



Cite this: *Chem. Commun.*, 2024, 60, 7950

Received 20th May 2024,
Accepted 5th July 2024

DOI: 10.1039/d4cc02442g

rsc.li/chemcomm

A photochemical acyl thiol–ene reaction can be used to rapidly cyclise fully unprotected peptides bearing both a thioacid and alkene to form peptide thiolactones. This strategy represents the first reported synthesis of peptide thiolactones under radical-mediated conditions.

Cyclic peptides have gained considerable interest in recent years both as drugs and as chemical probes due to their conformational rigidity, which results in improved metabolic stability, membrane permeability, selectivity and binding affinity compared to the linear counterparts.^{1,2} The majority of clinically approved cyclic peptide drugs to date have been derived from naturally occurring cyclic peptides such as hormones or antimicrobial agents.^{3,4} However, the *de novo* development of cyclic peptides is becoming increasingly common with the aid of computational design and high-throughput screening of synthetic cyclic peptide libraries.⁵

Common peptide cyclisation strategies include macrolactamisation, macrolactonisation, disulfide formation and ring closing metathesis.^{6,7} In addition, a variety of two-component peptide stapling methods have been developed whereby a bifunctional linker is used to crosslink two reactive groups on the linear peptide.⁸ Click reactions such as the copper-catalysed azide–alkyne cycloaddition (CuAAC) and thiol–ene reactions have emerged as rapid, highly efficient methods for the synthesis of cyclic peptides.^{9,10} The photochemical thiol–ene reaction involves the radical-mediated anti-Markovnikov addition of a thiol onto an alkene to form a thioether linkage.¹¹ The fast kinetics, high yields, mild reaction conditions, compatibility with aqueous conditions, and chemoselectivity of the thiol–ene reaction has prompted its widespread application for peptide bioconjugation and macrocyclisation.^{10,12} The absence of any metal catalysts is a notable advantage of the thiol–ene reaction

Synthesis of macrocyclic thiolactone peptides *via* photochemical intramolecular radical acyl thiol–ene ligation†

Alby Benny and Eoin M. Scanlan *

compared to the CuAAC reaction, as Cu(i) can be cytotoxic and complete removal of the metal can be labourious.¹³

Recently, we reported the radical-mediated acyl thiol–ene reaction to synthesise a variety of thioesters and small-molecule thiolactones through direct addition of thioacids onto alkenes.^{14–16} Thiolactones are found in several bioactive natural depsipeptides such as thiocoraline and verrucosamide.^{17,18} Another class of macrocyclic thiolactones known as autoinducing peptides (AIPs) are found in Gram positive bacteria such as *Staphylococcus aureus* and have a key role in the regulation of the quorum sensing system and the production of virulence factors.^{19–21} Although thioesters are unstable under basic conditions, analogues of AIPs have been successfully investigated as modulators of the quorum sensing system to attenuate and treat *S. aureus* infections.^{22–24}

Current methods for the chemical synthesis of macrocyclic thiolactone peptides have been reviewed by Gordon.²⁵ In summary, early approaches utilising coupling reagents to form the thiolactone bond between a cysteine (Cys) residue and the C-terminal carboxylic acid require fully protected peptides and long reaction times (Fig. 1a). More recent approaches whereby Cys displaces a latent thioester or activated leaving group (Dbz, MeDbz) to form the thiolactone still require reaction times of 2 h at elevated temperatures for good conversion (Fig. 1b). In this study, we describe a rapid photochemical acyl thiol–ene cyclisation strategy for fully unprotected peptides bearing a thioacid and alkene to furnish macrocyclic thiolactone peptides (Fig. 1c).

Previous work by Shimuzu *et al.* demonstrated that C-terminal peptide thioacid radicals undergo dethiocarboxylation *via* carbonyl sulfide (COS) elimination to form *N*-alkylamides.²⁶ In order to avoid this side reaction during acyl thiol–ene macrocyclisation, we utilised γ -thioaspartic acid bearing the required thioacid on the side chain of aspartic acid (Asp) as dethiocarboxylation should be unfavourable due to the requisite formation of a primary alkyl radical intermediate.²⁷ We envisaged introducing the γ -thioaspartic acid into the linear peptide as the protected *S*-triphenylmethyl (STr) thioester through standard Fmoc solid phase peptide synthesis (SPPS), with subsequent deprotection to release the free thioacid during resin

Trinity Biomedical Sciences Institute, Trinity College Dublin, 152–160 Pearse Street, Dublin 2, Ireland. E-mail: oin.scanlan@tcd.ie

† Electronic supplementary information (ESI) available: synthetic details, characterisation, HPLC traces and NMR spectra. See DOI: <https://doi.org/10.1039/d4cc02442g>



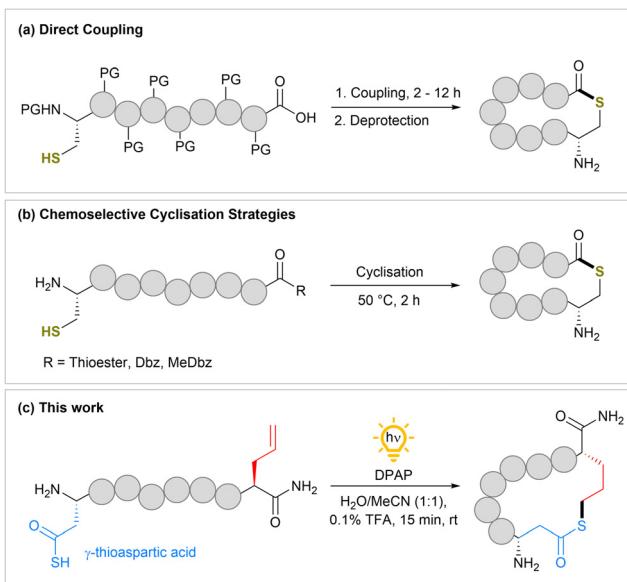


Fig. 1 Synthesis of peptide thiolactones. (a) Direct coupling of protected peptides to form thiolactones. (b) Chemoselective thiolactone formation *via* displacement of activated C-terminal groups. (c) This work; photochemical synthesis of peptide thiolactones *via* intramolecular radical-mediated acyl thiol-ene.

cleavage upon treatment with trifluoroacetic acid (TFA). To this end, the Asp(γ -STrt) derivative **1** was synthesised to serve as the building block for SPPS (Scheme 1). Previous work has shown that Asp(γ -STrt) thioesters are susceptible to aspartimide formation during Fmoc deprotection in SPPS; therefore the N-terminus was protected with the acid-labile *tert*-butoxycarbonyl (Boc) group which is removed during resin cleavage.^{28,29} Consequently, compound **1** must be incorporated in SPPS as the final amino acid at the N-terminal of the peptide sequence.

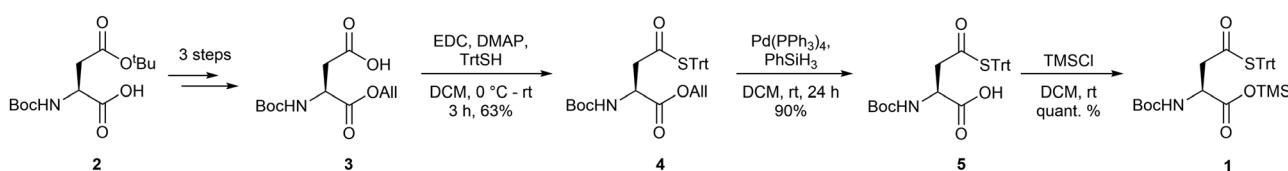
To commence our study, the allyl (All) ester Boc-Asp-OAll (**3**) was synthesised from commercially available Boc-Asp(OtBu)-OH (**2**) following the procedure reported by Kapurniotu and Taylor (Scheme 1).³⁰ Steglich thioesterification of the side chain carboxylic acid with triphenylmethanethiol (TrtSH) in dichloromethane (DCM) formed the Asp(STrt) thioester **4** in an isolated yield of 63%. Selective removal of the C-terminal allyl group *via* Pd-mediated deallylation furnished the carboxylic acid derivative **5** in 90% yield. The C-terminal carboxylic acid of side-chain trityl thioesters can undergo base-catalysed cyclic anhydride formation during activation *via* elimination of triphenylmethanethiol, making the C-terminal carboxylic acid **5** unsuitable for use in SPPS.^{28,29} However, Joseph *et al.* have demonstrated that this undesirable side-reaction can be

suppressed through formation of the corresponding C-terminal trimethylsilyl (TMS) ester which acts as a carboxylic acid surrogate while remaining sufficiently reactive for amide coupling with *N*, *N*'-diisopropylcarbodiimide (DIC).²⁹ Thus, compound **5** was reacted with trimethylsilyl chloride (TMSCl) for 30 min to form the required TMS ester **1** in quantitative yield. Overall, Boc-Asp(STrt)-OTMS (**1**) was prepared from compound **2** in a yield of 51% over 6 steps, requiring only two chromatographic purification steps. The compound is stable for several months at 0 °C if stored under a layer of inert gas and care is taken to exclude moisture.

Having prepared the requisite building block, we next set out to synthesise linear peptides bearing both an alkene and thioacid suitable for acyl thiol-ene cyclisation. The terminal alkene allylglycine (Agl) was selected for this study because it is commercially available and is stable to standard Fmoc SPPS conditions. The terminal alkene also ensures that only a single isomer of the cyclic peptide is formed upon acyl thiol-ene cyclisation because anti-Markovnikov addition of the thioacid radical to the alkene is highly favoured.¹⁶ Fmoc SPPS using Rink amide resin was used to synthesise the model linear peptide H-Asp(SH)-Ala-Ala-Agl-NH₂ **6** (Scheme 2). HATU/DIPEA in DMF was used for all amino acid couplings, except for Boc-Asp(STrt)-OTMS **1**, which was coupled using DIC/Oxyma Pure/DIPEA in dry DCM. Following cleavage, the fully unprotected linear peptide **6** was obtained in a yield of 83%. The peptide was obtained in sufficient purity for use in macrocyclisation studies without the requirement for further HPLC purification.

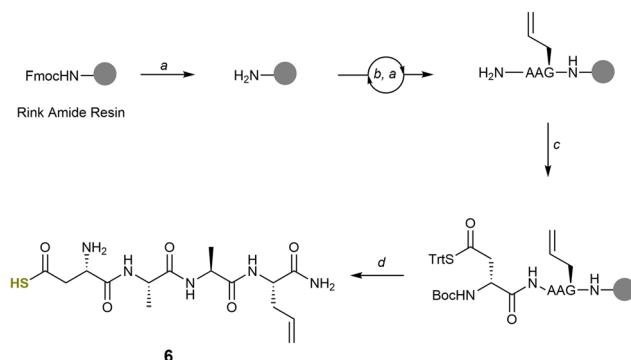
The linear peptide **6** was dissolved at 5 mM concentration in a solvent mix of H₂O/MeCN (1:1) containing 0.1% TFA and one eq. of the photoinitiator, 2,2-dimethoxy-2-phenylaceto-phenone (DPAP), and irradiated with UV light (354 nm) for 15 min. Gratifyingly, a conversion of 90% to the thiolactone **7** was observed by analytical HPLC (Fig. 2b) and the product was obtained in 54% yield following semi-preparative HPLC purification. Since the acyl thiol-ene macrothiolactonisation is an intramolecular hydrothioacetylation reaction, the mass of the linear and cyclised peptides are identical. Therefore, along with the shift to lower retention time observed in the analytical HPLC trace (Fig. 2b), NMR spectroscopy was used to confirm formation of the peptide thiolactone **7**. The HMBC spectrum of **7** provided in Fig. 2c shows unambiguous evidence for the formation of the thiolactone linkage. The HMBC spectrum shows correlation between the thiolactone carbonyl at position #2 (¹³C δ 194.8 ppm) and the diastereotopic methylene protons at position #3 (¹H δ 3.13–3.07 and δ 2.59–2.51 ppm).

The acyl thiol-ene cyclisation of **6** without the addition of 0.1% TFA in the solvent system resulted in a significantly



Scheme 1 Synthesis of Boc-Asp(STrt)-OTMS **1**.





Scheme 2 Solid phase peptide synthesis of **6**. (a) 20% Piperidine/DMF, 10 min \times 2. (b) Fmoc-AA-OH (4 eq.), HATU (3.9 eq.), DIPEA (8 eq.), DMF (0.2 M), 45 min. (c) Boc-Asp(STr)-OTMS **1** (3 eq.), DIC (3 eq.), Oxyma Pure (3 eq.), DIPEA (3 eq.), Dry DCM (0.2 M), 45 min \times 2 (d) TFA : DCM : TES : HOAc (90 : 5 : 2.5 : 2.5), 2 h.

reduced conversion of 35% to the thiolactone **7** (Fig. S2 in ESI[†]). As previously observed for the thiol-ene reaction, the addition of TFA in the solvent mixture is necessary to ensure that the thioacid remains protonated for efficient hydrogen atom transfer (HAT) to form the requisite thiyl radical to initiate the reaction.³¹ Screening of reaction time showed a moderate conversion of 59% at just 1 min irradiation time, and up to 80% at 5 min (Fig. S3 and S4 in ESI[†]). The reaction was conducted with 25 mM concentration of the linear peptide and a conversion of 82% was observed without significant intermolecular dimerisation or polymerisation reactions (Fig. S5 in ESI[†]).

To investigate the scope of the acyl thiol-ene mediated macrothiolactonisation reaction, a series of structurally diverse tetramer and pentamer linear peptides **S2–S4** were synthesised by SPPS (structures provided in ESI[†]). After cyclisation using the standard conditions, thiolactone peptides **8–10** were

obtained in moderate to good isolated yields (Fig. 3). In the case of **11**, no cyclisation was observed under the standard conditions. We theorised that intramolecular hydrogen bonding of the thioacid to the adjacent serine residue was preventing proton abstraction by the photoinitiator. Thus, the reaction was repeated with the addition of the chaotropic agent, 6 M guanidine hydrochloride, in the solvent mixture. Gratifyingly, under these modified conditions, full consumption of the linear peptide was observed by HPLC, and thiolactone **11** was isolated in a yield of 61%. Importantly, side-chain functional groups including phenols (**8**), amines (**9**), carboxylic acids (**10**) and alcohols (**11**) were all compatible with the acyl thiol-ene macrocyclisation protocol described. Furthermore, the sequence of thiolactone peptides **10** and **11** were based on the structure of the autoinducing peptides AIP-I and AIP-II, highlighting the utility of the reaction for the synthesis of biologically relevant peptides. Linear peptides **S5** and **S6** were also synthesised and cyclised to form thiolactones **S7** and **S8** (structures provided in ESI[†]). However, **S7** and **S8** were isolated with approximately 20% of a coeluting impurity corresponding to truncated or hydrolysed linear thioacid peptide carried through from the SPPS synthesis. Nonetheless, these peptides are still of sufficient purity (>75%) for initial, high-throughput drug screening studies.

In summary, we report the use of the photochemical acyl thiol-ene reaction for the synthesis of peptide thiolactones starting from unprotected linear peptides bearing γ -thio-aspartic acid and allylglycine residues. The cyclisation reaction is rapid (15 min) and chemoselective and represents the first reported synthesis of peptide thiolactones under photochemical, radical-mediated conditions. The rapid nature of the acyl thiol-ene thiolactonisation reaction would be amenable to the synthesis of peptide libraries, particularly with the

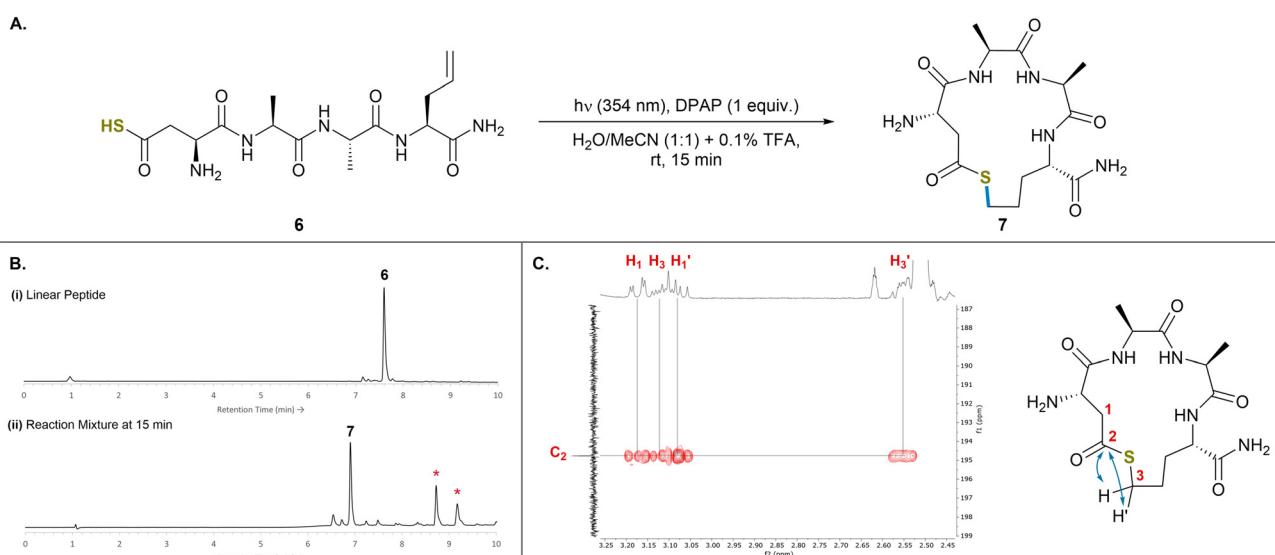


Fig. 2 (a) ATE macrocyclisation of **6**. Reaction conditions: peptide **6** (5 mM), DPAP (5 mM) in H₂O/MeCN (1:1) containing 0.1% TFA, UV light (354 nm), rt, 15 min. (b) (i) HPLC trace of linear peptide **6** (5–95% over 10 min, 220 nm) (ii) HPLC trace of reaction mixture at 15 min (5–95% over 10 min, 220 nm). *DPAP by-products. (c) HMBC spectrum of **7** showing correlation (blue arrows) across the thiolactone bond.



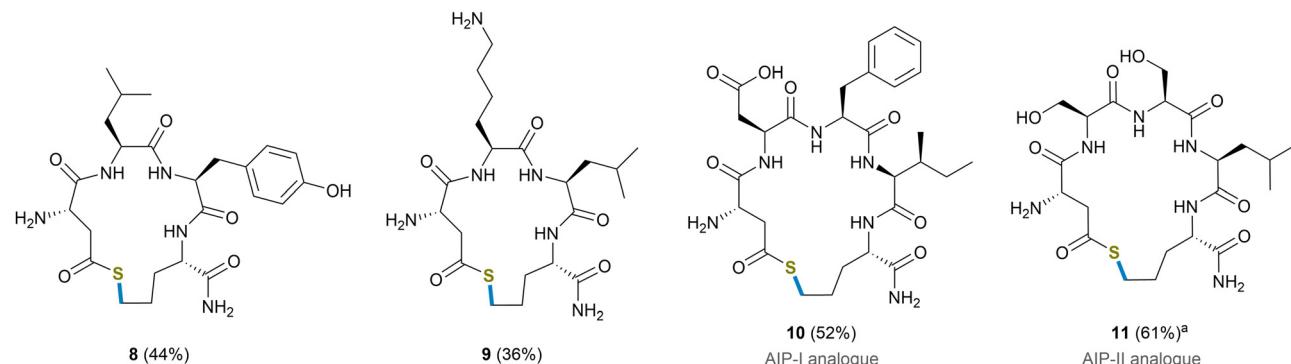


Fig. 3 Scope of the acyl thiol–ene cyclisation. Isolated yields after semi-preparative HPLC purification. Conditions unless otherwise stated for cyclisation: linear peptide (5 mM), DPAP (1 eq.) in $\text{H}_2\text{O}/\text{MeCN}$ (1:1) + 0.1% TFA, UV light (354 nm), rt, 15 min. ^a6 M guanidine hydrochloride added to solvent mixture.

development of on-resin cyclisation protocols. Further studies towards the application of this methodology for the preparation of libraries of novel thiolactone peptides suitable for high-throughput screening are currently ongoing in our labs.

Conceptualisation, E. M. S.; methodology, A. B.; writing – original draft preparation, A. B.; writing – review and editing, E. M. S.; supervision, E. M. S.; funding acquisition, E. M. S.

This work was funded by the Irish Research Council (Grant GOIPG/2020/813) and Science Foundation Ireland (19/FFP/6667). We are grateful to Dr John O'Brien and Dr Manuel Ruether for assistance with NMR spectroscopy and Dr Gary Hessman for assistance with mass spectrometry.

Data availability

The data supporting this article have been included as part of the ESI.†

Conflicts of interest

There are no conflicts to declare.

References

- 1 D. S. Nielsen, R.-J. Lohman, H. N. Hoang, T. A. Hill, A. Jones, A. J. Lucke and D. P. Fairlie, *ChemBioChem*, 2015, **16**, 2289–2293.
- 2 L. Mendive-Tapia, J. Wang and M. Vendrell, *Pept. Sci.*, 2021, **113**, e24181.
- 3 X. Ji, A. L. Nielsen and C. Heinis, *Angew. Chem., Int. Ed.*, 2024, **63**, e202308251.
- 4 H. Zhang and S. Chen, *RSC Chem. Biol.*, 2022, **3**, 18–31.
- 5 M. L. Merz, S. Habeshian, B. Li, J.-A. G. L. David, A. L. Nielsen, X. Ji, K. Il Khwildy, M. M. Duany Benitez, P. Phothirath and C. Heinis, *Nat. Chem. Biol.*, 2023, **20**, 634.
- 6 C. Bechtler and C. Lamers, *RSC Med. Chem.*, 2021, **12**, 1325–1351.
- 7 A. R. Paquette and C. N. Boddy, *Org. Biomol. Chem.*, 2023, **21**, 8043–8053.
- 8 Y. H. Lau, P. de Andrade, Y. Wu and D. R. Spring, *Chem. Soc. Rev.*, 2015, **44**, 91–102.
- 9 R. A. Turner, A. G. Oliver and R. S. Lokey, *Org. Lett.*, 2007, **9**, 5011–5014.
- 10 M. D. Nolan and E. M. Scanlan, *Front. Chem.*, 2020, **8**, 583272.
- 11 A. A. Aimetti, R. K. Shoemaker, C.-C. Lin and K. S. Anseth, *Chem. Commun.*, 2010, **46**, 4061–4063.
- 12 M. Ahangarpour, I. Kavianinia, P. W. R. Harris and M. A. Brimble, *Chem. Soc. Rev.*, 2021, **50**, 898–944.
- 13 L. Li and Z. Zhang, *Molecules*, 2016, **21**, 1393.
- 14 R. O. McCourt, F. Dénés, G. Sanchez-Sanz and E. M. Scanlan, *Org. Lett.*, 2018, **20**, 2948–2951.
- 15 R. O. McCourt and E. M. Scanlan, *Org. Lett.*, 2019, **21**, 3460–3464.
- 16 J. T. McLean, P. Milbeo, D. M. Lynch, L. McSweeney and E. M. Scanlan, *Eur. J. Org. Chem.*, 2021, 4148–4160.
- 17 V. Nair, M. C. Kim, J. A. Golen, A. L. Rheingold, G. A. Castro, P. R. Jensen and W. Fenical, *Mar. Drugs*, 2020, **18**, 549.
- 18 F. Romero, F. Espliego, J. Pérez Bas, T. García de Quesada, D. Grávalos, F. De la Calle and J. Luis Fernández-Puentes, *J. Antibiot. Res.*, 1997, **47**, 734–737.
- 19 B. Wang and Tom W. Muir, *Cell Chem. Biol.*, 2016, **23**, 214–224.
- 20 Y. Tal-Gan, M. Ivancic, G. Cornilescu, C. C. Cornilescu and H. E. Blackwell, *J. Am. Chem. Soc.*, 2013, **135**, 18436–18444.
- 21 B. H. Gless, M. S. Bojer, P. Peng, M. Baldry, H. Ingmer and C. A. Olsen, *Nat. Chem.*, 2019, **11**, 463–469.
- 22 J. K. Vasquez and H. E. Blackwell, *ACS Infect. Dis.*, 2019, **5**, 484–492.
- 23 C. P. Gordon, S. D. Olson, J. L. Lister, J. S. Kavanaugh and A. R. Horswill, *J. Med. Chem.*, 2016, **59**, 8879–8888.
- 24 K. H. J. West, C. G. Gahan, P. R. Kierski, D. F. Calderon, K. Zhao, C. J. Czuprynski, J. F. McAnulty, D. M. Lynn and H. E. Blackwell, *Angew. Chem., Int. Ed.*, 2022, **61**, e202201798.
- 25 C. P. Gordon, *Org. Biomol. Chem.*, 2020, **18**, 379–390.
- 26 T. Shimizu, R. Miyajima, N. Naruse, K. Yamaoka, K. Aihara, A. Shigenaga and A. Otaka, *Chem. Pharm. Bull.*, 2016, **64**, 375–378.
- 27 A. Benny, L. Di Simo, L. Guazzelli and E. M. Scanlan, *Molecules*, 2024, **29**, 1465.
- 28 M. J. Schöwe, O. Keiper, C. Unverzagt and V. Wittmann, *Chem. – Eur. J.*, 2019, **25**, 15759–15764.
- 29 R. Joseph, M. Morales Padilla and P. Garner, *Tetrahedron Lett.*, 2015, **56**, 4302–4304.
- 30 A. Kapurniotu and J. W. Taylor, *Tetrahedron Lett.*, 1993, **34**, 7031–7034.
- 31 M. D. Nolan, C. Shine, E. M. Scanlan and R. Petracca, *Org. Biomol. Chem.*, 2022, **20**, 8192–8196.

