precursor had a ratio of 5.45, the antibiotic should have a ratio of 7.49, if eight carbons are retained. An alternative version of the pathway could proceed via anthranilic acid. In this case, C-2 of the indole ring would be lost and only seven carbon atoms of tryptophan retained. The theoretical value for the ratio in this instance would be 8.56. The experimental value (7.96) falls between the two theoretical values, and so it does not allow one to decide whether seven or eight carbon atoms of tryptophan have been incorporated into sparsomycin. The results of expt 7 also do not prove that tryptophan has been incorporated into the antibiotic in the manner predicted by Scheme I. Nevertheless, the data clearly demonstrate that tryptophan is a specific precursor of sparsomycin. The validity of the hypothesis outlined in the scheme was proven in two ways. First, (2-^{13}C)-DL-tryptophan was synthesized by a combination of literature procedures[11] and administered to the producing organism. The results of this experiment were exceedingly gratifying, as a very strong enrichment appeared at C-8 of the antibiotic (Table I, expt 8). Second, the incorporation of (5-$^{2}H_1$)-DL-tryptophan[12] into sparsomycin was examined. The deuterium NMR spectrum of the antibiotic isolated in this experiment exhibited a high deuterium enrichment at 7.17 ppm demonstrating that C-5 of sparsomycin is derived from C-5 of tryptophan (Table I, expt 9).[13] We can therefore conclude that the uracil moiety of sparsomycin is biosynthesized from tryptophan in the unprecedented manner shown in Scheme I.

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Registry No. 1, 1404-64-4; Met, 63-68-3; Cys, 52-90-4; *S*-methyl-Cys, 1187-84-4; Trp, 73-22-3.

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(13) The resonance position of the corresponding proton in sparsomycin is at 7.11 ppm.

Synthesis of Inositol Phosphates

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The discovery that D-*myo*-inositol 1,4,5-tris(phosphate) (1,4,5-IP_3, **3b**) is involved in signal transduction in animal cells has led to intense interest in the biosynthesis, function, and metabolism of inositol phosphates.[1] To date, at least 15 inositol phosphates have been isolated from natural sources, including another putative "second messenger", D-*myo*-inositol-1,3,4,5-tetrakis(phosphate) (1,3,4,5-IP_4, **6**).[2] Research on the biological function of these compounds would be greatly aided by the availability of adequate amounts of synthetic inositol phosphates which are isomerically pure and/or radioactively labeled. Although a number of inositol poly(phosphates)[3-6] have been prepared by total synthesis, access to this family of compounds has generally been limited by difficulties in phosphorylation[7] and cumbersome routes to protected precursors. Phosphorylation of inositol derivatives is complicated by three well-known problems: (a) the relative unreactivity of conventional phosphorus(V) reagents, such as diphenylphosphorochloridate, toward the secondary alcohols of inositol;[3a,b,4a] (b) the tendency of the resulting phosphate triester intermediates to form cyclic phosphates;[6,8] and (c) the propensity of phosphate monoester groups to migrate to neighboring hydroxyl groups under acidic conditions via similar cyclic intermediates.[4a,9] Since chlorophosphites are remarkably more reactive than the corresponding phosphorus(V) phosphorylating agents,[10] we hoped that use of phosphitylating agents would alleviate the reactivity problem. We hypothesized that phosphorus(III) intermediates resulting from phosphitylation would also not suffer from cyclization or migration problems. Although a few reports of preparation of phosphate monoesters via phosphitylation[3c,d,11,12] have appeared recently, the full power of this methodology is not illustrated by these papers. We describe herein[13] (a) the use of the simple reagent dimethyl chlorophosphite[14] for the preparation of phosphate monoesters from alcohols, (b) the first successful bisphosphorylation of a cis vicinal diol, (c) a short synthesis of 1,4,5-IP_3 which uses a regioselective "partial" phosphoitylation as the key step, and (d) the first syntheses of 1,3,4,5-IP_4 and another naturally occurring inositol poly(phosphate), 1,4,5,6-IP_4 (**9**).[15,16]

D,L-1,4-Dibenzoyl-*myo*-inositol (**1**), which has both cis and trans vicinal diols, was selected as a convenient,[17] but challenging, model substrate for evaluation of our phosphitylation strategy. Treatment of tetraol **1** with excess dimethoxychlorophosphite and diisopropylethylamine followed by oxidation with hydrogen peroxide gave exclusively tetrakis(dimethyl phosphate) **2a**. This material was conveniently isolated in 73% yield by direct crystallization from the reaction mixture. Removal of the phosphate methyl groups was cleanly effected by exposure to bromotrimethylsilane[19] or hydrogen bromide in acetic acid. The resulting tetrakis(dihydrogen phosphate) was isolated by removing the volatile byproducts and precipitating the lithium salt from water at pH 10 with ethanol. Subsequent hydrolysis of the benzoates with lithium hydroxide gave the octalithium salt of 1,2,4,5-IP_4 (**3a**) in addition to about 10% of a mixture of inositol tris(phosphates)[20] which were

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(6) IP_5: Angyal, S. J.; Russell, A. F. *Aust. J. Chem.* **1969**, *22*, 391.

(7) For a general review of phosphorylation methodology, see: Slotin, L. A. *Synthesis* **1977**, 737.

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(9) Brown, D. M.; Hall, G. E.; Letters, R. *J. Chem. Soc.* **1959**, 3547.

(10) Letsinger, R. L.; Lunsford, W. B. *J. Am. Chem. Soc.* **1976**, *98*, 3655.

(11) (a) Bannwarth, W.; Trzeciak, A. *Helv. Chim. Acta* **1987**, *70*, 175. (b) Perich, J. W.; Johns, R. B. *Tetrahedron Lett.* **1987**, *28*, 101.

(12) Oligonucleotide 5'-phosphates: (a) Uhlmann, E.; Engels, J. *Tetrahedron Lett.* **1986**, *27*, 1023. (b) Horn, T.; Urdea, M. S. *Tetrahedron Lett.* **1986**, *27*, 4705.

(13) Syntheses of **3a** and **3b** were presented at the International Neurochemistry Society Meeting in LaGuaira, Venezuela, June 1987.

(14) Mazour, Z. U.S. Patent 4079103, **1978**.

(15) Mayr, G. W.; Dietrich, W. *FEBS Lett.* **1987**, *213*, 278.

(16) All compounds reported herein are racemic. IUPAC nomenclature rules number inositol derivatives beginning with the 3 syn hydroxyls according to the usual priority rules. See **1** and **2a**.

(17) Prepared in two steps from *myo*-inositol (21% yield) via TFA hydrolysis of 1,2:4,5-di-*O*-isopropylidene-3,6-di-*O*-benzoyl *myo*-inositol.[18]

(18) Gigg, J.; Gigg, R.; Payne, S.; Conant, R. *Carbohydr. Res.* **1985**, *142*, 132.

(19) (a) Chojnowski, J.; Cypryk, M.; Michalski, J. *Synthesis* **1978**, 777 and references therein. (b) Bartlett, P. A.; McQuaid, L. A. *J. Am. Chem. Soc.* **1984**, *106*, 7854.

(20) Since phosphate monoesters in general and product **3a** in particular are stable to basic hydrolysis, this unexpected phosphate hydrolysis may involve neighboring group participation by an intermediate arising during benzoate hydrolysis.

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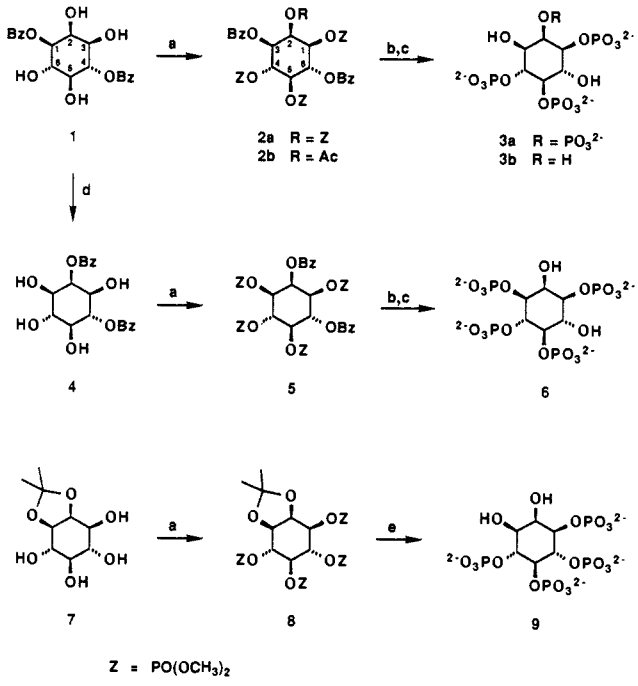
Scheme I[a]



[a] (a) excess $(CH_3O)_2PCl$, *i*-Pr_2NEt/DMF or CH_2Cl_2, 25 °C; then excess H_2O_2; except **1** → **2b**: 3.3 equiv of $(CH_3O)_2PCl$, 6 equiv of *i*-Pr_2NEt/DMF, −40 °C, then 2 equiv of AcCl, 0.13 equiv of DMAP, 25 °C, 30 min; then 10 equiv of H_2O_2, 25 °C; (b) 30% HBr/HOAc, 60 °C, 30 min; (c) LiOH or KOH, 60–80 °C, 1 h; (d) 6:4 pyridine–H_2O, 100 °C, 1 h; (e) 9 equiv of TMSBr, 25 °C, 30 min; then H_2O.

removed by ion exchange chromatography. This model study suggested that the above phosphorylation method has the potential to cleanly and efficiently convert all of the free hydroxyl groups in any appropriately protected inositol to phosphate monoesters.

Since the axial 2-hydroxyl group of *myo*-inositol derivatives is generally less reactive than equatorial ones,[21] preparation of **3b** by means of partial phosphitylation was investigated. When **1** was treated sequentially with (a) 3.3 equiv of dimethyl chlorophosphite and diisopropylethylamine at −40 °C, (b) acetyl chloride and dimethylaminopyridine,[22] and (c) hydrogen peroxide, the crude reaction mixture was shown by HPLC and ^{31}P NMR to contain the desired tris(dimethyl phosphate) (94%) along with about 4% of tetrakis(dimethyl phosphate) **2a** and approximately 2% of an unknown tris(dimethyl phosphate). No other products were detected. The 2-hydrogen of the major product showed no ^1H–^{31}P coupling, proving that the axial alcohol was the one which had not been phosphorylated. Demethylation of **2b** with hydrogen bromide in acetic acid and ester hydrolysis gave 1,4,5-IP_3 (**3b**) contaminated with about 5% bis(phosphates) in 88% yield.

Since the hydroxyl groups of *myo*-inositol have generally been differentiated by means of cyclic ketals, an appropriately protected precursor for preparation of 1,3,4,5-IP_4 (**6**) has not been previously reported. Such a precursor, 2,4-dibenzoate **4**, can be conveniently prepared in modest yield by base-catalyzed isomerization of the readily available 1,4-dibenzoates.[23] When heated in refluxing aqueous pyridine, 1,4-dibenzoate **1** was converted with negligible hydrolysis to a mixture of **1**, 2,4-dibenzoate **4**, 1,6-dibenzoyl-*myo*-inositol, and an unidentified dibenzoate. The desired dibenzoate **4** was most easily purified by fractional crystallization of the mixture produced when this isomerization was carried to less than 50% conversion.[24] As shown in Scheme I, 1,3,4,5-IP_4 (**6**) was then prepared from dibenzoate **4** by the same series of phosphitylation, oxidation, and deprotection steps used to make 1,2,4,5-IP_4. A third naturally occurring inositol tetraphosphate, 1,4,5,6-IP_4 (**9**), was synthesized in a similar manner from 1,2-*O*-isopropylidene-*myo*-inositol (**7**).[18] In this case, however, demethylation with hydrogen bromide in acetic acid caused the cyclic ketal of tetrakis(dimethyl phosphate) **8** to be removed prematurely and some phosphate migration occurred. In contrast, when **8** was deprotected with bromotrimethylsilane, the cyclic ketal was retained until the reaction mixture was quenched with water. The ketal of the resulting tetrakis(dihydrogen phosphate) then underwent self-catalyzed hydrolysis, and pure 1,4,5,6-IP_4 (**9**) was isolated as the free acid by removal of the volatile byproducts.

In each of these syntheses, the general strategy was to avoid phosphate triester intermediates with free hydroxyl groups since these species are prone to undergo cyclization and migration. Phosphitylating agents, especially the previously unappreciated reagent dimethyl chlorophosphite, are convenient and versatile reagents for bypassing such intermediates. The validity of this approach was proven when careful ^{31}P NMR, ^1H NMR, and ion exchange HPLC[25] analysis of the final inositol poly(phosphates) detected no isomeric impurities. This methodology should be useful for the preparation of phosphate monoesters of other complex carbohydrates, nucleotides, and peptides.

Note Added in Proof. The synthesis of **6** has now been reported by deSolms, et al. (deSolms, S. J.; Vacca, J. P.; Huff, J. R. *Tetrahedron Lett.* **1987**, *28*, 4503), Ozaki et al. (Ozaki, S.; Kondo, Y.; Nakahira, H.; Yamaoka, S.; Watanabe, Y. *Tetrahedron Lett.* **1987**, *28*, 4691), and Billington and Baker (Billington, D. C.; Baker, R. *J. Chem. Soc., Chem. Commun.* **1987**, 1011).

Supplementary Material Available: Experimental procedures for the preparation and characterization of compounds **1–9** (7 pages). Ordering information is given on any current masthead page.

(21) (a) For a review, see: Shvets, V. I. *Russ. Chem. Rev.* **1974**, *43*, 488. (b) The regioselectivity reported for P(V) phosphorylation of a simpler system was very low: Krylova, V. N.; Lyutik, A. I.; Gornaeva, N. P.; Shvets, V. I. *J. Gen. Chem. USSR* (*Engl. Trans.*) **1981**, *51*, 183; *Zh. Obschch. Khim.* **1979**, *51*, 210.

(22) If this protection step was omitted, product **3b** was contaminated with at least one additional isomeric inositol phosphate indicating that phosphate migration had taken place.

(23) Base-catalyzed isomerization of inositol acetates, however, occurs with little selectivity: Angyal, S. J.; Melrose, G. J. H. *J. Chem. Soc.* **1965**, 6494.

(24) The unreacted starting dibenzoate (and presumably the other dibenzoates) can be recycled.

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An Exceptionally Simple Method of Preparing Matrix Isolated Biradicals, Biradicaloids, and Carbenes

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Biradicals, biradicaloids, and carbenes are common reactive intermediates in organic[2] thermal and photochemical transformations.[3] A common objective in the study of these species is their direct detection and characterization by spectroscopic methods. Recent years have witnessed ever increasingly so-

(1) Camille and Henry Dreyfuss Teacher Scholar.

(2) See: Gajewski, J. J. *Hydrocarbon Thermal Isomerizations*; Academic Press: New York, NY, 1981.

(3) See: *Tetrahedron* **1982**, *38*, 735–867.

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