

Hydrogen-Bonding Cooperativity: Using an Intramolecular Hydrogen Bond To Design a Carbohydrate Derivative with a Cooperative Hydrogen-Bond Donor Centre

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Abstract: Neighbouring groups can be strategically located to polarise HO...OH intramolecular hydrogen bonds in an intended direction. A group with a unique hydrogen-bond donor or acceptor character, located at hydrogen-bonding distance to a particular OH group, has been used to initiate the hydrogen-bond network and to polarise a HO...OH hydrogen bond in a predicted direction. This enhanced the donor character of a particular OH group and made it a cooperative hydrogen-bond centre. We have proved that a five-membered-ring intramolecular hydrogen bond established between an amide NH group and a hydroxy group (1,2-e,a), which is additionally

located in a 1,3-*cis*-diaxial relationship to a second hydroxy group, can be used to select a unique direction on the six-membered-ring intramolecular hydrogen bond between the two axial OH groups, so that one of them behaves as an efficient cooperative donor. Talose derivative **3** was designed and synthesised to prove this hydrogen-bonding network by NMR spectroscopy, and the mannopyranoside derivatives **1** and **2** were used as models to demonstrate the presence in solution of the 1,2-

(e,a)/five-membered-ring intramolecular hydrogen bond. Once a well-defined hydrogen-bond is formed between the OH and the amido groups of a pyranose ring, these hydrogen-bonding groups no longer act as independent hydrogen-bonding centres, but as hydrogen-bonding arrays. This introduces a new perspective on the properties of carbohydrate OH groups and it is important for the de novo design of molecular recognition processes, at least in nonpolar media. Carbohydrates **1–3** have shown to be efficient phosphate binders in nonpolar solvents owing to the presence of cooperative hydroxy centres in the molecule.

Keywords: carbohydrates • hydrogen bonding • molecular recognition • supramolecular chemistry

Introduction

Carbohydrate recognition relies upon multiple weak interactions,^[1] of which hydrogen bonds (H-bonds) and van der Waals forces seem to be the most important.^[2] The high content of hydroxy groups in carbohydrates makes the study of carbohydrate OH...XH and OH...X H-bond energetics fundamental to understanding carbohydrate recognition.^[3] Moreover, hydrogen-bonding cooperativity, defined as the change in the strength of a hydrogen bond when a second

hydrogen bond is formed between the H-bond donor or acceptor of the existing hydrogen bond and a third hydrogen-bonding group, may enhance (positive cooperativity) or weaken (negative cooperativity) a hydrogen-bonding interaction.^[4] The relevance of a cooperative hydrogen-bonding network to the molecular recognition of a carbohydrate by a protein receptor has already been demonstrated by NMR spectroscopy in aqueous solution.^[4d] Previous studies by us^[5,6] and others^[7] have shown that the hydroxy groups of carbohydrates show different abilities to form intramolecular hydrogen bonds, depending on their relative position and configuration on the pyranose ring and on the nature of the adjacent functional groups. Their participation implies a polarisation of the hydrogen bonds between OH groups, which modulates the final directionality and strength of the intramolecular OH...OH hydrogen bonds of a given carbohydrate. Additionally, we showed that both the directionality and strength of the intramolecular hydrogen-bonding network of a carbohydrate determine the formation of cooperative or anticooperative hydrogen-bond centres, with conse-

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quent repercussions for the thermodynamics of the intermolecular hydrogen-bonding interactions of a given carbohydrate. This fact is extremely important for defining the nature of the molecular recognition processes in which a given carbohydrate is able to participate, because it determines whether or not “good hydrogen-bond acceptors and/or donors” are formed.

We have applied the concept of cooperativity to achieve effective dimerization of carbohydrates^[8] and binding of pyridine.^[6] Regarding the hydrogen-bonding patterns which are relevant for DNA groove binding, we have investigated the complementarity of sugar hydrogen-bond centres to GC base pair hydrogen-bond centres^[9] and phosphate hydrogen-bonding motifs.^[5] Furthermore, in the latter case, we have been able to quantify the effect of hydrogen-bonding cooperativity in this intermolecular process in apolar solvent. The most relevant results are that 1,2-*trans* cooperative diols and amido alcohols are very stable bidentate H-bonding motifs for this particular interaction.

From this study in apolar solvents, some general rules have been inferred for the prediction of the intramolecular hydrogen-bonding network characteristics of a given carbohydrate, and its influence on the energetics of intended intermolecular recognition processes.^[6] One conclusion was that neighbouring groups could be strategically located to polarise intramolecular hydrogen bonds in an intended direction. This will enhance the donor or acceptor character of a particular OH group and make it a cooperative hydrogen-bond centre.

Once a well-defined hydrogen bond is formed between the OH and amido groups of a sugar pyranose ring, these hydrogen-bonding groups no longer act as independent hydrogen-bonding centres, but as hydrogen-bonding arrays. This introduces a new perspective on carbohydrate OH groups and is extremely important for de novo design of molecular recognition processes, at least in nonpolar media.

Results and Discussion

Design: Our previous work has shown that the 1,3-*cis*-di-axial configuration of sugar diols and amido alcohols in a rigid framework (⁴C₁ pyranose ring) presents an efficient intramolecular six-membered-ring hydrogen-bond that even survives in aqueous solution to a certain extent^[10] (Figure 1 a). Moreover, this offers the possibility of establishing cooperative intermolecular hydrogen bonds.^[5] The intrinsic hydrogen-bonding donor and acceptor character of the OH

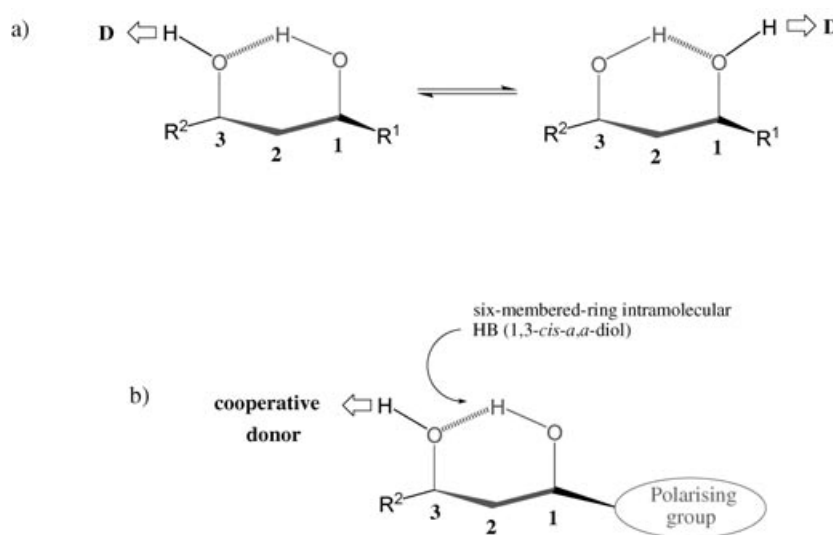


Figure 1. a) Hydrogen-bonding isomers in equilibrium for a 1,3-*cis*-diaxial diol. b) Schematic representation of the polarisation of the 1,3-diol-*cis*-diaxial hydrogen-bonding motif.

group results in a mixture of hydrogen-bonding isomers in solution (Figure 1 a). Thus, to be able to select a particular OH group to behave as a “cooperative donor or acceptor centre”, we envisaged the possibility of using functional groups strategically placed to interact within the hydrogen-bond network and to select a particular OH...OH hydrogen-bond, and thus obtain an energetic advantage from hydrogen-bonding cooperativity (Figure 1 b).

A group with a unique hydrogen-bond donor or acceptor character, located at hydrogen-bonding distance to a particular OH, can be used to initiate the hydrogen-bond network and to polarise it in the desired direction.

We now show that a five-membered-ring intramolecular hydrogen bond established between an amido NH group and a hydroxy group (Figure 2 b) that additionally is in a 1,3-*cis*-diaxial relationship to a second hydroxy group (Figure 2 c) can be used to select a unique direction on the six-membered-ring intramolecular hydrogen bond between di-axial hydroxy groups, so that one of them behaves as an efficient cooperative donor (Figure 2 a).

Hence, we selected two mannopyranoside derivatives (**1** and **2**) that have an amido NH group at the anomeric position (C1) with equatorial (β) orientation (Figure 3). Additionally, OH-2 in the pyranose ring has an axial orientation. We hypothesised on the establishment of a five-membered-ring intramolecular hydrogen bond between these amido NH and (OH-2) hydroxy groups (Figure 2 b). Sugar **2** is a 6-deoxy derivative of **1**, and was synthesized to simplify the hydrogen-bonding network.

Compound **3** is a talopyranose derivative^[11,12] which not only has an equatorial amido NH group at the anomeric position and an axial OH group at the 2-position (as in **1** and **2**), but also an axial hydroxy group in the 4-position. Thus, a 1,3-diaxial diol, involving the 2- and 4-positions of the pyranose ring, is additionally present here (see Figure 2 a).^[13] In this case we hypothesised the presence of the hydrogen-bonding network NH→OH-2→OH-4. According to our ex-

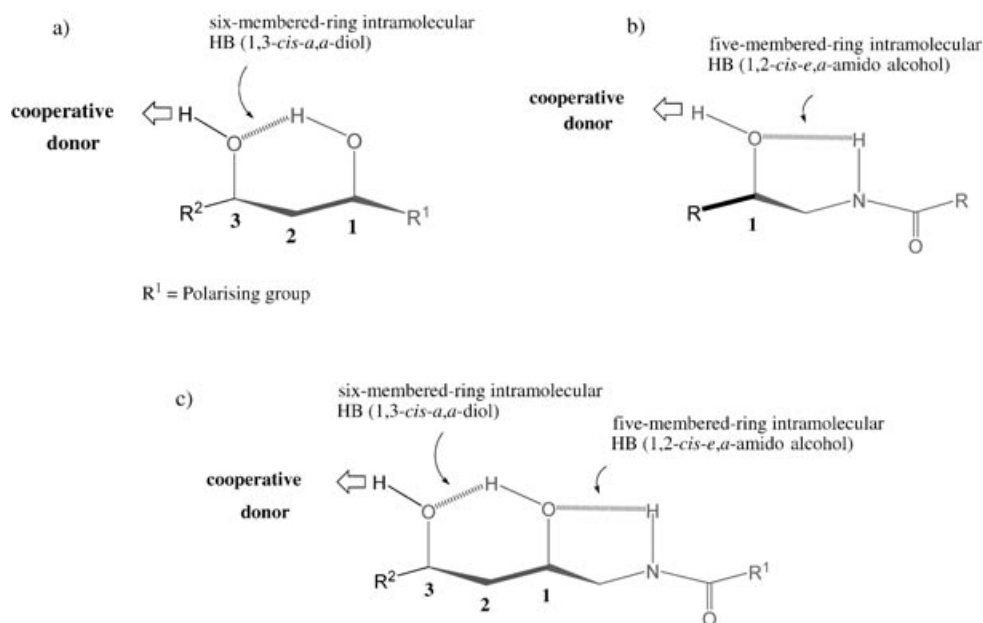


Figure 2. a) Six-membered-ring intramolecular hydrogen bond between a 1,3-*cis*-(*a,a*)-diol; b) five-membered-ring intramolecular hydrogen bond used as a polarizing group; c) cooperative hydrogen-bonding network

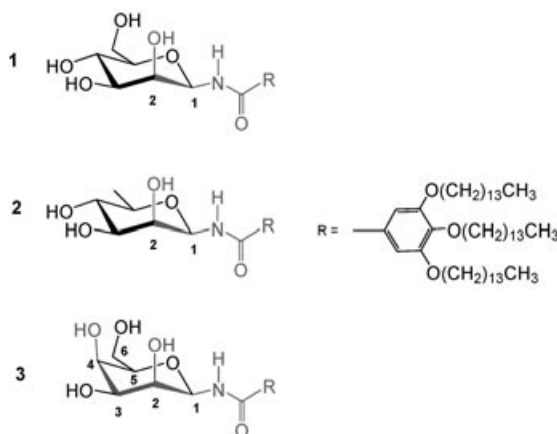


Figure 3. *N*-glycosylamides **1–3**.

pectations, the presence of the five-membered-ring intramolecular hydrogen bond established between an NH amido group and a hydroxy group in the 2-position should polarise the second hydrogen bond (six-membered-ring intramolecular hydrogen bond between the two diaxial OH groups) (Figure 2c), so that OH group at the 4-position behaves as an efficient cooperative donor.

On this basis, we carried out molecular mechanics calculations using the MM2* force field, as integrated in MACRO-MODEL 7.0, on **1**, **2**, and **3**. The GB/SA solvent model for chloroform was used. The first question was whether the structure of the minima predicted by the force field would allow the formation of the five-membered-ring intramolecular hydrogen bond between the NH group in the anomeric position and the axial OH-2 group.

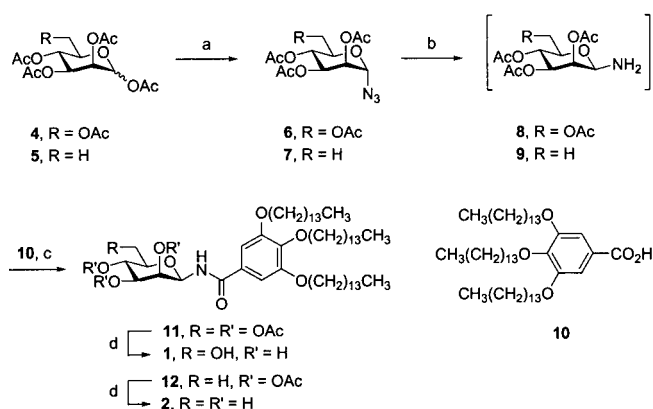
This was indeed the case. In the three compounds, and according to the calculations, the *anti* orientation around angle Φ (defined as H1-C1-N-HN) was always stabilised by more

than 6 kJ mol⁻¹ over the corresponding *syn* geometry. Values between 160 and 175° were found for this angle, corresponding to a NH...OH-2 distance of 2.61–2.62 Å, which suggests the possible presence of the five-membered-ring intramolecular hydrogen bond (see Supporting Information).

Additionally, in all cases, the different possible orientations of the hydroxy groups were considered. For both **1** and **2**, the clockwise orientation of the hydroxy groups (OH-2→OH-3→OH-4) was the most stable conformation. Our MM calculations for the talopyranoside derivative **3** in chloroform predicts the most stable conformer to be that in which O4 accepts a hydrogen atom from both OH-2 and OH-3. In addition, OH-4 acts as a donor to O6 (see Supporting Information).

The second more stable local minimum of **3** ($\Delta E = 1.5$ kJ mol⁻¹) exhibits a cooperative H-bond network of the type OH2→OH4→OH6, without involvement of OH-3 in the network. These results suggest that in talose derivative **3** the NH→OH-2 H-bond is directing or polarising the H-bonding equilibrium of the six-membered intramolecular hydrogen bond towards the presence of a unique OH-2→OH-4 H-bond isomer. Thus, this feature makes OH-4 act as a cooperative donor, which is able to form an additional intramolecular hydrogen bond to OH-6, or probably to establish strong intermolecular hydrogen bonds.

Synthesis: The synthesis of compounds **1** and **2** was achieved straightforwardly starting from D-mannose (Scheme 1). The amino group at the anomeric position was introduced via the corresponding azides **6**^[14,15] and **7**, prepared, respectively, by reaction of the pyranose peracetates **4** and **5**^[16] with TMSN₃ with catalysis by SnCl₄.^[17] Reduction of the azido group with hydrogen in the presence of Raney Ni^[18] took place quantitatively with concomitant almost complete anomerization to generate the corresponding β-aminoglycosides **8** and **9**.



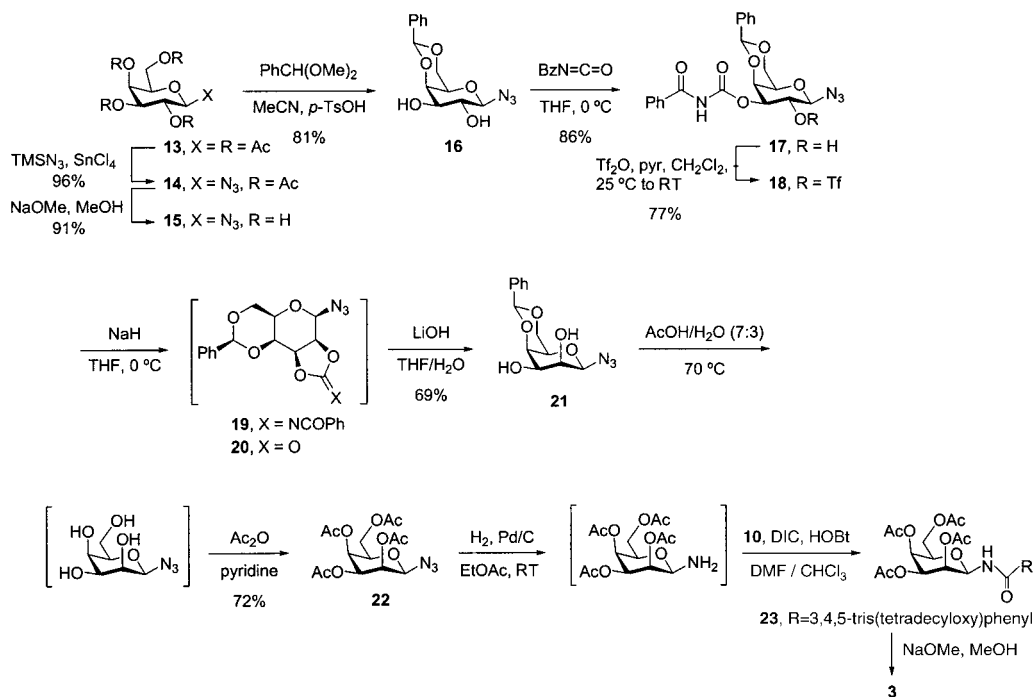
Scheme 1. Synthesis of **1** and **2**. a) TMSN_3 , SnCl_4 , CH_2Cl_2 , RT (**6**, 80%; **7**, 60%); H_2 , Raney Ni W-2, MeOH, RT; c) DIC, HOBT, DMF/ CHCl_3 (**17**), RT (**11**, 33%; **12**, 53%, 2 steps); d) NaOMe, MeOH, RT (**1**, 47%; **2**, 74%). DIC = diisopropylcarbodiimide, HOBT = hydroxybenzotriazole, TMS = trimethylsilyl.

N-Acylation with gallic acid derivative **10**^[19] was carried out with DIC and HOBT^[20] to give **11** and **12**, which were subsequently deacetylated under standard conditions to give the target β -D-mannosyl amides **1** and **2**.

The synthesis of **3** was more cumbersome. A number of synthetic routes could be conceived, the most straightforward of which proceeded via inversion of configuration of a single stereocentre of a readily available hexose, for example, D-galactose (inversion at C-2) or D-mannose (inversion at C-4). After assaying several routes, the most convenient in terms of number of steps and overall yield was found to be that shown in Scheme 2, which employs D-galactopyranose peracetate (**13**) as starting material. By using a descri-

bed procedure,^[21] β -D-galactosyl azide **14** was prepared in an amount of more than 25 g in high yield without requiring any purification. After attempting fruitlessly an oxidation–reduction protocol for the inversion of stereochemistry at C-2, we turned our attention to the protocol developed by Knapp et al.^[22] via intramolecular displacement of a trifluoromethanesulfonate ester by a vicinal carbamoyl group. The application of this protocol to our case would require installation of the carbamoyl group on the hydroxy group at C-3. This was easily performed after standard protecting-group manipulations that involved deacetylation of **14** with sodium methoxide to afford the pure azide **15** in high yield, followed by protection of the hydroxy groups at C-4 and C-6 in the form of a benzylidene acetal to give **16**. Treatment of **16** with a stoichiometric amount of benzoyl isocyanate at 0°C in THF gave selectively the monocarbamate **17** in 86% yield. By this route, **17** can be prepared in just four steps from β -D-galactose pentaacetate in 61% overall yield. Treatment of **17** with triflic anhydride and pyridine gave the unstable trifluoromethanesulfonic ester **18**, which was immediately used for the intramolecular displacement reaction.

Treatment of triflate **18** in THF at 0°C with an excess of NaH according to the procedure of Knapp et al. afforded a mixture of the *N*-benzoylimidate **19** and its hydrolysis product, the carbonate **20**. The crude reaction mixture was dissolved in THF and treated with a 2 M aqueous solution of lithium hydroxide monohydrate to afford D-talosyl azide **21** in 69% overall yield from **18**. The β -D-talo configuration of **21** could be readily confirmed from the ^1H NMR spectrum, which showed characteristic proton–proton coupling constants $^3J_{1,2}$ and $^3J_{2,3}$ of 3.5 Hz. Compound **21** was transformed into the corresponding tetraacetate **22** under standard conditions before reduction of the azide to the amine with hydro-



Scheme 2. Synthesis of **3**.

gen in the presence of 10% Pd/C and subsequent *N*-acylation with gallic acid derivative **10** to afford amide **23**. Deacetylation of **23** provided the final D-talose derivative **3**.

Structural studies: Conformational analysis of **1–3** was carried out by NMR spectroscopy in CDCl₃ solution. Full assignment of the NMR spectra was performed by standard 1D and 2D NMR experiments (selective decoupling, COSY, TOCSY and NOESY). The β configuration of the amido group at the anomeric position was established on the basis of NOESY data. The NOEs observed between H1, H3 and H5 resonances of the pyranose ring prove the presence of the β isomer for **1**, **2** and **3**.

The presence of hydrogen bonds in solution can be established^[11] by the study of 1) chemical shift values, 2) temperature coefficients ($\Delta\delta/\Delta T$), 3) vicinal coupling constants $^3J_{\text{OH,CH}}$ ^[23] d) rate of exchange with the solvent and e) isotope effects.

Tables 1 and 2 list the chemical shifts, vicinal coupling constants and temperature coefficients for the NH and OH resonances of **1–3** in CDCl₃. All the NMR parameters for **1–**

sembly. The lack of variation in the NMR parameters upon dilution showed that **1–3** do not aggregate in this concentration range.

Hydrogen-bond network in mannopyranoside derivatives 1 and 2: The NMR parameters of the NH amide resonance in both mannopyranoside derivatives **1** and **2** follow the same trend. The large *J* values are indicative of the presence of a *trans* relationship between the NH and CH-1 groups, and the small temperature coefficients are consistent with its involvement in an intramolecular hydrogen bond.

As mentioned above, we carried out molecular mechanics calculations by MM2* on both the *syn* and *anti* conformers of the N-glycosidic linkage of **1** and **2**. The *anti* orientation around the ϕ angle, defined as H1-C1-N-H, was always stabilised by more than 66 kJ mol⁻¹ relative to the corresponding *syn* geometry (ϕ values between 160 and 175° were found). Additionally, the distance between NH and OH-2 in the *anti* H1-C-N-H arrangement [*d* = 2.59 Å (**1**), 2.39 Å (**2**)] is in agreement with an intramolecular hydrogen bond, while in the *syn* conformer, the longer NH...OH-2 distance does not allow the formation of an intramolecular hydrogen bond.^[24]

The OH resonances of both **1** and **2** were detected as sharp doublets at low concentration (ca. 10⁻⁴ M for **1** and 10⁻³ M for **2**). The *J* values are in the range of those expected for OH groups involved in hydrogen bonding^[23] Small (1–4 Hz) and large (9–11 Hz) *J* values are indicative of protons fixed in an hydrogen bond. The same trend is observed for all the $\Delta\delta/\Delta T$ (within the range expected for an OH group involved in an hydrogen bond). The only exception is the OH-6 resonance in **1**, which has a temperature coefficient of -8 ppb K⁻¹ and a *J* value of 5 Hz, characteristic of a free OH group.

Thus, since according to the MM2* data the only OH at hydrogen-bonding distance to the amide NH is OH-2 and, significantly, in both **1** and **2**, OH-2 exhibits small *J* and $\Delta\delta/\Delta T$ values, its involvement in an intramolecular hydrogen bond seems to be granted.

To obtain additional experimental evidence for the presence of this NH→OH-2 intramolecular hydrogen bond, we also carried out partial deuteration experiments^[25] on **1** and **2** in CDCl₃ (Table 2). The NMR spectra were recorded 3 h after the addition of CD₃OD. In the case of **1**, it was not possible to observe the signal of the amide group, because it shifts and overlaps with the CDCl₃ signal upon addition of methanol.

The NH resonance of compound **2** showed an isotope effect of 5 ppb (see Supporting Information), which confirmed the presence of an intramolecular hydrogen bond between the amido β -NH group in the anomeric position and the axial OH-2 group in the pyranose ring. This is a five-membered-(e,a)-ring intramolecular hydrogen bond that makes the hydroxy group OH-2 a cooperative donor. This result is in accordance with the *J* value and temperature coefficients found for the NH resonance.

Additionally, in the case of **1**, an isotope effect was also detected for OH-2 (5 ppb), again consistent with its involvement in an intramolecular hydrogen bond (Table 2).

Table 1. Chemical shifts [ppm], coupling constants [Hz] and temperature coefficients $\Delta\delta/\Delta T$ of hydroxy and amide groups of **1–3** in CDCl₃.

		OH-2	OH-3	OH-4	OH-6	NH
1 ^[a]	δ	2.557	2.574	2.390	2.126	7.144
	<i>J</i>	3.5	4.0	3.0	5.0	9.0
	$\Delta\delta/\Delta T$	-2.2	-2.8	-2.7	-8.0	-2.2
2 ^[b]	δ	2.514	2.527	2.018	—	7.091
	<i>J</i>	3.6	4.2	2.7	—	9.0
	$\Delta\delta/\Delta T$	-2.5	-2.5	-2.9	—	-2.4
3 ^[c]	δ	3.735	3.006	3.937	2.301	7.316
	<i>J</i>	10	9.5	3.0	6.5	9.0
	$\Delta\delta/\Delta T$	-4.3	-2.3	-7.5	-3.9	-2.0

[a] 1.1 × 10⁻⁴ M, 40 °C, 500 MHz. [b] 1.66 × 10⁻³ M, 35 °C, 500 MHz. [c] 1.09 × 10⁻³ M, 22 °C, 500 MHz.

Table 2. Isotope effect [ppb] and level of deuteration [%] for the NH and OH resonances of **1–3**.

		OH-2	OH-3	OH-4	OH-6	NH
1 ^[a]	isotope effect	5	3	2	overlapping	overlapping
	deuteration level	31	31	39	—	—
2 ^[b]	isotope effect	broad	broad	broad	—	5
	deuteration level	—	—	—	—	61
3 ^[c]	isotope effect	6	3	overlapping	3	2
	deuteration level	74	35	—	27	69

[a] 1.1 × 10⁻³ M, 30 °C, 300 MHz. [b] 1.1 × 10⁻⁴ M, 32 °C, 500 MHz. [c] 1.5 × 10⁻³ M, 21 °C, 500 MHz.

3 were measured at relatively low concentrations (between 10⁻³ and 10⁻⁴ M), and additional dilution experiments were also carried out in order to exclude the possibility of self-as-

Thus, all experimental evidence is in agreement with the presence, in apolar solution, of the five-membered-ring intramolecular H-bond between the NH and the OH-2 groups (NH→OH-2) in **1** and **2** (Figure 4).

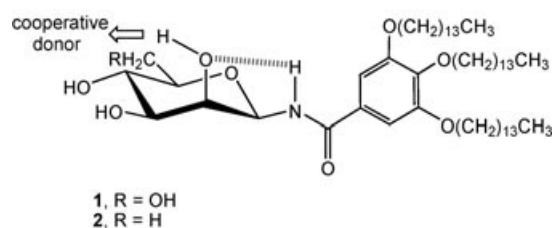


Figure 4. The predicted five-membered-ring intramolecular hydrogen bond 1,2-*cis*-(*a,e*) for **1** and **2**.

Smaller isotopic effects were also measured for the OH-3 and OH-4 resonances in **1**.^[26] This result, together with the small $\Delta\delta/\Delta T$ values of both resonances (Table 1), is also in accordance with the presence of the hydrogen-bond network suggested by molecular modelling, with an orientation OH-2→OH-3→OH-4.^[7b,6]

The hydroxy group OH-6 of **1** has a coupling constant of 5 Hz, which seems to indicate that this group is not involved in a hydrogen bond. Moreover, no isotope effect was observed for this resonance upon partial deuteration.

The NMR and molecular modelling data obtained for **1** and **2** suggest that in this apolar solvent, these *N*-glycosylamides can be described as a mixture of the hydrogen-bond isomers shown in Figure 5.

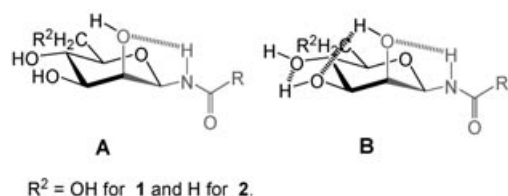


Figure 5. Intramolecular hydrogen-bonding isomers proposed for **1** and **2** in apolar solution.

Hydrogen-bonding network in talopyranoside derivative 3: Talopyranoside derivative **3** has the 1,2-amido alcohol-(*e,a*) motif of **1** and **2** (Figure 1b) and, additionally, the OH group in the 2-position has a 1,3-diaxial relationship to OH-4. Thus, a six-membered-ring intramolecular hydrogen bond is expected to be formed by this diol and polarised by the five-membered-ring intramolecular hydrogen bond, which makes OH-4 behave as a cooperative donor.

Molecular mechanics calculations were also carried out on both the *syn* and *anti* conformers of **3**. In this case, too, the *anti* orientation around the ϕ angle was more stable than the *syn* geometry, and the computed distance between NH and OH-2 (2.61 Å) is in agreement with an intramolecular hydrogen bond (see Supporting Information, part 3). The

NMR parameters for the hydroxy and amide resonances of β -D-talopyranoside derivative **3** in CDCl₃ are listed in Table 1.

The amide resonance in **3** shows the same *J* and $\Delta\delta/\Delta T$ values as were obtained for the β -D-mannopyranoside derivatives **1** and **2** (see Table 1). However, the chemical shift of the amide NH resonance, although in the same chemical environment, is more deshielded in **3** than in **1** and **2** ($\Delta\delta(\mathbf{3}-\mathbf{1})=0.154$ ppm and $\Delta\delta(\mathbf{3}-\mathbf{2})=0.195$ ppm at 30 °C). This difference suggests that the corresponding NH proton of **3** is more involved in an intramolecular hydrogen bond than those of the related mannopyranoside derivatives **1** and **2**.

The $\Delta\delta/\Delta T$ values of the OH resonances in **3** showed a clear difference between OH-2, OH-3 and OH-6 and OH-4. The last-named has a $\Delta\delta/\Delta T$ value characteristic of a free OH group (−7.5 ppb K^{−1}). The OH-2 and OH-3 groups have large *J* values (10 and 9.5 Hz) reflecting a preferred orientation, probably related to their involvement in intramolecular hydrogen bonds (dihedral angles larger than 150°). In contrast, the values for OH-4 and OH-6 are in the range of those expected for a free OH group.

These *J* values are in accordance with those reported by us for 1,6-anhydro-3-deoxy-3-palmitoylcarboxamide- β -D-glucopyranose,^[6] for which a six-membered-ring intramolecular hydrogen bond polarised in one direction was found.

Therefore, the previous NMR data are in agreement with the presence in solution of a detectable percentage of the hydrogen-bond isomer of **3** in which OH-4 is acting as a hydrogen-bond acceptor for OH-2 and OH-3, as predicted by MM2* calculations (see Supporting Information).

A partial deuteration experiment was also performed on an NMR sample of **3** under the same conditions described above for **1** and **2** (Table 2). The NH resonance of the amido group in the anomeric position shows an isotope effect (Table 2, Figure 6), which suggests its participation in one intramolecular hydrogen bond.

As for **1** and **2**, the NMR data of **3** support the presence of a five-membered-ring intramolecular hydrogen bond involving NH→OH-2 (the NH group of an amide can only be a hydrogen-bond donor). This feature is again only possible for the *anti* conformer of the *N*-glycosidic linkage (as predicted by the MM2* calculations). Moreover, we note that OH-2 of **3** has the largest isotope effect of the three compounds, while, the OH-3 and OH-6 resonances also showed smaller, but detectable, isotope effects. The NMR parameters for OH-3 (Table 1) are also in accordance with its participation in a hydrogen bond in solution. On the other hand, the OH-4 and OH-6 *J* and $\Delta\delta/\Delta T$ values are consistent with their being free in solution. Thus, **3** in chloroform solution may be described as equilibrium of hydrogen-bonding isomers **A**, **B** and **C** (Figure 7). In all three of them, the 1,3-diaxial six-membered-ring intramolecular H-bond between OH-2 and OH-4 is present. This feature seems to confirm the efficiency of the five-membered-ring intramolecular hydrogen bond in driving the OH...OH hydrogen-bond equilibrium between the 1,3-(*a,a*)-diol towards OH-2→OH-4.

To obtain further evidence, we explored the complexation of **3** with pyridine (Py). We have previously used the com-

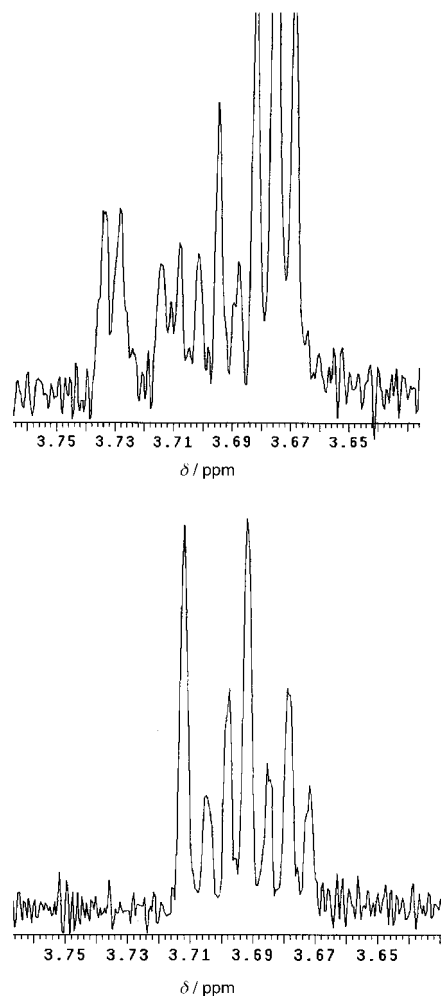


Figure 6. OH-2 proton resonances in the ^1H NMR spectra of **3** in CDCl_3 upon addition of 0 (a) and 74% (b) CD_3OD .

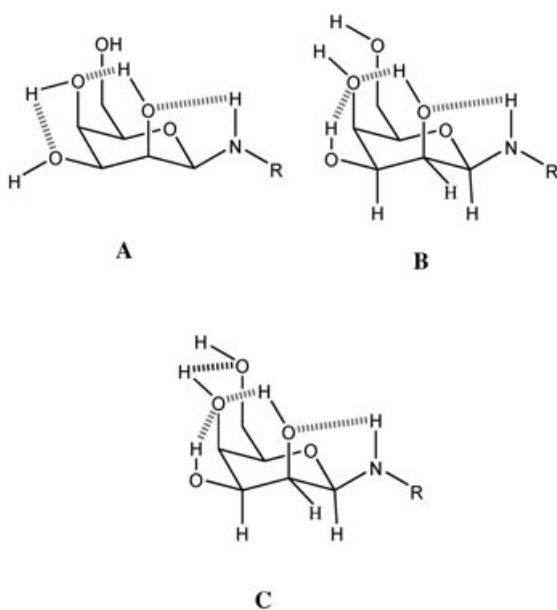


Figure 7. Hydrogen-bond isomers proposed for the talopyranoside derivative **3** in apolar solution.

plexation of a H-bonding acceptor such as $\text{Py}^{[6]}$ to detect cooperative donor centres and free hydroxy groups in carbohydrates. The NMR titration of **3** with Py allowed us to deduce a value of $K_a = 18\text{ M}^{-1}$ for the complex **3-Py**, which is in the range of those measured for sugars with cooperative donor centres. Furthermore, the largest chemical-induced shifts (CIS) were observed for OH-4 (CIS = +1.35 ppm) and OH-6 (CIS = +1.26 ppm), and this suggests that these two hydroxy groups are the most accessible to interaction with an acceptor. No CIS was observed for the NH resonance. This is in accordance with the presence of a high percentage of the hydrogen-bonding isomer **B** (Figure 7) of **3** in solution.

Phosphate complexes: Our previous work has shown that cooperative 1,2-(a,a)-*trans*-diol and amido alcohol hydrogen-bonding motifs are efficient binders of bidentate phosphate in apolar solvents. $^{[5]}$ Here, we decided to explore the phosphate binding as an additional proof of the effect that the presence of a hydrogen-bond cooperative centre may have on the recognition behaviour of our designed sugar derivatives **1–3**. We expected that the cooperative donor hydrogen-bonding motifs present in **1**, **2** and **3** could allow effective phosphate binding. Thus, NMR titrations were carried out with the chloroform-soluble phosphate salt tetrabutylammonium bis(3,5-di-*tert*-butyl)phenyl phosphate (**Phos**).

The titrations were carried out at constant concentration of sugar (**1**, **2** or **3**) by adding increasing concentrations of salt. The high-field NMR shifts of the methyne proton resonances were fitted to a 1:1 complex. The stoichiometries of all complexes were determined by Scatchard plots and Job plots based on chemical-shift variations. $^{[27]}$ The stability constants and ΔG° values of the complexes of **1–3** with **Phos** and those for selected model compounds **24–27** $^{[5]}$ (Figure 8) are given in Tables 3 and 4.

Significantly, phosphate complexes with our designed monosaccharides with cooperative donor centres (**1-Phos**, **2-Phos** and **3-Phos**) have ΔG° values which are between 1 and 2.2 kcal mol^{-1} higher than those reported in the literature

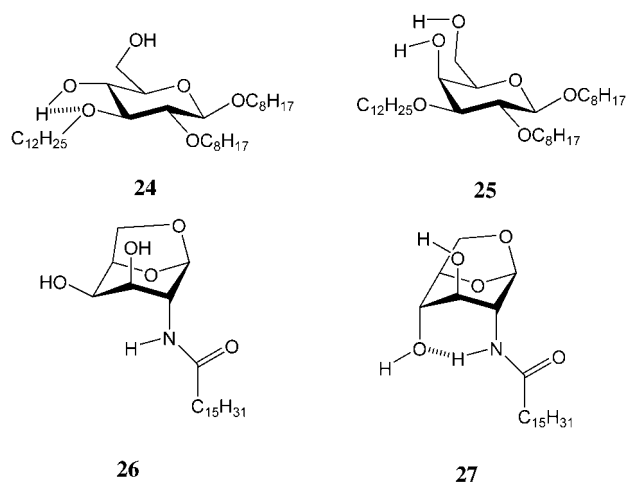


Figure 8. Model carbohydrates selected $^{[5]}$ for comparison with the phosphate complexes of **1–3**.

Table 3. K_a [M^{-1}] and ΔG° [$kcal\ mol^{-1}$] for the interaction between compounds **1**, **2**, **24** and **26** and the phosphate salt.

	1	2	24	26
K_a	16400	4880	96	554
ΔG° ^[a]	−5.8	−5.0	−2.7	−3.5

[a] Estimated error: $\pm 0.1\ kcal\ mol^{-1}$.Table 4. K_a [M^{-1}] and ΔG° [$kcal\ mol^{-1}$] for the interaction between compounds **3**, **25** and **27** and the phosphate salt.

	3	25	27
K_a	9610	111	1796
ΔG° ^[a]	−5.4	−2.8	−4.4

[a] Estimated error: $\pm 0.1\ kcal\ mol^{-1}$.

for chloroform-soluble gluco- and galactopyranoside derivatives.^[28]

Diols **24** and **25** were selected as models for **1** and **3**. These two molecules are the 4,6-diols of glucopyranose (**24**) and galactopyranose (**25**). The configuration at C-4 in **24** is the same as in the mannopyranose derivatives **1** and **2**. In turn, OH-4 is axial in **25**, as in the talopyranose compound **3**.

Regarding **1** and **2** (Table 3), the most stable complex is **1-Phos** ($\Delta G^\circ = -5.8\ kcal\ mol^{-1}$). It is $3\ kcal\ mol^{-1}$ more stable than its corresponding 1,3-flexible diol **24** with the same configuration at C-4. The lack of OH-6 in **2-Phos** results in a less stable complex (by $0.8\ kcal\ mol^{-1}$).

Regarding the observed CIS, all methyne resonances of the sugar are shielded upon complex formation, although both **1-Phos** and **2-Phos** showed higher induced shifts for the methyne CH-2 ($-0.1\ ppm$ in **1** and $-0.09\ ppm$ in **2**) and CH-3 ($-0.134\ ppm$ in **1** and $-0.09\ ppm$ in **2**) resonances, which suggests the involvement of their corresponding OH groups in the complexation process. This observation suggests the formation of a complex in which **Phos** is coordinated to the cooperative 1,2-(a,e)-*cis*-diol involving OH-2 and OH-3 of the pyranose ring.

In comparison, a 1,2-(a,e)-diol with an amide group in in the α position, but in a 1,2-*trans* relationship as in **26** (without the possibility of establishing an intramolecular hydrogen bond with the diol) gave a significantly less stable complex, with $K_a = 554\ M^{-1}$ ($\Delta G^\circ = -3.7\ kcal\ mol^{-1}$), that is, **2-Phos** displays an extra stabilisation of about $2\ kcal\ mol^{-1}$ compared to **26-Phos**.

For talose complex **3-Phos** (Table 4), higher CIS of the CH resonances upon complex formation were found for CH-4 ($-0.064\ ppm$) and CH-6,6' (due to overlap, the maximum CIS can not be accurately calculated). This observation also suggests the involvement of both hydroxy groups in complex formation.

These data are in agreement with CIS measured for the complex with pyridine **3-Py**. The CIS for this complex are largest for OH-4 and OH-6, which again suggests that these two hydroxy groups are the most accessible to interaction with an acceptor. No CIS was observed for the NH resonance.

We selected, for comparison, a flexible diol involving the 4- and 6-positions of the pyranose ring with the same configuration at C-4, namely, **25**. Additionally, the cooperative 1,2-*trans*-(a,a)-diol **27** was used as a reference for a cooperative diol. The ΔG° values show that **3-Phos** is about $2\ kcal\ mol^{-1}$ more stable than the corresponding complex of the flexible diol with the same configuration at C-4 **25-Phos**.

In the 1,2-*trans*-(a,a)-diol **27**, OH-4 is cooperative due to the presence of a six-membered-ring intramolecular hydrogen bond, which involves an NH group of an amide. The complex between **27-Phos** is $1\ kcal\ mol^{-1}$ less stable than that of **3-Phos**. All these results suggest that *N*-glycosylamides **1–3** with cooperative OHs centres are effective phosphate binders.^[29]

Conclusion

We have designed and synthesised sugars with cooperative hydrogen-bonding donor centres. The structural analysis of the *N*-glycosylamides **1** and **2** by NMR in apolar solution showed the presence of a five-membered-ring intramolecular hydrogen bond between the anomeric NH amide group in equatorial position and the axially oriented OH-2 group (NH→OH-2).

As a second step, the talose derivative **3** was designed and synthesised to prove that a group with a unique hydrogen-bond donor character, located at hydrogen-bonding distance to a particular OH group, can be used to initiate the hydrogen-bond network and to polarise a particular HO...OH hydrogen-bond in a desired direction.

We were able to prove that the presence in **3** of a five-membered-ring intramolecular H-bond, established between a *N*-glycosylamido group (NH) and a hydroxy group (in 1,2-(e,a) relationship) that additionally is in a 1,3-*cis*-diaxial relationship to a second hydroxy group (OH-4) can be used to select an unique direction on the six-membered-ring intramolecular hydrogen bond between the two diaxial OH groups, which makes one of them behave as an efficient cooperative donor (OH-4 in this case).

Finally, the presence of cooperative hydrogen-bond centres makes **1–3** more efficient phosphate binders in apolar solvent in comparison with model compounds **24–27**.

Experimental Section

The 1H NMR studies on carbohydrates **1–3** were performed with freshly prepared solutions in $CDCl_3$, which was always passed through basic alumina and collected over 4 Å molecular sieves.

1H NMR experiments: The binding abilities of compounds **1–3** to **Phos** were investigated by means of 1H NMR titration experiments. Carbohydrate solutions were obtained by diluting a known volume of a stock solution, previously prepared by dissolving a weighed amount of the sugar in $CDCl_3$, to 3 mL. Then, 0.5 mL of the resulting solution was placed in an NMR tube, and the remaining 2.5 mL was used to prepare the carbohydrate/phosphate titrant solution. The carbohydrate concentration was $10^{-4}\ M$. The concentration of **Phos** in the titrant solution was in the range 10^{-3} to $8 \times 10^{-4}\ M$. A minimum of 10 aliquots of the sugar/phosphate solution were added to the sugar solution, and a one-dimensional 1H NMR spectrum ($T = 298\ K$) was recorded after each addition. The binding con-

stants were evaluated from a least-squares fitting by using a well-established protocol.^[19]

The $\Delta\delta/\Delta T$ values of the exchangeable proton resonances of compounds **1–3** were obtained by means of variable-temperature ¹H NMR experiments. These were carried out at sugar concentrations ranging from 1.1×10^{-4} to 1.9×10^{-4} M, depending on the tendency for self-association of the carbohydrate investigated. The spectra were recorded at five different temperatures in the range 295–318 K, and the $\Delta\delta/\Delta T$ values were evaluated from a linear fit.

Compounds **1–3** were partially deuterated by adding a few microlitres of [D₄]methanol to an NMR sample at 303 K for **1**, 305 K for **2** and 294 K for **3**. After each addition, a one-dimensional ¹H NMR spectrum was recorded, and the percentage deuteration was calculated by comparison of the integrals of the resonances of the exchangeable protons (OH and NH resonances) with that of a nonexchangeable proton (e.g., H-1). Isotope effects, observable for NH, OH-2 and other OH groups, depending on the nature of compound (signal doubling), demonstrated the existence of the intramolecular hydrogen bond between the NH and OH-2 groups in the **1–3**. To observe NH signal doubling it was necessary to wait at least one hour after deuteration.

Molecular modelling: MM2* calculations were performed for **1–3** using MM2* as implemented in MacroModel 7.0. The GB/SA solvent model for chloroform was used. All the different combinations of staggered orientations of the hydroxy groups were used as input geometries. The results are given for the most stable ones in each case. Additionally, the three possible staggered orientations for the Φ angle at the N-glycosidic linkage (H1-C1-N-H) were also used as starting geometries. In all cases, the *anti*-type geometry was the most stable.

Synthesis: Compounds **5**,^[30] **6**,^[18] **10**,^[19] **13**^[31] and **14**^[17] were prepared by literature procedures.

N-(2,3,4,6-Tetra-O-acetyl- β -D-mannosyl)-3,4,5-tris(tetradecyloxy)benzamide (11): A solution of **6** (213 mg, 0.61 mmol) in EtOAc (3 mL) was treated with Raney Ni and shaken under an atmosphere of hydrogen for 6 h at room temperature. The catalyst was removed by filtration, and the solvent was evaporated under reduced pressure to afford **8** as a pale oil. Separately, a solution of DIC (140.0 μ L, 0.88 mmol), HOBt (120.0 mg, 0.88 mmol) and 3,4,5-tris(tetradecyloxy)benzoic acid (**10**; 560 mg, 0.74 mmol) in DMF/CHCl₃ (1/1, 14 mL) was stirred under argon at room temperature for 24 h before adding a solution of **8** in CHCl₃ (3 mL). After a further 24 h, the crude product was washed three times with water, the organic layer was dried over Na₂SO₄ and the solvent was removed under reduced pressure. The residue was purified by column chromatography (hexane/EtOAc 4/1) to afford compound **11** as a 7/93 mixture of α and β isomers (108 mg, 33 %); $R_f(\beta)$ = 0.35 (EtOAc/*n*-hexane 1/1); ¹H NMR (200 MHz, CDCl₃): β isomer: δ = 6.89 (s, 2H, *ortho*), 6.70 (d, ³J(H,H) = 9.2 Hz, 1H, NH), 5.69 (dd, ³J(H,H) = 1.0, 9.0 Hz, 1H, CH), 5.42 (dd, ³J(H,H) = 1.0, 3.0 Hz, 1H, CH), 5.28 (t, ³J(H,H) = 10.2 Hz, 1H, CH), 5.16 (dd, ³J(H,H) = 3.0, 10.0 Hz, 1H, CH), 4.35 (dd, ³J(H,H) = 5.0, ²J = 12.6 Hz, 1H, CH₂), 4.07 (dd, ³J(H,H) = 2.0, ²J = 12.6 Hz, 1H, CH₂), 3.96 (t, ³J(H,H) = 6.4 Hz, 6H, CH₂), 3.842 (ddd, ³J(H,H) = 2.0, 4.8, 9.6 Hz, 1H, CH), 2.23 (s, 3H, CH₃), 2.08 (s, 3H, CH₃), 2.04 (s, 3H, CH₃), 1.98 (s, 3H, CH₃), 1.76 (m, 6H, CH₂), 1.43 (brs, 6H, CH₂), 1.23 (m, 60H, CH₂), 0.85 (t, ³J(H,H) = 6.3 Hz, 9H, CH₃); visible signals of α isomer: 6.98 (s, 2H, *ortho*), 5.82 (dd, ³J(H,H) = 6.3, 8.4 Hz, 1H, CH), 5.37 (dd, ³J(H,H) = 3.3, 9.3 Hz, 1H, CH), 5.12 (dd, ³J(H,H) = 4.8, 6.0 Hz, 1H, CH), 4.49 ppm (dd, ³J(H,H) = 6.3 Hz, ²J = 12.0 Hz, 1H); ¹³C NMR (50 MHz, CDCl₃): β isomer: δ = 170.64 (COCH₃), 170.56 (COCH₃), 169.78 (COCH₃), 169.66 (COCH₃), 166.42 (CONH), 153.13 (C_m), 141.98 (C_q), 127.86 (C_p), 106.08 (C_o), 76.96 (C-1), 74.35, 73.53 53 (CH₂ sugar), 71.55, 70.65, 69.43 (2OCH₂), 65.27, 62.20 (OCH₂), 31.90 (CH₂), 29.66 (CH₂), 29.34 (CH₂), 26.03 (CH₂), 22.66 (CH₂), 20.86 76 (CH₃CO), 20.76 76 (CH₃CO), 20.66 76 (CH₃CO), 20.51 76 (CH₃CO), 14.08 76 ppm (CH₃); IR (KBr): $\tilde{\nu}$ = 3436, 2919, 2850, 1751, 1645, 1585, 1530, 1497, 1468, 1428, 1369, 1229, 1116, 1057 cm⁻¹; MS (APCI): m/z : 1088 [M+H]⁺; elemental analysis calcd (%) for C₆₃H₁₀₉NO₁₃: C 69.51, H 10.09, N 1.29; found: C 69.27, H 10.31, N 1.46.

N- β -D-Mannopyranosyl-3,4,5-tris(tetradecyloxy)benzamide (1): A solution of **11** (220.0 mg, 0.20 mmol) in MeOH (5 mL)/CHCl₃ (4 mL) was treated with 3 mL of a solution of sodium methoxide in MeOH prepared by dissolving Na (100 mg) in MeOH (10 mL). After stirring for 1 h at

room temperature, the solution was acidified to pH 6 with Amberlite IR-120H⁺ ion-exchange resin, and the resin removed by filtration. The solvent was removed under reduced pressure to give a mixture of α and β isomers. The β isomer **1** was obtained after column chromatography (SiO₂/CHCl₃) as a white amorphous solid (86.0 mg, 47 %). R_f = 0.27 (MeOH/EtOAc 5/95); $[\alpha]_D = -7.2$ (c = 0.50, CHCl₃); ¹H NMR (500 MHz, 40 °C, CDCl₃): δ = 7.11 (d, ³J(H,H) = 9.0 Hz, 1H, NH), 6.99 (s, 2H, *ortho*), 5.52 (d, ³J(H,H) = 9.0 Hz, 1H, CH), 4.03 (t, ³J(H,H) = 3.5 Hz, 1H, CH), 3.99 (t, ³J = 6.5 Hz, 6H, OCH₂), 3.90 (ddd, ²J(H,OH) = 1.5, ³J(H,H) = 3.5, 12.5 Hz, 1H, CH₂), 3.86 (ddd, ²J(H,OH) = 1.5 Hz, ³J(H,H) = 5.0, 12.5 Hz, 1H, CH₂), 3.85 (td, ²J(H,OH) = 3.0 Hz, ³J(H,H) = 9.5 Hz, 1H, CH), 3.75 (ddd, ²J(H, OH) = 4.0 Hz, ³J(H,H) = 5.0, 9.5 Hz, 1H, CH), 3.46 (ddd, ³J(H,H) = 3.5, 5.0, 9.5 Hz, 1H, CH), 2.57 (d, ²J(H,OH) = 4.0 Hz, 1H, OH-3), 2.56 (d, ²J(H,OH) = 3.5 Hz, 1H, OH-2), 2.39 (d, ²J(H,OH) = 3.0 Hz, 1H, OH-4), 2.13 (t, ²J(H,OH) = 5.0 Hz, 1H), 1.73 (m, 6H, CH₂), 1.44 (m, 6H, CH₂), 1.25 (brs, 60H, CH₂), 0.87 ppm (t, J = 6.5 Hz, 9H, CH₃); ¹³C NMR (125 MHz, CDCl₃): δ = 152.99 (C_m), 141.00 (C_p), 123.50 (C_q), 106.44 (C_o), 78.44 (C-1), 77.40 (C-5), 74.44 (C-3), 73.0 (C-4, C-6), 71.44 (C-2), 69.43 (2OCH₂), 60.30 (OCH₂), 31.90 (CH₂), 29.67 (CH₂), 29.52 (CH₂), 29.34 (CH₂), 26.11 (CH₂), 22.64 (CH₂), 14.02 ppm (CH₃); IR (KBr): $\tilde{\nu}_{\text{max}}$ = 2921, 2851, 1627, 1583, 1539, 1499, 1468, 1331, 1233, 1119 cm⁻¹; MS (APCI) (%): m/z : 920.5 (100) [M+H]⁺, 800.5, 758, 577; elemental analysis calcd (%) for C₅₅H₁₀₁NO₉: C 71.77, H 11.06, N 1.52; found: C 71.54, H 11.30, N 1.69.

2,3,4-Tri-O-acetyl-6-deoxy- α -D-mannopyranosyl azide (7): SnCl₄ (92 μ L, 0.78 mmol) and TMSN₃ (280 μ L, 2.10 mmol) were added to a solution of 6-deoxy-D-mannopyranose tetraacetate^[30c] **5** (349 mg, 1.05 mmol) in dry CH₂Cl₂ (6 mL), and the mixture was stirred at room temperature for 3 h. The mixture was washed successively with aqueous NaHCO₃ and water and dried over Na₂SO₄. The solvent was removed under reduced pressure to afford **7** as a colorless oil (197 g, 60 %). R_f = 0.52 (1/1 *n*-hexane/EtOAc); ¹H NMR (200 MHz, CDCl₃): δ = 5.29 (d, ³J(H,H) = 2.0 Hz, 1H, CH), 5.18 (dd, ³J(H,H) = 3.2, 10 Hz, 1H, CH), 5.12 (dd, ³J(H,H) = 2.0, 3.2 Hz, 1H, CH), 5.06 (t, ³J(H,H) = 10 Hz, 1H, CH), 4.01 (qd, ³J(H,H) = 6.2, 10 Hz, 1H), 2.14 (s, 3H, CH₃), 2.04 (s, 3H, CH₃), 1.97 (s, 3H, CH₃), 1.25 ppm (d, ³J(H,H) = 6.2 Hz, 3H, CH₃); ¹³C NMR (50 MHz, CDCl₃): δ = 169.85 (CO), 169.79 (CO), 169.76 (CO), 86.44 (C-1), 69.40, 68.40, 67.57, 67.24, 20.74 (CH₃CO), 20.70 (CH₃CO), 20.55 (CH₃CO), 17.36 ppm (CH₃); IR (KBr): $\tilde{\nu}_{\text{max}}$ = 2986, 2900, 2120, 1750, 1431, 1372, 1223, 1125, 1056, 957, 936 cm⁻¹; MS (ES): m/z (%): 274 (11) [M-N₃+H]⁺, 338 (100) [M+Na]⁺.

N-(2,3,4-Tri-O-acetyl-6-deoxy- β -D-mannosyl)-3,4,5-tris(tetradecyloxy)benzamide (12): A solution of **7** (110 mg, 0.35 mmol) in EtOAc (4 mL) was treated with Raney Ni and shaken under an atmosphere of hydrogen overnight. The catalyst was removed by filtration and the solvent evaporated under reduced pressure to afford a pale glassy film of **9**. Separately, a solution of DIC (80.0 μ L, 0.50 mmol), HOBt (67.0 mg, 0.50 mmol) and **10** (318 mg, 0.42 mmol) in DMF/CHCl₃ (1/1, 10 mL) was stirred under argon for 24 h at room temperature before addition of **9** dissolved in CHCl₃ (3 mL). After a further 24 h, the crude reaction mixture was washed thrice with water, the organic layer was dried with Na₂SO₄ and the solvent was removed under reduced pressure. The residue was purified by column chromatography (SiO₂, *n*-hexane/EtOAc 8/2) to afford **12** as a 36:64 mixture of α and β isomers (190 mg, 53 %). $R_f(\beta)$ = 0.15 (EtOAc/*n*-hexane 1/1); ¹H NMR (300 MHz, CDCl₃): β isomer: δ = 6.892 (s, 2H, *ortho*), 6.66 (d, ³J(H,H) = 9.0 Hz, 1H, NH), 5.64 (dd, ³J(H,H) = 1.0, 9.0 Hz, 1H, CH), 5.41 (dd, ³J(H,H) = 1.0, 3.0 Hz, 1H, CH), 5.12 (dd, ³J(H,H) = 3.0, 10.0 Hz, 1H, CH), 5.05 (t, ³J(H,H) = 10.0 Hz, 1H, CH), 3.96 (t, ³J(H,H) = 6.6 Hz, 6H, OCH₂), 3.71 (qd, ³J(H,H) = 6.3, 10.0 Hz, 1H, CH), 2.06 (s, 3H, CH₃), 2.22 (s, 3H, CH₃), 1.98 (s, 3H, CH₃), 1.78 (m, 6H, CH₂), 1.45 (m, 6H, CH₂), 1.24 (brs, 63H, 30CH₂ and CH₃ sugar), 0.86 (t, J = 6.6 Hz, 9H, CH₃); visible signals of α isomer: 6.95 (s, 2H, *ortho*), 6.79 (d, ³J(H,H) = 8.7 Hz, 1H, NH), 5.81 (dd, ³J(H,H) = 6.3, 8.4 Hz, 1H, CH), 5.30 (m, 2H, CH), 4.93 (t, 1H, CH), 2.11 (s, 3H, CH₃), 2.09 (s, 3H, CH₃), 2.07 ppm (s, 3H, CH₃); ¹³C NMR (75 MHz, CDCl₃): β isomer: δ = 170.67 (CO), 169.94 (CO), 169.87 (CO), 166.51 (CONH), 153.05 (C_m), 141.81 (C_p), 128.00 (C_q), 105.96 (C_o), 76.57 (C-1), 73.48 (OCH₂), 72.40 (C-5), 71.48 (C-3), 70.92 (C-2), 70.25 (C-4), 69.35 (2OCH₂), 31.90 (CH₂), 29.68 (CH₂), 29.35 (CH₂), 26.03 (CH₂), 22.66 (CH₂), 20.88 (CH₃CO), 20.78 (CH₃CO), 20.56 (CH₃CO), 17.51 (CH₃ sugar), 14.09 ppm (CH₃); IR (KBr): $\tilde{\nu}_{\text{max}}$ = 3436, 2920, 2851, 1752, 1644, 1584,

1527, 1496, 1468, 1427, 1369, 1225, 1116, 1055 cm⁻¹; MS (APCI): *m/z*: 1030 [M+H]⁺; elemental analysis calcd (%) for C₆₃H₁₀₉NO₁₃: C 71.13, H 10.39, N 1.36; found: C 71.00, H 10.60, N 1.51.

N-(6-Deoxy-β-D-mannopyranosyl)-3,4,5-tris(tetradecyloxy)benzamide (2):

A solution of **12** (260.0 mg, 0.25 mmol) in MeOH/CHCl₃ (1/1, 8 mL) was treated with 3 mL of a solution of sodium methoxide in MeOH prepared by dissolving Na (100 mg) in MeOH (10 mL). The reaction was monitored by TLC (*n*-hexane/EtOAc 1/1). The solution was acidified to pH 6 with IR-120 ion-exchange resin, and the resin removed by filtration. The solvent was removed under reduced pressure to give a mixture of α and β isomers. The β isomer **2** was obtained after column chromatography (SiO₂, acetone/toluene 1/1) as a white amorphous solid (37.0 mg of β isomer and 131 mg of an α/β mixture, 74%). *R_f*(β)=0.37 (acetone/toluene 1/1), *R_f*(α)=0.30 (acetone/toluene 1/1); [α]_D²⁵=+0.75 (*c*=0.57, CHCl₃); MS (ES): *m/z* (%): 904 (100) [M+H]⁺; ¹H NMR (500 MHz, 35°C, CDCl₃): δ = 7.09 (d, ³J(H,H)=9.0 Hz, 1H, NH), 6.98 (s, 2H, *ortho*), 5.47 (d, ³J(H,H)=9.0 Hz, 1H, CH), 4.02 (brs, 1H, CH-2), 3.67 (brs, 1H, CH-3), 3.46 (m, 2H, CH-4 and CH-5), 2.53 (d, ²J(H,OH-3)=4.2 Hz, 1H, OH-3), 2.51 (d, ²J(H,OH-2)=3.6 Hz, 1H, OH-2), 2.02 (d, ²J(H,OH-4)=2.7 Hz, 1H, OH-4), 1.74 (m, 6H, CH₂), 1.45 (m, 6H, CH₂), 1.37 (d, 3H, J=6.0 Hz, CH₃ sugar), 1.25 (brs, 60H, CH₂), 0.86 ppm (t, ³J(H,H)=6.3 Hz, 9H, CH₃); ¹³C NMR (125 MHz, CDCl₃): δ = 153.06 (C_m), 140.55 (C_p), 128.33 (C_q), 106.00 (C_o), 74.31 (C-1), 73.51 (C-4 or C-5), 73.22 (C-4 or C-5), 70.97 (C-3), 69.56 (C-2), 69.37 (2 OCH₂), 60.30 (OCH₂), 31.92 (CH₂), 29.67 (CH₂), 29.57 (CH₂), 29.36 (CH₃ sugar), 26.07 (CH₂), 22.67 (CH₂), 14.12 ppm (CH₃); IR (KBr): $\tilde{\nu}_{\text{max}}$ = 3435, 2919, 2850, 1626, 1583, 1536, 1495, 1468, 1338, 1236, 1121 cm⁻¹; elemental analysis calcd (%) for C₅₅H₁₀₁NO₉: C 73.04, H 11.26, N 1.55; found: C 72.75, H 11.41, N 1.63.

β-D-Galactopyranosyl azide (15): A solution of azide **14**^[17] (8.50 g, 23.09 mmol) in MeOH (137 mL) was treated with a solution of sodium (200 mg) in MeOH (10 mL). After 30 min, the solution was acidified with Amberlite IR-120H⁺ to pH 6. Evaporation of the solvent under reduced pressure afforded **15** as a white crystalline solid (280 mg, 91%). ¹H NMR (200 MHz, CDCl₃): δ = 5.29 (s, 1H, CH), 4.85 (s, 1H, CH), 4.66 (s, 1H, CH), 4.53 (s, 1H, CH), 4.35 (d, 1H, J=8.5 Hz), 3.66 (m, 1H), 3.50 (m, 3H), 3.32 ppm (m, 2H); ¹³C NMR (75 MHz, CDCl₃): δ = 90.7, 77.6, 73.3, 70.3, 68.2, 60.5 ppm; IR (KBr): $\tilde{\nu}_{\text{max}}$ = 3600–3000, 2891, 2138, 1140, 1102, 1073, 1056 cm⁻¹; MS (ES⁺): *m/z* (%): 433 (23) [2M+Na]⁺, 413 (52), 228 (100) [M+Na].

(S)-4,6-O-Benzylidene-galactopyranosyl azide (16): A solution of azide **15** (5.00 g, 24.4 mmol) in dry, distilled CH₃CN (30 mL) was treated with *p*-TsOH (232 mg, 1.22 mmol) and benzaldehyde dimethyl acetal (18.3 mL, 122 mmol). After 24 h at room temperature, the mixture was treated with Et₃N (1 mL) and evaporated under reduced pressure. The residue was purified by column chromatography (SiO₂, EtOAc) to afford **16** as a white solid (5.82 g, 81%). *R_f*=0.41 (EtOAc); ¹H NMR (200 MHz, CDCl₃): δ = 7.52–7.35 (m, 5H, H_{Arom}), 5.53 (s, 1H, CH-8), 4.55 (d, 1H, ³J(H,H)=8.2 Hz), 4.36 (dd, 1H, ³J(H,H)=1.6 Hz, ²J(H,H)=12.5 Hz, CH₂), 4.20 (dd, 1H, ³J(H,H)=1.2, 3.2 Hz, CH), 4.06 (dd, 1H, ³J(H,H)=1.8 Hz, ²J(H,H)=12.6 Hz, CH₂), 3.69 (s, 1H), 3.65 (s, 1H), 3.55 (dd, 1H, ³J(H,H)=1.6, 2.9 Hz), 2.88 ppm (m, 2H); ¹³C NMR (300 MHz, CDCl₃): δ = 139.0, 129.1, 128.4, 126.7, 100.3, 90.8, 76.1, 72.1, 70.1, 68.7, 68.3 ppm; IR (KBr): $\tilde{\nu}_{\text{max}}$ = 3600–3200, 2910, 2861, 2116, 1249, 1086, 1102, 1051, 1001 cm⁻¹; MS (ES⁺): *m/z* (%): 609 (20) [2M+H]⁺, 362 (25), 316 (100) [M+Na]⁺, 251 (57) [M–N₃]⁺; elemental analysis calcd (%) for C₁₃H₁₃N₃O₅: C 53.24, H 5.16, N 14.33; found: C 53.40, H 5.35, N 14.61.

(S)-4,6-O-Benzylidene-3-O-(N-benzoylcarbamoyl)-galactopyranosyl azide (17): A solution of **16** (4.30 g, 14.7 mmol) in dry, distilled THF (20 mL) under Ar at 0°C was treated with a solution of benzoyl isocyanate (2.59 g, 17.6 mmol) in THF (30 mL) dropwise. After 30 min, a further 600 mg of benzoyl isocyanate was added. After 1 h at 0°C, the mixture was evaporated under reduced pressure, and the residue purified by column chromatography (SiO₂, 50% EtOAc/hexane) to afford **17** as a white foam (5.54 g, 86%). Part of this material was recrystallised from chloroform to provide an analytical sample (small needles). *R_f*=0.48 (EtOAc/hexane 7/3); ¹H NMR (300 MHz, CDCl₃): δ = 9.00 (brs, 1H, NH), 7.74–7.69 (m, 2H, H_{Arom}), 7.49–7.43 (m, 3H, H_{Arom}), 7.38–7.24 (m, 5H, H_{Arom}), 5.48 (s, 1H, CH-8), 4.88 (dd, ³J(H,H)=3.7, 10.0 Hz, 1H, CH-3), 4.62 (d, ³J(H,H)=8.5 Hz, 1H, CH-1), 4.44 (d, ³J(H,H)=3.4 Hz, 1H, CH-4), 4.28 (dd, ³J(H,H)=1.4 Hz, ²J(H,H)=12.5 Hz, 1H, CH-6 or CH-

6'), 3.90 (dd, ³J(H,H)=1.6, ²J(H,H)=12.5 Hz, 1H, CH-6 or CH-6'), 3.88 (t, 1H, CH-2), 3.80 (brs, 1H, CH-5), 3.52 ppm (brs, 1H, OH); ¹³C NMR (75 MHz, CDCl₃): δ = 165.8, 150.7, 137.3, 133.1, 132.3, 129.3, 128.7, 128.3, 127.9, 126.5, 101.1, 90.3, 75.3, 73.3, 68.7, 67.9, 67.8 ppm; IR (KBr): $\tilde{\nu}_{\text{max}}$ = 3600–3200, 2981, 2873, 2118, 1774, 1510, 1485, 1197, 1093 cm⁻¹; [α]_D²⁵ = 17.6 (*c*=2.00, acetone); MS (ES⁺): *m/z* (%): 903 (46) [2M+Na]⁺, 731 (47), 559 (47), 463 (63) [M+Na]⁺, 398 (100) [M–N₃], 122 (99%), 105 (82%); elemental analysis calcd (%) for C₂₁H₂₀N₄O₇: C 57.27, H 4.58, N 12.72; found: C 57.27, H 4.58, N 12.51.

(S)-4,6-O-Benzylidene-3-O-(N-benzoylcarbamoyl)-2-O-trifluoromethane-sulfonate-galactopyranosyl azide (18):

A solution of carbamate **17** (100 mg, 0.227 mmol) in dry, distilled CH₂Cl₂ (1 mL) at –25°C was treated with dry, distilled pyridine (37 μL, 0.454 mmol) and triflic anhydride (44 μL, 0.261 mmol). After stirring for 2 h at –25°C, the mixture was warmed to room temperature, water was added (1 mL) and the mixture was extracted with CH₂Cl₂ (3 × 10 mL). The combined organic phases were dried over Na₂SO₄, filtered and evaporated at reduced pressure. The residue was purified by column chromatography (SiO₂, 50% EtOAc/hexane) to afford **18** as a clear glassy film (80 mg, 77%). ¹H NMR (200 MHz, CDCl₃): δ = 8.58 (s, 1H, NH), 7.75–7.84 (m, 2H, H_{Arom}), 7.35–7.61 (m, 6H, H_{Arom}), 5.27 (dd, 1H, ³J(H,H)=3.6, 10.2 Hz, CH-3), 5.52 (s, 1H, CH-8), 4.93–5.04 (m, 2H, CH-1 and 2), 4.61 (dd, 1H, ³J(H,H)=0.8, 3.6 Hz, CH-4), 4.39 (dd, 1H, ³J(H,H)=1.8 Hz, ²J(H,H)=12.8 Hz, CH-6 or CH-6'), 4.11 (dd, 1H, ³J(H,H)=1.6 Hz, ²J(H,H)=12.8 Hz, CH-6 or CH-6'), 3.83 ppm (d, J(H,H)=0.8 Hz, 1H, CH-5); ¹³C NMR (75 MHz, CDCl₃): δ = 164.57 (CO), 149.08 (CO), 136.92 (C_{q1}), 133.48 (C_{p1}), 132.07 (C_{q2}), 129.50 (C_{p2}), 128.00 (C_{o1}), 128.35 (C_{o2}), 127.61 (C_{m1}), 126.41 (C_{m2}), CF₃ signal was not observed, 101.44 (C-8), 86.93 (CH sugar), 80.22 (CH sugar), 73.73 (CH sugar), 71.86 (CH sugar), 68.33 (CH₂ sugar), 68.02 ppm (CH sugar); IR (KBr): $\tilde{\nu}_{\text{max}}$ = 3 429, 2127, 1784, 1691, 1602, 1504, 1484, 1417, 1244, 1197, 1141, 1093, 1023, 994, 963, 894, 869, 818, 765, 699, 623 cm⁻¹; MS (ES⁺): *m/z* (%): 1167.1 (18) [2M+Na]⁺, 573.1 (100) [M+H]⁺; elemental analysis calcd (%) for C₃₉H₄₃N₇O₁₃: C 46.15, H 3.32, N 9.79; found: C 46.31, H 3.60, N 10.03.

(R)-4,6-O-Benzylidene-talopyranosyl azide (21): A solution of triflate **18** (540 mg, 0.943 mmol) in dry, distilled THF (5 mL) at 0°C under argon was treated with NaH (34 mg, 1.41 mmol) in one portion and stirred for 45 min. A further portion of NaH (50 mg, 2.08 mmol) was added, and the mixture allowed to warm to room temperature. After the mixture had been stirred at room temperature overnight, saturated aqueous NaHCO₃ (10 mL) was added and the mixture was extracted with CH₂Cl₂ (3 × 50 mL). The combined organic layers were dried over Na₂SO₄, filtered and evaporated at reduced pressure to give a crude mixture of **19** and **20**. The residue was taken up in THF (5 mL) and treated with a saturated aqueous solution of LiOH·H₂O. After stirring at room temperature overnight, the mixture was extracted with CH₂Cl₂ (3 × 50 mL) and the combined organic layers were dried over Na₂SO₄, filtered and evaporated at reduced pressure. Purification of the residue by column chromatography (SiO₂, EtOAc/hexane 1/1) afforded **21** as a white amorphous solid (190 mg, 69%). *R_f*=0.24 (EtOAc); ¹H NMR (200 MHz, CDCl₃): δ = 7.78–7.80 (m, 1H), 7.37–7.52 (m, 4H), 5.51 (s, 1H), 4.49 (d, 1H, ³J(H,H)=1 Hz, CH-4), 4.45 (dd, 1H, ³J(H,H)=1.6, ²J(H,H)=12.8 Hz, CH-6 or CH-6'), 4.24 (td, 1H, ³J(H,H)=1.2, 3.8 Hz, CH-1), 4.09 (dd, 1H, ³J(H,H)=1.8 Hz, ²J(H,H)=12.6 Hz, CH-6 or CH-6'), 3.86 (tdd, 1H, ³J(H,H)=1, 3.4 Hz, ²J(H,OH)=12.0 Hz, CH-3), 3.68 (td, 1H, ³J(H,H)=3.4, ²J(H,OH)=11.6 Hz, CH-2), 3.51 (td, 1H, 1.8, 1.8, 1.2 Hz, CH-5), 3.06 (d, 1H, ²J(H,OH)=12.2 Hz, OH-3), 3.04 ppm (d, 1H, ²J(H,OH)=11.6 Hz, OH-2); ¹³C NMR (75 MHz, CDCl₃): 136.80 (C_q), 129.55 (C_p), 128.50 (C_o), 125.98 (C_m), 101.84 (C-8), 87.31 (CH sugar), 75.46 (CH sugar), 71.70 (CH sugar), 69.01 (CH₂ sugar), 68.82 (CH sugar), 68.63 ppm (CH sugar); IR (KBr): $\tilde{\nu}_{\text{max}}$ = 3504, 3370, 3176, 2929, 2876, 2109, 1660, 1411, 1253, 1141, 1102, 1081, 1037, 1025, 772, 743, 700 cm⁻¹; [α]_D²⁰ = –78.81 (*c*=1.01 g per 100 mL, DMSO); MS (ES⁺): *m/z* (%): 609 (35) [2M+Na]⁺, 463 (61), 458 (42), 316 (43) [M+Na]⁺, 311 (100) [M+18], 251 (31) [M–N₃]⁺.

2,3,4,6-Tetra-O-acetyl-talopyranosyl azide (22): A solution of **21** (60 mg, 0.24 mmol) in AcOH/H₂O (7/3, 8.5 mL) was heated at 70°C for 4 h. The crude mixture was concentrated and co-evaporated with toluene (3 × 15 mL). The resultant β-D-talopyranosyl azide was dissolved in pyridine (15 equiv) and acetic anhydride (4.5 equiv) and stirred at room temperature overnight. The reaction mixture was evaporated under reduced pres-

sure and purified by column chromatography (SiO₂, EtOAc/hexane 1/1) to give **22** as a white amorphous solid (64.50 mg, 72 %); R_f =0.24 (EtOAc); ¹H NMR (200 MHz, CDCl₃): δ =5.31 (dd, ³J(H,H)=1.2, 3.6 Hz, 1H, CH-2), 5.27 (dd, ³J(H,H)=1.2, 3.8 Hz, 1H, CH-4), 5.04 (t, ³J(H,H)=3.6 Hz, 1H, H₃), 4.72 (d, ³J(H,H)=1.8 Hz, 1H, CH-1), 4.23 (dd, ³J(H,H)=1.6, 7.4, 2H, CH-6 or CH-6'), 3.99 (td, ³J(H,H)=1.6, 7.4 Hz, 1H, CH-5), 2.15 (s, 1H, CH₃), 2.12 (s, 1H, CH₃), 2.04 (s, 1H, CH₃), 1.97 ppm (s, 1H, CH₃); ¹³C NMR (75 MHz, CDCl₃): δ =170.41 (CO), 170.02 (CO), 169.88 (CO), 169.45 (CO), 85.74 (C-1), 78.51 (C-5), 67.58 (C-4), 67.10 (C-2), 64.51 (C-3), 61.43 (CH₂, C-6), 20.65 (CH₃), 20.62 (CH₃), 20.55 (CH₃), 20.41 ppm (CH₃); IR (KBr): $\tilde{\nu}_{\text{max}}$ =3475, 2966, 2119, 1748, 1434, 1370, 1223, 1091, 1046, 969, 917, 746 cm⁻¹; [α]_D²⁰=−65.66 (c=1.06 g/100 mL, CHCl₃ (1 mL)); MS (ES+): m/z (%): 331 (38) [M -Ac+NH₄]⁺, 391 (100) [M +NH₄]⁺, 396 (53) [M +Na]⁺; elemental analysis calcd (%) for C₁₃H₁₅N₃O₅: C 45.0, H 5.10, N 11.26; found: C 45.21, H 4.79, N 10.98.

N-(2,3,4,6-Tetra-O-acetyl-talopyranosyl)-3,4,5-tris(tetradecyloxy)benzamide (23): A solution of **22** (108 mg, 0.30 mmol) in EtOAc (2 mL) was treated with 10 % Pd/C (8 mg, 0.07 mmol) and shaken under an atmosphere of hydrogen for 6 h. The catalyst was removed by filtration over Celite and the solvent evaporated at reduced pressure to afford a pale glassy film of β -D-talopyranosyl amine. Separately, a solution of DIC (62.0 μ L, 0.396 mmol), HOBt (54.0 mg, 0.396 mmol) and **10** (250 mg, 0.33 mmol)^[19] in DMF/CHCl₃ (4 mL/4 mL) was stirred under argon for 24 h at room temperature before addition to β -D-talopyranosylamine dissolved in CHCl₃ (2 mL). After a further 24 h, the crude mixture was washed three times with water, the organic layer was dried over Na₂SO₄ and the solvent was removed at reduced pressure. The residue was purified by column chromatography (SiO₂, hexane/EtOAc 8/2) to afford **23**(α/β) as an amorphous white solid (108 mg, 33 %); R_f (β)=0.35 (EtOAc/CH₂Cl₂ 1/9); ¹H NMR (300 MHz, CDCl₃): β isomer: δ =6.91 (s, 2H, *ortho*), 6.67 (d, ³J(H,H)=9.3 Hz, 1H, NH), 5.66 (d, ³J(H,H)=9.3 Hz, 1H, CH-1), 5.33 (brs, 2H, CH), 5.19 (t, ³J(H,H)=3.6 Hz, 1H, CH), 4.16 (m, 3H), 3.97 (t, ³J(H,H)=6.6 Hz, 6H, OCH₂), 2.19 (s, 3H, CH₃), 2.13 (s, 3H, CH₃), 2.03 (s, 3H, CH₃), 1.98 (s, 3H, CH₃), 1.76 (m, 6H, CH₂), 1.43 (brs, 6H, CH₂), 1.24 (m, 60H, CH₂), 0.86 ppm (t, ³J(H,H)=6.3 Hz, 9H, CH₃); α isomer: δ =6.92 (s, 2H, *ortho*), 6.68 (d, ³J(H,H)=9.0 Hz, 1H, NH), 5.80 (t, ³J(H,H)=8.7 Hz, 1H, CH-1), 5.64 (brs, 1H, CH), 5.21 (dd, ³J(H,H)=3.0, 6.3 Hz, 1H, CH), 5.06 (dd, ³J(H,H)=3.3, 8.7 Hz, 1H, CH), 4.69 (dd, ³J(H,H)=9.3, ²J(H,H)=12.6 Hz, 1H), 4.43 (dd, ³J(H,H)=3.0 Hz, ²J(H,H)=12.6 Hz, 1H), 4.36 (ddd, ³J(H,H)=3.0, 9.0, 6.0 Hz, 1H, CH-5), 3.98 (t, ³J(H,H)=6.3 Hz, 6H, OCH₂), 2.19 (s, 3H, CH₃), 2.09 (s, 3H, CH₃), 2.06 (s, 3H, CH₃), 2.01 (s, 3H, CH₃), 1.75 (m, 6H, CH₂), 1.44 (brs, 6H, CH₂), 1.24 (m, 60H, CH₂), 0.86 ppm (t, ³J(H,H)=6.3 Hz, 9H, CH₃); ¹³C NMR (75 MHz, CDCl₃) δ =170.32, 170.06, 169.62, 169.12, 166.488, 153.18, 141.99, 127.94, 106.20, 77.28, 73.36, 73.39, 69.51, 68.65, 68.40, 64.87, 61.33, 31.90, 29.68, 29.33, 26.042, 22.65, 20.75, 20.62, 20.59, 20.40, 14.06 ppm; MS (ES): m/z (%): 1088 (100) [M +H]⁺; IR (KBr): $\tilde{\nu}$ =3436, 2922, 2852, 1750, 1649, 1584, 1527, 1495, 1427, 1369, 1228, 1116, 1051, 721 cm⁻¹; elemental analysis calcd (%) for C₆₃H₁₀₉NO₁₃: C 69.51, H 10.09, N 1.29; found: C 69.38, H 10.21, N 1.39.

N-(Talopyranosyl)-3,4,5-tris(tetradecyloxy)benzamide (3): A solution of **23**(α/β) (86.0 mg, 0.08 mmol) in MeOH (6 mL) and CHCl₃ (4 mL) was treated with 3 mL of a solution of sodium methoxide prepared from Na (100 mg, 4.34 mmol) and MeOH (10 mL). The solution was acidified to pH 6 with Amberlite IR-120H⁺. After filtering to remove the resin, the solvent was evaporated at reduced pressure to give a mixture of α and β isomers. The pure β isomer **3** was obtained after column chromatography (SiO₂, 100 % CHCl₃ to MeOH/CHCl₃ 1/9) as a white amorphous solid (55.0 mg, 75 %). R_f =0.40 (MeOH/CHCl₃ 1/9); ¹H NMR (500 MHz, CDCl₃): δ =7.32 (d, ³J(H,H)=9.0 Hz, 1H, NH), 6.99 (s, 2H, *ortho*), 5.38 (d, ³J(H,H)=9.0 Hz, 1H, CH-1), 4.15 (t, ³J(H,H)=4.0 Hz, 1H, CH-4), 4.07 (dd, 1H, ³J(H,H)=4.0 Hz, ²J(H,H)=12.5 Hz, CH-6 or CH-6'), 4.04 (dd, 1H, ³J(H,H)=3.5, ²J(H,H)=12.5 Hz, CH-6 or CH-6'), 3.99 (m, 6H, OCH₂), 3.94 (d, J=3.0 Hz, 1H, OH-4), 3.86 (dd, ³J(H,H)=3.5, 9.5 Hz, 1H, CH-2), 3.73 (d, ²J(H,OH)=10.0 Hz, 1H, OH-2), 3.68 (dt, ³J(H,H)=3.5, 3.5, ²J(H,OH)=9.5 Hz, 1H, CH-3), 3.56 (t, ³J(H,H)=4 Hz, 1H, CH-5), 3.01 (d, ²J(H,OH)=9.5 Hz, 1H, OH-3), 2.30 (t, ³J(H,OH)=6.5 Hz, 1H, OH-6), 1.76 (m, 6H, CH₂), 1.45 (brs, 6H, CH₂), 1.24 (m, 60H, CH₂), 0.86 ppm (t, ³J(H,H)=6.5 Hz, 9H, CH₃); ¹³C NMR (125 MHz, CDCl₃): δ =166.94 (CONH), 153.12 (C_m), 141.81 (C_p), 128.13 (C_q), 106.05 (C_o),

78.46 (C-1), 74.60 (C-5), 73.52 (C-6), 71.51 (C-2 and C-4), 69.51 (C-3), 69.42 (2OCH₂), 64.29 (OCH₂), 31.92 (CH₂), 29.71 (CH₂), 29.64 (CH₂), 29.41 (CH₂), 29.37 (CH₂), 26.07 (CH₂), 22.69 (CH₂), 14.11 ppm (CH₃); IR (KBr): $\tilde{\nu}_{\text{max}}$ =3435, 2919, 2850, 1638, 1585, 1525, 1492, 1426, 1330, 1228, 1120 cm⁻¹; [α]_D²⁰=+11.1 (c=0.54, CHCl₃); MS (ES): m/z (%): 920 (100) [M +H]⁺; elemental analysis calcd (%) for C₅₅H₁₀₁NO₉: C 71.77, H 11.06, N 1.52; found: C 71.50, H 11.18, N 1.62.

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