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An Efficient and Selective 1-*N*-Monoethylation of Sisomicin: Process Development of Netilmicin

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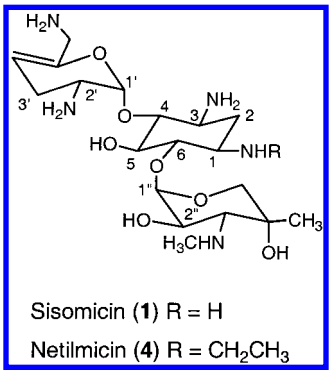
Abstract:

With a new reagent developed for the selective monoethylation at the 1-amino group of sisomicin (1), a new process suitable for the mass production of netilmicin under conditions less sensitive to air and moisture has also been developed. Three of the amino groups, at the C-3, C-2′, and C-6′ positions of the four amino groups of sisomicin, were selectively protected by using $Zn(OAc)_2$ and acetic anhydride in methanol. Development efforts focused on optimising the conditions for ethylation to give an improved product (96% yield) according to the new and concise synthetic route.

Introduction

Sisomicin (**1**) is a naturally occurring aminoglycoside antibiotic produced by *Micromonospora inyoensis*, while netilmicin (**4**) is a semisynthetic aminoglycoside derived from ethylation at the 1-amino group of sisomicin. The introduction of an ethyl group to the 1-amino group of sisomicin was known to inhibit the acetylation by acetylase,[2] resulting in an improvement not only in the stability against aminoglycoside-resistant strains but also in antibacterial activity. In clinical studies, netilmicin showed significantly less cytotoxicity than tobramicin, and less nephrotoxicity than either tobramicin or gentamicin.[3]



Antibacterial activity of netilmicin showed a generally broader antibacterial spectrum than that of sisomicin, tobramicin, dibekacin, or gentamicin. Since netilmicin (**4**) was originally developed by Schering Co., U.S.A. in 1976[4–6] and launched onto the U.S. market early in the 1980s, it has been widely used in Great Britain, Scandinavia, continental Europe, Southeast Asia, Australia, and New Zealand as a remedy for respiratory localized infections, urinary tract infections, and bacteremia originating from Gram-negative bacteria.

Even though there have been several efforts to develop more economical and effective synthetic routes, problems still exist in the selective monoethylation at the C-1 amino group of sisomicin and in the selective protection of the three amino groups of the C-3, C-2′, and C-6′ positions. Sisomicin has in total, five amino groups: four primary (C-6′, C-1, C-3, and C-2′ positions) and one secondary (C-3″ position). The reactivities of the four primary amino groups are similar to each other, but that of the secondary position is slightly different from these. It is difficult to undergo selective ethylation at the C-1 amino group. Two methods for selective ethylation have been reported. First, by controlling the pH of the reaction media due to the pH-dependent reactivity of amino groups,[4] it was possible to accomplish selective ethylation of the C-1 amino group through reductive alkylation with acetaldehyde and sodium cyanoborohydride. But the formation of byproducts could not be avoided because very precise pH control of the reaction media was necessary. They were identified as the diethylated compound at the C-1 position and an ethylated compound at another amino group. Second, by selective chelation of particular amino and hydroxy groups, it was possible to protect the remaining free amino groups and then to achieve the selective ethylation at the C-1 amino group.[5] However, a large excess of chelation reagent, such as $(Cu(OAc)_2$, $Ni(OAc)_2$ or $Co(OAc)_2)$ was required, and consequently, environmental and cost problems arise. In addition, the reductive alkylation with acetaldehyde and sodium cyanoborohydride produced the diethylated compound as a byproduct.

The formation of a diethylated side-product resulted in intensive purification, lowered the yield, and increased the cost of production. For this reason, we have focused the process development of netilmicin in two areas: One is to optimize the protection conditions of the three amino groups at the C-3, C-2′, and C-6′ positions with $Zn(OAc)_2$, and the

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Scheme 1

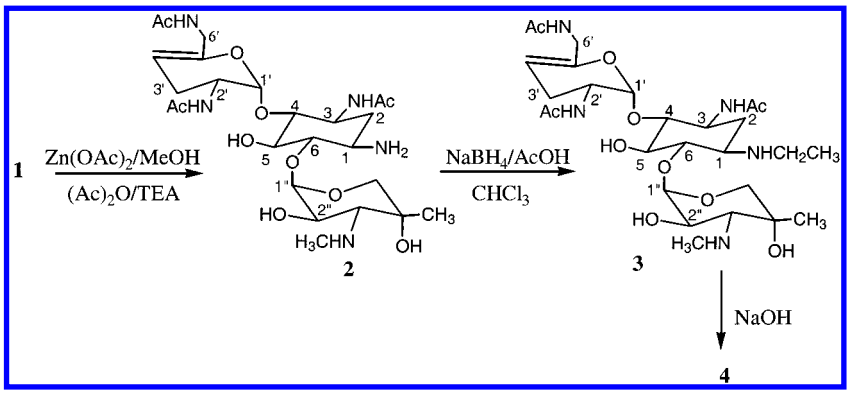

Table 1. **Protection of the 3,2′,6′-amino groups using $Zn(OAc)_2$ and $(Ac)_2O$**

entry	equiv of $Zn(OAc)_2$	equiv of $(Ac)_2O$	equiv of TEA	time (h)	yield[a] (%)
1	4.0	3.2	7.0	24.0	79
2	4.0	4.0	7.0	48.0	82
3	4.0	4.0	7.0	20.0	92
4	2.5	3.2	5.0	15.0	85
5	2.5	3.5	5.0	18.0	96
6	2.5	3.5	–	18.0	51

[a] Isolated yield.

other is to develop a selective monoethylation reagent to avoid diethylation at the C-1 position. In this report, we describe an optimized, efficient and selective 1-*N*-ethylation method which uses a mixture of carboxylic acid and boron metal hydride.[7−9]

Results and Discussion

The synthetic route for netilmicin can be divided into three steps, as shown in Scheme 1: protection of the amino groups at the C-3, C-2′, and C-6′ positions, ethylation at the C-1 amino group of the protected sisomicin (**2**), and deprotection of **3** to afford **4**.

Step I: Protection of the Amino Groups at the C-3, C-2′, C-6′ Positions. For the protection of the amino groups at the C-3, C-2′, and C-6′ positions, $Zn(OAc)_2$ was used as the chelating reagent. In early investigations, the desired product was obtained from the reaction of **1** with $Zn(OAc)_2$ and acetic anhydride in methanol in 51% yield; however, it contained tetraacetylated byproducts, which made the purification procedure tedious and lowered the yield. We therefore made an effort to improve the reaction conditions and found that the presence of triethylamine in the reaction inhibits the formation of byproducts. The results are summarized in Table 1. Use of 2.5 equiv of zinc acetate, 3.5 equiv of acetic anhydride, and 5 equiv of triethylamine significantly improved the yield of the desired product.

Ammonia water removed chelating metal from the protected sisomicin derivative, followed by chromatography on silica gel to afford the 3,6′,2′-tri-*N*-acetylated sisomicin in good yield and purity. (96% chemical yield; 99% chemical purity.)

Table 2. **Result of selective C-1 mono-ethylation of 3,2′,6′-tri-*N*-acetylsisomicin (2)**

entry	[a]ratio of AcOH	[b]ratio of $NaBH_4$	solvent	temp (°C)	time (h)	yield (%)
1	3.3	5.0	THF	rt	96	NR[c]
2	3.3	5.0	THF	40	18	59
3	3.3	5.0	THF	50	6	28
4	3.0	5.0	$CHCl_3$	40	48	80
5	3.0	5.0	$CHCl_3$	50	48	62
6	4.0	5.0	toluene	40	24	18
7	4.0	5.0	benzene	40	48	37
8	4.0	8.0	THF	40	20	68
9	4.0	8.0	$CHCl_3$	40	18	96

[a] Ratio of acetic acid to sodium borohydride. [b] Ratio of sodium borohydride to tri-acetylsisomicin. [c] NR means no reaction. Entry 9 is optimimum condition; ratio of AcOH/$NaBH_4$ is 4 and ratio of $NaBH_4$/triacetylsisomicin is 8; thus, the net molar ratio of net AcOH is 32-fold over triacetylsisomicin.

Step II: Monoethylation of the C-1 Amino Group. The monoethylation of the C-1 amine is the key step because of the potential formation of the diethylated byproduct. In general, a series of *N*-alkylamine is more reactive than the free amines for the reductive alkylation reaction. Even though monoethylation had occurred, it was difficult to maintain constant reaction conditions, such as pH, the amount of reagent (especially acetaldehyde), and so on. Therefore, it was inevitable that the diethylated compound was produced. Accordingly, a new method for the specific ethylation in the synthesis of 1-*N*-ethylsisomicin was employed. Gordon W. Gribble's[7] reductive alkylation method of amine was applied to this case. The reaction of sisomicin with a mixture of sodium borohydride and acetic acid in chloroform gave the monoethylated product in 96% yield. The results of these reactions are shown in Table 2. The sodium borohydride was added in several portions at room temperature, and the reaction was carried out under the presented conditions at several temperatures.

The optimized method in order to enhance the yield was the use of a 4 M ratio of acetic acid and an 8 M of sodium borohydride (see entry 8 in Table 2). Another way to improve the yield was achieved by using chloroform at 40 or 50 °C. Using other solvents, THF, toluene, or benzene lowed yields in the range of 20−60%. A temperature above 50 °C or room temperature also caused a reduction in the yield.

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Table 3. **Mono-1-*N*-ethylation using various reducing agents**

entry	reducing agent	solvent	temp (°C)	yield[e] (%)
1	$NaBH(OAc)_3$[a] (7 equiv)	$CHCl_3$	40	87
2	$NaBH(OAc)_3$[b] (7 equiv)	$CHCl_3$	40	78
3	KBH_4/AcOH[c]	$CHCl_3$	40	72
4	$NaBH_4$/AcOH[d]	$CHCl_3$	40	96

[a] It was synthesized in our laboratory by reference 10 and also contained $NaBH_2(OAc)_2$ and $NaBH_3OAc$. [b] It was purchased from Aldrich Chemicals.[c] The same ratio as entry 9 in Table 2. [d] The same ratio as entry 9 in Table 2. [e] Isolated yield.

Potassium borohydride or triacetoxy sodium borohydride was used in place of sodium borohydride as the reducing agent (Table 3). These reagents helped to reduce the environmental problems compared to sodium cyanoborohydride.

The desired product **2** was purified by extraction, and the *N*-deacylation of **2** with aqueous sodium hydroxide gave **4** after purification by silica gel column chromatography in over 90% yield. Each compound proposed during the investigation was elucidated by comparing the NMR spectra and the physical data of authentic samples.

Conclusions

Netilmicin **4** was synthesized from sisomicin (**1**) in a 73% overall yield in three steps. Discovery of a monoethylation reagent and optimization of monoethylation at the C-1 amino group of **2** was successfully achieved. In addition, reduction in the quantity of the chelating agent and the use of an inexpensive reagent ($Zn(OAc)_2$) together with simplification of the workup led to a successful, economical, and practical synthesis of netilmicin.

Experimental Section

Commercial reagent grade solvents were used. All reactions were monitored by analytical thin-layer chromatography (TLC, silica gel Baker-flex IB2-F plates) with fluorescent indicator (254 nm), and visualized by ultraviolet light and staining with anisaldehyde or ceric ammonium molybdate. ^{1}H NMR spectra were recorded on a Brüker AMX-300 (300 MHz) or AMX-500 (500 MHz), and δ values are given in ppm relative to trimethylsilane as the internal standard. ^{13}C NMR spectra were obtained with proton decoupling on a Brüker AMX-300 (75 MHz), or AMX-500 (125 MHz) spectrometer and are reported in ppm with residual solvent as the internal standard (77.0 for $CDCl_3$). Mass spectra were determined with a VG70-VSEQ mass spectrometer at 8 kV ionizing voltage (FAB-Gunmicrobeam-35 keV, Cs^+, matrix: nitrobenzylalchol). Melting points were measured on a Thomas-Hoover apparatus, and are not corrected.

Preparation of 3,2′,6′-Tri-*N*-acetylsisomicin (2). Sisomicin (**1**) (447 g, 1.00 mol) was dissolved in methanol (5 L), and zinc acetate dihydrate (447 g, 2.5 mol) was added. After the reaction mixture stirred at room temperature for 15 h, a solution of acetic anhydride (331 mL, 3.5 mol) and triethylamine (696 mL, 5.0 mol) in tetrahydrofuran (1.5 L) was added dropwise to the stirred solution at room temperature over 1 h. The reaction mixture was concentrated under vacuum, and the residue was dissolved in a mixture of 28% ammonia water (1 L) and ethanol (2 L). The organic layer was extracted with chloroform (2 L × 3) and then washed with saturated brine (1 L). The organic layer was concentrated under vacuum. The resultant residue was subjected to silica gel column chromatography (70−230 mesh, 1.5 kg) using an eluting solution comprising chloroform, methanol, and 14% ammonia water in the ratio of 2:1:1. Evaporation of the appropriate combined fractions gave the title compound (551 g, 0.96 mol) as an ivory solid: 96% yield. MS (FAB) 574 ($M + H^+$); ^{1}H NMR (D_2O, 300 MHz) δ 1.34 (s, 3H, C−CH_3), 1.95 (s, 3H), 2.00 (s, 3H), 2.02 (s, 3H), 2.19 (m, 3H), 2.91 (s, 3H, *N*-CH_3, 3.46−3.51 (m, 3H), 4.19 (dd, 1H, J = 10.86 Hz, 3.54 Hz), 5.05 (d, 1H, J = 4.61 Hz), 5.47 (d, 1H, J = 1.83 Hz); ^{13}C NMR (D_2O, 75 MHz) δ 176.88, 176.53, 176.11, 148.04, 103.72, 99.79, 98.72, 86.80, 80.51, 77.66, 72.62, 70.34, 69.03, 66.17, 57.06, 53.18, 49.74, 48.19, 43.82, 37.21, 32.48, 26.41, 24.58, 23.61, 21.78.

Preparation of 1-*N*-Ethyl-3,2′,6′-tri-*N*-acetylsisomicin (3). To glacial acetic acid (1.8 L, 32 mol) in chloroform (4 L) was slowly added sodium borohydride (300 g, 8 mol) at 20 °C. The addition rate was adjusted by observing the generation rate of hydrogen gas. To this solution, a solution of 3,2′,6′-tri-*N*-acetylsisomicin (573 g, 1 mol) solution in chloroform (5 L) was added, and the mixture was stirred for 20 h at 40 ± 5 °C. After being cooled to room temperature, the resulting solution was neutralized with saturated sodium hydroxide solution. This neutralized solution was diluted with ethanol (5 L), extracted with chloroform (5 L), and distilled in vacuo. The residue was chromatographed on a column of silica gel (2.5 kg) with 30:10:1 chloroform:methanol:14% ammonia water as eluant. Fractions were collected, concentrated, and lyophilized to give the title compound **3** as an ivory powder (578 g, 96%). MS (FAB) 602 ($M + H^+$); ^{1}H NMR (D_2O, 300 MHz) δ 1.27 (t, 3H, J = 6.79 Hz, $CH_2\underline{CH_3}$) 1.34 (s, 3H, C−CH_3), 1.68−1.78 (m, 2H), 1.96 (s, 3H), 2.00 (s, 3H), 2.02 (s, 3H), 2.12 (m, 1H), 2.31 (m, 1H), 2.91 (m, 1H), 2.92 (s, 3H, *N*-CH_3), 3.08 (m, 1H), 3.26 (m, 1H), 3.47−3.51 (m, 3H), 3.64−4.00 (m, 8H), 4.23−4.27 (dd, 1H, J = 3.36 Hz, 10.73 Hz), 5.05 (d, 1H, J = 3.41 Hz, H-1″), 5.48 (d, 1H, J = 1.82 Hz, H-1′); ^{13}C NMR (D_2O, 75 MHz): δ 174.53, 174.20, 173.74, 145.74, 101.87, 97.44, 96.36, 84.26, 77.98, 75.30, 70.27, 68.04, 66.89, 64.04, 57.11, 54.77, 47.40, 45.85, 41.47, 41.14, 35.04, 27.86, 22.56, 22.36, 22.22, 21.19, 11.25.; Anal. for $C_{27}H_{47}N_5O_{10}\cdot 15H_2O$. Calcd: C, 51.88; H, 8.02; N, 11.14. Found: C, 51.50; H, 8.13; N, 11.40.

Preparation of 1-*N*-Ethylsisomicin (4). 1-*N*-Ethyl-3,2′,6′-*N*-acetylsisomicin (**3**) (330 g, 0.5 mol) was dissolved in 10% sodium hydroxide solution (1 L), refluxed for 41 h under a nitrogen atmosphere. After being cooled to room temperature, the pH of the resulting mixture was adjusted to 9 with 1 N sulfuric acid in an ice bath. The resulting solution was concentrated under vacuum. 2-Propanol (4 L) was added to the resultant residue to precipitate a solid that was filtered off. The filtrate was subjected to column chromatography on silica gel using chloroform, methanol, and 7% ammonia water in the ratio of 40:20:7 as eluent. Lyophilizing of the concentrated fractions gave the title compound **4** (190 g,

80%) as an ivory powder; mp 100−103 °C. MS (FAB) 476 ($M + H^+$); ^{1}H NMR (D_2O, 500 MHz) δ 1.13 (t, 3H, J = 6.98 Hz, $CH_2\underline{CH_3}$), 1.28 (s, 3H, 6″-Me), 2.03 (m, 1H, 3′-H), 2.21−2.25 (m, 3H, 2-H, 3′-H), 2.52 (m, 1H, 2-H), 2.58 (s, 3H, 7″-*N*-CH_3), 2.64 (dd, 1H, J = 6.45 Hz, 0.83 Hz, 3″-H), 2.77−2.84 (m, 4H, 3′-H, -$\underline{CH_2}CH_3$), 3.14 (m, 1H, 2′-H), 3.38 (m, 3H, 6-H, 5″-H), 3.53 (t, 1H, J = 9.63 Hz, 4-H)), 3.63 (t, 1H, J = 9.28 Hz, 5-H), 3.70 (q, 1H, J = 7.08 Hz), 3.88 (dd, 1H, J = 4.06 Hz, 3.96 Hz, 2″-H), 4.06−4.10 (m, 1H, 5″-H), 4.95 (d, 1H, J = 3.86 Hz), 5.01 (d, 1H, J = 3.63 Hz, 1″-H), 5.42 (s, 1H, 1′-H); ^{13}C NMR (D_2O, 75 MHz) δ 11.85 (−$CH_2\underline{CH_3}$), 19.70 (6″-C), 22.76 (3′-C), 29.91 (2-C), 34.89 (7″-C), 40.40 (6′-C), 38.48 (−$\underline{CH_2}CH_3$), 44.63 (2′-C), 47.44 (3-C), 58.43 (1-C), 61.44 (3″-C), 67.44 (2″-C), 65.97 (5″-C), 70.50 (4″-C), 72.79 (5-C), 82.24 (4-C), 83.85 (6-HC), 98.01 (1′-C), 94.83 (4′-C), 99.49 (1″-C), 146.62 (5′-C).

Preparation of 1-*N*-Ethylsisomicin Sulfate. 1-*N*-Ethylsisomicin (**4**) (130 g, 270 mmol) obtained from the previous step was dissolved in water (1 L), and the pH of the solution was adjusted to 4.5 by slow addition of a 1 N aqueous sulfuric acid solution (682 mL, 680 mmol). To the resulting solution was added methanol (2.5 L) followed by filtration of a solid to give stoichiometrically the desired compound as a white powder; mp 182−183 °C dec. ^{1}H NMR (D_2O, 500 MHz) δ 1.33 (t, 3H, J = 7.35 Hz, $CH_2\underline{CH_3}$), 1.40 (s, 3H, 6′-CH_3), 2.10 (q, 2H, J = 12.5 Hz, $\underline{CH_2}CH_3$), 2.42 (m, 1H, 3′-H), 2.65 (m, 1H, 2-H), 2.74 (m, 1H, 3′-H), 2.94 (s, 3H, 3″-*N*-CH_3), 3.13 (m, 1H, 2-H), 3.34 (m, 1H, 2-H), 3.49−3.56 (m, 4H), 3.75−3.80 (m, 3H), 3.91−3.95 (m, 2H), 4.00 (d, 1H, J = 12.8 Hz, 5″-H), 4.14 (t, 1H, J = 9.7 Hz), 4.27 (dd, 1H, J = 10.77 Hz, 2.98 Hz, 2″-H), 5.15 (dd, 1H, J = 2.75 Hz, 1″-H), 5.20 (s, 1H, 4′-H), 5.62 (s, 1H, 1′-H); ^{13}C NMR (D_2O, 125 MHz) δ 12.09 ($CH_2\underline{CH_3}$), 21.97 (6″-C), 24.86 (3′-CH_3), 26.48 (2-C), 35.94 (7″-C), 41.75 (6′-C), 42.20 ($\underline{CH_2}CH_3$), 47.20 (2′-C), 49.34 (3-C), 57.41 (1-C), 64.84 (3″-C), 67.78 (2″-C), 68.79 (5″-C), 71.08 (4″-C), 74.71 (5-C), 80.01 (4-C), 84.01 (6-C), 99.40 (1′-C), 101.51 (4′-C), 102.64 (1″-C), 144.79 (5′-C). Anal. for $C_{21}H_{41}N_5O_7$ $2.5H_2SO_4$: Calcd C, 34.99; H, 6.43; N, 9.72; S, 11.12. Found C, 34.70; H, 6.41; N, 9.87; S, 11.34.

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