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Bicycle synthesis through peptide macrocyclization using aziridine aldehydes followed by late stage disulfide bond installation†

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We present a method that can be applied to generate medium-sized peptidomimetic macrocycles equipped with disulfide bonds. The reaction hinges on amphoteric aziridine aldehydes and their ability to bridge the ends of linear peptides in the presence of isocyanides. Aziridine aldehyde dimers enable the initial cyclization, which is followed by site-specific aziridine ring-opening with sodium azide. Subsequent to that, thallium-induced oxidative disulfide bond formation furnishes the final product. The NMR characterization of the molecules obtained using this method indicates that conformationally well-behaved systems are readily accessible. The site-specific introduction of azide functionality should open the doors to subsequent functionalization using well-established protocols.

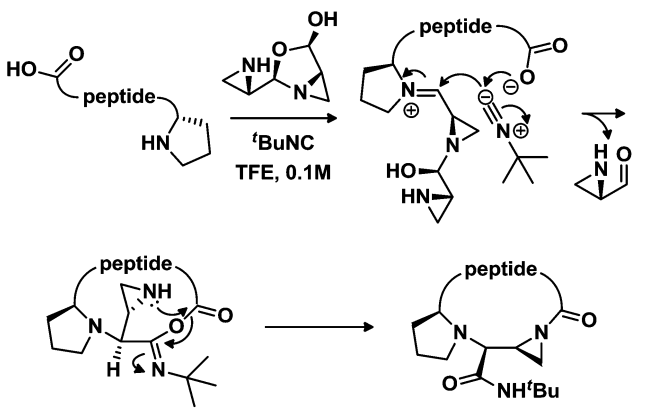
Introduction

Macrocycles are a therapeutically relevant class of molecules that often show increased binding affinity and selectivity to various biological targets,[1] as well as improved stability compared to the corresponding linear congeners.[2] Notable naturally occurring macrocycles include erythromycin, a broad-spectrum macrolide antibiotic, amphotericin B, a polyene antifungal, and cyclosporine A, a cyclic peptide immunosuppressant.[3] Cyclic peptides, as a subset of macrocycles, are particularly noteworthy for their ability to inhibit protein–protein interactions. Recent successes include the development of cyclic peptide inhibitors of the p53/MDM2 interaction,[4] αvβ3 integrin antagonists,[1a,5] and hydrocarbon stapled peptides for the disruption of the BID-Bcl-2 complex,[1b] among others.[6]

While there has been much development in contemporary strategies for the synthesis of cyclic peptides, peptide macrocyclization still proves to be challenging, especially for small- to medium-sized rings.[7] As increasing numbers of protein–protein interactions are validated as drug targets, there has been demand for new methods of peptide cyclization. Recently, we reported a robust process for head-to-tail peptide macrocyclization using aziridine aldehydes and isocyanides, enabling the synthesis of peptidomimetic macrocycles of various ring sizes and sequences (Scheme 1).[8]

As part of a program aimed at delineating drug-like properties of macrocycles, we became interested in creating additional post-cyclization constraints. Modifications such as *N*-methylation and intramolecular covalent bridge formation have been previously reported to improve potency and bioavailability of peptide macrocycles.[9] Conformationally restricted peptide macrocycles can also act as models for epitopes of folded proteins by conferring structural stability to peptides that would otherwise exist as random coils in solution.[10] Previous examples include the alkylation of cysteine residues within a peptide using tris-(bromomethyl)benzene, promoting internal hydrogen-bond formation within the structure and ultimately stabilizing the γ- and β-turns within the macrocycle.[11]

Inspired by the ubiquity of disulfides in biology, we sought to prepare synthetic peptidomimetic macrocycles containing a disulfide bridge. We also hoped to explore the compatibility of



Scheme 1 Mechanism of the macrocyclization of N-terminal proline peptides using (*S*)-aziridine-2-carboxaldehyde dimer and *tert*-butyl isocyanide. TFE = 2,2,2-trifluoroethanol.

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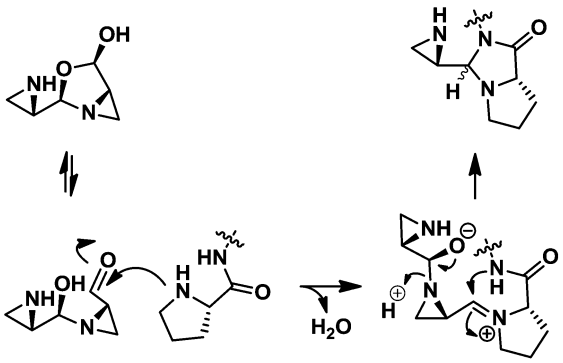
aziridine aldehyde-derived peptide macrocyclization with disulfide bridge formation. Ultimately, the intramolecular disulfide could serve as a structurally stabilizing element to further rigidify a macrocyclic scaffold. In this report, we outline a strategy for disulfide-bond formation in the synthesis of challenging medium-sized peptidomimetic macrocycles derived from the aziridine aldehyde-driven cyclization.

Synthesis

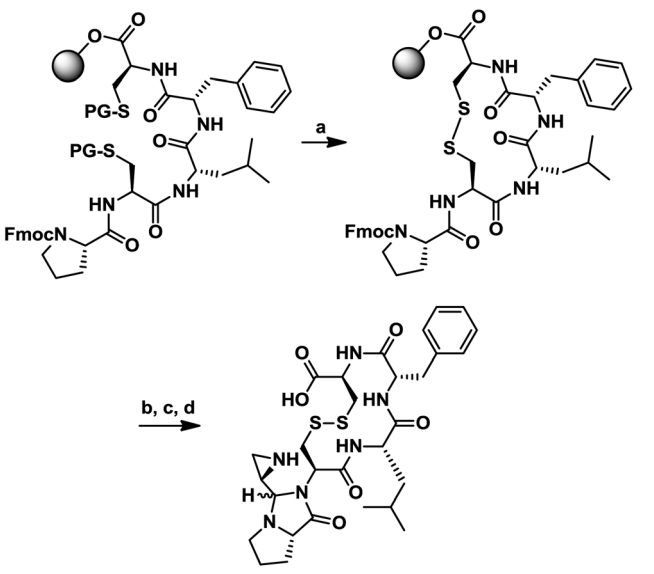
We envisioned the synthesis of disulfide-bridged macrocycles through the macrocyclization of peptides containing two cysteine residues. This approach can be realized by two different pathways – the first requiring formation of the disulfide bond followed by cyclization, while the second involving cyclization and subsequent disulfide bridge formation. For the first approach in which the disulfide is formed before cyclization (Scheme 2), the linear peptide Fmoc-PC(PG)LFC(PG)-OH (PG = Acm or Trt) was prepared on 2-chlorotrityl resin. The solid supported peptide was then subjected to oxidative deprotection using either iodine/base or thallium trifluoroacetate, $Tl(TFA)_3$. Oxidation was monitored by performing microcleavages of the resin-bound peptide followed by liquid chromatography-mass spectrometry (LC-MS) analysis. Out of four reactions, oxidative deprotection of the Acm-protected peptide by $Tl(TFA)_3$ gave the cleanest outcome. The disulfide-containing peptide then underwent Fmoc-deprotection and resin cleavage using 1,1,1,3,3,3-hexafluoroisopropan-2-ol/dichloromethane (HFIP/DCM, 1 : 3), followed by macrocyclization. The macrocyclization was monitored by LC-MS, which revealed a small amount of the desired product. However, the mass of an imidazolidinone product was observed to be the major component of the reaction mixture.



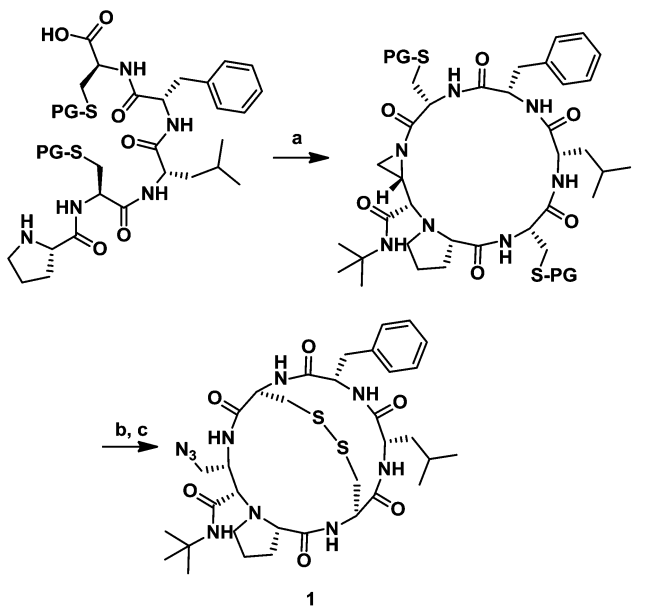
Scheme 3 Mechanism for formation of the imidazolidinone side product.

The mechanism of imidazolidinone formation (Scheme 3) first involves reversible dissociation of the aziridine aldehyde to the reactive open dimer form.[12] Next, condensation of the peptide N-terminal nitrogen with the open dimer form generates the iminium intermediate. Finally, nucleophilic attack of the $(n + 1)$ amide nitrogen on the resulting iminium gives the five-membered imidazolidinone. This process is hypothesized to occur because the disulfide forces the peptide to exist in a conformation where the C-terminal carboxylate is oriented away from the intermediate iminium and unfavourable for cyclization to proceed.

Since the presence of the disulfide appears to prohibit head-to-tail peptide macrocyclization and increases the possibility of imidazolidinone by-product formation, we wanted to see if macrocyclization before disulfide formation could form the desired disulfide-bridged peptidomimetic macrocycle. In the alternative approach, linear peptide PC(PG)LFC(PG) (PG = Acm or Trt) underwent cyclization first, followed by oxidation (Scheme 4). As head-to-tail macrocyclization of peptides using



Scheme 2 Disulfide formation prior to peptide macrocyclization. (a) I_2 (8 eq.), DIPEA (8 eq.), DCM/MeOH/H_2O (15 : 6 : 1, 18 mL g^{-1} resin), RT, 45 min. OR $Tl(TFA)_3$ (1.5 eq.), DMF (0.06 M), RT, 2 h; (b) 20% (%v/v) piperdine, DMF (10 mL g^{-1} resin), RT, 30 min; (c) HFIP/DCM (1 : 3, 10 mL g^{-1} resin), RT, 1 h; (d) (*S*)-aziridine-2-carboxaldehyde dimer (1 eq.), *tert*-butyl isocyanide (1 eq.), TFE (0.1 M), RT, 6h.



Scheme 4 PCLFC cyclization followed by disulfide formation. (a) (*S*)-aziridine-2-carboxaldehyde dimer (0.51 eq.), *tert*-butyl isocyanide (1.01 eq.), TFE (0.1 M), RT, 6 h; (b) NaN_3 (10 eq.), MeCN/DMF (0.04 M), 60 °C, 4 h; (c) Tl(TFA)3 (1.5 eq.), anisole (2 eq.), TFA (2 mM), RT, 5 h.

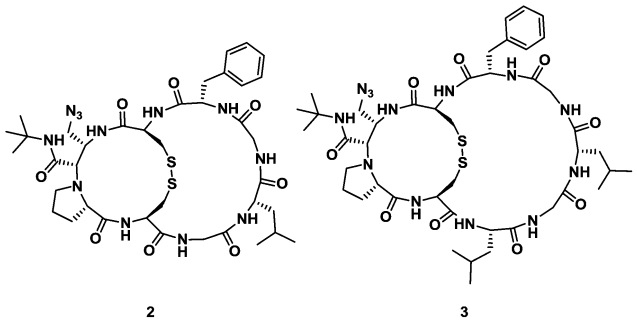


Fig. 1 Structures of disulfide-bridged peptidomimetic macrocycles derived from PC(Acm)GLGFC(Acm) (**2**) and PC(Acm)LGLGFC(Acm) (**3**).

Table 1 List of ^{1}H NMR chemical shifts for **2** (methanol-d_4). Chemical shifts are reported in ppm

Residue position	α	β	γ	δ
P1	3.63	2.19, 1.93	1.84, 1.67	3.30, 2.93
C2	4.37	3.69, 3.05	—	—
G3	4.09, 3.67	—	—	—
L4	4.57	1.57	1.48	0.92
G5	3.78, 3.41	—	—	—
F6	4.60	3.44, 2.85	—	—
C7	4.73	3.57, 3.35	—	—
Exocyclic amide	3.66 (CH)	1.40 (^{t}Bu)		
N_3	4.28 (CH)	3.33 (CH_2)		

aziridine aldehydes requires free N- and C-termini, the side-chain protected peptides must first be cleaved from resin after preparation on solid phase.

Cyclization of the Acm-protected peptide in solution proceeded cleanly, but the reaction with the Trt-protected peptide was slow and did not result in formation of the desired product. This can be attributed to steric bulk at the Trt-protected C-terminal cysteine residue which prohibited macrocyclization. The Acm-protected macrocycle was treated with sodium azide to afford the aziridine ring-opened product. Finally, oxidative Acm-deprotection with $Tl(TFA)_3$ yielded the final disulfide-bridged macrocycle **1** in 8% isolated yield.[13] Assay yields of the three step sequence were determined by LC-MS for the synthesis of **1** using caffeine as an external standard (details in ESI†). The first two steps (macrocyclization and azide functionalization) gave an assay yield of 92% while the final step (oxidative Acm-deprotection) gave an assay yield of 87%.

This method was used to generate two additional disulfide-bridged peptidomimetic macrocycles **2** and **3** derived from linear peptides PC(Acm)GLGFC(Acm) and PC(Acm)LGLGFC(Acm) (Fig. 1). 1- and 2-D NMR studies of the disulfide-bridged macrocycles demonstrate sharp peaks. A representative ^{1}H NMR spectrum and a list of ^{1}H resonances is shown for **2** (Fig. 2 and Table 1).

This spectrum indicates that the scaffolds are either structurally homogeneous and exist as one major conformation in solution, or have multiple conformations that interconvert rapidly in the NMR time scale. Given the size (from five to eight residues, medium length) of the peptidomimetic macrocycles as well as the presence of an intramolecular disulfide, the rapid interconversion is most likely but future work involves further experimentation in order to clarify this.

Summary

In summary, a method for the preparation of synthetically challenging medium-sized disulfide-bridged peptidomimetic macrocycles has been developed. The disulfide-containing compounds that were prepared illustrate an enabling approach to generate novel scaffolds whose bicyclic connectivities represent an underexplored area of structural space. In addition, the use of an azide nucleophile for late-stage aziridine ring-opening creates opportunities for downstream application of macrocycles. Given the tremendous interest in the development of macrocyclic scaffolds, the ability to generate disulfide-bridged macrocycles in a systematic fashion should facilitate the discovery of novel biological probes.

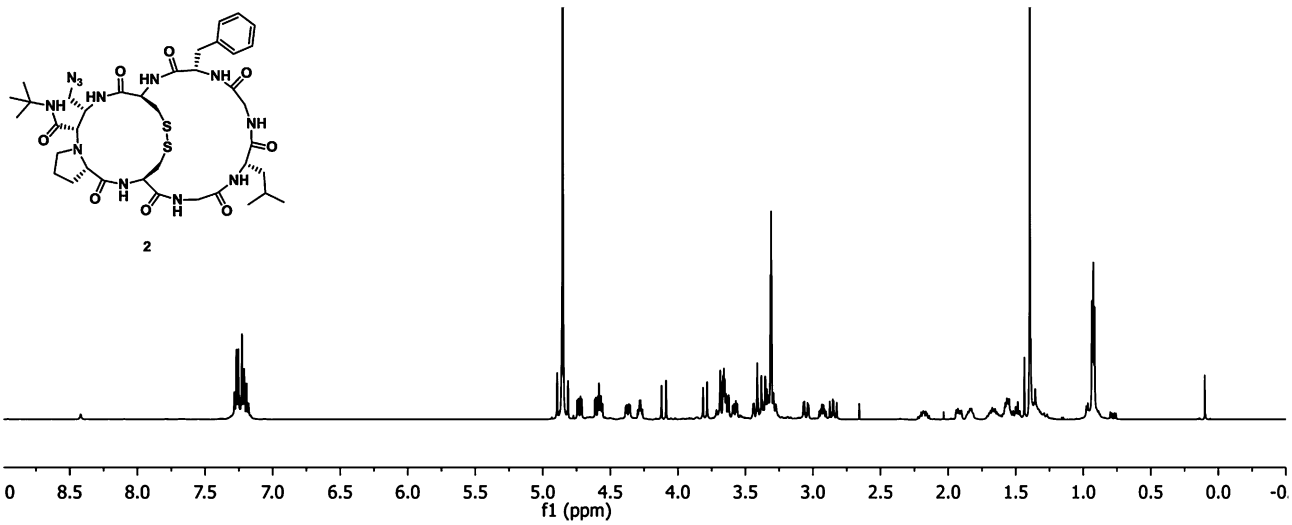


Fig. 2 ^{1}H NMR spectrum of **2**. Sample was prepared in methanol-d_4 and obtained on an Agilent DD2 500 MHz spectrometer with an Agilent HC 5-mm XSens cryogenically-cooled probe.

Experimental

2-Chlorotrityl chloride resin, coupling reagents, and all Fmoc-protected amino acids were purchased from AAPPTec Inc. (Louisville, KY, U.S.A.). 2,2,2-Trifluoroethanol was purchased from Oakwood Products (West Columbia, SC, U.S.A.). All other reagents and solvents were purchased from commercial sources and used as received, unless otherwise noted. All reactions involving chemical transformations on peptides were analyzed using analytical high-performance liquid chromatography-mass spectrometry (HPLC/MS) with an Agilent Technologies (Mississauga, ON, Canada) 1200 Series liquid chromatography system. Purification of final peptide products was performed with semi-preparative HPLC/MS using an Agilent 1260 Infinity Series liquid chromatography system. ^{1}H, ^{13}C, gCOSY, zTOCSY, and gc2HSQCse nuclear magnetic resonance (NMR) spectra were recorded on an Agilent DD2 500 MHz spectrometer. ^{1}H and ^{13}C NMR spectra chemical shifts (δ) are reported in parts per million (ppm) referenced to residual solvent peaks (MeOD, δ = 3.31 for ^{1}H, δ = 49.00 for ^{13}C).

General procedure for synthesis of compounds 1–3

Side-chain protected linear peptide (1 eq.) and (*S*)-aziridine-2-carboxaldehyde dimer (0.51 eq.) were weighed in a 20 mL glass vial. 2,2,2-Trifluoroethanol (0.1 M) was added, and the resultant solution/suspension stirred vigorously. *tert*-Butyl isocyanide (1.01 eq.) was added, and the reaction was stirred at room temperature for 3–5 hours until complete consumption of linear peptide as demonstrated by LC-MS analysis. The solvent was removed under reduced pressure and triturated once with *tert*-butyl methyl ether (TBME). The triturated aziridine-containing macrocycle was then dissolved in a mixture of acetonitrile : *N*,*N*-dimethylformamide (MeCN : DMF 1 : 1, 0.04 M) followed by the addition of sodium azide (10 eq.). The reactions were heated to 65 °C with stirring for 4 hours. The solvent was partially removed under reduced pressure. The remaining mixture in DMF was diluted with ten volumes of water, and extracted five times with ethyl acetate (EtOAc). The organic extracts were combined, dried with anhydrous sodium sulfate, filtered, and concentrated under reduced pressure. The residue containing azide ring-opened macrocycle was dissolved in trifluoroacetic acid (0.002 M), followed by addition of anisole (2 eq.) and thallium(III) trifluoroacetate (1.5 eq.). The reactions were stirred at room temperature for 4 hours. Next, the solvent was removed under reduced pressure and the macrocycles were triturated once with TBME. The crude material was then subjected to silica gel chromatography using a CombiFlash® Rf system equipped with a RediSep Rf Gold® Normal-Phase Silica 4 g column and a gradient of 0–50% methanol in EtOAc as eluent. After concentration under reduced pressure, the roughly purified product was subjected to semi-preparative HPLC/MS purification. The fractions containing the desired mass as determined by ESI-MS were combined and lyophilized overnight.

Disulfide 1. ^{1}H NMR (500 MHz, methanol-d$_4$) δ 7.30–7.18 (m, 5H), 4.22 (ddd, J = 7.0, 5.4, 4.0 Hz, 1H), 3.95 (t, J = 7.5 Hz, 1H), 3.75 (dd, J = 15.2, 5.3 Hz, 1H), 3.62–3.54 (m, 2H), 3.51–3.32 (m, 4H), 3.27–3.13 (m, 5H), 2.17–2.06 (m, 1H), 1.95 (m, 1H), 1.85 (m, 1H), 1.68 (m, 1H), 1.36 (s, 12H), 0.87 (d, J = 6.1 Hz, 1H), 0.81 (d, J = 6.2 Hz, 1H). ^{13}C NMR (126 MHz, methanol-d$_4$) δ 176.9, 174.4, 174.0, 172.2, 172.1, 171.9, 130.4, 129.5, 127.9, 115.8, 66.6, 66.3, 56.8, 54.33, 52.7, 52.6, 52.2, 51.6, 49.5, 49.3, 49.1, 49.0, 40.9, 40.4, 31.0, 28.8, 28.7, 25.7, 25.4, 22.9, 22.5. HRMS (ESI) calculated for $C_{38}H_{56}N_{12}O_8S_2$ (M + H$^+$): 759.3429; found: 759.3423. Retention time (analytical HPLC/MS): 6.99 min.

Disulfide 2. ^{1}H NMR (500 MHz, methanol-d$_4$) δ 7.31–7.15 (m, 5H), 4.73 (dd, J = 12.2, 4.9 Hz, 1H), 4.60 (dd, J = 11.2, 3.7 Hz, 1H), 4.57 (dd, 1H), 4.37 (dd, J = 12.0, 4.2 Hz, 1H), 4.28 (td, J = 7.0, 3.8 Hz, 1H), 4.10 (d, J = 16.9 Hz, 1H), 3.80 (d, J = 15.0 Hz, 1H), 3.68 (d, J = 10.0 Hz, 2H), 3.66 (d, J = 3.2 Hz, 1H), 3.65–3.61 (m, 1H), 3.58 (dd, J = 12.1, 4.9 Hz, 1H), 3.46–3.32 (m, 6H), 3.05 (dd, J = 15.1, 4.2 Hz, 1H), 2.93 (td, J = 9.7, 5.6 Hz, 1H), 2.85 (dd, J = 14.4, 11.1 Hz, 1H), 2.18 (tdd, J = 12.4, 9.9, 6.8 Hz, 1H), 1.96–1.89 (m, 1H), 1.88–1.80 (m, 1H), 1.72–1.61 (m, 1H), 1.61–1.53 (m, 2H), 1.49 (q, J = 8.4, 7.5 Hz, 1H), 1.40 (s, 9H), 0.93 (dd, J = 6.1, 4.3 Hz, 6H). ^{13}C NMR (126 MHz, methanol-d$_4$) δ 179.1, 174.4, 173.8, 172.9, 172.1, 171.9, 171.1, 170.9, 139.0, 130.0, 129.5, 127.7, 71.5, 68.2, 60.5, 56.2, 54.4, 53.5, 53.1, 52.9, 52.6, 44.9, 44.5, 44.0, 39.2, 37.3, 34.3, 32.3, 28.9, 28.8, 26.1, 25.6, 23.3, 23.0. HRMS (ESI) calculated for $C_{38}H_{56}N_{12}O_8S_2$ (M + H$^+$): 873.3858; found: 873.3831. Retention time (analytical HPLC/MS): 7.22 min.

Disulfide 3. ^{1}H NMR (500 MHz, methanol-d$_4$) δ 7.33–7.11 (m, 5H), 4.71 (t, J = 7.5 Hz, 1H), 4.66 (dd, J = 10.0, 4.9 Hz, 1H), 4.41 (td, J = 7.2, 4.4 Hz, 1H), 4.27 (dd, J = 9.5, 5.2 Hz, 1H), 4.20 (s, 2H), 4.14 (d, J = 17.2 Hz, 1H), 3.88–3.81 (m, 1H), 3.74–3.70 (m, 1H), 3.67 (dd, J = 3.9, 1.5 Hz, 1H), 3.65–3.60 (m, 1H), 3.57 (dd, J = 16.7, 1.6 Hz, 1H), 3.53–3.32 (m, 6H), 3.28–3.24 (m, 2H), 3.17 (dd, J = 13.9, 10.3 Hz, 1H), 3.01 (q, J = 7.8, 7.1 Hz, 1H), 2.23–2.10 (m, 1H), 1.95 (dt, J = 12.2, 4.4 Hz, 1H), 1.82 (ddd, J = 13.4, 6.8, 3.8 Hz, 2H), 1.80–1.60 (m, 5H), 1.59–1.50 (m, 1H), 1.40–1.36 (m, 9H), 0.97–0.90 (m, 12H). ^{13}C NMR (126 MHz, methanol-d$_4$) δ 177.4, 174.7, 172.9, 171.6, 171.1, 171.0, 170.7, 170.4, 169.7, 137.9, 128.8, 128.1, 126.2, 68.8, 66.3, 55.8, 54.3, 53.2, 53.0, 52.6, 52.4, 51.9, 51.4, 51.3, 43.3, 42.3, 39.5, 38.5, 34.7, 30.6, 27.5, 24.7, 24.6, 24.5, 22.3, 21.9, 20.7, 20.5. HRMS (ESI) calculated for $C_{38}H_{56}N_{12}O_8S_2$ (M + H$^+$): 986.4699 found: 986.4685. Retention time (analytical HPLC/MS): 7.39 min.

Acknowledgements

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13 Low isolated yields are due to product loss during semi-preparative HPLC-MS purification.

