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Novel chloroenyne-modified amino acid derivatives

M. Gredičak, A. Kolonić, and **I. Jerić**

Division of Organic Chemistry and Biochemistry, Ruđer Bošković Institute, Zagreb, Croatia

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Summary. Three groups of chloroenyne-modified amino acids were synthesized. Chloroenyne moiety was attached at the *N*- or *C*-terminal amino acid (Tyr, Phe, Val, Gly, Lys) position carrying different protecting groups. Prepared derivatives will be used as building blocks in the synthesis of enediyne-peptide conjugates. Furthermore, reactivity of modified amino acids in the peptide bond formation reaction was tested.

Keywords: Amino acid modifications – Sonogashira reaction – Enediynes – Chloroenynes

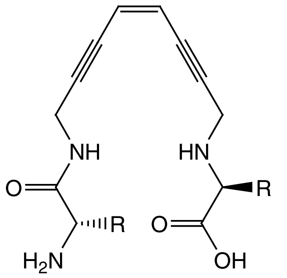
Introduction

Terminal and side-chain modification of amino acids is a useful approach towards new derivatives with targeted properties. Amino acids have been equipped with caged groups for the study of phosphorylation-dependent pathways (Rothman et al., 2003), modified by porphyrin moiety for the DNA-binding properties study (Biron and Voyer, 2005) and attached to different quinones for the post-chain assembly modifications (Bittner et al., 2002). Furthermore, *N*-alkylation is often used to affect proteolytic stability of peptides (Adesi et al., 2003), their conformational preferences (Tran et al., 2006) and for the structure–activity relationship studies (Lengyel et al., 2002). On the other hand, unnatural amino acids are versatile frames with a variety of potentials. They are widely used for the peptide secondary structure control (Yeo et al., 2006) and as targets for further functionalization (Mohamed and Brook, 2002; IJsselstijn et al., 2003). Amino acids carrying unsaturated appendages are exploited in polymer science for the synthesis of polyacetylenes with interesting optical and conformational properties (Rahim et al., 2006).

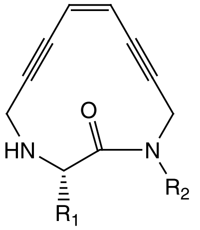
Our interest is focused on the synthesis of chloroenyne-substituted amino acids suitable for the construction of enediyne-related peptide derivatives. Naturally occurring enediyne compounds are among the most effective anticancer agents known, and synthetically modified enediynes are lead chemotherapeutics today. The mechanism of enediyne action involves the cycloaromatization of enediyne moiety and the formation of highly reactive diradical capable of double-strand DNA cleavage. The association of enediyne framework and peptides is expected to bring interesting properties to new enediyne-peptide conjugates, owing to the rigidity of enediyne group and a structural diversity of peptides. Recently, we have described a general synthetic route to enediyne-bridged amino acids (Jerić and Chen, 2007). This route has now been tested on a number of amino acids (Tyr, Phe, Val, Gly, Lys) with different protecting groups, and here we describe the synthesis and characterization of amino acids carrying penta-5-chloro-4-en-2-yne moiety at the *N*- or *C*-terminus. As outlined at the Scheme 1, prepared derivatives are building blocks in the synthesis of various enediyne-peptide adducts, acyclic bridged peptides (type **I**) and cyclic amino acid-related (type **II**) or peptide-related (type **III**).

Materials and methods

The melting points were determined on a Tottoli (Büchi) apparatus and were uncorrected. Optical rotations were measured at 20 °C with an Optical Activity LTD automatic AA-10 Polarimeter. Reactions were monitored by TLC on Silica Gel 60 F_{254} plates (Merck; Darmstadt, Germany) using detection with ninhydrin, chlorine-iodine reagent or mostain solution (1 g of $(NH_4)_6Mo_7O_{24} \times 4H_2O$ and 10 mg of $Ce(SO_4)_2$ in 20 ml of 10% H_2SO_4). NMR spectra were recorded on Bruker AV 600 spectrometer, operating at 75.47 MHz for ^{13}C and 600.13 MHz for ^{1}H nuclei. Spectra were assigned based on 2D homonuclear (COSY) and heteronuclear (HMQC and HMBC) spectra. Mass spectrometry measurements were performed on a Micromass Quattro micro^{TM} API Tandem Quadrupole



type I R = amino acid side-chain



type II R_1 = amino acid side-chain
R_2 = H

type III R_1 = amino acid side-chain
R_2 = -CHR$_1$COOH

Scheme 1

mass spectrometer, operating in positive electrospray ionization (ESI) mode (Case Western Reserve University, Cleveland, USA). Spectra were recorded from a 50 μg/ml compound solutions in methanol or methanol-0.1% TFA (1:1). Solution was infused into the ion source of the instrument from a syringe pump at a flow rate of 10 μl/min. For the collision-induced dissociation (CID) MS/MS measurements, argon was used as collision gas and cone voltage was set to 20 V. Compounds **1b**, **7a**, **7e** and **8b** were described previously (Jerić and Chen, 2007).

*Synthesis of Boc-Aaa-NH-CH$_2$-C≡CH (**1a–e**)*

Boc-Aaa-OH (4.68 mmol) was dissolved in 2 ml of dry DMF, solution was cooled to 0 °C, and NMM (0.50 ml, 4.68 mmol) and isobutyl chloroformate (0.60 ml, 4.68 mmol) were added. Propargylamine (0.93 ml, 4.68 mmol) and NMM (0.50 ml, 4.68 mmol) in 1 ml of dry DMF were added dropwise. Reaction was stirred for 30 min at 0 °C and then at room temperature overnight. Solvent was evaporated and the residue extracted with EtOAc. Product was crystallized from EtOAc/hexane.

Boc-Tyr(Boc)-NH-CH$_2$-C≡CH (**1a**)

Yield: 90%. White crystals; mp 122–125 °C. R_f 0.70 (petrol ether-EtOAc 1:1). $[\alpha]_D + 5.0°$ (*c* 1.2, MeOH). ^{13}C NMR (DMSO-d$_6$): δ = 27.77 (CH$_3$, OBoc), 27.97 (C1, propargylamide), 28.09 (CH$_3$, NHBoc), 36.73 (β Tyr), 55.50 (α Tyr), 73.05 (C3, propargylamide), 77.98 (C, NHBoc), 80.94 (C2, propargylamide), 82.93 (C, OBoc), 120.81 (ε Tyr), 130.21 (δ Tyr), 135.65 (γ Tyr), 149.13, 151.25 (CO NHBoc, OBoc), 155.18 (ζ Tyr), 171.29 (CO Tyr). ^{1}H NMR (DMSO-d$_6$): δ = 1.34 (s, 9H, CH$_3$ NHBoc), 1.48 (s, 9H, CH$_3$ OBoc), 2.72, 2.92 (dd, 2H, ββ′ Tyr, $^3J_{\alpha,\beta}$ = 3.90 Hz, $^3J_{\alpha,\beta'}$ = 10.60 Hz, $^2J_{\beta,\beta'}$ = 13.50 Hz), 3.13 (br s, 1H, H3 propargylamide), 3.88 (dd, 2H, H11′ propargylamide, $^3J_{NH,H11'}$ = 4.65 Hz, $^4J_{H11',H3}$ = 1.80 Hz), 4.12 (m, 1H, α Tyr), 6.94 (d, 1H, NH Tyr, $^3J_{\alpha,NH}$ = 8.02 Hz), 7.08 (d, 2H, ε Tyr, $^3J_{\delta,\varepsilon}$ = 8.28 Hz), 7.30 (d, 1H, δ Tyr, $^3J_{\delta,\varepsilon}$ = 8.28 Hz), 8.41 (t, 1H, NH propargylamide, $^3J_{NH,H11'}$ = 4.65 Hz).

Boc-Val-NH-CH$_2$-C≡CH (**1c**)

Yield: 76%. White crystals; mp 110–112 °C. R_f 0.80 (petrol ether-EtOAc 1:1). $[\alpha]_D$ −26.0° (*c* 0.93, MeOH). ^{13}C NMR (CDCl$_3$): δ = 19.45 (γ Val), 28.53 (CH$_3$ Boc), 29.22 (C1 propargylamide), 31.09 (β Val), 60.07 (α Val), 71.78 (C3 propargylamide), 79.50 (C2 propargylamide), 80.24 (C Boc), 156.15 (CO Boc), 171.70 (CO Val). ^{1}H NMR (CDCl$_3$): δ = 0.94, 0.98 (d, 6H, γγ′ Val, $^3J_{\beta,\gamma\gamma'}$ = 6.75 Hz), 1.46 (s, 9H, CH$_3$ Boc), 2.15 (m, 1H, β Val), 2.22 (t, 1H, H3 propargylamide, $^3J_{H11',H3}$ = 2.50 Hz), 3.95 (dd, 1H, α Val, $^3J_{\alpha,\beta}$ = 8.90 Hz), 4.06 (m, 2H, H11′ propargylamide), 5.12 (d, 1H, NH Val, $^3J_{\alpha,NH}$ = 6.90 Hz), 6.52 (bs, 1H, NH propargylamide).

Boc-Gly-NH-CH$_2$-C≡CH (**1d**)

Yield: 90%. White crystals; mp 102-105 °C. R_f 0.50 (petrol ether-EtOAc 1:1). ^{13}C NMR (CDCl$_3$): δ = 28.51 (CH$_3$ Boc), 29.31 (C1 propargylamide), 44.40 (α Gly), 71.94 (C3 propargylamide), 79.36 (C2 propargylamide), 80.76 (C Boc), 169.47 (CO Gly). ^{1}H NMR (CDCl$_3$): δ = 1.46 (s, 9H, CH$_3$ Boc), 2.24 (t, 1H, H3 propargylamide, $^4J_{H11',H3}$ = 2.50 Hz), 3.82 (d, 2H, α Gly, $^3J_{NH,\alpha}$ = 5.60 Hz), 4.07 (dd, 2H, H11′ propargylamide, $^3J_{NH,H11'}$ = 5.25 Hz), 5.22 (br t, 1H, NH propargylamide), 6.53 (br s, 1H, NH Gly).

Boc-Lys[Z(2-Cl)]-NH-CH$_2$-C≡CH (**1e**)

Yield: 86%. White crystals; mp 103–105 °C R_f 0.70 (petrol ether-EtOAc 1:1). $[\alpha]_D$ −7.0° (*c* 1.08, MeOH). ^{13}C NMR (CDCl$_3$): δ = 22.59 (γ Lys), 28.46 (CH$_3$ Boc), 29.26 (C1 propargylamide), 29.58 (δ Lys), 31.89 (β Lys), 40.58 (ε Lys), 54.31 (α Lys), 64.08 (CH$_2$ Z(2-Cl)), 71.79 (C3 propargylamide), 79.45 (C2 propargylamide), 80.50 (C Boc), 127.00 (C5 Z(2-Cl)), 129.49 (C3 Z(2-Cl)), 129.65 (C4 Z(2-Cl)), 129.90 (C6 Z(2-Cl)), 133.69 (C2 Z(2-Cl)), 134.45 (C1 Z(2-Cl)), 156.05 (C Boc), 156.55 (CO Z(2-Cl)), 172.03 (CO Lys). ^{1}H NMR (CDCl$_3$): δ = 1.43 (m, 11H, CH$_3$ Boc, γ,δ Lys), 1.53 (m, 2H, γ′,δ′ Lys), 1.63, 1.83 (m, 2H, ββ′ Lys), 2.21 (br t, 1H, H3 propargylamide), 3.20 (br d, 2H, εε′ Lys), 4.03 (br s, 2H, H11′ propargylamide), 4.10 (br s, 1H, α Lys), 5.00 (br t, 1H, NHε Lys), 5.21 (s, 2H, CH$_2$ Z(2-Cl)), 5.23 (br s, 1H, NHα Lys), 6.67 (br s, 1H, NH propargylamide), 7.25 (m, 2H, H4,5 Z(2-Cl)), 7.36 (m, 1H, H6 Z(2-Cl)), 7.41 (m, 1H, H3 Z(2-Cl)).

*Synthesis of oNbs-Aaa-NH-CH$_2$-C≡CH (**2a–e**)*

Boc-Aaa-NH-CH$_2$-C≡CH (100 mg) was treated with TFA/H$_2$O (9/1, 1 ml) for 1 h at room temperature. Solvent was evaporated and the residue triturated with hexane. TFA salt of amino acid propargylamide was dissolved in 3 ml of dry DCM, followed by the addition of TEA (2 eqv.) and oNbs-Cl (2 eqv.). Reaction was stirred at room temperature for 4 h. Solvent was evaporated and the residue purified by flash column chromatography.

oNbs-Tyr-NH-CH$_2$-C≡CH (**2a**)

Yield: 41%. White crystals; mp 95–100 °C. R_f 0.43 (EtOAc-petrol ether 2:1). $[\alpha]_D$ +47.0° (*c* 0.96, MeOH). ^{13}C NMR (DMSO-d$_6$): δ = 27.93 (C1 propargylamide), 37.37 (β Tyr), 58.58 (α Tyr), 73.20 (C3 propargylamide), 80.43 (C2 propargylamide), 114.79 (ε Tyr), 124.25 (C3 oNbs), 126.72 (C2 oNbs), 129.24 (C6 oNbs), 132.24 (C4 oNbs), 130.18 (δ Tyr), 133.23 (γ Tyr), 133.46 (C5 oNbs), 146.97 (C1 oNbs), 155.86 (ζ Tyr), 169.89 (CO Tyr). ^{1}H NMR (DMSO-d$_6$): δ = 2.65, 2.78 (dd, 2H, ββ′ Tyr, $^3J_{\alpha,\beta}$ = 4.70 Hz, $^3J_{\alpha,\beta'}$ = 9.80 Hz, $^2J_{\beta,\beta'}$ = 13.72 Hz), 3.12 (t, 1H, H3 propargylamide,

$^4J_{H11',H3} = 2.25$ Hz), 3.74 (dd, 2H, H11′ propargylamide, $^3J_{NH,H11'} = 5.10$ Hz), 3.97 (m, 1H, α Tyr), 6.52 (d, 2H, ε Tyr, $^3J_{\delta,\varepsilon} = 8.27$ Hz), 6.96 (d, 2H, δ Tyr, $^3J_{\delta,\varepsilon} = 8.27$ Hz), 7.58 (m, 2H, H5,6 oNbs), 7.74 (m, 1H, H4 oNbs), 7.84 (d, 1H, H3 oNbs, $^3J_{H3,H4} = 7.80$ Hz), 8.33 (d, 1H, NH Tyr, $^3J_{NH,\alpha} = 7.12$ Hz), 8.45 (t, 1H, NH propargylamide, $^3J_{NH,H11'} = 5.10$ Hz), 9.16 (s, 1H, OH Tyr).

oNbs-Phe-NH-CH$_2$-C≡CH (**2b**)

Yield: 78%. White crystals; mp 125–130 °C. *R*$_f$ 0.60 (EtOAc-petrol ether 2:1). $[\alpha]_D$ +16.0° (*c* 0.98, MeOH). ^{13}C NMR (DMSO-d$_6$): δ = 28.01 (C1 propargylamide), 38.13 (β Phe), 58.25 (α Phe), 73.40 (C3 propargylamide), 80.46 (C2 propargylamide), 124.35 (C3 oNbs), 126.50 (ζ Phe), 127.99 (ε Phe), 129.23 (C6 oNbs), 129.35 (δ Phe), 132.39 (C4 oNbs), 133.18 (C1 oNbs), 133.66 (C5 oNbs), 136.81 (γ Phe), 147.00 (C2 oNbs), 169.85 (CO Phe). ^{1}H NMR (DMSO-d$_6$): δ = 2.76, 2.88 (dd, 2H, ββ′ Phe, $^3J_{\alpha,\beta} = 4.80$ Hz, $^3J_{\alpha,\beta'} = 9.82$ Hz, $^2J_{\beta,\beta'} = 13.64$ Hz), 3.12 (t, 1H, H3 propargylamide, $^4J_{H11',H3} = 2.44$ Hz), 3.74 (dd, 2H, H11′ propargylamide, $^3J_{NH,H11'} = 5.23$ Hz), 4.06 (m, 1H, α Phe), 7.13 (m, 5H arom Phe), 7.59 (d, 2H, H5,6 oNbs), 7.73 (m, 1H, H4 oNbs), 7.84 (d, 1H, H3 oNbs, $^3J_{H3,H4} = 7.84$ Hz) 8.42 (d, 1H, NH Phe, $^3J_{NH,\alpha} = 8.41$ Hz), 8.50 (t, 1H, NH propargylamide, $^3J_{NH,H11'} = 5.23$ Hz).

oNbs-Val-NH-CH$_2$-C≡CH (**2c**)

Yield: 70%. White crystals; mp 103–105 °C. *R*$_f$ 0.65 (EtOAc-petrol ether 2:1). $[\alpha]_D$ −96.0° (*c* 1.15, MeOH). ^{13}C NMR (CDCl$_3$): δ = 17.32, 19.34 (γγ′ Val), 29.44 (C1 propargylamide), 31.15 (β Val), 63.60 (α Val), 72.17 (C3 propargylamide), 78.93 (C2 propargylamide), 125.86 (C3 oNbs), 131.01 (C6 oNbs), 133.20 (C5 oNbs), 133.45 (C1 oNbs), 134.21 (C4 oNbs), 148.00 (C2 oNbs), 169.86 (CO Val). ^{1}H NMR (CDCl$_3$): δ = 0.80, 0.92 (d, 6H, γγ′ Val, $^3J_{\beta,\gamma\gamma'} = 6.76$ Hz), 2.22 (t, 1H, H3 propargylamide, $^4J_{H11',H3} = 2.40$ Hz), 2.27 (m, 1H, β Val), 3.75 (dd, 1H, α Val, $^3J_{\alpha,\beta} = 7.40$ Hz), 3.93 (m, 2H, H11′ propargylamide), 6.00 (d, 1H, NH Val, $^3J_{NH,\alpha} = 7.40$ Hz), 6.47 (br t, 1H, NH propargylamide), 7.76 (m, 2H, H5,6 oNbs), 7.92 (m, 1H, H6 oNbs), 8.09 (m, 1H, H3 oNbs).

oNbs-Gly-NH-CH$_2$-C≡CH (**2d**)

Yield: 58%. White crystals; mp 142–145 °C (decomp). *R*$_f$ 0.34 (EtOAc-petrol ether 2:1). ^{13}C NMR (DMSO-d$_6$): δ = 27.95 (C1 propargylamide), 44.98 (α Gly), 73.18 (C3 propargylamide), 80.69 (C2 propargylamide), 124.46 (C3 oNbs), 129.66 (C6 oNbs), 132.68 (C4 oNbs), 133.04 (C1 oNbs), 133.97 (C5 oNbs), 147.45 (C2 oNbs), 167.18 (CO, Gly). ^{1}H NMR (DMSO-d$_6$): δ = 3.10 (t, 1H, H3 propargylamide, $^4J_{H11',H3} = 2.48$ Hz), 3.63 (br s, 2H, α Gly), 3.80 (dd, 2H, H11′ propargylamide, $^3J_{NH,H11'} = 5.45$ Hz), 7.85 (m, 2H, H4,5 oNbs), 7.97 (dd, 1H, H6 oNbs, $^3J_{H5,H6} = 5.90$ Hz, $^4J_{H4,H6} = 3.20$ Hz), 8.03 (dd, 1H, H3 oNbs, $^3J_{H3,H4} = 5.90$ Hz, $^4J_{H3,H5} = 3.30$ Hz), 8.29 (br s, 1H, NH Gly), 8.37 (t, 1H, NH propargylamide, $^3J_{NH,\alpha} = 5.15$ Hz).

oNbs-Lys[Z(2-Cl)]-NH-CH$_2$-C≡CH (**2e**)

Yield: 75%. Colourless oil. *R*$_f$ 0.65 (petrol ether-EtOAc 3:2). $[\alpha]_D$ −13.0° (*c* 1.0, MeOH). ^{13}C NMR (CDCl$_3$): δ = 21.83 (γ Lys), 29.03 (δ Lys), 29.26 (C1 propargylamide), 29.44 (β Lys), 40.01 (ε Lys), 57.61 (α Lys), 64.13 (CH$_2$ Z(2-Cl)), 71.88 (C3 propargylamide), 78.78 (C2 propargylamide), 125.59 (C3 oNbs), 126.95 (C5 Z(2-Cl)), 129.46 (C3 Z(2-Cl)), 129.54 (C4 Z(2-Cl)), 129.81 (C6 Z(2-Cl)), 130.91 (C6 oNbs), 132.96 (C4 oNbs), 133.16 (C1 oNbs), 133.54 (C2 Z(2-Cl)), 134.01 (C5 oNbs), 134.01 (C1 Z(2-Cl)), 147.76 (C2 oNbs), 156.64 (CO Z(2-Cl)), 170.30 (CO Lys). ^{1}H NMR (CDCl$_3$): δ = 1.45, 1.55 (m, 4H, γγ′δδ′ Lys), 1.80 (m, 2H, ββ′ Lys), 2.18 (br t, 1H, H3 propargylamide), 3.11 (m, 2H, εε′ Lys), 3.87 (br s, 2H, H11′ propargylamide), 4.00 (m, 1H, α Lys), 5.18 (s, 2H, Z(2-Cl)), 6.32 (d, 1H, NHα Lys, $^3J_{NH,\alpha} = 7.37$ Hz), 6.82 (br t, 1H, NH propargylamide), 7.27 (m, 2H, H4,5 Z(2-Cl)), 7.45 (m, 2H, H3,6 Z(2-Cl)), 7.74 (m, 2H, H4,5 oNbs), 7.85 (m, 1H, H6 oNbs), 8.08 (m, 1H, H3 oNbs).

*Synthesis of cis-Boc-Aaa-NH-CH$_2$-C≡C-CH=CHCl (**3a–e**)*

(C$_6$H$_5$CN)$_2$PdCl$_2$, (10% eqv.), CuI (10% eqv.), *cis*-ClCH=CHCl (2 eqv.) and BuNH$_2$ (2 eqv.) were dissolved in 1 ml of dry THF and the reaction was stirred for 30 min under argon. A solution of Boc-Aaa-NH-CH$_2$-C≡CH (1 eqv.) and 2 eqv. of BuNH$_2$ in 2 ml of THF was added dropwise over the syringe, and the reaction was stirred at room temperature until all acetylene was consumed. Solvent was evaporated and the residue purified by flash chromatography.

cis-Boc-Tyr(Boc)-NH-CH$_2$-C≡C-CH=CHCl (**3a**)

Yield: 57%. Beige crystals; mp 87–90 °C. *R*$_f$ 0.65 (petrol ether-EtOAc 1:1). $[\alpha]_D$ +5.0° (*c* 0.4, MeOH). MW 478.97 ESI-MS: *m/z* 501.23 $[M+Na]^+$ ^{13}C NMR (DMSO-d$_6$): δ = 27.24 (CH$_3$, OBoc), 28.13 (CH$_3$, NHBoc), 28.82 (C1, chloroenyne), 36.71 (Cβ, Tyr), 55.63 (α, Tyr), 76.14 (C2, chloroenyne), 78.03 (C, NHBoc), 82.99 (C, OBoc), 95.01 (C3, chloroenyne), 112.12 (C4, chloroenyne), 120.90 (ε, Tyr), 128.76 (C5, chloroenyne), 130.20 (δ, Tyr), 135.74 (γ, Tyr), 149.14, 151.31 (CO, NHBoc, OBoc), 155.26 (ζ, Tyr), 171.44 (CO, Tyr). ^{1}H NMR (DMSO-d$_6$): δ = 1.29 (s, 9H, CH$_3$ NHBoc), 1.48 (s, 9H, CH$_3$ OBoc), 2.72, 2.95 (dd, 2H, ββ′ Tyr, $^3J_{\alpha,\beta} = 3.95$ Hz, $^3J_{\alpha,\beta'} = 10.70$ Hz, $^2J_{\beta,\beta'} = 13.33$ Hz), 4.11 (m, 3H, H11′ chloroenyne, α Tyr), 6.18 (br td, 1H, H4 chloroenyne), 6.78 (d, 1H, H5 chloroenyne, $^3J_{H4,H5} = 7.33$ Hz), 6.97 (d, 1H, NH Tyr, $^3J_{NH,\alpha} = 8.75$ Hz), 7.07 (d, 2H, ε Tyr, $^3J_{\delta,\varepsilon} = 8.18$ Hz), 7.28 (d, 2H, δ Tyr, $^3J_{\delta,\varepsilon} = 8.37$ Hz), 8.50 (t, 1H, NH chloroenyne, $^3J_{NH,H11'} = 5.13$ Hz).

cis-Boc-Phe-NH-CH$_2$-C≡C-CH=CHCl (**3b**)

Yield: 40%. Yellow oil. *R*$_f$ 0.67 (petrol ether-EtOAc 1:1). $[\alpha]_D$ +3.0° (*c* 0.5, MeOH). MW 362.85 ESI-MS: *m/z* 363.65 $[M+H]^+$ ^{13}C NMR (CDCl$_3$): δ = 28.52 (CH$_3$, Boc), 30.25 (C1, chloroenyne), 38.72 (β, Phe), 56.02 (α, Phe), 77.60 (C2, chloroenyne), 92.95 (C3, chloroenyne), 111.75 (C4, chloroenyne), 127.32 (ζ, Phe), 129.02 (ε, Phe), 129.26 (C5, chloroenyne), 129.61 (δ, Phe), 136.72 (γ, Phe), 171.15 (CO, Phe). ^{1}H NMR (CDCl$_3$): δ = 1.41 (s, 9H, CH$_3$ Boc), 3.07 (d, 2H, ββ′ Phe, $^3J_{\alpha,\beta\beta'} = 6.60$ Hz), 4.18 (m, 2H, H11′ chloroenyne), 4.35 (m, 1H, α Phe), 5.00 (br s, 1H, NH Phe), 5.84 (td, 1H, H4 chloroenyne), 6.06 (br t, 1H, NH chloroenyne), 6.39 (d, 1H, H5 chloroenyne, $^3J_{H4,H5} = 7.46$ Hz), 7.26 (m, 5H, arom Phe).

cis-Boc-Val-NH-CH$_2$-C≡C-CH=CHCl (**3c**)

Yield: 52%. Yellow oil. *R*$_f$ 0.70 (petrol ether-EtOAc 1:1). $[\alpha]_D$ −36.0° (*c* 1.0, MeOH). MW 314.81 ESI-MS: *m/z* 315.29 $[M+H]^+$ ^{13}C NMR (CDCl$_3$): δ = 18.01, 19.49 (γγ′, Val), 28.52 (CH$_3$, Boc), 30.16 (C1, chloroenyne), 31.02 (β, Val), 60.13 (α, Val), 93.17 (C3, chloroenyne), 111.72 (C4, chloroenyne), 129.17 (C5, chloroenyne), 156.13 (CO, Boc), 171.56 (CO, Val). ^{1}H NMR (CDCl$_3$): δ = 0.93, 0.97 (d, 6H, γγ′ Val, $^3J_{\beta,\gamma} = 6.68$ Hz, $^3J_{\beta,\gamma'} = 8.30$ Hz), 1.45 (s, 9H, CH$_3$ Boc), 2.20 (m, 1H, β Val), 3.93 (m, 1H, α Val), 4.26 (m, 2H, H11′ chloroenyne), 5.06 (br s, 1H, NH Val), 5.86 (td, 1H, H4 chloroenyne, $^5J_{H11',H4} = 1.92$ Hz), 6.34 (br s, 1H, NH chloroenyne), 6.36 (d, 1H, H5 chloroenyne, $^3J_{H4,H5} = 7.48$ Hz).

cis-Boc-Gly-NH-CH$_2$-C≡C-CH=CHCl (**3d**)

Yield: 41%. Yellow oil. *R*$_f$ 0.35 (petrol ether-EtOAc 1:1). MW 272.73 ESI-MS: *m/z* 295.14 $[M+Na]^+$ ^{13}C NMR (CDCl$_3$): δ = 28.52 (CH$_3$, Boc), 30.21 (C1 chloroenyne), 44.61 (α Gly), 80.76, 80.81 (C2 chloroenyne, C

Boc), 93.00 (C3 chloroenyne), 111.68 (C4 chloroenyne), 129.32 (C5 chloroenyne), 156.31 (CO Boc), 169.37 (CO Gly). ^{1}H NMR (CDCl$_3$): δ = 1.46 (s, 9H, CH$_3$ Boc), 3.82 (d, 2H, α Gly), 4.26 (m, 2H, H11′ chloroenyne), 5.13 (br s, 1H, NH Gly), 5.86 (td, 1H, H4 chloroenyne, $^{5}J_{\mathrm{H11',H4}} = 2.00$ Hz), 6.40 (d, 1H, H5 chloroenyne, $^{3}J_{\mathrm{H4,H5}} = 7.47$ Hz), 6.44 (br s, 1H, NH chloroenyne).

cis-Boc-Lys[Z(2-Cl)]-NH-CH$_2$-C≡C-CH=CHCl (**3e**)

Yield: 40%. Beige powder; mp 85–89 °C. R_f 0.50 (petrol ether-EtOAc 1:1). [α]$_D$ −10.0° (*c* 0.7, MeOH). MW 512.43 ESI-MS: *m/z* 513.32 [M + H]$^+$ ^{13}C NMR (CDCl$_3$): δ = 22.66 (γ Lys), 28.52 (CH$_3$ Boc), 29.70 (δ Lys), 30.27 (C1 chloroenyne), 31.85 (β Lys), 40.63 (ε Lys), 54.46 (α Lys), 64.17 (CH$_2$ Z(2-Cl)), 77.58 (C2 chloroenyne), 80.51 (C Boc), 93.18 (C3 chloroenyne), 111.74 (C4 chloroenyne), 127.08 (C5 Z(2-Cl)), 129.17 (C5 chloroenyne), 129.58 (C3 Z(2-Cl)), 129.75 (C4 Z(2-Cl)), 130.01 (C6 Z(2-Cl)), 133.80, 134.53 (C1, C2 Z(2-Cl)), 156.60 (CO Z(2-Cl)), 171.91 (CO Lys). ^{1}H NMR (CDCl$_3$): δ = 1.47 (m, 11H, CH$_3$ Boc, γ,δ Lys), 1.54 (m, 2H, γ′δ′ Lys), 1.87 (m, 2H, ββ′ Lys), 3.21 (d, 2H, εε′ Lys), 4.09 (br s, 1H, α Lys), 4.24 (br s, 2H, H11′ chloroenyne), 4.91 (br s, 1H, NHε Lys), 5.12 (br s, 1H, NHα Lys), 5.24 (s, 2H, CH$_2$ Z(2-Cl)), 5.85 (td, 1H, H4 chloroenyne, $^{5}J_{\mathrm{H11',H4}} = 2.01$ Hz), 6.39 (d, 1H, H5 chloroenyne, $^{3}J_{\mathrm{H4,H5}} = 7.52$ Hz), 6.49 (br s, 1H, NH chloroenyne), 7.27 (s, 2H, H4,5 Z(2-Cl)), 7.38 (m, 1H, H6 Z(2-Cl)), 7.43 (m, 1H, H3 Z(2-Cl)).

*Synthesis of cis-oNbs-Aaa-NH-CH$_2$-C≡C-CH=CHCl (**4a–e**)*

(C$_6$H$_5$CN)$_2$PdCl$_2$, (10% eqv.), CuI (10% eqv.), *cis*-ClCH=CHCl (2 eqv.) and BuNH$_2$ (2 eqv.) were dissolved in 1 ml of dry THF and the reaction was stirred for 30 min under the argon. A solution of oNbs-Aaa-NH-CH$_2$-C≡CH (1 eqv.) and 2 eqv. of BuNH$_2$ in 2 ml of THF was added dropwise through the syringe and the reaction was stirred at room temperature until all acetylene was consumed. Solvent was evaporated and the residue purified by flash column chromatography.

cis-oNbs-Tyr-NH-CH$_2$-C≡C-CH=CHCl (**4a**)

Yield: 54%. Beige powder; mp 80–85 °C (decomp). R_f 0.17 (petrol ether-EtOAc 1:1). MW 463.89 ESI-MS: *m/z* 464.19 [M + H]$^+$ ^{13}C NMR (DMSO-d$_6$): δ = 28.71 (C1, chloroenyne), 37.68 (β Tyr), 58.87 (α Tyr), 76.17 (C2 chloroenyne), 94.52 (C3 chloroenyne), 112.07 (C4 chloroenyne), 114.75 (ε Tyr), 124.04 (C3 oNbs), 127.04 (C1 oNbs), 128.78 (C6 oNbs), 129.26 (C5 chloroenyne), 130.15 (δ Tyr), 132.02 (C4 oNbs), 133.03 (C5 oNbs), 147.10 (C2 oNbs), 155.83 (ζ Tyr), 170.52 (CO Tyr). ^{1}H NMR (DMSO-d$_6$): δ = 2.67, 2.74 (dd, 2H, ββ′ Tyr, $^{3}J_{\alpha,\beta} = 4.80$ Hz, $^{3}J_{\alpha,\beta'} = 8.85$ Hz, $^{2}J_{\beta,\beta'} = 13.55$ Hz), 3.91 (m, 3H, α Tyr, H11′ chloroenyne), 6.16 (br td, 1H, H4 chloroenyne), 6.52 (d, 2H, ε Tyr, $^{3}J_{\delta,\varepsilon} = 8.11$ Hz), 6.77 (d, 1H, H5 chloroenyne, $^{3}J_{\mathrm{H4,H5}} = 7.33$ Hz), 6.94 (d, 2H, δ Tyr, $^{3}J_{\delta,\varepsilon} = 8.32$ Hz), 7.58 (m, 2H, H5,6 oNbs), 7.71 (m, 1H, H4 oNbs), 7,78 (d, 1H, H3 oNbs, $^{3}J_{\mathrm{H3,H4}} = 7.81$ Hz), 8.45 (t, 1H, NH chloroenyne, $^{3}J_{\mathrm{NH,H11'}} = 5.24$ Hz).

cis-oNbs-Phe-NH-CH$_2$-C≡C-CH=CHCl (**4b**)

Yield: 44%. Yellow oil. R_f 0.50 (petrol ether-EtOAc 1:1). MW 447.89 ESI-MS: *m/z* 448.19 [M + H]$^+$ ^{13}C NMR (CDCl$_3$): δ = 30.49 (C1 chloroenyne), 38.60 (β Phe), 59.89 (α Phe), 68.36 (C2 chloroenyne), 92.58 (C3 chloroenyne), 111.70 (C4 chloroenyne), 126.22 (C3 oNbs), 127.53 (ζ Phe), 128.90 (ε Phe), 129.21 (δ Phe), 129.36 (C5 chloroenyne), 131.16 (C6 oNbs), 132.88 (C1 oNbs), 133.25 (C4 oNbs), 133.98 (C5 oNbs), 135.14 (γ Phe), 147.30 (C2 oNbs), 169.88 (CO Phe). ^{1}H NMR (CDCl$_3$): δ = 2.93, 3.25 (dd, 2H, ββ′ Phe, $^{3}J_{\alpha,\beta} = 5.11$ Hz, $^{3}J_{\alpha,\beta'} = 9.10$ Hz, $^{2}J_{\beta,\beta'} = 14.21$ Hz), 4.14 (m, 2H, α Phe, H1 chloroenyne), 4.27 (ddd, 1H, H1′ chloroenyne, $^{3}J_{\mathrm{H1',H4}} = 2.05$ Hz, $^{3}J_{\mathrm{NH,H1'}} = 5.90$ Hz, $^{2}J_{\mathrm{H1,H1'}} = 17.77$ Hz), 5.87 (td, 1H, H4 chloroenyne, $^{5}J_{\mathrm{H11',H4}} = 2.05$ Hz), 6.05 (br s, 1H, NH Phe), 6.42 (d, 1H, H5 chloroenyne, $^{3}J_{\mathrm{H4,H5}} = 7.48$ Hz), 6.74 (t, 1H, NH chloroenyne, $^{3}J_{\mathrm{NH,H11'}} = 5.06$ Hz), 7.02 (m, 5H, arom Phe), 7.69 (m, 2H, H5,6 oNbs), 7.78 (m, 1H, H6 oNbs), 7.96 (m, 1H, H3 oNbs).

cis-oNbs-Val-NH-CH$_2$-C≡C-CH=CHCl (**4c**)

Yield: 35%. Yellow powder; mp 134–138 °C. R_f 0.50 (petrol ether-EtOAc 1:1). MW 399.85 ESI-MS: *m/z* 400.20 [M + H]$^+$ ^{13}C NMR (CDCl$_3$): δ = 17.35, 19.37 (γγ′ Val), 30.34 (C1 chloroenyne), 31.20 (β Val), 63.63 (α Val), 92.50 (C3 chloroenyne), 111.60 (C4 chloroenyne), 125.87 (C3 oNbs), 129.50 (C5 chloroenyne), 130.97 (C6 oNbs), 133.16 (C4 oNbs), 124.21 (C5 oNbs), 169.76 (CO Val). ^{1}H NMR (CDCl$_3$): δ = 0.86, 0.97 (d, 6H, γγ′ Val, $^{3}J_{\beta,\gamma\gamma'} = 6.55$ Hz), 2.25 (m, 1H, β Val), 3.75 (dd, 1H, α Val, $^{3}J_{\alpha,\beta} = 5.00$ Hz, $^{3}J_{\alpha,\beta'} = 7.55$ Hz), 4.11 (m, 2H, H11′ chloroenyne), 5.86 (td, 1H, H4 chloroenyne, $^{5}J_{\mathrm{H1',H4}} = 2.01$ Hz), 6.00 (br d, 1H, NH Val), 6.38 (br t, 1H, NH chloroenyne), 6.42 (d, 1H, H5 chloroenyne, $^{3}J_{\mathrm{H4,H5}} = 7.47$ Hz), 7.75 (m, 2H, H4,5 oNbs), 7.90 (m, 1H, H6 oNbs), 8.09 (m, 1H, H3 oNbs).

cis-oNbs-Gly-NH-CH$_2$-C≡C-CH=CHCl (**4d**)

Yield: 38%. Beige powder; mp 145–150 °C (decomp). R_f 0.20 (petrol ether-EtOAc 1:1). MW 357.77 ESI-MS: *m/z* 358.18 [M + H]$^+$ ^{13}C NMR (CDCl$_3$): δ = 30.45 (C1 chloroenyne), 46.47 (α Gly), 78.04 (C2 chloroenyne), 92.33 (C3 chloroenyne), 111.58 (C4 chloroenyne), 126.03 (C3 oNbs), 129.61 (C5 chloroenyne), 131.40 (C6 oNbs), 132.93 (C1 oNbs), 133.30 (C4 oNbs), 134.45 (C5 oNbs), 148.28 (C2 oNbs), 167.06 (CO Gly). ^{1}H NMR (CDCl$_3$): δ = 3.81 (d, 2H, α Gly, $^{3}J_{\alpha,\mathrm{NH}} = 6.09$ Hz), 4.25 (br d, 2H, H11′ chloroenyne), 5.87 (td, 1H, H4 chloroenyne, $^{5}J_{\mathrm{H1',H4}} = 1.74$ Hz), 6.10 (t, 1H, NH chloroenyne, $^{3}J_{\mathrm{NH,H11'}} = 5.37$ Hz), 6.42 (d, 1H, H5 chloroenyne, $^{3}J_{\mathrm{H4,H5}} = 7.21$ Hz), 6.51 (br s, 1H, NH Gly), 7.79 (m, 2H H4,5 oNbs), 7.93 (m, 1H, H6 oNbs), 8.13 (m, 1H, H3 oNbs).

cis-oNbs-Lys[Z(2-Cl)]-NH-CH$_2$-C≡C-CH=CHCl (**4e**)

Yield: 30%. Yellow oil. $R_{f=}$0.20 (petrol ether-EtOAc 1:1). MW 597.47 ESI-MS: *m/z* 619.25 [M + Na]$^+$ ^{13}C NMR (CDCl$_3$): δ = 22.02 (γ Lys), 29.28 (δ Lys), 30.32 (C1 chloroenyne), 32.12 (β Lys), 40.19 (ε Lys), 57.85 (α Lys), 64.28 (CH$_2$ Z(2-Cl)), 92.63 (C3 chloroenyne), 111.69 (C4 chloroenyne), 125.78 (C3 oNbs), 127.14 (C5 Z(2-Cl)), 129.33 (C5 chloroenyne), 129.63 (C3 Z(2-Cl)), 129.71 (C4 Z(2-Cl)), 130.03 (C6 Z(2-Cl)), 131.05 (C6 oNbs), 133.11 (C4 oNbs), 133.37 (C1 oNbs), 133.74 (C2 Z(2-Cl)), 134.21 (C5 oNbs), 134.41 (C1 Z(2-Cl)), 147.95 (C2 oNbs), 156.75 (CO Z(2-Cl)), 170.33 (CO Lys). ^{1}H NMR (CDCl$_3$): δ = 1.40 (m, 4H, γγ′,δδ′ Lys), 1.80 (m, 2H, ββ′ Lys), 3.11 (m, 2H, εε′ Lys), 3.95 (dd, 1H, α Lys, $^{3}J_{\alpha,\beta} = 4.86$ Hz, $^{2}J_{\mathrm{H,H}} = 2.82$ Hz), 4.07 (br s, H11′ chloroenyne), 4.93 (t, 1H, NHε Lys, $^{3}J_{\mathrm{NH},\varepsilon\varepsilon'} = 5.60$ Hz), 5.22 (d, 2H, CH$_2$ Z(2-Cl), $^{3}J_{\mathrm{NH},\varepsilon\varepsilon'} = 5.60$ Hz), 5.83 (td, 1H, H4 chloroenyne, $^{5}J_{\mathrm{H1',H4}} = 1.74$ Hz), 6.39 (d, 1H, H5 chloroenyne, $^{3}J_{\mathrm{H4,H5}} = 7.45$ Hz), 6.81 (t, 1H, NH chloroenyne, $^{3}J_{\mathrm{NH,H11'}} = 5.00$ Hz), 7.29 (m, 2H, H4,5 Z(2-Cl)), 7.39 (m, 1H, H6 Z(2-Cl)), 7.43 (m, 1H, H3 Z(2-Cl)), 7.73 (m, 2H, H4,5 oNbs), 7.86 (m, 1H, H6 oNbs), 8.08 (m, 1H, H3 oNbs).

*Synthesis of oNbs(CH≡C-CH$_2$)-Aaa-OEt (**7a–e**)*

oNbs-Aaa-OEt was dissolved in dry DMF, K$_2$CO$_3$ (2 eqv.) and propargyl bromide (1 eqv.) was added and reaction mixture was stirred at room temperature. After 4 h solvent was evaporated and the residue purified by flash column chromatography on silica gel.

oNbs(CH≡C-CH$_2$)-Tyr-OEt (**7a**)

Yield: 93%. Colourless oil. R_f 0.50 (petrol ether-EtOAc-AcOH 7:5:0.5). [α]$_D$ −41.5° (*c* 1, MeOH). ^{13}C NMR (DMSO-d$_6$): δ = 13.56 (CH$_3$ OEt), 34.41 (C1 propargyl), 34.50 (β Tyr), 61.29 (α Tyr), 60.99 (CH$_2$ OEt), 75.76 (C3 propargyl), 79.11 (C2 propargyl), 115.15 (ε Tyr), 124.87 (C6 oNbs), 126.40 (C1 oNbs), 130.22 (δ Tyr), 130.43 (C6 oNbs), 131.52 (γ Tyr), 132.12 (C4 oNbs), 134.68 (C5 oNbs), 147.57 (C2 oNbs), 169.33 (CO Tyr). ^{1}H NMR (DMSO-d$_6$): δ = 0.96 (t, 3H, CH$_3$ OEt, $^{3}J_{H,H} = 7.00$ Hz), 3.01, 3.20 (dd, 2H, ββ′ Tyr, $^{3}J_{\alpha,\beta} = 6.80$ Hz, $^{3}J_{\alpha,\beta'} = 8.40$ Hz, $^{2}J_{\beta,\beta'} = 14.10$ Hz), 3.25 (br s, 1H, H3 propargyl), 3.92 (q, 2H, CH$_2$ OEt, $^{3}J_{H,H} = 7.00$ Hz), 4.25, 4.34 (dd, 2H, H11′ propargyl, $^{4}J_{H11',H3} = 2.05$ Hz), $^{2}J_{H1,H1'} = 18.90$), 4.69 (t, 1H, α Phe), 6.64 (d, 2H, ε. Tyr, $^{3}J_{\delta,\varepsilon} = 8.41$ Hz), 7.06 (d, 2H, δ Tyr, $^{3}J_{\delta,\varepsilon} = 8.41$ Hz), 7.82 (m, 4H, H3,4,5,6 oNbs), 9.28 (s, 1H, OH Tyr).

oNbs(CH≡C-CH$_2$)-Phe-OEt (**7b**)

Yield: 94%. Colourless oil. R_f 0.52 (toluene-EtOAc-10:1). [α]$_D$ −41.5° (*c* 1, MeOH). ^{13}C NMR (CDCl$_3$): δ = 13.98 (CH$_3$ OEt), 35.11 (C1 propargyl), 36.97 (β Phe), 61.56 (α Phe), 61.70 (CH$_2$ OEt), 73.57 (C3 propargyl), 79.03 (C2 propargyl), 124.39 (C6 oNbs), 127.26 (ζ Phe), 128.74 (ε Phe), 129.62 (δ Phe), 131.66 (C3 oNbs), 131.85 (C4 oNbs), 133.56 (C1 oNbs), 133.85 (C5 oNbs), 136.45 (γ Phe), 146.46 (C2 oNbs), 170.13 (CO Phe). ^{1}H NMR (CDCl$_3$): δ = 1.04 (t, 3H, CH$_3$ OEt, $^{3}J_{H,H} = 7.08$ Hz), 2.21 (t, 1H, H3 propargyl, $^{4}J_{H11',H3} = 2.40$ Hz), 3.24, 3.39 (dd, 2H, ββ′ Phe, $^{3}J_{\alpha,\beta} = 6.96$ Hz, $^{3}J_{\alpha,\beta'} = 8.40$ Hz, $^{2}J_{\beta,\beta'} = 13.86$ Hz), 4.01 (q, 2H, CH$_2$ OEt, $^{3}J_{H,H} = 7.08$ Hz), 4.35, 4.40 (dd, 2H, H11′ propargyl, $^{4}J_{H11',H3} = 2.40$ Hz, $^{2}J_{H1,H1'} = 18.78$ Hz), 4.89 (dd, 1H, α Phe, $^{3}J_{\alpha,\beta} = 6.96$ Hz, $^{3}J_{\alpha,\beta'} = 8.40$ Hz), 7.25 (m, 5H, arom Phe), 7.60 (m, 3H, H4,5,6 oNbs), 7.98 (dd, 1H, H3 oNbs, $^{3}J_{H3,H4} = 7.98$ Hz, $^{4}J_{H3,H5} = 1.02$ Hz).

oNbs(CH≡C-CH$_2$)-Val-OEt (**7c**)

Yield: 66%. Colourless oil. R_f 0.53 (toluene-EtOAc 10:2). [α]$_D$ −58.0° (*c* 1.0, MeOH). ^{13}C NMR (CDCl$_3$): δ = 14.10 (CH$_3$ OEt), 19.57, 20.03 (γγ′ Val), 28.98 (β Val), 34.79 (C1 propargyl), 61.28 (CH$_2$ OEt), 65.67 (α Val), 72.78 (C2 propargyl), 79.39 (C3 propargyl), 124.19 (C6 oNbs), 131.65, 131.80, 133.97 (C3,4,5 oNbs), 133.34 (C1 oNbs), 148.47 (C2 oNbs), 170.24 (CO Val). ^{1}H NMR (CDCl$_3$): δ = 1.02, 1.09 (d, 6H, γγ′ Val, $^{3}J_{\beta,\gamma} = 6.60$ Hz, $^{3}J_{\beta,\gamma'} = 6.80$ Hz), 1.11 (t, 3H, CH$_3$, OEt, $^{3}J_{H,H} = 7.20$ Hz), 2.21 (t, 1H, H3 propargyl, $^{4}J_{H11',H3} = 2.40$ Hz), 2.34 (m, 1H, β Val), 4.01 (q, 2H, CH$_2$ OEt, $^{3}J_{H,H} = 7.20$ Hz), 4.20 (d, 1H, α Val, $^{3}J_{\alpha,\beta} = 10.00$ Hz), 4.34, 4.46 (dd, 2H, H11′ propargyl, $^{4}J_{H11',H3} = 2.40$ Hz, $^{2}J_{H1,H1'} = 18.85$ Hz), 7.62, 7.69 (m, 3H, H4,5,6 oNbs), 8.15 (m, 1H, H3 oNbs).

oNbs(CH≡C-CH$_2$)-Gly-OEt (**7d**)

Yield: 89%. White powder; mp 62–63 °C. R_f 0.49 (toluene-EtOAc 10:2). ^{13}C NMR (CDCl$_3$): δ = 14.25 (CH$_3$ OEt), 38.19 (C1 propargyl), 47.41 (α Gly), 61.82 (CH$_2$ OEt), 74.76 (C3 propargyl), 76.59 (C2 propargyl), 124.67 (C6 oNbs), 131.16 (C3 oNbs), 132.11, 134.07 (C4,5 oNbs), 133.20 (C1 oNbs), 148.17 (C2 oNbs), 168.55 (CO Gly). ^{1}H NMR (CDCl$_3$): δ = 1.23 (t, 3H, CH$_3$ OEt, $^{3}J_{H,H} = 7.10$ Hz), 2.27 (t, 1H, H3 propargyl, $^{4}J_{H11',H3} = 2.30$ Hz), 4.15 (q, 2H, CH$_2$ OEt, $^{3}J_{H,H} = 7.10$ Hz), 4.31 (s, 2H, α Gly), 4.35 (br d, 2H, H11′ propargyl), 7.07 (m, 3H, H4,5,6 oNbs), 8.08 (m, 1H, H3 oNbs).

Synthesis of oNbs(cis-ClCH=CH-C≡C-CH$_2$)-Aaa-OEt (8a–e)

(C$_6$H$_5$CN)$_2$PdCl$_2$, (10% eqv.), CuI (10% eqv.), *cis*-ClCH=CHCl (2 eqv.) and BuNH$_2$ (2 eqv.) were dissolved in 1 ml of dry THF and stirred for 30 min under the argon. A solution of oNbs(CH≡CCH$_2$)-Aaa-OEt (1 eqv.) in 2 ml of THF was added dropwise through the syringe and the reaction stirred at room temperature until all acetylene was consumed. Solvent was evaporated and the residue purified by flash chromatography.

oNbs(cis-ClCH=CH-C≡C-CH$_2$)-Tyr-OEt (**8a**)

Yield: 30%. Yellow oil. R_f 0.54 (toluene-EtOAc 2:1). MW 492.93 ESI-MS: *m/z* 493.28 [M + H]$^+$ ^{13}C NMR (CDCl$_3$): δ = 14.05 (CH$_3$ OEt), 35.94, 36.17 (C1 chloroenyne, β Tyr), 61.74 (α Tyr), 61.75 (CH$_2$ OEt), 79.19 (C2 chloroenyne), 92.64 (C3 chloroenyne), 111.50 (C4 chloroenyne), 115.60 (ε Tyr), 124.33 (C3 oNbs), 128.36 (C1 oNbs), 129.51 (C5 chloroenyne), 130.87 (δ Tyr), 131.66 (C6 oNbs), 131.90 (C4 oNbs), 133.62 (γ Tyr), 133.77 (C5 oNbs), 148.27 (C2 oNbs), 154.92 (ζ Tyr), 170.32 (CO Tyr). ^{1}H NMR (CDCl$_3$): δ = 1.08 (t, 3H, CH$_3$ OEt, $^{3}J_{H,H} = 7.00$ Hz), 3.20, 3.34 (dd, 2H, ββ′ Tyr, $^{3}J_{\alpha,\beta} = 7.30$ Hz, $^{3}J_{\alpha,\beta'} = 8.02$ Hz, $^{2}J_{\beta,\beta'} = 13.95$ Hz), 4.04 (q, 2H, CH$_2$ OEt, $^{3}J_{H,H} = 7.00$ Hz), 4.53, 4.63 (dd, 2H, H11′ chloroenyne, $^{5}J_{H11',H4} = 2.03$ Hz, $^{2}J_{H1,H1'} = 19.11$ Hz), 4.06 (dd, 1H, α Tyr, $^{3}J_{\alpha,\beta} = 7.30$ Hz, $^{3}J_{\alpha,\beta'} = 8.02$ Hz), 5.74 (td, 1H, H4 chloroenyne, $^{5}J_{H11',H4} = 2.05$ Hz), 6.35 (d, 1H, H5 chloroenyne, $^{3}J_{H4,H5} = 7.48$ Hz), 6.72 (d, 1H, ε Tyr, $^{3}J_{\delta,\varepsilon} = 8.57$ Hz), 7.16 (d, 1H, δ Tyr, $^{3}J_{\delta,\varepsilon} = 8.57$ Hz), 7.64 (m, 3H, H4,5,6 oNbs), 8.00 (dd, 1H, H3 oNbs, $^{3}J_{H3,H4} = 7.75$ Hz, $^{4}J_{H3,H5} = 1.00$ Hz).

oNbs(cis-ClCH=CH-C≡C-CH$_2$)-Phe-OEt (**8b**)

Yield: 48%. Colourless oil. R_f 0.59 (toluene-EtOAc-10:2). [α]$_D$ −66.0° (*c* 0.5, MeOH). MW 476.93 ESI-MS: *m/z* 477.23 [M + H]$^+$ ^{13}C NMR (CDCl$_3$): δ = 13.71 (CH$_3$ OEt), 35.67 (C1 chloroenyne), 36.79 (β Phe), 61.25 (α Phe), 61.45 (CH$_2$ OEt), 78.96 (C2 chloroenyne), 92.32 (C3 chloroenyne), 111.98 (C4 chloroenyne), 124.06 (C6 oNbs), 126.93 (ζ Phe), 128.45 (ε Phe), 129.28, 128.35 (δ Phe, C5 chloroenyne), 131.3 (C3 oNbs), 131.67 (C4 oNbs), 133.60 (C5 oNbs), 136.16 (γ Phe), 147.98 (C2 oNbs), 169.93 (CO, Phe). ^{1}H NMR (CDCl$_3$): δ = 1.04 (t, 3H, CH$_3$ OEt, $^{3}J_{H,H} = 7.14$ Hz), 3.38 (m, 2H, ββ′ Phe), 4.02 (q, 2H, CH$_2$ OEt, $^{3}J_{H,H} = 7.14$ Hz), 4.55, 4.65 (dd, 2H, H11′ chloroenyne, $^{5}J_{H11,H4} = 1.90$ Hz, $^{2}J_{H1,H1'} = 19.10$ Hz), 4.92 (dd, 1H, α Phe, $^{3}J_{\alpha,\beta} = 7.16$ Hz, $^{3}J_{\alpha,\beta'} = 8.36$ Hz), 5.72 (td, 1H, H4 chloroenyne, $^{5}J_{H11,H4} = 1.90$ Hz), 6.34 (d, 1H chloroenyne, $^{3}J_{H4,H5} = 7.33$ Hz), 7.23 (m, 5H, arom Phe), 7.60 (m, 3H, H4,5,6 oNbs), 7.96 (m, 1H, H3 oNbs).

oNbs(cis-ClCH=CH-C≡C-CH$_2$)-Val-OEt (**8c**)

Yield: 32%. Colourless oil. R_f 0.65 (toluene-EtOAc 10:2). [α]$_D$ −57.0° (*c* 1.0, MeOH). MW 428.89 ESI-MS: *m/z* 429.25 [M + H]$^+$ ^{13}C NMR (CDCl$_3$): δ = 14.12 (CH$_3$ OEt), 19.60, 20.13 (γγ′ Val), 29.05 (β Val), 35.65 (C1 chloroenyne), 61.31 (CH$_2$ OEt), 65.69 (α Val), 78.43 (C2 chloroenyne), 93.09 (C3 chloroenyne), 111.66 (C4 chloroenyne), 124.15 (C6 oNbs), 129.13 (C5 chloroenyne), 131.69, 131.78, 133.91 (C3,4,5 oNbs), 133.39 (C1 oNbs), 148.05 (C2 oNbs), 170,29 (CO Val). ^{13}C NMR (CDCl$_3$): δ = 1.01, 1.10 (d, 6H, γγ′ Val, $^{3}J_{\beta,\gamma\gamma'} = 6.60$ Hz), 1.12 (t, 3H, CH$_3$ OEt, $^{3}J_{H,H} = 7.10$ Hz), 2.36 (m, 1H, β Val), 4.04 (q, 2H, CH$_2$ OEt, $^{3}J_{H,H} = 7.10$ Hz), 4.22 (d, 1H, α Val, $^{3}J_{\alpha,\beta} = 10.00$ Hz), 4.54, 4.64 (dd, 2H, H11′ chloroenyne, $^{5}J_{H1,H4} = 2.05$ Hz, $^{5}J_{H1',H4} = 1.80$ Hz $^{2}J_{H1,H1'} = 19.03$ Hz), 5.78 (td, 1H, H4 chloroenyne, $^{5}J_{H11',H4} = 2.05$ Hz), 6.34 (d, 1H, H5 chloroenyne, $^{3}J_{H4,H5} = 7.50$ Hz), 7.65 (m, 3H, H4,5,6 oNbs), 8.17 (m, 1H, H3 oNbs).

oNbs(cis-ClCH=CH-C≡C-CH$_2$)-Gly-OEt (**8d**)

Yield: 50%. Yellow oil. R_f 0.57 (toluene-EtOAc 10:2). MW 386.81 ESI-MS: *m/z* 387.20 [M + H]$^+$ ^{13}C NMR (CDCl$_3$): δ = 14.27 (CH$_3$ OEt), 39.06 (C1 chloroenyne), 47.53 (α Gly), 61.82 (CH$_2$ OEt), 68.36 (C2 chloroenyne), 80.41 (C3 chloroenyne), 111.29 (C4 chloroenyne), 124.64 (C6 oNbs), 130.04 (C5 chloroenyne), 131.13, 132.10 (C4,5 oNbs), 131.16 (C3 oNbs) 133.22 (C1 oNbs), 168.58 (CO Gly). ^{1}H NMR (CDCl$_3$): δ = 1.24 (t, 3H, CH$_3$ OEt, $^{3}J_{H,H} = 7.10$ Hz), 4.16 (q, 2H, CH$_2$ OEt, $^{3}J_{H,H} = 7.10$ Hz), 4.35 (s, 2H, α Gly), 4.56 (bs, 2H, H11′ chloroenyne), 5.79 (td, 1H, H4 chloroenyne, $^{5}J_{H11',H4} = 1.80$ Hz), 6.39 (d, 1H, H5 chloroenyne, $^3J_{H4,H5} = 7.40$ Hz), 7.70 (m, 3H, H4,5,6 oNbs), 8.10 (m, 1H, H3 oNbs).

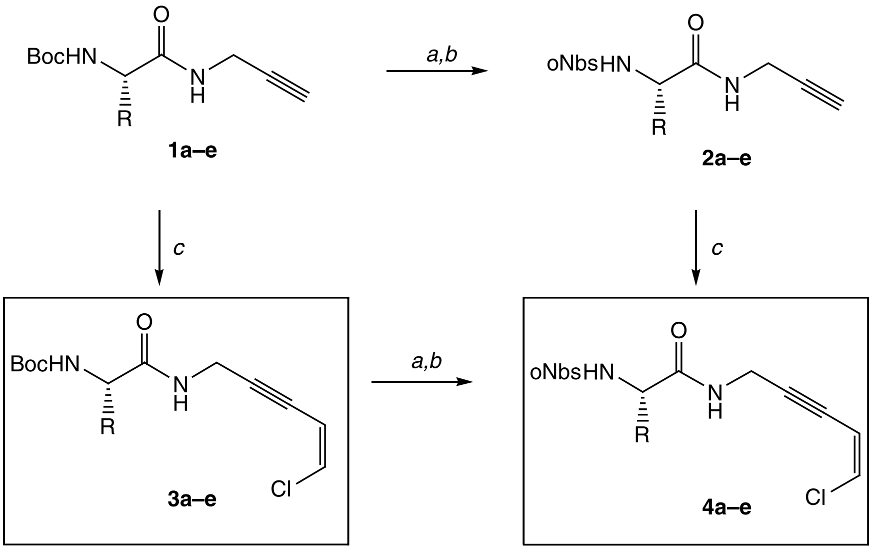
*Synthesis of oNbs(CH≡C-CH$_2$)-Gly-(cis-ClCH=CH-C≡C-CH$_2$)Gly-OEt (**11**)*

Solution of oNbs(CH≡C-CH$_2$)-Gly-OH (15 mg, 0.05 mmol) in 5 ml of dry DMF was cooled to 0 °C. NMM (15 µl, 0.10 mmol) and isobutyl chloroformate (8 µl, 0.05 mmol) were added and reaction left in ice-bath for 30 min. (*cis*-ClCH=CH-C≡C-CH$_2$)-Gly-OEt (14.5 mg, 0.05 mmol) was dissolved in 1 ml of dry DMF and added to the reaction mixture. Reaction was stirred at 0 °C for 1 h, and then at room temperature overnight. Solvent was evaporated and the residue purified by flash column chromatography.

Yield: 53%. Yellow oil. R_f 0.57 (toluene-EtOAc-AcOH 15:50.5). MW 487.91 ESI-MS: m/z 488.27 $[M+H]^+$ ^{13}C NMR, mixture of *trans* and *cis* (CDCl$_3$): 14.32 (CH$_3$ OEt), 36.99, 38.17, 38.29, 38.92 (C1 propargylamine, C1 chloroenyne), 47.40, 47.46, 47.60, 47.70 (α Gly^1, Gly^2), 61.64, 62.33 (CH$_2$ OEt), 74.57, 74.66 (C3 propargylamine), 79.47, 78.00 (C2 chloroenyne), 90.21, 91.23 (C3 chloroenyne), 111.31, 111.55 (C4 chloroenyne), 124.45, 124.52 (C6 oNbs), 129.71, 130.37 (C5 chloroenyne), 131.38, 131.47 (C3 oNbs), 132.03 (C4 oNbs), 132.98, 133.14 (C2 oNbs), 133.90, 133.95 (C5 oNbs), 148.27 (C2 oNbs), 167.29, 167.39, 168.66, 168.79 (CO Gly^1, Gly^2). ^{1}H NMR (CDCl$_3$): δ = 1.26, 1.33 (t, 3H, CH$_3$ OEt), 2.21, 2.24 (t, 1H, H3 propargylamide), 4.30 (m, 10H, H11′ propargylamide, chloroenyne, α Gly^1, α Gly^2, CH$_2$ OEt), 5.86, 5.91 (td, 1H, H4 chloroenyne), 6.43, 6.47 (H5 chloroenyne), 7.66 (m, 3H, H4,5,6 oNbs), 8.11 (m, 1H, H3 oNbs).

Results and discussion

A synthetic approach towards *C*-terminally modified amino acid derivatives **3a**–**e** and **4a**–**e** rely on pre-functionalized *tert*-butyloxycarbonyl (Boc) and 2-nitrobenzensulfonyl (oNbs) protected derivatives **1a**–**e** and **2a**–**e**, respectively (Scheme 2). Amino acid derivatives **1a**–**e** were prepared by simple reaction of Boc-protected amino acid and propargylamine. The deprotection of Boc-group under acidic conditions followed by the introduction of oNbs-group yields derivatives **2a**–**e**. Obtained propargylamides were subjected to the Pd/Cu catalysed Sonogashira-type cross-coupling reaction with *cis*-1,2-dichloroethene. Sonogashira reaction is one of the most useful reactions for the construction of C–C bond. However, it is evidenced throughout the literature that reaction conditions depend on the reactivity of partners involved. Generally, best results can be expected in reactions with at least one aromatic reactant (terminal acetylene or halide). Aliphatic substrates are far less reactive, regardless of the solvent, base or catalyst used. Also, generally poor reactivity of chlorides compared with bromides and iodides remains a problem to be solved (Sonogashira, 2002). In our attempts to find out the general and the most effective reaction conditions for the preparation of chloroenyne-related amino acid derivatives **3a**–**e** and **4a**–**e** and to minimize the parallel formation of unwanted diacetylene compound, different approaches were tested. THF, as a solvent, is more suitable than DCM or DMF. BuNH$_2$ gave better results compared to (Et)$_2$NH or TEA. Also, amount of



1a–e

2a–e

3a–e

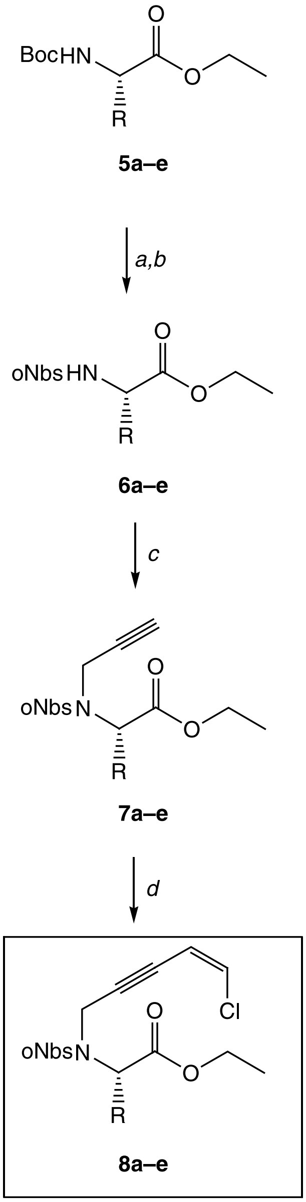
4a–e

a R = -CH$_2$-C$_6$H$_4$-OBoc
b R = -CH$_2$-C$_6$H$_5$
c R = -CH(CH$_3$)$_2$
d R = H
e R = -(CH$_2$)$_4$-NH[Z(2-Cl)]

a R = -CH$_2$-C$_6$H$_4$-OH
b R = -CH$_2$-C$_6$H$_5$
c R = -CH(CH$_3$)$_2$
d R = H
e R = -(CH$_2$)$_4$-NH[Z(2-Cl)]

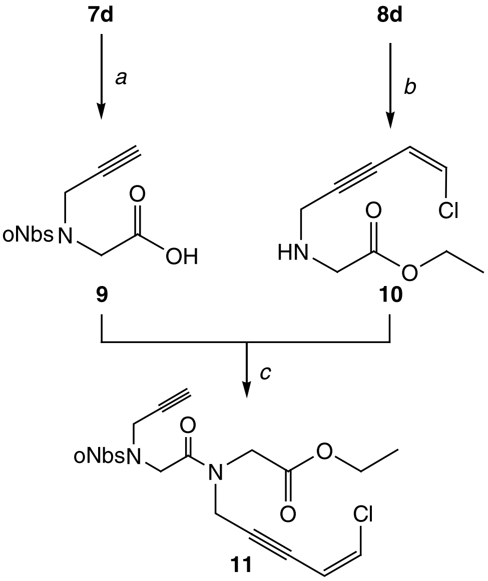
Scheme 2. Reagents and conditions: *a* TFA/H$_2$O (9/1); *b* oNbs-Cl, TEA, DCM; *c* (C$_6$H$_5$CN)$_2$PdCl$_2$, CuI, *cis*-ClCH=CHCl, BuNH$_2$, THF base added turned out to be of crucial importance. Finally, the best results were obtained when a solution of acetylene and 2 equivalents of $BuNH_2$ in THF is added dropwise over the syringe into the solution of Pd-catalyst, CuI, 2 equivalents of *cis*-1,2-dichloroethene and 2 equivalents of $BuNH_2$ in THF under the argon atmosphere. If less than 4 equivalents of a base is used, a lot of acetylene components are left in the reaction mixture even after prolonged reaction time (up to three days). Also, for the reproducibility of results great care must be given to the inert atmosphere as well as dryness of solvent and glassware. Finally, compounds **3a**–**e** and **4a**–**e** were obtained in fair yields, ranging between 40 and 52% for **3a**–**e** and 30 and 54% for **4a**–**e**. Prepared compounds **3a**–**e** are currently used in the synthesis of enediyne-bridged peptide units (type **I**, Scheme 1), while derivatives with oNbs group **4a**–**e** were prepared to allow further functionalization through *N*-alkylation reactions. The results will be published in due course.

For the synthesis of *N*-terminally modified amino acid derivatives **8a**–**e** carboxyl function was converted into ethyl ester group, while amino group was activated through oNbs moiety (Scheme 3). *N*-propargylation of compounds **6a**–**e** was performed in the presence of K_2CO_3 and propargyl bromide. Electron-withdrawing oNbs moiety increases the acidity of sulphonamide hydrogen and consequently the nucleophilicity of nitrogen in substitution reaction. Sonogashira reaction between derivatives **7a**–**e** and *cis*-1,2-dichloroethene was performed in a similar manner as described previously, but with 2 equivalents of a base instead of 4 equivalents. Compounds **8a**–**e** are currently involved in the synthesis of cyclic peptides carrying enediyne function (Type **III**, Scheme 1),



a R = $-CH_2-C_6H_4-OBoc$
b R = $-CH_2-C_6H_5$
c R = $-CH(CH_3)_2$
d R = H
e R = $-(CH_2)_4-NH[Z(2-Cl)]$

Scheme 3. Reagents and conditions: *a* TFA/H_2O (9/1); *b* oNbs-Cl, TEA, DCM; *c* $CH{\equiv}C-CH_2-Br$, K_2CO_3, DMF; *d* $(C_6H_5CN)_2PdCl_2$, CuI, *cis*-ClCH=CHCl, $BuNH_2$, THF



Scheme 4. Reagents and conditions: *a* 1 M NaOH in MeOH; *b* PhSH, K_2CO_3 in DMF; *c* $(CH_3)_2CHOCOCl$, NMM in DMF

and the results will be present separately. However, here we also described the efficiency test of such approach on the simplest model through the coupling of modified glycine derivatives. So, compound **7d** was treated with 1 M NaOH to yield free acid **9** (Scheme 4), while glycine derivative **8d** was converted into free amine **10** after oNbs-group deprotection by PhSH/K_2CO_3. Coupling of glycine derivatives **9** and **10** by mixed anhydride method gave dipeptide **11** in 53% yield.

NMR analysis

NMR spectra of all related compounds showed some common characteristics. One of the most prominent attributes of chloroenyne amino acid derivatives **3a**–**e**, **4a**–**e** and **8a**–**e** is facilitated magnetization flow along the triple–double bond system. One of the examples is shown in Fig. 1 for the tyrosine-related chloroenyne compound **8a**. The diastereotopic H11′ protons appeared as dd, due to the long-range coupling with H4 proton. The same phenomenon is evident at the H4 proton, which occurred as td (Fig. 1). Coupling constant $^5J_{H11',H4}$ varies from compound to compound, but is found in the range 1.70–2.50 Hz in all chloroenyne-modified amino acids.

^{13}C and ^1H NMR spectra of dipeptide **11** clearly showed two sets of signals arising from *cis-trans* isomerization of peptide bond. The ratio of two rotamers, determined from the integration of signals in ^1H spectra, was *trans*:*cis* = 50:50.

Mass spectrometry

The positive-ion ESI mass spectra of chloroenyne amino acid derivatives show molecular $[M+H]^+$ or pseudomolecular $[M+Na]^+$ ions at expected values. Tandem mass spectrometry (MS/MS) was used to search fragmentation patterns of related compounds and to identify common fragmentation behaviour, if present. MS/MS spectra of the molecular $[M+H]^+$ ion of chloroenyne derivatives **3a**–**e** are characterized by two main peaks: first, corresponding to the cleavage of the $(CH_3)_3COCO$ moiety from the *N*-terminal part (loss of 100 Da) and second, corresponding to the immonium-type ions, characteristic of amino acid presence.

The MS/MS spectra of the molecular $[M+H]^+$ ion of chloroenyne derivatives **4a**–**e** show different fragmentation preferences. As shown in Fig. 2a, for the valine derivative **4c**, the most abundant peak at m/z 257 is A-type ion and is consistent with the loss of 143 Da from the molecular $[M+H]^+$ ion. Also, an intensive ion at m/z 117 is Y″-type ion characteristic of penta-1-amino-5-chloro-4-ene-2-yne moiety. A low abundant ion at m/z 186 is attributed to the oNbs moiety. Contrary to that, the most informative fragmentation pattern is found for chloroenyne derivatives **8a**–**e**. As shown in Fig. 2b, for the valine-related derivative **8c**, along with the cleavage of the oNbs moiety (m/z 186) and the *N*-terminal substituent (m/z 99), A-type ion at m/z 355, A-43 at m/z 313 arising from the valine side-chain cleavage, and A-186 at m/z 168 are also present in the MS/MS spectrum of the molecular $[M+H]^+$ ion. The ion at m/z 343 (structure shown in Fig. 2b) is a precursor of ions at m/z 315 and m/z 271. Therefore, it is clear that amino acid derivatives described here showed different fragmentation patterns, depending on the type of protecting groups and the position of chloroenyne moiety.

In summary, a series of modified amino acids carrying chloroenyne moiety at the *N*-terminal or *C*-terminal position with different protecting groups was synthesized and characterized by NMR spectroscopy and mass spectrometry. They will be used for the construction of acyclic and cyclic enediyne-related peptide and amino acid derivatives. In addition, reactivity of such modified amino acids in peptide bond formation reaction was confirmed.

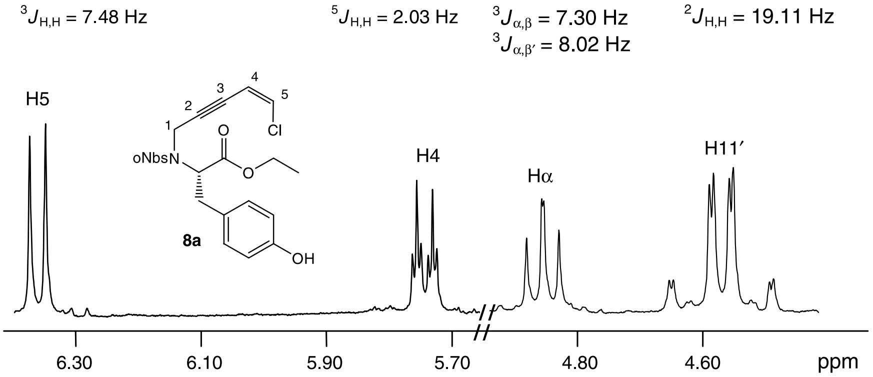


Fig. 1. Part of the ^1H NMR spectrum of compound **8a**

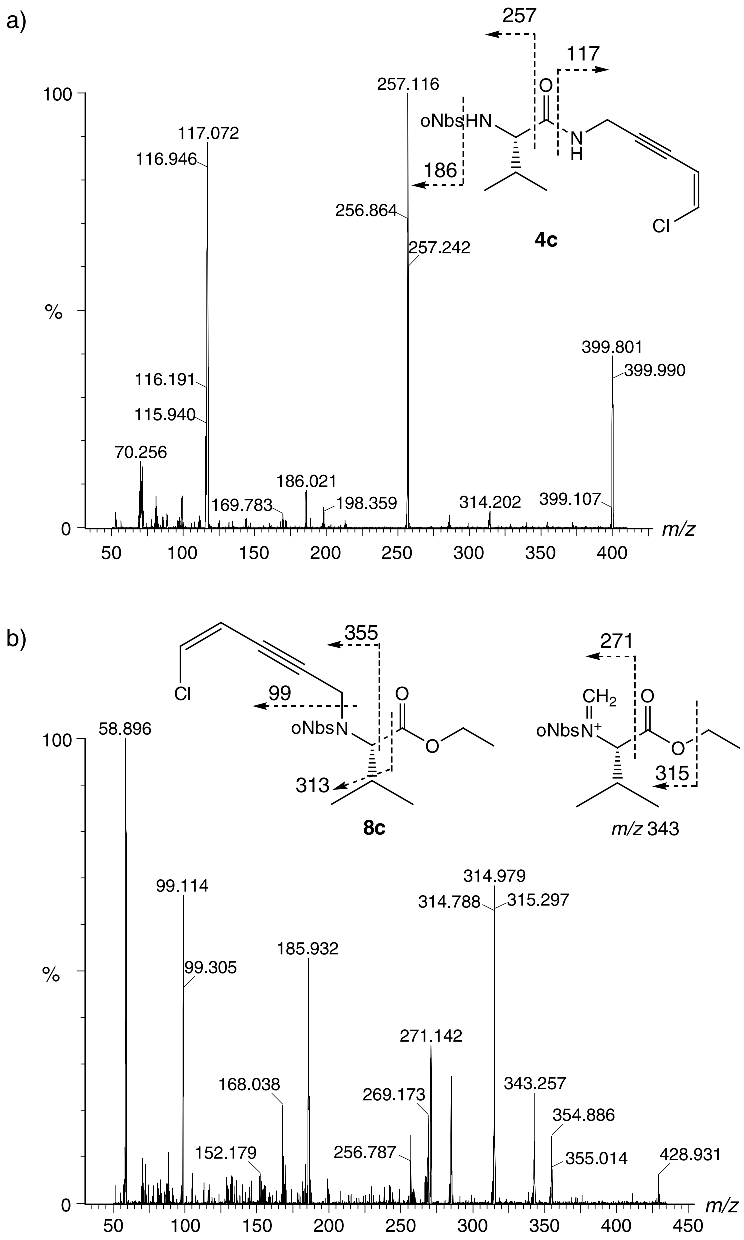


Fig. 2. MS/MS of the $[M+H]^+$ ions of *a*) **4c** and *b*) **8c**

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Authors' address: Dr. Ivanka Jerić, Division of Organic Chemistry and Biochemistry, Ruđer Bošković Institute, POB 180, 10002 Zagreb, Croatia, Fax: +385-1-4560-998, E-mail: ijeric@irb.hr