Supplementary Information

Diene Incorporation by a Dehydratase Domain Variant in Modular Polyketide Synthases

Christian Hobson[1†‡], Matthew Jenner[1, 2†], Xinyun Jian[1, 2], Daniel Griffiths[1‡], Douglas M. Roberts[1], Matias Rey-Carrizo[1‡] and Gregory L. Challis[1, 2, 3, 4*]

[1]Department of Chemistry, University of Warwick, Coventry CV4 7AL, UK
[2]Warwick Integrative Synthetic Biology Centre, University of Warwick, Coventry CV4 7AL, UK
[3]Department of Biochemistry and Molecular Biology, Biomedicine Discovery Institute, Monash University, Clayton, VIC 3800, Australia
[4]ARC Centre of Excellence for Innovations in Peptide and Protein Science, Monash University, Clayton, VIC 3800, Australia

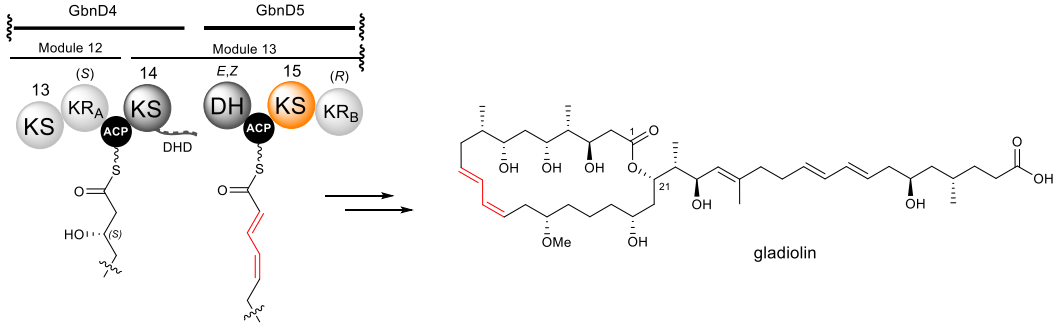
*Corresponding Author; Email: G.L.Challis@warwick.ac.uk

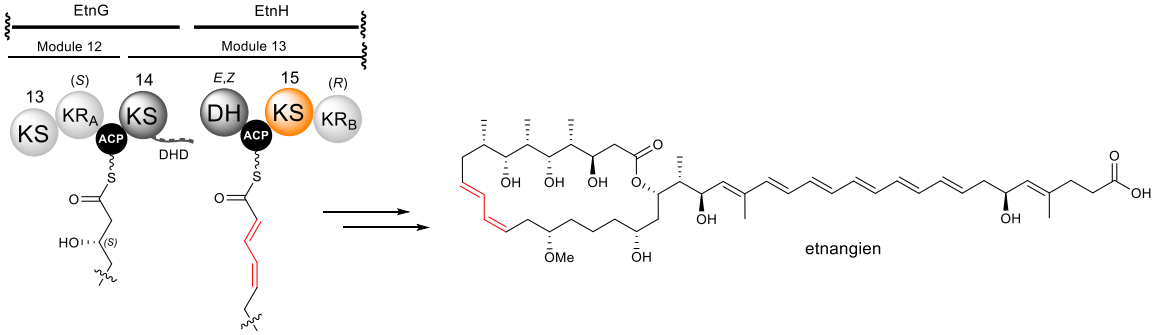
[†]These authors contributed equally
[‡]Current addresses: Willow Biosciences Inc, Vancouver, British Columbia, Canada (C.H.); Monash University Accident Research Centre, Clayton, VIC 3800, Australia (D.G.); BCN Medical Writing, Sabadell, Catalunya 08203, Spain (M.R.-C.); Department of Biochemistry and Molecular Biology, Biomedicine Discovery Institute, Monash University, Clayton, VIC 3800, Australia (X.J.)

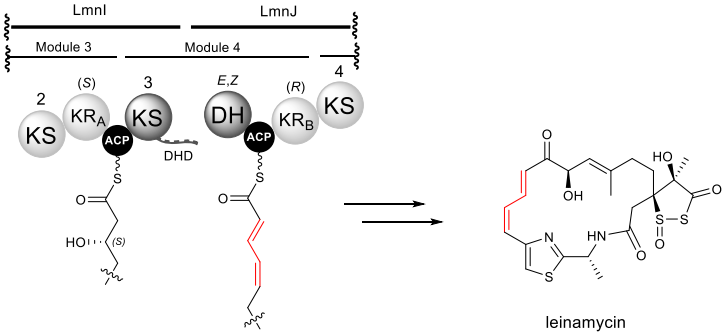
Supplementary Table 1: Measured and calculated (in parentheses) masses for the wild type and mutant DH-ACP di-domain from module 13 of the gladiolin PKS bearing substrate analogues products.

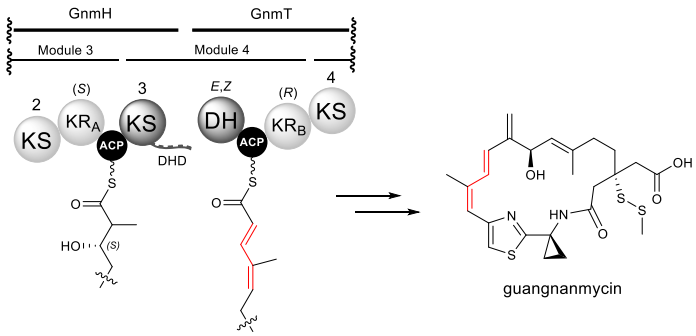
Substrate	Mass of di-domain bearing substrate / Da	Mass of di-domain bearing dehydration products / Da	Mass of di-domain bearing rehydration products / Da	Corresponding Figure
DH-ACP di-domain				
5	52706.6 (52706.5)	52687.4 (52688.5)	-	Fig. 2b
6	52705.3 (52706.5)	*N.D.* (52688.5)	-	Fig. 2c
7	52658.6 (52660.4)	-	52676.4 (52678.4)	Fig. 2d
8	52658.8 (52660.4)	-	*N.D.* (52678.4)	Fig. 2e
9	52693.6 (52694.5)	-1 x H_2O 52676.3 (52676.4) -2 x H_2O 52657.8 (52658.4)	-	Fig. 3a
10	52657.2 (52658.4)	-	+1 x H_2O 52674.6 (52676.4) +2 x H_2O 52695.6 (52694.5)	Fig. 3b
11	52692.9 (52694.5)	-1 x H_2O 52675.1 (52676.4) -2 x H_2O 52657.2 (52658.4)	-	Extended Data Fig. 1d
12	52693.6 (52694.5)	-1 x H_2O 52673.0 (52676.4) -2 x H_2O 52656.5 (52658.4)	-	Extended Data Fig. 1a (*top*)
13	52657.2 (52658.4)	-	+1 x H_2O 52675.8 (52676.4) +2 x H_2O 52695.7 (52694.5)	Extended Data Fig. 1a (*bottom*)
14	52692.9 (52694.5)	-1 x H_2O 52675.1 (52676.4) -2 x H_2O 52657.9 (52658.4)	-	Extended Data Fig. 1b
15	52675.1 (52676.4)	52657.5 (52658.4)	52693.6 (52694.5)	Fig. 4a
16	52675.1 (52676.4)	52657.2 (52658.4)	52694.3 (52694.5)	Extended Data Fig. 1e
DH-ACP (H158Y mutant)				
11	52701.2 (52702.4)	N.D.	52719.1 (52720.4)	Fig. 5b

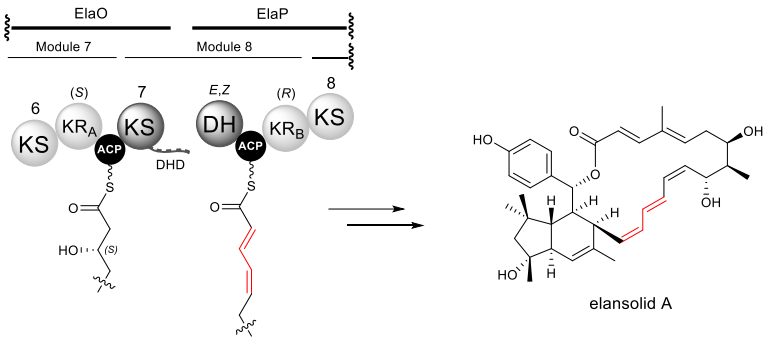
trans-AT PKSs

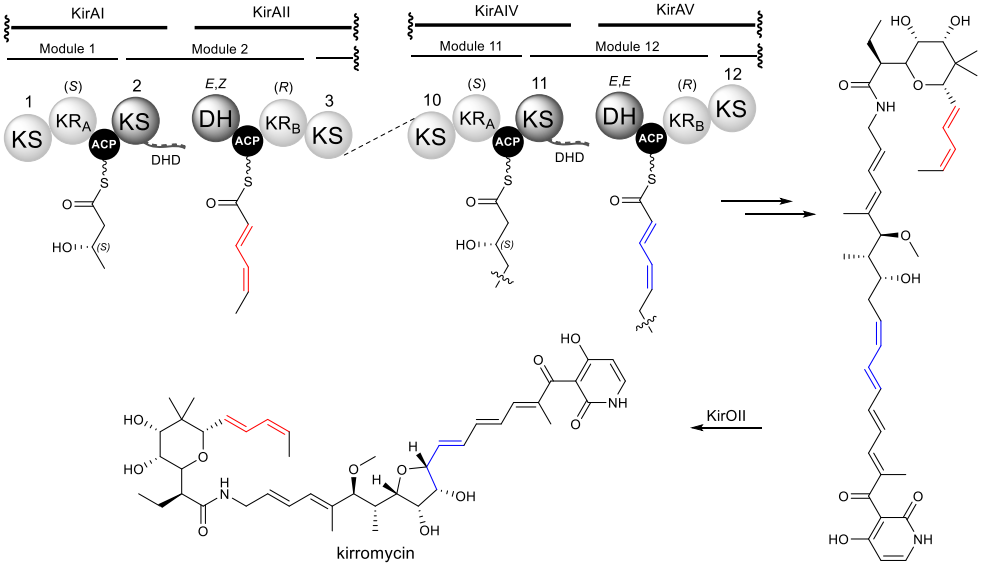


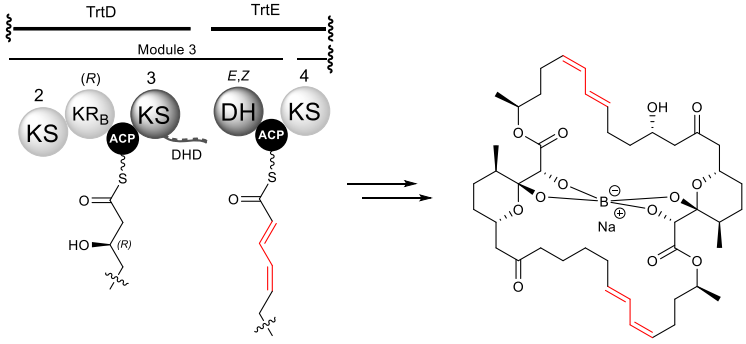


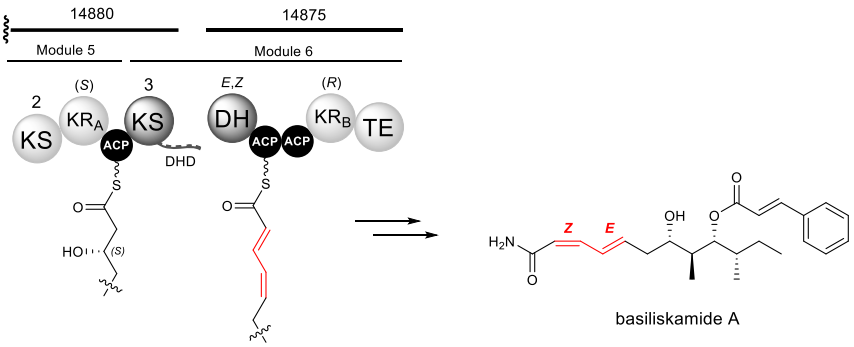


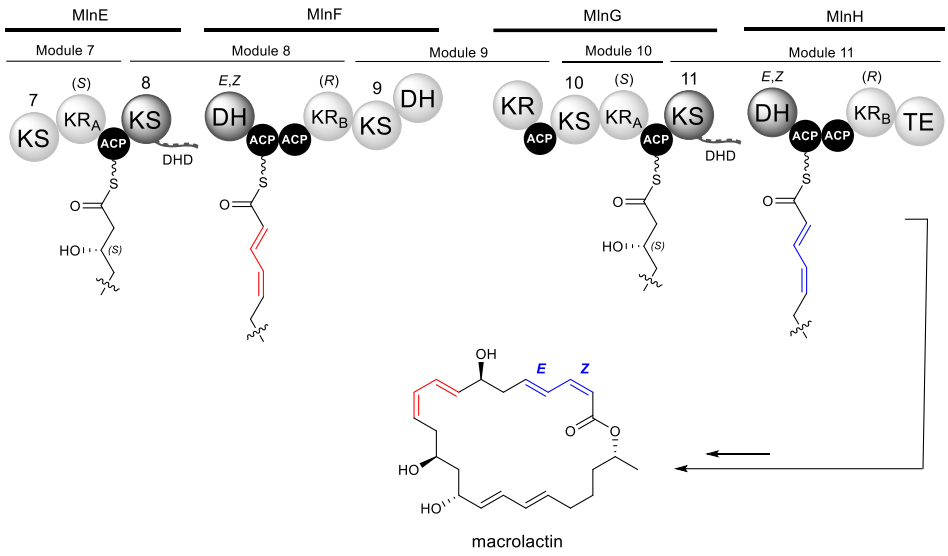


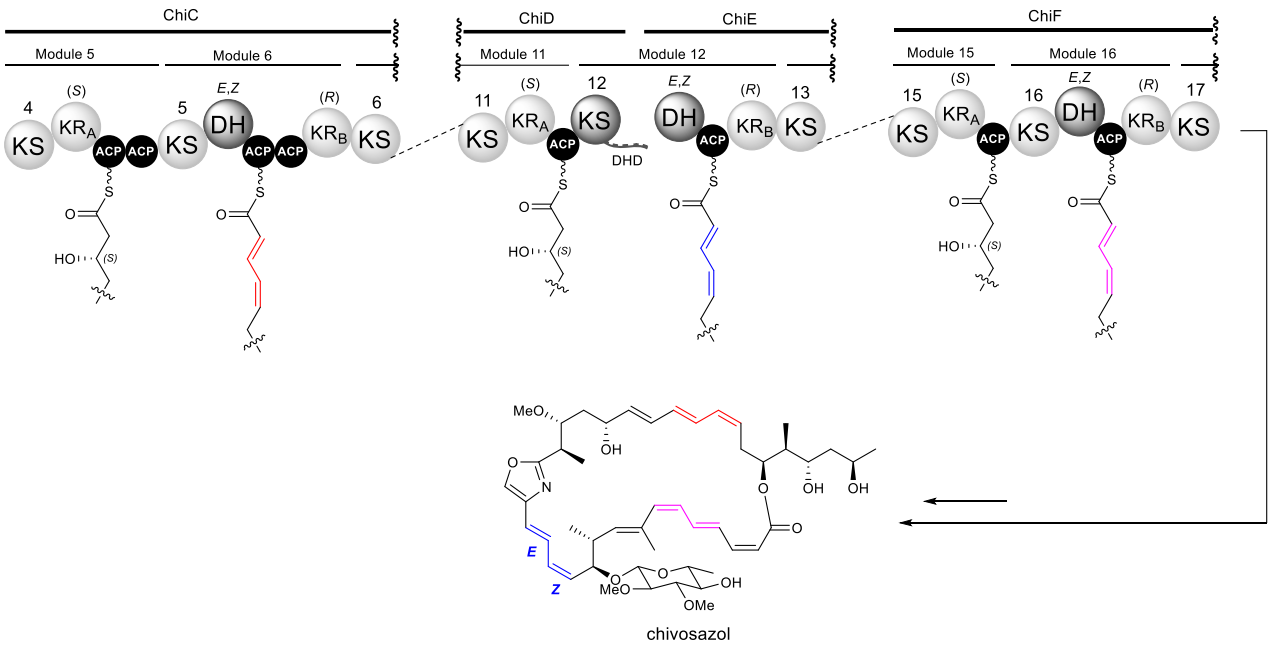

ElaO
ElaP
Module 7
Module 8
elansolid A

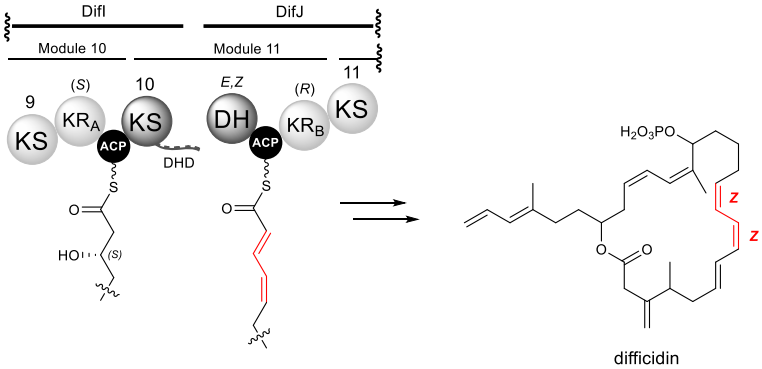

KirAI
KirAII
KirAIV
KirAV
Module 1
Module 2
Module 11
Module 12
KirOII
kirromycin


TrtD
TrtE
Module 3
tartrolon E

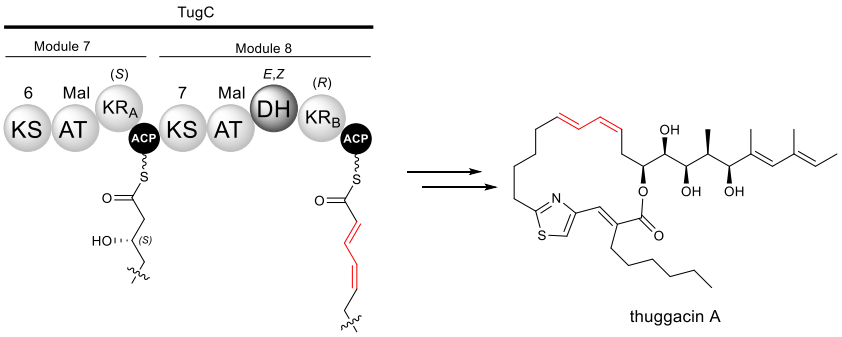

14880
14875
Module 5
Module 6
basiliskamide A

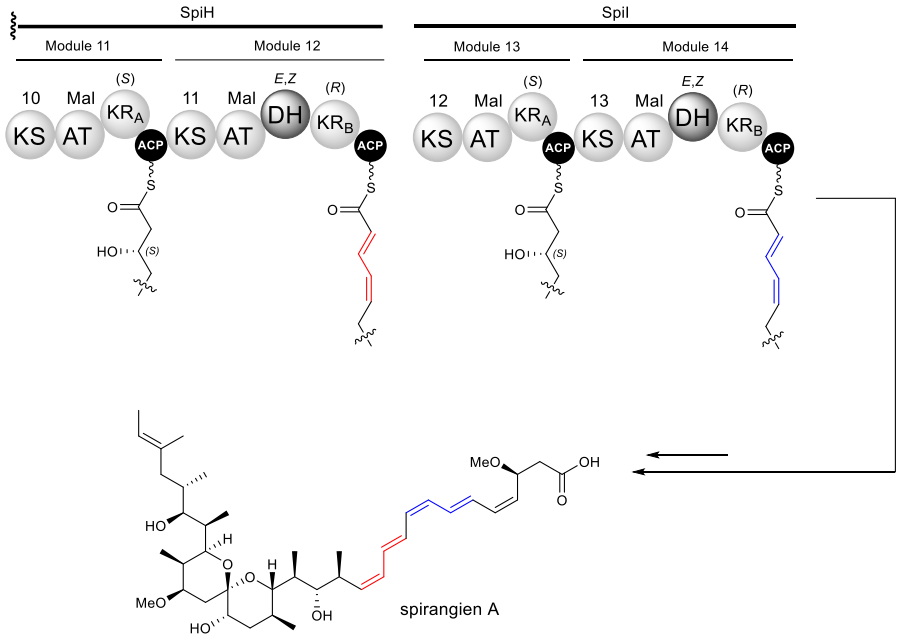

MlnE
MlnF
MlnG
MlnH
Module 7
Module 8
Module 9
Module 10
Module 11
macrolactin

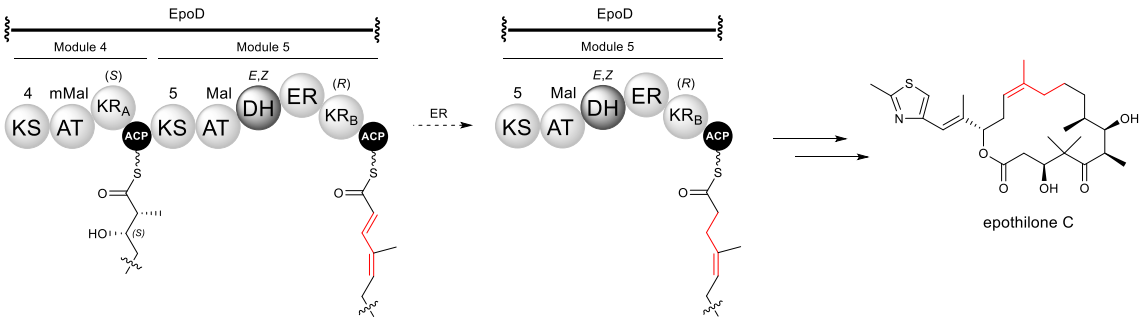

ChiC
ChiD
ChiE
ChiF
Module 5
Module 6
Module 11
Module 12
Module 15
Module 16
chivosazol


DifI
DifJ
Module 10
Module 11
difficidin

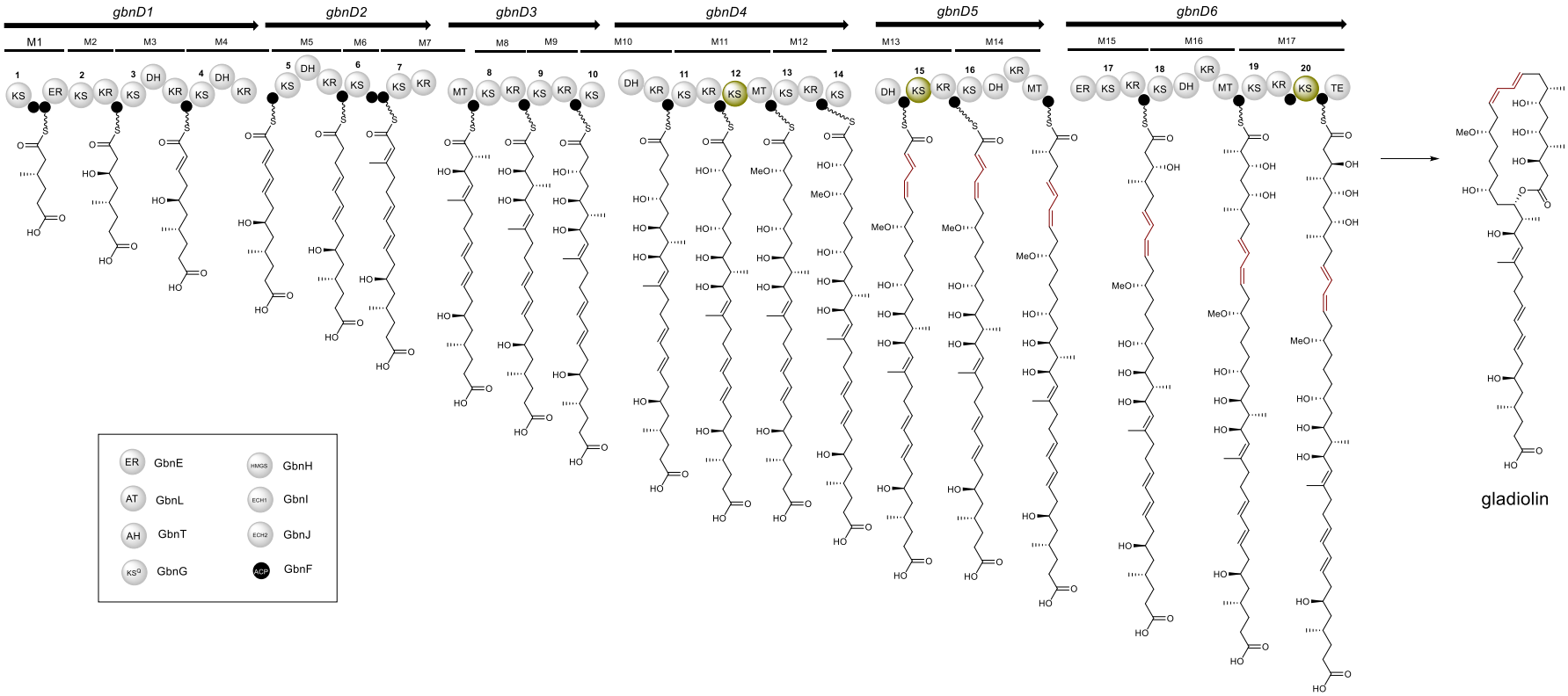
cis-AT PKSs


TugC
Module 7
Module 8
6
Mal
(S)
KR$_A$
7
Mal
E,Z
DH
(R)
KR$_B$
KS
AT
ACP
HO
(S)
OH
thuggacin A


SpiH
Module 11
Module 12
SpiI
Module 13
Module 14
10
11
12
13
Mal
(S)
KR$_A$
E,Z
DH
(R)
KR$_B$
KS
AT
ACP
HO
MeO
OH
spirangien A


EpoD
Module 4
Module 5
4
mMal
5
Mal
(S)
KR$_A$
E,Z
DH
ER
(R)
KR$_B$
KS
AT
ACP
HO
epothilone C

Supplementary Figure 1: Examples of DH-like domains in *trans*- and *cis*-AT PKSs proposed to be responsible for diene incorporation. Domains flanking the DH-like domains (as defined in the MIBIG database,[1] except the TE domain at the C-terminus of the basiliskamide assembly line, which was identified using PFAM, and the dehydratase docking (DHD) domains, which were identified as recently described[2]) are illustrated and the predicted stereospecificities[3] of the up- and downstream KR domains are indicated. To avoid ambiguity, KS domains are numbered sequentially from the N- to C-terminus of each PKS. This is unrelated to the module numbering. Note that the organization of domains in the *trans*-AT systems differ significantly from that in the canonical *cis*-AT PKSs: the second module is split across two subunits, with a DHD domain mediating communication between them, and the KR domain in the second module is positioned downstream of the ACP domain. In the case of the gladiolin and etnangien PKSs a putative non-elongating KS domain (highlighted in orange) is juxtaposed between the ACP and KR domains. In the case of tatrolon PKS, the second module lacks a KR domain and the KR domain in the first module is predicted to produce a (3*R*)-3-hydroxyacyl thioester. Dienes in the biosynthetic intermediates that correspond to those in the final products are highlighted in the same color. In most cases, the configuration of the diene in the final product matches the predicted 2*E*, 4*Z*-configuration of the diene in the corresponding biosynthetic intermediate. In a minority of cases (highlighted by stereochemical descriptors adjacent to double bonds), the configuration of the diene in the final product differs from the predicted 2*E*, 4*Z*-configuration of the diene in the corresponding biosynthetic intermediate. The reasons for this are currently unclear, but in the case of difficidin the triene is known to be configurationally labile.[4] In the case of kirromycin, one of the double bonds of the diene produced by the module 12 DH-like domain undergoes oxidative modification by the cytochrome P450 KirOII, resulting in formation of a dehydroxylated tetrahydrofuran.[5]



Supplementary Figure 2: Subunit, module and domain organization of the gladiolin *trans*-AT PKS. The proposed structures of the ACP domain-bound thioester intermediates following α- and β-carbon processing are shown. Note that although the PKS harbors 20 KS domains, only 17 chain elongation reactions are required for the assembly of the gladiolin backbone. No canonical non-elongating ketosynthase (KS^0) domains are present in the PKS, but the KS12, KS15 and KS20 domain (all highlighted in gold) are hypothesized not to elongate the polyketide chain based on comparisons with analogous PKS architectures containing canonical KS^0 domains. Domain abbreviations are as follows: KS, ketosynthase; KR, ketoreductase; DH, dehydratase; ER, enoyl reductase; MT, C-/O-methyltransferase; ACP, acyl carrier protein and TE, thioesterase. To avoid ambiguity, KS domains are numbered sequentially from the N- to C-terminus of the PKS. This is unrelated to the module numbering.

ACP domain

```
MHHHHHHGKP IPNPLLGLDS TENLYFQGLD PFT-

        10         20         30         40         50         60
VAAGYDGARA AALAAGESTR ASFDEALRRF VTDQLAAQGV ALAGRLGDDT PFFDAGLDST

        70         80         90        100        110
HLLALVRALE THCGRTFYPT LLFEHQTLRE LAAHLHRETP AAFGQAVPVW SESVAA
```

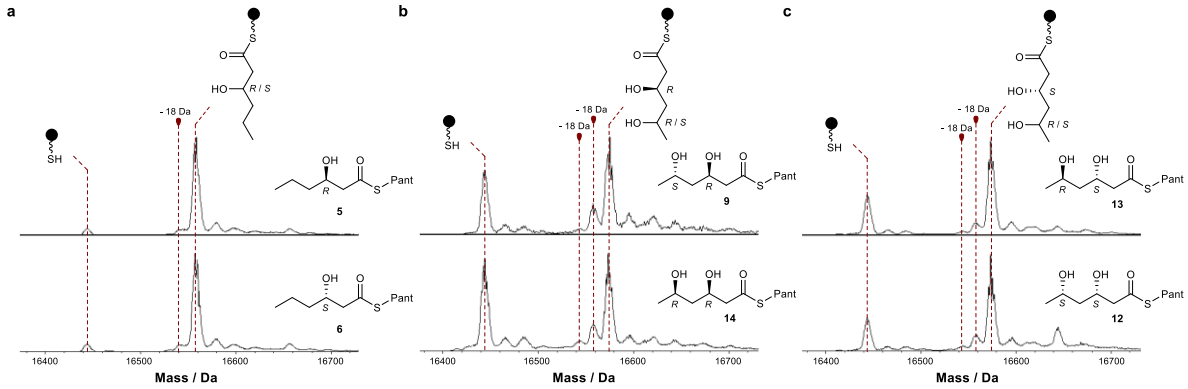
MW: 16,105 Da

DH-ACP di-domain

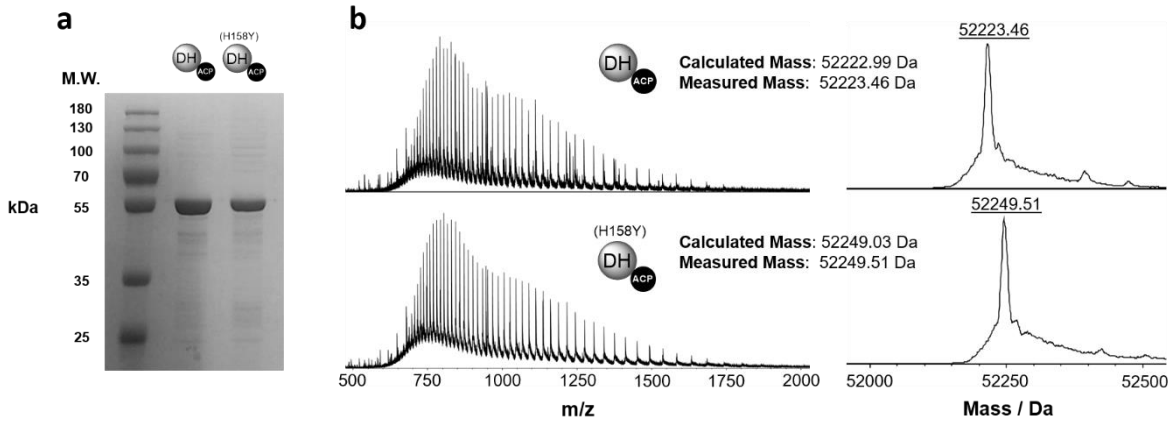
```
MHHHHHHGKP IPNPLLGLDS TENLYFQGLD PFT-

        10         20         30         40         50         60
MTHRHAASYE LDFEHDNLIL RDHRVHGVSI LPGVTLIDVV YRLGQHLLGH QRFELAQLLF

        70         80         90        100        110        120
RLPLATSGHL ARRMTVRFAP GADHGCWTVS LSSVPLRSGV PGTGRDLHAE CVLRELDAED

       130        140        150        160        170        180
LRDPAEADFD VAGFIASAER STRVDEVYRG VRELGVVHGP FMQTLGEIFH RGDEELMRLS

       190        200        210        220        230        240
LGPLAESLRE RFHAHPALLD GATFAGSAFK LVGEVAADFR DDRPHIPFSV ERVRLLRPFP

       250        260        270        280        290        300
ARILVASRHG DKLGGAGAAR REVTSSDLRI LDEEGRVLAL FERLSYKRVR QAADIVRLVD

       310        320        330        340        350        360
QAPGEAEGAV AGEAARSAGA DVGVGAVPAS TVAAGYDGAR AAALAAGEST RASFDEALRR

       370        380        390        400        410        420
FVTDQLAAQG VALAGRLGDD TPFFDAGLDS THLLALVRAL ETHCGRTFYP TLLFEHQTLR

       430        440
ELAAHLHRET PAAFGQAVPV WSESVAA-
```

MW: 52,223 Da

Supplementary Figure 3: Sequences and calculated molecular weights of the DH-ACP di-domain and the excised ACP domain from module 13 of the gladiolin PKS. The extra residues encoded by the expression vector appended to the N-termini of the proteins are in red. The Ser residue in the ACP domain that serves as the phosphopantheine attachment site and the active site His_1, His_2 and Asp residues in the DH domain are bold/underlined/blue.



Supplementary Figure 4: Intact protein MS analysis of the 3-hydroxy- and 3,5-dihydroxy-hexaonyl thioesters attached to the excised ACP(DH) domain from module 13 of the gladiolin PKS. Deconvoluted mass spectra of the excised ACP(DH) domain resulting from conversion of pantetheine thioesters to the corresponding coenzyme A thioesters and subsequent loading onto the *apo*-ACP domain. **a,** Using (3*R*)-3-hydroxyhexanoyl thioester **5** (top) and (3*S*)-3-hydroxyhexanoyl thioester **6** (bottom). **b,** Using (3*R,* 5*S*)-3,5-dihydroxyhexanoyl thioester **9** (top) and (3*R,* 5*R*)-3,5-dihydroxyhexanoyl thioester **14** (bottom) **c,** Using (3*S,* 5*R*)-3,5-dihydroxyhexanoyl thioester **13** (top) and (3*S,* 5*S*)-3,5-dihydroxyhexanoyl thioester **12** (bottom). In all cases, little or no spontaneous dehydration is observed. Experiments were performed in triplicate and representative data are shown.



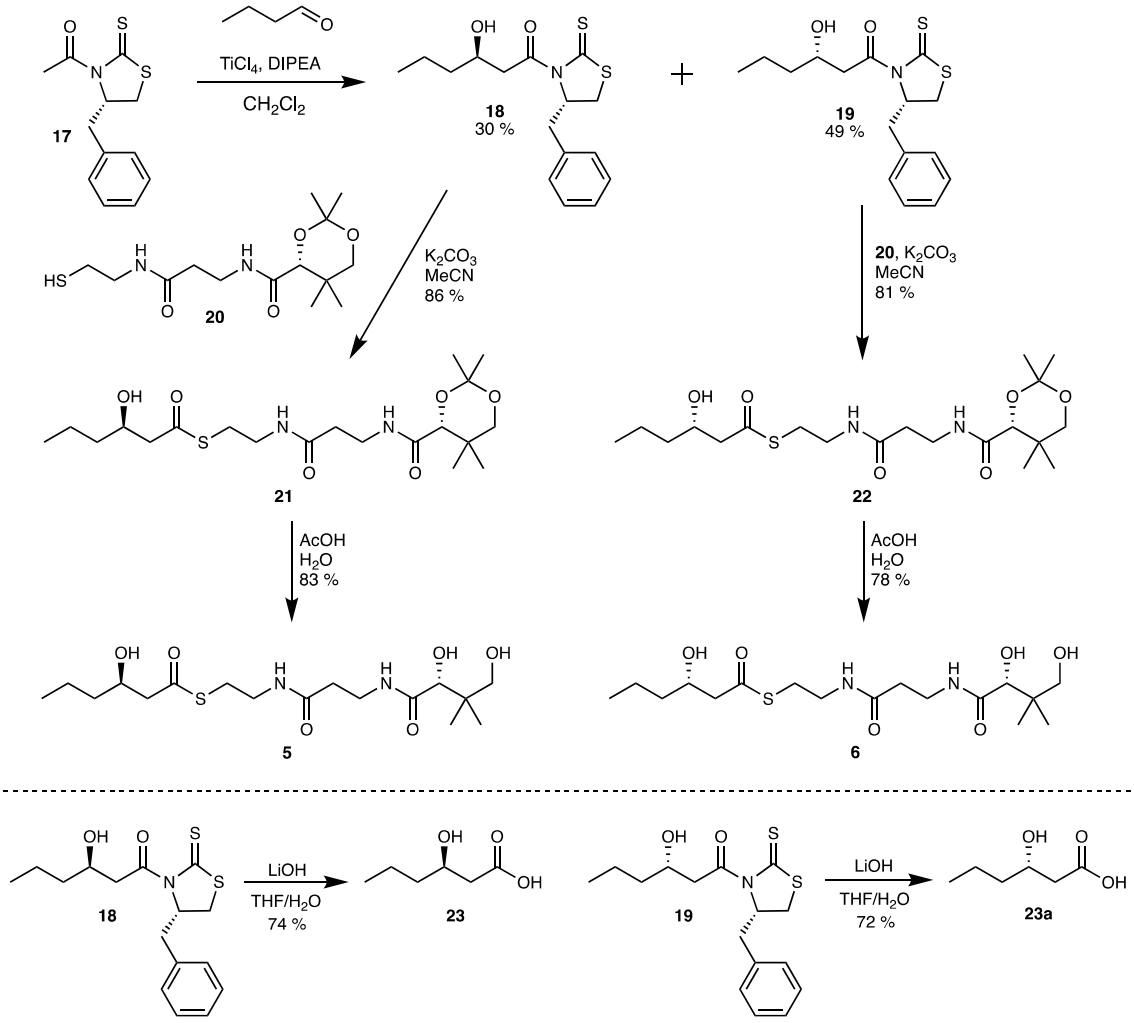
Supplementary Figure 5: Comparison of the H158Y mutant of the DH-ACP di-domain from module 13 of the gladiolin PKS with the wild type protein. a, 10% SDS-PAGE analysis of the purified wild type DH-ACP di-domain (middle lane) and the H158Y mutant (right lane). **b**, Mass spectra of the DH-ACP di-domain (top) and the H158Y mutant (bottom). The calculated and measured masses are given for each protein. Unprocessed (left) and deconvoluted (right) mass spectra are shown. The difference in mass between the wild type and mutant di-domain is consistent with the change of H158 to Y.

Supplementary Note: Synthesis of Pantetheine Thioesters

General Procedures

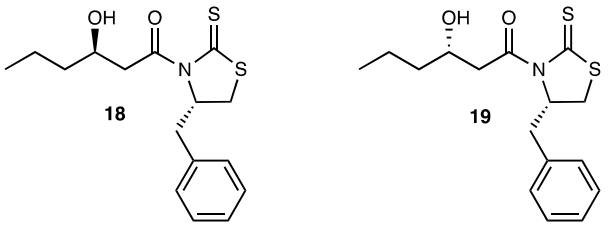
Room temperature refers to ambient temperature (298 K), 5 °C refers to a cold water bath and 0 °C refers to an ice slush bath. Heated experiments were conducted using thermostatically controlled oil baths. All chemicals were purchased from Sigma-Aldrich and were used without further purification. The stereochemistry of purchased chiral starting materials was confirmed using optical rotation. NMR spectra were recorded on Bruker Advance HD-500 and HD-700 MHz spectrometers at room temperature (298 K). Chemical shifts are reported in parts per million (ppm) referenced to either $CDCl_3$ (δ_H: 7.26 ppm and δ_C: 77.0 ppm) or CD_3OD (δ_H: 3.31 ppm and δ_C: 49.0 ppm). Coupling constants (*J*) are rounded to the nearest 0.5 Hertz (Hz). Multiplicities are given as multiplet (m), singlet (s), doublet (d), triplet (t), quartet (q), quintet (quin.), sextet (sext.), septet (sept.), octet (oct.) and nonet (non.). ^{1}H and ^{13}C assignments were established on the basis of COSY, DEPT, HSQC and HMBC correlations. Infra-red spectra were recorded using either a Perkin Elmer Spectrum 100 FT-IR spectrometer or an Alpha Bruker Platunium ATR single reflection diamond ATR module. Optical rotations were measured using an Optical Activity Ltd AA-1000 millidegree auto-ranging polarimeter (589 nm). Specific rotations are given in units of 10^{-1} deg cm^2 g^{-1}. Melting points were recorded on a Stuart scientific melting point apparatus and are uncorrected. Silica column chromatography was performed on 40-60 Å silica gel. Thin layer chromatography (TLC) was carried out on aluminium sheets coated with 0.2 mm silica gel 60 F_{254}. Plates were visualized using UV light (254 nm) or potassium permanganate solution followed by heating. Low resolution mass spectra (LRMS) were recorded using an Agilent 6130B single quadropole ESI-MS. High resolution mass spectra (HRMS) were obtained were obtained by either Dr Lijiang Song or Mr Philip Aston using a Bruker MaXis- ESI-Q-TOF-MS.

Synthesis of 3-hydroxy pantetheine thioesters



Synthesis of 3-hydroxy pantetheine thioesters 5 and 6. Auxiliary **17** and thiol **20** were synthesized according to literature procedures.[6,7] Confirmation of stereochemistry for known aldol products **18** and **19** was obtained by hydrolysis to corresponding acids **23** and **23a**.[8]

(*R*)-1-((*S*)-4-benzyl-2-thioxothiazolidin-3-yl)-3-hydroxyhexan-1-one (18) and (*S*)-1-((*S*)-4-benzyl-2-thioxothiazolidin-3-yl)-3-hydroxyhexan-1-one (19)[8]



(*R*)-1-((*S*)-4-benzyl-2-thioxothiazolidin-3-yl)-3-hydroxyhexan-1-one **18** and (*S*)-1-((*S*)-4-benzyl-2-thioxothiazolidin-3-yl)-3-hydroxyhexan-1-one **19** were synthesized according to the procedure described

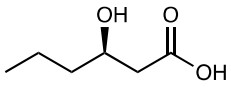
by Pompeo *et al*.[8] To a solution of (*S*)-1-(4-benzyl-2-thioxothiazolidin-3-yl)ethan-1-one **17** (648 mg, 2.58 mmol, 1.0 equiv.) in dry CH_2Cl_2 (10 mL) under an Ar atmosphere was added a 1 M solution of $TiCl_4$ in CH_2Cl_2 (2.58 mL, 2.58 mmol, 1.0 equiv.) dropwise at -78 °C. The orange suspension was allowed to stir for 10 min at the same temperature before *N,N*-diisopropylethylamine (0.90 mL, 5.16 mmol, 2.0 equiv.) was added dropwise resulting in an immediate colour change to dark burgundy. After 1 h, a solution of butyraldehyde (697 µL, 7.73 mmol, 3.0 equiv) in CH_2Cl_2 (4.0 mL) was added dropwise, and the solution stirred for a further 45 min at the same temperature. The resulting burgundy-orange solution was diluted with aqueous saturated NH_4Cl solution (20 mL) and deionized water (20 mL). The cooling bath was removed and the mixture was allowed to warm to 23 ºC. The layers were separated and the aqueous layer was extracted with CH_2Cl_2 (2 x 25 mL). The combined organic extracts were washed sequentially with an aqueous sodium bisulfite solution (1 M, 3 x 40 mL) and brine (40 mL), before being dried ($MgSO_4$), filtered, and concentrated *in vacuo*. The resulting orange residue was purified by silica chromatography (Et_2O : Petroleum ether, 30 : 70) to afford (*R*)-1-((*S*)-4-benzyl-2-thioxothiazolidin-3-yl)-3-hydroxyhexan-1-one **18** (250 mg, 30 %) as a bright yellow solid, and (*S*)-1-((*S*)-4-benzyl-2-thioxothiazolidin-3-yl)-3-hydroxyhexan-1-one **19** (409 mg, 49 %) as a bright yellow oil. Spectroscopic data for both compounds were consistent with that reported by Pompeo *et al*.[8] Further confirmation of stereochemistry for each aldol product was obtained by hydrolysis to the corresponding acid and comparison of spectral data to the literature.

(*R*)-1-((*S*)-4-benzyl-2-thioxothiazolidin-3-yl)-3-hydroxyhexan-1-one **18**: δ_H (700 MHz; $CDCl_3$) 7.37-7.34 (2H, m, *m*-Ar*H*), 7.30-7.27 (3H, m, *o*-Ar*H*, *p*-Ar*H*), 5.42 (1H, ddd, J 10.5, 7.0 and 4.0, C*H*N), 4.07 (1H, ddtd, J 11.5, 7.0, 4.5 and 2.5, C*H*OH), 3.46 (1H, dd, J 17.5 and 9.5, CH_2CON), 3.41 (1H, ddd, J 11.5, 7.5 and 1.0, CH_2S), 3.34 (1H, dd, J 17.5 and 2.5, CH_2CON), 3.23 (1H, dd, J 13.5 and 4.0, CH_2Ar), 3.09 (1H, d, J 4.0, O*H*), 3.05 (1H, dd, J 13.0 and 10.5, CH_2Ar), 2.91 (1H, d, J 11.5, CH_2S), 1.61-1.54 (1H, m, C$H_2$$CH_2CH_3$), 1.52-1.38 (3H, m, C$H_2CH_2CH_3$, CH_2CH_2CH_3), 0.94 (3H, t, J 7.0, CH_2CH_3); δ_C (175 MHz, $CDCl_3$) 201.7 (*C*S_2), 174.1 (*C*ON), 136.6 (ArC_{quat}), 129.6 (ArC), 129.1 (ArC), 127.5 (ArC), 68.4 (2C, *C*HN, *C*HOH), 46.7 (*C*H_2CON), 38.9 (*C*$H_2CH_2CH_3$), 37.0 (*C*H_2Ar), 32.2 (*C*H_2S), 18.9 (*C*H_2CH_3), 14.2 (CH_2*C*H_3)**;** HRMS (ESI) $C_{16}H_{21}NNaO_2S_2$ $[M + H]^+$ requires 346.0906, found 346.0905. $[\alpha]_D^{25}$ (c 0.5, $CHCl_3$): +97.

(*S*)-1-((*S*)-4-benzyl-2-thioxothiazolidin-3-yl)-3-hydroxyhexan-1-one **19:** δ_H (700 MHz; $CDCl_3$) 7.37-7.33 (2H, m, *m*-Ar*H*), 7.30-7.27 (3H, m, *o*-Ar*H*, *p*-Ar*H*), 5.40 (1H, ddd, J 11.0, 7.0 and 4.0, C*H*N), 4.17 (1H,

ddtd, J 11.5, 7.0, 4.5 and 2.5, C*H*OH), 3.65 (1H, dd, J 17.5 and 2.5, CH_2CON), 3.41 (1H, ddd, J 11.5, 7.0 and 1.0, CH_2S), 3.23 (1H, dd, J 13.5 and 4.0, CH_2Ar), 3.13 (1H, dd, J 17.5 and 9.5, CH_2CON), 3.05 (1H, dd, J 13.0 and 10.5, CH_2Ar), 2.91 (1H, d, J 11.5, CH_2S), 2.70 (1H, d, J 4.0, O*H*), 1.61-1.55 (1H, m, CH_2CH$_2$CH$_3$), 1.52-1.38 (3H, m, CH_2CH$_2$CH$_3$, CH$_2$CH_2CH$_3$), 0.94 (3H, t, J 7.0, CH$_2$CH_3); δ_C (175 MHz, $CDCl_3$) 201.6 (*C*S$_2$), 173.6 (*C*ON), 136.6 (ArC_{quat}), 129.6 (ArC), 129.1 (ArC), 127.5 (ArC), 68.5 (*C*HN), 67.8 (*C*HOH), 46.1 (*C*H$_2$CON), 38.7 (*C*H$_2$CH$_2$CH$_3$), 37.0 (*C*H$_2$Ar), 32.2 (*C*H$_2$S), 18.9 (*C*H$_2$CH$_3$), 14.2 (CH$_2$*C*H$_3$); HRMS (ESI) $C_{16}H_{21}NNaO_2S_2$ [M + H]$^+$ requires 346.0906, found 346.0906. $[\alpha]_D^{25}$ (c 0.5, $CHCl_3$): +126.

(3*R*)-Hydroxyhexanoic acid (**23**)

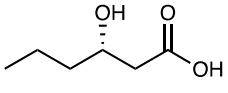


23

To a stirred solution of (*R*)-1-((*S*)-4-benzyl-2-thioxothiazolidin-3-yl)-3-hydroxyhexan-1-one **18** (81 mg, 0.25 mmol, 1.0 equiv.) in THF (2 mL) was added aqueous LiOH solution (1.0 M, 1.0 mL, 1.0 mmol, 4.0 equiv.) and the reaction stirred at room temperature for 18 h. The THF was then removed *in vacuo* and the resulting solution was washed with EtOAc (5 x 10 mL) to remove residual (*S*)-1-(4-benzyl-2-thioxothiazolidin-3-yl)ethan-1-one. The aqueous solution was then acidified to pH ~ 1 before being extracted with EtOAc (5 x 10 mL). The combined organics were then dried ($MgSO_4$), filtered and concentrated *in vacuo* to afford (3*R*)-Hydroxyhexanoic acid **23** as a colourless oil (24 mg, 74 %). Spectroscopic data was consistent with that reported by Pompeo *et al*.[8]

δ_H (700 MHz; $CDCl_3$) 4.05 (1H, tdd, J 8.0, 4.5 and 3.0, C*H*OH), 2.57 (1H, dd, J 16.5 and 3.0, CH_2COOH), 2.48 (1H, dd, J 16.5 and 9.0, CH_2COOH), 1.58-1.35 (4H, m, CH_2CH$_2$CH$_3$, CH_2CH$_3$), 0.94 (3H, t, J 7.0, CH_3); δ_C (175 MHz, $CDCl_3$) 177.9 (*C*OOH), 67.9 (*C*HOH), 41.2 (*C*H$_2$COOH), 38.8 (CH_2CH$_2$CH$_3$), 18.8 (CH_2CH$_3$), 14.1 (CH_3); $[\alpha]_D^{25}$ (c 2.2, $CHCl_3$): -27.1.[9]

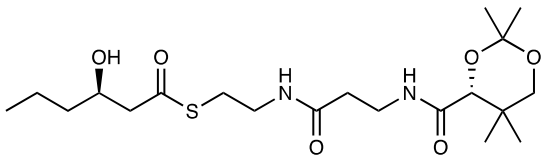
(3*S*)-Hydroxyhexanoic acid (**23a**)



23a

(3*S*)-Hydroxyhexanoic acid **23a** was synthesized using the same procedure as for the synthesis of (3*R*)-Hydroxyhexanoic acid **23** using (*S*)-1-((*S*)-4-benzyl-2-thioxothiazolidin-3-yl)-3-hydroxyhexan-1-one **19** (81 mg, 0.25 mmol, 1.0 equiv.) to afford the product as a colourless oil (23 mg, 72 %). Spectral data was identical to that of (3*R*)-Hydroxyhexanoic acid **23**. $[\alpha]_D^{25}$ (c 2.2, $CHCl_3$): +26.6.[10]

_S_-(2-(3-((_R_)-2,2,5,5-tetramethyl-1,3-dioxane-4-carboxamido)propanamido)ethyl) (_R_)-3-hydroxyhexanethioate (**21**)



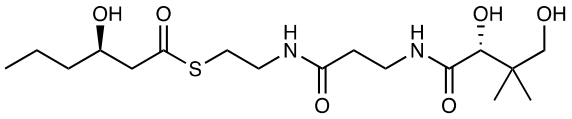
21

To a stirred solution of (*R*)-1-((*S*)-4-benzyl-2-thioxothiazolidin-3-yl)-3-hydroxyhexan-1-one **18** (150 mg, 0.47 mmol, 1 equiv.) in acetonitrile (10 mL), was added K_2CO_3 (64 mg, 0.47 mmol, 1 equiv.) and (*R*)-*N*-(3-((2-mercaptoethyl)amino)-3-oxopropyl)-2,2,5,5-tetramethyl-1,3-dioxane-4-carboxamide **20** (148 mg, 0.47 mmol, 1 equiv.). The mixture was stirred at room temperature for 1 h before being quenched by the addition of saturated NH_4Cl solution (1 mL). The acetonitrile was then removed *in vacuo* and the resulting solution extracted with EtOAc (3 x 10 mL). The combined organics were washed with brine (10 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo* to give a crude oil, which was purified by silica chromatography (CH_2Cl_2 : MeOH, 10 : 1) to give the product as a colourless oil (169 mg, 86 %).

ν_{max}/cm^{-1} (neat) 3428 (OH), 2935 (NH), 1653, 1533 (C=O), 1151 (C-O); δ_H (700 MHz; $CDCl_3$) 6.98 (1H, br. t, J 6.0, N*H*), 6.27 (1H, br. s, N*H*CH_2CH_2S), 4.14-4.09 (1H, m, C*H*OH), 4.08 (1H, s, C*H*CONH), 3.68 (1H, d J 11.5, C*H*$_2$OC$(CH_3)_2$), 3.59-3.33 (5H, m, NHC*H*$_2$, C*H*$_2CH_2S$, O*H*), 3.28 (1H, d J 11.5, C*H*$_2$OC$(CH_3)_2$), 3.12 (1H, ddd, J 14.0, 7.0 and 5.5, C*H*$_2$S), 2.98 (1H, ddd, J 14.0, 6.5 and 5.5, C*H*$_2$S), 2.72 (1H, dd, J 15.0 and 3.5, C*H*$_2$COS), 2.69 (1H, dd, J 15.0 and 9.0, C*H*$_2$COS), 2.42 (2H, td J 6.5 and 3.5, C*H*$_2$CONH), 1.55-1.35 (4H, m, CHOHC*H*$_2$C*H*$_2$), 1.46 (3H, s, OC(C*H*$_3)_2$), 1.42 (3H, s, OC(C*H*$_3)_2$), 1.03 (3H, s, CH_2C(C*H*$_3)_2$), 0.96 (3H, s, CH_2C(C*H*$_3)_2$), 0.93 (3H, t, J 7.5, CH_2C*H*$_3$); δ_C (175 MHz, $CDCl_3$) 199.4 (*C*OS), 171.3 (CH_2*C*ONH), 170.7 (CH*C*ONH), 99.3 (O*C*$(CH_3)_2$), 77.3 (*C*H), 71.6 (*C*H_2OC$(CH_3)_2$), 68.7 (*C*HOH), 51.4 (*C*H_2COS), 39.3 (*C*H_2CH_2S), 39.3 (C*H*$_2$CHOH), 36.5 (*C*H_2CONH), 35.4 (*C*H_2NH),

33.2 ($CH_2C(CH_3)_2$), 29.6 (($OC(CH_3)_2$), 28.8 (CH_2S), 22.3 ($CH_2C(CH_3)_2$), 19.0 ($CH_2C(CH_3)_2$), 18.9 ($OC(CH_3)_2$), 18.8 (CH_3CH_2), 14.1 (CH_2CH_3); HRMS (ESI) $C_{20}H_{36}N_2NaO_6S$ [M + Na]$^+$ requires 455.2192, found 455.2190; $[\alpha]_D^{25}$ (c 0.2, $CHCl_3$): +16.2.

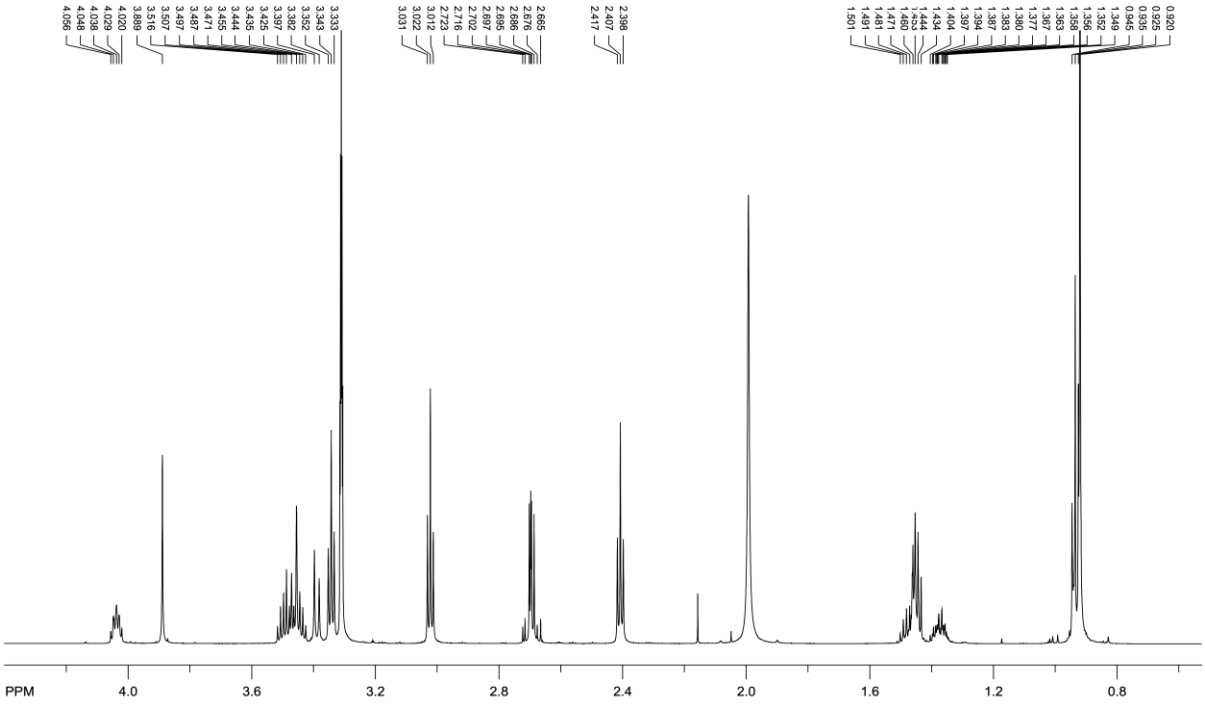
***S*-(2-(3-((*R*)-2,4-dihydroxy-3,3-dimethylbutanamido)propanamido)ethyl) (*R*)-3-hydroxyhexanethioate** (**5**)



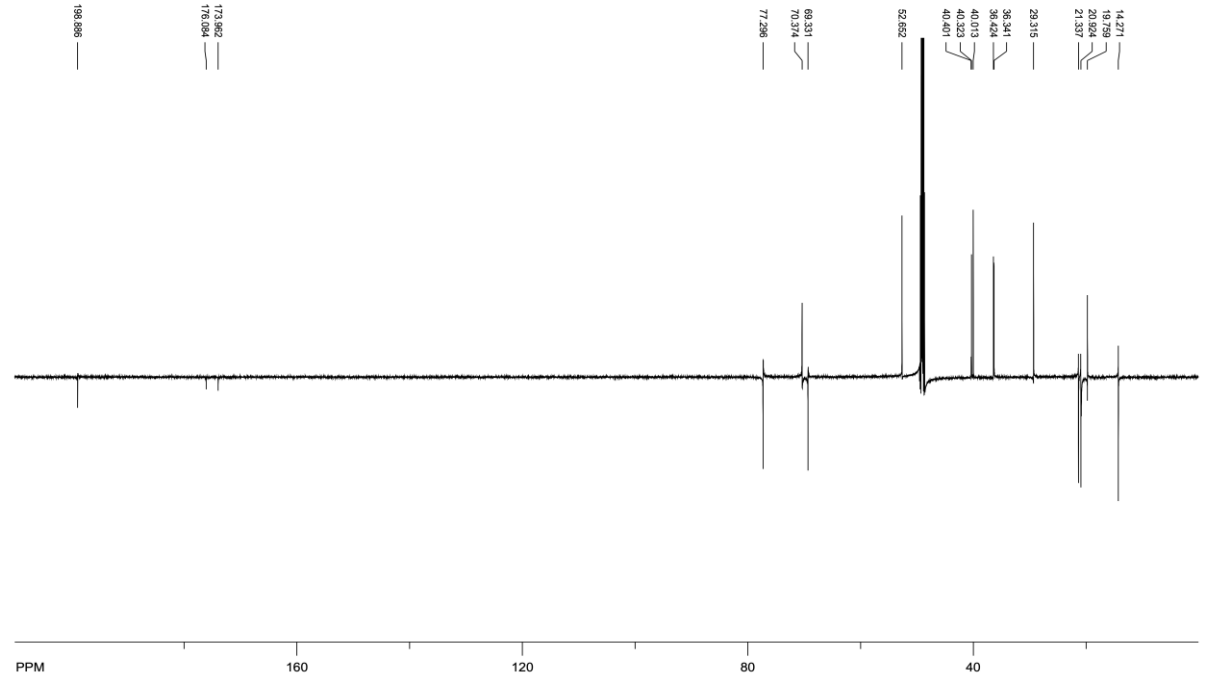
5

S-(2-(3-((*R*)-2,2,5,5-tetramethyl-1,3-dioxane-4-carboxamido)propanamido)ethyl) (*R*)-3-hydroxyhexanethioate **21** (100 mg, 0.23 mmol, 1.0 equiv.) was stirred in AcOH : H_2O (2 : 1, 3 mL), for 16 h at room temperature. The mixture was concentrated *in vacuo* and purified using silica chromatography (CH_2Cl_2 : MeOH, 85 : 15) to give the product as a colourless oil (75 mg, 83 %).

ν_{max}/cm^{-1} (neat) 3400 (OH), 2876 (NH), 1644, 1530 (C=O), 1065 (C-O); δ_H (700 MHz; CD_3OD) 4.06-4.02 (1H, m, C*H*OH), 3.89 (1H, s, C*H*), 3.52-3.42 (2H, m, NHCH_2), 3.46 (1H, d, J 11.0, CH_2OH), 3.39 (1H, d, J 11.0, CH_2OH), 3.34 (2H, t, J 6.5, CH_2CH_2S), 3.02 (2H, t, J 6.5, CH_2S), 2.71 (1H, dd, J 15.0 and 5.0, CH_2COS), 2.68 (1H, dd, J 15.0 and 7.5, CH_2COS), 2.41 (2H, t, J 6.5, CH_2CONH), 1.52-1.34 (4H, m, $CH_3CH_2CH_2$), 0.93 (3H, t, J 7.0, CH_2CH_3), 0.92 (6H, s, $CH_2C(CH_3)_2$); δ_C (175 MHz, CD_3OD) 198.9 (*C*OS), 176.0 (CH*C*ONH), 174.0 (CH_2*C*ONH), 77.3 (*C*H), 70.4 (*C*H_2OH), 69.3 (*C*HOH), 52.6 (*C*H_2COS), 40.4 ($CH_3CH_2CH_2$), 40.3 ($CH_2C(CH_3)_2$), 40.0 (*C*H_2CH_2S), 36.4 (*C*H_2CONH), 36.3 (NH*C*H_2), 29.3 (*C*H_2S), 21.3 ($CH_2C(CH_3)_2$), 20.9 ($CH_2C(CH_3)_2$), 19.8 (CH_3CH_2) 14.3 (CH_3CH_2); HRMS (ESI) $C_{17}H_{32}N_2NaO_6S$ [M + Na]$^+$ requires 415.1879, found 415.1880; $[\alpha]_D^{25}$ (c 0.2, MeOH): +10.4.

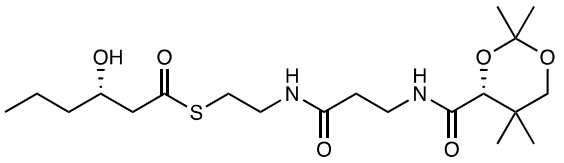


The ^{1}H NMR spectrum of *S*-(2-(3-((*R*)-2,4-dihydroxy-3,3-dimethylbutanamido) propanamido)ethyl) (*R*)-3-hydroxyhexanethioate **5** (d^{4}-MeOD).



The ^{13}C NMR spectrum of *S*-(2-(3-((*R*)-2,4-dihydroxy-3,3-dimethylbutanamido) propanamido)ethyl) (*R*)-3-hydroxyhexanethioate **5** (d^{4}-MeOD).

***S*-(2-(3-((*R*)-2,2,5,5-tetramethyl-1,3-dioxane-4-carboxamido)propanamido)ethyl) (*R*)-3-hydroxyhexanethioate** (**22**)

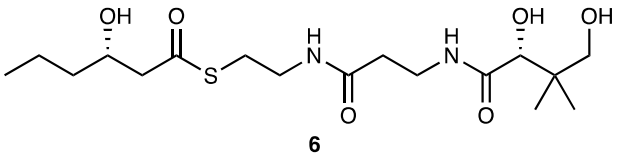


22

S-(2-(3-((*R*)-2,2,5,5-tetramethyl-1,3-dioxane-4-carboxamido)propanamido)ethyl) (*S*)-3-hydroxyhexanethioate **22** was synthesized using the same procedure as for the synthesis of *S*-(2-(3-((*R*)-2,2,5,5-tetramethyl-1,3-dioxane-4-carboxamido) propanamido)ethyl) (*R*)-3-hydroxyhexanethioate **21** using (*S*)-1-((*S*)-4-benzyl-2-thioxothiazolidin-3-yl)-3-hydroxyhexan-1-one **19** (150 mg, 0.47 mmol, 1 equiv.) to give the product as a colourless oil (159 mg, 81 %).

ν_{max}/cm^{-1} (neat) 3430 (OH), 2935 (NH), 1655, 1534 (C=O), 1147 (C-O); δ_H (700 MHz; $CDCl_3$) 6.99 (1H, br. t, J 6.0, N*H*), 6.35 (1H, t, J 5.5, N*H*CH_2CH_2S), 4.13-4.08 (1H, m, C*H*OH), 4.07 (1H, s, C*H*CONH), 3.68 (1H, d J 11.5, C*H*$_2$OC$(CH_3)_2$), 3.59-3.44 (4H, m, NHC*H*$_2$, C*H*$_2$CH$_2$S,), 3.38 (1H, br. s, O*H*), 3.28 (1H, d J 11.5, C*H*$_2$OC$(CH_3)_2$), 3.10 (1H, dt, J 14.0 and 6.0, C*H*$_2$S), 3.01 (1H, ddd, J 14.0 and 6.0, C*H*$_2$S), 2.73 (1H, dd, J 15.0 and 3.5, C*H*$_2$COS), 2.68 (1H, dd, J 15.0 and 9.0, C*H*$_2$COS), 2.42 (1H, dt, J 15.0 and 6.5, C*H*$_2$CONH), 2.40 (1H, dt, J 15.0 and 7.0, C*H*$_2$CONH), 1.56-1.35 (4H, m, CHOHC*H*$_2$C*H*$_2$), 1.46 (3H, s, OC(C*H*$_3$)$_2$), 1.42 (3H, s, OC(C*H*$_3$)$_2$**),** 1.03 (3H, s, CH$_2$C(C*H*$_3$)$_2$), 0.96 (3H, s, CH$_2$C(C*H*$_3$)$_2$), 0.93 (3H, t, J 7.0, CH$_2$C*H*$_3$); δ_C (175 MHz, $CDCl_3$) 199.4 (*C*OS), 171.3 (CH$_2$*C*ONH), 170.7 (CH*C*ONH), 99.3 (O*C*$(CH_3)_2$), 77.3 (*C*H), 71.6 (*C*H$_2$OC$(CH_3)_2$), 68.9 (*C*HOH), 51.4 (*C*H$_2$COS), 39.4 (C*H*$_2$CHOH), 39.3 (*C*H$_2$CH$_2$S), 36.5 (*C*H$_2$CONH), 35.4 (*C*H$_2$NH), 33.2 (CH$_2$*C*$(CH_3)_2$), 29.6 ((OC(*C*H$_3$)$_2$), 28.9 (*C*H$_2$S), 22.3 (CH$_2$C(*C*H$_3$)$_2$), 19.1 (CH$_2$C(*C*H$_3$)$_2$), 18.9 (OC(*C*H$_3$)$_2$), 18.9 (CH$_3$*C*H$_2$), 14.1 (CH$_2$*C*H$_3$); HRMS (ESI) $C_{20}H_{36}N_2NaO_6S$ $[M + Na]^+$ requires 455.2192, found 455.2193; $[\alpha]_D^{25}$ (c 0.1, $CHCl_3$): +38.1.

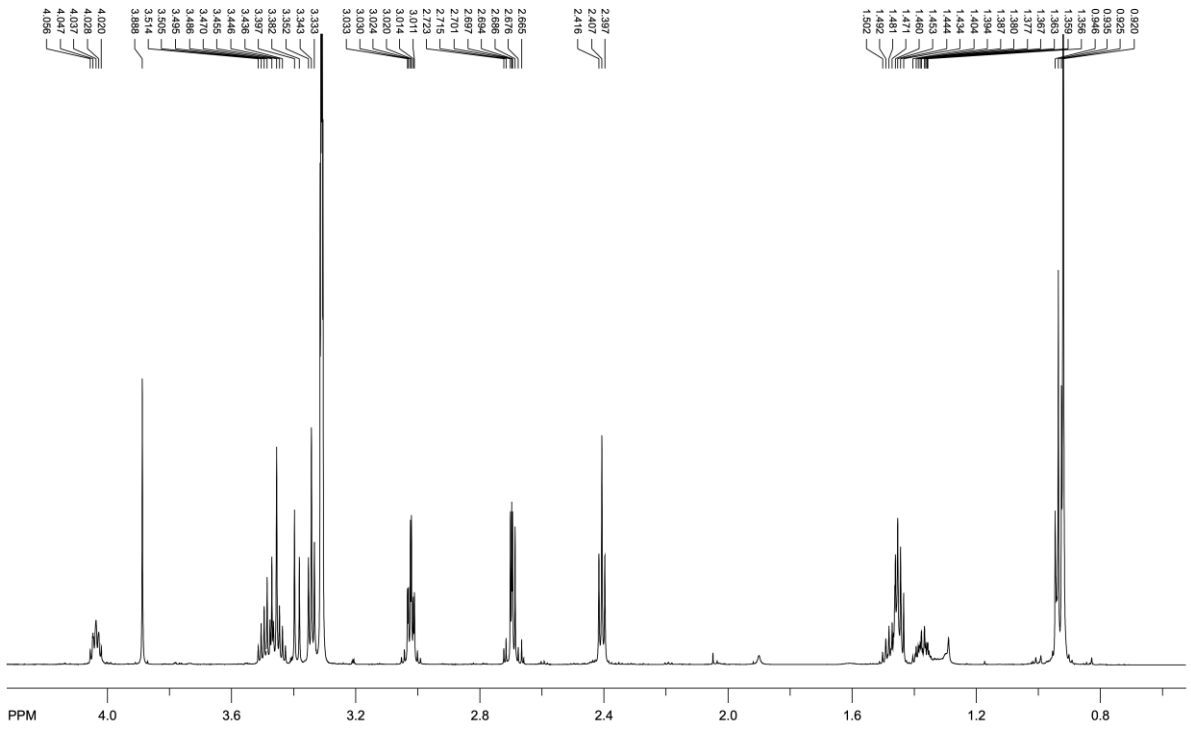
***S*-(2-(3-((*R*)-2,4-dihydroxy-3,3-dimethylbutanamido)propanamido)ethyl) (*R*)-3-hydroxyhexanethioate** (**6**)



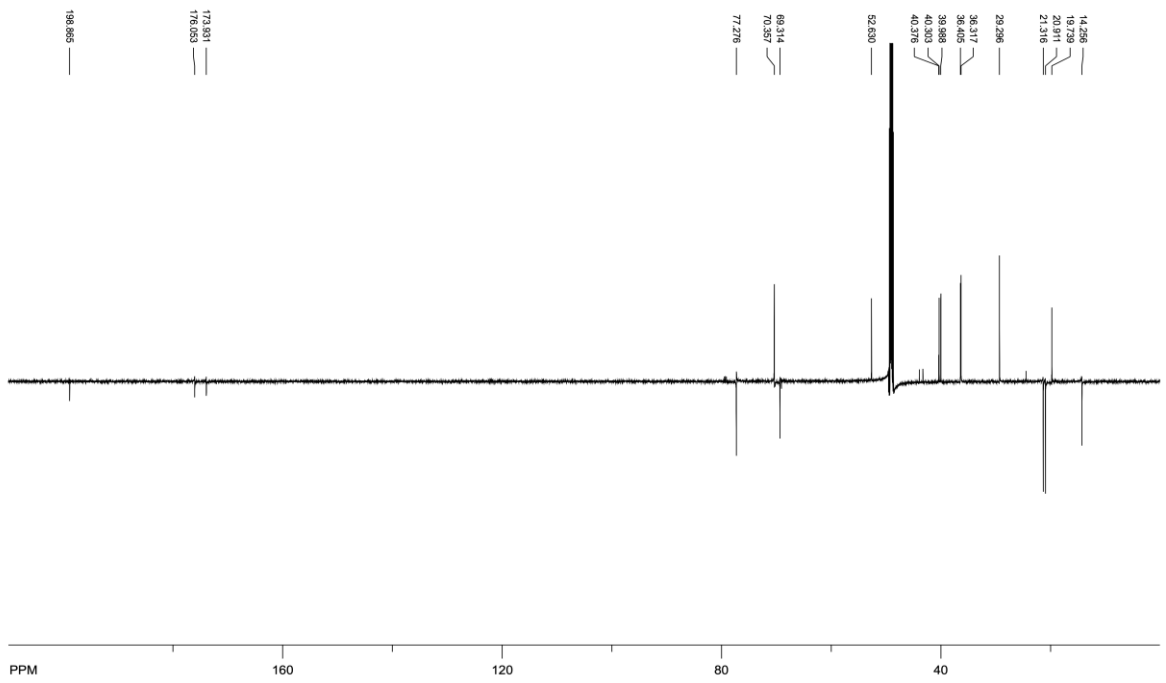
6

S-(2-(3-((*R*)-2,4-dihydroxy-3,3-dimethylbutanamido)propanamido)ethyl) (*S*)-3-hydroxyhexanethioate **6** was synthesized using the same procedure as for the synthesis of *S*-(2-(3-((*R*)-2,4-dihydroxy-3,3-dimethylbutanamido)propanamido)ethyl) (*R*)-3-hydroxyhexanethioate **5** using *S*-(2-(3-((*R*)-2,2,5,5-tetramethyl-1,3-dioxane-4-carboxamido)propanamido)ethyl) (*S*)-3-hydroxyhexanethioate **22** (100 mg, 0.22 mmol, 1 equiv.) to give the product as a colourless oil (72 mg, 85 %).

ν_{max}/cm^{-1} (neat) 3306 (OH), 2875 (NH), 1641, 1522 (C=O), 1074 (C-O); δ_H (700 MHz; CD_3OD) 4.06-4.02 (1H, m, C*H*OH), 3.89 (1H, s, C*H*), 3.52-3.42 (2H, m, NHCH_2), 3.46 (1H, d, J 11.0, CH_2OH), 3.39 (1H, d, J 11.0, CH_2OH), 3.34 (2H, t, J 6.5, CH_2CH$_2$S), 3.02 (2H, td, J 6.5 and 2.0 CH_2S), 2.71 (1H, dd, J 15.0 and 5.0, CH_2COS), 2.68 (1H, dd, J 15.0 and 7.5, CH_2COS), 2.41 (2H, t, J 6.5, CH_2CONH), 1.51-1.34 (4H, m, CH$_3$CH_2CH_2), 0.93 (3H, t, J 7.0, CH$_2$CH_3), 0.92 (6H, s, CH$_2$C(CH_3)$_2$); δ_C (175 MHz, CD_3OD) 198.9 (*C*OS), 176.1 (CH*C*ONH), 173.9 (CH$_2$*C*ONH), 77.3 (*C*H), 70.4 (*C*H$_2$OH), 69.3 (*C*HOH), 52.6 (*C*H$_2$COS), 40.4 (CH$_3$CH$_2$CH_2), 40.3 (CH$_2$*C*(CH$_3$)$_2$), 40.0 (*C*H$_2$CH$_2$S), 36.4 (*C*H$_2$CONH), 36.3 (NH*C*H$_2$), 29.3 (*C*H$_2$S), 21.3 (CH$_2$C(*C*H$_3$)$_2$), 20.9 (CH$_2$C(*C*H$_3$)$_2$), 19.8 (CH$_3$*C*H$_2$) 14.3 (*C*H$_3$CH$_2$); HRMS (ESI) $C_{17}H_{32}N_2NaO_6S$ $[M + Na]^+$ requires 415.1879, found 415.1881; $[\alpha]_D^{25}$ (c 0.2, MeOH): +13.2.

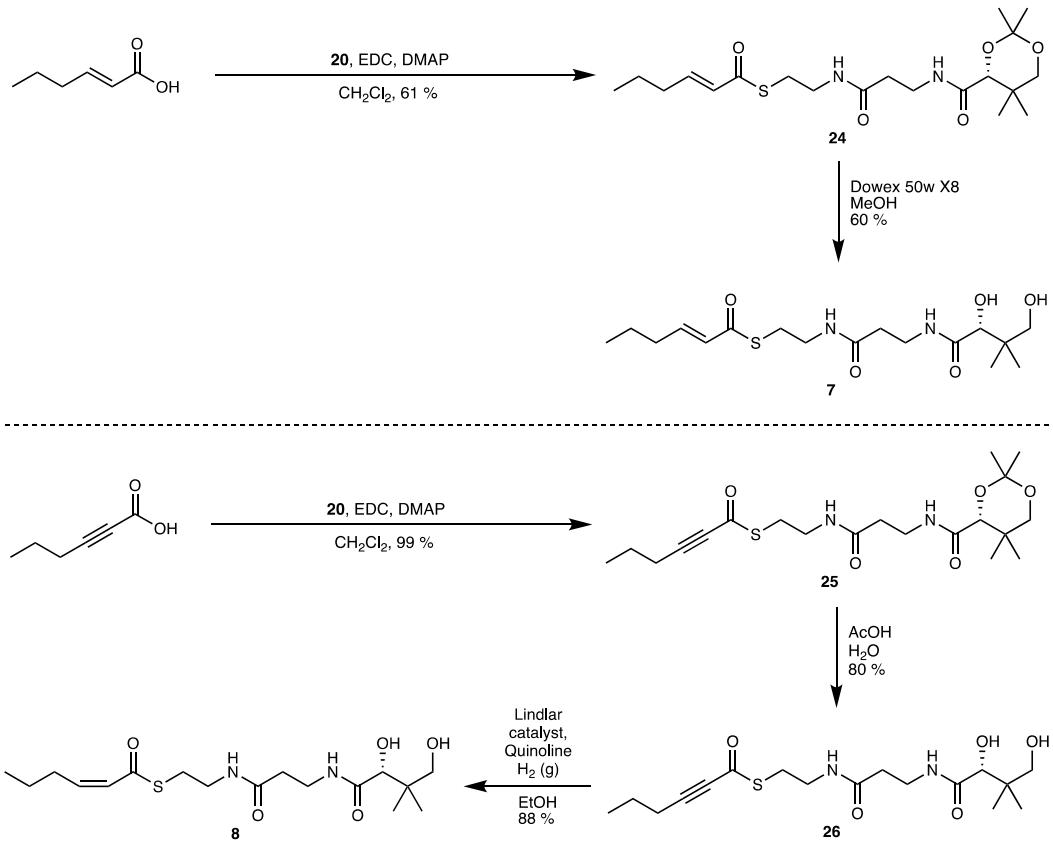


The ^{1}H NMR spectrum of *S*-(2-(3-((*R*)-2,4-dihydroxy-3,3-dimethylbutanamido) propanamido)ethyl) (*S*)-3-hydroxyhexanethioate **6** (d^4-MeOD).



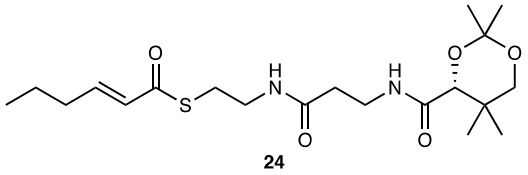
The ^{13}C NMR spectrum of *S*-(2-(3-((*R*)-2,4-dihydroxy-3,3-dimethylbutanamido) propanamido)ethyl) (*S*)-3-hydroxyhexanethioate **6** (d^4-MeOD).

Synthesis of 2-enoyl pantetheine thioesters



Synthesis of 2-enoyl pantetheine thioesters 7 and 8. The reduction of intermediate **26** afforded substrate **8** in a 95:5, *Z*/*E* ratio.

(*R*)-S-(2-(3-(2,2,5,5-tetramethyl-1,3-dioxane-4-carboxamido)propanamido)ethyl) (*E*)-hex-2-enethioate (**24**)

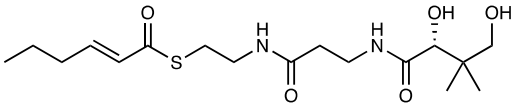


24

Procedure modified from Roberts *et al*.[11] To a solution of (2*E*)-2-hexenoic acid (76 mg, 0.67 mmol, 1.3 equiv.), (*R*)-*N*-(3-((2-mercaptoethyl)amino)-3-oxopropyl)-2,2,5,5-tetramethyl-1,3-dioxane-4-carboxamide **20** (235 mg, 0.74 mmol, 1.4 equiv.) and DMAP (18 mg, 0.16 mmol, 0.3 equiv.) in CH_2Cl_2 (5 mL), was added EDC (141 mg, 0.75 mmol, 1.4 equiv.) at 0 °C. The reaction was stirred at room temperature for 17 h and was quenched by the addition of 2 M HCl. The mixture was extracted with CH_2Cl_2 (3 x 10 mL), washed with brine (10 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo*. The residue was purified by silica chromatography (EtOAc) to afford the product as a colourless oil (167 mg, 61 %).

ν_{max}/cm^{-1} (neat) 2914 (NH), 1618, 1520 (C=O), 1159 (C-O); δ_H (500 MHz; $CDCl_3$) 7.01 (1H, br. t, J 6.0, N*H*), 6.92 (1H, dt, J 15.5 and 7.0, C*H*CHCOS), 6.18-6.07 (2H, br. m, N*H*CH_2CH_2S, C*H*COS), 4.07 (1H, s, C*H*), 3.67 (1H, d, J 12.0, C*H*$_2$OC$(CH_3)_2$), 3.62-3.40 (4H, m, NHC*H*$_2$, C*H*$_2CH_2$S), 3.27 (1H, d, J 12.0, C*H*$_2$OC$(CH_3)_2$), 3.07 (2H, t, J 6.0, C*H*$_2$S), 2.40 (2H, t, J 6.0, C*H*$_2$CONH), 2.17 (2H, q, J 7.0, CH_3CH_2C*H*$_2$), 1.50-1.44 (2H, m, CH_3C*H*$_2$), 1.44 (3H, s, OC(C*H*$_3)_2$), 1.40 (3H, s, OC(C*H*$_3)_2$), 1.03 (3H, s, CH_2C(C*H*$_3)_2$), 0.97-0.89 (6H, m, CH_2C*H*$_3$, CH_2C(C*H*$_3)_2$); δ_C (125 MHz, $CDCl_3$) 190.1 (*C*OS), 171.6 (CH_2*C*ONH), 170.1 (CH*C*ONH), 146.7 (*C*HCHCOS), 128.5 (C*H*COS), 99.2 (O*C*$(CH_3)_2$), 77.2 (*C*H), 71.5 (*C*H$_2$OC$(CH_3)_2$), 39.8 (*C*H$_2CH_2$S), 36.0 (*C*H$_2$CONH), 34.8 (*C*H$_2$NH), 34.2 (*C*H$_2$S), 33.0 (CH_2*C*$(CH_3)_2$), 29.5 (OC(*C*H$_3)_2$), 22.2 (CH_2C(*C*H$_3)_2$), 21.2 (CH_3CH_2*C*H$_2$), 21.1 (CH_3*C*H$_2$), 19.0 (CH_2C(*C*H$_3)_2$), 18.8 (OC(*C*H$_3)_2$), 13.6 (*C*H$_3CH_2$); HRMS (ESI) $C_{20}H_{34}N_2NaO_5S$ $[M + Na]^+$ requires 437.2086, found 437.2088; $[\alpha]_D^{26}$ (c 0.2, $CHCl_3$): +6.5.

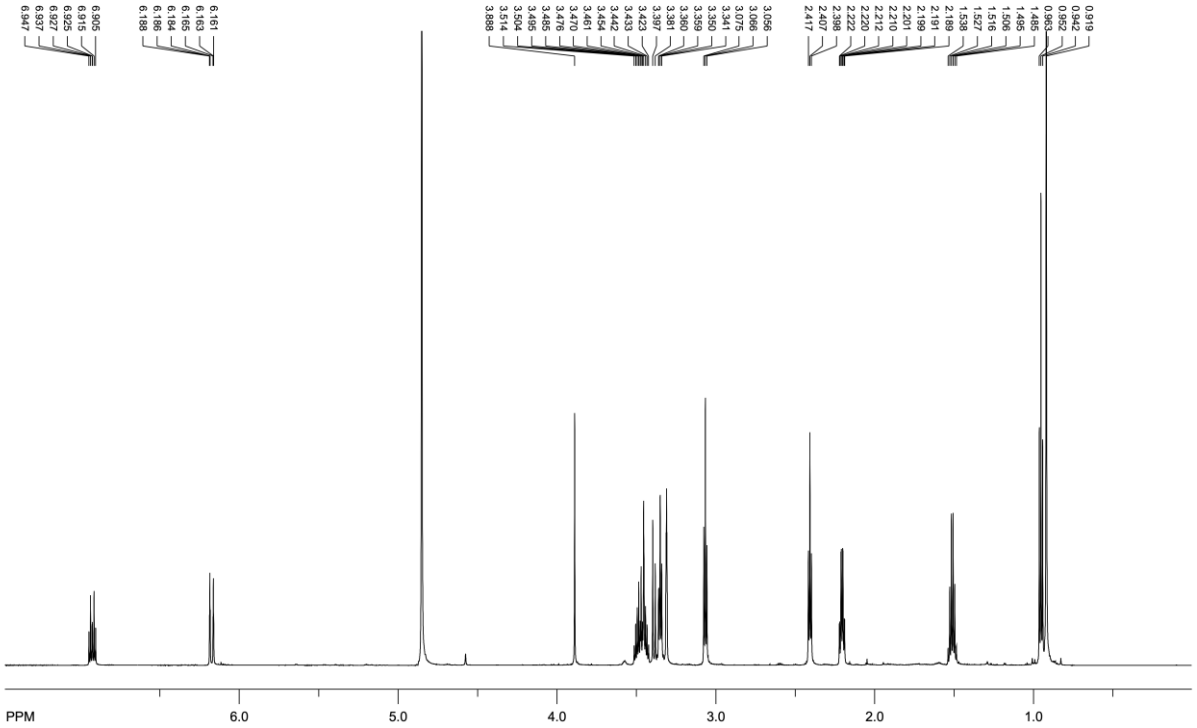
(*R*)-*S*-(2-(3-(2,4-dihydroxy-3,3-dimethylbutanamido)propanamido)ethyl) (*E*)-hex-2-enethioate (**7**)



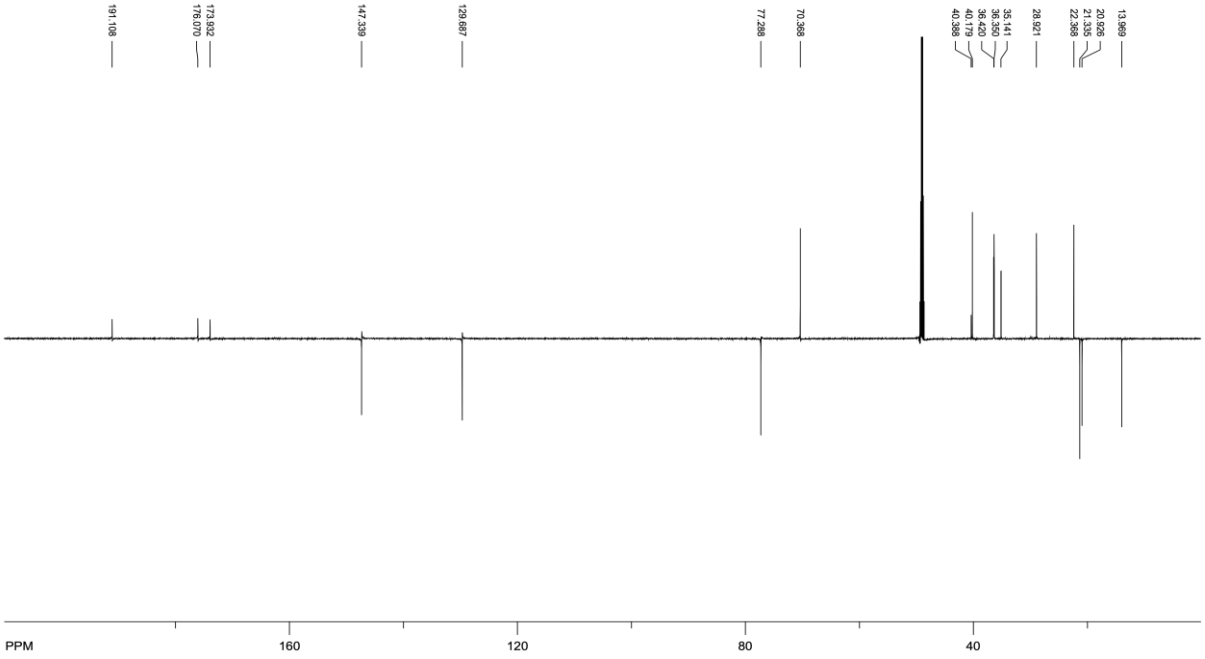
7

Procedure modified from Roberts *et al*.[11] To a solution of (*R*)-*S*-(2-(3-(2,2,5,5-tetramethyl-1,3-dioxane-4-carboxamido)propanamido)ethyl) (2*E*)-2-hexenethioate **24** (167 mg, 0.4 mmol, 1.0 equiv.) in MeOH (2 mL) was added Dowex® 50w X8 resin (85 mg) and the resulting mixture was stirred at room temperature for two days. The reaction mixture was then filtered and concentrated *in vacuo* to afford the product as a colourless oil (91 mg, 60 %).

ν_{max}/cm^{-1} (neat) 3301 (OH), 2946 (NH), 1642, 1530 (C=O), 1099 (C-O); δ_H (700 MHz; CD_3OD) 6.93 (1H, dt, J 15.5 and 7.0, C*H*CHCOS), 6.17 (1H, dt, J 15.5 and 1.5, C*H*COS), 3.89 (1H, s, C*H*), 3.51-3.33 (6H, m, NHC*H*$_2$, C*H*$_2$CH$_2$S, C*H*$_2$CHOH), 3.07 (2H, t, J 7.0, C*H*$_2$S), 2.41 (2H, t, J 6.5, C*H*$_2$CONH), 2.21 (2H, qd, J 7.0 and 1.5, CH$_3$CH$_2$C*H*$_2$), 1.51 (2H, sext., J 7.5, CH$_3$C*H*$_2$), 0.95 (3H, t, J 7.5, CH$_2$C*H*$_3$), 0.92 (6H, s, CH$_2$C(C*H*$_3$)$_2$); δ_C (175 MHz, CD_3OD) 191.1 (*C*OS), 176.0 (CH*C*ONH), 173.9 (CH$_2$*C*ONH), 147.3 (*C*HCHCOS), 129.7 (CH*C*HCOS), 77.3 (*C*H), 70.4 (*C*H$_2$OH), 40.4 (CH$_2$*C*(CH$_3$)$_2$), 40.2 (*C*H$_2$CH$_2$S), 36.4 (*C*H$_2$CONH), 36.3 (*C*H$_2$NH), 35.1 (CH$_3$CH$_2$*C*H$_2$), 28.9 (*C*H$_2$S), 22.3 (CH$_3$*C*H$_2$), 21.3 (CH$_2$C(*C*H$_3$)$_2$), 20.9 (CH$_2$C(*C*H$_3$)$_2$), 13.9 (*C*H$_3$CH$_2$); HRMS (ESI) $C_{17}H_{30}N_2NaO_5S$ [M + Na]$^+$ requires 397.1768, found 397.1770; $[\alpha]_D^{26}$ (c 0.2, MeOH): +8.3.

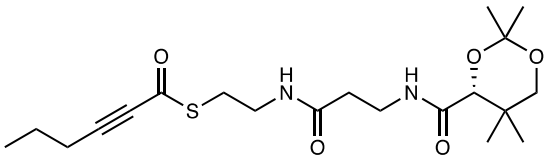


The ^{1}H NMR spectrum of (*R*)-S-(2-(3-(2,4-dihydroxy-3,3-dimethylbutanamido)propanamido)ethyl) (*E*)-hex-2-enethioate **7** (d^{4}-MeOD).



The ^{13}C NMR spectrum of (*R*)-S-(2-(3-(2,4-dihydroxy-3,3-dimethylbutanamido)propanamido)ethyl) (*E*)-hex-2-enethioate **7** (d^{4}-MeOD).

***(R)-S*-(2-(3-(2,2,5,5-tetramethyl-1,3-dioxane-4-carboxamido)propanamido)ethyl) hex-2-ynethioate** (**25**)

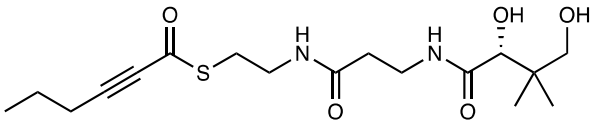


25

To a solution of hex-2-ynoic acid (0.12 mL, 0.75 mmol, 1.3 equiv.), (*R*)-*N*-(3-((2-mercaptoethyl)amino)-3-oxopropyl)-2,2,5,5-tetramethyl-1,3-dioxane-4-carboxamide **20** (255 mg, 0.80 mmol, 1.4 equiv.) and DMAP (21 mg, 0.23 mmol, 0.3 equiv.) in CH_2Cl_2 (10 mL), was added EDC (156 mg, 0.80 mmol, 1.4 equiv.) at 0 °C. The reaction was stirred at room temperature for 16 h and was quenched by the addition of 1 M HCl. The mixture was extracted with CH_2Cl_2 (3 x 10 mL), washed with saturated $NaHCO_3$ solution (10 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo* to give a viscous oil, which was purified by silica chromatography (EtOAc) to give the product as a colourless oil (305 mg, 99 %).

ν_{max}/cm^{-1} (neat) 3309 (OH), 2939 (NH), 1649, 1529 (C=O), 1158 (C-O); δ_H (500 MHz; $CDCl_3$) 7.01 (1H, br. t, J 6.0, N*H*), 6.20 (1H, br. s, N*H*CH_2CH_2S), 4.07 (1H, s, C*H*), 3.67 (1H, d, J 11.5, C*H*$_2$OC$(CH_3)_2$), 3.61-3.39 (4H, m, NHC*H*$_2$, C*H*$_2CH_2S$), 3.27 (1H, d, J 11.5, C*H*$_2$OC$(CH_3)_2$), 3.09 (2H, t, J 6.5, C*H*$_2$S), 2.43 (2H, t, J 6.0, C*H*$_2$CONH), 2.37 (2H, t, J 7.0, $CH_3CH_2CH_2$), 1.62 (2H, sextet, J 7.5, CH_3CH_2), 1.46 (3H, s, OC$(CH_3)_2$), 1.42 (3H, s, OC$(CH_3)_2$), 1.03 (3H, s, $CH_2C(CH_3)_2$), 1.02 (3H, t, J 7.5, CH_2CH_3), 0.96 (3H, s, $CH_2C(CH_3)_2$); δ_C (125 MHz, $CDCl_3$) 176.4 (*C*OS), 171.4 (CH_2*C*ONH), 170.3 (CH*C*ONH), 99.2 (O*C*$(CH_3)_2$), 96.7 (*C*CCOS), 78.9 (C*C*COS), 77.3 (*C*H), 71.6 (*C*H_2OC$(CH_3)_2$), 42.6 (*C*H_2CH_2S), 36.1 (*C*H_2CONH), 34.9 (*C*H_2NH), 33.1 (CH_2*C*$(CH_3)_2$), 29.6 (OC(*C*$H_3)_2$), 29.5 (*C*H_2S), 22.3 (CH_2C(*C*$H_3)_2$), 21.2 (CH_3CH_2*C*H_2), 21.1 (CH_3*C*H_2), 19.0 (CH_2C(*C*$H_3)_2$), 18.8 (OC(*C*$H_3)_2$), 13.6 (*C*H_3CH_2); HRMS (ESI) $C_{20}H_{32}N_2NaO_5S$ [M + Na]$^+$ requires 435.1924, found 435.1926; $[\alpha]_D^{26}$ (c 0.3, $CHCl_3$): +4.8.

(*R*)-*S*-(2-(3-(2,4-dihydroxy-3,3-dimethylbutanamido)propanamido)ethyl) hex-2-ynethioate (**26**)

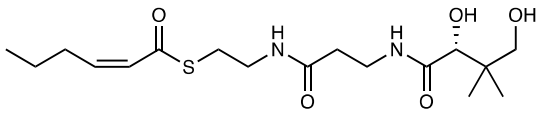


26

(*R*)-*S*-(2-(3-(2,2,5,5-tetramethyl-1,3-dioxane-4-carboxamido)propanamido)ethyl) hex-2-ynethioate **25** (300 mg, 0.73 mmol) was stirred in AcOH (4 mL) and water (2 mL) for 16 h at room temperature. The mixture was then concentrated *in vacuo* and purified using silica chromatography (MeOH : EtOAc 1 : 10) to give the product as a colourless oil (216 mg, 80 %).

ν_{max}/cm^{-1} (neat) 3299 (OH), 2964 (NH), 1643, 1533 (COS), 1037 (C-O); δ_H (500 MHz; CD_3OD) 3.89 (1H, s, C*H*), 3.61-3.39 (6H, m, NHC*H*$_2$, C*H*$_2CH_2S$, C*H*$_2$CHOH), 3.09 (2H, t, J 6.5, C*H*$_2$S), 2.42 (2H, t, J 7.0, $CH_3CH_2CH_2$), 2.41 (2H, t, J 6.5, C*H*$_2$CONH), 1.62 (2H, sextet, J 7.5, CH_3CH_2), 1.03 (3H, t, J 7.5, CH_2CH_3), 0.92 (6H, s, $CH_2C(CH_3)_2$); δ_C (125 MHz, CD_3OD) 177.2 (*C*OS), 176.1 (CH*C*ONH), 174.0 (CH_2*C*ONH), 96.9 (*C*CCOS), 79.6 (C*C*COS), 77.3 (*C*H), 70.4 (*C*H_2OH), 40.4 (CH_2*C*$(CH_3)_2$), 39.8 (*C*H_2CH_2S), 36.4 (*C*H_2CONH), 36.3 (*C*H_2NH), 30.1 (*C*H_2S), 22.2 (CH_3CH_2*C*H_2), 21.4 (CH_3*C*H_2), 21.2 (CH_2C(*C*$H_3)_2$), 19.0 (CH_2C(*C*$H_3)_2$), 13.7 (*C*H_3CH_2); HRMS (ESI) $C_{17}H_{28}N_2NaO_5S$ [M + Na]$^+$ requires 395.1611, found 395.1615; $[\alpha]_D^{26}$ (c 0.4, MeOH): +15.4.

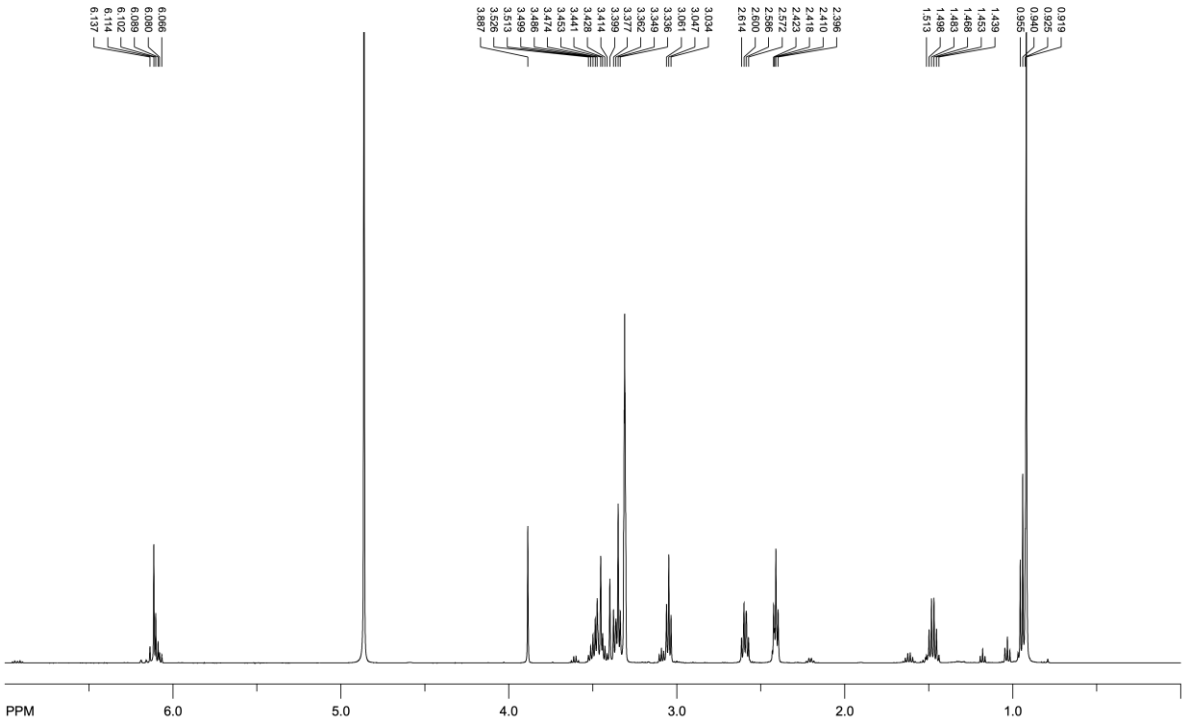
(*R*)-*S*-(2-(3-(2,4-dihydroxy-3,3-dimethylbutanamido)propanamido)ethyl) (*Z*)-hex-2-enethioate (**8**)



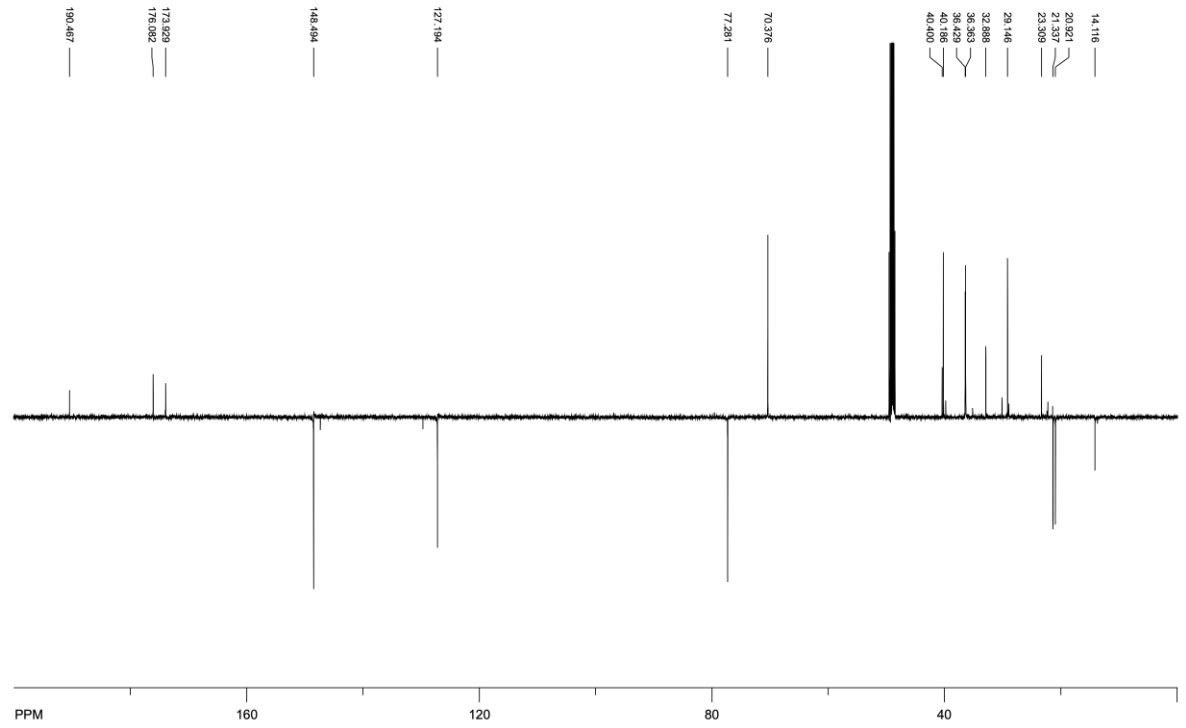
8

Procedure modified from Alhamadsheh *et al*.[12] A mixture of Lindlar catalyst (10 mg, 5% (w/w) of Pd on $CaCO_3$, poisoned with lead) and quinoline (5 µL, 0.1 equiv. 0.05 mmol) in anhydrous ethanol (5 ml) was stirred at room temperature for 20 min. A solution of (*R*)-*S*-(2-(3-(2,4-dihydroxy-3,3-dimethylbutanamido)propanamido)ethyl) hex-2-ynethioate **26** (200 mg, 0.51 mmol, 1 equiv.) in ethanol (5 ml) was added and the mixture was stirred for an additional 20 min at room temperature. The reaction mixture was placed under hydrogen atmosphere (balloon) and stirred for an additional 4 h. Mass spectrometric analysis indicated that the reaction had not yet reached completion. More catalyst was added (10 mg), and this process was repeated until the reaction was complete (8 days, 100 mg total catalyst). The catalyst was removed by filtration through celite and the filtrate was concentrated *in vacuo* to afford a yellow oil which was purified by silica chromatography (MeOH : EtOAc, 1 : 10) to give the product as a colourless oil (168 mg, 88 %, 95 : 5, *Z* : *E*).

ν_{max}/cm^{-1} (neat) 3287 (OH), 2951 (NH), 1640, 1539 (COS), 1070 (C-O); δ_H (500 MHz; CD_3OD) 6.14-6.06 (2H, m, C*H*CHCOS, CHC*H*COS), 3.89 (1H, s, C*H*), 3.53-3.33 (6H, m, NHCH_2, CH_2CH_2S, CH_2CHOH), 3.05 (2H, t, J 7.0, CH_2S), 2.59 (2H, q, J 7.0, $CH_3CH_2CH_2$), 2.41 (2H, t, J 6.5, CH_2CONH), 1.48 (2H, sextet, J 7.5, CH_3CH_2), 0.94 (3H, t, J 7.5, CH_2CH_3), 0.92 (6H, s, $CH_2C(CH_3)_2$); δ_C (125 MHz, CD_3OD) 190.4 (*C*OS), 176.1 (CH*C*ONH), 173.9 (CH_2*C*ONH), 148.5 (*C*HCHCOS), 127.2 (CH*C*HCOS), 77.3 (*C*H), 70.3 (*C*H_2OH), 40.4 ($CH_2C(CH_3)_2$), 40.2 (*C*H_2CH_2S), 36.4 (*C*H_2CONH), 36.3 (*C*H_2NH), 32.9 (*C*H_2S), 29.1 ($CH_3CH_2CH_2$), 23.3 (CH_3CH_2), 21.3 ($CH_2C(CH_3)_2$), 20.9 ($CH_2C(CH_3)_2$), 14.1 (*C*H_3CH_2); HRMS (ESI) $C_{17}H_{30}N_2NaO_5S$ $[M + Na]^+$ requires 397.1768, found 397.1767; $[\alpha]_D^{26}$ (c 0.1, MeOH): +7.

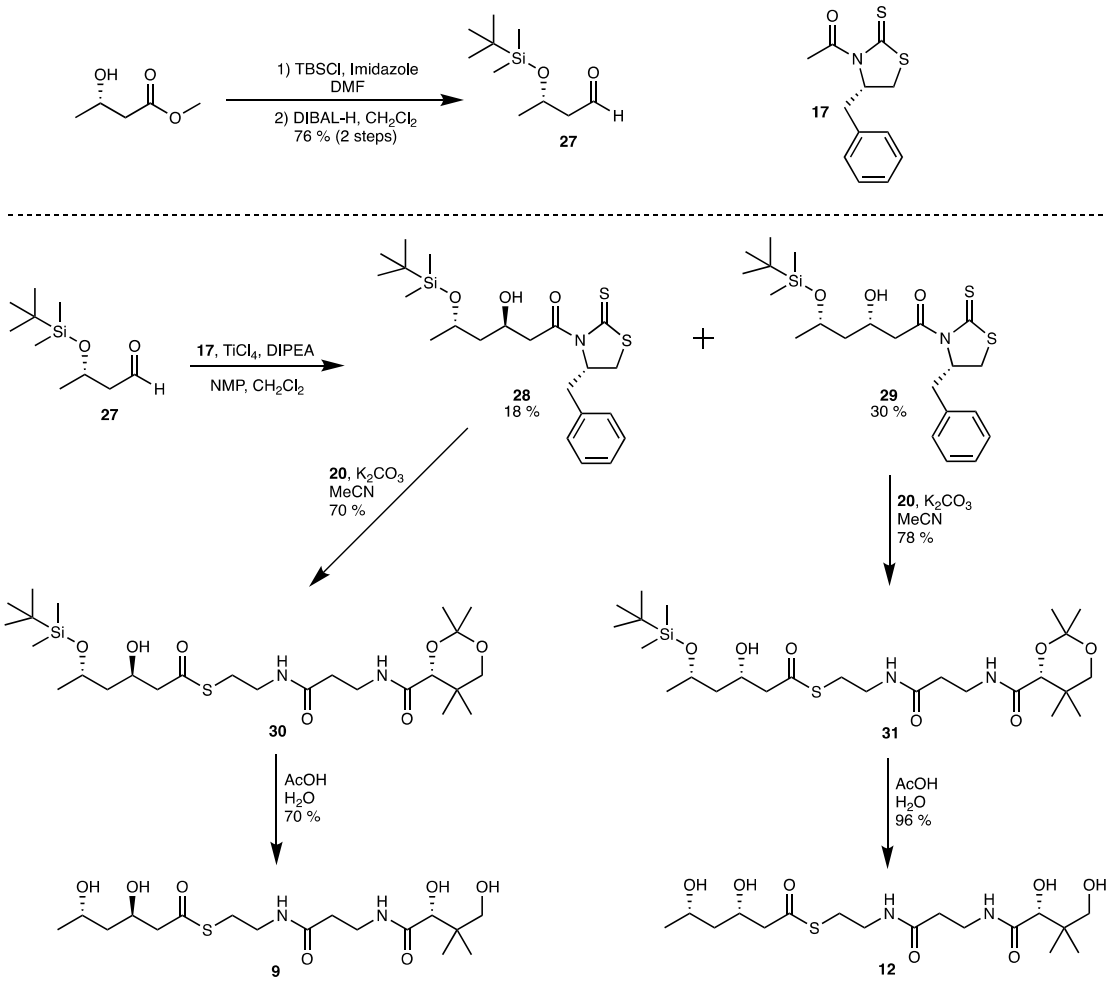


The ^{1}H NMR spectrum of (*R*)-S-(2-(3-(2,4-dihydroxy-3,3-dimethylbutanamido)propanamido)ethyl) (*Z*)-hex-2-enethioate **8** (d^{4}-MeOD).



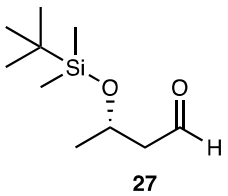
The ^{13}C NMR spectrum of (*R*)-S-(2-(3-(2,4-dihydroxy-3,3-dimethylbutanamido)propanamido)ethyl) (*Z*)-hex-2-enethioate **8** (d^{4}-MeOD).

Synthesis of 3,5-dihydroxyacyl pantetheine thioesters



Synthesis of thioesters 9 and 12. Aldehyde **27** was synthesized according to literature procedure.[13]

(*S*)-3-((*tert*-butyldimethylsilyl)oxy)butanal (27)

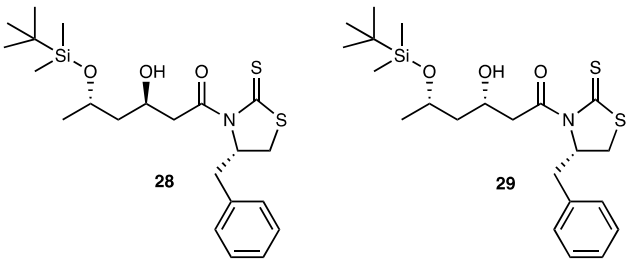


Procedure modified from Wang *et al*.[13] Under an Ar atmosphere, TBSCl (1.34 g, 8.88 mmol, 1.05 equiv.) was added to a solution of commercially available methyl (*S*)-3-hydroxybutanoate (1.0 g, 8.48 mmol, 1.00 equiv.) and imidazole (0.86 g, 12.7 mmol, 1.50 equiv.) in DMF (25 mL) at 0 °C, and the mixture was

stirred for 16 h at RT. The reaction mixture was then diluted with Et_2O, successively washed with HCl solution (10 %, 25 mL), saturated $NaHCO_3$ (25 mL), dried ($MgSO_4$) and concentrated *in vacuo* to afford a colourless oil (1.88 g) which was used without further purification. The resulting oil was dissolved in CH_2Cl_2 (50 mL) and 1 M solution DIBAL-H in hexane (9.33 mL, 9.33 mmol, 1.1 equiv.) was added dropwise at - 78 ^oC under an Ar atmosphere. After being stirred for 30 minutes at this temperature, MeOH (10 mL) was added, and the reaction was warmed to RT. A saturated solution of sodium potassium tartrate was then added (50 mL), and the mixture stirred until clear separation was observed. The organic layer was removed, and the aqueous layer further extracted with CH_2Cl_2 (2 × 50 mL), the combined organics were washed with brine (50 mL), dried ($MgSO_4$) and concentrated *in vacuo*. The resulting oil was then purified by silica chromatography (3:100 Et_2O : Petroleum ether) to afford the product as a colourless oil (1.30 g, 76 % (2 steps)).

δ_H (500 MHz; $CDCl_3$) 9.79 (1H, dd, *J* 3.0 and 2.0, C*H*O), 4.35 (1H, dqd, *J* 7.0, 6.0 and 5.0, C*H*), 2.55 (1H, ddd, *J* 15.5, 7.0 and 3.0, CH_2), 2.46 (1H, dd, *J* 15.5, 5.0 and 2.0, CH_2), 1.23 (3H, d, *J* 6.0, CH_3), 0.87 (9H, s, C(CH_3)$_3$), 0.07 (3H, s, SiCH_3), 0.06 (3H, s, SiCH_3); δ_C (125 MHz, $CDCl_3$) 202.6 (*C*HO), 64.9 (*C*H), 53.4 (*C*H_2), 26.1 (C(*C*H_3)$_3$), 24.6 (*C*H_3), 18.3 (*C*(CH$_3$)$_3$), -4.0 (Si*C*H$_3$), -4.6 (Si*C*H$_3$)$_3$; HRMS (ESI) cald. for $C_{11}H_{26}NaO_3Si$ (M + MeOH + Na^+) requires 257.1543, found 257.1543; $[\alpha]_D^{25}$ (c 1.0, CH_2Cl_2): +11.2. Spectroscopic data were consistent with those previously reported in the literature.[14]

(3*R*,5*S*)-1-((*S*)-4-benzyl-2-thioxothiazolidin-3-yl)-5-((*tert*-butyldimethylsilyl)oxy)-3-hydroxyhexan-1-one (28) and (3*S*,5*S*)-1-((*S*)-4-benzyl-2-thioxothiazolidin-3-yl)-5-((*tert*-butyldimethylsilyl)oxy)-3-hydroxyhexan-1-one (**29**)



Procedure taken from Gao *et al*.[15] To a solution of (*S*)-1-(4-benzyl-2-thioxothiazolidin-3-yl)ethan-1-one **17** (600 mg, 2.39 mmol, 1 equiv.) in dry CH_2Cl_2 (20 mL) under an Ar atmosphere was added a 1 M solution of $TiCl_4$ in CH_2Cl_2 (2.55 mL, 2.51 mmol, 1.05 equiv.) dropwise at 0 °C. The orange suspension

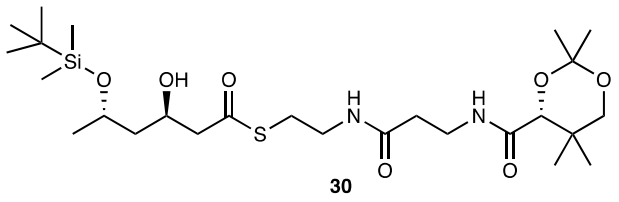
was allowed to stir for 15 min at the same temperature before diisopropylethylamine (0.46 mL, 2.63 mmol, 1.1 equiv.) was added dropwise. The resulting purple solution was allowed to stir for a further 40 min at 0 °C. 1-Methyl-2-pyrrolidinone (0.46 mL, 4.78 mmol, 2.0 equiv.) was then added at the same temperature and the reaction stirred for an additional 10 min before a solution of (*S*)-3-((*tert*-butyldimethylsilyl)oxy)butanal **27** (978 mg, 4.4 mmol, 2.0 equiv.) in dry CH_2Cl_2 (5 mL) was added. The reaction was left to stir for 2 h at 0 °C before the addition of saturated NH_4Cl (10 mL). The organic layer was separated and the aqueous layer extracted with CH_2Cl_2 (2 x 20 mL). The combined organics were dried ($MgSO_4$), filtered and concentrated *in vacuo* to give the crude product, which was purified by silica chromatography (EtOAc : Petroleum ether, 10 : 90) to give **(**3*R*,5*S*)-1-((*S*)-4-benzyl-2-thioxothiazolidin-3-yl)-5-((*tert*-butyldimethylsilyl)oxy)-3-hydroxyhexan-1-one **28** (196 mg, 18 %) and (3*S*,5*S*)-1-((*S*)-4-benzyl-2-thioxothiazolidin-3-yl)-5-((*tert*-butyldimethylsilyl)oxy)-3-hydroxyhexan-1-one **29** (326 mg, 30 %) as yellow oils respectively.

(3*R*,5*S*)-1-((*S*)-4-benzyl-2-thioxothiazolidin-3-yl)-5-((*tert*-butyldimethylsilyl)oxy)-3-hydroxyhexan-1-one **28**: δ_H (700 MHz; $CDCl_3$) 7.36-7.32 (2H, m, Ar*H*), 7.30-7.26 (3H, m, Ar*H*), 5.42 (1H, ddd, J 10.5, 7.0 and 4.0, C*H*N), 4.38 (1H, tq, J 9.5 and 3.0, C*H*OH), 4.18 (1H, dqd, J 7.5**,** 6.5 and 3.0, C*H*OSi), 3.57 (1H, d, J 3.0. O*H*), 3.48 (1H, dd, J 17.5 and 9.0, CH_2CON), 3.39 (1H, dd, J 11.5 and 7.5, CH_2S), 3.36 (1H, dd, J 17.5 and 3.5, CH_2CON), 3.24 (1H, dd, J 13.0 and 4.0, CH_2Ar), 3.04 (1H, dd, J 13.0 and 10.5, CH_2Ar), 2.89 (1H, d, J 11.5, CH_2S), 1.68 (1H, ddd, J 14.0, 10.0 and 3.0, CHCH_2CHOH), 1.53 (1H, ddd, J 14.0, 7.5 and 2.5, CHCH_2CHOH), 1.21 (3H, d, J 6.5, CH_3CH), 0.89 (9H, s, C(CH_3)$_3$), 0.10 (3H, s, SiCH_3), 0.09 (3H, s, SiCH_3); δ_C (175 MHz, $CDCl_3$) 201.3 (*C*S_2), 173.1 (*C*ON), 136.5 (ArC_{quat}), 129.4 (ArC), 128.9 (ArC), 127.2 (ArC), 68.3 (*C*HN), 66.2 (*C*HOSi), 65.1 (*C*HOH), 46.3 (*C*H_2CON), 44.9 (CH*C*H_2CHOH), 36.8 (*C*H_2Ar), 31.9 (*C*H_2S), 25.9 (C(*C*H_3)$_3$), 23.6 (CH*C*H_3), 18.0 (*C*(CH$_3$)$_3$), -4.5 (Si*C*H_3), -4.9 (Si*C*H_3); HRMS (ESI) $C_{22}H_{36}NO_3S_2Si$ $[M + H]^+$ requires 454.1900, found 454.1915. $[\alpha]_D^{24}$ (c 0.4, $CHCl_3$): +99.8.

(3*S*,5*S*)-1-((*S*)-4-benzyl-2-thioxothiazolidin-3-yl)-5-((*tert*-butyldimethylsilyl)oxy)-3-hydroxyhexan-1-one **29**: δ_H (700 MHz; $CDCl_3$) 7.36-7.33 (2H, m, Ar*H*), 7.30-7.25 (3H, m, Ar*H*), 5.40 (1H, ddd, J 11.0, 7.0 and 4.0, C*H*N), 4.36 (1H, tq, J 9.0 and 3.5, C*H*OH), 4.12 (1H, dqd., 8.5, 6.0 and 4.5, C*H*OSi), 3.50 (1H, d, J 2.0, O*H*), 3.50 (1H, dd, J 17.5 and 3.5, CH_2CON), 3.39 (1H, dd, J 11.5 and 7.0, CH_2S), 3.29 (1H, dd, J 17.5 and 8.5, CH_2CON), 3.23 (1H, dd, J 13.5 and 4.0, CH_2Ar), 3.04 (1H, dd, J 13.5 and 10.5, CH_2Ar), 2.88 (1H, d, J 11.5, CH_2S), 1.73 (1H, dt, J 14.0 and 9.0, CHCH_2CHOH), 1.62 (1H, ddd, J 14.0, 4.5 and 3.5, CHCH_2CHOH), 1.21 (3H, d, J 6.0, CH_3CH), 0.90 (9H, s, C(CH_3)$_3$), 0.12 (3H, s, SiCH_3), 0.10 (3H, s,

SiC*H*$_3$); δ_C (175 MHz, $CDCl_3$) 201.3 (*C*S_2), 172.4 (*C*ON), 136.5 (ArC_{quat}), 129.4 (ArC), 128.9 (ArC), 127.2 (ArC), 68.6 (*C*HOSi), 68.4 (*C*HN), 67.0 (*C*HOH), 46.1 (*C*H_2CON), 45.4 (CH*C*H_2CHOH), 36.7 (*C*H_2Ar), 32.0 (*C*H_2S), 24.8 (C(*C*H_3)$_3$), 24.2 (CH*C*H_3), 17.9 (*C*$(CH_3)_3$), -4.0 (Si*C*H_3), -4.7 (Si*C*H_3); HRMS (ESI) $C_{22}H_{35}NNaO_3S_2Si$ $[M + Na]^+$ requires 476.1720, found 476.1724. $[\alpha]_D^{24}$ (c 0.4, $CHCl_3$): +141.1. Spectroscopic data for both compounds were consistent with those reported by Gao et al.[15] The stereochemistry of both aldol products was further confirmed by comparison of diagnostic NMR signals relative to those previously reported in the literature (Supplementary Table 1) and Mosher's ester analysis (See 'Confirmation of the stereochemistry of aldol products').[16,17]

_S_-(2-(3-((_R_)-2,2,5,5-tetramethyl-1,3-dioxane-4-carboxamido)propanamido)ethyl) (3_R_,5_S_)-5-((*tert*-butyldimethylsilyl)oxy)-3-hydroxyhexanethioate (**30**)



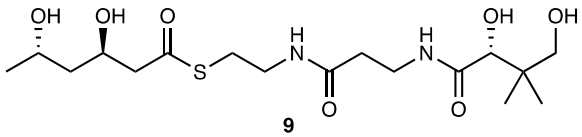
30

Procedure modified from Gao *et al.*[15] To a stirred solution of (3*R*,5*S*)-1-((*S*)-4-benzyl-2-thioxothiazolidin-3-yl)-5-((*tert*-butyldimethylsilyl)oxy)-3-hydroxyhexan-1-one **28** (150 mg, 0.33 mmol, 1 equiv.) in acetonitrile (5 mL), was added K_2CO_3 (63 mg, 0.33 mmol, 1 equiv.) and (*R*)-*N*-(3-((2-mercaptoethyl)amino)-3-oxopropyl)-2,2,5,5-tetramethyl-1,3-dioxane-4-carboxamide **20** (106 mg, 0.33 mmol, 1 equiv.). The mixture was stirred at room temperature for 1 h before being quenched by the addition of saturated NH_4Cl solution (1 mL). The acetonitrile was then removed *in vacuo* and the resulting solution extracted with EtOAc (3 x 10 mL). The combined organics were washed with brine, dried ($MgSO_4$), filtered and concentrated *in vacuo* to give a crude oil, which was purified by silica chromatography (MeOH : CH_2Cl_2, 1 : 10) to give the product as a colourless oil (128 mg, 70 %).

ν_{max}/cm^{-1} (neat) 3323 (OH), 2956 (NH), 1656, 1530 (C=O), 1099 (C-O); δ_H (500 MHz; $CDCl_3$) 7.02 (1H, br. t, J 6.0, CHCON*H*), 6.32 (1H, br. s, N*H*CH_2CH_2S), 4.43-4.38 (1H, m, C*H*OH), 4.17 (1H, quin.d, J 6.0 and 3.5, C*H*OSi), 4.06 (1H, s, C*H*CONH), 3.83 (1H, br. s, O*H*), 3.67 (1H, d J 11.5, C*H*$_2$OC$(CH_3)_2$), 3.60-3.35 (4H, m, NHC*H*$_2$, C*H*$_2$CH$_2$S), 3.27 (1H, d J 11.5, C*H*$_2$OC$(CH_3)_2$), 3.05 (1H, dt, J 14.0 and 6.5, C*H*$_2$S),

3.01 (1H, dt, J 14.0 and 6.5, CH_2S), 2.75 (1H, dd, J 15.0 and 8.5, CH_2COS), 2.65 (1H, dd, J 15.0 and 4.0, CH_2COS) 2.40 (2H, t, J 6.5, CH_2CONH), 1.65 (1H, ddd, J 14.0, 9.5 and 4.0, CH_2CHOSi), 1.50 (1H, ddd, J 14.0, 6.5 and 2.5, CH_2CHOSi), 1.45 (3H, s, OC(CH_3)$_2$), 1.41 (3H, s, OC(CH_3)$_2$), 1.21 (3H, d, J 6.0, CHCH_3), 1.02 (3H, s, CH_2C(CH_3)$_2$), 0.96 (3H, s, CH_2C(CH_3)$_2$), 0.88 (9H, s, C(CH_3)$_3$), 0.08 (3H, s, SiCH_3), 0.07 (3H, s, SiCH_3); δ_C (125 MHz, $CDCl_3$) 198.5 (CO_2S), 171.4 (CH_2CONH), 170.4 (CHCONH), 99.3 (OC(CH$_3$)$_2$), 77.3 (CH), 71.6 (CH_2OC(CH$_3$)$_2$), 70.0 (CHOSi), 66.0 (CHOH), 51.9 (CH_2COS), 44.4 (CH_2CHOSi), 39.4 (CH_2CH_2S), 36.2 (CH_2CONH), 35.0 (CH_2NH), 33.1 (CH_2C(CH$_3$)$_2$), 29.6 (OC(CH$_3$)$_2$), 28.9 (CH_2S), 26.0 (C(CH$_3$)$_3$), 23.3 (CHCH$_3$), 22.3 (CH_2C(CH$_3$)$_2$), 19.1 (CH_2C(CH$_3$)$_2$), 18.9 (OC(CH$_3$)$_2$), 18.1 (C(CH$_3$)$_3$), -4.3 (SiCH$_3$), -4.8 (SiCH$_3$); HRMS (ESI) $C_{26}H_{50}N_2NaO_7SSi$ $[M + Na]^+$ requires 585.3000, found 585.3004; $[\alpha]_D^{26}$ (c 0.3, $CHCl_3$): +40.

(2-(3-((*R*)-2,4-dihydroxy-3,3-dimethylbutanamido)propanamido)ethyl) (3*R*,5*S*)-3,5-dihydroxyhexanethioate (**9**)

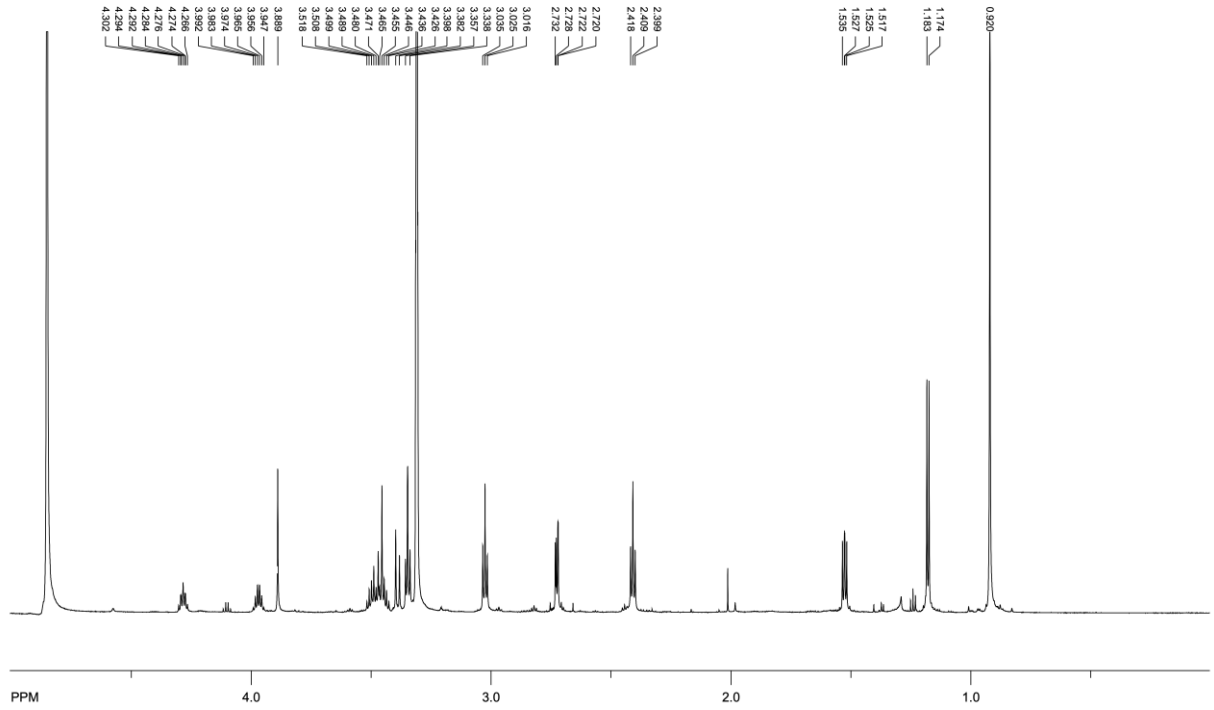


9

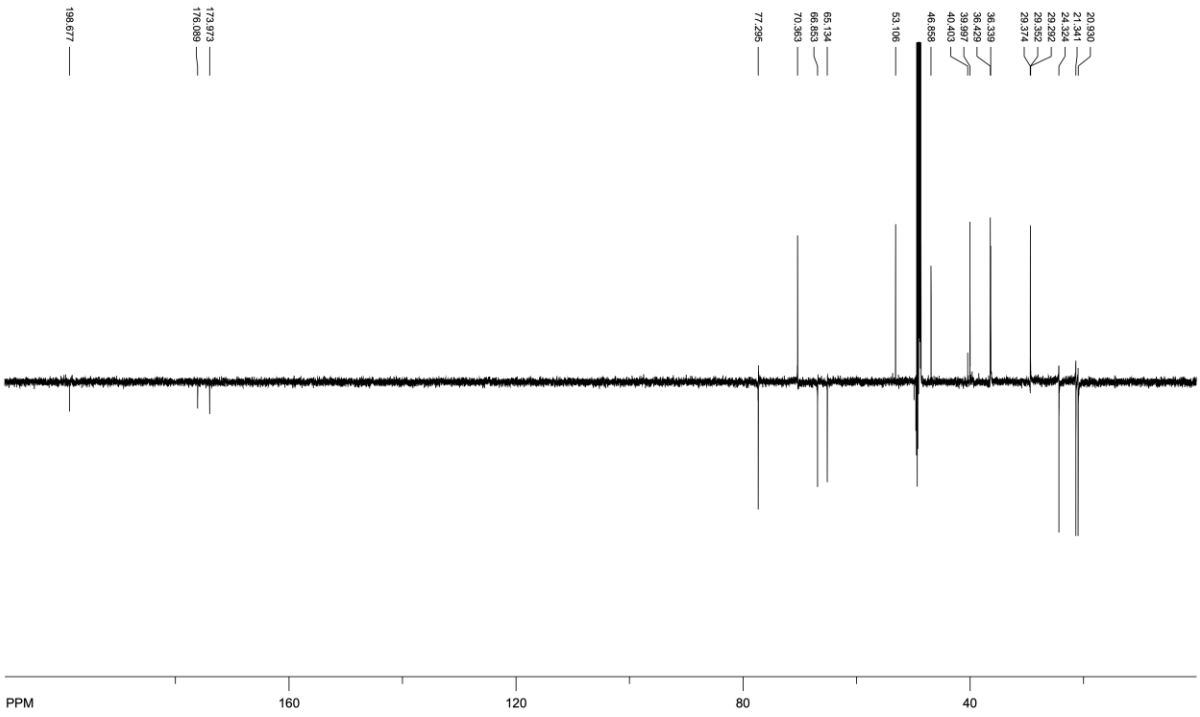
S-(2-(3-((*R*)-2,2,5,5-tetramethyl-1,3-dioxane-4-carboxamido)propanamido)ethyl) (3*R*,5*S*)-5-((*tert*-butyldimethylsilyl)oxy)-3-hydroxyhexanethioate **30** (100 mg, 0.18 mmol) was stirred in a 2:1 ratio of AcOH/H_2O (3 mL) at room temperature for 16 h. The mixture was then concentrated in *vacuo* and purified by silica chromatography (15:85 MeOH:CH_2Cl_2) to afford the desired product as a colourless oil (51 mg, 70 %).

ν_{max}/cm^{-1} (neat) 3380 (OH), 2960 (NH), 1654, 1528 (C=O); δ_H (500 MHz; CD_3OD) 4.28 (1H, quintet, J 6.5, CH_2CHOHCH_2), 3.97 (1H, sextet, J 6.0, CH_3CHOH), 3.89 (1H, s, CHCONH), 3.54-3.30 (6H, m, NHCH_2, CH_2CH_2S, CH_2OH), 3.02 (2H, t, J 6.5, CH_2S), 2.74 (1H, dd, J 15.0 and 7.5, CH_2COS), 2.71 (1H, dd, J 15.0 and 6.0, CH_2COS) 2.41 (2H, t, J 7.0, CH_2CONH), 1.53 (2H, dd, J 7.0 and 5.5, CH_3CH_2CHOH), 1.18 (3H, d, J 6.5, CHCH_3), 0.92 (6H, s, C(CH_3)$_2$); δ_C (125 MHz, CD3OD) 198.7 (CO_2S), 176.1 (CH_2CONH), 174.0 (CHCONH), 77.3 (CHOHC(CH$_3$)$_2$), 70.4 (CH_2OH), 66.9 (CH_2CHOHCH_2), 65.1 (CH_3CHOH), 53.1 (CH_2COS), 46.8 (CHOHCH_2CHOH), 40.4 (CH_2C(CH$_3$)$_2$), 40.0 (CH_2CH_2S), 36.4

(CH_2CONH), 36.3 (CH_2NH), 29.3 (CH_2S), 24.3 ($CHCH_3$), 21.3 ($CH_2C(CH_3)_2$), 20.9 ($CH_2C(CH_3)_2$); HRMS (ESI) $C_{17}H_{32}N_2NaO_7S$ $[M + Na]^+$ requires 431.1822, found 431.1824; $[\alpha]_D^{26}$ (c 0.3, MeOH): +8.2.

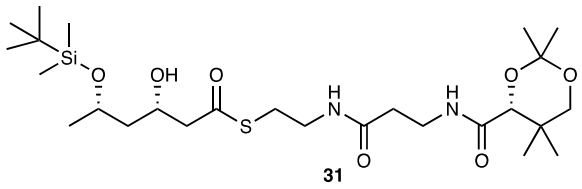


The ^{1}H NMR spectrum of *S*-(2-(3-((*R*)-2,4-dihydroxy-3,3-dimethylbutanamido) propanamido)ethyl) (3*R*,5*S*)-3,5-dihydroxyhexanethioate **9** (d^4-MeOD).



The ^{13}C NMR spectrum of *S*-(2-(3-((*R*)-2,4-dihydroxy-3,3-dimethylbutanamido) propanamido)ethyl) (3*R*,5*S*)-3,5-dihydroxyhexanethioate **9** (d^4-MeOD).

_S_-(2-(3-((_R_)-2,2,5,5-tetramethyl-1,3-dioxane-4-carboxamido)propanamido)ethyl) (3_S_,5_S_)-5-((_tert_-butyldimethylsilyl)oxy)-3-hydroxyhexanethioate (**31**)

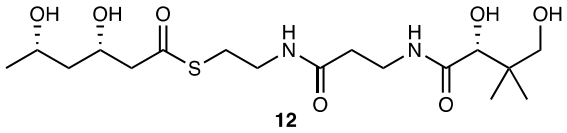


31

S-(2-(3-((*R*)-2,2,5,5-tetramethyl-1,3-dioxane-4-carboxamido)propanamido)ethyl) (3*S*,5*S*)-5-((*tert*-butyldimethylsilyl)oxy)-3-hydroxyhexanethioate **31** was synthesized using the same procedure as that used for the synthesis of *S*-(2-(3-((*R*)-2,2,5,5-tetramethyl-1,3-dioxane-4-carboxamido)propanamido)ethyl) (3*R*,5*S*)-5-((*tert*-butyldimethylsilyl)oxy)-3-hydroxyhexanethioate **30** using (3*S*,5*S*)-1-((*S*)-4-benzyl-2-thioxothiazolidin-3-yl)-5-((*tert*-butyldimethylsilyl)oxy)-3-hydroxyhexan-1-one **29** (200 mg, 0.44 mmol), to afford the product as a colourless oil (193 mg, 78 %).

ν_{max}/cm^{-1} (neat) 3320 (OH), 2953 (NH), 1655, 1529 (C=O), 1098 (C=O); δ_H (500 MHz; $CDCl_3$) 7.01 (1H, br. t, J 6.0, CHCON*H*), 6.20 (1H, br. t, J 6.0, N*H*CH_2CH_2S), 4.25 (1H, tt, J 8.0 and 4.0, C*H*OH), 4.11-4.04 (2H, m, C*H*OSi, C*H*CONH), 3.86 (1H, br. s, O*H*), 3.67 (1H, d J 11.5, C*H*$_2$OC$(CH_3)_2$), 3.60-3.41 (4H, m, NHC*H*$_2$, C*H*$_2$CH$_2$S), 3.28 (1H, d J 11.5, C*H*$_2$OC$(CH_3)_2$), 3.07 (1H, dt, J 14.0 and 6.0, C*H*$_2$S), 3.02 (1H, dt, J 14.0 and 6.0), C*H*$_2$S), 2.75 (1H, dd, J 15.0 and 8.0, C*H*$_2$COS), 2.68 (1H, dd, J 15.0 and 4.5, C*H*$_2$COS) 2.42 (1H, dt, J 15.0 and 6.5, C*H*$_2$CONH), 2.39 (1H, dt, J 15.0 and 6.5, C*H*$_2$CONH), 1.67 (1H, dt, J 14 and 9, C*H*$_2$CHOSi), 1.58 (1H, dt, J 14.0 and 4.0, C*H*$_2$CHOSi), 1.46 (3H, s, OC(C*H*$_3$)$_2$), 1.42 (3H, s, OC(C*H*$_3$)$_2$), 1.18 (3H, d, J 6.0, CHC*H*$_3$), 1.04 (3H, s, CH$_2$C(C*H*$_3$)$_2$), 0.97 (3H, s, CH$_2$C(C*H*$_3$)$_2$), 0.89 (9H, s, C(*C*H$_3$)$_3$), 0.11 (3H, s, SiC*H*$_3$), 0.10 (3H, s, SiC*H*$_3$); δ_C (125 MHz, $CDCl_3$) 198.4 (*C*O$_2$S), 171.3 (CH$_2$*C*ONH), 170.4 (CH*C*ONH), 99.3 (O*C*(CH$_3$)$_2$), 77.3 (*C*H), 71.6 (*C*H$_2$OC(CH$_3$)$_2$), 69.0 (*C*HOSi), 68.2 (*C*HOH), 51.6 (*C*H$_2$COS), 45.6 (*C*H$_2$CHOSi), 39.5 (*C*H$_2$CH$_2$S), 36.2 (*C*H$_2$CONH), 35.1 (*C*H$_2$NH), 33.1 (CH$_2$*C*(CH$_3$)$_2$), 29.7 (OC(*C*H$_3$)$_2$), 28.9 (*C*H$_2$S), 26.0 (C(*C*H$_3$)$_3$), 24.5 (CH*C*H$_3$), 22.4 (CH$_2$C(*C*H$_3$)$_2$), 19.1 (CH$_2$C(*C*H$_3$)$_2$), 18.9 (OC(*C*H$_3$)$_2$), 18.1 (*C*(CH$_3$)$_3$), -3.8 (Si*C*H$_3$), -4.6 (Si*C*H$_3$); HRMS (ESI) $C_{26}H_{50}N_2NaO_7SSi$ $[M + Na]^+$ requires 585.3000, found 585.3004; $[\alpha]_D^{26}$ (c 0.1, $CHCl_3$): +64.

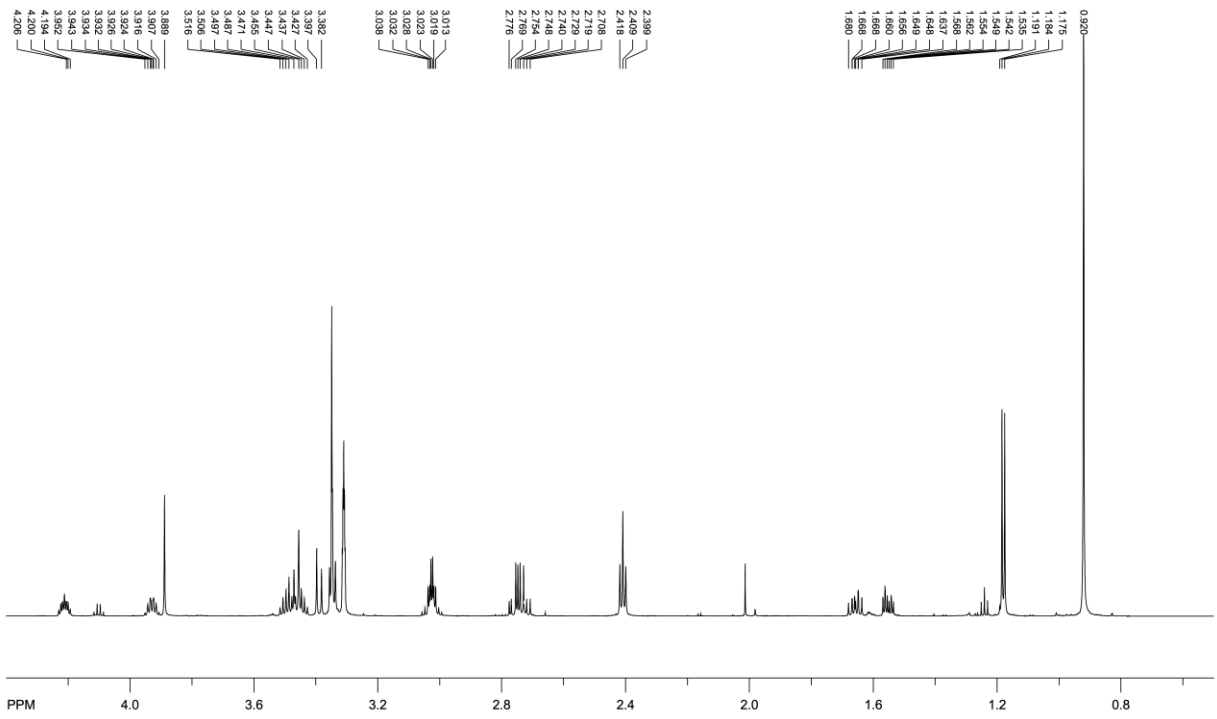
***S*-(2-(3-((*R*)-2,4-dihydroxy-3,3-dimethylbutanamido)propanamido)ethyl) (3*S*,5*S*)-3,5-dihydroxyhexanethioate** (**12**)



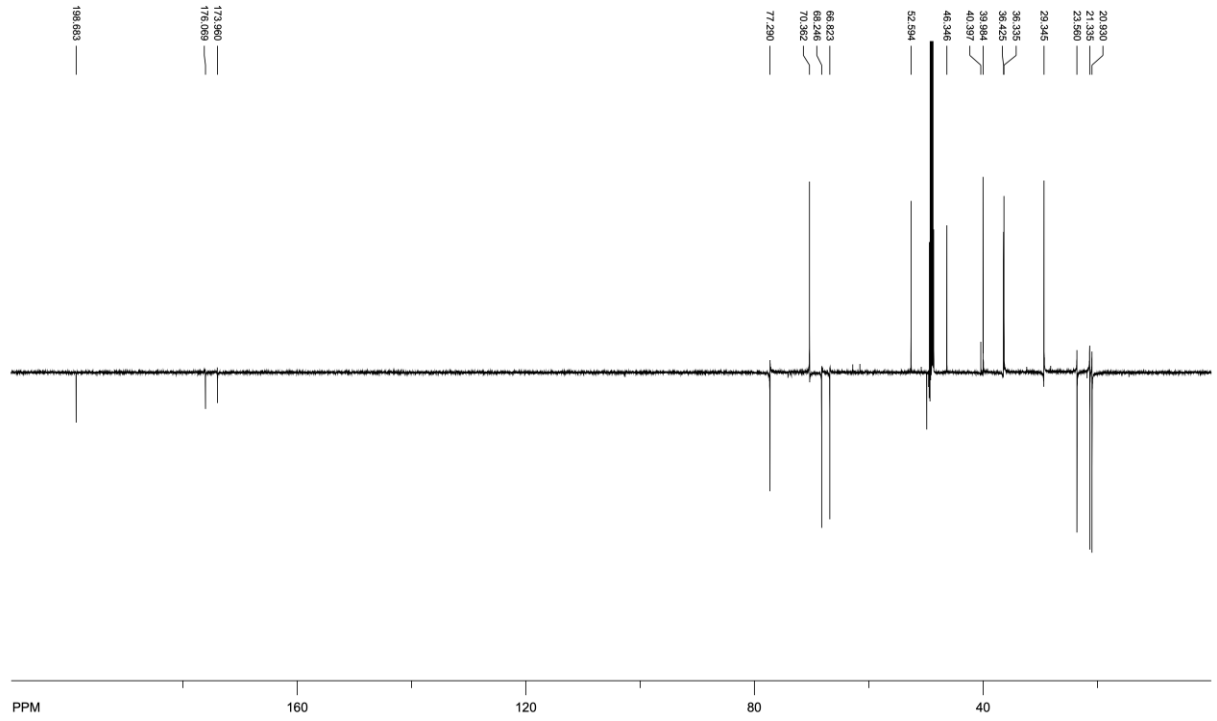
12

S-(2-(3-((*R*)-2,4-dihydroxy-3,3-dimethylbutanamido)propanamido)ethyl) (3*S*,5*S*)-3,5-dihydroxyhexanethioate **12** was synthesized using the same procedure as that used for the synthesis of (2-(3-((*R*)-2,4-dihydroxy-3,3-dimethylbutanamido)propanamido) ethyl) (3*R*,5*S*)-3,5-dihydroxyhexanethioate **9** using *S*-(2-(3-((*R*)-2,2,5,5-tetramethyl-1,3-dioxane-4-carboxamido)propanamido)ethyl) (3*S*,5*S*)-5-((*tert*-butyldimethylsilyl) oxy)-3-hydroxyhexanethioate **31** (100 mg, 0.18 mmol), to afford the product as a colourless oil (70 mg, 96 %).

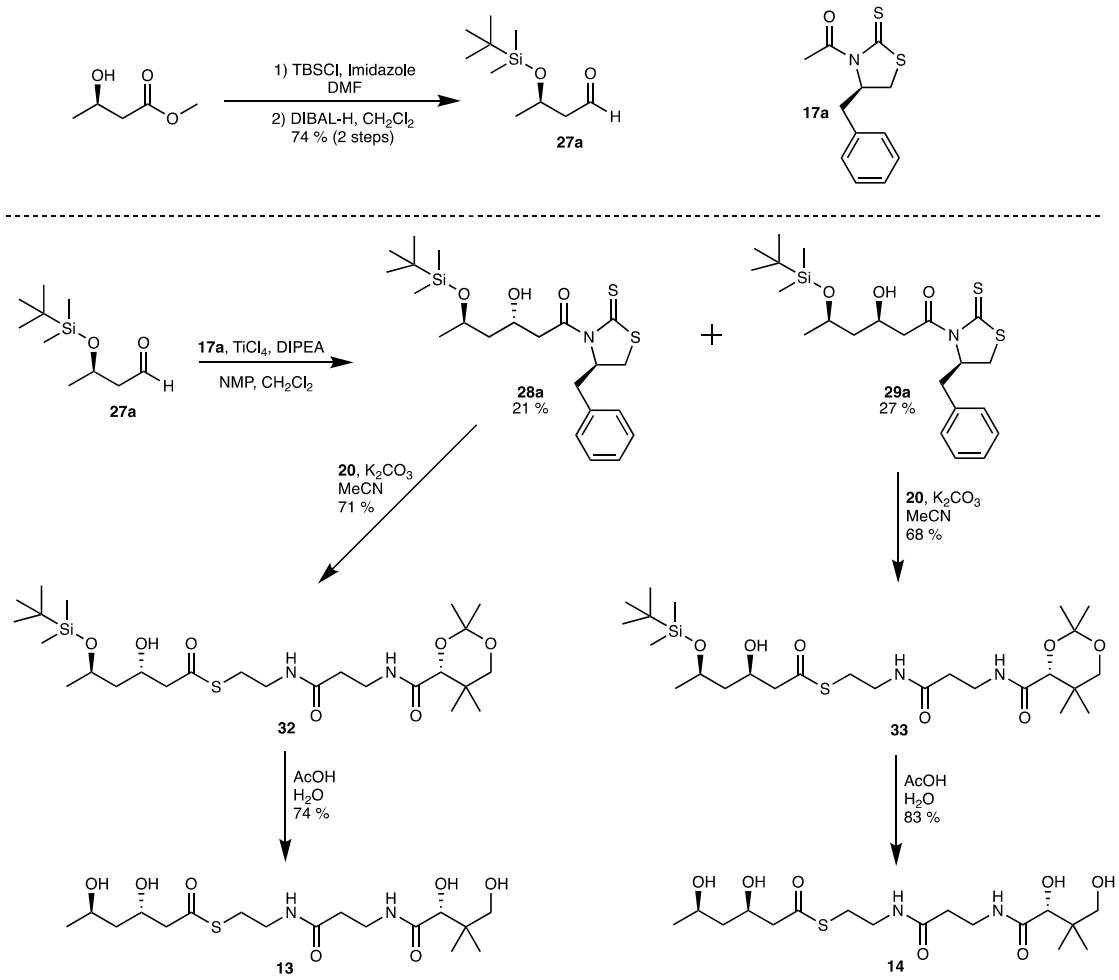
ν_{max}/cm^{-1} (neat) 3326 (OH), 2966 (NH), 1647, 1560 (C=O); δ_H (500 MHz; CD_3OD) 4.21 (1H, tt, J 8.5 and 5.5, $CH_2CHOHCH_2$), 3.93 (1H, dquin., J 7.5 and 6.0, CH_3CHOH), 3.89 (1H, s, $CHCONH$), 3.53-3.33 (6H, m, $NHCH_2$, CH_2CH_2S, CH_2OH), 3.04 (1H, dt, J 13.5 and 6.5, CH_2S), 3.01 (1H, dt, J 13.5 and 6.5, CH_2S), 2.76 (1H, dd, J 15.0 and 5.0, CH_2COS), 2.72 (1H, dd, J 15.0 and 7.5, CH_2COS) 2.41 (2H, t, J 7.0, CH_2CONH), 1.66 (1H, dt, J 14.0 and 8.0, CH_3CH_2CHOH), 1.55 (1H, dt, J 14.0 and 5.0, CH_3CH_2CHOH), 1.18 (3H, d, J 6.0, $CHCH_3$), 0.92 (6H, s, $C(CH_3)_2$); δ_C (125 MHz, CD_3OD) 198.7 (CO_2S), 176.1 (CH_2CONH), 174.0 ($CHCONH$), 77.3 ($CHOHC(CH_3)_2$), 70.3 (CH_2OH), 68.2 ($CH_2CHOHCH_2$), 66.8 (CH_3CHOH), 52.6 (CH_2COS), 46.3 ($CHOHCH_2CHOH$), 40.4 ($CH_2C(CH_3)_2$), 40.0 (CH_2CH_2S), 36.4 (CH_2CONH), 36.3 (CH_2NH), 29.3 (CH_2S), 23.5 ($CHCH_3$), 21.3 ($CH_2C(CH_3)_2$), 20.9 ($CH_2C(CH_3)_2$); HRMS (ESI) $C_{17}H_{32}N_2NaO_7S$ $[M + Na]^+$ requires 431.1822, found 431.1821; $[\alpha]_D^{26}$ (c 0.35, MeOH): +17.7.



The ^{1}H NMR spectrum of *S*-(2-(3-((*R*)-2,4-dihydroxy-3,3-dimethylbutanamido) propanamido)ethyl) (3*S*,5*S*)-3,5-dihydroxyhexanethioate **12** (d^{4}-MeOD).

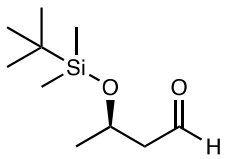


The ^{13}C NMR spectrum of *S*-(2-(3-((*R*)-2,4-dihydroxy-3,3-dimethylbutanamido) propanamido)ethyl) (3*S*,5*S*)-3,5-dihydroxyhexanethioate **12** (d^{4}-MeOD).



Synthesis of thioesters 13 and 14. Auxiliary **17a** was synthesized according to a literature procedure.[7]

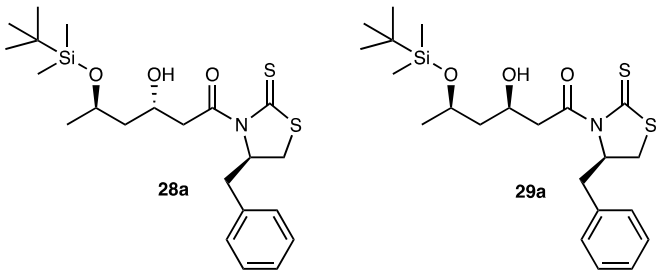
(*R*)-3-((*tert*-butyldimethylsilyl)oxy)butanal (27a)



27a

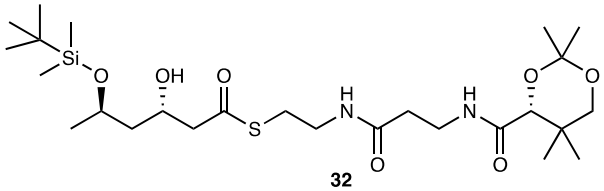
(*R*)-3-((*tert*-butyldimethylsilyl)oxy)butanal **27a** was synthesized using the same procedure as that used for the synthesis of (*S*)-3-((*tert*-butyldimethylsilyl)oxy)butanal **27**, using commercially available methyl (*R*)-3-hydroxybutanoate (1.0 g, 8.48 mmol, 1.00 equiv.) to afford (*R*)-3-((*tert*-butyldimethylsilyl)oxy)butanal **27a** (1.27 g, 74 %, $[\alpha]_D^{25}$ (c 1.0, CH_2Cl_2): -11.5). Spectroscopic data were consistent with those reported in the literature.[13]

(3*S*,5*R*)-1-((*R*)-4-benzyl-2-thioxothiazolidin-3-yl)-5-((*tert*-butyldimethylsilyl)oxy)-3-hydroxyhexan-1-one (28a) and (3*R*,5*R*)-1-((*R*)-4-benzyl-2-thioxothiazolidin-3-yl)-5-((*tert*-butyldimethylsilyl)oxy)-3-hydroxyhexan-1-one (**29a**)



(3*S*,5*R*)-1-((*R*)-4-benzyl-2-thioxothiazolidin-3-yl)-5-((*tert*-butyldimethylsilyl)oxy)-3-hydroxyhexan-1-one **28a** and (3*R*,5*R*)-1-((*R*)-4-benzyl-2-thioxothiazolidin-3-yl)-5-((*tert*-butyldimethylsilyl)oxy)-3-hydroxyhexan-1-one **29a** were synthesized using the same procedure as that used for the synthesis of (3*R*,5*S*)-1-((*S*)-4-benzyl-2-thioxothiazolidin-3-yl)-5-((*tert*-butyldimethylsilyl)oxy)-3-hydroxyhexan-1-one **28** and (3*S*,5*S*)-1-((*S*)-4-benzyl-2-thioxothiazolidin-3-yl)-5-((*tert*-butyldimethylsilyl)oxy)-3-hydroxyhexan-1-one **29**, using (*R*)-1-(4-benzyl-2-thioxothiazolidin-3-yl)ethan-1-one **17a** (600 mg, 2.39 mmol) and (*R*)-3-((*tert*-butyldimethylsilyl)oxy)butanal **27a** (978 mg, 4.78 mmol) to afford the products (3*S*,5*R*)-1-((*R*)-4-benzyl-2-thioxothiazolidin-3-yl)-5-((*tert*-butyldimethylsilyl)oxy)-3-hydroxyhexan-1-one **28a** (227 mg, 21 %, $[\alpha]_D^{24}$ (c 0.4, $CHCl_3$): -100.5) and (3*R*,5*R*)-1-((*R*)-4-benzyl-2-thioxothiazolidin-3-yl)-5-((*tert*-butyldimethylsilyl)oxy)-3-hydroxyhexan-1-one **29a** (292 mg, 27 %, $[\alpha]_D^{24}$ (c 0.4, $CHCl_3$): -140.7). Spectroscopic data for aldol products **28a** and **29a** were identical to that of their respective enantiomers **28** and **29**, with the exception of optical rotation which was inverted.

***S*-(2-(3-((*R*)-2,2,5,5-tetramethyl-1,3-dioxane-4-carboxamido)propanamido)ethyl) (3*S*,5*R*)-5-((*tert*-butyldimethylsilyl)oxy)-3-hydroxyhexanethioate** (**32**)

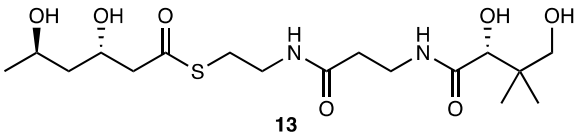


32

S-(2-(3-((*R*)-2,2,5,5-tetramethyl-1,3-dioxane-4-carboxamido)propanamido)ethyl) (3*S*,5*R*)-5-((*tert*-butyldimethylsilyl)oxy)-3-hydroxyhexanethioate **32** was synthesized using the same procedure as that used for the synthesis of *S*-(2-(3-((*R*)-2,2,5,5-tetramethyl-1,3-dioxane-4-carboxamido)propanamido)ethyl) (3*R*,5*S*)-5-((*tert*-butyldimethylsilyl)oxy)-3-hydroxyhexanethioate **30** using (3*S*,5*R*)-1-((*R*)-4-benzyl-2-thioxothiazolidin-3-yl)-5-((*tert*-butyldimethylsilyl)oxy)-3-hydroxyhexan-1-one **28a** (200 mg, 0.44 mmol), to afford the product as a colourless oil (176 mg, 71 %).

ν_{max}/cm^{-1} (neat) 3310 (OH), 2953 (NH), 1655, 1527 (C=O); δ_H (500 MHz; $CDCl_3$) 7.02 (1H, br. t, J 6.0, CHCON*H*), 6.19 (1H, br. t, J 6.0, N*H*CH_2CH_2S), 4.28 (1H, tt, J 8.5 and 3.5, C*H*OH), 4.12-4.04 (2H, m, C*H*OSi, C*H*CONH), 3.68 (1H, d J 11.5, C*H*$_2$OC$(CH_3)_2$), 3.60-3.40 (4H, m, NHC*H*$_2$, C*H*$_2$CH$_2$S), 3.28 (1H, d J 11.5, C*H*$_2$OC$(CH_3)_2$), 3.07 (1H, dt, J 14.0 and 6.0, C*H*$_2$S), 3.02 (1H, dt, J 14.0 and 6.0), C*H*$_2$S), 2.75 (1H, dd, J 15.0 and 8.5, C*H*$_2$COS), 2.67 (1H, dd, J 15.0 and 4.0, C*H*$_2$COS) 2.42 (1H, dt, J 15.0 and 6.5, C*H*$_2$CONH), 2.40 (1H, dt, J 15.0 and 6.5, C*H*$_2$CONH), 1.67 (1H, dt, J 14.0 and 8.5, C*H*$_2$CHOSi), 1.57 (1H, dt, J 14.0 and 3.5, C*H*$_2$CHOSi), 1.46 (3H, s, OC(C*H*$_3$)$_2$), 1.42 (3H, s, OC(C*H*$_3$)$_2$), 1.18 (3H, d, J 6.0, CHC*H*$_3$), 1.04 (3H, s, CH$_2$C(C*H*$_3$)$_2$), 0.97 (3H, s, CH$_2$C(C*H*$_3$)$_2$), 0.89 (9H, s, C(C*H*$_3$)$_3$), 0.11 (3H, s, SiC*H*$_3$), 0.10 (3H, s, SiC*H*$_3$); δ_C (125 MHz, $CDCl_3$) 198.5 (*C*O$_2$S), 171.3 (CH$_2$*C*ONH), 170.3 (CH*C*ONH), 99.3 (O*C*(CH$_3$)$_2$), 77.4 (*C*H), 71.5 (*C*H$_2$OC(CH$_3$)$_2$), 69.0 (*C*HOSi), 68.2 (*C*HOH), 51.6 (*C*H$_2$COS), 45.7 (*C*H$_2$CHOSi), 39.4 (*C*H$_2$CH$_2$S), 36.2 (*C*H$_2$CONH), 35.0 (*C*H$_2$NH), 33.1 (CH$_2$*C*(CH$_3$)$_2$), 29.8 (OC(*C*H$_3$)$_2$), 28.9 (*C*H$_2$S), 26.0 (C(*C*H$_3$)$_3$), 24.5 (CH*C*H$_3$), 22.4 (CH$_2$C(*C*H$_3$)$_2$), 19.1 (CH$_2$C(*C*H$_3$)$_2$), 18.9 (OC(*C*H$_3$)$_2$), 18.1 (*C*(CH$_3$)$_3$), -3.8 (Si*C*H$_3$), -4.6 (Si*C*H$_3$); HRMS (ESI) $C_{26}H_{50}N_2NaO_7SSi$ $[M + Na]^+$ requires 585.3000, found 585.3003; $[\alpha]_D^{26}$ (c 0.1, $CHCl_3$): +45.5.

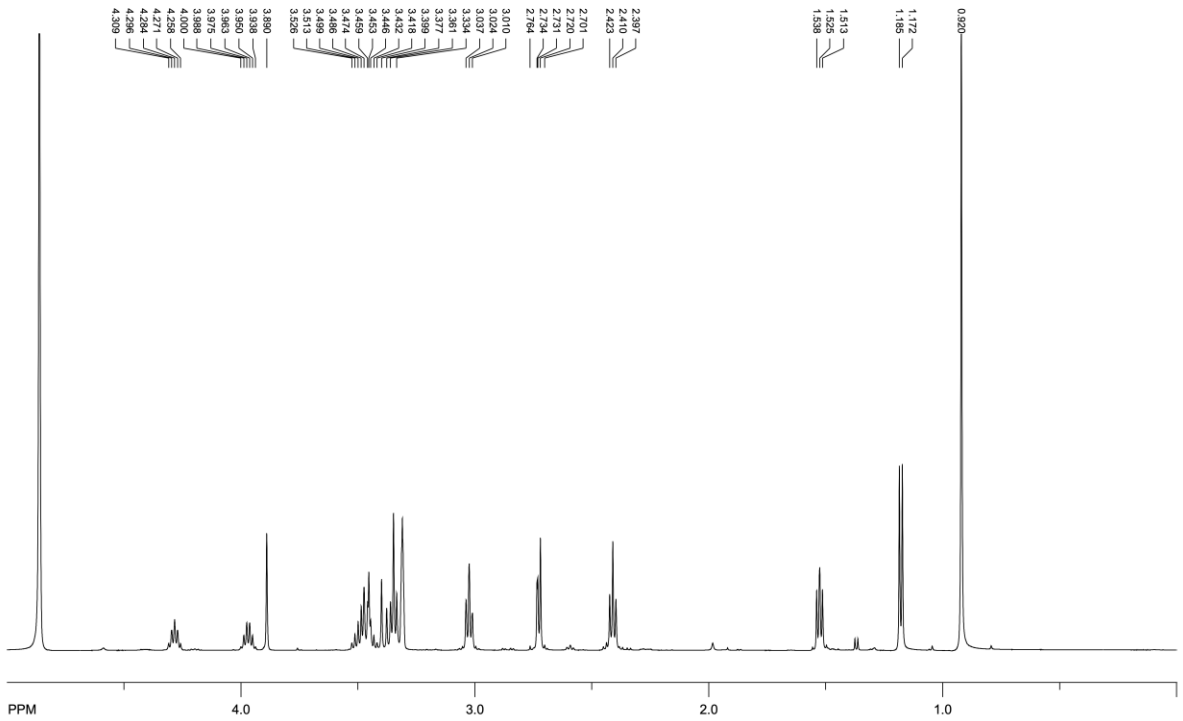
_S_-(2-(3-((_R_)-2,4-dihydroxy-3,3-dimethylbutanamido)propanamido)ethyl) (3_S_,5_R_)-3,5-dihydroxyhexanethioate (**13**)



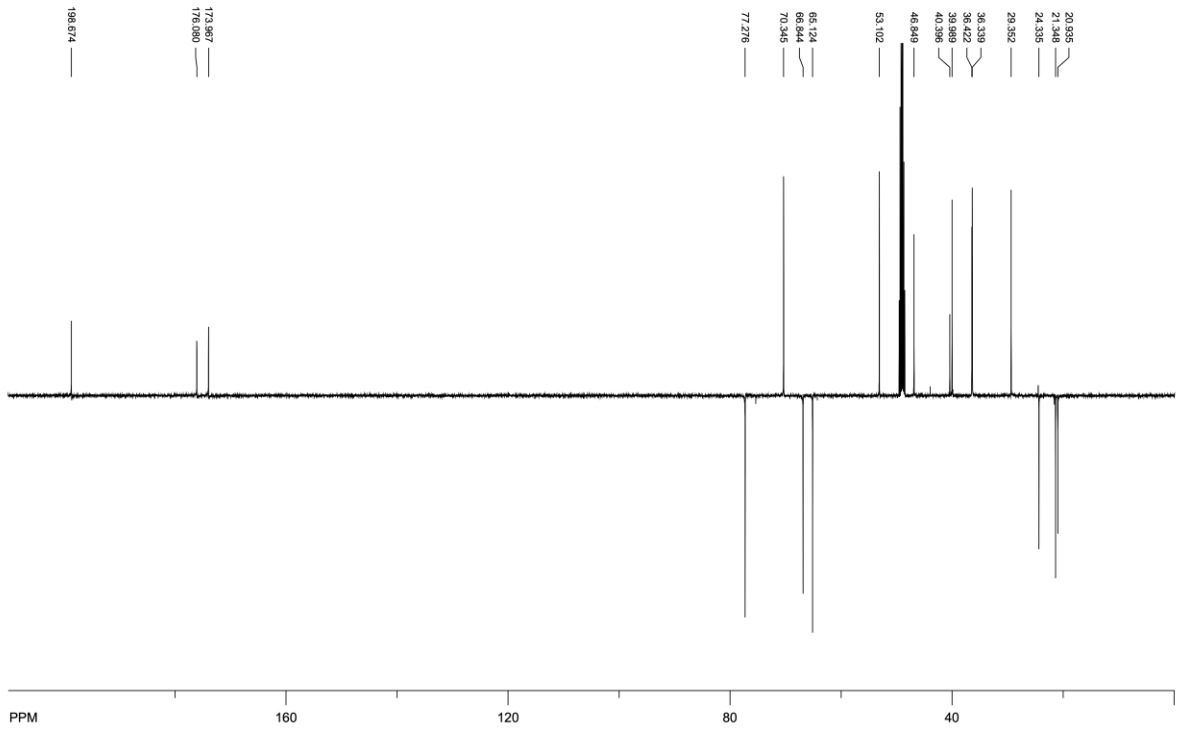
13

S-(2-(3-((*R*)-2,4-dihydroxy-3,3-dimethylbutanamido)propanamido)ethyl) (3*S*,5*R*)-3,5-dihydroxyhexanethioate **13** was synthesized using the same procedure as that used for the synthesis of (2-(3-((*R*)-2,4-dihydroxy-3,3-dimethylbutanamido)propanamido) ethyl) (3*R*,5*S*)-3,5-dihydroxyhexanethioate **9** using *S*-(2-(3-((*R*)-2,2,5,5-tetramethyl-1,3-dioxane-4-carboxamido)propanamido)ethyl)(3*S*,5*R*)-5-((*tert*-butyldimethylsilyl) oxy)-3-hydroxyhexanethioate **32** (100 mg, 0.18 mmol), to afford the product as a colourless oil (54 mg, 74 %).

ν_{max}/cm^{-1} (neat) 3328 (OH), 2964 (NH), 1648, 1543 (C=O); δ_H (500 MHz; CD_3OD) 4.28 (1H, quintet, J 6.5, $CH_2CHOHCH_2$), 3.97 (1H, sextet, J 6.0, CH_3CHOH), 3.89 (1H, s, CHCONH), 3.53-3.32 (6H, m, $NHCH_2$, CH_2CH_2S, CH_2OH), 3.02 (2H, t, J 6.5, CH_2S), 2.74 (1H, dd, J 15.0 and 7.5, CH_2COS), 2.71 (1H, dd, J 15.0 and 5.5, CH_2COS) 2.41 (2H, t, J 7.0, CH_2CONH), 1.53 (2H, dd, J 6.5 and 5.5, CH_3CH_2CHOH), 1.18 (3H, d, J 6.5, $CHCH_3$), 0.92 (6H, s, $C(CH_3)_2$); δ_C (125 MHz, CD3OD) 198.6 (CO_2S), 176.1 (CH_2CONH), 173.9 ($CHCONH$), 77.3 ($CHOHC(CH_3)_2$), 70.3 (CH_2OH), 66.8 ($CH_2CHOHCH_2$), 65.1 (CH_3CHOH), 53.1 (CH_2COS), 46.8 ($CHOHCH_2CHOH$), 40.4 ($CH_2C(CH_3)_2$), 40.0 (CH_2CH_2S), 36.4 (CH_2CONH), 36.3 (CH_2NH), 29.3 (CH_2S), 24.3 ($CHCH_3$), 21.3 ($CH_2C(CH_3)_2$), 20.9 ($CH_2C(CH_3)_2$); HRMS (ESI) $C_{17}H_{32}N_2NaO_7S$ $[M + Na]^+$ requires 431.1822, found 431.1822; $[\alpha]_D^{26}$ (c 0.5, MeOH): +5.3.

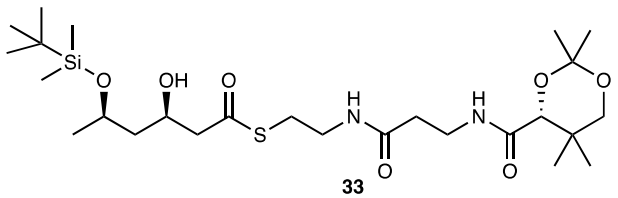


The ^{1}H NMR spectrum of *S*-(2-(3-((*R*)-2,4-dihydroxy-3,3-dimethylbutanamido) propanamido)ethyl) (3*S*,5*R*)-3,5-dihydroxyhexanethioate **13** (d^4-MeOD).



The ^{13}C NMR spectrum of *S*-(2-(3-((*R*)-2,4-dihydroxy-3,3-dimethylbutanamido) propanamido)ethyl) (3*S*,5*R*)-3,5-dihydroxyhexanethioate **13** (d^4-MeOD).

_S_-(2-(3-((_R_)-2,2,5,5-tetramethyl-1,3-dioxane-4-carboxamido)propanamido)ethyl) (3_R_,5_R_)-5-((_tert_-butyldimethylsilyl)oxy)-3-hydroxyhexanethioate (**33**)

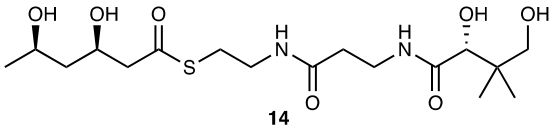


33

S-(2-(3-((*R*)-2,2,5,5-tetramethyl-1,3-dioxane-4-carboxamido)propanamido)ethyl) (3*R*,5*R*)-5-((*tert*-butyldimethylsilyl)oxy)-3-hydroxyhexanethioate **33** was synthesized using the same procedure as that used for the synthesis of *S*-(2-(3-((*R*)-2,2,5,5-tetramethyl-1,3-dioxane-4-carboxamido)propanamido)ethyl) (3*R*,5*S*)-5-((*tert*-butyldimethylsilyl)oxy)-3-hydroxyhexanethioate **30** using (3*R*,5*R*)-1-((*R*)-4-benzyl-2-thioxothiazolidin-3-yl)-5-((*tert*-butyldimethylsilyl)oxy)-3-hydroxyhexan-1-one **29a** (200 mg, 0.44 mmol), to afford the product as a colourless oil (168 mg, 68 %).

ν_{max}/cm^{-1} (neat) 3310 (OH), 2954 (NH), 1655, 1529 (C=O); δ_H (500 MHz; $CDCl_3$) 7.02 (1H, br. t, J 6.5, CHCON*H*), 6.33 (1H, br. t, J 6.0, N*H*CH_2CH_2S), 4.40 (1H, dddd, J 10.0, 8.5, 4.5 and 2.5 C*H*OH), 4.17 (1H, quin.d, J 6.5 and 3.5, C*H*OSi), 4.06 (1H, s, C*H*CONH), 3.81 (1H, br. s, O*H*), 3.67 (1H, d J 11.5, C*H*$_2$OC$(CH_3)_2$), 3.60-3.35 (4H, m, NHC*H*$_2$, C*H*$_2$$CH_2S$), 3.26 (1H, d J 11.5, C*H*$_2$OC$(CH_3)_2$), 3.05 (1H, dt, J 14.0 and 6.0, C*H*$_2$S), 3.01 (1H, dt, J 14.0 and 6.5, C*H*$_2$S), 2.74 (1H, dd, J 15.0 and 8.5, C*H*$_2$COS), 2.66 (1H, dd, J 15.0 and 4.0, C*H*$_2$COS), 2.42 (1H, dt, J 15.0 and 5.5, C*H*$_2$CONH), 2.38 (1H, dt, J 15.0 and 6.0, C*H*$_2$CONH), 1.65 (1H, ddd, J 14.0, 10.0 and 3.5, C*H*$_2$CHOSi), 1.51 (1H, ddd, J 14.0, 6.5 and 2.5, C*H*$_2$CHOSi), 1.45 (3H, s, OC(C*H*$_3$)$_2$), 1.41 (3H, s, OC(C*H*$_3$)$_2$), 1.20 (3H, d, J 6.5, CHC*H*$_3$), 1.02 (3H, s, CH_2C(C*H*$_3$)$_2$), 0.95 (3H, s, CH_2C(C*H*$_3$)$_2$), 0.88 (9H, s, C(*C*H_3)$_3$), 0.08 (3H, s, SiC*H*$_3$), 0.07 (3H, s, SiC*H*$_3$); δ_C (125 MHz, $CDCl_3$) 198.5 (*C*O_2S), 171.4 (CH_2*C*ONH), 170.4 (CH*C*ONH), 99.3 (O*C*$(CH_3)_2$), 77.3 (*C*H), 71.6 (*C*H_2OC$(CH_3)_2$), 66.9 (*C*HOSi), 66.0 (*C*HOH), 52.0 (*C*H_2COS), 44.5 (*C*H_2CHOSi), 39.4 (*C*H_2CH_2S), 36.2 (*C*H_2CONH), 35.1 (*C*H_2NH), 33.1 (CH_2*C*$(CH_3)_2$), 29.6 (OC(*C*H_3)$_2$), 28.9 (*C*H_2S), 26.0 (C(*C*H_3)$_3$), 23.3 (CH*C*H_3), 22.3 (CH_2C(*C*H_3)$_2$), 19.1 (CH_2C(*C*H_3)$_2$), 18.9 (OC(*C*H_3)$_2$), 18.1 (*C*$(CH_3)_3$), -4.3 (Si*C*H_3), -4.8 (Si*C*H_3); HRMS (ESI) $C_{26}H_{50}N_2NaO_7SSi$ $[M + Na]^+$ requires 585.3000, found 585.3007; $[\alpha]_D^{26}$ (c 0.15, $CHCl_3$): +27.7.

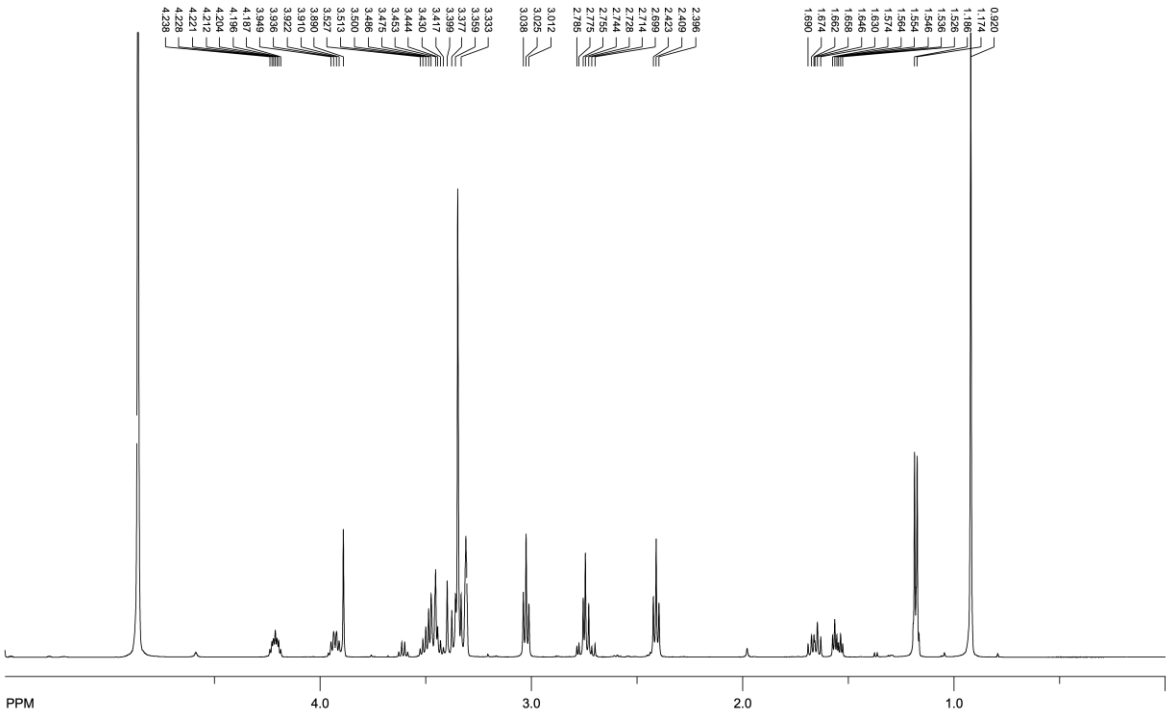
_S_-(2-(3-((_R_)-2,4-dihydroxy-3,3-dimethylbutanamido)propanamido)ethyl) (3_R_,5_R_)-3,5-dihydroxyhexanethioate (**14**)



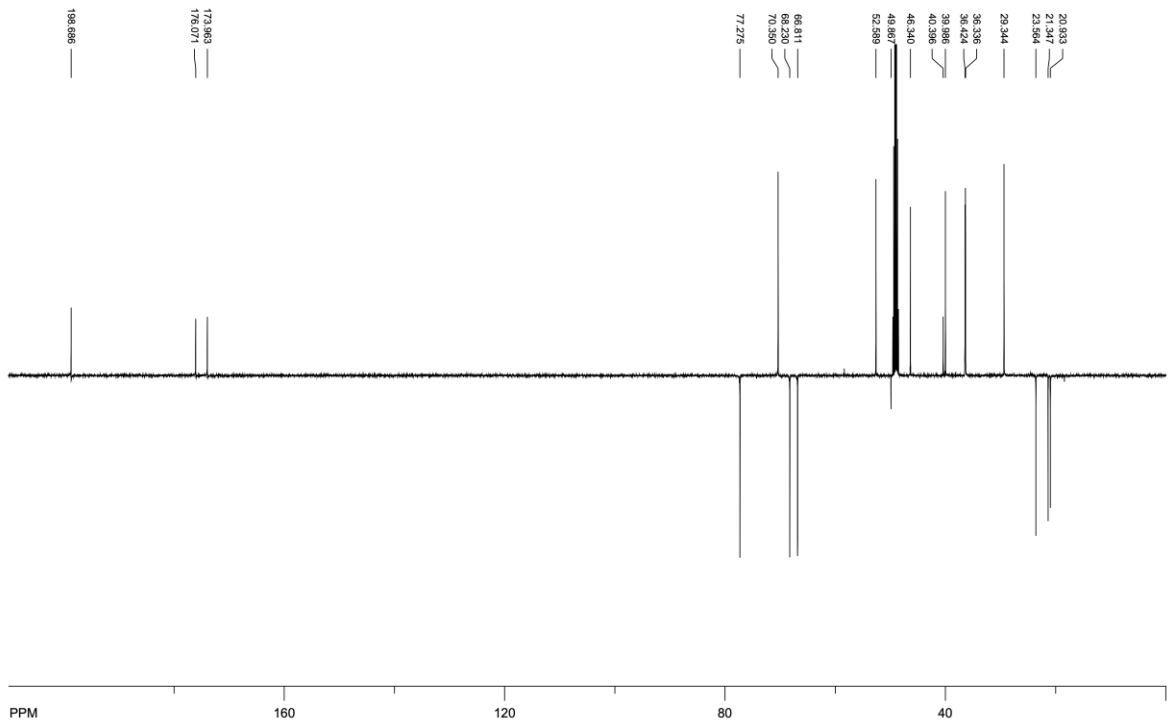
14

S-(2-(3-((*R*)-2,4-dihydroxy-3,3-dimethylbutanamido)propanamido)ethyl) (3*R*,5*R*)-3,5-dihydroxyhexanethioate **14** was synthesized using the same procedure as that used for the synthesis of (2-(3-((*R*)-2,4-dihydroxy-3,3-dimethylbutanamido)propanamido) ethyl) (3*R*,5*S*)-3,5-dihydroxyhexanethioate **9** using *S*-(2-(3-((*R*)-2,2,5,5-tetramethyl-1,3-dioxane-4-carboxamido)propanamido)ethyl) (3*R*,5*R*)-5-((*tert*-butyldimethylsilyl) oxy)-3-hydroxyhexanethioate **33** (100 mg, 0.18 mmol), to afford the product as a colourless oil (61 mg, 83 %).

ν_{max}/cm^{-1} (neat) 3344 (OH), 2960 (NH), 1647, 1560 (C=O); δ_H (500 MHz; CD_3OD) 4.21 (1H, tt, J 8.5 and 5.0, $CH_2CHOHCH_2$), 3.93 (1H, dquin., J 8.0 and 6.0, CH_3CHOH), 3.89 (1H, s, CHCONH), 3.53-3.32 (6H, m, $NHCH_2$, CH_2CH_2S, CH_2OH), 3.02 (2H, t, J 6.5, CH_2S), 2.76 (1H, dd, J 15.0 and 5.0, CH_2COS), 2.72 (1H, dd, J 15.0 and 7.5, CH_2COS), 2.41 (2H, t, J 6.5, CH_2CONH), 1.66 (1H, dt, J 14.0 and 8.0, CH_3CH_2CHOH), 1.55 (1H, dt, J 14.0 and 5.0, CH_3CH_2CHOH), 1.18 (3H, d, J 6.0, $CHCH_3$), 0.92 (6H, s, $C(CH_3)_2$); δ_C (125 MHz, CD3OD) 198.7 (CO_2S), 176.1 (CH_2CONH), 174.0 (CHCONH), 77.3 ($CHOHC(CH_3)_2$), 70.3 (CH_2OH), 68.2 ($CH_2CHOHCH_2$), 66.8 (CH_3CHOH), 52.6 (CH_2COS), 46.3 ($CHOHCH_2CHOH$), 40.4 ($CH_2C(CH_3)_2$), 40.0 (CH_2CH_2S), 36.4 (CH_2CONH), 36.3 (CH_2NH), 29.3 (CH_2S), 23.6 ($CHCH_3$), 21.3 ($CH_2C(CH_3)_2$), 20.9 ($CH_2C(CH_3)_2$); HRMS (ESI) $C_{17}H_{32}N_2NaO_7S$ $[M + Na]^+$ requires 431.1822, found 431.1822; $[\alpha]_D^{26}$ (c 0.45, MeOH): +3.9.

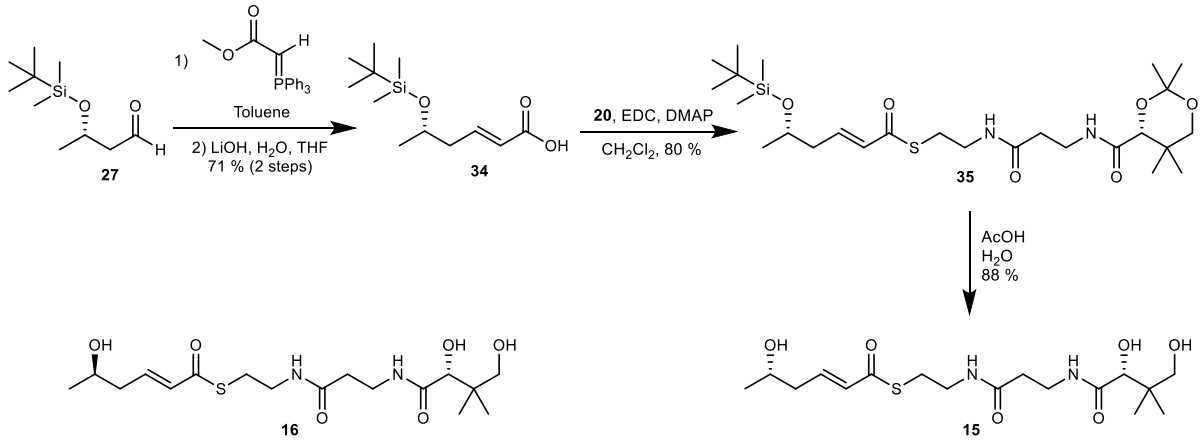


The ^{1}H NMR spectrum of *S*-(2-(3-((*R*)-2,4-dihydroxy-3,3-dimethylbutanamido) propanamido)ethyl) (3*R*,5*R*)-3,5-dihydroxyhexanethioate **14** (d^4-MeOD).



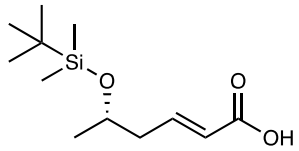
The ^{13}C NMR spectrum of *S*-(2-(3-((*R*)-2,4-dihydroxy-3,3-dimethylbutanamido) propanamido)ethyl) (3*R*,5*R*)-3,5-dihydroxyhexanethioate **14** (d^4-MeOD).

Synthesis of 5-hydroxy-2-enoyl pantetheine thioesters



Supplementary Fig. 32: Synthesis of 5-hydroxy-2-enoyl pantetheine thioester 15. Thioester **16** was synthesized via the same route from (*R*)-configured aldehyde **27a.**

(*S*,*E*)-5-((*tert*-butyldimethylsilyl)oxy)hex-2-enoic acid (34)

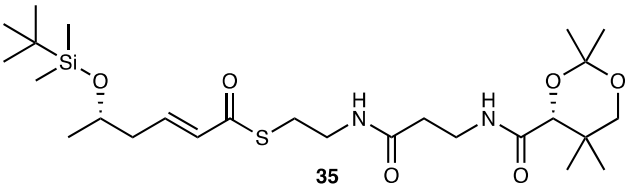


34

To a solution of (*S*)-3-((*tert*-butyldimethylsilyl)oxy)butanal **27** (610 mg, 3.0 mmol, 1.0 equiv.) in dry toluene (25 mL) was added methoxycarbonylmethylenetriphenylphosphorane (1.10 g, 3.3 mmol, 1.1 equiv.) and the mixture heated to reflux for 16 hours. The precipitate was removed by filtration and the filtrate concentrated *in vacuo* to afford a colourless oil, which was purified by silica chromatography (3:10 Et_2O/petroleum ether) to afford the corresponding methyl ester as a colourless oil (723 mg). The oil was then dissolved in a 5:3 mixture of THF (10 mL) and water (6 mL) before LiOH (120 mg, 6.0 mmol, 2.0 equiv.) was added and the reaction stirred at RT for 16 h. The THF was removed *in vacuo* and the resulting solution acidified to pH 2.0 using 1 M HCl. The mixture was then extracted with CH_2Cl_2 (3 x 10 mL), the combined organics washed with brine (10 mL), dried ($MgSO_4$) and concentrated *in vacuo* to afford the product as a colourless oil (520 mg, 71 % (2 steps)).

δ_H (500 MHz; $CDCl_3$) 7.07 (1H, dt, *J* 15.5 and 7.5, CH_2CHCH), 5.84 (1H, dt, *J* 15.5 and 1.5, CH_2CHCH), 3.94 (1H, sext., *J* 6.0, C*H*), 2.40-2.30 (2H, m, CH_2), 1.17 (3H, d, *J* 6.0, CH_3), 0.88 (9H, s, $C(CH_3)_3$), 0.05 (3H, s, $SiCH_3$), 0.04 (3H, s, $SiCH_3$); δ_C (125 MHz, $CDCl_3$) 171.4 (*C*OOH), 149.4 (CH_2CHCH), 122.8 (CH_2CHCH), 67.9 (*C*H), 42.9 (CH_2), 26.2 ($C(CH_3)_3$), 24.2 (CH_3), 18.5 ($C(CH_3)_3$), -4.2 ($SiCH_3$), -4.4 ($Si(CH_3)_3$; HRMS (ESI) cald. for $C_{12}H_{23}O_3Si$ (M - H+) requires 243.1422, found 243.1420; $[\alpha]_D^{24}$ (c 1.0, $CHCl_3$): +9.8.[18] Spectroscopic data were consistent with those previously reported in the literature.[19]

S-(2-(3-((*R*)-2,2,5,5-tetramethyl-1,3-dioxane-4-carboxamido)propanamido)ethyl) (*S*,*E*)-5-((*tert*-butyldimethylsilyl)oxy)hex-2-enethioate (**35**)



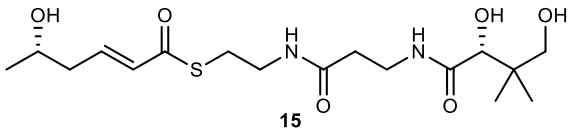
35

To a solution of (5*S*, 2*E*)-5-((*tert*-butyldimethylsilyl)oxy)-2-hexenoic acid **34** (60 mg, 1.3 equiv., 0.25 mmol), (*R*)-*N*-(3-((2-mercaptoethyl)amino)-3-oxopropyl)-2,2,5,5-tetramethyl-1,3-dioxane-4-

carboxamide **20** (85 mg, 1.4 equiv., 0.27 mmol) and DMAP (7 mg, 0.3 equiv., 0.06 mmol) in CH_2Cl_2 (4 mL) was added EDC (52 mg, 1.4 equiv., 0.267 mmol) at 0 °C, and the mixture allowed to stir at room temperature for 16 h. The mixture was diluted with CH_2Cl_2 (10 mL), washed with 1 M HCl (10 mL), saturated $NaHCO_3$ solution (10 mL), dried ($MgSO_4$) and concentrated *in vacuo* to give a colourless oil, which was purified by silica chromatography (1:99 MeOH:CH_2Cl_2) to afford the desired product as a colourless oil (108 mg, 80 %).

ν_{max}/cm^{-1} (neat) 3304 (NH), 2953 (C=C-H), 1664, 1525 (C=O); δ_H (500 MHz; $CDCl_3$) 7.03 (1H, br. t, J 5.5, N*H*), 6.92 (1H, dt, J 15.5 and 7.5, C*H*CHCOS), 6.14 (1H, br. s, N*H*CH_2CH_2S), 6.13 (1H, dt, J 15.5 and 1.5, CHC*H*COS), 4.07 (1H, s, C*H*CONH), 3.93 (1H, sextet, J 6.0, C*H*OSi), 3.68 (1H, d J 11.5, C*H*$_2$OC$(CH_3)_2$), 3.61-3.39 (4H, m, NHC*H*$_2$, C*H*$_2$CH$_2$S), 3.27 (1H, d J 11.5, C*H*$_2$OC$(CH_3)_2$), 3.08 (2H, t, J 6.5, C*H*$_2$S), 2.42 (2H, t, J 6.5, C*H*$_2$CONH), 2.32 (2H, td, J 6.5 and 0.5, C*H*$_2$CHOSi), 1.46 (3H, s, OC(C*H*$_3$)$_2$), 1.41 (3H, s, OC(C*H*$_3$)$_2$), 1.16 (3H, d, J 6.0, CHC*H*$_3$), 1.03 (3H, s, CH$_2$C(C*H*$_3$)$_2$), 0.97 (3H, s, CH$_2$C(C*H*$_3$)$_2$), 0.88 (9H, s, C(C*H*$_3$)$_3$), 0.05 (3H, s, SiC*H*$_3$), 0.04 (3H, s, SiC*H*$_3$); δ_C (125 MHz, $CDCl_3$) 190.3 (*C*O$_2$S), 171.6 (CH$_2$*C*ONH), 170.5 (CH*C*ONH), 144.0 (*C*HCHCOS), 130.5 (CH*C*HCOS), 99.5 (O*C*(CH$_3$)$_2$), 77.5 (*C*H), 71.9 (*C*H$_2$OC(CH$_3$)$_2$), 67.9 (*C*HOSi), 42.8 (*C*H$_2$CHOSi), 40.1 (*C*H$_2$CH$_2$S), 36.3 (*C*H$_2$CONH), 35.1 (*C*H$_2$NH), 33.4 (CH$_2$*C*(CH$_3$)$_2$), 29.9 (OC(*C*H$_3$)$_2$), 28.6 (*C*H$_2$S), 26.2 (C(*C*H$_3$)$_3$), 24.3 (CH*C*H$_3$), 22.5 (CH$_2$C(*C*H$_3$)$_2$), 19.3 (CH$_2$C(*C*H$_3$)$_2$), 19.1 (OC(*C*H$_3$)$_2$), 18.4 (*C*(CH$_3$)$_3$), -4.1 (Si*C*H$_3$), -4.4 (Si*C*H$_3$)**;** HRMS (ESI) $C_{26}H_{48}N_2NaO_6SSi$ $[M + Na]^+$ requires 567.2895, found 567.2901; $[\alpha]_D^{26}$ (c 0.2, $CHCl_3$): +37.5.

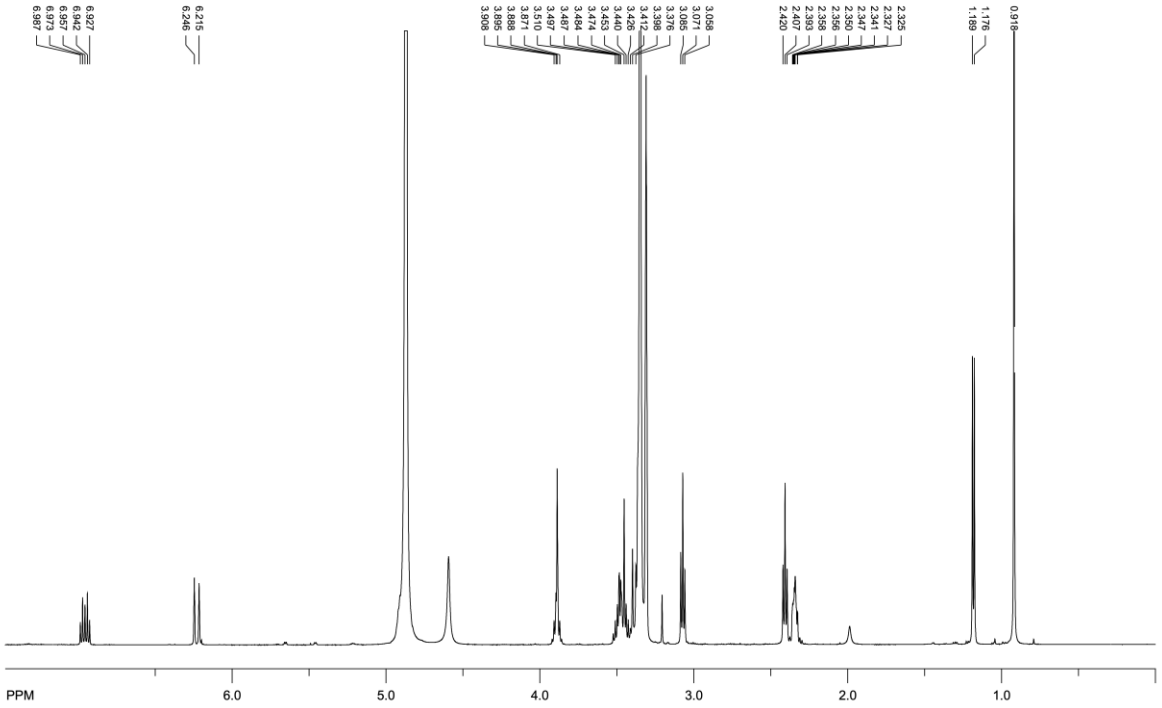
_S_-(2-(3-((_R_)-2,4-dihydroxy-3,3-dimethylbutanamido)propanamido)ethyl) (5_S_, 2_E_)-5-hydroxy-2-hexenethioate (**15**)



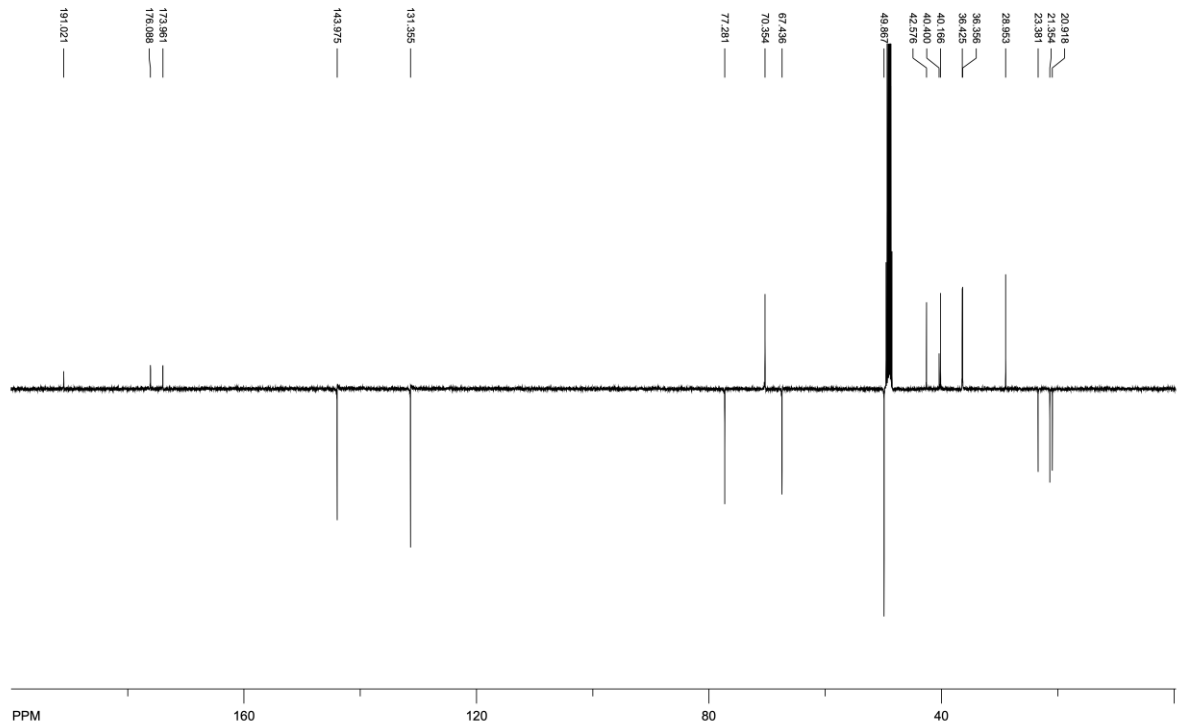
15

S-(2-(3-((*R*)-2,2,5,5-tetramethyl-1,3-dioxane-4-carboxamido)propanamido)ethyl) (5*S*, 2*E*)-5-((*tert*-butyldimethylsilyl)oxy)-2-hexenethioate **35** (100 mg, 0.18 mmol) was stirred in a 2:1 ratio of AcOH/H_2O

(3 mL) at room temperature for 16 h. The mixture was concentrated in *vacuo* and purified by silica chromatography (12:88 MeOH:CH_2Cl_2) to afford the desired product as a colourless oil (63 mg, 88 %). ν_{max}/cm^{-1} (neat) 3310 (OH), 2967 (C=C-H), 1644, 1534 (C=O); δ_H (500 MHz; CD_3OD) 6.96 (1H, dt, J 15.5 and 7.5, C*H*CHCOS), 6.23 (1H, dt, J 15.5 and 1.5, CHC*H*COS), 3.91 (1H, sextet, J 6.5, C*H*OH), 3.91 (1H, s, C*H*CONH), 3.55-3.32 (6H, m, CH_2OH, NHCH_2, CH_2CH_2S), 3.09 (2H, t, J 6.5, CH_2S), 2.43 (2H, t, J 6.5, CH_2CONH), 2.38-2.34 (2H, m, CH_2CHOH), 1.16 (3H, d, J 6.0, CHCH_3), 1.03 (3H, s, $CH_2C(CH_3)_2$), 0.94 (6H, s, $CH_2C(CH_3)_2$); δ_C (125 MHz, $CDCl_3$) 191.3 (CO_2S), 176.4 (CHCONH), 174.2 (CH_2CONH), 144.3 (*C*HCHCOS), 131.6 (CH*C*HCOS), 77.6 (*C*HONH), 70.6 ($CH_2OC(CH_3)_2$), 67.8 (*C*HOH), 42.9 (CH_2CHOH), 40.7 ($CH_2C(CH_3)_2$), 40.5 (CH_2CH_2S), 36.7 (CH_2NH), 36.6 (CH_2CONH), 29.2 (CH_2S), 23.7 ($CHCH_3$), 21.6 ($CH_2C(CH_3)_2$), 21.2 ($CH_2C(CH_3)_2$); HRMS (ESI) $C_{17}H_{30}N_2NaO_6S$ $[M + Na]^+$ requires 413.1717, found 413.1717**;** $[\alpha]_D^{26}$ (c 0.2, MeOH): +8.5.

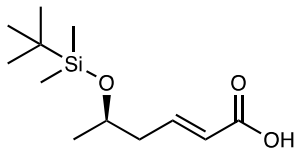


The ^{1}H NMR spectrum of *S*-(2-(3-((*R*)-2,4-dihydroxy-3,3-dimethylbutanamido) propanamido)ethyl) (*S*,*E)*-5-hydroxyhex-2-enethioate **15** (d^4-MeOD).



The ^{13}C NMR spectrum of *S*-(2-(3-((*R*)-2,4-dihydroxy-3,3-dimethylbutanamido) propanamido)ethyl) (*S*,*E)*-5-hydroxyhex-2-enethioate **15** (d^4-MeOD).

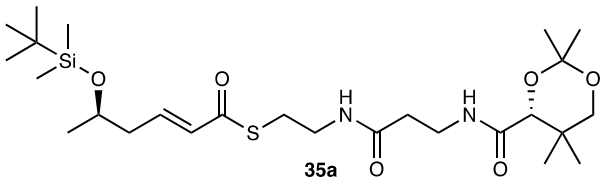
(*R*,*E*)-5-((*tert*-butyldimethylsilyl)oxy)hex-2-enoic acid (34a)



34a

(*R*,*E*)-5-((*tert*-butyldimethylsilyl)oxy)hex-2-enoic acid **34a** was synthesized using the same procedure as that used for the synthesis of (*S*,*E*)-5-((*tert*-butyldimethylsilyl)oxy)hex-2-enoic acid **34**, using (*R*)-3-((*tert*-butyldimethylsilyl)oxy)butanal **27a** (610 mg) to afford the product as a colourless oil (544 mg, 74 % (2 steps), $[\alpha]_D^{24}$ (c 1.0, $CHCl_3$): -9.2).[19] Spectroscopic data was consistent with that previously reported in the literature.[19]

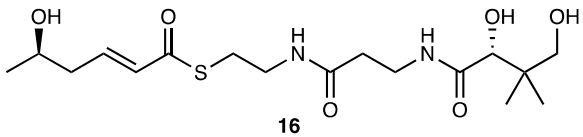
***S*-(2-(3-((*R*)-2,2,5,5-tetramethyl-1,3-dioxane-4-carboxamido)propanamido)ethyl) (5*R*, 2*E*)-5-((*tert*-butyldimethylsilyl)oxy)2-hexenethioate** (**35a**)



35a

S-(2-(3-((*R*)-2,2,5,5-tetramethyl-1,3-dioxane-4-carboxamido)propanamido)ethyl) (5*R*, 2*E*)-5-((*tert*-butyldimethylsilyl)oxy)2-hexenethioate **35a** was synthesized using the same procedure as that used for the synthesis of *S*-(2-(3-((*R*)-2,2,5,5-tetramethyl-1,3-dioxane-4-carboxamido)propanamido)ethyl) (5*S*, 2*E*)-5-((*tert*-butyldimethylsilyl)oxy)-2-hexenethioate **35** using (5*R*, 2*E*)-5-((*tert*-butyldimethylsilyl)oxy)-2-hexenoic acid **34a** (180 mg, 0.74 mmol), to afford the product as a colourless oil (296 mg, 73 %). ν_{max}/cm^{-1} (neat) 3339 (NH), 2953 (C=C-H), 1660 (C=O); δ_H (500 MHz; $CDCl_3$) 7.03 (1H, br. t, J 5.5, N*H*), 6.94 (1H, dt, J 15.5 and 7.5, C*H*CHCOS), 6.14 (1H, br. s, N*H*CH_2CH_2S), 6.12 (1H, dt, J 15.5 and 1.5, CHC*H*COS), 4.07 (1H, s, C*H*CONH), 3.93 (1H, sextet, J 6.0, C*H*OSi), 3.68 (1H, d J 11.5, C*H*$_2$OC$(CH_3)_2$), 3.60-3.39 (4H, m, NHC*H*$_2$, C*H*$_2CH_2S$), 3.29 (1H, d J 11.5, C*H*$_2$OC$(CH_3)_2$), 3.08 (2H, t, J 6.5, C*H*$_2$S), 2.40 (2H, t, J 6.5, C*H*$_2$CONH), 2.32 (2H, td, J 6.5 and 0.5, C*H*$_2$CHOSi), 1.46 (3H, s, OC(C*H*$_3)_2$), 1.41 (3H, s, OC(C*H*$_3)_2$), 1.16 (3H, d, J 6.0, CHC*H*$_3$), 1.03 (3H, s, CH_2C(C*H*$_3)_2$), 0.97 (3H, s, CH_2C(C*H*$_3)_2$), 0.88 (9H, s, C(*C*H$_3)_3$), 0.04 (3H, s, SiC*H*$_3$), 0.04 (3H, s, SiC*H*$_3$); δ_C (125 MHz, $CDCl_3$) 190.2 (*C*O$_2$S), 171.6 (CH_2*C*ONH), 170.4 (CH*C*ONH), 144.0 (*C*HCHCOS), 130.5 (CH*C*HCOS), 99.5 (O*C*$(CH_3)_2$), 77.4 (*C*H), 71.9 (*C*H$_2$OC$(CH_3)_2$), 67.9 (*C*HOSi), 42.8 (*C*H$_2$CHOSi), 40.0 (*C*H$_2CH_2S$), 36.3 (*C*H$_2$CONH), 35.1 (*C*H$_2$NH), 33.6 (CH_2*C*$(CH_3)_2$), 29.9 (OC(*C*H$_3)_2$), 28.6 (*C*H$_2$S), 26.2 (C(*C*H$_3)_3$), 24.3 (CH*C*H$_3$), 22.5 (CH_2C(*C*H$_3)_2$), 19.2 (CH_2C(*C*H$_3)_2$), 19.1 (OC(*C*H$_3)_2$), 18.4 (*C*(CH$_3)_3$), -4.1 (Si*C*H$_3$), -4.3 (Si*C*H$_3$)**;** HRMS (ESI) $C_{26}H_{48}N_2NaO_6SSi$ $[M + Na]^+$ requires 567.2895, found 567.2896; $[\alpha]_D^{26}$ (c 0.2, $CHCl_3$): +17.5.

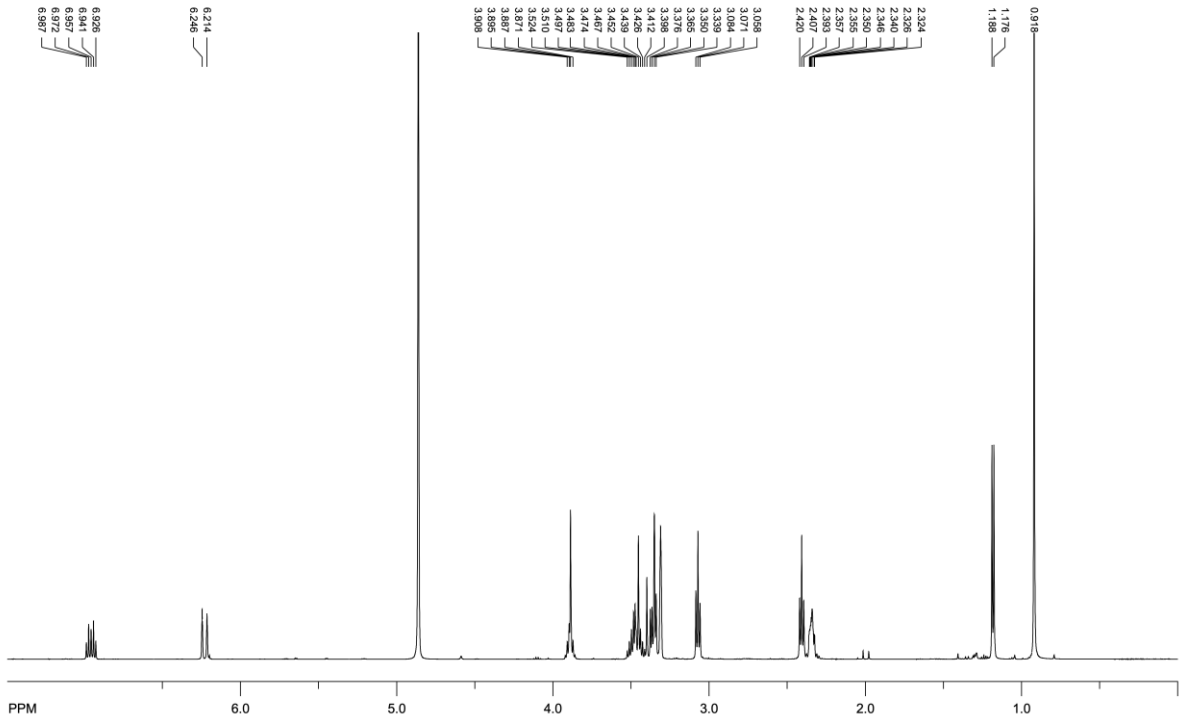
_S_-(2-(3-((_R_)-2,4-dihydroxy-3,3-dimethylbutanamido)propanamido)ethyl) (_R_,_E_)-5-hydroxyhex-2-enethioate (**16**)



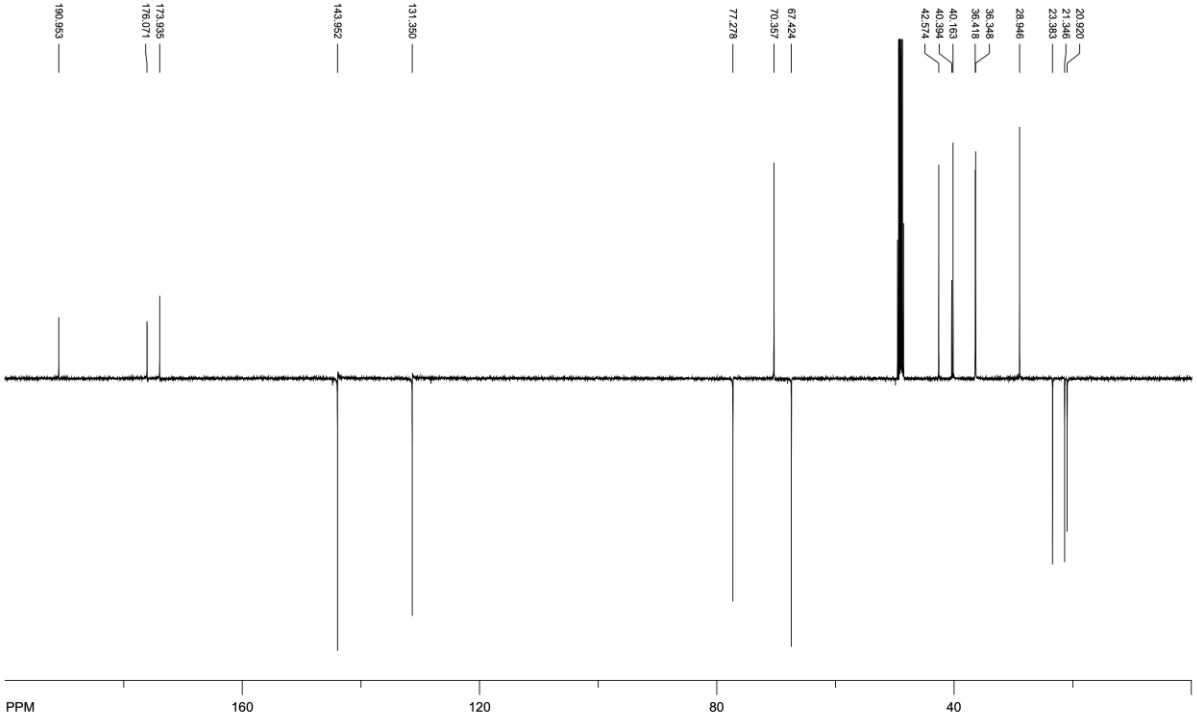
16

S-(2-(3-((*R*)-2,4-dihydroxy-3,3-dimethylbutanamido)propanamido)ethyl) (5*R*, 2*E*)-5-hydroxy-2-hexenethioate **16** was synthesized using the same procedure as that used for the synthesis of *S*-(2-(3-((*R*)-2,4-dihydroxy-3,3- dimethylbutanamido)propanamido) ethyl) (5*S*, 2*E*)-5-hydroxy-2-hexenethioate **11** using *S*-(2-(3-((*R*)-2,2,5,5-tetramethyl-1,3-dioxane-4-carboxamido)propanamido)ethyl) (5*R*, 2*E*)-5-((*tert*-butyldimethylsilyl)oxy)-2-hexenethioate **35a** (100 mg, 0.184 mmol), to afford the product as a colourless oil (60 mg, 84 %).

ν_{max}/cm^{-1} (neat) 3327 (OH), 2966 (C=C-H), 1655, 1561 (C=O); δ_H (500 MHz; CD_3OD) 6.96 (1H, dt, J 15.5 and 7.5, C*H*CHCOS), 6.23 (1H, dt, J 15.5 and 1.5, CHC*H*COS), 3.89 (1H, sextet, J 6.5, C*H*OH), 3.91 (1H, s, C*H*CONH), 3.53-3.33 (6H, m, CH_2OH, NHCH_2, CH_2CH$_2$S), 3.07 (2H, t, J 6.5, CH_2S), 2.41 (2H, t, J 6.5, CH_2CONH), 2.36-2.32 (2H, m, CH_2CHOH), 1.18 (3H, d, J 6.5, CHCH_3), 1.03 (3H, s, $CH_2C(CH_3)_2$), 0.92 (6H, s, $CH_2C(CH_3)_2$); δ_C (125 MHz, $CDCl_3$) 190.9 (CO_2S), 176.1 (CH*C*ONH), 173.9 (CH_2*C*ONH), 143.9 (*C*HCHCOS), 131.3 (CH*C*HCOS), 77.3 (*C*HONH), 70.3 ($CH_2OC(CH_3)_2$), 67.4 (*C*HOH), 42.6 (CH_2*C*HOH), 40.4 ($CH_2C(CH_3)_2$), 40.1 (CH_2CH_2S), 36.4 (CH_2NH), 36.3 (CH_2CONH), 28.9 (CH_2S), 23.4 ($CHCH_3$), 21.3 ($CH_2C(CH_3)_2$), 20.9 ($CH_2C(CH_3)_2$); HRMS (ESI) $C_{17}H_{30}N_2NaO_6S$ $[M + Na]^+$ requires 413.1717, found 413.1719; $[\alpha]_D^{26}$ (c 0.55, MeOH): +12.7.

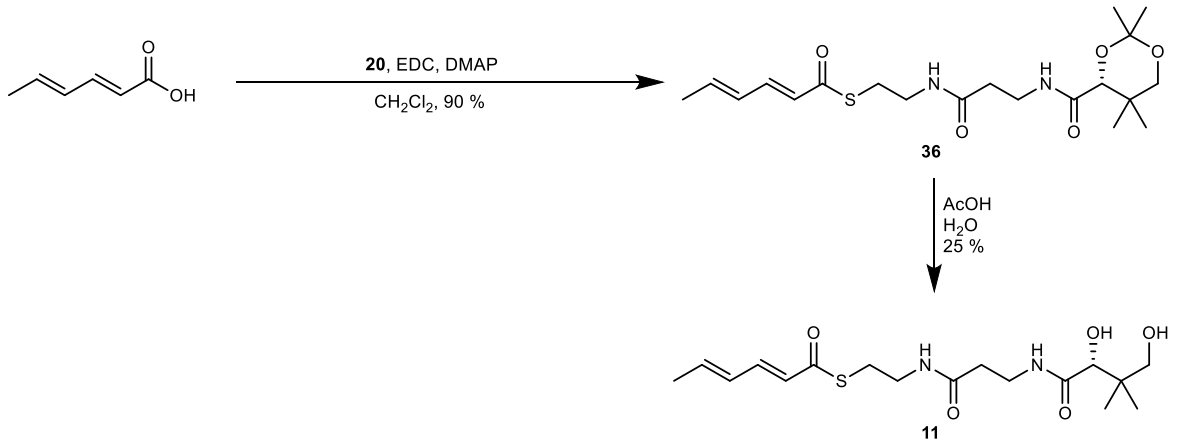


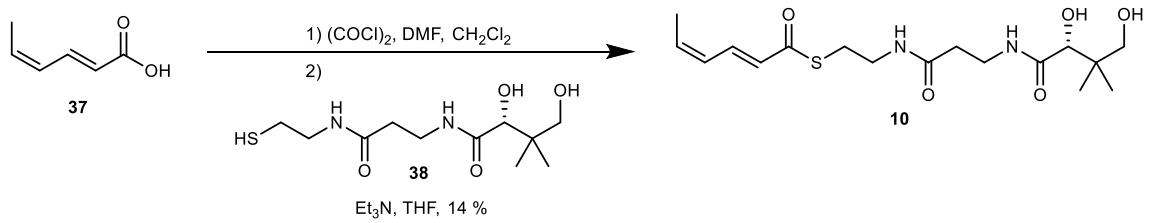
The ^{1}H NMR spectrum of *S*-(2-(3-((*R*)-2,4-dihydroxy-3,3-dimethylbutanamido) propanamido)ethyl) (*R*,*E)*-5-hydroxyhex-2-enethioate **16** (d^4-MeOD).



The ^{13}C NMR spectrum of *S*-(2-(3-((*R*)-2,4-dihydroxy-3,3-dimethylbutanamido) propanamido)ethyl) (*R*,*E)*-5-hydroxyhex-2-enethioate **16** (d^4-MeOD).

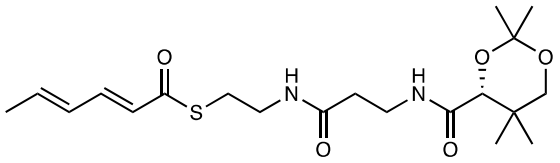
Synthesis of dienoyl pantetheine thioesters





Synthesis of dienoyl thioesters 11 and 10. Acid **37** and thiol **38** were synthesized according to literature procedures.[20,21]

***S*-(2-(3-((*R*)-2,2,5,5-tetramethyl-1,3-dioxane-4-carboxamido)propanamido)ethyl) (2*E*, 4*E*)-2, 4-hexadienethioate** (**36**)

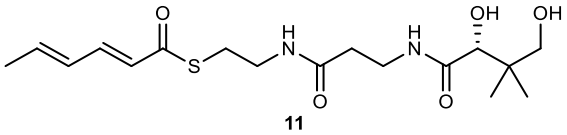


36

S-(2-(3-((*R*)-2,2,5,5-tetramethyl-1,3-dioxane-4-carboxamido)propanamido)ethyl) (2*E*, 4*E*)-2, 4-hexadienethioate **36** was synthesized using the same procedure as that used for the synthesis of *S*-(2-(3-((*R*)-2,2,5,5-tetramethyl-1,3-dioxane-4-carboxamido)propanamido)ethyl) (5*S*, 2*E*)-5-((*tert*-butyldimethylsilyl)oxy)-2-hexenethioate **35** using sorbic acid (61 mg, 0.54 mmol, 1.3 equiv.), to afford the product as a colourless oil (199 mg, 90 %).

ν_{max}/cm^{-1} (neat) 3328 (NH), 2940 (C=C-H), 1671, 1512 (C=O); δ_H (500 MHz; $CDCl_3$) 7.19 (1H, dd, J 15 and 10.5, C*H*CHCOS), 7.03 (1H, br. t, J 6.0, N*H*), 6.24 (1H, dq, J 15.0 and 7.0, CH_3CH), 6.23 (1H, br. m, N*H*CH_2CH_2S), 6.15 (1H, ddd, J 15.0, 11.0 and 1.0, CH_3CHCH), 6.06 (1H, d, J 15.0, C*H*COS), 4.06 (1H, s, C*H*CONH), 3.67 (1H, d J 11.5, $CH_2OC(CH_3)_2$), 3.60-3.40 (4H, m, NHCH_2, CH_2CH_2S), 3.26 (1H, d J 11.5, $CH_2OC(CH_3)_2$), 3.08 (2H, t, J 6.5, CH_2S), 2.41 (2H, t, J 6.5, CH_2CONH), 1.87 (3H, d, J 7.0, $CHCH_3$), 1.45 (3H, s, $OC(CH_3)_2$), 1.41 (3H, s, $OC(CH_3)_2$), 1.02 (3H, s, $CH_2C(CH_3)_2$), 0.96 (3H, s, $CH_2C(CH_3)_2$); δ_C (125 MHz, $CDCl_3$) 190.3 (CO_2S), 171.4 (CH_2CONH), 170.2 (CHCONH), 142.1 (CH_3CH), 142.0 (CHCHCOS), 129.7 (CH_3CHCH), 125.8 (CHCHCOS), 99.2 ($OC(CH_3)_2$), 77.3 (CH), 71.6 ($CH_2OC(CH_3)_2$), 40.0 (CH_2CH_2S), 36.1 (CH_2CONH), 34.9 (CH_2NH), 33.1 ($CH_2C(CH_3)_2$), 29.7 ($OC(CH_3)_2$), 28.5 (CH_2S), 22.3 ($CH_2C(CH_3)_2$), 19.1 ($OC(CH_3)_2$), 18.9 ($CH_2C(CH_3)_2$), 14.1 ($CHCH_3$)**;** HRMS (ESI) $C_{20}H_{32}N_2NaO_5S$ $[M + Na]^+$ requires 435.1924, found 435.1923; $[\alpha]_D^{28}$ (c 0.1, $CHCl_3$): +21.2.

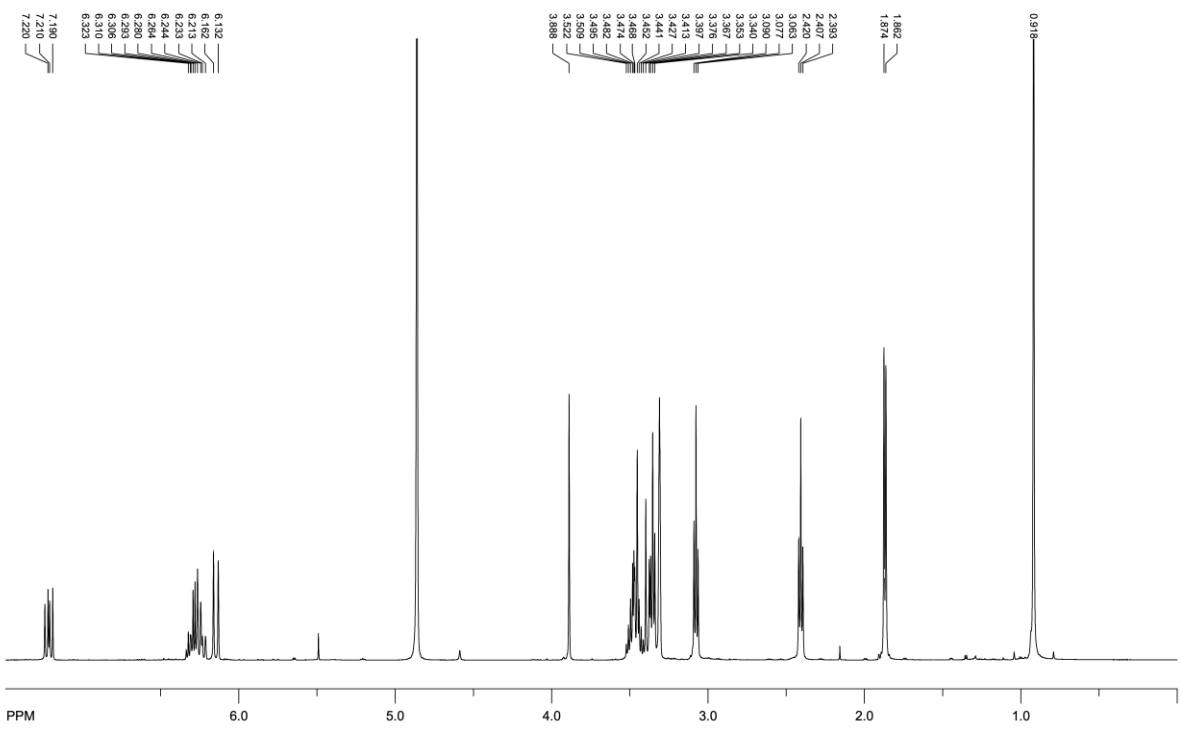
***S*-(2-(3-((*R*)-2,4-dihydroxy-3,3-dimethylbutanamido)propanamido)ethyl) (2*E*,4*E*)-hexa-2,4-dienethioate** (**11**)



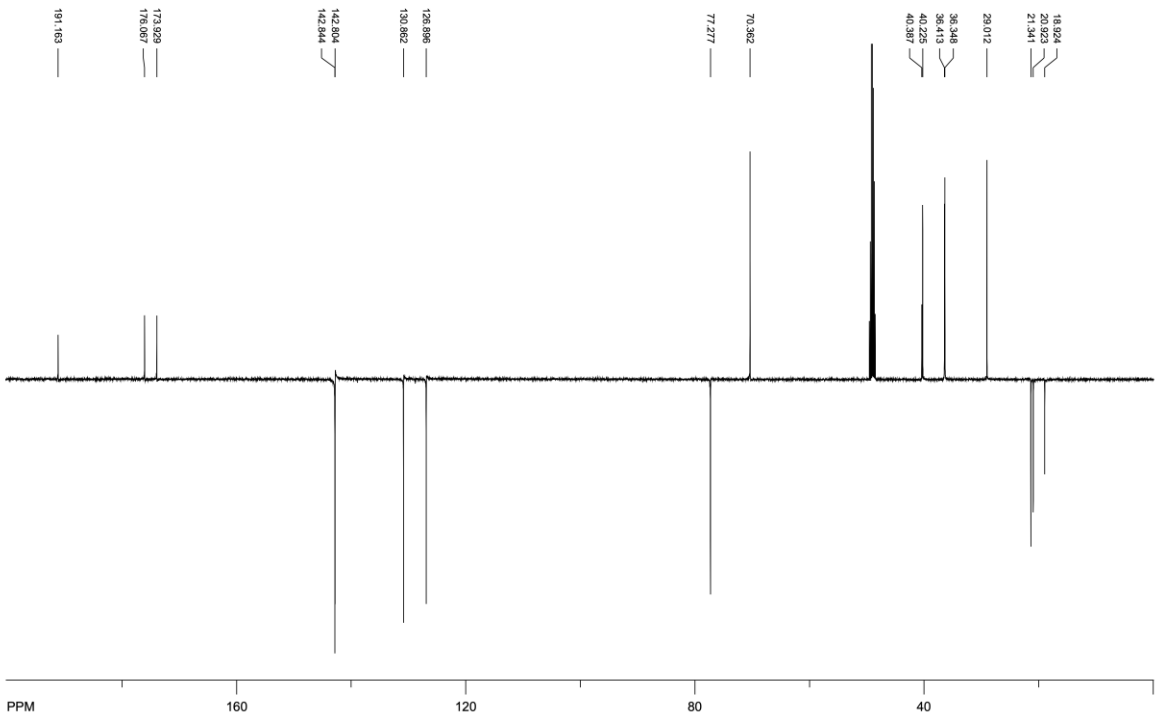
11

S-(2-(3-((*R*)-2,4-dihydroxy-3,3-dimethylbutanamido)propanamido)ethyl) (2*E*, 4*E*)-2, 4-hexadienethioate **11** was synthesized using the same procedure as that used for the synthesis of *S*-(2-(3-((*R*)-2,4-dihydroxy-3,3- dimethylbutanamido)propanamido) ethyl) (5*S*, 2*E*)-5-hydroxy-2-hexenethioate **8** using *S*-(2-(3-((*R*)-2,2,5,5-tetramethyl-1,3-dioxane-4-carboxamido)propanamido)ethyl) (2*E*, 4*E*)-2, 4-hexadienethioate **36** (180 mg, 0.45 mmol, 1.0 equiv.), to afford the product as a colourless oil (42 mg, 25 %).

ν_{max}/cm^{-1} (neat) 3340 (OH), 2969 (NH), 2940 (C=C-H), 1651, 1540 (C=O); δ_H (500 MHz; CD_3OD) 7.21 (1H, dd, J 15.0 and 10.0, C*H*CHCOS), 6.30 (1H, dq, J 15.0 and 6.5, CH_3CH), 6.24 (1H, dd, J 15.0 and 10.0, CH_3CHCH), 6.15 (1H, d, J 15.0, C*H*COS), 3.89 (1H, s, C*H*CONH), 3.53-3.33 (6H, m, $NHCH_2$, CH_2CH_2S, CH_2OH), 3.08 (2H, t, J 7.0, CH_2S), 2.41 (2H, t, J 6.5, CH_2CONH), 1.87 (3H, d, J 6.0, $CHCH_3$), 0.92 (6H, s, $C(CH_3)_2$); δ_C (125 MHz, CD_3OD) 191.2 (CO_2S), 176.1 (CH_2CONH), 173.9 (CHCONH), 142.8 (CH_3CH), 142.8 (CHCHCOS), 130.8 (CH_3CHCH), 126.9 (CHCHCOS), 77.3 (CH), 70.3 (CH_2OH), 40.4 ($CH_2C(CH_3)_2$), 40.2 (CH_2CH_2S), 36.4 (CH_2CONH), 36.3 (CH_2NH), 29.0 (CH_2S), 21.3 ($CH_2C(CH_3)_2$), 20.9 ($CH_2C(CH_3)_2$), 14.2 ($CHCH_3$)**;** HRMS (ESI) $C_{17}H_{28}N_2NaO_5S$ $[M + Na]^+$ requires 395.1611, found 395.1605; $[\alpha]_D^{28}$ (c 0.5, MeOH): +40.4.

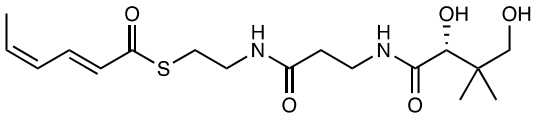


The ^{1}H NMR spectrum of *S*-(2-(3-((*R*)-2,4-dihydroxy-3,3-dimethylbutanamido)propanamido)ethyl) (2*E*,4*E*)-hexa-2,4-dienethioate **11** (d^{4}-MeOD).



The ^{13}C NMR spectrum of *S*-(2-(3-((*R*)-2,4-dihydroxy-3,3-dimethylbutanamido)propanamido)ethyl) (2*E*,4*E*)-hexa-2,4-dienethioate **11** (d^{4}-MeOD).

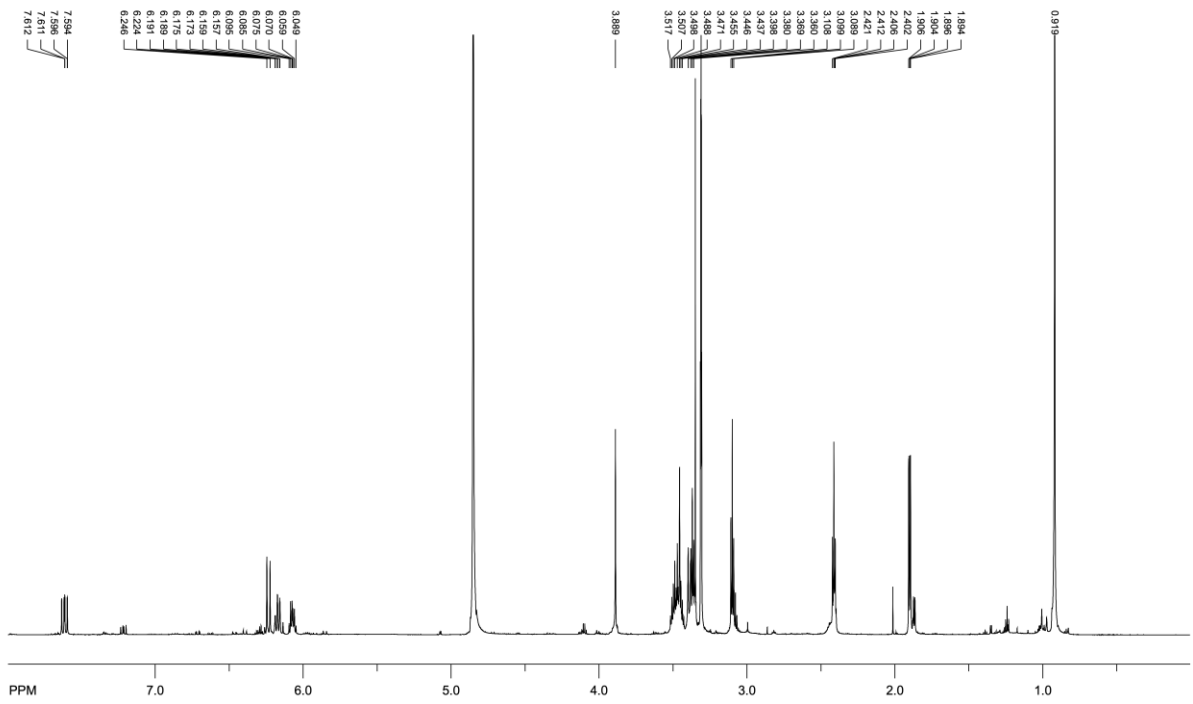
***S*-(2-(3-((*R*)-2,4-dihydroxy-3,3-dimethylbutanamido)propanamido)ethyl) (2*E*,4*Z*)-hexa-2,4-dienethioate** (**10**)



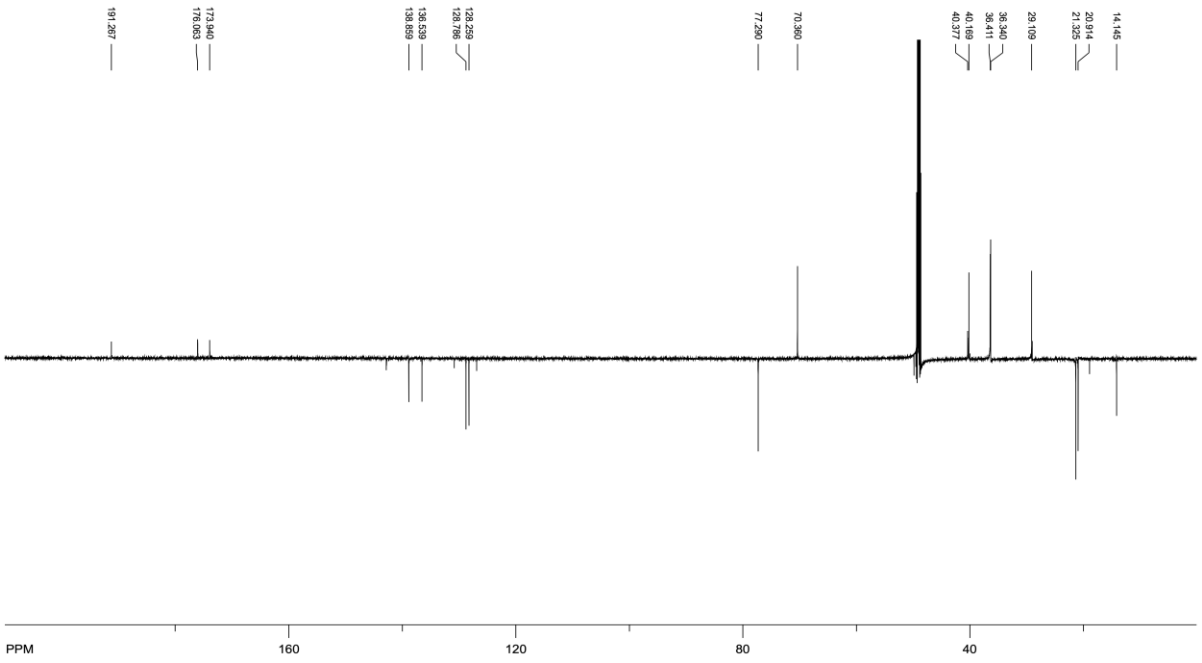
10

To a stirred solution of (2*E*, 4*Z*)-2, 4-hexadienoic acid **37** (50 mg, 0.45 mmol, 1 equiv., 94:6, *E/Z*:*E/E*) in DCM (1 mL) was added oxalyl chloride (0.04 mL, 0.45 mmol, 1 equiv.) drop-wise, followed by 2 drops of DMF. The reaction was stirred for 2 h after which time the reaction was added drop-wise to a pre-stirred mixture of (*R*)-2,4-dihydroxy-*N*-(3-((2-mercaptoethyl)amino)-3-oxopropyl)-3,3-dimethylbutanamide **38** (125 mg, 0.45 mmol, 1 equiv.) and triethylamine (0.06 mL, 0.45 mmol, 1 equiv.) in dry THF (1 mL). The reaction was then stirred at room temperature for 1 h under an inert atmosphere before being concentrated *in vacuo*. The resulting orange oil was purified by silica chromatography (MeOH/EtOAc, 1:10) to afford the product as a yellow oil (23 mg, 14 %, 80:20, *E,Z*:*E,E*).

ν_{max}/cm^{-1} (neat) 3355 (OH), 2976 (NH), 2921 (C=C-H), 1636, 1543 (C=O); δ_H (500 MHz; CD_3OD) 7.61 (1H, ddd, J 15.0, 11.5 and 1.0, C*H*CHCOS), 6.24 (1H, d, J 15.0, C*H*COS), 6.19-6.15 (1H, m, CH_3CHCH), 6.07 (1H, dqt, J 11.0, 7.0 and 1.0, CH_3CH), 3.89 (1H, s, C*H*CONH), 3.52-3.35 (6H, m, $NHCH_2$, CH_2CH_2S, CH_2OH), 3.10 (2H, t, J 7.0, CH_2S), 2.41 (2H, t, J 6.5, CH_2CONH), 1.90 (3H, dd, J 7.0 and 1.5, $CHCH_3$), 0.92 (6H, s, $C(CH_3)_2$); δ_C (125 MHz, CD_3OD) 191.3 (CO_2S), 176.1 (CH_2CONH), 173.9 (CHCONH), 138.9 (CHCHCOS), 136.5 (CH_3CH), 128.8 (CH_3CHCH), 128.3 (CHCOS), 77.3 (CH), 70.4 (CH_2OH), 40.4 ($CH_2C(CH_3)_2$), 40.2 (CH_2CH_2S), 36.4 (CH_2CONH), 36.3 (CH_2NH), 29.1 (CH_2S), 21.3 ($CH_2C(CH_3)_2$), 20.9 ($CH_2C(CH_3)_2$), 14.2 ($CHCH_3$)**;** HRMS (ESI) $C_{17}H_{28}N_2NaO_5S$ $[M + Na]^+$ requires 395.1611, found 395.1613; $[\alpha]_D^{28}$ (c 0.3, MeOH): +28.9.



The ^{1}H NMR spectrum of *S*-(2-(3-((*R*)-2,4-dihydroxy-3,3-dimethylbutanamido)propanamido)ethyl) (2*E*,4*Z*)-hexa-2,4-dienethioate **10** (d^{4}-MeOD).

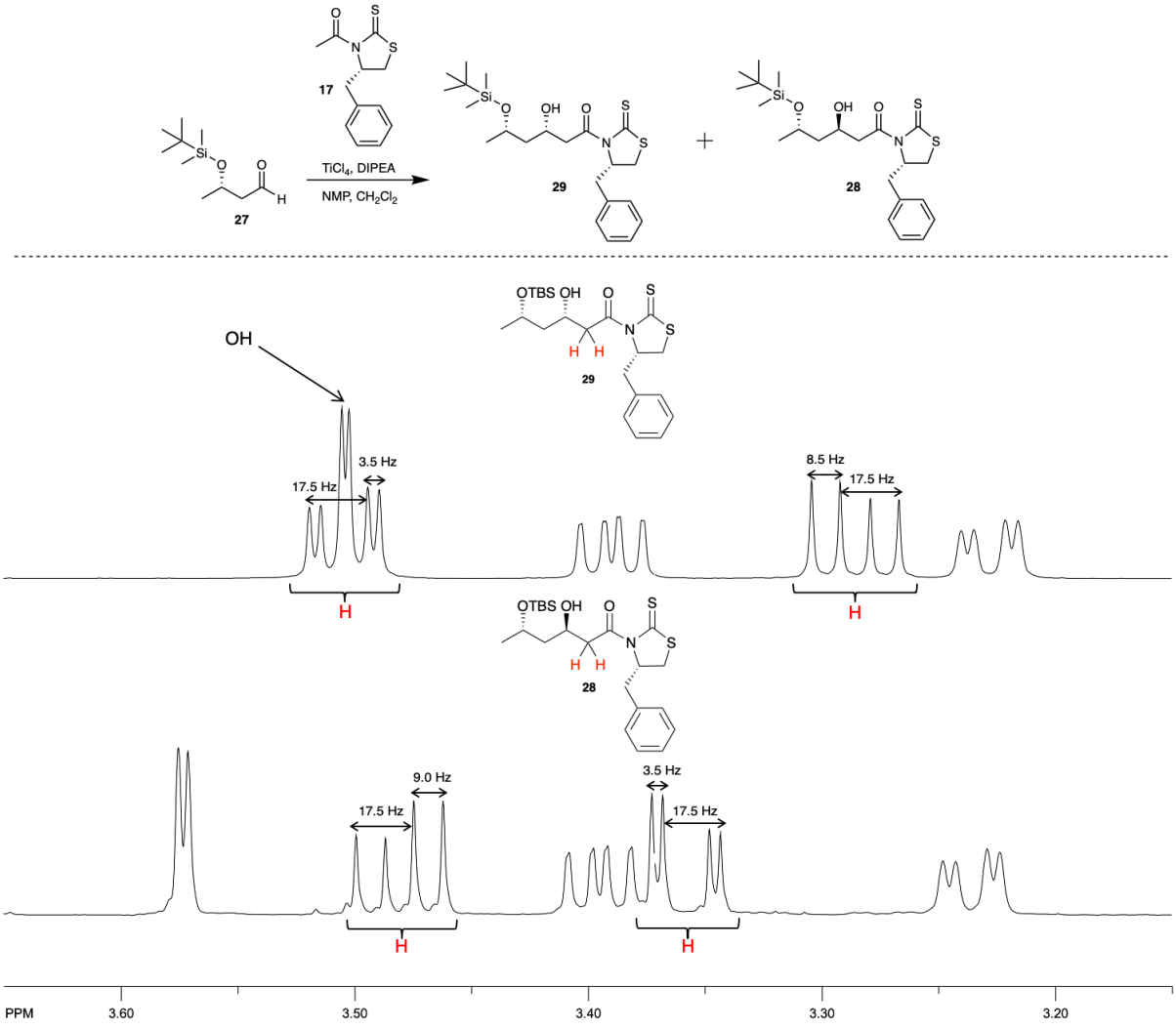


The ^{13}C NMR spectrum of *S*-(2-(3-((*R*)-2,4-dihydroxy-3,3-dimethylbutanamido)propanamido)ethyl) (2*E*,4*Z*)-hexa-2,4-dienethioate **10** (d^{4}-MeOD).

Confirmation of the stereochemistry of aldol products

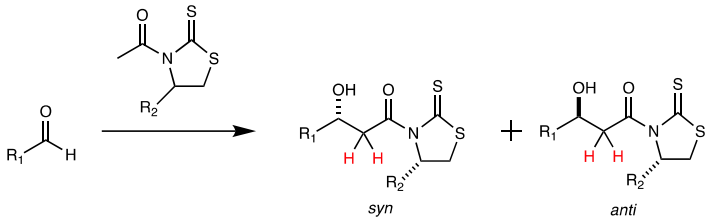
Relative stereochemistry of aldol products

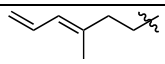
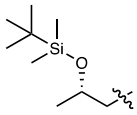
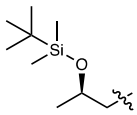
A study by Hodge and coworkers showed that the relative stereochemistry of aldol products from acetate aldol reactions can be assigned based on diagnostic coupling constants of the C-2 protons. They observed that the downfield C-2 proton consistently has a smaller vicinal (^3J) coupling constant (around 3.0 Hz) than the upfield C-2 proton (around 9.0 Hz) in the *syn*-aldol product, and this is reversed in the *anti*-aldol product; the downfield C-2 proton has a larger vicinal coupling constant (around 9.0 Hz) than the upfield C-2 proton (around 3.0 Hz). This trend was consistent for aldol products with a variety of side chains and was validated by crystallography.[16] The trend is also consistent with acetate aldol products from other studies (See table below)[8,22–24] and is in agreement with the predicted relative stereochemistry of aldol products from this study.



^{1}H NMR spectra of aldol products 29 and 28. The coupling constants of the C-2 protons (red) are in agreement with those reported in the literature for *syn*- and *anti*-aldol products, respectively. Note that the geminal coupling constant (^2J) for the C-2 protons is 17.5 Hz.

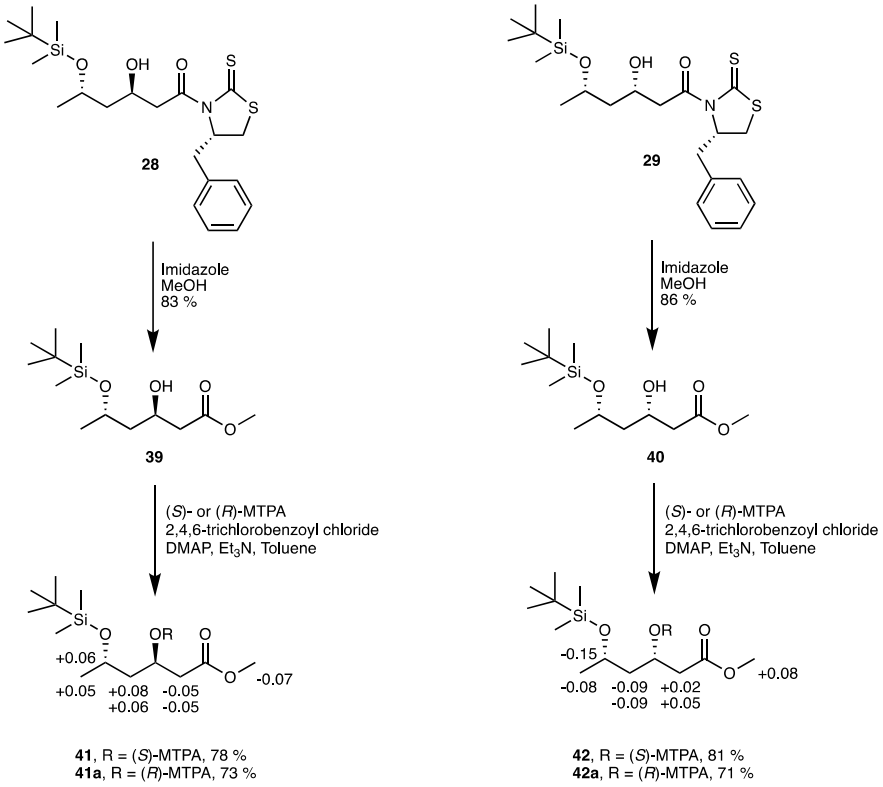
Coupling constant data for various *syn*- and *anti*-acetate aldol products. ^3J coupling constants observed for the C-2 protons (red).



		***Syn*-product**		***Anti*-product**		
R_1	**R_2**	**Downfield H *J* value (Hz)**	**Upfield H *J* value (Hz)**	**Downfield H *J* value (Hz)**	**Upfield H *J* value (Hz)**	**Ref.**
-CH=CH-C_6H_5	*i*-Pr	3.1	8.7	8.8	3.5	Hodge[16]
-CH=C(CH_3)$_2$	*i*-Pr	3.0	8.9	8.8	3.4	
-CH=CH-CH=CHBr	*i*-Pr	3.1	8.6	8.9	3.3	
-C_6H_5	*i*-Pr	2.7	9.3	9.5	3.3	
-C_6H_4Br	*i*-Pr	2.7	9.4	9.5	3.1	
-C(CH_3)$_3$	*i*-Pr	2.0	10.5	10.5	2.0	
-CH(C_6H_5)$_2$	*i*-Pr	2.6	9.2	9.4	2.8	
-CH_2CH_3	*i*-Pr	2.3	9.5	9.1	3.0	
-$CH_2CH_2C_6H_5$	*i*-Pr	2.5	9.2	9.4	2.7	
-CH=CH_2	*i*-Pr	3.0	8.8	8.9	3.4	
-$CH_2CH_2C_6H_5$	Bn	2.3	9.4	9.1	2.4	
-$CH_2(CH_2)_3CH(CH_3)_2$	Bn	2.2	9.5	9.3	2.6	Cochrane[22]
-$CH_2(CH_2)_6$OTBS	Bn	2.2	5.5	9.4	2.5	Yadav[23]
	Bn	2.0	9.4	9.3	2.5	Das[24]
	*i*-Pr	3.6	8.4	9.2	3.2	Prasad[25]
-$CH_2CH_2CH_3$	Bn	2.4	9.5	9.4	2.6	Pompeo[8]
-$CH_2CH_2CH_3$	Bn	2.5	9.5	9.5	2.5	This Study (**19** and **18**)
	Bn	3.5	8.5	9.0	3.5	This Study (**29** and **28**)
	Bn	3.5	8.5	9.0	3.5	This Study (**29a** and **28a**)

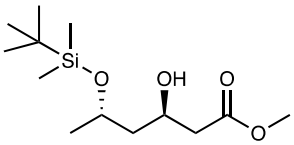
Absolute stereochemistry of aldol products

To confirm the absolute stereochemistry of aldol products **28** and **29**, they were first converted to methyl esters **39** and **40**, respectively, before being subjected to Mosher's ester analysis.[17] The C-3 hydroxyl group of each ester was acylated with (*S*)- and (*R*)-Mosher's acid to afford two pairs of Mosher's ester derivatives: **41** and **41a** (from **39**), and **42** and **42a** (from **40**). The $\Delta\delta^{SR}$ values ($\Delta\delta^{SR} = \delta_S - \delta_R$) for protons either side of the chiral C-3 carbon were calculated and used to assign the absolute stereochemistry of the C-3 hydroxyl group in both **39** and **40**. The negative $\Delta\delta^{SR}$ values of the C-2 protons, and positive $\Delta\delta^{SR}$ values of the C-4 protons of alcohol **39** clearly confirm the expected (*R*)-configuration of the C-3 hydroxyl group. Correspondingly, the positive $\Delta\delta^{SR}$ values of the C-2 protons, and negative $\Delta\delta^{SR}$ values of the C-4 protons of alcohol **40** confirm a (3*S*)-configured alcohol.[17,25]



Mosher's ester analysis of 28 and 29. The $\Delta\delta^{SR}$ values ($\Delta\delta^{SR} = \delta_S - \delta_R$) for each diastereomer confirm that the C-3 hydroxyl group is (*R*)-configured in aldol product **28**, and (*S*)-configured in **29**.

Methyl (3*R*,5*S*)-5-((*tert*-butyldimethylsilyl)oxy)-3-hydroxyhexanoate (**39**)

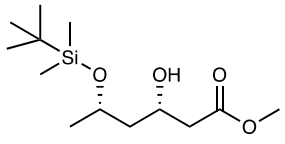


39

To a solution of (3*R*,5*S*)-1-((*S*)-4-benzyl-2-thioxothiazolidin-3-yl)-5-((*tert*-butyldimethylsilyl)oxy)-3-hydroxyhexan-1-one **28** (50 mg, 0.11 mmol, 1.0 equiv.) in anhydrous methanol (3.0 mL) at 0 °C was added imidazole (37.4 mg, 0.55 mmol, 5.0 equiv.) and the mixture was stirred for 16 h at RT. The reaction mixture was then quenched with a saturated solution of NH_4Cl (1 mL). Methanol was removed *in vacuo* and the mixture extracted with EtOAc, washed with water, brine, dried ($MgSO_4$), and concentrated *in vacuo*. The resulting oil was purified by silica chromatography (EtOAc : Hexane, 1 : 5) to afford the product as a colourless oil (25 mg, 83%).

δ_H (400 MHz; $CDCl_3$) 4.35 (1H, dddt, J 10.0, 7.0, 4.5 and 2.0, C*H*OH), 4.09 (1H, dquin., J 6.5 and 3.5, C*H*CH_3), 3.70 (3H, s, OC*H*$_3$), 3.62 (1H, br. d, J 2.0, O*H*), 2.49 (1H, dd, J 16.0 and 8.0, C*H*$_2$$CO_2CH_3$), 2.43 (1H, dd, J 16.0 and 4.5, C*H*$_2$$CO_2CH_3$), 1.65 (1H, ddd, J 14.0, 10.0 and 3.5, C*H*$_2$CHOSi), 1.51 (1H, ddd, J 14.0, 6.5 and 2.5, C*H*$_2$CHOSi), 1.22 (3H, d, J 6.5. CHC*H*$_3$), 0.89 (9H, s, SiC(C*H*$_3$)$_3$), 0.09 (3H, s, SiC*H*$_3$), 0.08 (3H, s, SiC*H*$_3$); δ_C (100 MHz; $CDCl_3$) 172.9 (*C*O_2CH_3), 66.7 (*C*HOSi), 65.0 (*C*HOH), 51.7 (CO_2*C*H_3), 44.3 (*C*$H_2$$CO_2CH_3$), 41.9 (*C*$H_2$CHOSi), 25.8 (SiC(*C*$H_3$)$_3$), 23.2 (CH*C*$H_3$), 18.0 (Si*C*$(CH_3)_3$), -4.5 ($SiCH_3$), -5.0 ($SiCH_3$); HRMS (ESI) $C_{13}H_{28}NaO_4Si$ $[M + Na]^+$ requires 299.1655, found 299.1656.

Methyl (3*S*,5*S*)-5-((*tert*-butyldimethylsilyl)oxy)-3-hydroxyhexanoate (**40**)



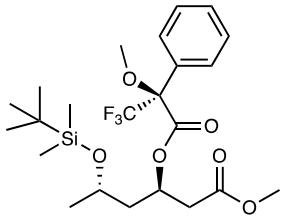
40

Methyl (3*S*,5*S*)-5-((*tert*-butyldimethylsilyl)oxy)-3-hydroxyhexanoate **40** was synthesized according to the same procedure as that used for the synthesis of methyl (3*R*,5*S*)-5-((*tert*-butyldimethylsilyl)oxy)-3-hydroxyhexanoate **39**, using (3*S*,5*S*)-1-((*S*)-4-benzyl-2-thioxothiazolidin-3-yl)-5-((*tert*-butyldimethylsilyl)oxy)-3-hydroxyhexan-1-one **29** (50 mg, 0.11 mmol, 1.0 equiv.) to afford the product as a colourless oil (26 mg, 86 %).

δ_H (400 MHz; $CDCl_3$) 4.18 (1H, ddddd, J 9.0, 7.5, 5.5, 3.5 and 2.0, C*H*OH), 4.09 (1H, dqd, J 8.5, 6.0 and 5.0, C*H*CH_3), 3.70 (3H, s, OC*H*$_3$), 3.36 (1H, br. d, J 2.0, O*H*), 2.51 (1H, dd, J 16.0 and 7.5,

C*H*$_2$CO$_2$CH$_3$), 2.45 (1H, dd, J 16.0 and 5.5, C*H*$_2$CO$_2$CH$_3$), 1.66 (1H, dt, J 14.0 and 8.5, C*H*$_2$CHOSi), 1.59 (1H, ddd, J 14.0, 4.5 and 3.5, C*H*$_2$CHOSi), 1.18 (3H, d, J 6.0. CHC*H*$_3$), 0.89 (9H, s, SiC(C*H*$_3$)$_3$), 0.10 (3H, s, SiC*H*$_3$), 0.09 (3H, s, SiC*H*$_3$); δ_C (100 MHz; $CDCl_3$) 172.6 (*C*O$_2$CH$_3$), 68.8 (*C*HOSi), 67.5 (*C*HOH), 51.7 (CO$_2$*C*H$_3$), 45.3 (*C*H$_2$CO$_2$CH$_3$), 41.7 (*C*H$_2$CHOSi), 25.8 (SiC(*C*H$_3$)$_3$), 24.2 (CH*C*H$_3$), 17.9 (Si*C*(CH$_3$)$_3$), -4.0 (SiCH$_3$), -4.8 (SiCH$_3$); HRMS (ESI) $C_{13}H_{28}NaO_4Si$ $[M + Na]^+$ requires 299.1655, found 299.1658.

(3*R*,5*S*)-5-((*tert*-butyldimethylsilyl)oxy)-3-(((*S*)-3,3,3-trifluoro-2-methoxy-2-phenylpropanoyl)oxy)hexanoate (41)



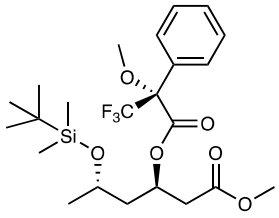
41

Procedure modified from Das *et al*.[24] To a solution of (*S*)-Mosher's acid (28 mg, 0.12 mmol, 3.0 equiv.) in anhydrous toluene (1 mL) was added DMAP (17 mg, 0.14 mmol, 3.5 equiv.), Et$_3$N (0.02 mL, 0.14 mmol, 3.5 equiv.) and 2,4,6-trichlorobenzoyl chloride (0.02 mL, 0.12 mmol, 3.0 equiv.) at RT. The white turbid mixture was stirred for 30 min, and a solution of methyl (3*R*,5*S*)-5-((*tert*-butyldimethylsilyl)oxy)-3-hydroxyhexanoate **39** (10 mg, 0.04 mmol, 1.0 equiv.) in dry toluene (0.5 mL) was then cannulated. After being stirred for 4 h at room temperature, the reaction mixture was quenched with saturated aqueous NH_4Cl (0.5 mL) and extracted with EtOAc. The combined organic extracts were washed with water and brine, dried (Na_2SO_4), and concentrated *in vacuo*. Purification by silica chromatography (EtOAc : hexane, 1 : 20) afforded ester **41** as a colourless oil (15 mg, 78 %).

δ_H (400 MHz; $CDCl_3$) 7.56-7.51 (2H, m, Ar*H*), 7.42-7.37 (3H, m, Ar*H*), 5.51 (1H, tt, J 7.0 and 5.0, C*H*OCO), 3.91 (1H, dqd, J 9.5, 6.0 and 3.5, C*H*OSi), 3.58 (3H, s, CO$_2$C*H*$_3$), 3.53 (3H, br. q, J 1.0, OC*H*$_3$), 2.72 (1H, dd, J 16.0 and 5.5, C*H*$_2$CO$_2$CH$_3$), 2.66 (1H, dd, J 16.0 and 7.0, C*H*$_2$CO$_2$CH$_3$), 1.85 (1H, ddd, J 14.0, 7.5 and 3.5, C*H*$_2$CHOSi), 1.73 (1H, ddd, J 14.0, 9.0 and 5.0, C*H*$_2$CHOSi), 1.15 (3H, d, J 6.0, CHC*H*$_3$), 0.88 (9H, s, SiC(C*H*$_3$)$_3$), 0.05 (3H, s, SiC*H*$_3$), 0.05 (3H, s, SiC*H*$_3$); δ_C (100 MHz; $CDCl_3$) 170.1 (*C*O$_2$CH$_3$), 165.8 (*C*O$_2$CH), 132.0 (Ar*C*$_{quat}$), 129.6 (Ar*C*), 128.4 (Ar*C*), 127.5 (Ar*C*), 124.7 (*C*F$_3$), 121.8 (*C*OCH$_3$), 71.9 (*C*HOCO), 65.5 (*C*HOSi), 55.4 (CO*C*H$_3$), 51.7 (CO$_2$*C*H$_3$), 44.0 (*C*H$_2$CO$_2$CH$_3$), 39.3

(*C*H_2CHOSi), 25.8 (SiC(*C*H_3)$_3$), 24.4 (CH*C*H_3), 17.9 (Si*C*(CH$_3$)$_3$), -4.0 (SiCH_3), -4.9 (SiCH_3); HRMS (ESI) $C_{23}H_{35}F_3NaO_6Si$ [M + Na]$^+$ requires 492.2155, found 492.2156.

(3*R*,5*S*)-5-((*tert*-butyldimethylsilyl)oxy)-3-(((*R*)-3,3,3-trifluoro-2-methoxy-2-phenylpropanoyl)oxy)hexanoate (41a)

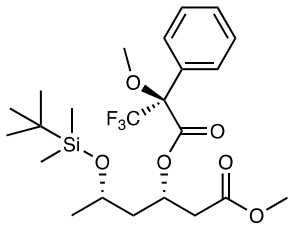


41a

(*R*), (*R,S*)-Ester **41a** was synthesized using the same procedure as that used for (*S*), (*S,R*)-Ester **41**, using (*R*)-Mosher's acid (28 mg, 0.12 mmol, 3.0 equiv.) to afford the product as a colourless oil (14 mg, 73 %).

δ_H (400 MHz; $CDCl_3$) 7.57-7.51 (2H, m, Ar*H*), 7.42-7.37 (3H, m, Ar*H*), 5.50 (1H, tt, J 7.0 and 5.0, C*H*OCO), 3.85 (1H, dqd, J 9.5, 6.0 and 3.5, C*H*OSi), 3.65 (3H, s, CO_2CH_3), 3.53 (3H, br. q, J 1.0, OCH_3), 2.77 (1H, dd, J 16.0 and 5.5, C$H_2CO_2CH_3$), 2.71 (1H, dd, J 16.0 and 7.0, C$H_2CO_2CH_3$), 1.77 (1H, ddd, J 14.0, 7.0 and 3.5, CH_2CHOSi), 1.67 (1H, ddd, J 14.0, 9.0 and 5.0, CH_2CHOSi), 1.10 (3H, d, J 6.0, CHCH_3), 0.88 (9H, s, SiC(CH_3)$_3$), 0.05 (3H, s, SiCH_3), 0.05 (3H, s, SiCH_3); δ_C (100 MHz; $CDCl_3$) 170.3 (CO_2CH_3), 165.9 (CO_2CH), 132.1 (ArC_{quat}), 129.6 (Ar*C*), 128.4 (Ar*C*), 127.4 (Ar*C*), 124.7 (CF_3), 121.9 ($COCH_3$), 72.0 (*C*HOCO), 65.5 (*C*HOSi), 55.3 (CO*C*H_3), 51.8 (CO_2CH_3), 43.9 ($CH_2CO_2CH_3$), 39.5 (*C*H_2CHOSi), 25.8 (SiC(*C*H_3)$_3$), 24.4 (CH*C*H_3), 17.9 (Si*C*(CH$_3$)$_3$), -4.0 (SiCH_3), -4.9 (SiCH_3); HRMS (ESI) $C_{23}H_{35}F_3NaO_6Si$ [M + Na]$^+$ requires 492.2155, found 492.2155.

(3*S*,5*S*)-5-((*tert*-butyldimethylsilyl)oxy)-3-(((*S*)-3,3,3-trifluoro-2-methoxy-2-phenylpropanoyl)oxy)hexanoate (42)

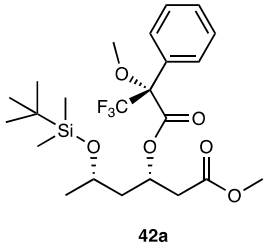


42

(*S*), (*S,S*)-Ester **42** was synthesized using the same procedure as that used for (*S*), (*S,R*)-Ester **41**, using methyl (3*S*,5*S*)-5-((*tert*-butyldimethylsilyl)oxy)-3-hydroxyhexanoate **40** (10 mg, 0.04 mmol, 1.0 equiv.) to afford the product as a colourless oil (16 mg, 81 %).

δ_H (400 MHz; $CDCl_3$) 7.56-7.50 (2H, m, Ar*H*), 7.42-7.37 (3H, m, Ar*H*), 5.57 (1H, dtd, J 8.0, 6.5 and 4.5, C*H*OCO), 3.75 (1H, sext., J 6.0, C*H*OSi), 3.66 (3H, s, CO_2CH_3), 3.54 (3H, br. q, J 1.0, OCH_3), 2.77 (1H, dd, J 16.0 and 4.5, $CH_2CO_2CH_3$), 2.70 (1H, dd, J 16.0 and 8.0, $CH_2CO_2CH_3$), 1.88 (1H, dt, J 14.0, 6.5, CH_2CHOSi), 1.67 (1H, ddd, J 14.0, 6.5 and 1.0, CH_2CHOSi), 1.13 (3H, d, J 6.0, $CHCH_3$), 0.85 (9H, s, $SiC(CH_3)_3$), -0.01 (3H, s, $SiCH_3$), -0.03 (3H, s, $SiCH_3$); δ_C (100 MHz; $CDCl_3$) 170.5 (CO_2CH_3), 165.7 (CO_2CH), 132.2 (ArC_{quat}), 129.6 (Ar*C*), 128.4 (Ar*C*), 127.3 (Ar*C*), 124.7 (CF_3), 121.9 ($COCH_3$), 71.3 (*C*HOCO), 65.0 (*C*HOSi), 55.4 ($COCH_3$), 51.9 (CO_2CH_3), 42.8 ($CH_2CO_2CH_3$), 39.9 (CH_2CHOSi), 25.8 ($SiC(CH_3)_3$), 23.2 ($CHCH_3$), 17.9 ($SiC(CH_3)_3$), -4.4 ($SiCH_3$), -5.0 ($SiCH_3$); HRMS (ESI) $C_{23}H_{35}F_3NaO_6Si$ $[M + Na]^+$ requires 492.2155, found 492.2157.

(3*S*,5*S*)-5-((*tert*-butyldimethylsilyl)oxy)-3-(((*R*)-3,3,3-trifluoro-2-methoxy-2-phenylpropanoyl)oxy)hexanoate (42a)



42a

(*R*), (*S,S*)-Ester **42a** was synthesized using the same procedure as that used for (*S*), (*S,R*)-Ester **41**, using (*R*)-Mosher's acid (28 mg, 0.12 mmol, 3.0 equiv.) and methyl (3*S*,5*S*)-5-((*tert*-butyldimethylsilyl)oxy)-3-hydroxyhexanoate **40** (10 mg, 0.04 mmol, 1.0 equiv.) to afford the product as a colourless oil (14 mg, 71 %).

δ_H (400 MHz; $CDCl_3$) 7.55-7.49 (2H, m, Ar*H*), 7.43-7.37 (3H, m, Ar*H*), 5.59 (1H, dtd, J 8.0, 7.0 and 4.5, C*H*OCO), 3.90 (1H, sext., J 6.0, C*H*OSi), 3.58 (3H, s, CO_2CH_3), 3.50 (3H, br. q, J 1.0, OCH_3), 2.75

(1H, dd, J 16.0 and 4.5, C*H*$_2$CO$_2$CH$_3$), 2.64 (1H, dd, J 16.0 and 8.0, C*H*$_2$CO$_2$CH$_3$), 1.97 (1H, dt, J 14.0, 6.5, C*H*$_2$CHOSi), 1.76 (1H, ddd, J 14.0, 6.5 and 1.0, C*H*$_2$CHOSi), 1.21 (3H, d, J 6.0, CHC*H*$_3$), 0.88 (9H, s, SiC(C*H*$_3$)$_3$), 0.04 (3H, s, SiC*H*$_3$), 0.03 (3H, s, SiC*H*$_3$); δ_C (100 MHz; $CDCl_3$) 170.2 (*C*O$_2$CH$_3$), 165.7 (*C*O$_2$CH), 132.0 (Ar*C*$_{quat}$), 129.6 (Ar*C*), 128.4 (Ar*C*), 127.5 (Ar*C*), 124.7 (*C*F$_3$), 121.9 (*C*OCH$_3$), 71.4 (*C*HOCO), 65.2 (*C*HOSi), 55.3 (CO*C*H$_3$), 51.8 (CO$_2$*C*H$_3$), 43.0 (*C*H$_2$CO$_2$CH$_3$), 39.7 (*C*H$_2$CHOSi), 25.8 (SiC(*C*H$_3$)$_3$), 23.4 (CH*C*H$_3$), 18.0 (Si*C*(CH$_3$)$_3$), -4.4 (SiCH$_3$), -5.0 (SiCH$_3$); HRMS (ESI) $C_{23}H_{35}F_3NaO_6Si$ $[M + Na]^+$ requires 492.2155, found 492.2155.

References

1. Kautsar, S. A. *et al.* MIBiG 2.0: a repository for biosynthetic gene clusters of known function. *Nucleic Acids Res.* **48,** (2020).
2. M. Jenner, *et al*. Mechanism of intersubunit ketosynthase–dehydratase interaction in polyketide synthases. *Nat. Chem. Biol.* **14**, 270–275 (2018).
3. Keatinge-Clay, A. Stereocontrol within polyketide assembly lines. *Nat. Prod. Rep.* 33, 141-149 (2016).
4. Wilson, K.E. *et al.* Difficidin and oxydifficidin: novel broad spectrum antibacterial antibiotics produced by Bacillus subtilis. II. Isolation and physico-chemical characterization. *J. Antibiot.* **40,** 1682–1691 (1987).
5. H . L. Robertsen *et al.* Filling the Gaps in the Kirromycin Biosynthesis: Deciphering the Role of Genes Involved in Ethylmalonyl-CoA Supply and Tailoring Reactions. *Sci. Rep.* **8**, 3230 (2018).
6. Agarwal, V. *et al.* Chemoenzymatic Synthesis of Acyl Coenzyme A Substrates Enables *in Situ* Labeling of Small Molecules and Proteins. *Org. Lett.* **17,** 4452–4455 (2015).
7. Skaanderup, P. R. & Jensen, T. Synthesis of the Macrocyclic Core of (−)-Pladienolide B. *Org. Lett.* **10,** 2821–2824 (2008).
8. Pompeo, M. M., Cheah, J. H. & Movassaghi, M. Total Synthesis and Anti-Cancer Activity of All Known Communesin Alkaloids and Related Derivatives. *J. Am. Chem. Soc.* **141,** 14411–14420 (2019).
9. Evans, D. A., Bartroli, J. & Shih, T. L. Enantioselective aldol condensations. 2. Erythro-selective chiral aldol condensations via boron enolates. *J. Am. Chem. Soc.* **103,** 2127–2129 (1981).
10. Hsiao, C. N., Liu, L. & Miller, M. J. Cysteine- and serine-derived thiazolidinethiones and oxazolidinethiones as efficient chiral auxiliaries in aldol condensations. *J. Org. Chem.* **52,** 2201–2206 (1987).
11. Roberts, D. M. *et al.* Substrate selectivity of an isolated enoyl reductase catalytic domain from an iterative highly reducing fungal polyketide synthase reveals key components of programming. *Chem. Sci.* **8,** 1116–1126 (2017).
12. Alhamadsheh, M. M., Palaniappan, N., Daschouduri, S. & Reynolds, K. A. Modular polyketide synthases and cis double bond formation: establishment of activated cis-3-cyclohexylpropenoic acid as the diketide

intermediate in phoslactomycin biosynthesis. *J. Am. Chem. Soc.* **129,** 1910–1 (2007).

13. Wang, G. *et al.* Highly stereoselective total synthesis of fully hydroxy-protected mycolactones A and B and their stereoisomerization upon deprotection. *Chemistry* **17,** 4118–4130 (2011).
14. Perlmutter, P., Selajerern, W. & Vounatsos, F. Enantioselective synthesis of the dioxabicyclo[3.2.1]octane core of the zaragozic acids via intramolecular Wacker-type cyclisation reactions. *Org. Biomol. Chem.* **2,** 2220–2228 (2004).
15. Gao, Z. *et al.* Investigation of Fungal Iterative Polyketide Synthase Functions Using Partially Assembled Intermediates. *J. Am. Chem. Soc.* **135,** 1735–1738 (2013).
16. Hodge, M. B. & Olivo, H. F. Stereoselective aldol additions of titanium enolates of N-acetyl-4-isopropyl-thiazolidinethione. *Tetrahedron* **60,** 9397–9403 (2004).
17. Hoye, T. R., Jeffrey, C. S. & Shao, F. Mosher ester analysis for the determination of absolute configuration of stereogenic (chiral) carbinol carbons. *Nat. Protoc.* **2,** 2451–2458 (2007).
18. Reber, K. P., Mease, J., Kim, J. Total Synthesis of Cladosins B and C. *J. Org. Chem.* **85,** 11571–11578 (2020).
19. Solladié, G., Gressot, L. & Colobert, F. Enantioselective Synthesis of the Unsymmetrical Bis(lactone) (−)-(3E,6R,9E,12S,14R)-Colletol Induced by Chiral Sulfoxides and an Approach to (+)-Colletodiol by Asymmetric Hydroxylation of an α,β-Hydroxy Lactone. *European J. Org. Chem.* **2000,** 357–364 (2000).
20. Davies, S. G. *et al.* Asymmetric total synthesis of sperabillins B and D via lithium amide conjugate addition. *Org. Biomol. Chem.* **2,** 2630 (2004).
21. Heine, D. *et al.* A widespread bacterial phenazine forms S-conjugates with biogenic thiols and crosslinks proteins. *Chem. Sci.* **7,** 4848–4855 (2016).
22. Cochrane, S. A., Surgenor, R. R., Khey, K. M. W. & Vederas, J. C. Total Synthesis and Stereochemical Assignment of the Antimicrobial Lipopeptide Cerexin A1. *Org. Lett.* **17,** 5428–5431 (2015).
23. Yadav, J. S., Ganganna, B., Dutta, P. & Singarapu, K. K. Synthesis and Determination of Absolute Configuration of α-Pyrones Isolated from Penicillium corylophilum. *J. Org. Chem.* **79,** 10762–10771 (2014).
24. Das, S. & Goswami, R. K. Stereoselective Total Synthesis of Ieodomycins A and B and Revision of the NMR Spectroscopic Data of Ieodomycin B. *J. Org. Chem.* **78,** 7274–7280 (2013).
25. Prasad, K. R. & Revu, O. Total Synthesis of (+)-Seimatopolide A. *J. Org. Chem.* **79,** 1461–1466 (2014).