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Cyclic Peptides as Scaffolds for Multivalent Presentation of Carbohydrates: A Combinatorial Approach

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Introduction

Carbohydrate–lectin interactions mediate a large variety of intercellular recognition events. Since the interactions of individual saccharide groups are usually of low affinity and broad specificity, subsite and subunit multivalency is often employed by nature in order to enhance the attractive binding forces [1]. Accordingly, the synthesis of multivalent carbohydrate ligands has recently attracted interest [2]. Binding studies with these multivalent ligands revealed the importance of the spacer between the monomeric epitopes. Attachment of the saccharides to rigid scaffolds can result in particularly pronounced affinity enhancements provided that the sugars are presented in the "correct" spatial arrangement. However, in contrast to the utilization of flexible linkers, a larger number of ligands has to be screened in order to find a ligand with the appropriate orientation of the carbohydrates.

Here we present a synthetic approach that enables the preparation of libraries of conformationally restricted cyclic peptides which can serve as scaffolds for the multivalent presentation of carbohydrate ligands [3]. Such libraries are useful to (1) identify high affinity lectin ligands based on multivalent interactions and (2) to determine the required orientation of the carbohydrates *via* conformational analysis of the cyclopeptide scaffold.

Strategy:

A library of cyclic peptides with regioselectively addressable side chain amino groups is synthesized by combinatorial solid phase synthesis (split and combine method [4]). Following peptide assembly, several copies of a carbohydrate ligand are attached to the side chain amino groups *via* a new Alloc-based urethane-type linker. In contrast to glycosylation reactions employing resin bound peptides, the formation of an urethane bond proceeds in virtually quantitative yields. The members of the obtained library differ in the conformation of the peptide scaffold and the number and location of carbohydrates attached to it.

The neoglycopeptide library can then be applied in on-bead lectin binding assays. Identification of high affinity ligands can be accomplished by standard peptide analytical methods (Edman degradation) after Pd(0) catalyzed cleavage of the carbohydrate-peptide linker. Attachment of the saccharides after the cyclopeptide synthesis has the conceptual advantage that once a library is prepared, it can be used to study several carbohydrate binding proteins by simply connecting different sugars to it.

Results and Discussion

Figure 1 outlines the synthetic strategy using a cyclic model neoglycopeptide. Solid phase peptide synthesis was carried out on TentaGel functionalized with the Sieber linker and followed the Fmoc strategy. Only in the last step, an N^{α}-Boc-protected amino acid was applied. Cyclization of the resin bound nonapeptide was carried out between the side-chain carboxyl group of glutamic acid (Allyl protection) and the ε-NH_2 group of lysine (Alloc protection) after simultaneous deprotection with catalytic amounts of tetrakistriphenylphosphine palladium(0) and morpholine in DMF/DMSO (1:1). Addition of HBTU/HOBt/DIEA resulted in clean cyclization product as judged by HPLC analysis of the crude cleavage product obtained from a small resin sample by treatment with dichloromethane/TFA/tri-*iso*-propyl silane (98:1:1) followed by ESIMS.

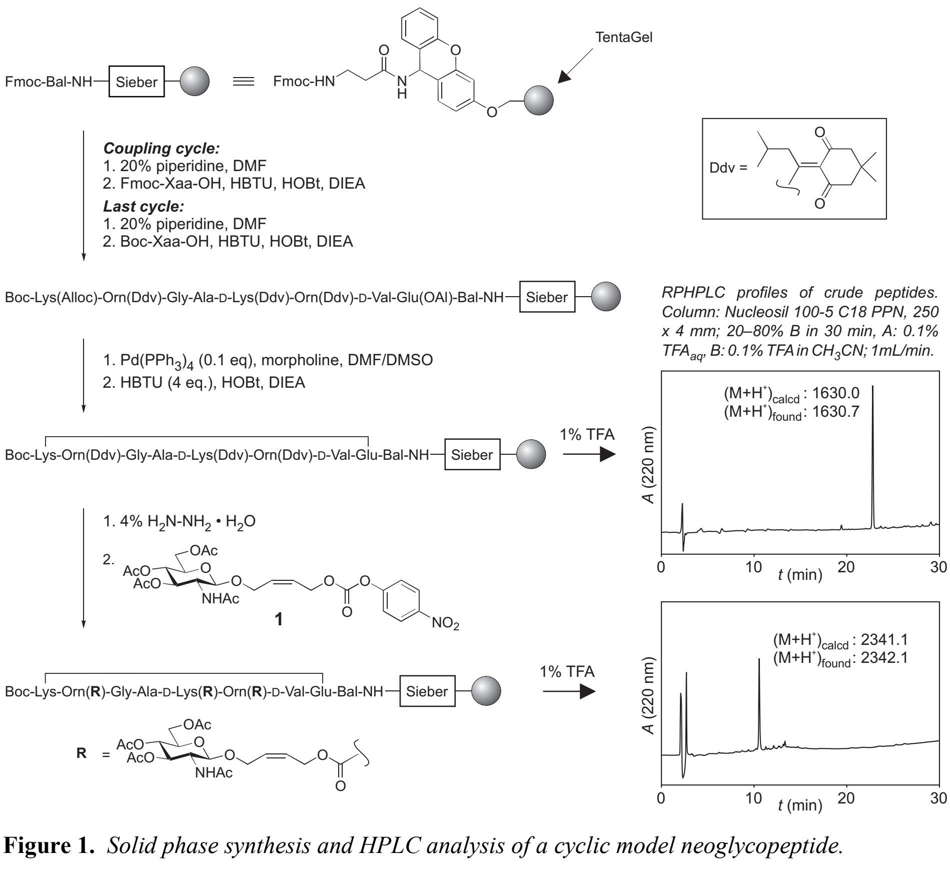

Figure 1. *Solid phase synthesis and HPLC analysis of a cyclic model neoglycopeptide.*

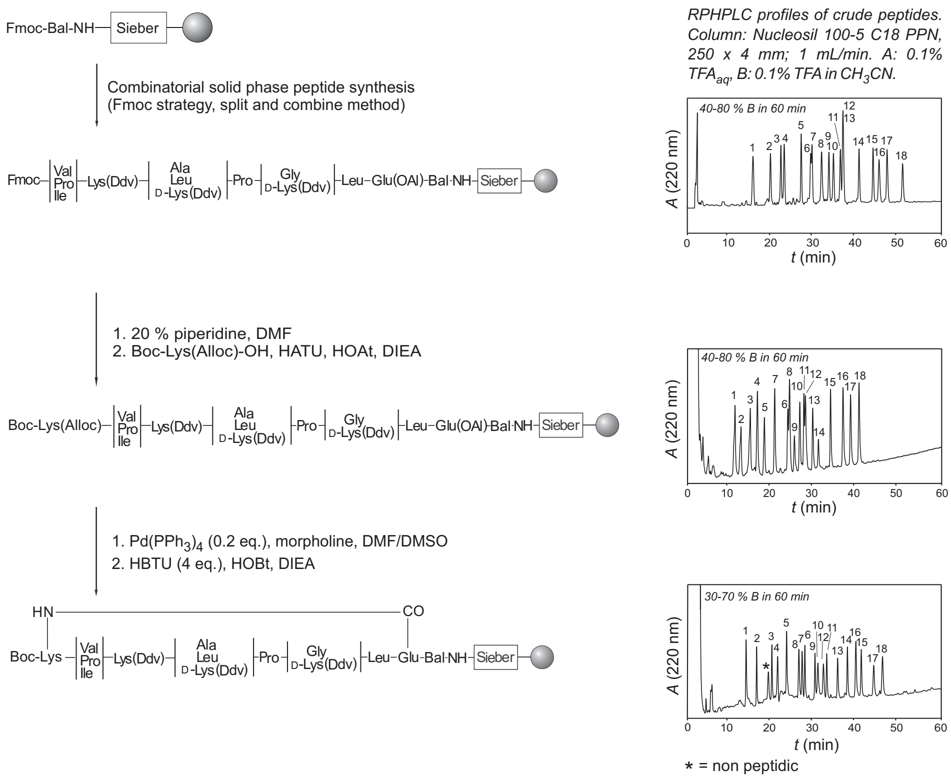

Figure 2. *Synthesis and HPLC analysis of an 18-membered cyclic-peptide library. Peaks of corresponding peptides are labeled with the same number.*

Subsequently, the 1-(4,4-dimethyl-2,6-dioxocyclohexylidene)isovaleryl (Ddv) groups [5] which were used to protect the attachment points of the carbohydrate residues were removed with 4% hydrazine hydrate in DMF. Treatment with the *p*-nitrophenyl active carbonate **1** finally gave the target neoglycopeptide which was cleaved from the solid support. HPLC analysis of the crude peptide confirmed the high efficiency of the overall process.

We next turned our attention to the question weather the reaction conditions, especially those of the critical cyclization step, would be successful with other peptide sequences. Therefore, we prepared an 18-membered cyclopeptide library (split and combine method) and followed the course of the synthesis by HPLC in combination with ESIMS (Figure 2).

It turned out that all 18 peptides underwent cyclization without forming major side products. Particularly, we were not able to detect any linear or cyclic peptide dimers. The assignment of each peak of the HPLC profile of the cyclopeptide library is given in Table 1.

Table 1. *Calculated and experimentally determined masses of the cyclic-peptide library.*

Peak	Compound		$[M + H]^+$ calcd	$[M + H]^+$ found
1	*cyclo*[Boc-Lys-Pro-Lys(Ddv)-Ala-Pro-Gly-Leu-Glu]-Bal-NH_2		1197.7	1198.3
2	*cyclo*[Boc-Lys-Val-Lys(Ddv)-Ala-Pro-Gly-Leu-Glu]-Bal-NH_2		1199.7	1200.1
3	*cyclo*[Boc-Lys-Ile-Lys(Ddv)-Ala-Pro-Gly-Leu-Glu]-Bal-NH_2		1213.8	1214.1
4	*cyclo*[Boc-Lys-Pro-Lys(Ddv)-Leu-Pro-Gly-Leu-Glu]-Bal-NH_2		1239.8	1240.4
5	*cyclo*[Boc-Lys-Val-Lys(Ddv)-Leu-Pro-Gly-Leu-Glu]-Bal-NH_2		1241.8	1242.2
8	*cyclo*[Boc-Lys-Pro-Lys(Ddv)-D-Lys(Ddv)-Pro-Gly-Leu-Glu]-Bal-NH_2		1460.9	1461.5
7	*cyclo*[Boc-Lys-Ile-Lys(Ddv)-Leu-Pro-Gly-Leu-Glu]-Bal-NH_2		1255.8	1256.2
6	*cyclo*[Boc-Lys-Pro-Lys(Ddv)-Ala-Pro-D-Lys(Ddv)-Leu-Glu]-Bal-NH_2		1474.9	1475.4
9	*cyclo*[Boc-Lys-Val-Lys(Ddv)-Ala-Pro-D-Lys(Ddv)-Leu-Glu]-Bal-NH_2 *cyclo*[Boc-Lys-Ile-Lys(Ddv)-D-Lys(Ddv)-Pro-Gly-Leu-Glu]-Bal-NH_2	or	1476.9	1477.5
10	*cyclo*[Boc-Lys-Val-Lys(Ddv)-D-Lys(Ddv)-Pro-Gly-Leu-Glu]-Bal-NH_2		1462.9	1463.5
12	*cyclo*[Boc-Lys-Ile-Lys(Ddv)-D-Lys(Ddv)-Pro-Gly-Leu-Glu]-Bal-NH_2 *cyclo*[Boc-Lys-Val-Lys(Ddv)-Ala-Pro-D-Lys(Ddv)-Leu-Glu]-Bal-NH_2	or	1476.9	1477.4
11	*cyclo*[Boc-Lys-Ile-Lys(Ddv)-Ala-Pro-D-Lys(Ddv)-Leu-Glu]-Bal-NH_2		1491.0	1491.6
13	*cyclo*[Boc-Lys-Pro-Lys(Ddv)-Leu-Pro-D-Lys(Ddv)-Leu-Glu]-Bal-NH_2		1517.0	1517.5
14	*cyclo*[Boc-Lys-Val-Lys(Ddv)-Leu-Pro-D-Lys(Ddv)-Leu-Glu]-Bal-NH_2		1519.0	1519.6
16	*cyclo*[Boc-Lys-Pro-Lys(Ddv)-D-Lys(Ddv)-Pro-D-Lys(Ddv)-Leu-Glu]-Bal-NH_2		1738.1	1739.2
15	*cyclo*[Boc-Lys-Ile-Lys(Ddv)-Leu-Pro-D-Lys(Ddv)-Leu-Glu]-Bal-NH_2		1533.0	1533.5
17	*cyclo*[Boc-Lys-Val-Lys(Ddv)-D-Lys(Ddv)-Pro-D-Lys(Ddv)-Leu-Glu]-Bal-NH_2		1740.1	1740.8
18	*cyclo*[Boc-Lys-Ile-Lys(Ddv)-D-Lys(Ddv)-Pro-D-Lys(Ddv)-Leu-Glu]-Bal-NH_2		1754.1	1755.0

Conclusion

We have demonstrated an efficient process for the generation of libraries of cyclic-peptide scaffolds suitable for a conformationally defined multivalent presentation of carbohydrate ligands. Currently, we are using this strategy to screen neoglycopeptide libraries for lectin binding properties.

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