SITE SELECTIVE ALKOXYMETHYLATION OF IMIDAZO[4,5-*b*]PYRIDINES: STRUCTURAL ANALYSIS BY HIGH FIELD NMR METHODS

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Abstract - The alkylation reactions of 2-aryl-1(3)*H*-imidazo[4,5-*b*]pyridines (equivalent to 1-deazapurines) with alkoxymethyl chlorides and bromoacetonitrile are described. The structural assignments of the products were made by the use of two-dimensional ^{1}H-^{1}H NOE (NOESY) and selective INEPT (INAPT) ^{13}C nmr experiments utilizing polarization transfer from carbon-bound hydrogens in the alkyl side chains to selected ^{13}C resonances via long-range $^{3}J_{CH}$ couplings. Although three isomeric *N*-alkyl derivatives could arise from a single heterocycle based on considerations of tautomeric equillibria, however, the reactions exhibit marked site selectivity even under quite different reaction conditions. Thus, *N*-3 alkyl derivatives are produced exclusively in basic (Et_3N/NaH) nonpolar media following an S_E2cB mechanism. Solvent effects are evident in a loss of N-3 *vs* N-1 selectivity for alkylation when the polar aprotic solvent DMF is used. Under neutral conditions direct alkylation occurs at the *N*-4 position following an S_E2' mechanism. The overall site selectivity appears to be governed by the relative reactivity of individual nucleophilic sites rather than the tautomeric composition in solution.

INTRODUCTION

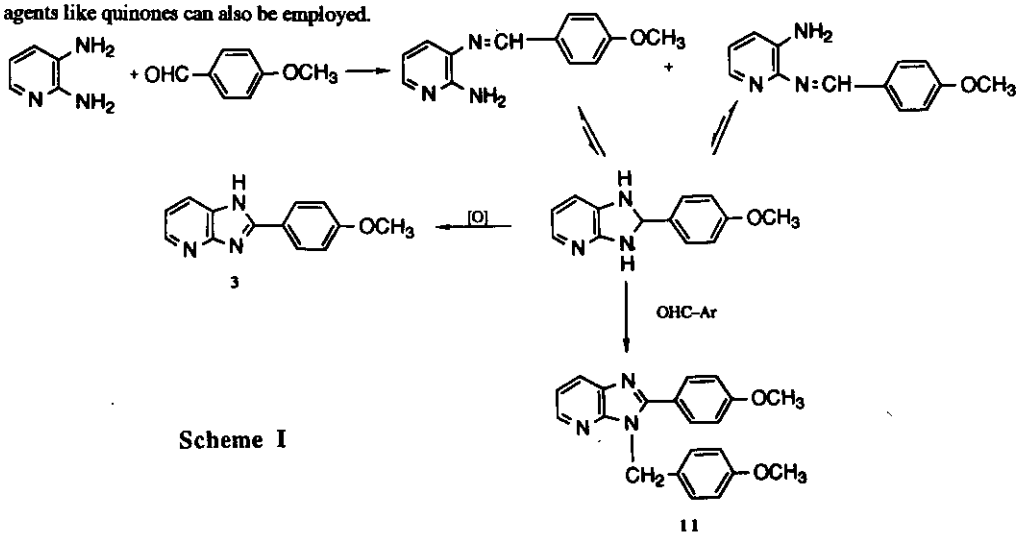
Recent publications have called attention to the rational design of DNA sequence selective agents based on structural modification of naturally occurring and existing synthetic DNA minor groove binding agents and their efficacy as anticancer, antiviral and antiretroviral agents.[1] As part of an ongoing program on such agents in our laboratories, we recently reported the synthesis and DNA binding characteristics of certain bis-imidazo[4,5-*b*]pyridine analogs of Hoechst 33258.[2] During the course of this synthesis, that required the use of an *N*-protected 2-methoxyphenyl-3*H*-imidazo[4,5-*b*]pyridine intermediate, we observed an unusually high site selectivity of imidazopyridine derivatives towards *N*-alkoxymethylation reaction. This prompted us to investigate further two related

issues of chemical interest. The first was the unambiguous determination of the structure of the products by appropriate nmr based methods which would obviate the need for preparing authentic samples via alternative chemical routes. The second issue was that, once we are able to correlate the structures with the chemistry of the site selectivity, it was of interest to assess the variations in the reaction conditions to provide the three possible isomers exclusively.

A number of imidazo[4,5-*b*]- and imidazo[4,5-*c*]pyridines have been recently prepared for medicinal use as cardiotonic drugs[3] and non-benzodiazepine anxiolytics.[4] In the latter case, a range of elaborate procedures is used for obtaining regioisomeric *N*-acetamido derivatives. The chemistry described here affords convenient and direct methods for achieving similar results for related compounds. Studies on the mechanism of alkoxymethylation reactions under different conditions are also warranted because such new *N*-substituted Hoechst 33258 compounds exhibit novel bifunctional DNA/protein binding activity.[5,6] Therefore such information would be useful for the design of more potent DNA sequence selective minor groove binding agents based on Hoechst 33258.[5c]

RESULTS

The imidazo[4,5-*b*]pyridine derivatives (**1**) and (**3**), and benzimidazole (**2**) were prepared *via* an oxidative cyclodehydrogenation strategy shown in Scheme I. Thus, the reaction of 2,3-diaminopyridine with *p*-methoxybenzaldehyde in nitrobenzene afforded 2-methoxyphenyl-1(3)*H*-imidazo[4,5-*b*]pyridine (**3**). Similarly, 6-methyl-2,3-diaminopyridine and 4-methyl-1,2-phenylenediamine provided **1** and **2** respectively.[2] It was observed that the use of two equivalents of aldehyde in these reactions led to the formation of *N*-methoxybenzyl substituted product (e.g. **11**). As depicted in Scheme I, the production of imidazopyridine might occur by a multistep process involving initial Schiff base formation followed by intramolecular cyclization and subsequent aromatization *via* nitrobenzene mediated oxidation to **3**. The role of nitrobenzene as an oxidant in the last step is supported by the fact that alternative oxidizing agents like quinones can also be employed.



Scheme I

The question of orientation of the reaction products expected for the reactions involving electrophilic attack at nitrogen atoms in imidazopyridine is analogous to the situation for unsymmetrically substituted imidazoles and benzimidazoles.[8] Alkoxymethylation reactions of the aforementioned heterocycles containing two or three potentially nucleophilic centers were studied with various alkoxymethyl chlorides and with bromoacetonitrile. The products isolated from these reactions were identified on the basis of their spectroscopic properties (nmr and mass spectral analyses).

The structural assignments of the isomeric products were made by NOE and selective polarization transfer based ^{13}C nmr experiments. The ^{1}H nmr spectra were first analysed by inspection and by considering relative chemical shift changes and matching spectral splittings. For instance, the upfield set of 2H doublets due to the methoxyphenyl substituent was assigned to the degenerate protons ortho to OCH_3, followed by identification of its mutually scalar coupled 2H doublet signal (due to C2'/C6'-H). Similarly the signal for H-7 was ascribed a position further downfield from H-6 based on analogy with assignments in pyridinic compounds. The ambiguity regarding the location of CH_2OR substituent was resolved on the basis of selective NOEs observed in the two-dimensional NOESY experiments. A typical NOESY spectrum shown in Figure 1 illustrates this strategy of structural assignment for compound

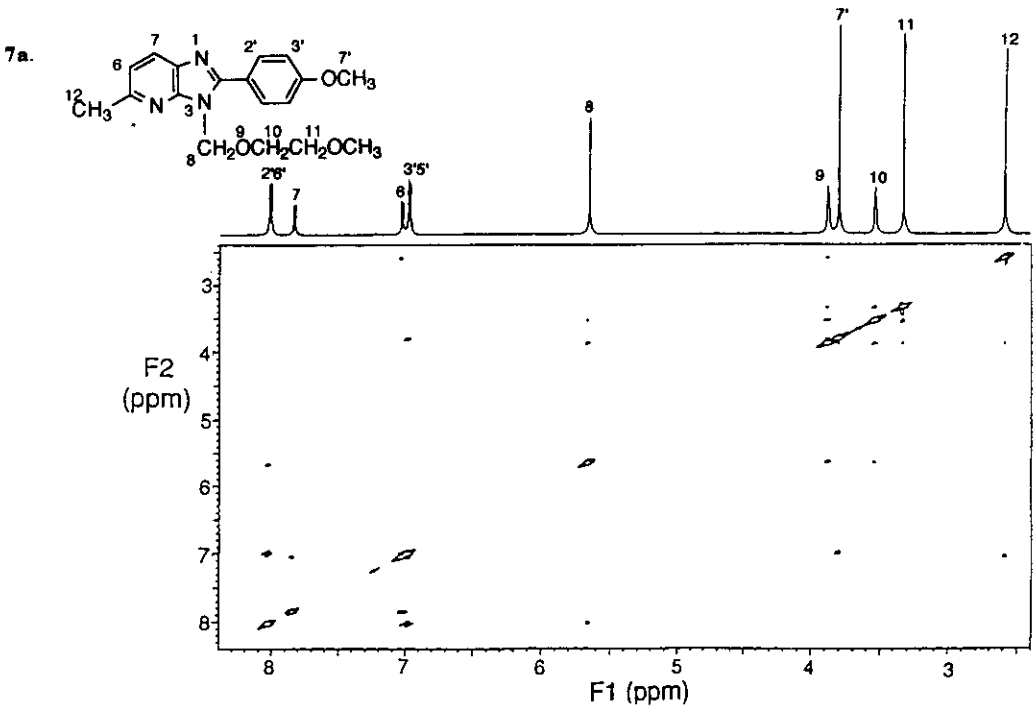


Figure 1. Contour plot of 500 MHz 2D-NOESY spectrum of compound (**7a**). Crosspeaks showing proton-proton through-space connectivities appear symmetrically with respect to the diagonal. Also shown is the proton 1D spectrum with resonance assignments.

The cross peak for the CH_2 protons at 5.65 ppm and C2'/C6'-H protons at 8.04 ppm confirms their spatial proximity as evidence for either N3- or N1-substitution in the structure for **7**. The absence of an NOE relationship in the same spectrum between the CH_2 group and proton H-7 further shows the position of the CH_2OR group to be N3 and not N1. Although one can argue against such inference based on a lack of NOE interactions, the distinction between each type of isomer based on characteristic NOESY spectra was unambiguous in the case of imidazopyridine (**3**) where each product (**10a-c**) could be obtained in pure form. The NOE results are summarized in Figure 2. Additional sets of protons in close proximity are also observed as indicated in Figures 1 and 2.

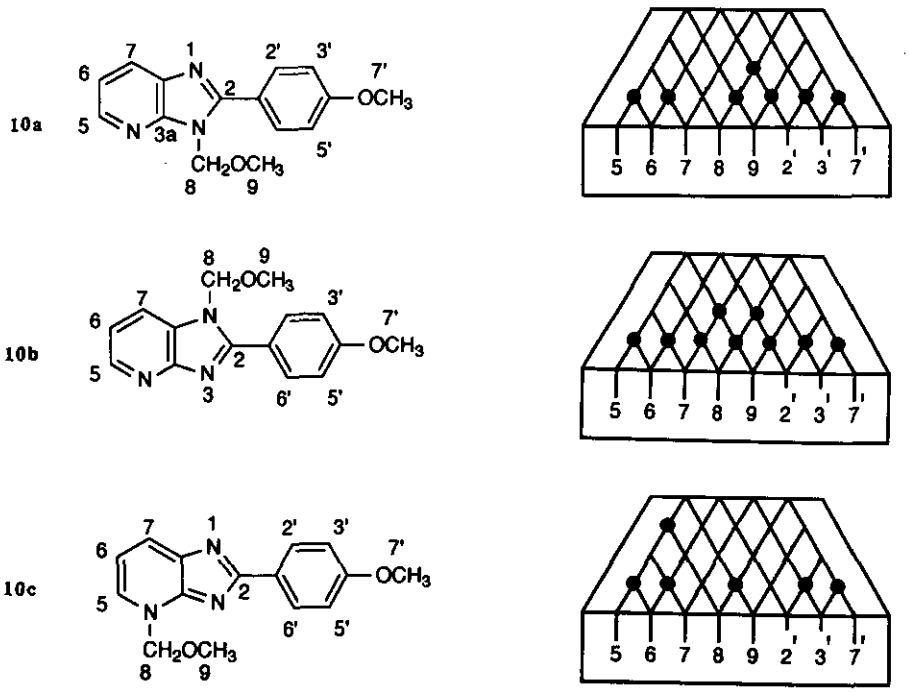


Figure 2. Summary of experimental NOEs used to establish the position of methoxymethyl substituent in three regioisomers (**10a-c**) obtained from the reactions of 2-arylimidazo[4,5-*b*]pyridine (**3**). Numbers 2' and 3' on the grid drawings represent the degenerate sets of protons corresponding to $C_{2'}/C_{6'}$-H and $C_{3'}/C_{5'}$-H. The NOE relationships characteristic of specific structures were: C_8-H $\leftrightarrow$ $C_{2'}/C_{6'}$-H for **10a**; C_8-H $\leftrightarrow$ $C_{2'}/C_{6'}$-H and C_7-H $\leftrightarrow$ C_8-H for **10b**; and C_5-H $\leftrightarrow$ C_8-H for **10c**.

Complementary structural information was provided by ^{13}C nmr experiments involving selective polarization transfer from the side chain CH_2 group protons to the carbon centers corresponding to $^3J_{CH}$ couplings of ~7 Hz. All ^{13}C nmr spectra were first assigned (Table II) using the reference data on 2-arylimidazo[4,5-*b*]pyridine described by previous workers.[9] Selective magnetization transfer

experiments, namely INAPT and devised originally by Bax,[10] were next perfomed which permitted the detection of ^{13}C resonances β to the proton signals selectively irradiated. For instance, C-3a, C-2, and C-9 resonances were observed for structure (**10a**) (Figure 3a), while irradiation of CH_2 protons in **10b** correlates exclusively with the C-7a, C-2, and C-9 signals (Figure 3b).

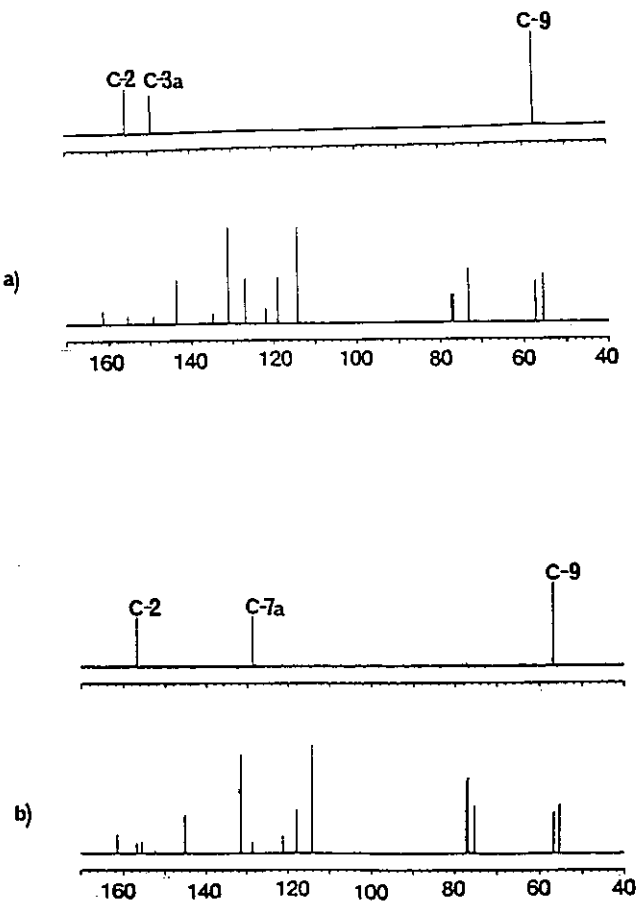
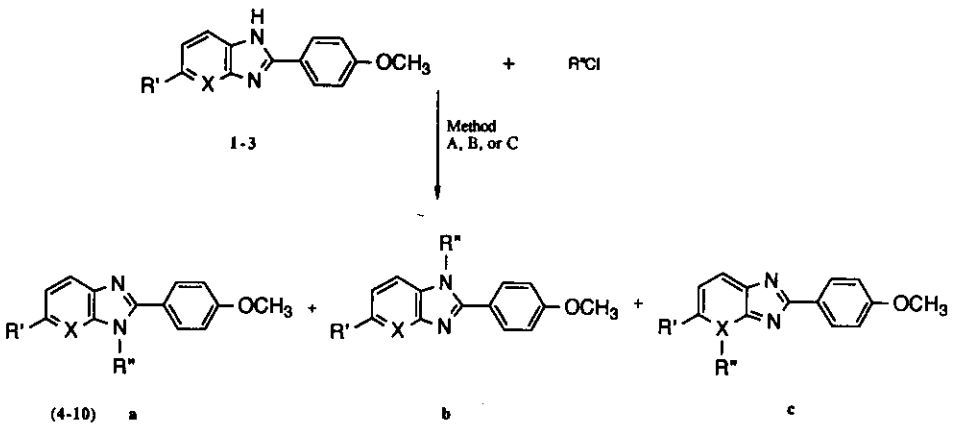


Figure 3. ^{13}C Nmr spectra of compounds [**10a** (a) and **10b** (b)] with the corresponding selective INAPT detection of the specific ^{13}C resonances that appear upon selective pulsing of CH_2 protons of the methoxymethyl substituent. The modified INEPT pulse sequence described in ref. 10 was optimized for polarization transfer *via* proton-carbon three-bond couplings of ~ 7 Hz. Detection of marked signals was used in conjunction with 2D-NOE data to confirm the structural assignments as described in the text.



Scheme II

Table I. Yields and relative proportions of isomeric products in the alkoxymethylation of imidazo[4,5-*b*]pyridines.

Entry No.	Reactant	X	R'	R"	Method	% Yield	Products*	[Ratio]
1	1	N	CH_3	$-CH_2OCH_3$	A	88	**4a/4b/4c**	[100:0:0]
2	1	N	CH_3	$-CH_2OCH_2CH_3$	A	92	**5a/5b/5c**	[100:0:0]
3	1	N	CH_3	$-CH_2O(CH_2)_7CH_3$	A	95	**6a/6b/6c**	[100:0:0]
4	1	N	CH_3	$-CH_2OCH_2CH_2OCH_3$	A	92	**7a/7b/7c**	[100:0:0]
5	1	N	CH_3	$-CH_2C{\equiv}N$	B	60	**8a/8b/8c**	[100:0:0]
6	2	CH	CH_3	$-CH_2OCH_3$	A	96	**9a/9b/9c**	[50:50:0]
7	3	N	H	$-CH_2OCH_3$	A	97	**10a/10b/10c**	[100:0:0]
8	3	N	H	$-CH_2OCH_3$	B	92	**10a/10b/10c**	[82:18:0]
9	3	N	H	$-CH_2OCH_3$	C	96	**10a/10b/10c**	[0:0:100]

*Numbers in boldface refer to the predominant products formed.

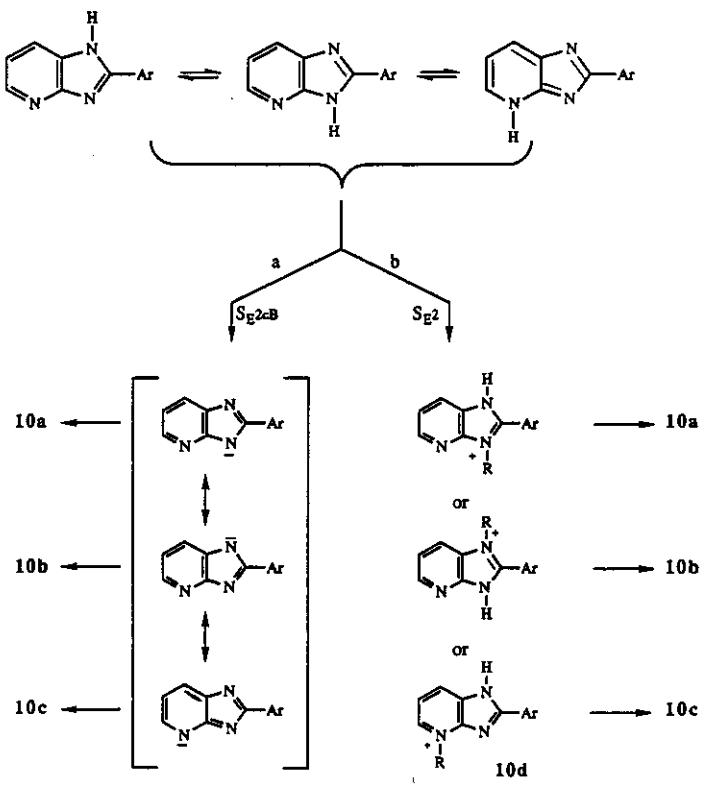
Table II. ^{13}C chemical shift assignments for compounds **(4-8)a, 10a-d,** and **11.**

Compound	C-2	C-3a	C-5	C-6	C-7	C-7a	C-8	C-9	C-1'	C-2',6'	C-3',5'	C-4'	C-7'
4a	154.45*	148.84*	153.20	118.87	126.86	132.64	72.87	57.16*	122.14	130.99	114.24	161.31	55.37
5a	154.45*	148.79*	153.04	118.74	126.77	132.63	71.28	64.96*	122.24	130.99	114.18	161.22	55.30
6a	154.21*	148.58*	153.09	118.78	126.53	132.24	71.45	69.42*	121.94	130.91	114.15	161.28	55.30
7a	154.29*	148.64*	152.93	118.67	126.65	132.46	71.33	68.46*	121.91	130.91	114.09	161.15	55.18
8a	152.52*	146.98*	154.39	119.83	127.38	132.10	31.32	114.53*	120.28	130.58	114.88	161.82	55.49
10a	155.35*	149.27*	143.67	118.94	126.83	134.87	73.06	57.18*	121.83	131.09	114.26	161.47	55.33
10b	155.65*	155.45	145.13	117.89	117.99	128.56*	75.28	56.63*	121.19	131.37	114.23	161.50	55.34
10c	169.74	155.23*	130.22*	112.79	127.89	146.77	83.44	58.02*	128.36	130.14	114.50	161.91	55.70
10d	164.29	150.55*	136.24*	118.64	128.63	134.27	90.68	58.59*	120.55	131.56	115.51	160.46	56.16
11	154.76*	148.90*	143.73	118.56	126.79	135.06	46.21	—	122.15	130.64	114.10	161.10	55.26

*Signals that are selctively detected *via* INAPT experiments as described in the text.

Most informative of the relative configurations of the side chain in the two structures therefore was the diagnostic detection of C_{3a} *vs* C_{7a} signals for **10a** and **10b** respectively. The ^{13}C INAPT experiments, in conjunction with 2D NOE data, have thus provided unambiguous proof of the relative configurations of the alkoxymethyl groups in all the products shown in Table I. The complete spectra (NOESY, ^{13}C, and ^{13}C-INAPT) for each compound have been submitted as supplementary material (Figures S1-S9), and the structural assignments are summarized in Scheme II and Table I.

The reaction of **1** in the presence of added base and in a nonpolar solvent (Method A) led to the formation of a single product in each case. As shown in Scheme III (path a, S_E2cB mechanism), the production of *N*-alkoxymethyl derivatives plausibly occurs by a two step process involving initial deprotonation followed by attack on the alkylating agent. In principle the possibility exists for reaction at any of the nitrogen centers in the reactants so that a mixture of products would be expected. However only one isomer is obtained under the conditions employed for method A in the six examples studied.



Scheme III

In order to explore the origin of the site selectivity observed for compound (**1**) a comparative study was undertaken of benzimidazole derivative (**2**), and imidazopyridine (**3**) under different reaction conditions. In the case of **2** (entry 6, Table I) a 50:50 mixture consisting of *N*3- and *N*1- alkylated products (**9a** and **9b**) was obtained. Consistent with the pK_a values of <10 for benzimidazoles,[11a] the basic conditions used for these reactions suggest the anionic form of the heterocycle to be the reactive species (i.e. an S_E2cB mechanism), but the product distribution ratio for the reaction of **2** correlates well with the original presence of N1H and N3H tautomers in equal amount based on K_T value of unity.[11] The use of base and nonpolar solvent conditions for **3** (Entry 7, Table I) led to a similar site selective reactivity as exemplified by **1** (Entry 1, Table I). However, upon changing the solvent medium to DMF, both N3- and N1- derivatives were obtained in 4:1 ratio as determined by the relative integration ratio of the characteristic well separated ^1H nmr signals of the two forms. The individual isomers were separated by chromatography and identified using characteristic NOEs as described above. The presence of two tautomers in a dilute solution of **3** in DMF-d_7 is indicated by two NH signals in a 2:1 intensity ratio. The predominant form was ascribed to the N1H derivative by analogy with the previous ^{13}C and ^{15}N nmr studies.[9] Thus, the presence of two tautomers leads to a loss in site selectivity towards methoxymethylation when DMF is used as solvent. The reaction involves the mesomeric anion forms and the product composition therefore appears to be a consequence of the relative nucleophilicity of the three nitrogen centers (i.e. N3>N1>>N4).

Under neutral conditions where an S_E2' type mechanism is expected to be operative the reaction of **3** with methoxymethyl chloride (Entry 9, Table I) followed an alternative pathway (path b, Scheme III), in contrast to the other examples. A ^1H nmr time study (in DMF-d_7) of direct reaction of **3** with methoxymethyl chloride in the absence of added base indicated quaternization at the pyridinyl nitrogen (N4) which was determined by the characteristic downfield shift of the protons H-5 and H-7. The structure of the quaternary species (**10d**) was further confirmed by 2D-NOESY and ^{13}C INAPT experiments. Subsequent addition of base followed by work-up provided pure **10e** as the sole product.

DISCUSSION

The two mechanisms of electrophilic reaction between imidazopyridines and alkoxymethyl halides, depending on the reaction conditions (S_E2cB and S_E2'), are summarized in Scheme III. The selectivity of alkylation does not appear to correlate with the initial tautomeric composition of the heterocycles, except in the case of benzimidazole (**2**). The nmr based methods described above for structural analysis have provided means of direct and unambigous determination of the isomeric structures thus obviating the need for elaborate procedures to obtain authentic samples. In particular, the ^{13}C INAPT experiments demonstrate how only those ^{13}C resonances that show long-range couplings to individual protons with $^3J(C,H) = 7$ Hz, can be detected selectively and further be ascribed to specific structures.

Although the difference in orientation towards electrophilic attack on multiple nitrogen sites can be related to the the presence of tautomeric forms of the reactant (e.g product ratio of **10a**/**10b** in DMF vs benzene), this is unlikely to be the only cause for observed site selectivity, for two reasons: (a) the reaction involves the mesomeric anionic species in the basic media, and (b) the original presence of two tautomers in 2:1 ratio in DMF does not correlate with the 4:1 product ratio. More plausibly, the course of the reaction appears to depend on the relative reactivity of the sites with secondary effects operative due to the change in the reaction conditions. The role of differing nucleophilicity of the three nitrogen centers in **3** is also evident at the stage of its preparative reaction (Scheme I) which, with the use of excess aldehyde, led to the formation of only the N3-methoxybenzyl product (**11**).

The product ratios of **10a** to **10b** may also depend on the electronic nature of the 2-aryl substituent of imidazopyridines. For example, a recent study[4] showed that the use of a 4-chlorophenyl (instead of 4-methoxyphenyl used in the present work) substituent in a similar electrophilic reaction led to the formation of N1-alkyl derivative (formally equivalent to **10b**) as the major product. This result can be explained in terms of the effects on the respective rates of the reaction (N1- vs N3-) due to the electron withdrawing chloro substituent on the aryl ring, directly conjugated with the reactive centers of the heterocycle. Such modulation of reactivity/selectivity further supports our conclusion that the tautomeric composition of the reactants is not the sole determinant in the orientation of such reactions involving equilibriating multiple reactive centers.

EXPERIMENTAL

Reactions were carried out under an inert atmosphere (N_2 or Ar) and the evaporations were performed in vacuo using a rotary evaporator. The ^1H nmr spectra were recorded at 400 or 500 MHz on Bruker WH-400 and Varian Unity 500 spectrometers. All ^1H and ^{13}C chemical shifts are reported in parts-per-million downfield from tetramethylsilane. One-dimensional NOE measurements and two-dimensional COSY/NOESY experiments were performed in degassed sample solutions. High-resolution mass spectra (HRms) were determined using the electron ionization technique on Associated Electrical Industries (AEI) MS-9 and MS-50 focusing mass spectrometers. Ir spectra were obtained with a Nicolet 7199 FT spectrophotometer, and only the principal absorptions are reported. All starting organic chemicals were obtained from Aldrich Chemical Co. and were used as recieved. Hplc grade solvents were used for chromatography. Anhydrous benzene and THF were distilled from sodium benzophenone ketyl. Anhydrous DMF was distilled under reduced pressure from CaH_2. Melting points were recorded on a Fisher-Johns capillary apparatus, and are uncorrected. Kieselgel 60 (230-400 mesh) obtained from E. Merck was used for flash chromatography.

General Synthetic Procedures. 2,3-Diamino-6-methylpyridine was prepared by catalytic hydrogenation of 2-amino-6-methyl-3-nitropyridine[12] (3.06 g, 20 mmol) dissolved in 100 ml EtOAc, using 10% Pd/C (320 mg). At the completion of the reaction, the catalyst was removed by filtration through Celite and the filtrate was evaporated to afford 2.34 g of the product **2** (95%

yield) which was used in the subsequent step without further purification: mp 70-71 °C (EtOH); ir (KBr) ν_{max} 3380, 3320, 1650, 1600, 1480 cm^{-1}; ^1H nmr ($CDCl_3$) δ 2.30 (3H, s, CH_3), 3.10 and 4.21 (2H each, 2 br s, 2 x NH_2, exch), 6.40 (1H, d, J = 7.5 Hz, Ar-H), 6.78 (1H, d, J = 7.5 Hz, Ar-H).

2-(4-Methoxyphenyl)-5-methyl-3*H*-imidazo[4,5-*b*]pyridine was prepared by heating a mixture of 2,3-diamino-6-methylpyridine (1.48 g, 12 mmol) and 4-methoxybenzaldehyde (1.63 g, 12 mmol) in 25 ml nitrobenzene at 150 °C for 18 h. The reaction mixture was then cooled and nitrobenzene was evaporated under reduced pressure. The residue obtained was purified by silica gel flash chromatography (1:1 EtOAc:Hexane eluant) to afford 2.04 g of the title compound (71% yield): mp 231-233 °C (MeOH); ir (KBr) ν_{max} 3440, 1610, 1490, 1440, 1390, 1250 cm^{-1}; ^1H nmr ($CDCl_3$) δ 2.56 (3H, s, CH_3), 3.84 (3H, s, OCH_3), 7.06 (1H, d, J = 8 Hz, C_6-H), 7.12 (2H, d, J = 8.5 Hz, $C_{3'}/C_{5'}$-H), 7.82 (1H, d, J = 8 Hz, C_7-H), 8.14 (2H, d, J = 8.5 Hz, $C_{2'}/C_{6'}$-H); HRms calcd for $C_{14}H_{13}N_3O$ m/z 239.1058, found m/z 239.1058 (M^+, 100%), 224 (34%), 196 (19%); Anal. Calcd for $C_{14}H_{13}N_3O$: C, 70.29; H, 5.44; N, 17.57. Found: C, 70.31; H, 5.42; N, 17.49.

2-(4-Methoxyphenyl)-1(3)*H*-imidazo[4,5-*b*]pyridine (3) was prepared following the same procedure as above using 2,3-diaminopyridine respectively. mp 187 °C (MeOH); ^1H nmr (DMSO-d_6) δ 3.80 (3H, s, CH_3), 6.93 (2H, d, J = 8.5 Hz, $C_{3'}/C_{5'}$-H), 7.06 (1H, dd, J = 5 and 8 Hz, C_6-H), 7.78 (1H, d, J = 8 Hz, C_7-H), 8.11 (2H, d, J = 8.5 Hz, $C_{2'}/C_{6'}$-H), 8.20 (1H, d, J = 5 Hz, C_5-H).

2-(4-Methoxyphenyl)-3-((4-methoxyphenyl)methyl)-3*H*-imidazo[4,5-*b*]pyridine (11) was isolated from the same reaction as above for **3**, when 2 mol equiv. of *p*-methoxybenzaldehyde was used. The product (**11**) was purified by silica gel flash chromatography (EtOAc eluent). mp 172-174 °C (EtOH); ^1H nmr (DMF-d_7) δ 3.75 (3H, s, Ar-OCH_3), 3.90 (3H, s, $C_{4'}$-OCH_3), 5.70 (2H, s, CH_2), 6.85 and 7.05 (2H each, 2d, J = 8.6 Hz, Ar-H), 7.15 (2H, d, J = 8.8 Hz, $C_{3'}/C_{5'}$-H), 7.35 (1H, dd, J = 5 and 8 Hz, C_6-H), 7.80 (2H, d, J = 8.8 Hz, $C_{2'}/C_{6'}$-H), 8.15 (1H, d, J = 8 Hz, C_7-H), 8.40 (1H, d, J = 5 Hz, C_5-H); ^{13}C nmr ($CDCl_3$) δ 46.21 (CH_2), 55.11, 55.26 ($C_{4'}$-OCH_3), 114.08 (Ar), 114.10 ($C_{3'}/C_{5'}$), 118.56 (C_6), 122.15 ($C_{1'}$), 126.79 (C_7), 127.76 (Ar), 128.94 (Ar), 130.64 ($C_{2'}/C_{6'}$), 135.06 (C_{7a}), 143.73 (C_5), 148.90 (C_{3a}), 154.76 (C_2), 158.91 (Ar), 161.10 ($C_{4'}$); HRms calcd for $C_{21}H_{19}N_3O_2$ m/z 345.1479, found m/z 345.1474 (M^+, 100%), 225 (32%); Anal. Calcd for $C_{21}H_{19}N_3O_2$: C, 73.04; H, 5.50; N, 12.17. Found: C, 73.12; H, 5.28; N, 12.33.

General methods for alkoxymethylation reactions were as follows: *Method A*. In a typical procedure, a solution of alkyl halide (10 mmol) in 5 ml benzene was added dropwise to a stirred suspension of the heteroaromatic derivative **1-3** (5 mmol) in 80 ml anhydrous benzene containing 2 ml Et_3N at 0 °C. The reaction mixture was allowed to warm to 25 °C and then heated under reflux for 4 h. At the completion of the reaction, the reaction mixture was partitioned between water and EtOAc, and the organic layer was removed,

washed with water, dried (Na_2SO_4) and evaporated to afford a solid residue which was analysed before and after purification by silica gel flash chromatography (2:1 EtOAc:Hexane eluant). *Method B.* This procedure was similar to method A in all respects except for the use of anhydrous DMF in place of benzene as the reaction solvent. *Method C* involved treatment of the heterocycle derivative with alkyl halide in DMF. At the completion of the reaction as monitored by nmr of the aliquots, triethylamine was added and the reaction mixture worked up as described under method A.

3-Methoxymethyl-2-(4-methoxyphenyl)-5-methyl-3*H*-imidazo[4,5-*b*]pyridine (4a) was obtained in 88% isolated yield from **1** and methoxymethyl chloride using method A. mp 109-111 °C (MeOH/Hexane); ir (KBr) ν_{max} 2980, 2940, 2830, 1600, 1500, 1460 cm^{-1}; ^1H nmr ($CDCl_3$) δ 2.64 (3H, s, C_5-CH_3), 3.54 (3H, s, CH_2OCH_3), 3.86 (3H, s, $C_{4'}$-OCH_3), 5.60 (2H, s, CH_2), 7.02 (2H, d, J = 8.5 Hz, $C_{3'}/C_{5'}$-H), 7.10 (1H, d, J = 8 Hz, C_6-H), 7.90 (1H, d, J = 8 Hz, C_7-H), 8.02 (2H, d, J = 8.5 Hz, $C_{2'}/C_{6'}$-H); ^{13}C nmr ($CDCl_3$) δ 24.49 (C_5-CH_3), 55.37 ($C_{4'}$-OCH_3), 57.16 (OCH_3), 72.87 (CH_2), 114.24 ($C_{3'}/C_{5'}$), 118.87 (C_6), 122.14 ($C_{1'}$), 126.86 (C_7), 130.99 ($C_{2'}/C_{6'}$), 132.64 (C_{7a}), 148.84 (C_{3a}), 153.20 (C_5), 154.45 (C_2), 161.31 ($C_{4'}$); HRms calcd for $C_{16}H_{17}N_3O_2$ m/z 283.1321, found m/z 283.1320 (M^+, 100%), 252 (97%), 238 (21%); Anal. Calcd for $C_{16}H_{17}N_3O_2$: C, 67.84; H, 6.00; N, 14.84. Found: C, 67.74; H, 6.21; N, 14.96.

3-Ethoxymethyl-2-(4-methoxyphenyl)-5-methyl-3*H*-imidazo[4,5-*b*]pyridine (5a) was obtained in 92% isolated yield from **1** and ethoxymethyl chloride using method A. mp 122-124 °C (EtOH/Hexane); ir (KBr) ν_{max} 2960, 2920, 2840, 1610, 1590, 1530, 1480, 1460, 1420, 1400, 1360, 1300, 1260 cm^{-1}; ^1H nmr ($CDCl_3$) δ 1.28 (3H, t, J = 6 Hz, CH_3), 3.83 (2H, q, J = 6 Hz, CH_2CH_3), 3.90 (3H, s, $C_{4'}$-OCH_3), 5.68 (2H, s, CH_2), 7.08 (2H, d, J = 8.5 Hz, $C_{3'}/C_{5'}$-H), 7.15 (1H, d, J = 8 Hz, C_6-H), 7.98 (1H, d, J = 8 Hz, C_7-H), 8.12 (2H, d, J = 8.5 Hz, $C_{2'}/C_{6'}$-H); ^{13}C nmr ($CDCl_3$) δ 15.08 (CH_3), 24.47 (C_5-CH_3), 55.33 ($C_{4'}$-OCH_3), 64.96 (CH_2), 71.28 (N-CH_2), 114.18 ($C_{3'}/C_{5'}$), 118.74 (C_6), 122.24 ($C_{1'}$), 126.77 (C_7), 130.99 ($C_{2'}/C_{6'}$), 132.63 (C_{7a}), 148.79 (C_{3a}), 153.04 (C_5), 154.45 (C_2), 161.22 ($C_{4'}$); HRms calcd for $C_{17}H_{19}N_3O_2$ m/z 297.1478, found m/z 297.1480 (M^+, 64%), 253 (42%), 252 (100%), 239 (20%), 238 (14%); Anal. Calcd for $C_{17}H_{19}N_3O_2$: C, 68.68; H, 6.39; N, 14.14. Found: C, 68.88; H, 6.61; N, 14.02.

2-(4-Methoxyphenyl)-3-octyloxy-5-methyl-3*H*-imidazo[4,5-*b*]pyridine (6a) was isolated in 95% yield from the reaction of octyloxymethyl chloride with **1** according to method A. mp 148-149 °C (EtOH/Hexane); ir (KBr) ν_{max} 2930, 2855, 1610, 1480, 1420, 1260 cm^{-1}; ^1H nmr ($CDCl_3$) δ 0.86 (3H, t, J = 6 Hz, CH_3), 1.24 (10H, br s, 5 x CH_2), 1.62 (2H, m, CH_2), 2.66 (3H, s, C_5-CH_3), 3.75 (2H, t, J = Hz, CH_2OCH_2), 3.88 (3H, s, $C_{4'}$-OCH_3), 5.67 (2H, s, CH_2), 7.05 (2H, d, J = 8.5 Hz, $C_{3'}/C_{5'}$-H), 7.12 (1H, d, J = 8 Hz, C_6-H), 7.94 (1H, d, J = 8 Hz, C_7-H), 8.10 (2H, d, J = 8.5 Hz, $C_{2'}/C_{6'}$-H); ^{13}C nmr ($CDCl_3$) δ 13.47, 21.99, 24.36 (C_5-CH_3), 25.45, 28.63, 28.85, 31.15, 55.30 ($C_{4'}$-OCH_3), 69.42 (OCH_2), 71.45 (N-CH_2), 114.15 ($C_{3'}/C_{5'}$),

118.78 (C_6), 121.94 ($C_{1'}$), 126.53 (C_7), 130.91 ($C_{2'}/C_{6'}$), 132.24 (C_{7a}), 148.58 (C_{3a}), 153.09 (C_5), 154.21 (C_2), 161.28 ($C_{4'}$); HRms calcd for $C_{23}H_{31}N_3O_2$ m/z 381.2417, found m/z 381.2414 (M^+, 100%), 268 (9%), 252 (93%), 239 (37%); Anal. Calcd for $C_{23}H_{31}N_3O_2$: C, 72.44; H, 8.13; N, 11.02. Found: C, 72.11; H, 8.36; N, 11.38.

3-Methoxyethoxymethyl-2-(4-methoxyphenyl)-5-methyl-3*H*-imidazo[4,5-*b*]-pyridine (7a) was obtained in 92% isolated yield from 1 and methoxyethoxymethyl chloride using method A. mp 118-119 °C (EtOH); ir (KBr) ν_{max} 2920, 2840, 1610, 1470, 1415, 1360, 1300, 1253, 1180, 1100 cm^{-1}; ^1H nmr ($CDCl_3$) δ 2.67 (3H, s, C_5-CH_3), 3.41 (3H, s, CH_2OCH_3), 3.62 (2H, t, J = 6 Hz, CH_2), 3.90 (3H, s, $C_{4'}$-OCH_3), 3.98 (2H, t, J = 6 Hz, CH_2), 5.76 (2H, s, CH_2), 7.06 (2H, d, J = 8.5 Hz, $C_{3'}/C_{5'}$-H), 7.15 (1H, d, J = 8 Hz, C_6-H), 7.98 (1H, d, J = 8 Hz, C_7-H), 8.14 (2H, d, J = 8.5 Hz, $C_{2'}/C_{6'}$-H); ^{13}C nmr ($CDCl_3$) δ 24.31 (C_5-CH_3), 55.18 ($C_{4'}$-OCH_3),58.82 (OCH_3), 68.47 (OCH_2), 71.33 (N-CH_2), 71.52 (OCH_2), 114.09 ($C_{3'}/C_{5'}$), 118.67 (C_6), 121.91 ($C_{1'}$), 126.65 (C_7), 130.91 ($C_{2'}/C_{6'}$), 132.46 (C_{7a}), 148.64 (C_{3a}), 152.93 (C_5), 154.29 (C_2), 161.15 ($C_{4'}$); HRms calcd for $C_{18}H_{21}N_3O_3$ m/z 327.1583, found m/z 327.1582 (M^+, 94%), 252 (97%), 239 (100%); Anal. Calcd for $C_{18}H_{21}N_3O_3$: C, 66.05; H, 6.42; N, 12.84. Found: C, 65.72; H, 6.18; N, 12.66.

3-Cyanomethyl-2-(4-methoxyphenyl)-5-methyl-3*H*-imidazo[4,5-*b*]pyridine (8a) was obtained in 60% yield from the reaction of 1 with bromoacetonitrile in the presence of NaH according to method B. *Note: the use of Et_3N failed to give any product in this case.* mp 111-112 °C (MeOH); ir (KBr) ν_{max} 1610, 1480, 1445, 1420, 1380, 1300, 1260 cm^{-1}; ^1H nmr ($CDCl_3$) δ 2.68 (3H, s, C_5-CH_3), 3.89 (3H, s, $C_{4'}$-OCH_3), 5.21 (2H, s, CH_2), 7.07 (2H, d, J = 8.5 Hz, $C_{3'}/C_{5'}$-H), 7.18 (1H, d, J = 8 Hz, C_6-H), 7.79 (2H, d, J = 8.5 Hz, $C_{2'}/C_{6'}$-H), 7.97 (1H, d, J = 8 Hz, C_7-H); ^{13}C nmr ($CDCl_3$) δ 24.33 (C_5-CH_3), 31.32 (CH_2), 55.49 ($C_{4'}$-OCH_3), 114.53 (CN), 114.88 ($C_{3'}/C_{5'}$), 119.83 (C_6), 120.28 ($C_{1'}$), 127.38 (C_7), 130.58 ($C_{2'}/C_{6'}$), 132.10 (C_{7a}), 146.98 (C_{3a}), 152.52 (C_2), 154.39 (C_5), 161.82 ($C_{4'}$); HRms calcd for $C_{16}H_{14}N_4O$ m/z 278.1168, found m/z 278.1160 (M^+, 100%), 277 (55%), 263 (8%); Anal. Calcd for $C_{16}H_{14}N_4O$: C, 69.06; H, 5.04; N, 20.14. Found: C, 69.32; H, 5.28; N, 19.88.

1(3)-Methoxymethyl-2-(4-methoxyphenyl)-5-methyl-1(3)*H*-benzimidazole (9a & 9b). An inseparable 1:1 mixture of **9a** and **9b** was obtained in 96% yield from **2** and methoxymethyl chloride according to method A. The individual assignments were made on the basis of characteristic NOEs in the 2D experiment on the mixture of two isomeric forms: *N*(1)-methoxymethyl-2-(4-methoxyphenyl)-1*H*-benzimidazole (9a): ^1H Nmr ($CDCl_3$) δ 2.50 (3H, s, CH_3), 3.43 (3H, s, OCH_3), 3.88 (3H, s, $C_{4'}$-OCH_3), 5.44 (2H, s, CH_2), 7.03 (2H, d, J = 8.5 Hz, $C_{3'}/C_{5'}$-H), 7.14 (1H, d, J = 8 Hz, C_6-H), 7.39 (1H, d, J = 8 Hz, C_7-H), 7.60 (1H, s, C_4-H), 7.86 (2H, d, J = 8.5 Hz, $C_{2'}/C_{6'}$-H); and for *N*(3)-methoxymethyl-2-(4-methoxyphenyl)-5-methyl-3*H*-benzimidazole (9b): ^1H Nmr ($CDCl_3$) δ 2.52 (3H, s, CH_3), 3.42 (3H, s, OCH_3), 3.88 (3H, s, $C_{4'}$-OCH_3), 5.43 (2H, s, CH_2), 7.03 (2H, d, J = 8.5 Hz, $C_{3'}/C_{5'}$-H), 7.14 (1H, d, J = 8 Hz, C_6-H), 7.29 (1H, s, C_4-H), 7.68 (1H, d, J = 8 Hz, C_7-H), 7.87 (2H, d, J = 8.5 Hz, $C_{2'}/C_{6'}$-H);

HRms calcd for $C_{17}H_{18}N_2O_2$ m/z 282.1369, found m/z 282.1365 (M^+, 100%), 251 (90%), 237 (30%); Anal. Calcd for $C_{17}H_{18}N_2O_2$: C, 72.34; H, 6.38; N, 9.93. Found: C, 72.66; H, 6.60; N, 9.68.

***N*(3)-methoxymethyl-2-(4-methoxyphenyl)-3*H*-imidazo[4,5-*b*]pyridine (10a)** was isolated in 97% yield from **3** and methoxymethyl chloride using method A. mp 98-100 °C (MeOH/Hexane); ^{1}H nmr (DMF-d_7) δ 3.53 (3H, s, OCH_3), 3.93 (3H, s, $C_{4'}$-OCH_3), 5.72 (2H, s, CH_2), 7.20 (2H, d, J = 8.8 Hz, $C_{3'}/C_{5'}$-H), 7.38 (1H, dd, J = 4.8 and 7.9 Hz, C_6-H), 8.10 (2H, d, J = 8.8 Hz, $C_{2'}/C_{6'}$-H), 8.12 (1H, dd, J = 1.5 and 7.9 Hz, C_7-H), 8.42 (1H, dd, J = 1.5 and 4.8 Hz, C_5-H); ^{13}C nmr ($CDCl_3$) δ 55.33 ($C_{4'}$-OCH_3), 57.18 (OCH_3), 73.06 (CH_2), 114.26 ($C_{3'}/C_{5'}$), 118.94 (C_6), 121.83 ($C_{1'}$), 126.83 (C_7), 131.09 ($C_{2'}/C_{6'}$), 134.87 (C_{7a}), 143.67 (C_5), 149.27 (C_{3a}), 155.35 (C_2), 161.47 ($C_{4'}$); HRms calcd for $C_{15}H_{15}N_3O_2$ m/z 269.1165, found m/z 269.1163 (M^+, 100%), 238 (92%); Anal. Calcd for $C_{15}H_{15}N_3O_2$: C, 66.91; H, 5.57; N, 15.61. Found: C, 67.16; H, 5.76; N, 15.80.

***N*(1)-methoxymethyl-2-(4-methoxyphenyl)-1*H*-imidazo[4,5-*b*]pyridine (10b)** was isolated from a mixture of **10a** and **10b** that formed in 92% total yield in the reaction of **3** with methoxymethyl chloride according to method B. The separation and purification was accomplished by silica gel flash chromatography (EtOAc eluent). mp 120-122 °C (MeOH/Hexane); ^{1}H nmr (DMF-d_7) δ 3.46 (3H, s, OCH_3), 3.93 (3H, s, $C_{4'}$-OCH_3), 5.71 (2H, s, CH_2), 7.20 (2H, d, J = 8.8 Hz, $C_{3'}/C_{5'}$-H), 7.35 (1H, dd, J = 4.7 and 8.2 Hz, C_6-H), 8.03 (2H, d, J = 8.8 Hz, $C_{2'}/C_{6'}$-H), 8.26 (1H, dd, J = 1.5 and 8.2 Hz, C_7-H), 8.50 (1H, dd, J = 1.5 and 4.7 Hz, C_5-H); ^{13}C nmr ($CDCl_3$) δ 55.34 ($C_{4'}$-OCH_3), 56.63 (OCH_3), 75.28 (CH_2), 114.23 ($C_{3'}/C_{5'}$), 117.89 (C_6), 117.99 (C_7), 128.56 (C_{7a}), 121.19 ($C_{1'}$), 131.37 ($C_{2'}/C_{6'}$), 145.13 (C_5), 155.45 (C_{3a}), 156.65 (C_2), 161.50 ($C_{4'}$); HRms calcd for $C_{15}H_{15}N_3O_2$ m/z 269.1165, found m/z 269.1164 (M^+, 100%), 238 (94%); Anal. Calcd for $C_{15}H_{15}N_3O_2$: C, 66.91; H, 5.57; N, 15.61. Found: C, 66.73; H, 5.22; N, 15.48.

***N*(4)-methoxymethyl-2-(4-methoxyphenyl)-4*H*-imidazo[4,5-*b*]pyridine (10c)** was obtained in 96% yield from **3** and methoxymethyl chloride using method C. mp 111-113 °C (EtOAc); ^{1}H nmr (DMF-d_7) δ 3.49 (3H, s, OCH_3), 3.89 (3H, s, $C_{4'}$-OCH_3), 6.12 (2H, s, CH_2), 7.10 (2H, d, J = 8.8 Hz, $C_{3'}/C_{5'}$-H), 7.25 (1H, dd, J = 6.4 and 7.3 Hz, C_6-H), 8.20 (1H, d, J = 7.3 Hz, C_7-H), 8.24 (1H, d, J = 6.4 Hz, C_5-H), 8.42 (2H, d, J = 8.8 Hz, $C_{2'}/C_{6'}$-H); ^{13}C nmr (DMF-d_7) δ 55.70 ($C_{4'}$-OCH_3), 58.02 (OCH_3), 83.44 (CH_2), 112.97 (C_6), 114.50 ($C_{3'}/C_{5'}$), 127.89 (C_7), 128.36 ($C_{1'}$), 130.14 ($C_{2'}/C_{6'}$), 130.22 (C_5), 146.77 (C_{7a}), 155.53 (C_{3a}), 161.91 ($C_{4'}$), 169.74 (C_2); HRms calcd for $C_{15}H_{15}N_3O_2$ m/z 269.1165, found m/z 269.1161 (M^+, 100%), 238 (70%); Anal. Calcd for $C_{15}H_{15}N_3O_2$: C, 66.91; H, 5.57; N, 15.61. Found: C, 66.80; H, 5.46; N, 15.88.

***N*(4)-methoxymethyl-2-(4-methoxyphenyl)-1(3)*H*-imidazo[4,5-*b*]pyridinium chloride (10d)** was characterized by nmr analysis of an aliquot obtained from the reaction mixture prior to the addition of Et_3N (cf preparation of **10c** according to method C). ^{1}H Nmr (DMF-d_7) δ 3.60 (3H, s, CH_3), 3.96 (3H, s, $C_{4'}$-OCH_3), 6.33 (2H, s, CH_2), 7.22 (2H, d, J = 8.8 Hz, $C_{3'}/C_{5'}$-

H), 7.83 (1H, dd, J = 6.4 and 7.8 Hz, C_6-H), 8.67 (2H, d, J = 8.8 Hz, $C_{2'}/C_{6'}$-H), 8.69 (1H, d, J = 7.8 Hz, C_7-H), 8.92 (1H, d, J = 6.4 Hz, C_5-H); ^{13}C nmr (DMF-d_7) δ 56.16 ($C_{4'}$-OCH_3), 58.59 (OCH_3), 90.68 (CH_2), 115.51 ($C_{3'}/C_{5'}$), 118.64 (C_6), 120.55 ($C_{1'}$), 128.63 (C_7), 131.56 ($C_{2'}/C_{6'}$), 134.27 (C_{7a}), 136.24 (C_5), 150.55 (C_{3a}), 160.46 ($C_{4'}$), 164.29 (C_2).

Nmr Spectroscopy. Two dimensional NOESY experiments were performed on non-spinning samples in the phase-sensitive mode according to the hypercomplex method[13a,b] on a Varian Unity 500 MHz nmr spectrometer by use of standard NOESY pulse sequence provided in the Varian vnmr software. Data were acquired with the carrier frequency at the center of the spectrum and quadrature detection for a sweep width of 4500 Hz (adjusted to cover the entire range of the resonances) in both the *t1* and *t2* dimensions. Datasets consisted of 512 FIDS (*t1*) and 2048 data points in *t2*. A relaxation delay interval of 2.0 s and mixing time of 0.5 s were used. In order to avoid truncation of the free induction decays, a skewed sinebell apodization function was set interactively to ensure that the interferrograms decayed to zero in both *t1* and *t2* dimensions. The baseline corrections were performed according to the procedure of Otting.[13c] The contour plots for 2D experiments were constructed for publication after symmetrization.

^{13}C Nmr spectra were obtained with and without gated broad-band proton decoupling at 125 MHz on a Varian Unity 500 spectrometer. Typically a 45° pulse was used to acquire 32K data points over a 10 kHz spectral window. Data were weighted with 1.2 Hz line broadening prior to Fourier transformation. Experiments for selective polarization transfer from ^1H to ^{13}C optimized for three-bond couplings [$^3J(C,H)$ = 7 Hz] were performed by utilizing the INAPT pulse sequence devised by Bax.[10] The transmitter for proton pulses was adjusted at the center of preselected proton resonance of interest and attenuated to deliver 'soft' 13.9 ms 90° pulses which corresponds to a 20 Hz excitation band width (narrow enough to selectively pulse only a single resonance). Dephasing and refocusing delays were each set to 15 ms to permit magnetisation transfer through long range coupling without any loss due to T_2 relaxation. Hard 8 μs 90° ^{13}C pulses were implanted in the centre of soft proton pulses and broadband proton decoupling was employed during acquisition. Data were Fourier transformed after weighting with 1.2 Hz line broadening.

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