Azide- and Alkyne-Functionalised α- and β^3-Amino Acids

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Abstract: The synthesis and full characterisation of bifunctional β^3-amino acids that have side chains functionalised with terminal azides (*S*)-**4** and (*R*)-**4** or acetylenes **5** and **6** is reported for the first time. The building blocks incorporate a turn-inducing β^3-segment and a side chain that can be functionalised further, for example, through copper-catalysed Huisgen cycloaddition. Moreover, the corresponding α-amino acids **1** and **3** have been synthesised and characterised. All amino acid building blocks were of high optical purity as demonstrated by derivatisation and subsequent NMR analysis.

Key words: alkynes, azides, amino acids, diazotransfer, Arndt–Eistert homologation, peptidomimetics

Fuelled by the discovery of the copper-catalysed azide alkyne cycloaddition (CuAAC)[1–3] azide- and alkyne-functionalised α-amino acids have had an ever-growing impact in virtually all chemistry disciplines.[4] In recent years we have had a growing interest in the use of CuAAC in medicinal chemistry,[5] in particular azide- and alkyne-functionalised α-amino acids as building blocks for medicinal chemistry[4,6] and in the synthesis of constrained peptidomimetics.[7,8] The use of β^2- and β^3-amino acids in peptidomimetic research pioneered by the groups of Dieter Seebach[9] and Samuel Gellman[10] provides an elegant and relatively simple method for the synthesis of peptidomimetics with secondary structure, that is, foldamers. We have found the work of Gellman and co-workers for the synthesis of helix mimetics employing heterogeneous α,β^3-peptidomimetics particularly interesting.[11,12] The method of Gellman and co-workers is appealing because it limits the number of unnatural amino acids required in peptidomimetic design whilst retaining high α-helical content in solution and significantly enhanced metabolic and proteolytic stability compared to natures α-peptides. We are currently engaged in several pharmacological research projects and envisage that α-helical peptidomimetics with heterogeneous backbones comprised of α- and β^3-amino acids could be designed as ligands for G protein-coupled receptors.[13] To achieve a high degree of helical content under physiological conditions we wish to explore the use of α- and β^3-amino acids in combination with macrocyclic clamps formed by intramolecular reaction of amino acid side chains via CuAAC. The use of macrocyclic clamps for preorganisation and stabilisation of peptide secondary structure elements is a well-established concept within peptidomimetic research for the synthesis of biologically relevant mimics of peptide secondary structures such as turns,[14–17] helices,[18–23] and sheets.[24–26] We set out to synthesise a series of azide- and alkyne-functionalised α-amino acids **1**–**3** (Figure 1)[4] and the corresponding bifunctional β^3-amino acids **4**–**6** that combine a turn-inducing β^3-amino acid segment with azide- and alkyne-functionalised side chains that allow further functionalisation.

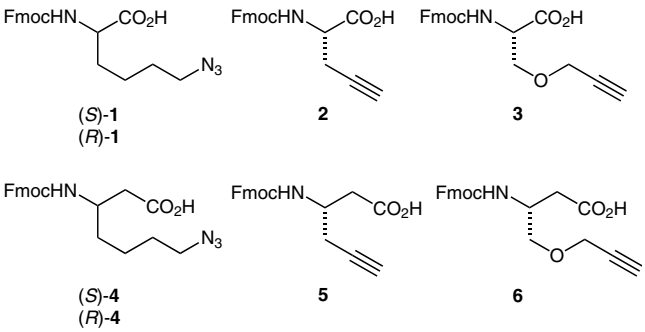

Figure 1 Azide- and alkyne-functionalised α- and β^3-amino acid target molecules

The synthesis of α-amino acids functionalised with a terminal azide in the side chain is commonly achieved from the corresponding amine by a diazotransfer reaction. The synthesis of Fmoc-protected ε-azido lysine (*S*)-**1** and the enantiomer (*R*)-**1** has previously been reported using a variety of diazotransfer conditions.[4] In 2007 Goddard-Borger and Stick reported a new shelf-stable diazotransfer reagent imidazole-1-sulfonyl azide hydrochloride (**8**) that has been widely adopted as a safe alternative to the commonly employed triflyl azide (Scheme 1).[27,28] Diazotransfer reaction on both enantiomers of lysine **7** using diazotransfer reagent **8** was accomplished on multigram scale in high yield to give crude products of excellent purity thus rendering further purification unnecessary. The pH value of the diazotransfer reaction was monitored carefully using a pH meter, and saturated aqueous $NaHCO_3$ was added during the course of the reaction to maintain pH 8–9. To evaluate the optical purity of azides **1**, (*S*)-**1** was derivatised with valine methyl ester. Analysis of the crude reaction mixture by NMR spectroscopy indicated the presence of a single diastereoisomer **10** suggesting that no significant stereochemical leakage had occurred. Activation of azides **1** by reaction with isobutyl chloroformate followed by treatment with diazomethane[31] gave diazoketones **9** in good yield. Wolff rearrangement of diazoketones **9** was accomplished by sonication in the

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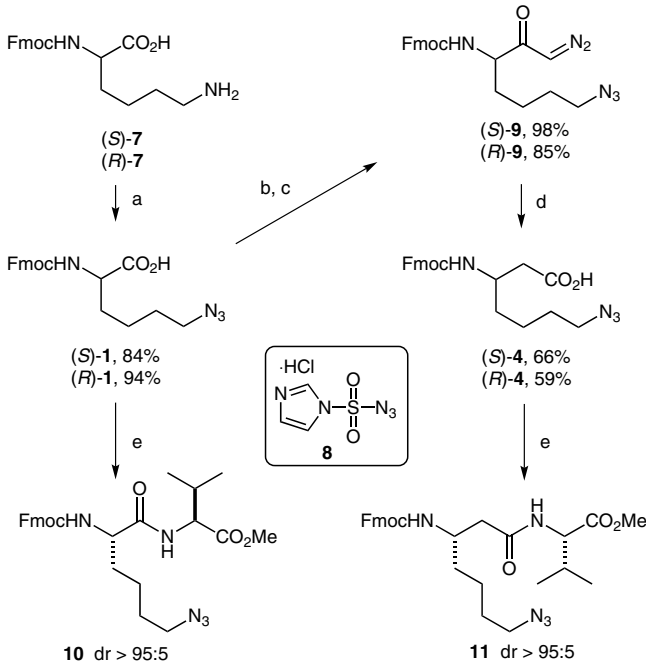
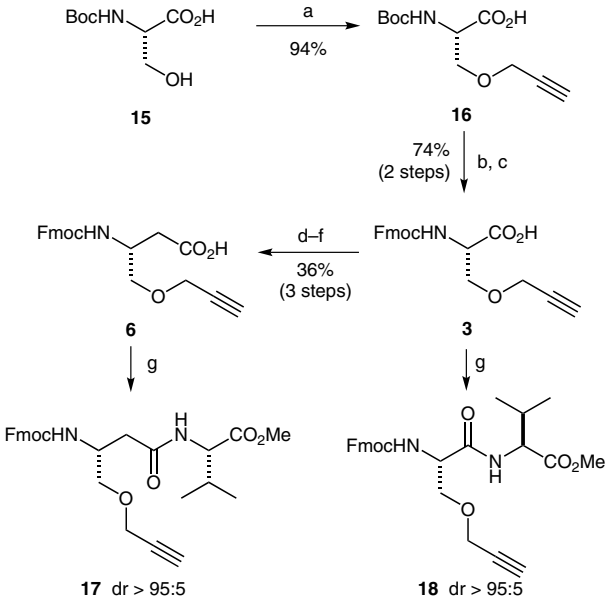
dark with a catalytic amount of silver trifluoroacetate to give β³-amino acids **4**. No stereochemical leakage could be detected after derivatisation and analysis of dipeptide **11** by NMR spectroscopy. The method should be broadly applicable to amines with varying side-chain lengths to produce a variety of optically pure azido β³-amino acids.[32]



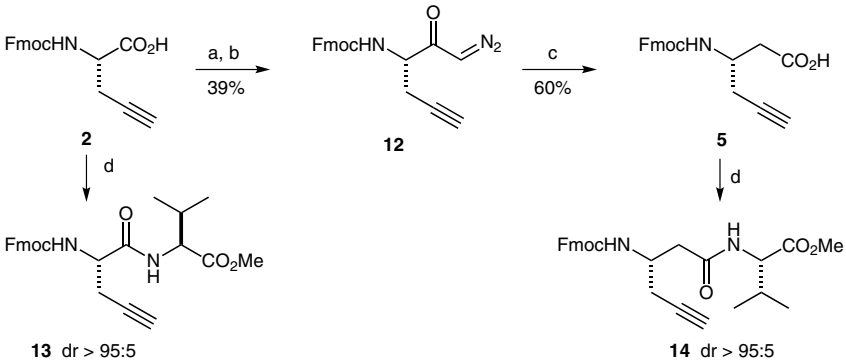
Scheme 1 Synthesis of both enantiomers of Fmoc-Lys(N_3)-OH [(*S*)- and (*R*)-**1**] and Fmoc-β³-Lys(N_3)-OH [(*S*)- and (*R*)-**4**] from Fmoc-protected L- and D-lysine [(*S*)- and (*R*)-**7**]. *Reagents and conditions*: a) **8**, $CuSO_4{\cdot}5H_2O$, MeOH, H_2O, $NaHCO_3$ (aq), pH 8–9, 5 h; b) isobutyl chloroformate, NMM, THF, –20 °C, 30 min; c) CH_2N_2, Et_2O, 0 °C to r.t., overnight; d) CF_3CO_2Ag, THF–H_2O (8:2), sonication, 3 h; e) (*S*)-**1** or (*S*)-**4**, L-valine methyl ester·HCl, HOBt, EDCI·HCl, (*i*-Pr)$_2$NEt, CH_2Cl_2, r.t., overnight.

Next we turned our attention to the synthesis of α- and β³-amino acids functionalised with terminal acetylenes in the side chain. Propargyl glycine **2** is commercially available and thus only the Arndt–Eistert homologation to produce acetylene β³-amino acid **5** was required (Scheme 2).[33] It is well-known that silver(I) coordinates strongly to alkynes, hence we anticipated that the Wolff rearrangement might prove problematic. Synthesis of the diazoketone **12** proved difficult producing numerous side products thus making column chromatography necessary prior to the Wolff rearrangement. As anticipated the Wolff rearrangement proved sluggish, requiring extended reaction time and a higher catalyst loading. Nevertheless, we found the protocol satisfactory providing **5** in two steps from propargyl glycine **2**. The optical purity of **5** was excellent as determined by NMR analysis of derivative **14**.



Scheme 3 Synthesis of α- and β³-acetylene amino acids **3** and **6** from Boc-Ser-OH **15**. *Reagents and conditions*: a) NaH, DMF, propargyl bromide, 0 °C to r.t., overnight; b) TFA, 0 °C, 1 h; c) Fmoc-succinimide, K_2CO_3, H_2O, dioxane, 0 °C to r.t., 3.5 h; d) isobutyl chloroformate, NMM, THF, –20 °C, 30 min; e) CH_2N_2, Et_2O, 0 °C to r.t., overnight; f) CF_3CO_2Ag, THF–H_2O (8:2), sonication, 6.5 h; g) L-valine methyl ester·HCl, HOBt, EDCI·HCl, (*i*-Pr)$_2$NEt, CH_2Cl_2, r.t., overnight.

The synthesis of propargylated serine **16** has been described in the literature several times starting from Boc-protected serine **15** (Scheme 3).[4] By the same approach we were able to synthesise **16** in excellent yield on multi-



Scheme 2 Synthesis of β³-acetylene amino acid **5** from Fmoc-propargyl glycine **2**. *Reagents and conditions*: a) isobutyl chloroformate, NMM, THF, –20 °C, 30 min; b) CH_2N_2, Et_2O, 0 °C to r.t., overnight; c) CF_3CO_2Ag, THF–H_2O (8:2), sonication, 8 h; d) L-valine methyl ester·HCl, HOBt, EDCI·HCl, (*i*-Pr)$_2$NEt, CH_2Cl_2, r.t., overnight.

gram scale. Using standard methods, **16** was converted in two steps into the Fmoc-protected amino acid **3** in good yield.[34] Unlike the synthesis of β³-propargyl glycine **5** formation of the diazoketone starting from **3** proceeded to give a crude product of reasonable purity that was exposed directly to the Wolff rearrangement. Again the Wolff rearrangement proved sluggish and required a higher catalyst loading and extended reaction time to go to completion. Moreover, the rearrangement produced numerous side products and consequently an acceptable but low yield for the three-step process. Derivatisation of **3** and **6** and analysis by NMR spectroscopy revealed that no significant stereochemical leakage had occurred.

In summary, we report concise synthetic procedures for the synthesis of a new class of bifunctional β³-amino acid building blocks starting from readily available starting materials. Importantly, all compounds have been carefully characterised and shown to be of high optical purity. The ready availability and high purity of the reported amino acids will be of considerable interest to the chemical community, and we anticipate they will have an impact in medicinal chemistry and within peptidomimetic research. Work is ongoing in our laboratory to expand the repertoire of bifunctional amino acid building blocks and to incorporate the reported building block in peptides and will be reported in due course.

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Supporting Information for this article is available online at http://www.thieme-connect.com/ejournals/toc/synlett. Included are detailed experimental procedures, ^{1}H and ^{13}C NMR spectra.

References and Notes

(1) Rostovtsev, V. V.; Green, L. G.; Fokin, V. V.; Sharpless, K. B. *Angew. Chem. Int. Ed.* **2002**, *41*, 2596.
(2) Tornoe, C. W.; Christensen, C.; Meldal, M. *J. Org. Chem.* **2002**, *67*, 3057.
(3) Tornoe, C. W.; Meldal, M. In *Peptides 2001*; American Peptide Society and Kluwer Academic Publishers: San Diego, **2001**, 263.
(4) Johansson, H.; Pedersen, D. S. *Eur. J. Org. Chem.* **2012**, 4267.
(5) Soares da Costa, T. P.; Tieu, W.; Yap, M. Y.; Pendini, N. R.; Polyak, S. W.; Sejer Pedersen, D.; Morona, R.; Turnidge, J. D.; Wallace, J. C.; Wilce, M. C. J.; Booker, G. W.; Abell, A. D. *J. Biol. Chem.* **2012**, *287*, 17823.
(6) Stanley, N. J.; Pedersen, D. S.; Nielsen, B.; Kvist, T.; Mathiesen, J. M.; Bräuner-Osborne, H.; Taylor, D. K.; Abell, A. D. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 7512.
(7) Buysse, K.; Farard, J.; Nikolaou, A.; Vanderheyden, P.; Vauquelin, G.; Pedersen, D. S.; Tourwè, D.; Ballet, S. *Org. Lett.* **2011**, *13*, 6468.
(8) Pedersen, D. S.; Abell, A. *Eur. J. Org. Chem.* **2011**, 2399.
(9) Seebach, D.; Gardiner, J. *Acc. Chem. Res.* **2008**, *41*, 1366.
(10) Horne, W. S.; Gellman, S. H. *Acc. Chem. Res.* **2008**, *41*, 1399.
(11) Horne, W. S.; Boersma, M. D.; Windsor, M. A.; Gellman, S. H. *Angew. Chem. Int. Ed.* **2008**, *47*, 2853.
(12) Horne, W. S.; Johnson, L. M.; Ketas, T. J.; Klasse, P. J.; Lu, M.; Moore, J. P.; Gellman, S. H. *Proc. Natl. Acad. Sci. U.S.A* **2009**, *106*, 14751.
(13) Gloriam, D.; Wellendorph, P.; Johansen, L.; Thomsen, A.; Phonekeo, K.; Pedersen, D. S.; Bräuner-Osborne, H. *Chem. Biol.* **2011**, *18*, 1489.
(14) Tomboly, C.; Ballet, S.; Feytens, D.; Kover, K. E.; Borics, A.; Lovas, S.; Al-Khrasani, M.; Furst, Z.; Toth, G.; Benyhe, S.; Tourwe, D. *J. Med. Chem.* **2008**, *51*, 173.
(15) Holland-Nell, K.; Meldal, M. *Angew. Chem. Int. Ed.* **2011**, *50*, 5204.
(16) Pinsker, A.; Einsiedel, J.; Harterich, S.; Waibel, R.; Gmeiner, P. *Org. Lett.* **2011**, *13*, 3502.
(17) Larregola, M.; Lequin, O.; Karoyan, P.; Guinvarc'h, D.; Lavielle, S. *J. Pept. Sci.* **2011**, *17*, 632.
(18) Garner, J.; Harding, M. M. *Org. Biomol. Chem.* **2007**, *5*, 3577.
(19) Cantel, S.; Isaad, A. L. C.; Scrima, M.; Levy, J. J.; DiMarchi, R. D.; Rovero, P.; Halperin, J. A.; D'Ursi, A. M.; Papini, A. M.; Chorev, M. *J. Org. Chem.* **2008**, *73*, 5663.
(20) Schafmeister, C. E.; Po, J.; Verdine, G. L. *J. Am. Chem. Soc.* **2000**, *122*, 5891.
(21) Ebert, M.-O.; Gardiner, J.; Ballet, S.; Abell, A. D.; Seebach, D. *Helv. Chim. Acta* **2009**, *92*, 2643.
(22) Ingale, S.; Dawson, P. E. *Org. Lett.* **2011**, *13*, 2822.
(23) Kawamoto, S. A.; Coleska, A.; Ran, X.; Yi, H.; Yang, C. Y.; Wang, S. *J. Med. Chem.* **2011**, *55*, 1137.
(24) Pehere, A. D.; Pietsch, M.; Gütschow, M.; Neilsen, P. M.; Callen, D. F.; Pedersen, D. S.; Nguyen, S.; Sykes, M.; Morton, J. D.; Abell, A. D.; manuscript submitted for publication.
(25) Chen, J. Y.; Nikolovska-Coleska, Z.; Yang, C. Y.; Gomez, C.; Gao, W.; Krajewski, K.; Jiang, S.; Roller, P.; Wang, S. M. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 3939.
(26) Abell, A. D.; Jones, M. A.; Coxon, J. M.; Morton, J. D.; Aitken, S. G.; McNabb, S. B.; Lee, H. Y. Y.; Mehrtens, J. M.; Alexander, N. A.; Stuart, B. G.; Neffe, A. T.; Bickerstaffe, R. *Angew. Chem. Int. Ed.* **2009**, *48*, 1455.
(27) Goddard-Borger, E. D.; Stick, R. V. *Org. Lett.* **2007**, *9*, 3797.
(28) We discovered that imidazole-1-sulfonyl azide hydrochloride (**8**) when stored in a desiccator at r.t. decomposed over the course of 1–2 months to form a viscous black tar. Later Goddard-Borger and Stick published a safety update addressing this and other reagent related issues, attributing the decomposition to slow water hydrolysis of **8** and formation of hydrazoic acid.[29] More recently, Goddard-Borger and co-workers evaluated the stability of a wide range of imidazole sulfonyl azide salts and found that the hydrogensulfate and tetrafluoroborate salts are safe alternatives to the hydrochloride salt **8**.[30] Herein we report the use of the hydrochloride salt **8** that we have found to be perfectly stable (>10 months) when dried rigorously in a vacuum desiccator over KOH and stored at –20 °C. However, based on the reports by Goddard-Borger and co-workers we recommend that the hydrogensulfate salt be used for reproducing the experiments reported herein.
(29) Goddard-Borger, E. D.; Stick, R. V. *Org. Lett.* **2011**, *13*, 2514.
(30) Fischer, N.; Goddard-Borger, E. D.; Greiner, R.; Klapötke, T. M.; Skelton, B. W.; Stierstorfer, J. *J. Org. Chem.* **2012**, *77*, 1760.
(31) Despite being a useful reagent diazomethane is underutilised due to safety concerns. Diazomethane is indeed highly toxic

and explosive. However, with careful use of a modern diazomethane distillation kit the synthesis of diazomethane in Et_2O is simple, safe, and fast. Diazomethane distillation kits with clear-seal joints are available from many glassware manufacturers. We commonly employ a mini-Diazald apparatus available from Sigma-Aldrich that produces up to 1 mmol of diazomethane.

(32) Many azido-functionalised α-amino acids are commercially available. However, to the best of our knowledge no optically active azido-functionalised β^3-amino acids are available.

(33) Both enantiomers of amino acid **5** are commercially available from a number of vendors and according to Chemical Abstract (CAS) have been reported in two Chinese papers: (a) Zhan, J.-R.; Chen, T.-Y.; Liu, B. *Huaxue Shiji* **2004**, *26*, 137. (b) Liao, B.-R.; Hong, Y.-Y.; Xu, J.; Liu, B. *Youji Huaxue* **2004**, *24*, 63.

(34) Attempting the reaction on the methyl ester derivative of **15** under the same reaction conditions led to the formation of several side products, reduced yields, and significant racemisation.

