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Synthesis of Orthogonally Protected Disulfide Bridge Mimetics

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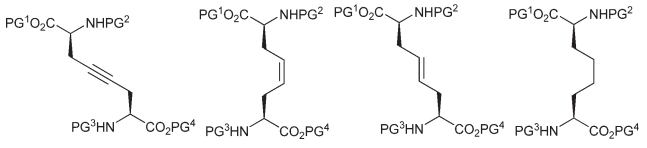

Concise routes to four orthogonally protected, enantiopure disulfide bridge mimetics are reported. These four dicarba analogues possess an alkyne, an (*E*)-alkene, a (*Z*)-alkene, and an alkane as substitutes for the disulfide bridge. Selective deprotection of one of these mimetics is also illustrated.

Cyclization stabilizes peptides and in certain cases may be essential for biological activity. Disulfide bridge formation is the most common mechanism for peptide cyclization. This stabilizing effect is exemplified by a unique subclass of biologically active peptides, known as cyclotides, which possess multiple interlinked disulfide bridges and are renowned for their resistance to thermal, chemical, and proteolytic decomposition.[1] Despite the stabilizing effect of disulfide bridges on many peptides, the disulfide bond may undergo different decomposition reactions and can also be easily reduced in vivo to the open chain peptide.[2] It has been reported that replacing a disulfide bridge with an isosteric carbon analogue (Figure 1, **2**−**5**) can considerably increase the bioavailabilty and in vivo stability of a peptide, without significantly reducing biological activity.[3] In addition, these isosteric carbon linkers may also be used to constrain a peptide in its biologically active conformation, as demonstrated by helical peptide stapling.[4]

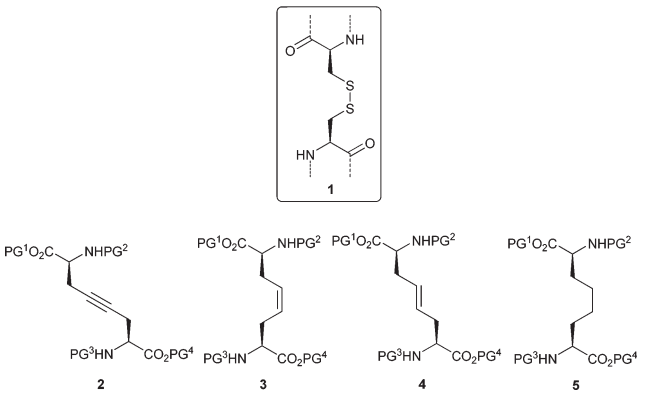

FIGURE 1. Internal disulfide bridge (**1**) and orthogonally protected disulfide bridge mimetics (**2**−**5**).

We are primarily interested in bicyclic peptides with nonterminal disulfide bridges. Interest in improving the in vivo stability of these peptides has led us to require structures **2**−**5** possessing inexpensive, orthogonal protecting groups.[5] To our knowledge, there is no procedure for the synthesis of orthogonally protected mimetics **2**−**4** in the literature. While routes to orthogonally protected **5** have been disclosed, they either require a cumbersome, lengthy synthesis or do not possess the appropriate protecting groups for our study.[6] Herein, we report convenient routes to orthogonally protected disulfide bridge mimetics **2**−**5** employing a copper-mediated organozinc/haloalkyne coupling and a ruthenium-catalyzed cross-metathesis reaction as key steps.

Our initial goal was the synthesis of mimetic **6**. The requirement of an orthogonally protected, enantiopure product severely limited the number of viable synthetic strategies. We envisaged that employing the copper-mediated organozinc/

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(6) Synthesis of orthogonally protected mimetic **5**: (a) Nutt, R. F.; Strachan, R. G.; Veber, D. F.; Holly, F. W. *J. Org. Chem.* **1980**, *45*, 3078. (b) Hiebl, J.; Kollmann, H.; Rovenszky, F.; Winkler, K. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 2963. (c) Lange, M.; Fischer, P. M. *Helv. Chim. Acta* **1998**, *81*, 2053. (d) Aguilera, B.; Wolf, L. B.; Nieczypor, P.; Rutjes, F. P. J. T.; Overkleeft, H. S.; van Hest, J. C. M.; Schoemaker, H. E.; Wang, B.; Mol, J. C.; Fürstner, A.; Overhand, M.; van der Marel, G. A.; van Boom, J. H. *J. Org. Chem.* **2001**, *66*, 3584. (e) Hernández, N.; Martín, V. S. *J. Org. Chem.* **2001**, *66*, 4934.

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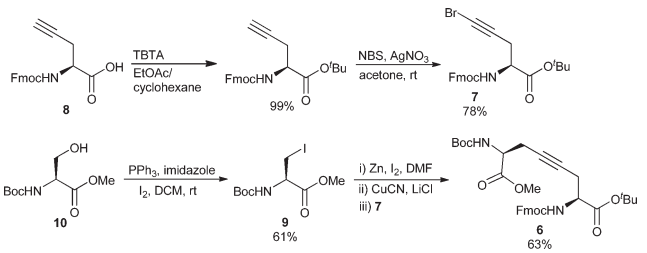
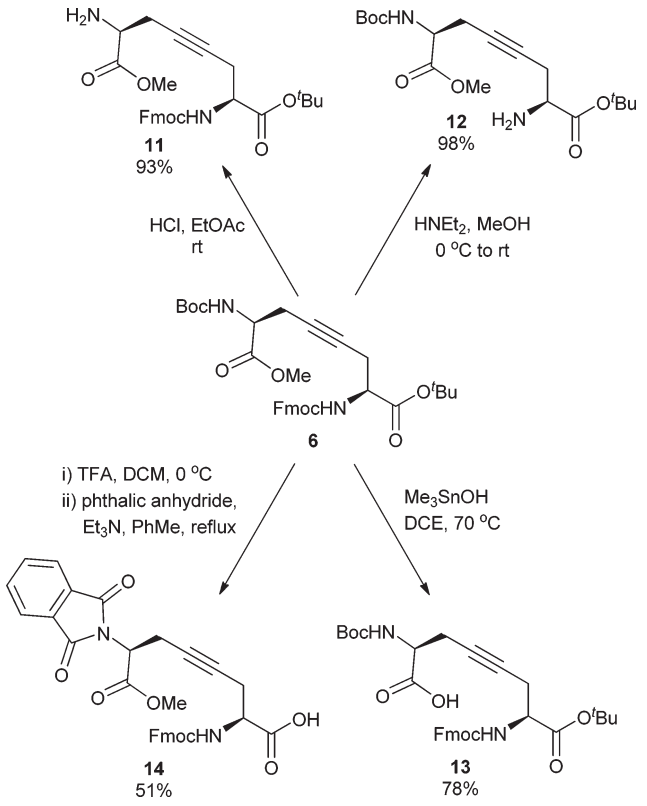
SCHEME 1. Synthesis of Mimetic 6



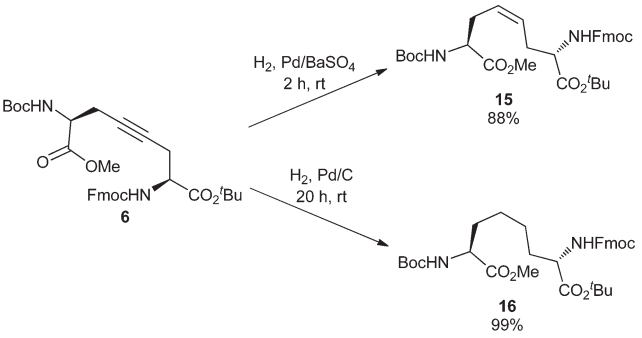
haloalkyne chemistry pioneered by Yeh and Knochel would provide an efficient route (Scheme 1).[7] Bromoacetylene **7** was prepared in two steps from protected propargyl glycine **8**[8] and serine iodide **9** was synthesized from the commercially available, protected serine **10**.[9] The key step, employing Zn/I_2 followed by CuCN with LiCl in DMF, provided mimetic **6** in 63% yield. Altering the copper source to CuBr·DMS (32% yield[10]) or changing the solvent to THF (< 10% yield) resulted in less efficient reactions.

SCHEME 2. Selective Deprotection of Mimetic 6



To demonstrate the full orthogonality of the chosen protecting groups, selective deprotection of **6** was investigated. Pleasingly, four different monodeprotected analogues can be easily accessed under standard conditions (Scheme 2). Cleavage of the two amine protecting groups proceeded smoothly using hydrochloric acid to remove the Boc group and diethylamine to remove the Fmoc moiety. Amines **11** and **12** were isolated in 93% and 98% yield respectively. Deprotection of the methyl ester to deliver carboxylic acid **13** initially proved challenging; treatment of **6** with LiOH resulted in partial cleavage of the Fmoc group and an unsatisfactory yield of the desired product. However, employing the conditions reported by Nicolaou and co-workers[11] resulted in a smooth transformation; subjecting **6** to Me_3SnOH at 70 °C allowed **13** to be cleanly isolated in 78% yield. Finally, carboxylic acid **14** was synthesized in two steps from **6** by using a double deprotection−reprotection strategy.

SCHEME 3. Partial and Full Reduction of Disulfide Bridge Analogue 6

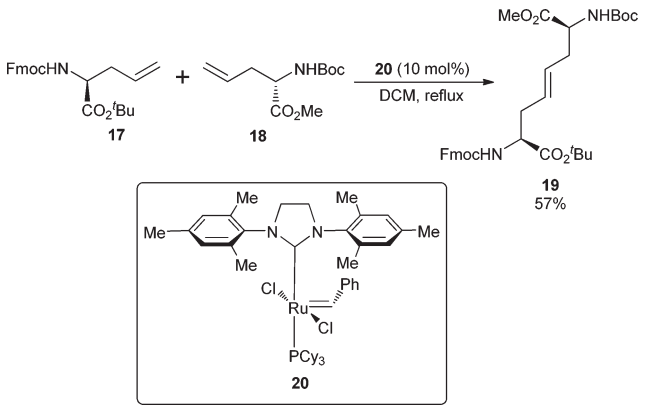


With isostere **6** in hand, it was predicted that partial and full hydrogenation of the alkyne would provide analogues **3** and **5**, respectively. Initial conditions to construct linker **15** by using Lindlar's catalyst gave poor results but, fortunately, switching the catalyst to $Pd/BaSO_4$ (Rosenmund's catalyst)[12] delivered an 88% yield of the unsaturated mimetic. Similarly, the fully saturated isostere **16** was synthesized in quantitative yield employing a Pd/C catalyst under a hydrogen atmosphere (Scheme 3).

To complete the set of disulfide bridge analogues, we also required a route to orthogonally protected isostere **4**, containing an (*E*)-alkene. As the selective reduction of functionalized alkynes to (*E*)-alkenes is still a synthetic challenge,[13] another approach was adopted. We predicted a ruthenium-catalyzed cross-metathesis reaction would provide access to the final mimetic from two differently protected allyl glycine units (Scheme 4, **17** + **18** → **19**).[14,15] Standard literature

(7) Yeh, M. C. P.; Knochel, P. *Tetrahedron Lett.* **1989**, *30*, 4799.
(8) Alkyne bromination adapted from: Hofmeister, H.; Annen, K.; Laurent, H.; Wiechert, R. *Angew. Chem., Int. Ed.* **1984**, *23*, 727.
(9) Huber, T.; Manzenrieder, F.; Kuttruff, C. A.; Dorner-Ciossek, C.; Kessler, H. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 4427.
(10) Procedure adapted from: Rodríguez, A.; Miller, D. D.; Jackson, R. F. W. *Org. Biomol. Chem.* **2003**, *1*, 973.
(11) Nicolaou, K. C.; Estrada, A. A.; Zak, M.; Lee, S. H.; Safina, B. S. *Angew. Chem., Int. Ed.* **2005**, *44*, 1378.
(12) Procedure adapted from: Hunt, T. A.; Parsons, A. F.; Pratt, R. *J. Org. Chem.* **2006**, *71*, 3656.
(13) Trost, B. M.; Zachary, B. T.; Jöge, T. *J. Am. Chem. Soc.* **2002**, *124*, 7922 and references cited therein.
(14) Key olefin metathesis articles/reviews (selected examples): (a) Chatterjee, A. K.; Choi, T.-L.; Sanders, D. P.; Grubbs, R. H. *J. Am. Chem. Soc.* **2003**, *125*, 11360. (b) Grubbs, R. H. *Angew. Chem., Int. Ed.* **2006**, *45*, 3760. (c) Hoveyda, A. H.; Zhugralin, A. R. *Nature* **2007**, *450*, 243. (d) Morris, T.; Sandham, D.; Caddick, S. *Org. Biomol. Chem.* **2007**, *5*, 1025. (e) Brik, A. *Adv. Synth. Catal.* **2008**, *350*, 1661.
(15) Examples of disulfide bridge mimetic formation by ruthenium-catalyzed ring closing metathesis (for synthesis of disulfide bridge mimetics using cross-metathesis see refs 5e, 5j, 5k, and 14d): (a) Miller, S. J.; Blackwell, H. E.; Grubbs, R. H. *J. Am. Chem. Soc.* **1996**, *118*, 9606. (b) Creighton, C. J.; Reitz, A. B. *Org. Lett.* **2001**, *3*, 893. (c) Stymiest, J. L.; Mitchell, B. F.; Wong, S.; Vederas, J. C. *Org. Lett.* **2003**, *5*, 47. (d) Ghalit, N.; Rijkers, D. T. S.; Kemmink, J.; Versluis, C.; Liskamp, R. M. J. *Chem. Commun.* **2005**, 192. (e) Derksen, D. J.; Stymiest, J. L.; Vederas, J. C. *J. Am. Chem. Soc.* **2006**, *128*, 14252. (f) Addona, D. D.; Carotenuto, A.; Novellino, E.; Piccand, V.; Reubi, J. C.; Cianni, A. D.; Gori, F.; Papini, A. M.; Ginanneschi, M. *J. Med. Chem.* **2008**, *51*, 512.

SCHEME 4. Synthesis of Mimetic 19



conditions were tested (10 mol % **20**, DCM, reflux), which gave a 57% yield of analogue **19**. Switching from thermal heating to microwave irradiation (36% yield[16]) or altering the catalyst loading (5 mol % **20**, 46% yield) was not advantageous.

In summary, we have developed efficient routes to four enantiopure, orthogonally protected disulfide bridge mimetics employing a copper-mediated organozinc/haloalkyne coupling and a ruthenium-catalyzed cross-metathesis reaction as key steps. This chemistry now enables these central peptidomimetic building blocks to be easily synthesized, selectively deprotected, and incorporated into peptides.

Experimental Section

Procedure for the Synthesis of Mimetic 6. Zinc dust (331 mg, 5.05 mmol) was weighed into a round-bottomed flask. Iodine (12.8 mg, 0.05 mmol) was added and the flask was heated with a heat gun, under vacuum, for 10 min and then flushed with argon. The flask was evacuated and flushed with argon a further 3 times and then cooled to 0 °C. Compound **9** (500 mg, 1.52 mmol) was dissolved in anhydrous DMF (1.5 mL) and added dropwise, via syringe, to the activated zinc at 0 °C. The reaction mixture was then allowed to warm to room temperature and stirred for 90 min to give the corresponding organozinc intermediate (TLC analysis was used to confirm the complete consumption of the starting material). In a separate flask, CuCN (118 mg, 1.32 mmol) and LiCl (112 mg, 2.63 mmol) were heated to 150 °C for 2 h under argon and then cooled to room temperature. DMF (2.2 mL) was added and the solution was stirred for 5 min to form the soluble CuCN·2LiCl complex. The copper complex was then cooled to −15 °C. Once the zinc insertion process was judged to have reached completion, stirring was ceased to allow the zinc powder to settle to the bottom of the flask. The supernatant was removed via syringe under argon (with care being taken to avoid the transfer of zinc) and added dropwise to the copper complex at −15 °C. Compound **7** (476 mg, 1.01 mmol) was then dissolved in DMF (1.5 mL) and also added dropwise to the copper complex at −15 °C. The cooling bath was removed and the reaction mixture was stirred at room temperature for 16 h under argon. Water (ca. 30 mL) was added and the suspension was extracted with diethyl ether (3 × 100 mL), washed with brine (ca. 60 mL), dried over anhydrous sodium sulfate, and concentrated in vacuo. The crude product was purified via flash column chromatography (5:1 hexane:ethyl acetate) to yield *alkyne* **6** (376 mg, 63%) as an amorphous white solid. ν_{max} (KBr)/cm^{-1} 3376, 3358, 3064, 3038, 2977, 2934, 1746, 1737, 1704, 1516, 1439, 1389, 1362, 1219, 1168, 1058, 1016, 845, 759, 743; δ_H (300 MHz; $CDCl_3$) 7.82−7.65 (4H, m), 7.45−7.26 (4H, m), 6.23 (1H, d, J=8.5 Hz), 6.03 (1H, d, J=8.7 Hz), 4.65−4.15 (5 H, m), 3.71 (3H, s), 2.78−2.52 (4H, m), 1.52 (9H, s), 1.46 (9H, s); δ_C (75 MHz; $CDCl_3$) 172.2, 170.4, 156.1, 155.5, 144.1, 141.5, 127.9, 127.4, 127.3, 125.6, 125.5, 120.2, 120.1, 82.9, 80.1, 78.5, 78.2, 67.3, 53.01, 52.95, 52.3, 47.4, 28.6, 28.3, 24.2, 24.1; m/z LRMS (EI) 592 (M^+, 20), 492 (25), 391 (50), 269 (45), 241 (55), 178 (100). Anal. Calcd for $C_{33}H_{40}N_2O_8$: C, 66.87; H, 6.80; N, 4.73. Found: C, 66.76; H, 6.78; N, 4.70.

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Supporting Information Available: Detailed experimental procedures and characterization data for new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

(16) Reaction conditions: **17** (1.0 equiv, 0.42 mmol), **18** (1.1 equiv, 0.46 mmol), **20** (10 mol %), DCM (4 mL), 100 °C (microwave irradiation), 10 min.