SYNTHESIS OF PHOSPHONIC ACID AND PHOSPHATE ANALOGUES OF NATURAL PHOSPHATES

by

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A dissertation submitted to the Graduate Faculty in Chemistry in partial fulfillment of the requirements for the degree of Doctor of Philosophy, The City University of New York.

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ABSTRACT

SYNTHESIS OF PHOSPHONIC ACID AND PHOSPHATE ANALOGUES

OF NATURAL PHOSPHATES

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A convenient route is described for the preparation of the isosteric phosphonic acid analogue of glycerol-3-phosphate, 3,4-dihydroxybutyl-1-phosphonic acid, in both enantiomeric forms and the racemic modifications, starting with commercially readily available materials. The (S)-enantiomer, that of absolute configuration corresponding to that of sn-glycerol-3-phosphate, has been found to be a growth inhibitor of several bacteria at low concentration. The synthetic route described is of particular value as it facilitates the preparation of a series of phosphonic acids and phosphates of related structure, in their enantiomeric forms, which are also of interest for metabolic regulation. These include the 1-methyl-3,4-dihydroxybutyl-1-phosphonic acid, (1RS,3S)-1,3,4-trihydroxybutyl-1-phosphonic acid, (R)-2,4-dihydroxybutylphosphoric acid, 3,4-epoxybutyl-1-phosphonate, and (S)-3,4-dihydroxybutylphosphoric acid with the related homodiglyceride and phosphatidic acid analogues. A method is also described for the synthesis of carbon-14 labelled 3-carboxy-3-hydroxybutyl-1-phosphonic acid, an analogue of phosphoglyceric acid known to serve as a substitute for the natural material in several biochemical process.

ACKNOWLEDGEMENTS

I sincerely thank Professor Robert Engel who helped me to develop my knowledge of phosphorus chemistry and my English. His patience, understanding and unwavering confidence in me made this thesis a reality.

I am grateful to the people whom I have met during this program of study and appreciate their help, especially the helpful suggestions in research from Professor Burton Tropp and Professor William Berkowitz and technical help from Mr. Randy Smith and Mr. Robert Wurman.

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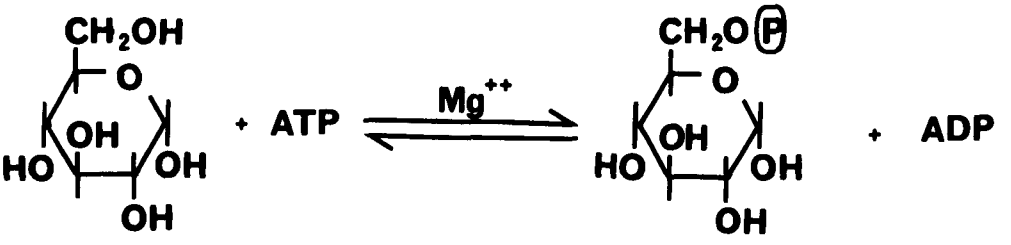
INTRODUCTION

The glycolytic pathway involves the conversion of a six-carbon carbohydrate to two three-carbon derivatives. This thesis is concerned with the synthesis of analogues of some of these three-carbon products as well as the closely related glycerol-3-phosphate which is at a branch point between glycolysis and phospholipid metabolism.

Earlier, in 1930's, Harden and Young demonstrated that the cell-free extracts of yeast or muscle could be separated by dialysis into a heat-labile fractions, the enzymes, and a heat-stable fraction, consisting of nicotinamide adenine dinucleotide (NAD), adenosine diphosphate (ADP), and adenosine triphosphate (ATP). Inorganic phosphate is necessary for glycolysis to proceed. A hexose diphosphate [i.e. fructose-1.6-diphosphate (3)] is an intermediate in the sequence and studies with the enzyme inhibitor, iodoacetate, caused an accumulation of this material (3) as well as two triose phosphates. Addition of fluoride to a crude extract leads to the accumulation of 3-phosphoglyceric acid and 2-phosphoglyceric acid. The elucidation of the details of the glycolytic pathway is a classic example of the use of inhibitors to determine metabolic sequences. The specifics of the inhibitory action is discussed later.

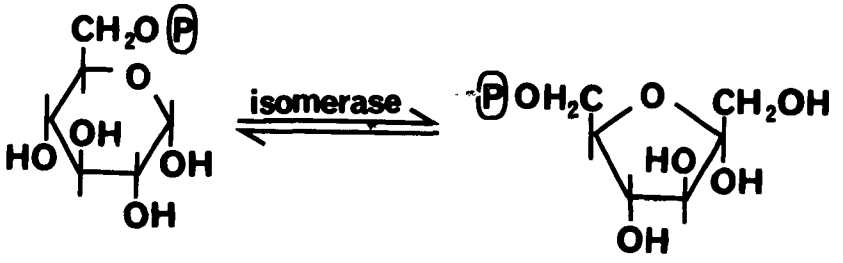
The first step in the fermentation of glucose is phosphorylation by ATP yielding glucose-6-phosphate (1) and ADP. Also needed is Mg^{+2} as a cofactor, a common situation for reactions involving ATP which is believed to react as its magnesium salt.



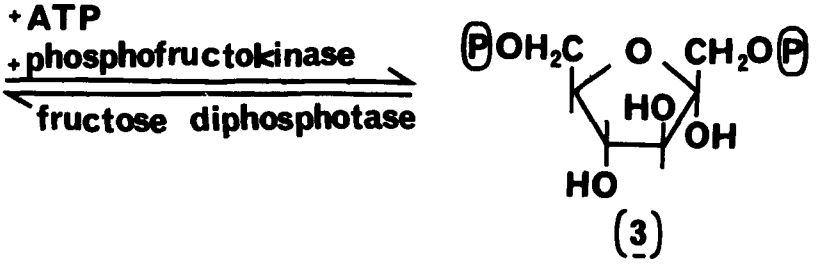
(1)

The enzyme commonly involved in this reaction is hexokinase, which is very widely distributed in nature; another enzyme, glucokinase (which has different kinetic properties) is found in a few tissues (especially liver) and catalyzes the same reaction. Compound (1), like most phosphate esters, does not pass readily across cell membranes. Animals have solved this problem by developing another enzyme, glucose-6-phosphatase, which in liver catalyzes the hydrolysis of the phosphate ester.

In the second step glucose-6-phosphate (1) is converted to its isomer, fructose-6-phosphate (2) in a reaction catalyzed by hexose phosphate isomerase. A second molecule of ATP is now used in further phosphorylation of (2) to give fructose-1,6-diphosphate (3) involving the enzyme phosphofructokinase. A highly negative free energy of reaction is involved here which is helpful in driving the entire reaction sequence to completion.



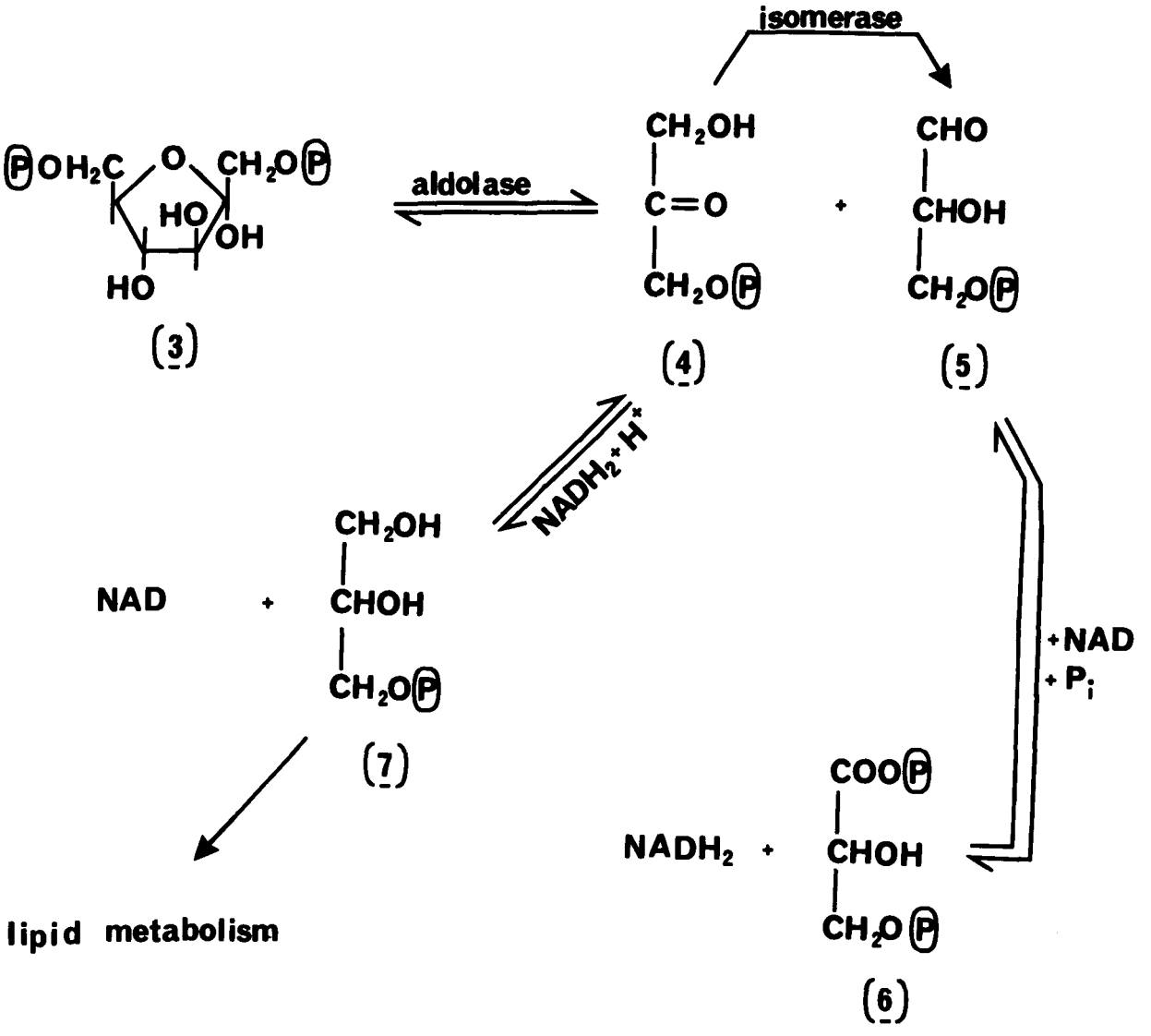
(1) (2)



(3)

The compound (3) thus generated can be split by the enzyme aldolase to yield two triose phosphate molecules, namely dihydroxyacetone phosphate (4) and glyceraldehyde-3-phosphate (5). Compound (4) is not further metabolized in the direct glycolysis pathway, but it can be converted to (5) in the presence of triose phosphate isomerase and oxidized immediately to glyceric acid-1,3-diphosphate (6) in the presence of NAD, triose phosphate dehydrogenase and a source of inorganic phosphate (P_i). The subsequent conversion of (4) to the isomeric glyceraldehyde-3-phosphate (5) forces the first cleavage reaction to completion.

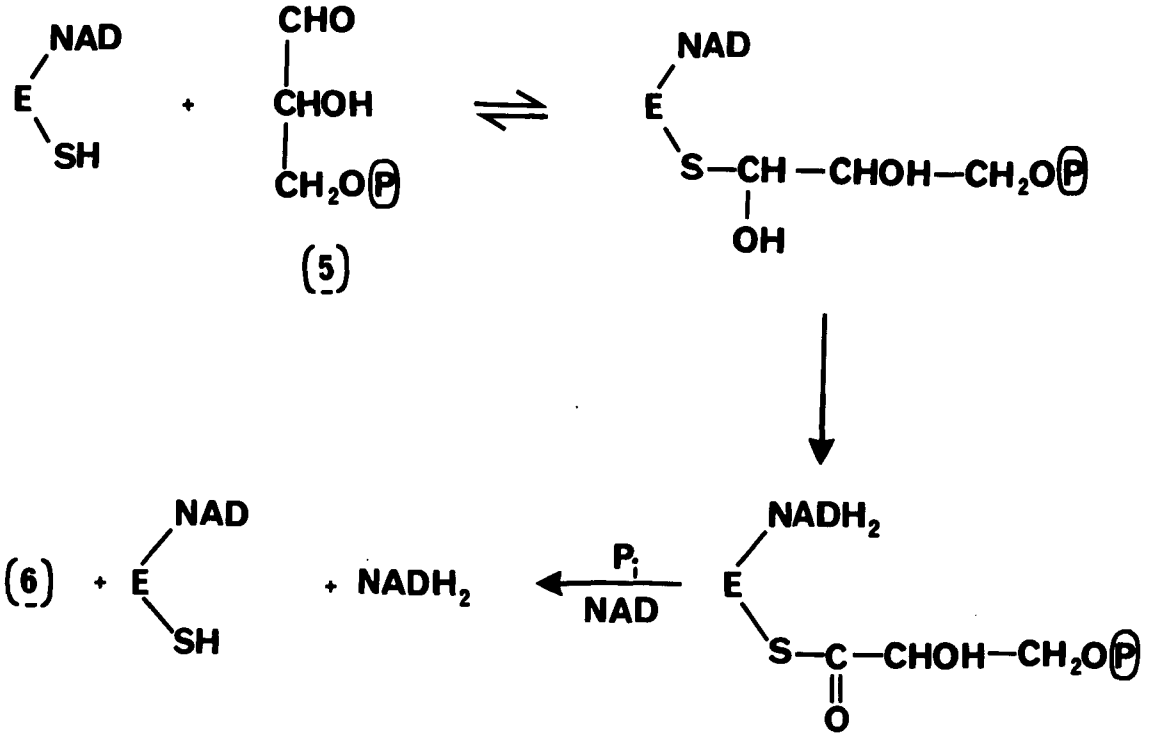
Compound (4) has available an alternative metabolic fate, one which is essential to lipid metabolism. This alternative results in the formation of glycerol-3-phosphate (7).



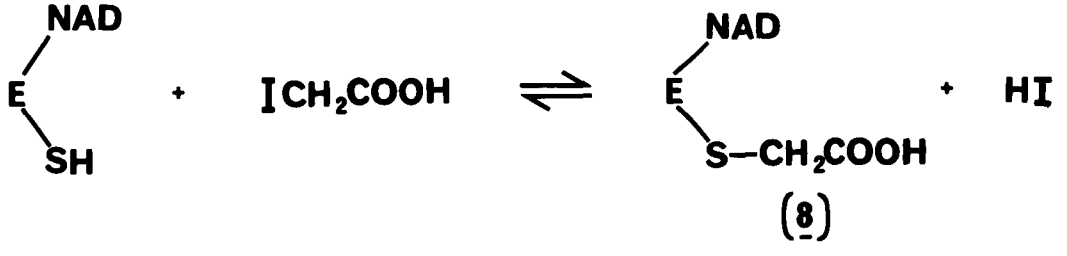
This reaction involves a substrate-level phosphorylation forming a high-energy compound in a process in which the respiratory chain is not involved. (The mechanism of this reaction has been studied in great detail in the hope that its elucidation would solve the problem of how the high energy phosphate compound ATP is formed during oxidative

phosphorylation. The hope has proven to be vain although the mechanism is of great interest.)

The detailed mechanism of the transformation of (5) to (6) involves triose phosphate dehydrogenase, the active site of which contains a sulfydryl group, to which a molecule of glyceraldehyde-3-phosphate (5) binds covalently. Nearby a molecule of NAD is also bound. Thus the reaction proceeds as follows and involves the subsequent hydrogen transfer steps.

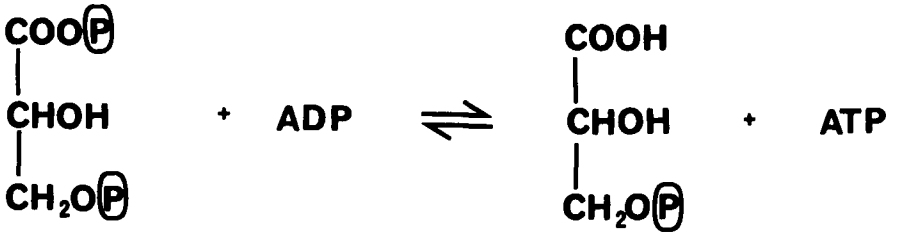


However, in the presence of iodoacetate, inhibition occurs which can be explained by the reaction shown below.



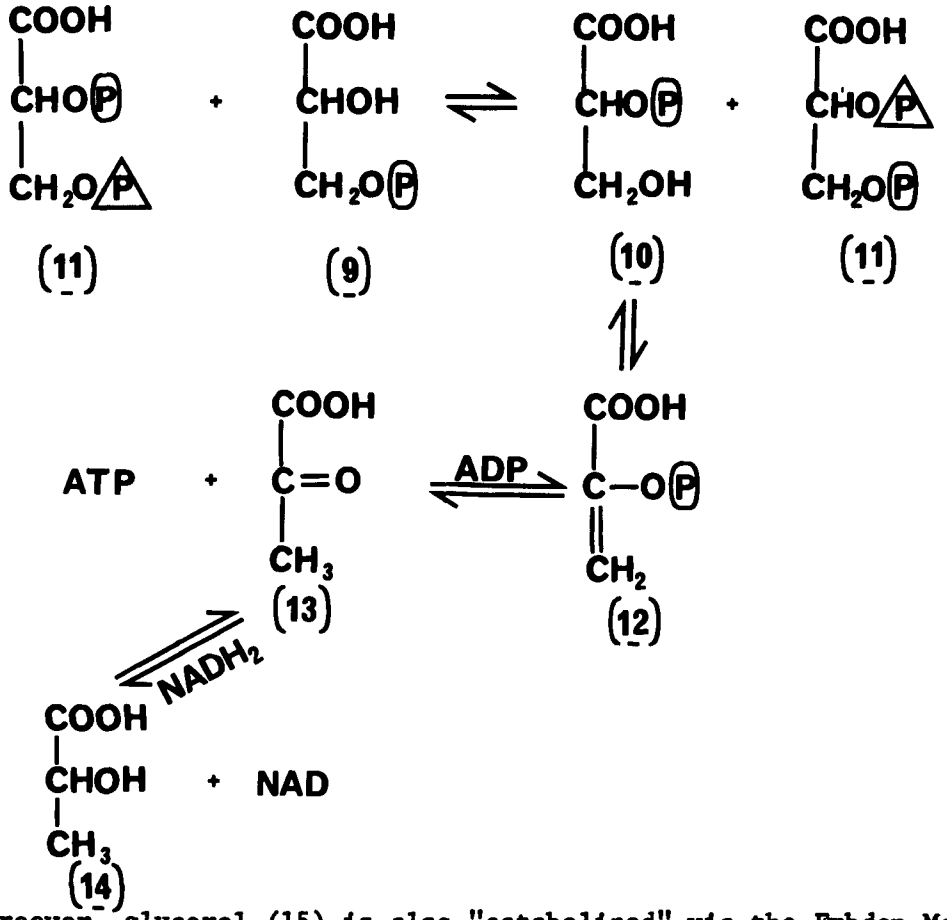
The complex (8) thus generated can not be oxidized by NAD. The consequent inhibition of the enzyme causes an accumulation of fructose-1,6-diphosphate (3) and the triose phosphates (4) and (5).

In the presence of phosphoglycerate kinase, compound (6) can transfer the phosphate group from C-1 to ADP and regenerate ATP yielding 3-phosphoglyceric acid (9).

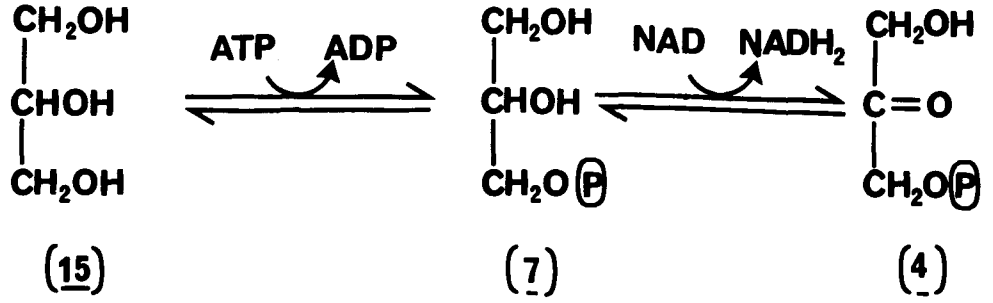


(6) (9)

3-Phosphoglyceric acid can be converted to 2-phosphoglyceric acid (10), a process catalyzed by phosphoglyceromutase requiring 2,3-diphosphoglyceric acid (11), as a cofactor. Compound (10), generated in this way, enolizes to phosphoenol pyruvic acid (12) in the presence of enolase and Mg^{+2} as cofactor. Subsequently, (12) can be converted to pyruvic acid (13) and lactic acid (14) as shown below.



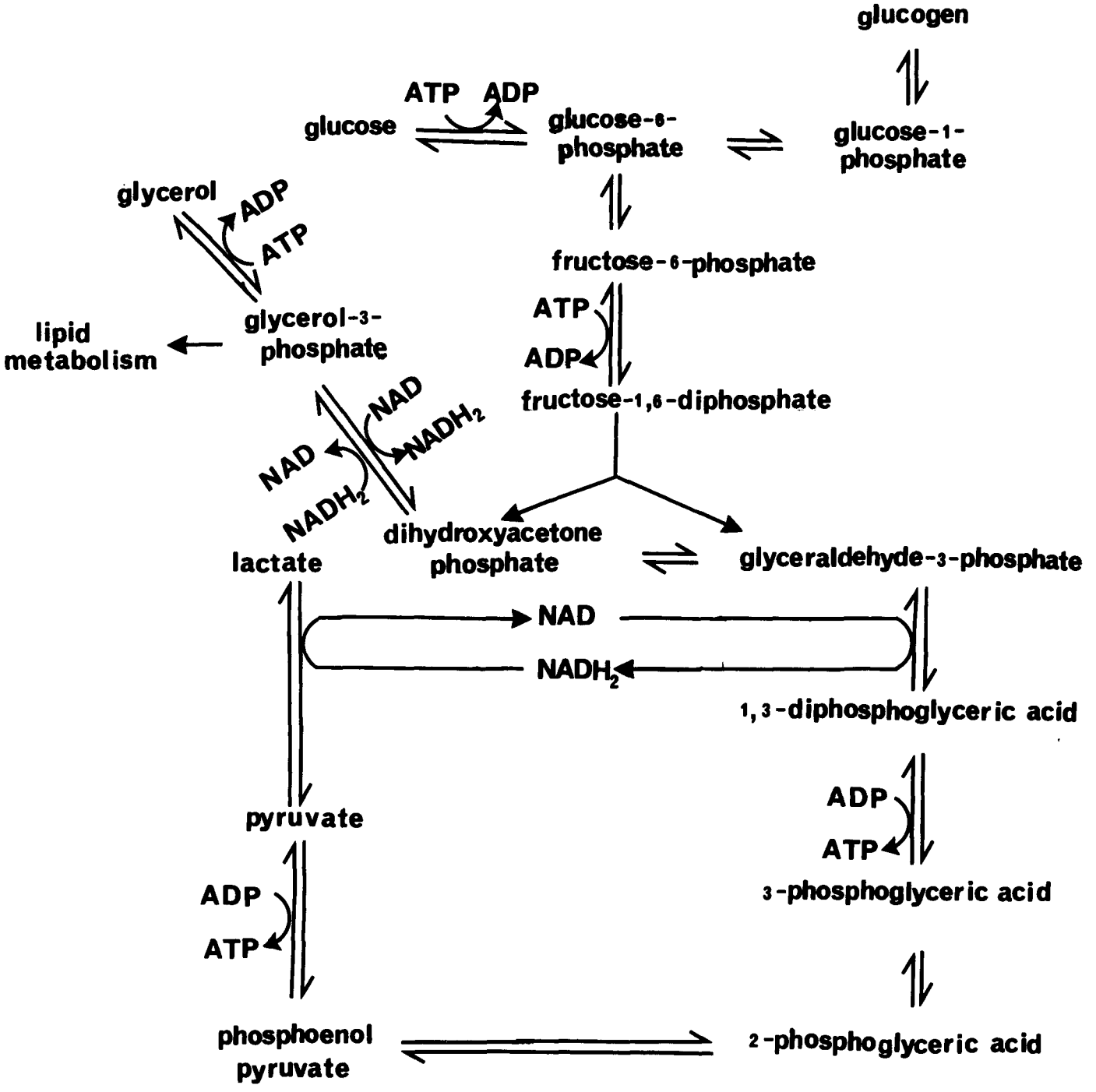
Moreover, glycerol (15) is also "catabolized" via the Embden Meyerhof pathway after conversion to dihydroxyacetone phosphate (4). This conversion involves reaction with ATP (catalyzed by glycerokinase) to form sn-glycerol-3-phosphate (7) which then is oxidized with glycerophosphate dehydrogenase.



It should be noted that in the breakdown of glycerol another molecule of NAD is reduced. In addition to the previously mentioned reduction reaction, there is a surplus of reducing power beyond that which can be disposed of in the other reactions in the pathway, and thus glycerol can not be metabolized in the complete absence of another electron acceptor.

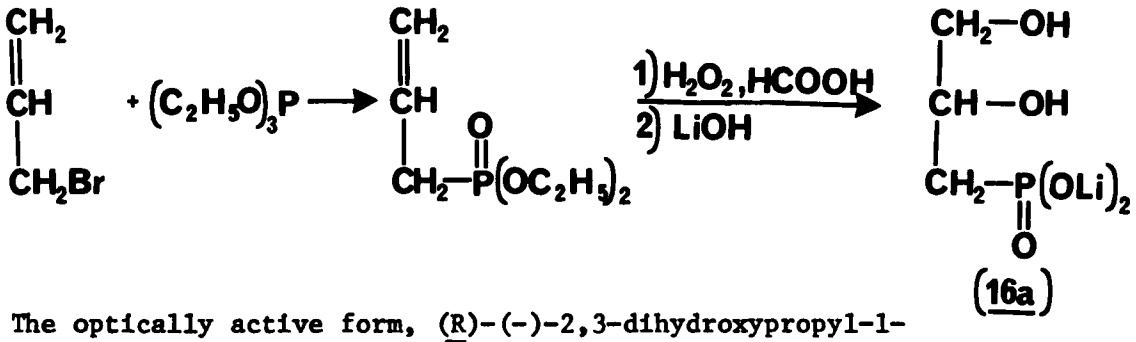
The reactions mentioned above can be summerized as shown in Scheme I. This illustrates the point that sn-glycerol-3-phosphate is on the branch point between phosphoglyceride(lipid) and carbohydrate metabolism. If one applied analogues of glycerol-3-phosphate and related compounds inattempts to regulate the metabolism of an organism, one would have reason to expect either pathway to be influenced.

A multitude of reports have shown regard for the possibilities for use of phosphonic acids as metabolic regulators. Analogues, both isosteric and nonisosteric for all simple phosphorous-containing products of carbohydrate degradation have been synthesized and investigated. The applicability of the term "isosteric" to analogues and natural compounds discussed herein is reasonable as they are almost identical in size and shape. This has been demonstrated by the use of available crystallographic data illustrating that the distances between the phosphoryl oxygen and some other position for the natural compound in comparison to its nominally isosteric phosphonic acid analogue varies by about 0.8%; suitable data has been obtained for the related compounds 2-aminoethylphosphate [1,2] and 2-aminoethylphosphonic acid[3] as well as for other simple phosphate esters[4,5,6].


glucogen
ATP ADP
glucose
glucose-6-phosphate
glucose-1-phosphate
glycerol
ADP
ATP
fructose-6-phosphate
ATP
ADP
lipid metabolism
glycerol-3-phosphate
fructose-1,6-diphosphate
NAD
NADH2
NAD
NADH2
lactate
dihydroxyacetone phosphate
glyceraldehyde-3-phosphate
NAD
NADH2
1,3-diphosphoglyceric acid
ADP
ATP
pyruvate
3-phosphoglyceric acid
ADP
ATP
phosphoenol pyruvate
2-phosphoglyceric acid

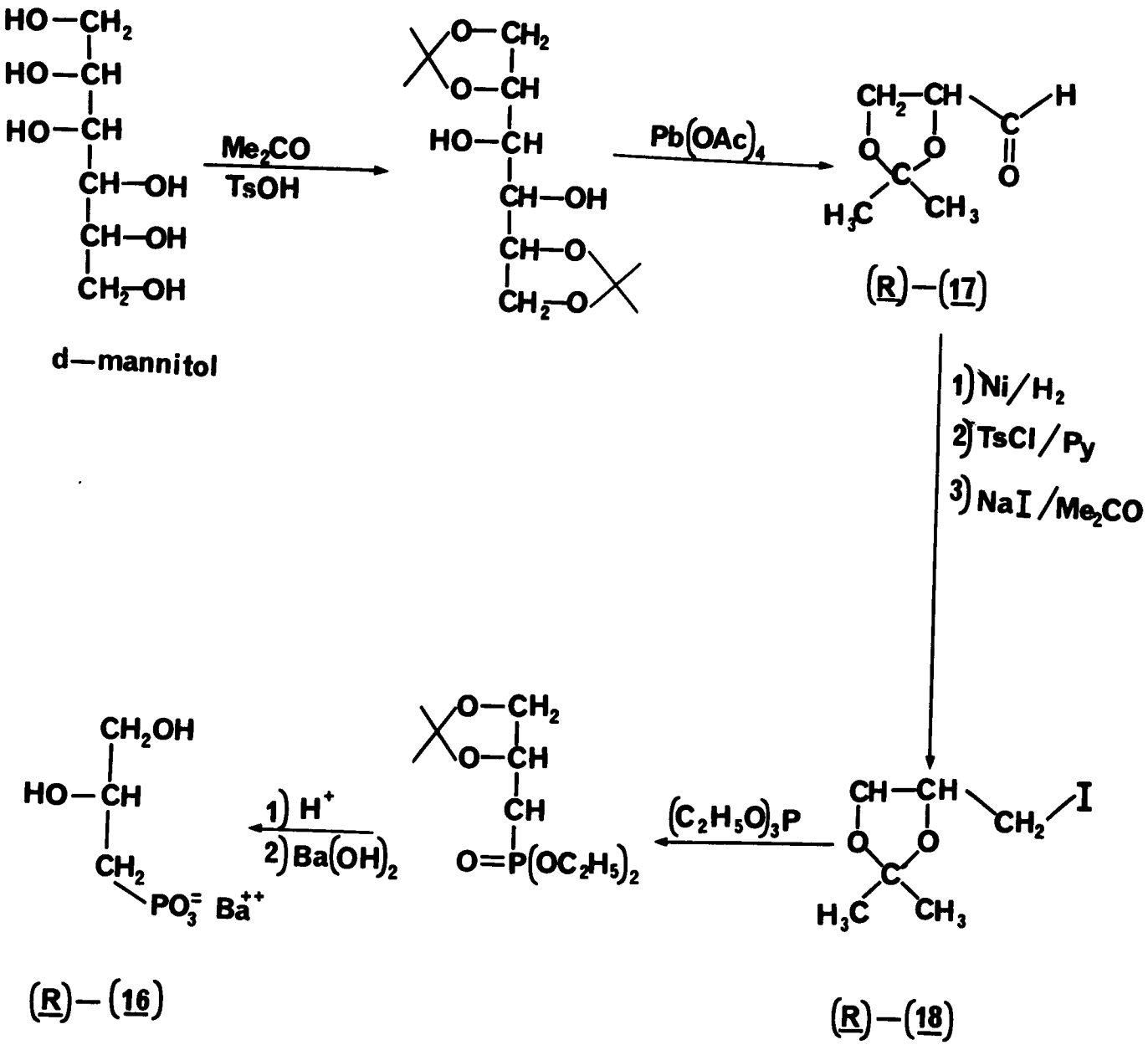
SCHEME I

Rosenthal and Geyer[7] first synthesized 2,3-dihydroxypropyl-1-phosphonic acid (16) as a nonisosteric analogue of glycerol-3-phosphate by an Arbuzov reaction on allyl bromide followed by hydroxylation and ester hydrolysis. It should be noted that the dilithium salts of this and subsequently synthesized phosphonic acids are particularly convenient forms for isolation and purification due to their particular solubility properties.



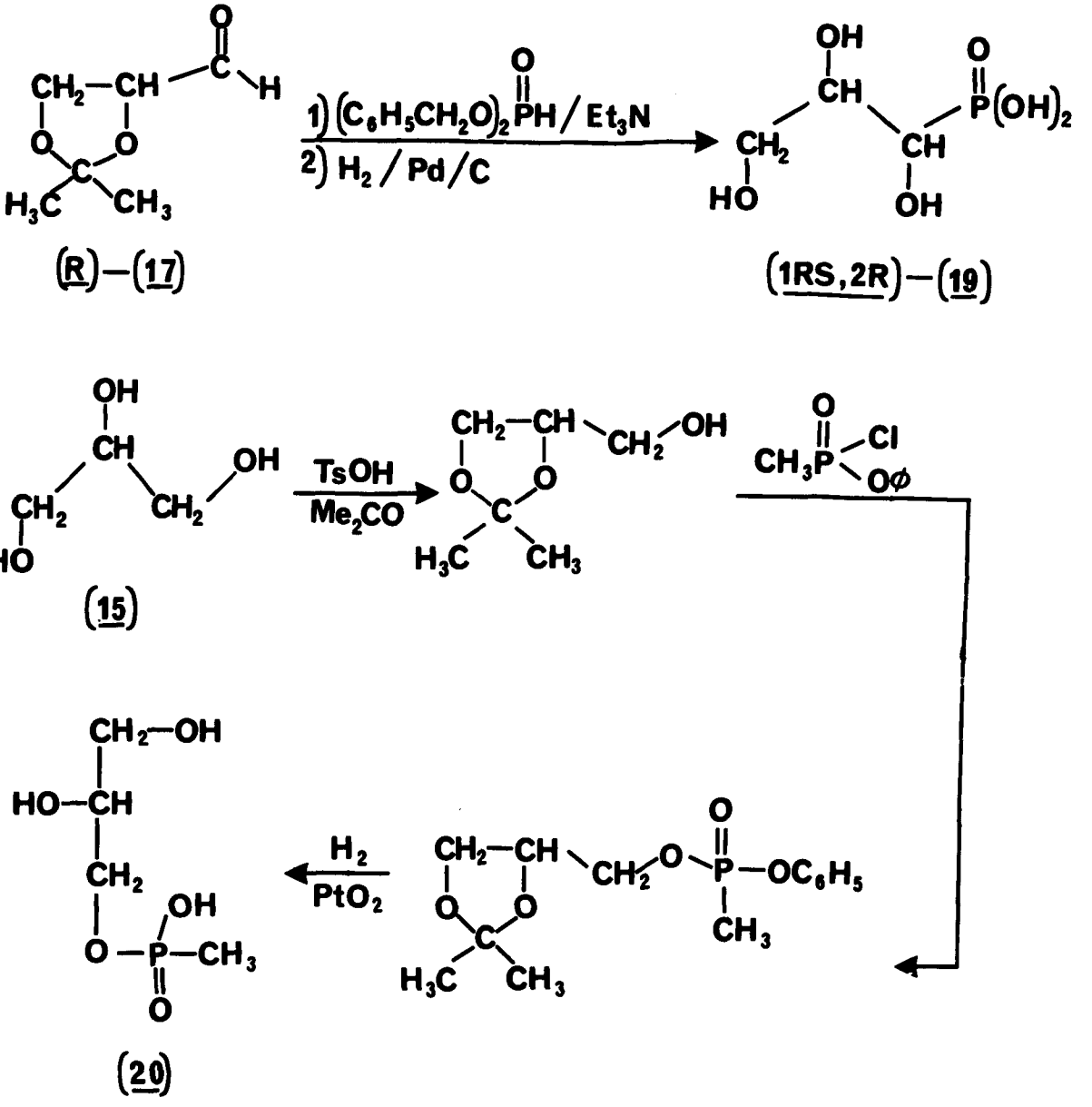
(16a)

The optically active form, (R)-(-)-2,3-dihydroxypropyl-1-phosphonic acid [(R)-(16)], bearing the same absolute configuration as the natural sn-glycerol-3-phosphate (7) about the internal hydroxyl was later reported by Baer and Basu[8]. This route began with D-mannitol diacetonide and involved an Arbuzov reaction on the iodide derived after cleavage. (Scheme II)


d—mannitol
Me2CO
TsOH
Pb(OAc)4
(R)—(17)
1) Ni/H2
2) TsCl/Py
3) NaI/Me2CO
(R)—(18)
(C2H5O)3P
1) H+
2) Ba(OH)2
(R)—(16)

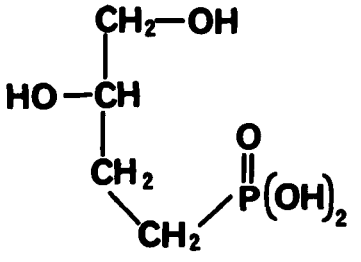
SCHEME II

Two other non-isosteric analogues of glycerol-3-phosphate have also been reported[9], these being 1,2,3-trihydroxypropyl-1-phosphonic acid (19) and the phosphonate (20) which can be derived from compound (17) and (15) respectively. (Scheme III)



SCHEME III

Because of the relatively major structural changes (relative to the natural material) in a small molecule, it might be expected that none of these would substitute well for glycerol-3-phosphate (7); in fact, Baer et al.[10] concluded that the ester oxygen was necessary for activity. However, the racemic isosteric analogue, 3,4-dihydroxybutyl-1-phosphonic acid (21), reported by Kabak et. al.[11] utilizing a route paralleling that of Rosenthal and Geyer[7] beginning with 4-bromo-1-butene, was used by Kabak et al.[11] and Shopsis et. al.[12-14] to demonstrate that it was capable of inhibiting the growth of mutant strains of E. coli at rather low concentration.

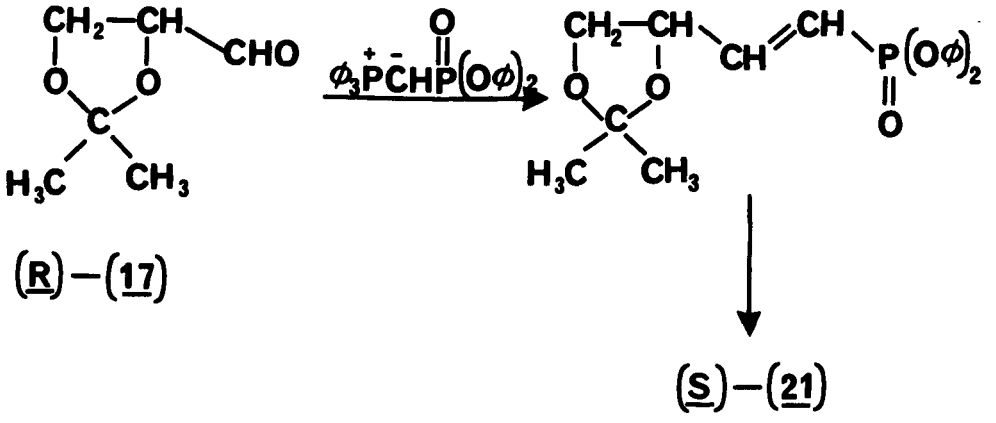


(21)

The bacteriostatic (growth inhibited without cell rupture) effect was accompanied by perturbation of normal phospholipid metabolism[15-17] resulting from an inability of the organism to cleave the phosphonate linkage now present in place of an ordinary phosphate. The organism is capable of being "outwitted" by (21) but not (16); that is, the analogue substitutes for glycerol-3-phosphate for a portion of the normal metabolic process but a point of inhibition is reached as a result of the inability to release phosphate.

Contrary to Baer's conclusion, the ester oxygen is not necessary for activity, but correspondence of size with the natural substrate is.

This result was also confirmed by Adams et al.[9] with the synthesis of the optically active form of (21) by a route beginning with phosphonylation by a Wittig reaction on the aldehyde followed by hydrogenation.

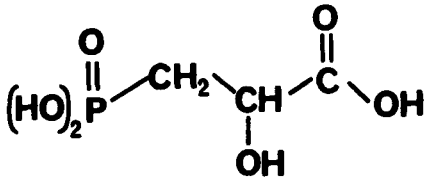


(R)–(17)

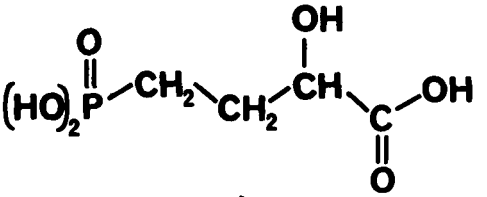
(S)–(21)

Paulsen and Bartsch[18] reported a similar sequence for the synthesis of the racemate of (21) starting with a Horner reaction using tetraethyl methylenediphosphonate on racemic (17). Phosphonate ester cleavage was accomplished using trimethylchlorosilane.

There are four syntheses of analogues of 3-phosphoglyceric acid which have been reported. Pfeiffer, et al.[19] described both the non-isosteric and isosteric analogues, (22) and (23). The syntheses

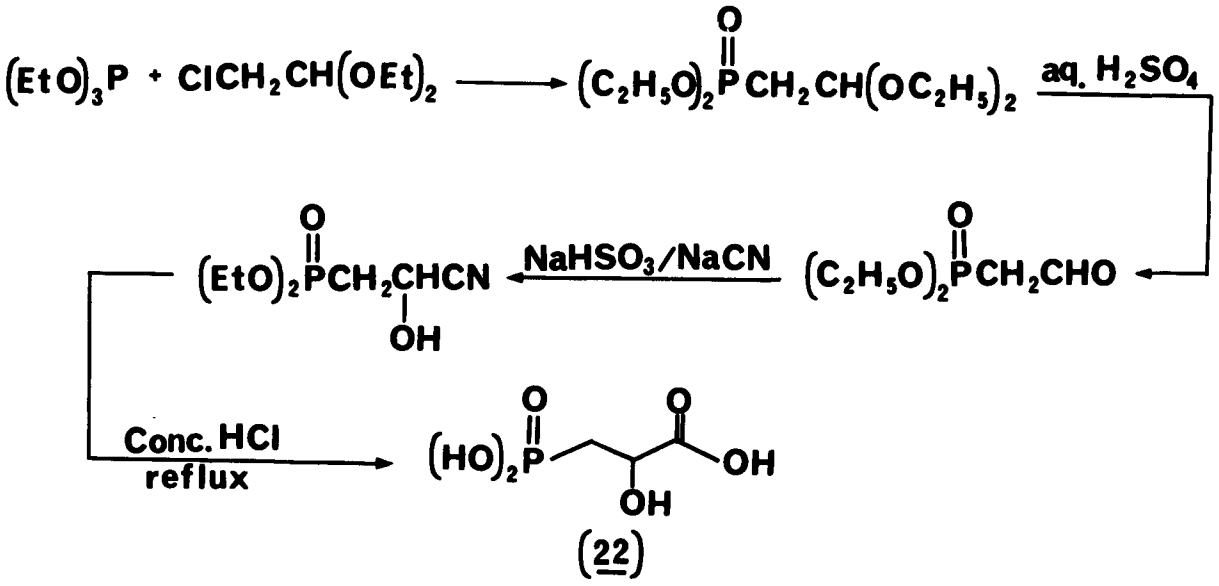


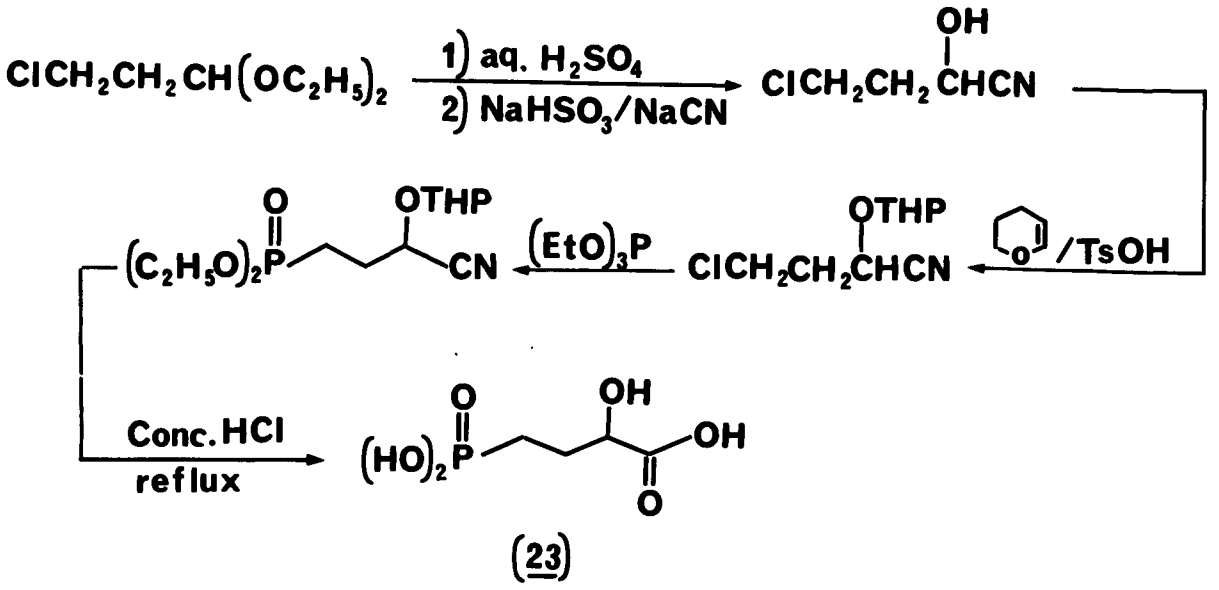
(22)



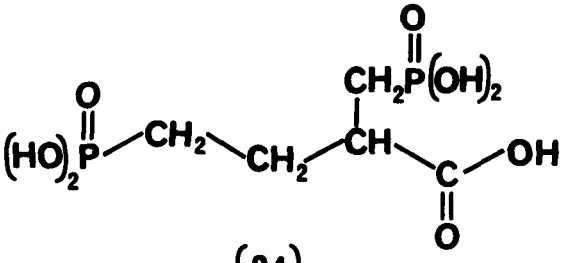
(23)

involve C-P bond formation by Arbuzov reactions followed by hydrolysis of the cyanohydrins as follows:



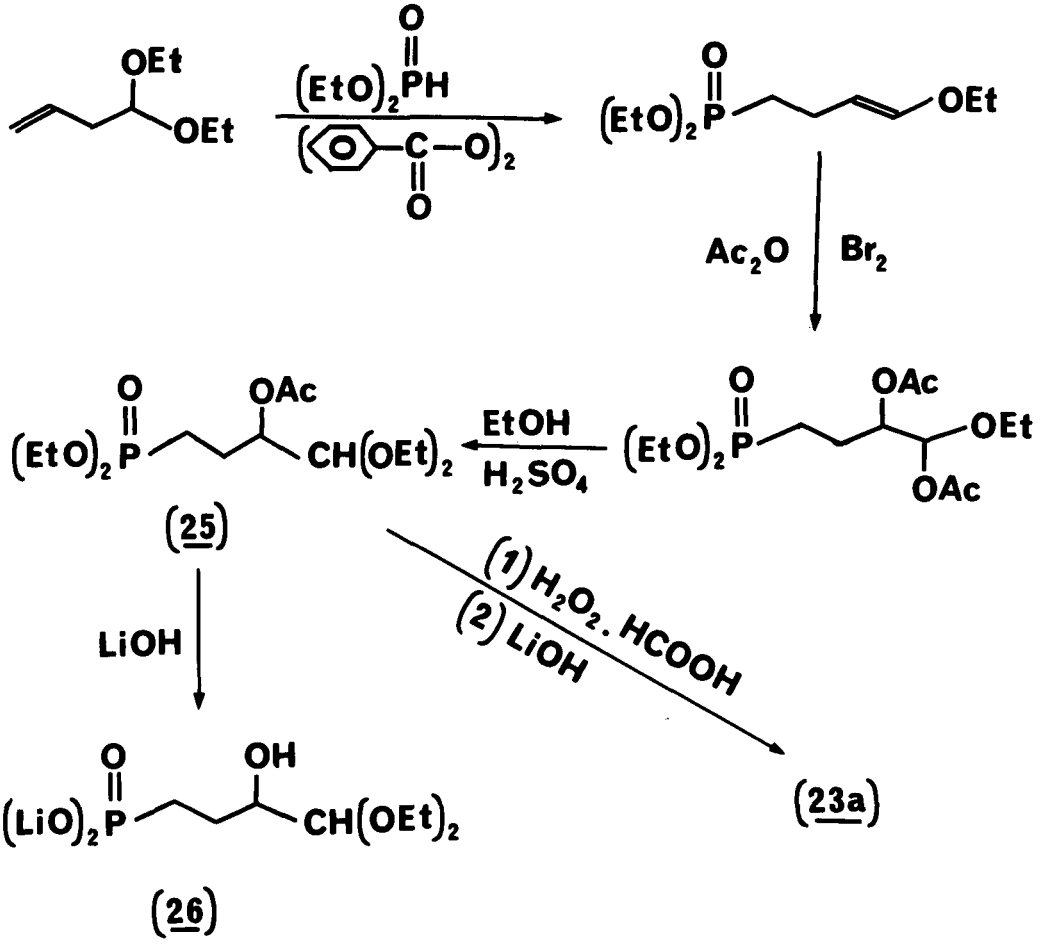


Also reported was the synthesis of the isosteric analogue (24) of 2,3-diphosphoglyceric acid.[19]



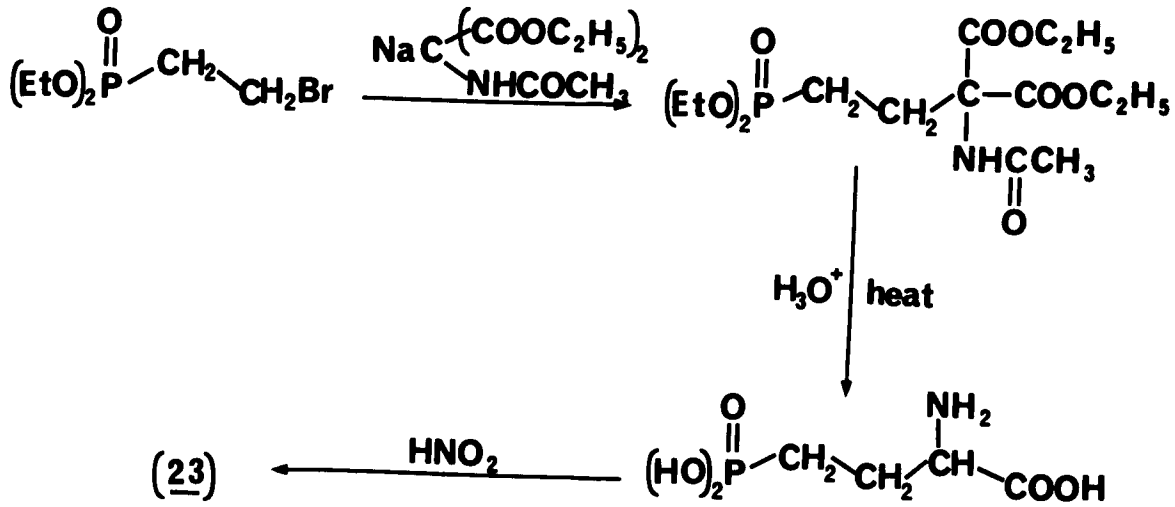
(24)

Goldstein, et al.[20] reported the preparation of the trilithium salt of (23) by the oxidation of an intermediate (25) in the preparation of the analogue (26) of glyceraldehyde-3-phosphate (Scheme IV).



SCHEME IV

Dixon and Sparkes[21] reported a multistep route for the preparation of (23) from diethyl 2-bromoethylphosphonate. (Scheme V)

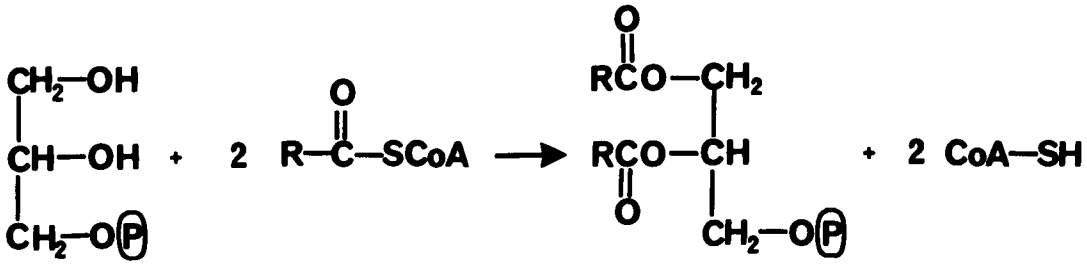


SCHEME V

Initial biochemical investigations[19] of (22) and (23) indicated neither to bear activity to increase or decrease the binding capabilities of human red blood cells. However, it was later found[21] that (23) was capable of replacing the natural material in oxidation of $NADH_2$. Orr and Knowles[22] observed it to be a substrate for phosphoglycerate kinase with a similar kinetic value (Km) to that of the natural material.

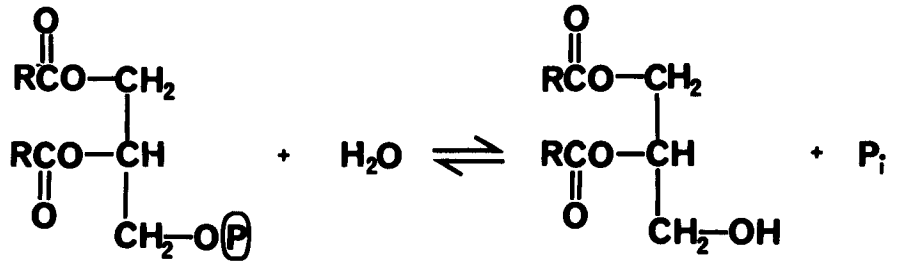
From the branch-point of the glycolytic pathway (Scheme I), dihydroxyacetone phosphate leads to an entirely different class of compounds, lipids. An intermediate in the synthesis of both triglycerides and phospholipids is phosphatidic acid (27). This is formed from

sn-glycerol-3-phosphate (7) and the coenzyme A derivatives of fatty acids.

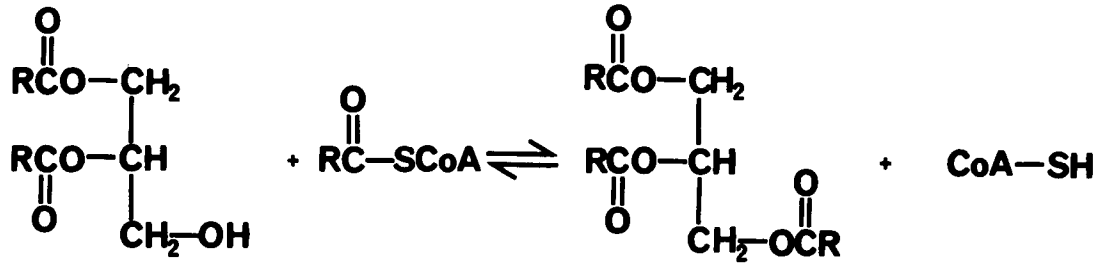


(7) (27)

The sn-glycerol-3-phosphate (7) required for this reaction may be supplied by either of two reactions mentioned previously in Scheme I. The formation of triglycerides (28) from phosphatidic acids (27) occurs by a sequence of two reactions. First, the acid is hydrolyzed in the presence of a phosphatase, and the resulting diglyceride (29) reacts with a third molecule of fatty-acyl coenzyme A.

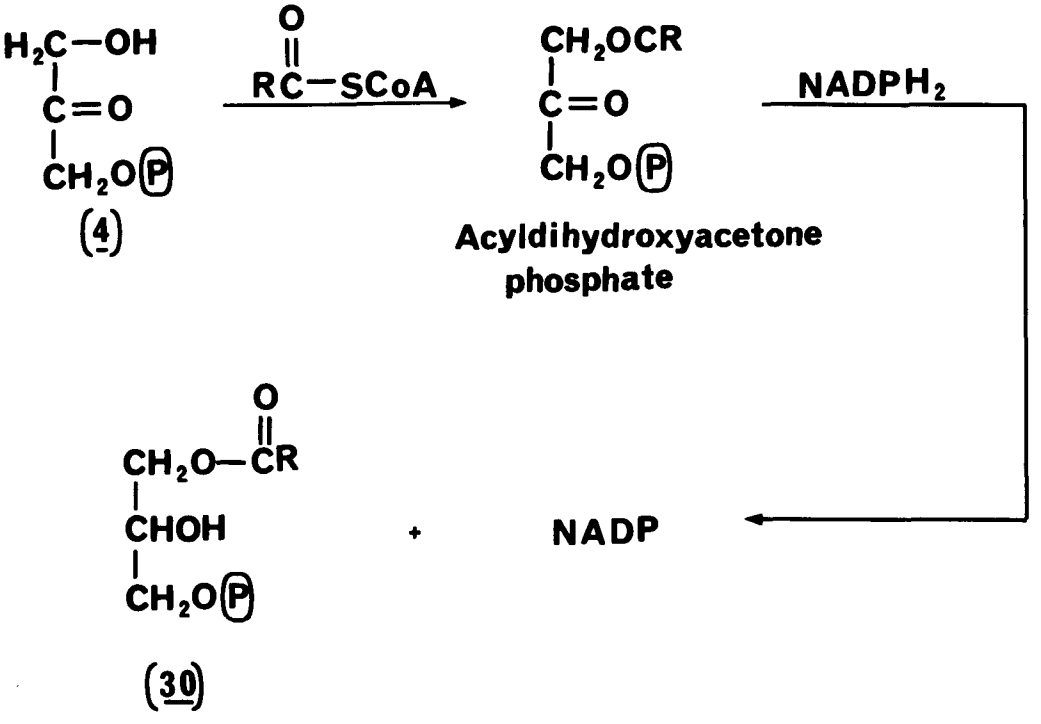


(27) (29)

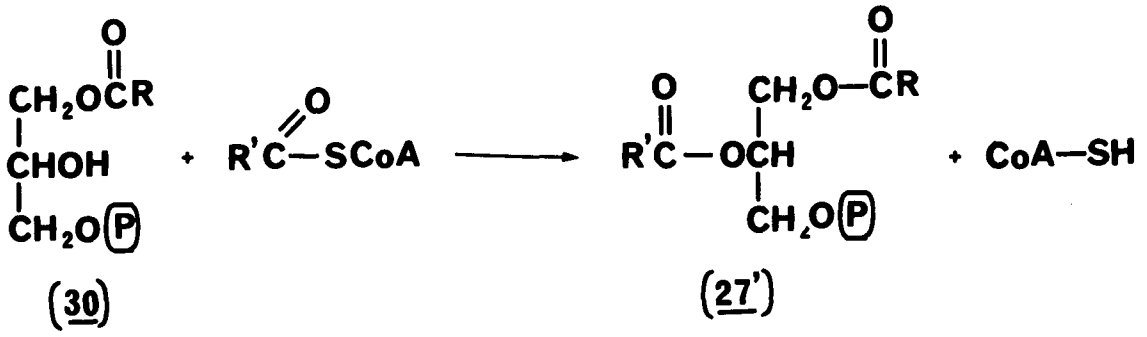


(29) (28)

In addition to the above pathway for the formation of (27), acylation of dihydroxyacetone phosphate (4) occurs in liver and is subsequently reduced in the presence of nicotinamide adenine dinucleotide phosphate ($NADPH_2$; reduced form) to give lysophosphatidic acid (30).



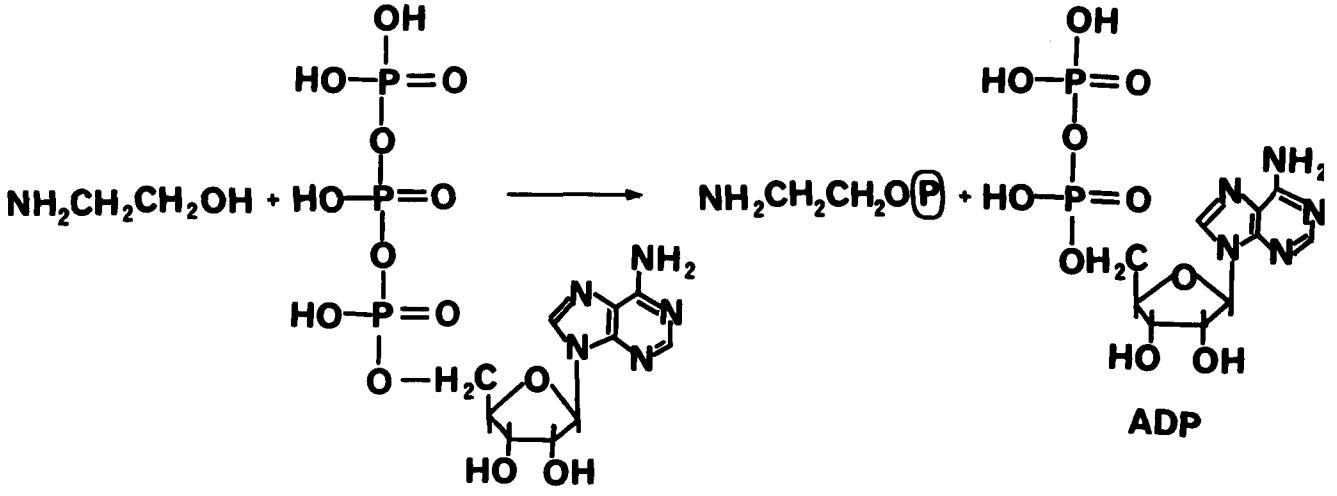
The transacylase is found in both mitochondria and microsomes and is specific for saturated fatty acid. Reduction of acyldihydroxyacetone phosphate is accomplished by a microsomal enzyme which uses an unsaturated fatty acyl CoA yielding (27').



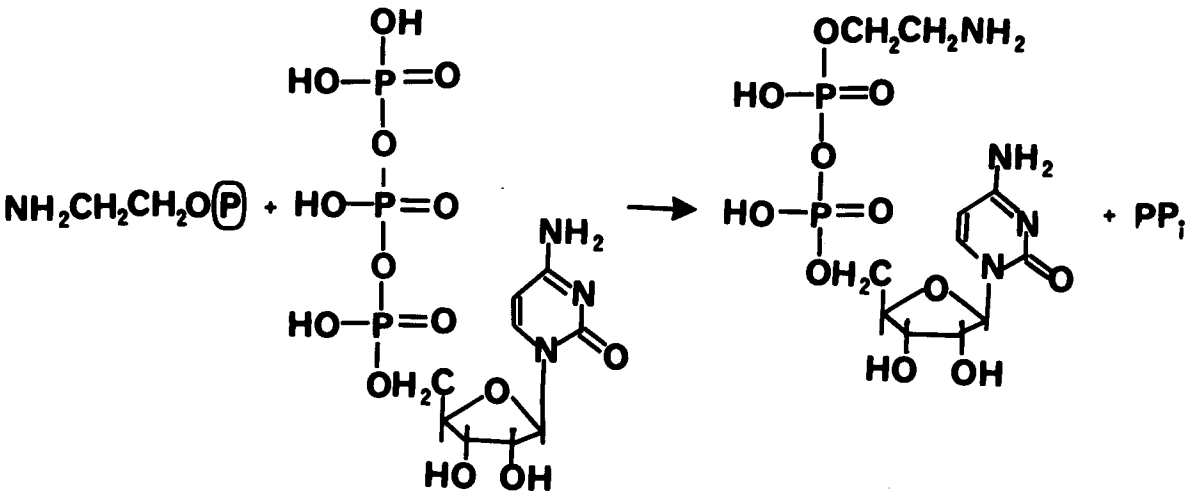
(30) (27')

The formation of other phospholipids is slightly more complicated. There are two pathways for the synthesis of phospholipids from phosphatidic acid, one of which occurs almost exclusively in plants and microorganisms. Both of them involve activation by cytidine triphosphate (CTP) and yield cytidine diphosphate (CDP).

In the first pathway, which occurs chiefly in animals, the base (ethanolamine or choline) that is to be inserted into the phospholipid is phosphorylated. The phosphoryl derivative then reacts with CTP to give the corresponding CDP derivative.

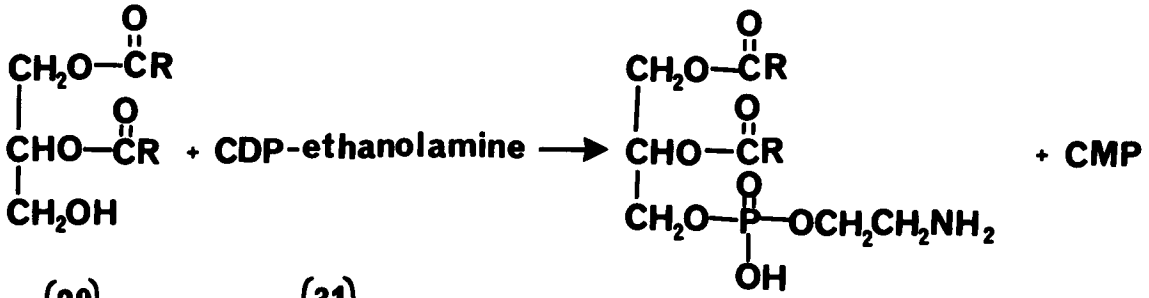


ATP ADP



CTP (31)

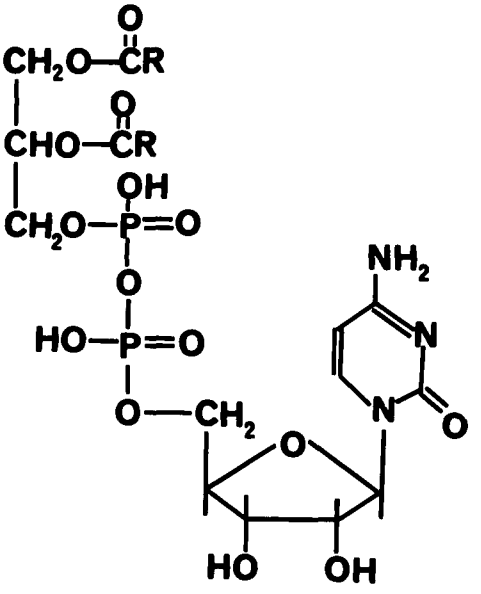
The CDP-ethanolamine or CDP-choline can now react with a diglyceride to form the corresponding phosphatidyl derivative, such as (32) and (36), and cytidine monophosphate (CMP).



(29) (31) (32)

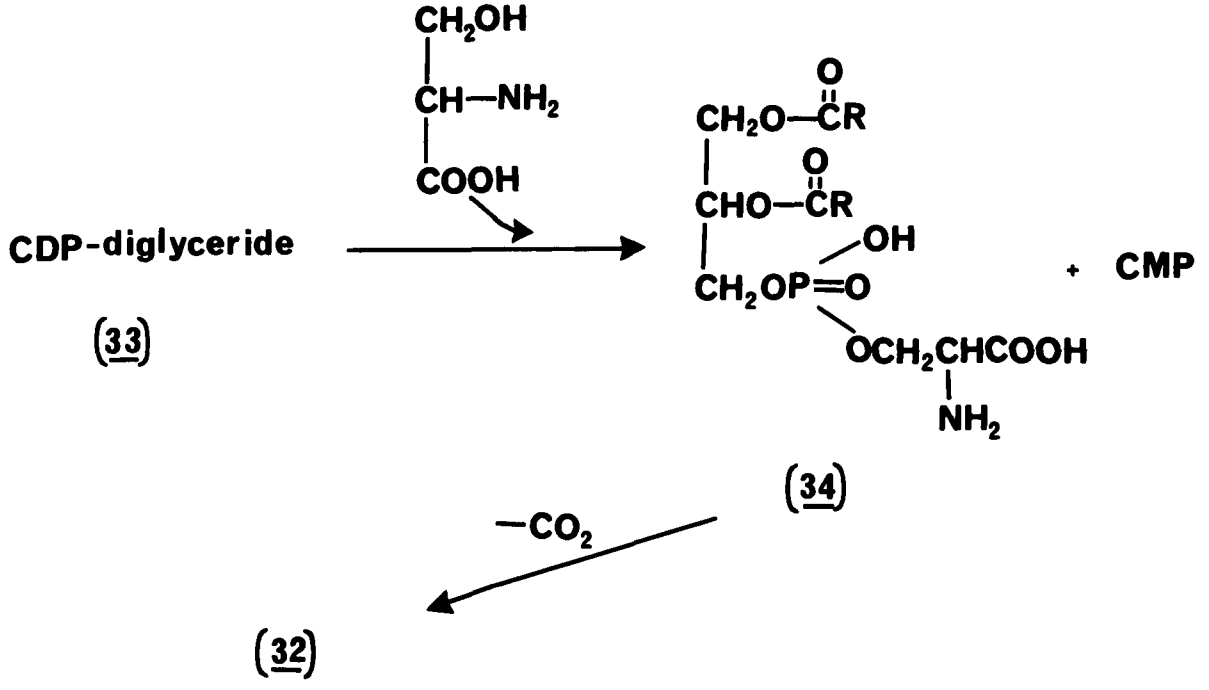
In the alternate pathway of synthesis of phospholipids, CTP is involved in activation of the phosphatidic acid (27). The compound thus formed is CDP-diglyceride (33), a compound believed to be the primary target site of the studies [13-17] of E. coli in vivo and in vitro with

3,4-dihydroxybutylphosphonic acid analogues.

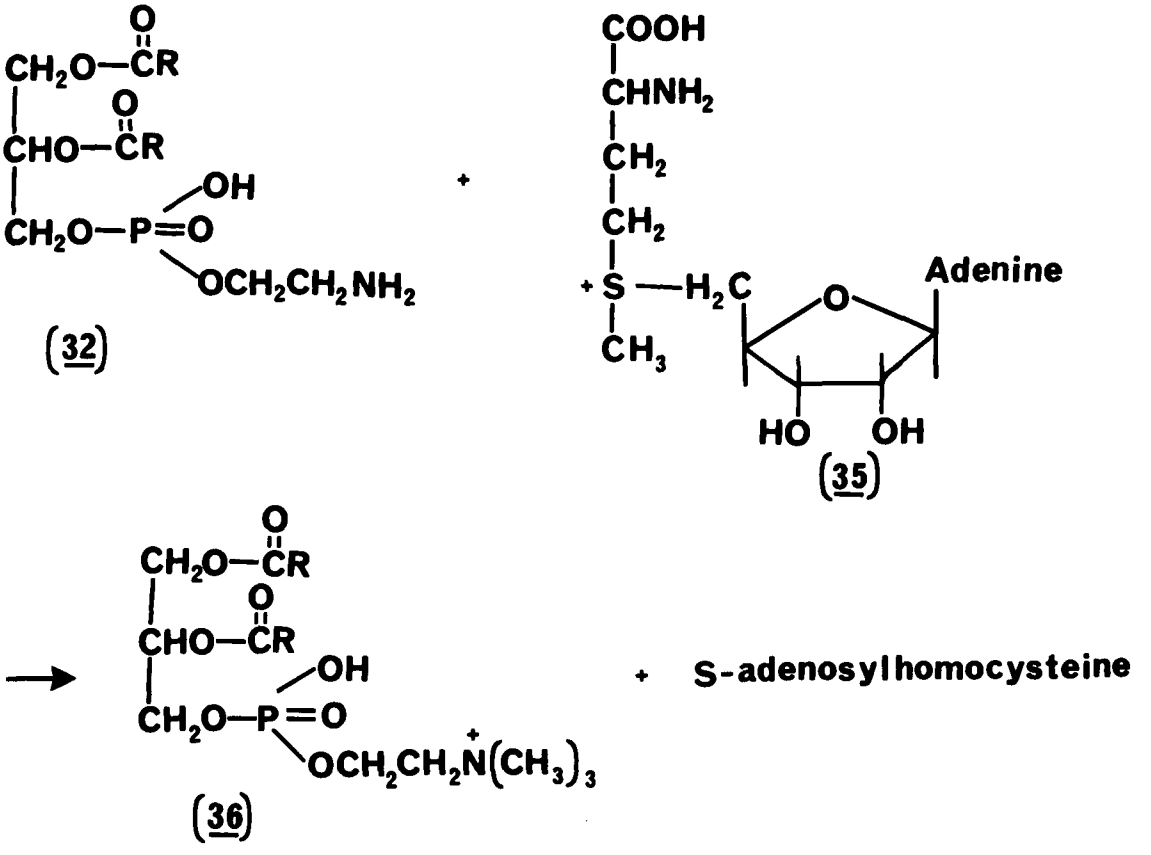


(33)

This can now react with serine to give phosphatidyl serine (34), which generates phosphatidyl ethanolamine (32) by decarboxylation.



In the organisms in which this pathway operates, (32) can accept methyl groups from a methyl donor, such as S-adenosylmethionine (35), and the ethanolamine group is methylated in stages to choline.

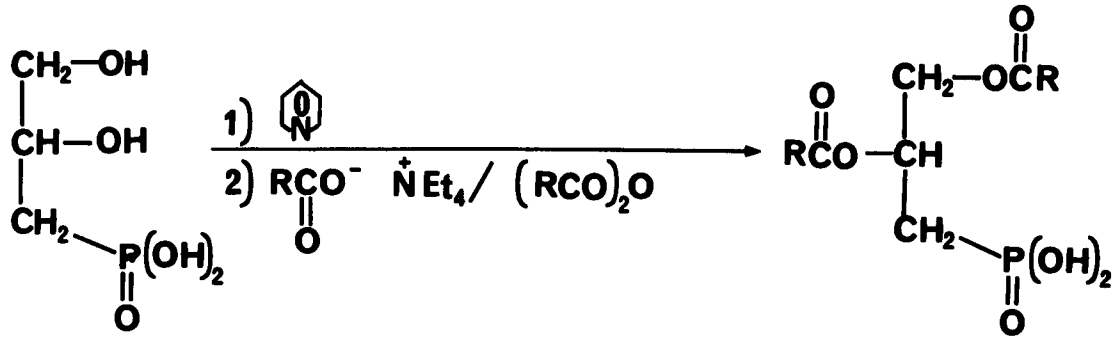


Actually, in some organisms (particularly bacteria) only one or two methyl groups are used, and phosphatidyl monomethyl or dimethyl ethanolamine occur in the phospholipid in place of phosphatidyl choline.

Baer introduced the general term phosphonolipids for analogues of phospholipids. One may consider here two main categories of phosphonolipids; one with structural changes in the "glycerol portion" (a C-P bond being present instead of the glycerol ester oxygen), and the other related to aminoethylphosphonic acid (a C-P bond being present

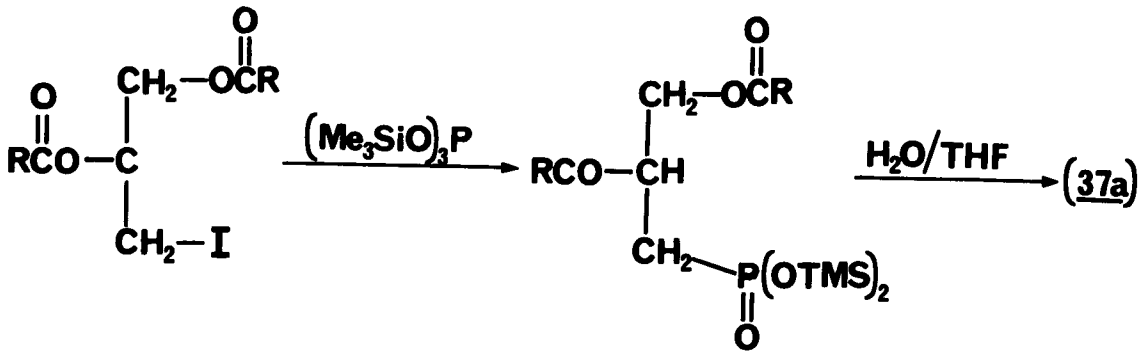
instead of the esteric oxygen of the head group). It should be noted that this latter category constitutes a "natural" system found in numerous organisms.[23-30]

For the first category, the phosphonolipid is derived from phosphotidic acids (37) analogues of phosphatidic acid. The non-isosteric analogues (37a) bearing saturated fatty acid functions were reported by Baer and Basu[31] and later by Bonsen et al.[32] by direct acylation of (16).

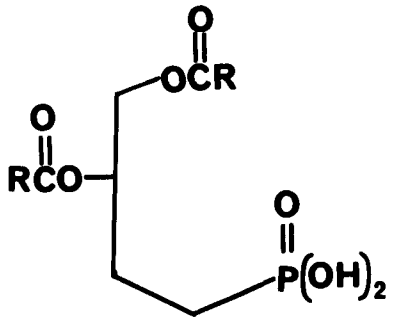


(16) (37a)

Rosenthal, et al. reported [33,34] the synthesis of (37a) using an Arbuzov reaction on 2,3-diacyl-1-iodopropanes with tris(trimethylsilyl) phosphite[35,36] followed by mild hydrolysis.

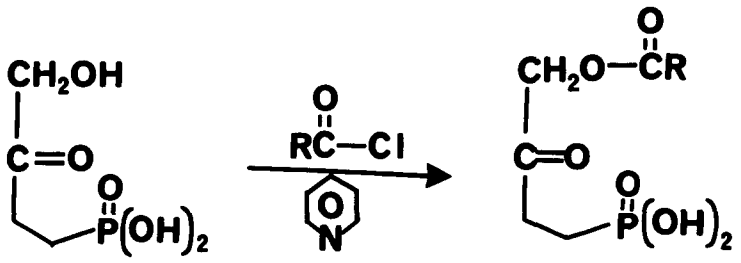


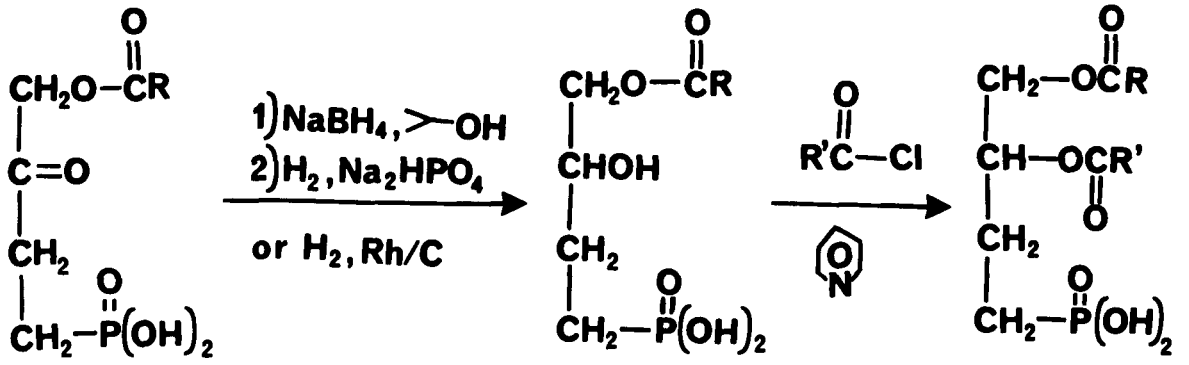
The isosteric phosphotidic acid (37b) bearing saturated and unsaturated fatty acid ester linkages have also been synthesized by the same route[37].



(37b)

In light of the in vivo biological path for formation of lysophosphatidic acid (30) from dihydroxyacetone phosphate (4), the isosteric phosphonic acid analogue of (30) appeared to be of interest for synthesis. This was accomplished by a chemical route similar to those previously used[38]. The analogue (38) thus prepared also promised a route to the mixed fatty acid analogue (39) of (27')[38]. That is:

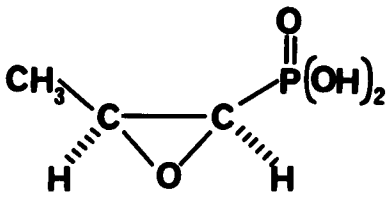




(38) (39)

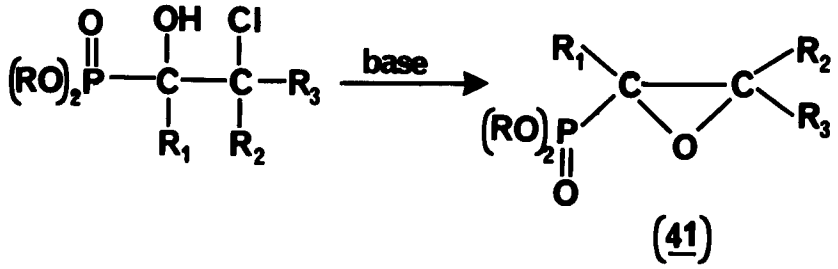
The analogues (38) have been found[38] to serve as substrates for acylation by lysophosphatidate: acyl CoA acyl transferase.

In relation to the phosphonic acids discussed above and their activity for inhibition of bacterial metabolism, it is also of interest to consider a naturally occurring phosphonic acid which is bactericidal. Phosphonomycin (40) is a promising new antibiotic of unusual structure originally isolated from fermentation broths of streptomyces fradiae. It has been found to be orally effective against both Gram-positive and Gram-negative infections in mice. Its bactericidal mode of action is via irreversible binding to the enzyme PEP: UDPGLcNAc enolpyruvyl transferase, thereby inhibiting cell wall synthesis[39]. It has been shown to have the structure (-)-(1R,2S)-1,2-epoxypropylphosphonic acid by synthesis together with a chemical determination of absolute configuration[40].



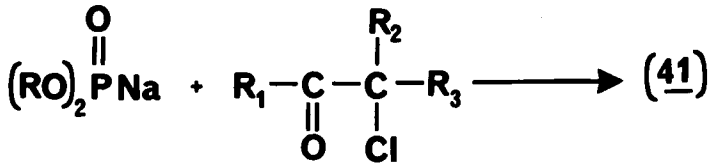
(40)

The methods leading to α-epoxyphosphonate (41) synthesis have recently been reviewed.[41] These methods include: the reaction of a dialkyl phosphonate halohydrin with base.

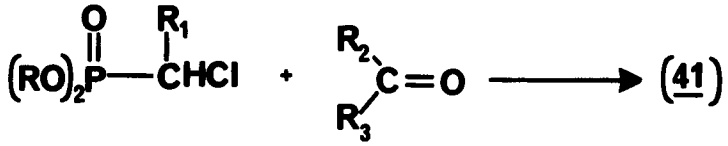


(41)

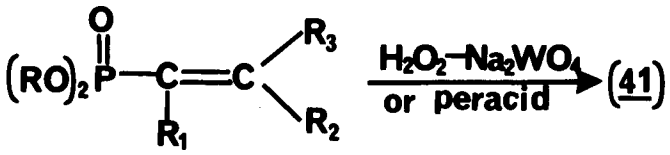
the reaction of sodium dialkylphosphonate with an α-haloketone.



Darzen's reactions of dialkyl chloromethylphosphonates with carbonyl compounds.



direct epoxidation of unsaturated phosphonates with a peroxide and catalyst or a peracid.



The yields for these reactions are at best 60-70% and are also subject to certain structural limitations as indicated below.

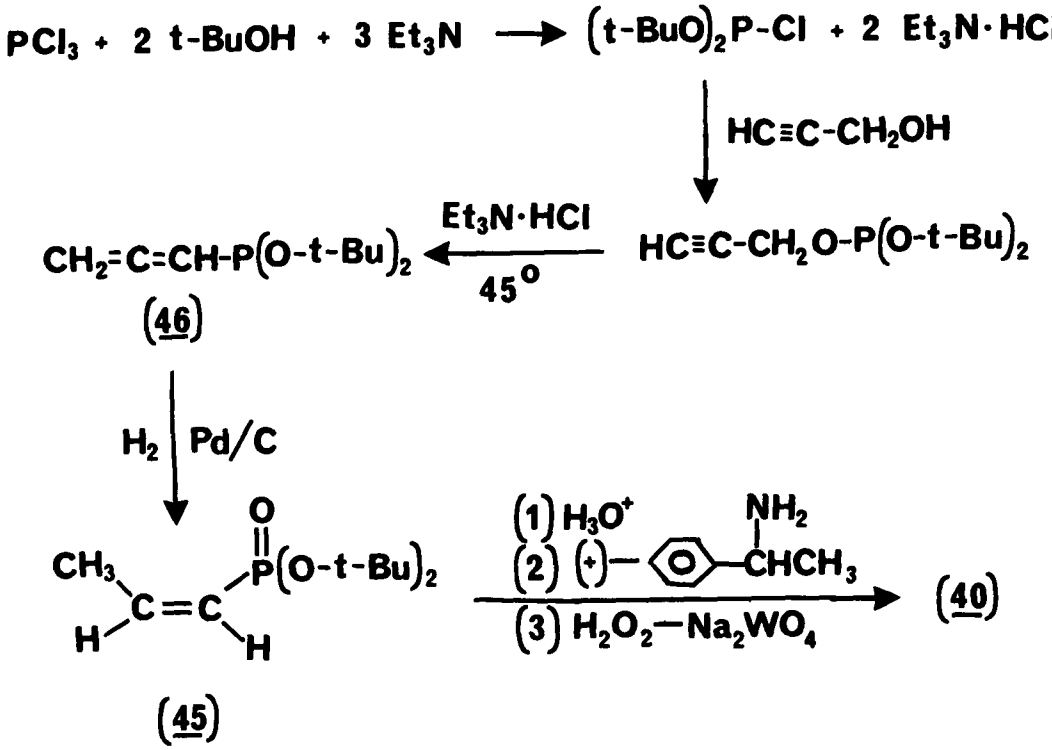
The reaction of a sodium dialkyphosphonate has been reported to give also the isomeric enol or vinyl phosphonate (42) via Perkow reaction and β-ketophosphonate (43) via Arbuzov reaction directly[42,43]; the α-halo carbon can not be tertiary[43].

The Darzen's reaction is without side reactions, but it is limited to ketones and aryl aldehydes, i.e. R_2 and R_3 = alkyl or R_3 = aryl and R_2 = H; in addition, this reaction has, as yet, been performed only with the methyl and ethyl esters of chloromethylphosphonic acid. (R_1 = H).

Epoxidation of the vinylic phosphonate can result in side reactions; in a buffered solution trifluoroperacetic acid, when used as the oxidant, may cause ring opening of the epoxide once formed[44], and the use of tert-butyl peroxide has been shown to result in the Michael addition product (44) of the butoxide to olefin[45].

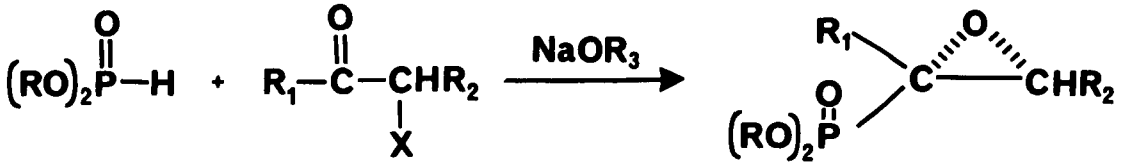
(44)

Epoxidation has two distinct advantages; first, the formation of the intermediate unsaturated phosphonate in the synthetic sequence permits an acid-catalyzed ester hydrolysis prior to epoxidation; once the epoxide is formed, as in the other reactions discussed, the ester can be removed by hydrogenation if R is a benzyl group[48]. Secondly, if R_2 or R_3 is not hydrogen, then there will be two isomeric unsaturated phosphonates formed which may be separable. Alternately, the stereospecific unsaturated phosphonate (45) can be obtained via catalytic hydrogenation of corresponding allenyl (46)[47] or alkynyl (47)[48] phosphonates. Epoxidation of the appropriate isomer can then take place in the presence of a resolving agent in order to isolate the product with the desired absolute stereochemistry[47]. (Scheme VI)



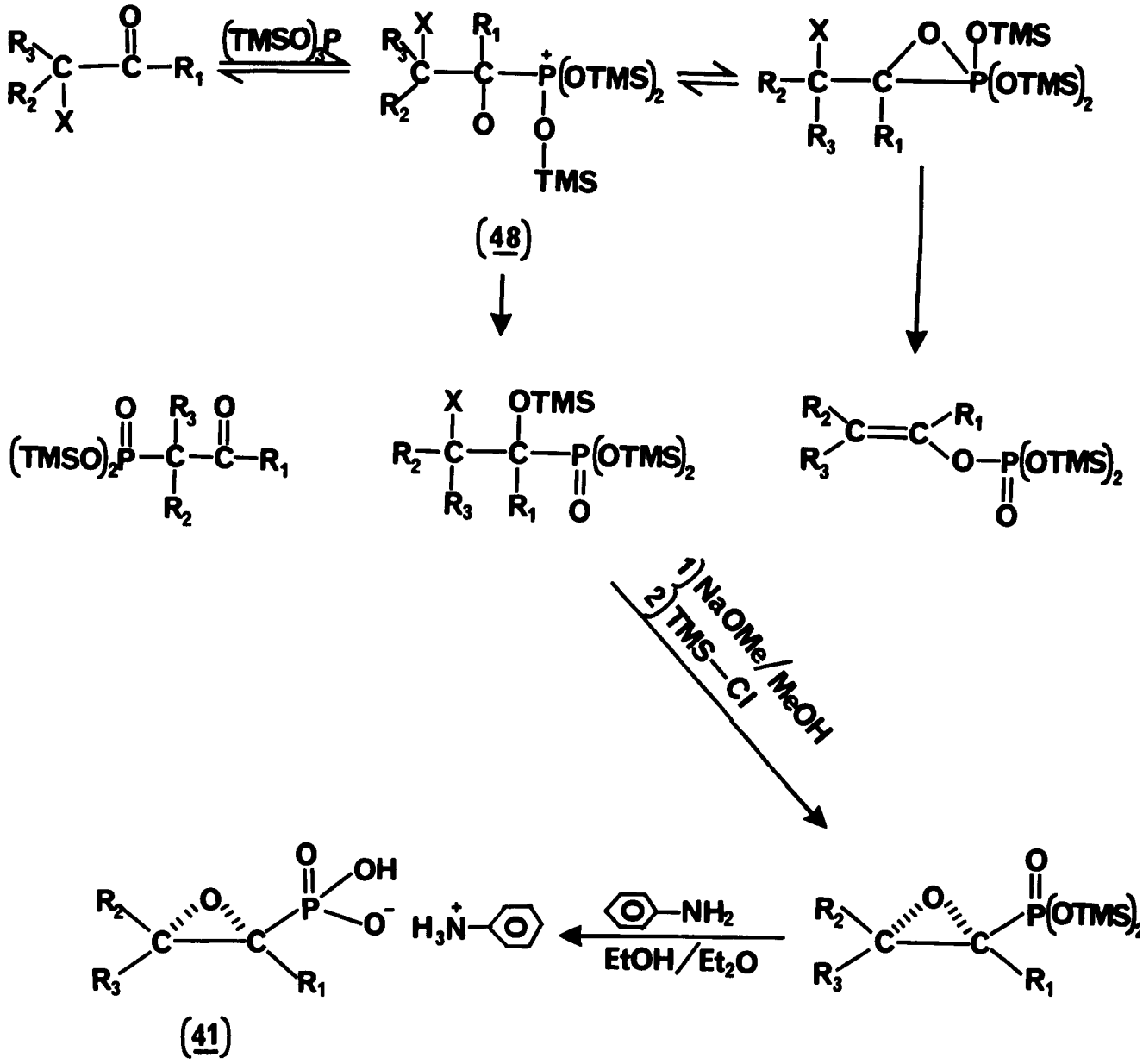
SCHEME VI

Haake et al.[46] also reported a facile one-step procedure to α-epoxyphosphonates which is a combination of two reactions previously mentioned[41].



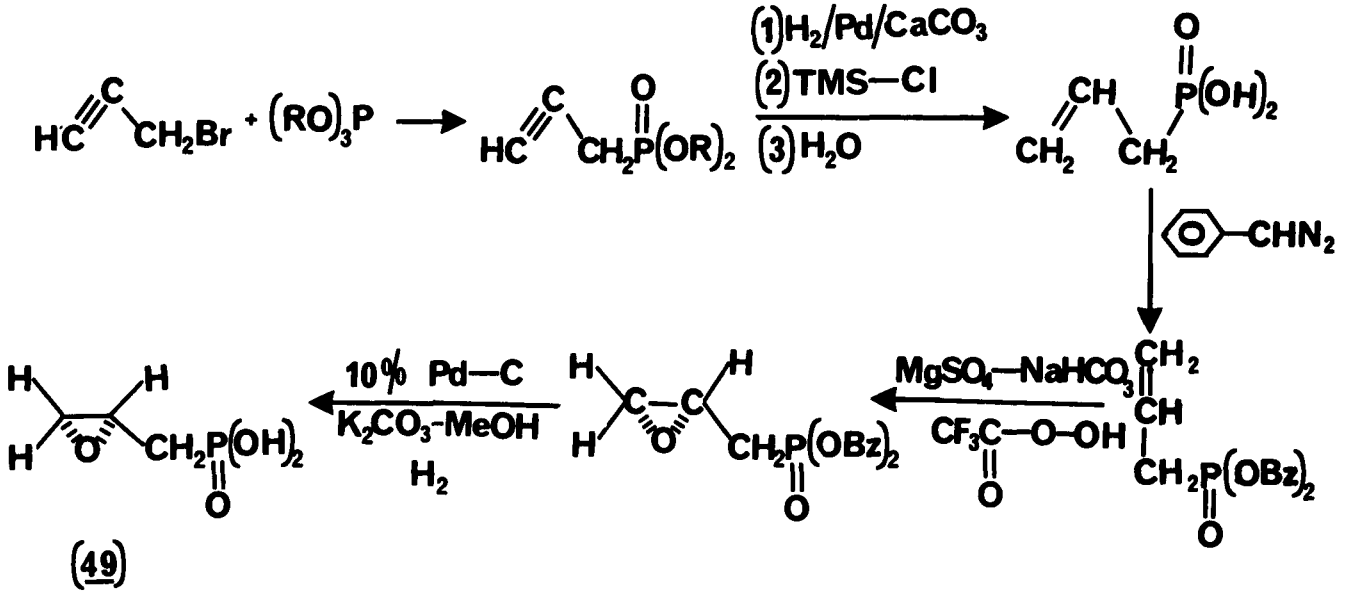
(41)

Recently, a Japanese group[49] reported the facile conversion of bis(trimethylsilyl) esters of phosphonic acids, generated via either a Perkow or Arbuzov reaction of a silyl phosphite with halocarbonyl compounds, to the corresponding free acid by simple addition of alcohols. It was also shown that the carbonyl adduct (48) can be converted to the free acid α-epoxyphosphonates by a sequence of reactions as shown in Scheme VII.



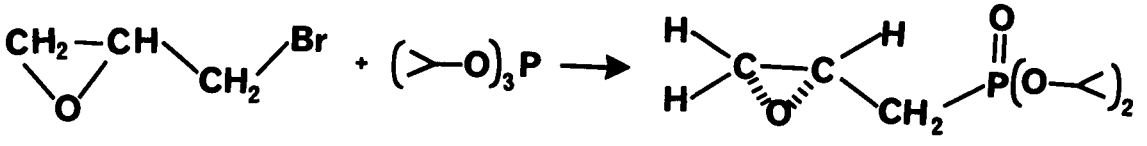
SCHEME VII

Two other β-epoxyphosphonates[48,50] have been reported in the literature, synthesized as shown below. The free β-epoxypropylphosphonic acid (49) was synthesized from propargyl bromide via an Arbuzov reaction.[48]



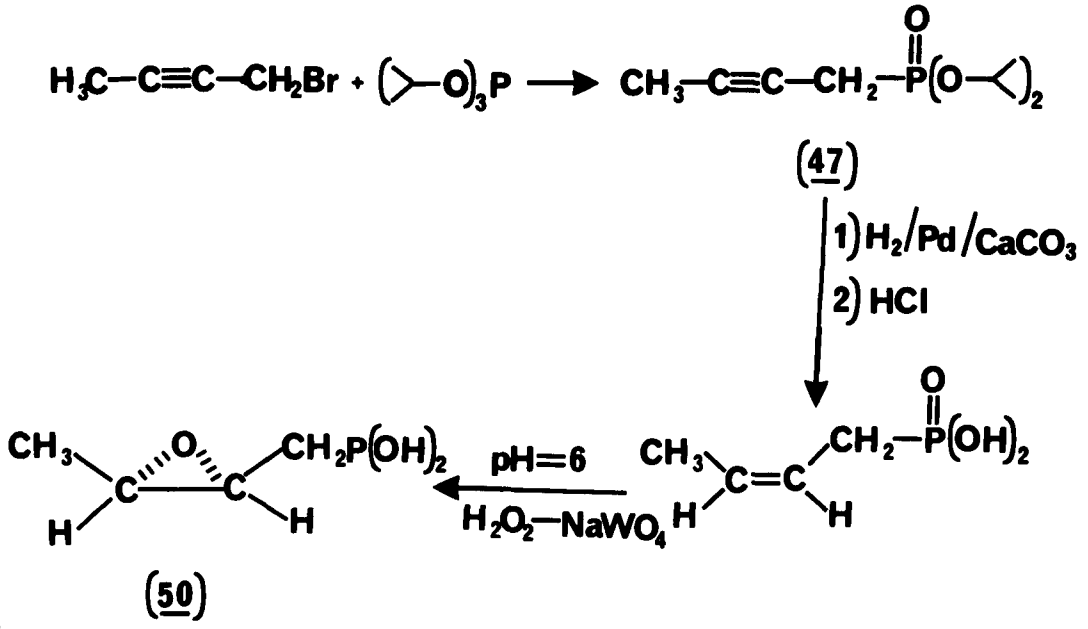
(49)

The isopropyl ester of β-epoxypropylphosphonate (49)was also obtained by a one-step reaction.[50]



(49)

Another β-epoxyphosphonate, 2,3-epoxybutylphosphonate (50), was synthesized as follows:[48]



As mentioned previously, the preparation of phosphonic acids having structures related to those of natural phosphates and their use as probes of biological mechanisms have been topics of interest for some time now.[51] Previous efforts have established the biological significance of isosteric analogues of the natural phosphate products of glycolysis.[11-17] 3,4-Dihydroxybutyl-1-phosphonic acid (21), the isosteric analogue of glycerol-3-phosphate, has been of particular value for *in vivo* as well as *in vitro* investigations.[12,52]

There are now routes for the synthesis of (21) noted in the literature.[9,11,18,53] The route[9] of Adams *et al.*, leads to the optically active form, but is not particularly applicable for the synthesis of large quantities of material. A further method was desired which would allow the preparation of the material as either of its pure enantiomers in sizable quantity and at the same time would

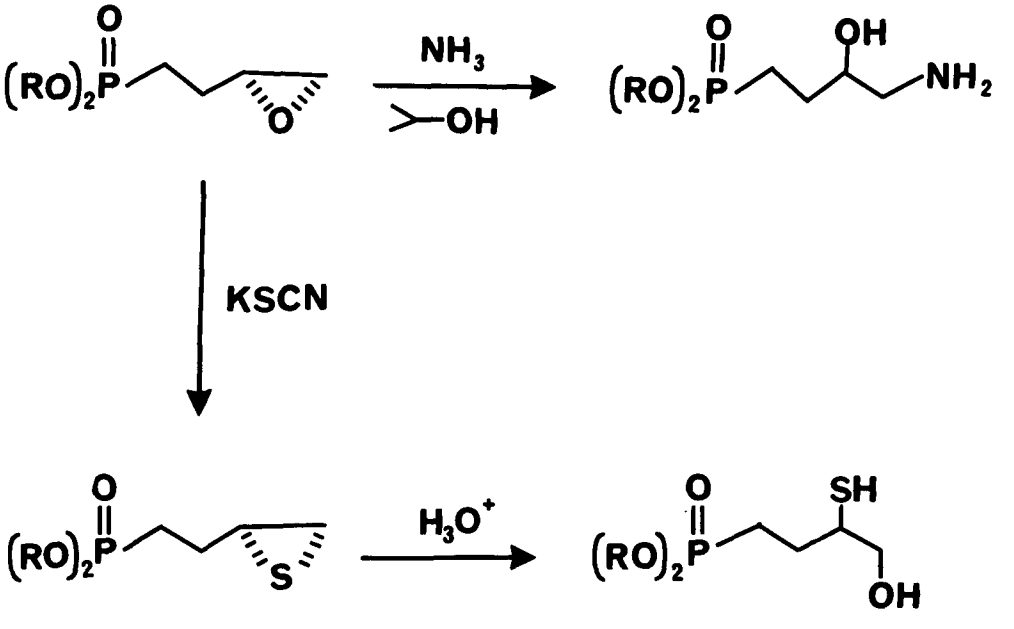
provide convenient intermediates for the syntheses, in optically active form, of related analogues of interest. In the course of biochemical investigations in our laboratory it became of interest to examine analogues other than the phosphonic acids isosteric with the natural phosphates. In particular, it was deemed desirable to explore the biological properties of analogues related to (21) in which a methyl or hydroxyl functional group was placed at C-1. We also wished to examine the effects of varying the distance between hydroxyl and phosphorous sites.

Prior efforts[7,8,12,13] indicated that the "shortened" non-isosteric phosphonic acid analogues of glycerol-3-phosphate, 2,3-dihydroxypropyl-1-phosphonic acid (16) and (1RS, 2S)-1,2,3-trihydroxypropylphosphonic acid (19), were not of significant value for *in vivo* studies, nor were they particularly good substrates when studied by *in vitro* enzymatic reactions. Presumably the compacted structure hindered interaction with the active site of the enzymes involved. However, little is known about the geometric requirements in regard to analogues "lengthened" as compared to the natural material, such as (S)-3,4-dihydroxybutylphosphate (74). It might be expected that the rotational flexibility of "lengthened" species would permit interaction in a manner similar to that of the natural material.

While there are also three different routes[19,20,21] reported in the literature for the preparation of 3-carboxy-3-hydroxypropyl-1-phosphonic acid (23), none of them are suitable for the preparation of 3-[^{14}C]-carboxy-3-hydroxypropyl-1-phosphonic acid (23') of high specific activity. A route incorporating the ^{14}C cleanly and late in the

overall sequence was desired.

In the light of the antibacterial activity of phosphonomycin (40), the introduction of an epoxide function in place of the vicinal diol of (21) appeared to be a reasonable structural choice for the generation of a new agent and should also provide a suitable intermediate for the preparation of other analogues such as those shown below.



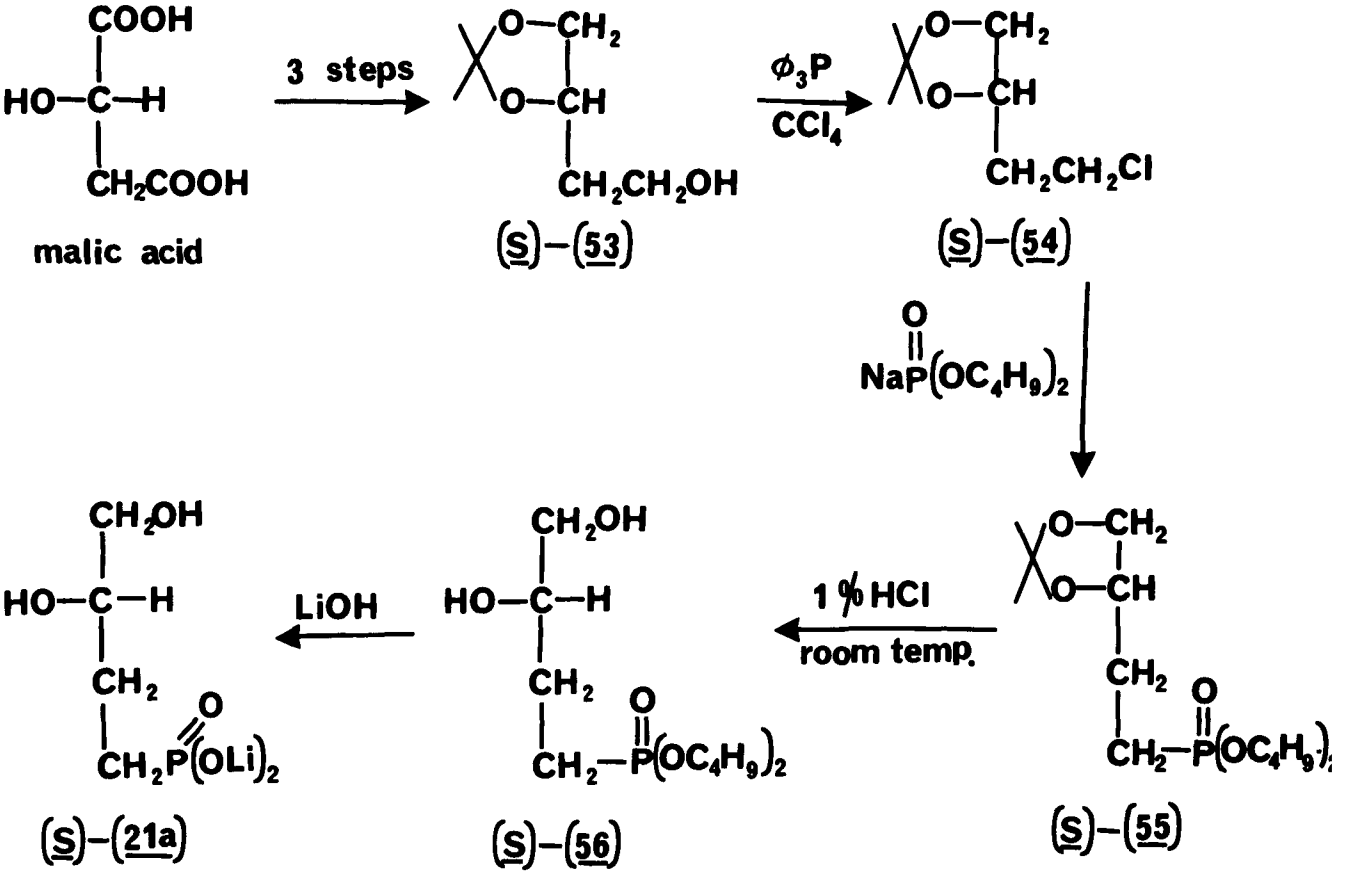
RESULTS AND DISCUSSION

Both enantiomers of (21) have been prepared starting with the commercially available optically active malic acids as illustrated in Scheme VIII. For the (S)-enantiomer of 3,4-dihydroxybutyl-1-phosphonic acid, [(S)-(21)], the l-malic acid was esterified using excess methanol in the presence of sulfuric acid and then neutralized with sodium bicarbonate under anhydrous conditions to yield the known dimethyl ester [(S)-(51)], the preparation of the ester is to facilitate reduction with lithium aluminum hydride generating (S)-1,2,4-butanetriol, [(S)-(52)]. This method proceeds much more favorably than the Fisher esterification mentioned by Hayashi et al.[54] for the water-soluble and acid-sensitive ester. The triol [(S)-(52)] thus obtained upon reduction was converted to its acetonide [(S)-(53)] according to the previously reported procedure of Hayashi et al.[54] Similar performance of esterification, reduction, and dioxolane formation starting with d-malic acid is reported here for the generation of the (R)-enantiomer of 3,4-dihydroxybutyl-1-phosphonic acid [(R)-(21)]; a synthesis of the racemic material, more convenient and proceeding in higher yields than those previously reported,[9,11,18] begins with the commercially available butane-1,2,4-triol (52). The triol acetonide (53) was chlorinated to (54) in high yield (86%) without the attendant difficulties noted[54] in the preparation of the corresponding bromide.

Initial attempts at the phosphonylation of [(S)-(54)] by a Michaelis-Arbuzov reaction using either triethyl phosphite or tris(trimethylsilyl) phosphite[33-36] gave results which were undesirable. In both attempts a significant amount of decomposition had occurred with the generation of extraneous by-products; in the latter attempt

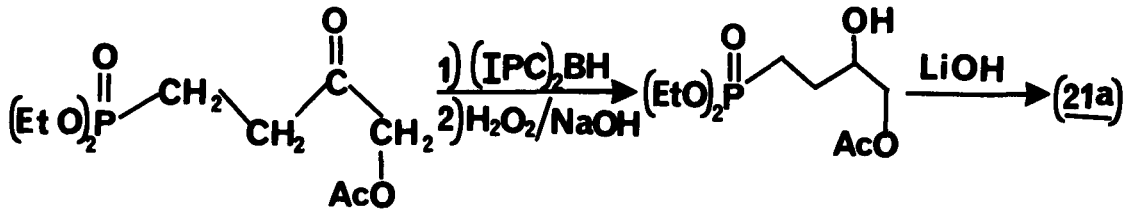
it appeared that the dioxolane ring had been opened followed by transesterification. Phosphonylation of [(S)-(54)] to generate [(S)-(55)] ultimately was attained in satisfactory yield through the use of a Becker reaction with the sodium salt of dibutyl phosphite. The butyl system was chosen due to the relatively favorable solubility properties of its salt compared to those of other alkyl phosphites[55]. The dibenzyl system, which was convenient for generating the free acid of 3,4-epoxybutylphosphonic acid (63) by hydrogenolysis[48], had been tried but due to a solubility problem gave a less satisfactory result.

Purification of [(S)-(55)] was hindered by the apparent partial decomposition of the dioxolane ring system upon attempts at distillation; as spectral data on the crude [(S)-(55)] indicated it to be approximately 95% pure, the reaction sequence was continued without further attempts at purification and [(S)-(55)] was deprotected in two steps to yield [(S)-(21a)], the dilithium salt of [(S)-(21)]. In this manner [(S)-(21a)] could be obtained with optical purity corresponding to that of a prior less convenient route.[9] It should be noted that while lithium hydroxide hydrolysis of phosphonate diester is often less desirable than other routes for the generation of free acid, heating either with mineral acid or silyl reagents led to significant racemization in this system.



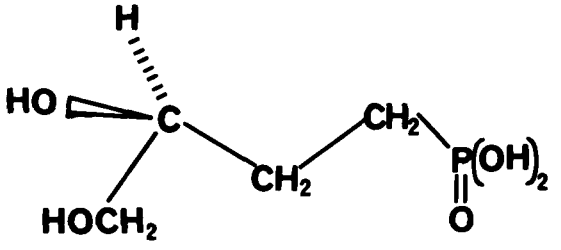
SCHEME VIII

An alternate initial approach involving asymmetric induction[56] of diethyl 4-acetoxy-3-oxobutyl-1-phosphonate[57] (57) with (-)- and (+)-diisopinocamphylboranes [$(IPC)_2BH$] had been tried and gave less desirable results (ca. 15% optical purity) with very inconvenient work-up processes.

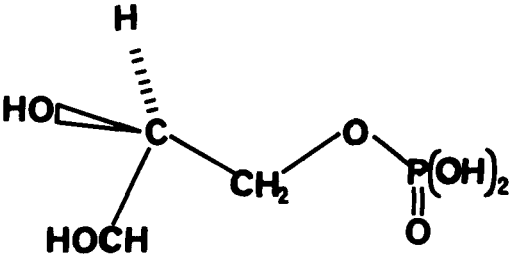


(57)

The compound [(S)-(21a)] obtained starting from l-malic acid is of the same absolute configuration about the internal hydroxyl as sn-glycerol-3-phosphate and would be expected to exhibit significantly different properties in biological systems as compared to its enantiomer. This in fact has been observed. Compound [(S)-(21a)] exhibits activity in the inhibition of growth of Escherichia coli strain 8 and Bacillus subtilus strains BD 170 and 1005 of twice the magnitude observed using racemic material.[58]



(S)-(21)



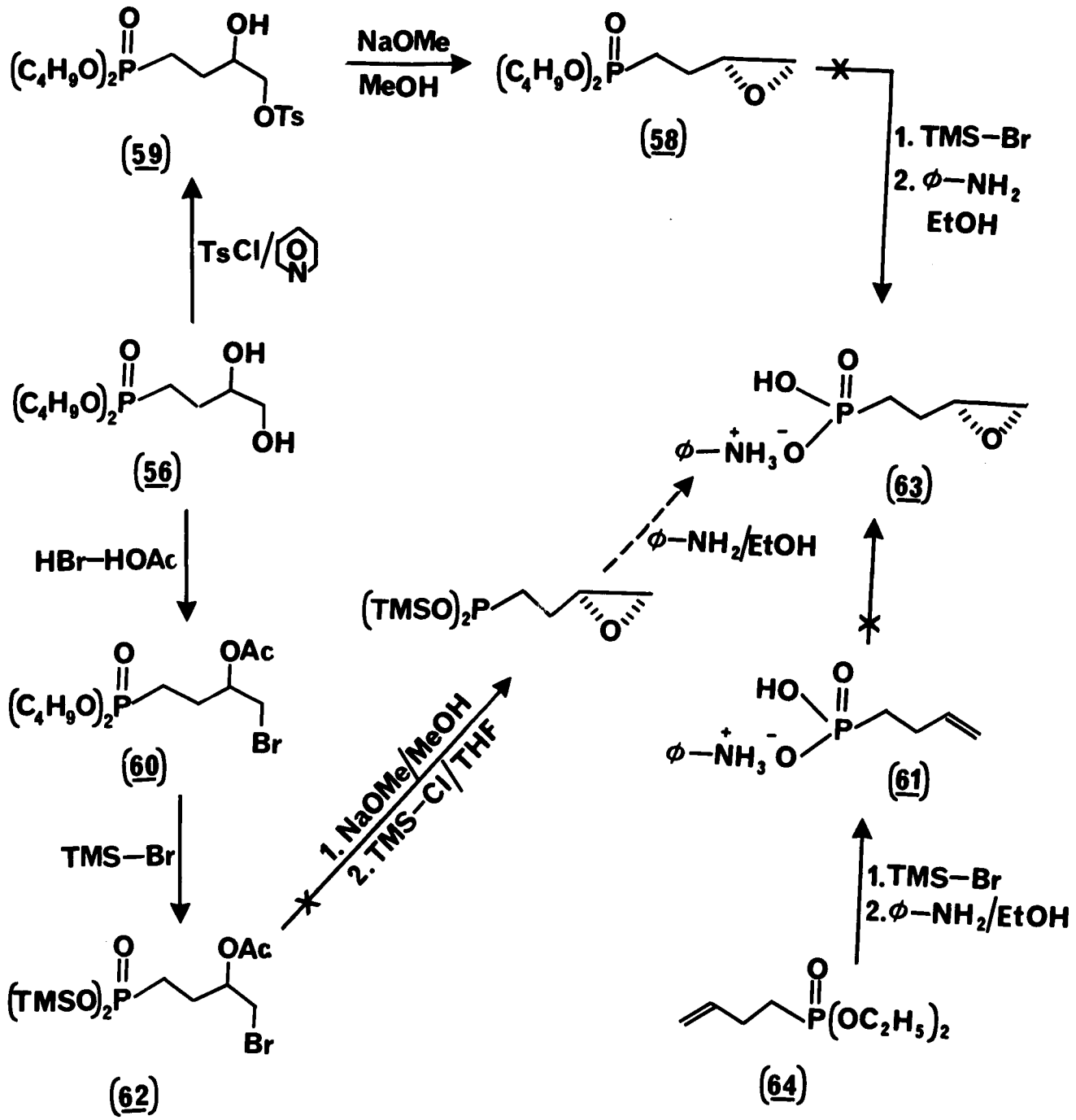
sn-glycerol-3-phosphate

It should be noted that the enantiomer[(R)-(21)]exhibits inhibition of about 8% of the magnitude as[(S)-(21)]. This is presumed to be due to

the presence of approximately 8% [(S)-(21)] in the [(R)-(21)] preparation resulting from the fact that the d-malic acid used as starting material contained approximately 8% l-malic acid as an impurity.

It has also been of interest to synthesize other phosphonates and phosphonic acids for use as analogues of glycerol-3-phosphate so that the effect on metabolic processes of further structural variations might be observed. In light of the activity of (1R,2S)-1,2-epoxypropylphosphonic acid (40), phosphonomycin, as an antibacterial agent,[39,59] the introduction of an epoxide function in place of the vicinal diol of (21) appeared to be a reasonable structural choice for the generation of a new agent. The routes toward the generation of the phosphonate diester (58) and phosphonic acid (63) are illustrated in Scheme IX. These procedures as performed with racemic material are entirely applicable for the generation of optically active material.[60,61]

For the initial investigations it was deemed most reasonable to prepare (58) and (63) as the recemates as it is not immediately obvious which enantiomer of (58) or (63) would exhibit biological activity. Epoxide (58) might be recognized by the interacting enzymes as a species of structural similarity to sn-glycerol-3-phosphate and [(S)-(21)] or [(S)-(56)], in which case the (S)-enantiomer would be expected to exhibit biological activity.

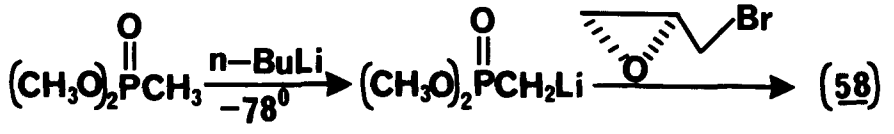

NaOMe
MeOH
TsCl/
HBr–HOAc
TMS–Br
1. NaOMe/MeOH
2. TMS–Cl/THF
1. TMS–Br
2. φ–NH2
EtOH
φ–NH2/EtOH
1.TMS–Br
2.φ–NH2/EtOH

SCHEME IX

It must also be considered that (58) might be recognized by the interacting enzymes as a species of structural similarity to D-glyceraldehyde phosphate (5) for which the (R)-enantiomer would be expected to correlate best. Finally, the epoxide function might be capable of acting without regard to stereochemistry.

Dibutyl dihydroxybutyl-1-phosphonate (56) was selectively monotosylated to (59) which was converted to epoxide (58) by treatment with sodium methoxide. Attempted hydrolysis of (58) to (63) by the trimethylsilyl bromide[62] route gave the bromohydrin. An alternate route involved conversion of (56) to the corresponding acetoxy-bromide (60) with 6 M hydrogen bromide in acetic acid. Subsequently, dealkylation of (60) to the bis(trimethylsilyl) phosphonate (62) with trimethylsilyl bromide and followed by epoxidation and re-trimethylsilylation[49] were tried but gave only an intractable mixture and none of the desired product (63). The direct epoxidation of 3-butenyl-1-phosphonate (61) with benzonitrile-hydrogen peroxide[63] and sodium tungstate-hydrogen peroxide[47,48] were attempted but only starting material was recovered. Direct bromohydrin formation of (64)[11] with N-bromosuccinimide (NBS) in moist dimethyl sulfoxide (DMSO)[64] also gave an unsatisfactory result; again an intractable mixture was obtained.

Furthermore, a one-step synthesis of (58) was proposed and attempted:

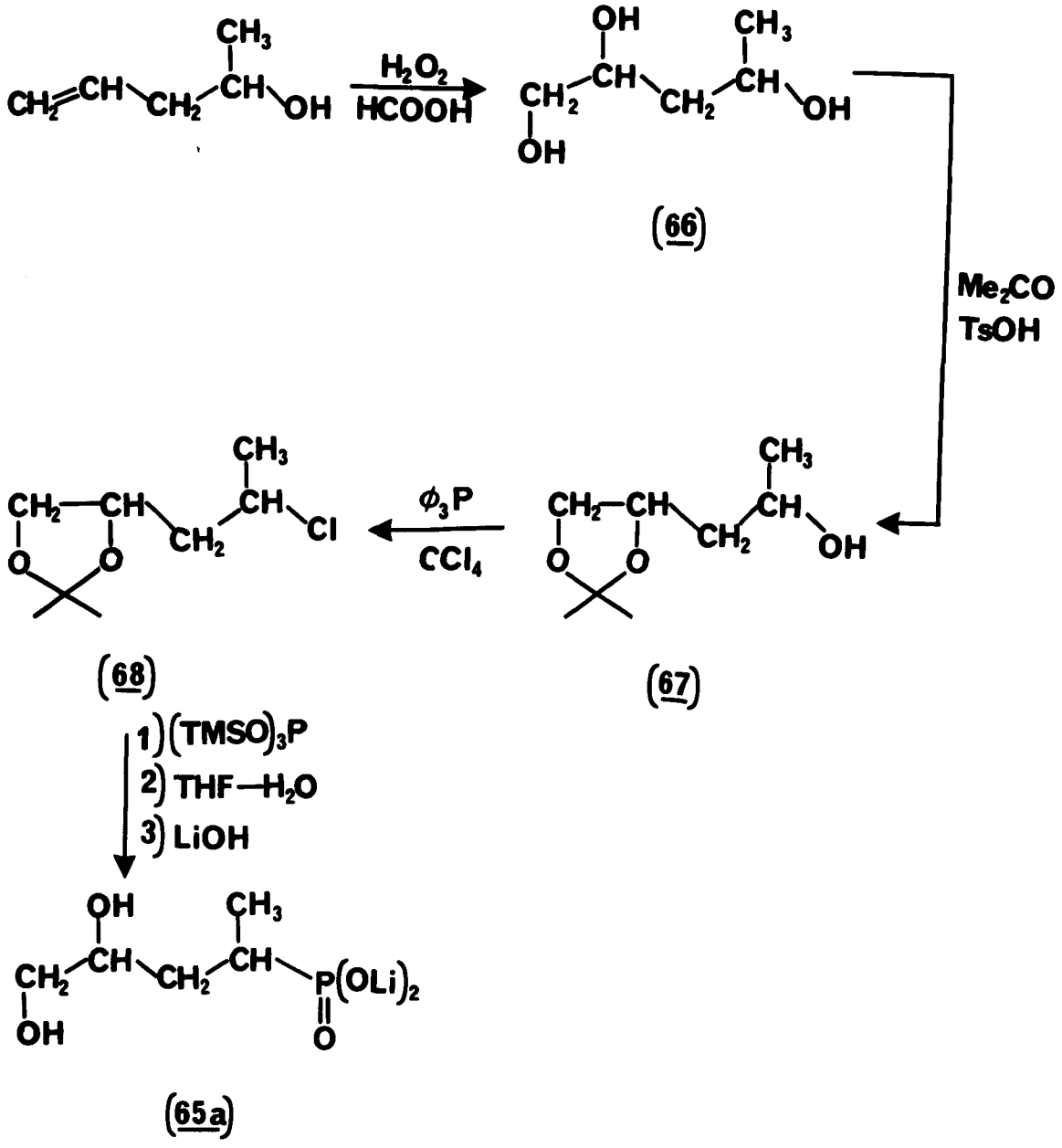


Dimethyl methylphosphonate was metalated by n-butyllithium in tetrahydrofuran[57a] or heptane at -78° under nitrogen to give ca. 0.5 M solution of the corresponding α-lithio derivative. The lithio reagent was allowed to react with epibromohydrin. When the reaction was run in tetrahydrofuran, the only material isolated was the unreacted starting material, dimethyl methylphosphonate, as the distillate and a yellow gummy residue, presumably the polymer of epibromohydrin. When the solvent was changed from tetrahydrofuran to heptane, the reaction mixture gave three unidentified fragmented products after distillation. The Corey-House reagent with cuprous bromide,[57b] when reacted with epibromohydrin also did not give the desired product (58); only an intractable gummy residue was obtained.

Preliminary evaluation of the *in vivo* activity of (58) has been performed with *E. coli* strains 8 and 4855.[65] It has been found to be an inhibitor of both strains and quantification of this result and investigation of the mechanism of inhibition is in progress.

One might also attempt to accomplish metabolic regulation through the introduction of structural entities capable of having selective steric interaction with the involved enzymes. In this direction we have prepared four molecular systems of interest. The first two of these incorporate an increase in bulk near the phosphorous site, such as methyl or hydroxy group at C-1, while maintaining the fundamental linear relationship between the phosphoryl and hydroxyl functions.

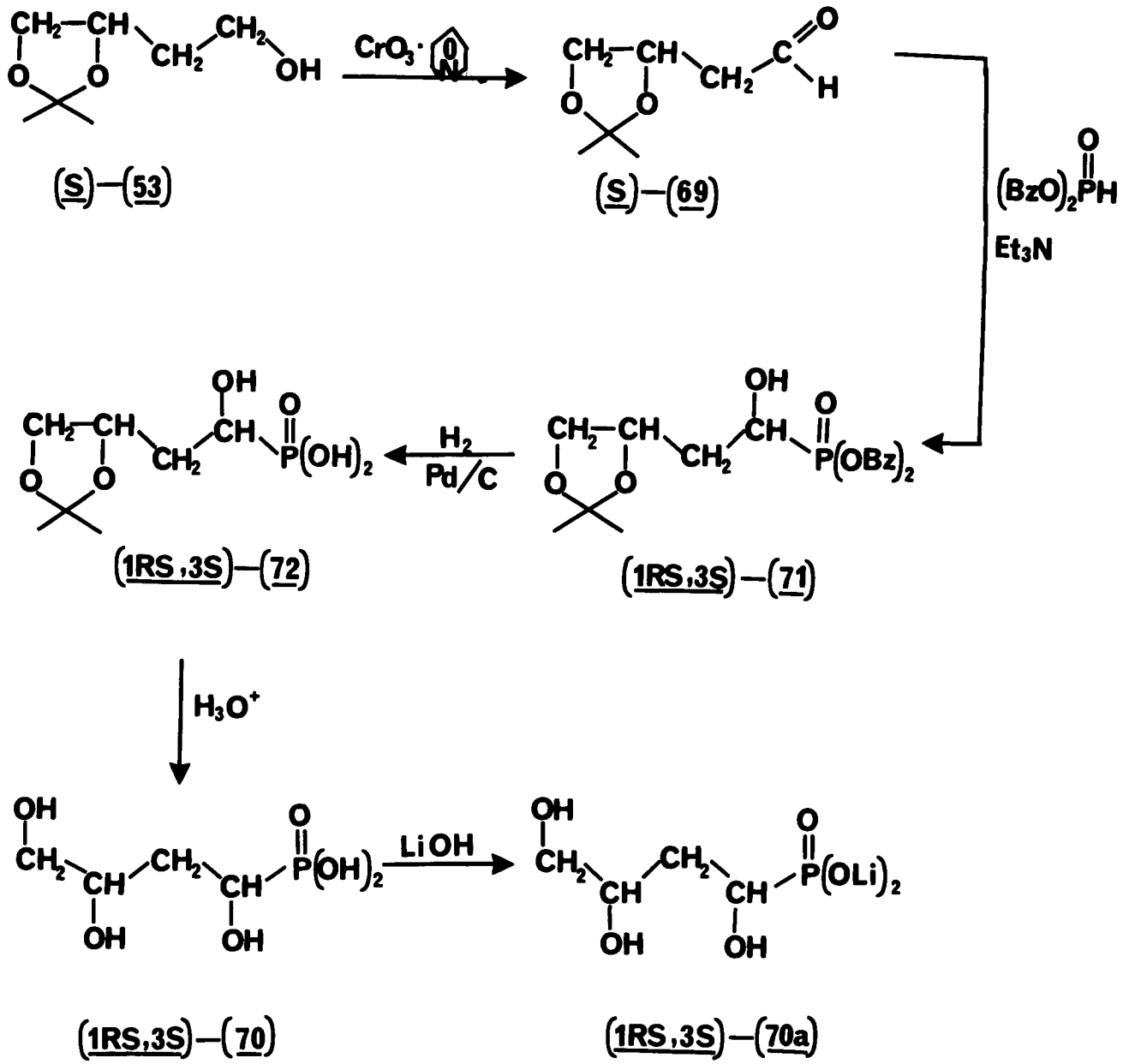
The general preparation of 1-methyl-3,4dihydroxybutyl-1-phosphonic acid, dilithium salt (65a), is illustrated in Scheme X.


H2O2
HCOOH
(66)
Me2CO
TsOH
(67)
ϕ3P
CCl4
(68)
1) (TMSO)3P
2) THF—H2O
3) LiOH
(65a)

SCHEME X

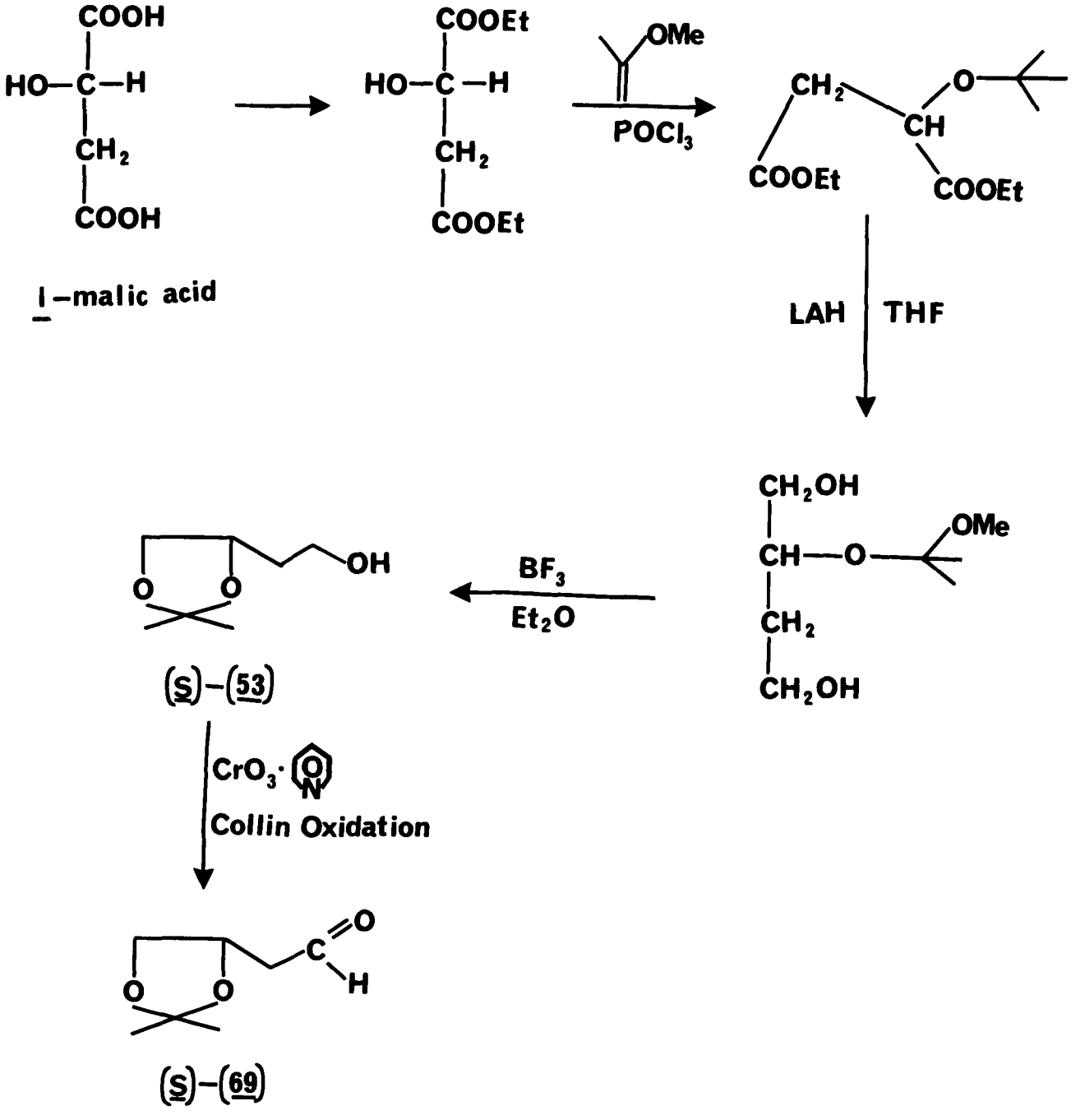
The route is fundamentally similar to that discussed above for the preparation of (21), although here the reaction of the alkyl chloride with tris(trimethysilyl) phosphite proceeded most favorable. Attempts to use a Becker reaction for introduction of the phosphorus resulted mainly in an elimination reaction. It should be noted that the approach used here generates (65) as two enantiomeric pairs. Should biological investigations indicate significant value for this structural system, optically active forms may be prepared readily starting from either the (R)- or (S)- form of aldehyde (69) which may be generated by oxidation of the corresponding enantiomer of (53) as shown in Scheme XI.

The second of these systems involves the introduction of a hydroxyl function α- to the phosphorus. The (1RS,3S)-1,3,4-trihydroxybutyl-1-phosphonic acid, dilithium salt (70), is generated from [(S)-(69)] as a pair of diastereoisomers as illustrated in Scheme XI. Phosphorylation was performed by the reaction of the aldehyde [(S)-(69)] with the anion of dibenzyl phosphite, followed by hydrogenolysis of the benzyl ester functions. For purification and analysis the resultant free acid was converted to the lithium salt [(S)-(70a)]. From the two sets of doublets for benzylic hydrogens of (71) in the NMR it is estimated that the two diastereoisomers are present in a 4:1 ratio.[66]

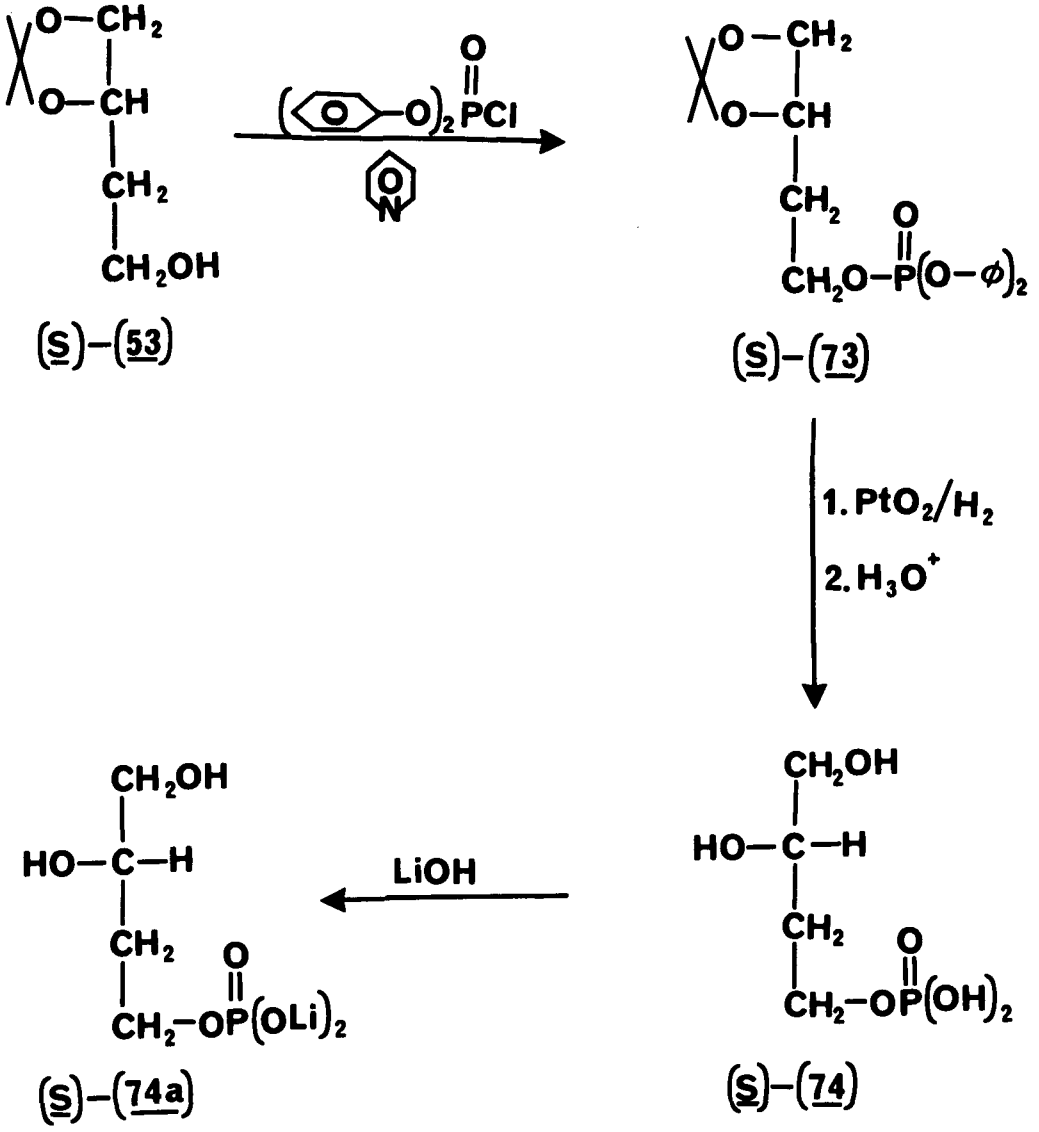

CrO3·
(S)–(53)
(S)–(69)
(BzO)2PH
Et3N
(1RS,3S)–(71)
H2
Pd/C
(1RS,3S)–(72)
H3O+
LiOH
(1RS,3S)–(70)
(1RS,3S)–(70a)

SCHEME XI

Another alternative route of synthesis of [(S)-(69)], also reported recently by E.J. Corey et al.,[67] is as follows:



The third of the systems incorporates steric variation from sn-glycerol-3-phosphate. The (S)-3,4-dihydrobutyl-1-phosphate [(S)-(74)] is lengthened as regards the relationship between the phosphoryl and hydroxyl functions. The synthesis of [(S)-(74)] from [(S)-(53)] is illustrated in Scheme XII.

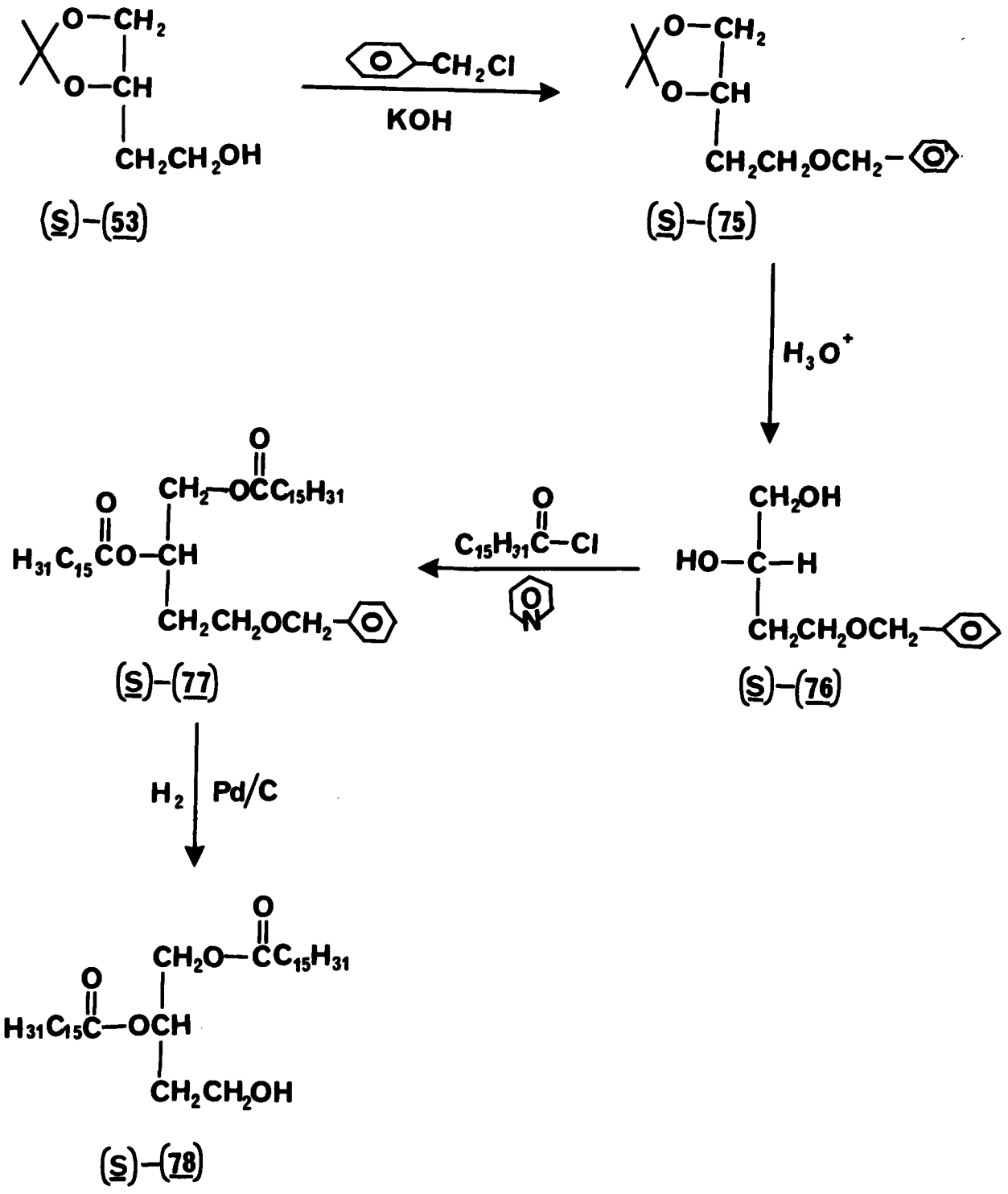


SCHEME XII

Phosphorylation was performed by reaction of the alcohol [(S)-(53)] with diphenyl phosphorochloridate and was followed by hydrogenolysis of the aryl ester functions. For purification and analysis the resultant free acid was converted to the lithium salt [(S)-(74a)]. An alternate route to the diester [(S)-(73)], displacement of chloride from [(S)-(54)] using the silver salt of diphenyl phosphoric acid according to the method of Posternak[68], gave results which were less than satisfactory, mostly starting material being recovered. The compound [(S)-(74)] is currently being evaluated with *in vivo* and *in vitro* systems.

Substitution of [(S)-(74)] for *sn*-glycerol-3-phosphate in enzymatic processes for which the latter is a natural substrate would be expected to generate a variety of new compounds having their own significance. One of the more interesting of these processes is that involving acyl CoA: *sn*-glycerol-3-phosphate acyltransferase, which, using [(S)-(74)] as a substitute, might be expected to generate a unique series of lipids, homologs of the usual glycerol-based lipids. In anticipation of their formation in biochemical systems, a homodiglyceride has been synthesized here.

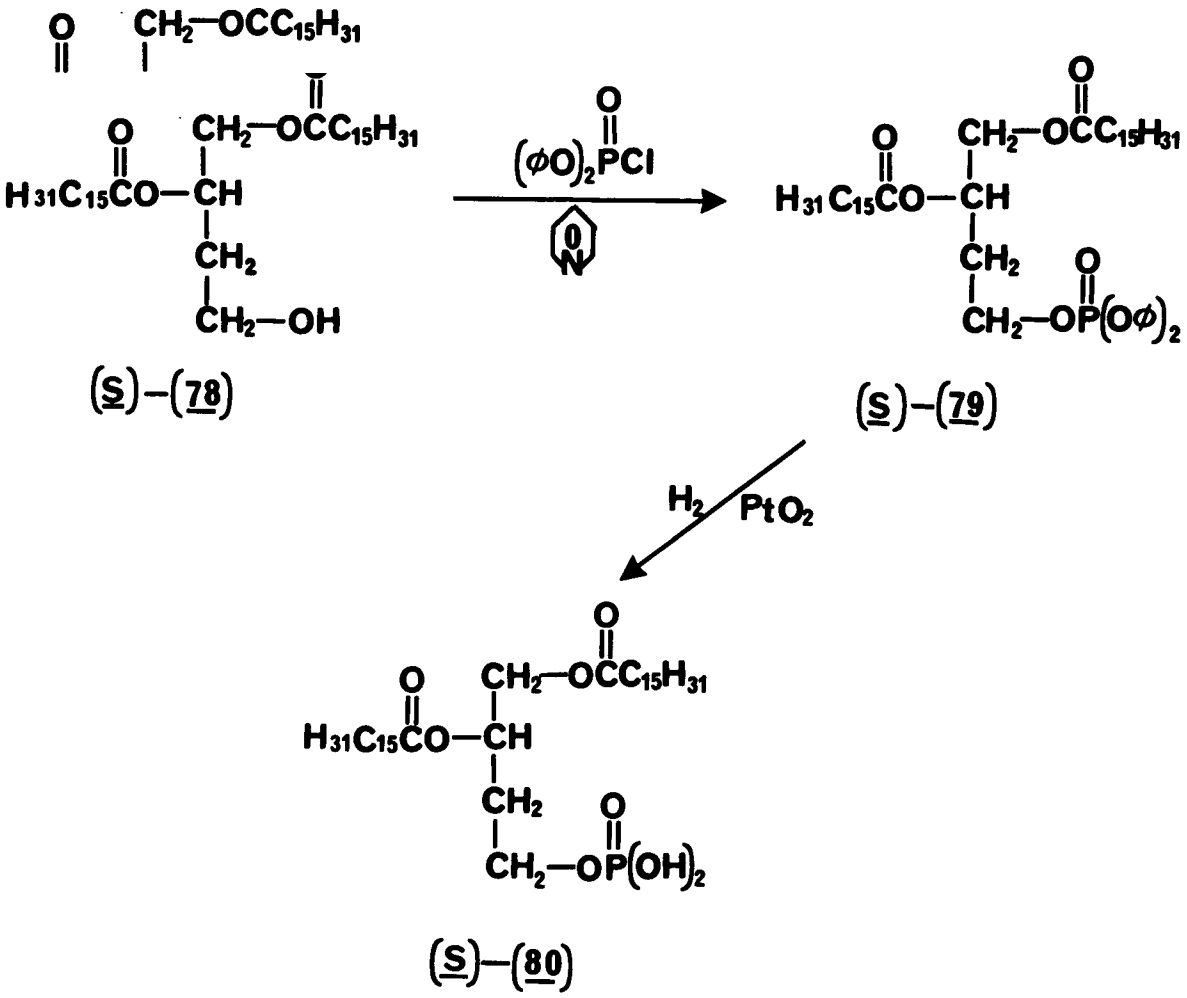
Unlike lipids related to the phosphonic acid analogue of glycerol-3-phosphate, previously synthesized in our laboratory,[37,38,69] lipids derived from [(S)-(74)] would be expected to be susceptible to phosphate hydrolysis under non-enzymatic conditions if not enzymatic as well. Thus it was of interest to prepare the homodiglyceride [(S)-(78)]. Its method of preparation is illustrated in Scheme XIII.


O—CH2
O—CH
CH2CH2OH
(S)-(53)
CH2Cl
KOH
O—CH2
O—CH
CH2CH2OCH2
(S)-(75)
H3O+
CH2OH
HO—C—H
CH2CH2OCH2
(S)-(76)
C15H31C—Cl
O
N
CH2—OCC15H31
H31C15CO—CH
CH2CH2OCH2
(S)-(77)
H2
Pd/C
CH2O—CC15H31
H31C15C—OCH
CH2CH2OH
(S)-(78)

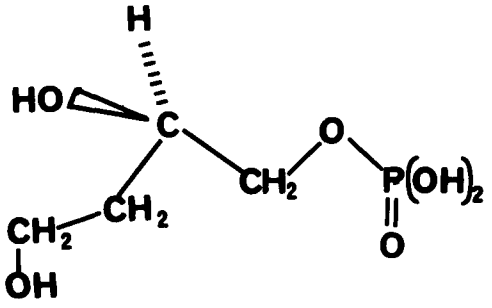
SCHEME XIII

Benzylation of [(S)-(53)] was performed using a modification of the technique described by Nelson, et al,[60] and followed by deprotection[60], acylation under standard conditions, and hydrogenolysis to give the homodiglyceride [(S)-(78)].

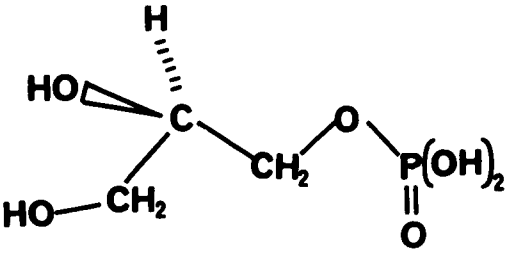
Furthermore, the homodiglyceride [(S)-(78)] can also be converted to the corresponding phosphatidic acid analogue [(S)-(80)] by phosphorylation of [(S)-(78)] with diphenyl phosphorochloridate and followed by hydrogenolysis of the aryl ester function as follows:



The fourth of the systems incorporates a positional variation with steric similarity to sn-glycerol-3-phosphate. The (R)-2,4-dihydroxybutyl-1-phosphate [(R)-(86)] has a similar configuration as regards the relationship between the phosphoryl and non-terminal hydroxyl group, but with a lengthened distance between the two hydroxyl groups.

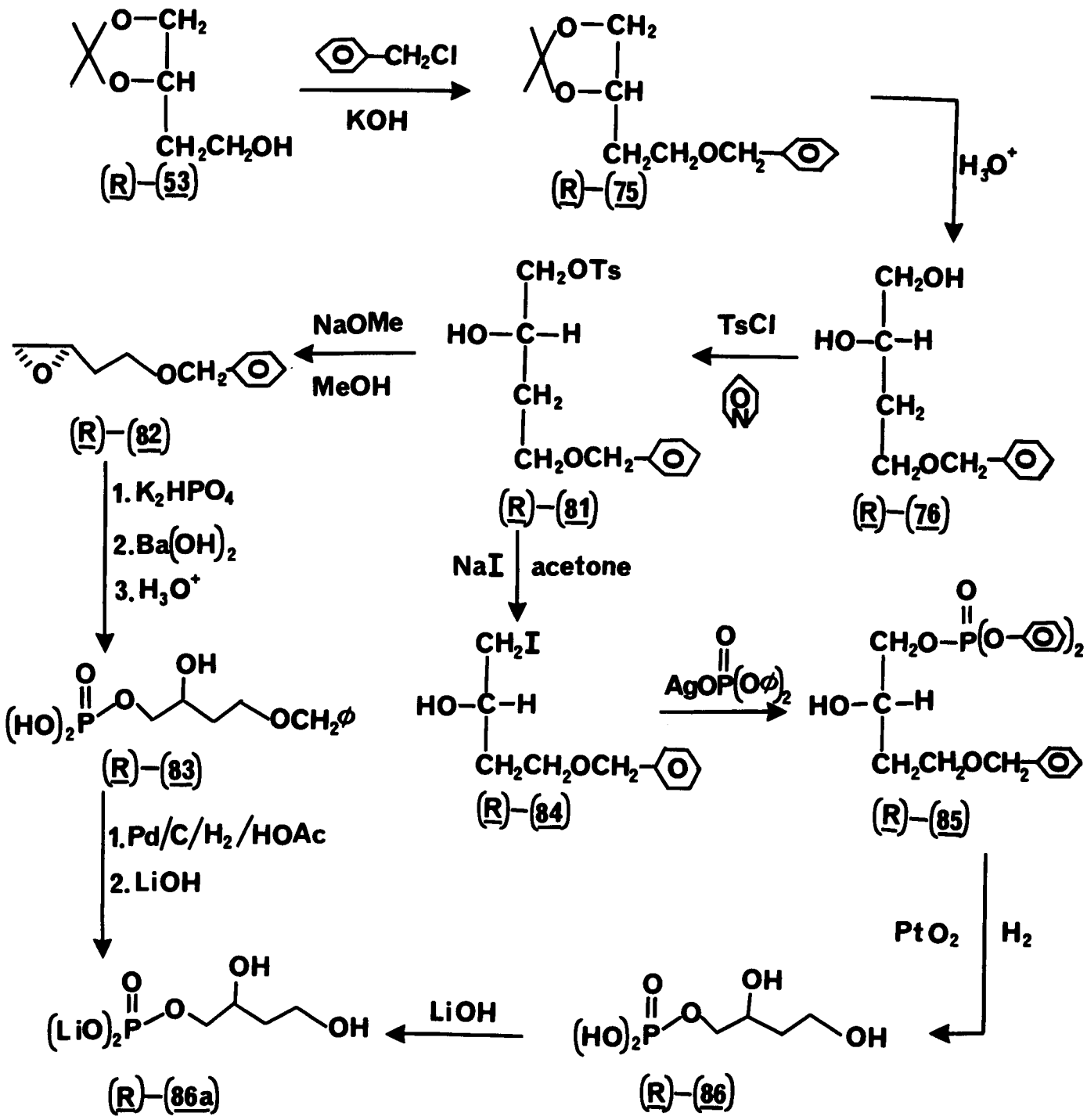


(R)—(86)



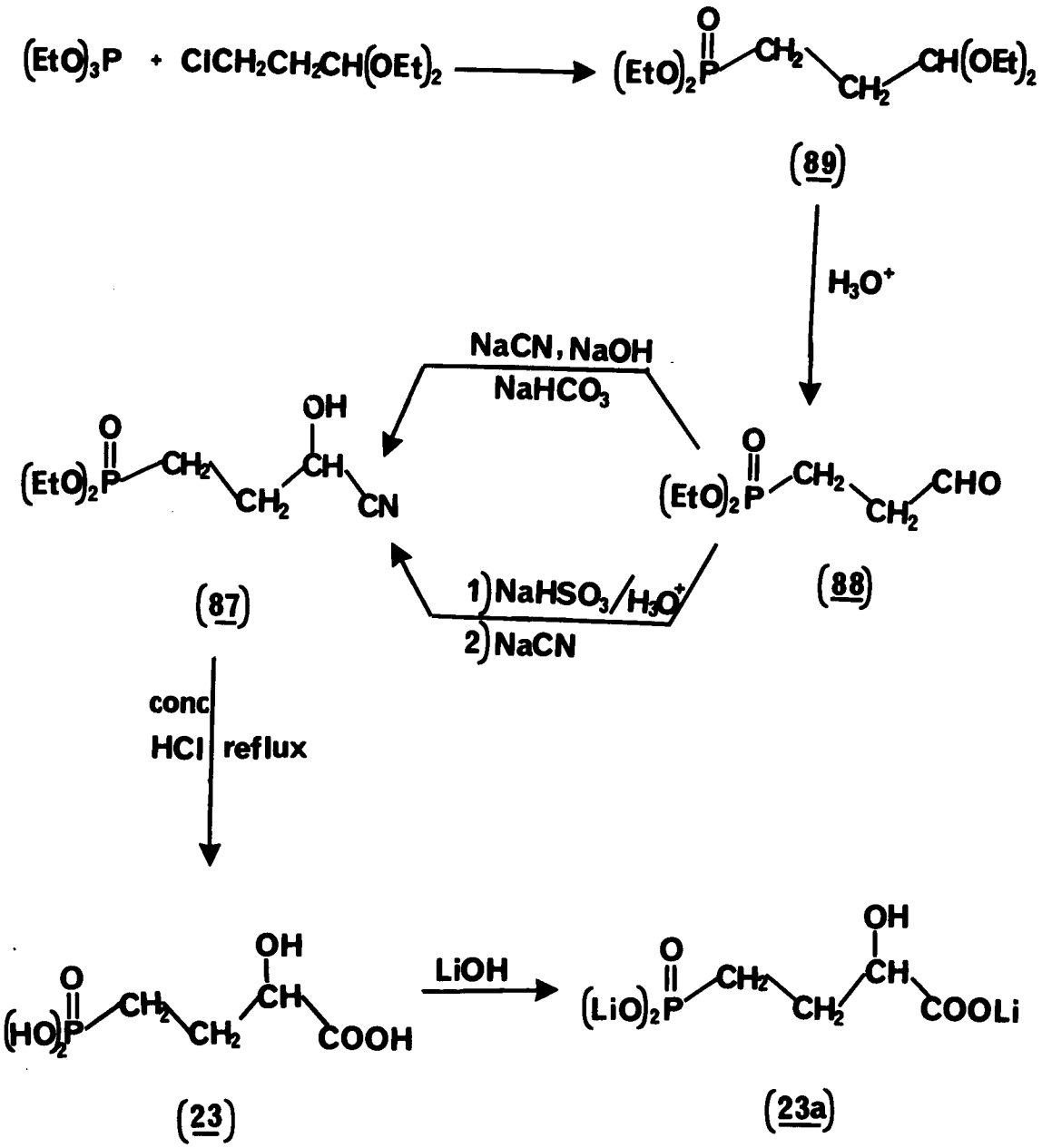
sn—glycerol—3—phosphate

The synthesis of [(R)-(86)] from [(R)-(53)] is illustrated in Scheme XIV. Benzylation, deprotection, and monotosylation were performed using a modification of the technique described by Nelson et al.[60] and followed by epoxidation[60], phosphorylation[70] and hydrogenolysis. An alternate route to the ester [(R)-(83)], displacement of iodide from [(R)-(81)] using the silver salt of diphenyl phosphoric acid according to the method of Posternak[68], gave results which again were less than satisfactory, principally unreacted starting material being recovered.


KOH
H3O+
TsCl
NaOMe
MeOH
1. K2HPO4
2. Ba(OH)2
3. H3O+
NaI
acetone
1. Pd/C/H2/HOAc
2. LiOH
LiOH
PtO2
H2

SCHEME XIV

The isosteric phosphonic acid analogue of phosphoglyceric acid, 3-carboxy-3-hydroxybutyl-1-phosphonic acid (23) has been found to interact with a variety of enzyme systems as a substitute for the natural substrate. These systems include the oxidation of $NADH_2$[21] and phosphorylation with phosphoglycerate kinase[22]. For the detailed investigation of the action of (23) with these and other enzyme systems, and *in vivo*, it is necessary for (23) to be available with a non-exchangable isotopic label. Unfortunately, the previously reported methods of preparation[19,20,21] are not convenient for the synthesis of the material bearing such a label. The synthesis of (23) bearing a carbon-14 label at the carboxyl carbon has been accomplished using a modification of the approach of Isbell *et al.*[71], the label being introduced in the form of carbon-14 cyanide by addition to the aldehyde (88) under basic conditions. A modification of the approach of Pfeiffer *et al.*[19] was not successful for cyanide addition although it works well with macroquantity, the major portion of label would be lost as a gaseous material under the standard acidic conditions. Isolation of product was performed by either preparative paper chromatography or gradient column chromatography with DEAE cellulose (DE-52; bicarbonate form); the latter is preferred. The route is outlined in Scheme XV.


(EtO)3P + ClCH2CH2CH(OEt)2
(EtO)2P(O)CH2CH2CH(OEt)2
(89)
H3O+
NaCN, NaOH
NaHCO3
(EtO)2P(O)CH2CH(OH)CH2CN
(87)
(EtO)2P(O)CH2CH2CHO
(88)
1) NaHSO3/H3O+
2) NaCN
conc HCl reflux
(HO)2P(O)CH2CH2CH(OH)COOH
(23)
LiOH
(LiO)2P(O)CH2CH2CH(OH)COOLi
(23a)

SCHEME XV

EXPERIMENTAL

General All chemicals were of reagent quality and used without further purification with the following exceptions: methylene chloride was distilled over phosphorous pentoxide and kept over molecular sieve 4A; pyridine and triethylamine were dried over potassium hydroxide pellets and distilled; benzene and hexane were dried over sodium; polmitoyl chloride (commercial source) was distilled immediately prior to use; tetrahydrofuran was distilled over lithium aluminum hydride. Thin layer chromatography was performed using Polygram Sil-N-HR sheets (Brinkman). Silica gel for preparative chromatography was from Baker (40-140 mesh). Infrared spectra were measured using a Perkin-Elmer 237-B spectrophotometer, and nmr spectra were measured with a Varian EM-360 instrument. Optical rotations were measured at 27°C using a Rudolph polarimeter (Sodium lamp) with a 1 dm cell.

Preparation of (S)-dimethyl malate [(S)-(51)] l-malic acid (25.0 g, 0.186 mole) was dissolved in 50 mL (1.23 mole)dried methanol, and 3 mL concentrated sulfuric acid were added carefully. The flask was equipped with a condenser and drying tube. The mixture was refluxed in an oil bath at 100 ± 5°C for 3 days. The cooled mixture was neutralized with anhydrous sodium bicarbonate until no more gas was evolved. The volatile components of the filtrate were removed on the rotatory evaporator under reduced pressure. The residue was vacuum distilled (b.p. = 104-5°/1.2 Torr) to yield 26.19 g (86.7%) of the ester and exhibited $[\alpha]_D^{27} = -8.36°$ (2.0 M in methanol).[72]

Preparation of (S)-1,2,4-butanetriol [(S)-(52)] l-Dimethyl malate (81 g, 0.5 mole) dissolved in 150 mL dry tetrahydrofuran was added dropwise

to a solution of lithium aluminum hydride (66 g, 1.9 mole) in 3.1 L dry tetrahydrofuran and refluxed overnight. After this time it was cooled and 500 mL of water was added carefully and the mixture filtered. The precipitate was washed four times with 500 mL absolute ethanol. The combined solution was evaporated to near dryness under reduced pressure. The inorganic material contained in the residual oil was removed by column chromatagraphy over 160 g (40-140 mesh) of silica gel which was eluated with 1.8 L (3:1 V:V) and 2.1 L (2:1 V:V) of chloroform-ethanol mixtures. These eluents were combined, the solvent was removed and the slightly yellow oil was submitted to fractional distillation to give a colorless oil (24.6 g, 46.4%): b.p. = 110-115° / 0.01 Torr (lit. 145-148° / 1.4 Torr)[54] which exhibited $[\alpha]_D^{27}$ = -25.0° (1.9 M in methanol).

Preparation of (S)-1,2-O-isopropylidenebutane-1,2,4-triol [(S)-(53)]. (S)-1,2,4-butanetriol (19.94 g, 0.188 mole) was stirred in 1.2 L acetone with 4 g of p-toluenesulfonic acid monohydrate at room temperature for 2.5 hours, after this time sodium bicarbonate was suspended in the solution and the stirring was continued for an additional 1/2 hr. The acetone was removed under reduced pressure to almost dryness; the residue was taken up in ethyl acetate and washed with aqueous solutions of sodium bicarbonate and sodium chloride, and dried over magnesium sulfate. After removal of the solvent, distillation of the residue gave 25.53 g (93%) of a colorless oil; b.p. = 93-94° / 3 Torr (lit. 87° C/ 2.2 Torr)[54] and exhibited $[\alpha]_D^{27}$ = -4.27°(1.5 M in methanol).

Preparation of (S)-1-Chloro-O-isopropylidenebutane-3,4-diol [(S)-(54)]. Triphenylphosphine (2.08 g, 7.94 m mole) in 3.0 mL of methylene chloride was added dropwise over a period of 4 hr. to a well stirred solution of 1.16 g (7.94 m mole) of (S)-1,2-O-isopropylidene-1,2,4-triol and 1.85 g (12 m mole) of carbon tetrachloride in 2.0 mL of methylene chloride at room temperature. The reaction mixture was stirred for one hr. at which time there was added 60 mL of n-pentane and the resultant precipitate of triphenylphosphine oxide was removed by filtration ahd washed with a further 60 mL of n-pentane. The combined n-pentane solutions were washed with saturated sodium bicarbonate solution, water, and brine, and dried over magnesium sulfate. After filtration to remove the drying agent the solvent was evaporated under reduced pressure and further 60 mL of n-pentane was added to precipitate the last traces of triphenylphosphine oxide. This was filtered through glass wool and the solvent removed under reduced pressure to yield 1.12 g (85.7%) of pure [(S)-(54)] as a light oil. Analysis indicated no further purification to be necessary; the material thus isolated exhibits a sharp single peak on gas-liquid-chromatography using a 14' X 1/4" column of 20% HPL on Chromosorb W; nmr ($CDCl_3$) 1.37δ(3H,s), 1.40δ(3H,s), 1.97δ(2H, m), 3.63δ(3H, m), 4.09δ(2H, m); ir ($CHCl_3$, cm^{-1}) 3010-2780 (3 peaks), 1452, 1385, 1370 1290, 1245, 1150, 1115, 1070; $[\alpha]_D^{27}$ = 14.3°(1 M, $CHCl_3$).

Anal. Calcd. for $C_7H_{13}O_2Cl$: C, 51.06; H, 7.90.

Found: C, 51.07; H, 7.87.

Preparation of dibutyl (S)-O-isopropylidene-3,4-dihydroxybutyl-1-phosphonate [(S)-(55)]. To metallic sodium (1.26 g, 0.055 g-atom) in a dried flask under an anhydrous atomsphere was added 150 mL of dry hexane. The hexane was heated to a gentle reflux, at which time there was added dibutyl phosphite (10.6 g, 55.0 m mole) dropwise over a period of 25 minutes. The heating was continued until all of the sodium metal had dissolved. At this time there was added (S)-1-chloro-O-isoproplidene-butane-3,4-diol (9.06 g, 55.0 m mole) and the reaction mixture continued to be heated at reflux for 48 hr. After cooling, the reaction mixture was washed with water, the organic layer being separated, dried over magnesium sulfate, filtered, and evaporated under reduced pressure leaving an oil which was vacuum distilled (134°/0.05 Torr) to yield 7.65 g (45%) of [(S)-(55)] as a colorless liquid. The material as isolated exhibited spectra in accord with the proposed structure although elemental analysis indicated the presence of a small impurity (ca. 5%) which could not be removed by distillation or chromatography. This possibly arises by cleavage of the dioxolane ring. The distilled material was deemed to be of sufficient purity to be used in the following step. Analytical data: nmr ($CDCl_3$) 0.98δ(3H, s), 1.44δ(3H, s), 1.21-2.56δ(12H, complex), 3.54δ(1H, m), 4.03δ(6H, complex); ir (between salts, cm^{-1}) 3025-2805, 1562, 1375, 1250, 1150, 1065, 1025, 975; $[\alpha]_D^{27}$ = -2.2°(1 M, methanol).

Preparation of dilithium (S)-3,4-dihydroxybutyl-1-phosphonic acid [(S)-(21a)]. The dibutyl (S)-O-isopropylidene-3,4-dihydroxybutyl-1-phosphonate (5.49 g, 17.9 m mole) was stirred overnight with 120 mL of 1% hydrochloric acid after which the solvent was removed under reduced

pressure without heating. The residual oil [(S)-(56)] was refluxed with 100 mL of 1.5 M lithium hydroxide solution for 48 hr. during which time a white precipitate began to form. To the filtrate of the reaction mixture was added 100 mL of absolute ethanol to complete the precipitation; the precipitate was filtered, washed with anhydrous ethanol and ether, and dried under vacuum to yield 2.48 gm (76.0%) of pure[(S)-(21a)]. Spectral data (nmr, ir) of [(S)-(21a)] thus isolated corresponded with that for previous preparations of recemic material.[53] The optical rotation was measured by dissolving 0.95 gm of [(S)-(21a)] in 4 mL of conc. hydrochloric acid and diluting with absolute ethanol to a total volume of 10 mL: $[\alpha]_D^{27}$ = +14.6 (vide supra, P. 40); the salt was insufficiently soluble in non-acidic media for measurements to be made.

Anal. Calcd. for $C_4H_9PO_5Li_2$: C, 26.40; H, 4.99;

Found: C, 26.42; H, 5.12.

Preparation of (R)-dimethyl malate [(R)-(51)]. Commercial d-malic acid (50 g, 0.372 mole) was converted to the dimethyl ester in 88% (53.1 g) yield by the procedure previously described for the (S)-enentiomer and exhibited $[\alpha]_D^{27}$ = +6.5° [72] after vacuum distillation (b.p. = 97° / 0.7 Torr). Spectral data (nmr, ir) corresponded with those for (S)-enantiomer.

Preparation of (R)-1,2,4-butanetriol [(R)-(52)]. The d-dimethyl malate (53.1 g, 327 m mole) was dissolved in 70 mL of tetrahydrofuran and added dropwise to 45.0 g (1.16 mole) of lithium aluminum hydride in 2100 mL of dry tetrahydrofuran and heated at reflux for 18 hr. The excess hydride was decomposed by the addition of 360 mL of water. The resultant mixture was filtered and the filtrate concentrated to a thick

oil under reduced pressure. This concentrate was purified by subjection to chromatography on a column of 120 gm of silica gel being eluted with 1400 mL (3:1 V:V) and 1600 mL (2:1 V:V) of chloroform-ethanol. The combined eluents were concentrated under reduced pressure and the residual oil vacuum distilled (120°-125° / 0.05 Torr) to yield 16.87 g (48.6%) of (R)-1,2,4-butanetriol which exhibited nmr and ir spectra identical to those for the (S)-enantiomer and $[\alpha]_D^{27}$ = +24.6°(2.8 M in methanol) which compared with a value of -25.0° as measured for the (S)-enantiomer under identical conditions.

Preparation of (R)-1,2-O-isopropylidenebutane-1,2,4-triol [(R)-(53)]. (R)-1,2,4-butanetriol (5.7 g, 54.0 m mole) was stirred in 450 mL of acetone in the presence of 3 g of p-toluenesulfonic acid monohydrate at room temperature for 3.5 hr. after which anhydrous sodium bicarbonate was added and the stirring continued. The reaction mixture was filtered and the solvent evaporated under reduced pressure to yield an oily residue which was taken up into ethyl acetate, washed succesively with aqueous sodium bicarbonate and brine and dried over magnesium sulfate. After filtration and removal of the solvent under reduced pressure the residue was vacuum distilled (82° / 3.2 Torr) to yield 6.12 g (77.6%) of [(R)-(53)] which exhibited nmr and ir identical to those of the (S)-enantiomer and $[\alpha]_D^{27}$ = 1.8°(2.0 M in methanol).

Preparation of (R)-1-chloro-O-isopropylidenebutane-3,4-diol [(R)-(54)]. (R)-1,2-O-isopropylidenebutane-1,2,4-triol (4.64 g, 31.8 m mole) was immediately treated in the same manner as described above for the (S)-enantiomer for conversion to the chloride [(R)-(54)]. There was thus obtained 3.0 gm (57%) of (R)-1-chloro-O-isopropylidenebutane-

3,4-diol [(R)-(54)] as a light oil which exhibited chromatographic and spectral properties identical to those of the (S)-enantiomer and $[\alpha]_D^{27}$ = +15.1° (1 M, chloroform) (vide supra, P. 40).

Preparation of dibutyl (R)-O-isopropylidene-3,4-dihydroxybutyl-1-phosphonate [(R)-(55)]. (R)-1-chloro-O-isopropylidenebutane-3,4-diol (3.0 gm, 18.0 m mole) was treated as described above for the (S)-enantiomer to yield on distillation (125° / 0.05 Torr) 2.50 g (45.0%) of [(R)-(55)] which exhibited ir and nmr spectra identical to those observed for the (S)-enantiomer.

Preparation of dilithium (R)-3,4-dihydroxybutyl-1-phosphonic acid [(R)-(21a)]. The dioxolane [(R)-(55)] combined from two preparations (5.74 g, 18.7 m mole) was deprotected and hydrolyzed as already described for the (S)-enantiomer to yield 3.00 g (88.2%) of [(R)-(21a)] which exhibited nmr and ir spectra identical to those of the (S)-enantiomer, and $[\alpha]_D^{27}$ = -13.5°.

Anal. Calcd. for $C_4H_9PO_5Li_2$: C, 26.40; H, 4.99;
Found: C, 26.38; H, 5.23.

Preparation of dilithium (R,S)-3,4-dihydroxybutyl-1-phosphonic acid(21). Racemic 1,2 4-butanetriol (63.6 g, 600 m mole) was treated in an identical manner as described above for the optically active enantiomers to yield on distillation (76° / 1 Torr) 60.0 g (68.5%) of (53) which corresponded in ir and nmr spectra with the enantiomeric forms described previously. Conversion of 40.6 g (278 m mole) of racemic dioxlane (53) to the chloride was preformed in an identical manner to that as described for the enantiomeric forms to yield 32.5 g (71.1%) of (54). The chloride (54), 23.0 g (140 m mole) was then phosphonylated using a solution of sodium dibutyl phosphite in hexane as described above for the optically active

enantiomers. In this way was obtained 25.6 g (59.7%) of (55) upon vacuum distillation (130°/0.1 Torr). The diester (55) was converted in the manner described for the enantiomeric forms in 71% yield to the racemic (21a) which corresponds in all spectral and chromatographic properties with that as prepared by other routes.[11,53]

Preparation of dibutyl (R,S)-4-tosyloxy-3-hydroxybutyl-1-phosphonate (59). The racemic dioxalane (55), 15.0 g (49.0 m mole), was stirred with 330 mL of 1% hydrochloric acid, after which the solvent was removed under reduced pressure. The residual oil was dissolved in 400 mL of chloroform, washed with water (3 X 100 mL) and dried over magnesium sulfate. After filtration the solvent was evaporated under reduced pressure to yield 11.0 g (41.35 m mole) of (56) as a yellow oil which was dissolved in 13 mL of pyridine, cooled to 0°C, and to which a solution of 7.90 g (41.4 m mole) of p-toluenesulfonyl chloride in 96 mL of benzene was added dropwise. The reaction mixture was allowed to come to room temperature and was stirred for 48 hr. after which there was added a further 120 mL of benzene and the reaction mixture was washed successively with 2 N hydrochloric acid (3 X 130 mL), saturated aqueous sodium bicarbonate (3 X 60 mL), water (4 X 140 mL)and dried over magnesium sulfate. The solvent was evaporated under reduced pressure and the residual oil taken up in a minimum volume of 1:1 ethyl acetate-chloroform and passed through a short (20 g) column of silica gel. After removal of the solvent under reduced pressure there was isolated 2.24 g (12.6%) of (59) as an oil which exhibited a single spot of R_f = 0.34 upon thin layer chromatography developed with 1:1 ethyl acetate-chloroform. Spectral analysis: nmr($CDCl_3$) 0.61-1.92δ(18H, complex, $CH_3CH_2CH_2$- and p-CH_2CH_2-), 2.15δ(3H, s), 3.30-4.02δ(7H, complex,

OCH- and OCH_2-), 4.39δ(1H, s), 6.86-7.60δ(4H, AA'BB'); ir (between salts, cm^{-1}) 3130-3750, 2825-3090, 1600, 1472, 1370, 1248, 1200, 1185 1105.

Anal. Calcd. for $C_{19}H_{33}PSO_7$: C, 52.28; H, 7.62;
Found: C, 52.22; H, 7.51.

Preparation of dibutyl (R,S)-3,4-epoxybutyl-1-phosphonate (58). To a solution of 2.24 g (5.34 m mole) of (59) freshly prepared in 8 mL of methanol was added a solution of 0.28 g (5.35 m mole) of sodium methoxide in 1.1 mL of water. The reaction mixture was refluxed for 3 hr., cooled, and the solvent removed under reduced pressure. To the residue was added 100 mL of ether and the resultant precipitate of sodium tosylate removed by filtration under suction. The solution was washed with water (3 X 25 mL), dried over magnesium sulfate, and the solvent removed under reduced pressure to yield an oil which was vacuum distilled (108° / 0.05 Torr) giving 0.90 g (63.7%) of pure (58) which exhibited a single spot of R_f = 0.46 upon thin layer chromatography with 1:1 ethyl acetate-chloroform and spectral data in accord with that for a terminal epoxide.[73] Spectral analysis: nmr ($CDCl_3$) 0.76-1.80δ (18H, complex, $CH_3CH_2CH_2$- and PCH_2CH_2-), 2.02δ(1H, dd), 2.27δ(1H, t), 2.55δ(1H, m), 3.59δ(4H, dt); ir (between salts, cm^{-1}) 2815-3050, 1470 1252, 1155, 1122, 1070, 1030, 980.

Anal. Calcd. for $C_{12}H_{25}PO_4$: C, 54.53; H, 9.53;
Found: C, 54.68; H, 9.37.

Preparation of monoanilinium salt of 3-butenyl-1-phosphonic acid (61). A 25 mL flask containing diethyl 3-butenyl-1-phosphonate (64)[11] 4.24 g (22.08 m mole), was flushed several times with nitrogen and 7.96 g (52.11 m mole) of bromotrimethysilane was injected by syringe and the reaction mixture was stirred under nitrogen atomsphere at room temperature for one day after which the excess bromotrimethylsilane was removed under high vacuum at room temperature. To the residue was added chlorotrimethysilane, 6.4 g (58.9 m mole), and refluxed for one day after which the excess reagent was removed under high vacuum at room temperature and the residual liquid was vacuum distilled (110°/ 3.2 Torr) to yield 4.74 g (77.8%) of pure bis(trimethysilyl) ester. Spectral analysis: nmr ($CDCl_3$) 0.58δ(18H, s), 1.40-2.80δ(4H, complex), 4.90-5.30δ(2H, m), 5.63-6.26δ(1H, complex); ir (between salts cm^{-1}) 2994, 1644, 1410, 1253, 1025, 910, 848, 761. To 2.76 g(10 m mole) of bis(trimethylsilyl) ester in 10 mL ether was added a mixture of 1.38 g (30 m mole) of absolute ethanol and 1.86 g (20 m mole) of aniline and stirred for one hour. The resultant precipitate was filtered and washed several times with ether to yield 2.15 g (94%) of pure (61) of mp 154-156°. Spectral analysis: nmr (5% CD_3COOD in D_2O) 1.36-2.70δ (4H, complex), 4.66-6.16δ(7H, complex), 7.25δ(5H, s); ir(KBr, cm^{-1}) 1941-3333, 1644, 1618, 1587, 1508, 1470, 1420 1333, 1300, 1233, 1189, 1140, 1112, 1074, 1019, 959, 931, 919, 846, 747, 691.

Anal. Calcd. for $C_{10}H_{16}PO_3N$: C, 52.40; H, 7.04;
Found: C, 52.62; H, 7.35.

Preparation of 1,2,4-pentanetriol (66). A solution of 25 g (0.29 mole) of 4-penten-2-ol with 200 mL of 88% formic acid and 66 g of 30% hydrogen peroxide was stirred overnight. The reaction tempterature was maintained below 50°. The reaction mixture was then heated at 50° for 5 hr. The volatile components were removed under reduced pressure and 200 mL saturated hydrogen chloride in methanol was added and refluxed for 3 hr. The volatile components were removed under reduced pressure and the residual liquid vacuum distilled (115°-120° / 0.01 Torr) to yield 21.36 gm (62%) of triol (66) (lit. 125° / 0.5 Torr)[75].

Preparation of 1.2-O-isopropylidenepentane-1,2,4-triol (67). The triol, 20.4 g (169 m mole), was stirred in 1000 mL of acetone with 2 g of p-toluenesulfonic acid monohydrate at room temperature for 14 hr. Anhydrous sodium bicarbonate was suspended in the reaction mixture, stirred for 2 hr., and filtered. The acetone was removed under reduced pressure and the residue dissolved in 150 mL of ethyl acetate which was then washed successively with saturated aqueous sodium bicarbonate solution, brine, and water and dried over magnesium sulfate. After evaporation of the solvent under reduced pressure the residual liquid was vacuum distilled (86° / 1.7 Torr) to yield 21.3 g (78.7%) of pure dioxolane (67). Spectral analysis: nmr ($CDCl_3$) 1.20δ(3H, d, J_A=6Hz), 1.35δ(3H, s), 1.41δ(3H, s), 1.69δ(2H, dd, J_B=J_C=5.5 Hz), 3.16δ(1H, s), 3.39-4.55δ(4H, m).

Anal. Calcd. for $C_8H_{16}O_3$: C, 59.97; H, 10.10;

Found: C, 59.89; H, 10.06.

Preparation of 4-chloro-O-isopropylidenepentane-1,2-diol (68). To a well stirred solution of 16.0 g (100 m mole) of alcohol (67) with 23.3 g (151 m mole) of carbon tetrachloride in 25 mL of methylene

chloride. The reaction mixture was stirred for 6 days after which 720 mL of n-pentane were added and the resultant precipitate of triphenylphosphine oxide was filtered. The filtrate was washed successively with 300 mL each of saturated aqueous sodium bicarbonate solution, brine, and water and then dried over magnesium sulfate. The solvent was removed under reduced pressure to yield 12.6 g (70.6%) of pure (68). Spectral analysis: nmr ($CDCl_3$) 1.00-2.38δ(11H, complex, $-CH_2-$, $-C(CH_3)_2$, $-CH_3$), 3.38-4.56δ(4H, complex, OCH, OCH_2, ClCH)[76]; ir(between salts, cm^{-1}) 2735-3110, 1430, 1370, 1250, 1195, 1052.

Anal. Calcd. for $C_8H_{15}O_2Cl$: C, 53.78; H, 8.46;

Found: C, 53.93; H, 8.20.

Preparation of dilithium 1-methyl-3,4-dihydroxybutyl-1-phosphonic acid (65a). A mixture of 8.3 g (35 m mole) of the chloride (68) and 42 g (140 m mole) of tris(trimethylsilyl) phosphite[33-36] was stirred under nitrogen atomsphere at 170° for 3 days. At this time the excess phosphite was removed under reduced pressure and a mixture of 40 mL of water and 230 mL of tetrahydrofuran was added and heated at reflux for 16 hours. After cooling, the reaction mixture was decolorized with charcoal and filtered. The filtrate was concentrated under reduced pressure and ethanolic lithium hydroxide solution was added to pH=8. The resultant precipitate was filtered, washed with ethanol and ether, and dried under vacuum to give 2.83 g of crude (65a). The crude material was reprecipitated from aqueous solution by addition of ethanol to yield 1.24 g (18.1%) of (65a) which exhibited a single spot of R_f=0.62 on thin layer chromatography with 10:1 methanol- 0.1 N hydrochloric acid and gave acceptable analytical data. Spectral analysis:

nmr (5% CD_3COOD in D_2O) 1.32δ(3H, broadened singlet), 1.42-2.29δ (3H, m), 3.41-4.48δ(3H, m); ir (KBr, cm^{-1}) 3200-3800, 2750-3080, 1650, 1430, 1320.

Anal. Calcd. for $C_5H_{11}PO_5Li_2$: C, 30.64; H, 5.66;
Found: C, 30.58; H, 5.64.

Preparation of (S)-1-oxo-O-isopropylidenebutane-3,4-diol [(S)-(69)]. The primary alcohol [(S)-(53)], 8.76 g (60.0 m mole), dissolved in 30 mL of methlene chloride, was added in a single portion to a solution of chromium trioxide-pyridine complex, prepared by the addition of 36.0 g (360 m mole) of chromium trioxide to 58.2 mL of pyridine with vigorous stirring over a 12 hr. period. The solvent was evaporated under reduced pressure and 1400 mL of ether were added to the residue. The insoluble salts were removed by filtration and the filtrate was washed successively with 5% aqueous sodium bicarbonate, cupric sulfate solution, brine, and water and dried over sodium sulfate. The ether was removed under reduced pressure and the residual liquid vacuum distilled (74° / 4.7 Torr) to yield 2.88 g (33.3%) of pure [(S)-(69)]. Spectral analysis: nmr ($CDCl_3$) 1.39δ(3H, s), 1.42δ(3H, s) 2.77δ(2H, dd, J_A= 6 Hz, J_B= 1.5 Hz), 3.60δ(1H, dd, J_C= 8 Hz, J_D= 6 Hz), 4.20δ(1H, dd, J_C= 8 Hz, J_E= 6 Hz), 4.59δ(1H, m), 9.80δ(1H, t, J_B= 1.5 Hz); ir ($CHCl_3$, cm^{-1}) 2840-3080, 2765, 1725, 1452, 1385, 1370, 1345, 1325, 1250, 1152, 1065; $[\alpha]_D^{27}$ = +13.3° (1 M in chloroform).

Anal. Calcd. for $C_7H_{12}O_3$: C, 58.32; H, 8.39;
Found: C, 57.95; H, 8.46.

Preparation of dibenzyl (1RS, 3S)-3,4-O-isopropylidene-1,3,4-trihydroxybutyl-1-phosphonate [(1RS,3S)-(71)]. A solution of 6.54 g

(26.6 m mole) of dibenzyl hydrogen phosphite[74] with 3.02 g (20.1 m mole) of [(S)-(69)] and 1 mL of triethylamine in 60 mL of dry benzene was heated at 60°-80° for 4 hours. The reaction mixutre was diluted with 400 mL of benzene and washed successively with saturated aqueous sodium bicarbonate (2 X 120 mL), 1 N hydrochloric acid (2 X 120 mL), water (2 X 120 mL) and dried over magnesium sulfate. Upon evaporation of the solvent there was obtained 5.72 g (69.8%) of [(1RS, 3S)-(71)] as an oil which exhibited a single spot of R_f= 0.36 on thin layer chromatography with 5:5:1 benzene-ethyl ether-ethanol. From the two sets of doublets for benzylic hydrogens in the nmr it is estimated that the two diastereomers are present in a 4:1 ratio.[66] Spectral analysis: nmr ($CDCl_3$) 1.30δ(6H, broad singlet), 2.00δ(2H, m), 3.15δ(1H, t), 3.84-4.49δ(3H, complex, OCH_2, OCH), 4.55δ(1H, s), 4.85-5.18δ(4H, pair of doublets for benzylic hydrogens), 7.30δ(10H, s); ir (between salts, cm^{-1}) 3160-3730, 2790-3010, 1505, 1460, 1375, 1370, 1225, 1160, 1035; $[\alpha]_D^{27}$ = +0.7° (0.4 M in chloroform).

Anal. Calcd. for $C_{21}H_{27}PO_6$: C, 62.06; H, 6.09;
Found: C, 62.33; H, 6.45.

Preparation of (1RS, 3S)-1,3,4-trihydroxybutyl-1-phosphonic acid [(1RS, 3S)-(70)]. The dibenzyl phosphonate [(1RS, 3S)-(71)], 5.0 g (12.3 m mole), was dissolved in 130 mL of absolute ethanol and hydrogenated on a Parr apparatus over 200 mg of 10% pd/C at 40 psig hydrogen at room temperature until no more hydrogen was absorbed. The catalyst was removed by filtration through Celite and the filtrate was evaporated under reduced pressure to yield 2.0 g (87%) of the free acid [(1RS, 3S)-(70)] which exhibited a singlet spot of R_f = 0 upon thin layer chromatography

with 5:5:1 benzene-ethyl ether-ethanol[9] and no benzylic or methyl protons in the nmr.[9] For analysis the free acid was converted to the dilithium salt by being dissolved in ethanol and treated with saturated ethanolic lithium hydroxide until basic (pH = 8). The resultant precipitate was filtered, washed several times with ethanol and ether and dried under vacuum. Spectral analysis: nmr (D_2O + CD_3COOD) 2.65δ(2H, broad), 3.85-5.10δ(4H, complex); ir (KBr, cm^{-1}) 3040-3750, 2850-3000, 1650, 1435; $[\alpha]_D^{27}$ = -6.92° (0.52 g in 2 mL conc. HCl diluted to 10 mL with ethanol).

Anal. Calcd. for $C_4H_9PO_6Li_2$: C, 24.27; H, 4.58;
Found: C, 24.34; H, 4.55.

Preparation of dilithium (S)-3,4-dihydroxybutyl-1-phosphate [(S)-(74a)]. To 2.92 g (22.0 m mole) of [(S)-(53)] in 12 mL of pyridine cooled to 0° was added dropwise 7.14 g (25.0 m mole) of diphenyl phosphorochloridate. After standing for 12 hr. at 0°, 1.2 mL of water was added and the reaction mixture concentrated under reduced pressure. The residual solid was dissolved in 150 mL of benzene and washed successively with 100 mL each of water, cold 1 N hydrochloric acid, cold 1 N potassium carbonate solution, and water and dried over magnesium sulfate. After evaporation of the solvent under reduced pressure the residual solid was dissolved in 150 mL of absolute ethanol and hydrogenated over 0.1 g of platinum oxide in a Parr apparatus at 52 psig of hydrogen until no more hydrogen was taken up. The catalyst was removed by filtration through Celite and the solvent evaporated under reduced pressure to yield the free acid [(S)-(74)] as a viscous oil. For analysis, the free acid was converted to its dilithium salt [(S)-(74a)] by

dissolution in 50 mL of ethanol and the addition of a saturated ethanolic solution of lithium hydroxide monohydrate to pH = 8. The resultant precipitate was filtered, washed with ethanol and ether, and dried under vacuum to yield 1.53 g (33.6%) of [(S)-(74a)] as a crystalline material which analyzed as hemihydrate; this water is firmly bound and could not be removed by heating the material under vacuum. The material exhibited a single spot of R_f = 0.50 on thin layer chromatography using 10:1 methanol-0.01 N hydrochloric acid. Spectral analysis: nmr (5% CD_3COOD in D_2O) 1.67δ(2H, m), 3.15-4.30δ(5H, complex); ir (KBr, cm^{-1}) 3050-3850, 2760-3015, 1650, 1455, 1430, 1390, 1340, 1260, 1210; $[\alpha]_D^{27}$ = -20.4° (free acid, 0.7 M in ethanol).

Anal. Calcd. for $C_4H_9PO_6Li_2$-1/2H_2O: C, 23.19; H, 4.34;

Found: C, 23.35; H, 4.46.

Preparation of (S)-4-benzyloxy-1,2-butanediol acetonide [(S)-(75)]. To 5.25 g (36.6 m mole) of [(S)-(53)] in 20 mL of dry dimethyl formamide was added 2.47 g (44 m mole) of finely powdered potassium hydroxide with stirring and cooling to 0°. Benzyl chloride, 5.80 g (45.9 m mole), was added dropwise and the reaction mixture allowed to warm, ultimately being heated at 70° for 6 hr. After cooling, 50 mL of water was added and the reaction mixture extracted with chloroform (4 X 35 mL). The combined extracts were washed with water (3 X 35 mL), dried over magnesium sulfate, and evaporated under reduced pressure; the residual yellow liquid was vacuum distilled (114° / 0.05 Torr) to yield 4.64 g (53.5%) of the benzyloxy derivative [(S)-(75)]. Spectral analysis: nmr ($CDCl_3$) 1.33δ(3H, s), 1.36δ(3H, s), 1.85δ(2H, dt, J_A = 6 Hz, J_B = 5.5 Hz), 3.36-4.33δ(5H, complex), 4.45δ(2H, s), 7.28δ(5H, s);

ir (between salts, cm^{-1}) 2816-3134, 1500, 1460, 1365, 1320, 1265, 1215 1169, 1095, 1035, 870, 740, 698; $[\alpha]_D^{27}$ = -5.2° (1.5 M in methanol).

Preparation of (S)-4-benzyloxybutane-1,2-diol [(S)-(76)]. The benzyloxy acetonide [(S)-(75)], (3 g, 12.66 m mole), was treated with 2 mL of 2 N hydrochloric acid and sufficient acetone to effect solution. After heating at reflux for 1.5 hr. the mixture was cooled, 20 mL of ethanol was added, and the solvent removed under reduced pressure. The residue was dissolved in 55 mL of chloroform, washed with water (3 X 12 mL), dried over sodium sulfate, and the solvent removed under reduced pressure to yield 2.53 g (68.1%) of pure [(S)-(76)] as a yellow oil. Spectral analysis: nmr ($CDCl_3$) 1.69δ(2H, dt, J_A= 6 Hz, J_B= 5.5 Hz), 3.18-3.87δ(5H, complex), 4.03δ(2H, broad singlet), 4.41δ(2H, s), 7.28δ(5H, s); ir (between salts, cm^{-1}) 3160-3740, 2755-3120, 1500, 1460, 1365, 1320, 1215, 1095, 1035, 910, 870, 740, 698; $[\alpha]_D^{27}$ = -15.6° (0.3 M in methanol).

Anal. Calcd. for $C_{11}H_{16}O_3$: C, 67.32; H, 8.22;
Found: C, 67.16; H, 8.32

Preparation of (S)-4-benzyloxy-1,2-dipalmitoyloxybutane [(S)-(77)]. To a solution of 1.00 g (5.10 m mole) of [(S)-(76)] in 2 mL of pyridine cooled to 0°C, was added dropwise 3.30 g (12.0 m mole) of palmitoyl chloride in 24 mL of benzene. The reaction mixture was allowed to come to room temperature and continue stirring for 24 hr. at which time a further 25 mL of benzene was added and the mixture washed successively with 2 N hydrochloric acid (3 X 7 mL) and water (4 X 7 mL) and dried over magnesium sulfate. Upon evaporation of the solvent under reduced pressure there was obtained a white solid which was twice recrystallized from 95% ethanol to yield 1.54 g (44.9%) of pure [(S)-(77)] of m.p. 40-41°.

Spectral analysis: nmr ($CDCl_3$) 0.59-2.04δ(60H, complex), 2.08-2.59δ (4H, complex), 3.57δ(2H, t, J = 6 Hz), 4.25δ(2H, m), 4.51δ(2H, s), 5.29δ(1H, m), 7.32δ(5H, s); ir (KBr, cm^{-1}) 2830-3075, 1745, 1470, 1422, 1405, 1365, 1285, 1251, 1230, 1205, 1185, 1080, 1030, 975, 943; $[\alpha]_D^{27}$ = -9.4° (0.074 M in chloroform).

Anal. Calcd. for $C_{43}H_{76}O_5$: C, 76.73; H, 11.38;

Found: C, 77.00; H, 11.17.

Preparation of (S)-4-hydroxy-1,2-dipalmitoyloxybutane [(S)-(78)]. A solution of 1.01 g (1.50 m mole) of [(S)-(77)] in 100 mL of 1:1 methanol-ethyl acetate with 200 mg of 10% palladium on carbon was hydrogenated in a Parr apparatus at 40 psig of hydrogen until no more hydrogen was taken up. The catalyst was removed by filtration through Celite and the solvent evaporated under reduced pressure to yield a solid material which upon recrystallization from methanol yielded 0.812 g (93%)of pure [(S)-(78)] of mp 58-60°. Spectral analysis: nmr ($CDCl_3$) 0.39-1.98δ(60H, complex), 2.03-2.70δ(4H, complex), 3.63δ(2H, m), 4.18δ(2H, m), 4.70δ(1H, s), 5.17δ(1H, m); ir ($CHCl_3$, cm^{-1}) 3240-3700, 3190, 2860-3050, 1740, 1472, 1425, 1385, 1320, 1280, 1185, 1065; $[\alpha]_D^{27}$ = -13.0° (0.05 M in chloroform).

Anal. Calcd. for $C_{36}H_{70}O_5$: C, 74.17; H, 12.10;

Found: C, 73.93; H, 12.27.

Preparation of diphenyl (S)-3,4-dipalmitoyloxybutyl-1-phosphate [(S)-(79)]. To 0.6 g (1.03 m mole) of [(S)-(78)] in 6 mL of pyridine cooled to 0° was added dropwise 0.52 g (1.83 m mole) of diphenyl phosphorochloridate. The reaction mixture was allowed to come to room temperature and continue stirring for 3 days at which time 2 mL of water

was added and the reaction mixture concentrated under reduced pressure. The residual solid was dissolved in 200 mL of benzene and washed successively with 80 mL each of water, cold 1 N hydrochloric acid, cold 1 N sodium bicarbonate, and water and dried over magnesium sulfate. After evaporation of the solvent under reduced pressure there was obtained a white solid which was recrystallized from methanol to yield 0.68 g (82%) of pure [(S)-(79)] of mp 39-41°. Spectral analysis; nmr ($CDCl_3$) 0.40-1.85δ(60H, complex), 1.90-2.40δ(4H, complex), 3.50-4.29δ(4H, m), 4.38-5.16δ(1H, m) 7.1δ(10H, broadened singlet); ir (KBr, cm^{-1}) 2793-3048, 1748, 1600, 1497, 1472, 1315, 1298, 1267, 1225, 1202, 1165, 1096, 1041, 1030, 1012, 959, 805, 720, 689; $[\alpha]_D^{27}$ = -32.9° (0.02 M in chloroform).

Anal. Calcd. for $C_{48}H_{79}PO_8$: C, 70.72; H, 9.77;
Found: C, 70.76; H, 9.99.

Preparation of (S)-3,4-dipalmitoyloxybutyl-1-phosphoric acid [(S)-(80)]. A solution of 1.0 g (1.22 m mole) of [(S)-(79)], combined from two preparations, in 100 mL of 1:1 methanol-ethyl acetate with 200 mg of platinum oxide in a Parr apparatus was hydrogenated at 44 psig of hydrogen until no more hydrogen was taken up. The catalyst was removed by filtration through Celite and the solvent evaporated under reduced pressure to yield a solid material which upon recrystallization twice from methanol-chloroform yielded 0.3 g (36.8%) of pure [(S)-(80)] of mp 72-74°. Spectral analysis: nmr ($CDCl_3$) 0.68-2.05δ(58H,complex), 2.12-2.65δ(4H, complex), 3.59-4.65δ(4H, m), 4.70-5.10δ(1H, m), 7.2δ(2H, s); ir (KBr, cm^{-1}) 3731-3154, 3106-2500, 1733, 1472, 1418, 1404, 1381, 1335, 1314, 1288,

1266, 1248, 1223, 1203, 1183, 1066, 1010, 961, 719; $[\alpha]_D^{27}$ = -10.8° (0.03 M in chloroform).

Anal. Calcd. for $C_{36}H_{71}PO_8$: C, 65.22; H, 10.79;
Found: C, 65.06; H, 11.11

Preparation of (R)-benzyloxy-1,2-butanediol acetonide [(R)-(75)]. To 21 g (143.2 m mole) of [(R)-(53)] in 80 mL of dry dimethyl formamide was added 9.88 g (176 m mole) of finely powdered potassium hydroxide with stirring and cooled to 0°. Benzyl chloride, 23.2 g (183.6 m mole), was added dropwise and the reaction mixture allowed to warm, ultimately being heated at 70° for 6 hr. After cooling, 200 mL of water was added and the reaction mixture extracted with chloroform (4 X 140 mL). The combined extracts were washed with water (3 X 140 mL) dried over magnesium sulfate, and evaporated under reduced pressure; the residual yellow liquid was vacuum distilled (115° / 0.10 Torr) to yield 20.6 g (60.7%) of benzyloxy derivative [(R)-(75)] which exhibited a single spot of R_f = 0.77 upon thin layer chromatography with 1:1 ethyl acetate-chloroform and spectral data in accord with those for the (S)-enantiomer and $[\alpha]_D^{27}$ = +6.33° (1.3 M in methanol).

Preparation of (R)-4-benzyloxybutane-1,2-diol [(R)-(76)]. The benzyloxy acetonide [(R)-(75)], 15.0 g (63.3 m mole), was treated with 10 mL of 2 N hydrochloric acid and sufficient acetone to effect solution.

After heating at reflux for 1.5 hr. the mixture was cooled, 100 mL of ethanol was added, and the solvent removed under reduced pressure. The residue was dissolved in 280 mL of chloroform, washed with water (3 X 60 mL), dried over sodium sulfate, and the solvent removed under reduced pressure to yield 8.6 g (69%) of pure [(R)-(76)] as a yellow oil which exhibited a single spot of R_f = 0.32 upon thin layer chromato-

graphy with 1:1 ethyl acetate-chloroform and spectral data in accord with those for (S)-enantiomer and $[\alpha]_D^{27}$ = +15.4° (0.5 M in methanol).

Anal. Calcd. for $C_{11}H_{16}O_3$: C, 67.32; H, 8.22;

Found: C, 67.59; H, 8.08.

Preparation of (R)-1-Tosyloxy-4-benzyloxy-2-butanol [(R)-(81)]. The diol [(R)-(76)], 8.5 g (43.1 m mole),was dissolved in 14 mL of pyridine, cooled to 0°, and a solution of 8.3 g (43.6 m mole) of p-toluenesulfonyl chloride in 100 mL of benzene was added dropwise, The reaction mixture was allowed to come to room temperature and was stirred for 48 hr. after which there was added a further 110 mL of benzene, and the reaction mixture was washed successively with 2 N hydrochloric acid (3 X 55 mL), saturated aqueous sodium bicarbonate (3 X 30 mL), water (4 X 60 mL) and dried over magnesium sulfate. The solvent was evaporated under reduced pressure to yield 11.39 g (75.3%) of pure [(R)-(81)] which exhibited a single spot of R_f = 0.68 upon thin layer chromatography with 1:1 chloroform-ethyl acetate, and gave acceptable spectral analyses although some difficulty was encountered in obtaining the elemental analysis; it appears that some decomposition of the tosylate occurs on standing. Spectral analysis: nmr ($CDCl_3$) 1.90δ(2H, dt, J_A = 6 Hz, J_B = 5.5 Hz), 2.46δ(3H, s), 3.26-3.87δ(3H, complex, $-CH_2O$ and -CHO), 3.90-4.33δ(3H, m, $-CH_2SO_3$ and -OH), 4.51δ(2H, s, benzylic), 7.13-7.60δ(7H, complex, phenyl and partial tosyl of AA'BB'), 7.66-8.00δ(2H, part of AA'BB'); ir (between salts, cm^{-1}) 3225-3846, 2777-3184, 1610, 1503, 1461, 1366, 1312, 1297, 1219, 1202, 1179, 1092, 1031, 1022, 970, 910, 819, 793, 743, 695, 667; $[\alpha]_D^{27}$ = +9.72° (0.4 M in methanol).

Anal. Calcd. for $C_{18}H_{22}SO_5$: C, 61.69; H, 6.33;

Found: C, 63.59, 63.15; H, 6.89, 6.50

Preparation of (R)-1-iodo-4-benzyloxy-2-butanol [(R)-(84)]. A mixture of 11.39 g (32.4 m mole) of [(R)-(81)], 4.85 g (32.4 m mole) of sodium iodide in 200 mL of acetone, which was freshly distilled over anhydrous potassium carbonate, was stirred at room temperature in the dark for 3 days after which it was poured into 600 mL of an equal mixture of saturated sodium bicarbonate and brine solution and extracted with ether (3 X 200 mL). The combined extracts were washed with 10% sodium thiosulfate solution (2 X 150 mL) and dried over magnesium sulfate. The solvent was removed under reduced pressure to yield 7.75 g (78%) of pure [(R)-(84)]. Spectral analysis: nmr ($CDCl_3$) 1.96δ(2H, dt, J_A = 6 Hz, J_B = 5.5 Hz), 3.19-4.01δ(6H, complex, OCH-, OCH_2-, OH and CH_2I), 4.57δ(2H, s) 7.33δ(5H, s); ir (between salts, cm^{-1}) 3194-3676, 2717-3144, 1503, 1462, 1422, 1369, 1218, 1194, 1183, 1103, 1081, 1033, 911, 813, 740, 685.

Preparation of diphenyl (R)-4-benzyloxy-2-hydroxybutyl-1-phosphate [(R)-(85)]. To a solution of 2.61 g (8.4 m mole) of [(R)-(84)] in 14 mL of dry benzene 3.1 g (8.68 m mole) of dry, powdered silver diphenylphosphate was added and refluxed for 1 hr. after which an additional 1.5 g (4.2 m mole) of the silver salt was added and refluxed for another one hour. After cooling, the reaction mixture was filtered and the silver salts were washed several times with dry benzene. The combined benzene layers were evaporated under reduced pressure and the residual oil taken up in a minimum volume of 1:1 ethyl acetate-chloroform and passed through a short (30 g) column of silica gel and eluted with an additional 2 L of the solvent mixture. After removal of the solvent under reduced pressure there was isolated 1.7 g (47%) of [(R)-(85)] as an oil which exhibited a single spot of R_f = 0.57 upon thin layer chromatography developed with

1:1 ethyl acetate-chloroform which was visualised by phospomolybdate spray[77] and iodine and gave acceptable spectral analyses although some difficulty was encountered in obtaining the elemental analysis. It appears that some decomposition of the phosphate occurs on standing. Spectral analysis: nmr (DMSO-d_6) 1.96δ(2H, dt, J_A = 6 Hz, J_B = 5.5 Hz), 3.28-4.25δ(6H, complex, -OCH_2, -OCH, -OH), 4.41δ(2H, s), 7.25δ(15H, broadened singlet); ir (between salts, cm^{-1}) 3194-3703, 2702-3174, 1597 1492, 1455, 1368, 1288, 1221, 1194, 1184, 1168, 1089, 1031, 957, 906, 815, 778, 752, 736, 699, 662.

Anal. Calcd. for $C_{21}H_{25}PO_6$: C, 62.37; H, 6.23;
Found: C, 69.63; H, 6.72.

Preparation of (R)-2,4-dihydroxybutyl-1-phosphoric acid [(R)-(86)]. A solution of 1.3 g (3.03 m mole) of [(R)-(85)] in 100 mL methanol with 0.3 g of platinum oxide in a Parr apparatus was hydrogenated at 46 psig of hydrogen until no more hydrogen was taken up. The catalyst was removed by filtration through Celite and the solvent evaporated under reduced pressure to yield 282 mg (50%) of pure [(R)-(86)]. For analysis, the free acid was converted to dilithium salt [(R)-(86a)] by dissolution in 50 mL of ethanol which was decolorized with charcoal and the addition of a saturated ethanolic solution of lithium hydroxide monohydrate to pH = 8. The resultant precipitate was filtered, washed with ethanol and ether, and dried under vacuum to yield 278 mg (46%) of [(R)-(86a)] which exhibited a single spot of R_f = 0.34 upon thin layer chromatography developed with 10:1 methanol-0.1 N hydrochloric acid and gave acceptable apectral analyses although some difficulty was encountered in obtaining the elemental analysis due to the contamination with lithium hydroxide. Spectral analysis: nmr (methanol-d_4, for free acid) 0.72-1.33δ(2H, m),

2.80-3.66δ(2H, m), 4.10-5.26δ(3H, m), 6.13-6.93δ(4H, broadened singlet); ir (KBr pellet of salt, cm^{-1}) 2703-3802, 1620, 1459, 1063, 870.

Anal. Calcd. for $C_4H_9PO_6Li_2$: C, 24.24; H, 4.54;
Found: C, 15.03; H, 3.22.

Preparation of (R)-1,2-epoxy-4-benzyloxybutane [(R)-(82)]. To a solution of 5.89 g (16.7 m mole) of [(R)-(81)] freshly prepared in 20 mL of methanol was added a solution of 0.90 g (16.7 m mole) of sodium methoxide in 1 mL of water, the reaction mixture was refluxed for 5 hr., cooled, and the solvent removed under reduced pressure. To the residue was added 300 mL of ether and the resultant precipitate of sodium tosylate removed by filtration under suction. The solution was washed with water (3 X 80 mL), dried over magnesium sulfate, and the solvent removed under reduced pressure to yield an oil which was vacuum distilled (100° / 0.05 Torr) giving 1.85 g (61.8%) of pure [(R)-(82)] and spectral data in accord with that for a terminal epoxide.[73] Spectral analysis; nmr ($CDCl_3$) 1.60δ(2H, m), 2.16δ(1H, dd), 2.40δ(1H, t), 2.76δ(1H, m), 3.30δ(2H, t), 4.23δ(2H, s), 7.05δ(5H, s); ir (between salts, cm^{-1}) 2776-3174, 1499, 1457, 1367, 1251, 1203, 1097, 1028, 910, 856, 836, 823, 742, 701.

Preparation of diethyl 3-oxopropylphosphonate (88)[78]: A solution of 62.7 g (0.376 mole) of fresh distilled β-chloropropionaldehyde diethyl acetal with 54.36 g (0.327 mole) of triethyl phosphite was heated at 185°-195° for 8 hr. in a distillation apparatus provided with a prograssively warmed reflux condenser. The volatile components were removed under reduced pressure and the residue was vacuum distilled (95° - 100° / 0.01 Torr) to yield 54.4 g (62%) of the corresponding phosphonate diethyl acetal (89). Spectral analysis: nmr ($CDCl_3$)

1.33δ(12H, m), 1.63-2.33δ(4H, m), 3.33-3.83δ(4H, m) 3.83-4.46δ(4H, m), 4.46-4.86δ(1H, t); ir (between salts, cm^{-1}) 2857-3086, 1481, 1445, 1392, 1375, 1255, 1218, 1162, 1126, 1041, 961, 855, 786.

A solution of 29.2 g (0.108 mole) of phosphonate diethyl acetal (89) in 142 mL of 3% hydrochloric acid in the presence of 1 g of hydroquinone was heated under nitrogen atomsphere at 75°-80° for 5 hr. The volatile components were removed under reduced pressure and the residue was vacuum distilled (74° - 76°/ 0.01 Torr) to yield 16.5 g (78.7%) of pure (88). Spectral analysis: nmr (CCl_4) 0.77δ(6H, t), 1.10-1.84δ (2H, m), 3.51δ(4H, m), 9.12δ(1H, t); ir (between salt, cm^{-1}) 2816-3076, 2762 (-CHO), 1739 (>C=O), 1490, 1451, 1422, 1400, 1375, 1234, 1164, 1098, 1036, 966, 893, 826, 790.

Preparation of trilithium 3-carboxy-3-hydroxypropyl-1-phosphonic acid (23).

Method A (in acidic media). A solution of 2.0 g (10.3 m mole) of diethyl 3-oxopropyl-1-phosphonate (88), 4 mL of dioxane, 7 mL of water, and 0.3 mL of concentrated sulfuric acid, cooled to 0°, was added a solution of 1.18 g (11.4 m mole) of sodium bisulfite in 1 mL of water in a period of 15 minutes after which 1.2 g (24.4 m mole) of sodium cyanide in 3 mL of water was added over 20 minutes to the suspension with the intermittent careful addition of 6 N sulfuric acid to maintain pH at 7.5. Stirring was continued at 0° for 2 hr. after which the reaction mixture was allowed to come to room temperature and the resultant solution was extracted continuously by liquid-liquid extraction with 600 mL of 5% methanol in ether for 3 days. The ether layer was separated and evaporated under reduced pressure to yield 1.60 g (70%) of crude (87) which after passage through 150 g of silica gel (40 - 140 mesh) with 5% methanol in ethyl

acetate and evaporation of the solvent under reduced pressure there was obtained 0.95 g (41%) of pure (87). The material exhibited a single spot of R_f = 0.46 on thin layer chromatography developed with 5% methanol in ethyl acetate. Spectral analysis: nmr (CCl_4) 0.77δ(6H, t), 1.03-1.86δ (4H, m), 3.20-4.08δ(5H, m, -OH, $-OCH_2$), 5.28δ(1H, d, J = 6 Hz, -OCH); ir (CCl_4, cm^{-1}) 3067-3846, 2793-3048, 2252 (-C≡N), 1633, 1597, 1445, 1392, 1369, 1226, 1164 (P=O), 1041, 966.

A solution of 0.7 g (31.5 m mole) of cyanohydrin (87) in 10 mL of concentrated hydrochloric acid was refluxed at 135° for 18 hr. after which charcoal was added, the mixture boiled, and charcoal removed by filtration through Celite and the yellow filtrate was concentrated to a small volume under reduced pressure and treated with saturated lithium hydroxide solution (under toluene) under a nitrogen atomsphere to pH = 8.5. After this, acetone was added and the resultant precipitate was filtered and dissolved in a minimum amount of water; the slightly cloudy solution was filtered and the filtrate was diluted with absolute ethanol to give a gelatinous solid. The solid was removed and more ethanol was added to filtrate to afford the crude trilithium salt. The precipitation procedure was repeated twice to yield 0.5 g (78%) of pure (23) which exhibited spectral chromatographic properties identical to those of previous preparations.[19, 20]

Method B (in basic media). A solution of 142.5 mg (2.908 m mole) of sodium cyanide and 116.4 mg(29.08 m mole) of sodium hydroxide in 5 mL of water was frozen in a 15 mL round bottom flask and to it was added 564.2 mg (2.908 m mole) of diethyl 3-oxopropyl-1-phosphonate (88) dissolved in 5 mL of 0.5 M aqueous sodium bicarbonate solution and frozen. The flask

was loosely stoppered, the frozen solution allowed to melt, after which time the flask was sealed and kept at room temperature for 4 days. After this time the reaction mixture was heated at 80° for 5 hr. and evaporated to near dryness under reduced pressure. To the residue was added 5 mL of concentrated hydrochloric acid and the mixture heated at 130° for 16 hr. after which it was again concentrated to near dryness. To the residue was added 10 mL of absolute ethanol, the mixture filtered, and saturated ethanolic lithium hydroxide added until precipitation was complete (pH = 8). The precipitate was filtered, washed with ethanol and ether, and dried under vacuum to yield 405.0 mg (53.5%) of (23) which exhibited spectral and chromatographic properties identical to those as previous preparations.[19, 20] This method has proven entirely suitable for the preparation of 3-[^{14}C]-carboxy-3-hydroxypropyl-1-phosphonic acid; the conditions preclude the loss of radioactive cyanide in the vapor form and allow the use of very small quantities necessary for preparations of high specific activity. For isolation of the labelled acid, paper chromatography (Whatman No. 1) and gradient column chromatography with DEAE cellulose (DE-52, bicarbonate form)[79] was used, the latter was preferred and the former being eluted concurrently with a sample of unlabelled material. The pertinent elution data is as follows: System No. 1; 2-butanol:ammonia:water 6:3:1, R_f = 0.11. System No. 2; 0.1 N hydrochloric acid:methanol 1:10, R_f = 0.78. System No. 3; n-Propyl alcohol: ammonia:water 6:8:1, R_f = 0.50.

SUGGESTIONS FOR FUTURE RESEARCH

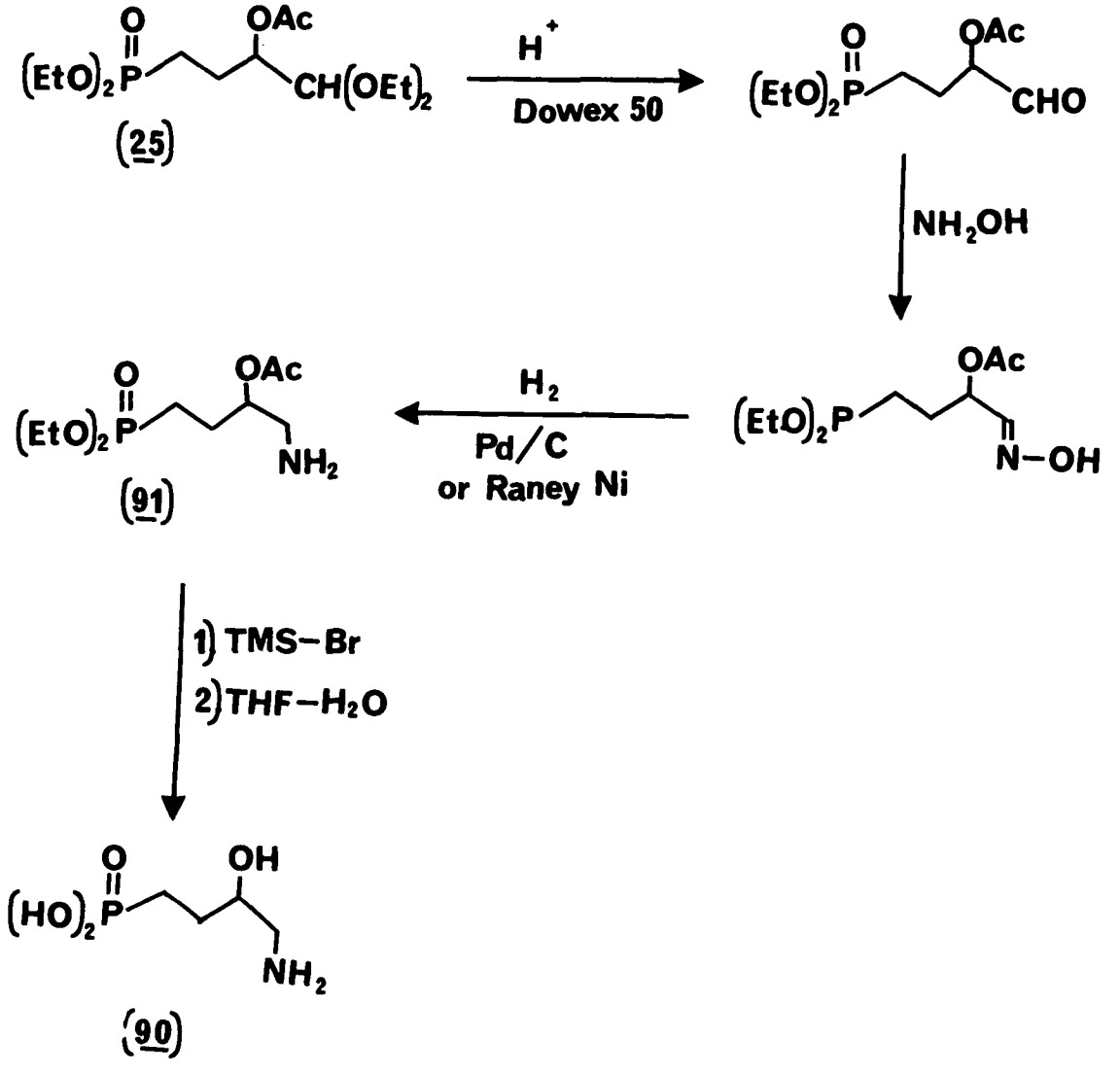
For more detailed study of the mechanism of action of 3,4-dihydroxybutyl-1-phosphonic acid (21), the isosteric analogue of sn-glyceral-3-phosphate, and 4-oxo-3-hydroxybutyl-1-phosphonic acid (26), the isosteric analogue of glyceraldehyde-3-phosphate, these materials should be synthesized bearing a carbon-14 label. Also, new functional group modifications of 3,4-dihydroxybutyl-1-phosphonate (21), such as the 3-amino and 4-amino compounds have potential as metabolic regulators and chemotherapeutic agents.

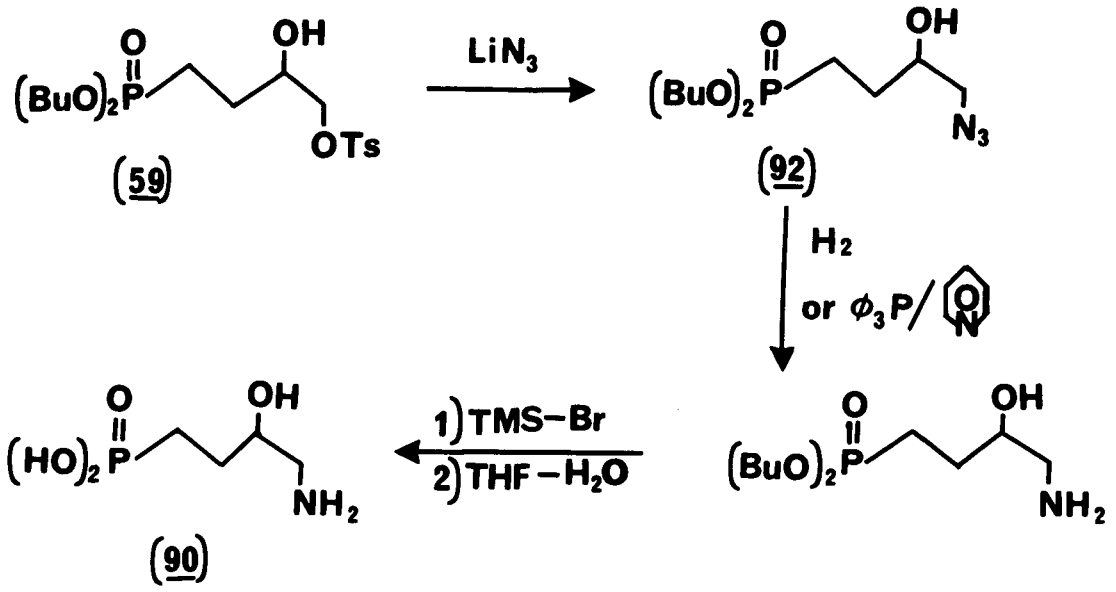
D-Glyceraldehyde-3-phosphate (5) has not been synthesized by chemical methods, but only by biochemical means. An approach to it is presented.

This is presented as a suggestion for future synthetic research, including the resolution (1RS, 3S)-1,3,4-trihydroxybutyl-1-phosphonate, (1RS, 3S)-(74); the latter is of interest due to the biological activity toward several bacteria. Further understanding of the stereochemical requirement at C-1 would be provided by the individual compounds thus isolated.

A. Preparation of 4-amino-3-hydroxybutyl-1-phosphonate (90): Earlier attempts for direct reduction of (87) with lithium aluminum hydride[80] to yield (90) found that the reagent can also reduce the phosphonate function to the phosphine.[81] The alternate routes proposing to prepare (90) start with the readily available intermediates, either (25), which can yield the corresponding oxime followed by catalytic hydrogenation to give the amine (90),[82,83] or (59), which can yield the corresponding 4-azido-3-hydroxybutyl-1-phosphonate (92) which then can be

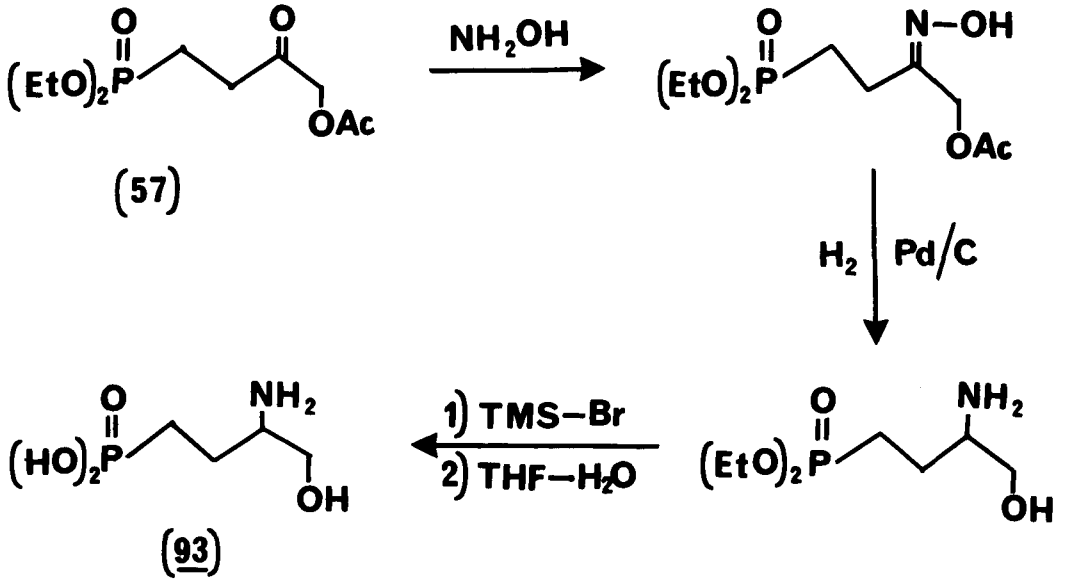
converted to (90) by either hydrogenation[84a] or allowed to react with triphenylphosphine in pyridine.[84b] The route starting with (59) will allow one to prepare the enantiomeric forms of (90) starting with optically active (59).





(59) (92) (90)

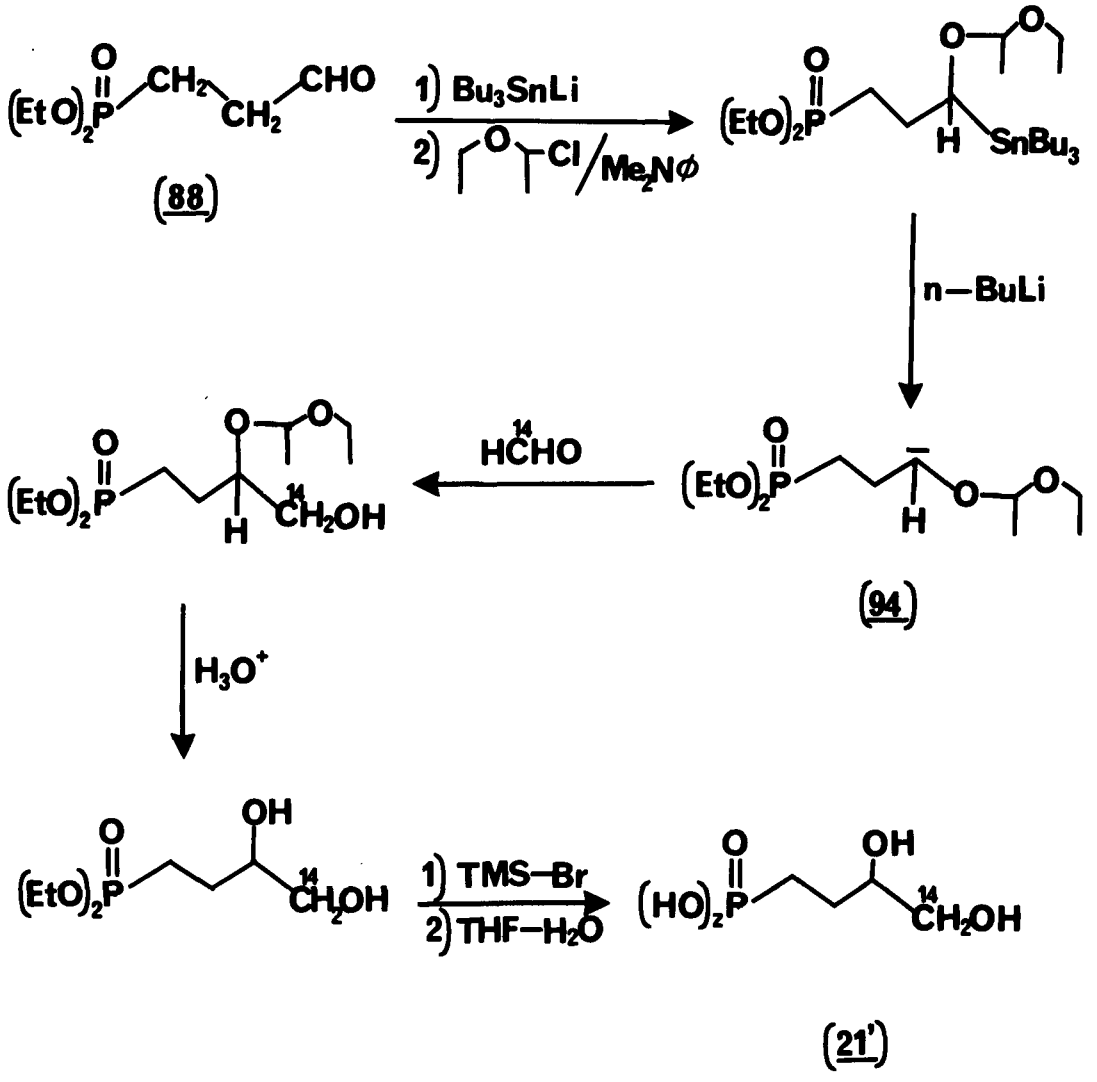
B. Preparation of 4-hydroxy-3-aminobutyl-1-phosphonate (93). The synthesis of this system could be performed by a parallel method to the one mentioned previously:



(57) (93)

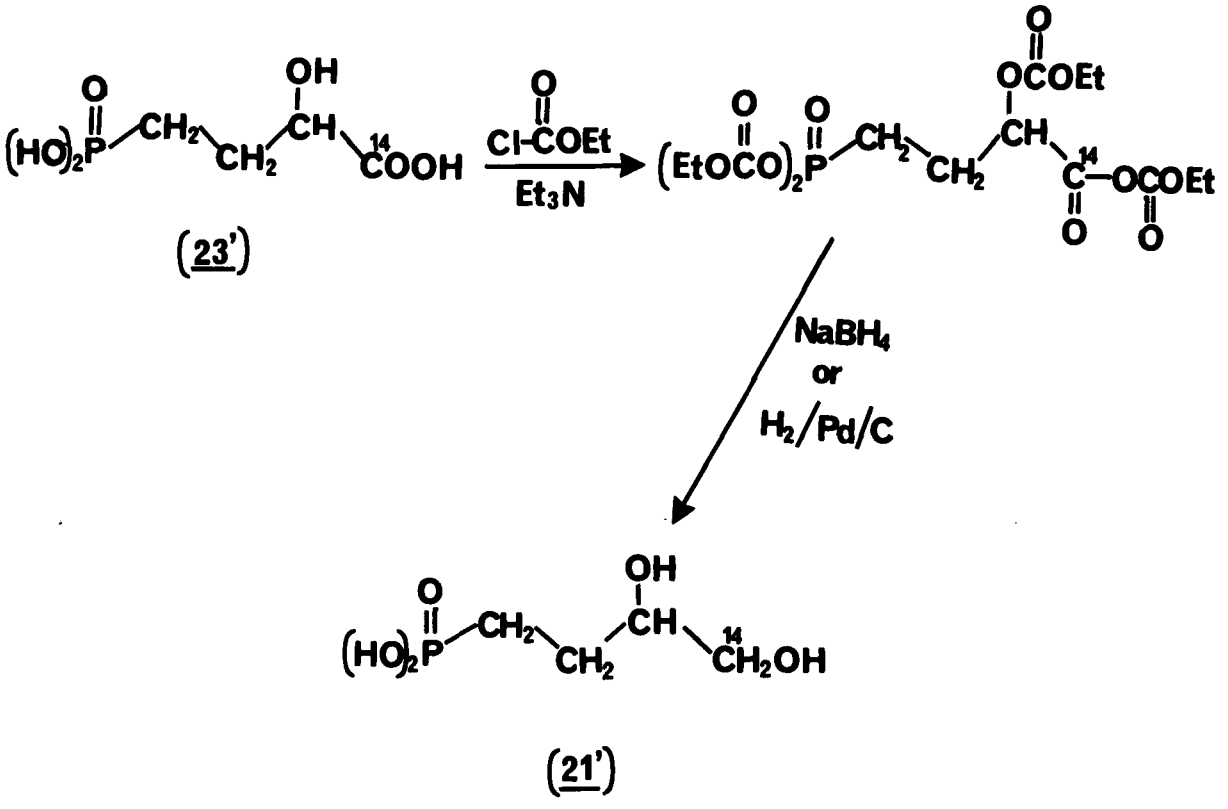
C. Preparation of 4-[^{14}C]-3, 4-dihydroxybutyl-1-phosphonate (21')

The synthesis of this system could be achieved[84] by generating the organolithium reagent (94) which then may react with [^{14}C]-formaldehyde.



An alternate route for the preparation of (21') involves generations of the mixed carbonate[86] to facilitate the reduction of the carboxyl group

by sodium boron hydride or hydrogenation as follows:

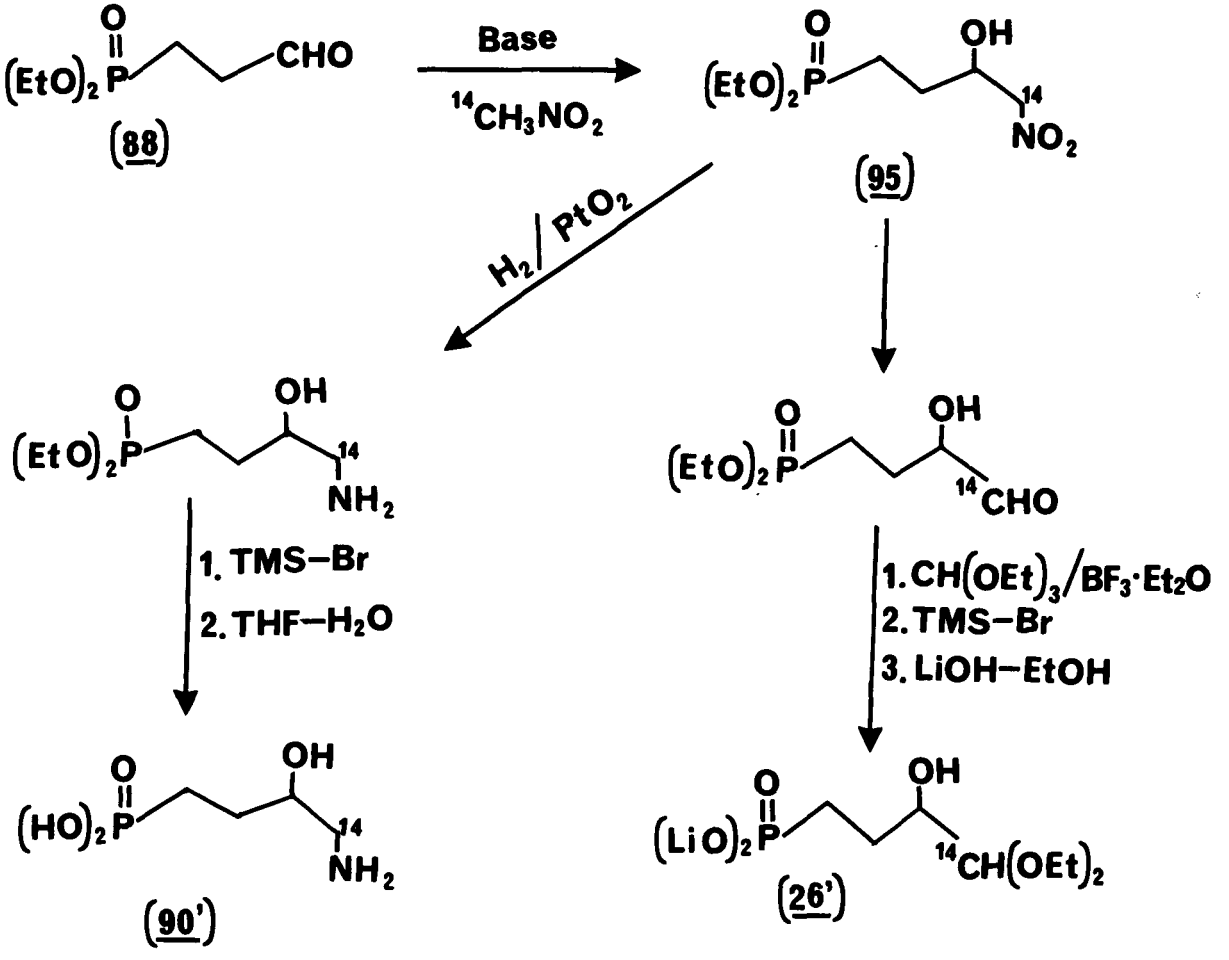


(23') (21')

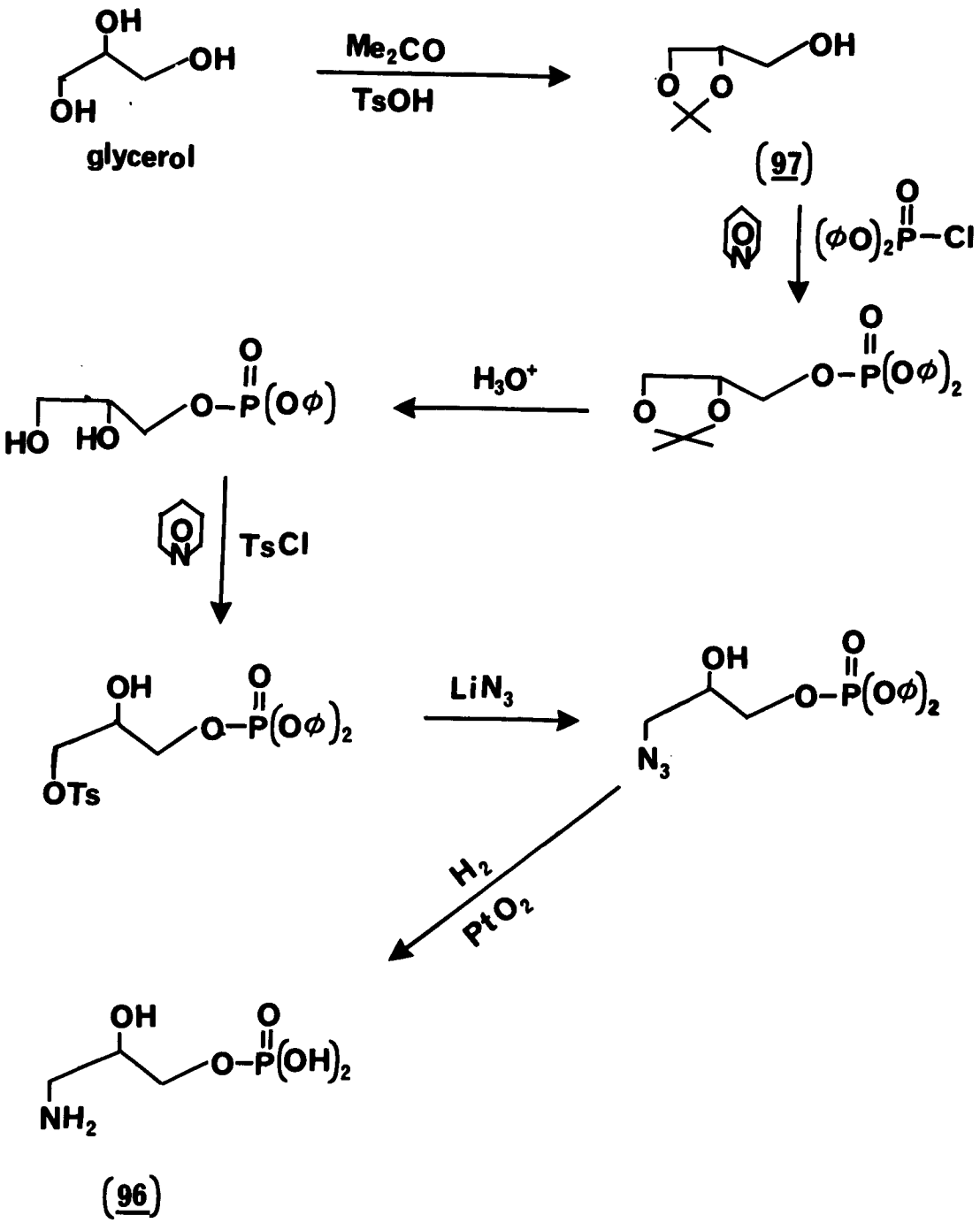
D. Preparation of 4-[^{14}C]-oxo-3-hydroxybutyl-1-phosphonate (26')

The synthesis of this system could be accomplished by nucleophilic attack of the anion of [^{14}C]-nitromethane on the aldehyde (88). The product (95) thus formed can be converted to the aldehyde by a Nef reaction as below.

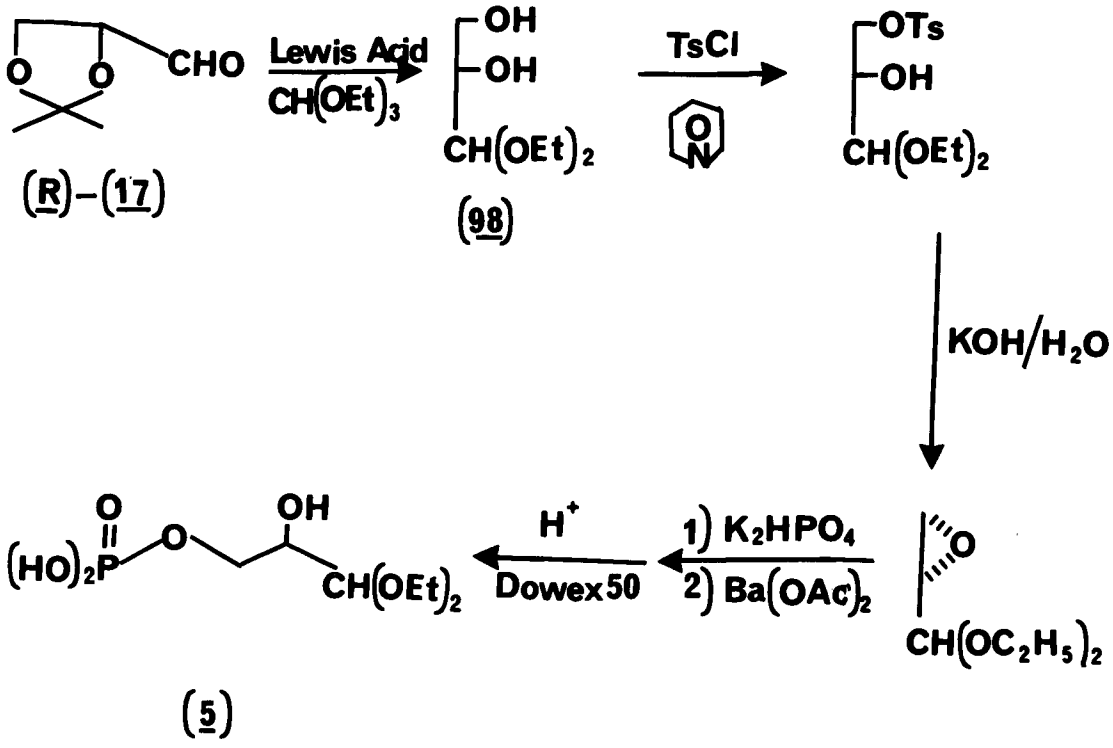
The hydrogenation of (95) will also allow one to obtain (90') containing a carbon-14 label.



E. Preparation of 3-amino-2-hydroxypropyl-1-phosphate (96). This material can be formed from glycerol through standard acetonide formation, phosphorylation with diphenyl phosphorochloridate, deprotection, selective monotosylation, azido-formation, and hydrogenation[9,84a] as below. This method should be suitable for the preparation of its enantiomers from the corresponding optically active (97); the latter can be prepared by reduction of (R)-(17) to (d)-(+)-acetone glycerol (97) which can also be converted to its enantiomer by the method reported by Baer et al.[85b]


OH
OH
OH
glycerol
Me2CO
TsOH
OH
O
O
(97)
O
(φO)2P–Cl
O–P(Oφ)2
H3O+
O–P(Oφ)
HO
HO
TsCl
OH
O–P(Oφ)2
OTs
LiN3
OH
O–P(Oφ)2
N3
H2
PtO2
OH
O–P(OH)2
NH2
(96)

F. Preparation of D-glyceraldehyde-3-phosphate (5). The diol diethyl acetal (98) could be obtained by the reaction of (R)-(17) with triethyl orthoformate in the presence of a catalytic amount of Lewis acid. The diol (98) thus formed could be selectively monotosylated,[60,88] epoxidized,[88] and phosphorylated[89] to yield (5).



(R)-(17) (98) (5)

G. Resolution of (1RS, 3S)-1.3.4-trihydroxybutyl-1-phosphonate (1RS, 3S)-(71). This should be possible either by the use of HPLC (high pressure liquid chromatography) on (71) directly or by conversion to a solid ester derivative which is then separated by fractional crystallization.

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(b) For 250 μCi of (23') (4mCi/m mole), a 2 X 15 cm column of DEAE cellulose (DE-52; bicarbonate form) was used and eluted with 400 mL of linear gradient of ammonium bicarbonate (0-0.2M). Fractions of 4 mL were collected and gave a satisfactory result.

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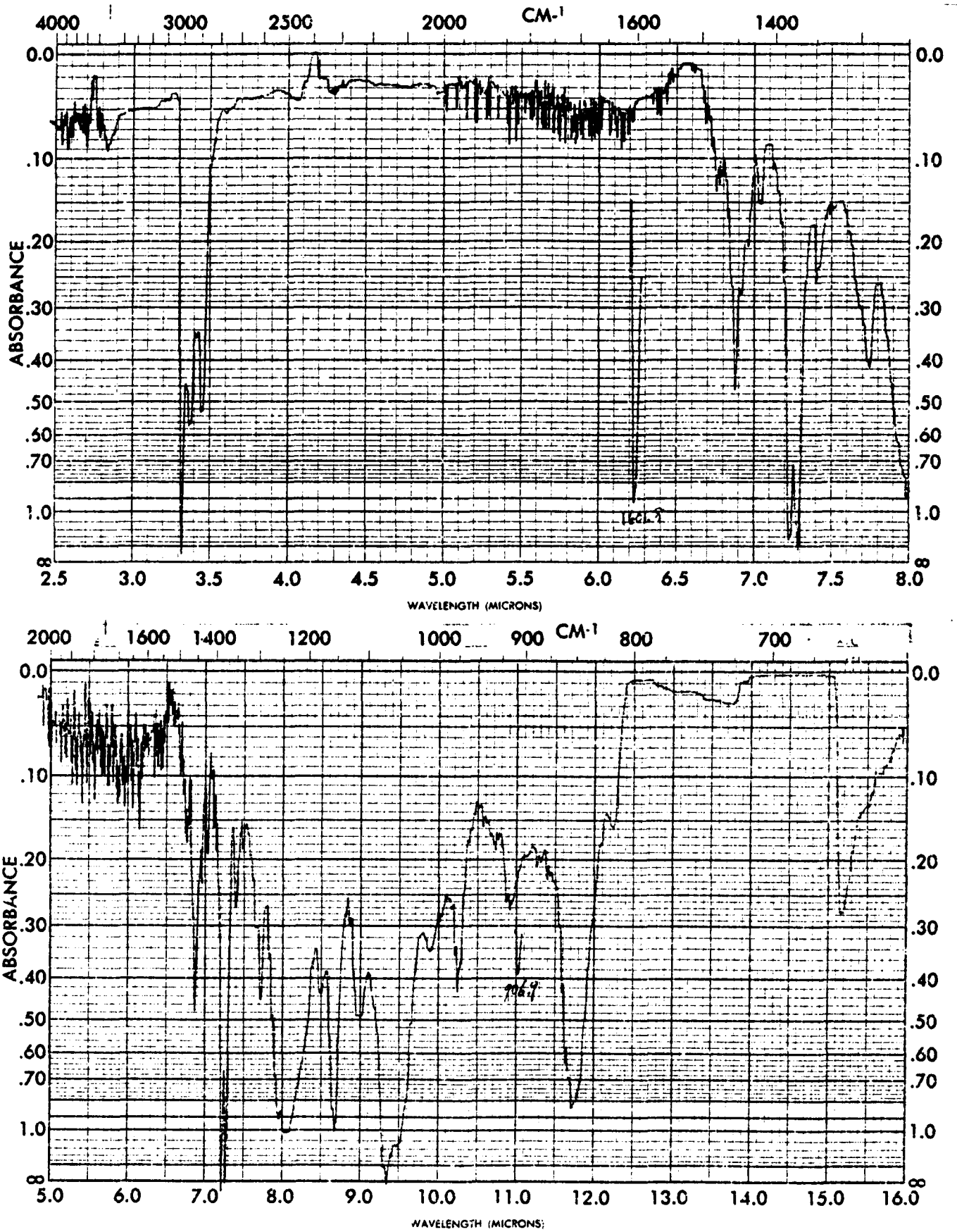
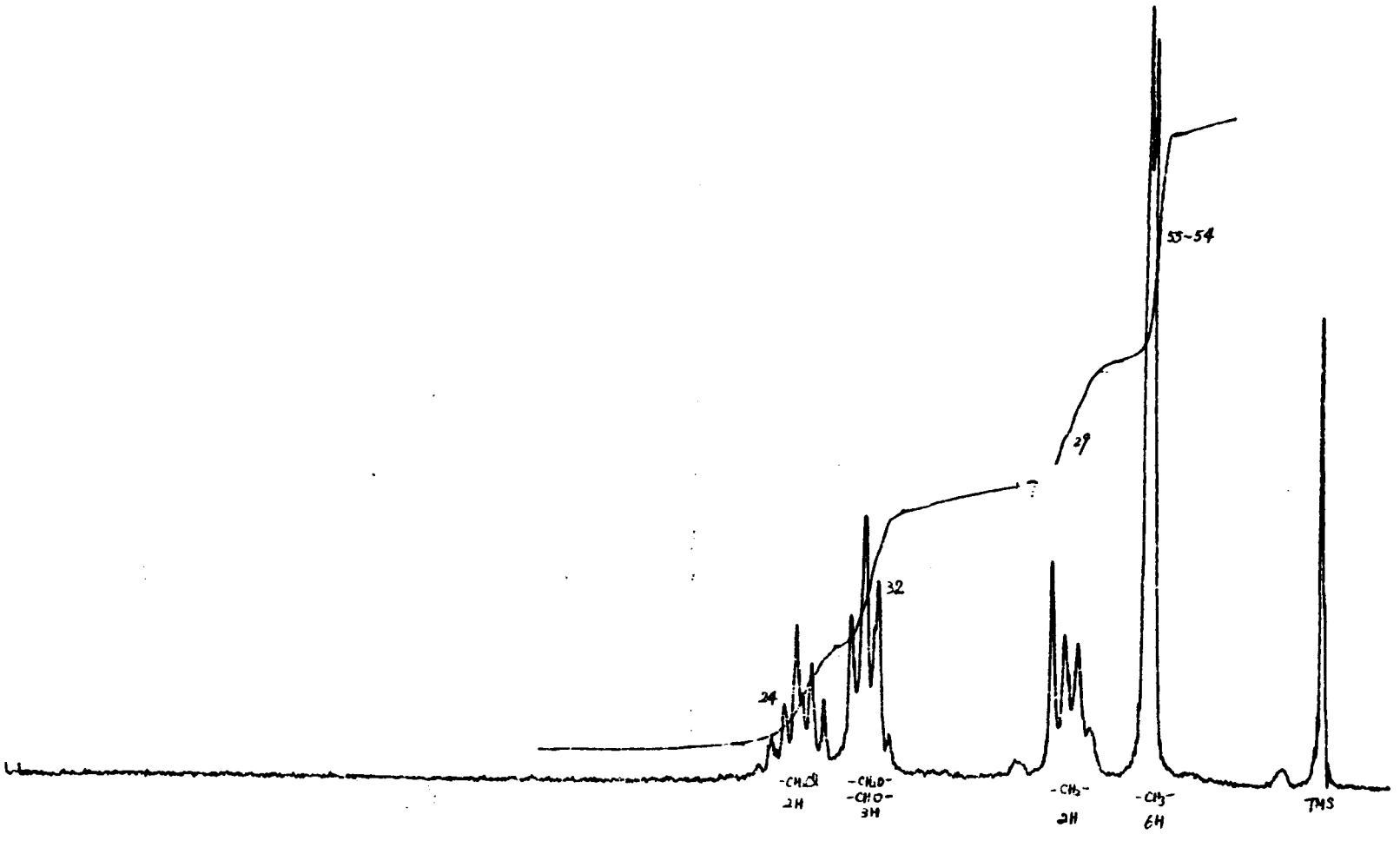
APPENDIX

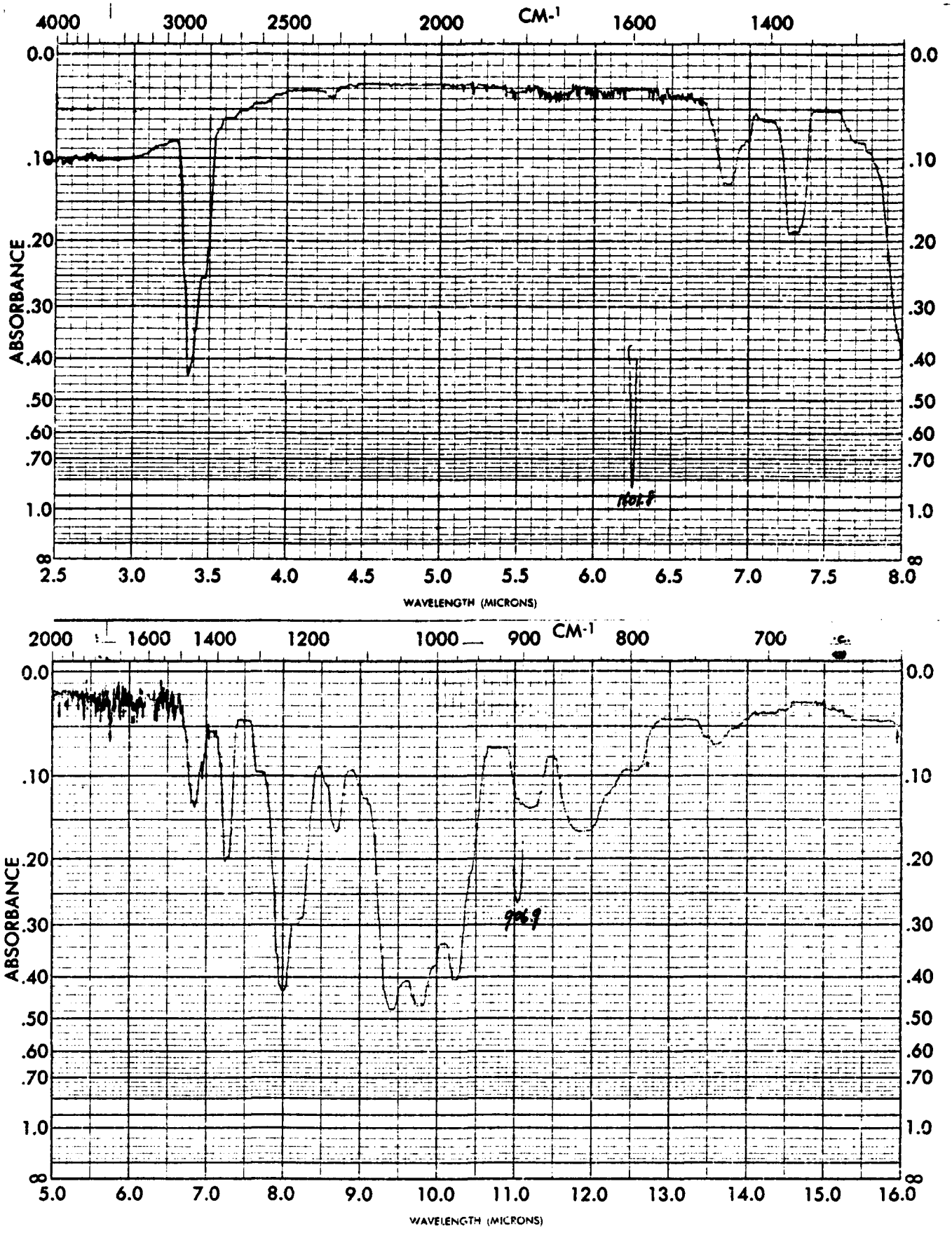
nmr and ir spectra



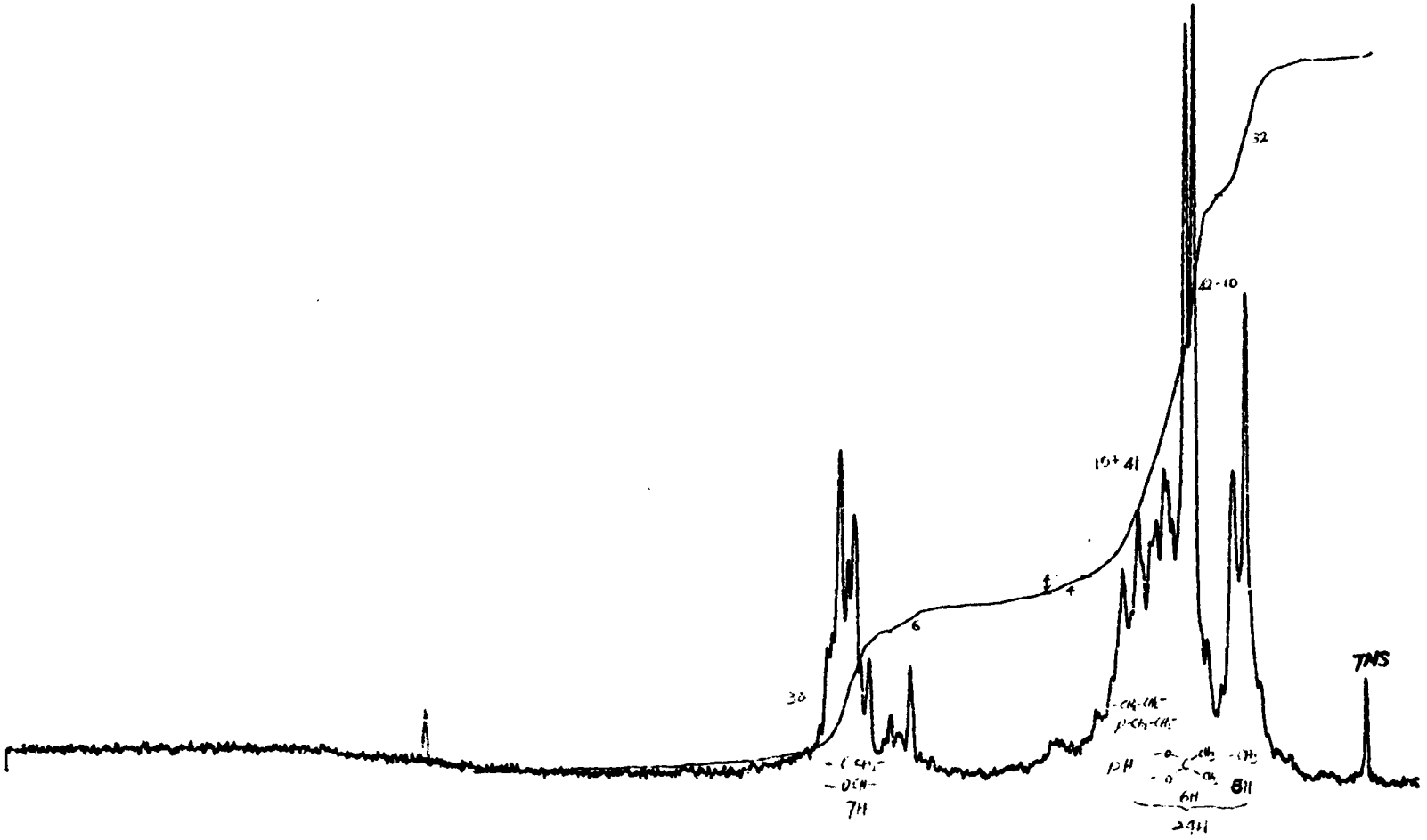
ir ($CHCl_3$) of (S)-(54)



nmr ($CDCl_3$) of (S)-(54)



ir (between salts) of (S)-(55)



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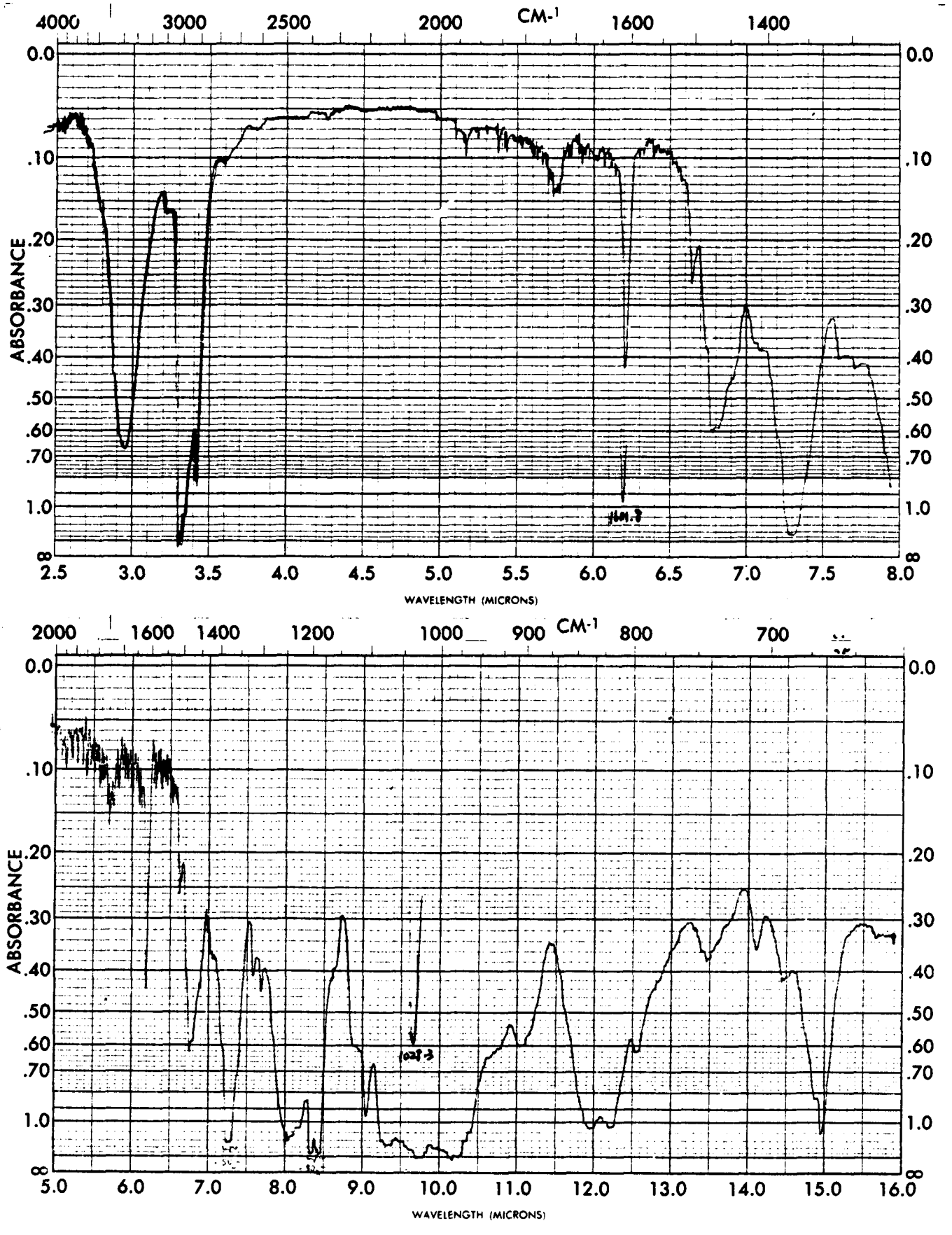
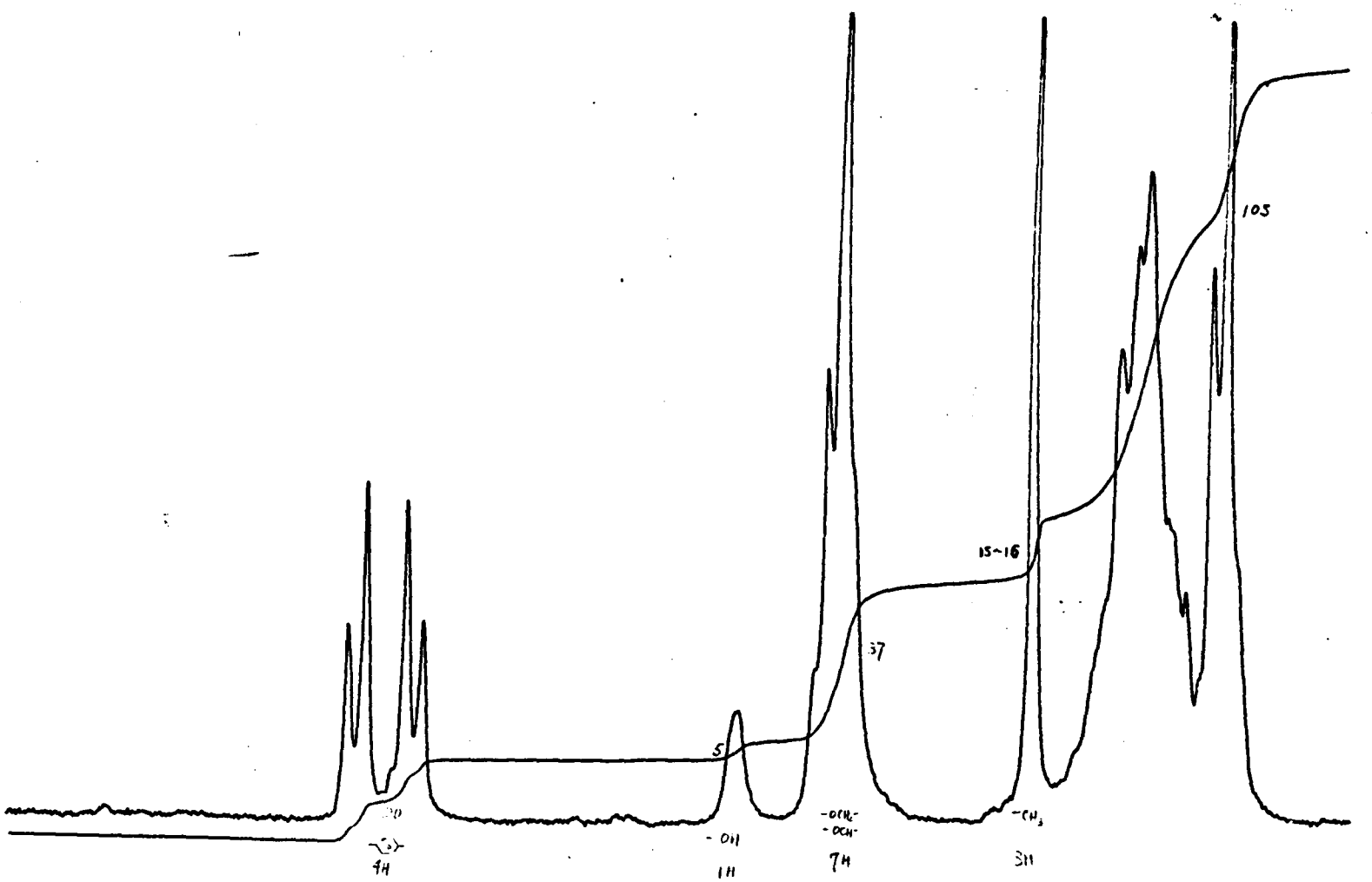


ir (between salts) of (59)



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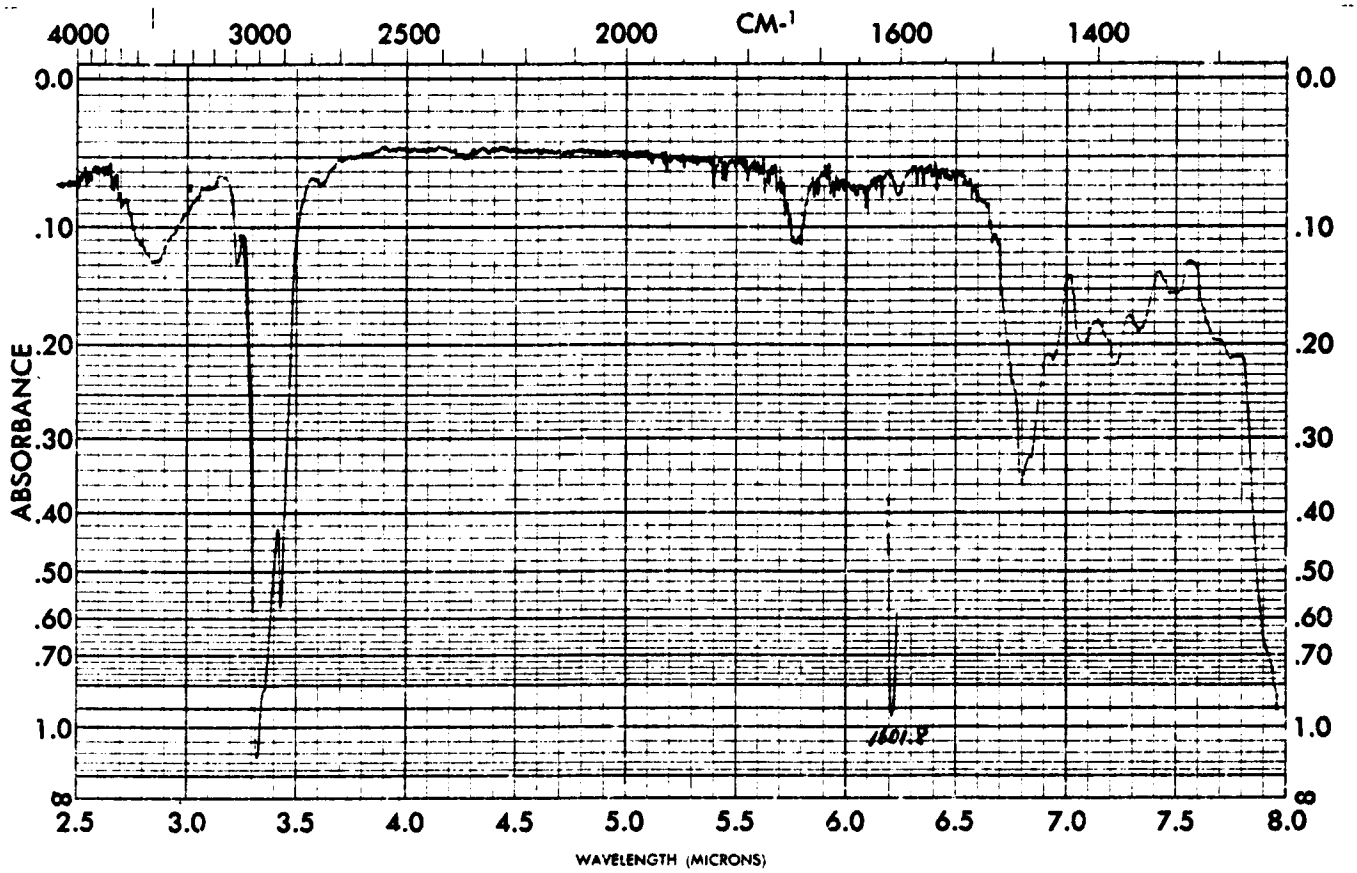
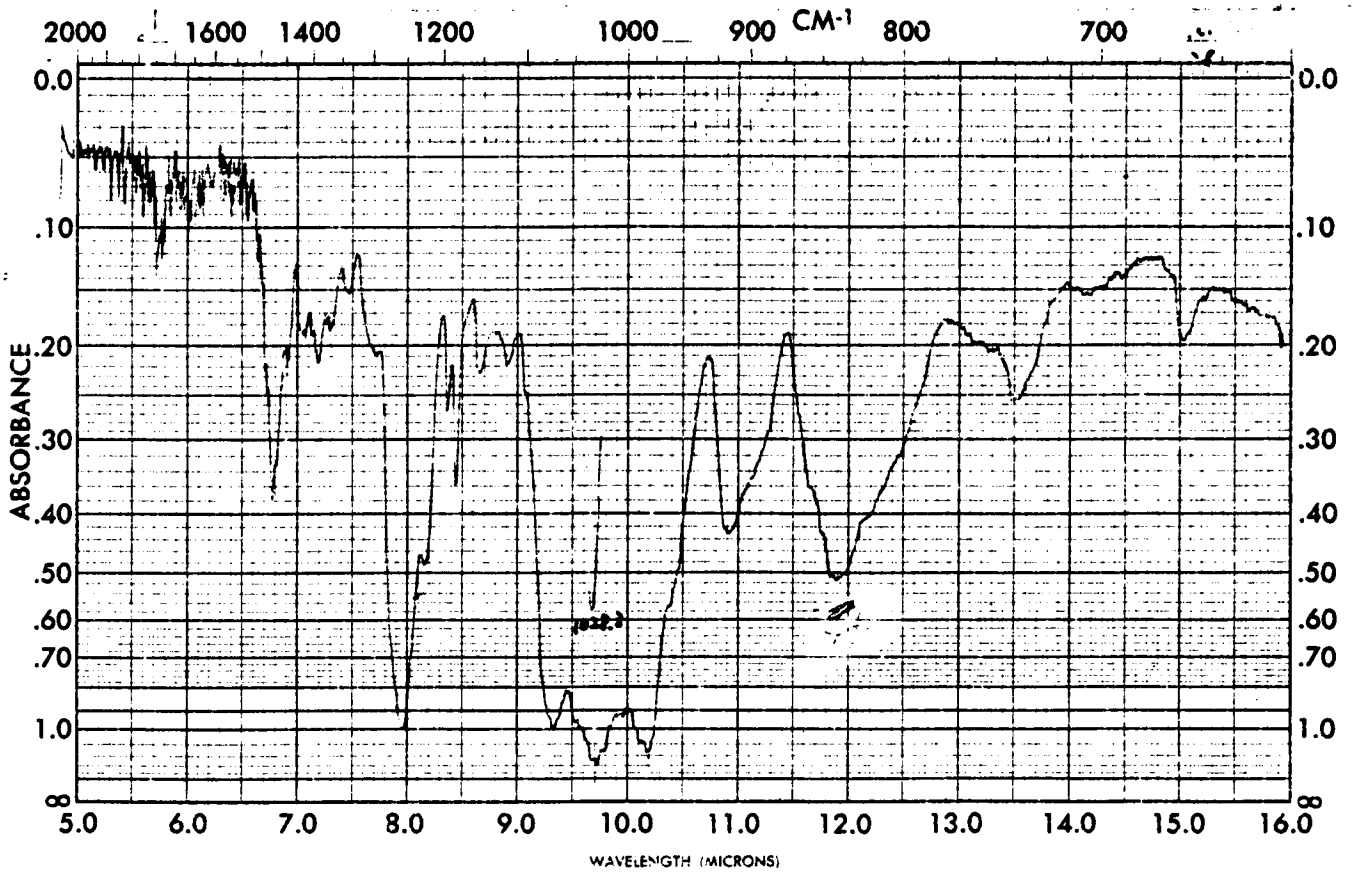
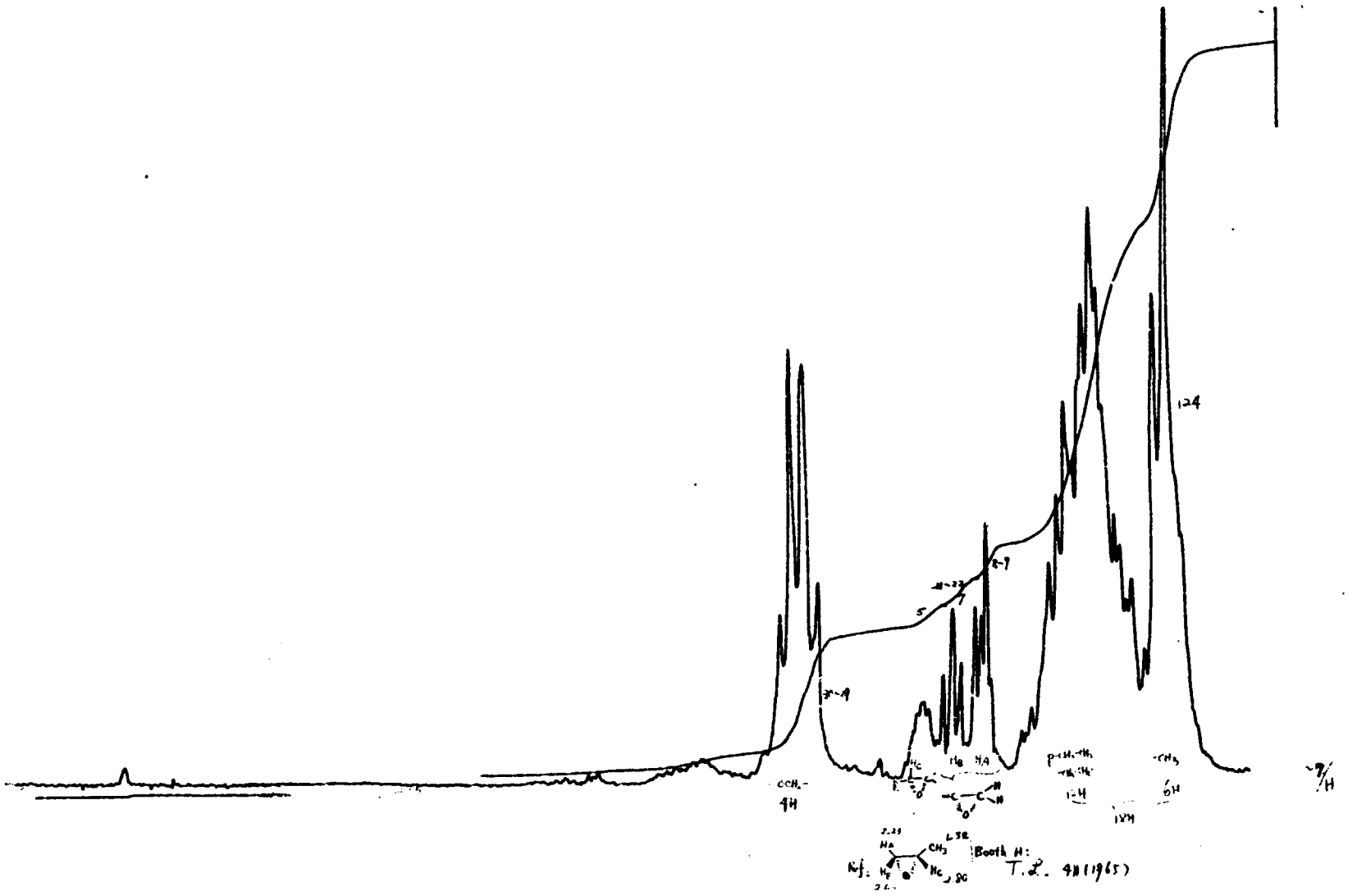




ir (between salts) of (58)



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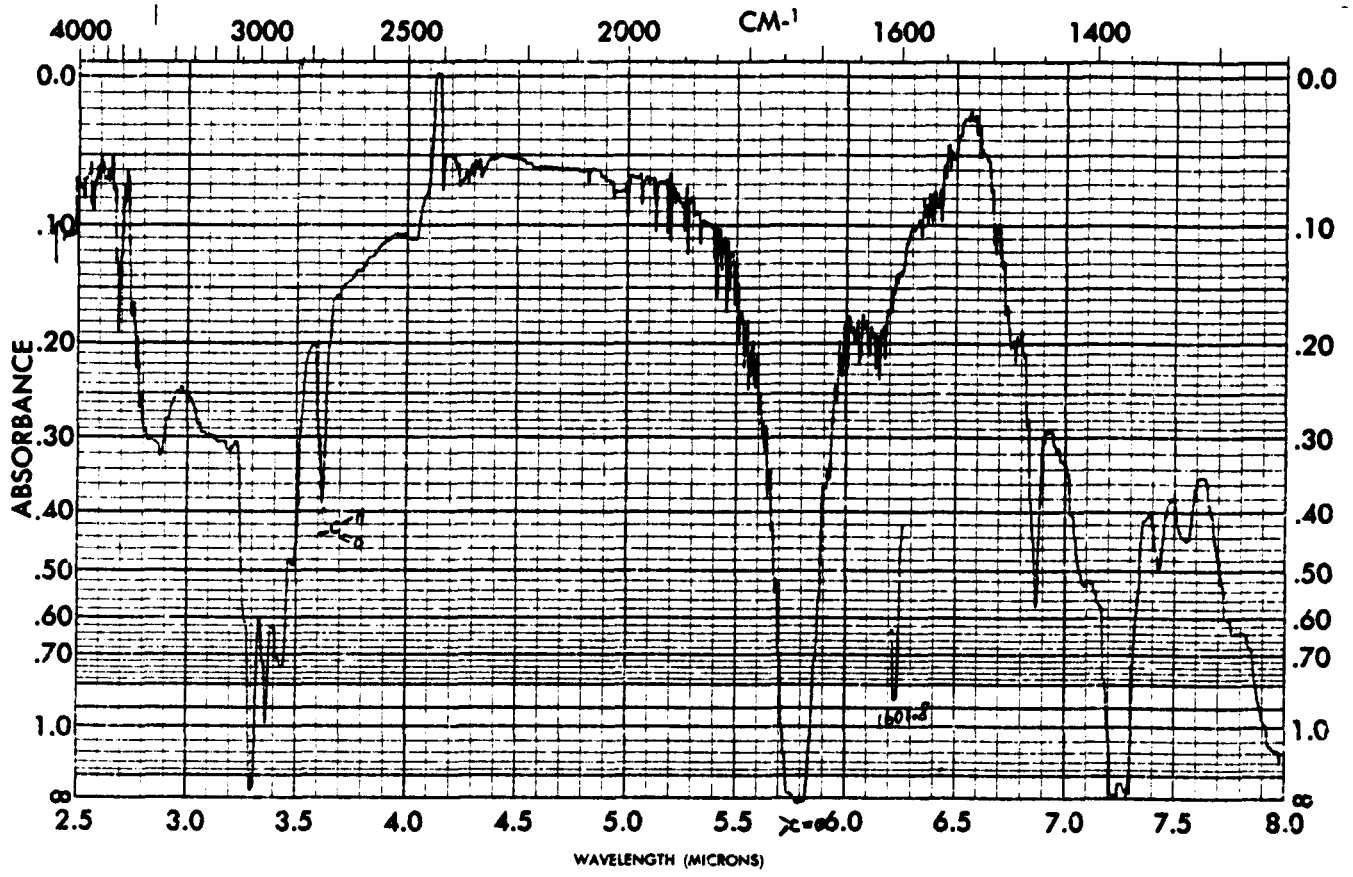
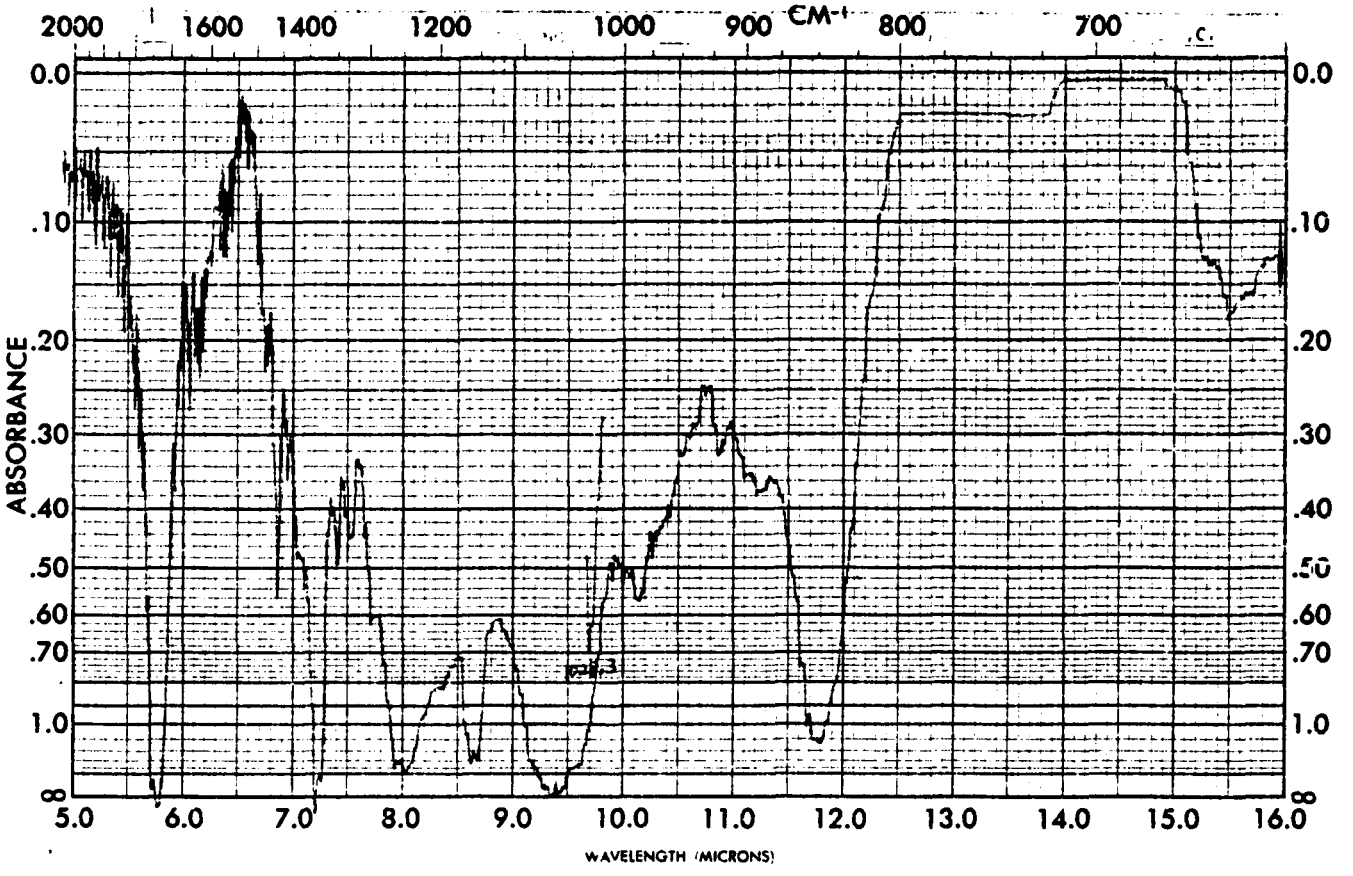
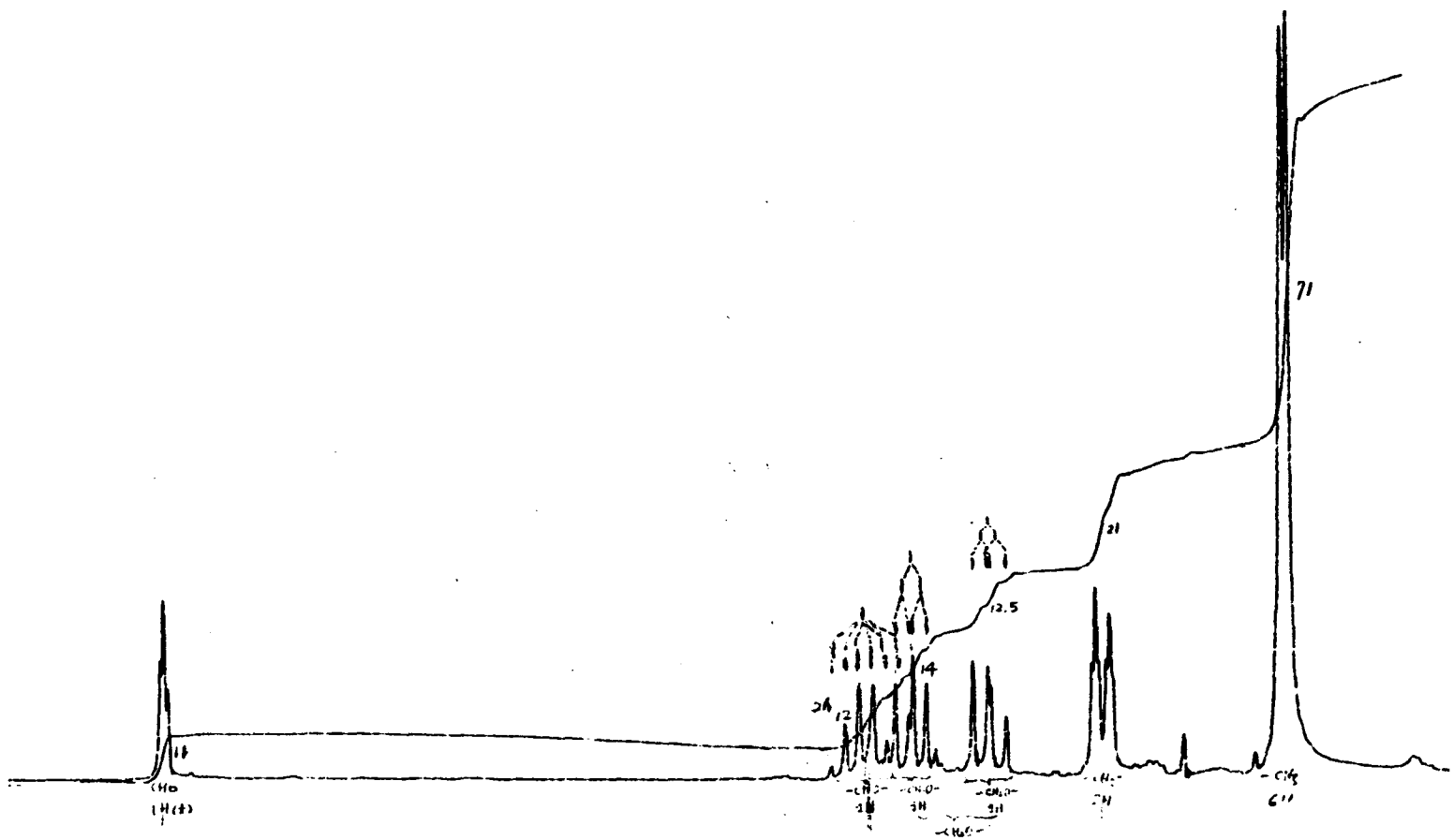




ir ($CHCl_3$) of (S)-(69)



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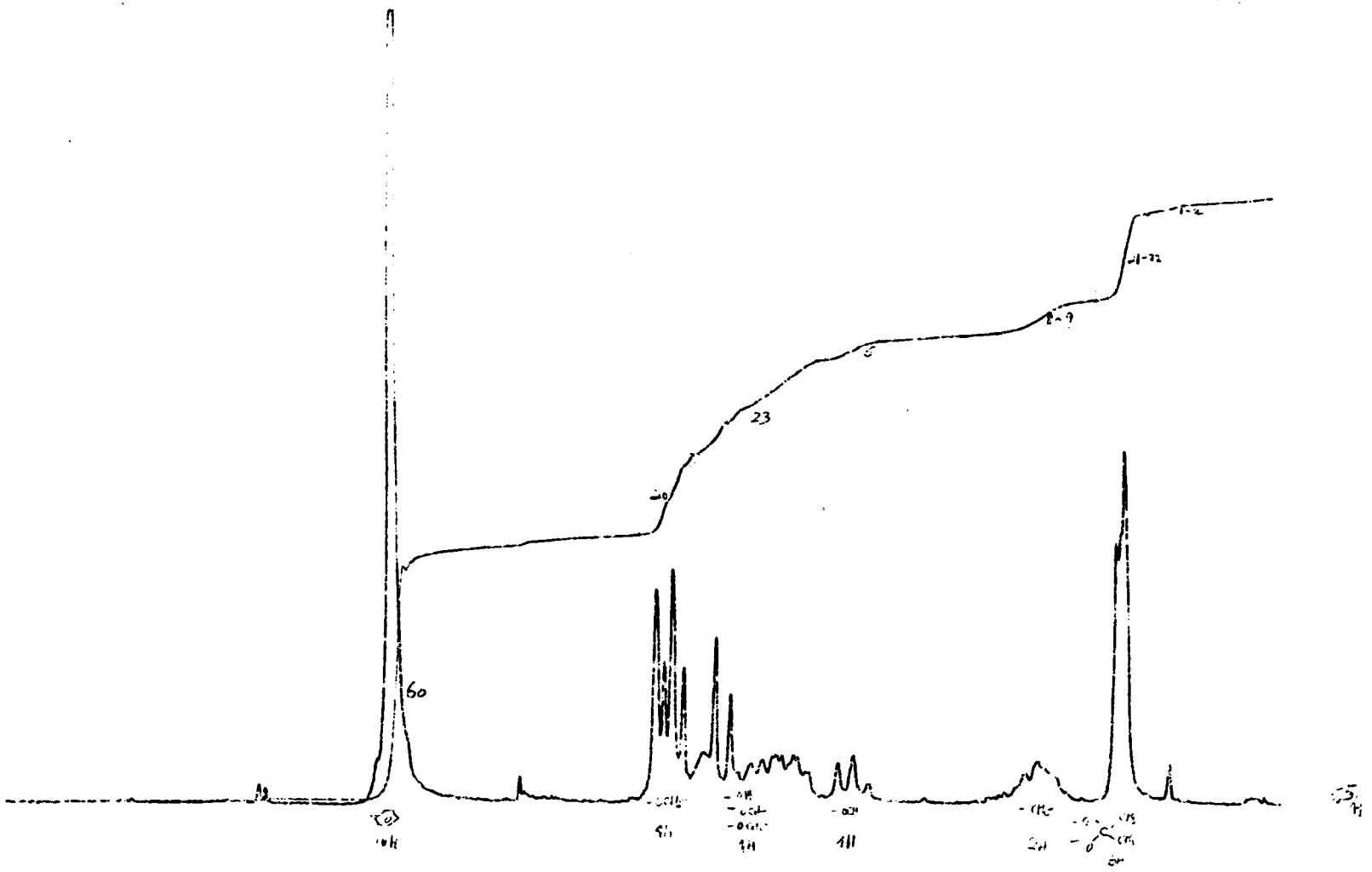
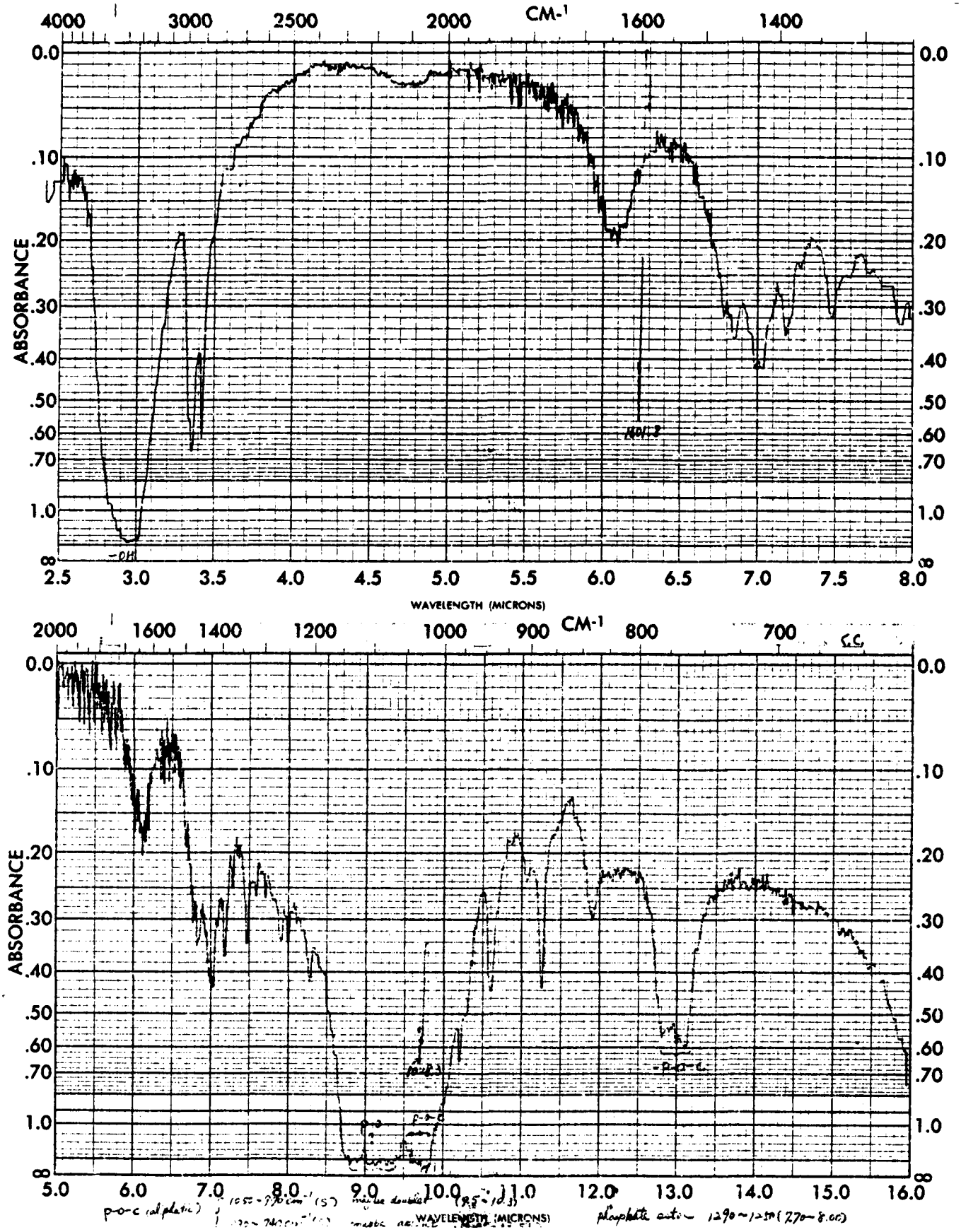


nmr ($CDCl_3$) of (1RS, 3S)-(71)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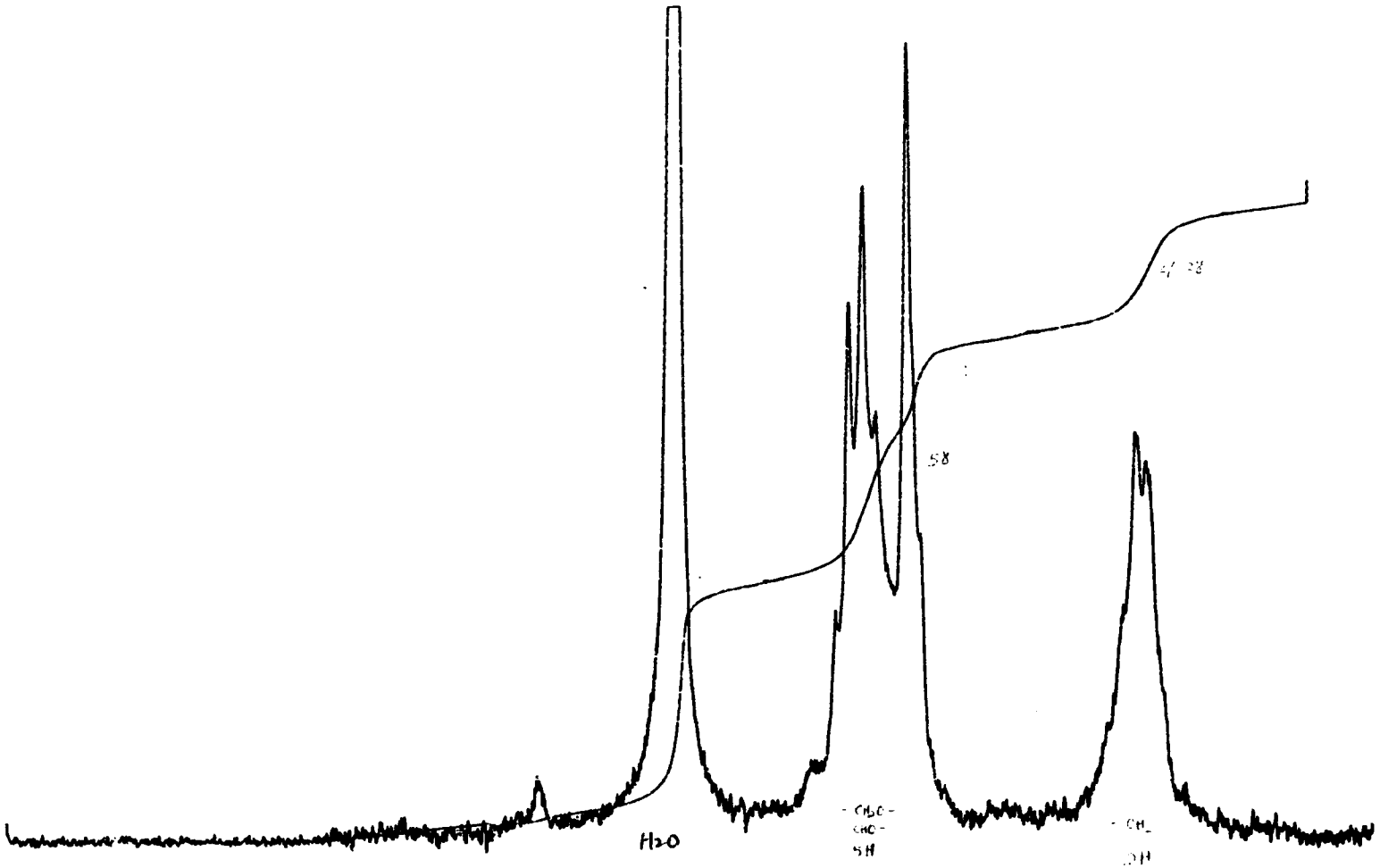
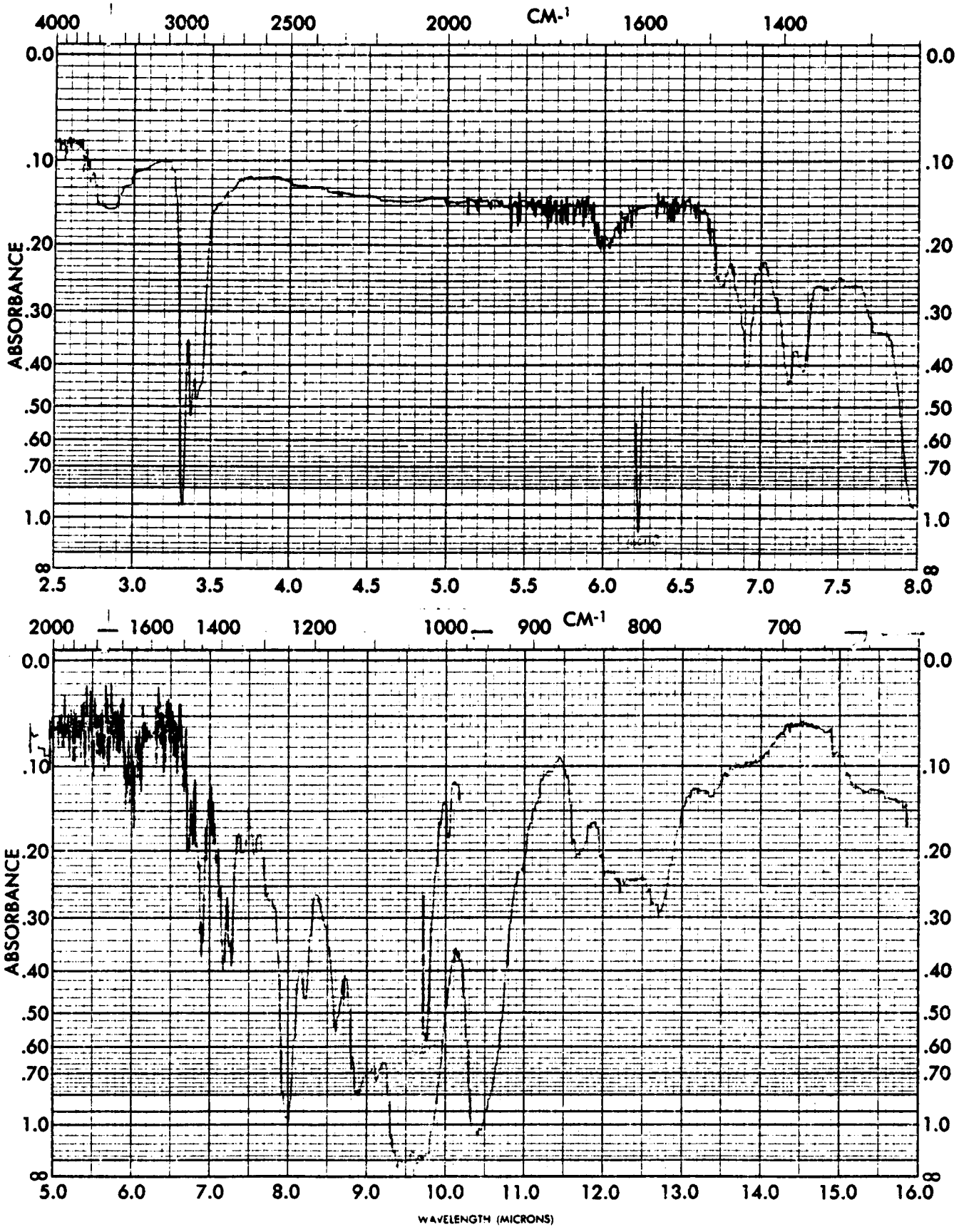
WAVELENGTH (MICRONS)

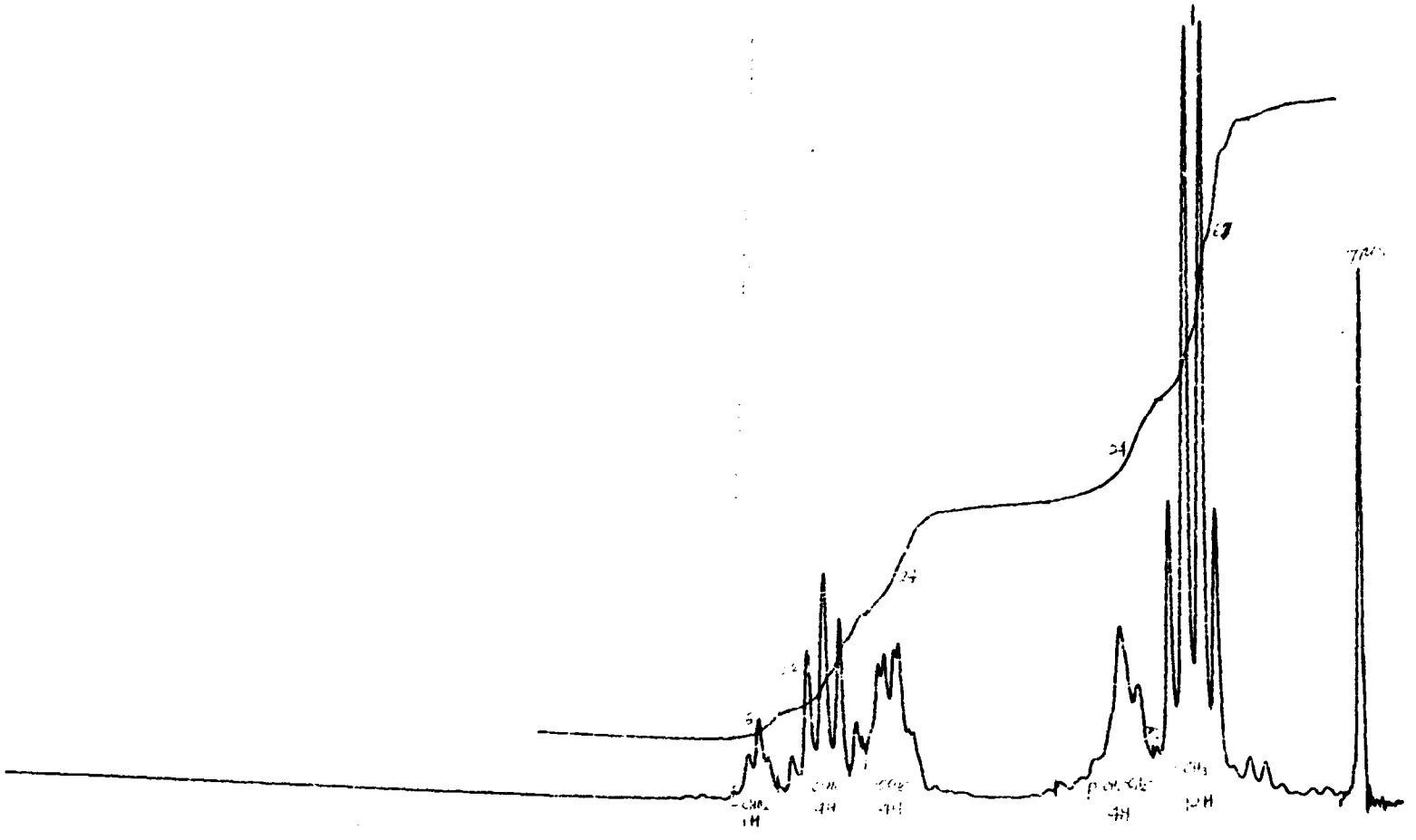
ir (KBr) of (S)-(74)



nmr (5% CD_3COOD in D_2O) of (S)-(74)



ir (between salts) of (89)



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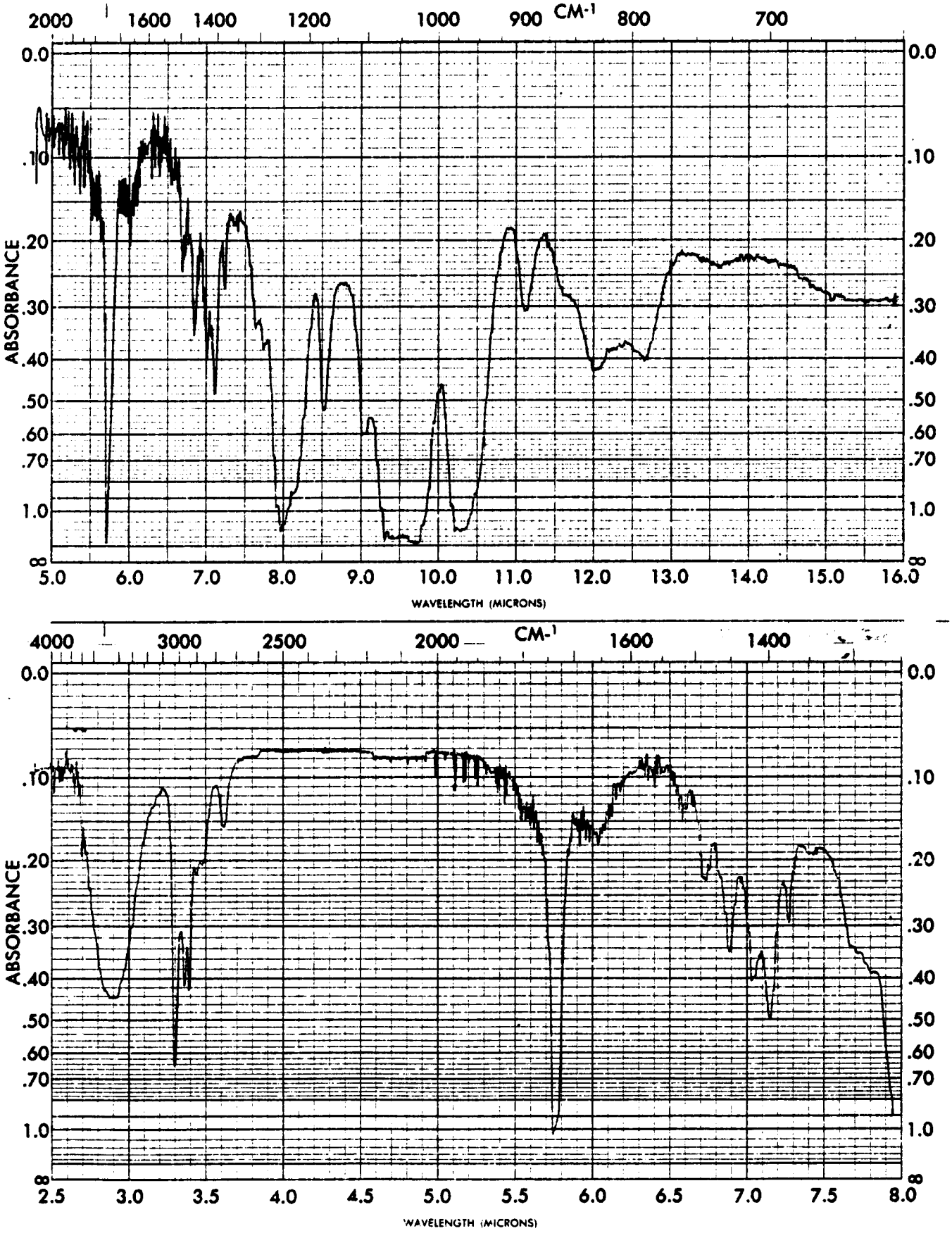
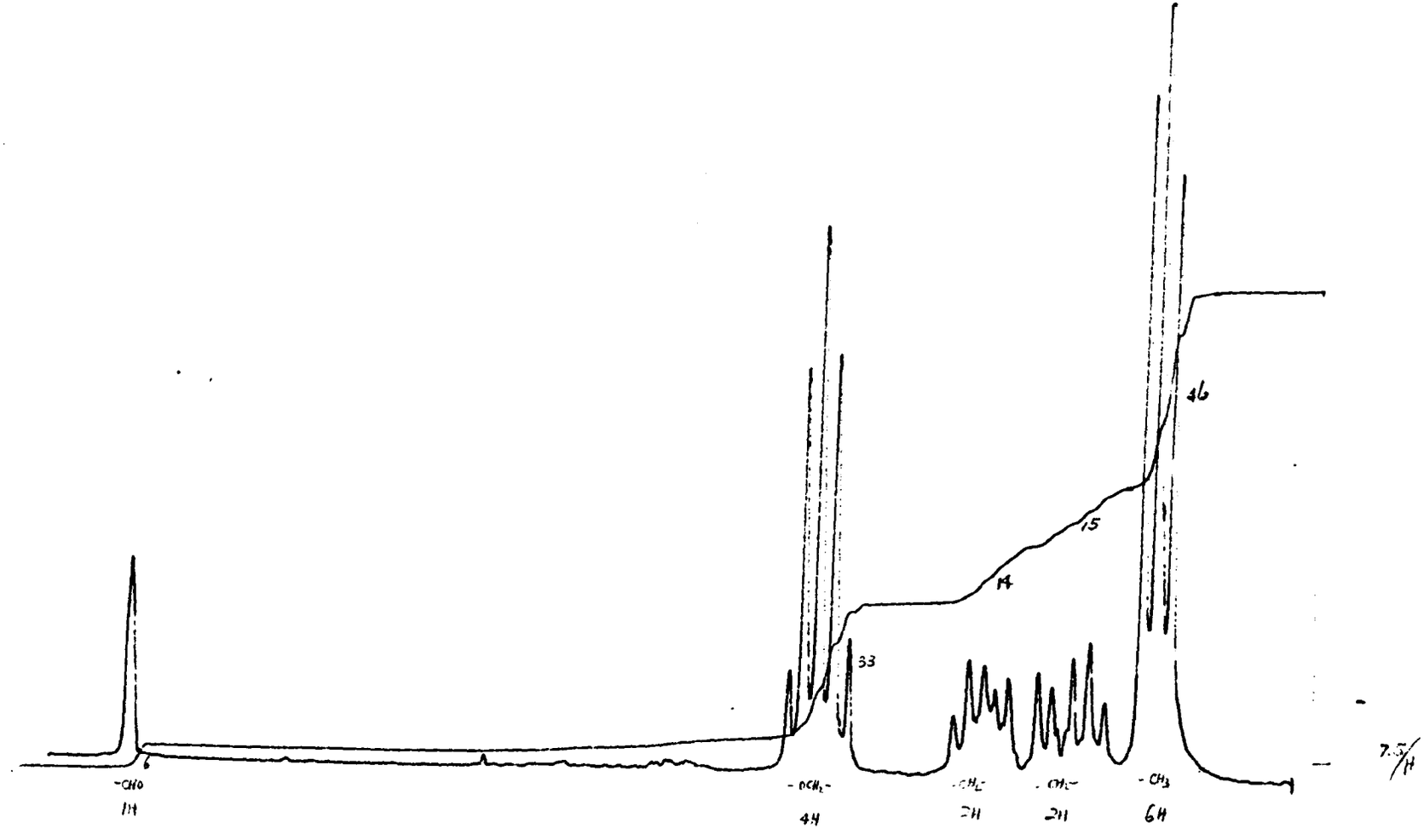
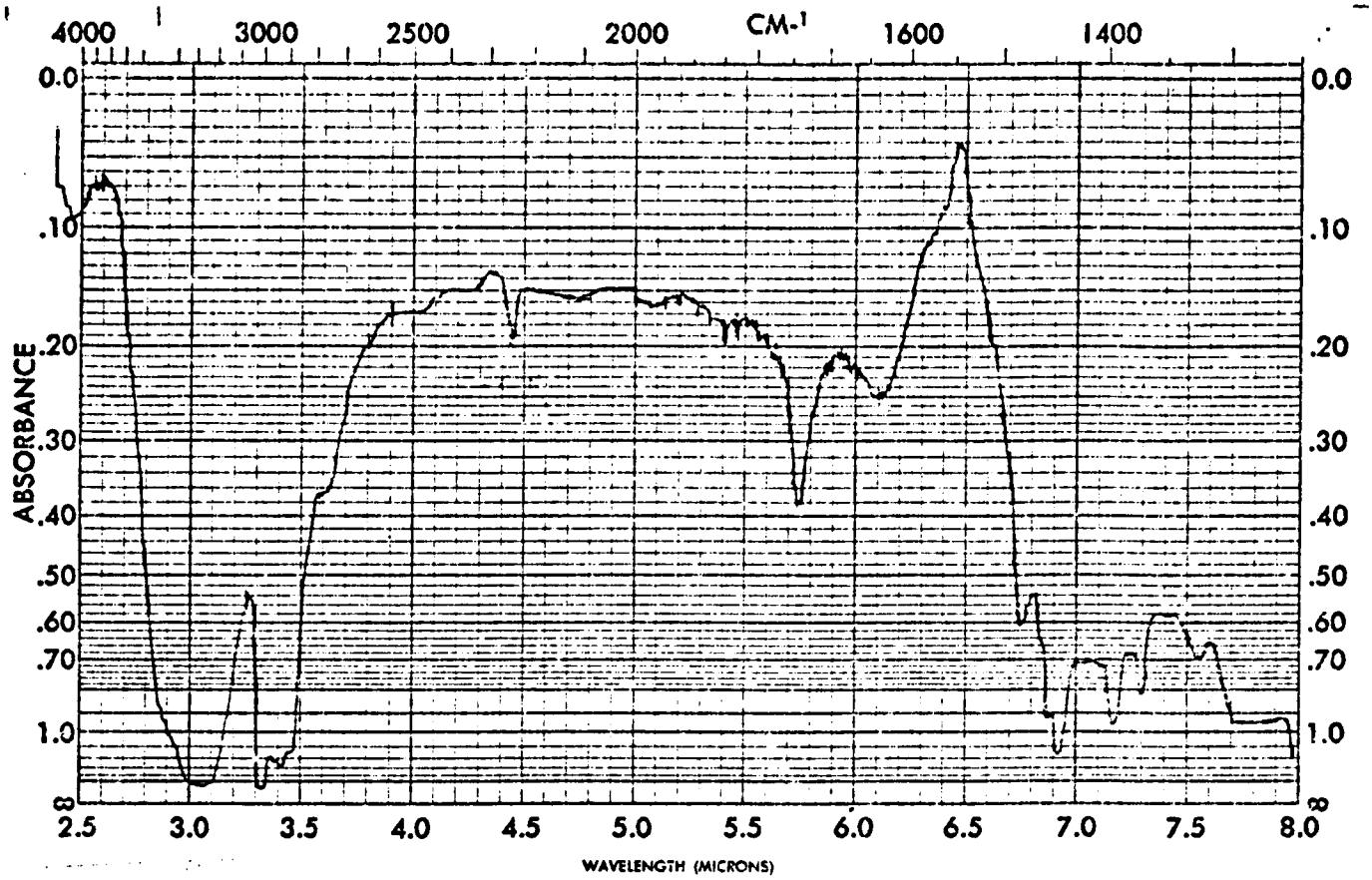


ir (between salts) of (88)



nmr (CCl_4) of (88)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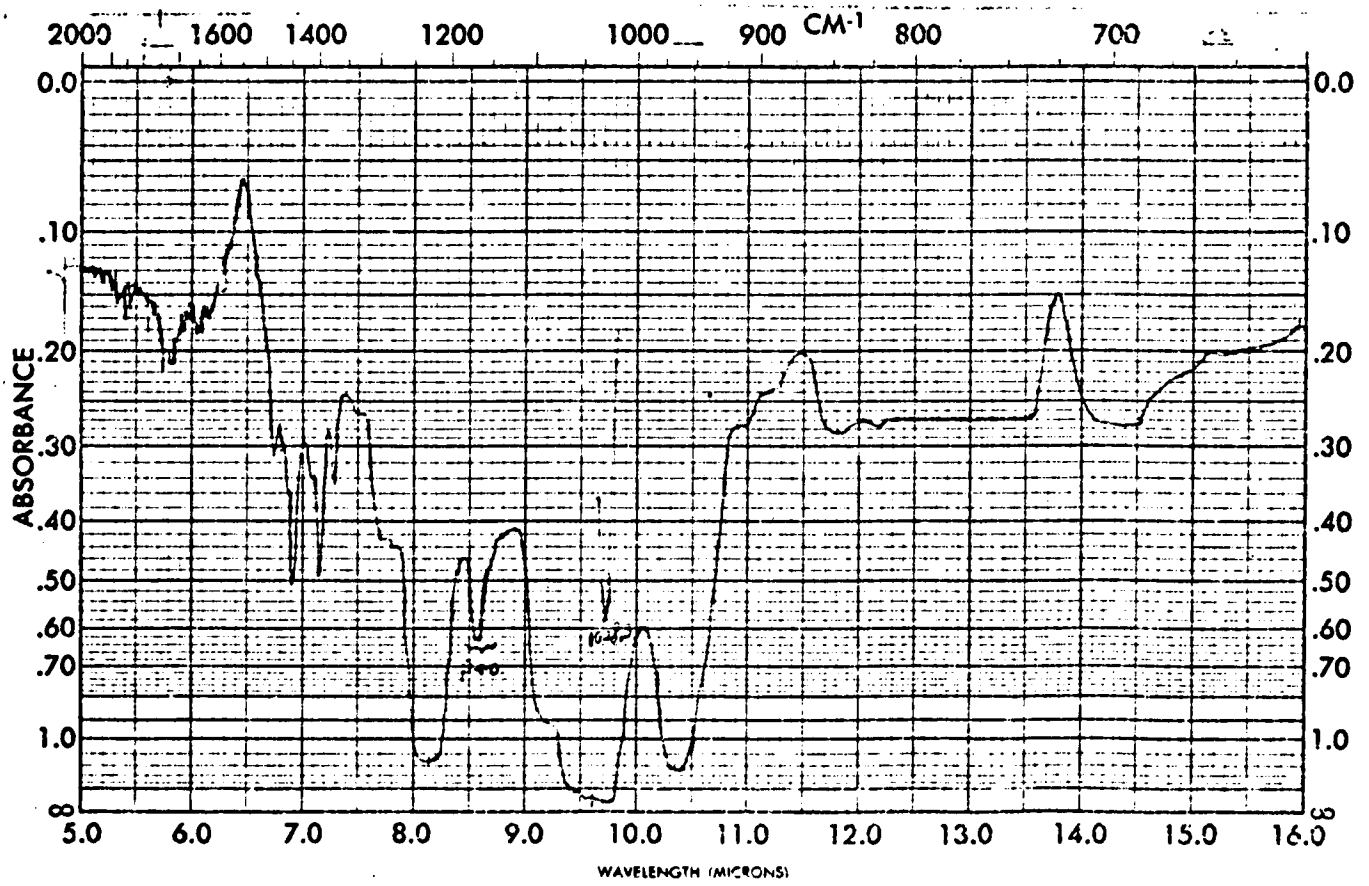
WAVELENGTH (MICRONS)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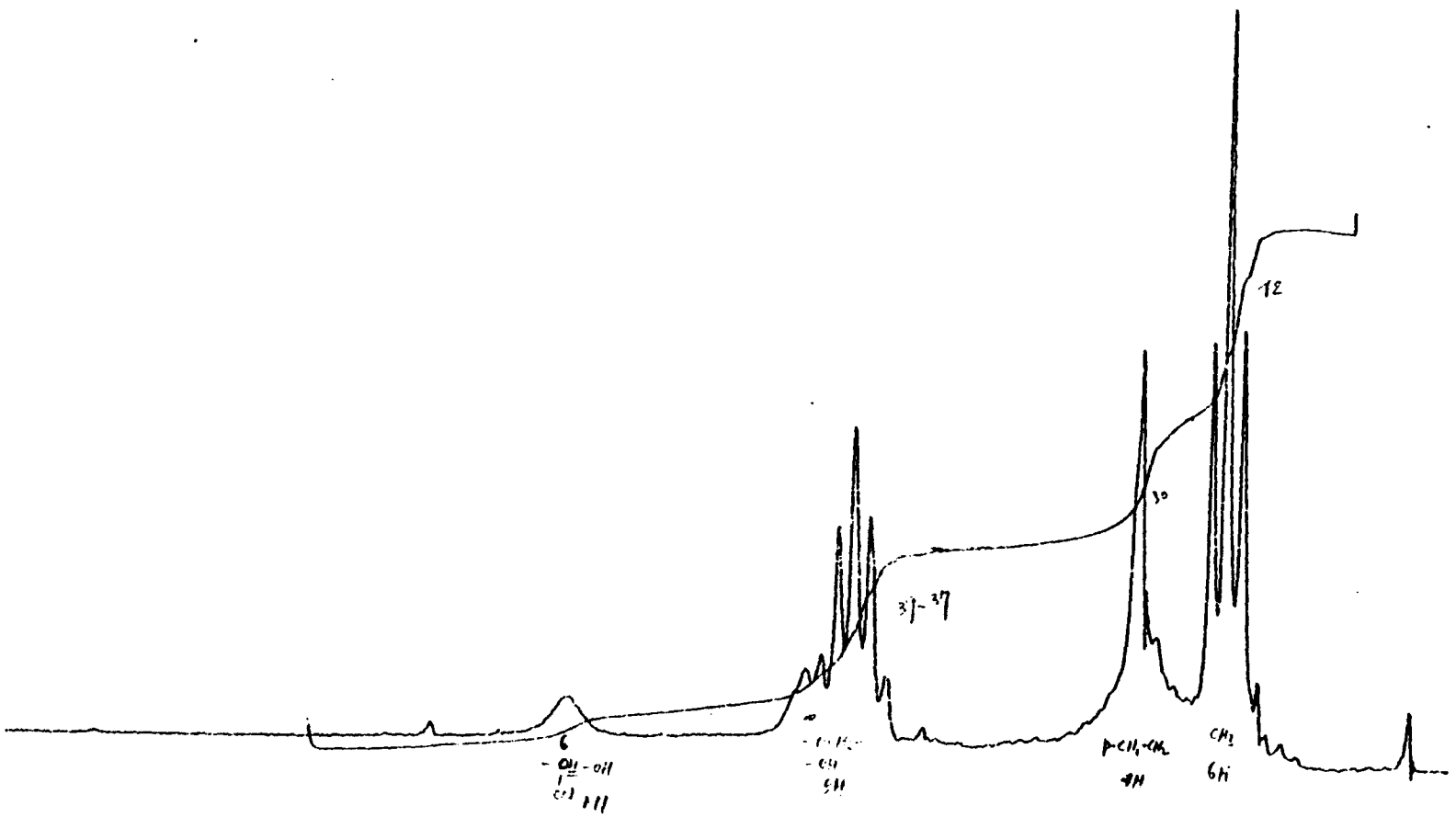
WAVELENGTH (MICRONS)

ir (CCl_4) of (87)



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