Total Synthesis of Minosaminomycin[1]

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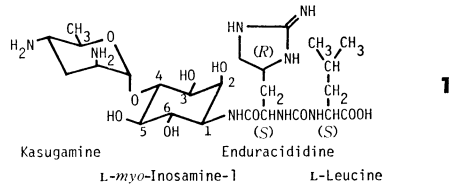
A component of the antibiotic minosaminomycin (**1**), (−)-1D-1-amino-1-deoxy-*myo*-inositol (**4**) was synthesized from D-inositol (**3**) by catalytic oxidation of an axial hydroxyl group followed by reduction of the ketoxime. Minobiosamine, 1D-1-amino-1-deoxy-4-*O*-(α-D-kasugaminyl)-*myo*-inositol (**2**), was synthesized from 2′,4′-di-*N*-acetylkasuganobiosamine (**9**) by a similar method, and separated from the two isomers of **2**. The total synthesis of **1** was accomplished by coupling 2′,4′-di-*N*-benzyloxycarbonylminobiosamine (**14**) and N^{α}-[(*S*)-1-benzyloxycarbonyl-3-methylbutylcarbamoyl]enduracididine (**15**) followed by removal of the protective groups. Compound **14** was derived from 1,2′,4′-tri-*N*-benzyloxycarbonylminobiosamine through the formation of the *cis*-1,2-carbamate. A diastereomer of **1** was also synthesized using alloenduracididine.

Minosaminomycin (**1**), produced by a streptomyces, inhibits the growth of mycobacteria.[2] The mechanism of action of **1** is similar to that of kasugamycin; that is, **1** inhibits the initiation of protein synthesis in a cell free system of *Escherichia coli* and exhibits stronger inhibition than kasugamycin.[3] The structure of **1** and its partial synthesis from natural minobiosamine (**2**) have been reported in a previous communication.[4] In this paper we report the synthesis of (−)-1D-1-amino-1-deoxy-*myo*-inositol (L-*myo*-inosamine-1) (**4**) from D-inositol (**3**), the synthesis of **2** from kasuganobiosamine[5] (**5**) and the total synthesis of **1**.



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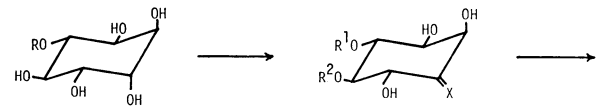
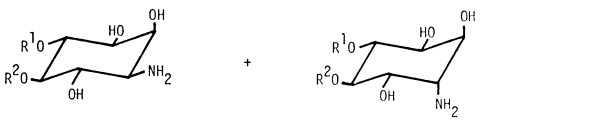
The absolute structure of the new aminocyclitol **4** determined by application of the TACu method,[2] was confirmed by the following synthesis. Catalytic oxidation of **3** with platinum black by the method of Post and Anderson[6] gave L-*myo*-inosose-1 (**6**).[7] The inosose **6** was treated with hydroxylamine to give a ketoxime (**7**) and then **7** was reduced with sodium amalgam to afford **4** in 16.5% yield from **3** by the method of Anderson and Lardy.[8] The identity of **4** with the natural material was confirmed in all respects. The synthesis of **4** also yielded the new aminocyclitol, (+)-1L-1-amino-1-deoxy-*chiro*-inositol (D-inosamine-2) (**8**) which was isolated by column chromatography on Amberlite CG-50 resin and silica gel in 12.4% yield. The structure of **8** was confirmed by the NMR spectrum of its *N*,*O*-hexaacetate in chloroform-*d*, which showed signals for an axial *O*-acetyl groups (δ 2.16), an axial *N*-acetyl and four equatorial *O*-acetyl groups (δ 2.05×3, 2.03, and 2.00)[9] and by application of the TACu method ($\Delta[M]_{TACu}$ −800°).[10]

Based on the successful synthesis of **4**, we designed a synthesis of **2** from **5** which had been synthesized in the total synthesis of kasugamycin.[11,12] Catalytic oxidation of 2′,4′-di-*N*-acetylkasuganobiosamine[5] (**9**) with platinum black followed by treatment with hydroxylamine gave a mixture of ketoximes **10** and **11**. Reduction of the mixture with sodium amalgam followed by alkaline hydrolysis with 2 M sodium hydroxide afforded a mixture of **2**, 1L-1-amino-1-deoxy-4-*O*-(α-D-kasugaminyl)-*chiro*-inositol (**12**) and 1D-1-amino-1-deoxy-5-*O*-(α-D-kasugaminyl)-*myo*-inositol (**13**) which were separated by column chromatography on Amberlite CG-50 resin in 7.9, 16.3, and 5.4% yields from **9**, respectively. The synthetic **2** was identical with the natural one in all respects. From the NMR spectra of **12** and **13** in deuterium oxide, the 1-amino group was confirmed to have an axial orientation in **12** (H-1: δ 3.76, multiplet having small coupling constants with two vicinal protons), and an equatorial orientation in **13** (H-1: δ 3.25, multiplet having a large coupling constant with one of vicinal protons). Acid hydrolysis of the *N*,*O*-heptaacetate of **12** gave **8**. The NMR spectra of the tri-*N*-acetyl-di-*O*-isopropylidene derivative (**12a**) of **12** (methyl signals of *trans*-*O*-isopropylidene: δ 1.37 and 1.39, *cis*: δ 1.32 and 1.48)[13] and the tri-*N*-acetyl-mono-*O*-isopropylidene derivative (**13a**) of **13** (methyl singals of *cis*-*O*-isopropylidene: δ 1.36 and 1.52) confirmed the structures of **12** and **13**.



3 R = H
5 R = α-D-kasugaminyl
9 R = di-*N*-acetyl-α-D-kasugaminyl

6 R^1, R^2 = H, X = O
7 R^1, R^2 = H, X = NOH
10 R^1 = di-*N*-acetyl-α-D-kasugaminyl, R^2 = H, X = NOH
11 R^1 = H, R^2 = di-*N*-acetyl-α-D-kasugaminyl, X = NOH



2 R^1 = α-D-kasugaminyl, R^2 = H
4 R^1, R^2 = H
13 R^1 = H, R^2 = α-D-kasugaminyl
14 R^1 = di-*N*-benzyloxycarbonyl-α-D-kasugaminyl, R^2 = H

8 R^1, R^2 = H
12 R^1 = α-D-kasugaminyl, R^2 = H

For the total synthesis of **1**, 2′,4′-di-*N*-benzyloxycarbonylminobiosamine[4] (**14**) was prepared from 1,2′,4′-tri-*N*-benzyloxycarbonylminobiosamine in good yield by fromation of a cyclic carbamate with sodium hydride in *N*,*N*-dimethylformamide followed by hydrolysis with 5% barium hydroxide octahydrate solution in 50%

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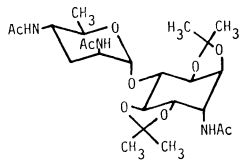
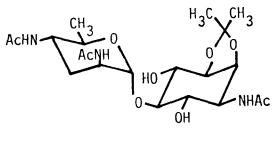
aqueous dioxane at 80 °C.[14] The structure of the cyclic carbamate was shown to be the *cis*-1,2-carbamate of **14** by the NMR spectrum of its mono-*O*-isopropylidene derivative (**14a**) which showed methyl signals for a *trans*-*O*-isopropylidene group at δ 1.38.

The total synthesis of **1** was accomplished in 21% yield by condensation of **14** with N^{α}-[(*S*)-1-benzyloxycarbonyl-3-methylbutylcarbamoyl]enduracididine (**15**) by the activated ester method using 1-hydroxybenzotriazole and dicyclohexylcarbodiimide in *N*,*N*-dimethylformamide[15] followed by catalytic hydrogenation with 5% palladium on carbon in a mixture of methanol, acetic acid and water, as described in a previous communication.[4] The ureylene compound **15** was prepared from L-leucine benzyl ester hydrochloride in 33% yield by treatment with trichloromethyl chloroformate in toluene followed by coupling with enduracididine.[16] Enduracididine found in the antibiotic enduracidin[17] has recently been synthesized from L-histidine by Shiba and coworkers.[18]

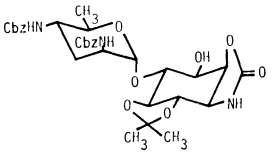
We synthesized also a diastereomer (2*R*-isomer) of **1** from alloenduracididine[16] in place of enduracididine. This isomer showed about 20% of the bacteriostatic activity of **1** by the cylinder plate method using *Mycobacterium smegmatis* ATCC 607 as the test organism.



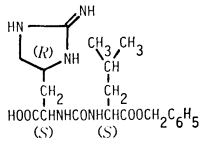
12a



13a



14a



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Experimental

Melting points were determined in capillary tubes and uncorrected. IR spectra were measured in KBr pellets with a Hitachi EPI-S2 spectrometer. NMR spectra were obtained on either a Varian A60-D, HA-100D, or XL-100 spectrometer. Chemical shifts in D_2O were recorded in ppm using tetramethylsilane as an external reference. All chemical shifts in other solvents were recorded in ppm downfield from internal tetramethylsilane. Optical rotations were measured with a Carl Zeiss LEP A2 polarimeter. Mass spectra were recorded with a Hitachi RMU-6M spectrometer. In all the experiments, TLC was carried out on silica gel plates (Merck, Art. 5721) and the spots on TLC were visualized with ninhydrin, 10% sulfuric acid, Rydon-Smith,[19] and HBr–ninhydrin (sprayed with 48% hydrobromic acid and heated at 105 °C, then with ninhydrin) reagents. High-voltage paper electrophoresis was carried out with a Model LT-48A from Savant Instruments under 3500 V for 15 min using formic acid–acetic acid–water (1 : 3 : 36) as an electrolyte solution.[19]

L-myo-*Inosamine-1* (**4**) *and* D-*Inosamine-2* (**8**) *from* D-*Inositol* (**3**). According to the method of Post and Anderson,[6] an aqueous solution (70 ml) of **3** (700 mg, 3.9 mmol) was mixed with an aqueous suspension (35 ml) of platinum black prepared from 350 mg of platinum dioxide just before the reaction, and then oxygen gas was bubbled through at 45 °C for 3 h with stirring. The catalyst was removed by filtration and the filtrate was concentrated to dyrness under reduced pressure, yielding a crude powder (752 mg) of L-*myo*-inosose-1 (**6**). By the method of Anderson and Lardy,[8] to an aqueous solution (12 ml) of the crude **6** (602 mg) and hydroxylamine hydrochloride (323 mg, 4.65 mmol), an aqueous solution (6 ml) of sodium acetate (318 mg, 3.88 mmol) was added dropwise under stirring, and the ketoxime (**7**) was formed by further stirring for 2.5 h at room temperature. To the reaction mixture diluted with water (5 ml), 5% sodium amalgam (total 25 g) was added at 10-min intervals for 1 h under stirring and cooling to below 25 °C in a water bath. The pH was held between 5.5 and 6.5 by the occasional addition of acetic acid (4 ml) and the reaction mixture was continuously stirred for 2 h, After removal of mercury by decantation, the supernatant was made up to 200 ml with water and charged on a column of Amberlite CG-50 (a mixture of 70% NH_4^+ form and 30% H^+ form, 80 ml). The column was washed with water (320 ml) and the eluate with 0.15 M ammonia (800 ml) was collected in 8-ml fractions. The ninhydrin-positive fractions (Nos. 59—66) were combined and concentrated to dryness yielding a slightly yellow powder (256 mg). An aqueous solution (4 ml) of the powder was adjusted to pH 8.2 by 1 M hydrochloric acid and rechromatographed on a column of Amberlite CG-50 (NH_4^+ form, 50 ml). The column was washed with water (200 ml) and eluted with 0.05 M ammonia (500 ml). The eluate was collected in 5-ml fractions. Evaporation of the ninhydrin-positive fractions (Nos. 12—31) afforded a white crystalline powder of **8** (69 mg, 12.4% from **3**); mp 192—193 °C (dec), $[\alpha]_D^{22}$ +55.2° (*c* 0.78, water) (lit,[20] L-isomer: mp 200—205 °C (dec), $[\alpha]_D^{23}$ −64.3°), $\Delta[M]_{TACu}$ −800°.[10] MS *m/e* 180 $[(M+1)^+]$, NMR (D_2O) δ 3.86 (1H t, J=3 Hz, H-1) and 3.9—4.6 (5H), TLC (chloroform–methanol–28% ammonia–water, 1 : 4 : 2 : 1) R_f 0.41. Found: C, 39.78; H, 7.19; N, 7.59%. Calcd for $C_6H_{13}NO_5$: C, 40.22; H, 7.31; N, 7.82%.

Evaporation of other ninhydrin-positive fractions (Nos. 49—59) from the rechromatography afforded a crude powder (156 mg) of **4**. The crude powder was chromatographed on a column of silica gel (Wakogel C-200, Wako Pure Chemicals, 15 g) developed with methanol–chloroform–17% ammonia (4 : 1 : 1), affording a white crystalline powder of **4** (91 mg, 16.5% from **3**); mp 207—212 °C (dec), $[\alpha]_D^{25}$ −4.2° (*c* 2.86, water). MS *m/e* 180 $[(M+1)^+]$, NMR (D_2O) δ 3.18 (1H m, H-1), 3.6—4.3 (4H) and 4.48 (1H br, H-2), TLC (chloroform–methanol–28% ammonia–water, 1 : 4 : 2 : 1) R_f 0.35. Found: C, 38.94; H, 7.04; N, 7.91%. Calcd for $C_6H_{13}NO_5 \cdot 1/2H_2O$: C, 38.30; H, 7.50; N, 7.44%.

The hydrochloride of **4** was crystallized from a mixrute of water and ethanol as colorless needles; mp 201—203 °C (dec), $[\alpha]_D^{25}$ −8.9° (*c* 1.35, water) (lit,[2] mp 201—203 °C, $[\alpha]_D^{26}$ −9.5°). This compound was identical with natural **4** hydrochloride ($C_6H_{13}NO_5 \cdot HCl \cdot 1/2H_2O$)[2] in all respects.

N,O-*Hexaacetates of* **4** *and* **8**. Treatment of **4** (47 mg) with acetic anhydride (0.25 ml) in pyridine (0.5 ml) at room temperature for 21.5 h gave mono-*N*-acetyl-penta-*O*-acetyl-L-*myo*-inosamine-1. Crystallization from a mixture of methanol and diethyl ether afforded colorless needles (97 mg); mp 212—214 °C (dec), $[\alpha]_D^{22}$ −15° (*c* 2.92, chloroform). MS *m/e* 432 $[(M+1)^+]$, IR (KBr) 1750 and 1230 (ester C=O), 1690, and 1530 cm^{-1} (amide), NMR ($CDCl_3$) δ 1.91 (3H s, eq NAc), 1.97 (3H s, eq OAc), 2.01 (6H s, eq OAc×2), 2.04 (3H

s, eq OAc), 2.21 (3H s, ax OAc), 4.51 (1H m, H-1), 5.52 (1H t, J=3 Hz, H-2), and 5.81 (1H d, J=9 Hz, amide), TLC (chloroform–ethanol, 10 : 1) R_f 0.65. Found: C, 50.31; H, 5.66; N, 3.65%. Calcd for $C_{18}H_{25}NO_{11}$: C, 50.11; H, 5.84; N, 3.25%.

Treatment of **8** (23 mg) with acetic anhydride (0.25 ml) in pyridine (0.5 ml) at room temperature for 18 h gave mono-*N*-acetylpenta-*O*-acetyl-D-inosamine-2. Crystallization from diethyl ether afforded colorless crystals (56 mg); mp 151—152 °C (dec), $[\alpha]_D^{22}$ −1.7° (*c* 0.67, chloroform). MS *m/e* 432 [(M+1)$^+$], IR (KBr) 1755 and 1230 (ester C=O), 1650 and 1550 cm^{-1} (amide), NMR ($CDCl_3$) δ 2.00 (3H s, eq OAc), 2.03 (3H s, eq OAc or ax NAc), 2.05 (9H s, eq OAc or ax NAc×3), 2.16 (3H s, ax OAc), 4.71 (1H m, H-1), and 6.51 (1H d, J=8 Hz, amide), TLC (chloroform–ethanol, 10 : 1) R_f 0.56. Found: C, 49.99; H, 5.62; N, 4.08%. Calcd for $C_{18}H_{25}NO_{11}$: C, 50.11; H, 5.84; N, 3.25%.

*Minobiosamine (**2**) and Its Isomers (**12** and **13**) from Kasuganobiosamine (**5**).* According to the method of Suhara *et al.*[5] 2′,4′-di-*N*-acetylkasuganobiosamine (**9**) was derived from **5**. An aqueous solution (200 ml) of **9** (2.0 g, 5.1 mmol) was mixed with an aqueous suspension (100 ml) of platinum black which was prepared from 1.0 g of platinum dioxide, and then oxygen gas was bubbled through at 45 °C for 2.5 h with stirring.[6] The catalyst was removed by filtration and the filtrate was concentrated to dryness yielding a mixture (1.75 g) of oxo compounds. To an aqueous solution (30 ml) containing the mixture (1.48 g) and hydroxylamine hydrochloride (396 mg, 5.7 mmol), an aqueous solution (15 ml) of sodium acetate (390 mg, 4.75 mmol) was added dropwise under stirring at room temperature, and stirring was continued for 2 h. The reaction mixture was concentrated to dryness yielding a mixture (1.946 g) of ketoximes **10** and **11**. The ketoxime (896 mg) in water (15 ml) was reduced with 25 g of 5% sodium amalgam in the same manner as described for **7**. After removal of mercury by decantation, the supernatant was concentrated to dryness. The residue was dissolved in 2 M sodium hydroxide (40 ml) and refluxed for 15 h in an oil bath at 125 °C to remove the acetyl groups. After neutralization with acetic acid (4.8 ml), the solution was made up to 400 ml with water and charged on a column of Amberlite CG-50 (a mixture of 70% NH_4^+ form and 30% H^+ form, 150 ml). The column was washed with water (600 ml) and the eluate with 900 ml of 0.5 M ammonia was collected in 15-ml fractions. Ninhydrin-positive fractions (Nos. 27—31) were combined and concentrated to dryness yielding a white powder (271 mg). It was dissolved in 5 ml of water and rechromatographed on a column of Amberlite CG-50 (NH_4^+ form, 80 ml). The column was washed with water (160 ml) and products were eluted with 0.1 M ammonia. The eluate was collected in 4-ml fractions which were examined by TLC with chloroform–methanol–28% ammonia–water (1 : 4 : 2 : 1). The fractions (Nos. 40—47) with R_f 0.69 were combined and concentrated to dryness yielding an epimer of **2**, 1L-1-amino-1-deoxy-4-*O*-(α-D-kasugaminyl)-*chiro*-inositol (**12**), as a white powder (81 mg, 16.3% from **9**); mp 104—115 °C (dec), $[\alpha]_D^{27}$ +117° (*c* 0.3, water). MS *m/e* 308 [(M+1)$^+$], NMR (D_2O) δ 1.72 (3H d, J=6 Hz, H-6′), 2.38 (2H m, H-3′), 3.44 (1H m, H-4′), 3.76 (2H, H-1 and -2′), and 5.43 (1H br, H-1′).

The fractions (Nos. 60—79) which contained material with R_f 0.64 were combined and concentrated to dryness yielding a positional isomer of **2**, 1D-1-amino-1-deoxy-5-*O*-(α-D-kasugaminyl)-*myo*-inositol (**13**), as a white powder (27 mg, 5.4% from **9**); mp 112—128 °C (dec), $[\alpha]_D^{27}$ +76° (*c* 0.59, water). MS *m/e* 308 [(M+1)$^+$], NMR (D_2O) δ 1.71 (3H d, J=6 Hz, H-6′), 2.36 (2H m, H-3′), 3.10—3.55 (2H, H-1 and -4′), 3.71 (1H m, H-2′), and 5.46 (1H d, J=2 Hz, H-1′).

The fractions (Nos. 80—102) with R_f 0.66 by TLC were combined and concentrated to dryness affording a white powder (39 mg, 7.9% from **9**) of **2**; mp 126—128 °C (dec), $[\alpha]_D^{32}$ +82.5° (*c* 1.0, water) (lit,[4] mp 126—128 °C (dec), $[\alpha]_D^{29}$ +81°). MS *m/e* 308 [(M+1)$^+$], NMR (D_2O) δ 1.69 (3H d, J=6 Hz, H-6′), 2.24 (2H m, H-3′), 2.95—3.38 (2H m, H-1 and -4′) 3.58 (1H m, H-2′), and 5.37 (1H d, J=2 Hz, H-1′). Found: C, 44.52; H, 8.38; N, 12.45%. Calcd for $C_{12}H_{25}N_3O_6 \cdot H_2O$: C, 44.29; H, 8.36; N, 12.93%.

N,O-*Heptaacetate of **2**.* Treatment of **2** (37 mg) with acetic anhydride (0.4 ml) in pyridine (0.8 ml) at room temperature for 18 h afforded the *N,O*-heptaacetate of **2**. Crystallization from a mixture of methanol and diethyl ether gave colorless needles (48 mg); mp 273—274 °C (dec), $[\alpha]_D^{25}$ +4.3° (*c* 2.55, chloroform). MS *m/e* 602 [(M+1)$^+$], IR (KBr) 1755 and 1230 (ester C=O), 1660 and 1550 cm^{-1} (amide), NMR ($CDCl_3$) δ 1.14 (3H d, J=6 Hz, H-6′), 1.75 (2H m, H-3′), 1.89 (3H s, 1-eq NAc), 1.95, 1.98, 2.01, 2.04, 2.15 (each 3H s, eq OAc×3, 2′-NAc and 4′-NAc), 2.18 (3H s, 2-ax OAc), and 6.1—6.8 (3H, amides), TLC (chloroform–ethanol, 10 : 1) R_f 0.26. Found: C, 51.52; H, 6.25; N, 6.56%. Calcd for $C_{26}H_{39}N_3O_{13}$: C, 51.91; H, 6.53; N, 6.99%. It was identical with the *N,O*-heptaacetate derived from natural **2** in all respects.

N,O-*Heptaacetates of **12** and **13**.* Compound **12** (79 mg) was treated with acetic anhydride (0.75 ml) in pyridine (1.6 ml) at room temperature for 18 h and the reaction mixture was concentrated to dryness. The residue was chromatographed on a column of silica gel (Wakogel C-200, 14 g) developed with chloroform–ethanol (10 : 1). Fractions containing the *N,O*-heptaacetate of **12** were combined and concentrated to dryness yielding a white crystalline powder (127 mg); mp 158—160 °C (dec), $[\alpha]_D^{24}$ +29° (*c* 0.74, chloroform). MS *m/e* 602 [(M+1)$^+$], NMR ($CDCl_3$) δ 1.17 (3H d, J=6 Hz, H-6′), 1.80 (2H m, H-3′), 1.97, 2.00, 2.02, 2.04, 2.05, 2.11 (each 3H s, eq OAc ×3, 1-ax NAc, 2′-NAc and 4′-NAc), 2.12 (3H s, 2-ax OAc), and 6.2—7.2 (3H, amides), TLC (chloroform–ethanol, 10 : 1) R_f 0.15.

Treatment of **13** (25 mg) with acetic anhydride (0.25 ml) in pyridine (0.5 ml) at room temperature for 18 h followed by column chromatography on silica gel (Wakogel C-200, 4.5 g) developed with chloroform–ethanol (20 : 1) gave a white crystalline powder of the *N,O*-heptaacetate of **13** (30 mg); mp 172—177 °C (dec). MS *m/e* 602 [(M+1)$^+$], NMR ($CDCl_3$) δ 1.12 (3H d, J=6 Hz, H-6′), 1.75 (2H m, H-3′), 1.89 (3H s, 1-eq NAc), 1.95, 1.97, 1.99, 2.07, 2.11 (each 3H s, eq OAc×3, 2′-NAc and 4′-NAc), 2.20 (3H s, 2-ax OAc), and 6.0—6.7 (3H, amides), TLC (chloroform–ethanol, 10 : 1) R_f 0.20.

Hydrolysis of the N,O-*Heptaacetate of **12**.* The *N,O*-heptaacetate of **12** (45 mg) was hydrolyzed with 6 M hydrochloric acid (2 ml) by refluxing in an oil bath at 100 °C for 5 h and the reaction mixture was concentrated to dryness. The residue was chromatographed on a column of Amberlite CG-50 (NH_4^+ form, 5 ml) eluted with 0.05 M ammonia (50 ml). The eluate containing **8** was concentrated to dryness yielding a white crystalline powder of **8** (11 mg, 82%).

*1L-1-Acetamido-1-deoxy-4-*O*-(di-*N*-acetyl-α-D-kasugaminyl)-2,3 : 5,6-di-*O*-isopropylidene-*chiro*-inositol (**12a**).* Compound **12** (20 mg, 0.067 mmol) was treated with acetic anhydride (0.3 ml) in methanol (0.6 ml) at room temperature for 6.5 h and the reaction mixture was concentrated to dryness yielding a slightly yellow powder (26 mg) of the tri-*N*-acetate of **12**. To a solution of this powder in dry *N,N*-dimethylformamide (0.6 ml) were added 2,2-dimethoxypropane (62 mg, 0.6 mmol) and *p*-toluenesulfonic acid monohydrate (1.4 mg

0.006 mmol). The reaction mixture was heated at 60 °C for 2 h under stirring. After neutralization with triethylamine (1.2 mg), the reaction mixture was concentrated to dryness. Purification by column chromatography on silica gel (Silic AR CC-7, Mallinckrodt, 4 g) developed with chloroform–ethanol (12 : 1) gave a white powder of **12a** (13 mg); mp 177—181 °C (dec), $[\alpha]_D^{23}$ +111° (*c* 0.73, methanol). IR (KBr) 1650 and 1545 cm^{-1} (amide), NMR (CD_3OD) δ 1.15 (3H d, J=6 Hz, H-6′), 1.32, 1.37, 1.39, 1.48 (each 3H s), 1.80 (2H m, H-3′), 1.91 (3H s, NAc), 1.99 (6H s, NAc×2), and 4.97 (1H br, H-1′), TLC (chloroform–ethanol, 1 : 1) R_f 0.81.

1-D-*1-Acetamido-1-deoxy-5-*O-*(di-*N-*acetyl-α-*D-*kasugaminyl)-2,3-*O-*isopropylidene-*myo-*inositol (**13a**).* Treatment of **13** (4.0 mg, 0.013 mmol) with acetic anhydride (0.05 ml) in methanol (0.15 ml) at room temperature for 7 h afforded a slightly yellow powder (5.0 mg) of the tri-*N*-acetate of **13**. Treatment of this powder in dry *N,N*-dimethylformamide (0.2 ml) with 2,2-dimethoxypropane (12 mg, 0.115 mmol) and *p*-toluenesulfonic acid monohydrate (0.2 mg, 0.001 mmol) at 60 °C for 2 h followed by neutralization with triethylamine (0.2 mg) and evaporation gave a crude powder. Purification by column chromatography on silica gel (Silic AR CC-7, 800 mg) developed with chloroform–ethanol (4 : 1) yielded a white crystalline powder of **13a** (2.9 mg); mp 157—162 °C (dec), $[\alpha]_D^{24}$ +64° (*c* 0.43, chloroform–ethanol (4 : 1)). IR (KBr) 1640 and 1540 cm^{-1} (amide), NMR (CD_3OD–$CDCl_3$ (1 : 1)) δ 1.16 (3H d, J=6 Hz, H-6′), 1.36, 1.52 (each 3H s), 1.85 (2H m, H-3′), 1.97, 2.02, 2.06 (each 3H s, NAc×3), and 5.01 (1H br, H-1′), TLC (chloroform–ethanol, 1 : 1) R_f 0.59.

*2′,4′-Di-*N*-benzyloxycarbonylminobiosamine (**14**).* To a solution of **2** (1.03 g, 3.35 mmol) and sodium hydrogencarbonate (1.1 g, 13 mmol) in water (15 ml), benzyloxycarbonyl chloride (1.88 g, 11 mmol) was added dropwise. The mixture was stirred for 1.5 h in an ice bath and then allowed to stand overnight at room temperature. The resulting precipitate was filtered and washed with water (30 ml) and diethyl ether (50 ml) to give a colorless powder of 1,2′,4′-tri-*N*-benzyloxycarbonylminobiosamine (2.02 g, 85%); mp 171—172 °C (dec), $[\alpha]_D^{24}$ +33° (*c* 1.0, *N,N*-dimethylformamide). IR (KBr) 1690 and 1525 cm^{-1} (amide), NMR (CD_3COCD_3) δ 1.15 (3H d, J=6 Hz, H-6′), 2.02 (2H m, H-3′), 5.06 (6H s, CH_2-ar), 5.16 (1H br, H-1′), and 7.34 (15H, ar), TLC (chloroform–methanol, 10 : 1) R_f 0.60. Found: C, 60.25; H, 6.41; N, 5.97%. Calcd for $C_{36}H_{43}N_3O_{12}$: C, 60.92; H, 6.11; N, 5.92%.

To a solution of 1,2′,4′-tri-*N*-benzyloxycarbonylminobiosamine (1.50 g, 2.1 mmol) in dry *N,N*-dimethylformamide (15 ml), 50% sodium hydride (273 mg, 5.7 mmol) was added, and the mixture was stirred for 4.5 h in an ice bath under a stream of nitrogen. After neutralization with acetic acid (0.36 ml), the solution was concentrated to dryness. A solution of the residue in ethyl acetate (50 ml) was washed with water (10 ml), dehydrated with anhydrous sodium sulfate, and evaporated to give a pale yellow oil (2.0 g). The oil was chromatographed on a column of silica gel (Silic AR CC-7, 100 g) developed with chloroform–methanol (10 : 1). Fractions containing 2′,4′-di-*N*-benzyloxycarbonylminobiosamine-1,2-carbamate were combined and concentrated to dryness yielding a white powder (970 mg, 77%); mp 115—118 °C (dec), $[\alpha]_D^{25}$ +45° (*c* 1.0, chloroform). IR (KBr) 1750 (C=O), 1690, and 1525 cm^{-1} (amide), NMR ($CDCl_3$) δ 1.14 (3H br, H-6′), 1.84 (2H m, H-3′), 4.98 (4H s, CH_2-ar ×2), and 7.22 (10H, ar), TLC (1-butanol–ethanol–chloroform–17% ammonia, 4 : 5 : 2 : 1) R_f 0.52. Found: C, 57.50; H, 6.96; N, 6.80%. Calcd for $C_{29}H_{35}N_3O_{11}$: C, 57.90; H, 6.86; N, 6.99%.

A solution containing the carbamate (914 mg, 1.52 mmol) and barium hydroxide octahydrate (765 mg, 2.34 mmol) in 50% aqueous dioxane (29 ml) was heated at 80 °C for 6 h. The reaction mixture was neutralized with carbon dioxide, and the resulting precipitates were removed by filtration. The filtrate was concentrated to give a solid (793 mg), which was chromatographed on a column of silica gel (Silic AR CC-7, 80 g) developed with 1-butanol–ethanol–chloroform–17% ammonia (8 : 10 : 4 : 1). Fractions containing **14** were combined and concentrated to dryness yielding a white powder of **14** (556 mg, 64%); mp 123—126 °C (dec), $[\alpha]_D^{24}$ +43° (*c* 1.0, *N,N*-dimethylformamide). IR (KBr) 1690 and 1520 cm^{-1} (amide), NMR (CD_3OD) δ 1.13 (3H d, J=6 Hz, H-6′), 1.88 (2H m, H-3′), 2.62 (1H m, H-1), 5.04 (5H, H-1′ and CH_2-ar), and 7.30 (10H, ar), TLC (1-butanol–ethanol–chloroform–17% ammonia, 4 : 5 : 2 : 1) R_f 0.24. Found: C, 56.77; H, 6.67; N, 7.12%. Calcd for $C_{28}H_{37}N_3O_{10}\cdot H_2O$: C, 56.65; H, 6.62; N, 7.08%.

*2′,4′-Di-*N*-benzyloxycarbonyl-5,6-*O*-isopropylideneminobiosamine-1,2-carbamate (**14a**).* To a solution of 2′,4′-di-*N*-benzyloxycarbonylminobiosamine-1,2-carbamate (17 mg, 0.029 mmol) in dry *N,N*-dimethylformamide (0.3 ml) were added 2,2-dimethoxypropane (31 mg, 0.3 mmol) and *p*-toluenesulfonic acid monohydrate (0.7 mg, 0.003 mmol). The mixture was heated at 60 °C for 2 h under stirring. After neutralization with triethylamine (0.6 mg), the reaction mixture was concentrated to dryness. Purification by column chromatography on silica gel (Silic AR CC-7, 3 g) developed with chloroform–ethanol (30 : 1) gave a white crystalline powder of **14a** (14.7 mg); mp 121—124 °C (dec), $[\alpha]_D^{23}$ +49.5° (*c* 0.85, methanol). IR (KBr) 1755 (C=O), 1700, and 1520 cm^{-1} (amide), NMR (CD_3OD) δ 1.19 (3H d, J=6 Hz, H-6′), 1.38 (6H s), 1.90 (2H m, H-3′), 4.92 (1H br, H-1′), 5.08 (4H s, CH_2-ar), and 7.32 (10H, ar), TLC (chloroform–ethanol, 10 : 1) R_f 0.50.

*Minosaminomycin (**1**).* To a solution of L-leucine benzyl ester hydrochloride (206 mg, 0.8 mmol) in dry toluene (4 ml), trichloromethyl chloroformate (336 mg, 1.7 mmol) was added. After refluxing for 4.5 h in an oil bath at 130 °C, the reaction mixture was evaporated to give a colorless oil of the isocyanate of L-leucine benzyl ester (189 mg, 95%); $n_D^{25.5}$ 1.4987, $[\alpha]_D^{26}$ −29° (*c* 1.55, toluene). IR (KBr) 2200 (N=C=O) and 1740 cm^{-1} (ester C=O), NMR ($CDCl_3$) δ 0.90 (6H d, J=5 Hz, CH_3×2), 1.4—2.0 (3H m, CH_2–CH), 4.10 (1H dd, J=7, 8 Hz, α-methine), 5.26 (2H s, CH_2-ar), and 7.42 (5H, ar).

A solution containing the isocyanate (189 mg, 0.77 mmol) and enduracididine (73 mg, 0.43 mmol) in dry dimethyl sulfoxide (4.5 ml) was stirred at room temperature for 17.5 h and then freeze dried to give a colorless oil (350 mg). Purification of the oil by column chromatography on silica gel (Silic AR CC-7, 36 g) developed with 1-butanol–ethanol–water (10 : 1 : 1) gave a white powder of N^{α}-[(*S*)-1-benzyloxycarbonyl-3-methylbutylcarbamoyl]enduracididine (**15**) (64 mg, 33% from enduracididine). To a methanolic solution (1 ml) of **15**, 0.5 M hydrochloric acid (0.28 ml) was added, and the solution was concentrated to dryness yielding a white powder (67 mg) of the monohydrochloride of **15**. To a solution containing the monohydrochloride (64 mg, 0.13 mmol) and 1-hydroxybenzotriazole[15] (22 mg, 0.16 mmol) in dry *N,N*-dimethylformamide (1.5 ml) was added a solution of **14** (77 mg, 0.13 mmol) in dry *N,N*-dimethylformamide (2 ml) and then dicyclohexylcarbodiimide (28 mg, 0.13 mmol). The mixture was stirred at 0 °C for 1 h and then at room temperature for 22.5 h. The dicyclohexylurea formed was removed by filtration and the filtrate was concentrated to dryness yielding a solid (202 mg). To remove the *N*-benzyl-

oxycarbonyl and benzyl ester groups, this solid in a mixture of methanol (3 ml), water (1 ml), and acetic acid (1 ml) was hydrogenated with 5% palladium on carbon (126 mg) under atmospheric pressure for 5 h. The catalyst was removed by filtration and the filtrate was concentrated to dryness yielding a white powder. An aqueous solution of the powder was charged on a column of Amberlite CG-50 (a mixture of 70% NH_4^+ form and 30% H^+ form, 5 ml). The column was washed with water (20 ml) and **1** was eluted with 0.15 M ammonia. Fractions containing **1** detected by high-voltage paper electrophoresis were combined and concentrated to dryness yielding a crude powder of **1** (35.8 mg). Purification by rechromatogrphy on a column of Amberlite CG-50 (NH_4^+ form, 5 ml) eluted with 0.05 M ammonia (50 ml) gave a white powder of **1** (17 mg, 21% from **15**); mp 225—260 °C (dec), $[\alpha]_D^{23}$ +28.4° (*c* 0.53, water) (lit,[2] mp 225—260 °C (dec), $[\alpha]_D^{22}$ +30°). It was identical with natural **1** ($C_{25}H_{46}N_8O_{10}\cdot 2H_2O$) in all respects including biological activity.

2R-Isomer of 1. A diastereomer (2*R*-isomer) of **1** was synthesized using alloenduracididine with the method described for **1**. To a solution of alloenduracididine (51 mg, 0.3 mmol) in dry dimethyl sulfoxide (4 ml) was added the isocyanate of L-leucine benzyl ester (146 mg, 0.6 mmol) in dry dimethyl sulfoxide (1 ml). The mixture was stirred at room temperature for 22.5 h, and freeze-dried to give a slightly yellow oil (280 mg). The oil was chromatographed on a column of silica gel (Wokogel C-200, 28 g) developed with 1-butanol–ethanol–water (10 : 1 : 1) and a white powder (23 mg, 17% from alloenduracididine) of the 2*R*-isomer of **15** was obtained. The monohydrochloride of the 2*R*-isomer of **15** was prepared by addition of 0.5 M hydrochloric acid (0.08 ml) in a methanolic solution (0.5 ml). To a solution of the monohydrochloride in dry *N,N*-dimethylformamide (0.5 ml) was added **14** (33 mg, 0.057 mmol) in dry *N,N*-dimethylformamide (0.5 ml), 1-hydroxybenzotriazole (11.5 mg, 0.085 mmol), and dicyclohexylcarbodiimide (10.7 mg, 0.052 mmol). The mixture was stirred at 0 °C for 1 h and then at room temperature for 26.5 h. The dicyclohexylurea formed was removed by filtration and the filtrate was concentrated to give a solid (83.4 mg). This solid in a mixture (2.5 ml) of methanol, water, and acetic acid (3 : 1 : 1) was hydrogenated with 5% palladium on carbon (50 mg) under atmospheric pressure for 6.5 h. After removal of the catalyst, the solution was concentrated to dryness. The residue was purified by column chromatography with Amberlite CG-50 (a mixture of 70% NH_4^+ form and 30% H^+ form, 5 ml) eluted with 0.15 M ammonia to yield a crude powder (15.6 mg). The crude powder was rechromatographed on a column of Amberlite CG-50 (NH_4^+ form, 5 ml) eluted with 0.05 M ammonia to give a white powder (2.9 mg, 9% from the 2*R*-isomer of **15**) of the pure 2*R*-isomer of **1**; mp 195—220 °C (dec), $[\alpha]_D^{27}$ +42° (*c* 0.95, water). IR (KBr) 1660 and 1570 cm^{-1} (amide), TLC (1-butanol–ethanol–chloroform–17% ammonia, 4 : 5 : 2 : 5) R_f 0.13.

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