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Development of sustainable synthesis of glucuronic acid glycodendrimers using ball milling and microwave assisted CuAAC reaction

Cecilia García-Oliva,[‡] Alejandro Merchán, [‡] Almudena Perona, [‡] Pilar Hoyos, [‡] Ángel Rumbero[‖] and María J. Hernáiz[‡*]

[‡]Departament of Chemistry in wPharmaceutical Sciences, Faculty of Pharmacy, Complutense University of Madrid, 28040 Madrid (Spain).

[‖]Department of Organic Chemistry, Faculty of Sciences, Autonomous University of Madrid, 28049 Madrid (Spain)

[*]Email: mjhernai@ucm.es

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1. Materials and methods

Commercially available reagents were used without further purification.

- Catalyst ascorbate sodium salt (CAS 134-03-2, Alfa Aesar (Germany).
- $CuSO_4.5H_2O$ (CAS 7758-99-8, Sigma-Aldrich, St. Louis, MO, US)
- Silica gel 40-60 µm, 60 Å, 230-400 mesh (CAS 7631-86-9, Acros Organics).
- Silica gel pore size 30 Å, 100-200 mesh (CAS 112926-00-8, Sigma-Aldrich)
- Pre-coated silica gel 60 F254 aluminium sheets were purchased from Merck.
- Sodium methoxide (CAS 124-91-4, Sigma Aldrich, St. Louis, MO, US).
- Amberlyst® 15 (CAS 39389-20-3, Sigma-Aldrich, St. Louis, MO, US)
- Glycerol (CAS 56-81-5, Sigma Aldrich, St. Louis, MO, US).
- DMA (CAS 35123-06-9, Sigma-Aldrich, St. Louis, MO, US)
- Na_2EDTA dehydrate (CAS 6381-92-6, Sigma-Aldrich, St. Louis, MO, US).
- Ethyl acetate (CAS 141-78-6, Fisher Scientific).
- Methanol (CAS 141-78-6, Fisher Scientific).
- Dichloromethane (CAS 141-78-6, Fisher Scientific).
- Na_2SO_4 anhydrous (CAS 7757-82-6, Fisher Scientific).

Planetary Ball Mill (PBM) model PM 100 from Retsch®, with a SS jar (12mL) and SS balls (30 ball of Φ = 5 mm and 200 balls of Φ = 3 mm) were employed to conduct the mechanochemical studies (Figure S1a).

Microwave (MW) heating was performed in a microwave reactor (Monowave 50, 315 W, Anton Paar) (Figure S1b).

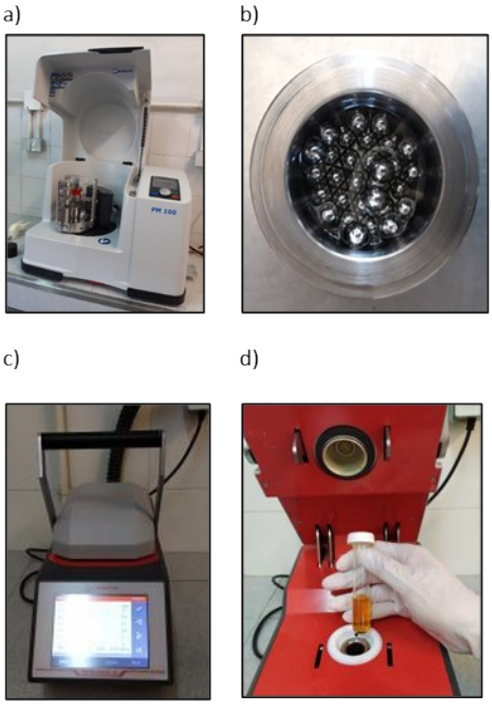


Figure S1. a) Planetary ball Mill (PBM); b) Stainless steel grinding jar and balls; c) Microwave (MW); d) Microwave vial.

NMR spectra were recorded at 293 K, with a 500 MHz spectrometer (Bruker AC). Shifts are referenced relative to deuterated solvent residual peaks. Complete signal assignments from 1D

and 2D NMR spectroscopy were based on COSY, HSQC, and HMBC correlation experiments. Spectra were consistent with previous references.[1]

2. Synthesis and Characterization of Glycodendrimers

Multivalent core scaffolds (compounds **1** and **6**) and glycosyl azide (compound **2**) were prepared as previously described in the literature from commercially available starting materials.[1]

2.1. Ball mill assisted synthesis of compound 4 and 3

Compound 4. Scaffold **1** (80 mg, 0.327 mmol), compound **2** (Table 1 entry 1-4: 300 mg, 0.720 mmol, 1.1 equiv./alkyne) or (Table 1 entry 5-7: 410 mg, 0.982 mmol, 1.5 equiv./alkyne), $CuSO_4.5H_2O$ (33.8 mg, 0.131 mmol, 0.2 equiv./alkyne) and sodium ascorbate (45.3 mg, 0.23 mmol, 0.35 equiv./alkyne) were introduced in a SS 12 mL jar in the presence of SS balls (200 Ø=3 mm ball or 30 Ø=5 mm balls). Filling the grinding jar with 1/3 of compounds, 1/3 of balls and 1/3 of free volume should be considered. 600 mg of silica gel were added as grinding auxiliar to reach the appropriate volume. Parameters such as speed (450 rpm), duration of each cycle (30 min), interval (4 min), inversion (every 4 min) and pause time (10 s) were selected. Intermediate additions were performed at t= 4 h and t= 8 h, incorporating each time **2** (273 mg, 0.654 mmol, 1 equiv./alkyne), $CuSO_4.5H2O$ (16.3 mg, 0.065 mmol, 0.1 equiv./alkyne) and sodium ascorbate (22.7 mg, 0.114 mmol, 0.175 equiv./alkyne). After each cycle of 30 min the system was readjusted, and samples were taken to follow the reaction by thin layer chromatography (TLC), using CH_2Cl_2/AcOEt 6:1 (v/v) as mobile phase to detect the substrates and CH_2Cl_2/MeOH 30:1 (v/v) to distinguish the products. When the reaction was finished (t= 11 h), compounds were dissolved in dichloromethane (30 mL) and washed with water (3x20 mL). The organic phase was dried with anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography, employing a solvent gradient from CH_2Cl_2/AcOEt 6:1 to CH_2Cl_2/MeOH 30:1 (v/v). After purification reaction yield was 65-70%. Unreacted substrate **2** was recovered unaltered to be reutilized in further reactions. Purified products were characterized by NMR.

In the optimization process, variations in speed (400-600), addition of solvent, equivalents of substrates and catalyst, frequency of addition and reaction times and ball sizes (200 Ø=3 mm ball and 30 Ø=5 mm balls) were performed (Table 1).

Structural determination was done by ^{1}H-NMR and ^{13}C-NMR (CDCl3, 500 MHz). Spectra were consistent with previous reference.[1]

Compound 3. It was isolated during the optimization process, monosubstituted product was founded in variable rates. It was purified by column chromatography.

2.2. Microwave assisted synthesis of compound 4, 3 and 7

Scaffold **1** (25 mg, 0.102 mmol) or **6** (72 mg, 0.128 mmol), was dissolved in 3 mL of biosolvent (glycerol or DMA1). Then, compound **2** (Table 2 entry 1-6: 94 mg, 0.224 mmol, 1.1 equiv./alkyne) or (Table 2 entry 7-10: 128 mg, 0.306 mmol, 1.5 equiv./alkyne), $CuSO_4{\cdot}5H_2O$ (10.2 mg, 0.0408 mmol, 0.2 equiv./alkyne) and sodium ascorbate (14.2 mg, 0.0714 mmol, 0.35 equiv./alkyne) were added to the solution and stirred at r.t. during 10 min. The resulting solution was irradiated at 80 °C for 90 min by using a MW reactor. After that time, the reaction carried out in DMA1 was diluted with 20 mL of ethyl acetate and washed with an aqueous solution of Na_2EDTA-NaOH

(2x20 mL) and brine (20 mL). When glycerol was used as biosolvent, dichloromethane (3x5mL) was added to extract the compounds from the glycerol. In both cases, the organic layer was dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by flash chromatography on silica gel (same procedure as BM explained above), obtaining **4** or **7** as white solids. After purification reaction yield was 80%. During the optimization process, temperature, reaction time and proportion of substrates and catalysts were evaluated (Table 2).

Compound 3. It was isolated in the optimization process. Data was consistent with the compound identified in ball mill reactions.

Compound 4. It was obtained as unique product (Table 7-10).

Structural determination was done by 1H-NMR and 13C-NMR (CDCl3, 500 MHz). Spectra were consistent with pre-vious reference.[1]

Compound 7. It was obtained as unique product.

Structural determination was done by 1H-NMR and 13C-NMR (CDCl3, 500 MHz). Spectra were consistent with previous reference.[1]

2.3. Preparation of compounds 5 and 8

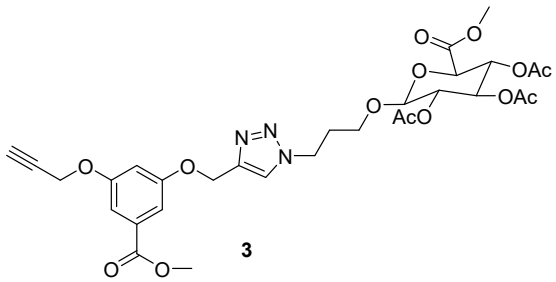
Peracetylated glycodendrimers (**4** and **7**) were dissolved in anhydrous methanol. Sodium methoxide (4 equiv.) was suspended in clean methanol and added dropwise over the previous solution. Stirring at 0 °C was maintained after complete consumption of the substrate. When the reaction was finished, it was quenched with Amberlite IRA-15. Then, filtration and solvent evaporation were performed. Compounds **5** and **8** were obtained as white solids after purification with silica gel in column chromatography, using CH_2Cl_2/MeOH/NH_3 (25%) (6:1:0.2).

Compound **5** was obtained with complete conversion. Structural determination was done by ^{1}H-NMR and ^{13}C-NMR ($CDCl_3$, 500 MHz). Spectra were consistent with previous reference.[1]

Compound **8** was obtained with complete conversion. Structural determination was done by ^{1}H-NMR and ^{13}C-NMR ($CDCl_3$, 500 MHz). Spectra were consistent with previous reference.[1]

3. Characterization and NMR Spectra

3.1. Characterization and ^{1}H and ^{13}C NMR spectra of compound 3



3

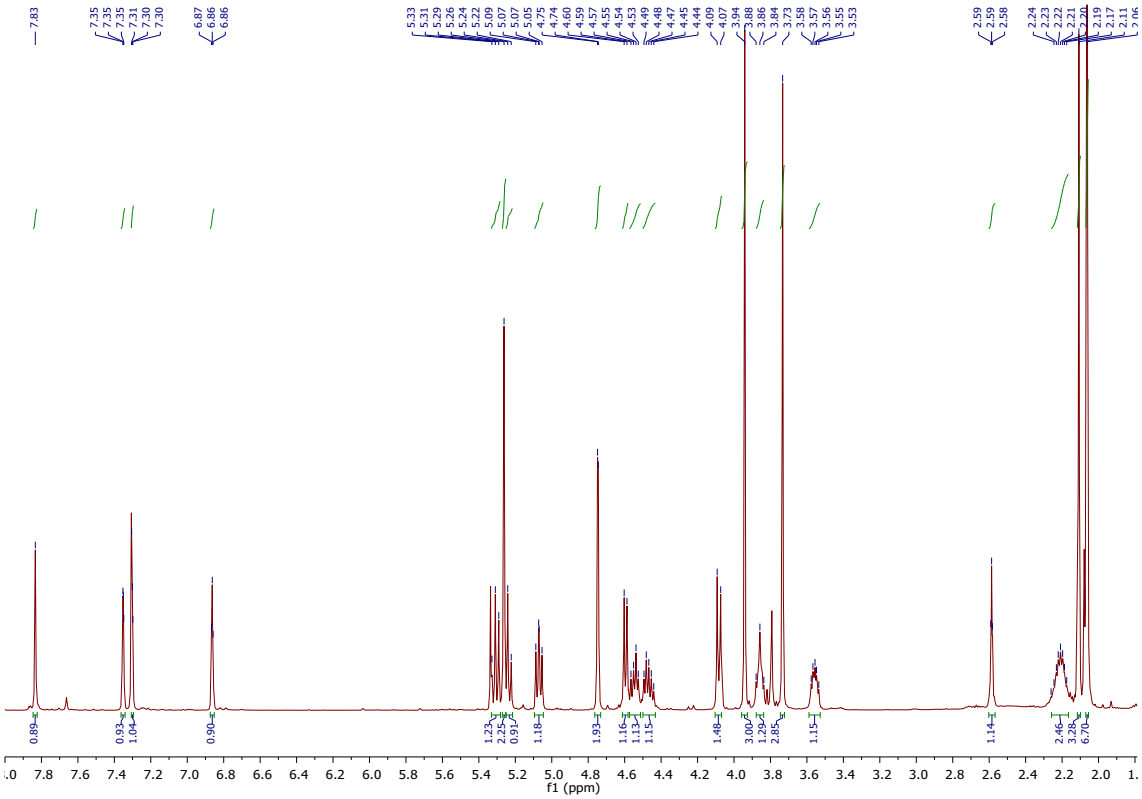
^{1}H-NMR (500 MHz, $CDCl_3$) δ (ppm): 7.83 (1H, s), 7.35 (1H, t, J= 2.4 Hz), 7.30 (1H, t, J= 2.4 Hz), 6.86 (1H, t, J= 2.4 Hz), 5.33 (1H, t, J= 9.4 Hz), 5.26 (2H, s), 5.24 (1H, t, J= 9.4 Hz), 5.07 (1H, dd, J= 9.4 Hz, J= 7.8 Hz), 4.75 (2H, d, J= 2.8 Hz), 4.60 (1H, d, J= 7.8 Hz), 4.6-4.5 (1H, m), 4.5-4.4 (1H, m), 4.08 (1H, d, J= 9.4 Hz), 3.94 (3H, s), 3.9-3.8 (1H, m), 3.73 (3H, s), 3.6-3.5 (1H, m), 2.58 (1H, t, J= 2.8 Hz), 2.30-2.15 (2H, m), 2.11 (3H, s), 2.06 (6H, s); ^{13}C-NMR (125 MHz, $CDCl_3$) δ (ppm): 170.1, 169.6,

169.5, 167.3, 166.6, 159.9, 158.7, 143.4, 132.3, 124.1, 109.0, 108.8, 107.5, 100.6, 78.2, 76.1, 72.4, 72.0, 71.3, 69.5, 65.8, 62.2, 56.2, 53.0, 52.5, 46.7, 30.2, 20.8, 20.7, 20.6.

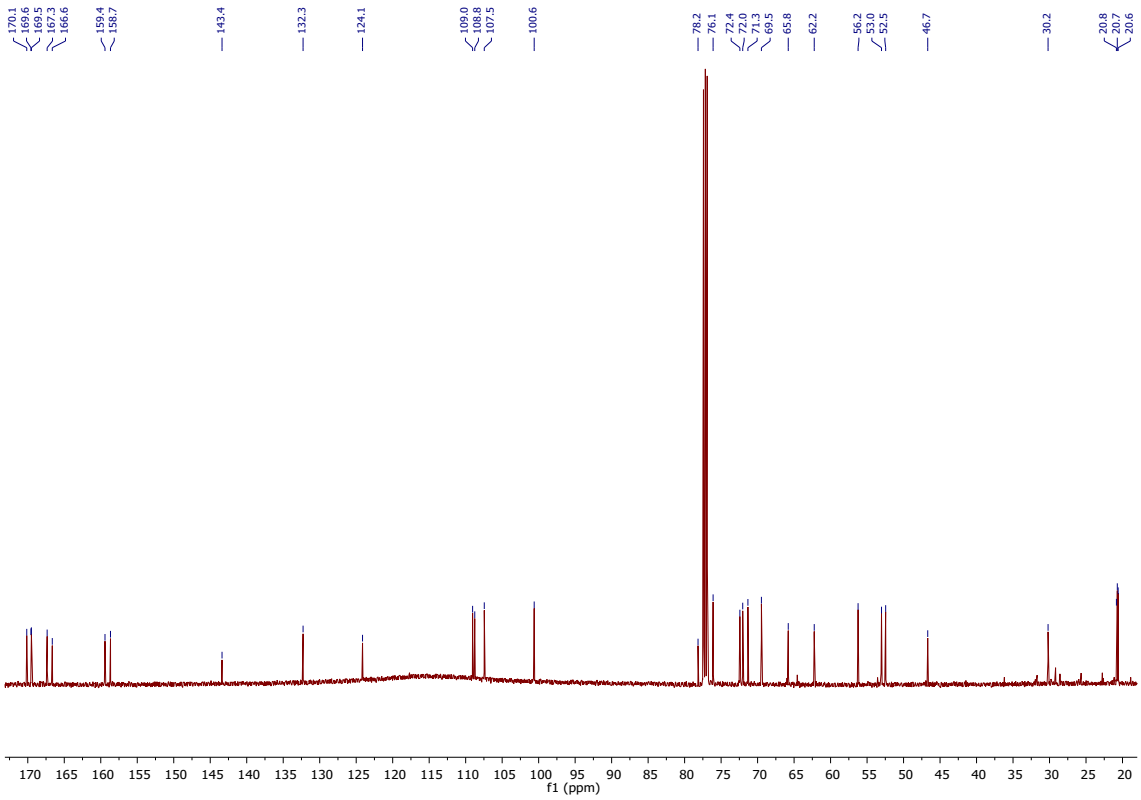
Anal. Calcd. for $C_{30}H_{35}N_3O_{14}$: C, 54.46; H, 5.33; N, 6.35; Found: C, 54.37; H, 5.32; N, 6.34.

HRMS for $[M+Na]^+$ $C_{30}H_{35}N_3O_{14}Na$ (m/z) calc 684.20; found 684.12

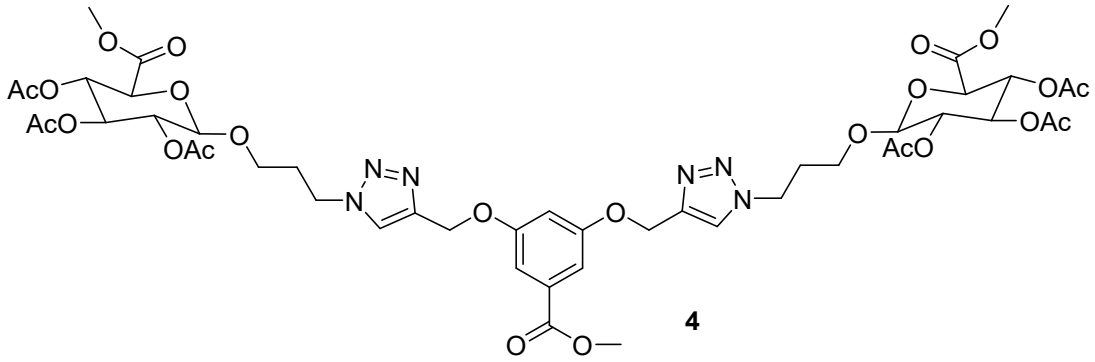
^{1}H-NMR Spectrum



^{13}C-NMR Spectrum



3.2 Characterization and ^{1}H and ^{13}C NMR spectra of compound 4 [1]



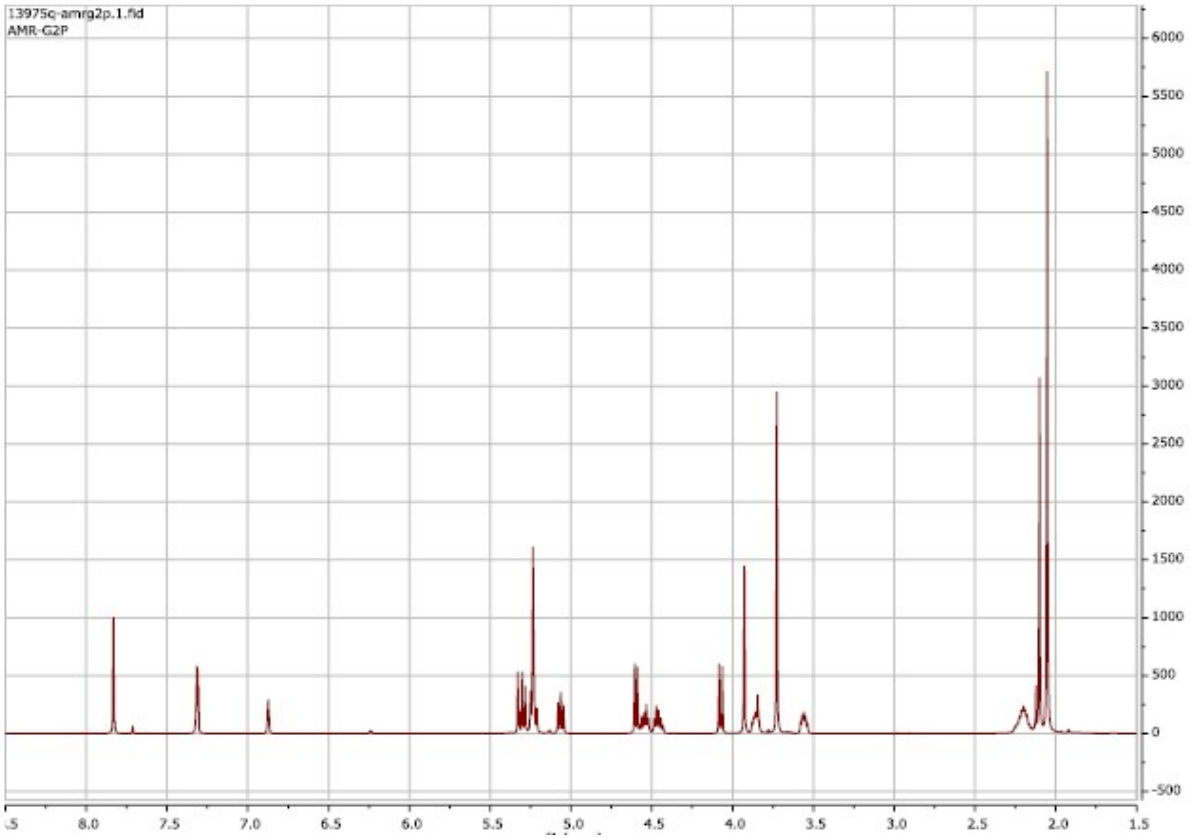
4

^{1}H-NMR (500 MHz, $CDCl_3$) δ (ppm): 7.77 (s br,2H, H-11), 7.25 (t, J=2.2 Hz, 2H,H-2 and H-6), 6.81 (t, J=2.2 Hz, 1H,H-4), 5.26 (t, J=9.6 Hz, 2H,H-3'), 5.24 (t, J=9.6 Hz, 2H,H-4'), 5.18 (s br,4H, H-9), 4.99 (dd, J=9.6, 7.7 Hz, 2H,H-2'), 4.54 (d, J=7.7 Hz, 2H,H-1'), 4.51–4.46 (m, 2H,H-12a), 4.43–4.37 (m, 2H,H-12b), 4.01 (d, J=9.6 Hz, 2H,H-5'), 3.87 (s, 3H,H-8), 3.82–3.78 (m, 2H,H-14a), 3.67 (s, 6H,H-7'), 3.52–3.48 (m, 2H,H-14b), 2.18–2.11 (m, 4H,H-13), 2.04 (s, 6H,OAc), 1.99 (s, 12H, OAc).^{13}C-NMR (125MHz,CDCl3) δ (ppm): 170.1 (CO), 169.5 (CO), 169.5 (CO), 167.4 (C-6'), 166.7 (C-7), 159.5 (C-3andC-5), 143.4 (C-10), 132.2 (C-1), 124.1 (C-11), 108.7 (C-2and C-6), 107.3 (C-4), 100.6 (C-1'), 72.4 (C-5'), 72.0 (C-3'), 71.3 (C-2'), 69.5 (C-4'), 65.8 (C-14), 62.2 (C-9), 53.0 (C-7'), 52.4 (C-8), 46.6 (C-12), 30.2 (C-13), 20.8 (AcO), 20.7 (AcO), 20.6 (AcO).

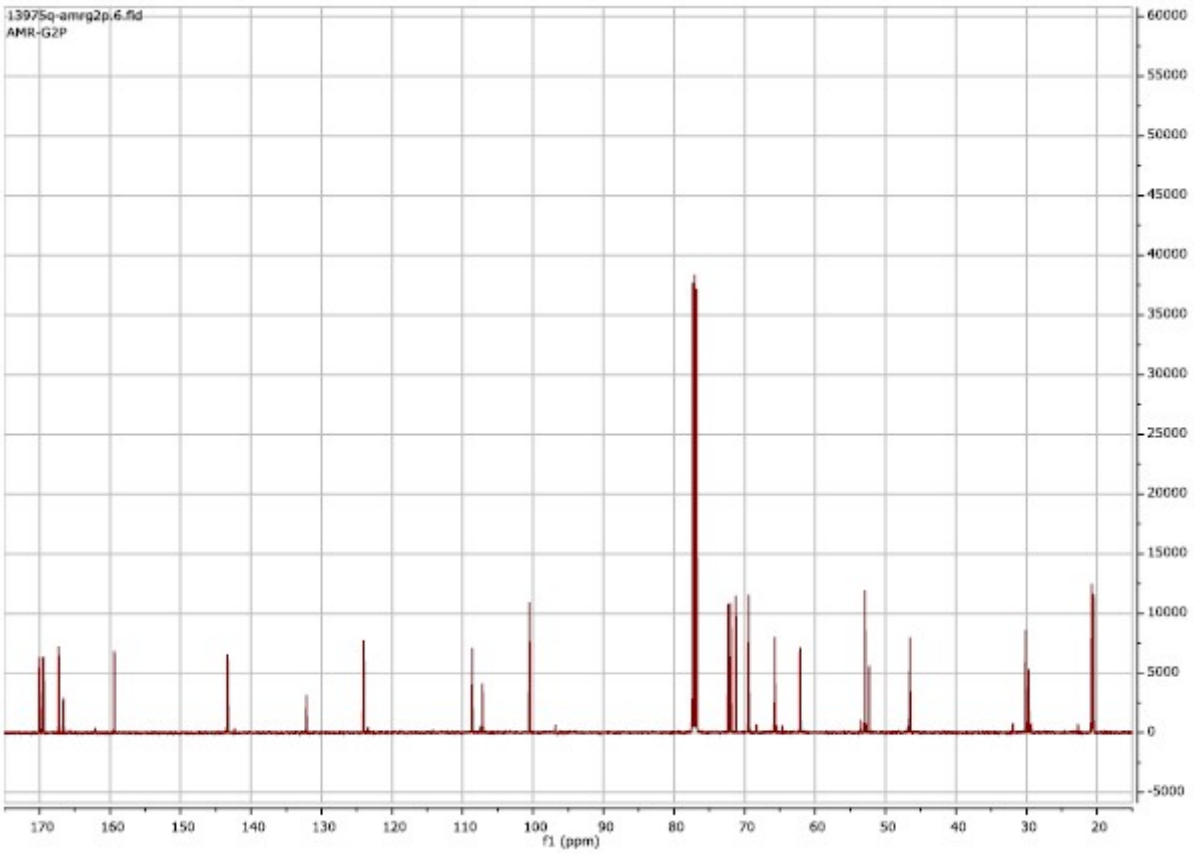
Anal. Calcd for $C_{46}H_{58}N_6O_{24}$:C,51.21; H,5.42; N,7.79. Found: C, 51.10; H,5.40; N,7.77

HRMS for $[M+Na]^+$ $C_{46}H_{58}N_6O_{24}Na$ (m/z) calc 1101,35; found 1101,15

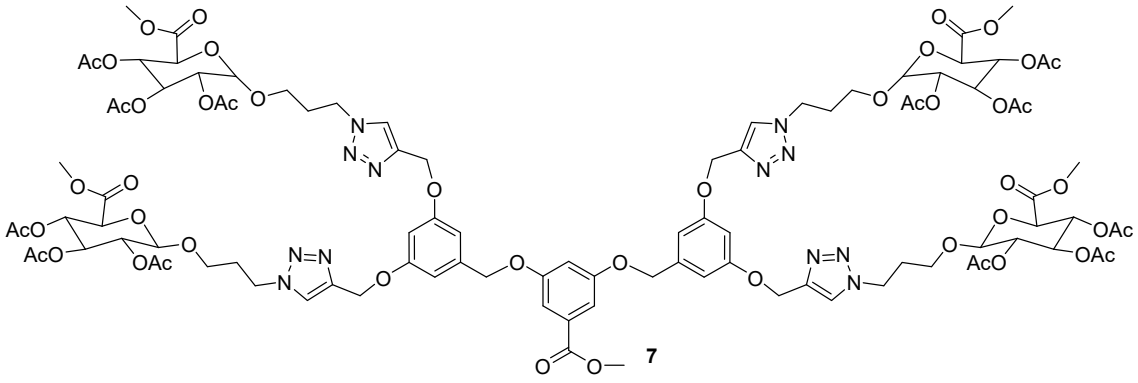
^{1}H-NMR Spectrum



^{13}C-NMR Spectrum



3.3 Characterization and ^{1}H and ^{13}C NMR spectra of compound 7 [1]

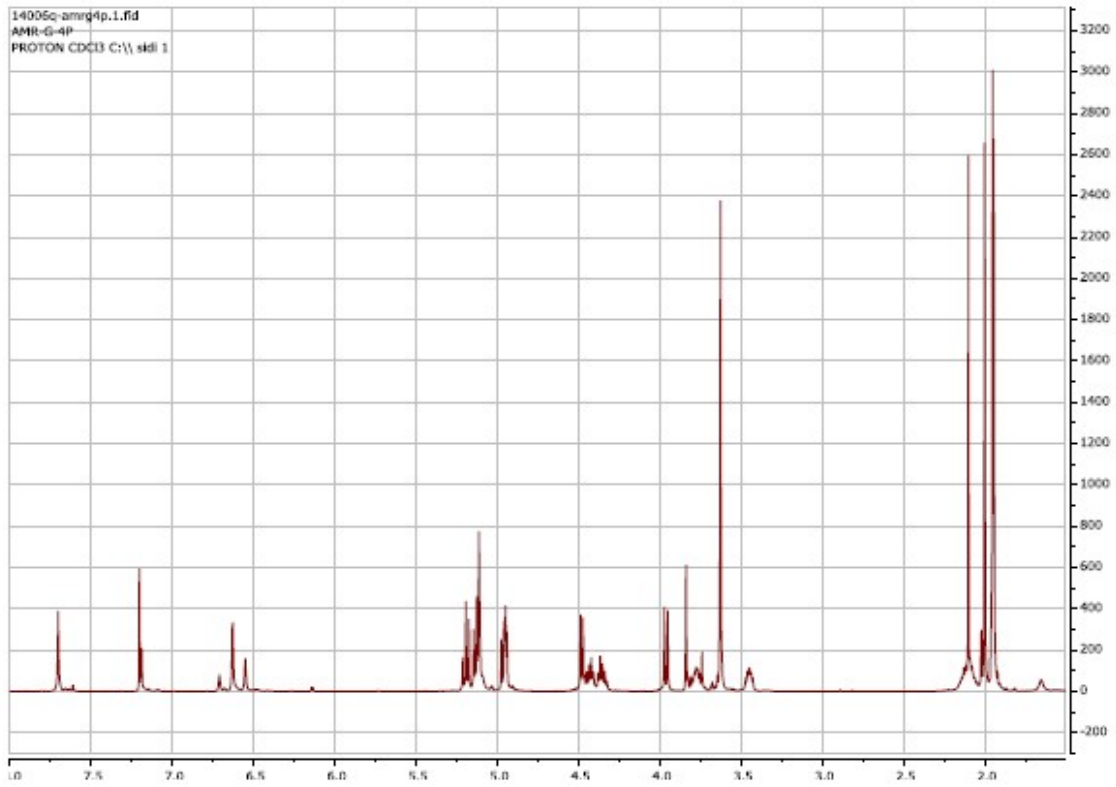


^{1}H NMR (500 MHz, CDCl3) δ (ppm): 7.77 (s br, 3H, H-9), 6.27 (s, 3H, H-2, H-4 and H-6), 5.25 (t, J=9.6 Hz, 3H, H-3'), 5.18 (t, J=9.6 Hz, 3H, H-4'), 5.12 (s br, 6H, H-7), 5.01 (dd, J= 9.6, 7.7 Hz, 3H, H-2'), 4.55 (d, J=7.7 Hz, 3H, H-1'), 4.52–4.46 (m, 3H, H-10a), 4.44–4.38 (m, 3H, H-10b), 4.02 (d, J=9.6 Hz, 3H, H-5'), 3.85–3.79 (m, 3H, H-12a), 3.75 (s, 9H, H-7'), 3.55–3.49 (m, 3H, H12b), 2.21–2.11 (m, 6H, H-11), 2.06 (s, 9H, AcO), 2.01 (s, 18H, AcO). ^{13}C NMR (125 MHz, CDCl3) δ (ppm): 170.1 (CO), 169.6 (CO), 169.5 (CO), 167.4 (C-6'), 160.2 (C-1, C-3 and C-5), 143.7 (C-8), 124.1 (C-9), 100.6 (C-1'), 95.2 (C-2, C-4 and C-6), 72.4 (C-5'), 72.0 (C-3'), 71.3 (C2'), 69.5 (C-4'), 65.9 (C-12), 61.9 (C-7), 53.0 (C-7'), 46.7 (C-10), 30.2 (C-11), 20.8 (AcO), 20.7 (AcO), 20.6 (AcO)

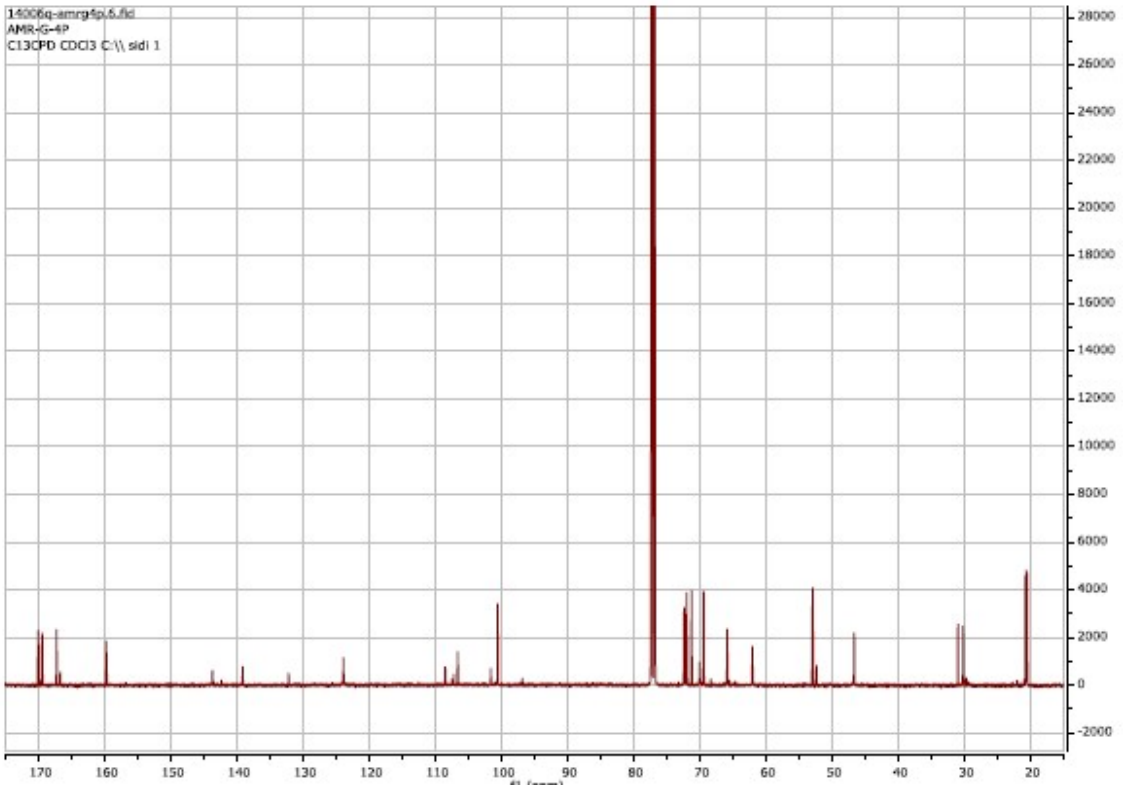
Anal. Calcd for $C_{98}H_{120}N_{12}O_{48}$:C,52.69; H,5.41; N,7.52. Found: C, 52.59; H,5.40; N,7.50

HRMS for $[M+Na]^{+}$ $C_{98}H_{120}N_{12}O_{48}Na$ (m/z) calc 2233,74; found 2233,57

^{1}H-NMR Spectrum

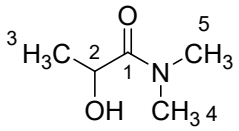


^{13}C-NMR Spectrum



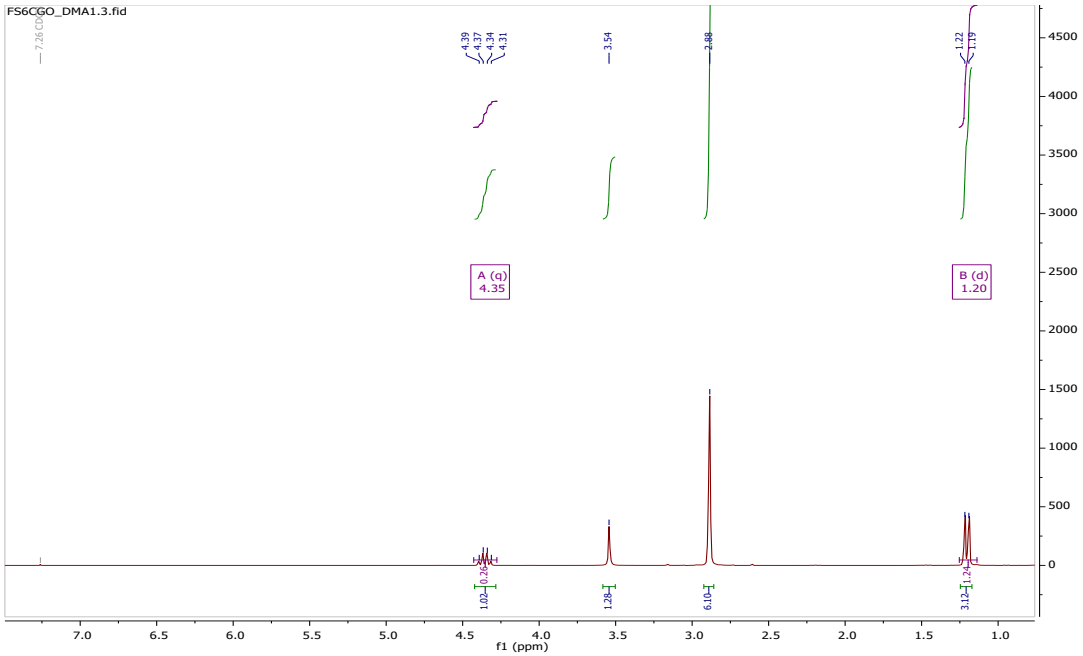
4. Biosolvent Recycling

The Na_2EDTA-NaOH solution, containing the biosolvent DMA and different salts, was evaporated under reduced pressure. The residue was collected in 20 mL of an ethyl acetate/EtOH (10:2) mixture. The organic phase containing the biosolvent and salts was filtered on a filter plate containing silica gel (pore size 30 Å, 100-200 mesh) to eliminate the salts and it was evaporated under vacuum to afford 95 % pure DMA. The biosolvent could be then recovered and reused in further reactions (Scheme 3). Structural determination was done by ^{1}H-NMR and ^{13}C-NMR ($CDCl_3$, 500 MHz).

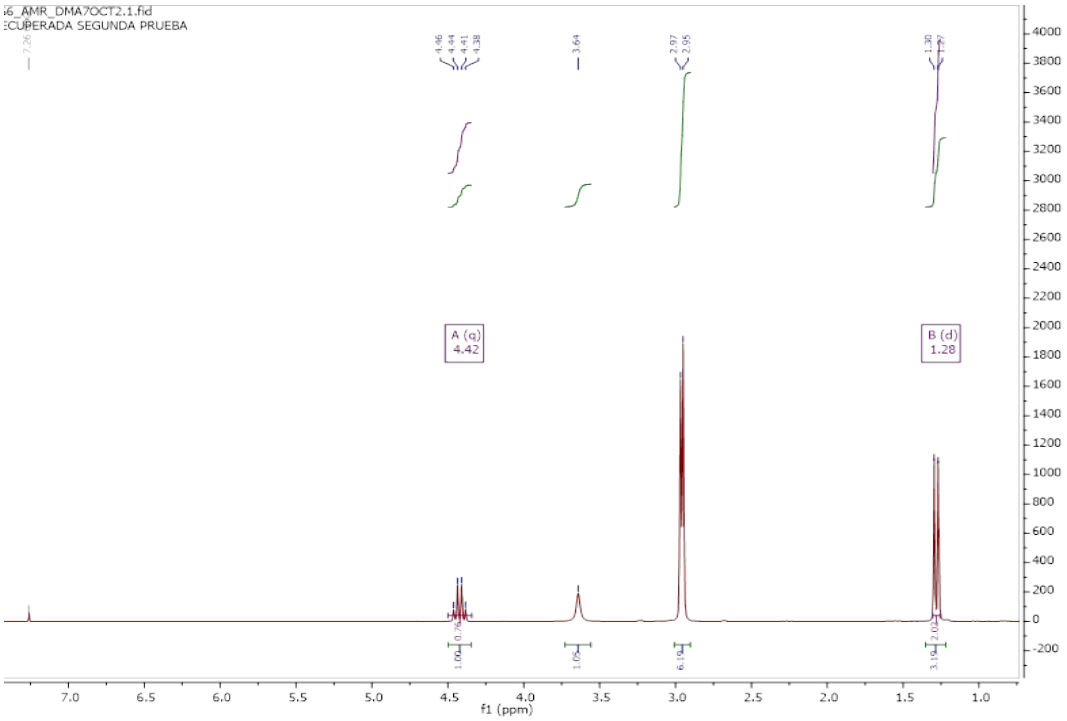


^{1}H NMR (500 MHz, CDCl3) δ (ppm): 4.35 (q, J = 6.6 Hz, 1H, H-2), 3.54 (s, 1H, OH), 2.88 (s, 6H, H-4 and H-5), 1.20 (d, J = 6.6 Hz,3H, H-3). ^{13}C NMR (125 MHz, CDCl3) δ (ppm): 174.9 (CO, C-1), 64.0 (C-2), 36.3 (CH_3-N), 35.7 (CH_3-N), 20.8 (C-3).

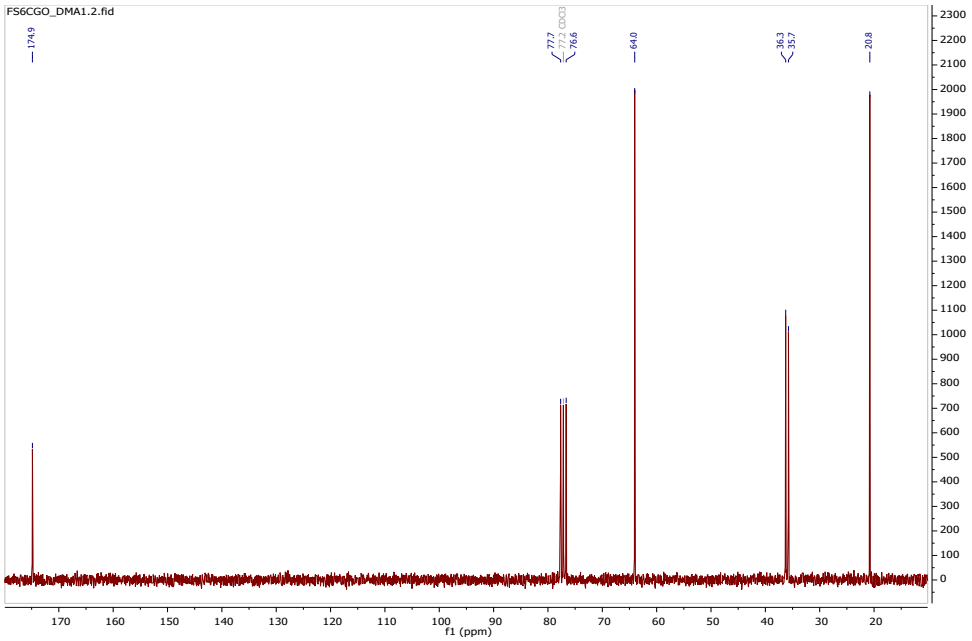
^{1}H-NMR Spectrum of DMA



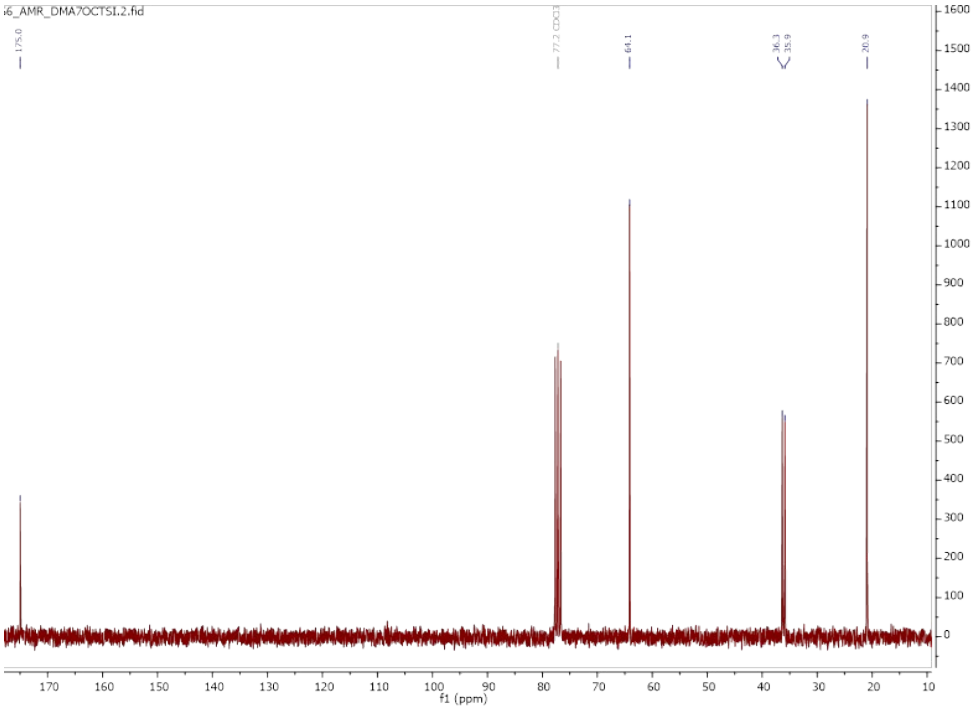
^{1}H-NMR Spectrum of recycled DMA



^{13}C-NMR Spectrum of DMA



^{13}C-NMR Spectrum of recycled DMA



4. References

1. García-Oliva, C.; Cabanillas, A. H.; Perona, A.; Hoyos, P.; Rumbero, Á.; Hernáiz, M. J., Efficient Synthesis of Muramic and Glucuronic Acid Glycodendrimers as Dengue Virus Antagonists. *Chem. Eur. J.* 2020, 26 (7), 1588-1596