Tetrahedron Letters,Vol.25,No.36,pp 3971-3974,1984 0040-4039/84 $3.00 + .00
Printed in Great Britain

2-(2-PYRIDYL-)ETHYL ESTERS
A NEW CARBOXYL PROTECTING GROUP IN PEPTIDE SYNTHESIS

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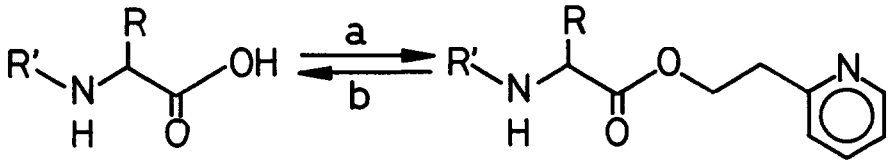
Abstract: 2-(2-pyridyl-)ethyl esters are used as chemically inert, highly selective protecting groups that are removable under mild conditions via a two step procedure.

In spite of the large number of existing protecting groups there still remains a great interest in variants, which are chemically inert over many steps in synthesis but selectively removable under mild conditions. During our work on cyclic peptides we encountered the problem of finding carboxyl protecting groups[1] which are orthogonal to other N-terminal, C-terminal, and side chain protecting groups with regard to stability and cleavage.

An elegant solution of the apparent contradiction between stability during peptide synthesis and deprotection under mild conditions was the N-terminal protecting group Pyoc[2]. We recommend here the same principle to be applied as a carboxyl protecting group. During the progress of our work we became aware that Prof. Kunz at the University of Mainz has independently done similar work with slightly different intentions and procedures[3]. The 2-(2-pyridyl-)ethyl ester (= Pet-ester) is easily introduced into an N-terminally protected amino

acid or peptide via DCC/HOBt[4] activated esterification with PetOH. It is stable under proteolytic conditions (deprotection of Boc or t-butyl esters), hydrogenolysis (deprotection of Z or benzyl esters), and on treatment with amines (deprotection of Fmoc or Fm-esters). Mild deprotection is achieved after alkylation by CH_3I with mild bases. As pointed out for the Pyoc-group[2], the use of Pet-esters is not recommended when the peptide contains methionine or histidine.

scheme I



a) introduction: 1.1 eq DCC; 0.25 eq HOBt; 3 eq PetOH; 0°C- RT, over night; CH_2Cl_2 or DMF

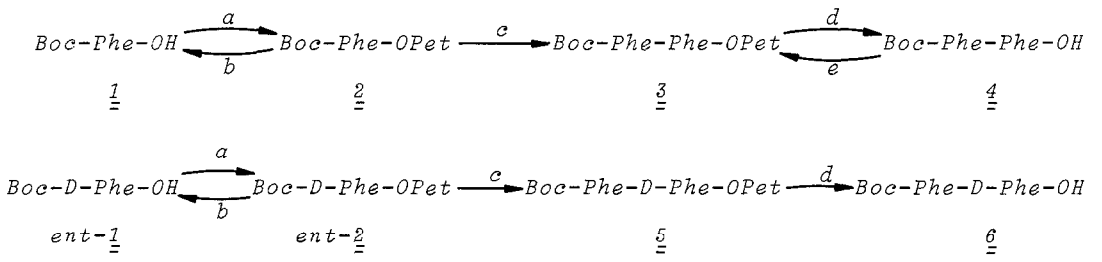
b) cleavage: 1.) 3 eq CH_3I; RT, 12h; acetonitrile

2.) remove excess CH_3I i.v., add 7-8 eq $HN(C_2H_5)_2$; RT, 12h; CH_2Cl_2 or acetonitrile

Advantages of the Pet-esters are also the higher solubility in protic solvents (H_2O) and the high UV absorption, which facilitates their chromatographic detection. To study racemization during formation and deprotection we synthesized *Boc-Phe-Phe-OPet* 3 and *Boc-Phe-D-Phe-OPet* 5 via different routes as shown in scheme II. We choose these peptides because of the known tendency of phenylalanine to racemization and the easy differentiation of the diastereomeric protected and deprotected Phe-Phe-peptides in their NMR spectra.

Racemization in peptide synthesis is often followed via the measurement of the optical activity of the crystallized and thus purified chiral product. The crystallization step itself however tends to separate the epimeric compounds, thus giving too low values of racemization. On the other hand, measurements of the crude oil that not only contains both epimers but also significant

scheme II



a) 83 %, b) 92 %, d) 90 %, e) 81 %: reaction conditions see scheme I.
c) 74 %, 1.) 10 % TFA in CH_2Cl_2; RT/ 2h; remove excess TFA i.v.
2.) 1.1 eq Boc-Phe-OH,DCHA; 1.1 eq DCC; 0.25 eq HOBt; 0°C-RT, over night.

amounts of impurities, result in too high values of racemization.

These shortcomings can be avoided by synthesizing dipeptides such as 3 and 4 as well as their diastereomers containing D-Phe in the C-terminal position (5 and 6). The differences of the NMR spectra of these epimeric compounds can be used for their identification in a crude oil, isolated without purification steps.

Crude *Boc-Phe-OH* 1 recovered from 1 —a)→ 2 —b)→ 1 as oil shows lowered optical activity corresponding to 2.9 % racemization and full optical activity after crystallization. *Boc-Phe-Phe-OH* 4 recovered as an oil from 4 —e)→ 3 —d)→ 4 again shows 3.1 % racemization when optical activity is used as criterion. NMR spectroscopy shows that the concentration of the epimeric *Boc-Phe-D-Phe-OH* 6 is lower than NMR detectibility (< 1 %).

Boc-Phe-Phe-OPet 3 prepared along route 1 —a)→ 2 —b)→ 3 and *Boc-Phe-D-Phe-O-Pet* 5 via ent-1 —a)→ ent-2 —c)→ 5 again show *no detectable traces of racemization in their NMR spectra.*

Comparison with various other Pet-esters of mono- and bifunctional amino acids and dipeptides, which we synthesized under analogous conditions, shows the above reactions to be fairly typical. A comprehensive report of this work is in preparation.

These results demonstrate the Pet-group to be a stable protecting group selectively removable under mild conditions via an E1cB mechanism[2]. Its application to solid phase peptide synthesis is currently under investigation.

Acknowledgements

We thank the Deutsche Forschungsgemeinschaft and the Fonds der Chemischen Industrie for financial support. Gifts of the amino acids were donated by Degussa, Frankfurt. H. Kogler thanks the Studienstiftung des Deutschen Volkes for a grant.

Significant physico-chemical data, which were used for the differentiation of the epimeres are the following:

Boc-Phe-OH (1): $(\alpha)_D^{20}$ = +9.5 (c = 1.2, CH_3OH); $(\alpha)_D^{20}$ = 24.9 (c = 1, C_2H_5OH).

Boc-Phe-OPet (2): $(\alpha)_D^{20}$ = -4.4 (c = 1, CH_3OH); ^1H NMR (D_6-DMSO δ*(ppm)*: 2.99 (t, 2H, $C\underline{H}_2$ Pet); 4.39 (m, 2H, $C\underline{H}_2$ Pet); 7.23 (m, 8H, two of those arom. H Pet); 7.7 (dt, 1H, arom. H Pet); 8.48 (d, 1H, arom. H Pet).

Boc-Phe-Phe-OPet (3): $(\alpha)_D^{20}$ = -10.3 (c = 1.1, CH_3OH); ^1H NMR (DMSO) δ*(ppm)*: 6.86 (d, 1H, N$\underline{H}$ urethane); 8.3 (d, 1H, N$\underline{H}$ Phe).

Boc-Phe-Phe-OH (4): $(\alpha)_D^{20}$ = -1.6 (c = 1.5, CH_3OH); ^1H NMR (DMSO) δ*(ppm)*: 6.87 (d, 1H, N$\underline{H}$ urethane); 8.11 (d, 1H, N$\underline{H}$ Phe).

Boc-Phe-D-Phe-OPet (5): $(\alpha)_D^{20}$ = +3.7 (c = 1.6, CH_3OH); ^1H NMR (DMSO) δ*(ppm)*: 7.07 (d, 1H, N$\underline{H}$ urethane); 9.82 (d, 1H, N$\underline{H}$ Phe).

Boc-Phe-D-Phe-OH (6): $(\alpha)_D^{20}$ = +2.2 (c = 0.6, CH_3OH); ^1H NMR (DMSO) δ*(ppm)*: 6.72 (d, 1H, N$\underline{H}$ urethane); 8.28 (d, 1H, N$\underline{H}$ Phe).

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(Received in Germany 25 May 1984)