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Probing the carbohydrate recognition domain of E-selectin: The importance of the acid orientation in sLe^x mimetics

Alexander Titz [a], John Patton [b], Martin Smiesko [a], Zorana Radic [a], Oliver Schwardt [a], John L. Magnani [b], Beat Ernst [a,*]

[a] Institute of Molecular Pharmacy, University of Basel, CH-4056 Basel, Switzerland
[b] GlycoMimetics Inc., Gaithersburg, MD 20878, USA

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ABSTRACT

The selectin–leukocyte interaction is the initial event in the early inflammatory cascade. This interplay proceeds via the terminal tetrasaccharide sialyl Lewis^x (sLe^x), present on physiological selectin ligands and E- and P-selectins located on the endothelial surface. Blocking this process is regarded as a promising therapeutic approach for inflammatory diseases where excessive leukocyte efflux is responsible for tissue damage. Selectin antagonists are generally based on sLe^x as lead structure, containing the essential pharmacophores pre-oriented in the bioactive conformation. In this work, we describe a set of competitive sLe^x mimetics possessing the carboxylic acid pharmacophore equipped with additional hydrophobic substituents as neuraminic acid (Neu5Ac) replacements. This small library of antagonists derived from Huisgen-1,3-dipolar cycloadditions allows to further probe the carbohydrate recognition domain of E-selectin.

1. Introduction

Selectins, a family of cell adhesion molecules, play a key role in the early inflammatory response, an essential mechanism of the immune defense.[1] In case of an inflammation, interactions of selectins with leukocytes lead to the characteristic tethering and rolling of the white blood cells on the vascular endothelium. In a second step, firm adhesion and migration through the endothelial monolayer, a process mediated by protein–protein interactions as for example of integrins with members of the IgG superfamily,[2] lead to the extravasation of leukocytes to the site of inflammation. However, in case of excessive extravasation of leukocytes, this mechanism originally intended as a defensive strategy can generate severe acute or chronic reactions as, for example, in reperfusion injury, stroke or rheumatoid arthritis.[3,4] Therefore, the antagonism of the selectins is considered to be a valuable approach for the treatment of inflammatory diseases.[5]

In the search for selectin antagonists, the terminal tetrasaccharide epitope sialyl Lewis^x (**1**, sLe^x, Fig. 1A),[6,7] present in physiological selectin ligands, served as lead structure.[8–10] The pharmacophores essential for binding to E-selectin are four hydroxy groups (3- and 4-OH of L-fucose and 4- and 6-OH of D-galactose) and the carboxylate of *N*-acetylneuraminic acid (Neu5Ac).[8] The bioactive conformation (Fig. 1B) of sLe^x and thus the spatial arrangement of the pharmacophores in the bound state, was determined by trNOE NMR experiments[11,12] and subsequently confirmed by X-ray crystallography.[13] To improve the low affinity of the sLe^x/E-selectin interaction (IC_{50} approx. 1 mM),[8] pre-organization of the pharmacophores in their bioactive orientation resulting in a reduction of entropy costs upon binding has been successfully explored.[14–16]

Whereas numerous modifications of the Le^x core have been studied,[8,9] only a few reports examined replacements of the Neu5Ac moiety (reviewed in [17]), for example by anionic substituents (sulphate[18–21] or phosphate[20]), lactic acid derivatives (e.g., **CGP69669A**, Fig. 1C)[14] or (*S*)-isoserine derivatives (Fig. 1D).[22] For the latter, the examined substituents R^1 and R^2 cover a broad range including aryl sulfonamides, fatty acid amides or polyhydroxylated alkyl chains, but do not include heterocyclic fragments. The reported relative affinities (rIC_{50} with sLe^x having a rIC_{50} of 1) range from 0.6 to 2.0 for 2° amines and amides, 0.2–0.6 for N-alkylated amides and 3° amines and 0.12–0.26 for sulfonamides (R^1 = H, $R^2 = S(O_2)R^3$). Overall, none of the analyzed isoserine derivatives showed an improved affinity compared to the (*S*)-cyclohexyllactate derivative **CGP69669A**[14,23] (rIC_{50} = 0.08, Fig. 1C).

In this communication, we report the synthesis and affinity data of a library of E-selectin antagonists **2** where Neu5Ac is replaced by (*S*)-isoserine modified by 1,3-dipolar cycloaddition (Fig. 1E).

2. Results and discussion

Molecular modeling studies with **2a** (Fig. 1E, R = H) containing an unsubstituted triazole predicted a limited pre-organization of the carboxylate in the bioactive conformation[11–13] (see acid orientation

* Corresponding author. Tel.: +41 61 267 1550; fax: +41 61 267 1552.
E-mail address: beat.ernst@unibas.ch (B. Ernst).

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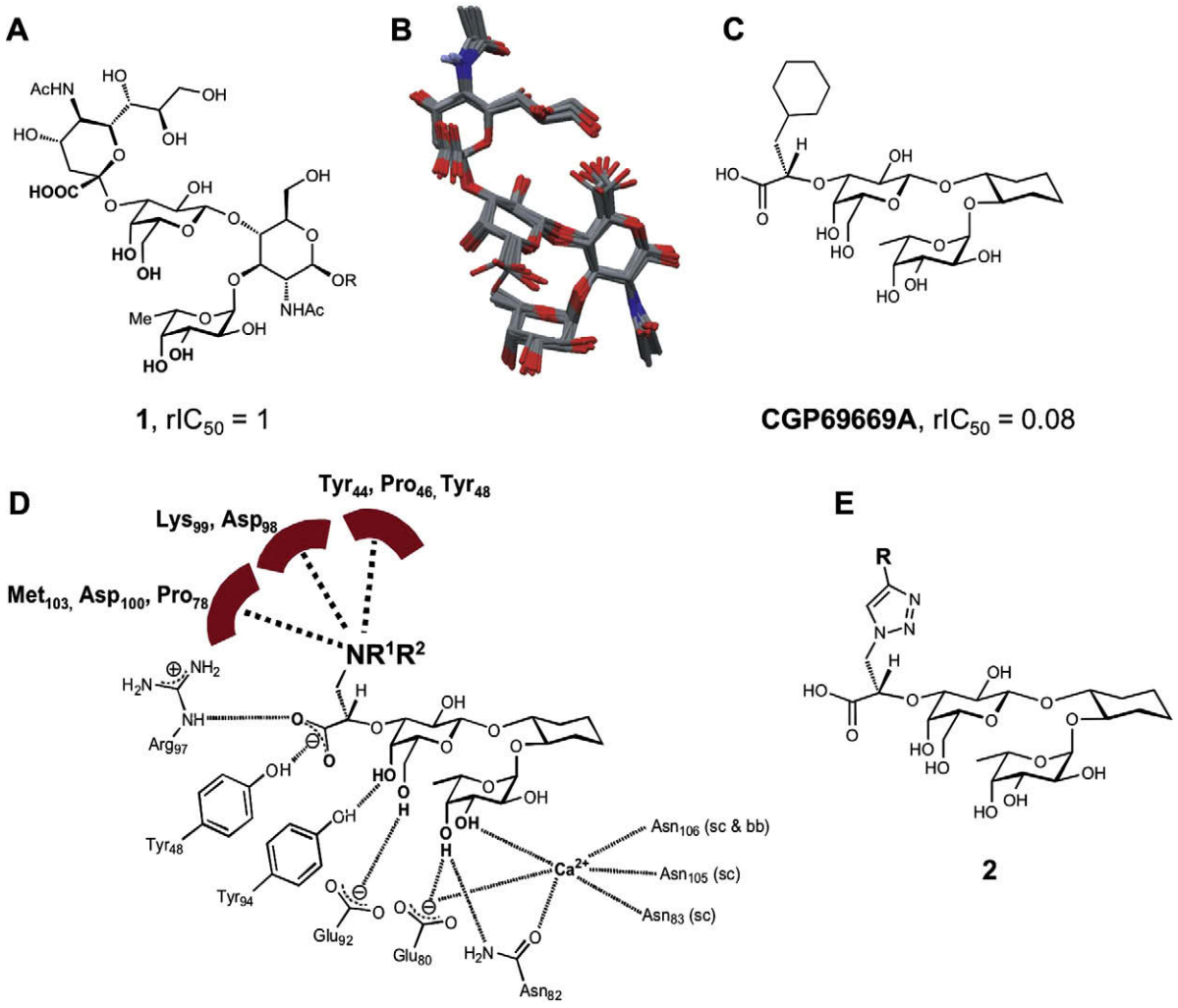


Figure 1. (A) The minimal E-selectin binding epitope: sialyl Lewis^x (**1**, sLe^x, essential pharmacophores in bold face); (B) the bioactive conformation of sLe^x as determined by trNOE NMR experiments;[11,12] (C) replacement of Neu5Ac by (*S*)-cyclohexyllactate led to **CGP69669A**[14,23] with a 12-fold improved affinity compared to **1**; (D) potential interactions of isoserine derivatives with the E-selectin binding site; sc: interactions with amino acid side chains; bb: interactions with the backbone of E-selectin; (E) E-selectin antagonists where Neu5Ac is replaced by (*S*)-isoserine modified by 1,3-dipolar cycloaddition.

in Fig. 2). Furthermore, a hydrophobic pocket formed by Tyr44, Pro46 and Tyr48 offers additional contacts for substituents of anti-substituted triazoles. Therefore, in a click chemistry approach[24,25] based on the Huisgen 1,3-dipolar cycloaddition,[26] the azido functionality in isoserine derivative **16** was reacted with an array of commercially available alkynes to obtain the desired triazoles **2** (Scheme 1).

For the synthesis of the Neu5Ac replacing moiety, the electrophile **12** is derived from (*R*)-isoserine (**9**), which was obtained from (*R*)-malic acid (**3**, Scheme 1). Acetal protection of the hydroxy group and the 1-carboxylate of **3** led to cyclohexyl-dioxolane **4** in 98% yield,[27] presenting the second carboxylic acid for a selective transformation into the acid chloride **5** and the corresponding acyl azide **6**, the precursor for a Curtius rearrangement. Under acidic conditions, the rearrangement product, the isocyanate **7**, cyclized to carbamate **8** and subsequently hydrolyzed to (*R*)-isoserine hydrochloride (**9**). The overall yield for the four steps from **4** to **9** was 51%. Carbamate **8**, formed as an intermediate, could clearly be verified by ^{1}H NMR (dd for H-2 at 5.00 ppm, corresponding to a downfield shift of 0.8 ppm compared to **9**). For the protection of the primary amine, **9** was transformed into an azide by a CH_2Cl_2-free diazo transfer (→**10**).[28] After the formation of a benzyl ester (→**11**), the triflate **12** was obtained by treatment with triflic anhydride.

The synthesis of **14** was achieved by a regioselective, Bu_2SnO-mediated alkylation of the core trisaccharide **13**[16] with triflate **12**. Deprotection by hydrogenolysis (→**15**) followed by reinstallation of the azido group with a CH_2Cl_2-free diazo transfer[28] yielded **16**. Finally, a small library of E-selectin antagonists (**2a–f**, Table 1) was obtained by click chemistry with the corresponding alkynes. Compound **2a** was obtained in low yield by cyloaddition with TMS–acetylene and subsequent fluoride mediated de-silylation.

The tetrahydro-benzotriazolyl compound **2g** was obtained by inverting the reaction sequence, that is, the fully protected azide **14** was first reacted with benzotriazolylpropyne to give **17**, followed by hydrogenolysis of the benzyl groups and partial hydrogenation of the benzotriazol moiety (Scheme 2).

The obtained sLe^x mimetics were tested in a static cell-free *in vitro* assay for their binding to E-selectin.[29] All compounds with the exception of **2e** are competitive antagonists (Table 1), with affinities in the range of sLe^x (**1**). Interestingly, azide **16** and the unsubstituted triazole **2a** show the best affinities in this series with rIC$_{50}$s of 0.29 and 0.48, respectively.

3. Conclusions

A small library of E-selectin antagonists was synthesized and biologically evaluated. The isoserine modifications were selected based on molecular modeling considerations, that is, the degree of pre-organization of the carboxylate in the bioactive conforma-

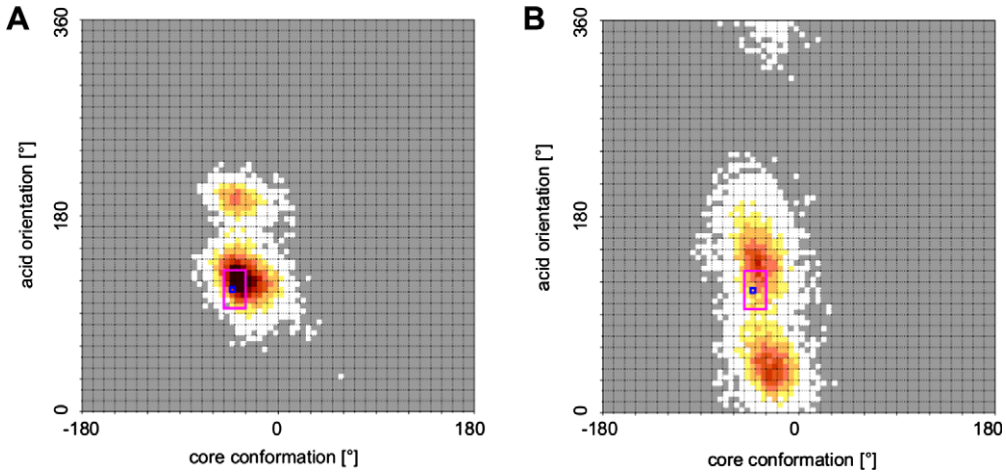
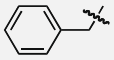
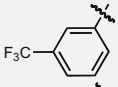
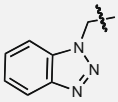
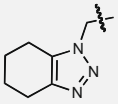


Figure 2. Histograms of the preferred core conformations/acid orientations for sLe^x (**1**, Fig. 2A) and triazole **2a** (Fig. 2B) as observed during 12 ns MD simulations using periodic boundary conditions and explicit solvent (TIP3P water, sampling frequency 1.2 ps). Area color coding: gray—unpopulated, white—low, yellow to orange—medium, red to black—high population. Pink frame indicates a bioactive region as established by transfer-NOE NMR experiment[11,12], blue frame indicates corresponding core conformation/acid orientation as observed in the crystal structure of sLe^x (**1**) complexed with E-selectin[13] (PDB-ID: 1G1T); for both compounds, the core conformation is approx. −40° and therewith in the range of the bioactive value; the acid orientation of the bound conformation of sLe^x (**1**) is approx. 110°, although it is populated by sLe^x (**1**) and triazole **2a**, some flexibility is predicted leading to entropy costs upon binding.

Table 1
Relative IC_{50}s (rIC_{50}) of the E-selectin antagonists **16** and **2a–g**

Entry	Compound	R =	rIC_{50}
1	**16**	—	0.29
2	**2a**	H	0.48
3	**2b**		1.03
4	**2c**		0.88
5	**2d**		3.13
6	**2e**		n.a.
7	**2f**		1.18
8	**2g**		1.22

Relative IC_{50}s (rIC_{50}s) were measured using **CGP69669A** (rIC_{50} = 0.08) as reference compound and are scaled on sLe^x (rIC_{50} = 1); n.a. = no competitive inhibition of **CGP69669A** binding observed.

tion and the possibility of additional contacts with a hydrophobic pocket formed by Tyr44, Pro46 and Tyr48. It was earlier reported[22] that a related set of antagonists did not substantially improve binding affinity, probably due to the zwitterionic character of an amine series and conformational restrictions of an amide series. Since triazole **2a** fulfilled the criteria of pre-organization of the carboxylate in the bioactive conformation, a library of triazoles was synthesized. Docking studies clearly showed that for anti-substituted triazoles, which are easily available by copper-catalyzed click chemistry, the above-mentioned hydrophobic pocket appeared accessible.

The library of the triazoles **2a–g** was tested in an *in vitro* assay. The affinities towards E-selectin, with the exception of **2e**, were only in the range of sLe^x (**1**). Only the unsubstituted triazole **2a** and the azido compound **16** showed slightly improved affinities. However, when substituents were added to the triazoles (**2b–g**), the observed inhibitory potency dropped. Overall, our results as well as the data published by Kolb[22] might allow the conclusion that the substituents of the triazole attached in close proximity to the essential carboxylate indeed establish additional interactions with E-selectin, but for the price of an alteration of the acid orientation. As a result, a weakening of the essential salt bridge, which was previously shown to be a prerequisite for binding,[8,13,30] and consequently a decrease in affinity is observed. Since this interpretation is based on a computational model, experimental structural data by X-ray crystallography or NMR spectroscopy of such a mimetic in complex with E-selectin is needed for a final proof of our hypothesis.

4. Experimental

4.1. General Methods

Nuclear magnetic resonance spectroscopy was performed on a Bruker Avance 500 UltraShield spectrometer at 500.13 MHz (^{1}H) or 125.76 MHz (^{13}C). Chemical shifts are expressed in ppm using residual solvent peaks[31,32] or tetramethylsilane (TMS) as references. Assignment of ^{1}H and ^{13}C NMR spectra was achieved using 2D DEPT-135, ^{1}H,^{1}H-COSY/TOCSY and ^{1}H,^{13}C-HSQC/HMBC experiments. For complex molecules, the following prefixes for substructures are used: Cy (cyclohexyl), Fuc (fucose), Gal (galactose), Lac (lactate), and Bt (benzotriazol). C^i indicates the *ipso* substituted carbons of aromatic systems. IR spectra were recorded on a Perkin–Elmer Spectrum One as KBr pellets or as films on NaCl plates. Optical rotations were measured on a Perkin–Elmer 341 polarimeter in the indicated solvents in p.a. quality. ESI mass spectra were recorded on a Waters micromass ZQ instrument. High resolution mass spectra were obtained on a ESI Bruker Daltonics micrOTOF spectrometer equipped with a TOF hexapole detector. TLC was performed using silica gel 60 coated glass plates containing fluorescence indicator (Merck KGaA, Darmstadt, Germany) and visualized by using UV light (254 nm) and/or by charring either in aqueous $KMnO_4$ solution or in a molybdate solution (a 0.02 M

solution of ammonium cerium sulfate dihydrate and ammonium molybdate tetrahydrate in aqueous 10% H_2SO_4) with heating to 140 °C for 5 min. Column chromatography was performed using silica gel 60 (0.040–0.063 mm, Fluka). Hydrogenation reactions were performed in a shaking apparatus (Parr Instruments Company, Moline, Illinois, USA) in 250 mL or 500 mL bottles with 4 bar H_2 pressure. Solvents were purchased from Fluka and dried prior to use. CH_2Cl_2 was dried by filtration through basic aluminum oxide (Fluka). Dioxane, DME, Et_2O and PhMe were dried by distillation from sodium/benzophenone. DMF was dried over activated molecular sieves and MeOH by distillation from sodium methoxide. Molecular sieves (4 Å) were activated in vacuo at 500 °C for 2 h immediately before use.

Biological data were obtained using the published ELISA procedure with **CGP69669A** as reference compound.[29]

4.1.1. *(5R)*-(2,2-Cyclohexylidene-4-oxo-1,3-dioxolan-5-yl)-acetic acid (4)

Compound **4** was prepared in analogy to Hanessian et al.[27] To a suspension of (*R*)-malic acid (**3**) (4.00 g, 29.8 mmol) in dry Et_2O (100 mL) was added cyclohexanone (3.09 mL, 29.8 mmol) at 0 °C followed by $BF_3{\cdot}Et_2O$ (5.62 mL, 44.8 mmol) via syringe. The mixture was stirred at 0 °C for 1 h and turned into a light red solution. After stirring for another 18 h at rt, Et_2O (100 mL) was added, and the organic phase was washed with aqueous sodium acetate (3 × 10 mL, 10% w/v). The combined aqueous phases were extracted with Et_2O (2 × 50 mL). The combined organic layers were dried over Na_2SO_4 and the solvent evaporated in vacuo affording **4** (6.25 g; 98%) as slightly red colored crystals. The analytical data were identical to those found in literature.[33]

$[\alpha]_D$ −2.9 (*c* 1.0, $CHCl_3$); ^{1}H NMR (500.1 MHz, $CDCl_3$): δ 10.60 (br s, RCO_2H), 4.72 (X of ABX, *J* = 3.9, 6.5 Hz, 1H, H-2), 3.00 (A of ABX, *J* = 3.9, 17.2 Hz, 1H, H-3a), 2.85 (B of ABX, *J* = 6.5, 17.2 Hz, 1H, H-3b), 1.90–1.81 (m, 2H, C_5H_{10}), 1.80–1.60 (m, 6H, C_5H_{10}), 1.55–1.35 (m, 2H, C_5H_{10}); ^{13}C NMR (125.8 MHz, $CDCl_3$): δ 175.1, 172.0 (2 CO), 112.2 (C-ketal), 70.0 (C-2), 36.2 (2C, C_5H_{10}), 35.3 (C-3), 24.4 (C_5H_{10}), 23.4 (2C, C_5H_{10}); IR (KBr): 3208 (m), 2939 (m), 1802 (s), 1733 (s), 1375 (m), 1291 (m), 1197 (s), 932 (m) cm^{-1}; ESI-MS Calcd for $C_{10}H_{13}O_5$ $[M-H]^-$: 213.1; found: 213.0.

4.1.2. (*R*)-3-Amino-2-hydroxy-propionic acid hydrochloride (9)

Compound **4** (6.25 g, 29.2 mmol) was dissolved in $SOCl_2$ (48 mL) with strong gas evolution. The solution was heated to reflux and the gas evolution ceased after 15 min. After refluxing the dark solution for 1 h, the solvent was evaporated and the residue dried under high vacuum over night. Compound **5** was obtained as a brownish oil, dissolved in acetone (56 mL) and cooled to −20 °C. A solution of sodium azide (2.56 g, 39.4 mmol) in water (16 mL) was added dropwise to the solution via syringe and the mixture was stirred for 1.5 h. After removal of the acetone in vacuo at 0 °C, water (150 mL) was added and the aqueous phase was extracted with PhMe (3 × 200 mL). The combined organic layers containing **6** were dried (Na_2SO_4) and concentrated to a final volume of ca. 75 mL. The remaining solution was slowly heated to reflux for 1 h to yield **7** after removal of the solvent. After addition of aqueous HCl (6 N, 35 mL) to crude **7**, the mixture was refluxed for 7 h, then the solvent removed in vacuo, the residue taken up in water (100 mL) and washed with CH_2Cl_2 (1 × 100 mL, 2 × 50 mL). The combined organic phases were extracted with aqueous HCl (3 N, 2 × 50 mL). The aqueous phases were combined and the water removed by evaporation to give a brownish oil. Precipitation from PhMe/EtOAc/MeOH gave **9** as off-white solid (2.10 g, 51%).

$[\alpha]_D$ +9.9 (*c* 0.48, H_2O); ^{1}H NMR (500.1 MHz, D_2O): δ 4.52 (X of ABX, *J* = 4.1, 8.4 Hz, 1H, H-2), 3.45 (A of ABX, *J* = 4.0, 13.3 Hz, 1H, H-3a), 3.23 (B of ABX, *J* = 8.4, 13.3 Hz, 1H, H-3b); ^{13}C NMR (125.8 MHz, DMSO-*d*6): δ 172.6 (C-1), 67.0 (C-2), 41.7 (C-3); IR (film): 3422 (s), 1735 (s), 1623 (m), 1234 (m), 1150 (m), 1079 (m) cm^{-1}; ESI-MS Calcd for $C_3H_8NO_5$ $[M-Cl]^+$: 106.1; found: 106.0.

4.1.3. *(R)*-3-Azido-2-hydroxy propanoic acid (10)

To a solution of **9** (363 mg, 2.56 mmol), $NaHCO_3$ (893 mg, 10.6 mmol) and $CuSO_4{\cdot}5H_2O$ (24 mg, 0.10 mmol) in water (2 mL) were added TfN_3 in PhMe (1 M, 3.1 mL) and MeOH (12 mL).[28] After stirring for 21 h, the organic solvents were removed, and the residue was taken up in water (20 mL). The olive green mixture (pH 8) was washed with CH_2Cl_2 (20 mL) and acidified with 6 N HCl. The resulting light red mixture was extracted with EtOAc (3 × 80 mL). The combined ester phases were dried over Na_2SO_4, filtered and the solvent was removed in vacuo to give 635 mg of a 1:1.1 mixture of **10** (83%) and $TfNH_2$. The material was used in the next step without further purification. The ^{1}H NMR data were identical to those found in literature.[34]

^{1}H NMR (500.1 MHz, DMSO-d_6): δ 4.22 (X of ABX, *J* = 3.6, 5.8 Hz, 1H, H-2), 3.47 (A of ABX, *J* = 3.5 12.8 Hz, 1H, H-3a), 3.40 (B of ABX, *J* = 5.9, 12.8 Hz, 1H, H-3b); ^{13}C NMR (125.8 MHz, DMSO-d_6): δ 173.2 (C-1), 69.8 (C-2), 53.5 (C-3); IR (KBr): 2117 (s, N_3), 1733 (s, CO) cm^{-1}; ESI-MS Calcd for $C_3H_4N_3O_3$ $[M-H]^-$: 130.0; found: 129.9.

4.1.4. Benzyl *(R)*-3-azido-2-hydroxy propanoate (11)

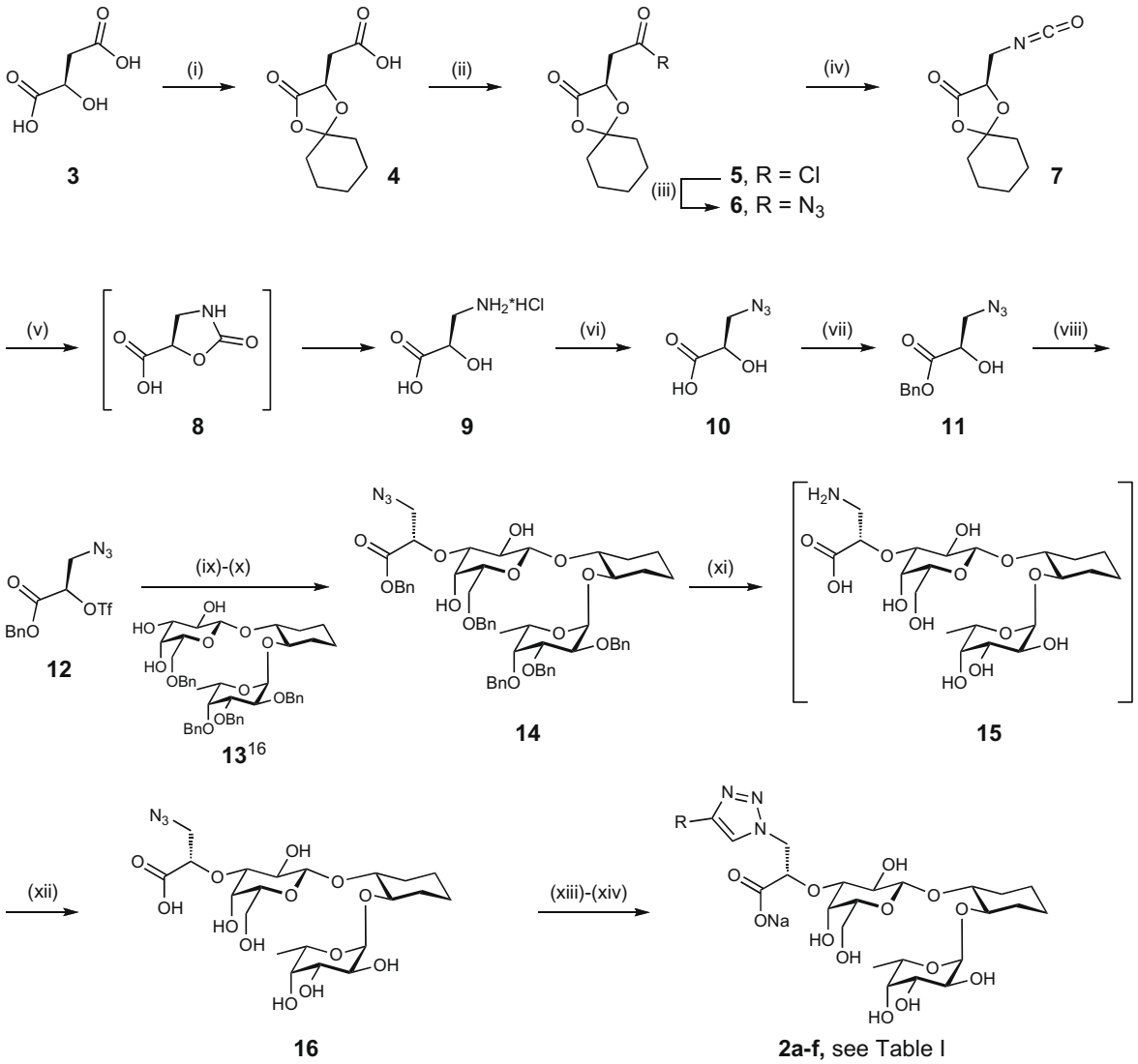
To a solution of crude **10** (3.97 g) in DMF (30 mL) was added benzyl bromide (13 mL, 110 mmol) followed by dropwise addition of NEt_3 (19 mL, 137 mmol) at rt. Upon addition of the base, the exothermic reaction needed to be cooled with a water bath at rt. After stirring for 1 d, the mixture was concentrated, diluted with water (100 mL) and extracted with CH_2Cl_2 (3 × 100 mL). The combined organic phases were dried over Na_2SO_4, filtered and the solvents removed in vacuo. The crude product was purified by column chromatography (petrol ether/EtOAc, 4:1) to give **11** (1.92 g, 63%) as white solid. The ^{1}H NMR data were identical to those found in literature.[22]

$[\alpha]_D$ +82.9 (*c* 1.04, $CHCl_3$); ^{1}H NMR (500.1 MHz, $CDCl_3$): δ 7.41–7.39 (m, 5H, Ar–H), 5.26 (s, 2H, PhCH_2), 4.40 (m, 1H, H-2), 3.64 (A of ABX, *J* = 3.3, 12.8 Hz, 1H, H-3a), 3.50 (B of ABX, *J* = 4.3, 12.8 Hz, 1H, H-3b); ^{13}C NMR (125.8 MHz, $CDCl_3$): δ 172.3 (C-1), 134.9 (Ar–C^i), 129.02, 128.96, 128.67 (5C, Ar–C), 70.4 (C-2), 68.3 (PhCH_2), 54.0 (C-3); IR (KBr): 3392 (m), 2110 (s, N_3), 1739 (s, CO), 1199 (s) cm^{-1}; ESI-MS Calcd for $C_{10}H_{11}N_3O_3Na$ $[M+Na]^+$: 244.1; found: 243.9.

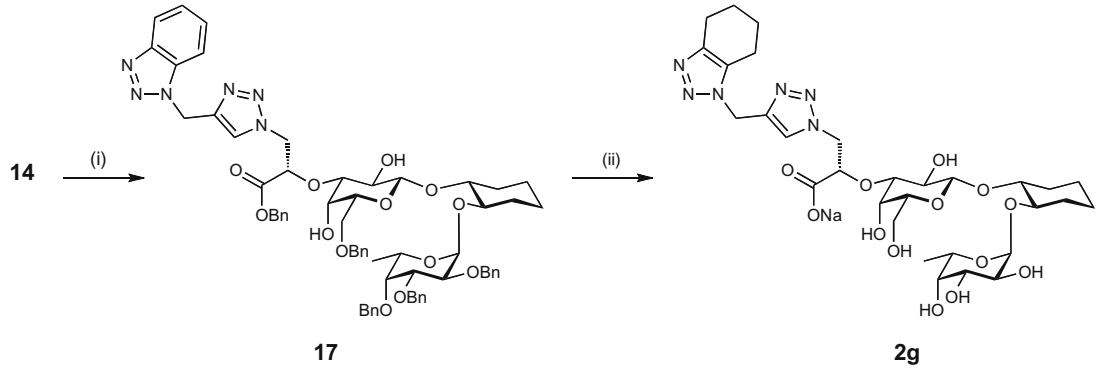
4.1.5. Benzyl *(R)*-3-azido-2-*O*-trifluoromethanesulfonyl-propanoate (12)

To a solution of **11** (1.00 g, 4.52 mmol) in dry CH_2Cl_2 (30 mL) under argon was added 2,6-di-*tert*-butylpyridine (1.73 mL, 7.69 mmol) at −20 °C. After dropwise addition of Tf_2O (1.29 mL, 7.69 mmol), the reaction was stirred for 3 h at −20 °C. Then, the reaction was allowed to warm to 0 °C. After stirring for 1 h, additional 2,6-di-*tert*-butylpyridine (1.73 mL, 7.69 mmol) and Tf_2O (1.29 mL, 7.69 mmol) were added at 0 °C. After stirring for 3 h, the reaction mixture was diluted with CH_2Cl_2 (100 mL), washed with ice-cold aqueous KH_2PO_4 (1 M, 50 mL) and the aqueous phase was extracted with CH_2Cl_2 (2 × 100 mL). The combined organic layers were dried over Na_2SO_4 and the solvent was evaporated in vacuo. Purification by column chromatography (petrol ether/EtOAc, 10:1) yielded **12** (1.40 g, 88%) as colorless oil. The ^{1}H and ^{13}C NMR data were identical to those found in literature.[22]

^{1}H NMR (500.1 MHz, $CDCl_3$): δ 7.35–7.41 (m, 5H, ArH), 5.31, 5.28 (A, B of AB, *J* = 12.0 Hz, 2H, PhCH_2), 5.23 (X of A′B′X, *J* = 3.6, 6.0 Hz, 1H, H-2), 3.84 (A′ of A′B′X, *J* = 3.6, 13.9 Hz, 1H, H-3a), 3.81 (B′ of A′B′X, *J* = 6.0, 13.9 Hz, 1H, H-3b); ^{13}C NMR (125.8 MHz, $CDCl_3$): δ 164.4 (C-1), 133.9 (Ar–C^i), 129.1, 128.8, 128.6 (5C, Ar–C), 118.4 (q, *J* = 320 Hz, CF_3), 81.0 (C-2), 69.0 (PhCH_2), 51.5 (C-3); ESI-MS Calcd for $C_{11}H_{10}F_3N_3O_5SNa$ $[M+Na]^+$: 376.0; found: 376.0.



Scheme 1. Reagents and conditions: (i) Cyclohexanone, $BF_3{\cdot}Et_2O$, Et_2O, 0 °C to rt, 1 d (98%); (ii) $SOCl_2$, reflux, 30 min (→**5**); (iii) NaN_3, H_2O, acetone, −20 °C, 1 h (→**6**); (iv) PhMe, reflux, 30 min (→**7**); (v) 6 M HCl, reflux, 6 h (→**9**, 51% over four steps); (vi) TfN_3, $CuSO_4$, $NaHCO_3$, PhMe/H_2O/MeOH, rt, 24 h; (vii) BnBr, NEt_3, DMF, rt, 1 d (63% over two steps); (viii) Tf_2O, 2,6-^{t}Bu$_2$py, CH_2Cl_2, −20 °C, 5 h (88%); (ix) **13**[16], Bu_2SnO, MeOH, mol. sieves 3 Å, reflux, 15 h; (x) **12**, CsF, DME, rt, 6 d (39% over two steps); (xi) $Pd(OH)_2$/C, H_2, THF, H_2O, 4 bar, rt, 72 h (95%); (xii) TfN_3, $CuSO_4$, $NaHCO_3$, PhMe/H_2O/MeOH, rt, 22 h (36%); for **2a**: (xiii) TMS–acetylene, DIPEA, CuCl, MeCN/H_2O, 9 d, rt; (xiv) (a) TBAF, HOAc/THF, rt, 30 min; (b) 1 equiv NaOH, (19% over two steps); for **2b–f**; (xiii) (a) alkyne, Cu, $CuSO_4$, EtOH/H_2O, 24 h, rt; (b) 1 equiv NaOH; yields for **2b** (97%), **2c** (97%), **2d** (42%), **2e** (94%), **2f** (91%).



Scheme 2. Reagents and conditions: (i) 1-Benzotriazolyl-prop-2-yne, Cu, MeOH/H_2O, 48 h, rt (86%); (ii) (a) $Pd(OH)_2$/C, H_2, dioxane/H_2O, 4 bar, rt, 24 h; (b) 1 equiv NaOH (39%).

4.1.6. Benzyl (2*S*)-3-azido-2-*O*-{1-*O*-[(*1R,2R*)-2-*O*-(2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)-cyclohexyl]-6-*O*-benzyl-β-D-galactopyranos-3-yl}-propionate (14)

Compound **13**[16] (667 mg, 0.85 mmol) and Bu_2SnO (235 mg, 0.94 mmol) were dried in high vacuum for 1 h and subsequently dissolved in dry MeOH (50 mL) under argon with warming. Activated molecular sieves (3 Å, 2.00 g) were added to the solution, which was then refluxed under argon for 15 h. Filtration of the suspension over Celite, evaporation of the solvent, co-evaporation with dry PhMe and drying for 8 h in high vacuum gave a yellow oily substance (880 mg). This residue was dissolved in dry DME (10 mL) under argon and a solution of dry **12** (384 mg, 1.09 mmol) in dry DME (4 mL) was added. When extensively dried CsF (168 mg, 1.11 mmol) was added, the solution turned slightly turbid. After stirring for 6 d at rt, TLC indicated a ratio of acceptor to product of ca. 1:1, but no triflate. A solution of KF (10%) in aqueous KH_2PO_4 (1 M, 50 mL) was added and after stirring for 1 h, the reaction was extracted with CH_2Cl_2 (3 × 50 mL). The combined organic phases were dried over Na_2SO_4 and the solvents were removed in vacuo. Purification of the residue by column chromatography (CH_2Cl_2/*i*PrOH, gradient 0–4%) yielded **14** (329 mg, 39%) as a white solid.

^{1}H NMR (500.1 MHz, $CDCl_3$): δ 7.37–7.23 (m, 25 H, Ar–H), 5.30 (m, 1H, Lac-H2), 5.23, 5.15 (A, B of AB, *J* = 12.1 Hz, 2H, PhC*H*$_2$OCO), 4.96–4.94 (m, 2H, Fuc-H1, 1H of PhC*H*$_2$), 4.80 (A″ of A″B″, *J* = 11.6 Hz, 1H, PhC*H*$_2$), 4.74 (A‴ of A‴B‴, *J* = 11.9 Hz, 1H, PhC*H*$_2$), 4.68 (B″ of A″B″, B‴ of A‴B‴, *J* = 11.7 Hz, 2H, PhC*H*$_2$), 4.60 (B′ of A′B′, *J* = 11.0 Hz, 1H, PhC*H*$_2$), 4.57 (M of ABM, *J* = 2.2, 5.2 Hz, 1H, Lac-H2), 4.52, 4.48 (A⁗, B⁗ of A⁗B⁗, *J* = 12.0 Hz, 1H, PhC*H*$_2$), 4.38 (q, *J* = 7.8 Hz, 1H, Fuc-H5), 4.30 (d, *J* = 7.7 Hz, 1H, Gal-H1), 4.02–3.97 (m, 3H, Fuc-H2, -H3, Gal-H4), 3.80 (t, *J* = 8.3 Hz, 1H, Gal-H2), 3.76–3.74 (m, 2H, 1H of Cy–CH, Gal-H6a), 3.67 (m, 1H, Fuc-H4), 3.65–3.50 (m, 5H, 1H of Cy–CH, Gal-H5, -H6b, Lac-H3), 3.43 (dd, *J* = 2.1, 9.3 Hz 1H, Gal-H3), 2.02–1.81 (m, 2H, Cy–CH_2), 1.67–1.60 (m, 2H, Cy–CH_2), 1.39–1.12 (m, 4H, Cy–CH_2), 1.10 (d, *J* = 6.3 Hz, 3H, Fuc-H6); ^{13}C NMR (125.8 MHz, $CDCl_3$): δ 170.5 (Lac-C1), 139.2, 138.9, 138.8, 138.0, 134.8 (5C, Ar–C^i), 128.8–127.2 (25C, Ar–CH), 100.2 (Gal-C1), 94.6 (Fuc-C1), 82.8 (Gal-C3), 79.7 (CH), 79.0 (Lac-C2), 78.2 (Fuc-C4), 77.2 (Gal-C2), 76.3 (Cy–CH), 76.1 (CH), 74.9, 73.7 (2 PhCH_2), 73.2 (CH), 73.01, 72.97 (2 PhCH_2), 71.1 (CH), 68.9 (Gal-C6), 67.6 (Ph–CH_2–OCO), 67.3 (CH), 66.3 (Fuc-C5), 52.8 (Lac-C3), 30.1, 29.7, 29.2, 23.3 (4C, Cy–CH_2), 16.6 (Fuc-C6); IR (KBr): 2923 (s), 2106 (s, N_3), 1742 (s, CO), 1453 (m) cm^{-1}; ESI-MS Calcd for $C_{56}H_{65}N_3O_{13}Na$ $[M+Na]^+$: 1010.4; found: 1010.5.

4.1.7. (2*S*)-3-Amino-2-*O*-{1-*O*-[(*1R,2R*)-2-*O*-(-L-fucopyranosyl)-cyclohexyl]-β-D-galactopyranos-3-yl}-propanoic acid (15)

To a solution of **14** (293 mg, 297 μmol) in THF/water (5 mL, 4:1) was added $Pd(OH)_2$/C (50 mg) and the reaction mixture was hydrogenated at 4 bar for 72 h at rt. Additional $Pd(OH)_2$/C (100 mg) was added and the mixture hydrogenated at 4 bar for another 48 h. After dilution with MeOH/water (1:1, 20 mL), the mixture was filtered and the solvents were removed to give crude **15** (144 mg), which was used without further purification.

^{1}H NMR (500.1 MHz, D_2O): δ 4.99 (d, *J* = 3.9 Hz, 1H, Fuc-H1), 4.63 (q, *J* = 6.6 Hz, 1H, Fuc-H5), 4.52 (d, *J* = 7.8 Hz, 1H, Gal-H1), 4.29 (dd, *J* = 3.9, 8.2 Hz, 1H, Lac-H2), 4.04 (d, *J* = 2.8 Hz, 1H, Gal-H4), 3.91 (dd, *J* = 6.6, 10.4 Hz, 1H, Fuc-H3), 3.79 (dd, *J* = 3.0, 9.3 Hz, 1H, Fuc-H2), 3.77–3.71 (m, 4H, Fuc-H4, Gal-H5, -H6a, 1H of Cy–CH), 3.65–3.60 (m, 2H, Gal-H2, -H6b), 3.55 (dd, *J* = 3.2, 9.6 Hz, 1H, Gal-H3), 3.51 (m, 1H, 1H of Cy–CH), 3.41 (dd, *J* = 3.9, 13.8 Hz, 1H, Lac-H3a), 3.22 (dd, *J* = 8.3, 13.4 Hz, 1H, Lac-H3b), 2.14–2.04 (m, 2H, Cy–CH_2), 1.69 (br s, 2H, Cy–CH_2), 1.33–1.21 (m, 4H, Cy–CH_2), 1.19 (d, *J* = 6.8 Hz, 3H, Fuc-H6); ^{13}C NMR (125.8 MHz, D_2O): δ 171.0 (Lac-C1), 100.1 (Gal-C1), 95.9 (Fuc-C1), 82.9 (Gal-C3), 79.0 (Cy–CH), 77.6 (2C, Lac-C2, Cy–CH), 74.7, 72.4, 70.8 (Fuc-C4, Gal-C2, -C5), 69.9 (Fuc-C3), 68.2 (Fuc-C2), 66.9 (Gal-C4), 66.8 (Fuc-C5), 61.8 (Gal-C6), 42.2 (Lac-C3), 29.9, 29.6, 29.5, 23.5 (4 Cy–CH_2), 15.6 (Fuc-C6); HR-MS Calcd for $C_{21}H_{36}NO_{13}$ $[M-H]^-$: 510.2192; found: 510.2197.

4.1.8. Sodium (2*S*)-3-azido-2-*O*-{1-*O*-[(*1R,2R*)-2-*O*-(-L-fucopyranosyl)-cyclohexyl]-β-D-galactopyranos-3-yl}-propionate (16)

To a solution of crude **15** (126 mg) in MeOH/water (3 mL, 7.5:1) was added a solution of TfN_3[28] in PhMe (0.91 mL, 0.36 M). After stirring for 22 h at rt, the reaction mixture was concentrated and purified by column chromatography (CH_2Cl_2/MeOH/water, 10:4:0.8) to give **16** (79 mg, contaminated with silica gel). This product was used for the 1,3-dipolar cycloaddition reactions without further purification. For analysis and biological testing, a small sample (6.6 mg) was purified by preparative HPLC to give pure **16** (4.7 mg, 35%, two steps) as free acid. The sodium salt was obtained by addition of an equimolar amount of aqueous NaOH (1 M, 87 μL) to an aqueous solution of the acid.

Analytical data for the free acid: $[\alpha]_D$ −112.1 (*c* 0.24, MeOH); ^{1}H NMR (500.1 MHz, CD_3OD): δ 4.85 (d, *J* = 3.8 Hz, 1H, Fuc-H1), 4.62 (q, *J* = 6.5 Hz, 1H, Fuc-H5), 4.51 (dd, *J* = 3.5, 5.4 Hz, 1H, Lac-H2), 4.30 (d, *J* = 7.7 Hz, 1H, Gal-H1), 4.00 (d, *J* = 2.7 Hz, 1H, Gal-H4), 3.87 (dd, *J* = 3.4, 9.9 Hz, 1H, Fuc-H3), 3.78–3.74 (m, 2H, Lac-H3a, Gal-H6a), 3.73–3.63 (m, 5H, Fuc-H2, -H4, Gal-H2, -H6b, 1H of Cy–CH), 3.57–3.51 (m, 2H, 1H of Cy–CH, Lac-H3b), 3.45–3.41 (m, 2H, Gal-H3, -H5), 2.05–2.03 (m, 2H, Cy–CH_2), 1.71 (br s, 2H, Cy–CH_2), 1.41–1.22 (m, 4H, Cy–CH_2), 1.18 (d, *J* = 6.4 Hz, 3H, Fuc-H6); ^{13}C NMR (125.8 MHz, CD_3OD): δ 175.1 (Lac-C1), 102.8 (Gal-C1), 97.5 (Fuc-C1), 84.8 (Gal-C3), 80.5 (Lac-C2), 79.4 (CH), 77.5 (Cy–CH), 76.2 (Gal-C5), 74.0 (CH), 72.4 (Gal-C2), 71.7 (Fuc-C3), 70.2 (CH), 68.6 (Gal-C4), 67.6 (Fuc-C5), 62.9 (Gal-C6), 54.3 (Lac-C3), 31.1, 30.2, 24.6 (4C, Cy–CH_2), 16.7 (Fuc-C6); IR (KBr, Na-form of **5**): 3412 (s), 2111 (s, N_3), 1606 (s, CO), 1447 (m) cm^{-1}; HR-MS Calcd for $C_{21}H_{34}N_3O_{13}$ $[M-H]^-$: 536.2097; found: 536.2091.

4.1.9. Sodium (2*S*)-3-*N*-(1,2,3-triazol-1-yl)-2-*O*-{1-*O*-[(*1R,2R*)-2-*O*-(-L-fucopyranosyl)-cyclohexyl]-β-D-galactopyranos-3-yl}-propionate (2a)

A solution of crude **16** (14 mg) and DIPEA (13.7 μL, 80 μmol) in MeCN/water (1.5 mL, 2:1) was thoroughly degassed by bubbling through argon. Subsequently, TMS–acetylene (7.4 μL, 54 μmol) and CuCl (5.3 mg, 80 μmol) were added and the mixture was stirred under argon. After 4 d, additional CuCl (5.3 mg, 80 μmol) and TMS–acetylene (15 μL, 110 μmol) were added and the reaction was stirred for another 5 d. The solvents were removed in vacuo, the residue was taken up in MeOH, filtered (0.2 μm) and evaporated. After drying for 2 h in high vacuum, the residue was taken up in TBAF in THF (1 M, 1 mL) and HOAc was added (0.2 mL). The reaction was stirred for 30 min at rt. Evaporation of the solvent and purification of the residue by preparative HPLC, followed by ion-exchange chromatography (Dowex-50, Na-form) and a second preparative HPLC gave 2.0 mg (19%, two steps) of pure **2a** as the free acid. The sodium salt was obtained by addition of equimolar amounts of aqueous NaOH to an aqueous solution of the acid.

Analytical data for the free acid: ^{1}H NMR (500.1 MHz, D_2O): δ 8.08 (s, 1H, triazol-H), 7.78 (s, 1H, triazol-H), 4.95 (d, *J* = 3.5 Hz, 1H, Fuc-H1), 4.90 (dd, *J* = 3.4, 14.1 Hz 1H, Lac-H3a), 4.76 (m, 1H, Lac-H3b), 4.63–4.57 (m, 2H, Fuc-H5, Lac-H2), 4.41 (d, *J* = 7.0 Hz, 1H, Gal-H1), 4.01 (d, *J* = 1.7 Hz, 1H, Gal-H4), 3.87 (dd, *J* = 3.4, 10.4 Hz, 1H, Fuc-H3), 3.77–3.65 (m, 5H, Fuc-H2, -H4, Gal-H6, 1H of Cy–CH), 3.54 (dd, *J* = 4.2, 7.4 Hz, 1H, Gal-H5), 3.50–3.43 (m, 3H, Gal-H2, -H3, 1H of Cy–CH), 2.09–2.02 (m, 2H, Cy–CH_2), 1.69–1.63 (m, 2H, Cy–CH_2), 1.27–1.17 (m, 4H, Cy–CH_2), 1.15 (d, *J* = 6.9 Hz, 3H, Fuc-H6); ^{13}C NMR (125.8 MHz, D_2O): δ 174.9 (Lac-

C1), 99.7 (Gal-C1), 95.5 (Fuc-C1), 82.1 (Gal-C3), 78.3 (CH), 78.1 (CH), 77.2 (Lac-C2), 74.3 (CH), 72.0 (Gal-C5), 70.2 (Fuc-C3), 69.5 (Fuc-C2), 67.9 (Gal-C4), 67.6 (Fuc-C5), 66.4 (2C, 2 CH), 61.3 (Gal-C6), 52.0 (Lac-C3), 29.6, 29.1, 23.1 (4C, 4 Cy–CH_2), 15.2 (Fuc-C6); HR-MS Calcd for $C_{23}H_{37}N_3O_{13}Na$ $[M+H]^+$: 586.2224; found: 586.2214.

4.1.10. Sodium (2*S*)-3-*N*-[4-benzyl-1,2,3-triazol-1-yl]-2-*O*-{1-*O*-[(*1R,2R*)-2-*O*-(-L-fucopyranosyl)-cyclohexyl]-β-D-galactopyranos-3-yl}-propionate (2b)

Crude **16** (7.1 mg) was dissolved in a solution of 1-phenyl-prop-2-yne (16.4 μL, 132 μmol) in EtOH/water (1 mL, 3:2). Copper powder (1.0 mg, 15.7 μmol) and $CuSO_4{\cdot}5H_2O$ (3.5 mg, 14 μmol) were added, and the reaction was vigorously stirred for 24 h at rt. Filtration through cotton, evaporation of the solvents, and microfiltration (0.2 μm) of a solution of the residue in MeOH/water (1:1) gave the crude product, which was purified by preparative HPLC. Pure **2b** was obtained as free acid (5.4 mg, 97%) and was subsequently transferred into the sodium salt by addition of equimolar amounts of NaOH.

Analytical data for the free acid: $[\alpha]_D$ −67.7 (*c* 0.27, MeOH); ^{1}H NMR (500.1 MHz, CD_3OD): δ 8.00 (s, 1H, triazol-H), 7.30–7.22 (m, 4H, Ar–H), 7.19 (m, 1H, *p*-Ar–H), 4.88–4.84 (m, 2H, Fuc-H1, Lac-H3a), 4.71 (m, 1H, Lac-H3b), 4.62–4.56 (m, 2H, Fuc-H5, Lac-H2), 4.26 (d, *J* = 7.7 Hz, 1H, Gal-H1), 4.03 (s, 2H, PhCH_2), 3.93 (m, 1H, Gal-H4), 3.86 (dd, *J* = 3.3, 10.0 Hz, 1H, Fuc-H3), 3.77–3.64 (m, 5H, Fuc-H2, -H4, Gal-H6, 1H of Cy–CH), 3.61–3.52 (m, 2H, Gal-H2, 1H of Cy–CH), 3.41 (m, 1H, Gal-H5), 3.33 (m, 1H, Gal-H3), 2.07–2.01 (m, 2H, Cy–CH_2), 1.73–1.67 (m, 2H, Cy–CH_2), 1.40–1.22 (m, 4H, Cy–CH_2), 1.18 (d, *J* = 6.5 Hz, 3H, Fuc-H6); ^{13}C NMR (125.8 MHz, CD_3OD): δ 174.5 (Lac-C1), 140.7 (triazol-C^i), 131.4 (Ar–C^i), 129.8, 129.7 (4 Ar–CH), 127.6 (2C, triazol-*C*H, Ar–CH), 102.8 (Gal-C1), 97.4 (Fuc-C1), 84.9 (Gal-C3), 79.5 (Lac-C2), 77.5 (Cy–CH), 76.1 (Cy–CH), 74.0 (Gal-C5), 72.1 (Fuc-C4), 71.7 (Gal-C2), 70.5 (Fuc-C3), 68.0 (Fuc-C2), 67.6 (2C, Fuc-C5, Gal-C4), 63.0 (Gal-C6), 53.7 (Lac-C3), 32.7 (Ph*C*H_2), 31.1, 30.9, 24.6 (4C, 4 Cy–CH_2), 16.8 (Fuc-C6); HR-MS Calcd for $C_{30}H_{43}N_3O_{13}Na$ $[M+H]^+$: 676.2694; found: 676.2696.

4.1.11. Sodium (2*S*)-3-*N*-[4-(3-thiophenyl)-1,2,3-triazol-1-yl]-2-*O*-{1-*O*-[(*1R,2R*)-2-*O*-(α-L-fucopyranosyl)-cyclohexyl]-β-D-galactopyranos-3-yl}-propionate (2c)

In analogy to **2b**, compound **2c** was prepared from crude **16** (7.1 mg) and 3-ethynyl-thiophene (12.7 μL, 129 μmol). After HPLC, 5.3 mg (97%) of the free acid were obtained, which was transferred into the sodium salt as described above.

Analytical data for the free acid: $[\alpha]_D$ −60.8 (*c* 0.27, MeOH); ^{1}H NMR (500.1 MHz, CD_3OD): δ 8.54 (s, 1H, triazol-H), 7.75 (m, 1H, Ar–H), 7.50–7.48 (m, 2H, Ar–H), 4.98 (dd, *J* = 2.9, 14.3 Hz, 1H, Lac-H3a), 4.84 (d, *J* = 3.9 Hz, 1H, Fuc-H1), 4.77 (dd, *J* = 6.8, 14.2 Hz, 1H, Lac-H3b), 4.64 (dd, *J* = 2.9, 6.7 Hz, 1H, Lac-H2), 4.58 (q, *J* = 6.6 Hz, 1H, Fuc-H5), 4.28 (d, *J* = 7.7 Hz, 1H, Gal-H1), 3.97 (d, *J* = 2.7 Hz, 1H, Gal-H4), 3.86 (dd, *J* = 3.4, 10.1 Hz, 1H, Fuc-H3), 3.75 (dd, *J* = 6.9, 11.4 Hz, 1H, Gal-H6a), 3.72–3.62 (m, 5H, Fuc-H2, -H4, Gal-H2, Gal-H6b, 1H of Cy–CH), 3.54 (dt, *J* = 4.5, 9.4 Hz, 1H, 1H of Cy–CH), 3.44–3.38 (m, 2H, Gal-H3, -H5), 2.07–2.01 (m, 2H, Cy–CH_2), 1.74–1.66 (m, 2H, Cy–CH_2), 1.43–1.22 (m, 4H, Cy–CH_2), 1.15 (d, *J* = 6.7 Hz, 3H, Fuc-H6); ^{13}C NMR (125.8 MHz, CD_3OD): δ 145.1 (triazol-C^i), 133.2 (Ar–C^i), 127.6, 127.0 (2C, Ar–C), 124.2 (triazol-*C*H), 122.2 (Ar–C), 102.7 (Gal-C1), 97.4 (Fuc-C1), 85.0 (Gal-C3), 79.5 (CH), 77.5 (Cy–CH), 76.0 (Gal-C5), 74.0 (CH), 72.0 (Gal-C2), 71.7 (Fuc-C3), 70.2 (CH), 67.9 (Gal-C4), 67.6 (Fuc-C5), 63.0 (Gal-C6), 54.1 (Lac-C3), 31.1, 30.2, 24.6 (4C, 4 Cy–CH_2), 16.8 (Fuc-C6); HR-MS Calcd for $C_{27}H_{39}N_3O_{13}SNa$ $[M+H]^+$: 668.2101; found: 668.2100.

4.1.12. Sodium (2*S*)-3-*N*-[4-(3-pyridyl)-1,2,3-triazolyl]-2-*O*-{1-*O*-[(*1R,2R*)-2-*O*-(α-L-fucopyranosyl)-cyclohexyl]-β-D-galactopyranos-3-yl}-propionate (2d)

In analogy to **2b**, compound **2d** was prepared from crude **16** (7.1 mg) and 3-ethynyl-pyridine (15 mg, 145 μmol). After HPLC, 2.2 mg (42%) of the free acid were obtained, which was transferred into the sodium salt as described above.

Analytical data for the free acid: $[\alpha]_D$ −44.7 (*c* 0.11, MeOH); ^{1}H NMR (500.1 MHz, CD_3OD): δ 9.04 (br s, 1H, Ar–H), 8.78 (s, 1H, triazol-H), 8.52 (br s, 1H, Ar–H), 8.30 (d, *J* = 7.6 Hz, 1H, Ar–H), 7.53 (m, 1H, Ar–H), 5.02 (dd, *J* = 2.8, 14.5 Hz, 1H, Lac-H3a), 4.83 (d, *J* = 3.5 Hz, 1H, Fuc-H1), 4.79 (dd, *J* = 7.1, 14.2 Hz, 1H, Lac-H3b), 4.72 (dd, *J* = 2.7, 7.0 Hz, 1H, Lac-H2), 4.59 (q, *J* = 6.7 Hz, 1H, Fuc-H5), 4.27 (d, *J* = 7.2 Hz, 1H, Gal-H1), 3.98 (d, *J* = 2.3 Hz, 1H, Gal-H4), 3.85 (dd, *J* = 3.1, 9.8 Hz, 1H, Fuc-H3), 3.75 (dd, *J* = 7.2, 11.7 Hz, 1H, Gal-H6a), 3.71–3.65 (m, 4H, Fuc-H2, -H4, Gal-H6b, 1H of Cy–CH), 3.62 (t, *J* = 8.0 Hz, 1H, Gal-H2), 3.52 (dt, J = 4.4, 9.4 Hz, 1H, 1H of Cy–CH), 3.44–3.38 (m, 2H, Gal-H3, -H5), 2.07–2.00 (m, 2H, Cy–CH_2), 1.72–1.65 (m, 2H, Cy–CH_2), 1.41–1.19 (m, 4H, Cy–CH_2), 1.13 (d, *J* = 6.5 Hz, 3H, Fuc-H6); ^{13}C NMR (125.8 MHz, CD_3OD): δ 147.2, 135.1, 126.1, 124.8 (4C, Ar–*C*H), 102.3 (Gal-C1), 97.0 (Fuc-C1), 84.3 (Gal-C3), 78.6 (Lac-C2), 77.0 (CH), 75.9 (CH), 73.1 (CH), 71.9 (CH), 71.3 (Fuc-C3), 71.2 (CH), 67.5 (Gal-C4), 66.7 (Fuc-C5), 62.8 (Gal-C6), 54.1 (Lac-C3), 31.1, 30.2, 24.6 (4C, 4 Cy–CH_2), 16.8 (Fuc-C6); HR-MS Calcd for $C_{28}H_{40}N_4O_{13}Na$ $[M+H]^+$: 663.2490; found: 663.2490.

4.1.13. Sodium (2*S*)-3-*N*-[4-(3-trifluoromethyl-phenyl)-1,2,3-triazol-1-yl]-2-*O*-{1-*O*-[(*1R,2R*)-2-*O*-(α-L-fucopyranosyl)-cyclohexyl]-β-D-galactopyranos-3-yl}-propionate (2e)

In analogy to **2b**, compound **2e** was prepared from crude **16** (7.1 mg) and 3-ethynyl-trifluoromethylbenzene (18.7 μL, 130 μmol). After HPLC, 5.9 mg (94%) of the free acid were obtained, which was transferred into the sodium salt as described above.

Analytical data for the free acid: $[\alpha]_D$ −52.9 (*c* 0.27, MeOH); ^{1}H NMR (500.1 MHz, CD_3OD): δ 8.75 (s, 1H, triazol-H), 8.16 (s, 1H, Ar–H), 8.10 (m, 1H, Ar–H), 7.65–7.61 (m, 2H, Ar–H), 5.01 (dd, *J* = 2.3, 13.7 Hz, 1H, Lac-H3a), 4.83 (d, *J* = 3.6 Hz, 1H, Fuc-H1), 4.82–4.76 (m, 2H, Lac-H2, -H3b), 4.59 (q, *J* = 6.7 Hz, 1H, Fuc-H5), 4.27 (d, *J* = 7.6 Hz, 1H, Gal-H1), 3.99 (d, *J* = 2.5 Hz, 1H, Gal-H4), 3.85 (dd, *J* = 3.3, 10.1 Hz, 1H, Fuc-H3), 3.75 (dd, *J* = 6.9, 11.4 Hz, 1H, Gal-H6a), 3.72–3.64 (m, 4H, Fuc-H2, -H4, Gal-H6b, 1H of Cy–CH), 3.63 (m, 1H, Gal-H2), 3.52 (dt, *J* = 4.3, 9.4 Hz, 1H, 1H of Cy–CH), 3.41–3.31 (m, 2H, Gal-H3, -H5), 2.04–1.99 (m, 2H, Cy–CH_2), 1.68 (br s, 2H, Cy–CH_2), 1.41–1.21 (m, 4H, Cy–CH_2), 1.13 (d, *J* = 6.6 Hz, 3H, Fuc-H6); ^{13}C NMR (125.8 MHz, CD_3OD): δ 174.5 (Lac-C1), 147.4 (triazol-C^i), 133.2 (Ar–C^i), 131.0, 130.4 (2 Ar–*C*H), 125.8 (triazol-CH), 125.0, 123.4 (2 Ar–C), 102.8 (Gal-C1), 97.5 (Fuc-C1), 84.7 (Gal-C3), 79.6 (Lac-C2), 79.0 (Cy–CH), 77.6 (Cy–CH), 76.1 (Gal-C5), 74.0 (Fuc-C4), 72.2 (Gal-C2), 71.7 (Fuc-C3), 70.2 (Fuc-C2), 68.2 (Gal-C4), 67.5 (Fuc-C5), 62.9 (Gal-C6), 53.8 (Lac-C3), 31.1, 30.3, 24.6 (4C, 4 Cy–CH_2), 16.7 (Fuc-C6); HR-MS Calcd for $C_{30}H_{40}F_3N_3O_{13}Na$ $[M+H]^+$: 730.2411; found: 730.2419.

4.1.14. Sodium (2*S*)-3-*N*-[4-(benzotriazolylmethyl)-1,2,3-triazol-1-yl]-2-*O*-{1-*O*-[(*1R,2R*)-2-*O*-(α-L-fucopyranosyl)-cyclohexyl]-β-D-galactopyranos-3-yl}-propionate (2f)

In analogy to **2b**, compound **2f** was prepared from crude **16** (7.1 mg) and 1-*N*-benzotriazolyl-prop-2-yne (20.7 mg, 132 μmol). After HPLC, 5.4 mg (91%) of the free acid were obtained, which was transferred into the sodium salt as described above.

Analytical data for the free acid: $[\alpha]_D$ −53.7 (*c* 0.27, MeOH); ^{1}H NMR (500.1 MHz, CD_3OD): δ 8.37 (br s, 1H, triazol-H), 7.98 (A of ABCD, *J* = 7.5 Hz, 1H, Bt–H), 7.81 (D of ABCD, *J* = 7.5 Hz, 1H, Bt–H), 7.55 (C of ABCD, *J* = 7.5 Hz, 1H, Bt–H), 7.43 (B of ABCD, *J* = 7.5 Hz, 1H, Bt–H), 6.03 (br s, 2H, Bt–*C*H_2), 4.92–4.86 (m, 2H, Fuc-H1, Lac-

H3a), 4.74 (dd, J = 6.5, 13.5 Hz, 1H, Lac-H3b), 4.62 (m, 1H, Lac-H2), 4.59 (q, J = 6.4 Hz, 1H, Fuc-H5), 4.24 (d, J = 7.8 Hz, 1H, Gal-H1), 3.93 (m, 1H, Gal-H4), 3.87 (dd, J = 3.5, 10.0 Hz, 1H, Fuc-H3), 3.77–3.65 (m, 5H, Fuc-H2, -H4, Gal-H6, 1H of Cy–CH), 3.61–3.53 (m, 2H, Gal-H2, 1H of Cy–CH), 3.40 (m, 1H, Gal-H5), 3.32 (m, 1H, Gal-H3), 2.08–2.01 (m, 2H, Cy–CH_2), 1.71 (br s, 2H, Cy–CH_2), 1.42–1.22 (m, 4H, Cy–CH_2), 1.18 (d, J = 6.7 Hz, 3H, Fuc-H6); ^{13}C NMR (125.8 MHz, CD_3OD): δ 176.8 (Lac-C1), 147.2 (Bt–C^i), 142.9 (triazol-C^i), 134.4 (Bt–C^i), 139.1 (Bt–CH), 127.4 (triazol-CH), 125.9 (Bt–CH), 120.1 (Bt–CH), 112.0 (Bt–CH), 102.7 (Gal-C1), 97.4 (Fuc-C1), 85.1 (Gal-C3), 79.5 (Cy–CH), 78.7 (Lac-C2), 77.4 (Cy–CH), 76.0 (Gal-C5), 74.0 (Fuc-C4), 72.8 (Gal-C2), 71.7 (Fuc-C3), 70.2 (Fuc-C2), 67.9 (Gal-C4), 67.6 (Fuc-C5), 63.0 (Gal-C6), 53.8 (Lac-C3), 44.6 (Bt–CH_2), 30.8, 24.4 (4C, 4 Cy–CH_2), 16.8 (Fuc-C6); HR-MS Calcd for $C_{30}H_{42}N_6O_{13}Na$ $[M+Na]^+$: 717.2708; found: 717.2705.

4.1.15. Benzyl (2*S*)-3-*N*-[4-(benzotriazolylmethyl)-1,2,3-triazol-1-yl]-2-*O*-{1-*O*-[(*1R,2R*)-2-*O*-(2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)-cyclohexyl] 6-*O*-benzyl-β-D-galactopyranos-3-yl}-propionate (17)

3-Benzotriazolyl-propyne (95 mg, 60.7 μmol) was dissolved in MeOH/water (1 mL, 9:1) and copper powder (1 mg) was suspended in this solution. A solution of **14** (10 mg, 10.1 μmol) in MeOH/water (1 mL, 9:1) was added and the reaction mixture was stirred for 24 h at rt. Additional copper powder (5 mg) was added and stirring continued for further 24 h before the solvent was removed in vacuo. Purification by column chromatography (PhMe/EtOAc 1:1) yielded **17** (10 mg, 86%) as a white solid.

^{1}H NMR (500.1 MHz, $CDCl_3$): δ 7.97–7.92 (m, 1H, Bt–H), 7.85 (br s, 1H, triazol-H), 7.59 (m, 1H, Bt–H), 7.38 (m, 1H, Bt–H), 7.32–7.13 (m, 26H, 25 Ar–H, 1 Bt–H), 5.80 (br s, 2H, Bt–CH_2), 5.13, 5.06 (A, B of AB, J = 11.9 Hz, 2H, PhCH_2OCO), 4.88–4.86 (m, 2H, Fuc-H1, 1H of PhCH_2), 4.74–4.70 (m, 2H, 1H of PhCH_2, Lac-H3a), 4.66 (A‴ of A‴B‴, J = 12.0 Hz, 1H, PhCH_2), 4.61 (B″ of A″B″, B‴ of A‴B‴, J = 11.7 Hz, 2H, PhCH_2), 4.53 (B′ of A′B′, J = 11.5 Hz, 1H, PhCH_2), 4.50–4.46 (m, 2H, Lac-H2, -H3b), 4.42, 4.38 (A‴, B⁗ of A⁗B⁗, J = 12.0 Hz, 2H, PhCH_2), 4.31 (q, J = 6.3 Hz, 1H, Fuc-H5), 4.07 (d, J = 7.7 Hz, 1H, Gal-H1), 3.94 (dd, J = 3.5, 10.1 Hz, 1H, Fuc-H2), 3.89 (dd, J = 2.7, 10.1 Hz, 1H, Fuc-H3), 3.77 (d, J = 2.5 Hz, 1H, Gal-H4), 3.66–3.60 (m, 3H, Fuc-H4, Gal-H6a, 1H of Cy–CH), 3.57–3.51 (m, 3H, Gal-H2, -H6b, 1H of Cy–CH), 3.31 (t, J = 6.0 Hz, 1H, Gal-H5), 3.39 (dd, J = 2.7, 9.3 Hz, 1H, Gal-H3), 1.97–1.87 (m, 2H, Cy–CH_2), 1.66–1.56 (m, 2H, Cy–CH_2), 1.35–1.13 (m, 4H, Cy–CH_2), 1.01 (d, J = 6.5 Hz, 3H, Fuc-H6); ^{13}C NMR (125.8 MHz, $CDCl_3$): δ 170.2 (Lac-C1), 139.4, 139.1, 139.0, 138.2, 134.5 (5C, Ar–C^i), 129.1–127.5 (Ar–C), 124.4, 120.0, 110.4 (3C, Bt–C), 100.7 (Gal-C1), 95.0 (Fuc-C1), 83.6 (Gal-C3), 79.9 (Fuc-C3), 78.4 (Fuc-C4), 77.9 (Cy–CH), 77.4 (Lac-C2), 76.6 (Fuc-C2), 76.3 (Cy–CH), 75.1, 73.8, 73.2 (4C, 4 PhCH_2), 73.0 (Gal-C5), 70.6 (Gal-C2), 69.0 (Gal-C6), 68.4 (PhCH_2–O–CO), 66.7 (Gal-C4), 66.4 (Fuc-C5), 52.3 (Lac-C3), 44.0 (Bt–CH_2), 30.2, 29.9, 29.4, 23.4 (4C, 4 Cy–CH_2), 16.9 (Fuc-C6); ESI-MS Calcd for $C_{65}H_{72}N_6O_{13}Na$ $[M+Na]^+$: 1167.5; found: 1167.7.

4.1.16. Sodium (2*S*)-3-*N*-[4-(tetrahydrobenzotriazolylmethyl)-1,2,3-triazol-1-yl]-2-*O*-{1-*O*-[(*1R,2R*)-2-*O*-(α-L-fucopyranosyl)-cyclohexyl]-β-D-galacto-pyranos-3-yl}-propionate (2g)

To a solution of **17** (10 mg, 8.7 μmol) in dioxane/water (3 mL, 4:1) was added $Pd(OH)_2$/C (18 mg) and the reaction mixture was hydrogenated at 4 bar for 48 h at rt. After filtration and removal of the solvents, the crude product was purified by preparative HPLC to give the free acid of **2g** (2.4 mg, 39%). The sodium salt was obtained as a white powder by addition of stoichiometric amounts of NaOH (1 N, 30 μL) to an aqueous solution of **2g** followed by lyophilization.

Analytical data for the free acid: $[\alpha]_D$ −40.8 (c 0.12, MeOH); ^{1}H NMR (500.1 MHz, CD_3OD): δ 8.27 (br s, 1H, triazol-H), 5.57 (br s, 2H, 4H-Bt–CH_2), 4.93–4.84 (m, 2H, Fuc-H1, Lac-H3a), 4.75 (dd, J = 5.9, 14.6 Hz, 1H, Lac-H3b), 4.62 (m, 1H, Lac-H2), 4.59 (q, J = 6.8 Hz, 1H, Fuc-H5), 4.27 (d, J = 8.0 Hz, 1H, Gal-H1), 3.94 (m, 1H, Gal-H4), 3.86 (dd, J = 3.6, 9.9 Hz, 1H, Fuc-H3), 3.77–3.65 (m, 5H, Fuc-H2, -H4, Gal-H6, 1H of Cy–CH), 3.61–3.52 (m, 2H, Gal-H2, 1H of Cy–CH), 3.41 (t, J = 5.6 Hz, 1H, Gal-H5), 3.35 (m, 1H, Gal-H3), 2.70–2.64 (m, 4H, 4H-Bt–H), 2.07–2.02 (m, 2H, Cy–CH_2), 1.87–1.77 (m, 4H, 4H–Bt–H), 1.73–1.68 (m, 2H, Cy–CH_2), 1.41–1.22 (m, 4H, Cy–CH_2), 1.18 (d, J = 6.6 Hz, 3H, Fuc-H6); HR-MS Calcd for $C_{30}H_{46}N_6O_{13}Na$ $[M+Na]^+$: 721.3021; found: 721.3021.

4.2. Molecular modeling

The three-dimensional structures of all compounds were generated using MacroModel.[35] Next, a conformational search was performed to identify the global minimum conformation by sampling a total of 10,000 structures (MacroModel, mixed torsional/low-mode sampling method, extended torsional sampling, OPLS 2005 force-field,[36] implicit water solvent model). A periodic boundary system was created by placing the global minimum in a box of pre-organized TIP3P water molecules. The system charge was neutralized and sodium and chloride ions were added to reach physiological electrolyte concentration of 0.15 M. Special attention was paid to building proper solvation shell around the solute: First, the solvent environment was minimized using a gradient criterion of 0.1 kcal/mol followed by a 24 ps molecular dynamics (MD) simulation, so that the water molecules could reorganize around the solute (geometry of the solute was kept fixed). The whole system was then completely minimized using a gradient criterion of 0.05 kcal/mol. A 12 ns MD simulation was performed using NPT ensemble and standard conditions (T = 300 K, p = 101.325 kPa) with frames sampled every 1.2 ps. All MD simulations were done using Desmond.[37–39] The statistical analysis of the structural data was performed from the 9950 frames collected during the MD run (first 50 frames were skipped due to equilibration of the system).

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2009.11.024.

References and notes

1. Kansas, G. S. *Blood* **1996**, *88*, 3259.
2. Varki, A. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 7390.
3. Mousa, S. A. *Drugs Future* **1996**, *21*, 283.
4. Mousa, S. A.; Cheresh, D. A. *Drug Discovery Today* **1997**, *2*, 187.
5. Ernst, B.; Magnani, J. L. *Nat. Rev. Drug Disc.* **2009**, *8*, 661.
6. Phillips, M. L.; Nudelman, E.; Gaeta, F. C.; Perez, M.; Singhal, A. K.; Hakomori, S.; Paulson, J. C. *Science* **1990**, *250*, 1130.
7. Walz, G.; Aruffo, A.; Kolanus, W.; Bevilacqua, M.; Seed, B. *Science* **1990**, *250*, 1132.
8. Ernst, B.; Kolb, H. C.; Schwardt, O. In *The Organic Chemistry of Sugars*; Levy, D. E., Fügedi, P., Eds.; CRC Press/Taylor & Francis: Boca Raton, 2006; p 828.
9. Kaila, N.; Thomas, B. E. I. V. *Med. Res. Rev.* **2002**, *22*, 566.
10. Simanek, E. E.; McGarvey, G. J.; Jablonowski, J. A.; Wong, C. H. *Chem. Rev.* **1998**, *98*, 833.
11. Rinnbauer, M.; Ernst, B.; Wagner, B.; Magnani, J.; Benie, A. J.; Peters, T. *Glycobiology* **2003**, *13*, 435.

12. Scheffler, K.; Ernst, B.; Katopodis, A.; Magnani, J. L.; Wang, W. T.; Weisemann, R.; Peters, T. *Angew. Chem., Int. Ed.* **1995**, *34*, 1841.
13. Somers, W. S.; Tang, J.; Shaw, G. D.; Camphausen, R. T. *Cell* **2000**, *103*, 467.
14. Kolb, H. C.; Ernst, B. *Chem. Eur. J.* **1997**, *3*, 1571.
15. Thoma, G.; Magnani, J. L.; Patton, J. T.; Ernst, B.; Jahnke, W. *Angew. Chem., Int. Ed.* **2001**, *40*, 1941.
16. Titz, A.; Patton, J.; Alker, A. M.; Porro, M.; Schwardt, O.; Hennig, M.; Francotte, E.; Magnani, J.; Ernst, B. *Bioorg. Med. Chem.* **2008**, *16*, 1046.
17. Titz, A.; Ernst, B. *Chimia* **2007**, *61*, 194.
18. Chandrasekaran, E. V.; Jain, R. K.; Matta, K. L. *J. Biol. Chem.* **1992**, *267*, 23806.
19. Nicolaou, K. C.; Bockovich, N. J.; Carcanague, D. R. *J. Am. Chem. Soc.* **1993**, *115*, 8843.
20. Yoshida, M.; Suzuki, T.; Ishida, H.; Kiso, M.; Hasegawa, A. *J. Carbohydr. Chem.* **1996**, *15*, 147.
21. Yuen, C. T.; Lawson, A. M.; Chai, W.; Larkin, M.; Stoll, M. S.; Stuart, A. C.; Sullivan, F. X.; Ahern, T. J.; Feizi, T. *Biochemistry* **1992**, *31*, 9126.
22. Kolb, H. C., WO 9701569, 1997.
23. Norman, K. E.; Anderson, G. P.; Kolb, H. C.; Ley, K.; Ernst, B. *Blood* **1998**, *91*, 475.
24. (a) Kolb, H. C.; Finn, M. G.; Sharpless, K. B. *Angew. Chem., Int. Ed.* **2001**, *40*, 2004; (b) Kolb, H. C.; Sharpless, K. B. *Drug Discovery Today* **2003**, *8*, 1128.
25. (a) Tornøe, C. W.; Meldal, M. Peptides: The Wave of the Future. In *Proceedings of the Second International and the Seventeenth American Peptide Symposium*; Lebl, M., Houghten, R. A., Eds.; Springer, 2001; p 263; (b) Tornøe, C. W.; Christensen, C.; Meldal, M. *J. Org. Chem.* **2002**, *67*, 3057.
26. Huisgen, R.; Knorr, R.; Moebius, L.; Szeimies, G. *Chem. Ber.* **1965**, *98*, 4014.
27. Hanessian, S.; Tehim, A.; Chen, P. *J. Org. Chem.* **1993**, *58*, 7768.
28. Titz, A.; Radic, Z.; Schwardt, O.; Ernst, B. *Tetrahedron Lett.* **2006**, *47*, 2383.
29. Thoma, G.; Magnani, J. L.; Oehrlein, R.; Ernst, B.; Schwarzenbach, F.; Duthaler, R. O. *J. Am. Chem. Soc.* **1997**, *119*, 7414.
30. Musser, J. H.; Rao, N.; Nashed, M.; Dasgupta, F.; Abbas, S.; Nematalla, A.; Date, V.; Foxall, C.; Asa, D. *Pharmacochem. Lib.* **1993**, *20*, 33.
31. Gottlieb, H. E.; Kotlyar, V.; Nudelman, A. *J. Org. Chem.* **1997**, *62*, 7512.
32. Hesse, M.; Meier, H.; Zeeh, B. In *Spectroscopic Methods in Organic Chemistry*; Stuttgart: New York: Thieme, 1995; p 364.
33. Song, H. Y.; Joo, J. M.; Kang, J. W.; Kim, D.-S.; Jung, C.-K.; Kwak, H. S.; Park, J. H.; Lee, E.; Hong, C. Y.; Jeong, S.; Jeon, K.; Park, J. H. *J. Org. Chem.* **2003**, *68*, 8080.
34. Dureault, A.; Tranchepain, I.; Depezay, J. C. *Synthesis* **1987**, *5*, 491.
35. MacroModel—MacroModel, version 9.6, Schrödinger, LLC, New York, NY, 2008.
36. Kaminski, G. A.; Friesner, R. A.; Tirado-Rives, J.; Jorgensen, W. J. *J. Phys. Chem. B* **2001**, *105*, 6474.
37. Desmond Molecular Dynamics System, version 2.0, D. E. Shaw Research, New York, NY, 2008.
38. Maestro-Desmond Interoperability Tools, version 2.0, Schrödinger, New York, NY, 2008.
39. Bowers, K. J.; Chow, E.; Xu, H.; Dror, R. O.; Eastwood, M. P.; Gregersen, B. A.; Klepeis, J. L.; IKolossvary, I.; Moraes, M. A.; Sacerdoti, F. D.; Salmon, J. K.; Shan, Y.; David E. Shaw, D. E. In Proceedings of the ACM/IEEE Conference on Supercomputing (SC06), Tampa, Florida, November 11–17, 2006.