RSC Medicinal Chemistry

Accepted Manuscript

This article can be cited before page numbers have been issued, to do this please use: E. Kim, J. Y. Hyun, J. H. Song, H. J. Lim and S. J. Park, *RSC Med. Chem.*, 2026, DOI: 10.1039/D5MD00948K.

ROYAL SOCIETY OF CHEMISTRY

rsc.li/medchem

Research articles RSC Medicinal Chemistry

N-Cyano Sulfoximine-Mediated Thiazole Ligation with *N*-Terminal Cysteine under Mild Aqueous Conditions

Communication
DOI: 10.1039/D5MD00948K

Eunsil Kim, [a,b] Ji Young Hyun, [a,b] Ji Ho Song, [a,b] Hwan Jung Lim *[a,b] and Seong Jun Park *[a,b]

Received 00th January 20xx,
Accepted 00th January 20xx

DOI: 10.1039/x0xx00000x

An *N*-terminal cysteine-selective click reaction employing *N*-cyano sulfoximines enables rapid thiazole formation under mild conditions. These three-demensional, hydrophilic scaffolds offer high selectivity, tunable reactivity, and improved drug-like properties. The platform holds promise for bioorthogonal conjugation and ligand design in drug discovery applications.

Efficient and selective chemical ligation methods have greatly advanced chemical biology and materials science.[1-3] Among these, click reactions such as the copper-catalyzed azide–alkyne cycloaddition (CuAAC)[4,5] and sulfur(VI) fluoride exchange (SuFEx) are powerful tools for modular synthesis. In particular, SuFEx using sulfuryl fluoride (SO_2F_2) affords stable linkages, though the resulting connectivities are typically limited to planar geometries.[6,7]

The shift from two-dimensional (2D) to three-dimensional (3D) molecular design is emerging as a promising strategy in medicinal chemistry to enhance aqueous solubility without compromising permeability or efficacy. Adoption of non-planar molecular architectures provides an effective strategy to address solubility challenges in drug development (Figure 1).[8–12]

Thionyl tetrafluoride (SOF_4) enables the formation of tetrahedral iminosulfur oxydifluorides with two reactive S–F handles, offering precise spatial control and polyvalency for constructing 3D architectures in biomolecular engineering and materials science.[13–15] However, SuFEx chemistry remains limited by reagent availability, substrate scope, and scalability.[11,16]

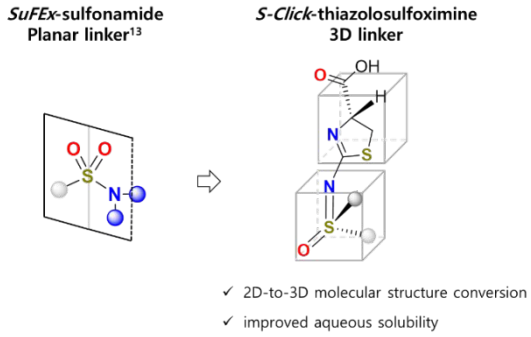


Figure 1. The thiazolosulfoximine 3D linker identified in this study

To establish a practical biomimetic approach, we selected readily accessible sulfoximines that enable the construction of three-dimensional molecular architectures.[17] Bioisosteric replacement of sulfone or sulfonamide groups with sulfoximine moieties significantly enhances aqueous solubility and has facilitated the clinical progression of a lead compound.[18–20]

N-cyanosulfoximines[21–32] are particularly attractive due to their ease of synthesis (Figure 2a), high aqueous solubility, three-dimensional molecular features, and the presence of a reactive cyano group amenable to click-type conjugation. Their potential as bioorthogonal platforms is further supported by the well-established reactivity of cyano groups—particularly cyanopyridines—with aminothiols.[33–42]

Importantly, *N*-terminal cysteine, a naturally encoded amino acid, was deliberately selected as the reaction partner[43,44] to ensure that the product more closely resembles a naturally occurring structure rather than a purely synthetic one. (Figure 2b). This biomimetic strategy provides a distinct advantage by aligning chemical reactivity with biological relevance and compatibility.

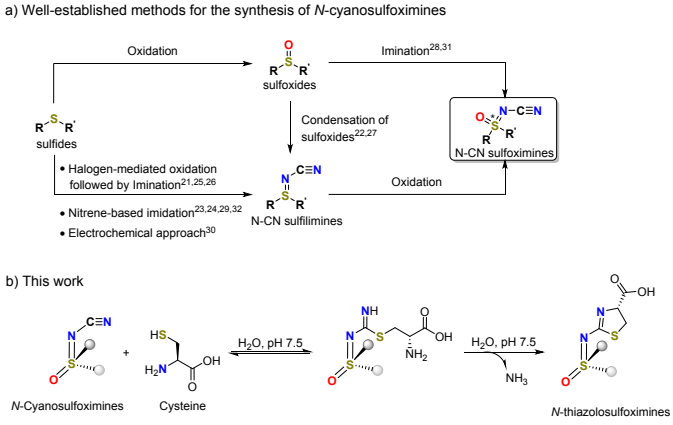


Figure 2. *N*-Cyanosulfoximine as a bioorthogonal conjugation handle

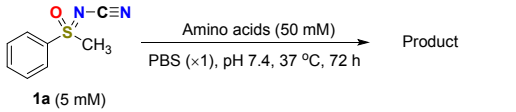
[a] *Department of Drug Discovery, Korea Research Institute of Chemical Technology (KRICT), 141 Gajeong-ro, Yuseong-gu, Daejeon 34114, Republic of Korea Tel.: +82-42-860-7175; fax: +82-42-860-7160; email: sjunpark@krict.re.kr.*
[b] *Pharmaceutical Chemistry, University of Science & Technology, Daejeon 34113, Republic of Korea.*

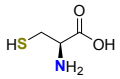
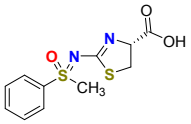
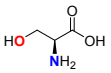
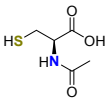
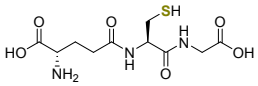
DOI: 10.1039/D5MD00948K

To evaluate the reactivity, we first examined the model reaction of phenylmethyl sulfoximine **1a** with cysteine. The desired click reaction proceeded smoothly to afford the corresponding thiazole **2a** when the reaction was carried out in PBS (pH 7.4) at 37 °C in the presence of 10 equivalents of cysteine (entry 1, Table 1).[45]

No reaction occurred when *N*-cyanosulfoximine **1a** was treated with serine, *N*-acetylcysteine, or glutathione (GSH), indicating high selectivity towards cysteine (entry 2 to 4, Table 1).

Table 1. Selectivity evaluation with other amino acids



Entry	Amino Acids	Product
1		 **2a**[a]
2		–[b]
3		–[b]
4		–[b]

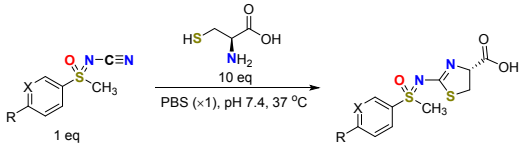
PBS(Phosphate Buffered Saline), [a]*N*-Cyanosulfoximine **1a** was completely converted to the desired thiazole **2a**, as confirmed by LC/MS analysis., [b]No reaction

To modulate the reactivity of the nitrile group in the electrophilic *N*-cyano sulfoximine, various electron-withdrawing or electron-donating groups were introduced onto the phenyl ring. Additionally, heteroatoms were incorporated into the aromatic system to further tune the electron density.

The reactions of **1a** and **1b** were completed after 72 h and 25 h, respectively (entry 1 and 2, Table 2). For *N*-cyanosulfoximine **1b**, which contains a heteroatom within the aromatic ring, a markedly shorter reaction time was observed (entry 2, Table 2).

For *para*-substituted *N*-cyano sulfoximines **1c** and **1d**, poor aqueous solubility prevented dissolution under standard buffer conditions; thus, 2.5% DMSO was added to achieve complete dissolution prior to the reaction. *N*-cyano sulfoximine **1d**, bearing an electron-withdrawing group, afforded the desired thiazole product **2d** with a higher conversion ratio compared to **1c**, which possesses an electron-donating group (entry 3 and 4, Table 2).

Table 2. Reactivity modulation of the electrophilic *N*-cyanosulfoximines **1a**-**1d**



Entry	*N*-Cyano Sulfoximines	R	X	Reaction Time (h)	Conversion (%) to thiazole **2**
1	**1a**	H	CH	72	**2a** (>99)
2	**1b**	H	N	25	**2b** (>99)
3	**1c**	OMe	CH	49	**2c** (9[b])
4	**1d**	NO_2	CH	49	**2d** (47[b])

[a]confirmed by LC/MS analysis, [b]after 49 hours, precipitation occurred

For comparison, the corresponding *N*-cyano sulfonamide **1ba** was also subjected to the reaction. As shown in the X-ray crystal structures in Figure 3, the sulfonamide **1ba** adopts the 2D conformation, whereas the sulfoximine **1b** exhibits the 3D structure. The sulfoximine **1b** also demonstrated approximately 1.7-fold higher solubility. Furthermore, in the case of the sulfonamide **1ba**, the desired thiazole product was not obtained upon reaction with cysteine.

Based on previous findings indicating high reactivity and good solubility under buffer conditions, *N*-cyano sulfoximine **1b** was selected to evaluate the effect of buffer pH on the reaction completion time for thiazole **2b** formation.

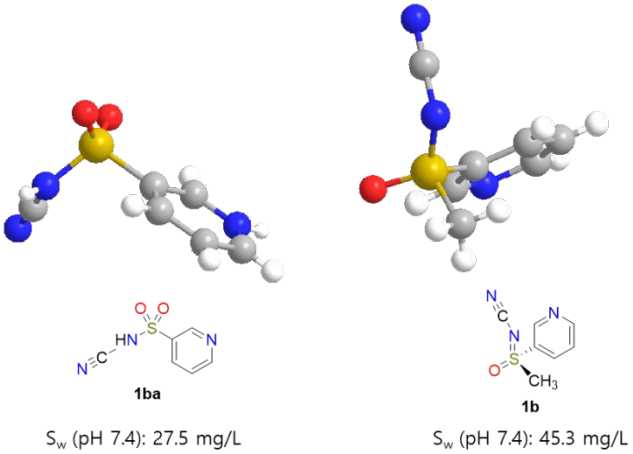


Figure 3. Comparison between *N*-cyano sulfonamide **1ba** and sulfoximine **1b**[46]

Under acidic conditions (pH 4), no reaction was observed, whereas under mildly acidic conditions (pH 6), the reaction reached completion after 72 hours. Under neutral conditions (pH 7.4), the reaction of *N*-cyano sulfoximine **1b** was complete after 24 h. Under basic conditions (pH 8.0–9.0), the reaction was found to reach completion within 7 hours. Interestingly, under strongly basic conditions (pH 10), the reaction was found to reach completion within just 1 hour (Figure 4).

To shorten the reaction completion time at neutral pH, the effect of varying the stoichiometric ratio between *N*-cyano sulfoximine **1b** and cysteine was investigated. As illustrated in Table 3, an excess of the electrophile *N*-cyano sulfoximine **1b** resulted in a faster reaction completion.[47]

Collectively, the results indicate that the use of 10 equivalents of *N*-cyano sulfoximine **1b** and 1 equivalent of cysteine at neutral pH leads to complete conversion within 3 hours, representing the optimised condition.

Figure 4. Investigation of reaction time as a function of pH (*N*-cyano sulfoximine **1b** to thiazole **2b**)

Figure 5. Prediction of the physicochemical properties of azidophiles and *N*-cysteinophile **1b**[49]

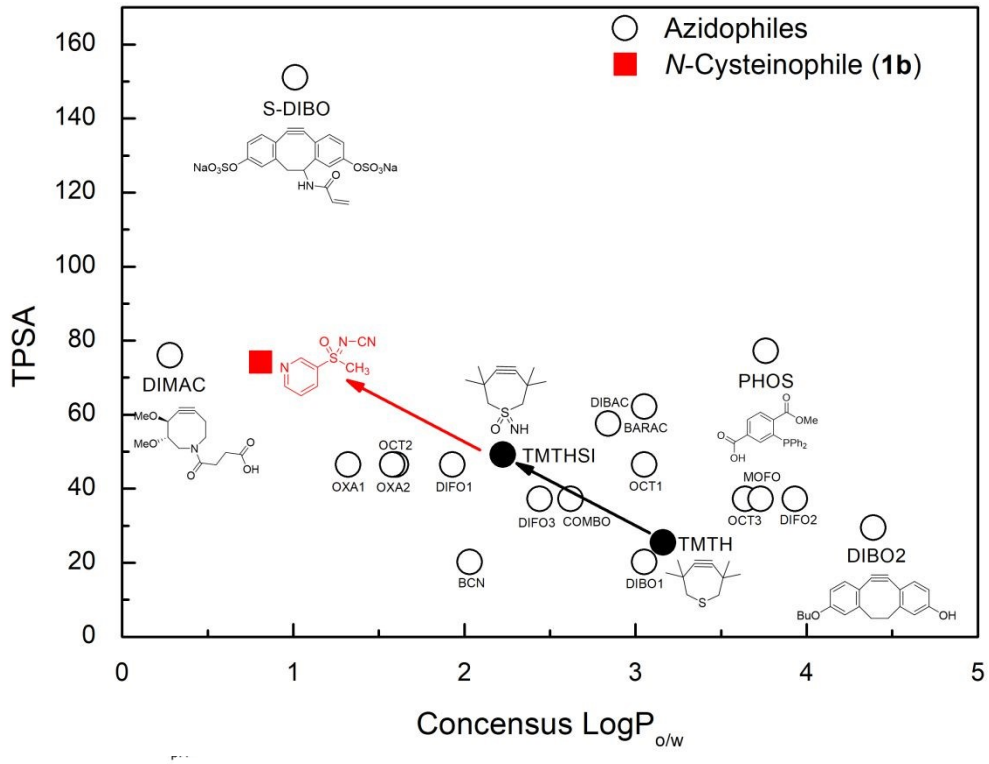
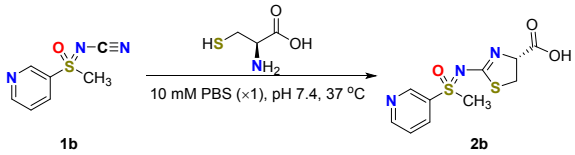


Table 3. Effect of Stoichiometry on Reaction Rate



Entry	**1b** (mM)	Cysteine (mM)	**2b** (Reaction completion time)[a]
1	10	1	3 h
2	5	1	7.5 h
3	1	10	21 h

[a]This was confirmed by ^{1}H NMR analysis

A comparison of the predicted physicochemical properties between the reported azidophile[48] and the *N*-cysteinophile **1b** developed in this study revealed that compound **1b** exhibits significantly more hydrophilic characteristics.[49] The compound **1b** was predicted to exhibit greater hydrophilicity than TMTHSI bearing a sulfoximine moiety, as reported by Liskamp and co-workers (Figure 5).[50]

To evaluate the practical applicability of the developed reaction, the transformation between *N*-cyano sulfoximine-derived methionine **1e** and *N*-terminal cysteine was carried out. Gratifyingly, the reaction reached completion within 1.5 h under mild aqueous conditions (Figure 6).[51] This result is significant as it demonstrates the ability to link the two naturally occurring sulfur-containing amino acids, methionine and cysteine.

To assess site selectivity under the optimized conditions, peptides bearing either *N*-terminal or internal cysteine residues were examined. **Peptide 1** (CGKSRF) bearing an *N*-terminal cysteine readily underwent ligation with *N*-cyano sulfoximine **1b**, affording the expected product after 28 h, as confirmed by LC-MS. In contrast, **Peptide 2** (KSCGRF), containing an internal cysteine, showed no reaction even after 72 h. Notably, efficient ligation was also observed with the longer **Peptide 3** (CGCGESGKSTIVKQMK), which features an *N*-terminal cysteine, completing the reaction within 3 h (Figure 7).[53]

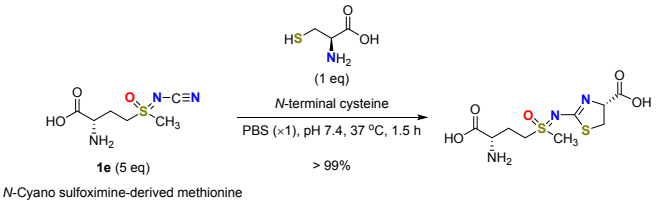


Figure 6. Practical Evaluation with *N*-cyano sulfoximine-derived methionine **1e**

In summary, we have developed a novel *N*-cyano sulfoximine-based click reaction that proceeds selectively with *N*-terminal cysteine to form thiazole linkages under mild, aqueous conditions. Systematic evaluation of structure–reactivity relationships revealed that electronic and solubility properties of the *N*-cyano sulfoximines significantly influence reaction kinetics. Among the tested analogs, compound **1b**, featuring a heteroaryl moiety, exhibited enhanced reactivity and superior aqueous solubility . These findings highlight the potential of *N*-cyano sulfoximines as versatile and biocompatible electrophilic warheads for bioorthogonal conjugation. The practical utility of this method was further demonstrated by its successful application to a methionine-derived *N*-cyano sulfoximine substrate **1e**. Notably, ligation occurred selectively at *N*-terminal cysteines, as shown by efficient conjugation of peptides 1 and 3, while no reaction was observed with the internal cysteine of peptide 2, highlighting the potential of this approach for bioconjugation and chemical biology applications.

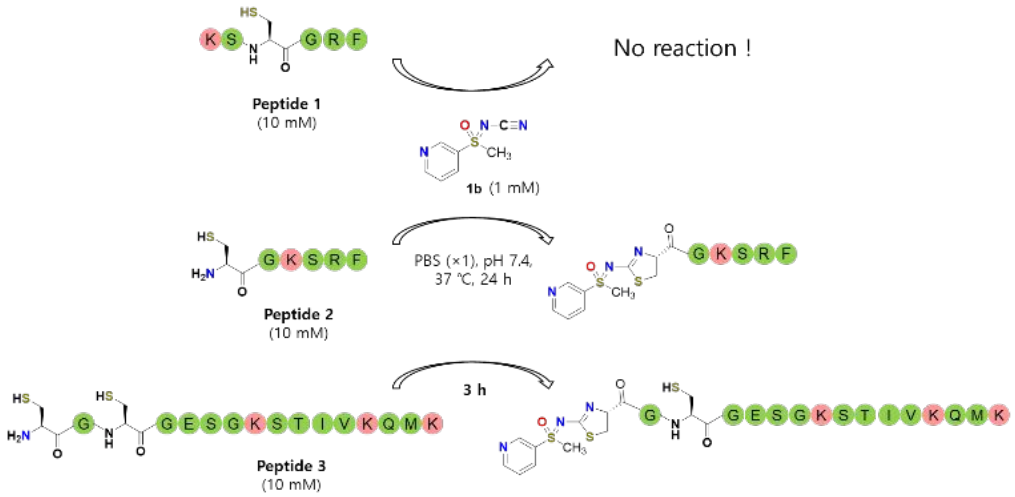


Figure 7. Reaction of **1b** with cysteine-containing peptides

View Article Online
DOI: 10.1039/D5MD00948K

Conflicts of interest

"There are no conflicts to declare".

Data availability

Data for this article, including [description of data types] are available at [name of repository] at [URL – format https://doi.org/DOI].
The data supporting this article have been included as part of the Supplementary Information.

Acknowledgements

This research was supported by the Korea Drug Development Fund funded by Ministry of Science and ICT, Ministry of Trade, Industry and Energy, and Ministry of Health and Welfare (RS-2025-02223190, Republic of Korea) and by KRICT (KK2431-20 and KK2531-20).

Notes and references

1 R. E. Bird, S. A. Lemmel, X. Yu, Q. A. Zhou, *Bioconjugate Chem*. 2021, **32**, 2457-2479.
2 K. M. Hartung, E. M. Sletten, *Chem* 2023, **9**, 2095-2109.
3 F. M. Zielke, F. P. J. T. Rutjes, *Topics in Current Chemistry*, 2023, **381**, 1-30.
4 J. E. Hein, V. V. Fokin, *Chem. Soc. Rev.*, 2010, **39**, 1302-1315.
5 E. Haldón, M. C. Nicasio, P. J. Pérez, *Org. Biomol. Chem.* 2015, **13**, 9528-9550.
6 J. Dong, L. Krasnova, M. G. Finn, K. B. Sharpless, *Angew. Chem. Int. Ed.* 2014, **53**, 9430-9448.
7 T. A. Fattah, A. Saeed, F. Albericio, *Journal of Fluorine Chemistry,* 2018, **213**, 87-112.
8 F. Lovering, J. Bikker, C. Humblet, *J. Med. Chem.* 2009, **52**, 6752-6756.
9 M. Ishikawa, Y. Hashimoto, *J. Med. Chem.* 2011, **54**, 1539-1554.
10 M. A. Walker, *Expert Opin. Drug Discov.* 2014, **9**, 1-13.
11 Z. He, W. Yang, F. Yang, J. Zhang, L. Ma, *Eur. J. Med. Chem.* 2024, **279**, 116842.
12 B. Das, A. T. K. Baidya, A. T. Mathew, A. K. Yadav, R. Kumar, *Bioorg. Med. Chem.* 2022, **56**, 116614.
13 S. Li, P. Wu, J. E. Moses, K. B. Sharpless, *Angew. Chem. Int. Ed.* 2017, **56**, 2903-2908.
14 B. Gao, S. Li, P. Wu, J. E. Moses, K. B. Sharpless, *Angew. Chem. Int. Ed.* 2018, **57**, 1939-1943.
15 D. Zeng, W.-P. Deng, X. Jiang, *Natl. Sci. Rev.* 2023, **10**, 1-19.
16 A. S. Barrow, C. J. Smedley, Q. Zheng, S. Li, J. dong, J. E. Moses, *Chem. Soc. Rev.,* 2019, **48**, 4731-4758.
17 M. Andresini, A. Tota, L. Degennaro, J. A. Bull, R. Luisi, *Chem. Eur. J.* 2021, **27**, 17293-17321.
18 U. Lücking, *Org. Chem. Front.* 2019, **6**, 1319-1324.
19 P. Mäder, L. Kattner, *J. Med. Chem.* 2020, **63**, 14243-14275.
20 Y. Han, K. Xing, J. Zhang, T. Tong, Y. Shi, H. Cao, H. Yu, Y. Zhang, D. Liu, L. Zhao, *Eur. J. Med. Chem.* 2021, **209**, 112885.
21 D. Swern, I. Ikeda, G. F. Whitfield, *Tetrahedron Lett.* 1972, **13**, 2635-2638.
22 T. E. Varkey, G. F. Whitfield, D. Swern, *J. Org. Chem.* 1974, **39**, 3365-3372.
23 J. E. G. Kemp, D. Ellis, M. D. Closier, *Tetrahedron Lett.* 1979, **20**, 3781-3784.
24 O. G. Mancheño, C. Bolm, *Org. Lett.* 2007, **9**, 2951-2954.
25 O. G. Mancheño, O. Bistri, C. Bolm, *Org. Lett.* 2007, **9**, 3809-3811.
26 A. Pandey, C. Bolm, *Synthesis* 2010, **17**, 2922-2925.
27 C. M. M. Hendriks, P. Lamers, J. Engel, C. Bolm, *Adv. Synth. Catal.* 2013, **355**, 3363-3368.
28 C. A. Dannenberg, L. Fritze, F. Krauskopf, C. Bolm, *Org. Biomol. Chem.* 2017, **15**, 1086-1090.
29 S. M. Kim, O. -Y. Kang, H. J. Lim, S. J. Park, *ACS Omega* 2020, **5**, 10191-10199.
30 M. Klein, S. R. Waldvogel, *Angew. Chem. Int. Ed.* 2021, **60**, 23197-23201.
31 N. Amri, T. Wirth, *J. Org. Chem.* 2021, **86**, 15961-15972.
32 Y. J. Seo, E. Kim, I. S. Oh, J. Y. Hyun, J. H. Song, H. J. Lim, S. J. Park, *RSC Adv.,* 2023, **13**, 24445-24449.
33 R. M. Oballa, J.-F. Truchon, C. I. Bayly, N. Chauret, S. Day, S. Crane, C. Berthelette, *Bioorg. Med. Chem. Lett.* 2007, **17**, 998-1002.
34 V. Ehmke, J. E. Q. Quinsaat, P. Rivera-Fuentes, C. Heindl, C. Freymond, M. Rottmann, R. Brun, T. Schirmeister, F. Diederich, *Org. Biomol. Chem.*, 2012, **10**, 5764-5768.
35 O. V. Maltsev, V. Walter, M. J. Brandl, L. Hintermann, *Synthesis* 2013, **45**, 2763-2767.
36 C. Nitsche, H. Onagi, J.-P. Quek, G. Otting, D. Luo, T. Huber, *Org. Lett.* 2019, **21**, 4709-4712.
37 Z. Chen, M. Chen, Y. Cheng, T. Kowada, J. Xie, X. Zheng, J. Rao, *Angew. Chem. Int. Ed.* 2020, **59**, 3272-3279.
38 F. -J. Chen, J. Gao, *Chem. Eur. J.* 2022, **28**, e202201843.
39 M. Proj, N. Strašek, S. Pajk, D. Knez, I. Sosic, *Bioconjugate Chem.* 2023, **34**, 1271-1281.
40 C. Nitsche, *Synlett* 2024, **35**, 1067-1071.
41 V. J. Thombare, Y. Wu, K. Pamulapati, M. Han, J. Tailhades, M. J. Cryle, K. D. Roberts, T. Velkov, J. Li, N. A. Patil, *Chem. Eur. J.* 2024, **30**, e202401674.
42 T. Yano, t. Yamada, H. Isida, N. Ohashi, T. Itoh, *RSC Adv.,* 2024, **14**, 6542-6547.
43 A. Istrate, M. B. Geeson, C. D. Navo, B. B. Sousa, M. C. Marques, R. J. Taylor, T. Journeaux, S. R. Oehler, M. R. Mortensen, M. J. Deery, A. D. Bond, F. Corzana, G. Jiménez-Osés, G. J. L. Bernardes, *J. Am. Chem. Soc.* 2022, **144**, 10396-10406.
44 R. Padanha, R. A. N. Cavadas, P. Merino, J. P. M. António, P. M. P. Gois, *Org. Lett.* 2023, **25**, 5476-5480.
45 The reaction was considered complete after 72 hours, as *N*-cyano sulfoximine **1a** was no longer detected by LC/MS analysis.
46 CCDC 2481304 (**1ba**) and CCDC 2481305 (**1b**) contain the supplementary crystallographic data for this paper. These data are provided free of by The Cambridge Crystallographic Centre.
47 The completion time was determined by ^{1}H NMR spectroscopy (Please see the supporting information).
48 M. F. Debets, J. C. M. van Hest, F. P. J. T. Rutjes, *Org. Biomol. Chem.,* 2013, **11**, 6439-6455.
49 A. Daina, O. Michielin, V. Zoete. *Sci. Rep.* 2017; **7**:42717.
50 J. Weterings, C. J. F. Rijcken, H. Veldhuis, T. Meulemans, D. Hadavi, M. Timmers, M. Honing, H. Ippel, R. M. J. Liskamp, *Chem. Sci.*, 2020, **11**, 9011-9016.
51 The synthetic procedure[52] for **1e**, together with full reaction details, is provided in the Supporting Information.
52 L. Buglioni, V. Bizet, C. Bolm, *Adv. Synth. Catal.* 2014, **356**, 2209-2213.
53 We would also like to point out that longer peptides may adopt an α-helical conformation, which could potentially reduce the reactivity of internal cysteines.

View Article Online
DOI: 10.1039/D5MD00948K

Data for this article, including [description of data types] are available at [name of repository] at [URL – format https://doi.org/DOI]. The data supporting this article have been included as part of the Supplementary Information.