A Circular Dichroic Method for determining the Linkage to Symmetric Hexocyclitols and Hexocyclitolamines: Structure of Two Antibiotics from *Nocardia* sp.

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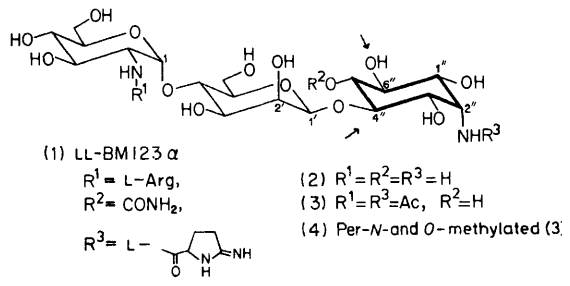
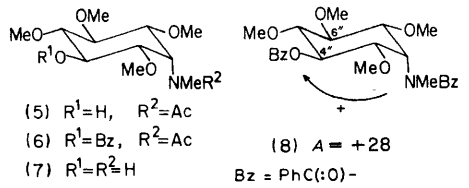
Summary A highly sensitive c.d. method which appears to have general applicability has been developed to determine the point of attachment of substituents on symmetric hexocyclitolamine and hexocyclitol residues; it has been applied in the structure determination of two antibiotics LL-BM 123α and LL-BM 782α_2.

HEXOCYCLITOLAMINE and hexocyclitol residues are present in a number of natural products such as the antibiotics (**1**) and (**9**) (thick lines). Because of the symmetry of these moieties, no chemical method has yet been described which resolves the point of attachment of other groups to available enantiotopic hydroxy-groups such as at positions 4 and 6 on the cyclitol subunits of (**1**) and (**9**). *X*-Ray analysis of course provides a solution but often these natural products, even in a highly purified form, are very difficult to crystallize. We describe here a practical and highly sensitive c.d. method which appears to have general applicability to solve such problems.

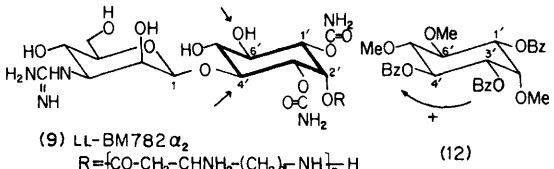




The antibiotic LL-BM 123α isolated from a species of *Nocardia* exhibits moderate *in vivo* activity against gram-negative bacteria and is remarkably nontoxic; structural studies led to the structure (**1**) where it was left undecided whether the mannose unit was linked to 4″ or 6″ (arrows) of the myoinosamine unit (and with the α-configuration at 1′).[1] The following evidence leads to the full structure (**1**). The trisaccharide (**2**)† (25 mg)[1] was *N*-acetylated with MeOH–Ac_2O (75 min, room temp.) to give (**3**),† which was permethylated (NaH–Me_2SO–MeI) to afford 9 mg of (**4**).† Methanolysis (MeOH–HCl, 1:1 v/v, 5 h) of (**4**) and column chromatography gave 4 mg of the *N*-acetyl-*O*,*N*-permethylated glucosaminylmannose derivative and four other products as detectable spots on t.l.c. with common spraying agents. However, since none of them showed a ^{1}H n.m.r. spectrum corresponding to the expected product (**5**), the remaining eluates were combined and benzoylated for spectroscopic detection; column chromatography and t.l.c. of the benzoylated eluate gave 0·5 mg of (**6**),† which was hydrolysed to (**7**) by dissolving it in a trace of MeOH and refluxing in aqueous $Ba(OH)_2$ for 3 h; (**7**)† showed OMe ^{1}H n.m.r. signals at δ 3·59, 3·49, 3·44, and 3·42 and one NMe signal at δ 2·57. Treatment of (**7**) with *p*-bromobenzoyl chloride in pyridine–Et_3N at 0 °C overnight gave 50 μg of the *p*-bromobenzoate (**8**)† which was purified by column chromatography and t.l.c.

Chemical ionization mass spectrometry with methane confirmed (**8**) to be the *O*,*N*-dibenzoyl compound. The amount of this compound, in a cell, was estimated from the standard u.v. ε value of 38,200 at 244·5 nm for di-*p*-bromobenzoates[2] without weighing of the sample, and from this it was possible to estimate‡ the Δε value of the exciton-split c.d. curve at 253/236 nm.[3,4]

Since (**8**) exhibits a positive split c.d. with an amplitude of +28,§ the OBz group is located at the 4″- and not the 6″-position. The point of attachment to the mannose unit is therefore also the 4″-position. In BM 123α $^1J_{CH}$ for C-1′ is 162·2 Hz. From the published values for α- and β-anomers, the $^1J_{CH}$[5–7] value shows that the anomeric configuration at 1′ is β. This leads to structure (**1**) for this antibiotic.



Structure (**9**) was assigned to the broad-spectrum antibiotic LL-BM 782α_2,[8] where an α-configuration was tentatively assigned to C-1 and attachment of the 3-deoxy-3-guanidino-D-mannose to myoinositol was left open (4′ or 6′, arrows).

† Compound which was purified by column chromatography and characterized by ^{1}H n.m.r. and mass spectroscopy, *etc.*

‡ The u.v. and c.d. properties of *N*-benzoates are similar to those of *O*-benzoates.

§ The difference in Δε values between the longer and shorter wavelength extrema of split Cotton effects.

The acid hydrolysis product (**10**)[9]† (10 mg) was permethylated to give (**11**),† the ^{1}H n.m.r. spectrum of which showed six OMe signals (δ 3·68—3·40) and five NMe signals¶ (δ 3·01—2·20). Permethylated (**11**) was submitted to methanolysis (20 h), $Ba(OH)_2$ hydrolysis (18 h) and *p*-bromobenzoylation to yield (**12**).† The above-mentioned m.s. → u.v. → c.d. sequence gave $\Delta\epsilon_{252} + 35{\cdot}3/\Delta\epsilon_{235} - 15{\cdot}9$ or $A = +51$. In the 1′,3′,4′- (or 1′,3′,6′-) tribenzoate system (**12**), the dibenzoate systems 1′,3′ and 1′,4′ (or 6′) do not lead to chirality. The positively split Cotton effects (+ 51) unambiguously show that the chirality is between the 3′,4′-dibenzoate system, and that therefore the original linkage is at 4′. The $^1J_{CH}$ 163·9 Hz (C-1) value again shows that the anomeric linkage is β.

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¶ Location of N-Me groups unassigned.

[1] G. A. Ellestad, J. H. Martin, G. O. Morton, M. L. Sassiver, and J. E. Lancaster, *J. Antibiot.*, 1977, **30**, 678.
[2] H. W. Liu and K. Nakanishi, *J. Am. Chem. Soc.*, in the press.
[3] N. Harada and K. Nakanishi, *Acc. Chem. Res.*, 1972, **5**, 257.
[4] N. Harada and K. Nakanishi, 'Circular Dichroic Spectroscopy—Exciton Coupling in Organic and Bioorganic Stereochemistry,' University Science Books, Mill Valley, California, 1981, in the press.
[5] K. Bock, I. Lundt, and C. Pedersen, *Tetrahedron Lett.*, 1973, 1037.
[6] K. Bock and C. Pedersen, *Acta Chem. Scand.*, 1975, **B29**, 258.
[7] R. Kasai, J. Okihara, K. Asakawa, K. Mitzutani, and O. Tanaka, *Tetrahedron*, 1979, **35**, 1427.
[8] W. J. McGahren, B. A. Hardy, G. O. Morton, F. M. Lovell, N. A. Perkinson, R. T. Hargreaves, D. B. Borders, and G. A. Ellestad, *J. Org. Chem.*, 1981, **46**, 792.