A New and Unequivocal Method for Establishing the Position of *N*-Glycosylation of Unsymmetrically *C*-Substituted Imidazoles

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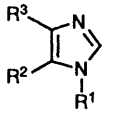
N-Substitution of an unsymmetrically *C*-substituted imidazole can give rise to a pair of structurally isomeric derivatives and to differentiate between such related compounds can be difficult. Two methods, one spectroscopic and one chemical, for ascertaining the orientation of such *N*-substitutions are described, with particular application to the establishment of the direction of *N*-ribosidation of a series of halogeno nitroimidazoles.

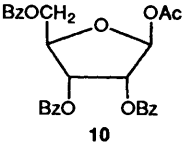
N-Unsubstituted imidazoles exhibit a tautomerism which can become evident upon the *N*-alkylation of an unsymmetrically *C*-substituted imidazole. In such circumstances, the reaction conditions and physicochemical properties of the reactants determine the ratio of structurally isomeric products,[1] whose subsequent separation and substituent-orientation elucidation can be potentially problematic. Whilst conducting a study on the *N*-ribosidation of some such above-mentioned imidazoles, an example of an *N*-alkylation which could give rise to a pair of structurally isomeric ribosides, it became necessary to assign unambiguously the orientation of *N*-ribosidation in each nucleoside product. We now wish to report a new chemical/chromatographic technique, which provides a rigorous confirmation of conclusions reached from UV spectroscopic analysis, by which this was achieved.

Results and Discussion

The halogeno nitroimidazoles **1–3**, each of which is in tautomeric equilibrium with the corresponding 5-halogeno-4-nitroimidazole, were treated with 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose **10** according to a literature procedure.[2] In every case only a single ribosidic product was isolated which was deprotected by treatment with saturated methanolic ammonia to afford the corresponding nucleoside. One of two structures, **11** or **12**, **13** or **14** and **15** or **16** are possible for each of these products, in all of which the β-anomeric conformation at C-1′ is assumed from earlier observations.[2,3] To permit the differentiation between each of these two possible structures, the isomeric pairs of *N*-methylimidazoles **4** and **5**, **6** and **7**, and **8** and **9**, of known substituent orientation, were synthesised for use as model compounds. 4(5)-Chloro-5(4)-nitroimidazole, **1**, prepared by heating 4(5)-bromo-5(4)-nitroimidazole **2**[4] (see below) with concentrated hydrochloric acid,[5] was *N*-methylated using dimethyl sulfate to afford a mixture of 4-chloro-1-methyl-5-nitroimidazole **4**[6–8] and 5-chloro-1-methyl-4-nitroimidazole **5**[7,8] which were separated by chromatography upon a column of neutral alumina. 4(5)-Bromo-5(4)-nitroimidazole, prepared from imidazole by sequential bromination to 2,4,5-tribromoimidazole, nitrate salt formation and heating with sulfuric acid,[4] was *N*-methylated using dimethyl sulfate[9] to afford a mixture of 4-bromo-1-methyl-5-nitroimidazole[9] **6** and 5-bromo-1-methyl-4-nitroimidazole **7**[9] which were separated chromatographically upon a column of neutral alumina. Iodination of imidazole gave 4,5-diiodoimidazole[10]† which upon reaction with a mixture of fuming nitric acid and concentrated sulfuric acid at −20 °C yielded 4(5)-iodo-5(4)-nitroimidazole **3**.[10] *N*-Methylation of this product with dimethyl sulfate in boiling 1,4-dioxane under reflux afforded 4-iodo-1-methyl-5-nitroimidazole **8**,[10] whereas when the reaction was effected in hot aqueous sodium hydroxide a mixture of the previous product, which was removed by washing the total reaction mixture with acetone, and the isomeric 5-iodo-1-methyl-4-nitroimidazole **9** was obtained.[10]



	R^1	R^2	R^3		R^1	R^2	R^3
1	H	NO_2	Cl	**6**	Me	NO_2	Br
2	H	NO_2	Br	**7**	Me	Br	NO_2
3	H	NO_2	I	**8**	Me	NO_2	I
4	Me	NO_2	Cl	**9**	Me	I	NO_2
5	Me	Cl	NO_2				



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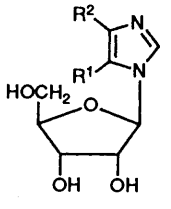

	R^1	R^2
11	Cl	NO_2
12	NO_2	Cl
13	Br	NO_2
14	NO_2	Br
15	I	NO_2
16	NO_2	I

Various spectroscopic techniques have been investigated as possible means of distinguishing between structurally isomeric pairs of *N*-substituted imidazoles. IR spectroscopy and mass spectrometry have been found to be of no significant diagnostic value in differentiating between isomeric pairs of 1-alkyl-4 (or 5)-monosubstituted or unsymmetrically C-substituted imidazoles[11] and, likewise, the IR and mass spectra of the present

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Table 1 UV spectroscopic details of compounds **4–9**

Compd.	λ_{max}/nm (ε)		λ_{min}/nm (ε)		Compd.	λ_{max}/nm (ε)	λ_{min}/nm (ε)	λ_{infl}/nm (ε)
	303	237	263	220		295	254	224
4	(9700)	(4550)	(2990)	(4050)	**5**	(8100)	(2350)	(5800)
	307	242	268	223		298	255	223
6	(6750)	(3600)	(2350)	(3400)	**7**	(6450)	(1500)	(5700)
	322	259	285	228		314	268	238
8	(6050)	(3850)	(3000)	(3100)	**9**	(9650)	(2400)	(6400)

All spectra obtained in 96% EtOH.

Table 2 UV spectroscopic details of compounds **11**, **13** and **15**

Compd.	λ_{max}/nm (ε)	λ_{min}/nm (ε)	λ_{infl}/nm (ε)
	294	255	225
11	(6400)	(2100)	(5900)
	299	258	223
13	(5940)	(1550)	(5250)
	314	269	238
15	(6950)	(2350)	(5200)

All spectra obtained in 96% EtOH.

three isomeric pairs of model compounds were all markedly similar with no distinguishing features. ^{1}H NMR spectroscopy has been employed to distinguish 1,4- from 1,5-disubstituted imidazoles by noting differences between the cross-ring coupling constants of 2-H and 5-H or 4-H, respectively,[11] but this technique is not applicable in the present study because the imidazoles under investigation possessed only one ring-proton. ^{13}C NMR spectroscopy has been successfully utilised to distinguish between some 1-alkyl-4 (or 5)-nitroimidazoles[12] but the feasibility of the technique has not been investigated in this study. However, significant differences between the UV spectra of isomeric pairs of *N*-alkylnitroimidazoles have been observed,[6,10] and, in relation to the present study, compounds **8** and **9** have previously been reported[10] to have λ_{max} 323 and 258 nm (ε 6870 and 4080), and λ_{max} 315 and λ_{infl} 240 nm (ε 7600 and 5000), respectively. These data have been largely confirmed, and extended, in the present study in which similar differences have also been discerned between the UV spectra of the isomeric pairs of compounds **4** and **5**, and **6** and **7** (Table 1).

The orientation of each of the present *N*-ribosidations was, therefore, determined, after the necessary removal of the three UV-absorbing benzoyl moieties, by comparison of the UV spectrum of the product with those of the above corresponding model isomeric pair (Table 2). Consequently, the three deprotected nucleosides were clearly assignable as the 5-halogeno-4-nitro-ribofuranosylimidazoles **11**, **13** and **15**, respectively.

However, since only one nucleosidic product could be isolated from each of the current ribosidations, it was not possible to effect a direct UV comparison between structurally isomeric pairs of such products. Furthermore, in the cases of the initially formed protected nucleosides, the orientation of *N*-ribosidation could not be ascertained because the UV absorption of the protecting benzoyl moieties completely masked that of the imidazoyl moiety. It was, therefore, considered desirable to establish rigorously the orientation of the above *N*-ribosidations by an alternative method and the following chemical/chromatographic procedure was, accordingly, developed.

Quaternisation of each nucleosidic product using dimethyl sulfate could be effected without the need to deprotect, although this permitted the use of milder reaction conditions in the subsequent quaternisations. In both situations, acid-catalysed hydrolysis of the quaternary product yielded the corresponding *N*-methylhalogenonitroimidazole having the opposite *C*-substituent orientation to that of the original nucleoside. The imidazolic product could be readily identified since it was found that a significant difference in R_F values exists between the isomers in each of the three pairs of model compounds **4** and **5**, **6** and **7**, and **8** and **9** (Table 3). Indeed, this is not surprising since each isomeric pair was preparatively separable by column chromatography. Furthermore, the imidazolic component resulting from hydrolysis was isolable by TLC and characterised by UV spectroscopy and melting point determination. Each of the three nucleosides yielded the corresponding 4-halogeno-1-methyl-5-nitroimidazoles **4**, **6** and **8**, thereby confirming their structures as **11**, **13** and **15**, respectively.

This procedure provides a facile general method by which the substituent orientation(s) in the product(s) resulting from the *N*-glycosylation of an unsymmetrically *C*-substituted imidazole may be unequivocally established, provided the corresponding pair of *N*-methylated imidazoles of known substituent orientation are available. Such an approach would be of value where the various spectral techniques are either inapplicable or give results which are open to doubt.

Experimental

M.p.s were determined on a Kofler hot stage apparatus and are corrected. Microanalyses were carried out on a Perkin-Elmer 240 analyser. UV spectra were recorded on a Pye-Unicam SP8-500 spectrophotometer and wavelengths are expressed in nm. Mass spectral data were obtained using an AEU-M530 mass spectrometer equipped with a DS-55 data system. Qualitative TLC was performed on either pre-coated silica gel (UV_{254}) or neutral aluminium oxide (UV_{254}) microplates (Camlab: polygram SIL G-UV_{254} and polygram ALOX N/UV_{254}) and R_F values, as appropriate, are quoted in the experimental text. Spots were detected by fluorescence quenching at 254 nm. Column chromatography was carried out using either neutral aluminium oxide (Merck: neutral aluminium oxide 90, activity 1, 70–230 mesh) or silica gel (Fisons, 60–120 mesh) as stationary phases and columns were prepared by wet packing. ^{1}H NMR spectra were recorded on a Bruker WP80 spectrometer operating at 80 MHz using tetramethylsilane (TMS) as an internal standard. All evaporations were carried out at less than 30 °C. All starting materials were purchased from the Aldrich Chemical Co. Anhydrous solvents were prepared according to standard procedures. 4(5)-Chloro-, bromo- and iodo-5(4)-nitroimidazoles (**1**,[5] **2**[4] and **3**[10]), 4-iodo-1-methyl-5-nitroimidazole **8**[10] and 5-iodo-1-methyl-4-nitroimidazole **9**[10] were all prepared according to the established literature procedures.

Chromatographic Systems.—Neutral aluminium oxide: (A) chloroform–diethyl ether (1:3) and (B) chloroform–diethyl ether (1:1).

Silica gel: (C) diethyl ether–light petroleum (b.p. 30–40 °C) (35:65), (D) diethyl ether–chloroform (3:1), (E) diethyl ether–

Table 3 R_F Values of compounds **4–9**

Compd.	R_F	Compd.	R_F	Chromatography system
4	0.47	**5**	0.28	A
6	0.33	**7**	0.18	B
8	0.77	**9**	0.44	A

chloroform (1:1), (F) diethyl ether–light petroleum (b.p. 30–40 °C) (85:15) and (G) chloroform.

4-Chloro-1-methyl-5-nitroimidazole **4** *and 5-Chloro-1-methyl-4-nitroimidazole* **5**.—These compounds were prepared by application of the literature procedure for the preparation of the corresponding bromo analogues,[9] but with modification of the work-up procedure to involve product separation by column chromatography (system A) instead of by fractional recrystallisation. Thus, treatment of 4(5)-chloro-5(4)-nitroimidazole (4.15 g, 28.1 mmol) with dimethyl sulfate (2.7 cm^3) afforded, after work-up, a brownish oil from which was initially eluted 4-chloro-1-methyl-5-nitroimidazole **4** (250 mg, 5.5%), m.p. 74–75 °C (from EtOH) (lit.,[6–8] 76–77, 77–78, 78 °C), followed by 5-chloro-1-methyl-4-nitroimidazole **5** (321 mg, 6.9%), m.p. 144–145 °C (from EtOH) (lit.,[7,8] 148, 147–148 °C).

4-Bromo-1-methyl-5-nitroimidazole **6** *and 5-Bromo-1-methyl-4-nitroimidazole* **7**.—These compounds were prepared from 4(5)-bromo-5(4)-nitroimidazole according to the literature method[9] but again employing column chromatography (system B) in place of fractional recrystallisation to separate the products. 4-Bromo-1-methyl-5-nitroimidazole **6** was eluted first (8.5 mg, 0.1%), m.p. 98–100 °C (from water) (lit.,[8] 101–102 °C), followed by 5-bromo-1-methyl-4-nitroimidazole **7** (21.5 mg, 0.3%), m.p. 187–189 °C (from water) (lit.,[9] 188 °C).

Preparation of Benzoylated Nucleosides: General Method.—All such nucleosides were prepared by application of a general literature procedure involving the stirring together, at room temperature for 2 h with exclusion of moisture, of the appropriate *N*-unsubstituted nitroimidazole (5 mmol), 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose (2.52 g, 5 mmol), hexamethyldisilazane (0.85 ml, 4 mmol), trimethylchlorosilane (0.5 ml, 4 mmol) and tin(IV) chloride (0.7 ml, 6 mmol) in absolute acetonitrile (75 cm^3) under dry nitrogen gas.[2]

Deprotection of Benzoylated Nucleoside.—The starting material was dissolved in a minimum volume of dry saturated methanolic ammonia and the solution stirred for 2 h at room temperature, with exclusion of moisture; after this time, the solvent was removed under reduced pressure to afford the crude deprotected nucleoside.

5-Chloro-4-nitro-1-β-D-ribofuranosylimidazole **11**. The benzoylated nucleoside was prepared as above, using 4(5)-chloro-5(4)-nitroimidazole **1** (750 mg, 5 mmol) as the imidazolic component. Column chromatography of the crude product (systems C, R_F 0.05, and D, R_F 0.85, respectively) yielded the pure acylated nucleoside as a foam (2.29 g, 77.3%) (Found: C, 58.6; H, 3.6; Cl, 16.2; N, 6.9. $C_{29}H_{22}ClN_3O_9$ requires C, 58.85; H, 3.75; Cl, 16.0; N, 7.1%). Deprotection furnished a yellowish syrup and column chromatography of this (system E, R_F 0.35) yielded the title compound as a pale yellow foam which could not be crystallised (405 mg, 43%); $\delta_H(CD_3SOCD_3)$ 5.60–5.82 (2 H, m, exchangeable-collapsed to symmetrical d, 1 H, 5.74, $J_{1',2'}$, 4.2 Hz, anomeric-H and 2′-OH), 8.36 (1 H, s, imidazoyl 2-H); (Found: *m/z* 280.0334 $C_8H_{10}ClN_3O_6$ requires 280.0337 for $M^+ + 1$).

5-Bromo-4-nitro-1-β-D-ribofuranosylimidazole **13**. The benzoylated nucleoside was prepared as above, using 4(5)-bromo-5(4)-nitroimidazole **2** (958 mg, 5 mmol) as the imidazolic component. Column chromatography of the crude product (systems C, R_F 0.05, and E, R_F 0.8, respectively) yielded the pure acylated nucleoside as a foam (2.66 g, 85%) (Found: C, 55.0; H, 3.55; Br, 12.7; N, 6.5. $C_{29}H_{22}BrN_3O_9$ requires C, 54.7; H, 3.48; Br, 12.55; N, 6.6%). Deprotection afforded the title compound as a brownish solid (612 mg, 50%), m.p. 172–173 °C (from EtOH) (lit.,[13] 174–175 °C) (Found: C, 29.7; H, 3.1; Br, 24.4; N, 13.0. $C_8H_{10}BrN_3O_6$ requires C, 29.6; H, 3.1; Br, 24.7; N, 13.0%); $\delta_H(CD_3SOCD_3)$ 5.56–5.79 (2 H, m, exchangeable—collapsed to a symmetrical d, 1 H, 5.71, $J_{1',2'}$, 4.7, anomeric-H and 2′-OH), 8.4 (1 H, s, imidazoyl 2-H); *m/z* 324 (M^+, 2% and exhibiting a typical bromine isotopic peak arrangement).

5-Iodo-4-nitro-1-β-D-ribofuranosylimidazole **15**. The benzoylated nucleoside was prepared as above, using 4(5)-iodo-5(4)-nitroimidazole **3** (1.2 g, 5 mmol) as the imidazolic component. Column chromatography of the crude product (systems F, R_F 0.6, C, R_F 0.0, and G, R_F 0.9, respectively) yielded the pure acylated nucleoside as a yellow foam (2.07 g, 61%) (Found: C, 51.3; H, 3.4; I, 18.2; N, 6.3. $C_{29}H_{22}IN_3O_9$ requires C, 50.9; H, 3.25; I, 18.6; N, 6.15%). Deprotection afforded the title compound as a cream coloured solid (256 mg, 27%), m.p. 178 °C (decomp.) (from acetone–water) (Found: C, 26.1; H, 2.7; I, 34.4; N, 11.1. $C_8H_{10}IN_3O_6$ requires C, 25.85; H, 2.7; I, 34.3; N, 11.3%); $\delta_H(CD_3SOCD_3)$ 5.48–5.79 (2 H, m, exchangeable—collapsed to symmetrical d, 1 H, 5.72, $J_{1',2'}$, 4.7 Hz, anomeric -H and 2′-OH), 8.46 (1 H, s, imidazoyl 2-H); *m/z* (CI) 372 ($M^+ + 1$, 5.6%).

General Quaternisation Procedures.—Of benzoylated (protected) nucleosides. The starting material (10 mg) was placed in a 5 cm^3 ampoule and methyl iodide (2 cm^3) added. After freezing by immersion in liquid nitrogen, the ampoule was immediately sealed and then heated in a steam-bath for 8 h (no reaction occurred after an earlier period of 2 days at room temperature). Subsequent removal of the excess of methyl iodide (allowed to evaporate at room temperature in an efficient fume cupboard) yielded the quaternary derivative.

Of deprotected nucleosides. The starting material (5 mg) was dissolved in anhydrous methanol (5 cm^3) and dimethyl sulfate (1 cm^3) added, care being taken to exclude moisture. The reaction mixture was stirred overnight (*ca.* 18 h) at room temperature after which time the solvent and excess of dimethyl sulfate were removed [the latter by co-evaporation with absolute EtOH (3 × 10 cm^3)] to yield the quaternary derivative.

General Procedure for Acid-catalysed Hydrolysis of Quaternary Derivatives.—To each quaternary derivative, hydrochloric acid (1 mol dm^{-3}; 5 cm^3) was added and the reaction mixture heated over a steam-bath for 6 h. After cooling, the crude product was made slightly alkaline (pH 8.5) by careful addition of aqueous sodium carbonate (10%) and shaken with chloroform (2 × 15 cm^3). The organic phases were pooled, dried, filtered and evaporated to furnish the hydrolysis product. The imidazolic component of this was initially identified by comparative TLC and UV absorption spectroscopy. Preparative TLC in the appropriate system followed by recrystallisation from the appropriate solvent yielded pure imidazolic material which was identified by its melting point and mixed melting point with authentic material.

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