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Total Synthesis of A54145 Factor D

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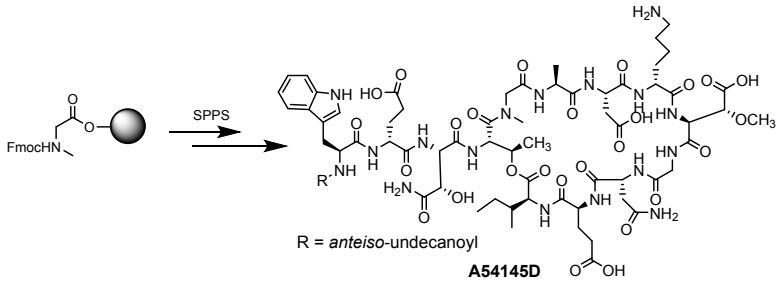
ABSTRACT: An efficient total synthesis of A54145 factor D (A5D), a member of the A54145 family of cyclic lipodepsipeptide antibiotics is reported. The peptide was constructed by attaching the peptide to the 2'-chlorotrityl polystyrene resin via Sar5, and developing conditions that avoided diketopiperazine formation upon subsequent elaboration using Fmoc SPPS. This route allowed for facile formation of the crucial depsi bond. A branched acyclic precursor was cyclized off-resin then globally deprotected to obtain A5D. Consistent with recent studies by others, we found that the MeOAsp residue has the 2*S*,3*R* configuration. We also established that the configuration of the stereocenter in the *anteiso*-undecanoyl lipid tail does not affect biological activity.

Graphical Abstract



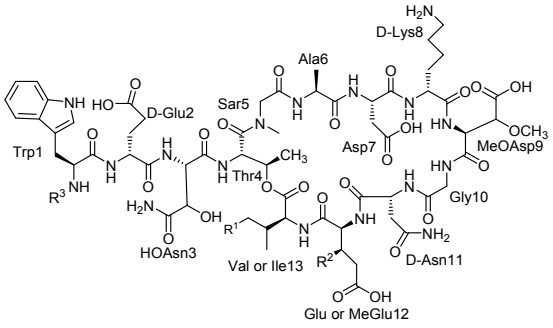
INTRODUCTION

A54145 (A5, Figure 1) is a family of cyclic lipodepsipeptide antibiotics (cLPAs) consisting of eight different factors isolated from *Streptomyces fradiae*.[1] This family is a member of the calcium-dependent cyclic lipopeptide class of antibiotics, of which the best known is daptomycin, which is used in clinic for treating serious infections caused by Gram-positive bacteria. Like daptomycin, each member of the A5 family consists of a 10 amino acid macrocycle closed with an ester (depsi) bond, to which is attached an exocyclic tripeptide bearing an *N*-terminal lipid. However, the A5 family differs from daptomycin in amino acids at eight positions. They also contain L-hydroxyasparagine (HOAsn3) and L-methoxyaspartate (MeOAsp9) which are not present in daptomycin. The macrocyclic nuclei of the A5 family differ from each other only at position 13 (Val or Ile) and/or position 12 (Glu or (2*S*,3*R*)-3-methylglutamate (MeGlu12)). The *N*-terminus is acylated with either an *iso*-decanoyl (*i*C10), *n*-decanoyl (*n*C10) or *anteiso*-undecanoyl (*a*C11) lipid.

No members of the A5 family have been developed into clinically useful drugs. The most potent natural factors (B, B1 and E) contain MeGlu12, and are only about 2-fold less active than daptomycin but are toxic to mice.[1b,2a,b] For example, the LD_{50} for A5B is low at only 28 mg/kg.[2b] Factors containing Glu12, such as A5D, are about 2-fold less potent than those containing MeGlu but are substantially less toxic.[1b,2a,b]

Daptomycin did not meet noninferiority standards in clinical trials for treating community-acquired pneumonia (CAP). A possible cause for this failure is its inhibition by lung surfactant.[3] Unlike daptomycin, A5D is not severely inhibited by lung surfactant.[2a,b,4] This characteristic of A5D prompted the development of combinatorial biosynthesis methodologies for preparing cLPAs based on the A5D peptide core.[2a,b,4] Although several A5D analogs were obtained that exhibited

good activity, low toxicity and low inhibition by surfactant, none were sufficiently active in mouse models for *S. pneumoniae* to pursue clinically.[2a] Nevertheless, these results are promising when one considers that key *in vitro* criteria (good activity, low toxicity and low inhibition by surfactant) were met even though the number of analogs prepared represented only a very small fraction of the potential chemical space. It is possible that the development of an efficient chemical approach to the synthesis of A5D and its analogs will enable researchers to access a larger portion of the available chemical space which could ultimately lead to the development of an cLPA for treating community acquired pneumonia (CAP) that is resistant to conventional therapy. The synthesis of A54145A, A1, B, B1 and F have very recently been reported.[5] Here we report the total synthesis of A5D.



Factor	R^1	R^2	R^3
A	CH_3	H	*iso*-decanoyl (*i*C_{10})
A_1	CH_3	H	*n*-decanoyl (*n*C_{10})
B	CH_3	CH_3	*n*-decanoyl (*n*C_{10})
B_1	CH_3	CH_3	*iso*-decanoyl (*i*C_{10})
C	H	CH_3	anteiso-undecanoyl (*a*C_{11})
D	CH_3	H	anteiso-undecanoyl (*a*C_{11})
E	CH_3	CH_3	anteiso-undecanoyl (*a*C_{11})
F	H	H	*iso*-decanoyl (*i*C_{10})

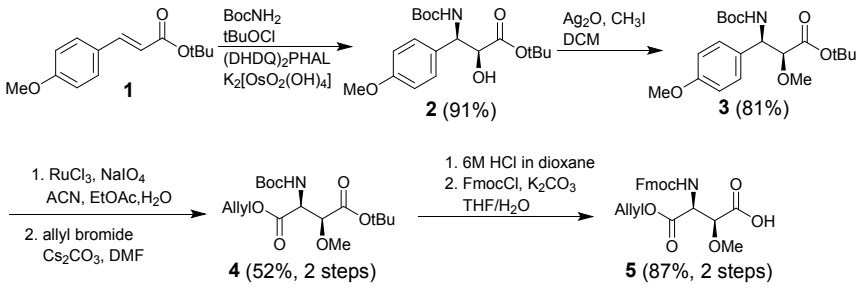
Figure 1. Structures of the A54145 family of antibiotics

RESULTS AND DISCUSSION

Although the absolute configurations of the side chains of the MeOAsp and HOAsn residues have been depicted in the literature as having the *S* configuration (L-*threo*-MeOAsp and L-*threo*-HOAsn),[6] in fact, at the time we began these studies, the absolute configuration of the side

chains of these residues had never been firmly established. While bioinformatic studies strongly suggested that the HOAsn residue is indeed the L-*threo* isomer,[2b,7] the same could not be said regarding the configuration of the MeOAsp residue. Nevertheless, we proceeded on the assumption that the amino acids were synthesized *in vivo* through a common intermediate and that both were L-*threo* amino acids.

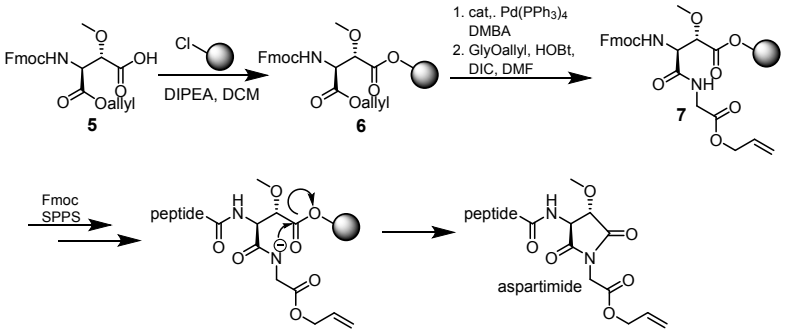
Our initial approach to A5D was to use an on-resin cyclization strategy similar to what we developed for the synthesis of daptomycin analogs.[8] This required that the peptide be attached to the resin via the side chain of MeOAsp9, and hence the synthesis of Fmoc-L-*threo*-MeOAspOallyl (Fmoc-*t*MeOAspOAllyl, **5** in Scheme 1) was required. Towards this end, an asymmetric aminohydroxylation reaction, using the conditions of von Eckardstein et al,[9] was performed on ester **1**[10] to give compound **2** in excellent yield. Methylation of the free hydroxyl group using MeI/Ag_2O gave compound **3** in good yield. Oxidation of the phenyl ring followed by esterification of the resulting acid using allyl bromide gave compound **4** in 52 % yield (2 steps). Finally, removal of the t-butyl and Boc protecting groups using acid followed by Fmoc protection of the free amino group gave compound **5** in 87% yield.



Scheme 1. Synthesis of Fmoc-*t*MeOAspOAllyl (5)

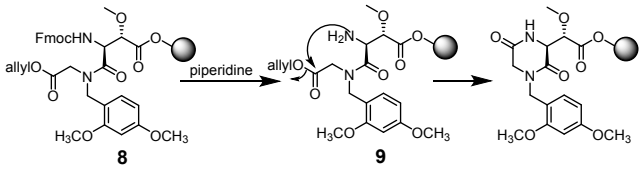
Amino acid **5** was attached to chlorotrityl-Tentagel (TG) resin via its side chain COOH group (Scheme 2). Removal of the allyl group in peptide **6** followed by installation of GlyOallyl

gave dipeptide **7** (Scheme 2). However, as the peptide was elongated using standard Fmoc SPPS, it was found that the amount of peptide remaining attached to the resin decreased with each successive amino acid coupling. We discovered that aspartimide formation was taking place during each successive Fmoc deprotection which resulted in cleavage of the peptide from the resin (Scheme 2). It is worth noting that for our daptomycin synthesis, we started with an Asp9-Gly10 dipeptide attached to the resin via the side chain of the Asp residue; however, no aspartimide formation was observed upon further elaboration of the peptide.[8] The finding that aspartimide formation occurs with L-*t*MeOAsp suggests that the presence of the electron withdrawing 3-MeO group was promoting aspartimide formation.



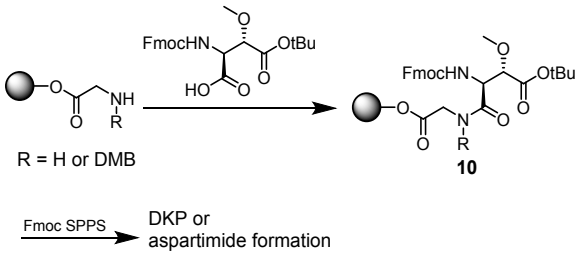
Scheme 2. Aspartimide formation during Fmoc SPPS of A5D via an on-resin cyclization approach.

To overcome the problem of aspartimide formation we prepared dipeptide **8** in which the amide nitrogen of Gly10 was protected with a dimethoxybenzyl (DMB) group (Scheme 3).[11] Unfortunately, we found that upon removal of the Fmoc group, the resulting dipeptide **9** underwent extremely rapid diketopiperazine (DKP) formation. Attempts to minimize this problem by using shorter deprotection times were unsuccessful.



Scheme 3. DKP formation upon deprotection of peptide 8.

Due to the difficulties encountered using the on-resin cyclization route, we decided to pursue an off-resin cyclization strategy. For this approach we preferred to attach the peptide to the resin via Gly10 or Sar5 in order to avoid potential epimerization issues during peptide cyclization. Hence, Fmoc-L-*threo*-MeOAsp(tBu)OH[12] was coupled to resin-supported Gly10 or DMB-protected Gly10 to give dipeptides of type **10** (Scheme 4). Not surprisingly, aspartimide or DKP formation were again major problems upon further elaboration via Fmoc SPPS.



Scheme 4. Attempted synthesis of A5D starting from Gly10.

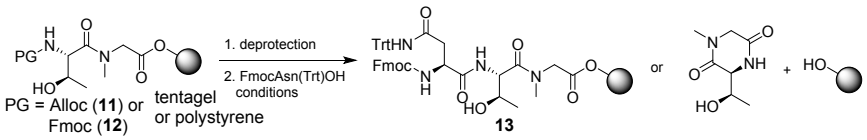
There are both advantages and disadvantages to attaching the peptide to the resin via Sar5. If the peptide is attached to the resin via Sar5 then the ester bond between Thr4 and Ile13 would be formed at an early stage in the synthesis and, therefore, on a relatively short peptide. This is an advantage, as we have shown that formation of the ester bond in daptomycin became more difficult as the length of the peptide containing the Thr residue increased, and was very difficult when the lipid tail was present.[13] Another advantage is aspartimide formation could be avoided through the use of DMB-Gly while also avoiding DKP formation between MeOAsp9 and Gly10. However, a disadvantage is that Fmoc-AA-Sar esters (AA is any amino acid) can undergo DKP formation

during deprotection of the terminal amino group.[14]

It has been reported that DKP formation can be minimized or eliminated by employing a one-pot, tandem deprotection/coupling protocol on resin bound *N*-Alloc dipeptides and activated Fmoc amino acids.[15a,b] This procedure was attempted using Alloc-protected peptide **11** and FmocAsn(Trt)OH as reactants and the conditions reported by Thieriet et al;[15a] however, none of the desired tripeptide product **13** was formed (Table 1, entry 1). Performing the reaction sequentially using DCM as solvent for both the deprotection and coupling reactions resulted in only trace amounts of peptide **13** (entry 2); however, when the reaction was performed sequentially using DMF as solvent for the coupling reaction, peptide **13** was obtained in 44 or 49 % yield depending upon whether TG or polystyrene (PS) resin was used (entry 3).

Siow et al reported that using 2-methylpiperidine (2-MP) for Fmoc removal can help reduce DKP formation during Fmoc SPPS.[16] Subjecting peptide **12** to 20% 2-MP in DMF for 3 x 10 min followed by coupling of FmocAsn(Trt)OH using DIC/HOBt gave peptide **13** in 26% yield on PS (entry 4, Table 1). Interestingly, no peptide **13** was obtained using TG resin. Decreasing the deprotection time to 2 x 5 min gave peptide **13** in 73 % yield on PS resin but in only 34% yield on TG (entry 5).[17] Surprisingly, decreasing the deprotection time to just 2 x 1 min (PS resin) resulted in almost complete deprotection of peptide **12** without DKP formation as evidenced by the fact that peptide **13** was obtained in almost quantitative yield (entry 6).

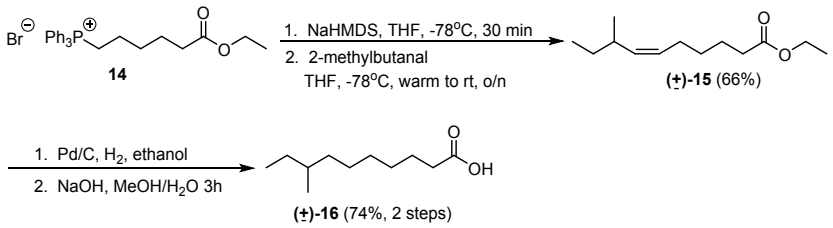
Table 1. Conditions examined for deprotecting and coupling FmocAsn(Trt)OH to peptides 11 or 12.



entry	PG	deprotection conditions	coupling reagents	sequential or tandem[a]	yield of **13** (%)
1	Alloc	12 eq DABCO or $PhSiH_3$, 0.2 eq $Pd(PPh_3)_4$, DCM	3 eq AA/HOBt/EDC or DIC	Tandem	0[b]
2	Alloc	10 eq DMBA[e], 0.2 eq $Pd(PPh_3)_4$, DCM, 1 h	4 eq AA/HOBt/DIC, DCM, 4 h	Sequential	trace[c]
3	Alloc	10 eq DMBA[e], 0.2 eq $Pd(PPh_3)_4$, DCM, 1 h	4 eq AA/HOBt/DIC, DMF, 4 h	Sequential	44[c], 49[d]
4	Fmoc	20% 2-MP, DMF, 3 x 10 min	4 eq AA/HOBt/DIC, DMF, 4 h	Sequential	0[c], 26[d]
5	Fmoc	20% 2-MP, DMF, 2 x 5 min	4 eq AA/ HOBt/DIC, DMF, 4 h	Sequential	34[c], 73[d]
6	Fmoc	20% 2-MP, DMF, 2 x 1 min	4 eq AA/ HOBt/DIC, DMF, 4 h	Sequential	**99%**[d]

[a]All tandem reactions were conducted for 2 h. [b]Tentagel or polystyrene resin. [c]Tentagel resin. [d]Polystyrene resin. [e]DMBA = dimethylbarbituric acid.

With conditions in hand that enabled us to remove the Fmoc group from peptide **12** without DKP formation, we were now ready to begin the synthesis of A5D starting from peptide **12**. First, (±)-8-methyldecanoic (**16**) was prepared (Scheme 5). This was achieved by performing a Wittig reaction between phosphonium salt **14**[18] and (±)-2-methylbutanal which gave alkene **15** in 66% yield. Lipid **16** was obtained in good yield by subjecting **15** to hydrogenolysis and then NaOH.



Scheme 5. Synthesis of (±)-8-methyldecanoic acid (16)

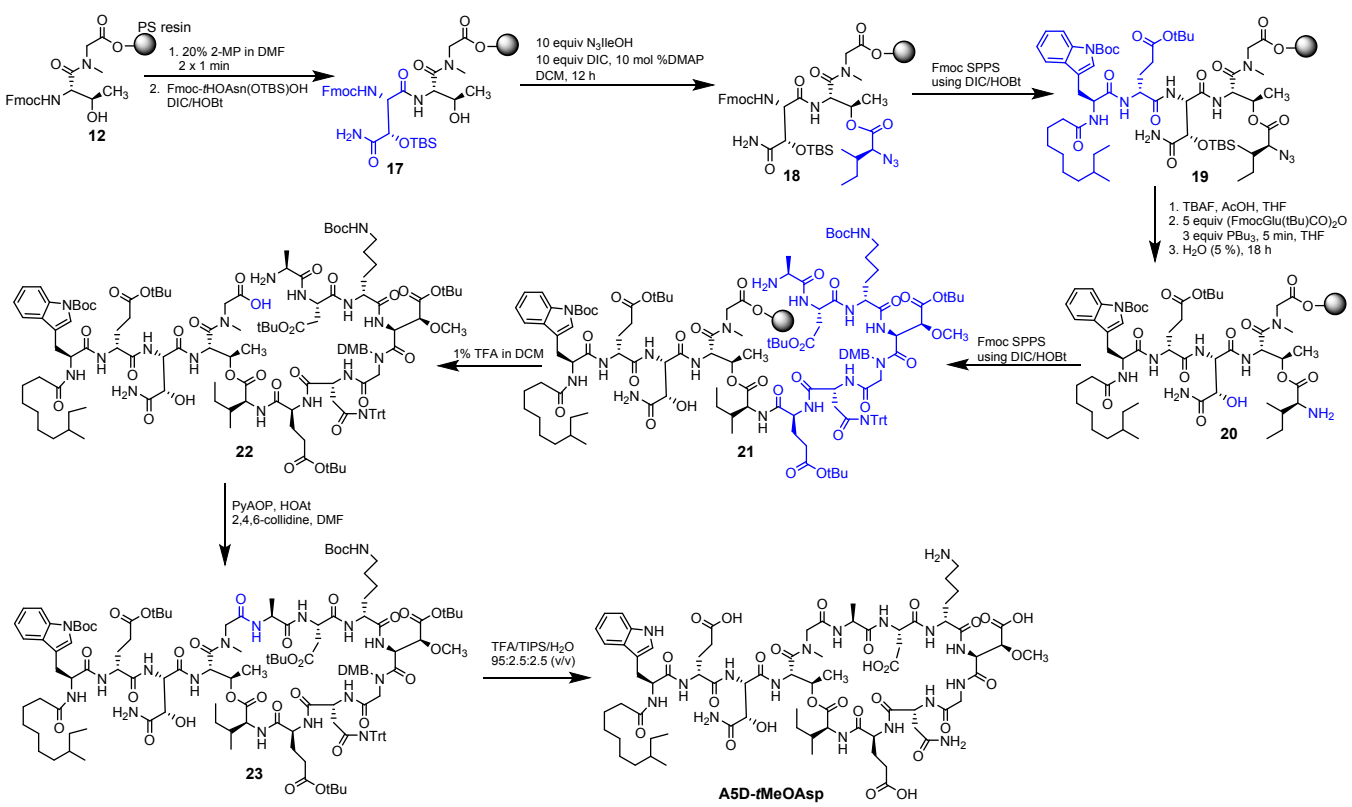
We initially used Fmoc-*t*HOAsn(OTBS,NTrt)OH[19] for the synthesis of A5D. Thus, peptide **12** was deprotected using the conditions outlined in entry 6 in table 1 and Fmoc-*t*HOAsn(OTBS,NTrt)OH was coupled to the resulting deprotected dipeptide using DIC/HOBt. However, the coupling was sluggish and, based upon the lower than expected signal intensities by analytical RP-HPLC of subsequent amino acid couplings, we suspected that increased DKP formation was again an issue. Therefore, we decided to try the less sterically encumbered Fmoc-*t*HOAsn(OTBS)OH building block.[12] In this case, the coupling proceeded smoothly and tripeptide **17** was formed in almost quantitative yield (Scheme 6).

Ester bond formation between peptide **17** and N_3IleOH proved to be straightforward using DIC and cat. DMAP, going almost to completion after one 12 h coupling. No epimerization was evident by RP-HPLC analysis of the resulting peptide **18** (Scheme 6). Fmoc SPPS was used to introduce Glu2, Trp1 and the racemic lipid **16** into peptide **18** to give peptide **19**.

Attempts to reduce the N_3 group in peptide **19** using PBu_3 in 95:5 THF/H_2O in the presence of the symmetric anhydride of FmocGlu(tBu)OH[8] gave a complex mixture of products. However, it was found that if the TBS group was removed prior to N_3 reduction then the desired peptide **20** could be obtained almost quantitatively. It is worth noting that, despite the presence of the symmetric anhydride of FmocGlu(tBu)OH, the major peptide product after the azide reduction was indeed peptide **20**, and not the Fmoc-Glu(tBu)-coupled product, consistent with our previous

studies.[8] The presence of the symmetric anhydride FmocGlu(tBu)OH during azido group reduction prevents cleavage of the ester bond during this step.[8] Accordingly, subsequent Fmoc-SPPS still required a standard coupling of Fmoc-Glu(tBu)OH using DIC/HOBt.

Standard Fmoc SPPS was used to introduce residues 12 to 6 to give peptide **21**. DMB-protected Gly was used to prevent aspartimide formation. Peptide **21** was cleaved from the resin using 1% TFA in DCM (v/v) to give peptide **22** which was cyclized using PyAOP, HOAt, and 2,4,6-collidine to give peptide **23**. Global deprotection of peptide **23** with a solution of TFA:TIPS:H_2O (95:2.5:2.5, v/v) and purification by semi-preparative RP-HPLC gave A5D-*t*MeOAsp in a 8% yield based on resin loading.

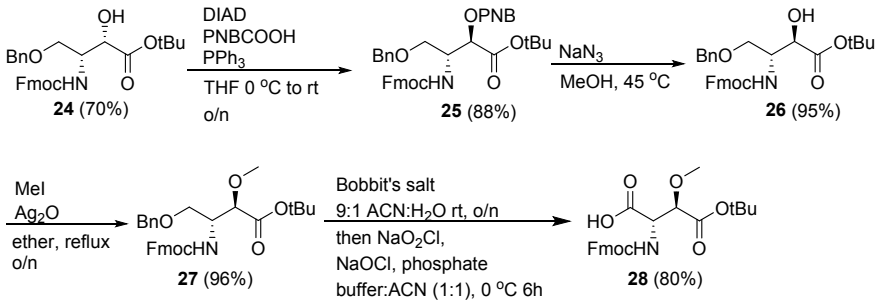


Scheme 6. Synthesis of A5D-*t*MeOAsp by attaching the peptide to the resin via Sar5.

The antimicrobial activities of authentic A5D and our synthesized A5D-*t*MeOAsp were determined against *Bacillus subtilis* 1046 (Table 2). The synthesized A5D-*t*MeOAsp exhibited an

MIC that was at least 64 times greater than that of authentic A5D which suggested to us that the configuration of the side chain of the HOAsn residue and/or the MeOAsp residue in the synthetic peptide was incorrect. As bioinformatic studies strongly suggested that the HOAsn residue is the L-*threo* isomer,[2b,7] we assumed that the problem was most likely with the MeOAsp residue.

To determine if the problem was with the MeOAsp residue, we prepared Fmoc-L-*erythro*-MeOAsp(tBu)OH (compound **28** in Scheme 7). The configuration of the alcohol in compound **24**[12] was inverted by first treating **24** with DIAD, PPh_3 and *p*-nitrobenzoic acid (PNBCOOH), and the resulting PNB ester **25** was then subjected to NaN_3.[20] This gave the corresponding *erythro* isomer **26** in excellent yield. Methylation of the alcohol in **26** with MeI/Ag_2O in refluxing ether gave ether **27** in almost quantitative yield. Removal of the benzyl group in **27** using Bobbit's salt[12] and oxidation of the *in situ* generated alcohol using NaO_2Cl/NaOCl gave acid Fmoc-L-*erythro*-MeOAsp(tBu)OH (**28**) in 80% yield.



Scheme 7. Synthesis of Fmoc-L-*erythro*-MeOAsp(tBu)OH

A5D-*erythro*-MeOAsp was prepared using the same route (Scheme 6) that was used for the *threo* isomer. The biological activity of A5D-*erythro*-MeOAsp was the same as authentic A5D (Table 2), and the two compounds co-eluted when analyzed by RP-HPLC (see Figure S4 in the Supporting Information). Comparing the NMR spectra of our synthesized A5D-*e*MeOAsp in D_2O to the literature spectrum (reported in D_2O) was not particularly helpful in terms of characterization

due to significant line broadening which was probably due to aggregation.[21] Therefore, the A5D nucleus was synthesized using the route described in Scheme 6 except that Boc-Trp(Boc)-OH was used instead of Fmoc-Trp(Boc)-OH, and no lipid tail was coupled. The synthetic A5D-nucleus was characterized by 1-D and 2-D NMR spectroscopy (see Figures S19-S23 and Table S2 in the Supporting Information) and its ^{1}H-NMR spectrum was consistent with the literature spectrum of the A5D nucleus (see Figure S18 in the Supporting Information).[22] These results indicated that the MeOAsp residue in A5D has the *2S,3R* (L-*erythro*) configuration and also strongly suggested that the HOAsn residue is indeed the L-*threo* isomer. On the basis that the literature ^{1}H NMR spectrum of A5D-nucleus matched our ^{1}H NMR spectrum, we also completely characterized A5-*e*MeOAsp by 1D and 2D NMR, with DMSO-d_6 as the solvent, which suppressed the line-broadening previously noticed when D_2O was used (See Figures S12-S17 and Table S1 in the Supporting Information).

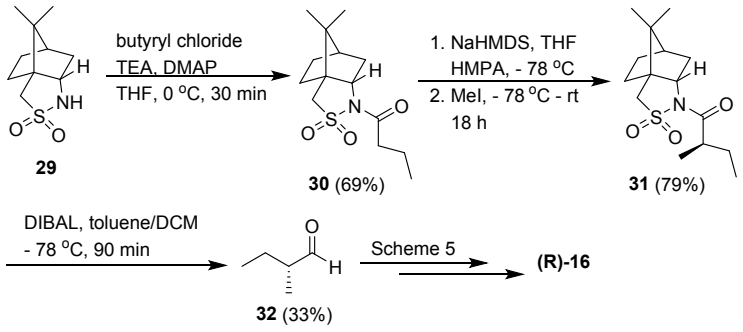
Table 2. MIC's of A5D peptides

entry	Peptide	MIC (μg/mL)[a]
1	Authentic A5D	0.5
2	A5D-*t*MeOAsp	>32
3	A5D-*e*MeOAsp	0.5
4	A5D-(*R*)-anteiso	0.5
5	A5D-(*S*)-anteiso	0.5

[a]Against *B. subtilis* 1046. 1.25 mM Ca^{2+} present.

The fact that our synthetic A5D, which contained a racemic lipid, exhibited the same activity as authentic A5D suggests that the stereochemistry of the lipid does not affect activity or that the lipid in A5D is racemic.[23] To determine if this is indeed the case, we prepared A5D (containing the correct L-*erythro*-MeOAsp isomer) bearing either (*R*)-8-methyldecanoic or (*S*)-8-

methyldecanoic acid (A5D-(8*R*)-anteiso and A5D-(8*S*)-anteiso). (8*R*)- and (8*S*)-methyldecanoic acid were prepared using the same route used to prepare (±)-8-methyldecanoic (Scheme 5) except (*R*) or (*S*)-2-methylbutanal was used instead of (±)-2-methylbutanal. (*S*)-2-methylbutanal was readily prepared by TEMPO oxidation of commercially available (*S*)-2-methylbutanol.[24] However, (*R*)-2-methylbutanol and (*R*)-2-methylbutanal are either not commercially available or prohibitively expensive. We prepared (*R*)-2-methylbutanal using a route similar to that developed by Yang et al for the synthesis of (*R*)-2-methylpentanal (Scheme 8).[25] (1*S*)-2,10-camphorsultam, **29**, was treated with butyryl chloride in the presence of TEA and DMAP which gave sulfonamide **30** in 69% yield. Methylation at the 2-position of the butyryl portion of **30** with NaHMDS/MeI gave compound **31** in 79 % yield.[26] Treatment of **31** with DIBAL gave (*R*)-2-methylbutanal (**32**) in 33 % yield.[27]



Scheme 8. Synthesis of (*R*)-2-methylbutanal

A5D-(8*R*)-anteiso and A5D-(8*S*)-anteiso were prepared using the same route outlined in Scheme 6 except Fmoc-L-*e*MeOAsp(tBu)OH, and (8*R*)- or (8*S*)-methyldecanoic acid was used. The biological activities of A5D-(8*R*)-anteiso and A5D-(8*S*)-anteiso were determined against *B. subtilis* (Table 2), and it was found that the biological activities of A5D-(*R*)-anteiso and A5D-(*S*)-anteiso were identical to those of authentic A5D and A5D-*e*MeOAsp revealing that the configuration of the lipid does not affect biological activity.

CONCLUSIONS

Very recently, and while the work described here was nearing completion, Chen et al reported the total synthesis of A54145A, A1, B, B1 and F, the stereochemical revision of the MeOAsp, and confirmed that the HOAsn residue is the L-*threo* isomer.[5] Their approach was to attach the peptide to the resin via Gly10 (as in Scheme 4); however, the yields were low (3%). Our studies suggest that the low yields may have been due to DKP and aspartimide formation. Their synthesis involved making the ester bond on a 10-mer peptide containing the lipid tail, which may also have contributed to the low yields. In contrast, we have devised a more efficient synthesis of A5D. Consistent with the studies of Chen et al,[5] we found that the MeOAsp residue has the 2*S,*3*R* configuration. We show that the configuration of the lipid does not affect biological activity. This opens the door for the synthesis of A5D analogs to establish SARs, which could lead to the development of novel antibiotics for treating CAP.

EXPERIMENTAL SECTION

General experimental information

All reagents used for peptide synthesis were obtained from commercial sources including coupling reagents, resins, and Fmoc amino acids: AllocThrOH was prepared according to a literature procedure.[28] N_3IleOH was prepared according to the procedure of Kim et al[29] except imidazole-1-sulfonyl azide hydrochloride[30] was used in place of triflic azide. ACS grade, *N,N'*-dimethylformamide (DMF), 4-methylpiperidine (4-MP), 2-methylpiperidine (2-MP), TFA, triisopropylsilane (TIPS) were purchased from commercial suppliers and used without further purification. CH_2Cl_2 (DCM) was distilled from calcium hydride under nitrogen. THF was distilled

from sodium metal and benzophenone under nitrogen. Peptide synthesis was performed manually using a rotary mixer for agitation.

Peptide syntheses were monitored by treating small aliquots of resin with 95:2.5:2.5 TFA/TIPS/H_2O for 1.5 h, removing the solvent by N_2 stream, re-dissolving the peptide in 1:1 CH_3CN/H_2O and analyzing by RP-HPLC and LRMS using a linear ion trap mass spectrometer.

Analytical HPLC was accomplished with a reversed-phase C18 column (10 μm, 250 mm × 4.6 mm, 1 ml/min flow rate). Peptides were purified by reversed-phase semi-preparative HPLC using a C18 column (10μm, 150 mm × 20 mm, 10 mL/min flow rate). High resolution positive ion electrospray (ESI+) mass spectra were obtained using a hybrid quadrupole-orbitrap mass spectrometer, , dissolving samples in 1:1 $MeOH/H_2O$ + 0.1% formic acid.

Resin loading was estimated using a procedure modified from Gude et al.[31] Resin containing the first amino acid, di- or tripeptide were subjected to 2 mL of 2% DBU in DMF (v/v) for 30 minutes. The suspension was diluted to 10 mL with acetonitrile, of which 1 mL was further diluted to 12.5 mL with acetonitrile. Absorbance of this solution was observed in triplicate at 304 nm with a reference solution as a blank. Resin loading was estimated by substitution of appropriate values into the equation below:

$$loading\ (mmole/g)_{304\,nm} = \frac{Abs_{304} * 0.0164}{m_{resin\,(g)} - Abs_{304} * \left(\frac{MW - 35.45}{1000}\right) * 0.0164}$$

where Abs_{304} is the average absorbance of the three trials, m_{resin} is the weight of the aliquot of resin, and MW is the molecular weight of the amino acid or peptide on the resin. The modified equation

above accounts for the mass of the amino acid or peptide on the resin as well as the displacement of chlorine.

Antibacterial Assays. The antibacterial activity of the synthetic peptides and authentic A54145-D was determined using a broth microdilution assay.[32] Overnight bacterial cultures were grown in LB broth then diluted to approximately 1×10^6 CFU/mL according to the measured optical density at 600 nm (OD600). Two-fold serial dilutions were used to prepare a series of peptide solutions in LB broth which were then inoculated with an equal volume of diluted bacterial culture in 96-well microplates. Plates were incubated for 24 h at 37 °C, then the MIC was determined by the lowest concentration at which there was no visible growth of bacteria.

tert-Butyl (2S,3R)-3-((tert-butoxycarbonyl)amino)-2-hydroxy-3-(4-methoxyphenyl)propanoate (2). This was prepared using the procedure that was reported by von Eckardstein et al.[9] To a solution of *tert*-butylcarbamate (3.67 g, 31.0 mmol) in nPrOH (40 mL) was added a solution of NaOH (1.2 g, 30 mmol) in water (70 mL) followed by the addition of *tert*-butylhypochlorite (3.74 mL, 33.0 mmol). The resulting mixture was stirred for 6 minutes then cooled to 0 °C (ice bath). A solution of $(DHQD)_2PHAL$ in iPrOH (36 mL) was added followed by a solution of **1**[10] (2.34 g, 10.0 mmol) in nPrOH (70 mL) which was followed by a solution of $K_2OsO_2(OH)_4$ in aq. NaOH (36 mg NaOH in 30 mL water). The ice bath was removed and the mixture was stirred for 1.5 h. The mixture was concentrated to 1/3 the volume by rotary evaporation and then diethyl ether (150 mL) and water (150 mL) was added with stirring. The layers were separated and the aqueous layer was extracted with diethyl ether (2 x 50 mL). The combined organics were washed with 0.05 M HCl (100 ml), sat. $NaHCO_3$ (100 mL) and sat. brine (100 ml). The organic layer was dried (Na_2SO_4), filtered and the solvent removed by rotary

evaporation. The resulting crude product was subjected to FC (20% EtOAc/80% hexane, $R_f = 0.2$) which gave pure **2** as a white foam (3.56 g, 91% yield). $[\alpha]^{22}_D = -32.3$ (c. 0.840, CH_2Cl_2). ^1H NMR (300 MHz, $CDCl_3$): δ 7.26 (2H, d, *J* = 9.0 Hz), 6.86 (2H, d, *J* = 8.3 Hz), 5.34 (2H, d, *J* = 10.5 Hz), 5.15 (2H, d, *J* = 9.0 Hz), 4.30 (1H, s), 3.78 (3H, s), 3.14 (1H, d, *J* = 2.9 Hz), 1.50 (9H, s), 1.39 (9H, s); $^{13}C\{^1H\}$ NMR (75 MHz, $CDCl_3$): δ 172.0, 158.8, 154.9, 131.9, 127.8, 113.7, 83.4, 79.3, 73.7, 55.1, 28.2, 27.7; HRMS (ESI+) m/z:: $[M + H]^+$ Calcd for $C_{19}H_{30}O_6N$ 368.2068; Found 368.2068.

tert-Butyl (2S,3R)-3-((tert-butoxycarbonyl)amino)-2-methoxy-3-(4-methoxyphenyl)propanoate (3). To a solution of **2** (0.500 g, 1.36 mmol) in dry dichloromethane (7 mL) was added a few beads of 4 Å MS followed by the addition of Ag_2O (0.42 g, 1.8 mmol) then methyl iodide (0.235 mL, 4.10 mmol). The mixture was stirred for 3 days with the exclusion of light. The mixture was filtered through Celite, then the filtrate was concentrated by rotary evaporation. The crude material was subjected to FC (5% EtOAc, 95% hexane, $R_f = 0.2$) which gave pure **3** as a white foam (421.5 mg, 81% yield). $[\alpha]^{22}_D = -21.6$ (c. 0.204, CH_2Cl_2). ^1H NMR (300 MHz, $CDCl_3$): δ 7.18 (2H, d, *J* = 7.8 Hz); 6.77 (2H, d, *J* = 7.8 Hz); 5.45 (1H, bs), 5.03 (1H, bs); 3.76 (1H, s), 3.67 (3H, s), 3.22 (3H, s), 1.40 (9H, s), 1.30 (9H, s); $^{13}C\{^1H\}$ NMR (75 MHz, $CDCl_3$): δ 169.0, 158.6, 154.7, 132.0, 127.6, 113.4, 83.0, 81.8, 79.0, 58.4, 55.1, 54.8, 28.0, 27.6; HRMS (ESI+)m/z: $[M + H]^+$ Calcd for $C_{20}H_{32}O_6N$ 382.2224; Found 382.2224.

1-Allyl 4-(tert-butyl) (2S,3S)-2-((tert-butoxycarbonyl)amino)-3-methoxysuccinate (4). To a solution of **3** (2.0 g, 5.2 mmol) in EtOAc/H_2O/CH_3CN (2:1:1, 125 mL) was added $NaIO_4$ (19 g, 89 mmol) then $RuCl_3$ (54 mg, 0.26 mmol). The mixture was stirred vigorously for 4 h. iPrOH (10 mL) was added and the mixture stirred for 10 min. The mixture was gravity filtered

through Celite twice and then filtered again without Celite (filter paper). The filtrate was concentrated to dryness. The residue was dissolved in dry methanol (25 mL) then gravity filtered (filter paper) and the filtrate concentrated. The residue was dissolved in dry methanol (25 mL) then $CsCO_3$ (1.15 g, 3.00 mmol) was added and the mixture stirred for 2 h. The mixture was concentrated to dryness. The residue was dissolved in dry DMF (20 mL) and allyl bromide (0.70 mL, 6.8 mmol) was added. The mixture was stirred for 16 h. Ether (200 mL) was added and then mixture was washed with water (3 x 100 mL) then concentrated to dryness. The crude material was subjected to FC (7.5 % EtOAc/92.5 % hexane then 10% EtOAc/90% hexane, $R_f = 0.2$) which gave pure **4** as a white foam (0.971 g, 52% yield). ^{1}H NMR (300 MHz, $CDCl_3$): δ 5.84-5.92 (1H, m), 5.30 (1H, d, J = 16.6 Hz), 5.21-5.25 (2H, m), 4.80 (1H, dd, J = 2.2, 10.3 Hz), 4.69 (1H, dd, J = 5.6, 13.3 Hz), 4.59 (1H, dd, J = 5.8, 13.3 Hz); 4.21 (1H, 2, J = 2.2 Hz), 3.38 (3H, s), 1.44 (9H, s), 1.37 (9H, s); ^{13}C{^{1}H} NMR (75 MHz, $CDCl_3$): δ 169.3, 167.9, 155.2, 131.4, 118.3, 82.5, 79.8, 79.7, 66.0, 58.8, 55.9, 28.0, 27.7; HRMS (ESI+) m/z: $[M + H]^+$ Calcd for $C_{17}H_{30}O_7N$ 360.2017; Found 360.2016.

Fmoc-*t*MeOAspOAllyl (5). To **4** (1.00 g , 2.87 mmol) was added a solution of 6 M HCl in dry dioxane (35 mL). The mixture was stirred for 16 h then concentrated to dryness. The residue was dissolved in THF (15 mL) then concentrated to dryness (2x). The residue was dissolved in THF (15 mL) and water (25 mL) then cooled (ice bath). A solution of Fmoc-Cl in THF (35 mL) was added and the mixture was stirred for 2 h. Most of the THF was removed by rotary evaporation. Water (30 mL) was added. The aqueous solution was washed with diethyl ether (3 x 50 mL). The aqueous layer was acidified to pH = 2 then extracted with EtOAc (4 x 50 mL). The combined organics were dried (Na_2SO_4), filtered, then concentrated to dryness. The residue was subjected to FC (30% EtOAc/68% hexane/2% AcOH, $R_f = 0.2$) which gave pure **5** as a white

foam (1.03 g, 87% yield). $[\alpha]^{22}_{D}$ = -18.3 (c. 1.00, CH_2Cl_2). ^{1}H NMR (300 MHz, $CDCl_3$): δ 10.64 (1H, bs), 7.76 (2H, d, *J* = 7.6 Hz), 7.62 (2H, d, *J* = 4.0 Hz), 7.41 (2H, app t, *J* = 7.4 Hz), 7.27-7.35 (2H, m), 5.80-5.97 (1H, m), 5.80 (1H, d, *J* = 9.9 Hz), 5.37 (1H, d, *J* = 17.0 Hz), 5.30 (1H, d, *J* = 10.3 Hz), 5.03 (1H, d, *J* = 9.9 Hz); 4.76 (1H, dd, *J* = 5.8, 12.9 Hz), 4.70 (1H, dd, *J* = 5.8, 13.5 Hz), 4.47 (1H, s), 4.36-4.41 (2H, m), 4.25 (1H, t, *J* = 7.4 Hz); 3.48 (3H, s); ^{13}C{^{1}H} NMR (75 MHz, $CDCl_3$): δ 172.2, 168.7, 156.8, 143.8, 143.6, 141.3, 131.2, 127.7, 127.1, 125.3, 119.9, 119.1, 79.3, 67.9, 66.7, 59.4, 56.3, 46.9; HRMS (ESI+)m/z: $[M + H]^+$ Calcd for $C_{23}H_{24}O_7N$ 426.1547; Found 426.1541.

Ethyl (Z)-8-methyldec-6-enoate (15). The following procedure was used to prepare (±)-**15**, (S)-**15** and (R)-**15** using either (±)-, (*S*)- or (*R*)-2-methylbutanal (**31**). To a 100 mL round-bottom flask was added **14**[18] (1.02 g, 2.10 mmol) and purged with argon gas. Dry THF (50 mL) was added and the mixture was cooled to -78 °C in a slurry of dry ice in acetone. A 1.0 M solution of NaHMDS in THF (2.1 mL, 2.1 mmol) was slowly added over five min and the resulting brown solution was stirred for 30 min at -78 °C. A solution of (±)-2-methylbutanal (231 μL, 2.00 mmol) in THF (5 mL) was transferred to the reaction mixture. The solution was allowed to warm to room temperature and stirred overnight. The reaction was quenched with saturated sodium bicarbonate and the product was extracted with ethyl acetate (2 x 50 mL). The organic layers were combined, dried with sodium sulfate, and concentrated *in vacuo*. The residue was subjected to FC (95% hexane/5% ethyl acetate, R_f = 0.3) which provided pure (±)-**15** as a colorless oil (0.305 g, 66% yield). The yields of (S)-**15** and (R)-**15** were 71% and 69% respectively. ^{1}H NMR (300 MHz, $CDCl_3$) *δ*: 5.22 (1H, dt, *J* = 7.2 Hz, 10.8 Hz), 5.04 (1H, app t, *J* = 9.9 Hz), 4.04 (2H, q, *J* = 7.2 Hz), 2.22 (2H, t, *J* = 7.5 Hz), 1.97 (2H, q, *J* = 7.3 Hz), 1.57 (2H, app quint, *J* = 7.6 Hz), 1.36-1.04 (7H, m), 0.85 (3H, d, *J* = 6.7 Hz), 0.76 (3H, t, *J* = 7.4 Hz). ^{13}C{^{1}H} NMR (75 MHz, $CDCl_3$) *δ*: 173.5,

136.4, 127.8, 60.0, 34.1, 33.3, 30.2, 29.3, 27.0, 24.5, 20.9, 14.1, 11.8. HRMS (ESI+)m/z: $[M + H]^+$ Calcd for $C_{13}H_{25}O_2$213.1849; Found 213.1849. The ^{1}H NMR spectra of (S)-**15** and (R)-**15** were identical to that of (±)-**15.**

(±)-8-methyldecanoic acid (16). The following procedure was used to prepare (±)-**16**, (S)-**16** and (R)-**16** using either (±)-**15**, (S)-**15** and (R)-**15**. Compound (±)-**15** (201 mg, 0.95 mmol) was added to a 50 mL round-bottom flask. To this was added ethanol (25 mL) and 10% Pd/C (40 mg) and the flask was purged with argon gas. The flask was then flushed with hydrogen gas and allowed to stir overnight at room temperature. The mixture was filtered over Celite and the filtrate was concentrated yielding a colorless oil (^{1}H-NMR (300 MHz, $CDCl_3$) δ: 4.06 (2H, q, J = 7.0 Hz), 2.22 (2H, t, J = 7.4 Hz), 1.56 (2H, m), 1.35-0.95 (15H, m), 0.88-0.72 (6H, m)). The oil (176 mg, 0.82 mmol) was dissolved in a solution of 1:1 0.6 M NaOH (aq) and MeOH (25 mL). After 3 h, the reaction was acidified to pH 1 with 1M HCl and the product extracted into ethyl acetate (2 x 50 mL). The organic layers were dried with Na_2SO_4 and the solvent removed by rotary evaporation yielding pure **16** as a colorless oil (153 mg, 77% yield). The yields of (S)-**16** and (R)-**16** were 78% and 79% respectively. ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 11.74 (1H, br s), 2.33 (2H, t, J = 7.5 Hz), 1.59 (2H, app. quint, J = 7.3 Hz), 1.39-1.00 (11H, m), 0.88-0.76 (6H, m); ^{13}C{^{1}H} NMR (75 MHz, $CDCl_3$) δ: 183.6, 39.4, 37.2, 37.0, 32.5, 32.3, 32.0, 29.7, 27.5, 22.0, 14.2; HRMS (ESI-) m/z: $[M-H]^-$ Calcd for $C_{11}H_{21}O_2$ 185.1547; Found 185.1552. The ^{1}H NMR spectra of (S)-**16** and (R)-**16** were identical to that of (±)-**16**. (S)-**16**: $[\alpha]^{22}_D$ +4.7 (c 0.20, $CHCl_3$) (lit.,[33] $[\alpha]^{25}_D$ +6.1 (1.14, $CHCl_3$)). (R)-**16:** $[\alpha]^{22}_D$ -4.4 (c 0.20, $CHCl_3$).

Fmoc-*t*HOAsn(OTBS,NTrt)OH. A solution of H_2SO_4:acetic anhydride (1:9, 300 µL, 0.557 mmol) was added dropwise to a suspension of trityl alcohol (5.37 g, 20.6 mmol, 10 equiv)

in acetic anhydride (8.3 mL) cooled in a room temperature water bath. After stirring for 5 min, the water bath was removed and after stirring for an additional 10 min at room temperature, Fmoc-*t*HOAsn(OTBS)OH[12] was added in a single portion. After 30 min, the reaction was diluted with ethyl acetate (20 mL), cooled in an ice water bath, and quenched through a dropwise addition of saturate aqueous $NaHCO_3$ (10 mL). After separation, the aqueous layer was cooled (ice-water bath) and acidified (pH ca. 3) with 1N HCl. The cold solution was extracted with ethyl acetate (2 x 30 mL) and the combined organic layer was dried over $MgSO_4$, filtered and concentrated. Silica gel column chromatography (5% ethyl acetate/94% hexanes/1% AcOH then 40% ethyl acetate/59% hexanes/1% AcOH) yielded Fmoc-*t*HOAsn(OTBS,NTrt)OH as a colorless solid (1.05 g, 70% yield). All spectroscopic data matched that previously reported.[34] $[\alpha]^{22}_{D}$ = -22.2 (c. 0.724, CH_2Cl_2). ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.31 and 8.20 (1H, s), 7.90-7.20 (24H, m) 4.68 and 4.64 (1H, s), 4.56-4.49 (1H, m), 4.42 (1H, d, J = 9.0 Hz), 4.30-4.16 (2H, m) 0.882 (9H, s) 0.084-0.020 (6H, m); ^{13}C{^{1}H} NMR (75 MHz, DMSO-d_6): 171.4, 168.7, 156.5, 144.3, 143.6, 143.6, 140.7, 140.6, 128.6, 128.5, 127.6, 127.5, 127.1, 127.0, 126.7, 125.6, 125.3, 120.0, 73.9, 69.5, 66.2, 57.5, 46.6, 25.6, 17.7, -5.0, -5.4; HRMS (ESI-) m/z: [M - H]- Calculated for $C_{44}H_{45}N_2O_6Si$ 725.3052; Found 725.3039.

(2R,3R)-3-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-4-(benzyloxy)-1-(tert-butoxy)-1-oxobutan-2-yl 4-nitrobenzoate(25). Compound **24**[12] (er = 95:5, 3.09 g, 6.14 mmol), *p*-nitrobenzoic acid (1.54 g, 9.20 mmol) and triphenylphosphine (2.41 g, 9.20 mmol) were dissolved in THF (31 mL) and this solution was cooled (ice-water bath). Diisopropyl azodicarboxylate (1.81 mL, 9.20 mmol) was added and the solution was left for 24 h at room temperature under an inert atmosphere. After concentrating the reaction mixture, a single re-crystallization of the crude solid in MeOH afforded **25** as a colorless, crystalline solid (3.52 g, 88%

yield). $[\alpha]^{22}_{D}$ = -7.8 (c. 1.03, CH_2Cl_2). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.20 (2H, d, *J* = 8.4 Hz), 8.10 (2H, d, *J* = 8.4 Hz), 7.75 (2H, m), 7.58 (2H, m), 7.41-7.25 (9H, m), 5.37-5.29 (2H, m), 4.58-4.42 (5H, m), 3.72-3.64 (2H, m) 1.45 (9H, s); ^{13}C{^{1}H} NMR (75 MHz, $CDCl_3$): 166.3, 163.8, 155.7, 150.6, 143.7, 141.3, 137.3, 134.5, 131.0, 128.5, 128.0, 127.7, 127.0, 125.0, 124.9, 123.5, 120.0, 83.3, 73.3, 67.8, 67.0, 51.1, 47.1, 27.9; HRMS (ESI+) m/z: $[M + H]^+$ Calcd for $C_{37}H_{37}O_9N_2$ 653.2494; Found 653.2507.

***tert*-Butyl (2R,3R)-3-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-4-(benzyloxy)-2-hydroxybutanoate (26).** Compound **25** (1.95 g, 3.00 mmol) and NaN_3 (584 mg, 9.00 mmol) were dissolved in MeOH (50 mL) at room temperature. The colorless suspension was stirred at 45 °C for 4 hours. The homogenous mixture was concentrated and the crude residue subjected to FC (20% ethyl acetate/80% hexanes) which provided pure **26** as a colorless foam (1.45 g, 95% yield). The er was determined to be 97:3 by chiral RP-HPLC (see Figure S24 in the Supporting Information). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.76 (2H, d, *J* = 6.9 Hz), 7.60 (2H, d, *J* = 6.9 Hz), 7.42-7.25 (9H, m), 5.45 (1H, m), 4.48-4.40 (4H, m), 4.23-4.22 (3H, m), 3.56-3.35 (3H, m), 1.44 (9H, s); ^{13}C{^{1}H} NMR (75 MHz, $CDCl_3$): 171.4, 155.8, 143.8, 141.3, 137.4, 128.4, 127.9, 127.9, 127.7, 127.0, 119.9, 83.0, 73.4, 71.7, 68.4, 66.9, 52.4, 47.1, 27.9; HRMS (ESI+) m/z: $[M + H]^+$ Calcd for $C_{30}H_{34}O_6N$ 504.2381; Found 504.2374.

***tert*-Butyl (2R,3R)-3-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-4-(benzyloxy)-2-methoxybutanoate (27).** To a solution of **26** (1.20 g, 2.38 mmol) in diethyl ether (48 mL) was added freshly prepared, dry silver oxide (1.65 g, 7.15 mmol) and MeI (2.97 mL, 47.7 mmol). The suspension was vortexed briefly then refluxed overnight. The suspension was cooled to room temperature and then filtered through Celite. The filtrate was concentrated which provided pure

27 as a white foam (1.18 g, 96% yield). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.76 (2H, d, *J* = 7.5 Hz), 7.60 (2H, d, *J* = 7.5 Hz), 7.40 (2H, t, *J* = 7.2 Hz), 7.33-7.25 (9H, m), 5.28-5.25 (1H, m), 4.51 (2H, m), 4.38 (2H, m), 4.23 (2H, m), 3.82 (1H, d, *J* = 5.7 Hz), 3.62 (1H, dd, *J* = 5.1, 9.6 Hz) 3.50-3.41 (5H, m), 1.43 (9H, s); ^{13}C{^{1}H} NMR (75 MHz, $CDCl_3$): 169.4, 155.8, 143.9, 141.3, 137.9, 128.4, 127.7, 127.7, 127.7, 127.0, 125.1, 120.0, 82.1, 80.4, 73.1, 68.0, 68.8, 58.7, 52.2, 47.2, 27.9; HRMS (ESI+) m/z: $[M + H]^+$ Calcd for $C_{31}H_{36}O_6N$ 518.2537; Found 518.2539.

Fmoc-*e*MeOAsp(tBu)OH (28). Bobbit's salt (2.33 g, 7.79 mmol) was added to a solution of **30** (1.30 g, 2.51 mmol) in MeCN (9 mL) and H_2O (1 mL) and the mixture was stirred overnight. The mixture was cooled (ice-water bath) and diluted with a solution of NaO_2Cl (80% w/w; 880 mg, 7.78 mmol) in phosphate buffer (0.67 N, pH ca. 7; 16.8 mL). The pH of the resulting solution was adjusted to 7 with 1 N NaOH, at which point MeCN (15.7 mL) was added, followed by a dropwise addition of NaOCl (0.81 N bleach; 2.17 mL, 1.76 mmol) over 30 min. After 8 hours, sat. aqueous $NaSO_3$ was added until the dark red color faded to a pale orange. The two layers were separated, and the aqueous layer was acidified to pH 2 with conc. aqueous HCl and extracted with DCM (3 x 50 mL). The combined organic layer was dried over $MgSO_4$, filtered and concentrated. The crude residue was subjected to FC (40% ethyl acetate/59% hexanes/1% AcOH) which provided pure **28** as a colorless foam (891 mg, 80% yield). $[\alpha]^{22}_D = 44.8^0$ (c. 0.800, CH_2Cl_2). ^{1}H NMR (300 MHz, $CDCl_3$): δ 11.30 (1H, br. s), 7.76 (2H, d, *J* = 7.5 Hz), 7.61 (2H, d, *J* = 7.2), 7.40 (2H, t, *J* = 7.5 Hz), 7.31 (2H, t, *J* = 7.5 Hz), 6.16 and 5.78 (1H, d, *J* = 8.4 Hz), 4.94 and 4.67 (1H, dd, *J* = 2.4, 8.4 Hz), 4.52-4.37 (2H, m), 4.25 (1H, t, *J* = 6.9 Hz), 4.07 (1H, d, *J* = 2.4 Hz), 3.53 and 3.46 (3H, s), 1.51 (9H, s); ^{13}C{^{1}H} NMR (75 MHz, $CDCl_3$): 172.7, 168.0, 155.9, 143.7, 143.5, 141.2, 127.6, 127.0, 125.0, 119.9, 83.1, 80.7, 67.3, 59.6, 55.8, 46.9, 27.8; HRMS (ESI+) m/z: $[M + H]^+$ Calcd for $C_{24}H_{28}O_7N$ 442.1860; Found 442.1857.

1-((3aR,6R)-8,8-dimethyl-2,2-dioxidotetrahydro-3H-3a,6-methanobenzo[c]isothiazol-1(4H)-yl)butan-1-one (30). (1S)-(−)-2,10-Camphorsultam (**29**, 10.0 g, 46.4 mmol) and DMAP (624 mg, 5.11 mmol) were dissolved in dry THF (60 mL) at room temperature. The solution was cooled (ice bath) and triethylamine (7.04 mL, 69.7 mmol) was added followed by the dropwise addition of butyryl chloride (5.3 mL, 51.1 mmol). The formed suspension was stirred for 30 min then quenched with 1N HCl (20 mL) and extracted with ethyl acetate (3 x 60 mL). The combined organic layer was washed once with 1N NaOH (100 mL), dried over $MgSO_4$ and filtered. The filtrate was concentrated, and the crude solid was purified by a single recrystallization in methanol, yielding **30** as a colorless, crystalline solid (9.2 g, 69% yield). ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.83 (1H, dd, J = 5.7, 6.9 Hz), 3.47 (1H, d, J = 13.8 Hz), 3.39 (1H, d, J = 13.8 Hz), 2.74-2.57 (2H, m), 2.06-2.01 (2H, m), 1.86-1.83 (2H, m), 1.68 (2H, sext, J = 7.2 Hz), 1.41-1.28 (2H, m), 1.12 (3H, s), 0.95-0.90 (6H, m); ^{13}C{^{1}H} NMR (75 MHz, $CDCl_3$): 171.9, 65.1, 52.9, 48.3, 47.7, 44.6, 38.5, 37.3, 32.8, 26.4, 20.8, 19.8, 17.9, 13.5; HRMS (ESI+) m/z: $[M + H]^+$ Calcd for $C_{14}H_{24}O_3NS$ 286.1471; Found 286.1465.

S(2R)-1-((3aR,6R)-8,8-dimethyl-2,2-dioxidotetrahydro-3H-3a,6-methanobenzo[c]isothiazol-1(4H)-yl)-2-methylbutan-1-one (31). NaHMDS (40.1 mL, 1M in THF, 40.1 mmol) was added dropwise to a solution **30** (8.81 g, 30.9 mmol) in dry THF (221 mL) cooled to -78 ^{o}C, under an inert atmosphere. The resulting solution was stirred for 1 h, then dry HMPA (16.1 mL) and MeI (5.76 mL, 92.6 mmol) were added and cooling was removed. The solution was stirred at room temperature for 18 h, then quenched with sat. aqueous NH_4Cl (40 mL) and extracted with ethyl acetate (3 x 100 mL). The combined organic layer was dried with $MgSO_4$, filtered and concentrated. The crude solid was purified by a single recrystallization in methanol which yielded **31** (7.30 g, 79% yield) as a colorless, crystalline solid. ^{1}H NMR (300 MHz, $CDCl_3$):

δ 3.85 (1H, t, J = 6.3 Hz), 3.47 (1H, d, J = 13.5 Hz), 3.39 (1H, d, J = 13.8 Hz), 2.93 (1H, sex., J = 6.9 Hz), 2.01-1.99 (2H, m), 1.89-1.70 (4H, m), 1.40-1.27 (3H, m), 1.16-1.11 (6H, m), 0.93-0.84 (6H, m); $^{13}C\{^{1}H\}$ NMR (75 MHz, $CDCl_3$): 176.2, 64.9, 53.1, 48.1, 47.6, 44.5, 41.7, 38.4, 32.7, 26.3, 25.6, 20.7, 19.8, 18.5, 11.7; HRMS (ESI+) m/z: $[M + H]^+$ Calcd for $C_{15}H_{26}O_3NS$ 300.1628; Found 300.1621. The absolute configuration of **31** was confirmed by X-ray crystallography (see Figure S25 in the Supporting Information).

(*R*)-2-methylbutanal (32). DIBAL (1M in toluene; 36.6 mL, 36.6 mmol) was added dropwise to a solution of **31** (7.31 g, 24.4 mmol) in dry DCM at -78 °C under an inert atmosphere. The resulting solution was stirred for 90 min, then quenched with a solution of $NaHSO_3$ (8.77 g) in H_2O (90 mL) and allowed to come to room temperature. The mixture was extracted with pentane (3 x 100 mL) and the combined organic layer concentrated by rotary evaporation (no heating) until only toluene remained. The residue was subjected to FC (30% DCM/70% pentane then 45% DCM/55% pentane). The combined fractions that contained **32** were carefully concentrated by rotary evaporation (no heating) until only a solution of **32** in DCM remained (40% w/w). The ^{1}H-NMR spectrum was identical to that of (*S*)-2-methylbutanal.[25] This solution was used immediately for the synthesis of (*R*)-**16**.

Synthesis of A5D-*t*MeOAsp. 2'-Cl-TrtCl polystyrene resin (theoretical substitution = 1.5 mmol/g, 66.7 mg, 0.1 mmol, 1 equiv) was activated in dry DCM with thionyl chloride (26 µL, 3.6 equiv) and pyridine (58 µL, 7.2 equiv) under reflux for 2 h. The resin was transferred to a disposable peptide cartridge and rinsed 6x with dry DCM, followed by loading with Fmoc-Sar-OH (4 equiv) and DIPEA (8 equiv) in 1 mL dry DCM for 2 h, twice. The resin was capped with 17:2:1 DCM/MeOH/DIPEA (3 x 10 min) and the loading efficiency determined to be 1.40 mmol/g.

Removal of the Fmoc group from Fmoc-Sar-OH was performed with four 30-minute iterations of 20% 4-methylpiperidine in DMF (v/v). All Fmoc-amino acids (4 equiv) and the lipid tail (4 equiv) were activated for 5 min using HOBt (4 equiv) and DIC (4 equiv) in DMF (2 mL) and then coupled for 4 h, except for Fmoc-*t*MeOAsp(tBu)OH, which was coupled for 12 h. Removal of the Fmoc-group from peptide **12** was performed with two 1-minute iterations of 20% 2-methylpiperidine in DMF (v/v), and all other Fmoc-removals were performed with three 10-minute iterations of 20% 2-methylpiperidine in DMF. Following installation of Fmoc-*t*HOAsn(OTBS)OH, yielding peptide **17**, peptide **18** was prepared by treating peptide **17** with N_3-Ile-OH (10 equiv), pre-activated with DIC (10 equiv) by stirring in DCM (2 mL) for 1 h, followed by the addition of DMAP (0.1 equiv), for 12 h. Depsi-bond completion was monitored by HPLC. The next amino acids, D-Glu2, Trp1, and 8-methyldecanoic acid, were introduced using the above general procedure, yielding peptide **19**. The TBS group was removed by treating peptide **19** with TBAF (3 equiv), and acetic acid (3 equiv) in THF (2 mL) for 1 h. The azide group in the resulting peptide was removed by treatment of the peptide with PBu_3 (3 equiv), and $(FmocGlu(tBu)CO)_2O$ (5 equiv) (formed by the addition of 10 equiv FmocGlu(tBu)OH and 5 eq DIC to 2 mL dry THF and stirring for 1 h) for 5 minutes, followed by the addition of 0.1 mL H_2O and further stirring for 16 h, yielding peptide **20**. The remaining amino acids were introduced using the coupling and deprotection protocol mentioned above, affording peptide **21**. The peptide was cleaved from the resin by treatment with 1% TFA in DCM (5 x 10 min) affording peptide **22**, which was converted to peptide **23** off-resin by cyclizing with PyAOP (5 equiv), HOAt (5 equiv), and 2,4,6-collidine (10 equiv) in DMF (100 mL, 1 mM) overnight. The solvent was removed by high-vacuum rotary evaporation, and the crude solid was treated with 95:2.5:2.5 TFA/TIPS/H_2O (v/v, 15 mL) for global deprotection of side-chain protecting groups. The TFA was removed under stream of N_2, and the crude product dissolved in

9:1 H_2O/CH_3CN. The target compound was purified by preparative RP-HPLC employing an isocratic gradient of 40% CH_3CN/60% H_2O (+0.1% TFA). Fractions containing A5D-*t*MeOAsp were pooled and lyophilized giving A5D-*t*MeOAsp as a white powder (12.3 mg, 8 % yield based on resin loading), judged to be > 95% pure by analytical RP-HPLC (See Figure S1 in the Supporting Information). HRMS (ESI+) *m/z*: $[M+2H]^{2+}$ Calcd for $C_{73}H_{113}N_{17}O_{27}$ 829.8990; Found 829.8992.

A5D-*e*MeOAsp. A5D-eMeOAsp was synthesized according to the procedure used for A5D-*t*MeOAsp, except that Fmoc-*e*MeOAsp(tBu)OH was used in place of Fmoc-*t*MeOAsp(tBu)OH. The desired compound was isolated as a white powder (12.4 mg, 8 % yield), judged to be >95% pure by analytical RP-HPLC (see Figure S3 in the supporting information). HRMS (ESI+) *m/z*: $[M+2H]^{2+}$ Calcd for $C_{73}H_{113}N_{17}O_{27}$ 829.8990; Found 829.9010. The peptide was also characterized by NMR (1-D ^1H, as well as 2-D: HSQC, HMBC, COSY, TOCSY, ROESY. See Figures S12-S17 and Table S1 in the Supporting Information).

A5D-(*S*)-anteiso. A5D-(*S*)-anteiso was synthesized according to the procedure used for A5D-*t*MeOAsp, except that Fmoc-*e*MeOAsp(tBu)OH was used in place of Fmoc-*t*-MeOAsp(tBu)OH, and (*S*)-8-methyldecanoic acid were used in place of (±)-8-methyldecancoic acid. A5D-(*S*)-anteiso was isolated as a white powder (16.6 mg, 11 % yield based on resin loading), judged to be >95% pure by analytical RP-HPLC (see Figure S6 in the supporting information). HRMS (ESI+) *m/z*: $[M+2H]^{2+}$ Calcd for $C_{73}H_{113}N_{17}O_{27}$ 829.8990; Found 829.8986.

A5D-(*R*)-anteiso. A5D-(*R*)-anteiso was synthesized according to the procedure used for A5D-*t*MeOAsp, except that Fmoc-*e*MeOAsp(tBu)OH was used in place of Fmoc-*t*MeOAsp(tBu)OH, and (*R*)-8-methyldecanoic acid were used in place of (±)-8-methyldecancoic

acid. A5D-(*R*)-anteiso was isolated as a white powder (16.7 mg, 11% yield based on resin loading), judged to be >95% pure by analytical RP-HPLC (see Figure S7 in the supporting information). HRMS (ESI+) *m/z*: $[M+2H]^{2+}$ Calcd for $C_{73}H_{113}N_{17}O_{27}$ 829.8990; Found 829.8991.

A5D-nucleus. The nucleus of A5D, used for characterization purposes, was synthesized according to the procedure used for A5D-*t*MeOAsp, except that Fmoc-*e*MeOAsp(tBu)OH was used in place of Fmoc-*t*MeOAsp(OBu)OH, and BocTrp(Boc)OH was used instead of FmocTrp(Boc)OH. No lipid tail was coupled to the peptide. The peptide was purified using preparative RP-HPLC employing an isocratic solution of 16% CH_3CN/84% H_2O (+ 0.1% TFA), yielding the A5D-nucleus as a white powder (12.0 mg, 9% based on resin-loading). The compound was judged to be >95% pure based on analytical HPLC (see Figure S10 in the supporting information). HRMS (ESI+) *m/z*: $[M+2H]^{2+}$ Calcd for $C_{62}H_{93}N_{17}O_{26}$ 745.8233; Found 745.8244. The peptide was also characterized by NMR (1-D ^1H, as well as 2-D: HSQC, HMBC, COSY, TOCSY, ROESY. See Figures S19-S23 and Table S2 in the Supporting Information).

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: HPLC chromatograms and HRMS spectra of all A5D peptides. 1-D and 2-D NMR spectra of the A5D nucleus and A5D-eMeOAsp and assignment of residue chemical shifts. Comparison of 1-D ^1H NMR spectrum of the synthetic A5D-nucleus to the 1-D ^1H-NMR spectrum of the A5D nucleus reported in the literature. Chiral HPLC of compound **26**. ^1H- and ^{13}C-NMR spectra for compounds **2-5**, (**±)15**, (**±)-16**, (**S)-16,** (**R)-16, 25-28**, **30**, **31** and Fmoc-*t*HOAsn(OTBS,NTrt)OH. X-ray crystal structure of compound **31**.

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Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

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