MD calculations, however, show that other factors could contribute to the efficiency of the enzyme. Indeed, the dynamics of the cavity can be important for increasing the affinity of SOD toward the substrate. In WT SOD, the active site cavity has a large mobility, and the surface exposed to the solvent, and therefore to the substrate, is larger than that observed in the crystallographic structure. This suggests a synergistic role for the active cavity, where the electrostatic effects are enhanced by the fluxionality of the cavity. The charged groups, due to their high mobility, could produce a large attraction for superoxide; the larger surface exposed to the solvent facilitates the entrance of O_2^-.

The relevance of structural fluctuations in ligand and substrate binding to proteins, especially if they are small molecules like in the present case, has been already extensively discussed.[28] A striking example of the effect of protein mobility in molecular binding is represented by myoglobin: energy calculations[54] have demonstrated that the CO and O_2 binding are strongly affected by the protein atoms fluxionality which determines the possible binding paths for these ligands in entering and leaving the heme pocket. The energy barriers for these paths are sizably reduced by protein motions. If myoglobin rigidly maintained its X-ray structure, no energetically feasible path was possible.

The additional role for the active site channel due to its fluxionality is speculative at the present stage of the investigation but is consistent with the behavior of the two mutants here investigated. The reduced catalytic rates of the Arg 143 → Glu mutant correlate, in addition to the electrostatic effect of a negative charge, also with a reduced mobility and a smaller open section of the active site channel. The Arg 143 → Ile mutant, in which the positive charge is neutralized and not reversed in sign, has intermediate activity rates and intermediate mobility and open section of the channel, between the WT and the Arg 143 → Glu SOD mutant. Therefore, the group on the 143 position may behave like a "gate keeper" at the reaction site.

The mobility of other residues in the channel is directly connected with their involvement in the catalytic process. For example, the equilibrium position of Lys 136 results to be far from the copper ion, pointing outside the channel. Deprotonation of this residue was invoked as resposible of the drop in activity at high pH;[55] however a biophysical and biochemical characterization of proteins with residue 136 mutated to neutral, not ionizable, residues has shown that Lys 136 has only a small electrostatic effect, without affecting sizably the overall enzymatic behavior.[56] This finding is in complete agreement with our results from MD calculations.

The present MD simulations provide further hints on the enzymatic behavior of SOD and can shed light on the role of the active site channel in determining the high efficiency of this enzyme.

Acknowledgment. The authors are grateful to Prof. Ivano Bertini for his encouragement and helpful discussions. We wish to thank also Prof. Kenneth M. Merz, Jr., for his precious suggestions and comments on the manuscript.

(54) Case, D. A.; Karplus, M. *J. Mol. Biol.* **1978**, *132*, 343-368.

(55) O'Neil, P.; Davies, S.; Fielden, E. M.; Calabrese, L.; Capo, C.; Marmocchi, F.; Natoli, G.; Rotilio, G. *Biochem. J.* **1988**, *251*, 41.

(56) Banci, L.; Bertini, I.; Luchinat, C.; Viezzoli, M. S. Submitted.

Enantioselective Total Synthesis of (-)-Decarbamoylsaxitoxin

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Abstract: An enantioselective total synthesis of (-)-decarbamoylsaxitoxin (**2**) has been accomplished, using the asymmetric trimolecular cyclization of **6**, i.e., **6** + (*R*)-glyceraldehyde 2,3-acetonide + $Si(NCS)_4$ → α-7, as the key step. The structure of the major product α-7 was determined by X-ray crystallographic analysis, with the C-6 configuration corresponding to the unnatural antipode of decarbamoylsaxitoxin. Acetonide α-7 was converted to thiourea **5**, an intermediate used in our previous racemic synthesis of saxitoxin. Thiourea **5** was further transformed to the tricyclic urea-thiourea **3**. At this stage, the ^1H NMR spectrum ($CDCl_3$) of optically active **3** was found to be dramatically different from that of racemic **3**. On the basis of concentration-dependent NMR spectroscopy and other studies, it was suggested that racemic **3** exists as a dimer of *d,l* pairs via two sets of hydrogen bonds whereas optically active **3** exists as a monomer because of a lack of such interactions due to geometric restriction. By following the previous synthetic route, tricyclic urea-thiourea **3** was converted to the unnatural antipode of decarbamoylsaxitoxin (**2**). Since the transformation of racemic, as well as optically active, decarbamoylsaxitoxin to saxitoxin has already been established, this synthesis constitutes a formal enantioselective total synthesis of saxitoxin. In addition, it was unambiguously demonstrated that the unnatural antipode of decarbamoylsaxitoxin exhibits no sodium channel blocking activity.

Introduction

The highly toxic nature of the red tide has been known and feared since ancient times. Periodic outbreaks of the red tide often occur during the summer months along the North Atlantic coasts of America and Europe, in particular along the North Pacific coast from California to Alaska, and the coastal areas of Japan and South Africa. Under certain tropical conditions, a toxic single-cell dinoflagellate *Gonyaulax catanella* grows at an abnormal rate. As these algae produce a red pigment(s), a bloom of dinoflagellates imparts a red color to the sea. Clams and mussels, feeding on these and other dinoflagellates by filtration of seawater through their siphon, concentrate the toxic principle of dinoflagellates in their organs and become poisonous to man, causing paralytic shellfish poisoning.[1]

In 1957, the toxic component of the paralytic shellfish poison was first isolated in the pure state by Schantz and co-workers. Extracts of the hepatopancreas of Alaskan butter clams (*Saxidomus giganteus*) yielded a highly toxic amorphous substance,

(1) For reviews on saxitoxin and related natural products, see: (a) Schantz, E. J. *Pure Appl. Chem.* **1980**, *52*, 183. (b) Shimizu, Y. In *Progress in the Chemistry of Organic Natural Products*: Herz, W., Grisebach, H., Kirby, G. W., Eds.; Springer-Verlag: New York, 1984; p 235. (c) Shimizu, Y. In *Marine Natural Products*; Scheuer, P. J., Ed.; Academic Press: New York, 1978; Vol. I, p 1.

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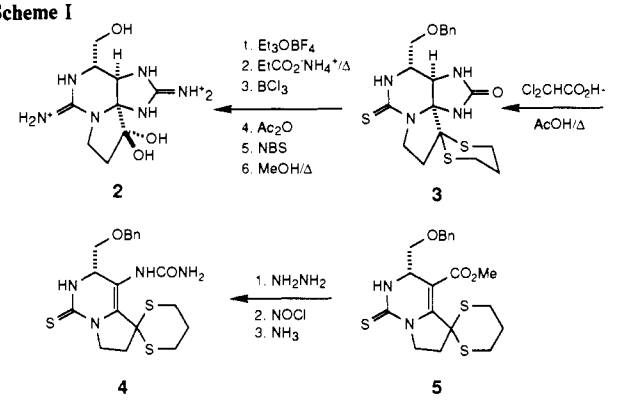
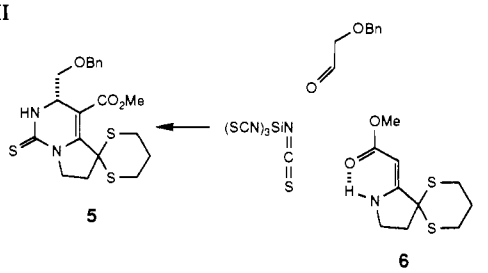
Scheme I



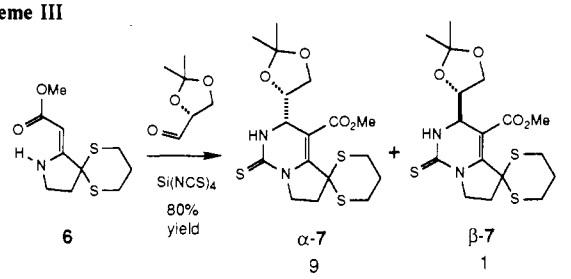
named saxitoxin.[2] Extensive structural studies by Rapoport[3] and by Schantz[4] over 2 decades narrowed down the number of plausible structures for saxitoxin. However, the complete structure was not elucidated until these two groups independently succeeded in obtaining crystalline derivatives of saxitoxin in 1975. The di-*p*-bromobenzenesulfonate salt of saxitoxin monohydrate and the dihydrochloride salt of the ethyl hemiketal of saxitoxin were analyzed by X-ray crystallography to establish the structure of saxitoxin as **1**.[5,6] Saxitoxin (STX) is the most historical and potent member of the gonyautoxins (GTXs), all of which have been isolated from dinoflagellates of the genus *Gonyaulax*.[7-12]

Saxitoxin is a challenging target for synthetic chemists. The unique backbone of this heterocycle is highly functionalized and very susceptible to oxidation. Chemical manipulation of saxitoxin and its synthetic intermediates is more difficult than the manipulation of normal target molecules because their high polarity limits the range of usable solvents for the chemical reactions. Nevertheless, the first total synthesis of saxitoxin was completed in this laboratory in 1977.[13] We also developed a much shorter and more efficient synthesis of the key intermediate of the original synthesis.[14] Jacobi and co-workers used a unique cycloaddition reaction to construct the tricyclic urea–thiourea, which was

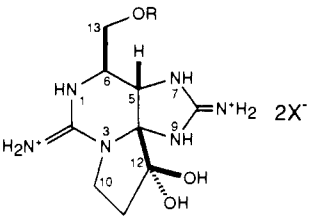
Scheme II



Scheme III



identical to the key intermediate of our synthesis except for the protecting group of the C-13 hydroxy group.[15]



1: R=$CONH_2$, saxitoxin
2: R=H, decarbamoyl saxitoxin

There is no enantioselective synthesis of saxitoxin reported to date, and this paper is concerned with the first enantioselective total synthesis of (−)-decarbamoylsaxitoxin. (+)-Decarbamoylsaxitoxin (**2**) was first obtained as an acid hydrolysis product of natural saxitoxin by Schantz.[16] This substance was later isolated as a minor toxic principle of the bivalve *Spondylus butleri*, collected at Arumizu Bay in Palau.[17]

It is worthwhile to mention the controversy concerning the biological activity of the unnatural antipodes of saxitoxin and decarbamoylsaxitoxin.[18] The potency of racemic saxitoxin and decarbamoylsaxitoxin was estimated to be close to that of the natural products, which suggests the unnatural antipodes of these toxins to be equally active. However, considering the technical difficulty in determining the accurate weight of samples, this conclusion may not be as definitive as it appears. This issue should be resolved by testing the unnatural antipode of saxitoxin and/or decarbamoylsaxitoxin.

Synthetic Plan

We chose to rely on our prior experience gained through the total synthesis of racemic saxitoxin. Since the transformation of tricyclic urea–thiourea **3** to decarbamoylsaxitoxin (**2**) has been

(2) (a) Schantz, E. J.; Mold, J. D.; Stanger, D. W.; Shavel, J.; Riel, F. J.; Bowden, J. P.; Lynch, J. M.; Wyler, R. S.; Riegel, B.; Sommer, H. *J. Am. Chem. Soc.* **1957**, *79*, 5230. (b) Schantz, E. J.; Lynch, J. M.; Vayvada, G.; Matsumoto, K.; Rapoport, H. *Biochemistry* **1966**, *5*, 1191.

(3) (a) Schuett, W.; Rapoport, H. *J. Am. Chem. Soc.* **1962**, *84*, 2266. (b) Wong, J. L.; Brown, M. S.; Matsumoto, K.; Oesterlin, R.; Rapoport, H. *J. Am. Chem. Soc.* **1971**, *93*, 4633. (c) Wong, J. L.; Oesterlin, R. J.; Rapoport, H. *J. Am. Chem. Soc.* **1971**, *93*, 7344.

(4) Schantz, E. J.; Mold, J. D.; Howard, W. L.; Bowden, J. P.; Stanger, D. W.; Lynch, J. M.; Wintersteiner, O. P.; Dutcher, J. D.; Walters, D. R.; Riegel, B. *Can. J. Chem.* **1961**, *39*, 2117.

(5) Schantz, E. J.; Ghazarossian, V. E.; Schnoes, H. K.; Strong, F. M.; Springer, J. P.; Pezzanite, J. O.; Clardy, J. *J. Am. Chem. Soc.* **1975**, *97*, 1238.

(6) Bordner, J.; Thiessen, W. E.; Bates, H. A.; Rapoport, H. *J. Am. Chem. Soc.* **1975**, *97*, 6008.

(7) GTX II and III: (a) Shimizu, Y.; Buckley, L. J.; Alam, M.; Oshima, Y.; Fallon, W. E.; Kasai, H.; Miura, I.; Gullo, V. P.; Nakanishi, K. *J. Am. Chem. Soc.* **1976**, *98*, 5414. (b) Boyer, G. L.; Schantz, E. J.; Schnoes, H. K. *J. Chem. Soc., Chem. Commun.* **1978**, 889.

(8) GTX I and IV: (a) Shimizu, Y.; Hsu, C.-P. *J. Chem. Soc., Chem. Commun.* **1981**, 314. (b) Wichmann, C. F.; Boyer, G. L.; Divan, C. L.; Schantz, E. J.; Schnoes, H. K. *Tetrahedron Lett.* **1981**, *22*, 1941.

(9) GTX VIII and epimer: (a) Kobayashi, M.; Shimizu, Y. *J. Chem. Soc., Chem. Commun.* **1981**, 827. (b) Wichmann, C. F.; Niemczura, W. P.; Schnoes, H. K.; Hall, S.; Reichardt, P. B.; Darling, S. D. *J. Am. Chem. Soc.* **1981**, *103*, 6977.

(10) C_3 and C_4 toxins: (a) Hall, S.; Darling, S. D.; Boyer, G. L.; Reichardt, P. B.; Liu, H.-W. *Tetrahedron Lett.* **1984**, *25*, 3537. (b) Noguchi, T.; Onoue, Y.; Maruyama, J.; Hashimoto, K.; Nishio, S.; Ikeda, T. *Bull. Jpn. Soc. Sci. Fish.* **1983**, *49*, 1931.

(11) GTX V and VI: (a) Harada, T.; Oshima, Y.; Yasumoto, T. *Agric. Biol. Chem.* **1982**, *46*, 1861. (b) Koehn, F. E.; Hall, S.; Wichmann, C. F.; Schnoes, H. K.; Reichardt, P. B. *Tetrahedron Lett.* **1982**, *23*, 2247.

(12) neo-STX: Shimizu, Y.; Hsu, C.-P.; Fallon, W. E.; Oshima, Y.; Miura, I.; Nakanishi, K. *J. Am. Chem. Soc.* **1978**, *100*, 6791.

(13) (a) Tanino, H.; Nakata, T.; Kaneko, T.; Kishi, Y. *J. Am. Chem. Soc.* **1977**, *99*, 2818. (b) Taguchi, H.; Yazawa, H.; Arnett, J. F.; Kishi, Y. *Tetrahedron Lett.* **1977**, 627. (c) Kishi, Y. *Heterocycles* **1980**, *14*, 1477.

(14) Hannick, S. M.; Kishi, Y. *J. Org. Chem.* **1983**, *48*, 3833.

(15) (a) Jacobi, P. A.; Brownstein, A.; Martinelli, M. J.; Grozinger, K. *J. Am. Chem. Soc.* **1981**, *103*, 239. (b) Jacobi, P. A.; Martinelli, M. J.; Polanc, S. *J. Am. Chem. Soc.* **1984**, *106*, 5594. (c) Martinelli, M. J.; Brownstein, A. D.; Jacobi, P. A. *Croat. Chem. Acta* **1986**, *59*, 267. (d) Jacobi, P. A. In *Strategies and Tactics in Organic Synthesis*; Lindberg, T., Ed.; Academic Press: New York, 1989; Vol. 2, p 191.

(16) Ghazarossian, V. E.; Schantz, E. J.; Schnoes, H. K.; Strong, F. M. *Biochem. Biophys. Res. Commun.* **1976**, *68*, 776.

(17) Harada, T.; Oshima, Y.; Yasumoto, T. *Agric. Biol. Chem.* **1983**, *47* (1), 191.

(18) Strichartz, G. R.; Rando, T.; Hall, S.; Gitschier, J.; Hall, L.; Magnani, B.; Hansen-Bay, C. In *Tetrodotoxin, Saxitoxin and the Molecular Biology of Sodium Channel*; Kao, C. Y., Levinson, S. R., Eds.; New York Academy of Science: New York, 1986.

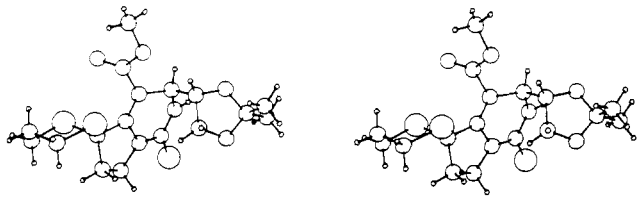


Figure 1. Stereoview of α-7.

established,[1,3] our first goal was the construction of the tricyclic urea–thiourea **3** in enantiomerically pure form (Scheme I; note that the structures correspond to the unnatural antipode). Tricyclic urea–thiourea **3** has asymmetric centers at the C-4, C-5, and C-6 positions. In the racemic synthesis,[13,14] we developed a stereoselective method to construct the thiourea **3**: upon heating in acid, urea **4** cyclized to yield the tricyclic compound **3** with 15:1 stereoselectivity, favoring the desired diastereoisomer. The bicyclic urea **4**, in turn, was prepared from thiourea **5**.

Thus our plan for the enantioselective synthesis became the development of an enantioselective synthesis of **5**, followed by the previous racemic synthesis. We synthesized racemic thiourea **5** via a trimolecular cyclization of vinylogous urethane **6** (Scheme II)[13] and noticed two possibilities to extend this cyclization to an enantioselective synthesis of **5**. First, the introduction of the C-6 stereocenter might be achieved enantioselectivity by incorporating a chiral alcohol on the ester moiety of the vinylogous urethane **6**. Second, an aldehyde with a chiral protecting group instead of benzyl might be employed to realize the enantioselective trimolecular cyclization. The vinylogous urethane **6** exists in an intramolecularly hydrogen-bonded form, thereby providing planarity to this molecule. It was therefore envisioned that a chiral auxiliary could have an effect on the developing C-6 stereocenter during the cyclization reaction. However, we also realized a potential limitation of this approach: the distance between the chiral center of the chiral auxiliaries and the developing C-6 stereocenter might not be close enough to realize high enantioselectivity. Nevertheless, we performed preliminary experiments which showed the degree of asymmetric induction in these two possibilities to be too low for our purposes.[19]

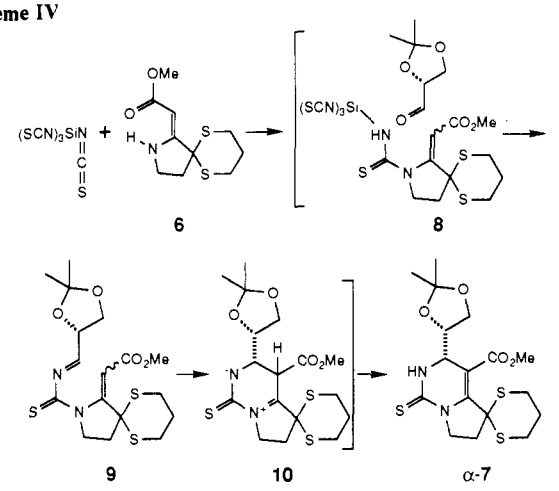
Results and Discussion

We were concerned that the distance between the chiral center of the auxiliaries and the developing C-6 stereocenter might be too great to influence the stereoselectivity of the cyclization. The major reason for exploring these two possibilities was that, if successful, these approaches would require minimal modifications of the previous synthetic route. However, the preliminary experimental results suggested that we needed to place two chiral centers in closer proximity in order to realize an efficient enantioselective cyclization, even though several extra steps might be required later.

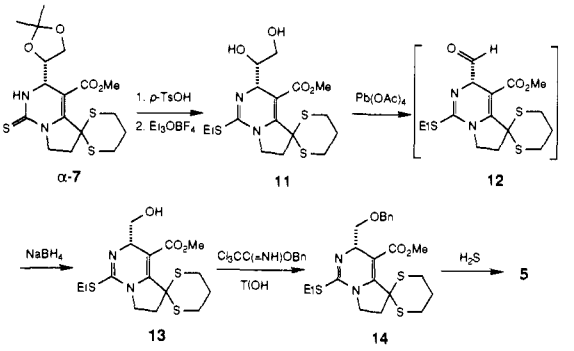
In this context, we tested (*R*)-glyceraldehyde 2,3-acetonide as the first candidate.[20] When the vinylogous urethane **6** was reacted with (*R*)-glyceraldehyde 2,3-acetonide in the presence of silicon tetraisothiocyanate in benzene at room temperature, a diastereomeric mixture of the expected acetonide **7** was formed with good stereoselectivity (Scheme III). The diastereomers were separated by silica gel column chromatography to furnish the major diastereomer α-**7** in 72% yield, along with the minor diastereomer β-**7** in 8% yield.

The structure of the major product α-**7** was determined by X-ray crystallographic analysis, establishing the C-6 configuration

Scheme IV



Scheme V



as corresponding to the unnatural antipode of decarbamoylsaxitoxin (Figure 1). The natural antipode should be obtainable by using (*S*)-instead of (*R*)-glyceraldehyde 2,3-acetonide. However, because of the reason described before, we opted to carry on the synthesis with the unnatural antipode.

Although not clearly established, one possible mechanism can be proposed for this trimolecular cyclization (Scheme IV). It is conceivable that the nucleophilic nitrogen of vinylogous urethane **6** could attack the electrophilic carbon of silicon tetraisothiocyanate, thereby generating the thiourea **8**. This intermediate could then condense with (*R*)-glyceraldehyde 2,3-acetonide to form the imine **9** after elimination of R_3SiOH.[21] The resulting imine **9** could cyclize to **10** by attack of the β-carbon of the enamine moiety. Finally, a 1,3-proton transfer of **10** would furnish the acetonide α-**7**.

Assuming this process involves the cyclization of **9** to **10**, the observed facial selectivity of cyclization could be explained by the Felkin–Ahn model[22] (Figure 2). Namely, the nucleophilic attack should take place from the less hindered face of this transition state to provide the observed configuration at the C-6 position.

The acetonide α-**7** was converted to the diol by acidic hydrolysis in quantitative yield (Scheme V). Because of the high reactivity of the thiourea group toward oxidants and electrophiles, this functional group was protected as its imino thioether **11** by treatment with the Meerwein reagent in dichloromethane at room temperature in 85% yield. The imino thioether **11** was then treated with lead tetraacetate in ethyl acetate at room temperature, and the resulting aldehyde was immediately reduced by sodium borohydride in methanol at 0 °C to provide the alcohol **13** in 70% overall yield.

The optical purity of the alcohol **13** was determined to be ≥96% by ^1H and ^{19}F NMR spectra of its Mosher's ester.[23] Because the optical purity of α-**7** was 100%, we reasoned that ≤4% ep-

(19) (-)-Menthol, (-)-8-phenylmenthol, and *exo*-(+)-borneol esters corresponding to **6** were prepared and subjected to the trimolecular cyclization to yield 62:38, 57:43, and 55:45 mixtures of C-6 α and β diastereomers corresponding to **5**. On the other hand, the trimolecular cyclization of **6** with α-(1-phenylethoxy)acetaldehyde, prepared from (-)-*sec*-phenethyl alcohol, gave a 56:44 mixture: Hong, C. Y. Ph.D. Dissertation, Harvard University, Cambridge, MA, 1991.

(20) For a review on the use of (*R*)- and (*S*)-glyceraldehyde 2,3-acetonides in organic synthesis, see: Jurczak, J.; Pikul, S.; Bauer, T. *Tetrahedron* **1986**, *42*, 447.

(21) The thiourea corresponding to **8** was shown to react with acetaldehyde to yield the expected bicyclic thiourea.[13c]

(22) (a) Cherest, M.; Felkin, H.; Prudent, N. *Tetrahedron Lett.* **1968**, 2199. (b) Cherest, M.; Felkin, H. *Tetrahedron Lett.* **1968**, 2205.

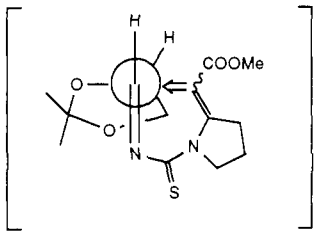


Figure 2.

imerization had occurred during the transformation from α-**7** to **13**, most likely at the stage of aldehyde **12**. The C-6 position of the aldehyde **12** was believed to be extremely susceptible to epimerization due to the highly acidic proton at the C-6 position.

The alcohol **13** was protected as its benzyl ether **14** under acidic conditions,[24] and the thio imino group of **14** was subsequently deprotected by treatment with hydrogen sulfide to furnish the thiourea **5** in 58% overall yield. On comparison of spectroscopic data (^{1}H NMR, IR, UV, and MS) and chromatographic behavior, the thiourea **5** was proven to be identical to the corresponding intermediate in the racemic synthesis of saxitoxin.

Hydrazide formation of **5**, followed by Curtius rearrangement, provided the urea **4** in 45% overall yield, and subsequent cyclization of **4** yielded the tricyclic urea–thiourea **3** in 56% yield (Scheme I).[13,14] At this stage, a rather unique behavior of tricyclic urea–thiourea **3** in solution was observed. In CD_3OD, both racemic and optically active **3** showed identical ^{1}H NMR spectra. However, in $CDCl_3$ their ^{1}H NMR spectra were completely different. In order to gain insight into this dramatically different behavior of **3** in $CDCl_3$, we examined the concentration dependence of the ^{1}H NMR spectra of **3** (Figure 3). The ^{1}H NMR spectrum of optically active **3** does not change in the concentration range from 5 to 0.02 mg in 1 mL of $CDCl_3$. On the contrary, the ^{1}H spectrum of racemic **3** was found to be sharply concentration dependent, and at high dilution (0.02 mg in 1 mL of $CDCl_3$) the ^{1}H NMR spectrum of racemic **3** becomes completely identical to that of optically active **3**.

We believe that this unique ^{1}H NMR behavior of the tricyclic urea–thiourea **3** can be rationalized by analogy to molecular recognition. In $CDCl_3$ at high concentration, the racemic **3** exists as a dimer of *d,l* pairs via two sets of hydrogen bonds between the urea and the thiourea groups (Figure 4), but this dimer breaks down to monomers when diluted. Even though we could not rule out the possibility of tetramer, hexamer, or polymer formation, we prefer the dimer formation because the signals in the ^{1}H NMR spectrum of racemic **3** are very sharp. In the case of optically active **3**, only one set of hydrogen bonds between urea and thiourea groups, two urea groups, or two thiourea groups is possible due to geometric restrictions. Therefore, optically active **3** does not form a dimer in $CDCl_3$ as racemic **3** does. In CD_3OD, both racemic and optically active **3** exist as monomers, because CD_3OD breaks all hydrogen bonds.

The chemical shifts of the urea and thiourea protons further support this explanation. Optically active **3** displays three NH resonances at high and low concentrations. However, racemic **3** shows only one broad NH resonance at high concentration. Upon dilution of racemic **3**, the NMR spectrum displays three NH resonances and the overall line shapes become much sharper.

In this context, it is interesting to examine the hydrogen-bonding pattern in the crystalline state. We had previously obtained X-ray data for the racemic compound **15**, a key intermediate of the gonyautoxin synthesis.[14] Excluding the C-11 α-OMe group, **15** has a structure identical to that of tricyclic urea–thiourea **3**. Therefore, we believe this compound should have properties similar to those of **3** and could provide relevant information about the solid-state structure of tricyclic urea–thiourea **3**. Indeed, the X-ray structure of **15** clearly displayed two sets of intermolecular hydrogen bonds between the urea and thiourea groups of alternating *d,l* pairs (Figure 5). As a result, this molecule formed a polymer-like structure via these intermolecular hydrogen bonds. Hydrogen bonds between *d,d* or *l,l* pairs were not observed.[25]

Following the previous racemic synthesis of saxitoxin,[13,14] tricyclic urea–thiourea **3** was converted to the unnatural antipode

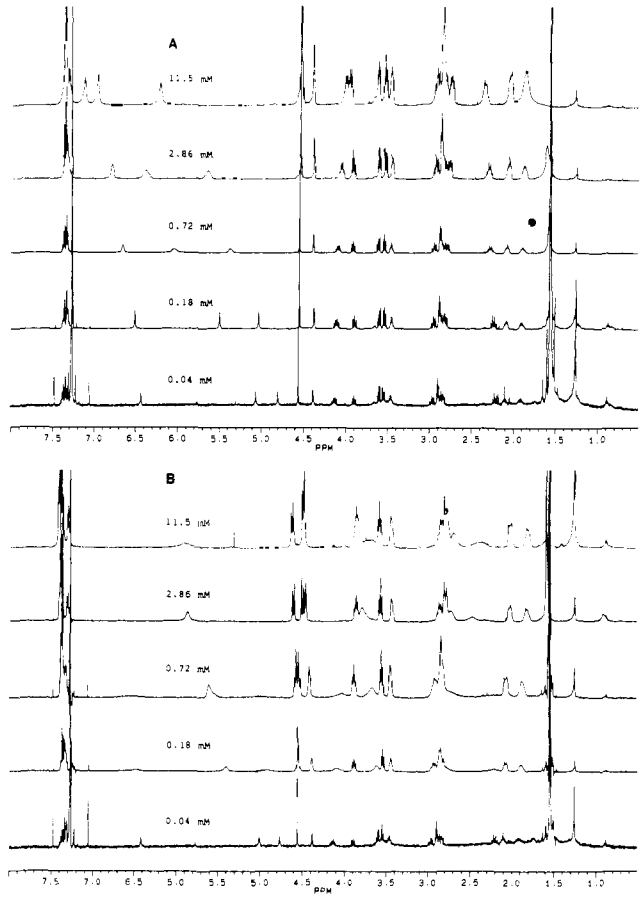


Figure 3. Concentration-dependent ^{1}H NMR (500 MHz) studies of **3** in $CDCl_3$ at room temperature: (A) optically active; (B) racemic.

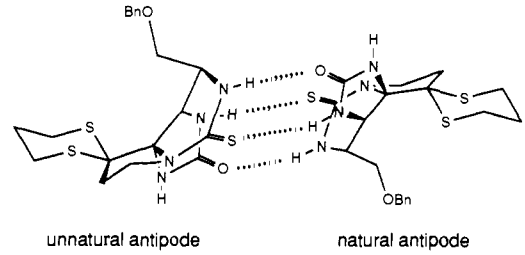


Figure 4. Proposed dimeric structure of racemic **3** in $CDCl_3$.

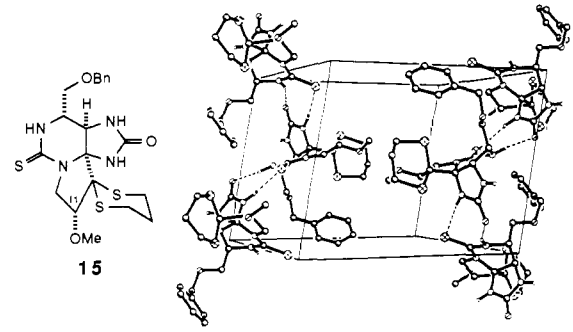


Figure 5. Unit cell structure of **15**.

(23) Dale, J. A.; Mosher, H. S. *J. Am. Chem. Soc.* **1973**, *95*, 512.
(24) Iversen, T.; Bundle, D. R. *J. Chem. Soc., Chem. Commun.* **1981**, 1240.

(25) We attempted to prepare a single crystal of the optically active **3** suitable for X-ray analysis without success.

of decarbamoylsaxitoxin (**2**) (Scheme I). On comparison of ^1H NMR spectra and TLC behavior in a variety of solvent systems, the synthetic unnatural antipode of decarbamoylsaxitoxin was identical to natural decarbamoylsaxitoxin, but it exhibited the opposite $[\alpha]_D$ value. Since the transformation of racemic, as well as optically active, decarbamoylsaxitoxin to saxitoxin is already established,[13] this synthesis constitutes a formal enantioselective total synthesis of saxitoxin.

Finally, the biological activity of the unnatural antipode of decarbamoylsaxitoxin was compared with that of natural decarbamoylsaxitoxin, demonstrating that the unnatural antipode has 3–4% of the sodium channel blocking activity of natural decarbamoylsaxitoxin. This residual activity can be attributed to a small amount of the contaminated natural antipode (≥4%), as shown at the stage of **13**. Therefore, this experiment unambiguously established that unnatural decarbamoylsaxitoxin does not exhibit sodium channel blocking activity.[26]

Experimental Section

General Procedures. ^1H NMR spectra were recorded at 500 MHz, and ^{13}C NMR spectra were recorded at 125 MHz. Chemical shifts are reported in parts per million. The residual solvent peak was used as an internal reference. Chemical ionization (CI) mass spectra were recorded using ammonia as a reagent gas. Fast atom bombardment (FAB) mass spectra were obtained with 3-nitrobenzyl alcohol or glycerol as the matrix. Sodium iodide was added when indicated. Fourier transform infrared spectra were obtained in solution using $CHCl_3$ as a solvent. Automatic base line corrections were performed as needed. Optical rotations were measured at room temperature using the sodium D line. Melting points (mp) are uncorrected. Analytical thin-layer chromatography (TLC) was performed with E. Merck precoated TLC plates (silica gel 60F-254, layer thickness 0.25 mm). Preparative TLC separations were carried out on E. Merck precoated TLC plates (silica gel 60F-254, layer thickness 0.50 nm). Flash chromatography separations were performed using E. Merck Kieselgel 60 (230–400 mesh) silica gel. Nonflash silica gel chromatography was conducted with E. Merck Kieselgel 60 (70–230 mesh) silica gel. Reagents and solvents are commercial grade and were used as supplied, with the following exceptions: Acetonitrile was distilled from calcium hydride. Benzene, ether, and tetrahydrofuran were distilled from sodium benzophenone ketyl. Methylene chloride was distilled from phosphorus pentoxide. Pyridine was distilled from calcium hydride. Toluene was distilled from sodium. All reactions sensitive to moisture or air were performed under either argon or nitrogen. Reaction vessels were flame-dried or oven-dried and allowed to cool under inert atmosphere for moisture-sensitive reactions.

Acetonides α-7 and β-7. To a stirred mixture of silicon tetraisothiocyanate (910 mg, 3.49 mmol) and freshly prepared (*R*)-glyceraldehyde 1,2-acetonide (1.97 g, 15.2 mmol) in dry benzene (100 mL) was added a solution of vinylogous urea **6** (3.10 g, 12.7 mmol)[14] in benzene (20 mL), and the resulting cloudy mixture was stirred for 10 h at room temperature. The solvent was removed in vacuo, and the residue was dissolved in dry toluene (100 mL). The resulting solution was refluxed for 0.5 h under N_2. The reaction mixture was cooled to room temperature, and filtered, and the filtrate was washed with 10% methanol in CH_2Cl_2. The combined extracts were concentrated in vacuo, and the residue was subjected to column chromatography (silica gel, 4:1 to 2:1 hexane/ethyl ether) to give bicyclic thiourea α-**7** (3.78 g, 72%) and epimer β-**7** (445 mg, 8%), each as white solids. An analytical sample of α-**7** was recrystallized from MeOH to afford colorless needles, mp 144–145 °C: ^1H NMR ($CDCl_3$) δ 1.36 (3 H, s), 1.48 (3 H, s), 1.95 (1 H, tq, *J* = 3.3, 13.5 Hz), 2.19 (1 H, m), 2.74 (1 H, td, *J* = 7.0, 13.8 Hz), 2.82 (1 H, m), 2.82 (2 H, m), 3.08 (2 H, dt, *J* = 2.1, 13.5 Hz), 3.83 (1 H, dd, *J* = 7.3, 8.7 Hz), 3.82 (3 H, s), 4.02 (1 H, dd, *J* = 6.5, 8.7 Hz), 4.04 (1 H, m), 4.20 (1 H, td, *J* = 6.9, 11.1 Hz), 4.27 (1 H, dt, *J* = 3.3, 7.1 Hz), 4.53 (1 H, t, *J* = 3.3 Hz), 6.81 (1 H, s); ^{13}C NMR ($CDCl_3$) δ 24.64, 25.45, 26.15, 28.30, 28.34, 39.66, 49.14, 51.67, 53.40, 56.68, 64.36, 77.45, 102.30, 110.01, 145.08, 164.95, 175.94; IR ($CHCl_3$) 3418 cm^{-1}, 2990, 1710, 1728, 1504, 1433, 1360, 1251; UV (λ_{max} in MeOH) 304 nm (ε = 8400); MS (DEI, NH_3) 417 (M + H, relative intensity 100), 385 (5), 343 (14), 311 (34), 253 (4), 211 (8); HRMS (DEI, NH_3) calcd for $C_{17}H_{24}N_2O_4S_3$ (M) 416.08981, found 416.09107; $[\alpha]_D$ +102.1° (*c* 1.2, $CHCl_3$). Anal. Calcd for $C_{17}H_{24}N_2O_4S_3$: C, 49.01; H, 5.81; N, 6.72. Found: C, 48.93; H, 5.85; N, 6.68.

Imino Thioether Diol 11. To a solution of acetonide α-**7** (3.50 g, 8.41 mmol) in MeOH (500 mL) was added *p*-TsOH (80 mg, 0.40 mmol), and the mixture was stirred for 24 h at room temperature. The solvent was evaporated in vacuo, and the residue was diluted with 10% MeOH in CH_2Cl_2 (500 mL). This solution was washed with saturated $NaHCO_3$ solution (2 × 100 mL) and brine (100 mL). The organic layer was dried ($MgSO_4$), filtered, and concentrated in vacuo to give the crude diol as a white solid (3.15 g, quantitative). An analytical sample of this diol was recrystallized from MeOH to afford fine white crystals, mp 219–220 °C: ^1H NMR (CD_3OD) δ 1.90 (1 H, m), 2.23 (1 H, m), 2.73 (1 H, ddd, *J* = 7.4, 8.0, 13.0 Hz), 2.80 (2 H, m), 2.99 (1 H, ddd, *J* = 4.4, 6.6, 11.0 Hz), 3.21 (2 H, m), 3.48 (1 H, dd, *J* = 7.3, 11.4 Hz), 3.63 (1 H, dd, *J* = 3.4, 11.3 Hz), 3.70 (1 H, ddd, *J* = 3.4, 5.0, 7.3 Hz), 3.80 (3 H, s), 3.90 (1 H, ddd, *J* = 4.4, 7.4, 11.0 Hz), 4.14 (1 H, ddd, *J* = 6.7, 8.1, 13.0 Hz), 4.21 (1 H, d, *J* = 5.0); ^{13}C NMR (CD_3OD) δ 26.12, 29.09, 29.13, 40.86, 50.31, 51.95, 56.52, 57.92, 62.91, 75.98, 104.34, 147.65, 168.08, 177.69; IR ($CHCl_3$) 3600–3000 cm^{-1} 3412, 2940, 1679, 1603, 1502, 1435, 1362; UV (λ_{max} in MeOH) 303 nm (ε = 9000); MS (FAB, NaI) 377 (M + H, relative intensity 12), 307 (25), 289 (15), 154 (100), 136 (73), 107 (28), 89 (30), 77 (38); HRMS (FAB, NaI) calcd for $C_{14}H_{21}N_2O_4S_3$ (M + H) 377.0662, found 377.0671; $[\alpha]_D$ +103.0° (*c* 1.3, MeOH). Anal. Calcd for $C_{14}H_{20}N_2O_4S_3$: C, 44.66; H, 5.35; N, 7.44. Found: C, 44.51; H, 5.46; N, 7.26.

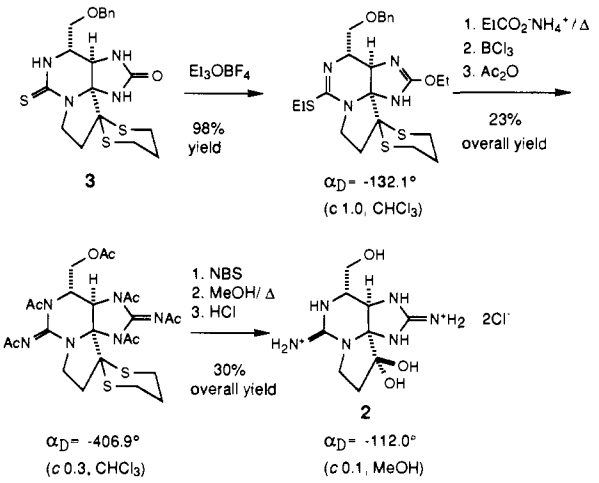
To a stirred suspension of the crude diol (902 mg, 2.40 mmol) and $NaHCO_3$ (2.12 g, 25.2 mmol) in CH_2Cl_2 (70 mL) was added dropwise $Et_3O{\cdot}BF_4$ in CH_2Cl_2 (1 M solution, 2.88 mL, 2.88 mmol) over 5 min, and the mixture was stirred at room temperature. After 0.5 h, the reaction mixture was quenched with 20% $KHCO_3$ (30 mL) and extracted with CH_2Cl_2 (3 × 100 mL). The combined organic layer was washed with H_2O (100 mL) and brine (100 mL), dried (K_2CO_3), filtered, and concentrated in vacuo. The residue was subjected to column chromatography (silica gel, 10:1 ethyl acetate/MeOH) to afford the imino thioether diol **11** (821 mg, 85%) as a pale yellow oil: ^1H NMR (CD_3OD) δ 1.31 (1 H, t, *J* = 7.4 Hz), 1.90 (1 H, m), 2.17 (1 H, m), 2.67 (1 H, td, *J* = 8.3, 12.9 Hz), 2.77 (2 H, tt, *J* = 4.0, 13.4 Hz), 2.93–3.12 (2 H, qq, *J* = 7.4, 13.0 Hz), 2.10 (1 H, m), 3.19 (2 H, m), 3.52 (2 H, m), 3.64–3.72 (2 H, m), 3.68 (1 H, m), 4.44 (1 H, d, *J* = 6.7 Hz); ^{13}C NMR ($CDCl_3$) δ 14.26, 25.03, 25.48, 28.27, 28.64, 40.36, 45.98, 51.66, 55.56, 59.63, 64.44, 73.36, 99.22, 149.58, 153.72, 168.52; IR (film) 3600–3000 cm^{-1}, 2927, 1720, 1668, 1578, 1433, 1366, 1259; UV (λ_{max} in MeOH) 315 nm (ε = 3700); MS (FAB, NaI) 405 (M + H, relative intensity 100), 343 (70), 307 (20), 289 (13), 154 (100), 136 (79), 107 (32), 89 (37), 77 (41); HRMS (FAB, NaI) calcd for $C_{16}H_{25}N_2O_4S_3$ (M + H) 405.0974, found 405.1001; $[\alpha]_D$ +4.4° (*c* 1.0, $CHCl_3$).

Imino Thioether Alcohol 13. To a stirred solution of diol **11** (500 mg, 1.24 mmol) in ethyl acetate (50 mL) was added $Pb(OAc)_4$ (423 mg, 1.31 mmol) at room temperature in small portions. The resulting orange solution was stirred at room temperature for 10 min, concentrated in vacuo to 5 mL, and filtered through a short silica gel column. The filtrate was concentrated in vacuo, and the residue was diluted with MeOH (50 mL) and cooled to 0 °C. $NaBH_4$ (234 mg, 6.19 mmol) was added to this solution at 0 °C in portions over 10 min, and the reaction mixture was stirred for 0.5 h at the same temperature. The reaction mixture was filtered through Celite and concentrated in vacuo, and the residue was dissolved in ethyl acetate (100 mL). The resulting solution was washed with water (50 mL) and brine (50 mL), dried ($MgSO_4$), filtered, and concentrated in vacuo. The residue was subjected to short column chromatography (silica gel, 1:2 hexanes/ethyl acetate) to give imino thioether alcohol **13** (380 mg, 82%) as a colorless oil: ^1H NMR (CD_3OD) δ 1.32 (3 H, t, *J* = 7.4 Hz), 1.91 (1 H, m), 2.17 (1 H, m), 2.61 (1 H, ddd, *J* = 7.7, 10.0, 12.8 Hz), 2.78 (2 H, m), 2.95–3.12 (2 H, qq, *J* = 7.4, 13.1 Hz), 3.16 (1 H, m), 3.19 (2 H, m), 3.52 (2 H, m), 3.65 (1 H, dt, *J* = 6.0, 9.5 Hz), 3.71 (1 H, dt, *J* = 2.2, 9.5 Hz), 3.76 (3 H, s), 4.38 (1 H, t, *J* = 5.1 Hz); ^{13}C NMR (CD_3OD) δ 14.47, 26.10, 26.36, 29.00, 29.23, 41.39, 47.26, 51.55, 56.91, 60.80, 65.69, 100.02, 150.85, 155.63, 167.96; IR ($CHCl_3$) 3500–3100 cm^{-1}, 2981, 1686, 1642, 1582, 1448, 1372, 1261; UV (λ_{max} in MeOH) 318 nm (ε = 7900); MS (DEI, NH_3) 375 (M + H, relative intensity 80), 343 (100), 269 (20), 154 (28), 136 (24), 95 (25), 69 (47), 55 (60), 43 (48); HRMS (DEI, NH_3) calcd for $C_{15}H_{23}N_2O_3S_3$ (M + H) 375.0869, found 375.0864; $[\alpha]_D$ +44.9° (*c* 1.4, MeOH).

Imino Thioether Benzyl Ether 14. To a stirred solution of the imino thioether alcohol **13** (330 mg, 0.882 mmol) in CH_2Cl_2 (50 mL) were added benzyl trichloroacetimidate (642 mg, 2.65 mmol) and ground 4-Å molecular sieves. Trifluoromethanesulfonic acid (124 mL, 0.927 mmol) was added dropwise, and the resulting reaction mixture was stirred for 10 h at room temperature. The reaction mixture was quenched with saturated $NaHCO_3$ solution (10 mL), and the aqueous layer was extracted with CH_2Cl_2 (3 × 30 mL). The combined organic layers were washed with brine (30 mL), dried ($MgSO_4$), filtered, and concentrated in vacuo. The residue was purified by column chromatography (silica gel, 4:1 hexanes/ethyl acetate) to give benzyl ether **14** (163 mg, 63%

(26) Strichartz, G. R.; Hall, S.; Magnani, B.; Hong, C. Y.; Kishi, Y.; DeBin, J. A.; Koschorke, G.-M. Manuscript in preparation.

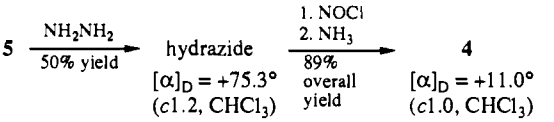
Scheme VI



based on the startig material recovery) as a colorless oil: ^{1}H NMR ($CDCl_3$) δ 1.32 (3 H, t, J = 7.4 Hz), 1.99 (1 H, m), 2.13 (1 H, m), 2.51 (1 H, ddd, J = 7.6, 9.6, 12.6 Hz), 2.78 (2 H, m), 2.95 (2 H, m), 2.96 (1 H, m), 3.03 (2 H, m), 3.53 (1 H, dd, J = 3.7, 9.7 Hz), 3.57 (1 H, dd, J = 5.7, 9.7 Hz), 3.62 (2 H, m), 4.53 (2 H, dd, J = 12.4 Hz), 4.72 (1 H, dd, J = 3.9, 5.6 Hz); ^{13}C NMR ($CDCl_3$) δ 14.17, 24.77, 25.49, 28.18, 28.48, 40.25, 45.72, 51.01, 55.76, 57.89, 73.03, 73.24, 98.55, 127.08, 127.19, 128.06, 128.18, 128.50, 139.02, 149.61, 152.51, 165.52; IR ($CHCl_3$) 2950 cm^{-1}, 1701, 1640, 1584, 1451, 1373, 1260, 1190; UV (λ_{max} in MeOH) 318 nm (ϵ = 8200); MS (FAB, NaI) 465 (M + H, relative intensity 7), 343 (10), 207 (15), 147 (43), 136 (28), 109 (20), 95 (36), 81 (47), 73 (100), 55 (86), 43 (68); HRMS (FAB, NaI) calcd for $C_{22}H_{28}N_2O_3S_3Na$ (M + Na) 487.1158, found 487.1183; $[\alpha]_D$ +68.4° (c 1.1, $CHCl_3$).

Thiourea 5. To the imino thioether benzyl ether **14** (151 mg, 0.325 mmol) in a precooled small autoclave was added H_2S (5 mL) via cannula at −78 °C. The autoclave was closed tightly and allowed to warm to room temperature. After 2 days, the reaction vessel was cooled to −78 °C, and the pressure was released carefully. The autoclave was warmed to room temperature, and the residue was diluted with CH_2Cl_2. The extracts were concentrated in vacuo, and the residue was purified by short column chromatography (silica gel, 5:1 hexanes/ethyl acetate) to give thiourea **5** as a white solid (129 mg, 91%). An analytical sample was recrystallized from hexanes/ethyl acetate to give white needles, mp 53–54 °C: ^{1}H NMR ($CDCl_3$) δ 1.95 (1 H, m), 2.18 (1 H, m), 2.63 (1 H, td, J = 8.0, 12.7 Hz), 2.82 (2 H, m), 2.91 (1 H, ddd, J = 3.9, 6.7, 12.8 Hz), 3.08 (2 H, m), 3.52 (1 H, dd, J = 8.3, 9.5 Hz), 3.59 (1 H, dd, J = 3.1, 9.6 Hz), 3.80 (3 H, s), 4.03 (1 H, ddd, J = 3.5, 7.5, 11.1 Hz), 4.11 (1 H, ddd, J = 6.7, 8.2, 11.1 Hz), 4.37 (1 H, td, J = 3.0, 8.2 Hz), 4.53 (2 H, s), 7.01 (1 H, s), 7.28–7.40 (5 H, m); ^{13}C NMR ($CDCl_3$) δ 24.70, 28.34, 39.56, 49.32, 51.53, 53.32, 56.60, 71.96, 73.44, 102.16, 127.59, 127.77, 128.45, 137.67, 146.54, 164.87, 176.23; IR ($CHCl_3$) 3418 cm^{-1}, 2929, 1704, 1636, 1503, 1434, 1363, 1250, 1120; UV (λ_{max} in MeOH) 309 nm (ϵ = 9200); MS (FAB, NaI) 437 (M + H, relative intensity 50), 419 (18), 405 (18), 315 (38), 307 (100), 289 (67), 275 (30), 223 (25); HRMS (FAB, NaI) calcd for $C_{20}H_{25}N_2O_3S_3$ (M + H) 437.1025, found 437.1034; $[\alpha]_D$ +85.2° (c, 1.1, $CHCl_3$). Anal. Calcd for $C_{20}H_{24}N_2O_3S_3$: C, 55.02; H, 5.54; N, 6.42. Found: C, 54.88; H, 5.62; N, 6.26.

The transformation of **5** to **4** was carried out as reported in the racemic synthesis of saxitoxin.[13] The spectroscopic data of the following intermediates were superimposable on those reported for the racemic series, except the optical rotations.



Tricyclic Urea–Thiourea 3. A solution of bicyclic urea–thiourea **4** (91.0 mg, 0.209 mmol) in a mixture of AcOH (10 mL) and dichloroacetic acid (1.5 mL) was stirred at 55–60 °C under N_2. After 2 days, the solvent was evaporated in vacuo, and the residue was dissolved in 10% MeOH in CH_2Cl_2 (50 mL). The resulting solution was washed with saturated $NaHCO_3$ solution (2 × 10 mL), dried ($MgSO_4$), filtered, and concentrated in vacuo. The residue was purified by PTLC (silica gel, 20:1 CH_2Cl_2/MeOH) to give tricyclic urea–thiourea **3** (51.0 mg, 56%) as a white solid. An analytical sample was recrystallized from hexanes/ethyl acetate to give fine white crystals, mp 134–136 °C: ^{1}H NMR ($CDCl_3$) δ 1.89 (1 H, m), 2.08 (1 H, m), 2.27 (1 H, td, J = 9.9, 13.6 Hz), 2.78 (1 H, dd, J = 7.7, 13.7 Hz), 2.82–2.98 (4 H, m), 3.45 (1 H, m), 3.53 (1 H, dd, J = 6.9, 9.3 Hz), 3.61 (1 H, dd, J = 6.7, 9.2 Hz), 3.90 (1 H, t, J = 10.3 Hz), 4.08 (1 H, dt, J = 9.1, 9.9 Hz), 4.37 (1 H, d, J = 4.7 Hz), 4.55 (2 H, s), 5.37 (1 H, s), 6.05 (1 H, s), 6.64 (1 H, s), 7.28–7.40 (5 H, m); ^{13}C NMR ($CDCl_3$) δ 24.47, 27.37, 27.64, 34.95, 47.49, 53.36, 56.09, 61.87, 69.53, 73.67, 82.98, 128.03, 128.53, 137.32, 160.24, 181.40; IR ($CHCl_3$) 3413 cm^{-1}, 1725, 1602, 1482, 1455, 1425, 1328, 1265, 1226; UV (λ_{max} in MeOH) 225 nm (ϵ = 18 500); MS (FAB, NaI) 459 (M + Na, relative intensity 15), 437 (M + H, 5), 329 (15), 199 (13), 176 (100), 154 (19), 91 (17), 77 (10); HRMS (FAB, NaI) calcd for $C_{19}H_{24}N_4O_3S_3Na$ (M + Na) 459.0957, found 459.0988; $[\alpha]_D$ −93.6° (c 1.1, $CHCl_3$).

The transformation of **2** to **3** was carried out as reported in the racemic synthesis of saxitoxin.[13] The spectroscopic data of the intermediates in Scheme VI were superimposed on those reported for the racemic series, except the optical rotations.

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Supplementary Material Available: Tables of X-ray crystallographic analysis data for α-**7** (10 pages); table of observed and calculated structure factors (14 pages). Ordering information is given on any current masthead page.