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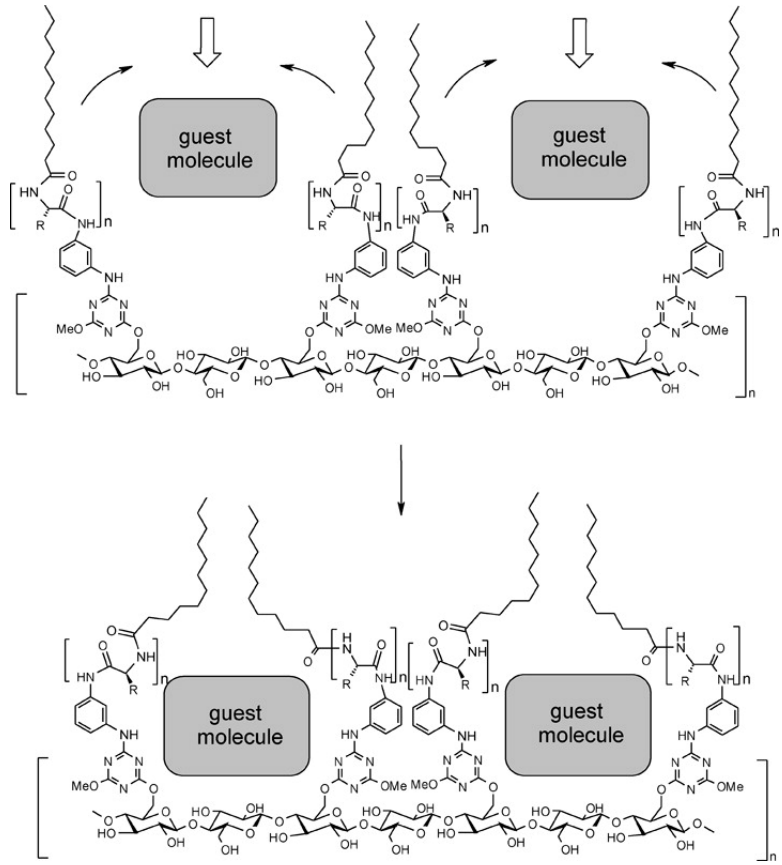
J. Comb. Chem., **2008**, 10 (6), 934-940• DOI: 10.1021/cc800114n • Publication Date (Web): 11 October 2008



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Design, Synthesis, and Application of a Library of Supramolecular Structures Formed by *N*-Lipidated Peptides Immobilized on Cellulose. Artificial Receptors

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Received July 4, 2008

An array of supramolecular structures formed from *N*-lipidated peptides attached to cellulose via aminophenylamino-1,3,5-triazine was synthesized. The structures thus prepared were prone to self-organization and to formation of monolayer of "holes" and "pockets" in dynamic equilibrium, structures which were capable of binding small guest molecules very efficiently recognizing the shape, size, and polarity of ligands, thus resembling artificial receptors. Because of the high flexibility of *N*-lipidated peptides, it is expected that the host adjusts its shape to wrap guest molecules most efficiently. The selectivity and rate of binding was studied by using triphenylmethyl dyes. It was found that the selectivity of binding depends on the structure of the peptide and the *N*-lipidic fragment of the receptor and varies with the structure of the analyte. Even tiny structural changes in guest molecules were detected by monitoring the alteration of the binding pattern.

Docking of ligands to receptors is one of the most fundamental interactions involved in regulatory processes in living organisms and, thus, is a crucial determinant of the activity of pharmaceuticals.[1,2] The relatively weak bonding forces and conformational flexibility of both partners make this phenomenon difficult to study, to categorize by any kind of empirical rules, or to predict based on molecular modeling. Even in the case of interactions between relatively simple molecules, the possible bonding and repulsive forces of mutual host−guest interactions are multifaceted, very numerous, and difficult in terms of molecular modeling.[3,4] Thus, a successful prediction of these interactions for the more advanced models involving flexible ligands and complex flexible receptor structures in many cases still exceeds our capabilities.[5]

On the other hand, quantitative structure−activity relations (QSAR) studies would be significantly advanced if additional parameters, more accurately representing complex interactions involving receptors, were taken into consideration. However, the data collected in the studies of receptors still remain incomplete, and moreover, there are no signs that it will be possible soon to attain this goal because of the size and complexity of receptor structures. In the search for a usable model adequate for the complex host−guest interactions described above, we focused our attention on the powerful potential presented by libraries of artificial receptors. A crucial advantage of measurements involving a library arises from the fact that the source of valuable data consists not only of the features of particular components of the library by themselves but also of the relations between them. Therefore, the amount of available and useful information increases much faster than the size of the library.

Recently, we hypothesized that *N*-lipidated peptides immobilized in a regular pattern on an appropriate solid support would undergo self-organization and create a highly ordered supramolecular structure with a composition of "holes" and "pockets" in dynamic equilibrium. Because of the high flexibility of peptide and lipid fragments, it is expected that the shape, size, and polarity of the holes and pockets could be adequately adjusted to fit the guest molecule most efficiently and thus to operate in a manner resembling that of artificial receptors (Figure 1).

Results and Discussions

Designing Receptor Structure. To obtain a strong, yet reversible binding force for the most of potential guest

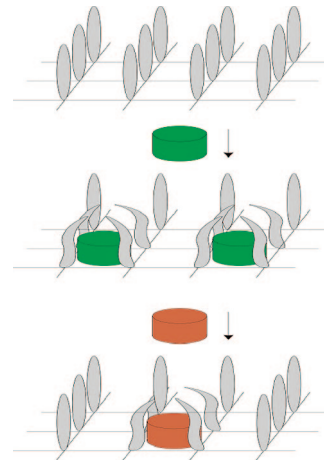

Figure 1. Molecular traps formed by podands regularly allocated on a support capable of reversible and competitive adsorption−desorption.

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10.1021/cc800114n CCC: $40.75
Published on Web 10/11/2008

Scheme 1. Structural Fragments Offering Bonding Potential Applied in the Synthesis of Podands Used for the Preparation of a Library of Artificial Receptors

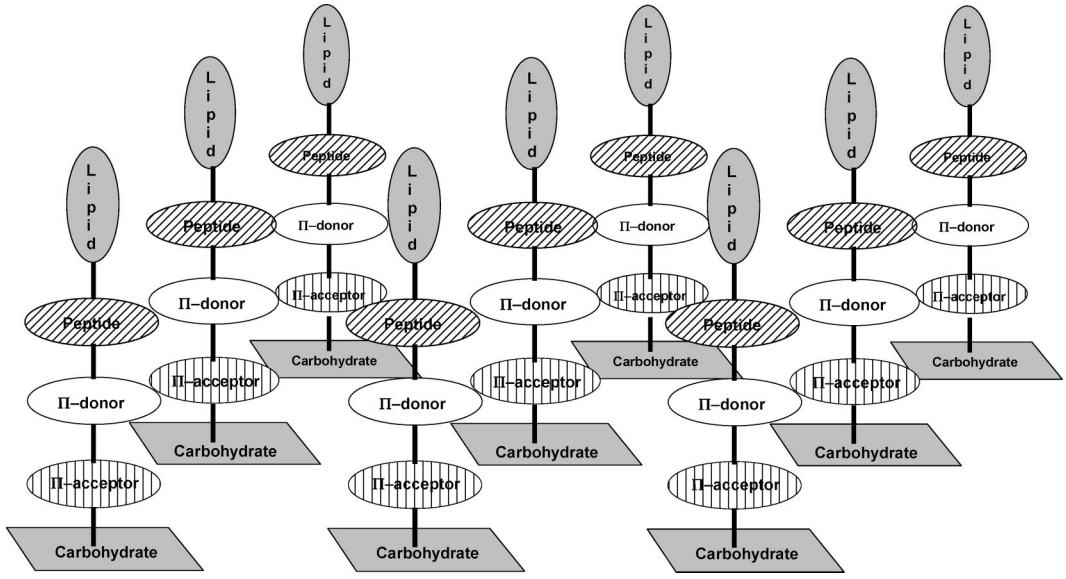
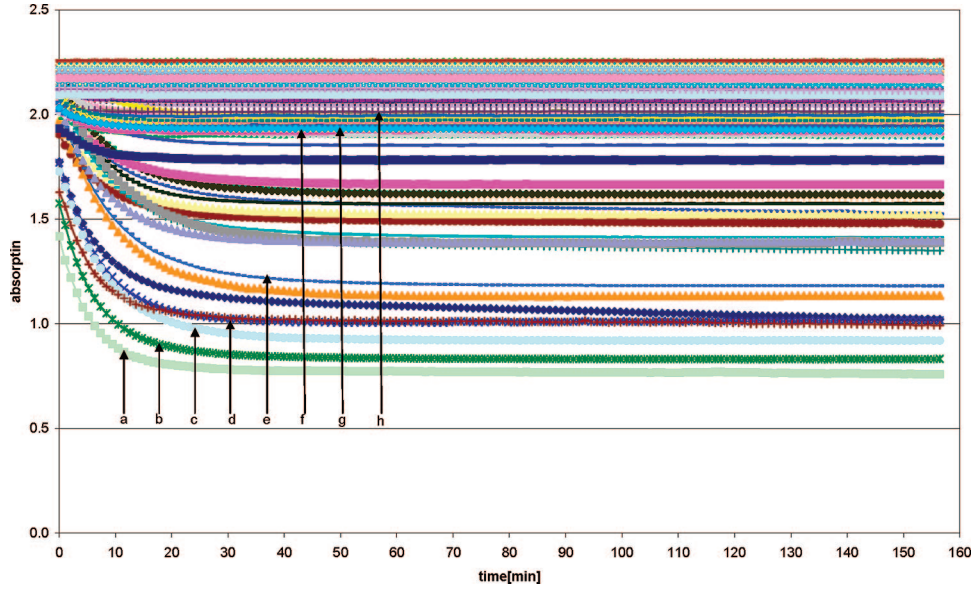


Chart 1. Rate of Guest Adsorption (Bromochlorophenol Blue) by Artificial Receptors (Determined by Sunrise Microplate Reader with Magellan Software)[a]



[a] Note that in most of the cases, saturation of binding pockets and cellulose support was reached within 20−30 minutes; then the residual concentration of ligand remained unchanged. The fastest and the strongest adsorption occurred with library members (a) 1B, (b) 1D, (c) 1E, and (d) 1C, moderately slowly binding with (e) 7I, and the slowest/weakest bonding was observed for (f) 8M, (g) 4G, and (h) 5H.

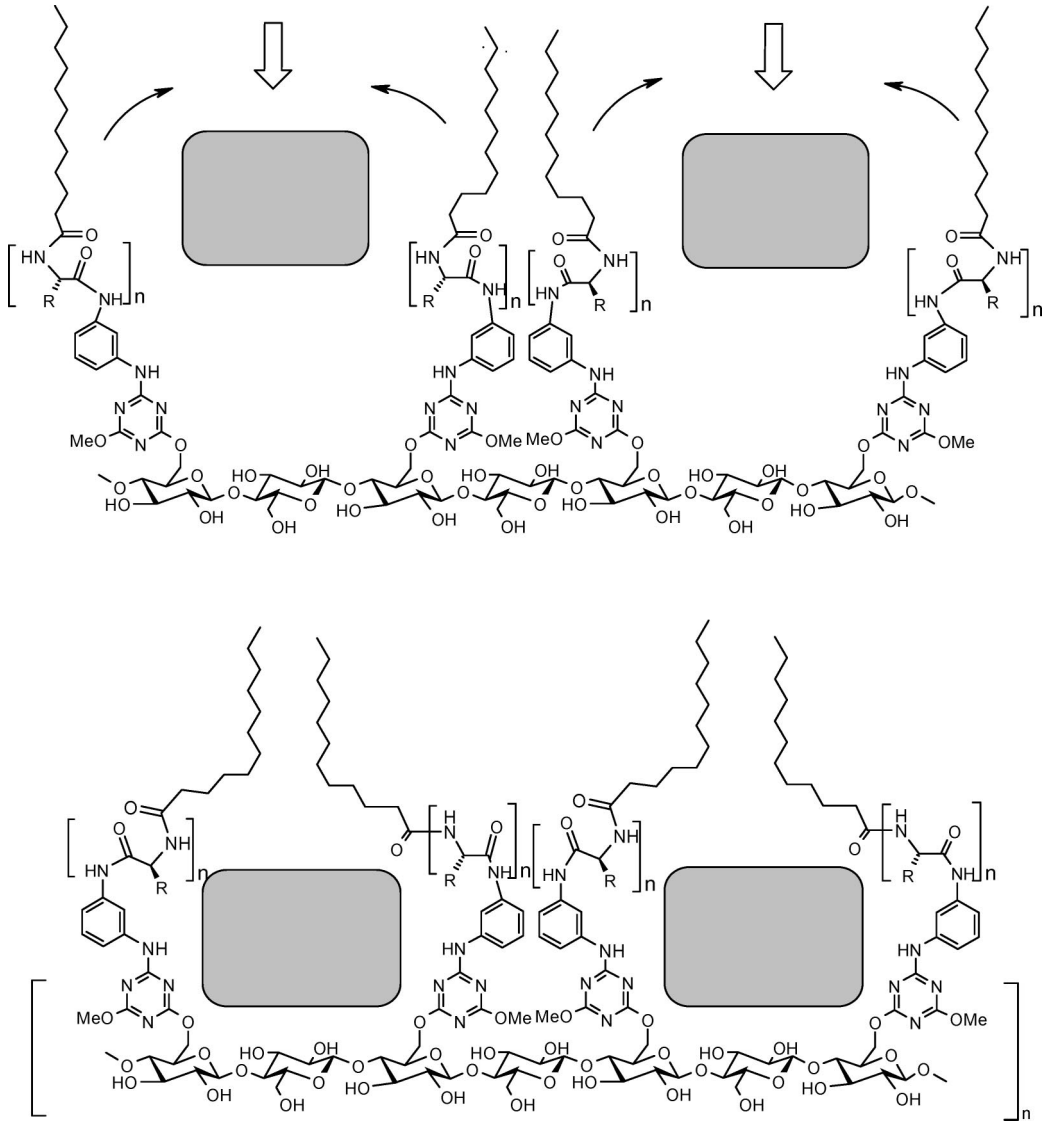
molecules,[6] we attempted to introduce into binding pockets most of the structural attributes responsible for weak intermolecular interactions.[7] These include hydrogen-bond donors and acceptors, as well as lipophilic and hydrophilic fragments supplemented with π-donors and π-acceptors (see Scheme 1).

The allocation of these elements inside the linear structure forming the matrix of podands was defined in such a way as to separate the flexible *N*-lipopeptide fragment from the solid support by relatively rigid, aromatic rings. Thus, a bonding "pocket" was composed from the tethered fragments of "walls" constructed from aromatic rings, expanded with a diversity of interactions offered by flexible peptide fragments, and finally closed with a "zipper" of hydrophobic chains of lipidic fragments.

Solid Support. The regularity of the pattern formed by immobilized podands on the surface of the solid carrier and the space between them was expected to be another crucial factor determining the discrimination of guest molecules on the basis of their size. After analyzing the structure of potential solid supports, we chose cellulose as the most promising support for the immobilization of podands. The structure of cellulose is very well-known,[8] and although not completely regular, microcrystalline regions outnumber anisotropic ones. This means that, after immobilization, most lipopeptides form a regular pattern on the surface of the carrier, although to some extent accompanied by irregularities located in anisotropic regions.

Cellulose is readily accessible in different forms (many of them very precisely standardized and convenient for

Scheme 2. Postulated Mechanism of Binding of Guest Molecules Inside the Pocket of an Artificial Receptor Formed in a Microcrystalline Region of Cellulose ($n = 1, 2, 3$, etc.)



miscellaneous applications) and with different percentages of crystalline regions. Moreover, there are known relatively simple procedures of destruction of anisotropic regions leading to an increase in the content of crystalline fragments, although accompanied by a substantial loss of the convenient fibrous form of the support.[9] Last, but not least, cellulose is inexpensive and prone to numerous chemical modifications[10] with verified procedures well documented in the literature.[11]

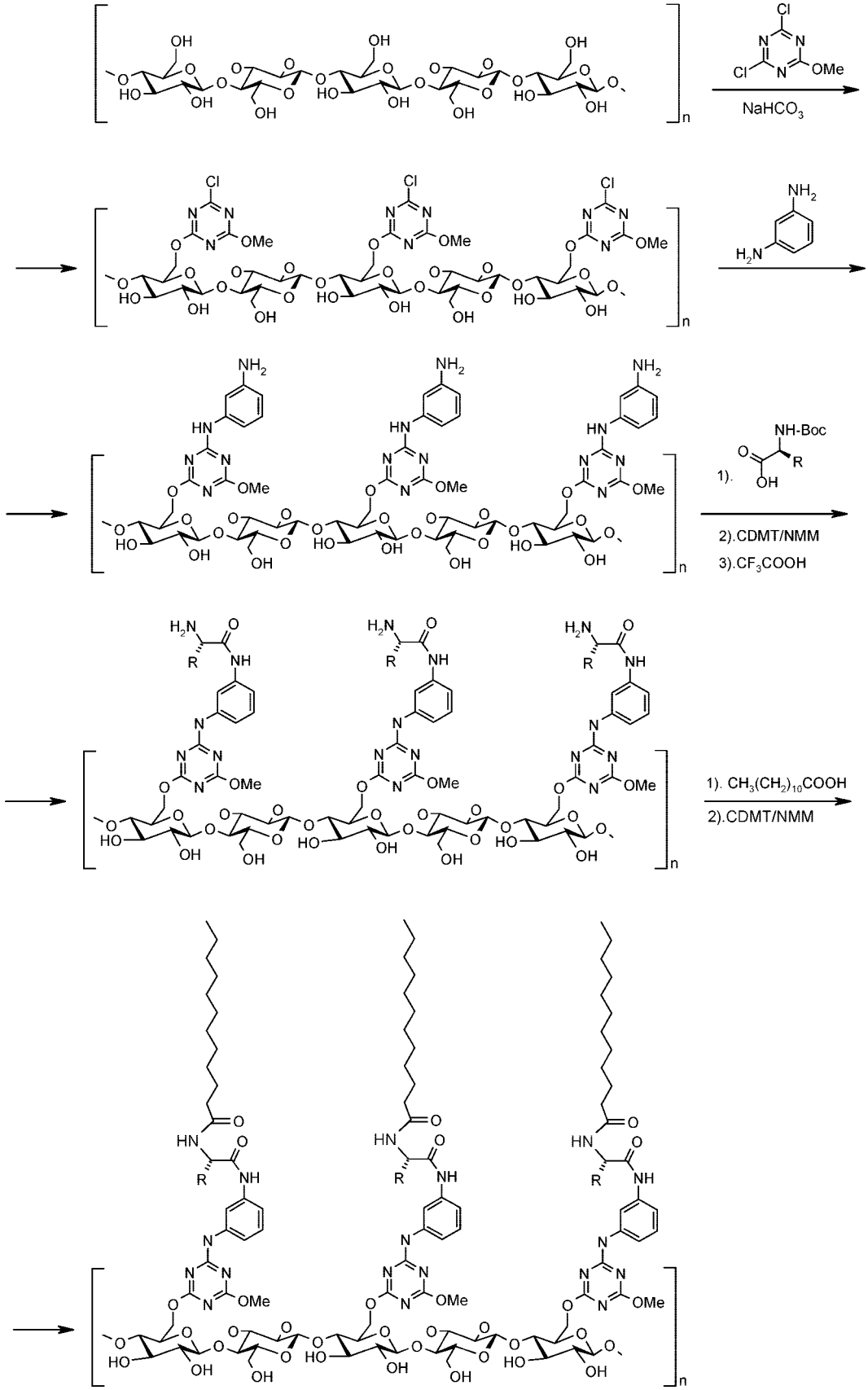
Synthesis of Libraries. Libraries were synthesized in a stepwise procedure involving immobilization of a 2,4-dichloro-6-methoxy-1,3,5-triazine scaffold on a sheet of cellulose support,[12] followed by a reaction with appropriate *ortho*-, *meta*-, or *para*-phenylenediamine,[13] acylation of the amino group with N-protected amino-acids, deprotection of amine functionality, and then coupling of appropriate carboxylic acids, previously activated by means of a triazine coupling reagent[14] (see Scheme 3).

After completion of the synthesis, the library consisted of a collection of rectangular cellulose sheets (pages) (100 cm^2 each). Every page acquired a unique lipophilic structure attached to a cellulose support via a triazine scaffold, resulting from an appropriate combination of carboxylic acids, amino acids, and a phenylenediamine linker.

It was found that libraries obtained with *ortho*- and *para*-phenylenediamine were highly susceptible to oxidation and formed a highly colored background. Therefore, in further studies involving tests with dyes, libraries more resistant to oxidation, synthesized with *meta*-phenylenediamine, were selected (see Scheme 3).

Loading of a triazine scaffold on a cellulose support was calculated on the basis of N and Cl content determined by elemental analysis. The progress of synthesis was also monitored by the determination of N, S, or Cl (Br) content in a functionalized cellulose matrix by elemental analysis. This means that, because of the limited scope of analytical methodology, the most precise measurements at all synthetic stages were available in experiments with sulfur amino acids (methionine and cysteine), in model experiments with halogen-substituted carboxylic acids used as lipophilic fragments. Using them as a probe, we were able to standardize the synthetic procedure. With Whatman 7

Scheme 3. Synthesis of a Library of *N*-Docosanoylated Amino Acids on *m*-Phenylenediamine Linker



filter papers as a support, the final products were obtained with a comparable loading (9−10 μmol/cm^2) and the anticipated ratio of molecular fragment triazine/*m*-phenylenediamine/amino acid/carboxylic acid.

In the synthesis of other components of the libraries, elemental analysis data were less comprehensive. Therefore, for the sake of neutralization of possible divergence and to secure the repeatability of docking experiments, each page

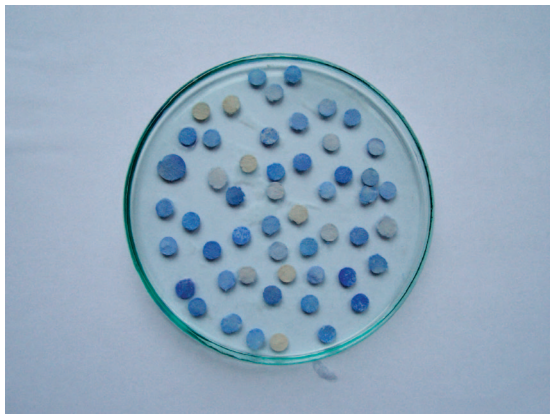


Figure 2. Picture of sublibraries A and B treated with bromochlorophenol blue.

of the library was divided into disks, each 6 mm in diameter. Every disk was permanently labeled by writing down the address of the relevant field (page) with a graphite pencil. This procedure multiplied the original library, supplied many clones of it, and provided, for all the tests, new and completely identical material.

For the study of host−guest interactions, a library was prepared from short-chain carboxylic acids (sublibrary A) and more hydrophobic long-chain carboxylic acids (sublibrary B). Sublibrary A ($7 \times 4 = 28$ fields) was prepared through the reaction of (a) 2,2-dimethylpropionic acid, (b) 2-ethylhexanoic acid, (c) pentanoic acid, (d) heptanoic acid, (e) hexanoic acid, (f) 10-undecenoic acid, and (g) 2-methylcynnamic acid with (1) His(Bzl), (2) Val, (3) Ala, and (4) Ser(Bzl), respectively.

Sublibrary B was prepared through the acylation of appropriate derivatives of (5) Glu(OBzl), (6) Gly, (7) Arg(NO_2), and (8) Trp with (h) 2-phenylbutyric acid, (i) (*Z*)-13-docosaenoic acid, (j) (*R*)-12-hydroxyoctadec-9c-enoic acid, (k) (*E*)-9-octadecaenoic acid, (l) octadecanoic acid, and (m) (*Z*)-9-octadecaenoic acid. In both sublibraries, *m*-phenylenediamine was used as a linker and π-donating constituent of the receptor.

Studies on Host−Guest Interactions. For preliminary experiments on binding, triphenylmethyl dyes were used as guest molecules. It was found that a lipophilic monolayer immobilized on cellulose was able to selectively adsorb a wide variety of dyes from the solution. The selectivity of adsorption depended on the structure of the adsorbed molecule, fatty acid, and (amino acid) oligopeptide sequence. The coloration of the discs after treatment with dyes was sufficiently diversified to be observed with the unaided eye (see Figure 2). The treatment of both sublibraries with a 1 μM/100 mL solution of bromochlorophenol blue caused the blue coloration of almost all disks. The most intensive coloration, characteristic of the basic environment, was observed in disks (Table 1, entry 5, row 1, a−g) bearing a strongly basic imidazole function in the side chain of histidine.

Less important was the initial concentration of the dye. Thus, both 1 mmol/L, as well as a hundred times more diluted, solutions of methylene blue (color change at 3.2−4.8 pH) (Figure 3a), after washing with water three times (Figure 3b, c), gave an identical coloration of the library (see Figure 3d).

Table 1. Structures of Guests (Left Column) and Diagrams Presenting the Intensity of Coloration

Entry	Guest structure	Binding pattern
1	Thymol Blue	not binding
2	Bromothymol Blue	
3	Bromocresol Purple	
4	Bromocresol Green	
5	Bromochlorophenol Blue	
6	Phenol Red	
7	Bromophenol Blue	
8	Chromeazurol S	

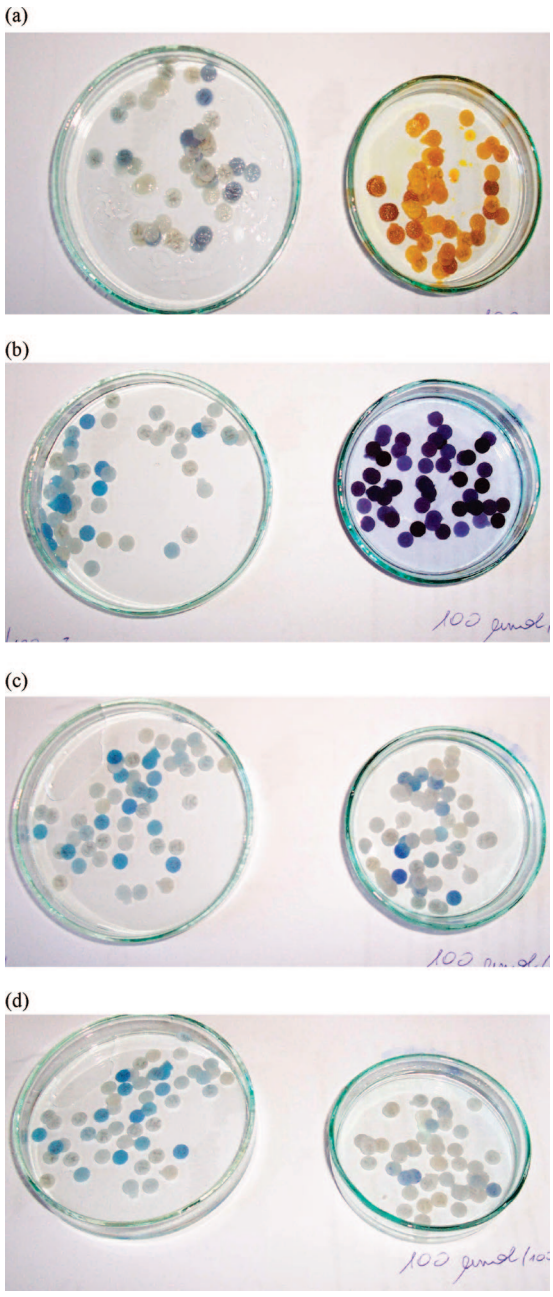


Figure 3. Pictures of library: (a) after treatment with 10 μmol/L solution of methylene blue (on the left), after treatment with 1000 μmol/L solution of methylene blue (on the right), (b) after the first washing with water, (c) after the second washing with water, and (d) after third washing with water.

We considered this result as strongly suggesting that host−dye binding occurs inside the receptor pocket, involving mechanisms of binding and equilibrium constants relatively unaltered by changes in the initial concentrations of the host at least by 2 orders of magnitude, if a sufficient excess of host solution was used in the experiment.

In quantitative measurements, all disks used in the experiment were scanned; then every color picture was transformed into a 286 gray scale digitalized picture by means of the Image-Quant program, and the digital data were stored as an Excel file. The collected data confirmed that a change in the initial concentration of methylene blue modified the intensity of bonding and bonding profile only insignificantly.

Rate of Guest Adsorption by Artificial Receptors. Rate measurements were performed using an ELISA plate reader. In the experiments, each page of the library was divided into uniform strips, each 3 × 16 mm in size, fastened around the sides of the wells of an ELISA plate in is such a way as to avoid disturbing light transmission. Then an equal volume of the stock solution of bromochlorophenol blue was added to the every microplate well to sink the strips, and the extinction of light was measured.

The obtained results, plotted on the graph presented in Chart 1, confirmed that the adsorption of dye proceeded relatively fast with all members of the library, in most cases reaching equilibrium within 20−30 min. Both the distribution of concentrations and the dispersion of time intervals required to reach equilibrium were sufficiently significant for analysis of the process. It was found that the fastest adsorption occurred with library members 1B [2-ethylhexanoyl-His-(Bzl)], 1D [heptanoyl-His(Bzl)], 1E [hexanoyl-His(Bzl)], and 1C [pentanoyl-His(Bzl)]. On the other hand, slow bonding was observed with 8M [(Z)-9-octadecaenoyl-Trp], (g) 4G [2-methylcynnamoyl-Ser(Bzl)], and (h) 5H [2-phenylbutyryl-Glu(OBzl)].

The most efficient bonding of dye corresponding to its lowest concentration in the solution in a state of equilibrium was observed for 1B [2-ethylhexanoyl-His(Bzl)]. This result was fully consistent with the strongest coloration of the disks 1A−1G and 7H−7M, as presented in Table 1, entry 5. The weakest bonding of dye was characteristic for 4A−4G, 5H, and 8M library members, which also gave less-colored disks in scanner measurements, respectively.

Studies on Molecular Recognition of a Guest Structure by a Library of Artificial Receptors. Supramolecular structures forming binding pockets are highly flexible and therefore prone to adjust their shape to fit efficiently various guest molecules. In spite of the potentially lowered selectivity of binding caused by the irregularity of the cellulose matrix and flexibility of the structure of *N*-lipidated peptides, a diagnostic member of library can be selected as a characteristic marker of a particular guest if a sufficiently large library of receptors is taken into consideration. However, even in the case of a small library, if a single receptor does not necessarily have selectivity for a particular analyte, a combined fingerprint response can be extracted as a diagnostic pattern visually or by using chemometric tools.[15] Thus, to study the ability of receptors to recognize tiny changes in analyte structures, we compared the binding pattern of 8 similar structures of triphenylmethyl dyes. As was done previously, the coloration of disks was scanned, transformed into a 286 point gray scale, and then plotted on diagrams presented in Table 1.

The steadily increased size of substituents in the triphenylmethyl ligand increased the diversification of binding. For relatively small phenol derivatives (Table 1, entries 5−7), efficient binding was observed in most receptors. For more space-demanding thymol derivatives (Table 1, entry 2), the docking of the ligand was observed only in the case of the most spacious glycine receptor (row 6), alanine (row 3), and receptors prepared from basic amino acids histidine (row 1) and arginine (row 7), with no binding by the tightest valine

receptors (row 2). For the most space-demanding thymol derivatives (Table 1, entry 1), no binding of the ligand was observed.

Conclusions

A new type of host structure was designed and prepared. A library of chiral "pockets" formed by supramolecular interactions between *N*-lipidated amino acids immobilized on cellulose via *m*-phenylenediamine and a 1,3,5-triazine scaffold was synthesized. The pockets were able to recognize the structure of the guest molecules and to selectively adsorb them from the solution, thus operating in a manner resembling receptors. The strength of bonding and selectivity of binding increased with the length of chain of the lipophilic fragment and also depended on the structure of amino acid fragments. A very strong control of the binding process by electrostatic interactions was noticed in the presence of basic functional groups inside the receptor pocket. The effect was so strong that, in most of cases, it overshadowed the diversification of the binding ability caused by the modification of the lipidic fragment of the receptor. The selectivity of binding of small molecules by an artificial receptor was sufficient for their application in the detection, as well as identification of guest molecules, as a new tool in studies of molecular recognition, medical diagnostics, intelligent drug delivery systems, and many other uses.

Acknowledgment. The study was supported by the Polish State Committee for Scientific Research under the Project 2 P05F 03130.

Supporting Information Available. Full experimental details of synthesis of library, binding procedure, and characterization of randomly selected library members. This material is available free of charge via the Internet at http://pubs.acs.org.

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CC800114N