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SYNTHETIC ROUTES FROM PAROMAMINE TO THE OCTODIOSE-CONTAINING PSEUDODISACCHARIDE PRESENT IN (OXY)APRAMYCIN*,†

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ABSTRACT

The synthesis of a 4-*O*-(2-amino-2-deoxyoctodiosyl)-2-deoxystreptamine, the first synthetic analog of the unusual pseudodisaccharide present in (oxy)apramycin was accomplished, starting from paromamine, by a two-carbon chain-elongation and a stereoselective *cis*-hydroxylation of the resulting *E*-unsaturated octuronate to give either ethyl [1-*N*-5,6-di-*O*-benzoyl-2-deoxy-1,3-di-*N*-*p*-tolylsulfonylstreptamin-4-yl) 3,4-di-*O*-benzoyl-2-deoxy-2-*p*-tolylsulfonamido-D-*threo*-α-D-*gluco*- (**12**) or -L-*threo*-α-D-*gluco*-octo-1,5-pyranosid] uronate. Methoxide-mediated deacylation of **12** afforded in one step a bicyclic, *trans*-decalone-like urono-8′,4′-lactone (**14**) that was highly sensitive to acid-catalyzed alcoholysis and had properties in sharp contrast to those of D-glucurono-6,3-lactone. Partial reduction of lactone **14** or of the corresponding methyl octuronate **12** with lithium aluminum hydride at low temperature gave the expected α-D-*threo*-D-*gluco*-octodialdo-1,5-pyranoside-8,4-pyranose, which was methanolyzed and *N*-detosylated to afford the free pseudodisaccharide 1-(2-deoxystreptamin-4-yl) 8-methyl (8*R*,*S*)-2-amino-2-deoxy-α-D-*threo*-D-*gluco*-octodialdo-1,5:8,4-dipyranodioside. All of the octodiose derivatives were found to adopt a rigid, dipyranoid structure.

INTRODUCTION

Apramycin[2] (**1**) (formerly called[3] nebramycin factor 2) and oxyapramycin[4] (**2**) (nebramycin factor 7) are unique aminocyclitol antibiotics produced by a strain of *Streptomyces tenebrarius*[5]. Both of these aminoglycosides have been shown[4,6] to contain an unusual, higher-carbon amino sugar, namely an aminooctodiose which adopts a *trans*-decalin-like, dipyranoid structure, and a nonreducing disaccharide linkage. As a probable consequence of its unique structure, apramycin exhibits a much stronger antimicrobial activity than other 4-*O*-(2-amino-2-deoxyglycosyl)-2-deoxystreptamines, such as neamine and its structural analogs[7], and was shown to

*Dedicated to Professor Raymond U. Lemieux.
†For a preliminary report of part of this work, see ref. 1.

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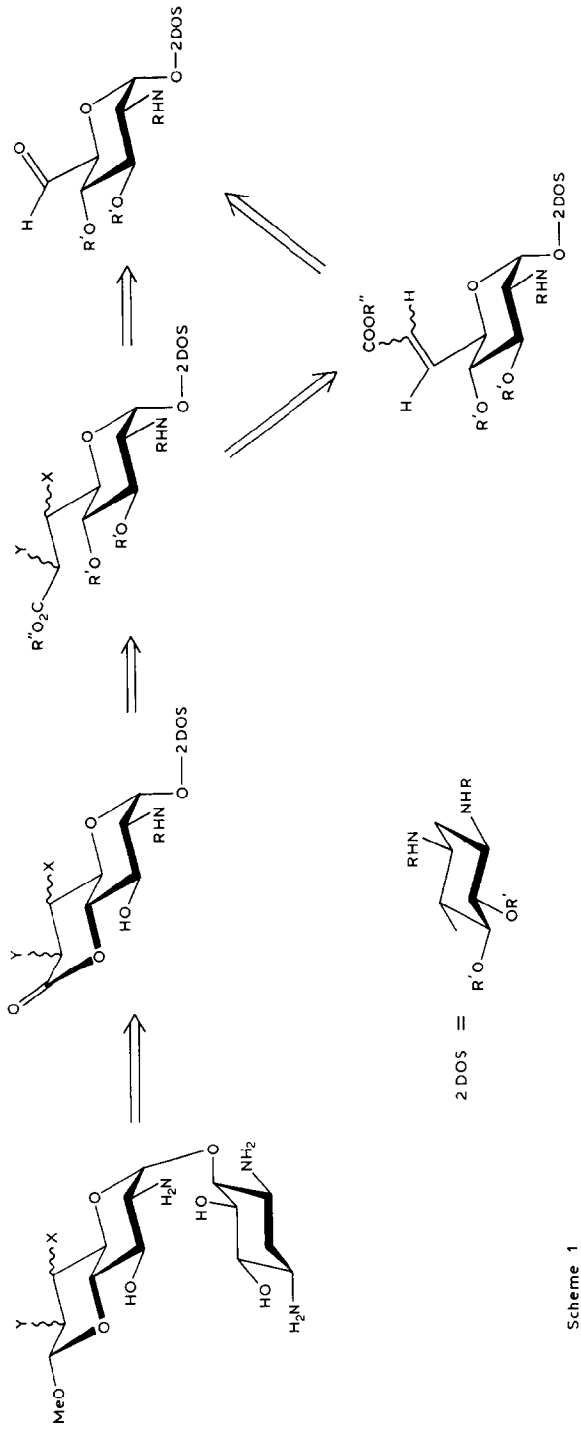


Scheme 1

be a potent inhibitor of protein synthesis in bacteria both *in vivo* and *in vitro*[8]. Furthermore, apramycin is not recognized by most of the known aminoglycoside-inactivating enzymes, the only enzyme as yet reported capable of modifying it being a 3-*N*-acetyltransferase[9].

Interestingly, replacement of the 4-amino-4-deoxy-D-glucosyl group in apramycin with a methyl group yields a compound having a very similar antimicrobial spectrum[7]. In view of this result, if was felt that synthetic pseudodisaccharides containing an aminooctodiose may prove to exhibit interesting biological and pharmacological properties, and we have undertaken investigations toward the preparation of apramycin-related pseudodisaccharides. The first examples of model octodioses existing as rigid dipyranoses have already been reported by this laboratory[10]. The preparation of a 4-*O*-(2-amino-2-deoxyoctodiosyl)-2-deoxystreptamine has now been achieved, and we describe herein the synthesis and some properties of the novel octuronic acid and octodiose derivatives.

Starting from paromamine (**3**), which contains the key glycosidic linkage between 2-deoxystreptamine and the aminoglycosyl residue, our general strategy for the elaboration of the bicyclic ring system (see Scheme 1) involved a two-carbon chain-elongation to an octuronic acid derivative with simultaneous (aldol-type chain-extension) or subsequent (by way of an unsaturated intermediate) functionalization of positions 6′ and 7′, lactonization to a bicyclic urono-8′,4′-lactone, and reduction of the lactone to an octodialdose derivative.

RESULTS AND DISCUSSION

Preparation of precursors. — The paromamine-derived precursor required for the chain-elongation reactions should possess *N*-protecting groups that are compatible with a hydride reducing agent, a 6′-aldehydo function, and easily removable *O*-protecting groups. Accordingly, paromamine (**3**) was selectively *N*-tosylated in excellent yield under conditions (*p*-toluenesulfonyl chloride, water–1,4-dioxane, sodium carbonate) similar to those described by Miyake *et al.*[11] for the preparation of penta-*N*-tosylkanamycin B. Selective silylation of the primary hydroxyl group of tri-*N*-tosylparomamine (**4**) with *tert*-butyldimethylsilyl chloride in *N,N*-dimethylformamide, in the presence of 4-dimethylaminopyridine (or imidazole–4-dimethylaminopyridine), afforded **5** which was exhaustively benzoylated with benzoyl chloride in pyridine to give **6**. Although benzenesulfonamide is known[12] to undergo acylation under these conditions, benzoylation of the relatively hindered *p*-toluenesulfonamido substituents of **5** was not expected to occur. However, as indicated by the analytical data, compound **6** contains, in addition to four benzoic esters, one *N*-benzoyl group. Examination of the ^{1}H-n.m.r. spectra of **6** and of the following compounds of the series revealed the presence of only two D_2O-exchangeable NH signals coupled with H-3 and -2′, respectively (for an example, see Fig. 1); this evidence together with that of the chemical-shift difference between H-1 (δ ~4.5) and H-3 (δ ~3.7) established definitely that **5** had undergone

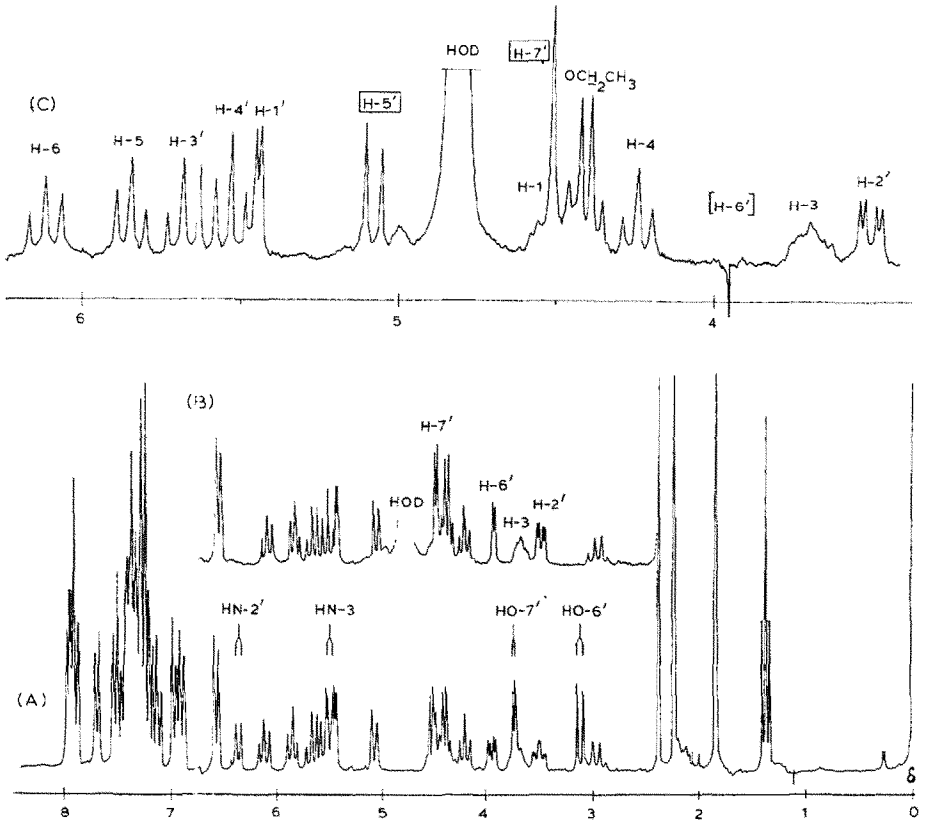
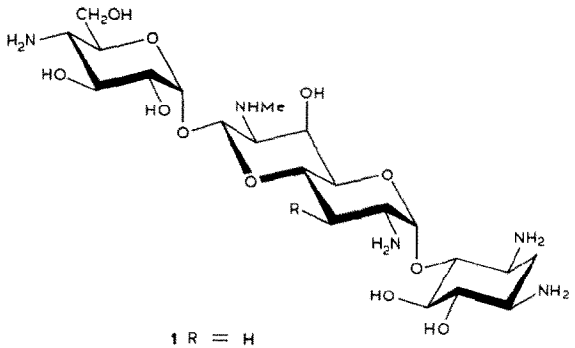


Fig. 1. ^{1}H-n.m.r. spectrum (chloroform-*d*) at 200 MHz of compound **12**: (A) normal; (B) after exchange with deuterium oxide; and (C) after exchange with deuterium oxide, expansion with irradiation of H-6'.



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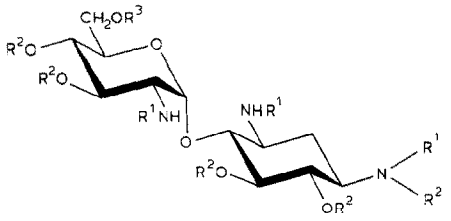
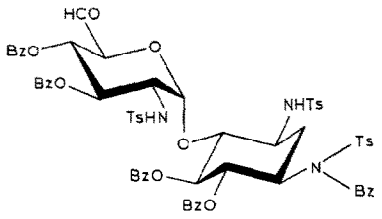
2 R = OH



3 $R^1 = R^2 = R^3 = H$
4 $R^1 = Ts, R^2 = R^3 = H$
5 $R^1 = Ts, R^2 = H, R^3 = SiMe_2{}^tBu$
6 $R^1 = Ts, R^2 = Bz, R^3 = SiMe_2{}^tBu$
7 $R^1 = Ts, R^2 = Bz, R^3 = H$
8 $R^1 = Ts, R^2 = Bz, R^3 = CH_2SMe$



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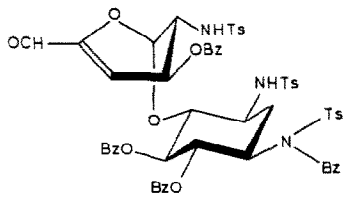

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a regiospecific benzoylation at N-1, undoubtedly the most accessible of the three *p*-toluenesulfonamido groups. The silyl group of **6** was then cleaved under mildly acidic conditions (acetic acid–oxolane–water, 90°), and the suitably protected paromamine derivative **7** was obtained in a 62% overall yield from paromamine (**3**).

Owing to the lability of 6-aldehydo derivatives of D-glucopyranoses having a good leaving group at C-4, oxidation procedures involving a base such as, for example, Swern's oxidation, cannot be employed, the reaction leading mainly to the corresponding α,β-unsaturated aldehydes (for example, see refs. 13, 14). However, the expected carbonyl compounds have been obtained in several instances by use of a dimethyl sulfoxide-based process under neutral[15] or acidic[16] conditions, and a partially acetylated pseudodisaccharide has been successfully converted into its 6′-aldehydo derivative by the dimethyl sulfoxide-*N,N*′-dicyclohexylcarbodiimide reagent in the presence of pyridinium trifluoroacetate in benzene[17]. These conditions were found to oxidize **7** efficiently to the key aldehydo intermediate **9** in over 85% yield, with only a small amount (5–10%) of the 6′-*O*-methylthiomethyl ether **8** being formed. Compound **9** was used without further purification for the subsequent steps. As expected, treatment of **9** with triethylamine in dichloromethane afforded almost instantaneously the corresponding α,β-unsaturated aldehyde **10** (71% from **7**, isolated yield). Amongst several other attempted oxidations of **7**, the dimethyl sulfoxide–phosphorus pentaoxide system (1.2 equiv. of P_4O_{10}, 5 h, 55°) was found to give **9** in acceptable yield (40%, isolated yield of **11**); with dimethyl sulfoxide and acetic anhydride, the reaction led to a mixture of **8** (13%) and **10** (>70%), and the methylthiomethyl ether **8** was the sole product of the reaction of **7** with dimethyl sulfoxide and thionyl chloride[18].

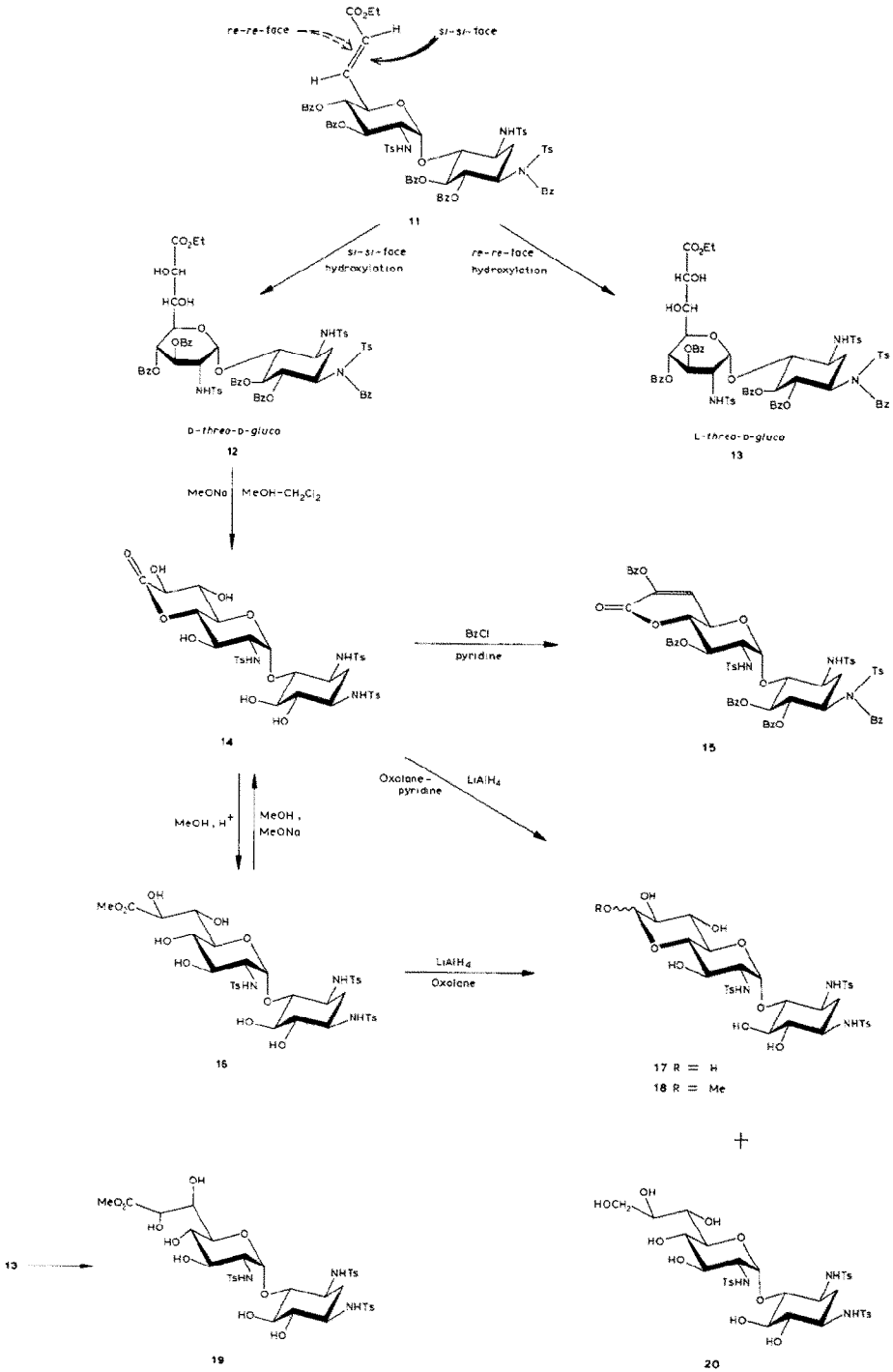
Preparation of octuronic acid derivatives. — With the aim of preparing a 7′-hydroxy analog of the pseudodisaccharide present in oxyapramycin, and of deter-

mining the feasibility of the subsequent steps of the scheme, compound **9** was treated by a sequence of a Wittig reaction and *cis*-hydroxylation. Thus, treatment of **9** with (ethoxycarbonylmethylene)triphenylphosphorane afforded exclusively the corresponding *E*-α,β-unsaturated octuronic ester **11** in 80–85% yield. *cis*-Hydroxylation of **11** was achieved by use of either pure osmium tetraoxide in pyridine (and then aqueous sodium hydrogensulfite to decompose the intermediate osmic esters; yield 80–90%) or osmium tetraoxide as a catalyst and *tert*-butyl hydroperoxide as the main oxidant under the conditions recently described by Akashi *et al.*[19] (yield 50–60%). Interestingly, the stereochemical outcome of the hydroxylation is strongly affected by the conditions; thus, the ethyl D-*threo*-D-*gluco* octuronate **12** resulting from an addition of the reagent to the *si-si* face of the double bond of **11** was the major isomer (3:1) of the noncatalytic reaction, whereas the L-*threo*-D-*gluco* isomer **13** was formed preponderantly (17:3) under the catalytic conditions.

The different reaction conditions and mechanisms, the different nature of the reactive species in the two hydroxylation techniques, as well as the relative freedom of rotation which seems to exist, as judged on the basis of the $J_{5',6'}$ value (5.9 Hz) around the C-5′–C-6′ bond of the starting unsaturated ester, make the observed change in diastereoselection very difficult to rationalize*; however, these results provide a useful way to prepare selectively one or the other of the epimeric ethyl octuronates, **12** or **13**. These compounds, as well as their debenzoylated compounds **16** and **19**, respectively exhibit some interesting structural features that will be discussed later.

Ethyl octuronate **12** was found to be ideally suited for a base-catalyzed debenzoylation and simultaneous lactonization to an octurono-8′,4′-lactone having a *trans*-decalin-like structure. Thus, treatment with sodium methoxide in methanol–dichloromethane gave a quantitative yield of a very polar product, namely **14** (R_F 0.05, solvent *K*), which could be isolated provided that a base was present throughout the processing (for example, by addition of 0.25 equiv. of sodium acetate to the reaction mixture immediately after its neutralization with an ion-exchange resin). The infrared spectrum of **14** shows the expected carbonyl absorption (ν_{max} 1735 cm^{-1}), and its ^1H- and ^{13}C-n.m.r. spectra were consistent with a fully debenzoylated (including the N-1 position), lactonized structure (absence of a signal for an alkoxy group), although a detailed analysis of its complex ^1H-n.m.r. spectrum was not possible. The presence of a base during the isolation process was necessary because of the extreme tendency of **14** to undergo alcoholysis; indeed, treatment of **14** in methanol containing a trace of an acidic catalyst gave rapidly the corresponding methyl octuronate **16**. The "hydroxy-ester" constitution of **16**, a compound that unexpectedly behaved as a compound less-polar than **14** in t.l.c. (R_F 0.50), was estab-

*Upon completion of the present study, it was reported[20] that the stereochemistry of the osmium tetraoxide *cis*-hydroxylation of allylic alcohols and derivatives is quasi-independent of the conditions (stoichiometric, or catalytic using *N*-methylmorpholine *N*-oxide).


re-re-face
si-si-face
11
si-si-face hydroxylation
re-re-face hydroxylation
D-threo-D-gluco
12
L-threo-D-gluco
13
MeONa
MeOH–CH2Cl2
14
BzCl
pyridine
15
Oxolane–pyridine
LiAlH4
MeOH, H+
MeOH, MeONa
16
17 R = H
18 R = Me
19
20

lished, in particular, by the presence of a signal attributable to a methoxycarbonyl group in its ^{1}H- and ^{13}C-n.m.r. spectra, and by the chemical shift of H-7′ (δ 4.44; δ H-7′ <3.80 for **14**; the deshielding effect of the carbonyl group on the α-hydrogen atom is weaker in lactones than in open-chain esters; see, for example, the values of δ H-2 for model aldono-1,5-lactones[21]). The chemical shift of the ester carbonyl carbon atom of **16** (δ 173.35, Me_2SO-d_6) appeared in the expected range for a saturated ester [for example[22], δ(^{13}CO) 174.2 for ethyl 5-hydroxyvalerate in $CDCl_3$]; however, as a consequence of the unusually large ^{13}C-chemical shift of the lactone carbonyl carbon atom of **14** [δ 175.78, Me_2SO-d_6; δ (^{13}CO) 171.8 for D-glucono-1,5-lactone in Me_2SO-d_6, and 171.2 for δ-valerolactone in $CDCl_3$ (ref. 22)], lactonization of **16** resulted in an increase of the chemical shift of the carbonyl carbon atom, an effect opposite to that normally observed upon 1,5-lactonization. Further tests confirmed the constitution of **14** and **16**; thus, treatment of **14** with three different alcohols (methanol, ethanol, or 2-propanol), each containing 1% of *p*-toluenesulfonic acid monohydrate, afforded three different esters having R_F-values in the same range [R_F of methyl ester (**16**) 0.50, ethyl ester 0.57, 2-propyl ester 0.61]; furthermore, lactonization of **16** was also found to occur, albeit very slowly, in an aprotic solvent in the presence of a basic (triethylamine) or an acidic (*p*-toluenesulfonic acid monohydrate) catalyst. Finally, lactone **14** was quantitatively regenerated from ester **16** in methanolic sodium methoxide.

Simple aldonolactones are known to undergo, in most cases, ring opening to the corresponding alkyl aldonates in acidic methanol[23,24], or even in neutral methanol[23,24] or ethanol[23–25], whereas the lactone form is favored in higher alcohols, such as 2-propanol, containing an acidic catalyst[23]. Under conditions of basic alcoholysis, sugar lactones are usually cleaved to the alkyl ester; in particular, the well-known D-glucofuranurono-6,3-lactone and its 5-*O*-acetyl-1,2-*O*-isopropylidene derivative have been reported to be esterified completely by alkaline methanol to the corresponding methyl D-gluco-pyranuronate[26] and -furanuronate[27] (the latter result being disputed, however, by Dax and Weidmann[28]). The behavior of lactone **14**, the first example of a bicyclic uronolactone having two fused six-membered rings, was, thus, in sharp contrast with that of simple analogs and of the hexurono-6,3-lactones, and its preferential formation over that of the methyl ester **16** under alkoxide-mediated alcoholysis conditions is worth emphasizing; thus, the *trans*-fused bicyclic system of **14** appears to be thermodynamically more stable than the "hydroxy-ester" structure under this set of conditions.

In an attempt to prepare a derivative suitable for spectral analysis, lactone **14** was benzoylated under standard conditions. The expected perbenzoate of **14** could not be isolated, but only the corresponding enollactone benzoate **15**; this compound arose undoubtedly from the saturated perbenzoate by β-elimination of benzoic acid from C-6′–C-7′, a well-documented reaction of peracylated aldonolactones[29,30]. The structure of compound **15** is supported, in particular, by the presence in its ^{1}H-n m.r. spectrum of a very low-field doublet (δ 6.82, $J_{5',6'}$ 1.5 Hz) characteristic of the vinylic proton of the enollactone–benzoate system (for exam-

ple, see refs. 29, 30); in addition, the small value for $J_{5',6'}$ and the unchanged couplings between H-1′–H-5′ indicated that the *trans*-fused, bicyclic ring system of **15** adopts a half-chair–chair conformation of the type $^4H_5$(L)–$^4C_1$(D). A further argument for the lactonized structure of **15** was given by the chemical shift of H-4′; the signal for this proton appeared, in the spectrum of **15**, at δ 4.42, a much higher field than that of the corresponding 4′-benzoate compounds, such as, for example, compound **12** (δ H-4′, 5.52). Indeed the carbonyl group of the lactone group exerts a much weaker deshielding effect on H-4′ than does a normal acyl group in which the carbonyl group is *syn*-parallel to the *H*COCOR proton[31].

Peracylation of aldonolactones without accompanying β-elimination has been performed under acidic conditions[23,32]. In a test experiment, ethyl octuronate **12** was treated with acetic anhydride containing perchloric acid, a reaction that afforded the corresponding diacetate in good yield; however, these conditions proved unsatisfactory for the acetylation of lactone **14**.

The behavior of **13** under methoxide-mediated debenzoylation conditions was very similar to that of isomer **12**, inasmuch as a new, very polar product (actually a ~3:1 mixture of two extremely-close components) was formed at a rate comparable to that of formation of **14**. However, detailed chemical and spectral analysis of the product revealed, surprisingly, the presence of a carboxylic acid group, as shown, in particular, by the strong carbonyl absorption at 1595 cm^{-1} (CO_2^-) of the product isolated as a base. The bicyclic uronolactone having the L-*threo*-D-*gluco* configuration, which is expected to be less stable than the D-*threo*-D-*gluco* analog because of the two axial hydroxyl groups at C-6′ and C-7′, may well have been formed under these conditions, but seems to have undergone an internal, lactone ring-opening by, for example, formation of a 4′,6′-anhydro ring, leading to a free carboxyl group. This transformation must be easily reversible under acidic conditions, since acidic methanolysis of the debenzoylated product led rapidly to the methyl octuronate **19** as the main compound, the constitution of which was unambiguously established by its ^{1}H-n.m.r. spectrum (H-1′–H-7′ sequence entirely analyzed; δ H-7′, 4.64; δ OMe, 3.79); however, the possibility of an epimerization at C-7′ in **19** has not been eliminated. The slow-moving compound was readily regenerated from **19** upon treatment with methanolic sodium methoxide and,

TABLE I

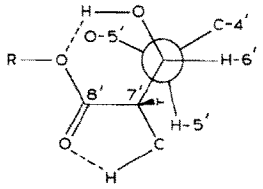
SELECTED ^{1}H-N.M R. PARAMETERS[a] FOR COMPOUNDS **12, 13, 16,** AND **19**

Compound	$J_{5',6'}$	$J_{6',7'}$	$J_{6',OH}$	$J_{7',OH}$	δ *HO-6′*	δ *HO-7′*
12[b]	<0.5	4.8	11.3	6	3.12	3.74
16[c]	1.2	4.0				
13[b]	9.5	<0.5	12.0	4.2	2.89	3.98
19[c]	5.7	1.5				

[a]Chemical shifts (δ) from signal of internal Me_4Si; coupling constants (J) in Hz. [b]In chloroform-*d*. [c]In methanol-d_4.

moreover, gave the same enollactone benzoate **15** upon perbenzoylation with benzoyl chloride in pyridine. There still remains further work to explain the puzzling behavior of compound **13** under debenzoylation conditions and to establish the structure of the final product.

Structural analysis of the octuronic acid derivatives. — The ^{1}H-n.m.r. spectra of the isomeric octuronates **12** (see Fig. 1) and **13**, as well as of the debenzoylated derivatives **16** and **19**, reveal some interesting HC–CH and HC–OH coupling constants in the open-chain portion of the molecule (see Table I). These values seem to indicate that, in all cases, the conformation of the side chain is determined by the existence of intramolecular hydrogen bonding between HO-7′ and the ester-carbonyl group, a well-known interaction in α-hydroxy esters[33], and by a weaker interaction between HO-6′ and the ester-alkoxy group (see Scheme 2); the observa-



Scheme 2 D-*threo*-D-*gluco* Isomers

tion that HO-7′ is involved in a stronger hydrogen-bond than is HO-6′ was further supported by the chemical shifts of the signals of these protons (see Table I). As shown by molecular models, the dihedral angles corresponding to the proposed conformation are $\phi_{H\text{-}6',H\text{-}7'}$ ~70–90, $\phi_{H\text{-}6',HO\text{-}6'}$~180, and $\phi_{H\text{-}7',HO\text{-}7'}$~120°, in good agreement with the observed couplings. Furthermore, the $J_{5',6'}$ values suggested that the conformation around the C-5′–C-6′ bond is governed by steric factors; for the L-*threo*- and D-*threo*-D-*gluco* isomers, the least-hindered conformer is indeed attained when H-5′ and H-6′ are in an antiparallel ($\phi_{H\text{-}5',H\text{-}6'}$ ~180°) and in a *gauche* ($60° < \phi_{H\text{-}5',H\text{-}6'} < 120°$; H-6′ pointing toward O-4′; see Scheme 2) relationship, respectively.

Preparation of octodipyranosides. — Three major, hydride reducing agents may be considered for the critical, partial reduction of uronolactone **14** to the corresponding octodiose derivative, namely, sodium borohydride in aqueous acid[34], disiamylborane[35], and lithium aluminum hydride at low temperature[36,37], all three having been used to convert *free* aldonolactones into the corresponding aldoses. The reaction of **14** with disiamylborane in oxolane was sluggish, even at room temperature, and led to only traces of the expected product **17**. The usefulness of the sodium borohydride system is limited by the poor solubility of **14** in water. A further attempt at reduction of **14** consisted of treating its per(trimethylsilyl) derivative (trimethylsilyl chloride–imidazole in dichloromethane, followed by rapid, aqueous processing in the cold; no change of the basic structure of **14** under these

TABLE II

SELECTED ^{1}H-N.M.R. PARAMETERS[a] OF OCTODIOSES **17** AND **18** AND RELATED COMPOUNDS

Parameter		**17**[b]	**18**[b]	**21**[c,d]	D-*Glucopyranose*[e,f]	
δ H-7′	8*R*	3.50			δ H-2	α 3.53
	8*S*	~3.25				β 3.24
δ H-8′	8*R*	5.09	4.67	4.81	δ H-1	α 5.23
	8*S*	4.45	4.16	4.25		β 4.64
$J_{6',7'}$	8*R*	9.0			$J_{2,3}$	α 9.9
	8*S*					β 9.9
$J_{7',8'}$	8*R*	3.5	3.5	3.3	$J_{1,2}$	α 3.8
	8*S*	7.5	7.8	7.5		β 8.0

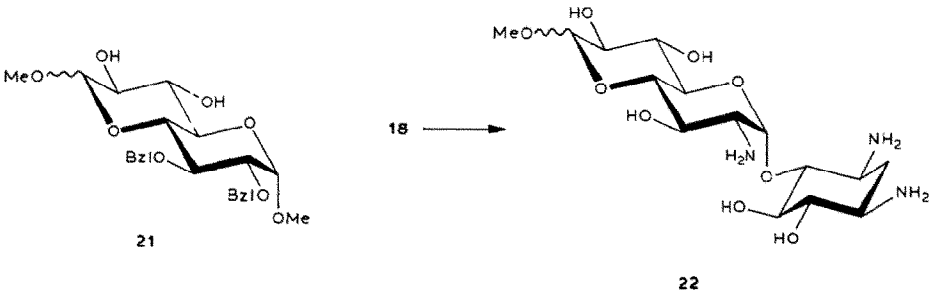
[a]Chemical shifts (δ) from signal of internal Me_4Si; coupling constants (J) in Hz. [b]In methanol-d_4. [c]In chloroform-*d*. [d]Data taken from ref. 10. [e]In deuterium oxide; chemical shifts (δ) from internal signal of 4,4-dimethyl-4-silapentane-1-sulfonic acid. [f]Data taken from ref. 40.

conditions) with diisobutylaluminum hydride in oxolane, a method recently reported[38] for the conversion of D-gulono-1,4-lactone into D-gulose; the reduction was found again to be too slow under these conditions. The best result was obtained with lithium aluminum hydride. The reaction of lactone **14** with this reagent in pyridine–oxolane at low temperature, as described by Němec and Jarý[37], was slow and incomplete, but led to a 1:1 mixture of the expected octodiose **17**, and of the completely reduced product, octose **20**. The presence of pyridine, known to react with lithium aluminum hydride to give a less-reactive reducing agent[39], is not compatible with the relatively low rate of reduction of **14**; starting with methyl octuronate **16**, which is soluble in oxolane alone, the reaction was much faster and led to a 5:4 mixture of **17** and **20** in 1 h at −78°, and in 20 min at −20°. Careful separation of the products by column chromatography afforded reducing octodiose **17** in 34% yield, and octose **20** in 23% yield. Methanolysis of **17** in methanolic hydrogen chloride gave the corresponding octodioside **18** as a 1:1 mixture of the two anomers (at position 8′).

The absence of a carbonyl absorption in the infrared spectrum of **17** indicated that this compound exists only in its *dipyranoid* form, a stable *trans*-decalin-like system having all of the nonanomeric substituents in an equatorial position. The configurations of **17** and **18**, and hence that of octuronate **12**, are definitively established by their ^{1}H-n.m.r. parameters (see Table II). Thus, the chemical shifts of H-7′ and H-8′ of both anomers at position 8′ (8′*R* =pseudo-α, 8′*S* = pseudo-β anomer) and, most important, the values of $J_{6',7'}$ (large, diaxial coupling) and of $J_{7',8'}$ are very close to the corresponding values reported for the anomers of D-glucopyranose[40] and for the model[10] D-*threo*-D-*gluco*-octodipyranosides **21**, and demonstrate that the newly created 8′,4′-pyranose ring has an "L-*gluco*" type of configuration (and not an "L-*altro*"), and hence that the octodiose itself has the D-*threo*-D-*gluco* configuration. It is worth noting that the upfield shifts of H-8′ observed upon glycosidation are entirely consistent with the glycosidation shift effects

on H-1 of D-glucopyranose (for example, δ H-1, 4.83 and 4.41, for methyl α- and β-D-glucopyranosides, respectively[41]).

Cleavage of the *N*-*p*-tolylsulfonyl groups of **18** with sodium in ammonia[11], and purification of the final product by ion-exchange chromatography afforded the free base **22** in good yield; compound **22** is the first example of a synthetic 2-deoxy-



streptamine-based pseudodisaccharide containing an aminooctodiose, and is a close analog of the pseudodisaccharide present in oxyapramycin. Despite the difficulties encountered with the partial reduction of the bicyclic lactone, the proposed procedure for the elaboration of *trans*-fused octodipyranosides proved to be an efficient one.

EXPERIMENTAL

General methods. — Melting points were determined on a Fisher–Johns apparatus and are uncorrected. Optical rotations were measured with a Perkin–Elmer model 141 automatic polarimeter for solutions in a 0.1-dm cell at 26 ±3°. I.r. spectra were recorded with a Perkin–Elmer 598 spectrophotometer. Proton (^{1}H-n.m.r.) and carbon (^{13}C-n.m.r.) nuclear magnetic resonance spectra were recorded with a Bruker CXP-200 spectrometer at 200 and 50.306 MHz, respectively, for solutions in chloroform-*d* with tetramethylsilane (Me_4Si) as the internal standard, unless otherwise stated. Chemical shifts (δ) are given downfield from the signal of Me_4Si. Exchanged samples were recorded at least 24 h after the addition of deuterium oxide. Chemical shifts and coupling constants were obtained from first-order analyses of the n.m.r. spectra. Prime numbers are related to the non-streptamine component.

Solvents were evaporated under reduced pressure and <40°. Analytical thin-layer chromatography (t.l.c.) was performed on precoated glass-plates with Merck silica gel 60F-254 as the adsorbent (layer thickness 0.25 mm). The developed plates were air dried and irradiated with u.v. light or sprayed (or both) with a solution of cerium sulfate (1%) and molybdic acid (1.5%) in 10% aqueous sulfuric acid, and heated at 150°. In specific cases, plates were sprayed with TTC-reagent (4% methanolic 2,3,5-triphenyl-2*H*-tetrazolium chloride and M sodium hydroxide

mixed in equal volumes before use; red spots for reducing compounds), with ninhydrin (0.3 g ninhydrin in 100 mL of 1-butanol and 3 mL acetic acid), or with 2,4-dinitrophenylhydrazine (0.4% in 2M hydrochloric acid), and heated at 100°. Column chromatography was performed on silica gel 60 (70–230 mesh, Merck). The following solvent systems (v/v) were used: (*A*) 6:1 and (*B*) 8:1 chloroform–methanol; (*C*) 10:10:1 toluene–ethyl acetate–methanol; (*D*) 3:1, (*E*) 2:1, (*F*) 1:1, (*G*) 6:1, (*H*) 7:2, and (*J*) 4:1 toluene–ethyl acetate; (*K*) 15:10:9:1 chloroform–ethyl acetate–methanol–water; (*L*) 16:12:3 chloroform–methanol–water; and (*M*) freshly prepared 1:3:2 chloroform–methanol–28% aqueous ammonia.

Materials. — Paromomycin pentahydrochloride was obtained from Warner–Lambert/Parke–Davis Pharmaceutical Research Division. Dry oxolane was distilled from lithium aluminum hydride immediately before use. Pyridine and *N,N*-dimethylformamide were distilled from calcium hydride. Methanol was dried by distillation from magnesium methoxide. The term "petroleum ether" refers to the fraction of b.p. 30–60°. Most of the *N*-*p*-tolylsulfonylated derivatives described in this article are amorphous solids melting over a rather broad range, even after careful purification and recrystallization.

1,3,2′-Tri-N-p-*tolylsulfonylparomamine* (**4**). — A solution of paromamine (**3**) (prepared from paromomycin pentahydrochloride under the conditions described by Umezawa and Koto[42]) (5.52 g, 17.07 mmol) in water (80 mL) was diluted with 1,4-dioxane (160 mL). To the cooled (0°) solution were added sodium carbonate (4.7 g) and then *p*-toluenesulfonyl chloride (10.45 g, 1.07 equiv./NH_2) in small portions. The mixture was stirred for 8 h at 0°, and for 9 h at room temperature. The solvents were removed under reduced pressure and the residue treated with water; the white solid was removed by filtration and carefully washed with cold water. Recrystallization of crude **4** from hot methanol afforded pure **4** (11.54 g, 86%) in three crops, m.p. 231–234°, $[\alpha]_D^{26}$ +25.8° (*c* 1.16, *N,N*-dimethylformamide); t.l.c. (*A*) R_F 0.28; ν_{max}^{KBr} 3460,3275 (OH, NH), 1325 and 1160 (SO_2), 1600, 1450, 1195, 818, 675, 555, and 545 cm^{-1}; ^{1}H-n.m.r. (Me_2SO-d_6): δ 0.94 (q, 1 H, $J_{1,2a} = J_{2a,2e} = J_{2a,3}$ = 12 Hz, H-2*a*), 1.41 (bd, 1 H, H-2*e*), 2.39 (s, 9 H, 3 $ArCH_3$), 2.66–3.84 (several m, methine H's), 4.14 (t, 1 H, *J* ~5 Hz, HO-6′), 4.44, 4.68, 4.86, and 4.89 (4 d, 4 H, H-1′, 3 OH), 5.57 (bs, 1 H, OH), 7.21 (d, 1 H, *J* ~9 Hz, NH), 7.31 (d, 2 H), 7.35 (d, 4 H), 7.56 (d, 2 H), 7.57 (d, 2 H), and 7.77 (d, 2 H) (3 $MeC_6H_4SO_2$); ^{13}C-n.m.r. (Me_2SO-d_6): δ 20.92 ($ArCH_3$), 34.12 (C-2), 52.37 (C-3), 53.29 (C-1), 58.67 (C-2′), 59.89 (C-6′), 69.64 (C-4′), 70.76, 72.60 (C-3′,5′), 73.96 (C-5), 75.85 (C-6), 83.28 (C-4), 99.88 (C-1′), 126.04, 126.28, 126.86 (C_{Ar}-2,2′), 129.14, 129.53 (C_{Ar}-3,3′), 138.56, 138.94, 139.24 (C_{Ar}-4), 141.95, and 142.39 (C_{Ar}-1).

Anal. Calc. for $C_{33}H_{43}N_3O_{13}S_3$ (785.91): C, 50.43; H, 5.52; N, 5.35; S, 12.24. Found: C, 50.12; H, 5.27; N, 5.17; S, 11.97.

6′-O-tert-*Butyldimethylsilyl-1,3,2′-tri*-N-p-*tolylsulfonylparomamine* (**5**). — To a solution of **4** (0.5 g, 0.64 mmol) in dry *N,N*-dimethylformamide (15 mL) were added 4-dimethylaminopyridine (0.4 g) and *tert*-butyldimethylsilyl chloride (142

mg, 0.94 mmol, 1.47 equiv.). After 64 h at room temperature, the solvent was evaporated and the residue filtered through a short column of silica gel (solvent *B*). Crude **5** was then dried under high vacuum to remove a silylated by-product, and purified by column chromatography (solvent *C*); yield 470 mg (81.6%) of pure **5**. Crystallization of **5** was achieved by addition of petroleum ether to a solution of **5** in a small volume of 1:1 chloroform–ether, m.p. 140–145°, $[\alpha]_D^{26}$ +21.4° (*c* 0.93, chloroform); t.l.c. (*A*) R_F 0.51; ν_{max}^{KBr} 3460 (OH), 3250 (NH), 1320 and 1155 (SO_2), 1595, 1440, 1085, 835, 815, 665, and 550 cm^{-1}; ^{1}H-n.m.r. ($CHCl_3$ as internal standard): δ −0.01 (s. 6 H, $SiMe_2$), 0.82 (s, 9 H, CMe_3), 1.06 (q, 1 H, J ~12 Hz, H-2*a*), 1.84 (bd, 1 H, H-2*e*), 2.33, 2.38 and 2.40 (3 s, 9 H, 3 $ArCH_3$), 4.54 (bs, 1 H, H-1′), 7.12, 7.25, 7.31, 7.45, 7.70, and 7.84 (6d, 12 H, 3 $MeC_6H_4SO_2$), other signals not interpretable.

Anal. Calc. for $C_{39}H_{57}N_3O_{13}S_3Si$ (900.16): C, 52.04; H, 6.38; N, 4.67; S, 10.69. Found: C, 52.28; H, 6.52; N, 4.72; S, 10.58.

For the preparation of larger amounts of **5**, an aqueous processing of the reaction mixture was performed. After evaporation of the solvent, the residue was dissolved in chloroform; the organic phase was successively washed with water, cold M aqueous hydrochloric acid, 5% aqueous sodium hydrogencarbonate, and water, dried ($MgSO_4$), and evaporated. The foamy residue was carefully washed with several portions of 1:1 ether–hexane. Crude **5** thus obtained was used without further purification for the next steps.

1-N-*3′,4′,5,6-Tetra*-O-*benzoyl-6′*-O-tert-*butyldimethylsilyl-1,3,2′-tri*-N-*p-tolylsulfonylparomamine* (**6**). — To a cooled (0°) solution of **5** (4.63 g, 5.14 mmol) in dry pyridine (100 mL) was added dropwise benzoyl chloride (14.45 g, 11.9 mL, 20 equiv.). After having been stirred for 2 h at 0° and 70 h at room temperature, the mixture was treated, at 0°, with water (15 mL). After 1 h at low temperature, the mixture was poured onto ice–water (300 g) and ether (200 mL). The separated aqueous layer was extracted with ether (2 × 150 mL), and the combined organic phases were washed with a saturated aqueous sodium hydrogencarbonate solution (60 mL) and then with water (60 mL), dried ($MgSO_4$) and evaporated under reduced pressure. The residue was submitted to short-column chromatography (solvent *D*) which afforded 6.96 g (95%) of crude **6**. Compound **6** was conveniently recrystallized as follows: a sample was dissolved in chloroform, most of the solvent was then evaporated, and the syrupy residue dissolved in ether; compound **6** slowly crystallized from that solution at 4°; yield 6.3 g (86.3%); m.p. 144–146°; $[\alpha]_D^{26}$ −2.4 ±1°, $[\alpha]_{365}^{26}$ −86.2° (*c* 1.28, chloroform); t.l.c. (*D*) R_F 0.51; ν_{max}^{KBr} 3290 (NH), 1730 (C=O), 1600, 1450, 1360, 1275, 1165, 1090, 710, 665, 590, and 550 cm^{-1}; ^{1}H-n.m.r. (after D_2O exchange): δ 0.11 and 0.13 (2 s, 6 H, $SiMe_2$), 0.96 (s, 9 H, CMe_3), 1.89, 2.24 and 2.42 (3 s, 9 H, 3 $ArCH_3$), ~2.2 (m, 1 H, $J_{2,e} \simeq J_{2e,3} \simeq 4$, $J_{2e,2a}$ ~12.5 Hz, H-2*e*), 2.87 (q, 1 H, $J_{1,2a} \simeq J_{2a,3}$ ~12.5 Hz, H-2*a*), 3.58 (dd, 1 H, $J_{1',2'}$ 3.5, $J_{2',3'}$ 10.5 Hz, H-2′), 3.67 (m, 1 H, $J_{3,4}$ 9.5 Hz, H-3), 3.80 (dd, 1 H, $J_{5',6'A}$ 6.5, $J_{6'A,6'B}$ 11.0 Hz, H-6′A), 3.89 (broad d, 1 H, $J_{5',6'B}$ 2.2 Hz, H-6′B), 4.22 (t, 1 H, $J_{4,5}$ 9.5 Hz, H-4), 4.52 (td, 1 H, $J_{1,6}$ 10 Hz, H-1), 4.90 (m, 1 H, $J_{4',5'}$ 9.5 Hz, H-

5'), 5.30 (t, 1 H, $J_{3',4'}$ 9.5 Hz, H-4'), 5.40 (d, 1 H, H-1'), 5.63 (t, 1 H, H-3'), 5.82 (t, 1 H, $J_{5,6}$ 9.5 Hz, H-5), 6.10 (t, 1 H, H-6), 6.61 (d, 2 H), 6.88 (d, 2 H), 6.96 (d, 2 H), 7.07–7.58 (m, 23 H), 7.69 (d, 2 H), and 7.92 (m, 6 H) (3 $MeC_6H_4SO_2$, 5 C_6H_5CO).

Anal. Calc. for $C_{74}H_{77}N_3O_{18}S_3Si$ (1420.71): C, 62.56; H, 5.46; N, 2.96; S, 6.77. Found: C, 62.45; H, 5.49; N, 3.04; S, 6.68.

*1-*N*-3',4',5,6-Tetra-*O*-benzoyl-1,3,2'-tri-*N*-p-tolylsulfonylparomamine* (**7**). — To a solution of **6** (32.2 g, 22.7 mmol) in freshly distilled oxolane (200 mL) were added glacial acetic acid (450 mL) and water (125 mL). The solution was heated at 90–92° until complete disappearance of **6** on t.l.c. (*E*) (3.25 h). The solvents were then removed under reduced pressure and the residue was dissolved in chloroform (350 mL). The organic solution of **7** was washed with a cold (0°), saturated aqueous sodium hydrogencarbonate solution and then with water (2 × 30 mL), dried (Na_2SO_4) and evaporated. The residue was dissolved in a small volume of acetone; ether and then 1:1 ether–petroleum were added to the point of incipient opalescence; compound **7** crystallized from that solution at 4°; yield 23.6 g (80%). The mother liquors were subjected to column chromatography (*E*) and the yield of **7** thus increased to 88%; m.p. 153–156°, $[\alpha]_D^{26}$ −32.8° (*c* 1.04, chloroform); t.l.c. (*E*) R_F 0.35, (*F*) 0.54; ν_{max}^{KBr} 3520, 3320 (OH, NH), 1730, 1695 (C=O), 1600, 1450, 1365, 1275, 1165, 1095, 1030, 712, 665, and 590 cm^{-1}; ^{1}H-n.m.r.: δ 1.89, 2.24 and 2.41 (3 s, 9 H, 3 ArCH_3), 2.00 (dt, 1 H, $J_{2a,2e}$ 12.5, $J_{1,2e} \simeq J_{2e,3}$ ~4 Hz, H-2*e*), 2.86 (q, 1 H, $J_{1,2a} = J_{2a,3}$ = 12.5 Hz, H-2*a*), 3.63 (td, dd after D_2O exchange, 1 H, $J_{1',2'}$ 3.5, $J_{2',3'}$ 10.5 Hz, H-2'), ~3.7 (m, 1 H, $J_{3,4}$ 9.5 Hz, H-3), 3.74 (dd, 1 H, $J_{5',6'A}$ 5.5, $J_{6'A,6'B}$ 12 Hz, H-6'A), 3.89 (bd, 1 H, $J_{5',6'B}$ 2 Hz, H-6'B), 4.22 (t, 1 H, $J_{4,5}$ 9.5 Hz, H-4), 4.50 (td, 1 H, $J_{1,6}$ 10.5 Hz, H-1), 4.91 (m, 1 H, $J_{4',5'}$ 9.5 Hz, H-5'), 5.31 (t, 1 H, $J_{3',4'}$ 9.5 Hz, H-4'), 5.44 [d, 1 H, *J* 8.5 Hz, exchanged in D_2O, HN-3 (or 2')], 5.47 (d, 1 H, H-1'), 5.72 (t, 1 H, H-3'), 5.77 [d, 1H, *J* 8.5 Hz, exchanged in D_2O, HN-2' (or 3)], 5.83 (t, 1 H, $J_{5,6}$ 9.5 Hz, H-5), 6.09 (t, 1 H, H-6), 6.56 (d, 2 H), 6.85 (d, 2 H), 6.95 (d, 2 H), 7.07–7.55) (m, 23 H), 7.68 (d, 2 H), and 7.94 (m, 6 H) (3 $MeC_6H_4SO_2$, 5 C_6H_5CO).

Anal. Calc. for $C_{68}H_{63}N_3O_{18}S_3$ (1306.45): C, 62.52; H, 4.86; N, 3.22; S, 7.36. Found: C, 62.39; H, 4.58; N, 3.37; S, 7.20.

*(1-*N*-5,6-Di-*O*-benzoyl-2-deoxy-1,3-di-*N*-p-tolylsulfonylstreptamin-4-yl) 3,4-di-*O*-benzoyl-2-deoxy-2-p-tolylsulfonamido-α-*D*-gluco-hexodialdo-1,5-pyranoside* (**9**). — To a solution of **7** (200 mg, 0.153 mmol) in 100:10:2:1 (v/v) dry benzene–dry dimethyl sulfoxide–dry pyridine–trifluoroacetic acid (1.5 mL) was added, at room temperature, dicyclohexylcarbodiimide (158 mg), and the mixture was stirred for 1.5 h. Ethyl acetate (3 mL) was then added, and the mixture cooled (0°) and treated with oxalic acid dihydrate (80 mg) in methanol (0.5 mL). After 0.5 h, ice (2 g) was added and the mixture stirred for 0.25 h. The dicyclohexylurea was removed by filtration and the organic phase diluted with benzene (3 mL); the aqueous layer was separated and the organic phase washed with cold water (3 × 2 mL), dried (Na_2SO_4), and evaporated. T.l.c. (*E*) of the remaining, glassy solid showed

an intense, elongated spot (**9**, R_F ~0.23, TTC positive) as well as a trace of 6'-*O*-methylthiomethyl ether **8** (R_F 0.54) and a weak, TTC-positive by-product (R_F 0.49); yield of **9**. ~85%. Larger quantities of **7** (up to 5 g) were oxidized under the same conditions without noticeable diminution of the yield. Compound **9** thus obtained was used without further purification.

1-N-*3',4',5,6-Tetra*-O-*benzoyl-6'*-O-*methylthiomethyl-1,3,2'-tri*-N-p-*tolylsulfonylparomamine* (**8**). — A 4% (v/v) solution of thionyl chloride in dry dichloromethane (0.76 mL, 0.42 mmol) was added to dichloromethane (2 mL) in a small, three-necked reaction vessel equipped with a low-temperature thermometer. To the cooled mixture (−60°, ethanol–Dry Ice) was added dropwise, and with exclusion of moisture, a solution of dimethyl sulfoxide (0.15 mL) in dichloromethane (2 mL), and the mixture was stirred for 0.25 h at low temperature. A solution of **7** (200 mg, 0.153 mmol) in dichloromethane (3 mL) was then added dropwise, and the mixture stirred for 1.25 h at −60°, 1.5 h at −20°, and 1 h at −10°. Triethylamine (0.115 mmol) in dichloromethane (1 mL) was then added at low temperature (−60°), and the mixture allowed to warm to room temperature. The mixture was washed with 0.4M aqueous hydrochloric acid (4 mL), then with water (5 × 3 mL), dried ($MgSO_4$), and concentrated. Column chromatography (*D*) of the residue afforded pure **8** (52 mg, 25%) and unreacted starting material (45 mg, 22%). Crystallization of **8** was obtained by the slow addition of hexane to a solution of **8** in ~1:1 ether–toluene at −10°, m.p. 133.5–138°, $[\alpha]_D^{26}$ 0°, $[\alpha]_{365}^{26}$ −87.3° (*c* 1.23, chloroform); t.l.c. (*E*) R_F 0.54; ν_{max}^{KBr} 3280 (NH), 1728, 1690, (C=O), 1600, 1450, 1360, 1270, 1160, 1090, 710, 660, 588, and 547 cm^{-1}; ^{1}H-n.m.r.: δ 1.89, 2.24 and 2.43 (3 s, 9 H, 3 ArCH_3), ~2.1 (m, 1 H, $J_{1,2e} \simeq J_{2e,3}$ ~4, $J_{2a,2e}$ ~12 Hz, H-2*e*), 2.18 (s, 3 H, SMe), 2.88 (q, 1 H, $J_{1,2a} \simeq J_{2a,3}$ ~12 Hz, H-2*a*), 3.60 (td, dd after D_2O exchange, 1 H, $J_{1',2'}$ 3.5, $J_{2',3'}$10.5 Hz, H-2'), ~3.65 (m, 1 H, $J_{3,4}$ 9.5 Hz, H-3), 3.77 (m, 2 H, 2 H-6'), 4.22 (t, 1 H, $J_{4,5}$ 9.5 Hz, H-4), 4.52 (td, 1 H, $J_{1,6}$ 10 Hz, H-1), 4.73 (AB, 2 H, *J* 11.5 Hz, OCH_AH_BS), 5.04 (m, 1 H, $J_{4',5'}$ 9.5 Hz, H-5'), 5.30 (d, 1 H, *J* 8.8 Hz, exchanged in D_2O, HN-2' or 3), 5.39 (t, 1 H, $J_{3',4'}$ 9.5 Hz, H-4'), 5.45 (d, 1 H, H-1'), 5.46 (d, 1 H, *J* 9 Hz, exchanged in D_2O, HN-3 or 2'), 5.66 (t, 1 H, H-3'), 5.84 (t, 1 H, $J_{5,6}$ 10 Hz, H-5), 6.11 (t, 1 H, H-6), 6.60 (d, 2 H), 6.87 (d, 2 H), 6.95 (d, 2 H), 7.05–7.58 (m, 23 H), 7.69 (d, 2 H), and 7.92 (m, 6 H) (3 $MeC_6H_4SO_2$, 5 C_6H_5CO).

Anal. Calc. for $C_{70}H_{67}N_3O_{18}S_4$ (1366.57): C, 61.52; H, 4.94; N, 3.07; S, 9.39. Found: C, 61.63; H, 4.96; N, 3.13; S, 9.46.

(1-N-*5,6-Di*-O-*benzoyl-2-deoxy-1,3-di*-N-p-*tolylsulfonylstreptamin-4-yl) 3*-O-*benzoyl-2,4-dideoxy-2*-p-*tolylsulfonamido-β*-L-threo-*hex-4-enodialdo-1,5-pyranoside* (**10**). — To a solution of aldehyde **9** [prepared from 200 mg (0.153 mmol) of **7** as just described] in dichloromethane was added triethylamine (0.5 mL). After 0.5 h at room temperature, the mixture was washed with M aqueous hydrochloric acid (5 mL), then with water (3 × 3 mL), dried, and evaporated. The residue was submitted to column chromatography (*G*) which afforded a small amount of **8** and then pure **10** (131 mg, 72% from **7**). Compound **10** crystallized from chloroform–

ether–hexane, m.p. 149–154°, $[\alpha]_D^{26}$ +77.8° (*c* 0.91, chloroform); t.l.c. (*E*, bright orange spot with 2,4-DNP spray reagent) R_F 0.49; ν_{max}^{KBr} 3300 (NH), 1730, 1700 (C=O), 1650 (C=C), 1600, 1450, 1360, 1340, 1270, 1160, 1090, 710, 660, 585, and 545 cm^{-1}; ^{1}H-n.m.r.: δ ~1.9 (m, 1 H, $J_{1,2e} \approx J_{2e,3}$ ~4, $J_{2e,2a}$ ~12.5 Hz, H-2*e*), 2.07, 2.22 and 2.36 (3 s, 9 H, 3 ArCH_3), 2.77 (q, 1 H, $J_{1,2a} = J_{2a,3}$ = 12.5 Hz, H-2*a*), 3.52 (bq, bt after D_2O exchange, 1 H, $J_{3,4}$ 10, $J_{3,NH}$ 8 Hz, H-3), 3.73 (bt, bd after D_2O exchange, 1 H, $J_{1',2'}$ 2.5, $J_{2',3'}$~8, $J_{2',NH}$ 8 Hz, H-2'), 4.22 (t, 1 H, $J_{4,5}$ 10 Hz, H-4), 4.46 (td, 1 H, $J_{1,6}$ 10 Hz, H-1), 5.02 (d, 1 H, exchanged in D_2O, HN-3), 5.45 (d, 1 H, H-1'), 5.56 (d, 1 H, exchanged in D_2O, HN-2'), 5.64 (t, 1 H, $J_{5,6}$ 10 Hz, H-5), 6.05 (m, 3 H, H-3',4',6), 6.83 (d, 2 H), 6.84 (d, 2 H), 6.91 (d, 2 H), 7.04–7.75 (m, 22 H), 7.81 (d, 2 H) and 7.93 (d, 2 H) (3 Me$C_6H_4SO_2$, 4 C_6H_5CO), and 9.34 (s, 1H, H-6').

Anal. Calc. for $C_{61}H_{55}N_3O_{16}S_3$ (1182.31): C, 61.97; H, 4.69; N, 3.55; S, 8.14. Found: C, 61.77; H, 4.83; N, 3.45; S, 8.17.

E-*Ethyl* [*(1*-N-*5,6-di*-O-*benzoyl-2-deoxy-1,3-di*-N-p-*tolysulfonylstreptamin-4-yl) 3,4-di*-O-*benzoyl-2,6,7-trideoxy-2*-p-*tolylsulfonamido-α*-D-gluco-*oct-6-enopyranosid*]*uronate* (**11**). — Recrystallized (benzene–petroleum ether) (ethoxycarbonylmethylene)triphenylphosphorane (2 equiv.) was added to a solution of crude aldehyde **9** in benzene (or directly to the solution of **9** obtained after processing of the oxidation reaction, see preparation of **9**), and the mixture stirred overnight at room temperature. The solvent was then evaporated and compound **7** separated from phosphorus-containing by-products and a trace of methylthiomethyl ether **8** (same mobility as **11**) by column chromatography (*H*) and recrystallization (chloroform–ether–hexane) (yield 80–85% from **7**); m.p. 143–146°, $[\alpha]_D^{26}$ +35.6° (*c* 1.01, chloroform); t.l.c. (*E*) R_F 0.54; ν_{max}^{KBr} 3280 (NH), 1725 (C=O), 1650 (C=C), 1600, 1450, 1362, 1270, 1165, 1090, 1030, 707, and 540 cm^{-1}; ^{1}H-n.m.r.: δ 1.30 (t, 3 H, *J* 7.2 Hz, OCH_2CH_3), 1.91, 2.24 and 2.43 (3 s, 9 H, 3 ArCH_3), ~2.25 (m, 1 H, $J_{1,2e} \approx J_{2e,3}$ ~4, $J_{2a,2e}$ 12.5 Hz, H-2*e*), 2.86 (q, 1 H, $J_{1,2a} = J_{2a,3}$ = 12.5 Hz, H-2*a*), 3.59 (td, dd after D_2O exchange, 1 H, $J_{1',2'}$ 3.4, $J_{2',3'}$10.2, $J_{2',NH}$ 9.0 Hz, H-2'), 3.65 (m, 1 H, $J_{3,4}$ 9.5, $J_{3,NH}$ 9.0 Hz, H-3), 4.14 (t, 1 H, $J_{4,5}$ 9.5 Hz, H-4), 4.19 (q, 2 H, OCH_2CH_3), 4.46 (td, 1 H, $J_{1,6}$ 10 Hz, H-1), 4.95 (d, 1 H, exchanged in D_2O, HN-3), 5.24 (t, 1 H, $J_{3',4'}$ 9.8, $J_{4',5'}$ 9.8 Hz, H-4'), 5.48 (d, 1 H, H-1'), 5.60 (d, 1 H, exchanged in D_2O, HN-2'), 5.63 (dd, 1 H, $J_{5',6'}$ 5.9 Hz, H-5'), 5.84 (t, 2 H, $J_{5,6}$ 10 Hz, H-3',5), 6.06 (t, 1 H, H-6), 6.32 (dd, 1 H, $J_{6',7'}$ 15.8, $J_{5',7'}$ 1.2 Hz, H-7'), 6.89 (hidden dd, 1 H, H-6'), 6.59 (d, 2 H), 6.81 (d, 2 H), 6.89 (d, 2 H), 7.05–7.45 (m, 23 H), 7.50 (d, 2 H), 7.65 (d, 2 H), and 7.90 (m, 4 H) (3 Me$C_6H_4SO_2$, 5 C_6H_5CO).

Anal. Calc. for $C_{72}H_{67}N_3O_{19}S_3$ (1374.52): C, 62.92; H, 4.91; N, 3.06; s, 7.00. Found: C, 63.16; H, 5.03; N, 2.98; S, 6.86.

cis-*Hydroxylation of* **11** *with osmium tetraoxide.* — *(a) Non-catalytic procedure.* To a solution of **11** (378 mg, 0.275 mmol) in dry pyridine (6 mL) was added a freshly prepared, 5% solution of osmium tetraoxide in pyridine (1.52 mL, then 0.4 mL after 6 h, and then 0.2 mL after 6.75 h, 0.42 mmol). After 7.05 h at room temperature, a solution of sodium hydrogensulfite (1.5 g) in water (13 mL) was

added and the mixture stirred overnight. Chloroform (25 mL) was added, the separated aqueous layer extracted with chloroform (2 × 10 mL), the combined organic phases washed with 1.5M aqueous hydrochloric acid (10-mL portions) until all of the pyridine had been removed, then with a saturated aqueous sodium hydrogencarbonate solution (8 mL), and water (2 × 5 mL), and dried ($MgSO_4$). The colorless solution was evaporated and the residue submitted to column chromatography (5:2 toluene–ethyl acetate, then 3:2 after the elution of isomer **13**) which afforded pure **13** (85 mg, 22%), an unidentified product of intermediate mobility (11 mg), and pure **12** (257 mg, 66%).

(b) Catalytic procedure. To a solution of **11** (194 mg, 0.141 mmol) in acetone (4 mL) were added tetra-*N*-methylammonium acetate (10 mg), a 70% aqueous solution of *tert*-butylhydroperoxide (50 μL), and osmium tetraoxide catalyst solution (50 μL, 0.25% OsO_4 in 2,2-dimethylpropanol stabilized with 3% H_2O_2). After 22 h at room temperature, most of the solvent was removed by applying a slight vacuum to the reaction flask; chloroform (10 mL) and a 3% aqueous solution of sodium hydrogensulfite (5 mL) were then added, and the two-phase system was stirred for 0.75 h. The organic layer was separated, washed with water (2 × 5 mL), dried ($MgSO_4$), and evaporated. Column chromatography (toluene–ethyl acetate 3:1, and then 2:1 after the elution of **13**) of the residue afforded **13** (89 mg, 45%) and isomer **12** (17 mg, 9%) as main products.

*Ethyl [(1-*N*-5,6-di-*O*-benzoyl-2-deoxy-1,3-di-*N*-p-tolylsulfonylstreptamin-4-yl) 3,4-di-*O*-benzoyl-2-deoxy-2-p-tolylsulfonamido-D-threo-α-D-gluco-octo-1,5-pyranosid]uronate* (**12**). — An analytical sample of **12** was obtained by recrystallization from hot ethanol, m.p. 184–187°, $[\alpha]_D^{26}$ −22.1°; $[\alpha]_{365}^{26}$ −156.6° (*c* 1.13, chloroform); t.l.c. (*F*) R_F 0.51; ν_{max}^{KBr} 3500 (OH), 3280 (NH), 1730 (C=O), 1600, 1450, 1360, 1270, 1190, 1020, 708, 660, 585, and 545 cm^{-1}; ^{1}H-n.m.r.: δ 1.38 (t, 3 H, *J* 7.0 Hz, OCH_2CH_3), 1.84, 2.25 and 2.38 (3 s, 9 H, 3 $ArCH_3$), 2.17 (dt, 1 H, $J_{1,2e} \simeq J_{2e,3}$ ~4.5, $J_{2e,2a}$ 12.5 Hz, H-2*e*), 2.97 (q, 1 H, $J_{1,2a} = J_{2a,3}$ 12.5 Hz, H-2*a*), 3.12 (d, 1 H, $J_{6',OH}$ 11.3 Hz, exchanged in D_2O, HO-6′), 3.51 (td, dd after D_2O exchange, 1 H, $J_{1',2'}$ 3.5, $J_{2',3'}$ 10.5, $J_{2',NH}$ 9.5 Hz, H-2′), 3.70 (m, 1 H, $J_{3,4}$ 9.5, $J_{3,NH}$ 10 Hz, H-3), 3.74 (d, 1 H, $J_{7',OH}$ 6 Hz, exchanged in D_2O, HO-7′), 3.95 (dd, d after D_2O exchange, 1 H, $J_{5',6'}$ <0.5, $J_{6',7'}$ 4.8 Hz, H-6′), 4.21 (t, 1 H, $J_{4,5}$ 9.5 Hz, H-4), 4.40 (~q, 2 H, $OCH_AH_BCH_3$, $\Delta\delta H_{A,B} \leq 2$ Hz), 4.52 (t, d after D_2O exchange, 1 H, H-7′), 4.52 (hidden td, 1 H, $J_{1,6}$ 10.5 Hz, H-1), 5.07 (d, 1 H, $J_{4',5'}$ 9.6 Hz, H-5′), 5.44 (d, 1 H, H-1′), 5.49 (d, 1 H, exchanged in D_2O, HN-3), 5.52 (t, 1 H, $J_{3',4'}$ 10 Hz, H-4′), 5.66 (t, 1 H, H-3′), 5.84 (t, 1 H, $J_{5,6}$ 9.2 Hz, H-5), 6.11 (t, 1 H, H-6), 6.36 (d, 1 H, exchanged in D_2O, HN-2′), 6.56 (d, 2 H), 6.88 (d, 2 H), 6.95 (d, 2 H), 7.06–7.46 (m, 21 H), 7.50 (d, 2 H), 7.68 (d, 2 H), and 7.91 (m, 6 H) (3 $MeC_6H_4SO_2$, 5 C_6H_5CO).

Anal. Calc. for $C_{72}H_{69}N_3O_{21}S_3$ (1408.54): C, 61.40; H, 4.94; N, 2.98; S, 6.83. Found: C, 61.55; H, 4.99; N, 3.22; S, 7.02.

*Ethyl [(1-*N*-5,6-di-*O*-benzoyl-2-deoxy-1,3-di-*N*-p-tolylsulfonylstreptamin-4-yl) 3,4-di-*O*-benzoyl-2-deoxy-2-p-tolylsulfonamido-L-threo-α-D-gluco-octo-1,5-py-*

ranosid]uronate (**13**). — Compound **13** was recrystallized from hot ethanol, m.p. 153–156° $[\alpha]_D^{26}$ −6.8 ±1.5°, $[\alpha]_{365}^{26}$ −109.3° (*c* 0.73, chloroform); t.l.c. (*E*) R_F 0.49; ν_{max}^{KBr} 3490 (OH), 3280 (NH), 1730 (C=O), 1600, 1450, 1360, 1275, 1160, 1090, 1025, 710, 665, 590, and 546 cm^{-1}; ^{1}H-n.m.r.: δ 1.32 (t, 3 H, *J* 7.2 Hz, OCH_2CH_3), 1.83, 2.24 and 2.42 (3 s, 9 H, 3 $ArCH_3$), 2.16 (dt, 1 H, $J_{1,2e} \simeq J_{2e,3}$ ~4, $J_{2e,2a}$ 12.5 Hz, H-2*e*), 2.82 (q, 1 H, $J_{1,2a} = J_{2a,3}$ = 12.5 Hz, H-2*a*), 2.89 (d, 1 H, $J_{6',OH}$ 12.0 Hz, exchanged in D_2O, HO-6′), 3.55 (td, dd after D_2O exchange, 1 H, $J_{1',2'}$ 3.7, $J_{2',3'}$ 10.7, $J_{2',NH}$ 9 Hz, H-2′), 3.77 (bq, bt after D_2O exchange, 1 H, $J_{3,4}$ 10, $J_{3,NH}$ 8.5 Hz, H-3), 3.98 (d, 1 H, $J_{7',OH}$ 4.2 Hz, exchanged in D_2O, HO-7′), 4.16 (t, 1 H, $J_{4,5}$ 9.5 Hz, H-4), 4.22 (dq, 1 H, J_{AB} 10.5 Hz, $OCH_AH_BCH_3$), 4.26 (t, d after D_2O exchange, 1 H, $J_{5',6'}$ 9.5, $J_{6',7'}$ <0.5 Hz, H-6′), 4.37 (dq, 1 H, $OCH_AH_BCH_3$), 4.50 (td, 1 H, $J_{1,6}$ 10.5 Hz, H-1), 4.79 (d, s after D_2O exchange, 1 H, H-7′), 5.15 (t, 1 H, $J_{4',5'}$ 9.5 Hz, H-5′), 5.43 (d, 1 H, exchanged in D_2O, HN-3), 5.44 (d, 1 H, H-1′), 5.54 (t, 1 H, $J_{3',4'}$ 9.5 Hz, H-4′), 5.66 (t, 1 H, H-3′), 5.88 (t, 1 H, $J_{5,6}$ 9.5 Hz, H-5), 6.08 (t, 1 H, H-6), 6.56 (d, 2 H), 6.88 (d, 2 H), 6.93 (d, 2 H), 7.05–7.55 (m, ~23 H), 7.66 (d, 2 H) and 7.91 (m, 6 H) (3 $MeC_6H_4SO_2$, 5 C_6H_5CO), and signal of HN-2′ hidden in aromatic region.

Anal. Calc. for $C_{72}H_{69}N_3O_{21}S_3$ (1408.54): C, 61.40; H, 4.94; N, 2.98; S, 6.83. Found: C, 61.54; H, 4.88; N, 3.12; S, 7.06.

[*(2-Deoxy-1,3-di*-N-p-*tolylsulfonylstreptamin-4-yl) 2-deoxy-2*-p-*tolylsulfonamido-*D-*threo-α*-D-gluco-*octo-1,5-pyranosid]urono-8,4-lactone* (**14**). — To a solution of ethyl octuronate **12** (600 mg, 0.426 mmol) in dry dichloromethane (6 mL) was added dry methanol (8 mL), and then a 0.19M sodium methoxide solution in methanol (9 mL, 4 equiv.). After 20 h at room temperature, the yellow solution was rapidly made neutral with methanol-washed Amberlite IR-120 (H^+) ion-exchange resin, the resin removed by filtration, and the nearly colorless filtrate stabilized with sodium acetate [0.25 equiv., formed *in situ* by the addition of 0.6 mL of 0.19M sodium methoxide in methanol and of the equivalent amount of 1% (v/v) acetic acid in methanol (0.32 mL)]. The solvents were evaporated, the methyl benzoate removed by trituration with ether (3 × 5 mL), and crude **14** containing sodium acetate obtained by filtration (yield quantitative); m.p. 187–195°, t.l.c. (*H*) R_F 0.05; ν_{max}^{KBr} 3470 (OH), 3280 (NH), 1735 (C=O), 1160 and 1325 (SO_2), 1600, 1450, 1090, 815, 670, and 550 cm^{-1}; ^{1}H-n.m.r. (Me_2SO-d_6): δ 1.24 (q, 1 H, $J_{1,2a} = J_{2a,2e} = J_{2a,3}$ ~12 Hz, H-2*a*), 1.63 (bd, 1 H, H-2*e*), 2.29, 2.38 and 2.41 (3 s, 9 H, 3 $ArCH_3$), 2.5–3.8 (several m, H-1,3,4,5,6,2′,3′,4′,5′,6′,7′), 5.07 (d, 1 H, $J_{1',2'}$ 3.2 Hz, H-1′), 7.28, 7.33, 7.38, 7.61, 7.68, and 7.73 (6 d, 12 H, 3 $MeC_6H_4SO_2$); ^{13}C-n.m.r. (Me_2SO-d_6): δ 20.87 ($ArCH_3$), 34.46 (C-2), 52.41 (C-3), 53.68 (C-1), 58.14 (C-2′), 69.16 (C-7′), 70.08, 70.37, 70.71 (C-3′,4′,5′,6′), 74.11 (C-5), 76.44 (C-6), 77.36 (C-4), 95.90 (C-1′), 126.13, 126.33, 126.57 (C_{Ar}-2,2′), 129.14 (C_{Ar}-3,3′), 138.99, 139.92 (C_{Ar}-4), 141.95, 142.29 (C_{Ar}-1), and 175.78 (C-8′).

Methyl [*(2-deoxy-1,3-di*-N-p-*tolylsulfonylstreptamin-4-yl) 2-deoxy-2*-p-*tolylsulfonamido-*D-*threo-α*-D-gluco-*octo-1,5-pyranosid]uronate* (**16**). — By treatment with 0.5M methanolic hydrogen chloride, lactone **14** was converted quantita-

tively into the corresponding methyl uronate **16** in 2 h at room temperature. After rapid neutralization of the acid with methanol-washed Amberlite IR-45 (OH^-) ion-exchange resin and removal of the resin by filtration, the solvent was evaporated and compound **16** recrystallized from chloroform containing a few drops of methanol, m.p. 160–167°, $[\alpha]_D^{26}$ +32.6° (*c* 0.95, methanol); t.l.c. (*K*) R_F 0.50; ν_{max}^{KBr} 3450 (OH), 3290 (NH), 1725 (C=O), 1320 and 1155 (SO_2), 1595, 1445, 1090, 1030, 815, 670, and 550 cm^{-1}; ^{1}H-n.m.r. (CD_3OD): δ 1.25 (q, 1 H, $J_{1,2a} = J_{2a,2e} = J_{2a,3}$ = 12.5 Hz, H-2*a*), 1.78 (dt, 1 H, $J_{1,2e} = J_{2e,3}$ ~4 Hz, H-2*e*), 2.39 (s, 3 H) and 2.44 (s, 6 H) (3 ArCH_3), 2.85 (td, 1 H, H-1 or 3), 3.0–3.6 (several m, H-3 or 1,4,5,6), 3.17 (dd, 2 H, $J_{1',2'}$ 3.6, $J_{2',3'}$ 9.5 Hz, H-2′), 3.43 and 3.54 (2 t, $J_{3',4'}$ 9.5, $J_{4',5'}$ 9 Hz, H-3′,4′), 3.73 (s. 3 H, CO_2Me), 4.12 (d, 1 H, $J_{5',6'}$ 1.2 Hz, H-5′), 4.19 (dd, 1 H, $J_{6',7'}$ 4.0 Hz, H-6′), 4.44 (d, 1 H, H-7′), 5.31 (d, 1 H, H-1′), 7.38 (m, 6 H), 7.61 (d, 2 H), 7.65 (d, 2 H), and 7.76 (d, 2 H) (3 $MeC_6H_4SO_2$); ^{13}C-n.m.r. (Me_2SO-d_6): δ 20.87 (ArCH_3), 34.99 (C-2), 51.30 ($COOCH_3$), 52.32 (C-3), 53.63 (C-1), 57.75 (C-2′), 69.35 (C-4′), 70.27 (C-5′), 70.27 (C-6′), 71.92 (C-7′), 73.81 (C-5), 76.43 (C-6), 78.52 (C-4), 97.25 (C-1′), 126.42 (C_{Ar}-2,2′), 129.14 (C_{Ar}-3,3′), 138.70, 138.84, 139.18 (C_{Ar}-4), 141.95, 142.19, 142.29 (C_{Ar}-1), and 173.35 (C-8′).

Anal. Calc. for $C_{36}H_{47}N_3O_{16}S_3$ (873.97): C, 49.47; H, 5.42; N, 4.81; S, 11.01. Found: C, 49.51; H, 5.31; N, 4.60; S, 11.11.

Debenzoylation of ethyl octuronate **13**. — To a solution of ethyl octuronate **13** (300 mg, 0.213 mmol) in dry dichloromethane (3 mL) were added dry methanol (7 mL) and a 0.19M sodium methoxide solution in methanol (4.45 mL, 4 equiv.). The mixture was stirred overnight at room temperature, and then made neutral with methanol-washed Amberlite IR-120 (H^+) ion-exchange resin. The resin was removed by filtration and the filtrate evaporated; the residue was triturated with ether (3 × 5 mL) to remove methyl benzoate, and dried; t.l.c. (*L*) of the white solid indicated the presence of two components (R_F 0.49, major; 0.52, minor); ν_{max}^{KBr} 3450 (OH), 3280 (NH), 1725 (C=O), 1595 (Ar), 1320 and 1150 (SO_2), 1450, 1280, 1080, 1055, 1015, 810, 665, and 550 cm^{-1}.

Conversion into conjugate base(s). A solution of these components in methanol was treated with 0.19M methanolic sodium methoxide until the pH reached ~8 (~1 equiv. added); the solvent was removed under reduced pressure and the residual white solid dried; t.l.c. identical to that just described; ν_{max}^{KBr} 3400 (OH), 3260 (NH), 1595 (very strong, CO_2^-), 1320 and 1155 (SO_2), 1450, 1280, 1080, 1055, 1020, 810, 665, and 550 cm^{-1}.

Methyl [*(2-deoxy-1,3-di-*N-p-*tolylsulfonylstreptamin-4-yl) 2-deoxy-2-*p-*tolylsulfonamido-*L*-threo-α-*D-gluco-*octo-1,5-pyranosid*]*uronate* (**19**). — Treatment of the debenzoylated product (prepared from **13** (400 mg, 0.28 mmol) as just described) with 0.33M methanolic hydrogen chloride (5 mL) for 2 h at room temperature afforded methyluronate **19** as the major product. The mixture was made neutral with methanol-washed Amberlite IR-45 (OH^-) ion-exchange resin, the resin removed by filtration, the filtrate evaporated, and the residue submitted to column chromatography (9:1 chloroform–methanol) to afford homogeneous (^{1}H-n.m.r.)

19 in ~70% yield. Compound **19** was recrystallized from 2-propanol or from ethyl acetate–ether, m.p. 148–153°, $[\alpha]_D^{26}$ +10.9° (*c* 0.82, methanol); t.l.c. (*K*) R_F 0.58; ν_{max}^{KBr} 3440 (OH), 3270 (NH), 1730 (C=O), 1315 and 1150 (SO_2), 1590, 1440, 1085, 1060, 1015, 810, 665, and 550 cm^{-1}; ^{1}H-n.m.r. (CD_3OD): δ 1.17 (q, 1 H, $J_{1,2a} = J_{2a,2e} = J_{2a,3}$ = 12.5 Hz, H-2*a*), 1.61 (dt, 1 H, $J_{1,2e} \simeq J_{2e,3}$ ~4 Hz, H-2*e*), 2.40 (s, 3 H), 2.42 (s, 3 H) and 2.45 (s, 3 H) (3 ArCH_3), 2.81 (m, 1 H, H-1 or 3), 3.04 (m, 2 H, H-4, H-3 or 1), 3.19 (dd, 1 H, $J_{1',2'}$ 3.7, $J_{2',3'}$ 9.8 Hz, H-2'), ~3.25 (m, 2 H, H-5,6), 3.48 (t, 1 H, $J_{3',4'} = J_{4',5'}$ = 9.0 Hz, H-4'), 3.60 (t, 1 H, H-3'), 3.75 (s, 3 H, CO_2Me), 4.04 (dd, 1 H, $J_{5',6'}$ 6.2 Hz, H-5'), 4.18 (dd, 1 H, $J_{6',7'}$ 1.5 Hz, H-6'), 4.56 (d, 1 H, H-7'), 5.03 (d, 1 H, H-1'), 7.35 (m, 6 H), 7.62 (d, 2 H), 7.68 (d, 2 H), and 7.82 (d, 2 H) (3 $MeC_6H_4SO_2$).

Anal. (see **16**). Found: N, 4.51; S, 11.22.

[*(1-*N*-5,6-di-*O*-benzoyl-2-deoxy-1,3-di-*N*-p-tolylsulfonylstreptamin-4-yl) 3,7-di-*O*-benzoyl-2,6-dideoxy-2-*p*-tolylsulfonamido-α-*D*-gluco-oct-6-eno-1,5-pyranosid]urono-8,4-lactone* (**15**). — To a solution of methyl uronate **16** (130 mg, 0.149 mmol) in methanol (2 mL) was added a 0.48M sodium methoxide solution in methanol (1.25 mL). After 24 h at room temperature, the solution was made neutral with methanol-washed Amberlite IR-120 (H^+) ion-exchange resin, the resin removed by filtration, sodium acetate (2 mg) added to the filtrate, the filtrate evaporated, and the residue dried under vacuum. Lactone **14** thus obtained was dissolved in pyridine (3 mL); benzoyl chloride (0.35 mL) was then added and the mixture stirred for 45 h at room temperature. After the usual processing, the residue was submitted to column chromatography (solvent *J*) to afford pure **15** (74 mg, 37%) which was recrystallized from *tert*-butyl methyl ether containing a trace of chloroform, m.p. 170–175°, $[\alpha]_D^{26}$ +34.7° (*c* 0.63, chloroform); t.l.c. (*D*) R_F 0.43; ν_{max}^{KBr} 330 (NH), 1730, 1690 (C=O, broad), 1595, 1445, 1360, 1260, 1155, 1085, 1040, 1020, 705, 655, 585, and 540 cm^{-1}; ^{1}H-n.m.r.: δ 1.91, 2.22 and 2.32 (3 s, 9 H, 3 ArCH_3), ~2.2 (m, 1 H, $J_{1,2e} \simeq J_{2e,3}$ ~4, $J_{2e,2a}$ 12.5 Hz, H-2*e*), 2.87 (q, 1 H, $J_{1,2a} = J_{2a,3}$ = 12.5 Hz, H-2*a*), 3.53 (td, dd after D_2O exchange, 1 H, $J_{1',2'}$ 3.6, $J_{2',3'}$ 10.5, $J_{2',NH}$ 9 Hz, H-2'), 3.72 (bq, td after D_2O exchange, 1 H, $J_{3,4}$ 9.5, $J_{3,NH}$ ~9 Hz, H-3), 4.18 (t, 1 H, $J_{4,5}$ 9.5 Hz, H-4), 4.38 (t, 1 H, $J_{3',4'}$ 10.5, $J_{4',5'}$ 10.5 Hz, H-4'), 4.58 (td, 1 H, $J_{1,6}$ 10.5 Hz, H-1), 4.95 (d, 1 H, exchanged in D_2O, HN-3 or 2'), 5.28 (d, 1 H, exchanged in D_2O, HN-2'or 3), 5.30 (dd, 1 H, $J_{5',6'}$ 1.6 Hz, H-5'), 5.35 (t, 1 H, H-3'), 5.44 (d, 1 H, H-1'), 5.82 (t, 1 H, $J_{5,6}$ 9.5 Hz, H-5), 6.16 (t, 1 H, H-6), 6.82 (d, 1 H, H-6'), 6.65 (d, 2 H), 6.89 (d, 2 H), 6.99 (d, 2 H), 7.08–7.78 (m, 25 H), 7.86 (d, 2 H), 8.01 (d, 2 H), and 8.13 (d, 2 H) (3 $MeC_6H_4SO_2$, 5 C_6H_5CO).

Anal. Calc. for $C_{70}H_{61}N_3O_{19}S_3$ (1344.45): C, 62.54; H, 4.57; N, 3.13; S, 7.15. Found: C, 61.97; H, 4.77; N, 2.99; S, 6.72.

Partial reduction of lactone **14** *with lithium aluminum hydride.* — To a solution of lactone **14** (100 mg, ~0.11 mmol, containing ~0.25 equiv. of sodium acetate) in dry pyridine (2 mL) was added, under nitrogen, dry oxolane (3 mL), and the mixture cooled to −78° (acetone–Dry Ice). A M solution of lithium aluminum

hydride in oxolane (0.6 mL, 0.6 mmol) was then added dropwise. The reaction was monitored in the following way: a sample was removed with a syringe, and diluted with oxolane, the reaction quenched with 10:1 methanol–water, and the solution treated with aqueous oxalic acid and analyzed by t.l.c. (*K*). The mixture was stirred for 1 h at −78°, 2 h at −10°, and 15 h at 0°, and then processed by the successive addition of 10:1 (v/v) methanol–water (2 mL), oxolane (4 mL), and M aqueous oxalic acid (2.5 mL). The solvents were removed under reduced pressure, the solid residue triturated with oxolane (5 × 3 mL), then dissolved in water (2 mL), and the resulting aqueous solution extracted with oxolane (3 × 2 mL). The combined, salt-free oxolane-extracts were evaporated. T.l.c. analysis (*K*) of the remaining solid indicated the presence of compounds **14**, **17** (TTC positive), and **20** in ~1:1:1 ratios.

Partial reduction of methyl uronate **16** *with lithium aluminum hydride.* — To a cooled (−78°, acetone–Dry Ice) solution of methyl uronate **16** (180 mg, 0.21 mmol) in dry oxolane (12 mL) was added dropwise, under nitrogen, a M solution of lithium aluminum hydride in oxolane (0.9 mL, 0.9 mmol). The reaction was monitored as described in the preceding experiment. The slightly cloudy mixture was stirred for 40 min at −78° and for 9 min at −20°, and cooled again to −78°. An additional volume of hydride solution (0.15 mL) was added and the mixture further stirred at −78° (15 min) and at −20° (10 min). Processing of the reaction mixture as just described afforded compounds **16**, **17** (TTC positive), and **20** in 1:5:4 ratios, approximately (t.l.c., *K*).

*1-(2-Deoxy-1,3-di-*N-p-*tolylsulfonyl-streptamin-4-yl) (8*R,S*)-2-deoxy-2-*p-*tolylsulfonamido-*D-*threo-α-*D-gluco-*octodialdo-1,5-pyranoside-8,4-pyranose* (**17**). — The mixtures of products arising from the partial reduction of lactone **14** (100 mg) and of methyl uronate **16** (280 mg) were combined and carefully resolved by column chromatography (10:1, gradually changed to 5:2 chloroform–methanol) to afford pure methyl uronate **16** (10 mg), octodiose **17** (122 mg, 34%), and octose **20** (83 mg, 23%). Compound **17** was recrystallized from methanol–ethyl acetate, m.p. 175–205°, $[\alpha]_D^{26}$ −25.9°, $[\alpha]_{365}^{26}$ −89.8° (*c* 0.58, methanol) (mixture of anomers); t.l.c. (*K*, TTC positive) R_F 0.42; m.p. 235–238° (8′*S* anomer, presumably); ν_{max}^{KBr} 3440 (OH), 3280 (NH), 1320 and 1150 (SO_2), 1595, 1450, 1080, 1055, 1005, 810, 665, and 550 cm^{-1}; ^{1}H-n.m.r. (CD_3OD): δ 1.11 (q, 1 H, $J_{1,2a} = J_{2a,2e} = J_{2a,3} = 12.5$ Hz, H-2*a*), 1.67 (dt, 1 H, $J_{1,2e} \simeq J_{2e,3}$ ~4 Hz, H-2*e*), 2.39, 2.44 and 2.46 (3 s, 9 H, 3 ArCH_3), 2.70–4.0 (several m and t, H-1,3,4,5,6,2′,3′,4′,5′,6′,7′), 3.50 (dd, 0.5 H, $J_{6',7'}$ 9.0, $J_{7',8'}$ 3.5 Hz, H-7′, 8′*R*-isomer), 4.45 (d, 0.5 H, $J_{7',8'}$ 7.5 Hz, H-8′, 8′*S*-isomer), 5.09 (d, 0.5 H, H-8′, 8′*R*-isomer), 5.28 and 5.33 (2 d, 2 × 0.5 H, $J_{1',2'}$ 3.7 Hz, H-1′s), 7.34 (d, 4 H), 7.40 (d, 2 H), 7.63 (d, 2 H), 7.69 (d, 2 H), and 7.82 (d, 2 H) (3 $MeC_6H_4SO_2$).

Anal. Calc. for $C_{35}H_{45}N_3O_{15}S_3$ (843.94): C, 49.81; H, 5.37; N, 4.98; S, 11.40. Found: C, 49.82; H, 5.41; N, 4.89; S, 11.35.

*(2-Deoxy-1,3-di-*N-p-*tolylsulfonyl-streptamin-4-yl) 2-deoxy-2-*p-*tolylsulfonamido-*D-*threo-α-*D-gluco-*octopyranoside* (**20**). — Octose **20**, isolated as just described, was recrystallized from hot 2-propanol, m.p. 190–195°, $[\alpha]_D^{26}$ −9.3 ±1.2°,

$[\alpha]_{365}^{26}$ −44.0° (*c* 0.86, methanol); t.l.c. (*K*) R_F 0.37; ν_{max}^{KBr} 3370 (OH, NH), 1320 and 1155 (SO_2), 1445, 1380, 1090, 1025, 815, 670, and 555 cm^{-1}; ^{1}H-n.m.r. (CD_3OD): δ ~1.17 (q, 1 H, $J_{1,2a} = J_{2a,2e} = J_{2a,3}$ = 12.5 Hz, H-2*a*), 1.58 (dt, 1 H, $J_{1,2e} \simeq J_{2e,3}$ ~4 Hz, H-2*e*), 2.78 (td, 1 H, H-1 or 3), 3.0–3.8 and 3.95–4.2 (several m, H-3 or 1,4,5,6,2′,3′,4′,5′,6′,7′,8′), 5.22 (d, 1 H, $J_{1',2'}$ 3.3 Hz, H-1′), 7.32 (d, 2 H), 7.34 (d, 2 H), 7.40 (d, 2 H), 7.61 (d, 2 H), 7.70 (d, 2 H), and 7.82 (d, 2 H) (3 $MeC_6H_4SO_2$).

Anal. Calc. for $C_{35}H_{47}N_3O_{15}S_3$ (845.96): C, 49.69; H, 5.60; N, 4.97; S, 11.37. Found: C, 49.80; H, 5.69; N, 5.29; S, 11.13.

*1-(2-Deoxy-1,3-di-*N*-p-tolylsulfonyl-streptamin-4-yl) 8-methyl (8*R,S*)-2-deoxy-2-*p*-toylsulfonamido-α-*D*-threo-*D*-gluco-octodialdo-1,5:8,4-dipyranodiosides* (**18**). — A solution of octodiose **17** (61 mg, 72 μmol) in 0.5M methanolic hydrogen chloride (9 mL) was boiled under reflux for 1 h, with exclusion of moisture. Methanol (7 mL) was then added, the cooled mixture made neutral with methanol-washed Amberlite IR-45 (OH^-) ion-exchange resin, the resin removed by filtration, the solvent evaporated, and the residue purified by passing through a short column of silica gel (5:1 chloroform–methanol) to afford pure **18** (8′*R*/*S* 1:1, ^{1}H-n.m.r.) (53 mg, 86%); a crystalline sample was obtained from 2-propanol, m.p. 289–295°, $[\alpha]_D^{26}$ −23.6°, $[\alpha]_{365}^{26}$ −87.4° (*c* 0.72, methanol); t.l.c. (*K*) R_F 0.55; ν_{max}^{KBr} 3440 (OH), 3280 (NH), 1315 and 1150 (SO_2), 1595, 1445, 1060, 1010, 815, 670, and 550 cm^{-1}; ^{1}H-n.m.r. (CD_3OD): δ 1.16 (q, 1 H, $J_{1,2a} = J_{2a,2e} = J_{2a,3}$ 12.5 Hz, H-2*a*), 1.65 (dt, 1 H, $J_{1,2e} \simeq J_{2e,3}$ ~4 Hz, H-2*e*), 2.40, 2.45 and 2.46 (3 s, 9 H, 3 ArCH_3), 2.63–4.06 (several m, H-1,3,4,5,6,2′,3′,4′,5′,6′,7′), 3.41 and 3.53 (2 s, 2 × 1.5 H, 8′-OMe), 4.16 (d, 0.5 H, $J_{7',8'}$ 7.8 Hz, H-8′, 8′*S*-isomer), 4.67 (d, 0.5 H, $J_{7',8'}$ 3.5 Hz, H-8′, 8′*R*-isomer), 5.28 and 5.30 (2 d, 2 × 0.5 H, $J_{1',2'}$ 3.6 Hz, H-1′s), 7.32 (d, 4H), 7.37 (d, 2 H), 7.62 (d, 2 H), 7.67 (d, 2 H), and 7.82 (d, 2 H) (3 $MeC_6H_4SO_2$).

Anal. Calc. for $C_{36}H_{47}N_3O_{15}S_3$ (857.97): C, 50.40; H, 5.48; N, 4.90; S, 11.21. Found: C, 49.76; H, 5.52; N, 4.95; S, 11.14.

*1-(2-Deoxystreptamin-4-yl) 8-methyl (8*R,S*)-2-amino-2-deoxy-*D*-threo-α-*D*-gluco-octodialdo-1,5:8,4-dipyranodioside* (**22**). — Ammonia (~15 mL) was condensed into a small, three-necked round-bottom flask containing **18** (124 mg, 0.145 mmol) and a magnetic-stirring bar, and maintained at −65° by use of a chloroform–Dry Ice bath. One of the necks of the reaction flask was connected to a small vial containing sodium (210 mg) by way of a piece of plastic tubing, the connection being kept closed (Mohr's clamp) during the condensation of ammonia. Ethylamine (~3 mL) and then the preweighed sodium metal were added to the solution of **18**. The dark-blue solution was stirred at −65° for 1.5 h. Methanol (0.9 mL) was then added and the resulting, colorless solution evaporated under slight vacuum. The residue was dissolved in water (40 mL) and the solution treated with Amberlite IR-120 (H^+) ion-exchange resin (~30 mL). The resin was poured into a column containing 10 mL of the same resin (NH_4^+), and the column was thoroughly washed with water. Elution of the column with M aqueous ammonia and evaporation of the fractions containing **22** afforded the crude aminoglycoside

(52 mg, 91%). Compound **22** was very efficiently separated from traces of partially detosylated by-products by chromatography on a Dowex 1-X2 (OH^-) ion-exchange resin column[43] with CO_2-free water as the eluent. Evaporation of the alkaline fractions afforded 28 mg (from 64 mg of crude **22**) of pure **22** as a colorless, glassy dihydrate: dec 200–250° without apparent melting; $[\alpha]_D^{26}$ +40.8° (*c* 0.64, water); t.l.c. (*M*, ninhydrin) R_F 0.49; ν_{max}^{KBr} 3600–2500 (OH, NH_2), 1590, 1450, 1365, 1045, 1005, and 600 cm^{-1}; ^{1}H-n.m.r. (D_2O, with Me_2CO as internal reference: δ 2.08 with respect to signal of Me_4Si): δ 1.09 (q, 1 H, $J_{1,2a} = J_{2a,2e} = J_{2a,3}$ = 12.5 Hz, H-2*a*), 1.85 (dt, 1 H, $J_{1,2e} = J_{2e,3} \sim 4$ Hz, H-2*e*), 2.61, 2.71 and 2.73 (3 m, 3 H, H-1,3,2′), 3.29 and 3.46 (2 s, 2 × 1.5 H, OMe-8′), 3.04 (t, 1 H, *J* 9.5 Hz), 3.15 (m, 2 H) and 3.30–3.71 (several m, 5 H, H-4,5,6,3′,4′,5′,6′,7′), 4.28 (d, 0.5 H, $J_{7',8'}$ 8.0 Hz, H-8′, 8′*S*-isomer), 4.72 (d partially hidden by DOH signal, $J_{7',8'}$ 3.5 Hz, H-8′, 8′*R*-isomer), 5.12 (d, 1 H, $J_{1',2'}$ 4 Hz, H-1′); (D_2O, after addition of CF_3CO_2D): δ 1.72 (q, 1 H, H-2*a*), 2.37 (dt, 1 H, H-2*e*), 3.30 and 3.46 (2 s, 2 × 1.5 H, OMe-8′), 3.13–3.83 (several m, 10 H), and 3.95 (t, 1 H) (H-1,3,4,5,6,2′,3′,4′,5′,6′,7′), 4.32 (d, 0.5 H, H-8′, 8′*S*-isomer), 4.75 (d, 0.5 H, H-8′, 8′*R*-isomer), and 5.51 (bt, 1 H, H-1′).

Anal. Calc. for $C_{15}H_{28}N_3O_9 \cdot 2H_2O$ (430.43): C, 41.86; H, 7.49; N, 9.76. Found: C, 41.43; H, 7.16; N, 9.54.

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