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A Synthetic Receptor for Nicotine from a Dynamic Combinatorial Library

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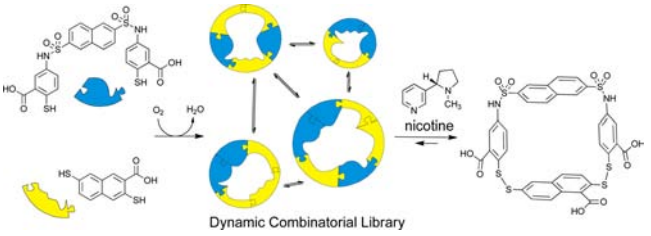
ABSTRACT



Designing synthetic receptors that bind biologically relevant guests in an aqueous solution remains a considerable challenge. We now report a new synthetic receptor for nicotine, selected from a dynamic combinatorial library, that binds this guest in water at neutral pH through a combination of hydrophobic and $\pi-\pi$ interactions.

(*S*)-Nicotine (Scheme 1) is a toxic alkaloid produced by the tobacco plant.[1] Nicotine has been used as a natural insecticide,[2] and its potential as a therapeutic agent for treatment of Alzheimer's,[3,4] Parkinson's,[5] and psychiatric diseases[6] and as a pain controller[7] has been investigated. Nicotine acts principally as a potent agonist on the nicotinic acetylcholine receptors such as $\alpha_4\beta_2$[8] and induces the release of several neurotransmitters.[9] Intake of nicotine often gives rise to addiction.[10] At present, smoke cessation treatments include nicotine replacement therapy[11] where nicotine is usually administered in a small quantity as a nicotine β-cyclodextrin complex.[12] Despite the important biological roles of nicotine, relatively few synthetic receptors for nicotine have been published to date.[13−15] Indeed, molecular recognition of nicotine in water is challenging given the relatively hydrophilic nature of this alkaloid (log D = 0.41 at pH 7.4).[16]

Here we report the use of dynamic combinatorial chemistry[17] as a simple and powerful tool for the discovery

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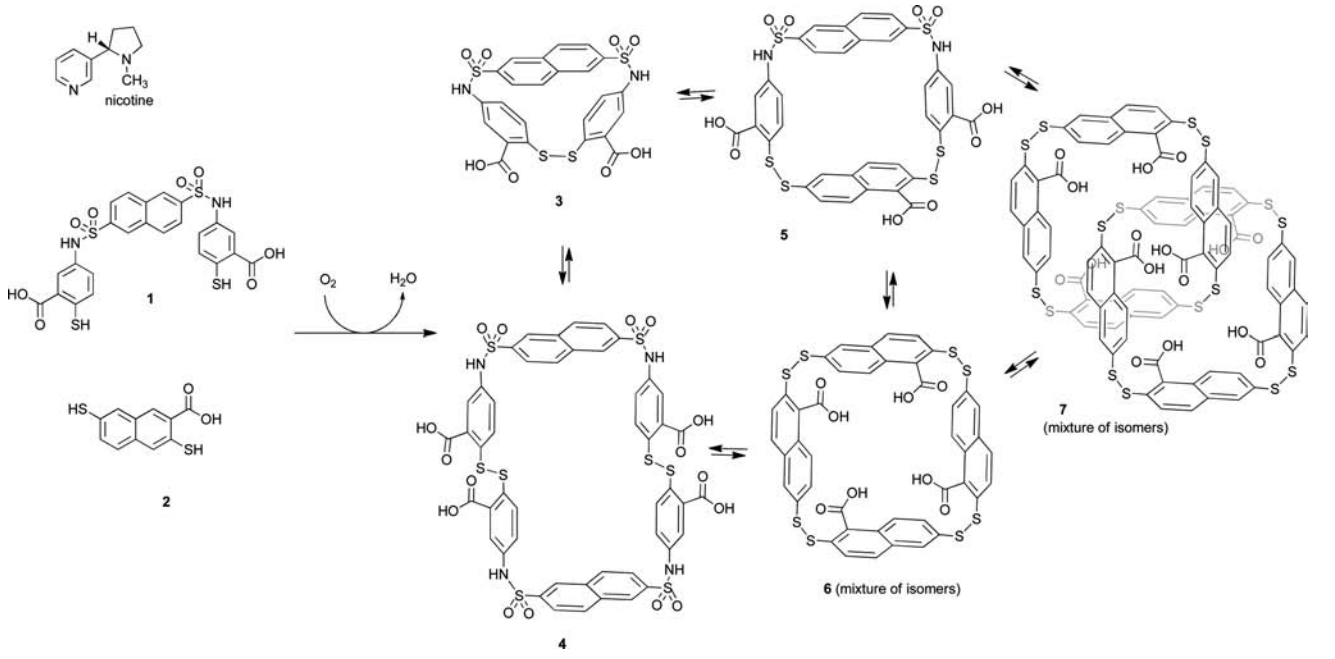
Scheme 1. Structure of Nicotine and the Main Constituents of the Dynamic Combinatorial Library Designed to Recognize Nicotine Made from Building Blocks **1** and **2**



of a new synthetic receptor that can bind nicotine in water at neutral pH.

Despite having only a few degrees of freedom, nicotine lacks a well-defined conformation in solution.[18] Therefore designing a receptor for it using classical organic synthesis is challenging. Dynamic combinatorial chemistry is a powerful alternative approach for providing potential receptors.[19] In this approach it is sufficient to know the functional groups of the target, while it is not necessary to know their relative orientations. In a dynamic combinatorial library (DCL), receptor fragments (building blocks) react reversibly with one another to form a mixture of interconverting library members that is under thermodynamic control. The libraries are adaptive, i.e. introducing a guest into a DCL of potential hosts will shift the equilibrium in favor of (ideally) the best receptor(s) for the guest. Thus, the guest drives the construction of the receptor from smaller fragments.

We selected building blocks **1** and **2** capable of reversible covalent associations, using thiol−disulfide exchange in water, and displaying motifs potentially suitable for nicotine recognition. Nicotine contains both hydrophobic and hydrophilic functionalities, and it is monoprotonated at physiological pH. Building block **1** features a hydrophobic surface area and exhibits the conformational flexibility required to produce a diverse product distribution.

For both building blocks **1** and **2**, aromatic moieties may provide $\pi-\pi$ and cation$-\pi$ interactions with the guest and the sulfonamide moieties of **1** may be able to interact with the protonated pyrrolidine moiety of nicotine through hydrogen bonding. Carboxylate groups in both building blocks serve as water solubilizing groups and may interact electrostatically with the protonated nicotine. The synthesis of building block **1** is outlined in Scheme 2. We first prepared amine **11** which carries protected thiol and carboxylic acid groups. This compound is a useful intermediate for preparing building blocks for reversible disulfide chemistry, as it may in principle be coupled to any carboxylic acid or sulfonyl chloride and can be prepared on a large scale (15 g). Coupling amine **11** to bis-sulfonyl chloride **13**, followed by deprotection, afforded dithiol **1** in moderate yield. Synthesis of building block **2** has been reported elsewhere.[20]

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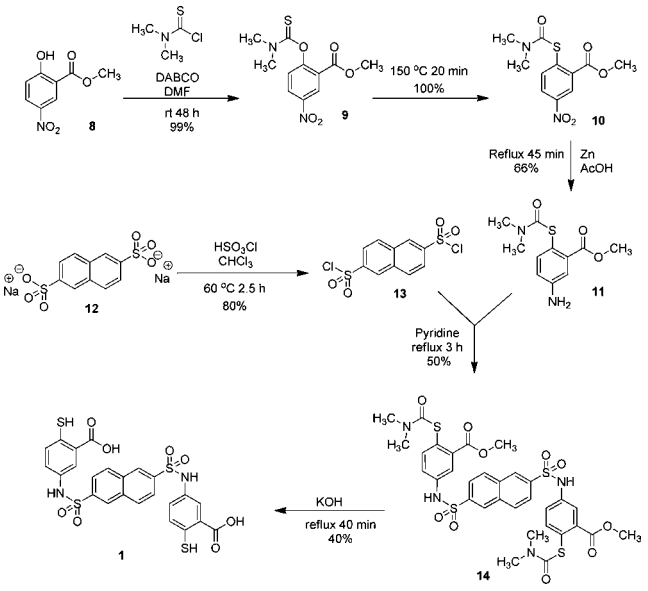
Scheme 2. Synthesis of Building Block **1**



Two libraries were prepared by mixing equimolar amounts of building blocks **1** and **2** (5 mM in total), with and without nicotine, and allowing these to oxidize and equilibrate in aqueous solution (50 mM borate buffer, pH 8.4) in the presence of air following standard protocols.[21]

The compositions of the resulting DCLs were analyzed by HPLC and mass spectrometry (Figure 1). Cyclic monomer **3**, homodimer **4**, heterodimer **5**, homotetramer **6**, and catenane **7** were the major products in the absence of nicotine. The asymmetric structure of **2** gives rise to four different isomers of **6** and many different isomers of catenane **7**.[20] When nicotine was introduced into the DCL, the area of the peak corresponding to heterodimer **5** increased by an amplification factor[22] of 3 relatively to the untemplated DCL, at the expense of most of the other library members.[23] Introduction of nicotine drives the conversion of 40% of dithiols **1** and **2** into the formation of this receptor. Receptor **5** was isolated using preparative HPLC and characterized using ^{1}H NMR, mass spectrometry, and elemental analysis.

The binding between **5** and nicotine was investigated using ^{1}H NMR spectroscopy. The signals of the pyridine protons f, g, h, and i of complexed nicotine are notably shielded relative to the corresponding signals for these protons in unbound nicotine (Figure 2). The observed shielding ($\Delta\delta \geq 0.29$ ppm) suggests that the pyridine is

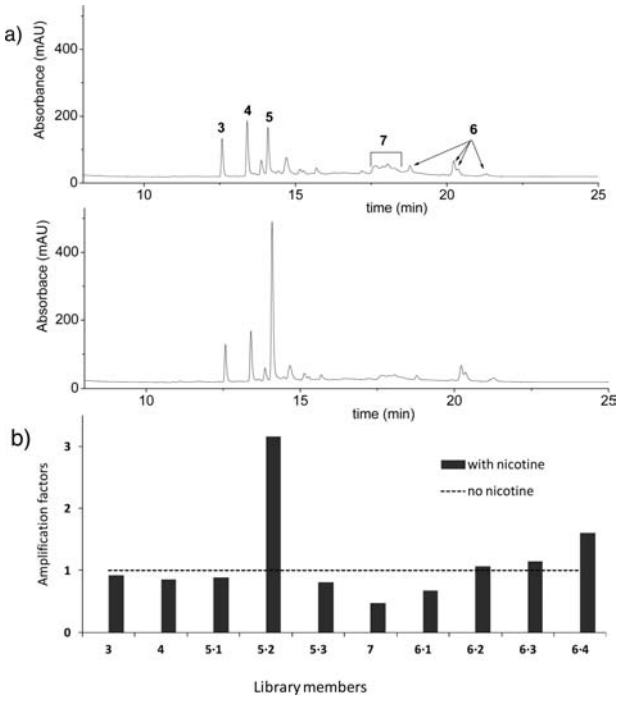


Figure 1. HPLC analysis of the libraries made from equimolar amount of dithiols **1** and **2** (5 mM in total), (a) in the absence (top) and in the presence (bottom) of nicotine (2.5 mM). (b) Amplification factors for the main library members.

located in the shielding cones of the aromatic rings of receptor **5**. In contrast, the protons close to the pyrrolidinyl nitrogen, including the *N*-methyl protons, show a downfield shift. These observations suggest that the pyridine moiety, and not the pyrrolidine group of nicotine, is located within the cavity of receptor **5**; i.e. the formation

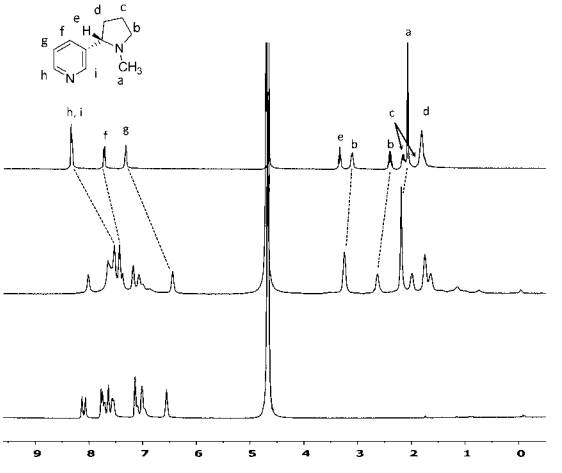


Figure 2. ^{1}H NMR analyses of nicotine (top), receptor **5** (18.2 mM) in the presence of 1 equiv of nicotine (middle), and receptor **5** (18.2 mM) (bottom). Spectra were recorded at 300 K in 200 mM borate buffer (pD 8.4).

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(22) The amplification factor of a library member corresponds to the ratio of the HPLC-UV peak areas in the presence and in the absence of the template.

(23) The fact that the homotetramer **6** was amplified in a library made from building blocks **1** and **2** suggests that it may also have affinity for nicotine. However **6** aggregates extensively, preventing its use as a synthetic receptor (cf. ref 20).

of $\pi-\pi$ interactions appears to be favored over cation$-\pi$ interactions.

The equilibrium constant for the binding of nicotine to receptor **5** in 50 mM borate buffer (pH 8.4) was found to be 1.81×10^3 M^{-1} as determined by isothermal titration calorimetry (ITC). This affinity is comparable to that for binding of nicotine to a cyclophane reported by Dougherty et al.[13] and significantly higher than the affinities of β-cyclodextrin and cucurbit[7]uril for (*S*)-nicotine (K_a = 20−250 and 360 M^{-1} respectively).[14,15]

Performing ITC titrations on the binding of receptor **5** to nicotine at pH 6.9 and 9.3 led to comparable binding constants: 3.43×10^3 and 3.52×10^3 M^{-1}, respectively.

This indicates that binding affinity is only weakly dependent on the protonation state of nicotine ($pK_A = 7.8$)[24] supporting the notion that cation$-\pi$ interactions do not play a major role in the binding. Instead, binding appears to be driven predominantly by $\pi-\pi$ and hydrophobic interactions. Our results show that it is possible, after the design of only building block receptor fragments, to use dynamic combinatorial chemistry to obtain a receptor that is able to bind nicotine in water at neutral pH. Disulfide-based receptors of this type may find application as carriers for nicotine that are able to release the alkaloid upon reduction of the disulfide linkages triggered by intracellular glutathione.[25]

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Supporting Information Available. Experimental details. This material is available free of charge via the Internet at http://pubs.acs.org.

The authors declare no competing financial interest.

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