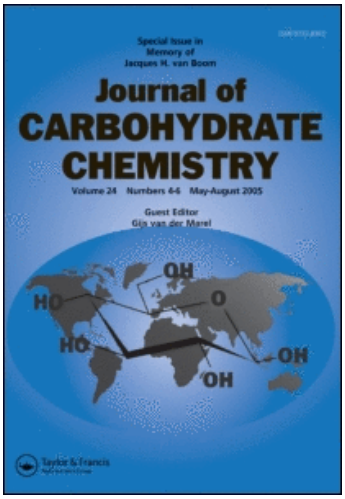

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SYNTHESIS OF AMINO ACID DERIVATIVES OF NEAMINE AND 2-DEOXYSTREPTAMINE TO BE USED AS MUTASYNTHONS

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ABSTRACT

6'-*N* derivatives of neamine with alanine, phenylalanine and lysine were synthesized either using an active esters method in one step under controlled conditions, or using a mixed anhydride method after blocking every functional group of neamine and leaving the 6'-amino group free to react. Similarly *N,N'*- diamino acid and monoamino acid derivatives of 2-deoxystreptamine were synthesized.

INTRODUCTION

Interest in aminocyclitol antibiotics, in spite of their long familiarity, remains high because of their clinical utility. Mutasynthetic techniques[2] as well as semisynthesis[3] have been used for producing aminocyclitols with a better and wider spectrum of biological activity. Among the most prominent aminocyclitols, natural or semisynthetic, are butirosin, amikacin, fortimycin, sporaricin and minosaminomycin which contain amino acids linked by an amide bond.[4] The

idea that it is easier to prepare amino acid containing mutasynthons rather than producing novel aminocyclitols by semisynthesis has stimulated us to develope procedures for preparing amino acid derivatives of neamine and 2-deoxystreptamine.

RESULTS AND DISCUSSION

Selective blocking of neamine (**1**) with the *p*-methoxy-benzyloxycarbonyl (Moz) group (Scheme 1), under controlled conditions and column purification using Amberlite CG-50 (NH_4^+) and 1,4-dioxane-water as eluent, yielded pure 6'-*N*-Moz-neamine (**2**) (TLC EtOH-H_2O-NH_3, 15:2.5:2.5). Treatment of 6'-*N*-Moz-neamine (**2**) with di-*tert*-butyl dicarbonate gave quantitatively 6'-*N*-Moz-tri-*N*-Boc-neamine (**3**), which with dimethoxypropane in the presence of molecular sieves and *p*-toluenesulphonic acid gave the 6'-*N*-Moz-1,3,2'-tri-*N*-Boc-5,6: 3',4'-di-*O*-isopropylideneneamine (**4**). The addition of molecular sieves secures the formation of the diisopropylidene derivative **4** of neamine instead of a mono and diisopropylidene mixture.[5] The addition of a small amount of triethylamine (Et_3N) prevents the transformation of the prepared diisopropylidene derivative **4** to a mixture on standing.

Hydrogenolysis of **4** results in the removal of the Moz group and formation of a neamine molecule where every functional group is blocked except the 6'-amino group which is free for a variety of chemical manipulations.

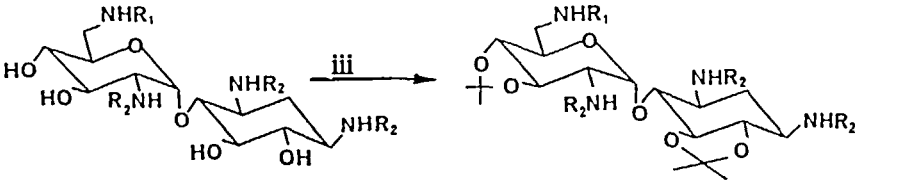
6'-*N*-alanylneamine (**8b**), prepared from **5** by coupling with Boc-L-Ala-OH using the mixed anhydride method[6] and total deblocking (Scheme 1), was found to be more reactive against *Gram*-negative bacteria of *Pseudomonas aeruginosa* 9027 than neamine itself. This prompted us to link directly amino acids to the 6'-*N* position of neamine.

Since the 6'-amino group of neamine has been proved to be the most reactive of the four amino groups,[7] we prepared 6'-*N* acylamino derivatives of neamine directly by selective reaction of neamine with the active esters (hydroxysuccinimide[8] or pentafluorophenyl[9]) of *N*-protected amino acids. Carbobenzoxy and *tert*-butyloxycarbonyl groups which were used for the protection of amino acids, were removed by hydrogenolysis over palladium/charcoal and by acidolysis (4 N HCl / THF) respectively. The 6'-*N* amino acyl derivatives **8** prepared by this simple direct derivatization were identical with those obtained by the first more tedious method.

According to the empirical rule of Nagabhushan and Daniels[10] protonation of amino groups of aminocyclitol antibiotics causes upfield shift of the α- and β-carbon signals due to shielding. However, in the 6'-*N* acyl derivatives the protonation shift for C-1' is about one-half of the ordinary value.[11] On the other hand 6'-*N* acylation causes an upfield shift by 1.1-1.4 ppm of the C-6' and about 1.7 ppm of the C-5'(β-carbon).[12]

^{13}C NMR data for the prepared compounds in Table 1, are in agreement with the above findings for the 6'-*N* acyl derivatives and prove that acylation was performed at the 6'-amino group.

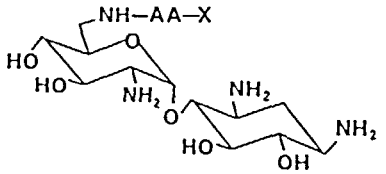
Acylation of **9** (Scheme 2) with *N*-hydroxysuccinimide (or pentafluorophenyl) esters of *N*-protected amino acids gave, after deprotection, the *N*,*N*'-diamino acid derivatives **12**. *N*-



1 R_1=R_2=H
2 R_1=Moz, R_2=H
3 R_1=Moz, R_2=Boc
i, ii

4 R_1=Moz, R_2=Boc
5 R_1=H, R_2=Boc
6 R_1=Boc-AA-, R_2=Boc
iv, v

i) Moz-S, 1,4-dioxane-water; ii) di-tert-butyl-dicarbonate; iii) 2,2-dimethoxypropane, p-toluenesulfonic acid; iv) H_2, Pd/$BaCO_3$; v) N-methylmorpholine, isobutyl chloroformate, Boc-AA-OH.



7 X=Boc or Z
8 X=H

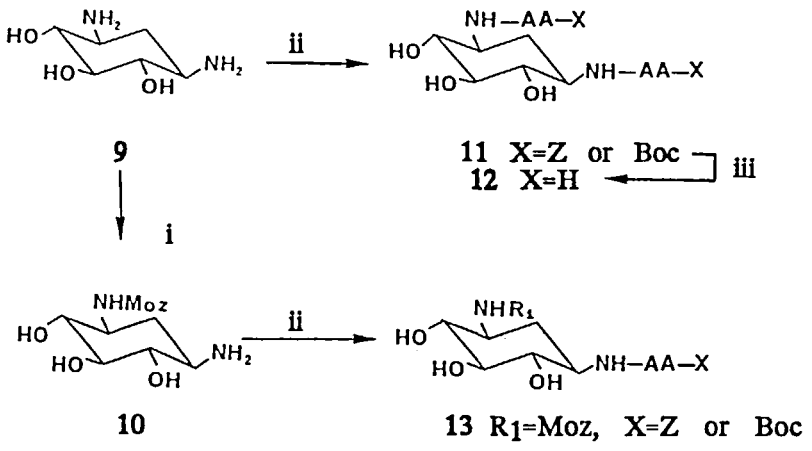
AA :a) Phe, b) Ala, c) Lys

SCHEME 1

TABLE 1.[13]C NMR Spectral Data for Neamine and Compounds 8a and 8b

	neamine[13]			8b		8a	
	base	H+	δc	H+	δc[a]	H+	δc[a]
C-2	36.7	29.2	7.5	30.7	6.0	30.7	6.0
C-4	88.2	77.9	10.3	81.0	7.2	80.9	7.3
C-1'	101.8	96.3	5.5	98.9	2.9	98.8	3.0
C-2'	56.3	54.7	1.6	56.1	0.2	56.0	0.3
C-5'	74.0	69.2	4.8	72.6	1.4	72.1	1.9
C-6'	42.8	41.5	1.3	41.7	1.1	41.4	1.4

a. δc=chemical shift of neamine (base)-chemical shift of **8a** (or **8b**).



i) Moz-S, 1,4-dioxane-water; ii) X-AA-OSu or X-AA-OPfp; iii) H_2, Pd/C or 4N HCl/THF.
AA: a) Phe, b) Ala, c) Lys, d) Gly

SCHEME 2

TABLE 2. ^{13}C NMR Spectral Data for Compounds 10 and 12a

	C-1	C-2	C-3	C-4	C-5	C-6
10	53.8	35.2	53.0	77.1	77.4	78.1
12a	51.8	34.1	51.8	76.2	78.2	76.2

Neamine and 2-deoxystreptamine derivatives with amino acids described here may be used as mutasynthons for the production of novel antibiotics.

monoprotected 2-deoxystreptamine **10** was obtained by treatment of an excess of **9** with *p*-methoxy-benzyl-*S*-(4,6-dimethyl-pyrimidin-2-yl)thiocarbonate (Moz-S) in solutions of 1,4-dioxane-water (3:1). The small amount of *N,N'*-di-Moz-2-deoxystreptamine which was simultaneously prepared and the excess of **9** were removed by column chromatography using Amberlite CG-50 (NH_4^+). Compound **10** was acylated by the active esters method to yield derivatives **13**.

Mono acyl derivatives of 2-deoxystreptamine give characteristically different ^{13}C NMR spectra, as expected,[14,15] than those of unsubstituted and disubstituted ones (Table 2).

EXPERIMENTAL

General Procedures. Melting points were determined on a Buchi micro melting point apparatus and are uncorrected. Specific rotations were determined with a Perkin-Elmer 141 polarimeter using a 10 cm cell. NMR spectra were recorded with a HFX 90 Bruker-Physic AG in D_2O containing sodium 4,4-dimethyl-4-silapentane-1-sulfonate, as the internal reference. IR spectra were recorded with a Perkin-Elmer 283B spectrophotometer. Tetrahydrofuran was passed through a column of aluminum oxide and distilled over CaH_2. Isobutyl chloroformate was distilled and stored over $CaCO_3$. *N*-Methylmorpholine was distilled from ninhydrin. All other solvents and chemicals were of reagent grade and used without further purification. All *N*-protected amino acids were of L-configuration and were purchased from Fluka. Pentafluorophenyl esters[9] and *N*-hydroxysuccinimide esters[8] of *N*-protected amino acids were synthesized as described in literature.

Neamine (1).[16] Neamine hydrochloride of ultimate purity (TLC one spot, EtOH-MeOH-H_2O-concd NH_3, 5:5:2.5: 2.5) was prepared by concentration of the methanolysis product (0.5 N HCl in MeOH) of neomycin sulfate, to a small volume and subsequent filtration. Neamine was collected as a white solid after passage over Amberlite IRA 410 (OH^-), following by lyophilization: mp 143 °C; $[\alpha]_D^{25}$ +69° (*c* 0.8, H_2O).

6'-N-p-Methoxybenzyloxycarbonylneamine (2). To a solution of neamine hydrochloride (4.48 g, 0.01 mol) in 4:2:1 1,4-dioxane- water-Et_3N (70 mL) was added *p*-methoxybenzyl-*S*-(4,6-dimethylpyrimidin-2-yl)thiocarbonate (Moz-S) (3.12g, 0.01 mol) in 2:1 1,4-dioxane-water (60 mL), dropwise at 3 °C. After 3 h stirring at room temperature, the solvents were removed under reduced pressure and the mixture was passed over Amberlite CG 50 (NH_4^+, 100 mL). The column was first eluted with a small amount of EtOH and then stepwise with 2:1 1,4-dioxane-H_2O and 66:33:2.5 1,4-dioxane-H_2O-concd NH_3. Fractions containing the title product were combined and concentrated to a small volume before they were freeze-dried (3g, 60%). Small amount was crystallized from EtOH-H_2O: mp 192-193 °C dec.; IR (KBr) 3500 (OH), 3400 (NH), 1700 cm^{-1} (NHCO); MS FAB (positive), m/z 487 $(M+H)^+$.

Anal. Calcd for $C_{21}H_{34}N_4O_9\ H_2O$: C, 49.99; H, 7.19; N, 11.10. Found; C, 49.96; H, 7.00; N, 11.01.

6'-N-p-Methoxybenzyloxycarbonyl-1,3,2'-tri-N-tert- butyloxycarbonylneamine (3). Di-*tert*-butyl dicarbonate (4.5g, 21 mmol) in 1,4-dioxane (100 mL) was added dropwise to a solution of **2** (3.4g, 7 mmol) in 3:1 1,4-dioxane-H_2O (400 mL). The reaction mixture after stirring for 12 h at 50 °C was concentrated to a small volume and **3** was obtained by filtration (5.0 g, 91%). Small amount was crystallized by EtOH: mp 228 °C; $[\alpha]_D^{25}$ + 46° (*c* 1.1, DMF); IR (KBr) 3500 (OH), 3400 (NH), 1700 cm^{-1} (NHCO); MS FAB (positive), m/z 787 $(M+H)^+$.

Anal. Calcd for $C_{36}H_{58}N_4O_{15}$: C, 54.95; H, 7.37; N, 7.12. Found: C, 54.71; H, 7.75; N, 6.9.

6'-N-p-Methoxybenzyloxycarbonyl-1,3,2'-tri-N-tert- butyloxycarbonyl-5,6:3',4'-di-O-isopropylideneneamine (4). A solution of **3** (0.786 g, 1 mmol) in dry *N,N*-dimethylformamide (DMF, 15 mL) was treated with 2,2-dimethoxypropane (20 mL) in the presence of anhydrous *p*-

toluenesulfonic acid (0.090 g) and activated molecular sieves 4-A^0. The mixture was allowed to stand at room temperature overnight. Then it was evaporated to a small volume, after adjusting pH at 8 with Et_3N. Column cromatography on silica gel (100 g) using 6:4 ether-AcOEt containing Et_3N (1%) as eluant, afforded the title compound (0.825 g, 95%): mp 185-187 °C; $[\alpha]_D^{25}$ + 39° (*c* 1, DMF).

1,3,2'-tri-N-Butyloxycarbonyl-5,6:3',4-di-O-isopropylideneneamine (5). A solution of **4** (0.295 g, 0.3 mmol) in 5:1 EtOH-H_2O (90 mL), after adjusting pH at 8, was hydrogenolyzed for 10 h in the presence of Pd/$BaCO_3$ 5% (0.1 g). After filtration of the catalyst, the solution was concentrated to a small volume and the compound **5** was obtained after lyophilization (0.225 g, 94%): IR (KBr) 3450 (NH_2), 1700 cm^{-1} (NHCO); MS FAB (positive) m/z 703 $(M+H)^+$.

6'-(N-tert-Butyloxycarbonyl)-L-phenylalanyl-1,3,2'-tri-N-tert-butyloxycarbonyl-5,6:3',4'-di-O-isopropylideneneamine (6a). To a cold solution of Boc-L-Phe-OH (0.032 g, 0.12 mmol) in anhydrous tetrahydrofuran (THF, 2.5 mL) at -10 °C, *N*-methylomorpholine (0.013 mL, 0.12 mmol) was added followed by isobutyl chloroformate (0.016 mL, 0.12 mmol). After 5 min a precooled solution of **5** (0.084 g, 0.12 mmol) in THF (5 mL) was slowly added. The reaction mixture was stirred for 30 min at -10 °C and 7 h in room temperature. The solvent was removed under reduced pressure and the resulting residue dissolved in ethyl acetate (AcOEt). The organic phase was washed consecutively with H_2O, 10% aq citric acid, H_2O, 5% aq $KHCO_3$, H_2O, dried ($MgSO_4$) and concentrated to a small volume. Upon addition of hexane the product was precipited and filtered to give **6** as a white solid (0.069 g, 61%): mp 135 °C; $[\alpha]_D^{25}$ +37^0 (*c* 1, DMF); IR (KBr) 3340 (NH), 1700 cm^{-1} (NHCO); MS FAB (positive) 951 $(M+H)^+$, 952 $(M+2H)^+$.

6'-(N-tert-Butyloxycarbonyl)-L-alanyl-1,3,2'-tri-N-tert-butyloxycarbonyl-5,6:3',4'-di-O-isopropylideneneamine (6b). **6b** was obtained following the described procedure for **6a** (0.066, 64%): mp 147-149 °C; $[\alpha]_D^{25}$ +47^0 (*c* 1, DMF); IR (KBr) 3360 (NH), 1705 cm^{-1} (Boc), 1660 cm^{-1}-(NHCO); MS FAB (positive), m/z 874 $(M+H)^+$.

General Procedure for the Preparation of 6'-N-Acylamino Derivatives 7. A cold solution of X-AA-OSu (or OPfp) (1.5 mmol) in 1,4-dioxane (10 mL) was added in portions to a cold solution of neamine (0.483 g, 1.5 mmol) in 1:1 H_2O-1,4-dioxane (20 mL). After stirring for 3 h the mixture was concentrated to a small volume and passed over Amberlite CG 50 (NH_4^+, 50 mL). The column was eluted with EtOH, 1,4-dioxane and 2:1 H_2O-1,4-dioxane. Pure fractions were obtained using 66:33:2 H_2O-1,4-dioxane-concd NH_3 as eluent followed by lyophilization.

6'-N-tert-Butyloxycarbonyl-L-phenylalanylneamine (7a): yield 0.513 g (61%); mp 135 °C; $[\alpha]_D^{25}$ +52^0 (*c* 0.4, 1:1 CH_3OH-AcOH); IR (KBr) 3600-3100 (NH,OH), 1700 (Boc), 1660 cm^{-1} (NHCO).

6'-N-tert-Butyloxycarbonyl-L-alanylneamine (7b): yield 0.473 g (64%); mp 148 °C; $[\alpha]_D^{25}$ +38^0 (*c* 1, MeOH); IR (KBr) 3600-3200 (NH, OH), 1700 (Boc), 1670 cm^{-1} (NHCO).

Anal. Calcd for $C_{20}H_{39}O_9N_5$: C, 48.67; H, 7.96; N,14.19. Found: C, 48.56; H, 7.73; N, 14.10.

6'-N-(Nα,Nε-di-p-Methoxybenzyloxycarbonyl-L-lysyl)neamine (7c): yield 0.712 g (62%); mp 130 °C; $[\alpha]_D^{25}$ +41^0 (*c* 1, DMF); IR (KBr) 3600-3100 (NH,OH), 1700 (Moz), 1660 cm^{-1} (NHCO).

General Deblocking Procedure for 6'-N Acylamino Derivatives of Neamine. 6 (7a, 7b) (1 mmol) was dissolved in 4N HCl/THF (14 mL). After stirring for 1 h at room temperature the solution was concentrated to dryness and the residue was solidified by treatment with MeOH-ether. The compound was obtained as an amorphous white solid (60-64%).

6'-N-L-Phenylalanylneamine Hydrochloride (8a). From **7a** or **6a**: mp 140 °C dec., $[\alpha]_D^{25}$ +56° (*c* 0.5, H_2O); IR (KBr) 3600-2800 (OH,NH_3^+), 1670 cm^{-1} (NHCO); ^1H NMR δ 1.85 (ddd, 1H, $J_{2a,2e} = J_{2a,1a} = J_{2a,3a}$ = 12 Hz, H-2a), 2.30 (ddd, 1H, $J_{2e,3a} = J_{2e,1a}$ = 4 Hz, H-2e), 2.95 (d, 2H, $CHCH_2C_6H_5$), 4.20 (t, 1H, J = 8 Hz, $CHCH_2C_6H_5$), 5.58 (d, 1H, $J_{1',2'}$= 4 Hz, H-1'); ^{13}C NMR δ 167.5 (C=O), 41.4 (C-6'), 72.1 (C-5'), 98.8 (C-1'), 56.9 ($CHCH_2C_6H_5$), 39.4 ($CHCH_2C_6H_5$).

6'-N-L-Alanylneamine Hydrochloride (8b). From **7b** or **6b**: mp 125 °C dec, $[\alpha]_D^{25}$ +51.3° (*c* 0.6, H_2O); IR (KBr) 3500-2800 (OH, NH_3^+), 1680 cm^{-1} (NHCO); ^1H NMR δ 1.30 (d, 3H, CH_3), 1.60 (ddd, 1H, $J_{2a,2e} = J_{2a,1a} = J_{2a,3a}$ = 12 Hz, H-2a), 2.30 (ddd, 1H, $J_{2e,1a} = J_{2e,3a}$ = 4 Hz, H-2e), 3.80 (q, 1H, J = 8 Hz, $CHCH_3$), 5.50 (d, 1H, $J_{1',2'}$ = 4 Hz, H-1'); ^{13}C NMR δ 173.5 (CO), 41.7 (C-6'), 72.6 (C-5'), 98.9 (C-1'), 51.1 ($CHCH_3$), 19.3 (CH_3).

6'-N-L-Lysylneamine Hydrochloride (8c). Compound **7c** was hydrogenated in the presence of 10% Pd/C and then the general deblocking procedure was followed: mp 132 °C dec.; $[\alpha]_D^{25}$ +16° (*c* 0.5, H_2O); IR (KBr) 3600- 2800 (OH, NH_3^+), 1670 cm^{-1} (NHCO); ^1H NMR δ 1.85 (ddd, 1H, $J_{2a,2e} = J_{2a,1a} = J_{2a,3a}$ = 12 Hz, H-2a), 2.30 (ddd, 1H, $J_{2e,3a} = J_{2e,1a}$ = 4 Hz, H-2e), 4.1 ($CHNH_2$), 5.60 (d, 1H, $J_{1',2'}$= 4 Hz, H-1').

2-Deoxystreptamine (9).[17] Neomycin sulfate (30 g, 0.064 mol) in HBr (200 mL, 48%) was stirring under reflux for 20 h. The solution was concentrated to dryness and the residue was dissolved in H_2O (100 mL). After decolorization through activated charcoal, the solvent was removed and 2-deoxystreptamine hydrobromide was obtained as a solid after addition of MeOH. Free 2-deoxystreptamine was obtained as an amorphous solid after passage over Amberlite IRA 410 (OH^-) resin followed by lyophilization: mp 210 °C dec; $[\alpha]_D^{25}$ +41.8° (*c* 1, H_2O).

(±) N-p-Methoxybenzyloxycarbonyl-2-deoxystreptamine (10). To a vigorously stirred solution of **9** (0.935 g, 5.5 mol) in 1:1 dist. H_2O-1,4-dioxane (100 mL) was added 4-methoxybenzyl-*S*-(4,6-dimethylpyrimidin-2-yl) thiocarbonate (1.520 g, 5 mol) in dioxane (100 mL) in portions during 1 h at 0 °C. After stirring for $2^1/_2$ h at 0 °C the solution was concentrated to a small volume and passed over Amberlite CG-50 (NH_4^+, 100 mL) resin. The *N,N'*-di-*p*-methoxybenzyloxycarbonyl-2-deoxystreptamine was obtained after using 2:1 1,4-dioxane-H_2O as eluent and the title compound using 66:33:1.5 1,4-dioxane-H_2O-concd NH_3 followed by lyophilization (yield 60%). The unreacted 2-deoxystreptamine was obtained using as eluant 66:33: 2.5 1,4-dioxane-H_2O-concd NH_3.

Compound **10**: mp 201 °C; MS FAB (positive) m/z 349,$(M+23)^+$, 327 $(M+H)^+$; ^1H NMR δ 1.45 (ddd, 1H, $J_{2e,2a} = J_{2a,1a} = J_{2a,3a}$ = 12 Hz, H-2a), 2.05 (1H, ddd, $J_{2e,1a} = J_{2e,3a}$= 4 Hz, H-2e), 4.94 (s, 3H, $CH_3OC_6H_4$), 4.98 (s, 2H, $CH_3OC_6H_4CH_2$), 7.25, 6.85 (4H, $CH_3OC_6H_4$); ^{13}C NMR δ 160.8 (NHCO), 78.1 (C-6), 77.4 (C-5), 77.1 (C-4), 69.5 ($CH_2C_6H_4OCH_3$), 53.8 (C-1), 53.0 (C-3), 58.0 (OCH_3), 35.2 (C-2).

N,N'-di-p-Methoxybenzyloxycarbonyl-2-deoxystreptamine: mp 221-222 °C; IR (KBr) 3460 (OH), 3320 (NH), 1695cm^{-1} (Moz); ^{1}H NMR δ 2.6 (m, 2H, H-1, H-3), 3.1-3.6 (m, 3H, H-4, H-5, H-6), 3.8 (s, 3H, OCH_3), 4.9 (2H, $CH_2C_6H_4$), 7.1, 6.8 (4H, $C_6H_4OCH_3$).

Anal. Calcd for $C_{24}H_{30}O_9N_2$; C, 58.77; H, 6.16; N, 5.71. Found: C, 58.42; H, 6.17; N, 5.60.

General Procedure for the Preparation of N- Moz-N'-aminoacyl Derivatives 13 of 2-Deoxystreptamine. A cold solution of Boc-AA-OPfp (or Boc-AA-OSu) (0.68 mmol) in 1,4-dioxane (10 mL) was added in portions to a precooled solution of **10** (221 mg, 0.68 mmol) in 30:10 1,4-dioxane-H_2O. The solution was stirring for 2 h at 0 °C and 24 h at room temperature. The solvents were removed under reduced pressure and the residue was washed with aq. citric acid (5%), H_2O, aq. $NaHCO_3$ (5%), H_2O, 1:10 EtOH-ether.

N-p-Methoxybenzyloxycarbonyl-N'-tert-butyloxycarbonyl-L-phenylalanyl-2-deoxystreptamine (13a): yield 0.232 g (62%); mp 211 °C; IR (KBr) 3460 (OH), 3320 (NH), 1690 (Moz, Boc), 1650 cm^{-1} (NHCO); MS FAB (positive), m/z 596 $(M+23)^+$,MS FAB (negative) m/z 572 $(M-H)^-$.

Anal. Calcd for $C_{29}H_{39}O_9N_3$: C, 55.49; H. 7.22; N, 6.69. Found: C, 55.26; H,6.87; N, 6.40.

N-p-Methoxybenzyloxycarbonyl-N'-tert-butyloxycarbonyl-L-alanyl-2-deoxystreptamine (13b). After the removal of solvents the residue was dissolved in AcOEt and the organic layer was washed with aq. citric acid (5%), H_2O, aq $NaHCO_3$ and the residue was crystallized by addition of EtOH-ether to give 0.222 g (66%): mp 156-158 °C; IR (KBr) 3440 (OH), 3320 (NH), 1680 (Moz, Boc), 1650 cm^{-1} (NHCO); MS FAB (positive) m/z 520 $(M+23)^+$, MS FAB (negative) m/z 496 $(M-H)^-$.

N-p-Methoxybenzyloxycarbonyl-N'-(Nα-tert-butyloxycarbonyl,Nε-benzyloxycarbonyl-L-lysyl)-2-deoxystreptamine (13c): yield 0.274 (59%); mp 136-139 °C; IR (KBr) 3500 (OH), 3315 (NH), 1690 (Moz, Z, Boc), 1650 cm^{-1} (NHCO); MS FAB (positive), m/z 689 $(M+H)^+$; ^{1}H NMR (DMSO-d_6) δ 1,3 (9H, Boc), 1.7-1.1 (2H, H-2a, H-2e), 2.5-3.6 (13H), 3.8 (s, 3H, OCH_3), 3.9 (1H, CHNH), 4.9 (s, 2H, $CH_2C_6H_4$), 5.0 (s, 2H, $CH_2C_6H_5$), 7.2, 6.9 (4H, C_6H_4), 7.3 (s, 5H, C_6H_5).

General procedure for the preparation of N,N'-di-aminoacyl derivatives 11 of 2-deoxystreptamine. A cold solution of Boc-L-AA-OSu (or Z-L-AA-OSu) (3 mmol) in 1,4-dioxane (10 mL) was added in portions to a cold solution of 2-deoxystreptamine (1.5mmol) in 1:1 H_2O-1,4-dioxane (20 mL). After stirring for 3 h the solvents were removed and the residue was washed with H_2O, MeOH, AcOEt and ether.

N-N'-di-(tert-Butyloxycarbonyl-L-phenylalanyl)-2-deoxystreptamine (11a): yield 0.274 (91%); mp 228-229 °C; $[\alpha]_D^{25}$ +3° (*c* 1, DMF); IR (KBr) 3500 (OH), 3320 (NH), 1690 (Boc), 1655 cm^{-1} (NHCO); MS FAB (positive), m/z 657 $(M+H)^+$.

Anal. Calcd for $C_{34}H_{48}O_9N_4$; C, 62.18; H, 7.37; N, 8.53. Found: C, 61.89; H, 7.52; N, 8.31.

N,N'-di-(tert-Butyloxycarbonyl-L-alanyl)-2-deoxystreptamine (11b). The residue was washed with AcOEt and the compound was obtained after passage over Amberlite CG-50 (NH_4^+) using H_2O as eluent, followed by lyophilization (0.704, 93%): mp 169-171 °C; $[\alpha]_D^{25}$ -6° (*c* 1, DMF); IR (KBr) 3500-3300 (OH,NH), 1695 (Boc), 1655 cm^{-1} (NHCO); MS FAB (positive), m/z 505 $(M+H)^+$.

N,N'-di-(Nα,Nε-di-p-Methoxybenzyloxycarbonyl)-L-lysyl-2-deoxystreptamine (11c): yield 0.887, 55%; mp 179-183 °C; $[\alpha]_D^{25}$ -2.5° (*c* 1, DMF); IR (KBr) 3500 (OH), 3320 (NH), 1680 (Moz), 1650 cm^{-1} (NHCO); MS FAB (positive) m/z 1075 $(M+H)^+$.

N,N'-di-(Benzyloxycarbonylglycyl)-2-deoxystreptamine (11d): yield 0.544 g, 68%; mp 230-232 °C; IR (KBr) 3500 (OH), 3320 (NH), 1700 (Z), 1650 cm^{-1} (NHCO); MS FAB (positive), m/z 545 $(M+H)^+$; ^1H NMR (DMSO-d_6) δ 1.7-1.1 (2H, H-2a, H-2e), 5.1 (s, 4H, CH_2CH_6), 7.3 (s, 10H, C_6H_5); MS FAB (positive) m/z 545 $(M+H)^+$.

Deblocking Procedure: The same as that described for the 6'-*N* derivatives of neamine. The compounds were obtained as white solids by treatment with ether (65-75%).

N,N'-di-L-Phenylalanyl-2-deoxystreptamine Dihydrochloride (12a): mp >220 °C; $[\alpha]_D^{25}$ +64° (*c* 1, H_2O); IR (KBr) 3300 (OH, NH), 1655 cm^{-1} (NHCO); ^1H NMR δ 1.0 (ddd, 1H, $J_{2a,2e} = J_{2a,3a} = J_{2a,1a} = 12$ Hz, H-2a), 1.7 (ddd, 1H, $J_{2e,1a} = J_{2e,3a} = 4$ Hz, H-2e), 3.0 (m, 6H, H-1, H-3, $CH_2C_6H_5$), 3.6 (m, 3H, H-4, H-5, H-6), 4.1 (m, 2H, $CHCH_2C_6H_5$), 7.1 (s, 10H, C_6H_5); ^{13}C NMR δ 170.7 (NHCO), 78.2 (C-5), 76.2 (C-4, C-6). 57.0 ($CHCH_2C_6H_5$), 51.8 (C-1, C-3), 39.2 ($CH_2C_6H_5$), 34.1 (C-2).

Anal. Calcd for $C_{24}H_{32}N_4O_5$ 2 HCl 3.5 H_2O; C, 48.65; H, 6.97; N, 9.45; Cl, 11.97. Found: C, 48.87; H, 6.61; N, 9.13; Cl, 11.62.

N,N'-di-L-Alanyl-2-deoxystreptamine Dihydrochloride (12b): mp >220 °C; $[\alpha]_D^{25}$ +5° (*c* 1, H_2O); IR (KBr) 3500-2900 (OH, NH, NH_3^+), 1670 cm^{-1} (NHCO); ^1H NMR δ 1.4 (d, 6H, CH_3), 1.5 (ddd, 1H, $J_{2a,2e} = J_{2a,3a} = J_{2a,1a} = 12$ Hz, H-2a), 2.0 (ddd, 1H, $J_{2e,1a} = J_{2e,3a} = 4$ Hz, H-2e), 3.3 (m, 2H, H-1, H-3), 3.8 (m, 3H, H-4, H-5, H-6), 4.0 (q, 2H, $CHCH_3$); ^{13}C NMR 173.3 (NHCO), 78.6 (C-5), 76.7 (C-4, C-6), 52.4 (C-1, C-3), 51.9 ($CHCH_3$), 34.9 (C-2), 19.2 ($CHCH_3$).

N,N'-di-L-Lysyl-2-deoxystreptamine Dihydrochloride (12c): mp >220 °C dec.; $[\alpha]_D^{25}$ +36° (*c* 0.5, H_2O); IR (KBr) 3600-2900 (OH, NH, NH_3^+), 1660 cm^{-1} (NHCO).

N,N'-di-Glycyl-2-deoxystreptamine Dihydrochloride (12d): mp >220 °C dec.; IR (KBr) 3600-2900 (OH, NH, NH_3^+), 1660 cm^{-1} (NHCO); ^1H NMR δ 1.7 (ddd, 1H, H-2a), 2.0 (ddd, 1H, H-2e), 3.1 (m, 2H, H-1, H-3), 3.7 (m, 7H, H-4, H-5, H-6, $OCOCH_2N$).

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18. Boc, *tert*-butyloxycarbonyl; Moz, *p*-methoxy-benzyloxycarbonyl; Moz-S, *p*-methoxybenzyl-*S*-(4,6-dimethylpyrimidin-2-yl) thiocarbonate; OPfp, pentafluorophenyl ester; OSu, *N*-hydroxysuccinimide ester; Z, benzyloxycarbonyl.