Phenazines and Natural Products; Novel Synthesis of Saphenic Acid

Lars Petersen, Knud J. Jensen, John Nielsen*

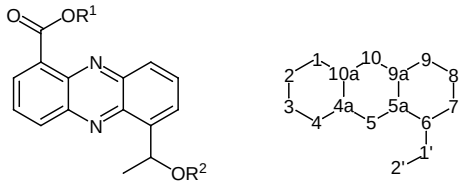
Technical University of Denmark, Department of Organic Chemistry, Building 201, DK-2800 Lyngby, Denmark
Fax +(45) 45933968; E-mail: okjni@pop.dtu.dk

Received 14 May 1999; revised 29 June 1999

Abstract: The natural product saphenic acid (6-(1-hydroxyethyl)-1-phenazinecarboxylic acid) was synthesized from readily accessible starting materials. The desired product was obtained in an overall yield of 22% for four steps with the key steps being formation of a diphenylamine, followed by cyclization under alkaline and reducing conditions. Assignments of ^{1}H NMR spectra were achieved by homo- and heteronuclear 1D and 2D correlations. Double pulsed field gradient spin-echo one-dimensional NOESY proved especially valuable for assignment of aromatic protons.

Key words: antibiotics, heterocycles, natural products, phenazine, DPFGSE-NOE

An interesting class of marine[1] compounds with antibiotic and antitumor activity contains heteroaromatic phenazine structures.[2] A sub-class of phenazine antibiotics contain 6-(1-hydroxyethyl)-1-phenazinecarboxylic acid, also referred to as *saphenic acid*, **1** (Figure 1, $R^1 = R^2 = H$). Naturally occurring **1** is optically active, but it has not been established whether it has the R or S configuration. It has been shown that **1** easily racemizes in the presence of either mild acids or bases.[3] Saphenic acid itself is reported to be biologically inactive, whereas proper substitutions at either the carboxy or at the secondary hydroxy group leads to biologically active compounds. For example, simple esters[4] as in *saphenamycin*[5] or the more complex *esmeraldines*[6] contain the saphenic acid scaffold. Likewise esters of the rare carbohydrate L-quinovose (6-deoxy-L-glucose),[3] are active against a broad range of bacteria (Figure 1).



1 (R^1=R^2=H)

Figure 1 Saphenic acid, **1**, (6-(1-hydroxyethyl)-1-phenazinecarboxylic acid ($R^1 = R^2 = H$) and derivatives: quinovosylsaphenates (R^1 = 2-*O*- or 3-*O*-quinovosyl, $R^2 = H$), simple esters ($R^1 = H$, $R^2 = C(O)CH_2Cl$ or $C(O)CH_2OH$), *saphenamycin* ($R^1 = H$, $R^2 = C(O)$-6-CH_3-2-OH-C_6H_3) or radical scavengers (a dimer or $R^1 = H$, R^2 = 2-phenazinyl). General numbering of atoms in phenazines.

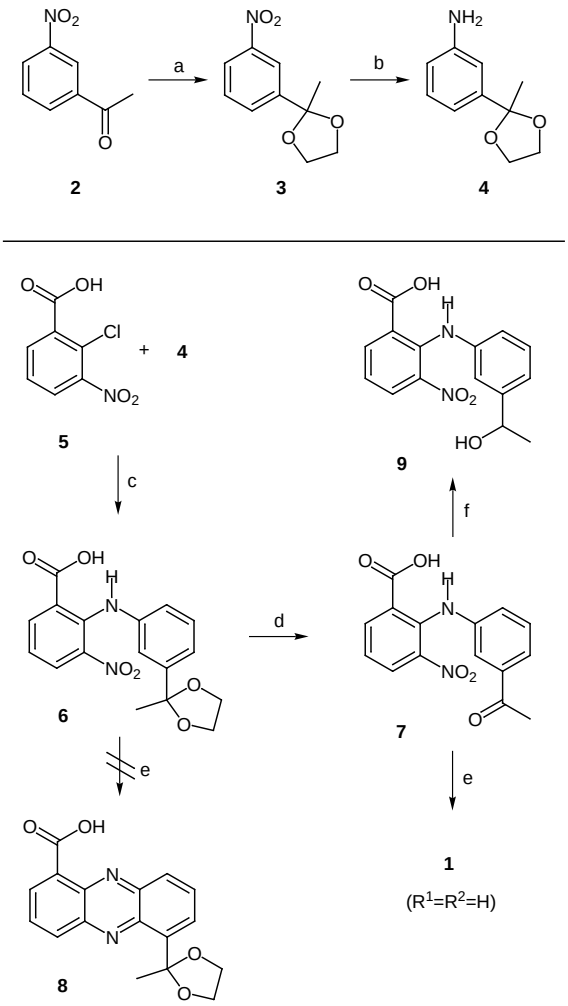
Thus, **1** is an interesting core structure in the search for novel potential antibiotics and antitumor agents. Combinatorial chemistry facilitates the synthesis of large numbers of structurally related compounds and plays a prominent role in modern drug discovery.[7] For the preparation of combinatorial libraries of saphenic acid derivatives, we needed an efficient synthesis of core structure **1**.

Several synthetic routes to phenazines have been reported but few are mild, efficient and simple to carry out.[8] Bahnmüller et al. have reported the preparation of saphenic acid in an overall yield of 3% using the *Wohl-Aue* reaction to fuse 2-aminobenzoic acid and a masked 2-nitroacetophenone at 200°C in sand and solid potassium hydroxide.[9] This synthesis failed in our hands. Ring closure of various diphenylamines is another route to phenazines. Breitmaier and Hollstein prepared 1,6-phenazinedicarboxylic acid by cyclization of a 2-nitrodiphenylamine.[10] This type of reaction is assumed to proceed via an attack of a resonance stabilized anion on the nitro group[11] yielding the 1,6-isomer rather than the 1,8-isomer.

Here we describe a novel and efficient synthesis of saphenic acid with the two key steps being formation of an appropriately substituted 2-nitrodiphenylamine, followed by ring closure under alkaline and reducing conditions. The first building block, protected 3-aminoacetophenone **4**, has previously been synthesized.[12] However, in an optimized protocol starting from easily available 3-nitroacetophenone[13] **2** which was masked as the ketal, **3**, to prevent polymerization, hydrogenation over Pd/C gave **4** in 83% overall yield. (Scheme 1). A second building block is a 2-halo-3-nitrobenzoic acid with 2-chloro-3-nitrobenzoic acid, **5**, being commercially available.

Copper metal or copper salts are often used to mediate the formation of diphenylamines.[14] However, an experiment without catalyst revealed no difference in rate of reaction, indicating that copper bronze or powder had no catalytic activity. Thus, in the absence of any catalyst, coupling of **4** and **5** in refluxing 1-pentanol in the presence of potassium carbonate for 18 h gave the diphenylamine ketal **6**. Acidic hydrolysis for 30 min yielded **7** in 81% for the two-step sequence (Scheme 1). An important issue is the complete deprotonation of **5** before adding **4**. The high yield of **7** is assured by treating **5** with potassium carbonate in 1-pentanol for 1 h. Otherwise the yield of **7** decrease dramatically because of cleavage of the ketal, followed by polymerization.

Attempted cyclization of nitro ketal **6** promoted by sodium ethoxide and sodium borohydride in ethanol to form phenazine ketal **8** was sluggish and required several days at reflux to yield only minor amounts of product. Han-



Reactions a-f: a) $(CH_2OH)_2$/*p*-toluenesulfonic acid in benzene, reflux 18h (89%); b) H_2/Pd(C) in EtOH, r.t. 30 min (93%); c) K_2CO_3 in 1-pentanol, reflux 18 h; d) 4M aq HCl, r.t. 30 min (81%, 2 steps); e) NaOEt and $NaBH_4$ in EtOH, reflux 18 h (32%); f) $NaBH_4$ in EtOH, r.t. 1h (81%).

Scheme 1

dling and isolation of compound **6** was difficult as it was easily hydrolyzed and non-crystalline.

We therefore decided to perform the cyclization on nitro ketone **7** assuming that it would proceed through intermediate **9**.[15] Treatment of **7** with sodium ethoxide and sodium borohydride at room temperature first yielded **9** which upon heating at reflux for 18 h reacted further to **1**. After extractive workup and recrystallization, **1** was obtained in an isolated yield of 32%. A critical issue in this synthesis is the use of a large excess of sodium borohydride. Without excess of borohydride the reaction mixture turns dark, and the colored impurities[16] formed hamper purification, as this type of compounds are poorly suited for chromatography on silica gel. Synthesis on a larger scale (17 mmol) showed no change in the isolated percentage yield.

The original structural elucidation of **1** by ^{1}H NMRwas carried out in $CDCl_3$ solution at 300 MHz.[5] We performed NMR studies in $CDCl_3$, DMSO-d_6, and pyridine-d_5 to develop fast and efficient NMR protocols for structural elucidation of library compounds related to **1**. The relative connectivity among the two sets of three aromatic protons was established by COSY spectra. Nuclear Overhauser enhancement spectroscopy (NOESY) showed strong cross peaks from H2' and H-1' to an aromatic proton thus identifying H-7 and yielding the relative orientation of the first set of correlated protons. Double pulsed field gradient spin-echo one-dimensional NOESY (DPFGSE-NOE)[17] experiments with selective excitation of H-2' gave the expected strong NOE at H-7 and a small NOE identifying H-4 in the other ring system. Thus, the second set of correlated protons could be orientated.

The good solubility of saphenic acid in DMSO-d_6 allowed for a gradient-selected heteronuclear multiple bond correlated (HMBC) spectrum which showed a three-bond cross peak between the single carbonyl and H-2, thus confirming the assignment. Carbon resonances were assigned following gradient-selected heteronuclear single-quantum coherence (HSQC) spectroscopy.[18]

In conclusion, we have succeeded in synthesizing the natural product saphenic acid in a yield of 32% from readily accessible 2-nitrodiphenylamine **7**. The obtained yield is about 10 times higher than previously reported and allows for large-scale synthesis of building blocks essential for the generation of combinatorial libraries on the saphenic acid scaffold. DPFGSE-NOE was a fast and powerful tool in structural elucidation by NMR. This protocol for NMR assignment should be extendable to derivatives of **1**.

Unless otherwise stated, all chemicals were obtained from commercial sources and used without further purification. TLC analysis was performed on Merck Silica Gel 60 F_{254} plates and visualized by UV-light of 254 nm. To visualize amines, TLC plates were dipped in a standard solution of ninhydrin (220 mg) in EtOH (200 mL) followed by heating. Melting points are uncorrected. HR-MS was performed by positive ion FAB and using a *m*-nitrobenzoic acid/PEG300 matrix. ^{1}H NMR spectroscopy was performed on a Varian Unity Inova 500 operating at 499.87 MHz equipped with a waveform generator and a z- (single axis) PFG inverse detection C-H-P probe. ^{13}C NMR spectroscopy was performed on a Varian Mercury 300 equipped with a 4-nuclei PFG probe and operating at 75.46 MHz or on a Bruker AC 200 operating at 50.32 MHz. NOESY spectra were acquired with a mixing time of 4.000 *sec* and a relaxation delay (d1) of 9.0 sec. DPFGSE-NOE was performed varying the mixing time from 1.0 to 5.0 sec. in steps of 0.5 sec.

2-Methyl-2-(3-nitrophenyl)-1,3-dioxolane (3)

Compound **2** (4.00 g; 24.2 mmol), ethylene glycol (1.88 g, 30.2 mmol), and *p*-toluenesulfonic acid (20 mg) were dissolved in benzene[19] (40 ml) in a round bottomed flask equipped with a Dean-Stark separator. After heating at reflux for 16 h, TLC indicated that **2** had been consumed and a new compound formed (R_f 0.52; EtOAc/hexane 1:3). The mixture was diluted with $CHCl_3$ (60 mL), washed with H_2O (3 × 20 mL), dried ($MgSO_4$) and concentrated to dryness. The remaining solid was recrystallized from acetone/hexane to yield 89% of a colorless, crystalline solid with identical melting point (71–72°C) and ^{1}H NMR data to literature data for **3**.[12b]

^{13}C NMR (50 MHz, $CDCl_3$): δ = 27.4 (CH_3), 64.7 (OCH_2CH_2O), 107.9, 120.5, 122.8, 129.3, 131.5, 145.9.

Table 1 ^{1}H NMR data (500 MHz) of saphenic acid (**1**) in $CDCl_3$, DMSO-d_6, and pyridine-d_5, δ-values in ppm (*J*-values in Hz).

	H-2	H-3	H-4	H-7	H-8	H-9	H-1'	H-2'
$CDCl_3$[a]	8.99	8.06	8.52	7.98	7.99	8.19	5.86	1.82
$^3J_{(H,H)}$ (Hz)	7.0	7.0 8.5	8.5	8.5	8.5 8.5	8.5	6.6	6.6
$^4J_{(H,H)}$ (Hz)	1.3		1.3			1.3		
NOE (%)[c]			0.41	9.57			19.32	
DMSO-d_6 [b]	8.56	8.08	8.51	8.13	8.08	8.24	5.98	1.56
$^3J_{(H,H)}$ (Hz)	6.8		8.5	6.4		8.5	6.4	6.4
$^4J_{(H,H)}$ (Hz)	1.3		1.3	1.3		1.7		
NOE (%)[d]			1.21	5.13			20.45	
Pyridine-d_5 [b]	8.89	7.89	8.46	8.51	7.97	8.10	6.52	1.95
$^3J_{(H,H)}$ (Hz)	6.7	8.5 6.7	8.9	7.0	8.9 6.7	8.9		
$^4J_{(H,H)}$ (Hz)	1.3		1.3	1.3		1.3		
NOE (%)[d]			0.46	1.93			7.89	

[a] 8 mg in ~0.7 mL, 303.1 K
[b] 10mg in ~0.7 mL, 303.1 K
[c] Excitation of H-2', mixing time 4.0 sec
[d] Excitation of H-2', mixing time 2.0 sec

3-(2-Methyl-1,3-dioxolane-2-yl)aniline (4)

To compound **3** (10.78 g; 51.5 mmol), dissolved in anhyd EtOH (400 mL), activated charcoal was added, and the suspension was refluxed for 5 min to remove any catalyst poisons. The solution was cooled to r.t. and filtered into a cylinder glass, flushed with N_2 and Pd-catalyst (0.5 g 5% Pd/C) was added. Then the mixture was hydrogenated at 50 bar. After 30 min, consumption of hydrogen ceased and the apparatus was disassembled. TLC showed only one spot which colored bright pink with ninhydrin (R_f 0.23; EtOAc/hexane 1:3). The reaction mixture was filtered, the filter cake was washed with more EtOH (2 × 30 mL) and the combined filtrates were evaporated to yield **4**, colorless crystals (8.59 g, 93%) with mp 86-88°C (lit.[12c] 80–81°C).

^{1}H NMR was identical with literature data.[12c]

^{13}C NMR (50 MHz, $CDCl_3$): δ = 27.5 ppm (CH_3), 64.4 (OCH_2CH_2O), 108.8, 112.1, 114.5, 115.6, 129.2, 144.6, 146.2.

2-(3-Acetylanilino)-3-nitrobenzoic Acid (7)

Compound **5** (4.40 g; 21.9 mmol) and K_2CO_3 (6.06 g; 43.9 mmol) were suspended in 1-pentanol (44 mL; dried over 4 Å molecular sieves), heated on an oil bath at 150 °C for 1 h whereby **5** dissolved. Compound **4** (3.93 g; 21.9 mmol) was added and the mixture heated at reflux for 16 h. Upon cooling, 4 M aq HCl (35.2 mL) was added and the two-phase system was stirred vigorously for 30 min to complete the deprotection. Aq NaOH (45 mL; 15% w/w) was added to pH >11, and the solution was diluted with H_2O to a total volume of 440 mL. This solution was washed with $CHCl_3$ (170 mL) and the organic phase extracted with 0.5 M aq NaOH (1 x 50 mL). The aqueous phases were combined and acidified with aq conc. HCl and after stirring for 30 min the yellow solid was collected by filtration (5.33 g, 81%). The product melted at 217–220°C and was used without further purification. An analytical sample was recrystallized from H_2O/EtOH to yield bright yellow blades (mp 223–224°C).

^{1}H NMR (300 MHz, DMSO-d_6): δ = 2.50 (s, 3H; CH_3), 7.20–7.12 (m., 2H), 7.36 (t, *J* = 8.0 and 7.8 Hz, 1H), 7.42 (t, *J* = 2.4 Hz, 1H), 7.54 (dt, *J* = 7.7 and 1.1 Hz, 1H), 8.09 (dd, *J* = 8.0 and 1.7 Hz, 1H), 8.18 (dd, *J* = 7.8 and 1.7 Hz; 1H), 9.77 (br s, 1H; NH), 13.7 (br s, 1H; COOH).

^{13}C NMR (50 MHz, DMSO-d_6): δ = 26.8 (CH_3) ppm, 116.7, 120.2, 122.6, 122.7, 129.7, 130.6, 136.7 (tertiary arom. CH), 121.9, 137.6, 137.9, 140.7, 142.4 (quaternary arom. C), 168.4 (COOH), 197.5 ($COCH_3$).

Anal. calcd. for $C_{15}H_{12}N_2O_5$: C, 60.00; H, 4.03; N, 9.32. Found: C, 59.85; H, 4.07; N, 9.18.

HR-MS: calcd. *m/z*: 300.0746, found:300.0746 $[M]^+$, calcd. *m/z*: 301.0824, found: 301.0815 $[M+H]^+$

2-[3-(1-Hydroxyethyl)anilino]-3-nitrobenzoic Acid (9)

Compound **7** (304 mg; 1.01 mmol) was suspended in anhyd EtOH (20 mL). $NaBH_4$ was added (190 mg; 5.05 mmol) and the red solution was stirred for 1 h at r.t. H_2O (20 mL) was added, EtOH evaporated under vacuum and the solution acidified with 4 M aq HCl to pH<1. The orange oil was extracted with $CHCl_3$ (20 and 4×10 mL), dried ($MgSO_4$) and evaporated to yield yellow crystals of compound **9** (248 mg, 81%; mp. 147–152°C). The crude product was recrystallized from EtOAc to constant mp of 159.5–160.5°C.

^{1}H NMR (500 MHz, DMSO-d_6): δ = 1.25 (d, *J* = 6.0 Hz, 3H; CH_3), 4.60 (q, *J* = 6.0 Hz, 1H; CH), 5.04 (br s, 1H; OH), 6.76 (d, *J* = 7.5 Hz, 1H), 6.86 (s, 1H), 6.96 (d, *J* = 7.3 Hz, 1H), 7.08 (t, *J* = 7.9 Hz, 1H), 7.16 (t, *J* = 7.6 Hz, 1H), 8.06 (d, *J* = 7.9 Hz, 1H), 8.20 (d, *J* = 7.3 Hz, 1H), 9.88 (s, 1H; NH), 13.7 (br s, 1H; COOH).

^{13}C NMR (75 MHz, DMSO-d_6): δ = 26.37 (CH_3), 68.54 (CHOH), 115.26, 117.04, 119.46, 120.98, 129.52, 131.50, 137.31, 139.55, 140.53, 141.76, 149.29 (C_{arom}), 169.30 (COOH).

Anal. calcd. for $C_{15}H_{14}N_2O_5$: C, 59.60; H, 4.66; N,9.26. Found: C, 59.06; H, 4.72; N, 9.03.

HR-MS: calcd. *m/z*: 303.0981, found: 303.0970 $[M+H]^+$.

6-(1-Hydroxyethyl)phenazine-1-carboxylic Acid (1)

A solution of NaOEt was prepared by dissolving Na (18.2 g, 0.76 mol) in anhyd EtOH (880 mL). $NaBH_4$ (16.74 g, 0.441 mmol) was added and the mixture stirred for 5 min, followed by addition of **7** (4.40 g, 14.6 mmol). After stirring for another 15 min, the mixture was heated at reflux for 18 h. Upon cooling, the bright yellow solution was evaporated partially under vacuum, H_2O was added (1200 mL) and remaining EtOH evaporated. The aq solution was acidified with 4 M aq HCl to pH 6.0 (pH-meter) and extracted with $CHCl_3$ (8 × 250 mL) until the organic phase was colorless. The combined organic phases were dried ($MgSO_4$) and evaporated which yielded a brown-yellow solid (3.1 g). This was dissolved in EtOAc by heating at reflux, which yielded yellow crystals with mp. 198–200 °C (lit. 202–204 °C) (1.26 g, 32%) after cooling overnight (5 °C) and filtration. TLC showed only one yellow spot (R_f 0.17, 2.5% MeOH/$CHCl_3$). The isolated product showed the same mp as previously reported[9] for racemic **1** as well as correct MS and microanalysis data.

^{1}H NMR (500 MHz, $CDCl_3$) was identical with literature data.[5]

^{13}C NMR (125 MHz, DMSO-d_6): δ = 25.548 (C-2'), 63.410 (C-1'), 126.824 (C-7 or C-9), 126.690 (C-7 or C-9), 127.675, 130.240 (C-3 or C-8), 132.812 (C-3 or C-8), 133.906 (C-4), 134.265(C-2), 139.250 (C-4a), 140.411, 140.624, 141.432, 146.186 (C-6), 166.205 (COOH).

Anal. calcd. for $C_{15}H_{12}N_2O_3$: C, 67.15; H, 4.50; N,10.44. Found: C, 66.90; H, 4.57; N, 10.27.

HR-MS: calcd. *m/z*: 269.0926, found: 269.0906 $[M+H]^+$.

References

(1) Fenical, W. *Chem. Rev.* **1993**, *93*, 1673.

(2) Activities arise from DNA intercalation thus inhibiting expression of certain proteins, see:
a) Hollstein, U.; van Gemert, R. J. jr. *Biochemistry* **1971**, *10*, 497.
b) Hollstein, U.; Butler, P. L. *Biochemistry* **1972**, *11*, 1345.
(c) Inhibitors of aquired multidrug resistance: van Rensburg, C. E. J.; Anderson, R.; Jooné, G.; Myer, M. S.; O'Sullivan, J. F. *Anti-Cancer Drugs* **1997**, *8*, 708.

(3) Pathirana, C.; Jensen, P. R.; Dwight, R.; Fenical, W. *J. Org. Chem.* **1992**, *57*, 740.

(4) Takahashi, K.; Takahashi, I.; Morimoto, M. *J. Antibiot.* **1986**, *39*, 624.

(5) Geiger, A.; Keller-Schierlein, W.; Brandl, M.; Zähner, H. *J. Antibiot.* **1988**, *41*, 1542.

(6) Keller-Schierlein, W.; Geiger, A.; Zähner, H.; Brandl, M. *Helv. Chim. Acta.* **1988**, *71*, 2058.

(7) a) Lam, K. S.; Lebl, M.; Krchnák, V. *Chem. Rev.* **1997**, *97*, 411.
b) Nefzi, A.; Ostresh, J. M.; Houghten, R. A. *Chem. Rev.* **1997**, *97*, 449.
c) *A Practical Guide to Combinatorial Chemistry*; Czarnik, A. W.; DeWitt, S. H., Eds.; American Chemical Society: Washington, DC, 1997.
d) *Solid-Supported Combinatorial and Parallel Synthesis of Small-Molecular-Weight Compound Libraries*; Obrecht, D.; Villalgordo, J. M., Eds.; Pergamon: Oxford, UK, 1998.

(8) In general about phenazines, see: Katritzky, A. R.; Rees, C. W. *Comprehensive Heterocyclic Chemistry*, Vol. 3, Boulton, A.J.; McKillop, A., Eds.; Pergamon Press: New York, 1984. For some of the older methods and historical preparations see: Swan, G.A.; Felton, D. G. I. *The Chemistry of Heterocyclic Compounds, Phenazines*; Interscience: New York and London, 1957.

(9) Bahnmüller, U.; Keller-Schierlein, W.; Brandl, M.; Zähner, H.; Diddens, H. *J. Antibiot.* **1988**, *41*, 1552.

(10) a) Flood, M. E.; Herbert, R. B.; Holliman, F. G. *J. Chem. Soc. Perkin Trans. I* **1972**, 622.
b) Breitmaier, E.; Hollstein, U. *J. Org. Chem.* **1976**, *41*, 2104.

(11) For the mechanism see: Cross, B.; Williams, P. J.; Woodall, R. E. *J. Chem. Soc. (C)* **1971**, 2085.

(12) a) Synthesis of **3** by a procedure similar to the present, but with no yield stated: Risley, J. M.; DeFrees, S. A.; Van Etten, R. L. *Org. Magn. Reson.* **1983**, *21*, 28.
b) Synthesis of **3** by nitration of 2-methyl-2-phenyl-1,3-dioxolane: Suzuki, H.; Yonezawa, S.; Mori, T. *Bull. Chem. Soc. Jpn.* **1995**, *68*, 1535.
c) Synthesis of **4** by different route: Zhang, M.Q.; Levshin, I.; Haemers, A.; Vanden Berghe, D.; Pattyn, S.R.; Bollaert, W. *J.Heterocycl.Chem*. **1991**, *28*, 673.

(13) 3-Nitroacetophenone is commercially available or can easily be prepared by nitration of acetophenone (Corson, B. B.; Hazen, R. K. *Org. Synth.* Coll. Vol II, 434).

(14) For a review on nucleophilic substitution of aromatic halogen assisted by copper see: Lindley, J. *Tetrahedron* **1984**, *40*, 1433.

(15) Standard reduction of **7** with $NaBH_4$ in EtOH gave nitro alcohol **9**, which we required as a reference compound.

(16) It is well known that this type of reaction can give by-products: Rewcastle, G. W.; Denny, W. A. *Synth. Commun.* **1987**, *17*, 1171.

(17) a) Stott, K.; Stonehouse, J.; Keeler, J.; Hwang, T.-L.; Shaka, A. J. *J. Am. Chem. Soc.* **1995**, *117*, 4199.
b) Stott, K.; Keeler, J.; Van, Q. N.; Shaka, A. J. *J. Magn. Res.* **1997**, *125*, 302.

(18) We observed a significant change of 0.43 ppm in the chemical shift for H-2 on going from $CDCl_3$ to DMSO-d_6. Here we should note that Geiger et al. in the study of phenazines from *Streptomyces antibioticus* used the chemical shift for H-2 to differentiate between derivatives with a free carboxylic acid or an ester at the neighboring position.[5]

(19) Substituting benzene with toluene was disadvantageous as the ethylene glycol under these conditions distilled into the water separator.

Article Identifier:
1437-210X,E;1999,0,10,1763,1766,ftx,en;xH02799SS.pdf