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σ Complexes as Biochemical and Biophysical Probes[1]

4. Development of a Novel Reporter Group Delivery System

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A novel reporter group delivery system for the chemical modification of proteins and the investigation of enzyme mechanisms is proposed. The design of this system is based on structural analogy with pyridoxal phosphate and σ-complex adduct formation. Progress is described toward the synthesis of suitable compounds, via three different approaches involving structural modification of the pyridine nucleus. A number of new compounds have been prepared, and other directions for future investigations are indicated.

INTRODUCTION

While chemical modification has become well established as a technique for the investigation of protein structure and function (*1–5*), the field has been supplemented more recently by the introduction of the concept of reporter group delivery systems (*6–8*). The reagents used combine affinity for a particular part of the protein with spectroscopic probe properties designed to reveal structural characteristics. The goal of the present work is the development of a new reporter group delivery system.

Work in our laboratory has been concerned with investigation of the covalent adducts known as Meisenheimer or σ complexes, which are formed from the interaction between nitroaromatic compounds and bases (*9–14*). Recently it has been shown that σ complexes are potentially useful tools for the elucidation of a number of biochemical and biophysical problems. Thus, Taylor and co-workers have demonstrated the accelerated decomposition of the σ complex 1,1-dihydro-2,4,6-trinitrocyclohexadienate (**1**) by bovine serum albumin (*15*), while (**2**) and related σ complexes have been implicated as key intermediates in the mode of action of 4-nitrobenzofuroxan and related compounds as antileukemic agents (*16–19*), being formed through the interaction of the heteroaromatic compound with intracellular thiol (Y = RS) and amino (Y = RNH) functions. Also, some authors have suggested the possible application of σ complexes as labels in enzyme studies (*20, 21*).

[1] Part 3 of the series: E. Buncel, N. Chuaqui-Offermanns, R. Y. Moir, and A. R. Norris, *Canad. J. Chem.* **57**, 494 (1979).

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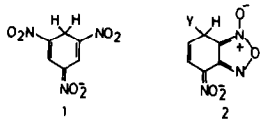
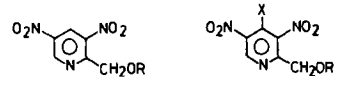


1 2

It is now evident that extension of these ideas toward the chemical modification of proteins and the investigation of enzyme mechanisms (*22*) would require the systematic development of σ complex-forming reagents especially suited for studies of enzyme reactivity and specificity. In view of this we have extended our studies to the pyridine series, with the intent of developing a reporter group delivery system capable of σ complexation with lysyl residues of pyridoxal phosphate-modifiable enzymes such as aldehyde reductase (*23*) and phosphoglyceromutase (*24, 25*). Such a system should possess both a side-chain phosphate and a high degree of electrophilicity. In addition, the spectral properties of the reporter group must be sufficiently distinct that data may be easily obtained and interpreted. These requirements are expected to be met by compound **3a** and **4a.**



3a (R = PO_3^{2-}) 4a (X = Cl, R = PO_3^{2-})
3b (R = H) 4b (X = Cl, R = H)

The anticipated reporter group properties of Type **3** are derived from its expected ability to form σ complexes. Since the aza function is well known to be activating in nucleophilic aromatic substitution (*26*), it is not surprising that reasonably stable anionic σ complexes can be obtained from the interaction of bases with electron-deficient pyridines or pyrimidines (*27–31*). In the trinitroaromatic series, σ-complex formation has been observed with a variety of nucleophiles including hydroxide, alkoxides, thiols, and amines (*9–14, 32–37*). Accordingly, σ-complex formation by **3** may occur with the phenolic hydroxyl group of tyrosyl residues, the mercapto group of cysteine, or the ϵ-amino group of lysine. The high extinction coefficients of such complexes ($\epsilon_{max} \sim 2 \times 10^4\ M^{-1}$ cm^{-1}) suggest that these compounds will be efficient spectral probes. In addition, it should be noted that σ complexation is reversible. This property should permit the establishment of the site of modification by competitive kinetics with substrate and should demonstrate the integrity of the system by regeneration of active enzyme.

The potential applications of Type **3** are complemented by those of **4**. When *X* is a displaceable group such as chlorine, the latter will undergo nucleophilic aromatic substitution to give dinitropyridyl derivatives. The use of halogenodinitropyridines for the determination of the N-terminal amino acid in a polypeptide chain has already been described (*38*). The dinitropyridyl amino acid derivatives produced are bright yellow, which is helpful for their micromanipulation, chromatography, and photometric estimation.

It should be possible to prepare compounds **3a** and **4a** by phosphorylation of the

corresponding alcohols **3b** and **4b**. Indeed, the phosphorylation of 2-pyridylcarbinol has already been described (*39*). A number of syntheses for the preparation of these alcohols have been considered in the present work.

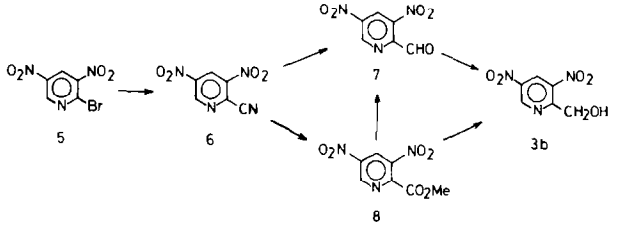
RESULTS AND DISCUSSION

The present paper describes three different approaches to the target compounds **3b** and **4b**. Scheme 1 is directed toward the synthesis of 2-substituted pyridine derivatives via functional group interconversion, using 2-cyano-3,5-dinitropyridine as the key intermediate. Scheme 2 aims at the direct introduction of suitable substituents in the 2-position via radical hydroxymethylation of certain dinitropyridine derivatives. Schemes 3 and 4 feature functionalization of the pyridine ring via directing groups.

The new compounds prepared during the course of this work, together with their numerical designations, are as follows: 2-cyano-3,5-dinitropyridine (**6**), 3,5-dinitro-4-phenoxypyridine (**10**), 2-methoxymethyl-4-hydroxypyridine (**12a**), 2-methoxymethyl-4-benzyloxypyridine (**14**), 2-methoxymethyl-4-benzyloxypyridine-*N*-oxide (**15**), 2-methoxymethyl-4-nitropyridine-*N*-oxide (**17**), 2-methoxymethyl-4-hydroxypyridine-*N*-oxide (**18**), and 2-methoxymethyl-3,5-dinitro-4-hydroxypyridine-*N*-oxide (**19**).

Synthetic Approaches via 2-Cyano-3,5-dinitropyridine (Scheme 1)

The synthesis of **3b** via 2-cyano-3,5-dinitropyridine offers the advantage of simultaneous replacement of the activating substituent required for dinitration and the introduction of a 1-carbon side chain, via the sequence –OH → –Br → CN. The nitrile function may then be converted to other groups from which the primary alcohol may be prepared (Scheme 1).



SCHEME 1

The preparation of 2-cyano-3,5-dinitropyridine (**6**) has previously been reported and involves heating 2-bromo-3,5-dinitropyridine (**5**) with cuprous cyanide in *p*-cymene at 175°C for 2 hr (*40*). In our hands, repeated attempts at this procedure resulted in a 1–3% yield of a product with mp 84°C, whereas the literature reports a 60% yield of a compound with mp 114°C (*40*). It is interesting to note that 4-cyano-3,5-dinitropyridine has been prepared by a similar method, but an attempted synthesis of 4-cyano-3,5-dinitropicoline failed (*41*).

We reasoned that active halides such as these might react with CuCN more readily in the presence of a phase transfer catalyst. One of the earliest applications of phase transfer catalysis was the cyanide ion displacement reaction of alkyl halides described by Starks (*42*). This type of reaction has led to the preparation of a variety of aliphatic nitriles but to our knowledge has not been extended to aromatic systems. Indeed only a few examples of nucleophilic aromatic substitution by phase transfer catalysis have been reported (*43–45*). Enhancement of reactivity has been observed in reactions between CuCN and aryl halides using dipolar aprotic solvents such as DMF and DMSO (*46, 47*).

When a mixture of **5**, CuCN and *n*-Bu_4NBr was heated under reflux in benzene, a 60% yield of a compound with mp 84°C was obtained on work-up. This compound was identified as **6** by NMR and elemental analysis. The NMR spectrum of **6** in $(CD_3)_2SO$ exhibited doublets at δ9.7 and δ9.3 ppm. This spectrum may be compared with that of the starting material (**5**), which showed doublets at δ9.4 and δ9.2. In contrast, the NMR spectra of 4-substituted 3,5-dinitropyridines (e.g., 4-chloro-3,5-dinitropyridine) show a single peak at ca. δ9.2. Thus, in the NMR spectrum of **6**, the doublet appearing at δ9.7 may be ascribed to the hydrogen at position 4, whereas the doublet at δ9.3 may be ascribed to the hydrogen at position 6.

As an additional point of interest it may be noted that a requirement for Cu^+ was also demonstrated, in that the reaction failed when NaCN, KCN, or *n*-Bu_4NCN was substituted for CuCN. The mechanism of reaction between aryl halides and CuCN is not certain and may not be a simple nucleophilic aromatic substitution. It has been suggested, however, that Cu^+ forms a complex with the halide, favoring CN^- attack (*48*).

Having obtained the nitrile **6**, we investigated methods of functional interconversion toward the synthesis of **3b**. Initially we attempted to prepare 3,5-dinitro-2-pyridinecarbaldehyde (**7**) by reduction of the nitrile with diisobutyl aluminium hydride (DIBAH). This reagent has proved especially useful for the reduction of nitriles to aldimines, which on work-up give rise to the corresponding aldehydes (*49*). Because of the low temperature at which nitriles are reduced by this reagent (−70 to +25°C), we hoped to selectively reduce this group without affecting the nitro substituents. In practice this was not found to be possible since reaction of DIBAH resulted in complete decomposition of the substrate. Although reduction of the nitro groups is a possibility, the reduction of the ring must also be considered since this electron-deficient system could be subject to hydride addition. Indeed, Signor *et al.* (*50*) have recently reported the reduction of some 3,5-dinitropyridine compounds with the relatively mild reducing agent $NaBH_4$.

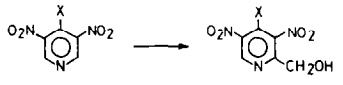
We also attempted to prepare the aldehyde via the ester **8**, but the direct acid methanolysis of **6** was unsuccessful, presumably because of the strong ortho effects of the nitro groups. This effect has also been noted in the benzene series, where *m*- and *p*-nitrobenzonitrile give the corresponding ester, while *o*-nitrobenzonitrile does not undergo reaction (*51*).

Synthetic Approaches via Radical Hydroxymethylation (Scheme 2)

The possibility of direct introduction of the hydroxymethyl substituent via

radical hydroxymethylation offers an attractive alternative to functional group interconversion in these systems. The radical hydroxymethylation would permit access to either **3b** or **4b**, as indicated in Scheme 2.

[1]



[2]

X = H, Cl, NH_2, OH

SCHEME 2

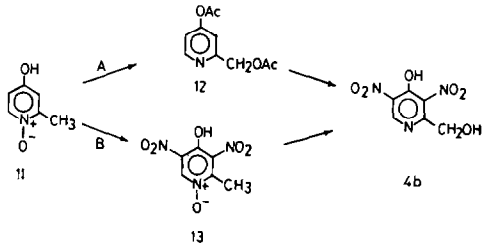
The synthesis of **4b** via the key intermediate 3,5-dinitro-4-phenoxypyridine (**10**) was envisaged. 2-Phenoxy-3,5-dinitropyridine has been prepared by refluxing phenol and 2-chloro-3,5-dinitropyridine in aqueous ethanol medium (*40*). This procedure was found unsatisfactory for the synthesis of **10** because of the susceptibility of the 4-chloro-3,5-dinitropyridine precursor **9** to hydrolysis. Accordingly, **10** was prepared by reaction of **9** with anhydrous potassium phenoxide in DMF, which afforded the desired product in 80% yield. The NMR spectrum of **10** showed a single peak (δ9.2) ascribable to the two equivalent hydrogens at the 2- and 6-positions, as well as the multiplet (δ6.8–7.4) due to the phenyl protons.

The radical hydroxymethylation (Eq. [2]) was attempted using compound **10** as well as other 3,5-dinitropyridine derivatives containing various substituents in the 4-position (H, Cl, NH_2, OH). The electron-deficient character of dinitropyridine should facilitate attack by the nucleophilic hydroxymethyl radical. Indeed, Sakamoto (*52*) has found pyrimidine derivatives to be superior in reactivity to pyridine derivatives, suggesting that electron-withdrawing groups promote the reaction. Surprisingly, we found that under a variety of conditions (see Experimental) the reaction failed with 3,5-dinitropyridine and the 4-substituted derivatives indicated. It is possible that though the nitro substituents may favor attack of the nucleophilic radical via electron withdrawal, the steric hindrance adjacent to the reaction site would inhibit the substitution. Therefore, it would be interesting to examine the radical hydroxymethylation of mononitropyridines and pyrimidines, since these compounds possess sterically unhindered positions.

Synthetic Approaches via Directed Nitration (Schemes 3 and 4)

As it was previously found that **4b** could not be produced directly via hydroxymethylation of a number of 3,5-dinitropyridine precursors, the following alternative synthesis was considered. Scheme 3 shows route A, in which the protected 4-hydroxy substituent is used to activate the ring toward dinitration and the 2-hydroxymethyl substituent would already be present in the critical step. The conversion of **11** to **12** by the acetic anhydride rearrangement was effected as previously described (*53*). However, the attempted formation of **4b** through

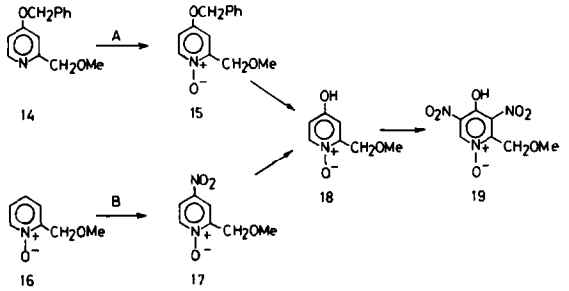
dinitration of **12** gave instead 4-hydroxy-3,5-dinitropyridine. Evidently, under the conditions required to effect dinitration, the hydroxymethyl side-chain was lost, probably by oxidation to the corresponding carboxylic acid followed by decarboxylation. Similar results (i.e., loss of side chain) were obtained with 2-methoxymethyl-4-hydroxypyridine (**12a**).



SCHEME 3

It was hoped that the difficulty involved with nitration in the presence of the 2-hydroxymethyl substituent, as in route A, could be circumvented by introduction of the side-chain alcohol following dinitration of **11**, as in route B. Accordingly, it was expected that the Ac_2O rearrangement of **13** would give the desired product **4b**, as this type of reaction has been generally successful with a number of picoline *N*-oxides. However, while the nitration of **11** to **13** was readily effected, the acetic anhydride-induced rearrangement of the latter could not be accomplished under a variety of conditions, only starting material and its 4-acetoxy derivatives being recovered.

The limited success in the scheme devised previously led us to consider a route based on modification of well-established procedures with analogous derivatives containing the desired structural features. Accordingly, a multistep synthesis of **19** via the intermediates 2-methoxymethyl-4-hydroxypyridine-*N*-oxide (**18**), 2-methoxymethyl-4-benxyloxypyridine-*N*-oxide (**15**), and 2-methoxymethyl-4-benzyloxypyridine (**14**), was pursued (route A in Scheme 4). The immediate precursor of **14**, 2-chloromethyl-4-benzyloxypyridine, was prepared by a five-step procedure starting with picoline *N*-oxide (*54*). The principal difficulty encountered with this scheme was the low yield accompanying not only the acetic anhydride rearrangement of 2-methyl-4-benzyloxypyridine-*N*-oxide to 2-acetoxymethyl-4-benzyloxypyridine, but also the peracetic acid oxidation of **14** to **15**.



SCHEME 4

It was found that compound **18** could be more readily prepared via 2-methoxymethyl-4-nitropyridine-*N*-oxide (**17**). The latter is obtained (Scheme 4, route B) by nitration of 2-methoxymethylpyridine-*N*-oxide (**16**), which in turn is available in two steps from 2-chloromethylpyridine (*55*). Although the nitration of **16** proceeds in only 20% yield (as found in the analogous nitration of bis(pyridyl-2-methyl-*N*-oxide)ether (*56*), route B was the preferred path for the preparation of **18**. The dinitration of **18** proceeds under relatively mild conditions and affords **19** in good yield. This step brings Scheme 4 to a successful conclusion.

CONCLUSION

Our work has shown the synthetic feasibility, in principle, of the reporter delivery system based on pyridoxal phosphate, with its built-in ability to form σ-complex adducts. The end product of our synthetic investigations, compound **19**, has the desired structural core, including the 2-alkoxymethyl substituent, nitro groups in the 3,5-positions, and a substituent in the 4-position which should be convertible to Cl (**4b**), H (**3b**), etc., following deoxygenation of the *N*-oxide function. Hence this core structure should enable, by relatively straightforward methods, the execution of the final stages in the development of the reporter group delivery system. Our work also points to certain advantages in the development of analogous reporter groups based on the pyrimidine nucleus. In this case, a number of key reactions which proved to be unsuccessful in the nitropyridine series (e.g., radical hydroxymethylation), should proceed with relative ease. It is hoped to undertake such studies in future work.

EXPERIMENTAL

General Methods

The solvents used in the preparative work were freshly distilled. Reagents used were of the highest quality commercially available. The pyridine derivatives used in this work were purchased from Aldrich Chemical Company. Thin-layer chromatography (TLC) was performed on BDH silica gel plates with fluorescent indicator. Compounds were detected by irradiation under a short-wavelength uv light from a 2537-Å Mineralight. Column chromatography was carried out using BDH silica gel (70–230 mesh). Elemental analyses were performed by Guelph Chemical Laboratories Ltd., Guelph, Ontario. Melting points were determined on a Fisher–Johns apparatus and are uncorrected. Infrared spectra were taken on a Beckman Acculab 6 or Perkin–Elmer 180 spectrophotometer, as neat smears (syrups) or in KBr pellets (solids). PMR spectra were recorded on a Varian EM 360 instrument in chloroform-*d* with tetramethylsilane (TMS) as internal standard, unless otherwise stated.

Syntheses Relating to Scheme 1

2-Bromo-3,5-dinitropyridine (**5**). Nitration of 2-hydroxypyridine by the method of Signor (*38*) gave 2-hydroxy-3,5-dinitropyridine which on reaction with PBr_5 according to the method of Talik (*40*) afforded 2-bromo-3,5-dinitropyridine (**5**).

2-Cyano-3,5-dinitropyridine (**6**). Ten grams of **5**, ten grams of CuCN, and thirteen grams of *n*-Bu_4NBr were suspended in 180 ml benzene and heated under reflux for 4 hr. After cooling, the solvent was removed under vacuum and the residue extracted with CH_2Cl_2. The combined extracts were washed with water and dried with $MgSO_4$. Removal of solvent gave a crude material containing a significant amount of tar. Extraction with hot benzene and crystallization from $CHCl_3$ gave 5 g of 2-cyano-3,5-dinitropyridine (64% yield), mp 90°C. PMR (DMSO-d_6): 9.7 (d, J = 2 Hz, 1H), 9.3 (d, J = 2 Hz, 1H). *Anal.* Calcd for $C_6H_2N_4O_4$: C, 37.13; H, 1.04; N, 28.86. Found: C, 36.85; H, 1.02; N, 29.27.

Attempted preparation of 3,5-dinitro-2-pyridine-carbaldehyde (**7**). DIBAH (1.9 ml) (1.0 *M* in hexane) was added dropwise to 0.5 g of **6** in 10 ml benzene and the reaction mixture was stirred for 8 hr at 45°C. The cooled reaction mixture was decomposed with methanol and the solid aluminium salts were filtered off. The filtrate was acidified with dil HCl and carefully neutralized with aq $NaHCO_3$. Extraction with $CHCl_3$ gave a mixture of decomposition products and starting material. No aldehyde was evident in the NMR spectrum or on addition of 2,4-dinitrophenylhydrazine.

Syntheses Relating to Scheme 2

3,5-Dinitro-4-phenoxypyridine (**10**). Nitration of 4-hydroxypyridine by the method of Crow (*57*) yielded 3,5-dinitro-4-hydroxypyridine, which with $POCl_3$ gave 4-chloro-3,5-dinitropyridine (**9**) (*38*). Due to its instability, **9** was reacted further immediately, as follows.

A solution of 0.4 g of PhOK in 1 ml DMF was added dropwise to a solution of 0.5 g of **9** in 1 ml DMF. The reaction mixture was stirred for 12 hr and then poured into water to give 0.3 g crude product (50% yield), which was recrystallized from $CHCl_3$/petroleum ether, mp 125–126°C. PMR: δ9.2 (s, 1H), 6.8–7.4 (m, 5H). *Anal.* Calcd for $C_{11}H_7N_3O_5$: C, 50.57; H, 2.68; N, 16.09. Found: C, 50.79; H, 2.78; N, 15.95.

Attempted radical hydroxymethylations. The reaction was performed with the substrates 3,5-dinitropyridine, 4-chloro-, 4-amino-, 4-hydroxy-, and 4-phenoxy-3,5-dinitropyridine, using the following general procedure. The substrate (5 mmol); $NH_4S_2O_3$ (10 mmol), and H_2O (4.5 ml) were dissolved in MeOH (9 ml) containing 1 eq 95% H_2SO_4. The reaction mixture was heated at 80°C for 24 hr, following which the volume was reduced under vacuum to one-half, the residue was neutralized with Na_2CO_3, and extracted with $CHCl_3$. The $CHCl_3$ extract was dried with $MgSO_4$ and examined by NMR and TLC. The aqueous layer was evaporated to dryness, the residue extracted with EtOH and also examined by NMR and TLC. In each case, only the starting material was recovered.

Syntheses Relating to Scheme 3

4-Hydroxy-2-picoline-*N*-oxide (**11**) was prepared by a three-step sequence from 2-picoline-*N*-oxide, that is, nitration to 4-nitro-2-picoline-*N*-oxide (*58*), conversion of the latter to 4-methoxy-2-picoline-*N*-oxide (*59*), and alkaline hydrolysis to **11** (*60*).

In route A, **11** was converted to **12** by the acetic anhydride rearrangement (*53*). Attempted dinitration of **12** by a modification of the method of Crowe (*57*) yielded only 4-hydroxy-3,5-dinitropyridine.

In route B, the dinitration of **11** to **13** was effected by adaptation of the method of Suzuki (*61*). Attempted Ac_2O rearrangement of **13** (cf. Ref. (*62*)) resulted in partial acetylation of the 4-hydroxy group and otherwise led to recovery of starting material.

Syntheses Relating to Scheme 4

2-Methoxymethyl-4-benzyloxypyridine (**14**). This compound was prepared by a six-step sequence from 2-picoline-*N*-oxide, that is nitration to 4-nitro-2-picoline-*N*-oxide, followed by its conversion to 4-benzyloxy-2-picoline-*N*-oxide (*54*), acid Ac_2O rearrangement to 2-acetoxymethyl-4-benzyloxypyridine (*54*), acid hydrolysis to 2-hydroxymethyl-4-benzyloxypyridine (*54*), thionyl chloride conversion to 2-chloromethyl-4-benzyloxypyridine hydrochloride (*54*), and displacement by methoxide to give **14**. The last step was effected by the following procedure.

Four grams of 2-chloromethyl-4-benzyloxypyridine hydrochloride in 40 ml MeOH was refluxed with 16 ml methanolic sodium methoxide (3 g Na/50 ml MeOH). The reaction was found to be complete within 3 hr by TLC. The cooled reaction mixture was filtered, solvent was removed under vacuum, and the residue poured into water and extracted with CH_2Cl_2. The combined extracts after drying ($MgSO_4$) and removal of solvent gave 2 g of product as a dark-brown oil (60% yield). A sample for analysis was prepared by bulb-to-bulb distillation under vacuum (120–140°C/3 mm). NMR: δ8.4 (d, J = 6 Hz, 1H), 7.4 (s, 5H), 7.1 (d, J = 3 Hz, 1H) 6.7–6.9 (m, 1H), 5.1 (s, 2H), 4.5 (s, 2H), 3.5 (s, 3H). *Anal.* Calcd for $C_{14}H_{15}NO_2$: C, 73.34; H, 6.59; N, 6.11. Found: C, 73.64; H, 6.57; N, 5.45.

2-Methoxymethyl-4-hydroxypyridine (**12a**). Compound **14** (0.5 g) and 0.2 g of 10% Pd-C in 25 ml MeOH were stirred under a hydrogen atmosphere. The reaction was followed by TLC and found to be complete within 4 hr. The reaction mixture was filtered and the solvent removed under vacuum to yield 0.21 g of product (80% yield). A sample for analysis was prepared by bulb-to-bulb distillation under vacuum (140°C/3 mm). PMR: δ7.7 (d, J = 7 Hz, 1H), 6.5 (m, 2H), 4.4 (s, 2H), 3.4 (s, 3H). *Anal.* Calcd for $C_7H_9NO_2$: C, 60.43; H, 6.47; N, 10.07. Found: C, 60.68; H, 6.45; N, 9.81.

2-Methoxymethyl-4-benzyloxypyridine-N-oxide (**15**). Two grams of **14** was heated at 80°C for 6 hr with 5 ml AcOH and 0.8 ml 30% H_2O_2, after which another 0.6 ml of 30% H_2O_2 was added and heating continued for 7 hr. The cooled reaction mixture was concentrated under vacuum, the solution was diluted with water and again concentrated. After neutralization with Na_2CO_3 the mixture was extracted

with $CHCl_3$, and the combined extracts dried ($MgSO_4$). The solvent was removed under vacuum and the residue washed twice with ether to give 0.65 g product (30% yield), mp 74–76°C. PMR: δ8.15 (d, J = 7 Hz, 1H), 7.4 (s, 5H), 7.1 (d, J = 3 Hz, 1H), 6.7–6.9 (m, 1H), 5.1 (s, 2H), 4.7 (s, 2H), 3.6 (s, 3H). *Anal.* Calcd for $C_{14}H_{15}NO_3$: C, 68.36; H, 6.15, N, 5.69. Found: C, 68.01; H, 6.32; N, 5.05.

2-Methoxymethyl-4-nitropyridine-N-oxide (**17**). Two grams of 2-methoxymethylpyridine-*N*-oxide (**16**), prepared by the peracetic acid oxidation of 2-methoxymethylpyridine (*55*), and 20 ml fuming HNO_3 were heated at 90°C for 15 hr. The reaction mixture was neutralized with Na_2CO_3 and extracted with $CHCl_3$. The extract was concentrated to give 0.53 g of **17** (20% yield), mp 125–126.5°C. PMR: δ7.8–8.4 (m, 3H), 4.6 (s, 2H), 3.6 (s, 3H). *Anal.* Calcd for $C_7H_8N_2O_4$: C, 45.65; H, 4.38; N, 15.22. Found: C, 45.85; H, 4.51; N, 15.04.

2-Methoxymethyl-4-hydroxypyridine-N-oxide (**18**). (A) A mixture of 2 g of **15** in 4 ml MeOH, 3.2 ml MeONa/MeOH (3 g Na/50 ml MeOH), and 125 mg 10% Pd-C was stirred under H_2. When the rapid reduction had ceased after consumption of 1 eq H_2, the solution was filtered and concentrated. The residue was washed with a little acetone, dissolved in a minimum volume of hot water and, after acidification with 6 *N* HCl, evaporated to dryness. Extraction with warm EtOH and removal of solvent under vacuum gave 0.9 g of product (73% yield), mp 198°C. PMR (D_2O): δ8.5 (d, 1H), 7.1–7.3 (m, 2H), 4.6 (s, 2H), 3.5 (s, 3H). *Anal.* Calcd for $C_7H_9NO_3$: C, 54.19; H, 5.85; N, 9.03. Found: C, 54.41; H, 5.79; N, 8.79.

(B) One gram of **17** in 2 ml water was treated at 0°C with a solution of 0.6 g NaOH in 2 ml 30% H_2O_2. The temperature was raised carefully and the reaction mixture was refluxed for 20 min. After cooling the solution was acidified with conc. HCl and concentrated under vacuum. The solution was diluted with additional water and then taken to dryness under vacuum. Extraction with EtOH gave 0.5 g product (53% yield).

2-Methoxymethyl-3,5-dinitro-4-hydroxypyridine-N-oxide (**19**). A solution of 0.5 g of **18** in 2.5 ml AcOH and 1 ml of 70% HNO_3 was heated at 70°C for 10 min. Cooling at 0°C for 20 min gave 0.4 g of crude material (50% yield). Recrystallization from MeOH afforded crystals of **19**, mp 208°C. PMR (DMSO-d_6): δ9.5 (s, 1H), 4.8 (s, 2H), 3.4 (s, 3H). *Anal.* Calcd for $C_7H_7N_3O_7$: C, 34.29; H, 2.86; N, 17.14. Found: C, 34.44; H, 2.97; N, 16.99.

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