

Developing high affinity oligosaccharide inhibitors: conformational pre-organization paired with functional group modification

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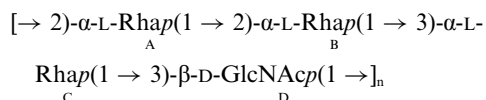
Intramolecular tethering combined with functional group modification has been investigated as an approach to design high affinity oligosaccharide ligands. The preceding paper reported successful tethering to constrain a trisaccharide in the conformation of its bound state with an antibody and thereby achieved a 15-fold increase in association constant. Here we report the synthesis of two β -alanyl tethered derivatives that employ monochlorination and monodeoxygenation strategies to create inhibitors that should enhance the binding affinity of the target molecules by an additional 10–25-fold, provided that free energy changes are additive when tethering is paired with functional group changes. The binding parameters of the new ligands **5** and **6** were measured by isothermal titration calorimetry and the results rationalized with molecular dynamics calculations and a simple docking analysis. The data indicate that while the alanine tether is a reasonable method to constrain trisaccharide **1**, free energy gains obtained by pairing it with functional group modification are not additive and in one case counter-productive.

Introduction

If carbohydrates are to find uses as therapeutics and practical affinity reagents, the weak attraction that dominates most oligosaccharide–protein interactions must be overcome. Efforts to design high-affinity carbohydrate epitopes have so far met with relatively few successes and, of these, free energy gains are frequently modest.

In the preceding paper we reported our attempts to tether a trisaccharide in its bound conformational state with an antibody. The active tethered trisaccharide exhibited a 15-fold affinity gain when compared with the native untethered trisaccharide **1**. The complex of **1** with the monoclonal antibody SYA/J6 has been extensively mapped by functional group modification with the introduction of either monodeoxy or monochlorodeoxy functions, creating ligands that exhibited 10- and 20-fold gains in affinity.¹ It was therefore of interest to investigate tethering of these derivatives and attempt to create significantly higher avidity ligands (possibly as high as 300-fold) through a combination of tethering and functional group changes, each of which individually yield affinity gains.

The monoclonal IgG antibody (mAb) SYA/J6 binds the linear tetrasaccharide repeating unit of the *O*-polysaccharide of *Shigella flexneri* variant Y:²



Chemical mapping determined the reading frame of the binding groove and identified a trisaccharide BCD as the optimal binding trisaccharide epitope (**1**). During these studies, it was found that deoxy-chloro (**2**)³ and deoxy-(**3**)⁴ congeners (Fig. 1) at the 2-position of the rhamnose C residue produced tighter binding trisaccharide derivatives with free energy gains ($\Delta\Delta G$ that ranged between approximately -1.4 to -1.7 kcal mol⁻¹), relative to the native trisaccharide **1**.

Detailed structures for the natural ABCDA' pentasaccharide and trisaccharide **3** (Fig. 1) in the binding site have been obtained from solved crystal structures and complement the data from epitope mapping by functional group replacement.^{5,6} The crystal structures revealed that the binding site is a deep groove, capable of binding internal residues of the natural *O*-polysaccharide. It

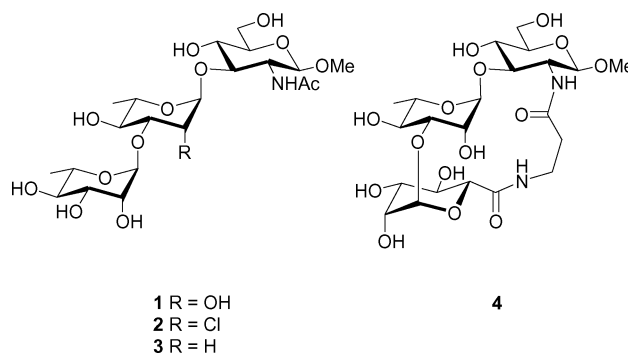


Fig. 1 Native trisaccharide **1** and previously synthesized derivatives thereof, including **2** and **3** containing modifications at the rhamnose C residue, along with tethered **4**.

also confirmed that indeed the BCD residues provide the major contribution to binding energy through polar and hydrophobic contacts. The Rha C residue was almost entirely buried in the deepest area of the combining site. The modes of binding between the ABCDA' pentasaccharide and **3** are similar, but differ in a few key aspects. For the **3**-SYA/J6 complex, it was noted that the 2-deoxy-Rha C residue sank even deeper in the binding groove and was accompanied by a change in the ϕ and ψ angles of the CD glycosidic bond that allowed the GlcNAc-D ring of **3** to make more polar contacts than pentasaccharide (**1**). The deoxygenated center also extends a hydrophobic area originating at the methyl group of the Rha B residue and the methyl moiety of the 2-acetamido group of the D residue, including Tyr L32 and His L27 of the protein. Although trisaccharide **2** has not been crystallized with SYA/J6, it has been proposed that its conformation in the bound state should be the same or similar to the conformation of the BCD portion of the ABCDA' pentasaccharide and would not penetrate the binding site as deeply as the monodeoxy congener **3** since the size and electronegativity of chlorine and hydroxyl groups are similar.⁷

Recent efforts to tailor the BCD epitope for high affinity binding have focused on intramolecular tethering to pre-organize the ligand in its bioactive conformation.¹ It has been postulated that immobilizing rotation upon binding to protein can incur a penalty of 0.5 to 2.0 kcal mol⁻¹ for each glycosidic

bond.^{8,9} Therefore, conformational restriction could lead to substantial favourable increases in entropic binding energy. This methodology has been used previously on several carbohydrate systems and resulted in both moderate gains and losses in binding energy.¹⁰ Crystal structure data for the SYA/J6 complex with **1** shows that the two methyl groups of residue D and residue B face away from the protein surface toward bulk solvent. This suggested that both sites are suitable for tethering that could provide a favourable entropic contribution for binding within the range 1–4 kcal mol⁻¹, provided that the tether preorganizes the trisaccharide in the bound conformation while it avoids unfavourable contacts with protein. We showed in the preceding paper that BCD trisaccharide derivative (**4**) containing a β -alanine (3-aminopropanoic acid) tether spanning these moieties effectively achieves this pre-organization, with an approximate 1.5 kcal mol⁻¹ gain in free energy compared to **1** (Fig. 1).⁸ However, we also concluded that the free energy gains from solvent reorganization at the periphery of the complex were of equal or greater significance to the gains in conformational entropy.

Here we test the hypothesis that the combination of tethering with specific functional group replacement could yield substantial affinity enhancement (Fig. 2). If free energy gains for pairing of individual modifications (Table 1, entries **2**, **3** and **4**) proved to be additive, the concept of combining intramolecular pre-organization with functional group modifications might become a useful strategy for the preparation of high-affinity carbohydrate inhibitors.

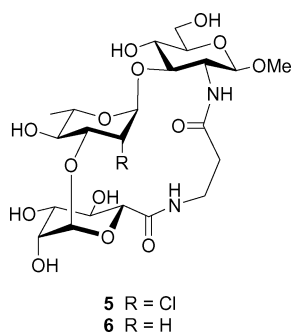


Fig. 2 Targets of this work, depicting the β -alanyl tether of **4** and functional group modifications at the rhamnose C residue.

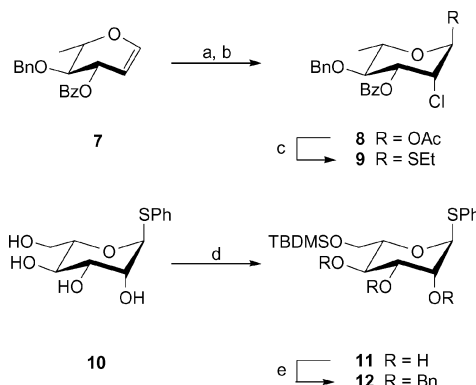
Results and discussion

Synthesis of cyclic trisaccharides **5** and **6**

The synthesis of **5** employed the same strategy as reported for **4**,¹ using standard thioglycoside methodology. It has been shown that 2-chloro-2-deoxy thioglycosides function well as thioglycoside donors and also as acceptors, since glycosylation of the 3-position of a terminal rhamnose residue containing a 2-chloro-2-deoxy functionality proceeded in good yield.³ Base labile protecting groups were employed at those hydroxyl and amino groups which were the sites of glycosylation and modification. Accordingly this required the deployment of orthogonal protecting groups, such as benzyl ethers and a benzylidene acetal, for those hydroxyl groups not involved in

glycosylation or tethering reactions. Given the relative ease of constructing 1,2-*trans*-linkages, the L-mannosyl donor was protected as a 2,3,4-tri-*O*-benzyl-6-*O*-silyl derivative **12**. The small amount of β -anomer that was likely to be formed as a consequence of the use of a non-participating group at C-2 was deemed acceptable so that the orthogonality of the benzyl protecting groups could be preserved and reduce the number of protecting group manipulations at advanced stages of the synthesis.

Installation of the chlorine atom at the 2-position of 1,5-anhydro-3-*O*-benzoyl-4-*O*-benzyl-2,6-dideoxy-hex-1-enitol (**7**)³ to afford the *rhamno*-configuration has previously been accomplished by chloromethoxylation, but suffers from poor selectivities, difficult purification and, consequently, low yield (30–35%).^{3,11} A modification of the Igarashi *et al.* protocol¹² led to highly diastereofacial *cis*-addition of chlorine gas to **9** in CCl₄, followed by the Koenig–Knorr glycosylation of acetic acid¹³ to afford **8** in 88% overall yield in a ratio of 4 : 1 *rhamno* : *quinovo* (6-deoxy-L-*gluco*) configurations (Scheme 1). It should be noted that the use of 1,2-propanediol cyclic carbonate as a solvent for the chlorination¹² leads to the opposite diastereofacial selectivity; a 6 : 1 mixture favoring the β -quinovosyl diastereomer of **8** in good yield. The stereochemistry of the 2-chloro-2-deoxy derivatives **8** and **9** were established by observation of ³J_{H-H} coupling constants. Lewis acid catalyzed thioglycosidation with ethanethiol produced donor **9** in excellent yield. The L-mannosyl thioglycoside donor **12** was synthesized from the known thiophenyl mannoside **10**¹⁴ *via* regioselective silylation and exhaustive benzylation, both in high yield (Scheme 1).

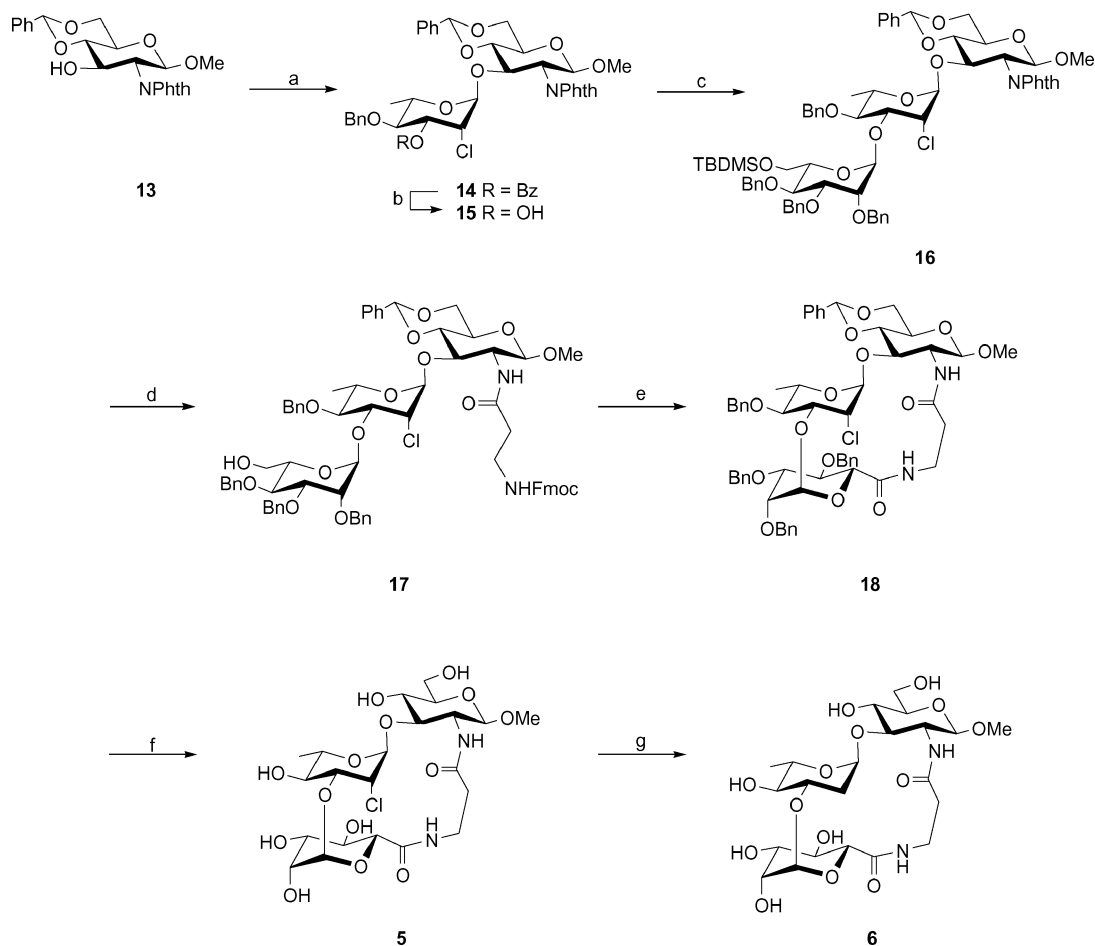


Scheme 1 Reagents and Conditions: (a) Cl₂, CCl₄, -10 °C; (b) AgOAc, AcOH, 70%, two steps; (c) EtSH, BF₃·OEt₂, CH₂Cl₂, 92%; (d) TBDMS-Cl, pyridine, Et₃N, 91%; (e) BnBr, NaH, DMF, 92%.

Glycosylation of acceptor **13**¹⁵ using **9**, NIS and AgOTf proceeded smoothly and rapidly to afford protected disaccharide **14** (92%) with no detectable trace of the β -anomer (Scheme 2). The anomeric configuration of the newly formed rhamnosyl linkage was determined by heteronuclear correlation spectroscopy, HMQC (¹J_{Cl-H1} = 173.2 Hz).¹⁶ Transesterification of the 3'-*O*-benzoate gave disaccharide acceptor **15**, which was glycosylated by **12** in good yield. Initial attempts using NIS–AgOTf to promote glycosylation gave very little trisaccharide product, but use of NIS–TfOH as promotor furnished **16** in 86% yield. HMQC experiments confirmed the assignment of

Table 1 Isothermal microcalorimetric data for native trisaccharide **1** and derivatives **2–6**

Compound	$K_{A/mol^{-1}}$	$\Delta G/Kcal\ mol^{-1}$	$\Delta H/Kcal\ mol^{-1}$	$-T\Delta S/Kcal\ mol^{-1}$
1 ¹⁰	1.1×10^5	-6.8 ± 0.2	-3.9 ± 0.1	-2.9 ± 0.1
2	1.1×10^6	-8.1	-6.3	-1.8
3	2.5×10^6	-8.5	-8.1	-0.5
4	$1.5 \pm 0.05 \times 10^6$	-8.3 ± 0.1	-4.2 ± 0.05	-4.1 ± 0.05
5	2.6×10^6	-8.6 ± 0.15	-4.4 ± 0.1	-4.2 ± 0.05
6	4.3×10^5	-7.4	-5.6	-1.8



Scheme 2 (a) **6**, NIS, AgOTf, CH₂Cl₂, 3 Å MS, 92%; (b) Na, MeOH, CH₂Cl₂, 81%; (c) **8**, NIS, TfOH (cat.), CH₂Cl₂, 86%; (d) (i) NH₂NH₂·H₂O, EtOH; (ii) TBAF, THF; (iii) Fmoc-β-ala-OH, TBTU, HOBT, NEM, DMF, 68%, 3 steps; (e) (i) TEMPO, KBr, NaHCO₃, NaOCl, MeCN (pH 10.5 with NaOH); (ii) 20% piperidine–DMF; (iii) PYBOP, DMF, 61%, 3 steps; (f) H₂, Pd(OH)₂, MeOH, H₂O, 79%; (g) *n*-Bu₃SnH, AIBN, THF, dioxane, 90%.

the linkage as α ($^1J_{\text{Cl-H1}} = 169.8$ Hz) and, as expected for the 2-*O*-benzyl-mannosyl donor, approximately 5% of the undesired β-anomer was formed.

The transformation of protected trisaccharide **16** to **17** was accomplished by using NH₂NH₂·H₂O to remove the phthalimido group, followed by TBAF to remove the 6''-*O*-TBDMS ether. The amino-alcohol was treated with *N*-Fmoc-β-alanine-OH under peptide coupling conditions (TBTU–HOBT) to afford **17** in 68% over 3 steps. This alcohol was oxidized using TEMPO–NaOCl¹⁷ and then filtered through a short pad of silica-gel and treated under standard Fmoc deprotection conditions. Initial attempts at macro-cyclization using the above stated peptide-coupling conditions resulted in a large amount of unreacted activated ester. Employing a single component coupling system of PyBOP in DMF¹⁸ at high dilution (1 mg mL⁻¹) afforded the protected lactam **18** (91% for the cyclization from the acid, 61% from **17**), which was hydrogenated over Pd(OH)₂ to afford deprotected **5** (79% yield).

The one and two dimensional proton NMR spectra of protected cyclic trisaccharide **18** indicated that tethering had restricted the solution conformation of the molecule. Resonances corresponding to the methylene protons of the tether had sharpened into diastereotopic signals with defined line structure. As expected, this fine structure was not present for acyclic precursor **17**, as rotamer conformations about the amide linkage averaged and broadened a number of signals in the spectrum. The gradient-enhanced COSY spectra (GCOSY) of **18** exhibited long-range intra-residue coupling (up to $^5J_{\text{H-H}}$) within the *rhamno*- and *manno*-ring systems. The deprotected compounds **5** and **6** also exhibited this long-range scalar coupling.¹⁹ The protected derivative **18** also exhibited $^4J_{\text{H-H}}$ inter-

residue coupling for both H-1' and H-1'' across the glycosidic bonds. While these extended coupling pathways have very small values and are only observed in two dimensional spectra of α -linked sugars, they were not observed for the less restricted open chain molecules described here or in the preceding paper.

Initially, it was intended to acetylate **5**, effect reduction of the chloride with tri-*n*-butyltin hydride (TBTH)²⁰ and deacetylate to get cyclic deoxy **6**. Standard acetylation conditions (Ac₂O, Pyr.) produced a mixture of hexa- and hepta-acetylated compounds. Although it is rarely observed that a lactam nitrogen is acetylated under these conditions it is the only available site for over-acetylation.²¹ Reduction of this mixture with TBTH–AIBN in toluene, followed by Zémpfen transesterification produced poor yields of **6** along with recovered **5** after HPLC. Direct reduction of the 2'-chloro substituent of **5** was facilitated with TBTH–AIBN in 3 : 1 dioxane : THF and produced **6** in good yield with little or no detectable **5** (Scheme 2).

Native trisaccharide **1** was synthesized based on a published procedure.²²

Binding with SYA/J6 and conformation

Microcalorimetry^{23,24} was carried out using HPLC-purified samples of trisaccharides **1** to **6**. Solutions of ligands (0.50 mM) were titrated against purified mAb SYA/J6 (30 μM, all of which were obtained from a single preparation of protein). Entries that report standard deviations were run in duplicate and on separate batches of purified protein.

The cyclic 2'-chloro tethered trisaccharide **5** exhibited the highest affinity of all trisaccharide ligands ($K_A = 2.6 \times 10^6$), a value that is essentially indistinguishable from the activity of

3 and **4**. However the origins of the free energy are quite different. Enthalpy and entropy are both favourable and contribute equally to the binding free energy of **5** and **4**. This suggests that functional group modification and tethering both contribute to the observed activity but the effects are in no way additive. Definitive proof for the conclusion that intramolecular pre-organization was not the only cause for affinity increases cannot be derived from thermodynamics since entropy–enthalpy compensation effects are always observed and mask the molecular basis for the observed activities.^{25,26} Surprisingly, cyclic **2**-deoxy **6** exhibited decreased affinity and bound with lower free energy than either cyclic **4** or acyclic deoxy **3**. When the data for **6** and **3** are compared a substantially reduced enthalpy of interaction occurs when **6** is bound. When compared to the binding of the tethered trisaccharide **4** comparable enthalpy it is seen but binding occurs with less favourable entropy. This is consistent with fewer attractive interactions between the protein and the tethered 2-deoxy compound **6**, which could arise if the tether prevents the deep penetration proposed to account for the activity of **3** (Table 1).

We sought to explain the binding data, especially with respect to **6**, by focusing our attention on modeling the solution conformation, dynamics and performing a docking analysis. Calculations were performed using Insight II with the AMBER Plus force field²⁷ containing an imported parameter designed to account for L-sugars in the ¹C₄ conformation.²⁸ Parallel calculations were performed on the disaccharides α -L-Rha-*p*-(1→3)- α -L-Rha-*p*-OMe and α -L-Rha-*p*-(1→3)- β -D-GlcNAc-*p*-OMe using the HSEA-based GeGOP²⁹ and Insight II. Both programs gave virtually identical results, encouraging us to use the Insight software for the remainder of our dynamics and conformational calculations. The only limitation of this force field is the inability to properly assign atom potentials for a covalent carbon–chlorine bond. Therefore, calculations could not be performed for compound **5**.

The dynamics calculations of **6** indicate highly restricted conformations around the glycosidic bonds that are almost identical to those of the solution state of the native trisaccharide epitope (Fig. 3). Superimposition of the calculated global minimum energy conformation of **6** on the structure of **3**, extracted from the co-crystallized complex of SYA/J6 with **3**, shows that the pre-organized conformation of **6** mimics the bound conformation of **3** (Fig. 4). By way of contrast, a slightly

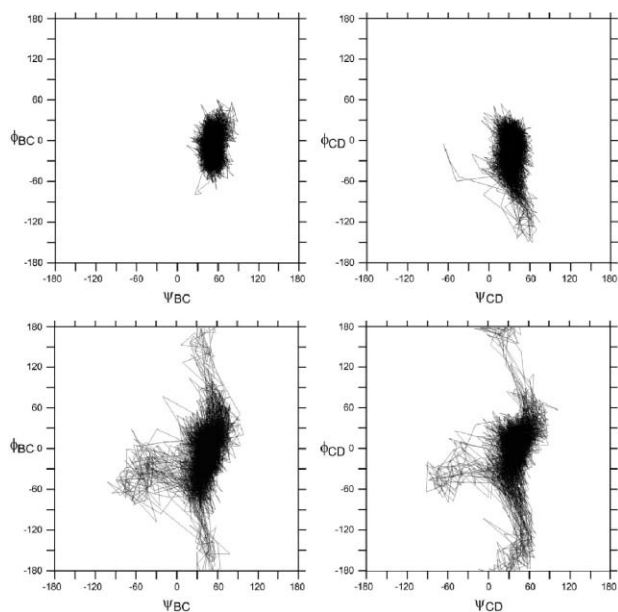


Fig. 3 ϕ vs. ψ plots for **1** (top) and **6** (bottom) derived from molecular dynamics simulations. Subscripts BC and CD refer to the glycosidic bonds between the B, C and D rings of the trisaccharides.

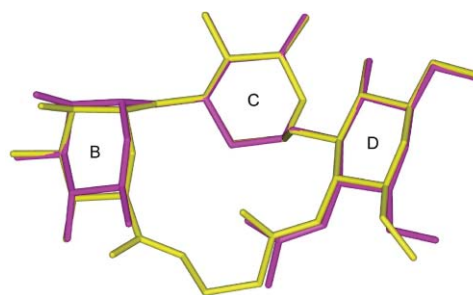


Fig. 4 Superimposition of the global energy minimum of **6** upon the bound conformation of **3** (taken from the co-complexed crystal structure of **3** and mAb SYA/J6).

more flexible bond was defined by the dihedral H-5''–C-5''–C-6''–O-6''. Calculations indicated that this bond sampled two conformations with the minimum energy structure being the preferred conformation approximately 90% of the time.

Docking analysis of SYA/J6 was performed by superimposing the global minimum conformer of **6** on the 3-SYA/J6 co-complex (Fig 5). If tethered **6** binds in the same mode as **3**, there does not appear to be any evident steric clash between the tether and the protein surface, although closer inspection shows that the nitrogen from the amide used for tethering at the L-mannosyl residue points directly towards a hydrophobic area of the protein surface, which is extended by deoxygenation of the 2'-position of the trisaccharide. It may also be the case that our paired modifications have induced a shift of the trisaccharide in the binding site that induces a significant clash of the tether and the protein.

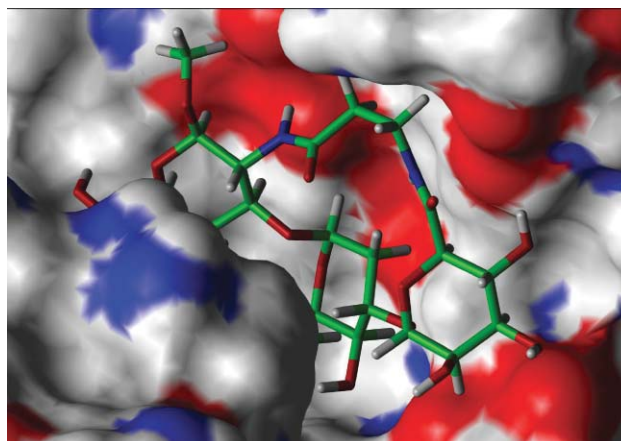


Fig. 5 Depiction of **6** docked on the bound structure of **3** in the antibody-binding site. No apparent clash of the ligand is visible with the protein surface.

Since we could not assign the atom potentials for **5** due to the lack of support for the carbon–chlorine covalent bond within the AMBER Plus force field, we can only hypothesize why it bound with the highest affinity. We have postulated that the binding of **2** would likely be the same as **1**, since the substitution of a hydroxyl for a chlorine is a conservative substitution in terms of size. Modeling indicates that tethered trisaccharide **4** can bind in the same mode as **1**. However, **5** binds with an affinity almost identical to that of **4**, which shows that the free energy changes are not additive. While we have successfully used intramolecular tethering to gain affinity, when this modification is combined with functional group replacement only a very small increase in affinity is observed.

Conclusions

Analysis of the MD calculations, docking and microcalorimetry data lead us to conclude that a more thorough analysis and

detailed computational model are required to investigate the optimum positions of the atoms of the tether for compounds **4** and **5** and their compatibility with the adjacent protein surface. We are investigating the binding of compounds **5** and **6** using the technique of STD NMR.³⁰ This technique reveals those ligand groups that make the most intimate contact with protein. This approach, together with MD simulations for the different trisaccharide complexes with SYA/J6, should be able to correlate modes of binding between our ligands. If this technique permits us to identify the ligand–protein contacts for the pairs **2** with **5** and **3** with **6**, we should be able to establish the origins for the absence of additive free energies and the disappointing affinities of **5** and **6**.

At present, the most likely explanation for the absence of strong additive free energy gains is the inherent flexibility of glycosidic linkages. In untethered oligosaccharides this could permit energy gains for single site modifications to be obtained by segmental movements that are permissible and create higher complementary, while in the tethered derivatives such mutually complementary movements are constrained by the presence of a tether. If this is the case, computational approaches that simulate and identify ligand intra-residue movements that result in enhanced activity offer the best hope to achieve high affinity carbohydrate ligands.

Experimental

General methods

Analytical thin-layer chromatography (TLC) was performed on silica gel 60 F₂₅₄ (Merck) and detected by UV light at 254 nm and charring with 5% sulfuric acid in ethanol. All reagents were used as supplied. Chlorine gas was purchased and used without any further purification. **Caution:** chlorine gas is extremely poisonous, corrosive and is strongly oxidizing—use proper backflow prevention devices in apparatus piping. When noted, solvents were distilled and dried according to common literature protocol. Column chromatography was performed with silica gel (230–400 mesh, 60 Å). High-performance liquid chromatography (HPLC) was performed using a Waters HPLC system using an absorbance detector. Separations were done using a C₁₈ semi-preparative reverse-phase column, with combinations of water and methanol as eluents. ¹H NMR spectra were recorded at 500 or 600 MHz and ¹³C spectra were recorded at 125 MHz. They were calibrated to residual solvent signals: δ_{H} 7.24 ppm and δ_{C} 77.0 ppm for samples in CDCl₃. Samples recorded in D₂O were referenced to 0.1% external acetone (δ_{H} 2.225 ppm and δ_{C} 31.07 ppm). Isothermal titration microcalorimetry was performed on a Microcal VPITC calorimeter. The optical rotations were measured with a Perkin-Elmer 241 polarimeter for samples in a 10 cm cell at 22 ± 2 °C. $[\alpha]_{\text{D}}$ values are given in units of 10⁻¹ deg cm² g⁻¹. Electro-spray mass spectrometry and microanalyses were performed by the analytical services of this department.

Acetyl 3-O-benzoyl-4-O-benzyl-2-chloro-2-deoxy- α -L-rhamnopyranose (8). Chlorine gas was slowly bubbled through a stirring solution of **7**⁴ (2.18 g, 6.74 mmol) in CCl₄ (6 mL) in a darkened flask at –10 °C, until a faint yellow color persisted. Excess chlorine gas was removed by bubbling argon through the solution for 1 h, the solvent was removed under a reduced pressure and evacuated under a high vacuum for 20 min. The opaque residue was dissolved in glacial acetic acid (5 mL) under an argon atmosphere, silver acetate (0.702 g, 4.20 mmol) was added and the solution was stirred for 12 h at rt. The solution was filtered through celite and concentrated. The light yellow syrup was dissolved in CH₂Cl₂, washed with saturated NaHCO₃ and water, then dried with Na₂SO₄ and evaporated to dryness. The product was purified by column chromatography (6 : 1 hexanes : ethyl acetate) to afford the target compound as a clear syrup (1.99 g, 88% overall yield; 3.9 : 1 distribution of

α -rhamno- and β -quinovo-isomers): $[\alpha]_{\text{D}}^{22} +1.98$ (*c* 1.0, CHCl₃); ¹H NMR (600 MHz, CDCl₃) δ 8.09–7.19 (m, 10H, Ar–H), 6.19 (d, 1H, *J* = 2.0 Hz, H-1), 5.61 (dd, 1H, *J* = 3.8, 9.1 Hz, H-3), 4.62 (ABq, 2H, *J* = 11 Hz, PhCH₂O–), 4.52 (dd, 1H, *J* = 2.0, 3.8 Hz, H-2), 3.99 (dq, 1H, *J* = 6.2, 9.5 Hz, H-5), 3.98 (dd \approx t, 1H, *J* = 9.3 Hz, H-4), 2.14 (s, 3H, –C(O)CH₃), 1.37 (d, 3H, *J* = 6.2 Hz, H-6); ¹³C NMR (125 MHz, CDCl₃) δ 168.6, 165.6, 137.4, 133.5, 129.8, 129.4, 128.6, 128.0, 93.1 (¹*J*_{C1–H1} = 180.19 Hz), 77.9, 75.5, 72.3, 70.5, 57.5, 20.9, 18.0; anal. calcd for C₂₂H₂₃ClO₆: C, 63.08; H, 5.53%, found C, 63.06; H, 5.57%. ES HRMS calcd for C₂₂H₂₃ClO₆Na (M + Na): 441.1080. Found 441.1072.

Ethyl 3-O-benzoyl-4-O-benzyl-2-chloro-2-deoxy-1-thio- α -L-rhamnopyranoside (9). To a solution of **8** (3.31 g, 7.90 mmol), 3 Å molecular sieves in EtSH (0.76 mL, 10.3 mmol) and distilled CH₂Cl₂ (50 mL) under Ar at 0 °C was added BF₃–OEt₂ (1.29 mL, 10.3 mmol). The reaction stirred for 2 h before the addition of Et₃N (4 mL). The solution was filtered and washed with NaHCO₃ and water. The organic layer was dried with Na₂SO₄ and concentrated under a vacuum. Column chromatography (9 : 1 hexanes : ethyl acetate) gave a clear syrup (3.04 g, 91%): $[\alpha]_{\text{D}}^{22} -62.3$ (*c* 2.56, CHCl₃); ¹H NMR (600 MHz, CDCl₃) δ 8.06–7.20 (m, 10 H, Ar–H), 5.53 (dd, 1H, *J* = 3.7, 9.2 Hz, H-3), 5.37 (s, 1H, H-1), 4.71 (dd, 1H, *J* = 1.2, 3.6 Hz, H-2), 4.69 (ABq, 2H, *J* = 10.9 Hz, PhCH₂O–), 4.26 (dq, 1H, *J* = 6.3, 9.4 Hz, H-5), 3.80 (dd \approx t, 1H, *J* = 9.3 Hz, H-4), 2.64 (m, 2H, SCH₂CH₃), 1.37 (d, 3H, *J* = 6.3 Hz, H-6); ¹³C NMR (125 MHz, CDCl₃) δ 165.3, 137.5, 133.3, 129.7, 129.5, 128.5, 128.3, 127.9, 127.8, 84.3 (*J*_{C1–H1} = 168.8 Hz), 78.6, 75.3, 73.2, 68.9, 61.1, 26.0, 18.0, 15.0; anal. calcd for C₂₂H₂₅ClO₄S: C, 62.77; H, 5.99%, found: C, 62.74; H, 5.89%. ES HRMS calcd for C₂₂H₂₅ClO₄SNa (M + Na): 443.1059, found 443.1056.

Phenyl 6-O-(tert-butylidimethylsilyl)-1-thio- α -L-mannopyranoside (11). To a stirred solution of **10**¹⁶ (1.72 g, 6.34 mmol) in pyridine (15 mL) was added TBDMS–Cl (1.43 g, 9.513 mmol). Et₃N (10 mL) was added in 1 mL portions over 10 min, whereupon the solvents were evaporated under a vacuum. The residue was dissolved in CH₂Cl₂, washed with water and dried with Na₂SO₄. Column chromatography (3 : 1 ethyl acetate : toluene) gave the title compound as a white foam (2.20 g, 90%): $[\alpha]_{\text{D}}^{22} -1.91$ (*c* 0.76, CHCl₃); ¹H NMR (600 MHz, CD₃OD) δ 7.54–7.23 (m, 5H, Ar–H), 5.41 (d, 1H, *J* = 1.2 Hz, H-1), 4.08–4.04 (m, 2H, H-2, H-5), 3.99 (dd, 1H, *J*_{gem} = 11.2 Hz, *J*_{vic} = 2.0 Hz, H-6_a), 3.82 (dd, 1H, *J*_{gem} = 11.2 Hz, *J*_{vic} = 6.7 Hz, H-6_b), 3.66 (dd, 1H, *J* = 2.8, 9.3 Hz, H-3), 3.63 (dd \approx t, 1H, *J* = 9.3 Hz), 0.89 (s, 9H, –C(CH₃)₃), 0.07 (s, 3H, –Si(CH₃)₂), 0.05 (s, 3H, –Si(CH₃)₂); ¹³C NMR (125 MHz, CD₃OD) δ 136.2, 132.7, 129.9, 128.3, 90.3 (¹*J*_{C1–H1} = 167.1 Hz), 76.1, 73.6, 73.2, 69.0, 64.5, 26.4, 19.3, –5.0, –5.1; anal. calcd for C₁₈H₃₀O₅SSi: C, 55.92; H, 7.82%, found: C, 55.55; H, 8.00%. ES HRMS calcd for C₁₈H₃₀O₅SSiNa (M + Na): 409.1475, found: 409.1474.

Phenyl 2,3,4-tri-O-benzyl-6-O-(tert-butylidimethylsilyl)-1-thio- α -L-mannopyranoside (12). A stirring solution of **11** (3.34 g, 8.65 mmol) in dry DMF (70 mL) was cooled to 0 °C under Ar and NaH (95% dry, 1.24 g, 51.9 mmol) was added. The reaction was allowed to stir for 30 min before the addition of BnBr (5.15 mL, 43.3 mmol). The mixture was stirred for 16 h before the slow addition of methanol (25 mL). The solvents were removed and the crude mixture was dissolved in CH₂Cl₂ and washed with water. The organic layer was dried with Na₂SO₄ and evaporated under a vacuum. Column chromatography (12 : 1 hexanes : ethyl acetate) gave a thin clear syrup (5.34 g, 94%): $[\alpha]_{\text{D}}^{22} -72.8$ (*c* 1.4, CHCl₃); ¹H NMR (600 MHz, CDCl₃) δ 7.47–7.23 (m, 20H, Ar–H), 5.57 (dd, 1H, *J* = 1.7 Hz, H-1), 4.96 (d, 1H, *J*_{gem} = 11.0 Hz, PhCH₂O–), 4.71–4.62 (m, 5H, PhCH₂O–), 4.12 (ddd, 1H, *J* = 1.7, 4.9, 9.6 Hz, H-5), 4.03 (dd \approx t, 1H, *J* = 9.4 Hz, H-4), 4.00 (dd, 1H, *J* = 1.7, 3.0 Hz, H-2), 3.93 (dd, 1H, *J*_{gem} = 11.5 Hz, *J*_{vic} = 5.1 Hz, H-6_a), 3.89–3.85 (m, 2H, H-3, H-6_b), 0.91 (s, 9H, –Si(CH₃)₃), 0.08 (s, 3H, –Si(CH₃)₂), 0.06 (s, 3H,

–Si(CH₃)₃); ¹³C NMR (125 MHz, CDCl₃) δ 138.7, 138.3, 138.0, 134.8, 131.5, 128.9, 128.4, 138.37, 138.31, 128.0, 127.9, 127.8, 127.7, 127.6, 127.2, 85.6, 80.2, 76.6, 75.2, 74.9, 74.3, 72.2, 71.8, 62.7, 26.0, 18.4, –5.1, –5.3; anal. calcd for C₃₉H₄₈O₅SSi: C, 71.30; H, 7.36%, found: C, 71.45; H, 7.33%. ES HRMS calcd for C₃₉H₄₈O₅SSiNa (M + Na): 679.2884. Found: 679.2885.

Methyl (3-*O*-benzoyl-4-*O*-benzyl-2-chloro-2-deoxy- α -L-rhamnopyranosyl)-(1 \rightarrow 3)-4,6-*O*-benzylidene-2-phthalimido- β -D-glucopyranoside (14). A solution of glucosamine acceptor **13**¹⁵ (2.31 g, 5.62 mmol) and rhamnosyl donor **9** (2.86 g, 6.79 mmol) were dissolved in dry CH₂Cl₂ (40 mL) and stirred with 3 Å molecular sieves for 1 h under Ar. The flask was darkened and NIS (1.53 g, 6.79 mmol) and AgOTf (1.395 g, 5.43 mmol) were added all at once. The reaction mixture was stirred for 15 min and neutralized with Et₃N (~1 mL) before filtration through celite. The CH₂Cl₂ solution was washed with Na₂S₂O₃ (10%), NaHCO₃ and water. The organic layer was dried with NaSO₄ and concentrated under a reduced pressure. Stepped gradient column chromatography (5 : 1 \rightarrow 4 : 1 \rightarrow 2 : 1 hexanes : ethyl acetate) gave a white foam (3.98 g, 92%): [α]_D²² +9.8 (c 1.1, CHCl₃); ¹H NMR (600 MHz, CDCl₃) δ 7.98–7.06 (m, 19H, Ar–H), 5.56 (s, 1H, PhCHO₂–), 5.48 (dd, 1H, *J* = 3.7, 8.7 Hz, H-3'), 5.18 (d, 1H, *J* = 8.5 Hz, H-1), 4.68 (d, 1H, *J* = 1.9 Hz, H-1'), 4.65 (dd, 1H, *J* = 8.9, 10.2 Hz, H-3), 4.51 (Abq, 2H, *J* = 11.1, 36.0 Hz, PhCH₂O–), 4.41 (dd, 1H, *J*_{gem} = 10.5 Hz, *J*_{vic} = 4.8 Hz, H-6_a), 4.30 (dd, 1H, *J* = 8.6, 10.3 Hz, H-2), 4.13 (dd, 1H, *J* = 1.9, 3.7 Hz, H-2'), 4.08 (dq, 1H, *J* = 6.2, 8.85 Hz, H-5'), 3.83 (dd \approx t, 1H, *J*_{5,6} \approx *J*_{vic} = 10.1 Hz, H-6_b), 3.72 (dd \approx t, 1H, *J* = 9.3 Hz, H-4), 3.67 (ddd, 1H, *J*_{5,6} \approx *J*_{gem} = 9.4 Hz, *J*_{vic} = 4.8 Hz, H-5), 3.49 (dd \approx t, 1H, *J* = 9.1 Hz, H-4'), 3.41 (s, 3H, OCH₃), 0.82 (d, 3H, *J* = 6.2 Hz, H-6'); ¹³C NMR (125 MHz, CDCl₃) δ 179.8, 165.5, 137.3, 136.9, 134.3, 133.2, 131.3, 129.7, 129.6, 129.0, 128.4, 128.2, 128.1, 127.7, 127.6, 126.3, 101.9, 100.2 (¹*J*_{C1-H1} = 165.9 Hz), 99.7 (¹*J*_{C1-H1} = 173.2 Hz), 80.6, 78.5, 74.6, 74.3, 71.9, 68.7, 68.4, 58.7, 57.0, 56.3, 17.3; anal. calcd for C₄₂ClH₄₀NO₁₁: C, 65.49; H, 5.23; N, 1.82%, found: C, 65.18; H, 5.24; N, 1.69%. ES HRMS calcd for C₄₂ClH₄₀NO₁₁Na (M + Na): 792.2187, found: 792.2180.

Methyl (4-*O*-benzyl-2-chloro-2-deoxy- α -L-rhamnopyranosyl)-(1 \rightarrow 3)-4,6-*O*-benzylidene-2-phthalimido- β -D-glucopyranoside (15). Protected disaccharide **14** (2.125 g, 2.76 mmol) was dissolved in 20 mL of dry CH₂Cl₂ under Ar at 0 °C. Methanol (40 mL) and sodium metal (88 mg) were added, the reaction warmed to rt and stirred for 12 h before neutralization with Rexyn 101 (H⁺) resin. The resin was filtered and the solution was concentrated under a vacuum. Column chromatography (2% acetone in CHCl₃) afforded a white powder (1.49 g, 81%): [α]_D²² –55.12 (c 2.1, CHCl₃); ¹H NMR (600 MHz, CD₂Cl₂) δ 7.91–7.23 (m, 14H, Ar–H), 5.56 (s, 1H, PhCHO₂–), 5.17 (d, 1H, *J* = 8.6 Hz, H-1), 4.64 (d, 1H, *J* = 11.3 Hz, PhCH₂O–), 4.62 (s, 1H, H-1'), 4.59 (dd, 1H, *J* = 8.9, 9.9 Hz, H-3), 4.52 (d, 1H, *J* = 11.3 Hz, PhCH₂O–), 4.40 (d, 1H, *J* = 4.3, 7.1 Hz, H-6_a), 4.19 (dd, 1H, *J* = 8.6, 10.1 Hz, H-2), 4.00 (ddd, 1H, 3.8, 7.2, 9.0 Hz, H-3'), 3.94–3.87 (m, H-2', H-5'), 3.85–3.79 (m, 1H, H-6_b), 3.70–3.63 (m, 2H, H-4, H-5), 3.41 (s, 3H, OCH₃), 3.14 (dd \approx t, 1H, *J* = 9.2 Hz, H-4'), 2.13 (d, 1H, *J* = 7.1 Hz, 3'-OH), 0.79 (d, 3H, *J* = 6.2 Hz, H-6'); ¹³C NMR (125 MHz, CD₂Cl₂) δ 168.3, 138.8, 137.7, 135.0, 131.6, 129.4, 128.7, 128.5, 128.09, 128.07, 126.8, 124.0, 102.3, 100.5, 100.1, 81.6, 81.0, 75.1, 75.0, 70.1, 69.1, 68.8, 66.9, 62.8, 57.5, 57.0, 17.7; anal. calcd for C₃₅H₃₆ClNO₁₀: C, 63.11; H, 5.45; N, 2.10%, found: C, 62.75; H, 5.34; N, 2.09%. ES HRMS calcd for C₃₅H₃₆ClNO₁₀Na (M + Na): 688.1925, found: 688.1924.

Methyl (2,3,4-tri-*O*-benzyl-6-*O*-tert-butylidimethylsilyl- α -L-mannopyranosyl)-(1 \rightarrow 3)(4-*O*-benzyl-2-chloro-2-deoxy- α -L-rhamnopyranosyl)-(1 \rightarrow 3)-4,6-*O*-benzylidene-2-phthalimido- β -D-glucopyranoside (16). Disaccharide acceptor **15** (1.33 g, 2.00 mmol) and thiomannosyl donor **12** (2.37 g, 3.60 mmol)

were dissolved in dry CH₂Cl₂ (40 mL), protected from light and cooled to –30 °C under Ar. NIS (0.81 g, 3.60 mmol) and a solution of CH₂Cl₂ (200 μ L) saturated with TfOH were added all at once. The reaction was warmed to –20 °C and allowed to stir for 2 h before neutralization with Et₃N (1.5 mL). The reaction mixture was washed with Na₂S₂O₃ (10%), NaHCO₃ and water. The organic layer was dried with Na₂SO₄ and concentrated under a vacuum. Stepped gradient column chromatography (7 : 1 \rightarrow 6 : 1 \rightarrow 4 : 1 hexanes : ethyl acetate) afforded a white foam (2.10 g, 86%): [α]_D²² –15.65 (c 0.69, CDCl₃); ¹H NMR (600 MHz, CDCl₃) δ 7.83–7.11 (m, 29H, Ar–H), 5.56 (s, 1H, PhCHO₂–), 5.20 (d, 1H, *J* = 8.4 Hz, H-1), 4.97 (d, 1H, *J* = 1.8 Hz, H-1'), 4.88 (d, 1H, *J*_{gem} = 10.8 Hz, PhCH₂O–), 4.67 (d, 1H, *J*_{gem} = 10.8 Hz, PhCH₂O–), 4.61 (d, 1H, *J*_{gem} = 11.4 Hz, PhCH₂O–), 4.58 (d, 1H, *J* = 1.8 Hz, H-1'), 4.56 (dd, 1H, *J* = 8.4, 10.2 Hz, H-3), 4.53–4.46 (m, 3H, PhCH₂O–), 4.43–4.35 (m, 3H, PhCH₂O–, H-6_a), 4.25 (dd, 1H, *J* = 8.5, 10.1 Hz, H-2), 4.08 (dd \approx t, 1H, *J* = 9.7 Hz, H-4'), 4.03 (dd, 1H, *J* = 3.7, 9.0 Hz, H-3'), 3.95–3.91 (m, 2H, H-5', H-6_a'), 3.90 (dd, 1H, *J* = 1.6, 3.7 Hz, H-2'), 3.87–3.81 (m, 3H, H-6_b, H-6_b', H-3'), 3.69–3.63 (m, 3H, H-4, H-5, H-5'), 3.59 (dd, 1H, *J* = 1.9, 2.9 Hz, H-2'), 3.42 (s, 3H, OCH₃), 3.19 (dd \approx t, 1H, *J* = 9.3 Hz, H-4'), 0.91 (s, 9H, (CH₃)₃CSi–), 0.77 (d, 3H, *J* = 6.1 Hz, H-6'), 0.12 (s, 6H, (CH₃)₂Si–); ¹³C NMR (125 MHz, CDCl₃) δ 167.9, 138.9, 138.7, 138.5, 138.3, 137.0, 134.4, 131.2, 129.0, 128.3, 128.2, 128.1, 127.8, 127.6, 127.5, 127.4, 127.2, 127.1, 126.3, 123.6, 101.8, 100.2 (¹*J*_{C1-H1} = 169.8 Hz, C-1'), 99.9 (¹*J*_{C1-H1} = 172.7 Hz, C-1'), 99.7 (¹*J*_{C1-H1} = 164.9 Hz, C-1), 80.5, 79.9, 79.3, 77.3, 76.2, 75.1, 74.6, 74.4, 73.9, 72.4, 72.3, 68.7, 68.6, 66.4, 61.2, 60.7, 57.1, 56.5, 26.0, 17.2, –5.0, –5.3; anal. calcd for C₆₈H₇₈ClNO₁₅Si: C, 67.34; H, 6.48; N, 1.15%, found: C, 67.46; H, 6.53; N, 1.13%. ESMS calcd for C₆₈H₇₈ClNO₁₅SiNa (M + Na): 1234.5, found: 1234.5.

Methyl (2,3,4-tri-*O*-benzyl- α -L-mannopyranosyl)-(1 \rightarrow 3)(4-*O*-benzyl-2-chloro-2-deoxy- α -L-rhamnopyranosyl)-(1 \rightarrow 3)-4,6-*O*-benzylidene-2-deoxy-2-(*N*-fluorenylmethoxycarbonyl- β -alanyl)-amido- β -D-glucopyranoside (17). To a solution of fully protected trisaccharide **16** (0.721g, 0.595 mmol) in EtOH (15 mL) was added NH₂NH₂·H₂O (1.0mL, 12.9 mmol) and the solution was heated to reflux under Ar for 24 h. Upon cooling the reaction was diluted with CHCl₃ and washed with water. The organic layer was evaporated to dryness and the resulting solid was suspended in boiling EtOH (100%), cooled, filtered and washed with cold EtOH to afford the free amine that needed no further purification. The white solid was dissolved in THF and TBAF (1.5 mL, 1.0 M in THF) was added. The solution stirred for 24 h and evaporated to dryness. The brown syrup was filtered through silica using toluene as the eluent and evaporated to dryness. The resultant white solid (0.467 g, 0.482 mmol) was dissolved in dry DMF (12 mL) and *N*-Fmoc- β -alanine (0.180, 0.578 mmol), TBTU (0.309 g, 0.964 mmol), HOBt (0.147 g, 0.964 mmol) and *N*-ethylmorpholine (0.24 mL, 1.880 mmol) were added. The reaction was allowed to stir for 2 h, the solvents were removed under a vacuum and the residue was dissolved in CH₂Cl₂ and washed with water. The organic layer was dried with Na₂SO₄, filtered and evaporated to dryness. Column chromatography (2 : 3 : 1 cyclohexane : ethyl acetate : acetone) afforded the target compound as a white powder (0.510 g, 68%): [α]_D²² –40.69 (c 1.02, CHCl₃); ¹H NMR (600 MHz, CDCl₃) δ 7.75–7.15 (m, 33H, Ar–H), 5.50 (s, 1H, PhCHO₂–), 5.11 (s, 1H, H-1'), 4.88 (s, 1H, H-1'), 4.84 (d, 1H, *J*_{gem} = 10.9 Hz, PhCH₂O–), 4.61 (d, 1H, *J*_{gem} = 11.7 Hz, PhCH₂O–), 4.59–4.40 (m, 8H, H-1, H-2', PhCH₂O–), 4.39–4.32 (m, 3H, H-6_a, –O–CH₂CHAR), 4.20–4.16 (m, 2H, H-3', –OCH₂CHAR), 4.01–4.09 (bm, 1H, H-3), 3.97–3.88 (m, 4H, H-5', H-3'', H-5'', H-6_a''), 3.77–3.72 (m, 2H, H-6_b, H-4''), 3.69–3.64 (m, 2H, H-2'', H-6_b''), 3.61–3.53 (m, 1H, H-2), 3.52 (dd \approx t, *J* = 9.3 Hz, H-4), 3.48–3.38 (m, 3H, H-5, tether- β _{a/b}), 3.38–3.33 (m, 4H, OCH₃, H-4'), 2.48–2.36 (m,

2H, tether- α H_{a/b}), 0.81 (d, 3H, $J = 6.0$ Hz, H-6'); ¹³C NMR (125 MHz, CDCl₃) δ 172.1, 157.0, 144.1, 143.8, 138.4, 138.3, 138.2, 138.1, 137.0, 129.0, 128.38, 128.35, 128.31, 128.26, 128.2, 128.1, 127.8, 127.7, 127.6, 127.55, 127.53, 127.51, 127.2, 127.1, 126.3, 125.2, 101.7, 101.4, 100.9, 100.8, 79.94, 79.87, 77.9, 77.7, 75.9, 75.4, 75.1, 75.0, 74.0, 72.7, 72.4, 68.9, 68.7, 66.8, 66.3, 63.0, 61.2, 57.2, 56.8, 37.1, 36.7, 17.3; anal. calcd for C₇₂H₇₇ClN₂O₁₆: C, 68.53; H, 6.15; N, 2.22%; found: C, 68.49; H, 6.20; N, 2.37%. ESMS calcd for C₇₂H₇₇ClN₂O₁₆Na (M + Na): 1283.4, found: 1283.4.

Methyl (2,3,4-tri-*O*-benzyl- α -L-mannopyranosyluronyl)-(1 \rightarrow 3)-(4-*O*-benzyl-2-chloro-2-deoxy- α -L-rhamnopyranosyl)-(1 \rightarrow 3)-2-(3-aminopropionamido)-4,6-*O*-benzylidene-2-deoxy- β -D-glucopyranoside lactam (18). To a solution of primary alcohol 17 (0.46 g, 0.36 mmol) in MeCN (3 mL) was added TEMPO (0.057 g, 0.36 mmol), 1 M KBr (0.365 mL), 0.50 M NaHCO₃ (1.83 mL) and NaOCl (5–6%, 2.20 mL) at –5 °C. The reaction was allowed to stir for 1 hr before the addition of methanol (0.5 mL). After 15 min 1 M HCl (15 mL) and CH₂Cl₂ (25 mL) were added and the organic layer was removed, washed with water and dried with Na₂SO₄. The crude syrup was passed through a small plug of silica using CH₂Cl₂ : MeOH (50 : 1) as eluent. The resultant off-white solid was stirred in 20% piperidine–DMF (5 mL) for 30 min. The solvents were evaporated under a vacuum and co-evaporated with toluene (3 \times 10 mL). The resulting light yellow syrup was dissolved in DMF (200 mL). PyBOP (0.360 g, 0.69 mmol) was added and the reaction stirred for 1 h before evaporation under a vacuum. The residue was chromatographed (4 : 2 : 1 hexane : ethyl acetate : acetone) to afford an amorphous white solid (0.22 g, 61%): [α]_D²⁵ –39.55 ($c = 0.89$, CHCl₃); ¹H NMR (600 MHz, CD₃C(O)CD₃) δ 7.51–7.21 (m, 27 H, Ar–H, 2 \times NH), 5.63 (s, 1H, PhCHO₂–), 5.25 (d, 1H, $J = 3.5$ Hz, H-1''), 4.94 (d, 1H $J = 1.3$ Hz, H-1'), 4.78 (d, 1H, $J = 10.3$ Hz, PhCH₂O–), 4.77 (d, 1H, $J = 11.0$ Hz, PhCH₂O–), 4.64–4.58 (m, 5H, PhCH₂O–), 4.52 (d, 1H, $J = 11.3$ Hz, PhCH₂O–), 4.46 (d, 1H, $J = 8.4$ Hz, H-1), 4.34 (dd, 1H, $J = 1.7, 3.7$ Hz, H-2'), 4.29 (d, 1H, $J = 8.8$ Hz, H-5''), 4.27–4.21 (m, 2H, H-6_a, H-2), 4.15 (dd, 1H, $J = 3.7, 9.3$ Hz, H-3'), 3.91–3.75 (m, 7H, tether- β H_a, H-3, H-6_b, H-5', H-2'', H-3'', H-4''), 2.67 (dd, 1H, $J_{3,4} \approx J_{4,5} = 9.5$ Hz, H-4), 3.44 (ddd, 1H, $J = 5.1, 9.7, 9.7$ Hz, H-5), 3.34 (dd, $J_{3,4} \approx J_{4,5} = 9.4$ Hz, H-4'), 3.24 (m, 1H, tether- β H_b), 2.48 (ddd, 1H, $J_{\text{gem}} = 16.5$ Hz, $J_{\text{vic}} = 3.0$ Hz, tether- α H_a), 2.40 (ddd, 1H, $J_{\text{gem}} = 16.5$ Hz, $J_{\text{vic}} = 12.0, 4.0$ Hz, tether- α H_b), 0.74 (d, 3H, $J = 6.2$ Hz, H-6'). ¹³C NMR (125 MHz, CDCl₃) δ 171.2, 169.2, 138.3, 138.1, 137.8, 137.1, 129.1, 128.32, 128.28, 128.26, 128.24, 127.9, 127.7, 127.63, 127.58, 127.55, 126.4, 102.6, 102.3, 101.7, 101.6, 82.2, 80.3, 19.1, 78.3, 77.5, 75.1, 73.6, 73.0, 72.8, 72.0, 68.8, 68.6, 66.5, 61.9, 56.7, 54.3, 35.3, 33.5, 17.6; anal. calcd for C₅₇H₆₃ClN₂O₁₄: C, 66.11; H, 6.13; N, 2.71%; found: C, 65.86; H, 6.03; N, 2.69%. ES MS calcd for C₅₇H₆₃ClN₂O₁₄Na (M + Na): 1057.4, found: 1057.4.

Methyl (α -L-mannopyranosyluronyl)-(1 \rightarrow 3)-(2-chloro-2-deoxy- α -L-rhamnopyranosyl)-(1 \rightarrow 3)-2-(3-aminopropionamido)-2-deoxy- β -D-glucopyranoside lactam (5). Protected lactam 18 (105 mg, 0.101 mmol) was suspended in methanol (10 mL) and water (1 mL). Pd(OH)₂ (Degussa type, 85 mg) was added and the reaction stirred under a hydrogen atmosphere (balloon) for 24 h. The reaction was filtered, evaporated and pre-purified with a Waters C-18 Sep-Pak. Further purification was performed using reverse-phase (C-18) HPLC utilizing a gradient from 0% to 15% methanol in water. The resultant clear glass was lyophilized to a white powder (47.2 mg, 79%). [α]_D²⁵ –27.31 ($c = 0.67$, H₂O), ¹H NMR (600 MHz, D₂O) δ 5.13 (d, 1H, $J = 1.1$ Hz, H-1''), 5.02 (d, 1H, $J = 1.1$ Hz, H-1'), 4.39 (d, 1H, $J = 8.5$ Hz, H-1), 4.12–4.06 (m, 4H, H-2', H-3', H-2'', H-5''), 4.03 (dq, 1H, $J = 9.7, 6.2$ Hz, H-5'), 3.95–3.85 (m, 3H, H-2, H-6_a, H-4''), 3.79 (dd, 1H, $J = 3.4, 9.3$ Hz, H-3''), 3.74 (dd,

1H, $J = 6.1$ Hz, $J_{\text{gem}} = 12.4$ Hz, H-6_b), 3.67–3.60 (m, 2H, H-4', tether- β H_a), 3.57–3.51 (m, 3H, H-3, H-4, tether- β H_b), 3.48 (s, 3H, OCH₃), 3.42 (ddd, 1H, $J = 2.0, 5.9, 8.5$ Hz, H-5), 2.64–2.51 (m, 2H, tether- α H_{a/b}), 1.28 (d, 3H, $J = 6.2$ Hz, H-6'). ¹³C NMR (125 MHz, D₂O) δ 174.5, 170.8, 104.3, 103.1, 102.7, 86.2, 78.7, 76.8, 73.2, 71.9, 71.2, 70.7, 70.4, 69.8, 68.9, 62.0, 61.6, 57.9, 55.1, 35.4, 34.7, 17.5. ES HRMS calcd for C₂₂H₃₅ClN₂O₁₄Na (M + Na): 609.1674, found 609.1673.

Methyl (α -L-mannopyranosyluronyl)-(1 \rightarrow 3)-(2-deoxy- α -L-rhamnopyranosyl)-(1 \rightarrow 3)-2-(3-aminopropionamido)-2-deoxy- β -D-glucopyranoside lactam (6). To a refluxing solution of 5 (19.4 mg, 33 μ mol) in a 1,4-dioxane–THF mixture (3 : 1, 2 mL) was added TBTH (18 μ L, 66 μ mol) and AIBN (6 mg, 33 μ mol) and was allowed to react for 12 h. The reaction was cooled to 40 °C, dichloromethane was added and refluxed for 30 min. The mixture was then concentrated under a vacuum. The reaction was pre-purified with a Waters C-18 Sep-Pak, followed by reverse-phase (C-18) HPLC utilizing a gradient from 0% to 15% methanol in water to give a clear glass that was lyophilized to a white solid (14.1 mg, 77%). [α]_D²⁵ = –36.15 ($c = 0.52$, H₂O). ¹H NMR (600 MHz, D₂O) δ 5.07 (s, 1H, H-1''), 4.94 (d, 1H, $J = 3.5$ Hz, H-1'), 4.40 (d, 1H, $J = 8.7$ Hz, H-1), 4.04 (dd, 1H, $J = 1.6, 3.5$ Hz, H-2'), 3.98–3.86 (m, 6H, H-2, H-6_a, H-3', H-5', H-4'', H-5''), 3.77–3.67 (m, 3 H, H-3'', H-6_b, tether- β H_a), 3.52–3.42 (m, 7H, tether- β H_b, H-3, H-4, H-5, OCH₃), 3.25 (dd, 1H $J_{3',4'} \approx J_{4',5'} = 9.5$ Hz, H-4'), 2.64–2.50 (m, 2H, tether- α H_{a/b}), 2.05 (dd, 1H, $J_{\text{vic}} = 12.5$ Hz, $J_{\text{gem}} = 4.9$ Hz, H-2_a'), 1.92 (ddd, 1H, $J_{\text{vic}} = 12.5$ Hz, $J_{\text{gem}} = 12.5, 4.9$ Hz, H-2_b'), 1.24 (d, 3H, $J = 6.2$ Hz, H-6'). ¹³C NMR (125 MHz, D₂O) δ 172.9, 169.6, 102.5, 101.3, 99.9, 84.5, 77.2, 75.4, 74.1, 70.9, 70.5, 69.3, 68.5, 67.5, 60.3, 56.4, 53.7, 37.2, 33.4, 16.4. ES HRMS calcd for C₂₂H₃₆N₂O₁₄Na (M + Na): 575.2064, found 575.2065.

Isothermal titration calorimetry

Titration microcalorimetry was performed for compounds 1–6 according to the procedure described in the preceding paper.¹

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