A Highly Chemoselective and Practical Alkynylation of Thiols

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Supporting Information Placeholder

ABSTRACT: A thiol-alkynylation procedure utilizing the hypervalent iodine alkyne transfer reagent TIPS-ethynyl-benziodoxolone (TIPS-EBX) has been developed. This scalable reaction proceeds in five minutes at room temperature in an open flask using commercially available reagents. The scope of the reaction is broad, with a variety of phenolic-, benzylic-, heterocyclic- and aliphatic thiols undergoing alkynylation in excellent yield. The method is highly chemoselective as a vast array of functional groups are tolerated. The utility of the thiol-alkynylation in post-synthetic elaboration has been demonstrated through the facile installment of a fluorophore tag on a cysteine containing peptide.

The development and implementation of highly modular reactions that provide near quantitative yields over a wide substrate range with perfect chemoselectivity had a transformational effect in areas extending from material and polymer science, to chemical biology, drug discovery and small molecule organic chemistry. These ideal reactions should also be experimentally simple to perform, utilize readily available starting materials, be insensitive to water and oxygen and should not require the use of expensive, sensitive or toxic metal catalysts. Driven by the exceptional reactivity of sulfur and its importance in biology, medicine and materials science,[1] recent research efforts targeted a series of thiol-based transformations including thiol alkylations, as well as the thiol-ene and thiol-yne reaction among others (Scheme 1).[2]

**Scheme 1. Thiol functionalization for the modification of drugs, biomolecules and materials.**
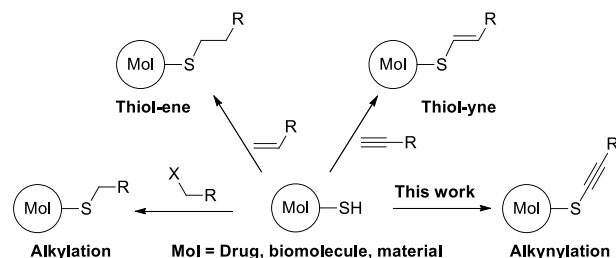
Unlike the well-established S-Csp[3] and S-Csp[2] bond forming processes, existing methods to construct S-Csp bonds are rare in number and often lack generality or require harsh conditions. Currently, the most common methods to form thio-alkynes require a pre-functionalization of the thiol (Scheme 2, **A**). These methods are generally based on nucleophilic substitutions between highly reactive lithium acetylide intermediates with pre-activated thiols or disulfide species.[3] Other methods utilize transition-metal cata-

lysts, such as the copper-catalyzed carbon sulfur coupling between terminal alkynes and disulfides,[4] or as in the elegant study by Yamaguchi, the use of catalytic rhodium to achieve C-S bond formation by C-H and S-S bond metathesis.[5] Alternatively, a range of processes utilize alkenyl[6] or alkynyl[7] halides bearing leaving groups that undergo elimination under strongly basic conditions to furnish the desired thio-alkyne (Scheme 2, **B**). Consequently, the limited functional group tolerance exhibited by these methods is not surprising as they require harsh conditions, proceed *via* highly reactive intermediates, or involve the use of sensitive catalytic systems.

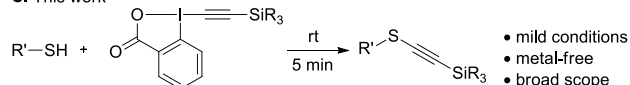**Scheme 2. Previously reported methods for the synthesis of thio-alkynes (A, B) and our new approach (C).**
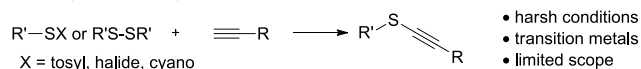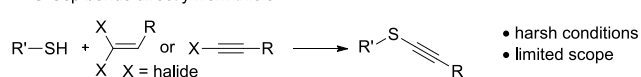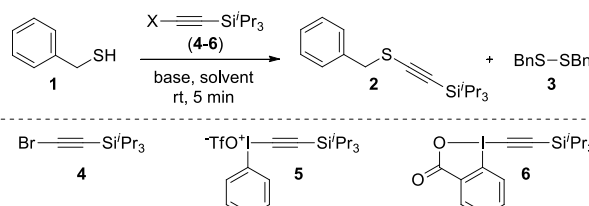

The absence of a broadly applicable thiol-alkynylation process in current literature is further highlighted by the non-existence of cysteine analogues derivatized with a terminal thio-alkyne at the side chain.[8] This motif would generate great interest as it could serve as a unique point of inception to, for instance, study post-translational modifications, install fluorophore or biotin tags for biotechnological purposes and broadly enable bioconjugation.[2a-b] In general, thio-alkynes represent an ideal platform for drug-diversification in light of the versatility of existing acetylene chemistry and, in particular, the extensively used copper-catalyzed azide-alkyne cycloaddition (CuAAC),[9] as well as the privileged position of organosulfur compounds among top-selling pharmaceutical drugs.[1a] Furthermore, the development of a robust, efficient and orthogonal thiol-alkynylation reaction is particularly attractive to the field of material and polymer science as an alternative tool to the current array of thiol-functionalization reactions.[2] In this context, we set out to investigate the feasibility of more facile and experimentally practical methods to gain access to thio-alkynes, with a particular focus on silyl acetylenes as a direct gateway to the most versatile terminal alkyne (Scheme 2, **C**).

Given the successful exploitation of hypervalent iodine alkyne transfer reagents by our group and others,[10] we speculated that

theses electrophilic acetylide equivalents could also be applied to the alkynylation of thiols. Unlike methods based on nucleophilic acetylide reagents, electrophilic alkynylation does not require pre-functionalization of the thiol with an activating group. We envisaged that such an *Umpolung* strategy would have the potential to proceed efficiently under mild conditions. Indeed, the mechanism of reactions involving the addition of nucleophiles to alkynyliodonium species is known to proceed *via* a fast succession of conjugate addition, α-elimination of the aryliodide followed by a 1,2-shift,[11] no long-living anionic or radical intermediates are formed, which in turn could lead to a broad functional group tolerance. However, such a thiol-alkynylation approach has, to the best of our knowledge, only been reported for the reaction between bis-alkynyl iodonium salts and a phenyl thiolate anion to give bis-thioalkynes.[12] The absence of additional reports could perhaps be attributed to the oxidative properties of hypervalent iodine reagents, which may lead to the facile oxidation of thiols, resulting in undesired disulfides.

We commenced our study with benzylthiol (**1**) as our model substrate while screening different alkynylation reagents under mildly basic conditions (Table 1). Less reactive halogenoacetylenes, such as triisopropylsilyl ethynyl bromide (**4**), did not undergo any reaction under these conditions (entry 1). The more reactive alkynyliodonium salt **5** furnished the desired thio-alkynylation (**2**), albeit as minor product (entry 2). Our initial reservations were confirmed as disulfide (**3**), resulting from oxidation, was identified as major product. However, to our delight, we discovered that benziodoxolone-derived hypervalent iodine reagent 1-[(tri*iso*propylsilyl)ethynyl]-1,2-benziodoxol-3(1*H*)-one (TIPS-EBX, **6**) furnished **2** as the main product (entry 3). This commercially available reagent is highly practical as it is a bench stable solid allowing for simple handling and long-term storage on the multi-gram scale.[13] While further optimizing our lead result, we discovered that the right choice of solvent and base was crucial to minimizing formation of the undesired disulfide product. We found that 1,1,3,3-tetramethylguanidine (TMG) was superior

to triethylamine and aqueous cesium carbonate or sodium hydroxide as base (entries 3-6). Similarly, we discovered that dimethyl sulfoxide (DMSO), or protic solvents such as methanol and ethanol, produced unsatisfactory levels of the oxidation side reaction while tetrahydrofuran (THF) was optimal (entries 6-9).[14]
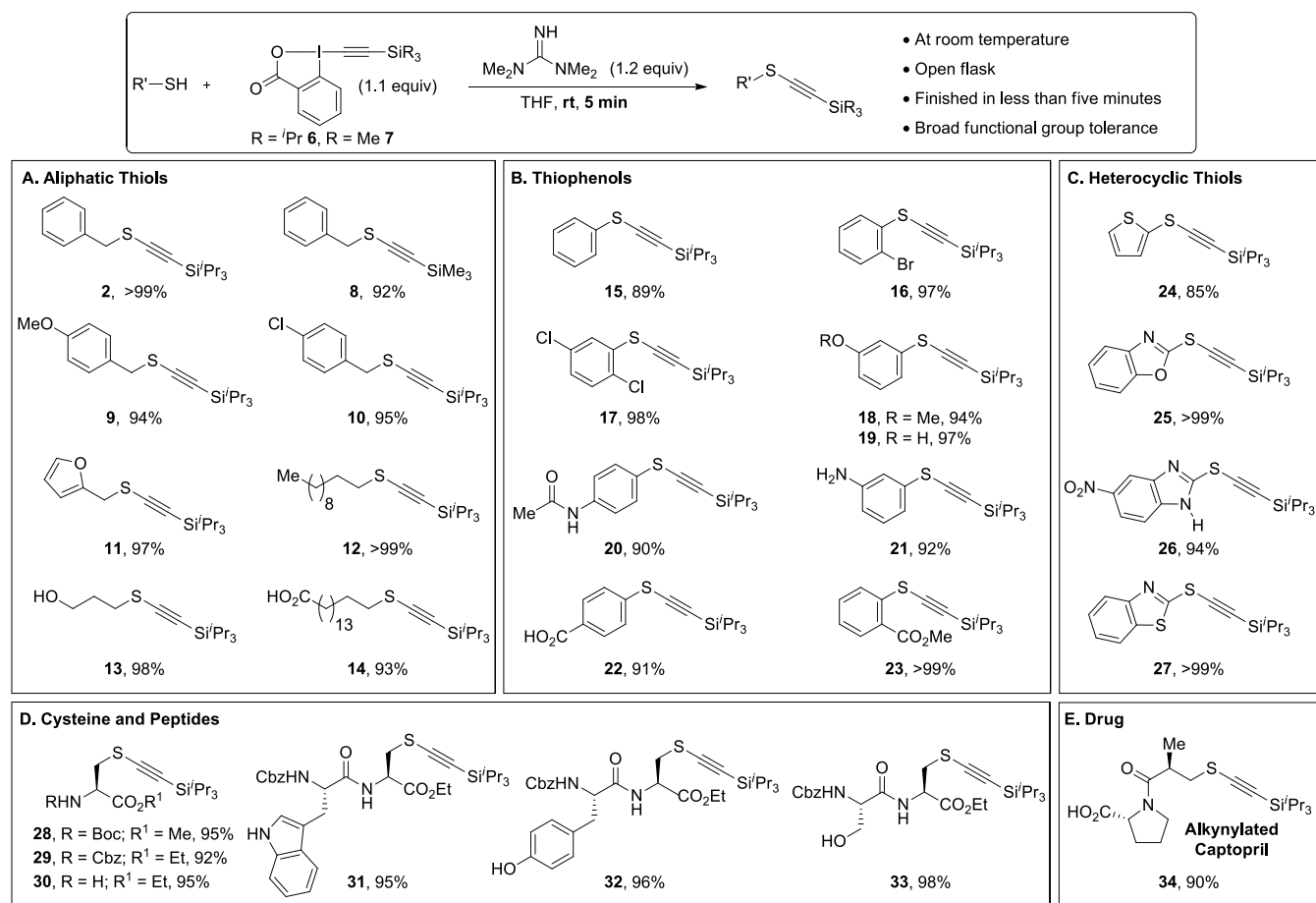
Under the optimized conditions, TIPS-EBX (**6**, 1.1 equiv) was added in one portion to a solution consisting of the corresponding thiol (1.0 equiv), TMG (1.2 equiv) and THF at room temperature and stirred for five minutes in an open flask to give the alkynylation product **2** in quantitative yield (Scheme 3).[15] The reaction was not affected by the addition of water, which was required in some cases for thiol-substrates producing THF insoluble salts with the guanidine base.[16] If desired, the formed 2-iodobenzoic acid co-product could also be recovered quantitatively and re-used to synthesize TIPS-EBX (**6**).[17] The transfer of trimethylsilyl ethyne utilizing benziodoxolone-derivative **7** was also successful and alkynylation product **8** was obtained in 92% yield (Scheme 3, **A**). Consequently, silyl-protected terminal alkynes with varying stability can be accessed. Due to its commercial availability, TIPS-EBX (**6**) was chosen to investigate the scope and limitations of this newly developed thiol-alkynylation reaction (Scheme 3).

Modulating the electronics of the benzylthiol did not change the reaction outcome as both **9** and **10**, bearing an electron-donating methoxy and an electron-withdrawing chloride group respectively, were obtained in excellent yields (Scheme 3, **A**). A furan heterocycle was also well tolerated to give product **11** in 97% yield. Furthermore, a highly hydrophobic aliphatic substrate was alkynylated in quantitative yield (product **12**). In addition, aliphatic thiols bearing a hydroxy or carboxylic acid functionality underwent the thio-alkynylation reaction with comparable results (products **13** and **14**), demonstrating that the method was tolerant to nucleophilic oxygen and acidic hydrogens.

Due to the significance of aromatic thiols as important building blocks in the synthesis of natural products, pharmaceutical and medicinal compounds,[18] a range of functionalized thiophenols were then subjected to the optimized conditions (Scheme 3, **B**). The successful formation of products **15**-**23** in 89-99% yield indicated that substituents such as halides (products **16** and **17**), methoxy and hydroxy groups (products **18** and **19**), protected and unprotected amines (products **20** and **21**), as well as carboxylic acids and esters (products **22** and **23**) were well tolerated. This broad functional group tolerance is unprecedented in the field of thiol-alkynylation. Shifting our focus to leading scaffolds of heterocyclic chemistry (Scheme 3, **C**), thiol-substituted thiophene (product **24**), benzoxazole (product **25**), benzimidazole (product **26**) and benzothiazole (product **27**) gave the desired thiol-alkynylation products in 85-99% yields. It is noteworthy that these heterocycles are omnipresent in medicinally relevant compounds, making their facile alkynylation a valuable tool to establish a platform for further diversification.[19] Furthermore, polymers with incorporated thiophene units play a crucial role in organic electronic materials, making alkynylation product **24** a useful building block.[20]

As previously stated, a cysteine side chain derivatized with a terminal thio-alkyne has not yet been reported.[8] At the outset, it was not clear if this was due to insufficient stability or the lack of methods to access such compounds. Thus, the mild thiol-alkynylation conditions permitted by TIPS-EBX (**6**) appeared very promising for the functionalization of such challenging and highly important substrates. In this context, *N*- and *C*-protected cysteine derivatives were examined as starting materials (Scheme 3, **D**). We were able to isolate the corresponding alkynylated products **28** and **29** in 95% and 92% yield, respectively. Moreover, the reaction is scalable as **28** could also be obtained in quantitative yield on the gram scale.[16] Selective alkynylation of the thiol group in the presence of a free amine also proceeded efficiently to

Table 1. Optimization of the Alkynylation Reaction[a]

entry	4-6	base	solvent	yield **2**[b]	yield **3**[b]
1	**4**	NEt₃	THF	no reaction	
2	**5**	NEt₃	THF	<10%	93%
3	**6**	NEt₃	THF	51%	46%
4	**6**	NaOH	THF	74%	24%
5	**6**	Cs₂CO₃	THF	71%	26%
6	**6**	TMG	THF	>99%	-
7	**6**	TMG	DMSO	81%	15%
8	**6**	TMG	MeOH	12%	85%
9	**6**	TMG	EtOH	80%	17%

[a] Benzylthiol (**1**, 0.40 mmol), alkyne transfer reagent (**4-6**, 0.44 mmol), base (0.48 mmol), solvent (5.0 mL), 23 °C, 5 min, open flask. [b] Isolated yield of spectroscopically pure product. TMG = 1,1,3,3-tetramethylguanidine.

Scheme 3. Scope of the thiol-alkynylation reaction.

A. Aliphatic Thiols

2, >99%

8, 92%

9, 94%

10, 95%

11, 97%

12, >99%

13, 98%

14, 93%

B. Thiophenols

15, 89%

16, 97%

17, 98%

18, R = Me, 94%
19, R = H, 97%

20, 90%

21, 92%

22, 91%

23, >99%

C. Heterocyclic Thiols

24, 85%

25, >99%

26, 94%

27, >99%

D. Cysteine and Peptides

28, R = Boc; R^1 = Me, 95%
29, R = Cbz; R^1 = Et, 92%
30, R = H; R^1 = Et, 95%

31, 95%

32, 96%

33, 98%

E. Drug

Alkynylated Captopril

34, 90%

give alkynylation product **30**, demonstrating that protection of the basic aliphatic free *N*-terminus of peptides is optional.

A subset of cysteine containing dipeptides was also alkynylated successfully, furnishing alkynylation products **31**, **32** and **33** in 95-98% yield. These results demonstrated that alkynylation of cysteine was possible in the presence of tryptophan, tyrosine and serine, respectively. Finally, amino acid-derived marketed drug captopril, an important angiotensin-converting enzyme inhibitor,[21] was successfully converted to the corresponding thio-alkyne **34** (Scheme 3, **E**). The reaction proceeded in 90% yield in the presence of the free carboxylic acid. This example demonstrated the potential utility of this new thiol-alkynylation reaction as a facile entry point to carry out drug diversification studies.

A final competition experiment was carried out to assess the extent of selectivity exhibited by TIPS-EBX (**6**) toward thiols (Eq. 1). For this purpose, unprotected L-histidine and L-lysine (amino acids bearing two of the most nucleophilic substituents present in biomolecules) along with dipeptide **35** were subjected to the optimized reaction conditions. The desired thiol-alkynylation product **31** was still obtained in 97% yield, showcasing the exceptional chemoselectivity of the developed reaction. It is also noteworthy that during the reaction scope exploration, we discovered that the thiol-alkynylation essentially takes place within 30 seconds upon
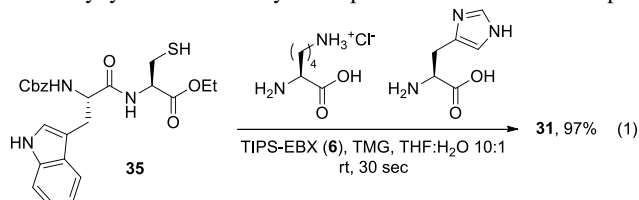
addition of TIPS-EBX (**6**), which was also the case for the competition experiment (Eq. 1).

To highlight the utility of the developed thiol-alkynylation process in post-synthetic elaboration of modified peptides, alkynylation product **31** was de-silylated using TBAF buffered with acetic acid, furnishing the corresponding terminal alkyne in 91% yield. We found that the free cysteine-acetylene derivative was stable as demonstrated by the complete lack of decomposition observed after stirring a solution of this compound in the presence of a pH = 7 buffer for several weeks at room temperature. Furthermore, the introduction of a dansyl fluorophore was accomplished *via* a standard CuAAC reaction to give conjugate **36** in 93% yield.[22] To the best of our knowledge, this is the first example of a terminal thio-alkyne participating in the powerful CuAAC process.[23]

1. TBAF, AcOH
   THF, rt, 10 h, 91%
2. Dans-(CH$_2$)$_3$-N$_3$
   CuSO$_4$·5H$_2$O
   Na ascorbate
   CHCl$_3$, H$_2$O
   rt, 24 h, 93%

36

(2)
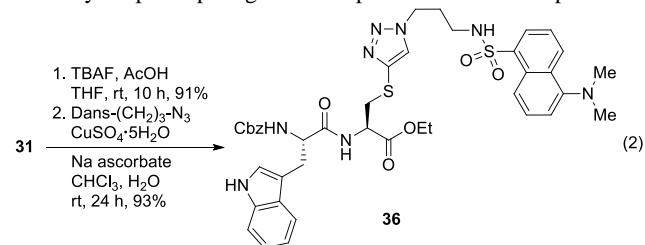
In conclusion, we have developed an operationally practical and highly efficient thio-alkynylation reaction utilizing the electrophilic alkyne transfer reagent TIPS-EBX (**6**). The mild reaction conditions and high chemoselectivity allow for the alkynylation of phenolic-, benzylic-, heterocyclic- aliphatic- and peptidic-thiols while tolerating a vast array of functional groups. Moreover, the potential utility for post-synthetic elaboration of the obtained alkynylated products was demonstrated through the first report of

35

TIPS-EBX (**6**), TMG, THF:H$_2$O 10:1
rt, 30 sec

31, 97% (1)

a CuAAC involving a terminal thio-alkyne. The developed method not only allows a highly efficient functionalization of thiols, but also provides access to unique alkynes with the potential for further derivatization. Consequently, the thiol-alkynylation presented herein has the potential to achieve a privileged position as a novel tool for various applications in synthetic chemistry, chemical biology and material science. Ongoing research efforts are aimed at investigating the applicability of other electrophilic alkyne transfer reagents and determining the precise mechanism of this thio-alkynylation process. Further studies to uncover unprecedented applications of terminal and silyl protected thio-alkynes are also progressing.

ASSOCIATED CONTENT

Supporting Information

Experimental procedures and analytical data for all new compounds. 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 interests.

REFERENCES

(1) (a) Zambrowicz, B. P.; Sands, A. T. *Nat. Rev. Drug. Discov.* **2003**, *2*, 38. (b) Paquette, L. A. *Sulfur-Containing Reagents*; Wiley: Chichester, 2009. (c) Masella, R. *Glutathione and Sulfur Amino Acids in Human Health and Disease*; Wiley: Hoboken, 2009. (d) Hoyle, C. E.; Lowe, A. B.; Bowman, C. N. *Chem. Soc. Rev.* **2010**, *39*, 1355.

(2) (a) Lundblad, R. L. *Chemical reagents for protein modification*; 3rd ed.; CRC Press: Boca Raton, 2005. (b) Hermanson, G. T. *Bioconjugate techniques*; 2nd edn. Academic Press: San Diego, 2008. (c) Hoyle, C. E.; Bowman, C. N. *Angew. Chem., Int. Ed.* **2010**, *49*, 1540. (d) Hoogenboom, R. *Angew. Chem., Int. Ed.* **2010**, *49*, 3415. (e) Lowe, A. B.; Hoyle, C. E.; Bowman, C. N. *J. Mater. Chem.* **2010**, *20*, 4745. (f) Stephanopoulos, N.; Francis, M. B. *Nat. Chem. Biol.* **2011**, *7*, 876. (g) Stenzel, M. H. *ACS Macro Lett.* **2012**, *2*, 14.

(3) Selected recent examples: (a) Godoi, B.; Speranca, A.; Back, D. F.; Brandao, R.; Nogueira, C. W.; Zeni, G. *J. Org. Chem.* **2009**, *74*, 3469. (b) Chandanshive, J. Z.; Bonini, B. F.; Gentili, D.; Fochi, M.; Bernardi, L.; Franchini, M. C. *Eur. J. Org. Chem.* **2010**, 6440.

(4) For selected examples: (a) Braga, A. L.; Reckziegel, A.; Menezes, P. H.; Stefani, H. A. *Tetrahedron Lett.* **1993**, *34*, 393. (b) Bieber, L. W.; da Silva, M. F.; Menezes, P. H. *Tetrahedron Lett.* **2004**, *45*, 2735.

(5) Arisawa, M.; Fujimoto, K.; Morinaka, S.; Yamaguchi, M. *J. Am. Chem. Soc.* **2005**, *127*, 12226.

(6) Selected examples: (a) Marchueta, I.; Montenegro, E.; Panov, D.; Poch, M.; Verdaguer, X.; Moyano, A.; Pericas, M. A.; Riera, A. *J. Org. Chem.* **2001**, *66*, 6400. (b) Ni, Z.; Wang, S.; Mao, H.; Pan, Y. *Tetrahedron Lett.* **2012**, *53*, 3907.

(7) Ziegler, G. R.; Welch, C. A.; Orzech, C. E.; Kikkawa, S.; Miller, S. I. *J. Am. Chem. Soc.* **1963**, *85*, 1648.

(8) Only two examples of alkyl substituted thio-alkyne cysteine analogs have been reported: (a) Carson, J. F.; Boggs, L. *J. Org. Chem.* **1965**, *30*, 895. (b) Kuligowski, C.; Bezzenine-Lafollee, S.; Chaume, G.; Mahuteau, J.; Barriere, J. C.; Bacque, E.; Pancrazi, A.; Ardisson, J. *J. Org. Chem.* **2002**, *67*, 4565.

(9) (a) Stang, P. J.; Diederich, F. *Modern acetylene chemistry*; VCH: Weinheim ; New York, 1995. (b) Diederich, F.; Stang, P. J.; Tykwinski, R. R. *Acetylene chemistry: chemistry, biology, and material science*; Wiley-VCH: Weinheim ; Great Britain, 2005. (c) Finn, M. G.; Fokin, V. V. *Chem. Soc. Rev.* **2010**, *39*, 1231.

(10) (a) Stang, P. J.; Boehshar, M.; Lin, J. *J. Am. Chem. Soc.* **1986**, *108*, 7832. (b) Ochiai, M.; Ito, T.; Takaoka, Y.; Masaki, Y.; Kunishima, M.; Tani, S.; Nagao, Y. *J. Chem. Soc. Chem. Comm.* **1990**, 118. (c) Brand, J. P.; Charpentier, J.; Waser, J. *Angew. Chem., Int. Ed.* **2009**, *48*, 9346. (d) Gonzalez, D. F.; Brand, J. P.; Waser, J. *Chem. Eur. J.* **2010**, *16*, 9457. (e) Nicolai, S.; Piemontesi, C.; Waser, J. *Angew. Chem., Int. Ed.* **2011**, *50*, 4680. (f) Shi, H.; Fang, L. C.; Tan, C. H.; Shi, L. L.; Zhang, W. B.; Li, C. C.; Luo, T. P.; Yang, Z. *J. Am. Chem. Soc.* **2011**, *133*, 14944; (g) T. Aubineau, J. Cossy, *Chem. Commun.* **2013**, *49*, 3303. Reviews: (h) Zhdankin, V. V.; Stang, P. J. *Tetrahedron* **1998**, *54*, 10927. (i) Brand, J. P.; Waser, J. *Chem. Soc. Rev.* **2012**, *41*, 4165.

(11) (a) Ochiai, M.; Kunishima, M.; Nagao, Y.; Fuji, K.; Shiro, M.; Fujita, E. *J. Am. Chem. Soc.* **1986**, *108*, 8281. A 1,2-sulfur shift has been proposed in the one-pot synthesis of thiazoles starting from alkynyliodonium salts: (b) Miyamoto, K.; Nishi, Y.; Ochiai, M. *Angew. Chem., Int. Ed.* **2005**, *44*, 6896.

(12) (a) Stang, P. J.; Zhdankin, V. V. *J. Am. Chem. Soc.* **1990**, *112*, 6437. (b) Stang, P. J.; Zhdankin, V. V. *J. Am. Chem. Soc.* **1991**, *113*, 4571.

(13) Commercially available TIPS-EBX (**6**) is routinely synthesized in our laboratory in 30-50 g batches. (a) Zhdankin, V. V.; Kuehl, C. J.; Krasutsky, A. P.; Bolz, J. T.; Simonsen, A. J. *J. Org. Chem.* **1996**, *61*, 6547. (b) Brand, J. P.; Waser, J. *Synthesis* **2012**, *44*, 1155.

(14) Under the optimized conditions, it was still not possible to suppress thiol oxidation when using alkynyliodonium salt **5**.

(15) The reaction yields the same outcome under anaerobic conditions using carefully degassed solvents. Please see the Supporting Information for more details.

(16) Please see the Supporting Information for more details.

(17) With the exception of the production of 2-iodobenzoic acid as co-product, we note that the developed thiol-alkynylation method meets all the standard of the extremely successful "click chemistry" approach: (a) Kolb, H. C.; Finn, M. G.; Sharpless, K. B. *Angew. Chem., Int. Ed.* **2001**, *40*, 2004. (b) Wang, Q.; Chan, T. R.; Hilgraf, R.; Fokin, V. V.; Sharpless, K. B.; Finn, M. G. *J. Am. Chem. Soc.* **2003**, *125*, 3192. (c) Tornoe, C. W.; Christensen, C.; Meldal, M. *J. Org. Chem.* **2002**, *67*, 3057. (d) Moses, J. E.; Moorhouse, A. D. *Chem. Soc. Rev.* **2007**, *36*, 1249.

(18) Metzner, P.; Thuillier, A. *Sulfur reagents in organic synthesis*; Academic Press: New York, 1994.

(19) *The chemistry of heterocyclic compounds*, Vol. 44, Wiley Interscience: New York, 1994.

(20) (a) Murphy, A. R.; Frechet, J. M. *Chem. Rev.* **2007**, *107*, 1066. (b) Allard, S.; Forster, M.; Souharce, B.; Thiem, H.; Scherf, U. *Angew. Chem. Int. Ed.* **2008**, *47*, 4070. (c) Mishra, A.; Ma, C. Q.; Bauerle, P. *Chem. Rev.* **2009**, *109*, 1141.

(21) Migdalof, B. H.; Antonaccio, M. J.; McKinstry, D. N.; Singhvi, S. M.; Lan, S. J.; Egli, P.; Kripalani, K. J. *Drug Metab. Rev.* **1984**, *15*, 841.

(22) Rostovtsev, V. V.; Green, L. G.; Fokin, V. V.; Sharpless, K. B. *Angew. Chem. Int. Ed.* **2002**, *41*, 2596.

(23) CuAAC reactions involving sulfur-substituted triple bonds have been reported only in the case of terminal sulfonyl-alkynes or pentafluorosulfanyl-alkynes. Selected examples: (a) Gonzalez-Vera, J. A.; Lukovic, E.; Imperiali, B. *J. Org. Chem.* **2009**, *74*, 7309. (b) Ye, C.; Gard, G. L.; Winter, R. W.; Syvret, R. G.; Twamley, B.; Shreeve, J. M. *Org. Lett.* **2007**, *9*, 3841.

Commercially Available

Mol—SH   +   O=⟨benziodoxolone⟩—I≡—Si^{i}Pr$_3$ →[Me$_2$N—C(=NH)—NMe$_2$ / THF, rt, 5 min]→   **Mol**—S—≡—Si^{i}Pr$_3$
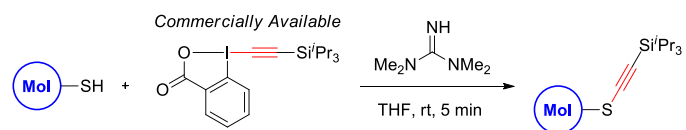
Mol = Aliphatic, Phenolic,
Heterocyclic, Peptidic

- At room temperature
- Open flask
- Finished in less than five minutes
- Broad functional group tolerance

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Supporting Information for

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Table of Contents

List of Abbreviations

AcOH	Acetic acid
Boc	*tert.*-Butyl carbonate
Cbz	Carboxybenzyl
CuAAC	Copper-catalyzed azide-alkyne cycloaddition
DCC	*N,N'*-Dicyclohexylcarbodiimide
DIPEA	*N,N*-Diisopropylethylamine
EDC	1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide
EtOAc	Ethyl acetate
HOBt	1-Hydroxybenzotriazole
NBS	*N*-Bromosuccinimide
ND	Not determined
NMM	*N*-Methylmorpholine
TBAF	Tetra-*n*-butylammonium fluoride
THF	Tetrahydrofuran
TIPS	Triisopropylsilyl
TIPS-EBX	1-[(Triisopropylsilyl)ethynyl]-1,2-benziodoxol-3(1*H*)-one
TLC	Thin layer chromatography
TMG	1,1,3,3-Tetramethylguanidine
TMS	Trimethylsilyl
Ultra	CH_2Cl_2:MeOH 3:1 (v/v) with 5% NH_3 (aq., 25%)

General Methods

Technical grade solvents were used for quantitative flash chromatography. HPLC grade solvents purchased from Sigma-Aldrich or freshly distilled solvents were used for flash chromatography for compounds undergoing full characterization. Reaction solvents were dried by passage over activated alumina under nitrogen atmosphere (H_2O content < 30 ppm, Karl-Fischer titration). We note; however, that the thiol-alkynylation reaction gives identical results when using HPLC grade THF purchased from Sigma-Aldrich or dried THF from the solvent system. Commercially available reagents were purchased from Acros, Aldrich, Fluka, VWR, Aplichem or Merck and used without any further purification. Chromatographic purification was performed as flash chromatography using Macherey-Nagel silica 40-63, 60 Å, using the solvents indicated as eluent with 0.1-0.5 bar pressure. TLC was performed on Merck silica gel 60 F254 TLC plates and visualized with UV light and permanganate stain. Melting points were measured on a calibrated Büchi B-540 melting point apparatus using open glass capillaries. ^{1}H NMR spectra were measured on a Brucker DPX-400 400 MHz spectrometer, all signals are reported in ppm with the corresponding internal solvent peak or TMS as standard. The data is being reported as (s = singlet, d = doublet, t = triplet, q = quadruplet, qi = quintet, m = multiplet or unresolved, br = broad signal, coupling constant(s) in Hz, integration; interpretation). ^{13}C NMR spectra were carried out with 1H-decoupling on a Brucker DPX-400 100 MHz. All signals are reported in ppm with the corresponding internal solvent signal or TMS as standard. Infrared spectra were obtained on a JASCO FT-IR B4100 spectrophotometer with an ATR PRO410-S and a ZnSe prisma and are reported as cm^{-1} (w = weak, m = medium, s = strong, sh = shoulder). High resolution mass spectrometric measurements were performed by the mass spectrometry service of ISIC at the EPFL on a MICROMASS (ESI) Q-TOF Ultima API.
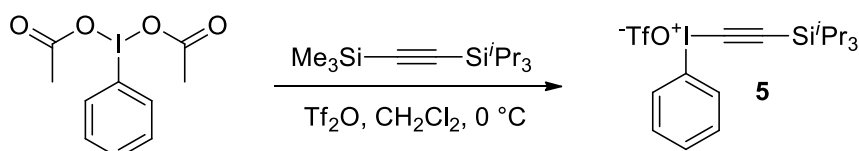
Preparation of Reagents

(Bromoethynyl)triisopropylsilane (4)

$$\equiv\!-\!Si^iPr_3 \xrightarrow[\text{Acetone, rt}]{\text{NBS, AgNO}_3} Br\!-\!\equiv\!-\!Si^iPr_3$$
4
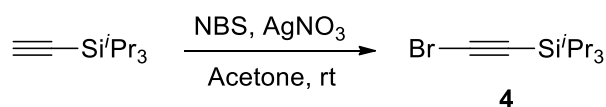
Following a reported procedure,[1] triisopropylsilylacetylene (813 mg, 4.45 mmol, 1.00 eq.) was dissolved in acetone (30 mL). To the clear colorless solution was added NBS (925 mg, 5.19 mmol, 1.16 eq.), followed by $AgNO_3$ (76 mg, 0.44 mmol, 0.1 eq.). The resulting mixture was stirred at room temperature for 3 h and then poured into ice water (30 mL). The aqueous layer was extracted with pentane (3 x 30 mL). The combined organic layers were dried over $MgSO_4$, filtered and concentrated *in vacuo*. The resulting oil was filtered twice through a short plug of silica gel using pentane as eluent affording pure (bromoethynyl)triisopropylsilane **4** (1.16 g, 4.43 mmol, 99%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.20-0.97 (m, 21 H, TIPS). ^{13}C NMR (100 MHz, $CDCl_3$) δ 83.5, 61.7, 18.5, 11.3. The values of the NMR spectra are in accordance with reported literature data.[1]

Phenyl(((triisopropylsilyl)ethynyl)iodonium trifluoromethanesulfonate (5)

$$\xrightarrow[\text{Tf}_2\text{O, CH}_2\text{Cl}_2, \, 0\,°C]{\text{Me}_3\text{Si}\!-\!\equiv\!-\!Si^iPr_3} \quad {}^-\text{TfO}^+\text{I}\!-\!\equiv\!-\!Si^iPr_3$$
5

Following a slightly modified reported procedure,[2] phenyliodonium diacetate (2.53 g, 7.85 mmol, 1.00 eq.) was diluted with CH_2Cl_2 (7 mL) and the mixture was stirred for 5 minutes. Next, triflic anhydride (0.600 mL, 3.90 mmol, 0.50 eq.) was added dropwise at 0 °C and the resulting yellow mixture was stirred for 30 min. To the mixture was added (trimethylsilyl)(triisopropylsilyl)acetylene (2.00 g, 7.86 mmol, 1.00 eq.) and stirring continued for 2 hours at 0 °C. Water was then added (30 mL) and the mixture was extracted with CH_2Cl_2 (2 x 30 mL). The combined organic layers were dried over $MgSO_4$, filtered and concentrated *in vacuo*. The resulting solid was triturated in hexane (10 mL). Filtration and removal of solvent *in vacuo* afforded the title compound **5** (2.90 g, 11.2 mmol, 70%) as a colorless solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.09 (m, 2 H, Ar*H*), 7.65 (m, 1 H, Ar*H*), 7.52 (m, 2 H, Ar*H*), 1.15-1.01 (m, 21 H, TIPS). ^{13}C NMR (100 MHz, $CDCl_3$) δ 133.7, 132.5, 132.4, 123.0 (J_{C-H} = 319 Hz), 117.6, 117.6, 44.9, 18.3, 11.1. IR ν 3288 (w), 3088 (m), 2949 (m), 2894 (m), 2869 (w), 1563 (m), 1467 (w), 1451 (w), 1388 (w), 1281 (s), 1236 (s), 1221 (s), 1174 (s), 1068 (w), 1028 (s), 988 (m), 916 (m), 884 (m), 736 (s), 679 (m), 639 (s). The characterization data is in accordance with reported literature values.[2]

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[1] Jiang, M. X. W.; Rawat, M.; Wulff, W. D. *J. Am. Chem. Soc.* **2004**, *126*, 5970.
[2] Brand, J. P.; Waser, J. *Angew. Chem. Int. Ed.* **2010**, *49*, 7304.

1-[(Trimethylsilyl)ethynyl]-1,2-benziodoxol-3(1*H*)-one (7)
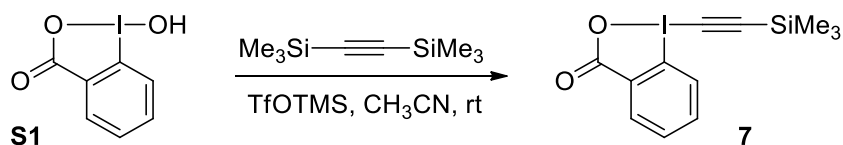
Following a reported procedure,[3] trimethylsilyl triflate (5.54 mL, 30.7 mmol, 1.10 eq.) was added to a suspension of **S1**[4] (7.36 g, 28.0 mmol, 1.00 eq.) in CH_2Cl_2 (85 mL) at room temperature. The resulting yellow mixture was stirred for 1 hour, followed by the dropwise addition of bis(trimethylsilyl)acetylene (6.98 mL, 30.7 mmol, 1.10 eq.). The resulting suspension was stirred for 6 hours at room temperature. A colorless solid formed, to which sat. aq. $NaHCO_3$ was added. The mixture was stirred vigorously until complete solubilization of the solid was achieved. The two layers were separated and the organic extract was washed with sat. aq. $NaHCO_3$, dried over $MgSO_4$, filtered and concentrated *in vacuo*. The resulting solid was purified by re-crystallization in acetonitrile (50 mL) affording title compound **7** (7.17 g, 20.8 mmol, 74%) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.42 (dd, 1 H, $J =$ 6.4, 1.9 Hz, Ar*H*), 8.19 (m, 1 H, Ar*H*), 7.78 (m, 2 H, Ar*H*), 0.32 (s, 9 H, TMS). ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.4, 134.9, 132.6, 131.7, 131.4, 126.1, 117.2, 115.4, 64.2, -0.5. IR ν 3389 (w), 2967 (w), 1617 (s), 1609 (s), 1562 (m), 1440 (w), 1350 (m), 1304 (w), 1254 (w), 1246 (w), 1112 (w), 1008 (w), 852 (s), 746 (m), 698 (m), 639 (m). The characterization data is in accordance with reported literature values.[3]

Representative Large-Scale Synthesis of TIPS-EBX (6)

Triisopropyl((trimethylsilyl)ethynyl)silane (S2)
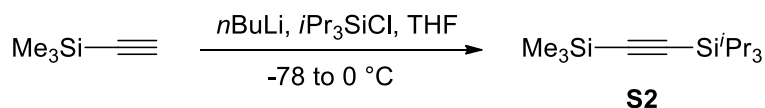
Following a reported procedure,[5] *n*-butyllithium (2.5 M in hexanes, 49.0 mL, 123 mmol, 0.98 eq.) was added dropwise to a stirred solution of ethynyltrimethylsilane (12.3 g, 125 mmol, 1.0 eq.) in THF (200 mL) at -78 °C. The mixture was then warmed to 0 °C and stirred for 5 min. The mixture was then cooled back to -78 °C and chlorotriisopropylsilane (26.7 mL, 125 mmol, 1.0 eq.) was added dropwise. The mixture was then allowed to warm to room temperature and stirred overnight. A saturated solution of ammonium chloride (165 mL) was added and the reaction mixture was extracted with diethyl ether (2 x 250 mL). The organic layer was washed with water and brine, then dried over $MgSO_4$, filtered and concentrated under reduced pressure to yield a colorless liquid, which was further purified by Kugelrohr distillation (56-57°C/0.25 mmHg) affording **S2** (29.9 g, 117 mmol, 95% yield) as a colorless liquid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.08 (m, 21 H, TIPS), 0.18 (s, 9 H, TMS). IR ν 2959 (m), 2944 (m), 2896 (w), 2867 (m), 1464 (w), 1385 (w), 1250 (m), 996 (w), 842 (s), 764 (s), 675 (m), 660 (m). The characterization data is in accordance with reported literature values.[5]

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[3] Brand, J. P.; Waser, J. *Synthesis* **2012**, *44*, 1155.
[4] The synthesis of **S1** is described below in the "Representative Large-Scale Synthesis of TIPS-EBX (**6**)" section.
[5] Helal, C. J.; Magriotis, P. A.; Corey, E. J. *J. Am. Chem. Soc.* **1996**, *118*, 10938.

1-Hydroxy-1,2-benziodoxol-3-(1*H*)-one (S1)
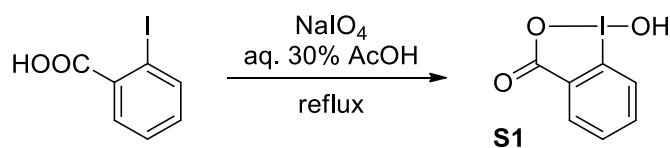
Following a reported procedure,[6] NaIO4 (25.5 g, 119 mmol, 1.05 eq.) and 2-iodobenzoic acid (28.5 g, 115 mmol, 1.00 eq.) were suspended in 30% (v:v) aq. AcOH (175 mL). The mixture was vigorously stirred and refluxed for 4 h. The reaction mixture was then diluted with cold water (460 mL) and allowed to cool to rt, while protecting it from light. After 1 h, the crude product was collected by filtration. The crystals were washed with ice water (3 x 115 mL) followed by acetone (3 x 115 mL) and then air-dried in the dark affording **S1** (29.7 g, 113 mmol, 98%) as a colorless solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.02 (dd, 1 H, J = 7.7, 1.4 Hz, Ar*H*), 7.97 (m, 1 H, Ar*H*), 7.85 (dd, 1 H, J = 8.2, 0.7 Hz, Ar*H*), 7.71 (td, 1 H, J = 7.6, 1.2 Hz, Ar*H*). ^{13}C NMR (100 MHz, (DMSO-d_6) δ 167.7, 134.5, 131.5, 131.1, 130.4, 126.3, 120.4. IR ν 3083 (w), 3060 (w), 2867 (w), 2402 (w), 1601 (m), 1585 (m), 1564 (m), 1440 (m), 1338 (s), 1302 (m), 1148 (m), 1018 (w), 834 (m), 798 (w), 740 (s), 694 (s), 674 (m), 649 (m). The characterization data is in accordance with reported literature values.[6]

1-[(Triisopropylsilyl)ethynyl]-1,2-benziodoxol-3(1*H*)-one (TIPS-EBX, 6)
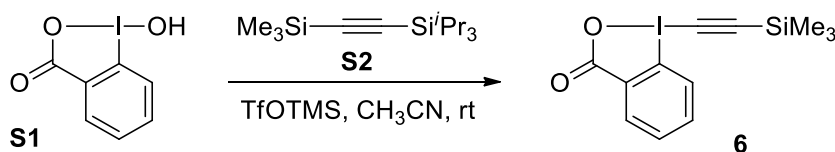
Following a reported procedure,[2] and oven-dried three-neck 1 L flask equipped with a magnetic bar was charged with **S1** (28.2 g, 107 mmol, 1.00 eq.). After three vacuum/nitrogen cycles, anhydrous acetonitrile (650 mL) was added *via* cannula and cooled to 0 °C. Trimethylsilyltriflate (21.3 mL, 117 mmol, 1.10 eq.) was added dropwise *via* a dropping funnel over a 30 min time period (no temperature increase was observed). After 15 min, (trimethylsilyl)(triisopropylsilyl)acetylene (**S2**, 29.9 g, 117 mmol, 1.10 eq.) was added *via* cannula over a 15 min time period (no temperature increase was observed) and the suspension became an orange solution. After 30 min of stirring, pyridine (9.50 mL, 117 mmol, 1.10 eq.) was added using a syringe. The reaction mixture was stirred for 15 minutes and then transferred to a one-neck 1 L flask and reduced under vacuum. The resulting solid was dissolved in CH₂Cl₂ (200 mL) and washed with 1.0 N aq. HCl (200 mL). The aq. layer was extracted with CH₂Cl₂ (200 mL) and the combined organic layers were washed with sat. aq. NaHCO₃ (2 x 200 mL), dried over MgSO₄, filtered and concentrated *in vacuo*. The resulting solid was purified by re-crystallization in acetonitrile (ca. 150 mL) affording TIPS-EBX (**6**) (38 g, 89.0 mmol, 83%) as a white crystalline solid. ^{1}H NMR (400 MHz, CDCl₃) δ 8.44 (m, 1 H, Ar*H*), 8.29 (m, 1 H, Ar*H*), 7.77 (m, 2 H, Ar*H*), 1.16 (m, 21 H, TIPS). ^{13}C NMR (100 MHz, CDCl₃) δ 166.4, 134.6, 132.3, 131.4, 131.4, 126.1, 115.6, 114.1, 64.6, 18.4, 11.1. IR ν 2943 (m), 2865 (m), 1716 (m), 1618 (m), 1604 (s), 1584 (m), 1557 (m), 1465 (m), 1439 (w), 1349 (m), 1291 (m), 1270 (w), 1244 (m), 1140 (m), 1016 (m), 999 (m), 883 (m), 833 (m), 742 (m), 702 (s), 636 (m). The characterization data is in accordance with reported literature values.[2]

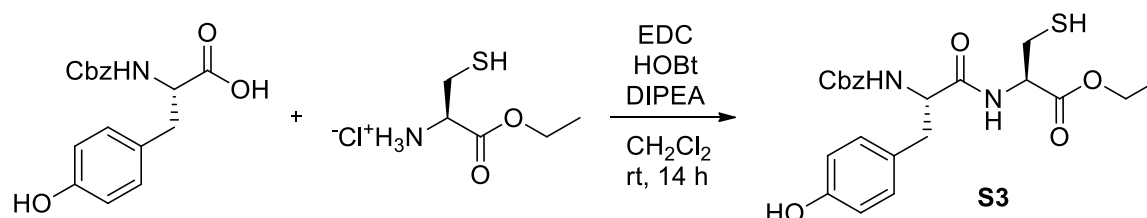[6] Kraszkiewicz, L.; Skulski, L. *Arkivoc* **2003**, 120.

Preparation of Substrates

**(*R*)-Ethyl 2-((*S*)-2-((1*H*-indol-3-yl)methyl)-3-(((benzyloxy)carbonyl)amino)-3-oxopropanamido)-3-mercaptopropanoate (35)**
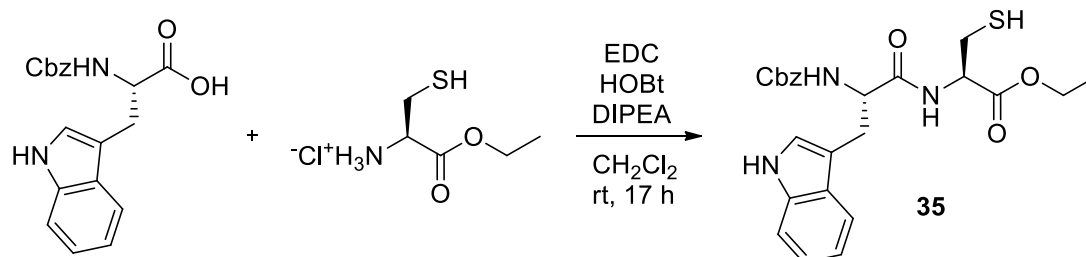
To a mixture of ʟ-cysteine ethyl ester hydrochloride (1.90 g, 10.0 mmol, 1.00 eq.), *N*-carbobenzyloxy-ʟ-tryptophan (4.06 g, 12.0 mmol, 1.20 eq.) and HOBt hydrate (2.37 g, 15.0 mmol, 1.50 eq.) in CH$_2$Cl$_2$ (100 mL) was added at 0 °C EDC hydrochloride (2.30 g, 12.0 mmol, 1.20 eq.) in one portion. The suspension was stirred for 10 minutes at 0 °C, after which DIPEA (5.24 mL, 30.0 mmol, 3.00 eq.) was slowly added. The ice bath was removed and the reaction mixture was stirred overnight at room temperature for 17 hours. Next, the solvent was evaporated under reduced pressure. The resulting oil was taken up in EtOAc (250 mL) and extracted with 5% aq. KHSO$_4$ (3 x 75 mL), 5% aq. NaHCO$_3$ (2 x 50 mL) and brine (50 mL). The organic layer was dried over MgSO$_4$, filtered and concentrated *in vacuo*. The crude white solid was purified by flash chromatography (pentane:EtOAc 2:1 to 3:2), followed by re-crystallization in EtOAc affording **35** (1.32 g, 2.81 mmol, 28%) as a white solid. R$_f$ (EtOAc:pentane 1:1) = 0.81. Melting point = 150.3-152.8°C. ^{1}H NMR (CDCl$_3$, 400 MHz): δ 8.21 (s, 1 H), 7.64 (d, 1 H, *J* = 7.9 Hz), 7.41-7.28 (m, 6 H), 7.23-7.16 (m, 1 H), 7.10 (t, 1 H, *J* = 7.5 Hz), 7.05 (s, 1 H), 6.67 (d, 1 H, *J* = 6.3 Hz), 5.51 (d, 1 H, *J* = 7.5 Hz), 5.13 (s, 2 H), 4.67 (dt, 1 H, *J* = 4.0, 7.4 Hz), 4.63-4.51 (m, 1 H), 4.24-4.05 (m, 2 H), 3.41 (dd, 1 H, *J* = 14.7, 5.4 Hz), 3.18 (dd, 1 H, *J* = 14.6, 7.0 Hz), 2.96-2.76 (m, 2 H), 1.24 (t, 3 H, *J* = 7.1 Hz, CO$_2$CH$_2$C*H$_3$*), 1.03 (t, 1 H, *J* = 9.0 Hz, S*H*). ^{13}C NMR (CDCl$_3$, 100 MHz) δ 171.3, 169.5, 156.1, 136.3, 136.1, 128.7, 128.4, 128.3, 127.5, 123.4, 122.6, 120.0, 118.9, 111.4, 110.2, 67.3, 62.1, 55.6, 53.9, 28.4, 26.7, 14.3. IR ν 3328 (w), 1739 (m), 1708 (m), 1660 (m), 1518 (m), 1212 (m), 1041 (m), 913 (w), 738 (s). HRMS (ESI) C$_{24}$H$_{27}$N$_3$NaO$_5$S$^+$ [M+Na]$^+$ calc. = 492.1564; [M+Na]$^+$ obs. = 492.1552.

(*R*)-Ethyl 2-((*S*)-2-(((benzyloxy)carbonyl)amino)-3-(4-hydroxyphenyl)propanamido)-3-mercaptopropanoate (S3)

To a mixture of ʟ-cysteine ethyl ester hydrochloride (1.90 g, 10.0 mmol, 1.00 eq.), *N*-carbobenzyloxy-ʟ-tyrosine (3.78 g, 12.0 mmol, 1.20 eq.) and HOBt hydrate (2.37 g, 15.0 mmol, 1.50 eq.) in CH$_2$Cl$_2$ (100 mL) was added at 0 °C EDC hydrochloride (2.30 g, 12.0 mmol, 1.20 eq.) in one portion. The suspension was stirred for 10 minutes at 0 °C, after which DIPEA (4.40 mL, 25.2 mmol, 2.52 eq.) was slowly added. The ice bath was removed and the

reaction mixture was stirred overnight at room temperature for 14 hours. Next, the solvent was evaporated under reduced pressure. The resulting oil was taken up in EtOAc (250 mL) and extracted with 5% aq. $KHSO_4$ (3 x 75 mL), 5% aq. $NaHCO_3$ (50 mL) and brine (50 mL). The organic layer was dried over $MgSO_4$, filtered and concentrated *in vacuo*. The crude white solid was purified by flash chromatography (pentane:EtOAc 3:2), followed by re-crystallization in EtOAc affording **S3** (1.46 g, 3.27 mmol, 33%) as a white solid. R_f (EtOAc:pentane 3:2) = 0.68. Melting point = 196.1-198.8 °C. ^{1}H NMR (MeOD, 400 MHz): δ 7.37-7.18 (m, 5 H, Cbz), 7.11-7.01 (m, 2 H, Ar*H*), 6.73-6.64 (m, 2 H, Ar*H*), 5.10-4.96 (m, 2 H), 4.58 (dd, 1 H, *J* = 6.4, 4.8 Hz), 4.37 (dd, 1 H, *J* = 9.0, 5.7 Hz), 4.18 (q, 2 H, *J* = 7.1 Hz, $CO_2CH_2CH_3$), 3.03 (dd, 1 H, *J* = 13.9, 5.8 Hz), 2.93 (dd, 1 H, *J* = 14.0, 8.9 Hz), 2.85 (dd, 1 H, *J* = 14.0, 6.5 Hz), 2.78 (dd, 1 H, *J* = 13.9, 9.0 Hz), 1.26 (t, 3 H, *J* = 7.1 Hz, $CO_2CH_2CH_3$). ^{13}C NMR (MeOD, 100 MHz) δ 174.3, 171.2, 158.2, 157.3, 138.2, 131.4, 129.4, 129.0, 128.9, 128.6, 116.2, 67.5, 62.7, 58.0, 56.1, 38.3, 26.6, 14.5. IR ν 3452 (w), 3295 (w), 2556 (w), 2433 (w), 1725 (m), 1687 (s), 1650 (s), 1428 (s), 1030 (m). HRMS (ESI) $C_{22}H_{27}N_2O_6S^+$ $[M+H]^+$ calc. = 447.1584; $[M+H]^+$ obs. = 447.1584.

(*R*)-Ethyl 2-((*S*)-2-(((benzyloxy)carbonyl)amino)-3-hydroxypropanamido)-3-mercaptopropanoate (S4)
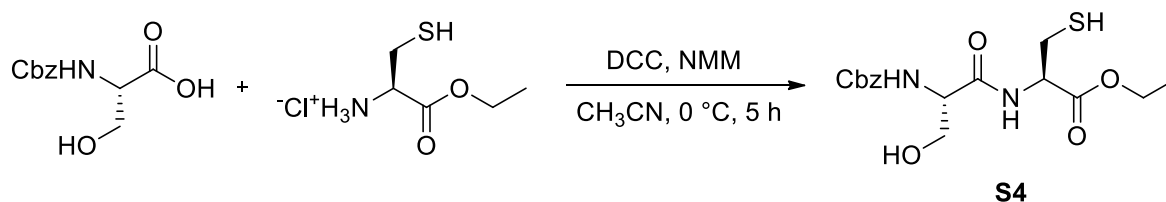
Following a slightly modified version of a reported procedure,[7] to a mixture of L-cysteine ethyl ester hydrochloride (1.90 g, 10.0 mmol, 1.00 eq.) and *N*-carbobenzyloxy-L-serine (2.39 g, 10.0 mmol, 1.00 eq.) in CH_3CN (25 mL) was added at 0 °C NMM (1.12 mL, 10.0 mmol, 1.0 eq.). The mixture was stirred at room temperature until it became a light yellow solution and cooled back to 0 °C. Next was added DCC (2.08 g, 10.0 mmol, 1.00 eq.) dissolved in CH_3CN (20 mL) *via* a dropping funnel over a 5 minute time period. The reaction mixture quickly became a white suspension and was stirred at 0 °C for 5 hours. The suspension was filtered and the filtrate was concentrated *in vacuo*. The resulting colorless oil was taken up in EtOAc (50 mL) and extracted with 0.5 N aq. HCl (50 mL), 5% aq. $NaHCO_3$ (25 mL) and brine (25 mL). The organic layer was dried over $MgSO_4$, filtered and concentrated *in vacuo*. The crude white solid was purified by flash chromatography (EtOAc:pentane 1:1), followed by re-crystallization in CH_2Cl_2 affording **S4** (1.82 g, 4.91 mmol, 49%) as a white solid. R_f (EtOAc:pentane 2:1 and 1% AcOH) = 0.47. Melting point = 117.2-119.3 °C. ^{1}H NMR (CDCl_3, 400 MHz): δ 7.44-7.28 (m, 6 H), 5.92 (d, 1 H, *J* = 7.5 Hz, N*H*), 5.20-5.07 (m, 2 H), 4.82 (dt, 1 H, *J* = 4.4, 8.3 Hz), 4.39-4.16 (m, 3 H), 4.13-3.99 (m, 1 H), 3.80-3.62 (m, 1 H), 3.30-3.12 (m, 1 H), 3.00 (d, 1 H, *J* = 4.5 Hz), 2.97 (d, 1 H, *J* = 4.4 Hz), 1.43 (t, 1 H, *J* = 9.0 Hz, S*H*), 1.29 (t, 3 H, *J* = 7.1 Hz, $CO_2CH_2CH_3$). ^{13}C NMR (CDCl_3, 100 MHz) δ 171.0, 170.0, 156.6, 136.1, 128.7, 128.4, 128.3, 67.5, 63.0, 62.4, 55.6, 54.0, 26.6, 14.3. IR ν 3314 (m), 2945 (w), 1723 (s), 1664 (s), 1530 (s), 1216 (s), 1030 (m), 737 (m). HRMS (ESI) $C_{16}H_{23}N_2O_6S^+$ $[M+H]^+$ calc. = 371.1271; $[M+H]^+$ obs. = 371.1269.

[7] Gotschi, E.; Jenny, C. J.; Reindl, P.; Ricklin, F. *Helv. Chim. Acta* **1996**, *79*, 2219.

Optimization of the Thiol-Alkynylation Reaction
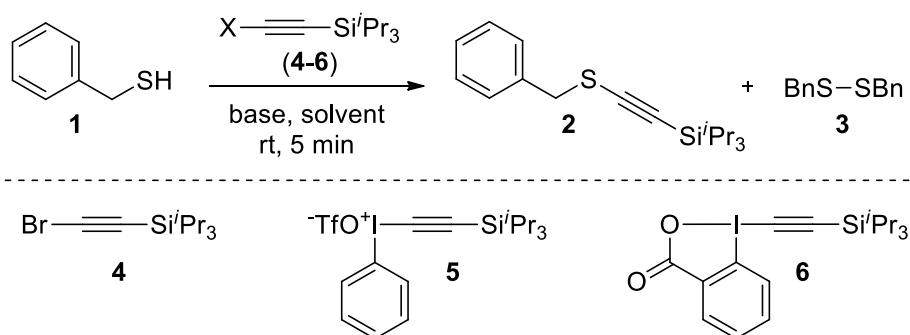
The following general procedure was utilized for the optimization of the thiol-alkynylation reaction. A 25 mL round bottom flask was charged with a magnetic stir bar, phenylmethanethiol (**1**, 49.7 mg, 0.400 mmol, 1.00 eq.), base (0.480 mmol, 1.20 eq.) and solvent (5.0 mL). After stirring the resulting solution for 5 minutes at room temperature, the alkynylating reagent (0.440 mmol, 1.10 eq.) was added in one portion. The resulting reaction mixture was stirred for 5 minutes at room temperature with the flask being open to the atmosphere. After 5 minutes of reaction time, the mixture was diluted with water (10 mL) and extracted with EtOAc (3 x 10 mL). The combined organic layers were dried over $MgSO_4$, filtered and concentrated *in vacuo*. The crude product was analyzed by ^1H NMR spectroscopy, calculating **2:3** NMR ratios using the integrated benzylic CH_2-peak areas. Both the desired and oxidation by-product were isolated by column chromatography for characterization.

((Benzylthio)ethynyl)triisopropylsilane (2): ^1H NMR (CDCl$_3$, 400 MHz): δ 7.42-7.26 (m, 5 H, Ar*H*), **3.95** (s, **2 H**, C*H₂*), 1.12-0.99 (m, 21 H, TIPS). Full characterization data (^{13}C NMR, R$_f$, IR, MS) for compound **2** is shown below in the reaction scope section.

1,2-Dibenzyldisulfane (3): ^1H NMR (CDCl$_3$, 400 MHz): 7.39-7.25 (m, 10 H, Ar*H*), **3.63** (s, **4 H**, ArC*H₂*SSC*H₂*Ar). The values of the ^1H NMR spectrum are in accordance with reported literature data.[8]

Table S1. Results of the thio-alkynylation optimization study.

Alkynylating Reagent	Base	Solvent	2:3 (NMR ratio)
4	NEt₃	THF	no reaction
4	TMG	THF	no reaction
5	NEt₃	THF	1 : 15
5	TMG	THF	1 : 4
6	NEt₃	THF	1.4 : 1
6	NaOH (in 0.5 mL H₂O)	THF	1 : 1.4
6	Cs₂CO₃ (in 0.5 mL H₂O)	THF	2.1 : 1
6	TMG	THF	>19 : 1
6	TMG	DMSO	9 : 1
6	TMG	MeOH	1 : 1.4
6	TMG	EtOH	9 : 1

[8] Oba, M.; Tanaka, K.; Nishiyama, K.; Ando, W. *J. Org. Chem.* **2011**, *76*, 4173.

Thiol-Alkynylation Procedure Employing TMS-EBX (7)

((Benzylthio)ethynyl)trimethylsilane (8)
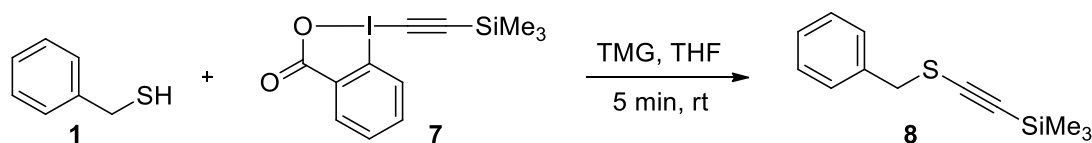
A 25 mL round bottom flask was charged with a magnetic stir bar, phenylmethanethiol (49.7 mg, 0.400 mmol, 1.00 eq.), TMG (60.4 µL, 0.480 mmol, 1.20 eq.) and THF (5.0 mL). After stirring the resulting solution for 5 minutes at room temperature, TMS-EBX (**7**, 188 mg, 0.440 mmol, 1.10 eq.) was added as a solid in one portion. The resulting reaction mixture was stirred for 5 minutes at room temperature. Next, the mixture was diluted with water (10 mL) and extracted with EtOAc (3 x 10 mL). The combined organic layers were dried over $MgSO_4$, filtered and concentrated *in vacuo*. The crude oil was purified by flash chromatography (pentane:EtOAc 200:1), affording **8** (81.0 mg, 0.368 mmol, 92%) as a colorless oil. R_f (pentane) = 0.35. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.37-7.27 (m, 5 H, Ar*H*), 3.94 (s, 2 H, ArC*H*$_2$S), 0.15 (s, 9 H, TMS). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 136.6, 129.3, 128.6, 127.9, 102.4, 94.4, 40.4, -0.3. The values of the NMR spectra are in accordance with reported literature data.[9]

Scope of the Reaction
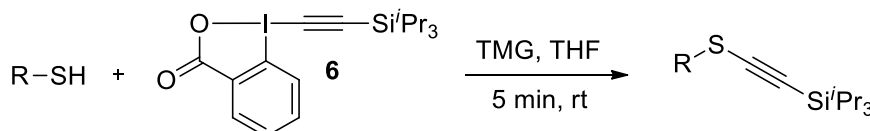
The following general procedure was utilized to determine the scope of thiol-alkynylation reaction with TIPS-EBX (**6**). A 25 mL round bottom flask was charged with a magnetic stir bar, the corresponding thiol derivative (0.400 mmol, 1.00 eq.), TMG (60.4 µL, 0.480 mmol, 1.20 eq.) and THF (5.0 mL). Several thiols were treated with water (0.5 or 1.0 mL as indicated) to ensure a homogenous reaction mixture. After stirring the resulting solution for 5 minutes at room temperature, TIPS-EBX (**6**, 188 mg, 0.440 mmol, 1.10 eq.) was added as a solid in one portion. The resulting reaction mixture was stirred with an open flask for 5 minutes at room temperature, worked-up and purified as indicated. It is noteworthy that TLC analysis indicates that the reaction is completed within 30 seconds upon TIPS-EBX (**6**) addition.
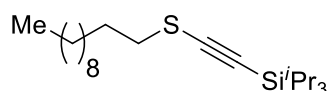
((Benzylthio)ethynyl)triisopropylsilane (2)
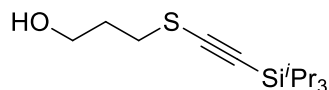

The reaction mixture was concentrated *in vacuo* and purified by flushing the crude oil dissolved in minimum amounts of CH_2Cl_2 through a small plug of silica gel using hexane:EtOAc 500:1 as mobile phase affording **2** (121 mg, 0.398 mmol, quant.) as a clear colorless oil. R_f (hexane) = 0.40. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.42-7.26 (m, 5 H, Ar*H*),

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[9] Seidel, W. W.; Meel, M. J.; Schaffrath, M.; Pape, T. *Eur. J. Org. Chem.* **2007**, 3526.

3.95 (s, 2 H, CH_2), 1.12-0.99 (m, 21 H, TIPS). ^{13}C NMR (CDCl$_3$, 100 MHz) δ 136.8, 129.1, 128.6, 127.8, 98.8, 95.4, 40.6, 18.7, 11.5. IR ν 2943 (s), 2865 (s), 2089 (s), 1461 (m), 1238 (w), 1072 (w), 996 (w), 882 (s), 857 (s). HRMS (ESI) C$_{18}$H$_{29}$SSi$^+$ [M+H]$^+$ calc. = 305.1754; [M+H]$^+$ obs. = 305.1753.

Triisopropyl(((4-methoxybenzyl)thio)ethynyl)silane (9)
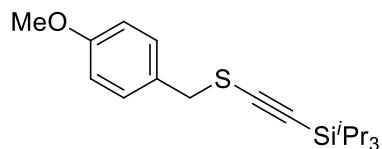
The reaction mixture was concentrated *in vacuo* and purified by flushing the crude oil dissolved in minimum amounts of CH$_2$Cl$_2$ through a small plug of silica gel using hexane:EtOAc 30:1 as mobile phase affording **9** (127 mg, 0.378 mmol, 94%) as a clear colorless oil. R$_f$ (hexane:EtOAc 19:1) = 0.71. ^{1}H NMR (CDCl$_3$, 400 MHz): δ 7.31-7.23 (m, 2 H, ArH), 6.90-6.79 (m, 2 H, ArH), 3.92 (s, 2 H, CH_2), 3.80 (s, 3 H, OCH_3), 1.11-0.96 (m, 21 H, TIPS). ^{13}C NMR (CDCl$_3$, 100 MHz) δ 159.3, 130.3, 128.9, 114.1, 98.7, 95.7, 55.4, 40.2, 18.7, 11.5. IR ν 2942 (m), 2864 (m), 2088 (w), 1511 (s), 1464 (w), 1251 (s), 1037 (w), 883 (m), 856 (s). HRMS (APPI) C$_{19}$H$_{30}$OSSi [M+] calc. = 334.1787; [M+] obs. = 334.1777.

((((4-Chlorobenzyl)thio)ethynyl)triisopropylsilane (10)
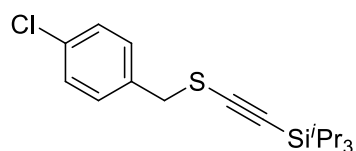
The reaction mixture was concentrated *in vacuo* and purified by flushing the crude oil dissolved in minimum amounts of CH$_2$Cl$_2$ through a small plug of silica gel using hexane:EtOAc 500:1 as mobile phase affording **10** (127 mg, 0.376 mmol, 95%) as a clear colorless oil. R$_f$ (hexane) = 0.48. ^{1}H NMR (CDCl$_3$, 400 MHz): δ 7.28 (s, 4 H, ArH), 3.88 (s, 2 H, CH_2), 1.06-0.99 (m, 21 H, TIPS). ^{13}C NMR (CDCl$_3$, 100 MHz) δ 135.4, 133.7, 130.5, 128.8, 99.4, 94.7, 39.7, 18.7, 11.4. IR ν 2943 (s), 2865 (s), 2088 (m), 1492 (w), 1464 (w), 1094 (w), 1016 (w), 883 (s), 856 (s), 828 (w). HRMS (ESI) C$_{18}$H$_{27}$ClSSi [M+] calc. = 338.1291; [M+H]$^+$ obs. = 338.1292.

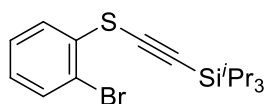## ((((Furan-2-ylmethyl)thio)ethynyl)triisopropylsilane (11)
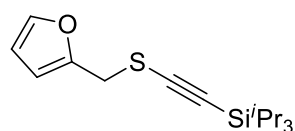
The reaction mixture was concentrated *in vacuo* and purified by flushing the crude oil dissolved in minimum amounts of CH$_2$Cl$_2$ through a small plug of silica gel using hexane:EtOAc 500:1 as mobile phase affording **11** (114 mg, 0.386 mmol, 97%) as a clear colorless oil. R$_f$ (hexane) = 0.52. ^{1}H NMR (CDCl$_3$, 400 MHz): δ 7.37 (dd, 1 H, J = 1.9, 0.9 Hz, ArH), 6.31 (dd, 1 H, J = 3.2, 1.9 ArH), 6.29 (dd, 1 H, J = 3.3, 0.9 ArH), 3.96 (s, 2 H, SCH_2), 1.10-1.01 (m, 21 H, TIPS). ^{13}C NMR (CDCl$_3$, 100 MHz) δ 149.7, 142.8, 110.7, 109.1, 99.5, 94.8, 33.2, 18.7, 11.4. IR ν 2942 (m), 2865 (m), 2090 (m), 1464 (w), 1012 (w), 883 (s), 856 (s), 737 (s). HRMS (ESI) C$_{16}$H$_{27}$OSSi$^+$ [M+H]$^+$ calc. = 295.1546; [M+H]$^+$ obs. = 295.1541.

((Triisopropyl((undecylthio)ethynyl)silane (12)

The reaction mixture was concentrated *in vacuo* and purified by flushing the crude oil dissolved in minimum amounts of CH$_2$Cl$_2$ through a small plug of silica gel using hexane as mobile phase affording **12** (147 mg, 0.399 mmol, quant.) as a clear colorless oil. R$_f$ (hexane, KMnO$_4$ staining) = 0.78. ^{1}H NMR (CDCl$_3$, 400 MHz): δ 2.71 (t, 2 H, J = 7.2 Hz, S-CH_2), 1.82-1.71

(m, 2 H), 1.47-1.36 (m, 2 H), 1.35-1.21 (m, 14 H), 1.11-1.02 (m, 21 H, TIPS), 0.87 (t, 3 H, J = 7.0 Hz, CH$_2$-CH_3). ^{13}C NMR (CDCl$_3$, 100 MHz) δ 96.9, 96.2, 36.0, 32.1, 29.8,[10] 29.6, 29.5, 29.4, 29.3, 28.4, 22.9, 18.8, 14.3, 11.5. IR ν 2927 (s), 2863 (s), 2090 (w), 1505 (w), 1453 (m), 1238 (w), 1133 (w), 997 (w), 883 (m), 856 (s), 742 (s). HRMS (ESI) C$_{22}$H$_{45}$SSi$^+$ [M+H]$^+$ calc. = 369.3006; [M+H]$^+$ obs. = 369.3009.

3-(((Triisopropylsilyl)ethynyl)thio)propan-1-ol (13)

The reaction mixture was concentrated *in vacuo* and purified by flash chromatography using hexane:EtOAc 20:1 to 10:1 as mobile phase affording **13** (107 mg, 0.393 mmol, 98%) as a clear colorless oil. R$_f$ (hexane:EtOAc 3:2, KMnO$_4$ staining) = 0.65. ^{1}H NMR (CDCl$_3$, 400 MHz): δ 3.78 (t, 2 H, J = 6.0 Hz, HO-CH_2), 2.84 (t, 2 H, J = 6.9 Hz, S-CH_2), 2.05-1.96 (m, 2 H, CH$_2$CH_2CH$_2$), 1.93 (bs, 1 H, OH), 1.12-0.94 (m, 21 H, TIPS). ^{13}C NMR (CDCl$_3$, 100 MHz) δ 97.4, 95.7, 60.6, 32.4, 31.7, 18.7, 11.4. IR ν 3353 (w), 2945 (s), 2865 (s), 2089 (s), 1513 (m), 1464 (m), 1251 (m), 1039 (m), 884 (s), 857 (s). HRMS (ESI) C$_{14}$H$_{29}$OSSi$^+$ [M+H]$^+$ calc. = 273.1703; [M+H]$^+$ obs. = 273.1704.

16-(((Triisopropylsilyl)ethynyl)thio)hexadecanoic acid (14)
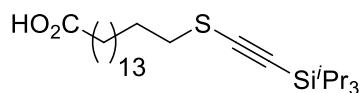
The 16-mercaptohexadecanoic acid starting material was dissolved in THF (5.0 mL) and water (0.5 mL) to ensure a homogenous mixture throughout the course of the reaction. The reaction mixture was concentrated *in vacuo* and purified by flash chromatography using hexane:EtOAc 20:1 and 1% AcOH as mobile phase affording **14** (174 mg, 0.370 mmol, 93%) as a white solid. R$_f$ (hexane:EtOAc 7:3, KMnO$_4$ staining) = 0.56. Melting point <35 °C. ^{1}H NMR (CDCl$_3$, 400 MHz): δ 2.70 (t, 2 H, J = 7.2 Hz, S-CH_2), 2.34 (t, 2 H, J = 7.5 Hz, CO$_2$H-CH_2), 1.82-1.70 (m, 2 H), 1.69-1.55 (m, 2 H), 1.46-1.36 (m, 2 H), 1.36-1.20 (m, 20 H), 1.10-0.99 (m, 21 H, TIPS). ^{13}C NMR (CDCl$_3$, 100 MHz)[11] δ 180.6, 96.9, 96.2, 36.0, 34.3, 29.8, 29.7, 29.6, 29.4, 29.3, 29.2, 28.3, 24.8, 18.8, 11.5. IR ν 2923 (s), 2855 (m), 2090 (w), 1704 (s), 1466 (w), 884 (w), 859 (m). HRMS (ESI) C$_{27}$H$_{53}$O$_2$SSi$^+$ [M+H]$^+$ calc. = 469.3530; [M+H]$^+$ obs. = 469.3531.

### Triisopropyl((phenylthio)ethynyl)silane (15)
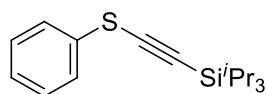
The reaction mixture was concentrated *in vacuo* and purified by flushing the crude oil dissolved in minimum amounts of CH$_2$Cl$_2$ through a small plug of silica gel using pentane as mobile phase affording **15** (103 mg, 0.355 mmol, 89%) as a clear colorless oil. R$_f$ (hexane) = 0.67. ^{1}H NMR (CDCl$_3$, 400 MHz): δ 7.51-7.44 (m, 2 H, ArH), 7.39-7.32 (m, 2 H, ArH), 7.26-7.20 (m, 1 H, ArH), 1.20-1.11 (m, 21 H, TIPS). ^{13}C NMR (CDCl$_3$, 100 MHz): δ 132.9, 129.3, 126.5, 126.1, 103.4, 91.2, 18.8, 11.5. The values of the ^{1}H NMR spectrum are in accordance with reported literature data.[12]

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[10] Signal at 29.8 ppm is not resolved and corresponds to two carbons.

[11] Several of the aliphatic carbon signals in the region around 30 ppm were not resolved at 100 MHz.

[12] Rücker, C.; Fritz, H. *Magn. Reson. Chem.* **1988**, *26*, 1103.

(((2-Bromophenyl)thio)ethynyl)triisopropylsilane (16)

The reaction mixture was concentrated *in vacuo* and purified by flushing the crude oil dissolved in minimum amounts of CH_2Cl_2 through a small plug of silica gel using pentane as mobile phase affording **16** (144 mg, 0.390 mmol, 97%) as a clear colorless oil. R_f (hexane) = 0.69. ^{1}H NMR (CDCl$_3$, 400 MHz): δ 7.77 (dd, 1 H, J = 8.0, 1.5 Hz, Ar*H*), 7.49 (dd, 1 H, J = 7.9, 1.3 Hz, Ar*H*), 7.40-7.32 (m, 1 H, Ar*H*), 7.12-7.05 (m, 1 H, Ar*H*), 1.22-1.06 (m, 21 H, TIPS). ^{13}C NMR (CDCl$_3$, 100 MHz) δ 134.6, 132.8, 128.2, 127.5, 127.1, 119.6, 105.3, 90.8, 18.8, 11.5. IR ν 2943 (w), 2865 (w), 2094 (w), 1447 (w), 1019 (w), 883 (m), 854 (s), 745 (s). HRMS (APPI) $C_{17}H_{25}^{79}BrSSi^+$ [M$^+$] calc. = 368.0630; [M$^+$] obs. = 368.0620.

(((2,5-Dichlorophenyl)thio)ethynyl)triisopropylsilane (17)
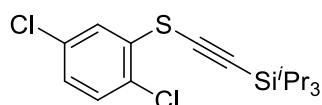
The reaction mixture was concentrated *in vacuo* and purified by running the crude dissolved in minimum amounts of CH_2Cl_2 through a small plug of silica gel using pentane as mobile phase affording **17** (143 mg, 0.393 mmol, 98%) as a white solid. R_f (hexane) = 0.80. Melting point = 45.3-47.1 °C. ^{1}H NMR (CDCl$_3$, 400 MHz): δ 7.77 (d, 1 H, J = 2.4 Hz, Ar*H*), 7.24 (d, 1 H, J = 8.4 Hz, Ar*H*), 7.12 (dd, 1 H, J = 8.5, 2.4 Hz, Ar*H*), 1.23-1.05 (m, 21 H, TIPS). ^{13}C NMR (CDCl$_3$, 100 MHz) δ 134.8, 133.9, 130.3, 128.5, 127.3, 126.8, 106.9, 89.2, 18.8, 11.4. IR ν 2943 (m), 2866 (w), 2097 (w), 1450 (s), 1096 (w), 1033 (m), 883 (m), 852 (s), 805 (s), 805 (s). HRMS (APPI) $C_{17}H_{24}Cl_2SSi$ [M+] calc. = 358.0745; [M+] obs. = 358.0737.

Triisopropyl(((3-methoxyphenyl)thio)ethynyl)silane (18)
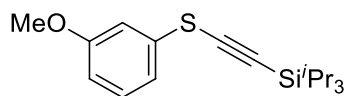
The reaction mixture was concentrated *in vacuo* and purified by flushing the crude oil dissolved in minimum amounts of CH_2Cl_2 through a small plug of silica gel using pentane:EtOAc 200:1 as mobile phase affording **18** (120 mg, 0.374 mmol, 94%) as a clear colorless oil. R_f (hexane:EtOAc 19:1) = 0.77. ^{1}H NMR (CDCl$_3$, 400 MHz): δ 7.24 (t, 1 H, J = 8.0 Hz, Ar*H*), 7.08-7.04 (m, 1 H, Ar*H*), 7.00 (ddd, 1 H, J = 7.8, 1.7, 0.9 Hz, Ar*H*), 6.77 (ddd, 1 H, J = 8.3, 2.5, 0.9 Hz, Ar*H*), 3.81 (s, 3 H, OC*H$_3$*), 1.21-1.08 (m, 21 H, TIPS). ^{13}C NMR (CDCl$_3$, 100 MHz) δ 160.4, 134.1, 130.0, 118.3, 113.0, 110.9, 103.8, 91.1, 55.4, 18.8, 11.5. IR ν 2943 (m), 2865 (m), 2093 (w), 1592 (m), 1463 (w), 1250 (m), 1044 (w), 883 (m), 855 (s), 735 (s). HRMS (ESI) $C_{18}H_{29}OSSi^+$ [M+H]$^+$ calc. = 321.1703; [M+H]$^+$ obs. = 321.1708.

3-(((Triisopropylsilyl)ethynyl)thio)phenol (19)
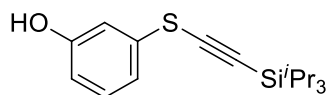
The reaction mixture was concentrated *in vacuo* and purified by flash chromatography using pentane:EtOAc 30:1 to 20:1 as mobile phase affording **19** (119 mg, 0.388 mmol, 97%) as a light yellow oil. R_f (hexane:EtOAc 9:1) = 0.33. ^{1}H NMR (CDCl$_3$, 400 MHz): δ 7.20 (t, 1 H, J = 8.0 Hz, Ar*H*), 7.01 (ddd, 1 H, J = 7.9, 1.8, 0.9 Hz, Ar*H*), 6.98-6.95 (m, 1 H, Ar*H*), 6.68 (ddd, 1 H, J = 8.1, 2.5, 0.9 Hz, Ar*H*), 5.24 (bs, 1 H, ArO*H*), 1.23-1.06 (m, 21 H, TIPS). ^{13}C NMR (CDCl$_3$, 100 MHz): δ 156.2, 134.5, 130.3, 118.5, 113.7, 113.0, 103.9, 90.9, 18.8, 11.5. IR ν 3388 (w), 2943 (m), 2865 (m), 2093 (m), 1587 (m), 1474 (m), 1219 (w), 996 (w), 883 (s), 856 (s), 771 (m). HRMS (ESI) $C_{17}H_{25}OSSi^-$ [M-H]$^-$ calc. = 305.1401; [M-H]$^-$ obs. = 305.1396.

### N-(4-(((Triisopropylsilyl)ethynyl)thio)phenyl)acetamide (20)
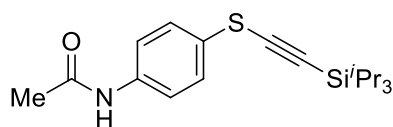
The N-(4-mercaptophenyl)acetamide starting material was dissolved in THF (5.0 mL) and water (0.5 mL) to ensure a homogenous mixture throughout the course of the reaction. The reaction mixture was concentrated *in vacuo* and purified by flash chromatography using pentane:EtOAc 5:1 to 3:1 as mobile phase affording **20** (125 mg, 0.358 mmol, 90%) as a white solid. R_f (pentane:EtOAc 2:3) = 0.57. Melting point = 95.8-98.9 °C. ^{1}H NMR (CDCl₃, 400 MHz): δ 7.55-7.45 (m, 3 H, Ar*H* and N*H*), 7.41-7.33 (m, 2 H, Ar*H*), 2.16 (s, 3 H, NHCOC*H₃*), 1.17-1.03 (m, 21 H, TIPS). ^{13}C NMR (CDCl₃, 100 MHz) δ 168.6, 136.7, 127.7, 127.1, 120.8, 103.1, 91.5, 24.7, 18.8, 11.5. IR ν 3300 (w), 2944 (s), 2865 (m), 2092 (w), 1667 (s), 1599 (m), 1534 (s), 1495 (s), 1317 (m), 1007 (w), 858 (m), 824 (m). HRMS (ESI) $C_{19}H_{30}NOSSi^+$ [M+H]$^+$ calc. = 348.1812; [M+H]$^+$ obs. = 348.1813.

3-(((Triisopropylsilyl)ethynyl)thio)aniline (21)
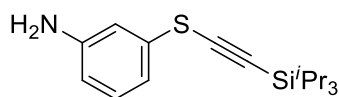
The reaction mixture was concentrated *in vacuo* and purified by flash chromatography using pentane:EtOAc 20:1 to 15:1 as mobile phase affording **21** (112 mg, 0.367 mmol, 92%) as a light yellow oil. R_f (hexane:EtOAc 7:3) = 0.56. ^{1}H NMR (CDCl₃, 400 MHz): δ 7.11 (t, 1 H, J = 7.8 Hz, Ar*H*), 6.87-6.74 (m, 2 H, Ar*H*), 6.57-6.49 (m, 1 H, Ar*H*), 3.70 (bs, 2 H, N*H₂*), 1.22-1.06 (m, 21 H, TIPS). ^{13}C NMR (CDCl₃, 100 MHz) δ 147.3, 133.7, 130.0, 116.1, 113.3, 112.2, 103.3, 91.4, 18.8, 11.5. IR ν 2942 (m), 2865 (m), 2092 (m), 1594 (m), 1464 (m), 1252 (w), 995 (w), 883 (s), 855 (s), 770 (m). HRMS (ESI) $C_{17}H_{28}NSSi^+$ [M+H]$^+$ calc. = 306.1706; [M+H]$^+$ obs. = 306.1706.

4-(((Triisopropylsilyl)ethynyl)thio)benzoic acid (22)
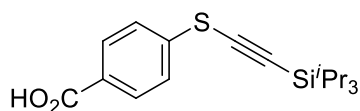
The 4-mercaptobenzoic acid starting material was dissolved in THF (5.0 mL) and water (1.0 mL) to ensure a homogenous mixture throughout the course of the reaction. The reaction mixture was concentrated *in vacuo* and purified by flash chromatography using pentane:EtOAc 40:1 and 1% AcOH as mobile phase affording **22** (121 mg, 0.362 mmol, 91%) as a white solid. R_f (pentane:EtOAc 4:1 and 1% AcOH) = 0.49. Melting point = 189.1-192.3 °C. ^{1}H NMR (CDCl₃, 400 MHz): δ 8.13-8.03 (m, 2 H, Ar*H*), 7.57-7.49 (m, 2 H, Ar*H*), 1.19-1.09 (m, 21 H, TIPS). ^{13}C NMR (CDCl₃, 100 MHz) δ 171.8, 141.1, 131.0, 127.2, 125.4, 105.7, 89.2, 18.8, 11.5. IR ν 2929 (w), 2862 (w), 2094 (w), 1688 (w), 1420 (w), 1292 (w), 909 (s), 855 (w), 735 (s). HRMS (ESI) $C_{18}H_{25}O_2SSi$ [M-H]$^-$ calc. = 333.1345; [M-H]$^-$ obs. = 333.1357.

Methyl 2-(((triisopropylsilyl)ethynyl)thio)benzoate (23)
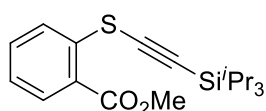
The reaction mixture was concentrated *in vacuo* and purified by running the crude oil dissolved in minimum amounts of CH₂Cl₂ through a small plug of silica gel using pentane:EtOAc 30:1 as mobile phase affording **23** (139 mg, 0.400 mmol, quant.) as a clear colorless oil. R_f (hexane:EtOAc 9:1) = 0.75. ^{1}H NMR (CDCl₃, 400 MHz): δ 8.10 (dd, 1 H, J = 8.2, 1.1 Hz, Ar*H*), 8.04 (dd, 1 H, J = 7.9, 1.6 Hz, Ar*H*), 7.58-7.49 (m, 1 H, Ar*H*), 7.29-7.21 (m, 1 H, Ar*H*), 3.92 (s, 3 H, CO₂C*H₃*), 1.17-1.10 (m, 21 H, TIPS). ^{13}C NMR (CDCl₃, 100 MHz) δ 166.4, 138.1, 133.1, 131.4, 127.3, 126.0, 125.5, 105.2, 92.9, 52.4, 18.8, 11.5. IR ν 2943 (w),

2865 (w), 2091 (w), 1717 (m), 1464 (w), 1261 (s), 883 (w), 846 (s), 742 (s). HRMS (ESI) $C_{19}H_{29}O_2SSi^+$ [M+H]$^+$ calc. = 349.1652; [M+H]$^+$ obs. = 349.1656.

Triisopropyl((thiophen-2-ylthio)ethynyl)silane (24)
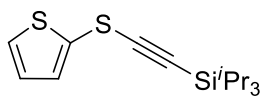
The reaction mixture was concentrated *in vacuo* and purified by flushing the crude oil dissolved in minimum amounts of CH_2Cl_2 through a small plug of silica gel using hexane:EtOAc 500:1 as mobile phase affording **24** (101 mg, 0.341 mmol, 85%) as a clear colorless oil. R_f (hexane) = 0.63. ^{1}H NMR (CDCl$_3$, 400 MHz): δ 7.35 (dd, 1 H, *J* = 5.3, 1.3 Hz, Ar*H*), 7.13 (dd, 1 H, *J* = 3.6, 1.3 Hz, Ar*H*), 6.98 (dd, 1 H, *J* = 5.3, 3.6 Hz, Ar*H*), 1.13-1.04 (m, 21 H, TIPS). ^{13}C NMR (CDCl$_3$, 100 MHz) δ 130.9, 129.9, 128.5, 127.7, 100.4, 92.9, 18.7, 11.5. IR ν 2942 (m), 2865 (m), 2094 (w), 1464 (w), 995 (w), 883 (m), 850 (s). HRMS (APPI) $C_{15}H_{24}S_2Si$ [M$^+$] calc. = 296.1089; [M$^+$] obs. = 296.1084.

2-(((Triisopropylsilyl)ethynyl)thio)benzo[*d*]oxazole (25)
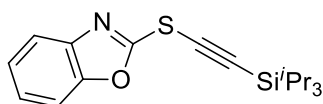
The reaction mixture was concentrated *in vacuo* and purified by flash chromatography using hexane:EtOAc 30:1 as mobile phase affording **25** (132 mg, 0.398 mmol, quant.) as a light yellow oil. R_f (hexane:EtOAc 9:1) = 0.76. ^{1}H NMR (CDCl$_3$, 400 MHz): δ 7.68-7.59 (m, 1 H, Ar*H*), 7.51-7.43 (m, 1 H, Ar*H*), 7.30-7.27 (m, 2 H, Ar*H*), 1.22-1.08 (m, 21 H, TIPS). ^{13}C NMR (CDCl$_3$, 100 MHz) δ 159.0, 152.3, 142.4, 124.8, 124.7, 119.3, 110.2, 106.7, 83.3, 18.7, 11.4. IR ν 2943 (m), 2865 (m), 2107 (w), 1504 (m), 1450 (s), 1238 (w), 1134 (m), 998 (w), 883 (m), 853 (s), 741 (s). HRMS (ESI) $C_{18}H_{26}NOSSi^+$ [M+H]$^+$ calc. = 332.1499; [M+H]$^+$ obs. = 332.1493.

5-Nitro-2-(((triisopropylsilyl)ethynyl)thio)-1*H*-benzo[*d*]imidazole (26)
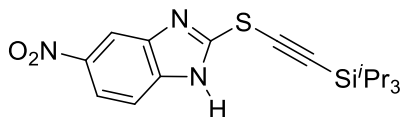
The reaction mixture was concentrated *in vacuo* and purified by flash chromatography using hexane:EtOAc 20:1 to 10:1 as mobile phase affording **26** (128 mg, 0.341 mmol, 94%) as a light yellow solid. R_f (hexane:EtOAc 4:1) = 0.44. Melting point = 131.5-132.9 °C. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 13.6 (bs, 1 H, N*H*), 8.39 (d, 1 H, *J* = 2.3 Hz, Ar*H*), 8.12 (dd, 1 H, *J* = 8.9, 2.3 Hz, Ar*H*), 7.70 (d, 1 H, *J* = 8.9 Hz, Ar*H*), 1.14-1.00 (m, 21 H, TIPS). ^{13}C NMR (DMSO-d_6, 100 MHz)[13] δ 149.6, 142.7, 118.1, 103.9, 86.2, 18.4, 10.7. IR ν 2944 (w), 2866 (w), 2101 (w), 1526 (m), 1425 (m), 1337 (s), 1278 (w), 1069 (w), 883 (m), 850 (m), 739 (s). HRMS (ESI) $C_{18}H_{26}N_3O_2SSi^+$ [M+H]$^+$ calc. = 376.1510; [M+H]$^+$ obs. = 376.1503.

2-(((Triisopropylsilyl)ethynyl)thio)benzo[*d*]thiazole (27)
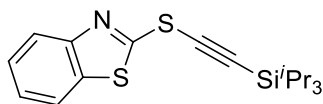
The reaction mixture was concentrated *in vacuo* and purified by flash chromatography using hexane:EtOAc 40:1 as mobile phase affording **27** (139 mg, 0.398 mmol, quant.) as light yellow oil. R_f (hexane:EtOAc 9:1) = 0.70. ^{1}H NMR (CDCl$_3$, 400 MHz): δ 7.91-7.79 (m, 2 H, Ar*H*), 7.44 (ddd, 1 H, *J* = 8.2, 7.3, 1.3 Hz, Ar*H*), 7.34 (ddd, 1 H, *J* = 8.2, 7.3, 1.2 Hz, Ar*H*), 1.28-1.06 (m, 21 H, TIPS). ^{13}C NMR (CDCl$_3$, 100 MHz) δ 165.7, 154.5, 135.9, 126.4, 124.7, 122.1, 121.2, 108.8, 87.6, 18.8, 11.4. IR ν 2943 (s), 2865 (m), 2099 (w), 1739 (w), 1465 (s), 1427 (s), 1239

[13] Presumably, due to rapid proton exchange of the delocalized imidazole ring system, some carbon signals were not observed.

(w), 1010 (s), 883 (s), 855 (s), 755 (s), 727 (w). HRMS (ESI) $C_{18}H_{26}NS_2Si^+$ [M+H]$^+$ calc. = 348.1270; [M+H]$^+$ obs. = 348.1264.

(**R**)-Methyl 2-((**tert**-butoxycarbonyl)amino)-3-((((triisopropylsilyl)ethynyl)thio)propanoate (28)
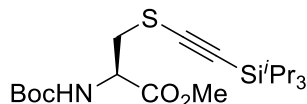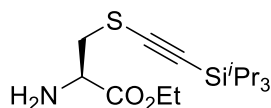
The reaction mixture was diluted with water (10 mL) and extracted with EtOAc (3 x 10 mL). The combined organic layers were dried over MgSO$_4$, filtered and concentrated *in vacuo*. The resulting crude oil was purified by flash chromatography using pentane:EtOAc 20:1 to 15:1 as mobile phase affording **28** (158 mg, 0.380 mmol, 95%) as a clear colorless oil. R$_f$ (Hexane:EtOAc 4:1) = 0.54. ^{1}H NMR (CDCl$_3$, 400 MHz): δ 5.47 (d, 1 H, *J* = 8.4 Hz, N*H*Boc), 4.73-4.58 (m, 1 H, C*H*NHBoc), 3.74 (s, 3 H, CO$_2$C*H$_3$*), 3.22 (dd, 1 H, *J* = 13.7, 4.2 Hz, SC*H$_2$*), 3.09 (dd, 1 H, *J* = 13.6, 6.0 Hz, SC*H$_2$*), 1.41 (s, 9 H, Boc), 1.09-0.98 (m, 21 H, TIPS). ^{13}C NMR (CDCl$_3$, 100 MHz) δ 170.8, 155.2, 98.2, 94.6, 80.3, 54.0, 52.7, 38.4, 28.3, 18.7, 11.3. IR ν 2945 (w), 2866 (w), 2092 (w), 1720 (s), 1502 (w), 1366 (w), 1163 (s), 1018 (w), 856 (s). HRMS (ESI) $C_{20}H_{38}NO_4SSi^+$ [M+H]$^+$ calc. = 416.2285; [M+H]$^+$ obs. = 416.2280.

(**R**)-Ethyl 2-((((benzyloxy)carbonyl)amino)-3-((((triisopropylsilyl)ethynyl)thio)propanoate (29)
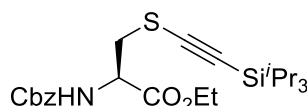
The reaction mixture was diluted with water (10 mL) and extracted with EtOAc (3 x 10 mL). The combined organic layers were dried over MgSO$_4$, filtered and concentrated *in vacuo*. The resulting crude oil was purified by flash chromatography using pentane:EtOAc 10:1 to 3:1 as mobile phase affording **29** (171 mg, 0.369 mmol, 92%) as a clear colorless oil. R$_f$ (Hexane:EtOAc 4:1) = 0.53. ^{1}H NMR (CDCl$_3$, 400 MHz): δ 7.40-7.27 (m, 5 H, Cbz), 5.81 (d, 1 H, *J* = 8.1 Hz, N*H*Cbz), 5.14 (d, 1 H, *J* = 12.1 Hz, CO$_2$C*H$_2$*Ar), 5.07 (d, 1 H, *J* = 12.3 Hz, CO$_2$C*H$_2$*Ar), 4.74 (ddd, 1 H, *J* = 8.1, 6.2, 4.1 Hz, C*H*NHCbz), 4.30-4.13 (m, 2 H, CO$_2$C*H$_2$*CH$_3$), 3.28 (dd, 1 H, *J* = 13.6, 4.1 Hz, SC*H$_2$*), 3.11 (dd, 1 H, *J* = 13.7, 6.2 Hz, SC*H$_2$*), 1.28 (t, 3 H, *J* = 7.2 Hz, CO$_2$CH$_2$C*H$_3$*), 1.12-0.94 (m, 21 H, TIPS). ^{13}C NMR (CDCl$_3$, 100 MHz) δ 169.9, 155.8, 136.1, 128.5, 128.2, 98.4, 94.5, 67.2, 62.0, 54.4, 38.3, 18.6, 14.2, 11.3.[14] IR ν 3350 (w), 2944 (m), 2866 (m), 2092 (m), 1729 (s), 1507 (m), 1463 (m), 1209 (s), 1055 (m), 1026 (m), 856 (m), 737 (w). HRMS (ESI) $C_{24}H_{38}NO_4SSi^+$ [M+H]$^+$ calc. = 464.2285; [M+H]$^+$ obs. = 464.2289.

(**R**)-Ethyl 2-amino-3-((((triisopropylsilyl)ethynyl)thio)propanoate (30)

The starting material, ʟ-cysteine ethyl ester hydrogen chloride, was dissolved in THF (5.0 mL) and water (0.5 mL) to ensure a homogenous mixture throughout the course of the reaction. The reaction mixture was diluted with water (10 mL) and extracted with EtOAc (3 x 10 mL). The combined organic layers were dried over MgSO$_4$, filtered and concentrated *in vacuo*. The resulting crude oil was purified by flash chromatography using CH$_2$Cl$_2$:Ultra 97:3 as mobile phase affording **30** (125 mg, 0.379 mmol, 95%) as a light yellow oil. R$_f$ (CH$_2$Cl$_2$:Ultra 9:1) = 0.24. ^{1}H NMR (CDCl$_3$, 400 MHz): δ 4.20 (q, 2 H, *J* = 7.2 Hz, CO$_2$C*H$_2$*CH$_3$), 3.87 (dd, 1 H, *J* = 8.6, 4.1 Hz, NH$_2$C*H*), 3.22 (dd, 1 H, *J* = 13.1, 4.1 Hz,

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[14] One ^{13}C signal could not be resolved.

SCH_2), 2.77 (dd, 1 H, J = 13.1, 8.6 Hz, SCH_2), 2.22 (bs, 2 H, NH_2), 1.27 (t, 3 H, J = 7.2 Hz, CO$_2$CH$_2$CH_3), 1.11-1.00 (m, 21 H, TIPS). ^{13}C NMR (CDCl$_3$, 100 MHz) δ 173.2, 98.2, 94.5, 61.6, 54.1, 41.1, 18.7, 14.3, 11.4. IR ν 3327 (w), 2941 (m), 2866 (m), 2091 (w), 1735 (s), 1464 (w), 1373 (w), 1196 (m), 1115 (w), 1020 (m), 882 (s), 741 (m). HRMS (ESI) C$_{16}$H$_{32}$NO$_2$SSi$^+$ [M+H]$^+$ calc. = 330.1918; [M+H]$^+$ obs. = 330.1927.

(*R*)-Ethyl 2-((*S*)-2-(((benzyloxy)carbonyl)amino)-3-(1*H*-indol-3-yl)propanamido)-3-((((triisopropylsilyl)ethynyl)thio)propanoate (31)
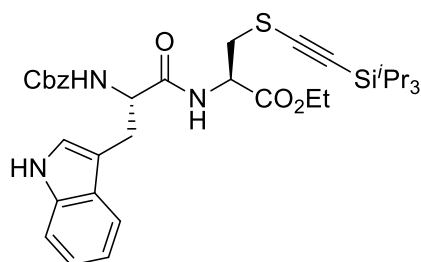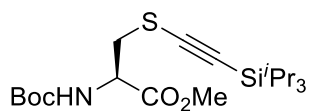

The reaction mixture was diluted with water (10 mL) and extracted with EtOAc (3 x 10 mL). The combined organic layers were dried over MgSO$_4$, filtered and concentrated *in vacuo*. The resulting crude oil was purified by flash chromatography using pentane:EtOAc 2:1 as mobile phase affording **31** (247 mg, 0.380 mmol, 95%) as a clear colorless oil. R$_f$ (pentane:EtOAc 1:1) = 0.81. ^{1}H NMR (CDCl$_3$, 400 MHz): δ 8.32 (d, 1 H, J = 2.7 Hz), 7.63 (d, 1 H, J = 7.9 Hz), 7.40-7.28 (m, 6 H), 7.22-7.15 (m, 1 H), 7.10 (t, 1 H, J = 7.5 Hz), 7.04 (s, 1 H), 6.61 (d, 1 H, J = 7.0 Hz), 5.58 (d, 1 H, J = 7.7 Hz), 5.11 (s, 2 H), 4.77-4.68 (m, 1 H), 4.64-4.50 (m, 1 H), 4.21-4.07 (m, 2 H), 3.35 (dd, 1 H, J = 14.7, 5.5 Hz), 3.27-3.08 (m, 2 H), 2.99 (dd, 1 H, J = 13.9, 6.2 Hz), 1.25 (t, 3 H, J = 7.2 Hz, CO$_2$CH$_2$CH_3), 1.13-1.01 (m, 21 H, TIPS). ^{13}C NMR (CDCl$_3$, 100 MHz) δ 171.4, 169.5, 156.0, 136.3, 136.2, 128.6, 128.2, 128.1, 127.5, 123.5, 122.3, 119.8, 118.7, 111.4, 110.1, 98.1, 94.3, 67.1, 62.1, 55.6, 51.9, 38.2, 28.5, 18.7, 14.1, 11.4. IR ν 3329 (w), 2943 (w), 2865 (w), 2092 (w), 1710 (m), 1670 (m), 1513 (m), 1211 (w), 1030 (w), 910 (m), 735 (s). HRMS (ESI) C$_{35}$H$_{47}$N$_3$NaO$_5$SSi$^+$ [M+Na]$^+$ calc. = 672.2898; [M+Na]$^+$ obs. = 672.2884.

(*R*)-Ethyl 2-((*S*)-2-(((benzyloxy)carbonyl)amino)-3-(4-hydroxyphenyl)propanamido)-3-((((triisopropylsilyl)ethynyl)thio)propanoate (32)
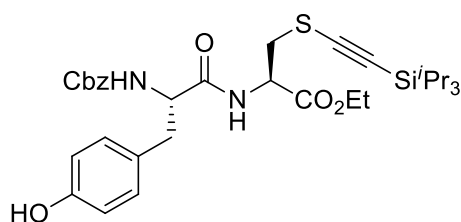
The reaction mixture was diluted with water (10 mL) and extracted with EtOAc (3 x 10 mL). The combined organic layers were dried over MgSO$_4$, filtered and concentrated *in vacuo*. The resulting crude oil was purified by flash chromatography using pentane:EtOAc 3:2 as mobile phase affording **32** (240 mg, 0.383 mmol, 96%) as a white solid. R$_f$ (pentane:EtOAc 3:2) = 0.72. Melting point = 104.7-106.1 °C. ^{1}H NMR (CDCl$_3$, 400 MHz): δ 7.39-7.23 (m, 5 H, Cbz), 6.97 (d, 2 H, J = 8.0 Hz, ArH), 6.85 (s, 1 H, OH), 6.82 (d, 1 H, J = 8.2 Hz, NH), 6.68 (d, 2 H, J = 8.3 Hz, ArH), 5.50 (d, 1 H, J = 8.0 Hz, NH), 5.08 (s, 2 H), 4.85-4.74 (m, 1 H), 4.52-4.38 (m, 1 H), 4.28-4.11 (m, 2 H), 3.22 (dd, 1 H, J = 13.4, 5.1 Hz), 3.12-2.86 (m, 3 H), 1.27 (t, 3 H, J = 7.1 Hz, CO$_2$CH$_2$CH_3), 1.13-0.99 (m, 21 H, TIPS). ^{13}C NMR (CDCl$_3$, 100 MHz) δ 171.4, 169.7, 156.2, 155.4, 136.0, 130.5, 128.6, 128.3, 128.1, 127.4, 115.8, 98.4, 94.0, 67.3, 62.3, 56.3, 52.0, 38.1, 37.8, 18.7, 14.1, 11.4. IR ν 3321 (w), 2944 (w), 2865 (w), 2092 (w), 1667 (m), 1516 (s), 1219 (s), 1024 (w), 911 (m), 856 (w), 734 (s). HRMS (ESI) C$_{33}$H$_{47}$N$_2$O$_6$SSi$^+$ [M+H]$^+$ calc. = 627.2919; [M+H]$^+$ obs. = 627.2905.

(*R*)-Ethyl 2-((*S*)-2-(((benzyloxy)carbonyl)amino)-3-hydroxypropanamido)-3-(((triisopropylsilyl)ethynyl)thio)propanoate (**33**)
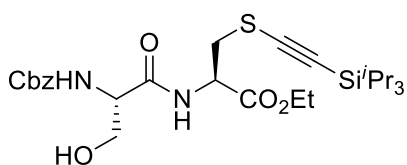
The reaction mixture was diluted with water (10 mL) and extracted with EtOAc (3 x 10 mL). The combined organic layers were dried over MgSO$_4$, filtered and concentrated *in vacuo*. The resulting crude oil was purified by flash chromatography using pentane:EtOAc 2:1 to 1:1 as mobile phase affording **33** (216 mg, 0.391 mmol, 98%) as a clear colorless oil. R$_f$ (EtOAc:pentane 3:2) = 0.56. ^{1}H NMR (CDCl$_3$, 400 MHz): δ 7.50 (d, 1 H, *J* = 7.7 Hz, N*H*), 7.39-7.25 (m, 5 H, Cbz), 6.01 (d, 1 H, *J* = 7.3 Hz, N*H*), 5.17-5.05 (m, 2 H), 4.91-4.80 (m, 1 H, *J* = 13.6, 4.1 Hz), 4.42-4.30 (m, 1 H), 4.28-4.13 (m, 2 H), 4.06-3.89 (m, 1 H), 3.79-3.58 (m, 2 H), 3.28 (dd, 1 H, *J* = 13.4, 4.6 Hz), 3.05 (dd, 1 H, *J* = 13.5, 7.4 Hz), 1.26 (t, 3 H, *J* = 7.1 Hz, CO$_2$CH$_2$C*H$_3$*), 1.13-0.98 (m, 21 H, TIPS). ^{13}C NMR (CDCl$_3$, 100 MHz) δ 170.9, 170.2, 156.5, 136.1, 128.6, 128.2, 128.1, 98.6, 94.0, 67.3, 63.1, 62.3, 55.7, 52.2, 37.8, 18.6, 14.1, 11.3. IR ν 3413 (w), 3322 (w), 2946 (w), 2867 (w), 2092 (w), 1721 (m), 1678 (m), 1515 (w), 1216 (m), 1062 (w), 909 (s), 733 (s). HRMS (ESI) C$_{27}$H$_{43}$N$_2$O$_6$SSi$^+$ [M+H]$^+$ calc. = 551.2606; [M+H]$^+$ obs. = 551.2611.

(*S*)-1-((*S*)-2-Methyl-3-(((triisopropylsilyl)ethynyl)thio)propanoyl)pyrrolidine-2-carboxylic acid (**34**)
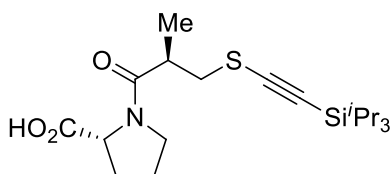
The natural product captopril was dissolved in THF (5.0 mL) and water (0.5 mL) to ensure a homogenous mixture throughout the course of the reaction. The reaction mixture was concentrated *in vacuo* and purified by flash chromatography using pentane:EtOAc 10:1 to 1:1 with 1% AcOH as mobile phase affording **34** (143 mg, 0.360 mmol, 90%) as a white solid. R$_f$ (EtOAc:AcOH 99:1) = 0.41. Melting point = 98.9-101.0 °C. ^{1}H NMR (CDCl$_3$, 400 MHz): δ 11.5 (bs, 1 H, COO*H*), 4.58 (dd, 1 H, *J* = 8.1, 3.7 Hz, NC*H*), 3.80-3.69 (m, 1 H), 3.67-3.55 (m, 1 H), 3.15 (dqd, 1 H, *J* = 5.3, 6.8, 8.9 Hz, CH$_3$C*H*CH$_2$S), 2.96 (dd, 1 H, *J* = 8.8, 13.0 Hz, CH$_3$CHC*H$_2$*S), 2.75 (dd, 1 H, *J* = 13.0, 5.5 Hz, CH$_3$CHC*H$_2$*S), 2.32-1.91 (m, 4 H), 1.25 (d, 3 H, *J* = 6.8 Hz, C*H$_3$*CHCH$_2$S), 1.10-0.99 (m, 21 H, TIPS). ^{13}C NMR (CDCl$_3$, 100 MHz) δ 175.1, 174.8, 97.6, 95.8, 59.4, 47.6, 39.0, 38.1, 28.4, 24.8, 18.7, 16.9, 11.4. IR ν 2943 (s), 2866 (m), 2089 (w), 1743 (m), 1613 (s), 1463 (s), 1188 (w), 915 (w), 883 (m), 856 (s), 734 (s). HRMS (ESI) C$_{20}$H$_{36}$NO$_3$SSi$^+$ [M+H]$^+$ calc. = 398.2180; [M+H]$^+$ obs. = 398.2175.

Scale-up Reaction for Thiol-Alkynylation Product 29

A 50 mL round bottom flask was charged with a magnetic stirring bar, N-(*tert*-butoxycarbonyl)- L-cysteine methyl ester (485 mg, 2.06 mmol, 1.00 eq.), TMG (0.314 mL, 2.47 mmol, 1.20 eq.) and THF (26 mL). After stirring the resulting solution for 5 minutes at room temperature, TIPS-EBX (**6**, 971 mg, 2.27 mmol, 1.10 eq.) was added as a solid in one portion. The resulting reaction mixture was stirred in an open flask for 5 minutes at room temperature. The reaction mixture was diluted with water (30 mL) and extracted with EtOAc (3 x 20 mL). The combined organic layers were dried over MgSO₄, filtered and concentrated *in vacuo*. The resulting crude product was purified by flash chromatography using pentane:EtOAc 12:1 as mobile phase affording **29** (851 mg, 2.05 mmol, quant.) as a clear colorless oil.[15]

Competition Experiment with Lysine and Histidine
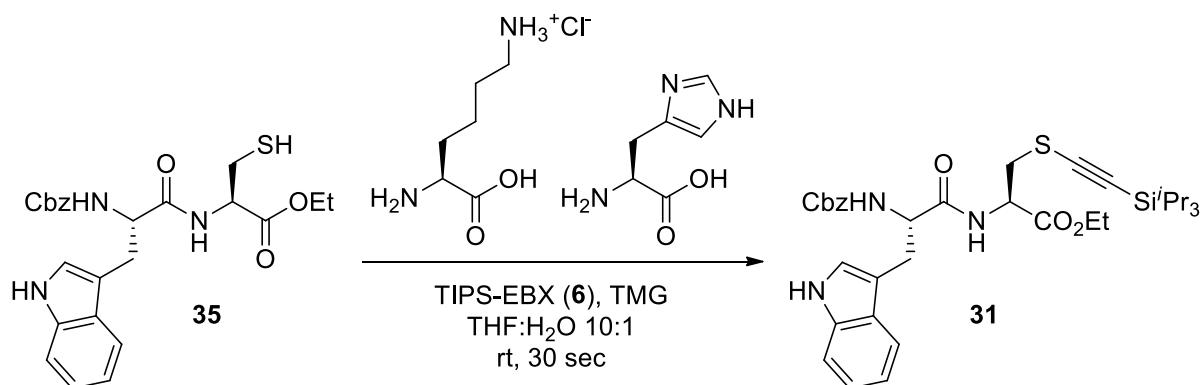
A 25 mL round bottom flask was charged with a magnetic stir bar, dipeptide **35** (188 mg, 0.400 mmol, 1.00 eq.), L-lysine (73.1 mg, 0.400 mmol, 1.00 eq.), L-histidine (62.1 mg, 0.400 mmol, 1.00 eq.), TMG (60.4 µL, 0.480 mmol, 1.20 eq.), THF (5.0 mL) and water (0.5 mL). After stirring the resulting solution for 5 minutes at room temperature, TIPS-EBX (**6**, 188 mg, 0.440 mmol, 1.10 eq.) was added as a solid in one portion. The resulting reaction mixture was stirred in an open flask for 30 seconds at room temperature. After exactly 30 seconds, the reaction mixture was diluted with water (10 mL) and extracted with EtOAc (3 x 10 mL). The combined organic layers were dried over MgSO₄, filtered and concentrated *in vacuo*. The resulting crude product was purified by flash chromatography using pentane:EtOAc 2:1 as mobile phase affording **31** (251 mg, 0.386 mmol, 97%) as a clear colorless oil.[16]

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[15] The ¹H and ¹³C NMR spectra matched those from **29** analyzed in the reaction scope section.

[16] The ¹H and ¹³C NMR spectra matched those from **31** analyzed in the reaction scope section.

Fluorophore Installment *via* the CuAAC

S6, Dans-(CH$_2$)$_3$-N$_3$
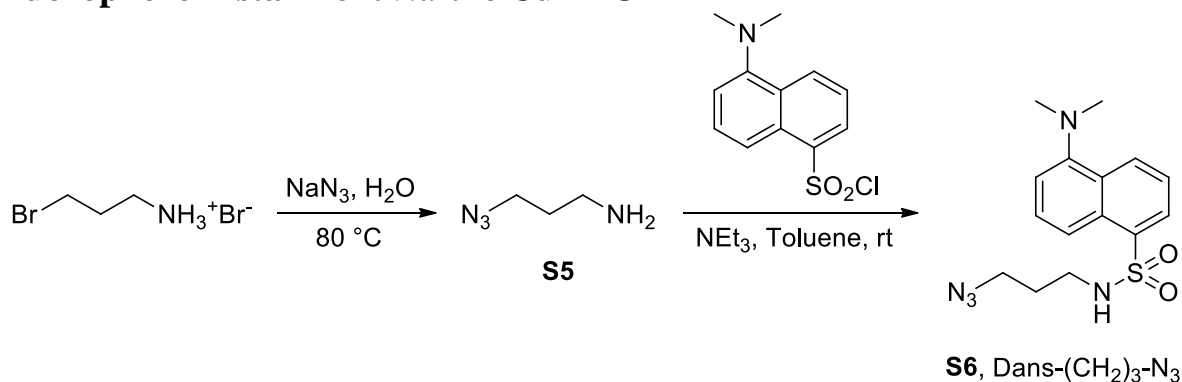
3-Azidopropylamine (**S5**)

Following a reported procedure,[17] a round bottom flask fitted with a condenser was charged with 3-bromopropylamine hydrobromide (4.98 g, 22.3 mmol, 1.00 eq.) and sodium azide (2.47 g, 37.9 mmol, 1.70 eq.). The two solids were dissolved in water (40 mL) and the resulting solution was stirred at 80 °C overnight for 17 hours. The reaction mixture was reduced to about 15 mL total volume under reduced pressure at 50 °C. The mixture was diluted with 5% (v:v) aq. NaOH (9.0 mL) and stirred at room temperature for 2 hours. The aq. mixture was extracted with toluene (5 x 20 mL). The combined organic layers dried over MgSO$_4$ and filtered. The volume was then reduced by rotary evaporation to 4.8 g of solution. The retained solution was found to contain 13.8 mol% of 3-azidopropylamine (**S5**) by NMR integration, corresponding to 15% by weight (0.72 g) of the desired product (32% yield). ^{1}H NMR (400 MHz, CDCl$_3$) δ 3.46 (t, 2 H, J = 6.7 Hz NH$_2$C*H*$_2$CH$_2$), 2.91 (t, 2 H, J = 6.8 Hz, N$_3$C*H*$_2$H$_2$), 1.83 (p, 2 H, J = 6.8 Hz, NH$_2$CH$_2$C*H*$_2$CH$_2$N$_3$), 1.15 (bs, 2 H, N*H*$_2$). The ^{1}H NMR data is in accordance with reported literature values.[17]

N-(3-Azidopropyl)-5-(dimethylamino)naphthalene-1-sulfonamide (**S6**, Dans(CH$_2$)$_3$-N$_3$)

Following a reported procedure,[17] a solution of triethylamine (1.12 mL, 7.99 mmol, 2.40 eq.) and 3-azidopropylamine (**S5**, 0.33 g as a 15% by weight solution in toluene, 3.33 mmol, 1.00 eq.) were added all at once to a stirred suspension of dansyl chloride (1.08 g, 3.99 mmol, 1.20 eq.) in toluene (25 mL). The resulting yellow mixture was stirred at room temperature overnight for 15 hours. Next, the reaction mixture was concentrated *in vacuo* and the resulting crude oil was purified by flash chromatography (pentane:EtOAc 3:1 to 1:2) affording (**S6**, Dans(CH$_2$)$_3$-N$_3$) (1.02 g, 3.06 mmol, 92%) as a yellow oil. ^{1}H NMR (400 MHz, CDCl$_3$): 8.56 (dt, 1 H, J = 8.6, 1.1 Hz), 8.31-8.23 (m, 2 H), 7.55 (ddd, 2 H, J = 15.9, 8.6, 7.4 Hz), 7.19 (dd, 1 H, J = 7.6, 0.9 Hz), 5.02 (t, 1 H, J = 6.3 Hz), 3.25 (t, 2 H, J = 6.4 Hz), 2.98 (q, 2 H, J = 6.4 Hz), 2.89 (s, 6 H), 1.65 (m, 2 H). ^{13}C NMR (100 MHz, CDCl$_3$): δ 152.4, 134.5, 130.8, 130.1, 129.9, 129.7, 128.7, 123.3, 118.6, 115.4, 48.9, 45.5, 40.9, 28.9. The NMR data is in accordance with reported literature values.[18]

[17] Lewis, W. G.; Magallon, F. G.; Fokin, V. V.; Finn, M. G. *J. Am. Chem. Soc.* **2004**, *126*, 9152.

[18] Chan, A. O.; Ho, C. M.; Chong, H. C.; Leung, Y. C.; Huang, J. S.; Wong, M. K.; Che, C. M. *J. Am. Chem. Soc.* **2012**, *134*, 2589.

(*R*)-Ethyl 2-((*S*)-2-(((benzyloxy)carbonyl)amino)-3-(1*H*-indol-3-yl)propanamido)-3-(ethynylthio)propanoate (S7)
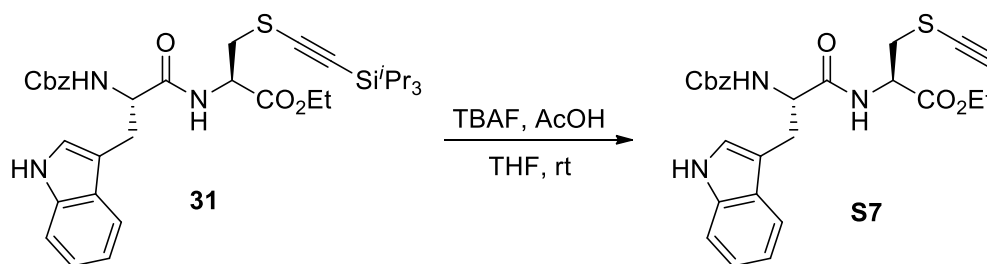
To a solution of TIPS protected alkyne **31** (0.25 g, 0.39 mmol, 1.00 eq.) and THF (5.0 mL) was added dropwise at 0 °C *via* a dropping funnel a mixture of tetra-*n*-butylammonium fluoride (1.0 M in THF, 770 µL, 0.770 mmol, 2.00 eq.) and AcOH (66.1 µL, 1.15 mmol, 3.00 eq.) diluted in THF (1.5 mL). The ice bath was removed and the reaction mixture was stirred at room temperature overnight for 10 hours. Next, the reaction mixture was diluted with water (10 mL) and extracted with EtOAc (3 x 10 mL). The combined organic layers were dried over MgSO$_4$, filtered and concentrated *in vacuo*. The resulting crude product was purified by flash chromatography (pentane:EtOAc 2:1 to 1:1) affording **S7** (173 mg, 0.351 mmol, 91%) as a white solid. R$_f$ (pentane:EtOAc 5:4 and 1% AcOH) = 0.64. Melting point = 153.8-156.7 °C. ^{1}H NMR (CDCl$_3$, 400 MHz): δ 8.13 (s, 1 H), 7.68 (d, 1 H, *J* = 8.0 Hz), 7.43-7.29 (m, 6 H), 7.22-7.15 (m, 1 H), 7.15-7.05 (m, 2 H), 6.65 (d, 1 H, *J* = 7.4 Hz), 5.53 (d, 1 H, *J* = 7.9 Hz), 5.20-5.09 (m, 2 H), 4.80 (dt, 1 H, *J* = 4.6, 7.4 Hz), 4.66-4.51 (m, 1 H), 4.24-4.06 (m, 2 H), 3.42 (dd, 1 H, *J* = 15.0, 4.9 Hz), 3.17 (dd, 1 H, *J* = 14.5, 7.6 Hz), 3.13-3.00 (m, 2 H), 2.32 (s, 1 H, SC$_2$*H*), 1.25 (t, 3 H, *J* = 7.1 Hz, CO$_2$CH$_2$C*H$_3$*). ^{13}C NMR (CDCl$_3$, 100 MHz) δ 171.2, 169.2, 156.0, 136.4, 136.3, 128.7, 128.3, 128.2, 127.5, 123.7, 122.4, 119.9, 118.9, 111.4, 110.2, 82.3, 73.4, 67.2, 62.2, 55.5, 52.6, 36.8, 28.7, 14.1. IR ν 3383 (w), 2252 (w), 1716 (w), 1686 (w), 1518 (w), 1322 (w), 1189 (w), 1025 (w), 907 (s), 729 (s). HRMS (ESI) C$_{26}$H$_{28}$N$_3$O$_3$S$^+$ [M+H]$^+$ calc. = 494.1744; [M+H]$^+$ obs. = 494.1747.

(*R*)-Ethyl 2-((*S*)-2-(((benzyloxy)carbonyl)amino)-3-(1*H*-indol-3-yl)propanamido)-3-((1-(3-(5-(dimethylamino)naphthalene-1-sulfonamido)propyl)-1*H*-1,2,3-triazol-4-yl)thio)propanoate (36)
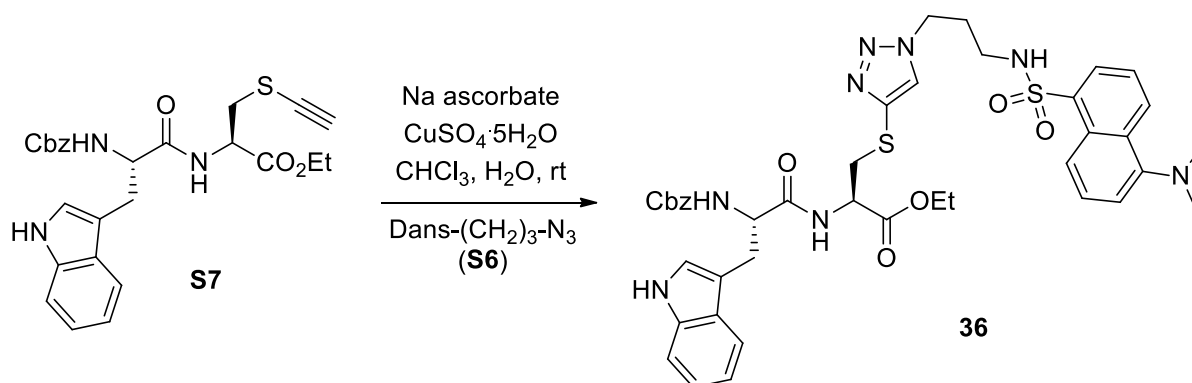
Terminal alkyne **S7** (99.0 mg, 0.200 mmol, 1.00 eq.) and Dansyl-(CH$_2$)$_3$-N$_3$ (**S6**, 66.7 mg, 0.20 mmol, 1.00 eq.) were suspended in chloroform (3.0 mL). To the mixture was added a solution of sodium ascorbate (7.90 mg, 40.0 µmol, 0.20 eq.) and copper(II) sulfate pentahydrate (1.00 mg, 4.00 µmol, 0.10 eq.) in water (200 µL). The heterogeneous reaction

mixture was stirred vigorously at room temperature for 24 hours, after which it was directly purified by column chromatography (EtOAc:pentane 1:1 to 3:1) affording **36** (153 mg, 0.185 mmol, 93%) as a yellow foam. R_f (EtOAc:pentane 4:1) = 0.44. Melting point = 75.7-80.1 °C. ^{1}H NMR (CDCl$_3$, 400 MHz): δ 8.62 (s, 1 H), 8.51 (d, 1 H, J = 8.5 Hz), 8.29 (d, 1 H, J = 8.6 Hz), 8.16 (d, 1 H, J = 7.2 Hz), 7.63-7.51 (m, 2 H), 7.46 (t, 2 H, J = 8.0 Hz), 7.33-7.14 (m, 7 H), 7.14-7.02 (m, 3 H), 6.98 (t, 1 H, J = 7.5 Hz), 6.14-5.99 (m, 1 H), 5.76 (d, 1 H, J = 8.0 Hz), 5.00 (s, 2H), 4.76-4.64 (m, 1 H), 4.63-4.50 (m, 1 H), 4.25-3.94 (m, 4 H), 3.34-3.06 (m, 4 H), 2.83 (s, 6 H, N(CH_3)$_2$), 2.76-2.68 (m, 2 H), 1.86 (p, 2 H, J = 6.5 Hz), 1.15 (t, 3 H, J = 7.1 Hz, CO$_2$CH$_2$CH_3). ^{13}C NMR (CDCl$_3$, 100 MHz)[19] δ 171.9, 169.9, 156.2, 152.1, 138.2, 136.4, 136.3, 134.5, 130.6, 129.9, 129.5, 128.6, 128.5, 128.0, 127.9, 127.6, 127.0, 123.9, 123.3, 121.9, 119.5, 118.7, 115.4, 111.4, 109.9, 66.9, 61.9, 55.6, 52.7, 47.3, 45.4, 39.8, 36.5, 29.7, 28.4, 14.1. IR ν 3301 (w), 2944 (w), 2252 (w), 1713 (w), 1668 (w), 1503 (w), 1457 (w), 1312 (w), 1205 (w), 1144 (m), 1048 (w), 908 (s), 793 (w), 729 (s). HRMS (ESI) C$_{41}$H$_{47}$N$_8$O$_7$S$_2$$^+$ [M+H]$^+$ calc. = 827.3004; [M+H]$^+$ obs. = 827.3002.

Spectra of New Compounds

Shown below are the corresponding ^{1}H, ^{13}C NMR and IR spectra of the above fully characterized new compounds.

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[19] Several signals were not resolve at 100 MHz.

10

12

13

HO2C $\left(\right)_{13}$ S $\equiv$ Si^{i}Pr$_3$

14

17

MeO—⟨ ⟩—S—≡—Si^{i}Pr$_3$

18

19

24

25

27

28

29

31

36

S3