Synthesis of (−)-3-Amino-3-deoxyquinic Acid

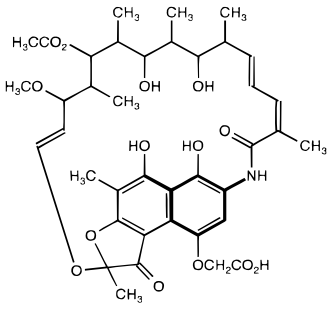
Michael Müller,† Rolf Müller,‡ Tin-Wein Yu, and Heinz G. Floss*

Department of Chemistry, Box 351700, University of Washington, Seattle 98195-1700

Received July 2, 1998

An efficient synthesis of 3-amino-3-deoxyquinic acid, starting from (−)-quinic acid, is reported and its importance for the biosynthesis of 3-amino-5-hydroxybenzoic acid, the precursor of mC_7N-units in ansamycin and mitomycin antibiotics is discussed.

A variety of antibiotics contain a biosynthetically unique structural moiety, called an mC_7N-unit, consisting of a six-membered carbocycle carrying an extra carbon and a nitrogen in a meta arrangement. This unit was first recognized as a structural element of the ansamycin antibiotic rifamycin B (**1**).[1] It is present in all the ansamycins[2] and ansamitocins[3] as well as in the structurally quite different mitomycins.[4]



Rifamycin B

Tracer and genetic experiments have demonstrated the shikimate pathway origin of this mC_7N unit via 3-amino-5-hydroxybenzoic acid (AHBA),[5] although none of the early pathway intermediates were incorporated.[6] Kim et al. have shown that labeled 5-deoxy-5-amino-3-dehydroshikimic acid (aminoDHS),[7] 5-deoxy-5-amino-3-dehydroquinic acid (aminoDHQ)[6] as well as 3,4-dideoxy-4-amino-D-*arabino*-heptulosonate 7-phosphate (aminoDAHP),[7,8] are converted efficiently (95, 41, and 45%, respectively) into AHBA upon incubation with cell free extracts of the rifamycin B producer, *Amycolatopsis mediterranei*, consistent with the proposed[9] pathway of AHBA formation shown in Scheme 1. The conversion of 5-deoxy-5-aminoshikimic acid (aminoSA) into AHBA under the same conditions is notably lower (0.9%) but nevertheless clearly detectable.[7] This is probably due to some transformation of aminoSA into aminoDHS catalyzed by a presumed aminoSA/aminoQA dehydrogenase (RifI)[10] present in *A. mediterranei* or by the normal shikimate dehydrogenase of the organism.

The question then arose whether 3-deoxy-3-aminoquinic acid (aminoQA) could be excluded as a biosynthetic precursor. In view of the low incorporation of aminoSA, we assumed that aminoQA would also be incorporated at a very low level into AHBA, if at all. For further investigations of the enzymatic dehydration step from aminoDHQ to aminoDHS, additional amounts of the former were needed as substrate. Our previous preparation of aminoDHQ proceeded by cyclization of aminoDAHP, catalyzed by dehydroquinate synthase from *Escherichia coli*.[11] However, aminoDAHP itself is only available through a multistep synthesis[8] similar to the DAHP synthesis described by Frost and Knowles.[12] A short nonenzymatic synthesis of aminoquinic acid would make possible a more efficient preparation of aminoDHQ, assuming that aminoQA can be oxidized by chemical means or by the $NADP^+$-dependent shikimate dehydrogenase from *E. coli*.

Starting from (−)-quinic acid (**1**) the known quinide **2** was obtained in 74% yield (Scheme 2).[13] Cleavage of the lactone with NaOMe−MeOH, protection of the 3-hydroxy group[14] and removal of the isopropylidine group with CF_3CO_3H in THF−H_2O gave diol **3**. These three steps can be carried out advantageously without purification of the intermediates to give the diol **3** in 85% yield. The elimination of methylsulfonic acid to the epoxide **4** could not be performed with DBU in THF at ambient temperature. After addition of a catalytic amount of LiBr,[15] the

† Present address: Institut für Biotechnologie, Forschungszentrum Jülich GmbH, D-52425 Jülich, Germany.

‡ Present address: Gesellschaft für Biotechnologische Forschung, Mascheroder Weg 1, D-38124 Braunschweig, Germany.

(1) (a) Brufani, M.; Kluepfel, D.; Lancini, G. C.; Leitich, J.; Mesentsev, A. S.; Prelog, V.; Schmook, F. P.; Sensi, P. *Helv. Chim. Acta* **1973**, *56*, 2315. (b) White, R. J.; Martinelli, E.; Gallo, G. G.; Lancini, G.; Beynon, P. *Nature* **1973**, *243*, 273.

(2) Rinehart, K. L., Jr.; Shields, L. S. *Fortschr. Chem. Org. Naturst.* **1976**, *33*, 231.

(3) Higashide, E.; Asai, M.; Ootsu, K.; Tanida, S.; Kozai, Y.; Hagesawa, T.; Kishi, T.; Sugino, Y.; Yoneda, M. *Nature* **1977**, *270*, 722.

(4) Hornemann, U. In *Antibiotics*; Corcoran, J. W., Hahn, F. E., Eds.; Springer-Verlag: Berlin, 1981; Vol. 4, p 297.

(5) Cf.: (a) Ghisalba, O. *Chimia* **1985**, *39*, 79. (b) Floss, H. G. *Nat. Prod. Rep.* **1997**, *14*, 433.

(6) Kim, C.-G.; Kirschning, A.; Bergon, P.; Zhou, P.; Su, E.; Sauerbrei, B.; Ning, S.; Ahn, Y.; Breuer, M.; Leistner, E.; Floss, H. G. *J. Am. Chem. Soc.* **1996**, *118*, 7486 and references therein.

(7) Kim, C.-G.; Kirschning, A.; Bergon, P.; Ahn, Y.; Wang, J. J.; Shibuya, M.; Floss, H. G. *J. Am. Chem. Soc.* **1992**, *114*, 4941.

(8) Kirschning, A.; Bergon, P.; Wang, J. J.; Breazeale, S. A.; Floss, H. G. *Carbohydr. Res.* **1994**, *256*, 245.

(9) Floss, H. G.; Beale, J. M. *Angew. Chem.* **1989**, *101*, 147; *Angew. Chem., Int. Ed. Engl.* **1989**, *28*, 146.

(10) August, P. R.; Tang, L.; Yoon, Y. J.; Ning, S.; Müller, R.; Yu, T.-W.; Taylor, M.; Hoffmann, D.; Kim, C.-G.; Zhang, X.; Hutchinson, C. R.; Floss, H. G. *Chem. Biol.* **1998**, *5*, 69.

(11) Medhi, S.; Frost, J. W.; Knowles, J. R. *Methods Enzymol.* **1987**, *142*, 306.

(12) Frost, J. W.; Knowles, J. R. *Biochemistry* **1984**, *23*, 4465.

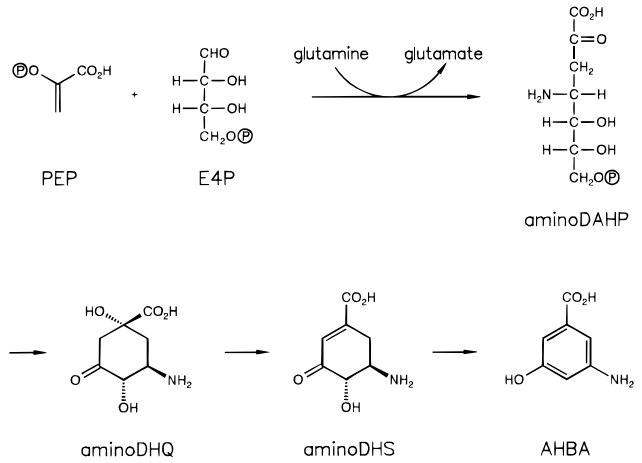
(13) Billen, G.; Karl, U.; Scholl, T.; Stroech, K. D.; Steglich, W. In *Natural Product Chemistry III*; Rahman, A., Le Quesne, P. W., Eds.; Springer-Verlag: Berlin, 1988; p 305.

(14) Sutherland, J. K.; Watkins, W. J.; Bailey, J. P.; Chapman, A. K.; Davies, G. M. *J. Chem. Soc., Chem. Commun.* **1989**, 1386.

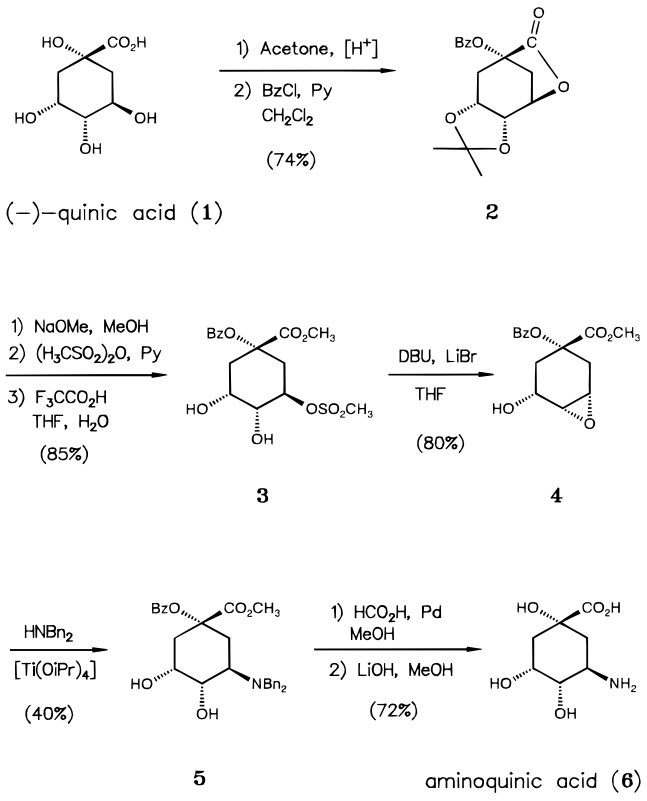
(15) Seebach, D.; Thaler, A.; Blaser, S.; Ko, Y. *Helv. Chim. Acta.* **1991**, *74*, 1102.

10.1021/jo981291l CCC: $15.00
Published on Web 12/04/1998

Scheme 1. Proposed Biosynthetic Pathway to AHBA



Scheme 2. Synthesis of 3-Deoxy-3-aminoquinic Acid

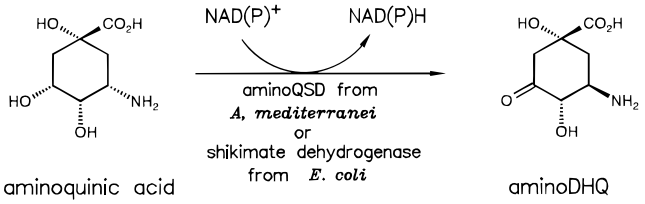


elimination proceeded smoothly at 0 °C. After 10 min at 0 °C, the reaction was almost complete. At higher temperatures or longer reaction times, migration of the 1-*O*-benzoyl group to the hydroxy group at C-3 took place.

The epoxide **4** can be opened nucleophilically at C-3 in the presence of Ti(O*i*Pr)$_4$ either with diallylamine or even more advantageously with dibenzylamine.[16] TLC analyses of the reaction mixture showed that the reaction with dibenzylamine is almost quantitative without the formation of any major side products. The rather low yield of product **5** (40%) is due to the harsh workup conditions (Kugelrohr distillation 120 °C/0.1 Torr), which are necessary to remove excess dibenzylamine. The deprotection

(16) Caron, M.; Sharpless, K. B. *J. Org. Chem.* **1985**, *50*, 1560.

Scheme 3. Enzymatic Formation of AminoDHQ



of the diallylamine group[17] could not be performed on a 100 mg scale so that for completion of the synthesis dibenzylamine was chosen as the nucleophile. Compound **5** can easily be deprotected by catalytic transfer hydrogenation with formic acid[18] and subsequent hydrolysis of the ester under classical conditions to give (−)-3-amino-3-deoxyquinic acid (**6**) in 14% overall isolated yield over nine steps.[19]

Although aminoquinic acid is a substrate for the NAD$^+$-dependent aminoquinate-shikimate dehydrogenase (RifI)[10] from *A. mediterranei*[21] and the NADP$^+$-dependent shikimate dehydrogenase from *E. coli* (Scheme 3),[22] the oxidation of aminoquinic acid to aminoDHQ could not be performed on a preparative scale with either of these enzymes. When about 5−10% of the NAD$^+$ or NADP$^+$, respectively, have been consumed, the reaction gradually comes to a halt. With the aminoquinate-shikimate dehydrogenase from *A. mediterranei*, after that amount of NAD$^+$ consumption in the oxidation of aminoquinic acid no further product formation can be detected by UV (formation of NADH, 340 nm) or by ^{1}H NMR spectroscopy. The same was observed by assay of NADPH formation in the oxidation of aminoquinic acid with shikimate dehydrogenase from *E. coli*. These observations are not due to the NADH (NADPH) concentration or the NADH:NAD$^+$ ratio. Addition of alcohol dehydrogenase and acetaldehyde to the reaction mixture with aminoquinate-shikimate dehydrogenase does not result in higher product formation, suggesting that the enzyme is not inhibited by NADH. Product inhibition by aminoDHQ, however, cannot be ruled out.

Nevertheless, formation of aminoDHQ by enzymatic oxidation of aminoquinic acid was unambiguously demonstrated, directly by proton NMR spectroscopy and indirectly after aromatization to protocatechuic acid and silylation by GC-MS.

Incubations of aminoquinic acid (**6**) with cell-free extracts of the rifamycin B producer, *A. mediterranei*, revealed that **6** is converted into AHBA to a similar extent as aminoSA (1−2%). This finding, as the earlier one with aminoSA,[6] does not answer the question whether aminoquinic acid, and/or aminoSA, are biosynthetic precursors of AHBA. Their conversions into AHBA are clearly less efficient than those of aminoDAHP, aminoDHQ, and aminoDHS. However, it cannot be ruled out

(17) (a) Gigg, R.; Conant, R. *J. Carbohydr. Chem.* **1983**, *1*, 331. (b) Laguzza, B. C.; Ganem, B. *Tetrahedron Lett.* **1981**, *22*, 1483. (b) Moreau, B.; Lavielle, S.; Marquet, A. *Tetrahedron Lett.* **1977**, *18*, 2591.

(18) Elamin, B.; Anantharamaiah, G. M., Royer, G. P., Means, G. E. *J. Org. Chem.* **1979**, *44*, 3442.

(19) After this work was completed, a publication appeared[20] which used a similar strategy to synthesize 3-azido-3-deoxyshikimic acid.

(20) Kim, C. U.; Lew, W.; Williams, M. A.; Liu, H.; Zhang, L.; Swaminathan, S.; Bischofberger, N.; Chen, M. S.; Mendel, D. B.; Tai, C. Y.; Laver, W. G.; Stevens, R. C. *J. Am. Chem. Soc.* **1997**, *119*, 681.

(21) Müller, R.; Müller, M.; Yu, T.-W.; Floss, H. G. Unpublished results.

(22) Chaudhuri, S.; Anton, I. A.; Coggins, J. R. *Methods Enzymol.* **1987**, *142*, 315.

that under different incubation conditions or with extracts of cells grown under different conditions these two substrates can be used more efficiently for the biosynthesis of AHBA and thus rifamycin B. The presence of an enzyme in *A. mediterranei*, encoded by the *rifI* gene in the rifamycin biosynthetic gene cluster, which specifically oxidizes aminoquinate and aminoshikimate, but not quinate, at C-3 suggests a physiological role for these compounds in rifamycin formation, for example in a possible salvage pathway of AHBA formation.

Experimental Section

(−)-Methyl (1*S*,3*R*,4*R*,5*R*)-1-(Benzoyloxy)-3-*O*-(methylsulfonyl)-4,5-dihydroxycyclohexane Carboxylate (3). To a solution of 486 mg (1.5 mmol) of 1-*O*-benzoyl-4,5-*O*-isopropylidenequinide (**2**) in 5 mL of MeOH was added a solution of 95 mg (1.75 mmol) NaOMe in 1 mL MeOH. After 30 min at room temperature, the solution was diluted with 50 mL of ether and washed with brine, NH_4Cl solution, $NaHCO_3$ solution, and brine. The organic layer was dried over Na_2SO_4, and the solvent was evaporated.

The residue was dissolved in 5 mL of dry CH_2Cl_2. Pyridine (300 μL, 4 mmol) and a solution of 350 mg of methanesulfonic anhydride (2 mmol) in 0.5 mL of dry CH_2Cl_2 were added. After 30 min at room temperature, the solution was diluted with 50 mL of ether and washed with brine, NH_4Cl, $NaHCO_3$, and brine. The organic layer was dried over Na_2SO_4, and the solvent was evaporated.

To a stirred solution of the residue in 5 mL of THF/H_2O 4:1 at 0 °C was added 0.45 mL of trifluoroacetic acid. The resulting solution was allowed to warm to room temperature and left overnight. The reaction mixture was diluted with 50 mL of EtOAc and washed with brine, NH_4Cl, $NaHCO_3$, and brine. The organic layer was dried over Na_2SO_4, and the solvent was removed under reduced pressure. The crude product was purified by flash chromatography (SiO_2, gradient of EtOAc−hexane 1:1 to 3:1) to give 495 mg (1.3 mmol, 85%) of **3** as a white foam.

^{1}H NMR ($CDCl_3$): 2.14 (m, 2H), 2.81 (d, J = 14.0 Hz, 2H), 3.08 (s, 3H), 3.69 (m, 1H), 3.71 (s, 3H), 4.25 (q, J = 3.0 Hz, 1H), 5.12 (ddd, J = 12.9, 4.0 Hz, 1H), 7.41 (t, J = 7.3 Hz, 2H), 7.55 (t, J = 7.3 Hz, 1H), 7.99 (d, J = 8.1 Hz, 2H). ^{13}C NMR ($CDCl_3$): 33.8, 37.85, 38.56, 52.96, 68.84, 72.65, 78.39, 79.81, 128.51, 129.44, 129.92, 133.5, 165.36, 170.97. $C_{16}H_{20}O_9S$ (388.4).

(−)-Methyl (1*S*,3*R*,4*R*,5*R*)-1-(Benzoyloxy)-3,4-epoxy-5-hydroxycyclohexane Carboxylate (4). To a stirred solution of 4.2 g of mesylate **3** (10.8 mmol) and 3.8 mL of DBU (25 mmol) in 100 mL of dry THF was added 50 mg of LiBr at −0 °C. The reaction mixture was allowed to warm to room temperature. After 10 min the reaction mixture was passed through a short silica gel column (4 × 4 cm, eluent 400 mL EtOAc−hexane 1:1, then 2 l EtOAc). Rechromatography (SiO_2, 30 × 3 cm, EtOAc−hexane 1:1, 800 mL) gave pure epoxide **4** (2.5 g, 8.5 mmol, 80%) as a colorless oil.

^{1}H NMR ($CDCl_3$): 2.01 (d, J = 15.5 Hz, 1H), 2.09 (dd, J = 12.6, 10.6 Hz, 1H), 2.28 (ddd, J = 12.6, 5.2, 1.5 Hz, 1H), 3.13 (ddd, J = 15.5, 4.9, 1.9 Hz, 1H), 3.39 (m, 1H), 3.47 (t, J = 4.3 Hz, 1H), 3.73 (s, 3H), 4.04 (ddd, J = 10.6, 5.5, 2.2 Hz, 1H), 7.43 (t, J = 7.5 Hz, 2H), 7.57 (t, J = 7.7 Hz, 1H), 7.98 (d, J = 8.1 Hz, 2H). ^{13}C NMR ($CDCl_3$): 31.37, 35.33, 52.82, 53.35, 55.13, 65.53, 78.81, 128.52, 129.29, 129.88, 133.55, 165.35, 171.72. $[\alpha]_D^{20}$: −68° (c = 0.01 acetone). MS (ES^+): 295 (2.7), 294 (16.6), 293 (100), 171 (24.8), 153 (22.2), 143 (16.6), 139 (10), 126 (15.5), 105 (18.8). R_f = 0.45 (EtOAc−hexane 1:1). $C_{15}H_{16}O_6$ (292.3).

(−)-Methyl (1*R*,3*R*,4*S*,5*R*)-1-(Benzoyloxy)-5-(*N*,*N*-dibenzylamino)-3,4-dihydroxycyclohexane Carboxylate (5). A mixture of 880 mg of epoxide **4** (3 mmol), 2 mL of dibenzylamine (10 mmol), and 0.2 mL of $Ti(OiPr)_4$ (0.68 mmol) was stirred at room temperature under argon for 48 h. After the mixture had been passed through a short column of silica gel (3 × 3 cm, EtOAc−hexane 1:1 400 mL, followed by 500 mL of EtOAc), the combined eluates were evaporated. The residue was subjected to a Kugelrohr distillation (120 °C/0.1 Torr, 15 min). The product in the remaining residue was purified by flash chromatography (SiO_2, EtOAc/hexane 1:1). Recrystallization from EtOAc−petroleum ether gave 587 mg **5** (1.2 mmol, 40%) of colorless needles, mp 184 °C. ^{1}H NMR ($CDCl_3$): 1.85 (t, J = 13.02 Hz, 1H), 1.98 (dd, J = 14.4, 1.5 Hz, 1H), 2.73 (d, br, 2H), 3.35 (d, J = 13.1 Hz, 2 H), 3.37 (m, 1H), 3.67 (dd, J = 10.5, 3.2 Hz, 1H), 3.76 (s, 3H), 3.87 (d, J = 13.1 Hz, 2H), 4.24 (m, br, 1H), 7.20−7.24 (m, 10H), 7.37 (t, J = 7.6 Hz, 2H), 7.55 (t, J = 7.4 Hz, 1H), 7.84 (d, J = 7.3 Hz, 2H). ^{13}C NMR ($CDCl_3$): 28.99, 33.50, 51.83, 52,62, 53.26, 67.41, 69.80, 79.96, 127.27, 128.21, 128.49, 128.85, 129.92, 130.01, 132.95, 138.50, 165.30, 172.08. $[\alpha]_D^{20}$ −51° (c = 0.0043 acetone). R_f = 0.7 (EtOAc−hexane 1:1). MS (FAB): 490 (100), 400 (7), 368 (12). HR-MS (FAB): $C_{29}H_{32}NO_6$ calcd 490.2229, obsd 490.2245. $C_{29}H_{31}NO_6$ (489.3).

(−)-Methyl (1*R*,3*R*,4*S*,5*R*)-5-Amino-1-(benzoyloxy)-3,4-dihydroxycyclohexane Carboxylate. To the protected aminoquinic acid **5** (800 mg, 1.4 mmol) in 2.5 mL of a 4.4% solution of formic acid in methanol was added 600 mg of palladium black (Aldrich). After complete reaction (30 min) the mixture was passed through a short silica gel column with 10 mL of MeOH. Purification was performed by preparative TLC (SiO_2, EtOAc−MeOH 3:1) to give 400 mg (90%, about 90% pure) of the debenzylated product, mp 175 °C. ^{1}H NMR ($CDCl_3$): 1.98 (t, br, 2H), 2.66 (t, br, 2H), 3.60−3.65 (m, br, 2H), 3.62 (s, 3H), 4.06 (s, br, 1H), 7.32 (t, 2H), 7.44 (t, 1H), 7.97 (d, 2H). R_f = 0.15 (EtOAc−MeOH 3:1). MS (FAB): 310 (100), 188 (20), 170 (15). HR-MS: $C_{15}H_{20}NO_6$ calcd 310.1290, obsd 310.1282. $C_{15}H_{19}NO_6$ (309.32).

(−)-(1*R*,3*R*,4*S*,5*R*)-5-Amino-1,3,4,-trihydroxy-cyclohexanecarboxylic Acid (Aminoquinic Acid) (6). To 400 mg (1.2 mmol) of the product from the previous step in 10 mL of methanol at 0 °C was added 100 mg of LiOH (2.4 mmol) in 5 mL of H_2O. After 12 h at room temperature more LiOH was added (50 mg in 2 mL of H_2O, total 3.6 mmol). The hydrolysis was complete after 5 h. The reaction mixture was loaded on a short ion exchange column (10 × 1 cm, Dowex AG 1 × 8, gradient H_2O to 3 M formic acid). The product eluted with 0.5 M formic acid, and evaporation of solvent gave 180 mg (80%) of a white powder, mp 201−203 °C. $[\alpha]_D^{20}$ −44° (c = 0.004 H_2O). ^{1}H NMR (D_2O): 1.93−2.10 (m, 4H), 3.42 (dt, J = 10.5 Hz, 4.1 Hz, 1H), 3.56 (dd, J = 10.5 Hz, 3.0 Hz, 1H), 4.07 (d, J = 3.0 Hz, 1H). ^{13}C NMR (D_2O): 37.89, 37.96, 48.85, 70.22, 72.29, 76.14, 181.23. MS (ES-):190.1 (100), 121.0 (22). HR-MS (FAB): $C_7H_{14}NO_5$ calcd 192.0872, obsd 192.0871. $C_7H_{13}NO_5$ (191.19).

Acknowledgment. This work was supported by a grant from the National Institutes of Health (AI20264 to H.G.F.). M.M. and R.M. thank the Deutsche Forschungsgemeinschaft for postdoctoral support.

JO981291L