Synthesis and Structure−Activity Relationship of Mannose-Based Peptidomimetics Selectively Blocking Integrin α4β7 Binding to Mucosal Addressin Cell Adhesion Molecule-1

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As part of our ongoing research in the development of α4β7 integrin antagonists, we are interested in peptidomimetics based on a rigid scaffold to allow the display of essential side chains in a suitable binding conformation while eliminating backbone amide bonds and therefore improving pharmacokinetic parameters of the drug. Except for a few examples, peptidomimetics scaffolds have only been moderately successful and often yield molecules that lack the potency of their peptide counterparts. However, we present herein a successful application of using a rigid scaffold. Starting from a mannopyranoside analogue previously discovered in our laboratory as an inhibitor of the α4β1/vascular cell adhesion molecule interaction, a biased library of functionalized carbohydrates was developed. One compound emerged from this library as an active and selective antagonist toward the α4β7/mucosal addressin cell adhesion molecule interaction. Conformational implications and the relevance of different pharmacophoric patterns will be discussed in order to explain the reverse selectivity and enhanced affinity.

Introduction

The infiltration of leukocytes to the site of inflammation contributes to the pathogenesis of a number of human autoimmune diseases such as multiple sclerosis and rheumatoid arthritis and results from a series of adhesive and activating events involving multiple receptor/ligand interactions. The inflammatory processes leading to tissue damage and disease are mediated in part by the α4 integrins, α4β1 and α4β7, expressed on the leukocyte cell surface. These glycoprotein receptors modulate cell adhesion via interaction with their primary ligands, vascular cell adhesion molecule-1 (VCAM-1) and mucosal addressin cell adhesion molecule-1 (MAdCAM-1), expressed in the affected tissue. Upon binding, the combined integrin/CAM interacts at the cell surface to result in firm adhesion of the leukocyte to the vessel wall followed by extravasation into the underlying tissue and subsequent activation and/or proliferation of inflammatory cells.

In particular, the gut-specific trafficking of lymphocytes from the vascular to gastrointestinal mucosa and lymphoid tissues is mediated by adhesive interactions with MAdCAM-1 and the homing receptor α4β7.[1−3] MAdCAM-1 specifically binds both human and mouse lymphocytes that express the homing receptor α4β7 and participates in the homing of these cells to the mucosal endothelium.[4,5] Organ-specific adhesion of normal lymphocytes and lymphoma cells to high endothelial venules of Peyer's patches is mediated by α4β7 integrins.[6−8] In mouse models it was demonstrated that antibodies specific for β7 integrins and/or MAdCAM-1 block recruitment of lymphocytes to inflamed colon and reduce significantly the severity of colonic inflammatory disease.[9] Moreover, antibodies against β7 integrins protected mice from the development of insulin-dependent diabetes.[10] Therefore, the α4β7/MAdCAM-1 adhesion pathway represents a potent and organ-specific target for therapeutic modulation of inflammatory diseases of the gastrointestinal tract and autoimmune diabetes.

Our approach to developing a MAdCAM-1 proteinomimetic uses a carbohydrate moiety as rigid scaffold, carrying the essential side chains in a suitable binding conformation. There are only a few research groups who succeeded in the transition from bioactive peptides to small-molecule inhibitors using rigid scaffolds such as cyclohexane.[11−16] In a pioneering work, Hirschmann, Nicolaou, and Smith developed somatostatin non-peptide peptidomimetics utilizing β-D-glucose as novel scaffolding.[11] We present here another successful application of this concept. On the basis of a D-mannose core, we developed in a rational combinatorial approach an active and selective inhibitor of the α4β7/MAdCAM interaction.

A peptide-based structure−activity study was carried out in our laboratory, leading to a number of potent and selective α4β7 inhibitors.[17] Cyclic hexapeptides were designed and synthesized in an effort to mimic the MAdCAM's LDT binding motif by incorporation into a rigid peptide core that adopts only specific conformations. These compounds were later used as templates for the design of a non-peptidic α4β7 antagonist based on β-D-mannose as the rigid scaffold, carrying the Leu, Asp, and Thr mimetics at 6, 1, and 2 positions of the pyranoside, respectively. The mannose core mimicked the bioactive conformation of the constrained peptides, displaying the aspartic acid at the $i + 1$ position of a

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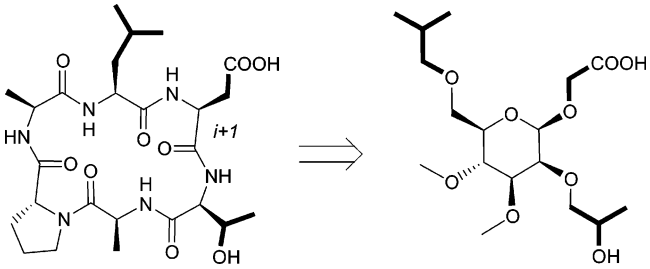


Figure 1. Comparison between a lead peptidic inhibitor of the α4β7/MAdCAM interaction and the mannose-based peptidomimetic.[18]

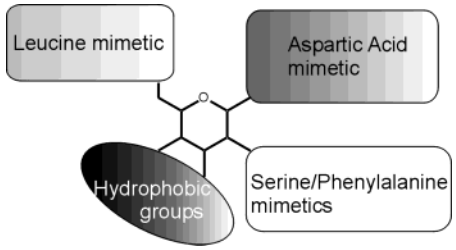


Figure 2. Development of a biased library of mannose peptidomimetics.

β-turn (Figure 1). Unfortunately, the designed mannopyranoside had no inhibitory activity against α4β7/MAdCAM interaction, but one analogue where the Thr side chain is replaced by the Ser side chain demonstrated inhibition of VCAM-mediated binding of α4β1 Jurkat cells ($IC_{50} \cong 3.7$ mM).[18] Our interest in α4β7 integrin antagonists prompted us to investigate the possibility of changing the selectivity and enhancing the potency of this carbohydrate peptidomimetic, as well as of shortening the synthetic strategy.

Results and Discussion

Design of a Biased Library of Peptidomimetics. In our strategy, we maintained the original D-mannose core and the Leu and Asp pharmacophores at the 6 and 1 positions of the sugar ring, respectively (Figure 2). For SAR studies, a spatial screening of the Asp pharmacophore was carried out by synthesizing and comparing the biological effects of the β- and α-anomers. The Ser side chain, which is known to be irrelevant for activity, was replaced at position 2 by the Phe side chain. In fact, a remarkable number of different research groups have discovered phenylalanine-based α4 antagonists.[19−24] The phenanthrene-9,10-diacetals (PDA) group was attached to the D-mannose core as a hydrophobic source at the 3 and 4 positions. The mannose scaffold was rigidified by a trans ring fusion between a dioxane ring and the pyranoside at the 3 and 4 positions. The Leu mimetic was introduced either via ether linkage or via a carbon−carbon bond-forming reaction to examine the effects on activity of changes in polarity and in the conformational space available for this pharmacophore.

Synthetic Strategies. The synthetic protocol of the desired functionalized mannopyranosides required an orthogonal set of protecting groups. The orthogonal protecting groups were successively cleaved, as specified, and replaced by pharmacophoric side chains.

We used the concept of 1,2-diacetals, developed by Ley et al.,[25] as a regioselective protecting group for the diequatorial 1,2-diol units in carbohydrates. In particular, the acid-catalyzed reaction of ethyl 1-thio-α-D-mannopyranoside with cyclohexane-1,2-diones resulted in selective protection of diequatorial, 3,4-diol functionalities as a cyclohexane-1,2-diacetal (CDA).[26]

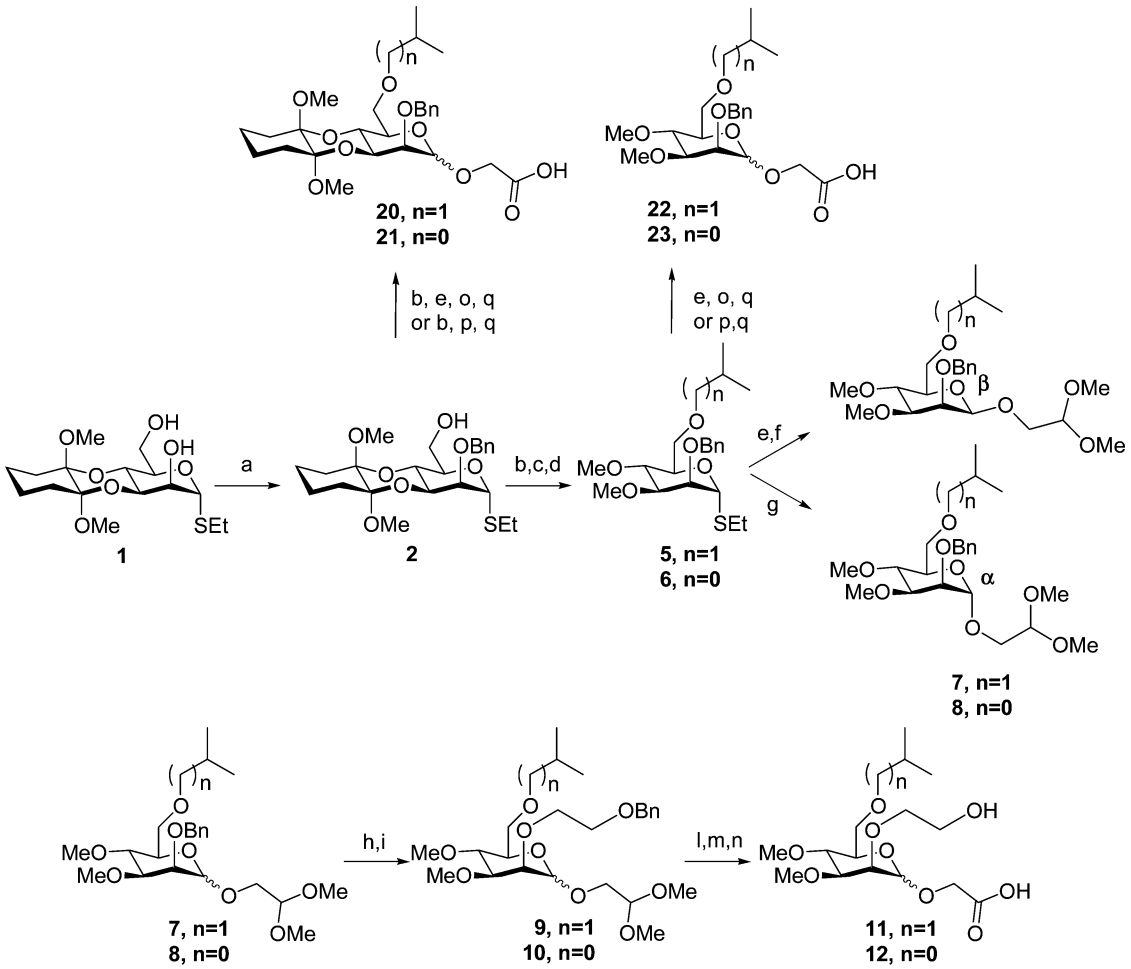
Rapid selective protection of the 2-position of CDA mannoside **1**, reported in Scheme 1, could be achieved by benzylation with benzyl bromide using KOH in DMSO, affording the 2-benzyl-protected sugar **2** in 75−85% yield. The regioselectivity was unambiguously assigned from the multiplicity of the hydroxyl proton in ^{1}H NMR and by the observation of a long-range ^{13}C−^{1}H correlation between the benzylic protons and C^2 of the sugar ring in the heteronuclear multiple-bond correlation (HMBC) spectrum. This reversal of selectivity (secondary versus primary hydroxyl group) was already observed by Ley and co-workers who attributed it to the higher basicity and reactivity of the oxyanion at the 2-position.[27] Direct protection of the 2-position of PDA mannoside **13** did not work, but it was achieved in two steps: regioselective protection of the primary alcohol with *tert*-butyldiphenylsilyl chloride (TBDPSCl) and subsequent benzylation of the secondary alcohol (Scheme 2).

To introduce the Ser and Leu side chains as well as the benzyl and methyl groups, we used the Williamson reaction, which is still the best general method for the preparation of unsymmetrical ethers. We used solid KOH in DMSO.[28] In the case of secondary halides, an excess of halide was needed because of elimination that occurred as a side reaction. Many other functional groups can be present in the molecule without interference.

The Leu side chain was also introduced by Wittig olefination of the sugar aldehyde with subsequent double bond reduction at the 6-position. The primary alcohol was oxidized to aldehyde according to known procedures using DMSO as an oxidizing reagent and sulfur trioxide pyridine complex as an alcohol activating reagent. As reported in Scheme 3, the in situ prepared ylide was then reacted with the crude aldehyde in cold toluene. The resulting double bond was subsequently reduced by *p*-toluenesulfonylhydrazide in dimethoxyethane to give sugars **26** and **27**. Another method we used for selective reduction of a double bond in the presence of a benzyl group was Pd/C catalyzed hydrogenation with a nitrogen-containing base or amine as poison.

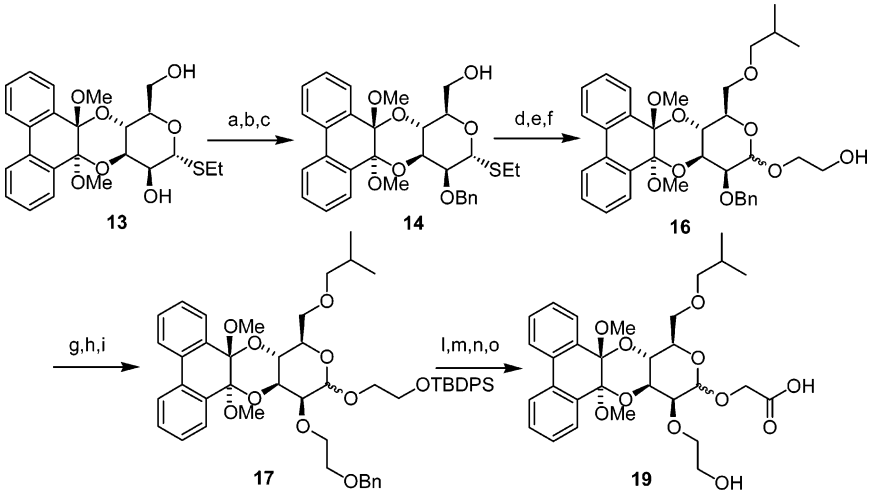
The Asp side chain was introduced via O-glycosidation. Attempts toward the synthesis of β-mannose linkages have been well reviewed recently by Gridley and Osborn.[29] The methodology we used to overcome the anomeric effect is a modified Koenigs−Knorr coupling using insoluble silver salt promoters to block the α-face of mannosyl halides and direct glycosidation to give β-mannosides (Scheme 1).[30] The β-anomeric configuration was verified by determination of the interproton distances between protons at positions 1, 3, and 5 derived from nuclear Overhauser effect spectrometry (NOESY) spectra and by ^{13}C NMR spectroscopy, based on the general rules[31,32] governing the stereochemical dependence of the $^1J(^{13}C\text{-}^1H)$ coupling constant at the anomeric center in hexopyranoses, which for the β-anomer was <160 Hz, while that for the α-anomer was >170 Hz. For the glycosyl donor, we used predominantly

Scheme 1[a]



[a] Reagents: (a) BnBr, KOH in DMSO, 75−85%; (b) ^{i}BuBr or ^{i}PrI, KOH in DMSO, 90−95%; (c) 90% TFA in DCM, 80%; (d) MeI, KOH in DMSO, 70%; (e) Br_2 in DCM at 0 °C; (f) $HOCH_2CH_2(OMe)_2$, Ag_2CO_3 in DCM at 0 °C, 75%, α/β 1:5; (g) $HOCH_2CH_2(OMe)_2$, NIS, AgOTf in DCM at 0 °C, 80%, α/β 6:1; (h) H_2, Pd/C in MeOH, 98%; (i) $BrCH_2CH_2OBn$, KOH in DMSO, 95%; (l) HCl in H_2O/THF; (m) $NaClO_2$, 2-methyl-2-butene in ^{t}BuOH; (n) H_2, Pd/C in MeOH, 98%; (o) $HOCH_2COOMe$, Ag_2CO_3 in DCM at 0 °C, 75%, α/β 1:5; (p) $HOCH_2COOMe$, NIS, AgOTf in DCM at 0 °C, 80%, α/β 6:1; (q) MeOH/NaOH 1 M.

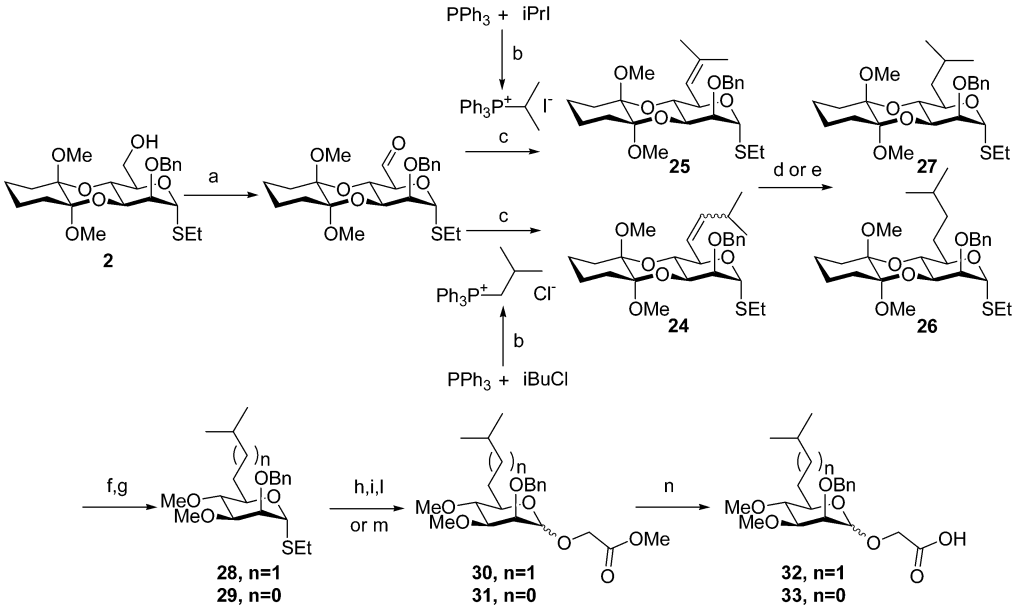
Scheme 2[a]



[a] Reagents: (a) TBDPSCl, imidazole in DMF, 85%; (b) BnBr, KOH in DMSO, 95%; (c) TBAF in THF, 96%; (d) ^{i}BuBr, KOH in DMSO, 95%; (e) Br_2 in DCM at 0 °C; (f) $HOCH_2CH_2OH$, Ag_2CO_3 in DCM/THF at 0 °C, 90%, α/β 1:1; (g) TBDPSCl, imidazole in DMF, 85%; (h) H_2, Pd/C in MeOH, 98%; (i) $BrCH_2CH_2OBn$, KOH in DMSO, 95%; (l) TBAF in THF, 96%; (m) $PySO_3$, TEA in DCM/DMSO 3:1; (n) $NaClO_2$, 2-methyl-2-butene in ^{t}BuOH; (o) H_2, Pd/C in MeOH, 98%.

mannosyl bromide, which was prepared via direct activation of the SEt group in ethyl 1-thio-α-D-mannosides with bromine in dichloromethane. In the case of less reactive *S*-glycosides, as for **28** and **29** reported in Scheme 3, we got the desired halide in two steps: hydrolysis of the SEt group with NBS/H^+ and subse-

Scheme 3[a]



[a] Reagents: (a) $PySO_3$, TEA in DCM/DMSO 3:1; (b) toluene, Δ; (c) $KN[Si(CH_3)_3]_2$ in toluene at 0 °C, 70% for **24**, *E*/*Z* > 1:10; (d) $TosNHNH_2$, NaOAc in DME at 80 °C, 60−65%; (e) H_2, Pd/C, TEA in MeOH, 80%; (f) 90% TFA in DCM, 80%; (g) MeI, KOH in DMSO, 90%; (h) NBS, HCl in ACN/H_2O, 90−95%; (i) $SOCl_2$; (l) $HOCH_2COOMe$, Ag_2CO_3 in DCM at 0 °C, 75%, α/β 1:5; (m) $HOCH_2COOMe$, NIS, AgOTf in DCM at 0 °C, 80%, α/β 6:1; (n) NaOH in MeOH/H_2O, 99%.

quent reaction with thionyl chloride leading to the mannosyl chloride almost quantitatively. The Asp side chain (glycosyl acceptor) was masqueraded as a methyl ester that was subsequently hydrolyzed to give the final sugars **20**−**23** or acetal-protected aldehyde as for compounds **7** and **8** (Scheme 1). The latter strategy was chosen to avoid the problematic C^{α} acidity of the carboxyl acid derivatives that might result in undesired byproducts during ether formation. After cleavage of the acetal, the free aldehyde was immediately oxidized to the corresponding carboxylic acid using sodium chlorite and 2-methyl-2-butene as scavenger for the hypochlorite that is produced during the oxidation.[33] For the PDA-protected sugar, the Asp side chain was introduced as a primary alcohol via the Koenigs−Knorr reaction using ethylene glycol as a glycosyl acceptor, leading to an α/β ratio of 1:1 (Scheme 2). In fact, hydrolysis of the dimethyl acetal, required by the previous protocol, in this case caused byproducts because of a concurrent aldol condensation. The alcohol functionality was finally oxidized to carboxylic acid **19** in a two-step reaction.

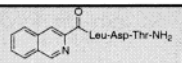
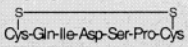
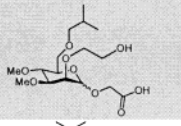
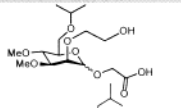
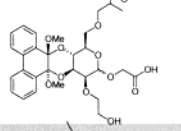
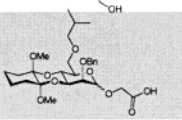
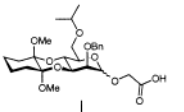
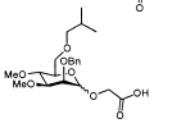
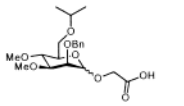
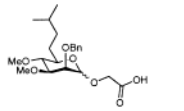
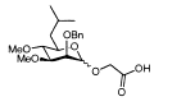
Stereoselective α-O-glycosidation was carried out using the thioglycoside methodology. This approach employs a thioglycoside donor (e.g., having an alkylthio aglycon), which is condensed directly with an alcohol acceptor in the presence of a thiophilic agent, where the latter is generated by a promoter system and transforms the aglycon into a good leaving group. In this case, a selectivity toward the more favorable α-anomer was expected. In fact, α-mannosides are thermodynamically and kinetically favored and are the major products when the oxonium ion is an intermediate in the glycosidation reaction. There are numerous promoter systems for thioglycoside activation, of which sulfenyl halides/silver trifluoromethanesulfonate, such as PhSCl/AgOTf and MeSBr/AgOTf, or *N*-iodosuccinimide (NIS) combined with either trifluoromethanesulfonic acid (TfOH) or silver trifluoromethanesulfonate appear to be the most commonly used. We chose the latter system to get the α-mannose glycosides (Scheme 1).

Biological Activity. The inhibition of 38-β7 ($\alpha4\beta1^-$, $\alpha4\beta7^+$) cell adhesion to MAdCAM-1 in the presence of 1 mg/mL of the sugar is reported in Table 1. In addition to biological activity, selectivity is a major goal in drug development. To verify selectivity (the ability of these compounds to inhibit specific interactions of integrins with their cognate ligands), binding of the structurally related integrin $\alpha4\beta1$ to VCAM-1 was investigated. Adhesion mediated by $\alpha4\beta1$ integrin was tested using Jurkat ($\alpha4\beta1^+$, $\alpha4\beta7^-$) lymphoma cells. To compare the potencies of our antagonists with literature known active compounds, the activity of a literature known $\alpha4\beta7$ antagonist (**34**)[34] and of a cyclic peptide (**35**), which was derived by Wang et al. from the crystal structure of VCAM,[35] was measured, and the IC_{50} curves were reported in Figure 3.

The β-anomer of compound **20** showed inhibitory activity toward the $\alpha4\beta7$/MAdCAM interaction with an IC_{50} of 420 μM (Figure 3C). This result is comparable to the activity of the literature known tripeptide $\alpha4\beta7$ antagonist (**34**), showing in our assay an IC_{50} of 280 μM (Figure 3A). In a previous report,[34] the IC_{50} of **34** was found to be 3 μM. However, when the potencies of different compounds are compared, it is important to consider that the measured IC_{50} values are highly dependent on reagent concentrations and assay conditions. Therefore, only a comparison between the relative potencies of antagonists measured in the same assay is informative.

Furthermore, the tripeptide **34** did not show any selectivity with respect to $\alpha4\beta1$ binding to VCAM-1 (Table 1). Considering that the two receptors share a common α4 chain, it is not surprising that many of the potent $\alpha4\beta7$/MAdCAM antagonists are inhibitors of $\alpha4\beta1$ as well. In contrast, the $\alpha4\beta1$/VCAM-1 interaction was not affected significantly by the β-anomer of **20**,

Table 1. Effect of Mannose-Based Peptidomimetics on 38-β7 Lymphoma and Jurkat Cell Binding to Immobilized VCAM-1 and MAdCAM-1

			38-β7 (α4β7) MAdCAM-1 adhesion [%][a]	Jurkat (α4β1) VCAM-1 adhesion [%]
34[b]			10 ± 8	10 ± 6
35[c]			n.d.	52 ± 12
11	β[d]		100 ± 19	60 ± 15
	α		100 ±21	100 ± 20
12	β		100 ±22	100 ± 19
	α		100 ± 20	98 ± 22
19	β		80 ±16	82 ± 15
	α		76 ±19	81 ± 19
20	β		15 ±6	77 ± 5
	α		90 ±11	89 ± 14
21	β		76 ± 10	100 ± 18
	α		100 ± 20	100 ± 22
22	β		100 ± 7	86 ± 10
	α		100 ± 20	100 ± 11
23	β		100 ± 14	86 ± 16
	α		100 ± 7	92 ± 15
32	β		100 ± 10	100 ± 21
	α		100 ± 10	97 ± 15
33	β		100 ± 11	100 ± 21
	α		100 ± 14	100 ± 18

[a] Cell adhesion is presented as percent of control medium in the presence of 1 mg/mL antagonist. Each percent value represents the average of at least four measurements ± the standard deviation. [b] Reference 34. [c] Reference 35. [d] Reference 18.

demonstrating its high selectivity for integrin α4β7 (Table 1). The development of selective inhibitors of the α4β7/MAdCAM interaction is important to in vivo migration of leukocytes to the gastrointestinal tract, where only MAdCAM expression has been linked to human disease,[36] without interfering with α4β1/VCAM-1 immune functions.

These present studies also reconfirmed the necessity of β-orientation of the Asp side chain for activity, as found for the β-anomer of **11** toward the α4β1/VCAM-1 interaction.[18] In fact, the α-anomer of **20**, as for the α-anomer of **11**, did not show any inhibitory activity. Besides, the original chain length for the Leu pharmacophore present in **11** also turned out to be a winning solution for blocking α4β7 binding to MAdCAM. For instance, the α4β7 activity of the β-anomer of **20** is almost completely lost as the ^{i}Bu group is replaced by the ^{i}Pr group as for **21** (Table 1).

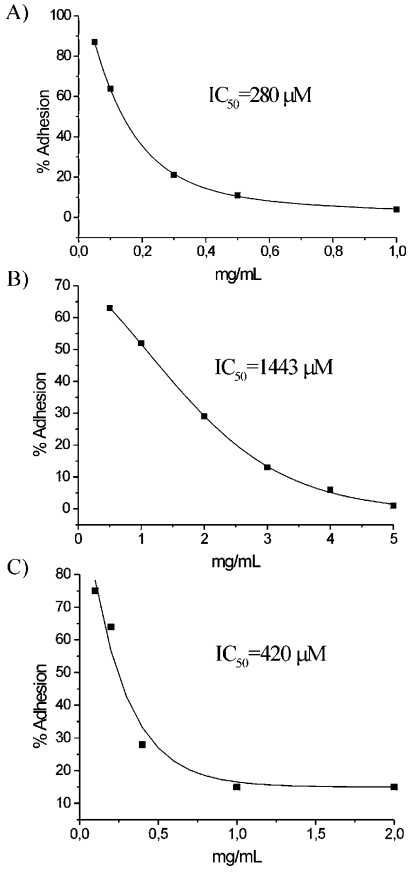


Figure 3. IC_{50} curves measured for (A) **34** toward the α4β7/MAdCAM interaction (IC_{50} = 280 ± 51 μM),[34] (B) **35** toward the α4β1/VCAM interaction (IC_{50} = 1443 ± 317 μM),[35] and (C) the β-anomer of **20** toward the α4β7/MAdCAM interaction (IC_{50} = 420 ± 84 μM). The standard deviations were determined on the basis of three independent measurements for each point in the curves.

Structural Studies. Conformational analysis was carried out for the β-anomer of **20** based on NMR data and molecular modeling. The NOESY spectrum acquired in CD_3CN is reported in Figure 4. Long-range connectivities involving aromatic protons of the Phe pharmacophore and protons of the Leu side chain indicated hydrophobic interactions between these residues. These proximities were also observed in a more polar solution (D_2O/CD_3CN, 1:1). A simulating annealing protocol generated 50 structures that were classified into five families based on the orientation of the Asp, Phe, and Leu pharmacophores. Representative structures for each family were superimposed in Figure 5. During the whole simulation, the sugar core remained rigid. The five structures were classified based on the NOE deviations, which of course have to be considered as average values because of the mobility of the side chains. However, the structure forming a hydrophobic region between the aromatic ring of Phe and the Leu side chain, which can be clearly seen in the side view of Figure 5, is in agreement with the NOE data, and it is by far the most populated conformation during simulated annealing.

Several possible mechanisms could explain the selectivity of the β-anomer of **20** for α4β7. This sugar lacks

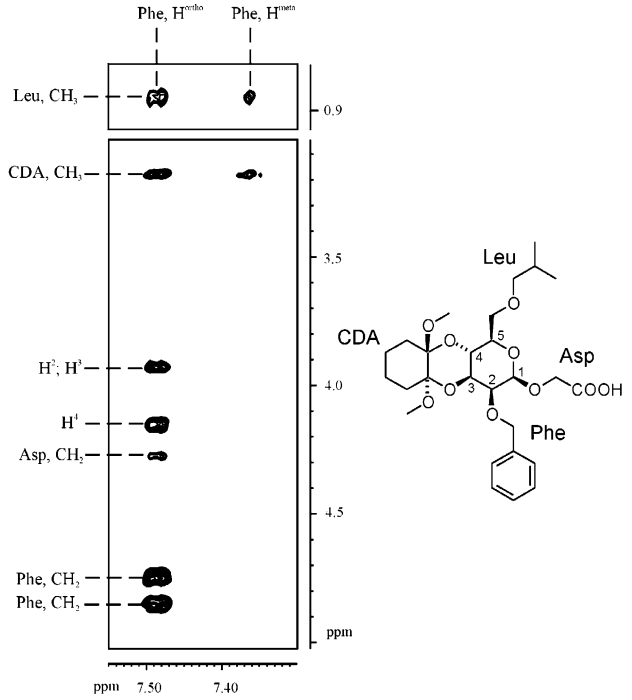


Figure 4. Region of the NOESY spectrum acquired for the β-anomer of **20** in CD_3CN at 275 K. Long-chain connectivities involving the aromatic protons of Phe are labeled.

a critical $\alpha4\beta1$ binding interaction, like the Ser side chain in **11**. This could also explain the negative effect on activity of the replacement of the ethylene glycol moiety at the 2-position of the original $\alpha4\beta1$ antagonist **11**-β by the benzyl group as for the β-anomer of **22**. Otherwise, the relatively bulky CDA group sterically hinders binding to $\alpha4\beta1$ but not to $\alpha4\beta7$. In fact, the incorporation of the PDA group, as source of aromaticity, at the 3 and 4 positions of **11**-β causes a decrease of $\alpha4\beta1$ activity in **19**-β as well. Besides, changes in antagonist conformation, due to the constraints introduced by the CDA group, can have a marked effect on binding affinity. An additional possibility exists that the activity and selectivity of **20**-β can be dictated by the hydrophobic region created by the Leu and Phe interactions, as confirmed by NMR spectroscopy and molecular modeling.

Conclusions

Interfering protein/protein interaction by small nonpeptidic molecules is one of the great challenges in medicinal chemistry. On the basis of a D-mannose core, we developed in a rational combinatorial approach a selective inhibitor of the $\alpha4\beta7$/MAdCAM interaction. The biased library of mannose peptidomimetics originated from an inhibitor of the $\alpha4\beta1$/VCAM interaction previously developed in our laboratory. In our strategy, we maintained the original mannose core and the Leu and Asp pharmacophores at the 6 and 1 positions of the sugar ring, respectively, occurring in the binding motif of $\alpha4$ natural ligands. A spatial screening for the Asp pharmacophore was carried out by measuring the activity for both α and β anomers. The Ser side chain at position 2 was replaced by the Phe side chain. Aromatic residues were introduced at the 3 and 4 positions to increase hydrophobicity of the mannose peptidomimetics. The mannose scaffold was rigidified by a trans ring fusion between a dioxane ring and the pyranoside at the 3 and 4 positions. The length, polarity, and conformational space available to the Leu side chain were modified by carbon−carbon linkage.

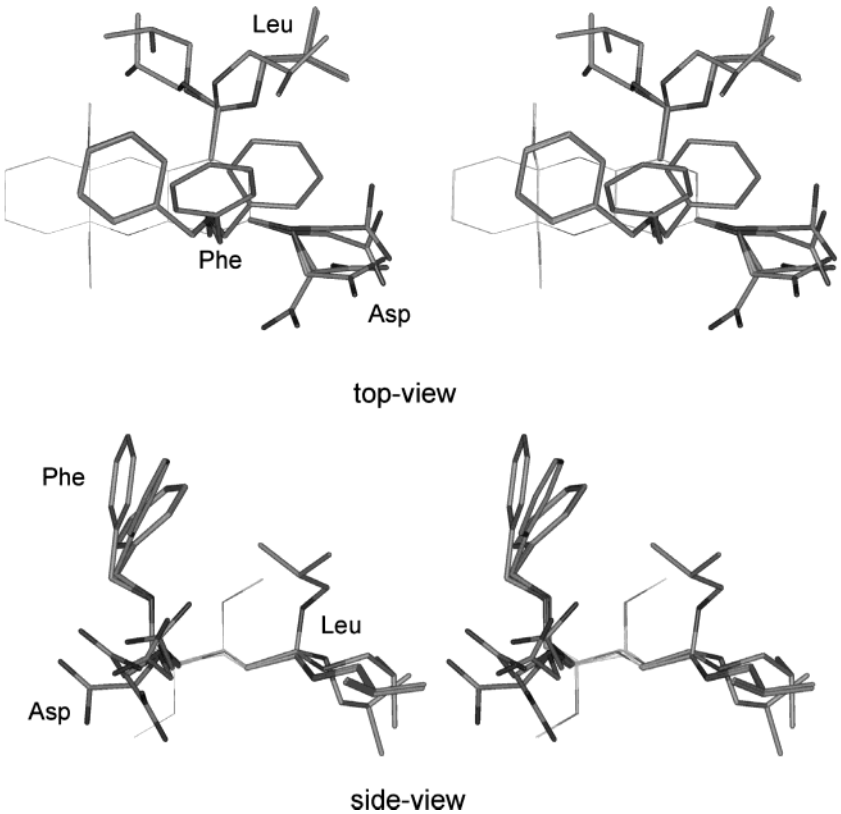


Figure 5. Stereoview of the superposition of representative structures of the β-anomer of **20** for each of the five cluster determined by molecular modeling.

SAR studies reconfirmed the β-orientation of the Asp side chain to be necessary for activity. The basis for affinity and selectivity toward the $\alpha4\beta7$/MAdCAM interaction is likely to be the association of the Leu and Phe pharmacophores, which might be accommodated in a hydrophobic pocket of the receptor, together with the conformational restraints introduced by the cyclohexane-1,2-diacetal group at the 3 and 4 positions. Besides, the mannose-based antagonist mimics the active conformation of $\alpha4\beta7$ selective cyclic peptides, previously developed in our group. Furthermore, compound **20** exhibits a $-1 < \log P < 5$ ($\log P = 3.35$), which is one requirement for orally availability according to Lipinski et al.[37] In conclusion, this class of peptidomimetics overcomes the limitations of peptidic drugs generally associated with mediocre absorption and poor metabolic stability, among other factors, and represents a promising candidate for drug development.

Experimental Section

General Methods. All chemicals were used as supplied without further purification. All organic solvents were distilled before use. Pd/C was donated by Degussa (Frankfurt/M., Germany). RP-HPLC analysis and semiscale preparations were carried out on a Waters (high-pressure pump 510, multiwavelength detector 490E, chromatography workstation Maxima 820), Beckman (high-pressure pump 110B, gradient mixer, controller 420, UV detector Uvicord from Knauer), and Amersham Pharmacia Biotech (Äkta Basic 10/100, autosampler A-900) instruments. RP-HPLC preparative separations were carried out on Beckman System Gold (high-pressure pump module 126, UV detector 166). C_{18} columns were used. Solvents were the following: (A) H_2O + 0.1% CF_3COOH and (B) CH_3CN + 0.1% CF_3COOH with UV detection at 220 and 254 nm. HPLC−ESI mass spectra were recorded on a Finnigan NCQ-ESI with HPLC conjunction LCQ (HPLC system Hewlett-Packard HP 1100, Nucleosil 100 $5C_{18}$). Degrees of purity given for final products refer to the latter HPLC system.

(I) General Procedure for Williamson Ether Synthesis. To DMSO (15 mL) was added powdered KOH (20 mmol per replaceable hydrogen of substrate). After the mixture was stirred for 10 min, the substrate alcohol (1 mmol) was added, followed immediately by the alkyl halide (10 mmol per replaceable hydrogen of substrate). Stirring was continued until completion of the reaction, monitored by TLC, after which the mixture was poured into water (20 mL) and extracted with diethyl ether or dichloromethane (3 × 20 mL). The combined organic extracts were washed with water (5 × 10 mL) and dried with Na_2SO_4, and the solvents were removed under reduced pressure. The residue was purified by flash column chromatography (gradient elution: hexane/EtOAc 9:1 to hexane/EtOAc 4:1) to give the title compounds in 70−95% yield.

(II) General Procedure for the SEt Cleavage of Ethyl-1-thio-α-D-mannopyranoside. The ethyl-1-thio-α-D-mannopyranoside substrate (4 mmol) was dissolved in 60 mL of CH_3CN/H_2O 2:1 solution. At room temperature, *N*-bromosuccinimide (8 mmol) and 1 mL of concentrated HCl were added. The solution was stirred for 3 h until completion of the reaction, monitored by TLC, after which CH_3CN was evaporated and the residue was extracted with dichloromethane (3 × 20 mL). The combined organic extracts were dried with Na_2SO_4 and evaporated and the residue was purified by flash column chromatography (gradient elution: hexane/EtOAc 5:1 to hexane/EtOAc 2:1) to give the title compounds in 90−95% yield.

(III) General Procedure for the Synthesis of Mannosyl Chloride. The α,β-D-mannopyranoside mixture (4 mmol) from general procedure II was dissolved at room temperature in pure thionyl chloride (10 mL), and the mixture was stirred for 2 h. After this time, the solution was evaporated and the residue was used immediately as a glycosyl donor in a Koenigs−Knorr type reaction.

(IV) General Procedure for the Synthesis of Mannosyl Bromide. A solution of ethyl-1-thio-α-D-mannopyranoside substrate (1 mmol) in 10 mL of dried dichloromethane was treated with bromine (53 μL, 164 mg, 1 mmol) at 0 °C for 10−15 min. The solution was successively washed with 10% aqueous $NaHSO_3$ and water, dried (Na_2SO_4), and concentrated. The residue was used immediately as a glycosyl donor in a Koenigs−Knorr type reaction.

(V) General Procedure for α-O-Glycosidic Linkage Formation. A solution of the ethyl-1-thio-α-D-mannopyranoside substrate (1 mmol) and alcohol (3 mmol), which was previously rotoevaporated with toluene and dried under vacuum, in dry dichloromethane (10 mL) containing freshly activated 4 Å molecular sieves (2 g) was treated with *N*-iodosuccinimide (1.2 mmol), followed by a solution of silver trifluoromethanesulfonate (1.2 mmol) in 1 mL of dry toluene. The reaction was essentially complete (as judged by TLC) after the addition of the triflate. The mixture was then diluted with CH_2Cl_2 and filtered. The filtrate was washed with 10% aqueous sodium thiosulfate, saturated aqueous sodium bicarbonate, and brine. The solution was dried (Na_2SO_4) and concentrated to an oil that was flash-chromatographed on silica gel (gradient elution: hexane/EtOAc 4:1 to hexane/EtOAc 1:1) to give the title compounds in 75−85% yield as an α/β mixture 6:1.

(VI) General Procedure for β-O-Glycosidic Linkage Formation. Solid Ag_2CO_3 (6 mmol) was added at 0 °C to a solution of mannosyl bromide (from step IV) or chloride (from step III) (3 mmol), the specified glycosyl acceptor (10 mmol), 4 Å molecular sieves (2 g), and 10 mL of dichloromethane, previously stirred at room temperature for 1 h. Stirring was continued overnight in the dark. Successively, the mixture was filtered through Celite and concentrated. The residue was purified by flash column chromatography (gradient elution: hexane/EtOAc 5:1 to hexane/EtOAc 2:1) to give the title compounds in 70−75% yield as an α/β mixture 1:5.

(VII) General Procedure for Primary Alcohol Oxidation to Aldehyde. A solution of 2,3,4-protected ethyl-1-thio-α-D-mannopyranoside (0.6 mmol) and Et_3N (380 μL, 2.72 mmol) in CH_2Cl_2/DMSO (3:1, 12 mL) was cooled to 0 °C and treated with sulfur trioxide pyridine complex (344 mg, 2.16 mmol). After being stirred at 0 °C for 30 min, the mixture was diluted with CH_2Cl_2 (100 mL), washed with H_2O (2×) and brine, dried (Na_2SO_4), and concentrated to give crude aldehyde, which was used in the following reaction without purification.

(VIII) General Procedure for the Synthesis of the Phosphonium Salt. A solution of triphenylphosphine (10 mmol) and the alkyl halide (10 mmol) in dry toluene (20 mL) was heated under reflux for 24 h. After this time the mixture was cooled with ice and filtrated. The white precipitate was washed with cold toluene, dried under vacuum, and used immediately.

(IX) General Procedure for Wittig Olefination of Sugar Aldehyde. A mixture of the phosphonium salt (0.5 mmol) obtained from general procedure VIII, activated 4 Å powdered molecular sieves (480 mg), and anhydrous toluene (5 mL) was cooled to −10 °C. To this suspension was added potassium bistrimethylsilylamide (1 mL of 0.5 M solution in toluene, 0.5 mmol) as base, followed by the aldehyde (0.5 mmol), obtained in step VII, dissolved in anhydrous toluene (2 mL). While being stirred, the suspension was allowed to slowly warm to room temperature (about 3 h), maintained for an additional 30 min at room temperature, and then filtered through Celite. The solvent was evaporated, and the residue was dissolved in CH_2Cl_2. The organic layer was washed with water (10 mL), dried (Na_2SO_4), and concentrated. Flash chromatography (12:1 hexane/EtOAc) of the residue furnished the olefin in 65−70% yield.

(X) General Procedure for CDA Deprotection. A sample of 20 mmol of 3,4-CDA protected 1-thiomannopyranoside was stirred at room temperature for 1 h in 90% TFA solution in CH_2Cl_2. At completion of the reaction, the solvents were evaporated and the residue was purified by flash chromatography (2:1 hexane/EtOAc) to give a colorless oil in 75−85% yield.

(XI) General Procedure for Alkene Reduction with *p*-Toluenesulfonylhydrazide. To a warmed (85 °C), stirred solution of alkene substrate (0.5 mmol) and freshly recrystallized *p*-toluenesulfonylhydrazide (285 mg, 1.53 mmol) in dimethoxyethane (10 mL) was added 1 M aqueous sodium acetate (1.53 mL) in six portions during 3 h. After an additional 3 h at 85 °C, the mixture was diluted with H_2O (5 mL) and extracted with CH_2Cl_2 (2 × 30 mL). The organic phase was dried (Na_2SO_4) and concentrated. The residue was eluted from a column of silica gel with hexane/AcOAc 12:1 to give the reduced product in 60−65% yield.

(XII) General Procedure for Selective Hydrogenation of Alkene in the Presence of the Benzyl Group. A solution of the alkene substrate (0.5 mmol), 30 mg of 10% Pd/C (50% water), and ethylenediamine as poison (0.25 mmol) in MeOH (10 mL) was stirred at room temperature under hydrogen atmosphere (50 mbar) for 12 h until completion of the reaction, monitored by TLC. The solution was then filtered through Celite, the solvent was evaporated, and the residue was used in the following reaction without purification (75−80% yield).

(XIII) General Procedure for Benzyl Group Cleavage. A solution of the benzyl-protected alcohol (0.5 mmol) and 60 mg of 10% Pd/C (50% water) in MeOH (15 mL) was stirred at room temperature under a hydrogen atmosphere (balloon) for 1 h until completion of the reaction, monitored by TLC. The solution was then filtered through Celite, the solvent was evaporated, and the residue was used in the following reaction without purification (90−98% yield).

Ethyl-2-*O*-benzyl-3,4-*O*-[1′,2′-dimethoxycyclohexan-1′,2′-diyl]-1-thio-α-D-mannopyranoside.[2] Ethyl-3,4-*O*-[1′,2′-dimethoxycyclohexan-1′,2′-diyl]-1-thio-α-D-mannopyranoside[26] **1** was benzylated at position 2 as outlined in general procedure I using 1.2 equiv of benzyl bromide (75−85% yield).

Ethyl-2-*O*-benzyl-3,4-*O*-[1′,2′-dimethoxycyclohexan-1′,2′-diyl]-6-*O*-isobutyl-1-thio-α-D-mannopyranoside.[3] Ethyl-2-*O*-benzyl-3,4-*O*-[1′,2′-dimethoxycyclohexan-1′,2′-diyl]-1-thio-α-D-mannopyranoside **2** was alkylated at position 6 as outlined in general procedure I using isobutyl bromide (95% yield).

Ethyl-2-*O*-benzyl-3,4-*O*-[1′,2′-dimethoxycyclohexan-1′,2′-diyl]-6-*O*-isopropyl-1-thio-α-D-mannopyranoside.[4] Ethyl-2-*O*-benzyl-3,4-*O*-[1′,2′-dimethoxycyclohexan-1′,2′-diyl]-1-thio-α-D-mannopyranoside **2** was alkylated at position 6 as outlined in general procedure I using isopropyl iodide (90% yield).

Ethyl-2-*O*-benzyl-6-*O*-isobutyl-3,4-di-*O*-methyl-1-thio-α-D-mannopyranoside.[5] CDA deprotection of ethyl-2-*O*-benzyl-3,4-*O*-[1′,2′-dimethoxycyclohexan-1′,2′-diyl]-6-*O*-isobutyl-1-thio-α-D-mannopyranoside **3** was carried out as outlined in step X. Subsequent methylation at the 3 and 4 positions following general procedure I using methyl iodide afforded **5** in 70% yield. Activation of the SEt group in the presence of MeI caused the formation of methylmannopyranoside as a byproduct. Before purification by flash column chromatography, the excess of toxic alkyl halide was destroyed with a 15% solution of ammonium hydroxide.

Ethyl-2-*O*-benzyl-6-*O*-isopropyl-3,4-di-*O*-methyl-1-thio-α-D-mannopyranoside.[6] CDA deprotection of ethyl-2-*O*-benzyl-3,4-*O*-[1′,2′-dimethoxycyclohexan-1′,2′-diyl]-6-*O*-isopropyl-1-thio-α-D-mannopyranoside **4** was carried out as outlined in step X. Subsequent methylation at the 3 and 4 positions following general procedure I using methyl iodide afforded **6** in 70% yield. Activation of the SEt group in the presence of MeI caused the formation of methylmannopyranoside as a byproduct. Before purification by flash column chromatography, the excess of toxic alkyl halide was destroyed with a 15% solution of ammonium hydroxide.

[Carboxylmethyl]-2-*O*-[2′-hydroxyethyl]-6-*O*-isobutyl-3,4-di-*O*-methyl-α,β-D-mannopyranoside.[11] Ethyl-2-*O*-benzyl-6-*O*-isobutyl-3,4-di-*O*-methyl-1-thio-α-D-mannopyranoside **5** was reacted with Br_2, as outlined in general procedure IV, and the freshly prepared mannosyl bromide as glycosyl donor was successively coupled to anhydrous glycol aldehyde dimethyl acetal (from step VI) to give preferentially the β-anomer, or **5** was reacted directly with glycol aldehyde dimethyl acetal as described in general procedure V to give preferentially the α-anomer. The benzyl group was then cleaved following general procedure XIII, and the residue was coupled with bromoethyl-2-benzyl ether under Williamson conditions as described in step I (95% yield). A solution of [2′,2′-dimethoxyethyl]-2-*O*-[2′-benzyloxyethyl]-6-*O*-isobutyl-3,4-di-*O*-methyl-D-mannopyranoside **9** (0.2 mmol, α and β anomers were kept in different flasks) in a mixture of 10 mL of 2 N aqueous HCl and 10 mL of THF was stirred at room temperature for 1 h until completion of the reaction (TLC). The solution was neutralized with $NaHCO_3$, the aldehyde was extracted with ether, and the organic extract was concentrated. The residue was then dissolved in 8 mL of *tert*-BuOH and 3 mL of 2-methyl-2-butene. An equivalent of $NaClO_2$ (18.0 mg, 0.2 mmol) and 0.3 g (2.52 mmol) of sodium dihydrogen phosphate were dissolved in 3 mL of H_2O, and the mixture was added dropwise to the previous solution. At completion of the reaction (3 h), the solution was treated with an aqueous Na_2SO_3 to destroy the excess $NaClO_2$. The solvent was evaporated, and the aqueous solution was extracted with EtOAc. The organic extract was dried (Na_2SO_4) and concentrated, and the residue was used in the following reaction without purification. The benzyl group was then cleaved as outlined in general procedure XIII, and both α and β anomers were purified by HPLC (80% yield).

[Carboxylmethyl]-2-*O*-[2′-hydroxyethyl]-6-*O*-isopropyl-3,4-di-*O*-methyl-α,β-D-mannopyranoside.[12] Ethyl-2-*O*-benzyl-6-*O*-isopropyl-3,4-di-*O*-methyl-1-thio-α-D-mannopyranoside **6** was reacted with Br_2, as outlined in general procedure IV, and the freshly prepared mannosyl bromide as glycosyl donor was successively coupled to anhydrous glycol aldehyde dimethyl acetal (step VI) to give preferentially the β-anomer, or **6** was reacted directly with glycol aldehyde dimethyl acetal as described in general procedure V to give preferentially the α-anomer. The benzyl group was then cleaved following general procedure XIII, and the residue was coupled with bromoethyl-2-benzyl ether under Williamson conditions described in step I (95% yield). A solution of [2′,2′-dimethoxyethyl]-2-*O*-[2′-benzyloxyethyl]-6-*O*-isopropyl-3,4-di-*O*-methyl-D-mannopyranoside **10** (0.2 mmol, α and β anomers were kept in different flasks) in a mixture of 10 mL of 2 N aqueous HCl and 10 mL of THF was stirred at room temperature for 1 h until completion of the reaction (TLC). The solution was neutralized with $NaHCO_3$, the aldehyde was extracted with ether, and the organic extract was concentrated. The residue was then dissolved in 8 mL of *tert*-BuOH and 3 mL of 2-methyl-2-butene. An equivalent of $NaClO_2$ (18.0 mg, 0.2 mmol) and 0.3 g (2.52 mmol) of sodium dihydrogen phosphate were dissolved in 3 mL of H_2O, and the mixture was added dropwise to the previous solution. At completion of the reaction (3 h), the solution was treated with an aqueous Na_2SO_3 to destroy the excess $NaClO_2$. The solvent was evaporated, and the aqueous solution was extracted with EtOAc. The organic extract was dried (Na_2SO_4) and concentrated, and the residue was used in the following reaction without purification. The benzyl group was then cleaved as outlined in step XIII, and both α and β anomers were purified by HPLC (80% yield).

Ehyl-2-*O*-benzyl-3,4-*O*-[9′,10′-dimethoxyphenanthrene-9′,10′-diyl]-6-*O*-isobutyl-1-thio-α-D-mannopyranoside.[15] Ehyl-3,4-*O*-[9′,10′-dimethoxyphenanthrene-9′,10′-diyl]-1-thio-α-D-mannopyranoside[26] **13** (13 mmol) and imidazole (39 mmol) were dissolved in 50 mL of DMF, and the solution was stirred at room temperature. Successively, *tert*-butyldiphenylsilyl chloride (TBDPSCl, 17 mmol) was added dropwise. Stirring was continued for 3 h until completion of the reaction, followed by TLC. The solvent was evaporated under vacuum, and the residue was purified by flash column chromatography (gradient elution: hexane/EtOAc 9:1 to hexane/EtOAc 4:1) to give the title compound as a colorless oil (11.0 mmol, 85% yield). The TBDPS-protected mannoside was then benzylated at position 2 following the general procedure I using benzyl bromide (95% yield). TBDPS removal was carried out overnight under stirring in 20 mL of THF using 2 equiv of tetrabutylammonium fluoride (TBAF) of a 1 M TBAF solution in THF. The solvent was evaporated, and the residue was

purified by flash column chromatography (gradient elution: hexane/EtOAc 6:1 to hexane/EtOAc 4:1) (96% yield). The 6 position was then alkylated following the general procedure I using isobutyl bromide (95% yield).

[2′-Hydroxyethyl]-2-*O*-benzyl-3,4-*O*-[9′,10′-dimethoxyphenanthrene-9′,10′-diyl]-6-*O*-isobutyl-α,*β*-D-mannopyranoside.[16] Ehyl-2-*O*-benzyl-3,4-*O*-[9′,10′-dimethoxyphenanthrene-9′,10′-diyl]-6-*O*-isobutyl-1-thio-α-D-mannopyranoside **15** was reacted with Br_2 as outlined in general procedure IV. Successively, the freshly prepared mannosyl bromide as glycosyl donor was coupled to anhydrous ethylene glycol in 5: 1 DCM/THF solution (step VI). The addition of THF was needed to dissolve the ethylene glycol and to create a homogeneous system. Purification by flash column chromatography afforded **16** in 90% yield with a 1:1 α/*β* ratio. The two conformers were collected together on purpose.

[Carboxylmethyl]-2-*O*-[2′-hydroxyethyl]-3,4-*O*-[9′,10′-dimethoxyphenanthrene-9′,10′-diyl]-6-*O*-isobutyl-α,*β*-D-mannopyranoside.[19] [2′-Hydroxyethyl]-2-*O*-benzyl-3,4-*O*-[9′,-10′-dimethoxyphenanthrene-9′,10′-diyl]-6-*O*-isobutyl-α,*β*-D-mannopyranoside **16** (6.5 mmol) and imidazole (19.5 mmol) were dissolved in 25 mL of DMF, and the solution was stirred at room temperature. Successively, *tert*-butyldiphenylsilyl chloride (TBDPSCl, 8.5 mmol) was added dropwise. Stirring was continued for 3 h until completion of the reaction, followed by TLC. The solvent was evaporated under vacuum and the residue was purified by flash column chromatography (gradient elution: hexane/EtOAc 9:1 to hexane/EtOAc 4:1) to give the title compound as a colorless oil (5.5 mmol, 85% yield). The benzyl group at position 2 was then cleaved as outlined in general procedure XIII, and the residue was coupled with bromoethyl-2-benzyl ether under Williamson conditions described in step I (95% yield). TBDPS removal was carried out overnight under stirring in 20 mL of THF using 2 equiv of tetrabutylammonium fluoride (TBAF) of a 1 M TBAF solution in THF. The solvent was evaporated, and the residue was purified by flash column chromatography (gradient elution: hexane/EtOAc 6:1 to hexane/EtOAc 4:1) (96% yield). The free primary alcohol present in [2′-hydroxyethyl]-2-*O*-[2′-benzyloxyethyl]-6-*O*-isopropyl-3,4-di-*O*-methyl-D-mannopyranoside **18** (0.2 mmol, α and *β* anomers were kept in different flasks) was then oxidized to aldehyde as described in general procedure VII. The residue was then dissolved in 8 mL of *tert*-BuOH and 3 mL of 2-methyl-2-butene. An equivalent of $NaClO_2$ (18.0 mg, 0.2 mmol) and 0.3 g (2.52 mmol) of sodium dihydrogen phosphate were dissolved in 3 mL of H_2O, and the mixture was added dropwise to the previous solution. At completion of the reaction (3 h), the solution was treated with an aqueous Na_2SO_3 to destroy the excess $NaClO_2$. The solvent was evaporated, and the aqueous solution was extracted with EtOAc. The organic extract was dried (Na_2SO_4) and concentrated, and the residue was used in the following reaction without purification. The benzyl group was then cleaved following the general procedure XIII, and both α and *β* anomers were purified by HPLC (80% yield).

[Carboxylmethyl]-2-*O*-benzyl-3,4-*O*-[1′,2′-dimethoxycyclohexan-1′,2′-diyl]-6-*O*-isobutyl-α,*β*-D-mannopyranoside.[20] Ethyl-2-*O*-benzyl-3,4-*O*-[1′,2′-dimethoxycyclohexan-1′,2′-diyl]-6-*O*-isobutyl-1-thio-α-D-mannopyranoside **3** was reacted with Br_2, as outlined in general procedure IV, and the freshly prepared mannosyl bromide as glycosyl donor was successively coupled to anhydrous glycolic acid methyl ester (step VI) to give preferentially the *β*-anomer, or **3** was reacted directly with glycolic acid methyl ester as described in general procedure V to give preferentially the α-anomer. Hydrolysis of the methyl ester in 10 mL of 1:1 solution of 1 M NaOH and MeOH for 1 h afforded quantitatively α- and *β*-**20**, which were purified by HPLC.

[Carboxylmethyl]-2-*O*-benzyl-3,4-*O*-[1′,2′-dimethoxycyclo-hexan-1′,2′-diyl]-6-*O*-isopropyl-α,*β*-D-mannopyranoside.[21] Ethyl-2-*O*-benzyl-3,4-*O*-[1′,2′-dimethoxycyclohexan-1′,2′-diyl]-6-*O*-isopropyl-1-thio-α-D-mannopyranoside **4** was reacted with Br_2, as outlined in general procedure IV, and the freshly prepared mannosyl bromide as glycosyl donor was successively coupled to anhydrous glycolic acid methyl ester (step VI) to give preferentially the *β*-anomer, or **4** was reacted directly with glycolic acid methyl ester as described in general procedure V to give preferentially the α-anomer. Hydrolysis of the methyl ester in 10 mL of a 1:1 solution of 1 M NaOH and MeOH for 1 h afforded quantitatively α- and *β*-**21**, which were purified by HPLC.

[Carboxylmethyl]-2-*O*-benzyl-6-*O*-isobutyl-3,4-di-*O*-methyl-α,*β*-D-mannopyranoside.[22] Ethyl-2-*O*-benzyl-6-*O*-isobutyl-3,4-di-*O*-methyl-1-thio-α-D-mannopyranoside **5** was reacted with Br_2, as outlined in general procedure IV, and the freshly prepared mannosyl bromide as glycosyl donor was successively coupled to anhydrous glycolic acid methyl ester (step VI) to give preferentially the *β*-anomer, or **5** was reacted directly with glycolic acid methyl ester as described in general procedure V to give preferentially the α-anomer. Hydrolysis of the methyl ester in 10 mL of a 1:1 solution of 1 M NaOH and MeOH for 1 h afforded quantitatively α- and *β*-**22**, which were purified by HPLC.

[Carboxylmethyl]-2-*O*-benzyl-6-*O*-isopropyl-3,4-di-*O*-methyl-α,*β*-D-mannopyranoside.[23] Ethyl-2-*O*-benzyl-6-*O*-isopropyl-3,4-di-*O*-methyl-1-thio-α-D-mannopyranoside **6** was reacted with Br_2, as outlined in general procedure IV, and the freshly prepared mannosyl bromide as glycosyl donor was successively coupled to anhydrous glycolic acid methyl ester (step VI) to give preferentially the *β*-anomer, or **6** was reacted directly with glycolic acid methyl ester as described in general procedure IV to give preferentially the α-anomer. Hydrolysis of the methyl ester in 10 mL of a 1:1 solution of 1 M NaOH and MeOH for 1 h afforded quantitatively α- and *β*-**23**, which were purified by HPLC.

α-D-Manno-non-6-enopyranoside, Ethyl-6,7,8,9-tetradeoxy-2-*O*-benzyl-3,4-*O*-[1′,2′-dimethoxycyclohexan-1′,2′-diyl]-8-methyl-1-thio.[24] The free primary alcohol in ethyl-2-*O*-benzyl-3,4-*O*-[1′,2′-dimethoxycyclohexan-1′,2′-diyl]-1-thio-α-D-mannopyranoside **2** was oxidized to aldehyde following the general procedure VII. The aldehyde was then reacted with isobutyltriphenylphosphonium chloride salt (step VIII) under conditions described in step IX with good *Z*/*E* selectivity (>10: 1).

α-D-Manno-oct-6-enopyranoside, Ethyl-6,7,8-trideoxy-2-*O*-benzyl-3,4-*O*-[1′,2′-dimethoxycyclohexan-1′,2′-diyl]-7-methyl-1-thio.[25] The free primary alcohol in ethyl-2-*O*-benzyl-3,4-*O*-[1′,2′-dimethoxycyclohexan-1′,2′-diyl]-1-thio-α-D-mannopyranoside **2** was oxidized to aldehyde following the general procedure VII. The aldehyde was then reacted with isopropyltriphenylphosphonium iodide salt (step VIII) under conditions described in step IX.

α,*β*-D-Manno-non-pyranoside, [Carboxylmethyl]-6,7,8,9-tetradeoxy-2-*O*-benzyl-3,4-di-*O*-methyl-8-methyl.[32] The double bond present in α-D-manno-non-6-enopyranoside, ethyl-6,7,8,9-tetradeoxy-2-*O*-benzyl-3,4-*O*-[1′,2′-dimethoxycyclohexan-1′,2′-diyl]-8-methyl-1-thio **24** was reduced with *p*-toluenesulfonylhydrazide following general procedure XI or by hydrogenation with poisoned Pd/C following general procedure XII. CDA deprotection was carried out as outlined in **X**. Subsequent methylation at the 3 and 4 positions as described in general procedure I using methyl iodide afforded the desired α-D-manno-non-pyranoside, ethyl-6,7,8,9-tetradeoxy-2-*O*-benzyl-3,4-di-*O*-methyl-8-methyl-1-thio **28** in 90% yield. Before purification by flash column chromatography, the excess of toxic alkyl halide was destroyed with a 15% solution of ammonium hydroxide. To obtain preferentially the *β*-anomer, **28** was reacted first with *N*-bromosuccinimide (step II) and second with thionyl chloride (step III), and the freshly prepared mannosyl chloride as glycosyl donor was successively coupled to anhydrous glycolic acid methyl ester (step VI). To obtain preferentially the α-anomer, **28** was reacted directly with glycolic acid methyl ester following general procedure V. Hydrolysis of the methyl ester in 10 mL of a 1:1 solution of 1 M NaOH and MeOH for 1 h afforded quantitatively α- and *β*-**32**, which were purified by HPLC.

α,*β*-D-Manno-oct-pyranoside, [Carboxylmethyl]-6,7,8-trideoxy-2-*O*-benzyl-3,4-di-*O*-methyl-7-methyl.[33] The double

bond present in α-D-manno-oct-6-enopyranoside, ethyl-6,7,8-trideoxy-2-*O*-benzyl-3,4-*O*-[1′,2′-dimethoxycyclohexan-1′,2′-diyl]-7-methyl-1-thio **25** was reduced with *p*-toluenesulfonylhydrazide following the procedure described in general procedure XI or by hydrogenation with poisoned Pd/C as outlined in step XII. CDA deprotection was carried out following general procedure X. Subsequent methylation at the 3 and 4 positions as described in general procedure I using methyl iodide afforded the desired α-D-manno-oct-pyranoside, ethyl-6,7,8-trideoxy-2-*O*-benzyl-3,4-di-*O*-methyl-7-methyl-1-thio **29** in 90% yield. Before purification by flash column chromatography, the excess of toxic alkyl halide was in this case reacted with a 15% solution of ammonium hydroxide. To obtain preferentially the β-anomer, **29** was reacted first with *N*-bromosuccinimide (step II) and second with thionyl chloride (step III) and the freshly prepared mannosyl chloride as glycosyl donor was successively coupled to anhydrous glycolic acid methyl ester (step VI). To obtain preferentially the α-anomer, **29** was reacted directly with glycolic acid methyl ester following general procedure V. Hydrolysis of the methyl ester in 10 mL of a 1:1 solution of 1 M NaOH and MeOH for 1 h afforded quantitatively α- and β-**33**, which were purified by HPLC.

Biological Evaluation. For MAdCAM-1 and VCAM supernatants, 293T cells (embryonic fibroblasts from kidney) are transfected with pCDNA3.1 IgG MAdCAM-1 and pCDNA3.1 IgG VCAM, respectively, and the supernatants are collected after 2−3 days. For cell adhesion assay, 96-well plates were coated with donkey antihuman IgG (Jackson ImmunoResearch Laboratories Inc.) (0.5 μg in 100 μL of PBS/well) overnight at 4 °C. The plates were washed with adhesion buffer (Click's RPMI, 1% BSA, 1.0 mM Ca^{2+}, 1.0 mM Mg^{2+}) and incubated for 30 min at room temperature with 25−150 μL/well MAdCAM or VCAM supernatant (depending on the concentration). After being washed with adhesion buffer, the plates were blocked with adhesion buffer for 1 h at 37 °C. The 38C13β7 lymphoma cells or Jurkat cells were labeled for 30 min at 37 °C with 6 μg/mL H33342 dye (Calbiochem, La Jolla, CA) in cell adhesion buffer and washed with PBS containing 1 mM EDTA and with PBS. The cells (8×10^5 cells/mL) were resuspended in cell adhesion buffer. The compounds were preincubated with the cells at the final concentration of 1 mg/mL for 10 min at 37 °C. Subsequently, cells were allowed to adhere for 30 min at 37°C and nonadherent cells were removed by inverse centrifugation for 10 min at 50 g. Adhesion assays were quantified by fluorimetry using a Cytofluor 2300 (Millipore, Bedford, MA).

NMR Spectroscopy. The spectra were acquired in CD_3CN or $CDCl_3$ at 300 K (unless otherwise indicated) with a Bruker DMX500 spectrometer and calibrated using the solvent as an internal reference. The assignment of all proton and carbon resonances was carried out via standard procedures[38,39] using double-quantum-filtered correlation spectroscopy (DQF-COSY)[40,41] and heteronuclear ($^1H-^{13}C$) single-quantum coherence (HSQC)[42−45] with echo/antiecho coherence selection and decoupling during acquisition. Sequential assignment was accomplished by through-bond connectivities from heteronuclear multibond correlation (HMBC)[46−48] with low-pass *J* filter to suppress one-bond correlations, no decoupling during acquisition, and echo/antiecho coherence selection and from HSQC-COSY experiments. Multiplicities are given (obtained from 1D spectra) as s (singlet), d (doublet), t (triplet), dd (doublet of doublets), or m (multiplet). Data were processed on a Bruker X32 workstation using the XWINNMR software. Proton distances were calculated using nuclear Overhauser enhancement (NOESY)[49] with $\tau_{mix} = 200$ ms, and phase sensitivity was calculated using the states time proportional phase increment (TPPI) method. The integration and calibration of the NOE cross-peaks were achieved using the program SYBYL (version 6.6, Tripos, Inc., St. Louis, MO). NOE connectivities were calibrated on the basis of fixed geometric distances between protons within the pyranose ring in the $^4C_1$ conformation. Homonuclear coupling constants were determined from one-dimensional spectra.

Computer Simulations. The structure calculations were performed on Silicon Graphics computers. Energy minimization (EM) and molecular dynamic (MD) calculations were carried out with the program DISCOVER using the CVFF force field.[50] After EM using steepest descent and the conjugate gradient, the system was gradually heated from 300 to 800 K and subsequently cooled to 300 K using 5 ps steps at every temperature, each by direct scaling of velocities. Configurations were saved every 25 ps for another 1250 ps. All the structures coming from MD simulations were minimized using again steepest descent and conjugate gradient algorithms. During molecular modeling simulations, no restraints were taken into account. The structures were evaluated at the end on the basis of the NOE data.

Other Physical Data. The log *P* values were calculated on the basis of Crippen's fragmentation.[51]

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Supporting Information Available: ESI mass spectra and ^1H and ^{13}C chemical shifts of compounds **2**−**6**, **11**, **12**, **15**, **16**, **19**−**25**, **32**, and **33**. This material is available free of charge via the Internet at http://pubs.acs.org.

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