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Identification of Regioisomers in a Series of N-Substituted Pyridin-4-yl Imidazole Derivatives by Regiospecific Synthesis, GC/MS, and ^{1}H NMR

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Abstract: The regiospecific synthesis of **2a** (Scheme 3), a novel and potent pyridinyl imidazole inhibitor of p38 MAP (mitogen-activated protein) kinase, and the regioselective preparation of its regioisomer **2b** (Scheme 4) are described. Chromatographic and spectroscopic data are presented, which in this class of compounds allow the unambiguous identification of regioisomers prepared by a nonregiospecific synthetic strategy. Biological data demonstrating the importance of the correct regiochemistry for inhibition of p38 are given.

Several small molecule inhibitors of cytokine release are currently being investigated for their potential as safe and efficient antiinflammatory drugs. Work carried out by various groups[1−3] as well as in our own laboratory[4,5] has identified polysubstituted imidazoles (Figure 1) as potent inhibitors of p38 MAP kinase (for a recent review, cf. Jackson and Bullington[6]). p38 is implicated in signal transduction events leading to the release of proinflammatory cytokines interleukin 1β (IL-1β) and tumor necrosis factor-α (TNF-α) from human monocytes,[7] hence the strong antiinflammatory effect exerted by p38 inhibitors. Structure−activity relationships in this class of p38 inhibitors have revealed that the vicinal 4-fluorophenyl and pyridin-4-yl moieties attached to an imidazole (or similar heterocyclic) scaffold are required for efficient binding to the target enzyme.[8,9]

Because of the different substituents at the 4- and 5-positions of the imidazole nucleus placement of an additional substituent at either one of the two imidazole ring nitrogens generates two different regioisomers. However, only at the ring nitrogen adjacent to the pyridin-4-yl moiety (like in SB 210313, Figure 1) are substituents tolerated without loss of p38 inhibition.[2] With beneficial properties such as enhanced oral activity[10] and reduced toxicity[11] being ascribed to substituents at the imidazole ring nitrogen, the regiospecific preparation of N1-substituted pyridin-4-yl imidazoles has been addressed.[1,12] However, if such N-substituted imidazoles are prepared via a nonregiospecific synthetic pathway, *both* regioisomers are usually formed[3,13] and the assignment of the correct regiochemistry becomes a challenging task. In the structurally related class of 2,3-dihydroimidazo[2,1-*b*]thiazole inhibitors of cytokine release, for example, the molecular structures published for the desfluoro congeners of regioisomers SK&F 86002 and SK&F 86055 (Scheme 1) had been assigned on the basis of spectroscopic data.[14] The initial assignment, however, was later disproved when X-ray crystallographic evidence became available for these compounds.[15] Like SK&F 86002 and SK&F 86055, methylsulfanylimidazoles ML 3375 and **1** (Figure 1) can be synthesized from suitable imidazole-2-thione precursors (Scheme 1). Following the discovery of 1*H*-imidazole **1** as a potent inhibitor of p38 MAP kinase and cytokine release,[16] we sought to prepare its N1-methylated analogue **2a** (Scheme 3). Herein we report the regiospecific synthesis of **2a** as well as a fast and convenient method to analytically distinguish the N-substituted regioisomers of 2-methylsulfanylimidazole derivatives using routine GC/MS and ^{1}H NMR techniques. This analytical protocol enabled us to unambiguously determine the regioselectivity for the direct con-

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(1) Boehm, J. C.; Smietana, J. M.; Sorenson, M. E.; Garigipati, R. S.; Gallagher, T. F.; Sheldrake, P. L.; Bradbeer, J.; Badger, A. M.; Laydon, J. T.; Lee, J. C.; Hillegass, L. M.; Griswold, D. E.; Breton, J. J.; Chabot-Fletcher, M. C.; Adams, J. L. *J. Med. Chem.* **1996**, *39*, 3929−3937.

(2) Gallagher, T. F.; Seibel, G. L.; Kassis, S.; Laydon, J. T.; Blumenthal, M. J.; Lee, J. C.; Lee, D.; Boehm, J. C.; Fier-Thompson, S. M.; Abt, J. W.; Soreson, M. E.; Smietana, J. M.; Hall, R. F.; Garigipati, R. S.; Bender, P. E.; Erhard, K. F.; Krog, A. J.; Hofmann, G. A.; Sheldrake, P. L.; McDonnell, P. C.; Kumar, S.; Young, P. R.; Adams, J. L. *Bioorg. Med. Chem.* **1997**, *5*, 49−64.

(3) Liverton, N. J.; Butcher, J. W.; Claiborne, C. F.; Claremon, D. A.; Libby, B. E.; Nguyen, K. T.; Pitzenberger, S. M.; Selnick, H. G.; Smith, G. R.; Tebben, A.; Vacca, J. P.; Varga, S. L.; Agarwal, L.; Dancheck, K.; Forsyth, A. J.; Fletcher, D. S.; Frantz, B.; Hanlon, W. A.; Harper, C. F.; Hofsess, S. J.; Kostura, M.; Lin, J.; Luell, S.; O'Neill, E. A.; Orevillo, C. J.; Pang, M.; Parsons, J.; Rolando, A.; Sahly, Y.; Visco, D. M.; O'Keefe, S. J. *J. Med. Chem.* **1999**, *42*, 2180−2190.

(4) Laufer, S.; Striegel, H.-G.; Neher, K. *PCT Int. Appl.* **2000** (Merckle GmbH, Germany), WO 0017192, 2002, p 53.

(5) Laufer, S.; Striegel, H.-G.; Wagner, G. *J. Med. Chem.* **2002**, *45*, 4695−4705.

(6) Jackson, P. F.; Bullington, J. L. *Curr. Top. Med. Chem.* **2002**, *2*, 1011−1020.

(7) Lee, J. C.; Laydon, J. T.; McDonnell, P. C.; Gallagher, T. F.; Kumar, S.; Green, D.; McNulty, D.; Blumenthal, M. J.; Heys, J. R.; Landvatter, S. W.; Strickler, J. E.; McLaughlin, M. M.; Siemens, I. R.; Fisher, S. M.; Livi, G. P.; White, J. R.; Adams, J. L.; Young, P. R. *Nature* **1994**, *372*, 739−746.

(8) Tong, L.; Pav, S.; White, D. M.; Rogers, S.; Crane, K. M.; Cywin, C. L.; Brown, M. L.; Pargellis, C. A. *Nat. Struct. Biol.* **1997**, *4*, 311−316.

(9) Wang, Z.; Canagarajah, B. J.; Boehm, J. C.; Kassisa, S.; Cobb, M. H.; Young, P. R.; Abdel-Meguid, S.; Adams, J. L.; Goldsmith, E. J. *Structure* **1998**, *6*, 1117−1128.

(10) Adams, J. L.; Boehm, J. C.; Gallagher, T. F.; Kassis, S.; Webb, E. F.; Hall, R.; Sorenson, M.; Garigipati, R. S.; Griswold, D. E.; Lee, J. C. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 2867−2870.

(11) Adams, J. L.; Boehm, J. C.; Kassis, S.; Gorycki, P. D.; Webb, E. F.; Hall, R.; Sorenson, M.; Lee, J. C.; Ayrton, A.; Griswold, D. E.; Gallagher, T. F. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 3111−3116.

(12) Laufer, S.; Wagner, G.; Kotschenreuther, D. *Angew. Chem., Int. Ed.* **2002**, *41*, 2290−2293.

(13) Chang, L. L.; Sidler, K. L.; Cascieri, M. A.; de Laszlo, S.; Koch, G.; Li, B.; MacCoss, M.; Mantlo, N.; O'Keefe, S. J.; Pang, M.; Rolando, A.; Hagmann, W. K. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 2549−2553.

(14) Klose, W.; Schwarz, K. *J. Heterocycl. Chem.* **1985**, *22*, 669−671.

(15) Shilcrat, S. C.; Hill, D. T.; Bender, P. E.; Griswold, D. E.; Bauers, P. W.; Eggleston, D. S.; Lantos, I.; Pridgen, L. N. *J. Heterocycl. Chem.* **1991**, *28*, 1181−1187.

(16) Laufer, S.; Wagner, G.; Kotschenreuther, D.; Albrecht, W. *J. Med. Chem.* Submitted for publication.

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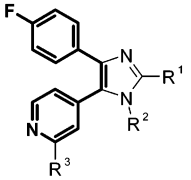
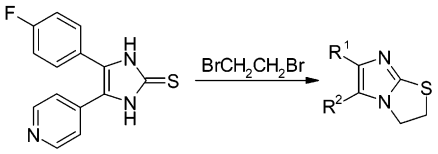


	R^1	R^2	R^3	IC_{50} (p38)
SB 203580	4-$H_3CS(O)$Ph-	H-	H-	0.36 μM
SB 210313	H-	3-morpholinopropyl-	H-	6.85 μM
ML 3375	H_3CS-	H-	H-	0.63 μM
1	H_3CS-	H-	$PhCH(CH_3)NH$-	0.38 μM

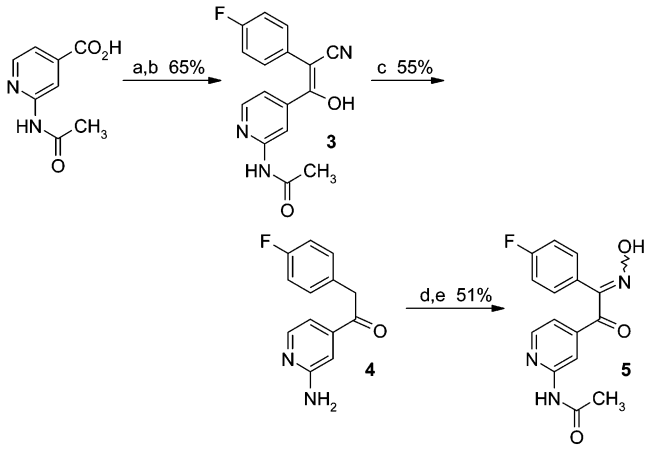
FIGURE 1. Inhibitors of p38 MAP kinase and cytokine release. The essential pharmacophore for inhibition of p38 is highlighted.

SCHEME 1. Synthesis of SK&F 86002 and SK&F 86055[17]



	R^1	R^2
SK&F 86002	4-F-ph	4-pyr
SK&F 86055	4-pyr	4-F-ph

SCHEME 2. Preparation of Key Intermediate 5[a]



[a] Reagents: (a) carbonyldiimidazole, absolute DMF, rt; (b) potassium *tert*-butoxide, 4-fluoro-phenylacetonitrile, absolute DMF, 120 °C; (c) 48% hydrobromic acid, reflux; (d) acetic anhydride, 4-(dimethylamino)pyridine, reflux; (e) isoamyl nitrite, sodium methoxide, methanol, rt.

version of several 1H-imidazoles into their N-substituted analogues.

Previously, we have established a regiospecific synthetic route toward N1-substituted pyridin-4-yl imidazole derivatives, starting from suitable α-hydroximinoketone precursors.[12] As a prerequisite to extending the scope of this strategy to the preparation of analogues bearing an acylamino or alkylamino side chain at the pyridine ring, acetylamino pyridine **5** was required (Scheme 2). The synthesis we had devised for **5** in turn relied on the availability of nitrile **3**. Attempts to prepare **3** from the corresponding ester of isonicotinic acid and 4-fluorophenylacetonitrile according to the standard protocol for this type of reaction (sodium alkoxide catalysis in alcoholic solvent)[18] failed. Under these conditions, 4-fluorophenylacetonitrile appeared to undergo polymerization, while the isonicotinic ester was saponified and the free acid was isolated as the main product. We finally managed to obtain nitrile **3** from the condensation of the activated isonicotinic acid analogue with 4-fluorophenylacetonitrile in an aprotic solvent. Compound **3** was converted into α-hydroximinoketone **5** via ketone **4** by standard procedures. However, the acid-catalyzed hydrolysis and subsequent decarboxylation of nitrile **3** was accompanied by the cleavage of the amide bond and therefore necessitated the reintroduction of the acetyl protecting group in a separate step prior to cyclization.

Target compound **2a** was obtained from α-hydroximinoketone **5** in a five-step synthesis (Scheme 3). Upon treatment of **5** with 1,3,5-trimethylhexahydro-1,3,5-triazine, the imidazole-*N*-oxide **6** was formed, which then was converted in situ into imidazole-2-thione **7** utilizing 2,2,4,4-tetramethylcyclobutane-1,3-dithione[19] as the sulfur source. *S*-Methylation of imidazole-2-thione **7** with excess iodomethane afforded methylsulfanylimidazole **8**, and subsequent acid-catalyzed cleavage of the amide bond in **8** furnished aminopyridine **9**. The nucleophilic displacement reaction of 1-phenylethylbromide with **9** provided target pyridinyl imidazole **2a**.

Although the requirement for an unambiguously established regiochemistry at the imidazole core was fully met by this reaction sequence, it suffered from several drawbacks. Most importantly, yields were low to moderate for the synthetic steps leading up to α-hydroximinoketone **5** and the protecting group approach was required, as the free amino group was not tolerated during the ring closure reaction. To facilitate the preparation of **2a** as well as the rapid synthesis of various analogues modified at the 2-position of the pyridine ring, the direct conversion of **1** and its analogue **10** into **2a** and **11a** was attempted (Scheme 4).

Upon treatment of **1**[16] with excess methyliodide in DMF, N-methylation was expected to take place at either imidazole ring nitrogen. Therefore, a reliable and fast analytical method was needed to determine the ratio by which regioisomers **2a** and **2b** were formed during the course of this reaction. In a series of N1-alkylated analogues of ML 3375, it was found that other than the parent ML 3375 (mp 263 °C), these compounds are comparatively low-melting (mp 100−170 °C, cf. Supporting Information for examples).[20] They were evaporated without decomposition at 250 °C and could thus be analyzed by GC/MS.[20] When monitoring the N-methylation of **1** by GC/MS, four peaks corresponding to two pairs of mass spectra, with identical molecular ions of *m*/*z* 418 and 432, respectively, were observed. We reasoned that not only one of the imidazole ring nitrogens but also the phenylethylamino group had reacted with the methylating agent under these conditions and that both possible

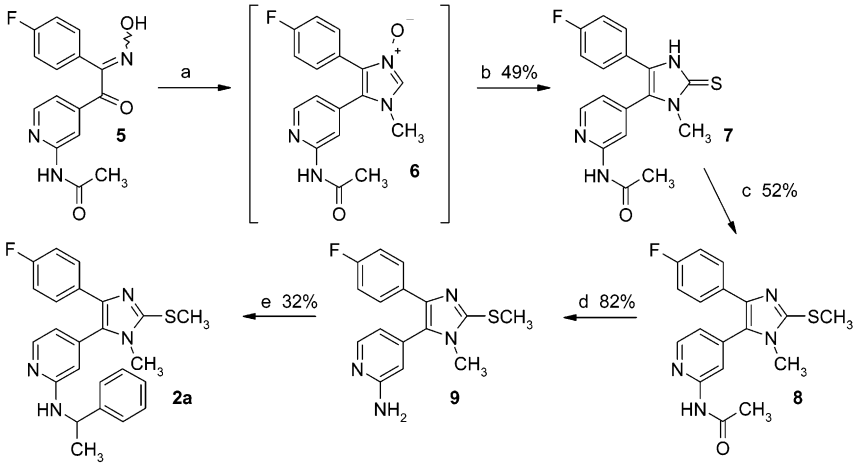
(17) Lantos, I.; Bender, P. E.; Razgaitis, K. A.; Sutton, B. M.; DiMartino, M. J.; Griswold, D. E.; Walz, D. T. *J. Med. Chem.* **1984**, *27*, 72−75.

(18) Lantos, I.; Gombatz, K.; McGuire, M.; Pridgen, L.; Remich, J.; Shilcrat, S. *J. Org. Chem.* **1988**, *53*, 4223−4227.

(19) Elam, E. U.; Davis, H. E. *J. Org. Chem.* **1967**, *32*, 1562−1565.

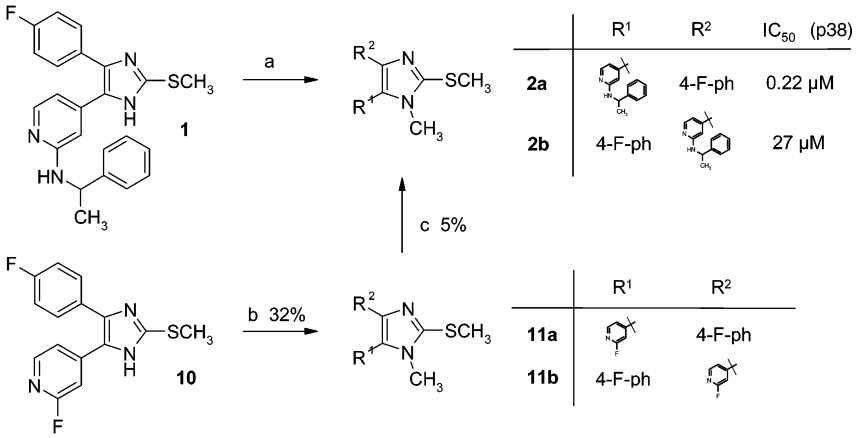
(20) Kotschenreuther, D. Ph.D. Thesis, Eberhard-Karls-Universität Tübingen, Tübingen, Germany, 2002.

SCHEME 3. Regiospecific Preparation of Regioisomer 2a[a]



[a] Reagents: (a) 1,3,5-trimethylhexahydro-1,3,5-triazine, ethanol, reflux; (b) 2,2,4,4-tetramethylcyclobutane-1,3-dithione, DCM, rt; (c) iodomethane, K_2CO_3, methanol, rt; (d) 10% HCl, reflux; (e) 1-phenylethylbromide, DMF, NaH, reflux.

SCHEME 4. Synthetic Approaches toward Regioisomers 2a and 2b[a]



[a] Reagents and conditions: (a) iodomethane, Cs_2CO_3, DMF, rt; (b) DMF−DMA, toluene, reflux (the yield is given for the conversion of **10** into **11b**); (c) 1-phenylethylamine, reflux (the yield is given for the conversion of **11b** into **2b**).

TABLE 1. Selected ^{1}H−^{13}C Connectivities Observed in the HMBC Spectra of Regioisomers 2a and 2b

2a	SCH_3	NCH_3	C3−H pyridine	C5−H pyridine
imidazole C2 (δ 144.2 ppm)[a]	**C**−S−C−**H**	**C**−N−C−**H**		
imidazole C5 (δ 128.3 ppm)[a]		**C**−N−C−**H**	**C**−C−C-**H**	**C**−C−C−**H**
2b	SCH_3	NCH_3	C3/C5−H 4-F−ph	
imidazole C2 (δ 146.4 ppm)[a]	**C**−S−C−**H**	**C**−N−C−**H**		
imidazole C4 (δ 134.6 ppm)[a]		**C**−N−C−**H**	**C**−C−C−**H**	

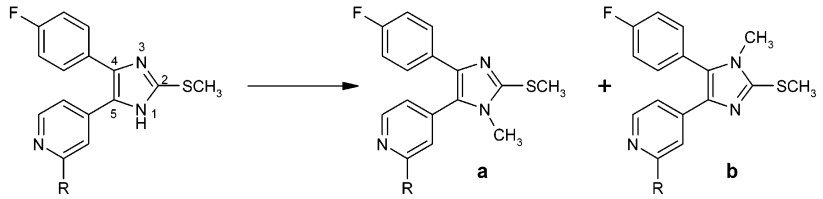
[a] Cf. Table 2 for the numbering of the imidazole nucleus.

imidazole regioisomers had been formed for both the secondary and the tertiary amine. However, the crude reaction mixture contained less than 20% of the desired 1-phenylethylamino pyridine **2a**, which eluted after 12.61 min, but contained 32% of the regioisomer **2b** (retention time 14.55 min). The former peak was unambiguously assigned to **2a** by comparison with the analytical data obtained for an authentic sample of **2a** from the regiospecific pathway.

Simultaneous alkylation at the imidazole ring and the phenylethylamino nitrogen was avoided by methylation of 2-fluoropyridine **10**[16] with excess dimethylformamide dimethylacetal (DMF−DMA) in toluene and subsequent introduction of the side chain by S_NAr (Scheme 4). Under these conditions, N-methylation proceeded with remarkable regioselectivity and isomer **11b** was the only reaction product that could be isolated, though a small amount of **11a** was detected by GC/MS (retention time 12.3 min, Table 2). Subsequent nucleophilic substitution of intermediate **11b** with 1-phenylethylamine afforded pyridinyl imidazole derivative **2b**. The biological results for **2a** and **2b** confirmed that placement of an additional substituent at the imidazole ring nitrogen adjacent to the 4-fluorophenyl moiety is detrimental for p38 inhibition. Compound **2a** exceeded its regioisomer in potency by 2 orders of magnitude (Scheme 4).

The regioselectivity of the methylation step providing **11b** was elucidated by comparison of the analytical data for the final product of this reaction sequence (i.e., **2b**) and for an authentic sample of **2a**. On the nonpolar

TABLE 2. Selectivity Ratios for the Alkylation of Various N-Unsubstituted Imidazoles



product code	**11**	**11**	**11**	**11**	**12**	**13**
R	−F	−F	−F	−F	−OH[a]	−OH[a]
conditions	DMF−DMA, reflux	MeI, Cs_2CO_3, DMF, rt	MeI, Na_2CO_3, DMF, rt	MeI, NaH, THF, rt	MeI, MeOH, reflux	BzBr, Na_2CO_3, EtOH, reflux
ratio of **a**:**b**	2:98[b]	7:93[b]	7:93[b]	0:100[c]	0:100[c]	0:100[c]

[a] IR and ^{13}C NMR data indicate that the pyridone is the preferred tautomeric form. [b] Ratio determined by GC/MS. [c] Isomers **11b**−**13b** were isolated as the only reaction products in the following yields: **11b** (36%), **12b** (8%), **13b** (3%).

stationary phase applied in GC/MS analysis, regioisomers **2a** and **2b** eluted over a time window of almost 2 min. The observed elution order corresponded to the order of melting points for **2a** and **2b**, as was expected, since separation on nonpolar capillary phases is mainly attributed to differences in vapor pressure of the solute. For the unequivocal assignment of peaks, it was crucially important to have a chromatographic system that clearly distinguished between both regioisomers, not the least because the mass spectra of **2a** and **2b** showed a very similar pattern of fragmentation. For both regioisomers, the base peak can be attributed to the molecular ion (*m*/*z* 418). The *m*/*z* 403 ion may result from demethylation at either the methylsulfanyl or methylamino group. Cleavage of the phenylethylamino substituent in various positions may account for the *m*/*z* 299, 120, and 105 fragments. Minor differences between the mass spectra of both regioisomers concerned the intensity of peaks, the most notable example being the relatively low abundance of the *m*/*z* 120 ion in the mass spectrum of **2b**.

With regard to the ^1H NMR spectra of **2a** and **2b**, regioisomeric substitution of the imidazole nucleus predominantly affected the chemical shifts for protons at the substituted pyridine ring (cf. Supporting Information). While an upfield shift by 0.59 and 0.32 ppm was observed for the C3- and C5-protons, respectively, in **2a** compared to **2b**, the signal assigned to the C6-proton in **2a** was shifted downfield by 0.61 ppm. As a result, the peaks attributed to the pyridine ring protons spread over a broad range of 2.06 ppm in the case of **2a** compared to only 0.87 ppm in the spectrum of **2b**. The significantly different distribution of electron density at the pyridine ring of regioisomers **2a** and **2b** can be explained by the different canonical forms induced by the presence of an additional substituent at either one of the two imidazole ring nitrogens. Therefore, we believe that in this class of compounds, the range covered by the signals assigned to the pyridine protons in the ^1H NMR spectra is a characteristic feature from which the regiochemistry of the imidazole ring can be determined. This view is supported by findings in a series of regioisomeric pyridinyl imidazoles and pyridinyl 2,3-dihydroimidazo[2,1-*b*]-thiazoles (cf. Supporting Information for examples).[20] It has to be noted that the differences in their ^1H NMR spectra are of particular value for the identification of regioisomers, as only one isomer is required for ^1H NMR analysis, whereas both isomers are needed for comparison of GC retention times.

The above assignment of the regioisomeric structures of **2a** and **2b** is supported by the results from two-dimensional NMR experiments. The NOESY spectrum of **2a** shows three cross-peaks for the *N*-methyl group (cf. Supporting Information). Two of them result from the interaction between the *N*-methyl protons and the C3- and C5-protons of the pyridine ring. As expected from the assigned structure, none of these cross-peaks is present in the NOESY spectrum of regioisomer **2b**. Here, a single cross-peak for the *N*-methyl substituent indicates its close proximity to the 4-fluorophenyl ring. The different connectivities observed in the respective HMBC spectra of **2a** and **2b** are also in agreement with the regiosomeric nature of these compounds (Table 1).

With a convenient GC/MS method at hand to monitor the regioselectivity of the direct alkylation approach and with the elution order of regioisomers **11a** and **11b** firmly established, we employed a variety of reaction conditions in order to improve the regioselectivity leading to the desired isomer **11a** (Table 2). However, a change in neither the methylating agent (DMF−DMA vs MeI) nor the strength of the base (NaH vs Cs_2CO_3) or the size of the counterion (Na_2CO_3 vs $CsCO_3$) considerably affected regioselectivity. When the influence of the substituent at the pyridine ring was tested in a series of pyridone analogues, elevated reaction temperatures suppressed N-substitution at the imidazole altogether. Here, **12b** and **13b** were the only isomers isolated in very poor yield, regardless of the alkylating agent and the solvent (Table 2). In summary, the direct N-alkylation of the substituted 2-methylsulfanylimidazoles presented herein appears to take place predominantly at the imidazole ring nitrogen adjacent to the 4-fluorophenyl substituent. This is in marked contrast to observations by other groups for related C2 carba-substituted imidazole derivatives.[3] However, the analytical protocol described above should facilitate ongoing efforts to alter the regioselectivity of this reaction.

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Supporting Information Available: Detailed synthetic procedures, spectroscopic data, and relevant examples from reference 20. This material is available free of charge via the Internet at http://pubs.acs.org.

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