A Concise Route to L-Azidoamino Acids: L-Azidoalanine, L-Azidohomoalanine and L-Azidonorvaline

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Dedicated to Prof. Gerry Pattenden on the occasion of his 70th birthday.

Abstract: A simple and highly efficient synthetic route to three homologous azidoamino acids, starting from inexpensive, commercially available, protected natural amino acids is reported. The products can be used to introduce bioorthogonal handles into proteins.

Key words: amino acids, azides, bioorganic chemistry, bioorthogonal chemistry, non-canonical

The creation of proteins that incorporate non-canonical (unnatural) amino acids bearing side chains that can be selectively modified has been the focus of increasing amounts of research over the past decade. A number of different methods have been devised including the use of the amber suppression codon,[1] the use of N-terminal modifying enzymes[2] and of auxotrophic bacteria [typically *E. coli* B834(DE3)] to introduce alkynyl,[3] azide[4] or ketone[5] bearing amino acids that can be used as handles for further modification via bio-orthogonal 'click chemistry'.[6] The azido-group has proved to be the most versatile of these because it can be used directly as an IR probe,[7] it can selectively undergo Huisgen [3+2] cycloadditions that can be either copper(I)-catalysed (with an alkyne functionalised probe),[8] or metal-free (with a strained cyclooctyne functionalised probe),[9] and can react in a Staudinger reaction with a phosphine-bearing probe.[10] In a collaborative project, the groups of Tirrell and Bertozzi were the first to demonstrate that an azide in the form of L-azidohomoalanine (L-Aha, **2**, Figure 1) could substitute for methionine as a substrate for *E. coli* methionyl tRNA-synthetase (MetRS) and be incorporated globally in place of methionine in the *E. coli* outer-membrane protein OmpC when expressed in auxotrophic *E. coli*.[4] In this study it was found that the MetRS adds L-Aha with a catalytic efficiency (k_{cat}/K_M) that was ~1/400th that of L-Met.

Later it was shown that other L-azidoamino acids such as: L-azidoalanine (**1**), L-azidonorvaline (**3**) (Figure 1) and L-azidonorleucine (not shown), could be incorporated into OmpC, albeit with much lower efficiency than L-Aha (**2**).[11] The development of an increasing variety of methods with which to incorporate azidoamino acids such as **2** into proteins has generated the need for a facile and cost effective method of preparing these compounds on larger scales (1–10 g). In this report we describe a generic and robust route to L-azidoalanine (**1**), L-azidohomoalanine (**2**), and L-azidonorvaline (**3**) from widely available, low cost, protected α-amino acids. This route would also be applicable to the synthesis of L-azidonorleucine from commercially available *N*-Boc-L-norleucine with an additional esterification step. There have been a number of previous syntheses of L-azidoalanine (**1**),[12] L-azidohomoalanine (**2**)[13,14,15] and L-azidonorvaline (**3**)[16] that give both the protected and fully deprotected amino acids. Of those that give the deprotected azidoamino acids, a number suffer from disadvantages such as starting materials that are either expensive or not readily available, the required use of hazardous reagents, or involve either a large number of transformations, or have a poor overall yield.

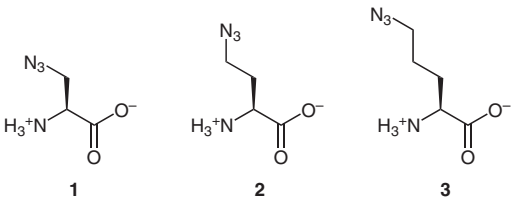

Figure 1 Three non-canonical azidoamino acids: L-azidoalanine (**1**), L-azidohomoalanine (**2**) and L-azidonorvaline (**3**)

Tirell and co-workers[13,14] have recently reported two syntheses for L-Aha (**2**) – the first being a modified version of Mangold's original synthesis.[15] Although starting from a readily available amino acid derivative (Boc-L-homoserine), this protocol uses a rather complicated protecting group strategy that requires the use of diazomethane, a hazardous reagent that requires specific handling and glassware. The alternative, improved synthesis they described avoids this step but starts from expensive Boc-Dab (Boc-diaminobutyric acid) and employs triflic azide, a relatively unstable reagent that has to be freshly prepared prior to use. For the preparation of larger quantities of proteins incorporating L-azidohomoalanine (**2**), Tirrell et al. have used α-amino-γ-butyrolactone[17] to give *rac*-azidohomoalanine in reasonable quantities.[15] Whilst only the L-enantiomer was incorporated into the proteins of interest, the effect of the D-enantiomer on cell growth and protein production was not reported, but could potentially be detrimental.

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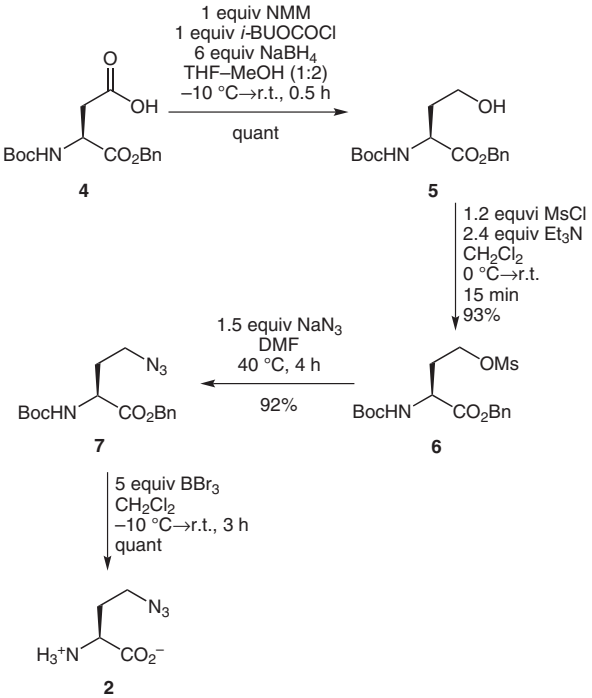
We proposed that the three homologous L-azidoamino acids **1**, **2** and **3** could be prepared starting from inexpensive, commercially available, protected amino acids employing a common synthetic scheme that avoids handling highly dangerous or toxic compounds such as diazomethane, or the use of the unstable triflic azide.

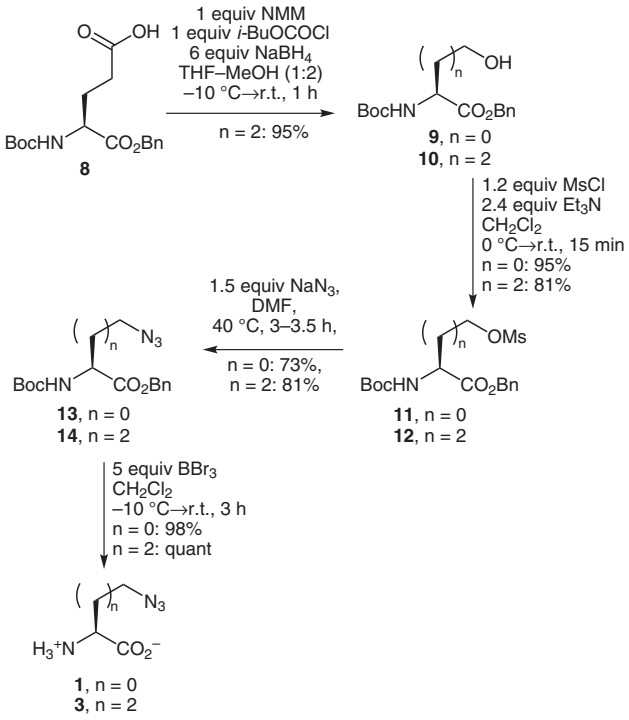
Initially, we developed the synthetic route shown in Scheme 1 for the synthesis of L-Aha (**2**) as this has been shown to be by far the most translationally active methionine surrogate[4,5] and, consequently, the azidoamino acid most widely employed. Starting from *N*-Boc- and *O*-benzyl-protected L-aspartic acid **4**, the γ-carboxylic acid was reduced directly via the mixed anhydride formed with isobutyl chloroformate.[18] This step was carried out on a multigram scale to give **5** reproducibly in quantitative yield and further purification was not required. The resulting alcohol **5** was then subjected to a standard mesylation reaction using mesylchloride and triethylamine to give mesylate **6**.[19] The mesylate function was then displaced with an azide group by treatment with sodium azide in DMF at slightly elevated temperature to give protected azide **7** in an excellent yield of 92%.[20] An initial attempt to deprotect the amino acid in two subsequent steps using an acid-mediated deprotection of the Boc group followed by a basic cleavage of the benzyl ester did result in the free amino acid, however, due to the additional purification step, the overall yield was reduced.



Scheme 1 Synthesis of L-azidohomoalanine (L-Aha, **2**)

We therefore applied a global deprotection strategy employing boron tribromide[21] that afforded **2** in quantitative yield after crystallisation; an overall yield of 86% from **4**.[22] The purity of the material obtained was ~98% (the major by-product resulting from a displacement of the azide group with a bromide), which could be enhanced to >99% by either recrystallisation from ethanol/water or ion-exchange chromatography.[5]

After establishing a viable route for L-Aha (**2**), we then turned our attention to the synthesis of the homologues **1** and **3** as single enantiomers. Applying the same strategy starting from either *N*-Boc- and *O*-benzyl-protected L-serine **9** or protected L-glutamic acid **8**, which are both low cost, commercially available compounds, the expected products **1** and **3** could be obtained in high overall yields of 68% (over three steps) and 62% (over four steps), respectively (Scheme 2).



Scheme 2 Synthesis of L-azidoalanine (**1**) and L-azidonorvaline (**3**)

It is noteworthy that, after reduction of the protected glutamic acid **8**, purification of the alcohol **10** by column chromatography was necessary, presumably to remove traces of boron-based compounds and any aldehyde present. If the alcohol is subjected to the mesylation reaction without prior purification, the yield of the mesylation reaction decreases from 81% to 42%.

In conclusion, a facile and robust synthesis of the three homologous azidoamino acids: L-azidoalanine (**1**), L-azidohomoalanine (**2**) and L-azidonorvaline (**3**), from inexpensive, commercially available starting materials has been developed. The synthetic route is high-yielding, applicable to all three compounds and could also be applied to the synthesis of L-azidonorleucine without modification. This route does not require highly hazardous or unstable reagents and so could be performed on multigram scales when large quantities of azidoamino acid bearing protein were required for further selective modification. The introduction of L-Aha (**2**) into proteins also allows mild fragmentation of peptides and proteins at specific positions.

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(19) To a solution of alcohol **5** (269 mg, 0.87 mmol, 1.0 equiv) in CH_2Cl_2 (5 mL) at 0 °C, was added Et_3N (288 μL, 2.09 mmol, 2.4 equiv) followed by dropwise addition of methylsulfonyl chloride (81 μL, 1.04 mmol, 1.2 equiv). The ice-bath was removed and the solution was stirred at r.t. for 15 min. Sat. $NaHCO_3$ (5 mL) was added and the layers were separated. The organic layer was washed with brine (2 × 5 mL) and the combined aqueous layer was back-extracted with CH_2Cl_2 (2 × 10 mL). The combined organic layer was dried over $MgSO_4$, the solids filtered off and the solvent removed in vacuo. The crude product was purified by column chromatography (silica; PE–EtOAc, 7:3) to give a colourless solid (312 mg, 0.81 mmol, 93%); mp 61–63 C ($CHCl_3$); $[\alpha]_D^{28}$ –37.1 (*c* 0.11, $CHCl_3$); IR: 3009, 1711, 1500, 1364, 1175 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ = 1.39 (s, 9 H, *t*Bu), 2.08 (m, 1 H, $H_{\beta1}$), 2.29 (m, 1 H, $H_{\beta2}$), 2.92 (s, 3 H, SO_2CH_3), 4.24 (m, 2 H, H_γ), 4.43 (m, 1 H, H_α), 5.15 (s, 2 H, CH_2Ph), 5.26 (bd, *J* = 7.0 Hz, 1 H, NH), 7.33 (m, 5 H, Ar); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 28.2 [C(*C*H_3)$_3$], 31.8 (C_β), 37.1 (SO_2CH_3), 50.4 (C_α), 65.8 (C_γ), 67.5 (*C*H_2Ph), 80.3 [*C*(CH_3)$_3$], 128.4, 128.5, 128.7, 135.0 (Ar), 155.3 (*t*-BuO*C*ONHR), 177.5 (*C*O_2Bn); MS (ESI+): *m*/*z* [M + Na]$^+$ calcd for $C_{17}H_{25}NNaO_7S$: 410.1244; found: 410.1225.
(20) To a solution of mesylate **6** (222 mg, 0.56 mmol, 1.0 equiv) in anhydrous DMF (2 mL), was added NaN_3 (54 mg, 0.84 mmol, 1.5 equiv) in one portion. The suspension was stirred at 40 °C for 4 h, then the solvent was removed in vacuo and the crude product was purified by column chromatography (silica; PE–EtOAc, 4:1). The azide **7** was obtained as a colourless oil (171 mg, 0.51 mmol, 92%); $[\alpha]_D^{28}$ +2.8 (*c* 0.71, $CHCl_3$); IR: 3434, 2981, 2104, 1712, 1499, 1160 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ = 1.41 (s, 9 H, *t*Bu), 1.89 (m, 1 H, $H_{\beta1}$), 2.08 (m, 1 H, $H_{\beta2}$), 3.34 (t, *J* = 6.7 Hz, 2 H, H_γ), 4.41 (m, 1 H, H_α), 5.13 (d, *J* = 12.3 Hz, 1 H, CH_2Ph), 5.18 (d, *J* = 12.3 Hz, 1 H, CH_2Ph), 5.19 (br s, 1 H, NH), 7.34 (m, 5 H, Ar); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 28.2 [C(*C*H_3)$_3$], 31.7 (C_β), 47.6 (C_γ), 51.5 (C_α), 67.3 (*C*H_2Ph), 80.2 [*C*(CH_3)$_3$], 128.3, 128.5, 128.6, 135.1 (Ar), 155.2 (*t*BuO*C*ONHR), 171.8 (*C*O_2Bn); MS (ESI+): *m*/*z* [M + Na]$^+$ calcd for $C_{16}H_{22}N_4NaO_4$: 357.1533; found: 357.1522.
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(22) To a solution of protected amino acid **7** (33 mg, 0.1 mmol, 1 equiv) dissolved in anhydrous CH_2Cl_2 (2.5 mL) at –10 °C under an N_2 atmosphere, boron tribromide solution (1 M in CH_2Cl_2, 0.5 mL, 0.5 mmol, 5 equiv) was added dropwise over 5 min. The resulting solution was stirred for 1 h at –10 °C and for 2 h at r.t. The reaction was quenched by careful addition of H_2O (2.5 mL), and then the layers were separated. The organic phase was washed with H_2O (3 × 5 mL) and the combined aqueous layer was evaporated to dryness. The crude product was re-dissolved in a minimum amount of ethanol and the pure product **2** was obtained by precipitation at 4 °C as a colourless crystalline solid (14 mg, 0.1 mmol, quantitative yield).