Pergamon

Tetrahedron Letters 41 (2000) 7239–7243

TETRAHEDRON
LETTERS

Total synthesis of a Pepstatin analogue incorporating two trifluoromethyl hydroxymethylene isosteres

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Received 31 May 2000; revised 11 July 2000; accepted 20 July 2000

Abstract

The total synthesis of a trifluoromethyl (Tfm) analogue of the aspartate protease inhibitor Pepstatin has been accomplished via incorporation of two α-Tfm-amino β-hydroxy peptide isosteres instead of the natural statine units. The title compound as well as several Tfm-substituted precursors did not show anti-HIV activity. © 2000 Elsevier Science Ltd. All rights reserved.

Keywords: peptide mimetics; enzyme inhibitors; Pepstatin; trifluoromethyl group.

Pepstatin[1] (Fig. 1) is a natural inhibitor of peptidic aspartate proteases, including pepsin,[1,2] renin,[3] HIV-1,[1b,4] and HIV-2 proteases.[1b,5] The central statine unit is believed to mimic the tetrahedral intermediate of peptide hydrolysis, acting as an isostere for a restricted conformation of a dipeptide unit,[2a] and its stereochemistry has a large effect on protease inhibition, a *syn* diastereomeric relationship between the amine and hydroxyl groups being required.[2a,6a] However, Pepstatin is rated only as 'moderately active' by NCI in a cell-based AIDS anti-viral screen.[6b] Much effort has been directed toward the synthesis of Pepstatin mimetics in order to discover analogues having improved properties.[1b]

The list includes a few fluorinated derivatives containing difluorostatine and difluorostatone units, which have been reported to be potent inhibitors of Penicillopepsin.[7] Replacement of the statine isobutyl residue in the P1 position with other groups has often led to analogues with greatly improved features.[8] However, its replacement with a fluoroalkyl residue has never been reported, despite the fact that fluoroalkyl groups are known to deeply modify physical–chemical properties such as lipophilicity, acidity/basicity, nucleophilicity and preferred conformation, and

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PII: S0040-4039(00)01244-2

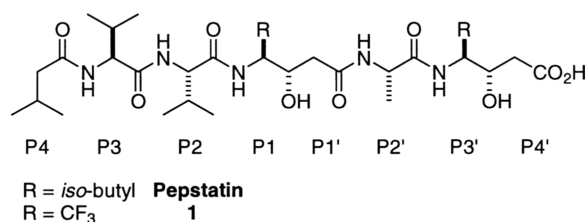
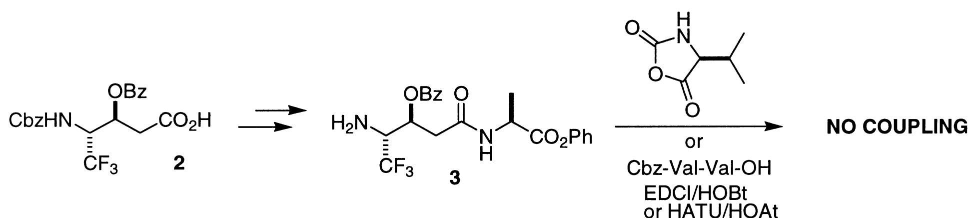


Figure 1.

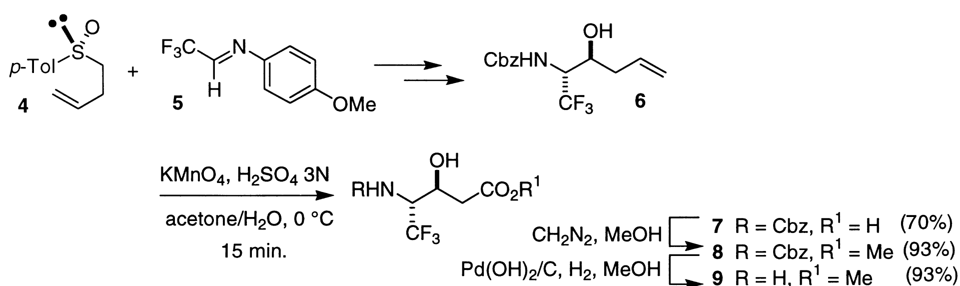
that useful spectroscopic data on the binding process might be obtained by ^{19}F NMR. This could be due to the following reasons: (1) the requisite stereodefined β-fluoroalkyl β-amino alcohol units[9] have been hitherto synthetically unavailable; and (2) incorporation of α-fluoroalkyl amino moieties into peptidic sequences via amide bond formation is a challenging endeavour, due to the low nucleophilicity of the NH_2 function.

This is particularly true for α-trifluoromethyl (Tfm) amino derivatives,[10a] since the Tfm group is strongly electron-withdrawing and 'sterically at least as large as $CH(CH_3)_2$'.[10b] Recently, we reported a stereocontrolled route to orthogonally protected *syn*-(3*S*,4*R*)-γ-(Tfm)GABOB **2** (Scheme 1), a new hydroxymethylene (statine) dipeptide isostere.[11] In this paper we describe the total solution-phase synthesis of the Pepstatin analogue **1** (Fig. 1), having two (Tfm)GABOB units in place of the natural *syn*-(3*S*,4*S*)-statines in the P1 and P3′ positions, as part of a project aiming at the investigation of the effect exerted by fluorine atoms belonging to fluoroalkyl groups on the binding process to aspartyl proteases.



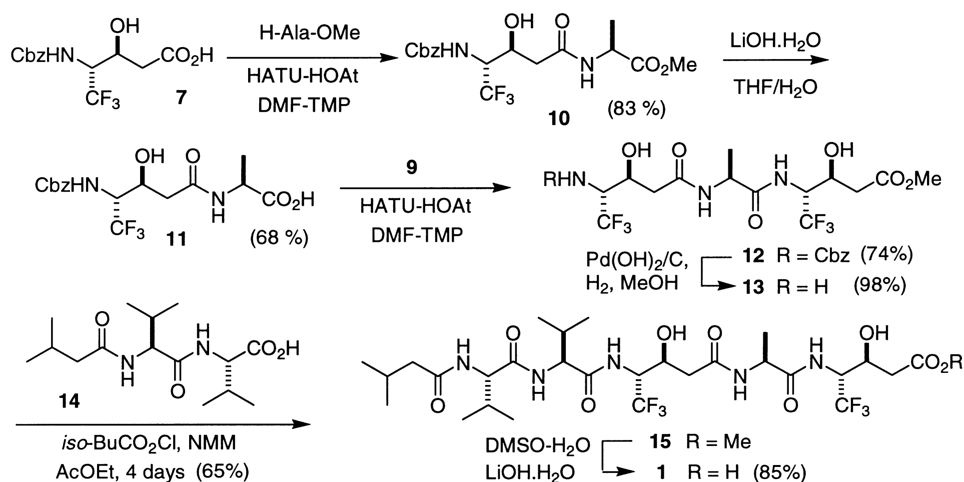
Scheme 1.

In our early attempts, we tried to build the peptide **1** starting from the central γ-(Tfm)GABOB (Scheme 1). Unfortunately, the dipeptide **3**, prepared by standard solution methods, failed to undergo coupling with Cbz-Val-Val-OH under a variey of conditions (for example EDCI/HOBt or HATU/HOAt[12] in DMF–TMP at 0°C) and also with Val derived Leuchs anhydride,[13] thus evidencing the expected poor reactivity of the H-(Tfm)GABOB fragment. However, a partial migration of the *O*-Bz protection to the amino group was occasionally observed under coupling conditions. This suggested to us that an unprotected β-OH group might favor the coupling.[14] Therefore, we decided to re-undertake the synthesis of **1** starting from *O*-unprotected statine isosteres **7** and **9** (Scheme 2). The former was prepared from the olefin **6**, accessible via a C–C bond forming reaction of the α-lithiated 3-butenyl-*p*-tolylsulfoxide **4** with the *N*-*p* methoxyphenylimine **5**, followed by stereoselective S_N2-type substitution of the sulfinyl with an hydroxy group.[11] Oxidative cleavage of **6** with $KMnO_4$, which occurred with excellent chemoselectivity, then treatment of **7** with diazomethane, followed by hydrogenolysis of the Cbz group of the resulting ester **8**, provided **9**.



Scheme 2.

Assembling of the tripeptide fragment H-(Tfm)GABOB-Ala-(Tfm)GABOB with Iva-Val-Val-OH was envisaged as a viable strategy to accomplish the synthesis of **1**, therefore the synthesis of the fluorinated tripeptide **13** was undertaken first (Scheme 3). Coupling of **7** with H-Ala-OMe (HATU/HOAt, DMF–TMP) afforded **10**, which was hydrolyzed with LiOH to the corresponding acid **11**. Satisfactorily, the key assembling of **11** with **9** afforded good yields of **12**,[15] which was hydrogenolyzed to the H_2N-tripeptide **13**.



Scheme 3.

The final assembling of **13** with Iva-Val-Val-OH (**14**), prepared by standard solution-phase technique, proved to be quite challenging (Scheme 3). In fact, under a variety of conditions (for example HATU/HOAt both in DMF and AcOEt, or *iso*-$BuCO_2Cl$/NMM in DMF) epimerization of the second Val unit took place, affording the target **15** as a mixture of epimers. This trouble was solved by using the exact conditions reported by Bartlett for the synthesis of a phosphorus-containing analogue of Pepstatin (*iso*-$BuCO_2Cl$/NMM in AcOEt)[16] which provided the stereochemically pure pentapeptide **15**, which was hydrolyzed with LiOH in excellent yield to the final target Pepstatin analogue **1**.[17]

The target **1** as well as precursors **7**–**13** were evaluated for their capacity to inhibit HIV-1 multiplication. The virus production was measured, in the presence or absence of test compounds, by quantification of the reverse transcriptase activity associated with virus particles

released from HIV-1 Lai-infected CEM-SS in culture medium or the cytotoxicity induced by HIV-1 IIIB replication in MT-4 cells. The cytotoxicity of the compounds was evaluated in parallel on uninfected cells.[18] At the highest concentration tested, 10 μg/ml in the case of **1**, there was no measurable antiviral or cytotoxic activity. The precursors **7–13** were used at 100 μg/ml or less but no activity was detected.

In summary, the challenging incorporation of an α-Tfm-amino β-hydroxy peptide isostere into a complex peptidic sequence has been successfully accomplished, producing the Tfm-Pepstatin analogue **1** on a hundredth of a milligram scale and very good overall yield. The solution and solid-phase synthesis of further fluorinated analogues of Pepstatin, as well as the elucidation of the effect of fluoroalkyl groups on aspartyl proteases inhibition are currently under investigation.[19]

Acknowledgements

MURST COFIN 1998 (Nuove metodologie e strategie di sintesi di composti di interesse biologica) and CNR–CSSON are gratefully acknowledged for financial support. C.P. thanks Politecnico di Milano for a biennial scholarship.

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14. To this end, *O*-debenzoylation of the dipeptide **5** with MeOH, K_2CO_3 was attempted, but the desired H-(Tfm)(OH)GABOB-Ala-OPh was not achieved.

15. To a solution of **11** (600 mg, 1.53 mMol) and **9** (308 mg, 1.53 mMol) in DMF (13 mL, stored overnight over 3 Å molecular sieves), TMP (371 mg, 0.406 mL, 3.06 mMol) was added and the resulting mixture was cooled with an ice-water bath. HATU (582 mg, 1.53 mMol) and HOAt (208 mg, 1.53 mMol) were added and the mixture was stirred for 25 min. A 1 M solution of HCl was added until pH 1–2 was reached, and the reaction mixture was extracted with ethyl acetate (3×10 mL). The collected organic layers were washed with a 5% aqueous $NaHCO_3$ solution, and then with brine. After drying over anhydrous Na_2SO_4, and filtration, the solvent was removed in vacuo, and the residue was purified by flash chromatography (*n*-hexane:AcOEt, 1:1) to give **12** in 74% yield after crystallization from *n*-hexane:AcOEt, 1:1: m.p. 205–206°C; $[\alpha]_D^{20}$ –52.04 (*c* 1.0, MeOH); ^1H NMR (acetone-d_6) δ 7.65 (d, J=6.6 Hz, 1H), 7.56 (d, J=9.9 Hz, 1H), 7.50–7.25 (m, 5H), 6.72 (d, J=9.9 Hz, 1H), 5.16 (br d, J=12.5 Hz, 1H), 5.13 (br d, J=12.5 Hz, 1H), 4.86 (d, J=4.9 Hz, 1H), 4.76 (d, J=5.8 Hz, 1H), 4.70 (ddq, J=9.9, 1.6 and 8.2 Hz, 1H), 4.57 (m, 1H), 4.50 (dq, J=6.6 and 7.2 Hz, 1H), 4.48 (m, 1H), 4.45 (ddq, J=9.9, 1.6 and 8.2 Hz, 1H), 3.62 (s, 3H), 2.51 (d, J=6.7 Hz, 2H), 2.49 (d, J=6.6 Hz, 2H), 1.33 (d, J=7.2 Hz, 3H); ^{19}F NMR (acetone-d_6) δ –71.72 (br d, J=8.2 Hz), –71.41 (br d, J=8.2 Hz); ^{13}C NMR (acetone-d_6) δ 173.9, 171.8, 171.7, 157.5, 137.7, 129.3, 128.8, 128.6, 126.1 (q, J=282.3 Hz), 126.0 (q, J=282.6 Hz), 67.6, 66.0, 65.3, 56.6 (q, J=28.7 Hz), 53.8 (q, J=28.7 Hz), 51.8, 50.3, 40.1, 39.1, 18.1. MS (DIS EI, 70 eV) *m*/*z* (%): 576 [$(M+H)^+$, 7], 575 [$M^{+\cdot}$, 9], 557 [$(M-H_2O)^{+\cdot}$, 11].
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17. (a) Coupling between **13** and **14**. A suspension of **14** (76 mg) in 3.6 ml of dry AcOEt was treated with 1.2 equiv. of NMM, cooled to –10°C, then 1.2 equiv. of *iso*-BuO_2CCl were added under stirring. After 5 min a suspension of **13** (1 equiv.) in 2.5 ml of AcOEt was added. The mixture was stirred 4 days at rt, then centrifuged and the solid was washed several times with AcOEt, MeOH and finally *n*-hexane to provide 100 mg of **15** (65%) as an amorphous solid. (b) Selected data for **1**: m.p. 265–270°C (dec.); $[\alpha]_D^{20}$ –32.16 (c 0.36, DMSO); ^1H NMR (DMSO-d_6) δ 8.08 (d, J=7.0 Hz, 1H), 8.06 (d, J=9.5 Hz, 1H), 8.00 (d, J=9.5 Hz, 1H), 7.90 (d, J=9.0 Hz, 1H), 7.79 (d, J=9.0 Hz, 1H), 5.35 (br signal, 2H), 4.65–4.50 (m, 2H), 4.44 (dq, J=7.0 and 7.1 Hz, 1H), 4.31 (dd, J=9.0 and 7.2 Hz, 1H), 4.35–4.25 (m, 2H), 4.20 (dd, J=9.0 and 7.1 Hz, 1H), 2.31 and 2.28 (m, 2H), 2.25 (dd, J=14.9 and 8.5 Hz, 1H), 2.19 (dd, J=14.9 and 4.4 Hz, 1H), 2.10–1.85 (m, 5H), 1.24 (d, J=7.1 Hz, 3H), 0.90–0.80 (m, 18H); ^{19}F NMR (DMSO-d_6) δ –71.20 (br d, J=8.3 Hz), –71.08 (br d, J=8.2 Hz); ^{13}C NMR (DMSO-d_6) δ 173.3, 171.8, 171.62, 171.59, 171.3, 169.5, 124.9 (q, J=284.3 Hz, 2C), 64.2, 63.8, 57.8, 57.7, 52.9 (q, J=26.9 Hz), 52.4 (q, J=27.7 Hz), 48.2, 44.4, 39.5, 38.5, 30.1, 29.9, 25.6, 22.2 (2C), 19.19, 19.17, 18.23, 18.18, 17.7. MS (DIS EI, 70 eV) *m*/*z* (%): 710 [$(M+H)^+$, 4], 692 [$(M+H-H_2O)^+$, 6]. (c) Epimerization of the second Val under different coupling conditions was assessed by ^1H and ^{19}F NMR, as witnessed by the fact that splitting of most of the signals of **15** was detected. For example, the undesired epimer showed the following ^{19}F NMR (DMSO-d_6) δ –71.15 (br d, J=8.3 Hz) and –70.95 (br d, J=8.3 Hz).
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19. Abbreviations: GABOB, γ-amino-β-hydroxybutyric acid; EDCI, *N*-Ethyl-*N*′-(3-dimethylaminopropyl)carbodiimide hydrochloride; HOBt, 1-Hydroxybenzotriazole; HATU, *N*,*N*,*N*′,*N*′-tetramethyl-*O*-(7-azabenzotriazol-1-yl)-uronium hexafluorophosphate; HOAt, 1-hydroxy-7-azabenzotriazole; TMP, 2,4,6-trimethylpyridine (*sym*-collidine); NMM, *N*-methylmorpholine.