–Electronic Supplementary Information (ESI) –

# Probing the Limitations of Fluorous Content for Tag-Mediated Microarray Formation

Heather D. Edwards, Sahana K. Nagappayya, and Nicola L. B. Pohl*
Departments of Chemistry and of Chemical and Biological Engineering, Hach Hall, Iowa State University, Ames, IA 50014

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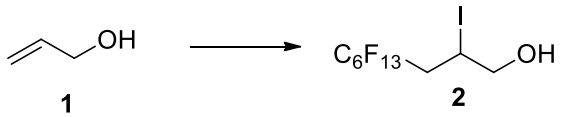
**General materials and methods.**

Reaction solvents were used directly from solvent tower (Swagelok). Amberlyst® 15 ion-exchange resin was washed repeatedly with methanol before use. All other commercial reagents and solvents were used as received without further purification. The reactions were monitored and the *R*f values determined using analytical thin layer chromatography (tlc) with Sorbent Technologies Silica gel HL TLC plates with UV 254 (250 $\mu$m). The developed tlc plates were visualized by immersion in *p*-anisaldehyde solution followed by heating on a hot plate. Flash chromatography was performed with ZeoPrep 60 Eco 40-63 $\mu$m silica gel unless otherwise specified. Fluorous phase chromatography using fluorous solid-phase extraction cartridges containing silica gel bonded with perfluorooctylethylsilyl chains (Fluorous Technologies, Inc.; Pittsburgh, PA). All other fluorous reagents were also obtained from Fluorous Technologies, Inc. All moisture-sensitive reactions were performed in flame- or oven-dried glassware under a nitrogen atmosphere. Bath temperatures were used to record the reaction temperature in all cases run without microwave irradiation. All reactions were stirred magnetically at ambient temperature unless otherwise indicated. Microwave heating was carried out with a CEM-Discover continuous wave microwave. $^{1}$H NMR and $^{13}$C NMR spectra were obtained with a Varian VXR at 400 MHz and162 MHz respectively. 1H NMR spectra were reported in parts per million relative to $CDCl_3$ as an internal reference. $^{13}$C NMR spectra were reported in parts per million relative to $CDCl_3$.

**Synthetic procedures.**

**• Synthesis of mono-$C_6F_{13}$-tag.**

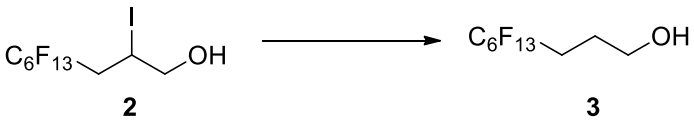
**4,4,5,5,6,6,7,7,8,8,9,9,9-tridecafluoro-2-iodononan-1-ol (2).**




**1** **2**

To a mixture of allyl alcohol (1.0 g, 17 mmol) and perfluorohexyl iodide (7.7 g, 17 mmol) was added AIBN (0.30 g, 1.7 mmol) at 20 ºC. The mixture was cooled to -78 ºC to freeze the contents and then degassed, warmed to 20 ºC, and then blanketed with argon. The reaction mixture was heated to 70 ºC and stirred for 20 h. The reaction was then dissolved in hexane and poured through a fritted glass funnel and immediately purified by flash column chromatography (hexane) (82%, 7.1 g, white solid). The $^{1}$H NMR data of compound **2** matched previously reported data.[1]

**4,4,5,5,6,6,7,7,8,8,9,9,9-tridecafluorononan-1-ol (3).**

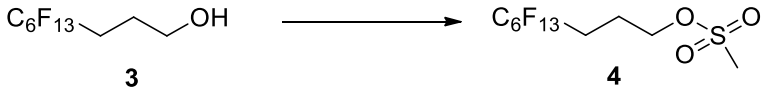



**2** **3**

Compound **2** (6.5 g, 13 mmol) was dissolved in tetrahydrofuran (150 mL) and cooled to 0 ºC under argon. Once cooled, lithium aluminum hydride (0.98 g, 26 mmol) was slowly added to the mixture. The reaction vessel was warmed to 22 ºC and stirred for 12 h. The reaction was

quenched very slowly with water (30 mL) by adding it dropwise. The solution was then filtered and washed with $NaHCO_3$ (50 mL) and extracted with ethyl acetate (2 x 80 mL). The organic layer was dried over $MgSO_4$. The solvent was removed under reduced pressure. The crude product was purified by flash column chromatography (2:1 hexane: ethyl acetate) (83%, 4.1 g, clear oil). The $^1$H NMR data of compound **3** matched previously reported data.[2]

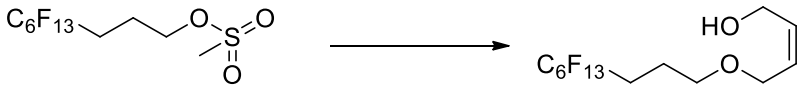
**4,4,5,5,6,6,7,7,8,8,9,9,9-tridecafluorononyl methanesulfonate (4).**



**3** **4**

To a solution of 3-(perfluorohexyl)propanol **3** (1.7 g, 4.6 mmol) in dichloromethane (20 mL) was added triethylamine (1.3 mL, 9.1 mmol) and the mixture was cooled to 0 °C. Mesyl chloride (0.7 mL, 9.1 mmol) was added dropwise over 10 min and the reaction mixture was allowed to warm to ambient temperature over 3 h. The reaction mixture was diluted with dichloromethane (20 mL) and the organic layer was washed with water (20 mL) and brine (20 mL), and dried over MgSO4. The solvent was removed under reduced pressure. The crude product was purified by flash column chromatography (2:1 hexane: ethyl acetate) to provide **4** (2.0 g, 98%, pale yellow oil).
**$^1$H NMR (400 MHz, CDCl3)**: δ (ppm) 4.34-4.30 (t, *J* =12, 2H), 3.05 (s, 3H), 2.32-2.19 (m, 2H), 2.13-2.06 (m, 2H)
**$^{13}$C NMR (400 MHz, CDCl3)**: δ (ppm) 117.6, 68.1, 37.7, 27.9, 27.6, 27.4, 23.6, 21.0, 20.9
**HRMS** calcd. for $C_{10}H_9F_{13}O_3SNa$: 479.00, found[M+Na]$^+$: 478.9959

**(Z)-4-((4,4,5,5,6,6,7,7,8,8,9,9,9-tridecafluorononyl)oxy)but-2-en-1-ol (5).**

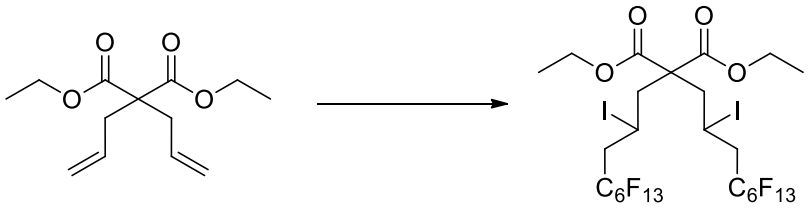


**4** **5**

A solution was made of *cis*-1,4-butenediol (3.6 mL, 44 mmol) and 3-(perfluorohexyl)propyl methyl sulfonate (2.0 g, 4.4 mmol) in DMF (20 mL). A second solution was made of tetrabutylammonium bromide (0.16 g, 0.5 mmol) in DMF (20 mL), to which powdered KOH (0.25 g, 4.4 mmol) was added. The first solution was added dropwise over 5 min to the second solution and the reaction mixture was stirred for 12 h at 22 ºC then poured into water (20 mL). The aqueous layer was extracted with ethyl acetate (40 mL) and the organic layer was washed with water (30 mL) and brine (30 mL), and dried over MgSO4. The solvent was removed under reduced pressure. The crude product was purified by flash column chromatography (2:1 hexane: ethyl acetate) to provide **3** (1.2 g, 60%, pale yellow oil).
**$^1$H NMR (400 MHz, CDCl3)**: δ (ppm) 5.85-5.79 (m, 1H), 5.72-5.66 (m, 1H), 4.22 (s, 2H), 4.07-4.06 (d, *J* = 6, 2H), 3.53-3.50 (t, *J* = 11.6, 2H), 2.25-2.12 (m, 2H), 1.92-1.85 (m, 2H), 1.67-1.66 (m, 0.5H), 1.58 (s, 0.5H)
**$^{13}$C NMR (400 MHz, CDCl3)**: δ (ppm) 132.4, 128.5, 123.9, 117.6, 69.1, 66.72, 59.0, 28.2, 23.6, 21.0,
**HRMS** calcd. for $C_{13}H_{13}F_{13}O_2Na$: 471.06, found [M+Na]$^+$: 471.06

**• Synthesis of di-$C_6F_{13}$-fluorous tag.**

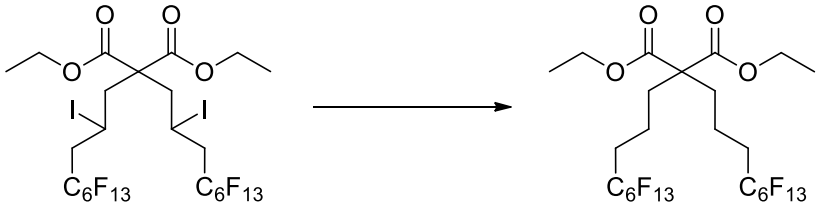
**Diethyl 2,2-bis(4,4,5,5,6,6,7,7,8,8,9,9,9-tridecafluoro-2-iodononyl)malonate (7).**




**6** **7**

To a mixture of 2,2-diallyl-diethylmalonate (0.10 g, 0.41 mmol) and perfluorohexyl iodide (0.37 g, 0.83 mmol) was added AIBN (13 mg, 82 µmol) at 20 ºC. The mixture was cooled to -78 ºC to freeze the contents and then degassed, warmed to 20 ºC, and then blanketed with argon. The reaction mixture was heated to 70 ºC and stirred for 20 h. The reaction was then dissolved in hexane and poured through a fritted glass funnel and immediately purified by flash column chromatography (hexane) to yield compound **7** (70%, 0.33 g, pale yellow oil).
**$^1$H NMR (400 MHz, CDCl3)**: δ (ppm) 4.23-4.18 (q, *J* = 7.2, 4H), 3.17-3.14 (m, 1H), 3.06-3.02 (t, *J* = 9.2, 1H), 2.58-2.51 (m, 3.5H), 2.33-1.99 (m, 4.5H), 1.28-1.23 (m, 6H)
**$^{13}$C NMR (400 MHz, CDCl3)**: δ (ppm) 172.5, 172.1, 62.1, 62.0, 58.5, 45.6, 39.9, 38.6, 35.6, 29.9, 14.2, 5.8
**HRMS** calcd. for $C_{25}H_{20}F_{26}I_2O_4K$: 1170.90, found $[M+K]^+$-I: 1048.89

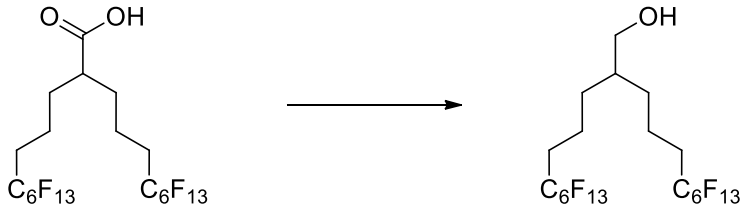
**Diethyl 2,2-bis(4,4,5,5,6,6,7,7,8,8,9,9,9-tridecafluorononyl)malonate (8):**




**7** **8**

To compound **7** (1.4 g, 1.3 mmol), a mixture of ethanol (20 mL) and hydrochloric acid (20 mL) was added to form a pale yellow-colored solution. Next the solution was heated in the microwave at 60 ºC for five min. Powdered zinc (2.0 g) was slowly added to the solution and reaction flask was placed back in the microwave at 60 ºC for 15 min to form a clear solution. Water (40 mL) was added to the reaction. The aqueous layer was extracted with ethyl acetate (2 x 60 mL) and the organic layer was washed with $NaHCO_3$ (100 mL) and dried over MgSO4. The solvent was removed under reduced pressure. The crude product was purified by flash column chromatography (2:1 hexane: ethyl acetate) to yield **8** (94%, 1.0 g, pale yellow oil). The $^1$H NMR data of compound **8** matched previously reported data.[3,4]

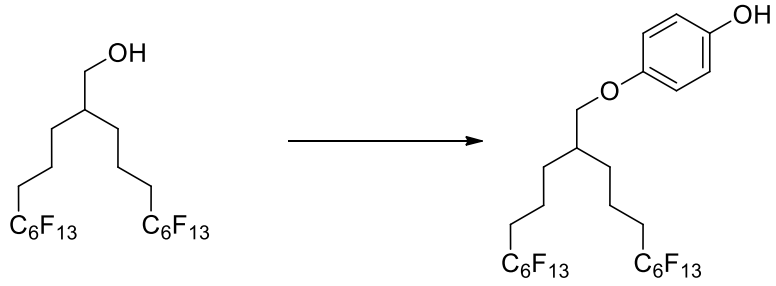
**6,6,7,7,8,8,9,9,10,10,11,11,11-tridecafluoro-2-(4,4,5,5,6,6,7,7,8,8,9,9,9-tridecafluorononyl)undecan-1-ol (10).**




**9** **10**

Ether (20 mL) was added to compound **9** (0.19 g, 0.25 mmol). The mixture was cooled to 0 ºC. Lithium aluminum hydride (20 mg, 0.53 mmol) was added to the reaction flask. The reaction vessel was warmed to 22 ºC and stirred for 12 h. The reaction was quenched very slowly with water (3 mL) by adding it dropwise. The solution was then filtered and washed with $NaHCO_3$ (40 mL) and extracted with ethyl acetate (2 x 40 mL). The organic layer was dried with $MgSO_4$. The solvent was removed under reduced pressure. The crude product was purified by flash column chromatography (2:1 hexane:ethyl acetate) (78%, 0.15 g, pale yellow oil). The $^1$H NMR data of compound **10** matched previously reported data. [3,4]

**4-((6,6,7,7,8,8,9,9,10,10,11,11,11-tridecafluoro-2-(4,4,5,5,6,6,7,7,8,8,9,9,9-tridecafluorononyl)undecyl)oxy)phenol (11).**




**10** **11**

To a mixture of compound **10** (0.60 g, 0.79 mmol) and hydroquinone (1.7 g, 16 mmol) in THF (20 mL) was added $PPh_3$ (0.41 g, 1.6 mmol) and DIAD (0.3 ml, 1.6 mmol) at 20 ºC. After stirring for 6 h at reflux condition, the reaction mixture was concentrated under reduced pressure. The crude product was purified by flash column chromatography (7:3 hexane: ethylacetate) to provide compound **11** (37%, 0.25 g, white solid).

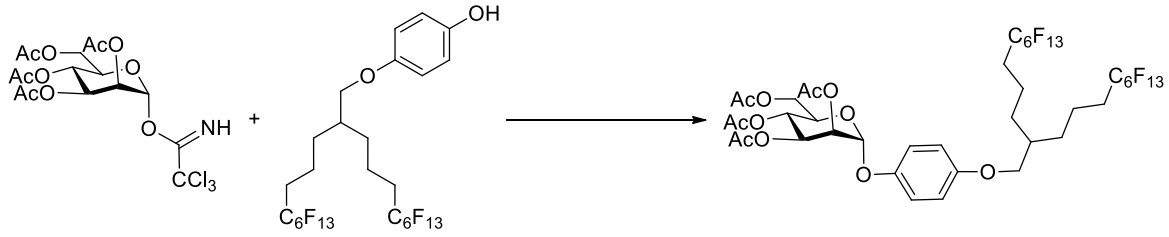
**$^1$H NMR (400 MHz, CDCl3)**: δ (ppm) 6.77-6.76 (d, *J* = 3.2, 4H), 4.63 (s, 1H), 3.82-3.80 (d, *J* = 8.8, 1H), 3.77-3.75 (d, *J* = 9.2, 1H), 2.58-1.01 (m, 10H), 0.91-0.81 (m, 3H)

**$^{13}$C NMR (400 MHz, CDCl3)**: δ (ppm) 153.5, 149.7, 116.3, 116.2, 115.9, 115.8, 73.4, 38.0, 37.3, 37.1, 36.8, 35.6, 35.1, 35.0, 33.4, 31.5, 31.2, 16.8, 14.7

**HRMS** calcd. for $C_{26}H_{20}F_{26}O_2$: 858.10, found 857.32

**• Synthesis of the mannose-linked tags.**

**4-((6,6,7,7,8,8,9,9,10,10,11,11,11-tridecafluoro-2-(4,4,5,5,6,6,7,7,8,8,9,9,9-tridecafluorononyl)undecyl)oxy)phenoxy-2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranoside (13).**



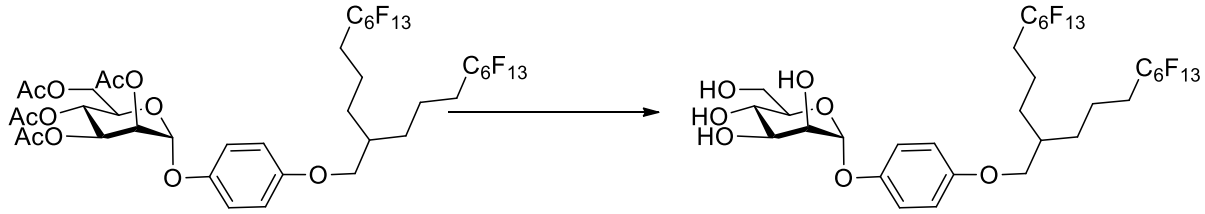

**12** **11** **13**

To a solution of 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl trichloroacetimidate (**12**) (27 mg, 55 µmol) and compound **11** (24 mg, 28 µmol) in toluene (5 mL) was added TMSOTf (5.0 µL, 1.4 µmol) at 0 ºC. The reaction mixture was stirred at 0 ºC for 20 min. The reaction mixture was quenched with triethylamine (0.20 mL) and concentrated under reduced pressure. The crude product was purified by fluorous solid-phase extraction (FSPE) using a fluorous solid-phase extraction cartridge. Non-fluorous compounds were eluted with 80% MeOH/water and the desired product was eluted by 100% MeOH. The solvent was removed under reduced pressure to provide **13** (27 mg, 83%, pale yellow oil).

**$^{1}$H NMR (400 MHz, CDCl3)**: δ (ppm) 7.01-6.99 (m, 1.5H), 6.82-6.76 (m, 2.5), 5.55-5.53 (m, 1H), 5.43-5.35 (2H), 4.30-4.20 (1H), 4.15-4.06 (2H), 3.81-3.76 (2H), 2.38-2.03 (16H), 1.80-1.55 (3.5H), 1.35-1.15 (4.5H) 0.87-0.85 (d, *J* = 6.4, 2H)

**$^{13}$C NMR (400 MHz, CDCl3)**: δ (ppm) 170.8, 170.2, 170.1, 170.0, 155.2, 149.9, 149.8, 118.0, 116.2, 115.8, 115.6, 115.6, 115.5, 96.6, 73.4, 73.1, 69.7, 69.2, 69.1, 66.3, 62.4, 37.9, 37.3, 37.1, 37.0, 36.8, 35.8, 35.6, 35.1, 35.0, 33.4, 31.5, 31.4, 29.9, 21.1, 21.0, 20.9, 20.8, 16.9, 14.8

**4-((6,6,7,7,8,8,9,9,10,10,11,11,11-tridecafluoro-2 (4,4,5,5,6,6,7,7,8,8,9,9,9tridecafluorononyl)undecyl)oxy)phenoxy-α-D-mannopyranoside (14).**




**13** **14**

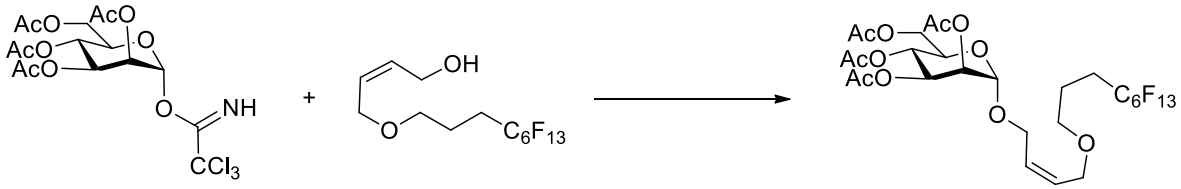
To a solution of compound **13** (27 mg, 23 µmol) in methanol (5.0 mL) was added $K_2CO_3$ (13 mg, 91 µmol). The reaction mixture was stirred at ambient temperature for 3 h. The reaction mixture was neutralized with Amberlyst® 15 ion-exchange resin and filtered over Celite®. The solvent was removed under reduced pressure to provide compound **14** (20 mg, 88%, white solid).

**$^{1}$H NMR (400 MHz, CDCl3)**: δ (ppm) 7.72-7.69 (m, 2H), 7.54-7.52 (m, 2H), 6.70 (s, 1H), 5.28-5.25 (1H), 4.31-4.12 (4H), 2.33-2.30 (5H), 1.89-1.83 (1H), 1.65 (2H), 1.62 (2H), 1.42-1.37 (6H)

**$^{13}$C NMR (400 MHz, CDCl3)**: δ (ppm) 131.09, 129.01, 69.03, 68.38, 62.39, 62.33, 57.30, 57.24, 57.03, 56.90, 54.57, 54.43, 38.95, 34.22, 31.92, 30.57, 29.95, 29.90, 29.61, 29.57, 29.44, 29.21, 29.16, 29.12, 29.09, 29.06, 28.87, 28.14, 28.12, 28.08, 28.06, 27.45, 27.42, 27.17, 26.82, 26.80, 26.72, 26.39, 25.02, 23.97, 23.24, 22.93, 22.82, 14.32, 14.25, 14.217, 11.21

**HRMS** calcd. for $C_{32}H_{30}F_{26}O_7Na$: 1043.15, found $[M+Na]^+$: 1043.15

**3-(perfluorohexyl)propanyloxybutenyl-2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranoside (15).**



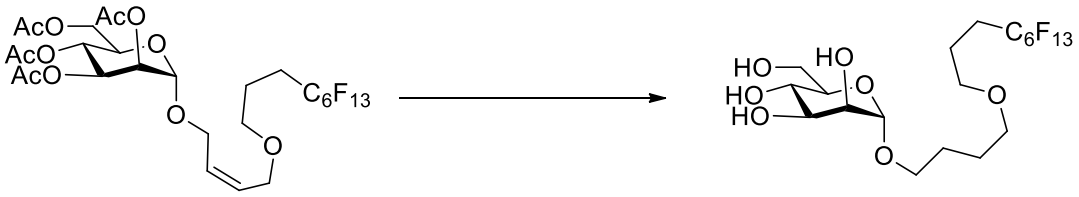
**12** **5** **15**

To a solution of 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl trichloroacetimidate (**12**) (21 mg, 47 µmol) and compound **5** (46 mg, 93 µmol) in toluene (5.0 mL) was added TMSOTf (0.80 µL, 4.6 µmol) at 0 ºC. The reaction mixture was stirred at 0 ºC for 30 min. The reaction mixture was quenched with triethylamine (0.2 mL) and concentrated under reduced pressure. The crude product was purified by fluorous solid-phase extraction (FSPE) using a fluorous solid-phase extraction cartridge. Non-fluorous compounds were eluted with 80%MeOH/water and the desired product was eluted by 100%MeOH. The solvent was removed under reduced pressure to provide **15** (28 mg, 77%, pale yellow oil).

**$^{1}$H NMR (400 MHz, CDCl3)**: δ (ppm) 5.78-5.67 (m, 2H) 5.36-5.31 (0.5H), 5.203-5.17 (0.5H), 4.69-4.63 (1H), 4.33-4.01 (6H), 3.51-3.49 (t, *J* = 10.8, 2H), 2.25-2.02 (15H), 1.91-1.84 (3H), 1.25 (s, 1H)

**$^{13}$C NMR (400 MHz, CDCl3)**: δ (ppm) 171.98, 170.88, 170.39, 16.92, 169.18, 157.35, 130.92, 130.90, 130.53, 128.03, 126.82, 99.83, 95.68, 71.60, 69.98, 69.22, 69.07, 66.68, 66.63, 63.22, 63.023, 60.36, 31.15, 31.11, 30.06, 29.94, 28.43, 28.20, 28.12, 27.97, 27.33, 21.05, 21.02, 20.95

**3-(perfluorohexyl)propanyloxybutanyl-α-D-mannopyranoside (16).**



**15** **16**

To a solution of compound **15** (25 mg, 33 µmol) in methanol (5 mL) was added 10% Pd/C (0.1 g). The reaction mixture was stirred at ambient temperature under hydrogen atmosphere for 2 h. The reaction mixture was then filtered over Celite® and partially concentrated under reduced pressure. Then $K_2CO_3$ (18.0 mg, 0.33 mmol) was added to the solution and stirred at ambient temperature for 3 h. The reaction mixture was neutralized with Amberlyst® 15 ion-exchange resin and filtered over Celite®. The solvent was removed under reduced pressure to provide compound **16** (16.1 mg, 82%, white solid).

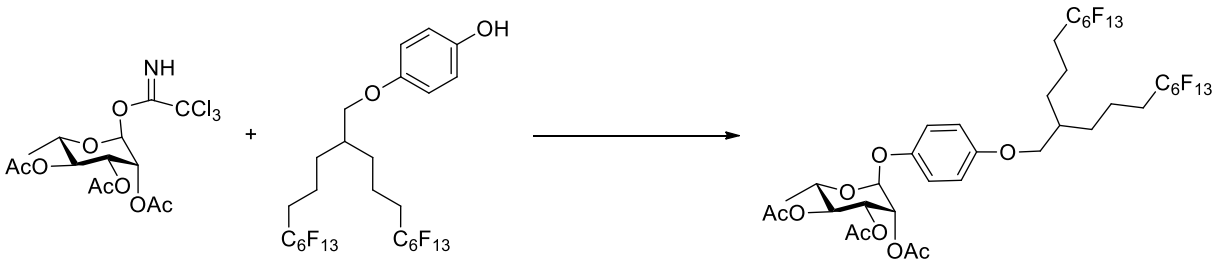
**$^{1}$H NMR (400 MHz, CDCl3)**: δ (ppm) 3.66 (s, 2H), 3.52-3.46 (4 H), 2.28-2.16 (4H), 1.98-1.86 (3H), 1.70-1.64 (5H), 1.25 (s, 3H)

**$^{13}$C NMR (400 MHz, CDCl3)**: δ (ppm) 115.55, 71.16, 69.52, 62.94, 30.28, 30.26, 30.22, 28.38, 28.21, 28.16, 27.94, 26.80, 26.76, 20.97, 20.94

**HRMS** calcd. for $C_{19}H_{25}F_{13}O_7Na$: 635.13, found $[M+Na]^+$: 635.13

**• Synthesis of the rhamnose-linked tags.**

**4-((6,6,7,7,8,8,9,9,10,10,11,11,11-tridecafluoro-2-(4,4,5,5,6,6,7,7,8,8,9,9,9-tridecafluorononyl)undecyl)oxy)phenyl-2,3,4-tri-*O*-acetyl-α-L-rhamnopyranoside (18).**



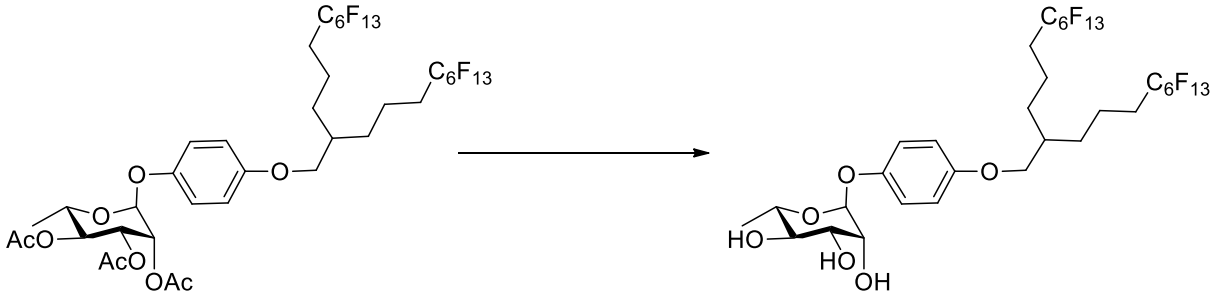

17 11 18

To a solution of 2,3,4-tri-O-acetyl-α-L-rhamnopyranosyl trichloroacetimidate (84 mg, 0.19 mmol) and compound **11** (81 mg, 0.98 mmol ) in toluene ( 4.0 mL), TMSOTf (0.35 mL, 1.9 μmol) was added at 0 ºC. The reaction mixture was stirred at 0 ºC for 20 min and then quenched with triethylamine (190 μL) and concentrated under reduced pressure. The crude product was purified by fluorous solid-phase extraction (FSPE) using a fluorous solid-phase extraction cartridge. Non-fluorous compounds were eluted with 80% MeOH/water and the desired product was eluted by 100% MeOH. The solvent was removed under reduced pressure to provide compound **18** (56 mg, 51%, oil).

**$^{1}$H NMR ($CD_3OD$, 400MHz)**: δ (ppm) 7.65-7.63 (dd, *J* = 12Hz, 2H), 7.26-7.23 (dd, *J* = 12 Hz, 2H), 5.37-5.35 (m, 1H), 5.26 (dd, 1H), 5.15 (dd, 1H), 5.09-5.07 (m, 1H), 4.12-4.10 (m, 2H), 3.16-3.14 (d, *J* = 8Hz, 2H), 2.34 (dd, 1H), 2.05 (s, 3H), 2.04 (s, 3H), 2.02 (s, 3H), 1.27-1.24 (m, 4H), 2.07-2.06 (m, 4H), 1.24-1.21 (m, 4H), 1.21-1.20 (d, 3H)

**$^{13}$C NMR ($CD_3OD$, 400MHz)**: δ (ppm): 170.4, 157.3, 154.1, 132.9, 132.3, 128.8, 128.6, 116.1, 115.8, 69.1, 68.0, 67.7, 51.0, 35.6, 33.4, 32.1, 29.9, 24.0, 16.6, 14.7, 14.0, 10.2

**4-((6,6,7,7,8,8,9,9,10,10,11,11,11-tridecafluoro-2-(4,4,5,5,6,6,7,7,8,8,9,9,9-tridecafluorononyl)undecyl)oxy)phenyl-α-L-rhamnopyranoside (19).**

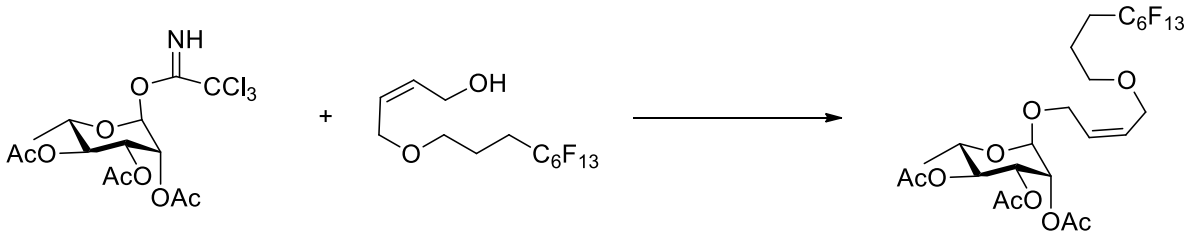



18 19

To a solution of compound **18** (42 mg, 37 μmol) in methanol (2.0 mL) $K_2CO_3$ (30 mg, 22 mmol) was added. The reaction mixture was stirred at ambient temperature for 2 h and then filtered over Celite®. The solvent was removed under reduced pressure to provide compound **19** (28 mg, 71%, oil).

**$^{1}$H NMR ($CD_3OD$, 400MHz)**: δ (ppm) 7.67-7.64 (dd, 12Hz, 2H), 7.49-7.46 (dd, 12Hz, 2H), 4.61-4.58 (m, 1H), 4.35-4.28 (m,1H), 4.12-4.10 (m, 1H), 3.75-3.73 (m, 1H), 3.52-3.54 (m, 1H), 3.47 (d, 2H), 2.21-2.10 (m, 4H), 1.72-1.70 (m, 4H), 1.23-1.21 (m, 4H), 1.21- 1.20 (d, 3H)
**$^{13}$C NMR ($CD_3OD$, 100 MHz)**: δ (ppm) 127.7, 123.9, 117.6, 114.8, 90.8, 69.9, 69.3, 69.1, 67.7, 65.0, 45.3, 28.4, 23.5, 19.5, 16.23
**HRMS** calcd. for $C_{32}H_{30}F_{26}O_6Na$: 1027.15, found $[M+Na]^+$2D: 1031.98

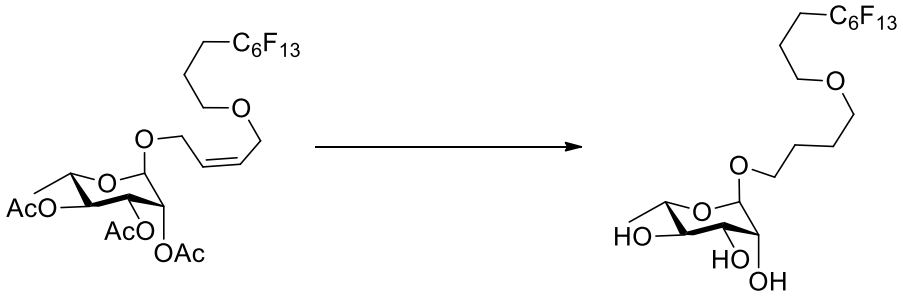
***cis*-4-(1H, 1H, 2H, 2H,3H, 3H-Perfluorononyloxy)-2-butenyl-2,3,4-O-tri-*O*-acetyl-α–L-rhamnopyranoside (20).**




**17** **5** **20**

To a solution of 2,3,4-tri-*O*-acetyl-α-L-rhamnopyranosyl trichloroacetimidate (21 mg, 49 μmol) and compound **5** (11 mg, 24 μmol) in toluene (3.0 mL), TMSOTf (9.0 μL, 0.49 μmol) was added at 0 ºC. The reaction mixture was stirred at 0 ºC for 20 min and then quenched with triethylamine (0.68 μL) and concentrated under reduced pressure. The crude product was purified by fluorous solid-phase extraction (FSPE) using a fluorous solid-phase extraction cartridge. Non-fluorous compounds were eluted with 80% MeOH/water and the desired product was eluted by 100%MeOH. The solvent was removed under reduced pressure to provide compound **20** (15 mg, 85%).
**$^{1}$H NMR (CD3OD, 400MHz)**: δ (ppm) 5.74-5.71 (m, 2H), 5.29 (dd, 1H), 5.07 (dd, 1H), 4.75 (dd, 1H), 4.19-4.17 (m, 1H), 4.13-4.10 (m, 1H), 4.06-4.05 (dd, 1H), 3.89-3.87 (m, 1H), 2.19-2.16 (m, 2H), 2.15 (s, 3H), 2.04 (s, 3H), 2.03 (s, 3H), 1.90-1.88 (m, 2H), 1.23-1.21 (d, 3H)
**$^{13}$C NMR ($CD_3OD$, 100 MHz)**: δ (ppm) 205.6, 184. 6, 167.3, 166.1, 137.1, 136.6, 116.5, 115.9, 103.2, 102.6, 71.5, 70.8, 70.3, 68.5, 66.6, 66.1, 46.2, 26.1, 25.6, 20.4, 17.2

**1H,1H,2H,2H,3H,3H-perfluorononyloxybutanyl-α-L-rhamnopyranoside (21).**




**20** **21**

To a solution of compound **20** (9.0 mg, 12 μmol) in methanol (1.5 mL) 10% Pd/C was added (4.0 mg). The reaction mixture was stirred at ambient temperature under hydrogen atmosphere for 2 h.

The reaction mixture was then filtered over Celite® and partially concentrated under reduced pressure. Then $K_2CO_3$ (10 mg) was added to the solution and stirred at ambient temperature for 3 h. The reaction mixture was neutralized with Amberlyst® 15 ion-exchange resin and filtered over Celite®. The solvent was removed under reduced pressure to provide compound **21** (6.2 mg, 83%, oil).

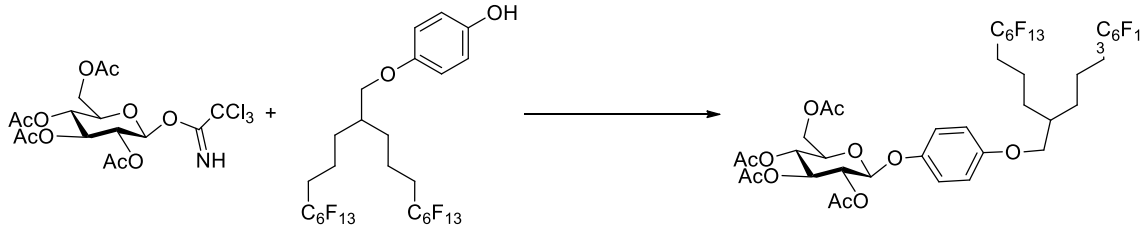
**$^1$H NMR ($CD_3OD$, 400MHz)**: δ (ppm) 4.15-4.31 (m, 1H), 3.45-3.98 (m, 11H), 1.62-1.68 (m, 4H), 1.23 (dd, 3H, *J* = 16.8, 6.4 Hz)

**$^{13}$C NMR ($CD_3OD$, 100 MHz)**: δ (ppm) 97.0, 77.3, 76.9, 76.6, 70.9, 70.4, 69.6, 69.1, 68.0, 67.7, 66.4, 26.6, 25.8, 20.7, 17.3

**HRMS** calcd. for $C_{19}H_{25}F_{13}O_6K$: 636.38, found $[M+K]^+$2D: 639.12

**• Synthesis of the glucose-linked tags.**

**4-((6,6,7,7,8,8,9,9,10,10,11,11,11-tridecafluoro-2-(4,4,5,5,6,6,7,7,8,8,9,9,9-tridecafluorononyl)undecyl)oxy)phenoxy-2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranoside (23).**



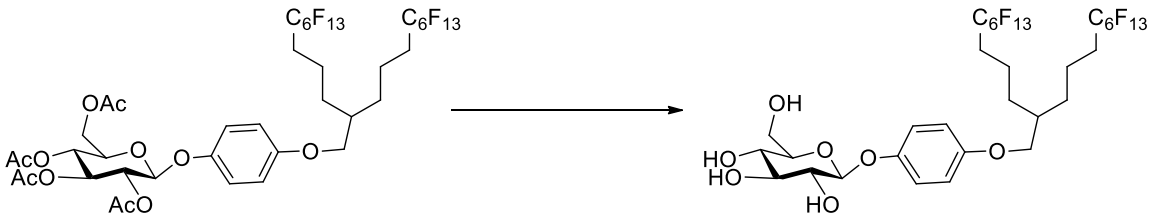

**22** **11** **23**

To a solution of 2,3,4,6-tetra-O-acetyl- β-D-glucopyranosyl trichloroacetimidate (**22**) (55 mg, 0.13 mmol) and compound **11** (19 mg, 22 µmol) in toluene (5 mL) was added TMSOTf (0.20 µL, 1.1 µmol) at 0 ºC. The reaction mixture was stirred at 0 ºC for 20 min. The reaction mixture was quenched with triethylamine (0.20 mL) and concentrated under reduced pressure. The crude product was purified by fluorous solid-phase extraction (FSPE) using a fluorous solid-phase extraction cartridge. Non-fluorous compounds were eluted with 80% MeOH/water and the desired product was eluted by 100% MeOH. The solvent was removed under reduced pressure to provide **23** (26 mg, 99%, pale yellow oil).

**$^1$H NMR (400 MHz, CDCl3)**: δ (ppm) 7.00-6.87 (2H), 6.81-6.74 (2H), 5.29-5.13 (2H), 4.95-4.93 (d, *J* = 8,1H), 4.30-4.04 (2H), 3.82-3.76 (3 H), 3.48 (12H) 2.07-2.03 (dd, *J* = 21.2, 12H), 1.66 (2H)

**$^{13}$C NMR (400 MHz, CDCl3)**: δ (ppm) 170.87, 170.53, 169.65, 169.57, 155.55, 151.07, 118.901, 115.51, 115.46, 100.54, 73.12, 72.98, 72.93, 72.15, 71.46, 68.61, 68.53, 68.06, 62.17, 57.01, 51.09, 40.58, 37.25, 37.12, 36.82, 35.81, 35.61, 35.56, 35.10, 34.94, 33.40, 31.53, 29.94, 29.91, 20.95, 20.93, 20.88, 20.85, 20.71, 16.86, 14.77, 14.38, 14.35

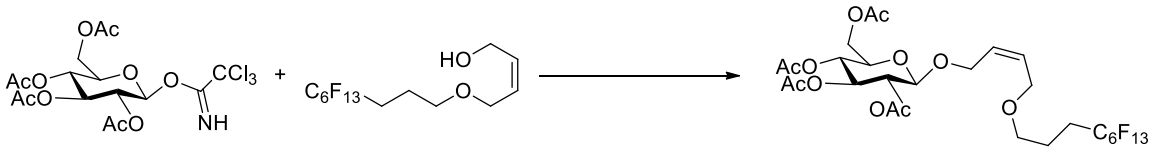
**4-((6,6,7,7,8,8,9,9,10,10,11,11,11-tridecafluoro-2-(4,4,5,5,6,6,7,7,8,8,9,9,9 tridecafluorononyl)undecyl)oxy)phenoxy-β-D-glucopyranoside (24).**




**23** **24**

To a solution of compound **23** (22 mg, 18 µmol) in methanol (5 mL) was added $K_2CO_3$ (13 mg, 92 µmol). The reaction mixture was stirred at ambient temperature for 3 h. The reaction mixture was neutralized with Amberlyst® 15 ion-exchange resin and filtered over Celite®. The solvent was removed under reduced pressure to provide compound **24** (14 mg, 75%, white solid).
**$^1$H NMR (400 MHz, CDCl3)**: δ (ppm) 7.01-6.97 (1.5H), 6.88-6.76 (2.5H), 3.83-3.76 (4H), 3.44 (2H), 3.38-3.37 (1H), 3.33 (1H), 2.34-2.17 (10H), 1.65 (2H), 1.56 (3H)
**$^{13}$C NMR (400 MHz, CDCl3)**: δ (ppm) 128.54, 128.30, 128.03, 127.94, 116.25, 116.24, 115.88, 115.85, 96.08, 96.04, 95.95, 80.80, 79.75, 73.38, 72.85, 68.11, 64.18, 51.81, 50.60, 37.31, 37.11, 37.08, 36.07, 35.66, 35.10, 33.46, 31.21, 29.95, 22.95, 18.10, 18.08
**HRMS** calcd. for $C_{32}H_{30}F_{26}O_7Na$: 1043.15, found $[M+Na]^+$2D: 1047.27

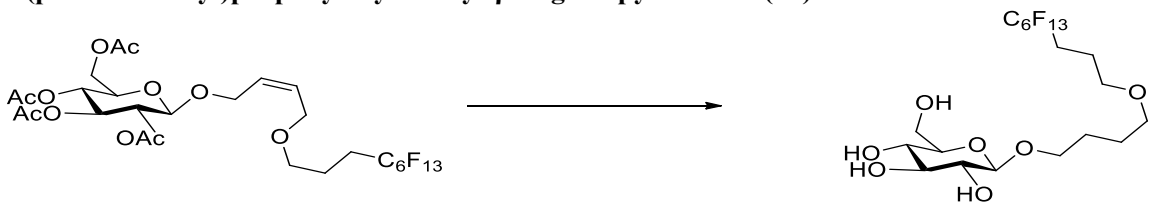
**3-(perfluorohexyl)propanyloxybutenyl-2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranoside (25).**




**22** **5** **25**

To a solution of 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl trichloroacetimidate (**22**) (39 mg, 78 µmol) and compound **5** (18 mg, 40 µmol) in toluene (5 mL) was added TMSOTf (0.40 µL, 2.0 µmol) at 0 ºC. The reaction mixture was stirred at 0 ºC for 20 min. The reaction mixture was quenched with triethylamine (0.40 mL) and concentrated under reduced pressure. The crude product was purified by fluorous solid-phase extraction (FSPE) using a fluorous solid-phase extraction cartridge. Non-fluorous compounds were eluted with 80%MeOH/water and the desired product was eluted by 100%MeOH. The solvent was removed under reduced pressure to provide **25** (26 mg, 83%, pale yellow oil).
**$^1$H NMR (400 MHz, CDCl3)**: δ (ppm) 5.76-5.64 (m, 2H), 5.23-4.98 (3H), 4.55-4.52 (1H), 4.39-4.33 (1H), 4.25-4.23 (2H), 4.16-4.12 (1H), 4.05-4.01 (2H), 3.70-3.66 (1H), 3.50-3.47 (2H), 2.18 (2H), 2.09 (3H), 2.04-2.01 (9H), 1.90-1.86 (2H)
**$^{13}$C NMR (400 MHz, CDCl3)**: δ (ppm) 132.32, 123.90, 123.87, 117.64, 111.37, 99.76, 95.68, 72.063, 71.42, 69.03, 66.72, 62.12, 31.85, 29.95, 20.93, 20.83

**3-(perfluorohexyl)propanyloxybutanyl-β-D-glucopyranoside (26).**



**25** **26**

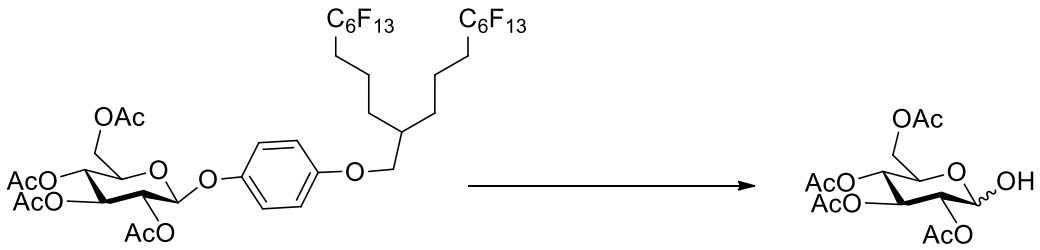
To a solution of compound **25** (21 mg, 27 µmol) in methanol (5 mL) was added 10% Pd/C (0.1 g). The reaction mixture was stirred at ambient temperature under hydrogen atmosphere for 2 h. The reaction mixture was then filtered over Celite® and partially concentrated under reduced pressure. Then $K_2CO_3$ (15 mg) was added to the solution and stirred at ambient temperature for 3 h. The reaction mixture was neutralized with Amberlyst® 15 ion-exchange resin and filtered over Celite®. The solvent was removed under reduced pressure to provide compound **26** (13 mg, 77%, white solid).

**$^1$H NMR (400 MHz, CDCl3)**: δ (ppm) 4.34-4.30 (2H), 3.66-3.65 (4H), 3.04 (3H), 2.20-2.15 (3H), 1.99-1.97 (2H), 1.88-1.85 (4H), 1.67-1.63 (4H), 1.25 (2H)

**$^{13}$C NMR (400 MHz, CDCl3)**: δ (ppm) 71.16, 71.12, 69.52, 69.47, 62.94, 62.90, 57.01, 37.88, 37.84, 37.80, 37.76, 37.74, 37.72, 30.26, 30.21, 30.16, 26.80, 26.75, 26.71, 21.037, 21.02, 20.93, 20.91

**HRMS** calcd. for $C_{19}H_{25}F_{13}O_7Na$: 635.13, found $[M+Na]^+$: 635.11

**2,3,4,6-tetra-*O*-acetyl-D-glucopyranoside (27):**



**23** **27**

To a solution of compound **23** (17 mg, 14 µL) in acetonitrile:water (4:1) (2 mL) was added ceric ammonium nitrate (16 mg, 27 µL). The reaction mixture was stirred for 2.5 h at 21 ºC. $NaHCO_3$ (10 mL) was added to the reaction. The aqueous layer was extracted with dichloromethane (2 x 10 mL) and the organic layer was dried over MgSO4. The solvent was removed under reduced pressure. The crude product was purified by flash column chromatography (2:1 hexane: ethyl acetate) to yield **27** (70%, 3.5 mg, pale yellow sticky solid). The $^1$H NMR data of compound **27** matched previously reported data.[5]

**Solubility study.**

The di-fluorous-tag could be made into 1M solutions in either pure dichloromethane or pure toluene with complete miscibility.

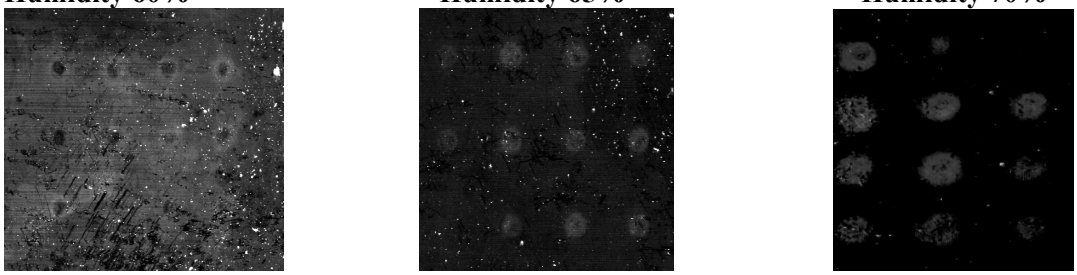
**Microarray preparation, screening and notes.**

Microarrays were printed by the Microarrayer**XactII™ (**LabNEXT Inc. 733 Beaver Road, Glenview, IL 60025**)** robotic pin (Xtend MP Microarray Pins 0.35 mm) deposition of the three

different fluorous-tagged sugars in methanol/DMSO/water (v:v:v = 2:6:2) (250 μM solution) from a 384-well plate onto commercially available fluorinated glass slides (Fluorous Technologies, Inc.; Pittsburgh, PA) at 70% humidity and a temperature of 22 ºC. (Note that the slide will work better if it is washed once with a 1:1 solution of dichloromethane:methanol.) The glass slide was allowed to dry for 15 h to 24 h inside a humidity chamber and for another 2 h outside of it. This drying procedure helped in avoiding the donut effect and allowed the molecules to orient themselves on the slides to obtain good spots. The ConA-FITC 200 μM solution was composed of the FITC-labeled ConA (Sigma, 1 mg/1 mL, 200 μL) in a 1X PBS (780 μL) 1 mM $CaCl_2$ (10 μL) and 1 mM $MnCl_2$ (10 μL) were used for the detecting protein-carbohydrate interactions. The arrays were incubated with the protein solution (150 μL) by using a PC500 CoverWell incubation chamber (Grace Biolabs, Bend, OR) for 1 h, and then washed twice with 1X PBS containing 1% BSA followed by washing once with distilled water. They were then dried and scanned at Iowa State University DNA facility. The slides were scanned using a General Scanning ProScanArray 5000 set at 488 nm. The fluorescent intensities were determined using the ImaGene 8.0 software.

During the microarray study we found that the spots had many inconsistencies depending on the environmental conditions. One of the major factors affecting the spot morphology was humidity. So, we did a comparative study by changing humidity and keeping the temperature constant at 22 ºC by using the already known mannose-$C_8F_{17}$ tags. We started with 60% humidity as most microarray studies were previously performed at 60% humidity condition. Then we tested 65% and 70% humidity conditions. From the slides shown below, we can conclude that the slide at 60% humidity has donut effect whereas the slide at 65% humidity has a slightly less donut effect, and the slide at 70% humidity has no donut effect at all. Also, consistently good spots were observed with the slides printed at 70% humidity. Based on these results, for all the studies in this paper, we performed the microarray at 70% humidity.

**Humidity 60%** **Humidity 65%** **Humidity 70%**



Also, we found that the use of new slides caused inconsistencies in the spots in terms of binding ability of the carbohydrate to the slide. The new slide always showed prominent donut effect and most of time the spot would be completely washed away. So, we washed the slides by adopting the method used by Spring and co-workers using 1:1 dichloromethane:methanol.[6] We decided to wash the new slide once with the 1:1 solution first before printing. Also, we were able to wash and reuse the slides at least five times.

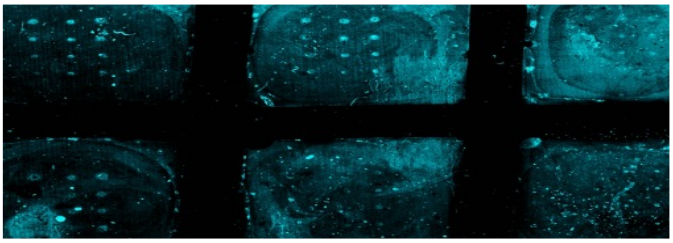
While printing we observed contamination of the spots from the pins. The washing protocol of the array was not very well set up for carbohydrate microarray studies. So, we had to wash the pins out by hand after each spot with acetone. We used the same pin for each spot to get reliable results.

To develop a good washing protocol for the fluorous tagged carbohydrates, we scanned the slide after each washing with 1XPBS containing 1% BSA. We found that the first wash with 1XPBS

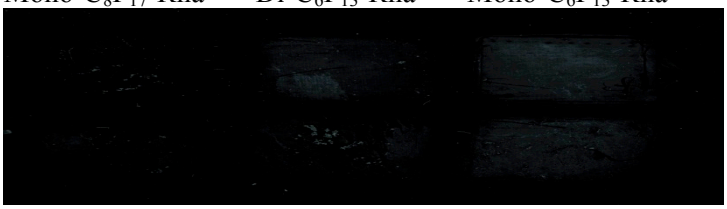
containing 1% BSA was most effective in clearing the protein from the slide whereas the second wash had little effect. Also, we found that washing with distilled water had no effect on the slide. Each slide was washed twice with 1XPBS containing 1% BSA and once with distilled water to remove any remaining salts that may have deposited on the slide.

The slides below are the results that we obtained from the microarray studies. The β-D-glucose and the α-L-rhamnose slide showed nothing, as expected. The α-D-mannose slide showed that the mono-$C_6F_{13}$-tag was washed away, while the di-$C_6F_{13}$-tag and mono-$C_8F_{17}$-tag were clearly noticeable. The last slide has all nine fluorous-tagged monosaccharides placed in one well. From this slide there are only two spots in each well indicating what the first three slides data shows.

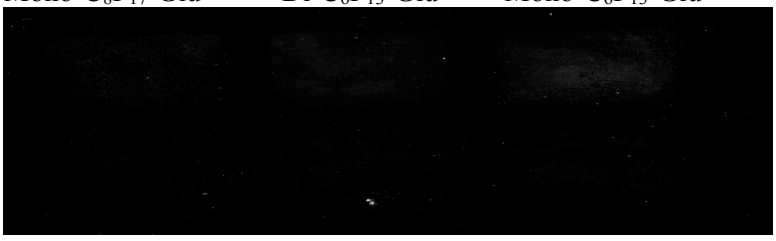
**Mannose Slide:**
Mono-$C_8F_{17}$-Man  Di-$C_6F_{13}$-Man  Mono-$C_6F_{13}$-Man



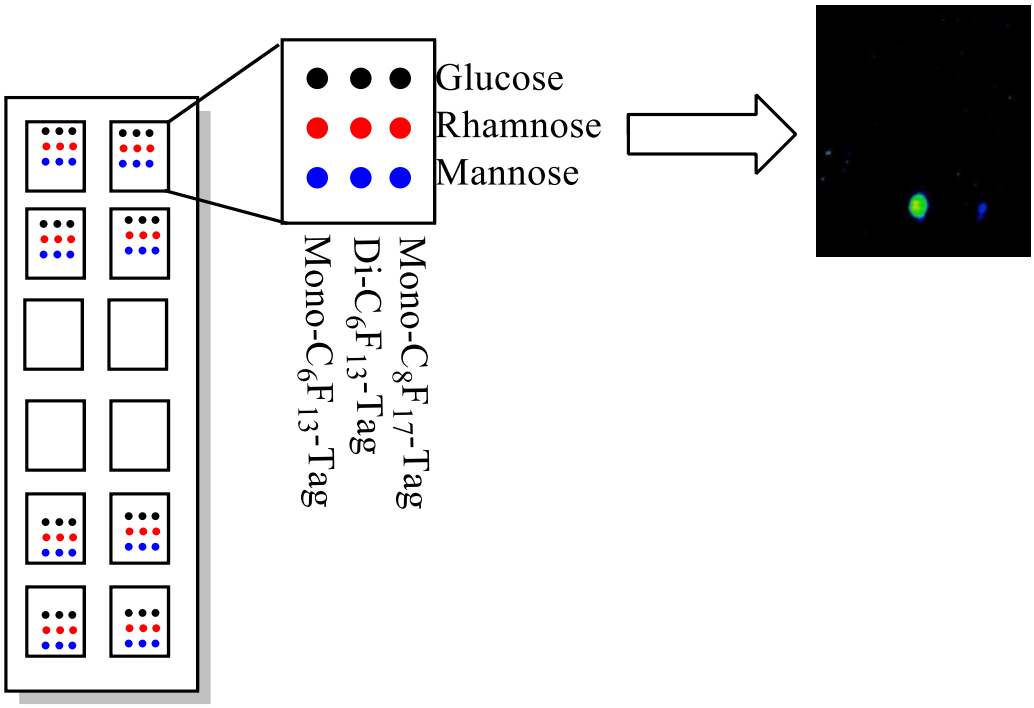
**Rhamnose Slide:**
Mono-$C_8F_{17}$-Rha  Di-$C_6F_{13}$-Rha  Mono-$C_6F_{13}$-Rha



**Glucose Slide:**
Mono-$C_8F_{17}$-Glu  Di-$C_6F_{13}$-Glu  Mono-$C_6F_{13}$-Glu



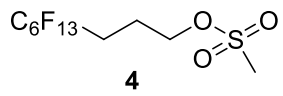
The pattern of the slide with all nine fluorous-linked monosaccharides and the actual slide:



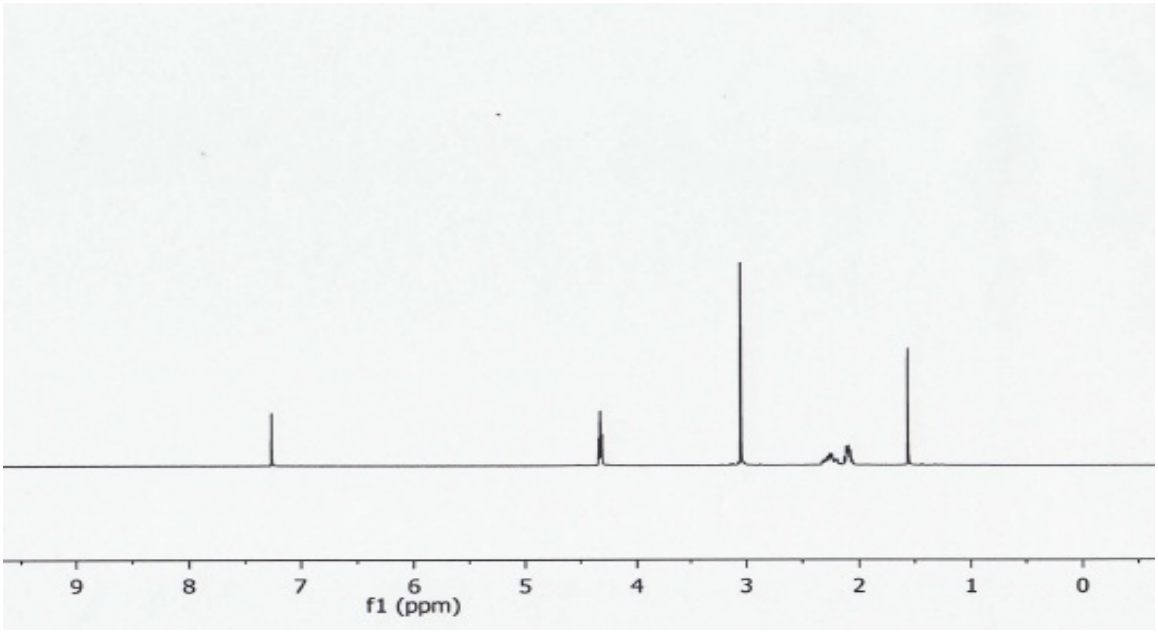

**References:**

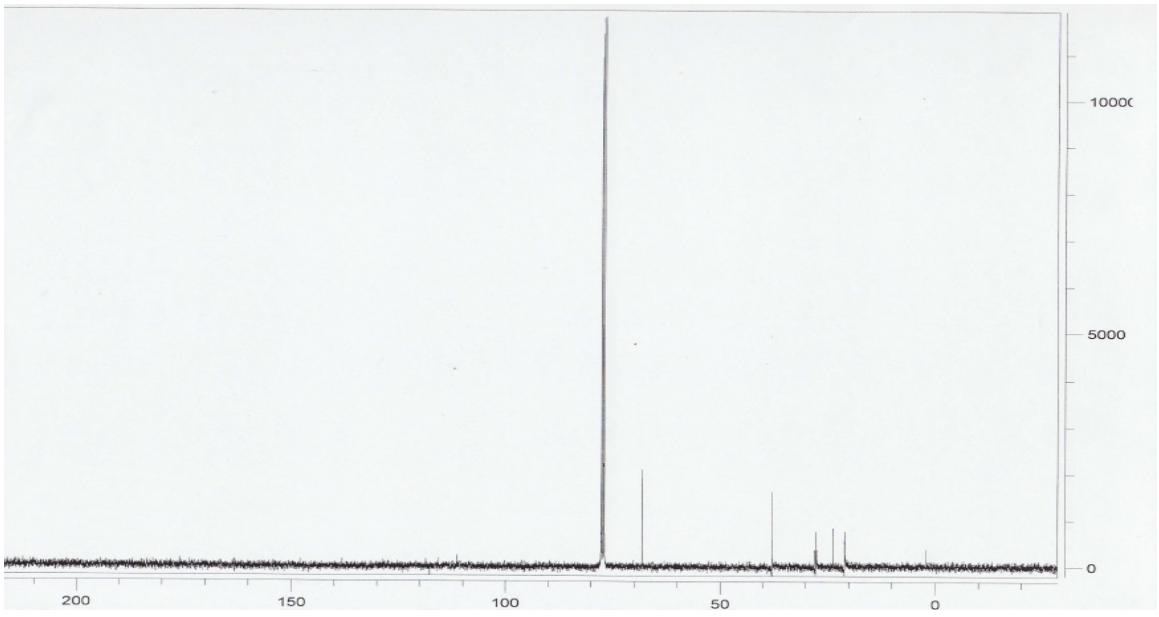
1. J. Rábai, C. Szíjjártó, P. Ivanko, D. Szabó, *Synthesis*, 2007, **16**, 2581-2584.
2. E. P. Go, W. Uritboonthai, J. V. Apon, S. A. Trauger, A. Nordstrom, G. O'Maille, S. M. Brittain, E. C. Peters, G. Siuzdak, *J. Proteome Res*., 2007, **6**, 1492-1499.
3. J. Loiseau, E. Fouquet, R. Fish, J.-M. Vincent, J.-B. Verlhac, *J. Fluorine Chem.* 2001, **108**, 195-197.
4. R. Kaplanek, T. Briza, M. Havlika, B. Dolensky, Z. Kejik, P. Martasek, V. Kral, *J. Fluorine Chem.* 2006, **129**, 386–390.
5. R. R. Schmidt, J. Michel, *Angew Chem Int Ed.* 1980, **19**, 731-732.
6. R. Nicholson; M. Ladlow; D. Spring; *Chem. Commun.* 2007, 3906–3908.

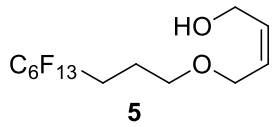
**$^{1}$H and $^{13}$C NMR's:**



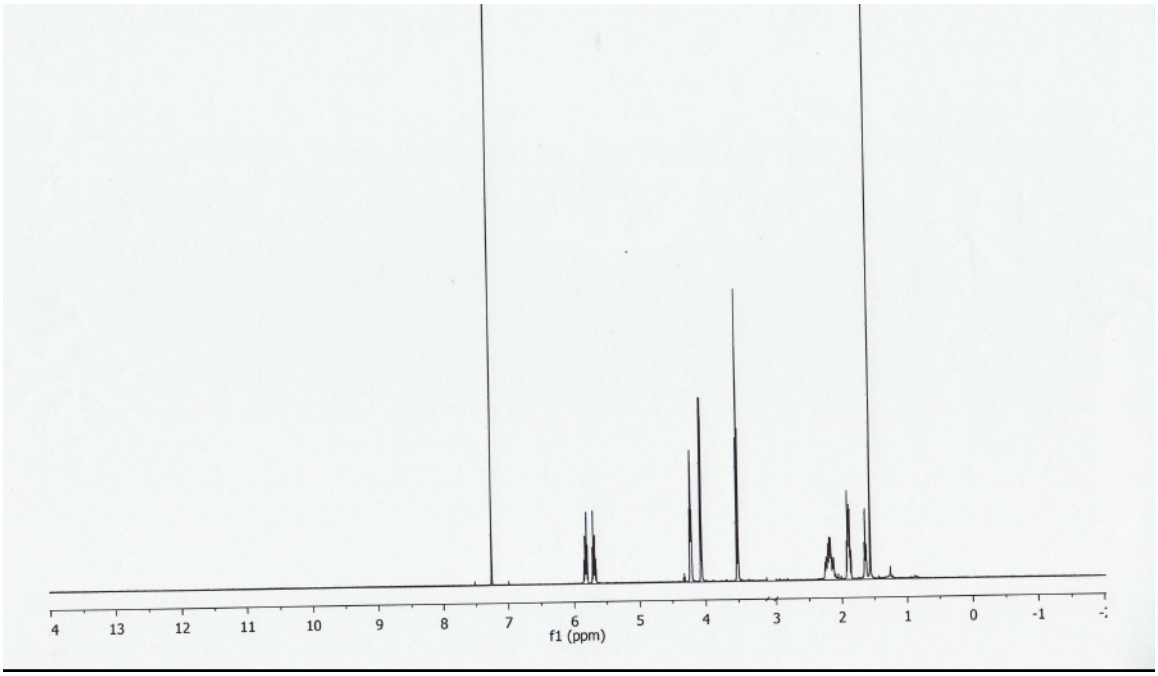

**4**




HO
$C_6F_{13}$
O

**5**


4
13
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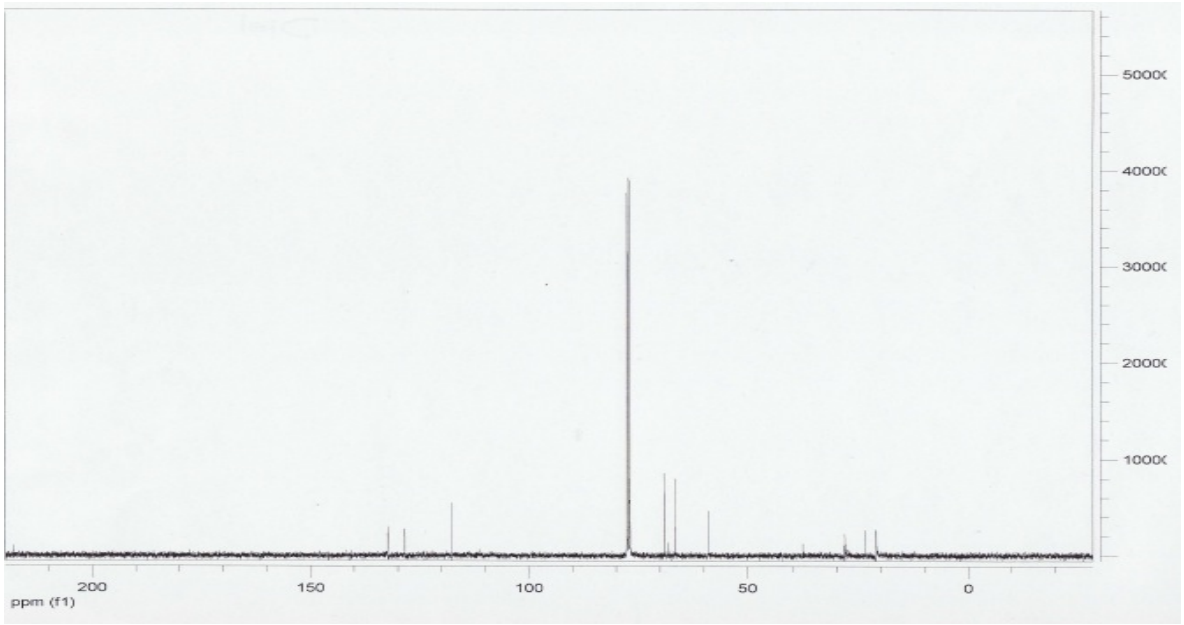
11
10
9
8
7
6
f1 (ppm)
5
4
3
2
1
0
-1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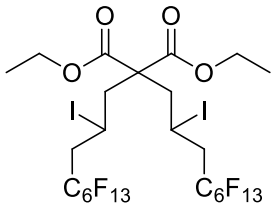
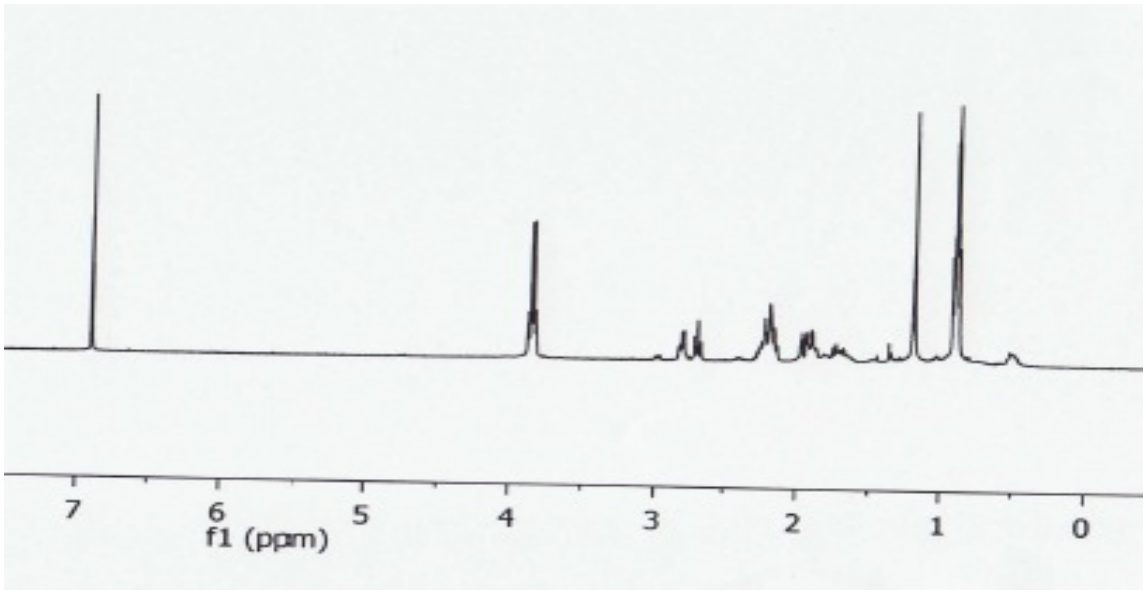
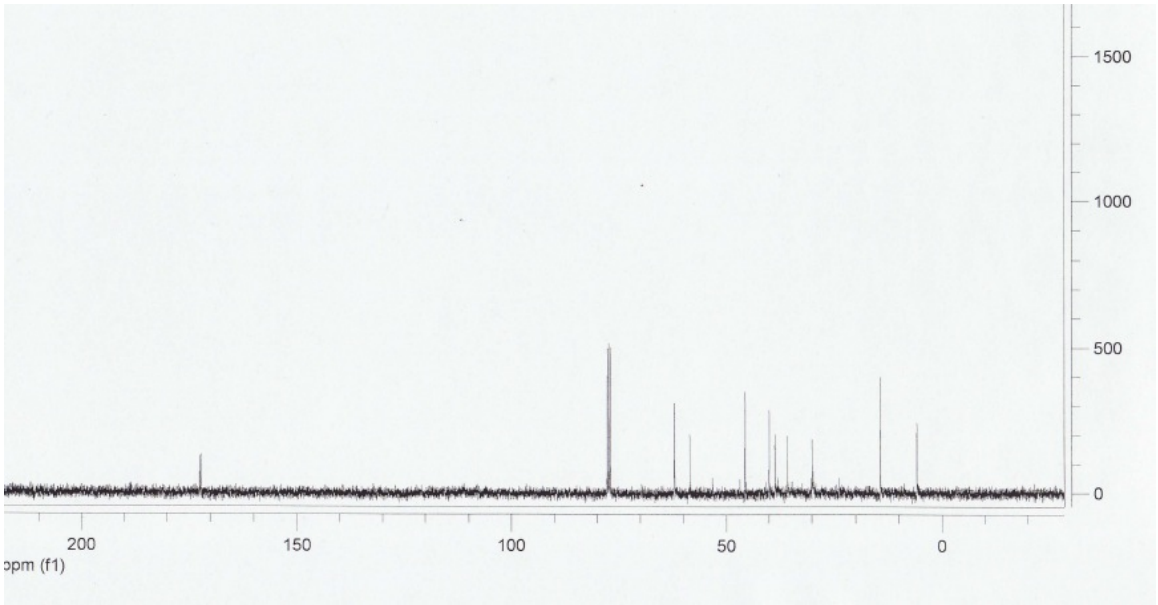
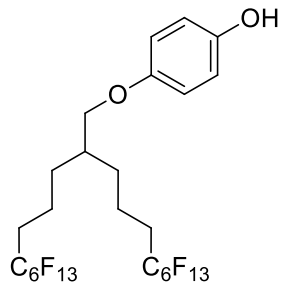
4000
3000
2000
1000
200
150
100
50
0
ppm (f1)


O
O
O
O
I
I
$C_6F_{13}$
$C_6F_{13}$

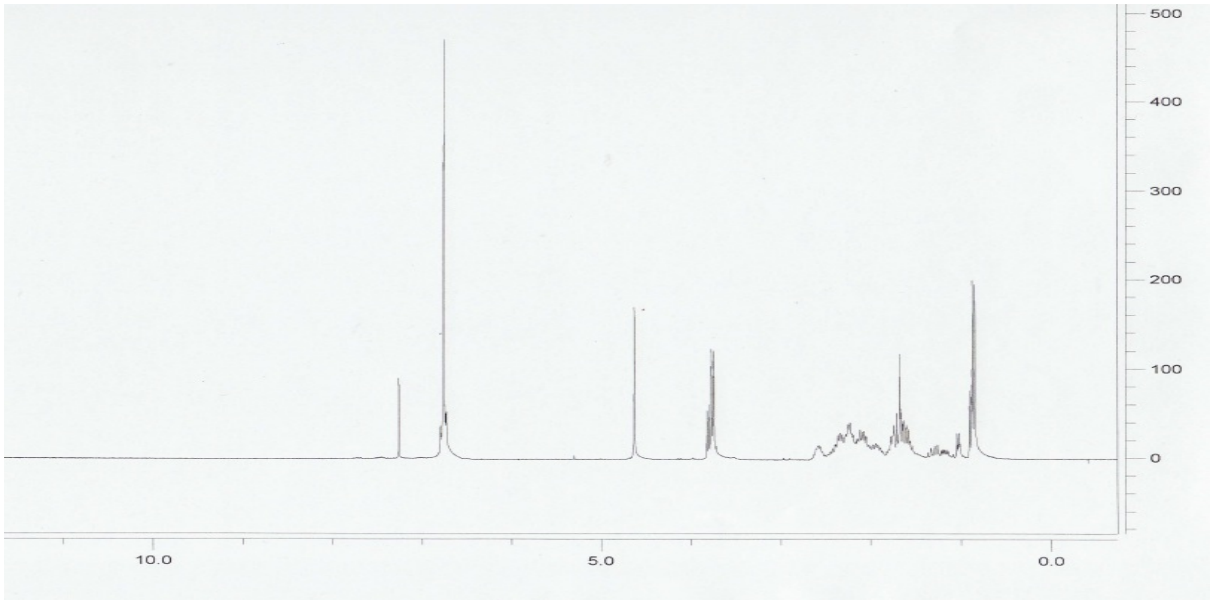
**7**

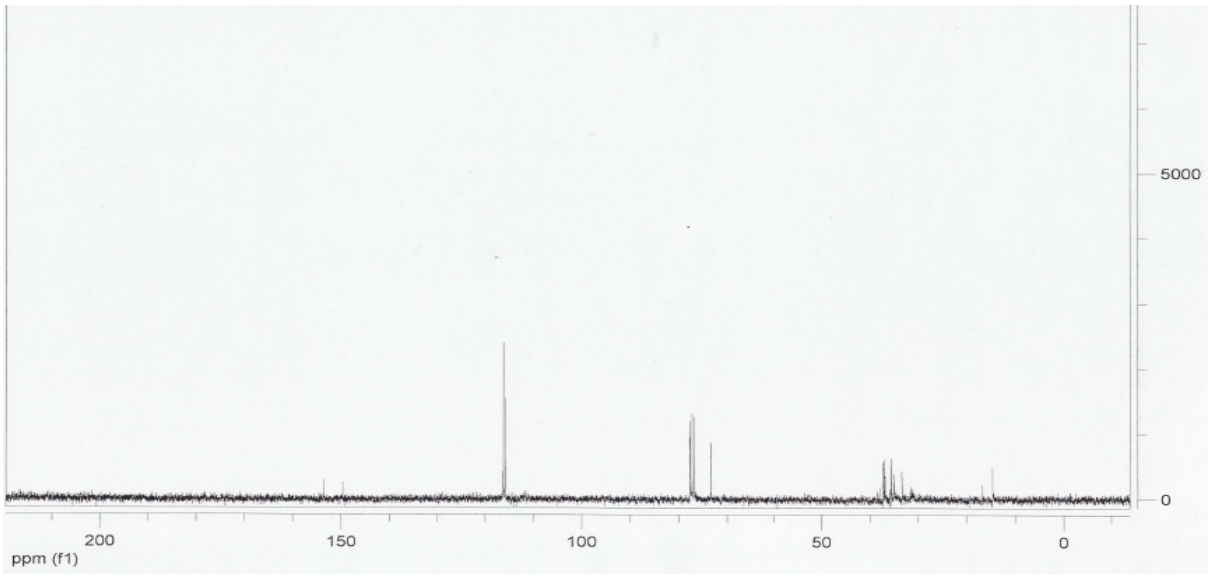

7
6
5
4
3
2
1
0
f1 (ppm)

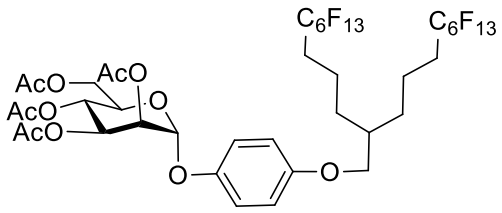

1500
1000
500
0
200
150
100
50
0
ppm (f1)


OH
O
C6F13
C6F13

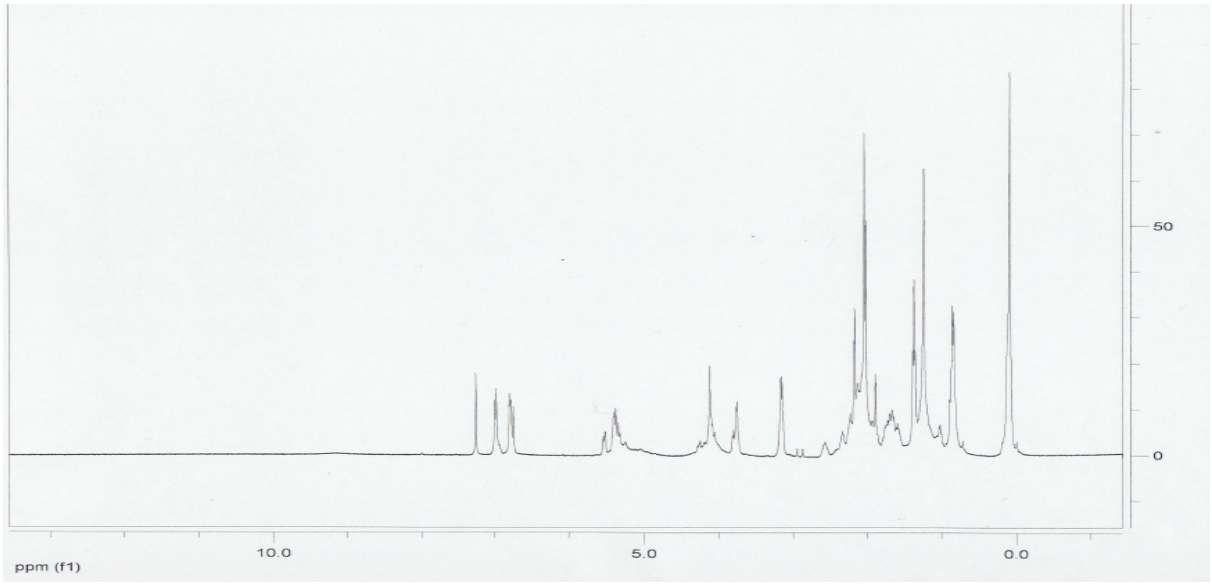
**11**

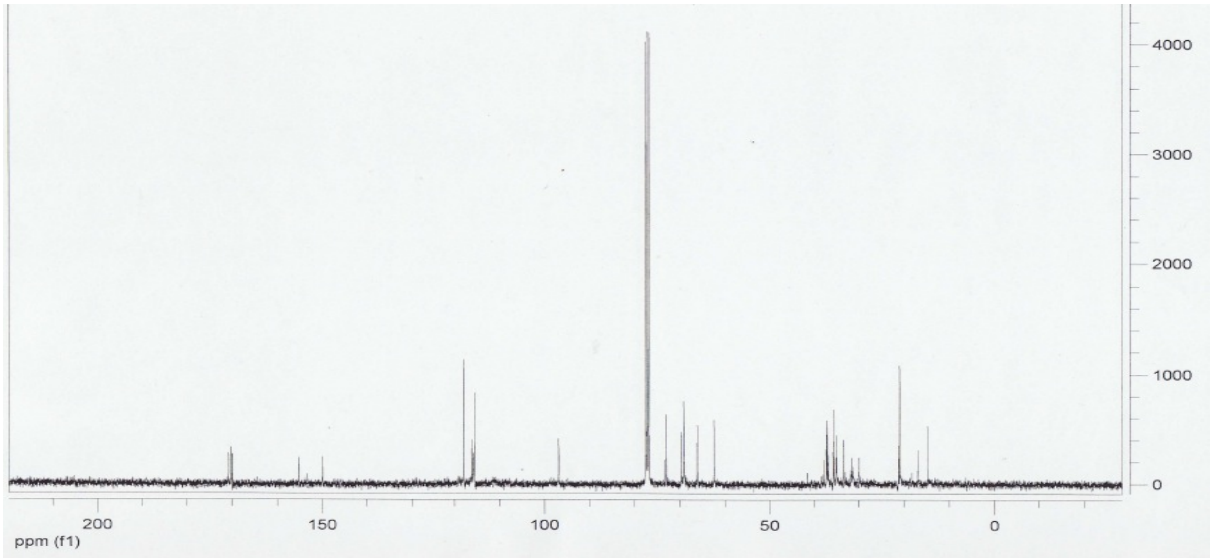

500
400
300
200
100
0
10.0
5.0
0.0

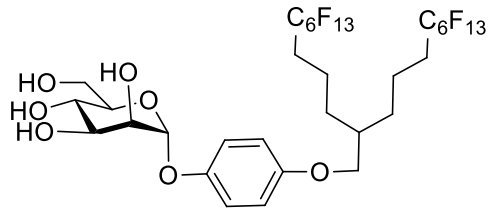

5000
0
200
150
100
50
0
ppm (f1)


C6F13
C6F13
AcO
AcO
AcO
AcO
O
O
O

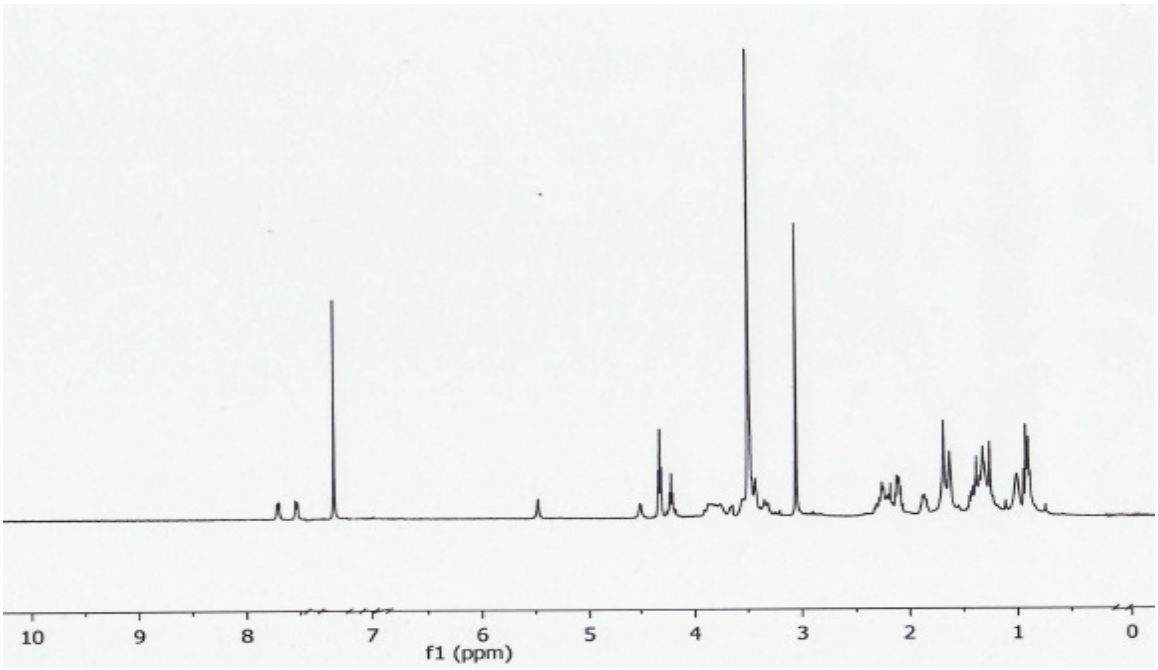
**13**

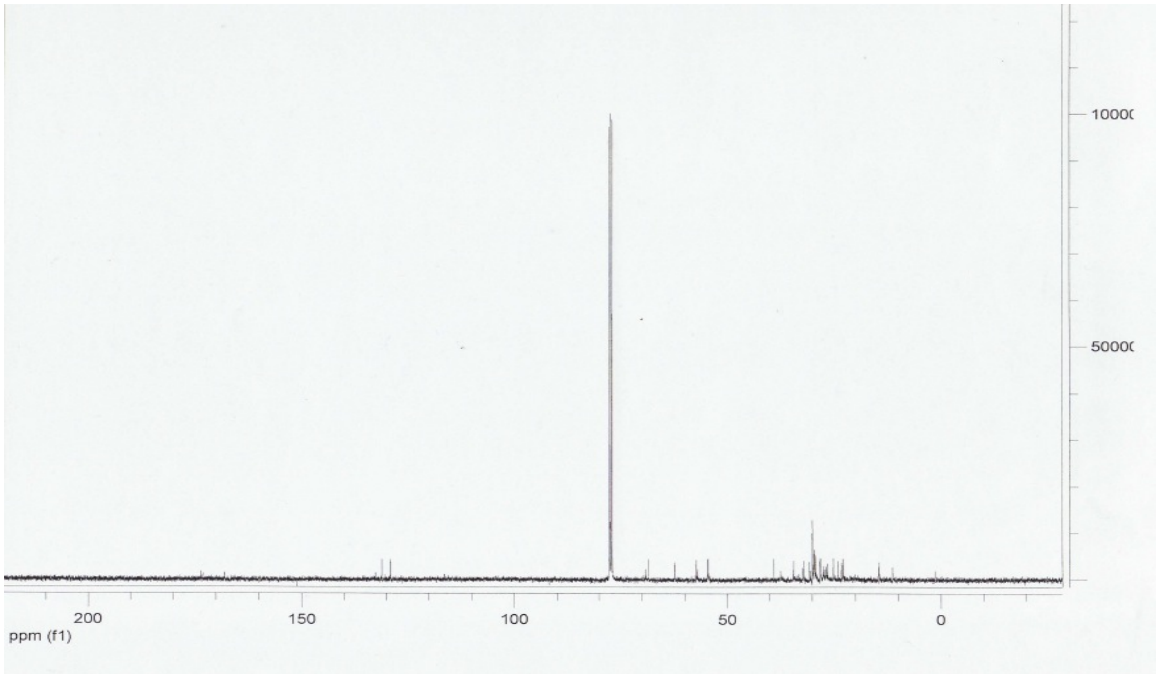

50
0
10.0
5.0
0.0
ppm (f1)

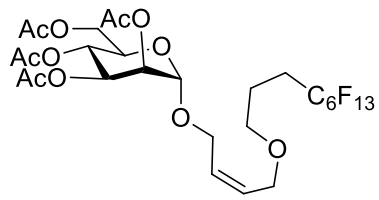

4000
3000
2000
1000
0
200
150
100
50
0
ppm (f1)


C6F13
C6F13
HO
HO
HO
HO
O
O
O

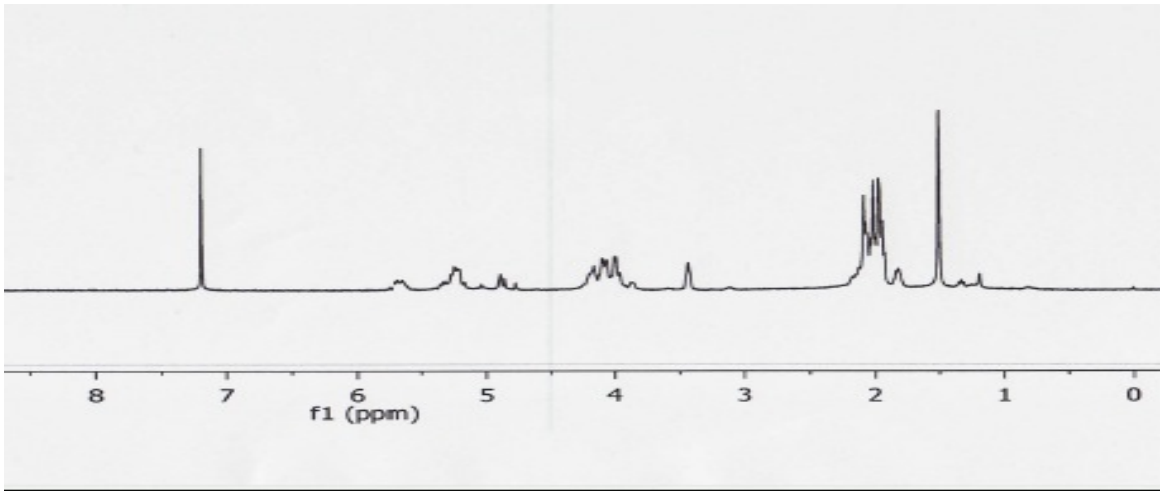
**14**

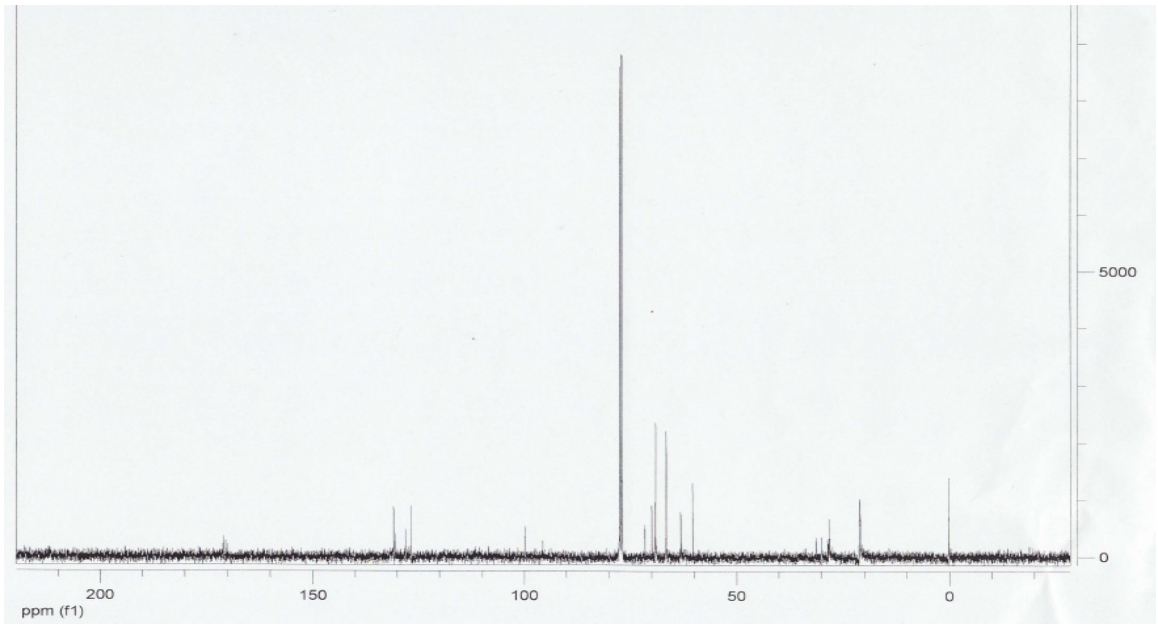

10
9
8
7
6
5
4
3
2
1
0
f1 (ppm)

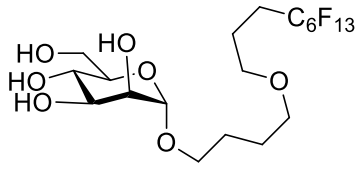

10000
50000
200
150
100
50
0
ppm (f1)


AcO
AcO
AcO
AcO
O
O
O
$C_6F_{13}$

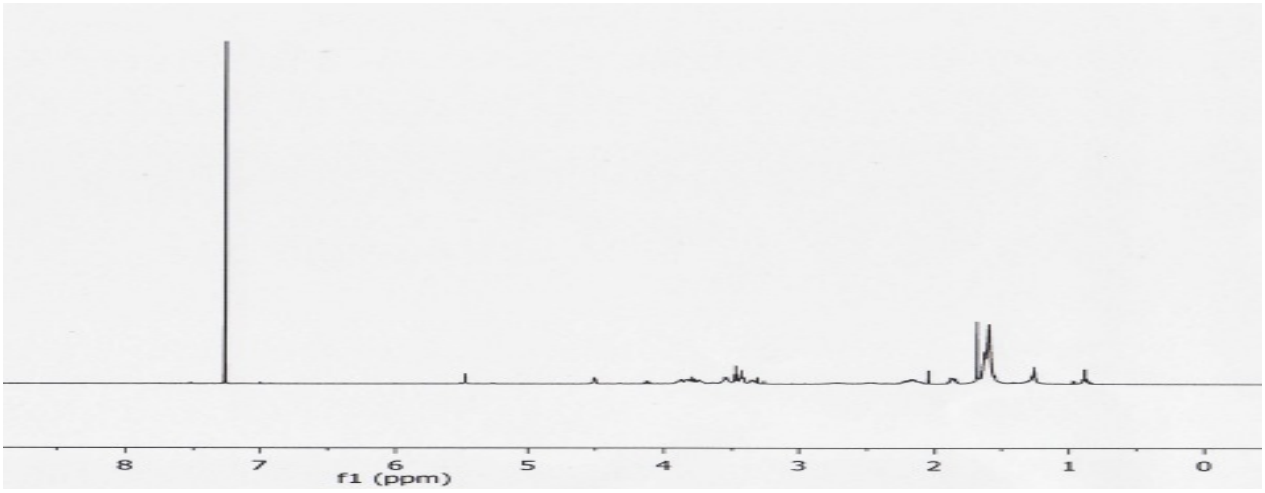
**15**

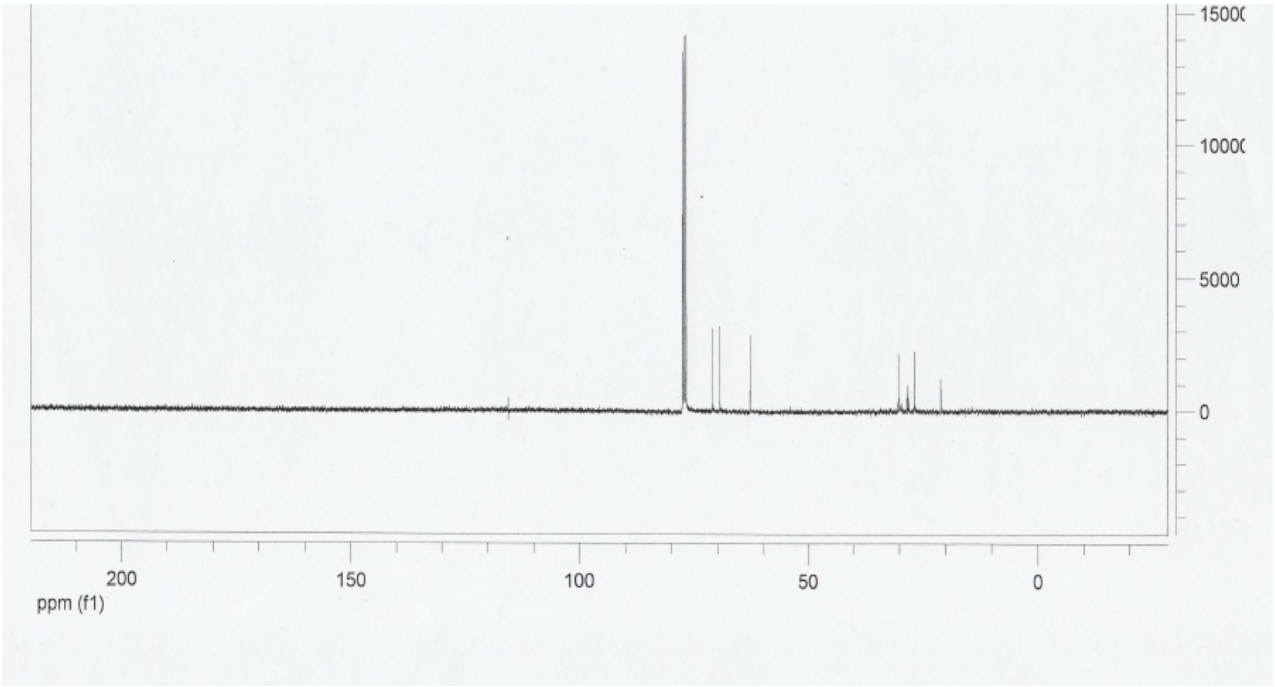

8
7
6
5
4
3
2
1
0
f1 (ppm)

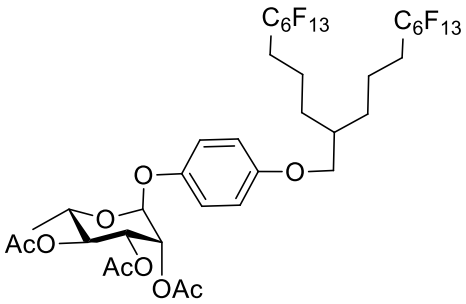

5000
0
200
150
100
50
0
ppm (f1)


HO
HO
HO
HO
O
O
O
$C_6F_{13}$

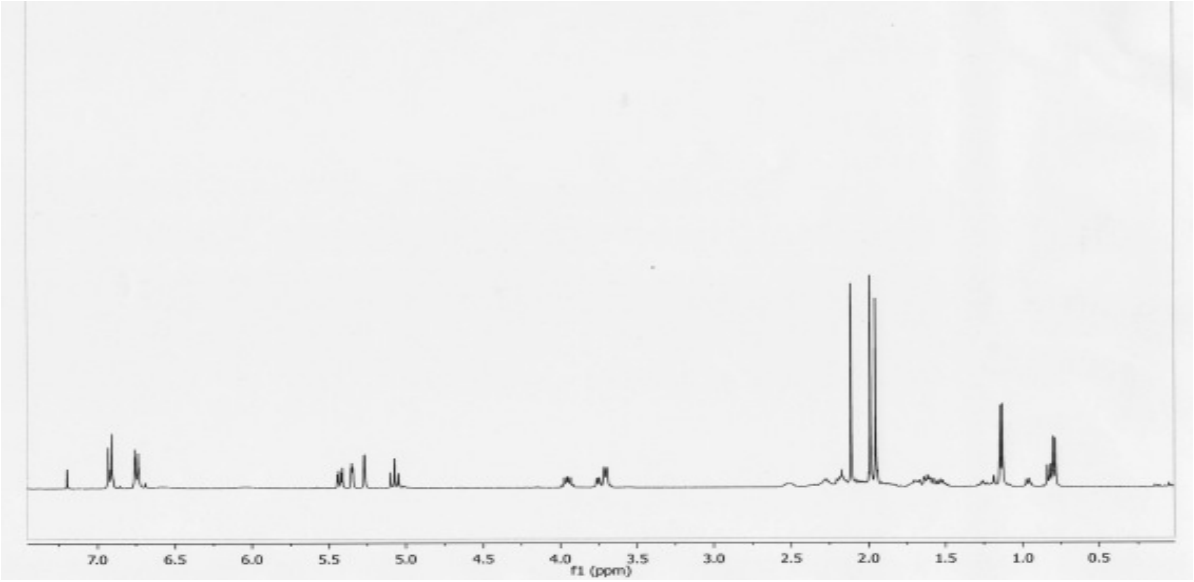
**16**

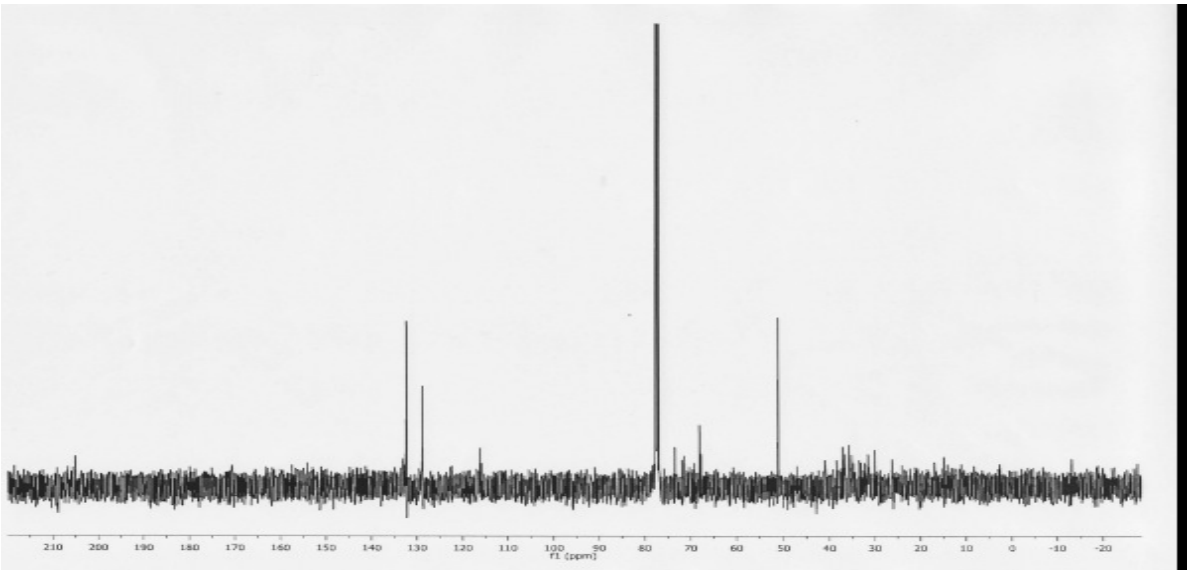

8
7
6
5
4
3
2
1
0
f1 (ppm)

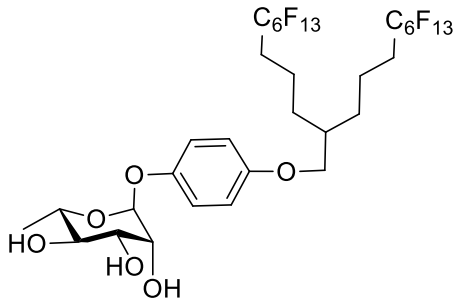

15000
10000
5000
0
200
150
100
50
0
ppm (f1)


C6F13
C6F13
O
O
O
AcO
AcO
OAc

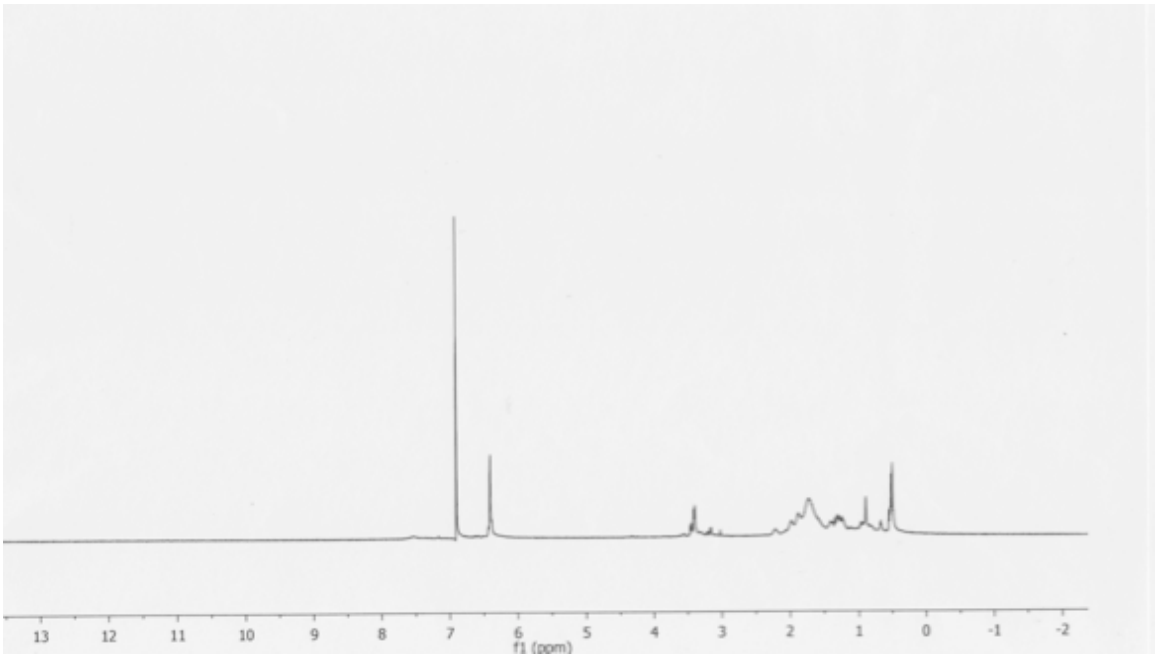
**18**

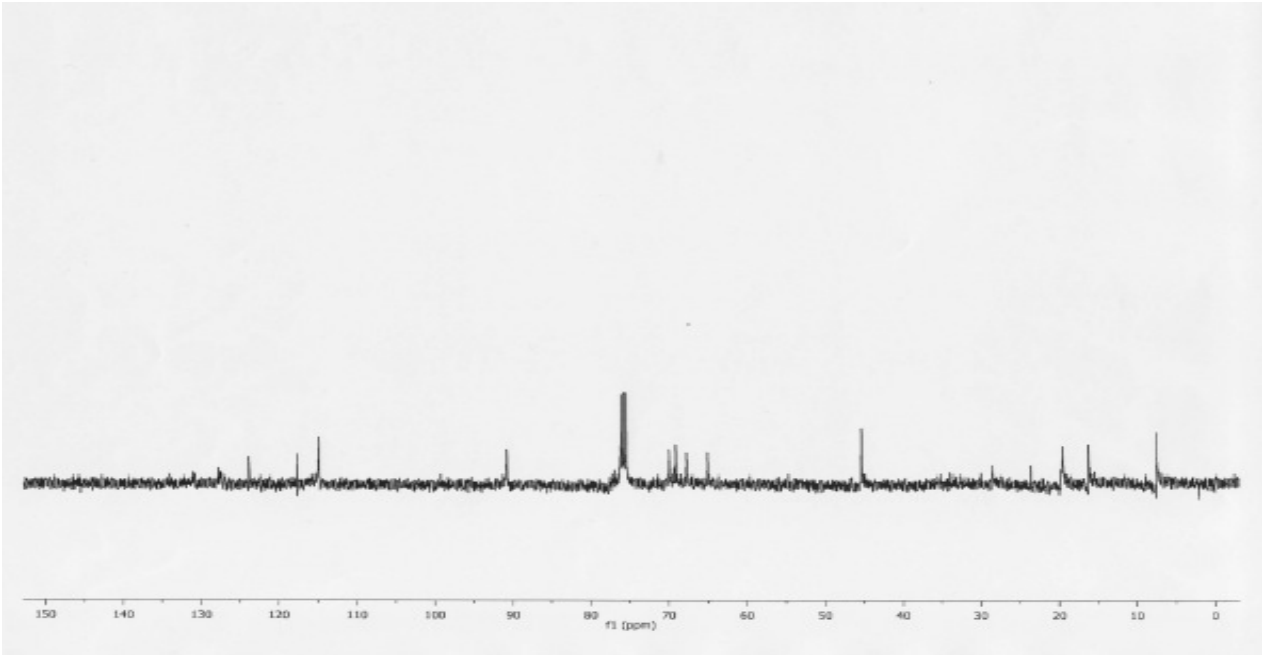

7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
f1 (ppm)

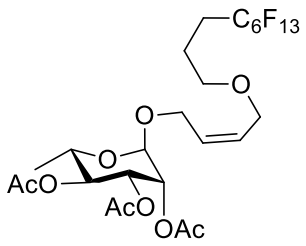

210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
-20
f1 (ppm)


C6F13
C6F13
O
O
O
HO
HO
OH

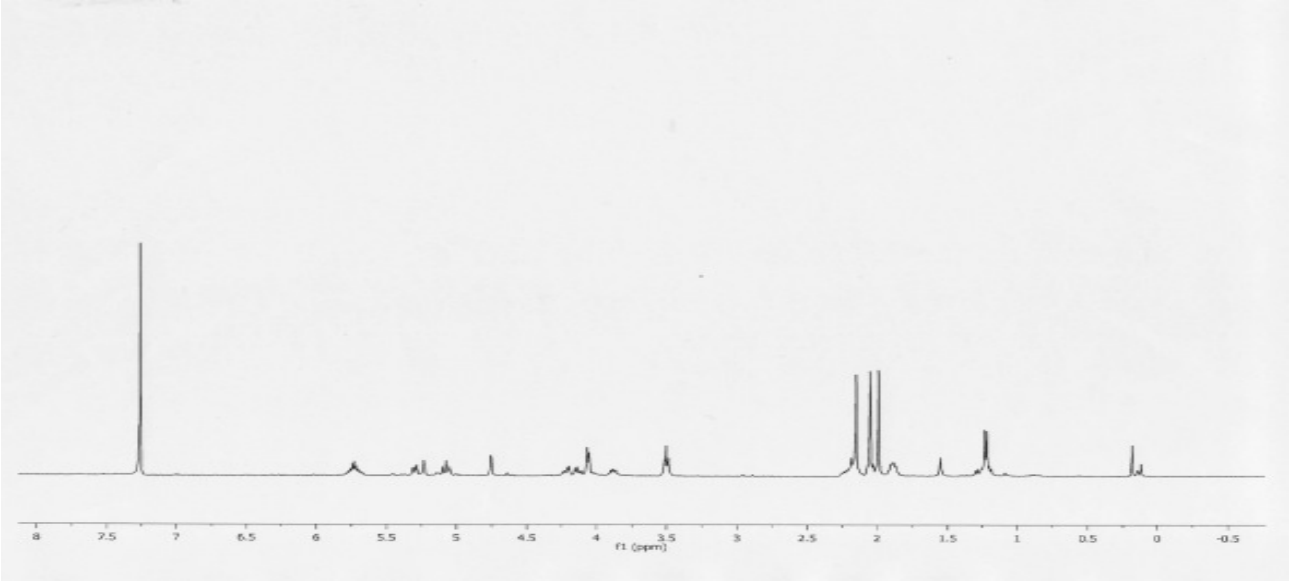
**19**

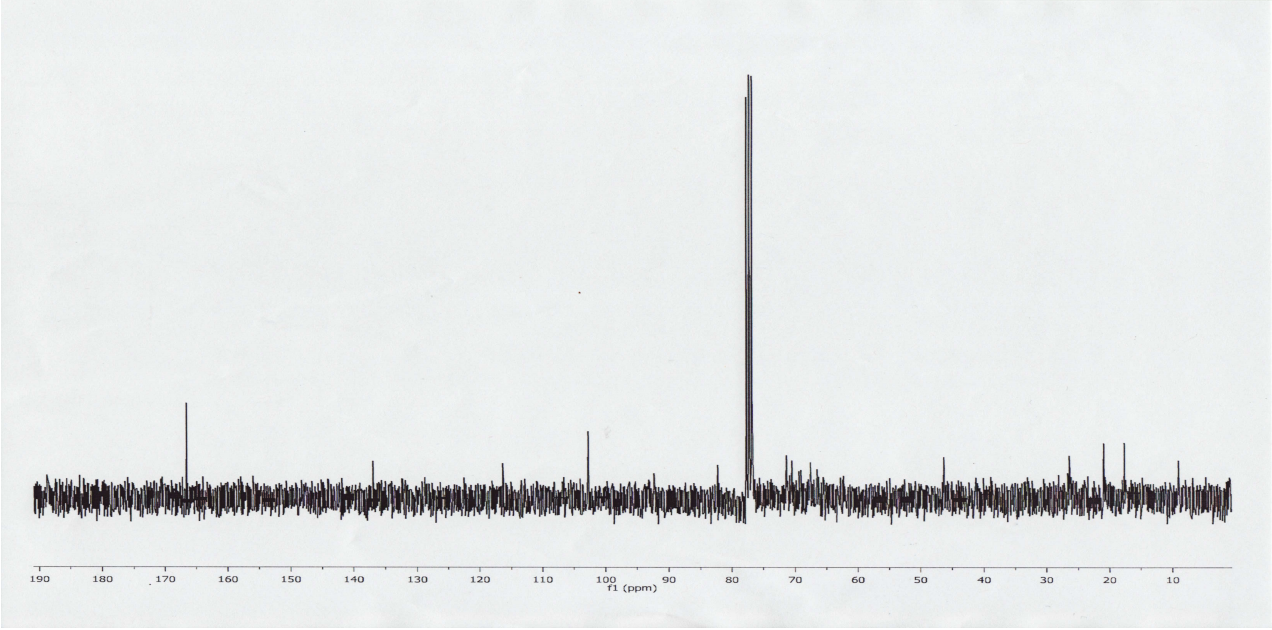

13
12
11
10
9
8
7
6
5
4
3
2
1
0
-1
-2
f1 (ppm)

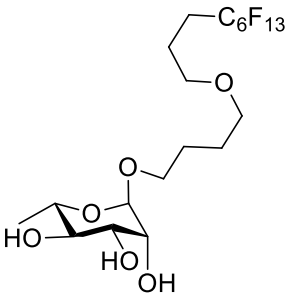

150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)


C6F13
O
O
O
AcO
AcO
OAc

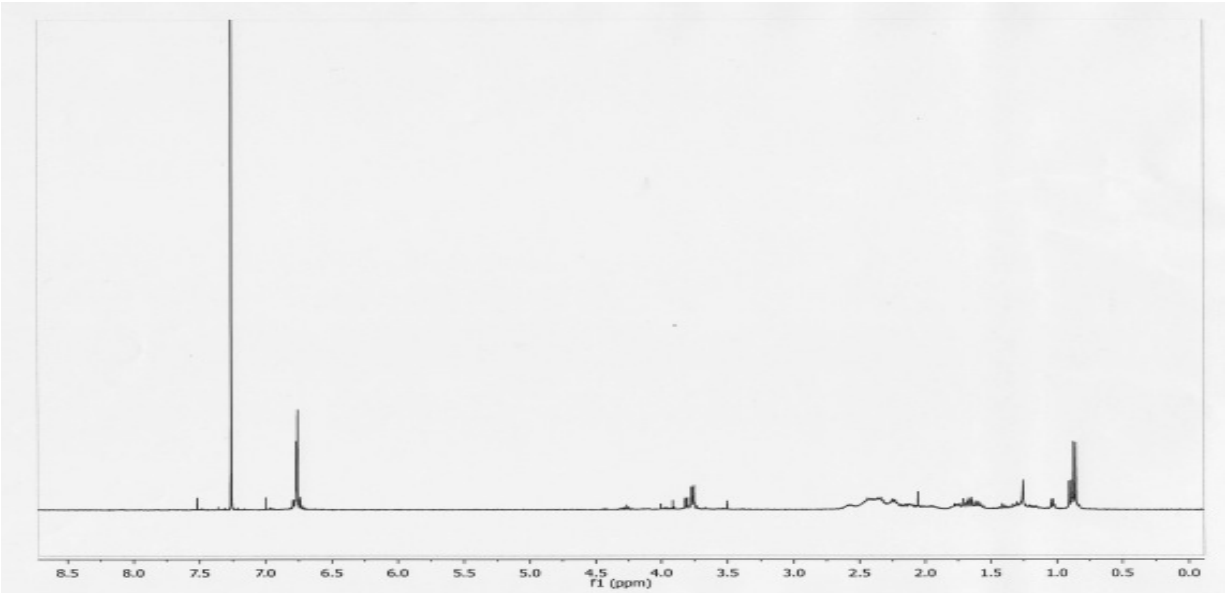
**20**

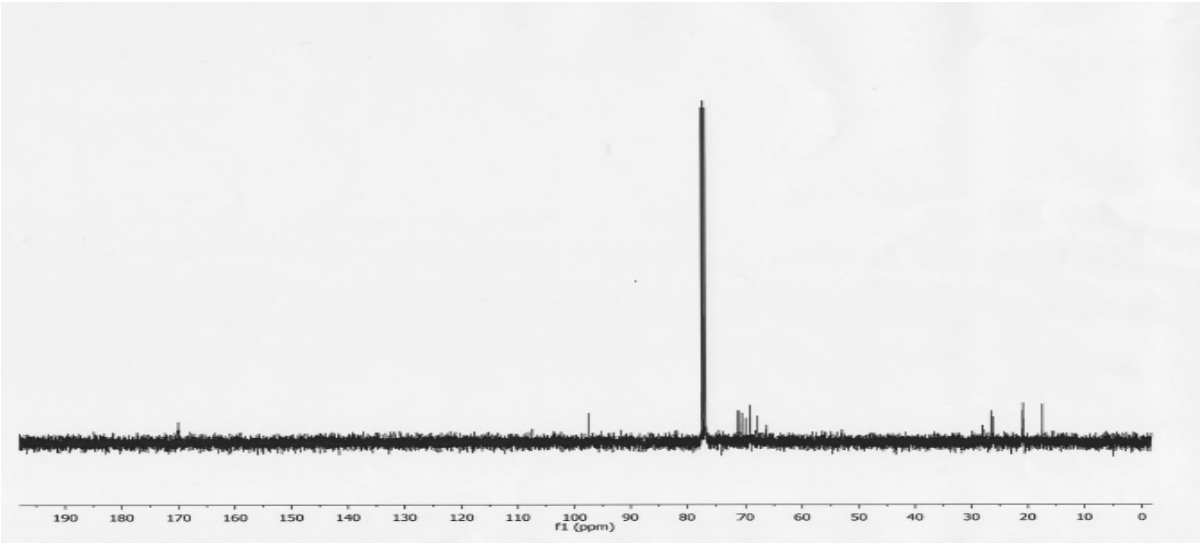

8
7.5
7
6.5
6
5.5
5
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
-0.5
f1 (ppm)

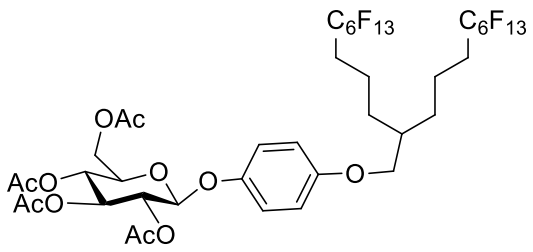

190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
f1 (ppm)


C6F13
O
O
O
HO
HO
OH

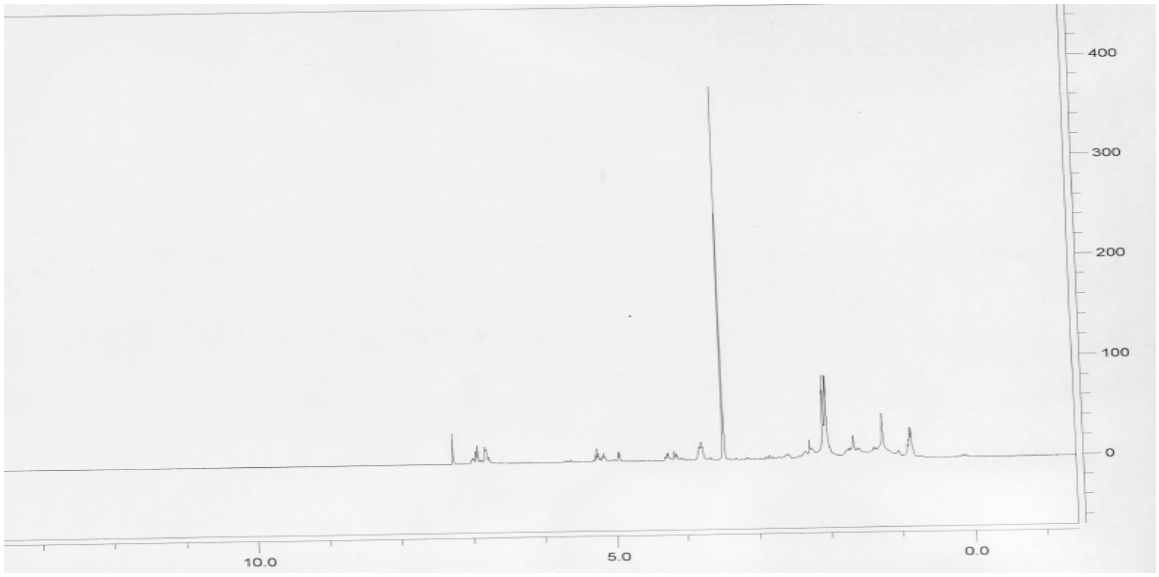
**21**

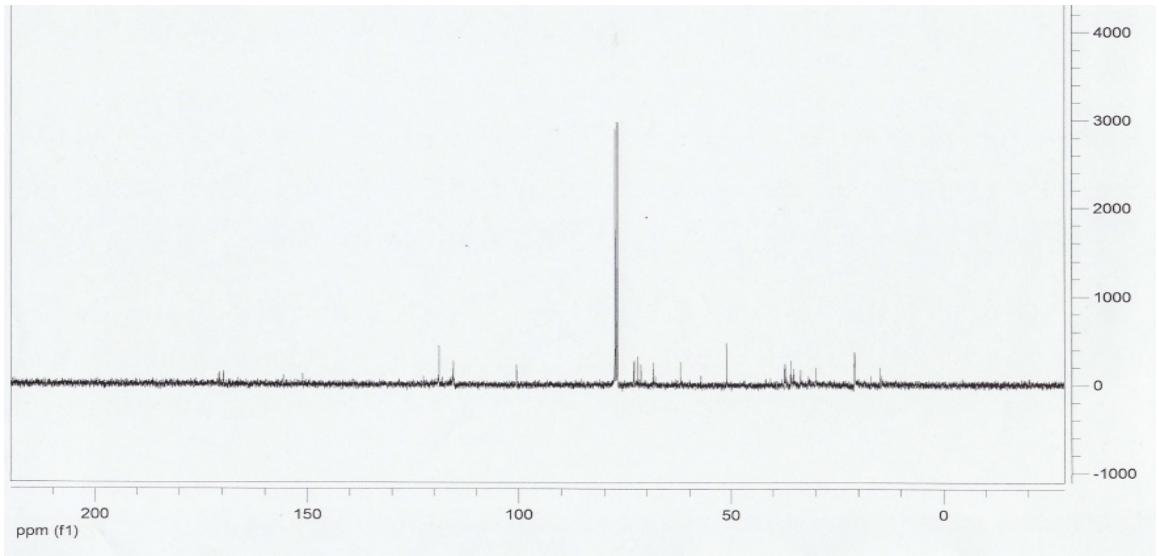

8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

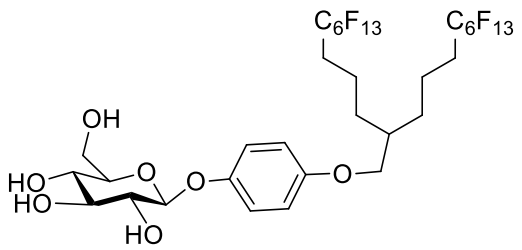

190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)


C6F13
C6F13
OAc
AcO
AcO
AcO
O
O
O

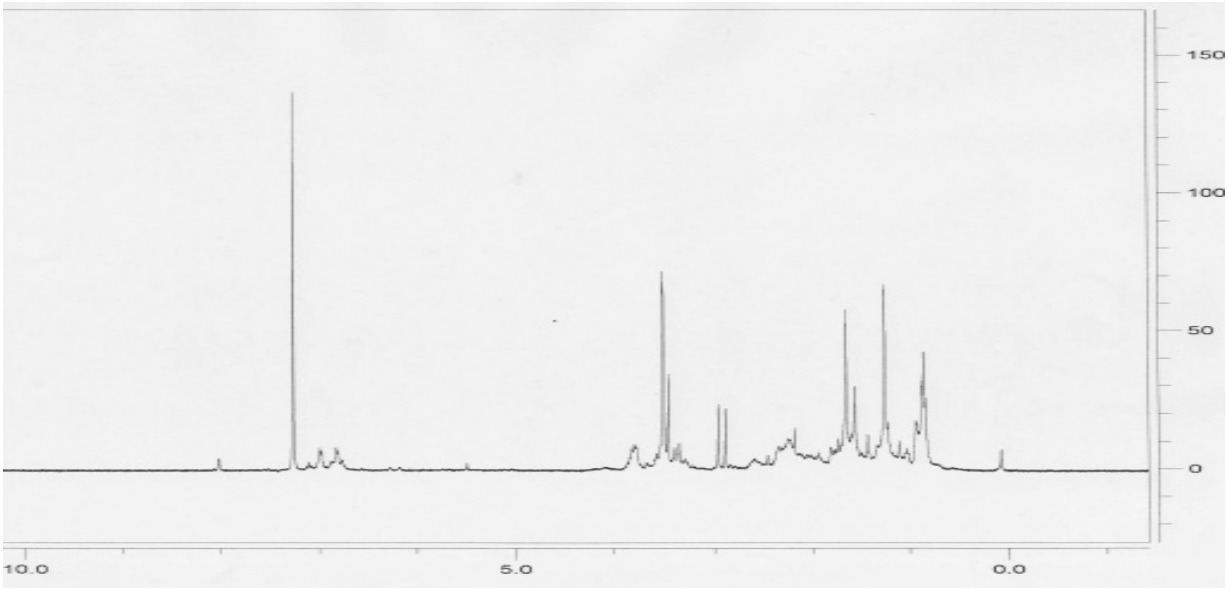
**23**

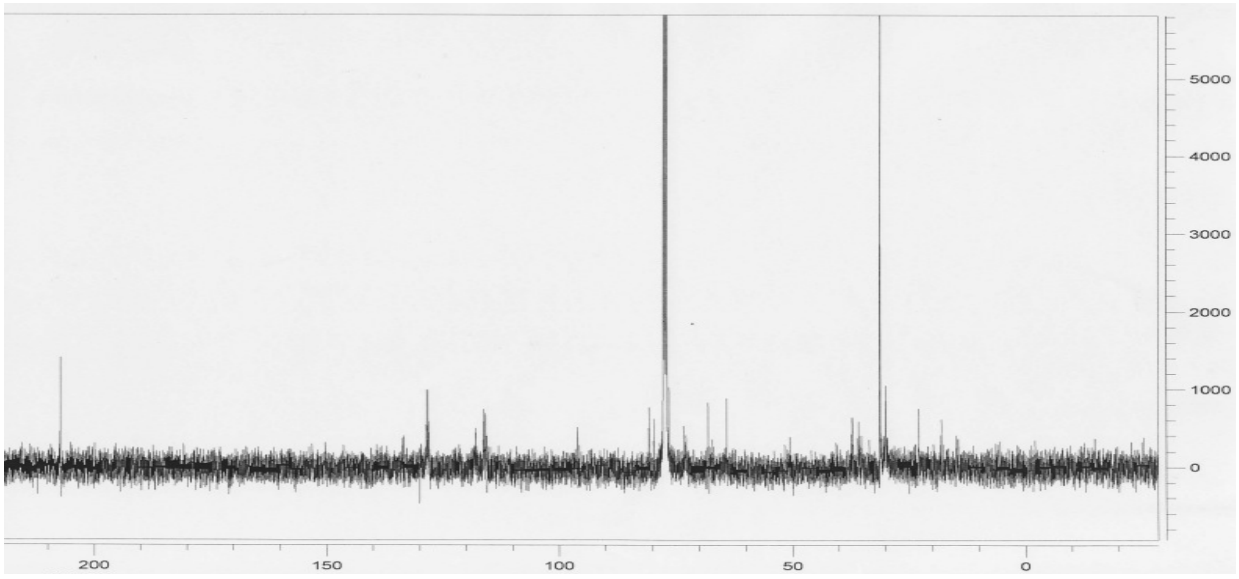

400
300
200
100
0
10.0
5.0
0.0

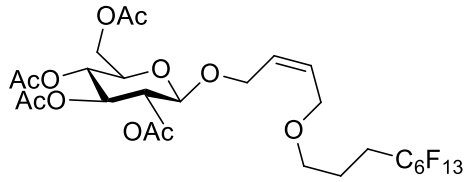

4000
3000
2000
1000
0
-1000
200
150
100
50
0
ppm (f1)



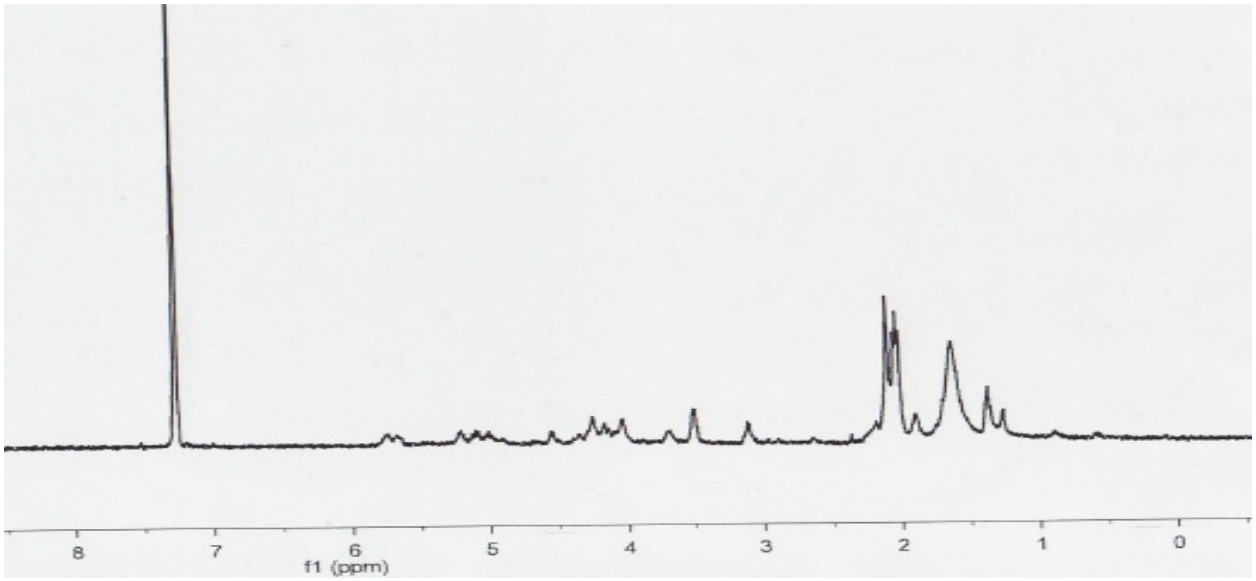

**24**

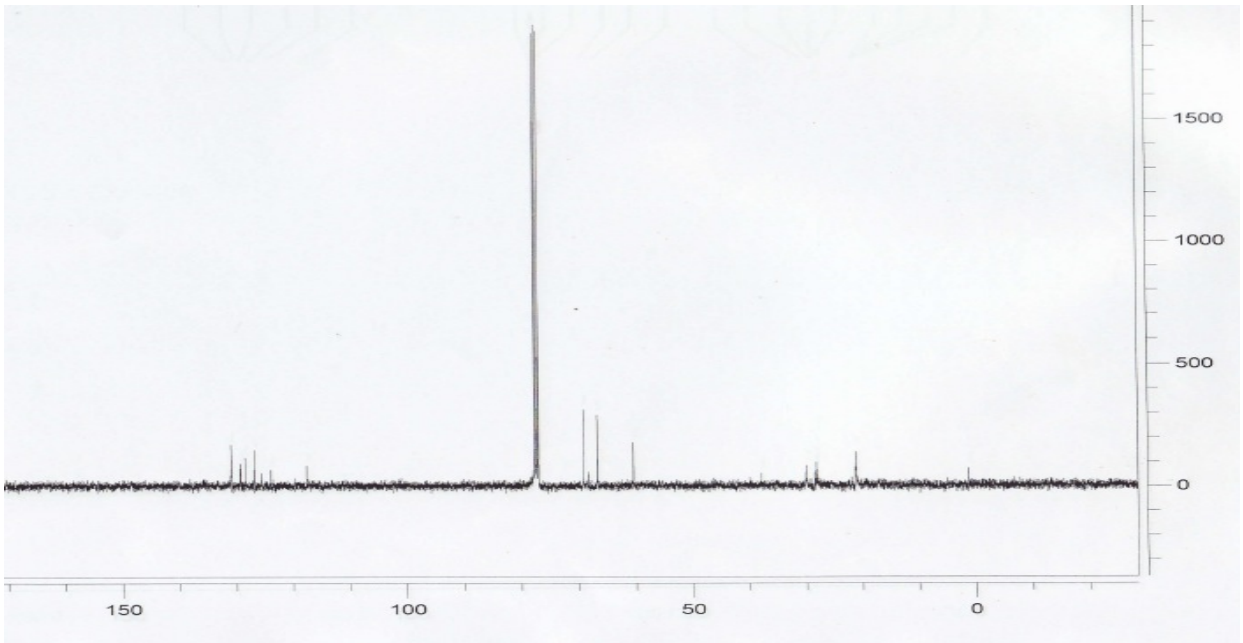


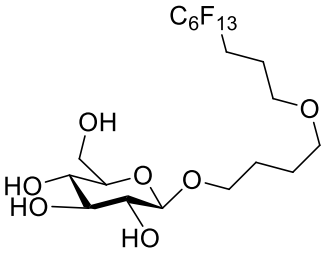




OAc
AcO
AcO
O
O
OAc
O
$C_6F_{13}$

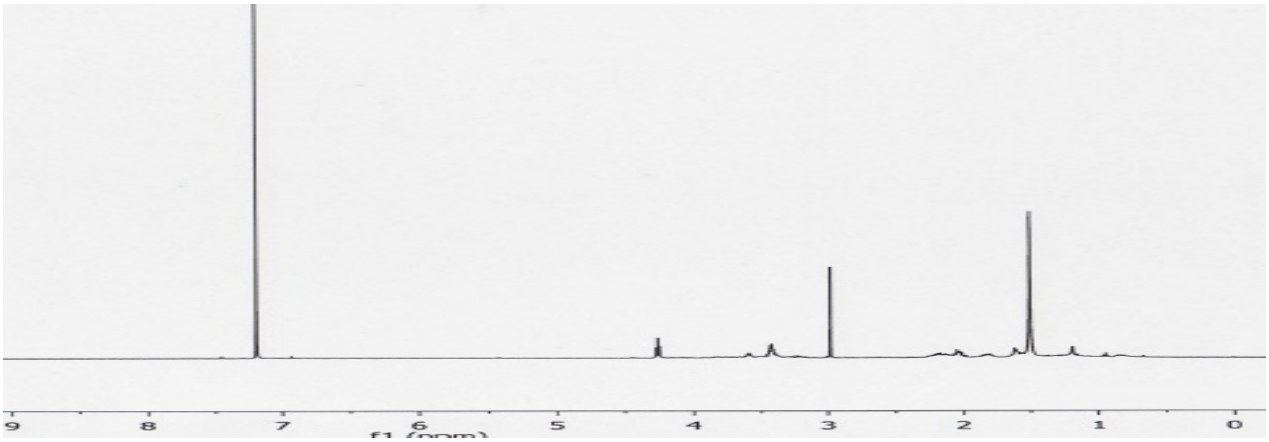
**25**

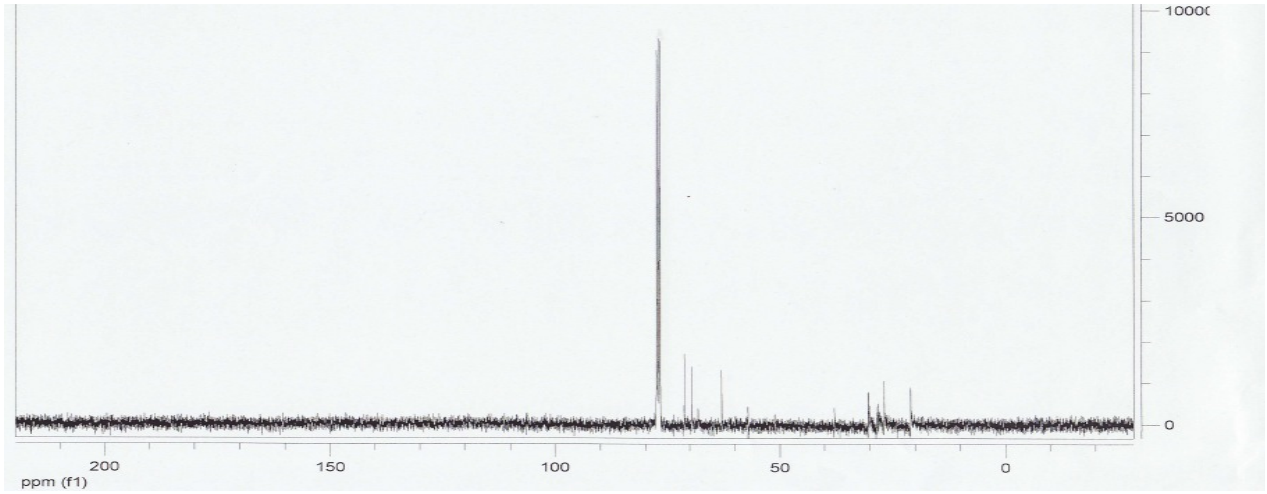

8
7
6
f1 (ppm)
5
4
3
2
1
0


1500
1000
500
0
150
100
50
0


C6F13
O
OH
HO
O
O
HO
HO

**26**


9
8
7
6
5
4
3
2
1
0


10000
5000
0
200
150
100
50
0
ppm (f1)