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A New Class of Macrocyclic Receptors from *iota*-Peptides

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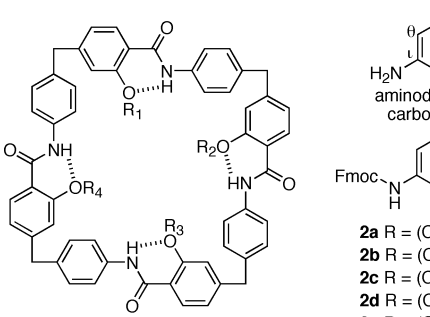
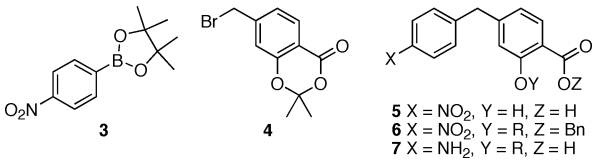
Macrocyclic oligomers, such as cyclodextrins, calixarenes, and porphyrins have for decades been among the preeminent receptors and catalysts of bioorganic and medicinal chemistry. These venerable macrocycles form as cyclohomooligomers via cyclooligomerization reactions or related processes and do not easily permit the presentation of a series of different substituents in sequence. It would be immensely challenging, if not completely impractical, to prepare a porphyrin with four different substituents in a certain order, a β-cyclodextrin with seven, or a resorc[4]arene with four. For this reason, contemporary applications of these and other important macrocycles must often be suited to symmetrical macrocycles or those bearing only one or two different substituents.[1,2] This paper presents a new class of macrocycles and demonstrates the potential of these macrocycles to bind guests and display different substituents in sequence. These macrocycles are based on *iota*-peptides (ι-peptides) and are comparable in size to cyclodextrins, calixarenes, resorcinarenes, and porphyrins.

The macrocyclic ι-peptides **1** are based on the family of ι-amino acids aminodiphenylmethanecarboxylic acid (Adc).[3] Adc can be thought of as an analogue of the α-amino acid glycine that has been enlarged fourfold, to 1.0 nm in length, by insertion of two benzene rings into the main-chain bonds. We have designed functionalized variants of Adc bearing β-alkoxy substituents (OR). These substituents enhance solubility and provide diversity. The OR groups intramolecularly hydrogen bond to the peptide amide groups, thus blocking intermolecular hydrogen bonding and reducing aggregation. When Adc is functionalized with an aminopropoxy group [$O(CH_2)_3NH_2$], which resembles the side chain of lysine, the resulting "Adc^K" peptides exhibit good water solubility. The aminopropoxy substituent can be further functionalized (e.g., with amino acids) to provide diversity in the structures. The macrocyclic ι-peptides **1** are readily prepared from the corresponding Fmoc-protected Adc variants **2**.



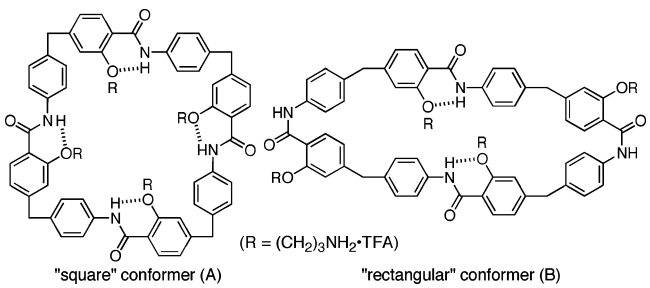
1a $R_1 - R_4 = (CH_2)_3NH\text{-Boc}$
1b $R_1 - R_4 = (CH_2)_3NH_2 \cdot TFA$
1c $R_1 = (CH_2)_3NH\text{-Tyr}\cdot TFA$, $R_2 = (CH_2)_3NH\text{-Ala}\cdot TFA$, $R_3 = (CH_2)_3NH\text{-Thr}\cdot TFA$, $R_4 = (CH_2)_3NH\text{-Val}\cdot TFA$
1d $R_1 = (CH_2)_3NH\text{-Tyr}\cdot TFA$, $R_2 = (CH_2)_3NH\text{-Thr}\cdot TFA$, $R_3 = (CH_2)_3NH\text{-Val}\cdot TFA$, $R_4 = (CH_2)_3NH\text{-Ala}\cdot TFA$

The Fmoc-protected Adc variants **2** are readily prepared in good yields on a multigram scale. Acetonide protection of commercially available 4-methylsalicylic acid, followed by benzylic bromination, provides bromide **4**. Suzuki cross-coupling reaction between boronic ester **3** and bromide **4**, followed by hydrolysis of the acetonide group, affords acid **5**. Selective benzylation of the carboxyl group and alkylation of the phenoxy group generates benzyl ester **6**. Finally, concomitant reduction of the nitro group and hydrogenolysis of the benzyl group, to give amine **7**, followed by Fmoc protection, affords Adc variants **2**. Each of these reactions gives a 60−96% yield.



The macrocyclic ι-peptides **1** are synthesized by solid-phase synthesis of protected linear Adc tetramers, followed by macrocylization, deprotection, and RP-HPLC purification. The linear tetramers are prepared by solid-phase synthesis on 2-chlorotrityl resin using HBTU and HOBt in DMF/CH_2Cl_2 solution. Monitoring the coupling reactions by analytical RP-HPLC establishes good coupling efficiencies with 4 equiv of **2** and 4−6 h reaction times. Cleavage from the resin with AcOH/TFE/CH_2Cl_2 solution affords linear tetramers with free amino and carboxyl termini and Boc-protected side chains. Macrocyclization proceeds smoothly with HCTU in DMF at 0.1−0.2 mM concentrations. Deprotection with CF_3CO_2H/H_2O/triisopropylsilane solution, followed by preparative RP-HPLC purification affords water-soluble macrocyclic ι-peptides **1** in about 20% overall yield.

The ^{1}H NMR spectrum of cyclo(Adc^K)$_4$ (**1b**) in DMSO-d_6 solution shows only one set of resonances, suggesting a single conformer with fourfold symmetry. The spectrum is more complex in D_2O, showing three sets of resonances in 1.0:1.4:1.4 ratio.[4] The minor set of resonances appears to correspond to the conformer with fourfold symmetry, while the two major sets of resonances appear to correspond to a conformer with twofold symmetry. These observations suggest the presence of two conformers in water in 1:2.8 ratio: a "square" conformer (A) with all *trans*-amide linkages and a "rectangular" conformer (B) with alternating *cis*- and *trans*-amide linkages.[4] EXSY NMR experiments confirm that these two conformers arise from the same species and establish that chemical exchange occurs on a hundred-millisecond time scale at 298 K. Variable-temperature ^{1}H NMR studies show that the three sets of resonances coalesce at ca. 358 K. ^{1}H NMR spectroscopic studies of model compounds containing *cis*- and *trans*-amide linkages corroborate the structures of the "square" and "rectangular" conformers (Supporting Information).[5]



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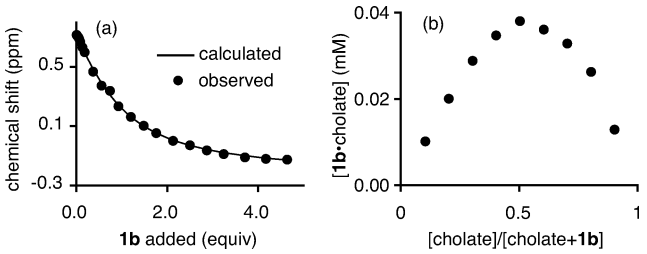
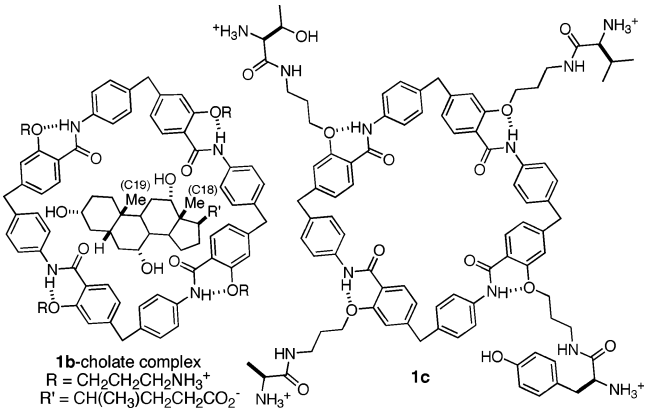


Figure 1. ^{1}H NMR titration (a) and Job plot (b) experiments illustrating the binding of cyclo(Adc^K)$_4$ to sodium cholate. In these experiments, D_2O solutions of cyclo(Adc^K)$_4$ were added to a solution of sodium cholate and the chemical shift of the cholate C18 methyl group was monitored.

^{1}H NMR mixing studies demonstrate that cyclo(Adc^K)$_4$ forms a 1:1 complex with sodium cholate in aqueous (D_2O) solution and that complexation shifts the equilibrium toward the "square" conformer. Substantial upfield shifting of the C18 and C19 methyl resonances of sodium cholate occurs upon complexation, indicating close interaction between these cholate methyl protons and the aromatic rings of **1b**. An ^{1}H NMR titration experiment shows that sodium cholate binds to cyclo(Adc^K)$_4$ with an association constant of ca. 10 000 M^{-1} (Figure 1a). Fitting a 1:1 binding isotherm to the NMR titration data indicates limiting chemical shifts of −0.355 ppm and −0.044 ppm, respectively, for the C18 and C19 methyl groups of the bound cholate. Consistent with the titration experiment, an ^{1}H NMR Job plot experiment shows 1:1 complexation of sodium cholate at tenth-millimolar concentrations (Figure 1b). Titration and Job plot experiments of cyclo(Adc^K)$_4$ with the zwitterionic cholate derivative CHAPS reveal much weaker 1:1 complexation, with an association constant of ca. 500 M^{-1}. The relative weakness of this complexation likely results from the absence of charge complementarity between cyclo(Adc^K)$_4$ and CHAPS.



Molecular modeling studies of cyclo(Adc^K)$_4$, using MacroModel, version 6.5, and the AMBER* force field with GB/SA water solvation, show that the cavity of the "square" conformer is complementary in size to sodium cholate, the hydrophobic cholate group can fit into the hydrophobic cavity of the "square" conformer, and the C18 and C19 methyl groups of the cholate can sit over the faces of the Adc^K aromatic rings in the resulting complex. These modeling studies also suggest that the ring of the "square" cyclo(Adc^K)$_4$ conformer is slightly strained, with an average C−C(O)−N(H)−C amide torsion angle of 167.5°. This ring strain, in conjunction with hydrophobic forces and aromatic interactions, may contribute to the formation of the "rectangular" conformer in D_2O solution and offset the formation of its otherwise unstable *cis*-amide linkages.

To demonstrate that cyclooligomers with a series of different substituents in sequence can be prepared, we synthesized isomeric macrocycles cyclo($Adc^{KY}Adc^{KA}Adc^{KT}Adc^{KV}$) (**1c**) and cyclo($Adc^{KY}Adc^{KT}Adc^{KV}Adc^{KA}$) (**1d**). These compounds present four different amino acids (Val, Ala, Thr, Tyr), tethered to the aminopropoxy side chains of the Adc^K units, in different order. These isomers illustrate the ability of the macrocyclic *ι*-peptides to achieve sequence and diversity: If one were to try to prepare a cyclic tetramer by one-pot cyclooligomerization of four different monomer units, a mixture of up to *64* different cyclotetramers would form. Titration experiments show significant differences between the strength, stoichiometry, and mode of binding of cholate by macrocycles **1c** and **1b** (Supporting Information). These differences illustrate that varying the Adc substituents can modify the binding properties of the receptor.

We envision that this new class of macrocyclic receptors based on *ι*-peptides may prove especially valuable in applications in which sequence and diversity are important. Current successes in the application of other macrocycles as ligands for protein surfaces, for example, have suffered the limitation that it is generally only practical to prepare most macrocycles with one or two types of substituents, rather than several in sequence.[1b,d,e,f,h,6,7] The unique combination of size and sequence of the macrocyclic Adc tetramers illustrates one advantage of these *ι*-peptides and opens the door to a variety of other applications.

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Supporting Information Available: Synthetic procedures, NMR spectroscopic data, and other experimental details. This material is available free of charge via the Internet at http://pubs.acs.org.

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(4) The ^{1}H NMR spectra of **1b** are sharp at low (<1 mM) concentration in D_2O at 298 K. At higher concentrations, the spectra broaden and the minor set of resonances grows, suggesting the onset of self-association and a shift in equilibrium toward "square" conformer A.

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