TOTAL SYNTHESIS OF d,l-SAXITOXIN

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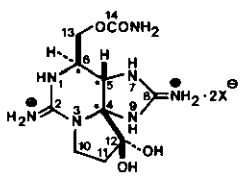
Saxitoxin is a potent toxic substance responsible for paralytic shellfish poisoning. Sporadic outbreaks of food toxication from mussels and clams along the Pacific coast of North America have long been known. During the 1920's it was shown that shellfish became toxic only during the time of the "red tides", which occur upon the blooming of the dinoflagellate, Gonyaulax catenella. Isolation, characterization and structure elucidation of saxitoxin was energetically carried out by Schantz and by Rapoport. However, the final conclusion on the structure had to await the results of X-ray analysis. Three new toxins in addition to saxitoxin were isolated from soft shell clams, Mya arenaria, collected during the red tide outbreaks on the New England coast. Two of the three new toxins, called gonyautoxins II and III, were revealed as 11α- and 11β-hydroxysaxitoxin, respectively.[1] In this article, we would like to review our efforts on the total synthesis of saxitoxin.[2,3]

Saxitoxin is now known to be formulated as Structure 1. The most remarkable

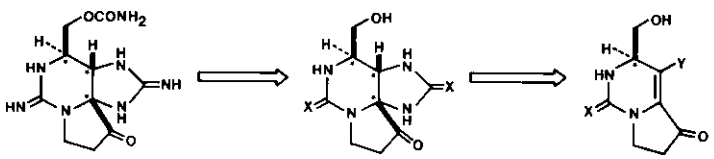


1 : SAXITOXIN

structural aspect of saxitoxin is the fact that all carbon atoms except the one at the C-11 position carry hetero atoms; thus, the proper control of the functional groups would be one of the major tasks in a saxitoxin synthesis. In addition, control of three asymmetric centers at the 4-, 5-, and 6-positions is necessary. One possible retrosynthetic route is depicted in Scheme 1. The two guanidino

groups were considered synthetically equivalent to the corresponding urea or thiourea groups. The reason for postponing the introduction of the guanidino groups

Scheme 1

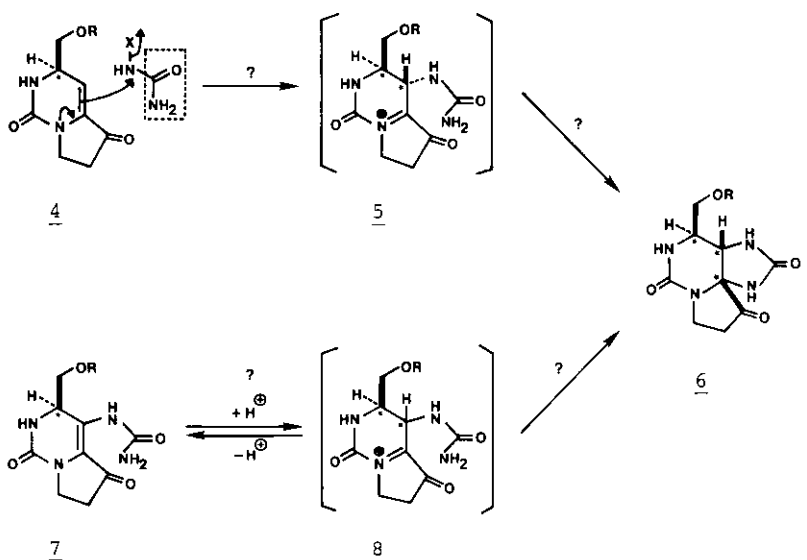


1 2 : X=O or S 3

to the very late stage of the synthesis was to avoid as much as possible technical difficulties in handling the very basic, polar guanidine derivatives. The carbamoyl group at the C-13 position was expected to be introduced by one of the known methods.

The next retrosynthetic step, i.e. 2 → 3, is explained in Scheme 2. One possibility was a sort of amination reaction of 4 with a properly protected and functionalized urea. The approach of the aminating reagent would take place

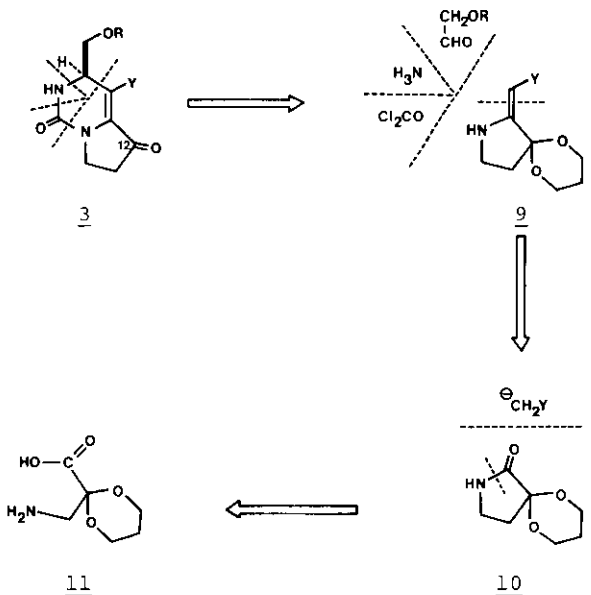
Scheme 2



preferentially from the opposite side of the hydroxymethyl group resulting in the desired stereochemistry at the C-4 and C-5 positions. An alternative possibility was the acid-catalyzed cyclization of the urea 7. Assuming that rapid protonation-deprotonation would be followed by slow cyclization, the desired stereochemistry at the C-4 and C-5 positions might be expected.

A possible disconnection of the urea 3 is shown in Scheme 3. In the first retrosynthetic step in this scheme, three carbon-nitrogen bonds and one vinylogous carbon-nitrogen bond were disconnected. Thus, we hoped that the urea 3 could be

Scheme 3



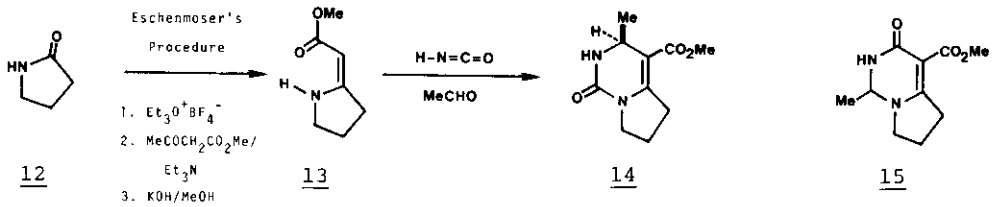
3 9 10 11

constructed from four indicated fragments. One of the encouraging factors in this disconnection was that the necessary enamine 9 could be synthesized by adapting the Eschenmoser procedure[4].

The feasibility of the retrosynthesis described above was first tested in the C-12 deoxo series, simply because of the availability of the starting material. Thus, 2-pyrrolidinone (12) was converted to the vinylogous urethane 13 by using Eschenmoser's procedure except for step 3, which is a straightforward cleavage reaction of a β-keto, β'-imino ester system under basic conditions. Treatment of the vinylogous urethane 13 with isocyanic acid in the presence of acetaldehyde in ether at room temperature yielded a product in excellent yield. This cyclization

reaction was found to work well with various aldehydes. The spectroscopic data for this product were consistent with the desired urea ester 14. However, the

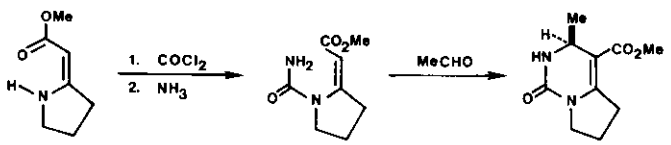
Scheme 4



12 13 14 15

alternative structure 15 was not necessarily eliminated. To establish the structure of the product conclusively, the following two experiments were carried out. The first experiment was the step-wise transformation of the vinylogous urethane 13 to the urea ester 14 as shown in Scheme 5. After the vinylogous urethane 13 was converted to the urea 16 under standard conditions, the cyclization was effected in acetic acid in the presence of acetaldehyde. The urea ester 14 thus obtained was identical with the product from the previous route. The

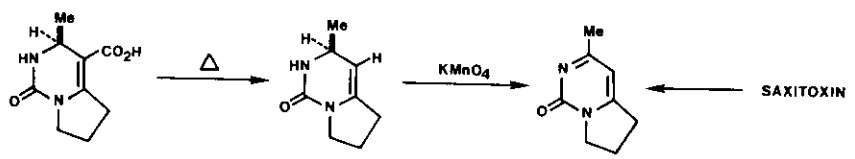
Scheme 5



13 16 14

second experiment was the derivatization of the urea ester 14 to the pyrimidine 19, known as a degradation product of saxitoxin, as shown in Scheme 6. After hydrolysis of the ester group, the urea ester 14 was subjected to a decarboxylation

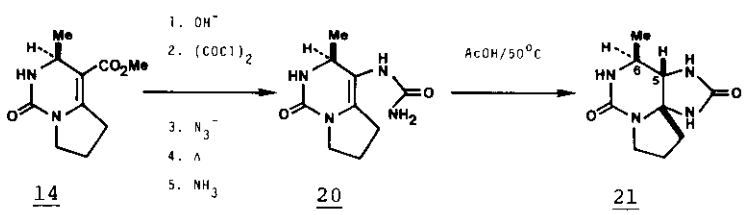
Scheme 6



17 18 19

reaction at 220°C, to afford the urea 18. Permanganate oxidation of 18 gave the pyrimidine 19, the spectroscopic data of which were superimposable on those reported by Rapoport[5]. There was one additional reason for the derivatization just described; that was to use the urea 18 in testing the feasibility of the cyclization reaction proposed in the upper half of Scheme 2. However, we soon realized that the proposed cyclization reaction was not experimentally feasible because of the instability observed for the urea 18. For this reason we studied the second possibility proposed in Scheme 2. The urea ester 14 was converted to the urea 20 in excellent overall yield by the standard procedures summarized in Scheme 7. The urea 20 was found to cyclize to the tricyclic urea 21 in acetic acid at 50°C in high yield. Examination of the NMR spectra of the crude cyclization product showed no signals corresponding to the other isomer (vide infra).

Scheme 7



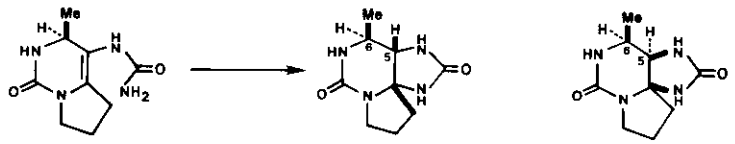
14 20 21

TRI-N-METHYL DERIVATIVE : $J_{5,6}$ = 1.0 Hz

cf. SAXITOXIN : $J_{5,6}$ = 1.3 Hz

The stereochemistry of 21 was concluded from analysis of the NMR spectra of the tri-N-methyl derivative of 21; the spin-spin coupling constant (1.0 Hz) between the C-5 and C-6 protons was almost identical with that (1.3 Hz) of saxitoxin. Cyclization of 20 was also effected in a boiling mixture of ethanol and toluene, to yield the tricyclic urea 21; again, no signals corresponding to its stereoisomer were detected in the NMR spectra of the crude product. On the other hand, treatment of 20 with neat trifluoroacetic acid at room temperature gave a 2:3 mixture of the tricyclic ureas 21 and 22 in high yield. The spin-spin coupling constant between the C-5 and C-6 protons of 22 was found to be 3.5 Hz, which provided further support for the previous assignment of the stereochemistry of 21.

Scheme 8



20 21 22

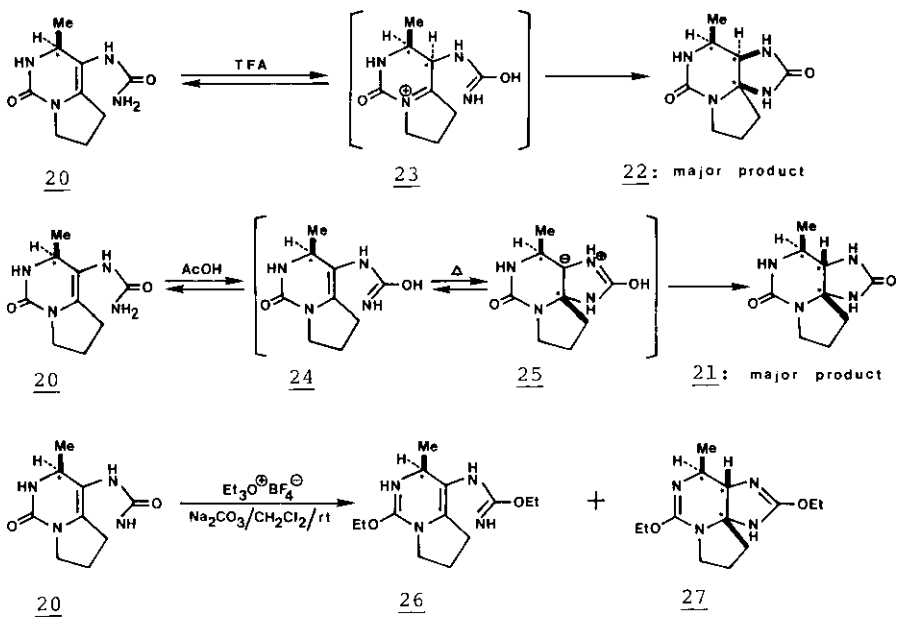
$J_{5,6}$ = 1.0 Hz* $J_{5,6}$ = 3.5 Hz

* J of tri-N-methyl derivative

Cyclization Conditions	Ratio
AcOH / 50°C	20 >> 1
EtOH - $C_6H_5CH_3$ / reflux	20 >> 1
TFA / rt	2 : 3

The tricyclic ureas 21 and 22 were not interconvertible under the trifluoroacetic acid or acetic acid conditions. These results may suggest that two different processes of cyclization are operating. Acetic acid could catalyze the enolization

Scheme 9



20 23 22: major product

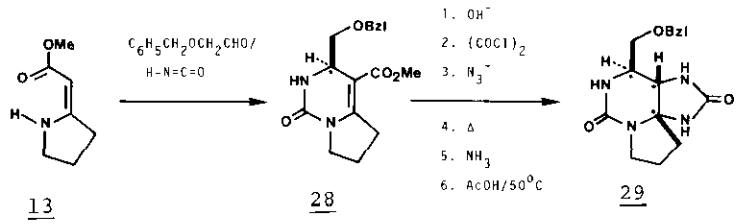
20 24 25 21: major product

20 26 27

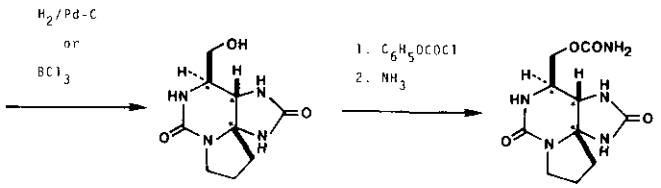
of 20 into 24, which would then cyclize to the zwitter ion 25. In the proposed electrocyclization process, the urea group would be expected to approach the C-4 position from the α side for steric reasons to yield 25, protonation-deprotonation of which yields the tricyclic urea 21. Supporting evidence for this proposed electrocyclization process is the fact that treatment of 20 with triethyloxonium tetrafluoroborate in methylene chloride in the presence of sodium carbonate at room temperature yields a mixture of 26 and 27. In strong acidic media such as trifluoroacetic acid, protonation of the unsaturated urea system of 20 might be taking place, followed by a cyclization process. The reason why a 2:3 mixture of 21 and 22 is produced in a strong acidic medium is not clear at this point; this could be due to a non-stereoselective protonation, due to a rapid protonation-deprotonation followed by a slow cyclization, or due to the operation of both ionic and electrocyclization processes in trifluoroacetic acid.

Having established an effective method to construct all three asymmetric centers existing in saxitoxin, we turned our attention to the functional group at the C-13 position. This problem was easily solved by replacing acetaldehyde with benzyloxyacetaldehyde for the cyclization reaction discussed before. This synthesis is summarized in Scheme 10. The tricyclic urea 29 was again the only detectable product under the acetic acid conditions.

Scheme 10



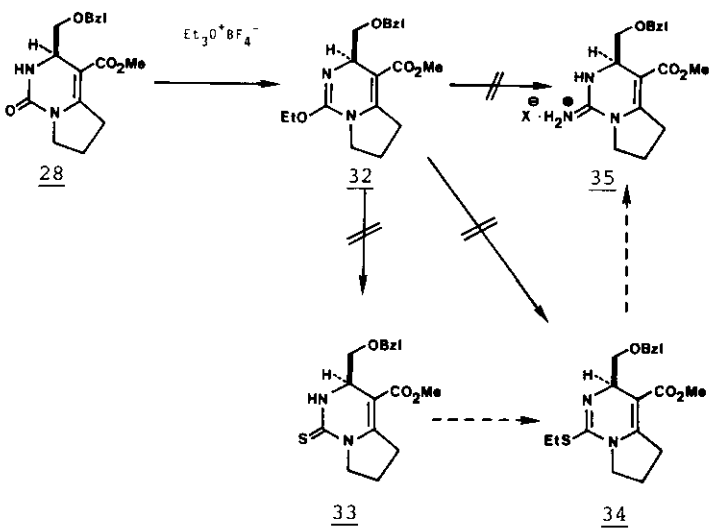
13 28 29



30 31

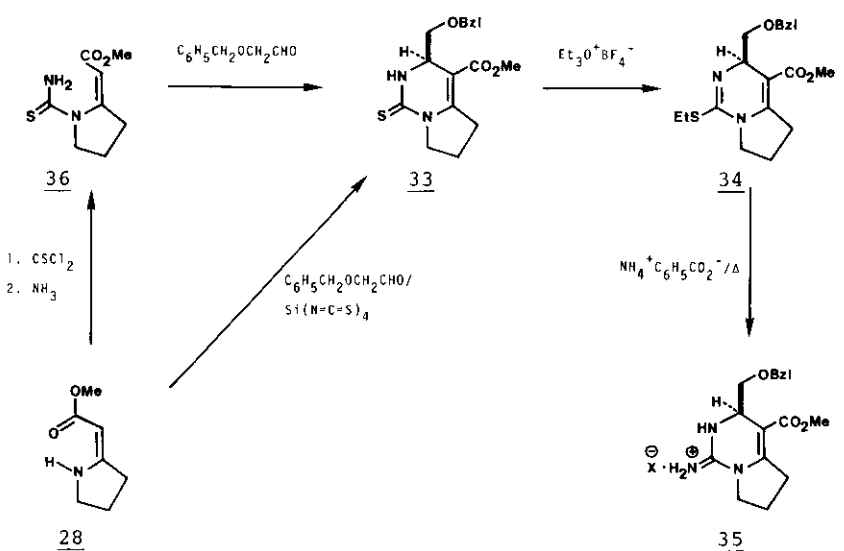
Functionalization of the C-2 position turned out more difficult than anticipated. For example, all attempts to transform the iminoether 32 to the guanidine 35 or the thioiminoether 34 by a variety of methods were uniformly fruitless.

Scheme 11



Under these circumstances, we naturally tried the preparation of the thiourea ester 33 directly from 28; indeed, this vinylogous urethane was successfully converted

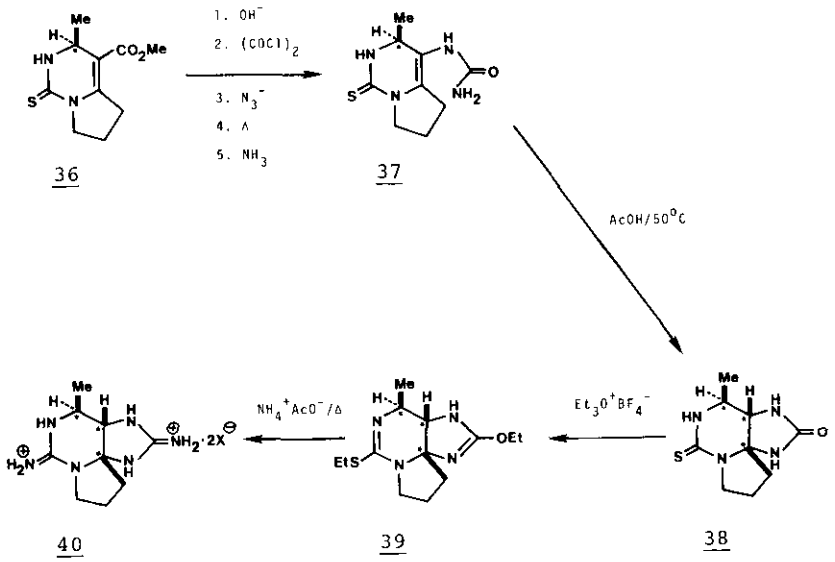
Scheme 12



to the thiourea ester 33 by two methods. The method using silicon tetraisothiocyanate[6] in benzene was excellent in terms of overall yield as well as simplicity of the operation. The thioiminoether 34, prepared from 33, was found to react smoothly with ammonium acetate or benzoate at 200°C to yield the guanidine 35.

Related to our next objective, functionalization of the C-8 position, we should describe briefly the technical difficulties encountered throughout this synthesis. As anticipated, almost all of the synthetic intermediates were extremely polar, hence we often experienced that the solvent choice for a reaction or the chromatographic choice for isolation of products were not trivial. However, we soon realized the more serious problem was that the NMR spectroscopy was very often useless in determining the structure of a product or of a product ratio, since almost all synthetic intermediates rarely gave easily recognizable signals. To overcome this problem, we used extensively the C-13 deoxy compounds such as 36 , which gave a nice doublet signal due to the methyl group. Greatly assisted by this small structural modification, the synthesis of the bisguanidine 40 was successfully carried out as shown in Scheme 13.

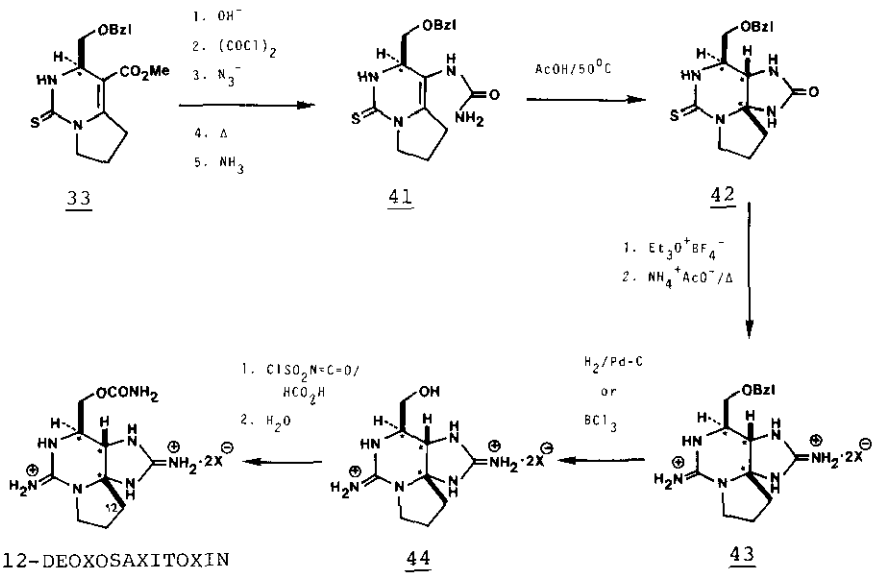
Scheme 13



Following the methods established in the C-13 deoxy series, 12-deoxosaxitoxin (45) was successfully synthesized by the method summarized in Scheme 14. No

special comments should be necessary for each of the steps used except for the choice of solvent in transforming the C-13 hydroxy group to the corresponding carbamoyl group. The solubility of the bisguanidine 44, as anticipated, was practically none except in water or methanol, which made it almost impossible to use a standard method for the necessary transformation. After many frustrating attempts, we finally discovered that the bisguanidine 44 was nicely soluble in

Scheme 14



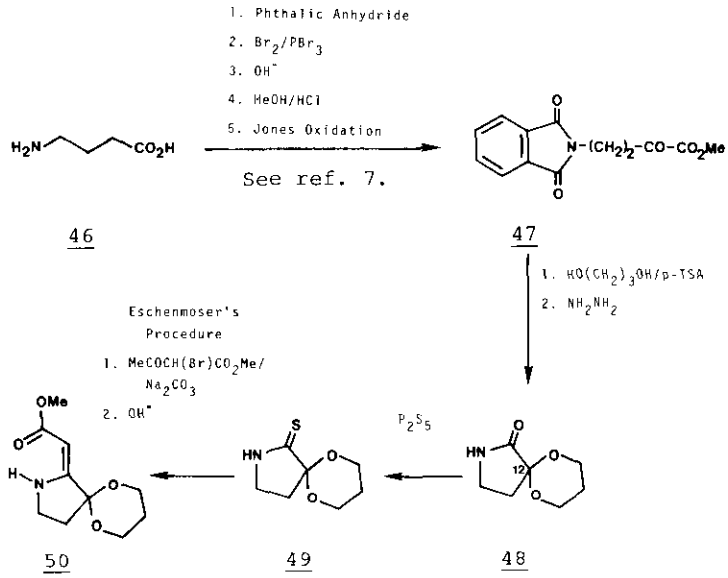
33 41 42

45 : 12-DEOXOSAXITOXIN 44 43

neat formic acid at 5°C. This choice of solvent sounds strange, since chlorosulfonyl isocyanate is known to react with carboxylic acids, but we had no other option. No toxicity against mice was observed for 12-deoxosaxitoxin (45).

Proper adjustment of the functional group at the C-12 position was necessary to achieve the total synthesis of saxitoxin. Thus, the lactam 48 was synthesized by the method summarized in Scheme 15. After the lactam 48 was converted to the corresponding thiolactam 49, the Eschenmoser procedure[4] was again used to prepare the vinylogous urethane 50. The preparation of 50 by this route was very effective in terms of reproducibility and overall yield, but there were three technical problems. First, the Hell-Volhard-Zelinskii reaction was laborious for a large-scale experiment. Second, the hydrolysis of the resultant β-bromoacid to the corresponding β-hydroxyacid under basic conditions took place sometimes too

Scheme 15

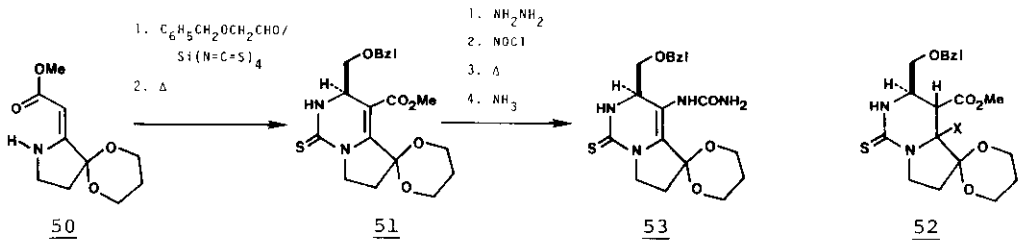


vigorously to control. Third, most serious, the solubility of the thiolactam 49 in organic solvent was poor so that an enormous amount of chloroform was required to work up the phosphorus pentasulfide reaction. These technical problems were solved by a new approach to the vinylogous urethane 50, which is discussed later.

The vinylogous urethane 50 was then converted to the thiourea ester 51 by the method established in the 12-deoxo series. Although 50 reacted similarly toward silicon tetraisothiocyanate and benzyloxyacetaldehyde, a part of the product existed as 52, which was smoothly transformed to 51 in refluxing toluene. The thiourea ester 51 was converted to the thiourea urea 53 by a sequence of reactions used previously (see Scheme 7), but the overall yield was unacceptably low, mainly because the reactivity of the methyl ester of 51 was poor and hence forcing conditions were necessary for its hydrolysis. This problem was solved by the reaction of 51 with hydrazine, followed by nitrosyl chloride oxidation.

The thiourea urea 53 was found, as we feared, very labile toward acids. For example, brief treatment of 53 with acetic acid or trifluoroacetic acid at room temperature yielded the pyrimidine 54 almost instantaneously. The observed acid-instability of 53 was a very serious problem since the best cyclization conditions discovered in the 12-deoxo series were "acetic acid at 50°C". In order to increase

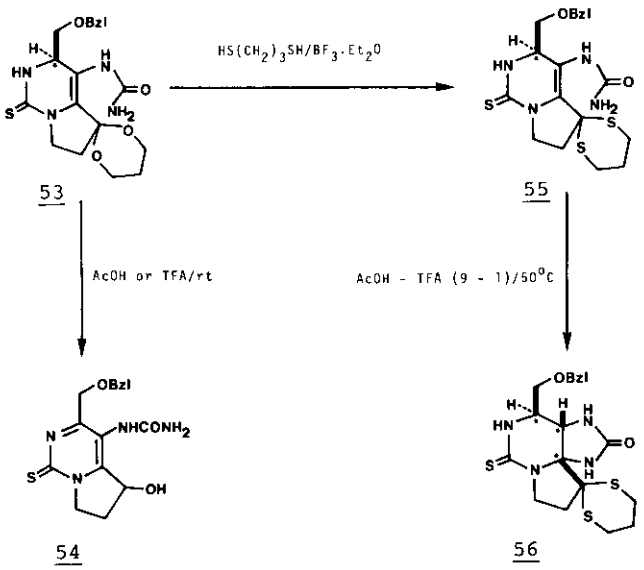
Scheme 16



50 51 53 52

the acid-stability of 53, we then planned to replace the ketal group of 53 with the thioketal group. This seemingly difficult transformation was achieved under carefully controlled conditions; 53 was first dissolved in acetonitrile containing 1,3-propanedithiol, then borontrifluoride etherate was carefully added to the solution at room temperature. The thiourea thioketal urea 55 was found, as anticipated, to be acid-stable. Thus, the best cyclization conditions, "acetic acid at 50°C", were applied to 55, and the desired tricyclic thiourea 56 was isolated. However, the rate of the cyclization under these conditions was too slow,

Scheme 17

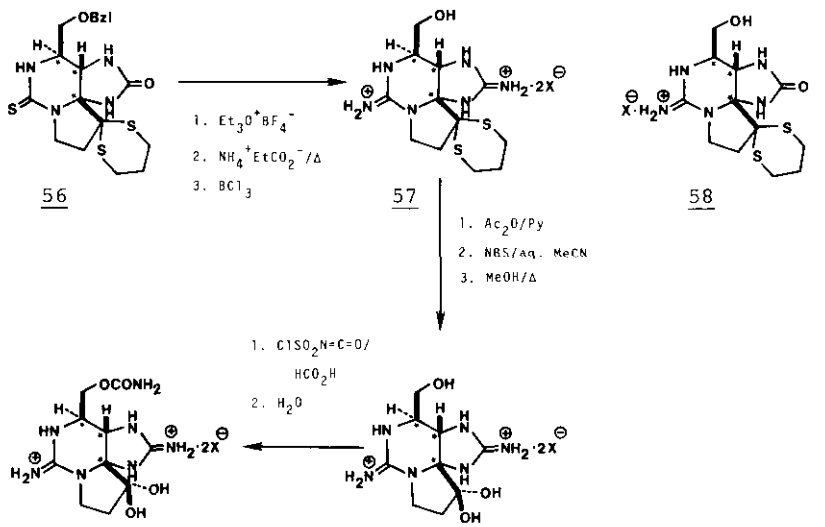


53 55 54 56

perhaps because of the steric hindrance of the thioketal group, for practical purposes. For this reason the cyclization was ultimately effected in a 9:1 mixture of acetic acid and trifluoroacetic acid at 50°C, resulting in a 12:1 mixture favoring the desired product 56. In neat trifluoroacetic acid, a 5:1 mixture favoring the undesired product was produced.

Functionalization of the C-2 and C-8 positions of 56 was achieved by the method used in the 12-deoxo series (see Scheme 13). Only one minor change, using ammonium propionate instead of ammonium acetate or benzoate, was made, because it was easier to prepare anhydrous ammonium propionate so that the amount of the by-product, monoguanidine monourea 58, was kept at a minimum. After complete acetylation, the bisguanidine 57 was treated with NBS in aqueous acetonitrile to hydrolyze the thioketal group. Hydrolysis of the acetyl group was conveniently carried out in methanol at 100°C to yield decarbamoylsaxitoxin (59). Decarbamoylsaxitoxin was then converted to saxitoxin (1) by using the method described previously. By comparison of NMR spectra, tlc, and toxicity against mice, the total synthetic saxitoxin was confirmed identical with natural saxitoxin.

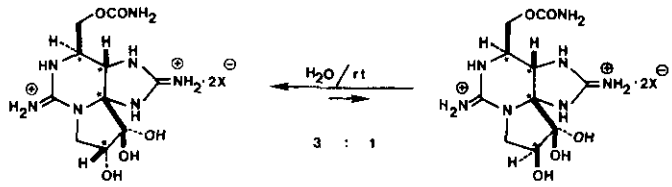
Scheme 18



56 57 58

1 : SAXITOXIN 59

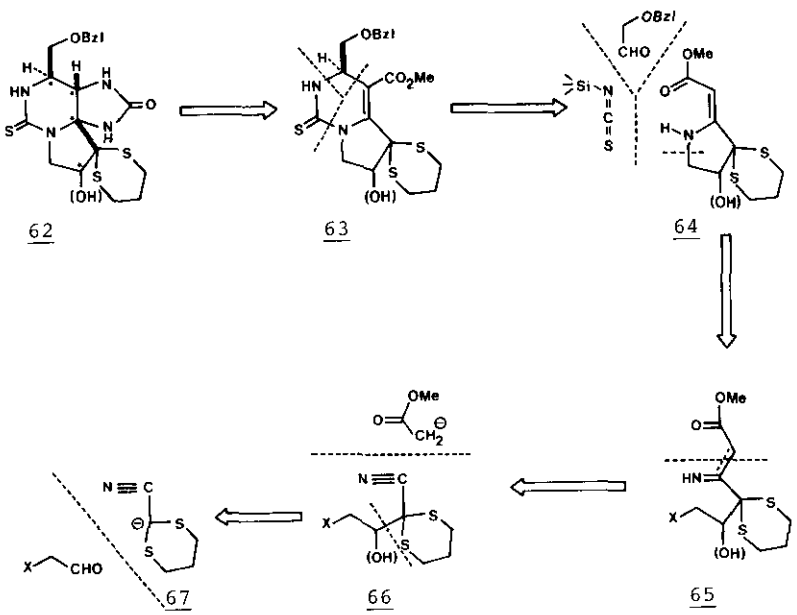
We would now like to turn our attention to synthetic efforts toward gonyautoxins II and III.[8] Two methylene hydrogens at the C-11 position of saxitoxin are known to undergo slow, reversible deuterium exchange in deuterium oxide at room temperature,[9] which would suggest the possibility of oxidizing saxitoxin to gonyautoxins. The stereochemistry of the oxidation, if realized, would not be a problem since gonyautoxins II and III are known to exist as a 3:1 equilibrium mixture in water at room temperature.[1,10] In spite of this preference, all our attempts have been fruitless up to this time. Under these circum-



60 : GONYAUTOXIN II 61 : GONYAUTOXIN III

stances, we decided to attack gonyautoxins by a total synthetic approach. One additional encouragement for this was that the three technical problems mentioned previously could be studied together.

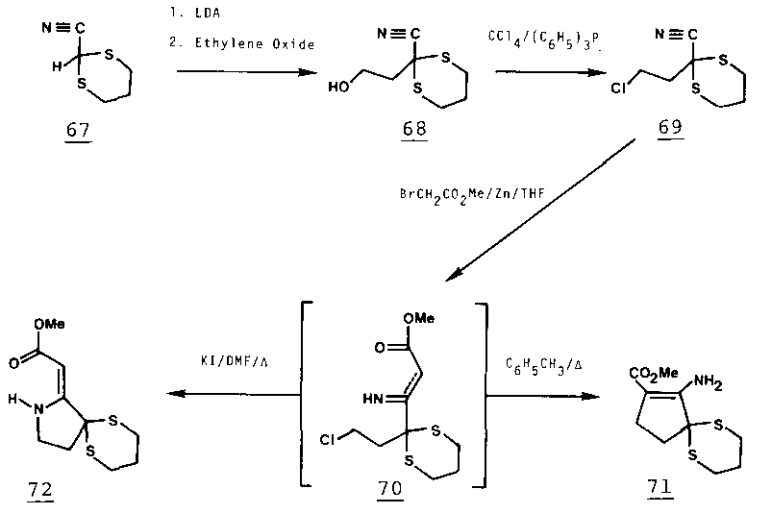
Scheme 19



A possible retrosynthetic route to gonyautoxins is shown in Scheme 19. Judging from the experience previously received, the key problem to be solved seemed the establishment of an efficient and practical synthesis of the vinylogous urethane 64. We decided to examine a new route to this substance depicted in the lower half of Scheme 19. There were two important questions to be examined; first, cyclization of 65 to 64, and second, Reformatsky-type reaction to make a carbon-carbon bond of 65 from 66.

The feasibility of this sequence was first studied in the saxitoxin series. The starting material necessary for this study, cyanodithiane (67), was prepared by Hayashi's method[11] with slight modification. The anion, prepared from the dithiane 67, reacted nicely with ethylene oxide to yield the alcohol 68, which was then converted to the chloride 69. Some examples for Reformatsky-type reactions between α-bromoester and nitrile are known.[12] Indeed, methyl bromoacetate and the chloride 69 reacted smoothly in tetrahydrofuran in the presence of zinc dust to yield an unstable product, 70. Without isolation, this product was refluxed in anhydrous toluene to yield exclusively the cyclopentene 71 in excellent yield. On the other hand, 70 behaved differently in dimethylformamide in the presence of potassium iodide at 140°C, to give the desired vinylogous urethane 72 in excellent yield. Thus, it is now possible to prepare the vinylogous urethane

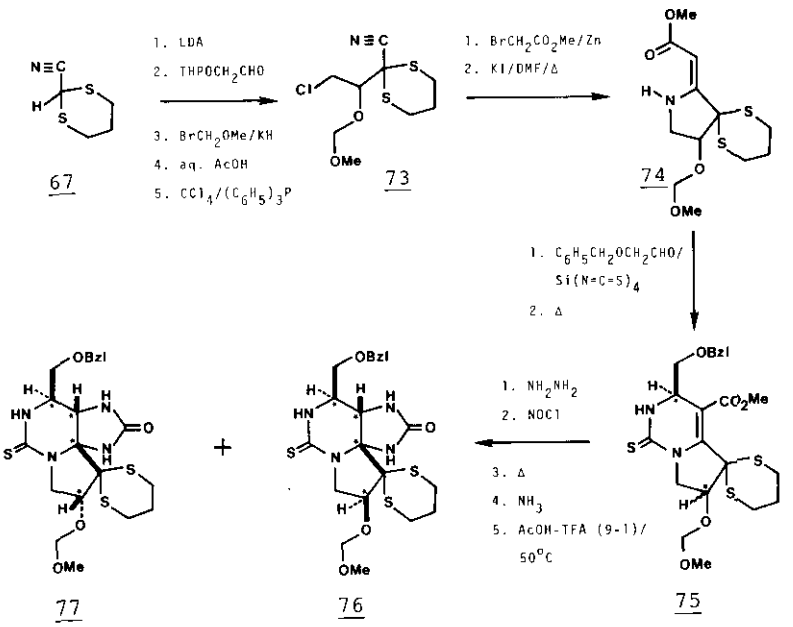
Scheme 20



72 in a fairly large quantity by a short route. There was no problem converting the vinylogous urethane 72 to the thiourea urea 55 of Scheme 17 by the method used previously.

The anion prepared from 67 reacted smoothly also with tetrahydropyranyloxyacetaldehyde as shown in Scheme 21. The Reformatsky-like reaction of the chloride, followed by cyclization in hot dimethylformamide, worked nicely to yield the vinylogous urethane 74, which turned out fortunately stable. Transformation of the vinylogous urethane 74 to the thiourea ester 75, isolated as an about 1:1 mixture of the two possible diastereomers, was effected as before. The thiourea ester 75 was then converted to the conjugated urea in four steps. At this stage,

Scheme 21



the diastereomers were separated by silica gel chromatography. Cyclization of the separated conjugated ureas was effected again by a 9:1 mixture of acetic acid and trifluoroacetic acid at 50°C to yield the tricyclic thiourea urea 76 and 77, respectively. Having the tricyclic thiourea ureas in hand, we now see a realistic possibility for a total synthesis of gonyautoxins II and III in the near future.

ACKNOWLEDGMENT

It is a great pleasure to conclude this presentation by expressing my sincere appreciation to Drs. H. Taguchi, H. Yazawa, J. F. Arnett, T. Kaneko, H. Tanino, T. Nakata, and Mr. S. M. Hannick, who worked on the various stages of this synthesis. Their devoted and skillful work made possible a very successful conclusion. Financial support from the National Institutes of Health (Grant No. NS 12108) is gratefully acknowledged.

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3. The numbering corresponds to that of saxitoxin (see Structure 1).
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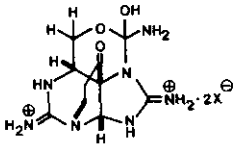
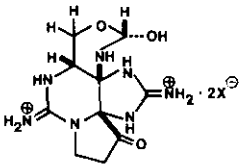
The following footnote, the content of which was mentioned in the opening remarks of a lecture delivered at the 6th Symposium on Heterocyclic Chemistry (Mulhouse, July 1-3, 1980), was added after the manuscript had been prepared.

From degradation and spectroscopic studies, Rapoport suggested the structure A for saxitoxin in 1971 (J. L. Wong, R. Oesterlin, H. Rapoport, J. Am. Chem. Soc., 93, 7344 (1971). However, it seemed for us difficult to explain chemical properties, for example the observed stability of saxitoxin under acidic conditions, if structure A was accepted as correct. We thought structure B might be an alternative as it better explained (1) the formation of the pyrimidine 19 from saxitoxin under phosphorous and hydriodic acid conditions, (2) the position (ν = 1770 cm^{-1}) of the carbonyl absorption of saxitoxin, and (3) the smooth Baeyer-Villiger oxidation observed for saxitoxin (note two positively charged guanidium groups attached at the α-position of the carbonyl group).

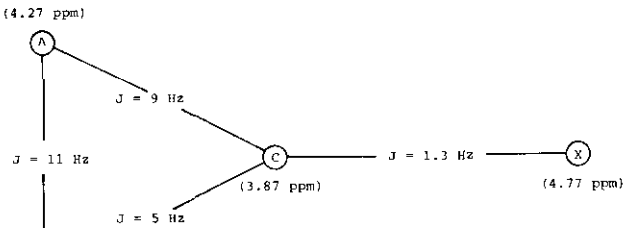


A



B

The proton NMR spectrum of saxitoxin in deuterium oxide was well analyzed by Rapoport. The unique four-spin system depicted below was assigned as $-OCH^{A}H^{B}-CH^{C}-C-CH^{X}-$ in which the spin-spin coupling constant of J = 11 Hz is



attributed to the geminal protons and J = 9 and 5 Hz to the vicinal. In the case of structure B, the assignment should be made as $-CH^{B}-CH^{A}H^{C}-O-CH^{X}-$, in which the spin-spin coupling constant of J = 9 Hz is attributed to the geminal protons, and J = 11 and 5 Hz to the vicinal. A spin-spin coupling constant due to geminal protons is known to have a negative sign, while that due to vicinal protons, a positive sign. Therefore, in the fall of 1972, spin-tickling experiments were carried out with the help of Mr. I. Miura at Columbia University to determine the relative sign of the three spin-spin coupling constants, J = 11, 9, and 5 Hz, and thus enable us to ascertain structure B, if correct. These experiments confirmed that the spin-spin coupling constant of J = 11 Hz has an opposite sign to those of J = 9 and 5 Hz; consequently, structure B was discarded. The correct structure 1, although considered in the early stage of these studies, had been mistakenly eliminated because it was difficult to explain the solvent effects on the dissociation constants of saxitoxin; the pK_a values of saxitoxin in water are known to be 8.24 and 11.60, while in 50% aqueous ethanol are 9.05 and above 11.