Tetrahydrofuran amino acids—versatile building blocks for unnatural biopolymers: lack of secondary structure in oligomeric carbopeptoids derived from a D-*galacto*-5-(aminomethyl) tetrahydrofuran-2-carboxylic acid

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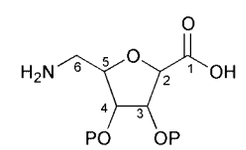
The efficient synthesis of a D-*galacto*-configured tetrahydrofuran amino acid from a seven-carbon lactone as a monomer for the generation of sequence-defined structured biopolymer mimics is described. Investigations into the characteristics of homooligomeric derivatives of this monomer indicate that they do not adopt well defined secondary structures. The unanticipated formation of a heterooligomeric derivative and investigations into its solution structure are also described.

Introduction

With a monomer alphabet of only twenty α-amino acids, the diversity of structure and function observed in proteins and polypeptides is breathtaking. When joined in specific sequences, physicochemical information inherent in these monomer units enables the formation of functional folded architectures. This property of biopolymers—that linear specific sequences possess properties greater than those of their monomer units—can provide valuable information in attempts to design materials that emulate these characteristics.[1] Implementation of this knowledge is exemplified by the synthesis of oligocarbamates[2] and ureas[3] composed of specific sequences of a range of monomer units. These unnatural oligomers have been shown to bind RNA sequences[4] and specific antibodies.[5] Due to their involvement in a plethora of biological processes, biopolymers such as peptides and proteins are attractive targets for mimicry,[6] although there are limitations of peptidic drugs, specifically those associated with mediocre absorption and poor metabolic stability. A successful approach for the mimicry of bioactive peptides is the use of peptidomimetics to induce a specific target conformation into a peptide.[7] A peptidomimetic can be broadly defined as an organic molecule (such as a peptide analogue or non-peptide ligand) that interacts with a receptor/acceptor in a similar chemical manner to the native peptide/protein ligand to elicit the same biochemical activities.[8] Most dipeptide analogues are based upon isosteric replacement of the amide bond with a suitable mimetic, or bridging between two neighbouring amino acids in a peptide. Bridging leads to a compound with limited conformational flexibility in comparison with that of regular dipeptides composed of proteinogenic amino acids.[9] One approach to the problem of *de novo* protein design is the use of stereochemically constrained[10] non-proteinogenic amino acids.[11]

An extension of this ideal involves the use of carbohydrate-like tetrahydrofuran (THF) frameworks **1** bearing both amino and carboxylic acid functionalities. This rigid scaffold can be formally derived from a conventional dipeptide **2** by introducing an ether linkage as an amide surrogate and also bridging between the two amino acids.[12] Investigations into the solution conformations of oligomeric derivatives of tetrahydrofuran amino acids such as **3** ('carbopeptoids'[13]) have revealed that one stereoisomer adopts a repeating β-turn-type secondary structure,[14] whilst another stereoisomer forms a left-handed helix stabilized by (i, $i-3$) hydrogen bonds.[15] Several biological applications of THF amino acids have been demonstrated. For example, a tetrahydrofuran sugar amino acid was incorporated into Leu-enkephalin as a Gly-Gly substitute to provide materials with equal pain-reducing properties to those of the natural peptide; CD studies indicated that such carbopeptoids may have a β-turn-like structure.[16] THF amino acids have been incorporated into a functional cation channel with a biomimetic channel entrance and exit; four THF amino acids continue the gramicidin β-helix.[17]



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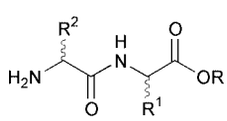

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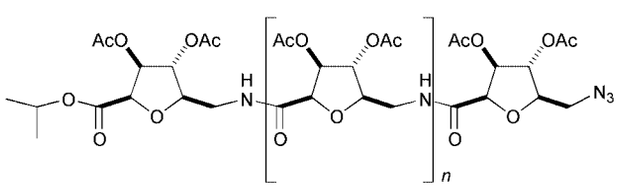

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This illustrates a general principle—that differing monomer-substitution patterns and backbone stereochemistries lead to different secondary structures in oligomeric derivatives.[18] This tenet has been elegantly exploited by a number of research groups, particularly in the formation of helices derived from α-,[19] β-[20] and γ-amino acids,[21] although other backbones have also been investigated.[22] Secondary structural elements in proteins serve primarily as templates or scaffolds for the presentation of specific side-chain groups. With this principle in mind, carbopeptoids have been proposed as a new generation of designer glycoconjugates in which carbohydrates provide not only the surface ligands but also an extremely well defined and

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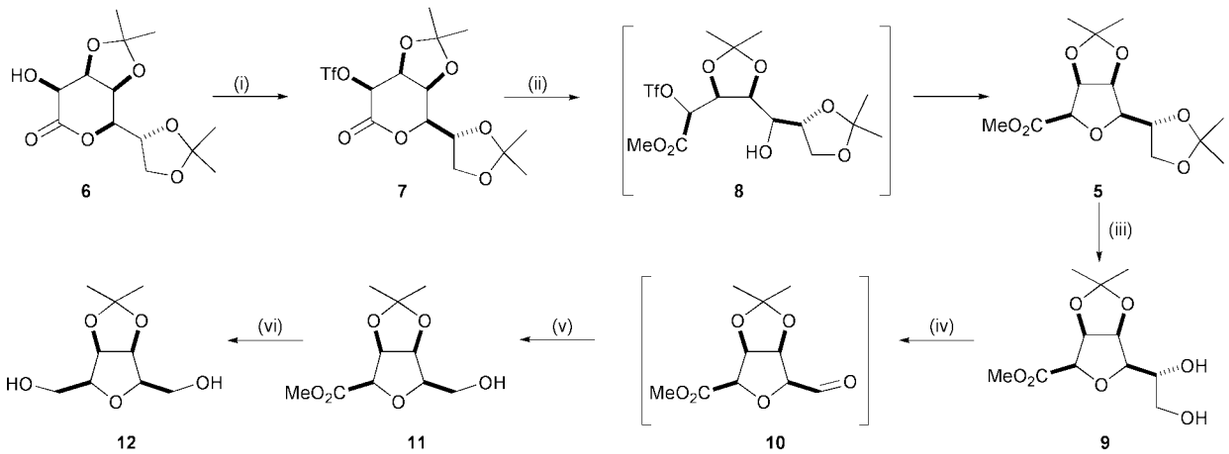


Scheme 1 *Reagents and conditions*: (i) Tf_2O, py, DCM, −30 °C; (ii) K_2CO_3, MeOH, 0 °C, 84% (2 steps); (iii) 80% AcOH (aq.), 98%; (iv) HIO_4, THF; (v) $NaBH_3CN$, AcOH, 88% (2 steps); (vi) $LiBH_4$, THF, 49%.

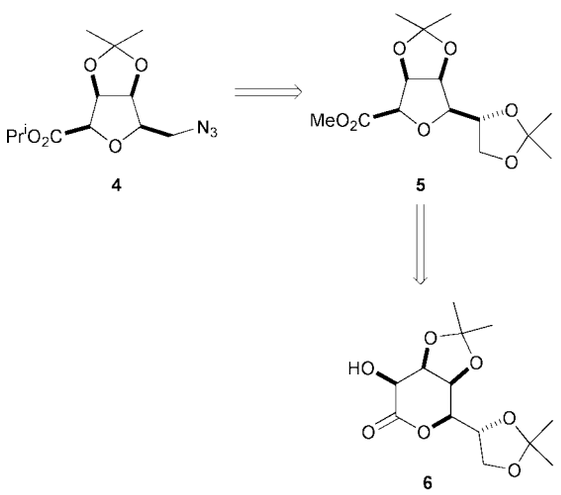


Fig. 1 Retrosynthesis of the tetrahydrofuran azido ester **4**.

predictable backbone.[23] The polyhydroxylated backbone also offers opportunities for the formation of bespoke hydrophobic or hydrophilic derivatives; this is significant, since the periodicity of polar and non-polar residues has been proposed to be one of the major determinants of secondary structure in proteins.[24]

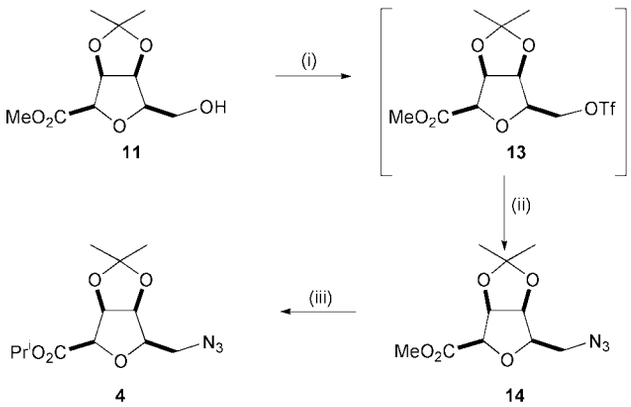
Results and discussion

It has previously been proposed that the 16 stereoisomers of the dipeptide isostere **1** constitute a family of monomeric units with a range of distinct conformational preferences suitable for foldamer generation.[25] The creation of an alphabet of biopolymer building blocks depends crucially on the efficient synthesis of these monomer units, combinations of which can be utilized to generate heteropolymers which may ultimately possess tertiary structure. Here we describe a synthesis of the all-*cis*-substituted tetrahydrofuran azido ester **4**, and subsequent formation of homooligomers as a demonstration of the utility of this building block. An investigation into the conformational preferences of these homooligomers is also described.

A strategy for the synthesis of the *C*-glycosyl azido THF **4** is shown in Fig. 1. The required D-*galacto* configuration **4** could be derived by a one-carbon degradation of the seven-carbon anhydro sugar **5** which has been previously reported in preliminary form.[26] The heptonolactone starting material **6** is available in kilogram quantities *via* a literature procedure from diacetone D-mannose;[27] the one-carbon extension at C-1 is achieved *via* the Kiliani–Fischer protocol.[28] Treatment of the heptonolactone **6** with trifluoromethanesulfonic anhydride (Tf_2O) in dichloromethane (DCM) in the presence of pyridine (py) at −30 °C gave the stable triflate **7**, which was stirred in methanol in the presence of potassium carbonate to afford the fully protected *C*-glycosyl derivative **5** in 84% yield over two steps, Scheme 1.[29] The mechanism of this transformation involves ring opening to form an open-chain hydroxy triflate **8**, with subsequent closure by S_N2-type intramolecular displacement of the C-2 triflate by the C-5 hydroxy group, with inversion of configuration at C-2. Stirring the diisopropylidene derivative **5** in 80% aq. acetic acid at 50 °C for two hours effected selective hydrolysis of the primary acetonide to afford the diol **9** in 98% yield. The resonance at 113.3 ppm in the ^{13}C NMR spectrum of **9** is characteristic of a quaternary carbon in a *cis*-fused five-membered dioxolane ring, and is consistent with removal of only the primary isopropylidene group.[30] Treatment of the diol **9** with periodic acid in THF effected oxidative cleavage to afford the C-6 aldehyde **10**, which was immediately reduced with sodium cyanoborohydride in acetic acid to the C-6 alcohol **11** in 88% yield (two steps). Ester functionalities can be reduced by sodium cyanoborohydride in systems with an α-oxygen, but here complete chemoselectivity for the C-6 aldehyde is observed. In order to confirm that the stereointegrity of the THF carboxylate **11** had not been compromised during this reaction sequence, the ester **11** was exhaustively reduced with lithium borohydride in THF to afford the known symmetric diol **12** (49% yield).[31]

Introduction of nitrogen at C-6 was achieved by a sulfonate ester activation–azide displacement protocol. Treatment of the methyl carboxylate **11** with trifluoromethanesulfonic anhydride in dichloromethane in the presence of pyridine gave the C-6 triflate **13**, which was stirred with sodium azide in dimethylformamide (DMF) to yield the azide **14** in 88% yield over the two steps, Scheme 2. In similar systems, intermolecular polymerization of a C-6 amine or intramolecular cyclization to bicyclic derivatives has occurred following reduction of the azido group.[32] To circumvent this possibility, it was decided to transesterify the ester **14** to the more sterically hindered isopropyl ester **4**.[33] Thus the azido ester **14** was treated with hydrogen chloride in propan-2-ol at 80 °C for 24 h, before being cooled and stirred in a large volume of acetone (to reprotect the 3,4-*cis*-diol unit). This gave the isopropyl azido ester **4** in 93% yield. This route gives access to the fully protected THF amino ester **4** in five preparative steps and 60% overall yield from the seven-carbon lactone **6**.

The strategy adopted for homooligomerization of **4** utilizes previously reported solution-phase methodology.[34] This involves reduction of the azide group of one azido ester unit, and hydrolysis of the ester group of another and subsequent peptide coupling. In principle the process is iterative, affording

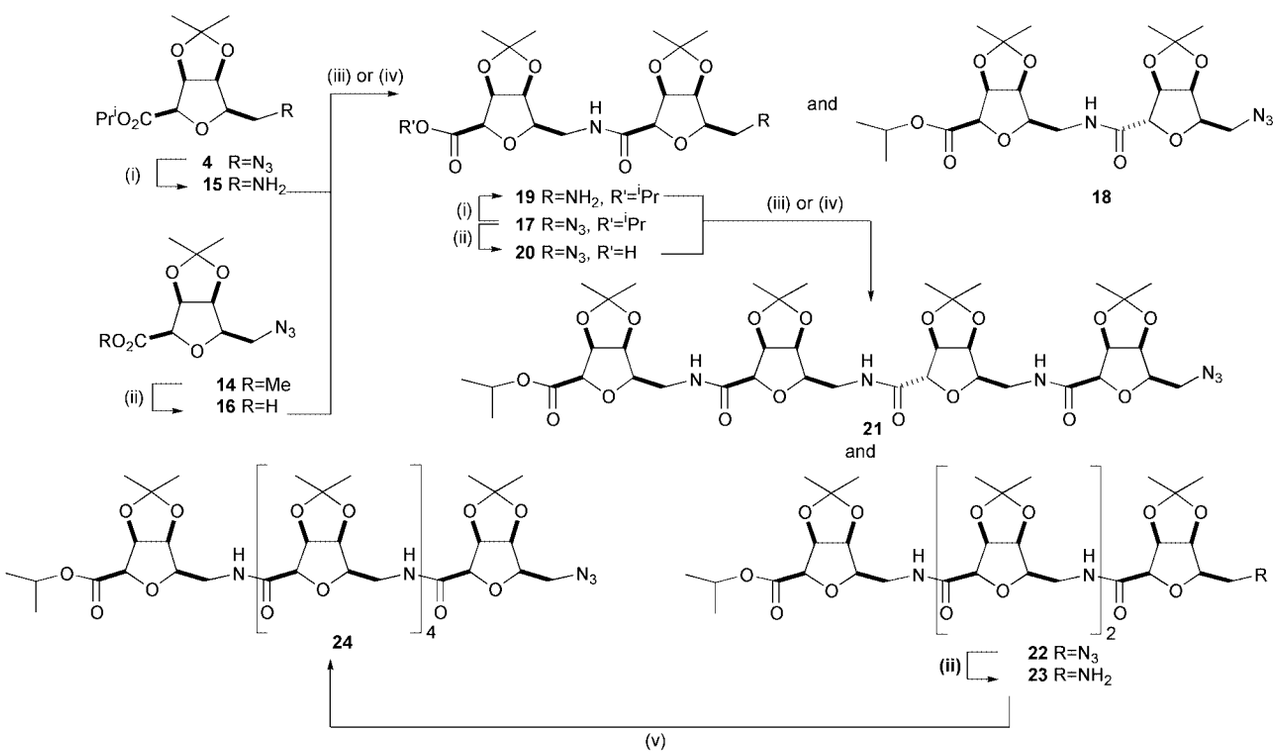


Scheme 2 *Reagents and conditions*: (i) Tf_2O, py, DCM, −30 °C; (ii) NaN_3, DMF, 88% (2 steps); (iii) ^{i}PrOH, HCl, 80 °C; then acetone, 93%.

successively larger oligomers. Thus, the azido functionality of the THF carboxylate **4** was reduced by catalytic hydrogenation in the presence of palladium black in propan-2-ol to afford the 6-amino derivative **15**, which was used without further purification. There was no evidence from ^{1}H NMR spectroscopy that intramolecular *N*-cyclization to a bicyclic lactam or uncontrolled intermolecular polymerization was occurring. Hydrolysis of the methyl ester in **14** with aq. sodium hydroxide in 1,4-dioxane and concomitant reprotonation with acidic ion-exchange resin gave the free acid **16**, which was coupled to the amine **15** under the auspices of 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (EDCI) and 1-hydroxy-1*H*-benzotriazole (HOBt)[35] in DCM in the presence of diisopropylethylamine (DIPEA) to give the dimer **17** in 63% yield. Alternatively, the coupling could be performed with *O*-(benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium tetrafluoroborate (TBTU) and DIPEA in DMF, *via* a modification of a literature procedure,[36] to afford the same dimer **17** in 84% yield, Scheme 3. A minor product, identified as the H-2^{A} epimer **18** by NMR spectroscopy, was also isolated, in 2% yield. The ^{1}H NMR spectra of this minor product **18** showed a single resonance shifted upfield when compared with the dimer **17**. This identified the resonance as arising from an H-2, whilst the phase-sensitive, two dimensional, total correlation (TOCSY) spectrum indicated it was part of the ring 'A' spin system.[37] For formation of the tetrameric derivative, the *N*-terminal azide functionality of the dimer **17** was reduced by hydrogenation in an analogous fashion to the azido ester **4** to afford the amine **19**. This was coupled to the acid **20** (derived from hydrolysis of the *C*-terminal ester of the dimer **17** with aq. sodium hydroxide) with EDCI and HOBt in DCM in the presence of DIPEA to afford the tetramer **22**, but in only 33% yield. Employing TBTU as the coupling reagent afforded the same tetramer **22** in 36% yield; an epimeric tetramer **21** was also isolable, in 6% yield.

It has been previously observed that oligomers of THF amino acids as short as a trimer[38] possessing a 2,5-*cis* arrangement of substituents across the THF ring adopt a repeating β-turn-type secondary structure in chloroform solution as determined by NMR spectroscopy. A similar investigation was conducted for the tetrameric derivative **22**. The partial ^{1}H spectrum is illustrated below, Fig. 2 (upper plot). This spectrum should be contrasted with that recorded for the previously observed 2,5-*cis* tetramer **3** ($n = 2$) (Fig. 2, lower plot) which showed well defined secondary structure and evidence for internal H-bonding. In this spectrum (Fig. 2, upper plot) there is poor dispersion of the amide NH and the tetrahydrofuran ring protons, consistent with a lack of H-bonding, as no amide NH protons are shifted to higher frequency and all the ring protons are grouped according to their locations within each monomer unit. The IR spectrum of the tetramer **22** in $CHCl_3$ solution (Fig. 3) provides additional evidence for the absence of H-bonding, with a single sharp N–H stretching band at 3431 cm^{-1} characteristic of a free, solvated amide.[39]

Correlations in TOCSY spectra, together with chemical-shift comparisons with previously assigned THF carboxylates readily identified the ring positions of each 'cluster' of resonances. Within these clusters, proton resonances were heavily overlapped, consistent with repeating monomeric units. 2D Rotating-frame NOE (ROESY) spectra demonstrated amide



Scheme 3 *Reagents and conditions*: (i) H_2, Pd, ^{i}PrOH; (ii) 1 M NaOH (aq.), 1,4-dioxane; (iii) EDCI, HOBt, DCM, DIPEA; (iv) TBTU, DMF, DIPEA; (v) TBTU, DMF, DIPEA, dimer **20**.

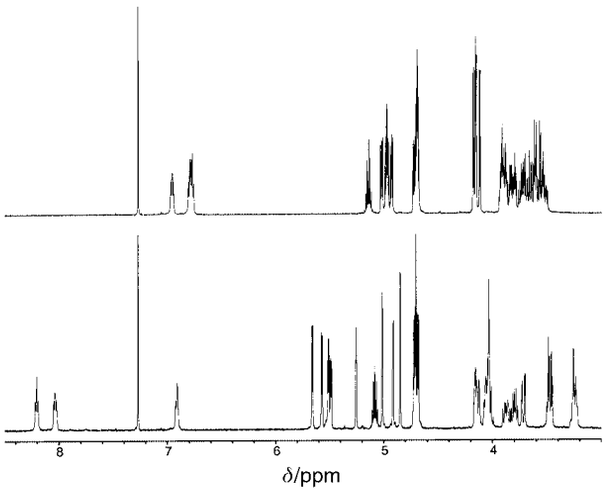


Fig. 2 Partial ^{1}H NMR spectrum of the tetramer **22** (upper plot: 500 MHz; $CDCl_3$; 298 K) and the previously observed tetramer **3** ($n = 2$) (lower plot: 500 MHz; $CDCl_3$; 298 K).

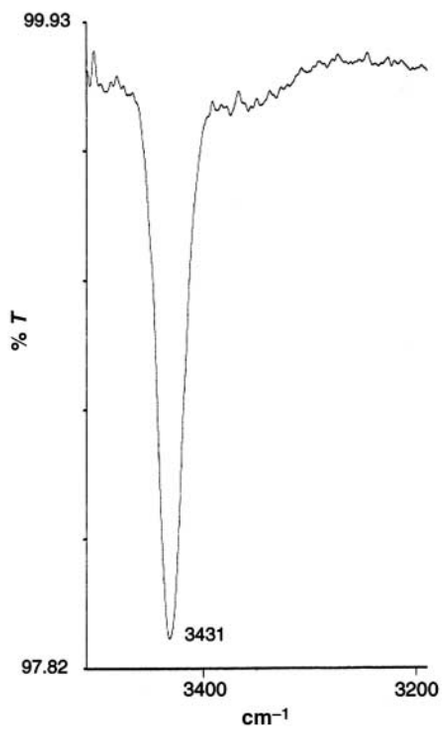


Fig. 3 IR spectrum of tetramer **22** showing the amide N–H stretch region (2 mM in $CHCl_3$).

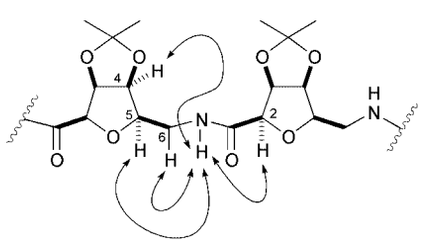


Fig. 4 Typical nuclear Overhauser enhancements observed for the tetramer **22**.

proton nuclear Overhauser enhancements (NOEs) to H-6 and H-5, plus weaker effects to H-4 of the same residue, together with NOEs to H-2 of the 'previous' residue, characteristic of short-range, sequential contacts, Fig. 4. The coincidence of two of the three amide NH resonances together with the general overlapping of the THF ring resonances prevented a complete proton assignment. Further studies on this tetramer were not pursued due to the lack of features that may be characteristic of secondary structure.

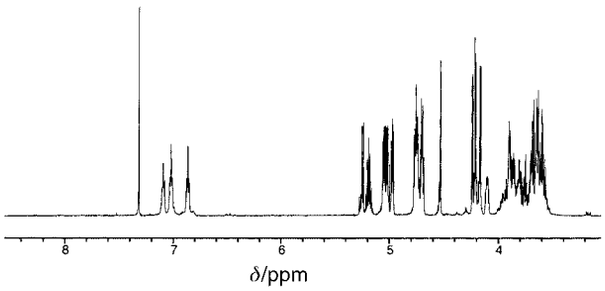


Fig. 5 Partial ^{1}H NMR spectrum of the tetramer **21** (500 MHz; $CDCl_3$; 298 K).

It is initially surprising that this material does not exhibit secondary structure, especially since it is epimeric at only a single centre per residue to oligomers **3** which adopt well defined secondary structures. It has previously been determined that the repeating β-turn structure adopted by oligomeric THF carboxylates bearing 2,5-*cis*-geometry across the THF ring is stabilized by (i, $i - 2$) hydrogen bonds. Simple models suggest that, in this derivative, one methyl group of the isopropylidene functionality could sit over the THF ring—potentially disfavouring the formation of hydrogen bonds of this type.

During formation of the tetramer **22**, a minor product **21** believed to be epimeric with the desired tetramer was isolated. It was necessary to determine which of the 16 stereogenic centres had undergone epimerization as this provides insight into the characteristics of the THF amino acid as a building block—and also serendipitously permits investigations into the conformational attributes of a heterooligomeric derivative. A partial proton spectrum of the epimeric tetramer **21** is illustrated below, Fig. 5.

Once again there was a general lack of dispersion throughout the spectrum and no high-frequency-shifted amide protons were evident. However, there was clearly a distinct set of resonances that were shifted away from the primary clusters (which fell in essentially identical locations to those in the desired tetramer **22**). These shifted resonances were readily identified as belonging to a single residue by correlations observed in TOCSY and proton-detected single-quantum coherence (HSQC) spectra. Analysis of these spectra indicated the greatest changes were observed in the proton spectrum (and to a lesser extent in the carbon spectrum) for positions 2, 3 and 5 in this residue. Significant proton changes of approximately +0.3, +0.2 and +0.2 ppm for H-2, H-3 and H-5, respectively, were observed for the epimerized residue relative to those shifts seen for the remaining three residues. Multiplet structures for these protons had also changed—the loss of structure to H-2 and H-3 indicating $J_{2,3} \cong 0$ Hz (now observed as a slightly broadened singlet and doublet, respectively, whereas others were doublet and double-doublet) is especially significant, whilst H-4 retained its double-doublet structure. The high-frequency chemical shift of H-3 (>5.1 ppm) and the singlet structure of H-2 has been observed previously in another THF-carboxylate, epimeric at C-2.[25]

All these data indicate epimerization at C-2 of one residue. NOEs observed in ROESY spectra are entirely consistent with this inversion—the unique observation of an H-5^i-to-NH^{i+1} NOE is only expected for a THF bearing 2,5-*trans*-geometry across the THF ring, Fig. 6. ROESY spectra were also used to assign the residues sequentially. Thus, H-2^i-to-NH^{i+1} NOEs established sequential contacts between those residues bound by an amide bond and these results indicated that epimerization had occurred only at C-2 of residue 'B' in the tetramer **21**.

In order to establish whether this epimerization had occurred during ester hydrolysis, the ^{1}H NMR spectrum of the

dimeric acid **20** was examined. This showed no evidence of any epimerization—suggesting that the C-2 inversion occurs during the activation procedure. It is possible that the steric constraints placed on the carbonyl group of ring 'B' in the acid **20** by the *cis* substituents on that ring slow the coupling reaction considerably. This suggests that epimerization does not proceed through any complex mechanism, but simply because attack of the amine **19** onto the activated acid **20** is slow. Furthermore, the resulting epimer is likely to couple more quickly because of its reduced steric hindrance.

The low reactivity of the dimeric acid **20** and its tendency to epimerize prompted the investigation of a convergent approach to the tetramer **22**. Hence the trimer **25** was synthesized in 69% yield by the TBTU-mediated coupling of the dimer amine **19** to the monomer acid **16**. Reduction of the azide group of the trimer **25**, and coupling of this trimeric amine **26** to the acid **16** with TBTU afforded the tetramer **22** in 60% yield from the azide **14**, Scheme 4. However, the convergent approach to the tetramer is not a viable synthetic alternative to the original divergent pathway, since the overall yield is only slightly improved (41% *versus* 36%).

Although secondary structure was not apparent in the tetramer **22**, it was still desirable to establish the conformational preferences of any of the higher order oligomers in this series. Therefore the hexamer was synthesized using a '4 + 2' strategy, from the tetrameric amine **23** and the dimeric acid **20**. TBTU-mediated coupling afforded the hexamer **24** in 40% yield. The electrospray ^{13}C isotope distribution is illustrated in Fig. 7.

The ^{1}H NMR spectrum in $CDCl_3$ showed no evidence of stable secondary structure, with very little dispersion of peaks, particularly in the amide region.

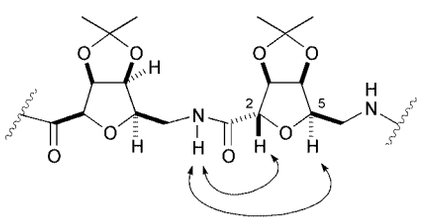
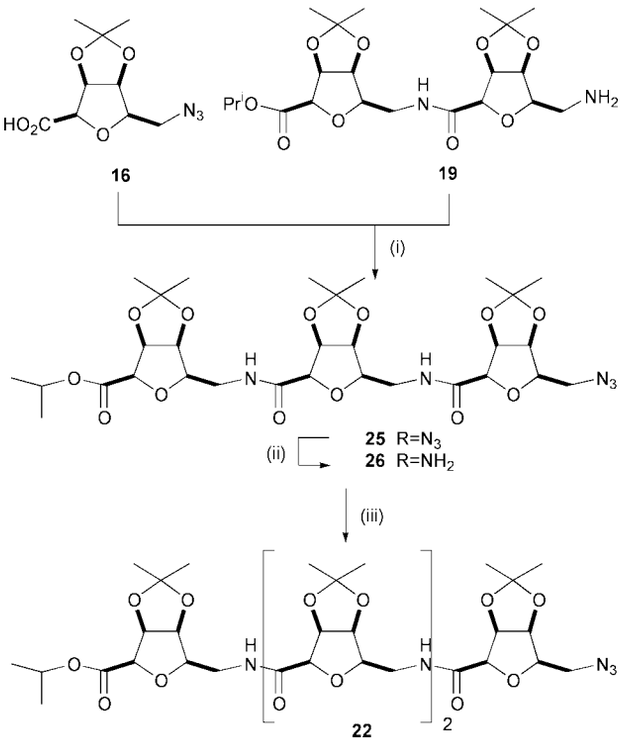


Fig. 6 Characteristic NOE enhancements observed for the tetramer **21**.



Scheme 4 *Reagents and conditions*: (i) TBTU, DMF, DIPEA; (ii) H_2, Pd, ^{i}PrOH; (iii) TBTU, DMF, DIPEA, monomer **16**.

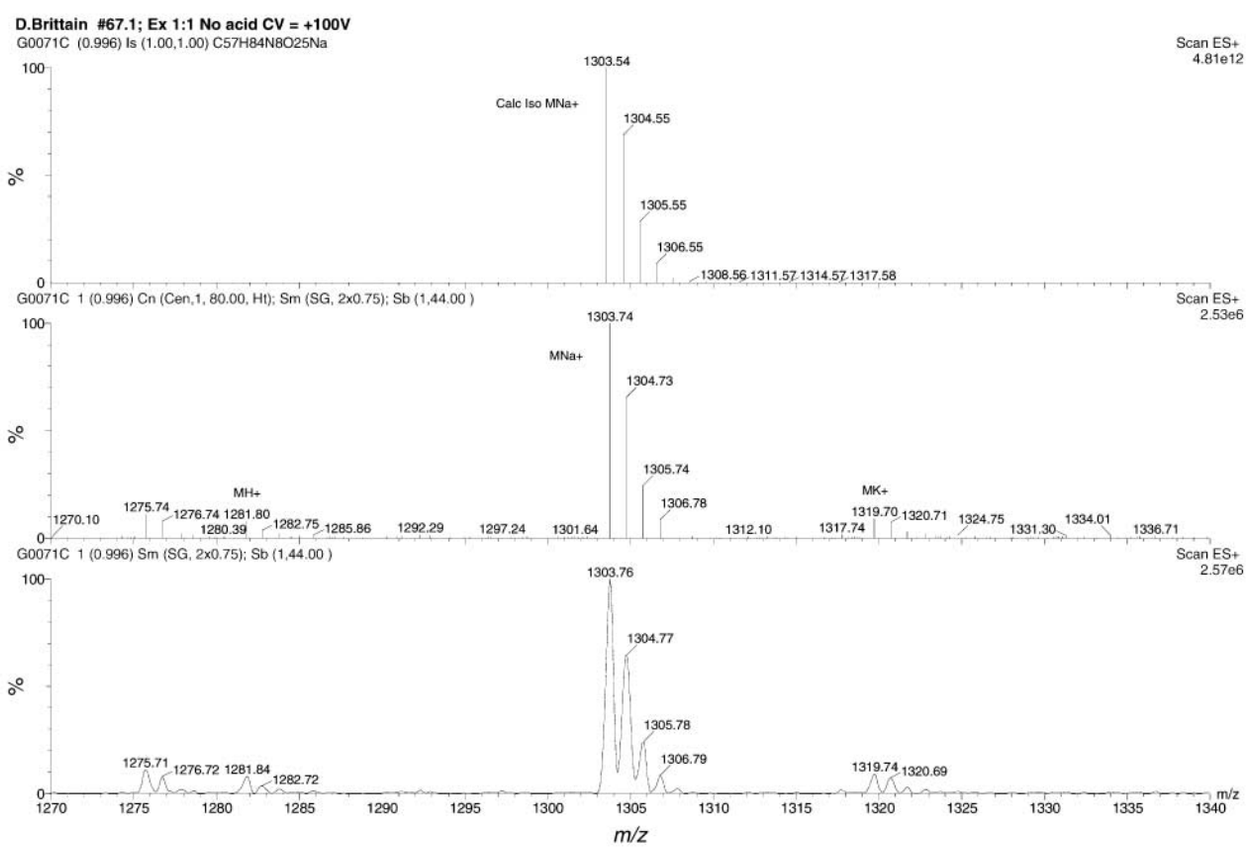
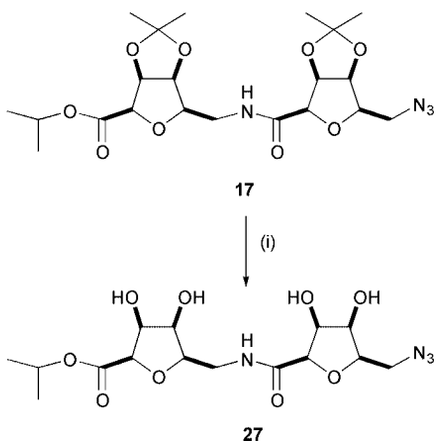


Fig. 7 Electrospray mass spectrum of the hexamer **24** illustrating calculated (top plot) and actual (centroid data, middle plot; raw data, bottom plot) ^{13}C isotope distribution for $[C_{57}H_{84}N_8NaO_{25}]^+$.

Removal of the 3,4-*O*-isopropylidene units of oligomeric derivatives should facilitate solubility in aqueous solutions and may also increase flexibility, possibly permitting formation of hydrogen bonds precluded by the presence of the protecting groups. However, care has to be taken that the conditions necessary for the efficient removal of the isopropylidene groups do not also cause amide or ester hydrolysis, or epimerization. A successful approach is illustrated by the deprotection of the dimer **17** under relatively mild conditions, Scheme 5.



Scheme 5 *Reagents and conditions*: (i) TFA–$CHCl_3$ (1 : 1) with trace of water.

Treatment of the dimer **17** at room temperature with a 1 : 1 mixture of trifluoroacetic acid (TFA)–chloroform in the presence of a trace of water gave the deprotected dimer **27** in 70% yield, which is isolable by standard chromatographic techniques.

Summary

THF amino acids are potentially important building blocks for the formation of unnatural biopolymeric materials with interesting properties. In this case, homooligomeric derivatives of an all-*cis* D-*galacto* monomer unit did not adopt well defined secondary structures. It has been demonstrated that the inversion of a single stereocentre on the THF ring can have profound effects for solution conformation, emphasizing the different characteristics of each THF amino acid building block. This inbuilt conformational diversity bodes well for the generation of a monomer alphabet tailored for the formation of foldameric materials which may ultimately possess tertiary structure. The following paper[40] provides an indication that a stereoisomeric *allo*-THF amino acid forms oligomers with a predisposition to act as a β-turn mimetic.[41]

Experimental

Melting points were recorded on a Kofler block and are uncorrected. ^{1}H nuclear magnetic resonance (δ_H) spectra were recorded on a Bruker DPX 400 spectrometer (at 400 MHz) at ambient probe temperatures (≈298 K). TOCSY spectra were collected using the MLEV-17 mixing sequence with mixing times of up to 100 ms. HSQC spectra were acquired with gradient selection. ROESY spectra were acquired with either a continuous-wave spin-lock or with the 180_x–180_{-x} composite spin-lock sequence to suppress TOCSY transfers (Tr-ROESY). Mixing times were 300 ms.[42] Coupling constants (J) were measured in Hz and are averaged. Carbon nuclear magnetic resonance (δ_C) spectra were recorded on a Varian Gemini 200 (at 50.3 MHz) or, where stated, on a Bruker DPX 400 spectrometer (at 100 MHz); multiplicities were assigned using a distortionless-enhancement-by-polarization-transfer (DEPT) sequence. All chemical shifts are quoted on the δ-scale using residual solvent as internal standard. IR spectra were recorded on a Perkin-Elmer 1750 IR FT spectrophotometer using a 1 mm cell for solution measurements. Mass spectra were recorded on VG Micromass 20-250, ZAB 1F, Micromass Platform 1 or Trio-1 GCMS (DB-5 column) spectrometers using chemical ionization (CI, NH_3), atmospheric pressure chemical ionization (APCI) or electrospray techniques (ES) as stated. Optical rotations were measured on a Perkin-Elmer 241 polarimeter with a path length of 1 dm. Concentrations are given in g 100 ml^{-1}. $[a]_D$-Values are in 10^{-1} deg cm^2 g^{-1}. Hydrogenations were executed under an atmosphere of hydrogen gas maintained by an inflated balloon. Microanalyses were performed by the microanalysis service of the Inorganic Chemistry Laboratory, Oxford. Thin layer chromatography (TLC) was carried out on aluminium sheets coated with $60F_{254}$ silica or glass plates coated with silica blend 41. Plates were developed using a spray of 0.2% w/v cerium(IV) sulfate and 5% ammonium molybdate in 2 M sulfuric acid. Flash chromatography was carried out using Sorbsil C60 40/60 silica. Solvents and commercially available reagents were dried and purified before use according to standard procedures; in particular, DCM was refluxed over, and distilled from, calcium hydride; pyridine was distilled from, and stored over, potassium hydroxide; and THF was distilled, under nitrogen, from a solution dried with sodium in the presence of benzophenone. A solution of KH_2PO_4 (85 g) and NaOH (14.5 g) in distilled water (950 ml) was used as a pH 7 buffer solution.

Methyl 2,5-anhydro-3,4-*O*-isopropylidene-D-*glycero*-D-*galacto*-heptonate 9

The diacetonide **5** (1.50 g, 5.00 mmol) was stirred in 80% v/v aq. acetic acid (50 ml) at 50 °C. After 2 h, TLC (ethyl acetate) indicated conversion of the starting material (R_f 0.8) to a single product (R_f 0.2). The solvent was removed *in vacuo* (co-evaporation with toluene) and the residue purified by flash chromatography (2% methanol in ethyl acetate) to yield *the monoacetonide* **9** (1.28 g, 98%) as a colourless oil; $[a]_D^{24}$ +40.1 (*c* 0.985 in $CHCl_3$) (Found: C, 50.32; H, 6.66. $C_{11}H_{18}O_7$ requires C, 50.38; H, 6.92%); ν_{max}(thin film)/cm^{-1} 3431 (OH), 1763 (C=O, ester); δ_H (400 MHz; $CDCl_3$) 1.34, 1.47 [6H, 2 × s, $C(CH_3)_2$], 3.68 (1H, dd, $J_{5,4}$ 3.7, $J_{5,6}$ 7.8, H-5), 3.81 (3H, s, CO_2CH_3), 3.81 (1H, dd, $J_{7,6}$ 5.1, $J_{7,7'}$ 11.5, H-7), 3.92 (1H, dd, $J_{7',6}$ 3.3, H′-7), 4.14 (1H, ddd, H-6), 4.27 (1H, d, $J_{2,3}$ 4.4, H-2), 4.87 (1H, dd, $J_{4,3}$ 6.1, H-4), 5.00 (1H, dd, H-3); δ_C [50.3 MHz; $(CD_3)_2CO$] 25.2, 26.1 [2 × q, $C(CH_3)_2$], 51.9 (q, CO_2CH_3), 64.9 (t, C-7), 69.3, 81.4, 81.6, 82.3, 82.5 (5 × d, C-2, -3, -4, -5, -6), 113.3 [s, $C(CH_3)_2$], 168.9 (s, C=O); *m/z* (CI, NH_3) 280 (M + NH_4^+, 100%), 263 (M + H^+, 85).

Methyl 2,5-anhydro-3,4-*O*-isopropylidene-D-galactonate 11

Periodic acid (4.38 g, 19.2 mmol) was added to a stirred solution of the diol **9** (4.58 g, 17.5 mmol) in THF (90 ml). After 10 min, TLC (ethyl acetate) indicated the absence of the starting material (R_f 0.2). The reaction mixture was pre-adsorbed onto silica gel, filtered through silica gel (eluted with ethyl acetate) and the solvent removed *in vacuo*. The crude residue was used without further purification.

Sodium cyanoborohydride (1.10 g, 17.5 mmol) was added to a stirred solution of the crude residue in glacial acetic acid (50 ml). After 20 min, TLC (ethyl acetate) indicated formation of a major product (R_f 0.2). The solvent was removed *in vacuo* (co-evaporation with toluene), and the residue was purified by flash chromatography (toluene–acetone 4 : 1) to yield *the primary alcohol* **11** (3.56 g, 88%) as a white crystalline solid, mp 66 °C (from ethyl acetate–hexane); $[a]_D^{20}$ +39.1 (*c* 1.015 in $CHCl_3$)

(Found: C, 51.68; H, 6.94. $C_{10}H_{16}O_6$ requires C, 51.72; H, 6.94%); ν_{max}(thin film)/cm^{-1} 3502 (OH), 1757 (C=O, ester); δ_H (400 MHz; $CDCl_3$ with D_2O) 1.37, 1.51 [6H, 2 × s, $C(CH_3)_2$], 3.82 (1H, ddd, $J_{5,4}$ 3.8, $J_{5,6}$ 4.8, $J_{5,6'}$ 6.6, H-5), 3.88 (3H, s, CO_2CH_3), 4.14 (1H, dd, $J_{6,6'}$ 12.0, H-6), 4.09 (1H, dd, H′-6), 4.34 (1H, d, $J_{2,3}$ 4.4, H-2), 4.84 (1H, dd, $J_{4,3}$ 6.0, H-4), 5.05 (1H, dd, H-3); δ_C (100.6 MHz; $CDCl_3$) 24.8, 25.7 [2 × q, $C(CH_3)_2$], 52.3 (q, CO_2CH_3), 60.5 (t, C-6), 80.4, 80.7, 81.6, 81.7 (4 × d, C-2, -3, -4, -5), 113.5 [s, $C(CH_3)_2$], 168.1 (s, C=O); *m*/*z* (APCI+) 233 (M + H$^+$, 60%), 175 (100).

Methyl 2,5-anhydro-6-azido-6-deoxy-3,4-*O*-isopropylidene-D-galactonate 14

Trifluoromethanesulfonic anhydride (1.99 ml, 11.8 mmol) was added dropwise to a stirred solution of the primary alcohol **11** (2.11 g, 9.10 mmol) in dichloromethane (75 ml) and dry pyridine (2.94 ml, 36.4 mmol) at −30 °C. After 20 min, TLC (ethyl acetate–hexane 1:1) indicated complete conversion of the starting material (R_f 0) to a single product (R_f 0.6). The reaction mixture was diluted with dichloromethane (100 ml), washed with 2 M hydrochloric acid (40 ml), and the aqueous layer was extracted with dichloromethane (40 ml). The combined organic layers were washed with pH 7 buffer (80 ml), dried ($MgSO_4$), filtered, and concentrated *in vacuo* to afford triflate **13**, which was used without further purification.

Sodium azide (0.77 g, 11.8 mmol) was added to a stirred solution of crude triflate **13** in dimethylformamide (120 ml). After 30 min, TLC (ethyl acetate–hexane 1:2) indicated complete conversion of the starting material (R_f 0.3) to a single product (R_f 0.4). The solvent was removed *in vacuo* (co-evaporation with toluene), and the residue was dissolved in ethyl acetate (300 ml), then washed with distilled water (100 ml) containing a trace of brine. The aqueous layer was extracted with ethyl acetate (50 ml), and the combined organic layers were dried ($MgSO_4$), filtered, and concentrated *in vacuo*. The residue was purified by flash chromatography (ethyl acetate–hexane 1:3) to yield *the azide* **14** (2.05 g, 88%) as a colourless oil; $[a]_D^{24}$ +29.3 (*c* 1.005 in $CHCl_3$) (Found: C, 46.80; H, 6.08; N, 16.04. $C_{10}H_{15}N_3O_5$ requires C, 46.69; H, 5.88; N, 16.33%); ν_{max}(thin film)/cm^{-1} 2105 (N_3), 1761 (C=O, ester); δ_H (400 MHz; $CDCl_3$) 1.33, 1.45 [6H, 2 × s, $C(CH_3)_2$], 3.67–3.75 (3H, m, H-5, H_2-6), 3.81 (3H, s, CO_2CH_3), 4.27 (1H, d, $J_{2,3}$ 4.3, H-2), 4.74 (1H, dd, $J_{4,3}$ 6.0, $J_{4,5}$ 3.1, H-4), 5.00 (1H, dd, H-3); δ_C (100.6 MHz; $CDCl_3$) 25.1, 25.7 [2 × q, $C(CH_3)_2$], 49.1 (t, C-6), 52.2 (q, CO_2CH_3), 79.9, 79.9, 81.0, 81.6 (4 × d, C-2, -3, -4, -5), 113.7 [s, $C(CH_3)_2$], 167.2 (s, C=O); *m*/*z* (APCI+) 230 (M − N_2 + H$^+$, 91%), 154 (100).

2,5-Anhydro-3,4-*O*-isopropylidene-D-galactitol 12

Lithium borohydride (2 M solution in THF; 0.65 ml, 1.3 mmol) was added to a stirred solution of methyl ester **11** (0.12 g, 0.50 mmol) in THF (5 ml) at 0 °C. The reaction mixture was allowed to warm to room temperature, and TLC (10% methanol in ethyl acetate) after 4 h indicated the absence of the starting material (R_f 0.6) and the formation of a major product (R_f 0.3). The reaction mixture was quenched by stirring with an excess of methanol and the solvent was removed *in vacuo*. The residue was purified by flash chromatography (8% methanol in ethyl acetate), followed by crystallization (from ethyl acetate) to yield 2,5-anhydro-3,4-*O*-isopropylidene-D-galactitol **12** (0.050 g, 49%) as a colourless crystalline solid, mp 129 °C (from ethyl acetate) [lit.,[31] mp 130 °C]; $[a]_D^{24}$ +0.0 (*c* 1.00 in $CHCl_3$) (Found: C, 53.17; H, 8.12. Calc. for $C_9H_{16}O_5$: C, 52.93; H, 7.90%); δ_H (400 MHz; $CDCl_3$) 1.31, 1.48 [6H, 2 × s, $C(CH_3)_2$], 2.59 (2H, br s, OH), 3.71–3.73 (2H, m, H-2, -5), 3.93–3.99 (4H, br m, H_2-1, -6), 4.76–4.79 (2H, m, H-3, -4); δ_C (100.6 MHz; $CDCl_3$) 24.3, 25.7 [2 × q, $C(CH_3)_2$], 61.0 (t, C-1, -6), 81.4, 81.4 (2 × d, C-2, -5 and C-3, -4), 112.8 [s, $C(CH_3)_2$]; *m*/*z* (CI, NH_3) 222 (M + NH_4^+, 100%), 205 (M + H$^+$, 80).

Isopropyl 2,5-anhydro-6-azido-6-deoxy-3,4-*O*-isopropylidene-D-galactonate 4

The methyl ester **14** (0.91 g, 3.5 mmol) was stirred in a 5% v/v solution of hydrogen chloride in propan-2-ol (20 ml) at 80 °C. After 24 h, TLC (ethyl acetate–hexane 2:1) indicated complete conversion of the starting material (R_f 0.7) to a major product (R_f 0.8) and a minor product (R_f 0.3). The reaction mixture was cooled and acetone (50 ml) was added. After 1 h, TLC (ethyl acetate–hexane 2:1) indicated complete conversion of the minor product (R_f 0.3) to the major product (R_f 0.8). Sodium hydrogen carbonate (5 g, excess) was added and the reaction mixture was stirred for 1 h, then filtered through Celite (eluted with acetone). The solvent was removed *in vacuo* and the residue was purified by flash chromatography (ethyl acetate–hexane 1:5) to yield *the isopropyl ester* **4** (0.93 g, 93%) as a colourless oil; $[a]_D^{24}$ +41.9 (*c* 1.02 in $CHCl_3$) (Found: C, 50.48; H, 6.75; N, 14.51. $C_{12}H_{19}N_3O_5$ requires C, 50.52; H, 6.71; N, 14.73%); ν_{max}(thin film)/cm^{-1} 2103 (N_3), 1756 (C=O, ester); δ_H (400 MHz; $CDCl_3$) 1.27, 1.28 [6H, 2 × d, *J* 6.3, $CH(CH_3)_2$], 1.31, 1.44 [6H, 2 × s, $C(CH_3)_2$], 3.63–3.73 (3H, m, H-5, H_2-6), 4.20 (1H, d, $J_{2,3}$ 4.4, H-2), 4.72 (1H, dd, $J_{4,3}$ 6.0, $J_{4,5}$ 2.9, H-4), 4.98 (1H, dd, H-3), 5.15 [1H, sept, $CH(CH_3)_2$]; δ_C (50.3 MHz; $CDCl_3$) 21.6, 21.7 [2 × q, $CH(CH_3)_2$], 25.2, 25.7 [2 × q, $C(CH_3)_2$], 49.1 (t, C-6), 68.9 [d, $CH(CH_3)_2$], 79.8, 79.9, 80.9, 81.7 (4 × d, C-2, -3, -4, -5), 113.5 [s, $C(CH_3)_2$], 166.3 (s, C=O); *m*/*z* (APCI+) 258 (M − N_2 + H$^+$, 100%).

Isopropyl 2,5-anhydro-6-(2,5-anhydro-6-azido-6-deoxy-3,4-*O*-isopropylidene-D-galactonamido)-6-deoxy-3,4-*O*-isopropylidene-D-galactonate 17 and isopropyl 2,5-anhydro-6-(2,5-anhydro-6-azido-6-deoxy-3,4-*O*-isopropylidene-D-talonamido)-6-deoxy-3,4-*O*-isopropylidene-D-galactonate 18

Method 1. A solution of the azide **4** (0.14 g, 0.48 mmol) in propan-2-ol (8 ml) was stirred under an atmosphere of hydrogen in the presence of palladium black (19 mg). After 2 h, TLC (ethyl acetate–hexane 2:1) indicated the absence of the starting material (R_f 0.8) and formation of a major product (R_f 0). The reaction mixture was filtered through Celite (eluted with propan-2-ol) and the solvent was removed *in vacuo* to give the crude amine **15**.

Aq. sodium hydroxide (1.38 ml; 1 M) was added to a stirred solution of the methyl ester **14** (0.180 g, 0.69 mmol) in 1,4-dioxane (6 ml). The reaction mixture was stirred at room temperature and TLC (ethyl acetate–hexane 2:1) after 2 h indicated complete conversion of the starting material (R_f 0.7) to a major product (R_f 0). The solvent was removed *in vacuo* (co-evaporation with toluene), the residue was dissolved in distilled water, and the solution was stirred with Amberlite IR-120(H$^+$) resin for 5 min. The resin was removed by filtration and the filtrate was concentrated *in vacuo* to give the crude carboxylic acid **16**.

1-[3-(Dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (0.20 g, 1.04 mmol) was added to a stirred solution of the crude acid **16**, 1-hydroxybenzotriazole (0.140 g, 1.04 mmol) and diisopropylethylamine (0.12 ml, 0.69 mmol) in dichloromethane (3 ml) at 0 °C. The mixture was stirred for 10 min and then a solution of the crude amine **15** in dichloromethane (3 ml) was added. The stirred reaction mixture was allowed to warm to room temperature. After 22 h, TLC (ethyl acetate–hexane 6:1) indicated the formation of a major product (R_f 0.2). The reaction mixture was diluted with dichloromethane (150 ml) and washed with 2 M HCl (30 ml). The aqueous layer was extracted with dichloromethane (60 ml) and the combined organic layers were washed with pH 7 buffer (60 ml), dried ($MgSO_4$), filtered, concentrated *in vacuo*, and the residue was purified by flash chromatography (ethyl

acetate–hexane 6:1) to yield *the galactonoyl-galactonate dimer* **17** (0.15 g, 63%) as an amorphous solid; $[\alpha]_D^{24}$ +73.3 (*c* 1.23 in $CHCl_3$); *m/z* (CI+) 485.2240 (M + H^+. $C_{21}H_{32}N_4O_9$ + H^+ requires *m/z*, 485.2248); ν_{max}(thin film)/cm^{-1} 3430 (NH), 2102 (N_3), 1755 (C=O, ester), 1678 (C=O, amide I), 1536 (C=O, amide II); δ_H (400 MHz; C_6D_6) 1.02, 1.07, 1.29, 1.42 [12H, 4 × s, 2 × $C(CH_3)_2$], 1.04, 1.08 [6H, 2 × d, *J* 6.3, $CH(CH_3)_2$], 3.01 (1H, m, H-5_A), 3.11–3.21 (2H, m, H_2-6_A), 3.56 (1H, ddd, $J_{5B,4B}$ 3.6, $J_{5B,6'B}$ 4.8, $J_{5B,6B}$ 7.5, H-5_B), 3.66 (1H, m, H′-6_B), 3.70 (1H, d, $J_{2B,3B}$ 4.4, H-2_B), 3.78 (1H, d, $J_{2A,3A}$ 3.9, H-2_A), 3.82 (1H, dd, $J_{4A,3A}$ 5.9, $J_{4A,5A}$ 3.8, H-4_A), 3.96 (1H, ddd, $J_{6B,6'B}$ 13.8, $J_{6B,NH}$ 5.1, H-6_B), 4.07 (1H, dd, $J_{4B,3B}$ 6.0, H-4_B), 4.40 (1H, dd, H-3_B), 4.51 (1H, dd, H-3_A), 5.10 [1H, sept, $CH(CH_3)_2$], 6.75 (1H, dd, $J_{NH,6'B}$ 6.4, NH); δ_C (100.6 MHz; $CDCl_3$) 21.7, 21.8 [2 × q, $CH(CH_3)_2$], 24.3, 25.2, 25.7, 25.8 [4 × q, 2 × $C(CH_3)_2$], 37.8 (t, CH_2NH), 49.4 (t, CH_2N_3), 68.8 [d, $CH(CH_3)_2$], 79.4, 79.7, 80.1, 80.2, 81.0, 81.2, 81.8 (7 × d, C-2A, -2B, -3A, -3B, -4A, -4B, -5A, -5B), 113.0, 113.3 [2 × s, 2 × $C(CH_3)_2$], 166.6, 167.2 (2 × s, 2 × C=O); *m/z* (APCI+) 485 (M + H^+, 100%).

Method 2. A solution of the azide **4** (0.332 g, 1.2 mmol) in propan-2-ol (15 ml) was stirred under an atmosphere of hydrogen in the presence of palladium black (35 mg). After 3 h, TLC (ethyl acetate–hexane 2:1) indicated conversion of the starting material (R_f 0.7) to a major product (R_f 0). The reaction mixture was filtered through Celite (eluted with propan-2-ol) and the solvent was removed *in vacuo* to give crude amine **15**.

Aq. sodium hydroxide (1.3 ml; 1 M) was added to a stirred solution of the methyl ester **14** (0.329 g, 1.3 mmol) in 1,4-dioxane (10 ml). The reaction mixture was stirred for 3 h at room temperature, when TLC (ethyl acetate–hexane 2:1) indicated complete conversion of the starting material (R_f 0.7) to a major product (R_f 0). The solvent was removed *in vacuo* (co-evaporation with toluene), the residue was dissolved in distilled water, and the solution was stirred with Amberlite IR-120(H^+) resin for 5 min. The resin was removed by filtration and the filtrate was concentrated *in vacuo* to give the crude acid **16**.

O-(Benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium tetrafluoroborate (0.50 g, 1.6 mmol) and diisopropylethylamine (0.227 ml, 1.3 mmol) were added to a stirred solution of the crude amine **15** and acid **16** in dimethylformamide (1 ml). After 16 h, TLC (ethyl acetate–hexane 6:1) indicated formation of a major product (R_f 0.3) and the solvent was removed *in vacuo* (co-evaporation with toluene). The residue was dissolved in ethyl acetate (250 ml), and washed successively with 2 M HCl (100 ml), pH 7 buffer (200 ml) and brine (200 ml), dried ($MgSO_4$), filtered, concentrated *in vacuo*, and the residue was purified by flash chromatography (ethyl acetate–hexane 8:1) to yield *the galactonoyl-galactonate dimer* **17** (0.473 g, 84%) as an amorphous solid; data as given above. Further elution yielded *the epimeric talonoyl-galactonate dimer* **18** (0.011 g, 2%) as an amorphous solid; $[\alpha]_D^{24}$ +30.4 (*c* 0.415 in $CHCl_3$); *m/z* (CI+) 485.2249 (M + H^+. $C_{21}H_{32}N_4O_9$ + H^+ requires *m/z*, 485.2248); ν_{max}(thin film)/cm^{-1} 3422 (NH), 2103 (N_3), 1756 (C=O, ester), 1674 (C=O, amide I), 1532 (C=O, amide II); δ_H (400 MHz; C_6D_6) 1.12, 1.18 [6H, 2 × d, *J* 6.3, 2 × $CH(CH_3)_2$], 1.13, 1.14, 1.42, 1.52 [12H, 4 × s, 4 × $C(CH_3)_2$], 3.33 (1H, dd, $J_{6A,5A}$ 5.8, $J_{6A,6'A}$ 12.6, H-6_A), 3.39 (1H, m, H-5_B), 3.46 (1H, dd, $J_{6'A,5A}$ 6.9, H′-6_A), 3.60 (1H, ddd, $J_{6B,5B}$ 6.9, $J_{6B,6'B}$ 13.9, $J_{6B,NH}$ 5.1, H-6_B), 3.86 (1H, d, $J_{2B,3B}$ 4.4, H-2_B), 3.92–3.99 (3H, m, H′-6_B, -4_B, -5_A), 4.15 (1H, m, H-4_A), 4.46 (1H, m, H-3_B), 4.55 (1H, m, H-2_A), 5.20 [1H, sept, $CH(CH_3)_2$], 5.35 (1H, m, H-3_A), 6.94 (1H, m, CH_2NH); δ_C (100.6 MHz; $CDCl_3$) 21.7, 21.8 [2 × q, 2 × $CH(CH_3)_2$], 24.8, 25.0, 26.0, 26.2 [4 × q, 4 × $C(CH_3)_2$], 38.4 (t, CH_2NH), 49.8 (t, CH_2N_3), 68.5 [d, $CO_2CH(CH_3)_2$], 78.9, 80.5, 80.7, 80.9, 80.9, 82.3, 84.1, 84.5 (8 × d, 8 × CH), 112.9, 113.3 [2 × s, 2 × $C(CH_3)_2$], 166.3, 169.2 (2 × s, 2 × C=O); *m/z* (APCI+) 485 (M + H^+, 100%).

Isopropyl 2,5-anhydro-6-[2,5-anhydro-6-azido-6-deoxy-3,4-*O*-isopropylidene-D-galactonamido-(*N*→6)-2,5-anhydro-6-deoxy-3,4-*O*-isopropylidene-D-galactonamido-(*N*→6)-2,5-anhydro-6-deoxy-3,4-*O*-isopropylidene-D-galactonamido]-6-deoxy-3,4-*O*-isopropylidene-D-galactonate 22 and isopropyl 2,5-anhydro-6-[2,5-anhydro-6-azido-6-deoxy-3,4-*O*-isopropylidene-D-galactonamido-(*N*→6)-2,5-anhydro-6-deoxy-3,4-*O*-isopropylidene-D-talonamido-(*N*→6)-2,5-anhydro-6-deoxy-3,4-*O*-isopropylidene-D-galactonamido]-6-deoxy-3,4-*O*-isopropylidene-D-galactonate 21

Method 1. A solution of dimer **17** (0.050 g, 0.10 mmol) in propan-2-ol (2 ml) was stirred under an atmosphere of hydrogen in the presence of palladium black (10 mg). After 6 h, TLC (ethyl acetate) indicated the absence of the starting material (R_f 0.3) and the formation of a major product (R_f 0). The reaction mixture was filtered through Celite (eluted with propan-2-ol) and the solvent was removed *in vacuo* to give crude dimer amine **19**.

Aq. sodium hydroxide (0.15 ml; 1 M) was added to a stirred solution of dimer **17** (0.050 g, 0.10 mmol) in 1,4-dioxane (2 ml). The reaction mixture was stirred at room temperature and TLC (ethyl acetate) after 24 h indicated complete conversion of the starting material (R_f 0.3) to a major product (R_f 0). The solvent was removed *in vacuo* (co-evaporation with toluene), the residue was dissolved in distilled water, and the solution was stirred with Amberlite IR-120(H^+) resin for 5 min. The resin was removed by filtration and the filtrate was concentrated *in vacuo* to give crude dimer acid **20**.

1-[3-(Dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (0.029 g, 0.15 mmol) was added to a stirred solution of the crude dimer acid **20**, 1-hydroxybenzotriazole (0.020 g, 0.15 mmol) and diisopropylethylamine (0.017 ml, 0.10 mmol) in dichloromethane (1 ml) at 0 °C. The mixture was stirred for 10 min and then a solution of the crude dimer amine **19** in dichloromethane (2 ml) was added. The stirred reaction mixture was allowed to warm to room temperature. After 16 h, TLC (10% methanol in ethyl acetate) indicated the formation of a major product (R_f 0.2). The reaction mixture was diluted with dichloromethane (50 ml) and washed with 2 M HCl (10 ml). The aqueous layer was extracted with dichloromethane (30 ml), and the combined organic layers were washed with pH 7 buffer (20 ml), dried ($MgSO_4$), filtered, and concentrated *in vacuo.* The residue was purified by flash chromatography (10% methanol in ethyl acetate) to yield *the tetragalactonoyl tetramer* **22** (0.029 g, 33%) as an amorphous solid; $[\alpha]_D^{24}$ +91.1 (*c* 1.00 in $CHCl_3$); *m/z* (CI+) 883.3969 (M + H^+. $C_{39}H_{58}N_6O_{17}$ + H^+ requires *m/z*, 883.3937); ν_{max}(thin film)/cm^{-1} 3431 (NH), 2102 (N_3), 1755 (C=O, ester), 1682 (C=O, amide I), 1538 (C=O, amide II); δ_H (400 MHz; $CDCl_3$) 1.25–1.35 [18H, m, 4 × $C(CH_3)$, $CH(CH_3)_2$], 1.41–1.51 [12H, m, 4 × $C(CH_3)$], 3.49–3.95 (12H, m, 4 × H-5, 4 × H_2-6), 4.12 (1H, d, $J_{2,3}$ 4.0, H-2), 4.15–4.16 (2H, m, 2 × H-2), 4.18 (1H, d, $J_{2,3}$ 3.9, H-2), 4.67–4.70 (3H, m, 3 × H-4), 4.72 (1H, dd, $J_{4,3}$ 6.0, H-4), 4.91–5.00 (3H, m, 3 × H-3), 5.02 (1H, dd, $J_{3,4}$ 5.9, H-3), 5.14 [1H, sept, *J* 6.3, $CH(CH_3)_2$], 6.77 (2H, m, 2 × NH), 6.95 (1H, dd, $J_{NH,6}$ 5.1, $J_{NH,6'}$ 7.5, NH); δ_C (100.6 MHz; $CDCl_3$) 21.6, 21.8 [2 × q, $CH(CH_3)_2$], 24.2, 24.2, 24.3, 25.3, 25.7, 25.8, 25.9 [7 × q, 4 × $C(CH_3)_2$], 37.7, 37.7, 37.8 (3 × t, 3 × CH_2NH), 49.6 (t, CH_2N_3), 68.8 [d, $CH(CH_3)_2$], 79.0, 79.2, 79.6, 79.7, 79.9, 79.9, 80.2, 80.4, 80.9, 81.3, 81.7, 81.8 (12 × d, 4 × C-2, 4 × C-3, 4 × C-4, 4 × C-5), 112.7, 112.7, 112.9, 113.2 [4 × s, 4 × $C(CH_3)_2$], 166.6, 167.1, 167.6, 167.6 (4 × s, 4 × C=O); *m/z* (APCI+) 883 (M + H^+, 100%).

Method 2. A solution of dimer **17** (0.116 g, 0.240 mmol) in propan-2-ol (3 ml) was stirred under an atmosphere of hydrogen in the presence of palladium black (12 mg). After 6 h, TLC (ethyl acetate) indicated the absence of the starting material (R_f 0.3) and the formation of a major product (R_f 0). The reaction

mixture was filtered through Celite (eluted with propan-2-ol) and the solvent was removed *in vacuo* to give crude dimer amine **19**.

Aq. sodium hydroxide (0.58 ml; 1 M) was added to a stirred solution of dimer **17** (0.139 g, 0.290 mmol) in 1,4-dioxane (3 ml). The reaction mixture was stirred at room temperature and TLC (ethyl acetate) after 15 h indicated complete conversion of the starting material (R_f 0.3) to a major product (R_f 0). The solvent was removed *in vacuo* (co-evaporation with toluene), the residue was dissolved in distilled water, and the solution was stirred with Amberlite IR-120(H^+) resin for 5 min. The resin was removed by filtration and the filtrate was concentrated *in vacuo* to give crude dimer acid **20**.

O-(Benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium tetrafluoroborate (0.100 g, 0.310 mmol) and diisopropylethylamine (0.050 ml, 0.29 mmol) were added to a stirred solution of the crude dimer amine **19** and dimer acid **20** in dimethylformamide (0.25 ml). After 17 h, TLC (15% methanol in ethyl acetate) indicated formation of a major product (R_f 0.3) and the solvent was removed *in vacuo* (co-evaporation with toluene). The residue was dissolved in ethyl acetate (100 ml), and the solution was washed successively with 2 M HCl (20 ml), pH 7 buffer (40 ml) and brine (40 ml). The organic layer was dried ($MgSO_4$), filtered, and concentrated *in vacuo*, and the residue was purified by flash chromatography (12% methanol in ethyl acetate) to yield *the pergalactonoyl tetramer* **22** (0.076 g, 36%) as an amorphous solid; data identical with those for the compound previously described. Further elution yielded *the talonoyl-trigalactonoyl tetramer* **21** (0.013 g, 6%) as an amorphous solid; $[\alpha]_D^{24}$ +74.8 (*c* 0.23 in $CHCl_3$); *m*/*z* (CI+) 883.3935 (M + H^+. $C_{39}H_{58}N_6O_{17}$ + H^+ requires *m*/*z*, 883.3937); ν_{max}(thin film)/cm^{-1} 3431 (NH), 2102 (N_3), 1755 (C=O, ester), 1682 (C=O, amide I), 1538 (C=O, amide II); δ_H (400 MHz; $CDCl_3$) 1.27, 1.28 [6H, 2 × d, $CH(CH_3)_2$], 1.29–1.36 [12H, m, 4 × $C(CH_3)$], 1.42–1.52 [12H, m, 4 × $C(CH_3)$], 3.51–3.93 (11H, m, 3 × H-5, 4 × H-6, 4 × H′-6), 4.06 (1H, m, H-5_B), 4.12 (1H, d, $J_{2C,3C}$ 4.1, H-2_C), 4.17 (1H, d, $J_{2D,3D}$ 4.5, H-2_D), 4.20 (1H, d, $J_{2A,3A}$ 3.9, H-2_A), 4.50 (1H, s, H-2_B), 4.64–4.73 (4H, m, 4 × H-4), 4.93 (1H, dd, $J_{3D,4D}$ 5.8, H-3_D), 4.98 (1H, dd, $J_{3C,4C}$ 5.9, H-3_C), 5.01 (1H, dd, $J_{3A,4A}$ 6.0, H-3_A), 5.15 [1H, sept, *J* 6.3, $CH(CH_3)_2$], 5.22 (1H, dd, $J_{3B,4B}$ 6.2, H-3_B), 6.82 (1H, t, *J* 6.2, N_BH), 6.96 (1H, t, *J* 5.9, N_CH), 7.04 (1H, m, N_DH); δ_C (100.6 MHz; $CDCl_3$) 21.7, 21.8 [2 × q, $CH(CH_3)_2$], 24.2, 24.3, 24.7, 25.3, 25.7, 25.8, 25.9, 26.1 [8 × q, 4 × $C(CH_3)_2$], 37.8, 37.9, 38.1 (3 × t, 3 × CH_2NH), 49.6 (t, CH_2N_3), 68.8 [d, $CH(CH_3)_2$], 78.9, 79.3, 79.8, 80.2, 80.3, 80.4, 81.0, 81.3, 81.7, 81.8, 83.4, 83.8 (12 × d, 4 × C-2, 4 × C-3, 4 × C-4, 4 × C-5), 112.8, 112.9, 113.0, 113.2 [4 × s, 4 × $C(CH_3)_2$], 166.7, 167.4, 167.5, 169.5 (4 × s, 4 × C=O); *m*/*z* (APCI+) 883 (M + H^+, 100%).

Method 3. A solution of trimer **25** (0.034 g, 0.050 mmol) in propan-2-ol (2 ml) was stirred under an atmosphere of hydrogen in the presence of palladium black (4 mg). After 16 h, TLC (8% methanol in ethyl acetate) indicated the absence of the starting material (R_f 0.2) and the formation of a major product (R_f 0). The reaction mixture was filtered through Celite (eluted with propan-2-ol) and the solvent was removed *in vacuo* to give crude trimer amine **26**.

Aq. sodium hydroxide (0.08 ml; 1 M) was added to a stirred solution of methyl 2,5-anhydro-6-azido-6-deoxy-3,4-*O*-isopropylidene-D-galactonate **14** (0.019 g, 0.074 mmol) in 1,4-dioxane (1 ml). The reaction mixture was stirred at room temperature and TLC (ethyl acetate–hexane 2 : 1) after 3 h indicated complete conversion of the starting material (R_f 0.7) to a major product (R_f 0). The solvent was removed *in vacuo* (co-evaporation with toluene), the residue was dissolved in distilled water, and the solution was stirred with Amberlite IR-120(H^+) resin for 5 min. The resin was removed by filtration and the filtrate was concentrated *in vacuo* to give crude monomer acid **16**.

O-(Benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium tetrafluoroborate (0.024 g, 0.075 mmol) and diisopropylethylamine (0.013 ml, 0.074 mmol) were added to a stirred solution of the crude trimer amine **26** and monomer acid **16** in dimethylformamide (0.5 ml). After 18 h, TLC (10% methanol in ethyl acetate) indicated formation of a major product (R_f 0.2) and the solvent was removed *in vacuo* (co-evaporation with toluene). The residue was dissolved in ethyl acetate (30 ml) and washed successively with 2 M HCl (20 ml), pH 7 buffer (20 ml) and brine (20 ml). The organic layer was dried ($MgSO_4$), filtered, and concentrated *in vacuo*, and the residue was purified by flash chromatography (10% methanol in ethyl acetate) to yield *the pergalactonoyl tetramer* **22** (0.026 g, 60%) as an amorphous solid; data identical with those previously described.

Isopropyl 2,5-anhydro-6-[2,5-anhydro-6-azido-6-deoxy-3,4-*O*-isopropylidene-D-galactonamido-(*N*→6)-2,5-anhydro-6-deoxy-3,4-*O*-isopropylidene-D-galactonamido]-6-deoxy-3,4-*O*-isopropylidene-D-galactonate 25

A solution of the dimer **17** (0.114 g, 0.230 mmol) in propan-2-ol (3 ml) was stirred under an atmosphere of hydrogen in the presence of palladium black (12 mg). After 6 h, TLC (ethyl acetate) indicated the absence of the starting material (R_f 0.3) and the formation of a major product (R_f 0). The reaction mixture was filtered through Celite (eluted with propan-2-ol) and the solvent was removed *in vacuo* to give crude dimer amine **19**.

Aq. sodium hydroxide (0.26 ml; 1 M) was added to a stirred solution of the isopropyl monomer **4** (0.066 g, 0.26 mmol) in 1,4-dioxane (3 ml). The reaction mixture was stirred at room temperature and TLC (ethyl acetate–hexane 2 : 1) after 3 h indicated complete conversion of the starting material (R_f 0.7) to a major product (R_f 0). The solvent was removed *in vacuo* (co-evaporation with toluene) and the residue was dissolved in distilled water and stirred with Amberlite IR-120(H^+) resin for 5 min. The resin was removed by filtration and the filtrate was concentrated *in vacuo* to give crude monomer acid **16**.

O-(Benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium tetrafluoroborate (0.098 g, 0.30 mmol) and diisopropylethylamine (0.045 ml, 0.26 mmol) were added to a stirred solution of the crude dimer amine **19** and monomer acid **16** in dimethylformamide (0.5 ml). After 24 h, TLC (8% methanol in ethyl acetate) indicated formation of a major product (R_f 0.2) and the solvent was removed *in vacuo* (co-evaporation with toluene). The residue was dissolved in ethyl acetate (100 ml), and the solution was washed successively with 2 M HCl (20 ml), pH 7 buffer (40 ml) and brine (40 ml), dried ($MgSO_4$), filtered, and concentrated *in vacuo* and the residue was purified by flash chromatography (8% methanol in ethyl acetate) to yield *the pergalactonoyl trimer* **25** (0.111 g, 69%) as an amorphous solid; $[\alpha]_D^{24}$ +97.7 (*c* 1.10 in $CHCl_3$); *m*/*z* (CI+) 684.3106 (M + H^+. $C_{30}H_{45}N_5O_{13}$ + H^+ requires *m*/*z*, 684.3092); ν_{max}(thin film)/cm^{-1} 3430 (NH), 2103 (N_3), 1755 (C=O, ester), 1682 (C=O, amide I), 1537 (C=O, amide II); δ_H (400 MHz; $CDCl_3$) 1.26 [6H, d, $CH(CH_3)_2$], 1.28, 1.31, 1.43, 1.46 [18H, 4 × s, 3 × $C(CH_3)_2$], 3.49 (1H, ddd, $J_{6'Z,5Z}$ 8.0, $J_{6'Z,6Z}$ 14.1, $J_{6'Z,NZH}$ 4.7, H′-6_Z), 3.53–3.61 (3H, m, H_2-6_A, H′-6_Y), 3.74 (1H, dt, $J_{5Z,4Z}$ 3.9, $J_{5Z,6Z}$ 8.0, H-5_Z), 3.79–3.89 (3H, m, H-5_A, -5_Y, -6_Y), 3.96 (1H, ddd, $J_{6Z,NZH}$ 8.0, H-6_Z), 4.14 (2H, m, H-2_Y, -2_Z), 4.24 (1H, d, $J_{2A,3A}$ 4.0, H-2_A), 4.67–4.70 (2H, m, H-4_Y, -4_Z), 4.73 (1H, dd, $J_{4A,3A}$ 5.9, $J_{4A,5A}$ 3.9, H-4_A), 4.92 (1H, dd, $J_{3Z,2Z}$ 4.5, $J_{3Z,4Z}$ 5.9, H-3_Z), 4.96 (1H, dd, $J_{3Y,2Y}$ 4.2, $J_{3Y,4Y}$ 6.0, H-3_Y), 5.01 (1H, dd, H-3_A), 5.14 [1H, sept, *J* 6.3, $CH(CH_3)_2$], 6.79 (1H, m, N_YH), 6.93 (1H, dd, N_ZH); δ_C (100.6 MHz; $CDCl_3$) 21.7, 21.8 [2 × q, $CH(CH_3)_2$], 24.2, 24.2, 25.2, 25.7, 25.8, 25.8 [6 × q, 3 × $C(CH_3)_2$], 37.6, 37.8 (2 × t, 2 × CH_2NH), 49.5 (t, CH_2N_3), 68.8 [d, $CH(CH_3)_2$], 79.0, 79.8, 79.8, 80.1, 80.2, 80.2, 80.9, 81.2, 81.3, 81.8 (10 × d, C-2A, -2B, -2C, -3A, -3B, -3C, -4A, -4B, -4C, -5A, -5B, -5C), 112.8, 112.9, 113.2 [3 × s, 3 × $C(CH_3)_2$], 166.6, 167.4, 167.4 (3 × s, 3 × C=O); *m*/*z* (APCI+) 684 (M + H^+, 15%).

Isopropyl 2,5-anhydro-6-[2,5-anhydro-6-azido-6-deoxy-3,4-*O*-isopropylidene-D-galactonamido-(*N*→6)-2,5-anhydro-6-deoxy-3,4-*O*-isopropylidene-D-galactonamido-(*N*→6)-2,5-anhydro-6-deoxy-3,4-*O*-isopropylidene-D-galactonamido-(*N*→6)-2,5-anhydro-6-deoxy-3,4-*O*-isopropylidene-D-galactonamido-(*N*→6)-2,5-anhydro-6-deoxy-3,4-*O*-isopropylidene-D-galactonamido]-6-deoxy-3,4-*O*-isopropylidene-D-galactonate 24

A solution of the tetramer **22** (0.041 g, 0.046 mmol) in propan-2-ol (4 ml) was stirred under an atmosphere of hydrogen in the presence of palladium black (22 mg). After 28 h, TLC (20% methanol in ethyl acetate) indicated the absence of the starting material (R_f 0.4) and the formation of a major product (R_f 0). The reaction mixture was filtered through Celite (eluted with propan-2-ol) and the solvent was removed *in vacuo* to give crude tetramer amine **23**.

Aq. sodium hydroxide (0.14 ml; 1 M) was added to a stirred solution of dimer **17** (0.034 g, 0.070 mmol) in 1,4-dioxane (2 ml). The reaction mixture was stirred at room temperature and TLC (ethyl acetate) after 18 h indicated complete conversion of the starting material (R_f 0.3) to a major product (R_f 0). The solvent was removed *in vacuo* (co-evaporation with toluene) and the residue was dissolved in distilled water and stirred with Amberlite IR-120(H^+) resin for 5 min. The resin was removed by filtration and the filtrate was concentrated *in vacuo* to give crude dimer acid **20**.

O-(Benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium tetrafluoroborate (TBTU) (0.023 g, 0.072 mmol) and diisopropylethylamine (0.012 ml, 0.069 mmol) were added to a stirred solution of the crude tetramer amine **23** and dimer acid **20** in dimethylformamide (0.5 ml). After 25 h, additional TBTU (0.023 g, 0.072 mmol) was added. After 1 h, TLC (15% methanol in ethyl acetate) indicated formation of a major product (R_f 0.2) and the solvent was removed *in vacuo* (co-evaporation with toluene). The residue was dissolved in ethyl acetate (40 ml), and the solution was washed successively with 2 M HCl (20 ml), pH 7 buffer (20 ml), and brine (20 ml). The organic layer was dried ($MgSO_4$), filtered, and concentrated *in vacuo* and the residue was purified by flash chromatography (20% methanol in ethyl acetate, then 6% methanol in chloroform) to yield the hexamer **24** (0.024 g, 40%) as an amorphous solid; $[\alpha]_D^{24}$ +78.1 (*c* 0.925 in $CHCl_3$); ν_{max}(thin film)/cm^{-1} 3430 (NH), 2102 (N_3), 1755 (C=O, ester), 1666 (C=O, amide I), 1536 (C=O, amide II); δ_H (400 MHz; $CDCl_3$) 1.27–1.36 [24H, m, 8 × $C(CH_3)$], 1.45 [6H, d, $CH(CH_3)_2$], 1.46–1.56 [12H, m, 4 × $C(CH_3)$], 3.53–3.94 (18H, m, 6 × H-5, 6 × H_2-6), 4.11–4.14 (3H, m, 3 × H-2), 4.16 (2H, m, 2 × H-2), 4.18 (1H, d, $J_{2,3}$ 3.9, H-2), 4.65–4.73 (6H, m, 6 × H-4), 4.93 (1H, dd, $J_{3,2}$ 4.5, $J_{3,4}$ 5.9, H-3), 4.96–5.00 (4H, m, 4 × H-3), 5.01 (1H, dd, $J_{3,2}$ 4.0, $J_{3,4}$ 6.0, H-3), 5.14 [1H, sept, *J* 6.3, $CH(CH_3)_2$], 6.75–6.85 (4H, m, 4 × NH), 6.95 (1H, m, NH); δ_C (100.6 MHz; $CDCl_3$) 21.7, 21.8 [2 × q, $CH(CH_3)_2$], 24.2, 24.3, 24.7, 25.3, 25.7, 25.8, 25.8, 25.9, 25.9, 26.1 [10 × q, 6 × $C(CH_3)_2$], 37.7, 37.7, 37.9, 38.0, 38.1 (5 × t, 5 × CH_2NH), 49.6 (t, CH_2N_3), 68.8 [d, $CH(CH_3)_2$], 79.0, 79.0, 79.1, 79.6, 79.8, 79.9, 80.3, 80.4, 81.0, 81.4, 81.7, 81.8, 81.8, 83.4, 83.9 (15 × d, 6 × C-2, 6 × C-3, 6 × C-4, 6 × C-5), 112.7, 112.7, 112.8, 112.8, 113.0, 113.0, 113.2 [7 × s, 6 × $C(CH_3)_2$], 166.6, 167.2, 167.6, 167.6, 167.7 (5 × s, 5 × C=O); *m*/*z* (ES+) 1303.74 (M + Na^+, 100%), 1304.73 (M + Na^+, 64), 1305.74 (M + Na^+, 23), 1306.78 (M + Na^+, 8).

Isopropyl 2,5-anhydro-6-(2,5-anhydro-6-azido-6-deoxy-D-galactonamido)-6-deoxy-D-galactonate 27

The protected dimer **17** (0.032 g, 0.066 mmol) was dissolved in a 1:1 v/v mixture of trifluoroacetic acid and chloroform (2 ml) with a trace of distilled water. The reaction mixture was sonicated and TLC (4% methanol in ethyl acetate) after 3 h indicated the formation of a major product (R_f 0.1). The solvent was removed *in vacuo* and the residue was purified by flash chromatography (4% methanol in ethyl acetate to 10% methanol in ethyl acetate) to yield *the deprotected dimer* **27** (0.018 g, 69%) as an amorphous solid; $[\alpha]_D^{24}$ +31.3 (*c* 0.675 in Me_2CO); *m*/*z* (CI+) 405.1612 (M + H^+. $C_{15}H_{24}N_4O_9$ + H^+ requires *m*/*z*, 405.1622); ν_{max} (thin film)/cm^{-1} 3393 (NH, OH), 2107 (N_3), 1736 (C=O, ester), 1676 (C=O, amide I), 1547 (C=O, amide II); δ_H [400 MHz; $(CD_3)_2CO$] 1.27 [6H, d, *J* 6.3, $CH(CH_3)_2$], 3.60–3.65 (3H, m, H′-6_Y, H_2-6_Z), 3.69 (1H, dd, $J_{6Y,5Y}$ 7.6, $J_{6Y,6'Y}$ 13.0, H-6_Y), 4.04 (1H, dd, $J_{5Z,6Z}$ 6.0, $J_{5Z,6'Z}$ 12.1, H-5_Z), 4.16 (1H, ddd, $J_{5Y,4Y}$ 4.6, $J_{5Y,6'Y}$ 9.4, H-5_Y), 4.29 (1H, dd, $J_{4Y,3Y}$ 4.3, H-4_Y), 4.36 (1H, dd, $J_{4Z,3Z}$ 5.6, $J_{4Z,5Z}$ 5.6, H-4_Z), 4.40 (1H, d, $J_{2Y,3Y}$ 6.6, H-2_Y), 4.45 (1H, d, $J_{2Z,3Z}$ 5.6, H-2_Z), 4.52 (1H, m, H-3_Z), 4.57 (1H, dd, H-3_Y), 5.08 [1H, sept, $CH(CH_3)_2$]; δ_C [100.6 MHz; $(CD_3)_2CO$] 21.9, 22.0 [2 × q, $CH(CH_3)_2$], 39.3 (t, CH_2NH), 51.7 (t, CH_2N_3), 69.3 [d, $CH(CH_3)_2$], 72.1, 72.2, 73.2, 73.9, 79.7, 80.6, 80.7, 81.0 (8 × d, C-2A, -2B, -3A, -3B, -4A, -4B, -5A, -5B), 170.8, 172.4 (2 × s, 2 × C=O); *m*/*z* (APCI+) 405 (M + H^+, 100%).

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