Large Increase in Cation Binding Affinity of Artificial Cyclopeptide Receptors by an Allosteric Effect

Stefan Kubik

Contribution from the Institut für Organische Chemie und Makromolekulare Chemie, Heinrich-Heine-Universität, Universitätsstrasse 1, D-40225 Düsseldorf, Germany

Received November 17, 1998. Revised Manuscript Received March 8, 1999

Abstract: The receptor properties of a cyclopeptide composed of L-glutamic acid and 3-aminobenzoic acid in an alternating sequence are described. ^{1}H NMR, NOESY NMR, and FT-IR spectroscopic investigations show that this cyclic peptide is relatively flexible in solution. Still, it is able to bind cations by cation−π interactions. For the *n*-butyltrimethylammonium iodide complex, for example, an association constant of 300 M^{-1} has been determined in chloroform. Besides cations, the cyclopeptide is also able to bind certain anions, such as sulfonates or phosphonates, at a second binding site. NMR and FT-IR spectroscopic investigations show that these anions are hydrogen bonded to the peptidic NH groups. Anion complexation results in an increase of the cyclic peptide's cation affinity by a factor of 10^3-10^4. The cyclopeptide−tosylate complex structure in solution was assigned by FT-IR , ^{1}H NMR, and NOESY NMR spectroscopic methods as well as molecular modeling. This structure shows that the drastic increase in cation binding affinity can be correlated with a preorganization of the cyclic peptide by the anion as well as electrostatic interactions between anion and cationic substrates in the final complex. Therefore, the influence of the anions on the complexing behavior of the cyclopeptide can be regarded as an *allosteric effect*. Association constants of the K^+−18-crown-6, Na^+−15-crown-5, and *n*-butyltrimethylammonium cation complexes have been determined by dilution and competitive NMR titrations.

Introduction

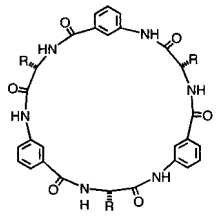
One of the fundamental principles in biochemistry is the regulation of enzyme activities by allosteric effects.[1] The interaction of an *effector* with the allosteric binding site of an enzyme causes a conformational change at the active center and, as a consequence, greatly improves the enzyme's substrate affinity or catalytic activity. The same principle has been transferred to a variety of different artificial receptors in recent years.[2] Many of these compounds have in common that they contain two conformationally coupled binding sites. When they bind a guest molecule, a conformational reorganization at the second binding site occurs and, similar to natural systems, the affinity toward another guest is altered. However, in contrast to enzymes, where effector and substrate binding sites are usually at remote positions in the molecule, in small artificial hosts both guests are bound in relatively close proximity. Especially when effector and substrate are charged, possible interactions between the two guests can influence the receptor cooperativity. For example, because of unfavorable Coulomb interactions, a positive cooperativity is relatively unlikely when effector and substrate are equally charged. We are aware of only a single artificial receptor with a positive cooperativity in the complexation of two metal cations.[2l] When, on the other hand, effector and substrate have opposite charges, one would expect that attractive electrostatic interactions between these compounds should even promote binding. Ditopic receptors which bind both components of an ion pair simultaneously have been described.[3] Yet, to our knowledge, so far there are no examples for systems in which the complexation of an ion results in a preorganization of the receptor as well as the introduction of a new binding site with which the substrate can interact. We have found that such unusual receptor properties can be realized with cyclopeptides

(1) Hervé, G. *Allosteric enzymes*; CRC Press: Boca Raton, FL, 1989. Perutz, M. F. *Mechanisms of cooperativity and allosteric regulation in proteins*; University Press: Cambridge, UK, 1990.

(2) (a) Traylor, T. G.; Mitchell, M. J.; Ciccone, J. P.; Nelson, S. *J. Am. Chem. Soc.* **1982**, *104*, 4986−4989. (b) Rebek, J., Jr. *Acc. Chem. Res.* **1984**, *17*, 258−264. (c) Rebek, J., Jr.; Costello, T.; Marshall, L.; Wattley, R.; Gadwood, R. C.; Onan, K. *J. Am. Chem. Soc.* **1985**, *107*, 7481−7487. (d) Beer, P. D.; Rothin, A. S. *J. Chem. Soc., Chem. Commun.* **1988**, 52−54. (e) Hunter, C. A.; Meah, M. N.; Sanders, J. K. M. *J. Chem. Soc., Chem. Commun.* **1988**, 694−696. (f) Tabushi, I. *Pure Appl. Chem.* **1988**, *60*, 581−586. (g) Ebmeyer, F.; Rebek, J., Jr. *Angew. Chem., Int. Ed. Engl.* **1990**, *29*, 1148−1150. (h) Petter, R. C.; Salek, J. S.; Sikorski, C. T.; Kumaravel, G.; Lin, F. *J. Am. Chem. Soc.* **1990**, *112*, 3860−3868. (i) Schneider, H.-J.; Ruf, D. *Angew. Chem., Int. Ed. Engl.* **1990**, *29*, 1159−1160. (j) Kobuke, Y.; Sumida, Y.; Hayashi, M.; Ogoshi, H. *Angew. Chem., Int. Ed. Engl.* **1991**, *30*, 1496−1498. (k) Sijbesma, R. P.; Nolte, R. J. M. *J. Am. Chem. Soc.* **1991**, *113*, 6695−6696. (l) Kobuke, Y.; Satoh, Y. *J. Am. Chem. Soc.* **1992**, *114*, 789−790. (m) Pierre, J.-L.; Gagnaire, G.; Chautemps, P. *Tetrahedron Lett.* **1992**, *33*, 217−220. (n) Schneider, H.-J.; Werner, F. *J. Chem. Soc., Chem. Commun.* **1992**, 490−491. (o) Inouye, M.; Konishi, T.; Isagawa, K. *J. Am. Chem. Soc.* **1993**, *115*, 8091−8095. (p) Haino, T.; Katsutani, Y.; Akii, H.; Fukazawa, Y. *Tetrahedron Lett.* **1998**, *39*, 8133−8136. (q) Lustenberger, P.; Welti, R.; Diederich, F. *Helv. Chim. Acta* **1998**, *81*, 2190−2200. (r) Takeuchi, M.; Imada, T.; Shinkai, S. *Angew. Chem., Int. Ed. Engl.* **1998**, *37*, 2096−2099.

(3) (a) Reetz, M. T.; Niemeyer, C. M.; Harms, K. *Angew. Chem., Int. Ed. Engl.* **1991**, *30*, 1474−1476. (b) Arafa, E. A.; Kinnear, K. I.; Lockhart, J. C. *J. Chem. Soc., Chem. Commun.* **1992**, 61−64. (c) Flack, S. S.; Chaumette, J. L.; Kilburn, J. D.; Langley, G. J.; Webster, M. *J. Chem. Soc., Chem. Commun.* **1993**, 399−401. (d) Rudkevich, D. M.; Verboom, W.; Reinhoudt, D. N. *J. Org. Chem.* **1994**, *59*, 3683−3686. (e) Rudkevich, D. M.; Brzozka, Z.; Palys, M.; Visser, H. C.; Verboom, W.; Reinhoudt, D. N. *Angew. Chem., Int. Ed. Engl.* **1994**, *33*, 467−468. (f) Savage, P. B.; Holmgren, S. K.; Gellman, S. H. *J. Am. Chem. Soc.* **1994**, *116*, 4069−4070. (g) Rudkevich, D. M.; Mercer-Chalmers, J. D.; Verboom, W.; Ungaro, R.; de Jong, F.; Reinhoudt, D. N. *J. Am. Chem. Soc.* **1995**, *117*, 6124−6125. (h) Scheerder, J.; van Duynhoven, J. P. M.; Engbersen, J. F. J.; Reinhoudt, D. N. *Angew. Chem., Int. Ed. Engl.* **1996**, *35*, 1090−1093. (i) Deetz, M. J.; Smith, B. D. *Tetrahedron Lett.* **1998**, *39*, 6841−6844. (j) Pelizzi, N.; Casnati, A.; Friggeri, A.; Ungaro, R. *J. Chem. Soc., Perkin Trans. 2* **1998**, 1307−1311.

10.1021/ja983970j CCC: $18.00
Published on Web 06/11/1999



1 R = CH_3

2 R = $CH_2CH_2COOCH(CH_3)_2$

Figure 1. Structures of cyclic hexapeptides **1** and **2**.

composed of natural amino acids and 3-aminobenzoic acid in an alternating sequence (Figure 1).

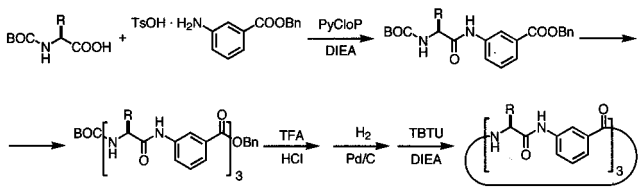
These compounds are interesting candidates for a new class of artificial receptors. They resemble conventional host molecules such as calixarenes or cyclophanes and can be easily obtained by a simple sequential synthesis. As a consequence, it is possible to vary their basic structure in a wide range by deliberate exchange of individual subunits. The rigid aromatic subunits not only cause a reduction of the conformational flexibility of these cyclic peptides, but they also allow easy modification of the peptide by introduction of functional groups with which substrates can interact. In addition, the side chains of the amino acid subunits may also serve as binding sites.

The complexation of phosphate by **1** has already been described.[4] Here, we will show for the first time that such cyclic peptides are also able to bind various cations. We have found that their cation affinity depends on the anion present in solution. With anions, such as phosphonates and sulfonates, which bind to NH groups of the cyclopeptides, allosteric effects have been observed. NMR and FT-IR spectroscopic investigations show that these anions stabilize a conformation of the originally relative flexible host which is ideally suited for an interaction with cations. Binding of the positively charged guests with the cyclopeptide anion complexes is due to cation$-\pi$ interactions with the aromatic subunits of the peptide as well as electrostatic interactions with the anion. We will show that the combination of both binding mechanisms leads to an increase of the complex stability by a factor of 10^3-10^4 in comparison to that of cation complexes without the influence of effector anions.

Results and Discussion

Design and Synthesis. Compound **1** has previously been synthesized on solid support, and its binding to disodium 4-nitrophenyl phosphate in DMSO-d_6 was demonstrated.[4] In this solvent, complexation of cations was not observed. On the other hand, it is known that artificial receptors such as, for example, calixarenes or cyclophanes are able to bind cations by cation$-\pi$ interactions.[5] Since **1** can, in principle, be regarded as a hybrid between a calixarene and a conventional cyclic peptide, one can speculate that it might also be able to bind positively charged guest molecules by the same binding mechanism. Investigations on the interactions of cations with uncharged artificial receptors are usually carried out in organic solvents of low polarity such as $CDCl_3$.[6] Because **1** is only poorly soluble in this solvent, we had to prepare derivatives with improved solubility. By replacing the natural amino acid L-alanine in **1** with esters of L-glutamic acid, it was possible to control the solubility of the products by varying the type of ester used. Whereas the chloroform solubility of a cyclic peptide with three L-glutamic acid 5-methyl ester subunits was still relatively low, sufficiently high solubility could be achieved by using the 5-isopropyl ester instead. We have performed the synthesis of the corresponding cyclopeptide **2** in solution because, in this way, the product could be obtained in higher purity and in better yields than by synthesis on solid support. The repeating dipeptide subunit was prepared by coupling the BOC-protected 5-isopropyl ester of L-glutamic acid with the benzyl ester of 3-aminobenzoic acid. This dipeptide was elongated successively, first to the tetrapeptide and then to the hexapeptide by using conventional peptide-coupling methods. The fully deprotected linear hexapeptide was cyclized under high dilution conditions (Scheme 1).

Scheme 1. Synthesis of the Cyclic Hexapeptide **2**



Cation Binding of the Cyclopeptide in the Absence of Effector Anions. Cation$-\pi$ interactions of quaternary ammonium ions and, e.g., calixarenes can be conveniently detected by NMR spectroscopy.[6] When cations are included into a cavity which is lined by aromatic subunits, they come in close contact with the faces of the aromatic systems. As a result, complexation causes an upfield shift of the guest protons in the ^{1}H NMR. The larger these shifts are, the better is the interaction of the guests with the aromatic systems or the fit of the guests inside the receptor cavity. In contrast, complexation by hydrogen bonding usually results in downfield shifts of the protons involved in binding.

We tested the binding affinity of **2** toward *n*-butyltrimethylammonium ($BTMA^+$) iodide. When this quaternary ammonium salt is added to a solution of **2** in $CDCl_3$, very small upfield shifts of the guest's *N*-methyl protons in the order of -0.02 ppm are observed. An interaction between $BTMA^+$ and the cyclopeptide obviously takes place, but the influence of the aromatic rings on the resonances of the guest molecule protons is relatively weak. An even smaller upfield shift can also be observed for all other protons of $BTMA^+$. Yet the more remote they are from the cationic headgroup, the less pronounced the effect is. This suggests a preferable inclusion of the trimethylammonium group into the host cavity. Rotation of the guest inside the cavity seems to be possible to a certain degree, which would account for the small shifts of the *n*-butyl protons. The resulting shift pattern is comparable to the one observed for cation$-\pi$ interactions between similar quaternary ammonium ions and calix[5]arenes,[6l] homooxacalixarenes,[6i] or cryptophanes.[6d] No significant change in the NMR spectrum of **2** was detected during complexation.

(4) Ishida, H.; Suga, M.; Donowaki, K.; Ohkubo, K. *J. Org. Chem.* **1995**, *60*, 5374−5375.

(5) (a) Lhotak, P.; Shinkai, S. *J. Phys. Org. Chem.* **1997**, *10*, 273−285. (b) Ma, J. C.; Dougherty, D. A. *Chem. Rev.* **1997**, *97*, 1303−1324.

(6) (a) Stauffer, D. A.; Dougherty, D. A. *Tetrahedron Lett.* **1988**, *29*, 6039−6042. (b) Araki, K.; Shimizu, H.; Shinkai, S. *Chem. Lett.* **1993**, 205−208. (c) De Iasi, G.; Masci, B. *Tetrahedron Lett.* **1993**, *34*, 6635−6638. (d) Garel, L.; Lozach, B.; Dutasta, J.-P.; Collet, A. *J. Am. Chem. Soc.* **1993**, *115*, 11652−11653. (e) Méric, R.; Lehn, J. M.; Vigneron, J. P. *Bull. Soc. Chim. Fr.* **1994**, *131*, 579−83. (f) Takeshita, M.; Nishio, S.; Shinkai, S. *J. Org. Chem.* **1994**, *59*, 4032−4034. (g) Casnati, A.; Jacopozzi, P.; Pochini, A.; Ugozzoli, F.; Cacciapaglia, R.; Mandolini, L.; Ungaro, R. *Tetrahedron* **1995**, *51*, 591−598. (h) Cattani, A.; Dalla Cort, A.; Mandolini, L. *J. Org. Chem.* **1995**, *60*, 8313−8314. (i) Masci, B. *Tetrahedron* **1995**, *51*, 5459−5464. (j) Arduini, A.; McGregor, W. M.; Paganuzzi, D.; Pochini, A.; Secchi, A.; Ugozzoli, F.; Ungaro, R. *J. Chem. Soc., Perkin Trans. 2* **1996**, 839−846. (k) Magrans, J. O.; Ortiz, A. R.; Molins, M. A.; Lebouille, P. H. P.; Sánchez-Quesada, J.; Prados, P.; Pons, M.; Gago, F.; de Mendoza, J. *Angew. Chem., Int. Ed. Engl.* **1996**, *35*, 1712−1715. (l) Arnecke, R.; Böhmer, V.; Cacciapaglia, R.; Dalla Cort, A.; Mandolini, L. *Tetrahedron* **1997**, *53*, 4901−4908.

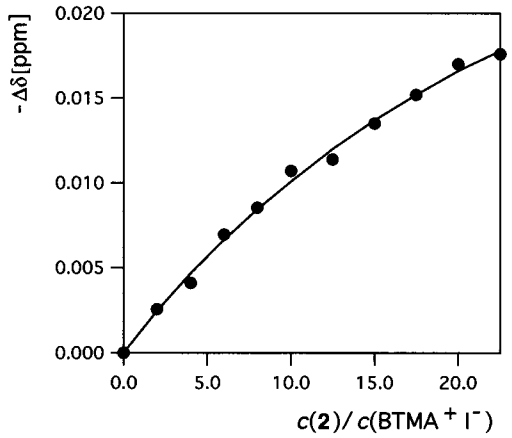


Figure 2. ^{1}H NMR titration curve of the complex of **2** with *n*-butyltrimethylammonium iodide.

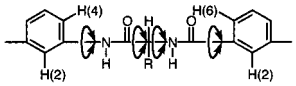


Figure 3. Rotating bonds in a peptide chain between the aromatic residues of cyclopeptide **2**.

A 1:1 stoichiometry of the complex of **2** with $BTMA^+$ iodide was determined by Job's method of continuous variations,[7,8] and an association constant K_a of 300 M^{-1} was determined by ^{1}H NMR titration (Figure 2).[7,9] The association constant is in the order of calixarene complexes with related quaternary ammonium ions.[6b,f,g,j,l] Despite the small influence of the receptor on the proton resonances of the guest, **2** obviously still forms reasonably stable complexes with $BTMA^+$.

For the determination of the binding mechanism of **2**, its conformation in the complex is an important indication. In the case of hydrogen bonding, all groups which are involved in binding must converge in such a way that they are able to bind the guest molecule at the suitable positions. In contrast, cation$-\pi$ interactions require that the aromatic subunits of **2** are arranged around a cavity so that the included guests are able to interact with the faces of the aromatic systems.[5b,10] In contrast to the case with simple calix[*n*]arenes,[11] possible conformations of **2** are not only determined by the relative orientations of the aromatic rings. Because there are six single bonds between the aromatic subunits of the cyclopeptides, four more than in calix[*n*]arenes, rotations around additional bonds have to be taken into account. For the secondary amide groups, energetically more favored trans conformations can be assumed. Rotations around the remaining bonds should be possible and cause different orientations of the protons at both nitrogens and the C(α) with respect to the aromatic rings (Figure 3).

The simple ^{1}H NMR (Figure 4a) and ^{13}C NMR spectra of **2** in DMSO-d_6 give no information whether these spectra represent a single C_3-symmetrical conformer of **2** or an average of different conformational isomers which rapidly interconvert in solution. In this respect, the NOESY NMR spectrum of **2** in DMSO-d_6 is more instructive. Both NH groups and H(α) possess strong

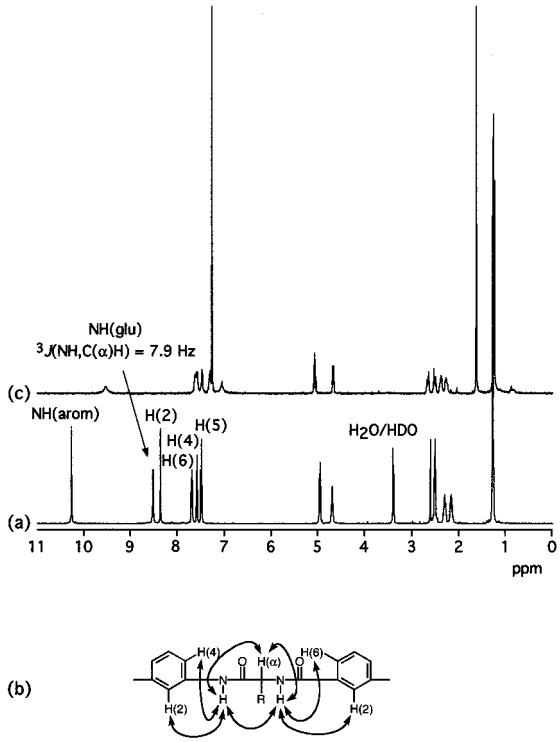


Figure 4. ^{1}H NMR spectra of **2** in DMSO-d_6 (a) and in $CDCl_3$ (c) as well as intramolecular NOE effects in the NOESY NMR spectrum of **2** in DMSO-d_6.

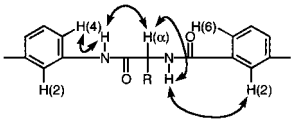


Figure 5. Intramolecular NOE effects in the NOESY NMR spectrum of **2** in 5% DMSO-d_6/$CDCl_3$.

NOE effects with each of their adjacent protons (Figure 4b). This large number of NOE effects shows that fast rotations around all bonds indicated in Figure 3 occur in DMSO at room temperature. The NOESY spectrum thus illustrates the high flexibility of cyclopeptide **2** in this solvent. Temperature-dependent ^{1}H NMR spectra of **2** have been recorded in DMF-d_7. Only broadening of the signals was observed down to -60 °C. Even at this temperature, the flexibility of **2** is obviously still too high for individual conformers to be resolved by NMR spectroscopy.

In $CDCl_3$, all signals in the ^{1}H NMR spectrum of **2** are somewhat broadened already at room temperature (Figure 4c). An explanation of this line broadening could be that the conformational flexibility of **2** is reduced in $CDCl_3$, possibly because some of its conformers are stabilized by intramolecular hydrogen bonds. Indeed, the NOESY NMR spectrum of **2** in 5% DMSO-d_6/$CDCl_3$ is much simpler than the one in DMSO-d_6. Increasing line broadening precluded measuring suitable spectra in solutions containing less DMSO-d_6. But even in 5% DMSO-d_6/$CDCl_3$, certain conformers of **2** seem to be favored. Cross-peaks can be detected in the NOESY NMR spectrum between the aromatic NH and H(4) of the adjacent aromatic residue as well as between the glutamic acid NH and H(2) (Figure 5). No cross-peak between an aromatic and a glutamic acid NH group is visible. This shows that the orientations of the amide groups with respect to the aromatic rings are relatively fixed, and the aromatic NH groups point "up" toward H(4) and

(7) Connors, K. A. *Binding Constants*; Wiley: New York, 1987.
(8) Gil, V. M. S.; Oliveira, N. C. *J. Chem. Educ.* **1990**, *67*, 473−478.
(9) Macomber, R. S. *J. Chem. Educ.* **1992**, *69*, 375−378.
(10) Mecozzi, S.; West, A. P., Jr.; Dougherty, D. A. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*, 10566−10571.
(11) Gutsche, C. D. *Inclusion compounds*, Vol. 4; Atwood, J. L., Davies, J. E. D., MacNicol, D. D., Eds.; Oxford University Press: Oxford, UK, 1991; pp 27−63. Shinkai, S. *Tetrahedron* **1993**, *49*, 8933−8968. Böhmer, V. *Angew. Chem., Int. Ed. Engl.* **1995**, *34*, 713−745.

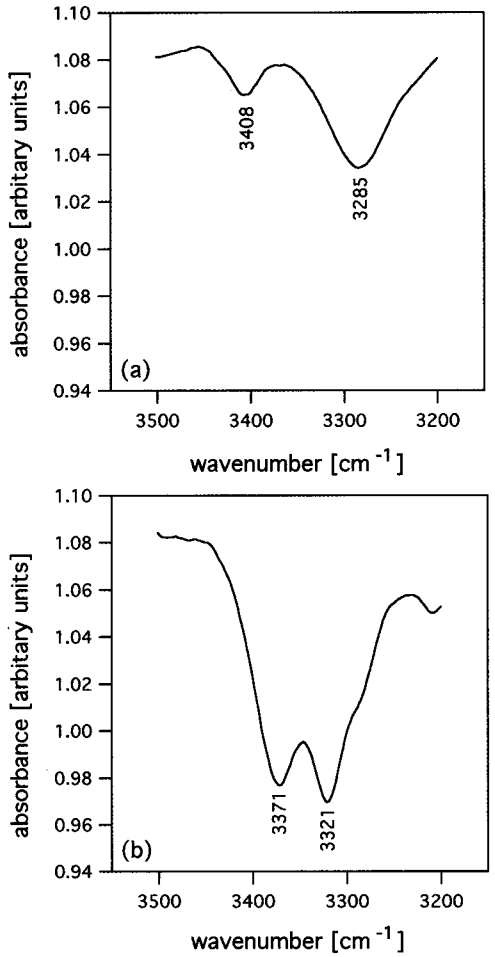


Figure 6. NH stretching region of the FT-IR spectra of free **2** (a) and its complex with K^+−18-crown-6 tosylate (b) (c = 1 mM in $CDCl_3$).

the glutamic acid NH "down" toward H(2). Indeed, only in such alternate orientations of the two NH groups are intramolecular hydrogen bonds possible. However, since both NH groups still possess NOE effects to H(α), rotations around the bonds at C(α) must still be possible, and the cyclopeptide is not completely rigid.

An alternative explanation for the NMR signal broadening may also be an intermolecular association of **2**. We could rule out this possibility, however, by FT-IR and NMR spectroscopic measurements. The IR spectrum of a 1 mM solution of **2** in $CDCl_3$ shows two N−H stretching bands, a weaker one at 3408 cm^{-1} (free NH) and a more intensive band at 3285 cm^{-1} (hydrogen-bonded NH) (Figure 6a).[12] The ratio of the areas under both bands remains constant over a concentration range from 2 to 0.2 mM. This, and the fact that the line broadening in the ^{1}H NMR spectrum of **2** is not affected upon dilution of the solution down to 0.1 mM, indicate that hydrogen bonding occurs intramolecularly. No significant intermolecular association could be detected in $CDCl_3$ at concentrations relevant for our investigations on the complexing behavior of **2**.

In summary, our results indicate that **2** is flexible in DMSO, whereas in $CDCl_3$ its flexibility is reduced and the conformational equilibrium is shifted toward conformers with intramolecular hydrogen bonds. The addition of $BTMA^+$ iodide does not affect the cyclopeptide's ^{1}H NMR spectrum or the NH vibration bands in its FT-IR spectrum, which would indicate an influence of complex formation on the solution structure of **2** or an interaction of the cyclic peptide with the iodide anions. Therefore, the complexing properties of **2** can best be explained when it is assumed that at least some of the conformers of **2** that are present in solution possess a cavity which is suitable for the interaction with cations. The upfield shifts of the $BTMA^+$ protons in the ^{1}H NMR suggest that binding is due to cation−π interactions.[13] The small influence of complexation on the chemical shifts of the guest protons may be attributed to the receptor's flexibility. Another reason for the small shift can also be that the diameter of the cyclopeptide cavity is too large for optimum interaction with $BTMA^+$. For the unexpected high stability of the **2**−$BTMA^+$ complex, electrostatic effects of the amide carbonyl groups may be responsible.[15] Besides the aromatic rings, also the peptide's carbonyl groups line the cyclopeptide's cavity and contribute to the overall negative electron density of its inner surface. As a consequence, the carbonyl groups might assist in the binding of the substrate. On the other hand, several groups have shown that, in the case of cation−π interactions, a tight fit of the guest inside the receptor is not necessary for the formation of stable complexes.[6d,15]

Cation Binding of the Cyclopeptide in the Presence of Tosylate Anions. We have found that cyclopeptide **2** behaves distinctly differently toward $BTMA^+$ cations when, instead of iodide, tosylate is the counterion in NMR titrations. In contrast to the iodide salt, a significant influence on the ^{1}H NMR spectra can be observed when $BTMA^+$ tosylate is added to solutions of **2** in $CDCl_3$. Mixtures of $BTMA^+$ tosylate and an excess of **2** possess two sets of signals in their NMR spectra: the broader signals of the free cyclopeptide and sharper ones of a new species composed of equimolar amounts of **2** and $BTMA^+$ tosylate. The latter can be assigned to a complex between **2** and the tosylate anion. At equimolar concentrations of **2** and $BTMA^+$ tosylate, only the spectrum of the complex is visible. A further increase of the salt concentration causes the appearance of the free $BTMA^+$ tosylate signals. The stability of the **2**−tosylate complex is obviously so high that the exchange of guest molecules is slow on the NMR time scale, and complexed and uncomplexed species can be observed separately.

The variation of the spectra of free cyclopeptide and its tosylate complex is most noticeable in the aromatic region (Figure 7). Complex formation between tosylate and **2** causes

(12) Vinogradov, S. N.; Ninnell, R. H. *Hydrogen Bonding*; Van Nostrand Reinhold Co.: New York, 1971. Néel, J. *Pure Appl. Chem.* **1972**, *31*, 201−225.

(13) Complexation of $BTMA^+$ iodide by **2** seems to be due to cation−π interactions, but other binding mechanisms may also be responsible for the complexation of this guest. For example, Reetz has shown that the α-methylene groups of a tetrabutylammonium cation can interact with carbonyl groups of enolates by hydrogen bonding.[14] In a recent investigation on the complexation of quaternary ammonium ions by uncharged macrocyclic "phane" esters, no interaction of the receptor's carbonyl groups with the guests was detected in $CDCl_3$.[15] The presented data suggest, however, that the ester functions may possess a cooperative role in cation−π interactions. Furthermore, one has to consider that amide carbonyls are stronger hydrogen bond acceptors than ester carbonyls.[16] FT-IR spectroscopic investigations on the complexes of **2** with $BTMA^+$ iodide showed no influence of complex formation on the vibration frequencies of the peptide's carbonyl groups. We take this as a further indication that the complexation of cations by **2** is due to cation−π interactions. More importantly, we recently were able to crystallize a complex of a quaternary ammonium ion and a cyclic hexapeptide which contains L-proline subunits instead of glutamic acid. We could detect no directed hydrogen bonds between the carbonyl groups of the peptide and the ammonium ion in the crystal structure. The properties of the L-proline-containing cyclopeptide will be published shortly.

(14) Reetz, M. T.; Hütte, S.; Goddard, R. *J. Am. Chem. Soc.* **1993**, *115*, 9339−9340.

(15) Roelens, S.; Torriti, R. *J. Am. Chem. Soc.* **1998**, *120*, 12443−12452.

(16) Arnett, E. M.; Mitchell, E. J.; Murty, T. S. S. R. *J. Am. Chem. Soc.* **1974**, *96*, 3875−3891. Spencer, J. N.; Garrett, R. C.; Mayer, F. J.; Merkle, J. E.; Powell, C. R.; Tran, M. T.; Berger, S. K. *Can. J. Chem.* **1980**, *58*, 1372−1375. Gellman, S. H.; Dado, G. P.; Liang, G.; Adams, B. R. *J. Am. Chem. Soc.* **1991**, *113*, 1164−1173.

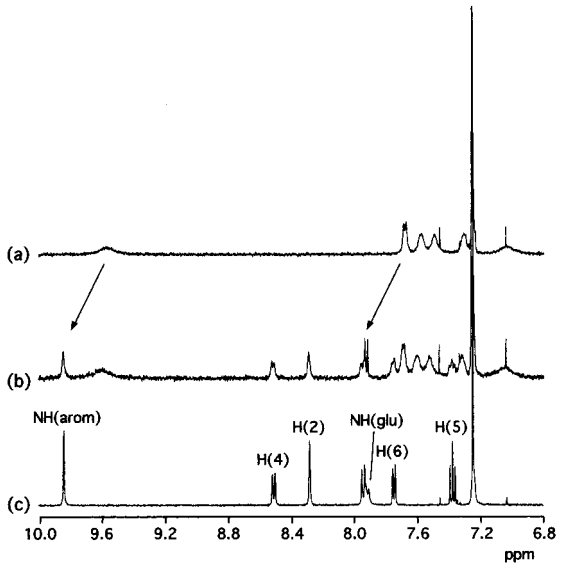


Figure 7. ^{1}H NMR spectra of **2** in $CDCl_3$ (a) and after addition of 0.25 (b) and 1.0 equiv (c) of K^+−18-crown-6 tosylate.

downfield shifts of the signals of both NH groups by ca. 0.25 ppm, but also all the signals of the aromatic ring protons of **2** are shifted in the same direction. The coupling constant $^3J_{NH-C(\alpha)H}$ in the **2**−tosylate complex amounts to 9.5 Hz, which is significantly larger than the one of free **2** in DMSO-d_6 ($^3J_{NH-C(\alpha)H} = 7.9$ Hz). From the coupling constant of the tosylate complex, a dihedral angle θ of ±158° can be calculated, whereas $^3J_{NH-C(\alpha)H}$ of the free cyclopeptide corresponds to the angles ±24° and ±143°.[17] It has to be considered, however, that $^3J_{NH-C(\alpha)H}$ of free **2** represents only a weighted averaged value of the coupling constants of all conformers present in solution.

Besides complexation of the tosylate, also that of the $BTMA^+$ cation can be observed in the NMR spectra from the characteristic upfield shift of the corresponding protons. In the spectrum of an equimolar mixture of **2** and $BTMA^+$ tosylate (1 mM in $CDCl_3$), the resonance of, e.g., the *N*-methyl protons of $BTMA^+$ is shifted by −0.34 ppm in comparison to that of the free salt. This shift is significantly larger than the one which is observed when **2** binds $BTMA^+$ iodide (−0.02 ppm). When more than 1 equiv of $BTMA^+$ tosylate is added to **2**, only averaged signals for the cations are observed. This fast guest exchange makes possible the NMR spectroscopic determination of the K_a of the $BTMA^+$ **2**−tosylate complex by dilution titration. No dissociation of **2**−tosylate could be detected in the ^{1}H NMR spectra upon dilution of a solution up to 10^{-6} mol·L^{-1}. Therefore, the K_a of this complex is at least in the order of 10^9 M^{-1}, and **2**−tosylate can be safely regarded as a stable compound in the investigated concentration region. On the other hand, dilution causes the dissociation of the complex between the $BTMA^+$ cation and the **2**−tosylate anion, as indicated by a downfield shift of the cation protons. When the variation of this shift is plotted against the total concentration of the complex, K_a can be calculated from the resulting saturation curve (Figure 8a).[7,9] An association constant of 3.88×10^6 M^{-1} has thus been determined, which shows that the change from an iodide to a tosylate salt causes an increased $BTMA^+$ complex stability by a factor of ca. 10^4. The tosylate anion behaves obviously highly cooperatively in the complexation of cations by **2**. The ^{1}H NMR spectrum of the **2**−tosylate complex clearly shows that complex formation is accompanied by a conformational reorganization

(17) Bystrov, V. F. *Prog. Nucl. Magn. Reson. Spectrosc.* **1976**, *10*, 41−81.

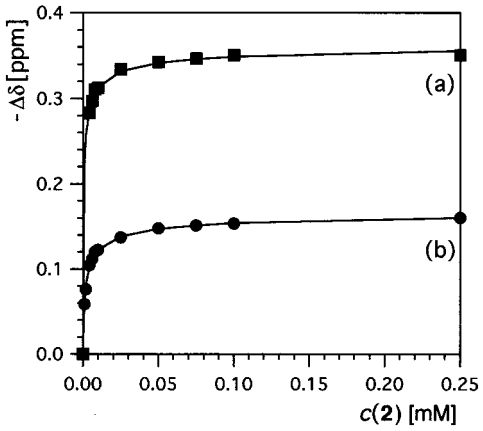


Figure 8. Saturation curves of NMR dilution titrations of the $BTMA^+$ **2**−tosylate complex (a) and of the K^+−18-crown-6 **2**·tosylate complex (b).

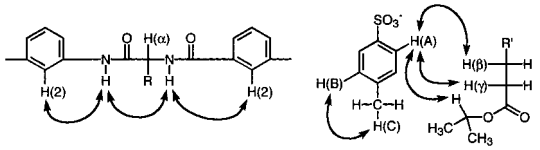


Figure 9. Intra- and intermolecular NOE effects in the NOESY NMR spectrum of the K^+−18-crown-6 **2**−tosylate complex in $CDCl_3$.

of the receptor. It is, therefore, reasonable to assume that the cyclopeptide conformation in the **2**−tosylate complex is much better preorganized for the complexation of cations than that of the more flexible free macrocycle. The preorganization is furthermore reflected in the increase of $\Delta\delta_{max}$. Preorganization of **2** causes a better interaction of the guests with the aromatic subunits and accordingly a larger value for $\Delta\delta_{max}$.

Again, NOESY NMR spectroscopy gives valuable information about the structure of the **2**−tosylate complex in solution. We used potassium tosylate instead of the $BTMA^+$ salt in these investigations. The K^+ salt is soluble in $CDCl_3$ when equimolar amounts of 18-crown-6 are added to the solution to bind the cations. The change of the cations does not seem to influence the complex conformation of **2**−tosylate because, apart from the cation signals, the spectra of $BTMA^+$ **2**−tosylate and K^+−18-crown-6 **2**−tosylate are identical.

The NOESY spectrum of the K^+−18-crown-6 **2**−tosylate complex shows cross-peaks between H(2) and both NH groups as well as between the two NH groups themselves (Figure 9). These effects show that **2** adopts a C_3-symmetrical conformation in the tosylate complex, with all NH groups as well as the side chains of the glutamic acids pointing "down" in the same direction as the H(2) of the aromatic rings. Clearly, the influence of the tosylate ion causes orientations of the NH groups and the amino acid side chains which are distinctly different from the ones in free **2**. Intermolecular NOE effects are visible between the aromatic ring of the tosylate and the glutamic acid side chains (Figure 9). Therefore, the aromatic ring of the tosylate must be arranged parallel to these residues.

The downfield shift of the NH groups in the ^{1}H NMR spectrum of the complex suggests that these groups interact with the tosylate anion by hydrogen bonding. This type of interaction can also be derived from the FT-IR spectrum. In this spectrum, two N−H vibration bands are visible which both possess the characteristic frequencies and intensities of hydrogen-bonded NH groups (Figure 6b).[12] A band for free NH groups as in Figure 6a cannot be observed. Therefore, all six NH groups of **2** must be involved in the binding of the tosylate anion. The

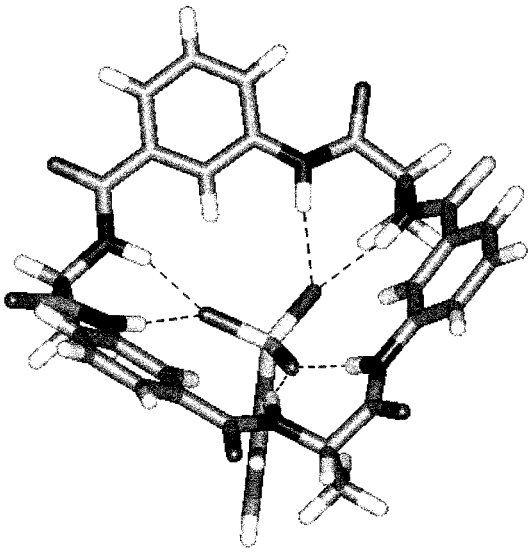


Figure 10. Energy-minimized structure of the **2**−tosylate complex (for reasons of clarity, the glutamic acid side chains have been replaced by methyl groups).

fact that two NH vibration bands are present indicates that, in the complex, two types of hydrogen bonds between anion and NH groups exist. Most probably, the six hydrogen bonds can be subdivided into three bonds between the anion and the glutamic acid NH and three between the anion and the aromatic NH. All of these hydrogen bonds are weaker than the intramolecular H-bonds in free **2**, as indicated by the higher NH absorption frequencies. A possible explanation for this observation may be that, in the **2**−tosylate complex, binding of three anion oxygens is distributed over six NH groups, and the corresponding interactions may therefore not have an optimal geometry. In contrast, each NH has one partner for a hydrogen bond in free **2**.

The sum of this structural information was used to calculate the structure of the **2**−tosylate complex by molecular modeling.[18] The energy-minimized conformation is depicted in Figure 10. In this structure, all six NH groups of **2** bind the three tosylate oxygens simultaneously, as predicted by IR spectroscopy. It has to be considered, however, that Figure 10 represents only a static symmetrical structure of the complex. Although the exchange of bound anions in solution is slow on the NMR time scale, a rotation of the guests in the complex may certainly be possible, and as a result, different orientations of the anion inside the cyclopeptide rapidly interconvert. The angle θ in the modeled cyclopeptide conformation amounts to −173°, which is in acceptable agreement with the one calculated from the observed coupling constant $^3J_{NH-C(\alpha)H}$ (±158°). The difference between calculated and observed values may be attributed to dynamical processes in the complex. The almost coplanar orientations of the carbonyl groups to the adjacent aromatic rings account for the downfield shift of the aromatic H(4) and H(6) upon complexation of tosylate by **2**. The downfield shift of H(2) can be attributed to the electric field of the anion which is bound in close proximity.

Figure 10 demonstrates that **2**−tosylate should, indeed, be ideally suited for the complexation of cations. The complex has the shape of a shallow dish with the anion located at its bottom. From there, the anion can attract cationic guests into the cavity.

(18) Molecular modeling was performed on a Silicon Graphics workstation using the program Cerius[2] (Molecular Simulations Inc.) with a Dreiding 2.21 force field (Mayo, S. L.; Olafson, B. D.; Goddard, W. A., III. *J. Phys. Chem.* **1990**, *94*, 8897−8909).

Table 1. Association Constants K_a and Maximum Chemical Shifts $\Delta\delta_{max}$ of Complexes of Preorganized and Non-preorganized **2** with Different Cations in $CDCl_3$ at 298 K

anion	cation	K_a (M^{-1})	$\Delta\delta_{max}$ (ppm)
iodide	K^+−18-crown-6	nd	<0.01
tosylate	K^+−18-crown-6	$8.09 \times 10^5 \pm 3.91 \times 10^4$	0.17
tosylate	Na^+−15-crown-5	$2.13 \times 10^5 \pm 2.07 \times 10^4$	0.07
tosylate	Li^+−12-crown-4	nd	<0.01
iodide	$BTMA^+$	$3.00 \times 10^2 \pm 0.40 \times 10^2$	0.05
tosylate	$BTMA^+$	$3.88 \times 10^6 \pm 1.95 \times 10^5$	0.37
tosylate	$\frac{K_a(BTMA^+)}{K_a(K^+-18\text{-crown-6})} = 0.210$	$K_a(BTMA^+) = 1.70 \times 10^5$	0.49
tosylate	$\frac{K_a(BTMA^+)}{K_a(Na^+-15\text{-crown-5})} = 0.773$	$K_a(BTMA^+) = 1.65 \times 10^5$	0.28
		1.68×10^5	0.39

In addition, negative electron density of the aromatic cyclopeptide subunits and of the peptide's carbonyl groups is distributed around the rim of the cavity. In this region of the receptor, binding should be due to cation−π interactions. The hydrogen bonding of the tosylate anions to the NH groups of the cyclopeptide may even increase the electron density of the aromatic rings and thus provide stronger cation−π interaction. This assumption is consistent with theoretical calculations, which showed that cation−π interactions between phenol and Na^+ are considerably enhanced when the phenolic OH is hydrogen-bonded to a formamide,[10] and with experimental results on an artificial receptor containing phenol subunits whose affinity toward cations also increases when anions are hydrogen-bonded at the OH groups.[19] Hydrogen bonding of the guest cations to the carbonyl groups of **2**−tosylate is unlikely. Figure 10 shows that all carbonyl groups of the receptor diverge from the center of the cavity and should not be able to cooperatively form defined 1:1 complexes with bound guest molecules.

In our NMR experiments with the K^+−18-crown-6 **2**−tosylate complex, we found a considerable upfield shift of the crown ether protons by −0.16 ppm in comparison to the resonance of free K^+−18-crown-6 tosylate (1 mM in $CDCl_3$). Obviously, also this cation, as possibly many others, can interact with the cyclopeptide. In this respect, the quaternary ammonium ions or the K^+−18-crown-6 only possess the advantage that their complexation can be easily followed by NMR spectroscopy. The interaction of K^+−18-crown-6 with **2**−tosylate can be rationalized by assuming that the whole cationic crown ether complex is embedded in the **2**−tosylate cavity, where it is attracted by the tosylate anions. This inclusion brings the crown ether protons in close proximity to the aromatic walls of the cyclopeptide, where they experience the shielding of their protons. No dissociation of the K^+−18-crown-6 complex has to occur for this type of complex formation. In fact, the K^+−18-crown-6 complex is so stable in $CDCl_3$ ($K_a = 1.6 \times 10^8$ M^{-1})[20] that, under the chosen conditions, its complexation by **2**−tosylate can almost be regarded as an interaction between two stable compounds. Consequently, it was possible to determine the stability of this complex by dilution titration analogously to the $BTMA^+$ complex (Figure 8b). By choosing other alkali cation crown ethers pairs, e.g., sodium tosylate and 15-crown-5, we could furthermore study the influence of the size of the guest on the K_a of the complexes systematically (Table 1). In all dilution titrations, the dissociation of the cation−crown ether complexes at low concentrations was taken into

(19) Jeong, K.; Hahn, K.; Cho, Y. *Tetrahedron Lett.* **1998**, *39*, 3779−3782.

(20) Iimori, T.; Still, W. C. *J. Am. Chem. Soc.* **1989**, *111*, 3439−3440.

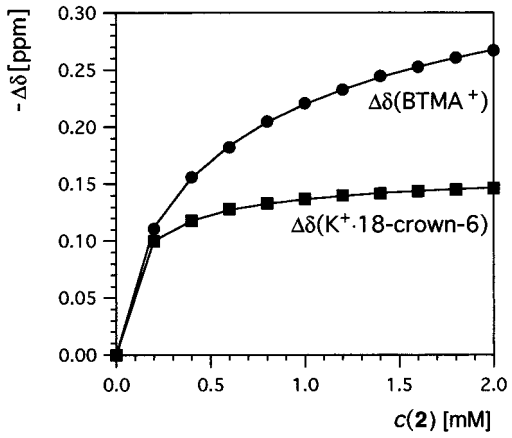


Figure 11. Saturation curves of a competitive NMR titration of the K^+−18-crown-6 **2**−tosylate complex and $BTMA^+$ iodide.

account by including the corresponding stability constants[20] in the calculations. With Li^+ and 12-crown-4, the upfield shift upon complexation by **2**−tosylate was not large enough to be followed quantitatively. Electrostatic interactions between Li^+−12-crown-4 and **2**−tosylate certainly also cause a complexation of this cation. But compared to the cyclopeptide cavity, the Li^+−12-crown-4 complex is probably too small for a significant influence of the aromatic systems on the resonance of the crown ether protons. Table 1 furthermore shows that a complexation of K^+−18-crown-6 by **2** can be also detected in the absence of effector anions, as indicated by the small upfield shift of the crown ether protons. Because of the small variation in the chemical shifts (<0.01 ppm), a quantitative determination of the K_a of this complex was difficult.

Knowing the cation−crown ether **2**−tosylate stability constants, we were able to determine the K_a of the corresponding $BTMA^+$ complex by an alternative method, namely by competitive NMR titrations.[21] For this, increasing amounts of $BTMA^+$ iodide were added to solutions of, e.g., K^+−18-crown-6 **2**−tosylate in $CDCl_3$. Under these conditions, complexes of **2**−tosylate with $BTMA^+$ and the cation−crown ether are formed simultaneously in relative amounts that depend on the ratio of their association constants. The relative concentrations of both complexes in solution are determined by following the upfield shift of the protons of both guests NMR spectroscopically. From the resulting pair of saturation curves (Figure 11), the K_a ratio of the complexes can be calculated and, by multiplying this ratio with the previously determined K_a of the corresponding cation−crown ether complex, a new association constant for the $BTMA^+$ complex is obtained. Such a titration has been performed with K^+−18-crown-6 as well as Na^+−15-crown-5. Table 1 shows that the association constants for the $BTMA^+$ complexes from the two experiments are in excellent agreement but more than 1 order of magnitude smaller than the K_a obtained in the dilution titration.

The deviation of the two association constants can, to a certain extent, be attributed to the different experimental conditions of dilution and competitive NMR titrations. A decrease of the K_a in competitive titrations could be expected because, due to the higher salt concentration and, hence, the increased ionic strengths of the solutions in these measurements, the electrostatic interaction between receptor and substrate should be weakened. However, the decrease is so pronounced that the $BTMA^+$ complex is now less stable than the cation−crown ether complexes. In our eyes, this indicates that the K_a of the $BTMA^+$ **2**−tosylate complex has been overestimated in the dilution titration. Under the conditions of this method, a dissociation of the complex is accompanied by an ion pair separation. Such a separation is not so problematic when the cations are stabilized by interactions with, e.g., crown ethers but is especially unfavorable when they are not sufficiently solubilized.[22] Therefore, the K_a which resulted from the competitive titrations seems to be a more realistic value for the stability of the $BTMA^+$ **2**−tosylate complex.

It appears as if the high stability of the cation **2**−tosylate complexes is mainly due to strong electrostatic interactions between the ionic guests. The K_a of the $BTMA^+$ **2**−tosylate complex ($1.68 \times 10^5\ M^{-1}$), for example, corresponds to a free energy of complex formation ΔG of $-29.6\ kJ \cdot mol^{-1}$. But already the complex of **2** with $BTMA^+$ iodide ($K_a = 300\ M^{-1}$) possesses a considerable binding energy of $\Delta G = -14.0\ kJ \cdot mol^{-1}$. Cation−$\pi$ interactions are certainly weaker than electrostatic interactions between two permanent charges.[5b] Still, the cooperativity of the subunits in **2** significantly contributes to the overall stabilities of the resulting complexes.

Cation Binding of the Cyclopeptide in the Presence of Phosphonate Anions. The complexation of phosphate by the cyclic peptide **1** has been reported.[4] Analogously to the **2**−tosylate complex, the binding of dipotassium 4-nitrophenyl phosphate by **1** in DMSO-d_6 causes a change of the cyclopeptide's ^1H NMR spectrum. Besides significant downfield shifts of the two NH signals, which were also interpreted in terms of hydrogen bonding of the phosphate anion to **1**, the aromatic protons shift similarly to the ones in the spectrum of the **2**−tosylate complex, and the coupling constants $^3J_{NH-C(\alpha)H}$ of both complexes even have the same value. Therefore, the side chains of the natural amino acid subunits do not seem to have a significant effect on the complexation of anions by **1** or **2**, and the cyclopeptide conformation of the phosphate and tosylate complexes should be comparable. Consequently, also phosphate anions might act as effectors for the complexation of cations by **2**.

To study the influence of the type of effector anion on the cation complex stabilities of **2**, we tested phosphate and related anions in our investigations, too. The monoesters of phosphate were only poorly soluble in $CDCl_3$, so we were unable to perform investigations with these anions. Instead, we could use dipotassium phenylphosphonate which dissolved in $CDCl_3$ when 2 equiv of 18-crown-6 was added to the solution for K^+ complexation. In the ^1H NMR spectrum of an equimolar mixture of $(K^+$−18-crown-6$)_2$ phenylphosphonate and **2** in 10% DMSO-

(21) Martin, M.; Raposo, C.; Almaraz, M.; Crego, M.; Caballero, C.; Grande, M.; Morán, J. R. *Angew. Chem., Int. Ed. Engl.* **1996**, *35*, 2386−2388. Almaraz, M.; Martin, M.; Hernández, J. V.; Caballero, M. C.; Morán, J. R. *Tetrahedron Lett.* **1998**, *39*, 1811−1814.

(22) In nonpolar solvents, salts are known to exist as close ion pairs even at low concentrations.[23] In a series of papers, Wilcox et al. have shown that, in nonpolar solvents, the rate of various reactions is significantly enhanced when a charged group is attached covalently close to the reactive site of a substrate.[24] This rate enhancement was attributed mainly to influences of the local electric field of the ion pair localized in close proximity to the substrate's reaction center. The effect was called the intramolecular salt effect and is strongest in chloroform, because in this solvent the most specific and most stable associations have been observed. These results indicate that cation−anion separation in chloroform is, indeed, unfavorable and can only be achieved, e.g., with ditopic receptors when both ions are stabilized by suitable binding sites.[3j] Therefore, the assumption that the dissociation of the $BTMA^+$ **2**−tosylate complex is less likely than the one of K^+−18-crown-6 **2**−tosylate seems to be reasonable.

(23) Davies, C. W. *Ion Association*; Butterworth: London, 1962.

(24) Smith, P. J.; Wilcox, C. S. *J. Org. Chem.* **1990**, *55*, 5675−5678. Smith, P. J.; Wilcox, C. S. *Tetrahedron* **1991**, *47*, 2617−2628. Smith, P. J.; Soose, D. J.; Wilcox, C. S. *J. Am. Chem. Soc.* **1991**, *113*, 7412−7414. Smith, P. J.; Reddington, M. V.; Wilcox, C. S. *Tetrahedron Lett.* **1992**, *33*, 6085−6088.

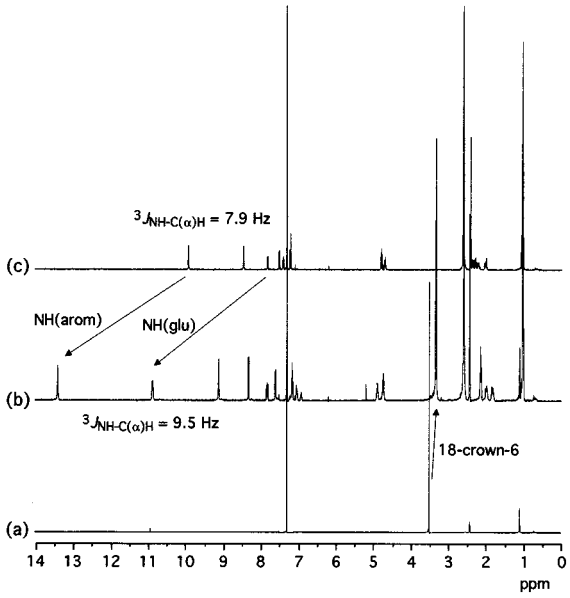


Figure 12. ^{1}H NMR spectra of $(K^+-18\text{-crown-}6)_2$ phenylphosphonate (a), **2** (c), and the $(K^+-18\text{-crown-}6)_2$ **2**−phenylphosphonate complex (b) in 10% DMSO-d_6/$CDCl_3$.

d_6/$CDCl_3$, the usual effects of anion complexation on the cyclopeptide signals and of cation complexation on the crown ether protons are visible (Figure 12). The most prominent effect of phosphonate complexation is the pronounced downfield shift of the NH protons by more than 3 ppm. This shift is significantly larger than the one which resulted from the complexation of tosylate by **2** (ca. 0.25 ppm) but is in the same order of magnitude as the shift of the **1**−phosphate complex.[4] Obviously, phosphates and phosphonates interact much more strongly with the cyclopeptides than tosylates. This can be also demonstrated by FT-IR spectroscopy. No defined NH stretching band is observed in the spectrum of the **2**−phosphonate complex in $CDCl_3$ above 3200 cm^{-1}. Instead, only a very broad band is visible between 2600 and 3400 cm^{-1} which overlaps with other bands in this region. The absorption frequency and width of this band account for the strong hydrogen bonding of the phosphonate oxygens to the cyclopeptide's NH groups. The stronger interaction of **2** with phenylphosphonate in comparison to tosylate can be attributed to the significantly larger basicity of the phosphonate dianion (pK_{a2}(phenylphosphonate) = 7.07;[25] pK_a(tosylate) = −1.3[26]), with which a higher affinity of the anion toward the NH protons is associated.

In the ^{1}H NMR spectrum of the $(K^+-18\text{-crown-}6)_2$ **2**−phosphonate complex, an upfield shift of the crown ether protons of −0.22 ppm was observed (1 mM in 10% DMSO-d_6/$CDCl_3$), which accounts for the interaction of the cations with the cyclopeptide moiety. However, we were unable to determine the K_a of the corresponding complex quantitatively. It was not possible to detect a dissociation of the complex NMR spectroscopically upon dilution of the solution. Obviously, the K_a of the $(K^+-18\text{-crown-}6)_2$ **2**−phosphonate complex is even larger than the one of the corresponding **2**−tosylate complex and cannot be determined by this method. Alternatively, we used competitive NMR titrations because it can be expected that, under the conditions of this method, the increased ionic strength of the solutions should reduce the K_a. But the saturation curves we obtained could not be fitted on the basis of a simple 1:1 complex formation.

The increased stability of the cation complexes of **2**−phosphonate in comparison to that of **2**−tosylate complexes is reasonable and can be attributed to the higher charge of the anion. Yet its 2-fold charge also has an influence on the complex structure. In solution, **2**−phosphonate interacts with two cations, but, because of their size, most probably only inclusion of one into the cyclopeptide cavity is possible. Such an equilibrium complicates quantitative investigations on complexes of **2**−phosphonate. Investigations in this respect are better carried out with monoanions, e.g., tosylates. We have shown that their complex equilibria can be analyzed in a straightforward manner by using 1:1 models.

Conclusion

In conclusion, we demonstrated the unusual allosteric receptor properties of **2**. This cyclopeptide is able to bind cations and anions by two different binding mechanisms. Independent of the type of the anion, cations can generally be bound by cation−π interactions. Yet certain anions such as sulfonates or phosphonates significantly increase the cyclopeptide affinity toward positively charged substrates. These anions are hydrogen-bonded to a second binding site of **2**, where they induce a preorganization of the receptor and are, furthermore, able to interact with the cationic substrates by electrostatic interactions. In contrast to many other artificial allosteric systems, the positive cooperativity of the effectors is not only an effect of a conformational reorganization of the receptor but also due to the proximity of the substrate cations and the effector anions in the final complex. The next step now should be to study whether such allosteric binding mechanisms could be transferred to more polar solvents. For this, we are synthesizing water-soluble cyclopeptides with the aim to investigate whether, e.g., phosphate or sulfate ions act as effectors also in this solvent. Furthermore, by introducing additional functional groups into the aromatic subunits of the cyclopeptides, a variety of new receptors for other substrates should be accessible. It should be possible to regulate their receptor properties by taking advantage of the allosteric effects of suitable anions.

Experimental Section

General Methods. Analyses were carried out as follow: melting points, Büchi 510; optical rotation, Perkin-Elmer 241 MC digital polarimeter (d = 10 cm); NMR, Varian VXR 300, Bruker DRX 500 equipped with an automatic sampler (the chemical shifts in the NMR titrations were calculated by using the $CHCl_3$ signal at δ = 7.27 ppm as internal standard); FT-IR, Bruker Vector 22 FT-IR, cuvette d = 5 mm (NaCl); elemental analysis, Pharmaceutical Institute of the Heinrich-Heine-University, Düsseldorf; mass spectrometry, Finnigan INCOS 50; chromatography, ICN silica gel 32-63 (ICN Biomedicals), MERCK LiChroprep RP-8 (40−63 mm) prepacked column size B (310-25). The following abbreviations are used: TsOH, toluene-4-sulfonic acid; Bn, benzyl; BOC, *tert*-butoxy carbonyl; DIEA, *N*-ethyldiisopropylamine; TFA, trifluoroacetic acid; PyCloP, chlorotripyrrolidinophosphonium hexafluorophosphate; TBTU, *O*-(1*H*-benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium tetrafluoroborate; Glu, glutamic acid; AB, 3-aminobenzoic acid.

Materials. All solvents were dried according to standard procedures prior to use. DMF p.A. was purchased from Fluka and was used without further purification. PyCloP[27] and the K^+−18-crown-6 4-toluenesulfonate salt[28] were prepared according to the literature procedures. TBTU was purchased from BACHEM.

Job Plot. Equimolar solutions (1 mM) of *n*-butyltrimethylammonium iodide and cyclopeptide **2** in 1% DMSO-d_6/$CDCl_3$ were prepared and

(25) Jaffé, H. H.; Freedman, L. D.; Doak, G. O. *J. Am. Chem. Soc.* **1953**, *75*, 2209−2211.
(26) Berkowitz, B. J.; Grunwald, E. *J. Am. Chem. Soc.* **1963**, *83*, 2956.
(27) Coste, J.; Frérot, E.; Jouin, P. *J. Org. Chem.* **1994**, *59*, 2437−2446.
(28) Dale, J.; Kristianssen, P. O. *J. Chem. Soc., Chem. Commun.* **1971**, 670−671.

mixed in various ratios. ^{1}H NMR spectra of the solutions were recorded, and the change in chemical shift of guest protons was analyzed.

K_a by Host−Guest Titrations. Stock solutions of the guest (0.1 μmol/200 μL) in 1% DMSO-d_6/$CDCl_3$ and of cyclopeptide **2** (2 μmol/800 μL) in $CDCl_3$ were prepared. Altogether, 11 NMR tubes were set up by adding increasing amounts of the host solution (0−800 μL) to 200 μL of the guest solution. All samples were made up to 1 mL with $CDCl_3$, and their ^{1}H NMR spectra were recorded. The chemical shifts of prominent guest protons were plotted against the host concentration. From the resulting saturation curves, K_a and $\Delta\delta_{max}$ were calculated by a nonlinear least-squares fitting method for 1:1 complexes[7,9] using the SIGMA Plot 3.0 (Jandel Scientific) software package.

K_a by Dilution of Complex Solution. A stock solution containing 1 mmol/mL cyclopeptide **2**, an equivalent amount of alkali 4-toluenesulfonate, and the corresponding crown ether in 0.1% DMSO-d_6/$CDCl_3$ was prepared. Altogether, 13 samples were prepared from this solution by dilution with 0.1% DMSO-d_6/$CDCl_3$ up to a concentration of 0.001 mmol/mL. ^{1}H NMR spectra were recorded, and the chemical shift of the crown ether protons was plotted against the concentration. From the resulting curve, K_a and $\Delta\delta_{max}$ were calculated analogously to the host−guest titration.

K_a by Competitive NMR Titrations. Stock solutions of *n*-butyltrimethylammonium iodide (0.4 mmol/200 mL) in 1% DMSO-d_6/$CDCl_3$ and of cyclopeptide **2** with an equivalent amount of alkali 4-toluenesulfonate and the corresponding crown ether (1 mmol/800 mL) in $CDCl_3$ were prepared. Altogether, 11 NMR tubes were set up by adding increasing amounts of the cyclopeptide solution (0−800 mL) to 200 mL of the *n*-butyltrimethylammonium iodide solution. All samples were made up to 1 mL with $CDCl_3$, and their ^{1}H NMR spectra were recorded. The chemical shifts of the protons of the trimethylammonium group and of the crown ether protons were plotted against the host concentration. From the two resulting saturation curves, the ratio of the association constants of the cation complexes and $\Delta\delta_{max}$ of the ammonium ion complex were calculated by a nonlinear least-squares fitting method based on eq 1[21] using SIGMA Plot 3.0. For the other δ_{max} (alkali ion−crown ether), the value which resulted from the dilution titration was used.

$$\frac{K_a(1)}{K_a(2)} = \frac{[\delta(1) - \delta_0(1)]}{[\delta(2) - \delta_0(2)]} \frac{[\delta_{max}(2) - \delta(2)]}{[\delta_{max}(1) - \delta(1)]} \quad (1)$$

General Procedure for Hydrogenation of Benzyl Esters. The ester is dissolved in methanol (50 mL/mmol). After addition of 10% Pd/C (100 mg), the resulting reaction mixture is hydrogenated at 1 atm for about 2 h. Completeness of reaction is checked by TLC. The catalyst is filtered off through a layer of Celite and washed with methanol. The solution is evaporated to dryness in vacuo.

General Procedure for Cleavage of *N-tert*-Butoxycarbonyl Groups. The carbamate is dissolved in CH_2Cl_2 (5 mL). The resulting solution is cooled with an ice bath, and trifluoroacetic acid (5 mL) is added dropwise. The reaction mixture is stirred for 1.5 h at 0−5 °C. Afterward, the solvent is evaporated in vacuo. The residue is dissolved in ethyl acetate, and the solution is extracted twice with 10% Na_2CO_3 solution and three times with water. The organic layer is dried, 1 N HCl (1 mL/mmol) is added, and the mixture is evaporated to dryness in vacuo.

3-Aminobenzoic Acid Benzyl Ester Toluene-4-sulfonate.[29] A mixture of 3-aminobenzoic acid (6.85 g, 50 mmol), dry toluene-4-sulfonic acid (8.61 g, 50 mmol), and toluene-4-sulfonyl chloride (11.44 g, 60 mmol) in benzyl alcohol (100 mL) is heated to 80 °C for 4 h under stirring. During this time, the benzoic acid completely dissolves. The product is precipitated by pouring the hot reaction mixture into diethyl ether (800 mL) under stirring. The suspension is kept in the refrigerator overnight. Afterward, the crude product is filtered off, washed with diethyl ether, and recrystallized from 2-propanol/diethyl ether: yield 11.8 g (59%); mp 176−178 °C; ^{1}H NMR (300 MHz, methanol-d_4, 25 °C, TMS) δ 2.30 (s, 3H; $TsCH_3$), 5.04 (s, b, ∼3H; NH), 5.36 (s, 2H; $PhCH_2$), 7.15 (d, ^3J(H,H) = 8.5 Hz, 2H; TsH), 7.37 (m, 5H; PhH + ABH), 7.65 (m, 4H; PhH + ABH + TsH), 8.11 (m, 2H; ABH).

(29) Arai, I.; Muramatsu, I. *J. Org. Chem.* **1983**, *48*, 121−123.

BOC-L-Glu(O*i*Pr)-OH.[30] L-Glutamic acid 5-isopropyl ester[31] (7.56 g, 40 mmol) is suspended in a mixture of 1,4-dioxane (100 mL) and water (60 mL). The resulting mixture is cooled with an ice bath, and the pH is adjusted to 9−10 by addition of triethylamine (5.56 mL, 40 mmol). Afterward, di-*tert*-butyl dicarbonate (10.03 g, 46 mmol) is added, and stirring is continued at room temperature for 4 h. During this time, the starting material slowly dissolves. The dioxane is removed in vacuo, and the remaining aqueous layer is extracted three times with diethyl ether. The organic layers are discarded. The aqueous layer is acidified to pH 3−4 by addition of aqueous $KHSO_4$ and extracted with ethyl acetate three times. The combined organic layers are washed with water and dried, and the solvent is evaporated in vacuo. The product slowly crystallizes in the refrigerator: yield 11.3 g (98%); mp 64−67 °C; $[\alpha]^{25}_D = -10.2$ (c = 2, methanol).

Dipeptide BOC-L-Glu(O*i*Pr)-AB-OBn. 3-Aminobenzoic acid benzyl ester toluene-4-sulfonate (2.39 g, 6.00 mmol) and BOC-L-Glu(O*i*Pr)-OH (2.43 g, 8.40 mmol) as well as PyCloP (3.54 g, 8.40 mmol) are dissolved in CH_2Cl_2 (120 mL). At room temperature, DIEA (3.96 mL, 22.8 mmol) is added dropwise, and the reaction mixture is stirred overnight. Afterward, the solvent is evaporated in vacuo, and the product is isolated chromatographically (ethyl acetate/hexane 1:2) from the residue. The product is triturated with petroleum ether 60/80 to afford a white solid: yield 2.60 g (87%); mp. 97−98 °C; $[\alpha]^{25}_D = -14.9$ (c = 2, methanol); ^{1}H NMR (300 MHz, DMSO-d_6, 25 °C, TMS) δ 1.15 + 1.17 (2d, ^3J(H,H) = 6.3 Hz, 6H; *i*PrCH$_3$), 1.39 (s, 9H; *t*BuCH$_3$), 1.87 + 1.96 (2m, 2H; GluC(β)H$_2$), 2.35 (m, 2H; GluC(γ)H$_2$), 4.10 (m, 1H; GluC(α)H), 4.87 (sept, ^3J(H,H) = 6.3 Hz, 1H; *i*PrCH), 5.37 (s, 2H; $PhCH_2$), 7.14 (d, ^3J(H,H) = 7.7 Hz, 1H; GluNH), 7.43 (m, b, 6H; PhH + ABH(5)), 7.70 (dt, ^3J(H,H) = 8.0 Hz, ^4J(H,H) = 1.2 Hz, 1H; ABH(6)), 7.94 (d, ^3J(H,H) = 8.0 Hz, 1H; ABH(4)), 8.28 (t, ^4J(H,H) = 1.4 Hz, 1H; ABH(2)), 10.26 (s, b, 1H; ABNH). Anal. Calcd for $C_{27}H_{34}N_2O_7$ (498.6): C, 65.04; H, 6.87; N, 5.62, Found: C, 64.99; H, 6.71; N, 5.55.

Tetrapeptide BOC-[L-Glu(O*i*Pr)-AB]$_2$-OBn. BOC-L-Glu(O*i*Pr)-AB-OBn (0.85 g, 1.70 mmol) is deprotected at the terminal amino group according to the general method. An equivalent amount of the same dipeptide is hydrogenated. Both components as well as PyCloP (1.2 equiv, 2.04 mmol, 0.86 g) are dissolved in CH_2Cl_2 (20 mL/mmol). At room temperature, DIEA (3.4 equiv, 5.78 mmol, 1.00 mL) is added dropwise, and the reaction mixture is stirred overnight. Afterward, the solvent is evaporated in vacuo, and the product is isolated chromatographically (ethyl acetate/hexane 1:1) from the residue. The product is triturated with hexane to afford a white solid: yield 1.17 g (88%); mp 67−70 °C; $[\alpha]^{25}_D = -7.2$ (c = 2, methanol); ^{1}H NMR (300 MHz, $CDCl_3$, 25 °C, TMS) δ 1.16 (m, 12H; *i*PrCH$_3$), 1.39 (s, 9H; *t*BuCH$_3$), 1.84−2.15 (m, b, 4H; GluC(β)H$_2$), 2.38 (m, 4H; GluC(γ)H$_2$), 4.11 (m, 1H; Glu^{1}C(α)H), 4.59 (m, 1H; Glu^{3}C(α)H), 4.85 (sept, ^3J(H,H) = 6.2 Hz, 2H; *i*PrCH), 5.37 (s, 2H; $PhCH_2$), 7.12 (d, ^3J(H,H) = 7.9 Hz, 1H; Glu^{1}NH), 7.40 (m, b, 7H; PhH + 2ABH(5)), 7.64 (d, ^3J(H,H) = 7.7 Hz, 1H; AB^{2}H(6)), 7.71 (dt, ^3J(H,H) = 8.0 Hz, ^4J(H,H) = 1.2 Hz, 1H; AB^{4}H(6)), 7.84 (d, ^3J(H,H) = 8.3 Hz, 1H; AB^{2}H(4)), 7.96 (2dd, ^3J(H,H) = 8.2 Hz, ^4J(H,H) = 1.0 Hz, 1H; AB^{4}H(4)), 8.05 (s, 1H; AB^{2}H(2)), 8.31 (t, ^4J(H,H) = 1.8 Hz, 1H; AB^{4}H(2)), 8.66 (d, ^3J(H,H) = 7.4 Hz, 1H; Glu^{3}NH), 10.15 + 10.39 (2s, 2H; ABNH). Anal. Calcd for $C_{42}H_{52}N_4O_{11}$ (788.9): C, 63.95; H, 6.64; N, 7.10. Found: C, 63.85; H, 6.55; N, 7.02.

Hexapeptide BOC-[L-Glu(O*i*Pr)-AB]$_3$-OBn. BOC-L-Glu(O*i*Pr)-AB-OBn (0.70 g, 1.40 mmol) is deprotected at the terminal amino group according to the general method. An equivalent amount of BOC-[L-Glu(O*i*Pr)-AB]$_2$-OBn (1.10 g, 1.40 mmol) is hydrogenated. Both components as well as TBTU (1.1 equiv, 1.54 mmol, 0.49 g) are dissolved in DMF (30 mL/mmol). At room temperature, DIEA (3.2 equiv, 4.48 mmol, 0.78 mL) is added dropwise, and stirring is continued for 2 h. Afterward, the reaction mixture is poured into water (150 mL/mmol). The pH is adjusted to ca. 4 with 1 N HCl, and the suspension is stirred for another 10 min. The precipitate is filtered off, washed with water, and dried. According to TLC, the product is usually obtained

(30) El Marini, A.; Roumestant, M. L.; Viallefont, P.; Razafindramboa, D.; Bonato, M.; Follet, M. *Synthesis* **1992**, 1104−1108.

(31) Albert, R.; Danklmaier, J.; Hönig, H.; Kandolf, H. *Synthesis* **1987**, 635−636.

in high purity by this procedure and can be used for the following step without further purification. A small amount was purified chromatographically (methanol/CH_2Cl_2 1:15) for characterization: yield 1.31 g (88%); mp 185−189 °C; $[\alpha]^{25}_D = +19.5$ ($c = 2$, DMF); ^{1}H NMR (300 MHz, DMSO-d_6, 25 °C, TMS) δ 1.15 (m, 18H; *i*PrCH$_3$), 1.38 (s, 9H; *t*BuCH$_3$), 1.80 + 2.20 (m, b, 6H; GluC(β)H$_2$), 2.40 (m, 6H; GluC(γ)H$_2$), 4.08 (m, 1H; Glu^{1}C(α)H), 4.58 (m, 2H; Glu^{3+5}C(α)H), 4.86 (m, 3H; *i*PrCH), 5.36 (s, 2H; PhCH$_2$), 7.11 (d, ^3J(H,H) = 7.7 Hz, 1H; Glu^{1}NH), 7.46 (m, b, 8H; PhH + 3ABH(5)), 7.63 (d, ^3J(H,H) = 8.2 Hz, 2H; AB^{2+4}H(6)), 7.70 (d, ^3J(H,H) = 7.8 Hz, 1H; AB^{6}H(6)), 7.87 (m, 2H; AB^{2+4}H(4)), 7.94 (d, ^3J(H,H) = 8.2 Hz, 1H; AB^{6}H(4)), 8.04 + 8.07 (2s, 2H; AB^{2+4}H(2)), 8.29 (t, ^4J(H,H) = 1.7 Hz, 1H; AB^{6}H(2)), 8.64 (2d, 2H; Glu^{3+5}NH), 10.15 + 10.29 + 10.38 (3s, 3H; AB^{2+4+6}NH). Anal. Calcd for $C_{57}H_{70}N_6O_{15}$ (1079.2): C, 63.44; H, 6.54; N, 7.79. Found: C, 63.16; H, 6.50; N, 7.60.

Cyclopeptide *cyclo*-[L-Glu(O*i*Pr)-AB]$_3$ (2). The linear hexapeptide BOC-[L-Glu(O*i*Pr)-AB]$_3$-OBn (1.19 g, 1.10 mmol) is first deprotected at the terminal amino group and then hydrogenated according to the general methods. For cyclization, the completely deprotected hexapeptide is dissolved in DMF (100 mL/mmol). Solid TBTU (1.1 equiv, 1.21 mmol, 0.39 g) is added, and the solution is stirred until complete dissolution. DIEA (3.2 equiv, 3.52 mmol, 0.61 mL) is added dropwise, and stirring is continued for 2 h. Afterward, the reaction mixture is poured into water (500 mL/mmol) under stirring. The pH is adjusted to ca. 4 with 1 N HCl, and the suspension is stirred for another 10 min. The precipitate is filtered off, washed with water, and dried. The product is isolated from this mixture chromatographically. At first, an initial purification step is carried out using a silica gel column (CH_2Cl_2/MeOH 10:1). The resulting material is further purified with a RP-8 column. For this, the product is dissolved in a small amount of DMF and subjected to a column conditioned with MeOH/H_2O 1:1. Gradually the eluent is changed to MeOH/H_2O 2.5:1 and then to MeOH/H_2O 5:1, with which pure product is eluted. The product is finally recrystallized from methanol/ethanol 1:2: yield 0.45 g (48%); mp 139−142 °C; $[\alpha]^{25}_D = +9.6$ ($c = 2$, DMF); ^{1}H NMR (300 MHz, DMSO-d_6, 25 °C, TMS) δ 1.16 (d, ^3J(H,H) = 6.6 Hz, 18H; *i*PrCH$_3$), 2.08 + 2.18 (2m, 6H; GluC(β)H$_2$), 2.41 (m, 6H; GluC(γ)H$_2$), 4.60 (m, 3H; GluC(α)H), 4.88 (sept, ^3J(H,H) = 6.3 Hz, 3H; *i*PrCH), 7.43 (t, ^3J(H,H) = 7.9 Hz, 3H; ABH(5)), 7.53 (d, ^3J(H,H) = 7.7 Hz, 3H; ABH(6)), 7.63 (d, ^3J(H,H) = 8.4 Hz, 3H; ABH(4)), 8.31 (t, 3H; ABH(2)), 8.50 (d, ^3J(H,H) = 7.9 Hz, 3H; GluNH), 10.25 (s, 3H; ABNH); ^{13}C NMR (75 MHz, DMSO-d_6, 25 °C, TMS) δ 21.5 (*i*PrCH$_3$), 26.3 (GluC(β)), 30.5 (GluC(γ)), 53.3 (GluC(α)), 67.1 (*i*PrC), 118.7 (ABC(2)), 122.0 (ABC(4)), 122.5 (ABC(6)), 128.6 (ABC(5)), 135.1 (ABC(1)), 138.7 (ABC(3)), 167.0 (ABCO), 170.0 (GluC(α)CO), 171.7 (GluC(γ)CO); CI-MS (NH_3) *m*/*z* (relative intensity) 888 (100) [M + NH_4^+], 828 (4) [M − *i*PrOH + NH_4^+]. Anal. Calcd for $C_{45}H_{54}N_6O_{12}\cdot 2H_2O$ (907.0): C, 59.59; H, 6.45; N, 9.27. Found: C, 59.89; H, 6.42; N, 9.20.

Acknowledgment. I thank Ms. D. Kubik for her assistance and Prof. G. Wulff for his interest and financial support.

Supporting Information Available: COSY and NOESY NMR spectra of the **2**−tosylate complex in $CDCl_3$ and of free **2** in DMSO-d_6 and in 5% DMSO-d_6/$CDCl_3$ (PDF). See any current masthead page for ordering information and Web access instructions.

JA983970J