ARTICLE

OBC
www.rsc.org/obc

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

Robert S. McGavin and David R. Bundle*

Alberta Ingenuity Centre for Carbohydrate Science, Department of Chemistry, University of Alberta, Edmonton, Alberta T6G 2G2. E-mail: Dave.Bundle@ualberta.ca

Received 18th October 2004, Accepted 9th May 2005
First published as an Advance Article on the web 10th June 2005

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 comapred 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]

$$[\rightarrow 2)\text{-}\alpha\text{-L-}\underset{A}{\text{Rha}}p(1\rightarrow 2)\text{-}\alpha\text{-L-}\underset{B}{\text{Rha}}p(1\rightarrow 3)\text{-}\alpha\text{-L-}\underset{C}{\text{Rha}}p(1\rightarrow 3)\text{-}\beta\text{-D-}\underset{D}{\text{GlcNAc}}p(1\rightarrow]_n$$

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 trisaccahride **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 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 moieties are similar.[7]

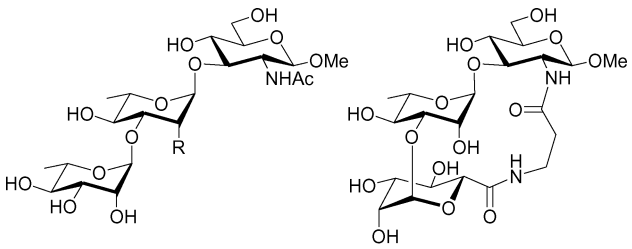


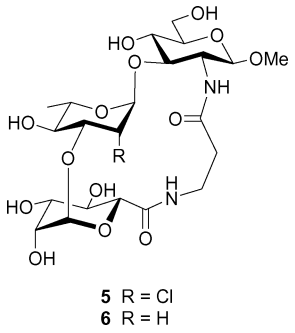
Fig. 1 Native trisaccharide **1** and previously synthesized derivatives thereof, including **2** and **3** containing modifications at the rhamnose C residue, along with tethered **4**.

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

DOI:10.1039/b416106h

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 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.



5 R = Cl
6 R = H

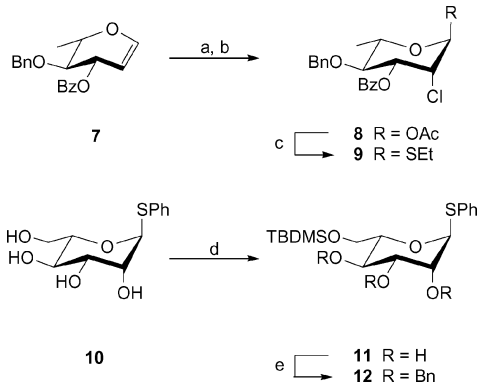
Fig. 2 Targets of this work, depicting the β-alanyl tether of **4** and functional group modifications at the rhamnose C residue.

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 likley to be formed as a consequence of the use of a non-partcipating 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.* protocol[12] led to highly diastereofacial *cis*-addition of chlorine gas to **9** in CCl_4, followed by the Koenig–Knorr glycosylation of acetic acid[13] 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 chlorination[12] 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).

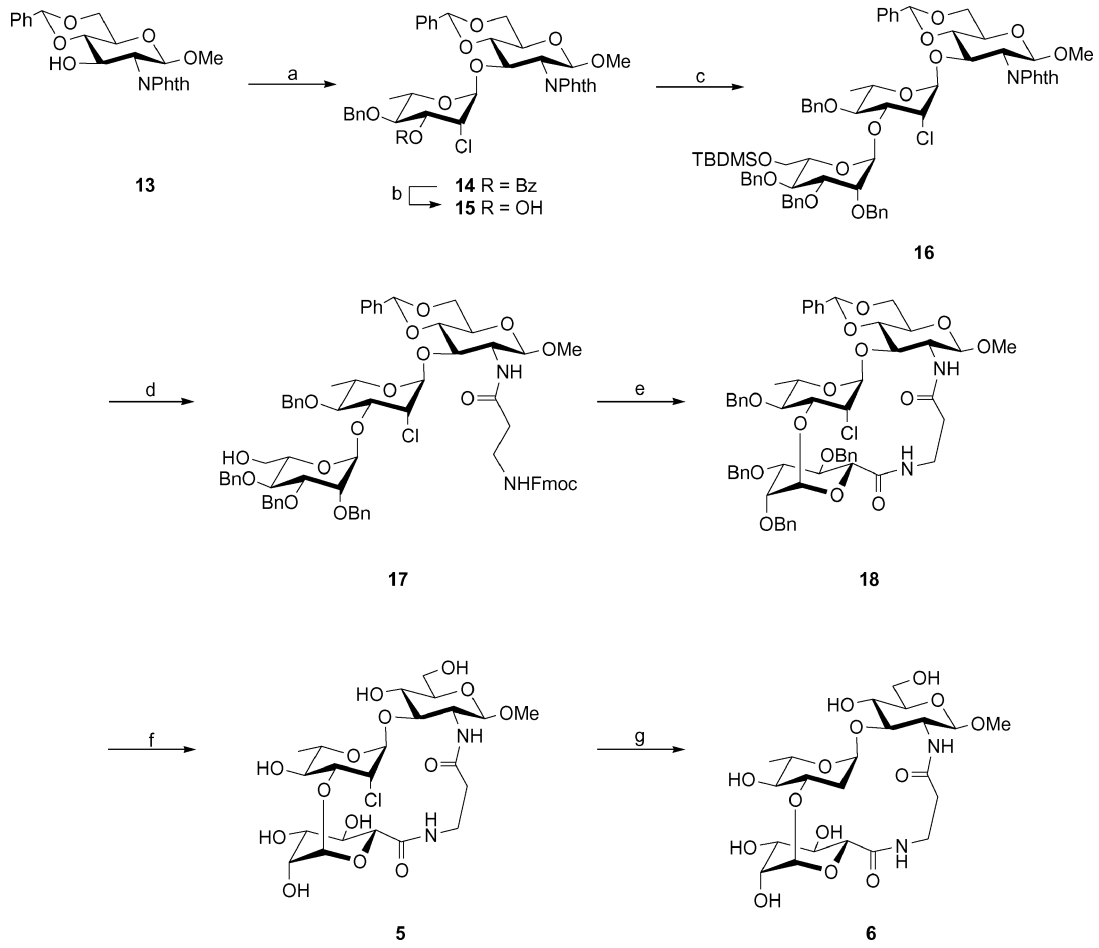


Scheme 1 *Reagents and Conditions*: (a) Cl_2, CCl_4, −10 °C; (b) AgOAc, AcOH, 70%, two steps; (c) EtSH, BF_3–OEt_2, CH_2Cl_2, 92%; (d) TBDMS–Cl, pyridine, Et_3N, 91%; (e) BnBr, NaH, DMF, 92%.

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 spectrsocopy, HMQC ($^1J_{C1-H1}$ = 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	$K_{A/mol^{-1}}$	ΔG/Kcal mol^{-1}	ΔH/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, CH_2Cl_2, 3 Å MS, 92%; (b) Na, MeOH, CH_2Cl_2, 81%; (c) **8**, NIS, TfOH (cat.), CH_2Cl_2, 86%; (d) (*i*) NH_2NH_2–H_2O, EtOH; (*ii*) TBAF, THF; (*iii*) Fmoc-β-ala-OH, TBTU, HOBt, NEM, DMF, 68%, 3 steps; (e) (*i*) TEMPO, KBr, $NaHCO_3$, NaOCl, MeCN (pH 10.5 with NaOH); (*ii*) 20% piperidine–DMF; (*iii*) PYBOP, DMF, 61%, 3 steps; (f) H_2, $Pd(OH)_2$, MeOH, H_2O, 79%; (g) *n*-Bu_3SnH, AIBN, THF, dioxane, 90%.

the linkage as α ($^1J_{C1-H1}$ = 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 NH_2NH_2–H_2O 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–NaOCl[17] 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 DMF[18] 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 $^5J_{H-H}$) 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 $^4J_{H-H}$ *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 (Ac_2O, Pyr.) produced a mixture of hexa- and hepta-acylated 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 Zémplen 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 esentially indistinguisable from the activity of

3 and **4**. However the orgins 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 field[27] containing an imported parameter designed to account for L-sugars in the $^1C_4$ 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 GeGOP[29] 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

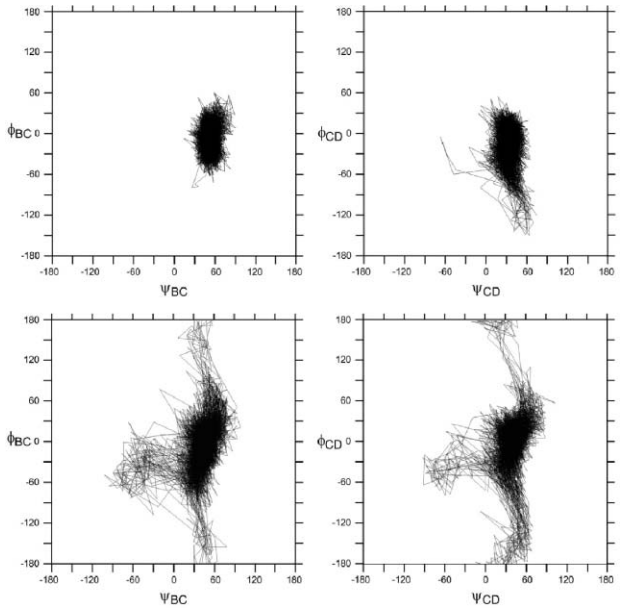


Fig. 3 ϕ *vs.* ψ plots for **1** (top) and **6** (bottom) derived from molecular dynamics simulations. Subscripts BC and CD refer to the glycosidic bonds between the B, C and D rings of the trisaccharides.

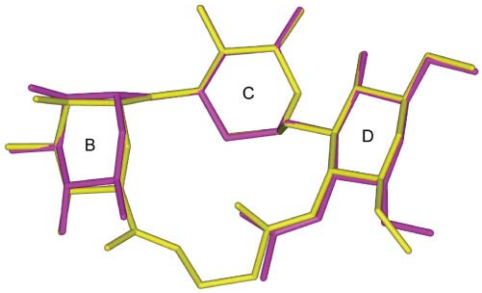


Fig. 4 Superimposition of the global energy minimum of **6** upon the bound conformation of **3** (taken from the co-complexed crystal structure of **3** and mAb SYA/J6).

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 hydophpobic 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.

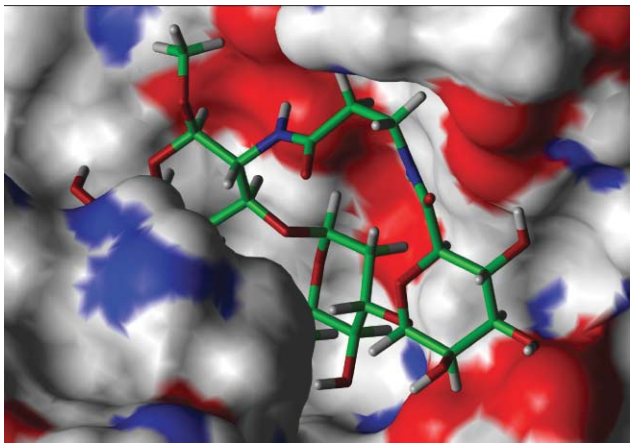


Fig. 5 Depiction of **6** docked on the bound structure of **3** in the antibody-binding site. No apparent clash of the ligand is visible with the protein surface.

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 succesfully 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 orgins for the absence of additive free energies and the disappointing affinites 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 complimentary, while in the tethered derivatives such mutually complementary movements are constrained by the presence of a tether. If this is the case, computional 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 F_{254} (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 preformed using a Waters HPLC system using an absorbance detector. Separations were done using a C_{18} semi-preparative reverse-phase column, with combinations of water and methanol as eluents. ^{1}H NMR spectra were recorded at 500 or 600 MHz and ^{13}C 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 $CDCl_3$. Samples recorded in D_2O 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 VP ITC calorimeter. The optical rotations were measured with a Perkin-Elmer 241 polarimeter for samples in a 10 cm cell at 22 ± 2 °C. $[a]_D$ values are given in units of 10^{-1} deg cm^2 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 CCl_4 (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 CH_2Cl_2, washed with saturated $NaHCO_3$ and water, then dried with Na_2SO_4 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): $[a]_D^{22}$ +1.98 (*c* 1.0, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$) 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, PhC*H*$_2$O–), 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 ≈ t, 1H, *J* = 9.3 Hz, H-4), 2.14 (s, 3H, –C(O)C*H*$_3$), 1.37 (d, 3H, *J* = 6.2 Hz, H-6); ^{13}C NMR (125 MHz, $CDCl_3$) δ 168.6, 165.6, 137.4, 133.5, 129.8, 129.4, 128.6, 128.0, 93.1 ($^1J_{C1-H1}$ = 180.19 Hz), 77.9, 75.5, 72.3, 70.5, 57.5, 20.9, 18.0; anal. calcd for $C_{22}H_{23}ClO_6$: C, 63.08; H, 5.53%, found C, 63.06; H, 5.57%. ES HRMS calcd for $C_{22}H_{23}ClO_6Na$ (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** (3.31g, 7.90 mmol), 3 Å molecular sieves in EtSH (0.76 mL, 10.3 mmol) and distilled CH_2Cl_2 (50 mL) under Ar at 0 °C was added BF_3–OEt_2 (1.29 mL, 10.3 mmol). The reaction stirred for 2 h before the addition of Et_3N (4 mL). The solution was filtered and washed with $NaHCO_3$ and water. The organic layer was dried with Na_2SO_4 and concentrated under a vacuum. Column chromatography (9 : 1 hexanes : ethyl acetate) gave a clear syrup (3.04 g, 91%): $[a]_D^{22}$ −62.3 (*c* 2.56, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$) 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, PhC*H*$_2$O–), 4.26 (dq, 1H, *J* = 6.3, 9.4 Hz, H-5), 3.80 (dd ≈ t, 1H, *J* = 9.3 Hz, H-4), 2.64 (m, 2H, SC*H*$_2$CH$_3$), 1.37 (d, 3H, *J* = 6.3 Hz, H-6), 1.27 (t, 3H, SCH$_2$C*H*$_3$); ^{13}C NMR (125 MHz, $CDCl_3$) 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 $C_{22}H_{25}ClO_4S$: C, 62.77; H, 5.99%, found: C, 62.74; H, 5.89%. ES HRMS calcd for $C_{22}H_{25}ClO_4SNa$ (M + Na): 443.1059, found 443.1056.

Phenyl 6-*O*-(*tert*-butyldimethylsilyl)-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). Et_3N (10 mL) was added in 1 mL portions over 10 min, whereupon the solvents were evaporated under a vacuum. The residue was dissolved in CH_2Cl_2, washed with water and dried with Na_2SO_4. Column chromatography (3 : 1 ethyl acetate : toluene) gave the title compound as a white foam (2.20 g, 90%): $[a]_D^{22}$ −1.91 (*c* 0.76, $CHCl_3$); ^{1}H NMR (600 MHz, CD_3OD) 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 ≈ t, 1H, *J* = 9.3 Hz), 0.89 (s, 9H, –C(C*H*$_3$)$_3$), 0.07 (s, 3H, –Si(C*H*$_3$)$_a$), 0.05 (s, 3H, –Si(C*H*$_3$)$_b$); ^{13}C NMR (125 MHz, CD_3OD) δ 136.2, 132.7, 129.9, 128.3, 90.3 ($^1J_{C1-H1}$ = 167.1 Hz), 76.1, 73.6, 73.2, 69.0, 64.5, 26.4, 19.3, −5.0, −5.1; anal. calcd for $C_{18}H_{30}O_5SSi$: C, 55.92; H, 7.82%, found: C, 55.55; H, 8.00%. ES HRMS calcd for $C_{18}H_{30}O_5SSiNa$ (M + Na): 409.1475, found: 409.1474.

Phenyl 2,3,4-tri-*O*-benzyl-6-*O*-(*tert*-butyldimethylsilyl)-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 CH_2Cl_2 and washed with water. The organic layer was dried with Na_2SO_4 and evaporated under a vacuum. Column chromatography (12 : 1 hexanes : ethyl acetate) gave a thin clear syrup (5.34 g, 94%): $[a]_D^{22}$ −72.8 (*c* 1.4, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$); 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, PhC*H*$_2$O–), 4.71–4.62 (m, 5H, PhC*H*$_2$O–), 4.12 (ddd, 1H, *J* = 1.7, 4.9, 9.6 Hz, H-5), 4.03 (dd ≈ 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, –SiC(C*H*$_3$)$_3$), 0.08 (s, 3H, –Si(C*H*$_3$)$_a$), 0.06 (s, 3H,

–Si(CH_3)$_b$); ^{13}C NMR (125 MHz, $CDCl_3$) 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 $C_{39}H_{48}O_5SSi$: C, 71.30; H, 7.36%, found: C, 71.45; H, 7.33%. ES HRMS calcd for $C_{39}H_{48}O_5SSiNa$ (M + Na): 679.2884. Found: 679.2885.

Methyl (3-*O*-benzoyl-4-*O*-benzyl-2-chloro-2-deoxy-α-L-rhamnopyranosyl)-(1→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 CH_2Cl_2 (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 Et_3N (~1 mL) before filtration through celite. The CH_2Cl_2 solution was washed with $Na_2S_2O_3$ (10%), $NaHCO_3$ and water. The organic layer was dried with $NaSO_4$ and concentrated under a reduced pressure. Stepped gradient column chromatography (5 : 1→4 : 1→2 : 1 hexanes : ethyl acetate) gave a white foam (3.98 g, 92%): $[\alpha]_D^{22}$ +9.8 (*c* 1.1, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.98–7.06 (m, 19H, Ar–*H*), 5.56 (s, 1H, PhC*H*O$_2$–), 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, Ph CH_2O–), 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 ≈ t, 1H, $J_{5,6} \approx J_{vic}$ = 10.1 Hz, H-6$_b$), 3.72 (dd ≈ 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 ≈ t, 1H, *J* = 9.1 Hz, H-4′), 3.41 (s, 3H, OCH_3), 0.82 (d, 3H, *J* = 6.2 Hz, H-6′); ^{13}C NMR (125 MHz, $CDCl_3$) 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 ($^1J_{C1-H1}$ = 165.9 Hz), 99.7 ($^1J_{C1-H1}$ = 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 $C_{42}ClH_{40}NO_{11}$: C, 65.49; H, 5.23; N, 1.82%, found: C, 65.18; H, 5.24; N, 1.69%. ES HRMS calcd for $C_{42}ClH_{40}NO_{11}Na$ (M + Na): 792.2187, found: 792.2180.

Methyl (4-*O*-benzyl-2-chloro-2-deoxy-α-L-rhamnopyranosyl)-(1→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 CH_2Cl_2 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 $CHCl_3$) afforded a white powder (1.49 g, 81%): $[\alpha]_D^{22}$ −55.12 (*c* 2.1, $CHCl_3$); ^{1}H NMR (600 MHz, CD_2Cl_2) δ 7.91–7.23 (m, 14H, Ar–*H*), 5.56 (s, 1H, PhC*H*O$_2$–), 5.17 (d, 1H, *J* = 8.6 Hz, H-1), 4.64 (d, 1H, *J* = 11.3 Hz, PhCH_2O–), 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, PhCH_2O–), 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, OCH_3), 3.14 (dd ≈ t, 1H, *J* = 9.2 Hz, H-4′), 2.13 (d, 1H, *J* = 7.1 Hz, 3′-O*H*), 0.79 (d, 3H, *J* = 6.2 Hz, H-6′); ^{13}C NMR (125 MHz, CD_2Cl_2) 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 $C_{35}H_{36}ClNO_{10}$: C, 63.11; H, 5.45; N, 2.10%, found: C, 62.75; H, 5.34; N, 2.09%. ES HRMS calcd for $C_{35}H_{36}ClNO_{10}Na$ (M + Na): 688.1925, found: 688.1924.

Methyl (2,3,4-tri-*O*-benzyl-6-*O*-*tert*-butyldimethylsilyl-α-L-mannopyranosyl)-(1→3)(4-*O*-benzyl-2-chloro-2-deoxy-α-L-rhamnopyranosyl)-(1→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 CH_2Cl_2 (40 mL), protected from light and cooled to −30 °C under Ar. NIS (0.81 g, 3.60 mmol) and a solution of CH_2Cl_2 (200 μL) 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 Et_3N (1.5 mL). The reaction mixture was washed with $Na_2S_2O_3$ (10%), $NaHCO_3$ and water. The organic layer was dried with Na_2SO_4 and concentrated under a vacuum. Stepped gradient column chromatography (7 : 1→6 : 1→4 : 1 hexanes : ethyl acetate) afforded a white foam (2.10 g, 86%): $[\alpha]_D^{22}$ −15.65 (*c* 0.69, $CDCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.83–7.11 (m, 29H, Ar–*H*), 5.56 (s, 1H, PhC*H*O$_2$–), 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, PhCH_2O–), 4.67 (d, 1H, J_{gem} = 10.8 Hz, PhCH_2O–), 4.61 (d, 1H, J_{gem} = 11.4 Hz, PhCH_2O–), 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, PhCH_2O–), 4.43–4.35 (m, 3H, PhCH_2O-, H-6$_a$), 4.25 (dd, 1H, *J* = 8.5, 10.1 Hz, H-2), 4.08 (dd ≈ 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, OCH_3), 3.19 (dd ≈ t, 1H, *J* = 9.3 Hz, H-4′), 0.91 (s, 9H, (CH_3)$_3$CSi–), 0.77 (d, 3H, *J* = 6.1 Hz, H-6′), 0.12 (s, 6H, (CH_3)$_2$Si–); ^{13}C NMR (125 MHz, $CDCl_3$) 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 ($^1J_{C1-H1}$ = 169.8 Hz, C-1″), 99.9 ($^1J_{C1-H1}$ = 172.7 Hz, C-1′), 99.7 ($^1J_{C1-H1}$ = 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 $C_{68}H_{78}ClNO_{15}Si$: C, 67.34; H, 6.48; N, 1.15%, found: C, 67.46; H, 6.53; N, 1.13%. ESMS calcd for $C_{68}H_{78}ClNO_{15}SiNa$ (M + Na): 1234.5, found: 1234.5.

Methyl (2,3,4-tri-*O*-benzyl-α-L-mannopyranosyl)-(1→3)(4-*O*-benzyl-2-chloro-2-deoxy-α-L-rhamnopyranosyl)-(1→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 NH_2NH_2–H_2O (1.0mL, 12.9 mmol) and the solution was heated to reflux under Ar for 24 h. Upon cooling the reaction was diluted with $CHCl_3$ 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 CH_2Cl_2 and washed with water. The organic layer was dried with Na_2SO_4, 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%): $[\alpha]_D^{22}$ −40.69 (*c* 1.02, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.75–7.15 (m, 33H, Ar–*H*), 5.50 (s, 1H, PhC*H*O$_2$–), 5.11 (s, 1H, H-1″), 4.88 (s, 1H, H-1′), 4.84 (d, 1H, J_{gem} = 10.9 Hz, PhCH_2O–), 4.61 (d, 1H, J_{gem} = 11.7 Hz, PhCH_2O–), 4.59–4.40 (m, 8H, H-1, H-2′, PhCH_2O–), 4.39–4.32 (m, 3H, H-6$_a$, –O–CH_2CHAr), 4.20–4.16 (m, 2H, H-3′, –OCH$_2$C*H*Ar), 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 ≈ t, *J* = 9.3 Hz, H-4), 3.48–3.38 (m, 3H, H-5,tether-$\beta_{a/b}$), 3.38–3.33 (m, 4H, OCH_3, H-4′), 2.48–2.36 (m,

2H, tether-$\alpha H_{a/b}$), 0.81 (d, 3H, J = 6.0 Hz, H-6′); ^{13}C NMR (125 MHz, $CDCl_3$) 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 $C_{72}H_{77}ClN_2O_{16}$: C, 68.53; H, 6.15; N, 2.22%, found: C, 68.49; H, 6.20; N, 2.37%. ESMS calcd for $C_{72}H_{77}ClN_2O_{16}Na$ (M + Na): 1283.4, found: 1283.4.

Methyl (2,3,4-tri-*O*-benzyl-α-L-mannopyranosyluronyl)-(1→3)-(4-*O*-benzyl-2-chloro-2-deoxy-α-L-rhamnopyranosyl)-(1→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 $NaHCO_3$ (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 CH_2Cl_2 (25 mL) were added and the organic layer was removed, washed with water and dried with Na_2SO_4. The crude syrup was passed through a small plug of silica using CH_2Cl_2 : 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 × 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%): $[\alpha]_D^{22}$ −39.55 (c = 0.89, $CHCl_3$); ^{1}HNMR (600 MHz, $CD_3C(O)CD_3$) δ 7.51–7.21 (m, 27 H, Ar–*H*, 2 × N*H*), 5.63 (s, 1H, PhC*H*O_2–), 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, PhCH_2O–), 4.77 (d, 1H, J = 11.0 Hz, PhCH_2O–), 4.64–4.58 (m, 5H, PhCH_2O–), 4.52 (d, 1H, J = 11.3 Hz, PhCH_2O–), 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-βH_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-βH_b), 2.48 (ddd, 1H, J_{gem} = 16.5 Hz, J_{vic} = 3.0 Hz, tether-αH_a), 2.40 (ddd, 1H, J_{gem} = 16.5 Hz, J_{vic} = 12.0, 4.0 Hz, tether-αH_b), 0.74 (d, 3H, J = 6.2 Hz, H-6′). ^{13}C NMR (125 MHz, $CDCl_3$) 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 $C_{57}H_{63}ClN_2O_{14}$: C, 66.11; H, 6.13; N, 2.71%, found: C, 65.86; H, 6.03; N, 2.69%. ES MS calcd for $C_{57}H_{63}ClN_2O_{14}Na$ (M + Na): 1057.4, found: 1057.4.

Methyl (α-L-mannopyranosyluronyl)-(1→3)-(2-chloro-2-deoxy-α-L-rhamnopyranosyl)-(1→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%). $[\alpha]_D^{22}$ −27.31 (c = 0.67, H_2O), ^{1}H NMR (600 MHz, D_2O) 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_{gem} = 12.4 Hz, H-6_b), 3.67–3.60 (m, 2H, H-4′, tether-βH_a), 3.57–3.51 (m, 3H, H-3, H-4, tether-βH_b), 3.48 (s, 3H, OCH_3), 3.42 (ddd, 1H, J = 2.0, 5.9, 8.5 Hz, H-5), 2.64–2.51 (m, 2H, tether-$\alpha H_{a/b}$), 1.28 (d, 3H, J, 6.2 Hz, H-6′). ^{13}C NMR (125 MHz, D_2O) 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 $C_{22}H_{35}ClN_2O_{14}Na$ (M + Na): 609.1674, found 609.1673.

Methyl (α-L-mannopyranosyluronyl)-(1→3)-(2-deoxy-α-L-rhamnopyranosyl)-(1→3)-2-(3-aminopropionamido)-2-deoxy-β-D-glucopyranoside lactam (6). To a refluxing solution of **5** (19.4 mg, 33 μmol) in a 1,4-dioxane–THF mixture (3 : 1, 2 mL) was added TBTH (18 μL, 66 μmol) and AIBN (6 mg, 33 μmol) 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%). $[\alpha]_D^{22}$ = −36.15 (c = 0.52, H_2O). ^{1}H NMR (600 MHz, D_2O) 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-βH_a), 3.52–3.42 (m, 7H, tether-βH_b, H-3, H-4, H-5, OCH_3), 3.25 (dd, 1H $J_{3',4'} \approx J_{4',5'}$ = 9.5 Hz, H-4′), 2.64–2.50 (m, 2H, tether-$a_{a/b}$), 2.05 (dd, 1H, J_{vic} = 12.5 Hz, J_{gem} = 4.9 Hz, H-2_a′), 1.92 (ddd, 1H, J_{vic} = 12.5 Hz, J_{gem} = 12.5, 4.9 Hz, H-2_b′), 1.24 (d, 3H, J = 6.2 Hz, H-6′). ^{13}C NMR (125 MHz, D_2O) 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 $C_{22}H_{36}N_2O_{14}Na$ (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]

Acknowledgements

Financial support was provided by research grants to D. R. Bundle from the Natural Science and Engineering Research Council (NSERC) and the University of Alberta. R. S. McGavin received NSERC, Alberta Heritage Foundation for Medical Research and Alberta Research Council postgraduate scholarships. The authors thank Mrs Joanna Sadowska for the preparation and purification of monoclonal antibody, and Dr C.-C. Ling for assistance with MD calculations.

References

1 R. A. Gagne, R. S. McGavin and D. R. Bundle, *Org. Biomol. Chem.*, DOI: 10.1039/b416105j.
2 N. I. A. Carlin, A. A. Lindberg, K. Bock and D. R. Bundle, *Eur. J. Biochem.*, 1984, **139**, 189.
3 F.-I. Auzanneau and D. R. Bundle, *Carbohydr. Res.*, 1993, **247**, 195.
4 H. R. Hanna and D. R. Bundle, *Can. J. Chem.*, 1993, **71**, 125.
5 M. N. Vyas, N. K. Vyas, P. J. Meikle, B. Sinnot, B. M. Pinto, D. R. Bundle and F. A. Quiocho, *J. Mol. Biol.*, 1993, **231**, 133.
6 N. K. Vyas, M. N. Vyas, M. C. Chervenak, M. A. Johnson, B. M. Pinto, D. R. Bundle and F. A. Quiocho, *Biochemistry*, 2002, **41**, 13575.
7 A. Maradufu and A. S. Perlin, *Carbohydr. Res.*, 1974, **32**, 93.
8 J. P. Carver, *Pure Appl. Chem.*, 1993, **65**, 763.
9 M. S. Searle and D. H. Williams, *J. Am. Chem. Soc.*, 1992, **114**, 10690.
10 (*a*) N. Navarre, A. H. van Oijen and G. J. Boons, *Tetrahedron Lett.*, 1997, **38**, 2023; (*b*) N. Navarre, N. Amiot, A. van Oijen, A. Imberty, A. Poveda, J. Jimenez-Barbero, A. Cooper, M. A. Nutley and G. J. Boons, *Chem. Eur. J.*, 1999, **5**, 2281; (*c*) M. C. Galan, A. P. Venot, J. Glushka, A. Imberty and G. J. Boons, *J. Am. Chem. Soc.*, 2002, **124**, 5964; (*d*) H. C. Kolb, *Bioorg. Med. Chem. Lett.*, 1997, **7**, 2629; (*e*) M. Wilstermann, J. Balogh and G. Magnusson, *J. Org. Chem.*, 1997, **62**, 3659; (*f*) D. R. Bundle, R. Alibes, S. Nilar, A. Otter,

M. Warwas and P. Zhang, *J. Am. Chem. Soc.*, 1998, **120**, 5317; (*g*) R. Alibes and D. R. Bundle, *J. Org. Chem.*, 1998, **63**, 6288; (*h*) S. A. Wacowich-Sgarbi and D. R. Bundle, *J. Org. Chem.*, 1999, **64**, 9080; (*i*) P. Zhang and D. R. Bundle, *Isr. J. Chem.*, 200, **40**, 189.
11 (*a*) B. Fraser-Reid and R. U. Lemieux, *Can. J. Chem.*, 1964, **42**, 532; (*b*) B. Fraser-Reid and R. U. Lemieux, *Can. J. Chem.*, 1965, **43**, 1460.
12 K. Igarashi, T. Honma and T. Imagawa, *J. Org. Chem.*, 1970, **35**, 610.
13 L. Vargha and J. Kuszmann, *Chem. Ber.*, 1963, **96**, 411.
14 T. Zeigler and G. Lemanski, *Eur. J. Org. Chem.*, 1998, **63**, 163.
15 N. K. Kochetkov, N. E. Byramova, Y. E. Tsvetkov and L. V. Backinowsky, *Tetrahedron*, 1985, **41**, 3363.
16 K. Bock, I. Lundt and C. Pedersen, *Tetrahedron Lett.*, 1973, **14**, 1037.
17 N. J. Davis and S. L. Flitsch, *Tetrahedron Lett.*, 1993, **34**, 1181.
18 J. Coste, D. Lenguyen and B. Castro, *Tetrahedron Lett.*, 1990, **31**, 205.
19 A. Otter and D. R. Bundle, *J.Magn. Reson.*, 1995, **109**, 194.
20 W. P. Neumann, *Synthesis*, 1987, **8**, 665.
21 (*a*) N. Hossain, A. Zapata, M. Wilstermann, U. J. Nilsson and G. Magnusson, *Carbohydr. Res.*, 2002, **337**, 569; (*b*) D. Scarpi, E. G. Occhiato, G. Danza, M. Serio and A. Guarnaa, *Bioorg. Med. Chem.*, 2002, **10**, 3455; (*c*) V. St Georgiev, R. G. van Inwegen and P. Carlson, *Eur. J. Med. Chem.*, 1990, **25**, 375.
22 F.-I. Auzanneau and D. R. Bundle, *Can. J. Chem.*, 1993, **71**, 534.
23 B. W. Sigurskjold, E. Altman and D. R. Bundle, *Eur. J. Biochem.*, 1991, **195**, 239.
24 D. R. Bundle and B. W. Sigurskjold, *Methods Enzymol.*, 1994, **247**, 288.
25 R. U. Lemieux, M.-H. Du and U. Spohr, *J. Am. Chem. Soc.*, 1994, **116**, 9803.
26 (*a*) R. U. Lemieux, *Acc. Chem. Res.*, 1996, **29**, 373; (*b*) R. U. Lemieux, L. T. J. Delbaere, H. Beierbeck and U. Spohr, *Host–Guest Molecular Interactions: From Chemistry to Biology*, Wiley, London, UK, 1991, p. 231.
27 S. W. Homans, *Biochemistry*, 1990, **29**, 9110.
28 D. Cameron and D. R. Bundle, unpublished results.
29 R. Stuike-Prill and B. Meyer, *Eur. J. Biochem.*, 1990, **194**, 903.
30 (*a*) M. Mayer and B. Meyer, *J. Am. Chem. Soc.*, 2001, **123**, 6108; (*b*) B. Meyer and T. Peters, *Angew. Chem., Int. Ed.*, 2003, **42**, 864.