Homochiral 4-Azalysine Building Blocks: Syntheses and Applications in Solid-Phase Chemistry[1]

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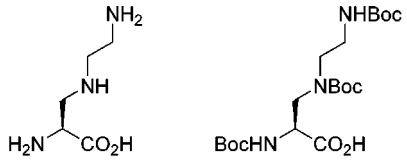
Anomalous amino acids not only play central roles as mimics of natural amino acids but also offer opportunities as unique building blocks for combinatorial chemistry. This paper describes the chiral syntheses and solid-phase applications of a versatile atypical amino acid, 4-azalysine (2,6-diamino-4-azahexanoic acid) **1**. The syntheses of differentially protected 4-azalysine derivatives **28a**−**e** have been developed by two efficient and inexpensive routes that start either from Garner's aldehyde **16** or the chiron (*S*)-N^{α}-Cbz-2,3-diaminopropionic acid **23**. Both approaches employ the convergent modular concept and exploit reductive amination of aldehydes with amines as the key step for the fusion of the two segments. In the first route, the overall process inverts the chirality of the starting material, L-serine, and thus provides an excellent route to the unnatural D-isomers. The alternative route starting from L-asparagine provides a shorter and high-yielding route to orthogonally protected 4-azalysine derivatives. The corresponding N^2-Fmoc-4-azalysines **31a**−**e**, readily derived from the key intermediate **27**, are compatible with the Fmoc-based solid-phase peptide synthesis (SPPS) and solid-phase organic chemistry (SPOC) protocols. Furthermore, the utility and versatility of another key structure, tris-Boc-4-azalysine **2** in the engineering of novel high-loading dendrimeric polystyrene resins **33** and **36**, have been demonstrated. Following derivatization with the Rink amide linker **34**, the stability and robustness of these resin-bound dendrimers **35** and **37** in the synthesis of small molecules using a range of reaction conditions (e.g., Mitsunobu and Suzuki reactions) have been effectively illustrated.

Introduction

Amino acids as one of nature's largest and most versatile pool of chiral molecules have been the source for the syntheses of many natural products and complex structures of biological and therapeutic importance.[2] Besides the 20 proteinogenic amino acids, over 700 atypical amino acids have been isolated from natural sources,[3] where these occur either in native states or as components of complex molecules such as peptides and pseudopeptides. The utility of these unique amino acids is 2-fold: first, many of these chiral molecules possess interesting pharmacological profiles, and second, they are useful chiral building blocks for the syntheses of novel molecular entities.[4] Moreover, amino acids can through structural modifications furnish further generations of unique amino acids. Many of these can serve as surrogates[5] of native amino acids and when incorporated in peptides may impart enhanced biological activities and stability to peptidases.

Recently, orthogonally protected multifunctional amino acids have found valuable applications in synthetic peptides and combinatorial chemistry.[6] For example, the N^{α} and side-chain amino functions of diamino acids, such as lysine and diaminopropionic acid, have been exploited in combinatorial chemistry for the construction of novel structures. The introduction of another amine functionality at the C-4 in the lysine framework would furnish 4-azalysine **1**. We envisaged that 4-azalysine **1** would not only to mimic the lysine residue but also offer opportunities for further diversification at the N-4 site to furnish unique building blocks for combinatorial chemistry.



1 **2**

Results and Discussion

Two routes for the synthesis of 4-azalysine have previously been described. One route by McCord[7] in-

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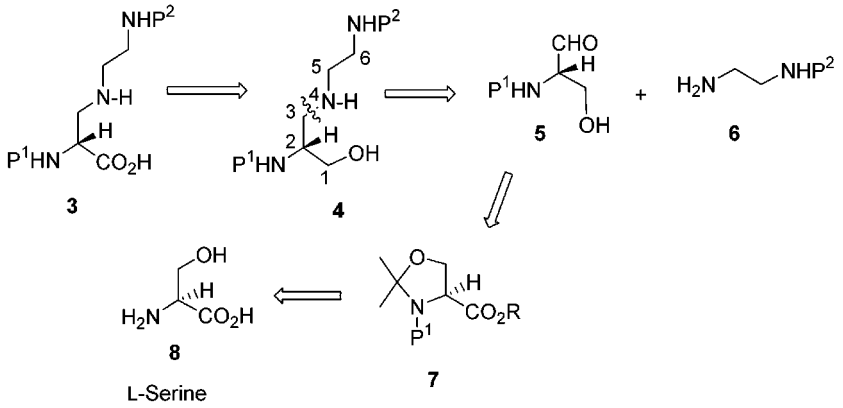
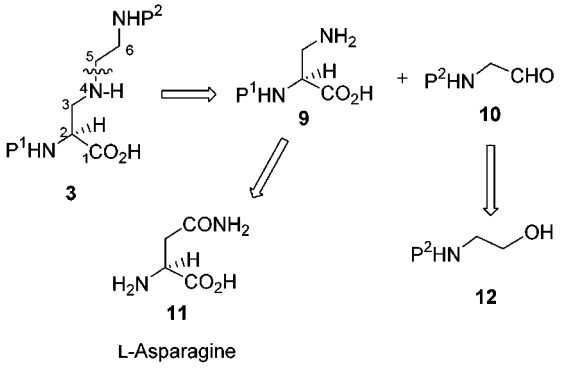
Scheme 1



Scheme 2



volved the conjugate addition of ethylenediamine to *N*-acetyldehydroalanine, which gave a racemic mixture. The second-described strategy[8] gave both enantiomers of 4-azalysine, starting from the corresponding N^{α}-tosyl-L- (or D)-α,*β*-diaminopropionic acid. It involved the cyanomethylation of the *β*-amino function, followed by the reduction of the nitrile with concomitant removal of the tosyl group, using Na in liquid NH_3 in the presence of MeOH, to afford the required 4-azalysine framework.

Herein we report the development of two flexible and inexpensive protocols for the assembly of differentially protected 4-azalysine **1** and its N^4-substituted derivatives. The potential of the synthesized 4-azalysine derivatives was established by their successful incorporation into an antibacterial hexapeptide sequence. Moreover, we envisaged that the N-protected triamino acid, tris-Boc-4-azalysine **2** could be exploited for the generation of higher order dendritic scaffolds on solid supports.

From the disconnection approach, two retrosynthetic routes were identified for the synthesis of the target molecule, N^{α},N^{ϵ}-diprotected 4-azalysine **3** (Schemes 1 and 2). Though both approaches employ the convergent modular concept, they differ by the disconnection at the C_3-N_4 and N_4-C_5 bond in Schemes 1 and 2, respectively. Of particular significance is that both strategies utilize readily available chirons, these being (*S*)-serine and (*S*)-asparagine. However, while (*S*)-asparagine ultimately affords (*S*)-4-azalysine (Scheme 2), (*S*)-serine in the contrasting approach outlined in Scheme 1 would result in the assembly of (*R*)-4-azalysine. Nevertheless, common in both cases is the fusion of the two core segments, an aldehyde and a primary amine, to afford the desired amines **3**.

First Strategy Using Garner's Aldehyde. The desired orthogonally $N^{\alpha,\epsilon}$-protected (*R*)-**3** is readily obtainable from the alcohol **4**. The latter when disconnected at the C_3-N_4 bond (Scheme 1) generates two fragments, an aldehyde fragment **5** and an amine fragment **6**. The aldehyde component **5** can in turn be derived from the fully protected methyl (*S*)-oxazolidine-4-carboxylate **7**, which is readily manufactured from the amino acid, (*S*)-serine. Controlled reduction of the ester **7** would afford an aldehyde module, commonly known as the Garner's aldehyde.[9-11]

Although most of the earlier works on Garner's aldehyde employ the Boc group as the N-protecting group,[9,10] we decided to employ the Cbz protection for the α-amino function, primarily due to its stability toward acidic conditions. Thus, *N*-Cbz-serine **13** was synthesized from serine by acylation with an excess of benzyl chloroformate, under basic conditions (2 M aqueous NaOH) in a good yield of 78% (Scheme 3). The free carboxyl function of **13** was next protected as a methyl ester. From the various available methods of preparing methyl esters, the initial attempts to synthesize **14** by esterification of *N*-CbzSerOH **13** with methanolic HCl gave low yield of the product. The base-catalyzed esterification[12] using methyl iodide in the presence of potassium carbonate in DMF was then attempted, which proceeded smoothly and was complete in 5 h. The product *N*-CbzSerOMe **14** was obtained as a pale amber colored oil in 79% yield, which solidified on refrigeration.

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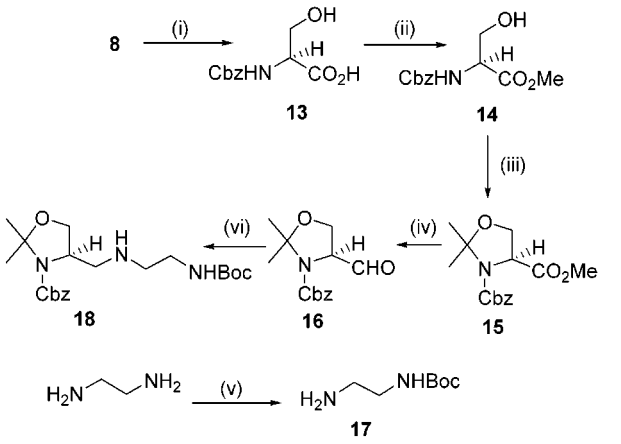
Scheme 3[a]



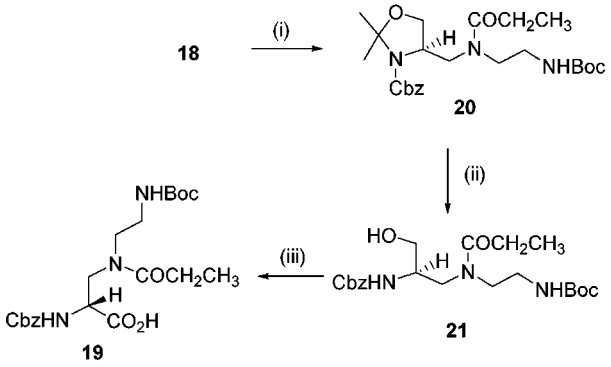
[a] Key: (i) CbzCl, aq NaOH; (ii) MeI, K_2CO_3; (iii) DMP, TsOH; (iv) DIBALH, toluene, −78 °C; (v) $(Boc)_2O$, TEA; (vi) **17**, $NaCNBH_3$, MeOH/AcOH, 99:1.

The protection of both the β-OH and the α-carbamate NH functionalities was then simultaneously achieved through oxazolidine formation, by refluxing a solution of the methyl ester **14** in 2,2-dimethoxypropane with catalytic amounts of TsOH monohydrate.[9,10] The ^{1}H NMR spectrum of oxazolidine methyl ester **15**, obtained in 81% yield, showed two sets of signals at ambient temperature for the ring Me_2, the methyl ester group, and the α-proton, indicating the existence of slowly interconverting conformers on the NMR time scale. The two conformers were seen to be in an approximate ratio of 2.5:1. Both the ring and the methylene protons of the Cbz group appeared as complex multiplets due to locked conformation. However, an average NMR spectrum could be obtained by raising the probe temperature to 75 °C when the signals merged into one set, suggesting the existence of a dynamic equilibrium. On the other hand, cooling the sample restored the spectrum to its original complexity. This is a general phenomenon that has previously been reported for the oxazolidine system[9a,13] and possibly indicative of tertiary carbamate rotamers.

The ester functionality of the oxazolidine methyl ester **15** was then reduced to the oxazolidine aldehyde **16**, using DIBALH in dry toluene at −78 °C under an inert atmosphere.[13a,14,15] On completion, the reaction mixture was quenched with methanol, and the crude aldehyde was purified by flash chromatography on a silica column affording the product in 58% yield. The ^{1}H NMR spectrum again showed the existence of conformers, as evident from two sets of signals at room temperature.

Having successfully synthesized the *N*,*O*-isopropylidene serinal **16**, the next task was to selectively monoprotect diaminoethane to afford **5**. Initial attempts to prepare N-protected diaminoethane **17** according to the method of Chan et al.[16] using $(Boc)_2O$ in MeOH gave a low yield (30−35%) of the desired monoprotected diaminoethane, with the formation of the bis-Boc derivative as the major product. Subsequently, mono-Boc protection of diaminoethane was accomplished according to the method of Huang et al.[17] to afford **17** in an improved yield of 64%.

Scheme 4[a]



[a] Key: (i) propanoic acid, DCC; (ii) (a) MeOH, TsOH, (b) $(Boc)_2O$, $NaHCO_3$; (iii) Jones reagent, acetone.

With the oxazolidine aldehyde **16** and the N-protected diaminoethane **17** in hand, condensation of the two components to deliver the 4-azalysine framework was investigated. This was successfully accomplished using $NaCNBH_3$ as the reducing agent, to give **18** in 35% yield. The synthesis of **18** completes the framework of 4-azalysine. The newly generated secondary amine can now be readily protected or derivatized before the unmasking of the carbamate NH and hydroxymethyl functionalities. Thus, a model compound **19** was prepared. This was achieved by, first, acylation of **18** with propionic acid in the presence of DCC as an activating agent to give the intermediate *N*-propionyl derivative **20** (Scheme 4). The ^{1}H NMR of the product revealed a complex pattern of multiplets arising in the regions of δ 0.90−1.40 and 2.10−2.50 corresponding to the CH_3 and CH_2 groups, respectively, of the propionyl substituent. These multiplets are a result of the restricted rotation imposed by the ring system.

Initial attempts were then made to carry out selective removal of the isopropylidene protection under mild conditions using methanolic TsOH[9,10] at ambient temperature. However, the deprotection reaction was found to be incomplete after 18 h. Furthermore, when the reaction mixture was refluxed for 1 h, partial formation of Boc-deprotected product was evident. Hence, it was apparent that the selective removal of the isopropylidene protection was not possible, and a global acidolytic deprotection was carried out. The crude Cbz-protected triamino alcohol was not isolated, and the terminal amine was reacylated with $(Boc)_2O$ to form the desired orthogonally protected triamino alcohol **21,** which was isolated in 78% yield. The alcohol **21** was then oxidized by Jones' reagent[18,19] to afford the desired differentially protected 4-azalysine derivative **19** in 64% yield.

This synthetic route, though efficient, suffers from the obvious limitations, in particular the difficulties encountered in the unmasking acidolysis step. Hence, an alternative strategy that is less cumbersome and provides an efficient route to 4-azalysine and its derivatives was developed.

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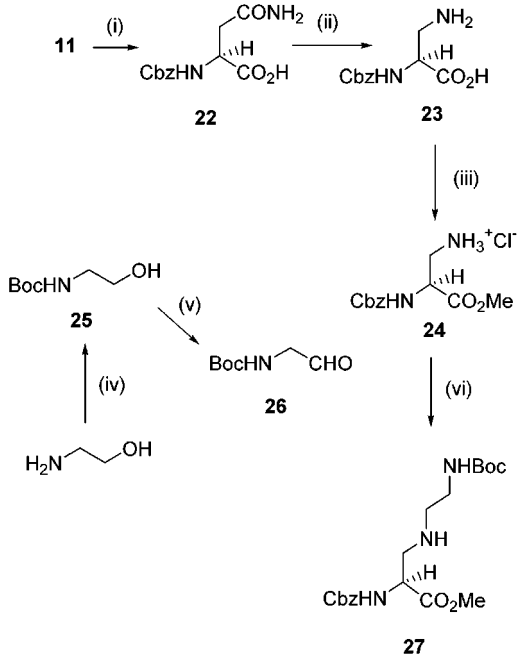
Second Strategy Using the Chiron (*S*)-2,3-Diaminopropionic Acid. In the first synthetic strategy to achieve **3**, the disconnection was made at the C_3-N_4 bond. Alternatively, the protected 4-azalysine may be disconnected at the N_4-C_5 bond (Scheme 2), generating the two components, an amine component **9**, and the aldehyde component **10**. Specifically, it was envisaged that reductive alkylation of methyl N^2-Cbz-2,3-diaminopropionate **24** with *N*-Boc-glycinal **26** would furnish the structural backbone for the target molecule **3**.

The N-protected 2,3-diaminopropionic acid **24** could be synthesized from the commercially available L-asparagine by, first, protection of the α-amino function, followed by oxidation of the side-chain amide functionality.[20−22] The aldehyde **26** is an N-protected glycinal that may be synthesized either from N-protected glycine via reduction of its hydroxamate derivative[23] or by oxidation of N-protected aminoethanol[24] **25**. In contrast to the first retrosynthetic route (Scheme 1), in this disconnection approach the source of chirality for the target molecule resides in the amine component **23**.

First, (*S*)-asparagine was acylated with benzyl chloroformate to yield *N*-CbzAsnOH **22**. The conversion of the amide functionality in **22** to the amine was effected by oxidation employing hypervalent iodine reagents. Thus, the desired product **23** was prepared by the use of either bis(trifluoroacetoxy)phenyl iodine[20] in DMF/H_2O or iodobenzene diacetate[21] in EtOAc/MeCN/H_2O solvent system (Scheme 5). Though both reagents gave excellent yields, the latter was preferred as it avoids the use of DMF and the unpleasant base, pyridine. The acid functionality of (*S*)-N^2-protected 2,3-diaminopropionic acid **23**[25]was then protected as a methyl ester by stirring overnight in methanolic HCl[26] at 5 °C, to afford the required chiron, methyl (*S*)-N^2-Cbz-2,3-diaminopropionate hydrochloride **24** as a white crystalline solid in 78% yield.

The synthesis of *N*-Boc-glycinal **26** was initially attempted using the method Salvi et al.,[23] which involved the $LiAlH_4$-mediated reduction of *N*,*O*-dimethyl N^{α}-*tert*-butoxycarbonylglycylhydroxamate. However, the desired derivative **26** was obtained in poor yield. Hence, *N*-Boc-2-aminoethanol **25**, prepared by acylation of 2-aminoethanol with $(Boc)_2O$ in 77% yield, was oxidized to the aldehyde **26** in 55% yield using *o*-iodoxybenzoic acid (IBX). The choice of IBX over the other oxidants was due to its versatility and mildness in the oxidation of alcohols to aldehydes.[27] During the course of our investigations, it was found that only dilute solution (5%) of the reactants in DMSO afforded a good yield (60−70%) of the required aldehyde **26**. Reactions carried out in concentrated solutions favored dimerization yielding the bis-Boc-diketopiperazine, which was established by the ^1H NMR spectrum of the isolated product.

Scheme 5[a]



[a] Key: (i) CbzCl, $NaHCO_3$; (ii) $PhI(COOCF_3)_2$, DMF/H_2O; (iii) methanolic HCl, 5 °C; (iv) $(Boc)_2O$; (v) IBX, DMSO; (vi) **26**, $NaCNBH_3$, MeOH/AcOH, 99:1.

With the two components in hand, reductive alkylation of the amine **24** with the aldehyde **26** in the presence of the reducing agent $NaCNBH_3$ was then carried out. The reaction was achieved using only 0.9 molar equiv of the aldehyde to avoid the possibility of dialkylation. The reaction proceeded smoothly in 1% AcOH in MeOH, with $NaCNBH_3$ being added in portions over a period of 45 min and was complete after overnight stirring at room temperature. The product **27** was isolated as a clear transparent oil in 65% yield. The three methylene groups in the ^1H NMR of the **27** appeared as three separate sets of multiplet signals at δ 2.40−2.70, 2.70−3.00, and 3.00−3.20. The identity of the multiplet at δ 2.70−3.00 for β-CH_2 was ascertained by spin decoupling of the α-proton signal.

It is apparent that this strategy provides a shorter and higher yielding (overall yield 45%) route to orthogonally protected 4-azalysine derivatives. Its merits over the first strategy are its convenience, chemical efficiency, and reproducibility in the multigram scale preparation of the key intermediate **27**. Having successfully synthesized the orthogonally protected 4-azalysine **27**, a series of chemical modifications, including N^4-derivatization, were carried out to afford derivatives suitable for Fmoc-based solid-phase chemistry.[28]

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(25) Since the ultimate aim was to produce N^2-Fmoc-4-azalysine derivatives in readiness for solid-phase synthesis, efforts were also made to prepare the N^2-Fmoc-2,3-diaminopropionic acid by $PhI(OAc)_2$-mediated oxidation of FmocAsnOH. However, the desired product was only obtained in very low yield and purity.
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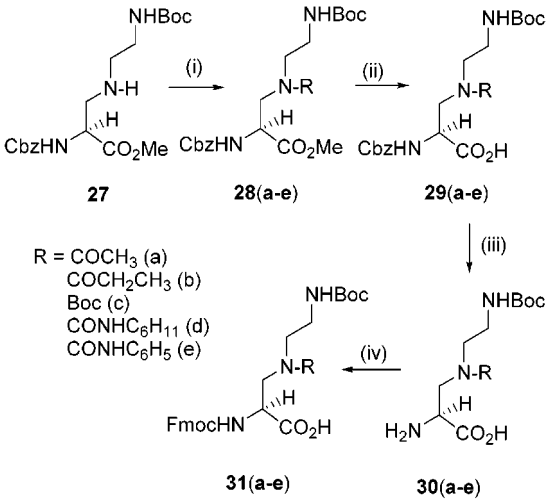
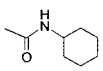
Scheme 6[a]



[a] Key: (i) N^4-derivatization; (ii) NaOH or LiOH, MeOH/H_2O; (iii) H_2, 10% Pd−C, MeOH; (iv) FmocOSu, $NaHCO_3$ (aq).

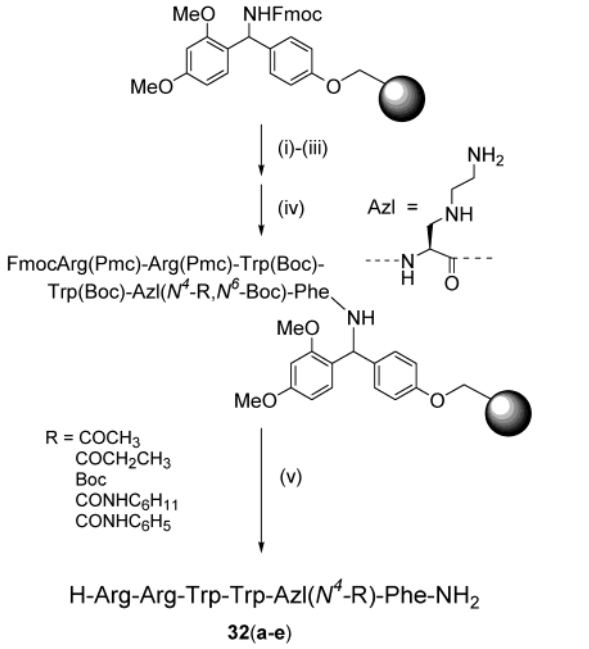
Table 1. Preparation of N^4-Substituted Methyl (*S*)-N^2-Cbz-N^6-Boc-4-azalysinate 28a−g

	N^4-substituents	Reaction conditions	Yield (%)
28a	$COCH_3$	Ac_2O, CH_2Cl_2, 4 h	62
28b	$COCH_2CH_3$	$EtCO_2H$, DCC, CH_2Cl_2, 4 h	51
28c	Boc	$(Boc)_2O$, CH_2Cl_2, 18 h	85
28d		$C_6H_{11}N{=}C{=}O$, Et_2O, 5 h	79
28e	CONHPh	$PhN{=}C{=}O$, Et_2O, 5 h	69
28f		$C_3H_5N{=}C{=}S$, MeOH, 18 h	59
28g	Me	HCHO, $NaCNBH_3$, MeCN, AcOH, 1 h	70

The N^4-secondary amine function in **27** was derivatized to produce a focused range of *N*-acyl, *N*-alkyl, *N*-carbamoyl, and *N*-thiocarbamoyl derivatives (Scheme 6), and the results are summarized in Table 1. Interestingly, the methylene protons of the Cbz group in **28a**,**b**,**d**−**f** appeared as an AA′BB′ quartet instead of a singlet as in **27**. This nonequivalency of the two methylene protons probably arises from the anisotropic effect of the carbonyl function of the N^4-substituent.

The preparation of the free acids **29** from **28** was straightforward and was effected by saponification with either NaOH or LiOH in aqueous methanol. Typically, the use of LiOH gave cleaner products in yields ranging from 61 to 98% (Scheme 6). The base-catalyzed hydrolysis of the *N*-methyl derivative **28g** to **29g**, however, involved difficult product isolation due to quaternization of the N^4-methyl upon acidification, giving very low yields of the desired product on extraction with an organic solvent; attempts of pH-controlled acidification were unsuccessful. The N^2-Cbz protection in **29a**−**e** was then removed by hydrogenolysis in the presence of 10% palladium over charcoal as a catalyst. The products **30a**−**e** were isolated as glassy solids, which on triturating with hexane gave the desired products as white solids in good to excellent yields (78−97%). Although the reaction was successful

Scheme 7[a]



[a] Key: (i) 20% piperidine/DMF; (ii) FmocPheOH, HOBt, TBTU, DIEA, DMF; (iii) FmocAzl(N^4-R-N^6-Boc)OH, HOBt, TBTU, DIEA, DMF; (iv) peptide synthesis; (v) 95% TFA/water.

for the derivatives **29a**−**e**, the *N*-cyclopropylthiocarbamoyl derivative **29f** failed to undergo hydrogenolysis even in the presence of high percentage (50%) of the catalyst due to the presence of sulfur in **29f** poisoning the catalyst.[29] Finally, the protection of the α-amino function of compounds **30a**−**e** with the Fmoc group was readily achieved by acylation with FmocOSu in the presence of $NaHCO_3$ in dioxane/water mixture affording compounds **31a**−**e** in 51−88% yields.

To demonstrate the utility and compatibility of the Fmoc-protected 4-azalysine derivatives in solid-phase peptide synthesis, the synthesized derivatives were incorporated in an antibacterial hexapeptide sequence,[30] Ac-Arg-Arg-Trp-Trp-Arg-Phe-NH_2. This sequence has been isolated from a synthetic peptide combinatorial library consisting of 34 million hexapeptides through an iterative selection process and exhibits potent antimicrobial activity. Hence, the sequence was chosen as a template, and the arginine residue at position-5 was replaced by the synthesized 4-azalysine derivatives. It was found that the novel amino acids could be readily introduced in the sequence using standard coupling conditions of TBTU/HOBt.[28]

Scheme 7 summarizes the various steps toward the assembly of the sequence. Cleavage of the assembled peptide amides from the resin using 95% TFA/water followed by lyophilization from water gave the required hexapeptide sequences **32a**−**e** in satisfactory yields. Analysis of the latter by ES-MS gave the expected molecular ions. HPLC analyses of the peptide amides in a 0.06% TFA−acetonitrile−water system revealed that the compounds were essentially pure (90−95%).

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(30) Houghten, R. A.; Blondelle, S. E.; Cuervo, J. H. In *Innovation and Perspectives in Solid-Phase Synthesis: Peptides, Polypeptides and Oligonucleotides*; Epton, R., Ed.; Intercept Ltd.: Andover, 1992; pp 237−239.

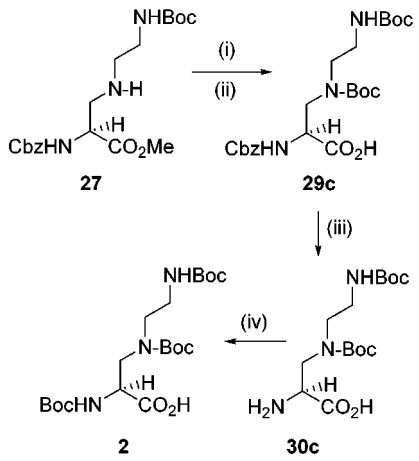
Chemically Engineered Dendrimeric Solid Supports. Dendrimer research in the recent years has flourished into an area of considerable interest.[31,32] The solution-phase synthesis of dendrimers is either by the "divergent approach" implying sequential monomer addition starting from a core and proceeding outward toward the macromolecular surface[33] or by the "convergent approach" whereby small fragments of the dendrimer are assembled on a multifunctional core.[34]

Both the convergent and divergent approaches in solution phase have been exploited in the development of a range of interesting and valuable dendritic systems. However, their solution-phase synthesis is very labor intensive due to complex purification procedures. Syntheses of dendrimers on solid supports leading to highly homogeneous structures obviate the drawbacks associated with the solution phase methods.[32,35] Dendrimerization on resin supports has also been reported as a practical and useful method to enhance bead loading. Furthermore, the use of orthogonally protected building blocks for dendrimer synthesis on solid supports offers a robust approach to unsymmetrical dendritic structures.

Since lysine has been used as a building block in the generation of dendrimeric structures and that dendrimers generated on solid supports could be used as high-loading inert scaffolds for solid-phase chemistry,[32] it was anticipated that the unique tetrafunctional triamino acid 4-azalysine would be an ideal starting material for dendritic systems. The tris-Boc-protected **2** could be exploited as the starter monomer in the synthesis of inert dendrimeric core with good homogeneity on solid supports. It was further envisaged that the presence of a third amine functionality at a strategic position in the lysine side chain would contribute to a rapid increase in the number of reactive amine functionalities, and the synthesized dendrimer core could serve as an excellent functionality enhancer.

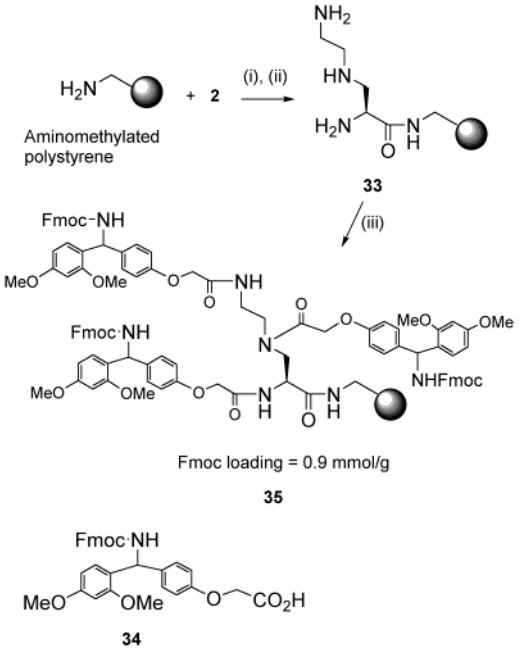
With this in mind, a robust route to $N^{\alpha},N^{\gamma},N^{\epsilon}$-tris-Boc-4-azalysine **2** was developed. An initial attempt to synthesize **2** from the bis-Boc derivative **29c** by catalytic hydrogenolysis in the presence of $(Boc)_2O$ in a "one-pot" procedure[36] was unsuccessful. The product obtained was either N^{α},N^{ϵ}-bis-Boc and/or N^{α},N^{α}-bis-Boc derivative of **30c**, which was established by ES-MS (observed MH^+ 548) and ^1H NMR. Thus, a two-step procedure was utilized. Catalytic hydrogenolysis of **29c** gave first the bis-Boc triamino acid **30c** in 97% yield, which was followed by acylation with $(Boc)_2O$ to afford the required tris-Boc-4-azalysine **2** (Scheme 8) in 42% yield.

Scheme 8[a]



[a] Key: (i) $(Boc)_2O$, TEA; (ii) NaOH (aq), MeOH; (iii) H_2, 10% Pd−C, MeOH; (iv) $(Boc)_2O$, TEA.

Scheme 9[a]



[a] Key: (i) HOAt, DIPCDI, DMF; (ii) 50% TFA in DCM, TES; (iii) Rink amide linker **34**, HOAt, DIPCDI, DMF.

Solid-phase synthesis of dendrimers started with the attachment of $N^{\alpha},N^{\gamma},N^{\epsilon}$-tris-Boc-4-azalysine **2** to the commercially available aminomethyl polystyrene (PS) resin (0.8 mmol g^{-1}) via the DIPCDI−HOAt-mediated coupling (Scheme 9). A negative amine test (2,4,6-trinitrobenzenesulfonic acid, TNBS test[37]) on the resin beads indicated that successful coupling had occurred after 3 h. Complete removal of the three Boc protecting groups was effected by treatment of the resin with 50% TFA in CH_2Cl_2 in the presence of triethylsilane (TES) as a scavenger, and was followed by neutralization of the resin material with 10% DIEA in DMF, giving the first-generation amines **33**. At this stage, a recovery yield of 97% was calculated. Based on the mass of 4-azalysine residue attached to the aminomethyl PS resin and the fact that there is a 3-fold increase in the number of

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(32) (a) Roberts, J. C.; Bhalgat, M. K.; Zera, R. T. *J. Biomed. Materials Res.* **1996**, *30*, 53−65. (b) Swali, V.; Wells, N. J.; Langley, G. J.; Bradley, M. *J. Org. Chem.* **1997**, *62*, 4902−4903. (c) Chow, H.-F.; Mong, T. K. K.; Nongrum, M. F.; Wan, C.-W. *Tetrahedron* **1998**, *54*, 8543−8660. (d) Wells, N. J.; Basso, A.; Bradley, M. *Biopolymers* **1998**, *47*, 381−396.

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(34) (a) Hawker, C. J.; Frechet, J. M. J. *J. Am. Chem. Soc.* **1990**, *112*, 7638−7647. (b) Zeng, F.; Zimmerman, S. C. *J. Am. Chem. Soc.* **1996**, *118*, 5326−5327.

(35) (a) Bielinska, A. U.; Kukowska-Lattallo, J. F.; Baker, J. R. *Biochim. Biophys. Acta* **1997**, *1353*, 180−190. (b) Cooper, R. G.; Etheridge, C. J.; Stewart, L.; Marshall, J.; Rudginsky, S.; Cheng, S. H.; Miller, A. D. *Chem. Eur. J.* **1998**, *4*, 17−42.

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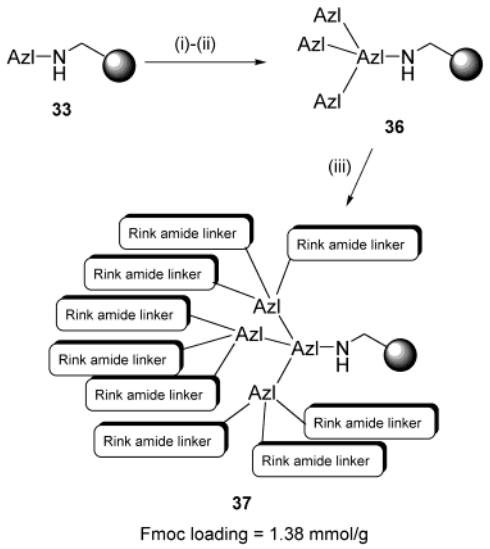
(37) Hancock, W. S.; Battersby, J. E. *Anal. Biochem.* **1976**, *71*, 260−264.

Scheme 10[a]



[a] Key: (i) **2**, HATU, DIEA, DMF; (ii) 50% TFA/DCM, TES; (iii) Rink amide linker **34**, HOAt, DIPCDI, DMF.

reactive amine functionalities, a theoretical loading of 2.18 mmol g^{-1} was calculated for the first-generation resin **33**. Compared to the unmodified resin with a loading of 0.8 mmol g^{-1}, the latter shows an increase of more than two-and-a-half times in just one single step of coupling and deprotection. To determine the substitution level by Fmoc-loading, the Fmoc-protected Rink amide linker[38] **34** was coupled onto the resin **33** using the DIPCDI−HOAt activation method giving the derivatized PS resin **35**. The efficiency of all the coupling steps was monitored using the TNBS test. Significantly, the resin **35** exhibited a substitution of 0.9 mmol/g (overall yield 89%) based on spectrophotometric determination of the Fmoc-derived chromophore[28] liberated upon treatment with 20% piperidine in DMF.

Repeated acylation of the resin **33** with tris-Boc-4-azalysine **2** using the aminium coupling reagent HATU followed by TFA-mediated acidolysis and neutralization with 10% DIEA in DMF gave the second-generation dendrimeric amines **36** in an overall recovery yield of 96% (Scheme 10). Again, based on the mass of three 4-azalysine residues attached onto the three amine functionalities of the first-generation resin **33** and the fact that now there is a 9-fold increase in the number of reactive amine functionalities compared to the starting unmodified resin, a theoretical loading of 3.38 mmol g^{-1} was calculated for the second-generation resin beads **36**. Compared to the underivatized resin, the second-generation dendrimeric beads show more than a 4-fold increase in the loading. Gratifyingly, attachment of the Rink amide linker to resin **36** afforded the resin **37**, which exhibited a considerably increased loading of 1.38 mmol g^{-1} (overall yield 84% in five steps) based on UV analysis of the Fmoc-deprotection product.

Thus, the high-loading dendrimeric resins of first- and second-generation were readily obtained, and the coupling of a linker at the secondary amine functionality was readily achieved. Thus, the method described serve both as an efficient means of enhancing bead loading and as a method of dendrimer synthesis. Although the loading

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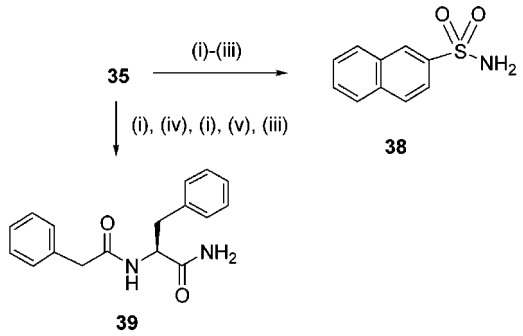
Scheme 11[a]



[a] Key: (i) 20% piperidine/DMF; (ii) 2-naphthalenesulfonyl chloride, DIEA, DMF; (iii) 90% TFA/water; (iv) FmocPheOH, HATU, DIEA, DMF; (v) $PhCH_2CO_2H$, HOBt, TBTU, DIEA.

per gram of the resin does not increase substantially due the mass of compound attached to the resin, the loading per reaction bead increases geometrically from one generation to the next. To assess the physical appearance of the resin beads, the unmodified aminomethyl resin beads and the second-generation dendrimer beads were swollen in CH_2Cl_2 and analyzed under an optical microscope with 100× magnification. The average diameter (number of beads examined = 25) of the second-generation dendrimer beads was found to be 110 μm while that of the unmodified resin beads was 65 μm.[1b] The second-generation solvated dendrimer beads are clearly larger in size compared to the parent aminomethylated polystyrene.

To evaluate the utility of the high-loading resins in SPOC[39] and the stability of these supports to a range of reaction conditions, small organic molecules were synthesized and characterized. We envisaged that the development of such methodologies would harness the innate advantages of dendrimers as high-yielding supports for SPOC. The high-loading efficiency of the dendrimeric resins was assessed by the synthesis of a representative range of small molecules such as 2-naphthalenesulfonamide **38**, N^{α}-phenylacetylphenylalaninamide **39**, N^{α}-4-methoxybenzenesulfonyl-N^{α}-benzylleucinamide **40** via the Mitsunobu[40] reaction, and N^{α}-(4-phenylbenzoyl)leucinamide **41** via the Suzuki[41] cross-coupling reaction.

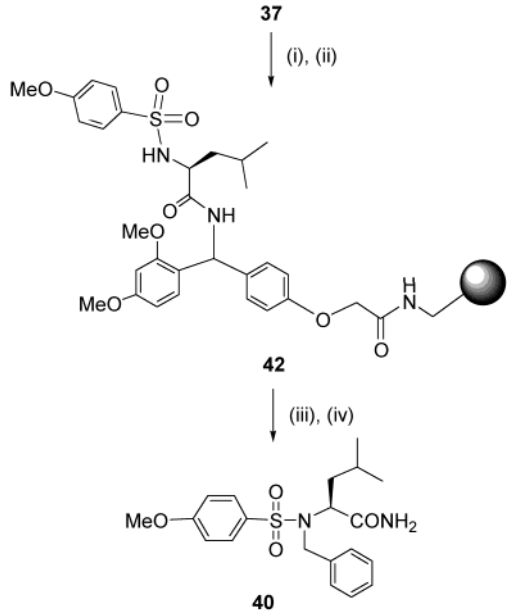
For the synthesis of **38**, resin **35** was subjected to Fmoc deprotection followed by reaction with 2-naphthalenesulfonyl chloride in the presence of DIEA to give the derivatized resin, which on treatment with 90% TFA−water yielded the desired product **38** in 85% yield (Scheme 11). For the synthesis of compound **39**, the resin **35** was treated with 20% piperidine−DMF followed by acylation with FmocPheOH using HATU−DIEA-mediated coupling conditions. The resin product showed a loading of 0.9 mmol g^{-1}. Fmoc deprotection followed by derivatization with phenylacetic acid afforded the desired resin product. Compound **39** was then released by aci-

(39) (a) Thompson, L. A.; Ellman, J. A. *Chem. Rev.* **1996**, *96*, 555−600. (b) Patel, D. A.; Gordon, E. M. *Drug Discovery Today* **1996**, *1*, 134−144. (c) Schultz, J. S. *Biotechnol. Prog.* **1996**, *12*, 729−743. (d) Bunin, B. A. *The Combinatorial Index*; Academic Press: London, 1998. (e) James, I. W. *Tetrahedron* **1999**, *55*, 4855−4946.

(40) Mitsunobu, O.; Yamada, M. *Bull. Chem. Soc., Jpn.* **1967**, *40*, 2380−2382.

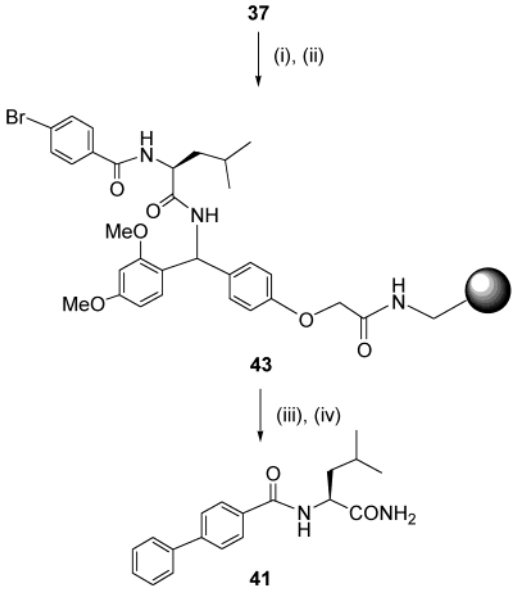
(41) (a) Miyaura, N.; Suzuki, A. *Chem. Rev.* **1995**, *95*, 2457−2483. (b) Miyaura, N.; Yanagi, T.; Suzuki, A. *Synth. Commun.* **1981**, *11*, 513−519.

Scheme 12[a]



[a] Key: (i) (a) 20% piperdine/DMF, (b) FmocLeuOH, HATU, DIEA, DMF; (ii) (a) 20% piperidine/DMF, (b) 4-methoxybenzenesulfonyl chloride, DIEA, DMF; (iii) $Me_2NCON{=}NCONMe_2$, Bu_3P, BnOH, THF; (iv) 95% TFA/water.

Scheme 13[a]



[a] Key: (i) (a) 20% piperdine/DMF, (b) FmocLeuOH, HATU, DIEA, DMF; (ii) (a) 20% piperdine/DMF, (b) 4-bromobenzoic acid, HOAt, DIPCDI, DMF; (iii) phenylboronic acid, $Pd(PPh_3)_4$, 2 M Na_2CO_3 (aq), DMF, reflux; (iv) 95% TFA/water.

dolysis in 71% yield (Scheme 11). The above reaction sequences were also repeated on the dendrimeric resin **37**, and compounds **38** and **39** were obtained in good yields on cleavage. To further validate the robustness of the high-loading dendrimeric resins in SPOC, compounds **40** and **41** were synthesized via two transformations widely used in organic synthesis, the Mitsunobu and Suzuki cross-coupling reactions.

Of the various azo reagents reported,[40,42] TMAD when used in combination with Bu_3P has been found to be highly effective in the Mitsunobu alkylation reactions.[43] Hence, resin **37** was Fmoc deprotected, washed extensively with DMF, and then N-acylated with FmocLeuOH using HATU−DIEA for activation (Scheme 12). Removal of the Fmoc group followed by N-sulfonation was achieved by overnight reaction with 4-methoxybenzenesulfonyl chloride in the presence of DIEA to give **42**. The dried resin **42**, suspended in dry THF, was treated overnight with TMAD, Bu_3P, and benzyl alcohol to give the derivatized resin. The desired product **40** was then selectively cleaved from the engineered solid support by treatment with TFA/water (95:5). Notably, from 50 mg of our linker-modified dendrimeric resin **37**, 23 mg of the target compound **40** was obtained in high purity.

Biaryl fragments represent one class of structural motif that has been the focus of considerable synthetic interest as an important pharmacophore present in many biologically active molecules.[44] Due to the mildness of the reaction and its tolerance of a wide scope of functionalities, the Suzuki coupling reaction has been extensively exploited on solid supports as a key C−C bond-forming reaction.[41,45] Hence, we subjected our new dendrimerized resin **37** to Suzuki reaction conditions (Scheme 13). Following standard coupling and deprotection steps, the functionalized resin **43** bearing a bromophenyl moiety was treated with phenylboronic acid in the presence of $Pd(PPh_3)_4$ and aqueous Na_2CO_3 in DMF. Acidolytic cleavage from the resin gave the desired product **41** in 78% yield and excellent purity when analyzed by RP-HPLC.

Conclusions

We have successfully elaborated the desired 4-azalysine framework **1** via two routes. In the first synthetic strategy, as the overall process inverts the chirality of the starting material, the approach provides an excellent route to the D-isomers starting from the naturally occurring L-serine. The methodology described utilizes Garner's aldehyde and the readily available diamines as the key components for the construction of **1**. This route has the advantage that by using higher diaminoalkanes, the distance between the secondary amine at N^4 and that of the side-chain amine functionality at N^6 can be easily exploited to generate new families of tri-amino acids. The alternative synthetic strategy starting from L-asparagine provided a shorter (six steps) and higher yielding (overall yield 45%) route to orthogonally protected 4-azalysine

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(44) For example: (a) Chang, L. L.; Ashton, W. T.; Flanagan, K. L.; Strelitz, R. A.; MacCoss, M.; Greenlee, W. J.; Chang, R. S. L.; Lotti, V. J.; Faust, K. A.; Chen, T. B.; Bunting, P.; Zingora, G. J.; Kivlighn, S. D.; Siegl, P. K. S. *J. Med. Chem.* **1993**, *36*, 2558−2568. (b) Pavia, M. R.; Cohen, M. P.; Dilley, G. J.; Dubuc, G. R.; Durgin, T. L.; Forman, F. W.; Hediger, M. E.; Milot, G.; Powers, T. S.; Sucholeiki, I.; Zhou, S.; Hangauer, D. G. *Bioorg. Med. Chem. Lett.* **1996**, *4*, 659−666. (c) Piettre, S. R.; Baltzer, S. *Tetrahedron Lett.* **1997**, *38*, 1197−1200.

(45) (a) Backes, B. J.; Ellman, J. A. *J. Am. Chem. Soc.* **1994**, *116*, 11171−11172. (b) Guiles, J. W.; Johnson, S. G.; Murray, W. V. *J. Org. Chem.* **1996**, *61*, 5169−5171. (c) Ruhland, B.; Bombrun, A.; Gallop, M. A. *J. Org. Chem.* **1997**, *62*, 7820−7826. (d) Han, Y.; Giroux, A.; Lepine, C.; Laliberte, F.; Huang, Z.; Perrier, H.; Bayly, C. I.; Young, R. N. *Tetrahedron* **1999**, *55*, 11669−11685. (e) Atkinson, G. E.; Fischer, P. M.; Chan, W. C. *J. Org. Chem.* **2000**, *65*, 5048−5056.

derivatives. The chirality of the target molecule synthesized via this route remains the same as that of the starting molecule L-asparagine. Clear merits of the second synthetic strategy over the first one are its convenience and chemical efficiency.

We have also successfully demonstrated the utility and versatility of the tris-Boc-4-azalysine **2** in the engineering of novel high-loading dendrimeric resins. Furthermore, the stability and robustness of these resin-bound dendrimers in the synthesis of small molecules using a range of reaction conditions has been effectively illustrated.

Experimental Section

General Methods. See the Supporting Information for detailed information. Routine ^{1}H NMR spectra were recorded on a spectrometer operating at 250 MHz. The spectra were recorded, unless otherwise stated, in deuteriochloroform, and the chemical shifts given in δ values are relative to an internal tetramethylsilane standard. Coupling constants are reported in hertz. ^{13}C NMR spectra were recorded in deuteriochloroform, unless otherwise stated, on a spectrometer operating at 62.9 MHz. The chemical shifts are reported relative to an internal deuteriochloroform standard on a broad band decoupled mode.

3-Benzyl 4-Methyl (*S*)-2,2-Dimethyloxazolidine-3,4-dicarboxylate (15). To a stirred solution of *N*-benzyloxycarbonyl-L-serine methyl ester **14** (3.0 g, 12 mmol) in 2,2-dimethoxypropane (25.4 g, 244 mmol) was added TsOH monohydrate (0.3 g, 1.5 mmol). The reaction mixture was refluxed for 3 h and then evaporated in vacuo. The residue was redissolved in ethyl acetate (75 mL) and washed with aqueous $NaHCO_3$ (2 × 30 mL). The organic layer was dried ($MgSO_4$) and evaporated in vacuo and the residue purified by column chromatography (10−50% ethyl acetate/hexane) to afford the oxazolidine methyl ester **15** as a yellow oil (2.8 g, 81%):[19] TLC (ethyl acetate/hexane, 1:1) $R_f = 0.76$; $[\alpha]^{22}_D$ −55.3 (*c* 1.02, MeOH) [lit.[46] $[\alpha^{27}]_D$ −55.9 (*c* 2.7, MeOH)]; ^{1}H NMR shows a mixture of conformational isomers in the ratio of 2.5:1 (A/B); δ_H isomer A 1.54, 1.71 (6H, 2 s), 3.64 (3H, s), 4.00−4.22 (2H, m), 4.48 (1H, dd, *J* 2.5, 7.5), 5.00−5.20 (2H, m), 7.25−7.39 (5H, m); isomer B 1.47, 1.64 (6H, 2 s), 3.73 (3H, s), 4.00−4.22 (2H, m), 4.56 (1H, dd, *J* 2.5, 7.5), 5.00−5.20 (2H, m), 7.25−7.39 (5H, m); δ_C 23.34, 24.24, 24.42, 25.37, 51.51, 51.57, 58.14, 58.89, 65.37, 65.80, 65.90, 66.66, 93.95, 94.59, 126.99, 127.27, 127.33, 127.47, 127.72, 127.86, 135.57, 135.85, 150.98, 151.96, 170.20, 170.46; *m*/*z* (ES-MS) 294 (MH^+), calcd 294.1.

Benzyl (*S*)-4-Formyl-2,2-dimethyloxazolidine-3-carboxylate (16). All glassware were dried overnight. To a stirred solution of oxazolidine methyl ester **15** (2.2 g, 7.6 mmol) in dry toluene (15 mL) under nitrogen was added DIBALH (25% solution in toluene, 8.86 mL) by means of a syringe at −78 °C over a period of 1 h. After the reaction mixture had been stirred for 2 h at −78 °C under nitrogen, it was slowly quenched with methanol (3 mL) and warmed to room temperature. The solvents were then removed in vacuo, and the residue was redissolved in ethyl acetate and washed with 1 M aqueous $KHSO_4$ (2 × 50 mL) followed by brine (1 × 50 mL). The organic extract was dried ($MgSO_4$) and concentrated in vacuo to yield the crude oxazolidine aldehyde **16** as a yellow oil. Purification by column chromatography (diethyl ether/hexane, 3:1) afforded the pure aldehyde **16** as a pale yellow oil (1.15 g, 58%) which consisted of a mixture of conformational isomers in the ratio of 2.5:1 (A/B): TLC (diethyl ether:hexane, 3:1) $R_f = 0.58$; $[\alpha]^{22}_D$ −60.2 (*c* 1.18, $CHCl_3$) [lit.[19] $[\alpha]^{25}_D$ −70.1 (*c* 1.01, $CHCl_3$)]; δ_H isomer A 1.58, 1.67 (6H, 2 s), 3.80−4.20 (2H, m), 4.25−4.40 (1H, m), 5.12 (2H, s), 7.24−7.40 (5H, m), 9.56 (1H, s); isomer B 1.51, 1.60 (6H, 2 × s), 3.80−4.20 (2H, m), 4.40−4.46 (1H, m), 5.12 (2H, m), 7.24−7.40 (5H, m), 9.63 (1H, s); *m*/*z* (ES-MS) 264 (MH^+), calcd 264.1.

(46) Cossu, S.; Conti, S.; Giacomelli, G.; Falorni, M. *Synthesis* **1994**, 1429−1432.

***N*-(*tert*-Butoxycarbonyl)-1,2-diaminoethane (17).** To a solution of ethylenediamine (2.67 mL, 40 mmol) and Et_3N (2.78 mL, 20 mmol) in chloroform (40 mL) was slowly added a solution of di-*tert*-butyl dicarbonate (4.36 g, 20 mmol) in chloroform (20 mL) over 4 h. The reaction mixture was then filtered and the filtrate concentrated in vacuo to give *N*-(*tert*-butoxycarbonyl)-1,2-diaminoethane **17** (4.1 g, 64%) as a clear transparent oil.[16,17] The compound was used as such without further purification: TLC (ethyl acetate/hexane, 1:10) R_f = 0.55; δ_H (250 MHz, $CDCl_3/D_2O$) 1.44 (9H, s), 2.78 (2H, t, *J* 6), 3.16 (2H, t, *J* 6); *m*/*z* (ES-MS) 161 (MH^+), calcd 161.1.

Benzyl (*R*)-2,2-Dimethyl-4-(2-aza-4-*tert*-butoxycarbonylamino)butyloxazolidine-3-carboxylate (18). *N*-(*tert*-Butoxycarbonyl)-1,2-diaminoethane **17** (0.36 g, 2.25 mmol) was added to the stirred solution of the oxazolidine aldehyde **16** (0.78 g, 3 mmol) in 1% acetic acid/methanol (15 mL). Sodium cyanoborohydride (0.18 g) was added portionwise over a period of 45 min and the reaction mixture stirred overnight. The mixture was cooled in an ice bath, and an aqueous $NaHCO_3$ solution (120 mL) was added under stirring followed by ethyl acetate (180 mL). The organic phase was separated, washed with water, dried ($MgSO_4$), and concentrated in vacuo. Silica column chromatography (ethyl acetate/MeOH, 95:5) gave the pure compound **18** as a gum (0.42 g, 35%): TLC (ethyl acetate) R_f = 0.10; $[\alpha]^{25}_D$ −25.2 (*c* 2.46, MeOH); ^{1}H NMR shows a mixture of two conformational isomers in the ratio of 2.5:1 (A/B); δ_H isomer A 1.44 (9H, s), 1.52, 1.61 (6H, 2 s), 2.52−2.80 (4H, m), 2.93−3.20 (2H, m), 3.94 (3H, br s), 4.87 (1H, br s), 5.14 (2H, s), 7.35 (5H, s); isomer B 1.44 (9H, s), 1.54, 1.65 (6H, 2 s), 2.52−2.80 (4H, m), 2.93−3.20 (2H, m), 3.94 (3H, br s), 4.87 (1H, br s), 5.19 (2H, s), 7.36 (5H, s); *m*/*z* (ES-MS) 408 (MH^+), calcd 408.25. Anal. Calcd for $C_{21}H_{33}N_3O_5$: C, 61.90; H, 8.16; N, 10.31. Found: C, 61.75; H, 8.18; N, 10.17.

Benzyl (*R*)-2,2-Dimethyl-4-(2-aza-4-*tert*-butoxycarbonylamino-2-propionyl)butyloxazolidine-3-carboxylate (20). To a stirred solution of **18** (0.09 g, 0.221 mmol) in dry CH_2Cl_2 was added propionic acid (0.018 mL, 0.243 mmol) followed by DCC (0.05 g, 0.243 mmol) and stirring continued for 4 h at room temperature. The reaction mixture was filtered and the filtrate concentrated in vacuo. The residue was redissolved in ethyl acetate and washed sequentially with 1 M aqueous $KHSO_4$ (2 × 30 mL), aqueous $NaHCO_3$ (2 × 50 mL), and brine (1 × 30 mL). The organic layer was dried ($MgSO_4$) and concentrated in vacuo. Purification of the residue by column chromatography (ethyl acetate) afforded the title compound **20** (0.06 g, 59%) as an oil: TLC (ethyl acetate) R_f = 0.63; $[\alpha]^{25}_D$ −6.9 (*c* 4.64, MeOH); ^{1}H NMR shows a mixture of two conformational isomers in the ratio of 2.5:1 (A/B); δ_H isomer A 0.85−1.35 (3H, m), 1.43 (9H, s), 1.54, 1.66 (6H, 2 × s), 2.14−2.52 (2H, m), 2.70−3.12 (2H, m), 3.15−3.60 (4H, m), 3.61−4.30 (3H, m), 4.41 (1H, br s), 4.95 (1H, br s), 5.05−5.40 (2H, s), 7.20−7.60 (5H, s); isomer B 0.85−1.35 (3H, m), 1.43 (9H, s), 1.52, 1.58 (6H, 2 × s), 2.14−2.52 (2H, m), 2.70−3.12 (2H, m), 3.15−3.60 (4H, m), 3.61−4.30 (3H, m), 4.41 (1H, br s), 4.95 (1H, br s), 5.05−5.40 (2H, s), 7.20−7.60 (5H, s); *m*/*z* (ES-MS) 464 (MH^+), calcd 464.3; HRMS (FAB) calcd for $C_{24}H_{38}N_3O_6$ (MH^+) 464.2761, found 464.2749.

(*R*)-N^2-Benzyloxycarbonyl-N^6-*tert*-butoxycarbonyl-N^4-propionyl-4-azalysinol (21). To a solution of **20** (0.056 g, 0.120 mmol) in methanol (5 mL) was added TsOH monohydrate (0.024 g, 0.120 mmol). The reaction mixture was refluxed for 3 h. The solvent was removed in vacuo to give the triamino alcohol as a tosylate salt, which was not isolated. The residue was redissolved in 50% dioxane/water (10 mL). To this were added Na_2CO_3 (0.020 g, 0.240 mmol) and $(Boc)_2O$ (0.026 g, 0.120 mmol), and the resultant mixture was stirred overnight. The solvents were removed in vacuo, and the residue was redissolved in ethyl acetate, washed with water (1 × 30 mL), 1 M aqueous $KHSO_4$ (2 × 30 mL), and finally with brine (1 × 30 mL). The organic extract was dried ($MgSO_4$) and concentrated in vacuo to give the title compound **21** (0.04 g, 78%) as a gum: TLC (ethyl acetate) R_f = 0.47; $[\alpha]^{25}_D$ +10.91 (*c* 2.75, MeOH); δ_H 1.13 (3H, t, *J* 7.3), 1.43 (9H, s), 2.42 (2H, q, *J* 7.3), 3.10−3.90 (8H, m), 3.90−4.15 (1H, m), 4.90−5.05 (1H, m), 5.08 (2H, s), 5.61(1H, d, *J* 7.8), 7.33 (5H, s); *m*/*z* (ES-MS) 424 (MH^+),

calcd 424.2; HRMS (FAB) calcd for $C_{21}H_{34}N_3O_6$ (MH^+) 424.2448, found 424.2425.

(*R*)-*N*^{2}-Benzyloxycarbonyl-*N*^{6}-*tert*-butoxycarbonyl-*N*^{4}-propionyl-4-azalysine (19). To a stirred solution of the triamino alcohol **21** (0.03 g, 0.07 mmol) in acetone (5 mL) was added dropwise chromic acid[18,19] (0.2 mL) and stirring continued for 2 h at room temperature. 2-Propanol (2 mL) was then added to the reaction mixture and stirring continued for an additional 30 min to consume the excess reagent. The resulting suspension was filtered through Celite to remove the chromium salt and the filtrate concentrated in vacuo to give the triamino acid **19** (0.02 g, 64%): TLC (chloroform/methanol/acetic acid 8:0.8:0.08) $R_f = 0.58$; $[\alpha]_D^{25}$ +13.33 (*c* 1.20, MeOH); δ_H ($CDCl_3/D_2O$) 1.13−1.25 (3H, m), 1.48 (9H, s), 2.22−2.28 (2H, m), 3.12−4.38 (7H, m), 5.18 (2H, s), 7.36 (5H, s); *m*/*z* (ES-MS) 437.7 (MH^+), calcd 438.2; HRMS (FAB) calcd for $C_{21}H_{32}N_3O_7$ (MH^+) 438.2240, found 438.2242.

(*S*)-*N*^{2}-Benzyloxycarbonyl-2,3-diaminopropionic Acid (23). A slurry of *N*^{2}-benzyloxycarbonylasparagine **22** (0.5 g, 1.88 mmol), ethyl acetate (4.6 mL), MeCN (4.8 mL), water (2.4 mL), and iodosobenzene diacetate (0.726 g, 2.25 mmol) was stirred at 15 °C for 30 min. The temperature was then allowed to rise to ambient temperature, and the reaction was stirred until completion (4 h) when the product separated out as a white solid. The reaction mixture was cooled to 5 °C, and the product was collected, washed with ethyl acetate (20 mL), and dried in vacuo to afford the title compound **23** (0.4 g, 89%): mp 227−228 °C (lit.[21] mp 228−230 °C); TLC (chloroform/methanol/acetic acid, 5:3:1) $R_f = 0.67$; $[\alpha]^{24}_D$ −7.70 (*c* 1.56, 1 M NaOH(aq)) [lit.[21] $[\alpha]^{20}_D$ −7.5 (*c* 0.4, 1 M NaOH(aq))]; δ_H (DMSO-d_6/TFA) 3.10−3.50 (2H, m), 4.30−4.60 (1H, m), 5.10 (2H, s), 7.10 (1H, d, *J* 6.0), 7.40 (5H, s), 7.90−8.20 (3H, br s); *m*/*z* (ES-MS) 239 (MH^+), calcd 239.1.

Methyl (*S*)-*N*^{2}-Benzyloxycarbonyl-2,3-diaminopropionate Hydrochloride (24). (*S*)-*N*^{2}-Benzyloxycarbonyl-2,3-diaminopropionic acid **23** (0.5 g, 2 mmol) was dissolved in methanolic HCl (15 mL) and the resulting solution stirred overnight at 5 °C. The solution was then evaporated in vacuo to dryness, and the white solid obtained was recrystallized from MeOH/diethyl ether to afford the methyl ester **24** (0.42 g, 78%): mp 156−157 °C; TLC (chloroform/methanol/acetic acid, 5:3:1) $R_f = 0.82$; δ_H (D_2O) 3.36 (1H, dd, *J* 9.06), 3.59 (1H, dd, *J* 5.07), 3.78 (3H, s), 4.62 (1H, m), 5.18 (2H, s), 7.46 (5H, s); δ_C (D_2O) 39.74, 51.99, 53.83, 67.92, 128.15, 128.89, 129.18, 136.31, 158.27, 170.98; *m*/*z* (ES-MS) 253 (MH^+), calcd 253.1.

***N*-(*tert*-Butoxycarbonyl)glycinal (26).** A solution of 2-(*tert*-butoxycarbonylamino) ethanol **25** (0.805 g, 5 mmol) in DMSO (3 mL) was added to a stirred solution of *o*-iodoxybenzoic acid (2.79 g, 10 mmol) in DMSO (20 mL) and the stirring continued at room temperature overnight. The reaction mixture was then diluted with water (200 mL) and filtered. The filtrate was extracted with ethyl acetate (2 × 100 mL), and the pooled extracts were washed with brine (1 × 30 mL), dried ($MgSO_4$), and concentrated in vacuo. The residue was chromatographed on a silica column using ethyl acetate as an eluent to yield the pure aldehyde **26** as a colorless oil (0.43 g, 55%):[1] TLC (ethyl acetate/hexane, 1:1) $R_f = 0.38$; δ_H 1.28 (9H, s), 3.80 (2H, d), 5.42 (1H, br s), 9.44 (1H, s); δ_C 28.55, 51.31, 80.04, 156.35, 198.89; *m*/*z* (ES-MS) 160 (MH^+), calcd 160.1.

Methyl (*S*)-*N*^{2}-Benzyloxycarbonyl-*N*^{6}-*tert*-butoxycarbonyl-4-azalysinate (27). Methyl (*S*)-*N*^{2}-benzyloxycarbonyl-2,3-diaminopropionate hydrochloride **24** (0.48 g, 1.68 mmol) was added to a solution of *N*-(*tert*-butoxycarbonyl)glycinal **26** (0.29 g, 1.85 mmol) in 1% acetic acid/methanol (12 mL). Sodium cyanoborohydride (133 mg, 2.1 mmol) was added portionwise over a period of 45 min. The reaction mixture was then stirred overnight. The mixture was cooled in an ice bath, and an aqueous 5% $NaHCO_3$ solution (80 mL) was added under stirring followed by ethyl acetate (150 mL). The organic extract was washed with water, dried, and concentrated in vacuo. Purification of the residual material by column chromatography using ethyl acetate gave the pure compound **27** (0.42 g, 65%) as an oil: TLC (ethyl acetate) $R_f = 0.23$; δ_H 1.37 (9H, s), 2.40−2.70 (2 H, m), 2.70−3.00 (2H, m), 3.00−3.20 (2H, m), 3.68 (3H, s), 4.30−4.50 (1H, br m), 5.05 (2H, s), 5.11 (1H, br t), 5.90−6.15 (1H, d, *J* 8), 7.10−7.40 (5H, m); δ_C 28.73, 40.33, 49.07, 50.38, 52.76, 54.42, 67.27, 79.35, 128.47, 128.81, 136.60, 156.59, 172.44; *m*/*z* (ES-MS) 396 (MH^+), calcd 396.2; HRMS (FAB) calcd for $C_{19}H_{30}N_3O_6$ (MH^+) 396.2103, found 396.2109.

Methyl (*S*)-*N*^{2}-Benzyloxycarbonyl-*N*^{6}-*tert*-butoxycarbonyl-*N*^{4}-acetyl-4-azalysinate (28a). Acetic anhydride (0.47 mL, 5 mmol) was added to a stirred solution of **27** (0.197 g, 0.5 mmol) in dry CH_2Cl_2 (15 mL), and the stirring was continued for 4 h at room temperature. The reaction mixture was concentrated in vacuo. The residue was redissolved in ethyl acetate and washed with aqueous $NaHCO_3$ (2 × 30 mL), 1 M aqueous $KHSO_4$ (2 × 30 mL), and brine (1 × 30 mL). The organic extract was then dried ($MgSO_4$) and concentrated in vacuo. The crude product was chromatographed on a silica column using ethyl acetate/hexane (1:1) as an eluant, to yield the desired product **28a** as a colorless oil (0.135 g, 62%): TLC (ethyl acetate/hexane, 1:1) $R_f = 0.48$; $[\alpha]^{23}_D$ −18.5 (*c* 2.60, MeOH); δ_H 1.41, 1.42 (9H, 2 × s), 2.06 (3H, s), 3.10−3.30 (2H, m), 3.30−3.50 (2H, m), 3.50−4.00 (2 H, m), 3.73 (3H, s), 4.40−4.65 (1H, m), 5.06, 5.12 (2H, 2 × d ($q_{AA'BB'}$), *J* 12.50, 12.25), 5.23 (1H, t, *J* 6.0), 6.15 (1H, d, *J* 7.75), 7.33 (5H, s); δ_C 21.04, 28.19, 38.69, 46.74, 48.83, 52.50, 53.45, 66.77, 79.56, 127.92, 128.33, 128.40, 136.14, 155.85, 156.09, 170.77, 172.65; *m*/*z* (FAB) 438 (MH^+), calcd 438.2; HRMS (FAB) calcd for $C_{21}H_{32}N_3O_7$ (MH^+) 438.2253, found 438.2259.

Methyl (*S*)-*N*^{2}-Benzyloxycarbonyl-*N*^{4},*N*^{6}-bis-*tert*-butoxycarbonyl-4-azalysinate (28c). Di-*tert*-butyl dicarbonate (0.24 g, 1.1 mmol) was added to a stirred solution of **27** (0.39 g, 1 mmol) in dry CH_2Cl_2 and stirring continued at room temperature overnight. The reaction mixture was then rotary evaporated, and the residue was redissolved in ethyl acetate (50 mL) and washed with 1 M aqueous $KHSO_4$ (2 × 30 mL) followed by brine (2 × 30 mL). The organic extract was dried ($MgSO_4$) and concentrated in vacuo to afford the title compound **28c**. Silica column chromatography (EtOAc) of the residue gave the pure product as a colorless oil (0.42 g, 85%): TLC (ethyl acetate) $R_f = 0.56$; $[\alpha]^{26}_D$ −23.33 (*c* 3.60, MeOH); δ_H 1.42, 1.44 (18H, 2 × s), 3.10−3.40 (4H, m), 3.40−3.70 (2H, m), 3.73 (3H, s), 4.30−4.70 (1H, m), 4.90−5.20 (1H, m), 5.09 (2H, s), 6.02 (1H, d, *J* 7.4), 7.33 (5H, s); δ_C 28.11, 28.25, 39.09, 47.84, 48.74, 52.47, 53.75, 66.88, 79.18, 80.70, 127.91, 128.33, 136.56, 155.94, 170.88; *m*/*z* (FAB) 496 (MH^+), calcd 496.3; HRMS (FAB) calcd for $C_{24}H_{37}N_3O_8Na$ (MNa^+) 518.2478, found 518.2477; calcd for $C_{24}H_{38}N_3O_8$ (MH^+) 496.2658, found 496.2674.

Methyl (*S*)-*N*^{2}-Benzyloxycarbonyl-*N*^{6}-*tert*-butoxycarbonyl-*N*^{4}-cyclohexylcarbamoyl-4-azalysinate (28d). Cyclohexyl isocyanate (0.169 mL, 1.33 mmol) was added to a stirred solution of **27** (0.480 g, 1.21 mmol) in sodium-dried ether and the stirring continued for 5 h at room temperature. The reaction mixture was concentrated in vacuo, residue redissolved in ethyl acetate, and washed with 1 M aqueous $KHSO_4$ (2 × 30 mL) followed by brine (50 mL). The organic extract was dried ($MgSO_4$) and concentrated in vacuo. The crude product was purified by column chromatography (EtOAc/hexane, 1:1) and recrystallized from ethyl acetate/hexane to afford **28d** (0.5 g, 79%) as colorless plates: mp 54 °C; TLC (ethyl acetate) $R_f = 0.68$; $[\alpha]^{28}_D$ −18.4 (*c* 2.39, MeOH); δ_H 0.90−2.00 (11H, m), 1.38 (9 H, s), 2.90−3.35 (4H, m), 3.35−3.90 (2H, m), 3.67 (3H, s), 4.30 (1H, m), 5.00, 5.07 (2H, 2 × d ($q_{AA'BB'}$), *J* 12.25, 12.50), 5.15−5.35 (1H, m), 6.08 (1H, d, *J* 7.04), 6.59 (1H, d, *J* 6.80), 7.27 (5H, s); δ_C 24.97, 25.01, 25.59, 33.16, 33.30, 28.23, 39.17, 47.13, 48.86, 49.81, 52.44, 54.61, 66.71, 79.80, 127.86, 127.91, 128.30, 136.26, 156.35, 156.67, 158.09, 171.18; *m*/*z* (FAB) 521 (MH^+), calcd 521.3; HRMS (FAB) calcd for $C_{26}H_{40}N_4O_7$ (MH^+) 521.2975, found 521.2950.

(*S*)-*N*^{2}-Benzyloxycarbonyl-*N*^{6}-*tert*-butoxycarbonyl-*N*^{4}-acetyl-4-azalysine (29a). A solution of ester **28a** (0.6 g, 1.37 mmol) in methanol (10 mL) was stirred with a solution of sodium hydroxide (0.06 g, 1.5 mmol) in water (5 mL) at room temperature for 1 h. The solvents were then evaporated in vacuo, the residue was redissolved in water and acidified to pH 2−3 with 1 M aqueous $KHSO_4$, and the precipitated product was extracted with ethyl acetate (3 × 50 mL). The combined organic extracts were dried ($MgSO_4$) and concentrated in vacuo. The residue was recrystallized from diethyl

ether/petroleum ether bp 40−60 °C to give the free acid **29a** (0.42 g, 72%) as an amorphous solid: mp 79−85 °C; TLC (chloroform/methanol/acetic acid, 2:0.2:0.02) $R_f = 0.68$; δ_H 1.38 (9H, s), 2.01 (3H, s), 3.00−3.90 (6H, m), 4.25−4.65 (1H, m), 4.90−5.15 (2H, m), 5.15−5.40 (1H, br m), 6.00−6.40 (1H, br d), 7.28 (5H, s), 10.96 (1H, br s); *m*/*z* (ES-MS) 423.62 (MH^+), calcd 424.2. Anal. Calcd for $C_{20}H_{29}N_3O_7 \cdot H_2O$: C, 54.42; H, 7.02; N, 9.52. Found: C, 54.44; H, 6.94; N, 9.65.

***N*²-Benzyloxycarbonyl-*N*⁴,*N*⁶-bis-*tert*-butoxycarbonyl-4-azalysine (29c).** Treatment of **28c** (1.301 g, 2.63 mmol) with aqueous LiOH (0.122 g, 2.9 mmol), as described in the procedure for **29a**, gave **29c** as colorless plates (1.0 g, 79%): mp 45−46 °C; TLC (chloroform/methanol/acetic acid, 2.0:0.2:0.02) $R_f = 0.4$; $[\alpha]^{26}_D$ −12.44 (*c* 2.09, MeOH); δ_H 1.42 (18H, s), 3.00−3.90 (6H, m), 4.20−4.80 (1H, m), 5.09 (2H, br s), 5.20−5.60 (1H, m), 5.90−6.50 (1H, m), 7.30 (5H, s), 9.75 (1H, s); δ_C 27.94, 28.09, 38.90, 47.60, 48.58, 53.41, 66.71, 79.30, 80.75, 127.75, 128.16, 135.94, 155.95, 156.28, 172.78; *m*/*z* (FAB) 482 (MH^+), calcd 482.2; HRMS (FAB) calcd for $C_{23}H_{36}N_3O_8$ (MH^+) 482.2503, found 482.2507.

(*S*)-*N*⁶-*tert*-Butoxycarbonyl-*N*⁴-acetyl-4-azalysine (30a). A solution of **29a** (0.384 g, 0.90 mmol) in methanol (10 mL) was hydrogenated at room temperature with stirring in the presence of 10% Pd−C (0.038 g). On completion, the reaction mixture was filtered through a Celite pad and the filtrate evaporated to dryness to give the product **30a** as a glassy solid, which was recrystallized from methanol/diethyl ether (0.262 g, 84%): mp 135−140 °C; TLC (methanol/acetic acid, 5:0.5) $R_f = 0.56$; δ_H 1.35 (9H, s), 2.14 (3H, s), 3.00−4.25 (6H, m), 4.30−4.75 (1H, br m), 8.12 (3H, br s); *m*/*z* (ES-MS) 290.18 (MH^+), calcd 290.17. Anal. Calcd for $C_{12}H_{23}N_3O_5 \cdot {}^1/_2H_2O$: C, 48.31; N, 8.10; N, 14.08. Found: C, 48.05; H, 7.95; N, 13.75.

***N*⁴,*N*⁶-Bis-*tert*-butoxycarbonyl-4-azalysine (30c).** Hydrogenation of **29c** (1.0 g, 2.07 mmol) using 10% Pd−C (0.10 g) in methanol (10 mL) in the procedure for **30a** gave the title compound **30c** (0.70 g, 97%) as a glassy solid that was recrystallized from $CHCl_3$/hexane: mp 170−172 °C; $[\alpha]^{27}_D$ −8.46 (*c* 1.04, MeOH); δ_H 1.38, 1.42 (18H, 2 × s), 3.10−4.30 (7H, m), 5.63 (1H, br s), 8.26 (3H, br s); δ_C 28.20, 28.33, 39.00, 48.74, 49.92, 54.84, 78.85, 81.08, 156.10, 157.33, 171.69; *m*/*z* (FAB) 348 (MH^+), calcd 348.2; HRMS (FAB) calcd for $C_{15}H_{30}N_3O_6$ (MH^+) 348.2134, found 348.2132.

(*S*)-*N*²-9-Fluorenylmethoxycarbonyl-*N*⁶-*tert*-butoxycarbonyl-*N*⁴-acetyl-4-azalysine (31a). To a stirred solution of **30a** (0.187 g, 0.65 mmol) in $NaHCO_3$ (0.163 g, 1.95 mmol) solution in water (10 mL) at 5 °C was added a solution of FmocOSu (0.240 g, 0.715 mmol) in dioxane (10 mL) over a period of 15 min. The reaction mixture was stirred for 5 h at room temperature, after which time it was evaporated to dryness and the residue redissolved with water (30 mL). The aqueous solution was washed with diethyl ether (2 × 30 mL), acidified with 2 M aqueous $KHSO_4$, and extracted with ethyl acetate (3 × 50 mL). The pooled extracts were dried and concentrated under reduced pressure to give the title compound as a white solid. Recrystallization of the crude product from ethyl acetate/hexane gave the title product **31a** (0.268 g, 81%): mp 145−149 °C; TLC (chloroform/methanol/acetic acid, 2:0.2:0.02) $R_f = 0.38$; $[\alpha]^{27}_D$ −11.9 (*c* 1.35, MeOH); δ_H 1.27 (9H, s), 2.16 (3H, s), 3.00−3.95 (6H, m), 4.10−4.30 (1H, m), 4.30−4.60 (3H, m), 5.00−5.30 (1H, m), 6.25 (1H, d, *J* 6.8), 7.20−7.50 (4H, m), 7.50−7.77 (2H, m), 7.70−7.90 (2 H, m); δ_C 21.10, 28.28, 39.06, 47.00, 47.09, 49.86, 54.08, 67.94, 81.04, 119.98, 125.06, 127.08, 127.74, 141.25, 143.76, 155.86, 156.09, 172.48, 174.90; *m*/*z* (ES-MS) 512.19 (MH^+), calcd 512.24. Anal. Calcd for $C_{27}H_{33}N_3O_7$: C, 63.46; H, 6.50; N, 8.22. Found: C, 63.11; H, 6.40; N, 8.00.

(*S*)-*N*²-9-Fluorenylmethoxycarbonyl-*N*⁴,*N*⁶-bis-*tert*-butoxycarbonyl-4-azalysine (31c). Using **30c** (0.60 g, 1.72 mmol), $NaHCO_3$ (0.433 g, 5.16 mmol), and FmocOSu (0.637 g, 1.89 mmol), in the procedure described for **31a**, and on recrystallization from ethyl acetate/hexane gave the title compound **31c** (0.5 g, 51%): mp 104−108 °C; TLC (chloroform/methanol/acetic acid, 2:0.2:0.02) $R_f = 0.4$; $[\alpha]^{28}_D$ −11.0 (*c* 1.09, MeOH); δ_H 1.41, 1.44 (18H, 2 × s), 3.00−3.90 (6H, m), 4.10−4.70 (4H, m), 4.90−5.40 (1H, m), 5.80−6.50 (1H, m), 7.10−7.45 (4H, m), 7.45−7.65 (2H, m), 7.65−7.85 (2H, m), 8.39 (1H, br s); δ_C 28.21, 28.30, 42.07, 46.98, 47.91, 49.01, 54.22, 67.28, 80.29, 81.25, 119.86, 125.14, 127.01, 127.62, 127.93, 141.17, 143.59, 143.82, 156.11, 156.53, 172.98; *m*/*z* (ES-MS) 571 (MH^+), calcd 570.3. Anal. Calcd for $C_{30}H_{39}N_3O_8$: C, 63.25; H, 6.90; N, 7.37. Found: C, 62.70; H, 6.84; N, 7.36.

(*S*)-*N*²-9-Fluorenylmethoxycarbonyl-*N*⁶-*tert*-butoxycarbonyl-*N*⁴-cyclohexylcarbamoyl-4-azalysine (31d). Using **30d** (0.32 g, 0.86 mmol), $NaHCO_3$ (0.216 g, 2.57 mmol), and FmocOSu (0.32 g, 0.94 mmol), in the procedure described for **31a**, on recrystallization from ethyl acetate/hexane gave the title compound **31d** (0.38 g, 75%): mp 110−112 °C; TLC (chloroform/methanol/acetic acid, 3.5:0.2:0.1) $R_f = 0.38$; $[\alpha]^{27}_D$ −3.48 (*c* 1.15, MeOH); δ_H 0.90−2.00 (11H, m), 1.36 (9H, s), 2.98−3.81 (6 H, m), 4.12−4.68 (4H, m), 5.42 (1H, br s), 6.39 (1H, br s), 6.52 (1H, d, *J* 6.62), 7.12−7.46 (4H, m), 7.46−7.69 (2H, m), 7.69−7.86 (2H, m); δ_C 25.04, 25.59, 33.25, 28.29, 39.20, 47.00, 47.79, 50.25, 51.61, 56.50, 67.29, 80.30, 119.94, 125.19, 127.71, 141.22, 143.55, 143.81, 156.40, 157.00, 158.86, 172.34; *m*/*z* (ES-MS) 595.3 (MH^+), calcd 595.31. Anal. Calcd for $C_{32}H_{42}N_4O_7 \cdot H_2O$: C, 62.71; H, 7.18; N 9.14. Found: C, 62.95; H, 6.89; N, 8.91.

H-Arg-Arg-Trp-Trp-Azl(*N*⁴-Ac)-Phe-NH₂ (32a). Rink amide aminomethyl polystyrene (0.050 g, 0.033 mmol; 0.66 mmol g^{-1}, 200−400 mesh) was swelled in DMF (2 mL) for 1 h and then Fmoc-deprotected using 20% piperidine in DMF (2.5 mL min^{-1}, 7 min). Using an LKB 4175 Biolynx manual peptide synthesizer, the peptide sequence Arg(Pmc)-Arg(Pmc)-Trp-(Boc)-Trp(Boc)-Azl(*N*⁴-Ac)-Phe was assembled. Sequential acylation reactions were carried out at ambient temperature for 3 h, except for the coupling of FmocAzl(*N*⁴-Ac)OH, which was left for 18 h. Appropriate Fmoc-amino acids [FmocPheOH, 0.051 g, 0.132 mmol; FmocTrp(Boc)OH, 0.069 g, 0.132 mmol; FmocArg(Pmc)OH, 0.087 g, 0.132 mmol; FmocAzl(*N*⁴-Ac,*N*⁶-Boc)OH **31a** (0.051 g, 0.099 mmol), HOBt (0.015 g, 0.099 mmol), TBTU (0.032 g, 0.099 mmol) and DIEA (0.034 mL, 0.198 mmol)] were used, and carboxyl-activated using TBTU (0.043 g, 0.132 mmol), HOBt (0.020 g, 0.132 mmol), and DIEA (0.046 mL, 0.264 mmol) in DMF (1 mL). Repetitive *N*-Fmoc-deprotection was achieved using 20% *v*/*v* piperidine in DMF (2.5 mL min^{-1}, 7 min). Following assembly, the resin product was filtered, washed with DMF (20 mL), CH_2Cl_2 (50 mL), and hexane (50 mL), and dried in vacuo to afford the required peptidyl-resin (98 mg, 98%). The resin (98 mg) was suspended in a mixture of TFA/H_2O/1,2-ethanedithiol/*i*-Pr_3SiH (9:0.7:0.1:0.2, 10 mL) for 3 h with occasional stirring. The mixture was filtered and the filtrate evaporated to dryness in vacuo. The residue was triturated several times with ether (3 × 10 mL), dissolved in water, and lyophilized to yield the title hexapeptide sequence as an amorphous powder (0.012 g). RP-HPLC conditions: solvent A, 0.06% aqueous TFA; solvent B, 0.06% TFA in acetonitrile/water, 90:10 v/v, gradient elution 20%-60% B in 25 min; t_R 9.38 min; *m*/*z* (ES-MS) 1018.8 (MH^+), calcd 1020.56.

H-Arg-Arg-Trp-Trp-Azl-Phe-NH₂ (32c). Use of FmocAzl-(*N*⁴-Boc,*N*⁶-Boc)OH **31c** afforded the title peptide as white powder (0.032 g): RP-HPLC analysis, t_R 8.30 min; *m*/*z* (ES-MS) 978.4 (MH^+), calcd 978.55.

H-Arg-Arg-Trp-Trp-Azl(*N*⁴-C₆H₁₁NHCO)-Phe-NH₂ (32d). Use of FmocAzl(*N*⁴-C_6H_{11}NHCO,*N*⁶-Boc)OH **31d** afforded the title peptide as a white amorphous powder (0.031 g): RP-HPLC analysis, t_R 12.56 min; *m*/*z* (ES-MS) 1104.0 (MH^+), calcd 1103.64.

***N*²,*N*⁴,*N*⁶-Tris-*tert*-butoxycarbonyl-4-azalysine (2).** Di-*tert*-butyl dicarbonate (0.627 g, 2.88 mmol) was added to a stirred solution of **30c** (1.00 g, 2.88 mmol) in dry CH_2Cl_2 followed by Et_3N (0.4 mL, 2.88 mmol) and the stirring was continued at room temperature for 6 h. Following evaporation in vacuo to dryness, the residue was redissolved in ethyl acetate (100 mL), washed with 1 M aqueous $KHSO_4$ (2 × 30 mL) and brine (1 × 30 mL). The organic phase was dried and concentrated in vacuo to give the crude product as a sticky solid. The crude compound was dissolved in aqueous $NaHCO_3$ (50 mL) and washed with ethyl acetate (1 × 30 mL), acidified with 1 M aqueous $KHSO_4$, followed by extraction with ethyl

acetate (3 × 50 mL). The pooled extracts were washed with brine, dried ($MgSO_4$), and rotary evaporated to compound **2** (0.539 g, 42%) as a frothy solid: mp 63−64 °C; $[\alpha]^{26}_D$ −9.52 (*c* 0.84, MeOH); δ_H 1.41, 1.42, 1.45 (27H, 3 × s), 2.85−3.89 (6H, m), 3.90−4.78 (1H, m), 4.88−5.50 (1H, m), 5.66−6.40 (1H, m), 8.57 (1H, br s); δ_C 28.18, 28.27, 39.17, 47.88, 48.95, 52.69, 79.46, 79.97, 80.94, 155.85, 173.33; *m*/*z* (FAB) 448 (MH^+), calcd 448.3; HRMS (FAB) 470.2472 calcd for $C_{20}H_{37}N_3O_8Na$ (MNa^+): 470.2478, found 470.2472; calcd for $C_{20}H_{38}N_3O_8$ (MH^+) 448.2659, found 448.2656.

***N*²,*N*⁴,*N*⁶-Tris-*tert*-butoxycarbonyl-4-azalysylaminomethylpolystyrene.** Aminomethylpolystyrene (0.250 g, 0.2 mmol; 0.8 mmol g^{-1}; 1% DVB, 200−400 mesh) was swollen in DMF (3 mL) for 1 h. N^2,N^4,N^6-Tris-Boc-4-azalysine **2** (0.268 g, 0.6 mmol) was activated with HOAt (0.082 g, 0.6 mmol) and DIPCDI (0.094 mL, 0.6 mmol) in DMF and was then added to the pre-swollen aminomethyl resin and the coupling reaction continued overnight with gentle stirring. A negative TNBS test confirmed the successful coupling of the protected triamino acid to the amine-functionalized resin. The resin was transferred to a sintered glass funnel, washed with DMF (50 mL) followed by CH_2Cl_2 (100 mL) and finally with hexane (100 mL), and dried in vacuo overnight to give the derivatized resin (0.351 g, 100% recovery yield).

4-Azalysylaminomethylpolystyrene (33). To the above derivatized resin (0.350 g) suspended in CH_2Cl_2 (6 mL) was added TFA (3 mL) and TES (0.1 mL), and the mixture was left for 1−2 h at ambient temperature. The resin was then transferred to a sintered glass funnel, washed with CH_2Cl_2 (100 mL), 10% v/v DIEA in DMF (20 mL) followed by CH_2Cl_2 (50 mL), and finally with hexane (20 mL). The resin was collected and dried overnight in vacuo (over KOH pellets) to afford the resin product **33** (0.270 g).

***N*²,*N*⁴,*N*⁶-Tris-(4-[(*RS*)-α-(9*H*-Fluoren-9-yl)methoxycarbonylamino-2,4-dimethoxybenzyl]phenoxyacetyl)-4-azalysylaminomethylpolystyrene (35).** Resin **33** (0.130 g, 0.282 mmol reactive amines) was swollen in DMF (3 mL) for 1 h. 4-[(*RS*)-α-(9*H*-Fluoren-9-yl)methoxycarbonylamino-2,4-dimethoxybenzyl]phenoxyacetic acid (Rink amide linker) **34** (0.755 g, 1.4 mmol) was activated with HOAt (0.190 g, 1.4 mmol) and DIPCDI (0.218 mL, 1.4 mmol) in DMF (1 mL). The activated linker was then added to the pre-swollen resin **33** and the resin gently stirred overnight. The resin was washed with DMF (100 mL), CH_2Cl_2 (50 mL), and finally with hexane (50 mL). It was then dried overnight in vacuo giving the derivatized resin **35** (0.298 g, 100% recovery yield): calcd Fmoc-substitution 1.014 mmol g^{-1}, found 0.90 mmol g^{-1} (90%).

***N*²,*N*⁴,*N*⁶-Tris(4-azalysyl)-4-azalysylaminomethylpolystyrene (36).** Resin **33** (0.092 g 0.2 mmol) was swollen in DMF (2 mL) for 1 h. Tris-Boc-4-azalysine **2** (0.223 g, 0.5 mmol) was activated using HATU (0.190 g, 0.5 mmol) and DIEA (0.174 mL, 1 mmol) in DMF. The activated amino acid was added to the pre-swollen resin **33** and the resin mixture gently stirred overnight. The resin was washed and dried as described before and obtained in a recovery yield of 96% (0.335 g). This was followed by TFA treatment, as described for the synthesis of resin product **33**, to afford dendrimer-tethered resin **36** (0.170 g).

***N*²,*N*⁴,*N*⁶-Tris-[*N*²,*N*⁴,*N*⁶-tris-(4-[(*RS*)-α-(9*H*-Fluoren-9-yl)methoxycarbonylamino-2,4-dimethoxybenzyl]phenoxyacetyl)-4-azalysyl]-4-azalysylaminomethylpolystyrene (37).** The Rink amide linker **34** (0.269 g, 0.5 mmol) was activated using HOAt (0.068 g, 0.5 mmol) and DIPCDI (0.078 mL, 0.5 mmol) and coupled to the resin-bound dendrimer **36** (0.019 g, 0.1 mmol theoretical reactive amines), previously swelled in DMF (2 mL), and the resin mixture gently stirred overnight. The resin mixture was then treated as described for **33** to give **37** in 92% recovery yield (0.066 g): calcd Fmoc-substitution 1.40 mmol g^{-1}, found 1.38 mmol g^{-1} (99%).

2-Naphthalenesulfonamide (38). Resin **35** (0.050 g, 0.045 mmol) was swollen in DMF (2 mL) for 1 h and treated with 20% piperidine in DMF. The resin was then washed with DMF (25 mL). 2-Naphthalenesulfonyl chloride (0.042 g, 0.2 mmol) was dissolved in DMF (1 mL) followed by DIEA (0.035 mL, 0.2 mmol), and the resultant mixture was added to the pre-swollen resin. The mixture was then gently agitated overnight. The resin was then transferred to a sintered glass funnel, washed with DMF (50 mL) followed by CH_2Cl_2 (50 mL) and hexane (50 mL), and then dried overnight over KOH pellets in vacuo. The dried resin, recovered in 97% yield, was treated with TFA/water (9:1, 10 mL) for 2 h. The mixture was filtered and the filtrate evaporated to dryness to yield 2-naphthalenesulfonamide **38** (7 mg, 85%) as a white solid: mp 213-215 °C (lit.[47] mp 211−212 °C); TLC (ethyl acetate/hexane, 1:1) R_f = 0.43; δ_H (CD_3OD) 7.60−7.80 and 7.90−8.15 (6H, m), 8.49 (1H, d, *J* 1.75); *m*/*z* (ES-MS) 207.5 (MH^+), calcd 208.04.

***N*^α-Phenylacetylphenylalaninamide (39).** Resin **35** (0.050 g, 0.045 mmol) was swollen in DMF for 1 h followed by treatment with 20% piperidine in DMF. The resin was then washed with DMF (25 mL). FmocPheOH (0.062 g, 0.16 mmol) and HATU (0.061 g, 0.16 mmol) were dissolved in DMF (1 mL) followed by DIEA (0.055 mL, 0.32 mmol). The activated amino acid mixture was added to the resin, and the resultant mixture was gently stirred overnight. The resin was then washed with DMF (50 mL), treated with 20% piperidine in DMF, washed with DMF (25 mL), and treated with a DMF solution (1 mL) of phenylacetic acid (0.22 g, 0.16 mmol), HOBt (0.024 g, 0.16 mmol), TBTU (0.056 g, 0.16 mmol), and DIEA (0.055 mL, 0.32 mmol), and the resin mixture stirred overnight at ambient temperature. The resin was then treated with TFA/water (9:1, 10 mL) for 2 h, to give compound **39** (0.009 g, 71%) as a white solid: mp 186−188 °C (lit.[48] mp 189−190 °C); δ_H 3.00 (2H, d, *J* 6.83), 3.55 (2H, s), 4.61 (1H, q, *J* 7.2), 5.24, 5.78 (2H, 2 × br s), 5.93 (1H, br d, *J* 5.87), 6.90−7.50 (10H, m); δ_C 37.20, 43.50, 54.50, 128.72, 129.09, 129.17, 170.00; *m*/*z* (ES-MS) 283.1 (MH^+), calcd 283.1; HRMS (FAB) calcd for $C_{17}H_{19}N_2O_2$ (MH^+) 283.1447, found 283.1448.

***N*^α-4-Methoxybenzenesulfonyl-*N*-benzylleucinamide (40).** The dendrimer-tethered resin **37** (0.050 g, 0.069 mmol) was swollen in DMF (2 mL) for 1 h, treated with 20% piperidine in DMF, washed with DMF (25 mL), and the excess DMF then removed. FmocLeuOH (0.121 g, 0.344 mmol) and HATU (0.130 g, 0.344 mmol) were dissolved in DMF (1 mL) followed by DIEA (0.120 mL, 0.688 mmol), and the resultant mixture was added to the resin and gently stirred overnight. The resin was then treated with 20% piperidine in DMF followed by a DMF wash (25 mL). A solution of 4-methoxybenzenesulfonyl chloride (0.056 g, 0.275 mmol) and DIEA (0.018 mL, 0.103 mmol) in DMF (1 mL) was added to the resin, which was then stirred for 24 h. The resin was collected and washed with DMF, CH_2Cl_2, and MeOH successively. The resin **42** was then dried in vacuo and recovered in 95% yield (0.050 g). The dried resin was swollen in THF (2 mL) for 10 min. The resin suspension was purged with nitrogen and cooled to 0 °C. Benzyl alcohol (0.143 mL, 1.376 mmol) was added to the resin followed by Bu_3P (0.171 mL, 0.688 mmol), and the mixture was stirred for 5 min. *N*,*N*,*N*,*N*-Tetramethylazodicarboxamide (TMAD; 0.118 g, 0.688 mmol) was then added to the resin mixture. The resin suspension, under N_2 atmosphere, was allowed to warm to room temperature and gently agitated for 24 h. The supernatant was decanted off and the resin re-treated as above for another 24 h. The resin product was collected, washed successively with DMF (20 mL), CH_2Cl_2 (50 mL), and MeOH (20 mL), and dried in vacuo. The resin was then treated with 95% TFA/water for 1 h, filtered, and washed with TFA (2 mL). The filtrate was evaporated to dryness in vacuo to afford the title compound **40** as a colorless oil (0.023 g, 96%): TLC (chloroform/MeOH, 8:2) R_f = 0.69; δ_H 0.62 (3H, d, *J* 6.49), 0.69 (3H, d, *J* 6.40), 1.17 (1H, m), 1.27 (1H, m), 1.80 (1H, m), 3.86 (3H, s), 4.26 (1H, t, *J* 7.2), 4.32, 4.61 (2H, 2 × d, *J* 15.25, 15.25), 6.00, 6.54 (2H, 2 × br s), 6.85−7.05, 7.65−7.85 (4H, 2 × m), 7.20−7.50 (5H, m); δ_C 22.12, 22.25, 24.56, 37.27, 48.65, 55.67, 57.44, 114.30, 127.91, 128.44, 128.87,

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129.46, 131.33, 136.35, 139, 173.70; *m*/*z* (ES-MS) 391 (MH^+), calcd 391.1, 413 (MNa^+), calcd 413.15.

***N*$^{\alpha}$-4-Phenylbenzoylleucinamide (41).** FmocLeuOH was coupled onto resin **37** (25 mg, 0.034 mmol) following Fmoc-deprotection. The resin, after 24 h coupling time, was washed with DMF (10 mL), treated with 20% piperidine in DMF (15 mL), and followed by DMF (20 mL) wash. A DMF (1 mL) solution of 4-bromobenzoic acid (0.069 g, 0.344 mmol), HOAt (0.047 g, 0.344 mmol), and DIPCDI (0.054 mL, 0.344 mmol) was added to the above resin and stirring continued overnight. The resin product **43** was washed with DMF and transferred to a round-bottomed flask along with DMF (5 mL). DMF was then degassed by bubbling N_2 for 10 min. A 2 M aqueous Na_2CO_3 solution (5 mL) was also thoroughly degassed and 0.1 mL added to the resin mixture. Phenylboronic acid (0.02 g, 0.165 mmol) was added to the resin mixture followed by $Pd(PPh_3)_4$ (0.007 g, 0.055 mmol). The reaction vessel was flushed with N_2, covered with aluminum foil, and then gently stirred for 3 days at 80 °C, with the palladium catalyst (0.007 g, 0.055 mmol) being added after every 24 h. The resin was then thoroughly washed with DMF (50 mL), CH_2Cl_2 (50 mL), and hexane (20 mL) and dried in vacuo. The resin was then treated with 95% TFA in water for 1 h and filtered, and the filtrate when evaporated to dryness afforded the compound **41** as a gum (8 mg, 78%): TLC (chloroform/methanol, 8:2) $R_f = 0.78$; δ_H (CD_3OD) 1.03, 1.04 (6H, 2 × d, *J* 5.0, 6.0), 1.60−2.00 (3H, m), 4.71 (1H, dd, *J* 4.6, 10.2), 7.39−7.54, 7.69−7.79, 7.98−8.01 (9H, 3 × m); *m*/*z* (FAB) 311 (MH^+), calcd 311.2; HRMS (FAB) calcd for $C_{19}H_{23}N_2O_2$ (MH^+) 311.1759, found 311.1758.

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Supporting Information Available: Experimental details and spectral data for the intermediate compounds **14**, **23**, and **25**, the azalysine derivatives **28**−**31b**, **29**−**31d**, and **28**−**31e**, and the synthetic peptides **32a**−**e**. This material is available free of charge via the Internet at http://pubs.acs.org.

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