Synthesis, Biological Activity, and Conformational Analysis of (2S,3R,4S)-MeBmt[1]-cyclosporin, a Novel 1-Position Epimer of Cyclosporin A[1]

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Cyclosporin A (CsA, 1), an immunosuppressive cyclic undecapeptide, contains a unique amino acid, (4*R*)-4-[(*E*)-butenyl]-4,*N*-dimethyl-L-threonine (MeBmt), that appears to be critically involved in the biological activity of CsA. In order to further explore the effect that structural elements in MeBmt have on the conformation and biological activity of CsA, the 4-epimer of MeBmt [(4*S*)-MeBmt, 2] and the corresponding CsA analogue [(4*S*)-MeBmt[1]-CsA, 3] have been synthesized. Biological assay using concanavalin A stimulated thymocytes indicated that (4*S*)-MeBmt[1]-CsA (3) has only 2–4% immunosuppressive activity relative to CsA. The NMR analysis by 1D and 2D NMR methods establishes the conformation of 3, of which the 33-membered cyclic peptide ring system in chloroform is very similar to that of CsA. However, the NMR analysis also reveals that the 1-position side chain orientation in (4*S*)-MeBmt[1]-CsA (3) is very different from that of CsA. Specifically, the (4*S*)-MeBmt α,β-torsion angle (χ_1) has been rotated approximately 120° relative to that of CsA, and the orientation of the butenyl side chain relative to the 33-membered peptide backbond is different. The orientation of the (4*S*)-MeBmt side chain is consistent with the possible conformations calculated for (4*S*)-MeBmt[1]-CsA (3) by using molecular mechanics (in vacuo) calculations. The conformational analysis suggests that the loss of biological activity for 3 results from an altered conformation of the 1-position side chain relative to the peptide backbond due to the changed chirality at C4 of MeBmt.

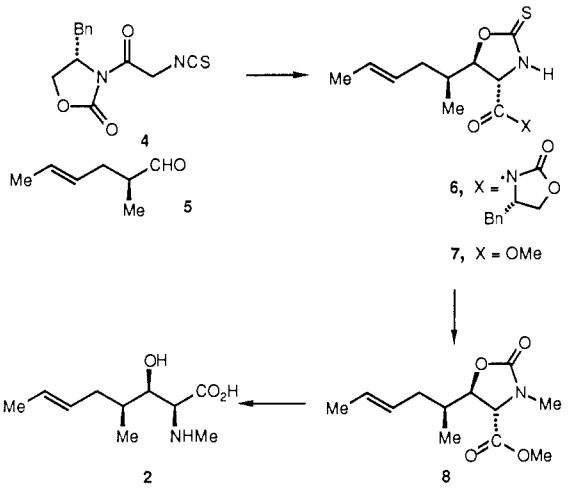
Cyclosporin A (CsA, 1)[2] is an unusually effective immunosuppressive drug currently marketed as Sandimmune for the prevention of rejection of transplanted human organs.[3] This cyclic undecapeptide is distinguished by the presence of seven N-methylated amino acids (Figure 1) in its structure together with a unique amino acid, (4*R*)-4-[(*E*)-butenyl]-4,*N*-dimethyl-L-threonine (MeBmt), in position 1.[4] Limited structure–activity studies have demonstrated that modification of the MeBmt moiety dramatically effects the immunosuppressive activity of the resultant CsA analogues[5-10] and that this amino acid appears to be critically involved in the biological activity of CsA. In conjunction with our continuing efforts[9,11] to explore the effect that structural elements in MeBmt have on the conformation and biological activity of CsA, we have synthesized the 4-epimer of MeBmt [(4*S*)-MeBmt, 2] and the corresponding CsA analogue [(4*S*)-MeBmt[1]-CsA, 3]. The immunosuppressive activity of this analogue has been determined and the solution conformation has been studied by NMR.

Results

Chemistry. The C4 epimer of MeBmt [(4*S*)-MeBmt, 2] was synthesized by using the asymmetric glycine enolate aldol reaction of Weber and Evans,[12] which was used previously to synthesize MeBmt and other chiral amino acids (Scheme I). The chiral glycine synthon isothiocyanate 4, as its derived stannous enolate, was reacted with the aldehyde 2(*S*)-methylhex-4-enal (5) to give the adduct 6 in 71% yield. (2*S*,4*E*)-2-Methyl-4-hexenal (5) was prepared in direct analogy to the procedure described for the *R* enantiomer.[12] Transesterification of 6 with a solution of magnesium methoxide in methanol gave the corresponding methyl ester 7, which was subjected to bismethylation by reaction with freshly prepared trimethyloxonium tetrafluoroborate. Hydrolysis of the methylated product afforded the oxazolidinone 8, which was saponified to the desired amino acid 2 in 49.5% overall yield after purification.

The synthesis of the peptide portion of (4*S*)-MeBmt[1]-CsA (3) closely followed the strategy first em-

Scheme I



ployed by Wenger[6,7] as modified here for the synthesis of CsA.[11] The tetra- and heptapeptide fragments 9 and 10b

(1) Abbreviations used: CsA, cyclosporin A; MeBmt, (2*S*,3*R*,4*R*,6*E*)-3-hydroxy-4-methyl-2-(methylamino)-6-octenoic acid; (4*S*)-MeBmt, (2*S*,3*R*,4*S*,6*E*)-3-hydroxy-4-methyl-2-(methylamino)-6-octenoic acid; XTL, conformational family evolving from a model built from the crystal structure of CsA; SOL, conformational family evolving from a model built from the apolar solution structure of CsA; XDV, conformational family evolving from a model built from the crystal structure of CsA but differing from said model by a rotation of ~120° about χ_2; DMSO, dimethyl sulfoxide; IR, infrared; NMR, nuclear magnetic resonance; NOE, nuclear Overhauser enhancement; NMM, *N*-methylmorpholine; DCC, *N*,*N*′-dicyclohexylcarbodiimide; NOESY, 2D nuclear Overhauser and exchange spectroscopy; Bn, benzyl; Abu, 2-aminobutyric acid; Sar, sarcosine.

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Table I. Chemical Shifts (δ) of the Amide, α-CH, *N*-Methyl Protons and Carbons of CsA (1) and Its 4*S*-Epimer 3

	NH		α-CH				*N*-CH_3				carbonyl	
			CsA (1)		4*S*-epimer 3		CsA (1)		4*S*-epimer 3			
residue	CsA (1)	4*S*-epimer 3	^1H	^{13}C	^1H	^{13}C	^1H	^{13}C	^1H	^{13}C	CsA (1): ^{13}C	4*S*-epimer 3: ^{13}C
1			5.47	58.75	5.13	59.1	3.51	34.0	3.42	32.1	169.65	170.5
2	7.96	8.43	5.03	48.8	4.92	48.5					173.0	173.0
3			3.23, 4.76	50.4	3.14, 4.65	49.8	3.40	39.4	3.40	39.2	170.5	170.4
4			5.34	55.5	5.3	55.0	3.11	31.3	3.05	31.1	169.35	169.0
5	7.48	7.60	4.66	55.4	4.65	55.0					173.1	172.9
6			5.02	55.3	5.17	54.1	3.25	31.5	3.26	31.2	170.9	171.5
7	7.68	7.91	4.52	48.7	4.41	48.3					170.4	171.15
8	7.17	7.42	4.83	45.2	4.81	44.8					172.9	173.6
9			5.70	48.3	5.63	47.8	3.12	29.65	3.16	29.6	169.75	170.6
10			5.10	57.5	5.07	57.2	2.70	29.8	2.65	29.7	169.45	170.2
11			5.14	57.9	5.02	58.4	2.71	29.8	2.65	30.0	172.85	173.2

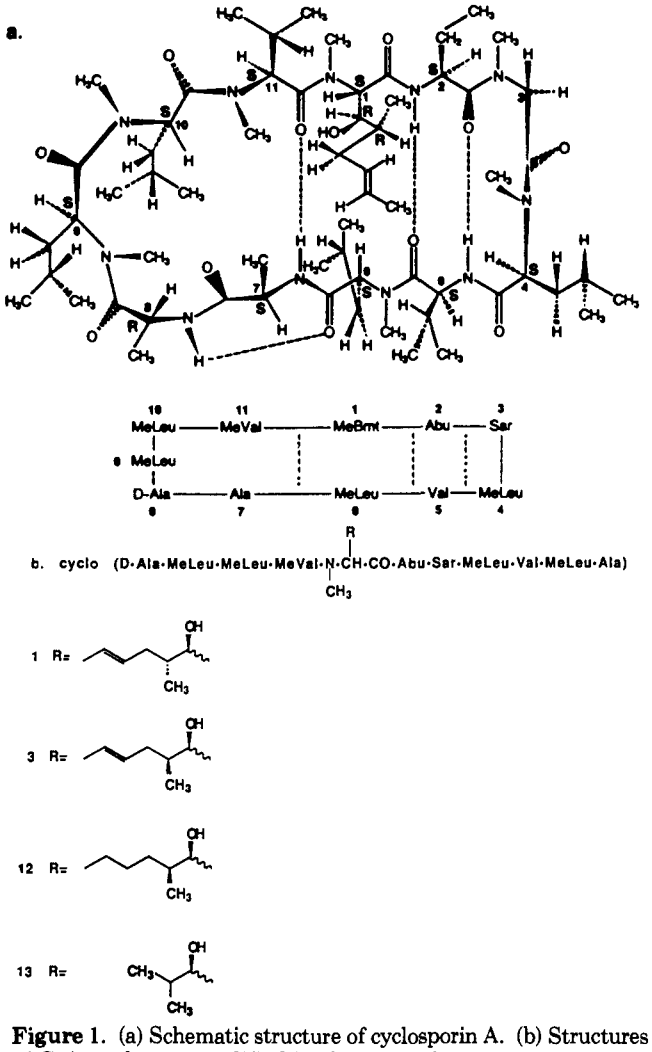

Figure 1. (a) Schematic structure of cyclosporin A. (b) Structures of CsA analogues modified in the 1-position.

were assembled by the stepwise coupling strategy (Figure 2) and coupled together to give the linear undecapeptide 11. Treatment of 11 with 0.2 N NaOH gave, after neutralization, fully deprotected linear undecapeptide, which was cyclized by the procedure developed by Wenger[7] to give **3** in 30% overall yield.

Biological Results. The biological activity of **3** was determined by using the inhibition of concanavalin A stimulated thymocytes as previously described.[9,13] CsA analogue **3** is only 2–4% as active as CsA in this assay.

NMR Results. In an attempt to determine whether the low biological activity of **3** was caused by a major change in the conformation of the cyclic peptide ring or by a different orientation of the MeBmt side chain due to the changed chirality at C4, we carried out the conformational analysis of **3** by NMR. Experiments were carried out in chloroform solution in which **3** is present essentially in only one conformation (>98%). The assignment of the protons and carbons (Table I) was carried out at 500 MHz (proton frequency) by using the methods described by Kessler et al.[14] for CsA and by us for the conformations of several CsA analogues.[11]

The 1D ^1H NMR spectrum of **3** differs in four major aspects from the spectra obtained for CsA. The amide protons of amino acid residues 2, 7, and 8 and (less markedly) 5 are downfield shifted in **3**. The chemical shift for the *N*-methyl group in (4*S*)-MeBmt resonates upfield (3.42 vs 3.51 ppm) relative to this group in CsA. Two upfield methyl doublets (at 0.62 and 0.71 ppm) are present in the spectrum for **3**. For CsA, only one upfield doublet (at $\delta = 0.70$ ppm) is observed, which corresponds to the protons on the methyl group attached to C4 of MeBmt. The α-carbon resonance in residue 1 is shifted upfield (5.13 vs 5.43 ppm) in **3** vs **1**.

These NMR-spectral differences could be the result of a different conformation for the 33-membered peptide ring system for **3** and/or they could result from a different orientation of the MeBmt side chain in the 1-position with respect to the peptide ring. To differentiate between these possibilities, we investigated the chloroform solution conformation of **3** by NMR. The small temperature coefficient for the chemical shift of Val[5]-NH ($\Delta\delta/\Delta T = 0.1 \times 10^{-3}$ ppm/°C) and the NOE observed between the MeLeu[4] *N*-methyl protons and the *si* proton of Sar[3] indicated that the type II′ β-turn found in CsA is retained in the epimeric analogue **3**. The cis amide bond between residues MeLeu-9 and MeLeu-10 is retained in **3**, which was established by the strong NOE between the two α-protons at these sites. All the other NOEs observed for the intracyclic protons of **3** are very similar to those reported for CsA (1), except for the NOE between the α-protons on residues 1 and 6

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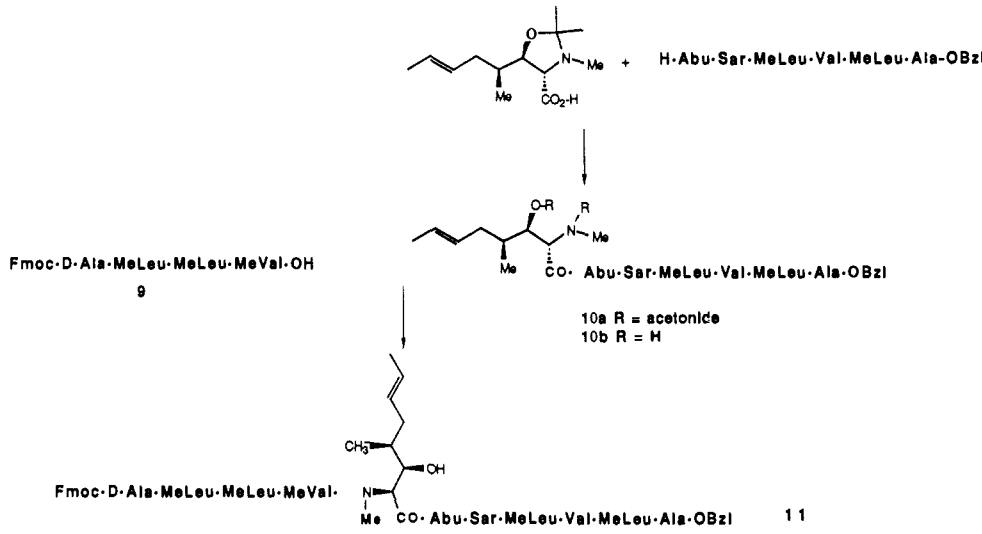

Figure 2. Strategy used to synthesize linear undecapeptide 11.

in CsA which could not be observed in 3 because the chemical shifts of these two protons overlap. Thus, the peptide ring conformation for 3 is very similar to the ring system conformation reported for CsA in chloroform.[14,15]

However, the chemical shift of the *N*-methyl and α-protons of MeBmt in analogue 3 are shifted upfield relative to the corresponding signals in CsA, which suggested that the (4*S*)-MeBmt side chain in 3 is oriented differently with respect to the peptide ring system than the (4*R*)-MeBmt epimer is orientated in CsA. In addition, there are two upfield doublets (0.62, 0.71 ppm) in analogue 3 but only one in CsA (0.70 ppm). The two upfield doublets in (4*S*)-MeBmt[1]-CsA were assigned by means of a total correlation spectroscopy experiment (HOHAHA).[16] One signal corresponds to the C4 methyl protons of the (4*S*)-MeBmt side chain (δ = 0.62 ppm) while the other resonance arises from one of the terminal methyl groups in the MeLeu[6] isobutyl side chain (δ = 0.71 ppm). The orientation of the MeBmt side chain in 3 was established from the coupling constant between the α- and β-protons. In CsA (1) this coupling constant is 5.7 Hz whereas it is 9.4 Hz for the 4*S*-epimer 3. This indicates that the torsion angle χ_1 and thus the side-chain orientation is significantly different in 3. By use of a Karplus-type curve, χ_1 is either 0–10° or 160–170°.[17] These two possibilities could be distinguished by NOE experiments because in 3 there is an NOE between the C4 methyl protons in MeBmt and an α-proton on either MeBmt or MeLeu-6. Although it was not possible to assign exactly the α-protons on these two residues (vide supra), an NOE to either (or both) proton is possible only when the C4 methyl group in MeBmt is folded under the peptide ring system. This arrangement is possible only when χ_1 is near to 180°.

In an attempt to define more precisely the orientation of the MeBmt side chain with respect to other side chains in 3, we tried to determine if the additional upfield shifted methyl of MeLeu[6] (δ = 0.71 ppm), which is not present in CsA, was caused by an anisotropic effect of the MeBmt double bond, which might approach the 6-position in a folded conformation. To test for this, we reduced the double bond in 3 by catalytic hydrogenation to afford the new compound, [(4*S*)-dihydro MeBmt][1]CsA[3] (12). Since both upfield resonances (0.71, 0.62 ppm) were present in the spectrum for the reduced analogue 3, the upfield resonance for the δ-methyl group in the MeLeu[6] residue is not caused by the double bond in (4*S*)-MeBmt. It is probable that the second upfield resonance is caused by a shielding effect from a neighboring carbonyl group, but this carbonyl has not been assigned. Thus, the conformation of the MeBmt side chain in (4*S*)-MeBmt[1]-CsA analogue (3) in chloroform with respect to torsion angles beyond χ_1 could not be determined more precisely.

Discussion

Previous results reported by the Sandoz group and from this laboratory have established that most of the side chain of the novel amino acid, MeBmt, appears to be needed for full immunosuppressive activity. Thus, [MeLeu(3-OH)][1]-CsA (13), which results from deletion of three carbons from MeBmt, has less than 0.1% of the immunosuppressive activity of CsA.[9] Similarly, deletion of the β-hydroxyl group from MeBmt produces an essentially nonimmunosuppressive CsA analogue. However, the importance of the *R* configuration of the 4-methyl group in CsA, either for immunosuppressive activity or for the solution conformation, had not been evaluated.

The synthesis of the (4*S*)-MeBmt was carried out in the same manner used to prepare MeBmt[12] except that the 4(*S*)-aldehyde was used (Scheme I). The resulting (4*S*)-MeBmt was incorporated into cyclosporin by using the modified route we have used to prepare other CsA analogues.[9,11] Biological assay using concanavalin A stimulated thymocytes[13] established that (4*S*)-MeBmt[1]-CsA (3) has only 2–4% biological activity relative to CsA. Thus, epimerization of this one chiral center reduces immunosuppressive activity by about 96–98%.

The dramatic loss in biological activity that results from epimerization of C4 in MeBmt could result from altered conformations of either the 1-position side chain or the 33-membered peptide ring system, or from steric hindrance between the 4(*S*)-methyl group and cyclosporin receptors. In order to address the first of these two possibilities, we have carried out the conformational analysis of (4*S*)-MeBmt[1]-CsA (3) in chloroform solution by nuclear magnetic resonance (NMR) by using the multiple approaches devised by Kessler and his colleagues in their definitive study of the solution conformation of CsA.[14]

The NMR analysis by 1D and 2D NMR methods establishes that the conformation of the 33-membered cyclic peptide ring system in chloroform is very similar to that

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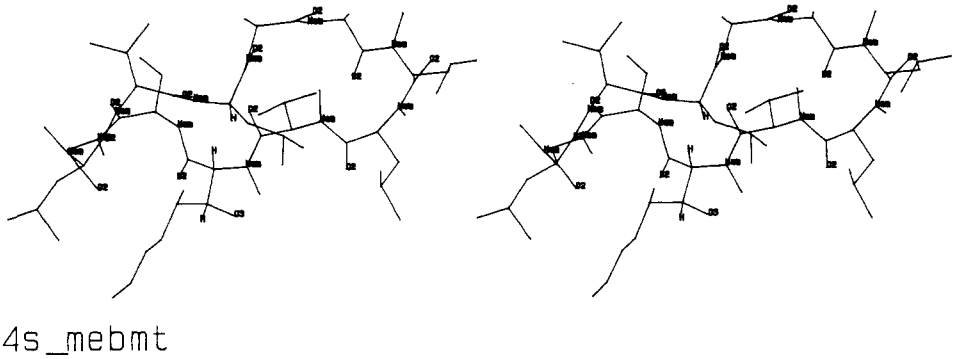

Figure 3. Crossed-stereo projection of the backbone and side-chain atoms in (4*S*)-MeBmt¹-CsA in conformation XDVA, which was obtained from molecular mechanics calculations.[20] Note that the hydrogens on C2 and C3 in MeBmt are antiperiplanar and that the 4*S*-methyl group lies equidistant between the α protons on MeBmt and MeLeu.[6]

of CsA. Specifically, the NOE experiments establish that the cis amide bond between MeLeu⁹-MeLeu¹⁰, the type II′ β-turn for the sequence Abu-Sar-MeLeu-Val⁵, and the four intramolecular hydrogen bonds involving all four NH groups in the molecule are retained in (4*S*)-MeBmt¹-CsA (**3**). The close proximity between the α-protons on MeBmt and MeLeu⁶ was not confirmed by the NOE experiment because the chemical shifts for these protons overlap in **3**. However, the NOESY spectrum for **3** is otherwise very similar to that obtained for CsA so that the solution conformations of **1** and **3** must be very similar, if not identical, with respect to the conformation of the cyclic peptide ring system.

However, the NMR analysis also reveals that there is a major difference in the orientations of the MeBmt side chains in these two analogues. For CsA in chloroform the MeBmt side chain is orientated away from the peptide ring system,[15] which is clearly evident from the C_α–C_β coupling constant for MeBmt in **1** that restricts χ_1 to 300–320°. Kessler and co-workers have shown that a hydrogen bond between the 3(*R*)-hydroxyl group and the MeBmt carbonyl oxygen exists in chloroform and most likely stabilizes this extended conformation.[14] Lautz et al. have shown by means of molecular dynamics calculations that the chloroform solution of CsA is destabilized when this hydrogen bond is broken by the hydrogen bonding solvent water,[18] and that the preferred conformation in water is closely related to the X-ray structure.

Our results show that the MeBmt side chain orientation in (4*S*)-MeBmt¹-CsA (**3**) is very different from that of CsA. The NMR data establish that the (4*S*)-MeBmt α–β coupling constant in **3** (9.4 Hz) and the NOE between the C4 methyl group and either the $C_\alpha{}^1$ or $C_\alpha{}^6$ protons are consistent with a torsion angle χ_1 of 170–190°. Thus, the α,β torsion angle (χ_1) has been rotated approximately 120° relative to CsA, and the orientation of the butenyl side chain relative to the 33-membered peptide backbone is very different.

The orientation of the (4*S*)-MeBmt side chain is consistent with the possible conformations calculated for (4*S*)-MeBmt¹-CsA by using molecular mechanics (in vacuo) calculations.[19] In work to be reported separately,[20] we have carried out a grid search of the possible conformations for the MeBmt side chain in CsA and several CsA analogues in which the MeBmt residue has been modified. These possible conformations were energy minimized by using the AMBER force field[21] in MACROMODEL,[22] and Boltzmann distributions for each analogue were calculated. In the case of (4*S*)-MeBmt¹-CsA, the calculations show that in the 11 lowest energy conformations that comprise 99% of the conformations, χ_1 lies between 178° and 197°. Nine of the 11 conformations, comprising 81% of the conformers, are derived from a new cyclosporin conformational family we have designated XDV. Only 18% of the conformations are related to the crystal (XTL) conformations of CsA, while less than 1% are related to the chloroform solution (SOL) conformations. The structure of the lowest energy conformation (XDVA) is shown in Figure 3. Note that in this conformation, the 4(*S*)-methyl group is approximately equidistant between the α-protons at the 1- and 6-positions, as required by our solution NOE data. Thus, epimerizing C4 in CsA from *R* to *S* causes a major change in the side-chain orientation of the 1-position residue without measurably perturbing the conformation of the 33-membered peptide ring system in chloroform.

Experimental Section

Optical rotations were measured on a Perkin-Elmer Model 241 polarimeter (1.000-dm cell) at room temperature. Infrared (IR) spectra were recorded on a Perkin-Elmer 599B spectrophotometer (data in cm^{-1}). Nuclear magnetic resonance (NMR) spectra were recorded on a Bruker WP-270 spectrometer, except for the full characterization of a CsA analogue which was carried out on a Bruker AM 500 or AC 300 spectrometer equipped with an Aspect 3000 computer.

Flash chromatography[23] was carried out under low pressure (5–15 psi) with Merck grade 60 silica, 230–400 mesh. Thin-layer chromatography (TLC) was run on Merck Kieselgel 60-F_{254} with fluorescent indicator visualized by ultraviolet (UV) or 7% phosphomolybdic acid (PMA) in ethanol.

Tetrahydrofuran and *N*-ethylpiperidine were distilled from sodium/benzophenone ketyl. Methylene chloride and *N*-methylmorpholine were distilled from calcium hydride. Methanol was distilled from magnesium methoxide. Acetone (for making acetonide) was dried with activated 4A molecular sieves and distilled prior to use. All other solvents and reagents were either ACS reagent or HPLC grade and used without further purification. All nonaqueous reactions were carried out under a dry nitrogen atmosphere in oven-dried (140 °C, 12 h) glassware.

(4*S*)-3-(((4′*S*,5′*R*)-5′-((1″*S*,3″*E*)-1″-Methyl-3″-pentenyl)-2′-thioxo-4′-oxazolidinyl)carbonyl)-4-(phenyl-

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methyl)-2-oxazolidinone (6). To the stannous enolate formed from 347 mg (1.26 mmol, 1.1 equiv) of isothiocyanate 4, 477 mg (1.14 mmol, 1.0 equiv) of stannous triflate,[27] and 168 mg (0.20 mL, 1.49 mmol, 1.3 equiv) of *N*-ethylpiperidine in 3 mL of THF was added via cannula a solution of 105 mg (0.938 mmol, 1.2 equiv) of (2*S*)-aldehyde **5**, prepared in direct analogy to the procedure described for the *R* enantiomer[12] in 3 mL of THF. After the reaction mixture was stirred at −78 °C for 1.5 h, the product was isolated according to the general procedure to give a white foam. HPLC analysis (Zorbax, 21% methylene chloride/30% *tert*-butyl methyl ether/49% isooctane, 2 mL/min, 244 nm) afforded a 0.57:96.6:2.5:0.29 mixture of diastereomers (t_r 2.46, 5.32, 9.31, 13.33 min, respectively). Purification by flash chromatography (30 × 250 mm silica gel, 25 and 40% ethyl acetate/hexane) yielded 259 mg (71%, >99% diastereomeric purity) of the title compound **6** as an oil: R_f 0.33 (35% ethyl acetate/hexane); $[\alpha]_D$ +217° (*c* 1.2, CH_2Cl_2); IR (CH_2Cl_2) 3430, 3070–2840, 1782, 1712, 1475, 1396, 1244, 1185 cm^{-1}; ^1H NMR (250 MHz, $CDCl_3$) δ 7.51 (br s, 1 H, N*H*), 7.43–7.16 (m, 5 H, aromatic H's), 5.55–5.30 (m, 3 H, C*H*=C*H*, C(S)OC*H*), 2.85 (dd, 1 H, *J* = 1.7, 2.9 Hz, C(S)NHC*H*), 2.79–2.70 (m, 1 H, C_4-*H*), 2.42–2.31 (m, 2 H, C_5-H_2), 3.21 (dd, 1 H, *J* = 3.5, 13.6 Hz, C*H*HPh), 2.92 (dd, 1 H, *J* = 8.6, 13.6 Hz, CH*H*Ph), 2.20–2.12 (m, 1 H, CHMeC*H*H), 2.05–1.88 (m, 2 H, C*H*MeCH*H*), 1.66 (d, 3 H, *J* = 6.0 Hz, CH=CHCH_3), 1.02 (d, 3 H, *J* = 6.5 Hz, CH(CH_3)CH_2); ^{13}C NMR (62.9 MHz, $CDCl_3$) δ 188.9, 166.6, 153.7, 134.1, 129.3, 129.0, 128.0, 127.6, 127.5, 86.7, 67.6, 60.7, 55.2, 37.5, 35.2, 17.7, 13.5. Anal. ($C_{20}H_{24}N_2O_4S$) C, H.

Methyl (4*S*,5*R*)-5-((1′*S*,3′*E*)-1′-Methyl-3′-pentenyl)-2-thioxooxazolidine-4-carboxylate (7). To a solution of 944 mg (2.43 mmol) of aldol adduct **6** in 20 mL of anhydrous methanol at 0 °C was added via cannula a suspension formed by the addition of 0.84 mL (2.67 mmol, 1.1 equiv, 3.2 M in diethyl ether) of methylmagnesium bromide to 5 mL of methanol. After the reaction mixture was stirred for 3 min, it was quenched by the addition of 10 mL of saturated aqueous ammonium chloride. Volatiles were removed in vacuo. The residue was dissolved in 1 N aqueous hydrochloric acid and extracted with three portions of methylene chloride. The combined organic phases were dried over anhydrous sodium sulfate and concentrated to give a pale yellow oil. Purification by flash chromatography (30 × 150 mm silica gel, 30% ethyl acetate/hexane) afforded 431 mg (100%) of the recovered chiral auxiliary and 565 mg (96%) of the title compound **7** as a clear oil: R_f 0.37 (35% ethyl acetate/hexane); $[\alpha]_D$ +110° (*c* 1.18, CH_2Cl_2); IR (CH_2Cl_2) 3440, 3040–2850, 1756, 1489, 1295, 1276, 1184 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.19 (br s, 1 H, N*H*), 5.60–5.48 (m, 1 H, C*H*=CH), 5.43–5.33 (m, 1 H, CH=C*H*), 2.94 (dd, 1 H, *J* = 2.2, 5.8 Hz, C_5-*H*), 2.29 (d, 1 H, *J* = 5.8 Hz, C_4-*H*), 3.83 (s, 3 H, OCH_3), 2.27–2.17 (m, 1 H, CHMeC*H*H), 2.04–1.83 (m, 2 H, C*H*MeCH*H*), 1.67 (dd, 3 H, *J* = 1.2, 6.2 Hz, CH=CHCH_3), 1.01 (d, 3 H, *J* = 6.3 Hz, CH(CH_3)CH_2); ^{13}C NMR (62.9 MHz, $CDCl_3$) δ 189.2, 169.1, 128.1, 127.2, 88.3, 59.9, 53.1, 37.6, 35.0, 17.7, 13.2. Anal. ($C_{11}H_{17}NO_3S$) C, H.

Methyl (4*S*,5*R*)-3-Methyl-5-((1′*S*,3′*E*)-1′-methyl-3′-pentenyl)-2-oxazolidine-4-carboxylate (8). To a suspension of 169 mg (1.19 mmol, 2.1 equiv) of trimethyloxonium tetrafluoroborate and 134 mg (0.625 mmol, 1.1 equiv) of 1,8-bis(dimethylamino)-naphthalene in 2 mL of methylene chloride at 0 °C was added via cannula a solution of 138 mg (0.568 mmol) of methyl ester **7** in 2 mL of methylene chloride at 0 °C. After the resultant white slurry was stirred for 3 h, it was concentrated in vacuo at 0 °C. The residue was suspended in 6 mL of THF at 0 °C, and 2 mL of pH 7 phosphate buffer was added. The reaction mixture was stirred at 0 °C for 1.5 h, poured into 50 mL of 1 N aqueous sodium bisulfate, and extracted with three 50-mL portions of methylene chloride. The combined organic extracts were dried over anhydrous sodium sulfate and concetrated to give a white solid and a yellow oil. Purification of the mixture by flash chromatography (20 × 100 mm silica gel; 35% ethyl acetate/hexane) afforded 115 mg (84%) of the title compound **8** as a clear oil: R_f 0.39 (40% ethyl acetate/hexane); $[\alpha]_D$ +59.2° (*c* 1.13, CH_2Cl_2); IR (CH_2Cl_2) 3060–2850, 1765, 1439, 1401, 1216 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 5.56–5.44 (m, 1 H, C*H*=CH), 5.40–5.30 (m, 1 H, CH=C*H*), 2.35 (t, 1 H, *J* = 2.9 Hz, C_5-*H*), 3.96 (d, 1 H, *J* = 5.0 Hz, C_4-*H*), 3.82 (s, 3 H, OCH_3), 2.93 (s, 3 H, NCH_3), 2.23–2.14 (m, 1 H, C*H*HCH=CH), 1.99–1.89 (m, 1 H, CH*H*CH=CH), 1.84–1.75 (m, 1 H, C*H*MeCH_2), 1.66 (dd, 3 H, *J* = 1.3, 6.2 Hz, CH=CHCH_3), 0.96 (d, 3 H, *J* = 6.7 Hz, CHCH_3); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 170.1, 157.3, 128.0, 127.5, 78.6, 62.1, 52.7, 37.6, 35.1, 30.0, 17.8, 13.0. Anal. ($C_{12}H_{19}NO_4$) C, H.

(2*S*,3*R*,4*S*,6*E*)-3-Hydroxy-4-methyl-2-(methylamino)-6-octenoic Acid (2). A solution of 101 mg (0.417 mmol) of methyl ester **8** in 1 mL of 2 N aqueous potassium hydroxide solution was heated at 75–80 °C overnight. The solution was allowed to cool to room temperature, and the pH was adjusted to 5 by the addition of 1 N aqueous hydrochloric acid. The aqueous solution was concentrated. The resultant white solid was suspended in methanol and sonicated to form a fine suspension, which was chromatographed (40 g of Sephadex LH-20, methanol) to give 70 mg (83% yield) of the title compound **2**. An analytical sample was prepared by recrystallization from ethanol/water: mp 254–256 °C; $[\alpha]_D$ +0.85° (*c* 0.59, 0.2 N aqueous HCl); IR (KBr pellet) 3500–2300 (br), 1626, 1605, 1575, 1465, 1430, 1415, 1356, 1121, 1001, 974, 851 cm^{-1}; ^1H NMR (250 MHz, D_2O) δ 5.49–5.24 (m, 2 H, C*H*=C*H*), 3.65 (dd, 1 H, *J* = 3.3, 8.6 Hz, C_3-*H*), 3.41 (d, 1 H, *J* = 8.6 Hz, C_2-*H*), 2.57 (s, 3 H, NCH_3), 1.98–1.81 (m, 2 H, C_5-H_2), 1.78–1.53 (m, 1 H, C_4-*H*), 1.50 (d, 3 H, *J* = 5.9 Hz, C_8-H_3), 0.79 (d, 3 H, *J* = 6.8 Hz, C_4-CH_3); ^{13}C NMR (75.5 MHz, d_3-MeOD, amino acid hydrochloride salt) δ 169.7, 129.7, 128.2, 73.1, 65.6, 37.7, 37.1, 33.3, 18.0, 13.6. Anal. ($C_{10}H_{19}NO_3$) C, H.

(((4*S*,5*R*,1′*S*,3′*E*)-2,2,3-Trimethyl-5-(1′-methyl-3′-pentenyl)-4-oxazolidinyl)carbonyl)-L-2-aminobutyryl-sarcosyl-*N*-methyl-L-leucyl-L-valyl-*N*-methyl-L-leucyl-L-alanine Benzyl Ester (*N*,*O*-isopropylidene-[(4*S*)-MeBmt]-Abu-Sar-MeLeu-Val-MeLeu-Ala-OBzl) (10a). A suspension of 60 mg of amino acid **2** (0.3 mmol) in 100 mL of anhydrous acetone was heated under reflux for 24 h until a clear solution was obtained. The solution was concentrated to 2 mL under vacuum and used immediately in the next step. The freshly prepared acetonide (0.3 mmol) in 2 mL of acetone was diluted with 4 mL of THF and 0.038 mL of NMM (0.345 mmol, 1.15 equiv) was immediately added. *N*-Hydroxybenzotriazole (101 mg, 0.66 mmol, 2.2 equiv), which was dehydrated by azeotropic distillation of H_2O with two 30-mL portions of toluene/THF, was added to the solution together with 238 mg of the hexapeptide H-Abu-Sar-MeLeu-Val-MeLeu-Ala-OBzl[8,11] (0.345 mmol, 1.15 equiv). The resulting mixture was cooled (0 °C) and 68 mg of DCC (0.33 mmol, 1.1 equiv) was added. The mixture was allowed to warm to room temperature and was stirred for 20 h under N_2. The mixture was diluted with 20 mL of CH_2Cl_2 and washed with saturated $NaHCO_3$ solution (15 mL). The aqueous phase was extracted with CH_2Cl_2 (3 × 15 mL). The combined CH_2Cl_2 was dried ($MgSO_4$) and evaporated. The residue was suspended in 10 mL of ethyl acetate and filtered. The filtrate was evaporated and the residue was flash chromatographed[23] on 20 g of silica gel with 10–20% acetone/hexane to give 240 mg (88%) of **10a**: R_f 0.31 (40% acetone/hexane); $[\alpha]_D$ −126° (*c* 1.5, $CHCl_3$); IR ($CHCl_3$) 3360, 2960, 1740, 1650 (shoulder), 1510, 1450, 1190, 1100, 760, 690 cm^{-1}; ^1H NMR ($CDCl_3$, 270 MHz, at room temperature one major conformer was observed) δ 0.8–1.0 (m, 24 H, CH_3-C(4^1), CH_3-C(3^2), $2CH_3$-C(4^4), $2CH_3$-C(3^5), $2CH_3$-C(4^6)), 1.18, 1.30 (2 s, 6 H, CH_3 of isopropylidene), 1.31 (d, *J* = 7.0 Hz, 3 H, CH_3-C(2^7)), 1.62 (d, *J* = 5.0 Hz, 3 H, CH_3-C(7^1)), 1.10–2.30 (m, 12 H, 2H-C(5^1), H-C(4^1), 2H-C(3^2), 2H-C(3^4), H-C(4^4), H-C(3^5), 2H-C(3^6), H-C(4^6)), 2.30 (s, 3 H, CH_3-N^1), 2.92, 3.00, 3.15 (3 s, 9 H, CH_3-N^3, CH_3-N^4, CH_3-N^6), 2.80–5.50 (m, 13 H, H-C(2^1), H-C(3^1), H-C(6^1), H-C(7^1), H-C(2^2), 2H-C(2^3), H-C(2^4), H-C(2^5), H-C(2^6), H-C(2^7), 2H (OCH_2Ph)), 7.32 (s, 5 H, aromat H), 6.40–7.80 (3 d, *J* = 9.0 Hz, 3 H, H-N^2, H-N^5, H-N^7); MS exact mass calcd for $C_{49}H_{82}N_7O_9$ 912.6174, found (HR-FAB) 912.6159.

((2*S*,3*R*,4*S*,6*E*)-3-Hydroxy-4-methyl-2-(methylamino)-6-octenoyl)-L-2-aminobutyryl-sarcosyl-*N*-methyl-L-leucyl-L-valyl-*N*-methyl-L-leucyl-L-alanine Benzyl Ester (H[(4*S*)-

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MeBmt]-Abu-Sar-MeLeu-Val-MeLeu-Ala-OBzl) (10b). A solution of 232 mg of the *N,O*-isopropylidene derivative **10a** (0.26 mmol) in 5 mL of CH_3OH was stirred for 12 h at room temperature in the presence of 1.05 mL of 1 N HCl (1.05 mmol, 4.0 equiv). The acid in the reaction mixture was neutralized with 300 mg of $NaHCO_3$ (3.57 mmol, 13.5 equiv) and the solvent was evaporated in vacuo (16 Torr) at room temperature. The residue was taken up into 2% CH_3OH/CH_2Cl_2 and flash chromatographed[23] on 20 g of silica gel with 2–3% CH_3OH/CH_2Cl_2 to give 192 mg (86%) of amine **10b**: R_f 0.28 (8% of CH_3OH/CH_2Cl_2); $[\alpha]_D$ −145.5° (*c* 1.6, $CHCl_3$); IR ($CHCl_3$) 3300 (br), 3000, 2950, 1740, 1640 (shoulder), 1500, 1400, 1220, 1190 cm^{-1}; ^1H NMR ($CDCl_3$, 270 MHz, at least two conformers present at room temperature) δ 0.68–1.10 (m, 24 H, CH_3-C(4^1), CH_3-C(3^2), $2CH_3$-C(4^4), $2CH_3$-C(3^5), $2CH_3$-C(4^6)), 1.30, 1.35 (2 d, *J* = 7.0 Hz, 3 H, CH_3-C(2^7)), 1.60 (d, *J* = 5.0 Hz, 3 H, CH_3-C(7^1)), 1.20–2.60 (m, 12 H, 2H-C(5^1), H-C(4^1), 2H-C(3^2), 2H-C(3^4), H-C(4^4), H-C(3^5), 2H-C(3^6), H-C(4^6)), 2.40 (s, 3 H, CH_3-N^1), 2.75–3.32 (m, 9 H, CH_3-N^3, CH_3-N^4, CH_3-N^6), 2.70–5.50 (m, 15 H, H-C(2^1), H-N^1, H-C(3^1), HO-C(3^1), H-C(6^1), H-C(7^1), H-C(2^2), 2H-C(2^3), H-C(2^4), H-C(2^5), H-C(2^6), H-C(2^7), 2 H (OCH_2Ph)), 7.32 (s, 5 H, aromat H), 6.70–8.15 (m, 3 H, H-N^2, H-N^5, H-N^7); MS exact mass calcd for $C_{46}H_{78}N_7O_9$ 872.5860, found (HR-FAB) 872.5854.

[[(9-Fluorenylmethyl)oxy]carbonyl]-D-alanyl-*N*-methyl-L-leucyl-*N*-methyl-L-leucyl-*N*-methyl-L-valyl-[(2*S*,3*R*,4*S*,6*E*)-3-hydroxy-4-methyl-2-(methylamino)-6-octenoyl]-L-2-aminobutyryl-sarcosyl-*N*-methyl-L-leucyl-L-valyl-*N*-methyl-L-leucyl-L-alanine Benzyl Ester (Fmoc-D-Ala-MeLeu-MeLeu-MeVal-[(4*S*)-MeBmt]-Abu-Sar-MeLeu-Val-MeLeu-Ala-OBzl) (11). To a solution of 152 mg of heptapeptide amine **10b** (0.175 mmol) and 136 mg of the tetrapeptide Fmoc-D-Ala-MeLeu-MeLeu-MeVal-OH[24] (0.2 mmol, 1.15 equiv) in 4.0 mL of CH_2Cl_2 was added sequentially 0.039 mL of NMM (0.35 mmol, 2.0 equiv) and 117 mg of BOP[25] (0.265 mmol, 1.5 equiv). The reaction mixture was stirred under N_2 for 60 h at room temperature. The solution was diluted with CH_2Cl_2 (20 mL) and washed with H_2O (15 mL). The aqueous phase was extracted with CH_2Cl_2 (3 × 10 mL) and the combined CH_2Cl_2 was dried over $MgSO_4$, filtered, and concentrated. The residue was flash chromatographed[23] on 18 g of silica gel with 10–25% acetone/hexane to give 161 mg (60%) of pure protected undecapeptide **11**: R_f 0.23 (40% acetone/hexane); $[\alpha]_D$ −151° (*c* 1.6, $CHCl_3$); IR ($CHCl_3$) 3400, 3300, 2950, 1710, 1640 (shoulder), 1500, 1460, 1405, 1190, 750, 720 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz, at room temperature more than one conformer was observed; the main conformer is described) δ 0.72–1.03 (m, 42 H, $2CH_3$-C(4^2), $2CH_3$-C(4^3), $2CH_3$-C(3^4), CH_3-C(4^5), CH_3-C(3^6), $2CH_3$-C(4^8), $2CH_3$-C(3^9), $2CH_3$-C(4^{10})), 1.25, 1.30 (2 d, *J* = 7.0 Hz, 6 H, CH_3-C(2^1), CH_3-C(2^{11})), 1.57 (d, *J* = 5.0 Hz, 3 H, CH_3-C(7^5)), 1.20–2.40 (m, 19 H, 2H-C(3^2), H-C(4^2), 2H-C(3^3), H-C(4^3), H-C(3^4), H-C(4^5), 2H-C(5^5), 2H-C(3^6), 2H-C(3^8), H-C(4^8), H-C(3^9), 2H-C(3^{10}), H-C(4^{10})), 2.93 (6 H), 2.95, 3.00, 3.08, 3.25, 3.33 (6 s, 21 H, CH_3-N^2, CH_3-N^3, CH_3-N^4, CH_3-N^5, CH_3-N^7, CH_3-N^8, CH_3-N^{10}), 3.75 (m, 1 H, H-C(3^5)), 2.70–5.50 (m, 20 H, H-C(2^1), H-C(2^2), H-C(2^3), H-C(2^4), H-C(2^5), HO-C(3^5), H-C(6^5), H-C(7^5), H-C(2^6), 2H-C(2^7), H-C(2^8), H-C(2^9), H-C(2^{10}), H-C(2^{11}), 2 H (OCH_2Ph), 3 H (Fmoc: H-C(9′), 2 H (CH_2O)), 5.72 (d, *J* = 9.0 Hz, 1 H, H-N^1), 6.80–7.78 (m, 16 H, H-N^6, H-N^9, H-N^{11}, aromat H); MS exact mass calcd for $C_{84}H_{130}N_{11}O_{15}$ 1532.9747, found (HR-FAB) 1532.9818.

Cyclo[((2*S*,3*R*,4*S*,6*E*)-3-hydroxy-4-methyl-2-(methylamino)-6-octenoyl)-L-2-aminobutyryl-sarcosyl-*N*-methyl-L-leucyl-L-valyl-*N*-methyl-L-leucyl-L-alanyl-D-alanyl-*N*-methyl-L-leucyl-*N*-methyl-L-leucyl-*N*-methyl-L-valyl] (Cyclo[[(4*S*)-MeBmt]-Abu-Sar-MeLeu-Val-MeLeu-Ala-D-Ala-MeLeu-MeLeu-MeVal]) (3). A solution of 150 mg of fully protected linear undecapeptide **11** (0.098 mmol) in 4 mL of EtOH (0 °C) was treated with 0.98 mL of 0.2 N aqueous NaOH solution (0.196 mmol, 2.0 equiv) and stirred at 0 °C. After 1.5 h, an additional 0.49 mL of 0.2 N aqueous NaOH (0.098 mmol, 1.0 equiv) was added and stirring was continued at 0 °C for another 3.5 h. The mixture was acidified with 1.47 mL of 0.2 N aqueous HCl (0.294 mmol, 3.0 equiv) to pH 6 and treated with 15 mL of saturated aqueous NaCl and 40 mL of CH_2Cl_2. The layers were separated, and the aqueous portion was extracted with CH_2Cl_2 (4 × 20 mL). The combined CH_2Cl_2 solution was dried over $MgSO_4$ and concentrated to an oil which was dried in vacuo. The residue was dissolved in 420 mL of CH_2Cl_2, and 60 mg of DMAP (0.5 mmol, 5.0 equiv), and 0.065 mL of propylphosphorus anhydride[26] (a 50% w/w solution in CH_2Cl_2, 0.39 mmol, 4.0 equiv) were added with vigorous stirring to this solution. The solution was stirred at room temperature under N_2 for 48 h and then concentrated to 2 mL and immediately applied to 20 g of silica gel. Flash chromatography with 10–20% acetone/hexane gave 77 mg (65.5%) of pure **3**: R_f 0.25 (40% acetone/hexane); $[\alpha]_D$ −278° (*c* 2.4, $CHCl_3$); IR ($CHCl_3$) 3300, 2960, 1630 (shoulder), 1525, 1470, 1410, 1200, 1090 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 0.62 (d, *J* = 6.5 Hz, 3 H, CH_3-C(4^1)), 0.71 (d, *J* = 6.5 Hz, 3 H, CH_3-C(4^6)), 0.78–1.03 (m, 36 H, CH_3-C(3^2), $2CH_3$-C(4^4), $2CH_3$-C(3^5), CH_3-C(4^6), $2CH_3$-C(4^9), $2CH_3$-C(4^{10}), $2CH_3$-C(3^{11})), 1.23 (d, *J* = 7.0 Hz, 3 H, CH_3-C(2^8)), 1.29 (d, *J* = 7.0 Hz, 3 H, CH_3-C(2^7)), 1.58 (d, *J* = 5.0 Hz, 3 H, CH_3-C(7^1)), 1.12–2.25 (m, 18 H, H-C(4^1), 2H-C(5^1), 2H-C(3^2), 2H-C(3^4), H-C(4^4), 2H-C(3^6), H-C(4^6), 2H-C(3^9), H-C(4^9), 2H-C(3^{10}), H-C(4^{10}), H-C(3^{11})), 2.42 (m, 1 H, H-C(3^5)), 2.65 (s, 3 H, CH_3-N^{10}), 2.65 (s, 3 H, CH_3-N^{11}), 3.05 (s, 3 H, CH_3-N^4), 3.16 (s, 3 H, CH_3-N^9), 3.26 (s, 3 H, CH_3-N^6), 3.40 (s, 3 H, CH_3-N^3), 3.42 (s, 3 H, CH_3-N^1), 3.14, 4.65 (2 d, *J* = 15.0 Hz, 2 H, 2H-C(2^3)), 4.04 (d, *J* = 9.4 Hz, 1 H, H-C(3^1)), 4.41 (m, 1 H, H-C(2^7)), 4.65 (t, *J* = 9.5 Hz, 1 H, H-C(2^5)), 4.81 (m, 1 H, H-C(2^8)), 4.92 (m, 1 H, H-C(2^2)), 5.02 (d, *J* = 11.0 Hz, 1 H, H-C(2^{11})), 5.07 (m, 1 H, H-C(2^{10})), 5.13 (d, *J* = 9.4 Hz, 1 H, H-C(2^1)), 5.17 (m, 1 H, H-C(2^6)), 5.30 (m, 1 H, H-C(2^4)), 5.41 (m, 2 H, H-C(6^1), H-C(7^1)), 5.63 (m, 1 H, H-C(2^9)), 7.42 (d, *J* = 9.0 Hz, 1 H, H-N^8), 7.60 (d, *J* = 9.0 Hz, 1 H, H-N^5), 7.91 (d, *J* = 9.0 Hz, 1 H, H-N^7), 8.43 (d, *J* = 9.0 Hz, 1 H, H-N^2); MS exact mass calcd for $C_{62}H_{112}N_{11}O_{12}$ 1202.8491, found (HR-FAB) 1202.8486.

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