11-Oxo-Δ^8-tetrahydrocannabinol (14). To a solution of 11-oxo-Δ^8-THC acetate (**13**)[18] (1.0 g, 2.7 mmol) in 100 mL of MeOH was added 10 mL of 5% K_2CO_3 solution, and the mixture was stirred for 2 h at room temperature under N_2. The reaction was quenched by the addition of 25 mL of NH_4Cl (saturated aqueous) with stirring for 15 min. The solvent was removed in vacuo and the residue was dissolved in 100 mL of CH_2Cl_2, which was washed successively with 100 mL each of aqueous concetrated NH_4Cl, H_2O, and aqueous, concetrated NaCl and then dried and evaporated in vacuo to give **14** (0.8 g; 91%): ^1H NMR ($CDCl_3$) δ 0.90 (br, t, 3 H), 1.05 (s, 3 H), 1.20 (s, 3 H), 2.0–3.0 (m, 10 H), 3.95 (br, d, 1 H), 6.03 (s, 1 H), 6.10 (s, 1 H), 6.80 (s, 1 H), 9.50 (s, 1 H). It was used without purification in the subsequent reaction.

11-[*N*,*N*-Bis(2-chloroethyl)amino]-Δ^8-tetrahydrocannabinol (12). To a solution of **14** (0.9 g, 2.7 mmol) in 200 mL of dry MeOH containing 3-Å molecular sieves (9 g; activated at 300 °C/0.1 mm) and stirred for 15 min at room temperature under N_2 were added *N*,*N*-bis(2-chloroethyl)amine hydrochloride (2.5 g, 13.8 mmol, 5 equiv) and $NaCNBH_3$ (0.28 g, 4.4 mmol, 1.6 equiv). This mixture was stirred for 14 h. After filtration through Celite and washing with 200 mL of MeOH, the solvent was removed in vacuo and the residue was dissolved in a mixture of 300 mL of CH_2Cl_2 and 100 mL of NH_4Cl (saturated aqueous). The organic layer was washed with brine, dried, and evaporated in vacuo. The residue was purified by flash chromatography (silica gel; 20% ethyl acetate/petroleum ether) to give 0.33 g (27%) of **12**: ^1H NMR ($CDCl_3$) δ 0.90 (t, 3 H), 1.05 (s, 3 H), 1.15 (s, 3 H), 1.60–2.60 (m, 10 H), 2.85 (t, 4 H), 3.05 (s, 2 H), 2.50 (t, 4 H), 4.90 (s, 1 H), 5.65 (s, 1 H), 6.07 (d, 1 H), 6.15 (d, 1 H); IR ν_{max} ($CDCl_3$) 1430, 1580, 1625, 2935, 3610 cm^{-1}. Anal. ($C_{25}H_{37}Cl_2NO_2$) C, H, N, Cl.

Pharmacology. Materials. Male ICR mice (22–30 g), obtained from Dominion Laboratories (Dublin, VA), were maintained on a 14/10-h light/dark cycle and received food and water ad libitum. Cannabidiol, Δ^8-THC, and Δ^9-THC were obtained from the National Institute on Drug Abuse.

Drug Preparation and Administration. The procedure of Olson et al.[10] was used to prepare suspensions suitable for injection, resulting in a final vehicle composition of ethanol/emulphor/saline (1:1:18), which was administered via tail-vein injection (0.1 mL/10 g), or intraventricularly in a volume of 5 μL by using previously described methods.[11]

Behavioral Evaluations. Spontaneous locomotor activity, antinociception (via tail-flick latency), hypothermia, and catalepsy (via the ring-test) were evaluated by previously reported methods.[6,12,13] Besides vehicle control, Δ^9-THC was administered as a positive control in each daily experiment, and a dose–response curve for Δ^9-THC was generated.

Possible antagonistic properties of the cannabinoids were also determined by previously reported methods.[6,12,13] Mice were pretreated with **2**, **3**, **4**, **5**, **8**, **9**, **10**, or **12** (at doses of 30, 1, 3, 1, 3, 3, 50, and 50 mg/kg intravenously, respectively) or 30 mg/kg 10-bromo-CBD diacetate or with **6** or **12** (at a dose of 150 μg intraventricularly) 10 min prior to administration of 6 mg/kg Δ^9-THC. Additionally, antagonism by **10** and **12** was also evaluated by administering Δ^9-THC at 24 and 48 h after nitrogen mustard treatment.

Statistical analysis was performed by using ANOVA (with Dunnett's *t* test for comparisons to control, and Scheffe's *F* test for multiple comparisons), and differences were considered significant at the $p < 0.05$ level (two-tailed).

Acknowledgment. This work was supported by NIDA Grants DA 03672 and DA 03959 and NIDA training Grants DA 07027 and DA 05330 and the Commonwealth of Virginia Center on Drug Abuse.

Registry No. **2**, 22972-55-0; **3**, 92974-69-1; **4**, 112812-70-1; **4**·HCl, 125590-92-3; **4a**, 125519-11-1; **5**, 112812-71-2; **6**, 112824-01-8; **7** (R = OH), 92464-59-0; **8**, 125519-07-5; **8** (R = N_3), 125519-14-4; **9**, 112812-74-5; **9**·HCl, 125519-12-2; **10**, 112812-75-6; **10**·HCl, 125519-13-3; **11**, 125519-08-6; **11** (R = HNAc), 125519-15-5; **12**, 112812-76-7; **13**, 51263-83-3; **14**, 53865-18-2; **15a**, 125519-09-7; **15b**, 57361-62-3; **15** (R = CH_2Br), 81780-81-6; **15** (R = CH_2N_3), 95647-69-1; **16**, 125519-10-0; $HN(C_2H_4Cl)_2$·HCl, 821-84-7; $H_2NC_2H_5$, 75-04-7; $H_2NC_3H_7$, 107-10-8; tetraacetylglycoluril, 10543-60-9.

Synthesis, Conformation, and Immunosuppressive Activity of Cyclosporines That Contain ϵ-Oxygen (4*R*)-4-[(*E*)-Butenyl]-4,*N*-dimethyl-L-threonine Analogues in the 1-Position[1]

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A series of CsA analogues that contain novel ϵ-oxygen isosteres of (4*R*)-4-[(E)-butenyl]-4,*N*-dimethyl-L-threonine (MeBmt) in the 1-position were synthesized. The key steps for the syntheses of enantiomerically pure ϵ-oxygen MeBmt analogues **4**–**7** were based on the stereoselective epoxidation of *cis*-allylic alcohol derivative **12** with a peracid, followed by the application of a base-catalyzed intramolecular rearrangement of epoxyurethane **15**, which was derived from the reaction of epoxy alcohol **14** and methyl isocyanate. All ϵ-oxygen MeBmt analogues have the same stereochemistry and the same functional groups as those on the α,β,γ-carbons of MeBmt except for the double bond of MeBmt, which is replaced by the $-OCH_2-$ group. The syntheses of the peptide portion of CsA analogues followed the strategy we reported previously.[10b,16] The immunosuppressive activities of CsA analogues **28a**–**e**, determined by inhibition of concanavalin A stimulated thymocytes, showed that **28b**, which has the closest structural resemblance to MeBmt, retains about 7–10% of activity of CsA, whereas the analogues **28a**, **28c**, and **28e** retain about 2–5% activity. It is interesting to note that **28d**, which has the larger benzyl group on the end of the side chain, is about 20–25% as active as CsA. Extensive conformational analyses by 1D and 2D NMR indicated that the conformation of the 33-membered peptide ring system for all CsA analogues was very similar to that of CsA. However, the NMR analyses revealed that the 1-position side chain of all these CsA analogues adopted a novel conformation in chloroform by forming a different intramolecular hydrogen bond between the β-OH and the ϵ-oxygen of the same residue. The NMR data also suggest that the chloroform conformation of these CsA analogues is similar to the conformation observed in the crystal structure of CsA in that the 1-position side chain is folded across the cyclic peptide ring system.

Since its introduction on the market only a few years ago, cyclosporine A (1; Sandimmune)[2] has become an important drug for preventing rejection of transplanted human organs.[3] In addition to its well-established immu-

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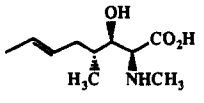
nosuppressive properties, CsA also exhibits potent antiparasitic, fungicidal, and chronic antiinflammatory activities that might be useful for treatment of other human diseases,[3] but more extensive clinical evaluation and use have been restricted by CsA's nephrotoxicity.[4] Therefore, the search for new cyclosporine analogues with improved biological specificities and reduced toxicities would greatly expand the clinical utility of this type of immunosuppressive agent.

The structure of CsA (Chart I) was established first by chemical degradation[5] of the natural peptide isolated from *Tolypocladium inflatum* Grams[6] and later by X-ray crystal-structure determination of both an iodo cyclosporine derivative[7] and CsA itself.[8] CsA is a neutral, homodetic, hydrophobic, cyclic undecapeptide that contains the novel amino acid (4R)-4-[(*E*)-butenyl]-4,*N*-dimethyl-L-threonine (MeBmt, **2**) along with several other



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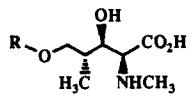
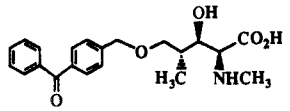


R=H **3**
=Me **4**
=Et **5**
=Bn **6**



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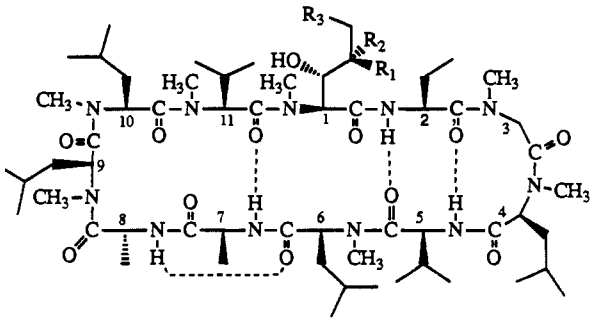
N-methylated amino acids. This new amino acid appears to be critically involved in the biological activities of CsA. Limited structure–activity studies have demonstrated that modification of almost any portion of MeBmt dramatically effects the immunosuppressive activity of the resultant CsA analogues.[9,10]

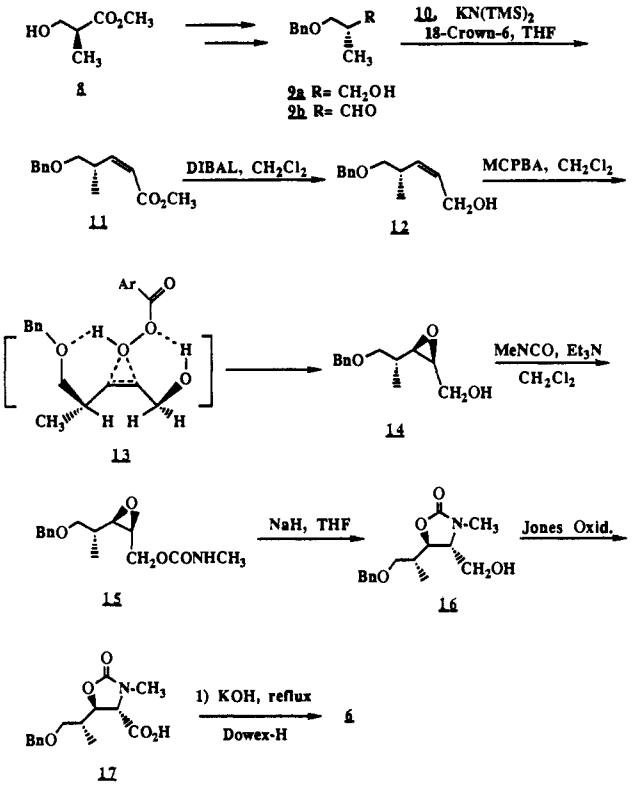
In order to further evaluate the structural requirements of MeBmt needed for full biological activity, we have synthesized a series of ϵ-oxygen isosteres of MeBmt. These new amino acids are alkylated derivatives of *N*-methyl

Chart I



	R_1	R_2	R_3
1	CH_3	H	$CH{=}CHCH_3$
28a	CH_3	H	OCH_3
28b	CH_3	H	OCH_2CH_3
28c	CH_3	H	O-*p*-benzoyl benzyl
28d	CH_3	H	OCH_2Ph
28e	CH_3	H	OH
30	H	H	$CH{=}CHCH_3$
31	H	CH_3	$CH{=}CHCH_3$
32	CH_3	CH_3	$CH{=}CHCH_3$
33a	CH_3	H	CH_2OH
33b	CH_3	H	CH_2OCH_3
33c	CH_3	H	$CH_2OCH_2CH_3$
34	CH_3	H	$CH_2CH_2CH_3$

Scheme I



(2*S*,3*R*,4*R*)-4-(hydroxymethyl)-4-methylthreonine, MeHOmt (**3**). The *O*-methyl, *O*-ethyl, *O*-benzyl, and *O*-*p*-benzoylbenzyl derivatives are designated as MeMOmt (**4**), MeEOmt (**5**), MeBOmt (**6**), and MeBBOmt (**7**), respectively. These ϵ-oxygen analogues of MeBmt have been utilized to prepare the corresponding CsA analogues. The synthesis, conformational analysis and immunosuppressive activity of these analogues are presented in this paper.

Results

Synthesis of ϵ-Oxygen MeBmt Analogues. The synthesis of enantiomerically pure ϵ-oxygen MeBmt ana-

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Table I. Physical Properties of CsA Analogues and Their Intermediates

compd	TLC R_f	$[\alpha]_D$[d] (c)	% yield	formula	analysis (FAB mass[c])
24a	0.20[a]	−134.5 (1.4)	46	$C_{47}H_{80}N_7O_{10}$	exact mass
24b	0.24[a]	−126.5 (1.9)	70	$C_{48}H_{82}N_7O_{10}$	exact mass
24c	0.19[a]	−102 (1.8)	80	$C_{60}H_{88}N_7O_{11}$	exact mass
24d	0.25[a]	−108 (1.3)	78	$C_{53}H_{84}N_7O_{10}$	exact mass
25a	0.18[b]	−130 (1.7)	94	$C_{44}H_{76}N_7O_{10}$	exact mass
25b	0.20[b]	−132 (1.8)	75	$C_{45}H_{78}N_7O_{10}$	exact mass
25c	0.34[b]	−120 (1.7)	64	$C_{57}H_{84}N_7O_{11}$	exact mass
25d	0.25[b]	−125 (0.9)	66	$C_{50}H_{80}N_7O_{10}$	exact mass
27a	0.21[a]	−131 (1.0)	40	$C_{82}H_{128}N_{11}O_{16}$	exact mass
27b	0.24[a]	−139 (1.1)	44	$C_{83}H_{130}N_{11}O_{16}$	exact mass
27c	0.15[a]	−113 (1.8)	55	$C_{95}H_{136}N_{11}O_{17}$	exact mass
27d	0.24[a]	−130 (1.8)	44	$C_{88}H_{131}N_{11}O_{16}Li$	exact mass (M^+Li)
28a	0.20[a]	−240 (0.8)	42	$C_{60}H_{109}N_{11}O_{13}$	exact mass
28b	0.21[a]	−239 (0.8)	49	$C_{61}H_{112}N_{11}O_{13}$	exact mass
28c	0.18[a]	−176 (0.8)	32	$C_{73}H_{118}N_{11}O_{14}$	exact mass
28d	0.21[a]	−205 (1.3)	33	$C_{66}H_{114}N_{11}O_{13}$	exact mass
28e	0.19[a]	−249 (0.5)	94	$C_{59}H_{108}N_{11}O_{13}$	exact mass

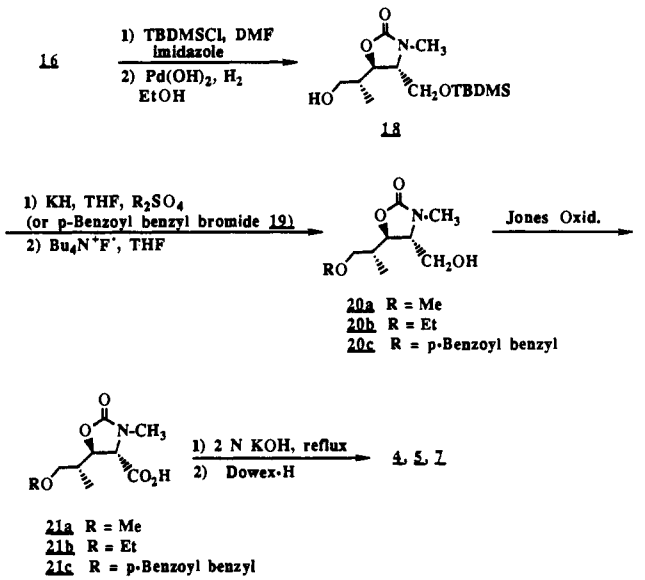
[a] 40% acetone/hexane. [b] 8% CH_3OH/CH_2Cl_2. [c] Formula (M^+H) except where noted. [d] Optical rotations were measured in chloroform.

logues **4–7** was based on the observation by Kishi[11] that epoxidation of the *cis*-allylic alcohol derivative **12** (Scheme I) with a peracid is highly stereoselective due to transition state **13**. The alcohol **9a**,[32] prepared in 84% yield over four steps from a commercial precursor **8**, was subject to Swern oxidation,[12] followed by the Still[13]-modified Hornor–Emmons reaction of **9b** with trimethyl phosphonoacetate (**10**) in the presence of potassium bis(trimethylsilyl)amide [$KN(TMS)_2$] and 18-crown-6 to give ester **11** (*cis*-ester > 10:1, *cis*-ester could be easily separated by chromatography). Reduction of *cis*-ester **11** with DIBAL, followed by epoxidation of **12** with 3-chloroperoxybenzoic acid (MCPBA), yielded almost exclusively the *anti*-epoxide **14** (*anti*-epoxide > 20:1). The reaction of **14** with methyl isocyanate under conditions devised by Roush[14] gave an epoxyurethane (**15**) which when treated with NaH rearranged to the expected *threo*-2-oxazolidinone **16**. Jones oxidation of **16** gave the corresponding acid **17**, which underwent basic hydrolysis to yield the desired amino acid **6**.

Methyl and ethyl ether analogues **4** and **5**, which can be considered isosteric analogues of MeBmt, were synthesized from 2-oxazolidinone derivative **16** (Scheme II). The hydroxy group of **16** was protected as the silyl ether and the benzyl group was removed by catalytic hydrogenation to give **18**. Alkylation of **18** with dimethyl sulfate, diethyl sulfate, or *p*-benzoylbenzyl bromide **19** and removal of the silyl protecting group with tetrabutylammonium fluoride gave **20a–c**. Oxidation to acids **21a–c** followed by saponification (vide supra) gave the final amino acids **4**, **5**, and **7** under the same conditions described for the preparation of **6**.

Synthesis of the Corresponding CsA Analogues. The synthesis of the peptide portion of CsA analogues **28a–d** closely followed the strategy successfully employed by Wenger[15] for the synthesis of CsA and modified here for the synthesis of CsA analogues[10b,16] (Scheme III). MeBmt analogues **4–7** were incorporated into hexapeptide **23**[10] as the N,O-protected dimethyloxazolidine derivative **22a–d**. These were readily formed by refluxing the amino

Scheme II



acids in acetone for 24 h. Removal of the isopropylidene protecting group by acidic hydrolysis of **24a–d** (4 equiv of 1 N aqueous HCl solution/MeOH) yielded **25a–d**, which were coupled with tetrapeptide **26**[17] by using Castro's reagent[18] to give **27a–d**. After double deprotection of **27a–d** with 0.2 N aqueous NaOH solution (3 equiv) in EtOH to remove the Fmoc and benzyl ester groups,[19] the fully deprotected undecapeptides were cyclized in the presence of propylphosphonic anhydride[20] to give the final CsA analogues **28a–d**. Removal of the benzyl group in **28d** by catalytic hydrogenation afforded **28e**, which has a free hydroxyl group at the terminus of the 1-position side chain. Table I lists physical properties for all fully characterized CsA analogues and their intermediates. The synthesis of [MeBOmt[1]]CsA (**28d**) is described in full detail in the Experimental Section. The remaining analogues **28a–c** were prepared by related procedures.

NMR Conformational Analysis of the CsA Analogues Modified at the 1-Position with Oxygen Isosteres of MeBmt. Solution NMR studies of the conformations of the CsA analogues reported here were carried

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Table II. Chemical Shifts of the Protons in CsA and CsA Analogues

compds		NH				NCH_3						
		2	5	7	8	1	3	4	6	9	10	11
CsA	1	7.96	7.48	7.68	7.17	3.51	3.40	3.11	3.25	3.12	2.70	2.71
[MeMOmt^1]CsA	**28a**	8.32	7.56	7.95	7.40	3.49	3.38	3.07	3.27	3.13	2.67	2.67
[MeEOmt^1]CsA	**28b**	8.39	7.55	8.02	7.43	3.50	3.39	3.11	3.28	3.17	2.69	2.68
[MeBBOmt^1]CsA	**28c**	8.40	*a*	8.00	*a*	3.53	3.41	3.11	3.35	3.18	2.71	2.72
[MeBOmt^1]CsA	**28d**	8.39	7.54	8.02	7.42	3.50	3.37	3.08	3.30	3.15	2.67	2.67
[MeHOmt^1]CsA	**28e**	8.25	7.46	7.85	7.41	3.48	3.41	3.10	3.28	3.16	2.67	2.67

compds		C_α-H											C_β-H:	C_δ-H:	
		1	2	3 *re*-H	3 *si*-H	4	5	6	7	8	9	10	11	1	1	
CsA	1	5.47	5.03	3.23	4.76	5.34	4.66	5.02	4.52	4.83	5.70	5.10	5.14	3.82		
[MeMOmt^1]CsA	**28a**	5.11	4.95	3.13	4.64	5.28	4.68	5.15	4.42	4.81	5.68	5.05	5.21	3.97	3.25	3.52
[MeEOmt^1]CsA	**28b**	5.12	4.96	3.15	4.67	5.29	4.72	5.17	4.45	4.85	5.71	5.07	5.25	4.05	3.37	3.52
[MeBBOmt^1]CsA	**28c**	5.20	4.97	3.18	4.68	5.20	4.76	5.20	4.47	4.84	5.68	5.06	5.20	4.05	3.55	3.76
[MeBOmt^1]CsA	**28d**	5.16	4.94	3.13	4.64	5.16	4.76	5.16	4.43	4.82	5.67	5.04	5.25	4.03	3.47	3.62
[MeHOmt^1]CsA	**28e**	5.35	4.97	3.19	4.67	5.17	4.67	5.17	4.45	4.83	5.67	5.08	5.03	4.08	3.49	3.57

[a] It is difficult to assign the chemical shifts for these two amide protons because of overlap with the aromatic protons.

Table III. Chemical Shifts of the Carbons in CsA and CsA Analogues

compds		C_α											C_β:	NCH_3						
		1	2	3	4	5	6	7	8	9	10	11	1	1	3	4	6	9	10	11
CsA	1	58.8	48.8	50.4	55.5	55.4	55.3	48.7	45.2	48.3	57.5	57.9	74.7	34.0	39.4	31.3	31.5	29.7	29.8	29.8
[MeMOmt^1]CsA	**28a**	60.6	48.5	49.9	55.2	55.0	54.4	48.4	44.7	47.8	57.1	57.7	73.3	32.8	39.2	31.4	31.2	29.6	29.7	29.6
[MeEOmt^1]CsA	**28b**	60.9	48.6	49.9	55.3	54.9	54.4	48.4	44.7	47.9	57.2	57.7	73.7	32.8	39.2	31.9	31.3	29.7	29.6	29.8
[MeBBOmt^1]CsA	**28c**	60.5	48.6	50.0	55.3	55.2	54.5	48.3	44.7	47.8	57.2	57.8	73.5	32.9	39.2	31.3	31.4	29.6	29.7	29.8
[MeBOmt^1]CsA	**28d**	60.8	48.6	49.9	55.2	54.9	54.4	48.3	44.7	47.8	57.1	57.7	73.5	32.8	39.2	31.3	31.3	29.6	29.8	29.8
[MeHOmt^1]CsA	**28e**	59.1	48.6	50.1	55.5	55.1	54.5	48.4	44.7	47.9	57.3	58.5	73.1	32.2	39.2	31.4	31.5	29.8	30.1	30.3

Table IV. Chemical Shifts of the Carbonyls in CsA and CsA Analogues

compds		carbonyl of amino acid residue										
		1	2	3	4	5	6	7	8	9	10	11
CsA	1	169.65	173.0	170.5	169.35	173.1	170.9	170.4	172.9	169.75	169.45	172.85
[MeMOmt^1]CsA	**28a**	169.7	173.4	170.9	170.28	173.2	171.47	171.0	173.4	170.6	169.5	173.2
[MeEOmt^1]CsA	**28b**	170.2	173.3	170.92	169.7	173.4	171.5	171.06	173.5	170.7	169.7	173.3
[MeBOmt^1]CsA	**28d**	170.2	173.21	170.75	169.8	173.48	171.44	171.04	173.48	170.69	169.75	173.35

out by using the 1D and 2D NMR methods developed by Kessler and colleagues in his definitive study of the conformation of CsA,[8,21] and used by us previously.[10b,16] The NMR analyses indicate that the conformation of the 33-membered peptide ring system for all CsA analogues is very similar to the ring system conformation reported for CsA in chloroform for the following reasons: (1) The small temperature coefficient for the chemical shift of NH(Val-5) ($\Delta\delta/\Delta T = 0.1 \times 10^{-3}$ ppm/°C) and the 2D rotating-frame nuclear Overhauser effect (ROE) observed between the *N*-methyl protons of the MeLeu-4 and the *si*-proton of Sar-3 indicate that the type II′ β-turn found in CsA is retained in all the CsA analogues. (2) The *cis*-amide bond between residues MeLeu-9 and MeLeu-10 is retained in all CsA analogues, which was established by the strong ROE between the two α-protons at these two residues. (3) All the other ROEs observed for the intracyclic protons of all CsA analogues are very similar to those reported for CsA. (4) The slow exchange of the amide protons in D_2O and the similar temperature coefficients of NH protons at low and high temperature with respect to CsA indicate that all the amide protons are involved in hydrogen bonds.

The chemical shifts of the amide protons, the *N*-methyl protons, the α-protons, the carbonyl carbons, the *N*-methyl carbons, and the α-carbons are very similar for all five CsA ether analogues (Table II–V), indicating that they adopt a similar conformation in chloroform solution. In comparison with the 1D ^1H NMR spectrum of CsA in chloroform, the ether analogues do show some characteristic differences: (1) the amide protons of amino acid residues 2, 7, 8, and (less markedly) 5 are shifted downfield; (2) the α-protons in residue 1 are shifted upfield (5.10–5.20 ppm vs 5.47 ppm) [A less affected shift (5.35 ppm) is observed for analogue **28e**, which has a terminal hydroxyl group in residue 1]; and (3) although an upfield doublet resonates near 0.70 ppm, as is found in the case of CsA, a total correlation spectroscopy experiment[22] established that this signal does not arise from the C-4 methyl of residue 1 but corresponds to one of the two methyl groups in the Me-

Table V. Coupling Constants of α,β-Protons in the Residue 1 of CsA and CsA Analogues (in Apolar Solvents)

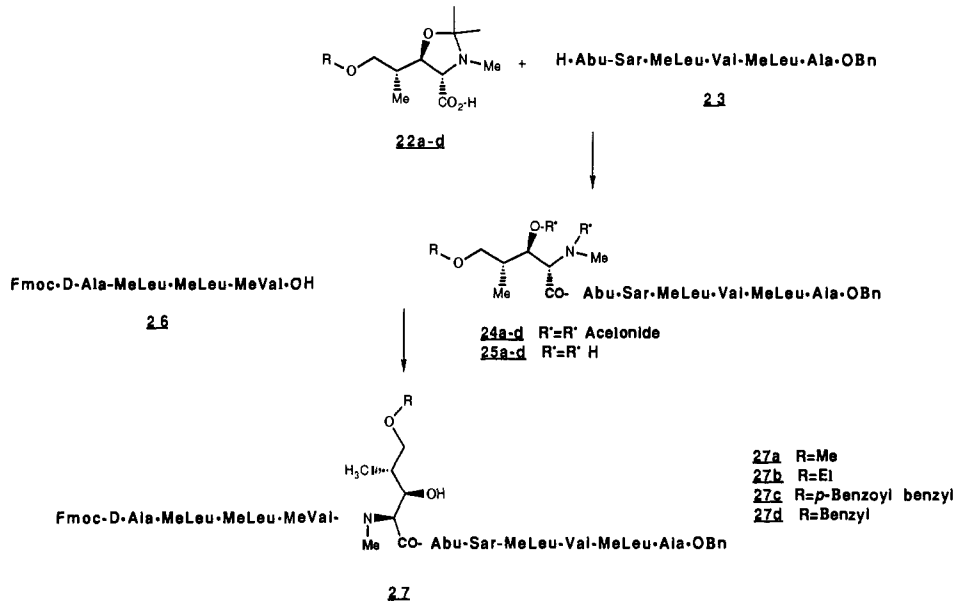
compd		*J*, Hz	
		$CDCl_3$	C_6D_6
CsA	1	5.7	6.8
OAc-CsA	**29**	10.0[a]	11.6
[(4*S*)-MeBmt^1]CsA	**31**	9.4	9.4
[MeBOmt^1]CsA	**28d**	9.0	10.0
[MeEOmt^1]CsA	**28b**	9.0	9.4
[MeMOmt^1]CsA	**28a**	9.3	11.0
[MeHOmt^1]CsA	**28e**	9.0	
[MeBBOmt^1]CsA	**28c**	9.0	

[a] The *J* value was measured with **29** dissolved in a mixed solvent of $CDCl_3$ (0.50 mL) and C_6D_6 (0.15 mL). In pure $CDCl_3$, the α,β-protons appear as a strongly coupled AB system from which no coupling constant could be deduced.

(21) Kessler, H.; Loosli, H. R.; Oschkinat, H. *Helv. Chim. Acta* **1985**, *68*, 661.

(22) Bax, A.; Davis, D. G. *J. Magn. Res.* **1985**, *65*, 355.

Scheme III. Strategy Used To Synthesize Linear Undecapeptides



Leu-6 isobutyl side chain. These minor spectral differences seemed to be the result of the structural modifications in residue 1, since the peptide rings retain ring-system conformations similar to that of CsA in chloroform (vide supra).

Further analyses suggested that the side chain of residue 1 in the ether analogues adopts a novel conformation in apolar solvents. The first indication of this came from the coupling constants of the α,β-protons in residue 1, which range between 9.0 and 9.3 Hz in $CDCl_3$ solution and 9.4 and 11.0 Hz in C_6D_6 (see Table V). These large coupling constants restrict the torsion angle χ_1 for these CsA ether analogues to either 0°–10° or 160°–180° in apolar solvents. The ROE data suggest the latter orientation for the 1-position side chain. Thus, the strong ROE between Abu-2-NH and the β-CH of residue 1 in CsA is not detected in the CsA ether analogues. Instead, a new ROE between the N-CH_3 and the β-CH in residue 1 is detected in all of the ether analogues. This latter ROE is possible only when χ_1 is equal to about 180° (compared with a $\chi_1 = -60°$ for CsA in apolar solvents).

The ether analogues in the 1-position of cyclosporin also permit the δ-protons in the side chain to be assigned unambiguously (in the range of 3.25–3.76 ppm, see Table II) due to their attachment to the carbon adjacent to the ether oxygen. An ROE between one of the δ-protons of residue 1 and the α-CH of MeLeu-4 indicated that these protons must be close to each other and thus that the side chain of residue 1 is folded back into the ply of the cyclic peptide ring in a manner similar to the conformation of MeBmt in the crystal structure of CsA. This arrangement prevents the formation of an intramolecular hydrogen bond between the C-3 hydroxyl group and the carbonyl oxygen in residue 1.

Finally, the C-3 hydroxyl proton in residue 1 resonates as a nicely resolved doublet (J = 9.0 Hz). This hydroxyl proton exchanges very slowly with D_2O and the chemical shift is insensitive to concentration, indicating that this hydroxyl proton is hydrogen bonded. These data are consistent with the formation of a six-membered ring in which the β-hydroxyl is hydrogen-bonded to the oxygen atom at ϵ-position of the side chain in residue 1. The observed coupling constants (J = 9.0 Hz) are consistent with the formation of the cyclic hydrogen bond system as depicted in Figure 1.

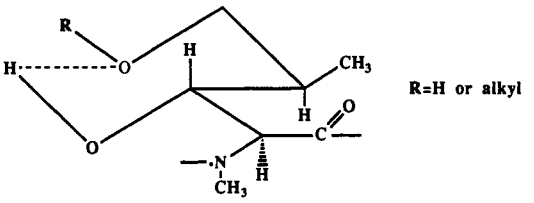


Figure 1. The postulated conformation of the side chain of residue 1 in CsA ether analogues (in apolar solvents) (partial structure).

The altered orientation of the 1-position ether analogues appears to result from the ready formation of the new hydrogen-bonding system between the β-hydroxyl and the ϵ-oxygen atom. If so, then other processes that destabilize the hydrogen bond between the β-hydroxyl and the carbonyl oxygen in MeBmt should cause the side chain to fold under the peptide ring system. To test this hypothesis, we synthesized the [*O*-acetyl-MeBmt]CsA (**29**) derivative. As shown in Table V, the α,β-coupling constant is consistent with χ_1 = 180° and with a folded conformation similar to that of the ether analogues.

The altered 1-position side chain orientation occurs in conjunction with subtle conformational changes in the side chain of MeLeu-6. This is apparent from the altered coupling constants between the α,β-protons of MeLeu-6 in the CsA ether analogues which are 4.2 and 10.0 Hz rather than 6.0 and 9.3 Hz in CsA in chloroform solution. Furthermore, one of the two terminal methyl groups of the MeLeu-6 side chain has been shifted upfield to 0.70 ppm. This shift seems to be induced by the carbonyl group in MeLeu-6 as a consequence of the conformational change in the side chain.

Biological Assay. The biological activities of CsA analogues **28a–e** vs CsA 1 were determined by using inhibition of concanavalin A stimulated thymocytes as previously described.[23] The bioassay data show that CsA analogue **28b**, bearing the closest structural analogy to MeBmt, gives only about 7–10% immunosuppressive activity relative to CsA. With smaller substituents on oxygen (**28e** and **28a**), the biological activities diminished to about

(23) Dunlap, B. E.; Dunlap, S. A.; Rich, D. H. *Scand. J. Immunol.* 1984, *20*, 237.

2–5%, whereas the larger substituent, e.g., the benzyl ether group, in **28d** gives surprisingly moderate (20–25%) activity. However, the significantly larger *p*-benzoylbenzyl ether[24] analogue **28c** is less active (5%).

Discussion

The structure of the immunosuppressive agent CsA is characterized by two novel features, the highly N-methylated cyclic undecapeptide ring system and the presence of the unique amino acid (4*R*)-4-[(*E*)-2-butenyl]-4,*N*-dimethyl-L-threonine (MeBmt) in the 1-position. Previous studies[9,10] of natural, semisynthetic, and synthetic CsA derivatives modified in the 1-position have demonstrated that MeBmt is important for biological activity. Removal of the nonpolar side chain of MeBmt as in [MeThr^1]CsA and [MeLeu(3-OH)^1]CsA reduces the immunosuppressive activity dramatically,[10a] as does modification of the hydroxy group to form *O*-acetyl- and deoxycyclosporines.[9] The geometry of the double bond also makes a contribution to the biological activity since dihydro[9] and acetylenic[25] CsA derivatives are generally less potent than CsA. Removal of the *N*-methyl group on the nitrogen of MeBmt to form [Bmt^1]cyclosporine[26] also reduces the immunosuppressive activity. Furthermore, we found recently that modifying the substituents on C-4 of MeBmt in CsA altered the immunosuppressive activity. For example, removal of the 4-methyl group to form [MeBth^1]CsA (**30**)[25] or epimerizing the stereochemistry at C-4 from *R* to *S* to form [(4*S*)-MeBmt^1]cyclosporine (**31**)[10b] significantly reduces the activity whereas an analogue with an extra methyl group added to C-4, [MeBm$_2$t]CsA (**32**), retains moderate activity.[25]

Conformational searches and energy minimization studies carried out on these analogues have suggested a possible bioactive conformation for cyclosporine when it is bound to its immunosuppressive receptor.[27] This conformation is closely related to the X-ray conformation of cyclosporine and differs from the chloroform conformation[8] primarily in the orientation of the MeBmt side chain with respect to the 33-membered peptide ring system. Quesniaux et al. have proposed a closely related structure for the bioactive conformation, based upon their studies of the binding of antibiotics to CsA in aqueous media.[28]

The importance of the structure in the 1-position side chain and its orientation vis a vis the ring system to immunosuppressive activity led us to explore other structural modifications of the MeBmt side chain. During our studies of the asymmetric synthesis of MeBmt analogues via epoxides,[29,30] we applied Kishi's chiral epoxidation reaction[11] to prepare chiral epoxide **14** by peracid epoxidation of *cis*-allylic alcohol derivative **12**, which can be synthesized conveniently from the commercial precursor **8**. Epoxide **14** was converted into an ϵ-oxygen-containing MeBmt analogue **6** over four steps in high yield by modifying the method we reported recently.[30] Amino acids **4** and **5**, which have a close structural resemblance to MeBmt, and amino acid **7** were synthesized by chemical modifications of intermediate **16**. All ϵ-oxygen MeBmt analogues **4–7** have the same stereochemistry and the same functional groups as those on the α,β,γ-carbons of MeBmt except for the double bond of MeBmt, which is replaced here by the $-OCH_2-$ group. The benzyl and *p*-benzoylbenzyl substituents on the oxygen atom in analogues **6** and **7** also markedly increase the steric bulk of this side chain in comparison with that of MeBmt or compounds **3–5**. The corresponding cyclosporine analogues **28a–d** were synthesized by following essentially the strategy developed by Wenger.[15]

The biological activity of CsA analogues **28a–e**, determined in an assay which measures inhibition of concanavalin A stimulated thymocytes,[23] showed that **28b**, which has the closest structural resemblance to MeBmt, retains about 7–10% of the activity of CsA, whereas the shorter analogues **28a** and **28e** retain only about 5% and 2% activity, respectively. It is interesting to note that **28d**, which has the larger benzyl group on the end of the side chain, is about 20–25% as active as CsA. Durette et al.[31] synthesized a related series of 1-position alcohol derivatives **33a–c** from natural CsA by a semisynthetic approach. These compounds differ in that the ether oxygen replaces C-7 in MeBmt, rather than C-6. Methyl ether **33b** retains about 8–12% immunosuppressive activity whereas ethyl ether derivative **33c** shows about 5–11% immunosuppressive activity. The activities are comparable to those obtained with analogues **28a,b**.

The activities of ether analogues **28a–e** should be compared with the fully saturated CsA derivative dihydro-CsA (**34**). Reduction of the double bond in CsA diminishes activity to about 25–50% of that of CsA.[10a,31] Replacement of one methylene group by an oxygen, either at C-6 or C-7 reduces activity further to about 5–12%. This additional loss in activity probably is a result of a reduced hydrophobic character in the 1-position of analogues **28a–e**, although an electrostatic or dipolar effect cannot be ruled out on the basis of the present data. However, benzyl analogue **28d**, which has a hydrophobic phenyl group added to what corresponds to the C-7 carbon in **4**, retains 20–25% immunosuppressive activity, an activity that is comparable to the activity of DH-CsA in our assay. The synthesis of additional related aromatic analogues is in progress to see if other hydrophobic groups will further restore immunosuppressive activity.

During the course of our NMR studies, we noticed that the CsA analogues that contained the oxygen-substituted MeBmt derivatives in the 1-position (CsA ether analogues) adopted a novel conformation for the 1-position side chain in chloroform. Instead of forming an intramolecular hydrogen bond between the β-OH and the carbonyl oxygen in the 1-position, as is found in the $CDCl_3$ conformation of CsA,[8] the ether analogues form a different intramolecular hydrogen bond between the β-OH and the ϵ-oxygen of the same residue (see Figure 1). A 9.0 Hz coupling constant for α,β-protons of residue-1, together with an ROE between the *N*-methyl group and the β-CH of the same residue restrict the torsion angle χ_1 to an angle near 180°. Furthermore, the ROE between one of the δ-protons at residue-1 and the α-CH of MeLeu-4 indicates that these two groups must be close to each other. These NMR data suggest that the $CDCl_3$ conformation of the CsA ether analogues is similar to the conformation observed in the crystal structure of CsA in that the 1-position side chain

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(30) Sun, C.-Q.; Rich, D. H. *Tetrahedron Lett.* **1988**, *29*, 5205.
(31) Durette, P. L.; Boger, J.; Dumont, F.; Firestone, R.; Frankshun, R. A.; Koprak, S. L.; Lin, C. S.; Melino, M. R.; Pessolano, A. A.; Pisano, J.; Schmidt, J. A.; Sigal, N. H.; Staruch, M. J., Witzel, B. E. *Transplant. Proc.* **1988**, *20* (Suppl. 2), 51.

is folded across the cyclic peptide ring system. This folded conformation probably is stabilized by the enhanced intramolecular van der Waals interactions made possible in the folded conformation at the same time that the hydrogen bond to the carbonyl oxygen has been exchanged for the hydrogen bond to the ε-oxygen of the MeBmt analogue.

Experimental Section

Melting points were determined on a Fisher-Johns melting point apparatus and are uncorrected. Optical rotations were measured on a Perkin-Elmer Model 241 polarimeter at room temperature. Infrared (IR) spectra were recorded on a Perkin-Elmer 599B spectrophotometer (data in cm^{-1}; br = broad, sh = shoulder). ^1H NMR spectra were recorded on a Bruker WP-200 or WP-270 spectrometer except for the full characterizations of CsA analogues, which were carried out on a Bruker AM 500 or AC 300 spectrometer equipped with an Aspect 3000 computer. Chemical shifts were reported as δ units (ppm) relative to tetramethylsilane as internal standard except for spectra in D_2O which were reported relative to DHO (4.63 ppm). Microanalyses were performed by Galbraith Laboratories, Inc., Knoxville, TN. Mass spectra (EI, at 70 eV) were recorded on a Finnegan 4000 quadrupole GC/MS spectrometer interfaced to a Finnegan M6000 data system. High resolution (HR) fast atom bombardment (FAB) mass spectra for CsA analogues and peptide intermediates were determined by the Midwest Center for Mass Spectrometry (University of Nebraska—Lincoln, Lincoln, NE.) Flash chromatography 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 UV and 7% phosphomolybdic acid (PMA) in ethanol.

Tetrahydrofuran (THF) was distilled from sodium benzophenone and hexane was distilled prior to use. Acetone (for making acetonides) was dried with activated 4-Å molecular sieves and distilled prior to use. All other solvents and reagents were either ASC 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*,2*Z*)-Methyl 5-(Benzyloxy)-4-methyl-2-pentenoate (11). A solution of trimethyl phosphonoacetate (**10**, 4.19 g, 23 mmol, 1.0 equiv) and 18-crown-6 (25 g, 94.5 mmol, 4.1 equiv) (used as received from Aldrich) in 350 mL of anhydrous THF was cooled to −78 °C under nitrogen and treated with $KN(TMS)_2$ (0.5 M solution in toluene, 23 mmol, 1.0 equiv). The resultant white suspension was stirred at this temperature for 30 min. Then, the freshly prepared aldehyde **9b** (made by Swern oxidation from **9a**;[32] 4.12 g, 23 mmol, 1.0 equiv) in THF was added dropwise. After addition, the mixture was stirred at −78 °C for another 1 h and then quenched with saturated aqueous NH_4Cl solution (130 mL). Most of THF was removed by a rotavapor and the residue was extracted with CH_2Cl_2 (4 × 100 mL). The combined organics were washed with brine, dried over Na_2SO_4, filtered, and concentrated. The residue was purified by flash chromatography (2% ethyl acetate/hexane) to give *cis*-ester **11** (R_f 0.41, 10% ethyl acetate/hexane; 4.80 g, 89%) and trans isomer (R_f 0.31, 10% ethyl acetate/hexane; 0.44 g, 8.2%). 2*Z* isomer **11**: $[\alpha]_D$ +61.7° (*c* 1.3, $CHCl_3$); IR ($CHCl_3$) 3035, 2995, 2950, 2860, 1715, 1635, 1440, 1215, 1190, 1180, 1090 cm^{-1}; ^1H NMR (270 MHz, $CDCl_3$) δ 1.07 (d, *J* = 6.8 Hz, 3 H, CH_3-C_4), 3.40 (d d, *J* = 1.4, 7.0 Hz, 2 H, 2 H-C_5), 3.72 (s, 3 H, CO_2CH_3), 3.86 (m, 1 H, H-C_4), 4.53 (s, 2 H, CH_2Ph), 5.80 (dd, *J* = 1.0, 11.6 Hz, 1 H, H-C_2), 6.13 (dd, *J* = 9.8, 11.6 Hz, 1 H, H-C_3), 7.34 (m, 5 H, aromatic H); MS (EI), 234 (M^+). Anal. ($C_{14}H_{18}O_3$) C, 70.77; H.

(4*S*,2*Z*)-5-(Benzyloxy)-4-methyl-2-penten-1-ol (12). To a solution of ester **11** (3.51 g, 15 mmol, 1.0 equiv) in CH_2Cl_2 (50 mL) cooled at −78 °C was added dropwise a solution of 1 M of DIBAL in CH_2Cl_2 (45 mL, 45 mmol, 3.0 equiv). After addition, the mixture was stirred at −78 °C for 2 h and then quenched carefully with CH_3OH (5 mL). The mixture was diluted with CH_2Cl_2 (400 mL) and shaken with 50% saturated Rochelle salt solution (400 mL). The CH_2Cl_2 phase was separated and aqueous phase was extracted with CH_2Cl_2 (4 × 50 mL). The combined CH_2Cl_2 solution was washed with 50% saturated Rochelle salt solution (100 mL) and brine (100 mL) and dried ($MgSO_4$). The solvent was removed, and the residue was purified by flash chromatography (25% ethyl acetate/hexane) to afford 2.99 g (96%) of **12** as a colorless oil: R_f 0.22 (33% ethyl acetate/hexane); $[\alpha]_D$ −1.17° (*c* 0.7, $CHCl_3$); IR ($CHCl_3$) 3430 (br), 3000, 2960, 2870, 1450, 1220, 1175, 1000 cm^{-1}; ^1H NMR (270 MHz, $CDCl_3$) δ 0.95 (d, *J* = 6.7 Hz, 3 H, CH_3-C_4), 2.87 (m, 1 H, H-C_4), 3.16 (t, *J* = 8.8 Hz, 1 H, H-C_5), 3.37 (dd, *J* = 5.1, 8.8 Hz, 1 H, H-C_5), 3.95 (ddd, *J* = 0.5, 6.5, 12.0 Hz, 1 H, H-C_1), 4.18 (ddd, *J* = 1.4, 7.7, 12.0 Hz, 1 H, H-C_1), 4.50 (s, 2 H, CH_2Ph); 5.33 (t, *J* = 10.8 Hz, 1 H, H-C_3); 5.77 (m, 1 H, H-C_2), 7.30 (m, 5 H, aromatic H); MS (EI), 206 (M^+). Anal. ($C_{13}H_{18}O_2$) C, H.

(2*S*,3*R*,4*R*)-5-(Benzyloxy)-2,3-epoxy-4-methylpenten-1-ol (14). A solution of **12** (0.412 g, 2 mmol) in CH_2Cl_2 (40 mL) was cooled at −10 °C and mCPBA (1.06 g, 6 mmol, 3.0 equiv) in CH_2Cl_2 (30 mL) was added via a dropping funnel. The mixture was stirred at −10 °C for 1 h and then at 0 °C for 1.5 h. The white suspension was washed with 1 N aqueous NaOH solution (20 mL) and water to pH 7. The organic solution was dried (Na_2SO_4), filtered, and concentrated. The residue was purified by flash chromatography (25% ethyl acetate/hexane) to yield 0.41 g (92.3%) of *anti*-epoxide **14** (R_f 0.35, 60% ethyl acetate/hexane) and 0.02 g (4.5%) of *syn*-epoxide (R_f 0.44, 60% ethyl acetate/hexane). *anti*-Epoxide **14**: $[\alpha]_D$ +17.6° (*c* 1.6, $CHCl_3$); IR ($CHCl_3$) 3440 (br), 3000, 2960, 2860, 1450, 1360, 1220, 1100, 1025 cm^{-1}; ^1H NMR (270 MHz, C_6D_6) δ 0.87 (d, *J* = 6.9 Hz, 3 H, CH_3-C_4), 1.55 (m, 1 H, H-C_4), 2.69 (dd, *J* = 4.3, 9.4 Hz, 1 H, H-C_3), 2.85 (m, 1 H, H-C_2), 3.40 (m, 4 H, 2 H-C_1, 2 H-C_5), 4.31 (s, 2 H, CH_2Ph), 7.05–7.30 (m, 5 H, aromatic H); MS (EI) 204 (M^+ − 18). Anal. ($C_{13}H_{18}O_3$) C, H.

(2*S*,3*R*,4*R*)-5-(Benzyloxy)-2,3-epoxy-4-methyl-1-[(*N*-methylcarbamoyl)oxy]pentane (15). To a solution of **14** (0.65 g, 2.92 mmol, 1.0 equiv) in CH_2Cl_2 (30 mL) were added sequentially Et_3N (0.62 g, 6.12 mmol, 2.1 equiv) and methyl isocyanate (0.37 mL, 6.12 mmol, 2.1 equiv). The mixture was stirred at room temperature for 18 h and then quenched with saturated aqueous NH_4Cl solution (30 mL). The organic layer was separated, dried (Na_2SO_4), and concentrated in vacuo. The residue was purified by chromatography (25% ethyl acetate/hexane) to afford 0.76 g (93%) of **15** as a colorless oil: R_f 0.38 (60% ethyl acetate/hexane); $[\alpha]_D$ +7.4° (*c* 2.0, $CHCl_3$); IR ($CHCl_3$) 3460, 3000, 2960, 2860, 1720, 1520, 1450, 1220, 1135, 1100 cm^{-1}; ^1H NMR (270 MHz, $CDCl_3$) δ 1.05 (d, *J* = 6.9 Hz, 3 H, CH_3-C_4), 1.67 (m, 1 H, H-C_4), 2.81 (d, *J* = 5.0 Hz, 3 H, NCH_3), 2.93 (dd, *J* = 4.4, 9.5 Hz, 1 H, H-C_3), 3.21 (m, 1 H, H-C_2), 3.56 (m, 2 H, 2 H-C_5), 4.02 (dd, *J* = 7.3, 12.0 Hz, 1 H, H-C_1), 4.38 (dd, *J* = 4.1, 12.0 Hz, 1 H, H-C_1), 4.56 (s, 2 H, OCH_2Ph), 4.71 (br, 1 H, NH), 7.35 (m, 5 H, aromatic H); MS (EI) 279 (M^+). Anal. ($C_{15}H_{21}NO_4$) C, H, N.

(1′*R*,4*R*,5*R*)-5-[2′-(Benzyloxy)-1′-methylethyl]-4-(hydroxymethyl)-3-methyl-2-oxazolidinone (16). A solution of **15** (0.13 g, 0.466 mmol, 1.0 equiv) in THF (5 mL) was added to a suspension of sodium hydride (0.045 g, 1.86 mmol, 4.0 equiv) in THF (15 mL). After stirring for 5.5 h, the reaction mixture was quenched by careful addition of saturated aqueous NH_4Cl solution (20 mL). The layers were separated and the aqueous phase was extracted with CH_2Cl_2 (3 × 20 mL). The combined organic solution was dried (Na_2SO_4), filtered, and concentrated. The residue was purified by chromatography (40% ethyl acetate/hexane) to give 0.11 g (84%) of **16** as a crystalline compound. An analytical sample was prepared by recrystallization from ethyl acetate/hexane: R_f 0.37 (5% methanol/methylene chloride; mp 81–82 °C; $[\alpha]_D$ +35.1° (*c* 1.0, $CHCl_3$); IR ($CHCl_3$) 3400 (br), 3000, 2960, 1740, 1450, 1435, 1405, 1240, 1080, 1040 cm^{-1}; ^1H NMR (270 MHz, $CDCl_3$) δ 0.99 (d, *J* = 7.0 Hz, 3 H, CH_3-C_1'), 2.15 (m, 1 H, H-C_1'); 2.85 (s, 3 H, NCH_3); 3.51 (d, *J* = 6.0 Hz, 2 H, 2 H-C_2'), 3.58 (m, 2 H, H-C_4, HC-C_4), 3.75 (dd, *J* = 5.0, 12.0 Hz, 1 H, HC-C_4), 4.39 (t, *J* = 5.5 Hz, 1 H, H-C_5), 4.47 (d, *J* = 2.4 Hz, 2 H, CH_2Ph), 7.33 (m, 5 H, aromatic H); MS (EI) 280 (M^+H). Anal. ($C_{15}H_{21}NO_4$) C, H, N.

(1′*R*,4*S*,5*R*)-5-[2′-(Benzyloxy)-1′-methylethyl]-3-methyl-2-oxooxazolidine-4-carboxylic Acid (17). To a vigorously

(32) Meyers, A. I.; Babiak, K. A.; Campbell, A. L.; Comins, D. L.; Fleming, M. P.; Henning, R.; Heuschmann, M.; Hudspeth, J. P.; Kane, J. M.; Reider, P. J.; Roland, D. M.; Shimizu, K.; Tomioka, K.; Walkup, R. D. *J. Am. Chem. Soc.* **1983**, *105*, 5015.

stirred solution of **16** (0.26 g, 0.93 mmol) in acetone (10 mL) was added dropwise Jones reagent to maintain the solution orange. After stirring for 1 h, the mixture was diluted with water (10 mL) and extracted with EtOAc (4 × 15 mL). The EtOAc extracts were washed with brine (2 × 30 mL), dried (Na_2SO_4), filtered, and concentrated. The crude acid was crystallized from ether/hexane to give 0.22 g (80%) of **17**: mp 82–83 °C; $[\alpha]_D$ +17.45° (*c* 1.0, $CHCl_3$); IR ($CHCl_3$) 3100–2900 (br), 2960, 2860, 1755, 1435, 1400, 1200, 1100, 1040 cm^{-1}; ^1H NMR (270 MHz, $CDCl_3$) δ 1.05 (d, *J* = 7.0 Hz, 3 H, CH_3-C_1'), 2.25 (m, 1 H, H-C_1'), 2.90 (s, 3 H, NCH_3), 3.55 (d, *J* = 5.5 Hz, 2 H, 2 H-C_2'), 4.25 (d, *J* = 5.1 Hz, 1 H, H-C_4), 4.47 (s, 2 H, CH_2Ph), 4.57 (t, *J* = 5.1 Hz, 1 H, H-C_5), 7.35 (m, 5 H, aromatic H); MS (EI) 294 (M^+H). Anal. ($C_{15}H_{19}NO_5$) C, H, N.

(2*S*,3*R*,4*R*)-5-(Benzyloxy)-3-hydroxy-4-methyl-2-(methylamino)pentanoic Acid (6). A solution of **17** (0.24 g, 0.82 mmol) in 2 N aqueous KOH solution (5 mL) was heated to reflux under N_2 for 6 h. The mixture was cooled to room temperature and treated with Dowex H^+ (50 W) to pH 4. The mixture was filtered through a Dowex H^+ column, eluted with 1.5 M aqueous NH_3 solution (375 mL). The aqueous solution was evaporated to dryness. The residue was crystallized in ethanol to give 0.18 g (81%) of crystalline **6**: mp 217–218 °C; $[\alpha]_D$ +3.9° (*c* 0.5, H_2O at pH 7 (phosphate buffer tritrisol, pH 7.00, from Merck)); IR (KBr) 3200, 3100 (br), 2980, 2920, 1635 (sh), 1400, 1380, 1330, 1135, 1080 cm^{-1}; ^1H NMR (270 MHz, D_2O) δ 0.85 (d, *J* = 7.0 Hz, 3 H, CH_3-C_4); 1.91 (m, 1 H, H-C_4); 2.42 (s, 3 H, NCH_3); 3.38 (d, *J* = 4.6 Hz, 1 H, H-C_2); 3.38, 3.55 (2 dd, *J* = 6, 10.0 Hz, 10, 2 H, 2 H-C_5); 3.77 (t, *J* = 5.1 Hz, 1 H, H-C_3); 4.40 (d, *J* = 1.2 Hz, 2 H, CH_2Ph); 7.25 (s, 5 H, aromatic H); MS (EI) 268 (M^+H). Anal. ($C_{14}H_{21}NO_4$) C, 62.11; H, N.

(1′*R*,4*R*,5*R*)-4-[[(*tert*-Butyldimethylsilyl)oxy]methyl]-5-(2′-hydroxy-1′-methylethyl)-3-methyl-2-oxazolidinone (18). To a solution of **16** (0.28 g, 1 mmol) in DMF (3.0 mL) were added *tert*-butyldimethylsilyl chloride (0.25 g, 1.5 mmol, 1.5 equiv) and imidazole (0.22 g, 3 mmol, 3.0 equiv). The mixture was stirred at room temperature for 6 h, diluted with ether (40 mL), and washed with water (3 × 50 mL). The ether phase was dried (Na_2SO_4), filtered, and concentrated in vacuo. The residue was dissolved in EtOH (10 mL) together with Pearlman's catalyst (palladium hydroxide on carbon, 100 mg) and a H_2 stream was passed through above the suspension for 1.5 h. The catalyst was removed by filtration via a pad of Celite and the filtrate was concentrated. The residue was purified by flash chromatography (2% CH_3OH/CH_2Cl_2) to give 0.27 g (89.3%) of **18**: R_f 0.22 (5% CH_3OH/CH_2Cl_2); $[\alpha]_D$ +24.8° (*c* 1.6, $CHCl_3$); IR ($CHCl_3$) 3470 (br), 3030, 2980, 2950, 2880, 1760, 1475, 1450, 1415, 1265, 1140, 1050, 850 cm^{-1}; ^1H NMR (270 MHz, $CDCl_3$) δ 0.07 (s, 6 H, $SiMe_2$), 0.88 (s, 9 H, $SiC(CH_3)_3$), 0.95 (d, *J* = 6.9 Hz, 3 H, CH_3-C_1'), 2.00 (m, 1 H, H-C_1'), 2.87 (s, 3 H, N-CH_3), 3.53 (q, *J* = 4.6 Hz, 1 H, H-C_4), 3.67 (m, 4 H, 2 H-C_2', C_4-CH_2O), 4.21 (dd, *J* = 4.6, 6.9 Hz, 1 H, H-C_5); MS (EI) 304 (M^+H). Anal. ($C_{14}H_{29}NO_4Si$) C, H, 9.09; N.

(1′*R*,4*R*,5*R*)-5-(2′-Methoxy-1′-methylethyl)-4-(hydroxymethyl)-3-methyl-2-oxazolidinone (20a). To a suspension of potassium hydride (0.13 g, 1.15 mmol, 1.5 equiv) in THF (5 mL) at 0 °C was added **18** (0.19 g, 0.73 mmol) and dimethyl sulfate (0.10 g, 1.46 mmol, 2 equiv). The mixture was stirred at 0 °C for 0.5 h then at room temperature for 1 h and quenched by careful addition of H_2O (2 mL). The mixture was diluted with ether (20 mL) and washed with H_2O (10 mL). The ether layer was dried ($MgSO_4$), filtered, and concentrated in vacuo.

The residue was dissolved in THF (5 mL) and stirred in the presence of tetrabutylammonium fluoride (1 M solution in THF, 2.2 mL, 3.0 equiv) at 0 °C for 40 min. The mixture was diluted with CH_2Cl_2 (10 mL) and washed with brine (2 × 10 mL). The organic layer was dried ($MgSO_4$), concentrated, and flash chromatographed (2% CH_3OH in CH_2Cl_2) to give 0.14 g (96%) of **20a** as a colorless oil: R_f 0.38 (5% methanol/methylene chloride); $[\alpha]_D$ +62.7° (*c* 9.4, $CHCl_3$); IR ($CHCl_3$) 3500–3300 (br), 2980, 2930, 2890, 1745, 1435, 1405, 1225, 1210, 1090, 1035 cm^{-1}; ^1H NMR (270 MHz, $CDCl_3$) δ 0.97 (d, *J* = 7.0 Hz, 3 H, CH_3-C_1'), 2.10 (m, 1 H, H-C_1'), 2.89 (s, 3 H, NCH_3), 3.32 (s, 3 H, OCH_3), 3.42 (d, *J* = 5.5 Hz, 2 H, 2 H-C_2'), 3.55–3.85 (m, 3 H, H-C_4, CH_2C_4), 4.37 (t, *J* = 5.5 Hz, 1 H, H-C_5); MS exact mass calcd for $C_9H_{17}NO_4$ 203.1158, found 203.1162 (M^+).

(1′*R*,4*R*,5*R*)-5-(2′-Ethoxy-1′-methylethyl)-4-(hydroxymethyl)-3-methyl-2-oxazolidinone (20b). Compound **20b** was prepared (85.5%) according to the procedure described for **20a** except diethyl sulfate was used as the alkylating agent. The analytical sample was obtained by crystallization in ether and pentane: mp 74–75 °C; $[\alpha]_D$ +44.6° (*c* 0.85, $CHCl_3$); IR ($CHCl_3$) 3420 (br), 2970, 2870, 1745, 1435, 1405, 1220, 1190, 1100, 1030 cm^{-1}; ^1H NMR (270 MHz, $CDCl_3$) δ 0.98 (d, *J* = 6.9 Hz, 3 H, CH_3-C_1'), 1.17 (t, *J* = 7.0 Hz, 3 H, OCH_2CH_3), 2.13 (m, 1 H, H-C_1'), 2.90 (s, 3 H, NCH_3), 3.45 (m, 4 H, $-CH_2OCH_2-$), 3.57–3.83 (m, 3 H, H-C_4, CH_2-C_4), 4.40 (t, *J* = 5.3 Hz, 1 H, H-C_5); MS (EI) 218 (M^+H). Anal. ($C_{10}H_{19}NO_4$) C, H, N.

(1′*R*,4*R*,5*R*)-5-[2′-[(4″-Benzoylbenzyl)oxy]-1′-methylethyl]-4-(hydroxymethyl)-3-methyl-2-oxazolidinone (20c). Compound **20c** was prepared (57%) according to the procedure described for **20a** except 4-benzoylbenzyl bromide (2.0 equiv) was used as the alkylating agent: R_f 0.24 (5% methanol/methylene chloride); $[\alpha]_D$ +32.3° (*c* 2.2 $CHCl_3$); IR ($CHCl_3$) 3420 (br), 3020, 2980, 2880, 1755, 1665, 1450, 1415, 1325, 1290, 1100 cm^{-1}; ^1H NMR (270 MHz, $CDCl_3$) δ 1.02 (d, *J* = 7.0 Hz, 3 H, CH_3-C_1'), 2.14 (m, 1 H, H-C_1'), 2.86 (s, 3 H, CH_3N), 3.56 (d, *J* = 5.6 Hz, 2 H, 2 H-C_2'), 3.54–3.84 (m, 3 H, H-C_4, CH_2-C_4), 4.36 (t, *J* = 5.5 Hz, 1 H, H-C_5), 4.55 (s, 2 H, OCH_2Ar), 7.36–7.83 (m, 9 H, aromatic H); MS (EI) 383 (M^+). Anal. ($C_{22}H_{25}NO_5$) C, 66.32; H, N.

(1′*R*,4*S*,5*R*)-5-(2′-Methoxy-1′-methylethyl)-3-methyl-2-oxooxazolidine-4-carboxylic Acid (21a). Compound **21a** was prepared (68%) from **20a** according to the procedure described for **17**. An analytical sample was prepared by recrystallization from ether/pentane: mp 89–90 °C; $[\alpha]_D$ +20.5° (c 1.0, $CHCl_3$). IR ($CHCl_3$) 3200–2800 (br), 2980, 2920, 1760 (sh), 1435, 1400, 1200, 1100, 1040 cm^{-1}; ^1H NMR (270 MHz, $CDCl_3$) δ 1.03 (d, *J* = 7.0 Hz, 3 H, CH_3-C_1'), 2.18 (m, 1 H, H-C_1'), 2.97 (s, 3 H, N-CH_3), 3.33 (s, 3 H, OCH_3), 3.45 (d, *J* = 5.5 Hz, 2 H, 2H-C_2'), 4.22 (d, *J* = 5.2 Hz, 1 H, H-C_4), 4.55 (t, *J* = 5.2 Hz, 1 H, H-C_5); MS (EI). 217 (M^+). Anal. ($C_9H_{15}NO_5$) C, H, N.

(1′*R*,4*S*,5*R*)-5-(2′-Ethoxy-1′-methylethyl)-3-methyl-2-oxooxazolidine-4-carboxylic Acid (21b). Compound **21b** was prepared (80%) from **20b** according to the procedure described for **17**. An analytical sample was prepared by recrystallization from ether/pentane: mp 70 °C; $[\alpha]_D$ +16.6° (*c* 1.7, $CHCl_3$); IR ($CHCl_3$) 3250–2800 (br), 2980, 2935, 2860, 1760 (sh), 1440, 1400, 1210, 1105, 1040 cm^{-1}; ^1H NMR (270 MHz, $CDCl_3$) δ 1.02 (d, *J* = 7.0 Hz, 3 H, CH_3-C_1'), 1.16 (t, *J* = 7.0 Hz, 3 H, OCH_2CH_3), 2.18 (m, 1 H, H-C_1'), 2.93 (s, 3 H, N-CH_3), 3.45 (m, 4 H, $-CH_2OCH_2-$), 4.28 (d, *J* = 5.1 Hz, 1 H, H-C_4), 4.57 (t, *J* = 5.1 Hz, 1 H, H-C_5); MS exact mass calcd for $C_{10}H_{17}NO_5$ 231.1106, found 231.1103 (M^+).

(1′*R*,4*S*,5*R*)-5-[2′-[(4″-Benzoylbenzyl)oxy]-1′-methylethyl]-3-methyl-2-oxooxazolidine-4-carboxylic Acid (21c). Compound **21c** was synthesized from **20c** according to the procedure described for **17** in 81% yield. The analytical sample was obtained by recrystallization from ether/pentane: mp 114–115 °C; $[\alpha]_D$ +17.0° (*c* 1.3, $CHCl_3$); IR ($CHCl_3$) 3100–2800 (br), 3020, 2980, 1760, 1660, 1450, 1410, 1280, 1210, 1050 cm^{-1}; ^1H NMR (270 MHz, $CDCl_3$) δ 1.07 (d, *J* = 7.0 Hz, 3 H, CH_3-C_1'), 2.22 (m, 1 H, H-C_1'), 2.88 (s, 3 H, CH_3-N), 3.58 (d, *J* = 5.5 Hz, 2 H, 2 H-C_2'), 4.21 (d, *J* = 5.1 Hz, 1 H, H-C_4), 4.54 (s, 2 H, OCH_2Ar), 4.56 (t, *J* = 5.1 Hz, 1 H, H-C_5), 7.35–7.85 (m, 9 H, aromatic H), 9.60 (br, 1 H, COOH); MS (EI) 397 (M^+). Anal. ($C_{22}H_{23}NO_6$) C, H, N.

(2*S*,3*R*,4*R*)-3-Hydroxy-5-methoxy-4-methyl-2-(methylamino)pentanoic Acid (4). Compound **4** was prepared (100%) from **21a** according to the procedure for **6**. An analytical sample was prepared by recrystallization from ethanol: mp 235 °C; $[\alpha]_D$ +3.5° (*c* 0.5, H_2O at pH 7 (phosphate buffer tritrisol, pH 7.00, from Merck)); IR (KBr) 3200, 3200–2500 (br), 2960, 2930, 2870, 1620, 1570, 1420, 1400, 1375, 1310, 1200, 1125, 1080, 1025 cm^{-1}; ^1H NMR (270 MHz, D_2O) δ 0.83 (d, *J* = 7.0 Hz, 3 H, CH_3-C_4), 1.85 (m, 1 H, H-C_4), 2.56 (s, 3 H, NCH_3); 3.19 (s, 3*H*, OCH_3), 3.28 (dd, *J* = 6.1, 9.8 Hz, 1 H, H-C_5); 3.43 (dd, *J* = 6.1, 9.8 Hz, 1 H, H-C_5), 3.45 (d, *J* = 5.4 Hz, 1 H, H-C_2), 3.72 (t, *J* = 5.5 Hz, 1 H, H-C_3); MS (EI) 192 (M^+H). Anal. ($C_8H_{17}NO_4$) C, H, N.

(2*S*,3*R*,4*R*)-5-Ethoxy-3-hydroxy-4-methyl-2-(methylamino)pentanoic Acid (5). Compound **5** was prepared (100%) from **21b** according to the procedure for **6**. An analytical sample was prepared by recrystallization from ethanol: mp 225 °C; $[\alpha]_D$ +1.6° (*c* 0.6, H_2O at pH 7 (phosphate buffer tritrisol, pH 7.00,

from Merck)); IR (KBr) 3300, 3200–2500 (br), 3030, 2970, 2850, 2430, 1630, 1570, 1460, 1410, 1360, 1130, 1100, 1020 cm^{-1}; ^1H NMR (270 MHz, D_2O) δ 0.84 (d, J = 7.0 Hz, 3 H, CH_3-C_4), 1.01 (t, J = 7.0 Hz, 3 H, OCH_2CH_3), 1.86 (m, 1 H, H-C_4), 2.56 (s, 3 H, NCH_3), 3.25–3.53 (m, 5 H, H-C_2, 2 H-C_5, OCH_2CH_3), 3.75 (t, J = 5.3 Hz, 1 H, H-C_3); MS (EI) 206 (M^+H). Anal. ($C_9H_{19}NO_4$) C, H, N.

(2*S*,3*R*,4*R*)-5-[(4′-Benzoylbenzyl)oxy]-3-hydroxy-4-methyl-2-(methylamino)pentanoic Acid (7). Compound **7** was prepared (50%) from **21c** according to the procedure for **6**. The analytical sample was obtained by recrystallization from ethanol: mp 181–182 °C; $[\alpha]_D$ −8.5° (*c* 0.4, EtOH); IR (KBr) 3430–2500 (br), 3020, 2960, 2880, 1660, 1610 (sh), 1415, 1370, 1280, 1080, 940, 925, 700 cm^{-1}; ^1H NMR (270 MHz, CD_3OD) δ 1.08 (d, J = 7.0 Hz, 3 H, CH_3-C_4); 2.15 (m, 1 H, H-C_4); 2.59 (s, 3 H, CH_3N); 3.49 (d, J = 4.8 Hz, 1 H, H-C_2); 3.57, 3.76 (dd, J = 6.2, 9.3 Hz, 2 H, 2 H-C_5); 3.96 (t, J = 4.9 Hz, 1 H, H-C_3); 4.64 (s, 2 H, OCH_2Ar); 7.46–7.80 (m, 9H, aromatic H); MS exact mass calcd for $C_{21}H_{24}NO_5Li_2$ 384.1975, found (HR-FAB) 384.1970 (M + 2 Li − H). Anal. ($C_{21}H_{25}NO_5$) C, H, N.

[[(4*S*,5*R*,1′*R*)-2,2,3-Trimethyl-5-[2′-(benzyloxy)-1′-methylethyl]-4-oxazolidinyl]carbonyl]-L-2-aminobutyryl-sarcosyl-*N*-methyl-L-leucyl-L-valyl-*N*-methyl-L-leucyl-L-alanine Benzyl Ester (24d). A suspension of amino acid **6** (70 mg, 0.26 mmol) in 120 mL of anhydrous acetone was heated at reflux for 24 h until a clear solution was obtained. The acetone was concentrated to 2 mL and used immediately in the next step.

The freshly prepared acetonide **22d** (0.26 mmol) in acetone (2 mL) was diluted with THF (4 mL), and *N*-methylmorpholine (0.035 mL, 0.315 mmol, 1.2 equiv) was immediately added. *N*-Hydroxybenzotriazole (92 mg, 0.6 mmol, 2.3 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 the hexapeptide H-Abu-Sar-MeLeu-Val-MeLeu-Ala-OBn (**23**), 207 mg, 0.3 mmol, 1.15 equiv). The mixture was cooled at 0 °C and DCC (60 mg, 0.288 mmol, 1.1 equiv) was added. The mixture was allowed to warm up to room temperature and stirred for 23 h under N_2. The mixture was diluted with CH_2Cl_2 (20 mL) and washed with a saturated aqueous $NaHCO_3$ solution (10 mL). The aqueous phase was extracted with CH_2Cl_2 (3 × 10 mL). The combined organic solution was dried (Na_2SO_4) and evaporated. The residue was suspended in 10 mL of EtOAc and filtered. After evaporation of the solvent, the residue was purified by flash chromatography (10–20% acetone/hexane) to give 0.20 g (78%) of **24d**: R_f 0.25 (40% acetone/hexane); $[\alpha]_D$ −108° (*c* 1.3, $CHCl_3$); IR ($CHCl_3$) 3380, 2960, 1740, 1650 (sh), 1510, 1455, 1190, 1070, 730, 670 cm^{-1}; ^1H NMR ($CDCl_3$, 270 MHz, at room temperature one major conformer was observed) δ 0.80–1.10 (m, 24 H, C-H_3-$C(4^1)$, CH_3-$C(3^2)$, 2 CH_3-$C(4^4)$, 2CH_3-$C(3^5)$, 2CH_3-$C(4^6)$); 1.20, 1.35 (2 s, 6 H, 2 CH_3 of isopropylidene); 1.37 (d, J = 7.0 Hz, 3 H, CH_3-$C(2^7)$); 1.30–2.80 (m, 10 H, H-$C(4^1)$, 2 H-$C(3^2)$, 2 H-$C(3^4)$, H-$C(4^4)$, H-$C(3^5)$, 2 H-$C(3^6)$, H-$C(4^6)$); 2.30 (s, 3 H, CH_3-N^1); 2.93, 3.00, 3.16 (3 s, 9 H, CH_3-N^3, CH_3-N^4, CH_3-N^6); 2.90–5.25 (m, 11 H, H-$C(2^1)$, H-$C(3^1)$, 2H-$C(5^1)$, H-$C(2^2)$, 2 H-$C(2^3)$, H-$C(2^4)$, H-$C(2^5)$, H-$C(2^6)$, H-$C(2^7)$); 4.50 (s, 2 H, OCH_2Ph); 5.13 (m, 2 H, OCH_2Ph (ester)); 7.32 (m, 10 H, aromatic 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 $C_{53}H_{84}N_7O_{10}$ 978.6279 (M^+H), found (HR-FAB) 978.6296.

[(2*S*,3*R*,4*R*)-5-(Benzyloxy)-3-hydroxy-4-methyl-2-(methylamino)pentanoyl]-L-2-aminobutyryl-sarcosyl-*N*-methyl-L-leucyl-L-valyl-*N*-methyl-L-leucyl-L-alanine Benzyl Ester (25d). A solution of **24d** (30 mg, 0.033 mmol) in CH_3OH (1 mL) was stirred for 12 h at room temperature in the presence of 1 N aqueous HCl solution (0.15 mL, 5 equiv). The mixture was neutralized with Na_2SO_4 (75 mg) and the solvent was evaporated in vacuo at 25 °C. The residue was purified by flash chromatography (2.5–5% CH_3OH/CH_2Cl_2) to give 18.8 mg (66%) of the amine **25d**: R_f 0.25 (8% CH_3OH/CH_2Cl_2); $[\alpha]_D$ −125° (*c* 0.8, $CHCl_3$); IR ($CHCl_3$) 3400, 3300, 2860, 1740, 1645 (sh), 1510, 1410, 1180, 1090, 740, 710 cm^{-1}; ^1H NMR (270 MHz, $CDCl_3$, at least two conformers at room temperature and the major one is described) δ 0.7–1.0 (m, 24 H, CH_3-$C(4^1)$, CH_3-$C(3^2)$, 2 CH_3-$C(4^4)$, 2 CH_3-$C(3^5)$, 2 CH_3-$C(4^6)$); 1.35 (d, J = 7.0 Hz, 3 H, CH_3-$C(2^7)$); 1.30–2.40 (m, 10 H, H-$C(4^1)$, 2 H-$C(3^2)$, 2 H-$C(3^4)$, 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); 2.94, 3.03, 3.17 (3 s, 9 H, CH_3-N^3, CH_3-N^4, CH_3-N^6); 2.70–5.25 (m, 13 H, H-N^1, H-$C(2^1)$, HO-$C(3^1)$, H-$C(3^1)$, 2H-$C(5^1)$, H-$C(2^2)$, 2H-$C(2^3)$, H-$C(2^4)$, H-$C(2^5)$, H-$C(2^6)$, H-$C(2^7)$); 4.51 (s, 2 H, OCH_2Ph); 5.15 (d, J = 3.0 Hz, 2 H, OCH_2Ph (ester)); 7.32 (m, 10 H, aromatic H); 6.47–8.10 (3 d, J = 9.0 Hz, 3 H, H-N^2, H-N^5, H-N^7); MS exact mass calcd for $C_{50}H_{80}N_7O_{10}$ 938.59661 (M^+H), found (HR-FAB) 938.5971.

[[(9-Fluorenylmethyl)oxy]carbonyl]-D-alanyl-*N*-methyl-L-leucyl-*N*-methyl-L-leucyl-*N*-methyl-L-valyl-[(2*S*,3*R*,4*R*)-5-(benzyloxy)-3-hydroxy-4-methyl-2-(methylamino)pentanoyl]-L-2-aminobutyryl-sarcosyl-*N*-methyl-L-leucyl-L-valyl-*N*-methyl-L-leucyl-L-alanine Benzyl Ester (27d). To a solution of **25d** (110 mg, 0.117 mmol) and tetrapeptide Fmoc-D-Ala-MeLeu-MeLeu-MeVal-OH (**26**, 96 mg, 0.14 mmol, 1.2 equiv) in CH_2Cl_2 (2.5 mL) were added *N*-methylmorpholine (23.7 mg, 0.234 mmol, 2.0 equiv) and BOP reagent[18] (78 mg, 0.176 mmol, 1.5 equiv). The mixture was stirred under N_2 for 2.5 days at room temperature, diluted with CH_2Cl_2 (30 mL), and washed with H_2O (15 mL). The aqueous phase was extracted with CH_2Cl_2 (3 × 10 mL). The combined CH_2Cl_2 extract was dried ($MgSO_4$), filtered, and concentrated. The residue was purified by flash chromatography (10–25% acetone/hexane) to give 82.3 mg (44%) of pure protected undecapeptide **27d**: R_f 0.24 (40% acetone/hexane); $[\alpha]_D$ −130° (*c* 1.8, $CHCl_3$). IR ($CHCl_3$) 3410, 3300, 3000, 2960, 1720, 1640 (sh), 1510, 1450, 1410, 1200, 1100, 730 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$, more than one conformer was observed at room temperature and the major one is described) δ 0.70–1.15 (m, 42 H, 2 CH_3-$C(4^2)$, 2 CH_3-$C(4^3)$, 2 CH_3-$C(3^4)$, CH_3-$C(4^5)$, CH_3-$C(3^6)$, 2 CH_3-$C(4^8)$, 2 CH_3-$C(3^9)$, 2 CH_3-$C(4^{10})$); 1.30, 1.35 (2 d, J = 7.0 Hz, 6 H, C H_3-$C(2^1)$, CH_3-$C(2^{11})$); 1.20–2.40 (m, 17 H, 2 H-$C(3^2)$, H-$C(4^2)$, 2 H-$C(3^3)$, H-$C(4^3)$, H-$C(3^4)$, H-$C(4^5)$, 2 H-$C(3^6)$, 2 H-$C(3^8)$, H-$C(4^8)$, H-$C(3^9)$, 2 H-$C(3^{10})$, H-$C(4^{10})$); 3.00, 3.02 (6 H), 3.04, 3.11, 3.22, 3.34 (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}); 2.60–5.50 (m, 23 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-$C(3^5)$, 2 H-$C(5^5)$, H-$C(2^6)$, 2 H-$C(2^7)$, H-$C(2^8)$, H-$C(2^9)$, H-$C(2^{10})$, H-$C(2^{11})$, 2 H (OCH_2Ph), 2 H (OCH_2Ph, ester), 3 H (Fmoc: H-C(9′), 2 H (CH_2O)); 5.70 (d, J = 8.5 Hz, 1 H, H-N^1); 6.40–8.00 (m, 21 H, H-N^6, H-N^9, H-N^{11}, aromatic H); MS exact mass calcd for $C_{88}H_{131}N_{11}O_{16}Li$ 1604.9852 (M^+Li), found (HR-FAB) 1604.9879.

Cyclo[[(2*S*,3*R*,4*R*)-5-(benzyloxy)-3-hydroxy-4-methyl-2-(methylamino)pentanoyl]-L-2-aminobutyryl-sarcosyl-*N*-methyl-L-leucyl-L-valyl-*N*-methyl-L-leucyl-L-alanyl-D-alanyl-*N*-methyl-L-leucyl-*N*-methylleucyl-*N*-methylvalyl] (28d). A solution of fully protected linear undecapeptide **27d** (76 mg, 0.047 mmol) in EtOH (2 mL) at 0 °C was stirred with 0.2 N aqueous NaOH solution (0.47 mL, 2.0 equiv). After 1.5 h, an additional 0.2 N aqueous NaOH solution (0.24 mL, 1.0 equiv) was added and the mixture continued to stir for another 3.5 h. The mixture was acidified with 0.2 N aqueous HCl solution (0.71 mL, 3 equiv) to pH 6 and treated with brine (20 mL) and CH_2Cl_2 (20 mL). The layers were separated and the aqueous phase was extracted with CH_2Cl_2 (4 × 15 mL). The combined organic solution was dried ($MgSO_4$) and concentrated in vacuo to dryness.

The residue was dissolved into CH_2Cl_2 (300 mL) and treated with vigorous stirring with 4-(dimethylamino)pyridine (29 mg, 0.235 mmol, 5.0 equiv) and propylphosphonic anhydride (a 50% w/w solution in CH_2Cl_2, 0.031 mL, 0.188 mmol, 4.0 equiv). The solution was stirred at room temperature under N_2 for 41 h, then concentrated to 2 mL, and directly applied to 20 g of silica gel. Flash chromatography (10–20% acetone/hexane) afforded 19.5 mg (33%) of **28d**: R_f 0.21 (40% acetone/hexane); $[\alpha]_D$ −205° (*c* 1.3, $CHCl_3$); IR ($CHCl_3$) 3320, 2960, 1640 (sh), 1510, 1470, 1410, 1200, 1080, 725 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 0.65 (d, J = 6.5 Hz, 3 H, CH_3-$C(4^6)$); 0.70–1.20 (m, 39 H, CH_3-$C(4^1)$, CH_3-$C(3^2)$, 2 CH_3-$C(4^4)$, 2 CH_3-$C(3^5)$, CH_3-$C(4^6)$, 2 CH_3-$C(4^9)$, 2 CH_3-$C(4^{10})$, 2 CH_3-$C(3^{11})$); 1.23 (d, J = 7.0 Hz, 3 H, CH_3-$C(2^8)$); 1.33 (d, J = 7.0 Hz, 3 H, CH_3-$C(2^7)$); 1.20–2.50 (m, 17 H, H-$C(4^1)$, 2 H-$C(3^2)$, 2 H-$C(3^4)$, H-$C(4^4)$, H-$C(3^5)$, 2 H-$C(3^6)$, H-$C(4^6)$, 2 H-$C(3^9)$, H-$C(4^9)$, 2 H-$C(3^{10})$, H-$C(4^{10})$, H-$C(3^{11})$); 2.67 (s, 6 H, CH_3-N^{10}, CH_3-N^{11}); 3.08 (s, 3 H, CH_3-N^4); 3.15 (s, 3 H, CH_3-N^9); 3.30 (s, 3 H, CH_3-N^6); 3.37 (s, 3 H, CH_3-N^3); 3.50 (s, 3 H, CH_3-N^1); 3.13, 4.64 (2 d, J = 15.0 Hz, 2 H, 2H-$C(2^3)$); 3.47, 3.62 (2 m, 2 H, 2 H-$C(5^1)$); 3.84 (d, J = 8.0 Hz, 1 H, HO-$C(3^1)$); 4.04 (m, 1 H, H-$C(3^1)$); 4.15, 4.41 (2 d, J = 12.0 Hz, 2 H, CH_2Ph); 4.43 (m, 1 H, H-$C(2^7)$); 5.04 (t, J = 6.5 Hz, 1 H, H-$C(2^{10})$); 5.16 (m, 3 H, H-$C(2^1)$, H-$C(2^4)$, H-$C(2^6)$); 5.25 (d, J = 10.5 Hz, 1 H, H-$C(2^{11})$); 5.67 (dd, J = 4.5, 11.0 Hz, 1 H, H-$C(2^9)$); 7.20–7.35 (m, 5 H, aromatic H); 7.42 (d, J = 8.0 Hz, 1 H, H-N^8); 7.54 (d, J = 9.0 Hz, 1 H, H-N^5); 8.02 (d, J = 9.0

Hz, 1 H, H-N^7); 8.39 (d, J = 10.0 Hz, 1 H, H-N^2); MS exact mass calcd for $C_{66}H_{114}N_{11}O_{13}$ 1268.8595 (M^+H), found (HR-FAB) 1268.8652.

Cyclo[[(2*S*,3*R*,4*R*)-3,5-dihydroxy-4-methyl-2-(methylamino)pentanoyl]-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-leucyl-*N*-methylvalyl] (28e). A hydrogen stream was bubbled through an ethanol suspension containing 6.3 mg of **28d** and 5 mg of 10% Pd/C for 5.5 h. The mixture was filtered via a pad of Celite and the filtrate was evaporated. The residue was flash chromatographed on 8 g of silica gel with 20% of acetone in hexane to give 5.5 mg (94%) of pure **28e**: R_f 0.19 (40% acetone/hexane); $[\alpha]_D$ -244° (c 0.5, $CHCl_3$); IR ($CHCl_3$) 3310, 2960, 1630 (sh), 1520, 1470, 1410, 1190, 1070 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.75 (d, J = 6.5, 3 H, CH_3-C (4^6)); 0.80–1.10 (m, 39 H, CH_3-C(4^1), CH_3-C(3^2), 2 CH_3-C(4^4), 2 CH_3-C(3^5), CH_3-C(4^6), 2 CH_3-C(4^9), 2 CH_3-C(4^{10}), 2 CH_3-C(3^{11})); 1.28 (d, J = 7.0, 3 H, CH_3-C(2^7)); 1.35 (d, J = 7.0, 3 H, CH_3-C(2^7)); 1.10–2.50 (m, 19 H, H-C(4^1), 2 H-C(3^2), 2 H-C(3^4), H-C(4^4), H-C(3^5), 2 H-C(3^6), H-C(4^6), 2 H-C(3^9), H-C(4^9), 2 H-C(3^{10}), H-C(4^{10}), H-C(3^{11}), HO-C(3^1), HO-C(5^1)); 2.67 (s, 6 H, CH_3-N^{10}, CH_3-N^{11}); 3.10 (s, 3 H, CH_3-N^4); 3.16 (s, 3 H, CH_3-N^9); 3.28 (s, 3 H, CH_3-N^6), 3.41 (s, 3 H, CH_3-N^3); 3.48 (s, 3 H, CH_3-N^1); 3.48, 3.57 (2 m, 2 H, 2 H-C(5^1)); 3.19, 4.17 (2 d, J = 15.0, 2 H, 2 H-(3^2)); 4.07 (m, 1 H, H-C(3^1)); 4.45 (m, 1 H, H-C(2^7)); 4.67 (m, 1 H, H-C(2^5)); 4.83 (m, 1 H, H-C(2^8)); 4.97 (m, 1 H, H-C(2^2)); 5.03 (d, J = 11.5, 1 H, H-C(2^{11})); 5.08 (t, J = 6.5, 1 H, H-C(2^{10})); 5.17 (m, 2 H, H-C(2^4), H-C(2^6)); 5.35 (d, J = 9.0, 1 H, H-C(2^1)), 5.67 (dd, J = 4.0, 11.0, 1 H, H-C(2^9)); 7.41 (d, J = 8.0, 1 H, H-N^8); 7.46 (d, J = 8.8, 1 H, H-N^5); 7.85 (d, J = 7.0, 1 H, H-N^7); 8.25 (d, J = 9.5, 1 H, H-N^2). MS exact mass calcd for $C_{59}H_{108}N_{11}O_{13}$ 1178.8127 (M^+H), found (HR-FAB) 1178.8114.

Acknowledgment. This work was supported by a grant from National Institutes of Health (Grant No. AR32007). High-resolution mass spectra were performed by the Midwest Center for Mass Spectrometry, a National Science Foundation Regional Instrumentation Facility (Grant No. CHE 8620177). We thank the Departments of Chemistry and Biochemistry (NMRFAM) for use of the 500-MHz NMR spectrometers.

Registry No. 4, 125782-34-5; **5**, 125782-35-6; **6**, 125782-36-7; **7**, 125782-37-8; **9a**, 63930-46-1; **6b**, 79026-61-2; (*E*)-**11**, 125782-33-4; (*Z*)-**11**, 125782-38-9; **12**, 114762-43-5; *anti*-**14**, 125873-80-5; *syn*-**14**, 125873-79-2; **15**, 125872-39-0; **16**, 125872-40-3; **17**, 125782-41-4; **18**, 125782-42-5; **20a**, 125782-43-6; **20b**, 125782-44-7; **20c**, 125782-45-8; **21a**, 125782-46-9; **21b**, 125782-47-0; **21c**, 125782-48-1; **22a**, 125782-49-2; **22b**, 125782-50-5; **22c**, 125782-51-6; **22d**, 125782-52-7; **23**, 81135-30-0; **24a**, 125782-53-8; **24b**, 125801-24-3; **24c**, 125782-54-9; **24d**, 125782-55-0; **25a**, 125782-56-1; **25b**, 125782-57-2; **25c**, 125782-58-3; **25d**, 125782-59-4; **26**, 115141-96-3; **27a**, 125782-60-7; **27b**, 125782-61-8; **27c**, 125782-62-9; **27d**, 125782-63-0; **28a**, 125782-64-1; **28b**, 125801-25-4; **28c**, 125782-65-2; **28c**, 125782-66-3; **28e**, 125782-67-4; trimethyl phosphonoacetate, 5927-18-4; 4-benzoylbenzyl bromide, 32752-54-8.

Synthesis and Anticonvulsant Activity of 1-Phenylcyclohexylamine Analogues

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Received July 24, 1989

Thirty-eight analogues of 1-phenylcyclohexylamine (PCA), a phencyclidine (PCP) derivative, were examined for their activities in the mouse maximal electroshock (MES) seizure test and in a motor-toxicity assay. In addition, we determined the binding affinities of the compounds for PCP acceptor sites in rat brain membranes labeled with [^{3}H]-1-[1-(2-thienyl)cyclohexyl]piperidine. Many of the analogues were protective against MES seizures (ED_{50}s of 5–41 mg/kg, ip) and all of these compounds caused motor toxicity. The potencies in the motor toxicity and MES seizure tests showed a moderate correlation with the affinities for PCP sites. Several analogues exhibited a greater separation of potencies in the motor toxicity and MES seizure tests than did the parent compound PCA. These were obtained by (i) 3-methylation of the cyclohexyl ring trans to the phenyl ring, (ii) methoxylation at the ortho position on the phenyl ring, and (iii) contraction of the cyclohexane ring to form the corresponding cyclopentane.

The effectiveness of phencyclidine (PCP) as an anticonvulsant agent has been largely overshadowed by its notoriety as a drug of abuse. Nevertheless, in rats and mice, PCP is protective in the maximal electroshock (MES),[1,2] pentylenetetrazol,[1,3] and audiogenic[1] seizure models. In addition, PCP increases the threshold of kindled seizures[4] and prolongs the latency of flurothyl-induced seizures.[5] Despite its effectiveness as an anticonvulsant, PCP has a variety of toxicities which limit its clinical usefulness in the treatment of seizure disorders[6] and these toxicities are shared by many PCP-related drugs.[2]

In a recent report by Leccese and co-workers,[7] in which the anticonvulsant actions of PCP and various PCP analogues were examined, it was noted that the primary amine analogue of PCP, 1-phenylcyclohexylamine (PCA, 1), had a particularly potent anticonvulsant action in the MES test. We subsequently demonstrated that PCA has a relatively reduced potency for inducing motor toxicity as compared to the parent compound PCP.[8] The relative potencies of anticonvulsant drugs in motor toxicity and

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