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Unnatural Amino Acid Derivatives through Click Chemistry: Synthesis of Triazolylalanine Analogues

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Abstract

A novel *tert*-butyl 2-(1-oxoisindolin-2-yl)acetate derivative is selectively alkylated with propargyl bromide in the presence of lithium hexamethyldisilazide. After removal of the *tert*-butyl protecting group, the resulting *N*-isindolinyl (ethynylalanine) derivative is reacted with a series of azides under 'click conditions'. The click reactions afford an array of *N*-isindolinyl-1,2,3-triazolylalanine derivatives as the free carboxylic acids. Following esterification, the *N*-isindolinone protecting group is then transformed into the more easily removable phthaloyl group by selective oxidation at the benzylic position.

Graphical Abstract

$R^1 = t\text{-Bu}$, $R^1 = \text{H}$, $R^2 = \text{alkyl, benzyl}$

Click conditions: $R^2\text{-N}_3/\text{CuSO}_4\cdot 5\text{H}_2\text{O}/\text{Na ascorbate}/\text{THF-H}_2\text{O}$, yields, 67–95%, 7 examples,

Benzylic oxidation condition: Oxone/KBr/MeCN- H_2O , yields, 72–91%, 7 examples.

Keywords

amino acids; click chemistry; histidine; metal complexes; peptides; triazoles

The synthesis and utilization of unnatural amino acids is a vital component of protein engineering, drug design, and enzyme modification.¹ Most frequently, the preparation of many unnatural or otherwise nonproteinogenic amino acids begins with the preparation of so-called glycine equivalents and further manipulation through the installation of side chains.² In general the term 'glycine equivalent' infers a two-carbon fragment whereby the carboxyl group and the amino group bear suitable protection so that the methylene group may be activated as a nucleophile or electrophile to facilitate side-chain installation. In instances where chirality is warranted, the conjunction of side chains may be facilitated by

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Supporting information

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mediation of chiral directing groups, ligands, or adjuvants.³ Unnatural amino acid precursors which have the requisite side chains already in place and contain a set of prochiral carbons, such as amino acrylates, need only to utilize enantioselective reducing agents to complete the scheme.⁴ In cases where the alkylation of protected glycine derivatives gives the racemic mixtures, the best recourse may be conversion into suitable substrates whereby resolution steps may employ chiral chromatography or enzymes.⁵ Considering the five-membered nitrogen heterocycles, the metal-complexing properties of the 1,2,3-triazolyl group is notable and has been the focus of development of diverse complexes bearing manganese, iron, nickel, copper, ruthenium, rhodium, iridium, palladium, and gold for use in catalysis.⁶ Accordingly, through affixing any of several nitrogen heterocycles with metal-complexing properties to a polypeptide backbone, one may tailor both unique and diverse peptide motifs with catalytic properties.⁷ One finds many interesting parallels of the 1,2,3-triazolyl group to the imidazole group in histidine (Figure 1). In the areas of coordination chemistry, the functional similarities between the two nitrogen heterocycles is noteworthy and has driven the evaluation of the C- or N-substituted triazolyl group.⁸

For example, functionalized polymers bearing imidazole and triazole residues can effectively coordinate Cu^{+1} and Cu^{+2} ions and are being explored as antifouling agents.⁹ Furthermore, triazole linkers which can be formed from alkynyl amino acid residues and functionalized azides may be used in the fluorogenic labeling of polypeptides.^{1a,10} Invariably, the most straightforward synthesis of 1,2,3-triazoles falls within one major area of 1,3-dipolar cycloaddition chemistry which is the so-called 'click' reaction.¹¹ Hence, the formation of the heterocycle only requires the reacting components of an azide and an alkyne and is facilitated under very mild conditions. Our synthesis of triazolyl analogues of histidine starts with the isoindolinone-protected *tert*-butyl glycine derivative **1** (Scheme 1). We elected to test the use of the isoindolinone group as N-protection of a glycine equivalent due to its increased robustness over the well-known *N*-phthaloyl group.

Furthermore, the employment of a strategic oxidative transformation converting the isoindolinoyl group into the easily removed phthaloyl group enhances or otherwise establishes the effectiveness of this 'more dormant' mode of protection. The sodium salt of isoindolinone is generated by treatment with sodium hydride (THF) followed by alkylation with *tert*-butyl bromoacetate (r.t., 16 h). The resulting *N*-isoindolinone-protected glycine ester **1** is then C-alkylated with propargyl bromide in the presence of lithium hexamethyldisilazide ($-78\text{ }^{\circ}\text{C}$, THF). The resulting *N*-isoindolinonoyl ethynylalanine-*tert*-butyl ester **2** was obtained in 84% yield after purification by silica gel column chromatography.¹² The reactions of *tert*-butyl ester **2** with benzyl-, 4-fluorobenzyl-, or 4-methoxybenzylazides under standard click conditions (CuSO_4 , ascorbate, THF, H_2O) gave less than modest yields of the corresponding click products.

Suspecting that the presence of the *tert*-butyl ester group was somewhat of a hindrance to the click reaction, it was removed (K_2CO_3 , MeOH, H_2O) and replaced with a methyl group (MeOH, acetyl chloride; Scheme 2). Surprisingly, the click reactions with the corresponding methyl ester **3a** did not show a marked improvement over similar reaction times and conditions as the *tert*-butyl substrate **3**. However, after removal of the *tert*-butyl group, the

corresponding ethynylalanine isoindolinone-protected free carboxylic acid **3** gave good to excellent yields of the corresponding click products **4–10** (Table 1).¹³

At this point we should note that, in principle, the base-sensitive *N*-phthaloyl protecting group, as opposed to the present *N*-isoindolyl protection, would not survive the ester cleavage and immediately suffer hydrolysis. The click products **4–10** were re-esterified with methanol (catalytic acetyl chloride, r.t., 16 h)¹⁴ which gave the more soluble corresponding methyl ester substrates **11–17** (64–87%) en route to the benzylic oxidation step. We have reported earlier that the interconversion of the isoindolinone (phthalimidine) group and the phthalimide (*N*-phthaloyl) group through a selective benzylic oxidation is a useful transformation, albeit with simple *N*-substituted isoindolinone substrates.¹⁵ While our earlier studies of the isoindolinone oxidation utilized an oxochromium(VI)–peroxide system, we now elect to explore a less toxic and more environmentally friendly oxidation system. Although not hitherto reported with nitrogen heterocycles and only with simple alkylarenes, an oxone/KBr-mediated direct benzylic oxidation appeared to be an ideally suited protocol for our purposes.¹⁶ Hence, using a modified procedure, treatment of ester substrates **11–17** with Oxone (5 equiv) and KBr (0.5 equiv) in MeCN/H₂O (9:1) under slightly elevated temperatures (55–60 °C) gave the corresponding phthalimides **18–23** (72–91%).¹⁷ Using the *N*-benzyltriazolyl alanine product **22** (Table 2), we demonstrate the potential application to triazole-linked peptide units by preparation of *N*-Boc **24** (see Supporting Information). Removal of the *N*-phthaloyl group (N₂H₄, MeOH) of **22** followed by direct installation of the *N*-Boc group (di-*tert*-butyl dicarbonate, NaHCO₃) gave **24** in 76% overall yield.^{18a}

In conclusion, we have described the preparation and utilization of a unique amino acid-based, *N*-isoindolinone-protected intermediate which undergoes base-mediated propargylation to give a protected ethynylalanine derivative. Ester protecting group adjustment then yields a click substrate which reacts with a diverse set of azides to afford the corresponding isoindolinone-protected triazoles. Re-esterification followed by selective benzylic oxidation converts the *N*-isoindolinone group into the more easily removable phthalimide group. Overall, the processes which involve isoindolinone protection and the benzylic oxidation are novel and will offer considerable synthetic latitude in preparing new amino acids. Separate studies in these laboratories have shown that selective alkylation of the isoindolinone-protected glycine equivalent **2** yields a wide range of unnatural or otherwise non-proteinogenic amino acid derivatives and the results will be reported in due course.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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- General Procedure for the Synthesis of Click Compounds 4–102-(1-Oxoisindolin-2-yl)pent-4-ynoic acid (3, 50 mg, 0.22 mmol, 1 equiv)** was dissolved in THF followed by the addition of CuSO₄·5H₂O (5.44 mg, 0.022 mol, 0.1 equiv) and the corresponding azides (0.24 mmol, 1.1 equiv) at r.t. A freshly prepared solution of sodium ascorbate (21.6 mg, 0.11 mmol, 0.5 equiv) in H₂O (1 mL) was added to the reaction mixture and stirring was continued (16 h). After completion of reaction, the mixture was concentrated and the resulting crude residue was purified by gravity column chromatography (CHCl₃/MeOH, 3:1) to provide pure triazoles **4–10.3-(1-Hexyl-1H-1,2,3-triazol-4-yl)-2-(1-oxaisoindolin-2-yl)propanoic Acid (4)** Off-white solid; 67 mg (86%): mp 138–140 °C; *R*_f = 0.44 (MeOH/CHCl₃, 2.5:7.5). FT-IR (neat): 3383, 2929, 2859, 1705, 1659, 1602, 1398, 1215 cm⁻¹. ¹H NMR (400 MHz, DMSO-*d*₆): δ = 7.77 (s, 1 H), 7.61–7.56 (m, 3 H), 7.47–7.43 (m, 1 H), 4.83 (d, *J* = 9.2 Hz, 1 H), 4.76 (d, *J* = 17.2 Hz, 1 H), 4.42 (d, *J* = 17.2 Hz, 1 H).

4.23 (t, J = 6.8 Hz, 2 H), 3.50 (dd, J = 4.0 Hz, 15.6 Hz, 1 H), 3.11 (dd, J = 12.0 Hz, 14.8 Hz, 1 H), 1.70-1.62 (m, 2 H), 1.17-1.10 (m, 4 H), 1.08-1.05 (m, 2 H), 0.80 (t, J = 7.6 Hz, 3 H) ppm. ^{13}C NMR (125 MHz, DMSO- d_6): δ = 173.9, 168.2, 145.2, 142.8, 133.3, 131.3, 127.8, 123.6, 122.9, 122.5, 56.5, 49.5, 4.73, 30.9, 30.1, 27.4, 25.8, 22.3, 14.3 ppm. HRMS: m/z calcd for $\text{C}_{19}\text{H}_{24}\text{N}_4\text{O}_3$, $[\text{M} + \text{H}]$: 357, 1921; found: 357.1919.

14. **General Procedure for the Preparation of Esters 11–17** The corresponding click acids **4–10** were dissolved in MeOH (20 mL) followed by the addition of a catalytic amount of acetyl chloride. The reaction mixture was stirred overnight at r.t. After completion of the reaction, the reaction mixture was concentrated, and the oily residue was partitioned between CH_2Cl_2 (20 mL) and H_2O (15 mL). The CH_2Cl_2 layer was separated, dried over Na_2SO_4 , and then concentrated to provide the corresponding esters **11–17** as clear colorless oils. **Methyl 3-(1-Hexyl-1H-1,2,3-triazol-4-yl)-2-(1-oxoisindolin-2-yl)propanoate (11)** Colorless oil; 50 mg (84%); R_f = 0.70 ($\text{CHCl}_3/\text{MeOH}$, 8:2). FT-IR: 2954, 2930, 1740, 1683, 1469, 1210 cm^{-1} . ^1H NMR (400 MHz, CDCl_3): δ = 7.74 (d, J = 7.2 Hz, 1 H), 7.49 (t, J = 7.2 Hz, 1 H), 7.41-7.37 (m, 3 H), 5.35 (dd, J = 4.8 Hz, 10.8 Hz, 1 H), 4.48 (d, J = 2.8 Hz, 2 H), 4.18 (td, J = 1.2 Hz, 7.2 Hz, 14.4 Hz, 2 H), 3.73 (s, 3 H), 3.50-3.44 (m, 2 H), 1.72 (q, J = 7.6 Hz, 2 H), 1.16-1.09 (m, 6 H), 0.78 (t, J = 6.8 Hz, 3 H) ppm. ^{13}C NMR (175 MHz, CDCl_3): δ = 170.7, 169.2, 143.2, 141.8, 131.6, 128.0, 123.9, 123.7, 123.2, 121.4, 53.4, 52.7, 50.3, 47.5, 31.0, 30.1, 26.6, 25.9, 22.3, 13.8 ppm. HRMS: m/z calcd for $\text{C}_{20}\text{H}_{26}\text{N}_4\text{O}_3$ $[\text{M} + \text{H}]$: 370.2005; found: 370.2003.
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17. **General Procedure for the Benzylic Oxidation of Isoindolinone-Protected Click Compounds 11–17 Giving Phthalimides 18–23** To a solution of isoindolinones **11–17** (1 equiv) in MeCN/ H_2O (9:1) was added Oxone (5.0 equiv) and KBr (0.5 equiv). The resulting reaction mixture was allowed to reflux (16 h). After completion of the reaction, the reaction mixture was concentrated and the residue was partitioned between H_2O (15 mL) and CH_2Cl_2 (15 mL). The organic layer was separated, dried over anhydrous Na_2SO_4 , and evaporated to obtain a crude residue which was further purified by column chromatography on silica gel ($\text{CHCl}_3/\text{MeOH}$, 9.5:0.5) to afford pure phthalimides **18–23**. **2-(13-Dioxoisindolin-2-yl)-3-(1-hexyl-1H-1,2,3-triazol-4-yl)propanoic Acid (18)** Colorless oil; 38 mg (91%); R_f = 0.57 (hexane/EtOAc, 1:1). FT-IR: 2925, 2856, 1775, 1744, 1711, 1386, 718, cm^{-1} . ^1H NMR (700 MHz, CDCl_3): δ = 7.80 (dd, J = 2.8 Hz, 5.6 Hz, 2 H), 7.70 (dd, J = 2.8 Hz, 5.6 Hz, 2 H), 7.32 (s, 1 H), 5.19 (dd, J = 4.9 Hz, 9.8 Hz, 1 H), 4.22-4.20 (m, 2 H), 3.76 (s, 3 H), 3.69-3.64 (m, 2 H), 1.77-1.74 (m, 2 H), 1.24-1.17 (m, 6 H), 0.83 (t, J = 7.0 Hz, 3 H) ppm. ^{13}C NMR (175 MHz, CDCl_3): δ = 169.1, 167.3, 143.1, 134.1, 131.7, 123.5, 121.7, 52.9, 51.7, 50.2, 31.0, 30.1, 25.9, 25.4, 22.3, 13.9 ppm. HRMS: m/z calcd for $\text{C}_{20}\text{H}_{24}\text{N}_4\text{O}_4$ $[\text{M} + \text{H}]$: 384.1798; found: 384.1608.
18. The spectral properties of **24** were identical with those previously reported: Mindt TL, Schlibi R. J Org Chem. 2007; 72:10247. [PubMed: 18044929] For a unique N-Boc-protected triazolyl alanine methyl ester derivative, see: Boibessot T, Benimelis D, Jean M, Benfodda Z, Meffre P. Synlett. 2016; 27:2685.

Figure 1.
Triazolylalanine vs. histidine residues

Figure 2.
N-Boc-protected benzyltriazole **24**

Scheme 1.

Preparation of the N-protected ethynylalanine click substrate **3**. *Reagents and conditions.* (a) NaH, *tert*-butylbromoacetate, THF, 0 °C to r.t., 16 h (97%); (b) LiHMDS, propargyl bromide, THF, -78 °C to r.t., 3 h, then HCl (84%); (c) K₂CO₃, MeOH, H₂O, 65–70 °C, then 5% HCl (75%).

Scheme 2.

Click reactions of **3** with various azides, esterification to substrates **11–17**, and oxidation to the phthaloyl-protected products **18–23**. *Reagents and conditions:* (a) $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, Na ascorbate, THF/ H_2O (2:1), r.t., 16 h (see Table 1); (b) MeOH, acetyl chloride (cat), r.t., 16 h; (c) Oxone, KBr, MeCN/ H_2O (9:1), 55–60 °C, 16 h.

Table 1Click Reactions of Ethynylalanine Analogue **3** with Various Azides^a

3, R¹ = H
3a, R¹ = Me

4–10

Azide	Product	Yield (%) ^b
N ₃ -C ₆ H ₁₃ - <i>n</i>	4 <i>n</i> -hexyl	86
N ₃ -C ₆ H ₁₇ - <i>n</i>	5 <i>n</i> -octyl	85
	6 cyclopentyl	67
	7 cyclohexyl	63
	8 benzyl	95
	9 4-fluorobenzyl	94
	10 4-methoxybenzyl	84

^a Reagents and conditions: CuSO₄·5H₂O, Na ascorbate, THF, H₂O, r.t., 16 h.^b Isolated chemical yield after silica gel column chromatography.

Table 2Esterification of Click Products **4–10** to Methyl Esters **11–17** and Oxidation of **11–17** to Phthalimides **18–23**^a

R²	Yield of 11–17 (%)^b	Yield of 18–23 (%)^{b,c}
4 <i>n</i> -hexyl	11 84	18 91
5 <i>n</i> -octyl	12 83	18 91
6 cyclopentyl	13 64	20 72
7 cyclohexyl	14 63	21 76
8 benzyl	15 86	22 87
9 4-fluorobenzyl	16 87	23 90
10 4-methoxybenzyl	17 87	NR

^a *Reagents* and conditions: (a) MeOH, acetyl chloride (cat.), r.t., 16 h; (b) Oxone, KBr, MeCN/H₂O (9:1), 55–60 °C, 16 h.^b Isolated chemical yield after silica gel column chromatography.^c NR = no reaction.