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ORIGINAL ARTICLE

Synthesis of orthogonally protected azahistidine: application to the synthesis of a GHK analogue

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Abstract The synthesis of various orthogonally protected azahistidine derivatives are obtained via 1,3-dipolar cycloaddition reactions. The newly obtained amino-acids can be selectively deprotected either at the side chain or at the *N*-terminus of the amino acid and should thus allow the use of these derivatives in (solid phase) peptide synthesis.

Keywords Click chemistry · Huisgen cycloaddition · Triazole · Azahistidine · GHK

Introduction

Unnatural α-amino-acids are key components for recent developments in peptides or proteins research. These synthetic compounds have been incorporated in biologically active peptides mostly to enhance proteolytic stability or to confer peculiar properties to these peptides. In addition, conformational flexibility, selectivity, pharmacokinetics and bioavailability can also be tuned based on well-designed amino-acids. In the field of nanotechnology, there is also a growing interest in synthetic amino-acids that can present specific properties (metal binding ability, polymerisation precursors, electron transfer…). Among the natural amino-acids, histidine is often strongly involved in the chemistry of enzymes due to the ability of the imidazole side chain to take part in acid-base catalysis. Indeed, histidine can act both as a donor or an acceptor of protons: developing analogues could pave the way to new applications. One of the most obvious parent compounds is azahistidine where the imidazole ring has been replaced by a 1,2,3-triazole (Fig. 1).

Recent applications based on this analogue include the synthesis of ^{99m}Tc radiolabelled amino-acids as well as metal chelating compounds (Mindt et al. 2006). It has also been demonstrated that histidine-auxotrophic bacteria (*E. coli*) can be supplemented with compound **1** leading to in vivo incorporation of this amino-acid in proteins (Ikeda et al. 2003).

Although the preparation of such a histidine surrogate has been already described (Losikina and Sokolov 1991), to the best of our knowledge, the synthetic routes published to date to obtain the fully deprotected amino-acid are usually rather long (seven steps) (Ikeda et al. 2003).

Materials and methods

Chemicals

Amino acid derivatives were purchased from Bachem, Fluka and Acros Organics. Reagents and solvents for synthesis and RP-HPLC were from Sigma, Fluka or Normapur products and used without further purification. Aryl-azide derivatives were prepared by standard protocol.

Equipment

LC/MS analysis

HPLC Waters system (2525 binary gradient module, in-line degasser, 2767 sample manager, 2996 Photodiode Array Detector), eluents: A: 99.9% water/0.1% HCOOH; B: 99.9% ACN/0.1% HCOOH.

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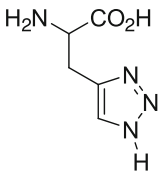
Fig. 1 Triazole analogue of histidine



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Analytical RP-HPLC column: X-bridge C18 column (100 × 4.6 mm, 3.5 µm particle size, 135 Å pore size), 1 ml/min flow rate, 20 µl sample is injected.

Preparative RP-HPLC column: X-bridge C18 column (150 × 19 mm, 5 µm particle size, 135 Å pore size), 17 ml/min flow rate.

Using a 8 or 25 min gradient of 95% A/5% B to 100% B for analysis and purification, respectively.

MS Waters Micromass ZQ system (electrospray ionization), ZQ2000 quadrupole analyser, Mass Lynx 4.0 software, source temperature 120°C, cone voltage 20 V, continuous sample injection at 0.3 ml/min flow rate, mass spectra were recorded in positive ion mode in the *m/z* 100–1,000 range.

NMR analysis

^{1}H- and ^{13}C-NMR spectra were recorded at room temperature at a frequency of 400 and 100 MHz, respectively, using a Bruker Avance 400 Ultrashield. Samples were dissolved in 400 µl of deuterated solvent. Chemical shifts are given in ppm and the coupling constants in Hz.

General method (A): synthesis of Boc-*β*-(1-benzyl)-1,2,3-triazol-4-yl)-Alanine 2

N-Boc-propargylglycine, measuring 107 mg (0.5 mmol) was introduced in a 100 ml round-bottom flask in 15 ml of ^{t}BuOH together with 70 mg (0.5 mmol, 1 eq.) of benzylazide. 10 mg (0.05 mmol, 0.01 eq.) of $Cu(OAc)_2 \cdot xH_2O$ dissolved in 3 ml of water and 20 mg (0.1 mmol, 0.02 eq.) of Na-ascorbate in 2 ml of water were further introduced. The mixture was stirred overnight at room temperature. ^{t}BuOH was then removed by rotary-evaporation and the pH of the resulting aqueous mixture was adjusted to 2 with a 1 M HCl solution. The aminoacid was extracted three times with ethylacetate (50 ml) and the organic phases, pooled together were washed with brine and dried over anhydrous $MgSO_4$. After filtration, the crude product was evaporated and dissolved in ethyl acetate. After crystallisation in cyclohexane and filtration 104.3 mg of the expected Boc-*β*-(1-benzyl)-1,2,3-triazol-4-yl-Alanine **2** were obtained (60% yield).

Purification conditions for the other derivatives were as follows:

Boc-*β*-(1-(4-methoxybenzyl)-1,2,3-triazol-4-yl)-Alanine **3** was purified by flash chromatography using an isocratic elution consisting in DCM/MeOH/acetic acid 90:10:1. The fractions were pooled, evaporated and dissolved in chloroform and crystallised from hexane.

Boc-*β*-(1-(3,5-dimethoxybenzyl)-1,2,3-triazol-4-yl)-Alanine **4** was obtained as a colourless oil without crystallization.

Boc-*β*-(1-pivaloyloxymethyl)-1,2,3-triazol-4-yl)-Alanine **5** was purified by flash chromatography using an isocratic elution consisting in DCM/MeOH/acetic acid 96:4:5%.

Fmoc-*β*-((1-benzyl)-1,2,3-triazol-4-yl)-Alanine **6** was dissolved in ethyl acetate and crystallised with pentane.

Fmoc-*β*-(1(-4-methoxybenzyl)-1,2,3-triazol-4-yl)-Alanine **7** was dissolved in chloroform and crystallised with hexane.

Fmoc-*β*-(1-(3,5-dimethoxybenzyl)-1,2,3-triazol-4-yl)-Alanine **8** was dissolved in ethyl acetate and crystallised with cyclohexane.

Fmoc-*β*-(1-pivaloyloxymethyl)-1,2,3-triazol-4-yl)-Alanine **9** was purified by flash chromatography using an isocratic elution consisting in DCM/MeOH/acetic acid 97:3:5%.

General method (B): synthesis of Ac-(1-benzyl)-1,2,3-triazol-5-yl)-Ala-OEt 10

In a 50 ml two-necked round bottom flask was introduced 10 ml of anhydrous toluene. This solvent was then degassed by three vacuum freeze/thaw cycles. 100 mg (0.54 mmol) of *N*-acetylpropargylglycine ethyl ester were then introduced together with 145 mg (1.09 mmol, 2 eq.) of benzyl azide and the mixture was degassed once more. Finally 21.7 mg (27.3 µmol, 0.05 eq.) of $Cp^{*}RuCl(PPh_3)_2$ were introduced and the mixture was degassed once again. The reaction mixture was then heated to 80°C under nitrogen for 15 h. The resulting mixture was then evaporated to afford 205.9 mg of crude product. This product was dissolved in acetonitrile and filtered. The liquid was further purified by preparative HPLC using a water/acetonitrile/formic acid gradient, yielding 97.12 mg of viscous oil for a yield of 44%.

Selective removal of BOC protecting group, affording H-*β*-((1-pivaloyloxymethyl)-1,2,3-triazol-4-yl)-Alanine 12 (Stahl et al. 1978)

HCl 35% measuring 2.2 ml was introduced on 100 mg (0.27 mmol) of Boc-*β*-(1-pivaloyloxymethyl)-1,2,3-triazol-4-yl)-Ala-OH **5** in 7.8 ml of ethyl acetate. Under

stirring at R.T. the reacting mixture, initially biphasic, turned rapidly to a homogenous yellowish liquid. After 3 h, the mixture is diluted in water and washed three times with dichloromethane. The aqueous phase was then freeze dried to obtain 94 mg of a yellowish solid.

The crude product was purified by preparative HPLC using a water/acetonitrile gradient with formic acid as ion pairing agent. 32.2 mg of pure white solid were obtained with a yield of 44%.

Selective removal of Fmoc protectivng group, affording H-*β*-((1-pivaloyloxymethyl)-1,2,3-triazol-4-yl)-Alanine 12

Ninety-nine milligram (0.20 mmol) of Fmoc-*β*-(1-pivaloyloxymethyl)-1,2,3-triazol-4-yl)-Ala-OH **9** were introduced in 5 mL of DMF with 13.5 µl (0.22 mmol, 1.1 eq.) of piperidine. The mixture was stirred at R.T. for 3 h 45 min and evaporated to obtain a white solid. The crude product was then triturated with diethyl ether to remove the dibenzofulvene-piperidine adduct. After drying, 29.3 mg (53%) of purified solid were obtained.

Selective removal of POM protecting group, affording Boc-*β*-(1, 2, 3-triazol-4-yl)-Alanine 13 (Bock et al. 2006)

150 mg (0.40 mmol) of Boc-*β*-(1-(pivaloyloxymethyl)-1,2,3-triazol-4-yl)-Ala-OH **5** were added to 2.1 ml of methanol with 2.5 ml of KOH 1 M (2.5 mmol, 6.2 eq.). The mixture was stirred for 1 day at R.T., then acidified to pH 3 and extracted with ethyl acetate. The organic fractions were pooled and dried with $MgSO_4$. After evaporation 92.6 mg of amorphous solid were obtained with a yield of 61%.

Selective removal of benzyl protecting group

- affording Ac-*β*-(1,2,3-triazol-5-yl)-Ala-OEt

30 mg (95 µmol) of Ac-*β*-(1-benzyl)-1,2,3-triazol-5-yl)-Ala-OEt **10** were introduced in 1.5 ml of MeOH into a one-neck round-bottom flask. 101.3 mg (95 µmol, 1 eq.) of palladium 10% on charcoal were added and the mixture was put under H_2 atmosphere and stirred at R.T. during 72 h. The crude mixture was then filtered through celite and a Millipore HVLP membrane, to yield to 22.9 mg of product. The yield was quantitative.

- affording Boc-*β*-(1,2,3-triazol-4-yl)-Alanine

In a pressure tank, 5 mg (14.4 µmol) of Boc-*β*-(1-benzyl)-1,2,3-triazol-4-yl)-Alanine **2** were introduced to 3.84 mg (3.6 µmol, 0.25 eq.) of Pearlman catalyst i.e. $Pd(OH)_2$ on charcoal (20% pure, 50% water in weight) in 2 ml of MeOH. The tank was flushed with N_2 for 5 min and then 10 bars of H_2 were admitted. The reaction was stirred at 45°C over 72 h.

The crude mixture was clarified on a syringe Millipore separation filter and analysed by LC/MS, revealing a peak of expected *m/z* accounting for more than 90% of the total UV signal area

Boc-*β*-(1-benzyl)-1,2,3-triazol-4-yl)-Alanine **2** ^{1}H NMR (400 MHz; $CDCl_3$): δ 1.27 (s, 9H, ^{t}Bu), 3.15 (br. s, 2H, $C_\beta HH'$), 4.32 (bs, 1H, $C_\alpha H$), 5.32 (d, $J = 15.0$, 1H, CH-Bzl), 5.38 (d, 1H, $J = 15.0$, CH′-Bzl), 5.97 (br. s, 1H, HNC_α), 7.13-7.19 (m, 2H, Bzl), 7.23-7.28 (m, 3H, Bzl), 7.32 (br. s, 1H, CH triazol).

MS (ESI): 347.1 $(M + H)^+$

Boc-*β*-(1-(4-methoxybenzyl)-1,2,3-triazol-4-yl)-Alanine **3** ^{1}H NMR (400 MHz; $CDCl_3$): δ 1.42 (s, 9H, ^{t}Bu), 3.15-3.25 (m, 1H, $C_\beta H$), 3.29-3.39 (m, 1H, $C_\beta H'$), 3.81 (s, 3H, *p*-methoxy), 4.49 (bs, 1H, $C_\alpha H$), 5.43 (s, 2H, CH_2-Bzl), 5.73 (br. s, 1H, HNC_α), 6.90 (d, $J = 8.4$, 2H, H-3,5 Bzl), 7.22 (d, $J = 8.4$ Hz, 2H, H-2,6 Bzl), 7.31 (br. s, 1H, CH triazol)

MS (ESI): 377.1 $(M + H)^+$

Boc-*β*-(1-(3,5-dimethoxybenzyl)-1,2,3-triazol-4-yl)-Alanine **4** ^{1}H NMR (400 MHz; CD_3OD): δ 1.36 (s, 9H, ^{t}Bu), 3.05 (dd, $J = 14.9$, $J = 4.5$, 1H, $C_\beta H$), 3.25 (dd, $J = 14.9$, $J = 8.9$, 1H, $C_\beta H'$), 3.74 (s, 6H, *m,m*-dimethoxy), 4.37 (dd, $J = 8.9$, J = 4.5, 1H, $C_\alpha H$), 5.48 (s, 2H, CH_2-Bzl), 6.43 (bs, 3H, Bzl), 7.73 (s, 1H, CH triazol)

MS (ESI): 407.1 $(M + H)^+$

Boc-*β*-(1-(pivaloyloxymethyl)-1,2,3-triazol-4-yl)-Alanine **5** ^{1}H NMR (400 MHz; d6-DMSO): δ 1.11 (s, 9H, ^{t}Bu), 1.45 (s, 9H, ^{t}Bu), 2.94 (dd, 1H, $J = 14.5$, $J = 9.7$, $C_\beta H$), 3.09 (dd, 1H, $J = 14.5$, $J = 4.1$, $C_\beta H$), 4.10–4.19 (m, 1H, $C_\alpha H$), 6.27 (s, 2H, CH_2 POM), 7.08 (d, 1H, $J = 7.9$, NH), 7.94 (s, 1H, CH triazol).

^{13}C NMR {^{1}H} (100 MHz, d6-DMSO): δ 26.87, 27.44, 28.53, 38.60, 53.80, 70.27, 78.52, 124.45, 144.14, 155.76, 173.52, 176.84.

MS (ESI): 371.7 $(M + H)^+$

IR (KBr): 3386, 3151, 2981, 2938, 2576, 1747, 1716, 1517 cm^{-1}

Fmoc-*β*-((1-benzyl)-1,2,3-triazol-4-yl)-Alanine **6** ^{1}H NMR (400 MHz; $CDCl_3$): δ 3.36 (dd, $J = 14.8$, $J = 5.4$, 1H, $C_\beta H$), 3.42 (dd, $J = 14.8$, $J = 3.8$, 1H, $C_\beta H'$), 4.16 (t, $J = 6.6$, 1H, CH Fmoc), 4.30–4.41 (m, 2H, CH_2 Fmoc), 4.57–4.66 (m, 1H, $C_\alpha H$), 5.37 (d, $J = 14.9$, 1H), 5.51 (d, $J = 14.9$, 1H, CH_2-Bzl), 5.93 (d, $J = 5.6$, 1H, HNC_α), 7.12–7.20 (m, 2H, Ar), 7.23 (bs, 1H, CH triazol), 7.27–7.33 (m, 5H, Ar), 7.34–7.44 (m, 3H, Ar), 7.55 (d, $J = 7.3$, 2H, Ar), 7.74 (t, $J = 8.2$, 2H, Ar).

^{13}C NMR {^{1}H} (400 MHz; $CDCl_3$): δ 27.51, 47.10, 53.25, 54.35, 66.78, 119.93, 123.18, 125.10, 127.06, 127.69, 127.92, 128.80, 129.08, 134.03, 141.19, 142.33, 143.67, 155.72, 172.55.

MS (ESI): 469.1 $(M + H)^+$

Fmoc-β-(1(-4-methoxybenzyl)-1,2,3-triazol-4-yl)-Alanine **7** ^{1}H NMR (400 MHz; $CDCl_3$): δ 3.28 (dd, $J = 14.8$, $J = 6.7$, 1H, $C_\beta H$), 3.40 (bd, $J = 14.8$, 1H, $C_\beta H'$), 3.74 (s, 3H, *p*-methoxy), 4.19 (t, $J = 6.9$, 1H, CH Fmoc), 4.37 (d, $J = 6.9$ Hz, 2H, CH_2 Fmoc), 4.59 (bs, 1H, $C_\alpha H$), 5.35 (d, $J = 14.8$, 1H, CH-Bzl), 5.46 (d, $J = 14.8$, 1H, CH-Bzl), 5.95 (d, $J = 5.4$, 1H, HNC_α), 6.84 (d, $J = 8.2$, 2H, Ar), 7.16 (d, $J = 8.6$, 2H, Ar), 7.21 (s, 1H, CH triazol), 7.28–7.34 (m, 2H, Ar), 7.37–7.43 (m, 2H, Ar), 7.55–7.61 (m, 2H, Ar), 7.74–7.80 (m, 2H, Ar).

MS (ESI): 499.1 $(M + H)^+$

Fmoc-β-(1-(3,5-dimethoxybenzyl)-1,2,3-triazol-4-yl)-Alanine **8** ^{1}H NMR (400 MHz; CD_3CN): δ 3.10 (dd, $J = 14.9$, $J = 7.6$, 1H, $C_\beta H$), 3.20 (dd, $J = 14.9$, $J = 4.7$, 1H, $C_\beta H'$), 3.70 (bs, 6H, *m,m*-dimethoxy), 4.20 (t, $J = 7.1$, 1H, CH Fmoc), 4.30 (d, $J = 7.1$, 2H, CH_2 Fmoc), 4.41–4.46 (m, 1H, $C_\alpha H$), 5.40 (s, 2H, CH_2-Bzl), 6.38 (bs, 3H, Bzl), 7.32 (t, $J = 7.5$, 2H, Fmoc), 7.42 (dd, $J = 7.5$, $J = 7.1$, 2H, Fmoc), 7.58–7.64 (m, 3H, Fmoc + CH triazol), 7.83 (d, $J = 7.5$, 2H, Fmoc).

MS (ESI): 529.2 $(M + H)^+$

Fmoc-β-(1-pivaloyloxymethyl)-1,2,3-triazol-4-yl)-Alanine **9** ^{1}H NMR (400 MHz; d6-DMSO): δ 1.07 (s, 9H, ^{t}Bu), 3.00 (dd, 1H, $J = 14.5$, $J = 9.6$, $C_\beta H$), 3.15 (dd, 1H, $J = 14.5$, $J = 4.6$, $C_\beta H'$), 3.30 (s, 1H, CH Fmoc), 4.10–4.30 (m, 3H, $C_\alpha H + CH_2$ Fmoc), 6.26 (s, 2H, CH_2 POM), 7.31 (bt, $J = 7.6$, 2H, Fmoc), 7.41 (bt, 2H, $J = 7.4$, Fmoc), 7.67 (bt, 2H, $J = 6.2$, Fmoc), 7.88 (d, 1H, $J = 7.6$ triazol), 7.96 (s, 1H, CH triazol).

^{13}C NMR {^{1}H} (100 MHz, $CDCl_3$): δ 26.72, 27.47, 38.73, 47.05, 53.18, 67.06, 69.81, 119.93, 124.57, 124.89, 125.08, 127.07, 127.68, 141.20, 141.21, 142.66, 143.64, 143.73, 155.89, 172.75, 177.65.

MS (ESI): 493.1 $(M + H)^+$

IR (KBr): 3411, 3129, 2966, 1738, 1725, 1529 cm^{-1}

Ac-β-(1-benzyl)-1,2,3-triazol-5-yl)-Ala-OEt **10** ^{1}H NMR (400 MHz; $CDCl_3$): δ 1.22 (t, $J = 7.2$, 3H, CH_3 ethyl), 1.96 (s, 3H, acetyl), 3.10 (dd, $J = 15.8$, $J = 5.9$, 1H, $C_\beta H$), 3.16 (dd, $J = 15.8$, $J = 6.2$, 1H, $C_\beta H'$), 4.12–4.24 (m, 2H, CH_2 ethyl), 4.73 (ddd, J = 7.1, $J = 6.2$, $J = 5.9$, 1H, HC_α), 5.46 (d, $J = 16.0$, 1H, CH-Bzl), 5.60 (d, $J = 16.0$, 1H, CH′-Bzl), 6.38 (d, $J = 7.1$, 1H, NH), 7.12–7.20 (m, 2H, Bzl), 7.30–7.38 (m, 3H, Bzl), 7.43 (s, 1H, CH triazol)

^{13}C NMR {^{1}H} (100 MHz, $CDCl_3$): δ 14.00, 22.95, 25.72, 51.28, 51.65, 51.18, 127.15, 128.37, 128.97, 132.20, 133.36, 134.70, 170.05, 170.51.

MS (ESI): 317.2 $(M + H)^+$

Ac-β-(1-(3,5-dimethoxybenzyl)-1,2,3-triazol-5-yl)-Ala-OEt **11** ^{1}H NMR (400 MHz; $CDCl_3$): δ 1.21 (t, $J = 7.1$, 3H, CH_3 ethyl), 1.96 (s, 3H, acetyl), 3.08 (dd, $J = 15.8$, $J = 5.5$, 1H, $C_\beta H$), 3.15 (dd, $J = 15.8$ Hz, $J = 6.4$ Hz, 1H, $C_\beta H'$), 3.71 (s, 6H, *m,m*-dimethoxy), 4.13 (qd, $J = 9.6$, $J = 7.1$, 1H, CH ethyl), 4.18 (qd, $J = 9.6$, $J = 7.1$, 1H, CH ethyl), 4.73 (ddd, $J = 7.1$, $J = 6.4$, $J = 5.5$, 1H, $C_\alpha H$), 5.38 (d, $J = 15.6$, 1H, CH-Bzl), 5.51 (d, $J = 15.6$, 1H, CH-Bzl), 6.27 (d, $J = 2.1$, 2H, Bzl), 6.35 (t, $J = 2.1$, 1H, Bzl), 6.59 (d, $J = 7.1$, 1H, NH), 7.41 (s, 1H, CH triazol).

MS (ESI): 377.1 $(M + H)^+$

H-β-(1-(pivaloyloxymethyl)-1,2,3-triazol-4-yl)-Alanine **12** ^{1}H NMR (400 MHz; D_2O): δ 1.26 (s, 9H, ^{t}Bu), 3.30 (dd, 1H, $J = 15.8$, $J = 6.8$, $C_\beta H$), 3.36 (dd, 1H, $J = 15.8$, $J = 5.2$, $C_\beta H$), 4.06 (dd, 1H, $J = 6.8$, $J = 5.2$, $C_\alpha H$), 6.33 (s, 2H, CH_2 POM), 8.06 (s, 1H, CH triazol).

^{13}C NMR {^{1}H} (100 MHz, D_2O): δ 25.81, 26.16, 38.41, 54.16, 71.22, 125.42, 142.14, 172.91, 179.74.

MS (ESI): 271.1 $(M + H)^+$

IR (KBr): 3142, 3044, 2969, 2591, 2088, 1740, 1617, 1586 cm^{-1}

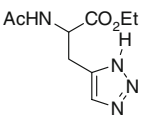
Boc-β-(1,2,3-triazol-4-yl)-Alanine **13** ^{1}H NMR (400 MHz; CD_3OD): δ 1.37 (s, 9H, ^{t}Bu), 3.06 (dd, 1H, $J = 14.9$, $J = 8.7$, $C_\beta H$), 3.24 (dd, 1H, $J = 14.9$, $J = 4.6$, $C_\beta H$), 4.38 (dd, $J = 8.7$, $J = 4.6$, $C_\alpha H$), 7.57 (s, 1H, CH triazol).

^{13}C NMR {^{1}H} (100 MHz, d6-DMSO): δ 26.72, 28.15, 53.39, 78.19, 131.5, 142.8, 155.30, 173.08

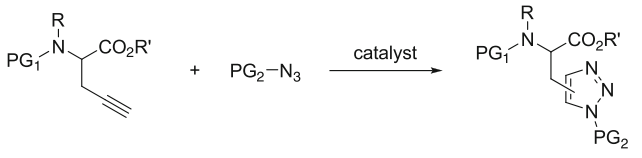
MS (ESI): 257.1

IR (KBr): 3159, 3981, 2602, 2360, 1694, 1520 cm^{-1}

Ac-β-(1,2,3-triazol-5-yl)-Ala-OEt from **10**



^{1}H NMR (400 MHz; $CDCl_3$): δ 1.19 (t, $J = 7.0$, 3H, CH_3 ethyl), 1.89 (s, 3H, acetyl), 3.10–3.30 (m, 2H, $C_\beta HH'$), 4.13 (q, $J = 7.0$, 2H, CH_2 ethyl), 4.86 (bs, 1H, $C_\alpha H$), 7.18 (bs, 1H, $C_\alpha NH$), 7.41 (s, 1H, CH triazol), 7.75 (bs, NH triazol).



Scheme 1 Azahistidine analogues via [3 + 2] Huisgen cycloaddition

Table 1 Synthesis of azahistidine analogues

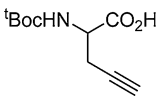
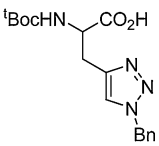
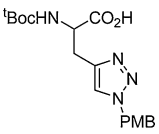
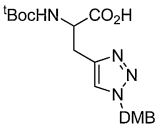
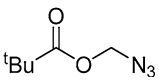
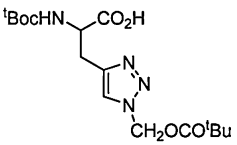
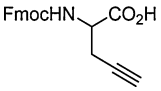
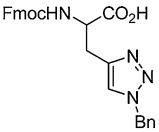
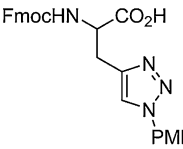
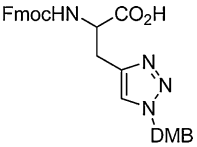
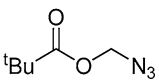
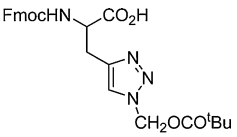
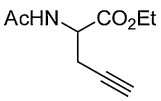
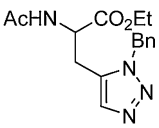
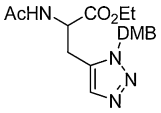
Entry	Propargyl glycine derivative	Azide	Conditions	Product	Yield (%)
1		Bn–N_3	A	 **2**	60
2		PMB–N_3	A	 **3**	40
3		DMB–N_3	A	 **4**	15
4			A	 **5**	73
5		Bn–N_3	A	 **6**	70
6		PMB–N_3	A	 **7**	40
7		DMB–N_3	A	 **8**	30
8			A	 **9**	57
9		Bn–N_3	B	 **10**	84

Table 1 continued

Entry	Propargyl glycine derivative	Azide	Conditions	Product	Yield (%)
10		$DMB–N_3$	B	 **11**	44

Conditions A $Cu(OAc)_2$/sodium ascorbate in $^tBuOH/H_2O$ at r.t.; *Conditions B* $Cp^*RuCl(PPh_3)_2$ in toluene at 80°C

Gly-D-azaHis-D-Lys 14

The synthesis of the tripeptide was achieved according to a literature protocol using DIC and HOBt as coupling reagents (Liakopoulou et al. 1997). Fmoc-Lys(Mtt)-OH and Fmoc-His(Mtt)-OH were replaced by Fmoc-D-Lys(-Boc)-OH and Fmoc-D-azaHis(POM)-OH, respectively. Cleavage from the resin was performed using 10 ml of a cocktail composed of TFA/TIS/H_2O (9.5/0.3/0.2) for 4 hours at room temperature. After filtration, TFA was removed by evaporation, the resulting solid was dissolved in water and the solution was lyophilized. About 100 mg of the peptide TFA salt was then subjected to deprotection (Fmoc and POM) by addition of 650 µl of a 1 M NaOH aqueous solution (2.2 eq.) in 3.5 ml of MeOH for 5 hours at room temperature. After addition of HCl (1 M) to reach pH 4, the solution was evaporated and the solid was taken up in MeOH. NaCl was filtrated and the resulting solution was evaporated. The resulting solid was triturated in cold diethyl ether yielding peptide **14** (yield 36%) as a white solid with a purity of 90% (estimated by LC/MS and ^1H NMR).

^1H NMR (400 MHz; D_2O): δ 1.19–1.31 (m, 2H, CH_2 δ Lys), 1.50–1.80 (m, 4H, CH_2 β and γ Lys), 2.88 (t, 2H, $J = 7.2$ CH_2 ε Lys), 3.13 (dd, 1H, $J = 7.7$, $J = 15.2$, CH_2 β azaHis), 3.20 (dd, 1H, $J = 6.2$, $J = 15.2$, CH_2 β azaHis), 3.74 (AB system, 2H, $J = 16.1$, CH_2 Gly), 4.13 (dd, 1H, $J = 5.4$, $J = 7.5$, CH α Lys), 4.51–4.82 (m, 1H superimposed with water, CH α azaHis), 7.68 (s, 1H, CH triazol).

^{13}C NMR {^1H} (100 MHz, D_2O): δ 21.84, 26.14, 26.69, 30.36, 39.08, 40.24, 53.26, 53.84, 127.29, 140.06, 166.8, 171.58, 176.73.

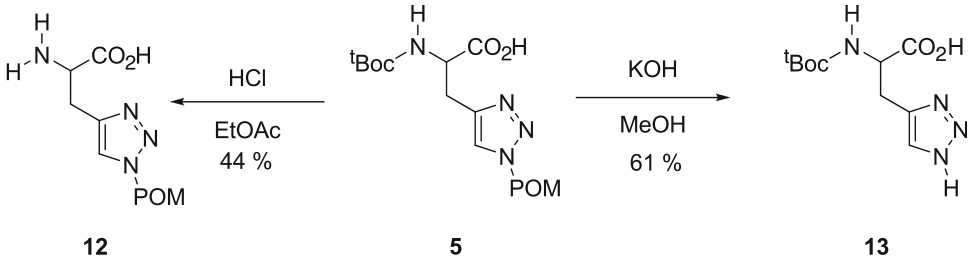
HRMS (ES-) calcd for $C_{13}H_{22}N_7O_4$:340.1733; found:340.1723.

Scheme 3 Selective deprotection of Fmoc-protected azahistidine derivative **9**

Results and discussion

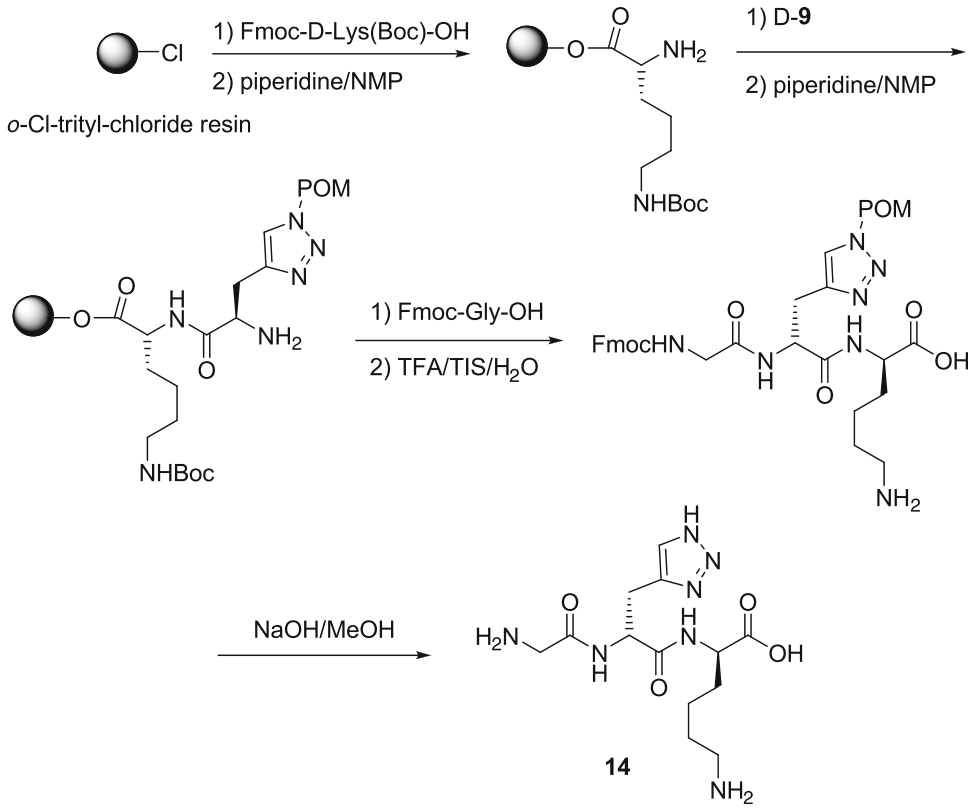
In order to efficiently use such a compound in (solid phase) peptide synthesis, easy access to orthogonally protected derivatives is strongly a necessity. Although there have been some routes to protected **1** or derivatives, none of them deals with the orthogonally protected analogues. The recent development of the copper catalysed version of Huisgen cycloaddition (so called click chemistry) allows the rapid access to 1,2,3-triazoles (Kolb et al. 2001; Lipshutz and Taft 2006; Bock et al. 2006). Using this approach, azahistidine derivatives have been obtained but no real attention has been paid to the removal of the protecting groups. In this paper, we describe the click chemistry based access to fully protected azahistidine, suitable for peptide synthesis and we briefly assay the deprotection steps (Scheme 1).

Azahistidines were obtained using either copper-catalysed click chemistry or the more recently described ruthenium-catalysed cycloaddition (Zhang et al. 2005; Oppilliart et al. 2007), affording the 1,5-disubstituted triazole. Nevertheless, the latter requires the fully protected propargylglycine as starting material, since the catalyst does not allow the use of free carboxylic acids. Using



Scheme 2 Selective deprotection of Boc-protected azahistidine derivative **5**

Scheme 4 SPPS of a GHK analogue, Gly-D-azaHis-D-Lys



either condition, rapid access to protected azahistidines was demonstrated and the expected regioisomers were obtained with fair to satisfactory yields (Table 1).

Using the copper-catalyzed version of the reaction, we were able to react either Fmoc- or ^{t}Boc- protected propargylglycine with a range of azides (Entries 1–8) yielding the expected triazoles. It was found that the purification of the acid products was sometimes difficult resulting in low yields. When the *N*-acetyl, ethyl ester of propargylglycine was used as a starting material, in conjunction with ruthenium catalyst, the expected 1,5-regioisomer was also cleanly obtained (Entries 9 and 10).

With the above histidine analogues in hand, the next step was to selectively remove the protective group. As expected the hydrogenolysis of benzyl, *p*-methoxybenzyl and dimethoxybenzyl derivatives turned out to be very difficult. By using harsh conditions we managed to remove the protecting groups on compounds **2** and **10** but the same protocols applied to compounds bearing a Fmoc protecting group (**6**–**8**) afforded debenzylated compounds along with partial hydrogenation of the benzyl ring as well as removal of Fmoc group. Alternative, oxidising conditions using CAN were not satisfactory. We then decided to test the recently proposed protected azide by Sharpless *id est* azidomethyl pivalate (Loren et al. 2005). Noteworthy is that this protecting group has been recently used in a very elegant synthesis of α-CF_3-substitued azahistidine (Shchetnikov et al. 2007). The cycloaddition starting from ^{t}Boc or Fmoc protected propargylglycine yielded the expected pivaloyloxymethyl (POM) azahistidine cleanly (Entries 4 and 8).

At this stage, selective deprotection of the ^{t}Boc group on compound **5** was achieved using HCl in ethyl acetate to furnish **12**. In parallel, selective removal of the pivaloyl group was cleanly achieved using 1 M NaOH in MeOH without affecting the ^{t}Boc group to afford compound **13** (Scheme 2).[1]

In a similar fashion, when compound **9** was treated under classical conditions for removal of the Fmoc protecting group (piperidine), we obtained compound **12**. This last result should permit the use of compound **9** for insertion of azahistidine derivatives in peptides using standard SPPS strategies (Scheme 3).

As an example, compound **9** was used for the solid phase synthesis of a Gly-His-Lys analogue, an endogenous tripeptide known as a growth-modulating factor, as a strong activator of wound healing and as a copper chelator. The

[1] As suggested by one of the referees, we checked the potential racemization of the azahistidine compounds under the deprotection conditions. Treatment of compound **5** (5 mg) overnight with NaOD/D_2O (100 µl of a 40% solution) in CD_3OD (400 µl) resulted in removal of the POM group without incorporation of deuterium at the alpha position (checked by ^{1}H NMR). This finding was moreover corroborated by the synthesis of the G-azaH-K peptide that resulted in no epimerisation of the two D-amino acids.

synthesis was performed on a chlorotrityl resin using Fmoc-glycine, compound **9** (D enantiomer) and Fmoc-D-lysine(Boc). After cleavage of the peptide from the resin using TFA, the two remaining protecting groups (*id est* Fmoc on the glycine and POM on the azahistidine side chain) were removed using NaOH in MeOH. After trituration in cold ether, GHK analogue **14** was obtained as a single isomer (Scheme 4).

In conclusion, we have described the synthesis of numerous protected azahistidine derivatives. Derivatives bearing a POM group on the triazole ring appeared to be good candidates for SPPS since selective deprotection of either the side chain or the amino-acid nitrogen group proved to be possible. Azahistidine bearing an Fmoc group and a POM group on the side chain is of particular interest since the deprotection of the Fmoc group takes place with piperidine while the POM deprotection requires more basic conditions thus allowing the use of compound **9** in SPPS as exemplified in the synthesis of a GHK analogue described herein.

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