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A semisynthesis of isepamicin by fragmentation method

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Abstract—Garamine derivative, key intermediate, was obtained from acid cleavage of sisomicin derivative. Its subsequent product was glycosylated with 6-azido-2,3,4-tri-*O*-benzyl-6-deoxy-α-D-glucopyranosyl chloride using silver triflate as a promoter to give isepamicin.

The aminoglycoside antibiotics are a large and diverse class of carbohydrate-based substance, which has shown the extensive clinical application for ailments such as tuberculosis and septicemia.[1] Various aminoglycoside antibiotics such as kanamycin[2] in 1957, gentamicin[3] in 1964, sisomicin[4] in 1970, netilmicin[5] in 1975, isepamicin[6] in 1978 and arbekacin[7] in 1987 have been vigorously investigated and marketed since the development of streptomycin[8] by Waksman in 1944. Also, many aminoglycosides exhibit inhibitory activity against HIV virus.[9]

Although resistance[10] and the risk of serious side-effects[11] in ototoxicity and nephrotoxicity have reduced the use of aminoglycoside drug in recent years, these drawbacks have been met with improved dosing regimens and have stimulated the development of semi-synthetic derivatives such as isepamicin and arbekacin.

Isepamicin developed by Schering–Plough[6] is a novel broad-spectrum aminoglycoside, which possesses a high level of stability to aminoglycoside inactivating enzymes and low level[12] of toxicity to the kidney and inner ear. Schering–Plough synthesized isepamicin from gentamicin B,[13] which was co-produced in the gentamicin fermentation. After then, the other synthetic method of isepamicin was not tried.

The ready availability of suitably protected garamine derivative[14,15] made it possible to contemplate the synthesis not only of isepamicin, but also other aminoglycoside derivatives. With these objectives in mind, we wish to describe the synthesis of isepamicin using 6-azido-2,3,4-tri-*O*-benzyl-6-deoxy-α-D-glucopyranosyl chloride (6-ABGC) as a glycosyl donor, 1-[(2-benzyloxy-3-*N*-benzyloxycarbonyl)-L-isoserinyl]-3,3″-di-*N*-benzyloxycarbonylgaramine as a glycosyl acceptor and silver triflate as a promoter.

Scheme 1 presents a retrosynthetic analysis leading to the key intermediate used for the synthesis of garamine derivative and isepamicin. Isepamicin can be divided into two moieties of glucose and garamine derivatives. Glucose derivative was synthesized by methyl-α-D-glucopyranoside, while garamine derivative was synthesized by sisomicin in a few steps.

Cbz-protected **1** was synthesized by selective acylation of sisomicin with *N*-(benzyloxycarbonyloxy)succinimide (N-BCSI) using the complexing method[16] between zinc acetate and available neighboring amino and hydroxy group pairs of sisomicin as shown in Scheme 2.

Compound **1** was reacted with (2*S*)-benzyloxycarbonyl-3-benzyloxycarbonylamino-propionic acid (BBPA, **9**)[15] in the presence of hydroxybenzotriazole (HOBT) and dicyclohexylcarbodiimide (DCC) to afford the coupling product **2** in 80% yield. The 3″-amino group of compound **2** was protected by *N*-(benzyloxycarbonyloxy)-succinimide and then hydrolyzed with sulfuric acid to

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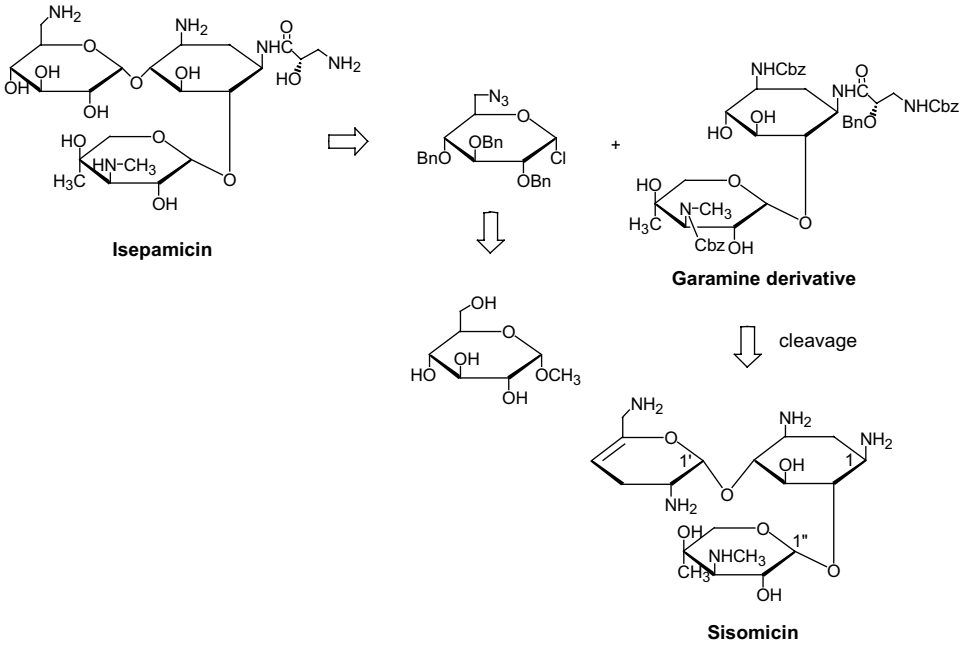
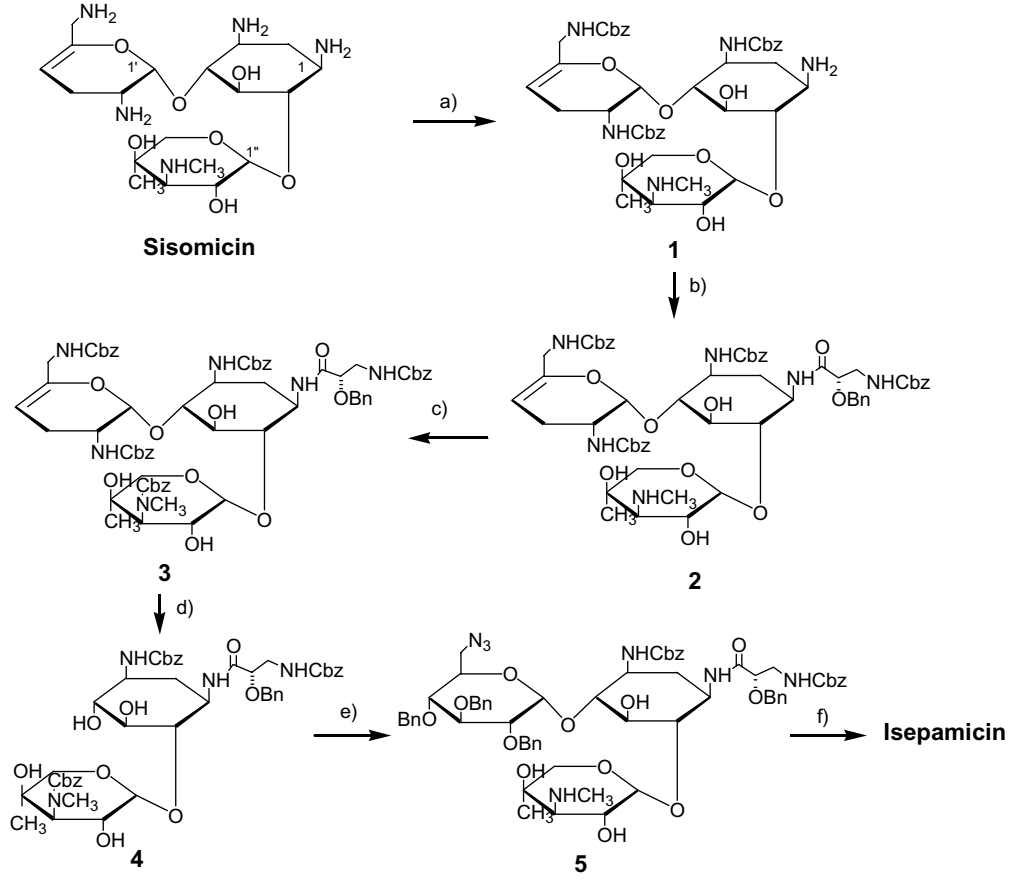


Scheme 1. Retrosynthetic analysis of isepamicin.



Scheme 2. Synthesis of isepamicin. Reagents and conditions: (a) $Zn(OAc)_2$, TEA, THF, N-BCSI, 65%; (b) BBPA, DCC, HOBT, MeOH, 80%; (c) N-BCSI, THF, NEt_3, 76%; (d) H_2SO_4, MeOH, 75%; (e) 6-ABGC, AgOTf, molecular sieve, benzene/1,4-dioxane (1:3, v/v), 52%; (f) 10% Pd/C, H_2, MeOH/THF (5:1, v/v), 57%.

afford the garamine derivative **4** in yield of 75%. Compound **3** was extremely labile towards sulfuric acid when pH reached 1 and the vinylic ether group of sisomicin derivative **3** was at first rendered susceptible to acid

hydrolysis, leading to the formation of garamine derivative **4**.[14] Subsequently glycosylation reaction of compound **4** with 6-azido-2,3,4-tri-*O*-benzyl-6-deoxy-α-D-glucopyranosyl chloride[17] gave the azide **5**. To perform the glycosidation reaction, Koenigs–Knorr[18] method and trichloroacetimidate method[19] were employed and the reactions were proceeded by the change of solvent, reaction temperature, catalyst and glycosyl donor. Regioselectivity can be generally achieved when glycosyl donor possesses selectively protected hydroxy groups and an activating group at the anomeric C atom. Therefore, it was more favorable than other protecting group to obtain α-glycosidic product that glycosyl donor in which the H atom on 2-OH was replaced by a benzyl group. To obtain α-glycosidic product diastereoselectively, the nucleophilic substitution with glycosyl acceptor **4** was conducted in such a way that it proceeded as completely as possible with retention in the sense of an S_N1 reaction. This can be done in a solvent of low polarity in the presence of an active catalyst such as AgOTf, $AgClO_4$ or $AgClO_4/Ag_2CO_3$. Among various reaction conditions, the best result was obtained by the following method. To the mixture of 6-azido-2,3,4-tri-*O*-benzyl-6-deoxy-α-D-glucopyranosyl chloride and 4 Å molecular sieves in dry benzene–dioxane (3:1, v/v), **4** and silver triflate were added at 0 °C. After stirring for 12 h at room temperature, silver triflate was again added at 0 °C. The reaction mixture was stirred for 5 h and quenched with water. The solution was evaporated and extracted with chloroform. The concentrated residue was purified by column chromatography ($CHCl_3$/MeOH, 60:1, v/v) to give stereoselectively only **5** in 52% yield. Deprotection of **5** was carried out using hydrogen gas in balloon in the presence of 10% palladium on charcoal to give isepamicin in 57% yield, which showed the specific ^{1}H NMR peak of 3.86 (J = 3.86 Hz, H-1′) and 3.05 ppm (J = 3.96 Hz, H-1″), respectively. The synthesized isepamicin was identified by comparison with commercial isepamicin using ^{1}H NMR, ^{13}C NMR and thin layer chromatography.

In conclusion, we successfully carried out the synthesis of new garamine derivative **4**[20] as intermediate for the synthesis of isepamicin. A stereocontrolled synthesis of the isepamicin has been achieved using the 6-azido-2,3,4-tri-*O*-benzyl-6-α-D-glucopyranosyl chloride as a glycosyl donor, silver triflate as a promoter and suitable protected garamine derivative **4** as a glycosyl acceptor. Also, the new garamine analogue **4** is capable to use for development of new aminoglycosides antibiotics.

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20. Compound **4**: R_f: 0.37 ($CHCl_3$/MeOH/NH_4OH = 5:1:0.1); mp: 172–172.5 °C; $[\alpha]_D^{20}$ + 26.3 (*c* 0.75, MeOH); ^{1}H NMR (600 MHz, MeOH–d_4) δ 7.35–7.23 (m, 20H), 4.62 (d, J = 12.0 Hz, 1H), 4.56 (d, J = 12.0 Hz, 1H), 4.26–4.19 (m, 2H), 4.15 (dd, J = 3.6, 9.4 Hz, 1H), 4.02 (m, 1H), 3.89 (m, 1H), 3.69 (dd, J = 4.2, 14.4 Hz, 1H), 3.60 (t, J = 9.6 Hz, 1H), 3.51–3.45 (m, 1H), 3.44–3.38 (m, 2H), 3.26 (m, 1H), 3.17 (dd, J = 12.6, 18.6 Hz, 1H), 2.91 (s, 3H), 1.95–1.88 (m, 1H), 1.44 (m, 1H), 1.00 (s, 2H), 0.95 (s, 1H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 173.32, 173.25, 159.93, 159.58, 158.97, 158.55, 138.78, 138.73, 138.35, 138.29, 138.14, 129.52, 129,45, 129.04, 129.00, 128.93, 128.87, 128.59, 100.90, 100.80, 81.70, 81.33, 79.51, 76.84, 76.73, 76.35, 76.28, 74.89, 74.56, 73.23, 70.75, 68.43, 68.31, 68.27, 67.52, 67.48, 65.92, 60.29, 60.17, 52.90, 50.77, 42.28, 42.20, 35.38, 30.99, 30.75, 22.49, 22.25; IR (KBr) 3282, 1684, 1544, 1274, 1036, 696 cm^{-1}; FAB MS *m/z* 901.38 (MH).