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Asymmetric Synthesis of a Protected Phosphonate Isostere of Phosphothreonine for Solid-Phase Peptide Synthesis

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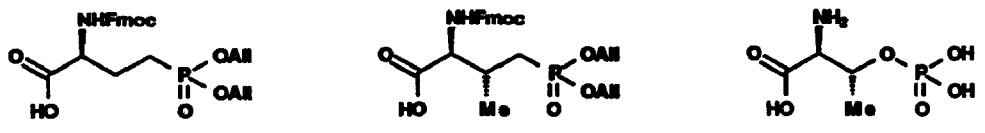
Abstract: The first enantiospecific synthesis of *2*, a phosphonate isostere of phosphothreonine suitably protected for solid-phase peptide synthesis, has been achieved by coupling the highly face-selective conjugate addition of the lithium salt of Schöllkopf's bislactim ether to *E*-prop-2-enyl-phosphonates with a selective enzymatic carboxylic ester hydrolysis. The absolute configuration of the products has been assigned from the X-ray structure of the adduct *9e*.

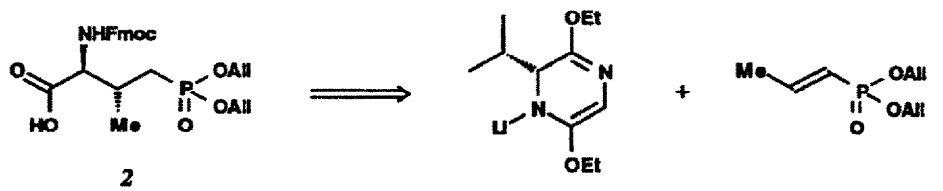
The reversible phosphorylation of proteins on serine, threonine or tyrosine residues constitutes a fundamental mechanism of biological regulation.[1] Thus, the synthesis of non-hydrolysable isosteres of naturally occurring phosphorylated biomolecules can provide useful substrates for biochemical and immunochemical studies.[2] In particular, we have been interested in the synthesis of peptides containing phosphonate mimics of natural phosphoamino acids. Although many syntheses of several non-hydrolyzable isosteres of phosphoserine[3] and phosphotyrosine[4] amino acids have been reported, no optically pure phosphothreonine mimic has been available for peptide synthesis. We have recently reported the synthesis of *N*-Fmoc-*O*,*O*-diallyl protected phosphonic acid *1*, the carbon isostere of phosphoserine, *via* Schöllkopf bislactim ether asymmetric synthesis coupled with an enzyme-mediated carboxylic ester hydrolysis.[3b] In this communication we present the stereospecific synthesis of *2*, the corresponding phosphonate analogue of naturally ocurring (2*S*, 3*R*)-phosphothreonine *3*, suitably protected for multigram solid-phase peptide synthesis (SPPS). This was achieved by adapting our asymmetric synthesis of 2-amino-3-methyl-phosphonobutyric acids [5] to the requirements of the *N*-Fmoc/*O*-allyl phosphonic ester protection strategy.



1 *2* *3*

Figure 1

The synthetic plan was as follows. By analogy with our previous results,[5] stereoselective addition of the lithium salt of Schöllkopf's bislactim ether *(-)-4* to *E*-*O*,*O*-diallyl-prop-2-enyl-phosphonate *5a* should provide a Michael adduct with the required (2*S*, 3*R*) stereochemistry (see Scheme 1). After hydrolysis of the bislactim ether and enzymatically-mediated carboxylic ester hydrolysis compatible with the chosen phosphonate allyl ester functionality, amino group protection should yield the target molecule *2*.



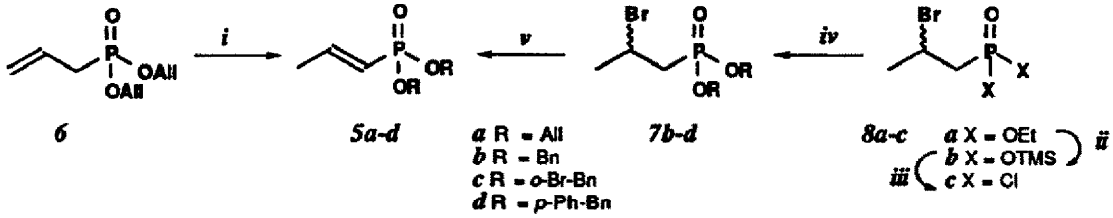
2
L-Fmoc-Ambu[PO(OAll)$_2$]-OH

Scheme 1

(-)-4

5a

The preparation of *E*-prop-2-enyl-phosphonate esters ***5a-d*** was achieved as depicted in Scheme 2. Treatment of known [6] allylphosphonate diallyl ester ***6*** with an excess of DBU at room temperature produced a 90:10 mixture of prop-2-enyl-phosphonate ***5a*** (>95% *E*-configuration after Kugelrohr distillation) and ***6***. The preparation of phosphonodiesters ***5b-d*** required other methodology. Transesterification of easily available *E*-*O,O*-diethyl-prop-2-enyl-phosphonate[7] *via* the corresponding phosphonic acid dichloride was unsuccessful, therefore a scheme involving transesterification followed by introduction of the insaturation was used. The ethyl 2-bromo-propyl phosphonate ***8a*** prepared *via* Arbuzov reaction[5] was converted (Scheme 2) in two steps to the corresponding phosphonic acid dichloride ***8c***. From this common intermediate the esters ***7b-d*** were readily available in good yield.[8] The desired *E*-prop-2-enylphosphonate esters ***5b-d*** were obtained by dehydrobromination with DBU with >98% *E*-configuration (^{1}H NMR analyses).

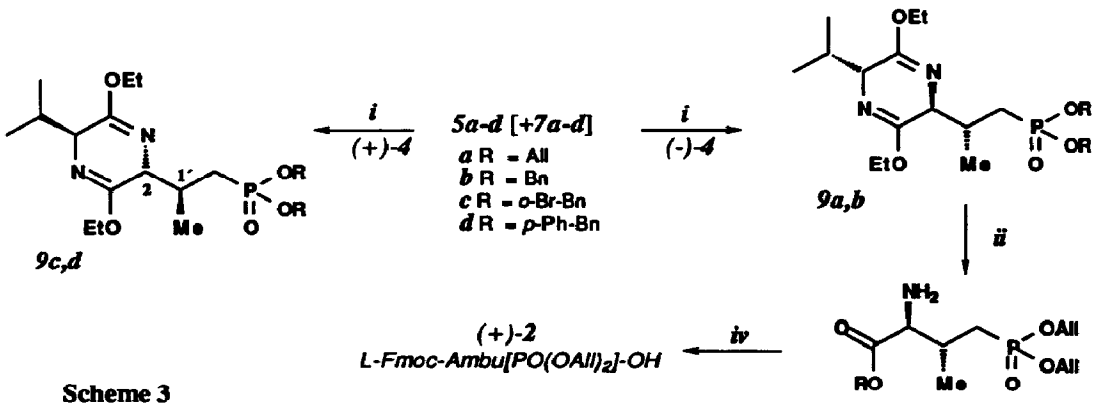


Scheme 2 *i* = 1.5 DBU, RT, 20min, 90%; *ii* = 2.5 TMSBr, CH_2Cl_2, -30 °C-RT, 14 h, 95 %; *iii* = oxalyl chloride, DMF(cat), CH_2Cl_2, 0 °C- RT, 3h, 85%; *iv* = 2.1 ROH, 2.1 pyridine, DMAP(cat), CH_2Cl_2, -30 °C-RT, 12h 85 %; *v* = 1.5 DBU, CH_2Cl_2, -20 °C, 85 %.

Lithium salts of Schöllkopf's bislactim ethers *(-)-4* or *(+)-4* rapidly added to *E*-vinyl phosphonates ***5a-d*** at low temperature with very high asymmetric induction in both new chiral centers (see Scheme 3). At C-2 the diasteromeric excess (*d.e.*) was greater than 98% while at C-1' the *d.e.* was greater than 85%, as previously found for the addition of ***4*** to *E*-*O,O*-diethyl-prop-2-enyl-phosphonates.[5] The slow addition of ***5a*** to three equivalents of *(-)-4* at -78 °C in THF followed by immediate acetic acid quenching and aqueous work-up gave a crude mixture containing *(+)-9a* along with the (*S*) C-1'epimer in *ca.* 93:7 ratio (^{31}P NMR analysis). The remaining phosphorylated side products were identified as resulting from the further addition of the Michael product anion to ***5a***. Chromatographic purification of the reaction mixture gave *(+)-9a* with isomeric purity greater than 95% in 65% yield. For the preparation of ***9b-9d*** a slightly modified protocol was followed. Based on our previous studies it was expected that the initial anionic Michael adduct would be effective in producing the vinyl phosphonate ***5*** *in situ*, *via* dehydrohalogenation of bromophosphonate ***7***, thus suppressing oligomerization.[9] When 1 equivalent of 2:1 ***7b-d*** / ***5b-d*** mixtures were added to 2 equivalents of ***4***, adducts ***9b-d*** were obtained in good yield with undiminished diastereoselectivity.[10] In all the cases, the excess of Schöllkopf's reagent could be recovered, and showed no racemization.[11]

The relative configuration of the adducts ***9a-d*** was readily assigned by comparison of their ^{1}H NMR spectra with those of the corresponding diethyl phosphonates previously reported.[12] Of the crystalline derivatives ***9b-d***, *(-)-9c* was amenable to X-ray structure determination.[13] (Fig. 2) The absolute configuration of *(-)-9c* follows from the known configuration of *(+)-4* which is in turn derived from L-(+)-valine. Thus, this confirmed the proposed stereochemical course for the Michael addition of ***4*** to *E*- and *Z*-prop-2-

enylphosphonates [14] and the corresponding assignment of the absolute configurations of the resulting 2-amino-3-methyl-4-phosphonobutanoic acids which were based on NMR studies of cyclic oxaphosphinane derivatives.[5]



Scheme 3

i = 0.33 eq *5a* or 0.5 eq (*5b-d*/*7b-d*, 1:2) THF, -78 °C, 10 min, 65-82%.
ii = 2.5 HCl 0.25 N, THF / H_2O 1:1, 24 h, 96 %.
iii = Lipase, K_2HPO_4 / NaH_2PO_4 / LiOH, pH = 7.7, 38 °C, 6 d, 55%.
iv = 1.1 Fmoc-O-*N*-succinimide, 1.1 $NaHCO_3$, acetone / H_2O 1 : 1, RT 14h, 75%.

To complete the synthesis, mild acid hydrolysis of *(+)-9a* gave amino ester *(+)-10* ($[\alpha]_D^{20}$ = + 8.9 (CH_2Cl_2, c = 1)) in 96% yield. Hydrolysis of the carboxylic ester with chymotrypsin was slow and inefficient. Porcine pancreatic lipase catalysed hydrolysis to amino acid *(+)-11* ($[\alpha]_D^{20}$ = + 9.7 (H_2O, c = 1), 55% yield) was satisfactory. Fmoc-protection of *(+)-11* under standard conditions gave *(+)-2*. [15] The incorporation of *(+)-2* into peptides by SPPS and the subsequent Pd^0-catalysed removal of the allyl moiety [16] to generate peptides containing free phosphonic acids has been successfully achieved, and will be reported separately.[17]

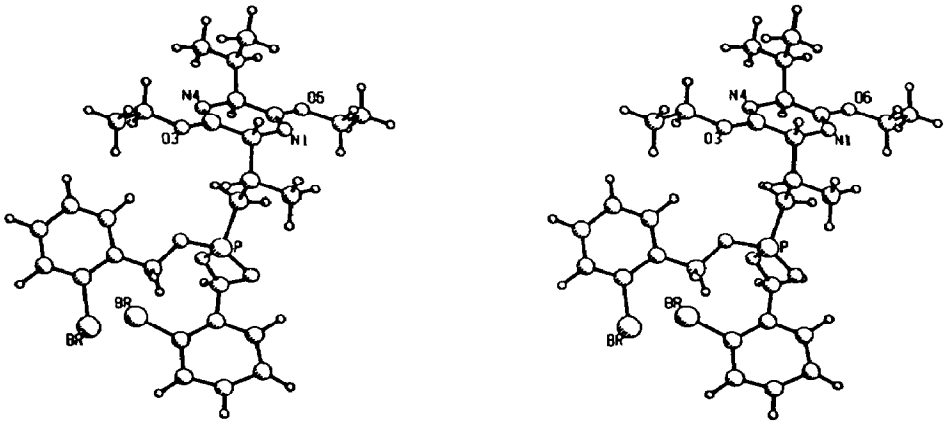


Figure 2. Relaxed stereoscopic plot of the crystal structure of *(-)-9c*.

ACKNOWLEDGEMENTS

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6. Carter, C. G.; Sterrenburg, D. R. U. S. Patent N° 5,142,084 (Aug 25, **1992**).
7. Multigram quantities of *E-O,O*-diethyl-prop-2-enyl-phosphonate were obtained from Sandoz Kilo Laboratory.
8. Direct preparation of ***8b*** by Arbuzov reaction between 1,2-dibropropane and tris(trimethylsilyl)phosphite (6 eq of 1,2-dibromopropane, 4h reflux under argon atmosphere) resulted in similar yields of the desired 3-bromo-phosphonopropanoic esters ***7b-d***.
9. Apparently, addition of the lithium salt ***4*** to ***5b-d*** is faster than elimination of ***7b-d***, and the initially formed *a*-phosphonate anion of the Michael adduct is more basic than ***4***.
10. ***(+)-9a***: 65% yield, oil, $[\alpha]_D^{20}$ = + 22.5 (Cl_2CH_2, c = 1); ***(+)-9b***: 76% yield, mp (pentane) = 51-53 °C, $[\alpha]_D^{20}$ = + 24.5 (Cl_2CH_2, c = 1); ***(-)-9c***: 82% yield, mp (pentane) = 52-54 °C, $[\alpha]_D^{20}$ = - 17.9 (Cl_2CH_2, c = 1.05); ***(-)-9d***: 63% yield, mp (hexane) = 107-108 °C, $[\alpha]_D^{20}$ = - 24.5 (Cl_2CH_2, c = 1).
11. Multigram quantities of Schöllkopf's reagents ***(-)-4*** and ***(+)-4*** were obtained from Sandoz Kilo Laboratory.
12. *Threo* adducts (*i.e.* (2*S*,3*R*) and enantiomer) showed a characteristic absorption with δ between 1.18 and 1.28 ppm as a doublet (J ~ 7 Hz) for CH_3 at C-(1'), whereas *erythro* adducts (*i.e.* (2*S*,3*S*) and enantiomer) showed the corresponding absorption at *ca.* δ = 0.77 ppm.
13. Crystallographic data: The crystals were obtained from a solution of ***(-)-9c*** in pentane, and belong to the monoclinic space group $P2_1$ with cell diemsions a=13.39, b=8.29,c=14.92 A, β = 113.0° V=1523 A^3, Z=2. Diffraction data were collected on a CAD4 diffractometer (CuKα radiation, Θ < 70°, ω/2Θ-mode) and corrected for absorption, radiation decay, and Lorenz/polarisation effects. The structure was solved and refined with SHELX86/77 to an R-factor of 0.123 for 3177 reflections. The absolute configuration was determined on the basis of the known *S*-configuration at C(5). The relevant crystallographic data is deposited at the Cambridge Crystallographic Data Centre.
14. The selective formation of the *threo* adducts in the reaction of ***4*** with *E*-prop-2-enylphosphonates is understood as a consequence of the almost exclusive interaction of the *Si*-face of ***(-)-4a*** with the *Si*-face of *E*-olefin (or the *Re*-face of ***(+)-4a*** with the *Re*-face of *E*-olefin).
15. ***(+)-2***: $[\alpha]_D^{20}$ = + 32.4 (Cl_2CH_2, c = 1); ^1H NMR (360 MHz, $CDCl_3$, *d*): 1.11 (d, J = 6.6 Hz, 3H); 1.82-2.05 (m, 2H); 2.57 (br s, 1H); 4.21 (t, J = 7.2 Hz, 1H); 4.37 (d, J = 7.2 Hz, 2H); 4.55-4.70 (m, 5H); 5.24-5.40 (m, 4H); 5.76 (br d, J = 7.8 Hz), 5.90-5.95 (m, 2H); 7.29 (td, J = 7.4, 1.2 Hz, 2H); 7.39 (t, J = 7.4 Hz, 2H); 7.58 (dd, J = 7.4, 3.3 Hz, 2H); 7.75 (d, J = 7.4 Hz, 2H).
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