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SYNTHESIS OF Leu-Abu(P) AND Glu-Abu(P)-Leu. ISOSTERES OF Ser(P)-PEPTIDES

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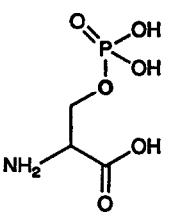
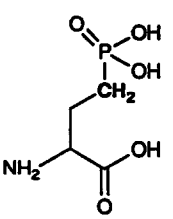
Abstract: Boc-Asp-O^{t}Bu was efficiently converted to Boc-Abu(PO$_3$Me$_2$)-O^{t}Bu in five steps and then to Boc-Abu(PO$_3$Me$_2$)-OH in two steps. Both the protected amino acids were used in the syntheses of Boc-Leu-Abu(PO$_3$Me$_2$)-O^{t}Bu and Boc-Glu(OBzl)-Abu(PO$_3$Me$_2$)-Leu-OBzl by the Boc mode of peptide synthesis. These were deprotected to Leu-Abu(P) and Glu-Abu(P)-Leu by hydrogenolysis followed by bromotrimethylsilane treatment.

Since 1981 we have been involved in the development of chemical methods for the synthesis of peptides containing O-phosphoserine {Ser(P)} (Fig. 1) and have subsequently described synthetic procedures for the synthesis of these peptides by the use of protected Boc-Ser(PO$_3$R$_2$)-OH derivatives (R = Ph, Bzl, ^{t}Bu) in the Boc mode of solution phase peptide synthesis.[1-3] In an extension of these studies and the recognition that C-phosphonate analogues are of use in the examination of many biological systems,[4] we realized that the methylene isostere of the Ser(P)-residue, 2-amino-4-phosphonobutanoic acid {Abu(P)} (Fig. 1), would be of particular use in the study of Ser(P)-based protein systems.[5] Unlike the susceptibility of the phosphate-serine linkage in Ser(P) to phosphatases, the C-P linkage of the Abu(P) residue is resistant to phosphatase action and stable under normal chemical conditions.[4] In this letter, we describe an improved seven step procedure for the synthesis of Boc-Abu(PO$_3$Me$_2$)-OH[5,6] (1) and its use in the solution phase synthesis of Boc-Glu(OBzl)-Abu(PO$_3$Me$_2$)-Leu-OBzl and its conversion to Glu-Abu(P)-Leu.



O-Phosphoserine
Ser(*P*)



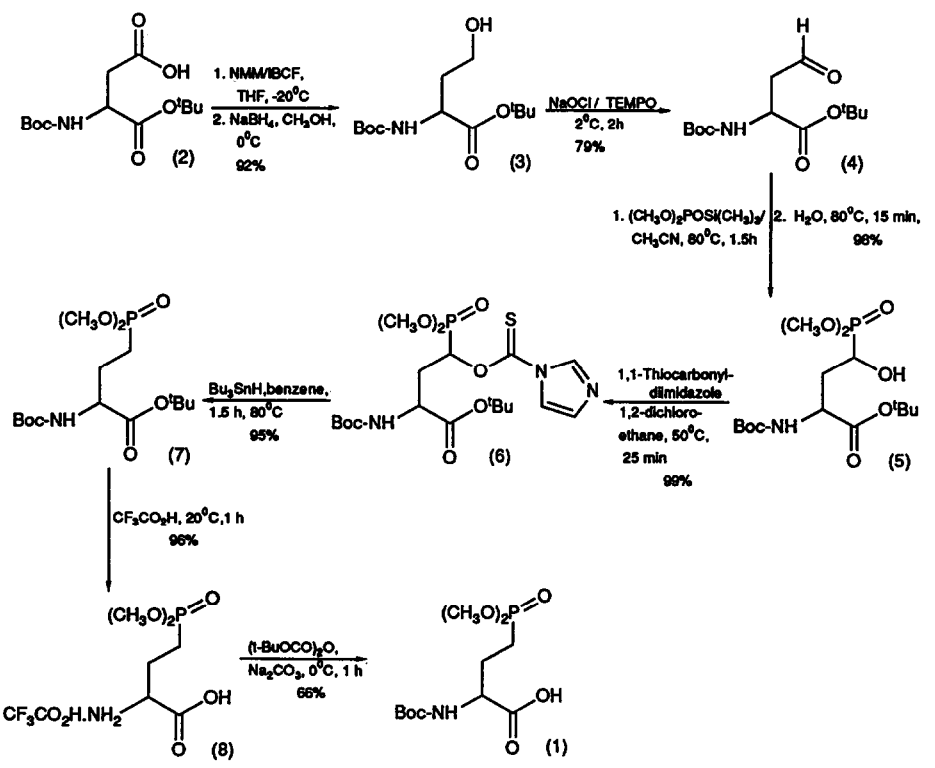
2-amino-4-phosphonobutanoic acid
Abu(*P*)

Figure 1

The synthesis of the optically pure Boc-L-Abu(PO_3Me_2)-OH, was accomplished in 42% overall yield from commercially available Boc-Asp-O^{t}Bu in seven steps and is based on the formation of dimethyl C-alkyl phosphonate group from the treatment of an aldehyde with dimethyl trimethylsilylphosphite[7] (Scheme 1). The synthetic route involved initial sodium borohydride reduction of the isobutoxycarbonyl mixed anhydride of Boc-Asp-O^{t}Bu (2) to give the homoserine derivative, Boc-Hse-O^{t}Bu (3), in 92% yield.[8] Subsequent TEMPO-catalysed hypochlorite oxidation[9] of Boc-Hse-O^{t}Bu (3) gave the aspartaldehyde derivative (4) as a homogeneous product in 79% yield. The treatment of aldehyde (4) with dimethyl trimethylsilylphosphite[7] for 1.5 h at 80°C followed by in situ aqueous hydrolysis (15 min, 80°C) gave the hydroxy dimethyl C-phosphonate (5) in 98% yield. Complete cleavage of the silyl group occurred during the short aqueous step. The hydroxy dimethyl C-phosphonate (5) was converted to Boc-Abu(PO_3Me_2)-O^{t}Bu (7) by initial conversion of the hydroxy dimethyl C-phosphonate (5) to its thioimidazolide (6) using thiocarbonyldiimidazole[10] followed by radical deoxygenation[10] with tributyltin hydride; these two steps proceeding in 99 and 95% yields respectively. The Boc and t-butyl ester group were quantitatively removed by a 1 h treatment with CF_3CO_2H and the Boc group was introduced to H-Abu(PO_3Me_2)-OH.CF_3CO_2H in 66% yield using di-t-butyldicarbonate.

In comparison to our previous 13-step synthesis of Boc-Abu(PO_3Me_2)-OH from aspartic acid,[6] the advantages of this synthetic route are that (a) the TEMPO-catalysed hypochlorite oxidation procedure gives aldehyde (4) in high yield and purity, and (b) the incorporation

Scheme 1

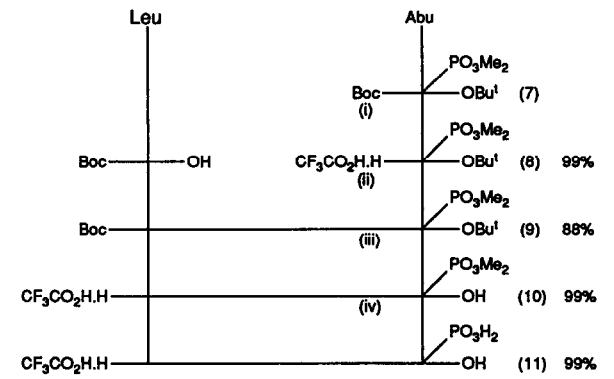


of the *in situ* hydrolysis step during the phosphorylation step obviates the isolation of the trimethylsilyl product.

A test of the optical purity of Boc-Abu(PO_3Me_2)-OH and its application in the Boc mode of peptide synthesis was demonstrated by the synthesis of Leu-Abu(P) as a test peptide (Scheme 2). The dipeptide Boc-Leu-Abu(PO_3Me_2)-O^{t}Bu (9) was obtained in 88% yield by the *iso*butoxycarbonyl mixed anhydride coupling of Boc-Leu-OH with H-Abu(PO_3Me_2)-O^{t}Bu.CF_3CO_2H {obtained by the treatment of (7) with 50% CF_3CO_2H/CH_2Cl_2 (20 min)}. The Boc and *t*-butyl groups were cleaved from dipeptide (9) by CF_3CO_2H treatment to give H-Leu-Abu(PO_3Me_2)-OH.CF_3CO_2H (10) in 99% yield. By C_{18} RP-h.p.l.c. comparison of dipeptide (10) with the diastereoisomeric dipeptide H-Leu-DL-Abu(PO_3Me_2)-OH, the optical purity of Boc-Abu(PO_3Me_2)-OH was established to be greater than 99.5% of a single isomer (we infer the L-isomer).

By the use of ^{31}P n.m.r. spectroscopy for monitoring methyl phosphate cleavage, it was observed that the treatment of the H-Leu-Abu(PO_3Me_2)-OH.CF_3CO_2H (10) with bromotrimethylsilane effected complete removal of both methyl groups after 15 min with the phosphorus signal at 27.3 ppm changing to 16.3 ppm. The peptide was obtained by the addition of water to the reaction solution followed by evaporation of all volatile products. The crude peptide obtained in this manner was established to be of greater than 99% purity by C_{18} RP-h.p.l.c. and, therefore, did not require any further purification. The structure of Leu-Abu(P) (11) was confirmed from its ^{13}C n.m.r. spectrum[12], its FAB mass spectrum (MH^+, m/z 315) and its ^{31}P n.m.r. spectrum (27.4 ppm).

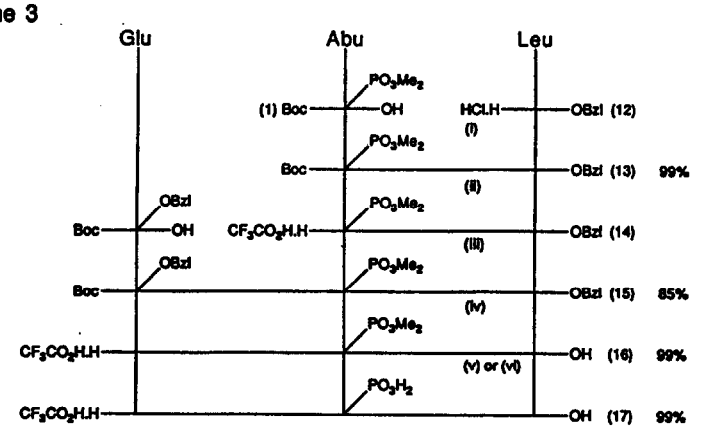
Scheme 2



(i) 50% CF_3CO_2H/CH_2Cl_2. (iii) CF_3CO_2H, 1 h.
(ii) NMM, IBCF (-20°C, 3 min), then amino acid (8). (iv) $(CH_3)_3SiBr/CH_3CN$ (20°C, 40 min).

By the use of Boc-L-Abu(PO_3Me_2)-OH in peptide synthesis, the tripeptide Boc-Glu(OBzl)-Abu(PO_3Me_2)-Leu-OBzl (15) (Scheme 3) was prepared in 84% yield (coupling yields of 99 and 85%) and converted to H-Glu-Abu(PO_3Me_2)-Leu-OH.CF_3CO_2H by hydrogenolysis in 50% CF_3CO_2H/CH_3CO_2H (99% yield) and then to H-Glu-Abu(P)-Leu-OH.CF_3CO_2H (17) by bromotrimethylsilane treatment (99% yield); this peptide being the isostere of the Ser(P)-containing peptide, Glu-Ser(P)-Leu. The structure of Glu-Abu(P)-Leu (17) was confirmed from its ^{13}C n.m.r. spectrum[13], its FAB mass spectrum (MH^+, m/z 426) and its ^{31}P n.m.r spectrum (27.6 ppm). Cleavage of the methyl phosphonate groups was also effected by the use of 1 M TMSBr/CF_3CO_2H/PhSMe, methyl cleavage being complete, by ^{31}P n.m.r. spectroscopy, after 12 h.

Scheme 3



In conclusion, we consider that the synthetic approach described here will be suitable for the synthesis of larger Abu(PO_3Me_2)- and multiple Abu(PO_3Me_2)-containing peptides (as demonstrated by the synthesis of Boc-Abu(PO_3Me_2)-Abu(PO_3Me_2)-NHMe[14]) and lead to the availability of a wide range of biochemically interesting Abu(P)-containing peptides.

Notes and References

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12. δ (^{13}C) (D_2O) (11) : 21.2, 21.6, 23.4 (d, $J_{P,C}$ 108.7 Hz, Abu(P) C4), 23.7, 23.9 (d, $J_{P,C}$ 3.66 Hz, Abu(P) C3), 39.7, 51.7, 52.9 (d, $J_{P,C}$ 18.3 Hz, Abu(P) C2), 170.4, 173.8.
13. δ (^{13}C) (D_2O) (17) : 20.4, 22.1, 22.6 (d, $J_{P,C}$ 136.4 Hz, Abu(P) C4), 24.2, 24.7, 25.8, 28.9, 39.1, 51.3, 52.1, 53.8 (d, $J_{P,C}$ 19.1 Hz, Abu(P) C2), 168.9, 172.2, 175.9.
14. Boc-Abu(PO_3Me_2)-Abu(PO_3Me_2)-NHMe was obtained in 80% yield from the isobutoxycarbonyl mixed anhydride coupling of Boc-Abu(PO_3Me_2)-OH with H-Abu(PO_3Me_2)-NHMe.CF_3CO_2H. This latter derivative was obtained in 88% yield from the mixed anhydride coupling of Boc-Abu(PO_3Me_2)-OH with N-methylamine followed by Boc-cleavage using 40% CF_3CO_2H/CH_2Cl_2.

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