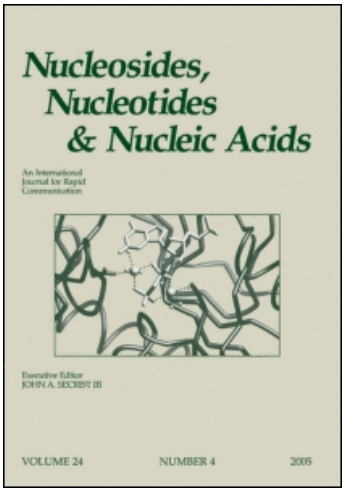

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Differentiation of Anomeric & Positional Isomers of Acyclic & Cyclic Nucleosides by FAB Tandem Mass Spectrometry & NMR

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DIFFERENTIATION OF ANOMERIC & POSITIONAL ISOMERS OF ACYCLIC & CYCLIC NUCLEOSIDES BY FAB TANDEM MASS SPECTROMETRY & NMR

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We have been interested in developing routine methods, using standard ^{1}H and ^{13}C NMR spectroscopy of assigning anomeric configuration for both acyclic and cyclic nucleosides of the types (1) &(2) respectively.In the latter class of compounds we have also sought to determine the site of glycosidation (viz N^{1} or N^{3} with respect to the imidazole ring).We have extended this study using FAB tandem mass spectrometry[1] to identify stereochemical differences.

Acyclic nucleosides

A range of NMR criteria have been examined for a series of N-acyl-D-ribosylamines (1) and the final assignment in any one case is made on the basis of the best fit to the set of criteria rather than rely on a single parameter.The criteria include:

i The α-anomeric proton generally has a more down field shift than the corresponding β-anomeric proton.

ii The C-1 in the β-anomers is shifted downfield (ca 5ppm) relative to the corresponding α-anomer.

iii Consistantly larger values of $J_{1,2}$ were observed for the α-anomers (mean 3.9Hz) compared with the β-anomers (mean 1.8Hz).

iv Larger values of $J_{3,4}$ were observed for the β-anomers (mean 0.9Hz) compared with the α-anomers (mean 2.0Hz).

v The observed values of $J_{1,NH}$ are in the range 9.0-9.6Hz in the α-anomers close to the value of 9.4Hz calculated from $^{3}J = 9.4\cos^{2}\theta - 1.1\cos\theta + 0.4^{2}$.Due to greater conformational freedom in the β-amomers smaller and variable couplings were observed (mostly 6.0-8.9Hz)

Assignments made on the best fit to these criteria were supported by an extensive NOE study of (1a) and (1b).

Previous FAB tandem mass spectrometric studies have proved to be of diagnostic value in differentiating α-and

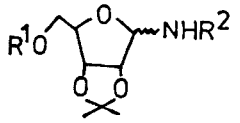
β-anomers.[3] The FAB mass spectra of, for example (1a) and (1b), showed similar fragmentation patterns.However the collision-activated dissocation-mass analysed kinetic energy sequential mass spectra (FAB CAD-MIKE) of the parent ion $[M+H]^+$ at m/z274 revealed different spectra.The daughter ions $[MH-CH_2CO]^+$ at m/z232 and $[MH-AcOH-CH_3COCH_3]^+$ at m/z156 were observed for the β-anomer (1b) but not for the α-anomer (1a).

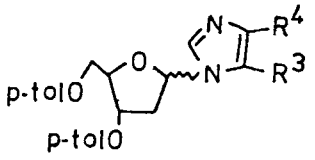
Cyclic nucleosides

^1H NMR spectroscopy proved reliable in identifying anomeric forms of the D-2'-deoxyribofuranosyl imidazole nucleosides (2)[4]. The splitting patern for H-1' was the best indication of anomeric configuration since the compling $J_{1',2''}$ is 2-3Hz in the α-anomers and 7Hz in the β-anomers. A larger value by 1Hz of $J_{2',2''}$ is also observed in the α-forms. The site of glycosylation can be determined by several features of both ^1H and ^{13}C spectra:

i The H-1' is deshielded by 0.4-0.6ppm in the N^3-isomers (2c) & (2d).
ii The nonequivalence (ca.0.04ppm) of the prochiral protons in the OEt group in the N^3-isomers (2c) & (2d).
iii The deshielding (0.3-0.7ppm) of the $-NH_2$ group in the N^1-isomers (2a) & (2b).
iv The C-4 and C-5 chemical shifts are also diagnostic since they are separated by ca.35ppm in N^1-isomers (2a & 2b) and ca.55ppm in N^3 isomers (2c & 2d).

The FAB MS/MS MIKE and CAD-MIKE and MS/MS hybrid sequential analysis of the positional isomers, cyclic nucleosides (2a) & (2c), showed characteristic differences. For example, [MH - 234]$^+$ at m/z 274 and $[MH-EtOH]^+$ at m/z 110 were absent for (2a) but present for (2c) in the CAD-MIKE spectra.





1 a R^1=Ac, R^2=Ac(α)
b R^1=Ac, R^2=Ac(β)
c R^1=H R^2=$COCH_2CO_2Et$(α)
d R^1=H R^2=$COCH_2CO_2Et$(β)

2 a R^3=NH_2, R^4=CO_2Et(α)
b R^3=NH_2, R^4=CO_2Et(β)
c R^3=CO_2Et, R^4=NH_2(α)
d R^3=CO_2Et, R^4=NH_2(β)

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