Application of a Library of Artificial Receptors Formed by the Self-Organization of N-Lipidated Peptides Immobilized on Cellulose in Studying the Effects of the Incorporation of a Fluorine Atom

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A library of artificial receptors formed by the self-organization of *N*-lipidated peptides attached to cellulose via *m*-aminophenylamino-1,3,5-triazine was used for docking pairs of small colorless *N*-phenylpiperazines with and without a fluorine atom in the phenyl ring. The interactions of guests with the receptors were visualized by using competitive adsorption−desorption of an appropriate reporter dye. Several library members demonstrated attributes characteristic of the detection of alterations in the guest structure caused by the substitution of one hydrogen atom with fluorine. Analysis of the binding pattern of *N*-phenylpiperazine derivatives showed two characteristic bonding patterns: one with stronger binding of fluorinated analogues and weaker binding of native phenyl substituted analogues by the most of the receptors studied and another one with stronger binding of native hydrogen substituted compounds and respectively weaker binding of fluorinated analogues of guest molecules by receptors with tryptophan inside the binding pocket.

Introduction

An application of libraries of artificial receptors that are capable of selectively binding a given quest molecules under physiological conditions are interesting not only as model systems for studying the principles of the underlying supramolecular chemistry but also as starting points for the development of sensors as diagnostic tools[1] or as molecular probes capable of interfering with an actual biological event.[2] Thus, the combinatorial chemistry has become an important tool not only for design and discovery of novel chemical molecules but also for exploration of interactions too complex for qualitative and quantitative rationalization. An important advantage of measurements using combinatorial library arises from the fact that the source of valuable data consists both the features of particular components of the library by themselves, as well as the relations between them. Therefore, the amount of available and useful information increases much faster than the size of the library. A systematic exploration of this information would be of the great importance if we are able to design and to synthesize the library of receptors which could emulate interactions between drug and living organism.

In Nature, molecular recognition events take place in clefts or cavities within proteins, which provide a less polar microenvironment for the binding than the aqueous solution of physiological fluids, allowing the effective exploitation of electrostatic interactions.[3] For most of the artificial chemical receptors, however, which in general are much smaller and therefore structurally less well-defined than proteins, this often represents a severe limitation in terms of both their design and development as well as for any potential application, which necessarily has to take place under physiological conditions. To surmount this restriction, we proposed to use as receptors N-lipidated peptides immobilized in a regular pattern on an appropriate solid support.[4] Because of the high flexibility of peptide and lipid fragments, it is expected that the shape, size, and polarity of binding pockets could be adequately adjusted to fit the guest molecule most efficiently, and moreover, because of the additional hydrophobic and aromatic interactions, the efficient substrate binding is possible also in aqueous solution. Thus, the molecular recognition take place in less polar microenvironment of the clefts with participation of diversified amino acid (peptide) fragment, just like in the case of natural receptors. To validate the recognition propensity of induced fit hosts we attempted to use them for sensing tiny modifications of structure of typical pharmacophore. In these preliminary studies, we attempted to use as ligands pairs of *N*-phenylpiperazine derivatives substituted with hydrogen and with one hydrogen atom substituted by fluorine respectively. The experiments should prove that one of the most popular strategies for altering the activity of biologically active products based on the incorporation of fluorine[5] what alters the electronic, lipophilic, and steric parameters and can critically increase intrinsic activity, chemical, and metabolic stability, and bioavailability can be detected by artificial

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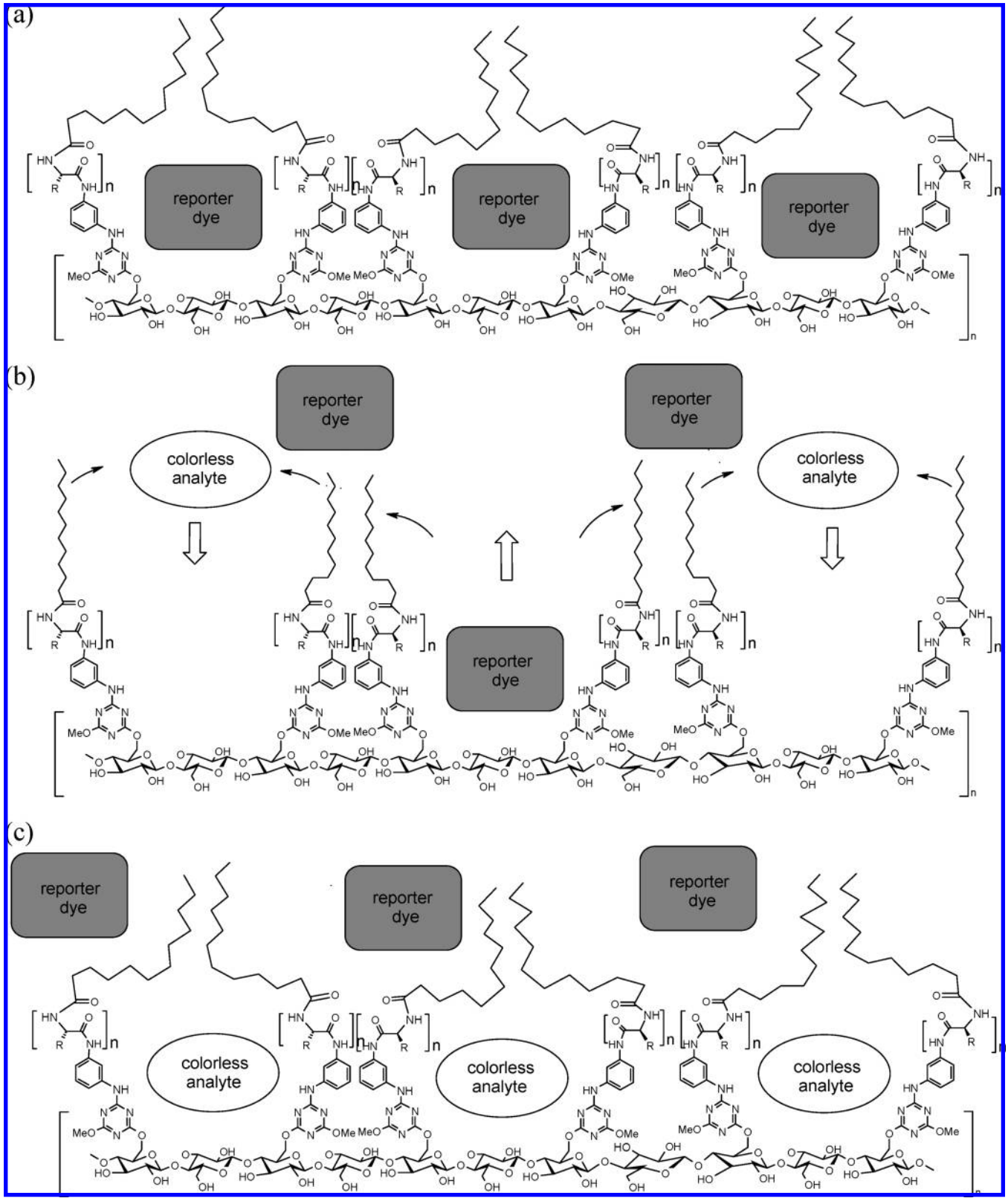
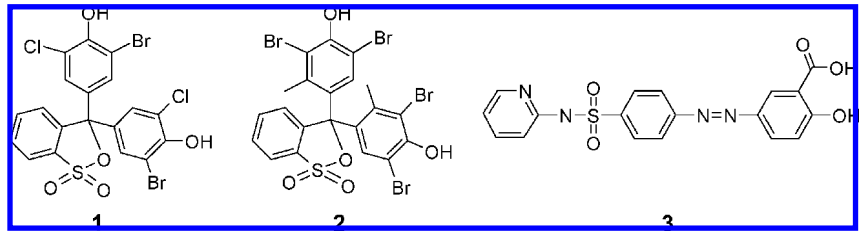
Scheme 1. Postulated Mechanism of Competitive Binding of the Host−Reporter Dye: (a) Binding of the Reporter Dye, (b) Competition between Ligands for the Binding "Pocket", and (c) Binding of the Colorless Ligand



receptors. The influence of the carbon−fluorine bond on docking interactions is of great importance considering that around a fifth of all the drugs that are currently in the market contain at least one fluorine substituent and fluorine-containing compounds account for more than 17% of all commercially available crop protection agents and many others are currently under development. Therefore, if this model of host−guest interaction could be useful at any stage of screening process of drugs (even at very early stage) it would be extremely beneficial concerning the high costs of selection experiments and strong demand for limitations of experiments with living animals.

Scheme 2. Structures of Reporter Dyes: Bromochlorophenol Blue (**1**), Bromocresol Green (**2**), and Sulphasalazine (**3**)



Results and Discussion

Spatially addressed libraries used as a tool for sensing closely analogous structure of fluorinated versus nonfluorinated guest molecules were synthesized in the previously described stepwise procedure, involving the immobilization of 2,4-dichloro-6-methoxy-1,3,5-triazine on the pages of cellulose support followed by treatment with an appropriate *m*-fenylenediamine, 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.[6] Expecting experiments with nonchromogenic ligands an approach based on the use of a reporter dyeing agent was developed (Scheme 1).

The prepared library was cloned into many copies by cutting every page of library members into Ø = 6 mm diameter disks. Every disk was then permanently labeled by writing the address of the relevant library member with a graphite pencil. Thus, all the tests were performed on new, exactly identical sets of disks.

Two libraries were used in the studies: library A, constructed mostly with short, branched chain carboxylic acid components, and library B with long chain fatty acids were prepared as described previously[7] with amino acid side chains remaining protected with a nitro or benzyl group. This made up the narrow and strongly hydrophobic interior of the binding pockets.

Library A (7 × 4=28 members) was prepared in reaction of (***a***) 2,2-dimethylpropionic acid, (***b***) 2-ethylhexanoic acid, (***c***) pentanoic acid, (***d***) heptanoic acid, (***e***) hexanoic acid, (***f***) 10-undecenoic acid, (***g***) 2-methylcynnamic acid with (***1***) His(Bzl), (***2***) Val, (***3***) Ala, and (***4***) Ser(OBzl), respectively.

Library B (6 × 4=24 members) was prepared by acylation by (***h***) 2-phenylbutyric acid, (***i***) (Z)-13-docosaenoic acid, (***j***) (R)-12-hydroxyoctadec-9c-enoic acid, (***k***) (E)-9-octadecaenoic acid, (***l***) octadecanoic acid, (***m***) (Z)-9-octadecaenoic acid of the appropriate derivative of (***5***) Glu(γBzl), (***6***) Gly, (***7***) ArgNO_2), and (***8***) Trp immobilized on cellulose support.

It was expected that artificial receptors were able do bind colorless guest molecules with efficiency comparable to that observed previously in the case of colored guests. Therefore, an approach based on the use of a reporter dyeing agent was used in primary experiments.

Competitive adsorption−desorption of bromochlorophenol blue (**1**), bromocresol green (**2**) (see Scheme 2), and S (+) naproxen[8] was studied in the disks of library A and library B.

In the case of the more lipophilic library B, desorption caused by 40 mmol/L S (+) naproxen solution was less substantial and depended on the structure of the dye and the structure of the lipophilic "pocket". The library members

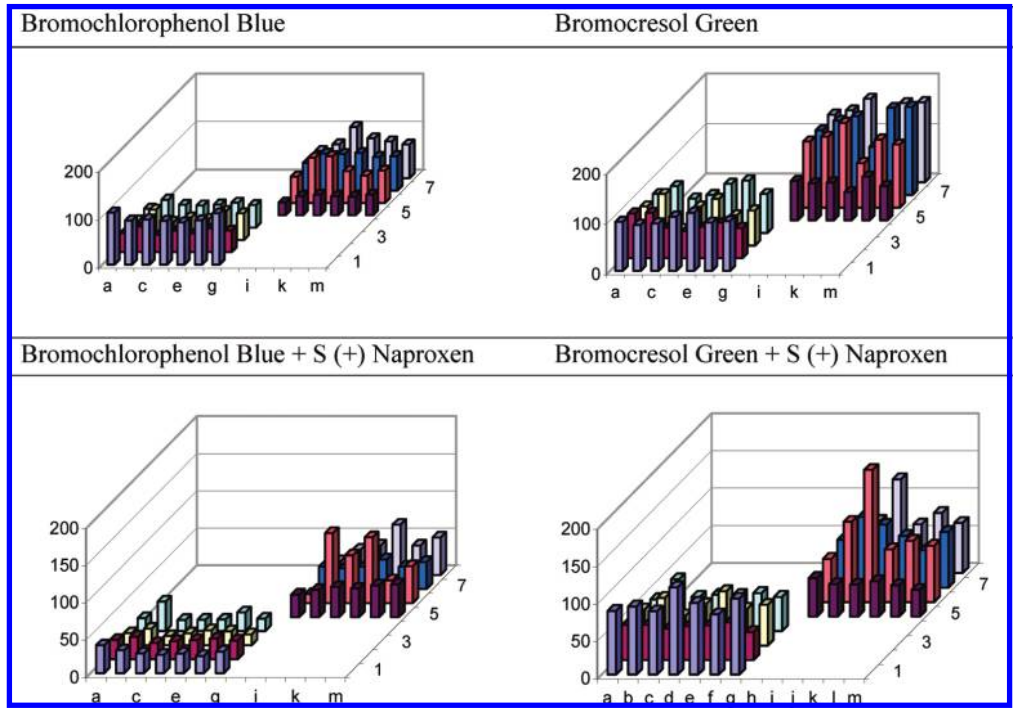
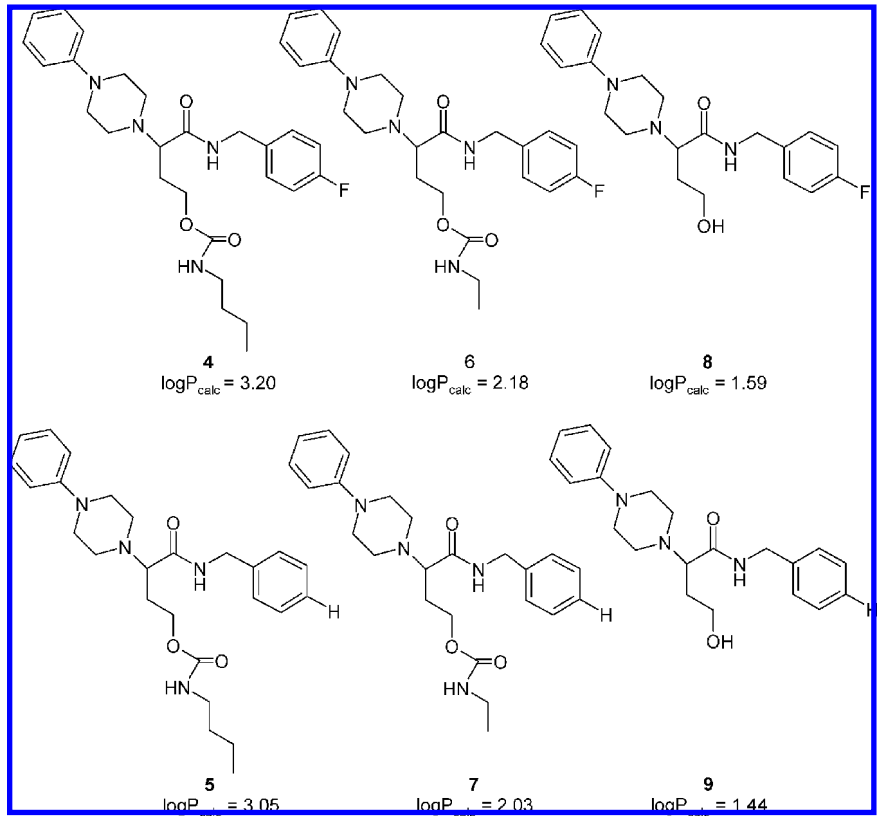


Figure 1. Intensity of coloration of disks in library A and library b after treatment with reporter dyes bromochlorophenol blue (left) and bromocresol green (right) and then with 20 mM/L S (+) naproxen aqueous solution (bottom).

Scheme 3. Structures of *N*-Benzylamide Derivatives of 4-Phenylpiperazines **4**−**9**



which most strongly bound bromochlorophenol blue were ***6-i*** (Gly + *cis*-13-docosenoic acid [erucic acid]), ***6-k*** (Gly + *trans*-9-octadecenoic acid [elaidic acid]), and ***8-k*** (Trp + *trans*-9-octadecenoic acid [elaidic acid]).

On the other hand, the more hydrophobic bromocresol Green bonded much stronger into "pockets" of both library A and B, and generally was less desorbed by S (+)naproxen with exception of ***7-l*** (Arg(NO_2) + octadecanoic acid; [stearic acid]), ***7-m*** (Agr(NO_2) + *cis*-9-octadecenoic acid; [oleic acid]), ***8-l*** (Trp + octadecanoic acid; [stearic acid]), and ***8-m*** (Trp + *cis*-9-octadecenoic acid; [oleic acid]). These results showed that the final results of competitive binding, measured as disk coloration in 256 tone gray scale, depend on the structure of the colorless ligand as well as on the structure of the reporter dye. Therefore, it was essential before any further experiments to choose the appropriate structure of

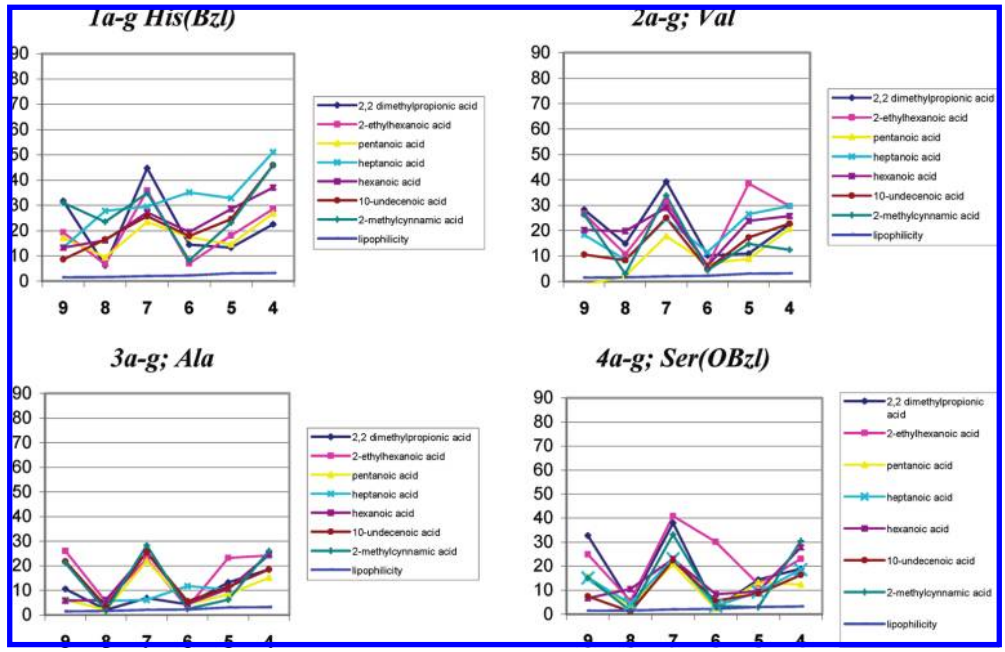


Figure 2. Bonding profile of **4**−**9** to receptors in library A. On the *x*-axis, compounds **4**−**9** are arranged numerically; on the *y*-axis, intense coloration remained after the treatment of each row of library A with sulphasalazine + *N*-phenylpiperazine **4**−**9** measured in 256 tone gray-scale.

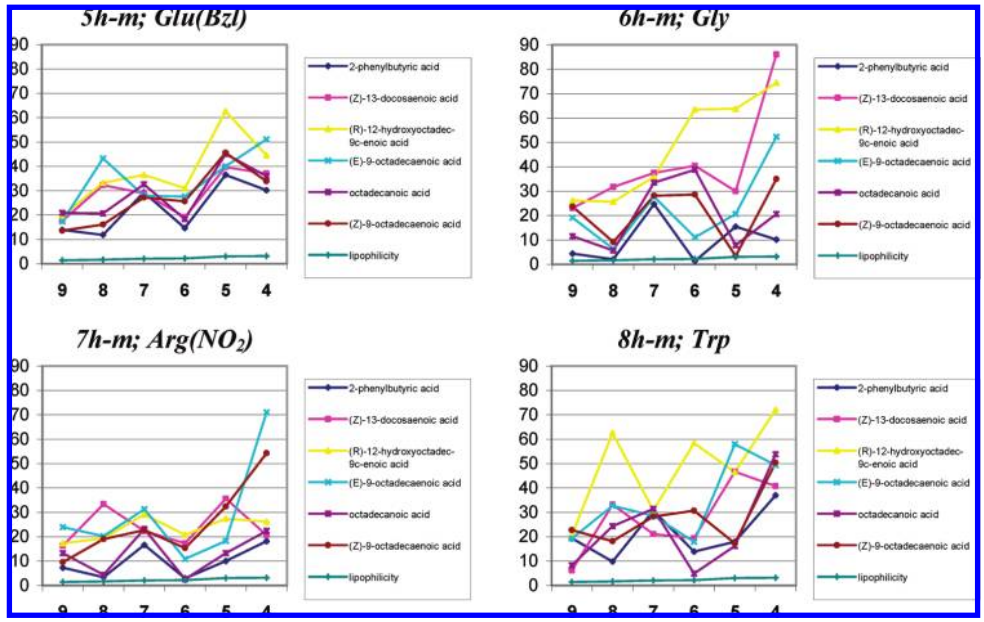


Figure 3. Bonding profile of **4**−**9** to receptors of library B. On the *x*-axis, compounds **4**−**9** are arranged numerically; on the *y*-axis, intense coloration, measured in 256 tone gray-scale, remained after treatment of each row of library B with sulphasalazine + *N*-phenylpiperazine **4**−**9**.

the reporter dye. As the most versatile reporter dye sulphasalazine (**3**) was selected because it binds to all library fields more uniformly than the trifluoromethane derivatives **1** and **2** used previously (Figure 1, poor binding in rows 2−5).

For a systematic investigation of competitive adsorption−desorption phenomena, we used a set of six *N*-benzylamide derivatives of arylpiperazine **4**−**9** with gradually decreased lipophilicity from log P_{calcd} = 3.20 to log P_{calcd} = 1.44 (Scheme 3). In this set of compounds, the attempts were made to find a correlation between the structure (fluorinated versus non-fluorinated analogues) and "recognition properties" of a library of artificial receptors. The structures of this set of compounds represent multifunctional molecules.[9] The compounds studied have a γ-hydroxybutyric acid skeleton and a 4-phenylpiperazine fragment characteristic of ligands to serotoninergic receptors, as well as of α_1-adrenoceptor antagonists. Compounds **6**−**9** are *N*-substituted carbamates, which are characteristic of acetylcholinesterase inhibitors. Therefore it has been expected that these multifunctional molecules are a good model series for studies involving a library of artificial receptors.[10]

In the previous study, the parameters of the relative lipophilicity of this set of compounds were determined using chromatographic and computational methods.[11] It was found that in all the cases the presence of a fluorine atom in the phenyl ring increased the lipophilicity of compounds **5**, **7**, and **9** in comparison to parent compounds **4**, **6**, and **8**; however, the increase was relatively small.

In these studies, we expected to observe some regularity in interactions between closely analogous compounds **4**−**9** and artificial receptors characteristic of the presence of a fluorine atom. The set of *N*-phenylpiperazines used in the study consisted of three pairs of compounds with even-numbered fluorinated derivatives **4**, **6**, and **8** and less lipophilic odd-numbered hydrogen analogues **5**, **7**, and **9**, respectively. Moreover, in the case of the last pair, the urethane group was replaced by a significantly more polar primary hydroxyl group attached to the flexible carbon chain.

The experiments were made with sulphasalazine (**3**) already loaded into the receptor pockets of library A and library B. Both libraries were subsequently treated with 2 mM/L *N*-phenylpiperazine **4**−**9** in aqueous buffered solution, and residual coloration was scanned and measured as previously. The resulting diagrams showed intensive replacement of reporter dye **3** by *N*-phenylpiperazines gradually increased with increasing hydrophilicity of **4**−**9**.

The binding properties of **4**, **6**, and **8** with a fluorine atom in the phenyl ring were compared to binding of nonfluorinated analogues **5**, **7**, and **9**, correlated with the structure of amino acid used for the construction of the binding pocket, then analyzed and presented in charts above (see Figure 2 for library A and Figure 3 for library B).

Very regular differences in the binding profile in fluorinated **4**, **6**, and **8** versus nonfluorinated ligands **5**, **7**, and **9** were observed as a W-shape zigzag for most of receptors accompanied sporadically by an M-shape pattern, and a mixed, less regular linear pattern found in all other cases. This gave an ample evidence that single substitution of hydrogen by fluorine in a molecule is detectable using both libraries of receptors. The regularity of pattern was usually distorted in the most bulky ligands **4** and **5**. In the other less bulky ligands fluorinated *N*-phenylpiperazines **6** and **8** competed in receptors of both libraries more efficiently than nonfluorinated analogues **7** and **9**. The most regular pattern was noticed in ***2-a*−*g,*** Val and most other receptors in library A. This shape was less abundant in receptors in library B, although found in ***5-h*,*i-m***; Glu(Bzl); ***6-h*,*k***, Glu; ***7-h*,*k*−*l,*** Arg(NO_2) and ***8-h*,*m*** Trp receptors.

A single example of an M-shape pattern characteristic of the stronger binding of nonfluorinated analogues than

fluorinated ones was detected among receptors in library B; ***8-j*** Trp. The less regular M-shape pattern was also observed in ***7-i***; Arg(NO_2) and ***8-i***,***k***, Trp.

In all the other cases, the regularity of the bonding pattern was considerably disturbed and even some correlation with the lipophilicity parameter was observed, particularly in receptors in library B with long chain carboxylic acid fragments. All this findings strongly suggests the complex nature of the process of ligand recognition and fluorine sensing. Yet, if the size and polarity of ligands is dominating, a substantial role of other binding forces modulating docking ability was documented even in the case of these small, randomly designed libraries A and B. The selectivity of recognition was found sufficient to unambiguously identify substitution of an aromatic hydrogen atom with fluorine. The most motivating, however, is the observation suggesting two different responses to the modification of the ligand structure with a fluorine atom. Depending on the structure of the amino acid fragment and the lipidic chain of the acyl fragment of the receptor, binding of fluorinated analogues can be improved (in most cases) or deteriorated (only in a few cases) as compared to nonfluorinated counterpart, although understanding biological consequences of this correlation requires further studies.

Conclusions

A library of artificial receptors formed by the self-organization of N-lipidated amino acids immobilized on cellulose support was found useful in studies into binding of colorless ligands with the use of competitive adsorption−desorption of reporter dyes. The binding profile has been found sufficiently specific to unequivocally identify a small structural alteration in the ligand structure, for example, exchange of aromatic hydrogen for fluorine. The most interesting, however, were preliminary results of fluorine sensing indicative of the presence of two opposite interactions between fluorinated ligands and artificial receptors. There is interesting, that basic information was not estimated directly by measurements of binding abilities of host−guest interactions, but refined as relation between small variation of interactions of different hosts with exactly the same receptors. These results strongly imply the application of this approach to the studies, rational design, screening, and measurement of the other molecular properties of bioactive compounds.

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