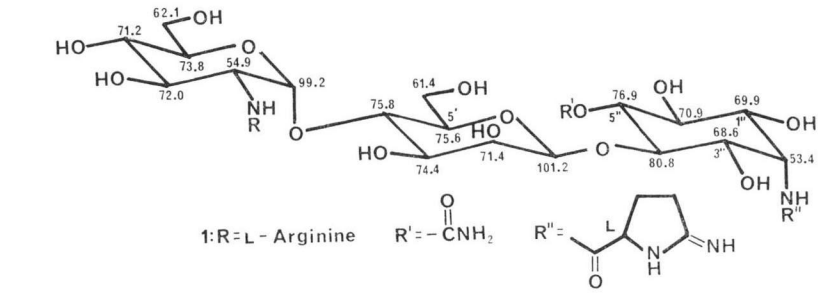
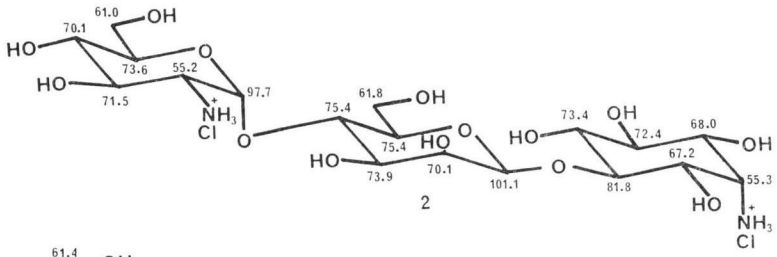
^{13}C NMR ANALYSIS OF LL-BM123α AND LL-BM782 ANTIBIOTICS

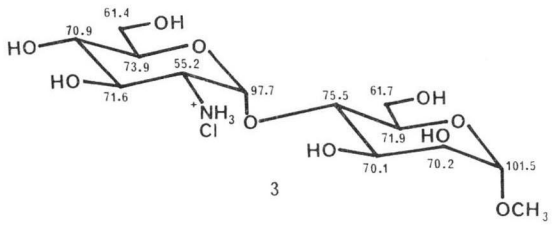
Sir:

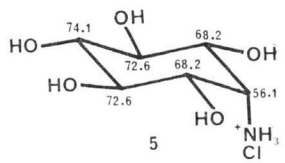
Recently we reported the isolation and structural characterization of two novel cyclitol antibiotics LL-BM123α (**1**)[1] and the LL-BM782 components (**6**)[2] which contain *myo*-inosamine-2 and *myo*-inositol respectively as the cyclitol moieties. Both antibiotics exhibit *in vivo* Gram-negative activity with LL-BM782 the more active of the two but also the more toxic. As seen in the structures, there are obvious differences between the two antibiotics but on closer inspection there are some commonalities as well. Both are acylated by strongly basic amino acids at the C_2 axial position of the cyclitol and both contain strongly basic functions on the terminal sugar: L-arginine in LL-BM123α and a guanidine on the 3-deoxy-3-aminomannose in the LL-BM782 series. NAKANISHI *et al.* recently showed the mannose and cyclitol subunits to be joined at the enantiotopic C_4 position of the cyclitol portion based on the CD benzoate chirality method.[3] In addition it was shown that the mannose glycosidic linkages are β instead of α based on the anomeric carbon-hydrogen coupling constants [$^1J(^{13}CH_1)$].

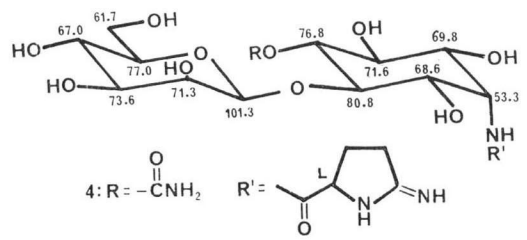
Our original α assignment of the mannose glycosidic linkage in LL-BM123α was based primarily on an erroneous ^{13}C NMR assignment which was influenced mistakenly by the assignments of the methanolysis product **3** which does











contain an α linkage at this point. Our initial assignment of an α linkage for the guanidinomannose unit in LL-BM782 followed similar reasoning due to the formation of an methyl 3-deoxy-3-guanidino-α-mannoside on methanolysis of **6**. Subsequently, we recorded the fully coupled proton ^{13}C NMR spectra of both antibiotics in D_2O and observed the mannose anomeric ^1J ($^{13}CH_1$) values to be 162.2 and 163.9 Hz for LL-BM123α and LL-BM782 respectively. Based on previous observations[4] on model methyl α- and β-glycosides (158~162 Hz for β glycosidic linkages; 169~171 Hz for α linkages measured in D_2O and $CDCl_3$), the above results caused us to reassign the mannose linkages as β in both antibiotics. Although the question seemed to be settled, some reservations remained in view of KASAI's more recent findings on a number of mannose and rhamnose glycosides where $^1J(^{13}CH_1)$ values of 153~156 Hz were observed for the β anomers and 164~166 Hz for the α anomers.[5] Since these spectra were recorded in deuteropyridine we attempted to record the coupled ^{13}C spectra of LL-BM123α and the LL-BM782 antibiotics in the same solvent. However, this was not possible due to the poor solubility of these compounds in deuteropyridine. In view of this and the previous misassignments of certain resonances, we present here a reevaluation of the ^{13}C NMR of these two antibiotics which is consistent only with the structural and stereochemical conclusions as depicted. ^{13}C NMR spectra were recorded at 20.0 and 25.1 MHz in D_2O. ^{13}C Chemical shifts were referenced to internal dioxane and reported in parts per million downfield from $Si(CH_3)_4$ (δ_c for dioxane 67.4 ppm).

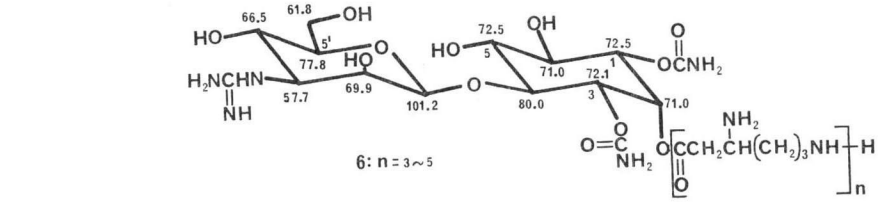
The ^{13}C spectra of LL-BM123α and key hydrolysis products were only briefly mentioned in the initial communication[1] and are discussed first. Assignments are given in the structural diagrams. Only the carbon atoms of the sugar moieties are presented. The carbon signals of the glucosamine subunit are readily identified based on the spectra of the methyl α-glycoside of glucosamine hydrochloride and the corresponding acetamide derivative.[6] The glycosidic linkage was originally defined as α by the $J_{1,2}$ of 3.5 Hz in the ^1H NMR spectrum of **1**[1] and subsequently confirmed by a fully coupled ^{13}C spectrum where the glucosamine anomeric $^1J(^{13}CH_1)$ is 173 Hz.[4] In addition, the C_1 and C_3 chemical shift differences between the intact antibiotic and the degradation products lacking the arginine moiety (**2** and **3**) are completely consistent with the expected β shifts on protonation or acylation of the C_2 nitrogen.

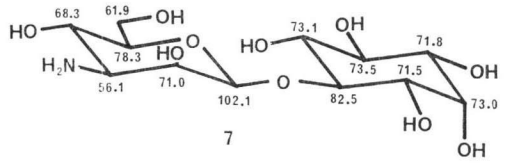
As mentioned above, the mannose carbons proved more difficult to assign due to confusion in assigning the $C_{5'}$ signal of mannose and the $C_{5''}$ signal of the *myo*-inosamine unit. The chemical shifts designated here are consistent only with a β glycosidic linkage at the mannose $C_{1'}$.[7] There is a shielding of 2 ppm at $C_{5'}$ in **1** and **2** compared to methyl β-mannoside due to glycosidation at $C_{4'}$ suggesting that the 4'-*O*-glycoside projects towards $C_{5'}$[8]. This is also apparent in the spectrum of the methanolysis product **3** where the $C_{5'}$ signal is shifted upfield to 71.9 ppm compared to 73.6 in methyl α-mannoside.[9] The $C_{3'}$ signal is essentially unaffected by the glycosidation at $C_{4'}$.

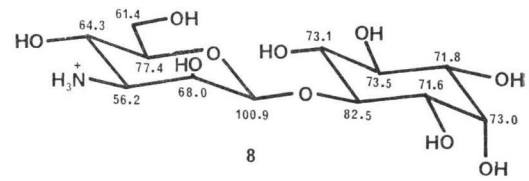
This leaves the assignments of the inosamine carbons. Those of carbons 1~4 and 6 are straightforward and are based on comparison with the corresponding values for *myo*-inosamine-2 hydrochloride (**5**) as well as with the *myo*-inosamine containing hydrolysis products **2** and **4**. Glycosidation at $C_{4''}$ causes a slight (0.7 ppm) shielding at $C_{5''}$ and a 1.0 ppm effect at $C_{3''}$ by comparing the spectra of **2** and **5**. Removal of the carbamoyl grouping results in the anticipated downfield shifts at $C_{4''}$ and $C_{6''}$ and an upfield shift of the $C_{5''}$ resonance in the spectrum of **2** compared with that of **1**.[9]

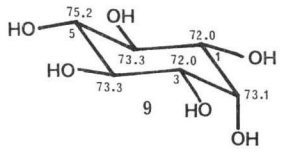
With regard to the LL-BM782 antibiotics, the assignments for the C_5 and $C_{5'}$ signals should be reversed from the previous ones.[2] This is based on the ^{13}C spectrum of the aminodecarbamoylated derivative **7** of the pseudodisaccharide LL-BM872ε.[2] The spectrum was recorded in both the free base form **7** and the protonated form **8**. The chemical shifts of the *myo*-inositol carbons are essentially unchanged by protonation whereas the signals of the aminomannose carbons are affected. Protonation results in the expected upfield shifts of $C_{2'}$ and $C_{4'}$ of 3 and 4 ppm respectively. A slight but significant upfield shift of 0.9 ppm is observed for the $C_{5'}$ resonance and a 1.2 ppm upfield shift is observed for the $C_{1'}$ signal.

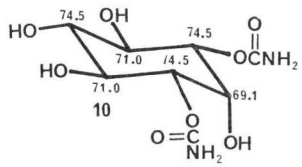
ANGYAL and ODIER have recently shown[10] that the original assignments[11] for the $C_{1,3}$ and $C_{4,6}$ signals of *myo*-inositol (**9**) are in error and should be reversed. This is based on the application of

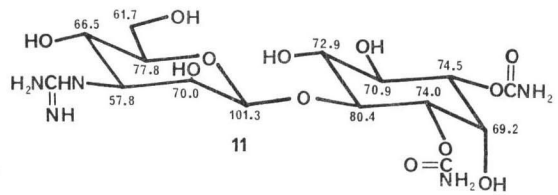












the KOCH-STUART method[12] of deuterium exchange to *myo*-inositol. This new finding has been taken into account in the present study and the assignments in structures **6**~**11** are in accord with this revision. All the chemical shift designations in this series are consistent with the anticipated changes due to carbamoylation,[9] esterification,[2] and glycosidation[6] starting from *myo*-inositol (**9**). Dicarbamoylation of **9** at C_1 and C_3 shifts these signals in **10** downfield by 2.5 ppm (α effect) whereas the C_4 and C_6 resonances are shifted upfield (β effect) by 2.3 ppm. The C_2 signal in **10** experiences an upfield β shift of a greater magnitude (4 ppm) due to the influence of two adjacent carbamate groupings. Glycosidation of **10** at C_4 to give **11** results in the expected large downfield shift of the C_4 signal from 71.0 to 80.4. This also results in a shielding at C_5 where that resonance is shifted upfield by 1.6 ppm. The C_3 resonance is only slightly moved upfield by 0.5 ppm. Chemical shifts at C_1, C_2, and C_6 are essentially unchanged by the glycosidation at C_4.

Esterification of **11** at C_2 with the oligopeptide side chain to give **6** results in almost no change in the shifts of C_4, C_5, and C_6. The C_1 and C_3 resonances, however, are now shifted upfield by 2 and 1.9 ppm respectively from the positions in **11** as anticipated.

The marked shielding at C_5 compared to C_3 on C_4 glycosidation as evidenced in the spectra of **6**, **8**, **9**, **10** and **11** strongly suggests that the 4-*O*-glycoside projects towards C_5 in the LL-BM782 antibiotics.[8]

In summary, the above ^{13}C NMR assignments provide strong support for a β glycosidic linkage connecting the mannose moiety with the cyclitol in both antibiotics consistent with the $^1J(^{13}CH_1)$ couplings. It remains unclear why KASAI's $^1J(^{13}CH_1)$ values are different from those recorded earlier by BOCK and PEDERSEN.

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