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RESEARCH ARTICLE

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N-Cyano sulfoximine-mediated thiazole ligation with N-terminal cysteine under mild aqueous conditions

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An N-terminal cysteine-selective click reaction employing N-cyano sulfoximines enables rapid thiazole formation under mild conditions. These three-dimensional, 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 (Fig. 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]

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-Cyano sulfoximines[21–32] are particularly attractive due to their ease of synthesis (Fig. 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 (Fig. 2b). This biomimetic strategy provides a distinct advantage by aligning chemical reactivity with biological relevance and compatibility.

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]

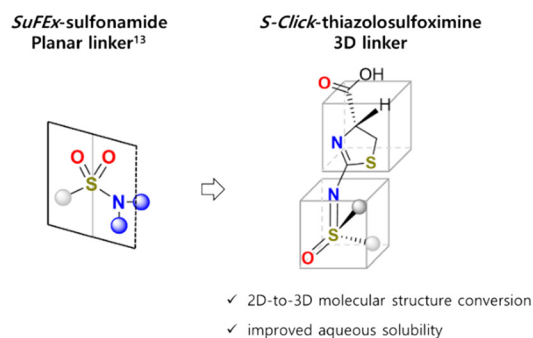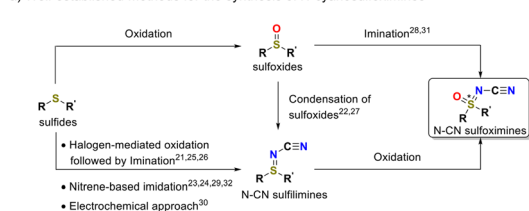No reaction occurred when N-cyano sulfoximine 1a was treated with serine, N-acetylcysteine, or glutathione (GSH),

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

SuFEx-sulfonamide Planar linker[13]

S-Click-thiazolosulfoximine 3D linker

✓ 2D-to-3D molecular structure conversion
✓ improved aqueous solubility

a) Well-established methods for the synthesis of N-cyanosulfoximines

b) This work
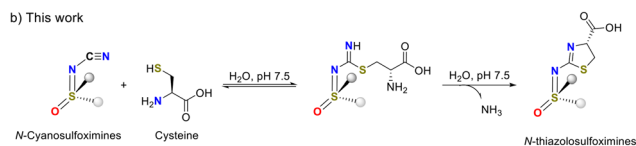
Fig. 2 N-Cyano sulfoximine as a bioorthogonal conjugation handle.

indicating high selectivity towards cysteine (entry 2 to 4, Table 1).

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-cyano sulfoximine **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

Table 1 Selectivity evaluation with other amino acids

Amino acids (50 mM) → Product
PBS (×1), pH 7.4, 37 °C, 72 h

1a (5 mM)

Entry	Amino acids	Product
1	(cysteine)	**2a**[a]
2	(serine)	—[b]
3	(N-acetylcysteine)	—[b]
4	(glutathione)	—[b]

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

Table 2 Reactivity modulation of the electrophilic N-cyano sulfoximines **1a**–**1d**

Entry	N-Cyano sulfoximines	R	X	Reaction time (h)	Conversion (%) to thiazole 2[a]
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]

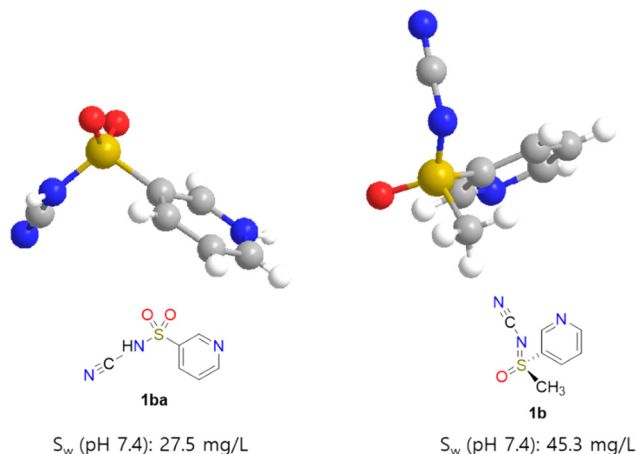[a] Confirmed by LC/MS analysis. [b] After 49 hours, precipitation occurred.

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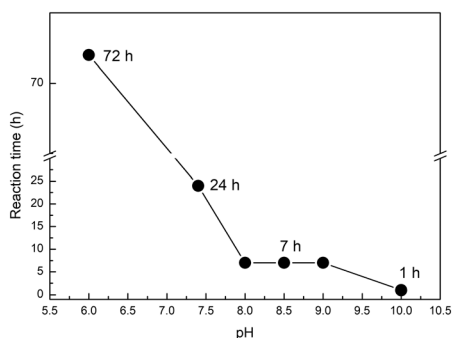

Fig. 4 Investigation of reaction time as a function of pH (*N*-cyano sulfoximine **1b** to thiazole **2b**).

conversion ratio compared to **1c**, which possesses an electron-donating group (entry 3 and 4, Table 2).

For comparison, the corresponding *N*-cyano sulfonamide **1ba** was also subjected to the reaction. As shown in the X-ray crystal structures in Fig. 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.

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 (Fig. 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.

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 (Fig. 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 (Fig. 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),

Table 3 Effect of stoichiometry on reaction rate

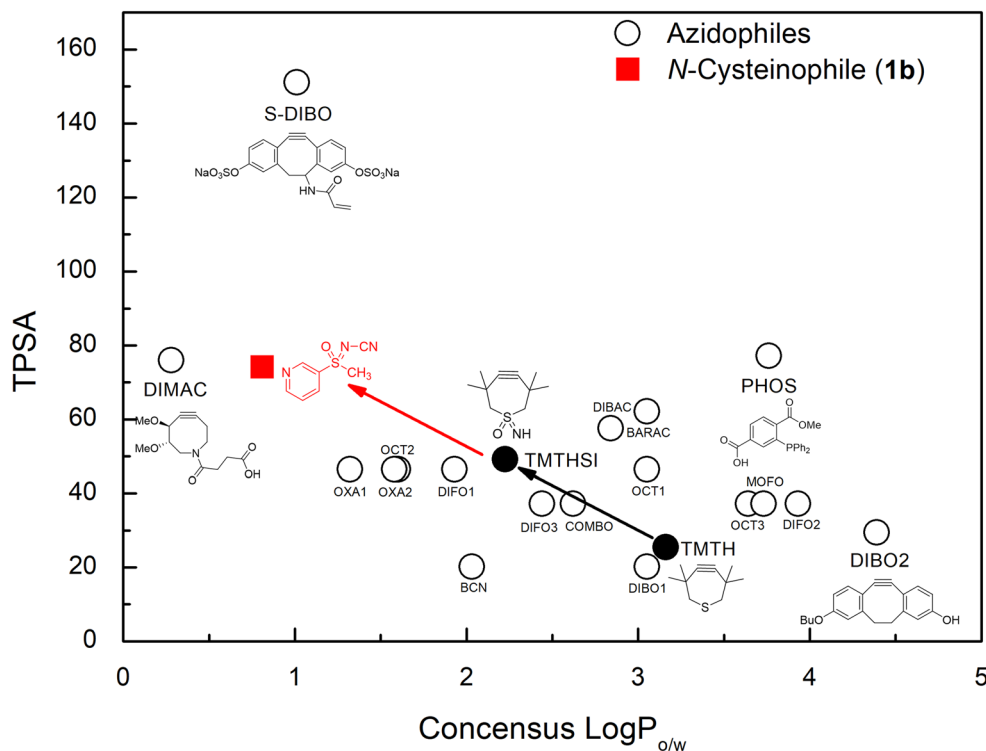| Entry	**1b** (mM)	Cysteine (mM)	**2b** (reaction completion time)[a]
1	10	1	3 h
2	5	1	7.5 h
3	1	10	21 h

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

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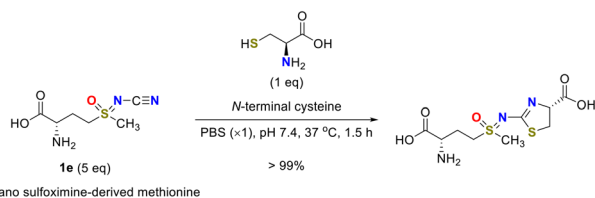

Fig. 6 Practical evaluation with *N*-cyano sulfoximine-derived methionine **1e**.

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 (Fig. 7).[53]

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
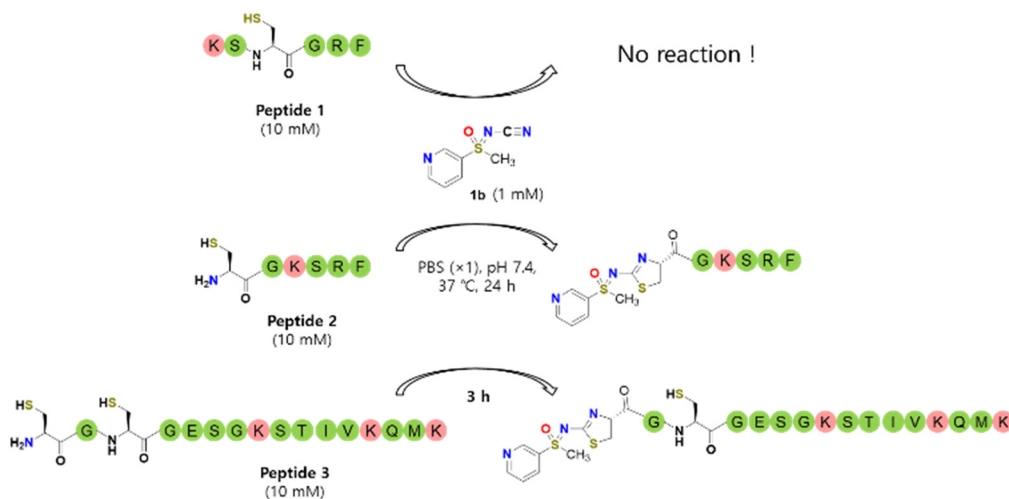

Fig. 7 Reaction of **1b** with cysteine-containing peptides.

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.

Conflicts of interest

There are no conflicts to declare.

Data availability

The data supporting this article have been included as part of the supplementary information (SI). Supplementary information is available. See DOI: https://doi.org/10.1039/d5md00948k.

CCDC 2481304 and 2481305 contain the supplementary crystallographic data for this paper.[54a,b]

Acknowledgements

Notes and references

1　R. E. Bird, S. A. Lemmel, X. Yu and Q. A. Zhou, *Bioconjugate Chem.*, 2021, **32**, 2457–2479.

2　K. M. Hartung and E. M. Sletten, *Chem*, 2023, **9**, 2095–2109.

3　F. M. Zielke and F. P. J. T. Rutjes, *Top. Curr. Chem.*, 2023, **381**, 1–30.

4　J. E. Hein and V. V. Fokin, *Chem. Soc. Rev.*, 2010, **39**, 1302–1315.

5　E. Haldón, M. C. Nicasio and P. J. Pérez, *Org. Biomol. Chem.*, 2015, **13**, 9528–9550.

6　J. Dong, L. Krasnova, M. G. Finn and K. B. Sharpless, *Angew. Chem., Int. Ed.*, 2014, **53**, 9430–9448.

7　T. A. Fattah, A. Saeed and F. Albericio, *J. Fluorine Chem.*, 2018, **213**, 87–112.

8　F. Lovering, J. Bikker and C. Humblet, *J. Med. Chem.*, 2009, **52**, 6752–6756.

9　M. Ishikawa and Y. Hashimoto, *J. Med. Chem.*, 2011, **54**, 1539–1554.

10　M. A. Walker, *Expert Opin. Drug Discovery*, 2014, **9**, 1–13.

11　Z. He, W. Yang, F. Yang, J. Zhang and L. Ma, *Eur. J. Med. Chem.*, 2024, **279**, 116842.

12　B. Das, A. T. K. Baidya, A. T. Mathew, A. K. Yadav and R. Kumar, *Bioorg. Med. Chem.*, 2022, **56**, 116614.

13　S. Li, P. Wu, J. E. Moses and K. B. Sharpless, *Angew. Chem., Int. Ed.*, 2017, **56**, 2903–2908.

14　B. Gao, S. Li, P. Wu, J. E. Moses and K. B. Sharpless, *Angew. Chem., Int. Ed.*, 2018, **57**, 1939–1943.

15　D. Zeng, W.-P. Deng and X. Jiang, *Natl. Sci. Rev.*, 2023, **10**, 1–19.

16　A. S. Barrow, C. J. Smedley, Q. Zheng, S. Li, J. Dong and J. E. Moses, *Chem. Soc. Rev.*, 2019, **48**, 4731–4758.

17　M. Andresini, A. Tota, L. Degennaro, J. A. Bull and R. Luisi, *Chem. – Eur. J.*, 2021, **27**, 17293–17321.

18　U. Lücking, *Org. Chem. Front.*, 2019, **6**, 1319–1324.

19　P. Mäder and 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 and L. Zhao, *Eur. J. Med. Chem.*, 2021, **209**, 112885.

21　D. Swern, I. Ikeda and G. F. Whitfield, *Tetrahedron Lett.*, 1972, **13**, 2635–2638.

22　T. E. Varkey, G. F. Whitfield and D. Swern, *J. Org. Chem.*, 1974, **39**, 3365–3372.

23　J. E. G. Kemp, D. Ellis and M. D. Closier, *Tetrahedron Lett.*, 1979, **20**, 3781–3784.

24　O. G. Mancheño and C. Bolm, *Org. Lett.*, 2007, **9**, 2951–2954.

25　O. G. Mancheño, O. Bistri and C. Bolm, *Org. Lett.*, 2007, **9**, 3809–3811.

26　A. Pandey and C. Bolm, *Synthesis*, 2010, **17**, 2922–2925.

27　C. M. M. Hendriks, P. Lamers, J. Engel and C. Bolm, *Adv. Synth. Catal.*, 2013, **355**, 3363–3368.

28　C. A. Dannenberg, L. Fritze, F. Krauskopf and C. Bolm, *Org. Biomol. Chem.*, 2017, **15**, 1086–1090.

29　S. M. Kim, O.-Y. Kang, H. J. Lim and S. J. Park, *ACS Omega*, 2020, **5**, 10191–10199.

30　M. Klein and S. R. Waldvogel, *Angew. Chem.*, 2021, **60**, 23197–23201.

31　N. Amri and 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 and 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 and 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 and F. Diederich, *Org. Biomol. Chem.*, 2012, **10**, 5764–5768.

35　O. V. Maltsev, V. Walter, M. J. Brandl and L. Hintermann, *Synthesis*, 2013, **45**, 2763–2767.

36　C. Nitsche, H. Onagi, J.-P. Quek, G. Otting, D. Luo and T. Huber, *Org. Lett.*, 2019, **21**, 4709–4712.

37　Z. Chen, M. Chen, Y. Cheng, T. Kowada, J. Xie, X. Zheng and J. Rao, *Angew. Chem., Int. Ed.*, 2020, **59**, 3272–3279.

38　F.-J. Chen and J. Gao, *Chem. – Eur. J.*, 2022, **28**, e202201843.

39　M. Proj, N. Strašek, S. Pajk, D. Knez and 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 and N. A. Patil, *Chem. – Eur. J.*, 2024, **30**, e202401674.

42 T. Yano, T. Yamada, H. Isida, N. Ohashi and 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 and 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 and 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 ¹H NMR spectroscopy (please see the SI).

48 M. F. Debets, J. C. M. van Hest and F. P. J. T. Rutjes, *Org. Biomol. Chem.*, 2013, **11**, 6439–6455.

49 A. Daina, O. Michielin and 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 and 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 SI.

52 L. Buglioni, V. Bizet and 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.

54 (*a*) CCDC 2481304: Experimental Crystal Structure Determination, 2025, DOI: 10.5517/fiz.icsd.cc2p9023; (*b*) CCDC 2481305: Experimental Crystal Structure Determination, 2025, DOI: 10.5517/fiz.icsd.cc2p9034.