A Concise Synthesis of Glycolipids Based on Aspartic Acid Building Blocks

Trinidad Velasco-Torrijos *, Lorna Abbey † and Roisin O’Flaherty †

Department of Chemistry, National University of Ireland Maynooth, Co. Kildare, Ireland

† These authors contributed equally to this work.

* Author to whom correspondence should be addressed; E-Mail: trinidad.velascotorrijos@nuim.ie; Tel.: +353-01-708-3747; Fax: +353-01-708-3815.

Received: 31 July 2012; in revised form: 15 September 2012 / Accepted: 21 September 2012 / Published: 25 September 2012

Abstract: L-Aspartic acid building blocks bearing galactosyl moieties were used to synthesise glycolipid mimetics of variable hydrocarbon chain length. The glycolipids were readily prepared through amide bond formation using the TBTU/HOBt coupling methodology. It was observed that, under these conditions, activation of the α-carboxylic acid of the intermediates led to near complete racemisation of the chiral centre if the reaction was carried out in the presence of a base such as triethylamine. The enantiomerically pure glycolipids were obtained after careful consideration of the synthetic sequence and by performing the coupling reactions in the absence of base.

Keywords: synthetic glycolipids; glycosylated amino acids; glycomimetics; aspartic acid; racemization

1. Introduction

Synthetic glycomimetics have been the subject of much research activity in the field of carbohydrate chemistry. The important role of carbohydrates in biological systems has prompted the development of different types of glycomimetics intended for diverse applications, such as therapeutic leads [1,2], novel materials [3], biosensors and diagnostic tools [4,5]. Glycolipid mimetics [6], in particular synthetic derivatives of biologically relevant ceramides (such as galactosyl ceramides, shown in Figure 1a), have attracted the attention of many carbohydrate chemists over recent years [7].
Amino acids that allow for side chain functionalization with glycosyl moieties, such as serine and aspartic acid, have been popular choices as the starting point for the preparation of glycolipid analogues [8,9]. The carboxylic acid present on the aspartic acid side chain offers the possibility for attachment of mono or oligosaccharides, while both the amino and carboxylic acid groups at the α-carbon allow for further functionalization. Due to the biological relevance of N-linked glycosides, this type of building blocks has been used predominantly in the synthesis of glycopeptides and glycopeptoids and hence, numerous examples of such compounds can be found in the literature [10–13]. In this study we report our investigations towards the synthesis of galactosylated building blocks based on: (i) orthogonally protected; (ii) enantiomerically pure and (iii) commercially available L-aspartic acid derivatives, as we intend to expand their application to the preparation of glycolipid mimetics. These non-natural glycolipids may be bioactive as neuroprotective agents [14] and/or may be used in materials or formulation science [15]. The nature of the building blocks should allow for a modular approach which could lead to the facile preparation of a small collection of glycolipids of different fatty acids chain lengths, such as 1–4, shown in Figure 1b. This feature of the glycolipid structure affects strongly its physicochemical characteristics, as well as its potential biological activity [16].

**Figure 1.** (a) Structure of the naturally occurring glycolipid β-galactosyl ceramide. (b) Structure of the galactosyl amines 5 [17,18] and 6 [19] and of the commercially available L-aspartic acid derivatives 7 and 8, used for the modular synthesis of glycolipid mimetics 1–4.
We have initially focused our attention on derivatives of decanoic acid (C-10), such as 1 and 3, and tetracosanoic acid (C-24), such as 2 and 4, as representative examples of medium and long fatty acid chain lengths. In glycolipids 1 and 2, the galactosyl moiety is connected to the aspartic acid by a flexible ethylene-type linker, while glycomimetics 3 and 4 resemble the native N-linked glycosides, as the acid conjugation occurs directly at the anomeric center. It is therefore expected that both sets of compounds would have different degrees of conformational freedom, which in turn may have an effect on their potential biological activities and physical properties.

2. Results and Discussion

Synthesis of the Glycolipids

Both the galactosyl amines 5 [17,18] and 6 [19] used in the syntheses described herein are readily prepared from D-galactose pentacetate following procedures described in literature. Our initial approach to the glycolipid mimetics 1–4 involved a convergent synthesis (Scheme 1), whereby the N-Boc-γ-benzyl ester protected L-aspartic acid 7 was coupled to tetradecylamine using standard TBTU/HOBt activation conditions in the presence of triethylamine. Subsequent removal of the N-Boc protecting group with TFA afforded the amine 9a, which was acylated with decanoic acid using the above mentioned TBTU/HOBt methodology. Hydrogenolysis of the side chain benzyl ester was carried out at 50 °C to enhance solubility and it afforded carboxylic acid 10a, which was then coupled to the primary amine of galactosyl derivative 6, to yield the acetyl protected glycolipid 11a. The 1H-NMR spectrum of 11a showed distinct duplication of every expected signal in a 1:1 ratio. To rule out possible conformational exchange equilibrium, variable temperature 1H-NMR spectra of compound 11a were recorded in d6-DMSO. No coalescence of the signals was observed at temperatures as high as 80 °C, which confirmed that glycolipid 11a was, in fact, a mixture of diastereoisomers.

Scheme 1. Synthesis of glycolipids 11a and 11b.

Reagents and conditions: (i) (1) TBTU, HOBt, C14H29NH2, NEt3, DMF, rt; (2) TFA, CH2Cl2, rt; 81% over 2 steps (9a), 61% over 2 steps (9b)*; (ii) (1) TBTU, HOBt, C10H20O2, NEt3, DMF, rt; (2) H2, Pd/C, EtOAc, 50 °C; 80% over 2 steps (10a), 47% over 2 steps (10b); (iii) TBTU, HOBt, 6, NEt3, DMF, 50 °C; 60% (11a), 23% (11b). * The coupling reaction was carried out in the absence of NEt3 for 9b.
The unexpected racemisation of the chiral α-carbon of the L-aspartic acid derivative 7 takes place in the first step of the synthesis. Although the use of TBTU and HOBr as coupling reagents is a very standard procedure in peptide synthesis [20], the activation of the α-carboxylic acid under these conditions is likely to increase the acidity of the α-proton in 7 and it may be abstracted in the presence of a base such as triethylamine. This is further supported by the disappearance of the optical activity of compound 9a \([\alpha]^{22}_D = 0\) (c 1.55, CHCl\(_3\)), while if the same coupling reaction is carried out in the absence of triethylamine, a specific optical rotation value is obtained for the L-enantiomer, compound 9b \([\alpha]^{22}_D = +2.5\) (c 1.55, CHCl\(_3\)). The effects on reaction yields and racemisation of the products, caused by different bases and activating reagents commonly used in peptide couplings, have been extensively reviewed in the literature [21]. Most of the published procedures reporting amide bond formation of N-Boc aspartic acid 7 involve the use of carbodiimide-type coupling reagents [22], formation of activated esters, such as pentafluorophenyl derivatives [23], or mixed anhydrides [24]. However, no compromise of the optical purity of the resulting aspartate derivatives when using uronium-type reagents (such as TBTU or HBTU) has been explicitly reported so far, to the best of our knowledge [25,26]. The mixture of D and L diastereoisomers of glycolipid 11a could not be separated by flash column chromatography or by recrystallization.

The same synthetic sequence as described above was carried out on the L-enantiomer 9b. Although this route allowed access to sufficient amounts of diastereomERICALLY pure 11b, we decided to investigate a different synthetic sequence that may result in an overall higher yield for the enantiomerically pure glycolipids, as outlined in Scheme 2. In the first step of the reviewed scheme, the free amino galactosyl derivative 6 was coupled to the N-Boc aspartic acid benzyl ester 8, which bears the free carboxylic acid at the side chain, to give the orthogonally protected compound 12. The benzyl ester on 12 was removed by hydrogenolysis and the resulting carboxylic acid at the α-carbon was then carefully reacted again with the TBTU/HOBt system, followed by the addition of tetradecylamine. To avoid racemisation of the chiral carbon in this crucial step, this reaction was carried out in the absence of base. Under these conditions, enantiomerically pure 13 was successfully obtained, albeit in a moderate yield (51% over two steps). This building block was then reacted with TFA to cleave the N-Boc group and the corresponding amine was acylated with pre-activated decanoic acid (stirred with TBTU/HOBt prior to addition) to lead to the protected glycolipid 11b. Acylation of the amine derived from 13 by treatment with TBTU/HOBt and tetracosanoic acid instead gave the longer C-24 compound 14. The hydrolysis of the acetyl protecting groups on the galactosyl moiety of both derivatives 11a and 14 was initially attempted following standard procedures, such as the Zemplén deprotection or reaction with hydrazine [27]. However, these conditions proved to be rather harsh, resulting in amide bond hydrolysis and degradation of the glycolipids. Enzyme catalysed acetylalysis was also considered, using both immobilized enzymes (such as CALB, Candida antarctica lipase [28], immobilized as Novazym 435) and soluble lipases (such as CRL, Candida rugosa lipase) [29]. These and many other lipases have been reported to chemoselectively achieve total or partial deacetylation of protected glycosides. However, the success of enzyme-catalysed reactions is often highly dependent on substrate structure, and we found that, perhaps due to the steric bulk imposed by the hydrocarbon chains, not even partial deacetylation of any of the glycolipids could be achieved. The deprotection of 11b and 14 was most successfully carried out with mild base catalysis in a
heterogenous mixture of triethylamine and dichloromethane/methanol/water at 40 °C, to give the corresponding glycolipids 1 and 2.

**Scheme 2.** Synthesis of O-glycolipids 1 and 2.

\[ \text{Scheme 2.} \]

\[ \text{Scheme 2.} \]

**Reagents and conditions:** (i) TBTU, HOBt, 6, NEt₃, DMF, rt; 76%; (ii) (1) H₂, Pd/C, EtOAc, rt; (2) TBTU, HOBt, C₁₂H₂₉NH₂, DMF, rt; 51% over 2 steps; (iii) (1) TFA, CH₂Cl₂, 50 °C; (2) TBTU, HOBt, C₁₀H₂₀O₂, DMF, rt; 47% over 2 steps (11b) or TBTU, HOBt, C₂₃H₄₇CO₂H, DMF, rt; 14% over 2 steps (14); (iv) NEt₃, CH₂Cl₂/MeOH/H₂O, 40 °C; 83% (1), 38%, (2).

Similar considerations regarding the preservation of the chirality of the L-aspartic acid asymmetric α-carbon were observed in the reviewed syntheses of the anomeric N-linked glycolipid analogues 3 and 4 (Scheme 3). In this case, the synthesis starts again with the direct coupling of galactosyl amine 5 with the N-Boc aspartic acid benzyl ester 8 to give building block 16 [30]. In order to avoid side reactions due to the increased acidity of the resulting anomic amide, the reaction was carried out with TBTU/HOBt but in the absence of an added base. The next step involved the removal of the N-Boc group of 15 with TFA, and the corresponding amine was acylated with either pre-activated decanoic or tetracosanoic acid, to give the corresponding intermediates 16 and 17, respectively. It was expected that the presence of the long hydrocarbon chains would introduce steric hindrance and minimize the risk of intramolecular cyclization to yield aspartimide-type by-products when attempting the coupling of the α-carboxylic acid, as this is a well known side reaction in glycopeptides and glycoprotein synthesis [31,32]. Indeed, after 16 and 17 underwent hydrogenolysis, the corresponding carboxylic acids were subjected to reaction with tetradeylamine mediated by TBTU/HOBt to give the enantiomerically pure glycolipids 18 and 19, and no significant formation of cyclic products could be observed. Since Zemplén deacetylation may have involved too harsh conditions for the final deprotection of the glycolipids 11b and 14 described earlier, we used again the mildly basic hydrolysis method described above to access derivatives 3 and 4. It must be noted that the solubility of the C-24 tetracosanoic acid derivatives, 2 and 4 is very poor (both in water and in most common solvents), when compared to that of the C-10 decanoic glycolipids 1 and 3. This is likely to hamper potential applications of the longer chain analogues.
Scheme 3. Synthesis of N-glycolipids 3 and 4.

Reagents and conditions: (i) TBTU, HOBt, DMF, rt; 72%; (ii) (1) TFA, CH₂Cl₂, rt; (2) TBTU, HOBt, C₁₂H₂₄O₂, DMF, rt; 63% over 2 steps (16) or TBTU, HOBt, C₂₃H₄₇CO₂H, DMF, 50 °C; 46% over 2 steps (17); (iii) (1) H₂, Pd/C, EtOAc, rt; (2) TBTU, HOBt, C₁₄H₂₉NH₂, DMF, rt; 34% over 2 steps (18) or (1) H₂, Pd/C, EtOAc, rt; (2) TBTU, HOBt, C₁₄H₂₉NH₂, DMF, rt; 25% over 2 steps (19); (iv) NEt₃, CH₂Cl₂/MeOH/H₂O, 40 °C; 21% (3), 79% (4).

3. Experimental

General Methods

All chemicals purchased were reagent grade and used without further purification unless stated otherwise. Dichloromethane was freshly distilled over CaH₂ prior use. Anhydrous dimethylformamide (DMF) was purchased from Sigma Aldrich. Molecular sieves (MS) used for glycosylation and coupling reactions were 8–12 mesh and were flame dried prior to use. Reactions were monitored with thin layer chromatography (TLC) on Merck Silica Gel F₂₅₄ plates, using mixtures of hexane/ethyl acetate unless otherwise stated. Detection was effected either by visualisation in UV light and/or charring in a mixture of 5% sulphuric acid-EtOH or phosphomolybdic acid-EtOH. NMR spectra were obtained on a Bruker Avance 300 spectrometer. Proton and carbon signals were assigned with the aid of 2D-NMR experiments and DEPT experiments for novel compounds. The 2D-NMR experiments included COSY and HCCOSW, which is an HSQC type of experiment. Better resolution of the signals was observed when using the HCCOSW experiments than with conventional HSQC experiments. Chemicals shifts for ¹H-NMR are reported in ppm relative to residual solvent proton. Flash chromatography was performed with Merck Silica Gel 60, using adjusted mixtures of hexane/ethyl acetate unless otherwise stated. Optical rotations were obtained using an AA-100 polarimeter. [α]²⁵ values are given in 10⁻¹ cm²·g⁻¹. The melting points were obtained using a Stuart Scientific SMP1
melting point apparatus and are uncorrected. High resolution mass spectrometry (HRMS) were performed on an Agilent-LC 1200 Series coupled to a 6210 Agilent Time-Of-Flight (TOF) mass spectrometer equipped with an electrospray source both positive and negative (ESI+/−) or in a MALDI-QTOF Premier MS SYSTEM, using an α-cyano-4-hydroxy cinnamic acid matrix. Infrared spectra were obtained as a film on NaCl plates in the region 4000–400 cm−1 on a Nicolet Impact 400D spectrophotometer.

N4-[2-O-(2,3,4,6-Tetra-O-acetyl-β-D-galactopyranosyl)-ethyl]-N2-tert-butoxycarbonyl-L-asparagine benzyl ester (12). HOBT (0.09 g, 0.68 mmol), followed by NEt3 (0.18 mL, 1.23 mmol), were added to a stirring solution of N-Boc-L-Asp-OBn 8 (0.2 g, 0.61 mmol) and TBTU (0.22 g, 0.6 mmol) dissolved in anhydrous DMF (10 mL), under N2 at rt. It was stirred for 30 min and 6 (0.29 g, 0.74 mmol) dissolved in anhydrous DMF (1.2 mL) was added dropwise. It was stirred for 18 h. The reaction mixture was concentrated under reduced pressure, diluted with ethyl acetate and washed successively with HCl 0.1 N, aqueous sat. NaHCO3 solution, and brine. Flash chromatography (hexane:ethyl acetate, 1:1) afforded 12 as a white solid (0.33 g, 76%). [α]22 D +6.9 (c 1.35, CH2Cl2); IR (NaCl film): 3374.7, 2978.0, 1750.7, 1665.8, 1499.3, 1368.8, 1224.3, 1167.9, 1124.3,1057.2 cm−1; 1H-NMR (300 MHz, CDCl3): δ 7.34 (bs, 5 H, H-Ph), 6.01 (t, J = 5.1 Hz, 1 H, CH2CH2NHCO), 5.76 (d, J = 8.1 Hz, 1 H, NHCOC(CH3)3), 5.39 (dd, J =0.6 Hz, J = 3.3 Hz, 1 H, H-4), 5.20–5.16 (m, 3 H, overlap of H-2, CH2Ph), 5.02 (dd, J = 3.3 Hz, J = 10.2 Hz, 1 H, H-3), 4.57–4.54 (m, 1 H, H-α), 4.44 (d, J = 7.8 Hz, 1 H, H-1), 4.18–4.13 (m, 2 H, overlap of H-6, H-6′), 3.93–3.89 (m, 1 H, H-5), 3.86–3.80 (m, 1 H, 1 H of OCH2CH2NH), 3.66–3.59 (m, 1 H, 1 H of OCH2CH2NH), 3.46–3.38 (m, 2 H, OCH2CH2NH), 2.91 (dd, J = 5.7 Hz, J = 17.4 Hz, 1 H, H-β), 2.71 (dd, J = 4.5 Hz, J = 15.9 Hz, 1 H, H-β), 2.15 (s, 3 H, O(CO)CH3), 2.05 (s, 6 H, O(CO)CH3 × 2), 1.99 (s, 3 H, O(CO)CH3), 1.42 (s, 9 H, COC(CH3)3); 13C-NMR (75 Hz, CDCl3): δ 171.38, 170.37, 169.76 (each CO), 155.55 (C-Ph), 128.51, 128.09 (CH-Ph), 101.42 (C-1), 79.01 (COC(CH3)3), 70.89 (C-5), 70.68 (C-2), 68.91 (C-3), 67.25 (CH2Ph), 66.96 (C-4), 61.35 (C-6), 50.47 (C-α), 39.18, 37.72 (each OCH2CH2NH), 37.12 (C-β), 28.29 (COC(CH3)3), 20.82, 20.57 (overlap of O(CO)CH3); HRMS (MS-TOF): [M+H]+ calcd. for C32H44N2O15: 697.2181 found 697.2800.

N4-[2-O-(2,3,4,6-Tetra-O-acetyl-β-D-galactopyranosyl)-ethyl]-N2-tert-butoxycarbonyl-L-asparagine tetradeaclylamine (13). To a solution of 12 (0.120 g, 0.17 mmol) in ethyl acetate (6 mL), Pd/C 10% w/w (0.012 g, 10% w/w) was added. The resulting slurry was stirred under H2 gas for 4 h. The mixture was then filtered through a Celite cake and the filtrate was concentrated under vacuum to afford the corresponding carboxylic acid as an off-white solid, which was used without further purification (0.094 g, 90%). 1H-NMR (300 MHz, CDCl3): δ 6.53 (bs, 1 H, CH2CH2NHCO), 5.86 (d, J = 5.4 Hz, 1 H, NHCOC(CH3)3), 5.41 (d, J = 3.3 Hz, 1 H, H-4), 5.17 (dd, J = 7.8 Hz, J = 10.2 Hz, 1 H, H-2), 5.00 (dd, J = 3.3 Hz, J = 13.8 Hz, 1 H, H-3), 4.49 (d, J = 7.8 Hz, 1 H, H-1), 4.45–4.42 (m, 1 H, H-α), 4.20–4.11 (m, 2 H, overlap of H-6, H-6′), 3.96–3.92 (m, 1 H, H-5) 3.90–3.85 (m, 1 H, 1 H of OCH2CH2NH), 3.75–3.68 (m, 1 H, 1 H of OCH2CH2NH), 3.54–3.39 (m, 2 H, OCH2CH2NH), 2.91 (d, J = 15.6 Hz, 1 H, H-β), 2.72 (dd, J = 8.49 Hz, J = 15.9 Hz, 1 H, H-β), 2.17, 2.13, 2.05, 1.98 (each s, 3 H, O(CO)CH3), 1.42 (s, 9 H, COC(CH3)3); HRMS (MS-TOF): [M+K]+ calcd. for C25H38N2O15: 645.1904 found 645.1899. HOBt (0.034 g, 0.25 mmol) was added to a stirring solution of the carboxylic acid obtained as described above (0.140 g, 0.23 mmol), tetradeaclylamine (0.06 g, 0.28 mmol), and TBTU (0.081 g,
0.25 mmol) dissolved in anhydrous DMF, (12 mL) at rt. It was stirred for 18 h. The reaction mixture was concentrated in vacuo, diluted with ethyl acetate and washed with brine. Flash chromatography (ethyl acetate) afforded 11b as a white solid (0.15 g, 63%). \([\alpha]^{22}_D = +8.8 (c 0.75, \text{CH}_2\text{Cl}_2); IR (\text{NaCl film}): 3289.5, 3098.3, 2919.3, 2850.8, 1750.8, 1681.1, 1646.5, 1542.4, 1467.4, 1370.4, 1225.5, 1174.9, 1058.5 \text{ cm}^{-1}; ^1\text{H}-\text{NMR} (300 \text{ MHz, CDCl}_3): \delta 7.43 (d, J = 6.9 Hz, 1 H, NHCO\text{C}_9\text{H}_19), 7.08 (t, J = 5.4 Hz, 1 H, \text{OCOC}_9\text{H}_19)\].

\[N^\text{d}-(2-O-(2,3,4,6-Tetra-\text{O}-acetyl-\text{D}-\text{galactopyranosyl})-\text{ethyl})-\text{N}^2-\text{decanoyl-L-asparagine tetradecylamide} \ (11b)\]. A solution of 13 (0.11 g, 0.13 mmol) in anhydrous CHCl\text{3}, (6 mL) was cooled in an ice bath and TFA (0.15 mL, 1.37 mmol) was added. The reaction mixture was heated to 50 °C for 1.5 h. The reaction mixture was concentrated in vacuo, diluted with ethyl acetate, washed with brine, dried (MgSO\text{4}) and concentrated. The residue obtained was purified by flash chromatography (ethyl acetate) to afford 11b as a white solid (0.12 g, 56%). [\(\alpha\)]\text{D} = +5.8 (c 0.8, CH\text{2Cl}_2); IR (\text{NaCl film}): 3289.5, 3098.3, 2919.3, 2850.8, 1750.8, 1681.1, 1646.5, 1542.4, 1467.4, 1370.4, 1225.5, 1174.9, 1058.5 cm\text{ }^{-1}; ^1\text{H}-\text{NMR} (300 \text{ MHz, CDCl}_3): \delta 7.43 (d, J = 6.9 Hz, 1 H, NHCO\text{C}_9\text{H}_19), 7.08 (t, J = 5.4 Hz, 1 H, \text{OCOC}_9\text{H}_19)\].
NHC13H26 (0.1 mL) was added to a stirring solution of COJ = concentrated. The residue obtained was purified by flash chromatography (ethyl acetate) to afford 2.05, 1.9 (each s, 3 H, O(CO)C66.98, 171.68, 170.61, 170.38, 170.20, 170.06, 169.90 (each CO), 101.33 (C-1), 70.81, 70.73 (C-5, C-3), 68.97, (C-2), 68.51 (NHC13H26), 66.98 (C-4), 61.30 (C-6), 49.72 (C-α), 39.67, 39.31 (each OCH2CH2NH), 36.98 (C-β), 36.62, 31.92, 29.70, 29.65, 29.56, 29.52, 29.36, 28.31, 26.88, 25.63, 22.62 (each CH2), 20.87–20.85 (overlap of O(CO)CH3), 14.12 (overlap of COC16H16CH3, NHC13H26CH3); HRMS (MS-TOF): [M+H]+ calcd. for C44H77N3O13: 855.5456 found 855.5492.

N4-[2-O-(2,3,4,6-Tetra-O-acetyl-β-D-galactopyranosyl)-ethyl]-N2-tetracosanoyl-L-asparagine tetradecylamide (14). HOBT (0.022 g, 0.16 mmol) was added to a stirring solution of tetracosanoic acid (0.056 g, 0.15 mmol) and TBTU (0.056 g, 0.16 mmol) dissolved in anhydrous DMF (6 mL), under N2 at rt. It was stirred for 10 min and the amine obtained from 13 as described above (0.128 g, 0.18 mmol) was dissolved in anhydrous DMF (8 mL) and added slowly. It was stirred for 3 h. The reaction mixture was concentrated in vacuo, diluted with ethyl acetate, washed with brine, dried (MgSO4) and concentrated. The residue obtained was purified by flash chromatography (ethyl acetate) to afford 14 as a white solid, (0.030 g, 18%). [α]D22 = +3.8 (c 0.83, CH2Cl2); IR (NaCl film): 3423.0, 2918.4, 2850.3, 1749.6, 1644.4, 1543.1, 1465.6, 1369.9, 1223.4, 1058.3 cm⁻¹; 1H-NMR (300 MHz, CDCl3): δ 7.44 (d, J = 6.9 Hz, 1 H, NHCOC25H47), 7.09 (t, J = 4.8 Hz, 1 H, CONHC14CH29) 6.26 (bs, 1 H, CH3CH2NHCOCO), 5.40 (d, J = 3 Hz, 1 H, H-4), 5.17 (dd, J = 7.8 Hz, J = 10.5 Hz, 1 H, H-2), 5.04 (dd, J = 2.7 Hz, J = 10.2 Hz, 1 H, H-3), 4.70–4.64 (m, 1 H, H-α), 4.53 (d, J = 7.8 Hz, 1 H, H-1), 4.21–4.10 (m, 2 H, overlap of H-6, H-6′), 3.97–3.92 (tm, 1 H, H-5) 3.90–3.85 (m, 1 H, 1 H of OCH2CH2NH), 3.71–3.64 (m, 1 H, 1 H of OCH2CH2NH), 3.54–3.44 (m, 2 H, OCH2CH2NH), 3.22–3.15 (m, 2 H, NHCH2C13H27), 2.77 (dd, J = 3.3 Hz, J = 15.3 Hz, 1 H, H-β), 2.45 (dd, J = 6.9 Hz, J = 15.6 Hz, 1 H, H-β′), 2.25–2.20 (t, J = 7.5 Hz, 2 H, COCH2C22 H45), 2.16, 2.09, 2.04, 1.90 (each s, 3 H, O(O)COCH3), 1.62–1.59 (m, 2 H, COCH2CH2C21H43), 1.46–1.45 (m, 2 H, NHCH2C16H21C12H25), 1.25 (bs, 62 H, overlap of COC2H4(CH2)20CH3, NHC2H4(CH2)11CH3, 0.89–0.85 (t, J = 6.3 Hz, 6 H, overlap of COC2H44CH3, NHC13H26CH3); 13C-NMR (75 Hz, CDCl3): δ 173.57, 171.72, 170.58, 170.38, 170.20, 170.07, 169.90 (each CO), 101.34 (C-1), 70.82, 70.73 (C-5, C-3), 68.98. (C-2), 68.54 (NHCH2C13H27), 66.98 (C-4), 61.31 (C-6), 49.72 (C-α), 39.66, 39.24 (each OCH2CH2NH), 36.95 (C-β), 36.64 (COCH2CH2C20H3), 31.92, 31.86, 29.66,29.56, 29.45, 29.36, 29.28, 26.89, 22.69, 22.66 (each CH2), 20.88 (overlap of O(O)COCH3), 14.12 (overlap of COC2H44CH3, NHC13H26CH3); HRMS (MS-TOF): [M+H]+ calcd. for C58H105N3O13: 1052.772 found 1052.775.

N4-[2-O-(β-D-Galactopyranosyl)-ethyl]-N2-decanoyl-L-asparagine tetradecylamide (1). Triethylamine (0.1 mL) was added to a stirring solution of 11b (0.120 g, 0.14 mmol) dissolved in
CH₂Cl₂/MeOH/H₂O (3 mL/6 mL/3 mL) at 40 °C. It was stirred for 18 h. The reaction mixture was concentrated under reduced pressure to afford 1 as a white solid (0.080 g, 83%). [α]D22 = −6.0 (c 0.33, C₅H₇N); ¹H-NMR (300 MHz, d₅-Pyr): δ 8.95 (d, J = 8.1 Hz, 1 H, NHCOOCH₃H₁₀), 8.87 (t, J = 5.4 Hz, 1 H, CH₂CH₂NHCO), 8.55 (t, J = 5.7 Hz, 1 H, CONHC₁₄H₂₉), 7.05, 6.79, 6.63, 6.39 (each bs, 1 H, OH), 5.55–5.53 (m, 1 H, H-α), 4.79 (d, J = 7.5 Hz, 1 H, H-1), 4.51–4.33 (m, 4 H, overlap of H-2, H-4, H-6, H-6'), 4.18–4.07 (m, 3 H, overlap of H-3, H-5, 1 H of OCH₂CH₂NH), 3.99–3.95 (m, 1 H, 1 H of OCH₂CH₂NH), 3.77–3.65 (m, 2 H, OCH₂CH₂NH), 3.22–3.16 (m, 2 H, NHCH₂CH₂C₁₂H₂₅), 3.18 (td, J = 6.6 Hz, J = 1.2 Hz, 2 H, overlap of H-β, H-β'), 2.39–2.34 (t, J = 7.5 Hz, 2 H, COCH₂C₈H₁₇), 1.79–1.69 (m, 2 H, COCH₂CH₂C₁₂H₁₄), 1.60–1.50 (m, 2 H, NHCH₂CH₂C₁₂H₂₅), 1.20 (bs, 34 H, overlap of COC₂H₄(CH₂)₆CH₃, NH₂CH₄(CH₂)₁₁CH₃), 0.89–0.82 (m, 6 H, overlap of COC₃H₁₆CH₃, NH₃CH₂C₁₃H₂₆CH₃); ¹³C-NMR (75 Hz, d₅-Pyr): δ 175.40, 173.98, 173.04 (each CO), 107.68 (C-1), 78.96, 77.22, 74.52, 72.25 (C-2, C-4, C-3, C-5), 71.68 (NHCH₂C₁₃H₂₇), 64.53 (C-6), 53.27 (C-α), 42.50, 41.80 (each OCH₂CH₂NH), 40.71 (C-β), 38.49, 38.49, 34.09, 34.01, 32.04, 31.95, 31.90, 31.68, 31.66, 31.63, 31.58, 31.49, 29.22, 28.09 (each CH₂), 18.63 (overlap of COC₃H₁₆CH₃, NH₃CH₂C₁₃H₂₆CH₃); HRMS (MS-TOF): [M+H]+ calcd. for C₅₀H₉₇N₃O₉: 883.7225 found 883.7278.

N°-[2-O-(β-D-Galactopyranosyl)-ethyl]-N²-tetracosanoyl-L-asparagine tetradecylamide (2). Triethylamine (0.1 mL) was added to a stirring solution of 1₄ (0.016 g, 0.015 mmol) dissolved in CH₂Cl₂/MeOH/H₂O/THF (1 mL/2 mL/1 mL/2 mL) at 40 °C. The reaction mixture was stirred and its progress was followed by ¹H-NMR spectra of aliquots. The reaction was deemed complete after 36 h. The reaction was concentrated under reduced pressure to afford 2 as a white solid (0.05 g, 38%). ¹H-NMR (300 MHz, d₅-Pyr): δ 9.02–8.96 (m, 1 H, NHCOO(CH₃)₃), 8.89–8.79 (m, 1 H, CH₂CH₂NHCO), 8.54–8.52 (m, 1 H, CONHC₁₄H₂₉), 5.54 (dd, J = 6.3, 12.9 Hz, 1 H, H-α), 4.80 (d, J = 7.8 Hz, 1 H, H-1), 4.52–4.36 (m, 4 H, overlap of H-2, H-4, H-6, H-6'), 4.18–4.10 (m, 3 H, overlap of H-3, H-5, 1 H of OCH₂CH₂NH), 3.99–3.95 (m, 1 H, 1 H of OCH₂CH₂NH), 3.77–3.65 (m, 2 H, OCH₂CH₂NH), 3.48–3.38 (m, 2 H, NHCH₂C₁₃H₂₆), 3.18 (m, 2 H, H-β, H-β'), 2.40–2.35 (m, 2 H, COCH₂C₁₂H₄₅), 1.79–1.69 (m, 2 H, COCH₂CH₂C₁₂H₄₃), 1.60–1.50 (m, 2 H, NHCH₂CH₂C₁₂H₂₅), 1.20 (bs, 62 H, overlap of COC₄H₄(CH₂)₂₀CH₃, NH₃CH₂(CH₂)₁₁CH₃), 0.89–0.82 (m, 6 H, overlap of COC₂H₄CH₃, NH₃CH₂C₁₃H₂₆CH₃); HRMS (MS-TOF): [M+H]+ calcd. for C₅₀H₇₁N₃O₉: 883.7225 found 883.7278.

N°-[2,3,4,6-Tetra-O-acetyl-β-D-galactopyranosyl]-N²-tert-butoxy carbonyl-L-asparagine benzyl ester (1₅) [30]. HOBt (1.30 g, 9.60 mmol) was added to a stirring solution of N-Boc-L-Asp-OBn 8 (1.55 g, 4.80 mmol) and TBTU (3.08 g, 0.720 mmol) in anhydrous DMF (25 mL) under N₂ at rt. It was stirred for 30 min and galactosyl amine 5 (2 g, 5.76 mmol) dissolved in anhydrous DMF (10 mL) was added dropwise to the solution. It was stirred for 18 h. The reaction mixture was concentrated in vacuo, diluted with ethyl acetate, washed with water, HCl 0.1 N and aqueous sat. NaHCO₃ solution, dried (MgSO₄) and concentrated. Flash chromatography (hexane/ethyl acetate 1:1) afforded 1₅ as a white solid (1.70 g, 72%). This was used without further purification. A small sample of 1₅ was recrystallised in CHCl₃/hexane to give white crystals used for characterisation. [α]D22 = +30 (c 1.2, CHCl₃); m.p. = 148–150 °C; IR (NaCl film): 3348.7, 2965.2, 1749.6, 1499.7, 1369.0, 1221.7, 1054.5 771.3 cm⁻¹; ¹H-NMR (300 MHz, CDCl₃): δ 7.34–7.33 (m, 5 H, Ph-H), 6.38 (d, J = 9 Hz, NHCO(CH₃)₃), 5.70 (d, J = 9 Hz, 1 H, NHCOCH₂), 5.43 (d, J = 3 Hz, 1 H, H-4), 5.21–5.04 (m, 5 H, overlap of CH₂Ph,
H-1, H-2, H-3), 4.58 (t, J = 6 Hz, 1 H, H-α), 4.15–3.97 (m, 3 H, overlap of H-5, H-6, H-6'), 2.95–2.84 (m, 1 H, H-β'), 2.71 (dd, J = 3 Hz, J = 15 Hz, 1 H, H-β), 2.13, 2.03, 1.99, 1.98 (each s, 3 H, O(CO)CH₃), 1.41 (9 H, O(CO)CH₃); ¹³C-NMR (75 MHz, CDCl₃): δ 171.53, 171.12, 170.54, 170.33, 169.95, 169.74 (each CO), 135.33 (C-Ph), 128.50, 128.26, 127.88 (CH-Ph), 80.10 (C(CH₃)), 78.44 (C-1), 72.40 (C-5), 70.67 (C-3), 68.17 (C-2), 67.29 (CH₂Ph), 67.06 (C-4), 61.08 (C-6), 50.09 (C-α), 37.85 (C-β), 28.25 (C(CH₃)), 20.66, 20.59, 20.57, 20.52 (each O(CO)CH₃); HRMS (MS-TOF): [M+H]+ calcd. for C₃₅H₅₀O₁₃N₂Na: 707.3386 found 707.3376.

N⁴-(2,3,4,6-Tetra-O-acetyl-β-D-galactopyranosyl)-N²-decanoyl-L-asparagine benzyl ester (16). TFA (1.34 mL, 17.93 mmol) in anhydrous CH₂Cl₂ (1.34 mL) was added dropwise to a solution of 15 (1.17 g, 1.79 mmol) in anhydrous CH₂Cl₂ (10 mL). It was stirred for 6 h. The reaction mixture was concentrated in the rotary evaporator and the residue was dissolved in CH₂Cl₂ and washed with aqueous sat. NaHCO₃ solution, brine and water. The organic phase was dried (MgSO₄) and concentrated in vacuo to yield the corresponding amine as a white foam (0.73 g, 74%). The compound was used without further purification. ¹H-NMR (300 MHz, CDCl₃): δ 8.10 (d, J = 9.3 Hz, 1 H, NHCOCH₂), 7.51–7.31 (m, 5 H, Ph-H), 5.40 (d, J = 1.4 Hz, 1 H, H-4), 5.22 (t, J = 9.3 Hz, 1 H, H-1), 5.12–5.07 (m, 4 H, overlap of CH₂Ph, H-2, H-3), 4.11–3.97 (m, 3 H, overlap of H-5, H-6, H-6'), 3.67 (bs, 1 H, H-α), 2.67–2.63 (m, 1 H, H-β), 2.39 (dd, J = 9.6 Hz, J = 5.3 Hz, 1 H, H-β'), 2.10, 2.00, 1.99, 1.95 (each s, 3 H, O(CO)CH₃); HRMS (MS-TOF): [M+Na]+ calcd. for C₂₅H₃₂O₁₂N₂Na: 553.2028 found 553.2024. TBTU (56 mg, 0.18 mmol) and HOBT (24 mg, 0.18 mmol) were added to a solution of decanoic acid (27 mg, 0.16 mmol) in anhydrous DMF (2 mL) under N₂ at rt. It was stirred for 20 min and the free amine obtained from 15 as described above (88 mg, 0.16 mmol) in anhydrous DMF (1 mL) was added dropwise to the solution. It was stirred for 18 h. The reaction mixture was concentrated in vacuo, diluted with ethyl acetate, washed with water and brine, dried (MgSO₄) and concentrated. The residue obtained was purified by flash chromatography (hexane/ethyl acetate 1:1) to yield 16 as a colourless oil (94 mg, 85%). [α]D²⁵ = +27.2 (c 1.76, CHCl₃); IR (NaCl film): 3330.9, 2926.4, 1751.0, 1674.3, 1530.8, 1370.1, 1222.5, 1179.4, 1052.8, 698.5 cm⁻¹; ¹H-NMR (300 MHz, CDCl₃): δ 7.33–7.31 (m, 5 H, Ph-H), 6.73 (d, J = 8.3 Hz, 1 H, NHCOCH₃)), 6.50 (d, J = 8.7 Hz, 1 H, NHCOCH₃)), 5.43 (d, J = 2.0 Hz, 1 H, H-4), 5.20–5.04 (m, 3 H, overlap of H-1, H-2, H-3, CH₂Ph), 4.93–4.87 (m, 1 H, H-α), 4.15–3.97 (m, 3 H, overlap of H-5, H-6, H-6'), 2.90 (dd, J = 4.1 Hz, J = 16.5 Hz, 1 H, H-β), 2.70 (dd, J = 4.4 Hz, J = 16.4 Hz, 1 H, H-β'), 2.22–2.17 (t, J = 7.3 Hz, 2 H, COCH₂C₈H₁₇), 2.13, 2.03, 1.99, 1.98 (each s, 3 H, O(CO)CH₃), 1.61–1.56 (m, 2 H, COCH₂CH₂C₇H₁₅), 1.24 (bs, 12 H, COC₂H₄(CH₂)₆CH₃), 0.88–0.84 (t, J = 7.0 Hz, 3 H, COC₈H₁₆CH₃); ¹³C-NMR (75 MHz, CDCl₃): δ 173.06, 171.49, 170.80, 170.28, 169.95, 169.76 (each CO), 135.23, (Ph-C), 128.53, 128.33, 127.92 (Ph-CH), 78.44 (C-1), 72.37 (C-5), 70.66 (C-3), 68.11 (C-2), 67.36 (CH₂Ph), 67.00 (C-4), 61.00 (C-6), 48.42 (C-α), 37.43 (C-β), 36.49, 31.81, 29.36, 29.27, 29.18, 25.49, 22.62 (each CH₃), 20.62, 20.55, 20.53, 20.49 (each O(CO)CH₃), 14.06 (COC₈H₁₆CH₃); HRMS (MS-TOF): [M+Na]+ calcd. for C₃₅H₅₀O₁₃N₂Na: 707.3386 found 707.3376.

N⁴-(2,3,4,6-Tetra-O-acetyl-β-D-galactopyranosyl)-N²-tetracosanoyl-L-asparagine benzyl ester (17). TBTU (59 mg, 0.183 mmol) and HOBT (25 mg, 0.183 mmol) were added to tetracosanoic acid (62 mg, 0.167 mmol) in anhydrous DMF (3 mL) containing 4 Å MS under N₂ at rt. It was stirred for 30 min
and the free amine obtained from 15 as described above (88 mg, 0.16 mmol) in anhydrous DMF (2 mL) was added dropwise to the solution. It was stirred for 2 h at 50 °C. The reaction mixture was concentrated in vacuo, diluted with ethyl acetate, washed with water and brine, dried (MgSO₄) and concentrated under reduced pressure. The residue obtained was purified by flash chromatography (hexane/ethyl acetate 1:1) to yield 17 as a white solid (93 mg, 62%). [α]D²⁵ = +18.9 (c 0.95, ethyl acetate); IR (NaCl film): 2918.7, 2918.7, 2850.5, 1750.5, 1371.1, 1231.8, 1054.9, 913.2, 743.7 cm⁻¹; ¹H-NMR (300 MHz, CDCl₃): δ 7.33–7.31 (m, 1 H, N COCH₂), 5.43 (d, J = 2.0 Hz, 1 H, H-(2)), 5.20–5.07 (m, 5 H, overlap of H-1, H-2, H-3, CH₂Ph), 4.93–4.88 (m, 1 H, H-α), 4.15–3.87 (m, 3 H, overlap of H-5, H-6, H-6'), 2.91 (dd, J = 3.9 Hz, J = 16.4 Hz, 1 H, H-β'), 2.71 (dd, J = 4.4 Hz, J = 16.4 Hz, 1 H, H-β'), 2.22–2.17 (t, J = 7.5 Hz, 2 H, COCH₂C₂H₄), 2.13, 2.04, 2.03, 1.99 (each s, 3 H, O(CO)CH₃), 1.61–1.56 (m, 2 H, COCH₂C₂H₅), 1.25 (bs, 40 H, COC₂H₄(CH₂)₂OCH₃), 0.89–0.85 (t, J = 7.0 Hz, 3 H, COC₂H₄(CH₂)₅CH₃); ¹³C-NMR (75 Hz, CDCl₃): δ 173.09, 171.53, 170.91, 170.82, 170.29, 169.97, 169.77 (each CO), 135.23, 128.54, 128.35 (CH-Ph), 127.93 (C-Ph), 78.46 (C-1), 72.38 (C-5), 70.66 (C-3), 68.12 (C-2), 67.39 (CH₂Ph), 67.00 (C-4), 61.00 (C-6), 48.43 (C-α), 37.45 (C-β), 36.52, 31.89, 29.67, 29.62, 29.45, 29.33, 29.31, 29.21, 25.51, 22.66 (each CH₃), 20.64, 20.57, 20.55, 20.51 (each O(CO)CH₃), 14.09 (COC₂H₄(CH₂)₅CH₃); HRMS (MS-TOF): [M+H]^+ calcd. for C₄₉H₇₅O₁₃N₂: 694.5610 found 694.5632.

N⁴-(2,3,4,6-Tetra-O-acetyl-β-D-galactopyranosyl)-N⁵-decanoyl-L-asparagine tetradecylamide (18). H₂ gas was bubbled through a suspension of 16 (56 mg, 0.079 mmol) in ethyl acetate (10 mL) and Pd/C 10% w/w (6 mg, 10% w/w) was added. It was left to stir for 3 h and then the reaction mixture was filtered through Celite washing with ethyl acetate and concentrated. The residue obtained was purified by flash chromatography (hexane/ethyl acetate 1:1:1) to yield 18 as a white solid (18 mg, 55%). [α]D²⁵ = +20.0 (c 0.75, CH₂Cl₂); IR (NaCl film): 3286.1, 2924.1, 2853.8, 1751.2, 1642.6, 1546.1, 1466.7, 1371.0, 1227.6, 1054.9 cm⁻¹; ¹H-NMR (300 MHz, CDCl₃): δ 7.58 (d, J = 7.6 Hz, 1 H, NHCOCH₂H₃), 6.79–6.74 (m, 2 H, overlap of NHCOCH₂, CONHC₁₄CH₂₀), 5.44 (d, J = 1.6 Hz, 1 H, H-(4)), 5.23–5.10 (m, 3 H, overlap of H-1, H-2, H-3), 4.71–4.67 (m, 1 H, H-u), 4.16–3.99 (m, 3 H, overlap of H-5, H-6, H-6'), 3.18–3.11 (m, 2 H, NHCH₂C₁₃H₂₇), 2.69 (dd, J = 3.4 Hz, J = 15.6 Hz, 1 H, H-β), 2.44 (dd, J = 5.6 Hz, J = 15.5 Hz, 1 H, H-β'), 2.25–2.21 (m, 2 H, COCH₂C₈H₁₇), 2.17, 2.14, 2.04, 2.00 (each s, 3 H, C₈H₁₇)}
O(CO)CH₃), 1.65 (bs, 2 H, COCH₂CH₂C₇H₅), 1.46–1.42 (m, 2 H, NHCH₂CH₂C₁₂H₂₅), 1.25 (bs, 34 H, overlap of COC₂H₄(CH₂)₆CH₃, NHCH₂(CH₂)₁CH₃), 0.90–0.85 (t, J = 6.9 Hz, 6 H, overlap of COC₃H₁₆CH₃, NHCH₂(CH₂)₆CH₃); ¹³C-NMR (75 MHz, CDCl₃): δ 173.77, 173.03, 172.24, 170.35, 170.29, 170.00, 169.78 (each CO), 78.47 (C-1), 72.30 (C-5), 70.72 (C-3), 67.84 (C-2), 67.05 (C-4), 61.06 (C-6), 49.75 (C-α), 39.59 (NHCH₂C₁₂H₂₇), 36.60 (C-β), 36.21, 31.91, 31.84, 29.68, 29.65, 29.61, 29.55, 29.44, 29.35, 29.31, 29.26, 26.89, 25.63, 22.68, 22.65 (each CH₂), 20.89, 20.67, 20.59, 20.54, (each O(CO)CH₃), 14.11 (overlap of COC₃H₁₆CH₃, NHCH₂(CH₂)₆CH₃); HRMS (MS-TOF): [M+Na⁺] calcd. for C₄₂H₇₃O₁₂N₃Na: 834.5086 found 834.5079.

N°-(2,3,4,6-Tetra-O-acetyl-β-D-galactopyranosyl)-N°-tetracosanoyl-L-asparagine tetradecylamide (19). H₂ gas was bubbled through a suspension of 17 (67 mg, 0.074 mmol) in ethyl acetate (5 mL) and Pd/C 10% w/w (7 mg, 10% w/w) was added. It was left to stir for 18 h and then the reaction mixture was filtered through Celite, washed with ethyl acetate and concentrated in vacuo to yield the corresponding carboxylic acid as a white solid, which was used without further purification (60 mg, 55%). ¹H-NMR (300 MHz, CDCl₃): δ 7.20 (d, J = 7.0 Hz, 1 H, NHCO₂C₂₃H₄₇), 6.62 (d, J = 9.2 Hz, 1 H, NHCOCH₂), 5.51 (d, J = 1.2 Hz, 1 H, H-4), 5.34–5.28 (m, 1 H, H-1), 5.12–5.10 (m, 2 H, overlap of H-2, H-3), 4.76–4.71 (m, 1 H, H-α), 4.22–4.02 (m, 3 H, overlap of H-5, H-6, H-6'), 2.96–2.84 (m, 1 H, H-β), 2.75 (dd, J = 5.0 Hz, J = 16.5 Hz, 1 H, H-β'), 2.37–2.26 (m, 2 H, COCH₂C₂₂H₄₃), 2.15, 2.06, 2.05, 2.00 (each s, 3 H, O(CO)CH₃), 1.67–1.58 (m, 2 H, COCH₂CH₂C₂₁H₄₃), 1.25 (bs, 40 H, COC₂H₄(CH₂)₂₀CH₃), 0.90–0.85 (t, J = 6.9 Hz, 3 H, COC₂₂H₄₄CH₃), HRMS (MS-TOF): [M+H⁺] calcd. for C₆₅H₁₀₂O₁₁N₂: 813.5107 found 813.5106. TBTU (12 mg, 0.037 mmol) and HOBt (5 mg, 0.037 mmol) were added to a solution of the carboxylic acid obtained from 17 as described above (27 mg, 0.033 mmol) in anhydrous DMF (3 mL), containing 4 Å MS, under N₂ and at rt. It was stirred for 20 min and tetradecyamine (7 mg, 0.033 mmol) was added. It was left to stir for 18 h and then the reaction mixture was diluted with ethyl acetate, washed with brine and water, dried (MgSO₄) and concentrated under reduced pressure. Flash chromatography (hexane/ethyl acetate 1:1) afforded 19 as a white solid (15 mg, 45%). [α]°D = +092.2 (c 0.65, CH₂Cl₂); IR (NaCl film): 3426.0, 2918.5, 2850.5, 1750.7, 1641.8, 1228.5 cm⁻¹; ¹H-NMR (300 MHz, CDCl₃): δ 7.58 (d, J = 7.8 Hz, 1 H, NHCO₂C₂₃H₄₇), 6.78–6.73 (m, 2 H, overlap of NHCOCH₂, CONHC₁₄H₂₀), 5.45 (d, J = 1.8 Hz, 1 H, H-4), 5.26–5.10 (m, 3 H, overlap of H-1, H-2, H-3), 4.71–4.66 (m, 1 H, H-α), 4.17–3.98 (m, 3 H, overlap of H-5, H-6, H-6'), 3.26–3.11 (m, 2 H, NHCH₂C₁₂H₂₇), 2.69 (dd, J = 3.5 Hz, J = 15.7 Hz, 1 H, H-β), 2.44 (dd, J = 5.6 Hz, J = 15.7 Hz, 1 H, H-β'), 2.24–2.21 (m, 2 H, COCH₂C₂₂H₄₃), 2.17, 2.14, 2.04, 2.00 (each s, 3 H, O(CO)CH₃), 1.66 (bs, 2 H, COCH₂CH₂C₂₁H₄₃), 1.46–1.42 (m, 2 H, NHCH₂CH₂C₁₂H₂₅), 1.25 (bs, 62 H, overlap of COC₂H₄(CH₂)₂₀CH₃, NHCH₂(CH₂)₁CH₃), 0.90–0.85 (t, J = 6.9 Hz, 6 H, overlap of COC₂H₄₄CH₃, NHCH₂(CH₂)₆CH₃); ¹³C-NMR (75 MHz, CDCl₃): δ 173.77, 173.03, 172.24, 170.35, 170.00, 169.78 (each CO), 78.47 (C-1), 72.30 (C-5), 70.72 (C-3), 67.83 (C-2), 67.04 (C-4), 61.04 (C-6), 49.73 (C-α), 39.57 (NHCH₂C₁₂H₂₇), 36.59 (C-β), 36.18,31.90, 29.68, 29.33, 26.87, 25.62, 22.66, (each CH₂), 20.87, 20.64, 20.57, 20.51, (each O(CO)CH₃), 14.09 (overlap of COC₂H₄₄CH₃, NHCH₂(CH₂)₆CH₃); HRMS (MS-TOF): [M+Na⁺] calcd. for C₅₆H₁₀₂O₁₂N₂: 1008.7458 found 1008.7429.
N4-β-D-Galactopyranosyl-N2-decanoyl-L-asparagine tetradecylamide (3). Triethylamine (0.05 mL) was added to a stirring solution of 18 (0.043 g, 0.053 mmol) dissolved in CH2Cl2/MeOH/H2O (1 mL/2 mL/1 mL) at 40 °C. It was stirred for 18 h. The precipitate formed was filtered through a vacuum to afford 3 as white crystals (7 mg, 21%). [α]22 D = −6.7 (c 0.6, C5H5N); 1H-NMR (300 MHz, d5-Pyr): δ 10.14 (d, J = 9.1 Hz, 1 H, NHCOCH2), 8.94 (d, J = 7.9 Hz, 1 H, NHCOC9H19), 8.53–8.48 (t, J = 9.2 Hz, 1 H, H-1), 5.45 (dd, J = 6.8, 14.3 Hz, 1 H, H-α), 4.58 (d, J = 2.9 Hz, 1 H, H-4), 4.56–4.49 (t, J = 9.2 Hz, 1 H, H-2), 4.38 (dd, J = 2.1 Hz, J = 6.1 Hz, 2 H, H-β, H-β'), 4.17 (dd, J = 3.0 Hz, J = 9.0 Hz, 1 H, H-3), 4.13–4.09 (t, J = 5.8 Hz, 1 H, H-5), 3.45–3.34 (m, 2 H, NHC2H2C13H27), 3.27 (d, J = 6.5 Hz, 2 H, H-β, H-β'), 2.36–2.31 (t, J = 7.5 Hz, 2 H, COCH2C8H17), 1.75–1.70 (m, 2 H, COCH2CH2C7H15), 1.58–1.50 (m, 2 H, NHCH2C12H25), 1.24 (bs, 34 H, overlap of COC2H4(C2H2)6CH3, NHC2H4(C2H11)11CH3), 0.88–0.81 (m, 6 H, overlap of COC8H16C3, NHC13H26C3); 13C NMR (d5-Pyr, 75 Hz): δ 173.41, 171.98, 171.83, (each CO), 81.74 (C-1), 78.35 (C-5), 76.19 (C-3), 70.42 (C-6), 62.37 (C-α), 39.89 (NHCH2C13H27), 39.05 (C-β), 36.46, 32.08, 32.00, 29.94, 29.88, 29.67, 29.64, 29.61, 29.57, 29.48, 27.25, 26.02, 22.89, 22.85 (each CH2), 14.22 (overlap of COC8H16CH3, NHCH13H26CH3); HRMS (MALDI MS-QTOF): [M+Na]+ calcd for C34H65O8N3Na: calcd: 666.4664 found 666.4689.

N4-β-D-Galactopyranosyl-N2-tetracosanoyl-l-asparagine tetradecylamide (4). Triethylamine (0.05 mL) was added to a stirring solution of 19 (0.035 g, 0.035 mmol) dissolved in CH2Cl2/MeOH/H2O (1 mL/2 mL/1 mL) at 40 °C. It was stirred for 18 h. The precipitate formed was filtered through a vacuum to afford 4 as a white solid (23 mg, 79%). 1H-NMR (300 MHz, d5-Pyr): δ 10.20–10.14 (m, 1 H, NHCOCH2), 9.02–8.90 (m, 1 H, NHCOCH2), 8.50 (t, J = 5.5 Hz, 1 H, CONJC14CH29), 5.90 (dd, J = 8.9 Hz, J = 17.9 Hz, 1 H, H-1), 5.65–5.61 (m, 1 H, H-α), 4.73 (d, J = 6.1 Hz, 1 H, H-4), 4.60 (d, J = 2.9 Hz, 1 H, H-2), 4.56–4.36 (m, 3 H, overlap of H-6, H-6', H-3), 4.20–4.13 (m, 1 H, H-5), 3.49–3.38 (m, 2 H, NHCH2C13H27), 3.29–3.21 (m, 2 H, H-β, H-β'), 2.35 (t, J = 7.5 Hz, 2 H, COCH2C22H43), 1.77–1.73 (m, 2 H, COCH2CH2C7H15), 1.59–1.54 (m, 2 H, NHCH2CH2C2H25), 1.26 (bs, 62 H, overlap of COC2H4(CH2)20CH3, NHCH24(CH2)11CH3), 0.89–0.85 (m, 6 H, overlap of COC8H16CH3, NHCH13H26CH3); HRMS (MALDI MS-QTOF): [M+Na]+ calcd for C48H93O8N3Na: calcd: 862.6860 found 862.6841.

4. Conclusions

In summary, we present a short and convenient route to access glycolipid mimetics from suitably protected and commercially available L-aspartic acid building blocks and easily synthesized galactosyl amines. A small collection of compounds of diverse structural characteristics has been prepared. The design of suitably assembled building blocks and careful consideration of the synthetic sequence, to avoid undesired side reactions, will allow for the next generation of glycolipid mimetics bearing different mono or oligosaccharides, as well as fatty acid derivatives of different chain lengths and saturation patterns.
Acknowledgments

The authors would like to thank IRC (Irish Research Council) for Ph.D funding, Barbara Woods and Orla Fenelon for their assistance with mass spectrometry data, John O’Brien (Trinity College Dublin) for his expertise and help with NMR experiments, and Martin Feeney (Trinity College Dublin) for his assistance with MALDI mass spectrometry data.

References

1. Ernst, B.; Magnani, J.L. From carbohydrate leads to glycomimetic drugs. *Nat. Rev. Drug Discov.* **2009**, *8*, 661–677.
2. Galan, M.C.; Benito-Alifonso, D.; Watt, G.M. Carbohydrate Chemistry in Drug Discovery. *Org. Biomol. Chem.* **2011**, *9*, 3598–3610.
3. Gorityala, B.K.; Ma, J.; Wang, X.; Chen, P.; Liu, X.W. Carbohydrate functionalized carbon nanotubes and their applications. *Chem. Soc. Rev.* **2010**, *39*, 2925–2934.
4. Papini, A.M. The use of post-translationally modified peptides for detection of biomarkers of immune-mediated diseases. *J. Pept. Sci.* **2009**, *15*, 621–628.
5. Zhang, J.; Pourceau, G.; Meyer, A.; Vidal, S.; Praly, J.P.; Souteyrand, E.; Vasseur, J.J.; Morvan, F.; Chevolot, Y. DNA-directed immobilisation of glycomimetics for glycoarrays application: Comparison with covalent immobilisation, and development of an on-chip IC₅₀ measurement assay. *Biosens. Bioelectron.* **2009**, *24*, 2515–2521.
6. Queneau, Y.; Chambert, S.; Besset, C.; Cheaib, R. Recent progress in the synthesis of carbohydrate-based amphiphilic materials: The examples of sucrose and isomaltulose. *Carbohydr. Res.* **2008**, *343*, 1999–2009.
7. Banchet-Cadeddu, A.; Henon, E.; Dauchez, M.; Renault, J.H.; Monneaux, F.; Haudrechy, A. The stimulating adventure of KRN 7000. *Org. Biomol. Chem.* **2011**, *9*, 3080–3104.
8. Huang, L.D; Lin, H.J.; Huang, P.H.; Hsiao, W.C.; Raghava Reddy, L.V.; Fu, S.L.; Lin, C.C. Synthesis of serine-based glycolipids as potential TLR4 activators. *Org. Biomol. Chem.* **2011**, *9*, 2492–2504.
9. Polidori, A.; Pucci, B.; Riess, J.G.; Zarif, L.; Pavia, A.A. Synthesis of double-chain glycolipids derived from aspartic acid: Preliminary investigation of their colloidal behavior. *Tetrahedron Lett.* **1994**, *35*, 2899–2902.
10. Elizabeth, C.; Maljaars, P.; Halkes, K.M.; de Oude, W.L.; van der Poel, S.; Pijnenburg, N.J.M.; Kamerling, J.P. Preparation of S- and N-Linked Glycosylated Amino Acid Building Blocks for Solid-phase Glycopeptide Library Synthesis. *J. Carbohydr. Chem.* **2005**, *24*, 353–367.
11. Nuti, F.; Paolini, I.; Cardona, F.; Chelli, M.; Lolli, F.; Brandi, A.; Goti, A.; Rovero, P.; Papini, A.M. Fmoc-protected iminosugar modified asparagine derivatives as building blocks for glycomimetics-containing peptides. *Bioorg. Med. Chem.* **2007**, *15*, 3965–3973.
12. Schips, C.; Ziegler, T. A Practical One-Pot Synthesis of New S-Glycosyl Amino Acid Building Blocks for Combinatorial Neoglycopeptide Synthesis. *J. Carbohydr. Chem.* **2005**, *24*, 773–788.
13. Mezzato, S.; Unverzagt, C. Synthesis of an Fmoc-Asn-heptasaccharide building block and its application to chemoenzymatic glycopeptide synthesis. *Carbohydr. Res.* **2010**, *345*, 1306–1315.
14. Biraboneye, A.C.; Madonna, S.; Laras, Y.; Krantic, S.; Maher, P.; Kraus, J.L. Potential neuroprotective drugs in cerebral ischemia: New saturated and polyunsaturated lipids coupled to hydrophilic moieties: Synthesis and biological activity. *J. Med. Chem.* **2009**, *52*, 4358–4369.

15. Zerkowski, J.A.; Solaiman, D.K.Y.; Ashby, R.D.; Foglia, T.A. Head group-modified sophorolipids: Synthesis of new cationic, zwitterionic, and anionic surfactants. *J. Surfactants Deterg.* **2006**, *9*, 57–62.

16. Lin, K.H.; Liang, J.J.; Huang, W.I.; Lin-Chu, S.Y.; Su, C.Y.; Lee, Y.L.; Jan, J.T.; Lin, Y.L.; Cheng, Y.S.E.; Wong, C.H. *In Vivo* Protection Provided by a Synthetic New Alpha-Galactosyl Ceramide Analog against Bacterial and Viral Infections in Murine Models. *Antimicrob. Agents Chemother.* **2010**, *54*, 4129–4136.

17. Stimac, A.; Kobe, J. Studies on the origin of stereoselectivity in the synthesis of 1,2-trans glycosfuranosyl azides. *Carbohyd. Res.* **2000**, *324*, 149–160.

18. Maier, M.A.; Yannopoulos, C.G.; Mohamed, N.; Roland, A.; Fritz, H.; Mohan, V.; Just, G.; Manoharan, M. Synthesis of Antisense Oligonucleotides Conjugated to a Multivalent Carbohydrate Cluster for Cellular Targeting. *Bioconjugate Chem.* **2003**, *14*, 18–29.

19. Orlandi, S.; Annunziata, R.; Benaglia, M.; Cozzi, F.; Manzoni, L. Synthesis of some oligopyridine-galactose conjugates and their metal complexes: A simple entry to multivalent sugar ligands. *Tetrahedron* **2005**, *61*, 10048–10060.

20. Wipf, P. *Handbook of Reagents for Organic Synthesis: Reagents for High-Throughput Solid-Phase and Solution-Phase Organic Synthesis*; Wiley-VCH: Berlin, Germany, 2005; pp. 40–43.

21. Han, S.Y.; Kim, Y.A. Recent developments of peptide coupling reagents in organic synthesis. *Tetrahedron* **2004**, *60*, 2447–2467.

22. Pramanik, D.; Majeti, B.K.; Mondal, G.; Karmali, P.P.; Sistla, R.; Ramprasad, O.G.; Srinivas, G.; Pande, G.; Chaudhuri, A. Lipopeptide with a RGDK tetrapeptide sequence can selectively target genes to proangiogenic alpha5beta1 integrin receptor and mouse tumor vasculature. *J. Med. Chem.* **2008**, *51*, 7298–7302.

23. Ziegler, T.; Rosseling, D.; Subramanian, L.R. Preparation of glycosyl amino acids as building blocks for the combinatorial synthesis of neoglycoconjugates. *Tetrahedron: Asymmetry* **2002**, *13*, 991–994.

24. Chaudhary, A.; Girgis, M.; Prashad, M.; Hu, B.; Har, D.; Repic, O.; Blacklock, T.J. Using mixed anhydrides from amino acids and isobutyl chloroformate in N-acylations: A case study on the elucidation of mechanism of urethane formation and starting amino acid liberation using carbon dioxide as the probe. *Tetrahedron Lett.* **2003**, *44*, 5543–5546.

25. Somogyi, L.; Haberhauer, G.; Rebek, J., Jr. Improved synthesis of functionalized molecular platforms related to marine cyclopeptides. *Tetrahedron* **2001**, *57*, 1699–1708.

26. Muller, H.M.; Delgado, O.; Bach, T. Total Synthesis of the Thiazolylpeptide GE2270 A. *Angew. Chem. Int. Ed. Engl.* **2007**, *46*, 4771–4774.

27. Ferrari, B.; Pavia, A.A. Artificial carbohydrate antigens: The synthesis of glycopeptidic haptons with TN specificity. *Bioorg. Chem.* **1982**, *11*, 85–95.

28. Nicolosi, G.; Spatafora, C.; Tringali, C. Enzymatic procedure catalysed by lipase from *Candida antarctica* for the regioprotection-deprotection of glucosamine. *Tetrahedron: Asymmetry* **1999**, *10*, 2891–2697.
29. Esmurziev, A.; Sundbø, E.; Hoff, B.H. Regioselective C-6 Hydrolysis of Methyl O-Benzoyl-
pyranosides Catalysed by Candida Rugosa Lipase. Eur. J. Org. Chem. 2009, 1592–1597.
30. Harrison, A.W.; Fisher, J.F.; Guido, D.M.; Couch, S.J.; Lawson, J.A.; Sutter, M.C.; William, M.V.;
DeGraaf, G.L.; Roger, J.E.; Pals, D.T.; et al. Appraisal of a glycopeptide cloaking strategy for a
therapeutic oligopeptide: Glycopeptide analogs of the renin inhibitor ditekiren. Bioorg. Med. Chem.
1994, 2, 1339–1361.
31. Seitz, O. Glycopeptide Synthesis and the Effects of Glycosylation on Protein Structure and
Activity. ChemBioChem 2000, 1, 214–246.
32. Conroy, T.; Jolliffe, K.A.; Payne, R.J. Synthesis of N-linked glycopeptides via solid-phase
aspartylation. Org. Biomol. Chem. 2010, 8, 3723–3733.

Sample Availability: Samples of the glycolipids 1–4 are available from the authors.

© 2012 by the authors; licensee MDPI, Basel, Switzerland. This article is an open access article
distributed under the terms and conditions of the Creative Commons Attribution license
(http://creativecommons.org/licenses/by/3.0/).