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A New Method for the Solid Phase Synthesis of Hydroxyethylamine Peptide Bond Isosteres : Synthesis of an HIV-1 Protease Inhibitor and of a β-Casomorphin-5 Analogue.

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Abstract : A solid phase synthesis procedure for the preparation of hydroxyethylamine peptide isosteres is described. Reductive amination of the resin-bound peptide with an α-hydroxyaldehyde, prepared via 2-(trimethylsilyl)thiazole addition to Boc-phenylalaninal or Boc-prolinal, produces the peptides in good yield.

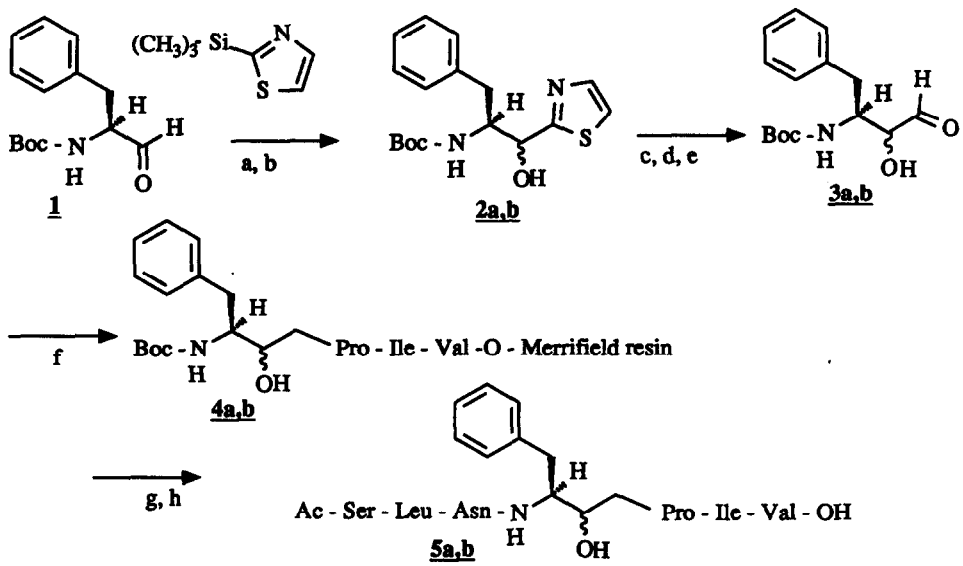
Potent transition state analogue inhibitors for aspartyl or zinc protease are obtained by replacing the scissile peptide bond by the hydroxyethylamine (HEA) isostere. This approach was very succesful for inhibition of renin (1,2), the HIV-1 protease (3,4,5,6) and ACE (7).

Peptides containing this isostere have been prepared by reaction of the P_1 α-amino acid-derived α-aminoepoxide with the P_1' aminoester (4,5,6), followed by incorporation of the pseudodipeptide in the peptide sequence. Alternatively, an alkylation of the C-terminal peptide sequence by the P_1-derived chloromethylketone leads to the ketomethyleneamine isostere, which is subsequently reduced using sodiumborohydride (3,6,7). Whereas the former method requires reaction conditions which are difficult to apply on a peptidyl-resin used in solid phase peptide synthesis, a solid phase protocol using the bromomethylketone alkylation has been described recently (8). The reduction step is however rather unselective and does not allow the preparation of the (R) or (S) aminoalcohols with good selectivity, while also the intermediate ketone is susceptible to racemization (3).

A solid phase procedure which allows for a rapid variation of the peptide sequence as well as for an easy and stereoselective introduction of the HEA isostere is highly desirable for the optimization of protease inhibitors. Since the development of a solid phase procedure for the preparation of "reduced" or $\psi(CH_2\text{-}NH)$ isosteres by Coy (9,10), the application of this type of isostere has increased enormously.

Our approach is based on the work of Dondoni to prepare α-hydroxyaldehydes by addition of 2-(trimethylsilyl)thiazole (2-TST) to α-amino aldehydes (11, 12). The resulting aldehydes are coupled to the resin-bound peptide using the same reductive amination technique as described for the "reduced" isosteres (9,10) (Scheme 1).

SCHEME 1

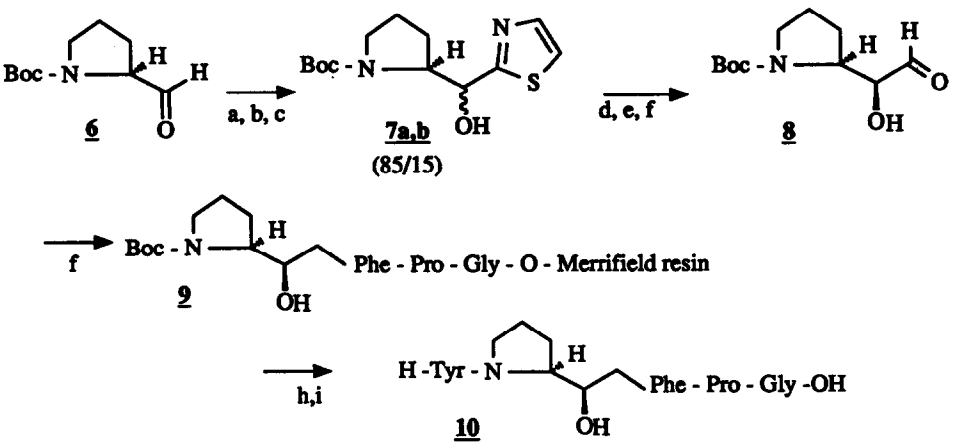


a. 2-TST, CH_2Cl_2, -30°C 17h; b. TBAF; c. CH_3I, CH_3CN reflux, d. $NaBH_4$; e. $HgCl_2$, CH_3CN/H_2O (4/1); f. $NaBH_3CN$, DMF, 1% AcOH, 4h; g. repeated cycles of deprotection/acylation; h. HF/anisole.

The synthesis of the hydroxy-aldehyde 3 derived from Boc-Phe was performed as described by Dondoni (12), and afforded an 8/2 mixture of (S)-alcohol and (R)-alcohol. The mixture was used in the subsequent reductive amination reaction, using sodium cyanoborohydride in DMF containing 1% acetic acid (10,14). After further peptide synthesis and acetylation, the peptide was cleaved from the resin and the epimeric (S) and (R) hydroxyethylamine isosteres 5 were isolated by RP-HPLC (13). A yield of 75% (5a,S) and 5% (5b,R) was obtained. The compounds were characterized by their FAB mass spectra ($M^{+}+H$: 847).

A similar procedure was used to prepare the HEA analogue of β-casomorphin-5 (Scheme 2). We reported that reduction of the Pro^2-Phe^3 peptide bond resulted in μ-selective opioid antagonist (14) and were therefore also interested in the corresponding HEA analogue 10. Addition of 2-TST to Boc-proline aldehyde 6, resulted in a 92% yield of 7a,b (85% /15%) from which the major epimer 7a was isolated by crystallization from hexane (71%) (15). In agreement with the proposed Felkin-Anh non-chelate model for asymmetric induction which operates on α-iminoaldehydes (11, 12), the configuration of the newly formed asymmetric carbon in 7a was assigned as (S), which leads to the (R) HEA configuration in 10. The thiazole was converted to the aldehyde 8a by the usual procedure (Scheme 2) (16). After the solid phase synthesis, and HF cleavage the peptide was purified by HPLC (17). A yield of 67% 10a was obtained. The biological properties of 10 will be reported elsewhere.

SCHEME 2



a. 2-TST, CH_2Cl_2, room temp., 17h; b. TBAF; c. crystallization : 71% 2a, mother liquor (20% 2a, 80% 2b); d. CH_3I, CH_3CN, reflux 12h; e. $NaBH_4$, MeOH; f. $HgCl_2$, CH_3CN/H_2O (4/1); g. $NaBH_3CN$, DMF, 1% AcOH; h. BocTyr, DCC, HOBt, then HF/anisole; i. HPLC : 10a 67%.

The procedure described here allows for the rapid synthesis of HEA using the solid phase synthesis. The peptides containing the epimeric alcohols can be easily separated during the final HPLC purification. Alternatively, stereochemically pure 2-TST adducts can be prepared (11,12), to obtain only the desired configuration of the analogues.

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13. Peptide synthesis was performed using DCC/HOBt activation. Semi-preparative HPLC was performed on a Vydac 218TP1022 (2.5x25 cm, 10µ) (C18 column, using a gradient of a 0.1% trifluoroacetic acid aqueous buffer containing 15% acetonitrile to 30% acetonitrile in 20 min, flow 20 ml/min). Retention times were 17 min (5a) and 18.5 min (5b).

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15. The crude reaction mixture was purified by chromatography through a short silica gel column, using petroluem ether/ethyl acetate (8/2) as eluent, followed by crystallization from hexane to give 71% of 7a.
7a : m.p. 105-109°C; R_f(SiO_2, petroleum ether/ethyl acetate 6/4) : 0.34, MS(FAB) : 285(M+-+1); NMR(250 MHz, $CDCl_3$) δ 1.45(s, 9H, Boc), 2.1(m, 2H), 2.3(m, 2H), 2.8(m, 1H, ProC$^{\alpha}$H), 3.35(t, 2H, ProC$^{\delta}$H), 4.35(t, 1H, CH-O), 5.15(d, 1H, OH), 7.25(d, 1H), 8.7(d, 1H).
Mixture 7a,b : TLC : same R_f as 7a, NMR(250 MHz, $CDCl_3$) : separated signals at δ 4.1(t, 1H, CH-O) and 4.9(1H, d, OH) which were used for quantification of the diastereomer ratio.

16. 8a : m.p. 63-65°C, R_f(SiO_2, petroleum ether/ethyl acetate 6/4) 0.34, MS(FAB) : 230(M+-+1), NMR(250 MHz, $CDCl_3$) δ 1.45(s, 9H, Boc), 1.6(m, 2H), 1.9(m, 2H), 3.35(t, 2H, ProC$^{\delta}$H), 3.95(m, 1H, Pro C$^{\alpha}$H), 5.2(m, 1H, CH-O), 5.4(s, 1H, OH), 9.6(s, 1H, CH=O).
Mixture 8a,b : TLC : same R_f as 8a, NMR(250 MHz, $CDCl_3$) : separated signals at δ 4.4(t, 1H, CH-O) and 5.2(s, 1H, OH) which were used for quantification of the diastereomer ratio.

17. Semi preparative HPLC was performed as for 5a,b, using a gradient of 10% to 27% acetonitrile in 20 min.
Retention time was 10 min (10) : MS(FAB) : 596 (M+-+1). Analysis of the 2D NMR spectra will be reported elsewhere.

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