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New Convenient Reagents for Chemoselective *N*-Alkoxycarbonylation of (*S*)-Isoserine: Application in the Isepamicin Synthesis

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Abstract: A synthesis of a series of *N*-alkoxycarbonyl mercaptobenzothiazoles (MBTs) and their application as reagents for chemoselective protection of amino group are presented herein. It was shown that all new reagents, Z-MBT, Fmoc-MBT, Phoc-MBT, and Tec-MBT, are highly effective in the selective *N*-alkoxycarbonylation of (*S*)-isoserine. The transformation is a simple, fast, and low-cost protocol, which is applicable in scale-up experiments. The starting MBT was fully recovered at the end of the process, which is an additional advantage of the method. The efficiency of the Z-reagent was also demonstrated by the selective protection of both gentamicin B and (*S*)-isoserine before their peptide-type coupling in the synthesis of the aminoglycoside antibiotic isepamicin.

Keywords: chemoselective, gentamicin B, isepamicin, mercaptobenzothiazole, *N*-protection, (*S*)-isoserine

INTRODUCTION

Isepamicin is an aminoglycoside antibiotic with activity against bacteria producing aminoglycoside-inactivating enzymes.[1,2] Its molecule is constructed by a peptide-type bonded (*S*)-isoserine and gentamicin B building

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blocks. Thus, the chemoselective *N*-protection of both moieties are important steps in the isepamicin synthesis.

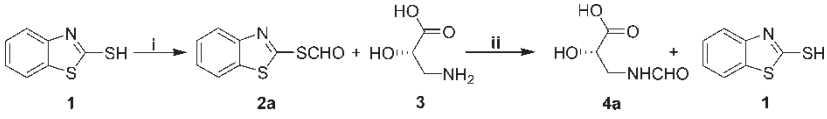
An *N*-formylating agent (**2a**) has been obtained from mixed acetic–formic anhydride and 2-mercaptobenzothiazole. We have applied this reagent in gentamicin B protection, whereas *N*-formyl isoserine have formed via a two-step protocol: total formylation of isoserine by a mixed anhydride followed by *O*-deprotection.[3,4]

Benzyloxycarbonyl *N*-protective group was introduced in 1932 by Bergman and Zervas and is widely applied in the field of peptide synthesis.[5] Since then, a variety of urethane-type protective groups have been developed.[6] Among them, fluorenylmethyloxycarbonyl (Fmoc), benzyloxycarbonyl (Z), and trichloroethoxycarbonyl (Tec) are vastly used, especially for the protection of amino acids. These protective groups are easily formed and can be readily removed under appropriate conditions. The latter present the main advantage of these compounds.[7] The classical Schotten–Baumann reaction,[5,8–10] which involves the higher reactive alkoxycarbonyl chlorides, leads to many side products. Recently, a variety of alternative reagents has been developed.[11,12] Particularly where *N*-selectivity has been desired, the mixed carbonates containing substituted succinimidyl groups were used.[13–15] Because the alkoxycarbonyl group is widely used for amine protection, the development of new reagents for its insertion is of current interest.

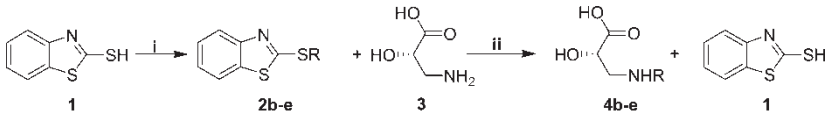
Synthesis of a series of new chemoselective and cost-effective reagents, based on mercaptobenzothiazol, for introduction of urethane-type *N*-protective groups in (*S*)-isoserine are presented herein. Benzyloxycarbonyl mercaptobenzothiazol thiocarbonate was further used for selective *N*-protection in gentamicin B as a step in isepamicin synthesis.

RESULTS AND DISCUSSION

The known formylating agent 2-formylmercaptobenzothiazole (**2a**) was prepared in a new way by condensation of formic acid and 2-mercaptobenzothiazole (**1**) with dicyclohexylcarbodiimide (DCC), and the obtained product was successfully used in the next step, a direct *N*-formylation of (*S*)-isoserine (**3**) (Scheme 1).[17]



Scheme 1. Preparation of *N*-formyl-(*S*)-isoserine: (i) formic acid (1.05 equiv), DCC (1.05 equiv), ethyl acetate; (ii) formic acid (2 equiv), **2a** (2 equiv), acetonitrile.



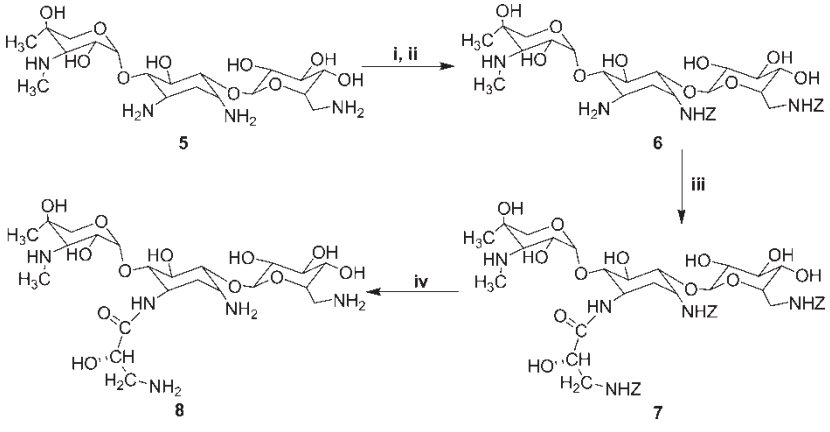
Scheme 2. Preparation of R-oxycarbonylmercaptobenzothiazoles: (i) alkylchloroformate (1.1 equiv), Na_2CO_3 (2 equiv), THF; (ii) reagent **2b–e** (1.1 equiv), Na_2CO_3 (1 equiv), H_2O/THF.

The use of ethyl acetate in the first step allowed the product dicyclohexylcarbamide to be quantitativily removed. The yields and reaction conditions are summarized in Table 1. At the end of the reaction, the staring material **1** and the unreacted reagent **2a** were almost fully recovered.

Further urethane-type protective reagents, Z-, Fmoc-, Phoc-, and Tec-mercaptobenzothiazolthiocarbonates (**2b–e**), were prepared in high yields throughout the acylation of **1** using the corresponding chloroformates in THF in the presence of dry sodium carbonate (Scheme 2).

Mercaptobenzothiazolthiocarbonates (**2b–e**) were used to obtain the desired *N*-protected (*S*)-isoserines (**4b–e**) in good yields (Scheme 2, Table 1). The starting material (**1**) was fully recovered because of its lipophilicity. The latter is very important in scale-up experiments.

In addition, the amino groups in position 3 and 6′ of gentamicin B (**5**) were selectively acylated according to the known procedure.[3] The reagent **2b** was used instead of the formylating agent, and a yield of



Scheme 3. Preparation of isepamicin: (i) zinc pivaloate e (3.5 equiv.), DMSO; (ii) **2b** (2.5 equiv.); (iii) **4b** (1.5 equiv.), HOBT · H_2O (0.5 equiv.), DCC (1.5 equiv.); (iv) Ra/Ni, HCl.

83% was achieved. The product **6** was used to prepare the isepamicin (**8**) (Scheme 3).

CONCLUSIONS

A synthesis of a series of new reagents for chemoselective *N*-alkoxycarbonylation based on the mercaptobenzothiazole scaffold is presented herein. The *N*-alkoxycarbonyl mercaptobenzothiazoles, Z-MBT, Fmoc-MBT, Phoc-MBT, and Tec-MBT, were obtained in excellent yields from the cheap and easily available mercaptobenzothiazole (MBT). It was shown that all new reagents are highly effective in the selective *N*-alkoxycarbonylation of (*S*)-isoserine. The transformation is a simple, fast, and low-cost protocol, which is applicable in scale-up experiments. The starting mercaptobenzothiazole was fully recovered at the end of the process, which presents an additional advantage of the method. The efficiency of the Z-reagent was also demonstrated by the selective protection of both gentamicin B and (*S*)-isoserine before their peptide coupling in the synthesis of the aminoglycoside antibiotic isepamicin.

EXPERIMENTAL

All reagents were purchased from Aldrich, Merck, and Fluka and were used without any further purification. Gentamicin B is a gift from Actavis AD, Sofia, and was purified by column chromatography. Thin-layer chromatography (TLC) was done on precoated 0.2-mm Merck silica-gel 60 F_{254} (0.040–0.060 mm) plates. Melting points (uncorrected) were determined on Mel-Temp capillary melting-point apparatus, model 1102D-230 VAC (Barnstead International). The IR spectra were taken on a Bruker IFS 113 v as KBr and are quoted in centimeters^{-1}. The NMR spectra were recorded on a Bruker Avance DRX 250 spectrometer; the chemical shifts were quoted in ppm in δ values against tetramethylsilane (TMS) as an internal standard, and the coupling constants were calculated in hertz. The microanalyses were carried out by the microanalyses service of the Institute of Organic Chemistry, Bulgarian Academy of Sciences. The optical rotation was recorded with a Perkin-Elmer 241 polarimeter.

(*S*)-Formylmercaptobenzothiazole (MBT-CHO, 2a)[3]

To a solution of **1** (44 g, 0.263 mol) in 1000 mL of ethyl acetate, 11.10 mL (0.289 mol) of formic acid was added and stirred for 15 min. A solution of 57.00 g (0.276 mol) DCC in 300 mL of ethyl acetate was added dropwise at room temperature, which was vigorously stirring for about 1 hour and

stirred for 5 h. The reaction mixture was filtered. The solvent was evaporated to dryness to obtain yellow crystals.

Mp 121–125°C; IR (KBr) 1720, 1450, 1300, 1270, 1130, 1000, 750; ^{1}H NMR ($CDCl_3$) 10.07 (1H, s), 8.56–8.52 (1H, m), 7.48–7.25 (3H, m); MS (m/z) 195 (M^+), 167.

N-Formyl-(*S*)-isoserine (IS-CHO, 3a)[17]

To a suspension of **3** (15 g, 0.143 mol) in 300 mL of acetonitrile, 10.80 mL (0.290 mol) formic acid was added and stirred until the solution was formed. Compound **2a** 56 g, (0.286 mol) was added at room temperature with vigorous stirring. It was stirred about 6 h. The reaction mixture was cooled in an ice bath for 1 h and filtered; mother liqueurs were concentrated about one-fourth of the volume and filtered again. The solvent was evaporated. The obtained white oil was boiled with ethyl acetate (2 × 50 mL) and acetone (2 × 50 mL) to remove the formic acid. The obtained hygroscopic white crystals were filtered and washed with cooled acetone.

$|\alpha|_D^{20} + 3.83°$ (C = 1, H_2O); mp 94–96°C; IR 3476, 3275, 1700, 1659, 1632, 1556; ^{1}H NMR (MeOD) 8.09 (1H, s), 4.24 (1H, t), 3.54 (2H, m); MS (m/z) 134 (M^+), 115, 88.

General Procedure for Preparation of *R*-Oxycarbonylmercapto benzothiazolyl-thiocarbonates (2b–e)

To a solution of **1** (5 g, 0.030 mol) in 45 mL of THF, 2 equiv. of sodium carbonate (6.50 g, 0.060 mol) were added, and the suspension was stirred at room temperature for 10 min. Solution of 1.1 equiv. of corresponding chloroformate (Table 1) in dry THF (15 mL) was added dropwise at room temperature with vigorous stirring. Sodium carbonate was filtered, and the solvent was removed by vacuum distillation. The obtained yellow oil was crystallized with 50 mL of petroleum ether. The crude product was recrystallized from methanol.

Data

(*S*)-1,3-Benzothiazol-2-yl-*O*-benzyl-thiocarbonate (Z-MBT, **2b**)

Mp. 91–93°C; R_f 0.45 (hexane–EtOAc, 9:1 v/v); IR 3417, 1742, 1454, 1423, 1368, 1313, 1255, 1213, 1142, 1076, 1022, 1011, 929, 912, 808, 753, 746, 726, 699, 667, 603, 492, 430; ^{1}H NMR ($CDCl_3$) 8.02 (ddd, J = 7.9, 1.5, 0.6 Hz, 1H), 7.88 (ddd, J = 7.9, 1.4, 0.6 Hz, 1H), 7.49 (ddd, J = 7.9, 7.2, 1.4 Hz, 1H), 7.41 (ddd, J = 7.9, 7.2, 1.5 Hz, 1H), 7.39 (m, 5H Ar-H), 5.36 (s, 2H,

-CH_2-Ph); ^{13}C NMR (62.89 Hz) 166.18, 157.76, 151.99, 136.42, 134.01, 129.02, 128.99, 128.83, 128.80, 128.77, 128,70, 126.39, 126.58, 123.02, 121.11, 70.64. Anal. calcd. for $C_{15}H_{11}NO_2S_2$:C, 59.78; H, 3.68; N, 4.65; S, 21.28. Found: C, 59.26; H, 3.58; N, 4.59, S, 21.01.

(*S*)-1,3-Benzothiazol-2-yl-*O*-(9*H*-fluoren-9-ylmethyl)-thiocarbonate (Fmoc-MBT, **2c**)

Mp. 98–100°C; R_f 0.48 (heptane–EtOAc, 5:1 v/v); IR 3050, 2944, 2870, 2280, 1700, 1545, 1418, 1311, 1224, 1144, 1070, 1016, 1003, 936, 829, 748, 732, 723, 621, 547, 541; ^{1}H NMR ($CDCl_3$) 8.04 (d, $J = 8.13$ Hz, 1H, benzothiazole), 7.88 (d, $J = 8.0$ Hz, 1H, benzothiazole), 7.73 (d, $J = 8.0$ Hz, 2H, fluoren), 7.54 (d, $J = 7.5$ Hz, 2H, fluoren), 7.43–7.29 (m, 2H, Ar-H, benzothiazole), 4.62 (d, 2H, -CH_2–), 4.29 (tr, 1H,=CH- from fluoren); ^{13}C NMR (62.89 Hz) 128.05, 127.25, 126.45, 125.71, 125.00, 123.16, 121.17, 120.12, 70.68, 46.47. Anal. calcd. for $C_{22}H_{15}NO_2S_2$:C, 67.84; H, 3.88; N, 3.60; S, 16.47. Found: C, 68.10; H, 3.72; N, 3.65; S, 16.38.

(*S*)-1,3-Benzothiazol-2-yl-*O*-phenylthiocarbonate (Phoc-MBT, **2d**)

Mp. 92–94°C (MeOH); R_f 0.40 (hexane–EtOAc, 9:1 v/v); IR 3060, 1735, 1589, 1489, 1455, 1426, 1314, 1242, 1178, 1161, 1124, 1020, 1003, 906, 856, 757, 737, 725, 681, 651, 598, 492, 429; ^{1}H NMR ($CDCl_3$) 8.21 (d, $J = 8.00$ Hz, 1H, benzothiazole), 7.90 (d, $J = 8.00$ Hz, 1H, benzothiazole), 7.30–7.21 (m, 7H, 5 Ar-H, 2H benzothiazole); ^{13}C NMR (62.89 Hz) 166.1, 157.8, 152.7, 151.5, 137.2, 130.4, 127.5, 127.2, 126.4, 123.8, 121.9, 121.8, 121.7, 121.6. Anal. calcd. for $C_{14}H_9NO_2S_2$:C, 58.528; H, 3.16; N, 4.87; S, 22.32. Found: C, 58.71; H, 3.03; N, 4.82; S, 22.30.

(*S*)-1,3-Benzothiazol-2-yl-*O*-(2,2,2-trichloropropyl)-thiocarbonate (Tec-MBT, **2e**)

Mp. 99–102°C (MeOH); R_f 0.52 (hexane–EtOAc, 9:1 v/v); IR 3051, 3017, 2957, 2213, 1720, 1450, 1420, 1357, 1310, 1260, 1064, 1020, 1003, 840, 800, 757, 710, 650, 564, 480; ^{1}H NMR ($CDCl_3$) 8.05 (d, $J = 7.50$ Hz, 1H, benzothiazole), 7.90 (d, $J = 7.50$ Hz, 1H, benzothiazole), 7.54–7.26 (m, 2H, Ar-H, benzothiazole), 4.94 (s, 2H, -CH_2-); MS (m/z) 343 (M^+); ^{13}C NMR (62.89 Hz) 166.6, 156.6, 152.8, 137.4, 127.3, 126.6, 124.0, 121.6, 94.3, 76.9. Anal. calcd. for $C_{10}H_6Cl_3NO_2S_2$:C, 35.05; H, 1.76; Cl, 31.04; N, 4.09; S, 18.72. Found: C, 35.28; H, 1.92; Cl, 31.18; N, 4.23; S, 19.00.

General Procedure for Preparation of *N*-Protected-(*S*)-isoserines (4b–e)

To a mixture of **3** (0.40 g, 0.0048 mol) in 8 mL of water and 1 equiv. of sodium bicarbonate, a solution of corresponding R-oxycarbonylmercapto-benzothiazolthiocarbonates (**2b–e**) (1.10 equiv.) in 24 mL THF was added dropwise at 50°C. After 1.5–2 h (Table 1) of vigorous stirring, the presence of **4** was not established (TLC). The reaction mixture was diluted with 30 mL of 20% aqueous sodium bicarbonate. The organic solvent was evaporated, and the precipitate of **1** was filtered off. The additional amount of starting material (**1**) was extracted with ethyl acetate (3 × 30 mL). Later, the aqueous layer was acidified (pH = 3) with 20% sulphuric acid and extracted with ethyl acetate (5 × 20 mL). The organic layer was dried (sodium sulphate) and filtered, and solvent was evaporated. The obtained *N*-protected-(*S*)-isoserine was recrystallized.

Data

(2*S*)-3-{[(Benzyloxy)carbonyl]amino}-2-hydroxypropanoic Acid (Z-IS, **4b**)

Mp. 125–127°C (acetone); Rf 0.46 ($CHCl_3$–MeOH–100% CH_3COOH, 18:3:1 v/v/v); $[\alpha]_D^{20}$+3.18°; IR 3432, 3340, 2950, 2129, 1745, 1690, 1535, 1495, 1456, 1377, 1271, 1186, 1149, 1116, 1088, 1079, 1001, 985, 950, 930, 911, 974, 780, 747, 731, 696, 636, 576, 466, 409; ^{1}H NMR (DMSO) 7.24–7.36 (m, 6H, Ar-H + -NH), 5.01 (s, 2H, Ar-CH_2-O-), 4.02 (dd, 1H, *J* 7.0, 4.8 Hz), 3.31 (m, 1H, 1/2 of -CH_2-N), 3.15 (m, 1H, 1/2 of -CH_2-N); ^{13}C NMR (62.89 Hz) 174.0, 156.2, 137.2, 128.4, 127.8, 127.7, 69.3, 65.3. Anal. calcd. for $C_{11}H_{13}NO_5$:C, 55.23; H, 5.48; N, 5.86. Found: C, 55.29; H, 5.46; N, 5.62.

Table 1. The yields and reaction conditions for the preparation of **2** and **4**

		Product **2**			Product **4**		
Reagent	R	Time, h	No	Yield, %	Time, h	No	Yield, %
HCOOH/DCC	CHO	6	**2a**	94	7	**4a**	80
Z-Cl	Z	1.5	**2b**	97	1.5	**4b**	93
Fmoc-Cl	Fmoc	2	**2c**	98	2	**4c**	91
Phoc-Cl	Phoc	1.5	**2d**	98	1.5	**4d**	94
Tec-Cl	Tec	2	**2e**	93	2	**4e**	92

[a]Before recrystalization.

(2*S*)-3-{[(9*H*-Fluoren-9-ylmethoxy)carbonyl]amino}-2-hydroxypropanoic Acid (Fmoc-IS, **4c**)

Mp. 166–168°C (MeOH); Rf 0.51 ($CHCl_3$–MeOH–100% CH_3COOH, 15:4:1 v/v/v); $[\alpha]_D^{20}$ −2.35° (C = 10, MeOH); IR 3274, 3061, 3026, 2928, 2868, 1946, 1908, 1742, 1695, 1606, 1477, 1445, 1418, 1371, 1363, 1314, 1208, 1190, 1152, 1097, 1072, 1049, 1025, 1006, 937, 867, 783, 757, 740, 726, 647, 621, 592, 562, 537, 429, 413; ^{1}H NMR (DMSO) 12.54 (brs, 1H, -COOH), 7.88 (d, 2H, *J* 7.5, from fluoren), 7.71 (d, 2H, *J* 7.5, from fluoren), 7.37 (m, 5H, from fluoren and -NH-), 4.25 (m, 3H, -CH_2-and =CH-from fluoren), 3.33 (m, 2H, 1/2 of -CH_2-N and OH), 3.17 (m, 1H, 1/2 of -CH_2-N); ^{13}C NMR (62.89 Hz) 174.0, 156.2, 143.9, 140.7, 127.6, 127.1, 125.2, 120.1, 69.3, 65.5, 46.6, 44.2. Anal. calcd. for $C_{18}H_{17}NO_5$:C, 66.05; H, 5.23; N, 4.28. Found: C, 66.15; H, 5.14; N, 4.42.

(2*S*)-2-Hydroxy-3-[(phenoxycarbonyl)amino]propanoic Acid (Phoc-IS, **4d**)

Mp. 89–91°C (EtOAc); Rf 0.41 ($CHCl_3$–MeOH–100% CH_3COOH, 18:4:1 v/v/v); $[\alpha]_D^{20}$ +5.29° (C = 10, MeOH); IR 3432, 3349, 2926, 1744, 1707, 1535, 1492, 1455, 1383, 1263, 1225, 1168, 1113, 1078, 942, 881, 749, 686, 640; ^{1}H NMR 12.10 (brs, 1H, -COOH), 7.77 (t, 1H, *J* 6.0, -NH-), 7.37 (t, 2H, *J* 7.5, m-Ar-H), 7.20 (t, 2H, *J* 7.5, p-Ar-H), 7.08 (d, 2H, *J* 7.5, o-Ar-H), 4.09 (dd, 1H, *J* 7.5, 4.8), 3.39 (m, 1H, 1/2 of -CH_2-N), 3.21 (m, 1H, 1/2 of -CH_2-N); ^{13}C NMR (62.89 Hz) 173.9, 154.5, 151.1, 129.2, 124.9, 121.7, 69.1, 44.4. Anal. calcd. for $C_{10}H_{11}NO_5$:C, 53.33; H, 4.92; N, 6.22. Found: C, 53.40; H, 4.97; N, 6.09.

(2*S*)-2-Hydroxy-3-{[(2,2,2-trichloroethoxy)carbonyl]amino}propanoic Acid (Tec-IS, **4e**)

Mp. 77–79°C (H_2O–MeOH, 5:1, v/v); Rf 0.47 (EtOAc–MeOH, 3:2 v/v); $[\alpha]_D^{20}$ 0° (C = 1, MeOH); IR 3421, 3405, 3315, 3101, 2954, 1743, 1690, 1564, 1533, 1430, 1374, 1353, 1282, 1245, 1205, 1169, 1145, 1120, 1080, 1061, 1049, 1011, 954, 870, 813, 773, 727, 660, 570; ^{1}H NMR 12.57 (brs, 1H, -COOH), 7.70 (t, 1H, *J* 6.0, -NH-), 4.78 (s, 2H), 4.04 (dd, 1H, *J* 7.0, 5.0), 3.33 (m, 1H, 1/2 of -CH_2-N), 3.17 (m, 1H, 1/2 of -CH_2-N); ^{13}C-NMR (62.89 Hz) 173.8, 154.5, 96.1, 73.4, 69.0, 44.4. Anal. calcd. for $C_6H_8Cl_3NO_5$:C, 25.69; H, 2.87; Cl, 37.92; N, 4.99. Found: C, 25.67; H, 3.03; Cl, 38.03; N, 5.15.

Preparation of Isepamicin

Preparation of 3,6′-*N*-Di-carbobenzoxygentamicin B[14,16] (**7**)

To a solution of **5** (4 g, 0.0083 mol) in 50 mL of DMSO, 3.5 equiv. zinc pivaloate (7.75 g, 0.0290 mole) were added and stirred at room temperature for 90 min. Compound **2b** 6.19 g, (3.5 equiv., 0.0205 mol) was added at room temperature and stirred for 180 min. The reaction mixture was precipitated into 400 mL of water and filtered through a small pad of Celite®. The filtrate was diluted with water to a final volume of 700 mL and charged onto a column containing 400 mL of Amberlite IRC-50 resin, which had been adjusted to a partial ammonium cycle. The product was eluted with 0.75M ammonium hydroxide. The fractions containing the product were pooled and lyophilized. Yield 5.17 g (83%).

Rf 0.35 (CH_2Cl_2–MeOH–NH_4OH (25%), 50:35:5 v/v/v); ^{1}H NMR (D_2O) 1.30 (s, 3H, C-$\underline{CH_3}$), 1.34–1.41 (dd, 1H*ax*, C_2-H), 1.92–2.22 (dd, 1H*eq*, C_2-H), 2.77 (s, 3H, N-$\underline{CH_3}$), 5.07 (s, 4H, -$\underline{CH_2}$-), 5.11 ($J = 4.2$ Hz, d, 1H, C_1'-H), 5.24 ($J = 3.6$ Hz, d, 1H, C_1'-H), 7.33–7.44 (m, 10H, Ar-$\underline{H}$).

Preparation of Isepamicin Base[3]

To a solution of **6** (5 g, 0.0093 mol) in 15 mL water, 40 mL of methanol and 0.72 g (0.063 mol) 1-hydroxybenzotriazole hydrate were added. To this solution, simultaneously solutions of 4.30 Γ (0.0208 mol) DCC in 35 mL of methanol and 3.56 Γ (0.0140 mol) **3b** in 35 mL of methanol were added slowly (for 45 min). The reaction mixture was stirred at room temperature for 60 min. The presence of **6** was not established (TLC). The reaction mixture was diluted with 30 mL of methanol and 30 mL of water, stirred for 20 min, and filtered. The filtrate was diluted with water to a final volume of 360 mL, and 60 mL of conc. HCl was added. After 5 min of stirring, 32 g of Ra/Ni in four portions were added over 120 min. The reaction was stirred for 24 h at room temperature. The catalyst was removed by filtration, and filtrate was neutralized (pH 7) with 20% NaOH. The solution containing isepamicin base was filtered and charched onto a column containing 300 mL of Amberlite CG-50 resin, which has been adjusted to a partial ammonium cycle. The product was eluted with 0.75M ammonium hydroxide. The fractions containing the product were pooled and lyophilized. Yield 3.03 g (82%).

Rf 0.31 (CH_2Cl_2–EtOH–n-BuOH–NH_4OH (25%), 5:5:4:2 v/v/v/v); ^{1}H NMR (D_2O) (1.33, s 3H, C-$\underline{CH_3}$), (1.74–1.84, dd 1H*ax*, C_2-H), (2.10–2.17, dd 1H*eq*, C_2-H), (2.90, s, 3H, N-$\underline{CH_3}$), (5.16, $J = 3.96$ Hz, d, 1H, C_1'-H), (5.62, $J = 3.83$ Hz, d, 1H, C_1'-H); $[\alpha]_D^{20} + 114^\circ$ (H_2O,C = 1).

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