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Total Synthesis of (±)-Surugatoxin[1]

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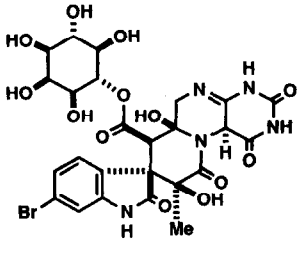
Abstract: Total synthesis of surugatoxin **1**, isolated from the toxic Japanese ivory shell (Babylonia japonica), was achieved from 6-bromoisatin by a stepwise ring construction involving two key steps: the stereospecific cyclization (**17** → **18**) and hydration (**33b** → **1**).

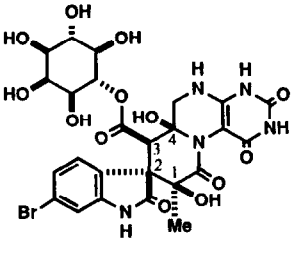
INTRODUCTION

Surugatoxin **1**, isolated from the toxic ivory shell (Babylonia japonica) by Kosuge et al.,[2] is a fascinating marine natural product. Its structure was determined by X-ray analysis in 1972.[2] However, structure **1'** first assigned to the toxin was corrected as **1** in 1981.[3] Neosurugatoxin **2**[3] reported in 1981 and prosurugatoxin **3**[4] in 1985 have been proven to be real causative agents of intoxication resulting from ingestion of the toxic shell, whereas **1** was found to be nontoxic.

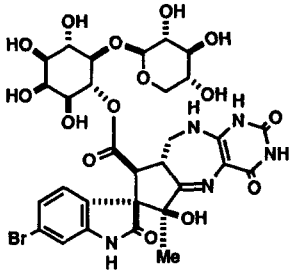
These three compounds **1**, **2**, and **3** have attracted much attention because of their quite new ring systems as well as their biological



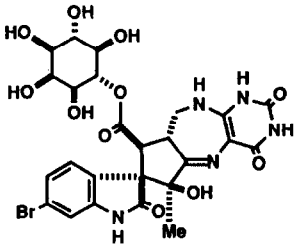
1'



***Surugatoxin* 1**



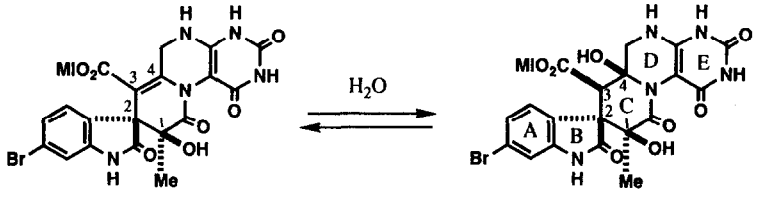
Neosurugatoxin 2



Prosurugatoxin 3

significance, all of them are worth attempting total synthesis. This paper deals with the first synthesis of (±)-surugatoxin **1**.[1]

The unique structure of surugatoxin **1** is characterized by a highly functionalized oxindolespiropiperidone moiety fused to a tetrahydropteridine ring, involving consecutive four asymmetric carbon atoms, which may vary their chiralities under certain reaction conditions. If 3,4-dehydrated surugatoxin **33b** is obtained, a hydroxyl group can be introduced properly to the C4-carbon atom in **33b** by taking advantage of the hydration-dehydration equilibrium between **33b** and **1**.

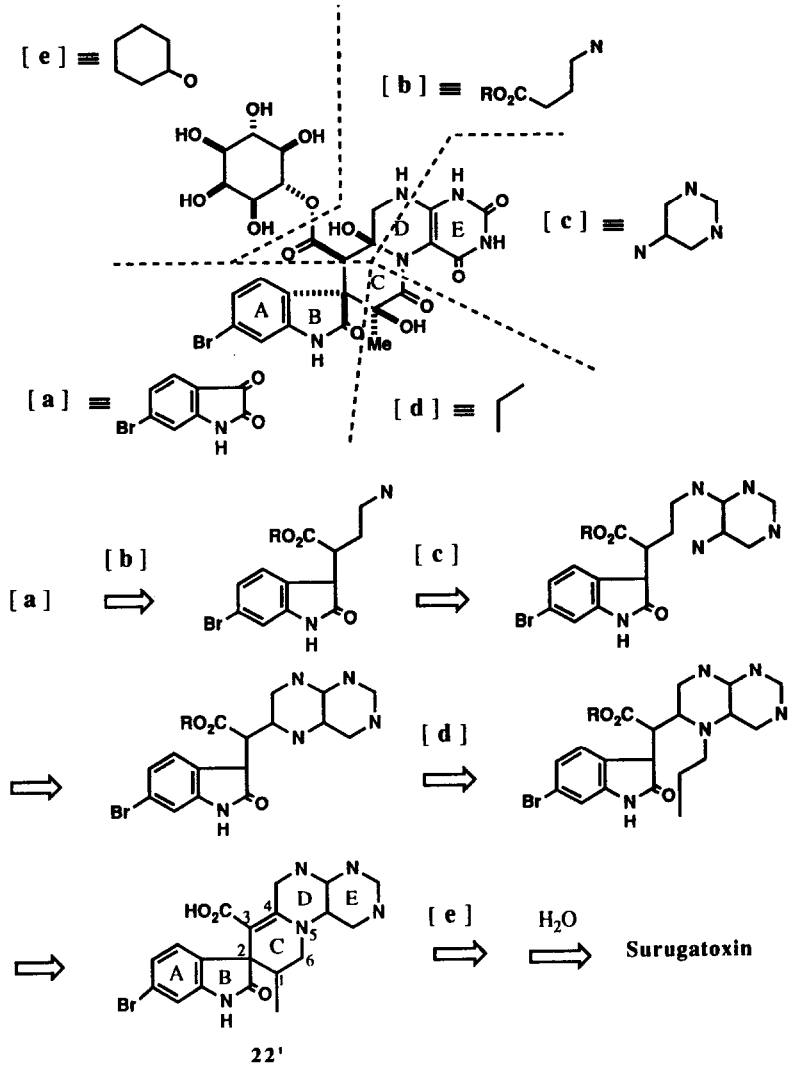


33b MI = *myo*-inositol **1**

Scheme 1

We focused on 3,4-dehydrated surugatoxin derivative **22** to be synthesized first so as to facilitate the construction of the pentacyclic framework. There will be several routes for constructing the pentacyclic ring system of the dehydrated surugatoxin derivative. To select the most appropriate route, many preliminary experiments were conducted, including the synthesis of the ethyl ester of four stereoisomeric aglycons of surugatoxin.[5-10] From these model experiments, it was confirmed that the chiralities of the consecutive four asymmetric carbons in ring C are variable and hydration-dehydration equilibrium occurs exactly giving a surugatoxin analogue and its dehydrated derivative.[5-10] Based on these findings, the following strategy was therefore designed for the synthesis

of surugatoxin.

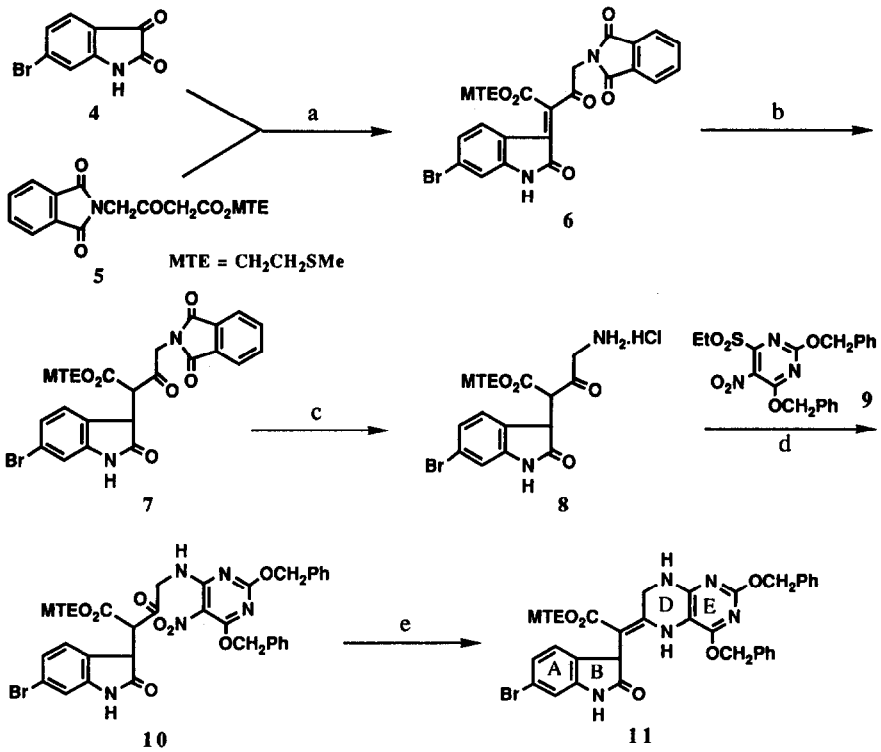


Scheme 2

The molecule of surugatoxin was divided into five moieties from [a] to [e]. According to our retrosynthetic analysis as shown with broken lines in Scheme 2, four segments shown as skeletal forms, [a], [b], [c], and [d] are combined one by one in alphabetical order to construct the surugatoxin framework **22'**, corresponding to the aglycon of the 3,4-dehydrated surugatoxin **22**. Esterification with alcohol [e] followed by hydration, at the final stage, leads to surugatoxin **1**.

RESULTS AND DISCUSSION

In accordance with the schematic diagram as described above, we first synthesized the aglycon of 3,4-dehydrated surugatoxin **22** as follows.



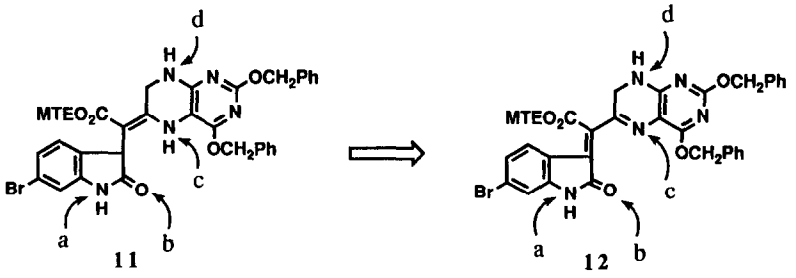
a) piperidine, AcOH, benzene; b) $Na_2S_2O_4$, EtOH; c) i) NH_2NH_2-H_2O; ii) 10% HCl; d) $NaHCO_3$, DMF; e) Zn-AcOH, THF.

Scheme 3

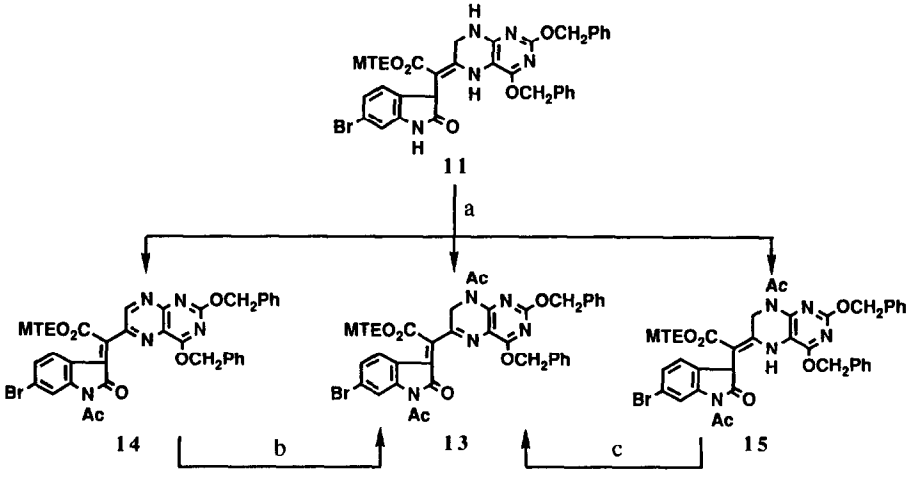
Condensation between 6-bromoisatin **4** and β-keto ester **5** under the Knoevenagel condition led to isatinylidene derivative **6** with a high degree of stereospecificity,[11] which was reduced with sodium hydrosulfite to form β-keto ester derivative **7**. To remove the protective phthalimide, the resultant mixture of diastereomers **7** was treated with hydrazine hydrate using a conventional method without protecting the adjacent ketone group. Since the free form of resultant aminoketone derivative **8** is very unstable, it was converted into its hydrochloride, which was then submitted to a reaction with a 4-ethylsulfonyl-5-nitropyrimidine derivative **9**,[7] corresponding to the part [c] shown in Scheme 2. Namely, compound **8** was reacted with **9** in the presence of an excess amount of

$NaHCO_3$ in DMF to yield nitroketone **10** in 90% yield. Then, the reduction of **10** with zinc-acetic acid to form ring D in the pentacyclic system gave tetrahydropteridine derivative **11** as a single product. Thus, compound **11** contains rings A, B, D, and E. To construct ring C to compound **11**, the pyruvoyl moiety as a three carbon unit should be introduced into the nitrogen atom in the enamine moiety of **11**.

As shown in Scheme 4, compound **11** may undergo acylation at three sites of **a**, **b**, and **d**, as well as at **c**. If the reactivity of site **b** can be nullified by oxidizing **11** into conjugated imine form **12**, the highly reactive sites of **a** and **d** can be distinguished from inactive site **c**. When **11** was acetylated with Ac_2O-pyridine (2:1) at 70 °C for 2 h, 1,4-dehydrogenation occurred simultaneously to give the desired dihydropteridine diacetate **13** (inseparable 5:1 E/Z mixture, in 50% yield)[8]



Scheme 4



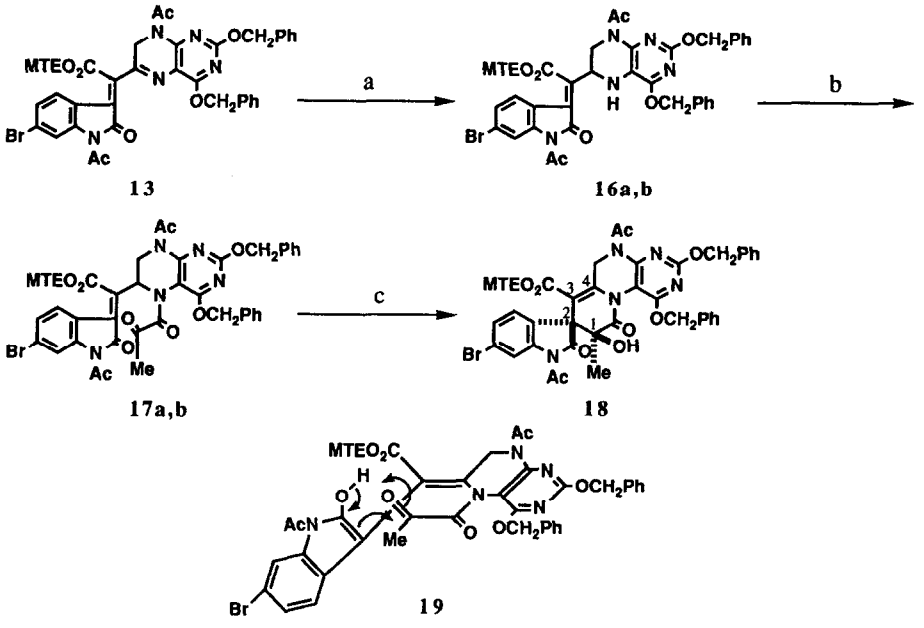
a) Ac_2O, pyridine, 70 °C; b) i) Zn, CSA, CH_2Cl_2; ii) Ac_2O, pyridine, 70 °C; c) pyridine, 70 °C.

Scheme 5

together with by-product **14** which was reduced with zinc-camphorsulfonic acid (CSA), and then acetylated to give **13**. Furthermore, another by-product **15** was heated in pyridine at 70 °C also to give **13**. Therefore, the combined yield of **13** from **11** was 70%.

The E/Z mixture of **13** was reduced with $NaBH_3CN$; the imine unit was selectively reduced in both isomers to a separable E/Z mixture of isatinylidene derivatives **16a** and **16b**[12] in 82% yield. Compound **16** was unstable and liable to dehydrogenation under basic conditions to restore the original imine **13**. Therefore, the subsequent acylation was carried out in the absence of the base. Thus, compound **16** dissolved in absolute benzene was cooled in an ice bath, and an excess amount of freshly purified pyruvoyl chloride was added dropwise to the solution to give pyruvoyl amide **17** with a yield of over 92%. Amide **17** was also obtained as a separable mixture of the E/Z isomers,[12] but the mixture gave a single product **18** in 83% yield when the solution of **17** in pyridine was allowed to stand overnight at room temperature. When the amide isomers **17a** and **17b** were separately reacted, the same product **18** was obtained.

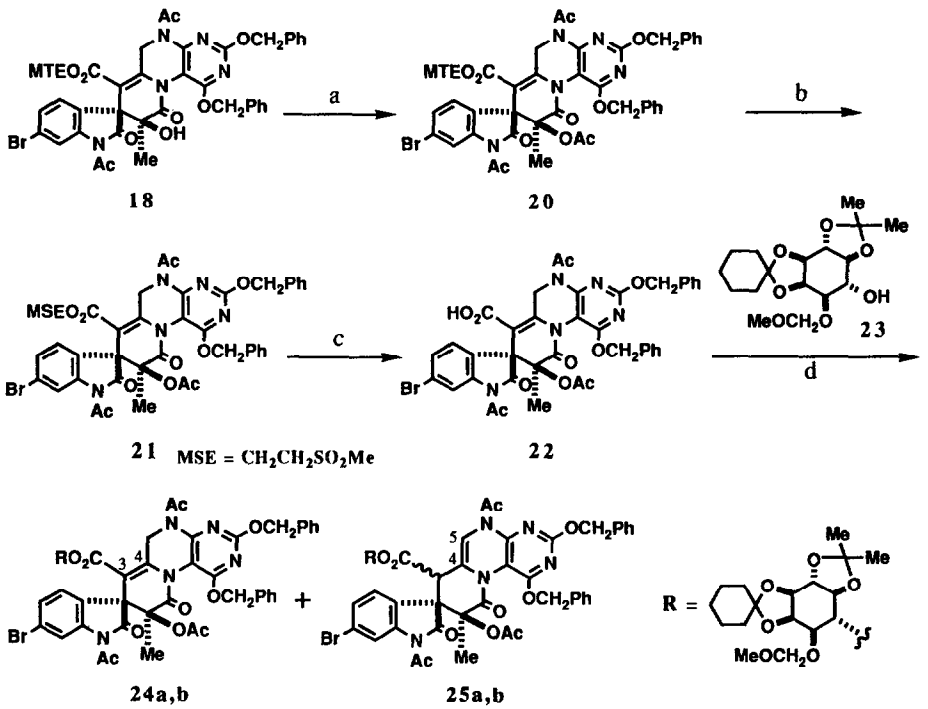
Spectral data supported the formation of the dehydrated surugatoxin



a) $NaBH_3CN$, 2N HCl, AcOEt; b) MeCOCOCl, benzene; c) pyridine, rt.

Scheme 6

framework in compound **18**. For example, in ^{1}H NMR spectrum, a signal at 2.04 ppm arising from the methylketone group in the pyruvoyl amide unit of **17a** was disappeared and a singlet at 1.32 ppm due to the methyl group attached to the C1 of **18** was observed. This appearance may indicate the shielding of the C1-methyl in ring C by the under lying oxindole unit. Therefore, compound **17** underwent cyclization to form a favorable structure for synthesizing natural surugatoxin. In facts, chemical shifts for the methyl groups attached to the C1 of surugatoxin **1** and its C1-epimer[13] are observed at 1.34 ppm and 1.54 ppm, respectively. This stereospecific cyclization to form ring C suggests that E/Z isomers **17a** and **17b** share the same intermediate **19** having an enamine type double bond prior to cyclization. Compound **18** resulting from the above cyclization underwent acetylation to protect the newly formed tert-hydroxyl group attached to C1. Resulting triacetate **20** was treated with m-chloroperbenzoic acid (m-CPBA) to convert the MTE ester group to the methylsulfonylethyl (MSE) ester group. The protective MSE ester group in **21** was removed under a mild condition so as not to affect the other protections; compound **21** was



a) Ac_2O, AcONa; b) *m*-CPBA, CH_2Cl_2; c) pH 10.2 buffer, acetone; d) picryl chloride, pyridine.

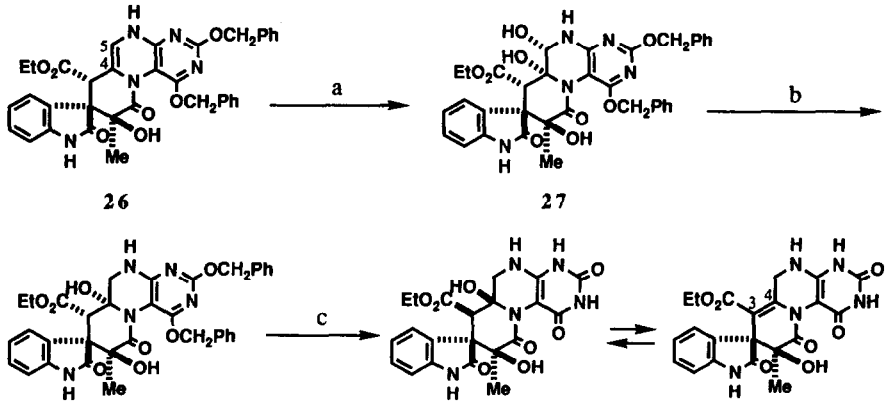
Scheme 7

dissolved in a 1:3 mixture of 0.1 M $NaHCO_3$-Na_2CO_3 buffer (pH 10.2) and acetone, and the solution was left at room temperature for 1 h, to give the desired 3,4-dehydrated surugatoxin carboxylic acid triacetate **22** in 82% corrected yield.

Esterification of the carboxyl group of aglycon **22**, synthesized as described above, was poorly reactive and could not undergo esterification under ordinary conditions. It was effected when **22** was treated with the myo-inositol derivative **23**[14] in pyridine via the agency of picryl chloride[15] at room temperature for 1.5 h.

An unexpected double bond isomerization occurred however during the esterification to give a 1:1 diastereomeric mixture of 4,5-dehydrated ester derivatives **25a,b** (55%) accompanied by 3,4-dehydrated esters **24a,b** (16%), each of **25a** and **25b** was easily separated by chromatography on silica gel (AcOEt-benzene=1:7). Compound **25b** can be led to natural surugatoxin **1** by the following experiments.

In our preliminary model experiments,[5,9,10] an unnatural surugatoxin analogue **28**, synthesized from 4,5-dehydrated derivative **26** as shown in Scheme 8, via diol derivative **27**, gave a 3:5 equilibrium mixture of natural surugatoxin analogue **29** and 3,4-dehydrated derivative **30** when it was heated in 90% trifluoroacetic acid (TFA) at 60 °C. This was an important finding.

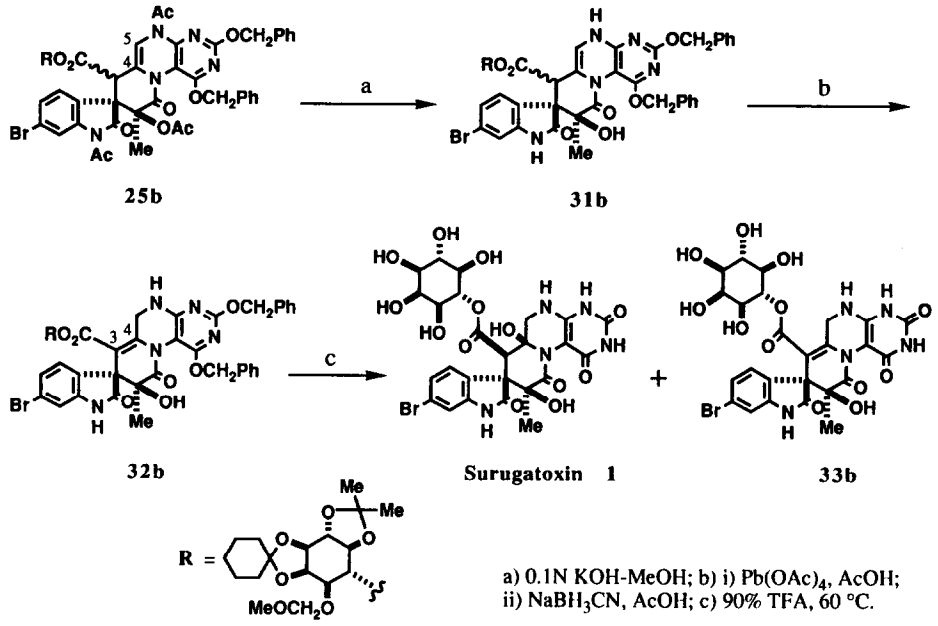


a) i) OsO_4, THF; ii) H_2S, THF; b) BH_3-pyridine, AcOH; c) 90% TFA, 60 °C.

Scheme 8

Therefore, the resulting 4,5-dehydrated isomer **25b** was deacetylated with 0.1 N KOH-MeOH to give **31b**, which was then converted into a desirable

3,4-dehydrated derivative **32b** in 76% yield by treatment with $Pb(OAc)_4$ in AcOH followed by $NaBH_3CN$ in AcOH. Finally, heating **32b** in 90% TFA at 60 °C for 7 h resulted in removal of the all protecting groups to give an equilibrium mixture of the hydrated compound, surugatoxin **1** and 3,4-dehydrated surugatoxin **33b**.



Scheme 9

The recovered dehydrated surugatoxin **33b** was redissolved in 90% TFA and heated for the stereospecific hydration. This recycling procedure was repeated three times to convert more than 40% of **33b** into natural surugatoxin. The chromatographical (TLC, HPLC) and spectral [^1H NMR, ^{13}C NMR, MS(SIMS), IR, UV] properties of the synthetic (±)-surugatoxin were found to be identical with those of natural surugatoxin.[2]

CONCLUSIONS

This study is the first effort to conduct the total synthesis of surugatoxin **1** which is sensitive to acids and alkalis.[2] A unique pentacyclic ring system bearing a hydroxyl group at the C/D ring junction in **1** and ester bond with myo-inositol may be responsible for its unstability. Its chemical properties were not yet clarified. Thus, the synthesis of **1** was started virtually without any information necessary to

design the synthetic route. We thus first studied the synthesis of a model compound of the ethyl ester analogue of **1** to find a practical route for making this new ring system.[5-10] It was considered that the chirality of four consecutive asymmetric carbon atoms in the spiropiperidone moiety, corresponding to the ring C in **1**, could be varied under certain conditions. In the case of the retroaldol-aldol reaction under basic conditions, the reaction at C1-C2 may give rise to an equilibrium mixture of two separable isomers. With the C3-C4 linkage in **1**, the stereochemistry can be varied by breaking and then reforming the C4-N linkage, or a hydroxyl group can be attached to the C4-carbon atom by taking advantage of the hydration-dehydration equilibrium of the 3,4-dehydrated derivative where the double bond conjugates to the ester carbonyl group.[5,10] According to the synthetic procedure developed based on the model experiments,[5-10] the 3,4-dehydrated surugatoxin framework corresponding to the compound **18** was made by stereospecific ring closure of pyruvoyl amide **17**. A solution of pyruvoyl amide **17a** or **17b** in pyridine was allowed to stand at room temperature overnight, with the result that each was efficiently cyclized to **18** as a single product whose stereochemistry corresponded to that of natural surugatoxin **1**. This stereospecific cyclization, proceeded by a chelation-controlled intramolecular aldol type reaction, indicates that the E/Z isomers **17a** and **17b** were transformed into the same intermediate **19** through allylic migration of the isatinyliden double bond in **17**. In a preliminary model experiment on the synthesis of the surugatoxin analogue **29**, when a single or a mixture of unnatural stereoisomers due to C3/C4 asymmetric centers such as **28** was heated in 90% TFA, equilibration occurred to give rise to natural isomer **29** and the 3,4-dehydrated derivative **30**. Data from the model experiments indicated key intermediate **32b** to have been transformed into **1** through the hydration-dehydration equilibrium. The structure of **1'** was found to **1** by Kosuge et al., and to be nontoxic.[3] The causative agents of neosurugatoxin **2**[3] and prosurugatoxin **3**[4] were isolated by the Kosuge group from the same toxic ivory shell, and **3** was convertible to surugatoxin **1**.[4,16] Using the data obtained in the present study toxins **2** and **3** were synthesized[16,17] from nitroketone **10**, an important intermediate in this total synthesis.

EXPERIMENTAL SECTION

Melting points were taken in capillary tubes and uncorrected. Spectra were recorded on the following instruments; IR spectra, JASCO IRA-1

spectrometer; UV spectra, Hitachi 323 spectrophotometer; MS spectra, Hitachi M-80B spectrometer; NMR spectra, JEOL JNM PS100 (100 MHz), JEOL JNM FX100 (100 MHz), and JEOL JNM GX400 (400 MHz) spectrometers. Chemical shifts of NMR spectra are given in ppm from tetramethylsilane as the internal standard. HPLC separation was carried out on a JASCO Trirotar II. Preparative thin-layer chromatography was conducted on a Kiselgel $60F_{254}$ (Merck, Art. 5744) or Kiselgel $60F_{254}S$ (Merck, Art. 13792) plates and column chromatographic separations were preformed on a silica gel (Kanto Chemical, Silica Gel, over 100 mesh).

2-(Methylthio)ethyl 1,3-Dihydro-β,1,3-trioxo-2H-isoindole-2-butanoate (5). A mixture of ethyl 1,3-dihydro-β,1,3-trioxo-2H-isoindole-2-butanoate[18] (40.0 g, 0.145 mol) and 2-(methylthio)ethanol (25 ml, 0.288 mol) was heated at 165 °C under nitrogen. After 5h, the mixture was diluted with a large volume of ice water, the separated crystals were collected, washed with H_2O and dried. Recrystallization of the crude product from CH_2Cl_2-MeOH (4:1) afforded colorless prisms (35.0 g, 75%): mp 100-101 °C; IR (KBr) 1785, 1745, 1700, 1410, 1320, 1260, 1170, 1095 cm^{-1}; ^1H NMR (100 MHz, $CDCl_3$) δ 2.12 (3H, s), 2.73 (2H, t, J=7 Hz), 3.59 (2H, s), 4.33 (2H, t, J=7 Hz), 4.66 (2H, s), 7.60-7.96 (4H, m). Anal. Calcd for $C_{15}H_{15}NO_5S$: C, 56.07; H, 4.71; N, 4.36. Found: C, 56.07; H, 4.66; N, 4.36.

2-(Methylthio)ethyl α-(6-Bromo-1,2-dihydro-2-oxo-3H-indol-3-ylidene)-1,3-dihydro-β,1,3-trioxo-2H-isoindole-2-butanoate (6). A mixture of 6-bromoisatin **4** (30.0 g, 0.133 mol), 2-(methylthio)ethyl 1,3-dihydro-β,1,3-trioxo-2H-isoindole-2-butanoate **5** (47.0 g, 0.146 mol), piperidine(10 ml, 0.10 mol), and acetic acid (40 ml, 0.70 mol) in benzene (1.2 l) was refluxed for 5 h using a Dean-Stark water separator. After being cooled, the separated crystals were collected, washed with benzene, MeOH then H_2O to give 57.0 g (82%) of greenish-yellow leaflets. The product was pure enough for further use. An analytical sample was prepared by recrystallization from DMF followed by washing with MeOH, and dried at 100 °C for 24 h in vacuo: mp 228-230 °C (decomp); IR (KBr) 3340, 1715, 1600, 1405, 1315, 1250, 1230, 1170, 1115, 935 cm^{-1}; ^1H NMR (100 MHz, DMSO-d_6) δ 2.08 (3H, s), 2.83 (2H, t, J=7 Hz), 4.42 (2H, t, J=7 Hz), 4.86 (2H, s), 7.04 (1H, d, J=2 Hz), 7.21 (1H, dd, J=8, 2 Hz), 7.87 (4H, s), 8.02 (1H, d, J=8 Hz), 11.06 (1H, s, NH). Anal. Calcd for $C_{23}H_{17}BrN_2O_6S$: C, 52.18; H, 3.24; N, 5.29. Found: C, 52.09; H, 3.12; N, 5.23.

2-(Methylthio)ethyl 6-Bromo-α-[(1,3-dihydro-1,3-dioxo-2H-isoindol-2-

yl)acetyl]-2,3-dihydro-2-oxo-1H-indole-3-acetate (7). $Na_2S_2O_4$ (11 g in 100 ml of H_2O, 63.2 mmol) was added to a suspension of Knoevenagel product **6** (10.0 g, 18.9 mmol) in EtOH (800 ml) and the mixture was refluxed for 5 min with vigorous stirring. The resulting colorless reaction mixture was filtered, and the filtrate was concentrated to one-fifth of the original volume. The residue was then poured into ice water and the separated crystals were collected, washed with H_2O and recrystallized from CH_2Cl_2-MeOH(4:1) to give 9.2 g (92%) of colorless crystalline solid (an enolizable diastereomeric mixture): mp 200-201 °C (decomp); IR (KBr) 3350, 1720, 1700, 1610, 1415, 1325, 1170 cm^{-1}; UV (MeOH) λ_{max} 241 (log ε 3.78), 259 (log ε 3.53), 290 (log ε 3.40) nm; ^1H NMR (100 MHz, DMSO-d_6) δ 2.00 (3H, s), 2.40-2.68 (2H, m), 3.90-4.30 (3H, m), 4.60-5.00 (3H, m), 6.80-7.10 (3H, m), 7.80 (4H, s), 10.53 and 10.57 (1H in total, s each, NH). Anal. Calcd for $C_{23}H_{19}BrN_2O_6S$: C, 51.99; H, 3.60; N, 5.27. Found: C, 51.69; H, 3.45; N, 5.14.

2-(Methylthio)ethyl α-(Aminoacetyl)-6-bromo-2,3-dihydro-2-oxo-1H-indole-3-acatate Hydrochloride (8). $NH_2NH_2.H_2O$ (100%, 5.0 ml, 103 mmol) was added gradually to an ice-cold suspension of **7** (10.0 g, 18.8 mmol) in 150 ml of a mixture of CH_2Cl_2-MeOH (4:1) and the mixture was stirred for 30 min so as to became a clear pale-yellow solution. To this solution, 10% HCl (90 ml, 0.26 mol) was added dropwise over a period of 20 min under ice cooling. After being stirred for 2 h at room temperature, the mixture was diluted with CH_2Cl_2 and stirred for additional 30 min. The separated crystals were collected, washed with CH_2Cl_2, then treated with 100 ml of H_2O (three times) to extract the resulting hydrochloride of aminoketone **8**. The aqueous extracts were concentrated below 30 °C *in vacuo* until the crystals began to separate. After being cooled, the separated crystals were collected, washed with ether and dried *in vacuo* to give 2.2 g (27%) of **8** as a mixture of inseparable diastereomers: mp 193-194 °C (decomp); IR (KBr) 3300-2800, 1735, 1710, 1615, 1485, 1350, 1330 cm^{-1}; ^1H NMR (100 MHz, DMSO-d_6) δ 2.05 (3H, s), 2.44-2.66 (2H, m), 4.00-4.40 (5H, m), 4.81 and 4.91 (1H in total, d each, J=4 Hz), 8.30-8.70 (3H, br s, NH_3^+), 10.80 and 10.83 (1H in total, s each, NH).

2-(Methylthio)ethyl 6-Bromo-2,3-dihydro-α-[[[5-nitro-2,6-bis(phenylmethoxy)-4-pyrimidinyl]amino]acetyl]-2-oxo-1H-indole-3-acetate (Nitroketone) (10). To a mixture of **8** (5.0 g, 11.4 mmol) and 4-(ethylsulfonyl)-5-nitro-2,6-bis(phenylmethoxy)pyrimidine **9** (4.46 g, 10.4 mmol) in DMF (60 ml) was added powdered $NaHCO_3$ (10 g, 119 mmol) in one portion and the

mixture was stirred vigorously at room temperature for 1 h. After being cooled, the separated precipitate was collected by filtration and the filtrate was diluted with ice water to precipitate the second crop of the product. Both precipitates were combined, washed with H_2O and dried. The pale-yellow crystalline solid thus obtained was recrystallized from CH_2Cl_2-MeOH to give 7.53 g of **10** (90%) as a colorless crystalline solid: mp 178-179 °C (decomp); IR (KBr) 3300, 1725, 1590, 1565, 1530, 1335, 1280, 1155 cm^{-1}; UV (MeOH) λ_{max} 238 (log ε 3.84), 260 (log ε 3.32), 265 (log ε 3.32), 331 (log ε 3.32) nm; ^1H NMR (100 MHz, DMSO-d_6) δ 1.94 (3H, s), 2.38-2.64 (2H, m), 3.90-4.30 (3H, m), 4.50-4.80 (3H, m), 5.22 and 5.28 (2H in total, s each), 5.45 (2H, s), 6.80-7.50 (13H, m), 9.13 (1H, br t, J=4 Hz, NH), 10.58 and 10.63 (1H in total, s each, NH). Anal. Calcd for $C_{33}H_{30}BrN_5O_8S$: C,53.81; H, 4.11; N, 9.51. Found: C, 54.03; H, 4.08; N; 9.59.

One-step Procedure of the Nitroketone (10) Synthesis from the Phthalimidobutyrate (7). $NH_2NH_2.H_2O$ (100%, 12.0 ml, 247 mmol) was added dropwise to a cold suspension (-15 °C) of **7** (20.0 g, 37.7 mmol) in THF (280 ml) and MeOH (120 ml). After the mixture was stirred for 30 min at -15 °C, 35% HCl (28.5 ml, 320 mmol) was added dropwise to precipitate the unreacted NH_2NH_2.HCl which was filtered off. To the filtrate, an additional 35% HCl (0.2 ml) was added under cooling, and the temperature was allowed to rise to room temperature. After 2 h, the mixture was cooled again to -15 °C and the separated phthaloyl hydrazide was filtered off. To the clear filtrate ethylsulfonylpyrimidine **9** (13.0 g, 30.3 mmol) and $NaHCO_3$ (30.0 g, 357 mmol) were added and the mixture was stirred vigorously at room temperature for 1 h. After being cooled, the mixture was filtered, the filtrate was evaporated to almost dryness in vacuo to give an oily residue which was solidified by treating with a small amount of MeOH. The product (15.8 g, 57%, 1:1 mixture of diastereomers) was identical with compound **10** in all respects.

2-(Methylthio)ethyl 6-Bromo-α-[7,8-dihydro-2,4-bis(phenylmethoxy)-6(5H)-pteridinylidene]-2,3-dihydro-2-oxo-1H-indole-3-acetate (11). Nitroketone **10** (5.0 g, 6.79 mmol) was dissolved in THF (250 ml) under a hot condition and then the solution was cooled to 0 °C. To this was added AcOH (50 ml, 0.87 mol) and zinc powder (15 g, 0.23 mol) and the mixture was stirred for 10 min, then filtered. The filtrate was evaporated to dryness in vacuo. Ice water was added to the residue and the insoluble portion was collected, washed with H_2O and dried. Recrystallization of the product

from CH_2Cl_2-MeOH gave a colorless crystalline solid (4.40 g, 94%): mp 166-167 °C; IR (KBr) 3200, 1710, 1655, 1590, 1410, 1250, 1145, 1050 cm^{-1}; UV (MeOH) λ_{max} 254 (log ε 3.72), 300 (log ε 3.72), 350 (log ε 3.97) nm; ^1H NMR (100 MHz, DMSO-d_6) δ 1.83 (3H, s), 2.14 (2H, m), 3.86 (2H, m), 4.29 (1H, s), 4.47 (2H, s), 5.25 (2H, s), 5.44 (2H, s), 6.80-7.50 (13H, m), 7.73 (1H, s, NH), 10.35 (1H, s, NH), 11.00 (1H, s, NH). Anal. Calcd for $C_{33}H_{30}BrN_5O_5S$: C, 57.56; H, 4.39; N, 10.17. Found: C, 57.79; H, 4.34; N, 10.20.

Acetylation of the Tetrahydropteridine (11). A mixture of **11** (3.0 g, 4.36 mmol), Ac_2O (60 ml) and pyridine (30 ml) was heated at 70 °C. After 2 h, the mixture was evaporated to dryness *in vacuo* and the residue was chromatographed on silica gel (200 g, AcOEt-benzene=1:7) to give the following three fractions.

Fraction 1: orange leaflets (from ether) of dihydropteridine diacetate **13** (1.66 g of inseparable 5:1 *E*/*Z* mixture, 50%); IR (KBr) 1725, 1590, 1550, 1415, 1340, 1285, 1250 cm^{-1}; UV (MeOH) λ_{max} 259 (log ε 4.35), 426 (log ε 3.96) nm; ^1H NMR (100 MHz, $CDCl_3$) δ 2.00 and 2.14 (3H in total, s each), 2.52 (3H, s), 2.61 (3H, s), 2.83 (2H, t, *J*=7 Hz), 4.28-4.70 (4H, m), 5.35 and 5.41 (2H in total, s each), 5.49 (2H, s), 6.12 (1H, dd, *J*=8, 2 Hz), 7.10-7.50 (10H, m), 8.34 and 8.42 (1H in total, d each, *J*=2 Hz), 8.57 (1H, d, *J*=8 Hz). Anal. Calcd for $C_{37}H_{32}BrN_5O_7S$: C,57.67; H, 4.19; N, 9.09. Found: C, 57.69; H, 4.27; N, 9.05.

Fraction 2: yellow leaflets (from ether) of pteridine monoacetate **14** (0.765 g of inseparable 5:1 *E*/*Z* mixture, 25%); IR (KBr) 1730, 1600, 1580, 1410, 1355, 1330, 1285, 1225, 1130 cm^{-1}; ^1H NMR (100 MHz, $CDCl_3$) δ 2.00 and 2.12 (3H in total, s each), 2.62 (3H, s), 2.82 (2H, t, *J*=7 Hz), 4.57 (2H, t, *J*=7 Hz), 5.65 (4H, s), 6.40 (1H, dd, *J*=8, 2 Hz), 7.28-7.60 (10H, m), 8.22 (1H, d, *J*=8 Hz), 8.40 (1H, d, *J*=2 Hz), 9.14 (1H, s). Anal. Calcd for $C_{35}H_{28}BrN_5O_6S.1/2H_2O$: C, 57.15; H, 3.97; N, 9.52. Found: C, 57.19; H, 3.84; N, 9.40.

Fraction 3: colorless prisms (from CH_2Cl_2-MeOH) of tetrahydropteridine diacetate **15** (0.384 g, 12%); mp 154 °C; IR (KBr) 1760, 1705, 1650, 1595, 1570, 1410, 1325, 1250, 1160, 1110 cm^{-1}; ^1H NMR (100 MHz, $CDCl_3$) δ 1.94 (3H, s), 2.20 (2H, t, *J*=7 Hz), 2.47 (3H, s), 2.65 (3H, s), 3.98 (2H, t, *J*=7 Hz), 4.44 (1H, s), 4.60 (1H, d, *J*=16 Hz), 5.12 (1H, d, *J*=16 Hz), 5.36 (2H, s), 5.56 (2H, s), 6.89 (1H, d, *J*=8 Hz), 7.20-7.60 (11H, m), 8.42 (1H, d, *J*=2 Hz), 11.05 (1H, s, NH). Anal. Calcd for $C_{37}H_{34}BrN_5O_7S$: C, 57.52; H, 4.44; N, 9.06. Found: C, 57.52; H, 4.39; N, 9.05.

Conversion of Pteridine Monoacetate (14) into Dihydropteridine Diacetate (13). Reduction of **14** (765 mg, 1.054 mmol) with zinc powder (2.0 g, 30.6 mmol) and CSA (1.0 g, 4.30 mmol) in CH_2Cl_2 (100 ml) followed by acetylation with Ac_2O (15 ml)-pyridine (5 ml) at 70 °C for 2 h gave **13** (255 mg, 31%).

Conversion of Tetrahydropteridine Diacetate (15) into 13. Compound **15** (384 mg, 0.497 mmol) was converted into **13** (194 mg, 51%) by the same treatment [Ac_2O (8 ml), pyridine (1.6 ml), 70 °C, 2 h] as in the case of **11**.

2-(Methylthio)ethyl 8-Acetyl-α-(1-acetyl-6-bromo-1,2-dihydro-2-oxo-3H-indol-3-ylidene)-5,6,7,8-tetrahydro-2,4-bis(phenylmethoxy)-6-pteridineacetate (16a and 16b). $NaBH_3CN$ (1.47 g, 23.4 mmol) was added to a solution of **13** (3.0 g, 3.90 mmol) in AcOEt (300 ml) containing 10 ml of 2 N HCl with vigorous stirring. After 10 min, the mixture was diluted with 300 ml of AcOEt, and the organic layer was washed successively with ice water, saturated aqueous $NaHCO_3$, and dried (Na_2SO_4), then concentrated. Purification of the residue using silica gel chromatography (200 g, AcOEt-benzene=1:7) afforded first 2.04 g (68%) of **16a** (yellow crystalline solid from MeOH, mp 175 °C) followed by 0.42 g (14%) of **16b** (yellow solid from MeOH, mp 89-89.5 °C).

16a: IR (KBr) 3340, 1750, 1720, 1660, 1580, 1420, 1345, 1265, 1120 cm^{-1}; UV (MeOH) λ_{max} 259 (log ε 3.84), 314 (log ε 3.67) nm; ^1H NMR (100 MHz, $CDCl_3$) δ 2.00 (3H, s), 2.50 (3H, s), 2.61 (3H, s), 2.61 (2H, t, J=8 Hz), 3.72 (1H, dd, J=12, 7 Hz), 4.30 (1H, s, NH), 4.31 (2H, t, J=8 Hz), 4.64 (1H, dd, J=12, 2 Hz), 4.76 (1H, dd, J=7, 2 Hz), 5.30 (2H, s), 5.43 (2H, s), 7.20-7.50 (12H, m), 8.45 (1H, d, J=2 Hz). Anal. Calcd for $C_{37}H_{34}BrN_5O_7S$: C, 57.52; H, 4.44; N, 9.06. Found: C, 57.48; H, 4.40; N, 9.01.

16b: IR (KBr) 3380, 1725, 1675, 1580, 1415, 1340, 1280, 1170, 1115 cm^{-1}; ^1H NMR (100 MHz, $CDCl_3$) δ 1.98 (3H, s), 2.51 (3H, s), 2.54 (2H, m), 2.66 (3H, s), 3.80-4.50 (5H, m), 5.30 (2H, s), 5.42 (2H, s), 5.62 (1H, br s, NH), 7.00 (1H, d, J=8 Hz), 7.10-7.50 (11H, m), 8.42 (1H, d, J=2 Hz). Anal. Calcd for $C_{37}H_{34}BrN_5O_7S$: C, 57.52; H, 4.44; N, 9.06. Found: C, 57.49; H, 4.40; N, 8.98.

2-(Methylthio)ethyl 8-Acetyl-α-(1-acetyl-6-bromo-1,2-dihydro-2-oxo-3H-indol-3-ylidene)-5-(1,2-dioxopropyl)-5,6,7,8-tetrahydro-2,4-bis(phenylmethoxy)-6-pteridineacetate (17a and 17b). A solution of pyruvoyl

chloride (9.0 g, 84.5 mmol) in anhydrous benzene (30 ml) was added dropwise to a solution of **16a** (3.0 g, 3.89 mmol) in anhydrous benzene (300 ml) under cooling (5 °C). After 10 min, the mixture was warmed to room temperature and stirring was continued for 5 h. Then, it was cooled to 5 °C, $NaHCO_3$ (13.7 g, 163 mmol) and MeOH (9 ml) were added gradually to decompose the excess pyruvoyl chloride. After 30 min, the mixture was shaken with H_2O and the benzene layer was dried (Na_2SO_4). Removal of the solvent *in vacuo* gave an oily residue which was solidified by adding CH_2Cl_2-MeOH. Recrystallization of the crude product from MeOH gave 3.01 g (92%) of **17a** as colorless prisms: mp 104-105 °C; IR (KBr) 3400, 1720, 1665, 1595, 1565, 1420, 1345, 1280, 1175, 1115 cm^{-1}; UV (MeOH) λ_{max} 269 (log ε 4.21), 292 (log ε 4.21) nm; ^1H NMR (100 MHz, $CDCl_3$) δ 1.95 (3H, s), 2.04 (3H, s), 2.49 (3H, s), 2.40-2.70 (2H, m), 2.64 (3H, s), 3.58 (1H, dd, *J*=14, 10 Hz), 4.08 (2H, m), 5.03 (1H, dd, *J*=14, 8 Hz), 5.37 (2H, s), 5.42 (2H, s), 5.87 (1H, dd, *J*=10, 8 Hz), 7.00-7.60 (12H, m), 8.55 (1H, d, *J*=2 Hz). Anal. Calcd for $C_{40}H_{36}BrN_5O_9S$: C, 57.01; H, 4.31; N, 8.31. Found: C, 57.05; H, 4.31; N, 8.26.

17b: prepared from **16b** by the same way as in the case of **17a**; mp 102-103 °C; IR (KBr) 3400, 1720, 1665, 1595, 1565, 1420, 1340, 1285, 1175 cm^{-1}; ^1H NMR (100 MHz, $CDCl_3$) δ 1.92 (3H, s), 2.05 (3H, s), 2.43 (2H, t, *J*=7 Hz), 2.51 (3H, s), 2.71 (3H, s), 3.51 (1H, dd, *J*=14, 10 Hz), 4.04 (2H, m), 5.10 (1H, dd, *J*=14, 8 Hz), 5.30 (1H, d, *J*=10 Hz), 5.37 (2H, s), 5.50 (1H, d, *J*=10 Hz), 6.68 (1H, dd, *J*=10, 8 Hz), 7.00-7.60 (12H, m), 8.49 (1H, d, *J*=2 Hz). Anal. Calcd for $C_{40}H_{36}BrN_5O_9S$: C,57.01; H, 4.31; N, 8.31. Found: C, 57.28; H, 4.28; N, 8.29.

2-(Methylthio)ethyl 1,5'-Diacetyl-6-bromo-1,2,5',6',9',10'-hexahydro-9'-hydroxy-9'-methyl-2,10'-dioxo-1',3'-bis(phenylmethoxy)spiro[3*H*-indole-3,8'-[8*H*]pyrido[1,2-*f*]pteridine]-7'-carboxylate (18). A solution of **17a** or **17b** (3.0 g, 3.56 mmol) in 120 ml of pyridine was allowed to stand at room temperature overnight. Pyridine was distilled off *in vacuo* and the residue was chromatographed on silica gel (200 g, AcOEt-benzene=1:7) to give a single product which was recrystallized from MeOH to give 2.48 g (83%) of **18** as colorless needles: mp 123 °C; IR (KBr) 3480, 1755, 1705, 1600, 1565, 1415, 1320, 1280, 1175, 1130 cm^{-1}: UV (MeOH) λ_{max} 264 (log ε 4.27), 289 (log ε 4.13) nm; ^1H NMR (100 MHz, $CDCl_3$) δ 1.32 (3H. s), 1.96 (3H, s), 2.13 (2H, m), 2.50 (3H, s), 2.56 (3H, s), 3.46 (1H, br d, *J*=16 Hz), 3.73 (1H, s, OH), 3.90 (2H, t, *J*=7 Hz), 5.41 (4H, s), 6.32 (1H, br d, *J*=16 Hz), 6.99 (1H, d, *J*=8 Hz), 7.16-7.52 (11H, m), 8.48 (1H, d, *J*=2 Hz). Anal. Calcd for $C_{40}H_{36}BrN_5O_9S.1/2H_2O$: C, 56.41; H, 4.38; N, 8.22. Found:

C, 56.61; H, 4.31; N, 8.13.

Acetylation of 18. A mixture of **18** (3.0 g, 3.56 mmol), AcONa (1.0 g, 12.2 mmol), and Ac_2O (60 ml) was heated at 120 °C for 2 h. After being cooled, the mixture was evaporated to dryness in vacuo. The product was taken up in CH_2Cl_2 (200 ml) and the CH_2Cl_2 layer was washed with aqueous $NaHCO_3$ (100 ml) and dried over Na_2SO_4. Removal of the solvent left an oily product which was solidified soon. Recrystallization of the product from MeOH gave 2.42 g (77%) of triacetate **20** as colorless needles: mp 173 °C; IR (KBr) 1765, 1710, 1600, 1570, 1415, 1360, 1310, 1280, 1170 cm^{-1}; UV (MeOH) λ_{max} 264 (log ε 4.27), 289 (log ε 4.13) nm; ^1H NMR (100 MHz, $CDCl_3$) δ 1.87 (3H, s), 1.93 (3H, s), 1.99 (3H, s), 2.27 (2H, m), 2.49 (3H, s), 2.63 (3H, s), 3.62 (1H, br d, J=16 Hz), 3.96 (2H, m), 5.40 (1H, d, J=12 Hz), 5.42 (2H, s), 5.65 (1H, d, J=12 Hz), 6.36 (1H, br d, J=16 Hz), 7.00-7.60 (12H, m), 8.51 (1H, d, J=2 Hz). Anal. Calcd for $C_{42}H_{38}BrN_5O_{10}S$: C, 57.02; H, 4.33; N, 7.92. Found: C, 57.07; H, 4.26; N, 7.79.

Triacetate (Methylsulfonyl)ethyl Ester (21). m-CPBA (80%, 1.62 g, 7.51 mmol) was added to a solution of **20** (3.0 g, 3.39 mmol) in CH_2Cl_2 (100 ml) under ice cooling. The mixture was warmed gradually to room temperature. When the reaction was completed (30 min as being judged by TLC), the mixture was diluted with CH_2Cl_2 (100 ml), it was shaken with saturated aqueous $Na_2S_2O_3$ (20 ml), aqueous $NaHCO_3$ (20 ml), and the CH_2Cl_2 layer was dried over Na_2SO_4. Removal of the solvent in vacuo left crude crystals which were recrystallized from MeOH to give 2.97 g (96%) of colorless plates: mp 171 °C; IR (KBr) 1770, 1700-1720, 1650, 1600, 1570, 1420, 1320, 1170 cm^{-1}; UV (MeOH) λ_{max} 263 (log ε 4.29), 285 (log ε 4.17) nm; ^1H NMR (100 MHz, $CDCl_3$) δ 1.80 (3H, s), 1.94 (3H, s), 2.46 (3H, s), 2.62 (3H, s), 2.82 (3H, s), 3.10 (2H, m), 3.40 (1H, br d, J=16 Hz), 4.29 (2H, m), 5.36 (1H, d, J=12 Hz), 5.37 (2H, s), 5.57 (1H, d, J=12 Hz), 6.30 (1H, br d, J=16 Hz), 6.90-7.50 (12H, m), 8.43 (1H, d, J=2 Hz). Anal. Calcd for $C_{42}H_{38}BrN_5O_{12}S$: C, 55.03; H, 4.18; N, 7.64. Found: C, 54.94; H, 4.04; N, 7.57.

1,5'-Diacetyl-9'-(acetyloxy)-6-bromo-1,2,5',6',9',10'-hexahydro-9'-methyl-2,10'-dioxo-1',3'-bis(phenylmethoxy)spiro[3H-indole-3,8'-[8H]pyrido[1,2-f]pteridine]-7'-carboxylic Acid (22). To a solution of **21** (1.83 g, 2.00 mmol) in acetone (160 ml) was added dropwise a solution of 0.1 M $NaHCO_3$-Na_2CO_3 (pH 10.2, 55 ml) at room temperature with stirring. After 30 min, an additional $NaHCO_3$-Na_2CO_3 solution (30 ml) and acetone (50 ml) were

added, and after 30 min, the reaction was quenched by addition of 1 N HCl under ice cooling, then basified with saturated aqueous $NaHCO_3$. The mixture was concentrated to one-third of the original volume, the basic solution was acidified again with 1 N HCl, extracted with CH_2Cl_2, dried (Na_2SO_4) and then concentrated in vacuo. Silica gel chromatography of the residue (100 g, CH_2Cl_2-MeOH=5:1) afforded the following three fractions.
Fraction 1: recovered starting material **21** (460 mg, 25%).
Fraction 2: colorless needles (from CH_2Cl_2-MeOH) of the desired carboxylic acid triacetate **22** (993 mg, 62%, corrected yield 82%); mp 158-159 °C; IR (KBr) 1765, 1715, 1640, 1600, 1570, 1420, 1325, 1015 cm^{-1}; ^1H NMR (100 MHz, $CDCl_3$) δ 1.69 (3H, s), 1.94 (3H, s), 2.43 (3H, s), 2.58 (3H, s), 3.50 (1H, br s), 5.30 (1H, br s), 5.36 (1H, d, J=12 Hz), 5.38 (2H, s), 5.56 (1H, d, J=12 Hz), 6.48 (1H, br s), 6.97 (2H, s), 7.20-7.60 (12H, m), 8.36 (1H, d, J=2 Hz). Anal. Calcd for $C_{39}H_{32}BrN_5O_{10}.2H_2O$: C, 55.33; H, 4.29; N, 8.27. Found: C, 55.40; H, 4.00; N, 7.99.
Fraction 3: a colorless crystalline solid (from CH_2Cl_2-MeOH) of carboxylic acid diacetate (107 mg, 7%, corrected yield 10%); mp 236-239 °C (decomp); IR (KBr) 1715, 1655, 1570, 1355, 1235, 1140 cm^{-1}; ^1H NMR (100 MHz, $CDCl_3$) δ 1.32 (3H, s), 1.90 (3H, s), 2.30 (3H, s), 3.30 (1H, br s), 5.36 (2H, s), 5.42 (2H, s), 6.20-7.60 (13H, m), 9.70 (1H, br s, NH). This compound was converted into triacetate **22** (83%) by treatment with Ac_2O at 80 °C for 7 h.

Esterification of 22. To a solution of **22** (1.20 g, 1.48 mmol) and (±)-2,3-O-cyclohexylidene-1-O-(methoxymethyl)-4,5-O-(1-methylethylidene)-myo-inositol **23** (0.53 g, 1.54 mmol) in pyridine (25 ml) was added picryl chloride (0.367 g, 1.48 mmol) and the mixture was stirred at room temperature. To this mixture, an additional amount of picryl chloride (0.367 g, 1.48 mmol then 0.184 g, 0.74 mmol) was added portion wise over 1 h.After being stirred for 30 min, the separated crystals were filtered off. The filtrate was evaporated to dryness in vacuo, the residue was taken up in CH_2Cl_2, and the extracts were washed with saturated aqueous $NaHCO_3$ (twice), dried (Na_2SO_4) and concentrated. The residue was chromatographed on silica gel (100 g, AcOEt-benzene=1:7) to afford the following two fractions.
Fraction 1: The eluents (813 mg) isolated as fraction 1 were rechromatographed on silica gel (100 g, AcOEt-benzene=1:2) to give 273 mg (16%) of a 1:1 mixture of inseparable diastereomeric 3,4-dehydrated myo-inositol esters **24a, b**[19] followed by 4,5-dehydrated derivative **25a** (445 mg, 27%).

24a: mp 166-167 °C (colorless needles from CH_2Cl_2-MeOH); IR (KBr) 1765, 1715, 1600, 1570, 1415, 1320, 1225, 1080 cm^{-1}; ^1H NMR (100 MHz, $CDCl_3$) δ 1.36 (6H, s), 1.62 (10H, br s), 1.78 (3H, s), 1.91 (3H, s), 2.49 (3H, s), 2.61 (3H, s), 3.07 (1H, t, J=8 Hz), 3.30 (2H, m), 3.36 (3H, s), 3.54 (1H, br d, J=16 Hz), 3.90-5.00 (5H, m), 5.34 (1H, d, J=12 Hz), 5.37 (2H, s), 5.61 (1H, d, J=12 Hz), 6.58 (1H, br d, J=16 Hz), 6.90-7.60 (12H, m), 8.45 (1H, d, J=2 Hz). Anal. Calcd for $C_{56}H_{58}BrN_5O_{16}\cdot 1/2H_2O$: C, 58.69; H, 5.19; N, 6.11. Found. C, 58.59; H, 5.08; N. 6.10.

24b: mp 197-198 °C (colorless needles from CH_2Cl_2-MeOH); IR (KBr) 1765, 1700, 1600, 1570, 1415, 1325, 1175 cm^{-1}; ^1H NMR (100 MHz, $CDCl_3$) δ 1.34 (3H, s), 1.38 (3H, s), 1.60 (10H, m), 1.88 (3H, s), 1.92 (3H, s), 2.46 (3H, s), 2.62 (3H, s), 3.32 (3H, s), 3.59 (1H, t, J=3 Hz), 3.80-4.30 (3H, m), 4.61 (2H, s), 5.00 (1H, dd, J=8, 3 Hz), 5.34 (1H, d, J=12 Hz), 5.36 (2H, s), 5.61 (1H, d, J=12 Hz), 6.30 (1H, br d, J=12 Hz), 7.00-7.60 (12H, m), 8.38 (1H, d, J=2 Hz). Anal. Calcd for $C_{56}H_{58}BrN_5O_{16}\cdot 1/2H_2O$: C, 58.69; H, 5.19; N, 6.11. Found: C, 58.54; H, 5.17; N, 6.07.

25a: mp 149-151 °C (a colorless crystalline solid from MeOH); IR (KBr) 1770, 1730, 1605, 1570, 1425, 1235, 1110 cm^{-1}; ^1H NMR (100 MHz, $CDCl_3$) δ 1.26 (3H, s), 1.34 (3H, s) 1.36 (3H, s), 1.59 (10H, br s), 2.03 (3H, s), 2.60 (3H, s), 2.71 (3H, s), 3.00-3.50 (3H, m), 3.17 (3H, s), 3.90-4.80 (5H, m), 4.58 (1H, s), 5.41 (2H, s), 5.42 (1H, d, J=12 Hz), 5.53 (1H, d, J=12 Hz), 5.72 (1H, dd, J=8, 2 Hz), 7.04 (1H, s), 7.10-7.60 (11H, m), 8.27 (1H, d, J=2 Hz). Anal. Calcd for $C_{56}H_{58}BrN_5O_{16}$: C, 59.16; H, 5.14; N. 6.16. Found: C, 58.97; H, 5.10; N, 6.10.

Fraction 2: colorless needles (from CH_2Cl_2-MeOH) of single product **25b** (470 mg, 28%); mp 214-216 °C; IR (KBr) 1765, 1730, 1600, 1560, 1420, 1230, 1105 cm^{-1}; UV (MeOH) λ_{max} 270_{br} (log ε 4.11) nm; ^1H NMR (100 MHz, $CDCl_3$) δ 1.26 (9H, s), 1.60 (10H, m), 2.06 (3H, s), 2.56 (1H, t, J=8 Hz), 2.62 (3H, s), 2.70 (3H, s), 3.31 (3H, s), 3.57 (1H, t, J=2 Hz), 3.72-4.20 (3H, m), 4.50 (1H, m), 4.55 (1H, d, J=7 Hz), 4.56 (1H, s), 4.65 (1H, d, J=7 Hz), 5.44 (2H, s), 5.48 (2H, s), 5.62 (1H, dd, J=8, 2 Hz), 7.08 (1H, s), 7.20-7.60 (11H, m), 8.30 (1H, d, J=2 Hz). Anal. Calcd for $C_{56}H_{58}BrN_5O_{16}\cdot 1/2H_2O$: C, 58.69; H, 5.19; N, 6.11. Found: C, 58.68; H, 5.08; N, 6.05.

Hydrolysis of 4,5-Dehydrated Derivative (25). Triacetate **25b** (850 mg, 0.748 mmol) was treated with 0.1 N KOH-MeOH (75 ml, 7.50 mmol) under nitrogen. After being stirred for 2 h at room temperature, the mixture was neutralized with AcOH , then evaporated to dryness in vacuo. The residue was dissolved in CH_2Cl_2, washed with saturated aqueous $NaHCO_3$, dried (Na_2SO_4) and then concentrated. The residue was chromatographed on

silica gel (100 g, CH_2Cl_2-MeOH=50:1) to give 542 mg (72%) of **31b**. Recrystallization from acetone afforded colorless crystalline solid: mp 188-189 °C (decomp); IR (KBr) 1705, 1610, 1580, 1450, 1350, 1155 cm^{-1}; UV (MeOH) λ_{max} 258_{br} (log ε 4.24), 302 (log ε 3.98) nm; ^1H NMR (100 MHz, $CDCl_3$) δ 1.16 (3H, s), 1.21 (3H, s), 1.24 (3H, s), 1.61 (10H, m), 2.66 (1H, t, J=8 Hz), 3.28 (3H, s), 3.45 (1H, br. s), 3.60-4.20 (3H, m), 4.10 (1H, s, OH), 4.24 (1H, s), 4.40-4.80 (3H, m), 5.30 (1H, d, J=12 Hz), 5.35 (2H, s), 5.48 (1H, d, J=12 Hz), 5.76 (1H, d, J=8 Hz), 5.88 (1H, d, J=6 Hz), 6.84 (1H, s), 7.04 (1H, d, J=8 Hz), 7.20-7.60 (10H, m), 8.49 (1H, br d, J=6 Hz, NH), 10.68 (1H, br s, NH). Anal. Calcd for $C_{50}H_{52}BrN_5O_{13}.H_2O$: C, 58.37; H, 5.29; N, 6.81. Found: C, 58.53; H, 5.16; N, 6.78.

Besides **31b**, 89.0 mg (12%) of diastereomer **31a** was obtained: mp 247-249 °C (decomp); IR (KBr) 1750, 1715, 1605, 1595, 1445, 1410, 1345, 1150, 1080 cm^{-1}; ^1H NMR (100 MHz, DMSO-d_6) δ 0.96 (3H, s), 1.28 (3H, s), 1.32 (3H, s), 1.54 (10H, br s), 3.04 (3H, s), 3.36 (1H, m), 3.60-4.60 (7H, m), 4.31 (1H, s), 5.22 (1H, d, J=12 Hz), 5.28 (2H, s), 5.49 (1H, d, J=12 Hz), 5.84 (1H, dd, J=8, 2 Hz), 5.87 (1H, s, OH), 6.21 (1H, d, J=4 Hz), 6.80 (1H, d, J=2 Hz), 7.00 (1H, d, J=8 Hz), 7.10-7.60 (10H, m), 9.06 (1H, d, J=4 Hz, NH), 10.65 (1H, s, NH). Anal. Calcd for $C_{50}H_{52}BrN_5O_{13}.H_2O$: C, 58.37; H, 5.29; N, 6.81. Found: C, 58.20; H, 5.32; N, 6.71.

Isomerization of 4,5-Dehydrated Derivative (31b) to 3,4-Dehydrated Derivative (32b). $Pb(OAc)_4$ (81.8%, 164 mg, 0.302 mmol) was added to a solution of **31b** (310 mg, 0.307 mmol) in AcOH (6 ml) at room temperature with stirring. After 15 min, $NaBH_3CN$ (19 mg, 0.302 mmol) was added and stirring was continued for 20 min. The mixture was evaporated *in vacuo*, the residue was taken up in CH_2Cl_2 (100 ml), washed with saturated aqueous $NaHCO_3$ and dried (Na_2SO_4). Concentration of the solvent *in vacuo* followed by silica gel chromatography of the residue (CH_2Cl_2-acetone=15:1) afforded 3,4-dehydrated derivative **32b** as a white solid. Recrystallization from MeOH gave 235 mg (76% from **31b**) of pure crystalline solid: mp 214-216 °C (decomp); IR (KBr) 1715, 1610, 1590, 1450, 1315, 1160 cm^{-1}; UV (MeOH) λ_{max} 270_{br} (log ε 4.25), 329_{br} (log ε 3.74) nm; ^1H NMR (100 MHz, $CDCl_3$) δ 1.00-2.00 (19H, m), 2.52 (1H, t, J=8 Hz), 3.37 (3H, s), 3.64 (1H, t, J=2 Hz), 3.80-5.20 (8H, m), 4.41 (1H, s, OH), 5.44 (4H, s), 6.07 (1H, br s, NH), 6.90-7.70 (13H, m), 10.41 (1H, br s, NH). Anal. Calcd for $C_{50}H_{52}BrN_5O_{13}.2H_2O$: ,57.36;, 5.39; N, 6.69. Found: C, 57.39; H, 5.16; N, 6.74.

31a was also isomerized to **32a** by the similar treatment as in the case of **31b**, colorless needles from MeOH: mp 182-183 °C; IR (KBr) 1710, 1605,

1450, 1415, 1350, 1310, 1150 cm^{-1}; ^1H NMR (100 MHz, 10%CD_3OD-$CDCl_3$) δ 1.36 (9H, s), 1.60 (10H, m), 2.70 (1H, d, J=3 Hz), 3.32 (3H, s), 3.70-5.00 (7H, m), 5.26 (1H, d, J=12 Hz), 5.32 (2H, s), 5.40 (1H, d, J=12 Hz), 7.10-7.50 (13H, m). Anal. Calcd for $C_{50}H_{52}BrN_5O_{13}.2H_2O$: C, 57.36; H, 5.39; N, 6.69. Found: C, 57.50; H, 5.13; N, 6.75.

(±)-6-Bromo-1,1',2,2',3',4',5',6',6'a,7',9',10'-dodecahydro-6'a,9'-dihydroxy-9'-methyl-1',2,3',10'-tetraoxospiro[3H-indole-3,8'-[8H]pyrido[1,2-f]pteridine]-7'-carboxylic Acid 6-myo-Inositol Ester [(±)-Surugatoxin] (1). A solution of **32b** (220 mg, 0.218 mmol) in 90% TFA (11 ml) was heated at 60 °C for 7 h under nitrogen to afford an equilibrium mixture of (±)-surugatoxin and its dehydrated form. The mixture was dried up *in vacuo* and the residue was fractionated by silica gel preparative TLC (AcOEt-MeOH-acetone-H_2O=3:1:1:1) to give the following two fractions.

Fraction 1: colorless prisms (from H_2O) of (±)-surugatoxin **1** (24 mg, 15%); mp >300 °C (decomp). Spectral data (IR, UV, MS, and NMR) of the product were completely identical with those of natural surugatoxin.

IR (KBr) 3400, 1740(sh), 1695, 1635, 1410, 1340, 1040 cm^{-1}; UV (H_2O) λ_{max} 277 (log ε 4.19) nm; UV (0.1N NaOH) λ_{max} 280 (log ε 4.22) nm; ^1H NMR (400 MHz, DMSO-d_6) δ 1.34 (3H, s), 3.06-3.18 (2H, m), 3.26-3.44 (2H, m), 3.38 (1H, dd, J=13.0, 1.4 Hz), 3.69 (1H, ddd, J=3.3, 2.4, 2.4 Hz), 3.77 (1H, s), 4.09 (1H, d, J=6.6 Hz, OH), 4.17 (1H, dd, J=13.0, 5.7 Hz), 4.30 (1H, d, J=5.1 Hz, OH), 4.42 (1H, d, J=5.9 Hz, OH), 4.71 (1H, d, J=3.3 Hz, OH), 4.75 (1H, t, J=9.8 Hz), 4.80 (1H, d, J=4.4 Hz, OH), 5.49 (1H, s, OH), 5.74 (1H, d, J=1.4 Hz, OH), 6.32 (1H, br d, J=5.7 Hz, NH), 6.89 (1H, d, J=1.8 Hz), 7.03 (1H, dd, J=8.1, 1.8 Hz), 7.10 (1H, d, J=8.1 Hz), 10.18 (1H, s, NH), 10.63 (1H, br s, NH), 10.68 (1H, s, NH); ^{13}C NMR (25 MHz, DMSO-d_6) δ 24.28 (q), 50.43 (d), 51.42 (t), 55.17 (s), 69.33 (d), 71.37 (d), 72.37 (d), 72.60 (d), 73.42 (s), 76.70 (d), 77.52 (s), 87.34 (s), 111.80 (d), 120.69 (s), 122.97 (d), 126.48 (d), 128.47 (s), 145.32 (s), 149.83 (s), 156.96 (s), 164.80 (s), 166.56 (s), 180.42 (s); MS (SIMS): m/z 686, 684 $(M+H)^+$; TLC: Rf=0.15 (silica gel, AcOEt-acetone-MeOH-H_2O=3:1:1:1); HPLC: 9.1 min (column: JASCO Finepak Sil C_{18}, 4.6x250 mm, solvent: 10% MeOH-H_2O, flow rate: 1 ml/min).

Fraction 2: a colorless crystalline solid (from MeOH) of 3,4-dehydrated surugatoxin **33b** (110 mg, 71%); mp 245-250 °C (decomp); IR (KBr) 1700, 1650, 1320, 1165, 1055 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 1.46 (3H, s), 3.00-3.20 (2H, m), 3.25-3.45 (2H, m), 3.69 (1H, br s), 3.79 (1H, br t, J=14.5 Hz), 4.29 (1H, d, J=6.6 Hz, OH), 4.42 (1H, d, J=5.9 Hz, OH), 4.73 (1H, d, J=2.9 Hz, OH), 4.80 (1H, t, J=9.9 Hz), 4.87 (1H, br d, J=14.5 Hz),

4.88 (1H, d, $\underline{J}$=4.8 Hz, OH), 4.95 (1H, d, $\underline{J}$=5.1 Hz, OH), 5.59 (1H, s, OH), 6.06 (1H, br d, $\underline{J}$=1.0 Hz, NH), 6.85 (1H, d, $\underline{J}$=1.8 Hz), 6.94 (1H, dd, $\underline{J}$=8.1, 1.8 Hz), 7.10 (1H, d, $\underline{J}$=8.1 Hz), 10.36 (1H, s, NH), 10.60 (1H, s, NH), 11.14 (1H, br s, NH); ^{13}C NMR (100 MHz, DMSO-$\underline{d}_6$) δ 23.08 (q), 42.85 (t), 61.02 (s), 69.11 (d), 69.49 (d), 71.34 (d), 72.13 (d), 72.76 (s), 75.62 (d), 93.29 (s), 104.58 (s), 111.49 (d), 120.45 (s), 120.50 (s), 122.76 (d), 126.48 (d), 127.72 (s), 145.35 (s), 145.95 (s), 149.47 (s), 155.91 (s), 164.65 (s), 167.95 (s), 176.98 (s). Anal. Calcd for $C_{25}H_{24}BrN_5O_{12}.4H_2O$: C, 40.66; H, 4.37; N, 9.48. Found: C, 40.55; H, 4.09; N, 9.28.

This compound was submitted again to the above equilibration. When the recycling was repeated for three times, the yield of (±)-surugatoxin 1 was raised up to 40%.

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11. The stereochemistry of Knoevenagel product **6** was assinged as E-configuration according to our knowledge reported in model experiments on surugatoxin synthesis. (See reference 6b.)
12. Assignment of E, Z stereochemistry of each isomer, **16a** and **16b**, has remained unknown. Since each E or Z isomer of **16** as well as **17** gave the same pentacyclic product **18**, we used the mixture for the subsequent ring closure without separation. In our model experiments of this series, we assigned the stereochemistry of the ethyl ester analog of these isatinylidene compounds corresponding to **16a** and **17a** as E-configuration by their NMR analysis. (See references 6b and 8.)
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19. Since the inseparable mixture of **24a,b** precluded the NMR analysis, a single isomer of **24a** and **24b** were prepared from the corresponding 4,5-dehydrated isomers of **25a** and **25b** by heating each of them in Ac_2O at 140 °C for 10 h followed by TLC purifications (AcOEt-benzene=2:7), respectively.

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