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Total synthesis of non-natural compounds for molecular recognition. The double challenge

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Abstract: Molecular recognition of biomolecules by synthetic receptors requires modular assembly of various components to complement the molecular characteristics (sizes, topologies, and functional groups) of the substrate. A number of receptors for biorelevant molecules containing oxoanions have been assembled from a bicyclic chiral guanidine subunit. Several receptors accelerate or catalyze reactions proceeding through anionic transition states. Among the structures recently prepared, a receptor incorporating a calix[6]arene subunit has been developed, showing high affinity for phosphocholine derivatives. Chains of tetraguanidinium sulfates form double helices in solution. These substances strongly induce formation of α-helical conformations in Asp rich peptides.

INTRODUCTION

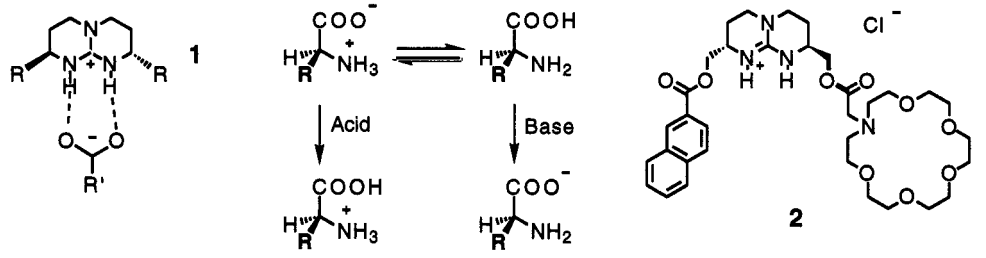
Many chemical developments, including the discovery of new reactions and procedures, have resulted from challenging total syntheses of natural compounds. Natural targets of increasing structural complexity constitute the best proof of the "state-of-the-art" achieved by modern synthetic chemistry. Most often, total synthesis is the only way to have access to large quantities of complex materials, as well as to chemically modified structures with novel biological properties. On the other hand, *de novo* design of artificial receptors for relevant biological molecules is a primary goal in bioorganic chemistry. These designs are based on the complementarity of molecular size, shape and charge, giving rise to reciprocal intermolecular forces that bind receptor and substrate to one another. The precise knowledge on how structures fit together is the basis of molecular recognition. As the substrates become structurally more complex, complexity must be incorporated as well into the receptors for a successful recognition. Thus, the synthesis of these non-natural compounds may result in a difficult and challenging problem, as is the synthesis of a complex natural substance. The experimental observation of the predicted property, i.e., selective complexation, catalysis, regulation or transport, is the best proof of a successful design, and constitutes a second important challenge in the creative process.

MOLECULAR RECOGNITION OF ZWITTERIONS

In our own research we have developed the high lipophilic, chiral bicyclic guanidinium subunit **1** for the molecular recognition of oxoanions. Anions display important roles in biology. Amino acids, peptides and nucleotides are representative examples of organic anions in living organisms (ref. 1). Guanidinium **1** ensures a strong substrate binding through simultaneous ion-pairing and two well oriented hydrogen bonds. The subunit can be conveniently prepared in multigram amounts from simple amino acids (ref. 2) and is therefore available in both pure enantiomeric forms (ref. 3). A simple naphthoyl ester derivative (**1**, R = 2-naphthoyl) showed remarkable affinity for aromatic carboxylic acids (ref. 4) and mononucleotides (ref. 5). In the case of *N*-tertbutoxycarbonyl protected tryptophan, a moderate selectivity (d.e. 30%) for the extraction of the L-enantiomer was shown by the receptor having (*S,S*) configuration (ref. 4).

The design of a receptor for the selective extraction of amino acids from water turns out to be more complicated. In neutral aqueous solutions, amino acids exist largely as strongly solvated zwitterionic structures. On the other hand, the lower electronic densities at the neighbouring carboxylate and ammonium functions with respect to the corresponding isolated groups causes the binding forces of complementary

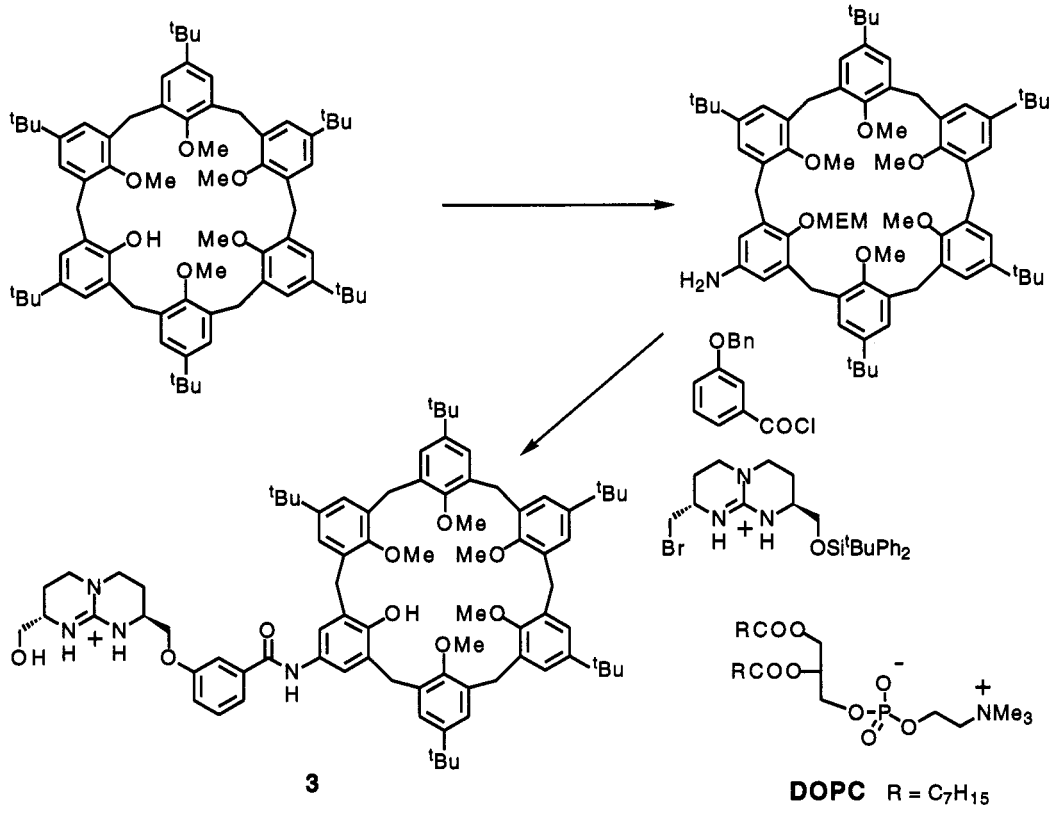
groups in the receptor to be less effective for the complexation. In addition, full complementarity, i.e. use of a cationic subunit for the carboxylate and an anionic subunit for the ammonium, should be avoided, because of the internal collapse of the receptor or of extensive self-association as a dimer. To overcome these difficulties, we designed lipophilic receptor **2** for the selective extraction of aromatic aminoacids, featuring: i) non self-complementary binding sites for carboxylate (the guanidinium function) and ammonium (a crown ether), preventing the receptor from internal collapse, ii) an aromatic planar surface (the naphthalene ring) for an additional stacking interaction with the side chain of aromatic amino acids (Trp, Phe), and iii) a chiral structure (*S,S*-isomer shown) to achieve enantioselective complexation. The affinity of **2** for aromatic amino acids was remarkable: *ca.* 40% of L-Trp or L-Phe was extracted from water into dichloromethane by a single shaking of a saturated aqueous solution of a mixture of amino acids with a solution of (*S,S*)-**2** (chloride) in dichloromethane, with a high enantioselectivity (ref. 6).



The principle of non self-complementarity of binding sites for zwitterion recognition applies also in **3** (ref. 7), a receptor that binds dioctanoyl-L-α-phosphatidylcholine (DOPC) making use of the key interactions found in the crystal structure of the F_{ab} domain of the McPC603 antibody complexed with phosphorylcholine (PC) (ref.8) In the McPC603-PC complex phosphorylcholine phosphate interacts with the positively charged Arg 52H whereas its quaternary ammonium head establishes both ion-ion (Asp 97L) and cation-π (Trp 107H, Tyr 33H and Tyr 100L) interactions. Thus, receptor **3** was designed to provide chemical complementarity to both phosphate and trimethylammonium groups of DOPC. It contains a bicyclic guanidinium salt for the selective complexation of phosphate monoanion and a calix[6]arene subunit (instead of the crown ether employed previously in the amino acid receptor **2**) for the complexation of the quaternary ammonium salt. Both subunits are linked through a short spacer containing an amide bond to provide an acidic hydrogen that could mimic the hydrogen bond between one of the phosphate oxygens and the hydroxyl group of Tyr 33H in the McPC603-PC complex.

The synthesis of **3** was achieved in five steps from a readily available mononitrocalix[6]arene (ref. 9) with an overall yield of 30%. A high affinity ($\Delta G°$ = -6.7 kcal/mol) was observed for the DOPC-**3** complex in chloroform, very similar to that observed for the complex of McPC603 with PC in water ($\Delta G°$ = -6.6 kcal/mol). However, the binding constant was still below the one estimated from model compounds of each isolated binding subunits, suggesting that there is still room for improvement in the design of the spacer unit.Van't Hoff plots showed that binding in DOPC-**3** was mostly enthalpically driven, as a result of the favorable electrostatic binding free energy in chloroform, whereas from calculations for the McPC603-PC complex in water the driving force appears to be the hydrophobic effect, as desolvation of the charged ligand in water greatly reduces the net electrostatic contribution to the binding free energy.

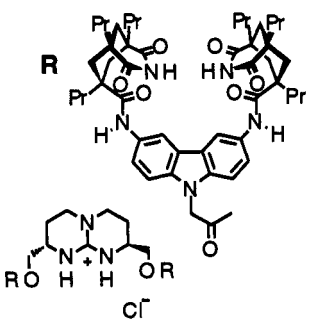
The structure of DOPC-**3** complex in chloroform solution, determined by 2D NMR and by extensive molecular dynamics simulations, showed a remarkable resemblance to the antigen binding site in the McPC603-PC complex: i) the phosphate group interacts strongly with one of the nitrogens of a cationic guanidinium group (Arg 52H in McPC603, the bicyclic guanidinium subunit in **3**), ii) a similarly oriented hydrogen bond is established between one of the phosphate oxygens and either the hydroxyl group of Tyr 33H or the amide nitrogen of the receptor's side arm, iii) the faces of the aromatic rings orientate their π-electron clouds toward the methyl groups on the choline head in such a way that each of the N-C vectors is perpendicular to the opposing ring plane, and (iv) the floor of the binding site is made up of electron-rich groups, the side chain of Asp 97L in the antibody, and methoxy groups and the additional hydroxyl group in the synthetic receptor. Thus, it is possible to design a synthetic non-peptidic receptor that displays the binding properties of a whole antibody by mimicking the key interactions found in the crystal structure of an hapten-antibody complex. Incorporation of suitable chemical reactivity into the recognition binding site should open the way towards synthetic catalytic antibodies or enzyme mimics.



RECEPTORS FOR NUCLEOTIDES AND ENZYME MIMICS

Receptor **4** was tailored to complement adenine nucleotides. The two Kemp's triacid imides linked to a carbazole subunit provides an almost ideal module for simultaneous aromatic stacking and complementary Hoogsteen and Watson-Crick hydrogen bond pairing to adenine. Attachment of this module to a guanidinium subunit provides a receptor for the selective extraction of adenine monophosphates, such as 2',3'-cAMP or 3',5'-cAMP (ref. 10). Similarly, a full equivalent of an adenine dinucleoside monophosphate, such as ApA or d(AA), is extracted from water into organic solvents with receptor **4**, containing two modules (ref. 11). This highly lipophilic receptor showed good activity for selective transport of short nucleotides across liquid membranes and extraction of *long* oligonucleotides into organic solvents, provided enough adenosines are present in the sequence (ref. 12). Thus, 68% of A_{20} (20-mer) was extracted, while sequences of comparable length but much lower adenosine and ApA content, such as the HH ribozyme ...GC (19-mer) and RNA PCR primer ...GG (20-mer) were extracted at 27% and 20% efficiencies, respectively, under the same conditions. Even a lengthly 76-mer (tRNA alanine ...CCA) was extracted to a detectable extent when exposed to excess carrier in the organic phase. Extension of this strategy to more complex receptors is illustrated by receptor **5**, with two guanidinium subunits and three adenine binding modules. Despite its structural complexity, **5** can be assembled readily by condensation of the appropriate modules. As predicted, extraction of d(AAA) or transport across a liquid membrane was more efficient with **5** than with an analogue with only two adenine modules and a simpler central aromatic spacer.

Introduction of reactive functions such as imidazole rings at specific positions of these receptors could favor phosphodiester cleavage under hydrolytic conditions. Thus, we have prepared compound **6**, with two histidine residues at the connecting side arms, although its potential use as a ribonuclease mimic remains to be evaluated. With the same aim, macrocycles **7** and **8** have been assembled readily, in a one-pot sequential isocyanate formation followed by a diphenyl phosphate templated cyclization. Interestingly, diphenyl phosphate is hydrolyzed during the synthesis of the "active" compound **8**.



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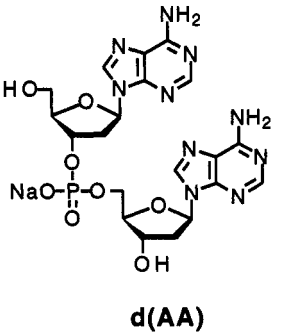
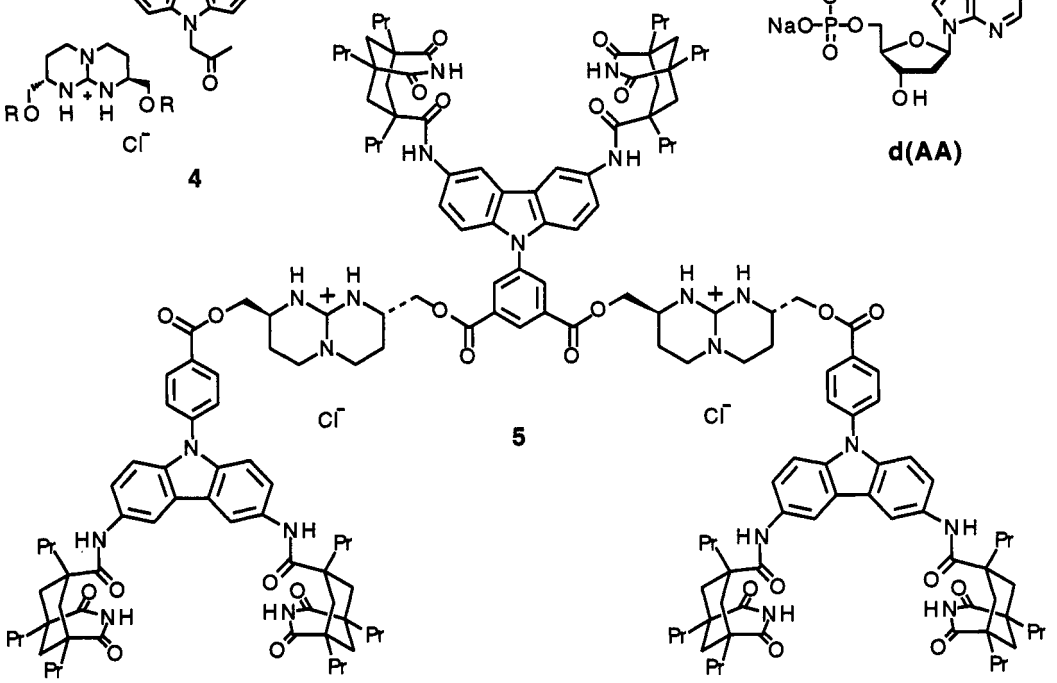


d(AA)



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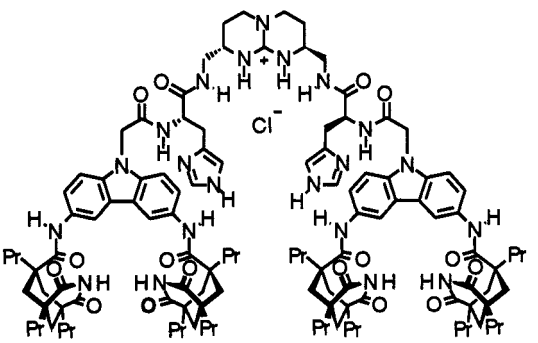

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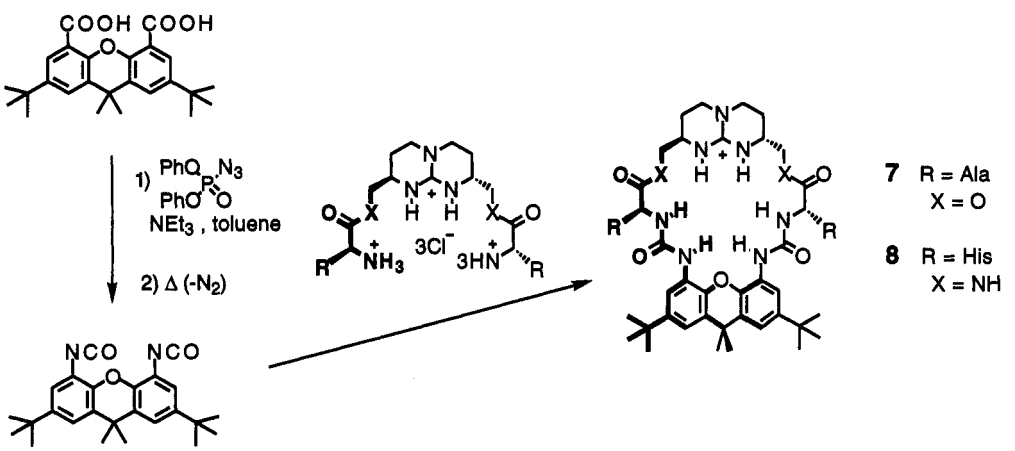
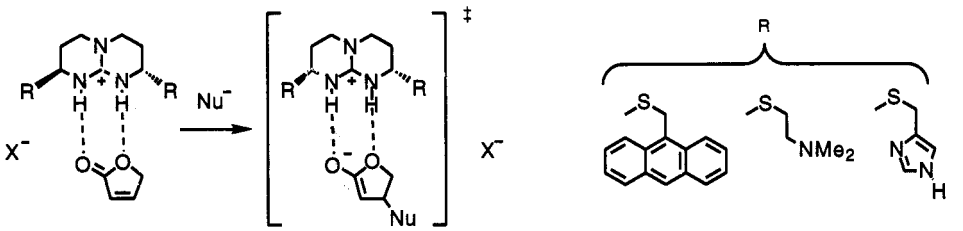


7 R = Ala
X = O

8 R = His
X = NH

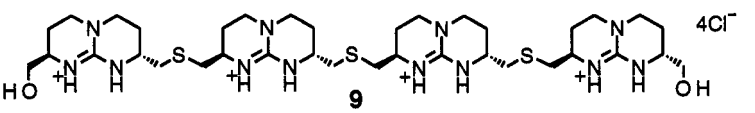
Ideally, a catalyst should be more complementary to the transition state than to both products or starting reactants. Tightly bound reaction products usually cause strong product inhibition. Guanidinium **1** could be used to stabilize negative charges developing in transition states, in processes where the charge is cancelled in a subsequent fast reaction step, resulting in neat rate accelerations and high turnovers. Michael addition of amines to α,β-unsaturated lactones provides a simple example of such an approach, because protonation of the enolate-like intermediate results in a non-charged saturated compound. Up to 8-fold reduction of half-life was observed with pyrrolidine as the reactant, in the presence of a catalytic amount (0.1 equiv.) of **1** (R = silyl protection) (ref. 13). More elaborated catalysts, with aromatic planar surfaces to fix the substrate in close proximity to the reagents and/or with amine or imidazole functions to provide an additional handle to the nucleophile give rise to even higher rates.



POLYGUANIDINIUM SYSTEMS. ANION HELICATES

More complex hosts result by chain concatenation of chiral bicyclic guanidinium subunits (i.e. **9**) These were designed for polianions such as DNA or α-helical peptides having aspartates every three residues. Thus, complexation of the hexadecapeptide Ac-Ala-Ala-Ala-*Asp*-Gln-Leu-*Asp*-Ala-Leu-*Asp*-Ala-Gln-*Asp*-Ala-Ala-Tyr-NH_2 with **9** in 10% water/methanol was studied by circular dicroism measurements (ref. 14). Binding constants were found to be surprisingly high (*ca.* 10^5 M^{-1}) for such a polar solvent mixture. However, the most remarkable observation was that the α-helix content of the peptide increased by *ca.* 150%.

Finally, circular dicroism measurements revealed increased helicities for the sulfate salts of bicyclic guanidinium dimers and tetramers, as compared with the corresponding chlorides. As the links between guanidines in **9** is too short to allow two cations to wrap around a single sulfate anion, two chains must thread in a double helix motif around four sulfate anions. The formation of two helical structures around the anion was confirmed by the observation of inter-chain NOE contacts in 2D NMR experiments (ref. 15).



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OUTLOOK

Supramolecular chemistry provides an infinite number of exciting challenges to synthetic chemists. In molecular recognition, a subtle expresion of information is necessary as substrates increase in complexity. On the other hand, encapsulation of large substrates requires the construction of big cavities by multistep covalent bond formation. In many of these three-dimensional surfaces, the windows allowing the encapsulation of complementary substrates are simply smaller than the inner volumes of the cavities. A new challenge is then to make cavities by self-assembly of smaller components whose curvature and complementarity at the edges results in the formation of three-dimensional capsules (ref. 16). Finally, introduction of functionality into specific sites of artificial receptors is a challenge towards the design of non-natural enzymes, complementary to transition states, without product inhibition and with high turnovers.

Acknowledgements

Most of the work on nucleotide recognition and transport was performed in collaboration with J. Rebek, Jr. and coworkers (MIT , USA). An ongoing collaboration is being carried out with the groups of A.D. Hamilton (Pittsburgh, USA) and E. Giralt (Barcelona, Spain) on the induction of helical conformations. NMR work has been carried.out mostly by M. Bruix (CSIC, Madrid, Spain), M. Pons (Barcelona, Spain), and their coworkers. We are deeply indebted to F. Gago and A.R. Ortiz (Alcalá de Henares, Spain) for the molecular modeling studies. Generous financial support was provided by Comisión Interministerial de Ciencia y Tecnología (Spain).

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