Assignment of the Liposidomycin Diazepanone Stereochemistry

Spencer Knapp,* Gregori J. Morriello, Santosh R. Nandan, Thomas J. Emge, George A. Doss,[§] Ralph T. Mosley,[§] and Lijian Chen

Department of Chemistry & Chemical Biology, Rutgers - The State University of New Jersey, P.O. Box 939, Piscataway, New Jersey 08854-8087, and Merck & Co., P.O. Box 2000, Rahway, New Jersey 07065-0900

knapp@rutchem.rutgers.edu

Received April 4, 2001

The liposidomycins comprise a family of complex nucleoside antibiotics that inhibit bacterial peptidoglycan synthesis. Their structures (**1**, **2**) feature nucleoside, ribofuranoside, diazepanone, and lipid regions. Several stereogenic centers remain unassigned, including three within the diazepanone region: C-6′, C-2‴, and C-3‴. An intramolecular reductive amination reaction has been used to prepare model diazepanones. Analysis of **40** and two of its diastereomers by NMR spectroscopy, X-ray crystallography, and molecular modeling indicates a close relative configurational and conformational match between **40** and the liposidomycin diazepanone degradation product **43** and allows the assignment of stereochemistry of the natural products as either [C-6′(*R*), C-2‴(*R*), C-3‴(*R*)] or [C-6′(*S*), C-2‴(*S*), C-3‴(*S*)].

Introduction

The liposidomycins are complex lipid-bearing nucleoside antibiotics (e. g., **1** and **2**) that selectively inhibit bacterial peptidoglycan synthesis.[1−4] They have been shown to specifically inhibit in vitro the *Escherichia coli* phospho-*N*-acetylmuramyl-pentapeptide translocase (translocase I), which catalyzes the first step in the peptidoglycan lipid cycle.[5,6] Additionally, **1** inhibits in rat liver microsomes the formation of polyprenyl (pyro)-phosphate *N*-acetylglucosamine, an intermediate in glycoprotein and teichoic/teichuronic acid biosynthesis.[7] Structures were ascribed to **1** and **2** based on degradation studies, and NMR and mass spectral evidence, but the relative and absolute stereochemistry at four carbons on or near the diazepanone ring (5′, 6′, 2‴, and 3‴) has not yet been established. Because of the flexibility of the seven-membered ring and the unusual nature of the ring atoms and substituents, vicinal ^{1}H−^{1}H coupling constants may not give a reliable indication of the relative disposition of the vicinal substituents found on C-2‴ and C-3‴, and NOE studies have likewise not yet afforded a complete picture of more distant stereochemical relationships. With the limited supply of material for further degradation or crystallographic studies, the synthesis of model compounds and degradation products becomes an important tool for structural assignment. Several research groups have engaged in this activity, among them Ubukata and co-workers,[8] Kim and co-workers,[9] Dini and co-workers,[10] Le Merrer and co-workers,[11] and ourselves,[12,13] resulting in the respective syntheses of a ribosyl-substituted diazepanone, a diazepanone nucleoside, a nucleoside disaccharide, an isoascorbic acid-derived diazepanone, and the 1,4-dimethyl-1,4-diazepan-2-ones (**3**) elaborated with carbethoxy and benzoyloxy substituents at C-2‴ and C-3‴. To this point, the cumulative analytical data have been insufficient to permit assignment of the diazepanone stereocenters. In this paper we present the experimental details of our earlier studies on **3**, the synthesis and characterization of three diastereomers of the valine-derived trisubstituted diazepanones **4**, and finally the comparison of the isomers of **4** with the degradation product of **2** and the evidence for assigning the stereochemistry of **1** and **2** as either [C-6′-(*R*), C-2‴(*R*), C-3‴(*R*)] or [C-6′(*S*), C-2‴(*S*), C-3‴(*S*)].

Results and Discussion

The Reductive Amination Approach to 1,4-Diazepan-2-ones. Searching in 1988 for literature precedent for the synthesis of 1,4-diazepan-2-ones, we were unable to find a method suitable for making the liposidomycin diazepanone itself. The reaction of 1,3-diamines with glyoxal to give simple diazepanones had been

[§] Merck & Co.

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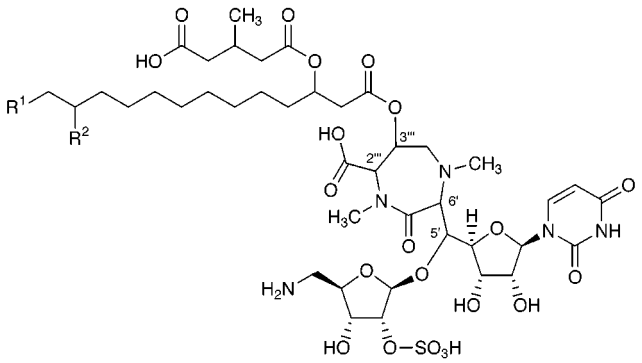
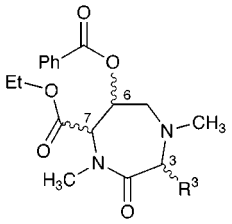
10.1021/jo010355g CCC: $20.00
Published on Web 07/26/2001



1, liposidomycin B: $R^1 = H$, $R^2 = CH_3$
2, liposidomycin C: $R^1 = CH_3$, $R^2 = H$



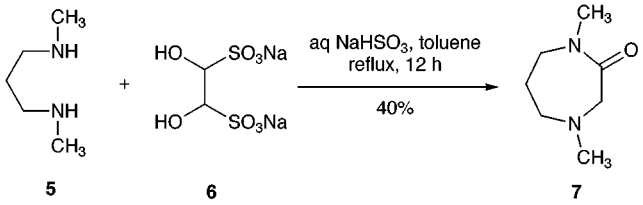
3: $R^3 = H$ (two possible diastereomers)
4: $R^3 = CH(CH_3)_2$ (four possible diastereomers)

demonstrated,[14] and when the symmetrical partners *N*,*N*′-dimethylpropane-1,3-diamine (**5**) and the sodium bisulfite adduct of glyoxal (**6**) were heated together at reflux, the expected 1,4-dimethyl-1,4-diazepan-2-one **7** was formed in modest yield (Scheme 1). The harsh conditions of this reaction, however, might well cause epimerization or elimination of the substituents of more elaborated diazepanones such as **3** and **4**, even if appropriate asymmetrical partner reagents could be identified. Because of these anticipated difficulties, this approach was not pursued further.

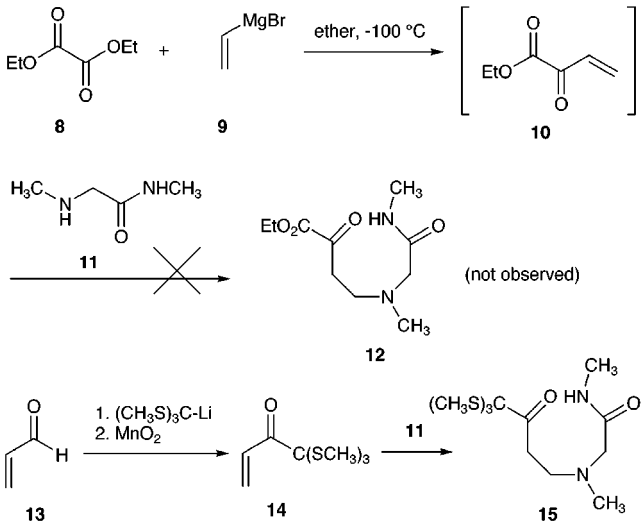
Two attempts were made to develop a diazepanone synthesis based on α-keto ester amination as a method for closing the N-1/C-7 bond (Scheme 2). Reaction of vinylmagnesium bromide (**9**) with ethyl glyoxylate (**8**) was presumed to give rise initially to the unsaturated keto ester **10**, but attempts to trap **10** in situ with sarcosine *N*-methylamide (**11**) were unsuccessful. The tris-thio-ortho ester **14** was then prepared in the hope that it would behave as a nonpolymerizing version of **10**. Thus tris(methylthio)methyllithium[15] was added to acrolein (**13**), and the resulting alcohol was oxidized to provide vinyl ketone **14**. Michael addition of **11** to **14** proceeded smoothly to afford the protected keto ester **15**, but several attempts to close the diazepanone ring by way of a carbinol amide were unsuccessful, and this approach was also abandoned. Vinyl ketone **14** is an interesting variant on methyl vinyl ketone and may have applications in other contexts as a Michael acceptor or dienophile.

Ring closure of the alternative N-4/C-5 bond by reductive amination proved to be more successful (Scheme 3). *N*-(*tert*-Butoxylcarbonyl)sarcosine ethyl ester (**16**) was converted to its lithium enolate, which was alkylated

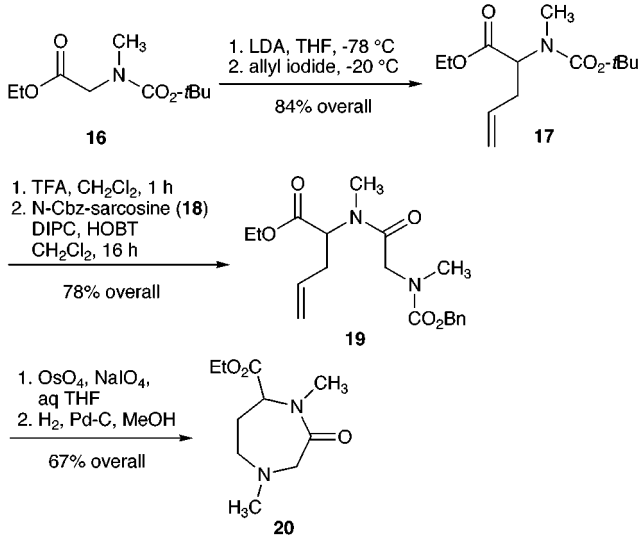
Scheme 1. One-Pot Diazepanone Synthesis



Scheme 2. α-Keto Ester Approaches



Scheme 3. Reductive Amination Approach



smoothly with allyl iodide. The resulting protected N-BOC amino ester **17** was N-deprotected, and the amine was coupled with *N*-(carbobenzyloxy)sarcosine to afford the dipeptide **19**. Oxidative cleavage of the vinyl group led to an aldehyde, which was not isolated, but rather was cyclized under reductive amination conditions (which also removed the N-Cbz) to afford the diazepanone **20** directly. The overall efficiency and mild conditions associated with this synthesis of **20** indicate that more complex diazepanones might also be made by using reductive amination as the key cyclization step.

The next level of diazepanone complexity was indeed reached by using an aldol condensation to assemble the initial amino ester (Scheme 4). The lithium enolate of **16** was condensed with acrolein to give a mixture of

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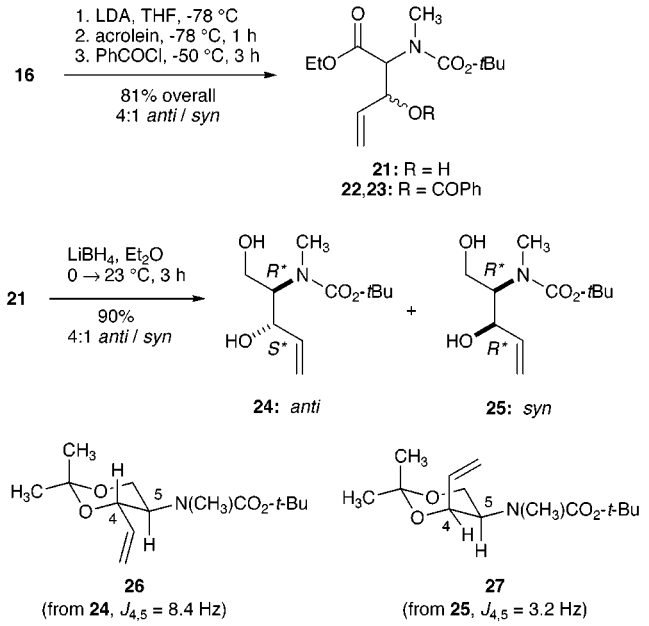
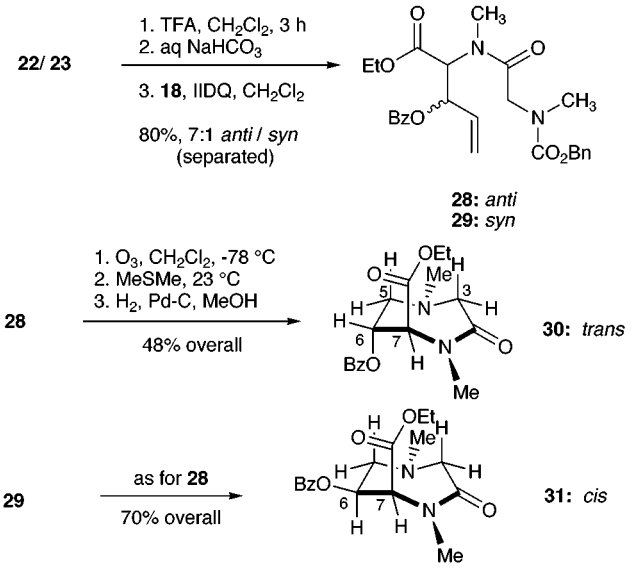
Scheme 4. Sarcosine Aldol Reaction and Proof of Stereochemistry



Scheme 5. Diazepanone Diastereomers Prepared by Reductive Amination



unstable aldol adducts **21**, which were immediately benzoylated in situ to provide two allylic benzoates **22**/**23** (4:1). Although the diastereomers could not be separated at this point, the mixture was reduced to the diols **24** and **25**, which were separated and independently converted to their acetonide derivatives (**26** and **27**, respectively). The major aldol adduct could be assigned the anti stereochemistry based on the H-4/H-5 coupling constant of 8.4 Hz, indicating a trans-diaxial disposition of these protons in **26**. The corresponding *J* for the cis H-4/H-5 protons of **27** is 3.2 Hz. Preference for the anti stereoisomer in the lithium aldol condensation is the expected result.[16]

The aldol benzoate mixture **22**/**23** was taken on to the dipeptides **28** and **29** by removal of the N-BOC protecting group, neutralization, and then peptide coupling with *N*-(carbobenzyloxy)sarcosine **18** (Scheme 5). The dipeptides were separable at this point and were isolated in 80% overall combined yield [70% **28** (anti) and 10% **29** (syn)]. The change in isomer ratio from 4:1 to 7:1 is possibly due to selective destruction of the syn amino benzoate by O-to-N benzoyl migration. Ozonolysis of the vinyl group, reduction of the ozonide, and then cyclization by reductive amination as before gave the diazepanone **30** exclusively from anti dipeptide **28**, and diazepanone **31** exclusively from syn dipeptide **29**. No crossover products were observed, demonstrating that the respective stereochemical relationships in **28** and **29** are maintained in **30** and **31**.

The structures of isomeric diazepanones **30** and **31** were established by ^{1}H NMR spectroscopic analysis. Trans isomer **30** exhibited an H-6/H-7 coupling constant of 5.2 Hz, which is consistent with pseudoequatorial H's, and pseudoaxial benzoyloxy and ethoxycarbonyl substituents at C-6 and C-7, respectively. Pseudoaxial H's at C-6 and C-7 would have been expected to show larger coupling, perhaps 8 Hz or greater. The preference of the ethoxycarbonyl substituent for pseudoaxial orientation may reflect its avoidance of the nearly eclipsing steric interaction with the amide *N*-methyl that would result if the two groups were to lie in the equatorial plane. A four bond "W" coupling ($J_{3,5}$ = 2.0 Hz) between H-3eq and H-5eq provides further evidence for the conformation shown for **30**. The cis diazepanone **31** shows an H-6/H-7 coupling constant of 2.2 Hz, which is consistent with a conformation analogous to that of **30**, but with the benzoyloxy substituent at C-6 pseudoequatorial. The pseudoaxial ethoxycarbonyl group in **31** likewise maintains its distance from the vicinal amide *N*-methyl. The chairlike diazepanone conformations with pseudoaxial ethoxycarbonyls shown as **30** and **31** are reproduced as minima by calculations using the MacroModel program (Amber subroutine),[17] which also predicts[18] H-6/H-7 coupling constants of 5.1 and 1.8 Hz, respectively.

Either diazepanone diastereomer, **30** or **31**, could be converted to the unsaturated ester **32** by treatment with DBU in toluene solution (Scheme 6). Hydrolysis of the ester gave the lithium salt **33**, and further treatment with anhydrous hydrochloric acid led to the ammonium salt **34**. The ability to generate this tetrahydro-1,4-diazepin-2-one carboxylate ring system from any of several stereoisomeric diazepanone esters should prove useful for eventual synthesis of the analogous liposidomycin degradation product **35** (Scheme 6, in box), and thus assignment of the stereochemistry at C-5′ and C-6′ of **1** and **2**.

Synthesis and Characterization of Valine-Derived Diazepanones. The diazepanones **30** and **31** lack a substituent at C-3 that would correspond to the carbohydrate chain attached at C-6′ of the liposidomycins, and thus they are inadequate as models for determining the stereochemistry and conformation of the diazepanone ring of the natural products. A bulky alkyl substituent at C-3 might, however, provide conformational bias similar to that in the natural system. Therefore, a route that was expected to lead to the four diastereomers of

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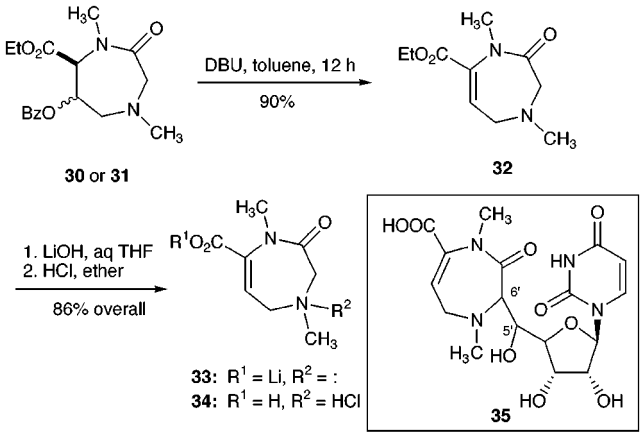
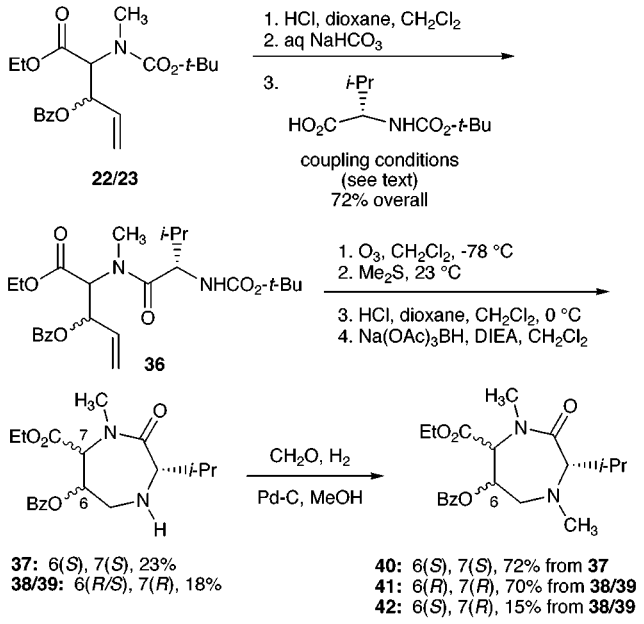
Scheme 6. Elimination and Hydrolysis Reactions of Diazepanones



Scheme 7. Synthesis of Valine-derived Diazepanones



the 3-isopropyl-1,4-diazepan-2-ones **4** was devised and implemented (Scheme 7).

The benzoylated acrolein adducts **22**/**23** (approximate 1:1 mixture) were N-deprotected, and the resulting amines were neutralized and then immediately coupled with the activated ester prepared from *N*-(*tert*-butoxycarbonyl)-valine and 2-chloro-4,6-dimethoxy-1,3,5-triazine.[19] Attempted coupling with the corresponding *N*-methylvaline derivative was unsuccessful, so *N*-methylation was postponed until after the cyclization. The dipeptides **36** were then taken through a four-step sequence consisting of ozonolysis of the vinyl group, reduction of the ozonide, removal of the N-BOC with anhydrous HCl, and finally reductive cyclization of the amino aldehyde under the action of sodium triacetoxyborohydride. Only three of the four possible diastereomeric cyclization products were detected: a "major" isomer **37**, isolated in pure form, and two "minor" isomers, **38** and **39**, isolated and analyzed as an inseparable 4:1 mixture. *N*-Methylation produced the desired pentasubstituted 1,4-diazepan-2-one models **40** (from **37**) and pure **41** and **42** (from the mixture **38/39**; compare **4**).

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The structures, stereochemistry, and conformations of **37**−**42** were established by ^{1}H and ^{13}C NMR spectroscopy (Tables 1 and 2), by NOE studies (Figure 1) and by X-ray crystallography (Supporting Information). The major diazepanone and its *N*-methylated derivative (**37** and **40**, respectively) have the configurations 3(*S*), 6(*S*), and 7(*S*). In chloroform-*d* or acetonitrile-d_3 solution they have very similar average conformations resembling a chair flattened at N-1/C-2 ("$^{1,2}C_5$", see also **30**), with pseudoaxial ethoxycarbonyl and benzoyloxy groups at C-7 and C-6, respectively, and a pseudoequatorial isopropyl group at C-3. In the major *N*-methylated diazepanone **40** the N-4 methyl substituent is pseudoaxial, as suggested by three NOE cross-peaks between this methyl and H-5eq, the benzoyl *o*-H's, and the isopropyl CH, respectively. This is also the *N*-methyl orientation that minimizes steric repulsion with the vicinal and pseudoequatorial isopropyl group. The isopropyl CH and H-3 are anti (J = 10.1 Hz, no NOE) in **40** but not in **37** (J = 6.2 Hz, strong NOE). An NOE cross-peak confirms the cis diaxial relationship of H-3 and H-5ax in both compounds. An important indication of ring conformation is the four-bond "W" coupling (≤1 Hz) observed in both **37** and **40** for the pseudoequatorial protons H-5eq and H-7. For **40**, this coupling is somewhat more prominent in acetonitrile-d_3 solution (J = 2 Hz). Irradiation of H-7 sharpened the broad signal for H-5eq to a clean dd (J = 15.8, 2.2 Hz), reflecting only the geminal coupling to H-5ax and the vicinal coupling to H-6. As in the case of the simpler diazepanone model **30**, the ethoxycarbonyl substituent at C-7 of **37** and **40** probably occupies a pseudoaxial position to avoid an eclipsing steric interaction with the pseudoequatorial N-1 methyl.

One minor diazepanone **38** and its *N*-methylated derivative **41** have configurations 3(*S*), 6(*R*), and 7(*R*). In other words, they are epimeric with **37**/**40** at C-6 and C-7, but nevertheless also have trans pseudodiaxial benzoyloxy and ethoxycarbonyl substituents at C-6 and C-7, respectively ($J_{6,7}$ = 4.8−5.3 Hz for the diazepanones **37**, **38**, **40**, and **41**). Unlike **37**, the minor diazepanone **38** exists in a completely different average conformation from its *N*-methylated derivative **41** in deuteriochloroform (or acetonitrile-d_3, where the absorbances are sharper) solution (Figure 1). The seven-membered ring appears to accommodate the cis 3(*S*),7(*R*) relationship (which would otherwise bring the isopropyl and ethoxycarbonyl substituents into close transannular contact; compare **37**), by deforming into a pseudoboat. Thus the H-3/H-5ax NOE cross-peak observed for **37**/**40** is retained in **38**, but the H-5ax/H-6 cross-peak is lost. No transannular cross-peaks involving the isopropyl group are observed. Upon *N*-methylation to **41**, the diazepanone ring returns to a pseudochair reminiscent of **37**/**40**, but with a pseudoaxial isopropyl group as evidenced by a new isopropyl CH/H-5ax NOE cross-peak. The pseudoaxial isopropyl comes into close transannular contact with the ethoxycarbonyl substituent at C-7. The change in conformation from pseudoboat (**38**) to pseudochair (**41**) upon *N*-methylation can be attributed to avoidance of the vicinal methyl/isopropyl steric interaction that would have developed at C-3/N-4 had the ring maintained the pseudoboat shape.

Overall, the conformations of these 1,4-diazepan-2-ones seem to be largely determined by the vicinal interactions, sometimes with the result that possibly unfavorable transannular interactions arise. The vicinal interactions

Table 1. ^{1}H NMR Spectra of Diazepanones

position	major N−H diazepanone **37** (in $CDCl_3$)		minor N-H diazepanone **38** (in $CDCl_3$)	
$CHMe_2$	2.21	sept, 6.6 Hz	2.67−2.75	m
H-3	2.66	d, 6.2 Hz	3.25	d, 3.4 Hz
H-7	4.56	dd, 5.3, 1.0 Hz	4.32	d, 5.3 Hz
H-6	5.55	dt, 2.5, 5.3 Hz	6.01	ddd, 7.8, 5.6, 2.1
H-5eq	3.45	ddd, 15.3, 2.9, 1.0 Hz	3.72	dd, 13.8, 8.0
H-5ax	3.22	dd, 15.4, 2.2 Hz	2.75	dd, 13.8, 2.1
$CONCH_3$	3.05	s	3.07	s
position	major N-Me diazepanone **40** (in $CDCl_3$)		minor N-Me diazepanone **41** (in $CDCl_3$)	
$CHMe_2$	2.22−2.32	m	1.68−1.79	m
H-3	2.64	3, 10.1 Hz	3.19	d, 11.2 Hz
H-7	4.51	d, 4.8 Hz	4.52	d, 5.0 Hz
H-6	5.63	**td, 2.3, 4.9 Hz**	5.76	**ddd, 5.6, 3.5, 2.4 Hz**
H-5ax	3.50	dd, 15.8, 2.5 Hz	3.47	dd, 16.0, 2.5 Hz
H-5eq	3.32	**br dd, 15.8, 2.2 Hz**	3.14	**dd, 16.0, 3.5 Hz**
$N-CH_3$	2.42	s	2.72	br s
$CONCH_3$	3.11	s	3.16	s
position	*all-cis* N-Me diazepanone **42** (in $CDCl_3$)			
$CHMe_2$	2.22	m		
H-3	3.19	d, 10 Hz		
H-7	4.94	br s		
H-6	5.62	**br t, 2.7 Hz**		
H-5ax	3.48	d, 14.5 Hz		
H-5eq	3.30	**br m**		
$N-CH_3$	2.38	br s		
$CONCH_3$	3.15	s		
position	lipo degradation product **43** (in D_2O)			
H-5′	4.18	dd, 10, 1.6 Hz		
H-6′	3.68	d, 10 Hz		
H-2‴	4.22	d, 4.8 Hz		
H-3‴	4.46	**td, 2.7, 4.8 Hz**		
H-4b‴	3.16	dd, 15.3, 2.7		
H-4a‴	3.21	**br dd, 15.3, 2.7**		
$N-CH_3$	2.41	s		
$CONCH_3$	3.09	s		

Table 2. ^{13}C NMR Data for Diazepanones[a]

position	**37**	**38**	**40**	**41**	**42**	**43** (D_2O)
$CHMe_2$	30.8	32.0	26.7	26.4	26.4	76.8
C-3	66.6	71.3	70.6	77.8	70.2	62.9
C-6	71.2	76.6	72.5	72.5	70.2	68.3
C-7	64.8	64.1	64.8	64.9	61.5	59.5
C-5	51.6	50.7	58.5	50.2	59.1	58.4
$N-CH_3$	−	−	36.6	38.8	37.2	38.5
$CONCH_3$	38.7	40.0	38.2	43.5	33.8	36.5

[a] Chemical shifts and assignments by HETCOR in CD_3CN solution (this work) except for **43** (data for the corresponding C's from ref 3).

in **40** and **41** appear to be comparable; however, **41** has a more destabilizing transannular interaction (isopropyl/ethoxycarbonyl). The upfield position of the isopropyl CH of **41** (−0.5 ppm relative to **40**) may be due to transannular anisotropic shielding by the nearby ethoxycarbonyl. The H-5eq/H-7 "W" coupling observed for **37**/**40** is not seen in **38**/**41**. As with **37**/**40**, the isopropyl CH and H-3 are anti (J = 11.2 Hz, no NOE) in **41** but not in **38** (J = 3.4 Hz, strong NOE).

X-ray crystal structures were obtained for the major and minor *N*-methylated diazepanones **40** and **41**, respectively, although the latter formed suitable crystals only as the hydrochloride salt. In addition, the absolute stereochemistry 3(*S*),6(*R*),7(*R*) was confirmed for **41**·HCl. Details of the crystallographic analysis including ORTEP drawings can be found in Supporting Information. The crystal structures fully confirm the NOE data obtained in CD_3CN solution; in fact, the conformations of **40**/**41**·HCl essentially match the solution phase conformations (Figure 1) despite the greater possibilities for H-bonding and other interactions in the solid state. This is further evidence that vicinal steric interactions are the main determinants of conformation in these isomers. The close transannular approach of the isopropyl CH and the ethoxycarbonyl in the minor diazepanone **41** can now be directly evaluated: the distances $Me_2CH\cdots CO_2$ and $Me_2CH\cdots CO_2$ are 3.25 and 2.4 Å, respectively. The N-4 methyl and the benzoyloxy oxygen are in close transannular contact in both isomers ($NCH_3\cdots OCOPh$ for **40**/**41** is 2.98 and 2.95 Å, respectively). In **41**, the chloride atom H-bonds to the (N-4) pseudoequatorial NH^+.

Mass spectral and ^{1}H NMR analysis of **42** indicate it to be a diastereomer of **40** and **41**, which must therefore have either the 3(*S*),6(*R*),7(*S*), or the 3(*S*),6(*S*),7(*R*) stereochemistry. Deuteriochloroform and acetonitrile solutions of **42** showed broadened signals for H-7, H-6, the H-5's, the isopropyl CH, and the N-4 methyl because of conformational changes, possibly including nitrogen inversion at N-4, that are slow on the NMR time scale. A deuteriobenzene solution of **42** at 60 °C, however, showed sharp and well-resolved signals for all protons, and NOE analysis could be carried out (Figure 1). Two transannular NOE cross-peaks (H-3/H-7, H-7/H-5ax), taken with an H-5ax/H-6 cross-peak, indicate that **42** is the all-cis isomer: 3(*S*),6(*S*),7(*R*). The average conformation under these conditions has apparent pseudoequatorial isopropyl and ethoxycarbonyl groups, and pseudoaxial N(4)-CH_3 and benzoyloxy groups. Little or no coupling (J < 1 Hz) is observed between H-6 and H-7 in any of the three solvents.

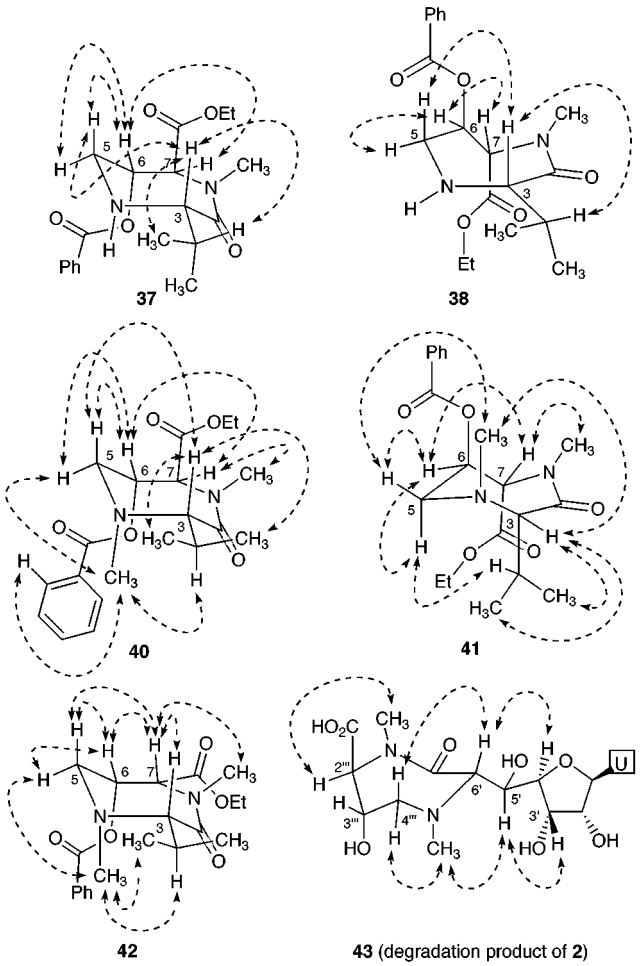

Figure 1. Key NOESY cross-peaks for model diazepanones (this work) and liposidomycin degradation product **43** (data from refs 8 and 20).

Comparison of Diazepanone Models 40−42 with the Liposidomycin Diazepanone. ^{1}H and ^{13}C NMR data reported[3] for the liposidomycin degradation product **43** (note liposidomycin numbering) in D_2O solution are shown in Table 1 along with those for the diazepanone models **40** and **41** (note diazepine numbering) in $CDCl_3$ solution, and the NOE cross-peaks reported[8,20] for **43** are shown in Figure 1. Comparisons of the configuration and conformation of the diazepanone models with **43** (and thus **2**) can be made only with the understanding that the solvent and certain substituents of the models are specifically different from those of **43**.[21] Clearly, the conformational preferences may also be different. Nevertheless, important observations have be made that in our view establish the relative configuration of **43** and **2** as [C-6′(*R*), C-2‴(*R*), C-3‴(*R*)], or, equivalently, [C-6′(*S*), C-2‴(*S*), C-3‴(*S*)], matching **40**.

The ^{1}H and ^{13}C NMR spectra of the models **40** and **41** (Tables 1 and 2) are qualitatively similar to the spectra of **43** in many respects, despite the differences in solvents and substitutents. The all-cis isomer **42**, however, can be declared stereochemically different from **43** in that it lacks the H-3/H-5 NOE cross-peak observed for **43**

(20) An NOE cross-peak for H-6′/N−CH_3 is not observed in an original DIFNOE analysis of **43**. Prof. M. Ubukata, personal communication.

(21) Attempts to hydrolyze the ethyl ester of the diazepanone models led instead to elimination of the benzoate.

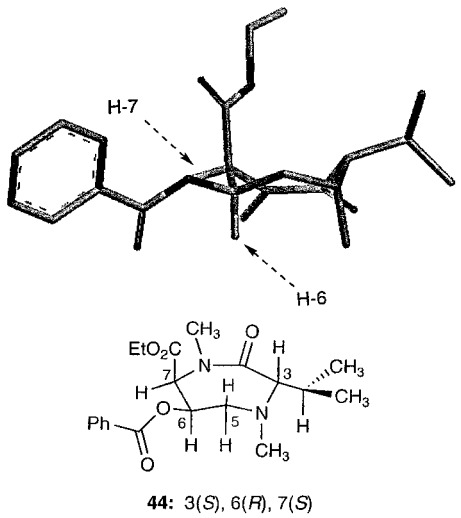

Figure 2. Most stable calculated conformation of diazepanone model **44**, showing pseudoaxial (ethoxycarbonyl and *N*(4)-methyl) and pseudoequatorial (benzoyloxy and isopropyl) substituents. The pseudoequatorial H-7 and pseudoaxial H-6 are indicated by arrows.

(Figure 1), and its H-6/H-7 coupling in several solvents is less than 1 Hz (compare *J* for H-2‴/H-3‴ at 4.8 Hz for **43**). The diazepanones **40** and **41** retain their pseudochair solution conformations in the solid state, even (for **41**) when the amino group is protonated. For diazepanones **40**, **41**, and **43**, H-3 (= H-6′ for **43**) is a wide doublet ($J \sim$ 10 Hz), indicating an H−C−C−H dihedral angle close to 180°. These observations provide circumstantial evidence that the steric interactions that govern the conformation of **40** and **41** in CD_3CN or $CDCl_3$ solution might also apply to the diazepanone carboxylic acid **43** in D_2O. Where there are differences in the ^{1}H NMR spectra of these two isomers (Table 1), the signals for **40** match more closely those of the liposidomycin degradation product **43**. In particular, the respective signals for H-6, shown in bold in Table 1, are (for **40**) td, J = 2.3, 4.9, and (for **41**) ddd, J = 2.4, 3.5, 5.6, compared with (for H-3‴ of **43**) td, J = 2.7, 4.8). Additionally, isomer **40** shows the H-3/H-5 NOE cross-peak observed for **43** (Figure 1), whereas **41** does not. Interestingly, the four bond W-coupling observed for the pseudoequatorial protons H-5eq and H-7 of **40**, which is manifest as a broadening of the H-5eq dd signal, is mimicked by broadening of the signal for H-4a‴ of **43**,[3] suggesting the possibility that **43** has the same configuration and pseudochair conformation. There is no corresponding broadening in **41** (H-5eq's are shown in bold in Table 1).

The fourth possible diazepanone isomer, **44** [3(*S*),-6(*R*),7(*S*), Figure 2], was not isolated from the reduction amination reaction described herein, and several other routes to **44** were unsuccessful, including attempts to invert the stereochemistry at C-6 of **40**. In the absence of a sample of **44**, the 3(*S**),6(*R**),7(*S**) stereochemistry cannot be categorically ruled out as the relative stereochemistry of the liposidomycin degradation product **43**. However, it is possible to make a reasonable guess as to the most probable conformation of **44**. This would be a pseudochair closely resembling diazepanone **31**, but with the additional pseudoequatorial isopropyl group, situated as in **40**. The transannular steric repulsion present in **40** between the benzoyloxy and the *N*-methyl groups would thereby be relieved, but a possible cost would be

increased steric repulsion between the gauche benzoyloxy and ethoxycarbonyl groups at C-6/7. Molecular modeling of possible conformations of **44** [energy minimization by using MMFF94 (s)[22]] led to the calculated lowest-energy conformation shown in Figure 2, matching the conformation observed for **31**. The H−C−C−H dihedral angle calculated for H-6/H-7 is 66.9°, corresponding to a calculated coupling constant of 2.88 Hz,[23] which is significantly less than the 4.8 Hz observed for **43**. Although this is an incomplete argument against the possibility of 3(*S**),6*R**),7(*S**) relative stereochemistry for **43**, the case against **44** is bolstered by the strong circumstantial evidence in favor of the 3(*S**),6(*S**),7(*S**) relative stereochemical and conformational match with **40**, examined in the context of five relevant diazepanone models **30**, **31**, **40**, **41**, and **42**.

Experimental Section

General. NMR spectra, reported in ppm downfield from TMS, were taken on $CDCl_3$ solutions at 200 (^1H) or 50 (^{13}C) MHz unless otherwise indicated. Coupling constants (*J*) are reported in Hz. FT-IR spectra were taken on thin films unless otherwise indicated; selected absorbances are reported in cm^{-1}. Organic solutions were dried over sodium sulfate unless otherwise indicated.

1,4-Dimethyl-hexahydro-1,4-diazepan-2-one (7). A solution of 1 mL (8 mmol) of *N*,*N*′-dimethyl-1,3-propanediamine (**5**) and 2.5 g (8.8 mmol) of glyoxal bisulfate adduct **6** in 30 mL of toluene was heated at reflux for 16 h. The reaction mixture was concentrated and then chromatographed with 19:1 dichloromethane/methanol as the eluant to give 0.467 g (40%) of the diazepanone **7** as an oil: ^1H NMR 3.44 (s, 2 H), 3.42 (t, $J = 5$, 2 H), 3.00 (s, 3 H), 2.85, (t, $J = 5.2$, 2 H), 2.42 (s, 3 H), 1.75−1.88 (m, 2 H); ^{13}C NMR 172.2, 61.5, 58.2, 50.1, 43.3, 35.4, 25.4; IR 1652. Anal. Calcd for $C_7H_{14}N_2O$: C, 59.12; H, 9.92; N, 19.70. Found: C, 59.47; H, 10.08; N, 19.42.

4,4,4-Tris(methylthio)-1-buten-2-one (14). *n*-Butyllithium (6 mL of a 1.3 M solution in hexanes, 7.8 mmol) was added to a solution of 1.16 g (7.52 mmol) of tris(methylthio)methane in 20 mL of tetrahydrofuran at −78 °C. The resulting white suspension was stirred at −78 °C for 1 h, and then 1.1 mL (16.4 mmol) of acrolein was added dropwise. The reaction mixture was warmed to room temperature and then stirred for an additional 2 h. The reaction was quenched with saturated ammonium chloride and extracted with ethyl acetate. The organic extract was dried, concentrated, and then chromatographed with 9:1 hexanes/ethyl acetate as the eluant to give 1.28 g (80%) of the carbinol adduct as a clear oil: ^1H NMR 6.17 (ddd, $J = 16.8$, 10.4, 5.8, 1 H), 5.47 (d, $J = 16.9$, 1 H), 5.33 (d, $J = 10.4$, 1 H), 4.23 (app t, $J = 5.4$, 1 H), 2.94 (d, $J = 4.9$, 1 H), 2.19 (s, 9 H); IR 3451, 1720, 1641, 1419.

A suspension of 1 g (5.5 mmol) of the carbinol adduct, 5.2 g (59.5) mmol of manganese dioxide, and 3 g of powdered activated 3 Å molecular sieves in 25 mL of ether was stirred at room temperature for 6 h, filtered, concentrated, and then chromatographed with 19:1 hexanes/ethyl acetate as the eluant to give 1.11 g (90%) of the enone **14** as a yellow oil: ^1H NMR 7.41 (dd, $J = 17$, 10.5, 1 H), 6.47 (dd, $J = 17$, 2.0, 1 H), 5.75 (dd, $J = 10.2$, 2.0, 1 H), 1.99 (s, 9 H); IR 1686.

***N*-Methyl-*N*-[2-(*N*′-methylacetamido)]-4-amino-1,1,1-tris(methylthio)-2-butanone (15).** A solution of 675 mg (3.24 mmol) of enone **14** in 5 mL of methanol was treated with a solution of 331 mg (3.24 mmol) of sarcosine *N*-methylamide (**11**) in 5 mL of methanol. The reaction mixture was stirred for 16 h, concentrated, and then chromatographed with ether as the eluant to afford 650 mg (65%) of the Michael adduct **15** as an oil: ^1H NMR 7.20 (br s, 1 H), 3.16 t, $J = 6.6$, 2 H), 3.01 (s, 2 H), 2.84 (d, $J = 4.0$, 3 H, 2.71 (t, $J = 6.6$, 2 H), 2.30 (s, 3 H), 2.0 (s, 9 H); IR 3378, 1672.

Ethyl 2-(*N*-*tert*-Butoxycarbonyl-*N*-methylamino)-4-pentenoate (17). A solution of 532 mg (2.46 mmol) of *N*-(*tert*-butoxycarbonyl)sarcosine ethyl ester (**16**) in 2 mL of tetrahydrofuran was added to a solution of lithium diisopropylamide [prepared from 0.375 mL (2.9 mmol) of diisopropylamine and 1.9 mL of 1.6 M *n*-butyllithium in hexanes] at −78 °C. This solution was stirred at −78 °C for 3 h, and then 0.340 mL (3.7 mmol) of allyl iodide was added. The reaction mixture was stirred for 5 h at −20 °C, quenched with saturated aqueous ammonium chloride, and then extracted with ethyl acetate. The organic layer was dried, concentrated, and then chromatographed by using 9:1 petroleum ether/ether as the eluant to give 530 mg (84%) of the allyl sarcosine **17** as an oil: ^1H NMR (1:1 mixture of rotamers) 5.68−5.82 (m, 1 H), 5.05−5.15 (m, 2 H), 4.81 and 4.42 (2 dd, $J = 10.4$, 5.0, 1 H), 4.17 (q, $J = 7.0$, 2 H), 2.80 and 2.85 (3 H each), 2.65−2.75 (m, 1 H), 2.42−2.65 (m, 1 H) 1.45 (s, 9 H), 1.35 (t, $J = 7.0$, 3 H); IR 1741, 1697. Anal. Calcd for $C_{13}H_{23}NO_4$: C, 60.67; H, 9.00; N, 5.44. Found: C, 60.57; H, 9.14; N, 5.23.

Ethyl 2-[*N*-(*N*′-Carbobenzyloxysarcosyl)-*N*-methyl]amino-4-pentenoate (19). A solution of 470 mg (1.83 mmol) of **17** in 5 mL of dichloromethane was treated with 2 mL of trifluoroacetic acid. The reaction mixture was stirred at room temperature for 30 min and then concentrated to a yellow oil. The crude deprotected amine was twice dissolved in 10 mL of toluene and concentrated to help remove traces of trifluoroacetic acid. The crude product was dissolved in 5 mL of dichloromethane, and then 450 mg (2 mmol) of N-carbobenzyloxysarcosine (**18**) was added. The solution was cooled to −10 °C, and 375 mg (2.8 mmol) of *N*-hydroxybenzotriazole and 0.345 mL (2.2 mmol) of *N*,*N*′-diisopropylcarbodiimide was added. The reaction mixture was stirred at −10 °C for 30 min and at room temperature for 16 h. Dichloromethane was added, and the organic solution was washed sequentially with saturated aqueous sodium bicarbonate, water, 10% aqueous hydrochloric acid, water, and finally brine. The organic layer was dried, concentrated, and chromatographed with 2:3 petroleum ether/ether as the eluant to afford 517 mg (78%) of the dipeptide **19** as a colorless oil: ^1H NMR (1:1 mixture of rotamers) 5.6−5.8 (m, 1 H), 5.0−5.3 (m, 2 H), 3.9−4.2 (m, 3 H), 2.80−2.85 (four s, 6 H), 2.7−2.8 (m, 1 H), 2.45−2.55 (m, 1 H), 1.50 and 1.55 (two s, 9 H), 1.2−1.3 (m, 3 H); IR 1739, 1703, 1666. Anal. Calcd for $C_{19}H_{26}N_2O_5$: C, 62.96; H, 7.23; N, 7.73. Found: C, 62.84; H, 7.36; N, 7.71.

7-Carbethoxy-1,4-dimethyl-hexahydro-1,4-diazepan-2-one (20). A solution of 500 mg (1.38 mmol) of dipeptide **19** in 5 mL of 4:1 tetrahydrofuran/water was treated with osmium tetraoxide (0.1 mL of a 0.01 M aqueous solution) and stirred for 10 min. Sodium metaperiodate (650 mg, 3 mmol) was added, and the reaction mixture was stirred for 3 h. The reaction was concentrated and treated with ether and water, and the aqueous layer was separated and washed with two additional portions of ether. The combined organic extract was dried, concentrated, and passed through a short pad of silica gel (10 g) while washing with ether. The filtrate was concentrated, dissolved in 10 mL of methanol and then treated with 100 mg of 10% palladium-on-carbon and stirred under an atmosphere of hydrogen for 24 h. The reaction was filtered through Celite, and the cake was washed with ethyl acetate. The filtrate was concentrated and then chromatographed with 9:1 ether/petroleum ether as the eluant to give 198 mg (67%) of the diazepanone **20** as a yellow oil: ^1H NMR 4.2 (q, $J = 8$, 2 H), 3.9 (dd, $J = 6.4$, 5.2, 1 H), 3.30 and 3.45 (Abq, $J = 17$, 2 H), 3.1 (s, 3 H), 2.7−2.9 (m, 1 H), 2.4−2.6 (m, 2 H), 2.32 (s, 3 H), 2.05−2.25 (m, 1 H), 1.3 (t $J = 8$, 3 H); ^{13}C NMR 172.46, 170.32, 62.68, 61.00 (2 C's), 52.90, 44.40, 38.01, 29.13, 14.28; IR ($CHCl_3$) 1736, 1643; CI-MS *m*/*z* 215 (MH^+).

Ethyl 2-(N-*tert*-Butoxycarbonyl-*N*-methyl)amino-3-benzoyloxy-4-pentenoate (22). A solution of 435 mg (2 mmol) of ethyl *N*-(*tert*-butoxycarbonyl)sarcosinate (**16**) in 2 mL of tetrahydrofuran was added to a solution of LDA [prepared from 0.275 mL (2.18 mmol) of diisopropylamine and 1.3 mL of 1.6 M *n*-butyllithium in hexanes] in 20 mL of tetrahydro-

(22) Halgren, T. A. *J. Comput. Chem.* **1996**, *17*, 490−519.

(23) Ramachandran, G. N.; Chandrasekaran, R. *Biopolymers* **1971**, *10*, 935−939. Ramachandran, G. N.; Chandrasekaran, R.; Kopple, K. D. *Biopolymers* **1971**, *10*, 2113-2131.

furan at −78 °C. The resulting solution was stirred at −78 °C for 3 h, 1 mL (15 mmol) of acrolein was added, and the reaction mixture, which presumably contained the adduct **21**, was allowed to warm to −50 °C over a period of 45 min. Benzoyl chloride (0.44 mL, 3.8 mmol) was then added, and the reaction mixture was stirred for 3 h at −50 °C. Saturated aqueous sodium bisulfite was added and then ethyl acetate. The aqueous layer was washed with additional ethyl acetate, and the combined organic extract was dried, concentrated, and chromatographed by using 19:1 petroleum ether/ether as the eluant to give 605 mg (81%) of the benzoates **22** and **23** as an oily mixture of diasteriomers (1:4 syn:anti): ^{1}H NMR (1:1 mixture of rotamers) 5.90−6.20 (m, 2 H), 5.52−5.28 (m, 2 H), 4.81 and 4.42 (two d's, J = 7.0, 1 H), 4.12−4.22 (m, 2 H), 2.85, 2.95, 2.98, and 3.04 (four s, 3 H), 1.43 and 1.45 [two s, 9 H), 1.13−1.22 (m, 3 H); IR 1731, 1694. Anal. Calcd for $C_{20}H_{27}NO_6$: C, 63.64; H, 7.21; N, 3.71. Found: C, 63.72; H, 7.34; N, 3.76.

4-(*N*-*tert*-Butoxycarbonyl-*N*-methylamino)butene-3,5-diol (24 and 25). Sarcosine derivative **16** (650 mg, 3 mmol) was converted to its enolate and condensed with acrolein as described above. The reaction was quenched with 10 mL of ammonium hydroxide/ammonium chloride buffer (pH = 8), and the aqueous layer was extracted with ether (2 × 20 mL). The combined organic extract was dried and concentrated to afford 510 mg (62%) of the unstable adduct **21** as a dark orange oil. This product was dissolved in 25 mL of dry ether, cooled to 0 °C, and then 77 mg (3.5 mmol) of lithium borohydride was added. The reaction was warmed to room temperature and stirred for 2 h. The reaction was quenched with saturated aqueous sodium bicarbonate, and the aqueous layer was further extracted with ether. The combined organic extract was dried, concentrated, and chromatographed by using 2:3 petroleum ether/ether as the eluant to afford 77 mg (18%) of a higher R_f syn diol **25** and 311 mg (72%) of a lower R_f anti diol **24**: ^{1}H NMR (**25**, 1:1 mixture of rotamers) 5.88 (ddd, J = 17, 10.5, 5.7, 1 H), 5.35 (dt, J = 17, 1.4, 1 H), 5.20 (d, J = 10.5, 1 H), 4.34−4.44 (m, 1 H of one rotamer), 4.12−4.19 (m, 1 H), 3.88−3.98 (m, 1 H), 3.76−3.85 (m, 1 H), 3.54−3.65 (m, 1 H), 2.90 (s, 3 H), 2.72 and 1.93 (two br s, 1 H), 1.45 (s, 9 H); ^{1}H NMR (**24**, 1:1 mixture of rotamers) 5.92 (ddd, J = 17, 10.4, 6.8, 1 H), 5.32 (dt, J = 17, 1.1, 1 H), 5.19 (d, J = 10.4, 1 H), 4.50−4.58 (br m, 1 H of one rotamer), 3.98 (br s, 2 H), 3.48−3.54 (m, 1 H), 3.23−3.37 (m, 1 H), 2.85 (s, 3 H), 1.73 (br s, 1 H), 1.45 (s, 9 H); IR ($CHCl_3$) 3398, 1669. Anal. (**24**) Calcd for $C_{11}H_{21}NO_4$: C, 57.12; H, 9.15; N, 6.05. Found: C, 57.32; H, 9.37; N, 5.92.

2,2-Dimethyl-5-(*R)-(*N*-*tert*-butoxycarbonyl-*N*-methylamino)-4-(*S**)-vinyl-1,3-dioxane (26).** A solution of 57 mg (0.25 mmol) of anti diol **24** and a catalytic amount (5 mg) of pyridinium *p*-toluenesulfonate in 3 mL of 2,2-dimethoxypropane was heated at reflux for 1 h. The reaction mixtue was concentrated and then chromatographed by using 1:1 petroleum ether/ether as the eluant to afford 56 mg (82%) of the trans acetal **26** as an oil: ^{1}H NMR (1:1 mixture of rotamers, assignments by decoupling) 5.75−5.85 (m, H-4a), 5.28−5.38 (m, two H-4b), 5.23 [d, J = 8.3 (at 400 MHz, $J_{4,5}$ = 8.4), H-4], 4.50−4.70 (m, 1 H), 4.0 and 4.1 (two t, J = 9.8, 1 H), 3.82 (dd, J = 9.3, 6.3, 1 H), 3.7−3.8 (m, 1 H), 2.7 and 2.8 (two s, 3 H), 1.4−1.6 (three overlapping app s, 15 H); IR 1701. Anal. Calcd for $C_{14}H_{25}NO_4$: C, 61.96; H, 9.29; N, 5.16. Found: C, 62.05; H, 9.43; N, 5.18.

2,2-Dimethyl-5-(*R)-(*N*-*tert*-butoxycarbonyl-*N*-methylamino)-4-(*R**)-vinyl-1,3-dioxane (27).** The syn diol **25** was converted to its acetonide **27** by following the procedure described for **24**, above: ^{1}H NMR (1:1 mixture of rotamers, assignments by decoupling) 5.76−5.86 (m, H-4a), 5.37 (d, J = 18, H-4b-*Z*), 5.20 (app t, J = 10, H-4b-*E*), 4.60 and 4.65 [two br s, H-4 (at 400 MHz with decoupling of H-4a, J = 3.2], 4.25 (dt, J = 12.5, 4.0, H-5), 4.13−4.20 (overlapping m, H-6ax), 3.9 (dd, J = 12.5, 3.5, H-6eq), 3.1 and 3.5 (two s, 3 H), 1.4−1.5 (three overlapping app s, 15 H); IR 1701.

Ethyl 2-[*N*-(Carbobenzyloxy)sarcosyl-*N*-methylamino]-3-benzoyloxy-4-pentenoate (28/29). A 4:1 mixture (600 mg, 1.59 mol) of benzoates **22** and **23** was N-deprotected with trifluoroacetic acid as for **19**. The crude amine was dissolved in 5 mL of dry dichloromethane, and then 530 mg (2.37 mmol) of *N*-(carbobenzyloxy)sarcosine (**18**) was added. The coupling reagent 2-isobutoxy-1-isobutoxycarbonyl-1,2-dihydroquinoline (0.89 mL, 3 mmol) was added, and then the reaction mixture was stirred for 48 h. Dichloromethane was added, and the solution was washed sequentially with saturated aqueous soldium carbonate, water, 10% aqueous hydrochloric acid, water, and finally brine. The organic extract was dried, concentrated, and chromatographed with 1:1 petroleum ether/ether as the eluant to give respectively 77 mg (10%) of the syn dipeptide **29** and 538 mg (70%) of the anti dipeptide **28** as oils: ^{1}H NMR (**29**, 1:1 mixture of rotamers) 8.05 (d, J = 8, 2 H), 7.60 (br t, 1 H), 7.45 (t, J = 8, two 2 H), 7.3−7.4 (m, 5 H), 5.75−6.05 (m, 2 H), 5.20−5.50 (m, 3 H), 5.12 and 5.18 (two s, 2 H), 4.07−4.25 (m, 4 H), 2.95−3.05 (three s, 3 H), 1.15 (t, J = 7, 3 H); ^{1}H NMR (**28**, 1:1 mixture of rotamers) 7.95 (d, J = 8, 2 H), 7.56 (br t, 1 H), 7.42 (t, J = 8, 2 H), 7.25−7.4 (m, 5 H), 6.20 (br s, 1 H), 5.70−5.92 (m, 2 H), 5.20−5.40 (m, 2 H), 5.04−5.15 (m, 2 H), 4.05−4.25 (m, 4 H), 2.90, 2.95, 3.12 and 3.18 (four s, 3 H), 1.15 (t, J = 7, 3 H); IR 1731, 1711, 1673. Anal. (**28**) Calcd for $C_{26}H_{30}N_2O_7$: C, 64.72; H, 6.27; N, 5.80. Found: C, 64.50; H, 6.19; N, 5.76.

6-(*R)-Benzoyloxy-7-(*R**)-carbethoxy-1,4-dimethylhexahydro-1,4-diazepan-2-one (30).** *Anti*-dipeptide **28** (64 mg, 0.13 mmol) was ozonized, cyclized, and chromatographed as for **31** (below), giving 21 mg (48%) of the *anti*-diazepanone **30** as an oil: ^{1}H NMR (400 MHz, assignments by decoupling) 8.00 (d, J = 7, two *o*-Bz-H), 7.60 (t, J = 7.3, *p*-Bz-H), 7.45 (t, J = 7.6, two *m*-Bz-H), 5.95 (ddd, J = 7.2, 5.1, 4.1, H-6), 4.30 (d, J = 5.2, H-7), 4.26 (q, J = 7, OCH_2CH$_3$), 3.56 (dd, J = 16, 2, H-3$_{eq}$), 3.45 (d, J = 17, H-3$_{ax}$), 3.32 (ddd, J = 14, 7.2, 2.0, H-5$_{eq}$), 3.08 (s, CONCH_3), 2.60 (dd, J = 14, 4.1, H-5$_{ax}$), 2.35 (s, CH$_2$NCH_3), 1.30 (t, J = 7, OCH$_2$CH_3); IR 1718, 1653; CI-MS m/z 335 (MH^+).

6-(*S)-Benzoyloxy-7-(*R**)-carbethoxy-1,4-dimethylhexahydro-1,4-diazepan-2-one (31).** *Syn*-dipeptide **29** (200 mg, 0.41 mmol) was ozonized and cyclized as for **19**. Chromatography with 3:7 petroleum ether/ether as the eluant gave 96 mg (70%) of the *syn*-diazepanone **31** as an oil: ^{1}H NMR (400 MHz, assignments by decoupling) 8.10 (d, J = 7.4, two *o*-Bz-H's), 7.60 (t, J = 7.2, *p*-Bz-H), 7.49 (t, J = 7.4, two *m*-Bz-H's), 5.45 (ddd, J = 8.8, 6.6, 2.1, H-6), 4.26 and 4.27 (two q, J = 7.1, OCH_2CH$_3$), 4.15 (d, J = 2.2, H-7), 3.53 and 3.35 (two d, J = 17, H-3), 3.15 (s, CONCH_3), 3.11 (dd, J = 12.3, 8.8, H-5), 2.95 (dd, J = 12.3, 6.6, H-5), 2.42 (s, CH$_2$NCH_3), 1.30 (t, J = 7.1, OCH$_2$CH_3); IR 1718, 1653; CI-MS m/z 335 (MH^+). Anal. Calcd for $C_{17}H_{22}N_2O_5$: C, 61.06; H, 6.63; N, 8.38. Found: C, 60.92; H, 6.59; N, 8.07.

7-Carbethoxy-1,4-dimethyl-1(*H*)-2,3,4,5-tetrahydro-1,4-diazepin-2-one (32). A solution of 60 mg (0.18 mmol) of diazepanone **31** (a mixture of **30** and **31** worked equally well) and 0.032 mL (0.21 mmol) of 1,8-diazabicyclo[5.4.0]undec-7-ene in 4 mL of toluene was stirred at room temperature for 12 h. The reaction mixture was concentrated and then partitioned between ethyl acetate and water. The organic layer was dried, concentrated, and chromatographed by using ether as the eluant to give 34 mg (90%) of **32** as an oil: ^{1}H NMR 6.84 (t, J = 7.3, 1 H), 4.29 (q, J = 7.2, 2 H), 3.30 (s, 2 H), 3.13 (d, J = 7.3, 2 H), 3.08 (s, 3 H), 2.50 (s, 3 H), 1.34 (t, J = 7.2, 3 H); ^{13}C NMR 170.0, 162.6, 138.6, 127.4, 61.8, 58.4, 51.2, 45.5, 33.0, 41.1; IR 1725, 1673, 1638; CI-MS m/z 213 (MH^+). Anal. Calcd for $C_{10}H_{16}N_2O_3$: C, 56.58; H, 7.60; N, 13.20. Found: C, 56.22; H, 7.46; N, 12.86.

7-Carboxy-1,4-dimethyl-1(*H*)-2,3,4,5-tetrahydro-1,4-diazepin-2-one Hydrochloride (34). A solution of 35 mg (0.165 mmol) of the diazepanone ester **32** in 3 mL of aqueous tetrahydrofuran (1:4) was treated with 10 mg (0.24 mmol) of lithium hydroxide. The reaction mixture was stirred at room temperature for 30 min and then concentrated to give 35 mg of crude lithium salt **33**. Purification was achieved by adding a solution of ethereal hydrochloric acid to a methanolic solution of **33**. The hydrochloride salt **34** precipitated upon cooling to −25 °C and was collected by filtration and pumped to dryness to afford 31 mg (86%) of a colorless powder: ^{1}H NMR (**33**, D_2O)

6.58 (t, J = 7.8, 1 H), 3.30 (s, 2 H), 3.15 (d, J = 7.8, 2 H), 3.05 (s, 3 H), 2.69 (s, 3 H); ^{13}C NMR (**33**, D_2O) 175.4, 172.5, 146.5, 126.0, 59.8, 52.7, 46.7, 35.7; ^{1}H NMR (**34**, D_2O) 6.9 (t, J = 7.6, 1 H), 4.02 (br s, 4 H), 3.29 and 3.19 (two s, 3 H each); ^{13}C NMR (**34**, D_2O) 167.9, 167.2, 145.0, 124.4, 57.9, 51.9, 45.5, 36.4.

Ethyl 2-[*N*-(2-*tert*-Butoxycarbonylamino-3-methylbutanoyl)-*N*-methylamino]-3-benzoyloxy-4-pentenoate (36). A mixture of 20 mL of 4 N HCl in dioxane and a solution of (2.50 g 6.07 mmol) of acrolein adducts **22**/**23** (approximate 3:2 mixture of anti/syn isomers) in 5 mL of dichloromethane was stirred for 1 h at room temperature and then concentrated. The residue was shaken with dichloromethane and saturated aqueous sodium bicarbonate. The aqueous layer was washed with dichloromethane. The combined organic layer was dried, filtered, and concentrated to give the crude deprotected amine, which was used immediately in the next reaction.

A solution of 1.34 mL (12.14 mmol) of *N*-methylmorpholine, 2.64 g (12.14 mmol) of *N*-*tert*-butyloxycarbonyl-L-valine, and 2.14 g (12.14 mmol) of 2-chloro-4,6-dimethoxy-1,3,5-triazine in 40 mL of dichloromethane was stirred at 0 °C for 2 h. A solution of the crude deprotected amine (~6.07 mmol) in 10 mL of dichloromethane was added, and the resulting solution was allowed to stir at room temperature overnight. The reaction was washed sequentially with 1 N HCl, saturated aqueous sodium bicarbonate, and brine. The organic layer was dried over magnesium sulfate and then concentrated. The oily residue was chromatographed by using a stepwise gradient of 1:9 to 1:1 ethyl acetate/hexane as the eluant to give 2.65 g (72%) of the dipeptide **36** as a yellow oil: ^{1}H NMR (500 MHz, approximate 3:2 mixture of diastereoisomers) 8.01 (overlapping dd, J = 1.6, 7.2; 2 H), 7.56 (app dt, J = 1.6, 7.4, 1 H), 7.44 (br t, J = 7.5, 2 H) 6.05 (t, J = 7.6, 0.4 H), 6.01 (t, J = 7.6, 0.6 H), 5.92−5.82 (m, 1 H), 5.52 (dd, J = 9.6, 17.2, 1 H) 5.47 (dd, J = 8.5, 17.2, 1 H), 5.29 (app dd, J = 6.3, 10.2, 1 H), 4.48 (dd, J = 5.3, 9.2; 0.4 H), 4.44 (dd, J = 6.7, 0.6 H), 4.24−4.09 (m, 2 H), 3.15 (s, 1.2 H), 3.12 (s, 1.8 H), 1.98 (app sext, J = 6.6, 0.4 H), 1.93 (app sext J = 6.6, 0.6 H), 1.44 (br s, 9 H), 1.17 (t, J = 7.1, 3 H), 0.99 (d, J = 5.0, 1.8 H), 0.97 (d, J = 5.0, 1.8 H), 0.93 (d, J = 6.6, 1.2 H), 0.86 (d, J = 6.6, 1.2 H).

6-Benzoyloxy-7-ethoxycarbonyl-3-isopropyl-1-methyl-1,4-diazepan-2-ones (37, 38/39). A solution of 750 mg (1.53 mmol) of the allylic benzoates **36** in 20 mL of dichloromethane was cooled to −78 °C and then treated with ozone until a slight blue color persisted. Nitrogen was bubbled through the solution to remove excess ozone, and then 2 mL of methyl sulfide was added to reduce the ozonide to the aldehyde. The mixture was warmed to room temperature and stirred for 2 h. The solution was concentrated, and the residue was taken up in ethyl acetate, which was washed with water and then brine. The organic layer was dried, filtered, and then concentrated to a viscous oil, 722 mg, 96% crude yield, which consisted mostly of two aldehyde isomers (approximately 3:2) according to ^{1}H NMR analysis. The crude aldehyde was used without further purification. ^{1}H NMR (500 MHz) 9.69 (d, J = 5.3, 1 H), 8.09 (br d, J = 7.3, 0.8 H), 8.06 (dd, J = 8.2, 1.1, 1.2 H), 7.64 (app tt, J = 1.1, 7.3, 1 H), 7.49 (app t, J = 7.6, 2 H), 5.80 (d, J = 4.3, 0.6 H), 5.76 (d, J = 3.8, 0.4 H), 5.53 (d, J = 3.8, 0.4 H), 5.25 (d, J = 4.3, 0.6 H), 5.19 (d, J = 9.4, 0.6 H), 5.15 (d, J = 9.4, 0.4 H), 4.50 (app q, J = 7.8, 1 H), 4.36−4.24 (m, 2 H), 3.27 (s, 1.2 H), 3.25 (s, 1.8 H), 2.03−1.92 (m, 1 H), 1.44 (s, 5.4 H), 1.42 (s, 3.6 H), 1.33−1.25 (m, 3 H), 1.01 (d, J = 6.9, 1.8 H) 0.99 (d, J = 7.1, 1.2 H), 0.93 (d, J = 6.9, 1.8 H), 0.91 (d, J = 7.1, 1.2 H).

A solution of the crude aldehyde in 2 mL of dichloromethane and 8 mL of a 4 N solution of HCl in dioxane was stirred for 1 h at 0 °C and then concentrated. The residue, which presumably contained the dipeptide amino aldehyde, was dissolved in 100 mL of dichloroethane and then treated with 400 mg of crushed activated 4 Å molecular sieves, 1.55 g (7.33 mmol) of sodium triacetoxyborohydride, and 279 μL (1.61 mmol) of diisopropylethylamine. The solution was stirred overnight at room temperature, then washed sequentially with 1 N sodium hydroxide and brine. The organic layer was dried and concentrated. Preparative TLC (1500 μm plates) of the residue with 2:3 ethyl acetate/hexane as the eluant gave 130 mg (23.5% overall yield) of a major diazepanone **37** and 100 mg (18.2% overall yield) of a 4:1 inseparable mixture of two additional diazepanone isomers, **38/39**, as oils: ^{1}H NMR of **37** (500 MHz, assignments by decoupling) 8.11 (dd, J = 8, 1, two *o*-Ar-H), 7.60 (t, J = 7.4, *p*-Ar-H), 7.47 (t, J = 8, two *m*-Ar-H), 5.55 (td, J = 5.3, 2.5, H-6), 4.56 (dd, J = 5.3, 1.0, H-7), 4.31 (app qd, J = 7.1, 1.8, OCH_2CH$_3$), 3.45 (ddd, J = 15.6, 2.4, 1.0, H-5eq), 3.22 (dd, J = 15.6, 2.2, H-5ax) 3.05 (s, CONCH_3), 2.66 (d, J = 6.2, H-3), 2.21 (hept, J = 6.6, CHMe$_2$), 1.33 (t, J = 7.1, OCH$_2$CH_3), 0.99 (d, J = 6.7, isopropyl CH_3), 0.96 (d, J = 6.6, isopropyl CH_3); ^{1}H NMR of **38/39** (500 MHz, signals of **38** unless indicated, assignments by decoupling) 8.09 (d, J = 7.1, two *o*-Ar-H of **39**), 7.97 (dd, J = 8.1, two *o*-Ar-H), 7.58 (t, J = 7.3, *p*-Ar-H), 7.44 (t, J = 8, two *m*-Ar-H), 6.01 (ddd, J = 7.8, 5.6, 2.1, H-6), 5.53 (app t, J = 6.6, H-6 of **39**), 4.32 (d, J = 5.3, H-7), 4.29 (dq, J = 10.8, 7.1, one OCH_2CH$_3$), 4.15 (dq, J = 10.8, 7.1, one OCH_2CH$_3$), 3.72 (dd, J = 13.8, 8, H-5eq), 3.35 (dd, J = 12.3, 8.0, H-5eq of **39**), 3.25 (d, J = 3.4, H-3), 3.23 (overlapped dd, J = 12.3, 6.5, H-5ax of **39**), 3.13 (d, J = 3.0, H-3a of **39**), 3.15 (s, CONCH_3 of **39**), 3.07 (s, CONCH_3), 2.75 (dd, J = 13.8, 2.1, H-5ax), 2.73−2.65 (m, CHMe$_2$), 1.30 (t, J = 7.1, OCH$_2$CH_3), 1.00 (d, J = 7.1, isopropyl CH_3), 0.85 (d, J = 7.0, isopropyl CH_3), the remaining peaks of **39** are buried beneath those of **38**; ^{1}H−^{13}C HETCOR of **37** (500 MHz, CD_3-CN) 135.5 (*p*-Ph), 129.8 (*m*-Ph), 130.3 (*o*-Ph), 71.2 (C-6), 66.6 (C-3), 64.8 (C-7), 63.5 (OCH_2CH$_3$), 51.6 (C-5), 38.7 (CONCH_3), 30.8 (CHMe$_2$), 22.4 (isopropyl CH_3), 18.8 (isopropyl CH_3), 16.0 (OCH$_2$CH_3); ^{1}H−^{13}C HETCOR of **38** (500 MHz, CD_3CN) 134.8 (*p*-Ph), 129.7 (*m*-Ph), 130.3 (*o*-Ph), 76.6 (C-6), 71.3 (C-3), 64.1 (C-7), 61.6 (OCH_2CH$_3$), 50.7 (C-5), 40.0 (CONCH_3), 32.0 (CHMe$_2$), 22.0 (isopropyl CH_3), 18.6 (isopropyl CH_3), 14.5 (OCH$_2$CH_3); LC−FAB-MS of **37** m/z 385 (MNa^+), 363 (MH^+); LC-FAB-MS of **38/39** m/z 385 (MNa^+), 363 (MH^+).

6-Benzoyloxy-1,4-dimethyl-7-ethoxycarbonyl-3-isopropyl-1,4-diazepan-2-ones (40, 41, 42). A solution of 40 mg (0.107 mmol) of the major diazepanone **37** in 1 mL of methanol and 0.2 mL of formalin was stirred for 30 min at room temperature. 10% palladium on carbon (12 mg) was added to the solution, and the resulting suspension was placed under hydrogen atmosphere. After 2 h, the catalyst was filtered and the filtrate was concentrated. Preparative TLC (1500 μm plates) of the residue with 35:65 ethyl acetate/hexane as the eluant gave the 28 mg (72%) of the *N*-methylated product **40** as a yellow oil. Crystallization from isopropyl ether gave colorless rods, mp 150−153 °C: ^{1}H NMR of **40** (500 MHz, assignments by decoupling) 8.02 (dd, J = 8, 1, two *o*-Ar-H), 7.60 (t, J = 7.3, *p*-Ar-H), 7.46 (br t, J = 8, two *m*-Ar-H), 5.63 (td, J = 4.9, 2.3, H-6), 4.51 (d, J = 4.8, H-7), 4.26−4.37 (m, OCH_2CH$_3$), 3.50 (dd, J = 15.9, 2.3, H-5ax), 3.32 (br dd, J = 15.8, 2.2, H-5eq), 3.11 (s, CONCH_3), 2.64 (d, J = 10.1, H-3), 2.42 (s, NCH_3), 2.22−2.32 (m, CHMe$_2$), 1.33 (t, J = 7.1, OCH$_2$CH_3), 0.90 (d, J = 6.4, isopropyl CH_3), 0.88 (d, J = 6.7 Hz; isopropyl CH_3); LC−FAB-MS of **40** m/z 399 (MNa^+), 377 (MH^+). The structure of **40** was confirmed by X-ray crystallographic analysis.

The minor diazepanone mixture **38/39** (50 mg) was *N*-methylated as above. Preparative TLC as before gave 34 mg (70%) of **41** and 7.3 mg of **42**, both as a yellow oils. Attempts to crystallize **41** were unsuccessful. Addition of 1 equiv of a 4 N solution of HCl in dioxane precipitated the hydrochloride salt of **41**, which was dissolved in 1 mL of isopropyl alcohol. The solution was filtered through a Pall Gelman 0.45 μm CR PTFE Acrodisc syringe filter and then placed into a crystallization dish and set in a desiccator containing isopropyl ether. Long rod-shaped crystals formed after 48 h, mp 124−126 °C. The structure of **41**·HCl including absolute configuration was confirmed by X-ray crystallographic analysis. ^{1}H NMR of **41** (as free base, 500 MHz, assignments by decoupling) 8.03 (dd, J = 8, 1.1, two *o*-Ar-H), 7.62 (t, J = 7.5, *p*-Ar-H), 7.48 (br t, J = 8, two *m*-Ar-H), 5.74−5.78 (m, H-6), 4.52 (d, J = 5.0, H-7), 4.35 (dq, J = 10.8, 7.1, one OCH_2CH$_3$), 4.15 (dq, J = 10.8, 7.1, one OCH_2CH$_3$), 3.47 (dd, J = 16.0, 2.5, H-5ax), 3.19 (d, J = 11.2, H-3), 3.16 (s, CONCH_3) 3.14 (dd, J = 16.0, 3.5, H-5eq), 2.72 (br s, NCH_3), 1.67−1.75 (m, CHMe$_2$), 1.33 (t, J = 7.1, OCH$_2$CH_3), 0.98 (d, J = 7, isopropyl CH_3), 0.96 (d, J = 7,

isopropyl CH_3); ^{1}H NMR of **42** (500 MHz, assignments by decoupling; peaks are broadened at 23 °C compared with those of **41**) 8.02 (d, J = 7.6, two *o*-Ar-H), 7.60 (t, J = 7.6, *p*-Ar-H), 7.46 (t, J = 7.6, two *m*-Ar-H), 5.62 (br t, J = 2.7, H-6), 4.94 (br s, H-7), 4.24 (app q, J = 7.1, OCH_2CH$_3$), 3.48 (d, J = 14.5, H-5ax), 3.30 (br m, H-5eq), 3.19 (d, J = 10.0, H-3), 3.15 (s, CONCH_3), 2.38 (br s, NCH_3), 2.22 (br m, CHMe$_2$), 1.27 (t, J = 7.1, OCH$_2$CH_3), 0.97 (d, J = 6.6, isopropyl CH_3), 0.95 (d, J = 6.6, isopropyl CH_3); LC−FAB-MS of **41** *m*/*z* 399 (MNa$^+$), 377 (MH$^+$); LC−FAB-MS of **42** *m*/*z* 399 (MNa$^+$), 377 (MH$^+$).

Molecular Modeling. One hundred fifty conformations of the three valine-derived diazepanones **41**, **41**, **42**, and the fourth possible diazepanone isomer **44**, with 3(*S*),6(*R*),7(*S*) absolute stereochemistry, were generated by using a distance geometry algorithm without any constraints. These conformations were then minimized by using MMFF94 (s) with a distance dependent dielectric of 2*r*. To simplify the subsequent calculations, the conformations of each structure were sorted by energy and then clustered on the basis of the selected pairwise atoms (the atoms that comprise the diazepanone ring and heavy atoms attached to them) with superposition rms of 0.25. Thus, the initial 150 conformations were reduced to 13 for **40**, 15 for **44**, 18 for **42**, and 20 for **41**. Coupling constants were then calculated for H-6/H-7 for each of these conformations. For **44**, energies ranged from 83.6 to 124.4 kcal/mol. The lowest energy calculated conformation is shown in Figure 2.

Acknowledgment. This work was supported by Merck & Co. and SynChem Research Inc. We are grateful to Prof. M. Ubukata for discussion and exchange of spectra.

Supporting Information Available: Details of the crystallographic structure determinations and NMR spectra of selected compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

JO010355G