Tetrahedron Letters, Vol.32, No.51, pp 7617-7620, 1991
Printed in Great Britain

0040-4039/91 $3.00 + .00
Pergamon Press plc

Formation of Peptide Thioamides by Use of Fmoc Amino Monothioacids and PyBOP.[1]

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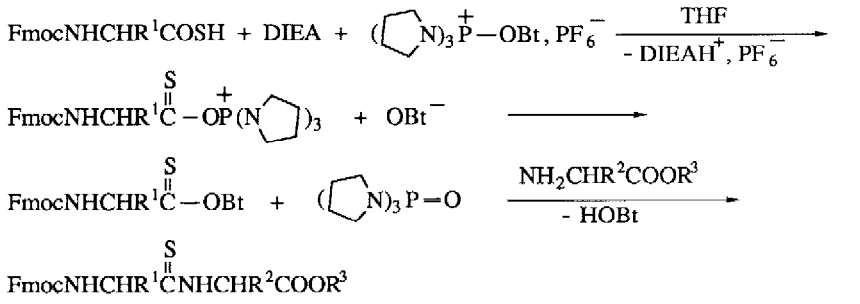
Abstract: Endothiopeptides have been obtained by using PyBOP® promoted coupling between Fmoc-protected amino monothioacids and amino acid or peptide esters. The protected endothiopeptides (Fmoc-Gly-ψ(CSNH)-Phe-OEt, Fmoc-Tyr(Bu^t)-ψ(CSNH)-Gly-Gly-Phe-Leu-OBu^t and Fmoc-Gln(Trt)-ψ(CSNH)-Phe-OEt) were formed (55, 45 and 37% yield) in admixture with the corresponding oxopeptides, from which they were easily separated chromatographically. Preliminary racemisation studies indicate that products of high optical purity are obtained. The mechanism for endothiopeptide formation is briefly discussed.

Peptide backbone modification by replacement of an amide bond by a thioamide bond (endothiopeptides) has attracted attention in recent years for several reasons. Receptor interactions of endothio-analogues of biologically active peptides may be more selective and/or potent than their parent compounds,[2] and enhanced stability against enzymatic hydrolyses can be expected.[3] Conformational studies of simple endothiopeptides have been undertaken by means of X-ray, IR, CD and NMR. The studies indicate that thioamides are reasonably good mimics of amides, although biological studies have shown that the behavior is unpredictable.[4] Endothiopeptides have been shown to be convenient starting points for the preparation of other pseudopeptides, for example desoxopeptides by reduction with Raney nickel or nickel boride.[5]

The most widely used reagents for the conversion of carbonyl compounds into thiocarbonyl analogues are 1,3-dithiadiphosphetane-2,4-disulfides. Lawesson and coworkers demonstrated the utility of 2,4-bis(4-methoxyphenyl)-1,3-dithiadiphosphetane-2,4-disulfide, which is now commonly referred to as Lawessons Reagent.[6] Thiopeptide linkages can be formed with this or modified reagents[7,8] from preformed protected peptides in a regioselective manner and in high yields. The regioselectivity is among other dictated by the steric environment of the individual amide function and thus certain thioamide bonds (Gly-ψ(CSNH)-Gly and others) may be formed while more sterically hindered bonds are not affected. Therefore, to prepare desired regioisomers of monothionated peptides, methods such as segment condensation between peptides and thionated peptides[9] or stepwise elongation from the N-terminal has been used in a number of cases. Attempts to elongate thionated dipeptides from their C-terminal resulted in low yields and serious racemisation, because of intermediary formation of a thioazlactone.[3,8] Acylation with thiono or dithio esters is an obvious method for

the preparation of thionated peptides,[10] but in practice the method suffers from lack of efficiently acylating thio derivatives.[3,9,11]

In the search for a specific method for potential use for solid phase peptide synthesis of endothiopeptides, we have considered using N-terminal protected amino monothioacids and, as coupling agent, BOP or PyBOP. This idea is based on the fact that phosphorus forms a stronger bond to oxygen than to sulfur (P-O, 142.6 kcal/mole; P-S, 106 kcal/mole[12]). The coupling reaction could be expected to proceed as depicted in Scheme 1. In this paper we wish to present our preliminary results with this new procedure, used in solution synthesis.



Scheme 1.

Fmoc and Boc are the most commonly used N-terminal protecting groups, but, although endothiopeptides are reasonably stable towards acids, repeated treatments with TFA may be troublesome.[13] We have therefore used Fmoc protected amino acids for the present experiments.[14] Fmoc amino monothioacids have, to the best of our knowledge, not been described hitherto, but we have found that they may be prepared in the same way as Boc- and Z-amino monothioacids, from the corresponding O-succinimide esters.[15,16] Thus Fmoc-Gly-SH (mp 109-10 °C; R_f 0.34 (0.19); yield 75%), Fmoc-Val-SH (mp 76-77 °C; R_f 0.54 (0.49); 56%); Fmoc-Tyr(Bu^t)-SH (mp 90-92 °C; R_f 0.58 (0.53); 49%) and Fmoc-Gln(Trt)-SH (mp 114-20 °C; R_f 0.51 (0.35); 94%) were obtained. R_f values refer to TLC analysis (CH_2Cl_2/AcOEt/AcOH, 90/10/5; silica gel sheets). R_f values for the corresponding oxygen acids are given in parenthesis. The thioacids obtained were purified by crystallization of their potassium salts (AcOEt) or by chromatography. They were found to be up to 97% pure, and their identity was confirmed by NMR and MS.[17] To avoid oxidation, they were stored as their potassium salts.[16]

The endothiopeptide Fmoc-Gly-ψ(CSNH)-Phe-OEt was prepared as follows: Fmoc-Gly-SH (0.25 mmol), H-Phe-OEt, HCl (0.25 mmol) and PyBOP (0.25 mmol) were dissolved in dried, N_2-purged THF (5ml). Use of other solvents, including DMF, resulted in lower yields of the endothiopeptide. DIEA (0.50 mmol) was added and the mixture left for 5 hr at room temperature. The solution was then evaporated to dryness *in vacuo* and the remaining material dissolved in AcOEt (5 ml). The solution was washed with 5% citric acid (2x3 ml), 5 % $NaHCO_3$ (2x3 ml) and H_2O (2x3 ml), dried over sodium sulfate and evaporated to dryness. TLC and HPLC analysis (comparing with reference material[18]) showed the crude product to consist of a mixture of the target peptide and the corresponding oxopeptide. For preparative purposes, the mixture was dissolved in a small

amount of CH_2Cl_2 and submitted to flash chromatography on silica gel (eluent: CH_2Cl_2/AcOEt, 9:1) to give Fmoc-Gly-ψ(CSNH)-Phe-OEt (55%, R_f 0.55) and Fmoc-Gly-Phe-OEt (39%, R_f 0.08) in a total yield of 94%. NMR and MS of the peptides were in agreement with the assignments.

As a further evaluation of the PyBOP promoted endothiopeptide formation, the protected pentapeptide Fmoc-Tyr(Bu^t)-ψ(CSNH)-Gly-Gly-Phe-Leu-OBu^t was synthesized. Similar Leu^5-enkephalin analogues have previously been prepared by condensation of Tyr methyl dithioesters with H-Gly-Gly-Phe-Leu-OR,[19,20] but in one case a 1:1 mixture of the L- and D-Tyr diastereomers was obtained (HPLC analysis).[20] In the present case H-Gly-Gly-Phe-Leu-OBu^t (0.1 mmol), prepared by *Fmoc amino acid solution technique* (FAAST),[21] was dissolved in dried, N_2-purged THF (2 ml). Fmoc-Tyr(Bu^t)-SH (0.1 mmol), PyBOP (0.1 mmol) and DIEA (0.1 mmol) were added, the mixture left for 6 hr at room temperature and worked up as described above. TLC ($CHCl_3$/Bu^tOH, 6:1) demonstrated the formation of Fmoc-Tyr(Bu^t)-Gly-Gly-Phe-Leu-OBu^t (comparing with reference material, R_f 0.35)[21] and Fmoc-Tyr(Bu^t)-ψ(CSNH)-Gly-Gly-Phe-Leu-OBu^t (R_f 0.46). The endothiopeptide was isolated by flash chromatography on silica gel ($CHCl_3$/Bu^tOH, 6:1) to give a yield of 45% and was characterized by NMR and MS. The yield of the oxopeptide was 44 %. The endothiopeptide showed only one major peak (>95%) in three different HPLC systems, indicating high optical purity.

In order to further investigate the stereospecificity of the endothiopeptide forming reaction, the L- and the D-enantiomers of Fmoc-Gln(Trt)-SH were prepared and coupled to H-Phe-OEt (0.25 mmol scale). The resulting crude products were submitted to HPLC analysis (55% EtOH, isocratic on C-18 column), and examined for possible racemisation. Analysis of the chromatograms revealed that the expected diastereomer was formed from Fmoc-L-Gln(Trt)-SH in better than 98 % purity and from Fmoc-D-Gln(Trt)-SH in better than 95%, demonstrating low racemisation under the prevailing conditions (in a first experiment, yields of endothiopeptides were 15-20%, but by adding 2-mercaptopyridine (1 eq.) to the reaction mixture, yields were raised to 37-38 %. Racemisation was similar in the two sets of experiments). Preparatively, Fmoc-L-Gln(Trt)-ψ(CSNH)-Phe-OEt (37%, R_f 0.53) and Fmoc-D-Gln(Trt)-ψ(CSNH)-Phe-OEt (38%, R_f 0.45) were separated from their respective oxo-analogues (R_f 0.14 and 0.13) by flash chromatography (CH_2Cl_2/AcOEt 9:1), and characterized by NMR and MS.

We have monitored the PyBOP promoted formation of Fmoc-Gly-ψ(CSNH)-Phe-OEt in both THF and DMF (25 mM) by means of ^{31}P-NMR, in a preliminary attempt to explain why the formation of the desired endothiopeptide is accompanied by formation of the corresponding oxopeptide. It turned out, that the reaction between the thioacid and PyBOP is a relatively slow reaction. Complete disappearance of the ^{31}P-signal from PyBOP took 4-5 hr in both solvents (even when an excess of DIEA (1/2 eq.) was employed). During the period a ^{31}P-signal from tris(pyrrolidino)phosphine oxide developed. When the reaction was carried out in DMF, a signal from tris(pyrrolidino)phosphine sulfide was also observed, integrating to approximately 10% of that of the oxo-analogue. The signal from the phosphine sulfide was not observed in THF. The slower activation of thioacids in comparison to oxoacids[22] is not altogether unexpected. Thus thioacids are stronger acids than the corresponding oxoacids,[16] and it is known that TFA is not activated by PyBOP/BOP.[23] In consideration of these observations, it is obvious that an oxoacid, in a mixture of the oxo- and the thioacid, is more rapidly ac-

tivated and consumed in a fast coupling step, before a significant amount of the activated thionoester is formed. Thus oxoacids (as impurities in the starting material or as a result of unintended hydrolysis / oxidation during coupling) will compete seriously with endothiopeptide formation, explaining, at least in part,[24] the co-formation of oxopeptide. This, of course, also leads to the conclusion that use of an excess of thioacid is problematic with this procedure.

References and notes:

1. Unusual abbreviations used: PyBOP®, benzotriazolyloxy-tris(pyrrolidino)-phosphonium hexafluorophosphate. Fmoc, 9-fluorenylmethoxycarbonyl. BOP, benzotriazolyl-tris(dimethylamino)-phosphonium hexafluorophosphate. HOBt, 1-hydroxybenzotriazole. DIEA, diisopropylethylamine. THF, tetrahydrofuran. Boc, tert-butyloxycarbonyl. Bu^t, tert-butyl. Z, benzyloxycarbonyl. DMF, dimethylformamide. TFA, trifluoroacetic acid. DCC, dicyclohexylcarbodiimide.
The use of ψ(CSNH) designates the presence of the thioamide function.
PyBOP® and amino acid derivatives were purchased from Novabiochem, Switzerland.
Unless otherwise indicated, all described amino acids are L-forms.
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(Received in UK 1 October 1991)