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Solid-phase submonomer synthesis of sequence-defined oligothioetheramides†

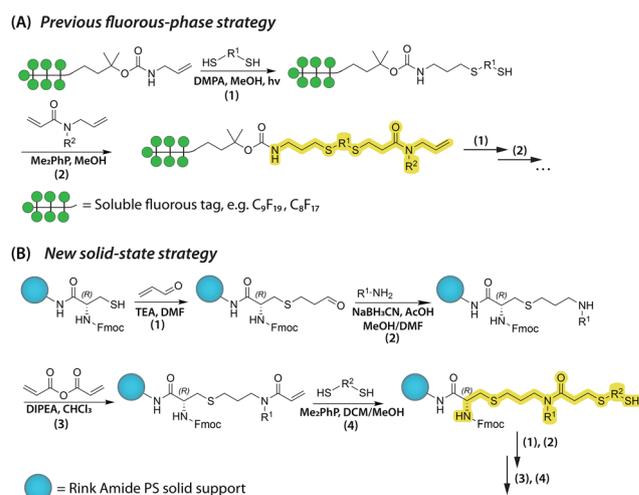Dana Abdullatif, [ID] [a] Adithya Rangamani [ID] [b] and Christopher A. Alabi [ID] ⋆[ab]

Sequence-defined polymers have been developed using various orthogonal iterative chemical techniques. Among these, oligothioetheramides (OligoTEAs), which are designed with antimicrobial, cell-penetrating, and other biological properties, are synthesized through iterative solution-phase monomer addition on a soluble fluorous support. To simplify and streamline the iterative process, this work investigates the feasibility of an alternative submonomer solid-phase approach to oligoTEAs.

Over the past decade, research on sequence-defined polymers has expanded significantly, leading to a diverse array of synthetic approaches. Some efforts in this field are fundamentally driven, focusing on incorporating novel chemical groups into sequence-defined backbones.[1–8] Others aim to introduce new chemistries that replicate the functional capabilities of natural biomolecules while overcoming some of their limitations, such as susceptibility to proteolytic degradation and immune recognition.[9–13] One such example in the latter category is sequence-defined oligothioetheramides (oligoTEAs), a novel class of oligomers introduced by the Alabi research group.[14] Over the past decade, oligoTEAs with varying chain compositions and lengths have been explored for applications as antimicrobial biomimetics, cell-penetrating agents, and linkers for antibody–drug conjugates.[15–20] The original oligoTEA synthesis approach employs an acid-cleavable fluorous-tagged initiating monomer to facilitate the solution-phase alternating thiol-Michael addition and thiol–ene reactions of dithiols and *N*-allylacrylamide building blocks, respectively, yielding sequenced-defined oligoTEAs (Scheme 1A). However, despite its success in producing functional oligomers, this synthesis method presents several challenges, including the need for pre-synthesis of *N*-allylacrylamide monomers, reliance on

expensive and toxic perfluoroalkyl fluorous tags, and a decline in fluorophilicity of fluorous-bound intermediates during fluorous solid-phase extraction (FSPE) as oligomer chain length increases.[21]

To address the challenges associated with the current synthesis method, we drew inspiration from the submonomer approach used in peptoid synthesis.[22] In this work, we investigate the feasibility of using a solid-phase submonomer synthesis (SPS) approach to produce oligoTEAs. Unlike the previous fluorous-tagged strategy, SPS simplifies intermediate purification on resins to a filtration step, in contrast to the multiple fluorophobic and fluorophilic washes required in FSPE. Additionally, by employing a submonomer chain extension strategy, functional groups are directly incorporated onto the solid phase, eliminating the need to pre-synthesize the *N*-allylacrylamide building block, as is typically required in the traditional approach.

We designed a synthetic scheme (Scheme 1B) to achieve a full thioetheramide unit in four submonomer steps on the solid

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Scheme 1 (A) Previous fluorous-phase oligoTEA synthesis strategy. (B) New solid-phase submonomer oligoTEA synthesis strategy explored in this work.

phase using commercially available building blocks. Specifically, our study began by using Fmoc-Cys(Mmt)-OH loaded onto a Rink amide AM resin, such that the oligoTEA synthesis propagates on the Mmt-liberated thiol side-chain. This cysteine protecting group (PG) was chosen for its ease of removal using mild acid treatment and its orthogonality with the fluorenylmethoxycarbonyl (Fmoc) protected amine.[23] As such, using the cysteine thiol as the functional site for oligoTEA chain extension, the reactions begin with a thiol-Michael addition of 2-propenal (acrolein), followed by reductive amination of a primary amine, N-acylation with an acrylic anhydride, and finally, a second thiol-Michael addition using a dithiol to complete the formation of a thioetheramide unit (Scheme 1B; see ESI† for complete synthetic information).

The selection of this submonomer transformation was driven by the use of commercially available building blocks, specifically primary amines and dithiols, to modify the pendant and backbone groups of the oligomer, respectively. This modularity is crucial, as previous studies have shown that variation in oligoTEA composition significantly impact physicochemical and biological properties.[24,25] In this study, we explore the accessibility of this new SPS protocol by employing butylamine (BA) or benzylamine (BzA) for the reductive amination step, and 3,6-dioxa-1,8-octanedithiol (DODT) or L-dithiothreitol (DTT) for the thiol-Michael addition step (Fig. 1A).

The optimized reaction conditions for the thiol-Michael addition of acrolein, reductive amination with benzylamine, acrylic anhydride acylation, and the second thiol-Michael with

L-DTT (see ESI† for detailed reaction conditions) successfully yielded the thioetheramide dimer, with each intermediate confirmed by MS (Fig. 1B). Despite obtaining the desired product, several by-products were detected. For example, during the second thiol-Micheal addition step catalyzed by dimethylphenyl-phosphine (DMPP) to give the 2 mer, a phosphonium adduct impurity was formed (Impurity I, Fig. 1C).[26,27] Since the Michael acceptor is resin-bound, this phosphonium impurity becomes localized on the support, resulting in an inert, terminated species observed in the crude product mixture. Given the critical role of DMPP as a catalyst, its loading was minimized to reduce the loss of resin-bound chains during each phosphine-catalyzed Michael addition in the iterative oligomer chain extension process.

Additionally, several impurities related to chain crosslinking were detected in the crude product mixture. During the reductive amination step, overalkylation led to the formation of an undesired tertiary amine (Impurity II, Fig. 1C).[28] A second crosslinked impurity was detected during the N-acylation step from an undesired reaction between the acrylamide product of one chain and the secondary amine intermediate of another, resulting in the formation of an aza-Michael product (Impurity III, Fig. 1C).[29] These intermolecular reactions lead to chain termination due to cross-linking. To address this, solvent choice and reaction duration were optimized to minimize resin-bound chain termination. Formation of the intermolecular crosslinking products persisted during SPS despite multiple efforts to achieve site isolation via low loading of the resin.[30]

Despite the presence of impurities, the overall efficiency of the four-step submonomer synthesis was promising, prompting us to explore the iterative synthesis of full oligomeric units through cyclic repetition of the submonomer reactions. This approach was validated using two distinct oligoTEA compositions, successfully tracking the formation of the 1-mer, 3-mer, and 5-mer species (Fig. 2A–D) as well as the 7- and 8-mer of the BzA/DTT oligoTEA (Fig. 3A, B and see Fig. S13, S14, ESI† for MS/MS structural characterization). Notably, this new SPS strategy offers versatility in end-group functionality, as the synthesis can be terminated at any submonomer step to give one of four distinct functional group sites. This feature is anticipated to be particularly advantageous for the post-synthetic modification of the oligomers.[17]

In our studies, we observed that the oligomers became increasingly hydrophobic with each cycle of chain extension, as evidenced by delayed elution during chromatographic analysis (available in Fig. S2–S11, ESI†). This trend occurred despite the use of a dihydroxyl L-DTT backbone monomer. Similar to challenges encountered with certain 'difficult' peptides, the growing hydrophobic chains on the solid support led to on-resin aggregation, which impaired both reaction conversion and cleavage efficiency.[31] While this increase in hydrophobicity may be a desired property for certain applications, it necessitated significant modifications to the cleavage protocol, as the conventional 95% trifluoroacetic acid (TFA) solution for the cleavage of Rink Amide handles proved insufficient. Inspired by a report of improved crude peptoid purity through reduced TFA concentrations in the cleavage solution, we explored alternative cleavage
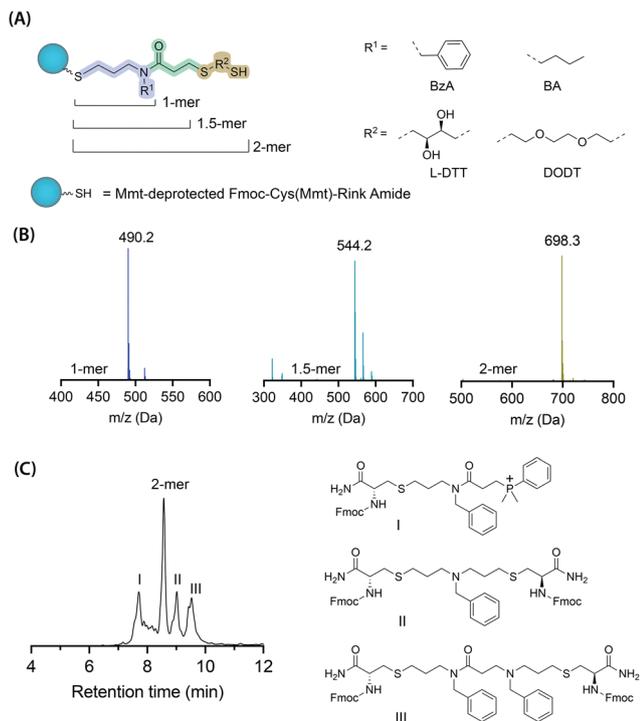

Fig. 1 (A) Compositional changes of oligoTEAs by interchangeable primary amine and dithiol building blocks. (B) Proof-of-concept of thioether-amide unit synthesized via submonomer SPS, with corresponding LC-MS spectra of 1-, 1.5-, and 2-mer intermediates assigned as [M + H]⁺. (C) HPLC profile (280 nm) of crude BzA/DTT 2-mer and sources of impurities.
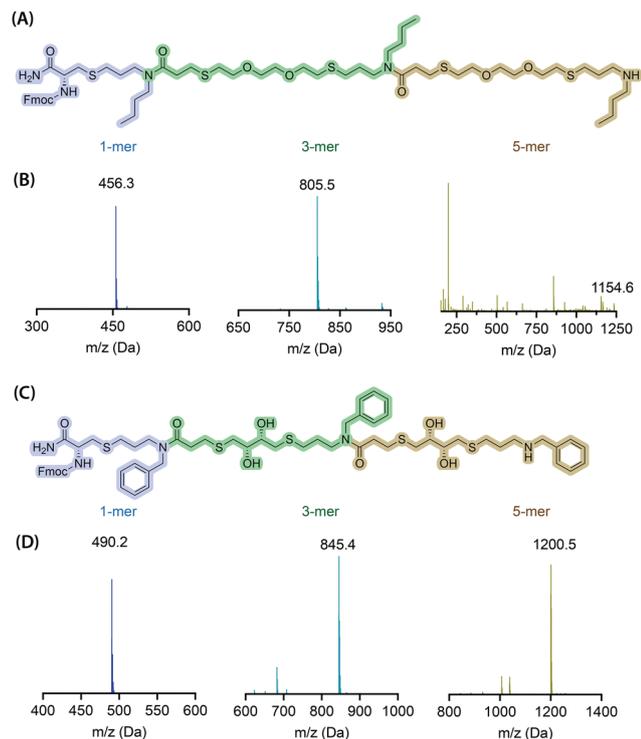
Fig. 2 Chain extension feasibility of (A) BA/DODT oligoTEA and (C) BzA/DTT oligoTEA up to 5-mer with corresponding LC-MS (B) and (D) of 1-, 3-, and 5-mer intermediates assigned as [M + H]$^+$.
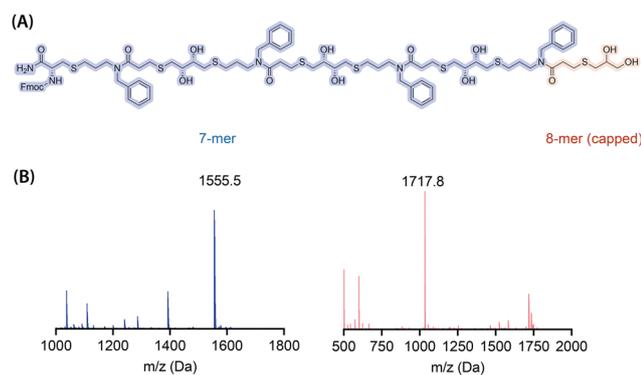

Fig. 3 (A) Structure of BzA/DTT oligoTEA 7-mer and capped 8-mer (B) chain extension of BzA/DTT oligoTEA 7-mer (left) and capped 8-mer (right) with corresponding LC-MS oligomers assigned as [M + H]$^+$ for both products.

conditions tailored to the hydrophobic nature of these oligoTEAs.[32] For example, cleavage of the BzA/DTT 8-mer (Fig. 3B; full characterization available in ESI†) required treating the resin with a modified cleavage cocktail consisting of HCl–HFIP–DCM (1:20:79 v/v), adapted from the protocol reported by Palladino and Stetsenko.[33] The successful cleavage and characterization of the BzA/DTT octamer suggest that the use of 1,1,1,3,3,3-hexafluoroisopropanol (HFIP) alongside increased amounts of DCM enhances solvation of the oligoTEA octamer during cleavage, thereby improving overall efficiency.[34] More specifically, the use of the solution may help overcome the oligomer's increased

hydrophobicity and possible chain aggregation, given HFIP's strong hydrogen donor ability combined with the increased DCM content to also facilitate in resin swelling during the process.[35] Therefore, change in cleavage cocktail allowed for the isolation of the 7-mer and 8-mers, both of which were previously unattainable using the classic 95% TFA cleavage cocktail.

In summary, we developed and characterized a novel stepwise solid-phase submonomer synthesis approach for oligo-TEAs. While issues with side reactions and low recovery persist, the mild reaction conditions and versatility of this SPS protocol are expected to support future optimization studies, ultimately leading to its use in generating expanded oligoTEA libraries for exploratory biological studies. A key feature of this method is the unconventional chain extension from the Cys thiol side chain, diverging from the traditional N-terminal extension typical of peptide synthesis. This distinctive strategy offers new opportunities for oligoTEA exploration, including streamlined oligoTEA-peptide conjugation directly at the N-terminus while on solid support. Moreover, the solid-phase synthesis platform provides a foundation for automating oligoTEA production, an appealing prospect for scaling up oligoTEA synthesis for broader applications.

Conflicts of interest

There are no conflicts to declare.

Data availability

The authors declare that the experimental data generated in this study to support its findings are available within the journal article and its ESI.† The experimental raw data, including ultraviolet-visible spectroscopy, high-performance liquid chromatography (HPLC), and liquid chromatography-mass spectrometry (LC-MS) total ion chromatograms are made available at: **https://doi.org/10.5281/zenodo.15359395**.

Notes and references

1 J. W. Grate, K. Mo and M. D. Daily, *Angew. Chem.*, 2016, **128**, 3993–3998.
2 R. Dong, R. Liu, P. R. J. Gaffney, M. Schaepertoens, P. Marchetti, C. M. Williams, R. Chen and A. G. Livingston, *Nat. Chem.*, 2019, **11**, 136–145.
3 P. Cwynar, P. Pasikowski and R. Szweda, *Eur. Polym. J.*, 2023, **182**, 111706.

4 K. S. Wetzel, M. Frölich, S. C. Solleder, R. Nickisch, P. Treu and M. A. R. Meier, *Commun. Chem.*, 2020, **3**, 63.

5 M. P. Kim, S. Kayal, C. Hwang, J. Bae, H. Kim, D. G. Hwang, M. H. Jeon, J. K. Seo, D. Ahn, W. Lee, S. Seo, J.-H. Chun, Y. Yu and S. Y. Hong, *Nat. Commun.*, 2024, **15**, 3381.

6 R. K. Weigel and C. A. Alabi, *Chem. Sci.*, 2024, **15**, 9138–9146.

7 I. De Franceschi, N. Badi and F. E. Du Prez, *Chem. Sci.*, 2024, **15**, 2805–2816.

8 N. F. König, S. Telitel, S. Poyer, L. Charles and J. Lutz, *Macromol. Rapid Commun.*, 2017, **38**, 1700651.

9 D. Karamessini, T. Simon-Yarza, S. Poyer, E. Konishcheva, L. Charles, D. Letourneur and J. Lutz, *Angew. Chem.*, 2018, **130**, 10734–10738.

10 A. Jose, S. N. Chathangad, P. Sivadas, D. Barik, K. Kannan, S. L. Das, S. Sadhukhan and M. Porel, *ACS Appl. Bio Mater.*, 2025, **8**, 1547–1558.

11 R. L. Kanasty, A. J. Vegas, L. M. Ceo, M. Maier, K. Charisse, J. K. Nair, R. Langer and D. G. Anderson, *Angew. Chem.*, 2016, **128**, 9681–9685.

12 E. E. Salcher, P. Kos, T. Fröhlich, N. Badgujar, M. Scheible and E. Wagner, *J. Controlled Release*, 2012, **164**, 380–386.

13 B. Mojsoska, G. Carretero, S. Larsen, R. V. Mateiu and H. Jenssen, *Sci. Rep.*, 2017, **7**, 42332.

14 M. Porel and C. A. Alabi, *J. Am. Chem. Soc.*, 2014, **136**, 13162–13165.

15 E. A. Hoff, C. M. Artim, J. S. Brown and C. A. Alabi, *Macromol. Biosci.*, 2018, **18**, 1800241.

16 N. N. Phan, C. Li and C. A. Alabi, *Bioconjug Chem.*, 2018, **29**, 2628–2635.

17 M. K. O'Leary, A. Ahmed and C. A. Alabi, *ACS Infect. Dis.*, 2023, **9**, 322–329.

18 J. S. Brown, Z. J. Mohamed, C. M. Artim, D. N. Thornlow, J. F. Hassler, V. P. Rigoglioso, S. Daniel and C. A. Alabi, *Commun Biol.*, 2018, **1**, 220.

19 C. M. Artim, M. Kunala, M. K. O'Leary and C. A. Alabi, *ChemBioChem*, 2021, **22**, 2697–2702.

20 M. Porel, D. N. Thornlow, C. M. Artim and C. A. Alabi, *ACS Chem. Biol.*, 2017, **12**, 715–723.

21 C. C. Tzschucke, C. Markert, W. Bannwarth, S. Roller, A. Hebel and R. Haag, *Angew. Chem., Int. Ed.*, 2002, **41**, 3964–4000.

22 R. N. Zuckermann, J. M. Kerr, S. B. H. Kent and W. H. Moos, *J. Am. Chem. Soc.*, 1992, **114**, 10646–10647.

23 R. J. Spears, C. McMahon and V. Chudasama, *Chem. Soc. Rev.*, 2021, **50**, 11098–11155.

24 C. M. Artim, N. N. Phan and C. A. Alabi, *ACS Infect. Dis.*, 2018, **4**, 1257–1263.

25 J. S. Brown, Y. M. Acevedo, G. D. He, J. H. Freed, P. Clancy and C. A. Alabi, *Macromolecules*, 2017, **50**, 8731–8738.

26 J. W. Chan, C. E. Hoyle, A. B. Lowe and M. Bowman, *Macromolecules*, 2010, **43**, 6381–6388.

27 G. Z. Li, R. K. Randev, A. H. Soeriyadi, G. Rees, C. Boyer, Z. Tong, T. P. Davis, C. R. Becer and D. M. Haddleton, *Polym. Chem.*, 2010, **1**, 1196–1204.

28 D. W. Gordon and J. Steele, *Bioorg. Med. Chem. Lett.*, 1995, **5**, 47–50.

29 B. C. Hamper, S. A. Kolodziej, A. M. Scates, R. G. Smith and E. Cortez, *J. Org. Chem.*, 1998, **63**, 708–718.

30 D. Comegna, A. Del Gatto, M. Saviano and L. Zaccaro, *Org. Lett.*, 2019, **21**, 4454–4458.

31 L. K. Mueller, A. C. Baumruck, H. Zhdanova and A. A. Tietze, *Front. Bioeng. Biotechnol.*, 2020, **8**, 162.

32 A. Lone, A. Arnous, P. R. Hansen, B. Mojsoska and H. Jenssen, *Front. Chem.*, 2020, **8**, 370.

33 P. Palladino and D. A. Stetsenko, *Org. Lett.*, 2012, **14**, 6346–6349.

34 H. F. Motiwala, A. M. Armaly, J. G. Cacioppo, T. C. Coombs, K. R. K. Koehn, V. M. Norwood and J. Aubé, *Chem. Rev.*, 2022, **122**, 12544–12747.

35 S. Rana, P. White and M. Bradley, *J. Comb. Chem.*, 2001, **3**, 9–15.