Synthesis, Molecular Modeling, 2-D NMR, and Biological Evaluation of ILV Mimics as Potential Modulators of Protein Kinase C

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Abstract: To study the structural determinants required for protein kinase C (PKC) activation by indolactam V (ILV) for purposes of arriving at simpler versions of this PKC activator, four simplified analogues of ILV (**4a–c** and **14a**) were synthesized. These analogues contain a benzene ring in place of the indole group of ILV and were designed for synthesis because molecular modeling studies revealed these simplified structures to possess readily accessible [ILV]-sofa-like conformations, thus mimicking the literature-reported bioactive conformation of ILV. During the course of designing these analogues, a more rigorous conformational search program (SysSearch) was developed to analyze the highly functionalized nine-membered lactam ring system present in ILV. The results of the molecular modeling studies using the SysSearch program on which the design of these analogues was based were confirmed by 2-D NMR and X-ray studies. The compounds of this series were constructed by use of the Mitsunobu reaction to generate the unique nine-membered lactam ring present in these structures. Two routes to compound **4a** are presented, one of which utilizes the amino acid building blocks, L-valine and L-phenylalanine, to fix the stereochemistry of its two asymmetric centers. The biological studies reveal that these new analogues fail to modulate PKC activity, and thus they exclude the possibility that a benzene ring can serve as a surrogate of the indole ring of ILV. The present work therefore indicates that the nine-membered lactam ring moiety of ILV in an [ILV]sofa conformation is not a sufficient structural determinant for PKC activation.

Introduction

Biological investigations have revealed the existence of at least nine isoforms of protein kinase C (PKC), an enzyme eliciting various important biological events through phosphorylation of its specific protein substrates. These isoforms bear closely related amino acid sequences[1] and exhibit a distinct pattern of tissue specific expression and intracellular localization. While the functional significance of these different isoforms remains to be elucidated,[2-7] the discovery of isoform-selective modulators (activators or inhibitors) of PKC undoubtedly constitutes an important biological goal. Such modulators would presumably aid in the functional characterization of these PKC isoforms allowing, for example, a study of their involvement in cellular differentiation.[8] Therefore, it is our aim to learn more about the structural determinants of PKC modulators that govern binding, activation, inhibition, translocation, and/or down-regulation of PKC and to thereby arrive at isoform-selective molecular probes of biological utility.

Two important known activators of PKC are teleocidin (**1**) and lyngbyatoxin A (**2a**). Teleocidin was first isolated from the mycelia of *Streptomyces mediocidicus* by Sakai et al.,[9] while lyngbyatoxin A was isolated from *Lyngbya majuscula* Gomont.[10] The structure of lyngbyatoxin A is closely related to that of the teleocidin family. The lyngbyatoxin series can be obtained together with the teleocidin B group from *S. mediocidicus*.[11] Therefore, they were also named as teleocidin A-1 (**2a**) and A-2 (**2b**) by Sakai. The structurally simplest and naturally occurring member of the teleocidin family is indolactam V or ILV (**3**), which also acts as an activator of PKC. Another structurally distinct PKC activator is the phorbol ester, 12-*O*-tetradecanoylphorbol 13-acetate (TPA). Investigations with TPA have provided considerable information on tumor promotion.[12,13]

TPA and the teleocidins are believed to serve as diacylglycerol (DAG) mimics, binding to the DAG site of PKC, thereby activating the enzyme (in terms of PKC activation, TPA ≅ teleocidins > ILV). Consistent with this related mode of action,

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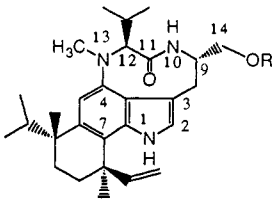
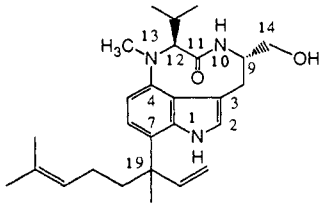
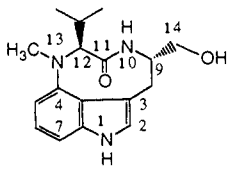


1, R = H: Teleocidin B-4 or Olivoretin D



2a, 19 R: Teleocidin A-1 or Lyngbyatoxin A
2b, 19 S: Teleocidin A-2



3, Indolactam V (ILV)

computer assisted molecular modeling studies have revealed similarities in certain hydrophobic regions and certain heteroatoms among TPA, the teleocidins, the lyngbyatoxins, and ILV. Some controversy exists in the modeling area, and presently, we consider the results reported by Itai et al.[14] to be the most reliable in which they suggest a correspondence between the CH_2OH at C-6 of TPA and C-9 of teleocidin (as donor), the C-11 carbonyl group of teleocidin and the C-3 carbonyl of TPA (as acceptor), and the amide NH of teleocidin and the C-4 OH of TPA (as H-bond donor). This correspondence suggests that the nine-membered lactam ring portion of ILV may serve as a sufficient structural determinant for PKC activation.

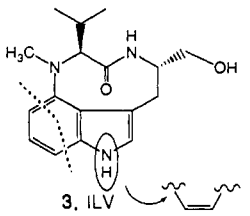
On the basis of both solution NMR studies and molecular modeling studies, it was reported that ILV can exist in two conformational states, the sofa-like or twist-like conformations (see Figure 3, Experimental Section). At equilibrium, the ratio of twist/sofa was 2.8.[15] Due to the relatively small energy difference between twist and sofa, considerable controversy also exists as to which of these conformational states is required for PKC activation. In the superposition studies of Itai, the three-dimensional correspondence between teleocidin B-4 and TPA was found to be best for the sofa form, thus providing support for this as the active conformer.

In an effort to obtain more evidence that the nine-membered lactam ring of ILV in its sofa conformation serves as a sufficient structural determinant for PKC activation, we undertook the synthesis of simplified ILV analogues in which their nine-membered lactam rings were found by molecular modeling studies to possess easily accessible [ILV]sofa-like conformations. An amalgamation of synthesis, molecular modeling, 2-D NMR, X-ray crystallography, and biological studies is thus detailed herein.

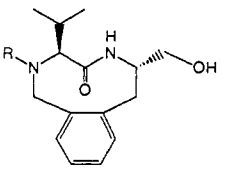
Design

We designed simplified versions of ILV, compounds **4** and **14**, in which the aromatic indole nucleus is replaced by a benzene ring while the nine-membered lactam ring is retained.

The reason for substitution of the indole nucleus by a benzene ring relates both to the ease of synthesis and to the results of molecular modeling studies employing the SysSearch program.[16] The modeling studies revealed readily accessible [ILV]sofa-like conformers equal to or about 2 kcal/mol higher in energy than their respective global minimum energy conformations (gmecs); these sofa forms were, however, 8 kcal/mol lower in energy than



3, ILV



4, a benzenoid mimic of ILV

the respective twist conformers (vide infra). As discussed above, and applying the ceteris paribus assumption, such analogues should serve as probes for defining the structural determinants of PKC activation. We also designed the bis(hydroxymethyl)analogue **14a**, for this compound would allow us to examine whether the presentation of additional H-bonding capabilities by the ligand might lead to altered PKC modulatory activity. Compound **14a** could be readily derived from an intermediate generated en route to compound **4**.

To confirm experimentally the results of the SysSearch program that was specifically developed to analyze the conformations of the highly functionalized nine-membered lactam ring of ILV and its analogues, 2-D NMR studies on the conformation of compound **4a** and X-ray studies on the structure of **14a** were carried out. Development of the SysSearch program became necessary since none of the currently available molecular modeling programs could be used to effectively analyze the conformations of complicated molecules like ILV and its analogues. The difficulty in carrying out conformational studies on these molecules relates to the fact that at least 1 million conformers must be minimized in energy for each molecule in order to obtain the possible gmec and most of the low-lying minimum energy conformations (llmec). From the NMR studies (see Experimental Section), the solution conformation of **4a** was found to be identical to the gmec calculated for **4a** employing the SysSearch program (Figure 1, right). The conformation of **14a** in its crystal structure was also found to be identical to its calculated gmec (Figure 1, left). The experimental confirmations of our molecular modeling studies thus gave credence to the design aspects of the present study.

Synthesis

To synthesize compound **4**, we examined two different synthetic routes. The first of these is shown in Scheme I and makes use of a single amino acid building block, while the second, albeit lengthier, scheme employs two amino acid building blocks to establish the absolute stereochemistry of the asymmetric centers. *N*-Tosyl-L-valine was thus coupled with diethyl aminomalonate to give **7**, which was in turn alkylated with the bromide **8**. The benzylic bromide **8** was prepared from phthalic anhydride by lithium aluminum hydride reduction, monosilylation of the resulting diol, and bromide formation by reaction of the derived mesylate with sodium bromide. At this stage, we were ready to try out an unprecedented reaction, this being the use of the Mitsunobu oxidation–reduction–condensation method[17] to form a nine-membered lactam ring. Accordingly, the *tert*-butyldimethylsilyl group was removed by *p*-toluenesulfonic acid treatment, and the Mitsunobu reaction was carried out at room temperature in THF. The ring closure reaction proceeded readily, and the desired lactam **10** was isolated in 75% yield. The ester groups were now hydrolyzed and decarboxylated to provide **11a** together with the undesired stereoisomer **11b**. The isomers were separated, and **11a** was transformed to the benzyl ester via the acid chloride intermediate. Benzyl ester formation was carried out in lieu of direct reduction of acid to alcohol in order to facilitate

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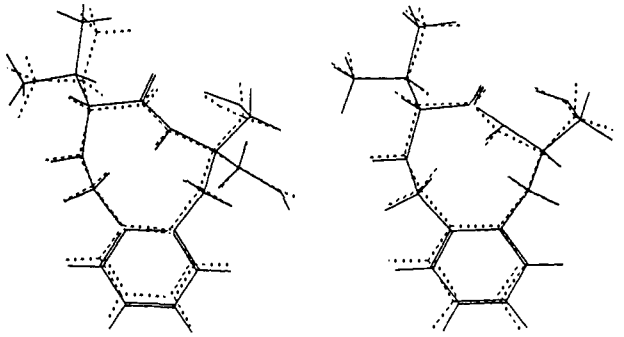

Figure 1. Right: superimposition structures of **4a**-gmec (dashed) and **4a**-NMR (solid); Left: superimposition structures of **14a**-gmec (dashed) and **14a**-X-ray (solid).

additional purification of the less polar ester. Ester reduction by sodium borohydride treatment followed by reductive detosylation[18] completed the construction of **4a**. *N*-Methylation of **4a** by use of methyl iodide and sodium bicarbonate furnished analogue **4b** while formic acetic anhydride treatment gave **4c**.

To synthesize **14a**, the two ester groups of **9** were reduced with LAH, the resulting diol was acetylated, and the silyl group was cleaved by the action of *p*-toluenesulfonic acid. The Mitsunobu reaction also proceeded well in this case with **13** being isolated in 86% yield. The acetate groups of **13** were cleaved by the action of potassium carbonate in methanol/water, and the tosyl group was removed by Na/NH_3 treatment. The bis(hydroxymethyl) compound **14a** proved to be a nicely crystalline compound that afforded us the opportunity to examine its solid-state conformation by X-ray crystallography (details provided below). Compound **14a** was converted to two additional analogues for biological study, the *N*-formyl derivative **14b** formed by reaction with formic acetic anhydride and its *N*-methyl derivative **14c** formed by reaction with methyl iodide/sodium bicarbonate.

As displayed in Scheme II, the second route to compound **4a** started from the isoquinoline intermediate **15** that was readily available from L-phenylalanine by a previously described procedure.[19] The isoquinoline was condensed with *N*-tosyl-L-valine, the ester group hydrolyzed with sodium hydroxide, and a potassium permanganate oxidation carried out to provide intermediate **16**. Borane reduction of the carboxylic acid groups provided a diol intermediate. In preparation for the Mitsunobu condensation, it was essential to selectively protect the nonbenzylic hydroxyl group of **17** in order to preclude ring closure to the six-membered ring in preference to closure to the nine-membered structure. Accordingly, the benzylic hydroxyl of **17** was oxidized to aldehyde with manganese dioxide, and then the other hydroxyl group was protected by acetylation. The aldehyde group of **18** was reduced, the Mitsunobu reaction carried out, and finally deprotection brought about by use of sodium and ammonia. The ^1H and ^{13}C NMR as well as the optical rotation of the final product **4a** obtained via Scheme II were identical to the data obtained for compound **4a** prepared by way of Scheme I. The stereochemical course of the decarboxylation step leading from **10** to **11** is thus confirmed by the identical nature of the final products emerging from these two independent synthetic sequences.

Biological Results

Compounds **4a**, **4b**, and **14a** were tested both for their ability to activate the isolated PKC enzyme by measuring the phosphorylation of histone H3 and for their ability to antagonize TPA activated phosphorylation of the same target. The methods were as described previously.[8] None of these compounds were found to show any significant activity as either PKC activators or inhibitors as is evident from the data shown in Table I. Additionally, the *N*-formyl derivative **4c** was evaluated in a separate set of experiments and was also found to be inactive. These data are not presented in Table I since the basal and TPA stimulated kinase activity measured in the second study varied from the first due to the use of a new batch of isolated enzyme.

Conclusions

Based upon the structural information obtained for compounds **4a–c** and **14a** together with the assays of PKC activity, we conclude that the nine-membered lactam ring portion of ILV in an [ILV]-sofa conformation is not a sufficient structural determinant for PKC activation. The lack of modulation of PKCs activity by these analogues may be due to their inability to adopt the bioactive conformation (perhaps the twist conformation) of the nine-membered lactam ring and/or to the replacement of the indole moiety by a benzene ring. The present findings therefore direct our attention to the design of simplified ILV analogues in which their nine-membered lactam moieties adopt the twist conformation.

Experimental Section

Molecular Modeling Studies. The structures of ILV and its analogues (**4a–c** and **14a**) were generated with the Sybyl program[20] employing the Tripos force field. The structures were then exported to the SysSearch program to carry out systematic conformational searches. The SysSearch program first systematically generates all possible rotamers by rotating about all rotatable bonds at increments of 30°, except for the increment of the amide bond which was set to 180° (Figure 2). The program then optimizes the rotamers, preserving the specified chiralities, and finally eliminates all duplicated conformers. The unique structures were then exported to MacroModel V3.5 for fine optimization with the MM3 force field.[21] The total number of unique conformations, the energy of the global minimum, the energy of the [ILV]sofa-like conformation, and the energy of the twist-like conformation of ILV, **4a–c**, and **14a** are listed in Table II. Ball and stick representations of the gmecs, the [ILV]sofa-like conformations, and the twist-like conformations of these five compounds are presented in Figure 3. It should be noted that the **[ILV]-sofa-like** conformation of **4a** (as well as of **14a**) differs from its **sofa*** conformation (its gmec) by the location of the "amide-flap" on the top face of the benzene ring rather than on the bottom face as depicted in Figure 3.

NMR Studies. Double quantum filtered COSY (DQFC)[22] and NOESY[23] experiments were performed on **4a**, which was dissolved in $CDCl_3$ at 500 MHz (Bruker AMX-500) and 20 °C. For the DQFC experiment; 32 scans of 4096 data t_2 points with 256 t_1 increments were acquired in the phase sensitive mode and Fourier transformed into 4K × 1K ReRe data points. Cosine/filter was used in both domains. For the NOESY spectrum, an experiment with τ_m = 1.2 s, 96 scans, 8192 t_2 points, and 512 t_1 increments was acquired and transformed into a 8192 × 512 ReRe matrix. Spectral assignments were based on the connectivities observed in the DQFC experiment together with the fine structure of the resolution enhanced 1-D spectrum from which coupling constants were measured as well.

All but the aromatic ring hydrogens can be uniquely assigned from the DQFC spectrum. The aromatic protons are strongly coupled and overlap with the solvent. The assignment of the geminal pairs 7/7′ and 16/16′ (for atom numbers, see Figure 4) together with their stereochemistry could be made from the observed NOE contacts. The chirality of carbon 13, which was known from the method of synthesis, was crucial in making these assignments, Proton H-13(β) exhibits a strong NOE to H-16 at 2.55 ppm and a weak NOE to H-16′ at 3.2 ppm from which the respective β and α configurations can be deduced. H-16(β) exhibits a strong NOE to the aromatic multiplet centered at 7.15 ppm while H-16-(α) does not show magnetization transfer to and from the aromatic protons.

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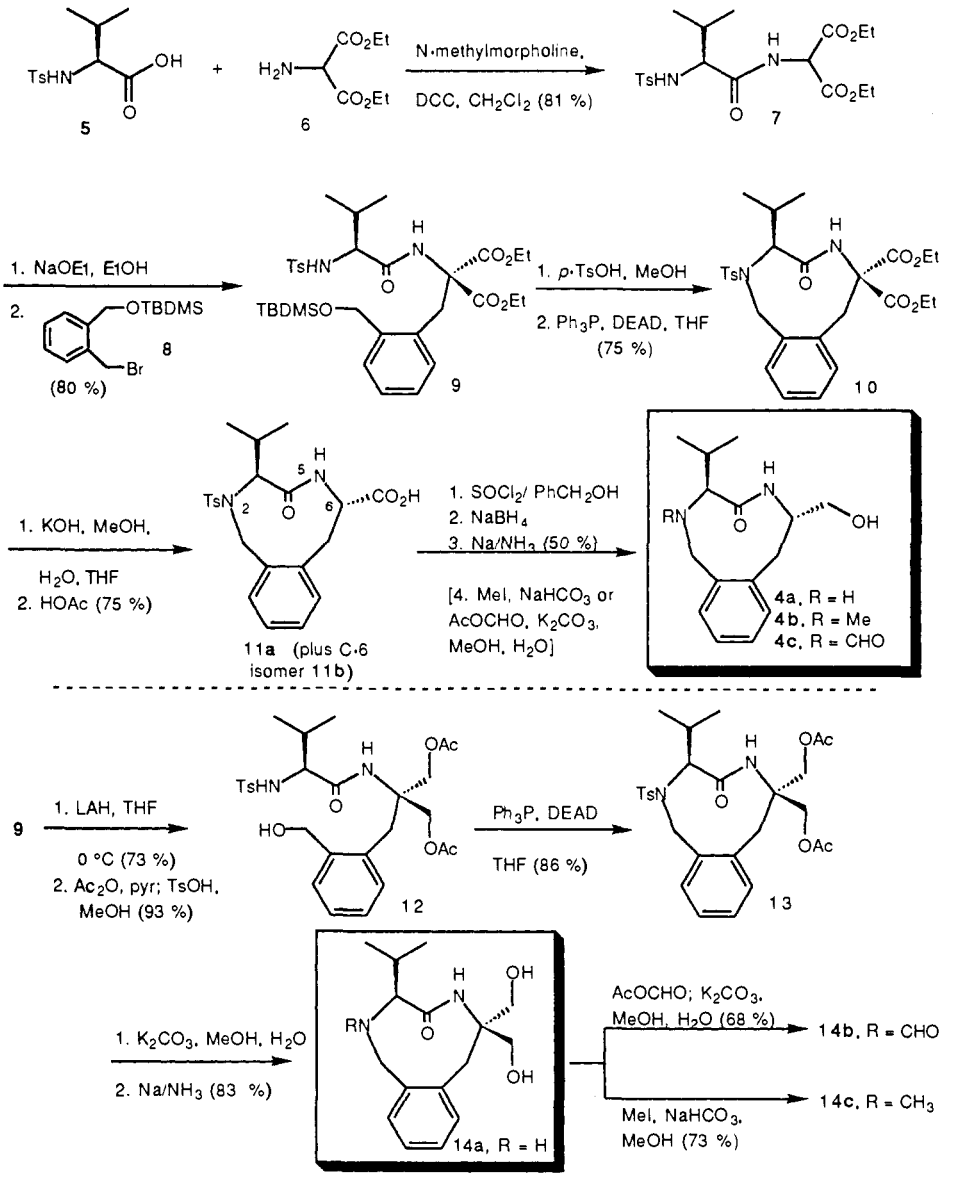
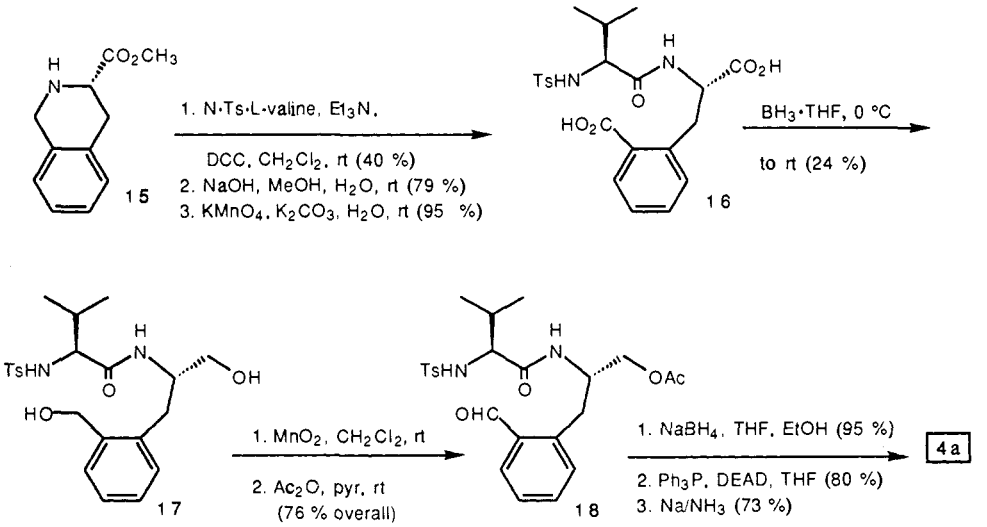
Scheme I. The Aminomalonate Route to Analogues **4a–c** and **14a–c**



Scheme II. The Phenylalanine Route to ILV Analogue **4a**



This connectivity, besides permitting the assignment of H-3, defines the position of the aromatic ring at 7.14 ppm, and H-7 at 3.97 ppm defines the configuration of the latter proton as β. The observed chemical shifts and coupling constants are listed in Table III.

From the contacts observed in the NOESY spectrum, interproton distances were divided into three categories: (a) strong NOESY cross-peaks, 2–3 Å; (b) weak NOESY cross-peaks, 2.5–3 Å; and (c) cross-peaks lost into noise (absent), 3–6 Å. This classification is based on the

Table I. PKC Agonist and Antagonist Activity of Compounds **4a**, **4b**, and **14a**

compd tested	PKC activity in the absence of TPA, pmol/min/mg protein[a,b]	PKC activity in the presence of TPA (1 μM), pmol/min/mg protein[c,d]
none	192 ± 17	859 ± 39
4a	213 ± 29	875 ± 14
4b	187 ± 10	850 ± 1
14a	243 ± 15	836 ± 29

[a] Protein kinase activity was determined in the presence of 25 μg/mL phosphatidylserine (PS) and 0.1 mM $CaCl_2$. [b] PKC activity in the presence of 1 μM TPA: 881 ± 82. [c] Protein kinase activity was determined in the presence of 25 μg/mL of PS and 0.5 mM $CaCl_2$. [d] Protein kinase activity in the absence of any PS and TPA: 104 ± 4.

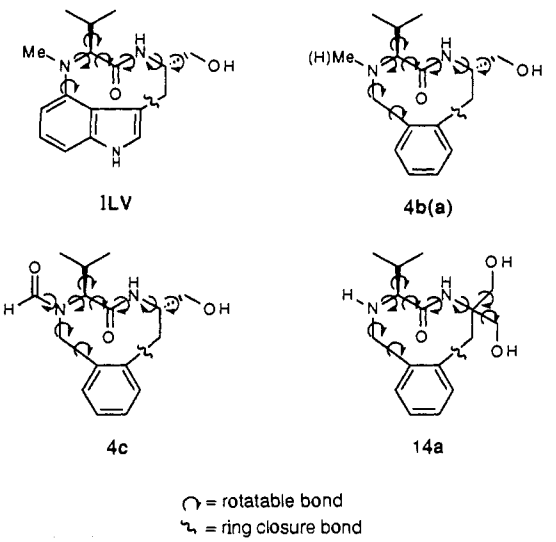


Figure 2. Definition of the rotatable bonds and ring closure bonds of ILV and the benzene mimics.

Table II. Conformational Analysis of ILV, **4a–c**, and **14a** Using SysSearch and MM3[a]

compound	$E_{sofa-like}$	$E_{twist-like}$	E_{gmec}	no. of confms
ILV	74.57	72.98	72.98	10
4a	37.58	45.80	34.05	40
4b	44.51	52.46	44.48	33
4c	40.02	48.90	40.02	50
14a	46.04	54.34	43.24	32

analysis of the buildup rates for geminal proton pairs (0.25 s^{-1}) for which the distance (1.75 Å) and the signal/noise ratio at a given mixing time are known. For proton pairs with distances longer than 3 Å, the cross-relaxation rate is 0.01 s^{-1} or less. With mixing times of the order of 1 s, such cross-relaxation rates give cross-peaks with intensities that are less than 1% of the corresponding diagonal, i.e., below the estimated noise level. The observed contacts are listed in Table IV.

The solution conformation of **4a** was determined by using the simulated annealing technique available in the DISCOVER (Biosym Technologies) program package. The annealing was performed with the protocol proposed for larger molecules with the observed NOE constraints and with the modified scaling of the force constants at high temperature. The results of the annealing study suggest that there is only one conformation of **4a** existing in solution, and this conformer is shown in Figure 4.

X-ray Study. To obtain further information in support of the results obtained from the molecular modeling studies, an X-ray crystal structure analysis was carried out on the only crystalline member of this series of compounds, namely, **14a**. The X-ray study was performed using Cu K_α radiation with a graphite crystal incident beam monochromator. The crystal was found to be orthorhombic, and the space group was determined to be $P2_12_12_1$ (a = 11.9658(9), b = 16.240(1), and c = 8.0479(8) Å; V = 1563.9 $Å^3$; Z = 4; d_c = 1.242 g cm^{-3}; μ = 6.58 cm^{-1}). As is apparent from the perspective view of **14a** shown in Figure 5, the solid-state conformation of this molecule possesses a trans amide bond with a C_3–C_4–C_5–C_6 torsional angle of 147.6°, a deviation of more than 30° from the ideal value of 180°. Deformation of the trans amide bond is an indicator of the geometric constraints imposed upon this group because of its incorporation into the nine-membered lactam ring structure.

Synthesis. Coupling of *N*-Tosyl-L-valine and Diethyl Aminomalonate Hydrochloride (7). To a suspension of *N*-Ts-L-Val[24] (3.00 g, 11 mmol) and diethyl aminomalonate hydrochloride (2.34 g, 11 mmol, Aldrich) in dried methylene chloride (75 mL) was added *N*-methylmorpholine (1.23 mL, 22 mmol) dropwise followed by dicyclohexylcarbodiimide (2.29 g, 11 mmol). The resulting mixture was stirred for 24 h at room temperature. At the end of the reaction, the suspension/solution was directly passed through a short column of silica gel using 300 mL of ethyl acetate as eluent. The eluate was concentrated to dryness, and the residual solid was recrystallized from the mixed solvent of ethyl acetate and *n*-hexane to afford 3.82 g (81%) of **7** as a white powder: IR (KBr) 3248, 2952, 1737, 1657, 1531 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 7.72 (d, J = 8.1 Hz, 2H), 7.24 (d, J = 8.1 Hz, 2H), 6.78 (d, J = 6.5 Hz, 1H), 5.21 (d, J = 8.4 Hz, 1H), 4.82 (d, J = 6.5 Hz, 1H), 4.22 (m, 4H), 3.61 (dd, J = 8.4, 5.1 Hz, 1H), 2.40 (s, 3H), 2.05 (m, 1H), 1.24 (t, J = 7.2 Hz, 6H), 0.89 (d, J = 6.9 Hz, 3H), 0.87 (d, J = 6.9 Hz, 3H); mass spectrum, m/z 428 (M^+).

1-(Bromomethyl)-2-[[[(1,1-dimethylethyl)dimethylsilyl]oxy]methyl]-benzene (8). A mixture of 1,2-benzenedimethanol (6.90 g, 50 mmol), *tert*-butyldimethylsilyl chloride (8.25 g, 55 mmol), and pyridine (7.91 g, 100 mmol) in 200 mL of methylene chloride was stirred for 8 h at room temperature. Workup by ether extraction followed by column chromatography of the residue using a stepwise gradient of ethyl acetate (0–67%) in hexane as eluent afforded 7.32 g of monoprotected alcohol (58% yield) together with 4.51 g of the diprotected product and 1.10 g of 1,2-benzenedimethanol. The monoprotected alcohol was converted to bromide **8** by the standard method involving mesylate formation and bromide displacement in 84% yield: ^1H NMR ($CDCl_3$, 300 MHz) δ 7.28–7.39 (m, 4H), 4.80 (s, 2H), 4.65 (s, 2H), 0.91 (s, 9H), 0.12 (s, 6H).

(*S*)-[[2-[[[(1,1-Dimethylethyl)dimethylsilyl]oxy]methyl]phenyl]methyl]-[[3-methyl-2-[[(4-methylphenyl)sulfonyl]amino]-1-oxobutyl]amino]propanedioic Acid Diethyl Ester (9). Sodium ethoxide (7.0 mmol, 0.9 M in EtOH) was added dropwise with cooling provided by an ice–salt bath to a suspension of the malonate ester **7** (3.0 g, 7.0 mmol) in 15 mL of absolute EtOH. After the addition was completed, stirring was continued for 2 min during which time the solution became clear. The bromide **8** (2.21 g, 7.0 mmol) was added by a syringe over a 5-min period. The resulting solution was stirred in the cold bath for 1 h, then stirred at room temperature for 1 h, and finally partitioned between 400 mL of ether and 200 mL of water. The organic layer was washed with 100 mL of saturated brine and dried over Na_2SO_4. Rotary evaporation and chromatography of the residue (silica gel, ethyl acetate–hexane, 1:3) afforded 3.8 g (82%) of **9**: $[\alpha]^{22}_D$ = +44.5° (c = 1.93, $CHCl_3$); IR (KBr) 3393 (br m), 3067 (m), 2958 (s), 1743 (s), 1678 (s), 1543 (m), 1465 (m), 1224 (s) cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 7.71 (d, J = 8.3 Hz, 2H), 7.40 (d, J = 7.5 Hz, 1H), 7.16–7.23 (m, 3H), 7.06 (m, 2H), 6.64 (d, J = 7.5 Hz, 1H), 5.32 (d, J = 8.1 Hz, 1H), 4.70 and 4.50 (AB q, J = 13.2 Hz, 2H), 3.98–4.25 (m, 4H), 3.72 and 3.27 (AB q, J = 15.3 Hz, 2H), 3.67 (dd, J = 8.1, 4.2 Hz), 2.20 (s, 3H), 2.03 (m, 1H), 1.20 (t, J = 7.2 Hz, 3H), 1.11 (t, J = 7.2 Hz, 3H), 0.93 (s, 9H), 0.90 (d, J = 6.6 Hz, 3H), 0.78 (d, J = 6.6 Hz, 3H), 0.10 (s, 3H), 0.08 (s, 3H); ^{13}C NMR ($CDCl_3$, 75.46 MHz) δ 169.3, 167.5, 167.2, 143.8, 140.1, 136.9, 131.9, 129.7, 129.5, 127.5, 127.3, 127.2, 128.9, 66.8, 63.0, 62.8, 62.6, 61.2, 33.1, 31.9, 26.0, 21.4, 19.1, 18.4, 16.7, 13.9, 13.7, 6.9; HRMS calcd for $C_{33}H_{51}N_2O_8SSi$ 663.313 ($M^+ + H^+$), found 663.315.

(*S*)-1,2,3,4,5,7-Hexahydro-3-(1-methylethyl)-2-[(4-methylphenyl)sulfonyl]-4-oxo-6*H*-2,5-benzodiazonine-6,6-dicarboxylic Acid Diethyl Ester (10). A solution of **9** (1.66 g, 2.45 mmol) and *p*-toluenesulfonic acid (50 mg, 0.28 mmol) in 25 mL of EtOH was stirred at room temperature for 3 h. The solvent was removed, and the residual oil was passed through a column of neutral aluminum oxide using 1:2 hexane–ethyl acetate as the eluent. The eluate was concentrated to dryness, and the residual solid was dissolved in 600 mL of THF. The resulting solution was cooled in an ice bath, and triphenylphosphine (760 mg, 2.91 mmol) and diethyl azodicarboxylate (507 mg, 2.91 mmol) were added sequentially. The reaction mixture was stirred overnight at room temperature and then evaporated under reduced pressure. The residual oil was chromatographed on silica gel with 1:5 ethyl acetate–hexane as eluent to provide 1.01 g (75%) of 10 that by NMR is comprised of an approximate 2.6:1 mixture of two conformers: $[\alpha]^{22}_D$ = −110.7° (c = 0.335, $CHCl_3$); IR (KBr) 3421 (m), 2980 (m), 1743 (s), 1689 (m), 1491 (m), 1151 (s) cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 7.90 (d, J = 7.2 Hz, 1H, major), 7.79 (d,

(24) Maurer, P. J.; Takahata, H.; Rapoport, H. *J. Am. Chem. Soc.* **1984**, *106*, 1095–1098.

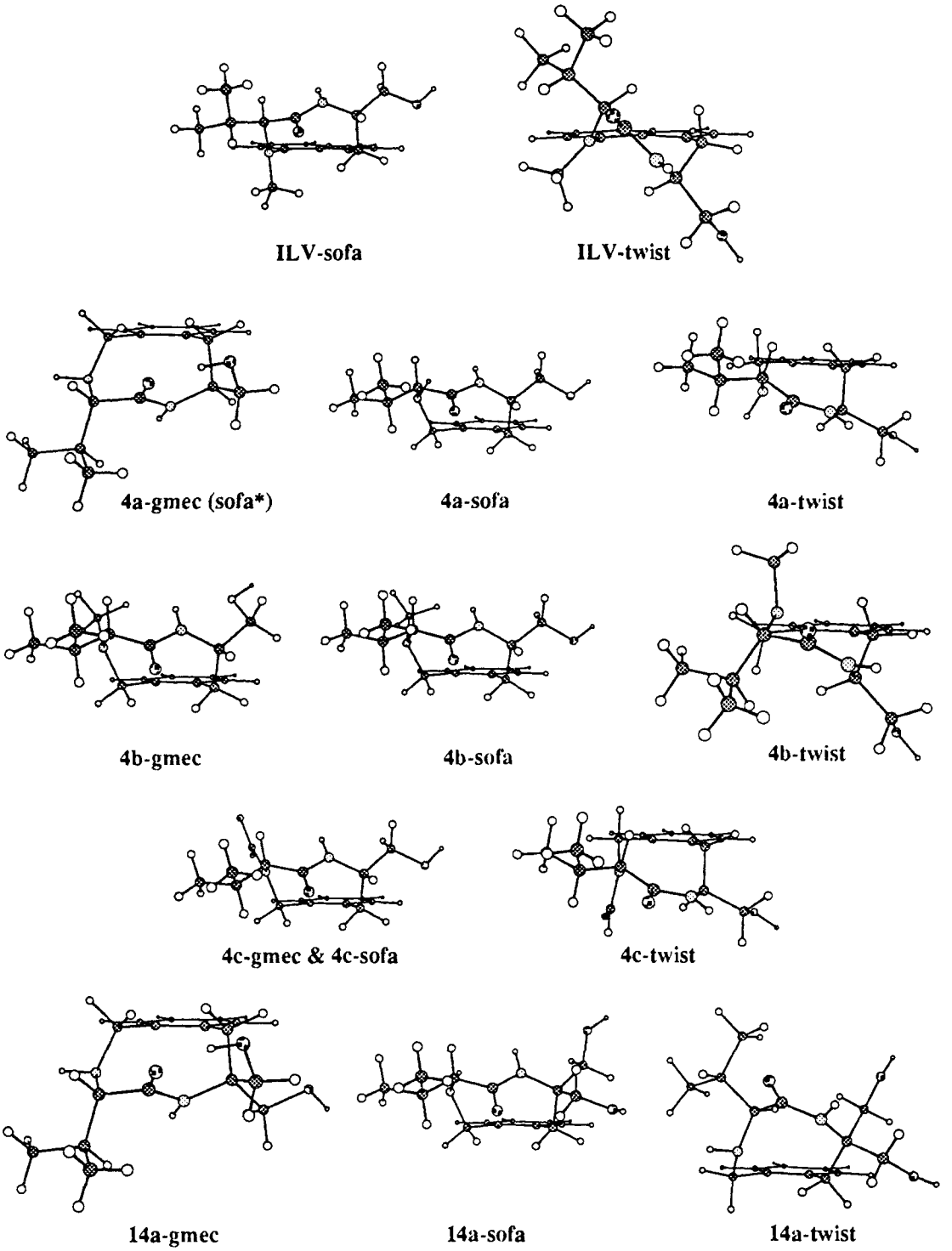


Figure 3. Ball and stick representations of ILV and the benzene analogues.

J = 8.1 Hz, 1H, major), 7.32 (d, J = 8.1 Hz, 2H, major), 7.33 (m, overlapping with previous signals, 2H, minor), 7.15–7.26 (m, 5H, minor), 7.17 (m, overlapping with previous signals, 1H, major), 7.05 (d, J = 8.3 Hz, 2H, major), 6.90 (d, J = 6.9 Hz, 1H, major), 6.78 (d, J = 7.6 Hz, 1H, minor), 6.19 (s, 1H, minor), 6.04 (s, 1H, major), 4.85 and 3.77 (AB q, J = 15.3 Hz, 2H, major), 4.48 and 3.52 (AB q, J = 14.7 Hz, 2H, minor), 4.04–4.36 (m, 5H, both), 3.80 and 3.14 (AB q, J = 14.3 Hz, 2H, minor), 3.57 and 3.25 (AB q, J = 14.3 Hz, 2H, major), 2.51 (m, 1H, major), 2.44 (s, 3H, minor), 2.31 (s, 3H, major), 1.63 (m, 1H, minor), 1.21–1.35 (m, 6H, both), 0.84–1.17 (m, 6H, both); HRMS calcd for $C_{27}H_{35}N_2O_7S$ 531.223 ($M^+ + H^+$), found 531.222.

[*S*-(*R,*R**)]-1,2,3,5,6,7-Hexahydro-3-(1-methylethyl)-2-[(4-methylphenyl)sulfonyl]-4-oxo-4*H*-2,5-benzodiazonine-6-carboxylic Acid (11a).** A solution of diester **10** (1.49 g, 2.81 mmol), KOH (0.62 g, 11.1 mmol), water (15 mL), EtOH (80 mL) and THF (40 mL) was stirred for 22 h at room temperature, and then 4 mL of HOAc was added. The acidified solution was stirred for 10 h at room temperature. Extraction with ether, concentration by rotary evaporation, and chromatography of the residual oil on silica gel with 1:3 hexane–ethyl acetate as eluent afforded 633 mg (52%) of trans isomer **11a** (less polar product) and 303 mg (25%) of cis isomer **11b** (more polar product).

11a: $[\alpha]^{22}_D$ = –77.7° (c = 0.70, $CHCl_3$); IR (KBr) 3500–2500 (br m), 3389 (s), 2766 (s), 1744 (s), 1691 (s), 1599 (m), 1494 (m), 1342 (s) cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 7.96 (m, 1H), 7.24–7.34 (m, 4H), 7.00–7.09 (m, 3H), 5.55 (m, 1H), 4.90 (m, 2H), 4.17 (d, J = 8.4 Hz, 1H), 3.74 (m, 1H), 3.31 and 2.84 (AB q, J = 14.1 Hz, both parts split into d with J = 5.9, 3.0 Hz, respectively, 2H), 2.50 (m, 1H), 2.31 (s, 3H), 1.19 (br s, 6H); HRMS calcd for $C_{22}H_{27}N_2O_5S$ 431.163 ($M^+ + H^+$), found 431.163.

11b: $[\alpha]^{22}_D$ = –116.0° (c = 0.72, $CHCl_3$); IR (KBr) 3500–2500 (br m), 3387 (s), 2988 (s), 1743 (s), 1688 (s), 1492 (m), 1342 (s), 1159 (s); HRMS calcd for $C_{22}H_{27}N_2O_5S$ 431.163 ($M^+ + H^+$), found 431.162.

Table III. Proton Chemical Shifts and Homonuclear Coupling Constants of **4a**

proton	chem shift, δ/ppm	multiplicity	coupling const,[a] J(Hz)
H-3	7.15	multiplet	
H-4, H-5	7.20–7.22	multiplet	
H-6	7.14	multiplet	
H-7α	3.19	AB doublet	14.8 (7β)
H-7β	3.97	AB doublet	14.8 (7α)
H-8α	3.18	doublet	7.4 (9)
H-9	1.64	octet	6.9 (8)
Me-10, Me-11	0.90, 0.91	doublet/doublet	6.6 (9)
H-13β	3.08	multiplet	
H-14α	3.99	doublet/doublet	12.4 (14β)
H-14β	3.89	doublet/doublet	12.4 (14α)
H-16α	3.21	doublet/doublet	13.7 (16β), 11.7 (13)
H-16β	2.54	doublet/doublet	13.7 (16α), 11.7 (13)
H(N)-1	2.21	very broad	
H(N)-2	6.99	broad doublet	3 (13)

[a] Numbers in parentheses denote a coupling partner for a given constant.

Table IV. Ranges of Interproton Distances from NOESY Experiment

interproton distance range (Å)	spin pairs					
2.0–3.0	1/7β	2/9	3/16β	6/7β	13β/16β	14β/16β
2.5–3.0	2/7α	2/8α	2/13β	3/13β	7α/16α	13β/16α
3.0–6.0	2/7β	2/14α	2/14β	2/16α	2/16β	3/14α
	3/14β	3/16α	7α/13β	8α/16α	8α/16β	

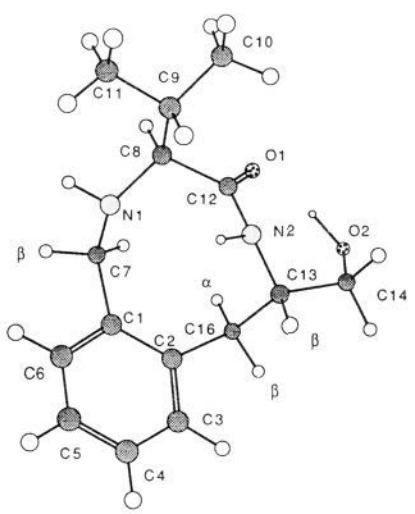


Figure 4. Structure of compound **4a** determined from the NOESY constraints and simulated annealing.

[*S*-(*R,*R**)]-1,2,3,5,6,7-Hexahydro-6-(hydroxymethyl)-3-(1-methylethyl)-4*H*-2,5-benzodiazonin-4-one (4a).** To a magnetically stirred solution of **11a** (630 mg, 1.46 mmol) and benzyl alcohol (2.54 g, 23.5 mmol) in 20 mL of dried benzene at room temperature was added dropwise by syringe thionyl chloride (52 μL, 0.71 mmol). The resulting solution was heated at reflux with continuous removal of water (Dean–Stark trap). After 18 h, the benzene was removed by rotary evaporation, and the excess benzyl alcohol was evaporated at 80 °C/0.4 Torr to afford a red oil. The oil was passed through a short column of silica gel using 10:1 hexane–ethyl acetate as eluent to yield 604 mg of the crude benzyl ester.

The ester (532.3 mg, 1.02 mmol) was dissolved in 10 mL of THF, and the resulting solution was cooled in an ice–salt bath. To this solution was added dropwise by syringe $LiBH_4$ (1.16 mmol, 2M in THF). After the addition was completed, stirring was continued for an additional 5 h at room temperature. Water (10 mL) was added to quench the reaction, and an extractive workup with ether followed by rotary evaporation and chromatography of the residue on silica gel with 1:1 hexane–ethyl acetate as eluent afforded 358.5 mg of the desired alcohol.

Following the same procedure as described below for the reductive detosylation of **13**, detosylation of 285.1 mg of the above alcohol afforded 132.8 mg (50% from **11**) of **4a**: $[\alpha]^{22}_D$ = +6.3° (c = 0.46, MeOH); IR (KBr) 3421 (br m), 3298 (s), 2953 (s), 1662 (s), 1543 (m), 1476 (m), 1246 (m) cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.18–7.34 (m, 4H), 7.13 (br s, 1H), 5.40 (m, 1H), 3.84–4.10 (m, 3H), 3.20–3.26 (m, 3H), 3.17 (m, 1H), 2.60 (m, 1H), 1.67 (m, 1H), 0.98 (d, J = 6.3 Hz, 3H), 0.97 (d, J = 6.3 Hz, 3H); ^{13}C NMR ($CDCl_3$, 75.46 MHz), δ 180.3, 141.3, 137.9, 131.7, 131.2, 128.1, 127.5, 72.9, 63.9, 59.0, 48.7, 36.0, 31.3, 19.6, 19.3; mass spectrum, m/z 262 (M^+), 231, 176, 162, 132, 104, 78, 72; HRMS calcd for $C_{15}H_{23}N_2O_2$ 263.175 ($M^+ + H^+$), found 263.175.

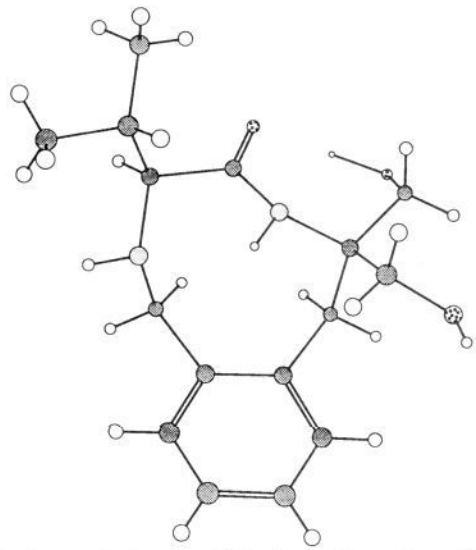


Figure 5. Perspective drawing of **14a** obtained from X-ray diffraction studies.

[*S*-(*R,*R**)]-1,2,3,5,6,7-Hexahydro-6-(hydroxymethyl)-2-methyl-3-(1-methylethyl)-4*H*-2,5-benzodiazonin-4-one (4b).** The compound **4b** was prepared by the same procedure as employed for the preparation of **14c**: 63% yield; $[\alpha]^{22}_D$ = 4.3° (c = 0.44, MeOH); IR (KBr) 3418 (br s), 2931 (s), 1672 (s), 1541 (m), 1458 (s); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.22–7.35 (m, 4H), 7.05 (br s, 1H), 5.55 (m, 1H), 4.06 and 3.43 (AB q, J = 13.5 Hz, 2H), 3.96 (m, 1H), 3.26 (m, 1H), 3.20 and 2.59 (AB q, J = 12.4 Hz, 2H), 3.18 (m, 1H), 3.06 (d, J = 4.5 Hz, 1H), 2.55 (s, 3H), 2.06 (m, 1H), 1.02 (d, J = 6.9 Hz, 3H), 0.83 (d, J = 6.9 Hz, 3H); ^{13}C NMR ($CDCl_3$, 75.46 MHz) δ 178.5, 139.2, 138.4, 132.1, 130.5, 127.3, 126.5, 63.9, 59.5, 58.7, 41.7, 34.7, 27.1, 20.1, 15.9; mass spectrum, m/z 276 (M^+), 245, 233, 190, 146, 132, 109, 86; HRMS calcd for $C_{16}H_{25}N_2O_2$ ($M^+ + H^+$) 277.192, found 277.192.

[*S*-(*R,*R**)]-2-Formyl-1,2,3,5,6,7-hexahydro-6-(hydroxymethyl)-3-(1-methylethyl)-4*H*-2,5-benzodiazonin-4-one (4c).** The compound **4c** was prepared by the same procedure as employed for the preparation of **14b**: 71% yield; $[\alpha]^{22}_D$ = 28.3° (c = 0.43, MeOH); IR (KBr) 3431 (br s), 2928 (s), 1678 (br s), 1548 (m), 1283 (m) cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.47 and 8.11 (each s, 1 H, cis and trans isomers), 7.11–7.32 (m, 4 H), 6.99 and 5.82 (each d, J = 10.2 and 9.7 Hz, 1 H, cis and trans isomers), 4.79 (br d, 1H), 4.67 and 3.52 (AB q, J = 8.7 Hz, 2H), 4.44 and 3.95 (AB q, J = 15 Hz, 2H), 4.31 (m, 1H), 3.80 (d, J = 7.8 Hz, 1H), 3.59–3.65 (m, 2H), 3.47 (m, 1H), 3.26 (m, 1H), 2.72 (m, 1H), 2.27 (m, 1H), 1.25–0.89 (m, 6H); ^{13}C NMR ($CDCl_3$, 75.46 MHz, two isomers) δ 173.6, 173.2, 164.8, 163.4, 136.8, 136.1, 133.7, 133.1, 132.2, 128.9, 128.5, 127.5, 127.0, 66.7, 62.4, 62.1, 60.8, 51.3, 47.3, 45.5, 34.4, 33.7, 29.1, 27.9, 20.9, 20.4, 19.7, 19.1.

(*S*)-2-[[2-(Hydroxymethyl)phenyl]methyl]-2-[[3-methyl-2-[[(4-methylphenyl)sulfonyl]amino]-1-oxobutyl]amino]propane-1,3-diol. The diester **9** (1.87 g, 2.84 mmol) was dissolved in 10 mL of THF, and the resulting solution was cooled in an ice–salt bath. To this magnetically stirred solution, lithium aluminum hydride (6.0 mmol, 1M in THF) was added slowly by syringe. Stirring was continued for 2 h at this temperature, and the reaction mixture was quenched by introducing 10 mL of H_2O at –78 °C. Extractive workup with ether gave a crude oil, which was chromatographed on silica gel using 2:3 ethyl acetate–hexane as eluent to afford 1.13 g (72%) of the desired diol as an amorphous powder: $[\alpha]^{22}_D$ = –12.8° (c = 0.46, $CHCl_3$); IR (KBr) 3365 (br s), 2958 (s), 1655 (s), 1543 (s), 1466 (m) cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.73 (d, J = 8.0 Hz, 1H), 4.87–4.98 (m, 2H), 4.73 and 4.66 (AB q, J = 11.8 Hz, 2H), 3.49 (dd, J = 8.4, 5.1 Hz, 1H), 3.27–3.41 (m, 2H), 3.03–3.11 (m, 2H), 3.02 and 2.90 (AB q, J = 13.8 Hz, 2H), 2.36 (s, 2H), 1.98 (m, 1H), 0.94 (s, 9H), 0.89 (d, J = 6.9 Hz, 3H), 0.85 (d, J = 6.9 Hz, 2H), 0.18

(s, 3H), 0.15 (s, 3H); ^{13}C NMR ($CDCl_3$, 75.46 MHz) δ 171.4, 143.9, 138.2, 136.5, 135.2, 131.8, 130.8, 129.7, 128.7, 127.6, 127.2, 64.4, 63.6, 63.4, 63.3, 62.4, 32.2, 31.8, 26.1, 21.6, 19.3, 18.4, 17.3, 6.6, 6.4; HRMS calcd for $C_{29}H_{47}N_2O_6SSi$ 579.292 ($M^+ + H^+$), found 579.290.

(*S*)-*O,O*-Diacetyl-2-[[2-(hydroxymethyl)phenyl]methyl]-2-[[3-methyl-2-[[(4-methylphenyl)sulfonyl]amino]-1-oxobutyl]amino]propane-1,3-diol (12). A mixture of the above diol (470 mg, 0.81 mmol), acetic anhydride (410 mg, 4.0 mmol), and pyridine (474 mg, 6.0 mmol) in THF (10 mL) was stirred overnight at room temperature. Extractive workup with ether afforded a crude oil, which was dissolved in 10 mL of MeOH. *p*-Toluenesulfonic acid (25 mg) was added, and the resulting solution was stirred at room temperature until TLC revealed the absence of starting material. The solvent was evaporated at reduced pressure, and the residual oil was chromatographed on silica gel with 1:1 ethyl acetate–hexane as eluent to afford 408 mg (91%) of **12** as an amorphous white powder: mp 137–138 °C (recrystallized from 1:2 ethyl acetate–hexane); $[\alpha]^{22}_D$ = −3.2° (*c* = 0.47, CH_2Cl_2); IR (KBr) 3447 (m), 3265 (m), 3198 (m), 3070 (m), 2968 (m), 1743 (s), 1676 (s), 1572 (s), 1230 (s) cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.90 (s, 1H), 7.45 (d, *J* = 8.1 Hz, 2H), 7.27–7.36 (m, 3H), 7.04 (d, *J* = 7.2 Hz, 1H), 6.67 (d, *J* = 8.1 Hz, 2H), 5.28 (d, *J* = 9.3 Hz, 1H), 4.69 (br s, 2H), 4.50 and 4.33 (AB q, *J* = 11.7 Hz, 2H), 4.32 and 3.39 (AB q, *J* = 11.7 Hz, 2H), 3.78 (br s, 1H), 3.36 (dd, *J* = 9.3, 6.0 Hz, 1H), 3.07 and 2.92 (AB q, *J* = 14.5 Hz, 2H), 2.29 (s, 3H), 2.13 (s, 3H), 2.07 (s, 3H), 1.88 (m, 1H), 0.91 (d, *J* = 6.7 Hz, 3H), 0.83 (d, *J* = 6.7 Hz, 3H); ^{13}C NMR ($CDCl_3$, 75.46 MHz) δ 170.8, 170.6, 170.0, 144.0, 139.1, 136.3, 134.1, 132.1, 131.2, 129.7, 128.8, 127.7, 127.1, 64.5, 63.2, 62.9, 61.9, 57.5, 34.2, 31.8, 21.5, 21.0, 20.9, 19.2, 17.4; HRMS calcd for $C_{27}H_{37}N_2O_8S$ 549.227 ($M^+ + H^+$), found 549.229.

(*S*)-1,2,3,5,6,7-Hexahydro-6,6-bis[(acetyloxy)methyl]-2-[(4-methylphenyl)sulfonyl]-3-(1-methylethyl)-4*H*-2,5-benzodiazonin-4-one (13). To an ice-cooled solution of **12** (2.85 g, 5.40 mmol) and triphenylphosphine (1.70 g, 6.48 mmol) in 1200 mL of THF was added dropwise by syringe diethyl azodicarboxylate (1.13 g, 6.48 mmol). The resulting solution was stirred for 16 h at room temperature, and the solvent was removed by rotary evaporation. The residual oil was purified by chromatography on silica gel using 1:5 ethyl acetate–hexane as eluent to afford 2.29 g (80%) of **13** as an amorphous white power: mp 129–130 °C (recrystallized from 1:3 ethyl acetate–hexane); $[\alpha]^{22}_D$ = −126.4° (*c* = 1.03, $CHCl_3$); IR (KBr) 3394 (s), 2968 (s), 1742 (s), 1703 (s), 1512 (m), 1248 (s) cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.81–7.93 (m, 1H), 7.38 (m, 1H), 7.31 (m, 1H), 7.17 (d, *J* = 8.4 Hz, 2H), 6.93–7.00 (m, 3H), 4.73 and 3.85 (AB q, *J* = 15.0 Hz, 2H), 4.66 and 4.62 (AB q, *J* = 11.3 Hz, 2H), 4.65 (s, 1H), 4.03 (d, *J* = 9.3 Hz, 1H), 3.33 and 2.52 (AB q, *J* = 14.1 Hz, 2H), 3.25 and 2.43 (AB q, *J* = 11.4 Hz, 2H), 2.42 (m, 1H), 2.27 (s, 3H), 2.04 (s, 6H), 1.18 (d, *J* = 6.9 Hz, 3H), 1.12 (d, *J* = 6.9 Hz, 1H); ^{13}C NMR ($CDCl_3$, 75.46 MHz) δ 174.8, 170.2, 169.8, 144.0, 139.1, 137.4, 135.5, 134.4, 133.7, 129.8, 128.9, 127.4, 126.4, 68.0, 64.2, 63.6, 58.1, 48.5, 35.6, 27.8, 21.3, 20.9, 20.7, 20.6, 19.9; HRMS calcd for $C_{27}H_{35}N_2O_7S$ 531.216 ($M^+ + H^+$), found 531.215.

(*S*)-1,2,3,5,6,7-Hexahydro-6,6-bis(hydroxymethyl)-3-(1-methylethyl)-4*H*-2,5-benzodiazonin-4-one (14a). A mixture of **13** (2.20 g, 4.15 mmol), potassium carbonate (1.20 g, 8.70 mmol), MeOH (30 mL), and water (15 mL) was stirred for 1.5 h at 0 °C and then partitioned between 400 mL of ether and 100 mL of water. The organic layer was washed with saturated brine and dried over Na_2SO_4. The solution was evaporated at reduced pressure to dryness. The residual white solid was dissolved in 20 mL of THF, and this solution was added to 100 mL of liquid ammonia at −78 °C. To this magnetically stirred solution was added Na metal (1.0 g, 43.5 mmol) in small pieces. The resulting deep blue mixture was stirred at −78 °C for 1 h. NH_4Cl (2.55 g, 48 mmol) was added to cause the blue color of the reaction mixture to turn to pale yellow. The ammonia was then allowed to evaporate, and the remaining solid was dissolved in 300 mL of ether and 150 mL of 1% aqueous sodium carbonate. The organic layer was separated, and the aqueous layer was extracted with ether (2 × 300 mL). The combined ether extracts were washed with saturated brine and dried over Na_2SO_4. The solution was concentrated to ca. 60 mL and allowed to stand for 10 min. The resulting precipitate was collected by suction filtration and washed with ether to afford 1.02 g (83%) of **14a**: mp 191–192 °C (recrystallization from 1:1 hexane–THF); $[\alpha]^{22}_D$ = −32.2° (*c* = 0.61, MeOH); IR (KBr) 3423 (s), 3342 (s), 3232 (m), 2966 (m), 1649 (s), 1532 (s), 1466 (m), 1061 (s) cm^{-1}; ^{1}H NMR ($CDCl_3$–D_2O, 300 MHz) δ 7.23–7.29 (m, 4H), 4.06 and 3.28 (AB q, *J* = 14.7 Hz, 2H), 3.97 and 3.93 (AB q, *J* = 12.3 Hz, 2H), 3.55 and 3.26 (AB q, *J* = 11.3 Hz, 2H), 3.22 (m, 1H), 3.19 and 2.63 (AB q, *J* = 13.9 Hz, 2H), 1.77 (m, 1H), 0.98 (d, *J* = 6.6 Hz, 3H), 0.96 (d, *J* = 6.6 Hz, 3H); ^{13}C NMR ($CDCl_3$, 75.46 MHz) δ 179.1, 141.4, 134.3, 132.7, 131.7, 127.9, 127.8, 72.1, 65.4, 64.8, 61.2, 49.0, 38.2, 31.0, 19.3; HRMS calcd for $C_{16}H_{25}N_2O_3$ 293.186 ($M^+ + H^+$), found 293.186.

(*S*)-2-Formyl-1,2,3,5,6,7-hexahydro-6,6-bis(hydroxymethyl)-3-(1-methylethyl)-4*H*-2,5-benzodiazonin-4-one (14b). A mixture of amine **14a** (15.1 mg, 0.052 mmol) and 1 mL of acetic formic anhydride was stirred for 20 min at room temperature, then diluted with 50 mL of ether, and washed with 10% aqueous $NaHCO_3$. The organic layer was concentrated to dryness, and the residue was mixed with 20 mg of K_2CO_3, 1 mL of water, and 2 mL of MeOH. The resulting solution was stirred for 3 h at room temperature, and then 50 mL of ether and 20 mL of water were added. The organic layer was separated and washed with saturated brine, dried over Na_2SO_4, and concentrated to afford an oil that was chromatographed to yield 11.2 mg (68%) of **14b**: $[\alpha]^{22}_D$ = −2.3° (*c* = 0.24, MeOH); IR (KBr) 3379 (br s), 3067 (m), 2962 (s), 1657 (s), 1560 (m), 1466 (m), 1398 (m) cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz, 1:1 mixture of two conformers) δ 8.62, and 8.18 (s and s, 1H), 7.22–7.35 (m, 4H), 6.50 and 6.12 (each s, 1H), 4.77–4.83 (m, 2H), 3.98–4.49 (m, 1H), 3.79–3.87 (m, 3H), 3.30–3.63 (m, 4H), 2.55 (m, 1H), 2.32–2.50 (m, 1H), 1.11 (d, *J* = 6.4 Hz, 6H of one conformer), 0.96 and 0.94 (each d, *J* = 6.3 Hz, 3H of one conformer); HRMS calcd for $C_{17}H_{25}N_2O_4$ 321.181 ($M^+ + H^+$), found 321.181.

(*S*)-1,2,3,5,6,7-Hexahydro-6,6-bis(hydroxymethyl)-2-methyl-3-(1-methylethyl)-4*H*-2,5-benzodiazonin-4-one (14c). A mixture of **14a** (9.6 mg, 0.033 mmol), iodomethane (45.6 mg, 0.32 mmol), and potassium carbonate (50 mg, 0.36 mmol) in MeOH (1 mL) was heated at reflux for 18 h. The cooled solution was partitioned between 30 mL of ether and 10 mL of water. The organic layer was washed with saturated brine and dried over Na_2SO_4. The solvent was evaporated at reduced pressure, and the residual oil was chromatographed on silica gel using 1:1 hexane–THF as eluent to afford 7.3 mg (73%) of **14c**: $[\alpha]^{22}_D$ = −35.9° (*c* = 0.44, MeOH); IR (KBr) 3423 (br s), 2926 (s), 1670 (s), 1543 (m), 1460 (s) cm^{-1}; ^{1}H NMR ($CDCl_3$–D_2O, 300 MHz) δ 7.28–7.36 (m, 4H), 3.88 (br s, 2H), 2.55 and 3.26 (AB q, *J* = 11.3 Hz, 2H), 2.47 and 3.31 (AB q, *J* = 14.9 Hz, 2H), 3.30 and 2.57 (AB q, *J* = 11.6 Hz, 2H), 3.06 (d, *J* = 4.5 Hz, 1H), 2.54 (s, 3H), 2.07 (m, 1H), 1.03 (d, *J* = 6.9 Hz, 3H), 0.84 (d, *J* = 6.9 Hz, 3H); ^{13}C NMR ($CDCl_3$, 75.46 MHz) δ 177.9, 139.9, 135.5, 132.6, 131.4, 128.2, 127.4, 66.0, 65.8, 61.0, 59.8, 40.0, 38.0, 27.1, 20.1, 15.9; HRMS calcd for $C_{17}H_{27}N_2O_3$ ($M^+ + H^+$) 307.202, found 307.203.

Synthesis of (*S*)-1,2,3,4-Tetrahydro-3-isoquinolinecarboxylic Acid Methyl Ester (15). (a) To a suspension of (*S*)-phenylalanine (100 g, 0.605 mol) and formalin (37%, 230 mL) was added concentrated HCl (770 mL). The reaction mixture was heated at 90–95 °C for 1 h. More formalin (100 mL) and concentrated HCl (200 mL) were added, and the reaction mixture was kept at this temperature for another 3 h. The reaction mixture was chilled in an ice bath, and the solid precipitate was collected by suction filtration. The crude product was dissolved in hot water, and hot methanol was added to the resulting solution. While the solution was still hot, concentrated ammonium hydroxide was added until Congo paper remained red. The resulting precipitate was collected by suction filtration and dried in vacuo at room temperature for 3 d to afford 76.8 g (72%) of the isoquinolinecarboxylic acid: ^{1}H NMR (D_2O + KOH) δ 2.67 (dd, *J* = 10.7, 16.6 Hz, 1H), 2.89 (dd, *J* = 4.6, 16.6 Hz, 1H), 3.28 (dd, *J* = 4.6, 10.6 Hz, 1H), 3.81 (AB q, *J* = 16.0 Hz, 2H), 6.85–7.15 (m, 4H).

(b) To a suspension of the above acid (76.8 g, 0.43 mol) in anhydrous methanol (600 mL) at 0 °C under an argon atmosphere was added thionyl chloride (47.4 mL, 0.65 mol) dropwise. After the addition was completed, the reaction mixture was refluxed for 4.5 h. After cooling, the solvent was removed by rotary evaporation, and the solid residue was dissolved in chloroform. The resulting solution was washed with saturated aqueous sodium bicarbonate, and the aqueous layer was twice re-extracted with chloroform. The combined organic layers were washed with brine, dried over $MgSO_4$, and concentrated in vacuo. The resulting oil was dissolved in diethyl ether, and HCl gas was bubbled into the solution. The resulting white solid was collected by suction filtration and dried in vacuo overnight to afford 74.5 g (76%) of the title ester: ^{1}H NMR (D_2O) δ 3.13 (dd, *J* = 10.9, 17.3 Hz, 1H), 3.35 (dd, *J* = 5.5, 17.4 Hz, 1H), 3.75 (s, 3H), 4.38 (AB q, *J* = 15.3 Hz, 2H), 7.08–7.27 (m, 4H); ^{13}C NMR (D_2O, 75.46 MHz) δ 33.0, 49.5, 59.1, 59.5, 131.9, 132.2, 132.8, 133.6, 134.2, 135.0, 175.0.

[*S*-(*R,*R**)]-2-Carboxy-α-[[3-methyl-2-[[(4-methylphenyl)sulfonyl]amino]-1-oxobutyl]amino]benzenepropanoic Acid (16).** (a) To a solution of **15** (8.50 g, 37 mmol), *N*-tosyl-L-valine (10.13 g, 37.3 mol), and triethylamine (5.2 mL, 37.3 mmol) in methylene chloride (200 mL) kept at 0 °C under an argon atmosphere was added DCC (8.47 g, 41 mmol). The reaction mixture was stirred at room temperature for 24 h. The urea was filtered off, the filtrate was diluted with methylene chloride, and the

resulting solution was washed sequentially with saturated sodium bicarbonate, water, 1 M HCl, water, and brine. The extract was dried over $MgSO_4$ and concentrated in vacuo. The oily residue was purified on silica gel with hexane–ethyl acetate (8:2 to 4:6) as eluent to give 6.55 g (40%) of the amide as a gum: IR (film) 3234, 2962, 1741, 1637, 1408, 1165 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 1.00 (d, J = 6.7 Hz, 3H), 1.14 (d, J = 6.8 Hz, 3H), 1.96–2.11 (m, 1H), 2.15 (s, 3H), 2.57 (dd, J = 6.3, 15.8 Hz, 1H), 3.02 (dd, J = 4.9, 15.8 Hz, 1H), 3.59 (s, 3H), 4.04 (dd, J = 5.7, 9.9 Hz, 1H), 4.44 (AB q, J = 15.4 Hz, 1H), 4.81 (dd, J = 5.0, 6.2 Hz, 1H), 5.55 (d, J = 9.9 Hz, 1H), 6.79 (d, J = 7.9 Hz, 2H), 7.12–7.18 (m, 2H), 7.25–7.35 (m, 2H), 7.50 (d, J = 8.23 Hz, 2H); ^{13}C NMR ($CDCl_3$, 75.46 MHz) δ 17.3, 19.1, 21.2, 29.9, 31.7, 45.2, 51.9, 52.3, 58.1, 125.9, 127.0, 127.2, 127.6, 128.1, 129.2, 131.3, 132.1, 138.3, 143.1, 170.5, 170.9; HRMS calcd for $C_{23}H_{28}N_2O_5S$ 444.174, found 444.174.

(b) To a solution of the above amide (6.55 g, 14.7 mmol) in MeOH/water (100 mL, 4:1) was added NaOH (0.95 M, 23 mL, 21.9 mmol). The reaction mixture was stirred at room temperature for 24 h. MeOH was removed by rotary evaporation, and the remaining solution was diluted with water and washed with ethyl acetate. The aqueous layer was adjusted to a pH of 1 with 3 M HCl and extracted with ethyl acetate. The organic layer was washed with brine, dried over $MgSO_4$, and concentrated in vacuo. The crude product was recrystallized from acetone/water to yield 4.99 g (79%) of the corresponding carboxylic acid as a white powder: ^1H NMR (CD_3OD, 300 MHz) δ 0.94 (d, J = 6.7 Hz, 3H), 0.99 (d, J = 6.8 Hz, 3H), 1.83–2.01 (m, 1H), 2.11 (s, 3H), 2.46 (dd, J = 6.3, 15.73 Hz, 1H), 2.93 (dd, J = 4.7, 15.7 Hz, 1H), 4.03 (d, J = 7.1 Hz, 1H), 4.47 (AB q, J = 15.6 Hz, 2H), 4.56 (dd, J = 4.6, 6.2 Hz, 1H), 6.79 (d, J = 8.03 Hz, 2H), 7.05–7.29 (m, 4H), 7.40 (d, J = 8.2 Hz, 2H); ^{13}C NMR (CD_3OD, 75.46 MHz) δ 18.5, 19.4, 21.4, 31.1, 33.0, 46.5, 53.3, 59.3, 127.3, 128.1, 128.4, 129.2, 129.2, 130.4, 133.5, 133.9, 139.1, 144.5, 172.5, 173.9.

(c) To a solution of the acid (4.99 g, 11.6 mmol) and $K_2CO_3 \cdot 1.5H_2O$ (1.92 g, 11.6 mmol) in water (100 mL) was added $KMnO_4$ (2.75 g, 17.4 mol) over a 1-h period. After the addition was completed, the reaction mixture was stirred at room temperature for an additional 1.5 h. The excess $KMnO_4$ was quenched by the addition of sodium bisulfite. The solid residue was filtered, and the filtrate was adjusted to a pH of 1 with 3 M HCl. The acidified solution was extracted twice with ethyl acetate. The combined extracts were washed with brine, dried over $MgSO_4$, and concentrated in vacuo to yield 5.09 g (95% crude yield) of the acid **16**, which was used in the next step without any further purification: ^1H NMR (D_2O + NaOD, 300 MHz) δ 0.09 (d, J = 6.74 Hz, 3H), 0.26 (d, J = 6.7 Hz, 3H), 1.15–1.31 (m, 1H), 2.00 (s, 3H), 2.65 (d, J = 6.9 Hz, 1H), 2.71 (dd, J = 10.2, 13.6 Hz, 1H), 2.86 (dd, J = 5.0, 13.9 Hz, 1H), 3.81 (dd, J = 5.0, 10.1 Hz, 1H), 6.86–7.25 (m, 8H); ^{13}C NMR (D_2O + NaOD, 75.46 MHz) δ 19.5, 19.6, 21.8, 33.7, 36.8, 57.9, 67.0, 127.5, 127.8, 128.51, 130.2, 130.4, 131.4, 135.9, 141.0, 142.0, 142.8, 178.6, 179.2, 179.8.

[*S*-(*R,*R**)]-2-(Hydroxymethyl)-β-[[3-methyl-2-[[(4-methylphenyl)sulfonyl]amino]-1-oxobutyl]amino]benzenepropanol (17).** To a solution of the acid **16** (5.09 g, 11 mmol) in THF (100 mL) at 0 °C under an argon atmosphere was added BH_3·THF (1.0 M, 33 mL, 33 mmol). The reaction mixture was gradually warmed to room temperature and stirred overnight. Methanol was added to quench the reaction, and the solvent was removed by rotary evaporation. The residue was dissolved in ethyl acetate, and the resulting solution was washed with saturated sodium bicarbonate. The organic layer was separated, washed with brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified on silica gel with hexane–ethyl acetate (1:1 to 3:7) as eluent to give 1.16 g (24%) of alcohol **17** as a colorless glassy solid: IR (film) 3290, 2965, 2876, 1653, 1541, 1456, 1325, 1161, 667 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 0.42 (d, J = 6.9 Hz, 3H), 0.62 (d, J = 6.8 Hz, 3H), 1.85–2.08 (m, 1H), 2.38 (s, 3H), 2.83 (dd, J = 7.1, 13.7 Hz, 1H), 2.92 (dd, J = 9.2, 13.7 Hz, 1H), 3.41 (dd, J = 4.7, 8.2 Hz, 1H), 3.53 (dd, J = 3.1, 11.5 Hz, 1H), 3.72 (dd, J = 3.3, 11.6 Hz, 1H), 4.06–4.19 (m, 1H), 4.65 (d, J = 11.1 Hz, 1H), 4.81 (d, J = 11.1 Hz, 1H), 5.44 (d, J = 8.3 Hz, 1H), 7.13–7.23 (m, 1H), 7.24–7.31 (m, 5H), 7.65–7.76 (m, 3H); ^{13}C NMR ($CDCl_3$, 75.46 MHz) δ 16.3, 19.0, 21.5, 30.6, 32.2, 53.4, 62.4, 63.6, 63.8, 126.7, 127.4, 128.9, 129.7, 130.1, 130.6, 135.9, 137.7, 143.9, 171.1; HRMS calcd for $C_{22}H_{31}N_2O_5S$ 435.195 ($M^+ + H^+$), found 435.194.

[*S*-(*R,*R**)]-*O*-Acetyl-2-formyl-β-[[3-methyl-2-[[(4-methylphenyl)sulfonyl]amino]-1-oxobutyl]amino]benzenepropanol (18).** A suspension of diol **17** (1.10 g, 2.53 mmol) and MnO_2 (1.10 g, 12.6 mmol) in 100 mL of dried methylene chloride was stirred for 12 h at room temperature. Additional MnO_2 (1.10 g, 12.6 mmol) was added, and stirring was continued for an additional 24 h. The reaction mixture was filtered, and the filter cake was washed with ethyl acetate (200 mL). The filtrate was concentrated to dryness, and the residual white solid was dissolved in 50 mL of THF. To this solution cooled in ice water were added pyridine (825 mg, 10.4 mmol) and acetic anhydride (639 mg, 6.26 mmol) sequentially. The resulting solution was stirred for 24 h at room temperature, and the reaction mixture was partitioned between 300 mL of ether and 100 mL of water. The organic layer was separated, washed with 100 mL of 5% HCl and 100 mL of saturated brine, and dried over Na_2SO_4. The solvent was removed by rotary evaporation, and the residual oil was chromatographed to afford 914 mg (76%) of **18** as a white solid: mp 167–168 °C (recrystallized from 1:1 ethyl acetate–hexane); $[\alpha]^{22}_D$ = –26.5° (c = 0.31, $CHCl_3$); IR (KBr) 3308 (s), 3264 (s), 3082 (m), 2962 (s), 2754 (m), 1743 (s), 1701 (s), 1649 (s), 1553 (s), 1442 (s), 1330 (s), 1238 (s), 1188 (s) cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 10.1 (s, 1H), 7.78 (d, J = 7.2 Hz, 1H), 7.63 (d, J = 8.2 Hz, 2H), 7.53 (t, J = 7.5, 1H), 7.47 (t, J = 7.5, 1H), 7.31 (d, J = 7.5 Hz, 1H), 7.18 (d, J = 8.4 Hz, 2H), 6.75 (d, J = 7.8, 1H), 4.93 (d, J = 7.8 Hz, 1H), 4.23 (m, 1H), 4.02 (m, 2H), 3.41 (dd, J = 7.8, 4.8 Hz, 1H), 3.22 and 3.05 (AB q, J = 13.5 Hz, both parts split into d with J = 9.6, 5.6 Hz, respectively, 2H), 2.35 (s, 3H), 2.09 (s, 3H), 1.98 (m, 1H), 0.72 (d, J = 6.9 Hz, 3H), 0.59 (d, J = 6.9 Hz, 3H); ^{13}C NMR ($CDCl_3$, 75.46 MHz) δ 194.6, 171.0, 170.2, 143.7, 139.5, 136.6, 134.7, 134.5, 134.0, 132.1, 129.6, 127.5, 127.3, 65.1, 62.1, 51.0, 33.6, 31.0, 21.5, 20.8, 18.9, 16.8; HRMS calcd for $C_{24}H_{31}N_2O_6S$ 475.190 ($M^+ + H^+$), found 475.191.

Reduction of 18. To an ice-cooled solution of aldehyde **18** (858 mg, 1.81 mmol) in THF–EtOH (40 mL, 1:1) was added $NaBH_4$ (344 mg, 9.05 mmol). Stirring was continued for 0.5 h at this temperature, and the solution was then poured into 200 mL of water. Extractive workup with ether followed by concentration and chromatography on silica gel with 2:1 hexane–ethyl acetate as eluent provided 825 mg (95%) of the desired alcohol as a white amorphous solid: $[\alpha]^{22}_D$ = –28.8° (c = 1.09, CH_2Cl_2); IR (KBr) 3547 (m), 3483 (m), 3298 (s), 3254 (s), 3080 (m), 2960 (s), 1738 (s), 1645 (s), 1447 (s), 1329 (s), 1240 (s), 1161 (s) cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 7.62 (d, J = 8.1 Hz, 2H), 7.39 (d, J = 7.2 Hz, 1H), 7.27 (m, 2H), 7.19–7.23 (m, 4H), 5.14 (d, J = 8.8 Hz, 1H), 4.83 and 4.59 (AB q, J = 11.4, both parts split into d with J = 5.4, 3.9 Hz, respectively, 2H), 4.21 (m, 1H), 4.03 (m, 2H), 3.56 (m, 1H), 3.47 (dd, J = 8.8, 4.8 Hz, 1H), 2.81 (d, J = 7.5 Hz, 2H), 2.38 (s, 3H), 2.11 (s, 3H), 1.94 (m, 1H), 0.66 (d, J = 6.9 Hz, 3H), 0.40 (d, 6.9 Hz, 3H); ^{13}C NMR ($CDCl_3$, 75.46 MHz) δ 171.2, 170.7, 143.9, 138.3, 136.7, 130.6, 130.3, 129.7, 128.7, 127.1, 65.2, 63.6, 62.2, 50.4, 33.5, 31.0, 21.6, 20.9, 19.1, 16.4; HRMS calcd for $C_{24}H_{33}N_2O_6S$ 477.206, found 477.207.

Conversion of 18 to 4a. To an ice-cooled solution of the alcohol prepared from **18** (809 mg, 1.70 mmol) in 450 mL of THF were added triphenylphosphine (579 mg, 2.21 mmol) and diethyl azodicarboxylate (350 μL, 2.21 mmol) sequentially. The resulting solution was stirred at 0 °C for 3 h. The solvent was evaporated at reduced pressure, and the residual oil was chromatographed to afford 623 mg (80%) of the cyclized product. The crude product was dissolved in 10 mL of THF, and the solution was added to 50 mL of liquid ammonia at –78 °C. Na metal was added in small pieces until the resulting deep blue color remained for at least 15 min. Stirring was continued at this temperature for an additional 30 min, and then sufficient NH_4Cl was added to discharge the blue color. The ammonia was allowed to evaporate, and the residual solid was dissolved in 200 mL of ether and 100 mL of water. The organic layer was separated, and the aqueous layer was extracted with ether (2 × 100 mL). The combined ether extracts were washed with brine and dried over Na_2SO_4. The solution was concentrated to ca. 30 mL and allowed to stand for 30 min. The resulting precipitate was collected by suction filtration, and the filter cake was washed with ether (20 mL) to afford 181 mg (73%) of **4a** as a white powder: $[\alpha]^{22}_D$ = +6.2° (c = 0.42, MeOH). The IR, ^1H NMR, and ^{13}C NMR spectral data were identical with those reported above.

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Supplementary Material Available: Complete X-ray structure report for **14a** (16 pages). Ordering information is given on any current masthead page. The author has deposited atomic coordinates for this structure with the Cambridge Crystallographic Data Centre. The coordinates can be obtained, on request, from the Director, Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge, CB2 1EZ, UK.