Amino Acids (2012) 43:857–874
DOI 10.1007/s00726-011-1145-2

ORIGINAL ARTICLE

Stable triazolylphosphonate analogues of phosphohistidine

Shin Mukai · Gavin R. Flematti · Lindsay T. Byrne · Paul G. Besant · Paul V. Attwood · Matthew J. Piggott

Received: 12 April 2011 / Accepted: 31 October 2011 / Published online: 22 November 2011

Abstract Histidine-phosphorylated proteins and the corresponding kinases are important components of bacterial and eukaryotic cell-signalling pathways, and are therefore potential drug targets. The study of these biomolecules has been hampered by the lability of the phosphoramidate functional group in the phosphohistidines and the lack of generic antibodies. Herein, the design and concise synthesis of stable triazolylphosphonate analogues of *N*1- and *N*3-phosphohistidine, and derivatives suitable for bioconjugation, are described.

Keywords Phosphohistidine · Azide-alkyne cycloaddition · Triazolylalanine · Stable analogues · Synthesis · Haptens

Abbreviations

Ac	Acetyl
All	Allyl
aq.	Aqueous
Ar	Aryl
Bn	Benzyl
Boc	*tert*-Butoxycarbonyl
br	Broad
Bu	Butyl
Cp*	Pentamethylcyclopentadienyl
DCM	Dichloromethane
d	Doublet
dd	Doublet of doublets
DEAD	Diethyl azodicarboxylate
DEPT	Distortionless enhancement by polarisation transfer
DIAD	Diisopropyl azodicarboxylate
DIPEA	Diisopropylethylamine
DMF	*N*,*N*-Dimethylformamide
E1cb	Elimination unimolecular conjugate base
ESI	Electrospray ionisation
Et	Ethyl
Fmoc	9-Fluorenylmethoxycarbonyl
FT	Fourier transform
HCTU	*O*-(6-Chlorobenzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate
HMBC	Heteronuclear multiple bond correlation
HHK	Histone H4 histidine kinase
HPLC	High-performance liquid chromatography
HR	High-resolution (MS)
i.d.	Internal diameter
i-Pr	Isopropyl
IR	Infrared
lit.	Literature
M	Multiplet
Me	Methyl
Mp	Melting point
MS	Mass spectrum
MWD	Multiple-wavelength detector
m/*z*	Mass:charge ratio
NDPK	Nucleoside diphosphate kinase
NMR	Nuclear magnetic resonance
Ph	Phenyl

S. Mukai · G. R. Flematti · L. T. Byrne · P. G. Besant · P. V. Attwood · M. J. Piggott (✉)
School of Biomedical, Biomolecular and Chemical Sciences, The University of Western Australia, Crawley, WA 6009, Australia
e-mail: matthew.piggott@uwa.edu.au

Present Address:
L. T. Byrne
Centre for Microscopy, Characterisation and Analysis, The University of Western Australia, Crawley, WA 6009, Australia

q	Quartet
R_f	Retention factor
Ser	Serine
sat.	Saturated
t	Triplet
t	Tertiary
TFA	Trifluoroacetic acid
THF	Tetrahydrofuran
TIPS	Triisopropylsilyl
TLC	Thin layer chromatography
Tza	Triazolylalanine
UV	Ultraviolet

Introduction

Histidine kinases are a family of enzymes that catalyse the phosphorylation of the imidazole *N*1 (**1**) or *N*3 (**2**) of specific histidine residues in proteins (Fig. 1) (Janiak-Spens and West 2004). Their better-known cousins, the serine/threonine and tyrosine kinases, have been implicated in the regulation of almost all eukaryotic cellular processes. In prokaryotes, fungi and plants, histidine kinases play critical roles in the response to environmental stimuli (Stock et al. 2000). There is also evidence that histidine kinases and their substrates are important components of mammalian cell-signalling pathways (Besant and Attwood 2005; Besant et al. 2003; Steeg et al. 2003). However, the only mammalian histidine kinase that has been well characterised is nucleoside diphosphate kinase (NDPK), which catalyses the interconversion of nucleoside di- and triphosphates via a phosphohistidyl-enzyme intermediate, as well as phosphorylating a number of proteins, including the beta subunit of some trimeric G-proteins (Attwood et al. 2007).

One mammalian histidine kinase that is of particular interest as a potential therapeutic target is histone H4 histidine kinase (HHK). The role of histone H4 phosphorylation is uncertain, but HHK activity is elevated in foetal, regenerating, and cancerous liver cells (Tan et al. 2004). Conversely, the enzyme activity is low in normal adult liver cells. The obvious inference is that HHK is intimately involved in liver cell proliferation.

In contrast to the robust serine, threonine and tyrosine phosphates, the hydrolytically labile phosphoramidate functional group in the phosphohistidines makes their identification, purification, and study challenging (Attwood et al. 2007). In particular, the availability of generic phosphotyrosine and phosphoserine/threonine antibodies has greatly facilitated the study of processes involving protein *O*-phosphorylation, but all attempts to generate an equivalent phosphohistidine antibody have thus far been unsuccessful. Where phosphohistidine itself, or the more acid-stable

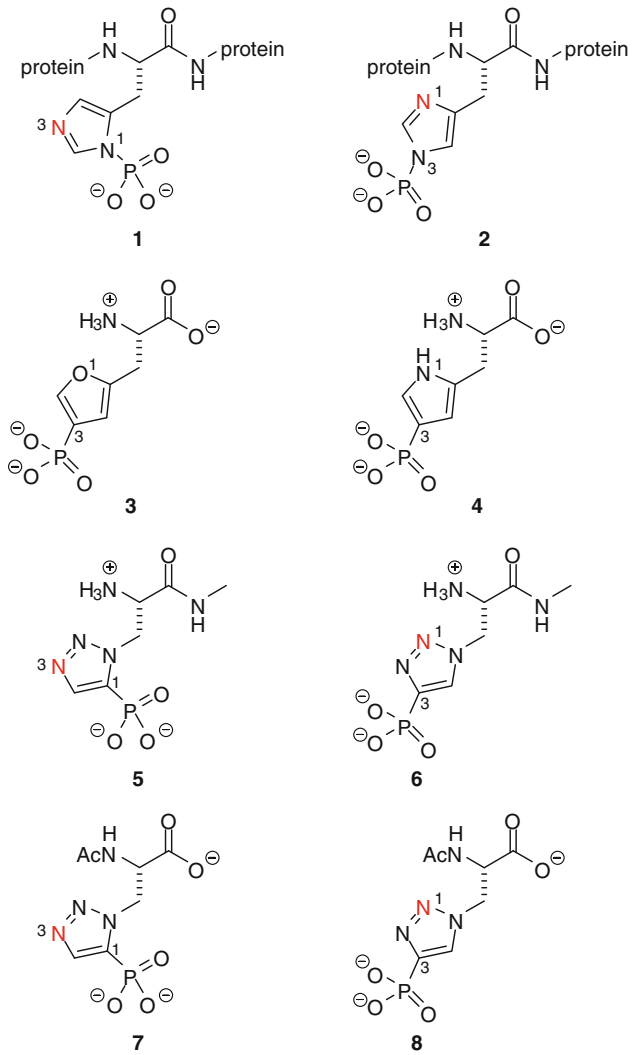


Fig. 1 *N*1- (**1**) and *N*3-phosphohistine (**2**) residues and stable phosphohistidine analogues, **3**–**8**, shown here in their predominant protonation state at physiological pH. The *numbering* shown reflects that used by biochemists for histidine

3-thiophosphohistidine (Pirrung et al. 2000), has been used to generate immunogens for this purpose, the failure has been attributed to rapid hydrolysis of the N–P bond in vivo (Besant PG and Attwood PV, Unpublished work).

Stable phosphonate analogues of *N*3-phosphohistidine, specifically furan (**3**) (Schenkels et al. 1999) and pyrrole (**4**) (Tan E, Pirrung MC, Attwood PV, Unpublished work) congeners, have been synthesised previously. Antibodies raised using **4** as a hapten recognised the immunogen and the hapten but had no affinity for phosphohistidine residues in a protein context (Tan E, Pirrung MC, Attwood PV, Unpublished work). One possible explanation for this failure is that the pyrrole analogue **4** lacks the hydrogen bond-accepting nitrogen atom present in phosphohistidine residues (red in Fig. 1). It is likely that this nitrogen would

be critical for molecular recognition in a phosphohistidine antibody–antigen complex.

Accordingly, we set out to design and synthesise better phosphohistidine analogues and apply them to the generation of generic phosphohistidine antibodies. Triazolylalaninephosphonates **5–8** (Fig. 1) were immediately appealing as the C–P bond is non-hydrolysable and they retain the spatial orientation of key functional groups likely to be important for molecular recognition, in particular, the hydrogen-bond-accepting ring nitrogen. The *N*-methylamide and acetamide functional groups in **5/6** and **7/8**, respectively, mimic the peptide bonds of native phosphohistidine residues, with the free amino and carboxyl groups ready for bioconjugation to a carrier protein through amide linkages with aspartate/glutamate and lysine residues, respectively. In addition, the robust azide-alkyne dipolar cycloaddition (Meldal and Tornoe 2008; Rostovtsev et al. 2002; Wu and Fokin 2007) offered the potential to rapidly access enantiopure analogues of both the *N*1- and *N*3-regioisomers of phosphohistidine, both of which are biologically relevant (Attwood et al. 2007; Besant and Attwood 2009).

Independently, the Muir group has recently adopted an almost identical core strategy and applied it to the solid phase synthesis of peptides containing phosphonotriazolylalanine residues (Kee et al. 2010). In a breakthrough for phosphohistidine research, they have used this approach to successfully generate antibodies specific for histidine-phosphorylated histone H4. Using native chemical ligation, they have also incorporated the phosphohistidine analogue into histone H4.

Similarly, Webb and co-workers have recently reported the synthesis of an Fmoc-protected triazolylalanine phosphonate analogue of *N*3-phosphohistidine, and its use in the solid phase synthesis of a heptapeptide based on the phosphocarrier domain of pyruvate, phosphate dikinase (McAllister et al. 2011).

Despite these recent advances, antibodies that recognise the phosphohistidine epitope in a broad protein context are yet to be discovered. Herein we describe the design and synthesis of haptens that may prove useful in achieving this goal.

Materials and methods

General

All solvents were distilled prior to use; anhydrous solvents and reagents were distilled under N_2. THF for organometallic reactions was distilled from sodium benzophenone ketyl. All reaction temperatures refer to bath temperatures. Organic extracts were dried over anhydrous $MgSO_4$ and then filtered. Solvents were evaporated under vacuum or a stream of N_2.

Flash chromatography was conducted with Merck silica gel 60. Analytical TLC was performed on Whatman flexible plates (250 μm layer, silica gel 60 F_{254}). Spots were visualised under UV light and by staining with ammonium molybdate or ninhydrin.

Melting points were measured on a Kofler hot-stage melting point apparatus. IR spectra were acquired using a Perkin-Elmer Spectrum One FTIR spectrometer. Mass spectra were acquired on an Applied Biosystems QSTAR pulsar I quadruple time-of-flight instrument. NMR spectra were acquired on Varian Inova 300 (300 MHz, ^{1}H; 75.5 MHz, ^{13}C; 120 MHz, ^{31}P), Bruker Avance 500 (500 MHz, ^{1}H; 125 MHz, ^{13}C) or Bruker AV600 (600 MHz, ^{1}H; 150 MHz, ^{13}C; 240 MHz, ^{31}P) spectrometers, as indicated. All spectra were recorded in $CDCl_3$ unless otherwise indicated. Chemical shifts are expressed in ppm, relative to $CHCl_3$ (^{1}H, 7.26 ppm), $CDCl_3$ (^{13}C, 77.0 ppm), CHD_2OD (^{1}H, 3.30 ppm), CD_3OD (^{13}C, 49.0 ppm), and 85% H_3PO_4 (external capillary, ^{31}P, 0 ppm), as appropriate. Routine assignments of ^{13}C NMR spectra were made with the assistance of DEPT 135 and DEPT 90 experiments.

HPLC

HPLC was conducted using a Hewlett Packard 1050 HPLC system equipped with a multiple wavelength detector (MWD) and a 250 × 10 mm i.d., 5 μm, Apollo C_{18} reversed-phase column (Grace-Davison), with a 33 mm × 7 mm guard column of the same material. The samples were eluted at 4 mL/min with 30% (v/v) MeCN–water. UV absorbance was measured at 220 nm.

Enantioselective chromatography of the carboxylic acids **31** and **32** was carried out using a 250 × 4.6 mm i.d., 5 μm Chiracel OD-H column (Diacel), eluted at 1.0 mL/min with 8% *i*-PrOH/hexanes, and detection at 220 nm.

Synthesis

General procedure for the synthesis of azidoalanine derivatives

A stirred solution of the serine derivative (5–15 mmol) and triphenylphosphine (1.2 eqv.) in anhydrous THF (20–50 mL) at −78°C, under N_2, was treated with a 2.5 M solution of HN_3 in toluene (Yeager and Finney 2004) (1.2 eqv.) and DEAD (1.2 eqv.). The resulting solution was allowed to warm to room temperature. After 5 h the solution was diluted with water (100 mL), the layers were separated, and the aqueous phase was extracted with Et_2O (3 × 100 mL). The combined organic phase was washed

with brine (50 mL), dried and evaporated, and the residue was subjected to flash chromatography.

*(S)-Methyl 3-azido-2-(t-butoxycarbonylamino)propanoate (**9a**)*

This compound was synthesised according to the method of Boger (Boger et al. 1994) with slight modifications. Following the general procedure with Boc-Ser-OMe (3.61 g, 16.5 mmol), flash chromatography and elution with 1:4 Et_2O/hexanes gave **9a** as a colourless oil (2.90 g, 72%). ^1H NMR (600 MHz, CD_3OD): δ 5.61 (br d, 1H, NH), 4.28 (m, 1H, α-CH), 3.55 (s, 3H, OCH_3), 3.49 (m [apparent br d], 2H, β-CH_2), 1.23 (s, 9H, *t*-Bu). ^{13}C NMR (150 MHz) δ 169.7 (CO_2), 154.6 ($HNCO_2$), 79.5, 77.2, 53.0, 51.9, 27.6 [$(CH_3)_3$]; MS (ESI) *m/z*: 267 $[M+Na]^+$. $[\alpha]_D = +36.4°$ (*c* 2.0, $CHCl_3$) [lit. (Dondoni et al. 2004) +36.0° (*c* 0.6, $CHCl_3$)]. The ^1H NMR data are similar to those reported (Dondoni et al. 2004).

*(S)-Methyl 2-acetamido-3-azidopropanoate (**9b**)*

Following the general procedure with Ac-Ser-OMe (**20**) (2.00 g, 12.4 mmol) and using DIAD (3.00 mL, 15.3 mmol) instead of DEAD, flash chromatography and elution with 1:1 Et_2O/hexanes gave **9b** as a yellow oil (1.39 g, 60%). ^1H NMR (300 MHz, $CDCl_3$): δ 6.51 (br d, 1H, *J* = 5.4 Hz, NH), 4.74 (m, 1H, α-H), 3.79 (s, 1H, OCH_3, 3H), 3.73 (dd, 2H, *J* = 3.6, 1.2 Hz, β-H), 2.05 (s, 3H, $OCCH_3$). ^{13}C NMR (75 MHz, $CDCl_3$): δ 170.1 (C=O), 169.5 (C=O), 53.8, 52.9, 52.2, 22.9 ($COCH_3$). MS (ESI) *m/z*: 187 $[M+H]^+$, 209 $[M+H]^+$. $[\alpha]_D = +79.3°$ (*c* 1.0, $CHCl_3$). [lit. (Davoli et al. 1995) +74.2° (*c* 1.4, $CHCl_3$)]. The ^1H NMR data are similar to those reported (Davoli et al. 1995).

*(S)-Allyl 2-acetamido-3-azidopropanoate (**9c**)*

Following the general procedure with Ac-Ser-OAll (**22**) (2.00 g, 10.7 mmol), flash chromatography and elution with 1:1 Et_2O/hexanes gave **9c** as a brown oil (1.38 g, 61%). $R_f = 0.40$ (Et_2O); IR (thin film) cm^{-1}: 3,274 (NH), 2,107 (N_3) 1,743 (OC=O), 1,660 (HNC=O). ^1H NMR (500 MHz, $CDCl_3$): δ 6.84 (d, 1H, *J* = 7.5 Hz, NH), 5.83 (m, 1H, *HC*=CH_2), 5.27 (dd, 1H, *J* = 17.0, 1.5 Hz, HC=C*H*$_2$ *trans*), 5.20 (dd, 1H, *J* = 10.5, 1.0 Hz, HC=C*H*$_2$ *cis*), 4.72 (m, 1H, α-H), 4.60 (dd, 2H, *J* = 4.5, 1.5 Hz, OCH_2), 3.68 (d, 2H, β-H), 1.99 (s, 3H, CH_3). ^{13}C NMR (125 MHz, $CDCl_3$): δ 170.2 (C=O), 169.2 (C=O), 130.9 (H*C*=CH_2), 119.0 (HC=*C*H_2), 66.3 (OCH_2), 52.1, 52.0, 22.6 (CH_3). MS (ESI) *m/z*: 235 $[M+Na]^+$. HRMS (ESI): observed, 235.0808, $[C_8H_{13}NO_4+H]^+$ requires 235.0802. $[\alpha]_D = +16.3°$ (*c* 1.0, CH_3Cl).

*(S)-Benzyl 2-acetamido-3-azidopropanoate (**9d**)*

Following the general procedure with Ac-Ser-OBn (**23**) (2.00 g, 12.4 mmol), and DIAD (2.00 mL, 10.2 mmol) instead of DEAD, flash chromatography and elution with 1:1 Et_2O/hexanes gave **9d** as a yellow oil (1.35 g, 61%). $R_f = 0.45$ (Et_2O). IR (thin film) cm^{-1}: 3,293 (NH), 2,106 (N_3), 1,743 (OC=O), 1,655 (HNC=O). ^1H NMR (500 MHz, $CDCl_3$): δ 7.26 (m, 5H, Ar), 7.15 (br d, *J* = 7.5 Hz, NH), 5.11 (d, 2H, OCH_2), 4.76 (m, 1H, α-H), 3.63 (dd, 2H, *J* = 4.0 Hz, 1.5 Hz, β-H), 1.95 (s, 3H, CH_3). ^{13}C NMR (125 MHz, $CDCl_3$): δ 170.1 (C=O), 169.2 (C=O), 134.5 (Ar), 128.2 (ArH), 128.1 (ArH), 127.8 (ArH), 67.2 (OCH_2), 51.9, 51.8, 22.2 (CH_3). MS (ESI) *m/z*: 263 $[M+H]^+$, 285 $[M+Na]^+$. HRMS (ESI): observed, 263.1133, $[C_{12}H_{14}N_4O_3+H]^+$ requires 263.1139. $[\alpha]_D = +22.8°$ (*c* 1.0, $CHCl_3$).

*(S)-Benzyl 3-azido-2-(t-butoxycarbonylamino)propanoate (**9e**)*

This compound has been prepared previously by a similar method (Kogan and Rawson 1992). Following the general procedure with Boc-Ser-OBn (1.50 g, 5.08 mmol), flash chromatography and elution with 1:4 Et_2O/hexanes gave **9e** as a colourless oil (1.18 g, 72%). $R_f = 0.20$ (1:4 Et_2O/hexanes). ^1H NMR (400 MHz, $CDCl_3$): δ 7.34 (m, 5H, Ar), 5.53 (br d, *J* = 7.6 Hz, 1H, NH), 5.19 (d, *J* = 2.8 Hz, 2H, OCH_2) 4.50 (m, 1H, α-H), 3.71 (d, *J* = 3.6 Hz, 2H, β-H), 1.44 (s, 9H, *t*-Bu). ^{13}C NMR (100 MHz, $CDCl_3$): δ 169.3 (CO_2), 154.7 (NCO_2), 134.6 (ArC), 128.24 (ArH), 128.17 (ArH), 127.9 (ArH), 80.0 [$OC(CH_3)_3$], 67.2 (OCH_2), 53.3 (α), 52.2 (β), 27.8 [$(CH_3)_3$]. MS (ESI) *m/z*: 267 $[M+Na]^+$. $[\alpha]_D = +8.2°$ (*c* 1.0, $CHCl_3$) [lit. (Kee et al. 2010) +8.0° (*c* 1.0, $CHCl_3$)]. The ^1H and ^{13}C NMR data are similar to those reported (Kee et al. 2010).

*(S)-Benzyl 2-(((9H-fluoren-9-yl)methoxy) carbonylamino)-3-azidopropanoate (**9f**)*

Following the general procedure with Fmoc-Ser-OBn (**36**) (1.51 g, 5.76 mmol), flash chromatography and elution with 1:4 Et_2O/hexanes gave **9f** as a white solid (1.38 g, 65%). mp 70–72°C. $R_f = 0.15$ (1:1 Et_2O/hexanes). IR (thin film) cm^{-1}: 3,337 (NH), 2,107 (N_3) 1,650–1,750 (br, C=O + NC=O). ^1H NMR (500 MHz, $CDCl_3$): δ 7.80 (d, *J* = 7.5 Hz, 2H, Ar), 7.64 (br d, *J* = 7.0 Hz, 2H, Ar), 7.33–7.46 (m, 9H, Ar), 5.85 (br d, *J* = 7.5 Hz, 1H, NH), 5.26 (d, *J* = 4.5 Hz, 2H, OCH_2Ph), 4.64 (m, 1H, α-H), 4.45 (m, 2H, NCO_2CH_2), 4.26 (t, *J* = 7.5 Hz, 1H, H9′), 3.79 (br s, 2H, β-H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 169.2 (CO_2), 155.6 (NCO_2), 143.6, 143.5, 141.2, 134.8 (ArC), 128.6 (ArH), 128.2 (ArH), 127.6 (ArH), 127.0, 125.0, 119.9, 67.8

(OCH_2), 67.2 (OCH_2), 53.9 (α), 52.4 (β), 46.9 (C9′). MS (ESI) *m/z*: 443 $[M+H]^+$, 465 $[M+Na]^+$. HRMS (ESI): observed, 465.1539, $[C_{25}H_{22}N_4O_4+Na]^+$ requires 465.1533. $[\alpha]_D = +25.7°$ (*c* 1.0, $CHCl_3$).

(S)-Benzyl 3-hydroxy-2-(t-butoxycarbonylamino) propanoate [Boc-Ser-OBn]

Benzyl bromide (1.80 mL, 15.2 mmol) was added to a solution of Boc-Ser-OH (3.00 g, 14.6 mmol) and DIPEA (1.30 mL, 15.2 mmol) in DMF (20 mL) under N_2 at 0°C. The resulting solution was allowed to warm to room temperature, stirred for 18 h, diluted with sat. aq. NH_4Cl (30 mL), and then extracted with EtOAc (3 × 50 mL). The organic extract was washed with brine (20 mL), dried and evaporated, and the residue was subjected to flash chromatography. Elution with 1:4 EtOAc/hexanes gave Boc-Ser-OBn as a colourless oil (3.66 g, 85%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.34–7.27 (m, 5H, Ar), 5.71 (d, *J* = 8.0 Hz, 1H, NH), 5.15 (d, *J* = 2.5 Hz, 2H, OCH_2Ph), 4.39 (m, 1H, α-H), 3.95 (m [apparent br d], 1H, β-Ha), 3.84 (dd, *J* = 11.0, 3.5 Hz, 1H, β-Hb), 3.50 (br s, 1H OH), 1.42 (s, 9H, *t*-Bu); ^{13}C NMR (125 MHz, $CDCl_3$): δ 170.8 (CO_2), 155.7 (NCO_2), 135.1 (ArC), 128.4 (ArH), 128.2 (ArH), 127.9 (ArH), 80.0 [$C(CH_3)_3$], 67.1 (OCH_2Ph), 62.9 (β), 55.7 (α), 28.1 [$C(CH_3)_3$]. MS (ESI) *m/z*: 296 $[M+H]^+$, 318 $[M+Na]^+$. $[\alpha]_D = -19.6°$ (*c* 1.0, MeOH) [lit. (Nakata et al. 1996) −19.0° (*c* 1.0, $CHCl_3$)]. The NMR data are similar to those reported (Tummatorn et al. 2007).

Diethyl (triisopropylsilyl)ethynylphosphonate

Ethyl bromide (1.00 mL, 13.7 mmol) was added dropwise to a suspension of stirred Mg turnings (416 mg, 17.0 mmol) in THF (10 mL) at 0°C under N_2 and the resulting mixture was heated under reflux under N_2 for 30 min. The ethynylmagnesium bromide solution thus prepared was added dropwise via canula to a solution of TIPS-acetylene (3.00 mL, 13.4 mmol) in THF (10 mL) at 0°C under N_2. The reaction mixture was allowed to warm to room temperature and stirring was continued for 1 h before being cooled to 0°C and treated dropwise with diethyl chlorophosphite (2.20 mL, 15.2 mmol). The resulting solution was allowed to warm to room temperature and after 1 h was diluted with sat. aq. NH_4Cl (30 mL) and extracted with Et_2O (3 × 50 mL). The organic extract was washed with brine (30 mL), dried and concentrated under reduced pressure, and the residue was subjected to flash chromatography. Elution with 1:4 EtOAc/hexane gave the title phosphonate as pale yellow oil (3.49 g, 80%). $R_f = 0.80$ (1:1 EtOAc/hexanes). ^{1}H NMR (600 MHz, $CDCl_3$) δ: 4.11 (m, 4H, OCH_2CH_3), 1.32 (dt, $J_{H,P} = 0.6$, *J* = 7.1 Hz, 6H, OCH_2CH_3), 1.03–1.10 (m, 21H, *i*-Pr). ^{13}C NMR (150 MHz, $CDCl_3$): δ 106.4 (d, $J_{C,P} = 37.6$ Hz, C2), 96.3 (d, $J_{C,P} = 269.4$ Hz, C1), 63.0 (d $J_{C,P} = 5.4$ Hz), 18.3 (*i*-Pr CH_3), 15.9 (d, $J_{C,P} = 6.8$ Hz, OCH_2CH_3), 10.7 (CSi). ^{31}P NMR (120 MHz): δ −7.38; MS (ESI) *m/z*: 319 $[M+H]^+$, 341 $[M+Na]^+$. This compound has been reported (Lecercle et al. 2006) but no characterisation data were given.

*Diethyl ethynylphosphonate (**10**)*

A solution of diethyl (triisopropylsilyl)ethynylphosphonate (1.00 g, 3.14 mmol) and KF (0.38 g, 6.5 mmol) in MeOH (10 mL) was stirred for 2 h and then concentrated under reduced pressure. The residue was diluted with water and extracted with Et_2O (3 × 40 mL). The organic extract was washed with brine (30 mL), dried, and concentrated under reduced pressure. The residue was subjected to flash chromatography. Elution with 1:1 Et_2O/hexane gave **8** as a pale yellow oil (310 mg, 61%). ^{1}H NMR (500 MHz, $CDCl_3$) δ: 4.04 (m, 4H, CH_2), 3.00 (d, *J* = 13.5 Hz 1H, CH), 1.24 (dt, $J_{H,P} = 0.5$ *J* = 7.0 sHz, 6H, 2 × CH_3). ^{13}C NMR (125 MHz, $CDCl_3$): δ 88.1 (d, *J* = 50.6 Hz, CH), 73.8 (d, *J* = 289 Hz, CP), 63.1 (d, *J* = 5.5 Hz, OCH_2CH_3), 15.7 (d, *J* = 7.0 Hz, OCH_2CH_3). MS (ESI) *m/z*: 163 $[M+H]^+$. The ^{1}H NMR data are similar to those reported (McAllister et al. 2011). This compound was also prepared using a published method (Vuilhorgne et al. 2003).

General procedure for thermal Huisgen cycloadditions

A stirred solution of the azide **9** and diethyl ethynylphosphonate **10** (1 eqv.) in toluene (5–10 mL), under N_2, was heated under reflux for 5 h. The toluene was evaporated and the residue was subjected to flash chromatography (details below).

*Thermal Huisgen cycloaddition of **9a***

Following the general procedure with azide **9a** (0.150 g, 0.614 mmol), flash chromatography and elution with (1:1 EtOAc/hexanes) gave **11a** as pale yellow oil (53 mg, 21%), identical with the material described below. Further elution with EtOAc gave **12a** as pale yellow oil (144 mg, 58%), identical with the material described below.

*Thermal Huisgen cycloaddition of **9b***

Following the general procedure with azide **9b** (1.00 g, 5.37 mmol), flash chromatography and elution with (7:3 EtOAc/hexanes) gave **11b** as pale yellow oil (486 mg, 26%), identical with the material described below. Further elution with EtOAc gave **12b** as pale yellow oil (1.12 g, 60%), identical with the material described below.

*Thermal Huisgen cycloaddition of **9c***

Following the general procedure with azide **9c** (1.00 g, 4.71 mmol), flash chromatography and elution with (7:3 EtOAc/hexanes) gave **11c** as pale yellow oil (722 mg, 41%), identical with the material described below. Further elution with EtOAc gave **12c** as pale yellow oil (774 mg, 44%), identical with the material described below.

*Thermal Huisgen cycloaddition of **9d***

Following the general procedure with azide **9d** (1.00 g, 3.81 mmol), flash chromatography and elution with 1:1 EtOAc/hexanes gave **11d** as pale yellow oil (697 mg, 43%), identical with the material described below. Further elution with EtOAc gave **12d** as a pale yellow oil (729 mg, 45%), identical with the material below.

*Thermal Huisgen cycloaddition of **9e***

Following the general procedure with azide **9e** (0.200 g, 0.624 mmol), flash chromatography and elution with (1:1 EtOAc/hexanes) gave **11e** as pale yellow oil (123 mg, 41%), identical with the material described below. Further elution with EtOAc gave **12e** as pale yellow oil (129 mg, 42%), identical with the material described below.

*Thermal Huisgen cycloaddition of **9f***

Following the general procedure with azide **9f** (0.100 g, 0.226 mmol), flash chromatography and elution with 2:3 EtOAc/hexanes gave the **11f** as a pale yellow oil (53 mg, 39%), identical with the material described below. Further elution with EtOAc gave the **12f** as pale yellow oil (56 mg, 41%), identical with the material described below.

General procedure for Ru(II)-catalysed azide-alkyne cycloadditions

Cp*RuCl(PPh_3)$_2$ (1.5 mol%) was added to a solution of the azide **9** (0.2–1.2 mmol) and **10** (1 eqv.) in toluene (5–20 mL) under N_2. The resulting solution was stirred at 60°C for 24 h, then diluted with water (10–20 mL), and extracted with EtOAc (3 × 30–100 mL). The extract was washed with brine (10–20 mL), dried and evaporated, and the residue was subjected to flash chromatography (details) below.

*(S)-Methyl 2-(t-butoxycarbonylamino)-3-(5-(diethoxyphosphoryl)-1H-1,2,3-triazol-1-yl)propanoate [Boc-5-PO(OEt)$_2$Tza-OMe] (**11a**)*

Following the general procedure with azide **9a** (0.10 g, 0.41 mmol), flash chromatography and elution with 1:1 EtOAc/hexanes gave **11a** as pale yellow oil (93 mg, 56%). $R_f = 0.15$ (EtOAc/hexanes 1:1); IR (thin film) cm^{-1}: 3,306 (NH), 1,747 (m, OC=O), 1,715 (s, NC=O); 1H NMR (600 MHz, $CDCl_3$): δ 7.95 (s, 1H, triazolyl), 5.69 (br d, J = 6.0 Hz, 1H, NH), 4.88–5.00 (m, 3H, α-CH and β-CH_2), 4.17 (m, 4H, OCH_2CH$_3$), 3.76 (s, 3H, OCH_3), 1.36 (s, 9H, *t*-Bu), 1.35 (apparent q J = 7.2 Hz, 6H, OCH$_2$CH_3). ^{13}C NMR (125 MHz, $CDCl_3$): δ 169.5 (CO_2), 154.8 (NCO_2), 140.0 (d, $J_{C,P}$ = 20.4 Hz, triazolyl CH), 126.7 (d, $J_{C,P}$ = 219.6 Hz, CP), 79.8 [O*C*CH$_3$)$_3$], 63.4 (d, $J_{C,P}$ = 5.9 Hz, O*C*H$_2$CH$_3$a), 63.3 (d, $J_{C,P}$ = 5.8 Hz, O*C*H$_2$CH$_3$b), 53.2 (α), 52.5 (OCH_3), 50.4 (β), 27.8 [C*C*H$_3$)$_3$], 15.9 (d, $J_{C,P}$ = 4.4 Hz, OCH$_2$*C*H$_3$a), 15.8 (d, $J_{C,P}$ = 4.5 Hz, OCH$_2$*C*H$_3$b); ^{31}P NMR (240 MHz): δ 4.03. MS (ESI) *m/z*: 407 [M+H]$^+$, 429 [M+Na]$^+$; HRMS (ESI): observed, 407.1688, [$C_{15}H_{27}N_4O_7P$+H]$^+$ requires 407.1690. $[\alpha]_D = -31.0°$ (*c* 1.7, EtOAc). Further elution with EtOAc gave **12a** as pale yellow oil (35 mg, 21%), identical with the material described below.

*(S)-Methyl 2-acetamido-3-(5-(diethoxyphosphoryl)-1H-1,2,3-triazol-1-yl)propanoate [Ac-5-PO(OEt)$_2$Tza-OMe] (**11b**)*

Following the general procedure with azide **9b** (0.200 g, 1.07 mmol), flash chromatography and elution with 7:3 EtOAc/hexanes gave **11b** as pale yellow oil (213 mg, 57%). $R_f = 0.35$ (EtOAc). IR (thin film) cm^{-1}: 3,283 (NH), 1,748 (OC=O), 1,671 (NC=O). 1H NMR (500 MHz, $CDCl_3$): δ 7.81 (s, 1H, triazolyl), 7.51 (br d, 1H, J = 8.0 Hz, NH), 4.99 (dd, 1H, J = 8.0, 4.0 Hz, α-H), 4.88 (dd, 1H, J = 14.0, 4.5 Hz, β-Ha), 4.75 (dd, 1H, J = 14.0, 8.0 Hz, β-Hb), 4.05 (m, 4H, 2 × OCH_2), 3.58 (s, 3H, OCH_3) 1.79 (s, 3H, $NCOCH_3$), 1.20 (m, 6H, 2 × CH_2CH_3). ^{13}C NMR (125 MHz, $CDCl_3$): δ 170.1 (C=O), 169.1 (C=O), 139.9 (d, $J_{C,P}$ = 20.4 Hz, triazolyl CH), 126.7 ($J_{C,P}$ = 220.4 Hz, CP), 63.6 (d, $J_{C,P}$ = 5.9 Hz, OCH_2), 52.5, 51.8, 50.0, 22.2 (acetyl CH_3), 15.81 ($J_{C,P}$ = 3.6 Hz, OCH$_2$*C*H$_3$a), 15.76 ($J_{C,P}$ = 3.8 Hz, OCH$_2$*C*H$_3$b). ^{31}P NMR (120 MHz, $CDCl_3$): δ 4.36. MS (ESI) *m/z*: 349 [M+H]$^+$, 371 [M+Na]$^+$. HRMS (ESI): observed, 371.1097, [$C_{12}H_{21}N_4O_6P$+Na]$^+$ requires 371.1091. $[\alpha]_D = +15.7°$ (*c* 1.0, CH_3Cl). Further elution with EtOAc gave **12b** as pale yellow oil (71 mg, 19%), identical with the material described below.

*(S)-Allyl 2-acetamido-3-(5-(diethoxyphosphoryl)-1H-1,2,3-triazol-1-yl)propanoate [Ac-5-PO(OEt)$_2$Tza-OAll] (**11c**)*

Following the general procedure with azide **9c** (250 mg, 1.2 mmol), flash chromatography and elution with 1:1 EtOAc/hexanes gave **11c** as pale yellow oil (323 mg, 73%). $R_f = 0.30$ (EtOAc); IR (thin film) cm^{-1}: 3,284

(NH), 1,747 (OC=O), 1,682 (NC=O). ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.88 (s, 1H, triazolyl), 7.19 (br d, 1H, J = 8.0 Hz, NH), 5.78 (m, 1H, *H*C=CH_2), 5.23 (ddd, 1H, J = 17.0, 2.5, 1.0 Hz HC=C*H*$_2$ *trans*), 5.15 (ddd, 1H, J = 10.5, 2.5, 1.0 Hz, HC=C*H*$_2$ *cis*), 5.11 (m, 1H, α-H) 4.95 (dd, 1H, J = 14.0, 4.5 Hz, β-Ha), 4.86 (dd, 1H, J = 14.5, 8.0 Hz, β-Hb), 4.56 (m, 2H, allyl OCH_2), 4.13 (m, 4H, 2 × O*C*H$_2$CH$_3$), 1.88 (s, 1H, NCO*C*H$_3$), 1.29 (m, 6H, 2 × OCH$_2$C*H*$_3$). ^{13}C NMR (125 MHz, $CDCl_3$): δ 170.1 (C=O), 168.5 (C=O), 140.1 (d, $J_{C,P}$ = 20.4 Hz, triazolyl CH), 131.0, 126.9 (d, $J_{C,P}$ = 220.4 Hz, CP), 118.8, 66.3, 63.73 ($J_{C,P}$ = 4.5 Hz, O*C*H$_2$CH$_3$a), 63.69 ($J_{C,P}$ = 4.7 Hz, O*C*H$_2$CH$_3$b), 52.1, 50.2, 22.5 (acetyl CH_3), 16.01 (d, $J_{C,P}$ = 3.6 Hz, OCH$_2$*C*H$_3$a), 15.96 (d, $J_{C,P}$ = 3.8 Hz, OCH$_2$*C*H$_3$b). ^{31}P NMR (120 MHz, $CDCl_3$): δ 4.48. MS (ESI) *m/z*: 375 $[M+H]^+$, 397 $[M+Na]^+$. HRMS (ESI): observed, 375.1422, $[C_{14}H_{23}N_4O_6P+H]^+$ requires 375.1428. $[\alpha]_D$ = +21.0° (*c* 1.0, CH_3Cl). Further elution with EtOAc gave **12c** as pale yellow oil (44 mg, 10%), identical with the material described below.

*(S)-Benzyl 2-acetamido-3-(5-(diethoxyphosphoryl)-1H-1,2,3-triazol-1-yl)propanoate [Ac-5-PO(OEt)$_2$Tza-OBn] (**11d**)*

Following the general procedure with azide **9d** (0.200 g, 0.763 mmol), flash chromatography and elution with 3:2 EtOAc/hexanes gave **11d** as pale yellow oil (262 mg, 81%). R_f = 0.40 (EtOAc). IR (thin film) cm^{-1}: 3,290 (NH), 1,747 (OC=O), 1,683 (NC=O). ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.83 (s, 1H, triazolyl), 7.44 (br d, 1H, J = 8.5 Hz, NH), 7.16 (m, 5H, Ar), 5.09 (dd, 1H, J = 8.0, 4.5 Hz, α-H), 5.02 (d, 2H, J = 6.5 Hz, O*C*H$_2$Ph), 4.90 (dd, 1H, J = 14.0, 4.5 Hz, β-Ha), 4.81 (dd, 1H, J = 14.0, 8.0 Hz, β-Hb), 4.00 (m, 4H, O*C*H$_2$CH$_3$), 1.81 (s, 1H, NCO*C*H$_3$), 1.18 (m, 6H, OCH$_2$C*H*$_3$) ^{13}C NMR (125 MHz, $CDCl_3$): δ 170.1 (C=O), 168.5 (C=O), 140.0 (d, $J_{C,P}$ = 20.6 Hz, triazolyl CH), 134.5, 128.1, 128.0, 127.8, 126.6 (d, $J_{C,P}$ = 220.4 Hz, CP), 67.2 (O*C*H$_2$Ph), 63.45 (d, $J_{C,P}$ = 6.2 Hz, O*C*H$_2$CH$_3$a), 63.40 (d, $J_{C,P}$ = 6.0 Hz, O*C*H$_2$CH$_3$b), 52.8, 50.0, 22.1 (acetyl CH_3), 15.70 (d, $J_{C,P}$ = 4.2 Hz, OCH$_2$*C*H$_3$a), 15.65 (d, $J_{C,P}$ = 4.2 Hz, OCH$_2$*C*H$_3$b). ^{31}P NMR (120 MHz, $CDCl_3$): δ 4.35. MS (ESI) *m/z*: 425 $[M+H]^+$, 447 $[M+Na]^+$. HRMS (ESI): observed, 425.1590, $[C_{18}H_{25}N_4O_6P+H]^+$ requires 425.1584 $[\alpha]_D$ = +14.0° (*c* 1.0, CH_3Cl).

*(S)-Benzyl 2-(t-butoxycarbonylamino)-3-(5-(diethoxyphosphoryl)-1H-1,2,3-triazol-1-yl)propanoate [Boc-5-PO(OEt)$_2$Tza-OBn] (**11e**)*

Following the general procedure with azide **9e** (0.200 g, 0.624 mmol), flash chromatography and elution with 3:2 EtOAc/hexanes gave **11e** as pale yellow oil (241 mg, 80%). R_f = 0.20 (1:1 EtOAc/hexanes). IR (thin film) cm^{-1}: 3,305 (NH), 1,747 (m, C=O), 1,716 (s, NC=O). ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.94 (s, 1H, triazolyl), 7.29 (m, 5H, Ar), 5.75 (br s, 1H, NH), 5.15 (s, 2H, OCH_2Ph), 4.88–5.00 (m, 3H, α-CH + β-CH_2), 4.14 (m, 4H, 2 × O*C*H$_2$CH$_3$), 1.34 (s, 9H, *t*-Bu), 1.27–1.36, (m, 6H, 2 × OCH$_2$C*H*$_3$). ^{13}C NMR (125 MHz, $CDCl_3$): δ 169.1 (CO_2), 155.0 (NCO_2), 140.3 (d, $J_{C,P}$ = 20.4 Hz, triazolyl CH), 134.8 (*Ar*C), 128.5 (ArH), 128.4 (ArH), 128.2 (ArH), 127.0 (d, $J_{C,P}$ = 219.6 Hz, CP), 80.1 [O*C*(CH$_3$)$_3$], 67.6 (O*C*H$_2$Ph), 63.64 (d, $J_{C,P}$ = 5.7 Hz, O*C*H$_2$CH$_3$a), 62.58 (d, $J_{C,P}$ = 5.8 Hz, O*C*H$_2$CH$_3$b), 53.5 (α), 50.6 (β), 28.1 [C(*C*H$_3$)$_3$], 16.09 (d, $J_{C,P}$ = 4.8 Hz, OCH$_2$*C*H$_3$a), 16.09 (d, $J_{C,P}$ = 4.9 Hz, OCH$_2$*C*H$_3$b). ^{31}P NMR (120 MHz, $CDCl_3$): δ 4.60. MS (ESI) *m/z*: 483 $[M+H]^+$, 505 $[M+Na]^+$. HRMS (ESI): observed, 505.1820, $[C_{21}H_{31}N_4O_7P+Na]^+$ requires 505.1823. $[\alpha]_D$ = +9.2° (*c* 1.0, $CHCl_3$).

*(S)-Benzyl 2-(((9H-fluoren-9-yl)methoxy)carbonylamino)-3-(5-(diethoxyphosphoryl)-1H-1,2,3-triazol-1-yl)propanoate [Fmoc-5-PO(OEt)$_2$Tza-OBn] (**11f**)*

Following the general procedure with azide **9f** (0.100 g, 0.226 mmol), flash chromatography and elution with 2:3 EtOAc/hexanes gave **11f** as pale yellow oil (107 mg, 78%). IR (thin film) cm^{-1}: 3,272 (NH), 1,700–1,750 (br, OC=O + NC=O). The NMR spectra showed the presence of two rotamers. ^{1}H NMR (500 MHz, $CDCl_3$): δ [7.99, 7.98 (2 s, 1H, triazolyl)], 7.74 (d, J = 7.5 Hz, 2H, Ar) 7.54 (d, J = 7.5 Hz, 2H, Ar), 7.23–7.43 (m, 9H, Ar), [6.42, 6.26 (2 br d, J = 7.5 Hz, 1H, NH)], 5.25 (d, J = 5.0 Hz, 2H, OCH_2Ph), 4.98–5.21 (m, 3H, α-CH + β-CH_2), 4.07–4.70 (m, 7H, NCO_2CH_2 + H9′ + 2 × O*C*H$_2$CH$_3$), 1.33 (t, J = 7.0 Hz, 3H, OCH$_2$C*H*$_3$a), 1.31 (t, J = 9.5 Hz, 3H, OCH$_2$C*H*$_3$b). ^{13}C NMR (125 MHz, $CDCl_3$): δ [168.7, 168.4 (CO_2)], [156.9, 155.8, (NCO_2)], 143.7, 143.5, 141.44, 141.39, 141.15, 141.14, 140.59, 140.56, [140.34, 140.27 (2 × d, $J_{C,P}$ = 20.3 Hz, triazolyl CH)], [134.7, 134.6 (*Ar*C)], 130.3, 128.3, 128.6 (ArH), 128.53 (ArH), 128.47 (ArH), 127.7, 126.63, 126.62, [127.3, 127.2 (2 d, $J_{C,P}$ = 221.4 Hz, CP)], 127.01, 126.99, 126.5, 126.4, 120.0, 119.9, 67.9, 67.4, 65.0, 64.0, [63.99, 63.92 (2 d, $J_{C,P}$ = 5.9 Hz, O*C*H$_2$CH$_3$a)], [63.8, 63.7 (2 d, $J_{C,P}$ = 5.5 Hz, O*C*H$_2$CH$_3$b)], [54.3, 54.0 (α)], [50.5, 50.4 (β)], 46.9 (C9′), 16.11 (d, $J_{C,P}$ = 6.0 Hz, OCH$_2$*C*H$_3$a), 16.07 (d, $J_{C,P}$ = 5.2 Hz, OCH$_2$*C*H$_3$b). ^{31}P NMR (120 MHz, $CDCl_3$): δ 4.54. MS (ESI) *m/z*: 605 $[M+H]^+$, 627 $[M+Na]^+$. HRMS (ESI): observed, 605.2168, $[C_{31}H_{33}N_4O_7P+H]^+$ requires 605.2160. $[\alpha]_D$ = +2.3° (*c* 1.0, $CHCl_3$).

General procedure for Cu(I)-catalysed azide-alkyne cycloadditions

$CuSO_4.5H_2O$ (10 mol %) was added to a solution of the azide **9** (0.6–1.3 mmol), **10** (1 eqv.) and sodium ascorbate (10 mol %) in 1:1 *t*-BuOH/water (5–15 mL). The resulting solution was stirred for 24 h, diluted with water (10–20 mL), and extracted with DCM or EtOAc (3 × 30–100 mL). The organic extract was washed with brine (10–20 mL), dried and evaporated, and the residue was subjected to flash chromatography (details below).

*(S)-Methyl 2-(t-butoxycarbonylamino)-3-(4-(diethoxyphosphoryl)-1H-1,2,3-triazol-1-yl) propanoate [Boc-4-PO(OEt)$_2$Tza-OMe] (**12a**)*

Following the general procedure with **9a** (0.200 g, 0.819 mmol), flash chromatography and elution with EtOAc gave **12a** as pale yellow oil (316 mg, 95%). $R_f = 0.10$ (EtOAc/hexanes 1:1); IR (thin film) cm^{-1}: 3,293 (NH), 1,744 (m, C=O), 1,716 (s, NC=O).^{1}H NMR (600 MHz, $CDCl_3$): δ 8.03 (s, 1H, triazolyl), 5.78 (br d, J = 7.7 Hz, 1H, NH), 4.76 (dd, J = 13.5, 4.5 Hz, 1H, β-Ha), 4.70 (dd, J = 14.0, 6.5 Hz, 1H, β-Hb), 4.55 (m, 1H, α-H), 3.99 (m, 4H, OCH_2CH$_3$), 3.59 (s, 3H, OCH_3), 1.21 (s, 9H, *t*-Bu), 1.144, (t, J = 7.2 Hz, 3H, OCH_2CH_3a), 1.140, (t, J = 7.2 Hz, 3H, OCH_2CH_3b); ^{13}C NMR (150 MHz, $CDCl_3$): δ 169.1 (CO_2), 154.8 (NCO_2), 136.7 (d, $J_{C,P}$ = 239 Hz, CP), 131.6 (d, $J_{C,P}$ = 32 Hz, triazolyl CH), 80.0 [O*C*(CH$_3$)$_3$], 62.59 (d, $J_{C,P}$ = 5.7 Hz, O*C*H$_2$CH$_3$a), 62.58 (d, $J_{C,P}$ = 5.9 Hz, O*C*H$_2$CH$_3$b), 53.3 (α), 52.5 (OCH_3), 50.1 (β), 27.7 [C*C*H$_3$)$_3$], 15.8 (d, $J_{C,P}$ = 6.5 Hz, OCH_2CH_3); ^{31}P NMR (240 MHz): δ 6.97. MS (ESI) *m/z*: 407 ([M+H]$^+$; HRMS (ESI): observed, 407.1696, [$C_{15}H_{27}N_4O_7P$+H]$^+$ requires 407.1690. $[\alpha]_D = -15.9°$ (*c* 2.2, EtOAc).

*(S)-Methyl 2-acetamido-3-(4-(diethoxyphosphoryl)-1H-1,2,3-triazol-1-yl)propanoate [Ac-4-PO(OEt)$_2$Tza-OMe] (**12b**)*

Following the general procedure with **9b** (0.250 g, 1.34 mmol), flash chromatography and elution with EtOAc gave **12b** as pale yellow oil (425 mg, 91%). $R_f = 0.10$ (EtOAc). IR (thin film) cm^{-1}: 3,272 (NH), 1,747 (OC=O), 1,673 (NC=O). ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.11 (s, 1H, triazolyl), 7.57 (br d, 1H, J = 7.5 Hz, NH), 4.78 (m, 2H, β-H), 4.69 (dd, 1H, J = 14.0, 7.0 Hz, α-H), 3.96 (m, 4H, 2 × OCH_2CH$_3$), 3.55 (s, 3H, OCH_3) 1.78 (s, 3H, acetyl CH_3), 1.121 (t, 3H, J = 7.0 Hz, OCH_2CH_3a), 1.118 (t, 3H, J = 7.0 Hz, OCH_2CH_3b). ^{13}C NMR (125 MHz,): δ 170.5 (C=O), 168.9 (C=O), 136.3 (d, $J_{C,P}$ = 240.2 Hz, CP), 131.6 (d, $J_{C,P}$ = 33.1 Hz, triazolyl CH), 62.7 (d, $J_{C,P}$ = 5.8 Hz, O*C*H$_2$CH$_3$), 52.4, 52.0, 49.8, 22.0 (acetyl CH_3), 15.70 (d, $J_{C,P}$ = 0.6 Hz, OCH_2CH_3a), 15.65 ($J_{C,P}$ = 0.75 Hz, OCH_2CH_3b). ^{31}P NMR (120 MHz, $CDCl_3$): δ 7.71. MS (ESI) *m/z*: 349 [M+H]$^+$, 371 [M+Na]$^+$. HRMS (ESI): observed, 371.1086, [$C_{12}H_{21}N_4O_6P$+Na]$^+$ requires 371.1091. $[\alpha]_D = +62.0°$ (*c* 1.0, CH_3Cl).

*(S)-Allyl 2-acetamido-3-(4-(diethoxyphosphoryl)-1H-1,2,3-triazol-1-yl)propanoate [Ac-4-PO(OEt)$_2$Tza-OAll] (**12c**)*

Following the general procedure with **9c** (250 mg, 1.2 mmol), flash chromatography and elution with EtOAc gave **12c** as pale yellow oil (371 mg, 84%). $R_f = 0.15$ (EtOAc); IR (thin film) cm^{-1}: 3,271 (NH), 1,745 (OC=O), 1,674 (NC=O). ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.10 (s, 1H triazolyl), 7.21 (br d, 1H, J = 7.5 Hz, NH), 5.80 (m, 1H, *H*C=CH$_2$), 5.25 (ddd, 1H, J = 17.5, 3.0, 1.5 Hz HC=C*H*$_2$ *trans*), 5.19 (ddd, 1H, J = 10.5, 2.5, 1.5 Hz HC=C*H*$_2$ *cis*), 4.93 (m, 1H, α-H), 4.85 (m, 2H, β-H), 4.57 (apparent dt, 2H, J = 5.5 Hz, 1.0 Hz, allylic CH_2), 4.09 (m, 4H, OCH_2CH$_3$), 1.92 (s, 1H, $NCOCH_3$), 1.251 (t, 3H, J = 7.5 Hz, OCH_2CH$_3$a), 1.248 (t, 3H, J = 7.5 Hz, OCH_2CH_3b). ^{13}C NMR (125 MHz, $CDCl_3$): δ 170.6 (C=O), 168.3 (C=O), 136.9 (d, $J_{C,P}$ = 239.8 Hz, CH), 131.8 (d, $J_{C,P}$ = 33.1 Hz, triazolyl CH), 130.9 (H*C*=CH$_2$), 119.2 (HC=*C*H$_2$), 66.6 (allylic), 63.0 (d, $J_{C,P}$ = 0.9 Hz, O*C*H$_2$CH$_3$a), 62.9 (d, $J_{C,P}$ = 0.8 Hz, O*C*H$_2$CH$_3$b), 52.4, 50.2, 22.5 (acetyl CH_3), 16.02 (d, $J_{C,P}$ = 1.3 Hz, OCH_2-*C*H$_3$a), 15.96 (d, $J_{C,P}$ = 1.1 Hz, OCH_2CH_3b). ^{31}P NMR (120 MHz, $CDCl_3$): δ 7.52. MS (ESI) *m/z*: 375 [M+H]$^+$. HRMS (ESI): observed, 375.1433, [$C_{14}H_{23}N_4O_6P$+H]$^+$ requires 375.1428. $[\alpha]_D = +25.3°$ (*c* 1.0, CH_3Cl).

*(S)-Benzyl 2-acetamido-3-(4-(diethoxyphosphoryl)-1H-1,2,3-triazol-1-yl)propanoate [Ac-4-PO(OEt)$_2$Tza-OBn] (**12d**)*

Following the general procedure with **9d** (0.200 mg, 0.763 mmol), flash chromatography and elution with EtOAc gave **12d** as pale yellow oil (282 mg, 87%). $R_f = 0.20$ (EtOAc). IR (thin film) cm^{-1}: 3,271 (NH), 1,744 (OC=O), 1,675 (NC=O). ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.10 (s, 1H, triazolyl), 7.67 (br d, 1H, J = 8.0 Hz, NH), 7.15 (m, 5H, Ar), 5.00 (s, 2H, OCH_2Ph), 4.88 (dd, 1H, J = 7.0, 4.5 Hz, α-H), 4.81 (dd, 1H, J = 14.0, 4.5 Hz, β-Ha), 4.71 (dd, 1H, J = 14.0, 7.5 Hz, β-Hb), 3.98 (m, 4H, OCH_2CH$_3$) 1.79 (s, 1H, $NCOCH_3$), 1.13 (t, 6H, J = 7.0 Hz, OCH_2CH_3) ^{13}C NMR (125 MHz, $CDCl_3$): δ 170.3 (C=O), 168.3 (C=O), 136.3 (d, $J_{C,P}$ = 239.8 Hz, CP), 134.4 (*Ar*C), 131.5 (d, $J_{C,P}$ = 33.1 Hz, triazolyl CH), 128.0 (ArH), 127.9 (ArH), 127.7 (ArH), 67.1 (benzylic), 62.5, (d, $J_{C,P}$ = 5.7 Hz, O*C*H$_2$CH$_3$) 52.1, 49.7, 22.0 (acetyl CH_3), 15.6 (d, $J_{C,P}$ = 6.4 Hz, OCH_2CH_3). ^{31}P NMR

(120 MHz, $CDCl_3$): δ 7.73. MS (ESI) *m/z*: 425 $[M+H]^+$, 447 $[M+Na]^+$. HRMS (ESI): observed, 447.1410, $[C_{18}H_{25}N_4O_6P+Na]^+$ requires 447.1404. $[\alpha]_D = +13.9°$ (*c* 1.0, CH_3Cl).

*(S)-Benzyl 2-(t-butoxycarbonylamino)-3-(4-(diethoxyphosphoryl)-1H-1,2,3-triazol-1-yl) propanoate [Boc-4-PO(OEt)$_2$Tza-OBn] (**12e**)*

Following the general procedure with **9e** (0.200 mg, 0.624 mmol), flash chromatography and elution with EtOAc gave **12e** as pale yellow oil (265 mg, 88%). $R_f = 0.10$ (1:1 EtOAc/hexanes). IR (thin film) cm^{-1}: 3,293 (NH), 1,744 (m, C=O), 1,716 (s, NC=O).^{1}H NMR (500 MHz, $CDCl_3$): δ 8.03 (s, 1H, triazolyl), 7.27 (5H, m, Ar) 5.71 (br d, J = 7.5 Hz, 1H, NH), 5.12 (d, J = 2.0 Hz, 2H, OCH_2Ph), 4.84 (m, 2H, β-H), 4.68 (m, 1H, α-H), 4.10 (m, 4H, OCH_2CH_3), 1.33 (s, 9H, *t*-Bu), 1.261, (t, J = 7.0 Hz, 3H, OCH_2CH_3a), 1.259, (t, J = 7.0 Hz, 3H, OCH_2CH_3b); ^{13}C NMR (125 MHz, $CDCl_3$): δ 168.6 (CO_2), 154.9 (NCO_2), 137.0 (d, $J_{C,P} = 239.4$ Hz, CP), 134.4 (*Ar*C) 131.2 (d, $J_{C,P} = 33.1$ Hz, triazolyl CH), 128.4 (ArH), 128.4 (ArH), 128.2 (ArH), 80.4 [$OC(CH_3)_3$], 67.8 (OCH_2Ph), 62.8 (d, $J_{C,P} = 5.8$ Hz, OCH_2CH_3), 53.5 (α), 50.5 (β), 27.9 [$CCH_3)_3$], 15.9 (d, $J_{C,P} = 6.5$ Hz, OCH_2CH_3). ^{31}P NMR (120 MHz, $CDCl_3$): δ 7.52. MS (ESI) *m/z*: 483 $[M+H]^+$, 505 $[M+Na]^+$. HRMS (ESI): observed, 505.1825, $[C_{21}H_{31}N_4O_7P+Na]^+$ requires 505.1823. $[\alpha]_D = +11.1°$ (*c* 1.0, $CHCl_3$).

*(S)-Benzyl 2-(((9H-fluoren-9-yl)methoxy)carbonylamino)-3-(4-(diethoxyphosphoryl)-1H-1,2,3-triazol-1-yl)propanoate [Fmoc-4-PO(OEt)$_2$Tza-OBn] (**12f**)*

Following the general procedure with **9f** (0.100 mg, 0.226 mmol), flash chromatography and elution with EtOAc gave **12f** as a white solid (116 mg, 85%). mp 100–101°C $R_f = 0.10$ (1:1 EtOAc/hexanes). IR (thin film) cm^{-1}: 3,271 (NH), 1,700–1,750 (br, C=O + NC=O). ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.10 (s, 1H, triazolyl), 7.73 (d, J = 7.5 Hz, 2H, Ar) 7.54 (d, J = 7.0 Hz, 2H, Ar), 7.22–7.39 (m, 9H, Ar), 6.31 (br d, J = 7.5 Hz, 1H, NH), 5.17 (br s, 2H, OCH_2Ph), 4.90 (m [apparent br d], 2H, β-H), 4.83 (m, 1H, α-H), 4.33 (m [apparent t], 2H, NCO_2CH_2), 4.09–4.22 (m, 5H, H9′ + 2 × OCH_2CH_3), 1.26–1.32 (m, 6H, 2 × OCH_2CH_3). The ^{13}C NMR spectra showed the presence of two rotamers. ^{13}C NMR (125 MHz, $CDCl_3$): δ 168.3 (CO_2), 155.7 (NCO_2), 143.3, 141.0, 137.2 (d, $J_{C,P} = 239.1$ Hz, CP), 134.4 (*Ar*C), 131.8 (d, $J_{C,P} = 33.5$ Hz, triazolyl CH), 128.5 (ArH), 128.46 (ArH), 128.3 (ArH), 127.6, 126.92, 126.88, 124.93, 124.85, 119.8, [68.0, 67.2 (benzylic CH_2)], 62.9 (d, $J_{C,P} = 5.7$ Hz, OCH_2CH_3), 54.0 (α), 50.4 (β), 46.8 (C9′) 16.03 (br s, OCH_2CH_3a), 15.98 (d, $J_{C,P} = 1.0$ Hz, OCH_2CH_3b). ^{31}P NMR (120 MHz, $CDCl_3$): δ 7.56. MS (ESI) *m/z*: 605 $[M+H]^+$, 627 $[M+Na]^+$. HRMS (ESI): observed, 627.1975, $[C_{31}H_{33}N_4O_7P+Na]^+$ requires 627.1979. $[\alpha]_D = +10.4°$ (*c* 1.0, $CHCl_3$).

*(S)-t-butyl 3-(5-(diethoxyphosphoryl)-1H-1,2,3-triazol-1-yl)-1-(methylamino)-1-oxopropan-2-ylcarbamate [Boc-5-PO(OEt)$_2$Tza-NHMe] (**13**)*

Thirty-three percentage aqueous methylamine (2.4 mL, 25 mmol) was added to a solution of **11a** (240 mg, 0.60 mmol) in MeOH (5 mL). The solution was stirred at room temperature for 4 h, the volatiles were evaporated, and the residue was subjected to flash chromatography. Elution with MeOH/DCM (1:20) gave **13** as pale yellow oil (207 mg, 85%). $R_f = 0.20$ (1:20 MeOH/DCM). IR (thin film) cm^{-1}: 3,307 (NH), 1,716 (OC=O), 1,672 (NC=O). ^{1}H NMR (500 MHz, CD_3OD): δ 8.11 (s, 1H, triazolyl), 5.12 (br d, 1H, J = 11.0 Hz, α-H), 4.74 (m, 2H, β-H), 4.25 (m, 4H, 2 × OCH_2CH_3), 2.75 (s, 1H, NCH_3), 1.38, (t, 6H, J = 7.5 Hz, 2 × OCH_2CH_3), 1.33 (s, 9H, *t*-Bu). ^{13}C NMR (125 MHz, CD_3OD): δ 171.5 (CO_2), 157.2 (NCO_2), 141.6 (d, $J_{C,P} = 20.8$ Hz, triazolyl CH), 127.9 (d, $J_{C,P} = 222$ Hz, CP), 80.9 [$OC(CH_3)_3$], 65.5 (d, $J_{C,P} = 5.8$ Hz, OCH_2CH_3a), 65.4 (d, $J_{C,P} = 5.9$ Hz, OCH_2CH_3b), 55.7, 52.3, 28.6, 26.5, 16.54 (d, $J_{C,P} = 2.9$ Hz, OCH_2CH_3a), 16.49 (d, $J_{C,P} = 3.0$ Hz, OCH_2CH_3b). ^{31}P NMR (120 MHz, CD_3OD): δ 4.74. MS (ESI) *m/z*: 406 $[M+H]^+$, 428 $[M+Na]^+$. HRMS (ESI): observed, 428.1673, $[C_{15}H_{28}N_5O_6P+Na]^+$ requires 428.1669. $[\alpha]_D = -33.8°$ (*c* 1.0, MeOH).

*(S)-t-Butyl 3-(4-(diethoxyphosphoryl)-1H-1,2,3-triazol-1-yl)-1-(methylamino)-1-oxopropan-2-ylcarbamate [Boc-4-PO(OEt)$_2$Tza-NHMe] (**14**)*

Thirty-three percentage aqueous methylamine (3.0 mL, 31 mmol) was added to a solution of triazole **12a** (0.30 g, 0.74 mmol) in MeOH (5 mL). The solution was stirred at room temperature for 4 h, then the volatiles were evaporated, and the residue was subjected to flash chromatography. Elution with MeOH/DCM (1:10) gave **14** as pale yellow oil (270 mg, 90%). $R_f = 0.20$ (MeOH/DCM 1:9). IR (thin film) cm^{-1}: 3,305 (NH), 1,712 (OC=O), 1,666 (NC=O). ^{1}H NMR (500 MHz, CD_3OD): δ 8.39 (s, 1H, triazolyl), 4.92 (dd, 1H, J = 12.5 Hz, 3.0 Hz, α-H), 4.63 (m, 2H, β-H), 4.14 (m, 4H, 2 × OCH_2CH_3), 2.70 (s, 1H, $CONHCH_3$), 1.32 (s, 9H, *t*-Bu), 1.288 (t, 3H, J = 7.0 Hz, OCH_2CH_3a), 1.287 (t, 3H, J = 7.0 Hz, OCH_2CH_3b). ^{13}C NMR (125 MHz, CD_3OD): δ 171.3 (CO_2), 157.1 (NCO_2), 137.4 (d, $J_{C,P} = 242.2$ Hz, CP), 133.5 (d, $J_{C,P} = 33.2$ Hz, triazolyl CH), 81.0, 64.45 (d, $J_{C,P} = 4.2$ Hz, OCH_2CH_3a), 64.50

(d, $J_{C,P} = 4.2$ Hz, OCH_2CH_3b), 55.7, 52.3, 28.6, 26.5, 16.6 (d, $J_{C,P} = 6.5$ Hz, OCH_2CH_3). ^{31}P NMR (120 MHz, CD_3OD): δ 8.73. MS (ESI) *m/z*: 406 $[M+H]^+$, 428 $[M+Na]^+$. HRMS (ESI): observed, 406.1855, $[C_{15}H_{28}N_5O_6P+H]^+$ requires 406.1850. $[\alpha]_D = -5.4°$ (*c* 1.0, MeOH).

*(S)-1-(Methylamino)-1-oxo-3-(5-phosphono-1H-1,2,3-triazol-1-yl)propan-2-aminium bromide [H_2N-5-$PO(OH)_2$Tza-NHMe.HBr] (**15**)*

A solution of **123** (0.10 g, 0.25 mmol) in 33% HBr in AcOH (2.0 mL, 8.1 mmol) was stirred for 48 h. The volatiles were evaporated and the residue was purified by Dowex cation-exchange chromatography. Elution with water gave **15** as a white solid (54 mg, 71%). $R_f = 1.0$ on reverse-phase TLC (H_2O), mp 141–143°C. IR (KBr) cm^{-1}: 3,000–3,500 (br, NH_3 + OHs), 1,685 (C=O). ^{1}H NMR (600 MHz, D_2O): δ 7.73 (s, 1H, triazolyl), 4.89 (dd, J = 6.6, 2.4 Hz, 2H, β-H), 4.37 (apparent t, J = 6.0 Hz, 1H, α-H), 2.51 (s, 1H, CH_3). ^{13}C NMR (125 MHz, D_2O): δ 166.4 (C=O), 138.4 (br s, triazolyl CH), 134.6 (d, $J_{C,P} = 192.1$ Hz, CP), 52.5, 49.0, 25.9 (CH_3). ^{31}P NMR (120 MHz, D_2O): δ −3.48 MS (ESI) *m/z*: 250 $[M+H]^+$ (where M = free base); HRMS (ESI): observed, 250.0703, $[C_6H_{11}N_5NaO_4P+H]^+$ requires 250.0700. $[\alpha]_D = +38.4°$ (*c* 1.0, H_2O).

*(S)-1-(Methylamino)-1-oxo-3-(4-phosphono-1H-1,2,3-triazol-1-yl)propan-2-aminium bromide [H_2N-4-$PO(OH)_2$Tza-NHMe.HBr] (**16**)*

A solution of **14** (0.10 g, 0.25 mmol) in 33% HBr in AcOH (2.00 mL, 8.1 mmol) was stirred for 48 h. The volatiles were evaporated and the residue was purified by Dowex cation-exchange chromatography. Elution with water gave **16** as a white solid (56 mg, 73%). $R_f = 1.0$ on reverse-phase TLC (H_2O). mp 145–146°C. IR (KBr disk) cm^{-1}: 3,100–3,500 (br, NH_3 + OHs), 1,646 (C=O). ^{1}H NMR (500 MHz, D_2O): δ 8.14 (s, 1H, triazolyl), 4.97 (d, J = 5.5 Hz, 2H, β-H), 4.54 (apparent t, J = 5.0 Hz, 1H, α-H), 2.69 (s, 1H, CH_3). ^{13}C NMR (125 MHz, D_2O): δ 167.0 (C=O), 144.5 (d, $J_{C,P} = 214.7$ Hz, CP), 129.7 (d, $J_{C,P} = 28.9$ Hz, triazolyl CH), 52.9, 49.8, 26.3 (CH_3). ^{31}P NMR (120 MHz, D_2O): δ 1.55 MS (ESI) *m/z*: 272 $[M+Na]^+$, 294 $[M+2Na-H]^+$ (where M = free base); HRMS (ESI): observed, 272.0522, $[C_6H_{12}N_5NaO_4P+Na]^+$ requires 272.0519. $[\alpha]_D = +27.2°$ (*c* 1.0, H_2O).

*(S)-Methyl 2-amino-3-(4-(diethoxyphosphoryl)-1H-1,2,3-triazol-1-yl)propanoate [H_2N-4-$PO(OH)_2$Tza-OMe.HBr] (**17**)*

TFA (1.0 mL, 13 mmol) was added to a solution of **12a** in DCM (10 mL) at 0°C. The solution was allowed to warm to room temperature and stirred for 5 h; then the volatiles were evaporated. The residue was diluted with sat. aq. $NaHCO_3$ (30 mL) and extracted with EtOAc (3 × 50 mL). The organic extract was washed with brine (30 mL), dried and evaporated, and the residue was subjected to flash chromatography. Elution with 1:10 MeOH/DCM gave **17** as pale yellow oil (302 mg, 80%). $R_f = 0.35$ (1:9 MeOH/DCM). IR (thin film) cm^{-1}: 3,392 (NH), 1,741 (C=O). ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.19 (s, 1H, triazolyl), 4.75 (dd, J = 13.5, 4.0 Hz, 1H, β-Ha), 4.54 (dd, J = 13.5, 7.0 Hz, 1H, β-Hb), 4.17 (m, 4H, 2 × OCH_2CH_3), 3.97 (br s, 1H, α), 3.74 (s, 3H, OCH_3), 1.312, (t, J = 7.0 Hz, 3H, OCH_2CH_3a), 1.311, (t, J = 7.0 Hz, 3H, OCH_2CH_3b). ^{13}C NMR (150 MHz, $CDCl_3$): δ 172.3 (CO_2), 136.9 (d, $J_{C,P} = 239.6$ Hz, CP), 132.0 (d, $J_{C,P} = 33.3$ Hz, triazolyl CH), 63.0 (d, $J_{C,P} = 5.8$ Hz, OCH_2CH_3), 54.2 (α), 53.3 (OMe), 52.6 (β), 16.0 (d, $J_{C,P} = 6.5$ Hz, OCH_2CH_3). ^{31}P NMR (120 MHz, $CDCl_3$): δ 7.73. MS (ESI) *m/z*: 307 $[M+H]^+$. HRMS (ESI): observed, 307.1160, $[C_{10}H_{19}N_4O_5P+H]^+$ requires 307.1166. $[\alpha]_D = -4.9°$ (*c* 1.0, $CHCl_3$).

*(S)-Allyl 2-acetamido-3-hydroxypropanoate [Ac-Ser-OAll] (**22**); (S)-Allyl 2-acetamido-3-acetoxypropanoate [Ac-Ser(OAc)-OAll] (**24**)*

Acetic anhydride (6.30 mL, 67.0 mmol) was added to a suspension of L-serine (5.90 g, 56.1 mmol) in AcOH (25 mL). The mixture was stirred for 18 h, and then the volatiles were evaporated. The residue was dissolved in DMF (25 mL), and allyl bromide (4.9 mL 56 mmol) and DIPEA (9.8 mL, 56 mmol) were added. The resulting solution was stirred for 18 h, diluted with sat. aq. NH_4Cl, and extracted with EtOAc (3 × 50 mL). The organic extract was washed with brine, dried and evaporated, and the residue was subjected to flash chromatography. Elution with EtOAc/hexanes (1:1) gave **24** as a white solid (5.79 g, 45%). mp 81–82°C $R_f = 0.40$ (EtOAc); IR (thin film) cm^{-1}: 3,294 (NH), 1,747 (OC=O), 1,661 (NC=O). ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.27 (d, 1H, J = 8.0 Hz, NH), 5.56 (m, 1H, $HC = CH_2$), 4.98 (dd, 1H, J = 17.0, 1.5 Hz, $HC = CH_2$ *trans*), 4.89 (dd, 1H, J = 10.0, 1.0 Hz, $HC = CH_2$ *cis*), 4.53 (1H, m, α-H), 4.30 (m, 2H, allylic), 4.04 (dd, 2H, J = 6.5, 5.0 Hz, β-H), 1.69 (s, 3H, OCH_3), 1.68 (s, 3H, acetyl CH_3). ^{13}C NMR (125 MHz, $CDCl_3$): δ 169.9 (C=O), 169.6 (C=O), 168.5 (C=O), 130.8 (vinylic CH), 117.7 (vinylic CH_2), 65.2, 62.9, 50.9, 21.7 (CH_3), 20.1 (CH_3); MS (ESI) *m/z*: 230 $[M+H]^+$, 252 $[M+Na]^+$. HRMS (ESI): observed, 230.1020, $[C_{10}H_{15}NO_5+H]^+$ requires 230.1023. $[\alpha]_D = +58.8°$ (*c* 1.0, CH_3Cl).

Further elution with EtOAc gave **22** as brown oil (2.21 g, 21%). $R_f = 0.15$ (EtOAc); IR (thin film) cm^{-1}: 3,366 (NH), 1,741 (OC=O), 1,655 (NC=O). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.28 (d, 1H, J = 7.6 Hz, NH), 5.77

(m, 1H, *H*C=CH_2), 5.20 (ddd, 1H, *J* = 17.6, 3.2, 1.6 Hz, HC=CH_2 *trans*), 5.11 (ddd, 1H, *J* = 10.4, 2.4, 1.2 Hz, HC=CH_2 *cis*), 4.51 (2H, dt, *J* = 6.0 Hz, 1.2 Hz, allylic CH_2), 4.47 (dd, 1H, *J* = 8.0, 4.0 Hz, α-H), 3.83 (dd, 1H, *J* = 11.2, 4.0 Hz, *β*-Ha), 3.71 (dd, 1H, *J* = 11.2, 3.2 Hz, *β*-Hb), 1.90 (s, 1H, acetyl CH_3). ^{13}C NMR (100 MHz, $CDCl_3$): δ 171.2 (C=O), 170.1 (C=O), 131.2 (vinylic CH), 118.3 (vinylic CH_2), 65.8, 62.0, 54.5, 22.3 (acetyl CH_3). MS (ESI) *m/z*: 188 $[M+H]^+$, 210 $[M+Na]^+$. HRMS (ESI): observed, 188.0914, $[C_8H_{13}NO_4+H]^+$ requires 188.0917. $[\alpha]_D = +33.6°$ (*c* 1.0, CH_3Cl).

*(S)-Benzyl 2-acetamido-3-hydroxypropanoate [Ac-Ser-OBn] (**23**); (S)-Benzyl 2-acetamido-3-acetoxypropanoate [Ac-Ser(OAc)-OBn] (**25**)*

Acetic anhydride (6.30 mL, 67.0 mmol) was added to a suspension of L-serine (5.90 g, 56.1 mmol) in AcOH (25 mL). The mixture was stirred for 18 h, and then the volatiles were evaporated. The residue was dissolved in DMF (25 mL), and benzyl bromide (6.70 mL 56.4 mmol) and DIPEA (9.80 mL, 56.3 mmol) were added. The resulting solution was stirred for 18 h, diluted with sat. aq. NH_4Cl, and then extracted with EtOAc (3 × 50 mL). The organic extract was washed with brine (30 mL), dried and evaporated, and the residue was subjected to flash chromatography. Elution with EtOAc/hexanes (1:1) gave **25** as a white solid (7.05 g, 45%). $R_f = 0.50$ (EtOAc). IR (thin film) cm^{-1}: 3,293 (NH), 1,746 (OC=O), 1,661 (NC=O). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.28 (m, 5H, Ar), 7.00 (br d, 1H, *J* = 8.0 Hz, NH), 5.13 (dd, 2H, *J* = 31.6 Hz, 12.4 Hz, OCH_2Ph), 4.87 (m, 1H, α-H), 4.42 (dd, 1H, *J* = 11.2 Hz, 4.0 Hz, *β*-Ha), 4.29 (dd, 1H, *J* = 11.2 Hz, 3.6 Hz, *β*-Hb), 1.97 (s, 1H, $NCOCH_3$), 1.87 (s, 1H, $COCH_3$). ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.1 (C=O), 169.9 (C=O), 169.1 (C=O), 134.7 (*Ar*C), 128.2 (ArH), 128.1 (ArH), 127.9 (ArH), 67.0, 63.5, 51.3, 22.4 (CH_3), 20.0 (CH_3). MS (ESI) *m/z*: 280 $[M+H]^+$, 302 $[M+Na]^+$. HRMS (ESI): observed, 302.1005, $[C_{14}H_{17}NO_5+Na]^+$ requires 302.0999. $[\alpha]_D = +24.5°$ (*c* 1.0, CH_3Cl).

Further elution with EtOAc gave **23** as a white solid (4.15 g, 31%). mp 60–62°C. $R_f = 0.20$ (EtOAc); IR (thin film) cm^{-1}: 3,313 (NH), 1,742 (OC=O), 1,657 (NC=O). ^{1}H NMR (400 MHz, $CDCl_3$): δ; 7.30 (m, 5H, Ar), 7.14 (br d, 1H, *J* = 7.6 Hz, NH), 5.14 (d, 2H, *J* = 1.2 Hz, OCH_2Ph), 4.63 (m, 1H, α-H), 3.95 (dd, 1H, *J* = 11.6 Hz, 4.0 Hz, *β*-Ha), 3.81 (dd, 1H, *J* = 11.2 Hz, 3.2 Hz, *β*-Hb), 1.97 (s, 3H, CH_3). ^{13}C NMR (100 MHz, $CDCl_3$): δ 171.1 (C=O), 170.3 (C=O), 135.0 (*Ar*C), 128.4 (ArH), 128.2 (ArH), 127.8 (ArH), 67.1 (OCH_2Ph), 62.4, 54.7, 22.6 (CH_3). MS (ESI) *m/z*: 238 $[M+H]^+$, 260 $[M+Na]^+$. HRMS (ESI): observed, 238.1077, $[C_{12}H_{16}NO+H]^+$ requires 238.1074. $[\alpha]_D = +14.3°$ (*c* 1.0, CH_3Cl).

*(S)-2-Acetamido-3-(4-(diethoxyphosphoryl)-1H-1,2,3-triazol-1-yl)propanoic acid [Ac-4-PO(OEt)$_2$Tza-OH] (**27**)*

Method A, saponification using LiOH: $LiOH.H_2O$ (60 mg, 1.44 mmol) was added to a solution of **12b** (0.500 g, 1.44 mmol) in 3:1 MeOH/water (15 mL) at 0°C. After 2 h, the reaction mixture was acidified to pH 4–5 with sat. aq. $KHSO_4$ and then extracted with EtOAc (3 × 50 mL). The organic extract was washed with brine (10 mL), dried and evaporated, and the residue was subjected to flash chromatography. Elution with 1:4 MeOH/DCM gave **27** as colourless oil (144 mg, 30%). Identical in every respect except optical activity with the material described below. $[\alpha]_D = +5.1°$ (*c* 1.0, MeOH).

Method B, saponification using K_2CO_3: K_2CO_3 (0.200 g, 1.44 mmol) was added to a solution of **12b** (0.500 g, 1.44 mmol) in 3:1 MeOH/water (15 mL) at 0°C. The solution was allowed to warm to room temperature and stirred for 2 h, then acidified to pH 4 with sat. aq. $KHSO_4$, and extracted with EtOAc (3 × 50 mL). The organic extract was washed with brine (10 mL), dried and evaporated, and the residue was subjected to flash chromatography. Elution with 1:4 MeOH/DCM gave **27** as colourless oil (226 mg, 47%). $R_f = 0.10$ (1:4 MeOH/DCM). IR (thin film) cm^{-1}: 3,293 (NH), 1,720 (C=O), 1,618 (NC=O).^{1}H NMR (500 MHz, CD_3OD): δ 8.47 (s, 1H, triazolyl), 5.06 (m, 1H, *β*-Ha), 4.78–4.86 (m, 2H, *β*-Hb+α-H), 4.20 (m, 4H, OCH_2CH_3), 1.96 (s, 3H, NCH_3), 1.361 (t, *J* = 7.0 Hz, 3H, OCH_2CH_3a), 1.360 (t, *J* = 7.2 Hz, 3H, OCH_2CH_3b); ^{13}C NMR (125 MHz, CD_3OD): δ 175.7 (CO), 173.9 (CO), 138.0 (d, $J_{C,P} = 242.3$ Hz, CP), 134.3 (d, $J_{C,P} = 33.3$ Hz, triazolyl CH), 65.47 (d, $J_{C,P} = 0.9$ Hz, OCH_2CH_3a), 65.43 (d, $J_{C,P} = 0.7$ Hz, OCH_2CH_3b), 57.0 (α), 53.8 (*β*), 23.5 (CH_3), 17.4 (d, $J_{C,P} = 6.5$ Hz, OCH_2CH_3). ^{31}P NMR (120 MHz, CD_3OD): δ 8.90. MS (ESI) *m/z*: 335 $[M+H]^+$, 357 [M+Na]. HRMS (ESI): observed, 357.1046, $[C_{11}H_{19}N_4O_6P+Na]^+$ requires 357.0934. $[\alpha]_D = +8.8°$ (*c* 1.0, MeOH).

*(S)-2-Acetamido-3-(5-phosphono-1H-1,2,3-triazol-1-yl)propanoic acid [Ac-4-PO(OH)$_2$Tza-OH] (**29**)*

A solution of **11d** (0.20 g, 0.47 mmol) in 33% HBr in AcOH (4.0 mL, 16 mmol) was stirred for 48 h. The volatiles were evaporated and the residue was purified by Dowex anion exchange chromatography. Elution with water gave **29** as a white solid (90 mg, 69%). mp 146–148°C. IR (KBr disk) cm^{-1}: 3,000–3,500 (br, NH_3 + OHs), 1,717 (C=O), 1,650 (NC=O). ^{1}H NMR (500 MHz, D_2O): δ 7.83 (s, 1H, triazolyl), 4.71 (dd, 1H, *J* = 14.0, 3.5 Hz, α-H), 4.54 (dd, 1H, *J* = 9.0, 3.5 Hz, *β*-Ha), 4.46 (dd, 1H, *J* = 14.0, 9.5 Hz, *β*-Hb), 1.38 (s, 3H,

CH_3). ^{13}C NMR (125 MHz, D_2O): δ 173.8 (C=O), 170.5 (C=O), 136.1 (d, $J_{C,P} = 197.9$ Hz, CP), 134.5 (br s, triazolyl), 51.6, 51.1, 21.5 (CH_3). ^{31}P NMR (120 MHz, D_2O): δ −2.65. MS (ESI) *m/z*: 279 $[M+H]^+$, 301 $[M+Na]^+$. HRMS (ESI): observed, 301.0305 $[C_7H_{11}N_4O_6P+Na]^+$ requires 301.0308; $[\alpha]_D = -23.8°$ (*c* 1.0, H_2O).

*(S)-2-Acetamido-3-(4-phosphono-1H-1,2,3-triazol-1-yl)propanoic acid [Ac-5-PO(OH)$_2$Tza-OH] (**30**)*

*Method A, from the benzyl ester **12d**:* A solution of **12d** (200 mg, 0.47 mmol) in 33% HBr in AcOH (4.0 mL, 16 mmol) was stirred for 48 h. The volatiles were evaporated and the residue was subjected to Dowex anion-exchange chromatography. Elution with water gave **30** as a white solid (88 mg, 67%). mp 135–137°C. IR (KBr disk) cm^{-1}: 3,100–3,500 (br, NH_3 + OHs), 1,732 (C=O), 1,637 (NC=O). ^1H NMR (500 MHz, D_2O): δ 8.10 (s, 1H, triazolyl), 4.71 (dd, 2H, *J* = 12.5, 5.5 Hz, β-H), 4.60 (dd, 1H, *J* = 13.5, 7.0 Hz, α-H), 1.67 (s, 3H, CH_3). ^{13}C NMR (125 MHz, D_2O): δ 173.9, 171.1, 139.9 (d, $J_{C,P} = 223.7$ Hz, CP), 131.5 (d, $J_{C,P} = 30.2$ Hz, triazolyl), 52.2, 50.5, 21.5 (CH_3). ^{31}P NMR (120 MHz, D_2O): δ 2.68. MS (ESI) *m/z*: 279 $[M+H]^+$, 301 $[M+Na]^+$. HRMS (ESI): observed 279.0491 $[C_7H_{11}N_4O_6P+H]^+$ requires 279.0489. $[\alpha]_D = +10.0°$ (*c* 1.0, H_2O).

*Method B, from the carboxylic acid **27**:* A solution of **27** (0.20 g, 0.60 mmol) in 33% HBr in AcOH (5 mL, 20 mmol) was stirred for 48 h. The volatiles were evaporated and the residue was subjected to Dowex anion-exchange chromatography. Elution with water gave **30** (117 mg, 70%) as a white solid, $[\alpha]_D = +6.1°$ (*c* 1.0, H_2O), identical in every other respect with the material described above.

*(S)-2-(t-Butoxycarbonylamino)-3-(5-(diethoxyphosphoryl)-1H-1,2,3-triazol-1-yl)propanoic acid [Boc-5-PO(OEt)$_2$Tza-OH] (**31**).*

LiOH.H_2O (5 mg, 0.1 mmol) was added to a stirred solution of **11a** (51 mg, 0.13 mmol) in 3:1 MeOH/water (4 mL) at 0°C. After 2 h, the reaction mixture was acidified to pH 4 with sat. aq. $KHSO_4$, and then extracted with DCM (3 × 40 mL). The organic extract was washed with brine (10 mL), dried and evaporated, and the residue was purified by flash chromatography. Elution with 1:9 MeOH/DCM gave 26 as colourless oil (41 mg, 86%). $R_f = 0.10$ (1:9 MeOH/DCM); 1H NMR (500 MHz, CD_3OD): δ 8.09 (s, 1H, triazolyl), 5.15 (m, α-CH), 4.74 (m, 2H, β-CH_2), 4.25 (m, 4H, OCH_2CH_3), 1.36 (t, *J* = 7.0 Hz, 6H, OCH_2CH_3), 1.30 (s, 9H, *t*-Bu). ^{13}C NMR (125 MHz, CD_3OD): 175.5 (CO_2), 158.0 (NCO_2), 142.3 (d, $J_{C,P} = 20.6$ Hz, triazolyl CH), 128.4 (d, $J_{C,P} = 222.5$ Hz, CP), 81.1 [$OC(CH_3)_3$], 66.3 (d, $J_{C,P} = 5.5$ Hz, OCH_2-CH_3a), 66.2 (d, $J_{C,P} = 5.5$ Hz, OCH_2CH_3b), 57.1 (α), 54.5 (β), 29.5 $(CH_3)_3$, 17.4 (d, $J_{C,P} = 6.3$ Hz, OCH_2CH_3a), 17.3 (d, $J_{C,P} = 5.9$ Hz, OCH_2CH_3b). MS (ESI) *m/z*: 393 $[M+H]^+$, 415 $[M+Na]^+$.

A sample that had partially degraded was further purified by HPLC to compare with the reported spectra in $CDCl_3$. ^1H NMR (500 MHz, $CDCl_3$): δ 8.00 (br s, 1H, triazolyl), 5.90 (br s, 1H, NH) 5.07 (br m, 1H, α-CH), 4.91 (br m, 2H, β-CH_2), 4.22 (m, 4H, OCH_2CH_3), 1.35–1.40 (m, 6H, OCH_2CH_3), 1.36 (s, 9H, *t*-Bu). ^{13}C NMR (125 MHz, $CDCl_3$): δ 171.1 (br, CO_2), 155.8 (NCO_2), 140.5 (br s, triazolyl CH), 126.7 (br d, $J_{C,P} = 231.5$ Hz, CP), 80.8 [$OCCH_3)_3$], 64.3 (br s, OCH_2CH_3), 53.8 (α), 51.1 (β), 28.2 [$CCH_3)_3$], 16.1 (br s, OCH_2CH_3); $[\alpha]_D = +1.2°$ (*c* 0.5, $CDCl_3$). The NMR data were similar to those reported (Kee et al. 2010). See Table 3 for enantiopurity.

*(S)-2-(t-Butoxycarbonylamino)-3-(4-(diethoxyphosphoryl)-1H-1,2,3-triazol-1-yl)propanoic acid [Boc-4-PO(OEt)$_2$Tza-OH] (**32**).*

LiOH.H_2O (5 mg, 0.1 mmol) was added to a stirred solution of **12a** (51 mg, 0.13 mmol) in 3:1 MeOH/water (4 mL) at 0°C. After 2 h, the reaction mixture was acidified to pH 4 with saturated aqueous $KHSO_4$, and then extracted with DCM (3 × 40 mL). The extract was washed with brine (10 mL), dried and evaporated, and the residue was subjected to flash chromatography. Elution with 1:4 MeOH/DCM gave 32 as colourless oil (42 mg, 88%). $R_f = 0.10$ (1:4 MeOH/DCM); ^1H NMR (500 MHz, CD_3OD): δ 8.39 (s, 1H, triazolyl), 5.01 (br dd, *J* = 17.5, 10.2 Hz, 1H, β-CH_2a), 4.71 (m [apparent dd], 1H, β-CH_2b), 4.51 (m, 1H, α-CH), 4.17 (m, 4H, OCH_2CH_3), 1.37 (s, 9H, *t*-Bu), 1.32 (t, *J* = 7.1 Hz, 6H, OCH_2CH_3). ^{13}C NMR (125 MHz, CD_3OD): δ 175.2 (CO_2), 158.2 (NCO_2), 138.0 (d, $J_{C,P} = 242.2$ Hz, CP), 134.2 (d, $J_{C,P} = 31.8$ Hz, triazolyl CH), 81.4 [$OCCH_3)_3$], 65.4 (d, $J_{C,P} = 3.4$ Hz, OCH_2CH_3a), 65.3 (d, $J_{C,P} = 3.3$ Hz, OCH_2CH_3b), 57.5 (α), 54.0 (β), 29.5 $(CH_3)_3$, 17.4 (d, $J_{C,P} = 6.5$ Hz, OCH_2CH_3). MS (ESI) *m/z*: 415 $[M+Na]^+$.

A sample that had partially degraded was further purified by HPLC to compare with the reported spectra in $CDCl_3$. ^1H NMR (600 MHz, $CDCl_3$): δ 8.28 (br s, 1H, triazolyl), 6.4 (v br s, OH), 5.55 (br s, 1H, NH), 4.98 (m, 2H, β-CH_2), 4.75 (br s, 1H, α-H), 4.20 (br m, 4H, OCH_2CH_3), 1.43 (s, 9H, *t*-Bu), 1.34 (2 × overlapping t at 1.34 and 1.33 [apparent q], *J* = 6.0 Hz, 6H, OCH_2CH_3). ^{13}C NMR (150 MHz, $CDCl_3$): δ 170.5 (br CO_2), 155.4 (NCO), 136.4 (br d, $J_{C,P} = 231.6$ Hz, CP), 132.5 (br s, triazolyl CH), 80.7 [$OCCH_3)_3$], 63.85 (br s, OCH_2CH_3a), 63.76 (br s, OCH_2CH_3b), 53.7 (br, α), 51.2 (β), 28.2

$(CH_3)_3$, 16.1 (br s, OCH_2CH_3); $[\alpha]_D = +40.5°$ (*c* 0.7, $CHCl_3$). The NMR data were similar to those reported (Kee et al. 2010). See Table 3 for enantiopurity.

*(S)-1-carboxy-2-(5-phosphono-1H-1,2,3-triazol-1-yl)ethanaminium bromide [H_2N-5-$PO(OH)_2$Tza-OH.HBr] (**33**)*

*Method A, from the carboxylic acid **31**:* A solution of **31** (0.090 g, 0.23 mmol) in 33% HBr in AcOH (2 mL, 8 mmol) was stirred for 48 h. The volatiles were evaporated and the residue was subjected to Dowex anion-exchange chromatography. Elution with water gave **33** as a white solid (51 mg, 70%), identical in every respect except optical rotation with the material described below.

*Method B, from the benzyl ester **11e**:* A solution of **11e** (0.100 g, 0.207 mmol) in 33% HBr in AcOH (2 mL, 8 mmol) was stirred for 48 h. The volatiles were evaporated and the residue was subjected to Dowex anion-exchange chromatography. Elution with water gave **33** as a white solid (43 mg, 65%), mp 138–140°C. IR (KBr disk) cm^{-1}: 3,200–3,500 (br, NH3 + OHs), 1,600–1,750 (br, obscures C=O); ^{1}H NMR (600 MHz, D_2O) δ: 7.86 (s, 1H, triazolyl), 5.11 (dd, J = 15.0, 3.6 Hz, 1H, β-Ha) 4.98 (dd, J = 15.6, 7.2 Hz, 1H, β-Hb), 4.27 (dd, J = 7.8, 3.6 Hz, 1H, α-H); ^{13}C NMR (125 MHz, D_2O): δ 169.6 (CO), 139.9 (d, $J_{C,P} = 18.4$ Hz, triazolyl CH), 135.6 (d, $J_{C,P} = 191.8$ Hz, CP), 53.2 (α), 49.0 (β); ^{31}P NMR (120 MHz): δ –3.14; MS (ESI) *m/z*: 237 $[M+H]^+$, 259 $[M+Na]^+$, 281 $[M+2\ Na]^+$ (where M = free base); HRMS (ESI): observed, 259.0201, $[C_5H_{10}N_4O_5P+Na]^+$ requires 259.0203; $[\alpha]_D = -7.7°$ (*c* 0.5, H_2O).

*(S)-1-Carboxy-2-(4-phosphono-1H-1,2,3-triazol-1-yl)ethanaminium bromide [H_2N-4-$PO(OH)_2$Tza-OH.HBr] (**34**).*

*Method A, from the carboxylic acid **32**:* A solution of **32** (0.090 g, 0.23 mmol) in 33% HBr in AcOH (2 mL, 8 mmol) was stirred for 48 h. The volatiles were evaporated and the residue was subjected to Dowex anion-exchange chromatography. Elution with water **34** as a white solid (52 mg, 72%), identical in every respect, except optical activity, with the material described below.

*Method B, from the benzyl ester **12e**:* Asolution of **12e** (0.100 g, 0.207 mmol) in 33% HBr in AcOH (2 mL, 8 mmol) was stirred at room temperature for 48 h. The volatiles were evaporated and the residue was subjected to Dowex anion-exchange chromatography. Elution with water gave **34** as a white solid (44 mg, 67%), mp 133–135°C. IR (KBr disk) cm^{-1}: 3,300–3,500 (br, NH3 + OHs), 1,716 (C=O); ^{1}H NMR (600 MHz, D_2O) δ: 8.12 (s, 1H, triazolyl), 4.98–5.00 (m, 2H, β-H), 4.47 (dd, J = 5.4, 4.8 Hz, 1H, α-H). ^{13}C NMR (125 MHz, D_2O): δ 170.7 (CO), 143.7 (d, $J_{C,P} = 217.6$ Hz, CP), 130.2 (d, $J_{C,P} = 31.7$ Hz, triazolyl CH), 54.5 (α), 50.0 (β). ^{31}P NMR (120 MHz): δ 1.95; MS (ESI) *m/z*: 237 $[M+H]^+$ (where M = free base); HRMS (ESI): observed, 237.0385, $[C_5H_{10}N_4O_5P]^+$ requires 237.0383. $[\alpha]_D = -25.0°$ (*c* 0.5, H_2O).

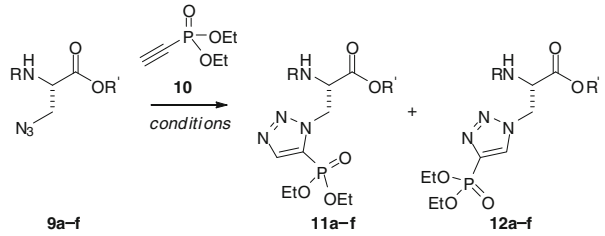
*(S)-Benzyl 2-(((9H-fluoren-9-yl)methoxy)carbonylamino)-3-hydroxypropanoate [Fmoc-Ser-OBn] (**36**)*

Benzyl bromide (1.40 mL, 11.8 mmol) was added to a solution of Fmoc-L-serine monohydrate (2.00 g, 5.79 mmol) and DIPEA (1.00 mL, 11.6 mmol) in DMF (20 mL) at 0°C. The solution was allowed to warm to room temperature, stirred for 18 h, diluted with sat. aq. NH_4Cl (30 mL), and extracted with EtOAc (3 × 50 mL). The organic extract was washed with brine (50 mL), dried and evaporated, and the residue was subjected to flash chromatography. Elution with 2:3 EtOAc/hexanes gave **36** as a white solid (2.18 g, 90%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.81 (d, J = 7.5 Hz, 2H, Ar), 7.70 (br d, J = 5.5 Hz, 2H, Ar), 7.46 (apparent t, J = 7.5 Hz, 4H, Ar), 6.47 (br d, J = 5.0 Hz, 1H, NH), 5.27 (s, 2H, OCH_2Ph), 4.66 (m, 1H, α-H), 4.53 (m [apparent t], 1H, NCO_2CH_2a), 4.43 (m [apparent t], 1H, NCO_2CH_2b), 4.66 (t, J = 7.0 Hz, 1H, H9′), 4.12 (m [apparent br d], 1H, β-Ha), 3.99 (m [apparent br d], 1H, β-Hb). ^{13}C NMR (125 MHz, CD_3OD): δ 170.4 (CO_2), 156.3 (NCO_2), 143.5 (Ar), 143.3 (Ar), 140.9 (Ar), 134.8 (Ar), 128.2 (Ar), 128.0 (Ar), 127.7 (Ar), 127.3 (Ar), 126.7 (Ar), 119.6 (Ar), 67.0, 66.9, 62.4, 56.0, 46.6. MS (ESI) *m/z*: 440 $[M+Na]^+$. $[\alpha]_D = +0.7°$ (*c* 1.0, DCM) [lit. (Huang et al. 2004) +0.6° (*c* 1.0, DCM)]. The ^{1}H and ^{13}C NMR data are similar to those reported (Huang et al. 2004).

Results and discussion

Our synthetic endeavours began with the thermal Huisgen cycloaddition (Dondoni et al. 2004) of the known phosphonate **10** (Vuilhorgne et al. 2003) and serine-derived azide **9a** (Boger et al. 1994). Gratifyingly, this reaction gave both of the desired triazole regioisomers **11a** and **12a** (Scheme 1; Table 1), which were easily separable by column chromatography. The constitution of the adducts was established by an HMBC experiment; specifically, a three-bond correlation between the triazolyl proton and the β-carbon identified the 1,4-substituted triazole **12a**.

As expected, the Cu(I)-catalysed 'click' reaction (Rostovtsev et al. 2002) afforded the 1,4-disubstituted isomer **12a** exclusively, in excellent yield, whereas Ru(II)

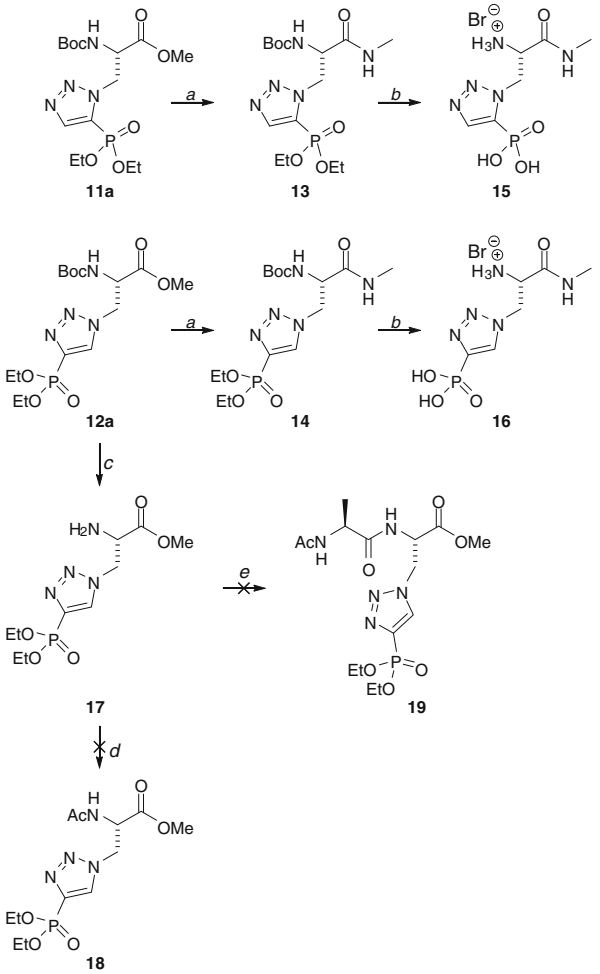


Scheme 1 Carboxyl-protecting groups in azidoalanine derivatives significantly affect the regioselectivity of dipolar cycloadditions to give the corresponding triazolylalanine phosphonates. See Table 1 for the nature of the R/R′ groups, conditions and yields

Table 1 Dipolar cycloadditions of **9a–f** and **10** under various conditions

Azide	R	R′	Product	Yield		
				Δ	Cu^{I}	Ru^{II}
9a	Boc	Me	**11a**	21	–	56
			12a	58	95	21
9b	Ac	Me	**11b**	26	–	57
			12b	60	91	19
9c	Ac	All	**11c**	41	–	73
			12c	44	84	10
9d	Ac	Bn	**11d**	43	–	81
			12d	45	87	–
9e	Boc	Bn	**11e**	41	–	80
			12e	42	88	–
9f	Fmoc	Bn	**11f**	39	–	78
			12f	41	85	–

Conditions: Δ: PhMe, 110°C; Cu^{I}: Cu_2SO_4, sodium ascorbate, 1:1 *t*-BuOH-H_2O; Ru^{II}: Cp*RuCl$(PPh_3)_2$, PhMe, 60°C



Scheme 2 Reagents, conditions and yields: **a** $MeNH_2$, MeOH/H_2O (**13**, 85%; **14** 90%); **b** 33% HBr/AcOH (**15**, 71%; **16**, 73%); **c** TFA, DCM (80%); **d** Ac_2O or AcCl, NEt_3, DCM (0%); **e** HCTU, *i*-Pr_2NEt, DCM (0%)

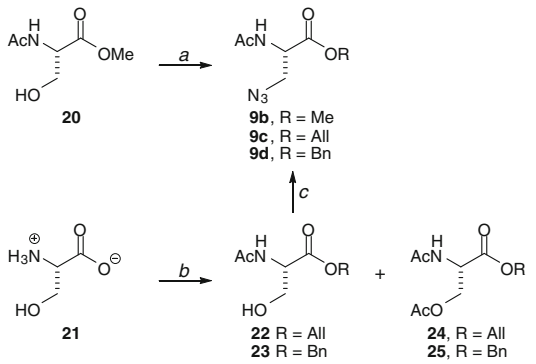
catalysis (Zhang et al. 2005) allowed a substantial improvement in the yield of the 1,3-isomer **11a** (Table 1).

Amidation (Lesma et al. 2007) of the methyl esters **11a** and **12a** proceeded smoothly, providing the *N*-methylamides **13** and **14**, respectively, in excellent yields (Scheme 2). Protonolysis then provided the first target haptens, ready for bioconjugation through the amino group, as the hydrobromides **15** and **16**.

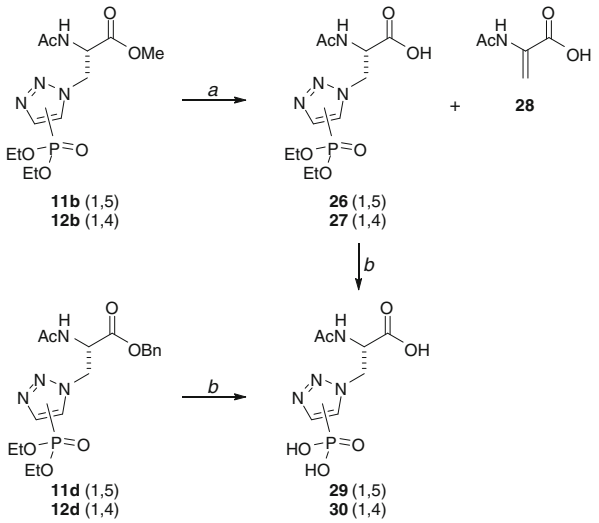
In contrast, although efficient and selective removal of the Boc group of **12a** was achieved, all efforts to acetylate the resulting amine **17** to give **18** were unsuccessful. An attempted coupling with *N*-acetylserine to give **19** also failed. It is unclear at this stage what prevents these seemingly simple transformations. Thus, to access haptens **7** and **8** (Fig. 1) it was necessary to acetylate at an earlier point.

To this end, the known azide **9b** (Davoli et al. 1995) was prepared in 60% yield from methyl *N*-acetylserinate (**20**) (Maruyama et al. 1992) using a Mitsunobu reaction with hydrazoic acid (Scheme 3). The azide-alkyne cycloadditions of **9b** under thermal, Cu(I)- or Ru(II)-catalysed conditions were very similar to those for the analogues carbamate **9a** (Scheme 1; Table 1).

Saponification of the resultant triazoylalanines **11b** and **12b** proved problematic (Scheme 4). The reaction of **12b** with LiOH at room temperature gave only *N*-acetyldehydroalanine (**28**). At lower temperature, **28** was not observed, but the yield of the desired carboxylic acid **27** was poor. This, coupled with the earlier observation of **28**, the product of an E1cb reaction, indicated the likely formation of an intermediate enolate and hence at least partial racemisation of **12b/27** under the reaction conditions. Indeed, when the saponification of **12b** was repeated with the weaker base K_2CO_3, at room temperature, the desired carboxylic acid **27** was isolated in improved, although still modest, yield, and the specific rotation of this material was greater than that of the LiOH-derived product (Table 2). At



Scheme 3 Reagents, conditions and yields: **a** PPh_3, HN_3, DIAD, THF, PhMe (**9b**, 60%); **b** (1) Ac_2O, AcOH; (2) AllBr or BnBr, *i*-Pr_2NEt, DMF (**22**, 21%; **24**, 45%; **23**, 31%; **25**, 45% [all over two steps]); **c** PPh_3, HN_3, DEAD (**9c**) or DIAD (**9d**), THF, PhMe (**9c**, 61%; **9d**, 61%)



Scheme 4 Reagents, conditions and yields: **a** base, MeOH, H_2O, then H_3O^+; **b** 33% HBr/AcOH. See Table 2 for further details

0°C, **12b** failed to react with K_2CO_3. The saponification of the regioisomer **11b** was also complicated by competing elimination, and in this case the desired product **26** had almost identical chromatographic mobility to the *N*-acetyldehydroalanine (**28**) and could not be isolated.

Protonolysis of the 1,4-triazolylphosphonate **27** derived from **12b** gave the target hapten **30** (the protonated form of **7**, Fig. 1) in 70% yield (Scheme 4), but the poor yields of the saponifications, coupled with the likelihood that partial racemisation had occurred, even with K_2CO_3 (which, as described below, was ultimately shown to be case), meant an alternative carboxyl-protecting group strategy had to be devised.

Allyl and benzyl esters were considered simultaneously. The azidoalanine derivatives **9c** and **9d** were prepared in three steps from L-serine (**21**) (Scheme 3). Acetylation

Table 2 Deprotection of *N*-acetyltriazolylalaninephosphonic acid derivatives (Scheme 4)

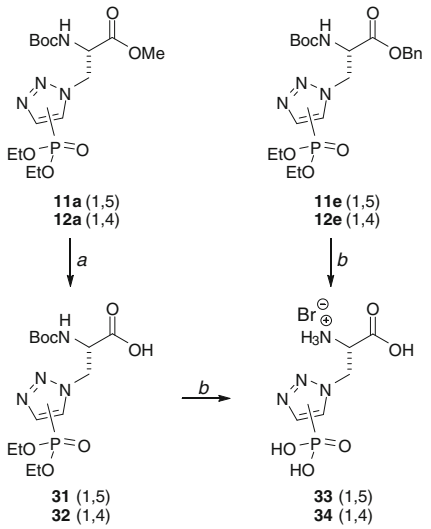
Compound	Conditions	Yield (%)	Specific rotation	Solvent (*c* 1.0)
27	LiOH, 0°C	31	+5.1°	MeOH
27	LiOH, RT	0	–	–
27	K_2CO_3, 0°C	0	–	–
27	K_2CO_3, RT	47	+8.8°	MeOH
29	from **11d**	69	−23.8°	H_2O
30	from **27**	70	+6.1°	H_2O
30	from **12d**	67	+10.0°	H_2O

followed by selective alkylation gave the allyl and benzyl esters, **22** and **23**, respectively, after separation from the *N*,*O*-diacetyl derivatives, **24** and **25**. Mitsunobu reactions of **22** and **23** with hydrazoic acid provided the desired azides.

The thermal Huisgen cycloadditions of **9c** and **9d** with alkyne **10** produced more of the 1,5-regiosiomers **11c** and **11d** relative to the reaction of the corresponding methyl ester **9b** (Scheme 1; Table 1). This innate tendency had little effect on the Cu(I)-catalysed reactions, with only the 1,4-disubstituted triazoles **12c** and **12d** observed in both cases. Conversely, relative to the methyl ester **9b**, the allyl-protecting group in **9c** improved the regioselectivity of the Ru(II)-catalysed reaction, giving the 1,5-isomer **11c** as the predominant product. The reaction of the still bulkier benzyl ester **9d** was completely regioselective, with only the desired 1,5-triazole **11d** observed, and isolated in excellent yield. At this stage the enhanced regioselectivity is attributed to steric effects; however, chelation of the catalyst by the pendant π-systems cannot be ruled out.

Attempts to remove the allyl-protecting groups in **11c**/**12c** under standard conditions [$Pd(PPh_3)_4$, morpholine, THF] (Schmittberger and Cotte 1998) returned only starting material. The failure of this normally reliable reaction could be due to the formation of a stable Pd complex with the abundant coordinating groups in the substrate. Alternative deprotection conditions were not pursued as the chemistry of the benzyl esters proved more fruitful. Thus, protonolysis of **11d** and **12d** gave the desired haptens **29** and **30** (the protonated forms of **7** and **8**), ready for bioconjugation through the carboxyl terminus (Scheme 4). The specific rotation of the hapten **30** derived in one step from **12d** was slightly larger than that of the material prepared in two steps from the analogous methyl ester **12b** (10.0° vs. 6.1°), indicating that partial racemisation had indeed occurred in the saponification step.

With the two desired haptens in hand, we then targeted the free phosphonoamino acids **33** and **34** (Scheme 5).



Scheme 5 Reagents and conditions: **a** LiOH, MeOH, H_2O, then H_3O^+; **b** 33% HBr, AcOH (see Table 3 for yields)

Although not particularly useful for synthesis or antibody generation, the free amino acids might possess interesting biological activity, such as histidine kinase inhibition. While extremely polar and almost certainly not able to passively cross cell membranes, it is possible that they may be substrates for an amino acid transporter.

Attempts to effect global deprotection of the methyl ester **11a** with aqueous hydrochloric or hydrobromic acid were unsuccessful, so a two-step saponification-protonolysis was investigated. Although partial racemisation of the acetamide **11b** was observed during saponification, we were optimistic that racemisation could be avoided in this case due to the decreased acidity of the α-protons in *t*-butyl carbamates compared with analogous *N*-acylamino esters. Careful saponification of **11a** and **12a** gave the carboxylic acids **31** and **32** reported by Muir (Kee et al. 2010) (Scheme 5). The NMR spectra of **31** and **32** matched those reported except that in our case, most signals in the ^{1}H NMR spectra, and several signals in the ^{13}C NMR spectra, in $CDCl_3$ solution, were significantly broadened in comparison with the published spectra, presumably due to some dynamic phenomenon. In contrast, the NMR spectra in CD_3OD exhibited sharp signals.

The specific rotations of both regioisomeric carboxylic acids were significantly lower in magnitude than those reported (Kee et al. 2010) (Table 3), suggesting that partial racemisation had again occurred during the saponification step. Enantioselective chromatography showed this to be the case; the enantiomeric excesses calculated from the HPLC peak integrals are close to those determined from the specific rotations. Although protonolysis afforded the 'free' phosphonoamino acids **33** and **34**, it was necessary to revise the protecting group strategy again to avoid the complication of partial racemisation.

The azide-alkyne cycloadditions of benzyl *N*-*t*-butoxycarbonylazidoalanine **9e** (Kogan and Rawson 1992) reflected those of the corresponding acetyl derivative **9d** (Scheme 1; Table 1), indicating that, while the ester group significantly influences the outcome of these reaction, they are largely unaffected by the *N*-protecting group. The Cu(I)-catalysed cycloaddition of the benzyl ester **9e** under aqueous conditions was higher yielding than the analogous reaction of the free carboxylic acid in DMF reported by Muir and co-workers (Kee et al. 2010). It is more difficult to compare the Ru(II)-catalysed reaction of **9e** in this work with that reported by Muir, as they immediately removed the benzyl-protecting group, providing acid **31** in 68% yield over two steps. Nevertheless, the desired 1,5-triazole **11e** was formed exclusively in excellent yield.

Global deprotection of **11e** and **12e** by protonolysis revealed the free amino acid hydrobromides **33** and **34** as

Table 3 Deprotections to give the free phosphonoamino acids (Scheme 6)

Compound	Yield (%)	$[\alpha]_D^a$		ee	
		Muir[b]	This work	$[\alpha]_D$	HPLC[e]
31	86	+1.8° (1.0)	+1.2° (0.5)	67%[c]	70%
32	88	+60° (1.0)	+40.5° (0.7)	68%[c]	74%
33 (from **31**)	70	–	−5.6° (0.8)	72%[d]	–
33 (from **11e**)	65	–	−7.7° (0.5)	–	–
34 (from **32**)	72	–	−18.8° (0.5)	75%[d]	–
34 (from **12e**)	67	–	−25.0° (0.5)	–	–

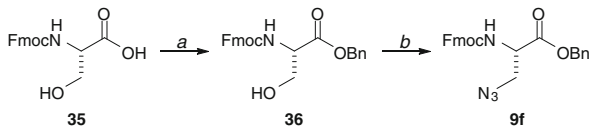
[a] $[\alpha]_D$ values were recorded at 21°C (Muir) and ambient temperature (this work). Concentrations in g 100 mL^{-1} in $CHCl_3$ (**31**, **32**) or H_2O (**33**, **34**) are given in brackets

[b] (Kee et al. 2010)

[c] Determined by comparison with the specific rotations reported by Muir and co-workers (Kee et al. 2010)

[d] Determined by comparison with the specific rotation of the same material synthesised by protonolysis of the benzyl esters **11e/12e**

[e] Determined from the relative integrals of the peaks corresponding to the enantiomers by enantioselective HPLC



Scheme 6 Reagents and conditions: **a** BnBr, *i*-Pr_2NEt, DMF (90%); **b** PPh_3, HN_3, DEAD, THF, PhMe (65%)

slightly hygroscopic solids, which were purified by ion exchange chromatography (Scheme 5). The specific rotations of the free amino acids **33** and **34** prepared in this way were consistent with those expected for enantiopure material, based on the specific rotations of **33** and **34** prepared from partially racemised carboxylic acids **31** and **32** (Table 3).

Finally, to provide access to derivatives suitable for use in solid-phase peptide synthesis, we targeted the Fmoc-protected triazolylalaninephosphonates **11f** and **12f** (Scheme 1). Fmoc-Ser-OBn (**36**), prepared by esterification of commercially available Fmoc-serine (**35**) with benzyl bromide, underwent a Mitsunobu reaction with hydrazoic acid to provide the novel azide **9f** (Scheme 6). Thermal cycloaddition of this compound with alkyne **10** gave a mixture of the regioisomeric triazoles **11f** and **12f** (Scheme 1; Table 1) and Cu(I)-catalysis provided the 1,4-regioisomer **12f** exclusively. Webb and co-workers have recently prepared the corresponding carboxylic acid by Cu(I)-catalysed cycloaddition of Fmoc-azidoalanine (McAllister et al. 2011). Ru(II)-catalysis is not compatible with a free carboxylic acid (Kee et al. 2010); the use of the benzyl ester-protecting group in the present work allows Ru(II)-catalysis, providing the 1,5-regioisomer **11f** exclusively, and in good yield (Table 1).

Conclusion

The synthesis of several stable triazolylphosphonate analogues of *N*1- and *N*3-phosphohistidine has been achieved. In addition to the 'free' amino acids, derivatives suitable for bioconjugation through amide linkages with lysine or aspartate/glutamate residues of carrier proteins were efficiently prepared.

A survey of the azide-alkyne cycloadditions of various L-azidoalanine derivatives with diethyl ethynylphosphonate has revealed that the amino-protecting group has little impact on the regioselectivity of the reactions. In contrast, bulkier carboxyl-protecting groups increase the relative proportion of the 1,5-disubstituted triazoles under thermal conditions, and with Ru(II) catalysis, allow exclusive and high-yielding formation of these products. Irrespective of the protecting groups, Cu(I) catalysis is regiospecific, providing the 1,4-disubstituted triazoles cleanly and in excellent yields.

The haptens described herein should prove valuable in the ongoing search for generic phosphohistidine antibodies and for otherwise exploring the biochemistry of histidine-phosphorylated proteins, the corresponding kinases, and associated cell-signalling pathways.

Acknowledgments SM was the recipient of a UWA full PhD scholarship.

Conflict of interest The authors declare that they have no conflict of interest.

References

Attwood PV, Piggott MJ, Zu XL, Besant PG (2007) Focus on phosphohistidine. Amino Acids 32:145–156

Besant PG, Attwood PV (2005) Mammalian histidine kinases. Biochim Biophys Acta, Proteins Proteomics 1754:281–290

Besant PG, Attwood PV (2009) Detection and analysis of protein histidine phosphorylation. Mol Cell Biochem 329:93–106

Besant PG, Tan E, Attwood PV (2003) Mammalian protein histidine kinases. Int J Biochem Cell Biol 35:297–309

Boger DL, Honda T, Menezes RF, Colletti SL, Dang Q, Yang W (1994) Total syntheses of (+)-P-3a, epi-(−)-P-3a, and (−)-desacetamido P-3a. J Am Chem Soc 116:82–92

Davoli P, Forni A, Moretti I, Prati F (1995) Stereochemistry of nucleophilic ring-opening reactions of optically active *N*-acetyl-2-methoxycarbonylaziridine. Tetrahedron Asymmetry 6:2011–2016

Dondoni A, Giovannini PP, Massi A (2004) Assembling heterocycle-tethered *C*-glycosyl and α-amino acid residues via 1,3-dipolar cycloaddition reactions. Org Lett 6:2929–2932

Huang Y, Dey S, Zhang X, Soennichsen F, Garner P (2004) The α-helical peptide nucleic acid concept: merger of peptide secondary structure and codified nucleic acid recognition. J Am Chem Soc 126:4626–4640

Janiak-Spens F, West AH (2004) Histidine kinases. Handbook of Cell Signaling 1:563–566

Kee J-M, Villani B, Carpenter LR, Muir TW (2010) Development of stable phosphohistidine analogues. J Am Chem Soc 132:14327–14329

Kogan TP, Rawson TE (1992) The synthesis of chiral 3-oxo-6-[(phenylmethoxy)carbonyl]-2-piperazineacetic acid esters designed for the presentation of an aspartic acid side chain. A subsequent novel Friedel Crafts reaction. Tetrahedron Lett 33:7089–7092

Lecercle D, Sawicki M, Taran F (2006) Phosphine-catalyzed α-P-addition on activated alkynes: a new route to P-C-P backbones. Org Lett 8:4283–4285

Lesma G, Meschini E, Recca T, Sacchetti A, Silvani A (2007) Synthesis of tetrahydroisoquinoline-based pseudopeptides and their characterization as suitable reverse turn mimetics. Tetrahedron 63:5567–5578

Maruyama K, Hashimoto M, Tamiaki H (1992) Intramolecular photoreaction of synthetic oligopeptide-linked anthraquinone molecules. J Org Chem 57:6143–6150

McAllister TE, Nix MG, Webb ME (2011) Fmoc-chemistry of a stable phosphohistidine analogue. Chem Commun 47:1297–1299

Meldal M, Tornoe CW (2008) Cu-catalyzed azide-alkyne cycloaddition. Chem Rev 108:2952–3015

Nakata T, Nakatani M, Takahashi M, Okai J, Kawaoka Y, Kouge K, Okai H (1996) Peptide synthesis in aqueous solution. V. Properties and reactivities of (*p*-hydroxyphenyl)benzylmethylsulfonium salts

for direct benzyl esterification of *N*-acylpeptides. Bull Chem Soc Jpn 69:1099–1106

Pirrung MC, James KD, Rana VS (2000) Thiophosphorylation of histidine. J Org Chem 65:8448–8453

Rostovtsev VV, Green LG, Fokin VV, Sharpless KB (2002) A stepwise Huisgen cycloaddition process: copper(I)-catalyzed regioselective "ligation" of azides and terminal alkynes. Angew Chem Int Ed 41:2596–2599

Schenkels C, Erni B, Reymond J-L (1999) Phosphofurylalanine, a stable analog of phosphohistidine. Bioorg Med Chem Lett 9:1443–1446

Schmittberger T, Cotte A (1998) Synthesis of characteristic lipopeptides of lipid modified proteins employing the allyl ester as protecting group. Chem Commun 937–938

Steeg PS, Palmieri D, Ouatas T, Salerno M (2003) Histidine kinases and histidine phosphorylated proteins in mammalian cell biology, signal transduction and cancer. Cancer Lett 190:1–12

Stock AM, Robinson VL, Goudreau PN (2000) Two-component signal transduction. Annu Rev Biochem 69:183–215

Tan E, Besant PG, Zu XL, Turck CW, Bogoyevitch MA, Lim SG, Attwood PV, Yeoh GC (2004) Histone H4 histidine kinase displays the expression pattern of a liver oncodevelopmental marker. Carcinogenesis 25:2083–2088

Tummatorn J, Albiniak PA, Dudley GB (2007) Synthesis of benzyl esters using 2-benzyloxy-1-methylpyridinium triflate. J Org Chem 72:8962–8964

Vuilhorgne M, Malpart J, Mutti S, Mignani S (2003) Preparative route to 2-ethoxycarbonylimidazole-4-phosphonate and diethyl imidazole-2,4-dicarboxylate. J Het Chem 40:159–162

Wu P, Fokin VV (2007) Catalytic azide-alkyne cycloaddition: reactivity and applications. Aldrichimica Acta 40:7–17

Yeager AR, Finney NS (2004) The first direct evaluation of the two-active site mechanism for chitin synthase. J Org Chem 69:613–618

Zhang L, Chen X, Xue P, Sun HHY, Williams ID, Sharpless KB, Fokin VV, Jia G (2005) Ruthenium-catalyzed cycloaddition of alkynes and organic azides. J Am Chem Soc 127:15998–15999