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SOLID-PHASE SYNTHESIS OF PEPTIDOSULFONAMIDE CONTAINING PEPTIDES DERIVED FROM LEU-ENKEPHALIN[1]

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Abstract: Using Boc or Fmoc-protected β-substituted aminoethanesulfonyl chlorides (2-substituted taurylchlorides) the solid-phase synthesis of dipeptidosulfonamides as well as peptidosulfonamide containing peptides derived from Leu-enkephalin is described. The binding activity of the peptidosulfonamide YGGFL derivatives is reported.

Oligo peptidomimetics are attracting considerable attention as starting points for the generation of new lead compounds for drug discovery.[2,3] Using oligo peptidomimetics, the desired selective binding of the parent peptide to a receptor may be preserved, while at the same time reducing or abolishing the disadvantageous properties of a peptide *e.g.* limited stability due to proteolytic degradation or fast clearance from the body. Oligo peptidomimetics are especially suitable for the preparation of combinatorial chemistry libraries because they can be constructed in a modular way from monomeric building blocks by solid-phase methods analogous to methods, which over the years have been highly optimized for solid-phase peptide synthesis.

Several oligo peptidomimetics, containing a modified backbone, have appeared in the literature by now *e.g.* peptoids[3a,l], oligopyrrolinones[3b], vinylogous peptides[3c], oligocarbamates[3d], peptidosulfonamides[4], hydrazinopeptides[3e], oligo ureas[3f,g,i,n], vinylogous sulfonopeptides[3h,o] oligosulfones[3j,k] and azatides[3m]. Recently, we have shown that the peptidosulfonamides introduced by us[4] can be prepared by solid-phase organic synthesis[4f], which is a prerequisite for combinatorial chemistry approaches[5] towards the development of libraries of these compounds, for example by multiple synthesis[6].

The thus reported solid-phase synthesis of peptidosulfonamides was based on the reaction of a sulfinyl chloride with an amino acid anchored to a solid support followed by oxidation of the resulting peptidosulf*i*namide to the corresponding peptidosulf*o*namide.[4f] Although this method is an attractive approach for the synthesis of small peptidosulfonamides, the yield of the oxidation step is somewhat dependent on the sequence of the peptidosulfonamide to be synthesized, which makes this approach less suitable as a general method for the solid-phase synthesis of peptidosulfonamides. In order to circumvent this oxidation step, we returned to the sulfonylchlorides, which we have previously used for the synthesis of peptidosulfonamides in solution[4a], in order to apply them now for the solid-phase synthesis of peptidosulfonamides.

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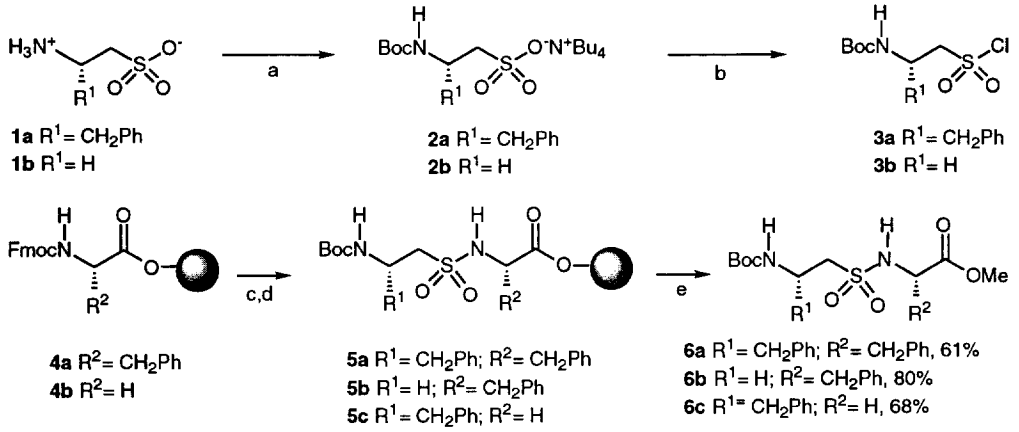
In addition to some illustrative examples for the solid-phase synthesis of dipeptidosulfonamides, the methodology was extended to peptidosulfonamide containing peptides derived from Leu-enkephalin (TyrGlyGlyPheLeu) in which specific amide bonds were replaced by sulfonamide moieties. One might consider these systematic replacements in Leu-enkephalin as a kind of "positional scan"[7] to determine the relative biological effect (*i.e.* on receptor affinity) of each subsequent replacement of the amide bond.

Sulfonylchorides can be prepared either by oxidation of a thioester or a disulfide with Cl_2/Ac_2O[4a,8], or by reaction of a sulfonate salt with triphosgene under Vilsmeier-Haack conditions.[9] The latter method is preferable, as the former resulted in a mixture of a sulfonyl and a sulfinyl chloride.[4a]

For the synthesis of the appropriate sulfonylchlorides **3a** or **3b** (Scheme 1) **1a**, prepared according to Higushiura *et al.*[10], or taurine (**1b**) was used as a starting material. Reaction of **1a** or **1b** with Boc_2O in the presence of tetrabutylammonium hydroxide resulted in the protected β-aminoethylsulfonates **2a,b** in yields of 96% and 99%, respectively. Reaction of **2a,b** with triphosgene in the presence of a catalytic amount of DMF yielded, after purification by filtration over a silica plug, sulfonylchlorides **3a,b** in 92% and 90% yield, respectively.

Scheme 1 Solid-phase synthesis of peptidosulfonamides employing sulfonylchlorides.



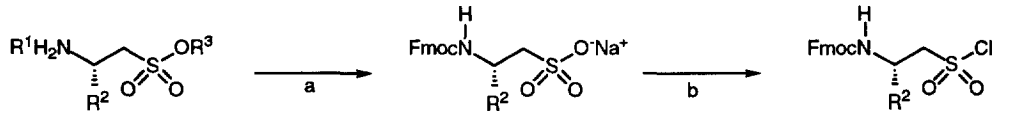
a) Boc_2O, *n*-Bu_4NOH; b) triphosgene, DMF; c) 20% piperidine in NMP; d) **3a** or **3b**, NMM, CH_2Cl_2; e) MeOH, Et_3N

For the solid-phase synthesis of peptidosulfonamides employing sulfonylchlorides **3a** and **3b**, Fmoc-Gly or Fmoc-Phe were attached to Tentagel® S-OH according to Sieber[11], yielding **4a** and **4b** (Scheme 1). After removal of the Fmoc protecting group with 20% piperidine in NMP (*N*-methyl-2-pyrrolidone), the resin was washed with NMP and CH_2Cl_2. Subsequently, a solution of the appropriate sulfonylchloride (3 eq.) in the presence of NMM (*N*-methylmorpholine, 6 eq.) in CH_2Cl_2 was added to the resin. To ensure completion of the reaction, as gauged by a Kaiser test[12], coupling was carried out twice resulting in peptidosulfonamides **5a-c**. Transesterification with methanol in the presence of triethylamine, followed by purification (silicagel chromatography) resulted in peptidosulfonamides **6a-c** in yields of 61%, 80% and 68%, respectively.

The solid-phase synthesis of dipeptidosulfonamides was further extended to the solid-phase synthesis of peptidosulfonamide containing peptides derived from Leu-enkephalin, in which specific amide bonds have been replaced by sulfonamide moieties, thus obtaining hybrid peptide-peptidosulfonamides. In order to prepare C-terminal amides as well as carboxylic acids, using Rink[13] or Wang[14] linkers, respectively, a Fmoc based solid-

phase synthesis strategy is crucial. Therefore, in addition to the Boc protected β-aminoethanesulfonyl chlorides **3a,b**, Fmoc protected β-aminoethanesulfonylchlorides **9a-c** were prepared as depicted in Scheme 2. Reaction of **1b**, **7a** or **7b**, either as zwitter-ion or sodium-hydrochloride salt,[15] with FmocOSu resulted in the protected aminosulfonates **8a-c** in good (81%-97%) yields. The corresponding Fmoc protected sulfonylchlorides **9a-c** were prepared (*vide supra*) by reaction with triphosgene in the presence of DMF and a phase transfer catalyst (Bu_4NCl) in yields of 80%, 79% and 94%, respectively.[16]

Scheme 2 Syntheses of Fmoc protected β-aminosulfonylchlorides **9a-c**



1b R^1= H^+, R^2= H, R^3= $^-$
7a R^1= HCl, R^2= CH_2Ph, R^3= $^-Na^+$
7b R^1= HCl, R^2= $CH_2CH(CH_3)_2$, R^3= $^-Na^+$

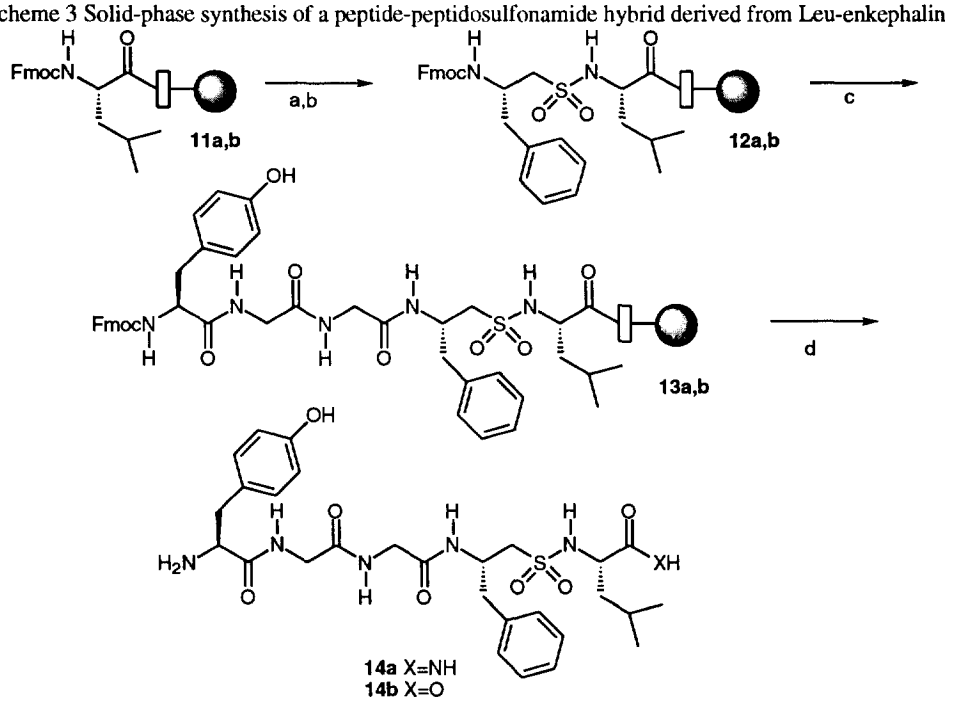
8a R^2= H
8b R^2= CH_2Ph
8c R^2= $CH_2CH(CH_3)_2$

9a R^2= H
9b R^2= CH_2Ph
9c R^2= $CH_2CH(CH_3)_2$

a) FmocOSu, 1 N NaOH; b) triphosgene, DMF

For the solid phase synthesis of the Leu-enkephalin peptide-peptidosulfonamide hybrids (scheme 3) having a C-terminal carboxylic acid, FmocLeu was attached to Tentagel® S-PHB resin according to the method of Sieber[11]. The Leu-enkephalin hybrids bearing a C-terminal amide were synthesized on a Tentagel® S-RAM

Scheme 3 Solid-phase synthesis of a peptide-peptidosulfonamide hybrid derived from Leu-enkephalin



11a,b **12a,b** **13a,b**

14a X=NH
14b X=O

▯ = Rink linker (**a** series) or Wang linker (**b** series)

a) 20% piperidine in NMP; b) **9b**, NMM; c) (i) 20% piperidine in NMP (ii) appropriate aminoacid (3eq.), BOP (3 eq.), 1-hydroxy benzotriazole (3 eq.), DiPEA (6 eq.); d) TFA/H_2O, 95/5, v/v

resin. These hybrids could be synthesized by a standard solid-phase synthesis protocol in which the Fmoc sulfonylchloride is substituted for the corresponding Fmoc-amino acid. This is illustrated by the synthesis of peptide-peptidosulfonamide hybrids **14a** and **14b**, starting from **11a** and **11b**, respectively (Scheme 3).

Coupling of the Fmoc protected sulfonyl chlorides **9a-c** was carried out analogous to the procedure described above for the preparation of dipeptidosulfonamides using Boc protected sulfonylchlorides. After removal of the Fmoc group the appropriate sulfonylchloride and NMM in CH_2Cl_2 were added. After 2.5 hr, the resin was washed with CH_2Cl_2 and the coupling was repeated to ensure completion of the reactions as was gauged by carrying out a Kaisertest[12]. The Fmoc protecting group on the sulfonamide residue enabled introduction of the remaining amino acids simply by continuing the Fmoc-protocol, resulting in *e.g.* **13a** and **13b** (Scheme 3). Deprotection and cleavage from the solid phase, followed by precipitation in ether/hexane and lyophilization resulted in sulfonamide containing Leu-enkephalin derivatives **14a,b - 17a** in good to excellent yields with high purity as was judged by HPLC (Table 1). Mass spectrometry as well as ^{13}C and ^{1}H NMR confirmed the structure of the newly synthesized compounds. NMR showed a small impurity, which probably a small amount of polyethylene glycol liberated from the resin during cleavage.

The high purity of the thus obtained peptidosulfonamide-peptide hybrids derived from Leu-enkephalin allowed determination of the IC_{50} by an inhibition ELISA for anti-β-endorphin monoclonal antibody[17] (Table 1) without the need of further purification. Leu-enkephalin **18b** and its amide **18a** were synthesized by the same solid-phase peptide synthesis protocol in order to compare the IC_{50} with those of the peptidosulfonamide containing peptides.

Table 1 yield, purity and receptor binding of peptidosulfonamide containing Leu-enkephalin derivatives.

Leu-enkephalin derivative	Yield (%)	Purity (HPLC)	IC_{50} (μM)
H-TyrGlyGlyPheLeu(ψCH_2SO_2)-NH_2 (**17a**)	84	100	0.1
H-TyrGlyGlyPhe(ψCH_2SO_2NH)Leu-NH_2 (**14a**)	52	99	0.9
H-TyrGlyGlyPhe(ψCH_2SO_2NH)Leu-OH (**14b**)	99	96	5
H-TyrGlyGly(ψCH_2SO_2NH)PheLeu-NH_2 (**15a**)	93	96	85
H-TyrGlyGly(ψCH_2SO_2NH)PheLeu-OH (**15b**)	67	94	>200
H-TyrGly(ψCH_2SO_2NH)GlyPheLeu-NH_2 (**16a**)	67	100	75
H-TyrGly(ψCH_2SO_2NH)GlyPheLeu-OH (**16b**)	96	96	>200
H-TyrGlyGlyPheLeu-NH_2 (**18a**)	78	100	0.07
H-TyrGlyGlyPheLeu-OH (**18b**)	95	100	0.1

As can be concluded from Table 1, introduction of a sulfonamide moiety at the C-terminus (**17a**) resulted in a nearly equal activity as for the Leu-enkephalin amide **18a**. When the sulfonamide moiety is shifted from the C-terminus towards the N-terminus of the Leu-enkephalin derivatives, a dramatic loss of activity was found. Replacement of the amide bond between Phe^4 and Leu^5 by a sulfonamide moiety (*viz.* **14a** and **14b**) gave rise to IC_{50} values which are 10 to 50 times larger compared to those of amide **18a** and Leu-enkephalin **18b**, respectively. When the amide bond between Gly^3 and Phe^4 (*viz.* **15a** and **15b**) or between Gly^2 and Gly^3 (*viz.* **16a** and **16b**) was replaced the peptidosulfonamide-peptide hybrid was virtually devoid of activity.

Although the biologically active conformation of enkephalins is unknown, it is assumed[18], based on X-ray diffraction data,[19] that YGGFL adopts a type I' 4→1 β-turn in order to form the active conformation with the

two glycines at the corners of the bend. The measured biological activities may be explained by the fact that this secondary structure may be partly or completely disrupted by introduction of a sulfonamide in compounds **14a,b**, **15a,b** and **16a,b**. This disruption may be caused by different bond angles between the sulfonamide function and the amide bound or by elongation of the backbone with one methylene unit. Introduction of the sulfonamide moiety at the C-terminal site resulted in a nearly equipotent compound (**17a**) as compared to **18a**, which can be rationalized by the fact that the leucine residue is not a part of the β-turn.

Although the peptidosulfonamides did not show in all cases the same binding activities as the corresponding peptides, they remain a highly attractive class of peptidomimetics because they are more stable towards proteolytic degradation. By constructing libraries of peptidosulfonamides, using combinatorial chemistry techniques,[5] compounds may be found with desired biological activities as well as having an enhanced stability towards proteolytic degradation.

In conclusion, we have described the solid-phase synthesis of peptidosulfonamides featuring reaction of a Boc or Fmoc-protected β-substituted aminoethanesulfonyl chloride (2-substituted taurylchloride) with an amino acid or peptide attached to a solid support. Treatment of glycine or phenylalanine, attached to the solid-phase, with β-aminosulfonyl chlorides resulted, after transesterification, in the corresponding peptidosulfonamides in good yields. Using this newly developed methodology for the introduction of a sulfonamide moiety on a solid support, a positional scan was carried out in which specific amide bonds in Leu-enkephalin were replaced by sulfonamide isosters. After deprotection and cleavage from the resin, followed by precipitation and lyophilisation, the peptidosulfonamide containing Leu-enkephalin derivatives were obtained in good to high yield and with an excellent purity. The binding activities of these peptidosulfonamide-peptide hydrides showed that replacement of the C-terminal amide resulted in a nearly equipotent compound but replacement of other amide bonds in the direction of the amine terminus resulted in a decreased and ultimately complete loss of biological activity.

In the near future, research will be directed towards the synthesis of peptidosulfonamides containing two or more sulfonamide moieties. Furthermore, using combinatorial chemistry techniques, libraries of peptidomimetics will be prepared containing one or more incorporated sulfonamide moieties.

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15. Although Fmoc β-aminosulfonates can be synthesized from the corresponding zwitterions of **7b** and **7c**, preparation starting from the HCl salts **7b** and **7c** is more convenient since tedious ion-exchange steps are avoided. The use of sodium salts of **8b** and **8c** is preferred since these compounds are solids whereas the corresponding tetrabutyl ammonium salts are oils.
16. Typical procedure: 210 mg of Fmoc protected leucine derivative **8c** was suspended in 5 mL dry CH_2Cl_2, followed by addition of 150 mg triphosgene (0.5 mmol), 100 μL dry DMF and a catalytic amount of tetrabutylammonium chloride. After completion of the reaction (1 h), as was demonstrated by TLC, the reaction mixture was filtrated over a small amount of silica 60 (eluent: EtOAc/hexanes, 1/3, v/v) and the solvent evaporated affording sulfonylchloride **9c** in a yield of 94% (198 mg).
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