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ψ(SO_2-NH) Transition State Isosteres of Peptides. Synthesis of the Glutathione Disulfide Analogue [Glu⌐└ψ(SO_2-NH)-Cys-Gly]$_2$

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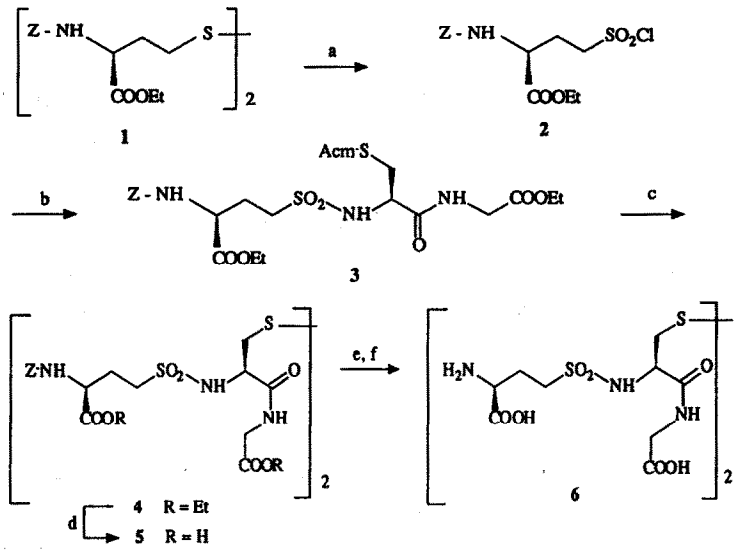
Abstract: [Glu⌐└ψ(SO_2-NH)-Cys-Gly]$_2$ **6** has been synthesized starting from ethyl (*S*)-2-benzyloxycarbonylamino-4-(chlorosulfonyl)butanoate and *S*-acetamidomethyl-L-cysteinyl-glycine ethyl ester. Compound **6** is a backbone modified analogue of glutathione disulfide containing the SO_2NH transition state mimic in place of the native γ-glutamyl-cystine CONH bond.

In recent years a variety of chemical strategies have been adopted in order to transform natural bioactive peptides into therapeutically useful agents[1]. Much interest is focused on the replacement of scissile peptide bonds with isosteric groups mimicking transition-states or high energy intermediates along the pathway of enzyme catalyzed hydrolytic reactions[2,3]. This approach represents the base for the rational design of tight binding enzymes inhibitors (transition state analogues, T.S.A.) as well as the development of catalytic antibodies. In this field several studies are currently dedicated to peptides containing the SO_2NH junction[4,5], the interest deriving from several promising features, *i.e.* T.S.A. potentiality, H-bonding capacity, hydrolytic stability and conformational preferences[4]. However, due to the intrinsic chemical instability of peptides containing the typical sequence -CONH-CH(R)-SO_2NH-CH(R')-CO-[6,7], studies are actually limited to models containing 2-amino-alkanesulfonic acid residues[4,5,8] or different sulfurated systems such as sulfinamido[7] and retrosulfonamido groups[9]. Analogues of natural peptides, characterized by the simple SO_2NH/CONH replacement and retaining all the other structural features of the corresponding bioactive parent, have not been examined.

In view of the biological relevance of γ-glutamyl peptides and γ-glutamyl transpeptidases[10], the enzymes involved in forming and breaking the γ-glutamyl amide bond, we started a research program aimed at studying -Glu⌐└ψ(SO_2-NH)-Xaa- pseudopeptides. These compounds offer in fact a valuable context to evaluate the potentiality of the SO_2NH replacement by using synthetically accessible and chemically stable analogues of bioactive natural peptides. It is worth noting that, although considerable attention is being devoted to glutathione analogues[11], very few data are available concerning isosteric replacement of peptide bonds[12]. Here we report the synthesis of [Glu⌐└ψ(SO_2-NH)-Cys-Gly]$_2$ **6**, a glutathione disulfide analogue characterized by the presence of the SO_2NH junction replacing the γ-glutamyl-cystine CONH bond.

As suitable intermediate to **6** the *S*-acetamidomethyl (Acm) derivative **3** was selected (Scheme 1). Compound **3** was obtained in 65% yield by acylating *S*-acetamidomethyl-L-cysteinyl-glycine ethyl ester with ethyl (*S*)-2-benzyloxycarbonylamino-4-(chlorosulfonyl)butanoate **2**, obtained by applying the procedure of ref. 13. Selective removal under mild conditions (I_2 in MeOH, room temperature) of the Acm-protecting group allowed direct conversion of **3** to the symmetrical disulfide **4** (80% yield). A critical step was the alkaline hydrolysis of **4**; good yields (90%) of the *N*-protected acid **5** were obtained when **4** (1 mmol) was treated with 1N NaOH



a) Cl_2, CCl_4-EtOH, O°C, 2h; b) Cys(Acm)-GlyOEt, Et_3N, $CHCl_3$, 5°C, 12h; c) I_2, MeOH, RT, 3h; d) 1N NaOH, DMF-H_2O-acetone (6:1:4), RT, 1.5h; e) 4N HBr-AcOH, RT, 2h; f) NH_3, EtOH, RT, 1h

Scheme 1

(6.2 mmol) in DMF-H_2O-acetone (6:1:4) solution (12ml) for 1.5 h at room temperature. *N*-deprotection, followed by treatment of the resulting *bis*-hydrobromide with NH_3 in EtOH, gave the title pseudopeptide **6**[14] (67% yield) as white solid. Studies are in progress in order to define the biochemical and pharmacological properties of **6** with particular reference to the activity on the enzymes controlling the γ-glutamyl cycle[10,15].

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14. Compound **6**: m.p. 185-190 °C (dec.) from EtOH-H_2O. IR (KBr): 3400, 3250, 1740, 1660-1610, 1530, 1230, 1145 cm^{-1}. ^{1}H-NMR (D_2O, 300 MHz): δ 2.35 (4H, m, $CHCH_2CH_2$), 3.0 and 3.25 (4H, AB part of ABX, J_{vic} = 9.0 and 4.6 Hz; J_{gem} = 14.4 Hz, Cys-CβH_2), 3.4 (4H, m, CH_2SO_2), 3.8 (4H, ABq, J=16.0 Hz, Gly-CH_2), 3.85 (2H, m, $CHCH_2CH_2$), 4.35 (2H, X part of ABX, Cys-CαH).^{13}C-NMR (D_2O, 75.43MHz): δ 27.41 ($CHCH_2CH_2$), 42.43 (Cys-Cβ), 45.73 (Gly-Cα), 51.75 (CH_2SO_2), 55.60 ($CHCH_2CH_2$), 58.33 (Cys-Cα), 174.64, 175.70, 178.13 (CO). FAB-MS *m/z*: 685 $[M+1]^+$.
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