N-(*tert*-Butoxycarbonyloxy)-5-norbornene-*endo*-2,3-dicarboximide, a Reagent for the Regioselective Introduction of the *tert*-Butoxycarbonyl (BOC) Protective Group at Unhindered Amines: Application to Aminoglycoside Chemistry

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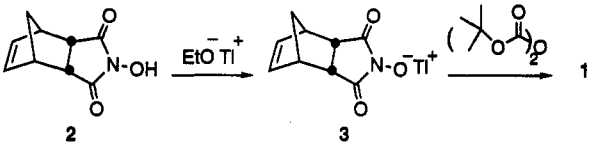
The *tert*-butoxycarbonyl (BOC) group has received much use as a versatile protective group for amines over the past several years. Its popularity stems from the fact that commercially available reagents for its introduction are widely available. Whereas typically carbamates are prepared by the reactions of their corresponding chloroformates with amines, the *tert*-butyl chloroformate is unstable at temperatures above −20 °C,[1] and as a consequence, has not been used often. However, the *tert*-butyl carbamate (BOC) group is formed readily by the reaction of an amine with di-*tert*-butyl-di-carbonate,[2] 2-[(*tert*-butoxycarbonyloxy)imino]-2-phenylacetonitrile ("BOC-ON"),[3] *tert*-butyl 2,3-dihydro-2-oxo-3-oxazolecarboxylate,[4] 1,2,2,2-tetrachloroethyl *tert*-butyl carbonate,[5] or *tert*-butyl azidoformate.[6] A second—and certainly not inconsequential—reason for the common use of the BOC group is the availability of methods for its selective removal. The BOC group is acid-labile, and as such, a large variety of acids (e.g., HCl, trifluoroacetic acid, sulfonic acids, etc.) have been used for its deprotection. Alternatively, reagents such as iodotrimethylsilane[7] or $AlCl_3$[8] have been used successfully for its removal.

In conjunction with our work with aminoglycoside antibiotics, we required a method for regioselective protection of amines in these molecules. This objective presented a challenge since these antibiotics often possess four to six amine groups. We report herein the use of *N*-(*tert*-butoxycarbonyloxy)-5-norbornene-*endo*-2,3-dicarboximide (1) for incorporation of the BOC group that greatly favors reaction at unhindered amines. The extent of selectivity shown by 1 is unprecedented, which makes this reagent ideally suited for application to aminoglycoside chemistry.

Compound 1 has been used previously in protection of amino acids.[9] Excess of the toxic phosgene gas was used in its synthesis. We report here a new synthesis for 1, in which the need for phosgene has been obviated. Compound 1 was readily synthesized in two steps, via an intermediary thallium salt[10] of *N*-hydroxy-5-norbornene-*endo*-2,3-dicarboximide, in an overall yield of 65% (2 → 3 → 1). The use of thallous ethoxide in this type of reaction finds precedent in peptide chemistry.[11] Whereas thallous ethoxide worked well in this reaction, in principle, it could be substituted with other strong bases. Compound 1 is a crystalline white solid, which can be stored at room temperature for months. The energy-minimized structure for 1 is depicted in Figure 1. The *tert*-butyl group folds toward the norbornene moiety; the nucleophile approach to the carbonate carbonyl is hindered from both the top and the bottom of the molecule, but more so from the top, as depicted in Figure 1. As shown in Figure 1, steric encumbrance provides the means for the observed regioselectivity shown by 1 (*vide infra*); both the *tert*-butyl and the norbornene[12] moieties play a key role in this function. To explore the full range of conformations that compound 1 may assume, we carried out 100 ps of dynamics simulation on compound 1. The molecule shows considerable structural flexibility. The *tert*-butyl group shows a tendency to be drawn to the norbornene function during the time course of simulation. At any given time, the approach of a nucleophile to the "BOC" carbonyl in 1 is possible—albeit sterically encumbered—only from one side of the molecule, with the other side being essentially blocked by the *tert*-butyl and the norbornene functions. These observations are supported by the IR spectrum of 1 which shows that the two carbonyl functions of the imide moiety are differentiated from one another; three carbonyl stretches are seen at 1801, 1773, and 1732 cm^{-1}. Figure 1 shows the energy minimum and three randomly selected conformations of 1 from the dynamics simulations.



The selectivity of 1 for unhindered amines is nicely shown with aminoglycosides. Neamine (4) has three primary amines at secondary carbons, and one primary amine at a primary carbon. Compound 1 afforded protection of the primary amine at the primary carbon in 65% isolated yield (4 → 5); 22% unreacted neamine was recovered from this reaction. The same reaction with kanamycin A gave a 70% isolated yield of the 6′-monocarbamoylated product (6 → 7). Isepamicin (8) undergoes reaction with 2 equiv of 1 to give the diprotected molecule 9 in 75% yield. The same reaction with 1 equiv

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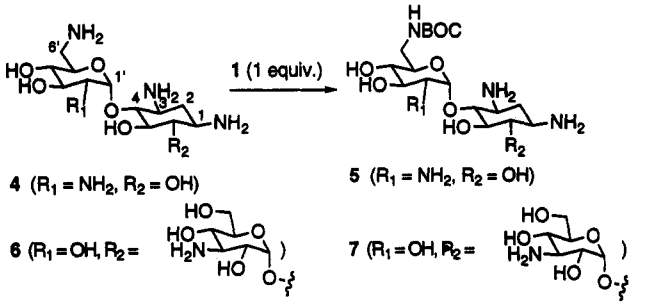
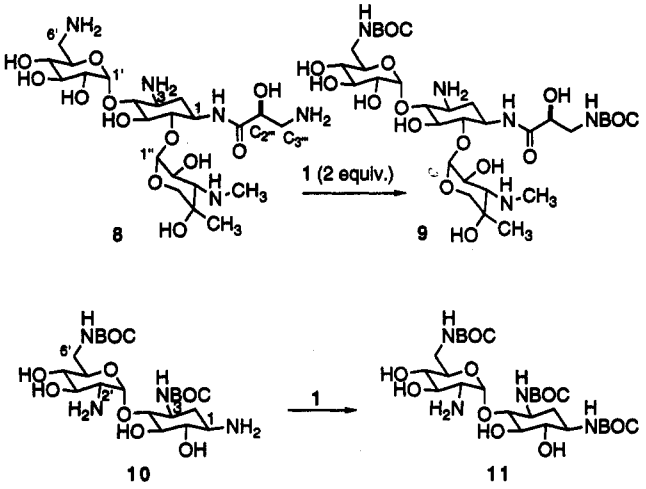


of compound 1 led to an inseparable mixture of the 6′- and 3‴-monoprotected isepamicin, as evidenced by the ^{13}C-NMR spectrum of the product mixture. Minute quantities of mono- and triprotected species were detected in the initial reaction mixture by thin-layer chromatography analysis prior to the workup of the reaction and purification by ion-exchange chromatography. Reaction of compound 10 with reagent 1 afforded a 60% yield of 1,3,6′-tri-BOC neamine (11).



Despite the complexity of the aminoglycoside structures, the regiochemistry of the products can be assigned unambiguously. For example, the mass spectra of compounds 5, 7, 9, and 11 gave molecular ions for the anticipated numbers of BOC groups in these structures, which were confirmed by intergration of their ^{1}H-NMR spectra. The exact positions of the BOC groups were then determined by the following techniques.

The position of the single BOC group in each of compounds 5 and 7, for example, was determined from their ^{1}H-NMR spectra in DMSO-d_6. The carbamic hydrogen ($NHCO_2$) is not exchanged in this solvent and it was, in both cases, observed as triplets in the expected region of the spectra (at 6.61 and 6.66 ppm, respectively). The multiplicity of these peaks indicates equal coupling of the carbamic hydrogen to each of the two methylene hydrogens (5.0 and 5.5 Hz, respectively). An inspection of the structures in question reveals that such a spin pattern could result only from carbamoylation at the 6′-amine.

Compound 9, on the other hand, showed a poorly resolved triplet at 6.65 ppm which corresponded to two hydrogens on integration of the ^{1}H-NMR spectrum, indicating carbamoylation at both 3‴- and 6′-amines. Additional proof for the structure of 9 was derived from the comparison of the ^{15}N-NMR spectra of 9 with that of the starting material 8. Thus, the signals for N-6′ and N-3‴[13] in the spectrum of 8 (at −8.8 and −8.2 ppm) were not present in the spectrum of 9, whereas two new signals appeared in this spectrum (at 58.1 and 58.5 ppm). In view of the fact that acylation of an aminoglycoside amine is associated with a 99–103 ppm downfield ^{15}N-shift,[14] the downfield chemical shifts we observed (66.3–67.3 ppm) confirm that the carbamoylation had taken place at positions N-3‴ and N-6′ of 8, producing the product 9.

Finally, the structure of 11 was assigned in a less straightforward manner, by the measurement of the β-carbon shift associated with the protonation of an amine. It has been shown that protonation of amines causes small chemical shifts for the α-, γ- and δ-carbons (0.5–1.5 ppm), but considerably larger chemical shifts for the β-carbons (up to 4.0 ppm in the upfield direction for the primary amines of aminoglycosides).[15] Comparison of the ^{13}C-NMR spectra of 11 and its hydrochloride[16] revealed β-carbon shifts upon protonation of 3.2 ppm in the upfield direction for both 1′- and 3′-carbon, indicative of the fact that the 2′-amine was not protected and available for protonation. On the other hand, the lack of any appreciable difference in the chemical shifts of 2- and 5′-carbons showed that all three amines at positions 1, 3, and 6′ were protected and confirmed the previous conclusion.

In summary, the hindered nature of reagent 1 affords good regioselectivity in these reactions. This property for 1 is unprecedented and should prove useful in applications to other types of molecules in the future.

Experimental Section

^{15}N-NMR spectra were recorded on a Varian U-500 spectrometer operating at 50.65 MHz. Concentrated aqueous solutions of the aminoglycosides were used (~250 mg/mL) and 10000–15000 FIDs were accumulated in each case. The pH was adjusted to 12 by using 1 M NaOH, and 15% D_2O was added for internal deuterium lock. The data acquisition was performed with a spectral window of 30 KHz and 32 K data points, 54° flip angle (15 μs) and a pulse repetition of 3.5 s. ^{15}N Chemical shifts were measured relative to external $^{15}NH_4Cl$ (2.9 M in 1 M HCl).[17] Proton and carbon NMR spectra were obtained at 300 and 75 MHz, respectively, using a Nicolet QE-300 spectrometer. Chemical shift values (δ) are given in ppm. Assignments for carbon chemical shifts were based on data reported for a series of analogous compounds;[18] however, assignments for methyl, methylene, and carbonyl carbons were confirmed by carrying out DEPT experiments.[19] Infrared and mass spectra were recorded on a Nicolet DX and a Kratos MS 80RFA spectrometers, respectively. Elemental analyses were performed by Galbraith Laboratories, Knoxville, TN. Melting points were taken on a Hoover UniMelt apparatus and are uncorrected. All the aminoglycoside carbamates sintered at approximately 60 °C and charred near 120 °C with concomitant release of CO_2. Thin layer chromatograms were made on silica gel. Isepamicin sulfate was a generous gift from the Schering-Plough Corp. Kanamycin A and neomycin sulfates were purchased from the Sigma Chemical Co. Neamine hydrochloride was prepared from neomycin sulfate

(13) These two signals are the most upfield signals in the spectrum of isepamicin (Schanck, A.; Brasseur, R.; Mingeot-Leclerq, M. P.; Tulkens, P. M. *Magn. Reson.* **1992**, *30*, 11). The differences, however, between the shift values reported by these authors and those of ours is mainly due to the difference in pH of the samples (5.4 vs 12).

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(16) The hydrochloride was prepared by suspending 11 in an ethereal solution of HCl (0.05 N) for a few minutes, followed by centrifugation of the suspension. The pelleted product was exhaustively washed by dry ether to remove the remaining traces of acid.

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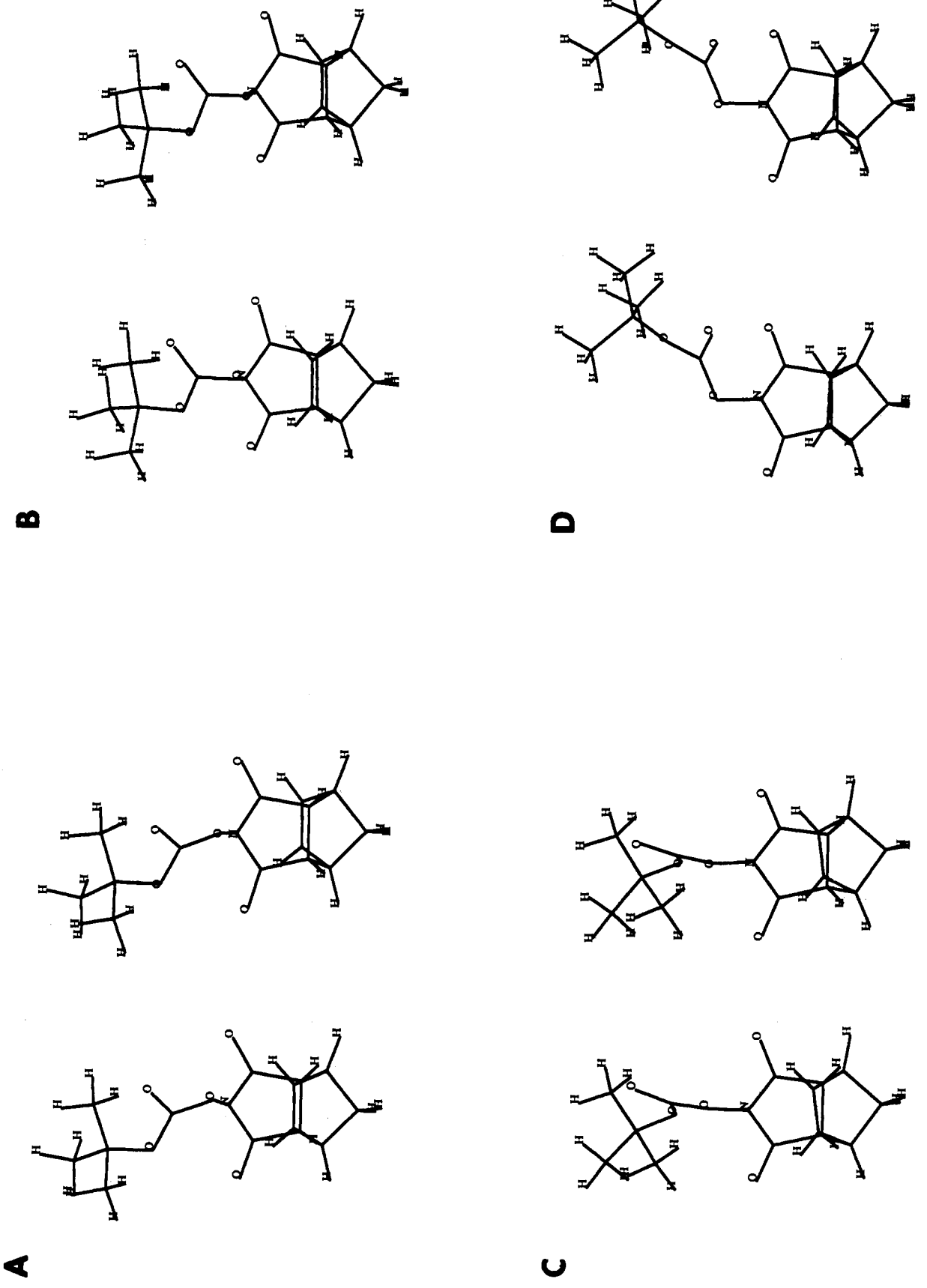

Figure 1. (A) Stereoview of the energy-minimized structure for compound 1; (B–D) Randomly selected conformers in the course of the 100-ps dynamics simulations. Hydrogens are shown.

by methanolysis.[20] All other reagents, were purchased from the Aldrich Chemical Co. All aminoglycosides were used in the free-base form, which were prepared from the corresponding ammonium salts by the use of Amberlite IRA 400 (OH^-) strongly basic ion-exchange resin.

Energy Minimization and Molecular Dynamics. Energy minimization of compound 1 was carried out on a Silicon Graphics Indigo R4000 computer, using the Sybyl software. Energy minimization with the Tripos force field was carried out until the change in energy was less than 0.001 kcal/mol between two successive iterations. Molecular dynamics simulations were performed in Sybyl. The minimized structure was heated to 300 °K over 100 ps, using a time step of 1 fs. Conformations were sampled from the collections of all conformers in the time course of simulation, and three randomly selected structures were selected for display in Figure 1.

***N*-(*tert*-Butoxycarbonyloxy)-5-norbornene-*endo*-2,3-dicarboximide (1).** *N*-Hydroxy-5-norbornene-2,3-dicarboximide (3.59 g, 20 mmol) was dissolved in absolute ethanol (100 mL), and was then evaporated to dryness *in vacuo* to remove the residual moisture from the reagent. The residue was redissolved in freshly prepared absolute ethanol (60 mL), and thallous ethoxide (1.42 mL, 20 mmol) was added dropwise with vigorous stirring. The solution was stirred for 3 h at room temperature and then overnight at 4 °C. The white precipitate which formed in the course of the reaction was collected by filtration, washed with cold ethanol, and dried under high vacuum. The solid material was suspended in methylene chloride (100 mL), and di-*tert*-butyl dicarbonate (4.37 g, 20 mmol) was added dropwise. The solution was stirred overnight at room temperature and was then washed with water (2 × 10 mL). The organic layer was dried over anhydrous $MgSO_4$, filtered, and evaporated to dryness to give the crude product as a white solid. The solid was suspended, with vigorous stirring, in anhydrous ether (60 mL) for 2 h, followed by filtration, to give 3.0 g of the pure product. Upon storage of the filtrate at −20 °C overnight, additional pure product precipitated to afford a total of 3.63 g of the title compound in two crops: yield, 65%; mp 121–122 °C; IR (KBr) cm^{-1} 1801, 1773, 1732; ^1H NMR ($CDCl_3$): δ 1.47 (m, 1H, $H_{7'}$), 1.48 (s, 9H, $OC(CH_3)_3$), 1.71 (dt, 1H, J = 9 Hz, J = 1.5 Hz, $H_{7''}$), 3.27 (dd, 2H, J = 1.5 and 2.1 Hz, H_1 and H_4), 3.40 (m, 2H, H_2 and H_3), 6.13 (s, 2H, H_5 and H_6); ^{13}C NMR ($CDCl_3$) δ 27.5 ($OC(CH_3)_3$), 43.1 (C_1 and C_4), 44.8 (C_2 and C_3), 51.3 (C_7), 87.4 ($OC(CH_3)_3$), 134.8 (C_5 and C_6), 170.1 (C=O); MS CI^- 278 (M − H, 1.6%), 177 [M − Me_3CO(C=O)−H, 38%], 161 [M − Me_3CO(C=O)O − H, 100%]. Anal. Calcd for $C_{14}H_{17}NO_5$: C, 60.20; H, 6.14; N, 5.02. Found: C, 60.54; H, 6.31; N, 4.99.

6′-*N*-(*tert*-Butoxycarbonyl)neamine (5). Neamine free base (322 mg, 1.0 mmol) was dissolved in dioxane/water (40 mL, 1:1), followed by the addition of a solution of reagent 1 (279 mg, 1.0 mmol) in 20 mL dioxane over 1 h. Subsequently, the reaction was stirred at room temperature for 5 h, at which time TLC analysis ($CHCl_3$/MeOH/concd NH_4OH 5:3:1) showed the presence of one major product (R_f 0.40), which proved to be the desired monocarbamoylated product, together with two minor polycarbamoylated materials (approximately R_f 0.60), and some remaining starting material (which remained at the base line of the TLC plate). Dioxane was removed under reduced pressure, and the remaining aqueous solution was diluted with water to 100 mL and was washed with *n*-butanol which had been saturated with water previously (2 × 50 mL). This treatment removed most of the polycarbamoylated byproducts. The *n*-butanol layer was washed with water (50 mL) and the combined aqueous layer and the washings were evaporated to dryness *in vacuo*. The oily residue was dissolved in 10 mL of dioxane/water (1:1) and was transferred to an Amberlite CG-50 (NH_4^+) column (1.5 cm × 30 cm) packed in dioxane/water (2:1). Elution with dioxane/water (2:1) removed the *N*-hydroxy-5-norbornene-2,3-dicarboximide from the column. Subsequent elution with dioxane/water (2:1) supplemented with 0.5% concd ammonia eluted the residual polycarbamoylated products, whereas the title compound was eluted from the resin when the ammonia concentration was increased to 1% (275 mg, 65%). A final wash of the column with dioxane/water (2:1) containing 5% concd ammonia recovered the unreacted neamine (70 mg, 22%): IR (KBr) cm^{-1} 1682; ^1H NMR (D_2O) δ 1.24 (m, 1H, H_{2ax}), 1.37 (s, 9H, $OC(CH_3)_3$), 1.98 (m, 1H, H_{2eq}), 2.60 to 3.80 (overlapping multiplets, 11H, various ring protons), 5.12 (d, 1H, J = 3 Hz, $H_{1'}$); ^{13}C NMR (D_2O): δ 27.8 ($OC(CH_3)_3$), 34.7 (C_2), 41.2 ($C_{6'}$), 49.6 (C_3), 50.6 (C_1), 55.4 ($C_{2'}$), 71.6 ($C_{5'}$), 72.0 ($C_{4'}$), 73.4 ($C_{3'}$), 75.8 (C_5), 76.3 (C_6), 81.2 ($OC(CH_3)_3$), 87.6 (C_4), 101.2 ($C_{1'}$), 158.4 (C=O); MS FAB$^+$ 423 (M+H, 63%).

6′-*N*-(*tert*-Butoxycarbonyl)kanamycin A (7). Kanamycin A free base (484 mg, 1.0 mmol) was dissolved in dimethyl sulfoxide (30 mL) and reagent 1 (279 mg, 1.0 mmol) was added. The solution was stirred at room temperature overnight, at which time TLC analysis ($CHCl_3$/MeOH/concd ammonia 5:3:1) indicated an almost total consumption of the starting material in favor of one major product (R_f 0.15, the title monocarbamoylated compound), accompanied by traces of two minor products (approximately R_f 0.30, polycarbamoylated). The solution was then poured into ethyl ether (400 mL) and was stirred vigorously until a colorless oil separated. Ether was carefully decanted from the flask and the residual oil was dissolved in 15 mL of dioxane/water (1:1) and was chromatographed on an Amberlite CG-50 (NH_4^+) column as described above for 6′-*N*-(*tert*-butoxycarbonyl)-neamine (408 mg): yield 70%; IR (KBr) cm^{-1} 1691; ^1H NMR (D_2O) δ 1.23 (q, 1H, J = 12.3 Hz, H_{2ax}), 1.42 (s, 9H, $OC(CH_3)_3$), 1.97 (partially resolved multiplet, 1H, J = 12.3 Hz, H_{2eq}), 2.70 to 4.00 (overlapping multiplets, 17H, various ring protons), 5.04 (d, 1H, J = 3.3 Hz, $H_{1''}$), 5.22 (d, 1H, J = 3.3 Hz, $H_{1'}$); ^{13}C NMR (D_2O) δ 27.00 ($OC(CH_3)_3$), 35.3 (C_2), 41.2 ($C_{6'}$), 49.6 (C_3), 50.5 (C_1), 54.4 ($C_{3''}$), 60.4 ($C_{6''}$), 69.2 ($C_{4''}$), 71.1 ($C_{5'}$), 71.8 ($C_{2''}$), 72.0 ($C_{2'}$ and $C_{5''}$), 72.2 ($C_{4'}$), 73.0 ($C_{3'}$), 74.3 (C_5), 81.1 ($OC(CH_3)_3$), 87.5 (C_4), 88.5 (C_6), 100.1 ($C_{1'}$), 100.6 ($C_{1''}$), 158.3 (C=O); MS FAB$^+$ 585 (M + H, 22%).

3‴,6′-di-*N*-(*tert*-Butoxycarbonyl)isepamicin (9). Isepamicin free base (569 mg, 1.0 mmol) was dissolved in dimethyl sulfoxide (30 mL), followed by the addition of reagent 1 (558 mg, 2.0 mmol). The solution was stirred at room temperature overnight, at which time a TLC analysis ($CHCl_3$/MeOH/concd ammonia 5:3:1) indicated the total consumption of the starting material in favor of one major product (R_f 0.45), which proved to be the title dicarbamoylated compound. A trace of a slower moving product (R_f 0.27, monocarbamoylated) could also be detected. The reaction was then worked up and the product was purified by chromatography on Amberlite CG-50 (NH_4^+) as described for compound 5. Elution with dioxane/water (1:1), supplemented with 0.5% concd ammonia, afforded the desired product (575 mg): yield 75%; IR (KBr) cm^{-1} 1691, 1660; ^1H NMR (D_2O) δ 1.21 (s, 3H, 4″-CH_3), 1.28 (m, 1H, H_{2ax}), 1.42 (s, 18H, $OC(CH_3)_3$), 1.91 (m, 1H, H_{2eq}), 2.57 (s, 3H, NCH_3), 2.60 to 4.40 (overlapping multiplets, 18H, various ring and side-chain protons), 5.08 (d, 1H, J = 3.3 Hz, $H_{1''}$), 5.20 (d, 1H, J = 2.4 Hz, $H_{1'}$); ^{13}C NMR (D_2O) δ 21.7 (4″-CH_3), 27.9 ($OC(CH_3)_3$), 34.6 (C_2), 36.7 (N-CH_3), 41.1 ($C_{6'}$), 43.3 ($C_{3'''}$), 49.3 (C_3), 49.6 (C_1), 63.8 ($C_{3''}$), 67.7 ($C_{5''}$), 68.4 ($C_{5'}$), 70.8 ($C_{4'}$), 71.1 ($C_{4''}$), 72.0 ($C_{2'}$ and $C_{2''}$ and $C_{2'''}$), 72.9 ($C_{3'}$), 74.8 (C_6), 79.4 (C_5), 81.1 and 81.3 ($OC(CH_3)_3$), 88.2 (C_4), 98.7 ($C_{1'}$), 100.8 ($C_{1''}$), 158.3 and 158.4 (NHCOO), 174.4 ($C_{1'''}$); MS FAB$^+$ 770 (M + H, 100%).

1,3,6′-tri-*N*-(*tert*-Butoxycarbonyl)neamine (11). 3,6′-di-*N*-(*tert*-Butoxycarbonyl)neamine (200 mg, 0.62 mmol) was dissolved in dioxane/water (30 mL, 2:1) and reagent 1 (173 mg, 0.62 mmol) was added to the mixture. The solution was stirred at room temperature for 48 h, at which time TLC analysis with $CHCl_3$/MeOH/concd ammonia (2:1:1)—the solvent mixture separated in two layers after thorough mixing, and the lower layer was used in TLC analysis—showed one major product (R_f 0.21), together with traces of higher moving products and some starting material (R_f 0.14). The solvent was evaporated to dryness, the residue was dissolved in 4 mL of methanol and was transferred

(20) Neamine was synthesized by methanolysis of neomycin sulfate by a slight modification of the method of Dutcher and Donin (Dutcher, J.; Donin, M. *J. Am. Chem. Soc.* **1952**, *74*, 3420). The literature method affords a neamine sample which is impure. The following modification in the procedure gives a sample of neamine which is chromatographically pure. The modification of the procedure is as follows: at the end of the reaction, the methanolic solution was concentrated to allow neamine hydrochloride to precipitate as a white solid. The precipitate was filtered and was washed repeatedly with anhydrous ether to remove the remaining traces of hydrogen chloride.

to a silica gel column. Elution with $CHCl_3$/MeOH/concd ammonia 2:1:1—prepared as described above—afforded the title product (230 mg): yield 60%; IR (KBr) cm^{-1} 1682; ^1H NMR (DMSO-d_6): δ 1.17 (m, 1H, H_{2ax}), 1.32, 1.34, and 1.35 (s, 27H, $OC(CH_3)_3$), 1.70 (m, 1H, H_{2eq}), 2.40 to 5.00 (unresolved multiplets, 19H, various ring protons and protons on heteroatoms), 6.41 (broad, 1H, NHCOO), 6.60 (broad, 2H, NHCOO); ^{13}C NMR (DMSO-d_6) : δ 28.6 and 28.7 ($OC(CH_3)_3$), 35.6 (C_2), 41.9 ($C_{6'}$), 49.3 (C_3), 51.5 (C_1), 55.5 ($C_{2'}$), 70.5 ($C_{4'}$), 71.4 ($C_{5'}$), 73.7 ($C_{3'}$), 77.7 (C_5), 77.9 (C_6), 78.1 and 78.2 ($OC(CH_3)_3$), 83.1 (C_4), 99.8 ($C_{1'}$), 155.3, 155.7, and 156.6 (C=O); MS FAB$^+$ 623 (M + H, 13%).

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Supplementary Material Available: Copies of ^1H NMR spectra of 1, 5, 7, 9, and 11 (5 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.