New Derivatives of Kanamycin B Obtained by Modifications and Substitutions in Position 6″. 1. Synthesis and Microbiological Evaluation

A. Van Schepdael,† J. Delcourt,† M. Mulier,† R. Busson,† L. Verbist,‡ H. J. Vanderhaeghe,† M. P. Mingeot-Leclercq,§ P. M. Tulkens,§ and P. J. Claes*,†

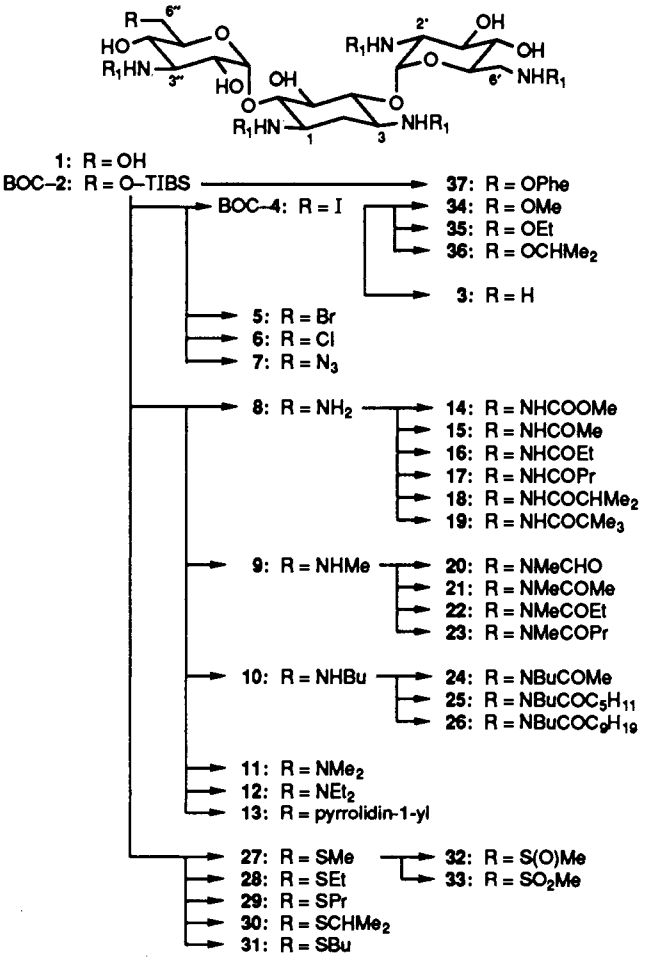
Laboratorium voor Farmaceutische Chemie (I.F.W.), Rega Instituut, Katholieke Universiteit Leuven, Minderbroederstraat, 10, B-3000 Leuven, Belgium, Laboratorium voor Medische Mikrobiologie, U.Z. St Rafaël, Kapucijnenvoer, 35, B-3000 Leuven, Belgium, and Laboratoire de Chimie Physiologique, Université Catholique de Louvain and International Institute of Cellular and Molecular Pathology, Avenue Hippocrate, 75, B-1200 Bruxelles, Belgium. *Received August 14, 1990*

The clinical use of the potent, wide-spectrum aminoglycoside antibiotics is limited by oto- and nephrotoxicities. The latter is related to the binding of these polycationic drugs to negatively charged phospholipids and to the subsequent inhibition of lysosomal phospholipases. In order to explore the influence of a modification of the hydrophobic/hydrophilic balance at a specific site of an aminoglycoside, kanamycin B has been chemically modified in position 6″ by substitution of the hydroxyl group with a halogen atom (or a pseudohalogen group), or an amino, an amido, a thioalkyl, or an alkoxy group, each series containing increasingly bulkier chains. Examination of the antibacterial activity of the synthesized compounds revealed a negative correlation between the size of the 6″-substituent and the antibacterial activity against kanamycin B sensitive Gram-positive and -negative organisms. Only derivatives with small substituents in position 6″, namely chloro, bromo, azido, amino, methylcarbamido, acetamido, methylthio, methylsulfinyl, *O*-methyl, *O*-ethyl, and *O*-isopropyl, showed acceptable activity (geometric mean of minimum inhibitory concentrations for Gram-negative strains ≤2.5 mg/L; value for kanamycin B, 0.5 mg/L). In vitro toxicological evaluation of all derivatives and computer-aided conformational analysis of selected compounds inserted in a phosphatidylinositol monolayer are presented in the following paper in this issue.

Introduction

Aminoglycosides are highly potent, broad-spectrum antibiotics active against most aerobic Gram-negative organisms, staphylococci, and Gram-positive bacilli. The main aminoglycosides used in clinical practice today belong to the group of the gentamicins and kanamycins which share a common pseudotrisaccharidic structure containing a diaminocyclitol (2-deoxystreptamine) associated with two amino sugar moieties[1] (see Scheme I for kanamycin B). Unfortunately, their use is associated with nephro- and ototoxic reactions, affecting on an average 8–30% of treated patients.[2] This has severely reduced the extent of aminoglycoside usage, while giving impetus for synthesis and/or screening of intrinsically less toxic derivatives. Efforts in these directions have met only with moderate success,[3] even though new, semisynthetic derivatives such as netilmicin, amikacin, or isepamicin appear useful in this respect.[2,4] Yet, these derivatives were primarily designed for resistance to bacterial aminoglycoside-inactivating enzymes,[1] and not for reduced toxicity. The molecular and cellular mechanisms by which aminoglycosides induce oto- and nephrotoxicity have been the subject of an intense series of investigations over the past several years.[5-7] The most critical aspects are the capacity of aminoglycosides to preferentially accumulate in kidney cortex[8] and inner ear tissue[9] and to interact with acidic phospholipids.[10,11] These interactions have been characterized by an array of biochemical and biophysical approaches,[12-15] which have shown that the drugs primarily establish electrostatic interactions with the polar head groups. Computer-aided conformational analysis has suggested that both the position and the degree of insertion of an aminoglycoside in acidic phospholipid layers play critical roles with respect to nephrotoxicity,[14,16,17] and this has rationalized the lesser intrinsic toxicity of 1-N-substituted derivatives of kanamycins (viz. amikacin, which is 1-*N*-(2-hydroxy-4-aminobutyryl)kanamycin A, and the 1-*N*-(2-hydroxy-4-aminobutyryl) derivative of 3″-*N*-methyl-4″,6″-dideoxykanamycin B), or of gentamicin B (viz. isepamicin, which is 1-*N*-(2-hydroxy-3-aminopropionyl)gentamicin

Scheme I. Structural Formula of Kanamycin B (1; R = OH; R_1 = H), 6″-Substituted Kanamycin B End Products (3, 5–37), and Key Intermediates (BOC-2, BOC-4) Used in Their Synthesis[a]



[a] TIBS, (2,4,6-triisopropylphenyl)sulfonyl; R_1 = H in unprotected, and R_1 = BOC (*tert*-butoxycarbonyl) in protected compounds. Numbers refer to those used to designate the corresponding compounds in the text. Numbers preceded by BOC refer to protected compounds. See refs 1, 14, and 17 for the structures of all other aminoglycosides mentioned in the text.

* Request for reprints should be addressed to Prof. P. J. Claes, Rega Instituut, Minderbroederstraat, 10, B-3000 Leuven, Belgium.
† Laboratorium voor Farmaceutische Chemie.
‡ Laboratorium voor Medische Mikrobiologie.
§ Laboratoire de Chimie Physiologique and International Institute of Cellular and Molecular Pathology.

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B).[17] Further reasoning suggested that changes in intrinsic toxicity could perhaps also be obtained by the addition of an hydrophobic group in the 3''-amino sugar of kanamycins or gentamicins. Since a free hydroxyl group in 2'' and a free amino group in 3'' are essential for activity on the one hand[1] and because the 4''-tertiary hydroxyl group is of low reactivity and gentamicins carry no other reactive group on this sugar on the other hand, only the 6''-hydroxyl group of kanamycins appeared suitable for modifications and substitutions. Actually, a limited number of modifications were already described in that position (deoxygenation,[18-21] fluorination,[22] or chlorination[18]) without marked loss of activity. Moreover, the naturally occurring 6''-*O*-carbamoylkanamycin B is also active.[23] We therefore decided to study, and we report here on, further and more systematic modifications of this position. We chose kanamycin B rather than kanamycins A or C on account of its availability and potential applications of the present work to the 3'-deoxy and 3',4'-dideoxy derivatives of kanamycin B (tobramycin and dibekacin, respectively) which show excellent activity against *Pseudomonas aeruginosa*, an important pathogen in diseases requiring the use of an aminoglycoside such as nosocomial infections or infections in immunodepressed and immunodeficient patients.[7]

Results

Chemical Syntheses. 6''-Modified kanamycin B derivatives can conveniently be obtained by nucleophilic displacement performed on a suitably *N*-protected precursor. For reasons of solubility we used *tert*-butoxycarbonyl (BOC) as N-protective group for the five amino functions. Numbers preceded by BOC in the text refer to the corresponding penta-*N*-BOC-protected compounds. The selective introduction of a leaving group in the 6''-position could successfully be accomplished in only two steps from kanamycin B (1) by reaction of the *N*-BOC-protected kanamycin B (BOC-1) with the bulky 2,4,6-triisopropylbenzenesulfonyl chloride (TIBS-Cl). The yield of the desired 6''-O-sulfonylated product (BOC-2) was about 70%, in contrast to the analogous but much less selective 6''-O-tosylation reported for kanamycin A,[24] which gave a yield of the 6''-O-substituted product of only 20%. The use of TIBS-Cl also nicely circumvented the more tedious way applied before for selective modification of the 6''-position.[25] In the latter procedure, the secondary hydroxyl groups have to be protected prior to introduction of the leaving group and this normally requires four additional steps (viz. 6''-O-tritylation, O-acetylation, detritylation, and deacetylation), and therefore gives a much lower total yield. 6''-Deoxy-6''-alkyl derivatives were considered as the most useful compounds to synthesize in order to selectively modify the hydrophobicity of that region of kanamycin B. We, however, were unsuccessful in obtaining 6''-deoxy-6''-alkyl compounds from an *N*-BOC-protected kanamycin B precursor. Indeed, substitution reactions of a 6''-iodo- or a 6''-(sulfonyloxy) group with lithium dialkylcuprates[26] using different reaction conditions, with or without blocking of the secondary hydroxyl groups, in all cases led to an intractable mixture of reaction products. The synthesis of this type of derivatives was therefore not further pursued, and efforts were directed at introducing the alkyl chains via an intermediate 6''-N, -S, or -O atom. This readily offered the possibility to obtain derivatives of variable hydrophobicity by choosing the appropriate alkyl group(s). In addition, we also elected to synthesize 6''-deoxy-6''-halogeno and 6''-deoxy-6''-azido analogues taken as derivatives carrying small-size hydrophobic substituents. Scheme I shows the structural formula of kanamycin B and of all final compounds as well as critical intermediates as they were obtained in this study. They are classified in groups according to the type of 6''-substitution of kanamycin B.

The halogen substituted derivatives BOC-4–BOC-6 were obtained by reaction of BOC-2 in DMF with NaI, LiBr, or LiCl. Likewise, treatment of BOC-2 with LiN_3 gave BOC-7 in a 80% yield. For reasons of solubility compound BOC-4 was used under its tetra-*O*-acetyl form as a starting material for the synthesis of BOC-3 via tributyltin hydride reduction of the iodo group and deacetylation. The O-acetylated iodide was easily prepared by iodination[27] of 3',4',2'',4''-tetra-*O*-acetyl-BOC-1, which was obtained according to the above-mentioned procedure described for kanamycin A.[25] The 6''-amino derivatives BOC-8–BOC-13 were all synthesized in high yield by heating at 40–70 °C a solution of BOC-2 in EtOH with the appropriate amine. Subsequently, three series of 6''-acylamino compounds

(1) Nagabhushan, T. L.; Miller, G. H.; Weinstein, M. J. In *The Aminoglycosides: Microbiology, Clinical Use and Toxicology*; Whelton, A., Neu, H. C., Ed.; Marcel Dekker: New York, 1982; pp 3–27.
(2) Kahlmeter, G.; Dahlager, J. I. *J. Antimicrob. Chemother.* **1984**, *13*, 9.
(3) Price, K. E. *Antimicrob. Agents Chemother.* **1986**, *29*, 543.
(4) Tulkens, P. M. *Toxicol. Lett.* **1989**, *46*, 107.
(5) Tulkens, P. M. *Scand. J. Infect. Dis.* **1990**, in press.
(6) Brummett, R.; Fox, K. *Antimicrob. Agents Chemother.* **1989**, *33*, 797.
(7) Lietman, P. S. In *Principles and Practice of Infectious Diseases*; Mandell, G. L., Douglas, R. G., Bennet, J. E., Ed.; John Wiley & Sons: New York, 1985; pp 192–206.
(8) Giuliano, R. A.; Paulus, G. J.; Verpooten, G. A.; Pattijn, V. M.; Pollet, D. E.; Nouwen, E. J.; De Broe, M. E. *Kidney Int.* **1984**, *26*, 838.
(9) Tran Ba Huy, P.; Bernard, P.; Schacht, J. *J. Clin. Invest.* **1986**, *77*, 1492.
(10) Laurent, G.; Kishore, B. K.; Tulkens, P. M. *Biochem. Pharmacol., in press.*
(11) Williams, S. E.; Zenner, H.-P.; Schacht, J. *Hear. Res.* **1987**, *30*, 11.
(12) Alexander, A. M.; Gonda, I.; Harpur, E. S.; Kayes, J. B. *J. Antiobiot. (Tokyo)* **1979**, *32*, 504.
(13) Chung, L.; Kaloyanides, G.; McDaniel, R.; McLaughin, A.; McLaughin, S. *Biochemistry* **1985**, *24*, 442.
(14) Brasseur, R.; Laurent, G.; Ruysschaert, J. M.; Tulkens, P. *Biochem. Pharmacol.* **1984**, *33*, 629.
(15) Au, S.; Weiner, N.; Schacht, J. *Antimicrob. Agents Chemother.* **1986**, *30*, 395.
(16) Mingeot-Leclercq, M. P.; Piret, J.; Tulkens, P. M.; Brasseur, R. *Biochem. Pharmacol.* **1990**, *in press.*
(17) Tulkens, P. M.; Laurent, G.; Mingeot-Leclercq, M. P.; Brasseur, R. In *Molecular Description of Biological Membrane Components by Computer-aided Conformational Analysis*; R. Brasseur, Ed.; CRC Press: Boca Raton, FL, in press.
(18) Tsuchiya, T.; Umezawa, S. *Bull. Chem. Soc. Jpn.* **1965**, *38*, 1181.
(19) Miyasaka, T.; Ikeda, D.; Kondo, S.; Umezawa, H. *J. Antibiot. (Tokyo)* **1980**, *33*, 527.
(20) Godfrey, J. *Ger. Offen.* 2, 550,429, 1976 [US Appl. 523,292, 13; Chem. Abstr. **1976**, *85*, P47017*f*].
(21) Carlier, M. B.; Brasseur, R.; Ruysschaert, J. M.; Tulkens, P. M. *Arch. Toxicol.* **1988**, *Suppl. 12*, 186.
(22) Albert, R.; Dax, K.; Stütz, A. E.; Hildebrandt, J. *J. Antibiot. (Tokyo)* **1985**, *38*, 275.
(23) Wick, W. E.; Welles, J. S. *Antimicrob. Agents Chemother.* **1968**, 341.
(24) Tsuchiya, T.; Iriyama, S.; Umezawa, S. *J. Antibiot. (Tokyo)* **1963**, *16*, 173.
(25) Albert, R.; Dax, K.; Stütz, A. E.; Weidmann, H. *J. Carbohydr. Chem.* **1983**, *2*, 279.
(26) Johnson, C. R.; Dutra, G. A. *J. Am. Chem. Soc.* **1973**, *95*, 7777.
(27) Albert, R.; Dax, K.; Gassner, N. A.; Stütz, A. E.; Weidmann, H. *Liebigs Ann. Chem.* **1985**, 644.

(BOC-14–BOC-19, BOC-20–BOC-23, and BOC-24–BOC-26) with gradually increasing lipophilic 6″-substituents were prepared by acylation of the 6″-amino derivative BOC-8 and of the two 6″-alkylamino derivatives BOC-9 and BOC-10, respectively. These reactions were carried out with the appropriate anhydride or acid chloride in EtOH at room temperature, except for the *N*-formyl derivative BOC-20, which was prepared by reaction of BOC-9 with *p*-nitrophenyl formate. The highly lipophilic BOC-26 was only sparingly soluble in water in its deprotected form, and henceforth was excluded from further experiments. The synthesis of compounds BOC-27–BOC-31 was performed through reaction of BOC-2 with the appropriate potassium alkanethiolates in DMF. Yields obtained in this series (ca. 40–60%) were significantly lower than those obtained with halogenides or amine nucleophiles. Oxidation of BOC-27 with *m*-chloroperbenzoic acid, in different molar ratios, gave sulfoxide BOC-32 and sulfone BOC-33. The 6″-*O*-alkyl derivatives BOC-34–BOC-36 were preparatively accessible only, albeit in low yield, by alcoholysis of the 6″-iodo precursor BOC-4 in the presence of silver trifluoroacetate. Indeed, nucleophilic displacement with basic alkoxides occurred in still lower yields and with the formation of side products, the structures of which will be reported elsewhere.[28] As could be anticipated, compound BOC-37, however, could be readily prepared from BOC-2 with a 30% yield through reaction with the much less basic potassium phenolate.

Compounds BOC-3 AND BOC-5–BOC-37 were N-deprotected by treatment with CF_3COOH at room temperature. The trifluoroacetate salts thus obtained were converted into the corresponding free bases by passing them over a column filled with Amberlite CG-50 resin in the NH_4^+ form and the bases were finally converted into the sulfate salts. As explicited in the Experimental Section, no deprotection was carried out for BOC-4, and special precautions had to be taken for BOC-5 due to the lack of stability of the free base form of this derivative. The purity of the end products, as estimated by silica gel thin-layer chromatography (system C), was at least 99% for all compounds tested. The free base content of each of the sulfates was determined by ^1H NMR analysis.[29] Structural proof of the modifications was obtained from ^{13}C NMR analysis at two stages in the synthetic sequence. Spectra of all penta-*N*-BOC-protected intermediates were recorded in pyridine-d_5, and those of the end products were run in D_2O at pH <1.5 and >10. In this way, by comparing the spectra for the N-acylated, protonated, and unprotonated form, highly reliable peak assignments could be made. Chemical shifts for each of the carbon atoms of the 2,6-diamino sugar and of the 2-deoxystreptamine moiety are expected to remain constant throughout the whole series of 6″-substituted compounds, and thus these resonance signals were easily recognized and assigned in accordance with literature data[30,31] for kanamycin B and related aminoglycosides. Assignments for the remaining carbon atoms, viz. those of the modified sugar moiety, were then straightforward, especially when characteristic protonation shifts[32] were taken into account. This is exemplified in Table I which contains the chemical shifts and the assignments for the unprotected and protected 6″-chloro compound (6 and BOC-6) in comparison with kanamycin B (1 and BOC-1). In the spectrum of 6, as could be anticipated, only the shifts for carbons 5″ and 6″ are significantly different from those of the parent compound kanamycin B. ^{13}C NMR data for all other end products are given in the experimental part and are limited to the diagnostic signals, viz. C-4″, C-5″, C-6″ and carbons of the 6″-O, -S, or -N substituents. As expected, shifts of the remaining carbons are, within experimental error, identical with those of the model substance in Table I. All shift changes were in perfect agreement with the proposed modifications. Spectra of derivatives 20–25, containing a tertiary amide function, revealed restricted rotation of the amide bond, as evidenced by the appearance of two signals for *N*-alkyl and *N*-acyl carbon atoms. These compounds showed splitted signals (especially at low pH) also for other carbon atoms in both sugar and deoxystreptamine moieties (data given in the Experimental Section are limited to the set of major signals). This effect may be explained by the existence of a slowly exchanging equilibrium mixture of a pair of conformational isomers, the rate of exchange and the proportion of which is markedly affected by pH. It should be noted that this phenomenon was not observed for compounds lacking a nitrogen substituent at 6″ and thus having other internal hydrogen-bonding preferences. Further confirmation of the proposed structures was obtained by FAB mass spectrometry of the sulfate salts of the end products (values are given in the Experimental Section). They all showed a significant peak with an m/z corresponding to the value calculated for the $(M + H)^+$ ion of the free base.

Table I. ^{13}C Chemical Shifts[a,b] and Peak Assignments[c] for Compounds 1 and 6

carbon	1	BOC-1	6	BOC-6
1	50.7 (0.6)	50.7	50.4 (0.9)	50.8
2	28.8 (7.6)	35.4	28.4 (7.6)	35.8
3	49.5 (0.7)	50.1	49.0 (1.0)	50.3
4	77.8 (9.9)	82.7	77.4 (9.8)	82.8
5	75.3 (0.0)	75.7	74.9 (0.0)	75.7
6	84.6 (4.2)	83.8	84.4 (4.0)	83.2
1′	96.3 (4.9)	100.6	96.1 (4.7)	100.9
2′	54.6 (1.6)	56.6	54.2 (1.8)	56.9
3′	70.3 (4.2)	71.8	69.9 (4.3)	72.0
4′	72.0 (0.2)	72.5	72.3 (0.1)	72.5
5′	69.2 (4.8)	72.5	68.8 (4.6)	72.0
6′	41.5 (1.0)	41.8	41.0 (1.2)	41.8
1″	101.5 (−0.8)	99.3	101.5 (−0.7)	99.2
2″	69.2 (3.5)	71.5	68.8 (2.6)	71.2
3″	56.0 (−0.9)	57.5	55.2 (−0.6)	57.2
4″	66.5 (3.7)	69.1	66.8 (3.6)	69.2
5″	73.9 (−1.0)	74.7	71.6 (0.5)	72.5
6″	61.0 (0.2)	61.6	44.6 (0.5)	46.1
3″-NHCO		157.8		157.7
6′-NHCO		157.2		157.2
3-NHCO		157.0		157.0
1-NHCO		156.3		156.2
2′-NHCO		155.6		155.7

[a] Expressed in ppm downfield from tetramethylsilane (TMS); peak positions were measured in D_2O solution (pD < 1.5) relative to external DMSO, set at 40.1 ppm vs TMS. [b] Protonation shifts, given in parentheses, are calculated as $\Delta\delta = \delta(pD > 10) - \delta(pD < 1.5)$. [c] Assignments for close-lying lines are tentative and may be interchanged.

Microbiological Evaluation. All unprotected end compounds shown in Scheme I proved of sufficient stability in aqueous solution at pH ~7 to withstand a 24-h incubation at 37 °C for microbiological evaluation, with the exception of 4, which could not be obtained from BOC-4 and converted into the sulfate without degradation

(28) Delcourt, J.; Busson, R.; Claes, P. J., *unpublished results.*
(29) Claes, P. J.; Busson, R.; Vanderhaeghe, H. *J. Chromatogr.* **1984**, *298*, 445.
(30) Koch, K. F.; Rhoades, J. A.; Hagaman, E. W.; Wenkert, E. *J. Am. Chem. Soc.* **1974**, *96*, 3300.
(31) Szilagyi, L. *Carbohydr. Res.* **1987**, *170*, 1.
(32) Kalinowski, H. O.; Berger, S.; Braun, S. In *Carbon-13 NMR Spectroscopy*; John Wiley & Sons: Chichester, 1988; pp 221–222.

and which, therefore, was not investigated, and **26**, which was only sparingly soluble in water. Microbiological evaluation was made by means of determination of minimum inhibitory concentrations in semisolid medium against an array of kanamycin B sensitive Gram-positive and -negative bacteria as well as against selected organisms resistant to kanamycin B but sensitive to gentamicin. Investigations were carried out independently by two investigators (L.V., M.P.M.) and yielded consistent results. Table II shows the most salient data obtained for all compounds tested, grouped by types of substitution, with respect to typical target organisms. Values for kanamycin B were in the range of those reported by others.[33] 6''-Deoxygenation of kanamycin B or substitution of the 6''-hydroxyl group by a halogen or a pseudohalogen did not modify the activities recorded to a significant extent. In contrast, substitutions involving an amino or an acetamido group resulted in a decrease of activity. Thus derivatives **8** and **15** showed higher mean geometric MIC values of 1 order of magnitude or more. Addition of an alkyl chain on the 6''-nitrogen, and lengthening of this chain in both the amino- and acetamido series, further decreased the activity. Increase of the chain length at the carbonyl side of the substituted amides caused still more of a loss of activity, and compounds **21–25** were almost totally inactive against all tested organisms with the noticeable exception of *Enterobacter cloacae*. Carbamate **14** showed an activity comparable to that of the 6''-acetamido-substituted derivative **15**. A slight retrieval of activity was obtained with compounds carrying branched alkyl chains (**18**, **19**), but not to the extent of yielding useful compounds. Alkylthio derivatives, up to an *S*-propyl chain, showed an activity similar to the activity of compounds **8** and **15**, slightly lower than that of kanamycin B, with the exception of that against *Bacillus subtilis*, and, perhaps more importantly, against *Serratia marcescens* and *Salmonella typhimurium*. Isopropyl and butyl chains caused a marked loss of activity and this effect, for isopropyl, is in contrast to the effect of chain branching observed in the amido series. The more polar methylthio derivatives in which the S atom was oxidized (**32**, **33**) showed a fair activity against most target organisms except *B. subtilis*, *S. marcescens*, and *S. typhimurium*, as for the other members of this series. Alkoxy derivatives with short alkyl chains showed an almost unaltered activity, but the lengthening of the chain reduced it toward *B. subtilis*, *S. marcescens*, and *S. typhimurium*, even though the bulkiness of this chain did not play such an important role as that observed in the other series. None of the derivatives studied showed activity against the kanamycin B resistant strains. In conclusion, only the deoxy, the halogeno, and to some extent the alkylthio and the alkoxy derivatives with short alkyl chains proved of microbiological interest. Compounds **8**, **14**, and **15** were considered of only marginal interest in this respect. Nevertheless, all compounds were kept for toxicological evaluation,[34] since the aim of the present work was to examine in parallel the changes in antibacterial and toxic potentials.

Discussion

The work reported in this paper represents a first systematic approach at modifying the position 6'' of kanamycins by introduction of increasingly hydrophobic moieties. This was achieved by substitution of the 6''-hydroxyl function with halogen atoms or with a pseudohalogen group (azido), or by adding increasingly bulkier alkyl chains via an intermediate N atom, yielding a 6''-amino or a 6''-amido function, or via a S or an O atom, yielding a thioalkyl or an alkoxy function, respectively. Sulfoxide and sulfone functions were obtained upon oxidation of the corresponding thiomethyl derivative. 6''-Deoxykanamycin B was also made for comparison. All these syntheses proved possible via nucleophilic displacement of a 6''-*O*-[(2,4,6-triisopropylphenyl)sulfonyl] (6''-*O*-TIBS) or a 6''-iodo group. The use of 2,4,6-triisopropylbenzenesulfonyl chloride to selectively sulfonylate carbohydrate moieties has been described for nucleoside chemistry.[35] For aminoglycosides, it greatly improved the final yield of the synthesized analogues under study over the other methods reported in the literature.[24,25] Our results show that substituents of limited size in C-6'' are compatible with the maintenance of the activity of kanamycin B. A first conclusion of our study is therefore that a free hydroxyl group in that position is indifferent with respect to the activity, and that the local hydrophilic character is also unimportant. Thus 6''-deoxy, 6''-halogeno (or 6''-azido), 6''-*S*-methyl, 6''-*O*-methyl, and 6''-*O*-ethyl show no or little difference with kanamycin B, especially concerning Gram-negative organisms. This confirms, but also extends, previous findings made with 6''-deoxy and 6''-halogeno derivatives of kanamycin A, kanamycin B, or amikacin.[18–20,22] Our results also show that the addition of an amino group to kanamycin B (viz. 6''-deoxy-6''-aminokanamycin B) leads to a decreased activity. This conclusion is, in our point of view, of critical importance since it has been suggested that activity and toxicity are directly related to the number of amino groups.[3] Our findings do not confirm this suggestion and are consistent with the observation that 3'-deoxy-3'-aminokanamycin A is not more active than kanamycin A.[36] The location of an additional amino group, however, may be critical, since amination of position 6' in gentamicin A was reported to slightly increase activity.[37]

Whereas the precise nature and physicochemical properties of the function carried by the C-6'' of kanamycin B are largely unimportant for, or affect only moderately, its activity, the size of the substituent appears very critical. Thus, all derivatives in all series consistently showed a decrease of activity toward most target organisms in direct relation to the length or, in many instances, the bulkiness of the chain. Interestingly enough, however, the loss of activity does not proceed in parallel for all organisms tested, with some of them such as *S. marcescens* showing a rapid increase of MIC's, whereas others such as *E. cloacae* remaining largely unaffected. We have not examined the reason for inactivity of these compounds, which could result from a reduced uptake by bacteria,[38] or from their inability to interact with the 30 S subunit of ribosomes.[39] These aspects would need further experimental approaches, including the use of radiolabeled derivatives. The possibility exists, however, that bulky side chains in C-6'' interfere with the ability of the molecule to adopt a

(33) Umezawa, H. *Asian Med. J.* **1968**, *11*, 291.
(34) Mingeot-Leclercq, M. P.; Van Schepdael, A.; Brasseur, R.; Busson, R.; Vanderhaeghe, H. J.; Claes, P. J.; Tulkens, P. M. *J. Med. Chem.*, following paper in this issue.
(35) Lohrmann, R.; Khorana, H. G. *J. Am. Chem. Soc.* **1966**, *88*, 829.
(36) Inouye, S. *Chem. Pharm. Bull.* **1968**, *16*, 573.
(37) Nagabhushan, T. L.; Daniels, P. J. L. *J. Med. Chem.* **1974**, *17*, 1030.
(38) Davis, B. D. *Microbiol. Rev.* **1987**, *51*, 341.
(39) Gale, E. P.; Cundliffe, E.; Reynolds, P. E.; Richmond; Waring, In *The Molecular Basis of Antibiotic Action*; Wiley Interscience: New York, 1980; pp 418–437.

Table II. Minimum Inhibitory Concentrations (mg/L) of 3, 5–25, and 27–37 against Selected, Kanamycin B Sensitive Gram-Positive

organism	strain ATCC number	kanamycin, B[g] 1	6″-deoxy[g] 3	halogeno and pseudohalogeno[g] 5[g]	6[g]	7[g]	amino[g] 8[g]	9[g]	10[g]	11[g]	12[g]	13[g]	amido[g] 14[g]	15[g]
Selected Gram-Positive Organisms														
Staphylococcus aureus	25923[a]	0.5	0.25	0.25	0.25	0.25	0.5	2	4	4	8	4	1	1
Staphylococcus epidermidis	12228	0.25	0.25	0.25	0.25	0.25	0.5	0.5	2	2	8	2	0.5	0.5
B. subtilis	6633	0.5	1	0.5	0.5	0.5	2	8	32	32	16	16	8	4
geometric mean[f]		0.4	0.4	0.3	0.3	0.3	0.8	2.0	6.3	6.3	10.1	5.0	1.6	1.3
Selected Gram-Negative Organisms														
Escherichia coli	25922[b]	1	1	1	1	0.5	4	8	16	16	64	32	2	4
E. aerogenes	13048	0.5	1	1	0.5	0.5	4	8	16	16	32	16	2	2
Enterobacter cloacae	23355	0.25	0.25	0.25	0.25	0.25	0.25	1	1	1	2	2	0.25	0.5
Citrobacter freundii	8090	0.5	0.5	0.5	0.5	0.5	4	8	16	16	32	16	2	2
Proteus vulgaris	13315	0.25	0.25	0.5	0.5	0.5	2	8	16	16	16	8	2	1
S. marcescens	8100[c]	1	1	2	2	2	4	8	32	64	>64	64	16	16
Klebsiella pneumoniae	13883	0.25	0.25	0.25	0.25	0.25	1	4	8	8	16	16	1	1
S. typhimurium	14028	1	1	2	1	1	4	16	32	32	64	64	8	8
geometric mean[f]		0.5	0.5	0.7	0.6	0.5	2.2	6.2	12.3	13.5	26.9	17.4	2.2	2.4
Kanamycin B Resistant Strains														
Staphylococcus faecalis	19431[d]	16	32	32	16	16	>64	>64	>64	>64	>64	>64	64	64
P. aeruginosa NCTC 10701[e]		32	>64	>64	>64	>64	16	>64	>64	>64	>64	>64	>64	>64

[a] Similar results with strains ATCC 15923, 29213. [b] Similar results for strain ATCC 37218. [c] MIC of kanamycin B = 1 mg/L for strain CDC 87457. [d] Similar results for strains ATCC 31186, 29212, 19423, 33186, 23186; MIC of gentamicin toward this strain = 8 mg/L. [e] MIC of kanamycin B = 16 mg/L for strain NCTC 10708 and 64 mg/L for strain ATCC 27853; MIC of gentamicin toward this strain = 1 mg/L. [f] Mean of all Gram-positive or -negative organisms tested. For mean calculation, values >64 mg/L were assumed to be 128 mg/L. [g] 6″-

crescent conformation with the O-5′ and the O-5″ atoms pointing to and interacting with the N-3 and O-5 atoms, respectively (see ref 1 for molecular description), which was claimed to be essential for activity. Whatever the precise molecular mechanism, however, it appears largely pointless to construct derivatives with a large, hydrophobic group appended to position 6″, since their activity will be defeated to an extent that probably may not be matched by decrease in toxicity.[34] Interestingly enough, a loss of activity was also observed for a small-sized substituent, namely 6″-deoxy-6″-acetamido, which is in contrast to the report pointing to an indifferent effect of O-carbamoylation in the same position.[23] Since these two substituents have approximately the same size and are both neutral, and assuming that the carbamoyl derivative remains metabolically stable, we may suggest that what defeats activity is the presence of a N atom directly linked to C-6″. In conclusion, substitutions can be made in position C-6″ of kanamycin B, and probably also of other kanamycins, without systematic loss of activity, which confirms the relative unimportance of this region of the molecule with respect to antibacterial properties. These substitutions, however, may not exceed a modest size and should preferably not involve a direct linkage between the C-6″ and a N atom.

Experimental Section

Chemistry. Kanamycin B was supplied by Meiji Seika Kaisha, Ltd. (Tokyo 104, Japan). Chemical reagents were obtained from Janssen Chimica, Beerse, Belgium. E. Merck precoated silica gel F254 plates were used for TLC, with the following mobile phases: system A, $CHCl_3$–MeOH–33% aqueous ammonia (70:10:2); system B, EtOAc–*i*-PrOH–33% aqueous ammonia (20:3:2); system C, $CHCl_3$–MeOH–33% aqueous ammonia (10:20:16), unless stated otherwise. Column chromatography was performed on silica gel (E. Merck, 0.040–0.063 mm). Infrared (IR) spectra were run on a Perkin-Elmer 257 spectrophotometer. ^{13}C and ^1H NMR spectra were run, at operating temperature, on a JEOL FX90Q spectrometer in 5-mm tubes using sample concentrations of 10–15%. Spectra of the N-protected compounds were run in pyridine-d_5 (chemical shift values were used for assignments but are not included here) and for the end products only diagnostically important signals are given. Shifts were measured in ppm relative to external DMSO set at 40.1 ppm vs TMS. Fast atom bombardment (FAB) spectra were recorded on a VG-70 SEQ and on a KRATOS concept IS mass spectrometer at 8 kV accelerating voltage. An Ion Tech saddle-field atom gun was used with Xe gas at 8 kV voltage and 1.0 mA current. The compounds were dissolved in thioglycerol or in thioglycerol acidified with methanesulfonic acid.

1,3,2′,6′,3″-Penta-*N*-BOC-kanamycin B (BOC-1). $(BOC)_2O$ (41.6 g, 185 mmol) was added to a stirred solution of kanamycin B free base (1; 13.5 g, 28 mmol) in H_2O–DMSO (1:6) (200 mL) and heated at 60 °C for 6 h. The reaction mixture was cooled, and the excess reagent was decomposed and BOC-1 precipitated by addition of 33% aqueous ammonia (35 mL). The precipitate was isolated, washed with water, and dried. The crude compound was stirred in toluene (150 mL) and anhydrous ether (400 mL) was added. The gel-like suspension was filtered and the filtrate containing less polar *O*-BOC contaminants was discarded. The precipitate was washed with ether and dried to yield 24.8 g (90%) of BOC-1: ^{13}C NMR; R_f (system A) 0.33.

6″-*O*-[(2,4,6-Triisopropylphenyl)sulfonyl]-1,3,2′,6′,3″-penta-*N*-BOC-kanamycin B (BOC-2). BOC-1 (9.84 g, 10 mmol), carefully dried by coevaporation with pyridine, was reacted with 2,4,6-triisopropylbenzenesulfonyl chloride (15.14 g, 50 mmol) in anhydrous pyridine (120 mL) for 70 h at room temperature. The reaction mixture was poured into 5% aqueous $NaHCO_3$ (150 mL), stirred for 30 min and evaporated to dryness. Crude BOC-2 was extracted by treatment of the residue with $CHCl_3$ (2 × 75 mL) and purified by column chromatography (eluent: $CHCl_3$–MeOH (98:2)), yielding 8.125 g (70%) of BOC-2: ^{13}C NMR; R_f (system A) 0.61.

6″-Deoxy-1,3,2′,6′,3″-penta-*N*-BOC-kanamycin B (BOC-3). BOC-1 (21 g, 21.34 mmol), dried by coevaporation with anhydrous pyridine under reduced pressure was dissolved in anhydrous pyridine and heated (50 °C) in the presence of monomethoxytrityl chloride (10.53 g, 34.11 mmol). After 12–24 h, Ac_2O (26.15 g, 256.1 mmol) was added, the reaction mixture was heated for 15 h at

and Gram-Negative Organisms and against Two Strains of Kanamycin-Resistant Gram-Negative Organisms

amido[g]										alkylthio[g]							alkoxy[g]			
16[g]	17[g]	18[g]	19[g]	20[g]	21[g]	22[g]	23[g]	24[g]	25[g]	27[g]	28[g]	29[g]	30[g]	31[g]	32[g]	33[g]	34[g]	35[g]	36[g]	37[g]
2	4	4	4	2	4	16	64	16	>64	0.5	1	1	1	4	0.5	1	0.5	0.5	1	2
1	1	1	1	1	2	8	16	2	>64	0.5	0.5	0.25	0.5	0.5	0.5	1	0.5	0.25	1	1
16	64	32	32	16	32	64	>64	64	>64	2	2	4	8	8	4	8	2	2	8	4
3.2	6.3	5.0	5.0	3.2	6.3	20.2	50.8	12.7	64	0.8	1.0	1.0	1.6	2.5	1.0	2.0	0.8	0.6	2.0	2.0
4	8	4	2	8	32	64	64	32	>64	1	2	2	8	8	2	8	1	1	2	2
4	8	4	8	8	32	64	64	32	>64	1	2	4	8	8	2	4	1	0.5	2	2
1	4	4	0.5	0.5	1	4	8	1	32	0.25	0.5	0.25	0.5	1	0.5	0.5	0.25	0.25	0.5	0.5
2	4	4	4	4	8	32	64	16	>64	0.5	2	1	2	4	2	4	1	0.5	2	2
4	8	8	8	4	8	32	>64	16	>64	1	2	1	4	8	0.5	2	0.5	0.5	2	4
32	>64	64	>64	64	>64	>64	>64	64	>64	8	32	>64	>64	>64	16	32	2	2	8	32
2	2	2	4	4	8	32	>64	32	>64	1	2	2	8	8	1	2	0.5	0.25	2	2
16	16	16	16	32	64	>64	>64	64	>64	4	4	8	16	16	8	16	2	2	8	8
4.4	8.7	6.7	6.2	6.7	16.0	41.5	>64	22.6	>64	1.2	2.6	2.8	6.7	8.7	2.0	4.4	0.8	0.6	2.4	3.1
64	>64	>64	>64	>64	>64	>64	>64	>64	>64	16	16	8	16	16	64	64	32	32	32	16
>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64

Substituents: **1**, OH; **3**, H; **5**, Br; **6**, Cl; **7**, N_3; **8**, NH_2; **9**, NHMe; **10**, NHBu; **11**, NMe_2; **12**, NEt_2; **13**, pyrrolidinyl; **14**, NHCOOMe; **15**, NHCOMe; **16**, NHCOEt; **17**, NHCOPr; **18**, $NHCOCHMe_2$; **19**, $NHCOCMe_3$; **20**, NMeCHO; **21**, NMeCOMe; **22**, NMeCOEt; **23**, NMeCOPr; **24**, NBuCOMe; **25**, $NBuCOC_5H_{11}$; **27**, SMe; **28**, SEt; **29**, SPr; **30**, $SCHMe_2$; **31**, SBu; **32**, S(O)Me; **33**, SO_2Me; **34**, OMe; **35**, OEt; **36**, $OCHMe_2$; **37**, OPhe.

50 °C, and a saturated aqueous solution, containing about 34 mmol of $NaHCO_3$, was added. After stirring for 3 h at room temperature, the reaction mixture was concentrated under reduced pressure to one-third of its original volume and added dropwise and under vigorous stirring to 1 L of ice water. The precipitate was isolated, dissolved in $CHCl_3$ (500 mL), and covered with water (400 mL). The stirred mixture was adjusted to pH 2.5 by addition of HCl; the organic layer was separated, washed with water, dried, and evaporated. The residue was triturated with petroleum ether, yielding about 26 g of crude 3′,4′,2″,4″-tetra-*O*-acetyl-6″-*O*-(monomethoxytrityl)-BOC-1 as an amorphous powder. The compound which is still contaminated with (monomethoxytrityl)-carbinol was used as such in the detritylation step. It can be purified by chromatography using $CHCl_3$–MeOH (99.5:0.5) as eluent. ^{13}C NMR; R_f (system A) 0.80.

A 2.4 M solution of $BF_3(MeOH)_2$ in MeOH (2.63 mL, 6.3 mmol) was added to 3′,4′,2″,4″-tetra-*O*-acetyl-6″-*O*-(monomethoxytrityl)-BOC-1 (15.0 g, 10.5 mmol) in anhydrous $CHCl_3$ (400 mL). The reaction mixture was kept for 1 min at room temperature, rapidly washed with ice–water until neutral, dried, and evaporated. The residue was dissolved in a minimal amount of anhydrous ether and precipitated by addition of an excess of petroleum ether, yielding 9.70 g (80%) of 3′,4′,2″,4″-tetra-*O*-acetyl-BOC-1: ^{13}C NMR; R_f (system A) 0.70.

When the detritylation was performed on crude 3′,4′,2″,4″-tetra-*O*-acetyl-6″-*O*-(monomethoxytrityl)-BOC-1 the reaction mixture was purified by chromatography using a stepwise gradient ($CHCl_3$ to $CHCl_3$–MeOH (98:2)) as eluent. The overall yield of 3′,4′,2″,4″-tetra-*O*-acetyl-BOC-1 (based on BOC-1) was 51%.

3′,4′,2″,4″-Tetra-*O*-acetyl-BOC-1 (4.61 g, 4 mmol) was further reacted with methyltriphenoxyphosphonium iodide (5.65 g, 12 mmol) in anhydrous DMF (60 mL) for 3 h. MeOH (10 mL) was added and the reaction mixture was evaporated. The residue was taken up in $CHCl_3$, washed (5% aqueous $NaHCO_3$, 10% $Na_2S_2O_3$ solution, and H_2O), dried, and evaporated. Chromatographic purification (eluent: 20–40% EtOAc in $CHCl_3$) yielded 4.0 g (80%) of 3′,4′,2″,4″-tetra-*O*-acetyl-BOC-4: ^{13}C NMR; R_f (system A) 0.86. A solution of this acetylated iodo derivative (3.53 g, 2.8 mmol), Bu_3SnH (2.35 mL, 8.4 mmol), and azobisisobutyronitrile (70 mg) in toluene (180 mL) was heated (80 °C) until complete conversion of the starting material. The mixture was evaporated to dryness and the residue was purified by column chromatography using a stepwise gradient (20–40% EtOAc in $CHCl_3$) as eluent. Yield of tetra-*O*-acetyl-BOC-3: 2.96 g (93%); R_f (system A) 0.79. Tetra-*O*-acetyl-BOC-3 (2.5 g, 2.2 mmol) in anhydrous MeOH (75 mL) containing MeONa (6.16 mmol) was kept for 1 h at 0 °C. The solution was neutralized with AG 50W-X8 (H^+, 100–200 mesh), filtered, and evaporated to dryness, yielding 1.8 g (86%) of BOC-3: ^{13}C NMR; R_f (system A) 0.44.

General Procedure for the Preparation of the Halogeno or Azido Analogues BOC-4 to BOC-7. NaI, LiBr, LiCl, or LiN_3 (5 mmol) and BOC-2 (1 mmol) were heated (90 °C) in DMF (50 mL) until complete conversion of the starting material. The solvent was evaporated under reduced pressure, the residue was taken up in $CHCl_3$, and the suspension was washed with water. The organic layer was dried and evaporated and the residue was purified by column chromatography (eluent: $CHCl_3$–MeOH (98:2)). The identity of the *N*-BOC-protected derivatives was checked by ^{13}C NMR, and for each compound, yield and TLC (system A) R_f values were, respectively, BOC-4, 83%, R_f 0.52; BOC-5, 72%, R_f 0.50; BOC-6, 75%, R_f 0.50; BOC-7, 80%, R_f 0.47; IR (KBr) ν_{max} 2140 cm^{-1} (N_3).

General Procedure for the Preparation of the Amine or Alkylamine Analogues BOC-8–BOC-13. A solution of BOC-2 (1 mmol) in EtOH (20 mL) containing a large excess of ammonia or of the appropriate amine was refluxed for 15 h (BOC-10, BOC-12, BOC-13) or (according to the boiling point of the amine) heated (60 °C) for 24 h (BOC-8, BOC-9, BOC-11) in a stainless steel pressure bottle. The reaction mixture was evaporated to dryness, and the residue dissolved in EtOAc (30 mL), washed (2 N NaOH and water), dried, and evaporated. The residue was purified by column chromatography (eluent: $CHCl_3$–MeOH (97:3)). The identity of the *N*-BOC-protected derivatives was checked by ^{13}C NMR, and for each compound, yield and TLC (system A) R_f value were, respectively, BOC-8, 89%, R_f 0.15; BOC-9, 81%, R_f 0.18; BOC-10, 76%, R_f 0.41; BOC-11, 65%, R_f 0.45; BOC-12, 69%, R_f 0.47; BOC-13, 70%, R_f 0.47.

General Procedures for N-Acylation BOC-14–BOC-26. Method A. A suspension or solution of the free amine derivative (1 mmol) in EtOH (60 mL) containing the appropriate anhydride (3 mmol) was stirred for 1 h at room temperature. A saturated $NaHCO_3$ solution (8 mL) was added and the solvent was evap-

orated. The residue was taken up in $CHCl_3$ and purified by column chromatography (eluent: $CHCl_3$–MeOH (98:2 or 99:1)).

Method B. A suspension or solution of the free amine derivative (1 mmol) in EtOH (60 mL) containing the suitable acid chloride (1.1 mmol to a 10-fold molar excess for the long chain acid chlorides) and an excess of triethylamine was stirred for 2 h at room temperature. The reaction mixture was worked up as in method A.

Method C. A solution of the free amine derivative (1 mmol) in dioxane (30 mL) containing *p*-nitrophenyl formate (500 mg, 3 mmol) was kept at room temperature for 1 h. The solvent was evaporated and the residue was purified as in method A.

The identity of the *N*-BOC-protected derivatives was checked by ^{13}C NMR, and for each compound, the method, yield, and TLC (system A) R_f value were, respectively, BOC-**14**, B, 88%, R_f 0.47; BOC-**15**, A, 80%, R_f 0.42; BOC-**16**, A, 76%, R_f 0.42; BOC-**17**, A, 88%, R_f 0.47; BOC-**18**, A, 93%, R_f 0.49; BOC-**19**, A, 80%, R_f 0.53; BOC-**20**, C, 61%, R_f 0.48; BOC-**21**, A, 67%, R_f 0.48; BOC-**22**, A, 77%, R_f 0.55; BOC-**23**, A, 80%, R_f 0.58; BOC-**24**, A, 80%, R_f 0.56; BOC-**25**, B, 82%, R_f 0.62; BOC-**26**, B, 74%, R_f 0.63.

General Procedure for the Preparation of the Alkylthio Derivatives BOC-27–BOC-31. Freshly prepared and dried potassium alkanethiolate (2 mmol) was added to a solution of BOC-**2** (1.25 g, 1 mmol) in anhydrous DMF (50 mL). The reaction mixture was kept at room temperature for 6 h under a nitrogen atmosphere and the reaction was quenched with HOAc. After neutralization with sodium bicarbonate and evaporation under reduced pressure, the residue was suspended in $CHCl_3$, freed from insoluble material, and purified by column chromatography using a stepwise gradient (from 40 to 60% EtOAc in $CHCl_3$) as eluent. The identity of the *N*-BOC-protected derivatives was checked by ^{13}C NMR, and for each compound, yield and TLC (system B) R_f value were, respectively, BOC-**27**, 50%, R_f 0.6; BOC-**28**, 69%, R_f 0.6; BOC-**29**, 51%, R_f 0.63; BOC-**30**, 41%, R_f 0.63; BOC-**31**, 55%, R_f 0.65.

6″-Deoxy-6″-(methylsulfinyl)-1,3,2′,6′,3″-penta-*N*-BOC-kanamycin B (BOC-32). A solution of BOC-**27** (1.02 g, 1 mmol) in $CHCl_3$ (50 mL) containing *m*-chloroperbenzoic acid (1.1 mmol) was kept at room temperature for 4 h. The solvent was removed; the residue was washed with saturated $NaHCO_3$ and extracted with $CHCl_3$. After drying and evaporation of the solvent, the residue was purified by column chromatography (eluent: $CHCl_3$–MeOH (99:1)), affording 0.82 g (80%) of BOC-**32**: ^{13}C NMR; R_f (system B) 0.2.

6″-Deoxy-6″-(methylsulfonyl)-1,3,2′,6′,3″-penta-*N*-BOC-kanamycin B (BOC-33). BOC-**33** was obtained by oxidation of BOC-**27** as described in the previous section but with a 1:5 molar ratio BOC-**27**/*m*-chloroperbenzoic acid, yielding 84% of BOC-**33**: ^{13}C NMR; R_f (system B) 0.35.

General Procedure for the Preparation of the Alkoxy Analogues BOC-34–BOC-36. A solution of the 6″-iodo derivative BOC-**4** (3.0 g, 2.8 mmol) in the appropriate anhydrous alcohol (60 mL) was refluxed for 5 h in the presence of silver trifluoroacetate (1.8 g, 8.4 mmol). The reaction mixture was evaporated to dryness, and the residue taken up in $CHCl_3$ (100 mL), filtered, and purified by column chromatography (eluent: CH_2Cl_2–EtOAc (1:1)). The identity of the protected derivatives was checked by ^{13}C NMR, and for each compound, yield and TLC (system B) R_f value were, respectively, BOC-**34**, 25%, R_f 0.3; BOC-**35**, 13%, R_f 0.4; BOC-**36**, 10%, R_f 0.45.

6″-*O*-Phenyl-1,3,2′,6′,3″-penta-*N*-BOC-kanamycin B (BOC-37). Anhydrous potassium phenolate (1.31 g, 9.92 mmol) was added to a solution of BOC-**2** (3 g, 2.45 mmol) in DMF (50 mL) and the solution was kept at room temperature for 4 days. The reaction was quenched with HOAc, neutralized with $NaHCO_3$, and evaporated. The residue was taken up in $CHCl_3$, filtered, and purified by column chromatography ($CHCl_3$–EtOAc (1:1)), yielding 800 mg (30%) of the title compound BOC-**37**: ^{13}C NMR; TLC (RP-18, MeOH–H_2O (85:15)) R_f 0.4.

Deprotection of *N*-BOC Derivatives—General Procedure. The penta-*N*-BOC derivative (0.2–0.5 mmol) was dissolved in 99% CF_3COOH (6 mL). The solution was kept at room temperature for 2–3 min. The reagent was removed by evaporation, and the residue was dissolved in water and adjusted to pH 9 with aqueous ammonia. The deprotected kanamycin derivative was charged onto a column of Amberlite CG-50 (100–200 mesh) (30 mL, adjusted to pH 9 with aqueous ammonia). After removal of the ammonium trifluoroacetate salt by washing of the column with water, the product was eluted with 0.1 M aqueous ammonia. The eluate containing the derivative was evaporated and freeze-dried. A 1% solution of the residue in water was adjusted to pH 5.5 with H_2SO_4, filtered (0.8 μm filter), concentrated, and freeze-dried. The solid was then triturated with absolute EtOH, dried over P_2O_5, and exposed to the laboratory atmosphere for 24 h. Free-base content was determined by ^1H NMR by using a procedure described earlier.[29] Yields were almost quantitative for all deprotections in this study. Deprotection was not carried out for the iodo analogue BOC-**4** which is highly unstable under these conditions. Since also the 6″-bromo derivative is not very stable in nonprotonated form, a modified procedure was used for deprotection of BOC-**5**. After treatment with CF_3COOH, the reagent was removed by evaporation under reduced pressure. The residue was taken up in water and the solution was adjusted to pH 7 with basic resin AG 3-X4A. The slurry was charged on a column with the AG 3 resin in the free base form, and the product was eluted with water. During elution, the effluent was cooled in an ice bath and continuously adjusted to pH 5.5 with dilute H_2SO_4. The solution was filtered, concentrated, and freeze-dried.

For each final compound, TLC (system C) R_f value, free base content (% m/m), FAB MS (m/z for the $(M + H)^+$ ion), and ^{13}C NMR chemical shifts for diagnostic signals at pH <1.5 are given below in the following order: 4″, 5″, 6″; α, β, ... of the 6″-X-R group; α, β, ... of the 6″-*N*-acyl group; for compound **32**, values for additional peaks due to diastereoisomerism are included in parentheses.

6″-Deoxykanamycin B (**3**): R_f 0.48; 53.4% free base; FAB m/z 468; ^{13}C NMR 71.8, 70.0, 17.5 ppm.

6″-Deoxy-6″-bromokanamycin B (**5**): R_f 0.47; 56.7% free base; FAB m/z 546; ^{13}C NMR 68.1, 71.6, 33.6 ppm.

6″-Deoxy-6″-chlorokanamycin B (**6**): R_f 0.44; 55.7% free base; FAB m/z 502; ^{13}C NMR 66.8, 71.6, 44.6 ppm.

6″-Deoxy-6″-azidokanamycin B (**7**): R_f 0.55; 57.5% free base; FAB m/z 509; ^{13}C NMR 67.3, 72.5, 51.5 ppm.

6″-Deoxy-6″-aminokanamycin B (**8**): R_f 0.28; 49.1% free base; FAB m/z 483; ^{13}C NMR 68.3, 69.7, 41.2 ppm.

6″-Deoxy-6″-(methylamino)kanamycin B (**9**): R_f 0.38; 57.6% free base; FAB m/z 497; ^{13}C NMR 68.7, 68.0, 50.2; 34.4 (N–R) ppm.

6″-Deoxy-6″-(butylamino)kanamycin B (**10**): R_f 0.71; 57.1% free base; FAB m/z 538; ^{13}C NMR 68.1, 69.0, 49.0; 48.5, 27.8, 19.8, 13.5 (N–R) ppm.

6″-Deoxy-6″-(dimethylamino)kanamycin B (**11**): R_f 0.45; 48.5% free base; FAB m/z 511; ^{13}C NMR 68.2, 68.2, 59.0; 45.8 ($N–R_2$) ppm.

6″-Deoxy-6″-(diethylamino)kanamycin B (**12**): R_f 0.66; 51.9% free base; FAB m/z 539; ^{13}C NMR 69.3, 68.6, 53.3; 48.6, 8.6 ($N–R_2$) ppm.

6″-Deoxy-6″-pyrrolidin-1-ylkanamycin B (**13**): R_f 0.56; 44.9% free base; FAB m/z 537; ^{13}C NMR 69.6, 68.7, 56.6; 55.5, 23.6 ($N–R_2$) ppm.

6″-Deoxy-6″-(methoxycarboxamido)kanamycin B (**14**): R_f 0.65; 60.3% free base; FAB m/z 541; ^{13}C NMR 67.4, 72.2, 41.4; 159.9, 53.3 (*N*-acyl) ppm.

6″-Deoxy-6″-acetamidokanamycin B (**15**): R_f 0.48; 41.2% free base; FAB m/z 525; ^{13}C NMR 67.9, 71.9, 40.5; 174.6, 22.9 (*N*-acyl) ppm.

6″-Deoxy-6″-propionamidokanamycin B (**16**): R_f 0.65; 56.1% free base; FAB m/z 539; ^{13}C NMR 67.5, 71.6, 40.1; 179.3, 29.7, 10.1 (*N*-acyl) ppm.

6″-Deoxy-6″-butyramidokanamycin B (**17**): R_f 0.70; 58.3% free base; FAB m/z 553; ^{13}C NMR 67.5, 71.6, 39.8; 178.4, 38.3, 19.5, 13.5 (*N*-acyl) ppm.

6″-Deoxy-6″-isobutyramidokanamycin B (**18**): R_f 0.67; 59.8% free base; FAB m/z 553; ^{13}C NMR 67.3, 71.5, 39.8; 182.5, 35.6, 19.5, 19.3 (*N*-acyl) ppm.

6″-Deoxy-6″-pivalamidokanamycin B (**19**): R_f 0.75; 63.3% free base; FAB m/z 567; ^{13}C NMR 67.5, 71.6, 40.1; 183.9, 39.2, 27.2 (*N*-acyl) ppm.

6″-Deoxy-6″-*N*-formyl-*N*-(methylamino)kanamycin B (**20**): R_f 0.60; 57.7% free base; FAB m/z 525; ^{13}C NMR 67.6, 70.5, 51.5; 32.0 (N–R); 166.8 (*N*-acyl) ppm.

6″-Deoxy-6″-*N*-acetyl-*N*-(methylamino)kanamycin B (**21**): R_f 0.65; 57.4% free base; FAB *m/z* 539; ^{13}C NMR 68.0, 71.2, 52.6; 35.5 (N–R); 175.9, 21.1 (*N*-acyl) ppm.

6″-Deoxy-6″-*N*-propionyl-*N*-(methylamino)kanamycin B (**22**): R_f 0.71; 59.8% free base; FAB *m/z* 553; ^{13}C NMR 67.9, 71.4, 51.7; 35.4 (N–R); 179.1, 26.7, 9.8 (*N*-acyl) ppm.

6″-Deoxy-6″-*N*-butyryl-*N*-(methylamino)kanamycin B (**23**): R_f 0.78; 56.2% free base; FAB *m/z* 567; ^{13}C NMR 68.0, 71.5, 51.9; 35.4 (N–R); 178.4, 35.3, 19.4, 13.8 (*N*-acyl) ppm.

6″-Deoxy-6″-*N*-acetyl-*N*-(butylamino)kanamycin B (**24**): R_f 0.76; 53.9% free base; FAB *m/z* 581; ^{13}C NMR 68.1, 72.1, 51.4; 47.1, 29.1, 20.0, 13.8 (N–R); 175.4, 21.7 (*N*-acyl) ppm.

6″-Deoxy-6″-*N*-hexanoyl-*N*-(butylamino)kanamycin B (**25**): R_f 0.87; 57.2% free base; FAB *m/z* 637; ^{13}C NMR 68.0, 72.4, 49.2; 47.0, 29.2, 20.0, 13.9 (N–R); 178.2, 33.7, 25.7, 31.4, 22.4, 13.9 (*N*-acyl) ppm.

6″-Deoxy-6″-(methylthio)kanamycin B (**27**): R_f 0.53; 54.3% free base; FAB *m/z* 514; ^{13}C NMR 68.9, 72.9, 35.6; 16.5 (S–R) ppm.

6″-Deoxy-6″-(ethylthio)kanamycin B (**28**): R_f 0.53; 57.8% free base; FAB *m/z* 528; ^{13}C NMR 68.9, 73.1, 32.6; 27.2, 14.8 (S–R) ppm.

6″-Deoxy-6″-(propylthio)kanamycin B (**29**): R_f 0.56; 59.5% free base; FAB *m/z* 542; ^{13}C NMR 68.9, 73.1, 33.0; 35.3, 23.0, 13.3 (S–R) ppm.

6″-Deoxy-6″-(isopropylthio)kanamycin B (**30**): R_f 0.56; 57.4% free base; FAB *m/z* 542; ^{13}C NMR 68.8, 73.1, 31.4; 36.5, 23.2 (S–R) ppm.

6″-Deoxy-6″-(butylthio)kanamycin B (**31**): R_f 0.60; 56.1% free base; FAB *m/z* 556; ^{13}C NMR 68.8, 73.1, 33.1; 32.9, 31.6, 21.9, 13.6 (S–R) ppm.

6″-Deoxy-6″-(methylsulfinyl)kanamycin B (**32**): R_f 0.39; 54.0% free base; FAB *m/z* 530; ^{13}C NMR 69.5 (68.8), 68.5 (67.4), 54.0 (52.6); 38.6 (37.7) (SO–R) ppm.

6″-Deoxy-6″-(methylsulfonyl)kanamycin B (**33**): R_f 0.42; 54.0% free base; FAB *m/z* 546; ^{13}C NMR 68.4, 68.4, 55.2; 42.8 (SO_2–R) ppm.

6″-*O*-Methylkanamycin B (**34**): R_f 0.42; 48.3% free base; FAB *m/z* 498; ^{13}C NMR 66.2, 72.3, 70.9; 59.4 (O–R) ppm.

6″-*O*-Ethylkanamycin B (**35**): R_f 0.51; 52.5% free base; FAB *m/z* 512; ^{13}C NMR 66.3, 72.5, 68.8; 67.9, 14.7 (O–R) ppm.

6″-*O*-Isopropylkanamycin B (**36**): R_f 0.56; 52.8% free base; FAB *m/z* 526; ^{13}C NMR 66.4, 72.6, 66.4; 73.9, 21.7, 21.5 (O–R) ppm.

6″-*O*-Phenylkanamycin B (**37**): R_f 0.58; free base content not determined; FAB *m/z* 560; ^{13}C NMR 66.0, 71.9, 66.8; 158.6, 115.6, 130.5, 122.4 (O–Ar) ppm.

Microbiology. Activity of the various compounds synthesized was assessed by the measurement of the minimum inhibitory concentration (MIC) in agar after 24-h incubation at 37 °C, according to standard techniques,[40] using 2-fold dilutions and with visual inspection of bacterial growth. Plates were seeded with a multipoint inoculator (Danley Tech. A400) at a density of 10^4–10^5 viable bacteria (colony forming units) per spot (21 spots/plate of 10–15 cm diameter). Results are given in mg/L of base, taking into account the free base content of the samples, as determined by ^{1}H NMR spectroscopy. All derivatives were prepared as sulfate salts and dissolved in sterile water. We used Mueller-Hinton Agar supplemented with $CaCl_2$ and $MgCl_2$ (BBL Microbiology Systems, Cockeysville, MD) according to the NCCLS standards.[41] Defined strains were obtained from the American Type Culture Collection, Rockville, MD, except one strain of *S. marcescens* (type 87457; Center for Disease Control, Atlanta, GA) and two strains of *Pseudomonas aeruginosa* (types 10708 and 10701; National Collection of Type Cultures, London, U.K.). All organisms were sensitive to gentamicin (MIC ≤ 1 mg/L), unless stated otherwise.

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Registry No. BOC-1, 64271-30-3; BOC-1, 3′,4′,2″,4″-tetra-*O*-acetyl-6″-*O*-(monomethoxytrityl) derivative, 132179-25-0; BOC-1, 3′,4′,2″,4″-tetra-*O*-acetyl derivative, 132179-26-1; BOC-**2**, 132204-58-1; **3**, 59864-27-6; BOC-**3**, 132179-28-3; BOC-**3**, 3′,4′,2″,4″-tetra-*O*-acetyl derivative, 132179-62-5; BOC-**4**, 132179-29-4; BOC-**4**, 3′,4′,2″,4″-tetra-*O*-acetyl derivative, 132179-27-2; **5**, 132102-37-5; BOC-**5**, 132179-30-7; **6**, 132102-38-6; BOC-**6**, 132179-31-8; **7**, 132102-39-7; BOC-**7**, 132179-32-9; **8**, 132102-40-0; BOC-**8**, 132179-33-0; **9**, 132102-41-1; BOC-**9**, 132179-34-1; **10**, 132102-42-2; BOC-**10**, 132179-35-2; **11**, 132102-43-3; BOC-**11**, 132179-36-3; **12**, 132102-44-4; BOC-**12**, 132179-37-4; **13**, 132102-45-5; BOC-**13**, 132179-38-5; **14**, 132102-46-6; BOC-**14**, 132179-39-6; **15**, 132102-47-7; BOC-**15**, 132179-40-9; **16**, 132102-48-8; BOC-**16**, 132179-41-0; **17**, 132102-49-9; BOC-**17**, 132179-42-1; **18**, 132102-50-2; BOC-**18**, 132179-43-2; **19**, 132102-51-3; BOC-**19**, 132179-44-3; **20**, 132102-52-4; BOC-**20**, 132204-59-2; **21**, 132102-53-5; BOC-**21**, 132179-45-4; **22**, 132102-54-6; BOC-**22**, 132179-46-5; **23**, 132102-55-7; BOC-**23**, 132179-47-6; **24**, 132102-56-8; BOC-**24**, 132179-48-7; **25**, 132102-57-9; BOC-**25**, 132179-49-8; BOC-**26**, 132179-50-1; **27**, 132102-58-0; BOC-**27**, 132179-51-2; **28**, 132102-59-1; BOC-**28**, 132179-52-3; **29**, 132102-60-4; BOC-**29**, 132179-53-4; **30**, 132102-61-5; BOC-**30**, 132179-54-5; **31**, 132102-62-6; BOC-**31**, 132179-55-6; **32**, 132102-63-7; BOC-**32**, 132179-56-7; **33**, 132102-64-8; BOC-**33**, 132179-57-8; **34**, 132102-65-9; BOC-**34**, 132179-58-9; **35**, 132102-66-0; BOC-**35**, 132179-59-0; **36**, 132102-67-1; BOC-**36**, 132179-60-3; **37**, 132102-68-2; BOC-**37**, 132179-61-4.

(40) Barry, A. L. In *The Antimicrobic Susceptibility Test: Principles and Practices*; Kimpton, H., Ed.; Lea & Febiger: London, 1976; pp 76–91.

(41) Moellering, R. C., Jr., In *The Aminoglycosides*; Whelton, A., Neu, H. C., Ed.; Marcel Dekker: New York, 1982; pp 65–95.