Bioorganic & Medicinal Chemistry 18 (2010) 7239–7251

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

Design, synthesis, biological evaluation, and modeling of a non-carbohydrate antagonist of the myelin-associated glycoprotein

Oliver Schwardt [a], Hendrik Koliwer-Brandl [b], Raphael Zimmerli [a], Stefanie Mesch [a], Gianluca Rossato [a], Morena Spreafico [a], Angelo Vedani [a], Sørge Kelm [b], Beat Ernst [a,*]

[a] Institute of Molecular Pharmacy, Pharmacenter, University of Basel, Klingelbergstrasse 50, CH-4056 Basel, Switzerland
[b] Department of Physiological Biochemistry, University of Bremen, D-28334 Bremen, Germany

ARTICLE INFO

Article history:
Received 26 May 2010
Accepted 12 August 2010
Available online 17 August 2010

Keywords:
Carbohydrate mimetics
Siglecs
Myelin-associated glycoprotein (MAG)
Binding affinity
Hapten inhibition assay
Surface plasmon resonance (SPR)
Molecular modeling
Key polar groups

ABSTRACT

Broad modifications of various positions of the minimal natural epitope recognized by the myelin-associated glycoprotein (MAG), a blocker of regeneration of neurite injuries, produced sialosides with nanomolar affinities. However, important pharmacokinetic issues, for example, the metabolic stability of these sialosides, remain to be addressed. For this reason, the novel non-carbohydrate mimic **3** was designed and synthesized from (−)-quinic acid. For the design of **3**, previously identified beneficial modifications of side chains of Neu5Ac were combined with the replacement of the ring oxygen by a methylene group and the substitution of the C(4)-OH by an acetamide. Although docking experiments to a homology model of MAG revealed that mimic **3** forms all but one of the essential hydrogen bonds identified for the earlier reported lead **2**, its affinity was substantially reduced. Extensive molecular-dynamics simulation disclosed that the missing hydrogen bond of the former C(8)-OH leads to a change of the orientation of the side chain. As a consequence, an important hydrophobic contact is compromised leading to a loss of affinity.

© 2010 Elsevier Ltd. All rights reserved.

1. Introduction

Siglecs[1,2] (sialic acid-binding immunoglobulin like-lectins) form a sub-group of the I-type lectins and function as cell signaling co-receptors primarily expressed on leukocytes to mediate acquired and innate immune functions. They can be divided into two subsets: the first, evolutionary conserved group consists of Siglec 1, 2, and 4, which show selective binding properties: Siglec 1 (also known as sialoadhesin) and Siglec 4 (myelin-associated glycoprotein, MAG) preferentially bind α(2 → 3)-linked *N*-acetylneuraminic acid (Neu5Ac), whereas Siglec 2 (CD22) is highly specific for α(2 → 6)-linked Neu5Ac. In contrast, members of the second Siglec 3-related group (Siglec 3 and Siglecs 5–13) are more promiscuous in their binding, often recognizing more than one presentation of Neu5Ac.

The most comprehensively characterized Siglecs are CD22, a regulatory protein on B lymphocytes that prevents the over-activation of the immune system and the development of autoimmune diseases, and MAG, one of several inhibitor proteins that block regeneration of injuries of the central nervous system (CNS).[2–10] MAG is located on the surface of neurons and interacts with two classes of targets: proteins of the family of Nogo receptors (NgR)[11,12] and brain gangliosides, specifically GD1a, GT1b, or GQ1bα.[2,13–15] Although the relative roles of gangliosides and NgRs as MAG ligands have yet to be resolved,[10,16] in some systems, MAG inhibition is completely reversed by sialidase treatment, suggesting that MAG uses sialylated glycans as its major axonal ligands.[17] Therefore, blocking MAG with potent antagonists may be a valuable therapeutic approach to enhance axon regeneration.[18]

SAR studies have revealed that the terminal tetrasaccharide epitope of GQ1bα (Fig. 1) shows superior binding to MAG compared with the terminal trisaccharide epitope present in GD1a or GT1b.[19,20] Furthermore, the MAG-affinity of tetrasaccharide **1** (Fig. 1), could clearly be correlated with its ability to reverse MAG-mediated inhibition of axonal outgrowth.[18]

To reduce the structural complexity of tetrasaccharide **1** numerous MAG antagonists have been synthesized.[21–23] Based on structural information obtained by trNOE NMR[24] and STD NMR,[25] the Galβ(1 → 3)Gal*N*Ac core was successfully replaced by non-carbohydrate linkers[22] and the α(2 → 6)-linked sialic acid by polar as

Abbreviations: DBU, 8-diazabicyclo[5.4.0]undec-7-en; DCM, dichloromethane; DMAP, 4-dimethylamino-pyridine; DMF, *N*,*N*-dimethylformamide; DMP, 2,2-dimethoxypropane; FAc, 2-fluoroacetyl; IgG, immunoglobulin G; K_D, dissociation constant; MAG, myelin-associated glycoprotein; Neu5Ac, *N*-acetylneuraminic acid; NgR, Nogo receptor; NMR, nuclear magnetic resonance; PDB, protein data bank; RP, reversed phase; SAR, structure-affinity relationship; SPR, surface plasmon resonance; TFA, trifluoroacetic acid; THF, tetrahydrofurane; *p*-Ts, *p*-tolylsulfonyl.

* Corresponding author. Tel.: +41 267 15 51; fax: +41 267 15 52.
E-mail address: beat.ernst@unibas.ch (B. Ernst).

0968-0896/$ - see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmc.2010.08.027

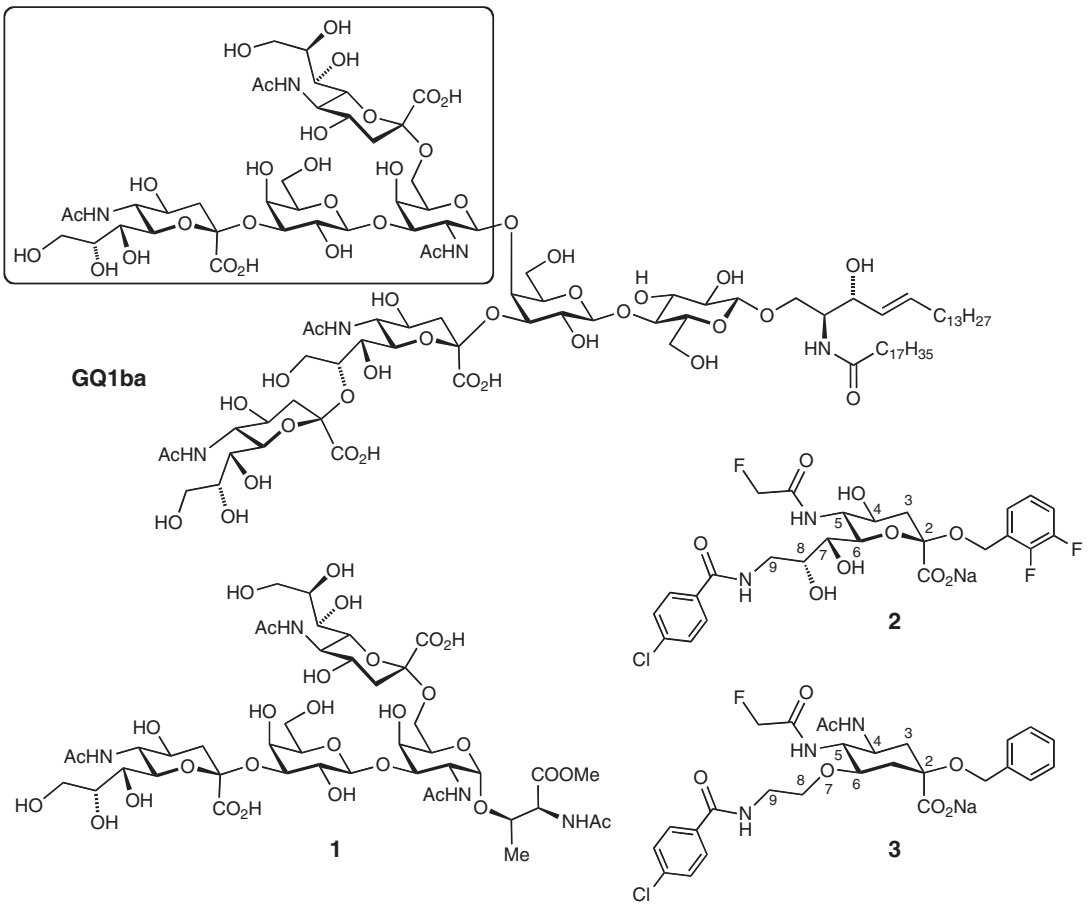


Figure 1. MAG antagonists; GQ1bα with the essential tetrasaccharide binding epitope highlighted, tetrasaccharide derivative 1,[18] sialic acid derivative **2**,[31] and the target molecule of this communication, the non-carbohydrate mimic **3** (for better comparability, the carbon atoms in **3** are numbered in analogy to compound **2**).

well as lipophilic substituents.[23] A further simplification of lead structure **1** was reported by Kelm and Brossmer who found that sialic acid derivatives modified in the 2-, 5-, or 9-position exhibit enhanced antagonistic activity.[26–28] Finally, when the best modifications found for the 2-,[29] 4-,[30] 5-,[31] and 9-position[32] were combined in one molecule, we identified the nanomolar low-molecular weight antagonist **2**.[31]

However, an important pharmacokinetic issue, namely the metabolic stability of sialosides like **2**, remains to be addressed. In general, the substrate specificity of mammalian sialidases is determined by the linkage type of the terminal sialic acid residue ($2 \rightarrow 3$, $2 \rightarrow 6$, or $2 \rightarrow 8$) and does not depend on the structure of the underlying oligosaccharide.[33] Hence, a fast metabolic cleavage of sialosides of type **2** by sialidases cannot be excluded.

To solve this metabolic challenge, we replaced the pyranose core in **2** by a cyclohexane moiety. Here, we describe design, synthesis, and biological evaluation of the corresponding non-carbohydrate lead compound **3**. It still contains the beneficial modifications identified for the 2-, 5-, and 9-position (see compound **2**), however a simplified glycerol side chain.

2. Results and discussion

By manual docking of sialoside **2** (K_D 500 nM)[31] to a homology model of MAG[23] the pharmacophores involved in this carbohydrate-lectin interaction were identified (see Fig. 3B). The most important contributions stem from a salt bridge formed by the carboxylic acid and Arg118[26,34,35] and a hydrogen bond of the C(9)-NH with the backbone carbonyl of Thr128. Additionally, hydrogen-bond formations of C(5)-NH with the backbone carbonyl of Gln126 and the backbone NH of Thr128 with the C(8)-OH were identified, whereas the C(4)- and C(7)-OH exhibit only minor contributions to binding. Overall, this hydrogen bond network nicely correlates with the SAR study published by Kelm and Schauer.[28] Furthermore, considerable contributions to the binding affinity result from two hydrophobic interactions: (i) the *p*-chlorobenzamide

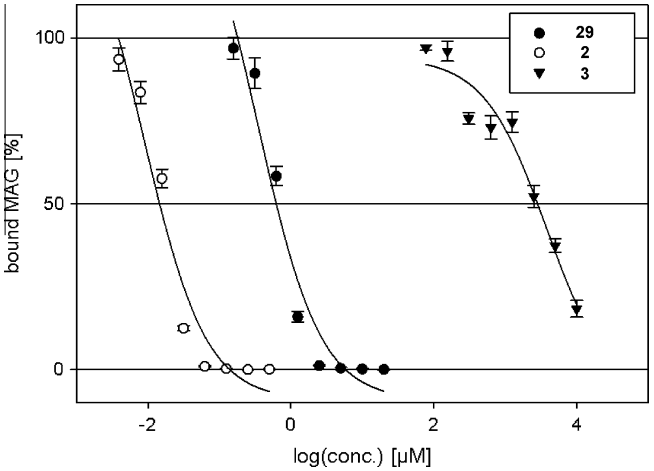


Figure 2. *Hapten inhibition assay with sialosides **29** and **2** and non-carbohydrate mimic **3**.* Fetuin-coated microtiter plates[26,32,41] were incubated with antibody-complexed MAGd1–3-Fc in the presence of the indicated compounds at different concentrations. Binding and inhibition were determined as described for the hapten inhibition assay in Section 4.2.

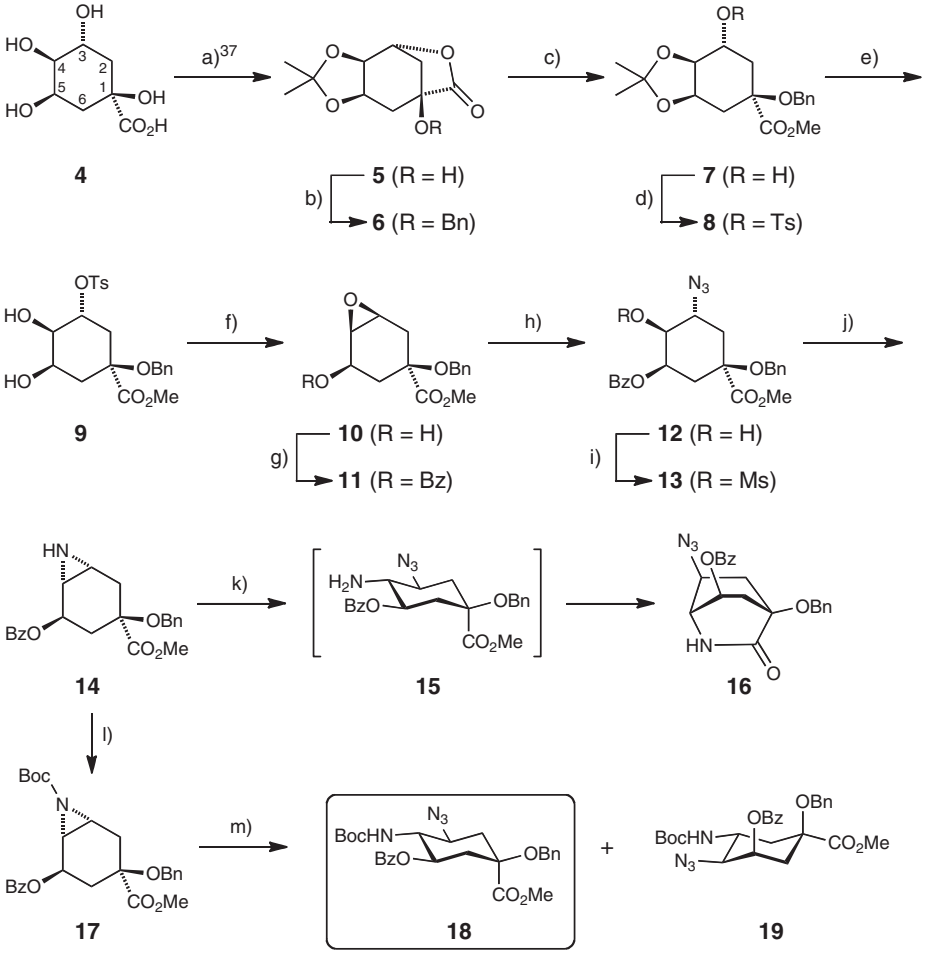
points into a hydrophobic pocket formed by Ser130 and the side chain methylenes of Glu131 and (ii) the 2,3-difluorobenzyl aglycone is hosted by a second hydrophobic pocket established by Trp59, Tyr60, Tyr69, and Tyr116. Most importantly, the pyranose ring oxygen was not found to play a significant role in the interaction of **2** and MAG.

Based on these findings, the non-carbohydrate mimic **3** (Fig. 1) was designed. Since the pyranose ring oxygen in **2** does not contribute to binding, it was exchanged by a methylene group. In addition, the hydroxyl group in the 4-position was replaced by an amide. By this modification, the H-bond with Gln126, which was shown to exhibit a minor contribution to binding,[28] is well preserved (see Fig. 3B and D). Furthermore, the additional acyl group allows the exploration of supplementary beneficial interactions with the target protein. Finally, the *p*-chlorobenzamido-substituted glycerol side chain was substituted by a simplified version, the 2-(*p*-chlorobenzamido)-ethoxy moiety. This modification maintains the essential H-bond originating from the C(9)-NH in **2**, but neglects the C(7)- and C(8)-OH, where at least the latter one was found to contribute to the binding to MAG.[28] However, the role of this functional group was only investigated with oligosaccharides and not in combination with other modifications in Neu5Ac derivatives,[26–28] and therefore is difficult to assess quantitatively.

2.1. Synthesis of sialic acid mimic 3

Because of the similar substitution pattern, that is, three ring substituents in the 4-, 5-, and 6-position and the α-hydroxy carboxylate function, (−)-quinic acid (**4**) is a feasible precursor for the synthesis of **3**. The main synthetic task is the inversion of the *trans*-C(3) and C(4) hydroxyls into the corresponding *trans*-C(3) and C(4) amino groups present in **3**.

The synthesis of the core building block **18** (Scheme 1) was performed in analogy to the synthesis of oseltamivir.[36,37] Treatment of (−)-quinic acid (**4**) with 2,2-dimethoxypropane (DMP) in acetone yielded lactone **5** in 87%. Benzylation of the free hydroxy function (→**6**) and subsequent methanolysis generated methyl ester **7**. Sulfonic ester **9** was obtained almost quantitatively by tosylation of **7** followed by cleavage of the acetonide function in **8**. Treatment of **9** with DBU in THF led to epoxide **10** in 96%. Nucleophilic ring opening of the benzoyl protected epoxide **11** with sodium azide in the presence of ammonium chloride generated azido alcohol **12** in 78% yield. The high regioselectivity of the epoxide opening can be attributed to the steric as well as the electron withdrawing influence of the adjacent benzoate. Conversion of azide **12** to aziridine **14** was achieved by a two-step procedure: first, mesylation of the free hydroxyl group in **12** (→**13**) and second, reduction of the azide using modified Staudinger conditions.[38] Unexpectedly, the



Scheme 1. Reagents and conditions: (a) DMP, cat. *p*-TsOH, acetone, 70 °C, 87%; (b) BnBr, NaH, DMF, 0 °C to rt, 94%; (c) NaOMe, MeOH, rt, 3 h, 73%; (d) TsCl, cat. DMAP, py, rt, 98%; (e) 80% aq AcOH, 60 °C, 3 h, 98%; (f) DBU, THF, 0 °C to rt, 18 h, 96%; (g) BzCl, cat. DMAP, py, 97%; (h) NaN_3, NH_4Cl, MeOH/H_2O (8:1), 70 °C, 78%; (i) MsCl, NEt_3, DCM, 0 °C to rt, 4 h, 94%; (j) PPh_3, THF then NEt_3, H_2O, rt, 5 h, 67%; (k) NaN_3, NH_4Cl, DMF, 65 °C, 50%; (l) Boc_2O, cat. DMAP, NEt_3, DCM, 0 °C to rt, 5 h, 96%; (m) NaN_3, NH_4Cl, DMF, 70 °C, **18**: 64%, **19**: 27%.

aziridine ring opening in **14** with sodium azide resulted exclusively in the bicyclic lactam **16**, resulting from a nucleophilic attack of the amine group in intermediate **15** to the carbonyl of the ester moiety. To avoid the lactame formation, the ring opening was performed with Boc-protected aziridine **17**. Again, an excellent chemical yield, but only a modest regioselectivity of 2.4:1 (**18**, 64% and **19**, 27%) was achieved.

When **18** was debenzoylated using Zemplén conditions, **20** was obtained in almost quantitative yield (Scheme 2). Since the subsequent allylation under phase transfer conditions afforded a mixture of allyl ester, methyl ester, and free acid, saponification with NaOH and re-esterification with TMS-CHN_2 was necessary to obtain uniform **21**. Conversion of its allyl group into the hydroxyethyl side chain (→**22**) was accomplished in 65% yield by oxidative cleavage using osmium tetroxide and sodium periodate in the presence of 2,6-lutidine[39] followed by reduction with sodium borohydride. The azido-group in **22** was then selectively reduced by catalytic hydrogenation in the presence of triethylamine[40] followed by O- and N-acetylation (→**23**). O-deacetylation (→**24**), tosylation (→**25**) and substitution of the tosylate by azide afforded **26**. The azido group was then converted into a *p*-chlorobenzamide (→**27**) using the previously applied strategy of catalytic hydrogenation in the presence of NEt_3 and subsequent acylation. The conversion of the carbamate in **27** into a fluoroacetamido group via a two-step procedure (Boc-deprotection with TFA, followed by acylation) gave **28** in 69%. After saponification of the ester, the sodium salt of the final compound **3** was obtained by ion exchange chromatography in 83%.

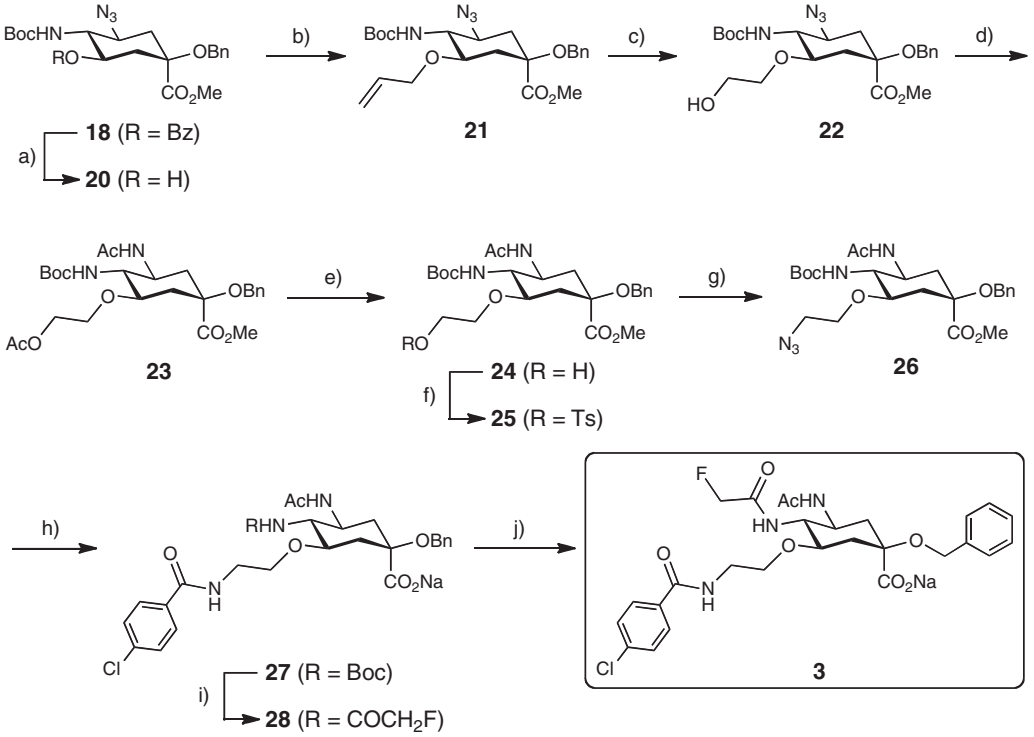
2.2. Biological evaluation

The interaction of MAG with sialoside **2** and the sialic acid mimetic **3** was analyzed by two previously reported assay formats; a fluorescent hapten binding assay[41] and a surface plasmon resonance (SPR) based biosensor (Biacore) experiment[23,31] (Table 1). For the hapten inhibition assay, a recombinant protein consisting of the three N-terminal domains of MAG and the Fc part of human IgG (MAG_{d1-3}-Fc) was produced by expression in CHO-Lec1 cells and affinity purification on protein A-agarose.[41] The relative inhibitory concentrations (rIC_{50}) of the test compounds were determined in microtiter plates coated with fetuin as binding target for MAG_{d1-3}-Fc. By complexing the Fc-part with alkaline phosphatase-labeled anti-Fc antibodies and measuring the initial velocity of fluorescein release from fluorescein diphosphate, the amount of bound MAG_{d1-3}-Fc could be determined. Four independent titrations were performed for each compound (Fig. 2). To ensure comparability with earlier results,[31,32] the affinities were measured relative to the reference compound **29**[32] (rIC_{50} of 1, Table 1, entry 1). For the K_D determination in the Biacore assay, MAG_{d1-3}-Fc was immobilized on a dextran chip modified with covalently bound protein A. A reference cell providing only protein A was used to compensate for unspecific binding to the matrix.

Whereas sialoside **2** (rIC_{50} 0.02, K_D 0.5 μM, entry 2) showed in both assays an approx. 50-fold improvement in affinity compared to the reference compound **29**, mimic **3** exhibited an IC_{50} of approx. 3 mM (Fig. 2) in the hapten inhibition assay (rIC_{50} 4300, entry 3), which corresponds to an about 200,000-fold reduced affinity for MAG compared to **2**. In the Biacore experiment compound **3** showed no binding up to a concentration of 800 μmol.

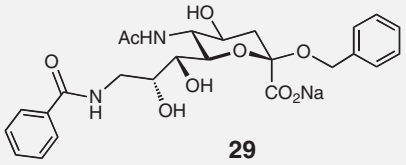
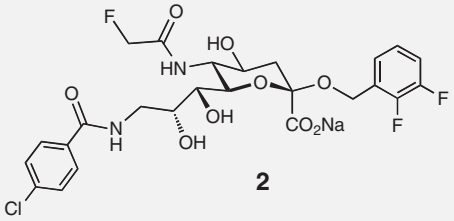
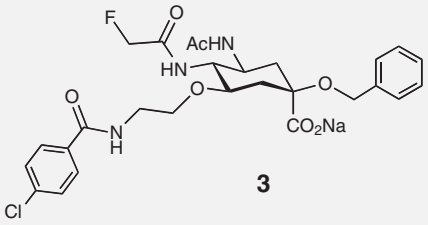
2.3. Molecular modeling

To elucidate this unexpected loss of binding affinity for mimic **3**, extensive molecular docking studies were performed. Since no crystal structure of MAG is yet available, the previously described homology model of the ligand-binding domain of MAG was used.[23,31] The homology model (mouse; UniProt P20917) is based on the three-dimensional structure of sialoadhesin[34] (mouse; Sig-



Scheme 2. Reagents and conditions: (a) NaOMe, MeOH, 94%; (b) (i) All-Br, 15-crown-5, DCM/50% NaOH, 60 °C; (ii) aq NaOH; (iii) TMS-CHN_2, toluene/MeOH, 79%; (c) (i) cat. OsO_4, $NaIO_4$, 2,6-lutidine, dioxane/H_2O; (ii) $NaBH_4$, MeOH, 0 °C; 65%; (d) (i) H_2 (1 atm), Pd/C, dioxane/NEt_3 (10:1); (ii) Ac_2O/py; 64%; (e) NaOMe, MeOH, 86%; (f) TsCl, NEt_3, cat. DMAP, DCM, 0 °C to rt, 84%; (g) NaN_3, 15-crown-5, DMF, 60 °C, 83%; (h) (i) H_2 (1 atm), cat. Pd/C, MeOH/NEt_3 (10:1); (ii) *p*-ClBzCl, cat. DMAP, NEt_3, DCM, 0 °C to rt, 66%; (i) (i) TFA/DCM (1:2), 0 °C to rt; (ii) FAcCl, cat. DMAP, NEt_3, DCM, 0 °C to rt, 69%; (j) (i) LiOH, THF/H_2O (9:1); (ii) Dowex 50X8 (Na+), 83%.

Table 1
Relative inhibitory concentrations (rIC_{50}) relative to reference compound **29**, K_D values of sialosides **2** and **29** and non-carbohydrate mimic **3**

Entry	Compound	rIC_{50}[a]	K_D (μM)
1	 **29**	1	26
2	 **2**	0.02	0.5
3	 **3**	4300	>800

[a] rIC_{50} is the concentration when 50% of the protein are inhibited, measured relative to reference compound **29**.

lec-1, PDB code 1QFP, 2.8 Å resolution) and was generated following standard protocols using the Fugue[42] and Orchestrar[43] concepts as implemented in SYBYL 7.3.[44] The homology between sialoadhesin and MAG was calculated using the Clustal W multiple sequence alignment program.[45] The primary sequences of the two proteins and the residues of their binding sites exhibit a homology of 52.5% and 50.0%, respectively. After generation, the homology model was subjected to a full refinement in aqueous solution using AMBER 7.0.[46]

The three-dimensional structures of compounds **2** and **3** were generated using MacroModel,[47] followed by a conformational search to identify low-energy conformations suited for binding (MacroModel,[47] OPLS force field,[48] aqueous solution, 5000 sampled structures). CM1 atomic partial charges were then generated using AMSOL.[49] In the main step, the ligands were manually docked to the binding site of the MAG model using the salt bridge to Arg118 as anchor point (Fig. 3). After rigorous molecular-dynamics relaxation (cf. below), each protein–ligand complex was fully minimized in aqueous solution.

A close inspection of the docking modes reveals slightly different binding modes for compounds **2** and **3** (Fig. 3). Beside the anchor point which was used for the docking of **2** and **3**, the hydrogens of the 5-NHFAc groups interact with the backbone carbonyl of Gln126, and the C(4)-OH (in **2**) or C(4)-NH (in **3**), respectively, are engaged in hydrogen bonds with the side chain carbonyl of Gln126. In addition, the 2,3-difluorobenzyl aglycone in **2** as well as the benzyloxy substituent in **3** are hosted by a hydrophobic pocket formed by Trp59, Tyr60, Tyr69, and Tyr116. However, whereas in **2** the C(9)-NH of the benzamido group establishes a H-bond with the backbone carbonyl of Thr128, in **3** the 9-benzamido carbonyl interacts with the backbone NH of Thr128, thus forming a much weaker H-bond than the parent compound **2**. Consequently, the orientations of the two side chains, the glycerol side chain in **2** and the 2-benzamido-ethyl side chain in **3**, are slightly modified, leading to a noticeably reduced interaction of the *p*-chlorobenzamide in **3** with the second hydrophobic pocket formed by Ser130 and Glu131.

Since the above discussed docking modes are the result of thermodynamic considerations, we also performed extended molecular-dynamics simulations to assess the dynamic stability of the protein–ligand complexes using Desmond[51] with an equilibrated water box (5458 and 4838 water molecules, respectively) and periodic boundary conditions. After an equilibration phase of $72 \times 10{-}12$ s, the structures were sampled for 4×10^{-9} s at 300 K and the essential interactions involved in the thermodynamic stabilization of the MAG-compound **2** complex were analyzed in time. An analogous study was done for the MAG-compound **3** complex. The results are summarized in Table 2.

The two complexes are characterized by obvious differences in respect to the hydrogen-bond network. While frequency and strength of the salt bridge to Arg118 is comparable for both **2** and **3** (entries 1 and 2), the hydrogen bonds of the 5-fluoroacetamido NH with the backbone carbonyl of Gln126 are comparable in frequency, but the interaction with **2** is slightly stronger (average distance 1.88 Å for **2** vs 1.93 Å for **3**, entry 3).

However, the major difference originates from the interaction patterns of the side chains, that is, the glycerol side chain in **2** and the 2-benzamidoethoxy side chain in **3**. In **2**, two important hydrogen bonds contribute to binding: (i) the 9-benzamido NH interacts with the backbone carbonyl of Thr128 (1.96 Å, entry 4) and (ii) the backbone NH of the same amino acid acts as donor in a H-bond with the oxygen of the C(8)-OH (1.94 Å, entry 6).

Since the benzamidoethoxy side chain in **3** is oriented in an inverted manner, a significantly weaker H-bond between the backbone NH of Thr128 and the 9-benzamido carbonyl (2.10 Å, frequency 0.1, entry 5) is formed. In addition, the hydroxy group at C(8) is absent, therefore the second H-bond is not present at all. As a consequence, the orientation of the side chain alters leading to a markedly reduced hydrophobic contact of the *p*-chlorobenzamide with Ser130 and Glu131.

Finally, the hydrogen bond of the C(4)-OH in **2** with the side chain carbonyl of Gln126, which was identified in the static docking studies, turned out to be irrelevant in the dynamic considerations. Although the hydrogen bond of the 4-acetamido NH in **3**

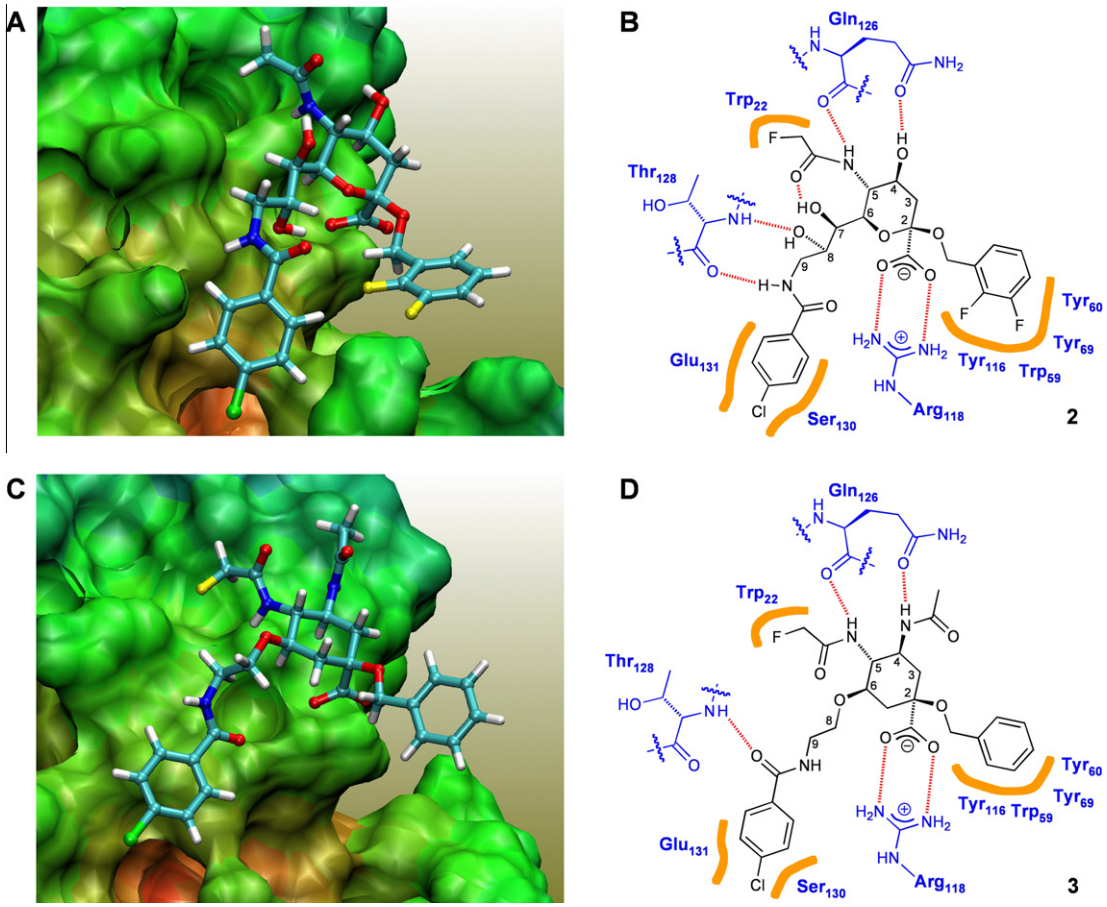


Figure 3. (A and C) The homology model of MAG complexed with **2** (A) and **3** (C). The images have been generated using VMD.[50] The ligands are depicted colored by atom (C: cyan, H: white, O: red, N: blue, Cl: green, F: yellow), the protein is represented by its van der Waals surface colored according to the depth. (B and D) Binding interactions of **2** (B) and **3** (D); hydrogen bonds are indicated as red dashed lines, hydrophobic interactions are shown as orange bars. For better comparability, the carbon atoms in **3** are numbered in analogy to compound **2**.

with the side chain carbonyl of Gln126 substantially contributes to the binding to MAG (1.93 Å, entry 7), this additional interaction unexpectedly does not compensate the experimentally observed loss of affinity caused by the reduced hydrophobic contact of the benzamido group in **3** compared to **2**.

3. Conclusion

The non-carbohydrate mimic **3** of the high-affinity MAG antagonist **2** was synthesized with the goal to improve its pharmacokinetic properties. Although the mimic **3** can establish all but one of the essential hydrogen bond interactions present in **2**, a 200,000-fold drop in affinity was observed. The docking modes of **2** and **3** to a homology model of MAG revealed a different orientation of the side chains in the 6-position. Obviously, the C(8)-OH and the C(9)-NH in sialoside **2** represent key polar groups,[52] that is, the recognition of both of these hydrogen bond donors is required for binding. Consequently, in the absence of the stabilizing H-bond formed between the C(8)-OH in **2** and the backbone NH of Thr128, the benzamidoethoxy side chain adopts an inverted orientation, leading to a less favorable interaction of its benzamido group with the hydrophobic pocket formed by Ser130 and Glu131 and therefore to the experimentally observed dramatic loss in affinity.

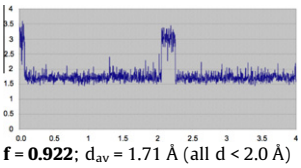
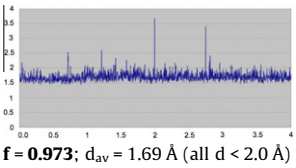
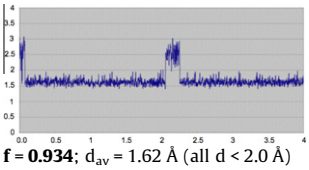
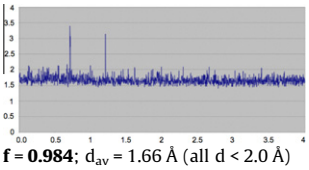
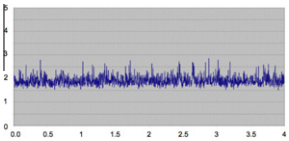
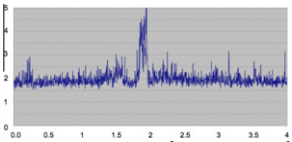
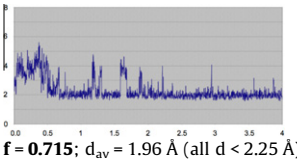
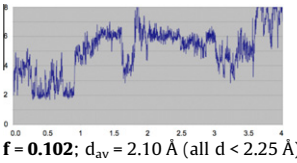
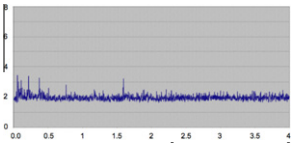
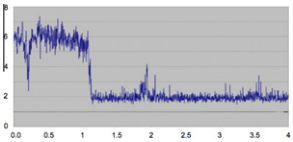
In summary, while docking studies based on a thermodynamic inspection of the binding event do only insufficiently depict the MAG-mimetic **3** interaction, dynamic considerations based on molecular-dynamics simulations gave a more detailed picture and allowed to interpret the experimental findings.

4. Experimental part

4.1. Chemistry

NMR spectra were recorded on a Bruker Avance DMX-500 (500 MHz) spectrometer. Assignment of ^{1}H and ^{13}C NMR spectra was achieved using 2D methods (COSY, HSQC). Chemical shifts are expressed in ppm using residual $CHCl_3$ and HDO as references. Optical rotations were measured using a Perkin–Elmer Polarimeter 341. Electron spray ionization mass spectra (ESI-MS) were obtained on a Waters micromass ZQ. The HRMS analyses were carried out using a Bruker QTOF. Reactions were monitored by TLC using glass plates coated with silica gel 60 F_{254} (Merck) and visualized by using UV light and/or by charring with a molybdate solution (a 0.02 M solution of ammonium cerium sulfate dihydrate and ammonium molybdate tetrahydrate in aqueous 10% H_2SO_4). Column chromatography was performed on silica gel (Fluka, 40–60 mesh). MPLC separations were carried out on a CombiFlash Companion from Teledyne Isco equipped with RediSep normal-phase flash columns. Tetrahydrofurane (THF) and dioxane were freshly distilled under argon over sodium and benzophenone. Methanol (MeOH) was dried by refluxing with sodium methoxide and distilled immediately before use. Pyridine was freshly distilled under argon over CaH_2. Acetone, dichloromethane (DCM), *N*,*N*-

Table 2
Time-resolved H-bond distances between key amino-acid residues of MAG and compounds **2** and **3**

Entry	Interaction	Compound **2**	Compound **3**
1	$-COO^{\ominus}\cdots H_2N^{\oplus}$-Arg118 (pair #1)	 **f = 0.922**; d_{av} = 1.71 Å (all d < 2.0 Å)	 **f = 0.973**; d_{av} = 1.69 Å (all d < 2.0 Å)
2	$-COO^{\ominus}\cdots H_2N^{\oplus}$-Arg118 (pair #2)	 **f = 0.934**; d_{av} = 1.62 Å (all d < 2.0 Å)	 **f = 0.984**; d_{av} = 1.66 Å (all d < 2.0 Å)
3	**5-NH–O=C–Gln126$_{MC}$**	 **f = 0.945**; d_{av} = 1.88 Å (all d < 2.25 Å)	 **f = 0.948**; d_{av} = 1.93 Å (all d < 2.25 Å)
4	**9-NH–O=C–Thr128**	 **f = 0.715**; d_{av} = 1.96 Å (all d < 2.25 Å)	absent
5	***p*-ClPh–CO–HN–Thr128**	absent	 **f = 0.102**; d_{av} = 2.10 Å (all d < 2.25 Å)
6	8-O(H)···HN-Thr128	 **f = 0.942**; d_{av} = 1.94 Å (all d < 2.25 Å)	absent
7	**4-NH–O=C–Gln126$_{SC}$**	absent	 **f = 0.693**; d_{av} = 1.93 Å (all d < 2.25 Å)

The horizontal axis represents the simulation time, the vertical axis the H···acceptor separation. *f*: frequency of observance of the H-bond throughout the molecular-dynamics simulation; d_{av}: average H···acceptor distance; MC: main chain; SC: side chain.

dimethylformamide (DMF), and toluene were dried by filtration over Al_2O_3 (Fluka, type 5016 A basic).

4.1.1. 3,4-*O*-Isopropylidene-1,5-quinic lactone (5)[37]

To a solution of (−)-quinic acid (**4**, 15.0 g, 78.1 mmol) and DMP (33.5 mL, 273 mmol) in acetone (75 mL) was added *p*-toluene sulfonic acid monohydrate (149 mg, 0.78 mmol). The reaction mixture was stirred at 70 °C for 3 h. After cooling to rt, NEt_3 (3 mL) was added with stirring and the solvent was evaporated. The residue was purified by chromatography on silica gel (petroleum ether/EtOAc 3:1 to 1:1, +0.5% NEt_3) to yield **5** (14.7 g, 87%) as a colorless solid. The analytical data correspond to reported values.[37]

4.1.2. 1-*O*-Benzyl-3,4-*O*-isopropylidene-1,5-quinic lactone (6)

NaH (3.53 g, 60% suspension in oil, 88.3 mmol) was washed with *n*-hexane (2 × 5 mL) under argon and suspended in DMF (10 mL) at 0 °C. Then a solution of **5** (9.46 g, 44.2 mmol) in DMF (50 mL) was added dropwise during 30 min with stirring. The reaction mixture was warmed to rt, stirred for 19 h and then quenched by slowly adding saturated aqueous NH_4Cl (20 mL) at 0 °C. The mixture was extracted with DCM (3 × 40 mL) and the combined organic layers were dried with Na_2SO_4. After filtration and evaporation of the solvents, the residue was purified by chromatography on silica (petroleum ether/EtOAc 10:1 to 5:1, +0.5% NEt_3) to yield **6** (12.6 g, 94%) as a colorless solid.

^{1}H NMR (500 MHz, $CDCl_3$): δ 1.34, 1.52 (2 s, 6H, C(CH_3)$_2$), 2.26 (dd, *J* = 2.7, 15.0 Hz, 1H, H-2a), 2.46–2.56 (m, 3H, H-2b, H-6), 4.32 (dt, *J* = 1.2, 6.4 Hz, H-4), 4.55 (dt, *J* = 2.7, 7.0 Hz, H-3), 4.58, 4.67 (A, B of AB, *J* = 10.8 Hz, 2H, CH_2Ph), 4.70 (dd, *J* = 2.4, 6.0 Hz, 1H, H-5), 7.29–7.40 (m, 5H, C_6H_5); ^{13}C NMR (125 MHz, $CDCl_3$): δ 24.3, 27.0 (C(CH_3)$_2$), 30.7 (C-6), 36.1 (C-2), 67.0 (CH_2Ph), 71.4 (C-3), 72.4 (C-4), 75.0 (C-5), 76.9 (C-1), 109.6 (C(CH_3)$_2$), 127.8, 127.9, 128.4, 137.4 (6C, C_6H_5), 175.8 (CO); ESI-MS Calcd for $C_{17}H_{20}NaO_5$ $[M+Na]^+$: 327.12. Found: 327.08.

4.1.3. Methyl (1*R*,3*R*,4*S*,5*R*)-1-benzyloxy-3,4,5-trihydroxy-4,5-*O*-isopropylidene-cyclohexanecarboxylate (7)

To a suspension of **6** (9.84 g, 32.3 mmol) in MeOH (170 mL) was added a freshly prepared solution of sodium (820 mg, 35.6 mmol) in MeOH (50 mL) at 0 °C under argon. The reaction mixture was stirred for 3 h at rt, then the solution was neutralized by adding Dowex 50X8 ion-exchange resin. After filtration and evaporation of the solvents, the residue was purified by chromatography on silica (petroleum ether/EtOAc 2:1 to 1:8, +0.5% NEt_3) to yield **7** (7.96 g, 73%) as a colorless solid.

^{1}H NMR (500 MHz, $CDCl_3$): δ 1.37, 1.48 (2 s, 6H, C(CH_3)$_2$), 1.81 (dd, J = 10.9, 13.6 Hz, 1H, H-6a), 2.25–2.31 (m, 2H, H-2a, H-6b), 2.54 (m, 1H, H-2b), 3.78 (m, 3H, OMe), 3.94 (t, J = 6.4 Hz, H-4), 4.16 (ddd, J = 4.1, 7.0, 10.9 Hz, 1H, H-5), 4.34 (A of AB, J = 10.2 Hz, 1H, CH_2Ph), 4.46 (dt, J = 3.6, 5.3 Hz, H-3), 4.60 (B of AB, J = 10.2 Hz, 1H, CH_2Ph), 7.27–7.39 (m, 5H, C_6H_5); ^{13}C NMR (125 MHz, $CDCl_3$): δ 25.9, 28.2 (C(CH_3)$_2$), 30.9 (C-2), 37.7 (C-6), 52.5 (OMe), 67.1 (CH$_2$Ph), 68.1 (C-5), 72.9 (C-3), 79.2 (C-1), 80.1 (C-4), 109.1 (C(CH$_3$)$_2$), 127.9, 128.2, 128.3, 138.0 (6C, C_6H_5), 174.1 (CO); ESI-MS Calcd for $C_{18}H_{25}O_6$ $[M+H]^+$: 337.17. Found: 337.14.

4.1.4. Methyl (1*S*,3*R*,4*R*,5*R*)-1-benzyloxy-3,4-dihydroxy-3,4-*O*-isopropylidene-5-(*p*-tosyloxy)-cyclohexanecarboxylate (8)

To a solution of **7** (5.55 g, 16.5 mmol) and DMAP (190 mg) in pyridine (40 mL) was added *p*-tosyl chloride (6.29 g, 33.0 mmol) under argon. The solution was stirred for 19 h, then the pyridine was evaporated and water (50 mL) was added. The aqueous phase was extracted with DCM (3 × 50 mL), then the combined organic layers were washed with brine (20 mL) and dried with Na_2SO_4. After evaporation of the solvent, the residue was purified by chromatography on silica (petroleum ether/EtOAc 3:1 to 1:1, +0.5% NEt_3) to give **8** (7.96 g, 98%) as a yellowish oil.

^{1}H NMR (500 MHz, $CDCl_3$): δ 0.93, 1.23 (2 s, 6H, C(CH_3)$_2$), 1.91 (t, J = 12.7 Hz, 1H, H-6a), 2.16 (dd, J = 5.0, 16.1 Hz, 1H, H-2a), 2.39 (s, 3H, PhCH_3), 2.59 (d, J = 16.1 Hz, 1H, H-2b), 2.68 (dd, J = 3.1, 13.6 Hz, 1H, H-6b), 3.76 (m, 3H, OMe), 4.01 (t, J = 6.5 Hz, H-4), 4.40–4.43 (m, 2H, H-3, CH_2Ph), 4.55 (B of AB, J = 10.5 Hz, 1H, CH_2Ph), 4.64 (m, 1H, H-5), 7.19 (AA′ of AA′BB′, J = 8.0 Hz, 2H, C_6H_4), 7.31–7.37 (m, 5H, C_6H_5), 7.71 (BB′ of AA′BB′, J = 8.0 Hz, 2H, C_6H_4); ^{13}C NMR (125 MHz, $CDCl_3$): δ 21.5 (PhCH_3), 25.8, 27.3 (C(CH_3)$_2$), 31.1 (C-2), 35.5 (C-6), 52.5 (OMe), 66.7 (CH$_2$Ph), 73.2 (C-3), 76.6 (C-4), 79.1, 79.2 (C-1, C-5), 109.2 (C(CH$_3$)$_2$), 127.4, 127.5, 128.1, 128.4, 129.6, 132.9, 137.9 (12C, C_6H_4, C_6H_5), 172.6 (CO); ESI-MS Calcd for $C_{25}H_{30}NaO_8S$ $[M+Na]^+$: 513.16. Found: 513.18.

4.1.5. Methyl (1*S*,3*R*,4*R*,5*R*)-1-benzyloxy-3,4-dihydroxy-5-(*p*-tosyloxy)-cyclohexanecarboxylate (9)

A suspension of **8** (7.96 g, 16.2 mmol) in 80% aqueous acetic acid (30 mL) was stirred for 2.5 h at 60 °C. After cooling to rt, the solvents were removed by co-evaporation with toluene (3 × 50 mL). The residue was dried in high vacuum to give **9** (7.13 g, 98%), which was used without further purification.

^{1}H NMR (500 MHz, $CDCl_3$): δ 1.94 (dd, J = 3.1, 15.1 Hz, 1H, H-2a), 2.16 (dd, J = 11.9, 14.2 Hz, 1H, H-6a), 2.40 (m, 1H, H-2b), 2.43 (s, 3H, PhCH_3), 2.89 (dt, J = 3.4, 14.3 Hz, 1H, H-6b), 3.56 (dd, J = 3.5, 9.3 Hz, H-4), 3.78 (m, 3H, OMe), 4.05 (m, 1H, H-3), 4.50, 4.52 (A, B of AB, J = 10.2 Hz, 2H, CH_2Ph), 4.59 (m, 1H, H-5), 7.31 (AA′ of AA′BB′, J = 8.1 Hz, 2H, C_6H_4), 7.33–7.39 (m, 5H, C_6H_5), 7.82 (BB′ of AA′BB′, J = 8.1 Hz, 2H, C_6H_4); ^{13}C NMR (125 MHz, $CDCl_3$): δ 21.7 (PhCH_3), 34.5 (C-6), 37.3 (C-2), 52.7 (OMe), 67.6 (CH$_2$Ph), 70.0 (C-3), 72.9 (C-4), 78.5 (C-5), 81.5 (C-1), 128.1, 128.1, 128.3, 128.7, 129.8, 133.2, 136.5, 145.0 (12C, C_6H_4, C_6H_5), 171.1 (CO); ESI-MS Calcd for $C_{22}H_{26}NaO_8S$ $[M+Na]^+$: 473.12. Found: 473.15.

4.1.6. Methyl (1*R*,3*S*,4*S*,5*R*)-1-benzyloxy-3,4-epoxy-5-hydroxy-cyclohexane-1-carboxylate (10)

To a solution of **9** (7.13 g, 15.8 mmol) in THF (50 mL) was added DBU (2.39 mL, 15.8 mmol) dropwise during 10 min at 0 °C under argon. The mixture was stirred at 0 °C for 1 h, then warmed to rt and stirred for another 17 h. The solvent was removed, and the residue was purified by MPLC on silica (petroleum ether/EtOAc) to yield **10** (4.23 g, 96%) as a colorless solid.

^{1}H NMR (500 MHz, $CDCl_3$): δ 1.86 (dd, J = 9.5, 12.6 Hz, 1H, H-6a), 2.13 (d, J = 15.1 Hz, 1H, H-2a), 2.23 (m, 1H, H-6b), 2.68 (ddd, J = 2.0, 4.8, 15.1 Hz, 1H, H-2b), 3.35 (m, 1H, H-4), 3.44 (t, J = 4.4 Hz, 1H, H-3), 3.77 (s, 3H, OMe), 4.03 (m, 1H, H-5), 4.31, 4.49 (A, B of AB, J = 10.6 Hz, 2H, CH_2Ph), 7.27–7.38 (m, 5H, C_6H_5); ^{13}C NMR (125 MHz, $CDCl_3$): δ 30.3 (C-2), 36.4 (C-6), 52.5 (C-3), 52.8 (OMe), 54.8 (C-4), 65.9 (C-5), 66.9 (CH$_2$Ph), 79.5 (C-1), 127.7, 127.8, 128.4, 137.4 (6C, C_6H_5), 173.2 (CO); ESI-MS Calcd for $C_{15}H_{18}NaO_5$ $[M+Na]^+$: 301.11. Found: 301.14.

4.1.7. Methyl (1*R*,3*S*,4*S*,5*R*)-5-benzoyloxy-1-benzyloxy-3,4-epoxy-cyclohexane-1-carboxylate (11)

To a solution of **10** (2.10 g, 7.55 mmol) in pyridine (20 mL) were subsequently added benzoyl chloride (1.78 mL, 15.1 mmol) and DMAP (120 mg) at 0 °C under argon. The mixture was stirred for 4 h at rt and then concentrated in vacuo. The residue was co-evaporated with toluene (3 × 10 mL), dissolved in DCM (20 mL), and washed with 1 N HCl (10 mL) and saturated aqueous $NaHCO_3$ (10 mL). The organic phase was dried (Na_2SO_4), filtered and concentrated in vacuo. The residue was purified by MPLC on silica (petroleum ether/EtOAc) to afford **11** (2.79 g, 97%) as a colorless solid.

$[\alpha]_D$ −33.4 (*c* 0.50, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ 2.06–2.15 (m, 2H, H-2a, H-6a), 2.42 (dd, J = 4.3, 11.9 Hz, 1H, H-6b), 2.85 (dd, J = 3.2, 14.9 Hz, 1H, H-2b), 3.43 (t, J = 4.5 Hz, 1H, H-3), 3.47 (m, 1H, H-4), 3.84 (s, 3H, OMe), 4.35, 4.50 (A, B of AB, J = 10.9 Hz, 2H, CH_2Ph), 5.39 (m, 1H, H-5), 7.28–7.35, 7.43–7.46, 7.56–7.59, 8.07–8.09 (m, 10H, 2 C_6H_5); ^{13}C NMR (125 MHz, $CDCl_3$): δ 31.2 (C-2), 32.2 (C-6), 51.8 (C-3), 52.7, 52.8 (C-4, OMe), 66.8 (CH$_2$Ph), 69.2 (C-5), 79.3 (C-1), 127.6, 127.8, 128.4, 128.4, 129.7, 129.8, 133.3, 137.5 (12C, 2C_6H_5), 166.0 (COPh), 173.2 (CO_2Me); ESI-MS Calcd for $C_{22}H_{22}NaO_6$ $[M+Na]^+$: 405.13. Found: 405.08.

4.1.8. Methyl (1*R*,3*R*,4*S*,5*R*)-5-azido-3-benzoyloxy-1-benzyloxy-4-hydroxy-cyclohexane-1-carboxylate (12)

To a solution of **11** (2.79 g, 7.30 mmol) in MeOH/water (8:1, 54 mL) were added sodium azide (2.47 g, 37.9 mmol) and ammonium chloride (859 mg, 16.1 mmol), and the mixture was stirred at 70 °C for 18 h under argon. The reaction mixture was diluted with saturated aqueous $NaHCO_3$ (10 mL) and water (20 mL), and the solution was carefully evaporated to remove methanol. The resulting aqueous phase containing an oily residue was extracted with ethyl acetate (3 × 50 mL). The combined organic extracts were dried (Na_2SO_4), filtered, and concentrated in vacuo. The residue was purified by MPLC on silica (1% gradient of MeOH in DCM) to yield **12** (2.45 g, 78%) as a colorless oil.

$[\alpha]_D$ −53.6 (*c* 0.45, $CHCl_3$); IR (film) 3479 (m b, OH), 2107 (vs, N_3), 1722 (vs, CO) cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.87 (dd, J = 11.7, 13.8 Hz, 1H, H-6a), 2.23 (dd, J = 3.5, 15.9 Hz, 1H, H-2a), 2.44 (d, J = 5.1 Hz, 1H, OH), 2.59 (m, 1H, H-6b), 2.67 (dt, J = 3.2, 15.9 Hz, 1H, H-2b), 3.76–3.80 (m, 4H, H-4, OMe), 4.10 (m, 1H, H-5), 4.31, 4.38 (A, B of AB, J = 10.5 Hz, 2H, CH_2Ph), 5.65 (q, J = 3.5 Hz, 1H, H-3), 7.20–7.28, 7.48–7.53, 7.90–7.93 (m, 10H, 2C_6H_5); ^{13}C NMR (125 MHz, $CDCl_3$): δ 32.3 (C-2), 36.8 (C-6), 52.6 (OMe), 58.4 (C-5), 67.4 (CH$_2$Ph), 70.8 (C-3), 73.7 (C-4), 79.4 (C-1), 127.7, 127.8, 128.3, 128.4, 129.4, 129.9, 133.3, 137.3 (12C, 2C_6H_5), 166.2 (COPh), 172.7 (CO_2Me); ESI-MS Calcd for $C_{22}H_{23}N_3NaO_6$ $[M+Na]^+$: 448.15. Found: 448.16.

4.1.9. Methyl (1*R*,3*R*,4*S*,5*R*)-5-azido-3-benzoyloxy-1-benzyloxy-4-(methylsulfonyloxy)-cyclohexane-1-carboxylate-1,4-lactam (13)

To a solution of **12** (2.43 g, 5.70 mmol) in DCM (25 mL) at 0 °C under argon was added NEt_3 (3.18 mL, 22.8 mmol) followed by the addition of methanesulfonyl chloride (887 μL, 11.4 mmol). The mixture was stirred at 0 °C for 1 h and warmed to rt with stirring for 3 h. The reaction was quenched with MeOH (2 mL) and concentrated in vacuo. Purification of the residue by MPLC on silica (petrol ether/EtOAc) yielded **13** (2.69 g, 94%) as a yellowish oil.

$[\alpha]_D$ −31.0 (*c* 0.50, $CHCl_3$); IR (film) 2111 (vs, N_3), 1732 (vs, CO) cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 2.01 (m, 1H, H-6a), 2.35 (d, *J* = 15.9 Hz, 1H, H-2a), 2.57–2.73 (m, 2H, H-2b, H-6b), 3.08 (s, 3H, SMe), 3.79 (s, 3H, OMe), 4.27–4.34 (m, 2H, H-5, C*H*$_2$Ph-H$_A$), 4.38 (B of AB, *J* = 10.5 Hz, 1H, C*H*$_2$Ph-H$_B$), 4.70 (dd, *J* = 3.4, 9.6 Hz, 1H, H-4), 5.84 (m, 1H, H-3), 7.18–7.32, 7.53–7.55, 7.94–7.95 (m, 10H, 2C_6H_5); ^{13}C NMR (125 MHz, $CDCl_3$): δ 31.8 (C-2), 37.4 (C-6), 38.7 (SMe), 52.7 (OMe), 55.9 (C-5), 67.4 (*C*H_2Ph), 68.5 (C-3), 79.8 (2C, C-1, C-4), 127.8, 127.9, 128.3, 128.5, 129.4, 129.9, 133.4, 137.0 (12C, 2C_6H_5), 166.3 (*C*OPh), 172.5 (*C*O$_2$Me); ESI-MS Calcd for $C_{23}H_{25}N_3NaO_8S$ $[M+Na]^+$: 526.13. Found: 526.09.

4.1.10. Methyl (1*R*,3*R*,4*R*,5*R*)-4,5-aziridino-3-benzoyloxy-1-benzyloxy-cyclohexane-1-carboxylate (14)

To a solution of **13** (2.69 g, 5.34 mmol) in THF (30 mL) at 0 °C was added PPh_3 (1.76 g, 6.68 mmol), initially adding a third of the amount while cooling and then after removing the ice bath adding the remaining amount over a period of 15 min. The reaction mixture was stirred at rt for 4 h, then NEt_3 (1.12 mL, 8.02 mmol) and water (2 mL) were added, and the mixture was stirred at rt for 16 h. The reaction mixture was concentrated to remove THF, and the residue was partitioned between DCM (50 mL) and brine (20 mL). The aqueous phase was extracted with DCM (3 × 20 mL), and the combined organic extracts were dried (Na_2SO_4), filtered, and evaporated. The residue was purified by MPLC on silica (petrol ether/EtOAc, +1% NEt_3) to give 2.20 g of a 3:2-mixture of **14** and triphenylphosphine oxide, which was directly used in the next step.

^1H NMR (500 MHz, $CDCl_3$): δ 2.13 (dd, *J* = 3.7, 14.8 Hz, 1H, H-2a), 2.37–2.44 (m, 3H, H-2b, H-6), 2.45–2.50 (m, 2H, H-4, H-5), 3.74 (s, 3H, OMe), 4.38, 4.53 (A, B of AB, *J* = 11.1 Hz, 1H, C*H*$_2$Ph), 5.60 (t, *J* = 4.9 Hz, 1H, H-3), 7.25–7.33, 7.53–7.56, 7.96–7.97 (m, 10H, 2C_6H_5); ^{13}C NMR (125 MHz, $CDCl_3$): δ 28.1 (C-6), 30.8 (C-2), 31.6, 32.5 (C-4, C-5), 52.2 (OMe), 66.5 (*C*H_2Ph), 68.1 (C-3), 76.9 (C-1), 127.5, 128.2, 128.3, 128.5, 129.7, 132.9, 138.2 (12C, 2C_6H_5), 166.1 (*C*OPh), 173.1 (*C*O$_2$Me); ESI-MS Calcd for $C_{22}H_{24}NO_5$ $[M+H]^+$: 382.17. Found: 382.10.

4.1.11. (1*R*,3*R*,4*R*,5*S*)-4-Amino-5-azido-1-benzyloxy-3-benzoyloxy-cyclohexane-1-carboxylate-1,4-lactam (16)

To a solution of **14** (105 mg, 0.275 mmol) in DMF (3 mL) was added NaN_3 (89.3 mg, 1.37 mmol) and NH_4Cl (29.4 mg, 0.55 mmol), and the mixture was heated at 65 °C under argon for 23 h. The reaction mixture was cooled to rt, diluted with ethyl acetate (10 mL), and filtered. The filtrate was washed with brine (5 mL) and the aqueous phase was extracted with EtOAc (3 × 10 mL). The combined organic phases were dried (Na_2SO_4), filtered, and concentrated in vacuo. The residue was purified by chromatography on silica (petrol ether/EtOAc 2:1 to 1:1) to yield **16** (53.5 mg, 50%) as colorless foam.

$[\alpha]_D$ −29.6 (*c* 1.24, $CHCl_3$); IR (film) 2099 (vs, N_3), 1719 (vs, CO) cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 1.99 (dd, *J* = 5.5, 13.4 Hz, 1H, H-6a), 2.04 (dd, *J* = 3.8, 13.7 Hz, 1H, H-2a), 2.57 (ddd, *J* = 3.5, 10.8, 13.3 Hz, 1H, H-6b), 2.81 (ddd, *J* = 3.5, 10.5, 13.7 Hz, 1H, H-2b), 4.04 (m, 1H, H-4), 4.09 (m, 1H, H-5), 4.78, 4.84 (A, B of AB, *J* = 11.0 Hz, 2H, C*H*$_2$Ph), 5.35 (m, 1H, H-3), 7.28–7.36, 7.55–7.61, 8.09–8.10 (m, 10H, 2C_6H_5); ^{13}C NMR (125 MHz, $CDCl_3$): δ 34.8 (C-6), 36.0 (C-2), 49.8 (C-4), 57.9 (C-5), 67.1 (*C*H_2Ph), 70.1 (C-3), 74.1 (C-1), 127.7, 128.4, 128.5, 129.3, 129.8, 133.4, 138.2 (12C, 2C_6H_5), 166.1, 175.2 (2 CO); ESI-MS Calcd for $C_{21}H_{20}N_4NaO_4$ $[M+Na]^+$: 415.14. Found: 415.16.

4.1.12. Methyl (1*R*,3*R*,4*R*,5*R*)-4,5-aziridino-3-benzoyloxy-1-benzyloxy-*N*-(*tert*-butoxycarbonyl)-cyclohexane-1-carboxylate (17)

Crude aziridine **14** (2.10 g, 3:2 mixture with PPh_3O) was dissolved in DCM (50 mL) and cooled to 0 °C under argon. Then NEt_3 (1.53 mL, 11.0 mmol), Boc_2O (1.80 g, 8.25 mmol), and DMAP (10 mg) were subsequently added and the solution was stirred at rt for 5 h. The solvent was evaporated and the residue purified by MPLC on silica (1% gradient of EtOAc in petrol ether) to yield **17** (1.66 g, 96%) as a colorless oil.

^1H NMR (500 MHz, $CDCl_3$): δ 1.47 (s, 9H, C(C*H*$_3$)$_3$), 2.26 (d, *J* = 15.3 Hz, 1H, H-2a), 2.35 (dd, *J* = 5.4, 15.4 Hz, 1H, H-2b), 2.40 (d, *J* = 15.3 Hz, 1H, H-6a), 2.52 (ddd, *J* = 1.6, 5.8, 15.4 Hz, 1H, H-6b), 2.83 (dd, *J* = 4.9, 5.7 Hz, 1H, H-5), 2.87 (m, 1H, H-4), 3.74 (s, 3H, OMe), 4.31, 4.50 (A, B of AB, *J* = 11.0 Hz, 2H, PhC*H*$_2$), 5.68 (m, 1H, H-3), 7.27–7.32, 7.50–7.53, 7.93–7.95 (m, 10H, 2C_6H_5); ^{13}C NMR (125 MHz, $CDCl_3$): δ 27.8 (C(*C*H$_3$)$_3$), 30.3 (C-2), 31.5 (C-6), 34.8 (C-5), 38.1 (C-4), 52.3 (OMe), 66.6 (C-3), 66.7 (*C*H_2Ph), 76.5 (C-1), 81.7 (*C*(CH$_3$)$_3$), 127.4, 127.5, 128.2, 128.3, 129.8, 129.8, 133.0, 137.9 (12C, 2C_6H_5), 161.6 (N*C*O), 165.8, 172.9 (2CO); ESI-MS Calcd for $C_{27}H_{31}NNaO_7$ $[M+Na]^+$: 504.20. Found: 504.19.

4.1.13. Methyl (1*R*,3*R*,4*R*,5*S*)-5-azido-1-benzyloxy-3-benzoyloxy-4-(*tert*-butoxycarbonylamino)-cyclohexane-1-carboxylate (18) and methyl (1*R*,3*R*,4*S*,5*R*)-4-azido-1-benzyloxy-3-benzoyloxy-5-(*tert*-butoxycarbonylamino)-cyclohexane-1-carboxylate (19)

To a solution of **17** (1.38 g, 2.87 mmol) in DMF (25 mL) was added sodium azide (1.86 g, 28.7 mmol) and ammonium chloride (1.23 g, 22.9 mmol), and the mixture was heated at 70 °C under argon for 18 h. The reaction mixture was cooled to rt, diluted with ethyl acetate (25 mL), and filtered. The filtrate was washed with brine (20 mL) and the aqueous phase was extracted with EtOAc (3 × 25 mL). The combined organic phases were dried (Na_2SO_4), filtered, and concentrated in vacuo. The residue was purified by MPLC on silica (petrol ether/EtOAc) to yield **18** (956 mg, 64%) and **19** (398 mg, 27%) as colorless foams.

Data for ***18***: $[\alpha]_D$ −49.0 (*c* 1.05, $CHCl_3$); IR (KBr) 3385 (m, NH), 2978 (m), 2100 (vs, N_3), 1722 (vs, CO), 1516 (m), 1454 (m), 1391 (m), 1301 (s), 1278 (vs), 1205 (m), 1157 (s), 1112 (s) cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 1.27 (s, 9H, C(C*H*$_3$)$_3$), 1.75 (t, *J* = 12.7 Hz, 1H, H-6a), 1.92 (t, *J* = 12.2 Hz, 1H, H-2a), 2.79 (d, *J* = 12.7 Hz, 1H, H-6b), 2.87 (d, *J* = 11.7 Hz, 1H, H-2b), 3.56 (m, 1H, H-5), 3.85 (s, 3H, OMe), 3.91 (q, *J* = 10.4 Hz, 1H, H-4), 4.42, 4.47 (A, B of AB, *J* = 10.8 Hz, 2H, PhC*H*$_2$), 4.51 (d, *J* = 9.9 Hz, 1H, NH), 5.09 (m, 1H, H-3), 7.27–7.36, 7.41–7.44, 7.55–7.58, 8.03–8.04 (m, 10H, 2C_6H_5); ^{13}C NMR (125 MHz, $CDCl_3$): δ 28.1 (C(*C*H$_3$)$_3$), 38.1 (C-2), 38.2 (C-6), 52.9 (OMe), 58.0 (C-4), 58.9 (C-5), 67.1 (*C*H_2Ph), 70.1 (C-3), 77.9 (C-1), 80.0 (*C*(CH$_3$)$_3$), 127.6, 127.9, 128.3, 128.4, 129.5, 129.9, 133.3, 137.2 (12C, 2C_6H_5), 155.5 (N*C*O), 166.2, 172.4 (2CO); ESI-MS Calcd for $C_{27}H_{32}N_4NaO_7$ $[M+Na]^+$: 547.22. Found: 547.29.

Data for ***19***: ^1H NMR (500 MHz, $CDCl_3$): δ 1.47 (s, 9H, C(C*H*$_3$)$_3$), 2.17–2.26 (m, 2H, H-2a, H-6a), 2.56 (d, *J* = 13.7 Hz, 1H, H-6b), 2.63 (d, *J* = 14.4 Hz, 1H, H-2b), 3.77 (s, 3H, OMe), 3.82 (m, 1H, H-4), 4.22 (m, 1H, H-5), 4.27, 4.39 (A, B of AB, *J* = 10.4 Hz, 2H, PhC*H*$_2$), 5.01 (br s, 1H, NH), 5.63 (m, 1H, H-3), 7.17–7.23, 7.27–7.30, 7.49–7.52, 7.94–7.95 (m, 10H, 2C_6H_5); ^{13}C NMR (125 MHz, $CDCl_3$): δ 28.4 (C(*C*H$_3$)$_3$), 33.1 (C-2), 36.4 (C-6), 46.8 (C-5), 52.7 (OMe), 62.8 (C-4), 67.2 (*C*H_2Ph), 69.9 (C-3), 79.5 (C-1), 80.1 (*C*(CH$_3$)$_3$), 127.6, 127.8,

128.1, 128.3, 129.3, 129.9, 133.2, 137.3 (12C, $2C_6H_5$), 155.1 (NCO), 165.8, 173.2 (2CO); ESI-MS Calcd for $C_{27}H_{32}N_4NaO_7$ $[M+Na]^+$: 547.22. Found: 547.28.

4.1.14. Methyl (1*R*,3*R*,4*R*,5*S*)-5-azido-1-benzyloxy-4-(*tert*-butoxycarbonylamino)-3-hydroxy-cyclohexane-1-carboxylate (20)

A solution of **18** (1.01 g, 1.93 mmol) in MeOH (15 mL) was treated with 1 M methanolic NaOMe (1.5 mL) under argon at rt for 3 h. The reaction mixture was neutralized with acetic acid and concentrated. The residue was purified by MPLC on silica (petrol ether/EtOAc) to give **20** (765 mg, 94%) as a colorless foam.

$[\alpha]_D$ +5.4 (*c* 1.01, $CHCl_3$); IR (KBr) 3268 (s, OH), 2982 (m), 2101 (vs, N_3), 1736 (vs, CO), 1675 (vs, CO), 1551 (vs), 1455 (m), 1393 (w), 1309 (vs), 1276 (m), 1199 (s), 1175 (s), 1126 (s) cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 1.46 (s, 9H, C(CH_3)$_3$), 1.71 (t, J = 12.5 Hz, 1H, H-6a), 1.72 (t, J = 12.3 Hz, 1H, H-2a), 2.75 (d, J = 12.5 Hz, 1H, H-6b), 2.76 (d, J = 12.3 Hz, 1H, H-2b), 3.34 (q, J = 9.2 Hz, 1H, H-4), 3.44 (m, OH), 3.50 (m, 1H, H-5), 3.62 (m, 1H, H-3), 3.77 (s, 3H, OMe), 4.41, 4.46 (A, B of AB, J = 10.8 Hz, 2H, PhCH_2), 4.77 (m, 1H, NH), 7.27–7.35 (m, 5H, C_6H_5); ^{13}C NMR (125 MHz, $CDCl_3$): δ 28.3 (C(CH_3)$_3$), 37.7 (C-6), 40.7 (C-2), 52.6 (OMe), 58.6 (C-5), 60.8 (C-4), 67.0 (CH_2Ph), 69.0 (C-3), 77.9 (C-1), 80.8 (C(CH_3)$_3$), 127.7, 127.9, 128.4, 137.3 (6C, C_6H_5), 156.8 (NCO), 172.5 (CO); HRMS Calcd for $C_{20}H_{28}N_4NaO_6$ $[M+Na]^+$: 443.1901. Found: 443.1902.

4.1.15. Methyl (1*R*,3*R*,4*R*,5*S*)-5-azido-1-benzyloxy-4-(*tert*-butoxycarbonylamino)-3-allyloxy-cyclohexane-1-carboxylate (21)

A solution of **20** (748 mg, 1.78 mmol) and 15-crown-5 (35.2 μL, 0.178 mmol) in DCM (10 mL) was added to 50% aqueous NaOH (25 mL, w/v). Allyl bromide (770 μL, 8.90 mmol) was then added, and the resultant mixture was refluxed with vigorous stirring for 16 h. The reaction mixture was concentrated to remove DCM, then MeOH (10 mL) was added and the solution stirred for 30 min at rt. After neutralization with 4 N aqueous HCl, the aqueous solution was extracted with EtOAc (4 × 50 mL). The combined organic layers were dried (Na_2SO_4) and concentrated in vacuo. The residue was dissolved in toluene (20 mL) and MeOH (15 mL) and treated with TMS-CHN_2 (2.5 mL, 2 M solution in hexane). After evaporation to dryness, the residue was purified by MPLC on silica (petrol ether/EtOAc) to give **21** (644 mg, 79%) as a colorless oil.

$[\alpha]_D$ −8.7 (*c* 1.02, $CHCl_3$); IR (film) 3375 (s, NH), 3066 (w), 2977 (s), 2098 (vs, N_3), 1719 (vs, CO), 1522 (vs), 1455 (m), 1391 (m), 1366 (s), 1300 (s), 1250 (s), 1203 (s), 1159 (vs) cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 1.46 (s, 9H, C(CH_3)$_3$), 1.58 (t, J = 12.1 Hz, 1H, H-2a), 1.59 (t, J = 12.6 Hz, 1H, H-6a), 2.70 (m, 1H, H-6b), 2.80 (m, 1H, H-2b), 3.25 (q, J = 10.1 Hz, 1H, H-4), 3.53 (m, 1H, H-3), 3.71 (m, 1H, H-5), 3.79 (s, 3H, OMe), 3.98 (dd, J = 5.9, 12.7 Hz, 1H, H-1′a), 4.12 (dd, J = 5.5, 12.7 Hz, 1H, H-1′b), 4.42 (s, 2H, PhCH_2), 4.63 (m, 1H, NH), 5.27 (dd, J = 1.1, 10.4 Hz, 1H, H-3′Z), 5.18 (dd, J = 1.5, 17.2 Hz, 1H, H-3′E), 5.87 (ddt, J = 5.7, 10.5, 17.1 Hz, 1H, H-2′), 7.27–7.35 (m, 5H, C_6H_5); ^{13}C NMR (125 MHz, $CDCl_3$): δ 28.3 (C(CH_3)$_3$), 38.2, 38.3 (C-2, C-6), 52.6 (OMe), 58.2 (C-5), 60.2 (C-4), 66.9 (CH_2Ph), 70.7 (C-1′), 73.7 (C-3), 77.9 (C-1), 79.7 (C(CH_3)$_3$), 117.4 (C-3′), 127.6, 127.9, 128.4, 137.3 (6C, C_6H_5), 134.6 (C-2′), 155.4 (NCO), 172.3 (CO); HRMS Calcd for $C_{23}H_{32}N_4NaO_6$ $[M+Na]^+$: 483.2214. Found: 483.2217.

4.1.16. Methyl (1*R*,3*R*,4*R*,5*S*)-5-azido-1-benzyloxy-4-(*tert*-butoxycarbonylamino)-3-(2-hydroxyethoxy)-cyclohexane-1-carboxylate (22)

To a solution of **21** (488 mg, 1.06 mmol) in dioxane/water (3:1, 10 mL) were added 2,6-lutidine (247 μL, 2.12 mmol), OsO_4 (2.5% in *tert*-butanol, 275 μL, 0.053 mmol), and $NaIO_4$ (907 mg, 4.24 mmol) under argon. The reaction was stirred at rt for 4 h, then water (5 mL) and DCM (20 mL) were added. The organic layer was separated, and the water phase was extracted by DCM (3 × 20 mL). The combined organic layers were washed with brine (20 mL) and dried over Na_2SO_4. The solvent was removed, and the remains were purified by MPLC on silica (1% gradient of MeOH in DCM) to afford the crude aldehyde intermediate (406 mg) as a yellow oil. The aldehyde was dissolved in MeOH (8 mL) under argon and sodium borohydride (66.4 mg, 1.76 mmol) was added in four portions at 0 °C. The solution was stirred for 45 min at 0 °C and quenched by the addition of saturated aqueous NH_4Cl (10 mL). The mixture was extracted with EtOAc (3 × 20 mL), and the combined organic layers were washed with brine (20 mL) and dried (Na_2SO_4). After concentration in vacuo, the residue was purified by MPLC on silica (1% gradient of MeOH in DCM) to give **22** (321 mg, 65%) as a colorless oil.

$[\alpha]_D$ −15.4 (*c* 0.80, $CHCl_3$); IR (film) 3365 (s, br, OH, NH), 2930 (m), 2098 (vs, N_3), 1695 (vs, CO), 1520 (m), 1455 (m), 1367 (s), 1301 (s), 1251 (m), 1204 (m), 1160 (s), 1095 (m) cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 1.46 (s, 9H, C(CH_3)$_3$), 1.57–1.66 (m, 2H, H-2a, H-6a), 2.26 (s br, 1H, OH), 2.72 (ddd, J = 3.0, 4.1, 12.8 Hz, 1H, H-6b), 2.82 (ddd, J = 3.0, 4.1, 12.5 Hz, 1H, H-2b), 3.34 (q, J = 9.8 Hz, 1H, H-4), 3.50–3.54 (m, 2H, H-3, H-1′a), 3.60 (m, 1H, H-5), 3.70 (m, 2H, H-2′), 3.74 (m, 1H, H-1′b), 3.79 (s, 3H, OMe), 4.42 (s, 2H, PhCH_2), 4.65 (d, J = 6.8 Hz, 1H, NH), 7.27–7.36 (m, 5H, C_6H_5); ^{13}C NMR (125 MHz, $CDCl_3$): δ 28.3 (C(CH_3)$_3$), 38.1 (C-2), 38.2 (C-6), 52.7 (OMe), 58.3 (C-5), 59.8 (C-4), 62.0 (C-2′), 67.0 (CH_2Ph), 71.0 (C-1′), 75.7 (C-3), 77.9 (C-1), 80.2 (C(CH_3)$_3$), 127.6, 127.9, 128.4, 137.2 (6C, C_6H_5), 155.8 (NCO), 172.4 (CO); ESI-MS Calcd for $C_{22}H_{32}N_4NaO_7$ $[M+Na]^+$: 487.22. Found: 487.23.

4.1.17. Methyl (1*R*,3*R*,4*R*,5*S*)-5-acetamido-3-(2-acetoxyethoxy)-1-benzyloxy-4-(*tert*-butoxycarbonylamino)-cyclohexane-1-carboxylate (23)

Compound **22** (220 mg, 0.473 mmol) and Pd/C (10%, 25 mg) were suspended in dioxane/NEt_3 (10:1, 11 mL) and hydrogenated (1 atm H_2) at rt for 3 h. The suspension was filtered over Celite and concentrated. To a solution of the residue in pyridine (6 mL) was added acetic anhydride (3 mL) at 0 °C under argon and the mixture was stirred at rt for 16 h. The solution was co-evaporated with toluene (3 × 5 mL) and the residue was purified by MPLC on silica (1% gradient of MeOH in DCM) to yield **23** (158 mg, 64%) as a colorless oil.

^1H NMR (500 MHz, $CDCl_3$): δ 1.43 (s, 9H, C(CH_3)$_3$), 1.62–1.67 (m, 2H, H-2a, H-6a), 1.90, 2.08 (2 s, 6H, 2COCH_3), 2.67 (d, J = 12.5 Hz, 1H, H-6b), 2.81 (d, J = 13.9 Hz, 1H, H-2b), 3.51 (m, 1H, H-3), 3.54 (q, J = 9.8 Hz, 1H, H-4), 3.69 (ddd, J = 3.1, 6.6, 11.5 Hz, 1H, H-1′a), 3.79 (m, 1H, H-5), 3.81 (s, 3H, OMe), 3.82 (m, 1H, H-1′b), 4.17 (ddd, J = 3.2, 6.6, 12.0 Hz, 1H, H-2′a), 4.24 (ddd, J = 3.1, 5.9, 12.0 Hz, 1H, H-2′b), 4.36, 4.50 (A, B of AB, J = 10.8 Hz, 2H, PhCH_2), 4.75 (d, J = 7.0 Hz, 1H, 4-NH), 6.53 (d, J = 7.4 Hz, 1H, 5-NH), 7.27–7.35 (m, 5H, C_6H_5); ^{13}C NMR (125 MHz, $CDCl_3$): δ 20.8, 23.1 (2COCH_3), 28.2 (C(CH_3)$_3$), 37.0 (C-2), 38.5 (C-6), 48.9 (C-5), 52.6 (OMe), 57.2 (C-4), 63.6 (C-2′), 66.8 (CH_2Ph), 67.3 (C-1′), 76.3 (C-3), 78.1 (C-1), 79.8 (C(CH_3)$_3$), 127.5, 127.7, 128.3, 137.5 (6C, C_6H_5), 157.3 (NCO), 170.0, 170.9, 172.4 (3CO); ESI-MS Calcd for $C_{26}H_{38}N_2NaO_9$ $[M+Na]^+$: 545.25. Found: 545.24.

4.1.18. Methyl (1*R*,3*R*,4*R*,5*S*)-5-acetamido-1-benzyloxy-4-(*tert*-butoxycarbonylamino)-3-(2-hydroxyethoxy)-cyclohexane-1-carboxylate (24)

A solution of **23** (176 mg, 0.337 mmol) in MeOH (5 mL) was treated with 1 M methanolic NaOMe (0.5 mL) under argon at rt for 6 h. The reaction mixture was neutralized with acetic acid and concentrated. The residue was purified by MPLC on silica (1% gradient of MeOH in DCM) to give **24** (139 mg, 86%) as a colorless foam.

$[\alpha]_D$ −19.2 (*c* 1.00, $CHCl_3$); IR (film) 3328 (s, br, OH, NH), 2930 (s), 1731 (s, CO), 1690 (vs, CO), 1662 (s, CO), 1537 (s), 1455 (m), 1367 (s), 1305 (s), 1250 (m), 1206 (m), 1162 (s), 1099 (s) cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 1.42 (s, 9H, C(CH_3)$_3$), 1.64–1.69 (m, 2H, H-2a, H-6a), 1.91 (s, 3H, COCH_3), 2.45 (s, 1H, OH), 2.62 (d, *J* = 12.5 Hz, 1H, H-6b), 2.82 (d, *J* = 11.5 Hz, 1H, H-2b), 3.47 (dt, *J* = 3.9, 10.2 Hz, 1H, H-3), 3.51–3.57 (m, 2H, H-4, H-1′a), 3.67 (m, 2H, H-2′), 3.73 (m, 1H, H-1′b), 3.78 (s, 3H, OMe), 3.84 (m, 1H, H-5), 4.36, 4.47 (A, B of AB, *J* = 10.8 Hz, 2H, PhCH_2), 5.00 (d, *J* = 8.0 Hz, 1H, 4-NH), 6.51 (s br, 1H, 5-NH), 7.27–7.34 (m, 5H, C_6H_5); ^{13}C NMR (125 MHz, $CDCl_3$): δ 23.1 (COCH_3), 28.3 (C(CH_3)$_3$), 37.4 (C-2), 38.7 (C-6), 48.6 (C-5), 52.7 (OMe), 58.0 (C-4), 62.1 (C-2′), 66.9 (CH_2Ph), 70.9 (C-1′), 76.9 (C-3), 78.1 (C-1), 80.0 (*C*(CH_3)$_3$), 127.6, 127.8, 128.4, 137.5 (6C, C_6H_5), 157.4 (N*C*O), 170.2, 172.5 (2CO); HRMS Calcd for $C_{24}H_{36}N_2NaO_9$ $[M+Na]^+$: 503.2364. Found: 503.2358.

4.1.19. Methyl (1*R*,3*R*,4*R*,5*S*)-5-acetamido-1-benzyloxy-4-(*tert*-butoxycarbonylamino)-3-(2-tosyloxyethoxy)-cyclohexane-1-carboxylate (25)

To a solution of **24** (117 mg, 0.243 mmol) in DCM were subsequently added NEt_3 (10.1 μL, 0.729 mmol), DMAP (10 mg) and *p*-toluenesulfonyl chloride (69.8 mg, 0.365 mmol) at 0 °C under argon. The mixture was stirred at rt for 4 h and then quenched by the addition of MeOH (0.3 mL). After evaporation of the solvents the residue was purified by MPLC on silica (1% gradient of MeOH in DCM) to yield **25** (130 mg, 84%) as a colorless oil.

$[\alpha]_D$ −14.2 (*c* 1.04, $CHCl_3$); IR (film) 3326 (m, br, NH), 3066 (w), 2926 (s), 1731 (vs, CO), 1690 (vs, CO), 1532 (s), 1455 (m), 1366 (vs), 1304 (s), 1248 (m), 1206 (m), 1189 (s), 1177 (vs), 1097 (s) cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 1.42 (s, 9H, C(CH_3)$_3$), 1.64–1.69 (m, 2H, H-2a, H-6a), 1.89 (s, 3H, COCH_3), 2.43 (s, 3H, PhCH_3), 2.61 (d, *J* = 12.4 Hz, 1H, H-6b), 2.72 (d, *J* = 11.5 Hz, 1H, H-2b), 3.44 (m, 1H, H-3), 3.49 (q, *J* = 9.4 Hz, 1H, H-4), 3.69 (m, 1H, H-1′a), 3.76 (m, 1H, H-1′b), 3.78–3.81 (m, 4H, H-5, OMe), 4.12 (m, 2H, H-2′), 4.33, 4.46 (A, B of AB, *J* = 10.7 Hz, 2H, PhCH_2), 4.70 (d, *J* = 8.0 Hz, 1H, 4-NH), 6.49 (d, *J* = 7.7 Hz, 1H, 5-NH), 7.27–7.34 (m, 7H, 2H of C_6H_4, C_6H_5), 7.78 (BB′ of AA′BB′, *J* = 8.3 Hz, 2H of C_6H_4); ^{13}C NMR (125 MHz, $CDCl_3$): δ 21.6 (PhCH_3), 23.2 (COCH_3), 28.2 (C(*C*H_3)$_3$), 36.8 (C-2), 38.4 (C-6), 48.9 (C-5), 52.6 (OMe), 57.1 (C-4), 66.9, 67.0 (C-1′, *C*H_2Ph), 69.2 (C-2′), 76.5 (C-3), 78.0 (C-1), 80.0 (*C*(CH_3)$_3$), 127.6, 127.8, 127.9, 128.4, 129.9, 132.9, 137.5, 144.9 (12C, C_6H_4, C_6H_5), 157.3 (N*C*O), 170.0, 172.6 (2CO); HRMS Calcd for $C_{31}H_{42}N_2NaO_{10}S$ $[M+Na]^+$: 657.2452. Found: 657.2450.

4.1.20. Methyl (1*R*,3*R*,4*R*,5*S*)-5-acetamido-3-(2-azidoethoxy)-1-benzyloxy-4-(*tert*-butoxycarbonylamino)-cyclohexane-1-carboxylate (26)

To a solution of **25** (129 mg, 0.204 mmol) in DMF (5 mL) were added 15-crown-5 (20.1 μL, 0.102 mmol) and sodium azide (133 mg, 2.04 mmol). The resulting suspension was stirred for 22 h at 60 °C under argon, then diluted with EtOAc (20 mL) and filtered over Celite. After co-evaporation with toluene (2 × 10 mL), the residue was purified by MPLC on silica (1% gradient of MeOH in DCM) to yield **26** (85.7 mg, 83%) as a colorless solid.

$[\alpha]_D$ −21.0 (*c* 0.99, $CHCl_3$); IR (KBr) 3363 (m, br, NH), 2926 (m), 2103 (s, N_3), 1732 (vs, CO), 1688 (vs, CO), 1661 (vs, CO), 1532 (m), 1455 (m), 1366 (s), 1305 (vs), 1250 (m), 1206 (m), 1163 (s), 1096 (m) cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 1.42 (s, 9H, C(CH_3)$_3$), 1.65 (t, *J* = 12.4 Hz, 1H, H-6a), 1.68 (t, *J* = 12.1 Hz, 1H, H-2a), 1.90 (s, 3H, COCH_3), 2.64 (d, *J* = 12.7 Hz, 1H, H-6b), 2.82 (d, *J* = 12.2 Hz, 1H, H-2b), 3.35 (m, 2H, H-2′), 3.49 (m, 1H, H-3), 3.57 (q, *J* = 9.6 Hz, 1H, H-4), 3.63 (m, 1H, H-1′a), 3.78 (m, 1H, H-1′b), 3.79 (s, 3H, OMe), 3.83 (m, 1H, H-5), 4.36, 4.48 (A, B of AB, *J* = 10.8 Hz, 2H, PhCH_2), 4.78 (d, *J* = 8.1 Hz, 1H, 4-NH), 6.58 (d, *J* = 7.7 Hz, 1H, 5-NH), 7.27–7.34 (m, 5H, C_6H_5); ^{13}C NMR (125 MHz, $CDCl_3$): δ 23.1 (COCH_3), 28.2 (C(CH_3)$_3$), 37.2 (C-2), 38.4 (C-6), 49.0 (C-5), 51.0 (C-2′), 52.6 (OMe), 57.4 (C-4), 66.9 (CH_2Ph), 68.5 (C-1′), 76.7 (C-3), 78.1 (C-1), 79.8 (*C*(CH_3)$_3$), 127.6, 127.8, 128.4, 137.5 (6C, C_6H_5), 157.2 (N*C*O), 170.1, 172.6 (2CO); HRMS Calcd for $C_{24}H_{35}N_5NaO_7$ $[M+Na]^+$: 528.2429. Found: 528.2430.

4.1.21. Methyl (1*R*,3*R*,4*R*,5*S*)-5-acetamido-1-benzyloxy-4-(*tert*-butoxycarbonylamino)-3-[2-(4-chlorobenzamido)ethoxy]-cyclohexane-1-carboxylate (27)

Compound **26** (29.0 mg, 57.5 μmol) and Pd/C (10%, 10 mg) were suspended in MeOH (2.5 mL). NEt_3 (150 μL) was added and the suspension was hydrogenated (1 atm H_2) at rt for 1 h. The mixture was filtered over Celite and concentrated. To a solution of the residue in DCM (2 mL) were subsequently added NEt_3 (31.8 μL, 0.230 mmol), DMAP (5 mg), and *p*-chlorobenzoyl chloride (14.7 μL, 0.115 mmol) at 0 °C under argon. The solution was stirred at rt for 2.5 h, then NEt_3 (50 μL) and MeOH (0.5 mL) were added and stirring was continued for 15 min. The solvents were evaporated and the residue was purified by MPLC on silica (1% gradient of MeOH in DCM) to yield **27** (23.4 mg, 66%) as a colorless solid.

$[\alpha]_D$ −37.9 (*c* 0.51, $CHCl_3$); IR (KBr) 3319 (m, br, NH), 2926 (m), 1732 (s, CO), 1690 (vs, CO), 1659 (vs, CO), 1537 (vs), 1486 (m), 1454 (m), 1366 (m), 1305 (s), 1250 (m), 1206 (m), 1161 (m), 1092 (s) cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 1.33 (s, 9H, C(CH_3)$_3$), 1.66 (t, *J* = 11.3 Hz, 1H, H-2a), 1.68 (t, *J* = 12.4 Hz, 1H, H-6a), 1.93 (s, 3H, COCH_3), 2.62 (ddd, *J* = 2.8, 3.4, 12.0 Hz, 1H, H-6b), 2.80 (ddd, *J* = 2.6, 3.9, 11.4 Hz, 1H, H-2b), 3.43 (ddd, *J* = 4.4, 9.9, 10.7 Hz, 1H, H-3), 3.53 (m, 2H, H-2′), 3.56 (q, *J* = 10.0 Hz, 1H, H-4), 3.64 (m, 1H, H-1′a), 3.75–3.80 (m, 4H, H-1′b, OMe), 3.89 (m, 1H, H-5), 4.35, 4.43 (A, B of AB, *J* = 10.7 Hz, 2H, PhCH_2), 4.94 (d, *J* = 8.9 Hz, 1H, 4-NH), 6.41 (d, *J* = 8.1 Hz, 1H, 5-NH), 6.78 (t, *J* = 5.2 Hz, 1H, 2′-NH), 7.24–7.31 (m, 5H, C_6H_5), 7.37, 7.74 (AA′, BB′ of AA′BB′, *J* = 8.6 Hz, 4H, C_6H_4); ^{13}C NMR (125 MHz, $CDCl_3$): δ 23.2 (COCH_3), 28.2 (C(*C*H_3)$_3$), 37.6 (C-2), 38.7 (C-6), 40.1 (C-2′), 48.0 (C-5), 52.7 (OMe), 58.6 (C-4), 67.0 (CH_2Ph), 68.4 (C-1′), 76.6 (C-3), 78.2 (C-1), 79.8 (*C*(CH_3)$_3$), 127.7, 128.0, 128.4, 128.6, 128.6, 132.6, 137.1, 137.5 (12C, C_6H_4, C_6H_5), 157.2 (N*C*O), 166.3, 170.2, 172.3 (3CO); HRMS Calcd for $C_{31}H_{40}ClN_3NaO_8$ $[M+Na]^+$: 640.2396. Found: 640.2400.

4.1.22. Methyl (1*R*,3*R*,4*R*,5*S*)-5-acetamido-1-benzyloxy-3-[2-(4-chlorobenzamido)ethoxy]-4-(2-fluoroacetamido)-cyclohexane-1-carboxylate (28)

A solution of **27** (30.5 mg, 49.3 μmol) was treated with DCM/TFA (2:1, 0.75 mL) for 3 h at 0 °C under argon. After concentration in vacuo the residue was dissolved in DCM (3 mL). NEt_3 (68.4 μL, 0.493 mmol), DMAP (5 mg), and 2-fluoroacetyl chloride (5.6 μL, 74.0 μmol) were subsequently added at 0 °C under argon and the solution was stirred for 2.5 h at rt. Then NEt_3 (20 μL) and MeOH (0.5 mL) were added and stirring was continued for 10 min. After evaporation to dryness the residue was purified by MPLC on silica (1% gradient of MeOH in DCM) to give **28** (19.7 mg, 69%) as a yellowish solid.

$[\alpha]_D$ −38.3 (*c* 0.84, $CHCl_3$); IR (KBr) 3292 (m, br, NH), 2927 (m), 1733 (s, CO), 1660 (vs, CO), 1652 (vs, CO), 1555 (vs), 1487 (m), 1455 (m), 1367 (m), 1310 (m), 1206 (m), 1150 (m), 1093 (s) cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 1.67 (t, *J* = 12.1 Hz, 1H, H-2a), 1.72 (t, *J* = 12.7 Hz, 1H, H-6a), 1.93 (s, 3H, COCH_3), 2.62 (m, 1H, H-6b), 2.87 (m, 1H, H-2b), 3.44 (ddt, *J* = 3.9, 7.6, 12.0 Hz, 1H, H-2′a), 3.54–3.59 (m, 2H, H-3, H-1′a), 3.65 (m, 1H, H-2′b), 3.74–3.80 (m, 4H, H-1′b, OMe), 3.94 (q, *J* = 9.8 Hz, 1H, H-4), 4.04 (m, 1H, H-5), 4.37, 4.44 (A, B of AB, *J* = 10.8 Hz, 2H, PhCH_2), 4.47, 4.62 (A, B of ABX, *J* = 14.4, 47.2 Hz, 2H, FCH_2), 6.29 (d, *J* = 8.5 Hz, 1H, 5-NH), 6.77 (t, *J* = 5.2 Hz, 1H, 2′-NH), 6.94 (dd, *J* = 1.9, 8.9 Hz, 1H, 4-NH), 7.26–7.35 (m, 5H, C_6H_5), 7.38, 7.75 (AA′, BB′ of AA′BB′, *J* = 8.5 Hz, 4H, C_6H_4); ^{13}C NMR (125 MHz, $CDCl_3$): δ 23.0 (COCH_3), 37.5 (C-2),

38.8 (C-6), 40.0 (C-2′), 47.1 (C-5), 52.8 (OMe), 57.5 (C-4), 67.0 (*C*H$_2$Ph), 68.3 (C-1′), 76.5 (C-3), 78.1 (C-1), 79.8 (d, *J* = 186.9 Hz, *C*H$_2$F), 127.6, 128.0, 128.4, 128.6, 128.6, 132.5, 137.2, 137.7 (12C, C$_6$H$_4$, C$_6$H$_5$), 166.3, 169.4, 170.5, 172.2 (4CO); HRMS Calcd for $C_{28}H_{33}ClFN_3NaO_7$ [M+Na]$^+$: 600.1883. Found: 600.1884.

4.1.23. Sodium (1*R*,3*R*,4*R*,5*S*)-5-acetamido-1-benzyloxy-3-[2-(4-chlorobenzamido)ethoxy]-4-(2-fluoroacetamido)-cyclohexane-1-carboxylate (3)

To a solution of **28** (16.5 mg, 28.5 μmol) in THF (4.5 mL) was added a solution of LiOH·H$_2$O (8 mg) in water (0.5 mL) at 0 °C. The mixture was stirred for 21 h at rt, then neutralized with acetic acid and concentrated in vacuo. The crude product was purified by reversed-phase chromatography (RP-18 column, 5% gradient MeOH in water), Dowex 50X8 ion-exchange chromatography (Na$^+$ type), and P2 size exclusion chromatography to afford **3** (13.8 mg, 83%) as a colorless solid after a final lyophilization from water.

[α]$_D$ −19.9 (*c* 0.49, MeOH); IR (KBr) 3430 (vs, br, NH), 2927 (m), 1645 (vs, CO), 1598 (vs, CO), 1554 (s), 1487 (m), 1454 (m), 1385 (m), 1314 (m), 1093 (s), 1015 (m) cm^{-1}; ^{1}H NMR (500 MHz, D$_2$O): δ 1.44 (t, *J* = 11.8 Hz, 1H, H-2a), 1.58 (t, *J* = 12.5 Hz, 1H, H-6a), 1.87 (s, 3H, COC*H*$_3$), 2.42 (ddd, *J* = 2.8, 3.6, 12.4 Hz, 1H, H-6b), 2.79 (ddd, *J* = 2.8, 3.9, 11.9 Hz, 1H, H-2b), 3.50 (m, 2H, H-2′), 3.63 (dt, *J* = 4.4, 10.6 Hz, 1H, H-3), 3.69 (ddd, *J* = 4.4, 6.4, 10.6 Hz, 1H, H-1′a), 3.76–3.83 (m, 2H, H-4, H-1′b), 3.94 (ddd, *J* = 4.0, 11.1, 12.4 Hz, 1H, H-5), 4.30, 4.34 (A, B of AB, *J* = 10.3 Hz, 2H, PhC*H*$_2$), 4.46, 4.63 (A, B of ABX, *J* = 14.4, 46.4 Hz, 2H, FC*H*$_2$), 7.29–7.38 (m, 5H, C$_6$H$_5$), 7.47, 7.69 (AA′, BB′ of AA′BB′, *J* = 8.6 Hz, 4H, C$_6$H$_4$); ^{13}C NMR (125 MHz, D$_2$O): δ 21.8 (COC*H*$_3$), 37.9 (C-2), 38.5 (C-6), 40.0 (C-2′), 47.7 (C-5), 56.9 (C-4), 66.8 (*C*H$_2$Ph), 67.9 (C-1′), 76.4 (C-3), 79.3 (d, *J* = 182.7 Hz, *C*H$_2$F), 80.3 (C-1), 128.2, 128.6, 128.6, 128.7, 128.8, 131.9, 137.2, 137.7 (12C, C$_6$H$_4$, C$_6$H$_5$), 169.7, 171.0, 173.4, 177.2 (4CO); HRMS Calcd for $C_{27}H_{30}ClFN_3Na_2O_7$ [M+Na]$^+$: 608.1546. Found: 608.1546.

4.2. In vitro binding assay

Murine MAG$_{d1-3}$-Fc was affinity purified from CHO-Lec 3.2.8.1 cell culture supernatant as described before[41], dialyzed against 10 mM phosphate buffer pH 7,4, sterile filtered and stored at 4 °C. Inhibition assays for Siglecs were performed as described previously.[26,32,41] In brief, fetuin was immobilized in microtiter plates and binding of MAG-Fc was determined in the presence of seven to eight different concentrations for each inhibitor using alkaline phosphatase-labeled anti-Fc antibodies. The half maximal inhibitory concentrations (IC$_{50}$) were determined from corresponding binding curves. In order to compare the results from different assays compound **29** was included in each test and used as a reference to calculate the relative inhibitory concentrations (rIC$_{50}$). At least four independent titrations were performed for each compound.

4.3. Surface plasmon resonance experiments (Biacore)[31]

Surface plasmon resonance experiments were performed on a Biacore 3000 machine using CM5 chips. Goat anti-human IgG antibody (Fc specific, Sigma) was immobilized following the standard Biacore EDC/NHS immobilization procedure. For this, 2 μL of a Fc-antibody solution of 2.1 mg/mL (in 50 mM phosphate buffer, pH 7.0) was added to 98 μL of 10 mM acetate buffer (pH 5.5). The immobilization yielded between 4.000 and 5.000 resonance units. A sample and a reference surface were prepared sequentially. For capturing MAG$_{d1-3}$-Fc onto the sample surface, MAG$_{d1-3}$-Fc in HBS-EP buffer (10 mM HEPES pH 7.4, 0.15 M NaCl, 3 mM EDTA, 0.0005% surfactant P20; Biacore) was injected. Ligands were dissolved in HBS-EP buffer. Eight ligand concentrations of a twofold dilution series starting from 50 μM were prepared. The samples were injected in a randomized order. Five blank buffer injections were performed before triplicate measurements and one between each single experiment. The flow rate was 10 μL/min for the immobilization, 1 μL/min for the capturing of MAG, and 20 μL/min for the injection of ligands. For the determination of K_D values and for the kinetics analysis, standard Biacore software, version 3.2, was used.

Acknowledgment

We thank the Volkswagen Foundation, the Swiss National Science Foundation and the German Federal Ministry for Education and Research (BMBF, project 031632A), and the Tönjes-Vagt Foundation (project XXI) for their support of this work.

Supplementary data

Supplementary data (HPLC data and NMR spectra of target compound **3**) associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2010.08.027.

References and notes

1. Crocker, P. R.; Paulson, J. C.; Varki, A. *Nat. Rev. Immunol.* **2007**, *7*, 255.
2. Kelm, S.; Pelz, A.; Schauer, R.; Filbin, M. T.; Tang, S.; de Bellard, M. E.; Schnaar, R. L.; Mahoney, J. A.; Hartnell, A.; Bradfield, P. *Curr. Biol.* **1994**, *4*, 965.
3. Schwab, M. E.; Bandtlow, C. E. *Nature* **1994**, *371*, 658.
4. Ramon y Cajal, S. *Degeneration and Regeneration of the Nervous System*; Oxford Univ. Press: London, 1928.
5. Schwab, M. E.; Caroni, P. *J. Neurosci.* **1988**, *8*, 2381.
6. Sandvig, A.; Berry, M.; Barrett, L. B.; Butt, A.; Logan, A. *Glia* **2004**, *46*, 225.
7. Filbin, M. T. *Nat. Rev. Neurosci.* **2003**, *4*, 703.
8. He, Z. G.; Koprivica, V. *Ann. Rev. Neurosci.* **2004**, *27*, 341.
9. Caroni, P.; Savio, T.; Schwab, M. E. *Prog. Brain Res.* **1988**, *78*, 363.
10. Quarles, R. H. *Neurochem. Res.* **2009**, *34*, 79.
11. Lauren, J.; Hu, F.; Chin, J.; Liao, J.; Airaksinen, M. S.; Strittmatter, S. M. *J. Biol. Chem.* **2007**, *282*, 5715.
12. Robak, L. A.; Venkatesh, K.; Lee, H.; Raiker, S. J.; Duan, Y.; Lee-Osbourne, J.; Hofer, T.; Mage, R. G.; Rader, C.; Giger, R. J. *J. Neurosci.* **2009**, *29*, 5766.
13. Yang, L. J. S.; Zeller, C. B.; Shaper, N. L.; Kiso, M.; Hasegawa, A.; Shapiro, R. E.; Schnaar, R. L. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*, 814.
14. Tang, S.; Shen, Y. J.; DeBellard, M. E.; Mukhopadhyay, G.; Salzer, J. L.; Crocker, P. R.; Filbin, M. T. *J. Cell Biol.* **1997**, *138*, 1355.
15. Vinson, M.; Strijbos, P. J. L. M.; Rowles, A.; Facci, L.; Moore, S. E.; Simmons, D. L.; Walsh, F. S. *J. Biol. Chem.* **2001**, *276*, 20280.
16. Wörter, V.; Schweigreiter, R.; Kinzel, B.; Mueller, M.; Barske, C.; Böck, G.; Frentzel, S.; Bandtlow, C. E. *PLoS One* **2009**, *4*, e5218. doi:10.1371/journal.pone.0005218.
17. Yang, L. J. S.; Lorenzini, I.; Vajn, K.; Mountney, A.; Schramm, L. P.; Schnaar, R. L. *Proc. Natl. Acad. Sci. U.S.A.* **2006**, *103*, 11057.
18. Vyas, A. A.; Blixt, O.; Paulson, J. C.; Schnaar, R. L. *J. Biol. Chem.* **2005**, *280*, 16305.
19. Collins, B. E.; Kiso, M.; Hasegawa, A.; Tropak, M. B.; Roder, J. C.; Crocker, P. R.; Schnaar, R. L. *J. Biol. Chem.* **1997**, *272*, 16889.
20. Collins, B. E.; Ito, H.; Sawada, N.; Ishida, H.; Kiso, M.; Schnaar, R. L. *J. Biol. Chem.* **1999**, *274*, 37637.
21. Ito, H.; Ishida, H.; Collins, B.; Fromholt, S.; Schnaar, R. L.; Kiso, M. *Carbohydr. Res.* **2003**, *338*, 1621.
22. Schwizer, D.; Gäthje, H.; Kelm, S.; Porro, M.; Schwardt, O.; Ernst, B. *Bioorg. Med. Chem.* **2006**, *14*, 4944.
23. Schwardt, O.; Gäthje, H.; Vedani, A.; Mesch, S.; Gao, G.; Spreafico, M.; von Orelli, J.; Kelm, S.; Ernst, B. *J. Med. Chem.* **2009**, *52*, 989.
24. Bhunia, A.; Schwardt, O.; Gäthje, H.; Gao, G.; Kelm, S.; Benie, A. J.; Hricovini, M.; Peters, T.; Ernst, B. *ChemBioChem* **2008**, *9*, 2941.
25. Shin, S.; Gäthje, H.; Schwardt, O.; Gao, G.; Ernst, B.; Kelm, S.; Meyer, B. *ChemBioChem* **2008**, *9*, 2946.
26. Kelm, S.; Brossmer, R.; Isecke, R.; Gross, H. J.; Strenge, K.; Schauer, R. *Eur. J. Biochem.* **1998**, *255*, 663.
27. Kelm, S.; Brossmer, R. PCT Patent WO 03/000709A2, 2003.
28. Strenge, K.; Schauer, R.; Bovin, N.; Hasegawa, A.; Kiso, M.; Kelm, S. *Eur. J. Biochem.* **1998**, *258*, 677.
29. Gao, G.; Smiesko, M.; Schwardt, O.; Gäthje, H.; Kelm, S.; Vedani, A.; Ernst, B. *Bioorg. Med. Chem.* **2007**, *15*, 7459.
30. Mesch, S.; Lemme, K.; Koliwer-Brandl, H.; Strasser, D. S.; Schwardt, O.; Kelm, S.; Ernst, B. *Carbohydr. Res.* **2010**, *345*, 1348.
31. Mesch, S.; Moser, D.; Strasser, D.; Kelm, A.; Cutting, B.; Rossato, G.; Vedani, A.; Koliwer-Brandl, H.; Wittwer, M.; Rabbani, S.; Schwardt, O.; Kelm, S.; Ernst, B. *J. Med. Chem.* **2010**, *53*, 1597.

32. Shelke, S. V.; Gao, G. P.; Mesch, S.; Gäthje, H.; Kelm, S.; Schwardt, O.; Ernst, B. *Bioorg. Med. Chem.* **2007**, *15*, 4951.
33. (a) Bieberich, E.; Liour, S. S.; Yu, R. K. *Methods Enzymol.* **2000**, *312B*, 339; (b) Kopitz, J.; Sinz, K.; Brossmer, R.; Cantz, M. *Eur. J. Biochem.* **1997**, *248*, 527.
34. May, A. P.; Robinson, R. C.; Vinson, M.; Crocker, P. R.; Jones, E. Y. *Mol. Cell* **1998**, *1*, 719.
35. Crocker, R. P.; Klem, S. *The Siglec Family of I-type Lectins*; Wiley-VCH: Weinheim, 2000, Vol. IV, p 579.
36. Kim, C. U.; Lew, W.; Williams, M. A.; Liu, H.; Zhang, L.; Swaminathan, S.; Bischofberger, N.; Chen, M. S.; Mendel, D. B.; Tai, C. Y.; Laver, G.; Stevens, R. C. *J. Am. Chem. Soc.* **1997**, *119*, 681.
37. Rohloff, J. C.; Kent, K. M.; Postich, M. J.; Becker, M. W.; Chapman, H. H.; Kelly, D. E.; Lew, W.; Louie, M. S.; McGee, L. R.; Prisbe, E. J.; Schultze, L. M.; Yu, R. H.; Zhang, L. *J. Org. Chem.* **1998**, *63*, 4545.
38. Boullanger, P.; Maunier, V.; Lafont, D. *Carbohydr. Res.* **2000**, *324*, 97.
39. Yu, W.; Mei, Y.; Kang, Y.; Hua, Z.; Jin, Z. *Org. Lett.* **2004**, *6*, 3217.
40. Pozsgay, V.; Ekborg, G.; Sampathkumar, S.-G. *Carbohydr. Res.* **2006**, *341*, 1408.
41. Bock, N.; Kelm, S.. In *Methods in Molecular Biology*; Brockhausen, I., Ed.; Humana Press Inc: Totowa, NJ, 2006; Vol. 347, p 359.
42. Shi, J. Y.; Blundell, T. L.; Mizuguchi, K. *J. Mol. Biol.* **2001**, *310*, 243.
43. (a) Deane, C. M.; Kaas, Q.; Blundell, T. L. *Bioinformatics* **2001**, *17*, 541; (b) Deane, C. M.; Blundell, T. L. *Protein Sci.* **2001**, *10*, 599.
44. Tripos Inc.: St. Louis, MO, 2007.
45. (a) Thompson, J. D.; Higgins, D. G.; Gibson, T. J. *Nucleic Acids Res.* **1994**, *22*, 4673; (b) Larkin, M. A.; Blackshields, G.; Brown, N. P.; Chenna, R.; McGettigan, P. A.; McWilliam, H.; Valentin, F.; Wallace, I. M.; Wilm, A.; Lopez, R.; Thompson, J. D.; Gibson, T. J.; Higgins, D. G. *Bioinformatics* **2007**, *23*, 2947; (c) http://www.ebi.ac.uk/tools/clustalw2.
46. General: Case, D. A.; Pearlman, D. A.; Caldwell, J. W. *AMBER 7*; University of California: San Francisco, 2002; AMBER* force field: Weiner, S. J.; Kollman, P. A.; Case, D. A.; Singh, U. C.; Ghio, C.; Alagona, G.; Profeta, S.; Weiner, P. *J. Am. Chem. Soc.* **1984**, *106*, 765.
47. Mohamadi, F.; Richards, N. G. J.; Guida, W. C.; Liskamp, R.; Lipton, M.; Caufield, C.; Chang, G.; Hendrickson, T.; Still, W. C. *J. Comput. Chem.* **1990**, *11*, 440.
48. Jorgensen, W. L.; Maxwell, D. S.; Tirado-Rives, J. *J. Am. Chem. Soc.* **1996**, *118*, 11225.
49. Cramer, C.; Truhlar, D. *J. Comput. Aided Mol. Des.* **1992**, *6*, 629.
50. Humphrey, W.; Dalke, A.; Schulten, K. *J. Mol. Graphics* **1996**, *14*, 33.
51. *Desmond Molecular Dynamics System*, 2.0109; D. E. Shaw Research: New York, NY, 2009.
52. Lemieux, R. U. *Chem. Soc. Rev.* **1989**, *18*, 347.