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"Click" Saccharide/β-Lactam Hybrids for Lectin Inhibition

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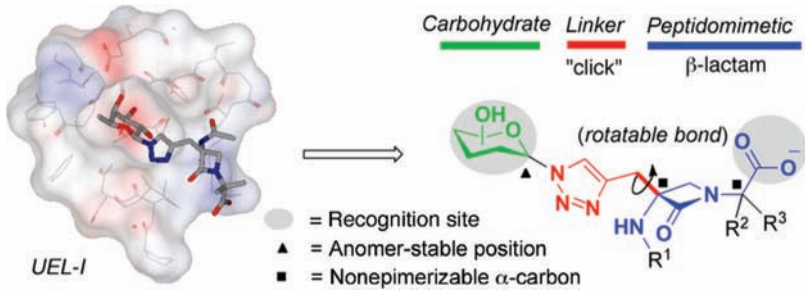
ABSTRACT



Hybrid glycopeptide β-lactam mimetics designed to bind lectins or carbohydrate recognition domains in selectins have been prepared according to a "shape-modulating linker" design. This approach was implemented using the azide−alkyne "click" cycloaddition reaction, and as shown by NMR/MD experiments, binding of the resulting mimetics to *Ulex Europaeus Lectin-1* (UEL-1) occurred after a "bent-to-extended" conformational change around a partially rotatable triazolylmethylene moiety.

Hybrid constructs[1] of natural or unnatural molecular entities are an important source of molecular diversity. This approach often takes advantage of the inherent biological activity of all or part of the components embodied in the conjugates. For a successful design, however, it is not enough to decide which fragments to incorporate into the hybrid but also to know where to place them. Therefore, a precision crafting is often necessary to generate hybrids permitting an efficient molecular recognition of each and every bioactive fragment, especially when the "rigid scaffold" design is used (Figure 1). We would like to outline here the advantages of an alternative "shape-modulating linker" design which provides partially flexible hybrids by connecting their rigid(ified) components with rotatable covalent bonds. As an example

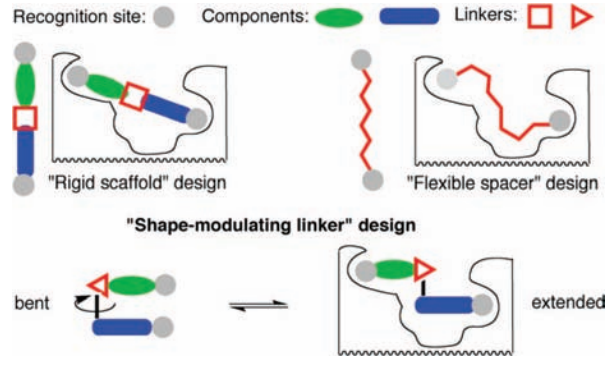


Figure 1. Semirigid hybrids: "shape-modulating linker" design.

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(1) For reviews, see: (a) Mehta, G.; Singh, V. *Chem. Soc. Rev.* **2002**, *31*, 324–334. (b) Tietze, L.; Bell, H. P.; Chandrasekhar, S. *Angew. Chem., Int. Ed.* **2003**, *42*, 3996–4028. (c) Meunier, B. *Chem. Rev.* **2008**, *104*, 69–77.

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of this design principle, we describe a novel family of saccharide/β-lactam hybrids for lectin inhibition.

Lectins[2] are naturally occurring nonenzymatic saccharide-binding proteins. They are involved in a variety of recognition events, including cell−cell interactions. For instance, selectins[3] gained attention a decade ago, when it was demonstrated that the adhesion of the tetrasaccharide sialylLewis-x (sLe^X) **1** (Figure 2)[4] to the carbohydrate-recogni-

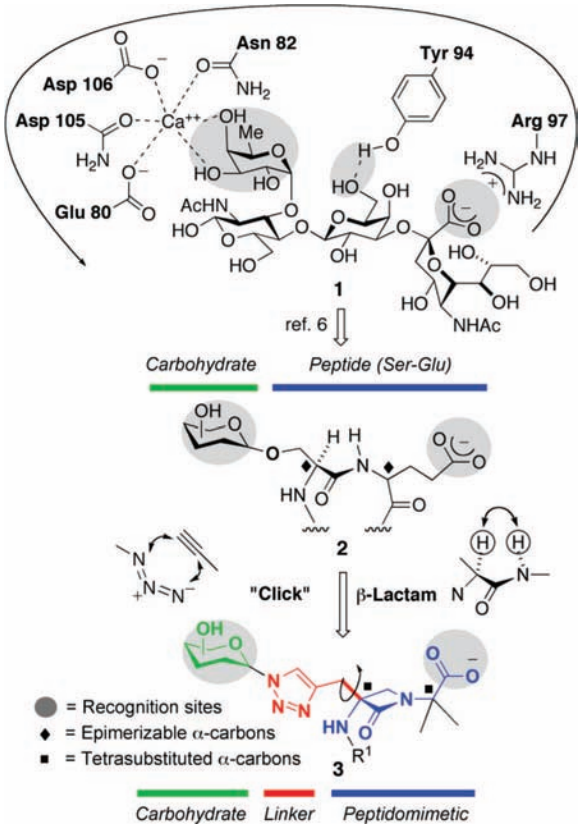
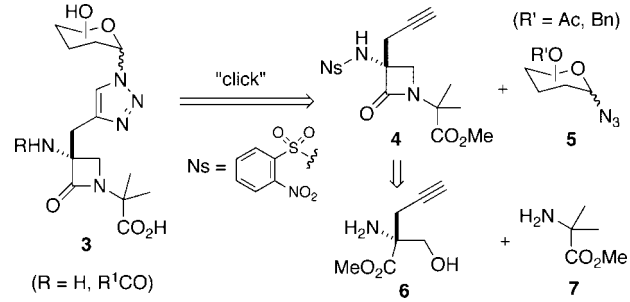


Figure 2. Design of Ser-Glu dipeptide-based mimetics **2** using the interaction pattern of sLe^x **1** with E-Selectin (top). "Shape-modulating linker"/β-lactam approach to semirigid saccharide/β-lactam hybrids **3** (bottom).

tion domain (CRD) of E-selectin regulated the rolling, tethering and transmigration of leukocytes on the vascular endothelium. Irregular and excessive infiltration of leukocytes is at the origin of acute and chronic inflammatory diseases such as asthma, psoriasis, and rheumatoid arthritis.[5]

Low molecular weight carbohydrate−peptide mimetics related to β-turned Ser-Glu dipeptide *O*-glycoside hybrids **2** have shown increased activity and good selectivity against several lectin receptors.[6] Unfortunately, excessive flexibility of the peptide backbone, biodegradability arising from glycosidic or peptidic linkages, and epimerization of the α-carbons are important drawbacks of this approach. Seeking for peptidomimetics with more favorable pharmacodynamic profiles, we have been focused over the past few years on the design and synthesis of peptidomimetics based on α-branched-α-amino-β-lactam scaffolds[7] which are expected to exhibit rigidified backbones and simultaneous enhanced resistance to chemical and enzymatic hydrolysis by proteases owing to the presence of the α,α-disubstitution pattern at the azetidin-2-one ring. Now, we report the synthesis of saccharide/β-lactam hybrids **3** incorporating the 1,2,3-triazolylmethyl moiety[8] as the shape-modulating linker.

Scheme 1. Retrosynthesis of Saccharide β-Lactam Hybrids **3**



Retrosynthetically (Scheme 1), compounds **3** were disconnected at the 1,2,3-triazole ring and divided into the readily available glycosyl azides **5**[9] and α-(*o*-nosylamino)-α-propargyl-β-lactam **4**. The synthesis of the latter was planned from the α-propargylserinate **6** and aminoester **7**, according to the procedure developed in our laboratory.[7b]

(2) Lis, H.; Sharon, N. *Chem. Rev.* **1998**, *98*, 637–674.

(3) Simanek, E.; McGarvey, G.; Jablonowski, J.; Wong, C. *Chem. Rev.* **1998**, *98*, 833–862.

(4) (a) Somers, W.; Tang, J.; Shaw, G.; Camphausen, R. *Cell* **2000**, *103*, 467–479. (b) Kranich, R.; Busemann, A. S.; Bock, D.; Schroeter-Maas, S.; Beyer, D.; Heinemann, B.; Meyer, M.; Schierhorn, K.; Zahtten, R.; Wolf, G.; Aydt, E. M. *J. Med. Chem.* **2007**, *50*, 1101–1115, (and references therein)

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(7) (a) Palomo, C.; Aizpurua, J. M.; Benito, A.; Miranda, J. I.; Fratila, R. M.; Matute, C.; Domercq, M.; Gago, F.; Martin-Santamaria, S.; Linden, A. *J. Am. Chem. Soc.* **2003**, *125*, 16243–16260. (b) Palomo, C.; Aizpurua, J. M.; Balentová, E.; Jiménez, A.; Oyarbide, J.; Fratila, R.; Miranda, J. I. *Org. Lett.* **2007**, *9*, 101–104.

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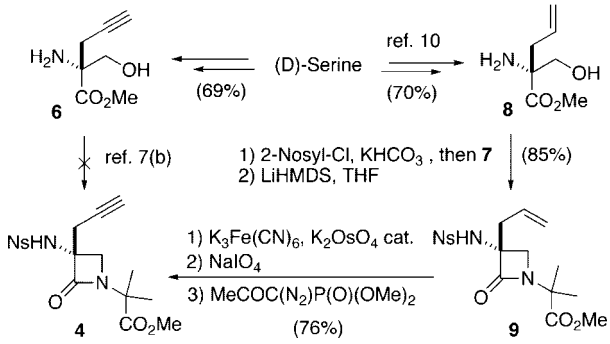
Scheme 2. Preparation of β-Lactam Alkyne **4** from D-Serine



α-Propargyl serinate **6** (Scheme 2) could be prepared from D-serine in 69% yield using Seebach's 1,3-oxazolidine enolate alkylation method.[10] However, attempts to transform it into the key α-propargyl-β-lactam method[7b] proved inefficient.[11]

Alternatively, we found that α-allyl serinate **8** cyclized with **7** to the α-allyl-β-lactam **9** in excellent yield after two synthetic operations. The allyl group of azetidin-2-one **9** was oxidized to the corresponding α-acetaldehyde which, in turn, was submitted "in situ" to the Ohira−Bestmann alkynylation[12] with dimethyl acetyldiazomethylphosphonate reagent, to afford the expected β-lactam alkyne **4** in 76% overall yield.

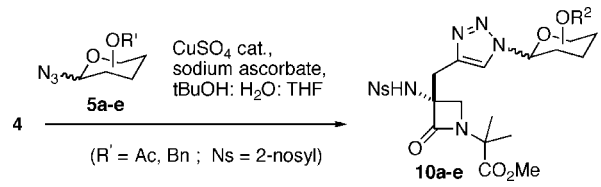
"Click" cycloaddition reaction of propargyl-β-lactam **4** with O-protected 2-azidosugars **5a**−**e** derived from D-mannose and L-fucose (Table 1) led to a clean and completely anomer-specific reaction to form the β-lactam glycoconjugates[13] **10a**−**e** in excellent yields. As shown in Table 1, this chemically effective and operatively simple method was applicable to the α- and β-anomers of both *O*-acetyl- and *O*-benzyl-protected 1-azido-D-mannoses **5a**−**c** (entries 1−3) and 1-azido-L-fucoses **5d**,**e** (entries 4 and 5).

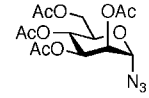
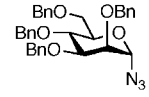
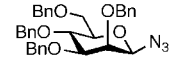
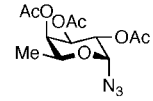
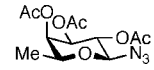
Conjugates **10a** and **10e** were submitted to N-denosylation[14] and "in situ" N-acylation, followed by O-deprotection to get the desired ligands (**15**−**18**) for lectin binding test (Table 2).

Importantly, the "click" cycloaddition method could also be extended to unprotected 1-azidosugars. As illustrated in Scheme 3, the reaction of β-1-azido-L-fucose **19** with the α-acetamido-α-propargyl-β-lactam resulting from the N-denosylation of **4** provided the glycopeptidomimetic **20** in excellent overall yield. Saponification of **20** with LiOH in aqueous THF gave a product identical to **17**.

The demonstration that these molecules could act as true glycomimetics of modulable shape was evaluated by a combined NMR/docking approach employing a model fucose-binding lectin, *Ulex Europaeus Lectin I* (UEL-I), as receptor. In fact, a clear interaction was found (Figure 3;

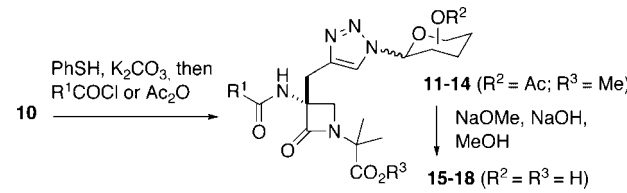
Table 1. "Click" Synthesis of α-(*N*-Glycosyltriazolyl)-β-lactams **10a**−**e** from α-Propargyl-β-lactam **4** and Glycosyl Azides **5**



entry	5		product	yield (%)[a]	$[\alpha]_D^{25}$ [b]
1		**5a**	**10a**	90	+5.2
2		**5b**	**10b**	60	+0.6
3		**5c**	**10c**	75	−35.1
4		**5d**	**10d**	98	−102.3
5		**5e**	**10e**	83	−23.3

[a] Yield of pure isolated products. [b] Measured in CH_2Cl_2 ($c = 1$).

Table 2. Synthesis of α-(*N*-Glycosyltriazolyl)-β-lactam Hybrids **15**−**18**

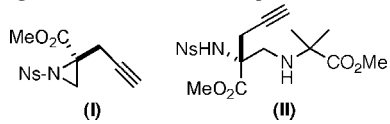


entry	β-lactam	R^1	product	yield[a] (%)	product	yield (%)
1	**10a**	Me	**11**	99	**15**	>98
2	**10a**	$CH_2CH_2C_8F_{17}$	**12**	55	**16**	>98
3	**10e**	Me	**13**	93	**17**	>98
4	**10e**	$CH_2CH_2C_8F_{17}$	**14**	72	**18**	>98

[a] Overall nonoptimized yields of pure isolated products. Three equivalents of acid chloride or 5 equiv of anhydride was used in all examples.

(10) Seebach, D.; Aebi, J. D.; Gander-Coquoz, M.; Naef, R. *Helv. Chim. Acta* **1987**, *70*, 1194–1216.

(11) Reaction of **6** with *o*-Ns-Cl and $KHCO_3$ in acetonitrile, followed by "in situ" treatment of the resulting intermediate *N*-nosylaziridine (**I**) with methyl α-aminoisobutirate **7** provided the *N*-peptidylazaserinate (**II**) in only 25% yield, along with several unidentified products.



(12) (a) Ohira, S. *Synth. Commun.* **1989**, *19*, 561. (b) Müller, S.; Liepold, B.; Roth, G. J.; Bestmann, H. J. *Synlett* **1996**, 521.

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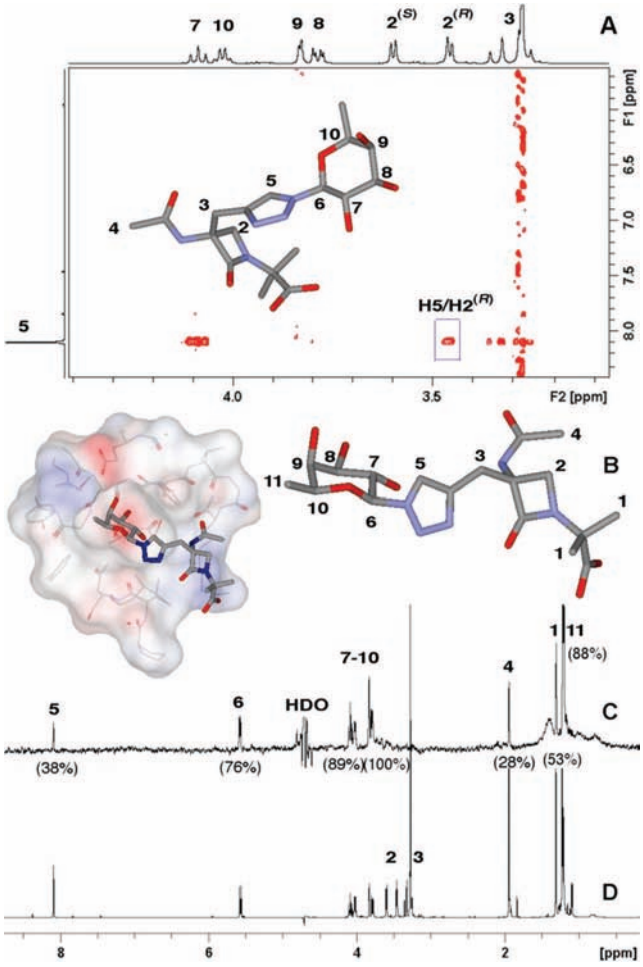
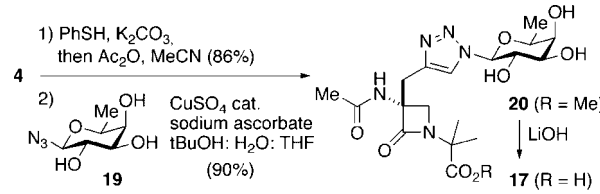


Figure 3. L-β-Fucose/β-lactam hybrid **17** is properly recognized by *Ulex Europaeus Lectin-I* after conformation change. (A) Solution conformation (bent) and ROESY magnification. (B) Complexed conformation from docking (extended). (C) STD spectrum measured at 298 K; signal enhancement normalized to H-10 in parentheses; (D) 500 MHz ^{1}H NMR spectrum measured in H_2O-D_2O 9:1 at 298 K.

see also the Supporting Information) between the L-β-fucose-substituted mimetic **17** and UEL-I. The conformational behavior of compound **17** in water was first checked by Molecular Dynamics, revealing a highly populated and homogeneous cluster of bent conformers, with a minor contribution of extended conformers. This prediction was confirmed experimentally by several long-range NOE cross-peaks and, more particularly, by the close spatial arrangement of the H5 triazole proton and the *pro*-R H2$^{(R)}$ proton of the β-lactam ring (see Figure 3A). However, a conformational selection process takes place upon binding to UEL-1. Indeed

Scheme 3. "Click" Reaction with Free Glycosyl Azides



(Figure 3C) STD-NMR[15] experiments unequivocally showed recognition of **17**, with a distinctive signal resonance intensity pattern. Indeed, all H atoms except those of methylene groups H2 and H3 experienced STD effects, with the relative intensities gathered in Figure 3C. Docking experiments with Autodock 3.0[16] were performed to explain the experimental results. Only the extended conformer (minor in solution) was able to account for the observed STD effects. This fact was also in agreement with the main cluster of minimum-energy conformers calculated by AutoDock, which showed the extended conformation of ligand **17** bind to UEL-1 by the carboxylate group at residue Arg-222 and by the fucose 8- and 9-OH groups to the Asp-87 and Glu-44 residues.

In conclusion, a short and practical procedure to obtain lectin antagonist saccharide/β-lactam hybrid peptido-mimetics has been developed by applying a "shape-modulating linker" design. The "click" reaction required to form saccharide/β-lactam hybrids displays full anomer and configuration control and is compatible with either *O*-protected or hydroxyl-free glycosyl azides. Owing to its simplicity, the extension of this design to further families of β-lactam hybrids is anticipated.

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Supporting Information Available: Preparation procedures, physical and spectroscopic data for compounds **4**−**20**, and detailed MD, Docking, and NMR experiments for compound **17**. This material is available free of charge via the Internet at http://pubs.acs.org.

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(16) Morris, G. M.; Goodsell, D. S.; Halliday, R. S.; Huey, R.; Hart, W. E.; Belew, R. K.; Olson, A. J. *J. Comput. Chem.* **1998**, *19*, 1639–1647. (pdb code: 1JXN)