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Traceless Azido Linker for the Solid-Phase Synthesis of *N*H-1,2,3-Triazoles via Cu-Catalyzed Azide−Alkyne Cycloaddition Reactions

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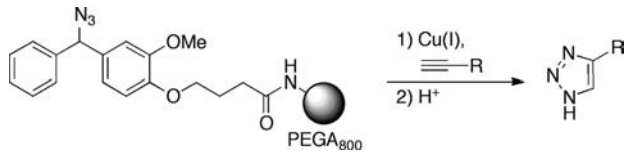
ABSTRACT



A broadly useful acid-labile traceless azido linker for the solid-phase synthesis of *N*H-1,2,3-triazoles is presented. A variety of alkynes were efficiently immobilized on a range of polymeric supports by Cu(I)-mediated azide−alkyne cycloadditions. Supported triazoles showed excellent compatibility with subsequent peptide chemistry. Release of pure material (typically >95%) from the solid support was readily achieved by treatment with aqueous TFA.

Since the introduction by Merrifield,[1] solid-phase synthesis has emerged as an increasingly important tool in modern chemical biology and medicinal chemistry research.[2] Being undisputedly beneficial in polypeptide synthesis, the field has developed significantly in recent years, where more emphasis has been given to the parallel synthesis of heterocycles,[3] such as diketopiperazines, benzodiazepines, and triazoles, but also to more complex ring systems, such as those made during diversity- and biology-oriented synthesis efforts.[4]

One of recent years' most significant accomplishments in solid-phase synthesis was reported by Meldal and co-workers on their discovery of the copper(I)-catalyzed azide−alkyne cycloaddition (CuAAC) reaction.[5] The reaction has been proven extremely reliable and robust for solid-phase applications, e.g., as evidenced by the successful synthesis and screening of a library containing over 400 000 peptidotriazoles[6] and very recently for the synthesis of antibody-like protein-capture agents.[7]

The *N*H-1,2,3-triazoles (*N*-unsubstituted 1,2,3-triazoles) possess many interesting biological properties.[8] However,

(1) Merrifield, B. R. *J. Am. Chem. Soc.* **1963**, *85*, 2149–2154.

(2) (a) Früchtel, J. S.; Jung., G. *Angew. Chem., Int. Ed.* **1996**, *35*, 17–42. (b) *Organic Synthesis on Solid Phase*; Dörwald, F. Z., Ed.; Wiley-VHC: Weinheim, 2002. (c) *Linker Strategies in Solid-Phase Organic Synthesis*; Scott, P., Ed.; Wiley-VHC: Weinheim, 2009. (d) *The power of functional resins in organic synthesis*; Tulla-Puche, J., Albericio, F., Ed.; Wiley-VCH: Weinheim, 2008.

(3) Bräse, S.; Gil, C.; Knepper, K. *Bioorg. Med. Chem.* **2002**, *10*, 2415–2437.

(4) (a) Feliu, L.; Vera-Luque, P.; Albericio, F. *J. Comb. Chem.* **2007**, *9*, 521–565. (b) Bunin, B. A.; Plunkett, M. J.; Ellman, J. A. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 4708–4712. (c) Lambert, J. N.; Mitchell, J. P.; Roberts, K. D. *J. Chem. Soc., Perkin Trans. 1* **2001**, *5*, 471–484. (d) Nielsen, T. E.; Meldal, M. *Curr. Opin. Drug Discovery Dev.* **2009**, *12*, 798–810. (e) Mitchell, J. M.; Shaw, J. T. *Angew. Chem., Int. Ed.* **2006**, *45*, 1722–1726. (f) Ludolph, B.; Waldmann, H. *Tetrahedron Lett.* **2009**, *50*, 3148–3150. (g) Izumi, M. *J. Pestic. Sci.* **2006**, *31*, 1–5. (h) Burke, M. D.; Schreiber, S. L. *Angew. Chem., Int. Ed.* **2004**, *43*, 46–58. (i) Nielsen, T. E.; Schreiber., S. L. *Angew. Chem., Int. Ed.* **2008**, *47*, 48–56.

(5) (a) Tornøe, C. W.; Christensen, C.; Meldal, M. *J. Org. Chem.* **2002**, *67*, 3057–3064. (b) For the first solution-phase CuAAC reaction, consult: Rostovtsev, V. V.; Green, L. G.; Fokin, V. V.; Sharpless, K. B. *Angew. Chem., Int. Ed.* **2002**, *41*, 2596–2599.

(6) Tornøe, C. W.; Sanderson, S. J.; Motrram, J. C.; Coombs, G. H.; Meldal, M. *J. Comb. Chem.* **2004**, *6*, 312–324.

(7) Agnew, H. D.; Rohde, R. D.; Millward, S. W.; Nag, A.; Yeo, W. S.; Hein, J. E.; Pitram, S. M.; Tariq, A. A.; Burns, V. M.; Krom, R. J.; Fokin, V. V.; Sharpless., K. B.; Heath, J. R. *Angew. Chem., Int. Ed.* **2009**, *48*, 4944–4948.

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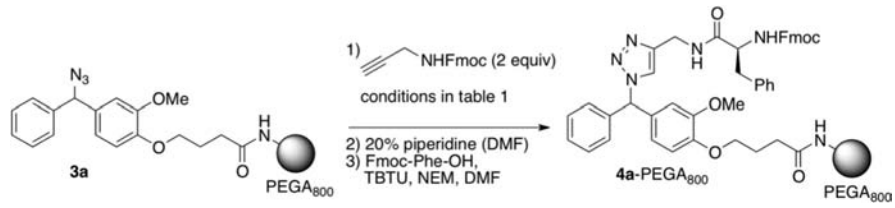
Table 1. Representative Solid-Phase Cu(I)-Catalyzed Azide−Alkyne Cycloaddition Reactions



entry	Cu source	base	reducing agent	solvent	conversion[b]
1	$CuSO_4{\cdot}H_2O$ (0.16 equiv)	-	Na ascorbate (3.4 equiv)	CH_2Cl_2:MeOH (1:1)	56%
2	CuI (2 equiv)	-	-	DMF:piperidine (4:1)	43%
3[a]	CuI (2 equiv)	-	-	DIPEA:pyridine:DMF (1:3:1)	38%
4	CuI (5 equiv)	DIPEA (10 equiv)	Na ascorbate (5 equiv)	DMF:piperidine (4:1)	49%
5	CuBr (2 equiv)	2,6-lutidine (10 equiv)	Na ascorbate (3 equiv)	DMF	77%
6	CuI (0.5 equiv)	2,6-lutidine (2 equiv)	Na ascorbate (1 equiv)	MeCN:DMSO:H_2O (8:2:1)	68%
7	CuI (1 equiv)	2,6-lutidine (2 equiv)	Na ascorbate (1 equiv)	NMP:H_2O (4:1)	>95%

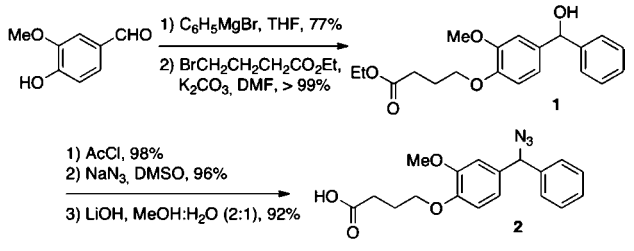
[a] 5 equiv of Fmoc-propargylamine was used. [b] Conversions were measured using RP-HPLC Fmoc quantification (254 nm).[14]

contrary to the formation of 1,4-disubstituted 1,2,3-triazoles, the general access to *N*H-1,2,3-triazoles on solid support is not described in the literature. Various methods are known for the solution-phase synthesis of this compound class, including the reaction of nitro-alkenes with sodium azide[9] and the application of various azide donors, such as azidomethyl pivalate and carbamates[10] and trimethylsilyl azide.[11] However, none of these methods have been reported for use on solid support. The direct synthesis of *N*H-1,2,3-triazoles from alkynes and inorganic azides is impractical on solid support, and copper(I)-catalyzed variants hereof are not easily optimized with sodium azide.

Intrigued by the potential of *N*H-1,2,3-triazoles, we envisioned a strategy that uses an acid-labile azido linker (**2**) compatible with common resins and reaction conditions typical for combinatorial chemistry.[12] The unique chemoselectivity of the CuAAC reaction would allow loading of the resin with multifunctional alkyne building blocks without resorting to an extensive protecting group scheme. The synthesis of **2** commenced with the addition of excess amounts of Grignard reagent derived from bromobenzene to vanillin followed by alkylation of the phenol with ethyl-4-bromobutyrate to yield **1** in 76% over two steps (Scheme 1).

Chlorination of **1** followed by azidation and hydrolysis of the ester moiety afforded the 3-(4-(azido(phenyl)-methyl)-2-methoxyphenoxy)propanoic acid linker (**2**) in 87% yield (three steps). In conclusion, the linker **2** can be made in only five steps with an overall yield of 66% on a +10 g scale, notably without resorting to chromatographic purification in any of the steps.

Scheme 1. Synthesis of the Azido Linker



To investigate the ability of the linker to function as a traceless linker in the formation of *N*H-1,2,3 triazoles,[13] **2** was readily coupled to the amino-functionalized $PEGA_{800}$ (polyethylene glycol dimethyl acrylamide) resin using TBTU (*O*-(benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium tetrafluoroborate). A range of procedures were attempted to find a quantitative protocol for the solid-phase synthesis of 1,2,3-triazoles (representative results are shown in Table 1). The efficiency hereof was evaluated by measuring the conversion of **3a** to **4a** via subsequent Fmoc quantification.[14] Copper(I) species generated in situ from $CuSO_4$ using sodium ascorbate in CH_2Cl_2/MeOH resulted in a moderate conversion of 56% (entry 1), and changing the copper(I) source to CuI gave a further decrease in conversion to 43% (entry 2). Increasing the amount of alkyne from 2 equiv to 5 equiv and use of

(8) (a) Kume, M.; Kubota, T.; Kimura, Y.; Nakashimizu, H.; Motokawa, K.; Nakano, M. *J. Antibiot.* **1993**, *46*, 177–192. (b) Cristalli, G.; Eleuteri, A.; Volpini, R.; Vittori, S.; Camaioni, E.; Lupidi, G. *J. Med. Chem.* **1994**, *37*, 201–205. (c) Nimkar, S. K.; Mabic, S.; Anderson, A. H.; Palmer, S. L.; Graham, T. H.; de Jonge, M.; Hazelwood, L.; Hislop, S. J.; Castagnoli, N. *J. Med. Chem.* **1999**, *42*, 1828–1835. (d) Fray, M. J.; Bull, D. J.; Car, C. L.; Gautier, E. C. L; Mowbray, C. E.; Stobie, A. *J. Med. Chem.* **2001**, *44*, 1951–1962. (e) Komeda, S.; Lutz, M.; Spek, A. L.; Yamanaka, Y.; Sato, T.; Chikuma, M.; Reedijk, J. *J. Am. Chem. Soc.* **2002**, *124*, 4738–4746.

(9) Quiclet-Sire, B.; Zard, S. Z. *Synthesis* **2005**, *19*, 3319–3326.

(10) Loren, J. C.; Krasinski, A.; Fokin, V. V.; Sharpless., K. B. *Synlett* **2005**, *18*, 2847–2850.

(11) Jin, T.; Kamijo, S.; Yamamoto, Y. *Eur. J. Org. Chem.* **2004**, *18*, 3789–3791.

(12) For previous work on *N*H-1,2,3-triazole synthesis on Wang resins, see: Harju, K.; Vahermo, M.; Mutikainen, I.; Yli-Kauhaluoma, J. *J. Comb. Chem* **2003**, *5*, 826–833.

(13) For a review on traceless linkers, see: Gil, C.; Bräse, S. *Curr. Opin. Chem. Biol.* **2004**, *8*, 230–237.

(14) The conversion of **4a** was measured by subjecting the formed Fmoc-protected peptide to a 2% DBU (DMF) solution containing 0.042 nM anthracene as internal standard for 30 min, followed by HPLC quantification of the anthracene/dibenzofulvene ratio. See also: Freeman, C. E.; Howard, A. G. *Talanta* **2005**, *65*, 574–577.

DIPEA (*N*,*N*-diisopropylethylamine) as a base gave an even lower conversion (entry 3). On the other hand, increasing the amount of copper to 5 equiv and addition of sodium ascorbate increased the conversion to 49% (entry 4).[15] A significant improvement in conversion to 77% was observed by switching to 2,6-lutidine as base (entry 5). Encouraged by this observation, we tried to decrease the amount of catalyst to 0.5 equiv as well as to change the solvent to prevent precipitation of Cu-salts, but conversion decreased to 68% (entry 6). Realizing that a stoichiometric amount of catalyst is needed, a full equivalent of CuI in NMP/H:O gave the desired conversion of >95% (entry 7), and no precipitation of Cu salts was observed.[15,16]

With an optimized CuAAC protocol at hand, we naturally turned our attention to the cleavage conditions. To measure the reactivity of immobilized **2**, small batches of the Fmoc-protected triazole linker derivative **4a** were subjected to 95% TFA(aq) for various lengths of time. After cleavage, the resin was washed with appropriate amounts of DMF and CH_2Cl_2, and residual Fmoc on the resin was quantified by standard HPLC methods (vide supra).[17] As seen in Figure 1, more than 90% of **4a** was released as the corresponding triazole after 24 h.

To test the versatility of the linker, the *N*H-1,2,3-triazole **5** was synthesized on seven commercially available and commonly used hydroxy- or amino-functionalized solid supports.[18]

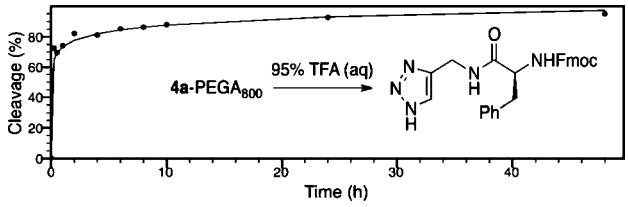
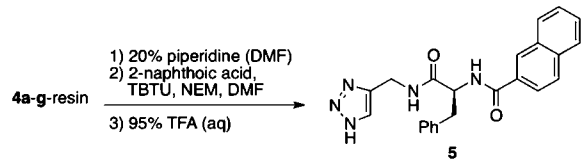


Figure 1. Acid-mediated release of triazole from **4a**-$PEGA_{800}$ as a function of time.

As seen in Table 2, the linker is well applicable with all polymeric supports examined, except for the Wang resin that appeared unstable under the reaction conditions. Confident with the solid-phase strategy, a small collection of aryl- and aliphatic *N*H-1,2,3-triazoles were synthesized.

As seen in Table 3, the solid-phase strategy is very robust and applicable to a range of alkynes containing a representative set of common functional groups, including free carboxyl acid moieties (entries 7 and 8). The purity was generally very high for ethynyl aryls, except for the pyridine derivative (entry 10). The yields are in most cases modest but acceptable. Crude aliphatic *N*H-1,2,3-triazoles were generally less pure, albeit with higher yields, except for the cyclopropyl derivative (entry 15). Aryl ethers, thioethers, and esters were synthesized in high purities and acceptable yields (entries 16−18).

Table 2. Test of Common Solid Supports for the Synthesis of *N*H-1,2,3-Triazole

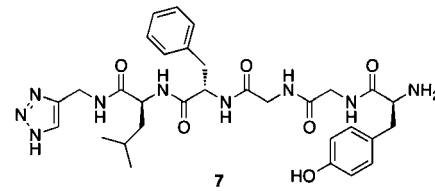


5

support	purity[a]	yield
4a−$PEGA_{800}$	>95%	71%
4b−polystyrene−OH	80%	57%
4c−polystyrene−NH_2	94%	54%
4d−tentagel−OH	>95%	67%
4e−tentagel−NH_2	94%	85%
4f−Chemmatrix−NH_2	94%	59%
4g−Wang−OH	38%	26%

[a] Purity was determined by RP-HPLC (215 and 254 nm).

The reliability of the linker in multistep peptide synthesis was explored by synthesizing an *N*H-1,2,3-triazole derivative of the opioid pentapeptide Leu-enkephalin (**7**) found in the brain.[19]



7

The peptide was synthesized using a standard Fmoc strategy, employing TBTU as the coupling reagent. The amino acids were coupled to the linker after an initial click reaction with Fmoc-propargylamine. After cleavage with 95% TFA(aq) for 24 h, the pentapeptide was obtained as the TFA salt in 85% purity and 80% yield.[20]

A complementary method that quantitatively assembles the azido linker on the solid support was also developed (Scheme 2). The alkylated benzhydrol **1** was hydrolyzed by LiOH and attached to the solid support as a TBTU activated ester (Scheme 2). After attachment, the linker was swollen

(15) Zhang, Z.; Fan, E. *Tetrahedron Lett.* **2006**, *47*, 665–669.

(16) Bock, V. D.; Hiemstra, H.; van Maarseveen, J. H. *Eur. J. Org. Chem.* **2006**, *1*, 51–68.

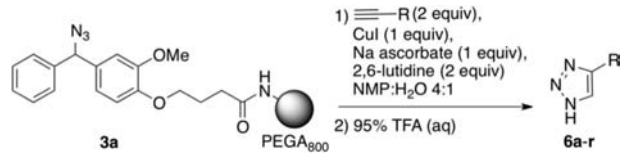
(17) Bernatowicz, M. S.; Daniels, S. B.; Köster, H. *Tetrahedron Lett.* **1989**, *30*, 4645–4648.

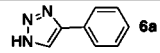
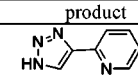
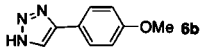
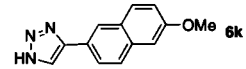
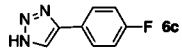
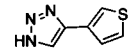
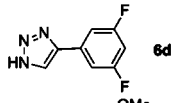
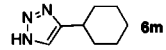
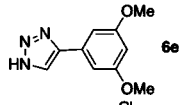
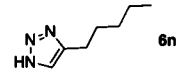
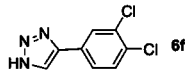
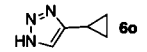
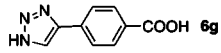
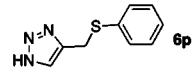
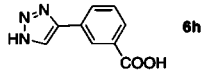
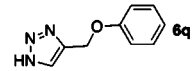
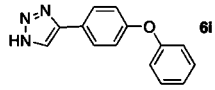
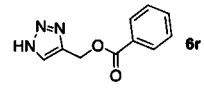
(18) (a) The following solid supports were tested: $PEGA_{800}$ (Polymer Labs), Hydroxymethyl polystyrene (Polymer Labs), Aminomethyl Polystyrene (Polymer Labs), Hydroxy Tentagel (Novasyn), Amino Tentagel (Fluka), Chemmatrix (Aldrich), Wang (Aldrich). (b) The linker **2** was attached to the solid support using TBTU (3 equiv of **2**, 4 equiv of NEM, and 2.88 equiv of TBTU in DMF for 2 h), or MSNT coupling (3 equiv of **1**, 2.25 equiv of MeIm, and 3 equiv of MSNT in CH_2Cl_2 1 h, repeated once). Fmoc-Phe-OH and 2-naphthoic acid were attached using standard TBTU coupling. Cleavage was mediated with 95% TFA(aq) for 24 h.

(19) (a) Hughes, J.; Smith, T. W.; Kosterlitz, H. W.; Fothergill, L. A.; Morgan, B. A.; Morris, H. R. *Nature* **1975**, *258*, 577–579. (b) Wilczynska, D.; Kosson, P.; Kwasiborskal, M.; Ejchart, A.; Olma, A. *J. Pept. Sci.* **2009**, *15*, 777–782.

(20) Determined by use of HPLC (215 and 254 nm) quantification and ^{1}H NMR.

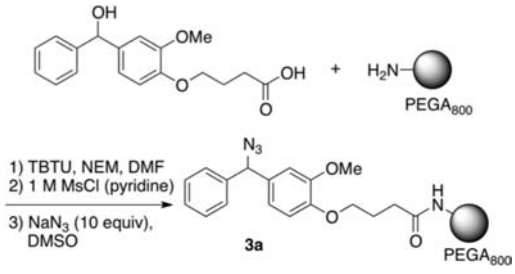
Table 3. Solid-Phase Synthesis of *N*H-1,2,3-Triazoles



entry	product	purity[a]	yield	entry	product	purity[a]	yield
1	 **6a**	>95%	44%	10	 **6j**	80%	62%
2	 **6b**	>95%	66%	11	 **6k**	>95%	66%
3	 **6c**	>95%	55%	12	 **6l**	>95%	50%
4	 **6d**	>95%	55%	13	 **6m**	79%	70%
5	 **6e**	>95%	68%	14	 **6n**	86%	66%
6	 **6f**	94 %	44%	15	 **6o**	78%	46%
7	 **6g**	89 %	53%	16	 **6p**	>95%	44%
8	 **6h**	>95%	83%	17	 **6q**	79%	53%
9	 **6i**	>95%	74%	18	 **6r**	81%	47%

[a] Purity was determined by RP-HPLC (215 and 254 nm).

Scheme 2. Solid-Phase Assembly of the Azido Linker



in concentrated MsCl in pyridine for 1 h. The mesylated resin was then shaken at 65 °C with 10 equiv of sodium azide in DMSO for 24 h. The *N*H-1,2,3-triazole **5** was synthesized in 88% purity and 44% yield using the conditions noted in Table 2.[20]

The observed yield and purity were almost identical to those obtained with linker **2**.

In summary, we have developed an acid-labile traceless azido linker for the synthesis of *N*H-1,2,3-triazoles on the solid phase. The linker can be synthesized in solution in five steps from readily available starting materials or directly on the solid phase. The linker is compatible with most commonly used solid supports and remains intact throughout the multipeptide synthesis. Products are ultimately released from the solid support in excellent purity using 95% TFA(aq).

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Supporting Information Available: Experimental procedures and full spectroscopic data for all new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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