ORGANIC LETTERS

2001
Vol. 3, No. 17
2637−2640

# Cyclic Hexapeptides with Free Carboxylate Groups as New Receptors for Monosaccharides

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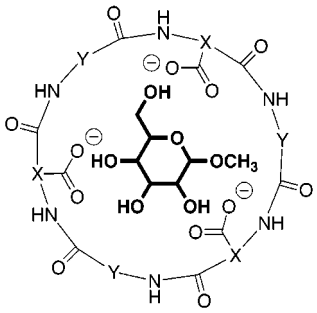
**Received May 23, 2001**

## ABSTRACT



**Cyclic hexapeptides composed of alternating L-proline and 3-aminobenzoic acid subunits with substituents on the aromatic subunits that contain free carboxylate groups are able to bind monosaccharides in 4% $CD_3OD/CDCl_3$. The binding selectivity of these peptides depends on the structure of the substituents on the aromatic subunits.**

Because of the importance of carbohydrate recognition in nature, the design of artificial receptors for saccharide complexation is currently intensively being pursued in supramolecular chemistry. The various approaches in this area of research have recently been reviewed by A. P. Davis.[1] In this paper, we add a new type of receptor to the number of systems studied so far that is based on a cyclic hexapeptide composed of alternating L-proline and 3-aminobenzoic acid residues. This macrocyclic host has currently only been used for the complexation of anions and cations,[2] but our structural assignment has shown that its cavity dimension should also be well suited for the inclusion of monosaccharides.[2a] The peptide certainly lacks functional groups with which sugars could interact, but these groups can be introduced either in the aromatic or the natural amino acid subunits along the peptide cavity.

In natural carbohydrate receptors, carboxylate groups in the side chains of glutamic acid or aspartic acid are important factors in the binding of the substrate by hydrogen bonding,[3] and we therefore decided to use the same functional groups to induce a carbohydrate affinity in our cyclopeptides. All derivatives tested (**2a**−**c**) contain a cyclic array of carboxylate groups around their cavity that stems from amino acid substituents attached to the aromatic cyclopeptide subunits. These macrocycles are thus related to the carbohydrate receptors, in which phosphonate groups are used for guest binding.[4]

To facilitate the synthesis of cyclopeptides with peripheral substituents, we used the cyclic hexapeptide **1** as a molecular scaffold, in which additional carboxyl groups on the 3-aminobenzoic acid subunits are available for further modifica-

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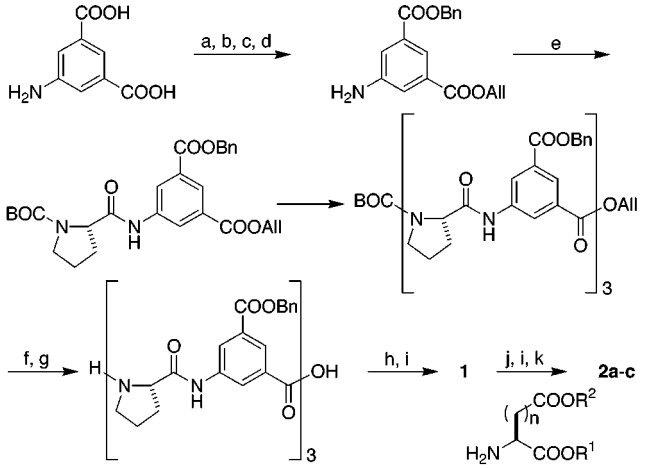
(2) (a) Kubik, S.; Goddard, R. *J. Org. Chem.* **1999**, *64*, 9475−9486. (b) Kubik, S.; Goddard, R. *Eur. J. Org. Chem.* **2001**, 311−322. Ishida et al. have reported on the phosphate binding of a similar peptide in DMSO: Ishida, H.; Suga, M.; Donowaki, K.; Ohkubo, K. *J. Org. Chem.* **1995**, *60*, 5374−5375.

(3) Lemieux, R. U. *Chem. Soc. Rev.* **1989**, *18*, 347−374. Quiocho, F. A. *Pure Appl. Chem.* **1989**, *61*, 1293−1306. Weis, W. I.; Drickamer, K. *Annu. Rev. Biochem.* **1996**, *65*, 441−473.

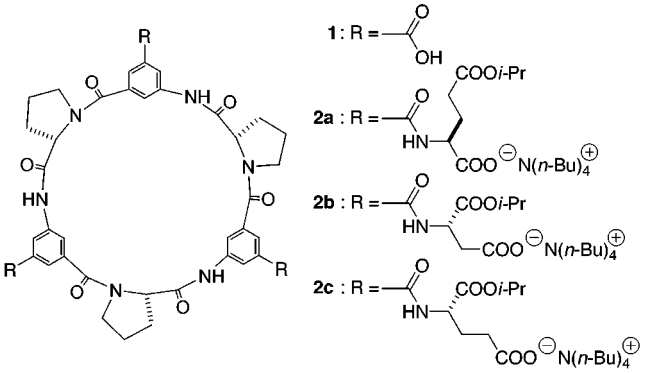
10.1021/ol016158l CCC: $20.00 
**Published on Web 07/25/2001**

**Scheme 1.** Synthesis of Cyclopeptides **1** and **2a**−**c**[a]




[a] (a) $BOC_2O$; (b) AllBr/$NaHCO_3$; (c) BnBr/$NaHCO_3$; (d) TFA; (e) BOC-L-proline/PyCloP/DIEA; (f) [$Pd(P(4\text{-}Me_2NPh)Ph_2)_4$]/morpholine; (g) HCl/1,4-dioxane; (h) TBTU/DIEA; (i) $H_2$/10% Pd/C; (j) **2a**: $n = 2$, $R^1$ = Bn, $R^2$ = *i*-Pr; **2b**: $n = 1$, $R^1$ = *i*-Pr, $R^2$ = Bn; **2c**: $n = 2$, $R^1$ = *i*-Pr, $R^2$ = Bn, PyCloP/DIEA; (k) $N(n\text{-}Bu)_4{}^+OH^-$.

tions. Because these groups line the wide rim of the peptide cavity, the overall conformation of **1** should be similar to the one we have determined for the unsubstituted parent compound.[2a]




Peptide **1** was prepared according to Scheme 1. For the chain elongation of the orthogonally protected dipeptide in solution, standard peptide methodology could be used, since the allyl ester and the *N*-BOC group can be cleaved selectively without affecting the benzyl ester. In the final step of the synthesis of **1**, the remaining benzyl esters were removed cleanly by hydrogenation.

The syntheses of peptides **2a**−**c** were completed by coupling suitably substituted amino acids with **1**, followed by deprotection and deprotonation of the carboxyl groups designated for substrate binding (Scheme 1). All products were fully characterized by $^1H$ and $^{13}C$ NMR spectroscopy, mass spectrometry, and elemental analysis.

The $^1H$ NMR spectra of **2a**−**c** in $d_6$-DMSO are consistent with averaged $C_3$-symmetrical conformations of the macrocycles. Interestingly, deprotonation of the carboxylic acids in the final step of the synthesis of **2c** caused a significant downfield shift of the signal of the glutamic acid NH protons, whereas the chemical shift of the ring NH protons is almost unaffected. The same effect is to a lesser extent also observed for **2b** (Figure 1).

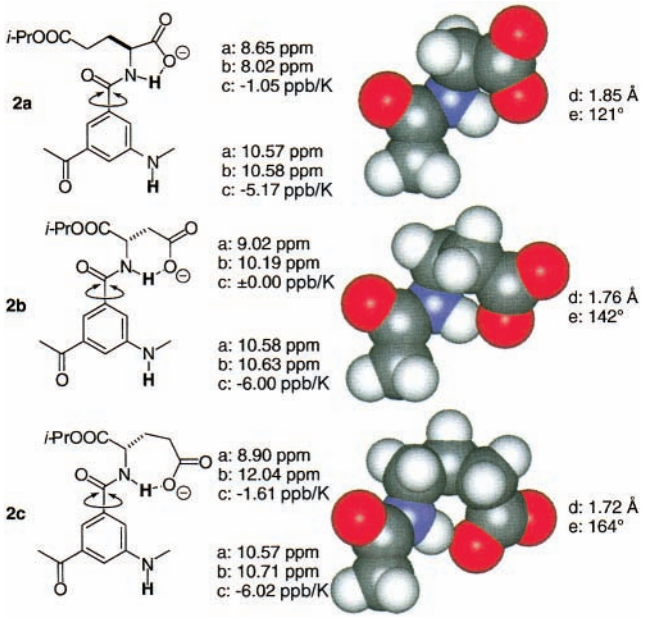



**Figure 1.** Possible cyclic arrangements of the amino acid substituents on the aromatic subunits of **2a**−**c**. The resonance of the NH protons before (a) and after (b) deprotonation of the carboxyl groups, the temperature dependency of the NH resonances in $d_6$-DMSO (c), and the calculated lengths (d) and angles (e) of the N−H···O hydrogen bonds are indicated.

Downfield shifts of NH signals usually indicate that the corresponding protons are involved in hydrogen bonding. The small temperature dependency of the NH resonance in the aromatic substituents of **2a**−**c** indicate that this type of interaction occurs in fact intramolecularly.[5] The corresponding hydrogen bonds are presumably formed between the NH groups and the neighboring carboxylate groups in which case they lock the aromatic substituents in a cyclic conformation. In Figure 1, energy minimized cyclic arrangements of the substituents in **2a**−**c** are depicted. These structures are in accordance with calculated conformations of α,ω aminocarboxylic acids that contain intramolecular hydrogen bonds.[6]

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The shortest hydrogen bond occurs in the seven-membered ring of the glutamic acid derivative **2c**, which explains the largest downfield shift of the NH signal upon deprotonation in this peptide. The intramolecular hydrogen bonds in the peripheral substituents reduce the flexibility of **2a**−**c** and stabilize certain orientations of the carboxylate groups. Both effects could contribute to receptor selectivity, but overall peptides **2a**−**c** should still be flexible enough to adapt to the steric demand of different guest molecules.

In the $^1$H NMR spectra of **2a**−**c** in $CDCl_3$, the signals of all peptide protons are significantly broadened, indicating either a slow conformational equilibrium or intermolecular association. We still carried out initial investigations on the monosaccharide affinity of the peptides in this solvent. A comparison of the $^1$H NMR spectrum of an equimolar mixture of α-D-octylglucopyranoside and **2c** with the spectra of the individual components at the same concentration clearly revealed an interaction of the cyclopeptide with the sugar. Most importantly, the prominent doublet of the anomeric proton of the glucoside is slightly but reproducibly shifted upfield in the presence of **2c** (ca. −0.015 ppm). Such upfield shifts are usually observed when guest molecules are included into a receptor cavity that is lined by aromatic subunits,[7] and we believe that they also indicate complex formation in the present case. No influence of sugar binding on the spectrum of **2c** could be detected. However, the signals of the tetra-*n*-butylammonium protons are also shifted upfield upon complex formation. This effect is strongest for the α-$CH_2$ protons of the cation and decreases with increasing distance of the protons from the ammonium nitrogen. It is reasonable to assume that in $CDCl_3$ the anionic receptor and the quaternary ammonium ion form a close ion pair with the α-$CH_2$ protons of the cation located in close proximity, possibly even hydrogen bonded to the receptor carboxylate groups. Hydrogen bonding capabilities of methylene groups adjacent to ammonium centers have in fact been demonstrated in solution and in the solid state.[8] Such hydrogen bonding would lead to a downfield shift of the α-$CH_2$ protons in the $^1$H NMR spectrum of **2c**, and the upfield shift that is observed when the guest is added therefore illustrates the dissociation of the cation−carboxylate ion pair that precedes sugar binding.

A competition between cation and glucoside complicates the complex equilibrium in $CDCl_3$, and we therefore tried to suppress the interaction of the ammonium cation with the peptides by increasing the polarity of the solvent. Indeed, no significant interactions between the ammonium ions and the carboxylate groups of the peptides were detected in $CDCl_3$ containing 4% $CD_3OD$. This solvent mixture has the additional advantages that the receptor signals in the $^1$H NMR spectra become sharper, and it is possible to use methyl glucosides as potential guests, saccharides that are insoluble in pure chloroform.

Even in this competitive solvent mixture, interactions between the cyclopeptides and carbohydrates are still detectable as illustrated by the effects of, e.g., **2c** on prominent signals of α-D-methylglucopyranoside in the $^1$H NMR spectrum (Figure 2). Again, a small upfield shift of the signal

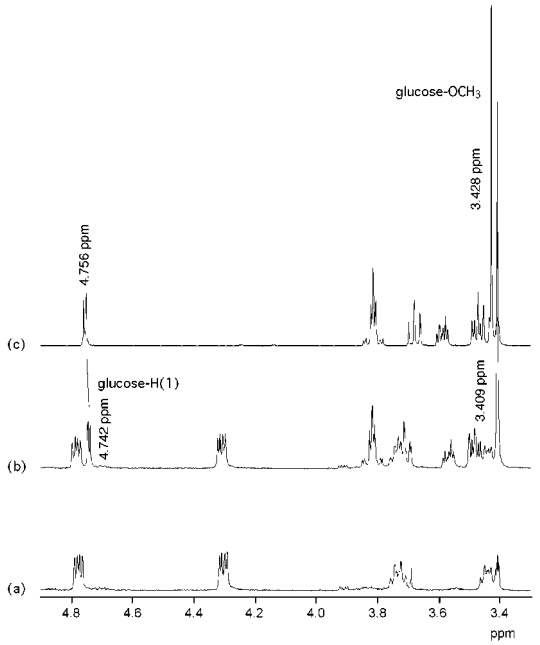


**Figure 2.** $^1$H NMR spectra of **2c** (a), α-D-methylglucopyranoside (c), and a 1:1 mixture of both compounds (b) in 4% $CD_3OD/CDCl_3$ ($c$ = 1 mM, 25 °C).

of the anomeric proton can be observed, the upfield shift of the resonance of the sugar $OCH_3$ group is significantly larger, however.

Receptor **2c** (and also the other two peptides) thus binds monosaccharides in the presence of a ca. 10 000-fold excess of methanol molecules that compete in binding to the carboxylate groups. A Job plot furthermore revealed a defined 1:1 stoichiometry for the complex between **2c** and α-D-methylglucopyranoside.[9] This indicates that the monosaccharide is included into the peptide cavity upon complex formation where it can interact with all three carboxylate groups simultaneously. Linear analogues of **2c** such as the hexapeptide **3** and the dipeptide **4** lack the preorganization of the cyclic derivative, and their interaction with monosaccharides is thus less specific. In the case of **4**, the interaction is in fact too weak to be determined quantitatively ($\Delta\delta$ < 0.001 ppm for the sugar protons), and in the case of **3**, a

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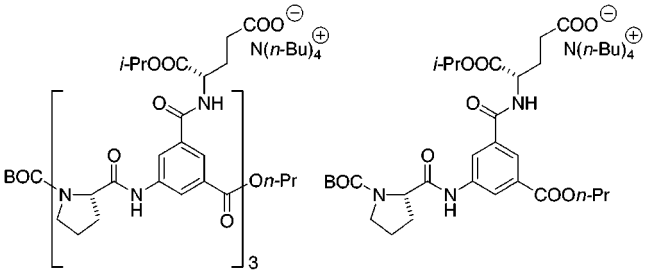
(9) We determined a 1:1 complex stoichiometry also for other receptor/monosaccharide combinations, e.g., **2b** and β-D-methylglucopyranoside. In addition, the excellent agreement of the saturation curves obtained experimentally in our NMR titrations with the ones calculated on the basis of 1:1 complex formation indicates that this stoichiometry holds for all complexes investigated (see Supporting Information).

peptide/sugar ratio of 2:3 was determined in the aggregate. This result clearly demonstrates the importance of the macrocyclic structure of peptides **2a**−**c** in carbohydrate complexation.




**3** **4**

The shift of the methylglycoside $OCH_3$ protons upon complex formation allows the quantitative determination of complex stability by NMR titrations. Since the $^1$H NMR spectra of **2a**−**c** are independent of the concentration in the region relevant for such quantitative investigations (0.2−2.0 mM), an intermolecular association of the peptides has not to be considered. The stability constants of peptides **2a**−**c** with a variety of glycosides are summarized in Table 1. They were calculated from the shift of prominent sugar signals by using the mathematical treatment for 1:1 complex equilibria.[10]

**Table 1.** D-Glycoside Association Constants of Complexes of Peptides **2a**−**c** in 4% $CD_3OD/CDCl_3$ at 298 K[a]

| | $K_a/\Delta\delta_{max}$ | | |
|---|---|---|---|
| | **2a** | **2b** | **2c** |
| α-methylglucopyranoside | 420/−0.06 | 660/−0.07 | 550/−0.05 |
| β-methylglucopyranoside | 550/−0.04 | 810/−0.07 | 650/−0.06 |
| β-octylglucopyranoside | | 560/+0.05 | |
| α-methylmannopyranoside | 450/−0.04 | 700/−0.04 | 440/−0.06 |
| α-methylgalactopyranoside | 300/−0.05 | 790/−0.06 | 390/−0.04 |
| β-methylgalactopyranoside | 400/−0.06 | 540/−0.08 | 430/−0.05 |
| α-methylribofuranoside | 160/−0.07 | 290/−0.08 | 190/−0.08 |

[a] $K_a$ stability constant in $M^{-1}$, error limits for $K_a$ < 20%; $\Delta\delta_{max}$ is the maximum chemical shift in ppm for the sugar $OCH_3$ protons except in the case of β-octylglucopyranoside, where the shift of the glucose C(3)H has been followed.

It has to be noted that reproducible results in the NMR titrations could only be obtained when the water content of the solvent mixture used was at least 0.002%. The presence of water is obviously important for complex formation. We can currently only speculate that water molecules contribute to the hydrogen bonding network between the hydroxyl groups of the monosaccharides and the peptide carboxyl groups and thus stabilize the whole aggregate. A similar effect of water has also been observed by Sanders et al. in the carbohydrate binding of another macrocyclic receptor.[11] The exact nature of the role of water in the interactions between carbohydrates and our peptides has still to be clarified, however.

Table 1 shows that the complexes between peptides **2a**−**c** and all glycosides tested possess stability constants (and maximum chemical shifts) of the same order of magnitude. The selectivity of the receptors with respect to a specific epimer or anomer is obviously only moderate. However, certain trends are evident. In general, peptide **2b** forms more stable complexes than the other two receptors. This indicates that the orientation of the carboxylate groups in the aspartic acid residues of **2b** seem to be somewhat better suited for monosaccharide binding than those in the substituents of the other two peptides. Furthermore, the hexopyranosides are significantly better bound than α-D-methylribofuranoside. This could be due to the larger ring size of the pyranosides but also to the additional hydroxyl group in hexoses with which the peptides can interact. In contrast to the $OCH_3$ protons of the methyl glycosides, the $OCH_2$ protons in the octyl chain of β-octylglucopyranoside are shifted *downfield* upon complex formation with **2b**. This most probably indicates that, as a result of the steric bulk of the octyl chain, the geometry of this complex differs from the ones of the methyl glycosides.

Sanders et al. have shown that the ability of sugars to form intermolecular hydrogen bonds to the solvent (methanol in $CDCl_3$) or to receptor binding sites increases in the order α-galactose < β-galactose ≈ α-glucose < β-glucose < β-mannose < α-mannose.[11] Carbohydrate receptors whose complex stabilities increase in the same order are therefore essentially nonselective. Table 1 shows that the complex stabilities of peptides **2a**−**c** deviate from the order proposed by Sanders in some instances, e.g., the β-methylglucopyranoside is bound best by all receptors. This suggests that the peptides do possess a certain selectivity in carbohydrate recognition.

A more detailed knowledge of the structure of the complexes would certainly help in the evaluation of the receptor properties of **2a**−**c**. However, in our eyes these initial investigations already show that the substituted cyclopeptides presented in this paper represent a promising basis for the design of a new type of monosaccharide receptors. By better preorganizing the peptides for substrate complexation, it should in principle be possible to increase the binding selectivity. Also, other functional groups can be used for the interactions with carbohydrates. Investigations in these directions are currently underway.

**Acknowledgment.** The funding of this project by the Deutsche Forschungsgemeinschaft is gratefully acknowledged. S.K. also thanks Prof. G. Wulff for his support.

**Supporting Information Available:** Syntheses of **2a**−**c**, **3**, and **4**. Selected $^1$H NMR spectra of compounds **2a**−**c**, saturation curves of NMR titrations, and Job plots. This material is available free of charge via the Internet at http://pubs.acs.org.

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