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The design, synthesis and evaluation of high affinity macrocyclic carbohydrate inhibitors†

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Carbohydrate–protein interactions have been investigated for a model system of a monoclonal antibody, SYA/J6, which binds a trisaccharide epitope of the *O*-polysaccharide of the *Shigella flexneri* variant Y lipopolysaccharide. The thermodynamics of binding for the methyl glycoside of the native trisaccharide epitope, Rha-Rha-GlcNAc (**1**) to SYA/J6 over a range of temperatures exhibits strong, linear enthalpy–entropy compensation and a negative heat capacity change ($\Delta C_p = -152$ cal mol^{-1} degree^{-1}). At 293 K the free energy of association is the sum of favourable enthalpy and entropy contributions ($\Delta H = -3.9$ kcal mol^{-1} and $-T\Delta S = -2.9$ kcal mol^{-1}). Crystal structures for SYA/J6 Fab detailed the position of the native trisaccharide epitope, Rha-Rha-GlcNAc (**1**), and facilitated a strategy to design a tighter binding, low molecular weight ligand. This involved pre-organization of the native trisaccharide **1** in its bound conformation by addition of intramolecular constraints (a β-alanyl or glycinyl tether). ELISA measurements indicated that the glycinyl tethered trisaccharide **2** was not an optimal candidate for further analysis, while microcalorimetry provided data showing that the β-alanyl tethered trisaccharide **3** displayed a 15-fold increase in affinity for SYA/J6. Tethering of **3** resulted in a favourable entropic contribution to binding, relative to the native trisaccharide **1** ($-T\Delta\Delta S = -1.2$ kcal mol^{-1}). Potential energy and dynamics calculations using the AMBER Plus force fields indicated that trisaccharide **3** adopted a rigid conformation similar to that of the bound conformation of the native trisaccharide epitope. While this strategy resulted in modest free energy gains by minimizing losses due to conformational entropy, thermodynamic data are consistent with significant contributions from solvent reorganization.

Introduction

Carbohydrate–protein interactions are found to have dissociation constants (K_D) in the milli- to micromolar range[1] with only a few exceptions that typically employ multivalent presentations.[2] While several physiologically significant interactions of this type could benefit from intervention by high affinity compounds, the K_D of the relevant carbohydrate–protein interaction must be significantly improved if design of small ligands as potential drug candidates is to be realized.[3]

The low intrinsic affinity of these interactions is general and its origins have been the topic of much speculation.[1,4] Several explanations have been advanced to account for such weak binding and include loss of conformational entropy when flexible glycosidic linkages are constrained in the bound state.[1] Several groups, including ours, have attempted to investigate this phenomenon and probe the validity of the latter explanation by pre-ordering ligands in their bound conformation.[5–8] Pre-ordering attempts have generally involved introducing conformational bias that favour the bound conformation[5] or intramolecular tethering between functional groups of the ligand that do not contact protein.[6,7] To date, increases in binding affinity reported as free energy gains have been limited to 0.5–1.0 kcal mol^{-1}. Some results failed to demonstrate any gains from entropy effects.

We have favoured investigation of ligand–protein complexes for which crystal structures are available and for which detailed titration microcalorimetry data has been collected. The combination of structural and thermodynamic data for a range of monodeoxy ligands provides a clear appreciation of those residues that generate crucial interactions. Our initial attempts utilized a trisaccharide–antibody Fab complex, wherein the branching 3,6-dideoxy hexose residue of the trisaccharide was buried and the hydroxymethyl groups of the other two hexose residues did not contact protein.[6a,b] A second antibody Fab–trisaccharide complex has been mapped in similar detail and, interestingly, introduction of either monodeoxy or chloro monodeoxy functions created ligands that exhibited 10–25-fold gains in affinity.[9] It was of interest to investigate tethering of such compounds and to attempt to create high avidity ligands.

The monoclonal antibody SYA/J6 was produced following hyper-immunization with a killed, whole cell vaccine.[10] The antibody recognizes the *O*-polysaccharide of the cell wall lipopolysaccharide of *Shigella flexneri* variant Y.[11] The *O*-polysaccharide is composed of linear tetrasaccharide repeating units (where the letters ABCD represent the positions of the monosaccharide residues within the repeat):[12]

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

Two crystal structures of SYA/J6 complexed with an ABCDA′ pentasaccharide and with a 2′-deoxy trisaccharide derivative (BC′D) have been described in a report that details the molecular interactions of the system.[11] Of the five residues of the pentasaccharide, it is chiefly the BCD residues that make the dominant interactions with the protein surface. This was also noticed during the refinement of the crystal structure, where a less optimised solution only provided electron densities for

† Electronic supplementary information (ESI) available: determination of ΔC_p for SYA/J6 bound to native trisaccharide and entropy–enthalpy compensation: $-\Delta H$ *vs.* $-T\Delta S$ for trisaccharide **1** and SYA/J6. See http://www.rsc.org/suppdata/ob/b4/b416105j/

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the BCD residues.[11] It was postulated that the flanking A and A′ residues were more mobile and thus did not participate in the key interactions between the SYA/J6 Fab and the pentasaccharide.[9c]

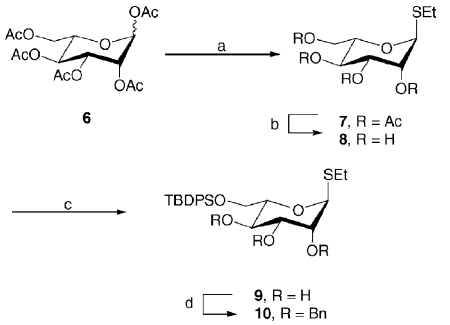
One proposal to explain the low intrinsic affinity of carbohydrate–protein interactions invokes the loss of rotational freedom about the glycosidic bond when an oligosaccharide ligand is bound by protein. The magnitude of this penalty has been estimated at about 0.6 kcal mol^{-1} per torsion angle, although work by Whitesides and co-workers suggests this number may be smaller.[13] Observation of the pentasaccharide in the binding groove of the SYA/J6 Fab indicates that the methyl groups belonging to the 2-acetamido moiety of residue D and the C-6 methyl group of residue B extend away from the protein surface into bulk solvent. We identified these sites as an optimal position at which to introduce intramolecular tethering. In order to achieve pre-organization in the bound conformation the size of the tether would have to be modified.

In this study, the native trisaccharide **1** was tethered by connecting residue B to residue D *via* glycinyl or β-alanyl tethers (**2** and **3**, respectively, Fig. 1). A linkage methodology was used that employed L-mannose instead of L-rhamnose as residue B to facilitate oxidation and amide bond formation to ligate the tether. Acyclic derivatives (**4** and **5**) are based on the β-alanyl-tethered **3**, but with "cut" tethers. These compounds were prepared to make available analogues with atom arrangements similar to their cyclic counterpart, especially the amide at the 6-position of residue B. The activity of these ligands were initially screened using a competitive solid phase assay, followed by more detailed analysis of the binding parameters using isothermal titration microcalorimetry (ITC) to determine the free energy, enthalpy and entropy of binding and also the heat capacity for the interaction of the native ligand with the antibody.[14] Molecular dynamics calculations and quantitative NOE measurements were used to evaluate the solution structure of the tethered trisaccharide **3**.

Results

The L-mannosyl thioglycoside donor **10** was prepared in five steps from L-mannose (Scheme 1). Peracetylated L-mannose (**6**) was reacted with ethanethiol and boron trifluoride diethyl etherate in CH_2Cl_2.[15] Acetate protecting groups were removed under Zemplen conditions (NaOMe–MeOH) to produce thioglycoside **8**, followed by regioselective protection with *t*-butylchlorodiphenylsilane and imidazole in DMF. Exhaustive benzylation by treatment of triol **9** with benzyl bromide and sodium hydride in THF afforded the fully blocked donor **10**.

Glycosylation of the known acceptor **11**[16] with the previously reported rhamnosyl thioglycoside donor **12**[17] initiated the linear synthesis of the core trisaccharide **17** (Scheme 2). This reaction proceeded smoothly and rapidly under promotion with *N*-iodosuccinimide (NIS) and silver triflate (AgOTf) to afford disaccharide **13** in 88% yield. Transesterification with a methanolic solution of sodium methoxide afforded disaccharide acceptor **14**. Glycosylation by the L-mannose donor **10**, again mediated by NIS–AgOTf, gave trisaccharide **15** (78% yield). The anomeric configuration of both newly synthesized glycosidic linkages were assigned as α based on $^1J_{CH}$ coupling constants (167.3 and 171.1 Hz).[18] Partial deprotection to afford intermediate core trisaccharide **17** was achieved by treatment of **15** with ethylene diamine and *n*-BuOH at reflux to afford the free amine **16**. Finally, tetrabutylammonium fluoride (TBAF)[19] mediated removal of silyl ether **16** gave the amino alcohol trisaccharide **17**.



Scheme 1 *Reagents and conditions*: (a) EtSH, BF_3–$O(Et)_2$, CH_2Cl_2, 77%; (b) NaOMe, MeOH, 100%; (c) TBDPS-Cl, imidazole, DMF, 81%; (d) BnBr, NaH, THF, 86%.

Parallel synthesis of trisaccharides **18** and **19** employed standard peptide coupling conditions (TBTU, HOBt, *N*-ethylmorpholine)[20] and *N*-Fmoc protected glycine *or N*-Fmoc protected β-alanine. Oxidation of the resultant primary alcohol of residue B used a TEMPO based system employing phase transfer conditions[21] to give the carboxylic acids **20** and **21** in moderate yield (Scheme 3). The Fmoc group was cleaved by 20% piperidine in DMF, followed by treatment with TBTU, HOBt and *N*-ethylmorpholine to effect macrolactamization in good yield (65% for **22** and 60% for **23**). Global deprotection *via* hydrogenolysis with $Pd(OH_2)$ in wet methanol afforded the deprotected cyclic trisaccharides **2** and **3**. Both were characterized by NMR and mass spectroscopy.

The synthesis of the acyclic variants of **3** originated from amine **16** (Scheme 4). Acylation of **16** with either acetic or propionic anhydride, followed by TBAF mediated desilylation afforded primary alcohols **24** and **25**. Oxidation was again accomplished with a TEMPO–NaOCl system and the crude uronic acids were each treated with carbonyldiimidazole (CDI) without further purification. The reactions were quenched with ammonia or methylamine to produce the protected bis-amides **26** and **27** respectively. Hydrogenolysis using $Pd(OH)_2$ produced the deprotected isomeric trisaccharides **4** and **5** (78% and 81%).

The inhibitory power of trisaccharides **2** to **5** were initially screened using a competitive ELISA.[22] Biotin labelled *O*-polysaccharide competed with increasing concentrations of inhibitors for binding to antibody immobilized on microtitre plates. Under the conditions of the assay the concentration of inhibitor required to reach 50% inhibition (IC_{50}) provides

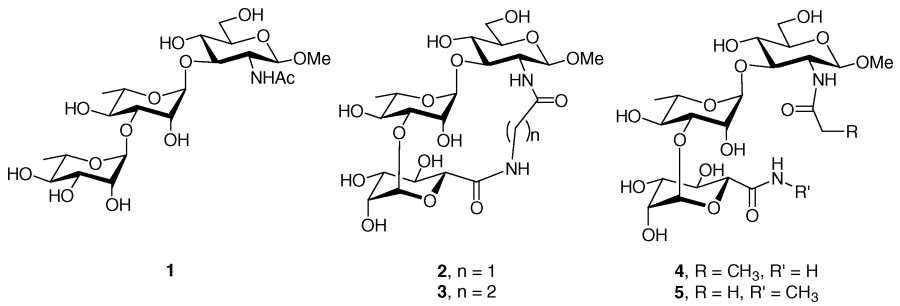
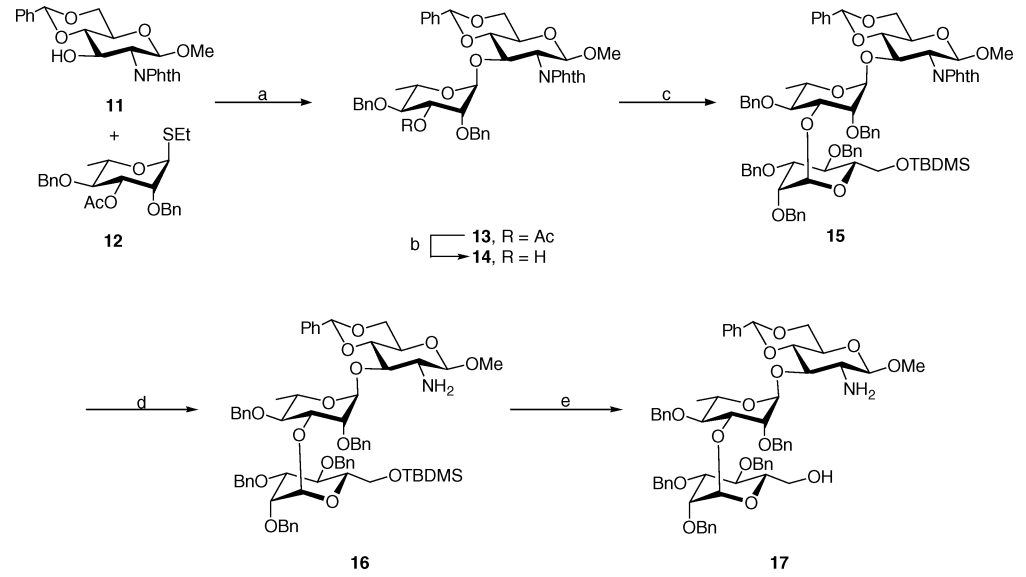
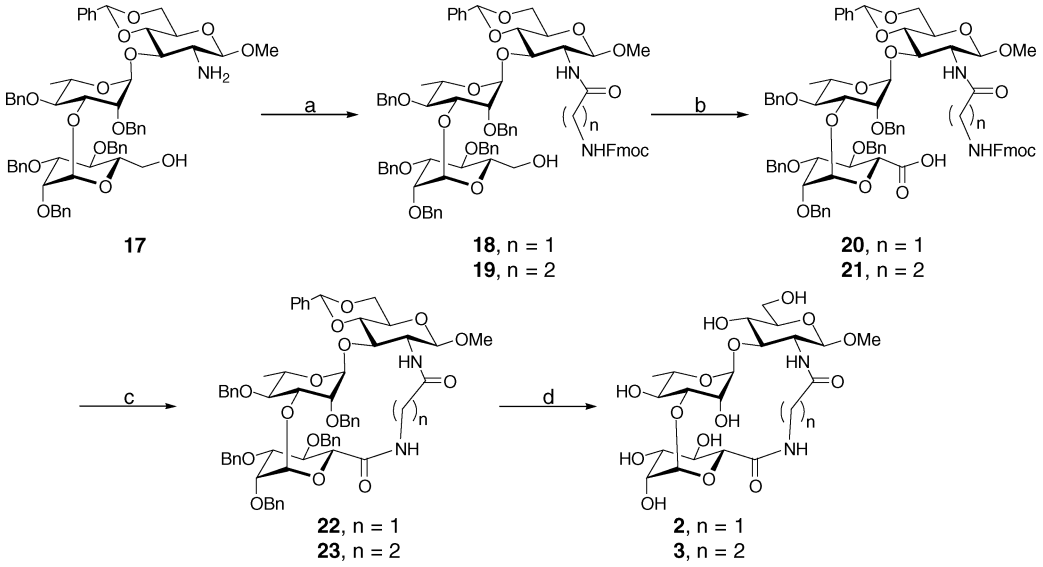


Fig. 1 Native trisaccharide (**1**), cyclic trisaccharides containing glycinyl- (**2**, n = 1) and β-alanyl- (**3**, n = 2) tethers. Acyclic derivatives **4** and **5**, with their "cut" tethers in isomeric positions, are also displayed. The rings are labelled BCD from the non-reducing end to the reducing end.



Scheme 2 *Reagents and conditions*: (a) NIS, AgOTf, CH_2Cl_2, 88%; (b) NaOMe, MeOH, 92%; (c) NIS, AgOTf, CH_2Cl_2, 78%; (d) $NH_2CH_2CH_2NH_2$, *t*-BuOH, 115 °C, 90%; (e) TBAF, THF, 60 °C, 86%.



Scheme 3 *Reagents and conditions*: (a) TBTU, HOBt, NEM, DMF *and* Fmoc-Gly-OH *or* Fmoc-β-Ala-OH, 86% for **18**, 91% for **19**; (b) TEMPO, NaOCl, *n*-Bu_4NCl, $NaHCO_3$, KBr, NaCl, 43% for **20**, 63% for **21**; (c) (i) 20% piperidine in DMF; (ii) TBTU, HOBt, NEM, DMF, 65% for **22**, 60% for **23**; (d) H_2, $Pd(OH)_2$, MeOH, 74% for **2**, 73% for **3**.

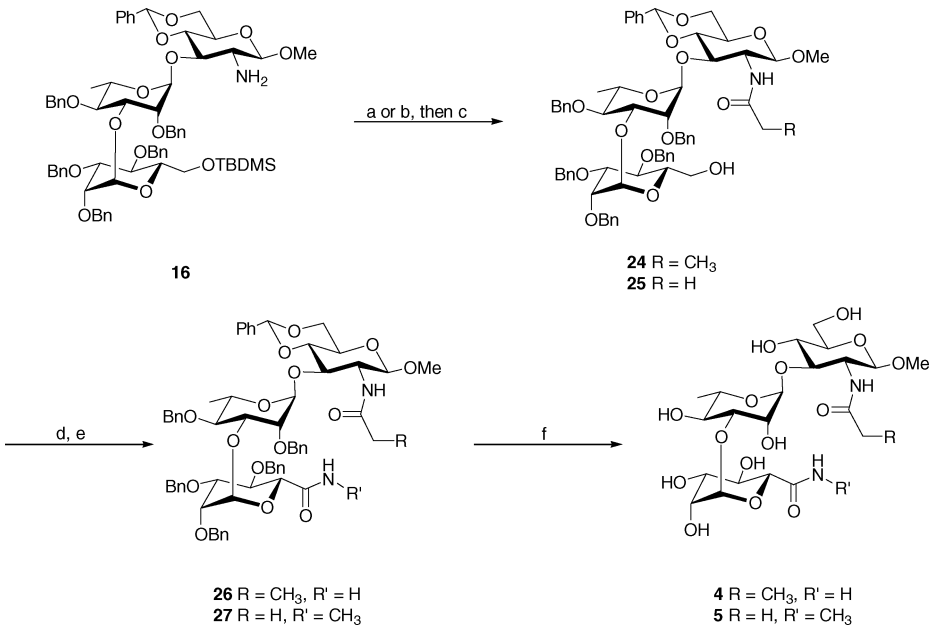
an estimate of the disassociation constant. The results are listed in the second column of Table 1. The β-alanyl-tethered trisaccharide **3** showed a marked increase in activity relative to **1**, consistent with the envisioned pre-organization of the ligand in the bound state conformation. Conversely, the glycinyl-tethered **2** lost approximately an order of magnitude of binding affinity, suggesting that the shorter tether induced a conformation that diverged substantially from that required in the bound state. Both acyclic variants **4** and **5** exhibited an almost equal 5-fold decrease in affinity relative to that of native trisaccharide **1**. The most likely explanations were either that a chemical incompatibility of the polar uronic amide installed on the L-mannosyl residue with the protein surface or a direct steric clash between the unligated forms of the tether (Table 1).

Microcalorimetry was employed to establish the thermodynamics of the ligand–antibody interactions. The binding of native ligand **1** was investigated over a range of temperatures. Constant pressure heat capacities, ΔC_p, were determined by

Table 1 Competitive ELISA and ITC data for the native trisaccharide (**1**) and synthesized trisaccharide derivatives (**2** through **5**)

	Compound	IC_{50}/μM	K_A/mol^{-1}	ΔG/kcal mol^{-1}	ΔH/kcal mol^{-1}	$-T\Delta S$/kcal mol^{-1}
1		17	1.1×10^5	-6.8 ± 0.2	-3.9 ± 0.1	-2.9 ± 0.1
2		137	—	—	—	—
3		5	$1.5 \pm 0.05 \times 10^6$	-8.3 ± 0.1	-4.2 ± 0.05	-4.1 ± 0.05
4		94	2.0×10^4	-5.8	-3.5	-2.3
5		92	2.9×10^4	-6.0	-3.1	-2.9

Standard deviations are calculated from duplicate measurements. Errors in the determination of ΔH are ±2.5%.



Scheme 4 Synthesis of acyclic trisaccharides **4** and **5**. *Reagents and conditions*: (a) acetic anhydride, MeOH; (b) propionic anhydride, MeOH; (c) TBAF, THF, 88% for **25**, 89% for **26** (two steps); (d) TEMPO, KBr, $NaHCO_3$, NaOCl, MeCN (pH 10.5 with NaOH); (e) CDI, CH_2Cl_2, then NH_3 *or* $MeNH_2$, 68% for **27**, 67% for **28** (two steps); (f) H_2, $Pd(OH)_2$, MeOH, H_2O, 81% for **4**, 78% for **5**.

evaluating ΔH between 285 to 309 K and were found to be negative ($\Delta C_p = -152$ cal mol^{-1} degree^{-1}).† The enthalpy and entropy exhibit strong linear compensation so that the entropic contribution to the binding interaction approaches zero as the temperature increases, and the enthalpy of binding equals the free energy of binding at a temperature of 318 K.†

Isothermal titration calorimetry was performed on the tightest binding trisaccharides (**1, 3** through **5**), since ELISA data provides only relative or approximate association constants.[13] The K_A of the tethered trisaccharide **3** determined by calorimetry was even larger than that predicted by the ELISA data and the free energy gain relative to **1** ($\Delta\Delta G$) was approximately -1.5 kcal mol^{-1}. In contrast to previously published data[11] from our group, the current measurements were recorded at 293 K (Table 1).

To explain the binding data, and to confirm that cyclic **3** was able to adopt a bioactive conformation, molecular dynamics calculations were performed using Insight II with the AMBER Plus force field.[23] A dielectric constant of 80 was used to simulate the presence of water. Fig. 2 illustrates the ϕ *vs.* ψ plots for both glycosidic bonds in the native trisaccharide **1**, tethered trisaccharide **3** and acyclic amide **5**. Compound **4** was omitted from the calculation due to its extensive similarity with **5** (Fig. 2). Tethered trisaccharide **3** shows remarkable similarity to the conformation of **1** and also indicates a narrower range of conformational freedom, despite a slight deviation on the ψ_2 axis away from the conformation of **1**. While maintaining a similar conformation to that of **1**, acyclic **5** (and by implication **4**) populated a broader range of conformational space. The shorter glycinyl tether resulted in a conformation for trisaccharide **2** that was distinctly different than that of native **1**.

Estimates of the solution conformation of unbound ligands were inferred from NOE data obtained from T-ROESY experiments. These data were used to calculate inter-proton distances. The values correlated well with distances found in the bound conformation of the BCD trisaccharide with SYA/J6 and conformations predicted by dynamics calculations. To illustrate this, the global minimum energy structure of **3** was superimposed on residue C of the BCD portion of the trisaccharide element of the ABCDA′ pentasaccharide–SYA/J6 complex taken from reference 11 (Fig. 3). This clearly shows that the conformation of **3** (blue) correlates closely with that of the bound BCD trisaccharide (red).

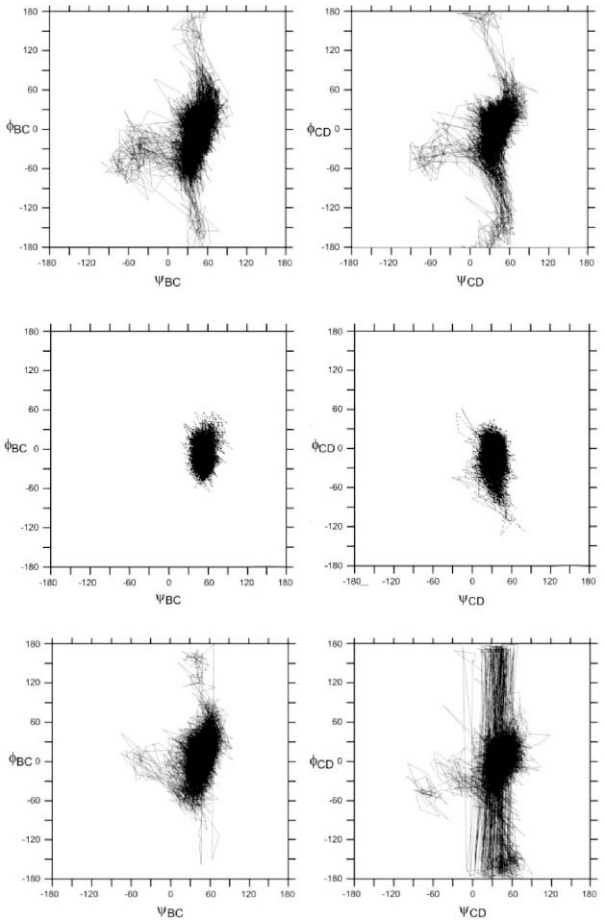


Fig. 2 ϕ *vs.* ψ plots for (from top) **1, 3** and **5** as measured from MD simulation. Subscripts BC and CD refer to the glycosidic bond between the BC and CD residues, respectively.

Discussion

We have previously shown that the native trisaccharide BCD essentially fills the binding site of the monoclonal antibody SYA/J6.[9c,11] Consistent with this interpretation, when the

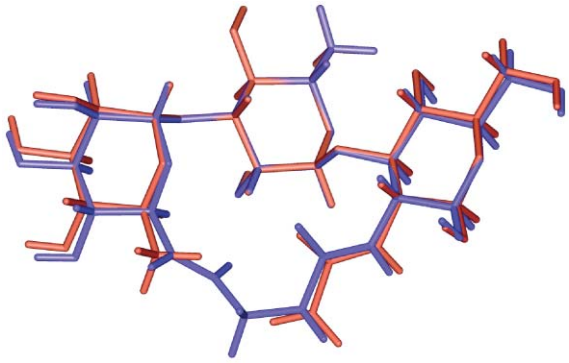


Fig. 3 Super-imposition of the conformation of **1**, taken from the SJA/J6 co-complex, and the global energy minimum of **3** as calculated by MD simulations.

epitope is extended by a single residue at either end to give first the tetrasaccharide (ABCD) corresponding to the biological repeating unit of the polysaccharide, the free energy of binding increased by −0.4 kcal mol^{-1} and by a further −0.2 kcal mol^{-1} as an additional rhamnose residue extends the oligosaccharide to give pentasaccharide ABCDA′. Interestingly, for each additional hexose residue the enthalpic change becomes increasingly detrimental and entropy changes are more favourable by ∼1.5 kcal mol^{-1}.[11] Significantly, the addition of two hexose residues yields no more than −0.6 kcal mol^{-1} of additional free energy for association. Of most interest to the following discussion are the increases in favourable entropy as the size of the epitope increased, with this phenomenon being ascribed to displacement of water molecules at the periphery of the binding pocket. This underscores the importance of solvent reorganization as a major factor in the association process. Similar conclusions have been reached for other carbohydrate–protein systems by Lemieux *et al*.[4,24] and from direct estimates of solvent effects by Chervenak and Toone.[25]

The free energy gain for the tethered trisaccharide **3** relative to **1** ($\Delta\Delta G$) was approximately −1.5 kcal mol^{-1}. At the temperature of the experiment, 293 K, this gain was of predominantly entropic origin (Table 1). Ignoring other competing effects, this would suggest that the maximum entropy gain to be achieved by pre-organizing a ligand would fall in the range 1–1.5 kcal mol^{-1}. However, the competing effects discussed below suggest this number should be regarded as an upper limit. The magnitude of these entropy gains for organizing four single bonds over two glycosidic linkages would tend to agree with the estimates suggested by Mammen *et al.*[13]

Molecular dynamics calculations confirmed that **3** was constrained in a conformation that is virtually super-imposable on the bound conformation of native ligand **1** (Fig. 2). Trisaccharide **2** is inactive since its tether is too short and it cannot adopt the bound conformation. Low activities of acyclic **5** (and by implication **4**) could be explained by the potential for significant steric interaction between the methyl groups of the NHAc and the *N*-methyl amide of **5**.

The role of water and the influence of the tether complicate the attempt to place an estimate on the entropy gains associated with successfully pre-ordering the oligosaccharide. It is generally impossible to de-convolute the competing enthalpy and entropy contributions and assign their origin to precise structural features of the carbohydrate–protein complex. To consider only a few of the likely processes, it could be anticipated that the tether might affect solvation of the complex, while the ligand also displaces water from the binding site. The negative heat capacity that characterizes binding of **1** is often correlated with hydrophobic effects and there is clear evidence that the favourable entropy of binding for trisaccharide **1** and larger structures is associated with displacement of water.[11] In this and other carbohydrate–antibody complexes[6a,9c] we have studied how water plays a crucial role in mediating protein–carbohydrate hydrogen bonds. The different roles of water can profoundly influence the thermodynamic parameters.[26–29] Antibody–protein complexes have been studied where water is either eliminated from the binding site or conserved to mediate antigen–antibody hydrogen bonds.[28,29] In addition, it seems probable that the tether itself may induce solvent re-organization in the bound state, since the tether will certainly displace water at the interface of the ligand and the protein complex[24] and possibly also make new van der Waals contacts with the protein. While examination of the crystal structure of bound oligosaccharide complexes with Fab offered no suggestions that the amide used for ligation of the tether to residue B of the trisaccharide **3** would make any significant contacts with the protein surface, there is some evidence that the tether in the vicinity of the amide group of residue D could make contacts with the protein and generate either favourable or unfavourable interactions with the binding. Both types of interactions could result in favourable enthalpic effects.[4c,24,25]

Conclusions

We have demonstrated that a pre-organized trisaccharide inhibitor with an optimized tether length is able to mimic the conformation of the bound, native acyclic trisaccharide epitope. A relative free energy change ($\Delta\Delta G = -1.5 \pm 0.3$ kcal mol^{-1}) determined by ITC measurements arose from a favourable entropy effect ($-T\Delta\Delta S = -1.2 \pm 0.15$ kcal mol^{-1}). These data suggest that the inherent flexibility of saccharides is not the primary cause of the low intrinsic affinity of carbohydrate–protein interactions. The successful design of the tethered form of this carbohydrate epitope provides us with an opportunity to probe potential strategies to create higher affinity carbohydrate ligands by combining tethering with functional group modifications. These findings are reported in our following paper.

Experimental

General methods

Analytical thin layer chromatography (tlc) was performed on silica gel 60-F_{254} (Merck). Detection of compounds on TLC plates was achieved by charring with 5% sulfuric acid in ethanol. The optical rotations were measured with a Perkin-Elmer 241 polarimeter for samples in a 10 cm cell at 22 ± 2 °C. $[\alpha]_D$ values are given in units of 10^{-1} deg cm^2 g^{-1}. All commercial reagents were used as supplied. Column chromatography used silica gel (SiliCycle) and solvents that were distilled. High performance liquid chromatography (HPLC) was performed using a Waters HPLC system that consisted of a Waters 600S controller, 626 pump and 486 tuneable absorbance detector. HPLC separations were performed on a Beckmann C_{18} semi-preparative reversed-phase column with acetonitrile and water as eluents. ^{1}H NMR spectra were recorded at either 300, 360, 500 or 600 MHz, and are referenced to internal standards of the residual protonated solvent peaks; δ_H 7.24 ppm for solutions in $CDCl_3$ or to 0.1% external acetone (δ_H 2.225 ppm) for solutions in D_2O. ^{13}C NMR spectra (HMQC) were recorded at 125 MHz and are referenced to internal $CDCl_3$ (δ_C 77.0 ppm) or to external acetone (δ_C 31.07 ppm). Electro-spray mass spectrometry and microanalyses were performed by the analytical services of this department.

Ethyl 2,3,4,6-tetra-*O*-acetyl-1-thio-α-L-mannopyranoside (7). Anhydrous CH_2Cl_2 (100 mL) was added to L-mannose pentaacetate (**6**) (2.1 g, 5.38 mmol) along with ethanethiol (1.20 mL, 16.2 mmol, 0.839 g mL^{-1}). Boron trifluoride diethyl etherate (2.00 mL, 15.8 mmol, 1.12 g mL^{-1}) was added and stirred under an argon atmosphere for 2 h at 0 °C, followed by 16 h at rt. Saturated $NaHCO_3$ (aq.) was added and the solution was stirred for 2 h. The organic layer was dried over Na_2SO_4, concentrated and the product was purified by silica gel chromatography using

toluene–ethyl acetate, 3 : 1, as the eluent to afford as a white solid (1.6 g, 77%): $[\alpha]_D^{22}$ −110.6 (*c* 0.9, CH_2Cl_2). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 5.34–5.25 (m, 4H, H-1, H-2, H-3, H-4), 4.38 (ddd, 1H, $J_{4,5} = 9.9$ Hz, $J_{5,6a} = 5.3$ Hz, $J_{5,6b} = 2.3$ Hz, H-5), 4.29 (dd, 1H, $J_{5,6a} = 5.3$ Hz, $J_{6a,6b} = 12.1$ Hz, H-6a), 4.08 (dd, 1H, $J_{5,6b} = 2.3$ Hz, $J_{6a,6b} = 12.1$ Hz, H-6b), 2.60–2.66 (m, 2H, SCH_2CH_3), 2.16 (s, 3H, OAc), 2.03 (s, 3H, OAc), 2.02 (s, 3H, OAc), 1.97 (s, 3H, OAc), 1.28 (t, 3H, SCH_2CH_3). Anal. calcd for $C_{16}H_{24}O_9S$: C, 48.97; H, 6.16; S, 8.17%. Found: C, 48.86; H, 6.17; S, 8.24%. ES HRMS: (M + Na): 415.1039, found: 415.1033.

Ethyl 1-thio-α-L-mannopyranoside (8). Distilled methanol (100 mL) was added to the tetraacetate **7** (1.5 g, 3.82 mmol) along with sodium (105 mg, 4.56 mmol) and stirred for 18 h at rt. Strongly-acidic cationic exchange resin was added and stirred until the solution became neutral. The solution was filtered through celite and concentrated. Alcohol **8** was obtained without purification as a white solid (0.86 g, 100%): ^{1}H NMR (600 MHz, D_2O) δ: 5.33 (d, 1H, $J_{1,2} = 1.6$ Hz, H-1), 4.05 (dd, 1H, $J_{1,2} = 1.6$ Hz, $J_{2,3} = 3.3$ Hz, H-2), 4.00 (ddd, 1H, $J_{4,5} = 9.7$ Hz, $J_{5,6a} = 2.3$ Hz, $J_{5,6b} = 6.2$ Hz, H-5), 3.89 (dd, 1H, $J_{5,6a} = 2.3$ Hz, $J_{6a,6b} = 12.4$ Hz, H-6a), 3.79 (dd, 1H, $J_{5,6b} = 6.1$ Hz, $J_{6a,6b} = 12.4$ Hz, H-6b), 3.78 (dd, 1H, $J_{2,3} = 3.3$ Hz, $J_{3,4} = 9.7$ Hz, H-3), 3.68 (dd, 1H, $J_{3,4} = J_{4,5} = 9.7$ Hz, H-4), 2.66–2.71 (m, 2H, SCH_2CH_3), 1.28 (t, 3H, SCH_2CH_3). ES HRMS: (M + Na): 247.0616, found: 247.0619.

Ethyl 6-*O*-*tert*-butyl-diphenylsilyl-1-thio-α-L-mannopyranoside (9). Dry DMF (30 mL) was added to alcohol **8** (700 mg, 3.12 mmol) along with *t*-butylchlorodiphenylsilane (1.60 mL, 6.15 mmol) and imidazole (425 mg, 6.24 mmol) and stirred for 20 h at rt. The volatiles were then removed and CH_2Cl_2 (100 mL) was added to the residue. The solution was then washed with 5% HCl (aq.) (50 mL), dried over Na_2SO_4 and filtered. The solvent was evaporated and the product was purified by silica gel chromatography (toluene–acetone–methanol, 7.5 : 1 : 0.5). Silyl ether **9** was isolated as a colourless oil (1.2 g, 81%): $[\alpha]_D^{22}$ −81.7 (*c* 1.0, MeOH). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.69–7.34 (m, 10H, aromatic), 5.28 (d, 1H, $J_{1,2} = 2.8$ Hz, H-1), 4.00–4.06 (m, 2H, H-2, H-5), 3.94–3.78 (m, 4H, H-3, H-4, H-6a, H-6b), 2.50–2.58 (m, 2H, SCH_2CH_3), 1.21 (t, 3H, SCH_2CH_3), 1.04 (s, 9H, *t*-Bu). Anal. calcd for $C_{24}H_{34}O_5SSi$: C, 62.30; H, 7.41%. Found: C, 61.83; H, 7.49%. ES HRMS: (M + Na): 485.1794, found: 485.1790.

Ethyl 2,3,4-tri-*O*-benzyl-6-*O*-*tert*-butyl-diphenylsilyl-1-thio-α-L-mannopyranoside (10). Anhydrous THF (100 mL) was added to alcohol **9** (950 mg, 2.05 mmol) along with sodium hydride (250 mg, 10.4 mmol) and stirred under an argon atmosphere for 15 min. Benzyl bromide (1.20 mL, 10.1 mmol) was added and the solution was stirred at 60 °C under an argon atmosphere for 24 h. The resulting yellow, cloudy solution was then cooled, methanol (6 mL) was carefully added and the solution was stirred for 15 min. The solvent was evaporated and the residue was taken up in CH_2Cl_2, washed with water and dried with Na_2SO_4. After concentration the crude product was purified by silica gel chromatography (hexane–ethyl acetate, 15 : 1). Compound **10** was isolated as a yellow oil (1.3 g, 86%): $[\alpha]_D^{22}$ −52.6 (*c* 1.2, CH_2Cl_2). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.75–7.15 (m, 25H, aromatic), 5.37 (d, 1H, $J_{1,2} = 1.3$ Hz, H-1), 4.92–4.56 (m, 6H, CH_2–Ph), 4.00–4.07 (m, 3H, H-4, H-5, H-6a), 3.89 (dd, 1H, $J_{5,6b} = 1.2$ Hz, $J_{6a,6b} = 10.4$ Hz, H-6b), 3.88 (dd, 1H, $J_{2,3} = 3.2$ Hz, $J_{3,4} = 9.7$ Hz, H-3), 3.68 (dd, 1H, $J_{1,2} = 1.6$ Hz, $J_{2,3} = 3.1$ Hz, H-2), 2.56–2.60 (m, 2H, SCH_2CH_3), 1.20 (t, 3H, SCH_2CH_3), 1.04 (s, 9H, *t*-Bu). Anal. calcd for $C_{45}H_{52}O_4SSi$: C, 73.73; H, 7.15%. Found: C, 73.85; H, 7.20%. ES HRMS: (M + Na): 755.3202, found: 755.3202.

Methyl 3-*O*-(3-*O*-acetyl-2,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-4,6-*O*-benzylidene-2-deoxy-2-phthalimido-β-D-glucopyranoside (13). Anhydrous CH_2Cl_2 (125 mL) was added to glycosyl donor **12**[17] (710 mg, 1.65 mmol), acceptor **11**[16] (523 mg, 1.27 mmol) and 4 Å molecular sieves (1.5 g) and stirred under an argon atmosphere for 14 h at rt. The solution was cooled to −78 °C, silver trifluoromethanesulfonate (85 mg, 0.33 mmol) and *N*-iodosuccinimide (742 mg, 3.30 mmol) were added and the solution was allowed to reach rt. The resulting dark purple solution was then filtered through celite and the resulting solution was washed with saturated $Na_2S_2O_3$ (100 mL) and dried with Na_2SO_4. The solvent was evaporated and the product was purified using silica gel chromatography (hexane–ethyl acetate, 2 : 1). The protected disaccharide **13** was isolated as a white solid (872 mg, 88%): $[\alpha]_D^{22}$ −24.0 (*c* 0.9, CH_2Cl_2). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.85–7.68 (m, 4H, Phth), 7.32–7.14 (m, 13H, aromatic), 6.90–6.95 (m, 2H, aromatic), 5.55 (s, 1H, C*H*–Ph), 5.19 (d, 1H, $J_{1,2} = 8.6$ Hz, H-1), 5.08 (dd, 1H, $J_{2',3'} = 3.5$ Hz, $J_{3',4'} = 9.5$ Hz, H-3′), 4.66 (dd, 1H, $J_{2,3} = 9.7$ Hz, $J_{3,4} = 10.3$ Hz, H-3), 4.59 (d, 1H, $J_{1',2'} = 1.8$ Hz, H-1′), 4.46–4.50 (m, 2H, CH_2–Ph), 4.41 (dd, 1H, $J_{5,6a} = 4.0$ Hz, $J_{6a,6b} = 12.5$ Hz, H-6a), 4.30 (dd, 1H, $J_{1,2} = 8.5$ Hz, $J_{2,3} = 10.2$ Hz, H-2), 3.96 (dq, 1H, $J_{4',5'} = 9.7$ Hz, $J_{5',6'} = 6.2$ Hz, H-5′), 3.94 (d, 1H, CH_2–Ph), 3.83–3.86 (m, 1H, H-6b), 3.76 (d, 1H, CH_2–Ph), 3.68–3.73 (m, 2H, H-4, H-5), 3.51 (dd, 1H, $J_{1',2'} = 2.0$ Hz, $J_{2',3'} = 3.5$ Hz, H-2′), 3.42 (s, 3H, OMe), 3.35 (t, 1H, $J_{3',4'} = J_{4',5'} = 9.5$ Hz, H-4′), 1.90 (s, 3H, OAc), 0.79 (d, 3H, $J_{5',6'} = 6.2$ Hz, H-6′). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 99.5 ($J_{C\text{-}1,H\text{-}1} = 164.6$ Hz, C-1), 97.7 ($J_{C\text{-}1',H\text{-}1'} = 167.3$ Hz, C-1′). Anal. calcd for $C_{44}H_{45}NO_{12}$: C, 67.77; H, 5.82; N, 1.80%. Found: C, 67.40; H, 5.83; N, 1.74%. ES HRMS: (M + Na): 802.2839, found: 802.2832.

Methyl 3-*O*-(2,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-4,6-*O*-benzylidene-2-deoxy-2-phthalimido-β-D-glucopyranoside (14). Distilled methanol (100 mL) was added to the acetate **13** (535 mg, 0.686 mmol), sodium (50 mg, 2.17 mmol) was added and the reaction was allowed to stir for 18 h at rt. Strongly-acidic cationic exchange resin was then added to neutralize the solution that was subsequently filtered through celite. The solvent was evaporated and the product was purified by silica gel chromatography (toluene–ethyl acetate, 5 : 1). Alcohol **14** was isolated as a white solid (466 mg, 92%): $[\alpha]_D^{22}$ −37.2 (*c* 1.0, CH_2Cl_2). ^{1}H NMR (600 MHz, $CDCl_3$) δ: 7.88–7.68 (m, 4H, Phth), 7.32–7.14 (m, 13H, aromatic), 6.86–6.91 (m, 2H, aromatic), 5.56 (s, 1H, C*H*–Ph), 5.19 (d, 1H, $J_{1,2} = 8.5$ Hz, H-1), 4.70 (d, 1H, CH_2–Ph), 4.64 (d, 1H, $J_{1',2'} = 1.3$ Hz, H-1′), 4.60 (dd, 1H, $J_{2,3} = 9.6$ Hz, $J_{3,4} = 10.4$ Hz, H-3), 4.46 (d, 1H, CH_2–Ph), 4.41 (dd, 1H, $J_{5,6a} = 4.3$ Hz, $J_{6a,6b} = 10.3$ Hz, H-6a), 4.26 (dd, 1H, $J_{1,2} = 8.5$ Hz, $J_{2,3} = 10.2$ Hz, H-2), 3.78–3.82 (m, 4H, H-6b, H-3′, H-5′, CH_2–Ph), 3.63–3.67 (m, 3H, H-4, H-5, CH_2–Ph), 3.42 (s, 3H, OMe), 3.35 (dd, 1H, $J_{1',2'} = 1.6$ Hz, $J_{2',3'} = 3.8$ Hz, H-2′), 3.05 (t, 1H, $J_{3',4'} = J_{4',5'} = 9.5$ Hz, H-4′), 2.01 (d, 1H, OH), 0.72 (d, 3H, $J_{5',6'} = 6.2$ Hz, H-6′). Anal. calcd for $C_{42}H_{43}NO_{11}$: C, 68.37; H, 5.87; N, 1.90%. Found: C, 68.14; H, 6.02; N, 1.88%. ES HRMS: (M + Na): 760.2734, found: 760.2748.

Methyl 3-*O*-(2,4-di-*O*-benzyl-3-*O*-[2,3,4-tri-*O*-benzyl-6-*O*-*tert*-butyl-diphenylsilyl-α-L-manno-pyranosyl]-α-L-rhamnopyranosyl)-4,6-*O*-benzylidene-2-deoxy-2-phthalimido-β-D-glucopyranoside (15). Anhydrous CH_2Cl_2 (70 mL) was added to mannosyl donor **10** (425 mg, 0.593 mmol) and acceptor **14** (336 mg, 0.455 mmol) and stirred for 10 h under argon with 4 Å molecular sieves (1 g). The solution was cooled to −78 °C, silver trifluoromethanesulfonate (30 mg, 0.12 mmol) and *N*-iodosuccinimide (267 mg, 1.19 mmol) were added and the solution was allowed to reach rt. The resulting dark purple solution was then filtered through celite and the resultant solution was washed with saturated $Na_2S_2O_3$, dried with Na_2SO_4 and filtered. The solvent was evaporated and the product was purified by silica gel chromatography (hexane–ethyl acetate, 2 : 1). Trisaccharide **15** was isolated as a white solid (500 mg, 78%): $[\alpha]_D^{22}$ −112.3 (*c* 1.1, CH_2Cl_2). ^{1}H NMR (600 MHz, $CDCl_3$) δ: 7.82–6.78 (m, 44H, aromatic), 5.54 (s, 1H, C*H*–Ph), 5.19 (d, 1H, $J_{1,2} = 8.5$ Hz, H-1), 5.12 (bs, 1H, H-1″), 4.92 (d, 1H,

CH_2–Ph), 4.65 (d, 1H, CH_2–Ph), 4.56 (dd, 1H, $J_{2,3}$ = 9.6 Hz, $J_{3,4}$ = 10.4 Hz, H-3), 4.53 (d, 1H, $J_{1',2'}$ = 1.3 Hz, H-1′), 4.51 (dd, 1H, $J_{5,6a}$ = 4.3 Hz, $J_{6a,6b}$ = 10.3 Hz, H-6a), 4.36–4.44 (m, 6H, CH_2–Ph), 4.36 (t, 1H, $J_{3'',4''} = J_{4'',5''}$ = 9.6 Hz, H-4″), 4.18 (dd, 1H, $J_{1,2}$ = 8.5 Hz, $J_{2,3}$ = 10.1 Hz, H-2), 3.97 (dd, 1H, $J_{5'',6a''}$ = 2.5 Hz, $J_{6a'',6b''}$ = 11.5 Hz, H-6a″), 3.76–3.86 (m, 7H, H-6b, H-3′, H-5′, H-3″, H-5″, H-6b″, CH_2–Ph), 3.60–3.70 (m, 4H, H-4, H-5, H-2″, CH_2–Ph), 3.42 (s, 3H, OMe), 3.26 (m, 2H, H-2′, H-4′), 1.04 (s, 9H, *t*-Bu), 0.74 (d, 3H, $J_{5',6'}$ = 6.2 Hz, H-6′). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 99.5 ($J_{C\text{-}1,H\text{-}1}$ = 165.0 Hz, C-1), 99.5 ($J_{C\text{-}1'',H\text{-}1''}$ = 171.1 Hz, C-1″), 97.6 ($J_{C\text{-}1',H\text{-}1'}$ = 168.0 Hz, C-1′). Anal. calcd for $C_{85}H_{89}NO_{16}$: C, 72.47; H, 6.37; N, 0.99%. Found: C, 72.24; H, 6.24; N, 1.01%. ES HRMS: (M + Na): 1430.5838, found: 1430.5848.

Methyl 2-amino-3-*O*-(2,4-di-*O*-benzyl-3-*O*-[2,3,4-tri-*O*-benzyl-6-*O*-*tert*-butyl-diphenylsilyl-α-L-mannopyranosyl]-α-L-rhamnopyranosyl)-4,6-*O*-benzylidene-2-deoxy-β-D-glucopyranoside (16). Ethylenediamine (3 mL) and *t*-butyl alcohol (30 mL) were added to **15** (450 mg, 0.319 mmol) and stirred under an argon atmosphere for 20 h at 115 °C. The resulting pale yellow solution was then cooled to rt. The solvent was evaporated and the product was purified by silica gel chromatography (toluene–ethyl acetate, 5 : 1). Amine **16** was isolated as a white solid (368 mg, 90%): $[\alpha]_D^{22}$ −75.2 (*c* 0.9, CH_2Cl_2). ^{1}H NMR (600 MHz, $CDCl_3$) δ: 7.82–7.14 (m, 40H, aromatic), 5.50 (s, 1H, C*H*–Ph), 5.22 (bs, 1H, H-1″), 5.08 (d, 1H, $J_{1',2'}$ = 1.7 Hz, H-1′), 4.97 (d, 1H, CH_2–Ph), 4.40–4.71 (m, 9H, CH_2–Ph), 4.34 (dd, 1H, $J_{5,6a}$ = 4.5 Hz, $J_{6a,6b}$ = 10.6 Hz, H-6a), 4.20 (t, 1H, $J_{3'',4''} = J_{4'',5''}$ = 9.4 Hz, H-4″), 4.08 (d, 1H, $J_{1,2}$ = 8.0 Hz, H-1), 3.95–4.07 (m, 3H, H-3′, H-5′, H-3″), 3.90 (dd, 1H, $J_{5'',6a''}$ = 4.2 Hz, $J_{6a'',6b''}$ = 11.4 Hz, H-6a″), 3.73–3.77 (m, 3H, H-6b, H-2″, H-5″), 3.61–3.69 (m, 2H, H-2′, H-6b″), 3.56 (t, 1H, $J_{2,3} = J_{3,4}$ = 9.0 Hz, H-3), 3.50 (s, 3H, OMe), 3.45–3.51 (m, 3H, H-4, H-5, H-4′), 2.62 (t, 1H, $J_{1,2} = J_{2,3}$ = 8.4 Hz, H-2), 1.02 (s, 9H, *t*-Bu), 0.82 (d, 3H, $J_{5',6'}$ = 6.1 Hz, H-6′). Anal. calcd for $C_{77}H_{87}NO_{14}Si$: C, 72.33; H, 6.86; N, 1.10%. Found: C, 71.80; H, 6.77; N, 1.09%. ES HRMS: (M + Na): 1278.5974, found: 1278.5968.

Methyl 2-amino-3-*O*-(2,4-di-*O*-benzyl-3-*O*-[2,3,4-tri-*O*-benzyl-α-L-mannopyranosyl]-α-L-rhamnopyranosyl)-4,6-*O*-benzylidene-2-deoxy-β-D-glucopyranoside (17). Anhydrous THF (50 mL) was added to silyl ether **16** (310 mg, 0.242 mmol) along with tetrabutylammonium fluoride (480 μL, 0.480 mmol, 1.0 mol L^{-1} in THF) and stirred under an argon atmosphere for 15 h at 60 °C. The resulting pale yellow solution was then cooled to rt and evaporated. The solution was then dissolved in CH_2Cl_2 and washed with water. The organic phase was dried with Na_2SO_4 and filtered. The solvent was evaporated and the product was purified by silica gel chromatography using (toluene–ethyl acetate–methanol, 7.5 : 2 : 0.5). Alcohol **17** was isolated as a white solid (217 mg, 86%): $[\alpha]_D^{22}$ −59.5 (*c* 0.8, CH_2Cl_2). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.45–7.16 (m, 30H, aromatic), 5.52 (s, 1H, C*H*–Ph), 5.30 (d, 1H, $J_{1'',2''}$ = 1.6 Hz, H-1″), 5.14 (d, 1H, $J_{1',2'}$ = 1.6 Hz, H-1′), 4.88 (d, 1H, CH_2–Ph), 4.60 (m, 9H, CH_2–Ph), 4.31 (dd, 1H, $J_{5,6a}$ = 4.9 Hz, $J_{6a,6b}$ = 10.5 Hz, H-6a), 4.19 (d, 1H, $J_{1,2}$ = 7.8 Hz, H-1), 3.96–4.02 (m, 2H, H-2″, H-3″), 3.85–3.89 (m, 2H, H-5′, H-6a″), 3.61–3.69 (m, 5H, H-4, H-6b, H-2′, H-5″, H-6b″), 3.53–3.58 (m, 3H, H-3, H-4′, H-4″), 3.51 (s, 3H, OMe), 3.40 (ddd, 1H, $J_{4,5}$ = 9.7 Hz, $J_{5,6a}$ = 4.9 Hz, $J_{5,6b}$ = 1.2 Hz, H-5), 2.79 (t, 1H, $J_{1,2} = J_{2,3}$ = 8.7 Hz, H-2), 1.10 (d, 3H, $J_{5',6'}$ = 6.1 Hz, H-6′). Anal. calcd for $C_{61}H_{69}NO_{14}$: C, 70.43; H, 6.69; N, 1.35%. Found: C, 70.06; H, 6.68; N, 1.31%. ES HRMS: (M + Na): 1062.4616, found: 1062.4628.

Methyl 3-*O*-(2,4-di-*O*-benzyl-3-*O*-[2,3,4-tri-*O*-benzyl-α-L-mannopyranosyl]-α-L-rhamnopyranosyl)-4,6-*O*-benzylidene-2-deoxy-2-(*N*-α-fluorenylmethoxycarbonyl-glycinyl)-amido-β-D-glucopyranoside (18). Dry DMF (20 mL) was added to amine **17** (70 mg, 0.067 mmol) along with *N*-α-Fmoc-L-glycine (40 mg, 0.14 mmol), TBTU (43 mg, 0.134 mmol), HOBt (18 mg, 0.13 mmol) and *N*-ethylmorpholine (34 μL, 0.26 mmol, 0.905 g mL^{-1}) and stirred for 18 h at rt. The solvents were evaporated and the product was purified by silica gel chromatography (toluene–ethyl acetate–methanol, 7.5 : 2 : 0.5). Amide **18** was isolated as a white solid (76 mg, 86%): $[\alpha]_D^{22}$ −13.2 (*c* 0.7, CH_2Cl_2). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.69–7.08 (m, 38H, aromatic), 6.43–6.45 (m, 1H, N*H*-Glc-NH_2), 5.68 (bt, 1H, N*H*-Fmoc), 5.48 (s, 1H, C*H*-Ph), 5.18 (bs, 1H, H-1″), 4.96 (bs, 1H, H-1′), 4.80 (d, 1H, CH_2–Ph), 4.60–4.24 (m, 10H, H-1, CH_2–Ph), 4.28 (dd, 1H, $J_{5,6a}$ = 5.7 Hz, $J_{6a,6b}$ = 10.5 Hz, H-6a), 4.12 (bt, O–CH_2–C*H*[Fmoc]), 4.01 (dd, 1H, $J_{2',3'}$ = 2.8 Hz, $J_{3',4'}$ = 9.7 Hz, H-3′), 4.00 (t, 1H, $J_{3'',4''} = J_{4'',5''}$ = 9.3 Hz, H-4″), 3.92 (dd, 1H, $J_{2',3'}$ = 2.9 Hz, $J_{3',4'}$ = 9.0 Hz, H-3″), 3.80–3.88 (m, 5H, H-2′, H-5′, H-6a″, linker), 3.70 (t, 1H, $J_{1'',2''} = J_{2',3'}$ = 1.9 Hz, H-2″), 3.61–3.69 (m, 4H, H-3, H-4, H-6b, H-6b″), 3.52 (t, 1H, $J_{3',4'} = J_{4',5'}$ = 9.3 Hz, H-4′), 3.40–3.45 (m, 6H, H-2, H-5, O–CH_2–CH[Fmoc], H-5″, H-6b″), 3.38 (s, 3H, OMe), 0.73 (d, 3H, $J_{5',6'}$ = 6.0 Hz, H-6′). ES HRMS: (M + Na): 1341.5511, found: 1341.5512.

Methyl 3-*O*-(2,4-di-*O*-benzyl-3-*O*-[2,3,4-tri-*O*-benzyl-α-L-mannopyranosyl]-α-L-rhamnopyranosyl)-4,6-*O*-benzylidene-2-deoxy-2-(*N*-β-fluorenylmethoxycarbonyl-β-alanyl)-amido-β-D-glucopyranoside (19)

Amine **17** was prepared under similar conditions as for the synthesis of **18**, employing Fmoc-protected β-alanine as the amino acid. The product was purified by silica gel chromatography (toluene–ethyl acetate–methanol, 7.5 : 2 : 0.5) to give the title compound as a white solid (105 mg, 91%): $[\alpha]_D^{22}$ −21.0 (*c* 0.7, CH_2Cl_2). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.69–7.08 (m, 38H, aromatic), 6.00 (bt, 1H, N*H*–Fmoc), 5.52 (s, 1H, C*H*–Ph), 5.11 (d, 1H, $J_{1'',2''}$ = 2.2 Hz, H-1″), 4.86 (bs, 1H, H-1″), 4.80 (d, 1H, CH_2–Ph), 4.60–4.24 (m, 10H, H-1, CH_2–Ph), 4.27 (dd, 1H, $J_{5,6a}$ = 5.5 Hz, $J_{6a,6b}$ = 10.5 Hz, H-6a), 4.08 (bt, O–CH_2–C*H*[Fmoc]), 4.01 (dd, 1H, $J_{2',3'}$ = 2.8 Hz, $J_{3',4'}$ = 9.7 Hz, H-3″), 4.01 (t, 1H, $J_{3'',4''} = J_{4'',5''}$ = 9.3 Hz, H-4″), 3.80–3.90 (m, 6H, H-2′, H-5′, H-3″, H-6a″, linker), 3.73–3.77 (m, 3H, H-3, H-6b, H-6b″), 3.66–3.69 (m, 2H, H-4, linker), 3.67 (t, 1H, $J_{1'',2''} = J_{2',3''}$ = 2.6 Hz, H-2″), 3.56–3.58 (m, 1H, linker), 3.52 (t, 1H, $J_{3',4'} = J_{4',5'}$ = 9.3 Hz, H-4′), 3.37–3.42 (m, 5H, H-5, O–CH_2–CH[Fmoc], H-5″, H-6b″), 3.30 (s, 3H, OMe), 3.24–3.27 (m, 1H, H-2), 2.16–2.19 (m, 2H, linker), 0.73 (d, 3H, $J_{5',6'}$ = 6.0 Hz, H-6′). Anal. calcd for $C_{79}H_{84}N_2O_{17}$: C, 71.15; H, 6.35; N, 2.10%. Found: C, 71.27; H, 6.24; N, 2.20%. ES HRMS: (M + Na): 1355.3667, found: 1355.3675

Methyl 3-*O*-(2,4-di-*O*-benzyl-3-*O*-[2,3,4-tri-*O*-benzyl-α-L-mannopyranosyluronic acid]-α-L-rhamnopyranosyl)-4,6-*O*-benzylidene-2-deoxy-2-(*N*-α-fluorenyl-methoxycarbonyl-glycinyl)-amido-β-D-glucopyranoside (20). Distilled CH_2Cl_2 (4 mL) and TEMPO (4 mg, 0.02 mmol) were added to alcohol **18** (60 mg, 0.045 mmol) and a solution of potassium bromide (8 mg, 0.07 mmol) and tetrabutylammonium chloride (10 mg, 0.036 mmol) in saturated $NaHCO_3$ (aq.) (1.5 mL) was added. The biphasic solution was cooled to 0 °C and a solution of sodium hypochlorite (1.5 mL, 5% solution in water), saturated $NaHCO_3$ (aq.) (0.6 mL) and saturated NaCl (aq.) (1.3 mL) was added dropwise over 45 min. The CH_2Cl_2 solution was acidified with 5% HCl (aq.) (5mL), washed with water and dried with Na_2SO_4. The solvents were removed and the product was purified by silica gel chromatography (toluene–ethyl acetate–methanol, 4 : 4 : 1). Carboxylic acid **20** was isolated as a white solid (26 mg, 43%): $[\alpha]_D^{22}$ +0.4 (*c* 0.6, MeOH). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.75–7.00 (m, 38H, aromatic), 5.42 (bs, 1H, C*H*–Ph), 5.18 (bs, 1H, H-1″), 5.04 (bs, 1H, H-1′), 4.83 (bd, 1H, CH_2–Ph), 4.63–4.34 (m, 10H, H-1, CH_2–Ph), 4.30–3.20 (m, 19H, H-2, H-3, H-4, H-5, H-6a, H-6b, H-2′, H-3′, H-4′, H-5′, H-2″, H-3″, H-4″, H-5″, linker, O–CH_2–C*H*[Fmoc], O–CH_2–CH[Fmoc]), 3.32 (s, 3H, OMe), 0.73 (d, 3H, $J_{5',6'}$ = 5.7 Hz, H-6′). Anal. calcd for $C_{78}H_{80}N_2O_{18}$: C, 70.26; H, 6.05; N,

2.10%. Found: C, 69.90; H, 6.44; N, 2.01%. ES HRMS: (M + Na): 1355.5304, found: 1355.5314.

Methyl 3-*O*-(2,4-di-*O*-benzyl-3-*O*-[2,3,4-tri-*O*-benzyl-α-L-mannurono]-α-L-rhamnopyranosyl)-4,6-*O*-benzylidene-2-deoxy-2-(2-*N*-fluorenylmethoxycarbonyl-β-alanyl)-amido-β-D-glucopyranoside (21). Alcohol **19** (85 mg, 0.064 mmol) was treated in an identical fashion to **18**, to afford the title compound that was purified by silica gel chromatography (toluene–ethyl acetate–methanol, 4 : 4 : 1) to give carboxylic acid **21** as a white solid (54 mg, 63%): $[\alpha]_D^{22}$ +13.6 (*c* 1.0, MeOH). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.69–7.06 (m, 38H, aromatic), 5.42 (bs, 1H, C*H*-Ph), 5.18 (bs, 1H, H-1″), 4.85 (bs, 1H, H-1′), 4.80 (d, 1H, CH_2–Ph), 4.60–4.24 (m, 10H, H-1, CH_2–Ph), 4.30–3.20 (m, 19H, H-2, H-3, H-4, H-5, H-6a, H-6b, H-2′, H-3′, H-4′, H-5′, H-2″, H-3″, H-4″, H-5″, linker, O–CH$_2$–C*H*[Fmoc], O–CH_2–CH[Fmoc]), 3.20 (s, 3H, OMe), 2.16–2.21 (m, 2H, linker), 0.73 (d, 3H, $J_{5',6'}$ = 5.7 Hz, H-6′). Anal. calcd for $C_{79}H_{82}N_2O_{18}$: H, 6.13; N, 2.08%. Found: H, 5.59; N, 2.11%. ES HRMS: (M + Na): 1369.5460, found: 1369.5464.

Methyl 2-(2-aminoacetamido)-3-*O*-(2,4-di-*O*-benzyl-3-*O*-[2,3,4-tri-*O*-benzyl-α-L-mannopyranosyl-uronic acid]-α-L-rhamnopyranosyl)-4,6-*O*-benzylidene-2-deoxy-β-D-glucopyranoside lactam (22). Dry DMF (12 mL) and piperidine (2 mL) were added to carboxylic acid **20** (36 mg, 0.027 mmol) and stirred for 1 h at rt. The solvents were evaporated and dry DMF (10 mL) was added to the residue along with TBTU (17 mg, 0.054 mmol), HOBt (7 mg, 0.054 mmol) and *N*-ethylmorpholine (14 μL, 0.11 mmol, 0.905 g mL^{-1}) and stirred for 24 h at rt. The solvents were evaporated and the product was purified by silica gel chromatography (toluene–ethyl acetate–methanol, 7.5 : 2 : 0.5). Lactam **22** was isolated as a white solid (19 mg, 65%): $[\alpha]_D^{22}$ −27.1 (*c* 0.4, CH_2Cl_2). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.50–7.13 (m, 30H, aromatic), 7.00 (bt, 1H, N*H*–Fmoc), 6.20 (d, 1H, N*H*–Glc–NH$_2$), 5.51 (s, 1H, C*H*–Ph), 5.24 (d, 1H, $J_{1'',2''}$ = 2.7 Hz, H-1″), 5.03 (d, 1H, $J_{1',2'}$ = 1.6 Hz, H-1′), 4.98 (d, 1H, $J_{1,2}$ = 8.3Hz, H-1), 4.71–4.38 (m, 10H, CH_2Ph), 4.30 (dd, 1H, $J_{5,6a}$ = 5.0 Hz, $J_{6a,6b}$ = 10.7Hz, H-6a), 4.23–4.26 (m, 1H, linker), 4.14 (d, 1H, $J_{4'',5''}$ = 9.3 Hz, H-5″), 4.08 (dd, 1H, $J_{2',3'}$ = 3.0 Hz, $J_{3',4'}$ = 9.7 Hz, H-3′), 3.90–4.00 (m, 4H, H-6b, H-5′, H-3″, H-4″), 3.59 (m, 2H, H-3, H-2″), 3.86 (t, 1H, $J_{3,4}$ = $J_{4,5}$ = 9.1 Hz, H-4), 3.79 (bt, 1H, $J_{1',2'}$ = $J_{2',3'}$ = 2.3 Hz, H-2′), 3.54–3.57 (m, 2H, H-5, H-4′), 3.45 (s, 3H, OMe), 3.02–3.07 (m, 1H, linker), 2.92 (bq, 1H, $J_{1,2}$ = $J_{2,3}$ = $J_{2,NH}$ 8.6 Hz, H-2), 0.83 (d, 3H, $J_{5',6'}$ = 6.2 Hz, H-6′). Anal. calcd for $C_{63}H_{68}N_2O_{15}$: C, 69.22; H, 6.27; N, 2.56%. Found: C, 69.35; H, 6.02; N, 2.64%. ES HRMS: (M + Na): 1115.4517, found: 1115.4527.

Methyl 2-(3-aminopropionamido)-3-*O*-(2,4-di-*O*-benzyl-3-*O*-[2,3,4-tri-*O*-benzyl-α-L-mannopyranosyluronic acid]-α-L-rhamnopyranosyl)-4,6-*O*-benzylidene-2-deoxy-β-D-glucopyranoside lactam (23). Carboxylic acid **21** (40 mg, 0.030 mmol) was treated in an identical fashion that produced **22** to afford the title compound that was purified by silica gel chromatography (toluene–ethyl acetate–methanol, 7.5 : 2 : 0.5). Lactam **23** was isolated as a white solid (20 mg, 60%): $[\alpha]_D^{22}$ +12.1 (*c* 0.5, CH_2Cl_2). ^{1}H NMR (600 MHz, $CDCl_3$) δ: 7.50–7.13 (m, 30H, aromatic), 6.89 (bs, 1H, N*H*–Fmoc), 5.48 (s, 1H, C*H*–Ph), 5.42 (d, 1H, N*H*-Glc-NH$_2$), 5.14 (d, 1H, $J_{1'',2''}$ = 2.3 Hz, H-1″), 4.91 (d, 1H, $J_{1',2'}$ = 1.3 Hz, H-1′), 4.71–4.38 (m, 10H, CH_2Ph), 4.30 (dd, 1H, $J_{5,6a}$ = 5.0 Hz, $J_{6a,6b}$ = 10.7Hz, H-6a), 4.22 (d, 1H, $J_{1,2}$ = 8.6 Hz, H-1), 4.04 (d, 1H, $J_{4'',5''}$ = 9.3 Hz, H-5″), 3.92 (bt, 1H, $J_{1,2}$ = $J_{2,3}$ = 8.6 Hz, H-2), 3.96 (dd, 1H, $J_{2',3'}$ = 3.0 Hz, $J_{3',4'}$ = 9.7 Hz, H-3′), 3.89 (t, 1H, $J_{3'',4''}$ = $J_{4'',5''}$ = 9.3 Hz, H-4″), 3.70–3.80 (m, 4H, H-6b, H-5′, H-3″, NH–CH_2–CH$_2$–NH), 3.59–3.69 (m, 2H, H-3, H-2″), 3.76 (t, 1H, $J_{3,4}$ = $J_{4,5}$ = 9.1 Hz, H-4), 3.79 (bt, 1H, $J_{1',2'}$ = $J_{2',3'}$ = 2.3 Hz, H-2′), 3.52–3.58 (m, 4H, H-4′, H-5, NH–CH_2–CH$_2$–NH), 3.45 (s, 3H, OMe), 2.37–2.39 (m, 2H, NH–CH$_2$–C*H*–NH), 0.81 (d, 3H, $J_{5',6'}$ = 6.2 Hz, H-6′). Anal. calcd for $C_{64}H_{70}N_2O_{15}$: H, 6.37; N, 2.53%. Found: H, 6.48; N, 2.62%. ES HRMS: (M + Na): 1129.4674, found: 1129.4684.

Methyl 2-(2-aminoacetamido)-3-*O*-(3-*O*-[α-L-mannopyranosyl-uronic acid]-α-L-rhamnopyranosyl)-2-deoxy-β-D-glucopyranoside lactam (2). Distilled methanol (15 mL) and distilled water (1 mL) were added to the protected cyclic trisaccharide **22** (16 mg, 0.014 mmol) along with palladium(II) hydroxide (23 mg, 20% on carbon) and the suspension was stirred under a hydrogen atmosphere for 24 h at rt. The resulting solution was then filtered through celite and the solvent was evaporated. Distilled water (5 mL) was added to the residue and the solution was passed through an RP-18 Sep-Pak cartridge. The product was purified by reversed-phase HPLC using water–acetonitrile, (0–10%). The deprotected cyclic trisaccharide **2** was lyophilized to a white solid (6 mg, 74%): ^{1}H NMR (600 MHz, D_2O) δ: 5.22 (d, 1H, $J_{1'',2''}$ = 1.9 Hz, H-1″), 4.99 (d, 1H, $J_{1',2'}$ = 1.5 Hz, H-1′), 4.76 (d, 1H, $J_{1,2}$ = 8.4 Hz, H-1), 4.14 (d, 1H, $J_{4'',5''}$ = 9.5 Hz, H-5″), 3.95–4.00 (m, 2H, H-5′, H-6a), 3.92–3.94 (m, 3H, H-2, H-3″, H-4″), 3.84 (bt, 1H, $J_{1'',2''}$ = $J_{2'',3''}$ = 3.2 Hz, H-2″), 3.83 (dd, 1H, $J_{2',3'}$ = 3.3 Hz, $J_{3',4'}$ = 9.9 Hz, H-3′), 3.79 (dd, 1H, $J_{1',2'}$ = 2.0 Hz, $J_{2',3'}$ = 3.2 Hz, H-2′), 3.76 (dd, 1H, $J_{5,6b}$ = 5.8 Hz, $J_{6a,6b}$ = 12.2 Hz, H-6b), 3.63–3.67 (m, 1H, linker), 3.50–3.58 (m, 4H, H-4′, H-3, H-4, linker), 3.50 (s, 3H, OMe), 3.46 (ddd, 1H, $J_{5,6a}$ = 2.3 Hz, $J_{5,6b}$ = 5.2 Hz, $J_{4,5}$ = 10.1 Hz, H-5), 1.29 (d, 3H, $J_{5',6'}$ = 6.4 Hz, H-6′). ES HRMS: (M + Na): 577.1857, found: 577.1863.

Methyl 2-(2-aminopropionamido)-3-*O*-(3-*O*-[α-L-mannopyranosyluronic acid]-α-L-rhamnopyranosyl)-2-deoxy-β-D-glucopyranoside lactam (3). The protected cyclic trisaccharide **23** (32 mg, 0.029 mmol) was deprotected and purified in an analogous fashion as **2** to give the title compound as a lyophilized white solid (12 mg, 73%): $[\alpha]_D^{22}$ −36.3 (*c* 0.5, H_2O). ^{1}H NMR (600 MHz, D_2O) δ: 5.11 (d, 1H, $J_{1'',2''}$ = 1.5 Hz, H-1″), 4.77 (d, 1H, $J_{1',2'}$ = 2.2 Hz, H-1′), 4.41 (d, 1H, $J_{1,2}$ = 8.6 Hz, H-1), 4.09 (dd, 1H, $J_{1'',2''}$ = 1.5 Hz, $J_{2'',3''}$ = 3.2 Hz, H-2″), 4.04 (d, 1H, $J_{4'',5''}$ = 9.5 Hz, H-5″), 3.95–3.99 (m, 2H, H-5′, H-6a), 3.92 (dd, 1H, $J_{1,2}$ = 8.5 Hz, $J_{2,3}$ = 10.3 Hz, H-2), 3.89 (t, 1H, $J_{3'',4''}$ = $J_{4'',5''}$ = 9.5 Hz, H-4″), 3.85 (dd, 1H, $J_{3'',4''}$ = 9.3 Hz, $J_{2'',3''}$ = 3.3 Hz, H-3″), 3.83 (dd, 1H, $J_{2',3'}$ = 3.3 Hz, $J_{3',4'}$ = 9.9 Hz, H-3′), 3.79 (dd, 1H, $J_{1',2'}$ = 2.2 Hz, $J_{2',3'}$ = 3.2 Hz, H-2′), 3.76 (dd, 1H, $J_{5,6b}$ = 5.8 Hz, $J_{6a,6b}$ = 12.2 Hz, H-6b), 3.65–3.70 (m, 1H, NH–CH_2–CH$_2$–NH), 3.52–3.57 (m, 4H, H-4′, H-3, H-4, NH-CH_2–CH$_2$–NH), 3.50 (s, 3H, OMe), 3.46 (ddd, 1H, $J_{5,6a}$ = 2.2 Hz, $J_{5,6b}$ = 6.1 Hz, $J_{4,5}$ = 8.1 Hz, H-5), 2.58–2.61 (m, 2H, NH–CH$_2$–CH_2-NH), 1.27 (d, 3H, $J_{5',6'}$ = 6.4 Hz, H-6′). ES HRMS: (M + Na): 591.2013, found: 591.2006.

Methyl 4,6-*O*-benzylidene-2-propionamido-3-*O*-(2,4-di-*O*-benzyl-3-*O*-(2,3,4-tri-*O*-benzyl-α-L-mannopyranosyl)-α-L-rhamnopyranosyl)-β-D-glucopyranoside (24). To a suspension of amine **16** (0.457 g, 0.39 mmol) in MeOH (15 mL) was added propionic anhydride (56 μL, 0.44 mmol). The reaction was stirred for 2 h until clear, and K_2CO_3 (100 mg) was added. The solvent was removed under a vacuum and the residue was dissolved in CH_2Cl_2 treated with excess TBAF in THF (5 mL). After 6 h the residue was evaporated and washed with water. The organic layer was dried with Na_2SO_4, filtered and evaporated to dryness. The residue was chromatographed (6 : 4 : 1 cyclohexane–ethyl acetate–acetone) to afford a white foam (0.383 g, 88%): $[\alpha]_D^{22}$ −38.68 (*c* = 0.99, $CHCl_3$). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.49–7.15 (m, 30H, Ar–*H*), 6.14 (bd, 1H, *J* = 7.4 Hz, EtC(O)N*H*–), 5.49 (s, 1H, PhC*H*O$_2$–), 5.21 (s, 1H, H-1″), 4.92 (d, 1H, J_{gem} = 14.4 Hz, PhCH_2O–), 4.91 (s, 1H, H-1′), 4.61–4.51 (m, 7H, PhCH_2O–), 4.39 (d, 1H, J_{gem} = 12.2 Hz, PhCH_2O–), 4.36–4.27 (m, 3H, PhCH_2O-, H-6$_a$, H-3), 4.14 (dd, 1H, *J* = 2.8, 9.8 Hz, H-3″), 3.99 (m, 1H, H-3″), 3.88–3.81 (m, 2H, H-5′, H-6$_a$″), 3.78 (dd, 1H, $J_{1,2} \approx J_{2,3}$ = 2.3 Hz, H-2′), 3.77–3.71 (m, 4H, H-6$_b$, H-2″, H-4″, H-5″), 3.68–3.62 (m, 1H, H-6$_b$″), 3.53–3.47 (m, 3H, H-4, H-5, H-4′), 3.43 (s, 3H, OCH_3), 3.16 (ddd ≈ q, *J* = 9.3 Hz, H-2), 2.04–1.91

(m, 2H, –CH_2CH$_3$), 1.01 (t, 3H, 7.6 Hz, –CH$_2$CH_3), 0.84 (d, 3H, J = 7.62 Hz, H-6′). Anal. calcd for $C_{64}H_{73}NO_{15}$: C, 68.68; H, 6.57; N, 1.25%. Found C, 68.53; H, 6.64; N, 1.30%. ES MS: (M + Na): 1118.5, found 1118.5.

Methyl 4,6-*O*-benzylidene-2-acetamido-3-*O*-(2,4-di-*O*-benzyl-3-*O*-(2,3,4-tri-*O*-benzyl-α-L-mannopyranosyl)-α-L-rhamnopyranosyl)-β-D-glucopyranoside (25). Amine **16** (0.496 g, 0.429 mmol) was treated analogously for the production of **22** using acetic anhydride (42 μL, 0.451 mmol) as the acylation agent. Similar work-up and chromatography gave the target compound (0.413 g, 89%) as white foam: $[\alpha]_D^{22} = -36.79$ ($c = 1.08$, $CHCl_3$). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.52–7.14 (m, 30H, Ar–H), 6.39 (bd, 1H, J = 7.3 Hz), 5.51 (s, 1H, PhCHO$_2$–), 5.22 (d, 1H, J = 1.0 Hz, H-1″), 4.94 (d, 1H, J = 1.7 Hz, H-1″), 4.92 (d, 1H, J_{gem} = 11.2 Hz, PhCH_2O–), 4.78 (d, 1H, J = 8.33 Hz, H-1), 4.66–4.52 (m, 7H, PhCH_2O–), 4.43–4.32 (m, 3H, H_2O-, H-6$_a$), 4.21 (dd, 1H, $J_{2,3} \approx J_{3,4}$ = 9.2 Hz, H-3), 4.14 (dd, 1H, J = 2.8, 9.7 Hz, H-3′), 3.91 (dd, 1H, J = 3.0, 8.6 Hz, H-3″), 3.88–3.84 (m, 2H, H-5′, H-6$_a$″), 3.81 (dd, 1H, $J_{1',2'} \approx J_{2',3'}$ = 2.5 Hz, H-2′), 3.78–3.72 (m, 4H, H-6$_b$, H-2″, H-4″, H-5″), 3.65 (dd, 1H, J_{gem} = 11.5 Hz, J_{vic} = 7.3 Hz, H-6$_b$″), 3.55–3.47 (m, 3H, H-4, H-5, H-4″), 3.42 (s, 3H, OCH_3), 3.27 (bddd, 1H, $J_{1,2} \approx J_{2,3} \approx J_{2,N-H}$ = 9.0 Hz, H-2), 2.74 (bs, 1H, 6″-OH), 1.76 (s, 3H, NHCOCH_3), 0.86 (d, 3H, J = 6.2 Hz, H-6′). Anal. calcd for $C_{63}H_{71}NO_{15}$: C, 69.92; H, 6.61; N, 1.29%. Found C, 69.80; H, 6.38; N, 1.35%. ES HRMS: (M + Na): 1104.4721, found 1104.4719.

Methyl 4,6-*O*-benzylidene-2-propionamido-3-*O*-(2,4-di-*O*-benzyl-3-*O*-(2,3,4-tri-*O*-benzyl-6-amino-α-L-mannopyranuronyl)-α-L-rhamnopyranosyl)-β-D-glucopyranoside (26). To a stirred solution of alcohol **24** (0.217 g, 0.198 mmol) in acetonitrile (5 mL) at 0 °C was added TEMPO (0.031 g, 0.198 mmol) and an aqueous solution (1.30 mL, pH 10.5 with NaOH) containing KBr (0.198 mmol) and $NaHCO_3$ (0.040 mmol). NaOCl (5–6%, 1.50 mL) was added dropwise and the reaction stirred for 1 h before the addition of HCl (1 M, 15 mL) and CH_2Cl_2 (15 mL). The organic layer was removed, washed with water, dried with Na_2SO_4 and evaporated to dryness. The residue was dissolved in dry CH_2Cl_2 (5 mL) under Ar, carbonyldiimidazole (0.064 g, 0.396 mmol) was added and the mixture was stirred for 45 min before the introduction of ammonia gas (XS). After an additional hour, the ammonia was allowed to evaporate and the solvent was removed under a vacuum. Column chromatography (2 : 3 : 1 cyclohexane–ethyl acetate–acetone) afforded the target compound as a clear glass (0.149 g, 68%): $[\alpha]_D^{22} = -22.72$ (c = 1.08, $CHCl_3$). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.48 7.11 (m, 30H, Ar–H), 6.58 (bs, 1H, –CONH_{2a}), 6.26 (bd, 1H, J = 7.6 Hz, –NHCOEt), 5.51 (s, 1H, PHCHO$_2$–), 5.46 (bs, 1H, –CONH_{2b}), 5.39 (d, 1H, J = 1.8 Hz, H-1″), 4.97 (d, 1H, J = 1.7 Hz, H-1′), 4.82 (d, 1H, J_{gem} = 10.7 Hz, PhCH_2O–), 4.69 (d, 1H, J = 8.3 Hz, H-1), 4.59–4.49 (m, 6H, PhCH_2O–), 4.39–4.32 (m, 3H, PhCH_2O-, H-6$_a$), 4.18–4.13 (m, 2H, H-3, H-3′), 4.09 (dd, 1H, $J_{3',4'} \approx J_{4',5'}$ = 9.0 Hz, H-4′), 4.02 (d, 1H, J = 9.2 Hz, H-5″), 3.89 (dq, 1H, J = 6.1, 9.5 Hz, H-5′), 3.85 (dd, 1H, J = 2.9, 8.5 Hz, H-3″), 3.77–3.72 (m, 2H, H-6$_b$, H-2′), 3.69 (dd, 1H, $J_{1'',2''} \approx J_{2'',3''}$ = 2.6 Hz, H-2″), 3.54 (dd, 1H, $J_{3'',4''} \approx J_{4'',5''}$ = 9.3 Hz, H-4″), 3.51–3.44 (m, 3H, H-4, H-5, H-4′), 3.42 (s, 3H, OCH_3), 2.10, 2.01 (m, 2H, –NHCOCH_2CH$_3$), 1.01 (t, 3H, –NHCOCH$_2$CH_3), 0.84 (d, 3H, J = 6.1 Hz, H-6). Anal. calcd for $C_{64}H_{72}N_2O_{15}$: C, 69.30; H, 6.54; N, 2.53%. Found: C, 69.15; H, 6.48; N, 2.82%. ES MS: (M + Na): 1131.5, found: 1131.5.

Methyl 4,6-*O*-benzylidene-2-acetamido-3-*O*-(2,4-di-*O*-benzyl-3-*O*-(2,3,4-tri-*O*-benzyl-6-*N*-methylamino-α-L-mannopyranuronyl)-α-L-rhamnopyranosyl)-β-D-glucopyranoside (27). Using the same protocol for the production of **26**, alcohol **25** (0.179 g, 0.182 mmol) was oxidized and converted to its corresponding amide using CDI (0.061 g, 0.377 mmol) and methylamine (0.10 mL, 2.0 M solution in THF) to afford the title compound (0.136 g, 67%) as a white foam. $[\alpha]_D^{22} = -32.99$ ($c = 0.97$, $CHCl_3$). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.48–7.09 (m, 30 H, Ar–H), 6.49 (bd, 1H, J = 7.1 Hz, –NHAc), 6.44 (bd, 1H, NHCH$_3$), 5.52 (s, 1H, PhCHO$_2$–), 5.24 (d, 1H, J = 1.4 Hz, H-1″), 5.04 (d, 1H, J = 1.50 Hz, H-1′), 4.81 (d, 1H, J_{gem} = 10.9 Hz, PhCH_2O–), 4.70 (d, 1H, J = 8.3 Hz, H-1), 4.64–4.49 (m, 7H, PhCH_2O–), 4.37–4.30 (m, 3H, PhCH_2O-, H-6$_a$), 4.13 (dd, 1H, J = 2.7, 9.7 Hz, H-3′), 4.08 (bdd, 1H, $J_{2,3} \approx J_{3,4}$ = 9.2 Hz, H-3), 4.03 (dd, 1H, $J_{3,4} \approx J_{4,5}$ = 9.0 Hz, H-4), 3.90–3.85 (m, 2H, H-5′, H-5″), 3.83 (dd, 1H, J = 2.9, 9.7 Hz, H-3″), 3.76 (dd, 1H, $J_{vic} \approx J_{gem}$ = 10.36 Hz, H-6$_b$), 3.72 (dd, 1H, $J_{1',2'} \approx J_{2',3'}$ = 2.2 Hz, H-2′), 3.68 (dd, 1H, $J_{1'',2''} \approx J_{2'',3''}$ = 2.6 Hz, H-2″), 3.56 (dd, 1H, $J_{3,4} \approx J_{4,5}$ = 9.2 Hz, H-4), 3.53–3.41 (m, 6H, OCH_3, H-2, H-5, H-4′), 2.68 (d, 3H, J = 4.9 Hz, –NHCH_3), 1.84 (s, 3H, –NHCOCH_3), 0.91 (d, 3H, J = 6.2 Hz, H-6′). Anal. calcd for $C_{64}H_{72}N_2O_{15}$: C, 69.30; H, 6.54; N, 2.53%. Found: C, 69.27; H, 6.63; N, 2.71%. ES HRMS: (M + Na): 1131.4830, found: 1131.4837.

Methyl 2-propionamido-3-*O*-(3-*O*-(6-amino-α-L-mannopyranuronyl)-α-L-rhamnopyranosyl)-β-D-glucopyranoside (4). The hydrogenolysis of amide **26** (56.1 mg, 50.6 μmol) was performed as described for that of protected lactam **22**, using identical workup and purification to afford a clear glass was lyophilized to white powder (23.4 mg, 81%): $[\alpha]_D^{22} = -63.54$ ($c = 0.54$, H_2O). ^{1}H NMR (600 MHz, D_2O) δ 8.22 (bd, 1H, –NHCOEt), 5.02 (s, 1H, H-1″), 4.81 (s, 1H, H-1′), 4.12 (d, 1H, J = 8.7 Hz, H-1), 4.12 (d, 1H, J = 9.8 Hz, H-5″), 4.08 (dd, 1H, $J_{1'',2''} \approx J_{2'',3''}$ = 1.7 Hz, H-2″), 4.03 (dq, 1H, J = 9.8, 6.2 Hz, H-5′), 3.93 (d, 1H, J_{gem} = 12.3 Hz, H-6$_a$), 3.91 (d, 1H, J = 3.3, 9.7 Hz, H-3″), 3.87 (dd, 1H, $J_{1',2'} \approx J_{2',3'}$ = 1.4 Hz, H-2′), 3.84–3.77 (m, 3H, H-2, H-3′, H-4″), 3.74 (dd, 1H, J_{gem} = 12.3 Hz, J_{vic} = 6.0 Hz, H-6$_b$), 3.59 (dd, 1H, $J_{3,4} \approx J_{4,5}$ = 8.5 Hz, H-3), 3.55–3.43 (m, 6H, OCH_3, H-4, H-5, H-4′), 2.27 (q, 2H, J = 7.7 Hz, COCH_2CH$_3$), 1.24 (d, 3H, J = 6.2 Hz, H-6′), 1.11 (t, 2H, J = 7.7 Hz, COCH$_2$CH_3). ES HRMS: (M + Na): 593.2164, found: 593.2159.

Methyl 2-acetamido-3-*O*-(3-*O*-(6-*N*-methylamino-α-L-mannopyranuronyl)-L-(rhamnopyranosyl)-β-D-glucopyranoside (5). The hydrogenolysis of amide **27** (92.4 mg, 83.3 μmol) was performed as described for that of protected lactam **22**, using identical workup and purification to afford a clear glass that was lyophilized to white powder (37.0 mg, 78%): $[\alpha]_D^{22} = -50.9$ ($c = 0.64$, H_2O). ^{1}H NMR (600 MHz, D_2O) 5.21 (d, 1H, J = 1.7 Hz, H-1″), 4.85 (d, 1H, J = 1.7 Hz, H-1′), 4.52 (d, 1H, J = 8.6 Hz, H-1), 4.14 (d, 1H, J = 9.2 Hz, H-5″), 4.12 (dd, 1H, J = 1.7, 3.3 Hz, H-2″), 4.06 (dq, 1H, J = 6.22, 9.81 Hz, H-5′), 3.98 (dd, 1H, J_{gem} = 10.2 Hz, J_{vic} = 2.2 Hz, H-6$_a$), 3.95 (dd, 1H, J = 3.3, 9.7 Hz, H-3″), 3.88 (dd, 1H, J = 2.0, 2.30 Hz, H-2′), 3.87–3.77 (m, 4H, H-2, H-6$_b$, H-3′, H-4″), 3.62 (dd, 1H, J = 8.5, 10.0 Hz, H-3), 3.59–3.48 (m, 6H, OCH_3, H-4, H-5, H-4′), 2.86 (s, 3H, –NHCH_3), 1.24 (d, 3H, J = 6.2 Hz, H-6′). ES HRMS: (M + Na): 593.2170, found 593.2171.

Purification of monoclonal antibody SYA/J6. Antibody was purified from ascites fluid by centrifugation (30 min, 64 000 g) to pellet cells and fatty tissue. After filtration first through a Millex AP 20 prefilter (Millipore) and then through a Millex-GV 0.22 μm low-binding sterilization filter (Millipore), the filtrate was loaded onto a Sepharose Protein-A (Pharmacia Biotech) column equilibrated with running buffer (50 mM Tris, 150 mM NaCl, 0.02% NaN_3, adjusted to pH 8.0). The column was washed with running buffer until serum proteins were eluted (absorbance at 280 nm below 0.1). Antibody was then eluted with citrate buffer (100 mM citric acid, 150 mM NaCl, 0.02% NaN_3, adjusted to pH 3.0). Fractions with an absorbance greater than 0.1 were collected, pooled and dialyzed (24 h) against the initial Tris buffer for calorimetry measurements, or against phosphate buffer saline (PBS) for ELISA measurements.

Enzyme-linked immunosorbent assays. Direct ELISA was carried out with a PBS solution of protein A purified SYA/J6 antibody coated on microtitre plates (1 μg mL^{-1}) and

O-polysaccharide coupled to biotin.[21] Streptavidin–HRP conjugate (Sigma Chemical Co., St. Louis, Mo.) served as the disclosing reagent. Inhibition experiments with increasing concentration of synthetic inhibitors were done in triplicate according to a previously reported protocol.[22,30]

Isothermal titration microcalorimetry. Dialyzed antibody was concentrated and equilibrated against the buffer used for calorimetry in CentriPrep units (Amicon). Antibody concentration was determined spectrophotometrically, using a calculated extinction coefficient of 1.53 mg mL^{-1}.[22] Dry saccharide samples were prepared as a 0.50 mM stock solution and assayed for carbohydrate concentration as previously described.[25a] Isothermal titration measurements were made using the Microcal VP-ITC titration microcalorimeter.[6a,25a] Purified antibody (IgG, 30 μM) was placed in the cell and titrated with ligand. In all cases, the value C, defined as the product of the binding constant K and the concentration of binding sites, was in the range of 1–500 and ligand concentrations were such that the final ligand concentration was at least $10K_D$. Titrations of the antibody and data processing were carried out as previously described.[6a,25a] The measurements were evaluated by the ORIGIN software package (MicroCal Inc., Northampton, MA) and data were processed using a single binding site model that assumes no cooperativity between sites.

Heat capacity measurements were made using the Microcal MCS titration microcalorimeter. The calorimeter cell held a volume of 1.3215 mL. For all titrations, solubilized antibody was placed in the cell and titrated with ligand. Antibody concentrations ranged from 20 to 100 μM. Titrations of the antibody were carried out at pH 8.0 in 50 mM Tris buffer augmented with 150 mM NaCl and 0.02% NaN_3. All ligands were dissolved in an identical buffer. Typically, for antibody–carbohydrate concentrations, 30 8 μL injections of ligand, 20 s in duration, were made, with 4 min intervals between injections. The data was processed as previously described using the ORIGIN software package.[6a] Non-negligible heats of dilution were subtracted prior to data processing. Constant pressure heat capacities, ΔC_p, were determined by evaluating ΔH as a function of T. Heat capacity is rigorously defined as the partial derivative of ΔH with respect to temperature at constant pressure, *i.e.* $\Delta C_p = (\delta H/\delta T)P$. Over short temperature ranges ΔC_p is predicted to be linear, and can be approximated as $\Delta(\Delta H)/\Delta T$, or $(\Delta H_1 - \Delta H_2)/(T_1 - T_2)$.

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