A New Method for the Synthesis of N^{ϵ}-Acetyl-N^{ϵ}-hydroxy-L-lysine, the Iron-Binding Constituent of Several Important Siderophores†

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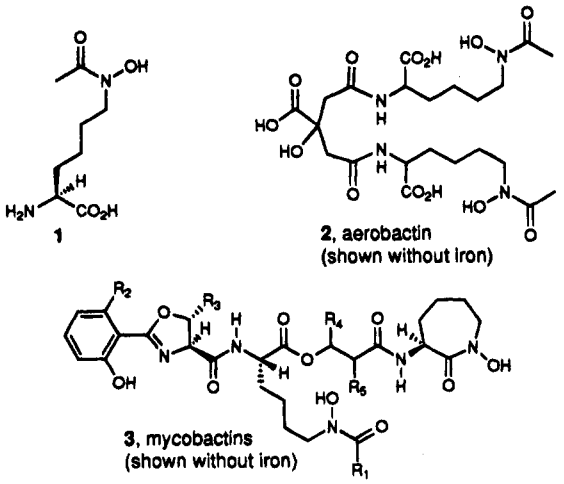
Oxidation of the ϵ-amino group of N^{α}-(carbobenzyloxy)-L-lysine methyl ester with dimethyldioxirane in acetone gave the acetone-derived nitrone of the desired hydroxylamine. Acidic hydrolysis, acetylation, and subsequent deprotection provided a short synthesis of N^{ϵ}-acetyl-N^{ϵ}-hydroxy-L-lysine, the iron-binding constituent of important microbial siderophores.

Siderophores are low molecular weight iron-sequestering agents produced by most microorganisms, especially under iron-deficient conditions.[1-4] The overall structures of siderophores vary depending on the microbial source. In fact, over 200 different siderophores have been identified. This variety may be a consequence of evolutionary pressure to give certain organisms selective growth advantages in a given iron-deficient environment. Interestingly, the iron-binding functional groups incorporated into siderophores by most microbes have been conservative, limited primarily to hydroxamic acids, catechols, and α-hydroxy carboxylic acids. N^{ϵ}-Acetyl-N^{ϵ}-hydroxy-L-lysine (**1**) is the essential iron-binding constituent of aerobactin (**2**)[5] and the mycobactins (**3**). Mycobactins contain varying lipophilic side chains and serve as important growth factors for various strains of pathogenic *Mycobacteria*, including *M. tuberculosis*, *M. paratuberculosis*, and *M. leprae*.[6] The recent resurgence of antibiotic resistant forms of tuberculosis, especially among immunocompromised patients, has prompted considerable concern about the development of new methods of controlling or inhibiting the growth of the causative strains of *Mycobacteria*. Since iron is essential for growth and survival of these microbes, studies related to inhibition of iron assimilation and metabolism may lead to the design of new antimycobacterial agents. One of our goals is to synthesize mycobactin analogs and various drug conjugates and determine if such molecules can either interfere with natural siderophore-mediated iron uptake and thus inhibit microbial growth by iron starvation or if the siderophore–drug conjugates can facilitate drug delivery by an active iron transport-mediated process. The initial focus of this work is to develop effective syntheses of the iron-chelating components of the appropriate siderophores. Herein we describe a new method for the synthesis of N^{ϵ}-acetyl-N^{ϵ}-hydroxy-L-lysine (**1**).

We reported the first and only total synthesis of a mycobactin, mycobactin S-2, in 1983.[7] As indicated then, previous syntheses of N^{δ}-hydroxy-L-ornithine and N^{ϵ}-hydroxyl-L-lysine included reduction of the respective ω-nitro compounds and hydrolysis of nitrones, usually in racemic form.[8-11] We utilized alkylation of preformed hydroxamates with ω-halo or ω-hydroxy amino acids.[12] However, as shown in Scheme 1, application of that methodology (**7** + **8** → **9**) first required synthesis and enzymatic resolution of L-ϵ-hydroxynorleucine (**6**). Conceptually, oxidation of the ϵ-amino group of L-lysine could provide forms of N^{ϵ}-hydroxy-L-lysine (**1**) directly, as apparently occurs biosynthetically.[13] Chemically indirect oxidation of the corresponding δ-amino group of ornithine derivatives, by imine formation, epoxidation, and hydrolysis of the resulting oxaziridine, was introduced in the 1970's.[14] Direct oxidation of primary amines by benzoyl peroxide was reported by Mileweska and Chimiak.[15] These methods are either multistep or byproduct rich, giving low yields of the final product or poor optical purity. Thus, alternative methodology for the oxidation of primary amines is of considerable interest. Dimeth-



† In memory of J.H.'s dear mother, Wenzhu Zhao.

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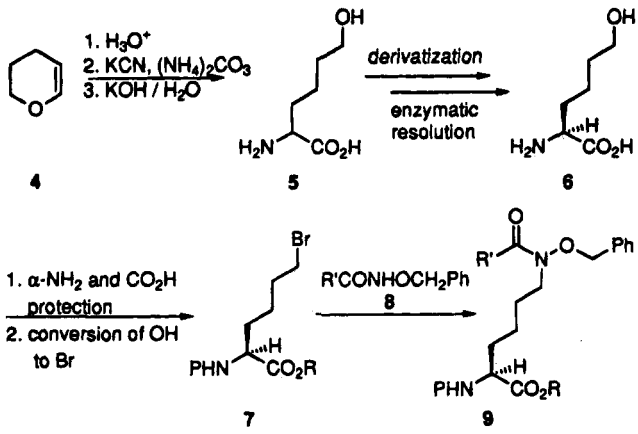
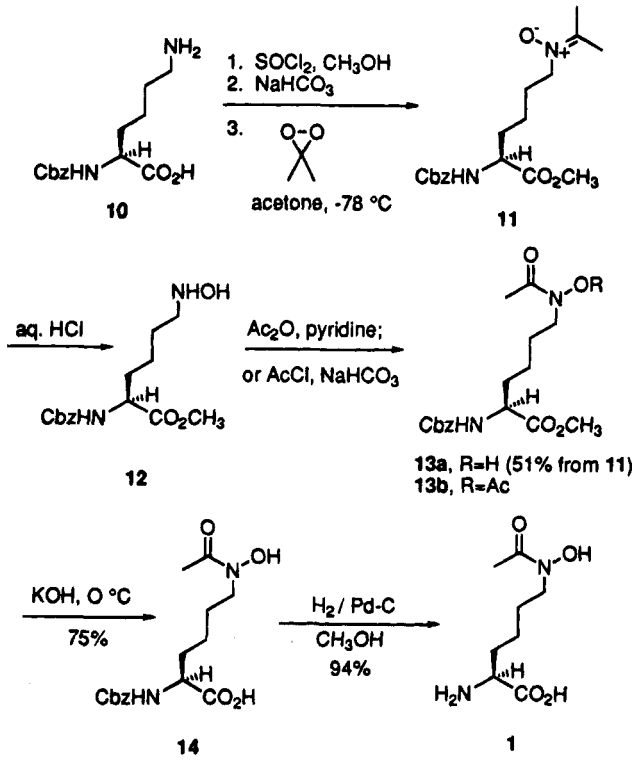
Scheme 1



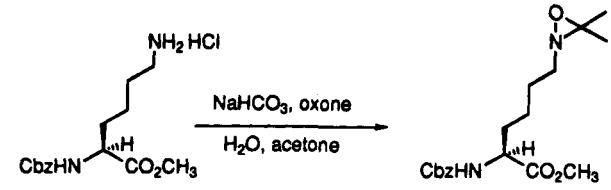
Scheme 2



yldioxirane[16] has recently been reported to oxidize the primary α-amino groups of α-amino acids directly to hydroxylamines without overoxidation.[17a] Thus, we decided to study the use of dimethyldioxirane for the direct oxidation of the ϵ-amino group of derivatives of L-lysine and their eventual conversion to derivatives of N^{ϵ}-acetyl-N^{ϵ}-hydroxy-L-lysine (**1**).

As shown in Scheme 2, commercially available N^{α}-(carbobenzyloxy)-L-lysine (**10**) was converted to the respective methyl ester and then treated with freshly prepared dimethyldioxirane in acetone at −78 °C. Interestingly, workup did not provide the expected hydroxylamine **12**, but instead gave the corresponding nitrone **11**, in 48% isolated yield. Oxidations with cyclohexanone dioxirane, pinacolone dioxirane, and hydrogen peroxide with a tungstate[18] catalyst gave complex mixtures. Alternatively, oxidizing N^{α}-(carbobenzyloxy)-L-lysine methyl ester hydrochloride (**15**) with oxone in the presence of sodium bicarbonate, water, and acetone provided oxaziridine **16**, the isomer of nitrone **11** (Scheme 3). Nitrone **11** and oxaziridine **16** were easily differentiated by ^{13}C NMR. The chemical shift of the quarternary carbon on the oxaziridine **16** is 80.70 ppm, whereas the chemical shift of the corresponding sp^2 carbon on the nitrone **11** is 145.09 ppm. Treatment of nitrone **11** with dilute hydrochloric acid[19] gave the desired, but apparently unstable, hydroxylamine **12** in 41% yield. The hydrolysis of oxaziridine **16** was attempted many times but in no case could the desired hydroxylamine **12** be isolated. Subsequent acetylation of hydroxylamine **12** with acetic anhydride in pyridine gave a mixture of N^{α}-(carbobenzyloxy)-N^{ϵ}-acetyl-N^{ϵ}-hydroxy-L-lysine methyl ester (**13a**) in low yields along with 43% of diacetylated product **13b** and 9% of the carbonyl *O*-acetylated hydroxylamine. The low overall yields again reflected the apparent instability of hydroxylamine **12**. Repetition of the acetylation with acetyl chloride and bicarbonate as the base gave a comparable mixture of acetylation products in only 44% total yield.[20] Alternatively, to avoid isolation of hydroxylamine **12**, the crude nitrone product was hydrolyzed and directly acetylated to give a 51% isolated yield of the desired hydroxamic acid **13a**. Saponification gave the corresponding free acid **14** in 75% yield, and hydrogenolytic removal of the Cbz group gave free N^{ϵ}-acetyl-N^{ϵ}-hydroxy-L-lysine (**1**) in 94% yield.

Scheme 3



The production of nitrone **11**, rather than hydroxylamine **12**, from the oxidation of the ϵ-amino group of lysine derivative **10** contrasts the reported direct oxidation of α-amino groups of amino acids to the corresponding hydroxylamines.[17a] Nitrone **11** could be produced by subsequent rapid reaction of hydroxylamine **12** with acetone (Scheme 4). Alternatively, oxidation of imine **17** is also reasonable since oxidation of imines to nitrones by dimethyldioxirane has been reported[17b] and oxaziridine **16** (Scheme 3) could be formed directly from the imine.

Despite the generation of an intermediate nitrone, the direct oxidation of the ϵ-amino group of N^{α}-protected lysine with dimethyldioxirane and subsequent hydrolysis and acetylation provides a significantly shortened synthesis of N^{ϵ}-acetyl-N^{ϵ}-hydroxy-L-lysine with no required solution.

Experimental Section

General Methods. Melting points were measured on a Thomas-Hoover capillary melting point apparatus and are uncorrected. Infrared (IR) spectra were recorded on a Perkin-

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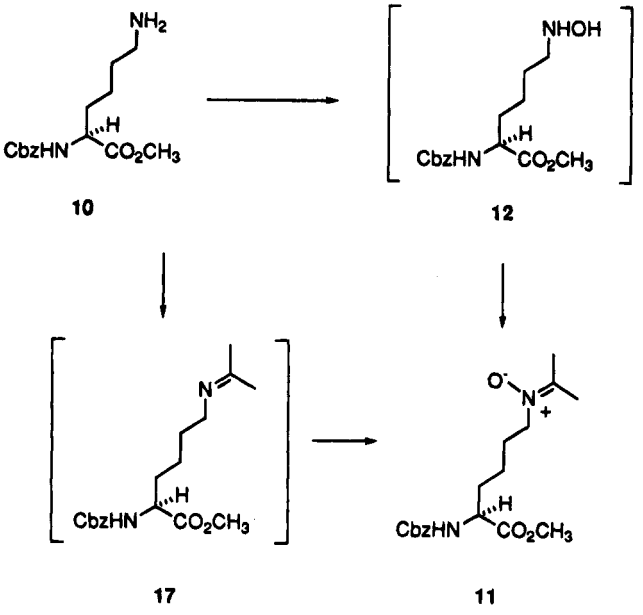
Scheme 4



Elmer 1420 spectrophotometer and were calibrated with the 1601 cm^{-1} band of polystyrene. ^1H and ^{13}C NMR spectra were obtained on a General Electric GN-300 spectrometer. For ^1H NMR spectra, chemical shifts are reported in ppm relative to tetramethylsilane (deuteriochloroform) or 1,4-dioxane (3.55 ppm in deuterium oxide and methyl-d_3 alcohol). ^{13}C NMR spectra were referenced to the center peak of deuteriochloroform (77.0 ppm) or the signal for tetramethylsilane (0.0 ppm) in deuteriochloroform, the signal for 1,4-dioxane (66.5 ppm) in deuterium oxide, and the center peak (49.0 ppm) of methyl-d_3 alcohol. Fast atom bombardment (FAB) mass spectra were recorded on a Finnigan MAT Model 8400 spectrometer. Optical rotations were measured on a Rudolf Research Autopol III polarimeter. Analytical TLC was performed using commercially available aluminum-backed 0.2-mm silica gel 60 F_{254} plates (EM Science) and was visualized with ultraviolet light (254 nm) and by treatment with 10% phosphomolybdic acid in ethanol or 0.3% ninhydrin in AcOH:EtOH (3:200) followed by heating. The ferric chloride test was performed by treating the TLC plate with 0.3% ferric chloride in 0.5 N HCl aqueous solution. Flash column chromatography was conducted on silica gel 60 (EM Science, 230–400 mesh ASTM). Acetone was distilled over 3-Å molecular sieves.

Dimethyl dioxirane was prepared and titrated by the procedures of Murray[16] and Adam.[21]

Nitrone 11. To precooled (ice–salt bath) absolute methanol (50 mL) was added slowly thionyl chloride (4.54 mL, 63.2 mmol). N^{α}-(Carbobenzyloxy)-L-lysine (**10**) (5 g, 17.8 mol) was added to the above mixture in one portion. The solution was allowed to warm to rt slowly and stirred overnight. The methanol was removed *in vacuo*, and a clear oil was obtained, 5.9 g (100%). Part of the oil (210 mg, 0.635 mmol) was dissolved in saturated $NaHCO_3$ solution (20 mL) and was extracted with CH_2Cl_2. The organic layer was dried over Na_2SO_4, filtered, and evaporated to give 183 mg of an oil. The oil was dissolved in acetone (20 mL) and stirred at −78 °C. Enough dimethyldioxirane/acetone was added in one portion of the above mixture. After being stirred for 5–10 min, the reaction mixture was concentrated and chromatographed eluting with MeOH:EtOAc (3:8) to give 107 mg (48%) of nitrone **11** as a white solid. The same reaction was repeated several times, and nitrone **11** was accumulated: mp 93–95 °C (recrystallized from EtOAc and Et_2O); IR (neat) 3350–3200, 1745, 1720 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.37–7.33 (m, 5H), 5.49–5.47 (d, J = 7.8 Hz, 1H), 5.11 (s, 2H), 4.41–4.34 (m, 1H), 3.85–3.81 (t, J = 7 Hz), 3.74 (s, 3H), 2.14 (s, 3H), 2.09 (s, 3H), 1.97–1.38 (m, 6H); ^{13}C NMR ($CDCl_3$) δ 172.75, 156.00, 145.09, 136.25, 128.53, 128.19, 128.11, 66.96, 58.45, 53.65, 52.46, 32.16, 26.74, 22.55, 20.47, 20.05; HRFABMS calcd for $C_{18}H_{27}N_2O_5$ 351.1920, found 351.1926. Anal. Calcd for $C_{18}H_{26}N_2O_5$: C, 61.70; H, 7.48; N, 7.99. Found: C, 61.81; H, 7.35; N, 8.05.

Oxaziridine 16. To a stirred solution of 500 mg (5.7 mmol) of $NaHCO_3$ in 7 mL of H_2O and 7 mL of acetone was added 500 mg (1.5 mmol) of N^{α}-(carbobenzyloxy)-L-lysine methyl ester hydrochloride (**15**) and 1.43 g (2.3 mmol) of oxone at rt under argon. After being stirred for 30 min the reaction was quenched by the addition of 50 mL of H_2O and then extracted with CH_2Cl_2, dried, concentrated, and chromatographed eluting with CH_2Cl_2:EtOAc (1:5) to give 354 mg (67%) of oxaziridine **16** as a clear oil (note, **16** exists as a pair of diastereomers due to the asymmetry at the oxaziridine nitrogen): R_f = 0.34 (CH_2Cl_2:EtOAc, 1:5); IR (neat) 3340 (br), 1750–1690 (br) cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.36–7.32, (m, 5H), 5.41–5.39, (d, J = 8.1 Hz, 1H), 5.11, (s, 2H), 4.42–4.35, (m, 1H), 3.74, (s, 3H), 2.86–2.55, (m, 2H), 1.88–1.44, (m, 6H), 1.52, (s, 3H), 1.41 (s, 3H); ^{13}C NMR ($CDCl_3$) δ (mixture of diastereomers) 172.69, 155.70, 136.04, 128.22, 127.85, 127.80, 80.70, 66.61, 53.71, 53.59, 53.55, 52.07, 32.09, 32.00, 27.84, 27.82, 25.58, 22.89, 22.85, 16.92; HRFABMS calcd for $C_{18}H_{27}N_2O_5$ 351.1920, found 351.1932.

N^{ϵ}-Acetyl-N^{ϵ}-hydroxy-N^{α}-(carbobenzyloxy)-L-lysine Methyl Ester (13a).[19,22] Nitrone **11** (777 mg, 2.22 mmol) was dissolved in 1 N HCl (50 mL) and water (10 mL), and the mixture was stirred at rt for 20 min. The solution was washed with CH_2Cl_2, and the aqueous layer was neutralized with $NaHCO_3$ to pH 8 and then extracted with CH_2Cl_2. The combined organic layer was dried over Na_2SO_4. $NaHCO_3$ (373 mg, 4.44 mmol) was added to the CH_2Cl_2 solution after filtering out the Na_2SO_4, and AcCl (0.158 mL, 2.22 mmol) in CH_2Cl_2 (5 mL) was added to the above solution slowly under argon after cooling to −20 °C. The mixture was stirred cold for 1 h, allowed to warm to rt, filtered, and concentrated to an oil. The desired product **13a** (400 mg, 51%) was obtained as an oil after flash chromatography with ethyl acetate as a solvent. Ferric chloride test: positive; IR (neat) 3500–3000, 1690 (br), 1600 (br) cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.35–7.34 (m, 5H), 5.08 (s, 2H), 4.20–4.15 (m, 1H), 3.70 (s, 3H), 3.62–3.55 (m, 2H), 2.07 (s, 3H), 1.90–1.32 (m, 6H); ^{13}C NMR (CD_3OD) δ 172.93, 172.22, 156.48, 136.02, 128.49, 128.18, 127.94, 67.00, 53.51, 52.40, 47.14, 31.98, 25.61, 22.03, 20.30; HRFABMS calcd for $C_{17}H_{25}N_2O_6$ 353.1712, found 353.1699.

Hydroxylamine **12** could be isolated eluting with MeOH–EtOAc (1:10) as a clear oil after hydrolysis of nitrone **11** (41%): IR (neat) 3500–3100, 1770–1660 (br) cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.36–7.33 (m, 5H), 5.57–5.55 (d, J = 8.1 Hz, 1H), 5.7–5.0 (br, 2H), 5.10 (s, 2H), 4.42–4.35 (m, 1H), 3.73 (s, 3H), 2.91–2.86 (m, 2H), 1.88–1.32 (m, 6H); ^{13}C NMR ($CDCl_3$) δ 173.00, 155.97, 136.18, 128.49, 128.15, 128.09, 66.99, 53.65, 53.21, 52.35, 32.45, 26.33, 22.63; HRFABMS calcd for $C_{15}H_{23}N_2O_5$ 311.1607, found 311.1604.

N^{ϵ}-Acetyl-N^{ϵ}-acetoxy-N^{α}-(carbobenzyloxy)-L-lysine Methyl Ester (13b). Nitrone **11** (100 mg, 0.29 mmol) was stirred in 10 mL of 1 N HCl at rt for 30 min. The aqueous solution was extracted with CH_2Cl_2 after it was basified to pH 8 with $NaHCO_3$. The combined CH_2Cl_2 was dried, concentrated to 10 mL, and then added 0.067 mL (0.71 mmol, 2.5 equiv) of acetic anhydride, and 1 drop of pyridine was added. After being stirred for 30 min, the reaction mixture was washed with 1 N HCl, 5% $NaHCO_3$, and H_2O, dried, concentrated, and chromatographed eluting with EtOAc–AcOH (1:20) to give compound **13b** (47.9 mg, 43%) and carbonyl *O*-acetylated product (9.3 mg, 9%), which was tentatively identified by its ^1H NMR and mass spectra. Compound **13b**: IR (neat) 1790, 1720 (br) cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.36–7.35 (m, 5H), 5.45–5.42 (d, J = 8.4 Hz, 1H), 5.11 (s, 24), 4.40–4.32 (m, 1H), 3.74 (s, 3H), 3.66–3.64 (br, 2H), 2.19 (s, 3H), 1.99 (s, 3H), 1.85–1.34 (m, 6H); FABMS 395 (M + 1).

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N^{ϵ}-Acetyl-N^{ϵ}-hydroxy-N^{α}-(carbobenzyloxy)-L-lysine (14).[23] N^{ϵ}-Acetyl-N^{ϵ}-hydroxy-N^{α}-(carbobenzyloxy)-L-lysine methyl ester (280 mg, 0.8 mmol) was dissolved in 0.2 M KOH (20 mL) and stirred in an ice bath for 30 min. The reaction mixture was acidified to pH 1 with 1 N HCl. The acidic solution was extracted with ethyl acetate. The extracts were dried over $NaSO_4$ and evaporated to give compound **14** as a clear oil, 200 mg (75%): ferric chloride test, positive; IR (neat) 3600–2600, 1725 (br), 1615 (br) cm^{-1}; ^1H NMR (CD_3OD) δ 7.36–7.33 (m, 5H), 4.16–4.11 (m, 1H), 3.57–3.54 (m, 2H), 2.08 (s, 3H), 1.95–1.34 (m, 6H); ^{13}C NMR (CD_3OD) δ 175.90, 173.49, 158.61, 138.09, 129.41, 128.93, 128.91, 67.55, 55.14, 48.41, 32.22, 27.10, 23.85, 20.20; FABMS 339 (M + 1).

N^{ϵ}-Acetyl-N^{ϵ}-hydroxy-N^{α}-L-lysine (1).[24] To a solution of methanol (10 mL) and compound **14** (107 mg, 0.32 mmol) was added 10% Pd/C (10 mg). The mixture was stirred under H_2 (1 atm) at rt for 30 min. The solution was filtered, the solvent was removed *in vacuo*, and the solid residue was recrystallized from deionized water and ethanol to give white crystals of **1**, 61 mg (94%): ninhydrin and ferric chloride tests: positive; mp 215–217 °C; $[\alpha]^{23}_D = +2.2°$ (c = 1.1, H_2O) [lit.[25] mp 209–210 °C, $[\alpha]^{20}_D = +2°$ (c = 5.6, H_2O)]; IR (KBr) 3600–2500 (br), 1600 (br) cm^{-1}; ^1H NMR (D_2O) δ 3.58–3.54 (dd, apparent t, partially obscured by 1,4-dioxane as a internal standard in D_2O, J = 6 Hz, 1H), 3.46–3.41 (t, J = 7 Hz, 2H), 1.91 (s, 3H), 1.72–1.17 (m, 6H); ^{13}C NMR (D_2O) δ 174.45, 173.67, 54.39, 47.34, 29.90, 25.42, 21.41, 19.20; HRFABMS calcd for $C_8H_{17}N_2O_4$ 205.1188, found 205.1187.

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Supplementary Material Available: ^1H and ^{13}C NMR spectra of **11**, **16**, **13a**, **14**, and **1** (13 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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