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Synthesis of the Liposidomycin Diazepanone Nucleoside

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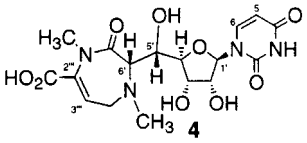
ABSTRACT



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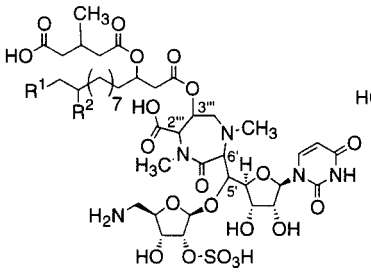
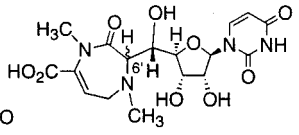
The synthesis of the liposidomycin degradation product 4 from D-glucose establishes its stereochemistry as 5′*S*,6′*S* and, by incorporation of the earlier diazepanone relative stereochemical assignment, establishes the absolute stereochemistry of the liposidomycins 1 and 2 as 5′*S*,6′*S*,2‴*S*,3‴*S*.

The liposidomycins,[1−4] including B, **1**, and C, **2**, constitute a family of complex nucleoside antibiotics[5] that inhibit phospho-*N*-acetylmuramylpentapeptide transferase (translocase I),[6,7] which catalyzes the first membrane step in the peptidoglycan lipid cycle.[8] High in vitro specificity and potency (ID_{50} 0.03 μg/mL) were observed for **2**, reflecting its structural resemblance to uridine diphosphate *N*-acetylmuramylpentapeptide, undecaprenyl phosphate, their transition state, and/or their product, Lipid I. Extensive NMR and mass spectral studies by Isono, Ubukata, McCloskey, and their co-workers led to the liposidomycin structural assignments illustrated, but these stopped short of specifying the relative and absolute stereochemistry at C-5′, C-6′, C-2‴, and C-3‴. Model studies on 3,6,7-trisubstituted-1,4-dimethyldiazepan-2-ones[9] showed close NMR correlation of a 6′*S*,2‴*S*,3‴*S* isomer (or its enantiomer) with **1**/**2** and their degradation products, leaving only the absolute stereochemistry at C-5′ and C-6′ to be determined to completely assign the diazepanone nucleoside portion of **1**/**2**. Fortunately, the degradation studies[3] had produced a nucleoside triol acid (see **3**/**4**) that features C-5′ and C-6′ as the only unassigned stereogenic centers, and thus an exact match of this compound with synthetic material of known structure would provide the missing stereochemical information.



1, liposidomycin B: $R^1 = H$, $R^2 = CH_3$
2, liposidomycin C: $R^1 = CH_3$, $R^2 = H$



3: H-6' is α (= 6'*R*)
4: H-6' is β (= 6'*S*)

Our analysis of the liposidomycin degradation product structures compatible with the vicinal proton coupling

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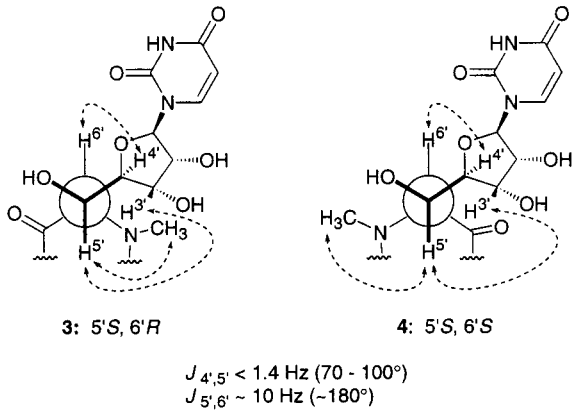

Figure 1. C-5′/C-6′ Newman projections of two proposed structures, **3** and **4**, for the liposidomycin nucleoside triol acid, showing selected NOEs and vicinal coupling constants observed for the degradation product and the related C-3‴ hydrate, a nucleoside tetrol acid. ^{1}H NMR data are taken from refs 3 and 10.

constants[3] and NOE's[10] reported by Ubukata and co-workers is shown in Figure 1. Given the uridine C-4′ *S* stereochemistry, an average H-4′/H-5′ dihedral angle close to 80° (dictated by the small *J*), and the proximity of H-3′ and H-5′ (from NOE measurements), one may logically assign C-5′ the *S* configuration. However, the stereochemistry at C-6′ cannot, in our view, be determined from the data available. Both structures **3** (with C-6′ as *R*) and **4** (with C-6′ as *S*) would seem to conform to the requirements set by an H-5′/H-6′ dihedral angle close to 180° and an NOE for H-5′ and NC*H*$_3$. We therefore undertook the synthesis of **3** and **4** by unambiguous routes.

The synthesis of **3** (see Figure 2) began with commercially available 1,2:5,6-di-*O*-isopropylidene-α-D-allofuranose, **5**. Protection of O-3 as its TBS ether[11] was followed by selective hydrolysis of the 5,6-*O*-isopropylidene with aqueous HOAc and then cleavage of the resulting 5,6-diol with $NaIO_4$ in aqueous THF to afford the C-5 carboxaldehyde. Direct treatment with methyl (triphenylphosphoranylidene)acetate in CH_2Cl_2 solution provided the *trans* unsaturated ester **6** in 66% overall yield from **5**. Reduction of **6** with DIBAL in THF gave the allylic alcohol (67% + 10% recovered **6**), and then Sharpless epoxidation[12] mediated by (−)-diethyl D-tartrate delivered the 5*S*,6*R* epoxy alcohol **7**. The primary hydroxyl of **7** was oxidized with $RuCl_3$ and $NaIO_4$,[13] and then the crude 5*S*,6*S* epoxy acid was treated with NaN_3 in refluxing methanol to give the 5*S*,6*R* azido acid **8** (84% for two steps). No other isomer of **8** was detected by NMR

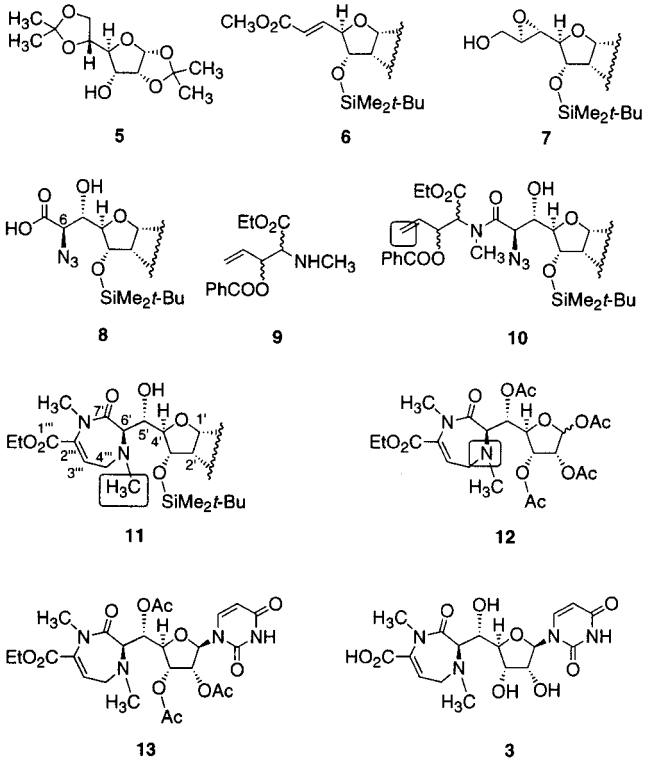

Figure 2.

analysis, indicating that the ring-opening reaction is highly site selective.

The azido acid **8**, which matches the stereochemistry of **3** at C-5′ and C-6′, was coupled to the sarcosine−acrolein adducts[9] **9** under the action of the peptide coupling reagent EEDQ in CH_2Cl_2.[14] A mixture of all four stereoisomers of **9** was used to ensure the formation of at least one stereoisomer of the dipeptide **10**, and in fact, only two isomers of **10** (2:1, 28%), of the four possible, were isolated. The efficiency of this coupling could undoubtedly be improved by selecting the best-matching amine partner **9**, but for the purpose at hand the mixture **10** proved to be a workable intermediate.

Cyclization of **10** to the desired diazepanone **11** was accomplished under conditions related to previous reductive amination sequences,[9] but with some surprising and helpful features. Exposure of **10** to ozone converted it to a stable azido ozonide, which was separately combined with triphenylphosphine in THF solution. The presumed imine intermediate was reduced in THF solution by treatment with excess sodium triacetoxyborohydride.[15] A single cyclized (and, under the basic conditions, eliminated) product (**11**, 28%) was formed. Astonishingly, the C-4 amine arrived *N*-methylated in **11** (-CH_3 in box), undoubtedly as the result of its reaction at some point with the formaldehyde liberated by reduction of the ozonide derived from **10** (=CH_2 in box).

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Both features (the in situ elimination and the *N*-methylation) advanced the cyclization product along the route toward **3**, and neither over-reduction nor dehydration interfered to any great extent.

Acetonide **11** was converted to its anomeric acetate **12** (72%, 4:1 mixture of C-1 isomers) by careful treatment with acetic anhydride and methanesulfonic acid (other acids, such as sulfuric or trifluoroacetic, were ineffective). Under these conditions, the hydroxyls at C-2 and C-5 were also acetylated, and the O-TBS group at C-3 was cleaved and the hydroxyl there acetylated as well. The resulting tetra-*O*-acetate **12** superficially resembles a wide variety of other anomeric acetates previously used for nucleoside (even "complex" nucleoside[5]) synthesis.[16] However, application of the standard conditions [bis(trimethylsilyl)uracil, TMS-OTf, refluxing acetonitrile, or 1,2-dichloroethane] to **12** gave no nucleoside product whatsoever. The problem was determined to be the nucleophilic N-4 (in box), which is positioned just six atoms away from the anomeric center. Lewis basic atoms have previously been observed to interfere with *O*- and *N*-glycosylations,[17] and the usual solution is to use an excess of the glycosyl donor to temporarily block the more Lewis basic site. As this is not an option here, the N-4 amino was instead pretreated with each of several different acids, including $SnCl_4$, methanesulfonic acid, BF_3, and HCl. Only as the hydrochloride salt did **12** provide any of the corresponding uracil nucleoside **13** (one isomer, 18%). The structure of **13** follows from the nearly first order ^{1}H NMR spectrum and its spectroscopic resemblance to similar compounds.[3,9] Deprotection with 0.5 N LiOH in ethanol solution and then purification by reverse-phase HPLC afforded the nucleoside triol acid **3** (64%).

For the synthesis of the 6′*S* diastereomer **4** (Figure 3), the C-5 carboxaldehyde derived from **5** as above was chain extended with the Still−Gennari reagent[10,18] to provide the *cis* unsaturated ester **14** (60%). The ester was reduced with DIBAL as before (55%), and the resulting primary alcohol was subjected to Sharpless epoxidation. Although some of the desired 5*S*,6*S* epoxy acid **15** was obtained in the presence of (+)-diethyl L-tartrate, the reaction was slow and did not proceed to completion. A more satisfactory solution was to epoxidize with *m*-chloroperoxybenzoic acid (CH_2Cl_2, 15 h), which led to the same diastereomer (67%). Oxidation as before gave the 5*S*,6*R* epoxy acid **16** (92%); however, the reaction of **16** with sodium azide was nonselective, giving rise to a 3:2 mixture of azido acids (78%). That the major product is the desired 5*S*,6*S* isomer **17** was established by subsequent transformations. The mixture of azido acids was coupled with amine **9** as before, leading to a mixture of two peptide isomers **18** (48%, theoretical 60%) with the same configuration at C-5′ and C-6′ (matching the major azido acid isomer) but differing in the C-2‴ and C-3‴ (liposidomycin numbering) configurations. The minor azido acid evidently did not couple. Cyclization of **18** to the diazepanone **19** was carried out as follows. A solution in CH_2Cl_2 was treated with ozone, concentrated, and then catalytically hydrogenated (10% Pd−C) in ethanol solution in the presence of HCl. The intermediate amino ozonide (*m*/*z* 685) was reduced with sodium triacetoxyborohydride as for **11** to give a 3:1 mixture of two diazepanone isomers (**19**, 8−21%) as the only cyclization products isolated. Neither elimination nor *N*-methylation occurred in this case, and the diazepanone ring stereochemistries could be assigned as 2‴*S*,3‴*S* and 2‴*R*,3‴*R*, respectively, by comparison to closely related model compounds (isopropyl replaces C-1′−C-5′ as the diazepanone substituent) with very similar proton coupling patterns.[9] Conversely, the match of the model systems with the more elaborate diazepanones **19** justifies somewhat further the earlier use of the models to assign relative stereochemistry. The C-2‴ and C-3‴ stereocenters are of no consequence here, however, as both isomers were readily converted to the same unsaturated diazepanone by treatment with 1,8-diazabicyclo[5.4.0]undec-7-ene (toluene, 1 h).[19] Separate *N*-methylation (formalin, sodium triacetoxyborohydride, CH_3CN, HOAc, 60 °C, 12 h) gave **20** (83% for two steps), the diazepanone epimeric at C-6′ with **11**. The reductive *N*-alkylation that provides **20** requires more forcing conditions than that for **11** owing to the formation of a stable intermediate oxazolidine (*m*/*z* 513) involving a methylene-bridged C-5′/C-6′ *erythro* amino alcohol. The situation where the C-6′ amino and C-5′ hydroxy are in close proximity (small dihedral angle) is seen in the Newman projection of **4** (Figure 1); *O*-cyclization of an (*N*-meth-

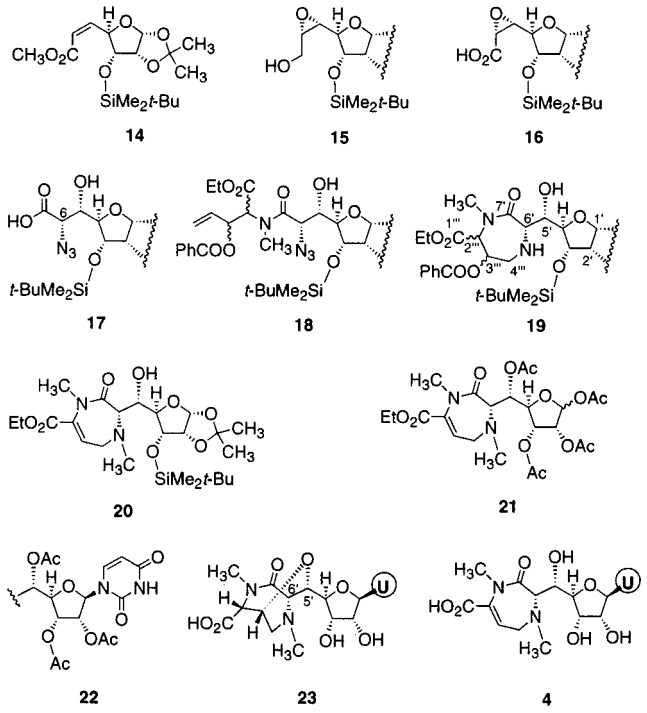

Figure 3.

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ylidene)iminium intermediate to an oxazolidine can more readily occur from a stable conformation when C-6′ has the *S* (rather than *R*) configuration.

Conversion of **20** to the tetraacetate **21** (1:1 anomeric mixture, 52%) was carried out as for **12**, and then **21** was pretreated with HCl and subjected to *N*-glycosylation as before to give the nucleoside **22** (15−20%). Deprotection of **22** with ethanolic 0.5 N LiOH gave after HPLC purification two nucleoside products: the desired nucleoside triol acid **4** (24%) and an isomeric cyclic ether **23** (60%), each as the trifluoroacetate salt. NMR analysis of **23** revealed reasonable upfield resonances for C-2‴ and C′3‴, and the appearance of an H-2‴/H-3‴ coupling ($J = 4.8$ Hz). H-6′ is a singlet, reflecting the profound change in the H-5′/H-6′ dihedral angle as the result of the O-5′/C-3‴ cyclization.

Comparison of the ^{1}H NMR spectra of the nucleoside triol acids **3** and **4** with the published spectra[3] of the liposidomycin degradation product in D_2O solution reveals a close match with **4** but not **3** (see tabulated data in Supporting Information). Furthermore, direct comparison of the spectrum of **4** at 600 MHz with that of an authentic sample of the liposidomycin degradation product under the same conditions of measurement confirms their identity down to the peak shapes. Therefore, the liposidomycins possess the 5′*S*,6′*S* absolute stereochemistry, and given the relative stereochemical determinations made previously,[9] C-2‴ (*S*) and C-3‴ (*S*) of **1** and **2** may also be assigned with confidence. At least seven research groups have published papers describing synthetic approaches to liposidomycin components;[9] clarification of the synthetic targets should sustain these efforts.

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Supporting Information Available: Experimental details and spectral characterization for all new compounds and ^{1}H NMR comparisons for **3** and **4**. This material is available free of charge via the Internet at http://pubs.acs.org.

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