Earlier studies prompted by our interests in potentially neuroprotective tetrahydropyridinyl “prodrugs” of nitroindazoles led to the development of synthetic routes to indazolylpyridinium derivatives, precursors to the targeted “prodrugs”. We were able to achieve the regiospecific syntheses of the 4-(1\textit{H}-indazolyl)-1-methylpyridinium iodide 110 (in the presence of base) and the 4-(2\textit{H}-indazolyl)-1-methylpyridinium iodide 112 (in the absence of base). Also an interesting rearrangement of the 2\textit{H}-indazolylpyridinium derivative 112 to the corresponding 1\textit{H}-isomer 110 was documented. In the first part of the present work, the results of our studies on the chemistry of indazole and indazolylpyridinium derivatives are reported with particular focus on the mechanism of the rearrangement reaction.

The rearrangement reaction of the 2\textit{H}-indazolylpyridinium derivative 112 to the corresponding 1\textit{H}-isomer 110 was found to be catalyzed by TMP. Our interpretation of the reaction pathway led us to propose that TMP attacked C-3 of the indazolyl system to form a spirodiaziridinyl intermediate 121. Collapse of this intermediate resulted in the formation of the 1\textit{H}-indazolylpyridinium rearrangement product 110. Kinetic studies were undertaken to gain additional information on the proposed pathway. Consistent with the proposed pathway, we found that the rearrangement was first order in TMP as well as in the 2\textit{H}-indazolylpyridinium.

When TMP was replaced with piperidine, a less hindered but more nucleophilic amine with comparable basicity to TMP, the expected rearrangement reaction was not observed. Instead, an aminolysis reaction took place resulting in the formation of indazole (44) and 4-piperidin-1-yl-1-methylpyridinium iodide (132), the product expected from the attack of piperidine at C-4 of the pyridinium moiety. When TMP was replaced with triethylamine or DABCO, more hindered, less nucleophilic amines with comparable basicity to TMP, a significant decrease in the rate of the rearrangement reaction was observed. These results support a nucleophilic role for TMP in the rearrangement reaction.
Although experimental evidence was in agreement with the proposed pathway, the site of attack by TMP was not clear. Among the possible sites of attack (C-3, C-4, C-6, C-7a), C-3 appeared to be the most likely site due to the formation of the energetically favored intermediate 121 (intrinsic aromatic feature of the 6-π electron system). This proposal was examined via 3-substituted-indazolylpyridinium derivatives. The increase in steric hindrance by substitution at C-3 was expected to slow the rate of the rearrangement reaction. The regiospecific synthetic route which had been employed successfully for the preparation of the 2H-indazolylpyridinium derivative 112 was attempted as a means to obtain 2H-3-methyl and 2H-3-bromoindazolylpyridinium derivatives 140 and 141, respectively. However, due to the resulting steric hindrance, the reaction between 4-chloro-1-methylpyridinium iodide (55) and 3-methyl and 3-bromoindazole in the absence of TMP proceeded too slowly to make this approach synthetically feasible. In the case of the 3-methylindazole (142), a very low yield of the 1H-isomer 144, rather than the expected 2H-isomer 140, was obtained. The 2H-isomer was present in even lower amounts. The formation of 144 was unlikely to take place through the attack by N-1 of neutral indazole due to the high energy of the charge localized intermediate 152 that would result from such an attack. Consequently, we speculated that a unimolecular rearrangement reaction of the 2H-isomer 140 to the 1H-isomer was taking place with the driving force being the stabilization of the hypothetical spirodiaziridinyl intermediate 153 by the 3-methyl substituent. The 2H-isomer, the minor product of the reaction between 3-methylindazole (142) and 55 in the absence of TMP, was isolated via preparative HPLC. The pure (by NMR) 2H-isomer readily rearranged in the absence of TMP. However, 140, with iodide as the counter ion, which was prepared by an independent synthesis, proved to be stable under the same conditions that led to rearrangement of the HPLC purified 140. Eventually, we discovered that the pyridinium compound from the HPLC column was isolated with acetate as the counter ion. The role of acetate in catalyzing the rearrangement reaction was confirmed when the otherwise stable 2H-protoindazolylpyridinium derivative 112 was shown to rearrange in the presence of potassium acetate.

The stability of the 2H-isomer 140 in the absence of base leaves open the question of the pathway leading to the 1H-isomer 144 in the reaction between 3-methylindazole
and 55 in the absence of base. Two possible pathways were proposed to account for the formation of the 1$H$-isomer in the absence of base, one involving disproportionation and the second one, tautomerization of 3-methylindazole.

The rearrangement of the 2$H$-3-methylindazolylpyridinium derivative 140 in the presence of TMP was found to proceed at a rate comparable to that observed with the 2$H$-proteindazolylpyridinium derivative 112. This was unexpected since attack by TMP at C-3 should be energetically disfavored due to the steric hindrance exerted by the presence of the methyl group at this position. This observation led to the consideration that the attacking species was actually hydroxide ion that would form in situ via an acid-base equilibrium reaction between TMP and the trace amount of water present in DMSO. Subsequently, it was shown that hydroxide ion did catalyze the rearrangement reaction. This observation is of particular interest since, based on the results obtained with piperidine, hydroxide, a small nucleophilic species, was expected to attack at C-4 of the pyridinium moiety leading to indazole and 1-methyl-4-pyridone (169). However, the poor acidity of OH proton compared to the piperidinium proton of the analogous intermediate 131 could retard this cleavage reaction and favor the rearrangement reaction.

After studying the effect of C-3 methyl substituent on the rate of the rearrangement reaction, 2$H$-3-bromoindazolylpyridinium derivative 141 was also prepared and its TMP catalyzed rearrangement to the corresponding 1$H$-isomer 145 was studied. This time, a significant increase in the rate of the rearrangement was observed. The rate enhancing effect was attributed to the electronegativity of the bromine atom and the resulting increased electropositivity at C-3 which would facilitate the attack by TMP.

The results obtained for the 3-substituted indazolylpyridinium derivatives were in agreement with the proposed rearrangement pathway initiated by the attack of TMP at C-3. Nevertheless, attack at C-6, another possible site, was also studied via the preparation of 6-substituted indazolylpyridinium derivatives. Preparation of both 1$H$- and 2$H$-6-bromo and 6-methoxyindazolylpyridinium derivatives were achieved via the regiospecific pathways described for the synthesis of protioindazolylpyridinium derivatives. The studies on the TMP catalyzed rearrangement of the 2$H$-6-bromoindazolylpyridinium derivative 174 showed that compound 174, like the 2$H$-3-bromoindazolylpyridinium species 141, underwent rearrangement at room temperature.
The rate, however, was slower than that observed for 141. This was attributed to the increased electropositivity of C-3 due to the electronegative inductive effect of the bromine atom present at C-6. On the other hand, the rearrangement of the 2H-6-methoxyindazolylpyridinium derivative 171 proceeded only at elevated temperatures and at a rate comparable to that observed for the 2H-protio analog 112. The 2H-5-bromo and 2H-5-methoxyindazolylpyridinium derivatives 193 and 194 were also prepared as a means to investigate the effect of substituents at a carbon atom where the attack by TMP will not lead to the rearrangement reaction. The rearrangement of the 2H-5-bromoindazolylpyridinium derivative 194 proceeded in the presence of TMP at room temperature at a comparable rate to that observed with the 2H-6-bromoindazolylpyridinium derivative 174. If the enhanced rate of rearrangement observed for the 2H-6-bromoindazolylpyridinium derivative were due to the attack by TMP at C-6, a similar rate of rearrangement for 2H-5-bromoindazolylpyridinium derivative 193 would not be expected. Therefore, the observation that the 2H-isomers 174 and 193 rearrange at similar rates provided additional evidence to rule out the possibility of C-6 attack by TMP. The rate of rearrangement observed for the 2H-5-methoxyindazolylpyridinium derivative 194 was comparable to that observed for the protio analog 112. This outcome suggested that the increase in electron density at C-3 via resonance is not significant.

The results of our studies on the rearrangement of the 2H-indazolylpyridinium derivatives bearing substituents at C-3, C-5 and C-6 positions were in agreement with the attack of TMP, most likely at C-3, leading to the formation of a spirodiaziridinyl intermediate, collapse of which results in the rearrangement of the 2H-isomer to the corresponding 1H-isomer. However, no direct evidence was available yet to document the site of attack. Therefore, the possibility of displacing bromide from the 2H-3-bromoindazolylpyridinium derivative 141 during the course of the rearrangement reaction was explored with the use of cyanide, a strong nucleophile with poor leaving group properties. When the 2H-3-bromoindazolylpyridinium derivative 141 was treated with cyanide, the rearrangement of 141 to the corresponding 1H-isomer 145 was observed with the retention of bromine despite the better leaving group properties of bromide compared to cyanide. This result forced us to consider a radical pathway as an
alternative mechanism for the rearrangement reaction. No experimental evidence, however, could be obtained to support a non-polar mechanism. The retention of bromine following attack by cyanide was rationalized by the stereospecific formation of one of the two possible diastereomeric spirodiaziridinyl intermediates 197a and 197b via a concerted Michael type 1,4-addition reaction.

The cyanide mediated rearrangement reaction of 2H-indazolylpyridinium derivatives was investigated in greater detail. It was found that the rearrangement reaction was not catalytic in cyanide and that cyanide was consumed during the course of the reaction. Both 1H- and 2H-isomeric indazolylpyridinium derivatives were shown to undergo cleavage reactions to form the corresponding parent indazoles and a complex mixture of cyano containing products. The identification of some of the decomposition products with the aid of 13CN− provided some insights into the pathways responsible for cyanide consumption. The effects of the presence of water and dioxygen on the cyanide mediated decomposition reaction were investigated. The comparison of the cyanide-mediated rate of rearrangement in DMSO, DMF and water also supported a nucleophilic pathway.

Finally, fully consistent with a polar reaction pathway involving nucleophilic attack of the 2H-indazolylpyridinium species was the observation that the rates of the rearrangement reaction paralleled the nucleophility of the attacking species with cyanide> hydroxide>azide>acetate> perchlorate.

In the second part of this work, we focused our attention on the biological evaluation of the nitroindazolyl “prodrugs”. The synthesis and MAO-B substrate and inhibitor properties of these compounds have been described previously.255

The 1H-5-nitro and 1H-6-nitro “prodrugs” are MAO-B substrates. The major metabolites observed in the incubation mixtures were the corresponding pyridinium derivatives. In addition to the pyridinium metabolites, the expected release of the parent indazoles also was observed suggesting the feasibility of the “prodrug” approach. However, the main target, the 2H-7-nitroindazolyl “prodrug”, was found not to be an MAO-B substrate but rather an MAO-B inhibitor.

255 Reference 1.
In the present study, the MAO-A substrate properties of these “prodrugs” were investigated. The $1H$-5-NI, $1H$-6-NI “prodrugs” and the $2H$-7-NI “prodrug” showed MAO-A substrate properties. According to the results of preliminary studies, the 6-NI “prodrug” appears to be the best MAO-A substrate among the three “prodrugs”. The extent of turnover by MAO-A was small for the 5-NI and 7-NI “prodrugs”. LC-MS analyses of metabolites confirmed the formation of the corresponding pyridinium species and the parent indazoles. Also, the presence of 1-methyl-2,3-dihydro-4-pyridone (32) was detected in the incubation mixtures providing additional support for the validity of the proposed bioactivation pathway.

Finally, with the availability of the x-ray crystal structure of MAO-B, docking studies were carried out to rationalize the observed MAO-B substrate properties of the $1H$-5-NI and 6-NI “prodrugs” and the non-substrate property of the $2H$-7-NI “prodrug”. During the docking studies, some of the key interactions between the MAO-B and the investigated ligands were identified. It was concluded that the length of the $2H$-7-NI “prodrug” is likely to be a determining factor leading to its MAO-B non-substrate properties. This conclusion is consistent with a previously developed model of MAO-B active site. The interactions lead to an alternative orientation of the $2H$-7-NI analog that accounts for the observed enzyme inhibition. Docking models suggested that in this alternative arrangement, the $2H$-7-NI “prodrug” was occupying both the entrance and substrate cavities of the active site. A similar observation has been reported previously for 1,4-diphenyl-2-butene (259), another competitive inhibitor of MAO-B. The reported docking studies demonstrate the applicability of such an approach to understand better the interactions of previously investigated tetrahydropyridinyl derivatives with the active site of MAO-B. Furthermore, the involvement of the entrance cavity in the inhibition of the enzyme may help to explain isoform selectivities of recently reported competitive inhibitors with large backbone structures.

After the determination of the kinetic parameters for the MAO-A and MAO-B substrate properties of nitroindazolyl “prodrugs”, in vivo neuroprotection studies on the selected “prodrugs” as well as the parent nitroindazoles will have to be carried out. The MAO-A and MAO-B mediated release of the parent compounds and the previously observed time dependent inhibition of MAO-B by the $1H$-6-NI “prodrug” are
encouraging for the application of the "prodrug" approach as a neuroprotective strategy. Although, the 2H-7-NI “prodrug” was shown not to be an MAO-B substrate, the MAO-A substrate properties of this compound leading to the release of 7-NI suggest that it should be a candidate for the neuroprotection studies. Due to the differences in their MAO-B and nNOS inhibition properties of 5-, 6-, and 7-nitroindazoles, the results of the neuroprotection studies involving nitroindazole “prodrugs” and the parent nitroindazoles may provide clues for the involvement of MAO-B versus nNOS in the pathways leading to neurotoxicity.

In addition to nitroindazole “prodrugs”, the MAO-A and MAO-B substrate properties of the other tetrahydropyridinyl analogues of the indazolylpyridinium derivatives described in the first part of this work, should be investigated. Accompanied with docking studies, these 1H- and 2H-tetrahydropyridinyl derivatives may provide additional information regarding the active sites of both isoforms of MAO. Among these tetrahydropyridinyl derivatives, additional candidates for neuroprotection studies may be identified.
CHAPTER 7
EXPERIMENTAL

7.1. General

All reactions were carried out using glassware that had been flame dried under an inert atmosphere of dry nitrogen or in sealed reaction vials. All chemicals were reagent or HPLC grade. Melting points were determined using a Thomas-Hoover melting point apparatus and are uncorrected. The $^1$H-NMR and $^{13}$C-NMR spectra were recorded with an INOVA 400 MHz or JEOL 500 spectrometer. DPFGSE-NOE $^1$H NMR experiments were carried on JEOL 500. $^1$H and $^{13}$C chemical shifts ($\delta$) are reported in parts per million (ppm) relative to the internal standard tetramethylsilane (TMS). Spin multiplicities are given as s (singlet), d (doublet), t (triplet), q (quartet). Coupling constants (J) are given in hertz (Hz). UV-Vis absorption spectra were recorded on a Beckman DU Series 7400 spectrophotometer. Gas chromatography-electron ionization mass spectrometry (GC-EIMS) was performed on a Hewlett Packard (HP) Model 6890 gas chromatography fitted with a HP-1 column (25 m x 0.20 mm x 0.33 μm thickness) which was coupled to an HP 5973 mass selective detector. Helium was the carrier gas (1 mL/min). Unless otherwise stated all GC-EIMS data were obtained using an initial oven temperature of 60 °C, holding at 60 °C for 3 minutes, ramping at 25 °C/min to a final temperature of 275 °C, holding at 275 °C for 4 minutes with a solvent delay of 4.1 min and injection port temperature of 250 °C. Normalized peak heights are reported as a percentage of the base peak. High pressure liquid chromatography (HPLC) was performed on a HP model 1100 HPLC system equipped with quaternary pump, degasser, diode array detector and a 250 mm x 4.6 mm Zorbax SB-C8 5 μm column (reverse phase) with an in-line pre-column filter (2 μM, Upchurch Scientific Inc.). LC-MS analysis was performed on API 365 LC/MS/MS system (Applied Biosystems) using APCI as the ionization source.
7.2. Chemistry

The following compounds were prepared according to the literature and showed the expected $^1$H NMR and mp behavior: 4-chloro-1-methylpyridinium iodide (55), $^{256}$ 1-methyl-4-piperidin-1-ylpyridinium iodide (132), $^{257}$ diazonium tetrafluoroborate salts $^{150,258}$ 184, $^{258}$ 3-bromoindazole (143), $^{259}$ 3-methylindazole (142), $^{260}$ 6-methoxyindazole (173), $^{260}$ 5-methoxyindazole (192). $^{260}$

6-Bromoindazole (172).

A solution of 5-bromo-2-methylaniline [178 (1.92 g, 10 mmoles) in 30 mL of glacial acetic cooled to 5 °C in an ice bath. To the solution of 178 in acetic acid, sodium nitrite (690 mg, 10 mmole in 1 mL of water) was added dropwise. The temperature was kept below 5 °C during the addition. After stirring the reaction mixture at 5 °C for 45 minutes, the solvent was evaporated via lyophilization. The resulting residue was partitioned between water and ethyl acetate. The organic layer was washed with first 10 % aqueous sodium hydroxide solution then with brine. The organic layer was dried over MgSO$_4$ and evaporated in vacuo. The resulting product was recrystallized from toluene to give 1.24 g (6.3 mmoles, 63 %) of 172 as an orange solid: mp 161-162 °C; $^1$H-NMR (DMSO-$d_6$, 500 MHz): $\delta$ 8.10 (s, 1H), 7.76 (d, $J$ = 1.6 Hz, 1H), 7.74 (d, $J$ = 8.5 Hz 1H), 7.24 (dd, $J$ = 1.6 Hz, $J$ = 8.5 Hz, 1H); $^{13}$C-NMR (DMSO-$d_6$, 100 MHz) 134.4, 123.9, 122.9, 122.3, 119.9, 113.1; GC-EIMS m/z (relative intensity) 198 (M$^+$, 100), 196 (M$^+$, 100), 169 (6), 117 (58), 90 (83), 63 (48), 62 (29), 52 (15); HR-FABMS. Calculated for C$_7$H$_6$BrN$_2$ (based on $^{79}$Br isotope): 196.97143. Found: 196.97083.

$^{259}$ Reference 193.
$^{260}$ Reference 195.
4-(1H-indazolyl)-1-methylpyridinium iodide (110).

A mixture of indazole [44 (240 mg, 2 mmole)], 4-chloro-1-methylpyridinium iodide [55 (510 mg, 2 mmole)] and TMP (0.68 mL, 4.0 mmole) in 3 mL DMF was stirred at 60 °C for 36 h. The reaction mixture was cooled to room temperature and the resulting solid was collected and recrystallized from methanol to give 510 mg (1.51 mmol, 76 %) of 110 as a tan solid: mp 261-263 °C; 1H-NMR (DMSO-d6, 360 MHz): δ 8.94 (m, 2H), 8.77 (d, J = 0.9 Hz, 1H), 8.53 (m, 2H), 8.36 (dddd, J = 0.9 Hz, J = 0.9 Hz, J = 0.9 Hz, J = 8.6 Hz, 1H), 8.02 (ddd, J = 0.9 Hz, J = 0.9 Hz, J = 8.0 Hz, 1H), 7.73 (ddd, J = 0.9 Hz, J = 7.1 Hz, J = 8.5 Hz, 1H), 7.49 (ddd, J = 0.9 Hz, J = 7.2 Hz, J = 8.0 Hz, 1H), 4.3 (s, 3H); 13C-NMR (DMSO-d6, 90 MHz) 150.9, 146.6, 142.1, 138.2, 129.8, 127.2, 124.6, 122.7, 115.2, 112.8, 46.6; Anal. Calculated for C13H12IN3: C, 46.31; H, 3.59; N, 12.47. Found: C, 46.41; H, 3.62; N, 12.40.

General method for the preparation of 1H-Indazolylpyridinium derivates 144, 145, 176. 177, 196, 197.

The starting indazole (1 mmol) and 4-chloro-1-methylpyridinium iodide [55 (260 mg, 1 mmol)] were dissolved in 2 mL of DMF in a sealed reaction vial. To the solution, 2,2,6,6-tetramethylpiperidine [56 (190 µL 1 mmol)] was added in one portion. The separation of product as crystalline material was observed between 15-30 min depending on the starting indazole. The reaction mixture was stirred for 24 hours at 22 °C and cooled to 0 °C. The resulting solid was collected via vacuum filtration and washed with DMF cooled to 5 °C. The product was recrystallized from appropriate solvent to give the 1H-indazolylpyridinium species.

The progress of the reactions was monitored by injecting aliquots (10 µL of reaction mixture added to 490 µL of acetonitrile) onto the HPLC-DA. Mobile phase (isocratic) consisted of 50 % acetonitrile and 50 % pH 4.7 aqueous containing 1 % triethylamine and 0.6 % acetic acid and the flow rate was 1 mL/min. Disappearance of the starting materials and the appearance of products were monitored at 269 nm, 290 nm, 335 nm, 350 nm and 375 nm.
4-(1H-3-Bromoindazolyl)-1-methylpyridinium iodide (145).

The product was crystallized from MeOH to give 330 mg (0.79 mmol, 79 %) of 145 as a gray solid: mp 270-271 °C; $^1$H-NMR (DMSO-$d_6$, 400 MHz): $\delta$ 8.99 (m, 2H), 8.56 (m, 2H), 8.43 (dd, $J$ = 1 Hz, $J$ = 6.0 Hz, 1H), 7.63 (dt, $J$ = 1 Hz, $J$ = 7.5 Hz, 1H), 7.88 (m, 2H), 4.32 (s, 3H); $^{13}$C-NMR (DMSO-$d_6$, 125 MHz) 150.3, 147.5, 139.8, 132.0, 131.9, 127.1, 126.1, 122.0, 116.1, 114.0, 47.2; Anal. Calculated for C$_{13}$H$_{11}$BrI$_3$: C, 37.53; H, 2.66; N, 10.10. Found: C, 37.77; H, 2.73; N, 9.92.

4-(1H-3-Methylindazolyl)-1-methylpyridinium iodide (144).

The product was crystallized from MeOH to give 260 mg (0.74 mmol, 74 %) of 144 as a pink solid: mp 281.5-282 °C; $^1$H-NMR (DMSO-$d_6$, 500 MHz): $\delta$ 8.89 (m, 2H), 8.49 (m, 2H), 8.36 (dt, $J$ = 0.7 Hz, $J$ = 8.6 Hz, 1H), 8.00 (dt, $J$ = 1.1, $J$ = 7.9 Hz, 1H), 7.75 (ddd, $J$ = 1.1 Hz, $J$ = 7.1 Hz, $J$ = 8.6 Hz, 1H), 7.52 (ddd, $J$ = 0.7 Hz, $J$ = 7.1 Hz, $J$ = 7.9 Hz, 1H), 4.28 (s, 3H), 2.66 (s, 3H); $^{13}$C-NMR (DMSO-$d_6$, 125 MHz) 151.4, 150.8, 147.0, 139.4, 130.5, 127.9, 125.0, 122.6, 115.1, 113.6, 46.6, 12.4; Anal. Calculated for C$_{14}$H$_{14}$I$_3$: C, 47.88; H, 4.02; N, 11.97. Found: C, 47.43; H, 4.00; N, 11.80.

4-(1H-6-Bromoindazolyl)-1-methylpyridinium iodide (176).

The product was crystallized from MeOH to give 235 mg (0.56 mmol, 56 %) of 176 as orange crystals: mp 301-302 °C (dec.); $^1$H-NMR (DMSO-$d_6$, 400 MHz): $\delta$ 8.98 (m, 2H), 8.81 (s, 1H), 8.62 (m, 3H), 8.01 (d, $J$ = 8.5 Hz, 1H), 7.70 (dt, $J$ = 0.8, $J$ = 8.5 Hz, 1H), 4.34 (s, 3H); $^{13}$C-NMR (DMSO-$d_6$, 125 MHz) 163.0, 147.3, 142.4, 139.6, 128.9, 126.9, 124.8, 124.2, 116.5, 116.0, 47.2; Anal. Calculated for C$_{13}$H$_{11}$BrI$_3$: C, 37.53; H, 2.66; N, 10.10. Found: C, 37.40; H, 2.61; N, 9.95.

4-(1H-6-Methoxyindazolyl)-1-methylpyridinium iodide (177).

The product was crystallized from MeOH to give 260 mg (0.71 mmol, 71 %) of 177 as a yellow solid: mp 259-260 °C; $^1$H-NMR (DMSO-$d_6$, 400 MHz): $\delta$ 8.94 (m, 2H), 8.66 (d, $J$ = 0.4 Hz, 1H), 8.56 (m, 2H), 7.91 (d, $J$ = 8.8 Hz, 1H), 7.69 (d, $J$ = 0.4 Hz, 1H), 7.15 (dd, $J$ = 2 Hz, $J$ = 8.8 Hz, 1H), 4.32 (s, 3H), 3.99 (s, 1H); $^{13}$C-NMR (DMSO-$d_6$, 125 MHz) 161.9, 151.2, 147.1, 142.5, 140.3, 124.1, 122.0, 116.0, 115.2, 96.4, 56.7,
47.2; Anal. Calculated. for C_{14}H_{14}IN_3O: C, 45.79; H, 3.84; N, 11.44. Found: C, 45.77; H, 3.82; N, 11.24. HR-FABMS. Calculated for C_{14}H_{14}N_3O^+: 240.11369. Found: 240.11348.

4-(1H-5-Bromoindazolyl)-1-methylpyridinium iodide (195).

The product was crystallized from MeOH to give 250 mg (0.60 mmol, 60 %) of 195 as yellow crystals: mp 280-281 °C; ^1H-NMR (DMSO-\textit{d}_6, 400 MHz): δ 8.96 (m, 2H), 8.75 (s, 1H), 8.54 (m, 2H), 8.33 (d, J = 9.0 Hz, 1H), 8.30 (m, 1H), 7.88 (d, J = 9.0 Hz, 1H), 4.32 (s, 3H); ^13C-NMR (DMSO-\textit{d}_6, 100 MHz) 150.8, 147.2, 141.6, 137.7, 132.7, 129.5, 125.6, 117.2, 116.2, 115.1, 47.2; Anal. Calculated for C_{13}H_{11}BrIN_3: C, 37.53; H, 2.66; N, 10.10. Found: C, 37.64; H, 2.55; N, 10.08.

4-(1H-5-Methoxyindazolyl)-1-methylpyridinium iodide (196).

The product was crystallized from acetonitrile to give 245 mg (0.67 mmol, 67 %) of 196 as a light pink solid: mp 266-267 °C; ^1H-NMR (DMSO-\textit{d}_6, 400 MHz): δ 8.92 (m, 2H), 8.69 (d, J = 0.8 Hz, 1H), 8.51 (m, 2H), 8.30 (dt, J = 0.8 Hz, J = 9.2 Hz, 1H), 7.49 (d, J = 2.5 Hz, 1H), 7.35 (dd, J = 2.5 Hz, J = 9.2 Hz, 1H), 7.49 (ddd, J = 0.9 Hz, J = 7.2 Hz, J = 8.0 Hz, 1H), 4.29 (s, 3H), 3.88 (s, 3H); ^13C-NMR (DMSO-\textit{d}_6, 125 MHz) 157.1, 150.9, 147.1, 142.5, 134.0, 129.2, 120.6, 115.3, 114.5, 103.7, 56.3, 47.0; Anal. Calculated for C_{14}H_{14}IN_3O: C, 45.79; H, 3.84; N, 11.44. Found: C, 45.80; H, 3.78; N, 11.41.

4-(2H-Indazolyl)-1-methylpyridinium iodide (112).

A solution of indazole [44 (120 mg, 1 mmol)] and 4-chloro-1-methylpyridinium iodide [55 (255 mg, 1 mmol)] in 2 mL of DMF was stirred at 90 °C for 24 hours in a sealed reaction vial. The solid which separated upon cooling was recrystallized from MeOH to give 180 mg (0.53 mmol, 53 %) of 112 as yellow crystals: mp 241-243 °C; ^1H NMR (DMSO-\textit{d}_6, 360 MHz): δ 9.55 (d, J = 1.0 Hz, 1H), 9.13 (m, 2H), 8.77 (m, 2H), 7.83 (ddd, J = 1.1 Hz, J = 1.1 Hz, J = 8.7 Hz, 1H), 7.74 (dddd, J = 1.1 Hz, J = 1.1, J = 1.1 Hz, J = 8.9 Hz, 1H), 7.44 (ddd, J = 1.1 Hz, J = 6.5 Hz, J = 8.9 Hz, 1H), 7.2 (ddd, J = 1.1 Hz, J = 6.5 Hz, J = 8.7 Hz, 1H), 4.4 (s, 3H); ^13C-NMR (DMSO-\textit{d}_6, 90 MHz) δ 151.0, 150.2, 147.3, 129.8, 124.7, 124.3, 123.4, 121.5, 117.8, 116.4, 47.1; Anal.
General method for the preparation of the 2H-Indazolpyridinium derivatives 174, 175, 193, 194.

A solution of the starting indazole (1 mmol) and 4-chloro-1-methylpyridinium iodide [55 (260 mg, 1 mmol)] in 2 mL of DMF was stirred at 90 °C for 24 hours in a sealed reaction vial. Diethyl ether (2 mL) was added and the reaction mixture was cooled to 0 °C. The resulting solid was collected via vacuum filtration and washed with diethyl ether. The product was recrystallized from appropriate solvent to give the 2H-indazolpyridinium species.

The progress of the reactions was monitored by injecting aliquots (10 µL of the reaction mixture added to 490 µL of acetonitrile) onto the HPLC-DA. Mobile phase (isocratic) consisted of 50 % acetonitrile and 50 % pH 4.7 aqueous containing 1 % triethylamine and 0.6 % acetic acid and the flow rate was 1 mL/min. Disappearance of the starting materials and the appearance of products were monitored at 269 nm, 290 nm, 335 nm, 350 nm and 375 nm.

4-(2H-6-Bromoindazolyl)-1-methylpyridinium iodide (174).

The product was crystallized from MeOH to give 300 mg (0.72 mmol, 72 %) of 174 as yellow crystals: mp 275.5-276 °C; ¹H NMR (DMSO-d₆, 400 MHz): δ 9.81 (d, J = 0.9 Hz, 1H), 9.65 (m, 2H), 9.21 (m, 2H), 7.98 (dt, J = 0.9 Hz, J = 9.5 Hz, 1H), 7.44 (dd, J = 2.4 Hz, J = 9.5 Hz, 1H), 7.37 (d, J = 2.4 Hz, 1H), 4.34 (s, 3H); ¹³C-NMR (DMSO-d₆, 100 MHz) δ 151.8, 150.6, 148.0, 128.2, 126.4, 124.3, 123.9, 122.4, 120.4, 117.2, 47.7; Anal. Calculated for C₁₃H₁₁BrN₃: C, 37.53; H, 2.66; N, 10.10. Found: C, 37.84; H, 2.66; N, 10.10.

4-(2H-6-Methoxyindazolyl)-1-methylpyridinium iodide (175).

The product was crystallized from acetonitrile to give 210 mg (0.57 mmol, 57 %) of 175 as bright yellow crystals: mp 272.5-273.5 °C; ¹H NMR (DMSO-d₆, 400 MHz): δ 9.43 (d, J = 0.7 Hz, 1H), 9.08 (m, 2H), 8.67 (m, 2H), 7.72 (dd, J = 0.7 Hz, J = 9.3 Hz,
1H), 6.87 (dd, J = 2.2 Hz, J = 9.3 Hz, 1H), 6.99 (d, J = 2.2 Hz, 1H), 4.32 (s, 3H), 3.88 (s, 3H); 13C-NMR (DMSO-d6, 100 MHz) δ 161.3, 153.1, 147.7, 125.2, 123.0, 121.0, 120.4, 116.2, 94.3, 65.4, 56.0, 47.4; Anal. Calculated for C14H14IN3O: C, 45.79; H, 3.84; N, 11.44. Found: C, 45.85; H, 3.85; N, 11.51.

4-(2H-5-bromoindazolyl)-1-methylpyridinium iodide (193).

The resulting solid was crystallized from MeOH to give 280 mg (0.67 mmol, 67%) of 193 as orange-red crystals: mp 257-258 °C; 1H NMR (DMSO-d6, 400 MHz): δ 9.55 (s, 1H), 9.16 (m, 2H), 8.80 (m, 2H), 8.19 (dd, J = 0.8 Hz, J = 1.8 Hz, 1H), 7.78 (dt, J = 0.9 Hz, J = 9.4 Hz, 1H), 7.55 (dd, J = 1.8 Hz, J = 9.5 Hz, 1H), 4.36 (s, 3H); 13C-NMR (DMSO-d6, 125 MHz) δ 150.6, 149.7, 148.0, 133.6, 125.0, 124.9, 124.1, 120.7, 117.5, 117.2, 47.7; HR-FABMS. Calculated for C13H11BrN3+ (based on 79Br isotope): 288.01363. Found: 288.01310.

4-(2H-5-Methoxyindazolyl)-1-methylpyridinium iodide (194).

The resulting solid was crystallized from MeOH to give 230 mg (0.63 mmol, 63%) of 194 as a yellow solid: mp 266-266.5 °C; 1H NMR (DMSO-d6, 400 MHz): δ 9.31 (d, J = 0.8 Hz, 1H), 9.04 (m, 2H), 8.71 (m, 2H), 7.68 (dt, J = 0.8 Hz, J = 9.5 Hz, 1H), 7.13 (dd, J = 2.4 Hz, J = 9.5, 1H), 7.03 (d, J = 2.4 Hz, 1H), 4.33 (s, 3H), 3.84 (s, 3H); 13C-NMR (DMSO-d6, 100 MHz) δ 156.5, 149.2, 147.7, 126.5, 124.5, 123.5, 119.9, 116.4, 96.7, 55.9, 47.5; 13C-NMR (D2O, 100 MHz) δ 155.7, 150.2, 149.0, 146.4, 126.2, 123.7, 122.8, 118.7, 116.0, 95.5, 55.4, 47.1; Anal. Calculated for C14H14N3O: C, 45.79; H, 3.84; N, 11.44. Found: C, 46.37; H, 4.20; N, 11.17. HR-FABMS. Calculated for C14H14N3O+: 240.11369. Found: 240.11308.

Acetate salt of 4-(2H-3-methylindazolyl)-1-methylpyridinium (140).

A solution of 3-methylindazole [142] (130 mg, 1 mmol) and 4-chloro-1-methylpyridinium iodide [55] (260 mg, 1 mmol) in 2 mL of DMF was stirred at 90 °C for 24 hours in a sealed reaction vial. After diluting 100 µL of the reaction mixture by adding 400 µL of water, the solution (50 µL) was applied to HPLC-DA. Isocratic conditions were used with a mobile phase consisting of 50 % acetonitrile and 50 % pH 4.7 aqueous
containing 1% triethylamine and 0.6% acetic acid and a flow rate of 1 mL/min. The effluent (3.1-3.4 minutes) from 10 injections was collected and the mobile phase was evaporated via freeze drying to give 1 mg of 144 as the acetate salt: $^1$H NMR (DMSO-$d_6$, 400 MHz): $\delta$ 9.16 (m, 2H), 8.56 (m, 2H), 7.85 (dd, $J = 1$ Hz, $J = 8.5$ Hz, 1H), 7.67 (dd, $J = 0.8$ Hz, $J = 8.5$ Hz, 1H), 7.41 (dt, $J = 0.8$ Hz, $J = 7.2$, 1H), 7.14 (dd, $J = 1$ Hz, $J = 7.6$ Hz, 1H), 4.33 (s, 3H), 2.84 (s, 3H), 1.53 (s, 3H). HR-FABMS Calculated for C$_{14}$H$_{14}$N$_3$+: 224.11877. Found: 224.11990.

4-(2H-Indazolyl)pyridine (157).

NaH (6 g, 150 mmol) was added portionwise with stirring to a solution of indazole [44 (11.81 g, 100 mmol)] and 4-chloropyridine (11.35 g, 100 mmol) in 200 mL of DMF. The reaction mixture was stirred under reflux for 18 hours. After cooling, DMF was extracted with 50 % aqueous ethyl acetate. The organic layer was dried over MgSO$_4$ and evaporated in vacuo to yield crude 157 in a mixture with the oily 1H-isomer. The mixture was cooled to 0 oC, the precipitate was collected and washed with hexane. Crystallization from hexane gave 6.8 g (35 mmol, 35%) of 157 as white crystals: mp 120-121 oC; $^1$H NMR (CDCl$_3$, 360 MHz) $\delta$ 8.76 (m, 2H), 8.54 (d, $J = 0.9$ Hz, 1H), 7.89 (m, 2H), 7.76 (dddd, $J = 0.9$ Hz, $J = 0.9$ Hz, $J = 0.9$, $J = 8.5$ Hz, 1H), 7.70 (ddd, $J = 1.1$ Hz, $J = 1.1$ Hz, $J = 8.5$ Hz, 1H), 7.35 (ddd, $J = 1.1$ Hz, $J = 6.6$ Hz, $J = 8.8$ Hz, 1H), 7.13 (ddd, $J = 0.9$ Hz, $J = 6.6$ Hz, $J = 8.5$ Hz, 1H); $^{13}$C NMR (CDCl$_3$, 90 MHz) $\delta$ 151.5, 150.8, 146.9, 128.0, 123.5, 123.2, 120.6, 120.1, 118.3, 114.3; GC-EIMS m/z (relative intensity) 195 (M$^+$, 100), 168 (21), 155 (1), 140 (8), 118 (8), 91 (6), 78 (15), 51 (35); Anal. Calculated for C$_{12}$N$_3$H$_9$: C, 73.83; H, 4.65; N, 21.52. Found: C, 73.32; H, 4.77; N, 21.22.

4-(2H-3-Bromoindazolyl)pyridine (156).

To a solution of 4-(2H-Indazolyl)pyridine [157 (490 mg, 2.5 mmoles)] in 10 mL of 1,4-dioxane, bromine/1,4-dioxane complex (65 µL of bromine, 1.25 mmole in 3 mL of 1,4-dioxane) was added dropwise. After stirring at room temperature for 3 hours, the resulting precipitate was filtered to give the hydrobromide salt of 156 (390 mg, 1.4 mmol) as an orange-red solid. mp 249-250 oC; $^1$H NMR (DMSO-$d_6$, 400 MHz) $\delta$ 9.41 (d,
The filtrate was partitioned between dichloromethane and water. The organic layers were combined, dried over MgSO₄ and evaporated in vacuo to yield crude 156. The product was purified by column chromatography on neutral alumina eluting with hexane:ethyl acetate (5:5) followed by recrystallization from hexane to give 220 mg of 156 (0.8 mmole, 32%). mp 110-111 °C; ¹H NMR (CDCl₃, 500 MHz) δ 8.82 (m, 2H), 7.78 (m, 2H), 7.72 (dt, J = 0.5 Hz, J = 8.9 Hz, 1H), 7.57 (dt, J = 1.0 Hz, J = 8.7 Hz, 1H), 7.38 (ddd, J = 1.0 Hz, J = 6.6 Hz, J = 8.9 Hz, 1H), 7.19 (ddd, J = 0.5 Hz, J = 6.6 Hz, J = 8.7 Hz, 1H); ¹³C NMR (CDCl₃, 125 MHz) δ 151.1, 149.9, 146.2, 128.5, 123.9, 123.8, 119.9, 119.8, 118.4, 105.7; GC-EIMS m/z (relative intensity) 275 (M⁺, 86), 273 (M⁺, 86), 194 (47), 167 (25), 140 (27), 117 (13), 90 (17), 78 (100), 63 (11), 51 (100); Anal. Calculated for C₁₃H₁₁BrN₃: C, 37.53; H, 2.66; N, 10.10. Found: C, 37.89; H, 2.75; N, 9.63; HR-FABMS. Calculated for C₁₃H₁₁N₃Br⁺ (based on ⁷⁹Br isotope): 288.01363. Found: 288.01250.

4-(2H-3-Bromoindazolyl)-1-methylpyridinium iodide (141).

To a solution of 4-(2H-3-bromoindazolyl)pyridine [156 (220 mg, 0.8 mmole)] in 3 mL of acetone, iodomethane (200 µL, 3.2 mmoles) was added. After stirring at room temperature for 24 hours, the solvent was evaporated and the residue was crystallized from methanol to give 290 mg of 141 (0.7 mmole, 87%). mp 225-226 °C; ¹H NMR (DMSO-d₆, 500 MHz) δ 9.22 (m, 2H), 8.70 (m, 2H), 7.77 (dt, J = 0.8 Hz, J = 8.9 Hz, 1H), 7.67 (dt, J = 1.1 Hz, J = 8.7 Hz, 1H), 7.50 (ddd, J = 1.1 Hz, J = 6.6 Hz, J = 8.9 Hz, 1H), 7.30 (ddd, J = 0.8 Hz, J = 6.6 Hz, J = 8.7 Hz, 1H); Anal. Calculated for C₁₃H₁₁BrN₃: C, 37.53; H, 2.66; N, 10.10. Found: C, 37.89; H, 2.75; N, 9.63; HR-FABMS. Calculated for C₁₃H₁₁N₃Br⁺ (based on ⁷⁹Br isotope): 288.01363. Found: 288.01250.
4-(2H-3-Methylindazolyl)pyridine (155).

A solution of 4-(2H-3-bromoindazolyl)pyridine [156 (70 mg, 0.25 mmole)] in 2.5 mL of THF was titrated with \(t\)-butyllithium (1.32 M in pentane) keeping the reaction temperature below –75 °C. The addition of \(t\)-butyllithium was stopped after color of the reaction mixture turned from yellow-green to orange (15 \(\mu\)L, 0.25 mmole \(t\)-butyllithium). After stirring the reaction mixture at –75 °C for 45 minutes, the reaction was stopped by pouring over ice. The crude product was extracted into dichloromethane, organic layers were combined, washed with brine and dried over MgSO\(_4\) and evaporated in vacuo. The resulting product was subjected to column chromatography on neutral alumina eluting with hexane:ethyl acetate (9:1) to give a an approximately 1 to 1 mixture (30 mg) of the desired product 155 and 4-(2H-indazolyl)pyridine (157). GC-EIMS m/z (relative intensity) 209 (M\(^+\), 100), 208 (93), 181 (52), 154 (11), 131 (18), 118 (14), 104 (11), 91 (15), 77 (8), 65 (22), 64 (20), 63 (23), 51 (8).

Iodide salt of 4-(2H-3-methylindazolyl)-1-methylpyridinium (140).

The mixture (25 mg, 0.12 mmole based on a 1 to 1 mixture) of 4-(2H-3-methylindazolyl)pyridine (155) and 4-(2H-indazolyl)pyridine (157) was dissolved in 1 mL of acetone. To this solution, iodomethane (30 \(\mu\)L, 0.48 mmole) was added at room temperature. After 24 hours at room temperature, acetone was removed using a syringe and remaining precipitate was washed with small amounts of acetone. Evaporation of the resulting solvent in vacuo resulted in a mixture (12 mg) of the desired product 140 and 4-(2H-indazolyl)-1-methylpyridinium iodide (112). The spectral characterization of compound 140 was achieved disregarding the signals present in the spectrum corresponding to compound 112. The \(^1\)H NMR (DMSO-\(d_6\), 400 MHz) spectrum was identical to the spectrum of 140 obtained via HPLC separation except for the absence of the signal at 1.53 ppm corresponding to the protons of the methyl group of acetate ion. HR-FABMS Calculated for C\(_{14}\)H\(_{14}\)N\(_3\)\(^+\): 224.11877. Found: 224.11940.

Rate dependence of the rearrangement reaction on TMP concentration.

Solutions of 4-(2H-6-bromoindazolyl)-1-methylpyridinium iodide [174 (2.08 mg, 0.005 mmol)] in 490 \(\mu\)L DMSO were prepared in conical micro reaction vials equipped
with caps containing septa to allow aliquot taking using a micro syringe and pre-equilibrated at 90 °C for 30 minutes. Stock solutions of TMP (0.05, 0.01, 0.015, 0.025, 0.5 and 1 M) were prepared in 1 mL DMSO and were pre-equilibrated at 90 °C for 30 minutes. The reactions were started by adding 10 µL of the stock solutions of TMP to the solutions of 174 in DMSO. The final concentration of 174 was 0.01 M and the final concentrations of TMP were 0.001, 0.002, 0.003, 0.005, 0.01 and 0.02 M. The reaction mixtures were stirred at 90 °C and aliquots (10 µL) were taken and added immediately onto 490 µL of acetonitrile. Aliquots were taken at 1, 5, 10, 15, 20, 25, 30, 45, 60, 90, 120 minutes when the final TMP concentration was 0.001, 0.002, 0.003, 0.005 M and at 1, 2, 3, 4, 5, 10, 15, 30, 45, 60 minutes when the final TMP concentration was 0.01, 0.02 M. The aliquots in acetonitrile were analyzed by HPLC. The injection volume was 225 µL and the injection loop size was 200 µL. Mobile phase consisted of 40 % acetonitrile and 60 % pH 4.7 aqueous containing 1 % triethylamine and 0.6 % acetic acid and the flow rate was 1 mL/min. The appearance of the 4-(1H-6-bromoindazolyl)-1-methylpyridinium iodide [176 (tR = 6.70 min)] was monitored at 335 nm. The height of the peak (mAU) corresponding to 176 was plotted versus time and initial rates of rearrangement were obtained from the slope of the linear part of the graphs. The logarithm of initial rates of rearrangement were plotted against the logarithm of TMP concentration in a secondary plot and the reaction was shown to be first order in TMP.

The above experiment was also attempted in DMF. However, the formation of the 1H-isomer 176 with time was not linear and the results were not reproducible. Therefore the stability of TMP in DMF was evaluated as follows. To a solution of 112 (1.70 mg, 0.005 mmol) in 490 µL DMSO, 10 µL of the stock solution of TMP (1 M) was added after pre-equilibrating both solutions at 90 °C for 30 minutes. An aliquot (10 µL) was taken after stirring the reaction mixture for 30 minutes at 90 °C, added immediately onto 490 µL acetonitrile and analyzed by HPLC-DA as described above. The same experiment was repeated two more times with the same stock solution of TMP, this time pre-equilibrating TMP solution at 90 °C for 1 hour prior to the addition. The total heating times for the TMP solution for the three experiments were was 0.5, 1.5, and 2.5 hours, respectively. A significant decrease in the rate of the rearrangement reaction (based on
the height of the peak corresponding to the 1H-isomer 176) was observed as the heating time for the TMP solution was increased.

**Rate dependence of the rearrangement reaction on the concentration of the 2H-isomer 174.**

Solutions of 4-(2H-6-bromoindazolyl)-1-methylpyridinium iodide [174 (1.04 mg, 0.0025 mmol; 4.16 mg, 0.01 mmol) in 490 µL DMSO were prepared. After pre-equilibrating at 90 °C for 30 minutes, 10 µL of the stock solution of TMP (0.5 M) was added to the solutions of 174 in DMSO. The final concentrations of 174 were 0.005, 0.02 M and the final concentration of TMP was 0.01 M. The reaction mixtures were stirred at 90 °C and aliquots (10 µL) were taken and added immediately onto 490 µL of acetonitrile. Aliquots were taken at 5, 10, 15, 20, 15, 30, 45, 60 minutes when the final concentration of 112 was 0.005 M and at 1, 2, 3, 4, 5, 10, 15, 30, 45, 60 minutes when the final concentration of 112 was 0.02 M. The aliquots in acetonitrile were analyzed by HPLC and the plots were constructed as described above.

**Stability of 112 in the presence of piperidine.**

A mixture of 112 (10 mg, 0.03 mmol) in 0.3 mL DMF containing piperidine (3 µL, 0.03 mmol) was stirred at 22 °C. Aliquots (10 µL) of the reaction mixture in 0.5 mL acetonitrile were analyzed by HPLC-DA (350 nm) using isocratic conditions of 50 % acetonitrile and 50 % pH 4.7 aqueous containing 0.6 % acetic acid and 1% triethylamine with a flow rate of 1 mL/min. The intensity of the peak (t_R = 3.14 min) corresponding to 112 decreased with time and was replaced with two peaks corresponding to 1-methyl-4-piperidin-1-ylpyridinium iodide [132 (t_R = 3.50 min)] and indazole [44 (t_R = 4.37 min)]. By 30 min, the peak corresponding to 112 had disappeared completely and the only peaks remaining corresponded to 132 and 44. Ether addition led to the precipitation of 132. Its spectral properties were shown to be identical to those of synthetic 132.

**Monitoring the TEA and DABCO catalyzed rearrangement of 112 by HPLC-DA.**

Solutions of 112 (10 mg, 0.03 mmol) in 0.3 mL DMF containing triethylamine (4 µL, 0.03 mmol) or DABCO (3 mg, 0.03 mmol) were stirred at 90 °C. Aliquots (10 µL) of
the reaction mixture in 0.5 mL acetonitrile were analyzed by HPLC-DA (350 nm) using isocratic conditions of 50 % acetonitrile and 50 % pH 4.7 aqueous containing 0.6 % acetic acid and 1% triethylamine with a flow rate of 1 mL/min. The intensity of the peak (t<sub>R</sub> = 3.14 min) corresponding to 112 decreased with time and was replaced with a peak corresponding to 110 (t<sub>R</sub> = 3.28 min). By 48 h, the peak corresponding to 112 had disappeared completely and the only peak remaining corresponded to 110.

**Monitoring the TEA and DABCO catalyzed rearrangement of 112 by 1H NMR.**

To a solution of 112 (1.70 mg, 0.5 mmol) in 490 µL DMSO-d<sub>6</sub>, 10 µL of triethylamine [133 (0.05 M in DMSO-d<sub>6</sub>)] was added. The reaction mixture was stirred at 90 °C and 1H NMR spectra were acquired at 3, 5 and 18 hours.

In a separate experiment, to a solution of 112 (1.70 mg, 0.005 mmol) in 300 µL DMSO-d<sub>6</sub>, 200 µL of DABCO [135 (0.025 M in DMSO-d<sub>6</sub>)] was added. The reaction mixture was stirred at 90 °C and 1H NMR spectra were acquired at 3, 5 and 18 hours.

**General procedure for monitoring the TMP catalyzed rearrangement reaction by HPLC-DA. (Rearrangement of the 2H-indazolpyridinium derivatives 112, 141, 174 and 193).**

A solution of the 2H-indazolpyridinium derivative (0.03 mmol) in 0.3 mL of DMF containing TMP (5 µL, 0.03 mmol) was stirred at 60 °C (compound 112) or at room temperature (compounds 141, 174, 193). Aliquots (10 µL) of the reaction mixture was added onto 490 µL acetonitrile were analyzed by HPLC-DA (335 nm and 350 nm) using isocratic conditions of 50 % acetonitrile and 50 % pH 4.7 aqueous containing 0.6 % acetic acid and 1% triethylamine with a flow rate of 1 mL/min. The intensity of the peak corresponding to the 2H-indazolpyridinium derivative decreased with time and was replaced with a peak corresponding to the expected 1H-indazolpyridinium derivative.
General procedure for monitoring the TMP catalyzed rearrangement reaction by $^1$H NMR. (Rearrangement of the 2$H$-indazolylpyridinium derivatives 112, 175, 194 and the mixture of 112 and 140).

To a solution of the 2$H$-isomer (0.005 mmol) in 490 µL DMSO-$d_6$, TMP solution (10 µL, 0.5 M in 1 mL DMSO-$d_6$) was added and $^1$H NMR spectrum was obtained. The reaction mixture was transferred to a conical reaction vial and stirred at 90 °C. After 1 hour, 2, and 4 hours, the reaction mixture was transferred back to an NMR tube and the $^1$H NMR was acquired. The rearrangement reactions of 175 and 194 were carried out in parallel with compound 112 as a control. In the case of the mixture of 112 and 140, compound 112 was present as an internal control.

Monitoring the rearrangement reaction in the absence of TMP. (Stability of the 2$H$-indazolylpyridinium derivatives 112, 140, 175 and the mixture of 112 and 140).

The stability of 4-(2$H$-3-methylindazolyl)-1-methylpyridinium iodide [140 (obtained via HPLC separation)] at room temperature in the absence of TMP in DMF and DMSO-$d_6$ was monitored by HPLC-DA as described above (general procedure for monitoring the TMP catalyzed rearrangement reaction by HPLC-DA). The structure of the rearranged product was assigned unambiguously to the corresponding 1$H$-isomer 144 by obtaining the $^1$H NMR of the reaction mixture in DMSO-$d_6$ after the completion of the rearrangement reaction. The stability of 4-(2$H$-indazolyl)-1-methylpyridinium iodide (112) and 4-(2$H$-6-methoxyindazolyl)-1-methylpyridinium iodide (175) was monitored in a similar way. However, the temperature of the solutions was increased gradually and no change was observed in the HPLC-DA tracings even at 150 °C. The stability of the mixture of 112 and 140 in the absence of TMP was monitored by $^1$H NMR as described above (general procedure for monitoring the TMP catalyzed rearrangement reaction by $^1$H NMR). No change was observed in the $^1$H NMR spectrum after 12 hours at 90 °C.

Monitoring the stability of 112 in the presence of 1 equivalent of KOAc at 22 °C by $^1$H NMR.

A solution of 4-(2$H$-indazolyl)-1-methylpyridinium iodide [112 (2.08 mg, 0.005 mmol)] in 300 µL DMSO-$d_6$ and a solution of KOAc (4.90 mg, 0.05 mmol) in 2 mL of
DMSO-d$_6$ were prepared. To the solution of **112**, KOAc solution (200 µL) was added. The final concentrations of **112** and KOAc in the reaction mixture were 0.01 M. The reaction was monitored by acquiring $^1$H NMR spectra at 0, 30, 90, 270 minutes and 10, 48 hours.

**Monitoring the rearrangement of 112 in the presence of 1 equivalent of TMP in “dry” and “wet” DMSO-d$_6$ at 90 °C by NMR.**

DMSO-d$_6$ was stirred for 24 hours over CaH$_2$ and distilled under vacuum immediately before the experiment. In a conical micro reaction vial, 4-(2$H$-6-bromoindazolyl)-1-methylpyridinium iodide [**112** (1.70 mg, 0.005 mmol)] was weighed and dried in a vacuum oven at 50 °C for 3 hours. A solution of **112** was prepared via adding anhydrous DMSO-d$_6$ (490 µL) through the septum of the reaction vial. A solution of TMP (0.5 M) was prepared in 1 mL anhydrous DMSO-d$_6$ in a separate conical reaction vial equipped with a cap containing a septum. To the solution of **112**, TMP solution (10 µL) was added. After stirring the reaction mixture at 90 °C for 4 hours, $^1$H NMR spectrum was acquired. The final concentrations of **112** and TMP in the reaction mixture were 0.01 M. A separate reaction mixture containing the same concentrations of **112** and TMP as described for the “dry” experiment was prepared this time using undistilled DMSO-d$_6$. After stirring the reaction mixture at 90 °C for 4 hours, $^1$H NMR spectrum of the “wet” experiment was acquired and compared with the spectrum obtained for the “dry” experiment.

**Monitoring the rearrangement of the 4-(2$H$-indazolyl)-1-methylpyridinium iodide (112) in the presence of 1 equivalent of KOH at 22 °C by $^1$H NMR.**

A solution of 4-(2$H$-indazolyl)-1-methylpyridinium iodide [**112** (2.08 mg, 0.005 mmol)] in 300 µL DMSO-d$_6$ and a solution of KOH (2.81 mg, 0.05 mmol) in 2 mL of DMSO-d$_6$ were prepared. After sonicating the KOH solution for 15 minutes, some insoluble particles remained. To the solution of **112**, KOH solution (200 µL) was added. The final concentration of **112** and KOH in the reaction mixture was 0.01 M (due to the presence of insoluble particles, the concentration of the KOH solution is only an estimate.
based on the amount of KOH used). The reaction was monitored by acquiring $^1$H NMR spectra at 5, 10, 15, 20, 25, 30 minutes and 1, 2, 2.5, 3, 17 hours.

**Stability of 4-(2H-3-bromoindazolyl)-1-methylpyridinium iodide (141) in the presence of 1 equivalent of KCN at 22 °C.**

A 0.05 M solution of 141 in 500 µL of DMSO-d$_6$ was prepared and $^1$H NMR spectrum was obtained. To the solution of 141 in DMSO-d$_6$, 500 µL of KCN solution (0.05 M in DMSO-d$_6$) was added, contents were mixed by inverting the NMR tube and $^1$H NMR spectrum was obtained after 15 minutes.

**Stability of 4-(2H-5-bromoindazolyl)-1-methylpyridinium iodide (193) in the presence of 0.1, 0.2 equivalent of KCN and 1 equivalent of TEMPO at 22 °C.**

To a 0.05 M solution of 193 in 500 µL of DMSO-d$_6$, 200 µL of TEMPO solution (0.125 M in DMSO-d$_6$) was added. The $^1$H NMR spectra were obtained before and after TEMPO addition and no change was observed. To the NMR tube, 100 µL of KCN solution (0.025 M in DMSO-d$_6$) was added, contents were mixed by inverting the tube and $^1$H NMR spectrum was obtained after 15 minutes. Another 100 µL KCN solution was added and $^1$H NMR spectrum was obtained after 15 minutes. Control experiments were carried out in the absence of TEMPO and no significant difference was observed in the $^1$H NMR spectra compared with the TEMPO experiments.

**Stability of 4-(2H-6-bromoindazolyl)-1-methylpyridinium iodide (174) in the presence of NaHSO$_3$ at 22 °C.**

To a solution of 174 (2.04 mg, 0.005 mmol) in 500 µL of DMSO-d$_6$, NaHSO$_3$ (3 mg, 0.03 mmole) was added. The reaction mixture was sonicated for 10 minutes to dissolve the remaining insoluble NaHSO$_3$ particles. The $^1$H NMR was obtained after 24 hours and no change was observed.

**Monitoring the stability of the 4-(2H-6-bromoindazolyl)-1-methylpyridinium iodide (174) in DMSO-d$_6$ in the presence of 0.1, 0.5, 1 and 2 equivalents of KCN.**

To solutions of 174 (2.04 mg, 0.005 mmol) in 500 µL DMSO-d$_6$, 500 µL of KCN solutions (0.001, 0.005, 0.01 and 0.02 M in DMSO-d$_6$) were added. The reaction
mixtures were stirred at room temperature and aliquots (10 μL) were taken at 15 minutes and added onto 490 μL of acetonitrile. The aliquots in acetonitrile were analyzed by HPLC-DA. The injection volume was 50 μL. Mobile phase consisted of 50 % acetonitrile and 50 % pH 4.7 aqueous containing 1 % triethylamine and 0.6 % acetic acid and the flow rate was 1 mL/min. The reaction mixture with a final cyanide concentration of 0.005 M was also analyzed by LC-MS using APCI as the ionization source. The mobile phase for LC-MS analysis consisted of solvent A (acetonitrile containing 1% formic acid) and solvent B (aqueous containing 1% formic acid). A concentration gradient was applied starting with 30% A for 5 minutes, increasing to 50% A between 5-10 minutes, holding at 50 % A for 5 minutes and increasing to 100 % A between 16-20 minutes.

An aliquot from the reaction mixture with a final cyanide concentration of 0.005 M was partitioned between water and ethyl acetate. The organic layer was dried over MgSO₄ and analyzed by GC-MS. GC-EIMS data were obtained using an initial oven temperature of 60 °C, holding at 60 °C for 3 minutes, ramping at 25 °C/min to a final temperature of 290 °C, holding at 290 °C for 10 minutes with a solvent delay of 4.1 min and injection port temperature of 250 °C.

**Monitoring the stability of 4-(2H-6-bromoindazolyl)-1-methylpyridinium iodide (174) in DMSO-d₆ in the presence of 2 equivalents of KCN over time.**

To a solution of 174 (2.04 mg, 0.005 mmol) in 500 μL DMSO-d₆, 500 μL of KCN solution (0.02 M in DMSO-d₆) was added. The reaction mixture was stirred at room temperature and aliquots (20 μL) were taken at 1, 2, 3, 4, 5, 6, 7, 8, 10 minutes and added onto 380 μL of acetonitrile. The aliquots in acetonitrile were analyzed by HPLC-DA as described above. The injection volume was 20 μL.

**Monitoring the stability of 4-(1H-6-bromoindazolyl)-1-methylpyridinium iodide (176) in DMSO-d₆ in the presence of 2 equivalents of KCN over time.**

To a solution of 176 (2.04 mg, 0.005 mmol) in 500 μL DMSO-d₆, 500 μL of KCN solution (0.02 M in DMSO-d₆) was added. The reaction mixture was stirred at room temperature and aliquots (20 μL) were taken at 1, 2, 3, 4, 5, 6, 7, 8, 10 minutes and added
onto 380 µL of acetonitrile. The aliquots in acetonitrile were analyzed by HPLC-DA as described above. The injection volume was 20 µL.

After the completion of the reaction, an aliquot from the reaction mixture was partitioned between water and ethyl acetate. The organic layer was dried over MgSO₄ and analyzed by GC-MS. GC-EIMS data were obtained using an initial oven temperature of 60 °C, holding at 60 °C for 3 minutes, ramping at 25 °C/min to a final temperature of 290 °C, holding at 290 °C for 10 minutes with a solvent delay of 4.1 min and injection port temperature of 250 °C.

**Comparison of the stability of 4-(1H-6-bromoindazolyl)-1-methylpyridinium iodide (176) in “dry” and “wet” DMSO-d₆ in the presence of 2 equivalent of KCN at 22 °C.**

KCN (1.81 mg, 0.028 mmol in an oven dried vial) and 176 (0.0044 mmol in an oven dried NMR tube) were kept in a vacuum oven at 55 °C for 2 hours. DMSO-d₆ was kept over 3 Å molecular sieves over night. The vial containing KCN was capped with a septum immediately after removing from the oven and placed in dessicator. The NMR tube containing 176 was placed under vacuum immediately after removing from the oven. A 0.01 M solution of KCN was prepared by adding 2.8 mL of DMSO-d₆ to the vial containing KCN. The KCN/DMSO-d₆ solution (880 µL) was added to the NMR tube containing 176. After capping and sealing the NMR tube with parafilm, the contents were mixed by inverting the tube (t = 0). The ¹H NMR spectra were obtained at t = 6, 11, 16, 30, 45, 60 and 70 minutes. The reaction mixture was mixed by inverting the NMR tube immediately before acquiring the NMR spectrum at each time point.

The reaction was carried out for the second time after adding 0.05 % (v/v) H₂O to the DMSO-d₆ prior to the preparation of the KCN solution. The ¹H NMR spectra were obtained at t = 7, 11, 16 and 30 minutes and compared with the spectra obtained under “dry” conditions.

**Monitoring the fate of ¹³CN in the presence of 0.1 equivalent 4-(2H-5-bromoindazolyl)-1-methylpyridinium iodide (193) by ¹³C NMR at 22 °C.**

A 0.05 M solution of K¹³CN in 500 µL DMF-d₇ and a 0.05 M solution of 193 in 500 µL DMF-d₇ was prepared. After obtaining the ¹³C NMR spectrum of the KCN
solution, 50 µL of the solution of 193 in DMF-d$_7$ was added to the NMR tube. The disappearance of the $^{13}$C signal of K$^{13}$CN was monitored by obtaining the $^{13}$C NMR spectrum after 10, 15, 20 and 30 minutes.

**Monitoring the stability of 4-$(1H$-6-bromoindazolyl)$-1$-methylpyridinium iodide (176) in DMSO-d$_6$ in the presence of 2 equivalents of KCN at 22 °C under anaerobic conditions.**

A 0.01 M solution of KCN in 980 µL of DMSO-d$_6$ was prepared, transferred to an NMR tube and degassed under vacuum via 3 freeze-thaw cycles. After the third cycle, the solution was frozen again and 176 (1.87 mg, 0.0045 mmole) was added to the NMR tube under a blanket of nitrogen. The solution was degassed once again under vacuum and the NMR tube was kept under nitrogen for 30 minutes until complete thawing of the solution. Immediately after complete thawing the NMR tube was capped under a blanket of nitrogen, sealed with parafilm and contents were mixed by inverting the NMR tube (t = 0), $^1$H NMR spectrum was obtained at t = 5, 15, 30 minutes. The resulting spectra were compared with the analogous experiment carried under aerobic conditions.

**Analysis of the cyano adducts derived from the reaction between 4-cyano-1-methylpyridinium iodide (214) and K$^{13}$CN.**

To a suspension of K$^{13}$CN (200 mg, 3.2 mmol) in 5 mL DMF, 4-cyano-1-methylpyridinium iodide [214 (390 mg, 1.6 mmol) was added. The reaction mixture turned black/green after 5 minutes. After 15 minutes at room temperature, 10 mL of water was added slowly onto the reaction mixture while cooling in water bath. The reaction mixture was extracted with dichloromethane, organic layers were combined, dried and the solvent was evaporated in vacuo to give 152 mg of a black residue. After filtering the residue through an alumina column eluting first with dichloromethane than with EtOAc:MeOH (9:1), the collected fractions were analyzed by GC-MS.
Monitoring the stability of 4-(2\textit{H}-6-bromoindazolyl)-1-methylpyridinium iodide (174) in D$_2$O in the presence of 0.5 equivalent of KCN over time.

To a 0.0025 M solution of 174 in 2 mL of D$_2$O, 500 µL of 0.005 M KCN solution in D$_2$O was added. Aliquots (10 µL) were taken at t = 0, 30 minutes and 52 hours and were added onto 490 mL of acetonitrile. The aliquots in acetonitrile were analyzed by HPLC-DA as described above (See: Monitoring the stability of the 4-(2\textit{H}-6-bromoindazolyl)-1-methylpyridinium iodide (174) in DMSO-d$_6$ in the presence of 0.1, 0.5, 1 and 2 equivalents of KCN). The injection volume was 50 µL.

7.3. Biology

Investigation of MAO-A substrate properties of nitroindazolyl “prodrugs”.

MAO-A substrate properties of test compounds 28, 45, and 46 were studied using HPLC-DA and HPLC-MS. Human placental preparations which contain only the A form of MAO were used as the enzyme source. Stock solutions of the test compounds (200 µM) were prepared in pH 7.4 0.1 M sodium phosphate buffer. Enzyme (25 µL, 6 mg protein/mL for 28 and 3 mg protein/mL for 45, 48) was added to incubation mixtures (pre-equilibrated at 37 °C) consisting of pH 7.4 0.1 M phosphate buffer (375 µL) and the test compound (100 µL) to yield a final substrate concentration of 40 µM. The final volume of the incubation mixtures was 500 µL and the final protein concentration was 0.3 mg/mL for incubations containing the “prodrug” 28 and 0.15 mg/mL for the incubations containing the “prodrugs” 45 and 48. These mixtures were incubated with gentle agitation in a water bath at 37 °C for 0, 5, 10, 15, 30, 45 and 60 minutes. Acetonitrile (500 µL) was added and the resulting mixture was vortex agitated. The denatured protein was sedimented by centrifugation at 10,000 g for 6 minutes. The supernatants were analyzed by HPLC-DA. Control incubations were conducted in the absence of enzyme and no metabolite formation was detected.

HPLC-DA analysis of “prodrug” incubation mixtures.

Supernatants (200 µL) were applied to HPLC equipped wit a 250 mm x 4.6 mm Zorbax SB-C8 5 µm column (reverse phase) with an in-line pre-column filter (2 µM,
Upchurch Scientific Inc.). The analysis was carried out using isocratic conditions with a mobile phase consisting of 40 % acetonitrile and 60 % pH 4.7 aqueous containing 1 % triethylamine and 0.6 % acetic acid and the flow rate was 1 mL/min. DA detector is set at 5 different wavelengths (269, 290 335, 350, 375 nm) for monitoring of the substrate and the metabolites.

**HPLC-MS analysis of “prodrug” incubation mixtures.**

Supernatants (250 µL) were concentrated by evaporating the solvent completely *in vacuo* and redissolving the residue in 50 µL of Milli Q water. Concentrated samples (20 µL) were applied to HPLC-MS equipped with a 150 mm x 4.6 mm XDB-C8 5 µm (reverse phase) column. The mobile phase consisted of solvent A (acetonitrile containing 1% formic acid) and solvent B (aqueous containing 1% formic acid). A concentration gradient (total run time was 20 minutes) was applied starting with 5% A for 5 minutes, increasing to 50% A between 5-6 minutes, holding at 50 % A for 9 minutes, increasing to 100 % A between 15-16 minutes and holding at 100 % A between 16-20 minutes. The flow rate was 0.5 mL/min. The column outflow was discarded for the first 5 minutes. APCI is used as the ionization source for the mass spectral analysis.

**7.3. Computational Studies**

Energy calculations were carried out using the MacSpartan Pro program (version 1.0.4; Wavefunction, Irvine, CA). Structures were energy minimized using the semi empirical AM1 calculations. Single point *ab initio* energy calculations were carried out on the minimized structures at the 6-31G* level.

Docking studies were carried out on a Silicon Graphics (Mountain View, CA) Octane workstation using the FlexiDock program, a part of the Biopolymer module of the SYBYL software (Version 6.9, Tripos Inc., St. Louis, MO). The ligands were minimized using the Conjugate Gradient minimizer with a minimum gradient change of 0.05 kcal/mol. The automatically assigned atom types were corrected when necessary. Atomic charges of the ligands were calculated using the Gasteiger-Hückel method. The coordinates of the x-ray crystal structure of MAO-B in complex with isatin (247) and *trans*-1,4-diphenyl-2-butene (259) are downloaded from the Protein Data Bank.
(www.rcsb.org/pdb/) in the pdb format. The pdb file was modified by removing the water molecules and one of the subunits of the homodimeric structure using the editor program, nedit. After uploading the ligands and enzyme in different molecular areas, the FlexiDock program was initiated. The ligand present in the x-ray crystal structure was removed after defining the active site using the coordinates of the ligand. Atom types of the flavin of the enzyme were checked and corrected where necessary. After adding the hydrogen atoms to the protein, the atomic charges were computed for the enzyme using the Kollman method. The Kollman method is not applicable for the calculation of the atomic charges of flavin. Therefore flavin is extracted, atomic charges are calculated using the Gasteiger-Hückel method and re-merged with the protein. The rotatable bonds of the ligand are defined whereas the protein was kept rigid. After manually placing the ligand within the vicinity of the active site, FlexiDock input file was created for the current ligand-protein pair. FlexiDock algorithm was run on the input file using the default parameter file. The maximum number of generations to be allowed was initially set to 3,000. However, it was increased to 10,000 due to the limited diversity among the generated binding conformers. Among the generated docking models, only those models which satisfy the criteria of the carbon atom undergoing oxidation being close to the N5 atom of flavin, were considered for further evaluation.
APPENDIX 1. SEQUENCE COMPARISON OF MAO-A AND MAO-B FROM DIFFERENT SPECIES


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The Favorskii rearrangement\textsuperscript{262} is an example of a rearrangement reaction involving the formation of a three-membered ring intermediate. The first step of the Favorskii rearrangement is the deprotonation of an $\alpha$-haloketone (260) by an alkoxide (or hydroxide or an amine) to form the corresponding carbanion 261. The intramolecular displacement of the halide leads to the formation of cyclopropanone (262) as shown in Scheme 100.

### Scheme 100. Formation of cyclopropanone during the Favorskii rearrangement.

![Scheme 100](image)

After the formation of cyclopropanone, the attack of alkoxide at the carbonyl carbon results in the ring-opened carbanion 264 via the formation of the tetrahedral intermediate 263. The corresponding ester 265 forms as the final product of the Favorskii rearrangement upon protonation of 264 (Scheme 101).

### Scheme 101. Formation of the carboxylic ester 264 as the final product of the Favorskii rearrangement.

![Scheme 101](image)

One example for the synthetic utility of the Favorskii rearrangement is the synthesis of the cubane derivative 267 from the bishaloketone 266 in the presence of KOH (Scheme 102).\textsuperscript{263}

\textsuperscript{262} Favorskii, A. (1895) \textit{J. Prakt. Chem.} 51, 533-563.

Scheme 102. Formation of the cubane derivative 267 via the Favorskii rearrangement.

In addition to α-haloketones, α-haloketimines were also shown to undergo a Favorskii rearrangement. The treatment of α-chloroketimine derivative 269 with potassium $t$-butoxide in THF under reflux has been reported to give the rearrangement product 270 together with the substitution product 271 (Scheme 103).264

Scheme 103. The Favorskii rearrangement of the α-chloroketimine derivative 269.

VITA

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After the completion of his Doctor of Philosophy degree under the direction of Professor Neal Castagnoli, Jr., Emre M. Işın will start his post-doctoral studies at the Biochemistry Department of Vanderbilt University Medical Center under the direction of Professor F. Peter Guengerich.