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Simple Plate-Based, Parallel Synthesis of Disulfide Fragments using the CuAAC Click Reaction

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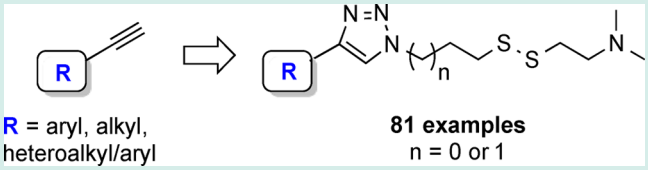
Supporting Information

ABSTRACT: Disulfide exchange screening is a site-directed approach to fragment-based lead discovery that requires a bespoke library of disulfide-containing fragments. Previously, we described a simple one-pot, two-step synthesis of disulfide fragments from amine- or acid-bearing starting materials. Here, we describe the synthesis of disulfide fragments that bear a 1,4-substituted-1,2,3-triazole linkage between disulfide and molecular diversity element. This work establishes the compatibility of copper(I)-catalyzed azide−alkyne cycloaddition (CuAAC) chemistry with a one-pot, two-step reaction sequence that can be readily parallelized. We performed 96 reactions in a single deep-well microtiter plate, employing 48 alkynes and two different azide linker reagents. From this effort, a total of 81 triazole-containing disulfide fragments were obtained in useful isolated yields. Thus, CuAAC chemistry offers an experimentally convenient method to rapidly prepare disulfide fragments that are structurally distinct from fragments accessed via amide, sulfonamide, or isocyanate chemistries.



KEYWORDS: *tethering, disulfide exchange screening, click chemistry, disulfide synthesis*

Fragment-based lead discovery has been widely adopted in the pharmaceutical industry as a complementary approach to high-throughput screening (HTS). Fragments typically have a molecular weight of less than 250 Da and binding affinities in the micromolar to millimolar range. The ligand efficiency (binding free energy per heavy atom) of fragments is generally higher than for compounds identified by HTS, making fragments excellent starting points for further optimization of binding affinity.[1] Composed of many fewer atoms than typical HTS compounds, fragments can more effectively sample chemical space at a given library size.[2,3] Because of the low intrinsic binding affinity of fragments, biophysical screening techniques, such as NMR, surface plasmon resonance (SPR), X-ray crystallography, or isothermal calorimetry (ITC) are preferred over more traditional biochemical assays.[2,4]

One approach to address the low intrinsic affinity of fragments is to introduce a reversible covalent bond (e.g., disulfide or imine) between a fragment and its target. Disulfide exchange has been successfully exploited in a variety of dynamic synthetic processes, including the self-assembly of synthetic receptors and supramolecular architectures,[5,6] and in the generation of dynamic combinatorial libraries.[7−9] Disulfide-exchange screening or "tethering"[10,11] is based upon disulfide exchange between thiol-containing fragments and native or engineered cysteine residues on a protein. The exchange reaction is performed under reducing conditions so that only fragments that also form noncovalent binding interactions with the target protein will persist in a disulfide tethered state. The thermodynamically favored protein-fragment adducts can then be detected by mass spectrometry or other methods. Fragments identified in this way can be further optimized for binding affinity until, ultimately, the disulfide tether can be removed to afford free-standing lead molecules.[12] A key advantage of tethering is that it is site-directed; putative binding sites on a target protein can be probed at will by preparing the appropriate cysteine mutants. Tethering has been successfully applied to probe both catalytic and allosteric sites on kinases and proteases.[13−16] One drawback of tethering is that it requires a bespoke library of disulfide-containing fragments. This apparent "barrier to entry" has prevented wider adoption of the approach, a situation we have aimed to remedy by developing robust and parallelizable synthetic methods for disulfide fragment synthesis.[17]

The structure of a typical disulfide fragment is shown in Figure 1. The chemical diversity element is small, drug-like, and joined to a disulfide via an amide, sulfonamide, urea, or other linker. A dimethylamino "cap" improves aqueous solubility and allows formulation in salt forms (e.g., TFA), which we find improves disulfide stability on long-term storage in screening plates.

Previously, we described a simple and robust two-step, one-pot method to prepare disulfide fragments **4** from simple amines or acids (Figure 2).[17] We have since synthesized ∼1500 disulfide fragments using this chemistry in a parallel, 96-well plate-based format. To further expand our disulfide fragment library and increase its structural diversity, we sought to

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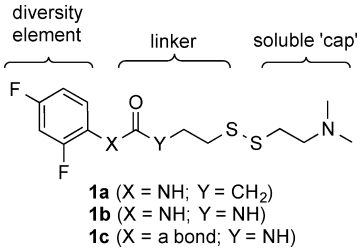


Figure 1. Chemical structure and components of a typical disulfide fragment.

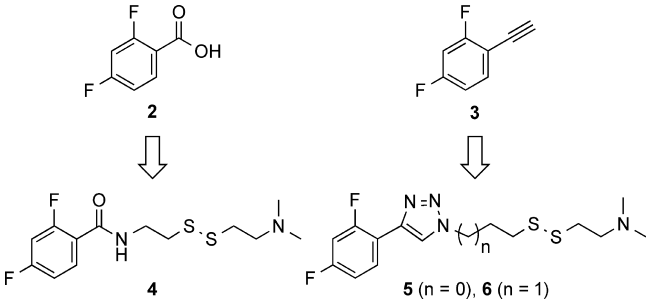


Figure 2. Comparison of a typical amide-based fragment **4** with the 1,4-substituted 1,2,3-triazole-based fragments **5** and **6** described in this work.

investigate new linkers not based on amide, sulfonamide, or urea chemistry. Heterocyclic linkers were of particular interest given their ubiquity in medicinal chemistry and common use as bioisosteres of esters, amides, and other carbonyl-containing functionality. Herein, we describe a convenient route to 1,2,3-triazole-containing disulfide fragments, such as **5** and **6** from diverse alkyne starting materials (Figure 2).

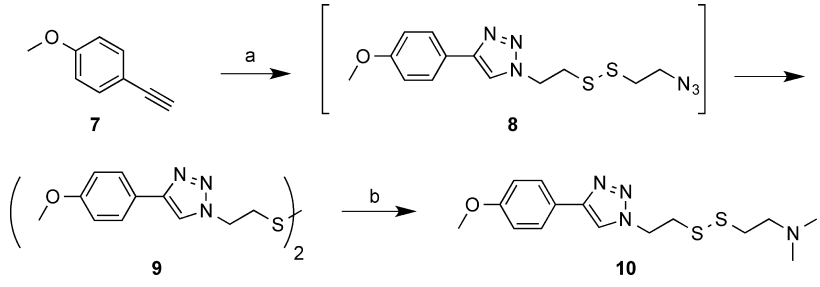
Much interest and many diverse applications of 1,2,3-triazoles have been reported since the copper(I)-catalyzed azide−alkyne cycloaddition (CuAAC) reaction was first described in 2002.[18,3] The 1,2,3-triazole ring can be viewed as a peptide bond surrogate, with regioisomeric 1,4- and 1,5-substituted systems mimicking trans- and cis-like amide conformers, respectively.[19−21] With respect to disulfide fragments, we appreciated that fragments based on 1,4- or 1,5-substituted triazoles would afford very different geometries between diversity element and disulfide tether. We therefore sought to prepare 1,2,3-triazole-based fragments from commercial alkynes and focused initially on the well-established CuAAC reaction leading to the 1,4-substitution pattern.

Following the precedent of our two-step synthesis of amide-based fragments, we set out to explore an analogous two-step, one-pot process involving initial CuAAC reaction followed by disulfide exchange to introduce the cap moiety (Scheme 1). Our earliest attempts at CuAAC utilized Cu(II) sulfate in combination with sodium ascorbate following the literature procedure.[22] 4-Ethynylanisole (**7**) was chosen as alkyne substrate for these initial studies as its reaction products were easily monitored by LC/MS. Unfortunately, the CuAAC reactions were slow and after 24 h a large quantity of the initial triazole product **8** remained alongside only a small amount of the desired symmetrical product **9**. We tentatively ascribed this negative result to disulfide reduction by ascorbic acid and poisoning of the copper catalyst by liberated thiol.[23]

To circumvent this issue, we attempted the reaction with copper(I) salts, thus eliminating the need for an added reductant. Copper(I) trifluoromethylsulfonate toluene complex (CuOTf) was examined initially with favorable results. Unlike with the Cu(II)/ascorbate system, reactions now proceeded to completion; however, multiple unidentified side-products were formed under these conditions. Upon exploring alternate sources of Cu(I), we found that copper(I) iodide promoted the reaction effectively, with few side products, and thus became the preferred copper catalyst for the reaction. The choice of solvent was also found to be critical. Polar aprotic solvents, such as DMF or DMSO, were preferable to acetonitrile or toluene as the former better solubilized reaction mixtures and generally led to higher conversions. We also evaluated the addition of basic amines to the reaction, which are thought to aid the formation of a copper acetylide complex resulting in Cu(I) preactivation.[24] Addition of one equivalent of *N,N*-diisopropylethylamine (DIPEA) to the reaction mixture caused rapid product formation (as observed by LC/MS), but also led to the formation of multiple unidentified side products and was therefore deemed unnecessary. Thus, the optimized conditions for the initial CuACC reaction involve 2.1 equiv of alkyne, one equivalent of diazide (**16** or **17**), and 0.1 equiv of CuI in DMSO.

The second step of the putative two-step, one-pot sequence requires disulfide exchange between the CuACC reaction products **12**/**13** and the symmetrical disulfide **18** (Scheme 2). Gratifyingly, our previously described procedure[17] for this step proved effective with the CuACC derived products, after some refinement. Thus, tris(2-carboxyethyl)phosphine hydrochloride (TCEP) was again employed to initiate the disulfide exchange reaction, which was carried out with a 5-fold excess of **18** to help drive the reaction toward to desired products **14**/**15**. While not necessary with earlier amide-based fragments, we found in the case of the triazoles that heating to 60 °C during the exchange reaction was beneficial as it helped to maintain

Scheme 1. CuAAC Trial Reactions with 4-Ethynylanisole (7)[a]



[a]Conditions (a) 0.48 equiv of **16**, 0.1 equiv of CuI, DMSO, RT, 18 h; (b) 5 equiv of **18**·2HCl, 0.1 equiv of TCEP, Et_3N, H_2O, 60 °C, 18 h DMSO.

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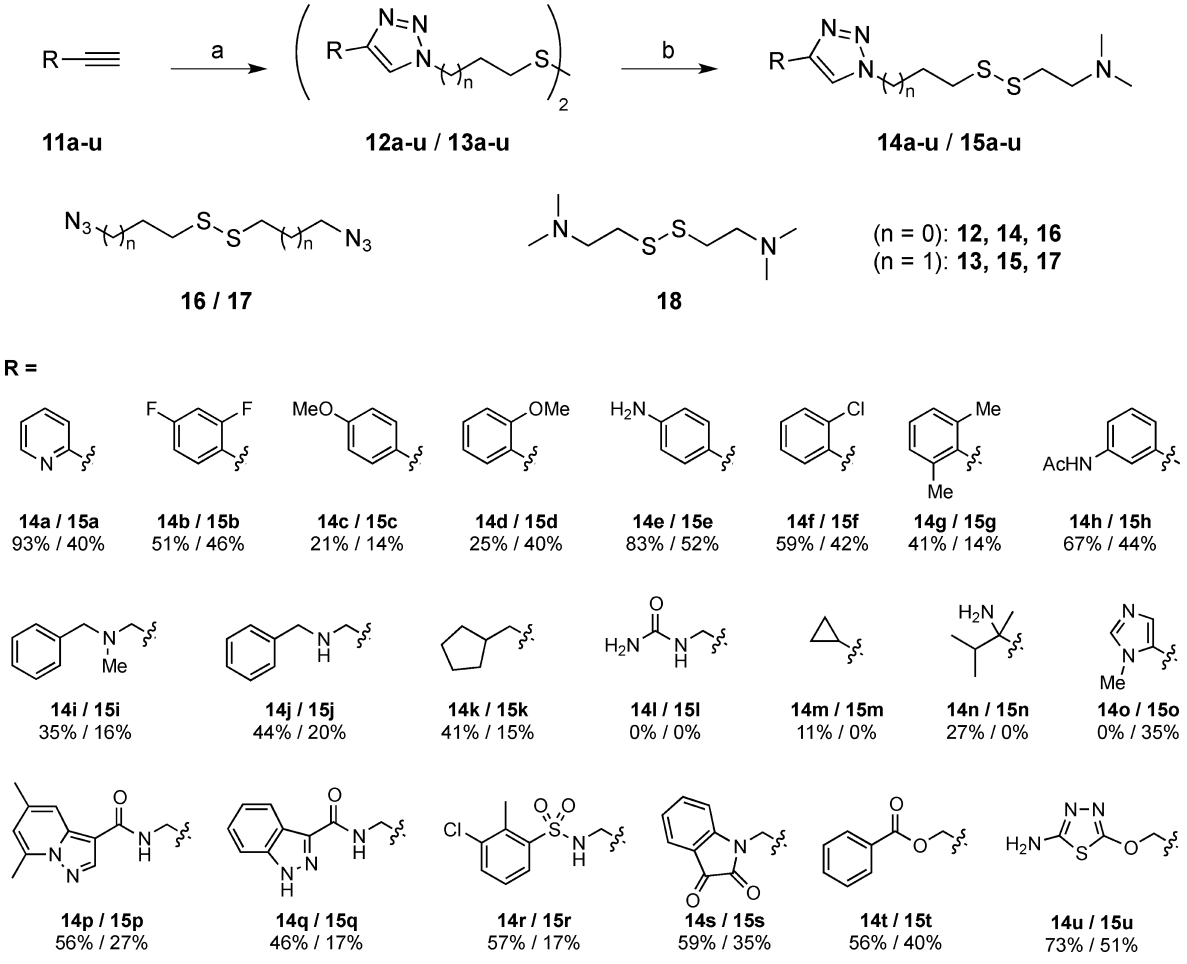
Scheme 2. One-Pot Synthesis of 1,2,3-Triazole Fragments and Representative Library Members (See Supporting Information for the Complete Library)[a]



[a]Conditions (a) 0.48 equiv of **16** or **17**, 0.1 equiv of CuI, DMSO, RT, 18 h; (b) 5 equiv of **18**·2HCl, 0.1 equiv of TCEP, Et_3N, H_2O, 60 °C, 18 h DMSO. Yields are over two steps and represent isolated HPLC purified material.

homogeneous reaction mixtures. The exchange reaction proceeds to afford a thermodynamic mixture of reaction products, which usually favors the desired unsymmetrical disulfide products **14**/**15** over the symmetrical starting material **12**/**13**. Separation of **14**/**15** from disulfide starting material, side-products, and excess reagents is readily accomplished by HPLC and using a mobile phase containing trifluoroacetic acid (TFA). Under these acidic conditions, further disulfide exchange is prevented and the final purified disulfide fragments **14**/**15** can be isolated as stable TFA salts. In our experience, disulfide fragments can be stored as TFA salts as neat powders or in DMSO solution for months to years without fear of degradation due to disulfide exchange.

To explore the scope and utility of this two-step one-pot method, we performed 96 reactions in a parallel reaction format. Hence, 48 commercial alkynes were reacted with diazide reagents **16** or **17**, respectively (for step 1), and disulfide **18** (for step 2). The reactions were carried out in a 2 mL 96-deep-well polypropylene plate and the crude reaction mixtures were purified by semipreparative reverse phase HPLC using a 19 × 50 mm C18 reverse phase column and gradient elution in water−methanol (0.05% TFA). Reaction wells in which precipitation was observed (approximately 5−10% of wells) were filtered prior to injection and generally resulted in lower yields. A representative set of the disulfide products obtained is provided (Scheme 2). The yields shown are over two-steps based on **16**/**17** as limiting reagent and account for the theoretical production of two moles of product for every mole of **16**/**17**. The desired products were obtained from reactions of various aromatic, benzylic, and aliphatic alkynes. Even highly hindered alkynes such as **11g** afforded useful yields of **14g**/**15g**. The two-step sequence succeeded with alkynes bearing primary aromatic or aliphatic amines and for an electrophilic isatin analog **14s**/**15s**. The low yields obtained for **14l**/**15l** and **14m**/**15m** may be due to poor solubility and high volatility of the starting materials, respectively. With a few exceptions, the products **14** (derived from **16**) were obtained in higher yields than the homologated analogs **15** (from **17**). This trend is consistent with our observation that reactions of **17** generally produced a greater number of side products and thus required more challenging purification of the final product. Despite this, 81 of the 96 reactions provided sufficient quantities of the desired disulfide fragment product for incorporation into screening master plates, thus meeting our original objectives.

We have described the parallel synthesis of 1,4-substituted 1,2,3-triazole containing disulfide fragments in synthetically useful yields from a diverse selection of functionalized alkynes.

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These libraries should prove useful for application in disulfide based screening and possibly also for use in dynamic combinatorial libraries. We are currently exploring the ruthenium-catalyzed azide−alkyne cycloaddition (RuAAC) reaction for the preparation of 1,5-substituted 1,2,3-triazole based fragments, a topic that will be detailed in a future publication.

■ ASSOCIATED CONTENT

Supporting Information

Copper-catalyzed azide−alkyne cycloaddition (CuAAC) and disulfide exchange, general information, experimental procedures, analytical data, and selected ^{1}H NMR spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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