proceeded cleanly to give >90% yields of oxidized products with >90% mass balance. In the case of DMA, the single primary product was 9-(hydroxymethyl)-10-methylanthracene (**1**, 31%) as well as the secondary products 9,10-bis(hydroxymethyl)-anthracene (**2**, 43%) and 10-methylanthracene-9-carboxaldehyde (**3**, 21%). Similarly, oxidation of EMA yielded, as a primary product, 9-ethyl-10-(hydroxymethyl)anthracene (**4**, 25%), as well as the secondary oxidation product 10-ethyl-9-anthracenecarboxaldehyde (**5**, 6%). Surprisingly, no 10-methyl-9-(1-hydroxyethyl)anthracene (**6**) was detected. Rather, an unprecedented reaction product, 10-methylanthrone (**7**), was produced in 63% yield (see Figure 2). The relative yield of products was solvent dependent, with the yield of 9-ethyl-10-(hydroxymethyl)anthracene (**4**) directly proportional to water concentration and dominating the reaction mixture at >20% water (see Table I). Finally, 9,10-diethylanthracene produced 10-ethylanthrone (**8**) only.

The ratio of hydroxymethyl **4** to anthrone product **7** from oxidation of EMA is thus a sensitive function of reaction environment for a common radical cation and provides us with a test for the nature of microsomal oxidation. Indeed, when the oxidation was carried out in a 20% dimethylformamide–water suspension of freshly prepared rat-liver microsomes, hydroxylation to form **4** was observed in only 3% yield, together with an 83% yield of 10-methylanthrone (**7**). In contrast, a cytosol fraction from the same tissue preparation yielded the hydroxylation product **4** in 86% yield along with 9% of anthrone **7**.

Formation of these products is readily interpreted as involving the intermediacy of the dialkylanthracene radical cation, in accord with the recent work of Kochi et al.[7] When a methyl group is present, rapid deprotonation followed by a second one-electron oxidation and hydration of the resulting cation occurs, provided a suitable proton carrier (water) is available. With an ethyl group, stereoelectronic effects inhibit deprotonation. Rather, elimination of ethylene via an unprecedented seven-member transition state appears likely, facilitated by conversion of a benzhydryl radical to an α-hydroxybenzhydryl radical (see Figure 2). In confirmation, ethylene was isolated from the reaction mixture and identified both by mass spectroscopy and trapping with bromine.

The decay of aryalkyl radical cations such as toluene is known to be accelerated in water,[8] a phenomenon we can now directly associate with deprotonation by water acting as a specific base. The paucity of this product of one-electron oxidation in rat-liver microsomes can be attributed to the hydrophobic environment of this oxidative system, a selectivity which is reversed in the (aqueous) cytosolic environment.

Dual pathways involving ring oxidation on the one hand and side-chain oxidation on the other involving a reversibly formed cyclohexadienyl radical have been invoked to explain the divergent oxidation chemistry of methylated benzenes under a variety of conditions.[9] Elimination of ethylene is, we believe, unique to 9-ethylanthracenes and their derivatives, arising as it does from the steric inhibition provided by two peri hydrogens.[10] This result provides a compelling explanation for the enzymic deactivation of 7,12-diethylbenz[*a*]anthracene and 6-ethylbenzo[*a*]pyrene, since the resulting anthrone derivatives are known to be innocuous. It further provides powerful permissive evidence for the intermediacy of radical cations in the biooxidation of these compounds, which, in the hydrophobic microsomal environment, undergo ring oxygenation rather than deprotonation and side-chain oxidation.

Acknowledgment. The preparation of rat-liver microsomes by Lydia C. Fowler and valuable discussions with Dr. Heath Herman and Professor James W. Flesher are gratefully acknowledged.

(7) (a) Schlesener, C. J.; Amatore, C.; Kochi, J. K. *J. Am. Chem. Soc.* **1984**, *106*, 7472. (b) Despite the observation of Kochi et al.,[7a] we were able to carry out efficient oxidation in aqueous acetonitrile.

(8) Green, M. M.; Mielke, S. L.; Mukhopadhyay, T. *J. Org. Chem.* **1984**, *49*, 1276.

(9) Walling, C. *Acc. Chem. Res.* **1975**, *8*, 125.

(10) In a related reaction, Dr. Donald Camaioni has observed oxidative elimination of formaldehyde from anthracenemethanol: Camaioni, D. M., Report No. GRI-86/0100, Gas Research Institute, 1986.

Total Synthesis of D- and L-*myo*-Inositol 1,4,5-Trisphosphate

Joseph P. Vacca,* S. Jane deSolms, and Joel R. Huff

Merck Sharp & Dohme Research Laboratories
West Point, Pennsylvania 19486

Received January 26, 1987

One of the control mechanisms for regulation of intracellular Ca^{2+} ion concentration involves activation of membrane receptors followed by signal transduction and the release of a modulatory substance.[1] Recent studies[2] provide compelling evidence that activation of these Ca^{2+} mobilizing receptors results in hydrolysis of phosphatidylinositol 4,5-diphosphate, giving rise to D-*myo*-inositol 1,4,5-trisphosphate (IP_3, **1**). IP_3 directly mediates release of intracellular calcium stores. The currently used method for preparing **1** involves chemical hydrolysis of membrane phosphoinositides followed by a tedious purification procedure.[3] Probing the fundamental biochemical processes related to IP_3 requires ready access to **1**, as well as its enantiomer and other derivatives which are not available from natural sources. Furthermore, no radiolabeled derivatives of **1** with high specific activity are currently available. For these reasons, we have developed an efficient chemical synthesis of **1** that may be adapted to the preparation of a variety of phosphorylated inositols and radiolabeled derivatives.

Two general problems impinge on any strategy for the synthesis of polyphosphoinositols. The first is differentiation of the hydroxyl substituents. Phosphorylation of the resultant protected cyclitol in a fashion which allows for efficient deprotection has historically been the second and hitherto unsolved problem.[4] With regard to differentiating the alcohols, a suitably protected triol such as **2** represents a valuable intermediate in the synthesis of **1**. The preparation of such a derivative in racemic form, in which the blocking groups were benzyl ethers, was first reported by Gigg[5] and was accomplished in 10 steps from *myo*-inositol. Ozaki[6] and co-workers have recently reported a synthesis of both enantiomers of triol **2** by a similar route and the conversion of the triol to the antipodes of **1**. In agreement with previous literature,[4] Ozaki et al. found it necessary to employ *N',N'*-diphenylphosphorodiamidic chloride[7] to effect polyphosphorylation of **2**. This reagent provided a trisphosphoranilide intermediate in about 40% yield. Removal of the six aniline protecting groups required strenuous conditions which resulted in a reportedly poor yield of **1**. We have corroborated the unsatisfactory deprotection of inositol phosphoranilides in prior work in our own laboratory (unpublished data).

We wish to report a practical, eight-step synthesis of both enantiomers of *myo*-inositol 1,4,5-trisphosphate which demonstrates a potentially general solution to the phosphorylation problem and subsequent deprotection. Furthermore, we have developed a considerably shorter and more efficient route to a suitably protected, enantiomerically pure triol similar to **2**.

4-Benzyl-1,2:5,6-di-*O*-cyclohexylidene-*myo*-inositol[8] (**3**) is readily obtained in two steps from commercially available *myo*-inositol by the method of Garegg. Esterification of the free

(1) Somlyo, A. V.; Bond, M.; Somlyo, A. P.; Scarpa, A. *Proc. Natl. Acad. Sci. U.S.A.* **1985**, *82*, 5231-5235.

(2) Berridge, M. J.; Irvine, R. F. *Nature (London)* **1984**, *312*, 315-321. Abdel-Latif, A. A. *Pharmacol. Rev.* **1986**, *38*, 227-272 and references cited therein.

(3) Grado, C.; Ballou, C. E. *J. Biol. Chem.* **1961**, *236*, 54-60.

(4) Krylova, V. N.; Gornaeva, N. P.; Oleinik, G. F.; Shvets, V. I. *Zh. Org. Khim.* **1980**, *16*, 315-322. Krylova, V. N.; Lyutik, A. I.; Gornaeva, N. P.; Shvets, V. I. *Zh. Obshch. Khim.* **1980**, *51*, 210-214.

(5) Gigg, J.; Gigg, R.; Payne, S.; Conant, R. *Carbohydr. Res.* **1985**, *140*, c1-c3.

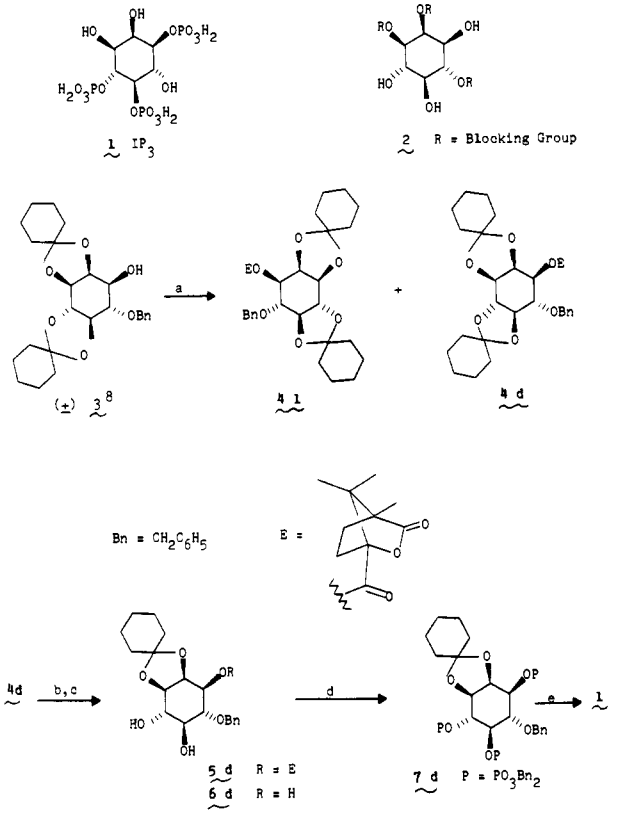
(6) Ozaki, S.; Watanabe, Y.; Ogasawara, T.; Kondo, Y.; Shiotani, N.; Nishi, H.; Matsuki, T. *Tetrahedron Lett.* **1986**, *27*, 3157-3160.

(7) Cremlyn, R. J. W.; Dewhurst, B. B.; Wakeford, D. H. *J. Chem. Soc. C* **1971**, 300-303.

(8) Garegg, P. J.; Iversen, T.; Johansson, R.; Lindberg, B. *Carbohydr. Res.* **1984**, *130*, 322-326.

0002-7863/87/1509-3478$01.50/0

Scheme I[a]



[a] (a) (S)-(-)-camphanic acid chloride, NEt_3, DMAP, CH_2Cl_2, 25 °C, 1 h; (b) MeOH/EtOAc, (1:5) catalytic HCl (gas), room temperature, 3 h; (c) 10 equiv of LiOH, DME/H_2O (2:1), room temperature 1 h; (d) 4 equiv of KH, THF, 60 °C, 30 min then 4 equiv of tetrabenzyl pyrophosphate, room temperature 4 h; (e) H_2, 50 psig, 10% Pd/C, 95% EtOH, room temperature 3 h then AcOH/H_2O, room temperature 16 h.

hydroxy substituent with (*S*)-(-)-camphanic acid chloride[9] (DMAP, Et_3N, CH_2Cl_2, 1 h, 25 °C) yielded a mixture of two diastereomers (combined yield = 90%) that were chromatographically separated (CH_2Cl_2/Et_2O, 98:2; SiO_2) to give **4d** (mp 152-153 °C)[10] and **4l** (mp 121-123 °C).[10] The diastereomeric purity of each compound exceeded 98% as determined both by HPLC and ^1H NMR. Selective hydrolysis of the trans ketal of **4d** with a catalytic amount of gaseous HCl in a mixture of ethyl acetate-methanol (5:1, 3 h) gave diol **5d** (mp 176-178 °C, $[\alpha]^{20}_D$ -32°, $CHCl_3$, c = 1 mg/mL)[10] in 76% yield. Basic hydrolysis of the ester group of **5d** (10 equiv of LiOH, DME/H_2O 2:1, 25 °C, 1 h) afforded triol **6d** (88%, mp 137-139 °C, $[\alpha]^{20}_D$ +21°, $CHCl_3$, c = 1 mg/mL).[10]

The crucial step of the synthesis relied on successfully phosphorylating triol **6d**, which contains the troublesome 4,5-vicinal diol. It was also essential that the phosphate groups be introduced in a form easily deprotected prior to hydrolysis of the 1:2-cyclohexylidene ketal in order to minimize the facile migration of phosphate esters from the 1- to the 2-hydroxyl group.[11] We explored a variety of methods to trisphosphorylate **6d** under neutral conditions, including the use of phosphite triesters,[12] but none were satisfactory. Phosphorylation of the third hydroxyl oxygen is kinetically very slow, allowing side reactions to predominate. In order to enhance the reaction rate, we explored the possibility of phosphorylating alkoxide salts. The preformed tripotassium alkoxide of triol **6d** (4 equiv of KH, THF, 60 °C, 30 min) reacted cleanly with tetrabenzyl pyrophosphate[13,14] (4 equiv of tetrabenzyl pyrophosphate, THF, 4 h, 25 °C) to give compound **7d** (oil, $[\alpha]^{20}_D$ -4.2°, $CHCl_3$, c = 1 mg/mL)[10a] in 65% yield. No trace of a 4,5-cyclic phosphate, often the predominant product in other phosphorylation procedures,[4] could be found under these conditions (Scheme I).

Hydrogenolysis of the seven benzyl groups of trisphosphate **7d** (H_2, 50 psig, 10% Pd/C, 95% EtOH, 3 h) and subsequent hydrolysis of the cyclohexylidene ketal (AcOH/H_2O 1:1, 16 h, 25 °C) in a one-pot procedure gave D-*myo*-inositol 1,4,5-trisphosphate (**1**) in 95% isolated yield. The compound was characterized as its hexasodium salt: $[\alpha]^{20}_D$ -30°, H_2O (pH 9.5), c = 1.6 mg/mL (lit.[3] $[\alpha]^{20}_D$ -28°, H_2O (pH 9.5), c = 1.6 mg mL); FAB mass spectrum, m/e M + H = 421, M + 22 = 443; C, H anal. Both the ^1H (300 MHz) and ^{31}P NMR of **1** was identical with those recently reported by Lindon[15] and Williamson[16] for natural **1**. None of the 2,4,5-trisphosphate was detected. Ester **4l** was transformed in the same manner as described for ester **4d** to give L-*myo*-inositol 1,4,5-trisphosphate which was characterized as its free acid $[\alpha]^{20}_D$ + 35°, H_2O (pH 10.0), c = 1 mg/mL; FAB mass spectrum, m/e M + 1 = 421, M + 22 = 443; C, H anal.).

We have successfully used this phosphorylation procedure for the synthesis of (±)-*myo*-inositol 1,3,4-trisphosphate and (±)-*myo*-inositol 1,3,4,5-tetrakisphosphate. The details will be published elsewhere.

Acknowledgment. We thank W. M. Sanders and C. A. Hunt for technical assistance, J. P. Moreau for combustion analysis, Dr. Harri J. Ramjitt for mass spectral analysis, C. Homnick for HPLC data, Dr. S. M. Pitzenberger for NMR spectra, and C. A. Gerson for manuscript preparation.

(9) For the use of this reagent for resolving a similar *myo*-inositol derivative, see: Billington, D. C.; Baker, R.; Kulagowski, J. J.; Mawer, I. M. *J. Chem. Soc., Chem. Commun.* **1987**, 314-316.

(10) (a) The compound was characterized by ^1H NMR, mass spectra, and (b) combustion analysis.

(11) Angyal, S. J.; Tate, M. E. *J. Chem. Soc.* **1961**, 4122-4128. Kiely, D. E.; Abruscato, G. J.; Baburao, V. *Carbohydr. Res.* **1974**, *34*, 307-313.

(12) Beaucage, S. L.; Caruthers, M. H. *Tetrahedron Lett.* **1981**, *22*, 1859-1862. McBride, L. J.; Caruthers, M. H. *Tetrahedron Lett.* **1983**, *24*, 245-248.

(13) Khorana, H. G.; Todd, A. R. *J. Chem. Soc.* **1953**, 2257-2260.

(14) Chouinard, P. M.; Bartlett, P. A. *J. Org. Chem.* **1986**, *51*, 75-78.

(15) Lindon, J. C.; Baker, D. J.; Farrant, R. D.; Williams, J. M. *Biochem. J.* **1986**, *233*, 275-277.

(16) Cerdan, S.; Hansen, C. A.; Johanson, R.; Inubushi, T.; Williamson, J. R. *J. Biol. Chem.* **1986**, *261*, 14676-14680. We are grateful to Dr. Williamson for a preprint of his manuscript.

^{57}Fe NMR of Heme Proteins. The Chemical Shift Anisotropy of Ferrocytochrome *c*

Lars Baltzer

Department of Organic Chemistry
University of Göteborg, S-412 96 Göteborg, Sweden

Received November 19, 1986

Iron-57 has a spin $I = {}^1/_2$ and a large chemical shift range that makes it very useful for the study of heme-ligand interactions. We have recently demonstrated the correlation between the ^{57}Fe NMR chemical shift and the free energy of ligand binding in carbonyl iron porphyrin complexes.[1] Also, we and others, have shown by direct[2,3] and indirect[4] determination of the ^{57}Fe NMR chemical shift[2-4] and relaxation parameters[2,3] of myoglobin-CO (MbCO) that ^{57}Fe NMR is a powerful tool for the study of heme proteins. In this paper, we report the first ^{57}Fe NMR observation of a cytochrome, ferrocytochrome *c*, together with its chemical

(1) Baltzer, L.; Landergren, M. *J. Chem. Soc., Chem. Commun.* **1987**, 32.

(2) Baltzer, L.; Becker, E. D.; Tschudin, R. G.; Gansow, O. A. *J. Chem. Soc., Chem. Commun.* **1985**, 1040.

(3) Lee, H. C.; Gard, J. K.; Brown, T. L.; Oldfield, E. *J. Am. Chem. Soc.* **1985**, *107*, 4087.

(4) LaMar, G. N.; Dellinger, C. M.; Sarkar, S. S. *Biochem. Biophys. Res. Commun.* **1985**, *128*, 628.

0002-7863/87/1509-3479$01.50/0