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Synthetic Receptors Based on Peptidosulfonamide Peptidomimetics

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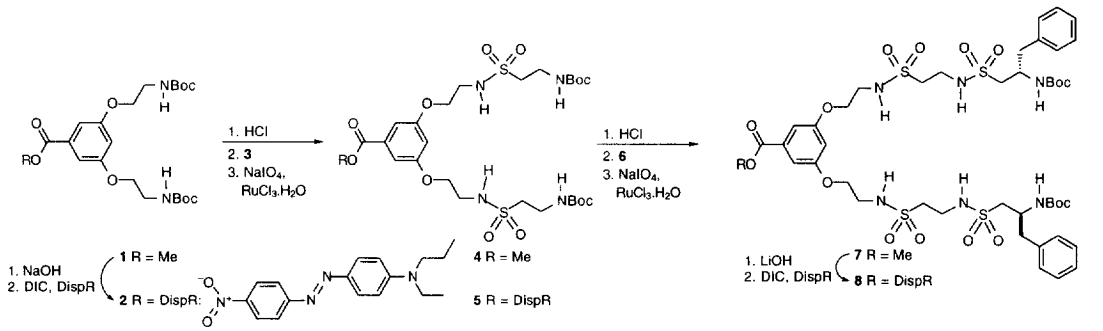
Abstract: We have synthesized tweezer-like receptor molecules based on peptidosulfonamide peptidomimetics. These compounds were screened for binding against a ~25,000 member encoded tripeptide library. One of these synthetic receptors showed a remarkable binding selectivity.

Combinatorial chemistry has offered many new methodologies for the generation of libraries consisting literally of several ten thousands of compounds which can be subjected to high-throughput screening for lead-finding and lead-optimization.[1] In addition, it has restructured our thoughts about our ability to design a molecule with predicted molecular interactions and/or a predicted biological activity. Furthermore, combinatorial chemistry offers tremendous possibilities to move beyond the generation of compounds for pharmaceutical screening purposes, since it may also be of great significance for the preparation of new synthetic receptors,[2] catalytic cavities and catalysts.[3] The area which is now emerging might be referred to as "combinatorial supramolecular chemistry". Employing combinatorial chemistry for this purpose is in fact a very "biomimetic" approach, since in nature for example many antibodies -biological receptors- are generated against a particular antigen, from which then antibodies with optimal binding properties are selected. We reasoned that combinatorial chemistry methods might offer attractive tools to probe the structure and interactions of peptidomimetics with peptides and proteins. We are interested in peptidosulfonamide peptidomimetics,[4] because the sulfonamide moiety might act as a transition-state isostere of the hydrolysis of the amide bond. It is more resistant to degradation by proteases, more flexible than the amide bond,[4e] and its more acidic hydrogen may give rise to stronger hydrogen bonds.[5]



Scheme 1

Following the successful concept of creating tweezer-like, two armed molecules,[6] we embarked on the construction of synthetic receptors based on our earlier described peptidosulfonamides, using diamino acid derivative **1** as a "hinge" molecule, which is accessible on a multigram scale and is used for the preparation of a new type of amino acid based dendrimers.[7] Saponification of the methylester and coupling to Disperse Red 1

gave the colored derivative **2**. After removal of the Boc-groups, receptor **5** was obtained by treatment with sulfinylchloride **3** (Figure 1) in the presence of Et_3N, followed by oxidation using $NaIO_4/RuCl_3$ (overall yield 14-33%, Scheme 1).[8] Peptidosulfonamide receptor **8** was obtained by removal of the Boc-groups in **4** - prepared from **1** (Scheme 1) - followed by coupling with sulfinylchloride **6** (Figure 1), oxidation to **7** and attachment of the dye.[9] Presence of the dye from the very start *i.e.* using **2** as a starting material for receptor **8** diminished the yields after the oxidation steps considerably, probably because of competing oxidation of the dye-part (Scheme 1).[10]

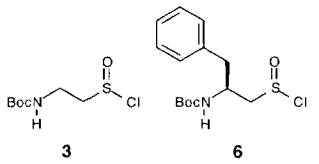
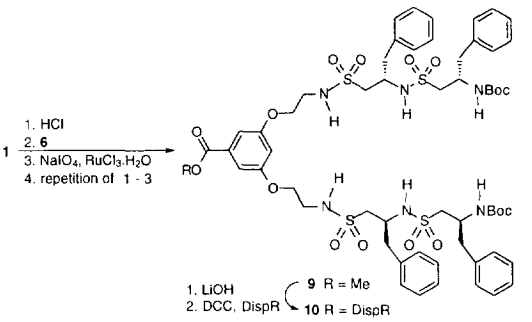


Figure 1



Scheme 2

Red colored peptidosulfonamide receptors **5** and **8** were screened for binding against a 24,389 ($=29^3$) member encoded tripeptide library on TentaGel® *i.e.* AA_3-AA_2-AA_1-N(H)-TentaGel ($AA_{1\text{-}3}$ are specified in Fig.2, the letters correspond to the one letter symbols of the various amino acids; a prefix 'd' indicates that the particular amino acid in the tripeptide was a D-amino acid). Synthetic receptor **5** did not show any binding whatsoever with either the deprotected (both side chains and amino terminus) or protected library at concentrations as high as 500 μM. In contradistinction, with peptidosulfonamide receptor **8**, containing an additional amino sulfonamide moiety at each arm, binding was observed with the *deprotected* library in chloroform. At a concentration of 100 μM approximately 1% of the library beads bound. However, no binding was observed with the *protected* library. Furthermore, the binding depended strongly on the charged state of the library in chloroform with the best selectivity around neutrality, since no binding was observed with added TFA, and addition of DBU or a weaker base (Et_3N) decreased the selectivity of binding. In order to try to obtain the best selectivity the bead-supported tripeptide library was equilibrated in a chloroform solution of the receptor having a slightly lower concentration (~70 μM). After 24 h equilibration, the colored beads were separated and decoded by photolytic release of the molecular tags, followed by EC-GC (electron capture gas chromatographic) analysis.[11] A total of 34 beads were decoded.[12] The frequencies of different amino acid residues in tripeptide library members, that bound to peptidosulfonamide receptor **8** are shown in Fig. 2. All decoded sequences contain an acidic amino acid residue, which is practically always (found in 32 out of 34 decoded beads: 94%) accompagnied by a second polar amino acid residue, especially Asn or His, sometimes Ser, and in six sequences an acidic amino acid residue. In almost all cases (found in 32 out of 34 decoded beads: 94%) the site for the acidic residue is AA_3 or AA_2 in which AA_3 is strongly biased towards being a Glu residue (found in 8 out of 34 decoded beads: 24%) and AA_2 an Asp residue (23 out of 34 beads: 68%). For AA_3 an Ala residue was found in 17 out of 34 decoded beads (50%). AA_2 is almost invariably a polar (His, Asn, Glu or Asp) amino acid residue (found in 33 out of 34 decoded beads: 97%). Interestingly, most variability is found in the third amino acid residue AA_1, which -for recognition by this receptor- might be designated as a 'wobble' amino acid residue. It is also noteworthy that in a number of cases (D)Ala-(L)Asp or its enantiomeric sequence (L)Ala-(D)Asp is found (found in 17 out of 34 decoded beads: 50%). None of the corresponding diastereomeric sequences ((D)Ala-(D)Asp or (L)Ala-(L)Asp) are found, indicating some degree of stereoselectivity of this receptor. Most tripeptide sequences, which were recognized by this receptor, have

consensus sequence (D)Ala-(L)Asp-(D)Xxx (or the enantiomeric sequence, found in 17 out of 34 decoded beads: 50%) or (D/L)Glu-(D/L)Asn/His-(D/L)Xxx (found in 7 out of 34 decoded beads: 20%). Finally, it is worth mentioning that some full tripeptide sequences were found multiple times,[12] *e.g.* (D)Ala-(L)Asp-(D)Asn and (D)Ala-(L)Asp-(D)Ser were found four times each, *i.e.* in 8 of 34 decoded beads (24%) and interestingly, Asn and Ser are quite similar.

In order to increase the selectivity of binding, we decided to prepare the less flexible peptidosulfonamide receptor **10**. This compound was prepared from **1** using the sulfinyl chloride **6** as is depicted in Scheme 2. Unfortunately, this less flexible peptidosulfonamide receptor **10** did not show binding with any of the libraries. The additional sulfonamide residue derived from phenylalanine clearly suppressed binding since no binding was observed using concentrations as high as 500 μM!

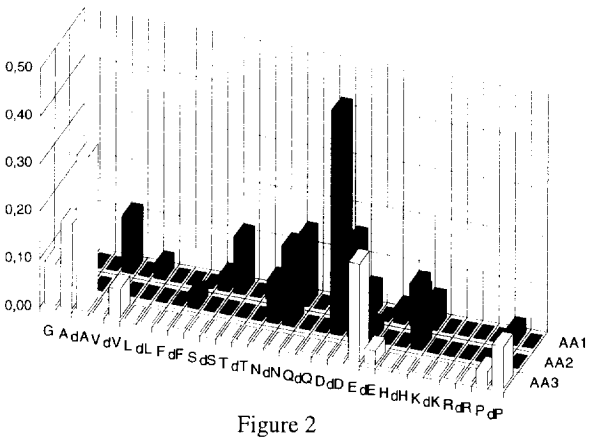


Figure 2

For a quantitative estimation of the binding constant one of the peptides was selected, that bound to the receptor *viz.* (D)Ala-(L)Asp-(D)Ser-N(H)-TentaGel and belongs to one of the consensus sequences. No binding was observed when the N-terminus was either acetylated or the TFA-salt was used. In chloroform a binding constant (K_a) of 310 ± 20 M^{-1} (ΔG = - 14 kJ mol^{-1}) was determined. This binding was reduced to a K_a of ca. 15 M^{-1} in dichloromethane and was very sensitive to traces of a protic solvent such as methanol. The presence of only 1% methanol was sufficient to reduce the binding to almost zero in both chloroform and dichloromethane. The enantiomer of the tripeptide *i.e.* (L)Ala-(D)Asp-(L)Ser-N(H)-TentaGel showed a comparable binding to the peptidosulfonamide receptor, which is in agreement with the aforementioned screening results. Interestingly, if a diastereomeric sequence of these peptides was prepared *e.g.*, (D)Ala-(L)Asp-(L)Ser-N(H)-TentaGel virtually no binding was observed by the same method, of the beads containing this sequence after equilibration with receptor **8**. As expected an arbitrary sequence such as (L)Ala-(L)Lys-(L)Phe-N(H)-TentaGel did not show any detectable binding with receptor **8**.

We have described the synthesis of a tweezer-like synthetic receptor based on peptidosulfonamide peptidomimetics with a remarkable binding selectivity for such a simple system. The preference of the synthetic receptor for interacting with acidic amino acid residues is interesting and probably points to strong hydrogen bond donor properties of the sulfonamide N-H.[13] The absence of any binding capability of the less flexible receptor **10** for tripeptides from the used libraries was somewhat surprising. This may be caused by hindrance of one of the sulfonamides and therefore **10** may have reduced binding properties. Alternatively, if the intramolecular interactions of the arms are too favorable, binding with a tripeptide from the library will be less likely to occur. Therefore, in the near future we will look for less flexible hinges to which the peptidosulfonamide arms can be attached thus avoiding intramolecular interactions of the arms as might be present in receptor **10**. Under present investigation is also the preparation of combinatorial libraries of these tweezer-like synthetic receptors based on peptidosulfonamide peptidomimetics.

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8. Procedure: see reference 4c.
9. ^{1}H-NMR (300 MHz, $CDCl_3$, TMS): δ 8.34, 7.94, 7.91 (6H, 3*d, ArH) 7.34-7.18 (10H, m, ArH) 7.12 (2H, d, ArH) 6.89 (2H, d, ArH) 6.82 (1H, t, ArH) 5.85, 5,61 (4H, bs, $NHSO_2$) 4.98 (2H, bs, NHCO) 4.52 (2H, t, CH_2OCO) 4.25 (2H, bs, $CHCH_2$) 4.05 (4H, t, OCH_2) 3.82 (2H, t, CH_2CH_2NAr) 3.58 (2H, q, CH_2CH_3) 3.49 (8H, m, CH_2NHSO_2) 3.30 (4H, m, SO_2CH_2CH) 3.19 (4H, m, CH_2Ph) 2.94 (4H, bs,$SO_2CH_2CH_2$) 1.38 (18H, s, $C(CH_3)_3$) 1.26 (3H, t, CH_2CH_3). ^{13}C-NMR ($CDCl_3$ + CD_3OD): d 166.0 ($OCOAr$) 159.3 (NHCOO) 156.7 ($C^{Ar}NN$) 155.9 ($C^{Ar}O$) 151.4 ($C^{Ar}NCH_2$) 147.5 ($C^{Ar}NO_2$) 143.8 ($C^{Ar}NN$) 136.6 ($C^{Ph}CH_2$) 131.7 ($C^{Ar}COO$) 129.3, 128.8, 127.0, ($C^{Ph}H$) 126.3, 124.7 122.7, 111.6, 108.4 ($C^{Ar}H$) 80.4 ($C(CH_3)_3$) 67.3 ($ArOCH_2$) 62.2 (CH_2OCO) 54.5 (SO_2CH_2CH) 52.5 ($SO_2CH_2CH_2$) 48.6 (CH_2CH_2NAr) 48.4 ($CHCH_2$) 45.5 (CH_2CH_3) 42.3 (CH_2NHSO_2) 40.3 (CH_2Ph) 38.0 ($SO_2CH_2CH_2$) 28.3 ($C(CH_3)_3$) 12.3 (CH_2CH_3) . MS: $(M+H)^+$: m/z 1345.3
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12. The sequences found by decoding the beads where:
(D)Glu-(D)Phe-(D)Asn (1x); (D)Pro-(L)Asp-(L)His (3x); (D)Val-(D)Asp-(D)Asp(1x); (D)Val-(L)Asp-(L)Asp (1x); (D)Ala-(L)Asp-(D)Asn (4x); (D)Ala-(L)Asp-(D)His (2x); (D)Ala-(L)Asp-(D)Ser (4x); (D)Ala-(L)Asp-(D)Thr (1x); Gly-(D)His-(D)Asp (1x); Gly-(L)Asp-(L)Asp (1x); Gly-(L)Asn-(L)Asp (1x); (L)Glu-(D)Asn-(L)Val (2x); (L)Glu-(D)His-(D)Glu (1x); (L)Glu-(D)His-(L)Asn (1x); (L)Glu-(D)His-(L)Val (1x); (L)Glu-(L)Asn-(L)Asn (1x); (L)Glu-(L)Asn-(L)Leu (1x); L)Ala-(D)Asp-(L)Asn (2x); (L)Ala-(D)Asp-(L)Asp (2x); (L)Ala-(D)Asp-(L)Val (1x); (L)Ala-(D)Asp-(L)Ser (1x); (L)Pro-(L)Glu-(L)Pro (1x).
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