 (m, 1H; H_4), 2.09 (s, 3H; Ac), 2.08 (d, J=3.5Hz; OH-4), 1.03 (s, 9H; t-Bu) ; ^{13}C NMR (75MHz, $CDCl_3$) δ= 143.2, 141.2, 135.8, 135.6,133.2, 133.1, 132.4, 132.1, 131.3, 130.3, 130.2, 129.0, 128.9, 128.1, 128.0, 127.9, 127.8, 127.1 (Ph, Fmoc) 125.1, 129.0 (Fmoc), 86.0 (C_1), 73.6 (C_2), 72.5 (C_3) 71.1 (C_4), 69.9 (Fmoc) , 68.3 (C_6), 66.9 (C_5), 46.7 (Fmoc) 26.8 (t-Bu), 21.0 (CH_3, Ac), 19.4 ($C_{quat.}$, TBDPS) FAB-MS *m/z* 797 (MNa^+)

Phenyl 2-O-Acetyl-3-O-t-butyldiphenylsilyl-6-O-(9-fluorenylmethoxycarbonyl)-4-O-levulinoyl-1-thio-α-D-manno-pyranoside (19)

To mixture of **18** (810mg, 1.046mmol) and DCC (1078mg, 5.23mmol) dissolved in DCM (9ml) levulinic acid (1.08ml, 10.5mmol) was added by syringe. After a few seconds a white precipitate was observed. DMAP (20mg) dissolved in DCM (150µl) was added and the reaction mixture stirred for 1.5 h, tlc-analysis indicating the consumption of starting material and the formation of a new slower running product. The suspension was diluted with DCM (30ml) and filtered over a pad of celite. After evaporation of the solvent, the residue was subjected to chromatographic purification (hexane/ethylacetate 6:1 → 5:1) affording **19** as a colorless foam (779mg, 0.89mmol, 85%) next to a small amount of starting material.

R_f 0.52 (hexane/AcOEt 3:1), $[\alpha]_D^{20}$ =+43.1° (c=3.43 in $CHCl_3$), ^1H NMR (500Mhz, $CDCl_3$, 25°C): δ= 7.78-7.17 (m, 23H, Fmoc; Ph),5.39 (t, J=19Hz, 1H; H_4), 5.33 (s, 1H; H_1),4.98 (bs, 1H; H_2), 4.43-4.20 (m, 7H; $H_{6a,b}$, H_3, H_5, H_9 (Fmoc), CH_2(Fmoc)), 2.75-2.64 (m, 1H; Lev), 2.52-2.37 (m, 2H; Lev), 2.13 (s, 3H; Lev), 2.11 (s, 3H; Ac), 2.00-1.92 (m, 1H; Lev), 1.03 (s, 9H; t-Bu) ; ^{13}C NMR (75MHz, $CDCl_3$) δ= 206.7 (COO, Lev) 172.6 (CO, Lev), 170.5 (CO, Ac), 155.3 (CO, Fmoc), 143.9, 143.7, 141.7, 136.3, 136.1, 133.5, 133.3, 132.7, 132.5, 130.8, 130.3, 129.4, 128.4, 128.3, 128.2, 128.1, 127.6, 127.5, 125.7, 125.6, 120.4 (TBDPS, Fmoc, SPh) 86.1 (C_1), 70.3, 70.1, 70.0, 66.9, 47.1 (Fmoc), 38.3 (CH_2, Lev), 30.1 ($COCH_3$, Lev), 28.2 (CH_2COO, Lev), 27.0 (t-Bu), 21.4 (CH_3, Ac), 19.7 ($C_{quat.}$, TBDPS) FAB-MS *m/z* 895 (MNa^+)

2-O-Acetyl-3-O-t-butyldiphenylsilyl-6-O-(9-fluorenylmethoxycarbonyl)-4-O-levulinoyl-α/β-D-manno-pyranoside (20)

19 (1.77g, 2.03mmol) is dissolved in acetone (25ml) cooled to –20°C and 1/3 of the total amount of N-Bromosuccinimide (144mg, 0.81mmol) added under the absence of light. After addition of water (110µl, 6.1mmol) and subsequent addition of the remaining reagent over an period of 1h, the solution is left stirring at low temperature until the consumption of the starting material is completed. The reaction is quenched with 10ml of sat. aqu. $NaHCO_3$-solution, stirred until the solution becomes colorless, and diluted with DCM 100ml.The organic phase is separated , washed with sat. aqu. $NaHCO_3$-solution and brine, dried over $MgSO_4$, filtered and the solvent removed under reduced pressure. Purification of the residue by absorption chromatography (hexane/ethylacetate 3:1) yields **19** (1.43g, 1.83mmol 90%) as a colorless stable foam.

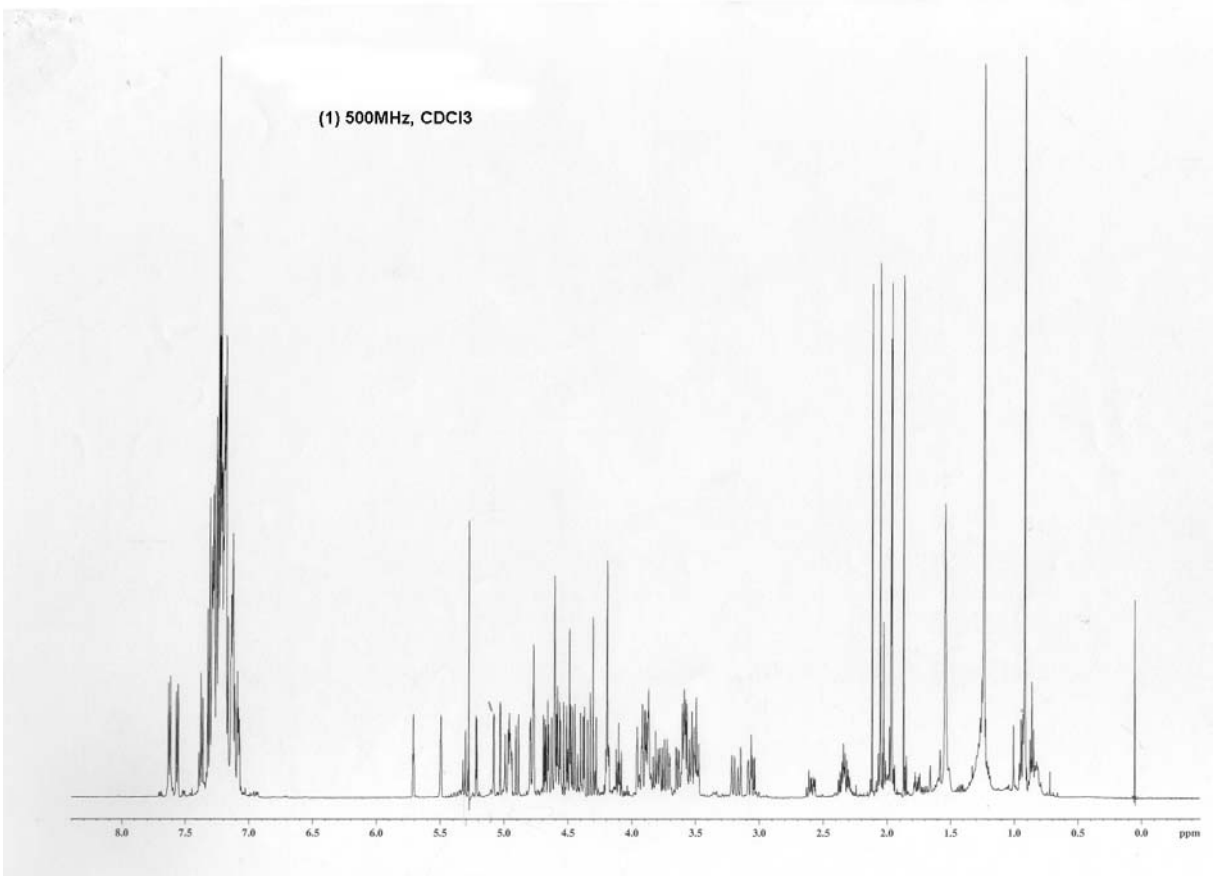
R_f 0.31 (hexane/AcOEt 2:1), ^1H NMR (500Mhz, $CDCl_3$, 25°C): δ= 7.77-7.74 (d, J=7.5Hz, 2H; Ph), 7.67-7.61 (m, 6H; Ph), 7.48-7.27 (m, 10H; Ph), 5.32 (t, J=19Hz, 1H; H_4), 5.11 (s, 1H; H_1), 4.81 (bs, 1H; H_2), 4.48-4.33 (m, 3H; H_3, CH_2(Fmoc)), 4.29-4.23 (m, 3H; $H_{6a,b}$, H_9(Fmoc)), 4.05-4.00 (m, 1H; H_5), 2.90 (d, J=4.0Hz, 1H; OH-1), 2.71-2.62 (m, 1H; Lev), 2.46-2.32 (m, 2H; Lev), 2.12 (s, 3H; Ac), 2.08 (s, 3H, CH_3; Lev), 1.92-1.82 (m, 1H; Lev), 1.02 (s, 9H; t-Bu) ; ^{13}C NMR (75MHz, $CDCl_3$) δ= 206.8 (COO, Lev) 172.6 (CO, Lev), 170.8 (CO, Ac), 155.3 (CO, Fmoc), 144.1, 143.9, 143.8, 143.7, 141.8, 141.7, 141.6, 141.5, 136.3,

136.1, 133.9, 132.9, 130.6, 130.5, 130.2, 128.5, 128.4, 128.3, 128.1, 128.0, 127.6, 127.5, 127.4, 125.7, 125.6, 120.4, 120.3 (TBDPS, Fmoc) 92.2 (C_1), 72.9, 70.3, 70.1, 69.1, 68.8, 67.1,(sugar core, Fmoc) 47.1 (Fmoc), 38.5 (CH_2, Lev), 30.1 ($COCH_3$, Lev), 28.1 (CH_2COO, Lev), 27.0 (t-Bu), 21.4 (CH_3, Ac), 19.7 ($C_{quat.}$, TBDPS) FAB-MS *m/z* 803 (MNa^+)

O-(2-O-Acetyl-3-O-t-butyldiphenylsilyl-6-O-(9-fluorenylmethoxycarbonyl)-4-O-levulinoyl-α-D-manno-pyranosyl) trichloroacetimidate (11)

20 (1.43g, 1.83mmol) was dissolved in trichloroacteonitrile (12ml) and treated with NaH (7.3mg on paraffin oil, 0.183mmol) at 0°C . After 40 min tlc analysis indicated the full consumption of the starting material whereupon the solution was absorbed onto silica and purified over a short filter of silica gel (hexane/ethylacetate 3:1+1% Et_3N) yielding **11** (1.33g, 1.44mmol, 79%) as a colorless foam (α/β=5.5:1).

R_f 0.41 (hexane/AcOEt 2:1), $[\alpha]_D^{20}$ (not measured), ^1H NMR (300Mhz, $CDCl_3$, 25°C) only α-anomer δ= 8.59 (s, 1H; NH), 7.75 (d, J=7.5Hz, 2H; Ph), 7.68-7.28 (m, 16H; Ph, Fmoc)), 6.16 (d, J=1.99Hz, 1H; H_1), 5.45 (t, J=19.5Hz, 1H; H_4), 5.03-5.01 (m, 1H; H_2), 4.42-4.26 (m, 6H; H_3, $H_{6a,b}$, H_9, CH_2(Fmoc)), 3.97-3.91 (m, 1H; H_5), 2.69-2.60 (m, 1H; Lev), 2.44-2.31 (m, H; Lev), 2.19 (s, 3H; Ac), 2.08 (s, 3H, CH_3; Lev), 1.77-1.67 (m, 1H; Lev), 1.01 (s, 9H; t-Bu) FAB-MS *m/z* 947 (MNa^+)



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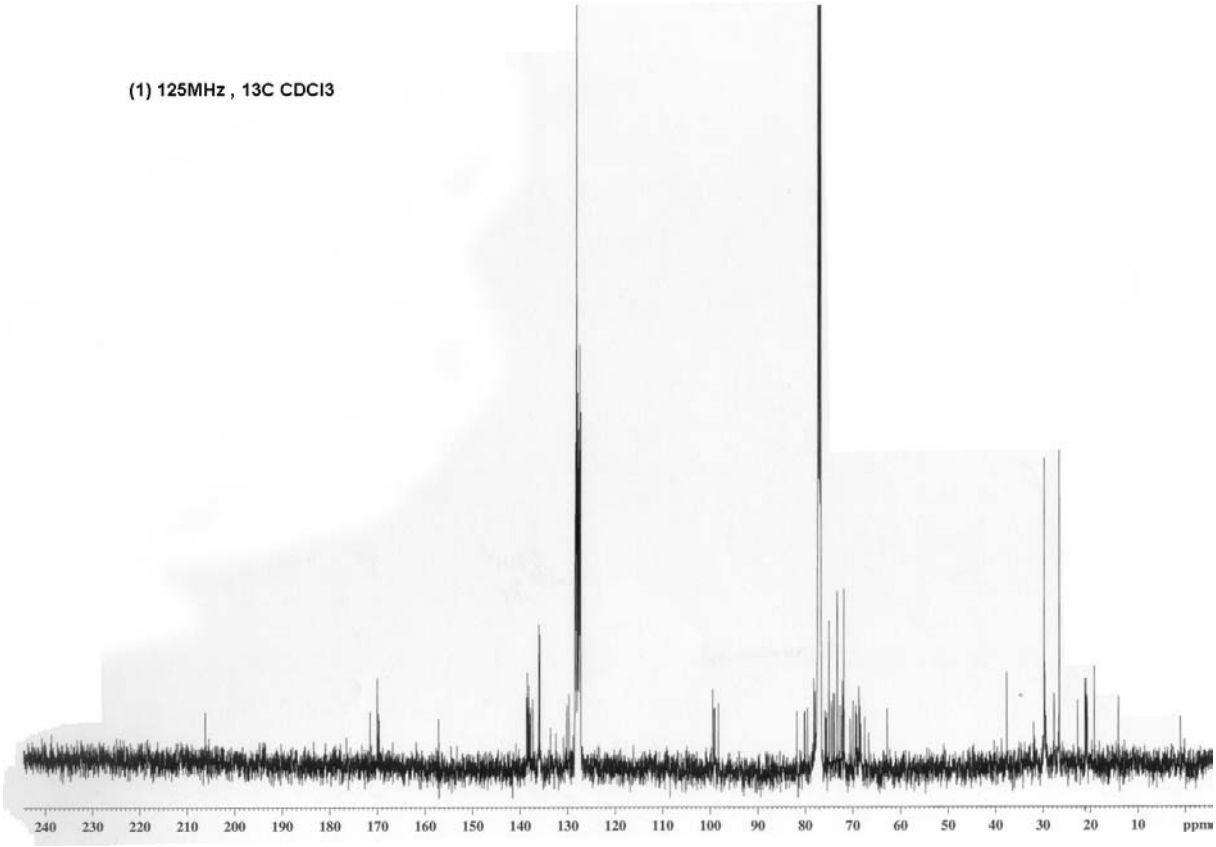

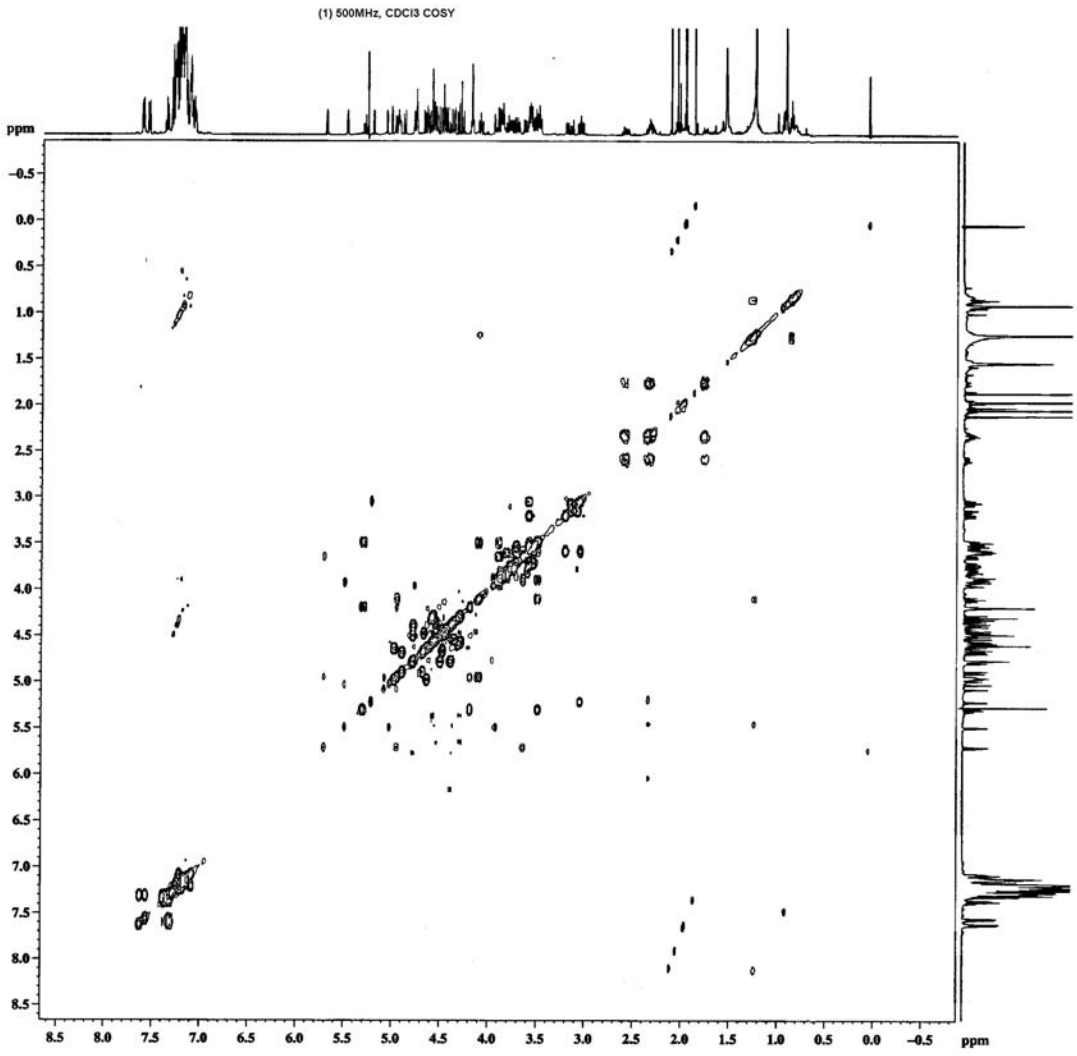
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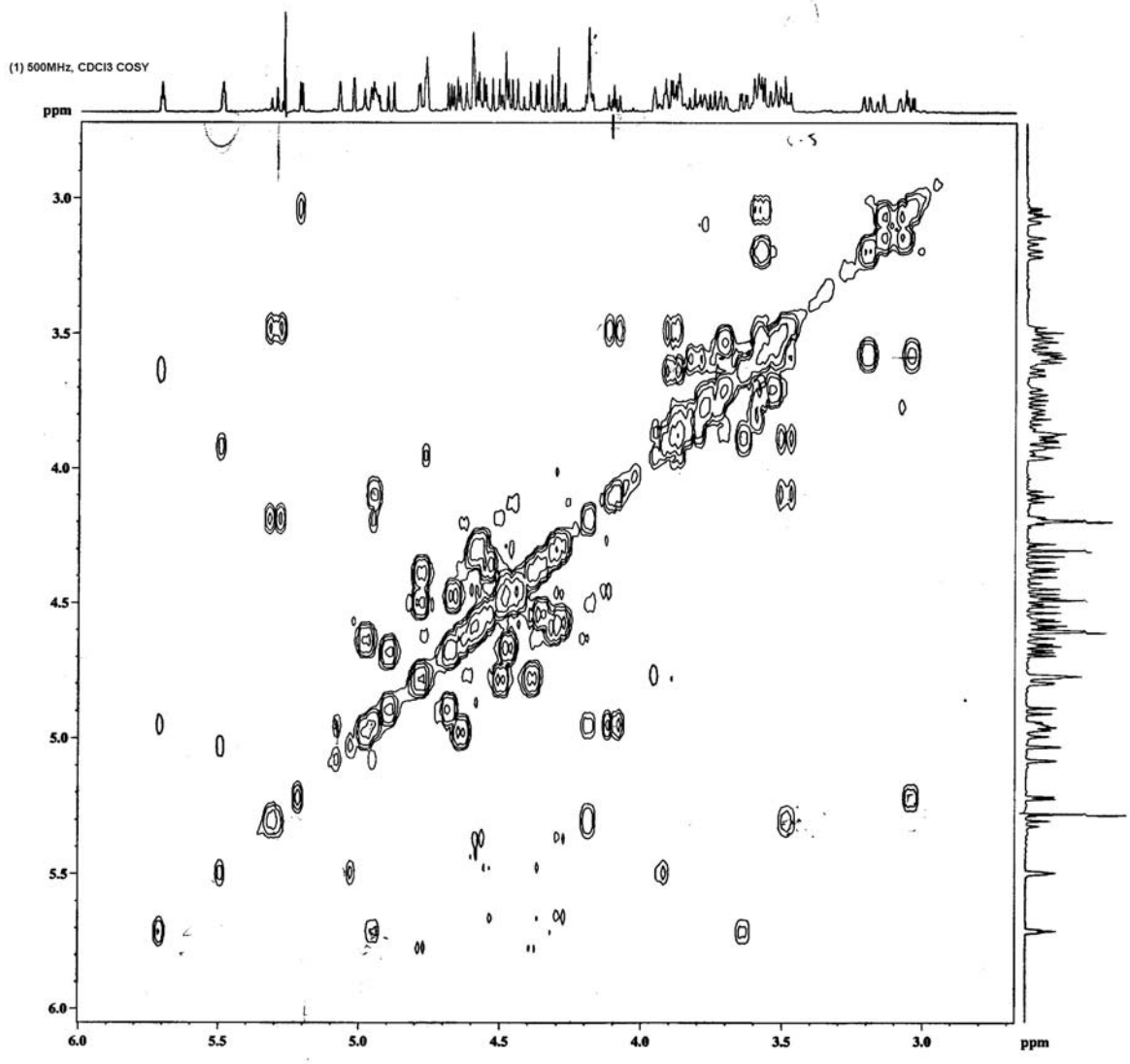
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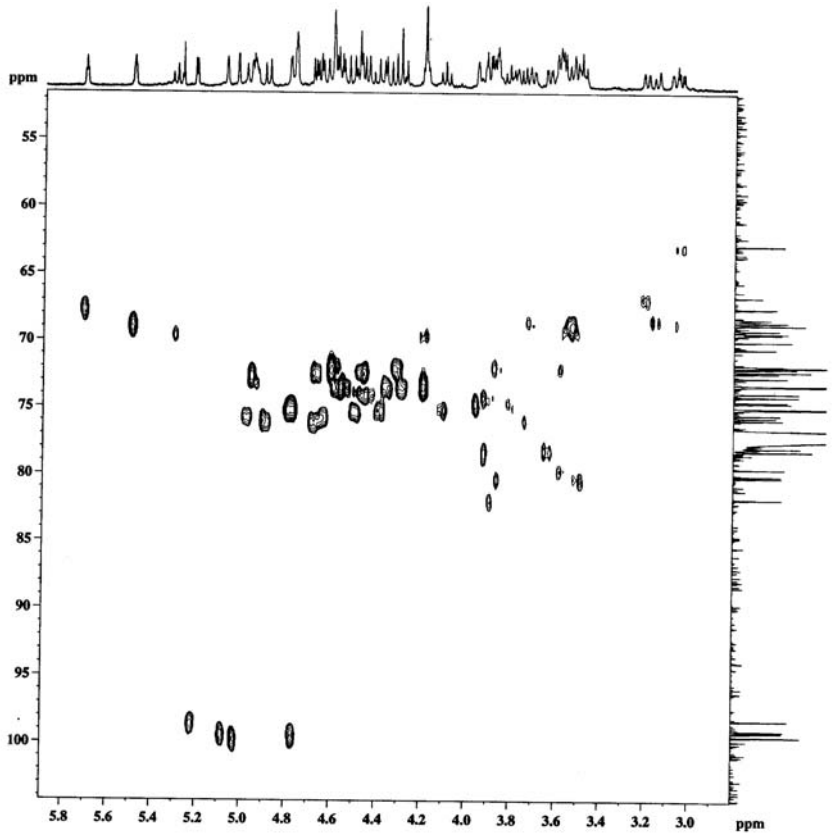
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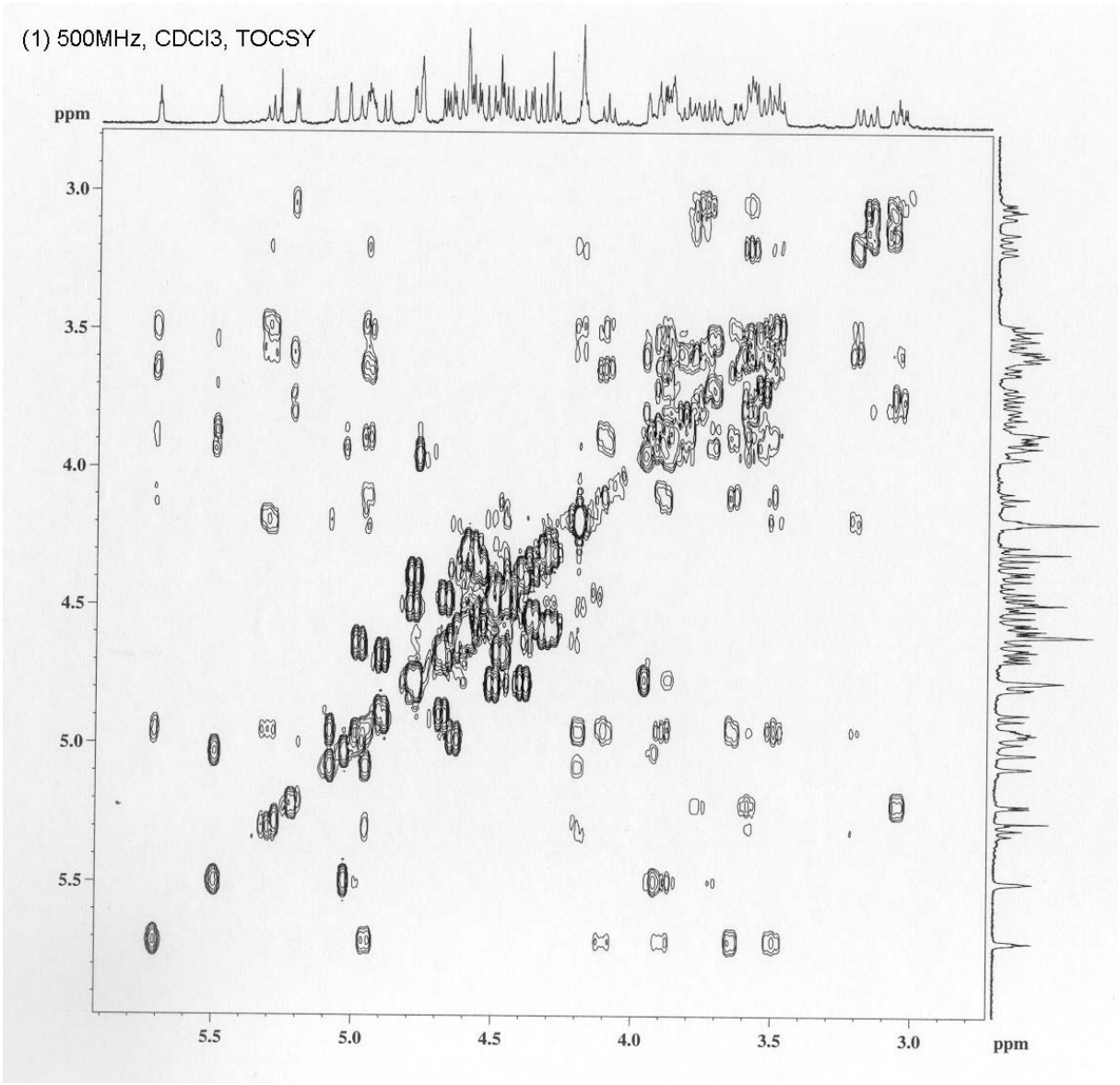
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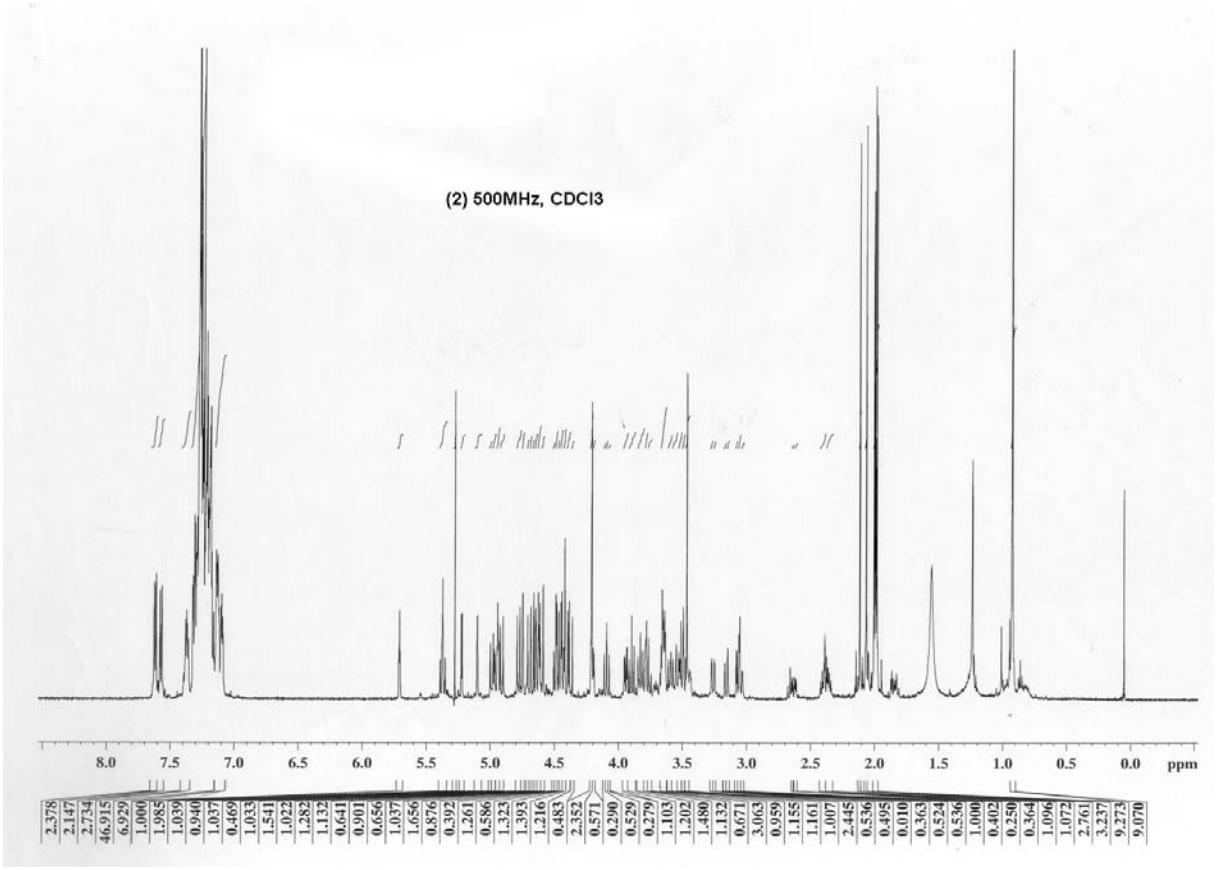
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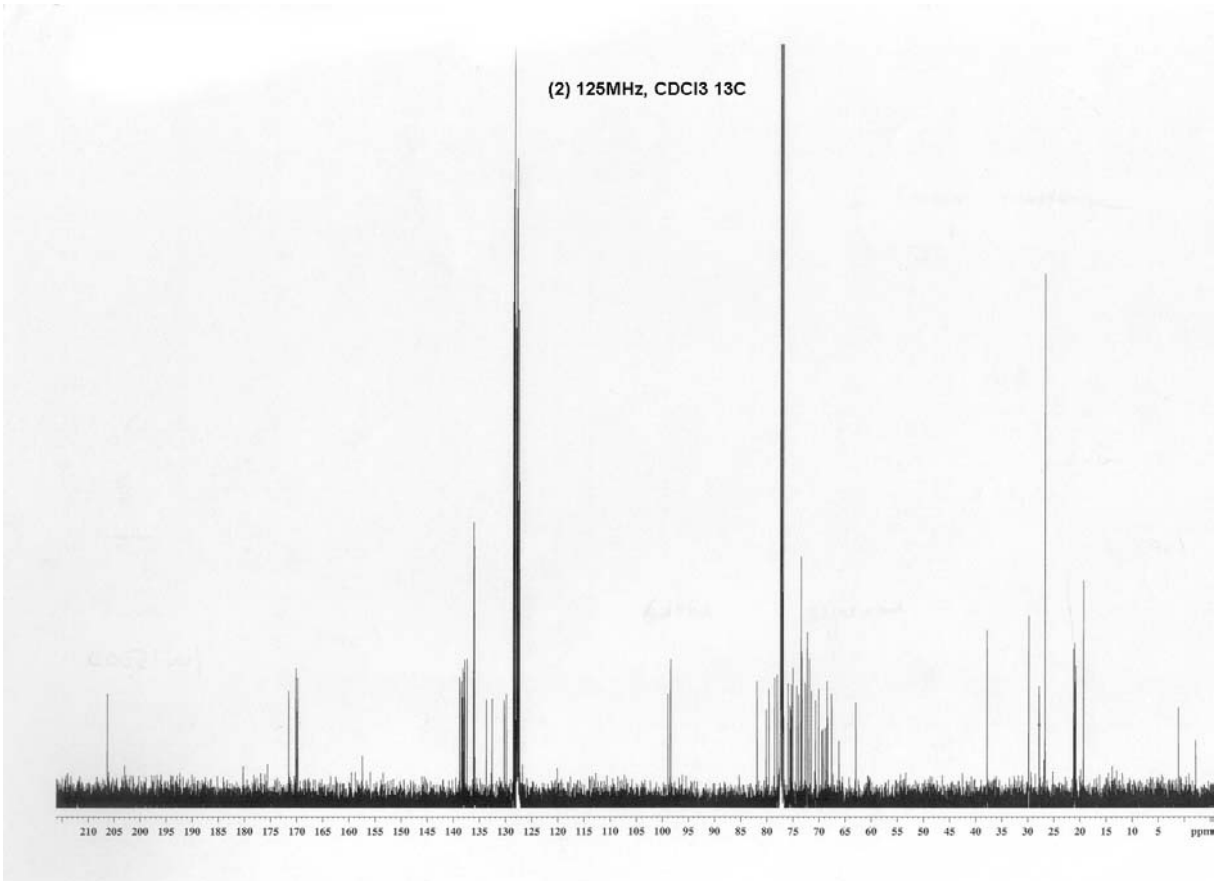
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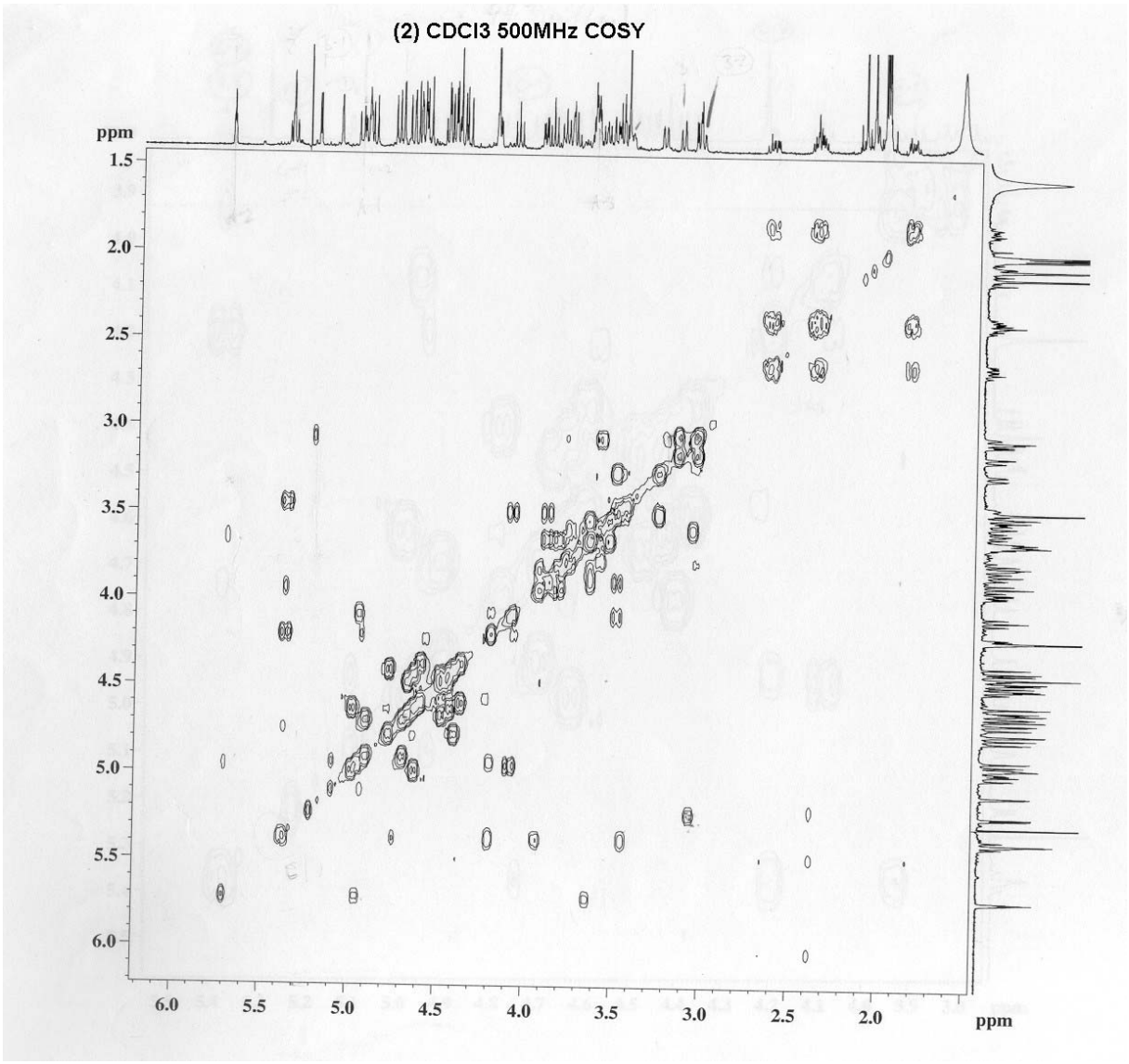
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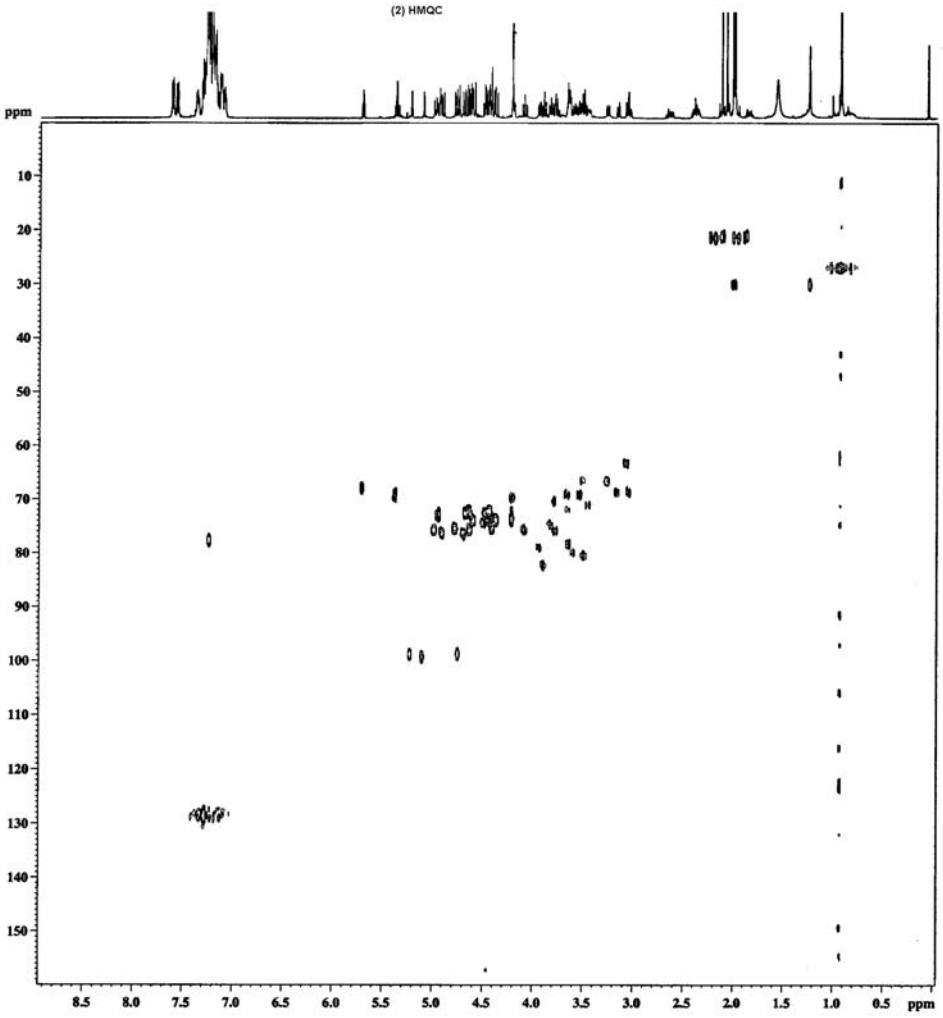
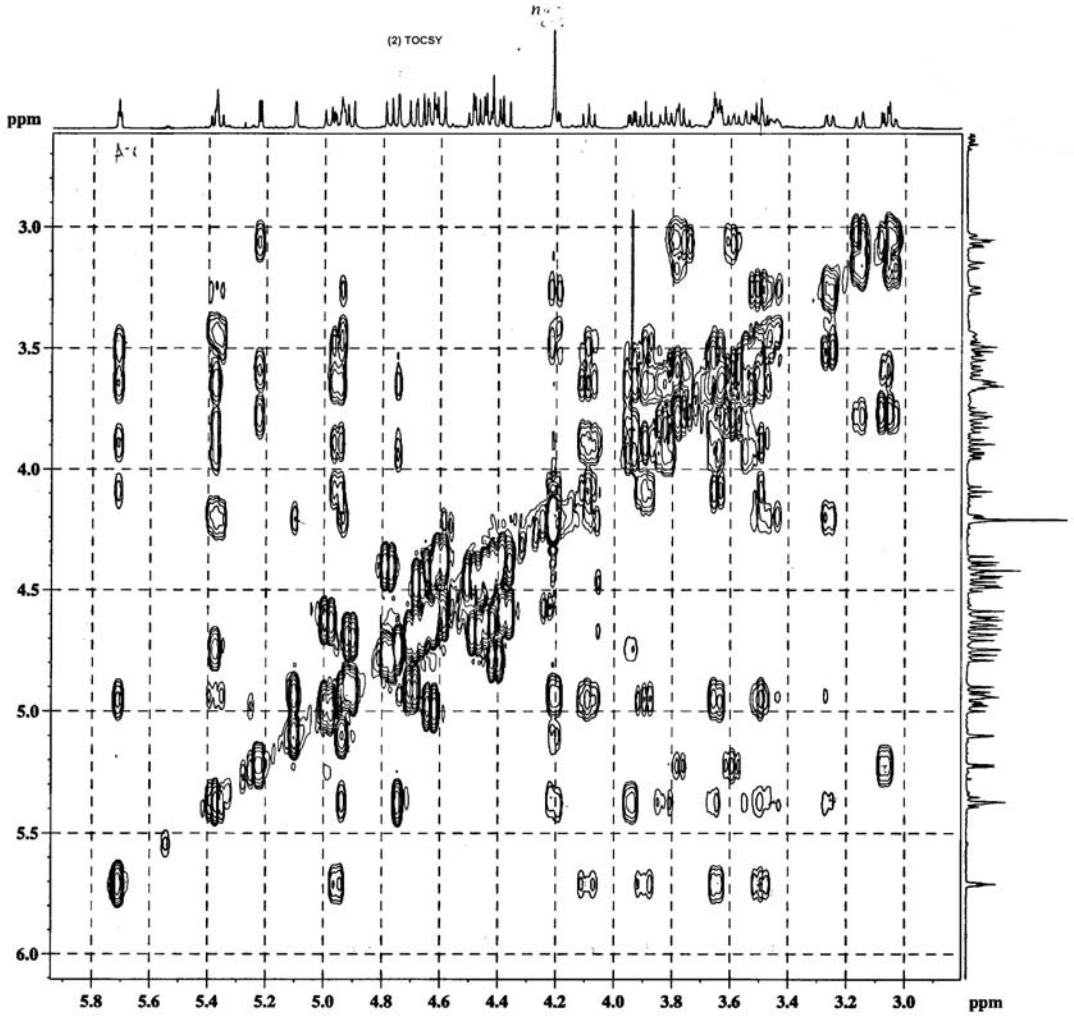
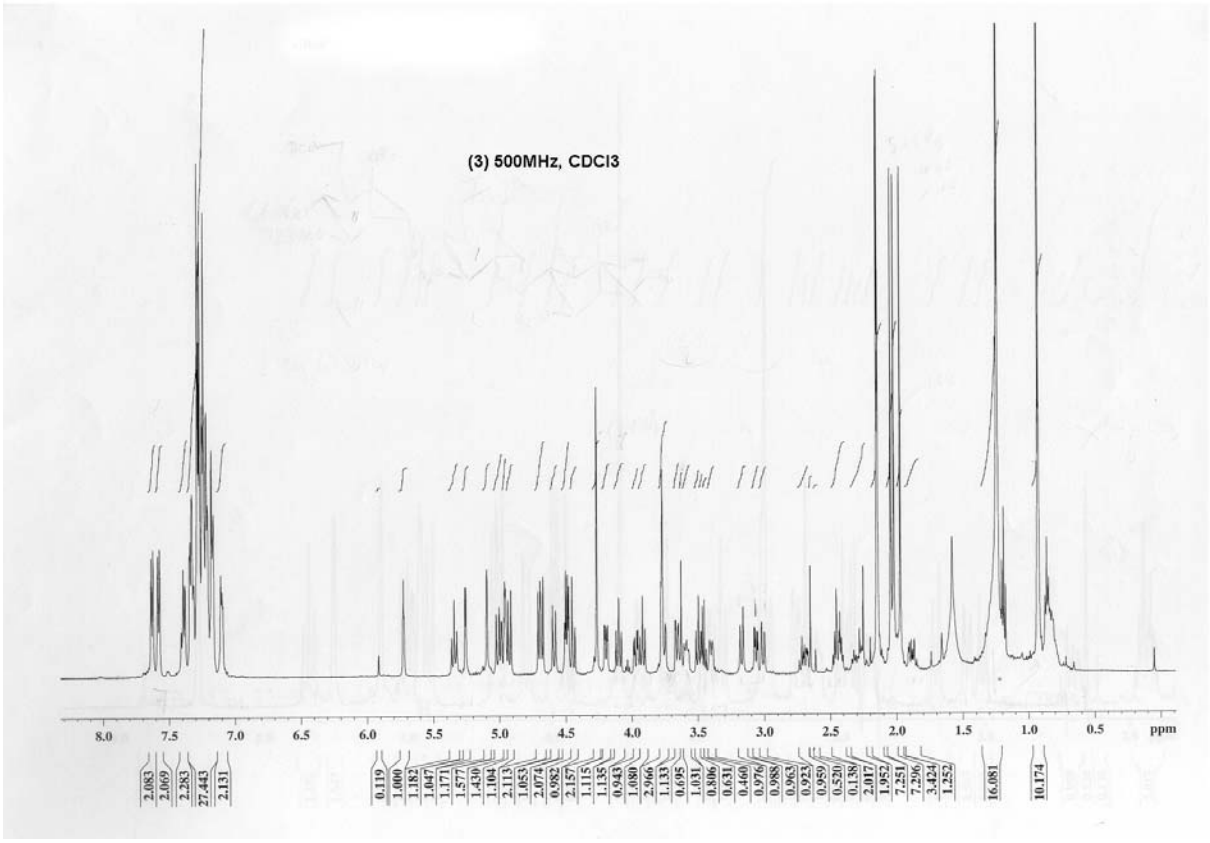
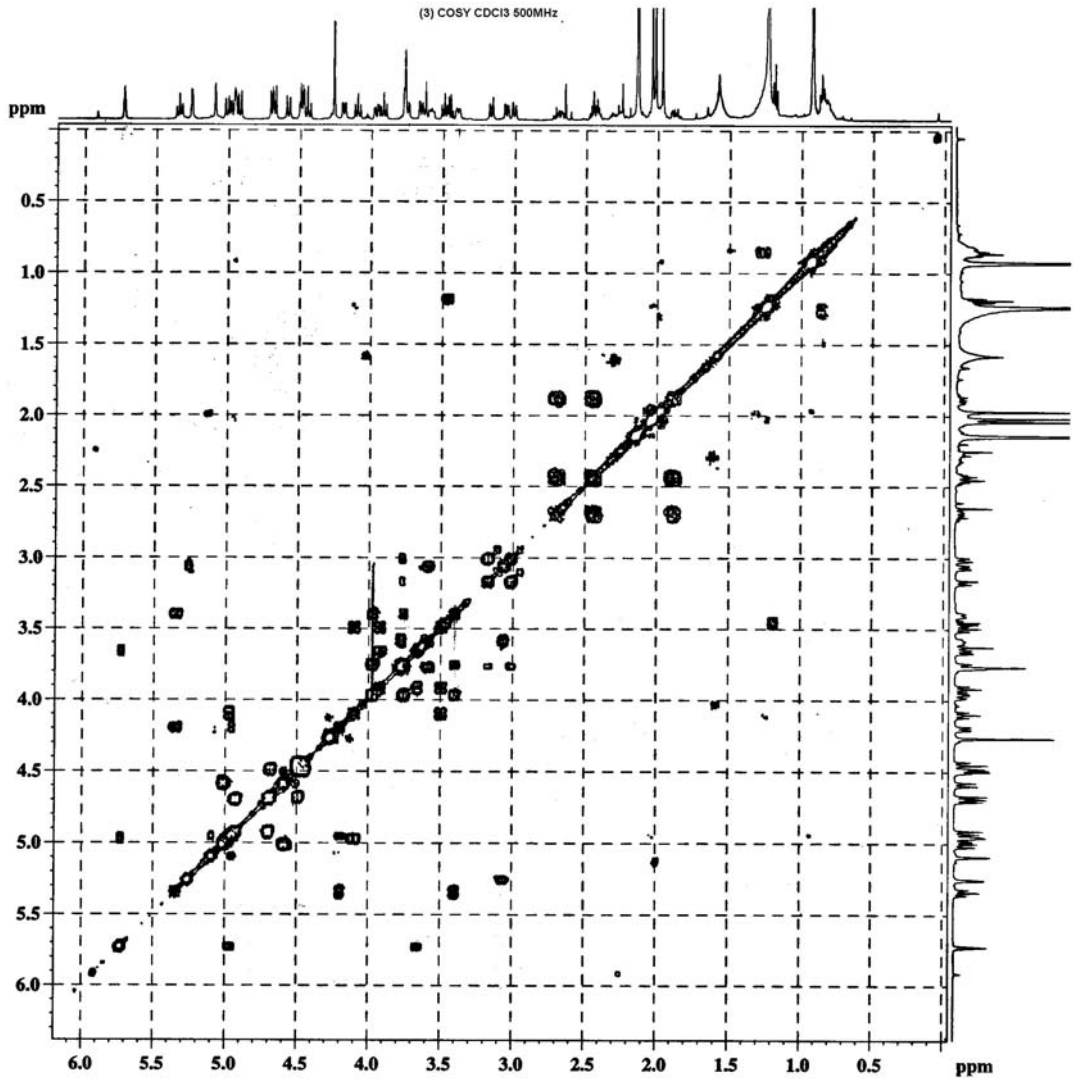
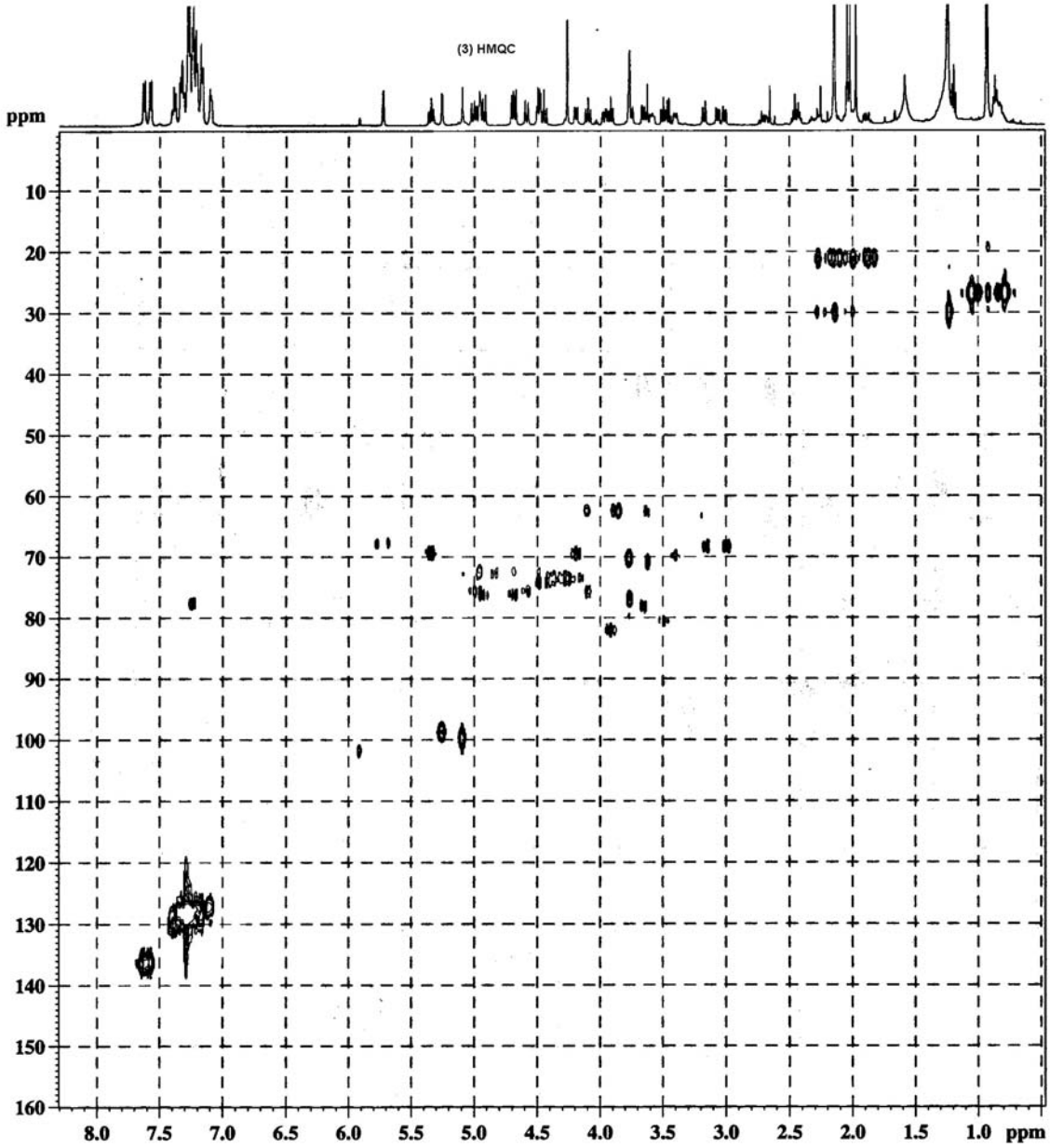
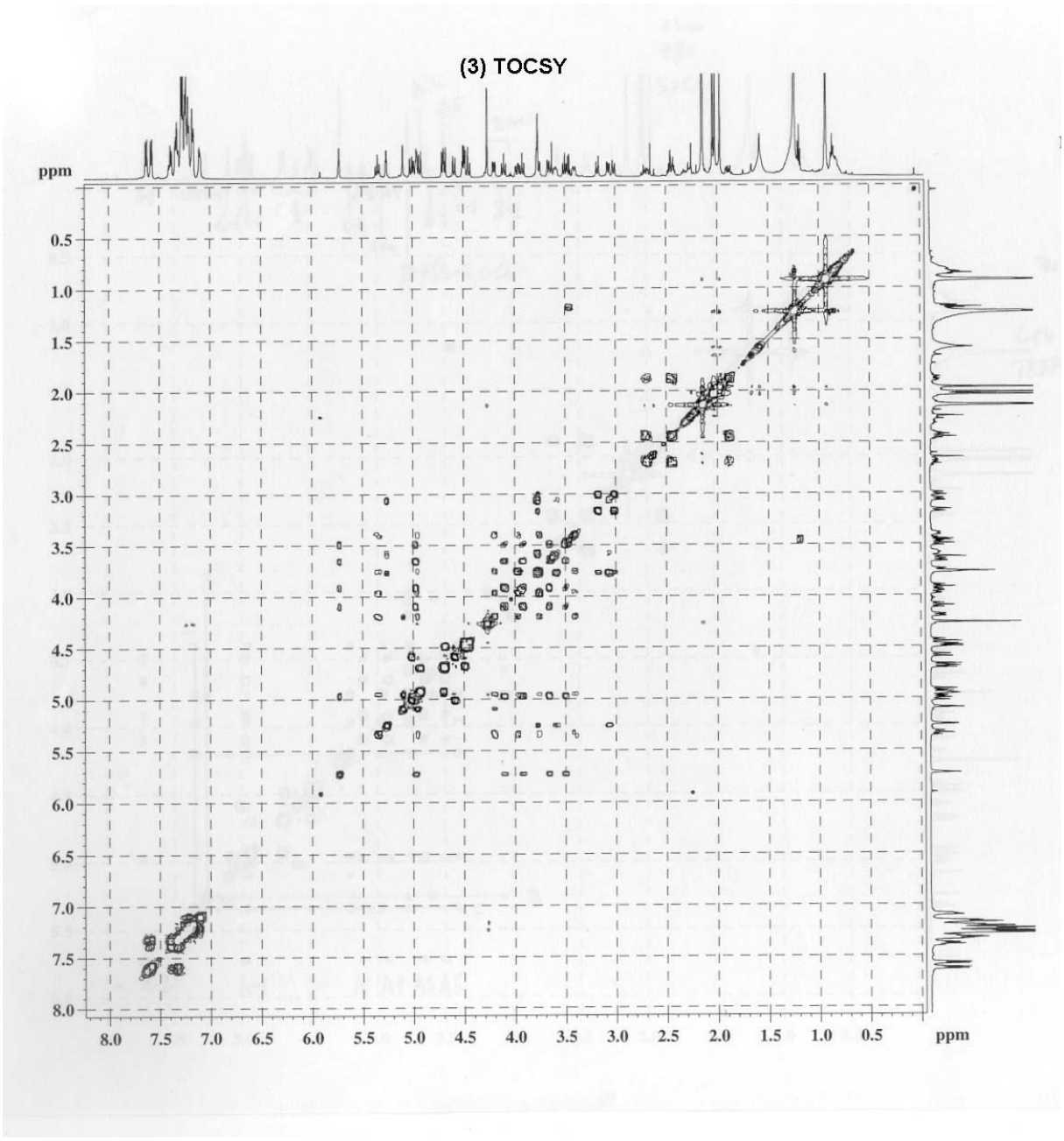

(2) HMQC
ppm
10
20
30
40
50
60
70
80
90
100
110
120
130
140
150
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
ppm


(2) TOCSY
ppm
3.0
3.5
4.0
4.5
5.0
5.5
6.0
5.8
5.6
5.4
5.2
5.0
4.8
4.6
4.4
4.2
4.0
3.8
3.6
3.4
3.2
3.0
ppm

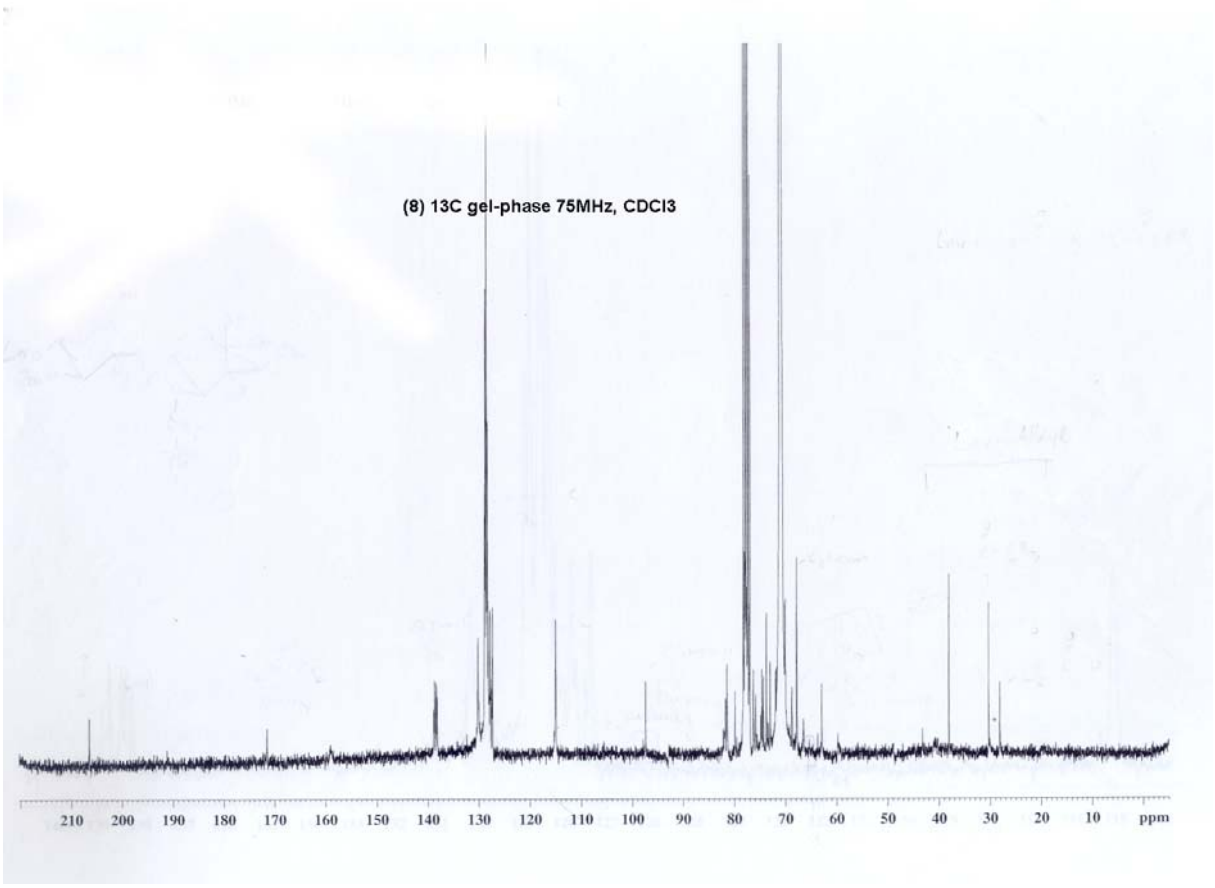

(3) 500MHz, CDCl3
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
ppm
2.083
2.069
2.283
27.443
2.131
0.119
1.000
1.182
1.047
1.171
1.577
1.439
1.104
2.113
1.053
2.074
0.982
2.157
1.115
1.135
0.943
1.080
2.966
1.133
0.695
1.031
0.806
0.631
0.460
0.976
0.988
0.963
0.923
0.959
0.520
0.138
2.017
1.952
7.251
7.296
3.424
1.252
16.081
10.174


(3) COSY CDCl3 500MHz
ppm
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
5.5
6.0
6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5
ppm

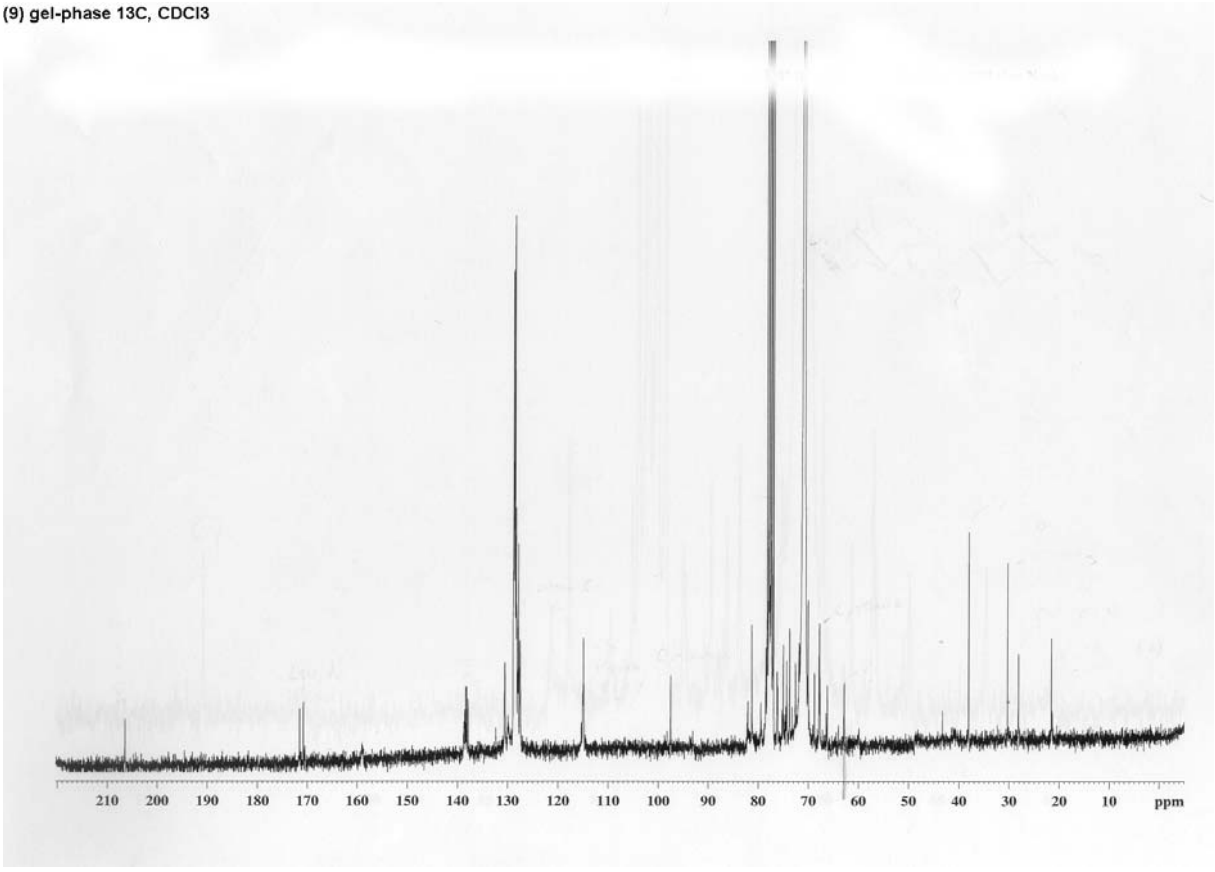

(3) HMQC
ppm
10
20
30
40
50
60
70
80
90
100
110
120
130
140
150
160
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
ppm

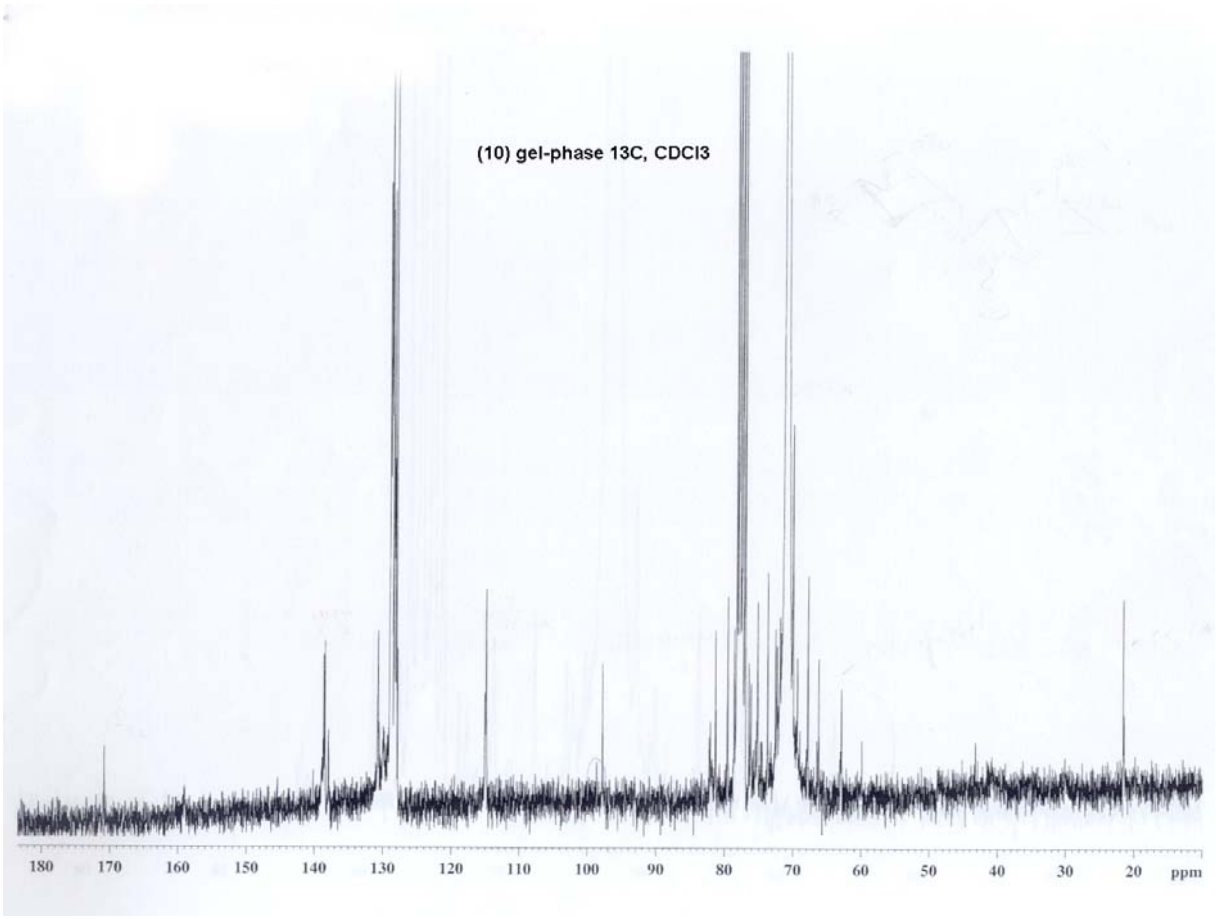

(3) TOCSY
ppm
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
5.5
6.0
6.5
7.0
7.5
8.0
8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5
ppm

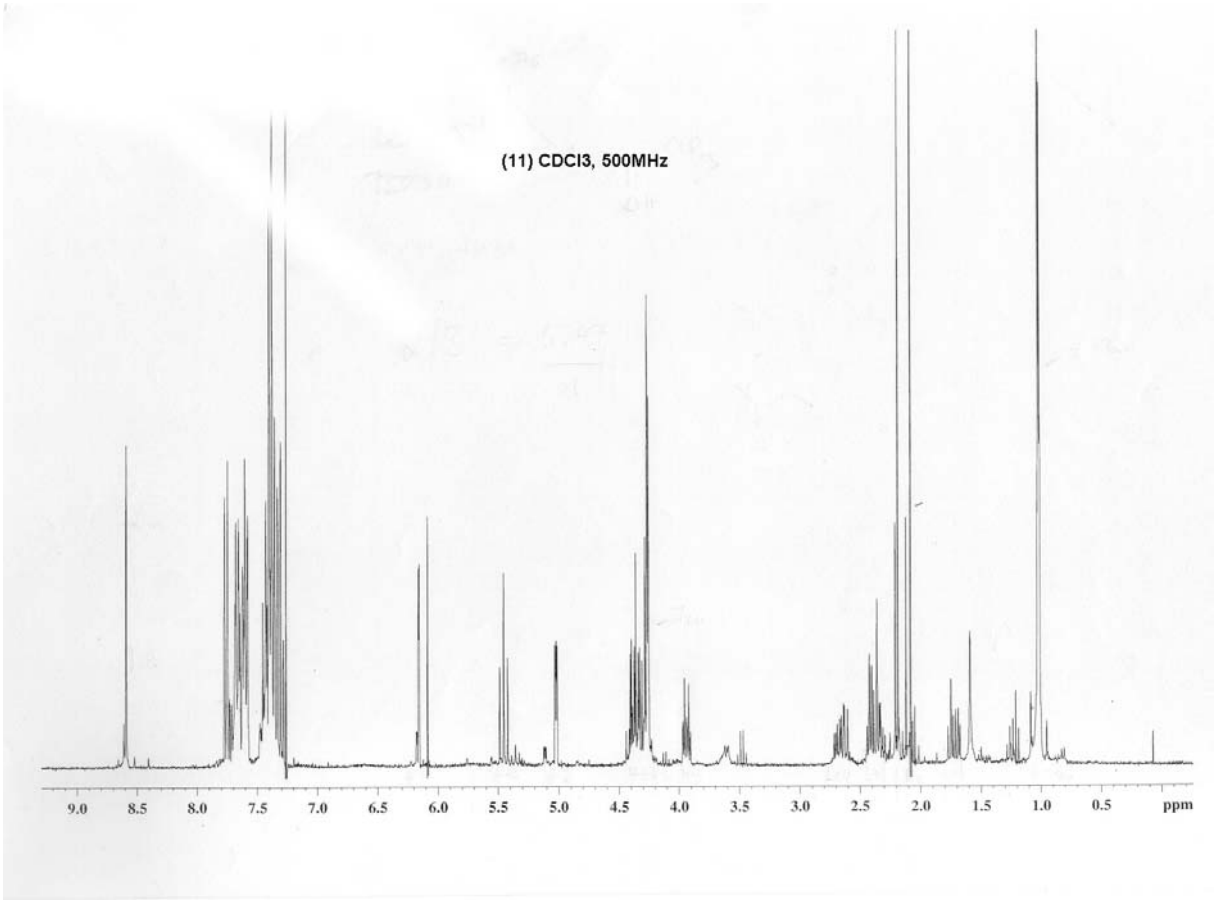
(8) 13C gel-phase 75MHz, CDCl3

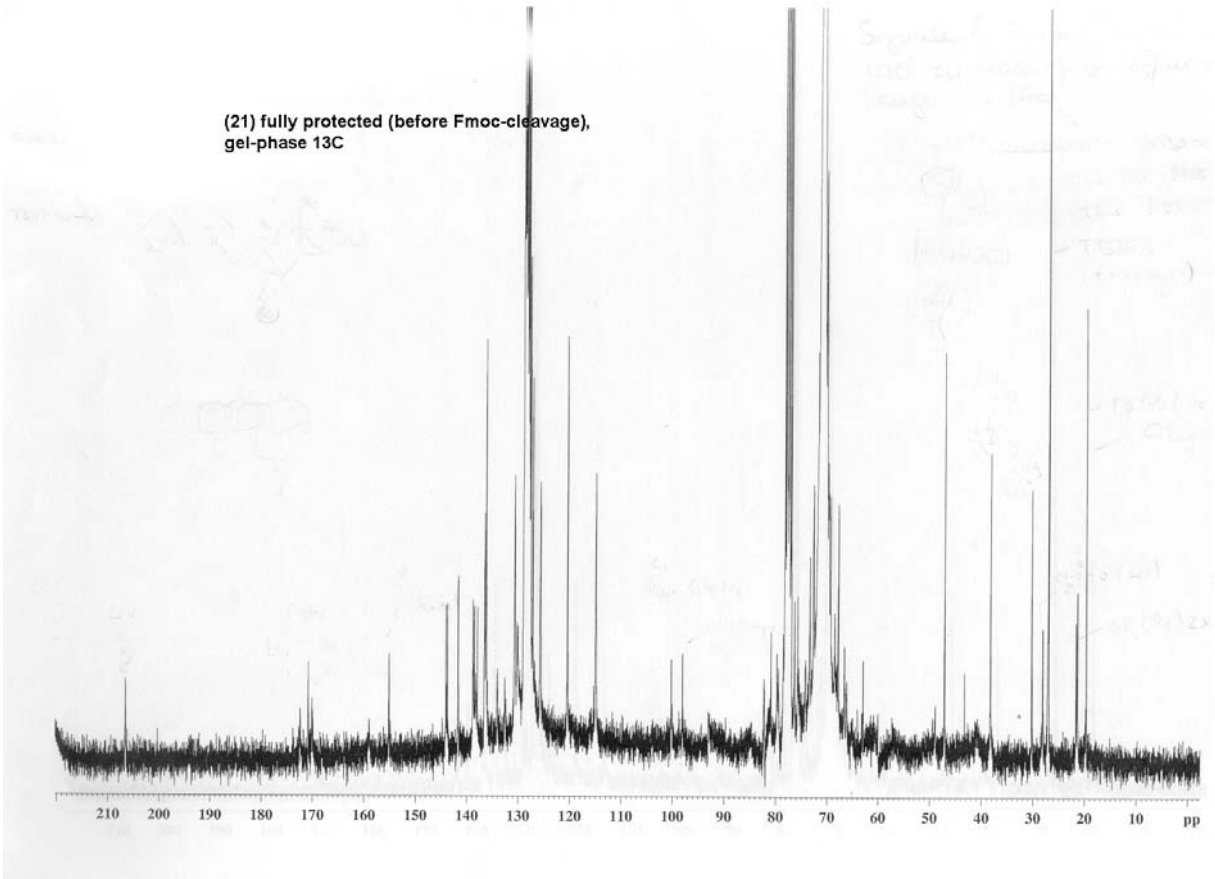


(9) gel-phase 13C, CDCl3




(10) gel-phase 13C, CDCl3
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
ppm


(11) CDCl3, 500MHz
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
ppm


(21) fully protected (before Fmoc-cleavage),
gel-phase 13C
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
pp