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SYNTHESIS OF BOTH D- AND L-FMOC-ABU[PO(OCH$_2$CH=CH$_2$)$_2$]-OH FOR SOLID PHASE PHOSPHONOPEPTIDE SYNTHESIS

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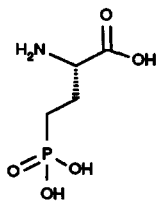
Summary: The Schöllkopf bislactim ether asymmetric amino acid synthesis was coupled with a subsequent enzyme mediated ester hydrolysis to generate a practical synthesis of both D and L enantiomers of Fmoc-Abu[PO(OCH$_2$CH=CH$_2$)$_2$]-OH (6). With this building block phosphonopeptide isosteres of serine phosphopeptides are accessible by Fmoc-solid phase peptide synthesis.

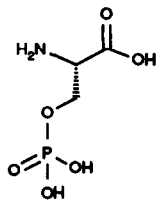
Due to the importance of protein phosphorylation in biology there is currently significant interest and effort toward synthesizing phosphopeptides and hydrolysis stable analogs thereof.[1] We have been particularly interested in the synthesis of peptides incorporating phosphatase stable analogs of phosphoserine. The incorporation of the phosphonate isostere (1) (L-2-amino-4-phosphonobutanoic acid = Abu(P)) of O-phosphoserine 2 into the tripeptide sequence H-Leu-Abu(P)-Glu-OH (3) via solution phase peptide synthesis using t-Boc strategy has recently been reported.[2] This synthesis employed optically pure L-Boc-



1

L-2-amino-4-phosphonobutanoic acid

Abu(P)



2

L-O-phosphoserine

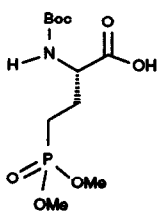
Ser(P)

H-Leu-Abu(P)-Glu-OH

3

Abu(PO$_3$Me$_2$)-OH (4),[3] prepared in seven steps from L-Boc-Asp-OtBu, as a building block. After assembly of the protected peptide Boc-Leu-Abu((PO$_3$Me$_2$)-Glu(OBn)-OH (5) the Boc and benzyl ester groups were removed concommitantly via acidolytic hydrogenolysis. The methyl phosphonate ester was then cleaved with excess trimethylsilyl bromide (TMSBr) to give phosphonopeptide 3 in excellent yield.[4] This solution phase methodology was unsuitable to our purposes of preparing diverse phosphonopeptides of significant complexity and length.[5] To this end we clearly required the use of a solid phase peptide synthesis (SPPS) method and decided upon the simpler and more practical Fmoc-SPPS strategy.[6] This then mandated the synthesis of the protected Fmoc amino acid phosphonate 6. Adapting the described synthesis of 4 for 6a simply involves

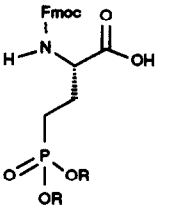
introduction of the Fmoc group instead of Boc in the final step. Upon repeating the reported synthesis, however, certain drawbacks became apparent.[7] As a result we turned our efforts to an asymmetric synthesis which could provide multigram quantities of both enantiomers of Fmoc amino acid phosphonate 6. A method



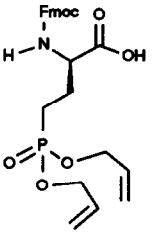
4

L-6a R = Me

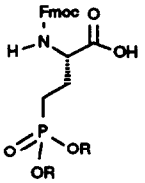
L-6b R = $CH_2CH{=}CH_2$



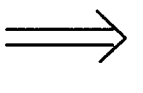
D-6b

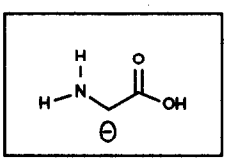


was desired which would also provide flexibility with regard to the nature of the ester protection for the phosphonic acid function. The allyl group was deemed most desirable since allyl phosphate esters can be removed under very mild conditions with palladium catalysis.[8] Thus, **6b** became our synthetic target molecule. Simple retrosynthetic analysis indicated that the fundamental elements of **6b** would be available by a route involving the coupling of a glycine anion synthon with a bromoethylphosphonate ester **7b**. Among the various chiral glycine equivalents we found Schöllkopf's bislactim ether to be very attractive.[9] Both enantiomers (-)-**8**



L-6





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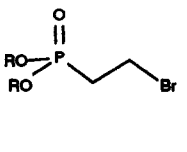
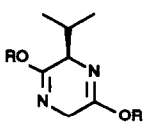


7a R= CH_2CH_3

7b R= $CH_2CH{=}CH_2$

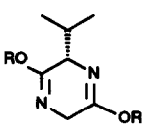
(-)-**8** R=Me

(-)-**9** R=Et



(+)-**8**

(+)-**9**

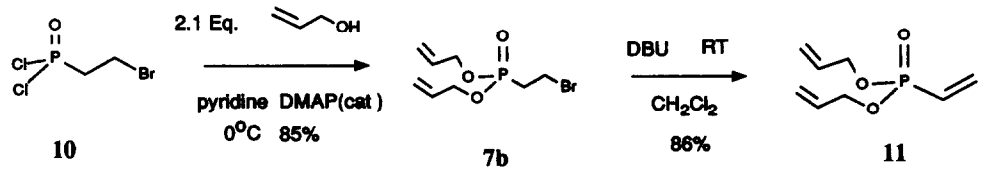


Scheme 1.

(derived from D-valine) and (+)-**8** (derived from L-valine) can be purchased[10] or readily synthesized making respectively both L and D- amino acids equally accessible. The hydrolysis of the bislactim ether function to the corresponding amino ester can be performed with very mild acid. However, the subsequent vigorous acid hydrolysis of the carboxylic ester typically used to form the amino acid is not compatible with maintaining phosphonic ester functionality. In fact, Schöllkopf has described the synthesis of the antipode of **1** from **7a** and (+)-**8**.[11] Nevertheless, we anticipated that, instead of the standard acid catalyzed hydrolysis, an enzymatically mediated carboxylic ester hydrolysis could be performed which would be compatible with phosphonate ester functionality.

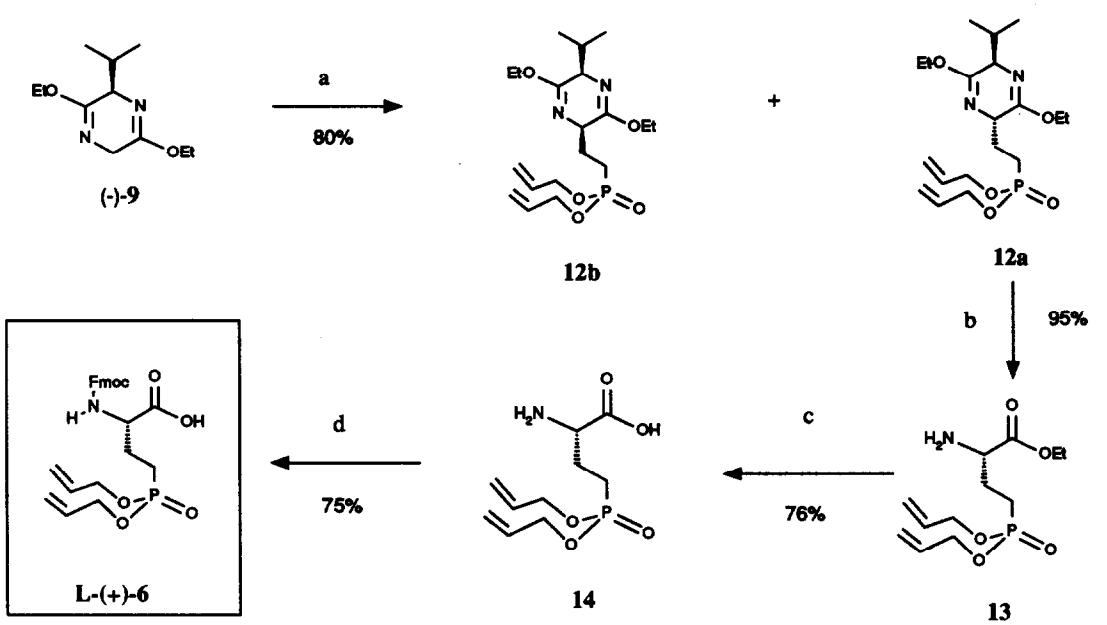
Our strategy for preparing both L and D enantiomers of **6b** has been realized as depicted in Scheme 3. An elegant and very practical synthesis of simple and mixed esters of starting material **7** had already been described.[12] From the known dichloride **10** the diallyl ester **7b** was easily prepared (Scheme 2).[13]

Addition of 7b to the lithium salt of (-)-9[14] under the described conditions[11] in THF gave the two diastereomers 12a and 12b in ca. 85:15 ratio.[15] Although the ratio could be improved to 93:7 after chromatographic purification, we were intrigued by the reported[11] diastereoselectivity of >98% in the coupling of 7a with 8b. We reasoned that the reactive species might be the vinyl phosphonate 11 (Scheme 2).[16] Attempts to couple the lithium salt of (-)-9 with 11 under various conditions were unsuccessful due to competing polymerization.[17] However, when an equimolar solution of 7b containing 10% of 11 was added to the lithium salt of (-)-9, diastereomer 12a ($[\alpha]_D^{20}$= -1.7, c=1.2 in MeOH) obtained in good yield to the virtual exclusion of 12b.[18] Mild acid hydrolysis of the bislactim ether function of 12a afforded amino ester 13 ($[\alpha]_D^{20}$= +11.0, c=1.7 in MeOH) in excellent yield. When 13 was subjected to chymotrypsin in phosphate buffer at pH 5.5 over several days at



Scheme 2.

37°C the slow but clean hydrolysis to amino acid 14 ($[\alpha]_D^{20}$= +9.7, c=0.65 in H_2O) was virtually complete. Using standard conditions 14 was converted to the corresponding N-Fmoc derivative L-(+)-6[19] in good yield. D-(-)-6 was prepared in identical fashion employing the enantiomeric bislactam ether, (+)-9.



Scheme 3. a. 1.0 eq. BuLi, THF -78°C, 1.0 eq. .7b/11 (90:10) -78°C b. 0.25 N HCl -THF:CH_3CN 3:1,RT c. chymotrypsin KH_2PO_4/Na_2PO_4, pH 5.5, 37 °C d. FmocO-N-succinimide acetone :H_2O, RT

With this synthesis both enantiomers of Fmoc-Abu[PO($OCH_2CH=CH_2$)$_2$]-OH are easily available in multigram quantity. The facile incorporation of Abu(P) into peptides using these building blocks in Fmoc-SPPS has been achieved and will be reported separately.

REFERENCES AND NOTES

1. de Bont, D. B. A.; Moree, W. J.; van Boom, J. H. and Liskamp, R. M. J. *J. Org. Chem.* **1993**, *58*, 1309. and references therein.
2. a. Tong, G.; Perich, J. W. and Johns, R. B. *Tetrahedron Lett.* **1990**, *31*, 3759. b. Tong, G., Perich, J. W. and Johns, R. B. *Aus. J. Chem.* **1992**, *45*, 1225.
3. The optical purity of **4** was determined to be >99.5% by its conversion to the dipeptide H-Leu-Abu(PO_3Me_2)-OH followed by HPLC analysis using a H-Leu-DL-Abu(PO_3Me_2)-OH standard.
4. The use of the phosponate-methylester protection was reported[2] to be preferred over the corresponding ethyl protection due to the more facile TMSBr deprotection of the former.
5. An uncomplicated general method (no particular amino acid limitations) was desired to prepare phosphonopeptides up to 20 residues in length.
6. Atherton, E. and Sheppard, R. C. *Solid Phase Peptide Synthesis*; Richwood, D., Hamas, B. D. Eds.: The Practical Approach Series; Information Press Ltd. Oxford, 1989.
7. The starting material Boc-Asp-OtBu is moderately expensive and must otherwise be prepared from aspartic acid. Multigram quantities of **6** were not readily available on a simple laboratory scale in our hands.
8. a. Hayakawa, Y; Kato, H.: Uchiyama, M.; Rajino, H and Noyori, R. *J. Org. Chem.* **1986**, *51*, 2402. b. Hayakawa, Y; Wakabashi, S.; Kato, H., and Noyori, R. *J. Am. Chem. Soc.* **1990**, *112*, 1691.
9. Williams, R. W *Synthesis of Optically Active α-Amino Acids*, Pergamon Press (1989).
10. Merck-Suchardt supplies **8a** and **8b** in 25g quantity at moderate cost.
11. Schöllkopf, U.; Busse, U.; Lonsky, R. and Hinrichs, R. *Liebigs Ann. Chem.* **1986**, 2150.
12. van der Klein, P. A. M.; Dreef, C. E.; van der Marel G. A.; van Boom; J. H., *Tetrahedron Lett.* **1989**, *31*, 5473.
13. Compound **10** has been prepared from the corresponding bis-trimethylsilyl phosphonate which was prepared *via* Arbuzov reaction of tris-trimethylsilylphosphite with dibromoethane. We have found that **7b** can also be readily prepared on a multigram scale from the corresponding ethyl phosphonate **7a**. This simply involves an extra step in which **7a** is converted to bis-trimethylsilyl phosphonate by treatment with TMSBr.
14. The equally effective Schöllkopf reagents (-)-**9** and (+)-**9** were available to us in multigram quantities from the Sandoz Kilo Laboratory.
15. The ratio was determined by integration of the baseline resolved methyl group signals in the 360MHz ^{1}H-NMR spectrum of the crude product.
16. Very high facial selectivity has been reported for Michael addition reactions of the Schöllkopf reagent. a. Schöllkopf, U., Pettig, D.; Busse, U. *Synthesis* **1986**, 737. b. Schöllkopf U.; Kuhnle, W.; Egert, E.; Dyrbusch, M. *Angew. Chem. Int. Ed. Engl.* **1987**, *26*, 480. c. Pettig, D.and Schöllkopf, U. *Synthesis* **1988**, 173.
17. Adding **11** to two equivalents of the lithium salt of **9**, the further addition of the initial Michael adduct to the reactive acceptor **11** could not be suppressed.
18. Presumably the addition of the lithium salt of **9** to **11** is much faster than the alkylation with **7b**. The Michael adduct is then trapped by **7b** in a dehydrohalogenation reaction regenerating the reactive species **11**. We have reproduced this fascinating reaction repeatedly always with high selectivity and good yield.
19. The corresponding amides of (+)-**6** and (-)-**6** derived from S-(-)-phenylethylamine were prepared. These were well resolved by normal phase HPLC and showed the compounds to be of high optical purity (>98%).All new compounds were satisfactorily characterized by ^{1}H-NMR (360MHz) and FAB-MS spectroscopy. Compound D-(-)-**6**: mp. 136-138°C (hexane-CH_2Cl_2), $[\alpha]_D^{20}$= -20.1 (c=1.1 in CH_2Cl_2). ^{1}H-NMR (360MHz, $CDCl_3$) δ= 7.75 (d, 2H, J= 7.2Hz), 7.58 (t, 2H, J= 7.2Hz), 7.37 (t, 2H, J= 7.2Hz), 7.31 (t, 2H, J= 7.2Hz), 5.83-5.98 (9 line multiplet, 2H), 5.76 (br. d, NH), 5.35 (d with additional fine coupling, 2H, J= 16Hz), 5.24 (d with additional fine coupling, 2H, J= 10Hz), 4.51-4.60 (m, 4H), 4.33-4.45 (m, 3H), 4.20 (t, 1H, J= 6.0Hz), 1.72-2.30 (m, 4H).

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