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APPROACHES TO THE SYNTHESIS OF PHOSPHINIC ACID ANALOGUES OF NUCLEIC ACID FRAGMENTS

by

AMAR P. GUPTA

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Abstract

APPROACHES TO THE SYNTHESIS OF PHOSPHINIC ACID ANALOGUES OF NUCLEIC ACID FRAGMENTS

by

AMAR P GUPTA

Adviser: Professor Robert Engel

The work described here represents considerable progress toward developing a convenient route to the synthesis of phosphinic acid analogues of oligonucleotides. The intermediates, LXXVII (Scheme XIV) and XCI (Scheme XXII) have been obtained in excellent overall yields from a cheap starting material, namely D-glucose. Both intermediates are promising precursors to phosphinic acid analogues of oligonucleotides and hopefully will be of great value in achieving that goal.

Several useful byproducts of this investigation are described below.

The phosphonium salts LVI and XLIX have been prepared, which are potentially valuable Wittig-reagents, and must be further investigated for their usefulness in phosphinate generation by reaction with aldehydes.

An improved method for the generation of vinyl phosphonates by using methylene-bisphosphonates in a Wadsworth-Emmons reaction with ketones, has evolved which

promises to be of great value.

An accidental discovery of phosphonic acid polymers obtained by simple pyrolysis of isopropyl esters of vinyl phosphonic acids is reported. Such polymers should prove to be of great value in industrial applications.

A convenient and cheap reagent, namely triphenylphosphine diiodide, has been discovered to be an excellent reagent for dealkylation of phosphonate esters under mild conditions.

An indirect method for achieving partial dealkylation of phosphonate diesters using a reduction-oxidation sequence, employing hexachlorodisilane-iodine, has been developed; although the reaction conditions are yet to be standardized for optimum yields.

In addition, several synthetic methods have been modified and improved (Experimental Section) to optimize yields and apply them successfully to our system.

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HISTORICAL

Over one hundred years ago a young Swiss physician, Friedrich Miescher published the first paper on "nuclein" and thus launched chemical research on nucleic acids (1). Miescher's work centered around chemical isolation of nuclei from cells. As source material for this investigation, Miescher chose pus cells collected from discarded bandages. Using strong salt solution he obtained a gelatinous substance. This gelatinous substance turned out to be composed mostly of long chain DNA. In the absence of a centrifuge, Miescher had no way to handle such viscous material and had to use other procedures involving successive alkali and acid treatments which yielded DNA in highly degraded form but which could be studied at that time. The material termed "nuclein" by Miescher turned out to contain 14% nitrogen and 2.5% phosphorus at a time when organic phosphorus was known to exist only in lecithin. "Nuclein" consisted of somewhat less than 30% DNA.

Subsequently, in the spring of 1872, Miescher notified the National History Society in Basel that he had isolated "nuclein" of high molecular weight from sperm heads obtained from sexually mature Salmon fish. This preparation had a phosphorus content of 9.59%, corresponding to the phosphorus content of pure nucleic acid.

The most important immediate successor to Miescher was Albrecht Karl Ludwig Martin Leonhard Kossel (2). Among his

achievements were the studies on purines, such as the isolation of xanthine and adenine (3), the identification of thymine (4), and the isolation and identification of cytosine (5). During Kossel's productive years he discovered histones from many sources, rediscovered protamines and studied the basic amino acids of these two nucleoproteins as well as the structure of nucleic acid bases.

In 1889 Altman proposed the term "nucleic acid" to describe the protein free nucleic acid which he isolated from yeast as well as from animal tissue (6). He is credited with having provided the general and convenient method for the preparation of nucleic acid at that time.

As for the pioneers in the field of the chemistry of the bases, the term "pyrimidine" was coined by Pinner in 1884-1885 from a combination of the words "pyridine" and "amidine" (7). The term "purine" was coined by Emil Fischer in 1884 (8) when he gave a rational basis to the nomenclature of compounds of this ring system by naming them derivatives of "purin". Adenine was isolated from acid hydrolysate of nuclein of beef pancreas in 1885 by Kossel (9) and was converted into hypoxanthine by a nitrous acid reaction (10). The proof of the adenine structure was provided by Fischer in his partial synthesis in 1897 (11) and the total synthesis of adenine and hypoxanthine was achieved by Traube in 1904 (12). Guanine was found in 1844 by Magnus (13), and by Unger in 1846 (14) in the excreta

(guano) of birds before it was recognized as a nucleic acid component by Kossel in 1879-1883 (15). The relationship of guanine to purine was elucidatd by Fischer in 1897 (16) and the total synthesis of guanine and xanthine was first described by Traube in 1900 (17). Thymine was first isolated in 1893 by Kossel and Newmann (4) from the acid hydrolysates of nucleic acids of calf-thymus and beef spleen. Stendel and others in 1900-1901 had established the correct structure of this compound by degradation and synthesis (18). In 1894 Kossel and Newmann also discovered that cytosine is a cleavage product from calf thymus DNA (4). In 1902 the correct structure of cytosine was proposed by Kossel and Stendel (19) and the total synthesis by Wheeler and Johnson in 1903 (20) confirmed it. Ascoli in 1901 (21) isolated uracil from yeast nucleic acid and its structure was confirmed by the synthesis of uracil by Fischer and Roeder (22).

As for the pioneers in the field of sugar components of nucleic acids, Kossel in 1891 (23) recognized from acidic hydrolysis a carbohydrate component of what is now called RNA which was identified three years later by Hammarsten as a pentose (24). Some fifteen years elapsed before Levene and Jacobs (25) succeeded in isolating the sugar in crystalline form and identifying it as D-Ribose, an unknown sugar at that time. The first synthesis of ribose was achieved by Fischer and Piloty (26) in the production of L-Ribose in syrupy form from L-arabonic acid via L-ribonic

acid. Blanksma and Alberda van Ekenstein repeated this procedure and isolated the L-ribose in crystalline form via purification as the phenyl hydrazone derivative (27). These workers repeated the synthesis in the D-series in converting D-arabonic acid to D-ribonic acid. It was then reduced by sodium amalgam and crystalline D-ribose was obtained via the p-bromophenylhydrazone purification procedure (28).

Significant advances in the chemical studies on 2-deoxy ribose were made at a much later date. In 1929-1930, Levene and coworkers obtained deoxy nucleosides from DNA by enzymic degradation (29) and mild acid hydrolysis of the purine deoxy-riboside yielded the 2'-deoxy-D-ribose in crystalline form (29-31). The chemical synthesis and characterization of 2'-deoxy-D-ribose took place as recently as from 1935-1950 (32).

The first definitive proof of the role of nucleic acids as genetic carrier of information was provided by Avery, Macleod and McCarthy (33) in 1944, when they showed conclusively that DNA was the bacterial transforming factor. However, it wasn't until the publication of the classical experiments of Hershey and Chase in 1953 (34), in which the genetic substance of bacteriophage was clearly shown to be the 32P labeled DNA, that the scientific world began to accept the biological role of nucleic acid as the physical basis of heridity. During the same period, Watson and Crick (35) had provided a model of DNA conformation as a complementary double helix, through which a molecular

process of self replication of DNA could be readily envisaged.

Since 1955, the advances in nucleic acid chemistry and molecular genetics have been tremendous, and substantial insight has been obtained about the complex phenomena of the cell. A brief summary of these advances, particularly with regard to structural aspects of nucleic acids and their role in protein biosynthesis, will be in order.

It is now well established that RNA molecules can possess secondary and tertiary structures. The macromolecular properties of polynucleotides are greatly influenced by the negatively charged electrostatic field arising from the phosphodiester groups and by the ability of bases to interact to form helical structures. These two forces tend to oppose each other and consequently conformation depends on ionic strength, temperature and pH (36). Three conformations of single stranded polyribonucleotides have been characterized and are as follows: (a) an amorphous form in which the bases have no preferred orientation with respect to one another, (b) a stacked conformation in which the flat purine and pyrimidine rings tend to pile one upon another to form a single helix, and (c) a "hairpin loop" conformation stabilized by hydrogen bonding between complementary bases to give a double-helical structure below the loop. In such a conformation, sequences that cannot find appropriate bases for pairing tend to be looped out. Double helices also form between RNA strands.

The resulting structure resembles that of DNA in that it consists of two antiparallel polynucleotide chains stabilized by Watson-Crick type base-pairing. Natural double-stranded RNA occurs in certain viruses.

Prior to 1950, by use of histochemical techniques, Bracnett and Caspersson independently established tnat cells active in protein-synthesis possessed a high RNA content. The suggestion was made that there was an intimate connection between protein synthesis and RNA. It was not until cell-free protein-synthesizing systems were developed, however, that real progress was made in establishing the role of RNA in the synthesis of polypeptide chains. In 1954 Zamecnik and associates in Boston had developed such a system from rat liver. Essential components of this cell-free system were amino acids, ATP, GTP, the ribonucleoprotein fraction of the microsomes (i.e., the ribosomes) and factors including enzymes in the 105,000-g supernatant. By the use of this system it was soon established that the ribosome was the probable site of synthesis of peptide chains (37-38) and that formation of "activated" amino acids (i.e. aminoacyl-adenylates), was the first step in the synthesis of protein from amino acids (39). In 1957 and 1958, again by the use of the in vitro protein-synthesizing system, the Zamecnik group made a dramatic advance that established the second step in the biosynthetic pathway. The activated amino acids were shown to become covalently bound to a type of RNA, termed soluble

RNA (s-RNA), in the 105,000-g supernatant. Next it was demonstrated that aminoacyl-sRNA substituted for free amino acid in the cell-free system and that the transfer of amino acid from sRNA (later termed "transfer RNA" or "tRNA") to the peptide chain on the ribosome was dependent on GTP (40). Evidence soon followed that tRNA was a complex mixture of polynucleotides, and that each tRNA species was specific for a particular amino acid.

Prior to the elucidation of the function of tRNA, it was thought by some that DNA might act directly as a template for assembly of amino acids into protein, a concept introduced by Gamow (41) after consideration of the Watson-Crick double helical structure for DNA. Each amino acid was considered to make direct steric fit with a sequence of bases. Later it was proposed that amino acids were held directly on RNA templates and then linked enzymically. Crick (42) rejected these concepts and proposed that "each amino acid would combine chemically, at a special enzyme, with a small molecule which, having a specific hydrogen bonding surface, would combine specifically with the nucleic acid template."

This became known as the "adaptor hypothesis". The molecules, or adaptors, to which the amino acids become attached were considered to be mediators between the encoded information of the nucleic acid and the extremely variable chemical structures of amino acids. The discovery of tRNA and its specificity completely vindicated the adaptor

hypothesis; tRNA possessed all the properties of Crick's adaptor.

By 1958 the ribosome was established as the site of cytoplasmic protein synthesis, and many assumed that ribosomal RNA was the carrier of genetic information transcribed from DNA. It was also considered that rRNA (ribosomal RNA) was the template that combined, not with amino acids, but, by hydrogen bonding, with specific groups of bases on the tRNA portion of aminoacyl-tRNA molecules. By 1961, however, this concept was generally discarded. It was replaced by a new concept supported by the elegant experiments of Brenner et al. (43). These workers found that after infection of the bacterium E. coli with T2-bacteriophage no new ribosomes were formed for synthesis of viral protein, but in fact a new RNA species with rapid turnover and a base composition complementary to that of phage DNA was produced and attached to pre-existing E. coli ribosomes. Such RNA with template function termed "messenger RNA" or "mRNA" was postulated by Jacob and Monod (44) in their theory of protein synthesis. RNA species that labeled very rapidly, attached to ribosomes and possessed a DNA like base composition were soon detected in normal uninfected bacterial cells (45).

Further support for the mRNA concept was provided by the classic in vitro experiments of Nirenberg and Matthaei (46) who, using a cell-free protein synthesizing system from E. coli, showed that addition of synthetic polyuridylic acid

resulted in the formation of polyphenylalanine. This observation together with similar experiments using other synthetic polymers as templates, convincingly established tne mRNA concept and also provided important information regarding the now familiar genetic code. In recent years the existence of mRNA has been demonstrated unequivocally. The work of Lockard and Lingrel (47) merits special mention. Treatment of mouse reticulocyte with detergent yielded an RNA species (9S) that sedimented between tRNA and rRNA in sucrose gradients. Although several properties of this RNA were consistent with it being the mRNA for globin, demonstration of ability to direct the synthesis of the globin chains was required to establish this unequivocally. Lockard and Lingrel showed that the 9S RNA from mouse polysomes did direct the formation of mouse globin β-chains in a rabbit reticulocyte cell-free protein-synthesizing system. it has also been shown that an RNA fraction from rabbit reticulocytes can direct the synthesis of globin in a cell-free system from E. coli (48). The concept that the mRNA carries information in the form of nucleotide sequences from the gene to the protein synthesizing mechanism is now clearly established. On the ribosome, the codons (sequences of three adjacent nucleotides that code for an amino acid) of mRNA pair sequentially with the anticodons of tRNA molecules, and since each tRNA is specific for a particular amino acid, amino acid sequence is dictated. In eukaryotic cells the mRNA synthesized on the DNA template passes from

the nucleus to the cytoplasm for translation into protein by cytoplasmic ribosomes, and transcription and translation are essentially independent processes. However, in bacteria, mRNA appears to be translated by a cluster of ribosomes that follow closely behind the RNA polymerase involved in transcription. Thus in bacteria both transcription and translation of an mRNA chain occur simultaneously, and the two processes may be coupled (49-50).

During the past few years, knowledge of tRNA biosynthesis, structure and function has advanced greatly and merits special comment. Our understanding of tRNA exceeds that of any other RNA species. The development of cell-free systems for tRNA biosynthesis, the first being devised by Zubay et al. (51), contributed greatly to the understanding of tRNA transcription and the processing of tRNA precursors. These (precursors) may contain several potential tRNA molecules linked by oligonucleotide segments and several tRNA precursors have been completely sequenced (52). The first functional tRNA molecule was sequenced by Holly et al. (53); today about 80 tRNA sequences are known, and the list includes tRNAs for all 20 amino acids except asparagine. The determination, in 1975, of the three dimensional (tertiary) structure of crystalline yeast-phenylalanine-tRNA by X-Ray diffraction analysis at 2.5 A resolution was a monumental achievement of great significance (54-55). A diversity of techniques including laser-Raman and NMR spectroscopy indicate that basically the

same structure exists in solution (56-57). The four common nucleoside bases in tRNA undergo sequence specific enzymic modification to yield a diversity of minor nucleosides, 43 of which have been identified unequivocally. This extensive nucleoside modification distinguishes tRNA from all other RNA species.

In recent years it has become clear that tRNA has multiple functions and is not merely an adaptor molecule in protein synthesis. In addition to certain biosynthetic roles which do not involve ribosomes but result in transfer of amino acids (58), tRNAs appear also to have certain regulatory functions. The best understood of these is the repression by aminoacyl-tRNAs of enzymes involved in aminoacid metabolism. The first significant work in this area was the observation by Schlesinger and Magasanik (59), which indicated that the concentration of charged histidine-tRNA and not the concentration of free histidine controlled repression of histidine biosynthetic enzymes in Salmonella typhimurium. The function of some polynucleotide polymerase systems, including reverse transcriptase, appears to depend on tRNA as cofactors (60).

Similarly, it now seems that rRNA is not simply an inert structural component of the active ribosome. The recent identification of sequences in 3'-termini of the rRNA of the small ribosomal subunit in prokaryotes that are partly complementary to initiation sequences within ribosomal binding sites in mRNA (61) indicates that interaction

between rRNA and mRNA may be important in determining the relative translation efficiencies of different mRNAs, including individual cistrons of polycistronic mrna.

A brief account of the present day knowledge about protein biosynthesis follows:

The translation process in both prokaryotes and eukaryotes has three basic steps: (a) initiation (b) elongation, and (c) termination. The initiation and termination steps are unique events in the synthesis of any protein, and signals of these events occur at particular sites on the mRNA molecule in the form of specific triplet codons. Elongation involves the repetitive synthesis of a peptide bond between the C-terminal of the growing peptide chain and the a-amino groups of the incoming amino acid. The order of addition of the amino acids is determined by the mRNA that moves, relative to the ribosome, by three nucleotides (i.e. one codon) toward its 5'-end after the attachment of each aminoacid.

The initiation of protein synthesis in E. coli requires free ribosomal subunits, mRNA, GTP, the special initiator tRNA, formylmethionyl tRNA (fmet-tRNA) and three protein initiation factors. Initiation factors are defined as factors essential for the initiation of the translation of mRNA. In prokaryotes there are three protein factors termed IF-1, IF-2, and IF-3 (previously f1, F1, or A; f2, FIII or C; f3, FII, or B respectively). All three factors are located on native small ribosomal subunits (62) from which

they can usually be dissociated with 0.5-1.0 M ammonium chloride buffers (63). The molecular weights of IF-1, IF-2 and IF-3 are 9,400 (64-65), 80,000 (66-68) and 22,600 (69), respectively.

The initiation process begins with the spontaneous binding of IF-1 and IF-3 to a a free 30S subunit released from a 70S ribosome, following the termination of translation (Fig.1). The [30S.IF1.IF3] particle is believed to bind a preformed ternary complex consisting of [fmet-tRNA.IF2.GTP] (70-72); fmet-tRNA cannot bind to 30S subunits in the absence of IF2 (73-75). The initiator tRNA is positioned on the 30S subunit such that when the 30S initiation complex associates with the 50S ribosomal subunit, it enters the peptidyl site on 70S ribosome (76-77).

Following the addition of the ternary complex, mRNA is bound to form a complete 30S initiation complex (73,75,78). Messenger RNA appears to complex with several components in the 30S initiation complex, including IF-3 (75), the ribosomal proteins S1, S7, S12 and S21 (79-80), and a region of 16S rRNA at or near its 3' terminus (81-83).

The complete 30S initiation complex rapidly associates with a 50S subunit to form a 70S initiation complex.It is not clear whether a joining factor is required (84). Three large subunit ribosomal proteins appear to be essential for the interaction between a 30S initiation complex and a 50S particle (85). At some stage during or immediately

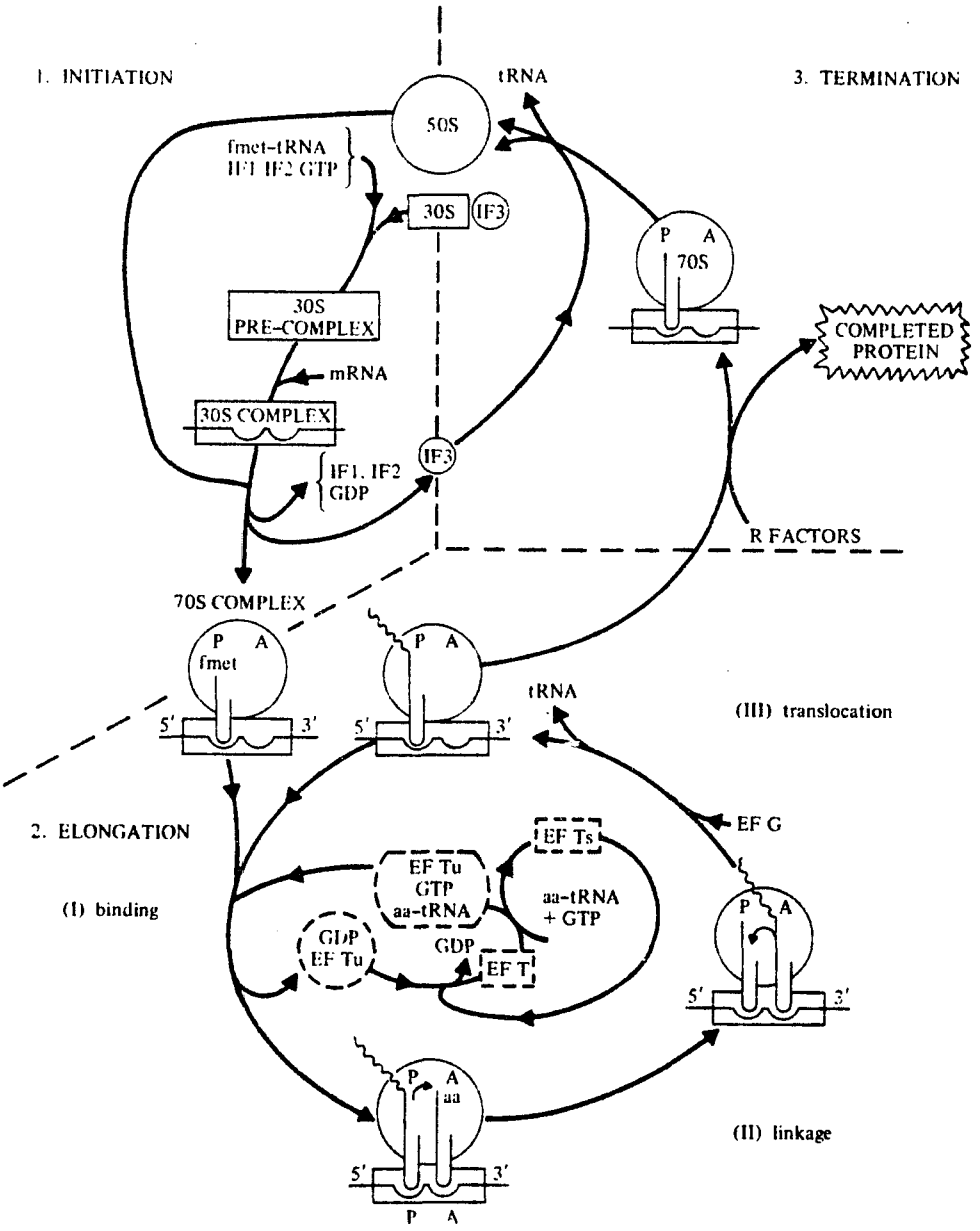


Fig. 1. Diagrammatic representation of the three general steps in the translation process in prokaryotes, namely, initiation, elongation, and termination.

following association, the three initiation factors are released by a process requiring the hydrolysis of GTP (86), the latter reaction apparently being promoted by IF-1 (67). Accordingly, the following is a summary of the steps

involved in the initiation process in E. coli:

(1) 30S + IF1 + IF3 ----→[30S.IF1.IF3];

(2) fmet-tRNA + IF2 + GTP ----→[fmet-tRNA.IF2.GTP]

(3) [30S.IF1.IF3] + [fmet-tRNA.IF2.GTP] ----→
[30S.IF1.IF3.fmet-tRNA.IF2.GTP]

(4) [30S.IF1.IF3.fmet-tRNA.IF2.GTP] + mrna ----→
[30S.IF1.IF3.fmet-tRNA.IF2.GTP.mRNA];

(5) [30S.IF1.IF3.fmet-tRNA.IF2.GTP.mRNA] + 50S ----→
[70S.mRNA.fmet-tRNA] + IF-(1+2+3) + GDP + Pi

Following the formation of the 70S initiation complex, fmet-tRNA is located at the "P" site (peptidyl or puromycin reactive site, also termed the "D" or "donor" site; Fig.2) on the 50S subunit and the "A" site (aminoacyl or acceptor site) is free for occupation by by an aminoacyl-tRNA (charged tRNA). The ribosome is now ready to commence peptide bond synthesis and polypeptide chain elongation.

The process of elongation, which is repetitive, can be divided into three stages: (a) binding of the charged tRNA to the "A" site of the ribosome, (b) linking of the incoming amino acid to the nascent peptide chain (or in the first instance to formylmethionine) by formation of a peptide bond and (c) translocation of the newly extended peptidyl-tRNA from the "A" site to the "P" site with concommitant movement of the ribosome along the mRNA by three bases (Fig.2). During this cycle, protein elongation factors play an

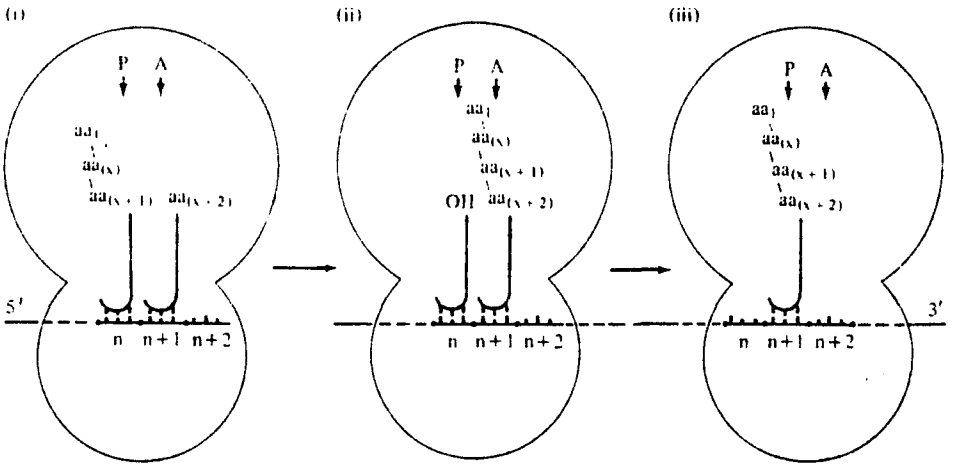


Fig. 2. Diagrammatic representation of translocation. The prepeptide bond complex (i) is converted into the pretranslocation complex (ii) by the formation of a peptide bond between the incoming aa-tRNA (A site) and peptidyl-tRNA (P site). Translocation involves the movement of the ribosome along the mRNA and the shift of the elongated peptidyl-tRNA from the A site to the P site, giving rise to the posttranslocation complex (iii).

important role. These factors, termed "EF-G", "EF-Tu" and "EF-Ts", occur in the soluble fraction of the cell. The "Tu" and "Ts" abbreviations refer to heat- unstable and -stable components of the EF complex respectively. The elongation factors have been purified and have molecular weights of 42,000-47,000 daltons for EF-Tu, 28,500-35,000 daltons for EF-Ts, and 72,000-84,000 daltons for EF-G (86,88).

The first step in the binding of charged tRNA to 70S ribosomes is the formation of a ternary complex between IF2, GTP and fmet-tRNA (Fig.1). The binding of GTP to EF-Tu components of the EF-T complex is thought to induce a conformational change in EF-Tu, causing its dissociation from EF-Ts and favoring its association with aminoacyl-tRNA (89). This ternary aminoacyl-tRNA complex binds to the ribosome so that the aminoacyl-tRNA is positioned in the "A" site. Following binding of the ternary complex, the

hydrolysis of GTP occurs with the release of [EF-Tu.GDP]. Upon release of the [EF-Tu.GDP] complex, the factor EF-Ts binds to the EF-Tu moiety of the complex displacing GDP, thereby generating EF-T, which may then interact once more with GTP and charged tRNA as described above (90-92).

After the charged tRNA is bound to the "A" site, peptide bond synthesis occurs between the free α-amino group of peptidyl-tRNA or fmet-tRNA located at the "P" site. This reaction is catalysed by the enzyme peptidyltransferase, which is an integral part of the 50S subunit (93). The peptidyltransferase center involves several ribosomal proteins (94-95). soluble or supernatant factors are required in this reaction, and GTP hydrolysis is unnecessary as the free-energy change is sufficiently negative to drive the reaction.

Following peptide bond synthesis, the newly extended peptidyl-tRNA is at the "A" site and uncharged tRNA is at the "P" site; at this stage the ribosome complex has been termed a "pretranslocation" complex (96). Translocation involves the movement of the ribosome complex along the mRNA, accompanied by transfer of the peptidyl-tRNA, from the "A" site to the "P" site giving rise to a "posttranslocation" complex (Fig.2). During this process the uncharged tRNA at the "P" site is released and hydrolysis of GTP occurs (97).

The elongation factor EF-G which has ribosome dependent GTPase activity, is necessary for translocation and binds to

tne larger ribosomal subunit. Proteins L7 and L12, which occur on the large subunit, are involved in GTP hydrolysis, but it is not known whether they have intrinsic GTPase activity or whether they promote this activity of EF-G (98).

During translocation the ribosome complex moves along the mRNA and at the same time EF-G catalyzes the hydrolysis of one GTP molecule (99). Although the distance moved by the ribosome complex relative to the mRNA has long been assumed to be three nucleotides in length, because of the triplet nature of the genetic code, positive evidence establishing this has only recently been presented. Using synthetic polynucleotides and bacteriophage f2 RNA as mRNAs, pre- and post-translocation complexes, separated by a single translocation step, were isolated and treated with ribonuclease to digest those parts of the mRNA that were not protected by the attached ribosome. The sequences of the nucleotide fragments that were subsequently isolated from the two complexes differed by three nucleotides at each end (76,96).

Termination involves the hydrolysis of the ester link between tRNA and the polypeptide chain and occurs while the ribosome is still attached to the mRNA (100-101). This event is signaled by any of three base triplets (termination codons) UAG, UAA and UGA, in the messenger. These codons were first identified by studies of the genetic code in bacteria, which indicated that none of them specified an amino acid. Subsequently these codons were shown to be

involved in premature chain termination of mRNA translation in nonsense mutants (102). Direct evidence that these nonsense triplets are natural termination signals has been presented in the case of bacteriophage R17 RNA in which a tanden arrangement of UAA is present at the end of the coat protein cistron (103).

In addition to the termination signals on mRNA, protein supernatant factors have been implicated in the release of completed polypeptide chains (101,104). Two factors with molecular weights of 44,000 and 47,000 daltons, respectively, have been purified from bacteria (105-106). They differ in the recognition of the nonsense triplets, RF1 being more specific for UAA and UAG, while RF2 recognizes UAA and UGA. A third factor (R3) stimulates the binding and release of RF1 and RF2 in the presence of GTP. It seems possible although evidence is lacking that the release factors in conjugation with the peptidyltransferase enzyme are actively involved in the hydrolysis of the peptidyl-tRNA link (88). This may not be the sole function of the release factors at termination since in the absense of these factors in vitro, ribosomes can run through termination codons (107) suggesting that release factors are involved in specific recognition of terminator codons.

A tentative sequence of events in termination has been presented by Tate and Caskey (101). The first event following translocation of the peptidyl-tRNA to the "P" site is recognition by the ribosome of the terminator codon at

the "A" site. This recognition event is possibly by the way of an interaction between the terminator codon and a complementary site at or near the 3'-OH end of 16S or 18S rRNA (81,108) and may be prompted by one of the release factors. Either RF1 or RF2 in association with RF3 and GTP then binds to the ribosome at a site that overlaps with the EF-G binding site on the large subunit. Release factor and peptidyltransferase interact to facilitate hydrolysis and the release of free polypeptide, and finally the release factor dissociates from the ribosome after hydrolysis of GTP. This last step has not been identified in the bacterial ribosomes, but is found to occur in mammalian systems (101).

Following the release of completed polypeptides, the vacated ribosomes separate from mRNA, dissociate into subunits, and become available again for initiation reactions. Ribosomes are thus recycled continuously during protein synthesis (109-110). The general order of events in the recycling process in both prokaryotes and eukaryotes is similar, although some minor points are disputed (109-110).

In any attempt to correct or treat genetic diseases using chemical agents (i.e. drugs), one or more fundamental difficulties may be expected. For example, if some substance foreign to the organism is introduced there is a high probability that the organism will recognize it as foreign and degrade it; thus it is necessarily a short range treatment requiring constant renewal, the incidence of

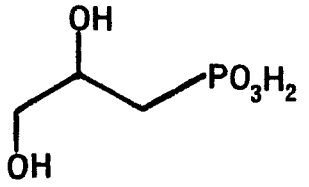
renewal dependent on the rate of degradation. A similar result might be expected were a corrected natural agent introduced, such as an ordinary nucleic acid; both mRNA & comparatively longer lived species are subject to eventual cleavage of the phosphate ester linkages. Moreover, there always exists the problem of introduction of the agent to the organism.

The first of these problems could presumably be overcome if one were to use an agent which bore such structural similarity to the natural materials that it could perform the normal (corrected) functions in place of the natural material, yet be impervious to the available modes of degradation. Specifically, an agent would be of potential value if a substitution were made for the normal phosphate ester oxygens of a nucleic acid or nucleic acid fragment by a methylene linkage. This type of substitution has been made sucessfully over the past fifteen years, in a variety of biologically active compounds including molecules such as products of glycolysis, carbohydrates, phospholipids and nucleotides (111). Briefly consider some of these efforts.

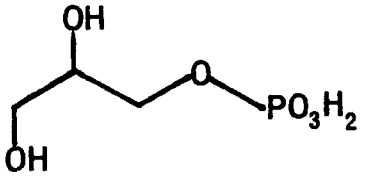
The compounds of interest are those in which a direct carbon to phosphorus bond is present in place of the usual carbon-oxygen-phosphorus linkage. In some of the systems investigted, the analogues devised have involved the exclusion of the oxygen of the natural phosphate ester linkage, an example being 2,3-dihydroxypropyl-1-phosphonic acid (I) prepared as an analogue of glycerol-3-phosphate

(II) (112-113). While such analogues have been found to have biological activity with certain systems, a more interesting class of analogues are those which are isosteric with the natural phosphates. An isosteric non-hydrolysable (phosphonic acid) analogue of (II) is 3,4-dihydroxybutyl-1-phosphonic acid (III) (114). It should be noted that while the term isosteric strictly refers to compounds of identical size and shape, and the phosphonic acid (III) compared to the natural glycrol-3-phosphate (II) does not meet these requirements most rigorously, crystallographic data for a series of phospates and related phosphonic acids indicate differences to be minimal unless a phosphorus-carbon-phosphorus linkage is present (115-121).

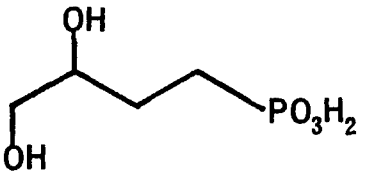
With the fundamental working postulate that the isosteric phosphonic acid analogue of a natural phosphate



I



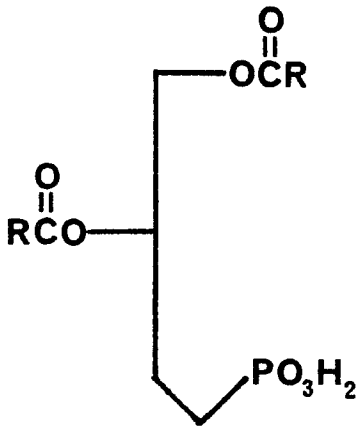
II



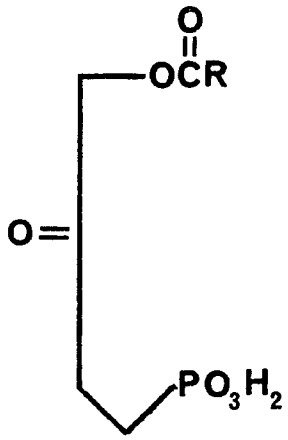
III

might be expected to substitute for that natural phosphate in all processes except cleavage of the phosphorus from the remaining organic function (noting all caveat pertaining to differences in acidity at the phosphorus site, the lack of binding potential of the esteric oxygen etc.), it was predicted that the analogue (III) could serve as a metabolic regulator by substitution for (II). In fact (III) is recognized by many of the enzymes that catalyze reactions normally involving (II) (122-123). The mechanism of inhibition of growth of E. coli appears to be consistent with the above mentioned postulate; the analogue participates in certain biosynthetic reactions as the natural material until the point where cleavage of the phosphorus is normally required. The accumulation of the resultant non-natural product, unable to divest itself of its phosphonic acid phosphorus, and the absence of the natural metabolic end product then perturbs the cell such that further growth does not occur.

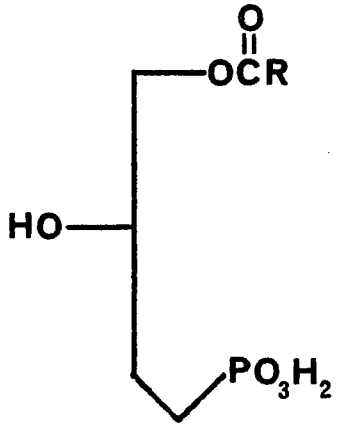
Some analogues of phospholipids, the isosteric phosphatidic acids (IV) bearing saturated and unsaturated fatty acid ester linkages, have been prepared by Tang et. al (124). Also, a lipid derivative of the analogue of dihydroxyacetonephosphate, compound (V), a reduced form of it, lyso-phosphatidic acid (VI), and the differentially substituted compound (VII) have been prepared (125). Compound (VI) has been found to be a substrate for lyso-phosphatidate acyltransferase.



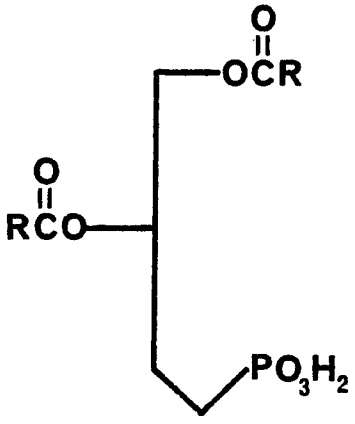
IV



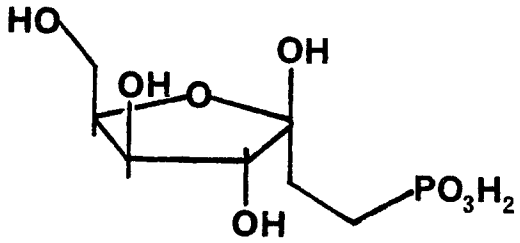
V



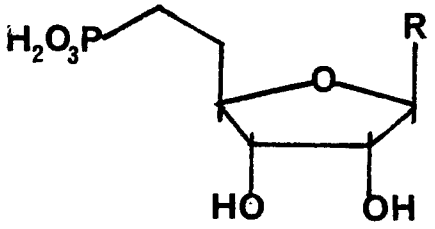
VI



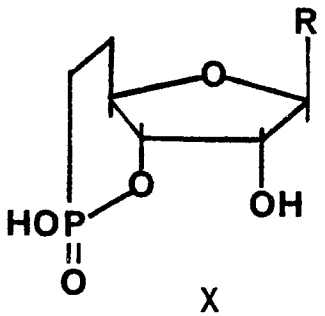
VII



VIII



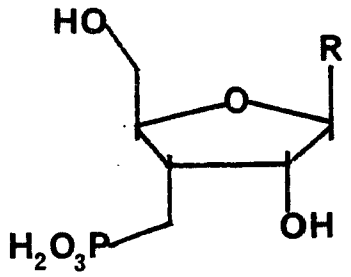
IX



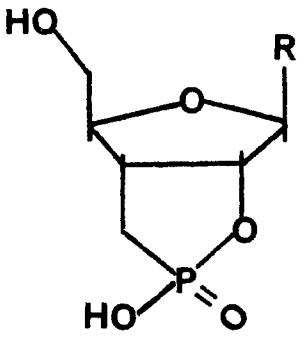
X

The isosteric phosphonic acid analogue of fructose-1-phosphate, (VIII), was also synthsized by Tang et. al, and found to be an in vivo inhibitor of growth of E. coli strains which are constitutive for the hexose phosphate transport system (126).

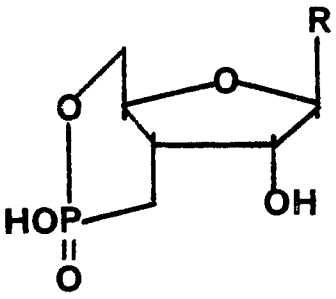
A number of isosteric phosphonic acid analogues of nucleotides related to the concern of this work have been prepared and investigated (111). Jones and Moffatt at Syntax, have reported the preparation of the 5'-deoxy-5'-(dihydroxyphosphonylmethyl) nucleosides (IX), where R is uracil or adenine (127). Patent claims were made later (128-129) for other nucleotide analogues including the



XI



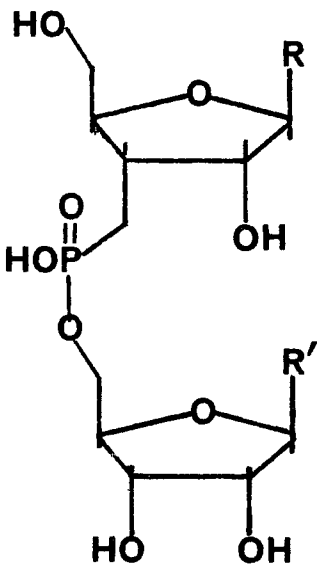
XII



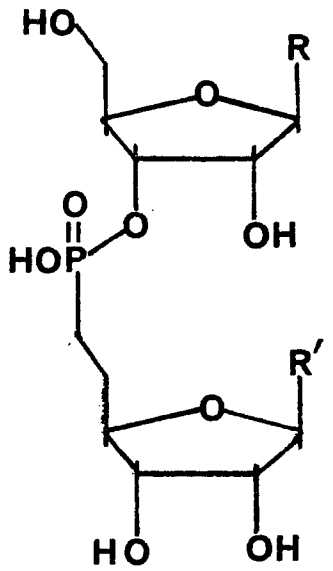
XIII

cyclic system (X). It was reported that (X), where R is adenine, exhibits pharmacological activity equivalent to that of the natural compound, but owned a longer half life due to a reduced susceptibility to hydrolysis. Activity for polymerase reactions was also claimed.

A series of phosphonic acids (XI), isosteric with the 3'-nucleoside phosphates, has also been reported (130). These and the related cyclic systems (XII) and (XIII) are reported to exhibit activity in controlling metabolic processes and in producing metabolic deficiencies (131-133).



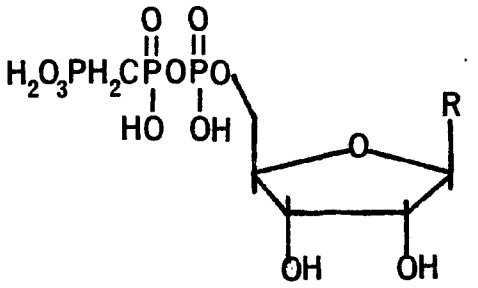
XIV



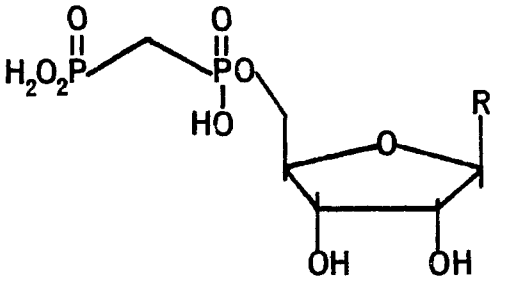
XV

A most important aspect of this work by the Syntex group has been the synthesis of isosteric analogues of dinucleoside phosphates (131). Two groups of compounds were prepared, one with the natural phosphate linkage at the 5'-position (XIV), and the other with it at the 3'-position (XV). While the full utility of these species has not yet been explored, their preparation represents a significant advance in the area of synthesis of analogues of nucleotides.

Several carbon to phosphorus linked analogues of nucleotides have been of great value in elucidation of the mechanism of protein biosynthesis (111). The most important of these are the analogues of nucleoside- pyrophosphates.



XVI R=Guanine
XVII R=Adenine



XVIII R=Adenine

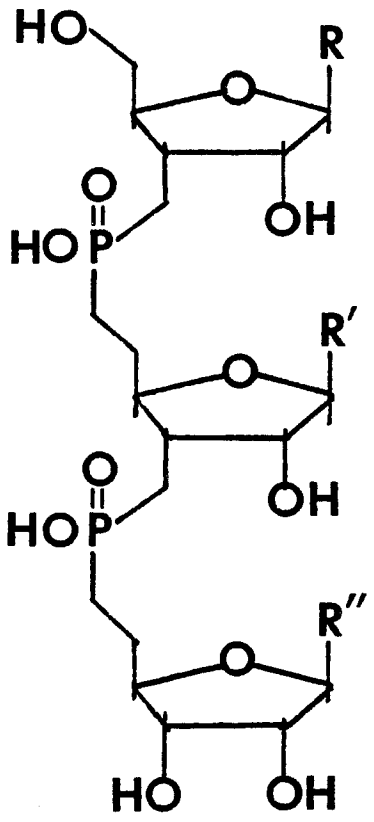
The analogue of GTP (XVI), in which the β,γ-pyrophosphate oxygen is replaced by a methylene group, was early discovered to serve as an overall inhibitor of peptide synthesis for several systems (134-139). With the use of (XVI), the role of GTP in initial binding of an aminoacyl-tRNA to the ribosomal complex was determined.

The analogue (XVI) has found use in investigations of several other systems as well. It has been used to define the role of GTP in specific binding of glycogen with plasma membranes (138), the stimulation of adenyl cyclase (141-144) and RNAse activity derived from E. coli (145), and has been found to be a competitive inhibitor of GTP in the action of adenylsuccinate-synthetase from E. coli (146).

Because of the wide range of biochemical processes in which ATP participates, the phosphonic acid analogue (XVII), often in conjunction with isomer (XVIII), has been employed

extensively. Briefly illustrating some of these investigations, (XVII) has been found capable of replacing ATP with RNA polymerase (147), adenylate deaminase, both at high and low concentrations (148), and 5-phosphoribosyl pyrophosphate synthetase (149). Moreover, the analogue (XVII) was found to be an order of magnitude better than ATP in inhibition of the incorporation of inorganic phosphate into ADP by polynucleotide phosphorylase (150). In studies of aspartate transcarbamylase from Neurospora crassa, it was found that (XVII), but not (XVIII) could substitute for ATP, indicating that the activity of ATP involved release of inorganic pyrophosphate (151). For other systems, strong inhibition of activity has resulted upon replacement of ATP by (XVII), indicating a necessity for binding or cleavage at the substituted site. These systems include phosphoenolpyruvate synthetase (152), adenylate cyclase (153-154), adenosine kinase from Ehrlich ascites-tumor cells (155), formylglycinamide ribonucleotide amidotransferase (156), and ATPase from carrot juice (157).

In spite of the evident usefulness of phosphonic acid analogues in studying the mechanism of protein synthesis, no attempts have been made previously to substitute such analogues for codon triplets or portions thereof. Such analogues bearing carbon to phosphorus bonds would be of value for further understanding of the details of protein synthesis and would have potential for practical utilization. For instance, if a totally non-hydrolysable



XIX

phosphinic acid analogue (XIX) of a suitable trinucleotide were incorporated in a natural mRNA and the molecule introduced into a cell-free protein synthesizing system, valuable information about the binding sites may be obtained.

While the codon-anticodon base pairing interaction is reasonably well understood, other structural subtleties are not. For example, how is the binding of mRNA to the 30S subunit of bacterial ribosome dependent on the phosphoric acid linkage of the individual nucleoside units? One might consider as a rational hypothesis that the nucleoside bases

of the mRNA, since they are to become involved in a codon-anticodon interaction, might not be in a position to bind to the ribosome, but rather might lie in a rather "open" positon. In such a situation, binding to ribosome might be expected to involve one or more of the three remaining potential binding sites: the 2'-hydroxyl group, the oxygen of the furanyl ring, and the phosphoric diester linkage.

Interactions involving esteric oxygens can be of sufficient magnitude so that their absence prevents substrate-enzyme binding. In particular, for a variety of phosphatases, binding using an esteric type oxygen (annydride) has been found to be more important than correspondence of size and shape (158-162). Interestingly, for many other systems, such binding capability has been found to be of negligible significance (111,114,115-116). For the case of oligo- and polynucleotides, the binding requirements (or hydrophilic interactions) of phosphate esteric oxygens are not known. Obviously there is no fundamental predictive theory for such interactions and each system must be tested individually before a pattern of activity may be deduced.

One other very significant use of non-hydrolysable RNA analogues might be in the generation of interferon. In recent years, synthetic homonucleosidic RNAs, as double stranded species, have come of importance for potential application in chemotherapy. It is well documented (163),

that these species serve, in vivo, in mammals including humans, as interferon inducers. Interferon is currently believed to be of great significance in the control, both by prophylaxis and attack, of viral infections of a wide variety. A fundamental problem, however, arises in the inherent lability of these substances introduced as drugs; at 25 C they are quite vulnerable to cleavage by RNAse (164), and it has been found that mammalian serum in general contains enzymes which rapidly hydrolyze double stranded RNA (165).

This problem could be circumvented if non-hydrolysable, phosphinic acid analogues of homonucleosidic RNA chains could be made and substitued, as double stranded species, for the normal compounds. These analogues might be expected to be superior in interferon producing activity, since they will not be subject to phosphate cleavage and might be expected to continue functioning for extended periods of time, under conditions where the normal material would be subject to degradation.

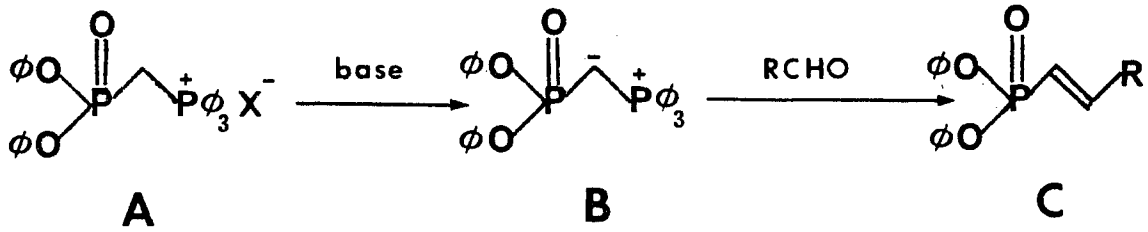
This work deals with the approaches toward the generation of such phosphinic acid analogues of oligonucleotides in both, a stepwise manner and by polymerization reactions.

DESIGN OF SYNTHESIS

The synthesis of phosphinic acid analogues of natural phosphate diesters is a considerably more complex endeavor than the synthesis of phosphonic acid analogues of phosphate monoesters. No simple standard method exists at present for the synthesis of unsymmetrical phosphinates which could be applied satisfactorily toward the solution of the present problem. Therefore, such a method must be developed. Two approaches are proposed, one of them is an extention of a widely employed method for the synthesis of phosphonates (developed by the Syntex group), and the other one is based on an existing method for the synthesis of phosphinates. Both methods are expected to be capable of both stepwise and polymeric type application for the generation of oligonucleotide type chains.

The first of these methods involves the synthesis of a species such as XX [Scheme I], which would be expected to undergo Wittig type polymerization reaction following generation of an aldehyde function at 5'position and treatment with base. Species XX could also be used in a stepwise sequence of Wittig type reactions to combine nucleotide analogues with different bases [Scheme I].

This approach evolves from the prior use of species (A) for generating phosphonates (166):

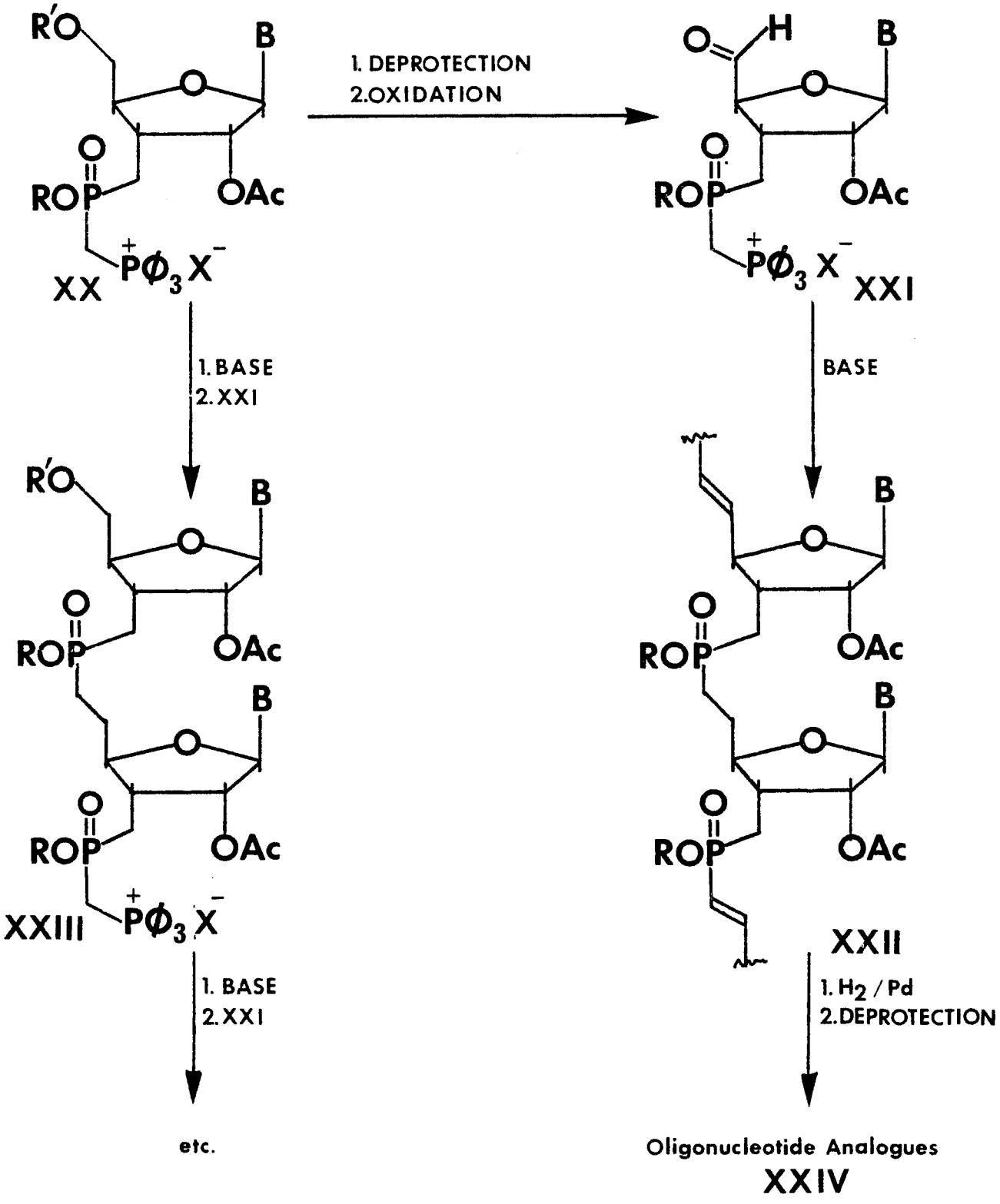


There is a fundamental similarity between (A) and (XX), the main difference being that (XX) is a phosphinic phosphonium salt while (A) is a phosphonic phosphonium salt.

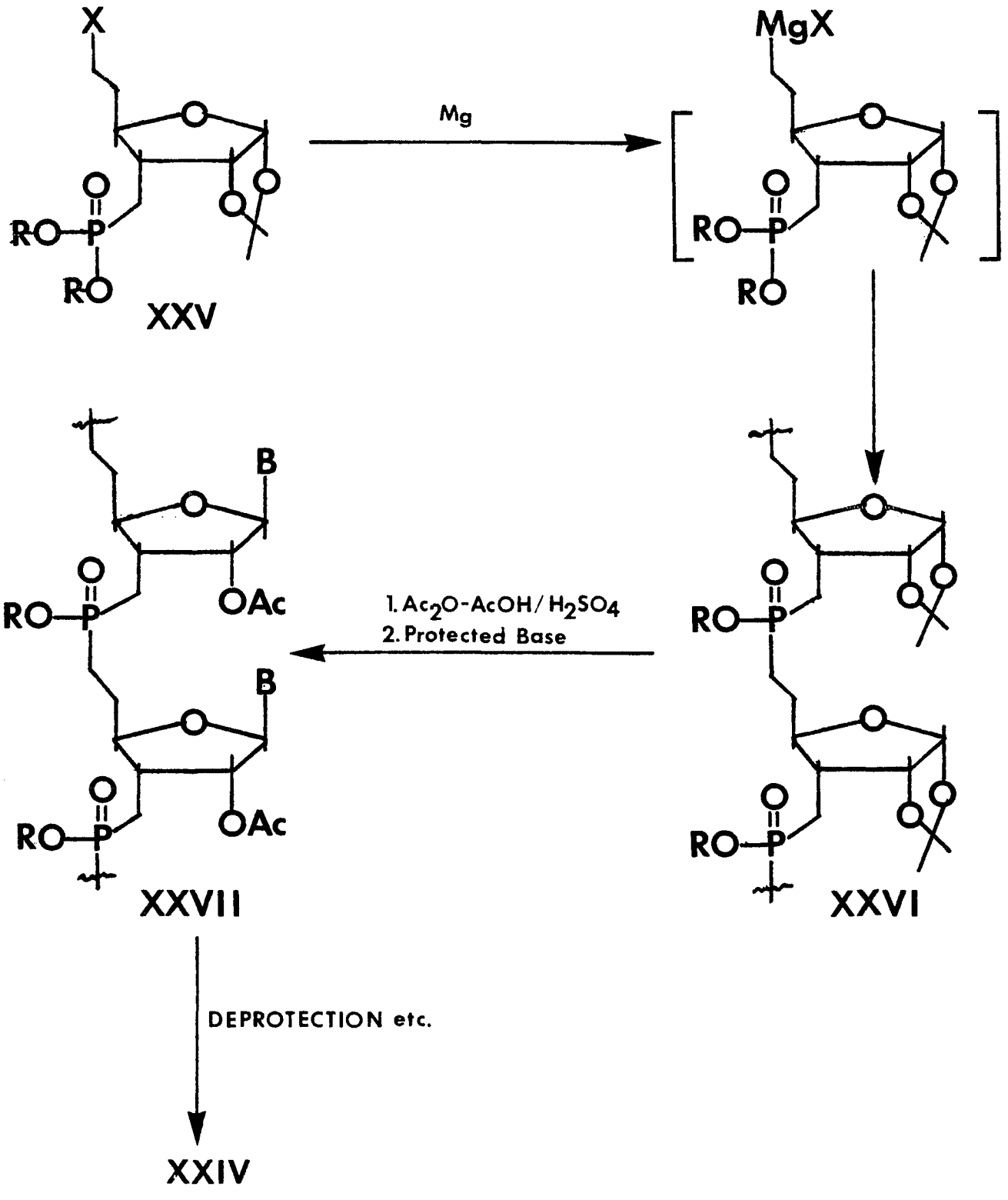
The second approach involves the generation of species (XXV) (Scheme II), which when treated with magnesium would give a Grignard reagent which would be expected immediately to undergo a polymerization reaction involving the phosphonate ester function at 3'position. To adapt this method for a stepwise addition of nucleotide analogues, the phosphonite (XXX) [Scheme III] must be generated and allowed to react in an Arbuzov process with (XXV) to give the phosphinate (XXXI); this may then be converted into the halide (XXXII) capable of undergoing further reaction with (XXX). When the required length of carbohydrate units has been obtained, the protecting groups could be removed and the nucleoside base introduced.

Both methods are discussed in detail in the next section.

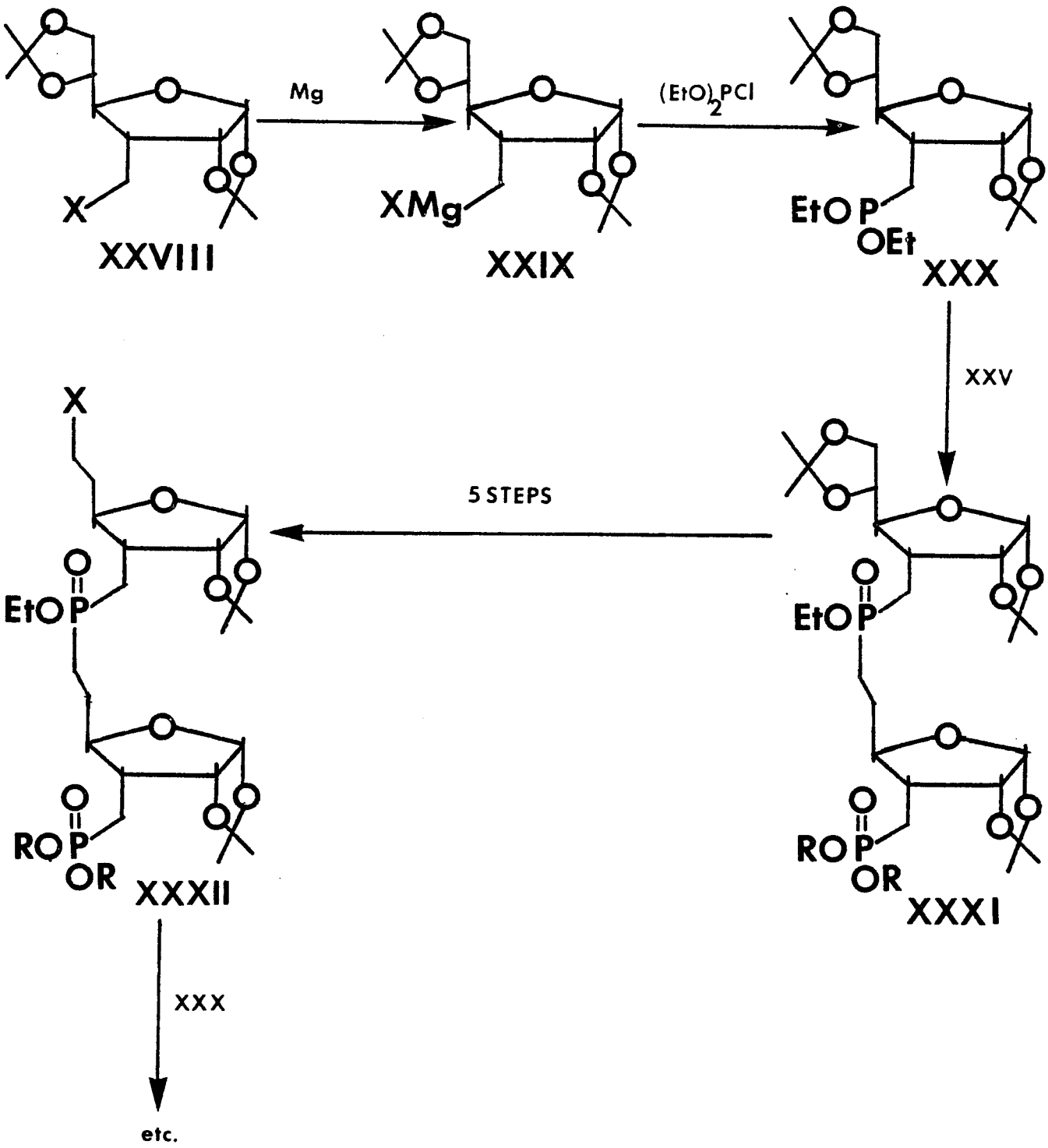
SCHEME I


R'O
B
ROP
OAc
XX
$P\phi_3 X^-$
1. DEPROTECTION
2.OXIDATION
H
XXI
1. BASE
2. XXI
BASE
XXIII
XXII
1. H_2 / Pd
2. DEPROTECTION
etc.
Oligonucleotide Analogues
XXIV

SCHEME II


X
RO
P
RO
XXV
Mg
MgX
RO
P
RO
B
OAc
RO
P
1. Ac2O-AcOH/ H2SO4
2. Protected Base
B
OAc
RO
P
XXVII
RO
P
XXVI
DEPROTECTION etc.
XXIV

SCHEME III


Mg
(EtO)2PCl
X
XXVIII
XMg
XXIX
EtO P
OEt
XXX
XXV
5 STEPS
EtO P
XXXI
RO P
OR
XXXII
XXX
etc.

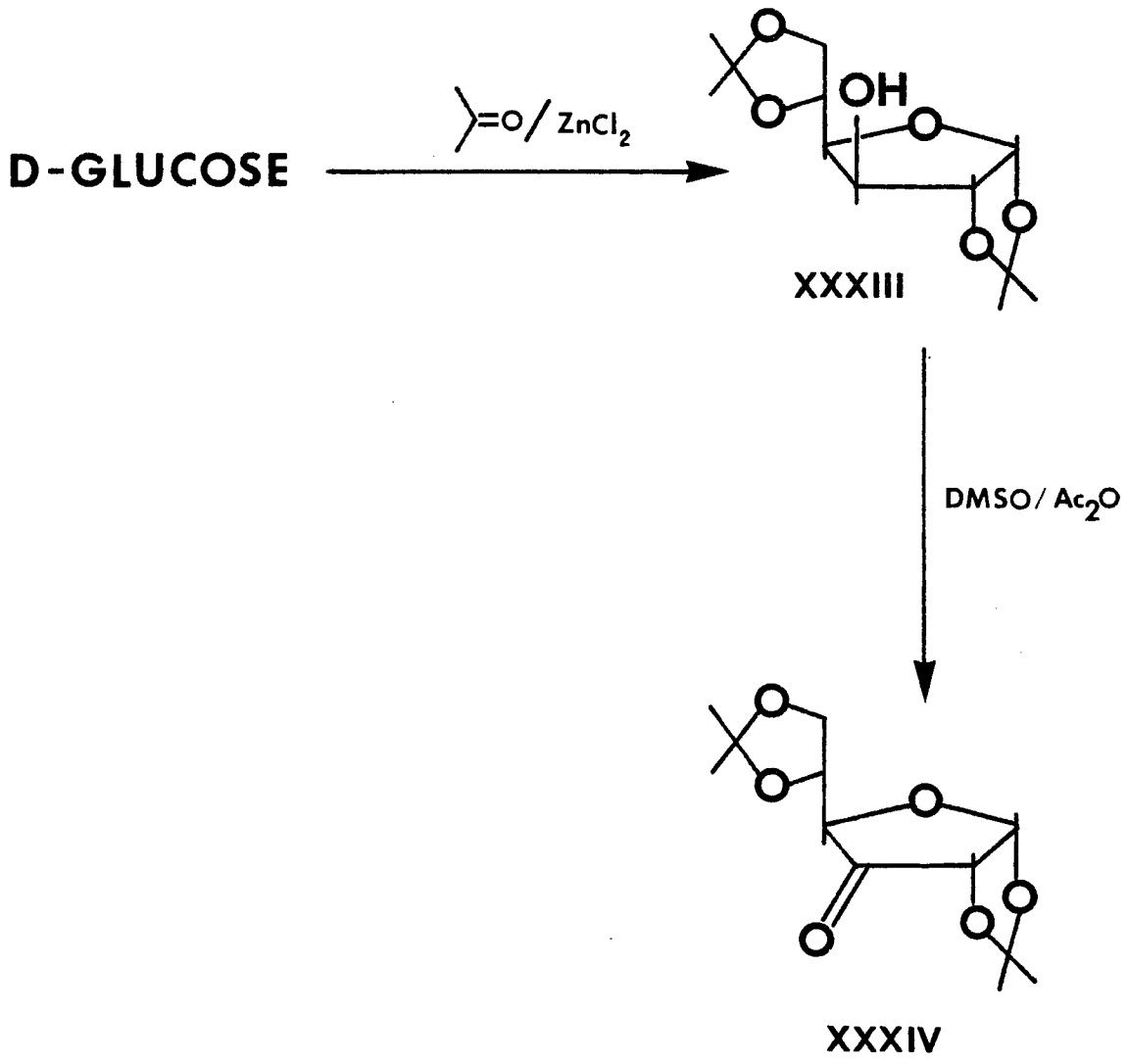
RESULTS AND DISCUSSION

The starting material, D-glucose, was converted [Scheme IV] into diacetone glucose (XXXIII) by the method of Glen et al. (167) using an excess of anhydrous acetone with annydrous zinc chloride and phosphoric acid as catalysts. The crystalline diacetone glucose was oxidized with DMSO/acetic anhydride (168), to give the versatile ketone (XXXIV) which was purified by repeated distillations (169).

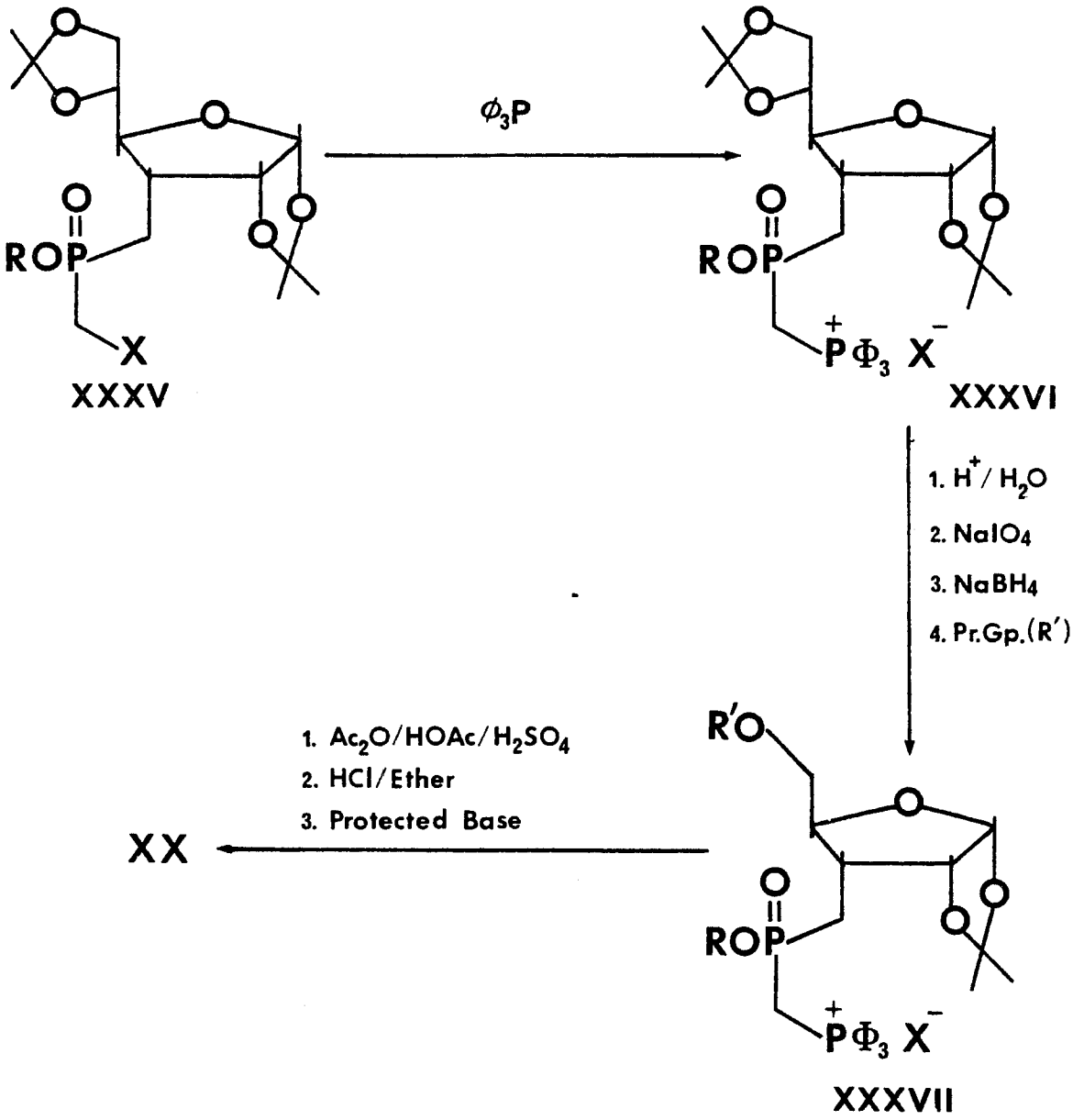
The ketone (XXXIV), by a series of transformations, was to be converted into (XX). These transformations basically involved, (a) introducing a halomethyl-methylene phosphinate group at 3' position (XXXV, Scheme V), which could then be converted into a phosphonium salt (XXXVI, Scheme V); (b) selectively removing the 5,6-isopropylidine group, cleaving off carbon 6 and protecting the 5'oxygen function (XXXVII, Scheme V); (c) removing the 1,2-isopropylidine group and introducing the nucleoside base at 1'position to give the intermediate (XX).

Several approaches were tried to introduce the methylene-halomethyl-phosphinate group at position 3 to give the intermediate (XXXV). Scheme VI represents our first efforts in this direction. Chloromethyl triphenyl phosphonium chloride (XXXIX) was prepared by the action of thionyl chloride on hydroxymethyl-triphenyl phosphonium chloride (XXXVIII), which in turn was obtained from triphenyl phosphine and paraformaldehyde (170). The

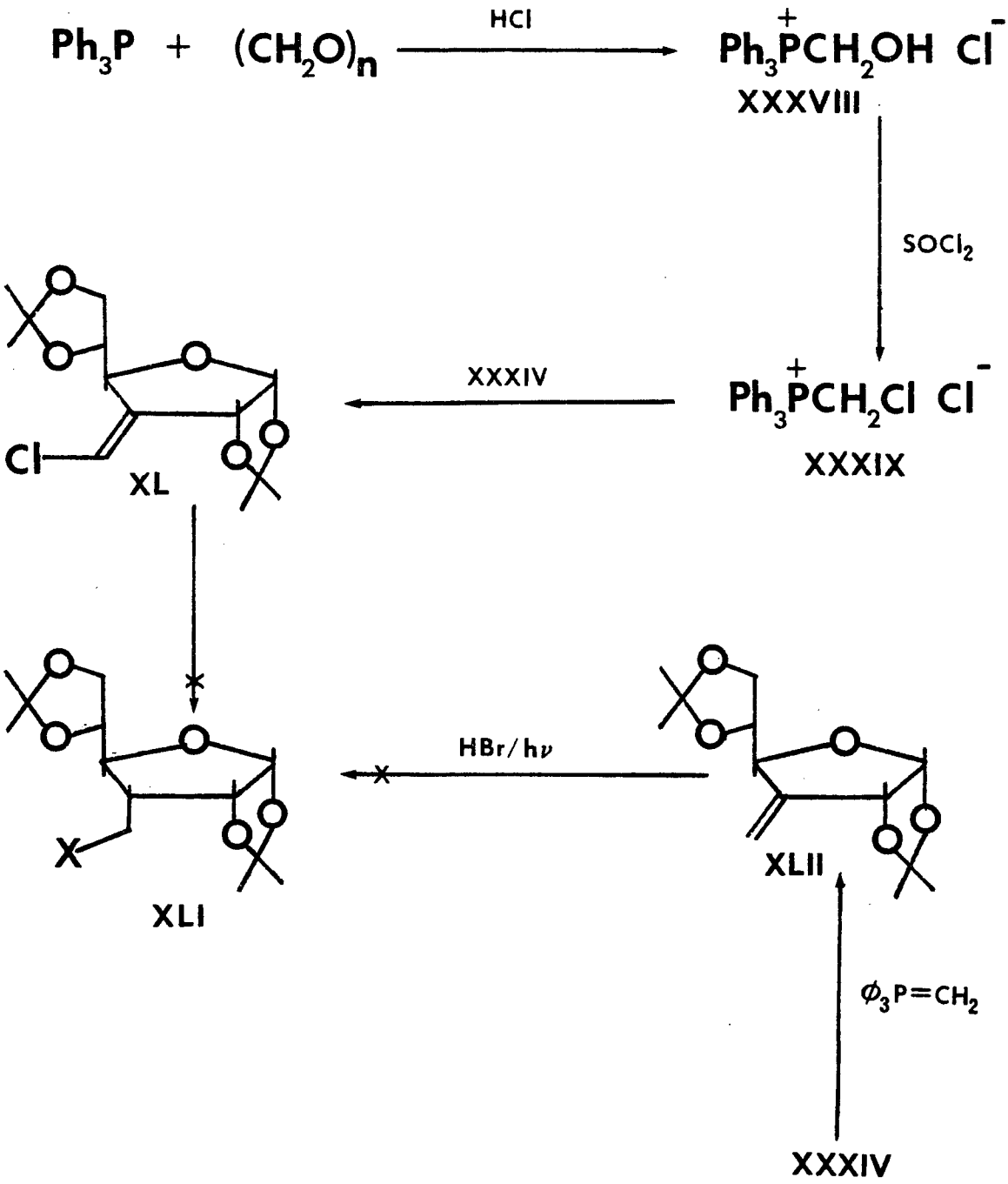
SCHEME IV



SCHEME V


ϕ_3P
ROP
X
XXXV
ROP
$P\Phi_3$ X^-
XXXVI
1. H^+/H_2O
2. $NaIO_4$
3. $NaBH_4$
4. Pr.Gp.(R')
R'O
ROP
$P\Phi_3$ X^-
XXXVII
1. $Ac_2O/HOAc/H_2SO_4$
2. HCl/Ether
3. Protected Base
XX

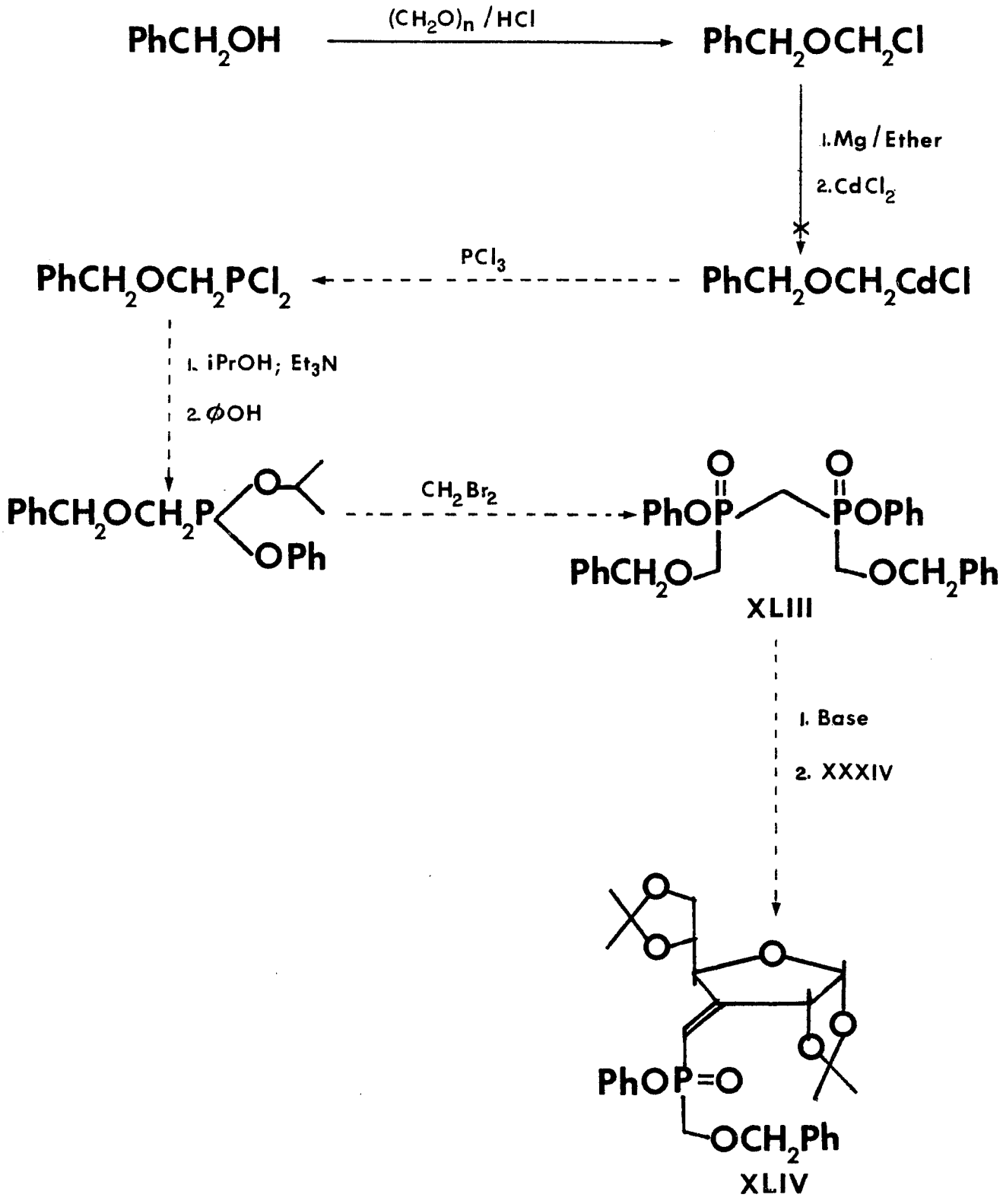
SCHEME VI


Ph3P + (CH2O)n
HCl
Ph3PCH2OH Cl
XXXVIII
SOCl2
Ph3PCH2Cl Cl
XXXIX
XXXIV
Cl
XL
X
XLI
HBr/hν
XLII
ϕ3P=CH2
XXXIV

compound XXXIX was treated with phenyllithium to form an ylid and the vinylic chloride (XL) was obtained by a Wittig reaction with the ketone (XXXIV). It was hoped that the double bond could be reduced without losing the halogen; the resulting primary halide (XLI) was to be converted into XXXV by a series of reactions. Among the systems used toward achieving this were diimide (171), diborane followed by protonolysis (172), and homogeneous catalytic hydrogenation using tris(triphenyl phosphine)-chlororhodium. None of the methods was successful, primarily owing to the extreme lability of the halogen in a reduction of this type, and the relatively hindered nature of the double bond. An alternative approach to make the halide XLI (Scheme VI) by an anti-Markownikoff addition of HBr (173) to the olefin XLII was equally unsuccessful; only an intractable black tar was obtained.

Next, several attempts were directed toward generating a reagent, which, when coupled with the ketone (XXXIV), would be expected to yield directly XXXV or XXXVI. The first such approach is outlined in Scheme VII. The bisphosphinate XLIII, was the desired reagent capable of undergoing a Wadsworth-Emmons type reaction (174) to give the olefin XLIV which could be converted readily into XXXV. It was necessary to abandon this route because the benzylchloromethyl ether (175), no matter how rigorously purified, was contaminated with benzyl chloride which seemed to catalyze further decomposition. Our attempts to use the

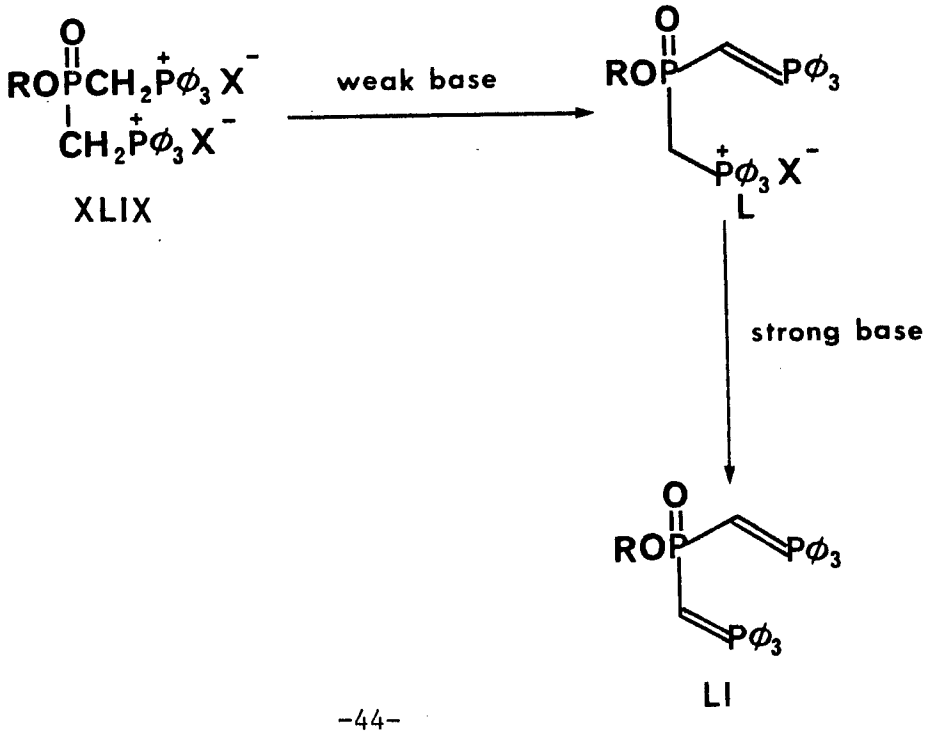
SCHEME VII


PhCH2OH
(CH2O)n / HCl
PhCH2OCH2Cl
1. Mg / Ether
2. CdCl2
PhCH2OCH2CdCl
PCl3
PhCH2OCH2PCl2
1. iPrOH; Et3N
2. ØOH
PhCH2OCH2P
OPh
CH2Br2
PhOP
POPh
PhCH2O
OCH2Ph
XLIII
1. Base
2. XXXIV
PhOP=O
OCH2Ph
XLIV

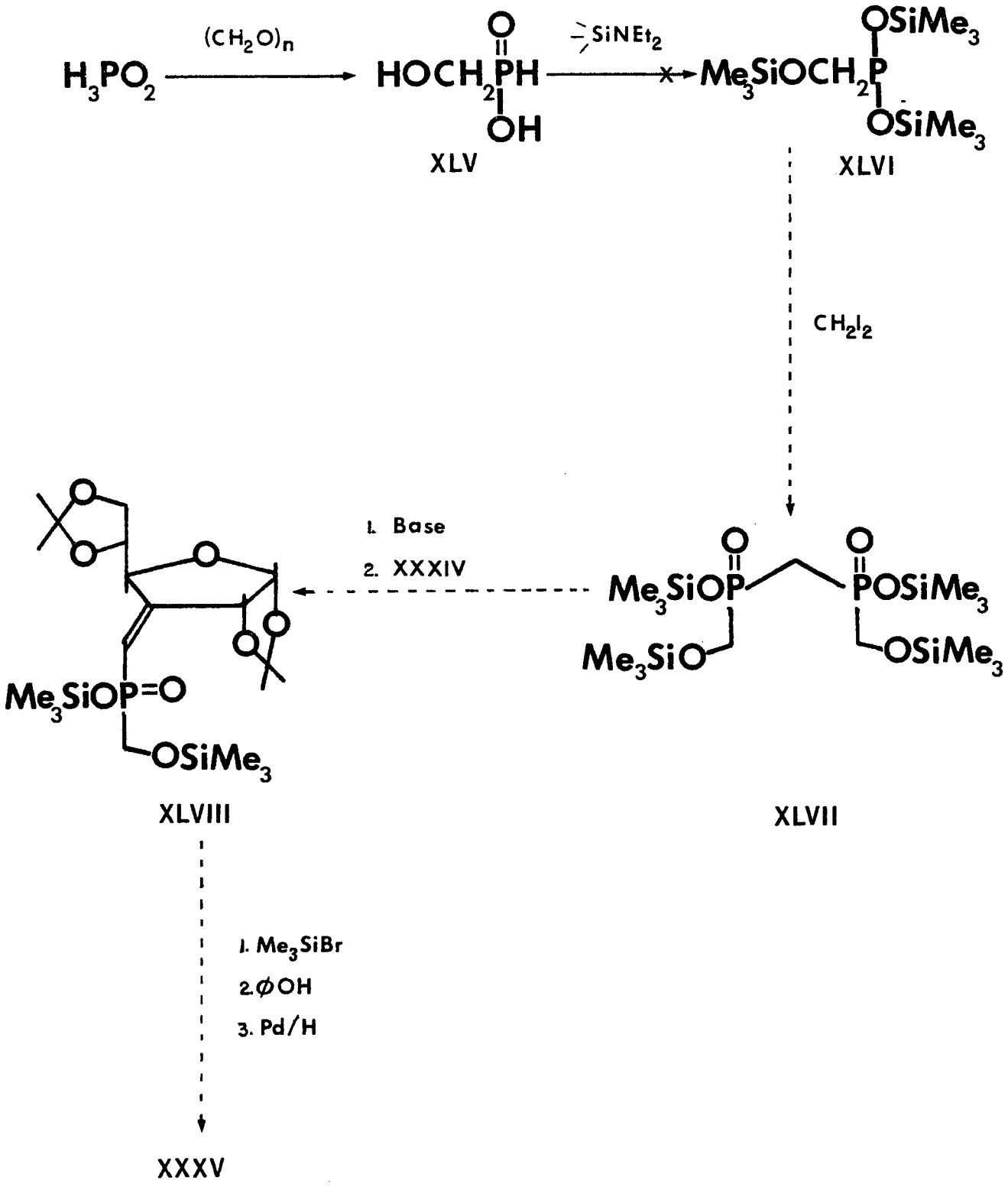
contaminated product for reaction with magnesium were completely unsuccessful.

Scheme VIII outlines the next effort toward generating XXXV via the bisphosphinate reagent (XLVII). Several attempts were made to make the phosphonous acid derivative (XLVI) without success, although it has been reported (176). Apparently, the reaction of hypophosphorus acid with paraformaldehyde (177), gave a mixture of products which resulted in an even more complex mixture upon silylation. The separation of this mixture by repeated fractionations using a spinning band column was attempted, but to no avail. Also, no evidence of the desired product was detected in an nmr of the mixture.

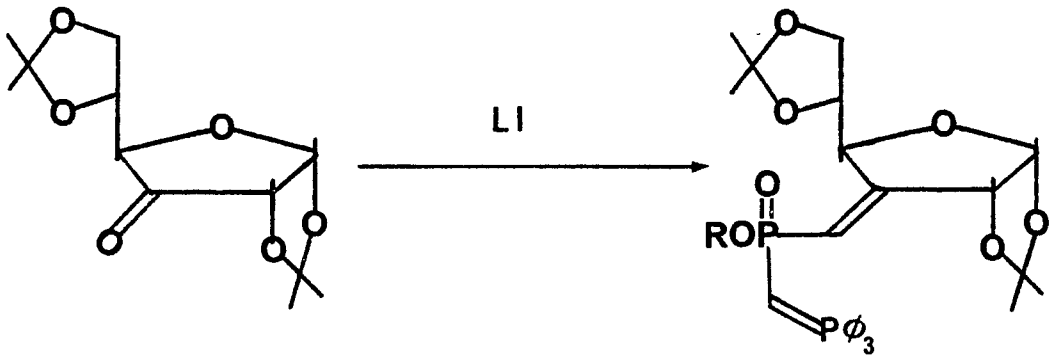
At this point, the obvious choice for another type of reagent capable of reacting with the ketone (XXXIV) to give XXXVI was a Wittig reagent such as XLIX:



SCHEME VIII


H_3PO_2
$(CH_2O)_n$
$HOCH_2PH$
OH
XLV
$SiNEt_2$
Me_3SiOCH_2P
OSiMe$_3$
OSiMe$_3$
XLVI
CH_2I_2
1. Base
2. XXXIV
Me_3SiOP
$POSiMe_3$
Me_3SiO
OSiMe$_3$
XLVII
Me_3SiOP=O
OSiMe$_3$
XLVIII
1. Me_3SiBr
2. ϕOH
3. Pd/H
XXXV

At first, such a reagent would seem like an unwise choice since phosphoranes stabilized by the d-orbitals of a neighboring phosphoryl-phosphorus are usually unreactive toward ketones (178) because of reduced electron density on the carbanion. However, it seems reasonable that if a bis-phosphorane is formed from a reagent such as XLIX (i.e. LI), the stabilization by the phosphoryl group might be restricted to only one carbanion, and the other carbanion might be fairly reactive and capable of reacting with our ketone (XXXIV) in the following manner:

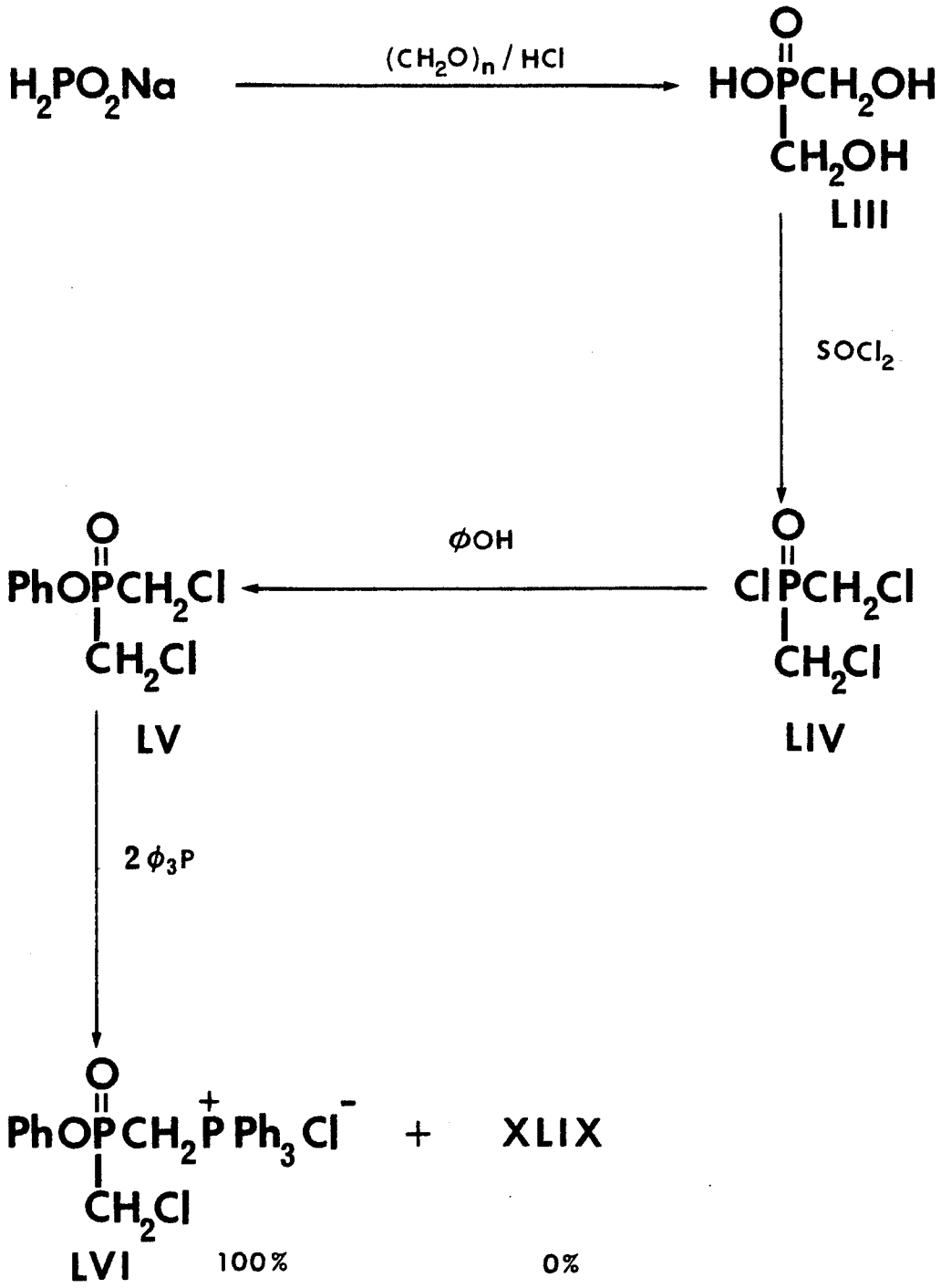


XXXIV LII

The resulting phosphorane (LII), on the other hand would be expected to be a stable phosphorane, incapable of reacting with the ketone (XXXIV). Working on these assumptions, the syntnesis of XLIX was attempted as represented in Scheme IX.

Bishydroxymethyl phosphinic acid (LIII) was obtained as a very viscous colorless syrup from sodium hypophosphite by

SCHEME IX


H2PO2Na
(CH2O)n / HCl
HOPCH2OH
CH2OH
LIII
SOCl2
ClPCH2Cl
CH2Cl
LIV
ϕOH
PhOPCH2Cl
CH2Cl
LV
2 ϕ3P
PhOPCH2P+Ph3Cl-
CH2Cl
LVI
100%
+
XLIX
0%

treatment with paraformaldehyde and hydrochloric acid at 95-100 C for 72 hours (179). The product was converted into bischloromethyl phosphinyl chloride (LIV) by treatment with thionyl chloride (179). Treatment with phenol gave the phenyl ester (LV) in good yield, which was purified by distillation. All attempts to quarternize both chloromethyl carbons in LV using vigorous conditions and a large excess of triphenyl phosphine resulted in the formation of a mono-phosphonium salt (LVI). The extreme inertness of the second chlorine is unusual but it could probably be attributed to steric constraints imposed by the bulky triphenylphosphonium group on the β-position, making it difficult for another bulky triphenylphosphine nucleophile to approach the carbon atom.

Treatment of LVI with aqueous sodium carbonate produced a gummy substance (rather than a crystalline phosphorane), which could be isolated by extraction with ether or chloroform. Surprisingly, different experiments gave slightly different products as evidenced by nmr, probably due to decomposition. When the reaction was performed in the presence of chloroform so that the product would be transferred immediately into the organic layer (it is practically insoluble in water) and prolonged contact with the base would be minimized, a consistent product was obtained. Experimentally, this was achieved by shaking the mixture vigorously on a mechanical shaker for ten minutes. The product was again a pale yellow gummy substance whose

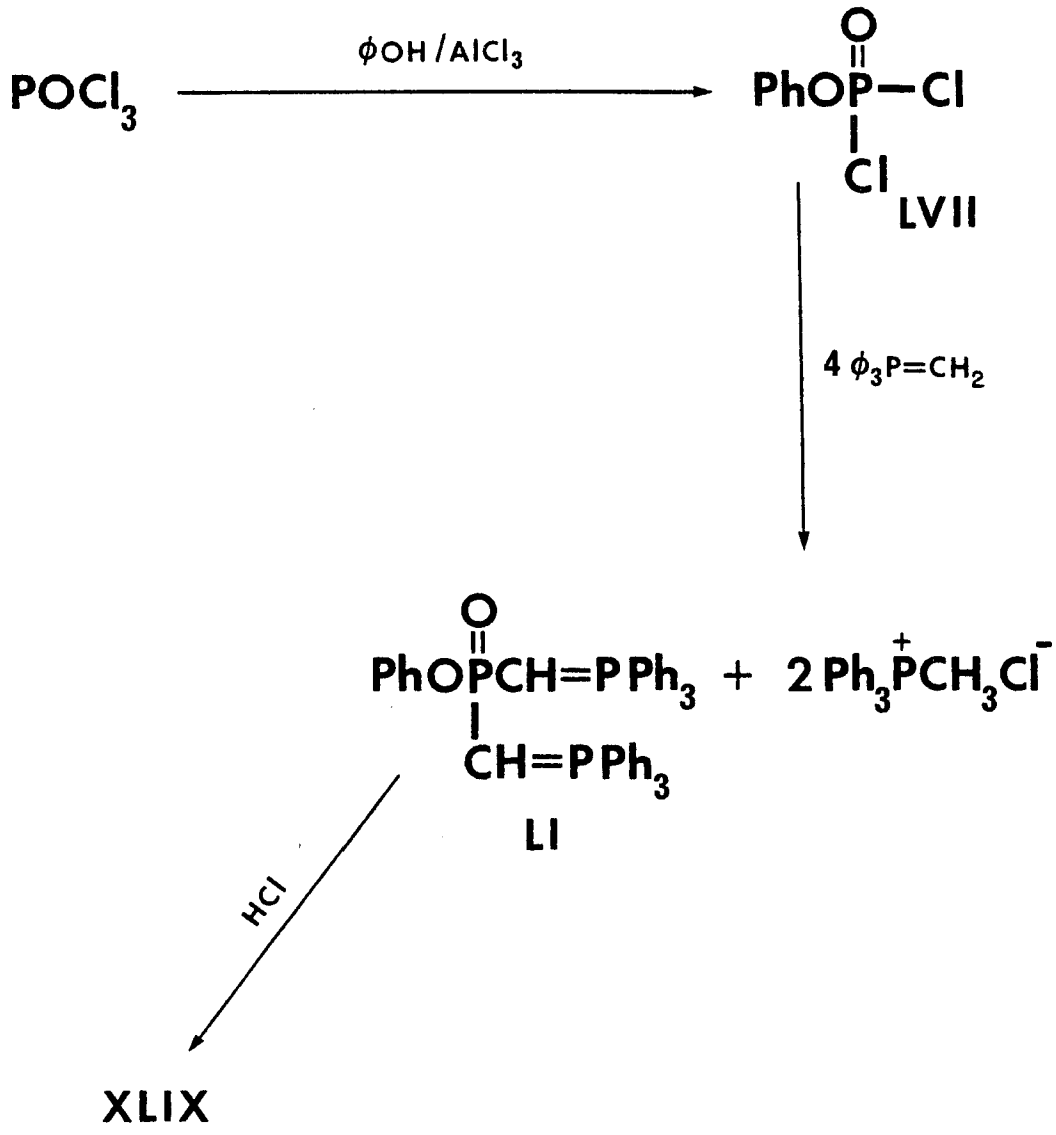
nmr was quite unexpected. Normally, the proton on the carbanion carbon in a stable phosphorane appears between 0.0-1.0δ; nothing was observed in that region. There was a singlet for one proton at 1.6δ which disappeared when the $CDCl_3$ solution was shaken with a few drops of D_2O. The doublet for the chloromethyl protons in the parent compound (LVI) was changed into a complex multiplet. Treatment of the $CDCl_3$ solution with a drop of conc. hydrochloric acid however, immediately transformed the nmr into the perfectly reasonable original nmr of LVI.*

In an attempt to explore the possibility of using LVI as a potential one step precursor to phosphinates, attempts were made to accomplish reaction of this gummy substance with simple aliphatic and aromatic aldehydes (such as butyraldehyde, valeraldehyde, benzaldehyde etc.) under a variety of conditions involving different solvents, temperatures, and bases for the generation of the ylid. The results were unsatisfactory and attempts to isolate pure products were not successful. Further persuit of this interesting system was abandoned at the time, but it holds definite promise with the use of preparative scale HPLC.

Having failed to obtain the bisphosphonium salt (XLIX) by the above mentioned method, another approach toward its synthesis was attempted. As described in Scheme X, phenyl phosphorodichloridate (LVII) was added to four molar equivalents of methylene-triphenylphosphorane (178) generated in ether solution from methyl triphenylphosphonium

* See page 81

SCHEME X


POCl3
ϕOH/AlCl3
PhOP(O)Cl2
LVII
4 ϕ3P=CH2
PhOP(O)(CH=PPh3)CH=PPh3
+ 2 Ph3PCH3Cl
LI
HCl
XLIX

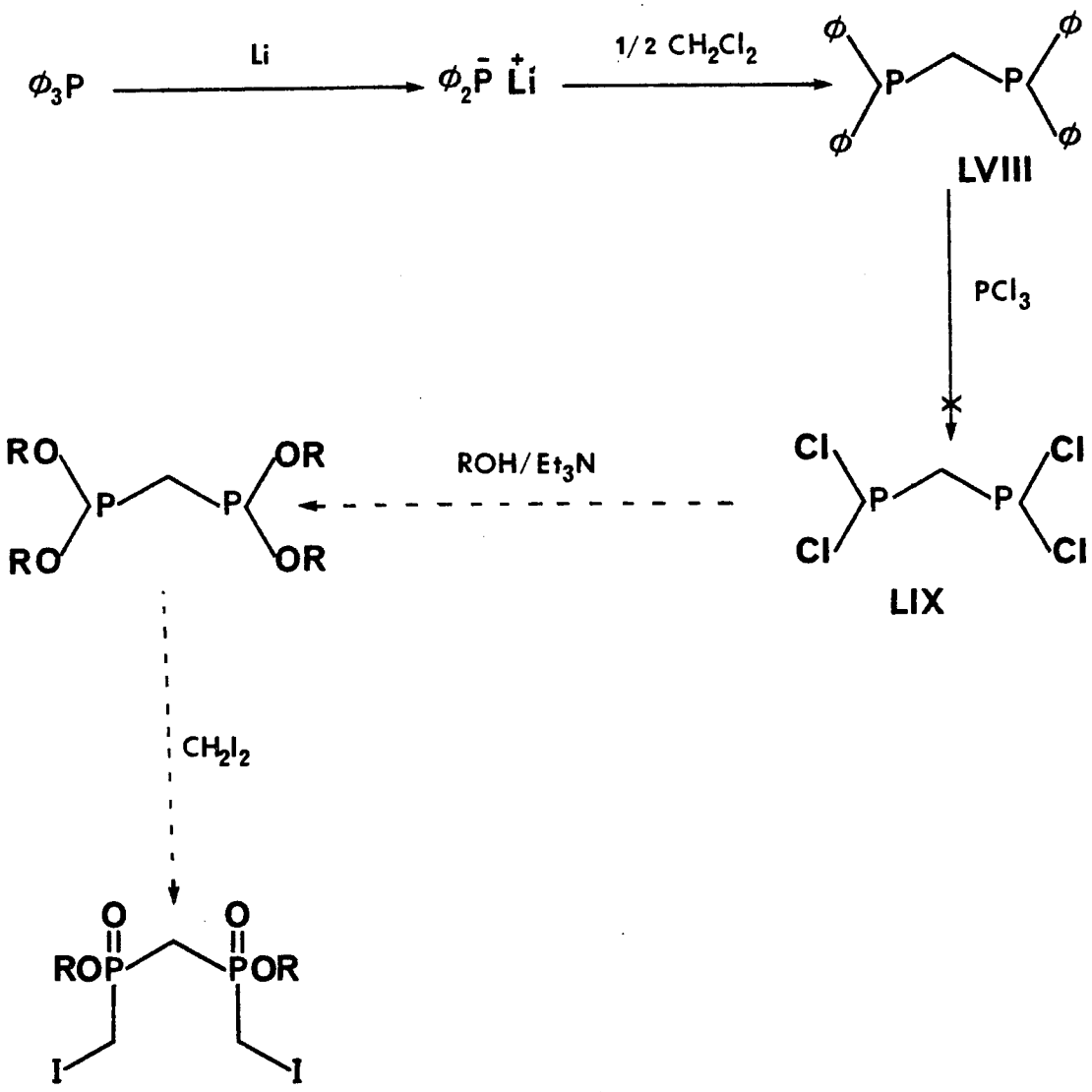
bromide and n-butyl lithium. After filtration, the ether solution was extracted with 0.1N HCl from which it could be isolated either as XLIX or as the bisphosphorane (LI) by neutralizing with sodium hydroxide. Contrary to predictions the bisphosphorane (LI) turned out to be a perfectly stable crystalline material, which was quite unreactive toward ketones.

At this point, another approach toward the synthesis of a bisphosphinate type reagent was tried [Scheme XI]. Triphenylphosphine was treated with two molar equivalents of lithium and the lithium diphenylphosphide thus generated was allowed to react with dichloromethane to produce crystalline methylene-tetraphenylbisphosphine (LVIII) (180). Several attempts were made to convert LVIII to tetrachloro-methylene-bisphosphine (180), by treating it with PCl_3 at 280 C in an autoclave, although it met with no success.

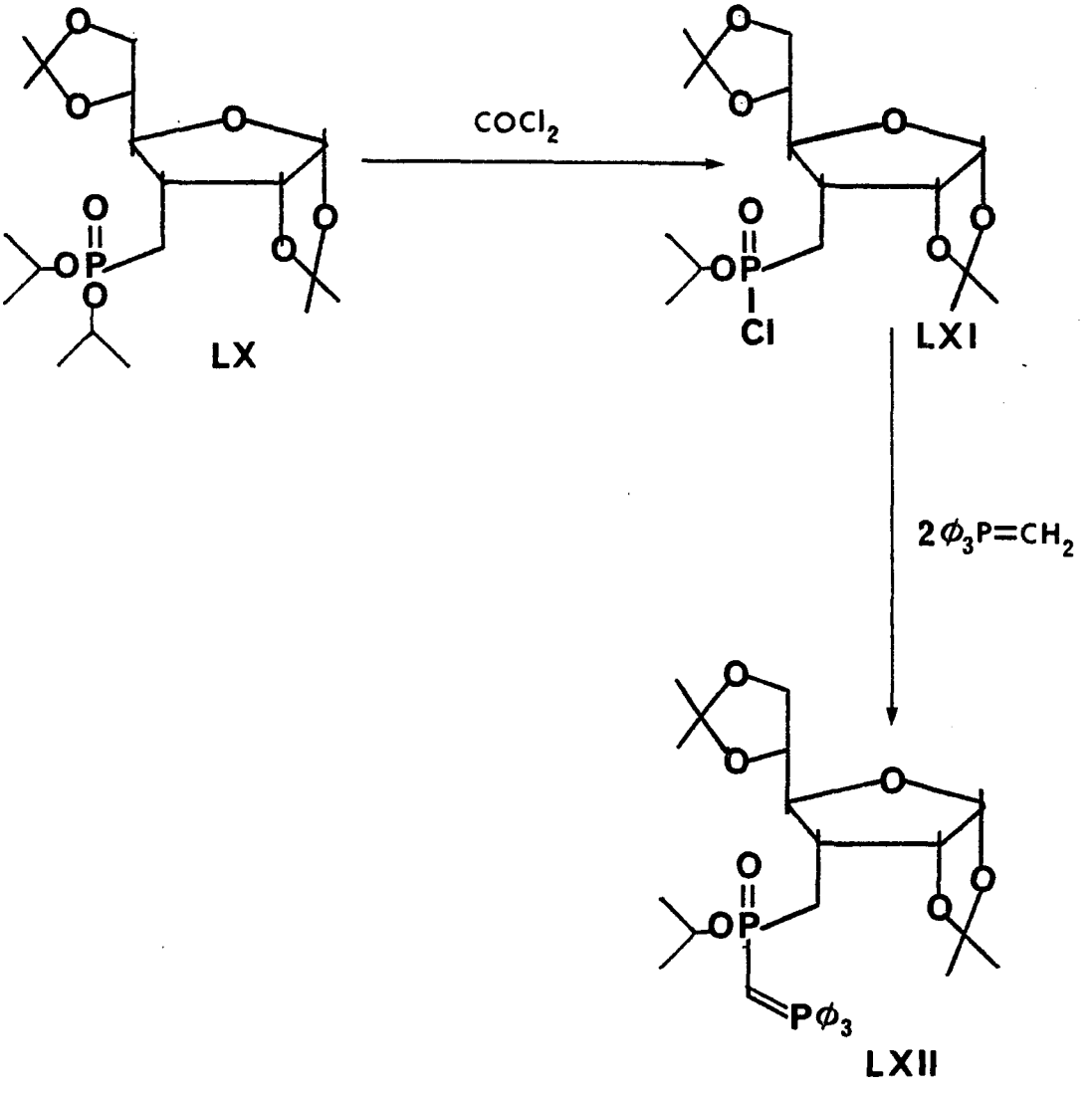
At this point a procedure was chosen such as shown in Scheme XII. The phosphonate (LX) was obtained [Scheme XIII] by reaction of the ketone (XXXIV) with a carbanion obtained from tetraisopropyl-methylene-bisphosphonate (LXIII), followed by hydrogenation. The bisphosphonate was conveniently prepared by the procedure of Roy et al. (181) which involved heating triisopropyl phospnite at 145-185 C with dibromomethane, in a flask equipped with a 24"column filled with glass helices and maintained at 60-65 C.

The initial results in the preparation of LXIV were very

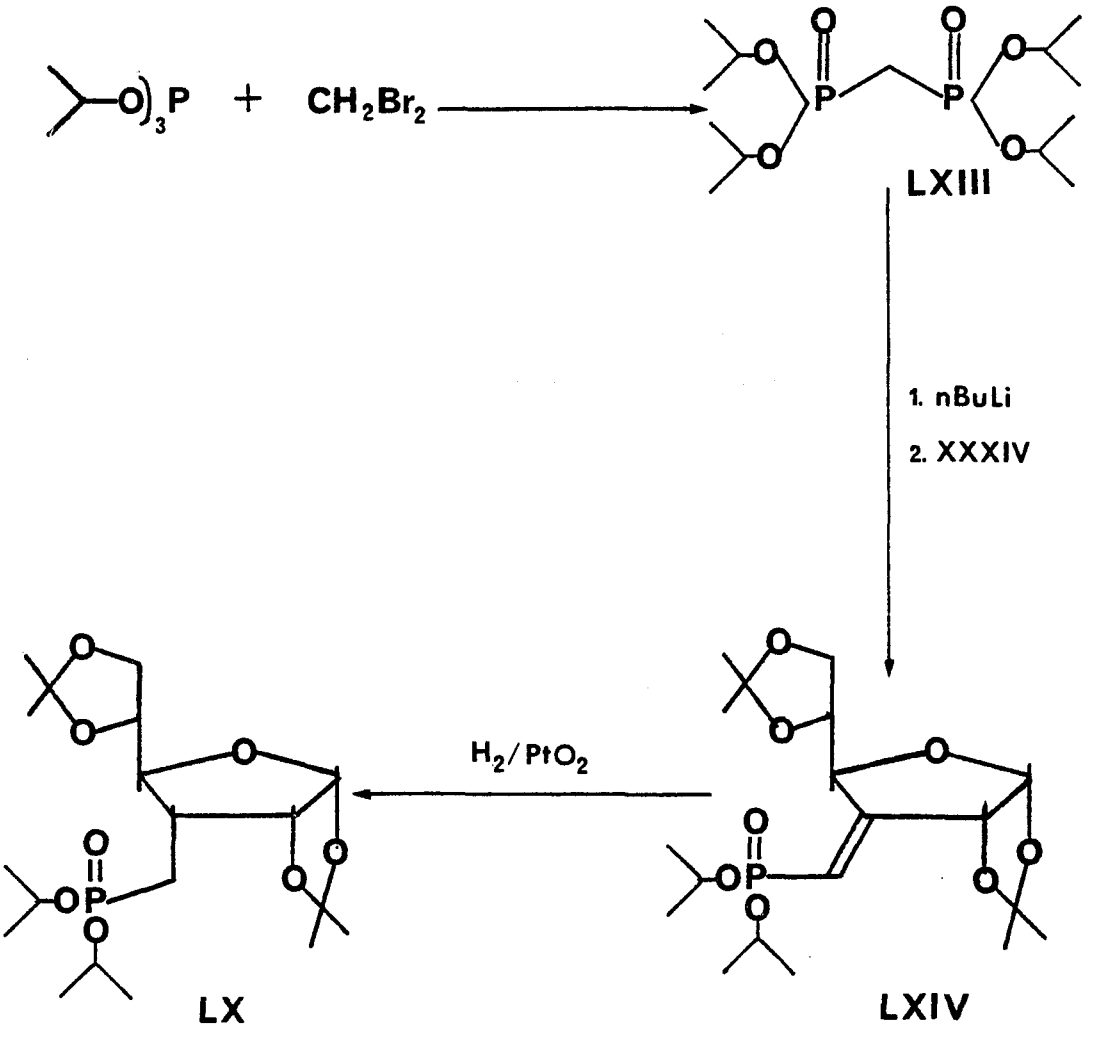
SCHEME XI



SCHEME XII



SCHEME XIII

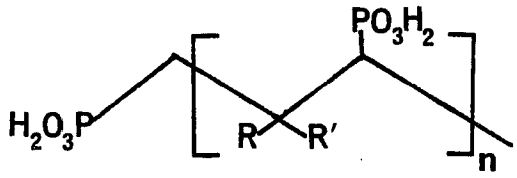


discouraging. Only a 5-10% yield of the vinyl phosphonate was obtained when ether was used as a solvent to generate the carbanion from the bisphosphonate (LXIII). In addition, the product was extremely impure and it took at least three successive separations by column chromatography, to obtain the pure product. All attempts to improve the yield and purity of the product by using solvents such as THF, DME or DMSO, or using sodium hydride as base to generate the carbanion met with failure. Similarly, the change of reaction temperature had no effect. When the reaction was carried out in n-heptane, however, using n-butyllithium as base, almost pure LXIV was obtained in 74% yield.

Encouraged by this remarkable success of the reaction run under these conditions, we prepared a number of vinyl phosphonates to test the generality of the method and evaluate its merits as a standard method for generating phosphonates (182). We found that the reaction worked very well with a number of aldehydes and ketones; the significant feature was that sterically hindered ketones not readily amenable to Wittig type reactions could be used to yield vinyl phosphonates readily. These results are summarized in Table I.

Another special feature of the system is that the products are isopropyl esters, which presents the possibility of removing the esteric groups by pyrolysis to yield the corresponding phosphonic acids:

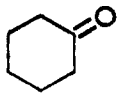
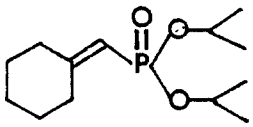
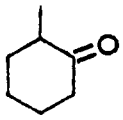
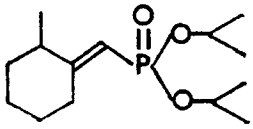
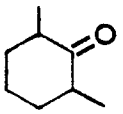
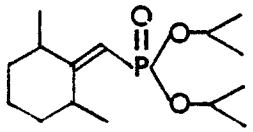
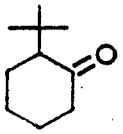
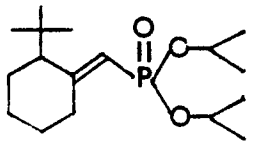
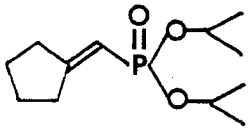
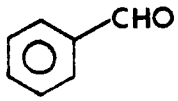
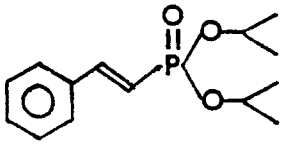
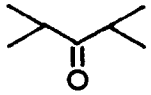
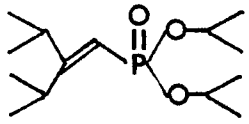
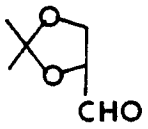
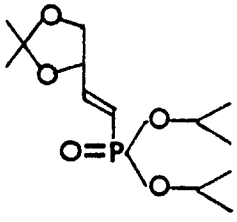
Several vinyl phosphonates were heated in a nitrogen atmosphere at 220-250 C. The products in all cases were insoluble in common organic solvents and water but moderately soluble in DMSO or DMF. While the complete characterization of these materials has not yet been done, they appear to be polymers of the type,



apparently formed by acid catalyzed polymerization of the double bond. To test this hypothesis, the double bonds in several of the vinyl phosphonates were hydrogenated and the reduced phosphonates subjected to pyrolysis. As expected, the corresponding phosphonic acids were obtained in good yield.

Hydrogenation of LXIV at atmospheric pressure with several catalysts was extremely slow and very little reaction was observed even after a week to ten days. Under

TABLE I

CARBONYL COMPD	PRODUCT	% YIELD
		91
		80
		36
		30
		41
		95
		28
		96

high pressure, however, hydrogenation was essentially complete in 24-36 hours and practically pure LX was obtained in quantitative yield.

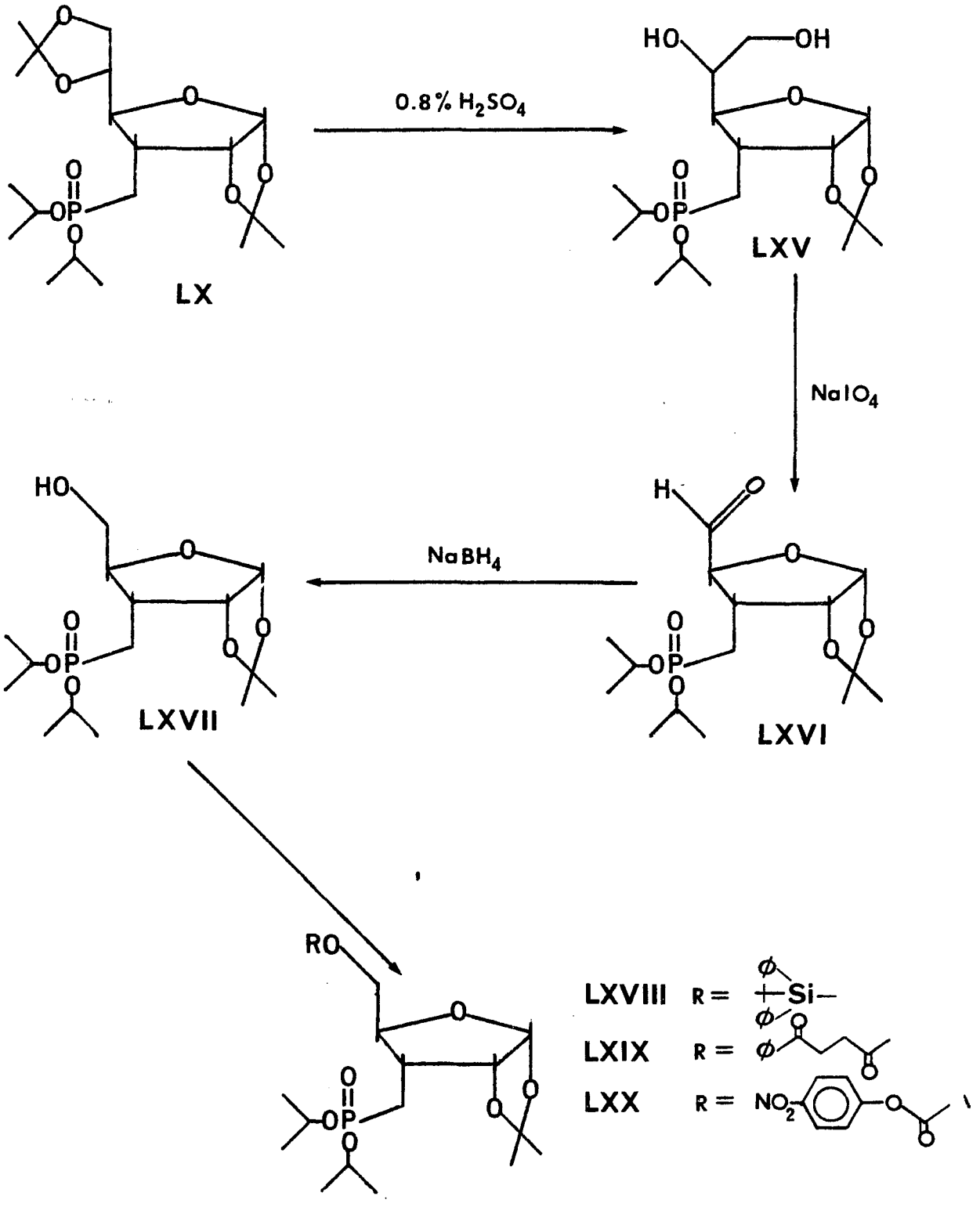
All attempts to obtain LXI [Scheme XII] met with failure. Apparently, the 5,6-isopropylidine group was being chopped off in preference to the phosphonate ester. Therefore we decided to follow Scheme XIV where this particular step is postponed until a later stage.

The phosphonate (LX) was treated with 0.8% sulfuric acid in methanolic solution to cleave the 5,6-isopropylidine group (183). The reaction was monitored on tlc and when no more starting material was indicated, the mixture was diluted and treated with 0.05M solution of sodium metaperiodate (184) to cleave the carbon-6. No attempt was made to isolate the aldehyde LXVI and it was reduced <u>in situ</u> to the corresponding alcohol (LXVII) with sodium borohydride (185). The compound LXVII was isolated by extraction with chloroform. The overall yield in going from LX to LXVI was 88%.

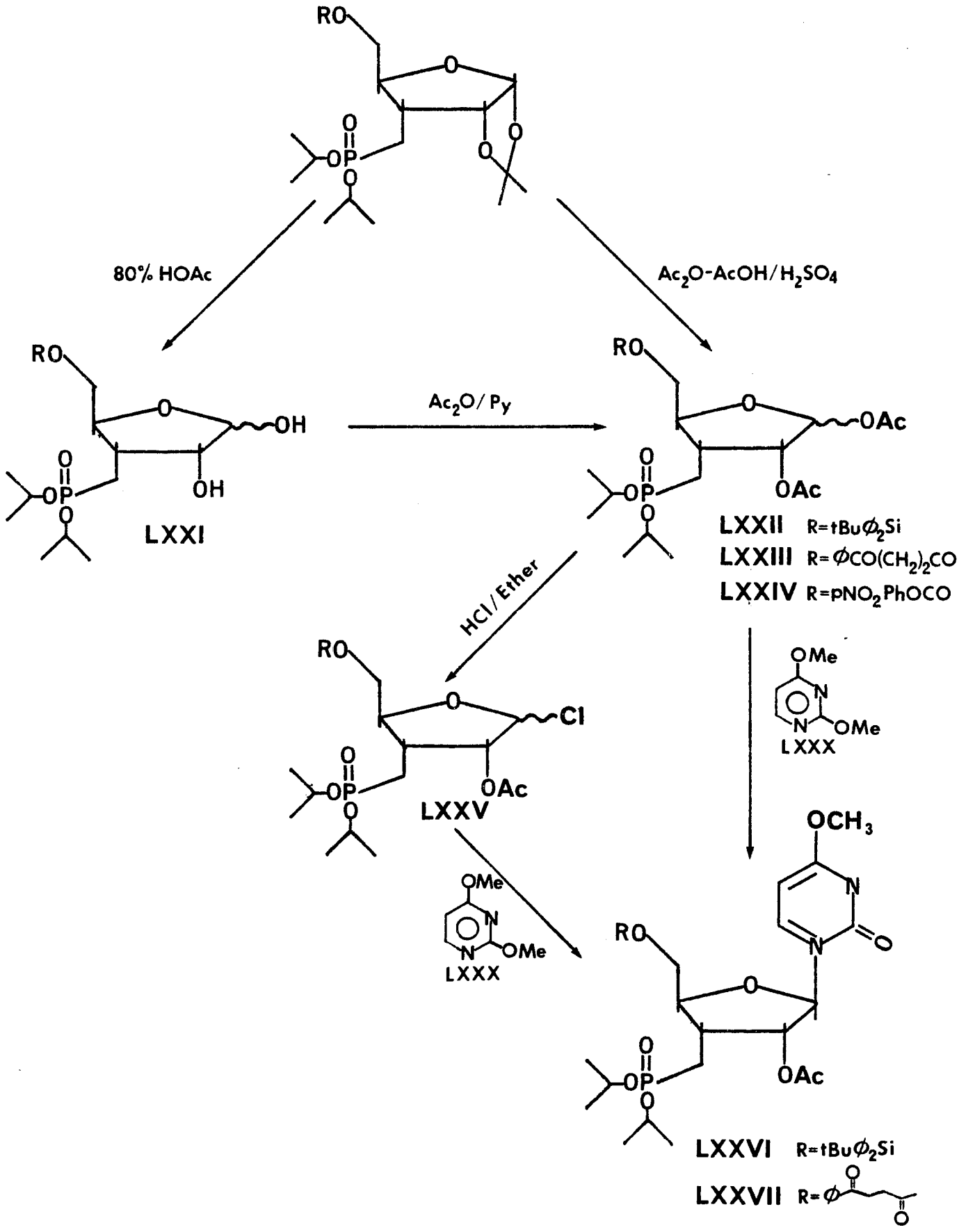
This material was next treated with t-butyl-diphenyl-silyl chloride (LXXVIII, Scheme XV) and imidazole in DMF to protect the 5-OH. The reagent (LXXVIII) was obtained by treating diphenyl-dichlorosilane with t-butyllithium (186).

The sequence LXVIII-----→LXXVI (Scheme XIV) was next attempted. After a few attempts, the best conditions for the hydrolysis of the 1,2-acetonide linkage (to give LXXI) were found to be treatment of LXVIII with 80% acetic acid

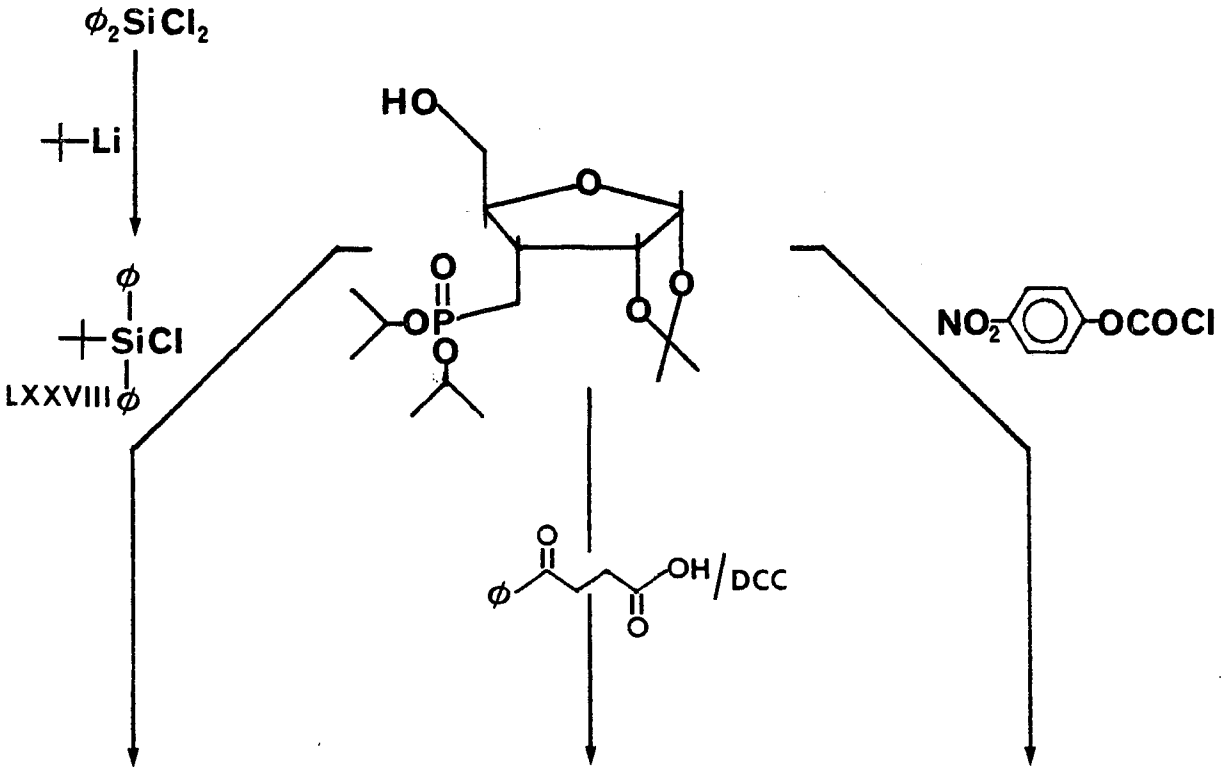
SCHEME XIV



continued


RO
80% HOAc
Ac2O-AcOH/H2SO4
Ac2O/Py
OH
OAc
LXXI
LXXII R=tBuΦ2Si
LXXIII R=ΦCO(CH2)2CO
LXXIV R=pNO2PhOCO
HCl/Ether
Cl
LXXV
OMe
LXXX
OCH3
LXXVI R=tBuΦ2Si
LXXVII R=Φ

SCHEME XV



(187) at 80 C for 24 hours. After removal of the liquids in vacuo, the nmr of the residue indicated loss of the acetonide linkage and a tlc examination indicated a single compound. Acetylation was then carried out (to give LXXII) in pyridine/acetic anhydride without purification of LXXI. After removal of solvents, the residue was treated with dry HCl in ether at -10 C for 8 days (188). Ether was

evaporated and the residue treated with excess of 2,4-dimethoxypyrimidine (LXXX) at 70 C and 20 mm pressure for 10 minutes (189). Excess LXXX was distilled off and the residue chromatographed. None of the phosphorus containing fractions contained either the base or the silyl protecting group as determined by nmr.

The conversion of LXVIII into LXXI was repeated and this time the product was chromatographed. To our surprise, the 5- position had been deprotected along with the acetonide cleavage. This was contrary to the claims of Hanessian et al. (186) that the diphenyl-t-butyl-silyl group is stable to 80% acetic acid. We tried to find conditions for selective hydrolysis of the acetonide linkage but deprotection was always easier.

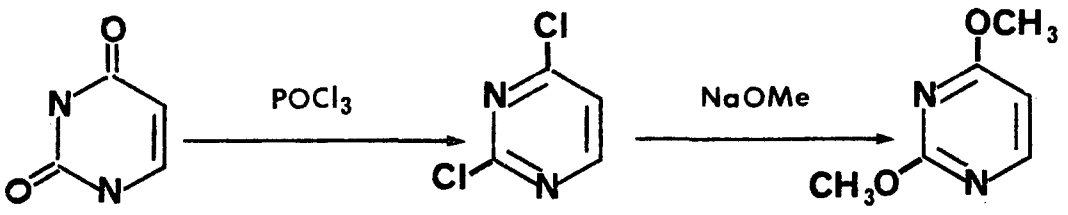
This observation called for using some other suitable protecting group for the 5-position which would be fairly stable to acid treatment but could be removed selectively under mild conditions at a later stage. A 3-benzoyl propionyl group seemed to satisfy these requirements since it is claimed to be stable to 80% acetic acid at steambath temperatures (190) and can be selectively removed in the presence of acetate groups under essentially neutral conditions, i.e., using hydrazine hydrate in pyridine buffered with acetic acid. The initial attempts to obtain LXXIII [Scheme XV], using equimolar amounts of 3-benzoyl propionic acid and DCC for extended periods of time, were unsuccessful. However, when LXVII was treated with a three

molar excess of 3-benzoyl propionic acid and a four molar excess of DCC, the reaction took place readily. The removal of the excess reagents could not be accomplished by simple purification methods and the product was purified by column chromatography. The overall yields were low.

The compound LXIX was then converted into LXXIII by hydrolysis of the acetonide linkage and acetylation. The product was again purified by chromatography.

Among the standard methods available for converting carbohydrate derivatives into nucleosides, two are noteworthy. The first of these involves a condensation of the carbohydrate-1-chloromercury derivatives with suitably protected nucleoside bases (191). This method is applicable toward the synthesis of both purine and pyrimdine nucleosides. The second method, known as the Hilbert-Johnson reaction (192), is used only for preparing pyrimidine nucleosides, and involves treatment of glycosyl halides with 2,4-dialkoxy or 2,4-bistrimethylsilyl pyrimidines. The Hilbert-Johnson reaction was chosen since there was an interest in placing a uracil moeity on the intermediate and this method offered certain advantages over the chloromercury-method. Specifically, the reaction setup and manipulation is much simpler and the reaction temperature much lower in a Hilbert-Johnson reaction. The chloromercury method requires reaction temperatures in excess of 150 C which might be detrimental for the present system in as much as isopropyl esters tend to undergo

SCHEME XVI



LXXIX LXXX

pyrolysis at high temperatures.

Accordingly, 2,4-dimethoxypyrimidine was prepared from uracil via 2,4-dichloropyrimidine [Scheme XVI], and treated with the glycosyl halide (LXXV) (Scheme XIV) at 70 C and 20 mm pressure. The glycosyl halide (LXXV) was obtained by treating (LXXIII) with dry HCl in ether at -10 C for 8 days. These attempts toward the preparation of LXXVII resulted only in the production of complex mixtures from which no LXXVII could be isolated. The reactive glycosyl- halide intermediate was hard to purify and it was hard to track down the origin of the problem. At this point, a paper was noted by Niedballa and Vorbruggen (193), in which there was reported a modification of the Hilbert-Johnson reaction. Regarding the reaction as a kind of Friedel-Crafts reaction, leading to the formation of a C-N bond, the authors successfully employed several standard Friedel-Crafts catalysts such as $SnCl_4$, $ZnCl_2$, $TiCl_4$, BF_3 and $AlCl_3$ to

improve the reaction considerably. The yield and the purity of the product was improved, lower reaction temperatures and shorter reaction times could be used and most importantly, more stable sugar derivatives such as 1-alkoxy and 1-acetoxy compounds could be employed.

The initial attempts to make LXXVII using this improved method were unsuccessful when LXXIII was treated with an excess of 2,4-dimethoxypyrimidine (LXXX) in dichloroethane at room temperature using $SnCl_4$ as catalyst. No reaction was observed under such conditions for extended periods of time (72 hours). Using an excess of the catalyst had no effect but when the mixture was refluxed, the reaction was complete in 4 hours as determined by monitoring the reaction on tlc. The catalyst was destroyed with sodium bicarbonate solution and the product recovered in good yield. The crude product was purified by chromatography.

There were certain problems associated with the use of 3-benzoyl propionyl group for protection of the 5'-position. The use of a large excess of 3-benzoyl propionic acid and DCC made the purification of the product difficult and the yield of LXIX was low. Also the protecting group contained a ketonic function which must be protected since a Wittig reaction was to be performed at a later stage. To circumvent these problems, the possibility of using some other suitable protecting group which might provide an improvement over the 3-benzoyl propionyl group was considered. There may be noted to be available two other

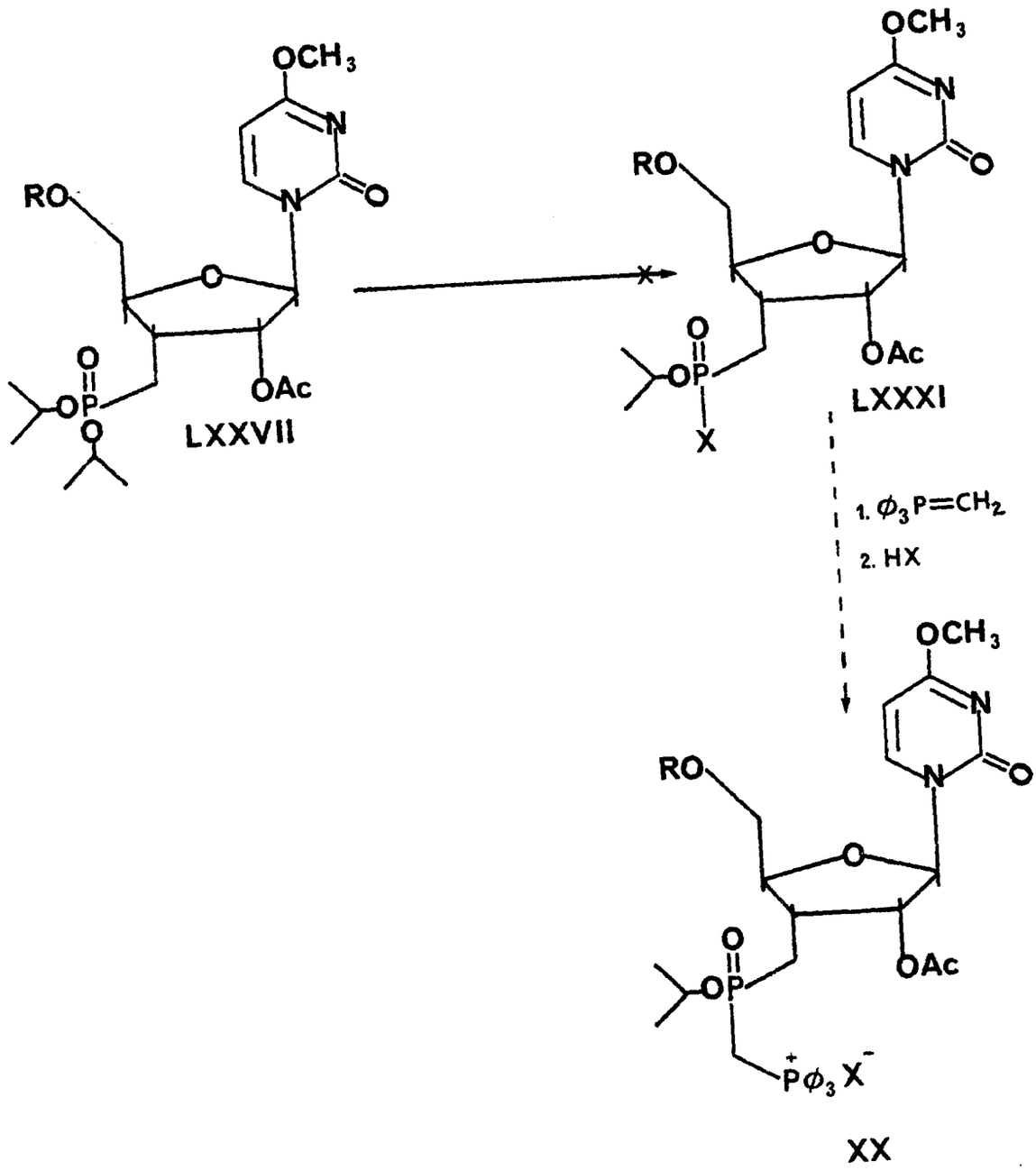
protecting groups which are reasonably stable to acid treatment and could be removed under relatively mild conditions. These are, the 2,4-dinitro benzene sulfenyl group (194) and the p-nitrophenyl carbonate group (195). Preliminary investigations showed that the latter was a superior choice in that it gave a purer product in better yield and was more stable to acid treatment. Thus LXX was prepared from LXVII by treatment with p-nitrophenyl chloroformate in pyridine and was converted into LXXIV without purification.

An improvement in yield and purity of the product was also observed in going from LXX to the 1,2-diacetate (LXXIV) (Scheme XIV) when it was accomplished in a single step by acetolysis using acetic anhydride containing 5% acetic acid and 2% sulfuric acid (196).

Having accomplished the desired transformations at the 1- and 5- positions of the molecule, its conversion into the intermediate XX was only two steps away [Scheme XVII]. The first step involved replacement of one phosphonate ester group by a halogen to give the reactive phosphonyl halide (LXXXI) which could be treated with methylene-triphenylphosphorane followed by acid to give XX.

Normally, the phosphate or phosphonate esters can be converted into the corresponding phosphoryl or phosphonyl halides by a variety of reagents, the most notable being phosgene, phosphorus pentahalides, thionyl chloride and sulfuryl chloride etc. (197). Preliminary investigations

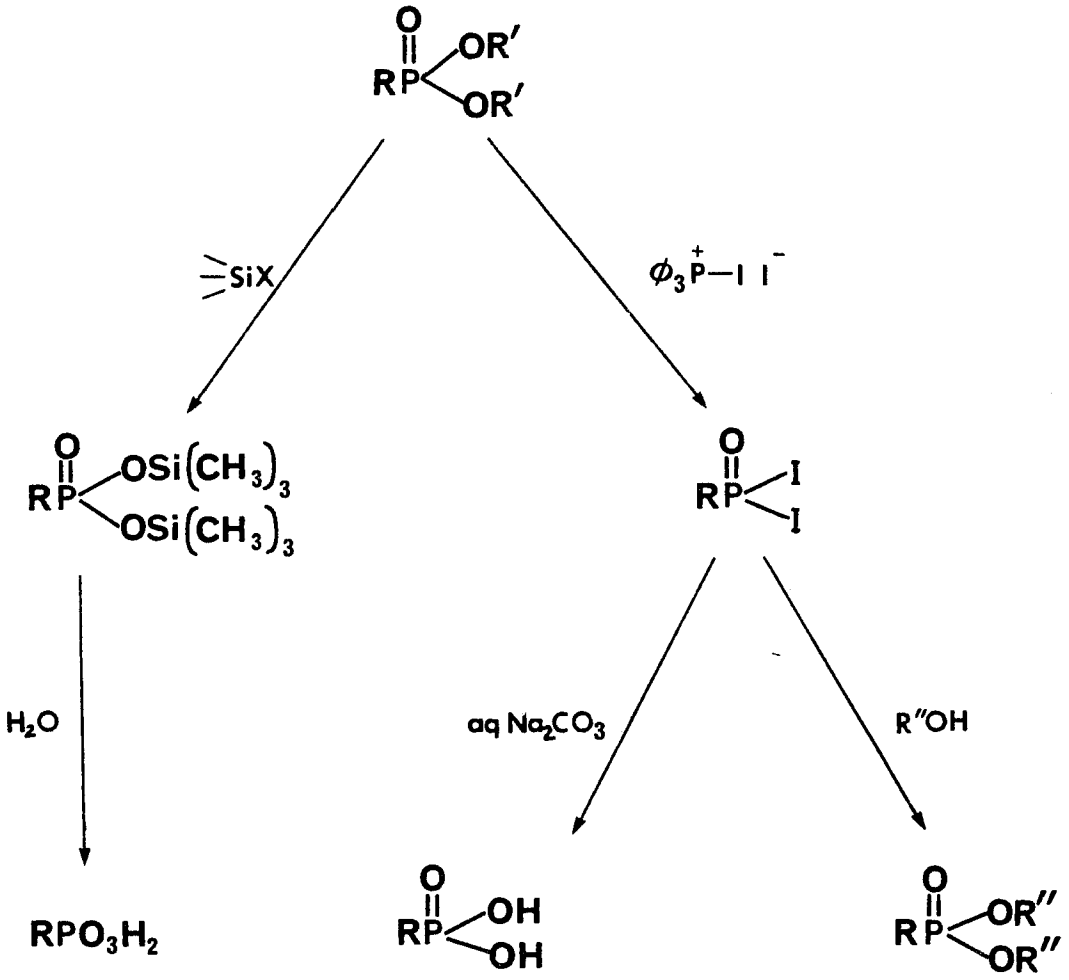
SCHEME XVII


OCH3
N
N
O
RO
O
O
P
O
OAc
LXXVII
OCH3
N
N
O
RO
O
O
P
X
OAc
LXXXI
1. ϕ3P=CH2
2. HX
OCH3
N
N
O
RO
O
O
P
OAc
+
Pϕ3 X-
XX

employing these reagents on LXXVII or precursors such as LX, however, showed that these reagents, while ineffective or mildly effective under the desired conditions (such as low reaction temperatures) for the intended purpose, were much too drastic for the other sensitive parts of the molecule. Here, hence was a need for a specific reagent capable of selectively removing one ester group from the phosphorus under very mild conditions.

In recent years, there has been a lot of interest in developing reagents capable of dealkylating phosphonates under mild conditions since a major problem accompanying the synthesis of phosphonic acid analogues of natural phosphates was that the drastic cnditions required in the classical methods of hydrolysis (i.e. prolonged treatment with acid or base) would tear the sensitive molecule apart. The most successful of these efforts has been the use of bromo- and iodo-trimethylsilanes (198) (Scheme XVIII). Phosphonic esters can be dealkylated with the use of these reagents in essentially neutral conditions at room temperature in a matter of hours. There was undertaken a study of these reagents to explore the possibility of partial dealkylation of phosphonate esters. It was expected that the monoester of the phosphonic acid thus obtained would be converted into the monochloro ester (LXXXI) by reaction with triphenylphosphine/CCl_4 (199). Studies on model compounds using several different solvents, low reaction temperatures and insufficient amounts of reagents, however, indicated

SCHEME XVIII



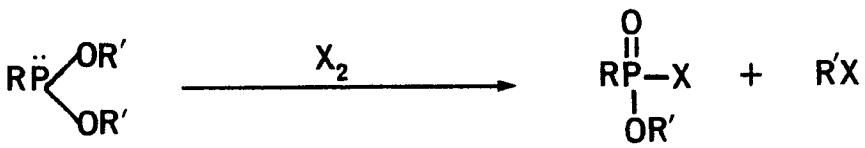
that the dealkylation of the esters was not an easily interrupted process and under any conditions that would result in the dealkylation of one ester group, the second ester group would also come off.

Continuing efforts in the search of a suitable reagent for the partial dealkylation of phosphonate diesters there was discovered a new and inexpensive reagent for

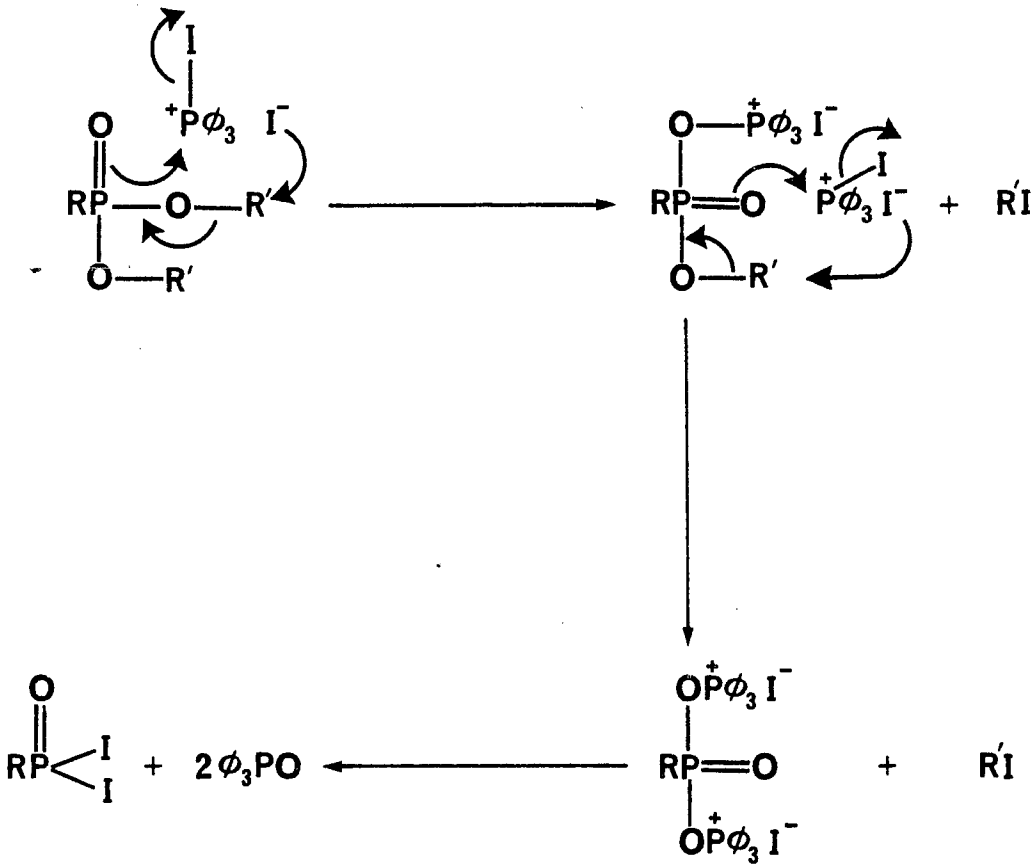
dealkylation of the phosphonate diesters. It was found that when phosphonate diesters were stirred with triphenylphosphine diiodide, either generated in situ from triphenylphosphine and iodine or freshly prepared as a yellow crystalline material, in chloroform solution, the ester groups were gradually replaced by iodine [Scheme XVIII]. The course of the reaction could be followed by nmr examination of samples withdrawn from the reaction mixture. The phosphonyl diiodide thus generated could either be hydrolysed (with sodium bicarbonate solution to protect acid sensitive functions), or reacted with an alcohol to replace the original ester groups by ester groups of choice. Again partial dealkylation could not be achieved in spite of repeated attempts.

A tentative mechanism for the reaction is proposed in Scheme XIX.

The next attempt to achieve the partial dealkylation was based on the observation that the trivalent phosphorus esters such as phosphites and phosphonites react readily and under mild conditions with halogens to give the corresponding phosphoryl halides as follows:



SCHEME XIX

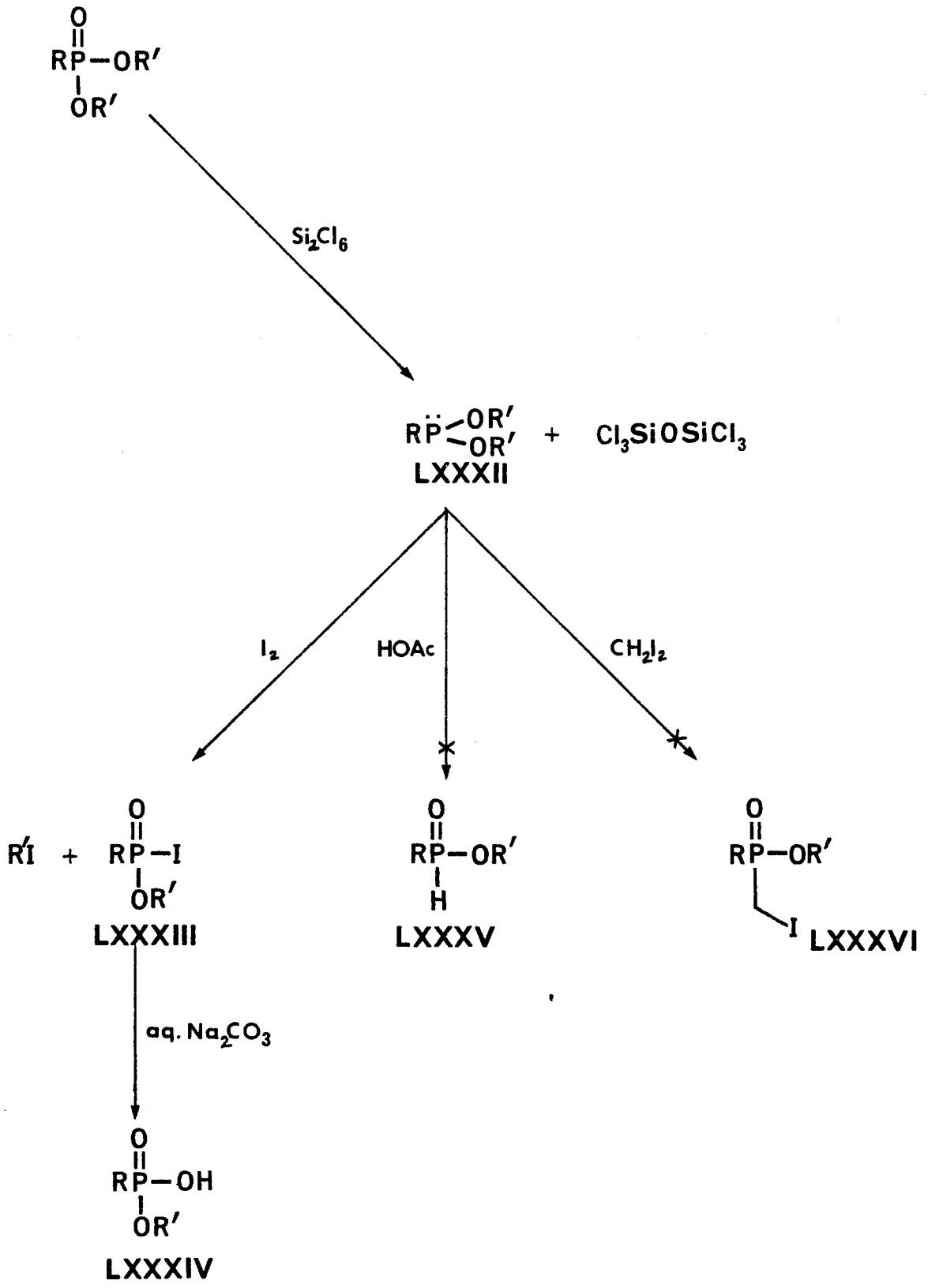


It was obvious therefore, that if we could reduce the phosphonate ester to the corresponding phosphonite ester, we could get the desired phosphonyl halide in quantitative yield by a reagent as mild as iodine. There was no precedent for such a reduction in the literature; most of the powerful reducing agents (such as lithium aluminum hydride) reduce phosphonates into primary phosphines. On the other hand, a relatively mild reducing agent, hexachlorodisilane, was reported to convert tertiary

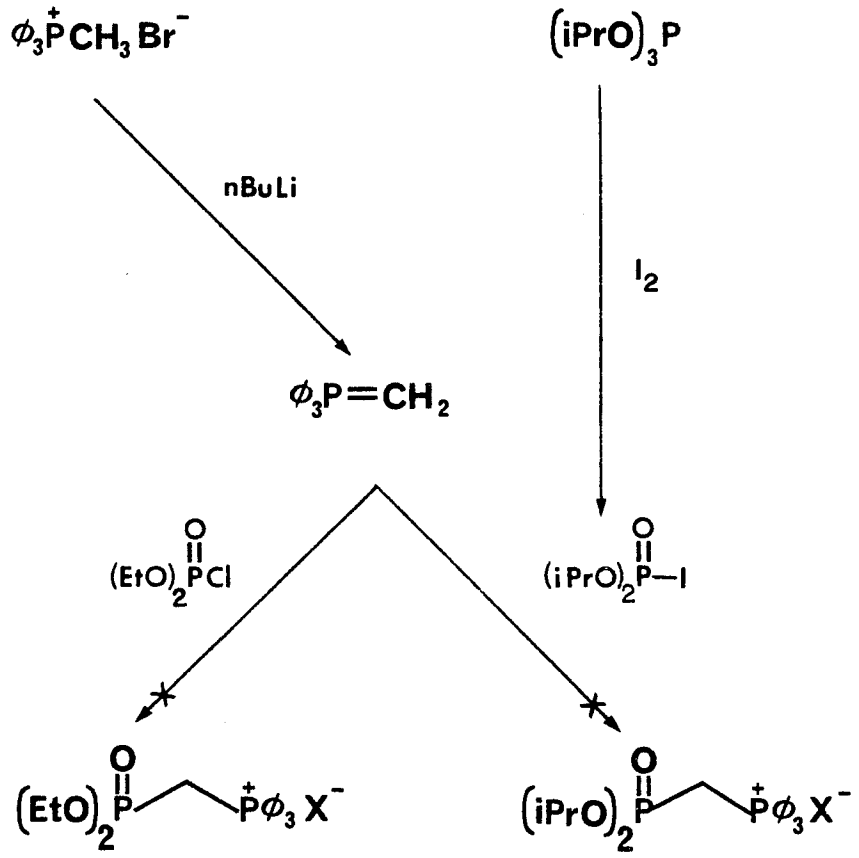
phosphine-oxides into tertiary phosphines at room temperature in nearly quantitative yields (200). The reaction was considered to involve deoxygenation of the phosphoryl linkage to form the energetically favorable Si-O-Si linkage. Hoping to be able to extend this useful reaction to phosphonates, we carried out model studies by treating simple phosphonate esters with hexachlorodisilane in chloroform solution at room-temperature in an argon atmosphere. Care was taken to eliminate all oxygen from the reaction system; the solvent was deoxygenated by bubbling argon through it. A visble reaction was observed as a considerable precipitate was formed upon a few hours of stirring. An attempt was made to isolate the products by working up the reaction with sodium carbonate solution and filtering off the siloxane polymers. However, extensive cleavage of the ester groups was observed, presumably due to the relative lability of phosphonite esters in acidic or alkaline aqueous media. Attempts were also made to control the ester cleavage by refluxing with acetic acid [Scheme XX] prior to workup to give the monoalkylphosphonite (LXXXV) but no success was realized. Similarly, the attempts to react LXXXII <u>in situ</u> with methylene iodide in an Arbuzov manner were unsuccessful. When a solution of iodine in chloroform was added however, it was decolorized until an excess was added. Subsequent workup using aqueous sodium carbonate produced the monoester (LXXXIV) in low yields.

At this point, model studies were performed [Scheme

SCHEME XX


RP(OR′)OR′
Si2Cl6
RP(OR′)2
LXXXII
+ Cl3SiOSiCl3
I2
HOAc
CH2I2
R′I + RP(O)(I)OR′
LXXXIII
RP(O)(H)OR′
LXXXV
RP(O)(CH2I)OR′
LXXXVI
aq. Na2CO3
RP(O)(OH)OR′
LXXXIV

SCHEME XXI

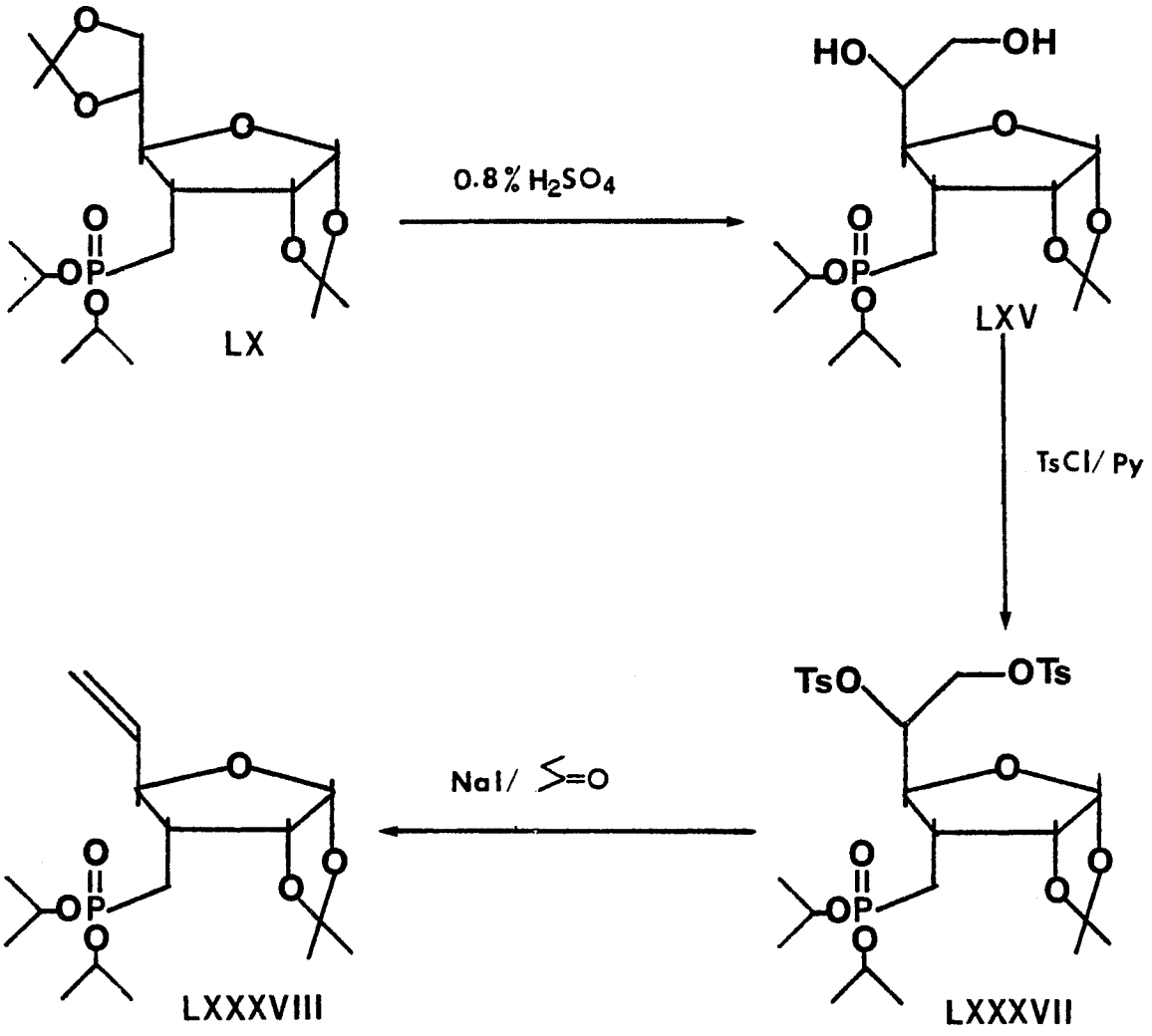


XXI], of the reaction of alkyl-phosphoryl halides with methylenetriphenylphosphorane. A variety of reaction conditions involving changes in solvents, temperature and workup procedures were used but none of the desired phosphonium salts were obtained. This was most puzzling since when diphenylphosphoryl chloride is employed under these conditions the corresponding phosphonium salt is obtained in moderate yields (166). Further investigation of this curious observation would be useful.

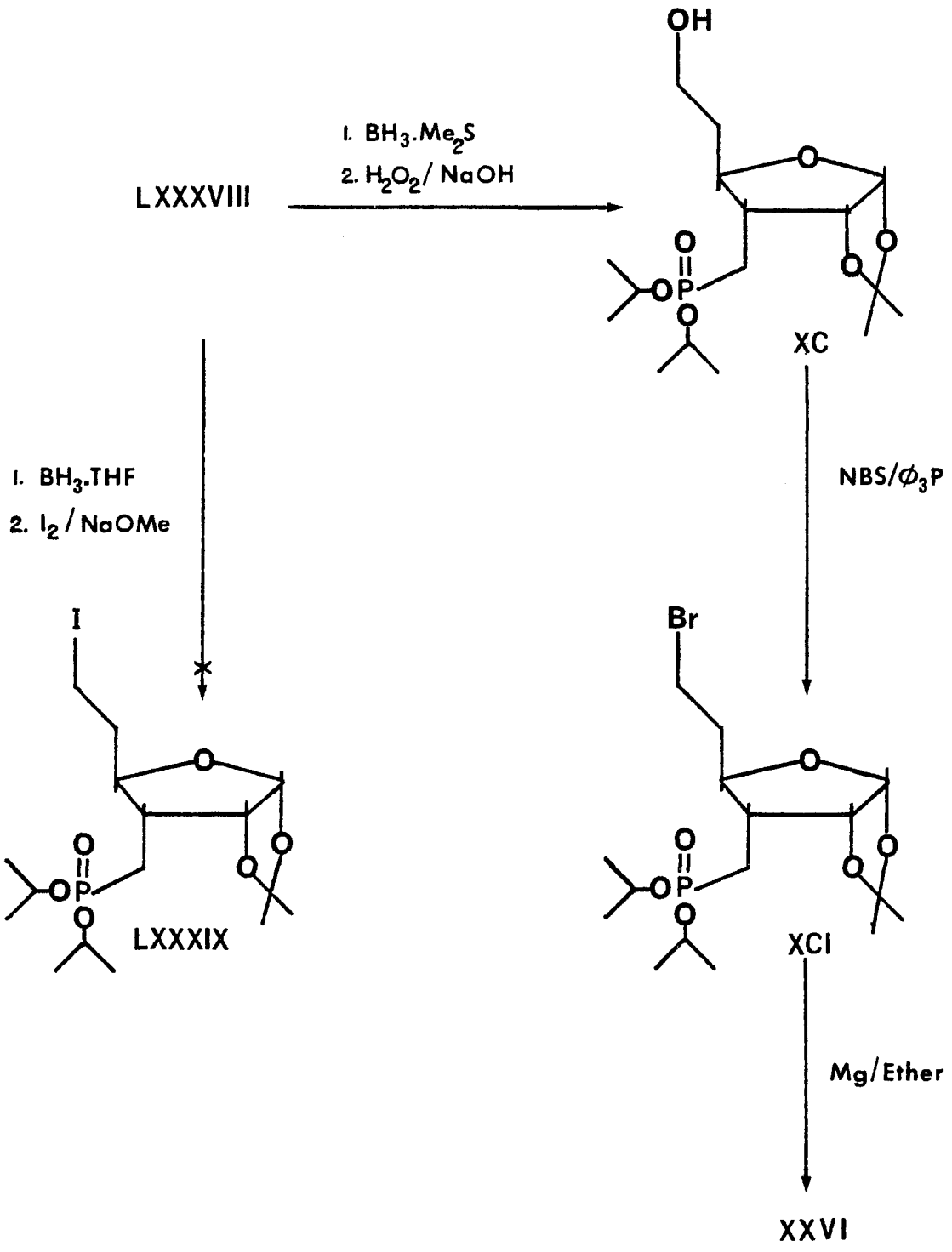
Next, it was decided to follow Scheme XXII in order to explore an alternative approach (Scheme II) to the target molecules. Accordingly, intermediate LX was hydrolysed with 0.8% sulfuric acid in methanolic solution [Scheme XXII] to give the glycol (LXV) which was isolated by extraction with chloroform. LXV was converted into the ditosylate (LXXXVII) by treating with p-toluenesulphonyl chloride in pyridine. The initial attempts, employing 2 moles of the reagent for one mole of LXV, resulted in incomplete reaction and an excess of p-toluenesulphonyl chloride had to be employed for complete esterification of the secondary alcohol function. The ditosylate was treated with an excess of sodium iodide in refluxing methylethyl ketone for 4 hours to produce the 5,6-alkene (LXXXVIII) (201).

In an attempt to convert LXXXVIII into the iodide (LXXXIX) by a single-pot synthesis, it was treated with borane-dimethylsulfide adduct followed by a mixture of iodine and sodium methoxide according to the procedure of Brown and DeLue (202). None of the desired product was obtained and a detailed investigation showed two problems. It was discovered that the use of a stoichiometric amount of the borane adduct resulted in very little hydroboration of the alkene; a problem easily overcome by employing a large excess of the reagent. The other problem was not so easily overcome since the alkyl-borane thus obtained refused to undergo iodinolysis over extended periods of time employing large excess of the reagents.

SCHEME XXII



continued


LXXXVIII
1. $BH_3.Me_2S$
2. H_2O_2/ NaOH
OH
XC
1. BH_3.THF
2. I_2 / NaOMe
NBS/ϕ_3P
I
LXXXIX
Br
XCI
Mg/Ether
XXVI

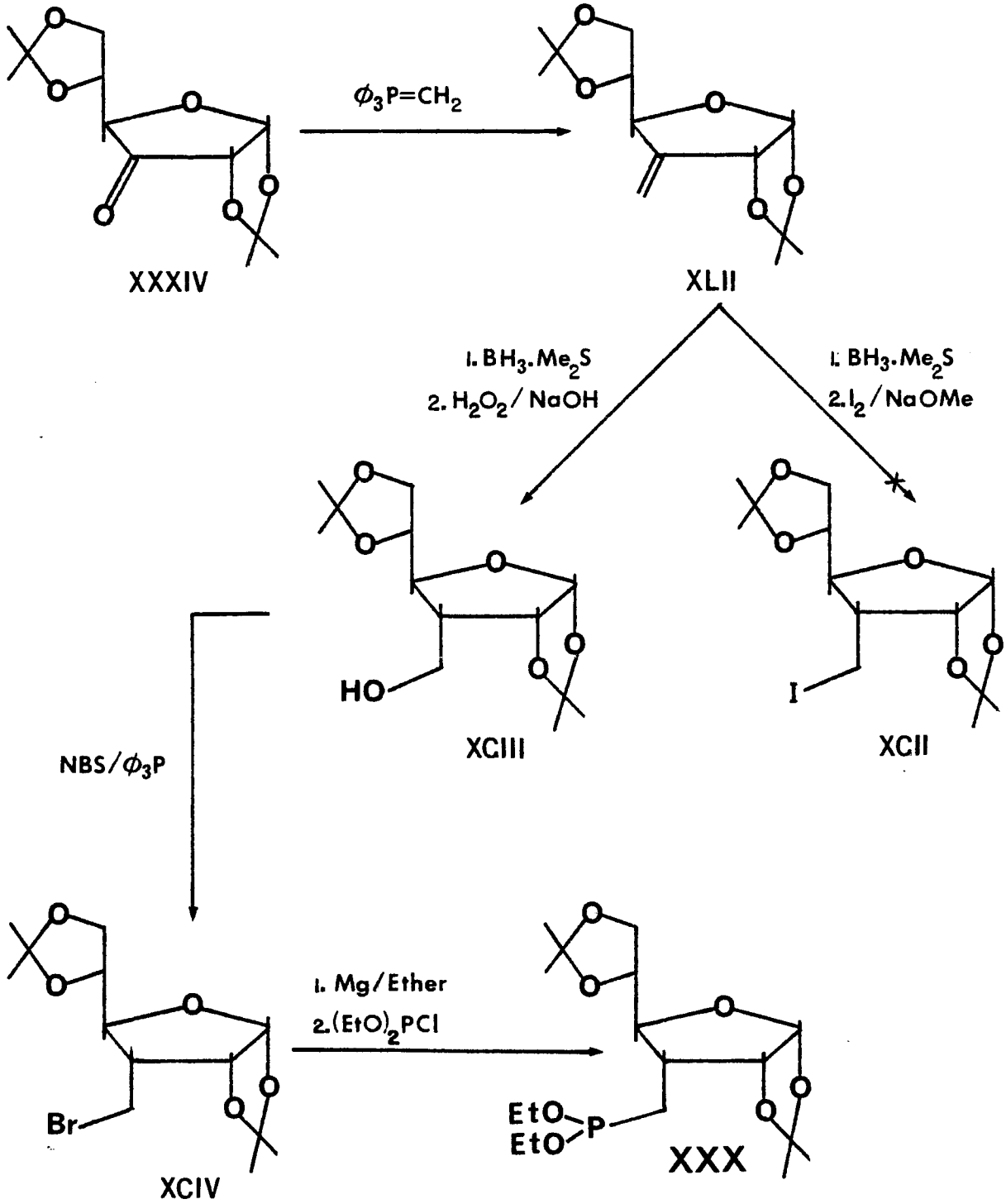
The alkylborane, however, easily underwent oxidation with alkaline hydrogen peroxide to afford the primary alcohol (XC) in good yield; XC was conveniently converted into the bromide (XCI) by treating with NBS/Ph_3P (203) in dimethylformamide. The product was purified by HPLC.

The attempts to prepare compound XXX [Scheme III] are outlined in Scheme XXIII. The alkene XLII was obtained by a Wittig reaction from the ketone XXXIV and purified by distillation. Again, the attempts to convert it into the iodide (XCII) by the method of Brown and DeLue (202) were unsuccessful. The initial attempts to generate the alcohol (XCIII) by hydroboration-oxidation resulted in a mixture of products when THF was used as a solvent. It was discovered that a partial opening of the 5,6-acetonide linkage was taking place under these conditions. However, XCIII was obtained in good yield when the reaction was carried out in hexane using neat borane.dimethylsulfide complex as the hydroborating agent.

Finally, XCIII was converted into the bromide (XCIV) using NBS/Ph_3P in DMF. The product was purified by running through a silica column using 10% ethylacetate in hexane as eluent whereupon a colorless oil was obtained which crystallized in a few minutes.

The attempts to react either XCI or XCIV with magnesium have been unsuccessful so far but work is continuing in that direction. Attempts are also being made to convert these substrates into the corresponding

SCHEME XXIII



alkyllithiums either by direct reaction with lithium metal or by transmetalation.

In conclusion, the work described herein represents considerable progress toward developing a convenient route to the synthesis of phosphinic acid analogues of oligonucleotides. The intermediates, LXXVII (Scheme XVII) and LXLI (Scheme XXII) have been obtained in excellent overall yields from a cheap starting material, namely D-glucose. Both intermediates are promising precursors to the target molecules and hopefully will be of great value in achieving that goal.

Mention must also be made in summary, of the following important byproducts of this investigation.

The phosphonium salts LVI and XLIX have been prepared, which are potentially valuable Wittig-reagents, and must be further investigated for their usefulness in phosphinate generation by reaction with aldehydes.

An improved method for the generation of vinyl phosphonates by using methylene-bisphosphonates in a Wadsworth-Emmons reaction with ketones, has evolved which promises to be of great value.

An accidental discovery of phosphonic acid polymers obtained by simple pyrolysis of isopropyl esters of vinyl phosphonic acids is reported. Such polymers should prove to be of great value in industrial applications.

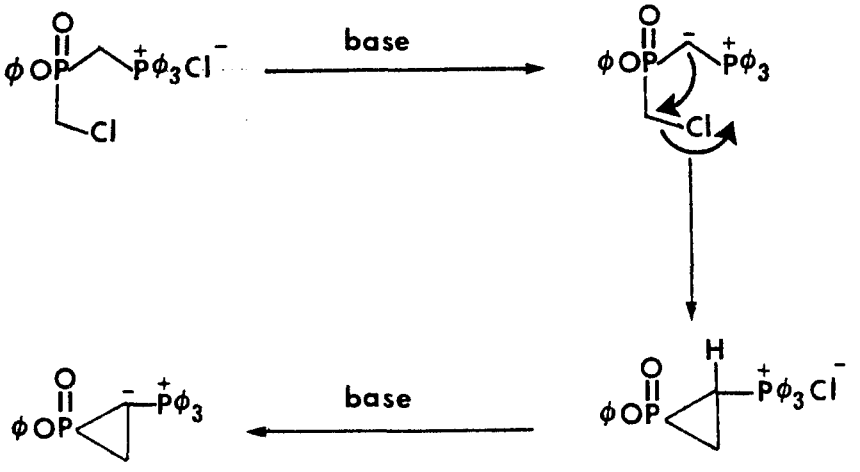
A convenient and cheap reagent, namely triphenylphosphine diiodide, has been discovered to be an

excellent reagent for dealkylation of phosphonate esters under mild conditions.

An indirect method for achieving partial dealkylation of phosphonate diesters using a reduction-oxidation sequence, employing hexachlorodisilane-iodine, has been developed; although the reaction conditions are yet to be standardized for optimum yields.

In addition, several synthetic methods have been modified and improved (Experimental Section) to optimize yields and apply them successfully to our system.

Note : It should be mentioned that the monophosphonium salt (LVI, Scheme IX) might be undergoing an intramolecular reaction to displace chlorine; the resulting intermediate would then lose a second proton as shown below :



EXPERIMENTAL

General

All chemicals were of reagent quality and used without further purification with the following exceptions: pyridine was refluxed with barium oxide overnight, distilled and stored over KOH pellets; triethylamine was dried over KOH pellets and distilled; benzene, hexane and heptane were dried over sodium metal; THF was distilled over lithium aluminum hydride and stored over molecular sieves; dimethyl sulfoxide was distilled over calcium hydride and stored over molecular sieves; acetonitrile was distilled over phosphorus pentoxide; DMF and methyl ethyl ketone were distilled immediately prior to use and kept over molecular sieves. Thin layer chromatography was performed using Polygram Sil-N-HR sheets (Brinkman); visualization was effected using spray reagents such as 20% methanolic sulfuric acid and molybdate spray (for phosphorus containing compounds). Silica gel for column chromatography was bought from Baker Chemicals (40-140 mesh). Samples for analysis were often purified by analytical HPLC (Waters Associates) and some purifications were carried out on Waters Associates-Prep500 HPLC machine. Infrared spectra were measured using a Perkin-Elmer 237-B spectrophotometer, and nmr spectra were obtained with a Varian EM-360 instrument. Optical rotations were measured at 27 C using a Rudolph polarimeter (sodium lamp) with a 1 dm cell.

Synthesis of Diacetoneglucose (XXXIII) (ref167): D-glucose (450g,2.5m) was stirred mechanically with 360g of anhydrous zinc chloride and 22.5g of 85% phosphoric acid in a 4L erlenmeyer flask. After 36 hours of stirring at room temperature the mixture was filtered and the undissolved glucose washed with acetone several times; 290g of glucose was recovered. The filtrate was brought to pH=8 by addition of 50% KOH solution while stirring the mixture mechanically and cooling in an ice-bath. The precipitated zinc-oxide was filtered off and washed thoroughly with acetone. The combined washings and the filtrate was concentrated on a rotatory evaporator. The viscous syrup thus obtained was diluted with 450ml of water and extracted with chloroform (4 X 300mL). The chloroform extract was washed three times with water, dried over anhydrous magnesium sulfate and finally concentrated on a rotatory evaporator to produce a near white solid. The crude product was purified by recrystallization from ligroin (bp 60-90 C). Yield=116g (50.2%); mp 108 C (lit 110). Analytical data; nmr(CCl_4,δ): 1.35 (d of d, 12H); 2.93 (s,1H); 3.7-4.46 (m,6H); 5.73 (d,1H,J=4Hz); ir($CHCl_3$,cm-1): 3413 (broad), 2985.1, 1449.2, 1379.3, 1369.7, 1250, 1162.8, 1063.8, 1010.1, 840.3, 806.5, 680.3.

Synthesis of 1,2:5,6-di-O-isopropylidine-α-D-ribo-hexos-3-ulose (XXXIV) (ref168,169): Diacetoneglucose (52g,0.2m) was mixed with 600mL of DMSO and 400mL of acetic anhydride in a

round bottom flask and the mixture was allowed to stir at room temperature after stoppering the flask tightly. The progress of the reaction was monitored on tlc at 4 hour intervals. None of the starting material could be detected after 24 hours. The solution turned yellow during this period and developed a foul odor due to the generation of methyl sulfide in the reaction. The volatile materials were removed from the reaction mixture by vacuum distillation in a hood (2.5-5 Torr; 50-60 C). Two Dry-Ice traps connected in series were placed between the pump and the distillation setup to prevent the dimethyl sulfide from entering the pump and escaping into the atmosphere. The product was recovered by distillation through a short fractionating column (at 95-125 C/0.005mm) and purified by redistillation (bp 95-100 C/0.005 Torr). Yield=30g (58%). Analytical data; nmr($CDCl_3$,δ): 1.4 (d,12H,J=7Hz); 3.9-4.5 (m,5H); 6.15 (d,1H,J=4.4Hz); ir(CCl_4,cm-1): 3012, 1776, 1369.8, 1234.6, 1162.8, 1052.2, 854.7.

Synthesis of Hydroxymethyl-triphenylphosphonium chloride (XXXVIII) (ref170): Triphenylphosphine (131g,0.5m) and 16.5g of paraformaldehyde were taken in 250 mL of dry ether contained in a 500mL 3-neck flask protected from the atmospheric moisture by a drying tube. The mixture was stirred with a mechanical stirrer and dry hydrogen chloride was bubbled through the mixture. A white precipitate appeared immediately. The hydrogen chloride was

continuously passed until no further precipitation was observed. The crystalline white solid was filtered off and washed several times with ether. The crude product was dried overnight under high vacuum and converted into chloromethyl-triphenylphosphonium chloride (XXXIX). A small sample was recrystallized from chloroform (mp 75-80 C) and chloroform-ethylacetate (mp 188-190 C; lit. 192 C). Yield=135.5g (82.5%). Analytical data; nmr(TFA,δ): 2.0 (s, 1H); 5.06 (s, 2H; Jp-c=0); 7.3 (m,15H); ir($CHCl_3$,cm-1): 3571-3030 (broad), 2958.6, 1438.8 1116.1, 869.5, 689.6.

Synthesis of Chloromethyl-triphenylphosphonium chloride (XXXIX) (ref170): The crude hydroxymethyl-triphenylphosphonium chloride was taken up with 115g of thionyl chloride in 250mL methylene chloride and the mixture refluxed for 40 minutes. The solvent and the excess thionyl chloride was stripped off on a rotatory evaporator and the residue stirred overnight under high vacuum to remove the last traces of thionyl chloride. The thick yellow-brown liquid so obtained was mixed with 150mL of methylene chloride and the solution boiled with 15g activated charcoal for 10 minutes. The solution was filtered and ether was added gradually until a white turbidity appeared. The solution was kept in a refrigerator to let the product crystallize out but only a brown oil separated. The oil was separated from the solvent and triturated with large amounts of ether. The oil finally solidified when it was kept in a

large excess of ether for a few days. The solid was filtered off and examined by nmr. The nmr indicated that it was a mixture of XXXIX and XXXVIII. The solid was then dissolved in chloroform and refluxed with an excess of thionyl chloride for two hours. An nmr examination of the reaction mixture at this stage indicated complete conversion into XXXIX. The volatile materials were removed under vacuum whereupon the residue readily solidified. After decolorization and recrystallization (chloroform-ether), a white crystalline solid was obtained in 75% yield (mp 252-256 C). Analytical data; nmr(TFA,δ): 4.57 (d,2H,J=6Hz); 7.25 (m,15H); ir($CHCl_3$,cm-1): 2976.2, 1445.1, 1250, 1204.8, 1113.6, 1098.9, 1000, 711.7, 687.3, 657.9

Synthesis of 1,2:5,6-di-O-isopropylidine-3-deoxy-3-chloromethylidine- -D-ribo-hexofuranose (XL): Chloromethyl-triphenylphosphonium chloride (17.35g,0.05m) was taken in a 500mL 3-neck flask fitted with a mechanical stirrer, a reflux-condenser and an addition funnel. After flushing the system with nitrogen for two hours, 250mL of anhydrous ether was introduced and the suspension was stirred rapidly. Phenyllithium (0.05m) was next added in a dropwise manner. The solution turned yellow at first, then turned bright orange with some precipitate formation. The mixture was allowed to stir at room temperature for one hour; 12.95g (0.05m) of the ketone (XXXIV) in 50 mL of ether was then added dropwise over a period of one hour while cooling the

mixture in an ice-bath. After the addition was complete, the icebath was removed and the mixture allowed to stir at room temperature for 2 hours. The reaction mixture was next filtered; the ether solution was washed with water, dried over anhydrous magnesium sulfate and evaporated to give a yellow-brown syrup. The syrup was chromatographed on a silica column (ethylacetate-hexane; 1:1) to afford XL in 30% yield. Analytical data; nmr($CDCl_3$,δ): 1.2-1.7 (m,12H); 3.8-4.5 (m,4H); 5.0-5.4 (m,1H); 5.9 (m,1H); 6.57 (m,1H); ir(between salts,cm-1); 3012, 1658.4, 1481.5, 1449.3, 1379.3, 1369.8, 1212.1, 1162.8, 1069.5, 1020.4, 843.9, 796.8, 699.3.

Synthesis of Benzyl-chloromethyl ether (ref175): Benzyl alcohol was freed from benzylchloride by conversion into crystalline calciumchloride-adduct and washing generously with benzene. The adduct was then decomposed with water, the benzyl alcohol was extracted with ether and pure benzyl alconol was obtained by distillation after drying and stripping off the ether. The purified benzyl alcohol was taken with formaldehyde in 1:3 ratio in a 3-neck flask. Hydrogen chloride gas was passed through the ice-cooled mixture to saturation. The mixture separated into two layers at this point; the upper layer was separated, dried over calcium chloride and distilled (bp 125 C/40 Torr; yield 77%).

Synthesis of hydroxymethyl-phosphonous acid (XLV) (ref177): To 83mL of 50% hypophosphorus acid under nitrogen was added with stirring 23g of trioxymethylene during 2 hours at 40-45 C. After two hours more, the temperature was raised to 50 C and the mixture stirred overnight. The clear colorless solution was evaporated several times with isopropanol to remove the water and the viscous colorless product was finally dried over phosphorus pentoxide in a vacuum dessicator; nmr(D_2O, δ): 1.3 (d,J=6Hz); 1.5 (d,J=6Hz); 4.0 (m); 5.32 (s,DOH).

Synthesis of Bishydroxymethyl phosphinic acid (LIII) (ref179): A mixture of 200g of sodium hypophosphite, 1.1 litre of water, 400mL of conc. hydochloric acid and 130g of paraformaldehyde was taken in a 2-litre round-bottom flask. The mixture was heated under reflux on a steam-bath for 50 hrs and then water, HCl, and excess formaldehyde were removed, first on a rotatory evaporator, and then under high vacuum. The precipitated sodium chloride was filtered off, the syrup diluted with half the volume of water and reevaporated. This operation was repeated several times, until the smell of formaldehyde disappeared. The thick colorless syrup thus obtained was finally dried under high vacuum (.001 Torr) at 50 C for several hours. The product was used without further purification for conversion into LIV. Yield=190g (66%). nmr (D_2O, δ): 4.0 (d,4H,J=5Hz); 5.16 (s,DOH).

Synthesis of Bischloromethyl-phosphinyl chloride (LIV) (ref179): Bishydroxymethyl-phosphinic acid (126g,1.0m) (LIII) was added gradually with stirring to a flask containing 416.5g (253mL; 3.5 moles) of thionyl chloride. The reaction flask immediately became cold, indicating an endothermic reaction. The mixture was stirred overnight. Samples were withdrawn periodically and examined by nmr. The hydroxymethylene peaks diminished and the chloromethylene peaks rose in height with time. The reaction was complete after 18 hours of stirring. The excess thionyl chloride was removed on a rotatory evaporator and the product was purified by distillation (bp 75-82 C/0.002 Torr). Yield=140g (77%); nmr($CDCl_3$,δ): 4.13 (d,J=7Hz).

Synthesis of Phenyl-bischloromethyl-phosphinate (LV): Bischloromethyl-phosphinyl chloride (90.75g,0.5m) (LIV) was taken with 51.7g (0.55m) of phenol and the mixture heated in an oil-bath at 80-85 C for 4 hours. The hydrogen chloride generated during the reaction was led to a gas-trap and dissolved in water. After 4 hours of heating, the esterification was essentially complete as determined by an nmr examination of a sample taken from the reaction mixture. The heating was continued for another two hours and then the reaction-flask was placed on a rotatory evaporator for several hours to remove the dissolved HCl gas. The brown viscous liquid was next subjected to vacuum distillation.

The residual phenol was the first to distil over and had to be melted down (as it tended to block the condenser) with the help of a heat-gun. The product was collected as a colorless liquid at 118-125 C and 0.01 Torr pressure. Yield=81g (67.8%); nmr($CDCl_3$,δ): 3.82 (d,4H,J=8Hz); 7.25 (s,5H).

Synthesis of Phenyl-(chloromethyl,methylene)-phosphinyl-triphenylphosphonium chloride (LVI): Triphenylphosphine (57.77g,0.22m) was taken up with 23.9g (0.1m) of phenyl-bischloromethyl phosphinate (LV) in 100mL of benzene and the mixture was subjected to reflux. The reaction flask was protected from moisture with a drying-tube. A white turbidity appeared at first, as the heating was continued and then white crystalline LVI started separating out. After 4 hours, the precipitate was filtered off and washed several times with benzene and ether to remove excess triphenylphosphine. The product was finally dried under high vacuum. Yield=85%, mp 232 C. Analytical data; nmr(TFA,δ): 3.47 (d,2H,J=8Hz); 4.03 (t,2H,J=15Hz); 6.2-8.1 (m,20H); ir($CHCl_3$,cm-1): 3030.3, 2941.2, 2398.1, 1587.3, 1515.1, 1488.1, 1432.6, 1204.8, 1098.9, 1020.4, 925.9, 746.2.

Synthesis of the ylid from LVI: The phosphonium salt (LVI) was dissolved in a minimum amount of water and the solution placed in a wide-mouthed bottle. An equal volume of chloroform was added, followed by the same volume of satd.

sodium cabonate solution. The bottle was stoppered and placed in a mechnical shaker. The mixture was shaken vigorously for 10-15 minutes. The chloroform layer was separated and washed several times with water. After drying over annydrous sodium sulfate, the chloroform solution was concentrated on a rotatory evaporator to produce a pale-brown gummy substance in almost quantitative yield. Analytical data; nmr($CDCl_3$,δ): 1.6 (s,1H,disappears with D O); 3.1-3.8 (m,2H); 6.5-8.2 (m,20H); ir($CHCl_3$,cm-1): 3020, 2400, 1600, 1525, 1500, 1450, 1220, 800, 730, 660.

Synthesis of Phenyl-phosphorodichloridate (LVII) (ref204): A mixture of 1.33g (0.01m) of aluminum chloride and 206.6g (2.0m) of phosphoryl chloride was heated to 90-95 C in a 3-neck flask equipped with a reflux condenser, an addition funnel and a gas-trap. 94g (1.0m) of phenol was added dropwise with stirring over a period of 30 minutes. The temperature was allowed to rise rapidly to 100-105 C. The refluxing was continued for 2 hours, the reflux-condenser was removed and the excess phosphoryl chloride was distilled off initially at 25 Torr pressure and finally at 1 Torr pressure. The product was purified by distillation through a short fractionating column (bp 95-100 C/5 Torr). Yield=127g (60%); ir(CCl_4,cm-1): 3125, 1597.4, 1492.5, 1312.3, 1190.4, 1168.2, 952.4, 775.2.

Synthesis of Phenyl-bis(methylene-triphenylphosphonium chloride)-phosphinate (LI), and Phenyl-bis(methylene-triphenylphosphorane)-phosphinate (XLIX): Methyl triphenyl-phospnonium bromide (28.5g,0.08m) was suspended in 600mL of dry ether and the mixture stirred mechanically in a 3-neck flask flushed with nitrogen. 50mL of 1.6M n-butyllithium (0.08m) was added dropwise whereupon the mixture turned bright yellow. The mixture was stirred for two hours at room temperature. 4.22g (0.02m) of phenyl phosphorodichloridate was dissolved in 100mL of ether and added dropwise. A thick white precipitate began to form immediately. When the addition was complete, the yellow color had been discharged and the solution was very thick due to substantial white precipitate. The mixture was stirred for an additional two hours and filtered. The ether solution was extracted with 0.1M HCl and the extract was placed in a refrigerator overnight. A crystalline white precipitate consisting of LI was obtained which was filtered and dried. Yield=1.3g, mp=182 C. The filtrate was brought to pH 8 by a slow addition of 6M sodium hydroxide solution. A crystalline white precipitate was obtained which was filtered under suction, washed with water and dried. Yield=3.5g (25.4%); nmr for LI ($CDCl_3$,δ): 4.77-5.37 (4H,d of d,J=20Hz); 7.07 (s,15H); 7.37-8.17 (m,20H); nmr for XLIX ($CDCl_3$,δ): 0.53-1.57 (broad,2H); 6.9-8.0 (m,35H).

Synthesis of methylene-tetraphenyl-bisphosphine (LVIII) (ref180): Triphenylphosphine (131g,0.5m) was dissolved in

600mL of dry THF charged with 7.5g of lithium ribbon in a 3-neck flask fitted with a mechanical stirrer and flushed with argon. The mixture was stirred vigorously. A red coloration was produced and the flask became quite warm; intermittent cooling was required to moderate the reaction. The stirring was continued for 4 hours and the thick red solution filtered through glasswool (in a nitrogen atmosphere) to get rid of the unconsumed lithium, into another 3-neck flask flushed with nitrogen. With stirring and cooling, 41g of freshly distilled t-butyl chloride was added dropwise to neutralize the phenyllithium produced in the reaction. With stirring continued, 21.25g (0.25m) of metnylene chloride in 100mL THF was added dropwise. After the addition was complete, the mixture was warmed on a water-bath for 30 minutes. The solvents were removed on a rotatory evaporator and 80mL of methanol and 500mL of water were added. The mixture was shaken thoroughly in a separatory funnel whereupon an organic layer consisting mainly of the bisphosphine product separated out. The organic layer was separated and dissolved in minimum amount of hot ethanol. Upon cooling, essentially pure LVIII separated out as white crystalline needles (mp 118-120 C). Yield=46g (47.9%); nmr($CDCl_3$,δ): 2.73 (t,2H,J=1Hz); 7.23 (m,20H).

Synthesis of Tetraisopropyl-methylene-bisphosphonate (ref181): Triisopropyl phosphite (312.4g,1.5m) and

dibromomethane (87g,0.5m) were combined in a 500mL 3-neck flask fitted with a thermometer, a magnetic stirrer and a 24" long fractionating column for separating the isopropyl bromide byproduct from the refluxing mixture. The fractionating column was constructed from a liebig condenser packed with glass helices. Hot water at 60-65 C was circulated through the condenser to maintain the column at that temperature. This temperature was sufficient to retain the unreacted starting material in the reaction system and allow the isopropyl bromide to be distilled off. A Barrett-receiver was connected to the top of the fractionating column and to the top of the Barrett-receiver was fitted a Dewar condenser cooled with dry-ice/isopropanol mixture. The system was protected from the atmospheric moisture by a drying tube. The mixture was heated in an oil-bath until the reaction started at 140 C. The temperature was slowly raised to 185 C over a period of 7 hrs. The temperature was held constant at 185 C for the next 2 hours with close monitoring. After cooling the reaction mixture to room-temperature, the excess triisopropyl phosphite was distilled off (0.1mm/50 C). The residue was subjected to further vacuum distillation to afford pure product (bp 90-100 C/0.05mm) in 86% yield. Analytical data: nmr($CDCl_3$,δ): 1.4 (d,24H,J=6Hz); 2.43 (t,2H,J=23Hz); 4.6 (m,4H); ir($CHCl_3$,cm-1): 3003, 1464.1, 1449.3, 1383.1, 1373.6, 1250, 1176.5, 1098.9, 1000, 970.8, 884.9, 819.7, 719.4, 709.2, 657.9.

Synthesis of 1,2:5,6-di-O-isopropylidine-3-deoxy-3-(diisopropoxyphosphinylmethylidine)-α-D-ribo-hexofuranse (LXIV): Tetraisopropyl-methylene-bisphosphonate (17.2g, 0.05m) was taken in 600mL of sodium-dry heptane in a 1L 3-neck flask fitted with a reflux condenser, an addition funnel and a mechanical stirrer. The flask was flushed with nitrogen prior to addition of the reagents; 31.2mL of 1.6m n-butyllithium (0.05m) was added dropwise with stirring. After the addition was complete, the mixture was stirred at room temperature for 2 hours. The ketone (XXXIV) (12.9g,0.05m) was dissolved in 100mL of dry heptane and transferred to the addition funnel. The reaction flask was cooled in an ice-bath and the ketone was added dropwise. The solution turned yellow to orange to finally brown. After the addition was complete the icebath was removed and the solution allowed to warm up to room temperature. A fine gummy-brown precipitate started to appear at this stage. The mixture was stirred overnight and finally refluxed for two hours the next morning. The red solution with the brown precipitate was transferred to a separatory funnel and mixed with 600mL of water. Upon shaking well, the heptane layer became much lighter in color; additional washings resulted in a colorless heptane layer. After drying over magnesium sulfate and concentrating on a rotatory evaporator a colorless syrup was obtained which was dried over phosphorus pentoxide. Yield=14.2g (74.5%); $[\alpha]_D^{27}$ +140.3 (c=0.1,chl);

nmr(CCl_4,δ): 1.3 (d,24H,J=6Hz); 3.65-4.86 (m,7H); 5.2-5.8 (m,2H); ir(CCl_4,cm-1): 3030.3, 1652.9, 1459.8, 1379.3, 1234.5, 985.2, 892.8.

Anal. calcd. for C(19)H(33)O(8)P: C,54.28; H,7.91

found: C,52.16; H,8.04 (Analyzes for LXIV.H_2O)

Synthesis of 1,2:5,6-di-O-isopropylidine-3-deoxy-3-(diisopropoxyphosphinylmethyl)-α-D-hexofuranose (LX): Compound LXIV (14.2g,0.034m) was dissolved in 400mL of absolute methanol and the solution filtered through a millipore filter. Platinum oxide (600mg) was added and the solution was transferred to a pressure-bottle which was then placed on a Paar-hydrogenator under a hydrogen pressure of 60psi. The hydrogen pressure in the bottle continued to decrease for 24 hours and then leveled off. Shaking under hydrogen was continued for an additional 12 hours and then the product was checked on tlc. No trace of the starting material could be detected. The catalyst was filtered off first on a celite pad and finally on a millipore filter. The solution was concentrated on a rotatory evaporator to give a slightly colored syrup. The color was due to some colloidal catalyst still persisting. The syrup was dissolved in a small quantity of methanol, filtered through a millipore filter and reconcentrated. This operation was repeated several times until a colorless viscous syrup was obtained. Yield=13.9g (98%); $[\alpha]_D^{27}$ +49.5 (c=0.04,chl). Analytical data: nmr($CDCl_3$,δ): 1.12-1.45 (m,24H);

1.65-2.45 (m,3H); 5.55 (d,1H,J=3.6Hz); ir(CCl_4,cm-1): 3030.3; 1457.7; 1388.8, 1373.6, 1250, 1219.5, 1010.1, 985.2, 892.8, 847.4.

Anal. calcd. for C(19)H(35)O(8)P: C,54.02; H,8.35.
found: C,54.59; H,8.61.

Synthesis of 1,2-O-isopropylidine-3-deoxy-3-(diisopropoxy-phosphinylmethyl)-α-D-hexofuranose (LXV): Compound LX (10.55g,0.025m) was dissolved in 50mL of methanol and mixed with 40mL of 0.8% sulfuric acid in a round bottom flask fitted with a magnetic stirrer. The reaction was monitored on tlc and found to be complete in 36 hours. The solution was diluted and treated with sodium metaperiodate to convert LXV into LXVI. For isolation of LXV, the solution was neutralized with solid sodium carbonate and extracted with chloroform. The extract was washed with water, dried and evaporated under reduced pressure to give a 95% yield of LXV. The product was converted into the ditosylate (LXXXVII) without purification; nmr($CDCl_3$,δ): 1.2-1.6 (m,18H); 1.9-2.9 (m,4H); 3.5-3.7 (m,4H); 4.5-4.9 (m,4H); 5.73 (d,1H,J=3Hz).

Synthesis of 1,2-O-isopropylidine-3-deoxy-3-(diisopropoxy-phospninylmethyl)-5-oxo-α-D-ribofuranose (LXVI): The reaction mixture from the preparation of LXV was transferred to a 2L round bottom flask and the solution diluted to 250mL with distilled water. Sodium metaperiodate (600mL; 0.05M) was added dropwise through an addition funnel while stirring

and cooling the mixture in an ice-bath. The outside of the flask was covered with aluminum foil to protect from light. After the addition was complete, the mixture was allowed to warm up to the room temperature and stirred overnight. No attempt was made to isolated the product and it was reduced in situ with sodium borohydride to give LXVII.

Synthesis of 1,2-O-isopropylidine-3-deoxy-3-(diisopropoxy-phosphinylmethyl)-α-D-ribofuranose (LXVII): Sodium borohydride (8.0g,0.2m) was dissolved in 250mL of water and added to the reaction mixture obtained from the preparation of LXVI in a dropwise manner. The solution was cooled in an ice-bath during the addition. The solution was warmed up to the room temperature after the addition was complete and stirred overnight. The solution was brought to pH 5 by a dropwise addition of 50% acetic acid. After stirring for an additionl hour, the solution was extracted a number of times with chloroform. The combined extracts were washed with water, dried over magnesium sulfate and concentrated to yield 7.8g of a colorless syrup (yield=88% overall from LX). Analytical data; nmr($CDCl_3$,δ): 1.05-1.45 (m,18H); 1.7-2.35 (m,3H); 3.4-3.78 (m,4H); 4.15-4.75 (m,3H); 5.52 (d,1H,J=3.6Hz); ir($CHCl_3$,cm-1): 3703.7-3125 (broad,-OH), 2985, 1639.3, 1449.3, 1373.6, 1219.5, 1010.1, 985.2, 884.9, 803.2.

Synthesis of t-Butyldiphenylsilyl chloride (LXXVIII) (ref186): Diphenyldichlorosilane (100g,0.395m) was taken in

500mL of sodium-dry hexane in a 1L 3-neck flask flushed with nitrogen and fitted with a reflux-condenser, a mechanical stirrer and a 250mL addition funnel. A rubber septum was placed on the addition funnel and 212.3mL of 1.86m solution of t-butyllithium (0.395m) transferred with the help of a double ended needle and argon pressure. The attempts to transfer the t-butyllithium with the help of a syringe were unsuccessful because of the extremely pyrophoric nature of t-butyllithium. The t-butyllithium was added with vigorous stirring at room temperature. A turbidity appeared when the addition was complete. The mixture was then refluxed for 4-5 hours and finally stirred overnight at room temperature. The precipitated lithium chloride was filtered off under nitrogen. The filtrate was concentrated and fractionated under vacuum to yield 77g (71%) of LXXVIII; bp 115-130 C/0.02 mm. Analytical data; nmr($CDCl_3$,δ): 1.37 (s,9H); 7.1-8.0 (2m,10h); ir(between salts, cm-1): 3105.6-2873.6, (several peaks); 1472.7, 1466.3, 1428.6, 1111.1, 1000, 819.6, 740.7, 696.8.

Synthesis of 1,2-O-isopropylidine-3-deoxy-3-(diisopropoxy-phosphinylmethyl)-5-(t-butyldiphenylsilyl)-α-D-ribofuranose (LXVIII): Compound LXVII (0.6g,0.0017m) was stirred with 0.515g (0.0018m) of t-butyldiphenylsilyl chloride (LXXVIII) and 0.255g (0.0039m) of imidazole in 10mL of dry DMF at room temperature. The mixture was allowed to stir overnight. A tlc examination showed complete conversion into LXVIII. The

reaction mixture was poured into 50mL of distilled water and extracted 4 times with 20mL portions of ether. The combined ether extracts were washed twice with water, dried over sodium sulfate and concentrated on a rotatory evaporator. A very viscous colorless liquid was obtained (0.92g, 91.5%) which was purified on a silica gel column using a 1:1 mixture of ethylacetate and hexane. The pure material solidified within a few hours to give a crystalline white solid (mp 72 C). Analytical data; nmr($CDCl_3$,δ): 1.04 (s,9H); 1.04-1.47 (m,18H); 1.47-2.64 (m,3H); 3.44-4.84 (m,6H); 5.57 (d,1H,J=3.6Hz); 6.94-7.64 (m,10H); ir(CCl_4,cm-1): 2985, 1470.6, 1434.7, 1383.1, 1234.6, 1117.3, 1015.2, 990, 877.2, 826.4, 743.5, 704.2.

Anal. calcd for C(31)H(47)O(7)PSi: C,63.02; H,8.02

found: C, 63.21, H,8.02

Synthesis of 1,2-O-isopropylidine-3-deoxy-3-(diisopropoxy-phosphinylmethyl)-5-(3-benzoyl-propionyl)-α-D-ribofuranose (LXIX): Compound LXVII (0.7g,0.002m) was taken with 1.07g (0.006m) of 3-benzoyl propionic acid and 1.65g (0.008m) of DCC in 15mL of pyridine and stirred overnight. A tlc examination at this stage indicated a complete conversion into LXIX. 2mL of water was added to the mixture cautiously to destroy the excess DCC and the mixture stirred for an additional 2 hrs. The precipitated dicyclohexylurea was filtered off and the filtrate concentrated on a rotatory evaporator. The last traces of pyridine were removed by

repeated evaporations with benzene. The residue was dissolved in chloroform and washed several times with sodium carbonate solution in an attempt to remove the excess 3-benzoylpropionic acid. An nmr examination after the usual treatment of the chloroform solution indicated a large excess of 3-benzoylpropionic acid. The product was finally purified by column chromatography using a 1:1 mixture of ethylacetate and hexane. Yield=45%; Analytical data; nmr($CDCl_3$,δ): 1.1-1.6 (m,18H); 1.6-2.5 (m,3H); 2.83 (t,2H,J=6Hz); 3.3 (t,2H,J=6Hz); 3.8-5.0 (m,6H); 5.75 (d,1H,J=3Hz); 7.8-8.0 (m,2H); ir($CHCl_3$,cm-1): 3012, 1736.1, 1689.2, 1449.3, 1383.1, 1373.6, 1234.6, 1162.8, 1000, 884.9, 701.7, 684.9.

Anal. calcd for C(25)H(37)O(9)P: C,58.58; H,7.27
found: C,58,72; H,7.57.

Synthesis of 1,2-O-isopropylidine-3-deoxy-3-(diisopropoxy-phosphinylmethyl)-5-(p-nitrophenylcarbonate)-α-D-ribofuranose (LXX): Compound LXVII (3.76g,0.0107m) was taken up with 2.5g (0.0124m) of p-nitrophenyl chloroformate in 75mL of dry pyridine and the mixture was stirred for 27 hours at room temperature with exclusion of moisture. Pyridine was then removed under reduced pressure on a rotatory evaporator, the last traces were removed by repeated evaporations with benzene. The residue (5.8g) was dried under vacuum and purified by column chromatography on a silica column using ethylacetate to give 4.7g (85%) of LXX. Analytical data;

nmr($CDCl_3$,δ): 1.16-1.66 (m,18H); 1.66-2.56 (m,3H); 3.76-5.16 (m,6H); 5.76 (d,1H,J=4Hz); 7.06-7.56 (m,2H); 7.96-8.36 (m,2H); ir($CHCl_3$,cm-1): 2985.1, 1760.6, 1592.3, 1515.2, 1381.2, 1369.9, 1336.9, 1234.6, 1162.8, 1098.9, 1005, 985.2, 862.1, 813, 675.7.

Anal. calcd. for C(22)H(32)O(11)PN: C,51.06; H,6.23.
found: C,51.24; H,6.42

Synthesis of 1,2-di-O-acetyl-3-deoxy-3-(diisopropoxy-phosphinylmethyl)-5-(3-benzoyl-propionyl)-α-D-ribofuranose (LXXIII): Compound LXIX (0.5g,0.001m) was heated with 20mL of 80% acetic acid at 80 C in a 50mL round bottom flask fitted with a reflux condenser. The reaction was monitored on tlc; all the starting material disappeared at the end of 24 hours and a new slow moving spot was visible near the origin (eluent__ethylacetate). The solution was evaporated first on a rotatory evaporator and finally under high vacuum to produce a gummy substance which showed loss of the 1,2-acetonide protecting group when examined by nmr. The product was converted into LXXIII without purification by stirring with 5mL acetic anhydride in 10mL of dry pyridine overnight. A tlc examination indicated complete conversion into LXXIII. The solvents were removed on a rotatory evaporator and the residue dried under vacuum. Purification was achieved by chromatography on silica gel using 1:1 ethylacetate/hexane. Yield=50%. Analytical data; nmr($CDCl_3$,δ): 1.3 (d,12H,J=6Hz); 1.5-1.9 (m,1H); 2.1

(d,6H,J=2Hz); 2.77 (t,2H,J=6Hz); 4.0-5.0 (m,7H); 5.2 (d,1H,J=4Hz); 6.03 (s,1H); ir($CHCl_3$,cm-1): 3003, 1748.2, 1689.2, 1449.3, 1369.8, 1234.6, 1169.6, 1010.1, 990, 892.8, 684.9.

Anal. calcd. for C(26)H(37)O(11)P: C,56.11; H,6.70.
found: C,55.91; H,6.83.

Synthesis of 1,2-di-O-acetyl-3-deoxy-3-(diisopropoxy-phosphinylmethyl)-5-(p-nitrophenyl-carbonate)-α-D-ribofuranose (LXXIV): 0.52g of LXX was treated with 20mL of acetic anhydride containing 1mL water and 0.4g conc. sulfuric acid. After 4 hours of stirring, the mixture was poured into 25g of ice and stirred for a half hour. The entire mixture was extracted with chloroform. The chloroform extract was washed with sodium bicarbonate solution and water, dried over anhydrous sodium sulfate and evaporated under reduced pressure. The resulting syrup was purified by column chromatography. Yield=65%. Analytical data; nmr($CDCl_3$,δ): 1.33 (d,12H,J=6Hz); 2.1 (d,6H,J=2Hz); 1.6-3.2 (m,3H); 3.8-5.0 (m,5H); 5.2 (d,1H,J=5Hz); 6.0 (s,1H); 7.23,8.15 (ABq,4H); ir($CHCl_3$,cm-1): 3003, 1754.4 (broad), 1519.7, 1369.7, 1345.9, 1204.8, 1098.9, 990.1, 925.9, 854.7, 763.3, 722, 662.3.

Anal. calcd. for C(23)H(32)O(13)PN: C,49.20; H,5.74.
found: C,48.93; H,5.70.

Synthesis of 2,4-dichloropyrimidine (LXXIX) (ref205): A suspension of 40g (0.36m) of uracil in 160mL of phosphoryl

chloride in a 250mL round bottom flask fitted with a reflux condenser and a drying tube was heated in an oilbath at 100-110 C for 2.5 hours. The resulting brown solution was cooled to room temperature and the excess phosphoryl chloride was removed at 30 C/20 mm. The viscous mixture was very slowly poured, with stirring, onto 350g of crushed ice; 50mL of ether was added to facilitate decomposition of the complex. After removal of the ether layer, the aqueous layer was extracted by five 100mL portions of ether. The combined ether extracts were washed with two 125mL portions of satd. sodium carbonate solution followed by drying with anhydrous sodium sulfate and removal of ether. The crude product was recystallized from pentane. Yield=32.1g (60%).

Synthesis of 2,4-dimethoxypyrimidine (LXXX) (ref205): A solution of 25g (0.17m) of 2,4-dichloropyrimidine (LXXIX) in 125mL of dry methanol was added slowly with stirring, to a solution of 8g of sodium in 150mL of absolute methanol. The mixture was refluxed for 30 minutes, cooled and filtered by suction. The filtered sodium chloride was rinsed with ether and the combined filtrate and washings were evaporated under reduced pressure. The oily residue was dissolved in 100mL of ether and the solution was washed with two 50mL portions of 30% sodium hydroxide and then with 50mL of water. The ether layer was dried over anhydrous sodium sulfate and concentrated on a rotatory evaporator. The crude product was purified by distillation (40 C/0.1 mm); yield=18g

(76.1%); nmr($CDCl_3$,δ): 3.45 (d,6H,J=2Hz); 5.82 (d,1H,J=5.6Hz); 7.65 (d,1H,J=5.6Hz).

Synthesis of 4-O-methyl-1'-O-acetyl-3'-deoxy-3'-(diisopropoxy-phosphinylmethyl)-5'-(3-benzoyl-propionyl)-uridine (LXXVII) (ref193): Compound LXXIII (0.55g,0.001m) was taken in 20mL of dry 1,2-dichloroethane with 1mL of 2,4-dimethoxypyrimidine in a 50mL round bottom flask fitted with a reflux-condenser and a drying tube. 0.5 mL of freshly distilled stannic chloride was added carefully and the mixture allowed to stir at room temperature. The progress of the reaction was monitored on tlc; no reaction was observed even after 72 hours of stirring. Heat was applied next and the mixture was refluxed for 4 hours. The reaction mixture turned deep red at this point and a tlc examination indicated the loss of all the starting material and a major new spot near the origin (solvent ethylacetate). 20 mL of satd. sodium bicarbonate solution was added carefully after cooling to room temperature and the mixture stirred for 10 minutes. The precipitated stannic hydroxide was filtered off on a Celite pad and washed with 1,2-dichloroethane. The organic layer was separated and the aqueous layer extracted with chloroform. The combined organic layers were dried over anhydrous sodium sulfate and concentrated on a rotatory evaporator. The crude product was purified by column chromatography on silica using 2% methanol in chloroform as solvent. Yield=56%. Analytical

data; nmr($CDCl_3$,δ): 1.5 (m,12H); 2.3 (s,3H); 2.87-3.27 (m,2H); 3.47-3.67 (m,3H); 4.2 (s,3H); 4.32-5.27 (m,5H); 5.54-6.37 (m,5H); 7.42-8.37 (m,6H); ir($CHCl_3$,cm-1): 2985.1, 2347.4, 1733.1, 1677.8, 1634, 1538.5, 1477.1, 1366.1, 1310.6, 1199, 1098.9, 1005, 990.1, 925.9, 775.2, 714.3, 666.7.

Synthesis of 1,2-O-isopropylidine-3-deoxy-3-(diisopropoxy-phosphinylmethyl)-5,6-dideoxy-5,6-ditosyloxy-α-D-hexofuranose (LXXXVII): Compound LXV (3.82g,0.01m) was taken with 6.85g (0.033m) of p-toluenesulfonyl chloride in 50mL of dry pyridine and the mixture was stirred for 48 hours. A tlc examination indicated complete conversion into the ditosylate. 2mL of water was added cautiously and the mixture allowed to stir for another half hour. Pyridine was evaporated first on a rotatory evaporator with repeated additions of benzene and finally under high vacuum. The residue was dissolved in 50mL of chloroform and washed several times with small portions of sodium bicarbonate and water. The chloroform solution was then dried and evaporated under reduced pressure to produce a very viscous oil which was purified by column chromatography on silica using ethylacetate as solvent. Yield=90%. Analytical data; nmr($CDCl_3$,δ): 1.34 (d,18H,J=6Hz); 1.6-2.3 (m,3H); 2.46 (s,6H); 3.7-4.3 (m,3H); 4.4-5.03 (m,4H); 5.55 (d,1H,J=3Hz); 7.2-7.4 (m,4H); 7.5-7.85 (m,4H); ir($CHCl_3$,cm-1): 2958.5, 1368, 1262.6, 1176.5, 1075.3, 1005.1, 813, 701.7.

Anal. calcd. for C(30)H(43)O(12)S(2)P: C,52.16; H,6.27.
found: C,52.14; H,6.18.

Synthesis of 1,2-O-isopropylidine-3-deoxy-3-(diisopropoxy-phosphinylmethyl)-5,6-dideoxy-5,6-dehydro-α-D-hexofuranose (LXXXVIII): Compound LXXXVII (6.9g,0.01m)) was taken with 9.0g of sodium iodide in 50mL of dry methylethylketone in a 100mL round bottom flask fitted with a reflux condenser and a drying tube. The mixture was refluxed for four hours. The solution turned reddish brown due to production of iodine and a white precipitate of sodium tosylate was formed. The mixture was cooled to room temperature and the sodium tosylate was filtered off and washed with acetone. The combined filtrate and washings were concentrated on a rotatory evaporator and the residue was fractionated between 100mL of chloroform and 50mL of satd. sodium thiosulfate soln. The chloroform fraction was washed sucessively with further 25mL portions of sodium thiosulfate soln and water followed by drying over anhydrous sodium sulfate and concentration on a rotatory evaporator. The residue was checked by nmr; in some preparations residual ditosylate was indicated in which case the product was treated with sodium iodide and the whole procedure was repeated. The product was finally purified on Prep-500 HPLC using ethylacetate as solvent. Yield=81%. Analytical data; nmr($CDCl_3$,δ): 1.2-1.57 (m,18H); 1.57-2.47 (m,3H); 3.77-4.23 (m,1H); 4.4-5.67 (m,6H); 5.8 (d,1H,J=3Hz); ir($CHCl_3$,cm-1): 3003, 1449.3, 1428.6, 1383.1 1371.7, 1234.6, 1105, 1000, 934.6,

892.8.

Anal. calcd. for C(16)H(29)O(6)P: C,55.32; H,8.12.
found: C,55.14; H,8.35).

Synthesis of 1,2-O-isopropylidine-3-deoxy-3-(diisopropoxy-phosphinylmethyl)-5-deoxy-α-D-hexofuranose (XC): Compound LXXXVIII (1.16g,0.0033m) was taken in 5mL of sodium-dry hexane in a 50mL 3-neck flask fitted with an addition funnel and a reflux condenser. The system was flushed with nitrogen and 5mL of borane.methylsulfide complex (1.0M in methylene chloride) was added dropwise with cooling in an icebath over a period of 30 minutes. The icebath was removed and the mixture stirred at room temperature for two hours. 10mL of absolute ethanol was cautiously added to destroy the excess borane complex. 5mL of 3M sodium hydroxide was next added and the mixture was cooled in an icebath. 2mL of 30% hydrogen peroxide was added dropwise over a period of 15 minutes. The icebath was removed and the mixture stirred overnight. 50mL of ether was added and the aqueous phase was saturated with sodium chloride. The ether phase was separated, aqueous phase extracted two more times with ether and the combined ether extracts were washed with water. Following drying over anhydrous sodium sulfate and evaporation on a rotatory evaporator, 0.7g (58%) of XC was obtained which was converted into XCI without purification. A small sample was purified by analytical HPLC. Analytical data; nmr($CDCl_3$,δ): 1.2-1.6 (m,18H); 1.6-3.2 (m,6H); 3.6-4.1 (m,3H); 4.4-5.1 (m,3H); 5.8

(d,1H,J=4Hz); ir(between salts, cm-1): 3333.3, 2985.1, 1449.3, 1369.8, 1315.8, 1234.6, 1162.8, 1098.9, 1010.1, 990, 884.9, 816.3, 800, 735.3, 680.3.

Anal. calcd. for C(16)H(31)O(7)P: C,52.45; H,8.53.

found: C,52.48; H,8.81.

Synthesis of 1,2-O-isopropylidine-3-deoxy-3-(diisopropoxy-phosphinylmethyl)-5,6-dideoxy-5-bromo-α-D-hexofuranose (LXLI): Compound XC (0.66g,0.0018m) was taken in 10mL of dry DMF in a 25mL round bottom flask protected with a drying tube. 0.96g (0.0037m) of triphenylphosphine was added and the mixture cooled in ice. 0.64g (0.0036m) of N-bromosuccinimide was next added in small portions over a period of 10 minutes. The icebath was removed and the mixture was stirred at room temperature for 2 hours. A tlc examination at this point showed complete conversion of the alcohol into the bromide. The mixture was heated to 50 C for 30 minutes; after cooling to room temperature, 10mL of methanol was added and the solvents evaporated. The residue was fractionated between ether and water, dried over magnesium sulfate and evaporated to give a white solid. 50mL of dry pentane was added and the mixture kept in a refrigerator overnight. After filtration, the clear pentane solution was evaporated a slightly yellow oil (0.54g, 70%). Although a tlc examination (ethylacetate) showed a single spot, slight contamination by triphenylphosphine oxide was indicated by nmr. Purification was achieved by Prep-500

HPLC using ethylacetate as solvent. Analytical data; nmr($CDCl_3$,δ): 1.2-1.6 (m,18H); 1.77-2.7 (m,5H); 3.1-4.1 (m,3H); 4.4-5.0 (m,3H); 5.77 (d,1H,J=3.8Hz); ir($CHCl_3$,cm-1): 2985, 1383.1, 1369.8, 1234.6, 1162.8, 1105, 1000, 877.2, 806.4, 680.3.

Anal. calcd. for C(16)H(30)O(6)PBr: C,44.76; H,7.04.
found: C,44.90; H,7.03.

Synthesis of 1,2:5,6-di-O-isopropylidine-3-deoxy-3-methylene-α-D-hexofuranose (XLII): Methyltriphenylphosphonium bromide (9.55g,0.0267m) was stirred with 100mL of dry ether in a 3-neck flask flushed with nitrogen. 16.7mL of n-butyllithium was added dropwise and the mixture allowed to stir for one hour. 6.9g (0.0267m) of XXXIV in 25mL dry ether was added dropwise and the mixture stirred overnight. The ether solution was filtered, washed with water, dried and evaporated. The crude product was purified by distillation under reduced pressure (bp 75-80 C;.005-.01 mm). Yield=4.45g (65%). Analytical data; nmr($CDCl_3$,δ): 1.3-1.6 (m,12H); 3.8-4.15 (m,2H); 4.3-4.75(broad s, 1H); 4.83 (d,1H,J=3Hz); 5.4 (m,2H); 5.77 (d,1H,J=3Hz); ir($CHCl_3$,cm-1): 3012, 1436.8, 1385, 1370, 1250, 1162.8, 1075.3, 1010.1, 840.3, 692, 649.3.

Synthesis of 1,2:5,6-di-O-isopropylidine-3-deoxy-3-hydroxymethyl-α-D-hexofuranose (XCIII): Compound XLII (1.3g,0.005m) was taken in 10mL of sodium-dry hexane in a 50mL 3-neck flask fitted with an addition funnel and a

reflux condenser. The system was flushed with nitrogen and 12mL of borane.methylsulfide complex (1.0M in methylene chloride) was added dropwise with cooling in an icebath over a period of 30 minutes. The icebath was removed and the mixture stirred at room temperature for two hours. 25mL of absolute ethanol was cautiously added to destroy the excess borane complex. 6mL of 3M sodium hydroxide was next added and the mixture was cooled in an icebath. 2mL of 30% hydrogen peroxide was added dropwise over a period of 15 minutes. The icebath was removed and the mixture stirred overnight. 50mL of ether was added and the aqueous phase was saturated with sodium chloride. The ether phase was separated, aqueous phase extracted two more times with ether and the combined ether extracts were washed with water. Following drying over anhydrous sodium sulfate and evaporation on a rotatory evaporator, a viscous colorless liquid was obtained which was dried over phosphorus pentoxide. Yield=1.05g (75.4%). Analytical data; nmr($CDCl_3$,δ): 0.7-1.6 (m,12H); 1.7-3.3 (m,3H); 3.3-4.2 (m,5H); 4.6 (t,1H,J=4Hz); 5.6 (d,1H,J=3Hz); ir($CHCl_3$,cm-1): 3472.2, 2941.2, 1449.3, 1379.3, 1369.8, 1307.2, 1242.2, 1149.4, 1058.2, 1005, 869.5, 840.3, 680.2, 645.2.

Anal. calcd. for C(13)H(22)O(6): C,56.92; H,8.08.

found: C,57.04; H,8.49

Synthesis of 1,2:5,6-di-O-isopropylidine-3-deoxy-3-bromomethyl-α-D-hexofuranose (XCIV): Compound XCIII

(2.74g,0.01m) was taken in 40mL of dry DMF in a 100mL round bottom flask protected with a drying tube. 5.24g (0.02m) of triphenylphosphine was added and the mixture cooled in ice. portions over a period of 10 minutes. The icebath was removed and the mixture was stirred at room temperature for 2 hours. A tlc examination at this point showed the presence of a new spot but the reaction was far from completion. The mixture was stirred at room temperature overnight. A tlc examination now showed complete conversion into XCIV. 10mL of methanol was next added and the solvents were evaporated. The residue was fractionated between ether and water, dried over magnesium sulfate and evaporated to give a white solid. 50mL of dry pentane was added and the mixture kept in a refrigerator overnight. After filtration, the clear pentane solution was evaporated to give a yellow oil which was purified by column chromatography on silica using 10% ethylacetate in hexane. A viscous colorless oil was obtained in 50% yield which readily solidified into a crystalline material (mp 60 C). Analytical data; nmr($CDCl_3$,δ): 1.2-1.6 (m,12H); 2.1-2.6 (m,2H); 3.3-4.2 (m,5H); 4.75 (t,1H,J=4Hz); 5.76 (d,1H,J=4Hz); ir($CHCl_3$,cm-1): 3012, 1503.7, 1369.8, 1204.8, 1162.8, 1105, 1052.6, 1010.1, 763.3, 719.4.

Anal. calcd. for C(13)H(21)O(5)Br: C,43.95; H,6.52.

found: C,44.42; H,6.24

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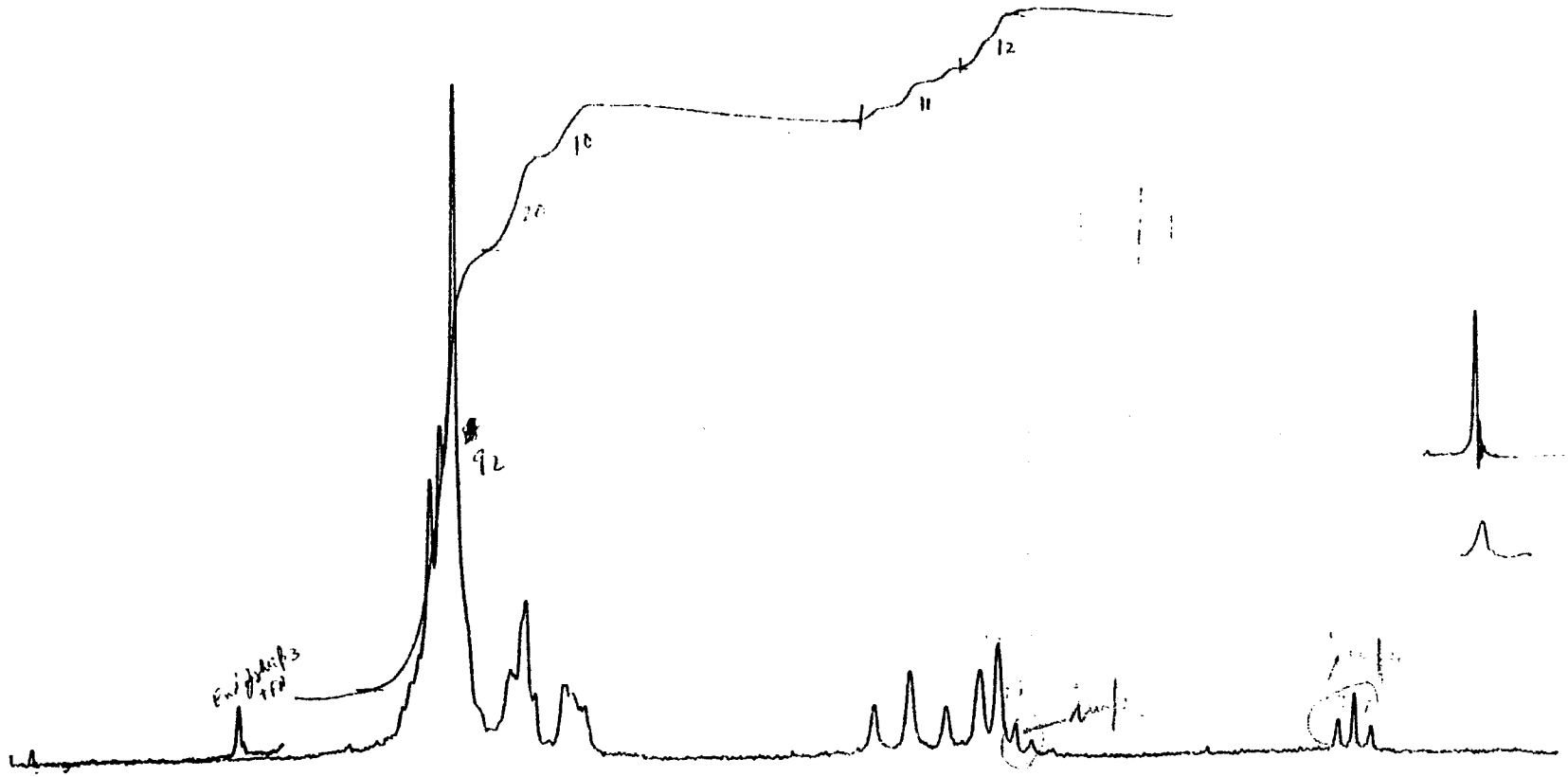
APPENDIX



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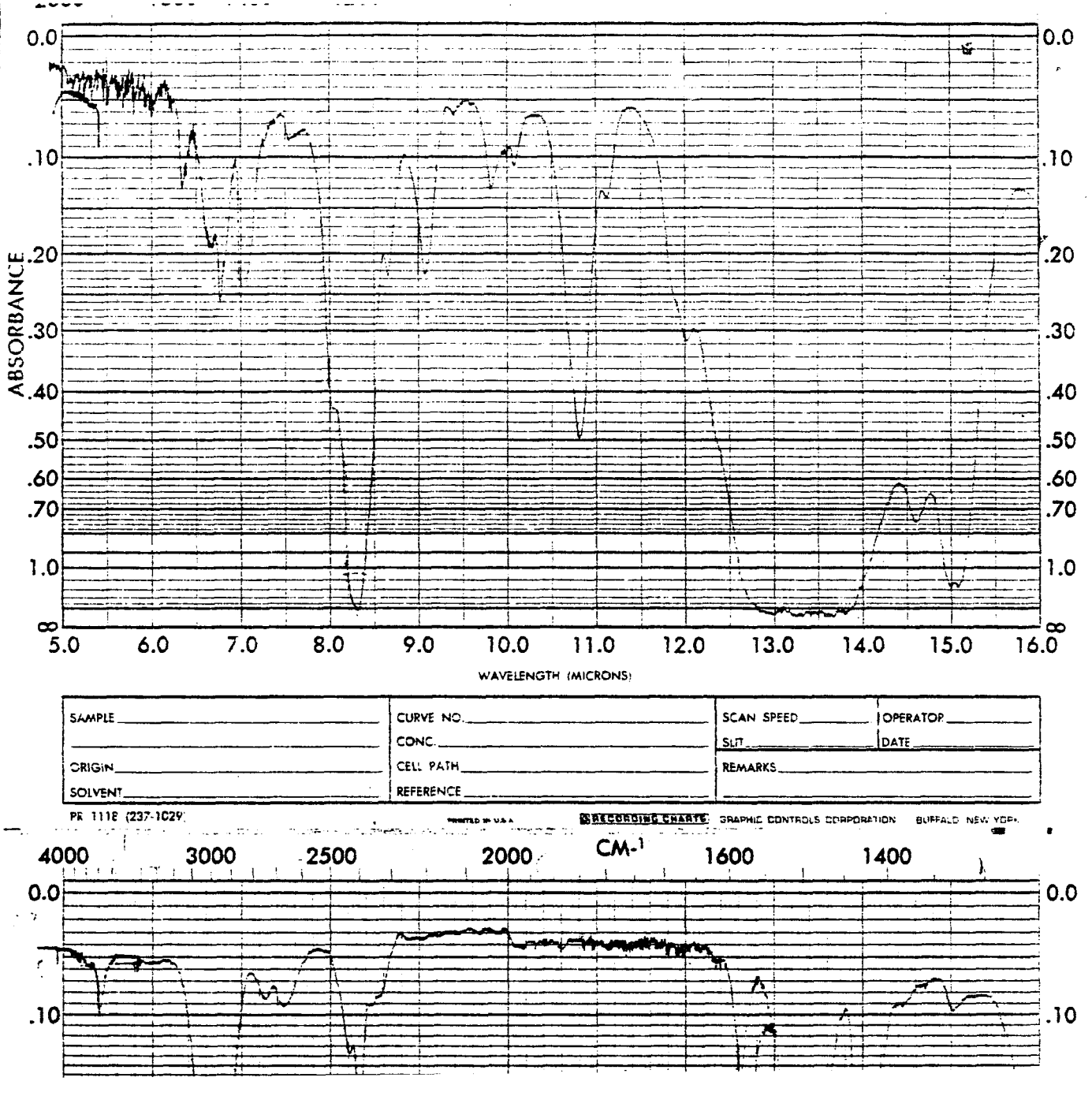
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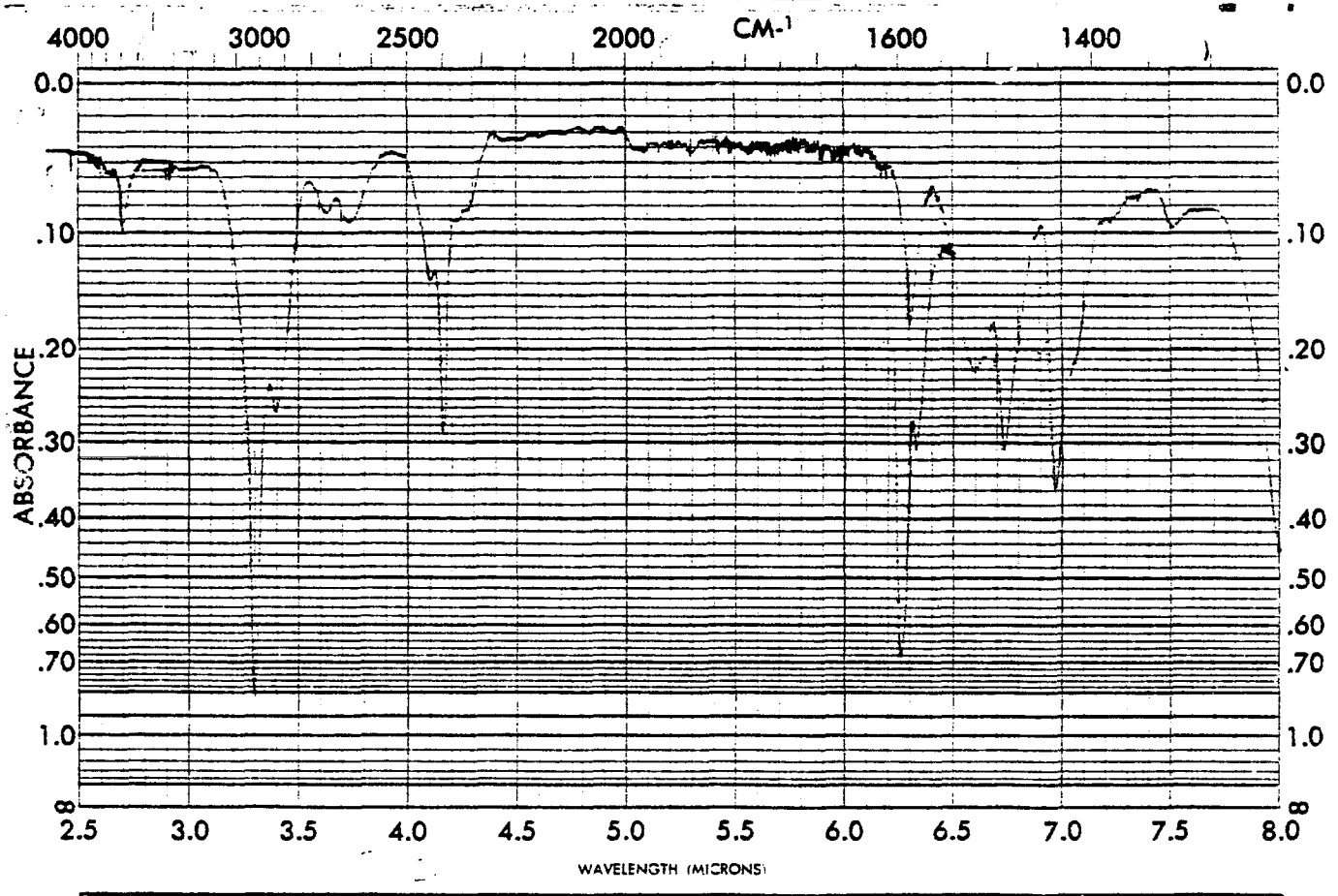

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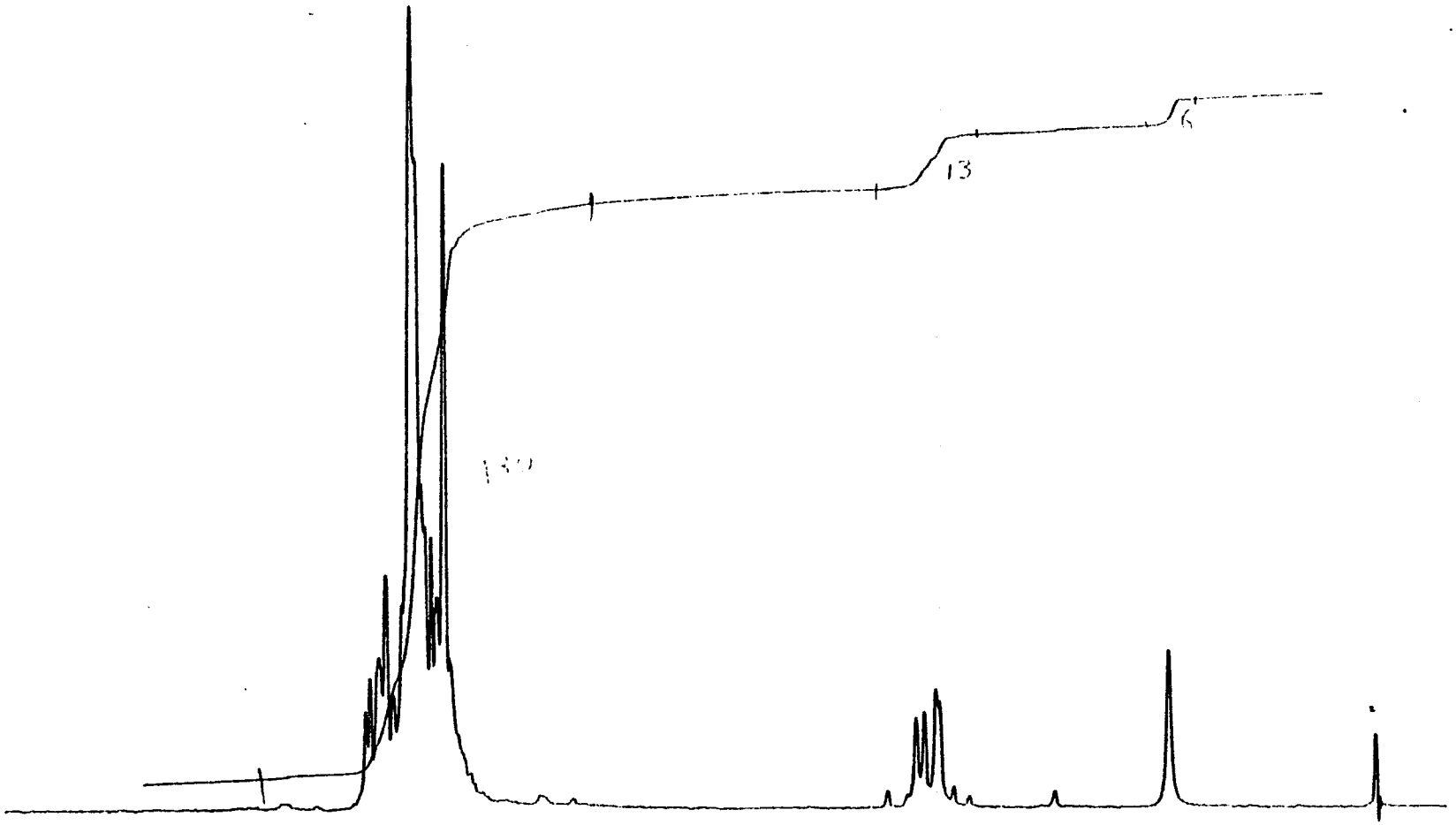

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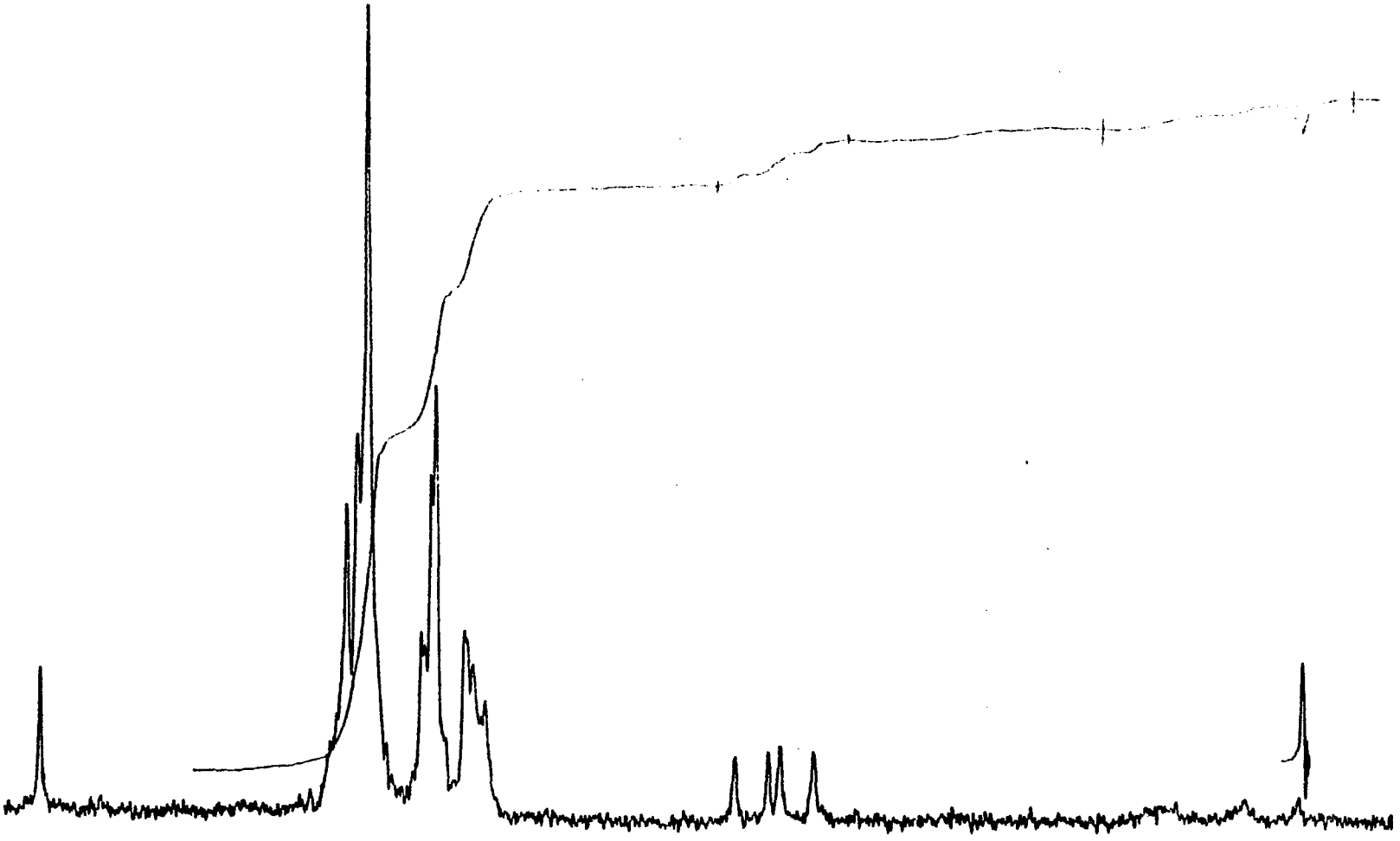

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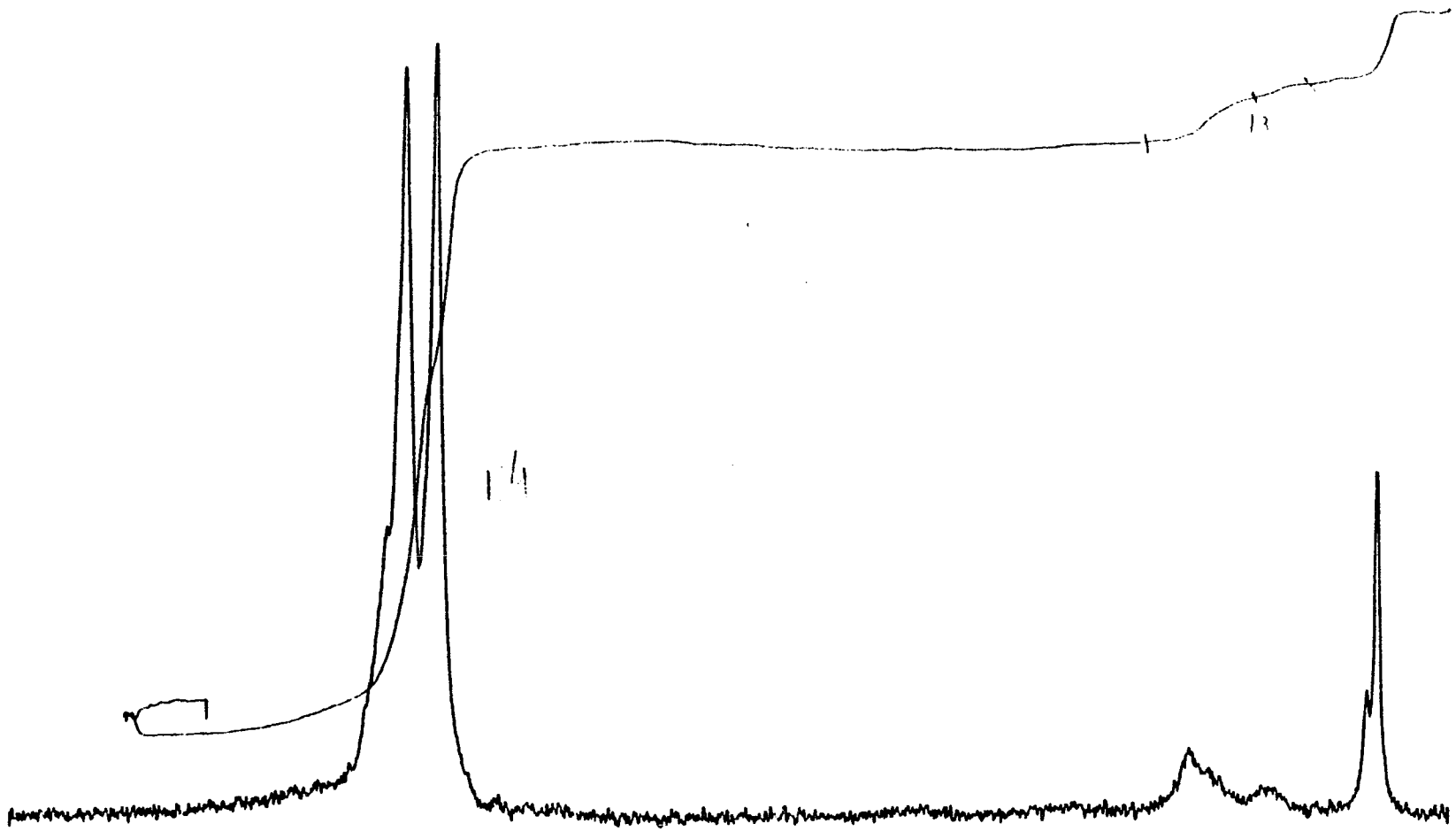

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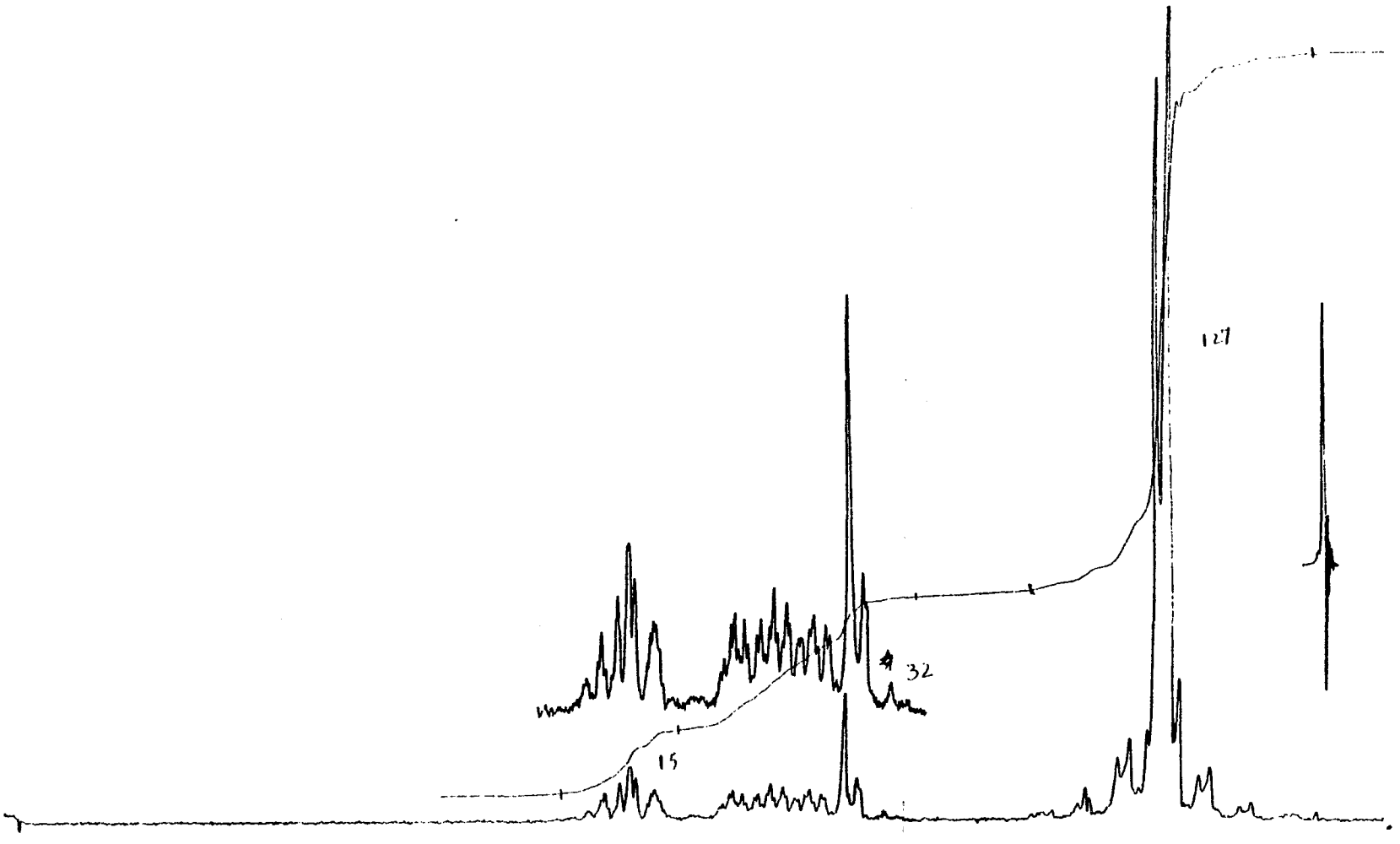

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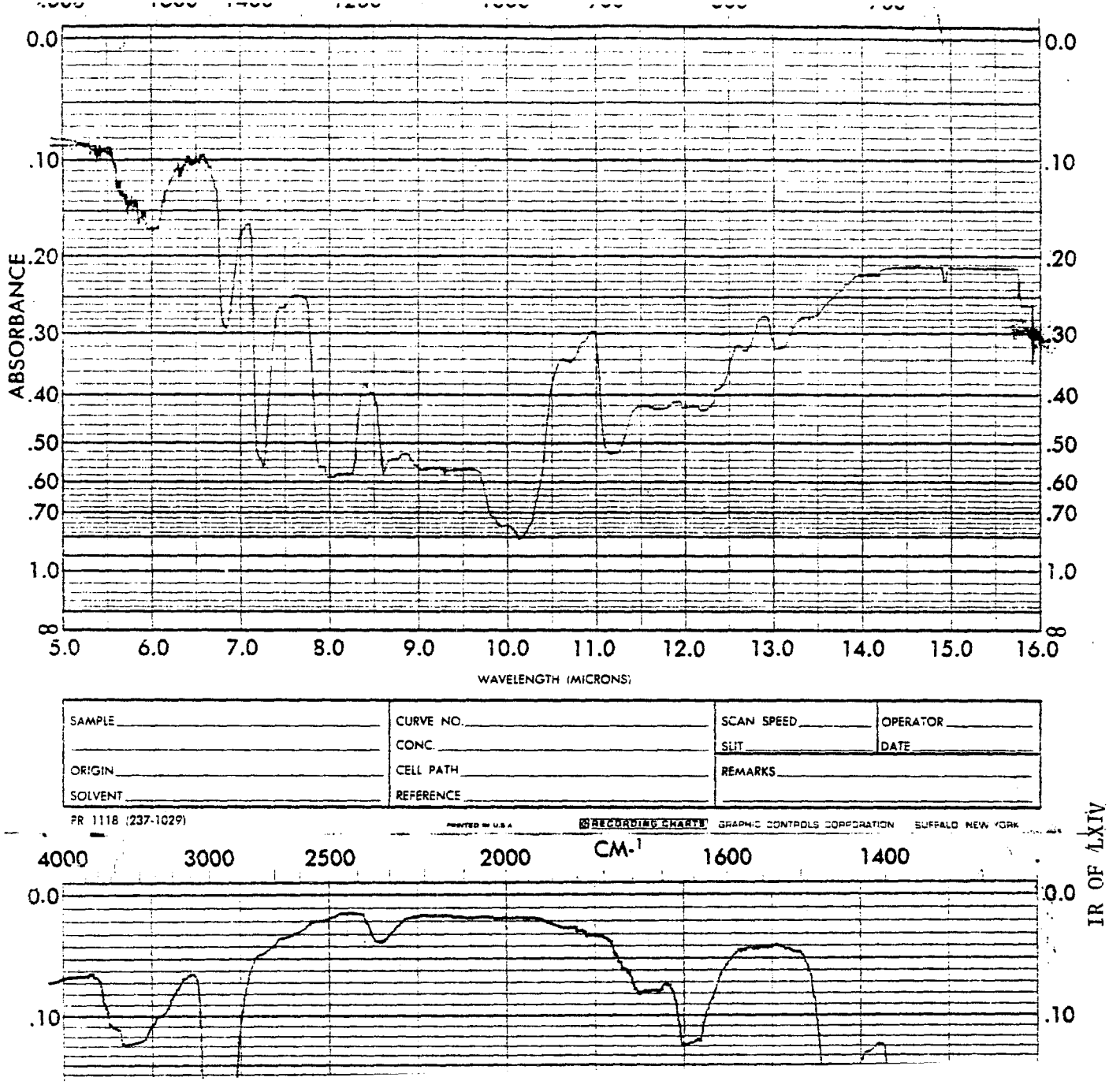
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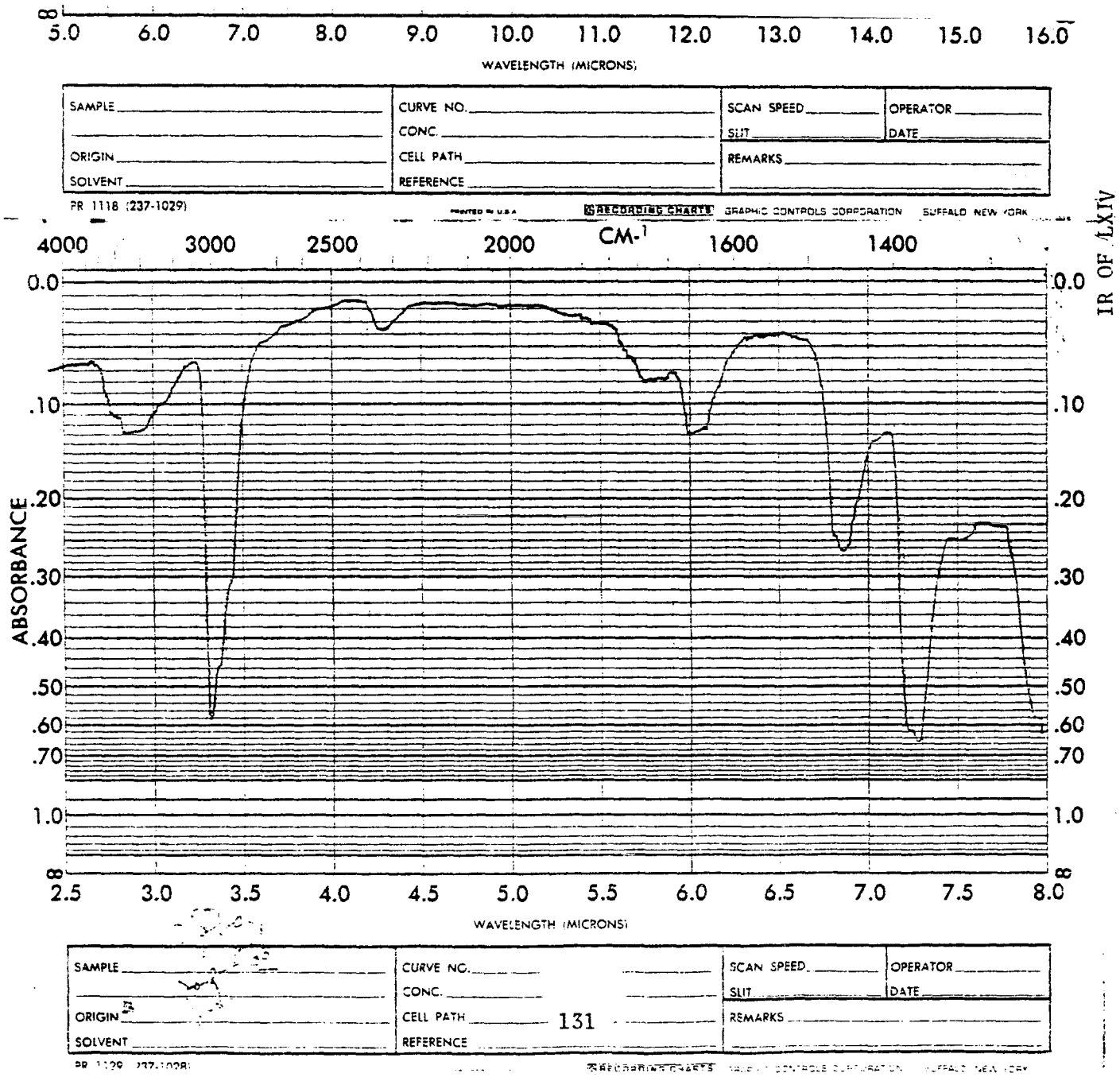
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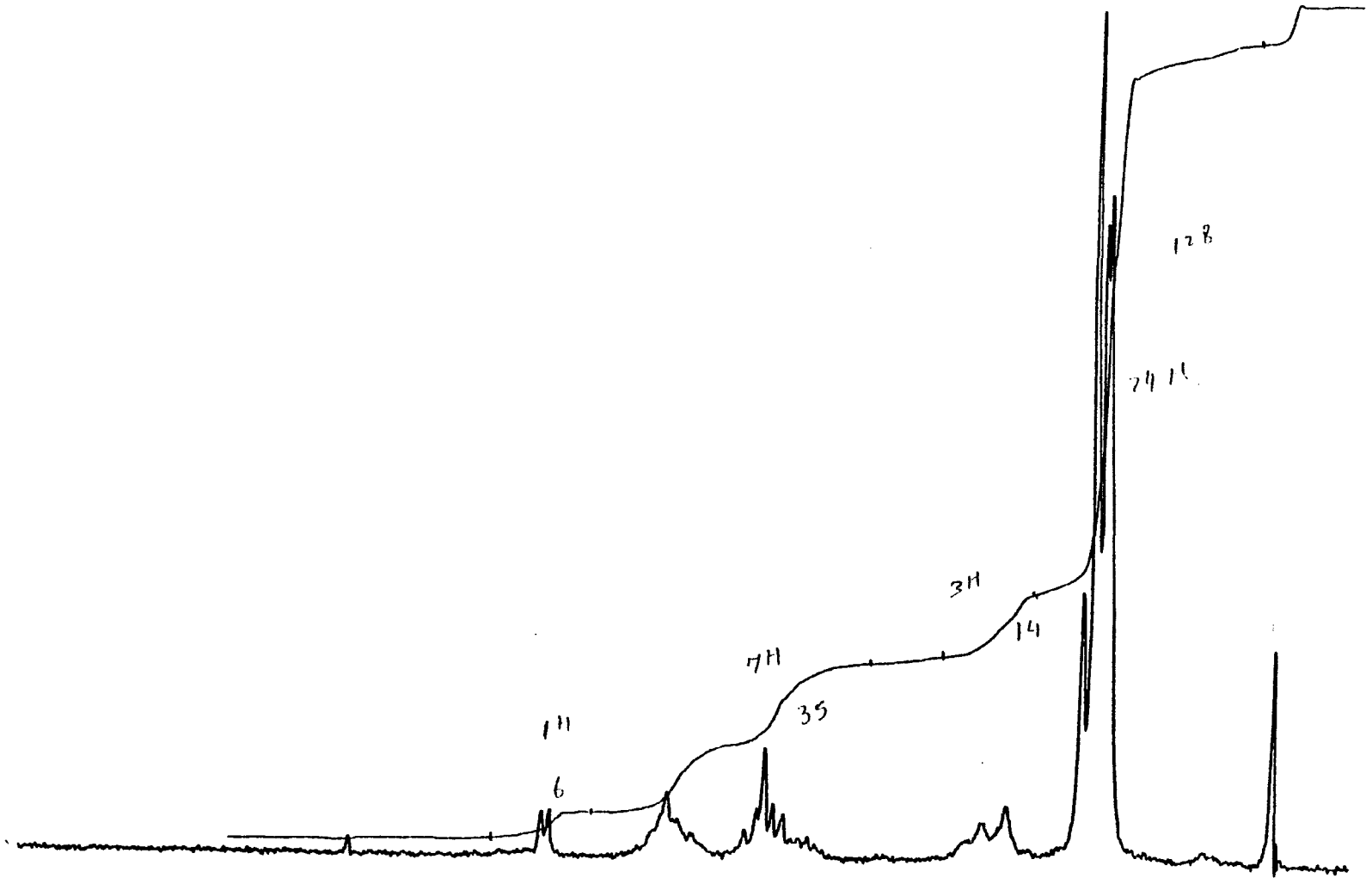

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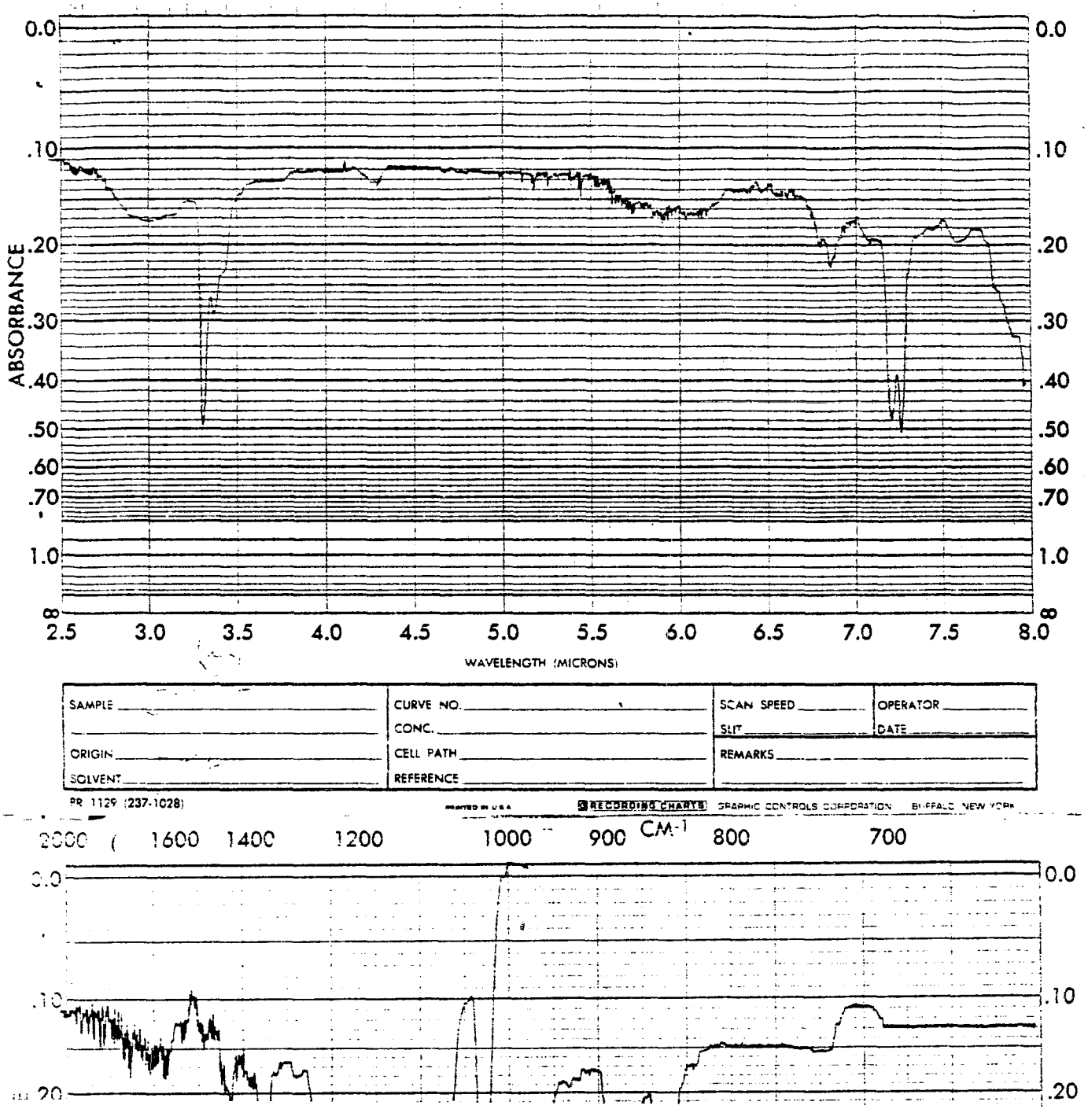
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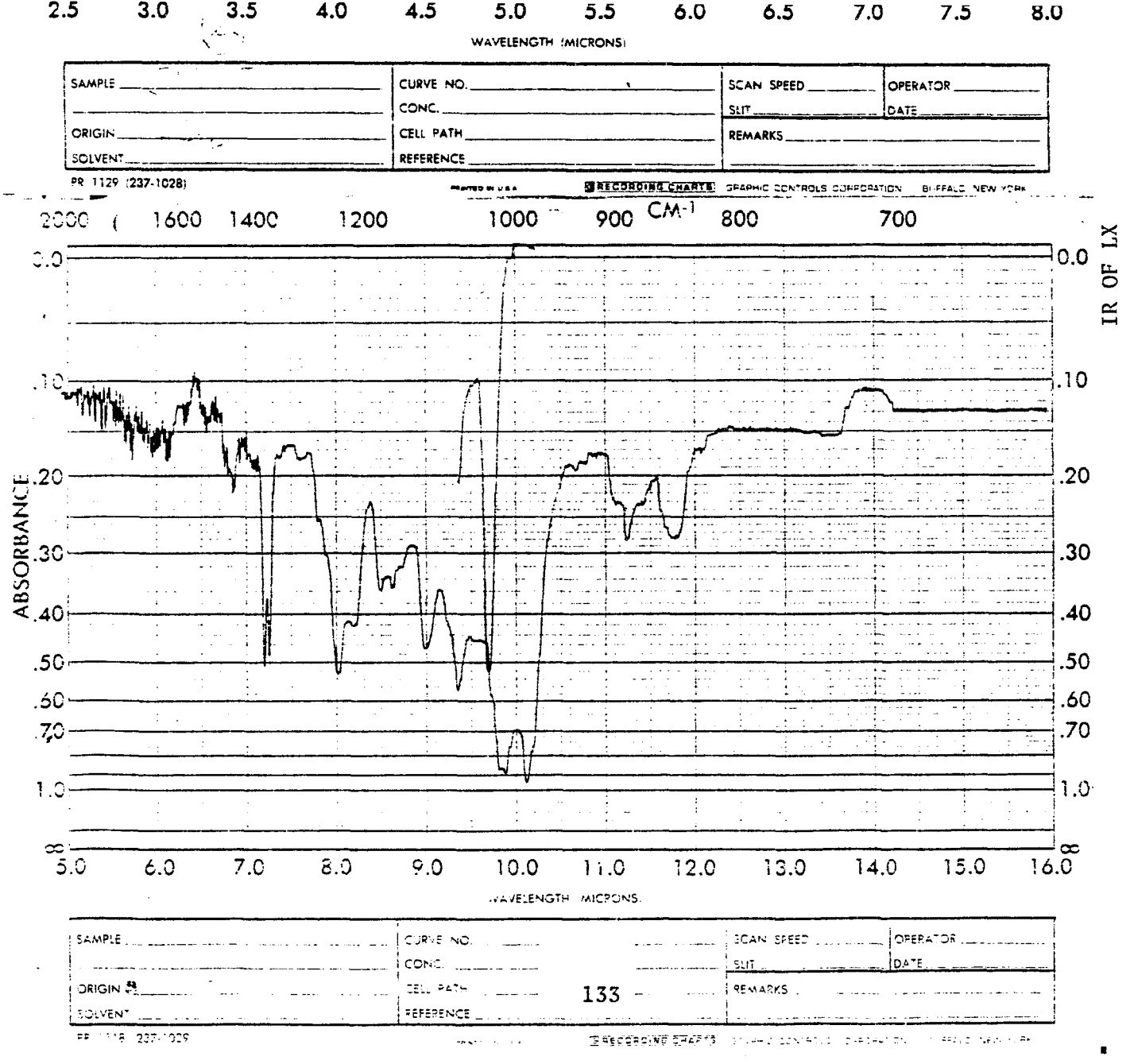
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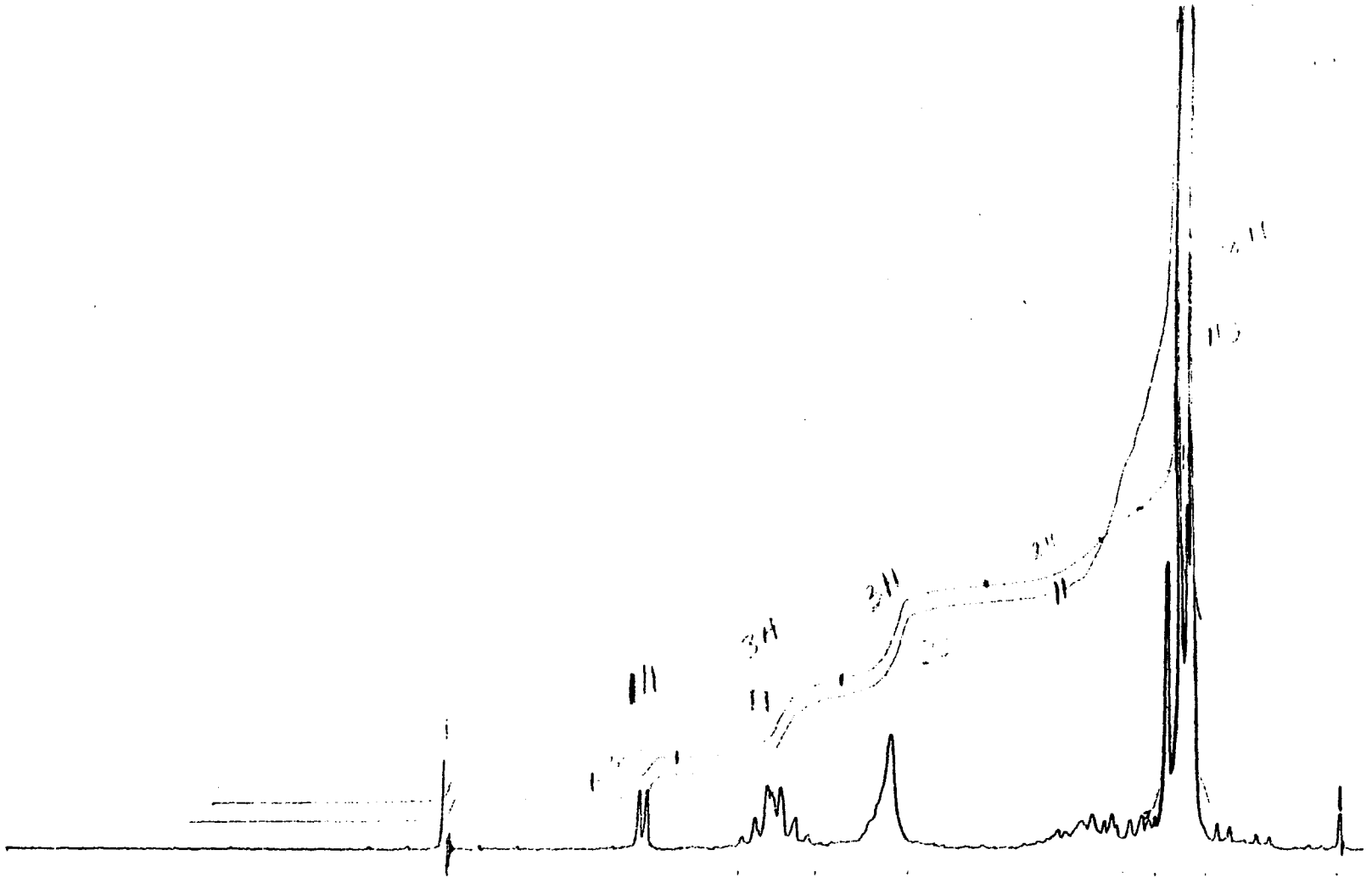

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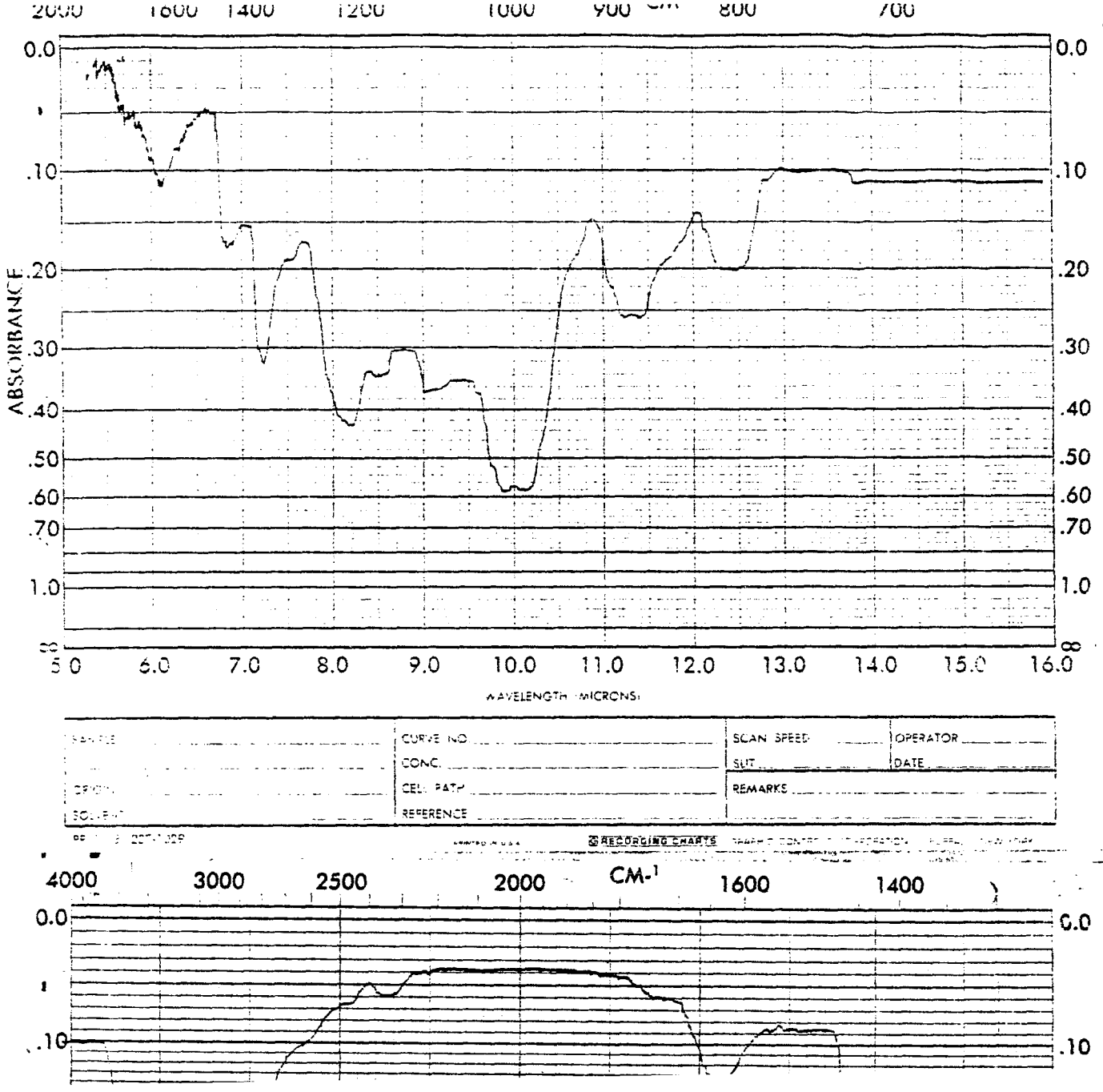
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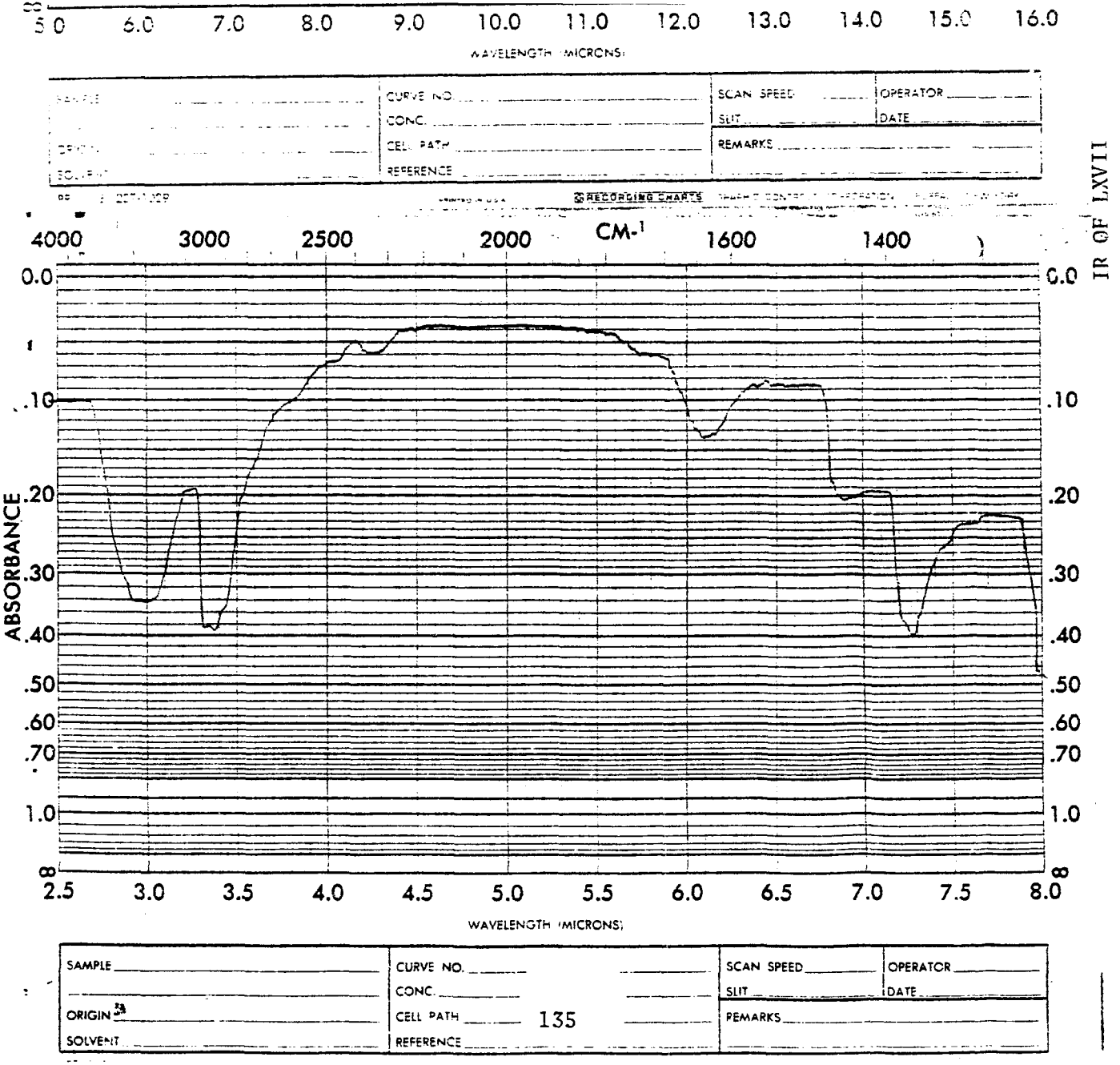
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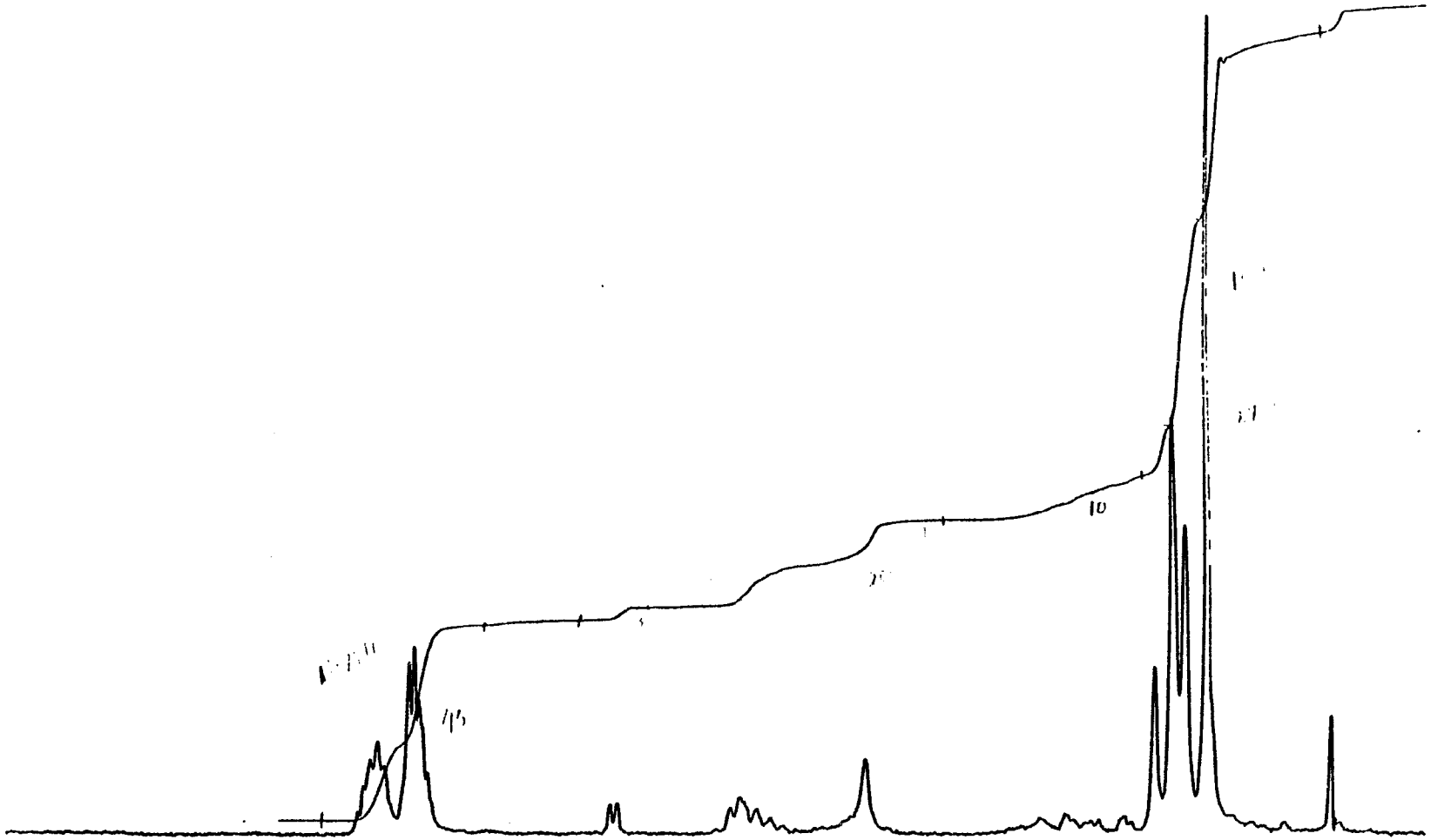

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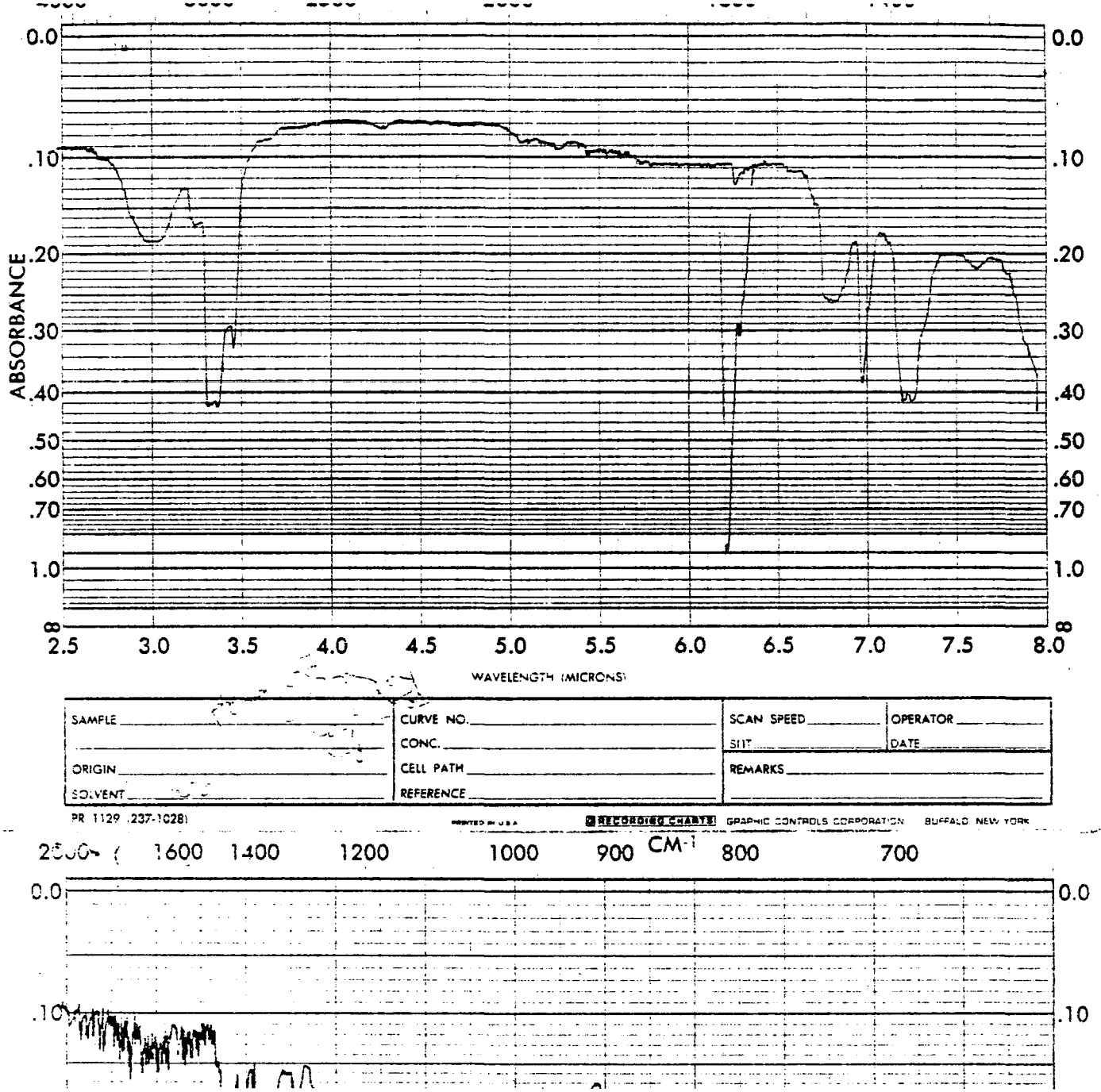
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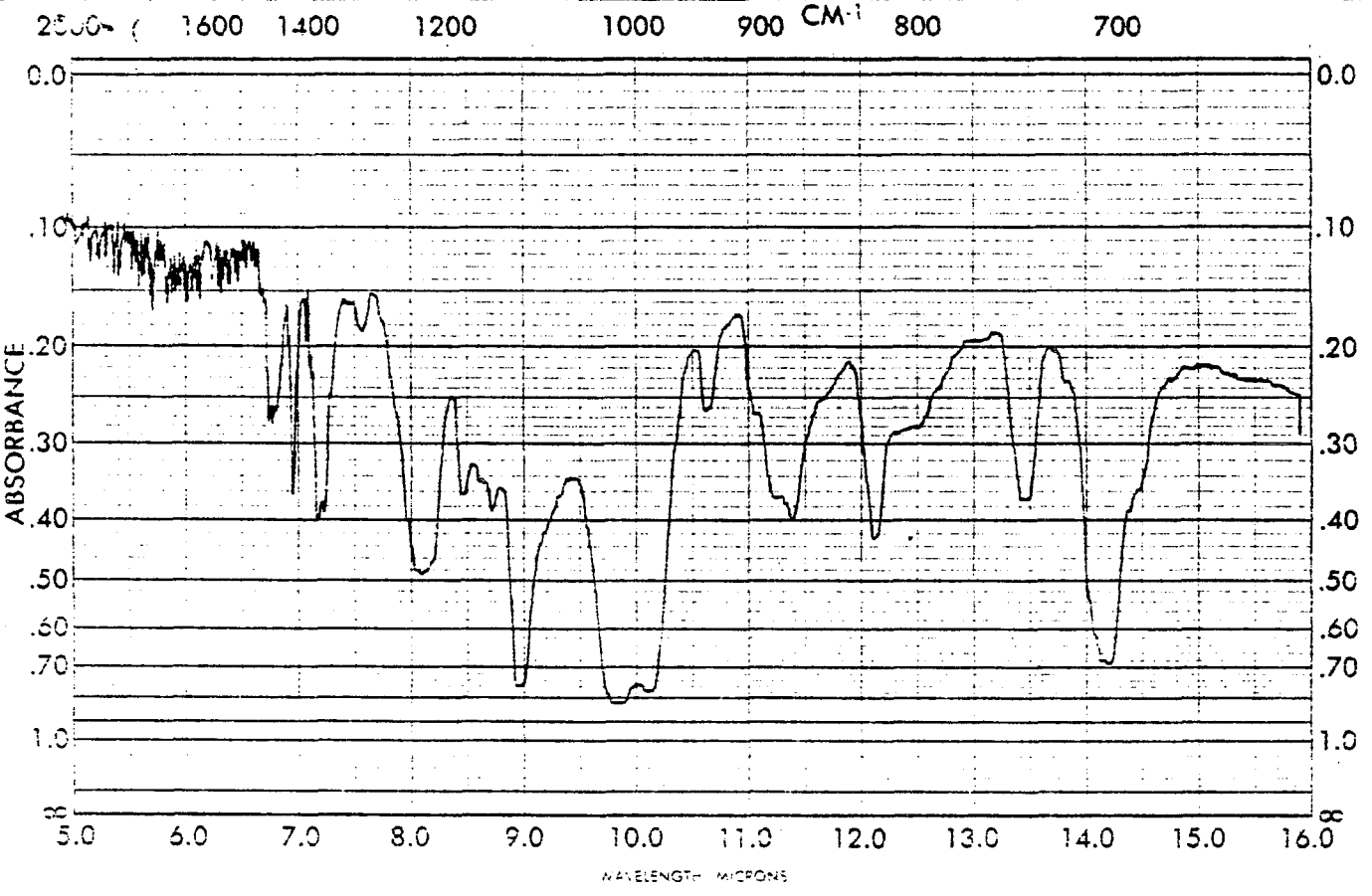

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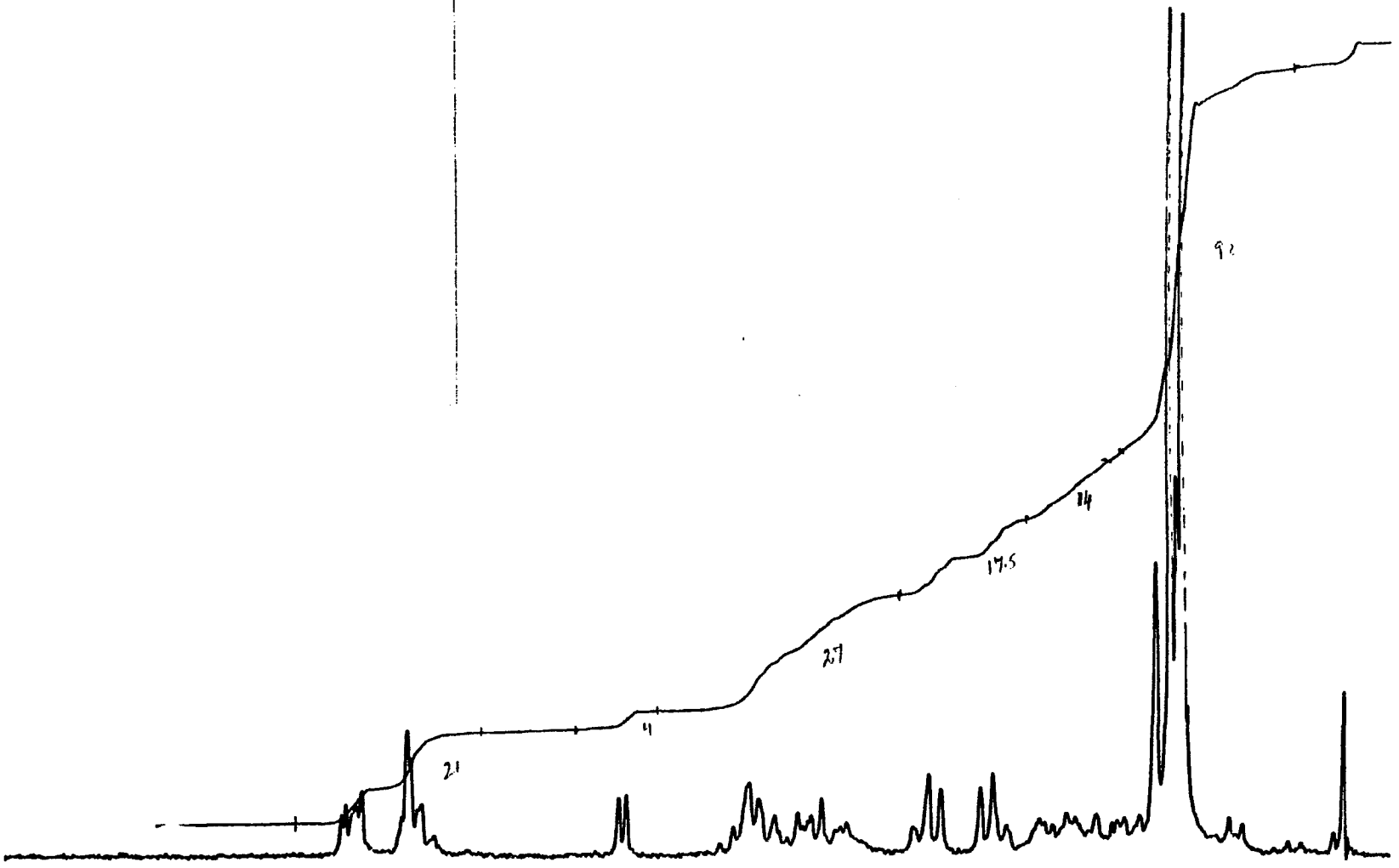

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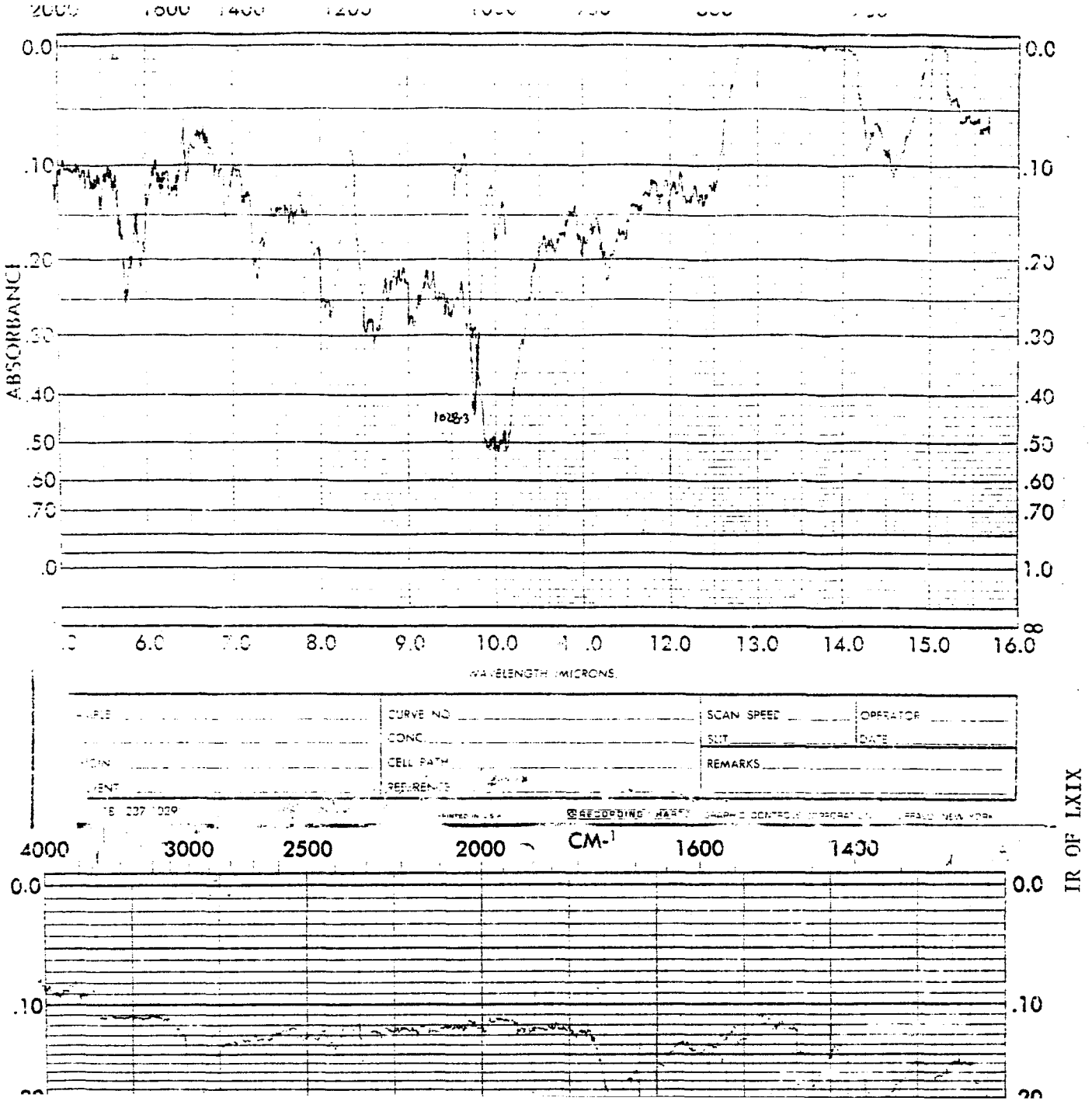
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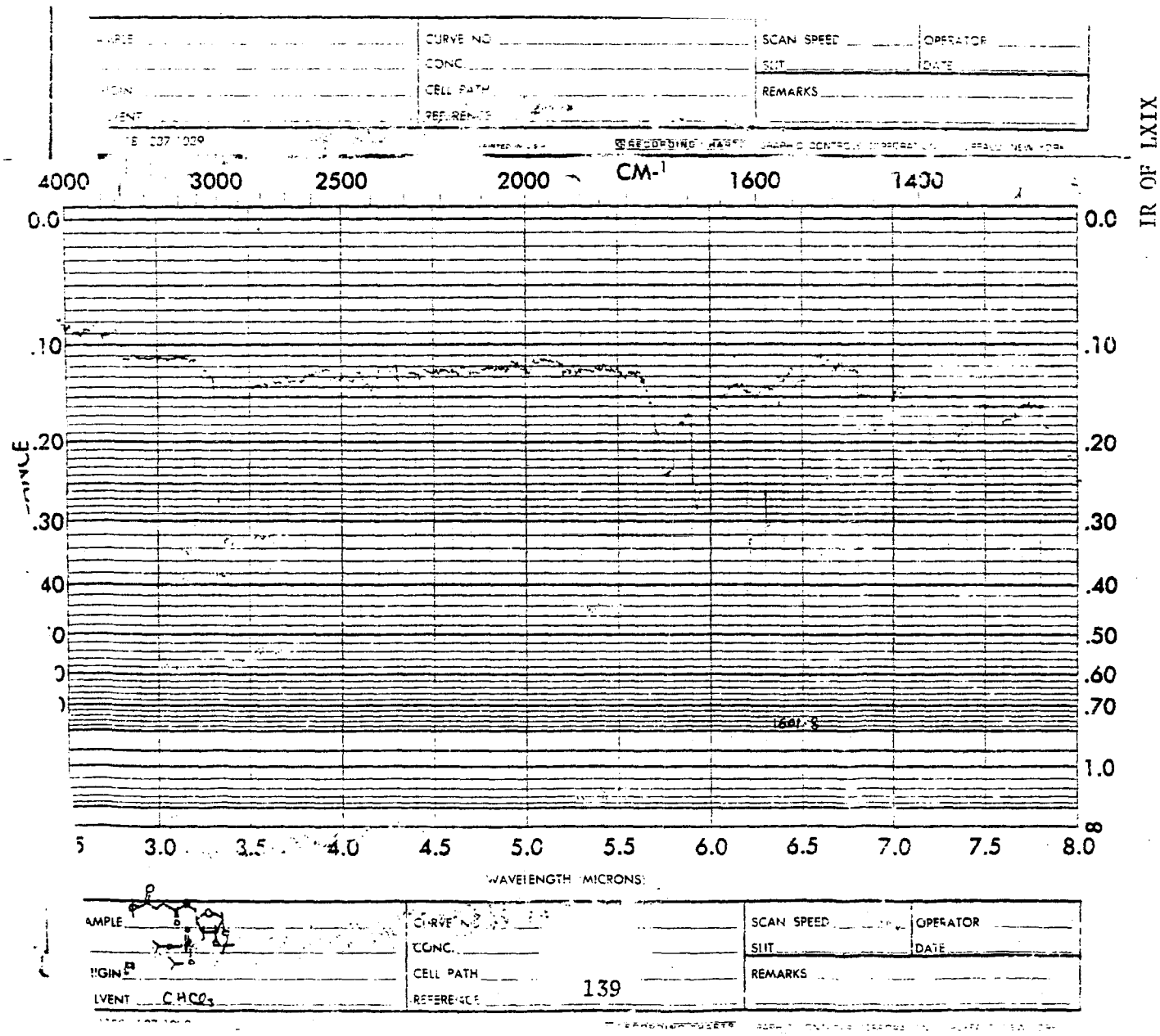
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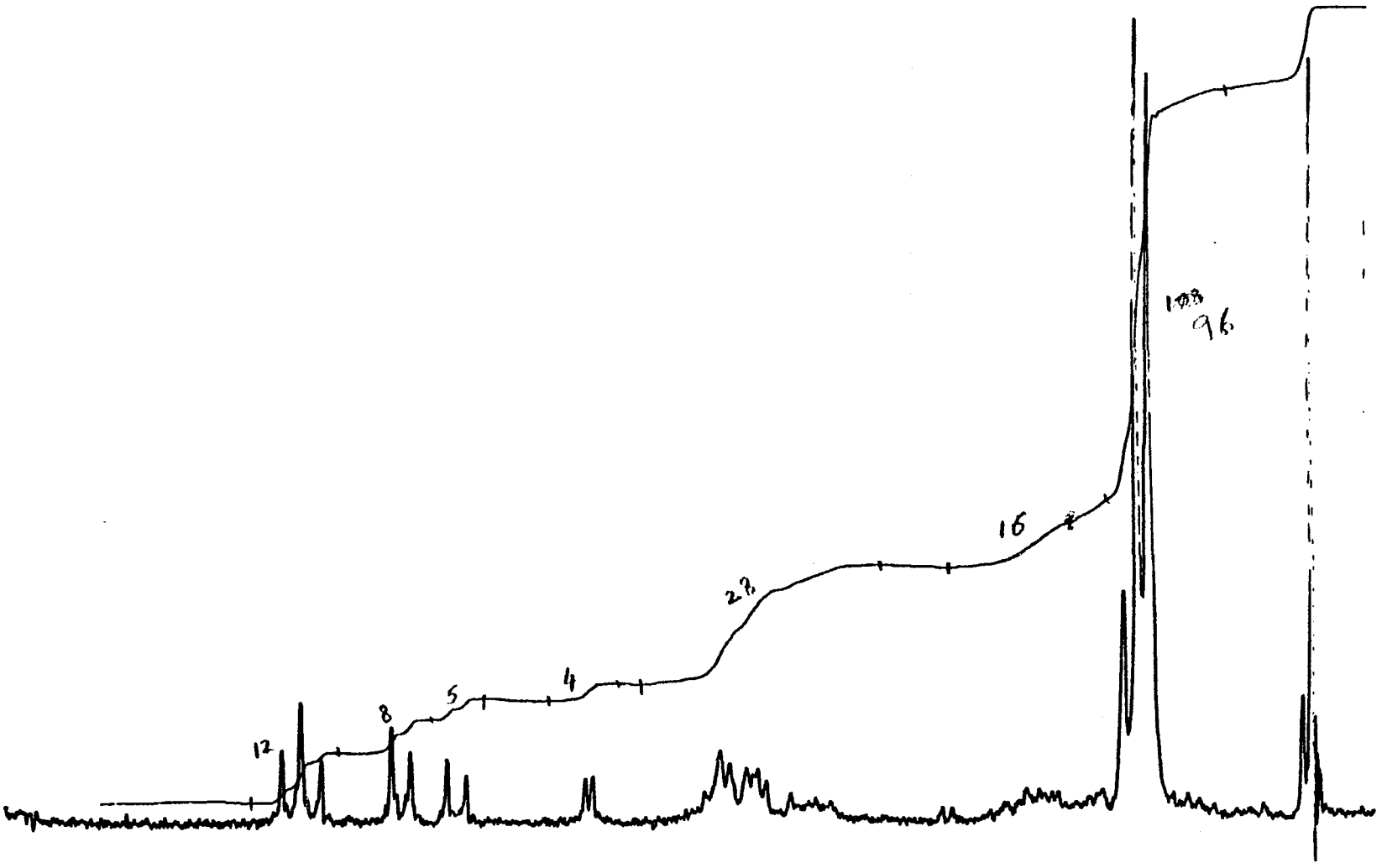

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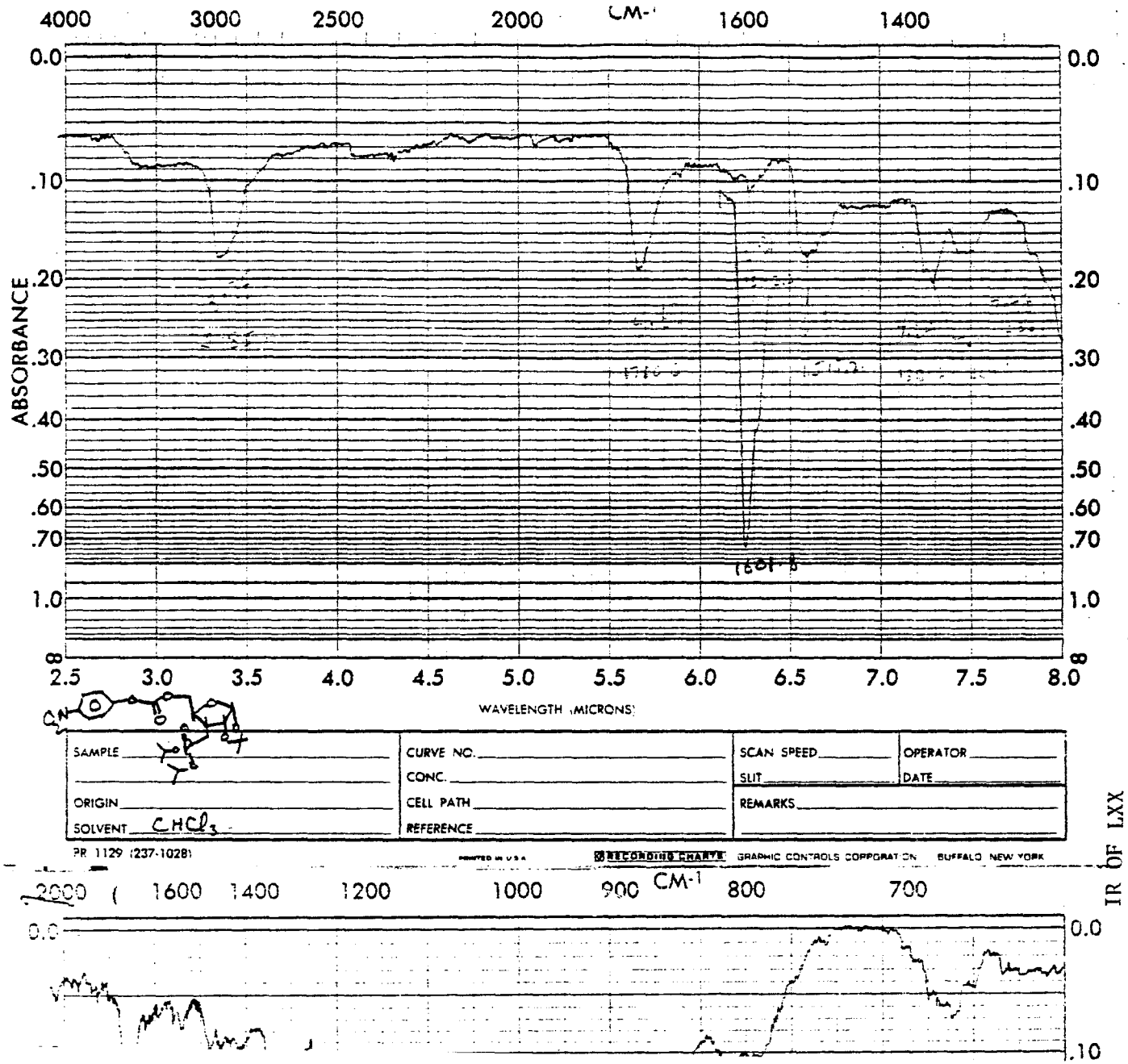
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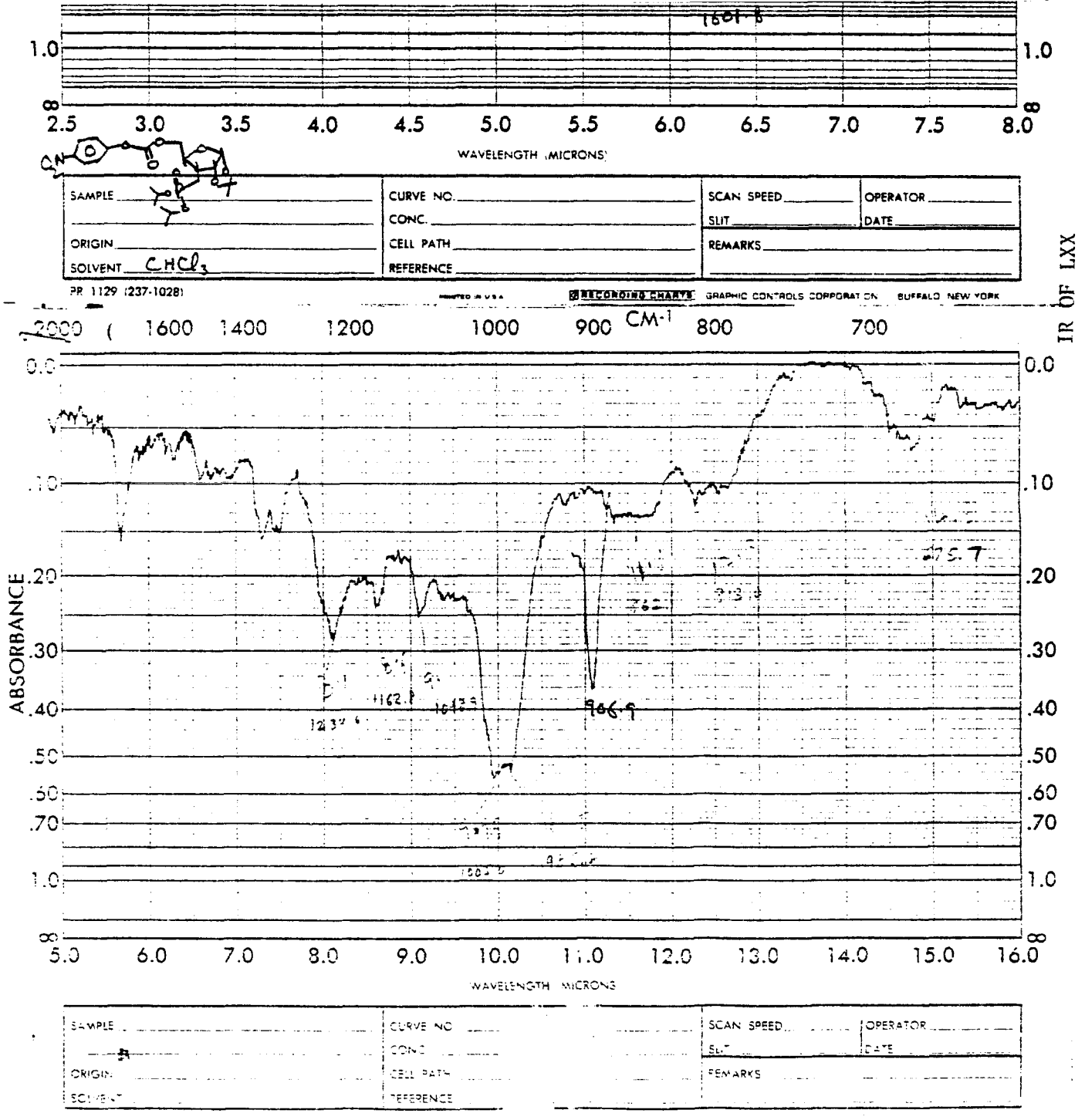
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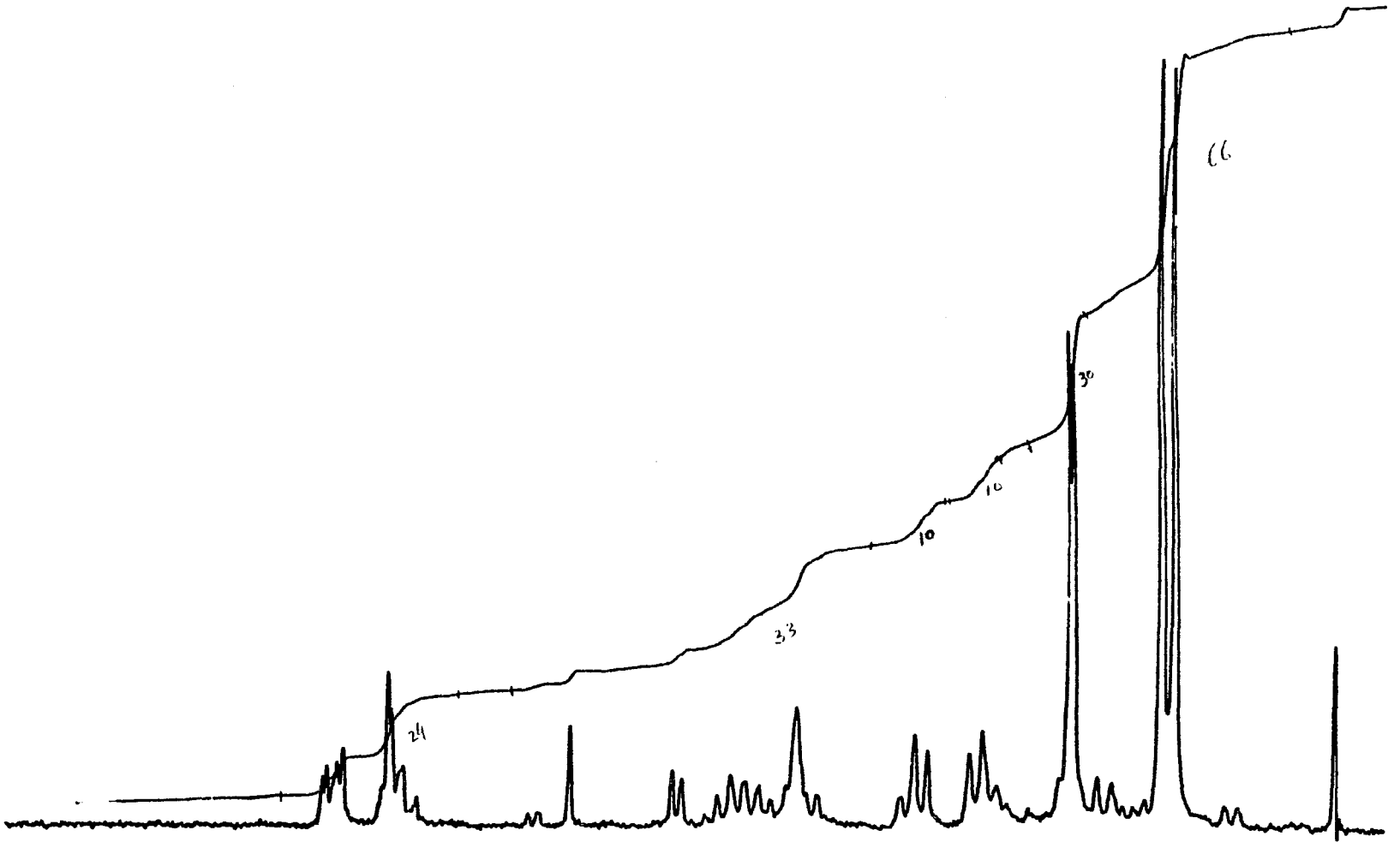

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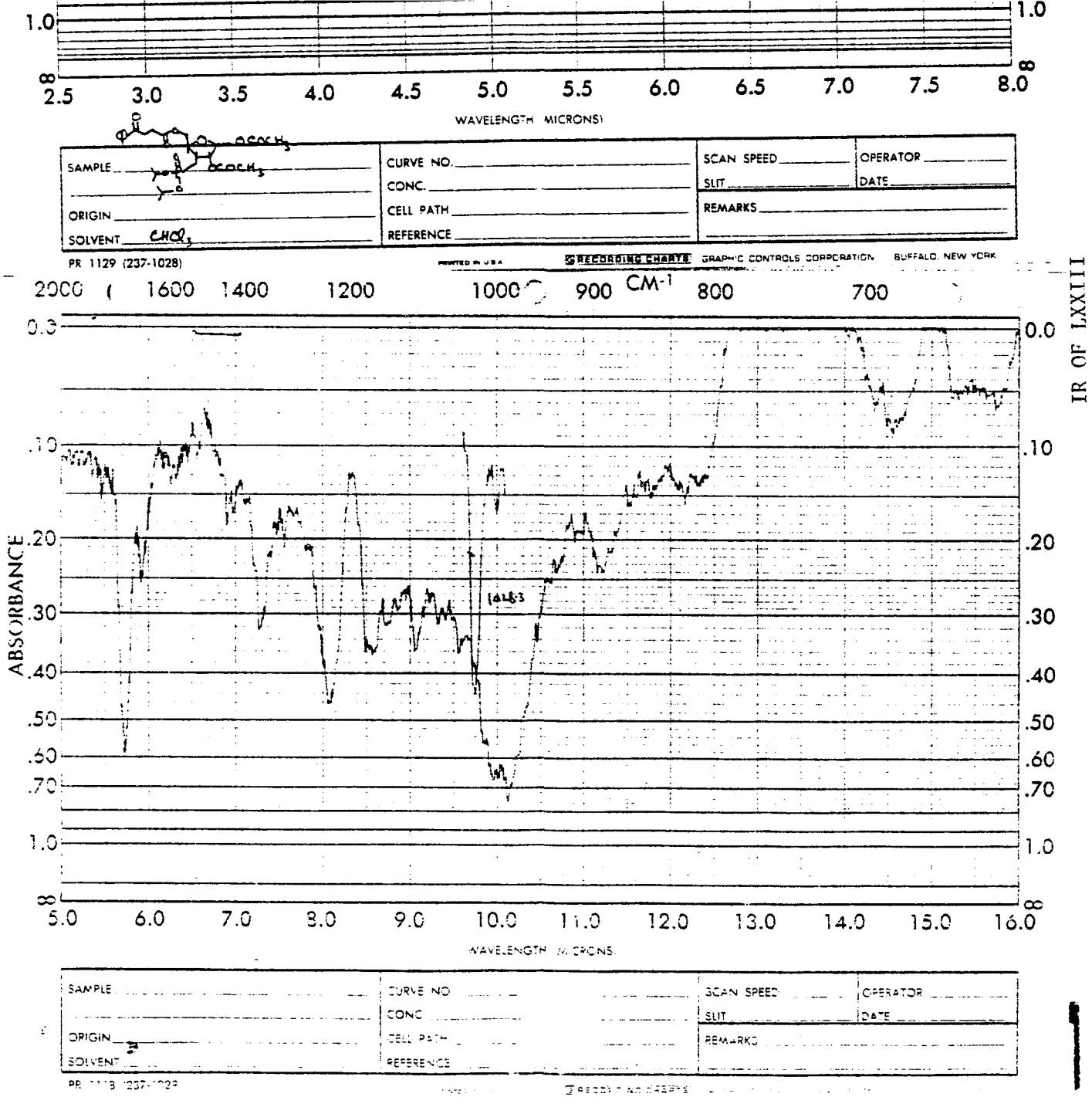
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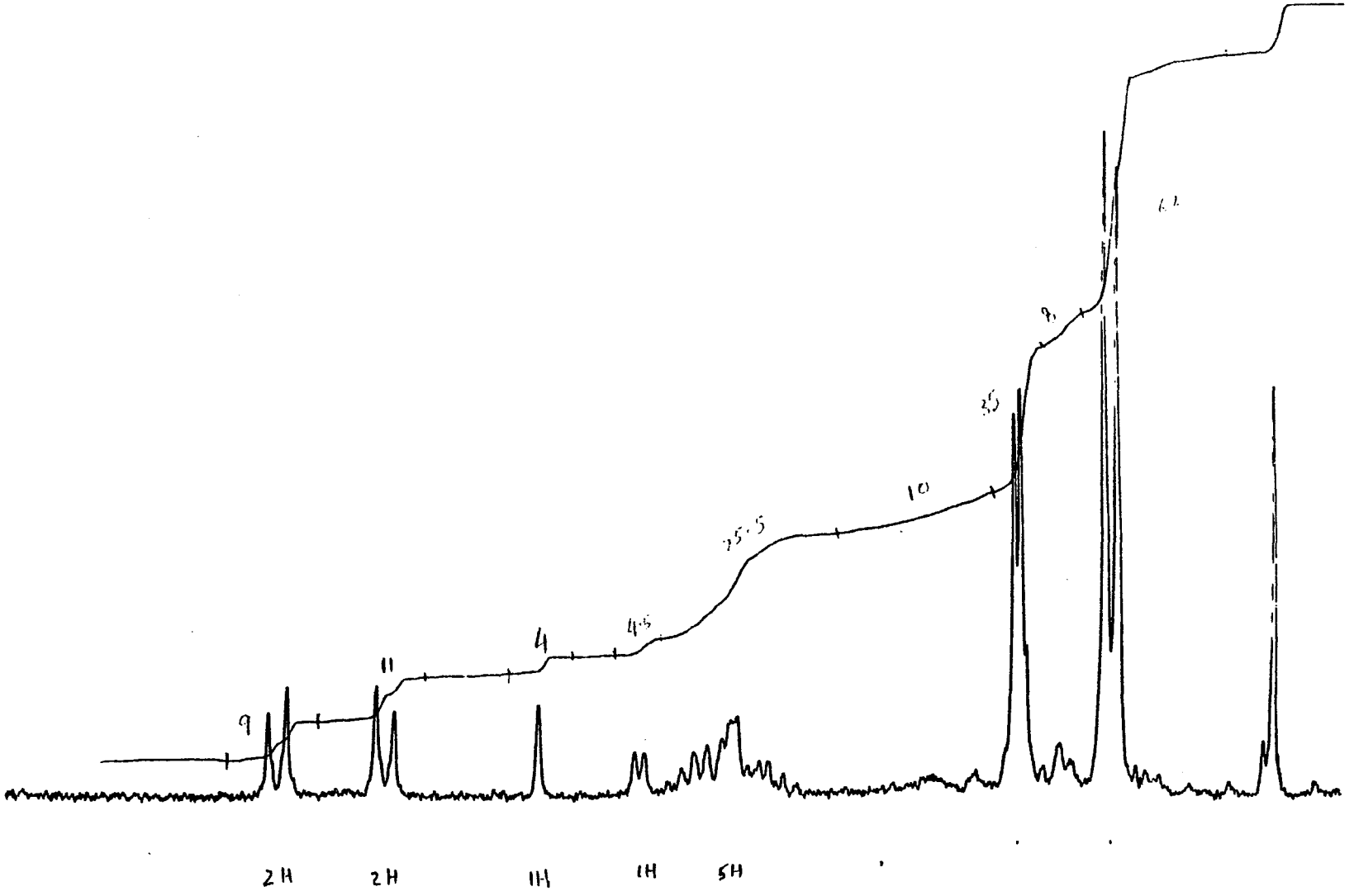
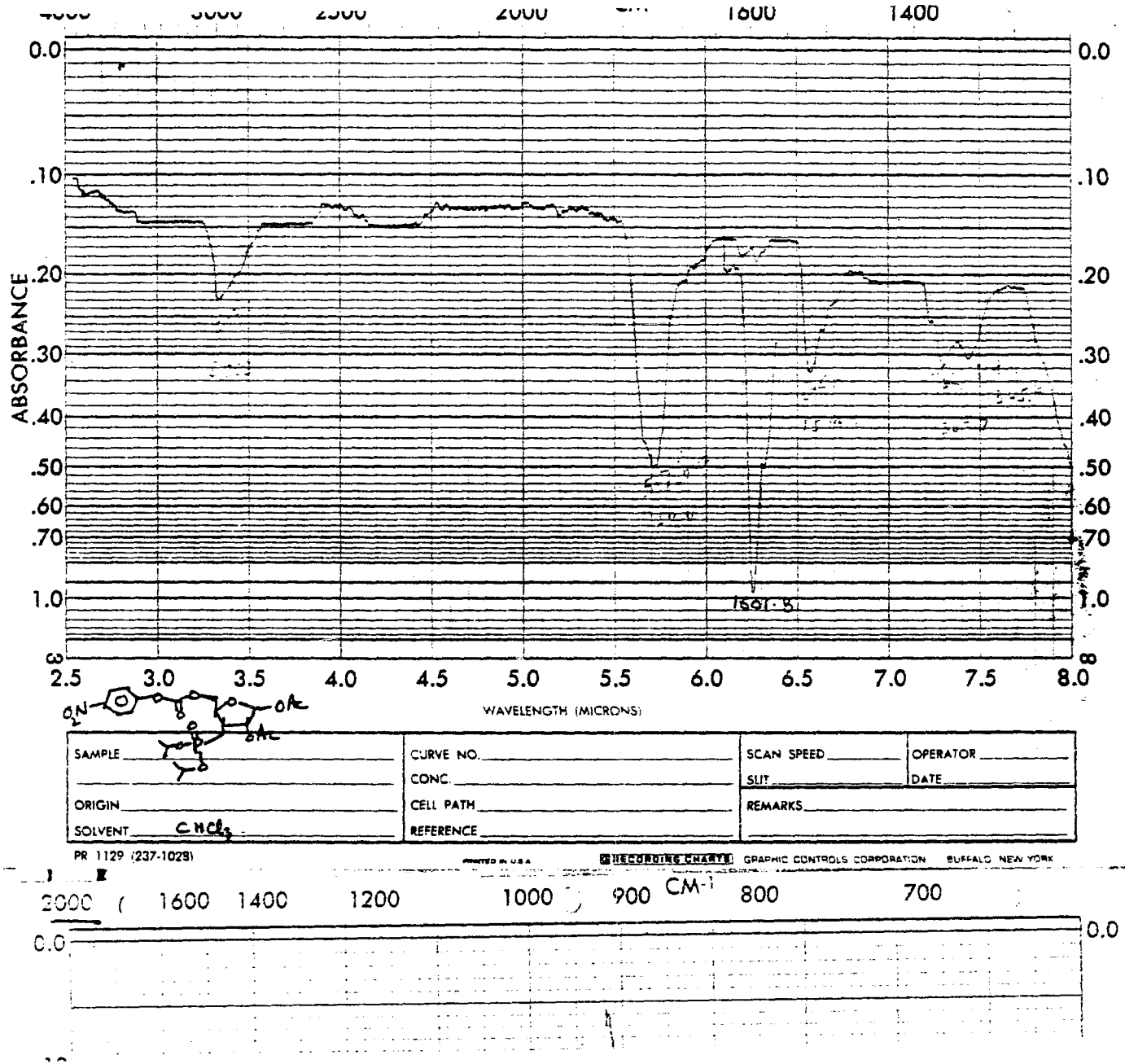
10.0
11.0
12.0
13.0
14.0
15.0
16.0
WAVELENGTH MICRONS
SAMPLE
ORIGIN
SOLVENT
CURVE NO.
CONC.
CELL PATH
REFERENCE
SCAN SPEED
SLIT
OPERATOR
DATE
REMARKS
PR 1129 (237-1028)
RECORDING CHARTS
GRAPHIC CONTROLS CORPORATION
BUFFALO, NEW YORK

IR OF LXXIII



NMR OF LXXIV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.40
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.70
1.0
2.5
3.0
3.5
4.0
4.5
5.0
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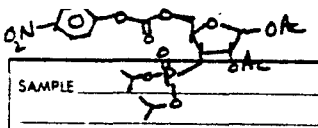
6.0
6.5
7.0
7.5
8.0
WAVELENGTH (MICRONS)
1601.5
SAMPLE
CURVE NO.
SCAN SPEED
OPERATOR
CONC.
SLIT
DATE
ORIGIN
CELL PATH
REMARKS
SOLVENT CHCl3
REFERENCE
PR 1129 (237-1029)
RECORDING CHARTS
GRAPHIC CONTROLS CORPORATION
BUFFALO NEW YORK
3000
1600
1400
1200
1000
900
CM-1
800
700

IR OF LXXIV

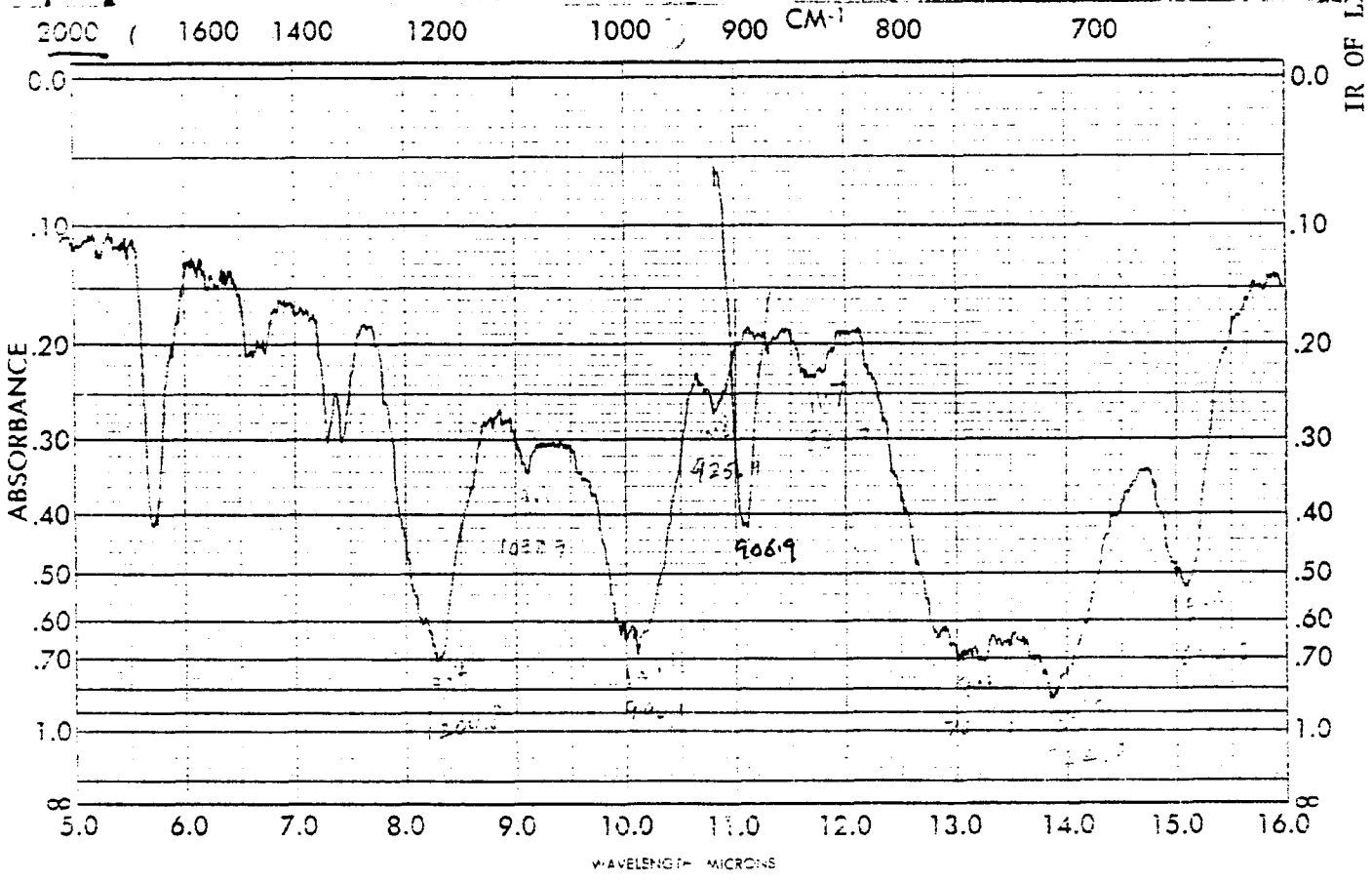


WAVELENGTH (MICRONS)

SAMPLE	CURVE NO.	SCAN SPEED	OPERATOR
	CONC.	SLIT	DATE
ORIGIN	CELL PATH	REMARKS	
SOLVENT $CHCl_3$	REFERENCE		

PR 1129 (237-1029) PRINTED IN U.S.A. RECORDING CHARTS GRAPHIC CONTROLS CORPORATION BUFFALO NEW YORK

IR OF LXXIV



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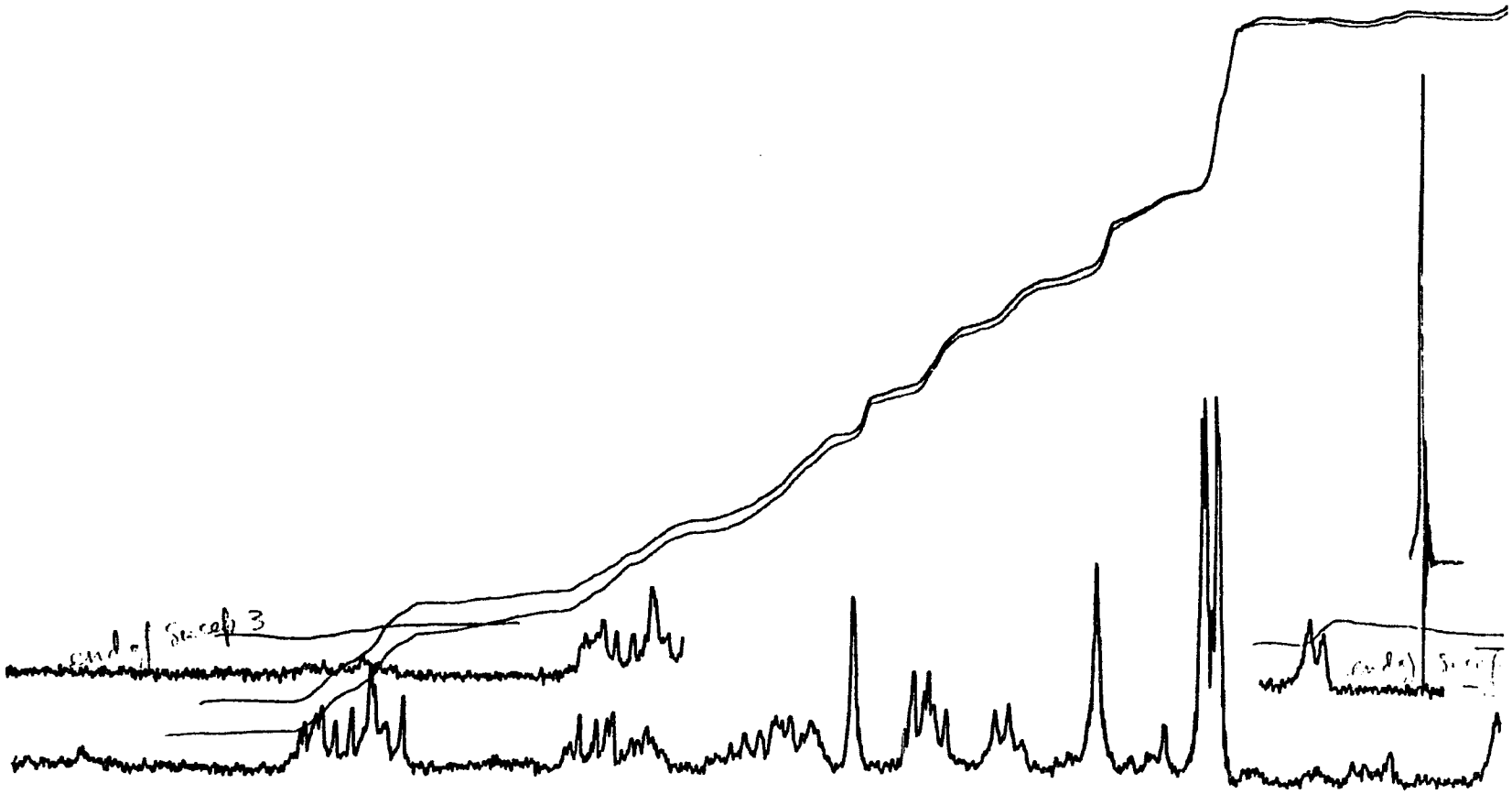
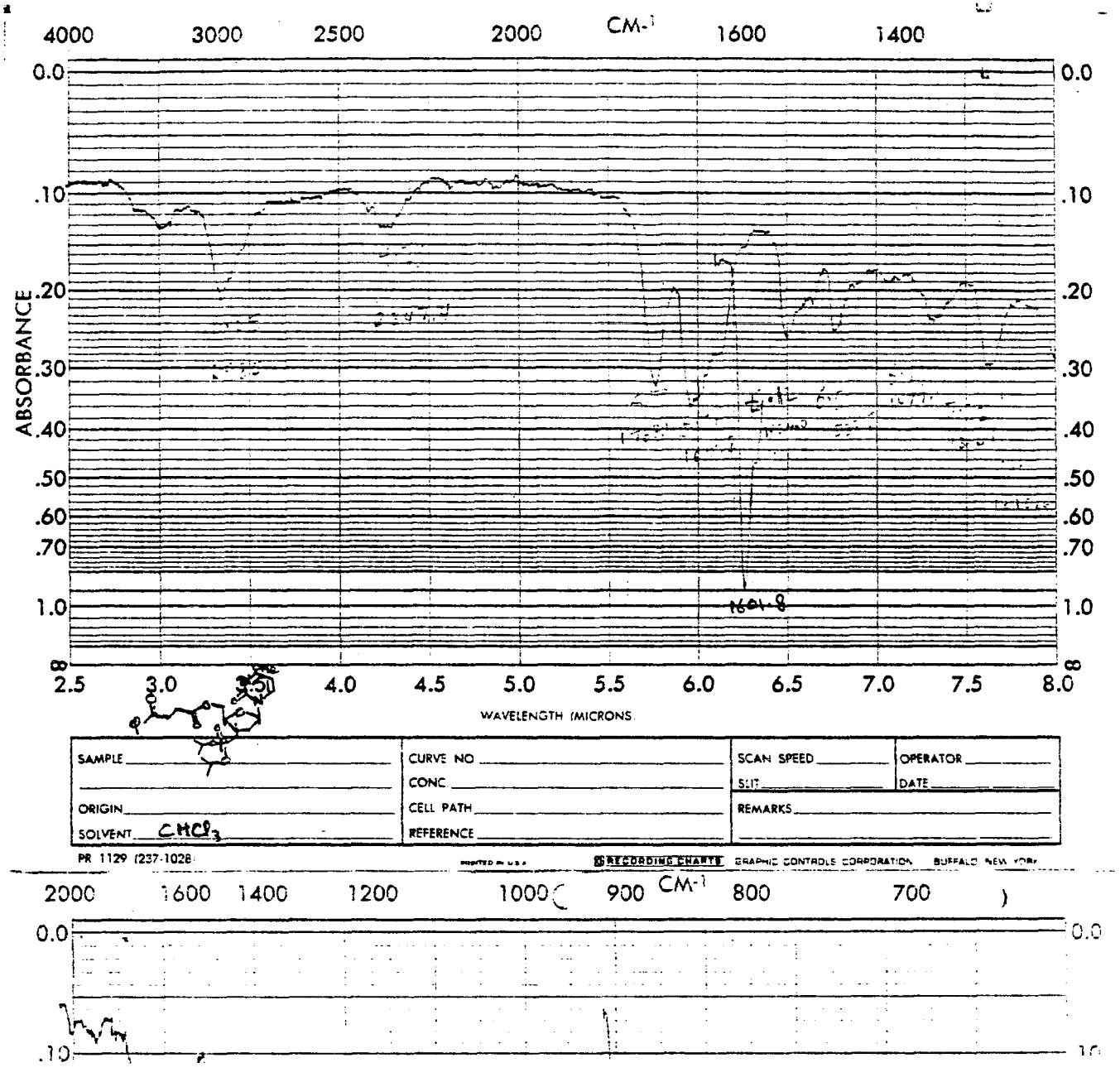
	CONC.	SLIT	DATE
ORIGIN	CELL PATH	REMARKS	
SOLVENT	REFERENCE		



NMR OF LXXVII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ABSORBANCE
1601.8
2.5
3.0
4.0
4.5
5.0
5.5
6.0
6.5
7.0
7.5
8.0
WAVELENGTH (MICRONS)
SAMPLE
ORIGIN
SOLVENT CHCl3
CURVE NO
CONC
CELL PATH
REFERENCE
SCAN SPEED
SLIT
OPERATOR
DATE
REMARKS
PR 1129 (237-1028)
RECORDING CHARTS GRAPHIC CONTROLS CORPORATION BUFFALO NEW YORK
2000
1600
1400
1200
1000
900
CM-1
800
700
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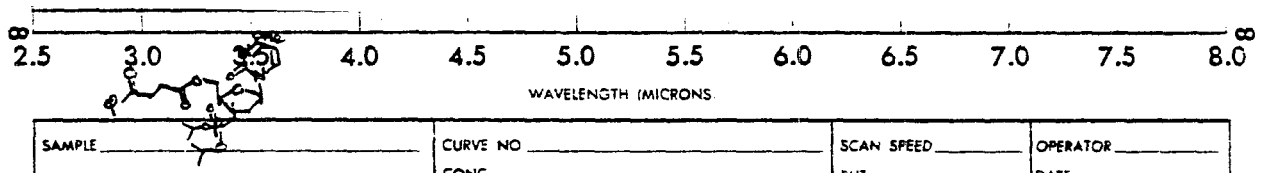
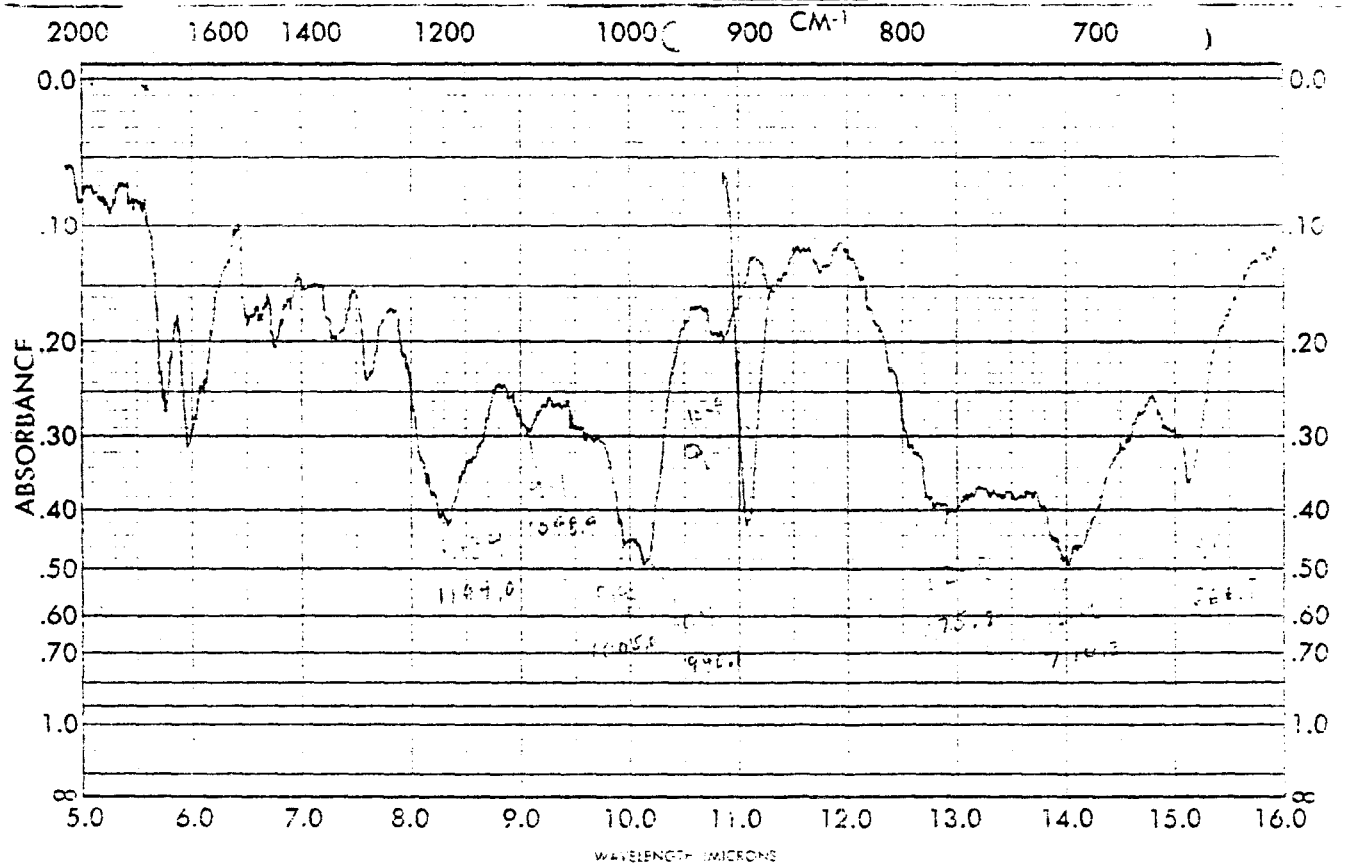
IR OF LXXVII



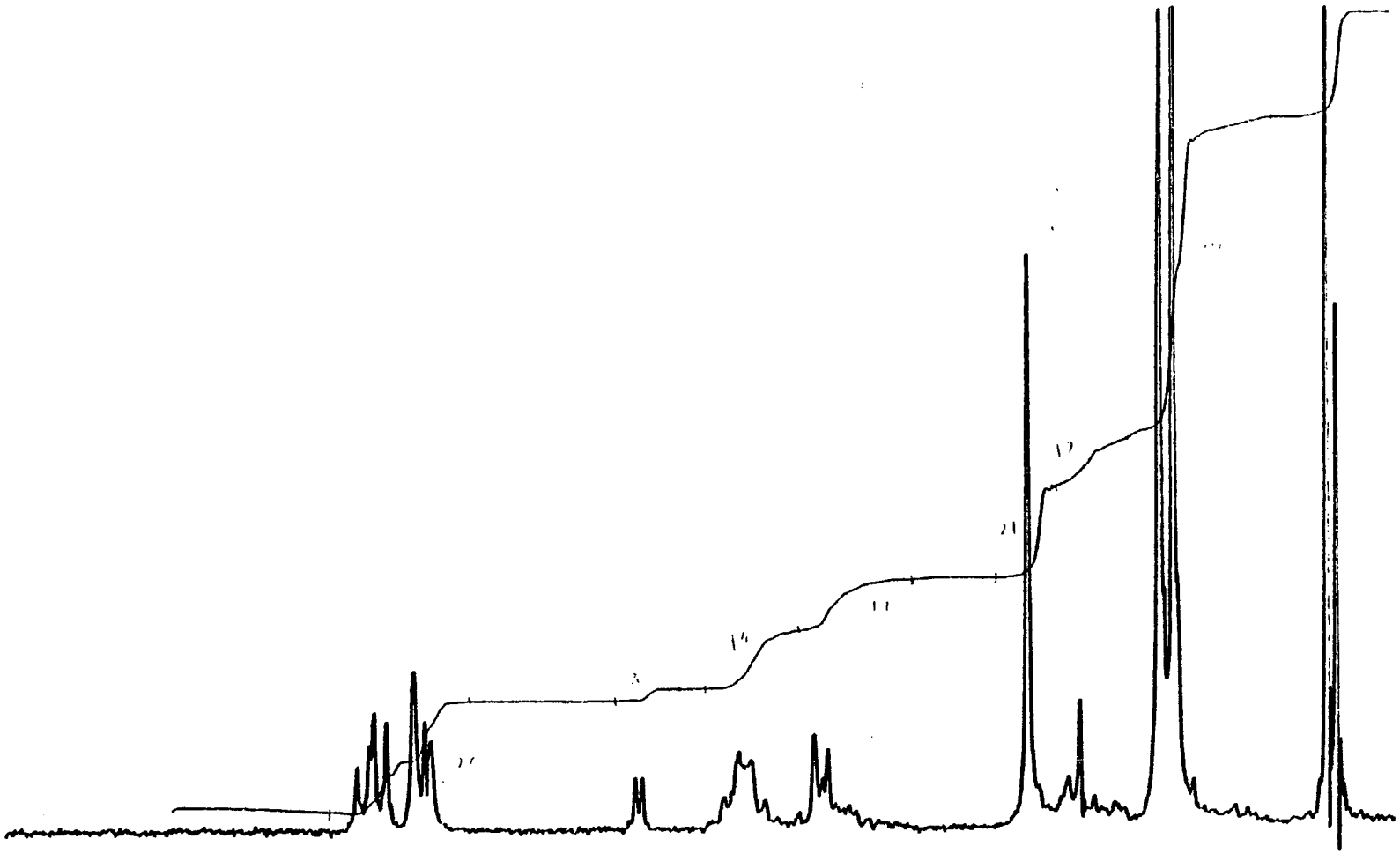
SAMPLE	CURVE NO	SCAN SPEED	OPERATOR
	CONC	SLIT	DATE
ORIGIN	CELL PATH	REMARKS	
SOLVENT $CHCl_3$	REFERENCE		

PR 1129 (237-1028) PRINTED IN U.S.A. RECORDING CHARTS GRAPHIC CONTROLS CORPORATION BUFFALO NEW YORK

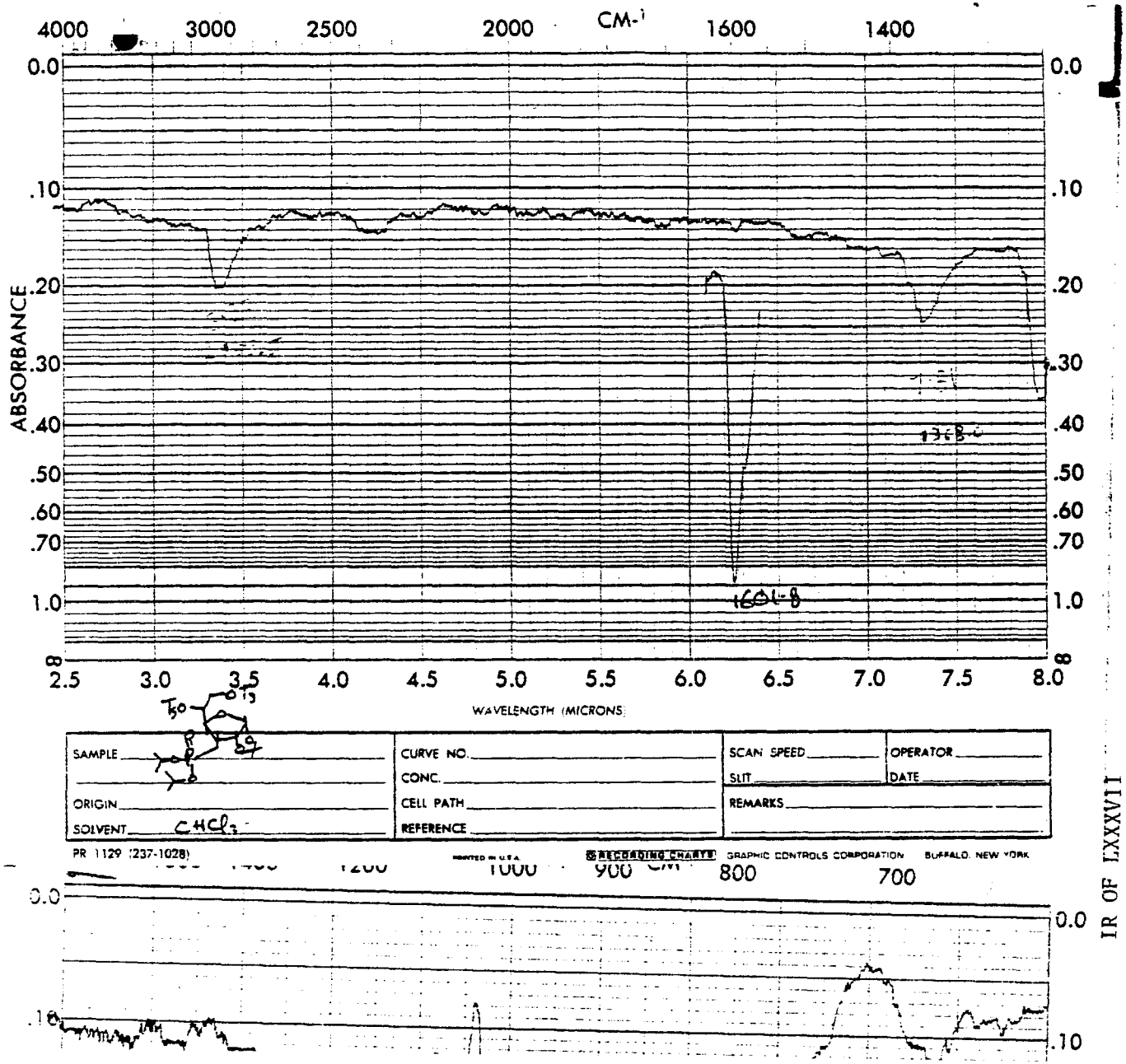


SAMPLE	CURVE NO	SCAN SPEED	OPERATOR
	CONC	SLIT	DATE
ORIGIN	CELL PATH	REMARKS	
SOLVENT	REFERENCE		

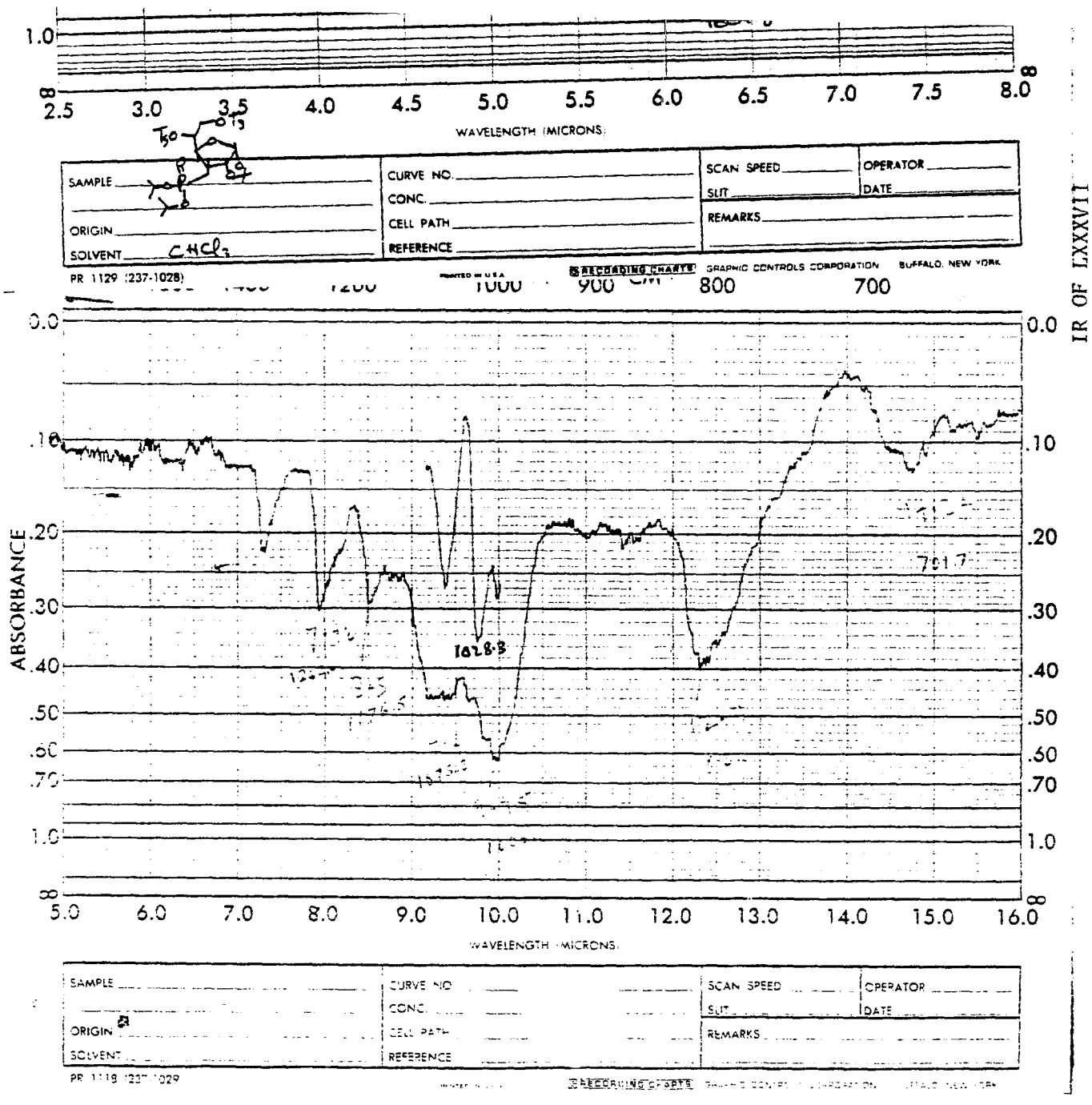
IR OF LXXVII



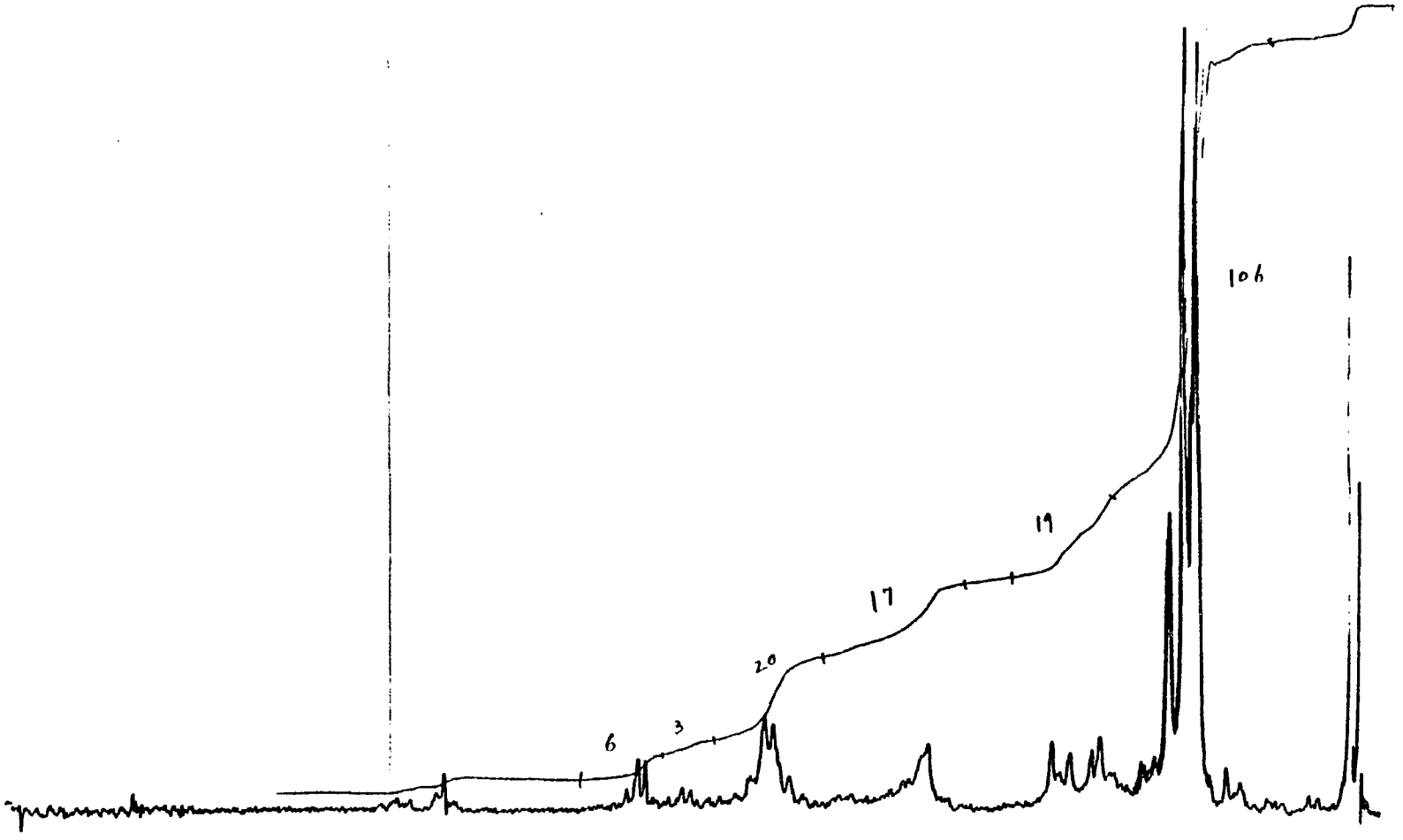
NMR OF LXXXVII



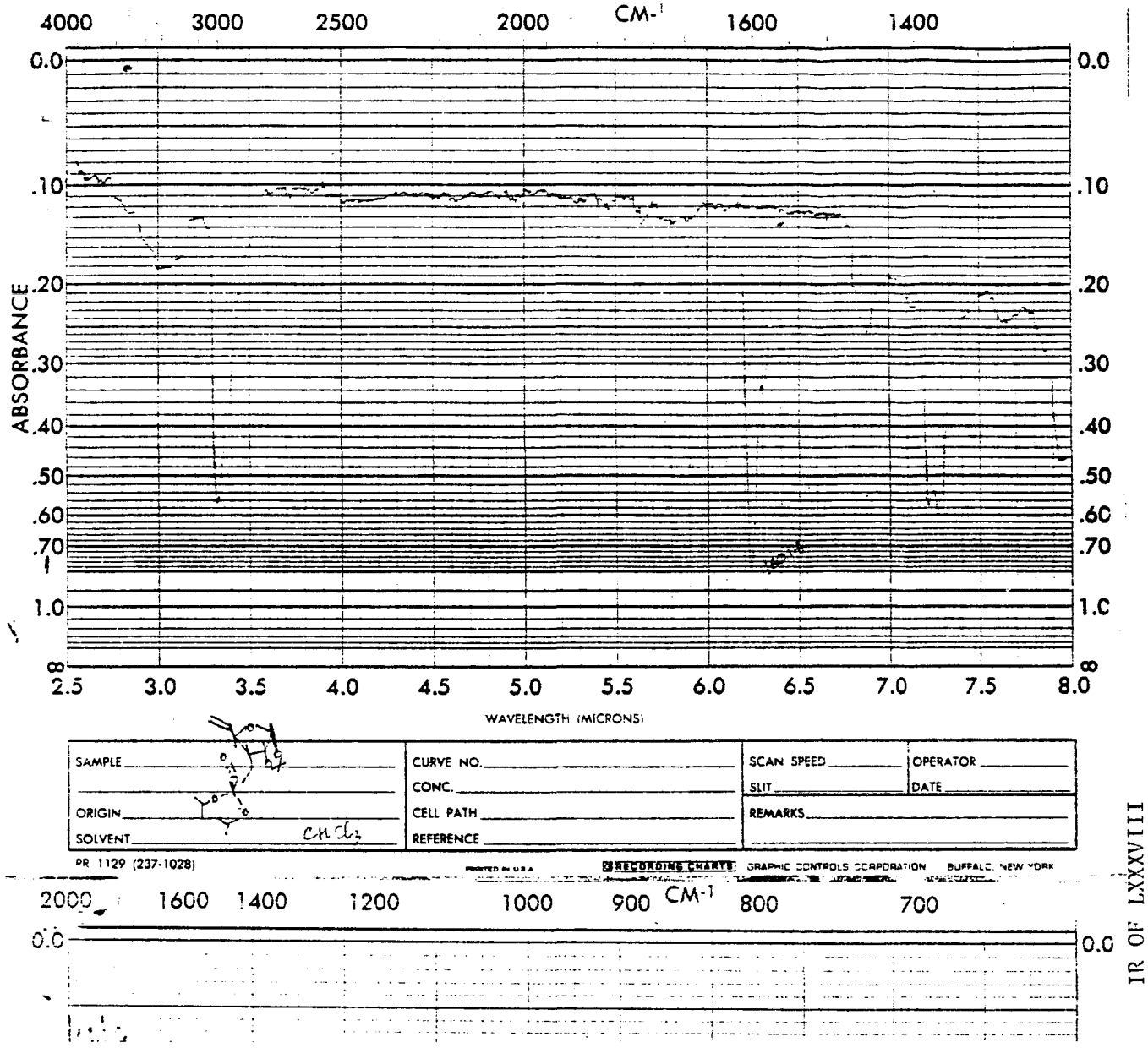
IR OF LXXXVII



IR OF LXXXVII



NMR OF LXXXVIII



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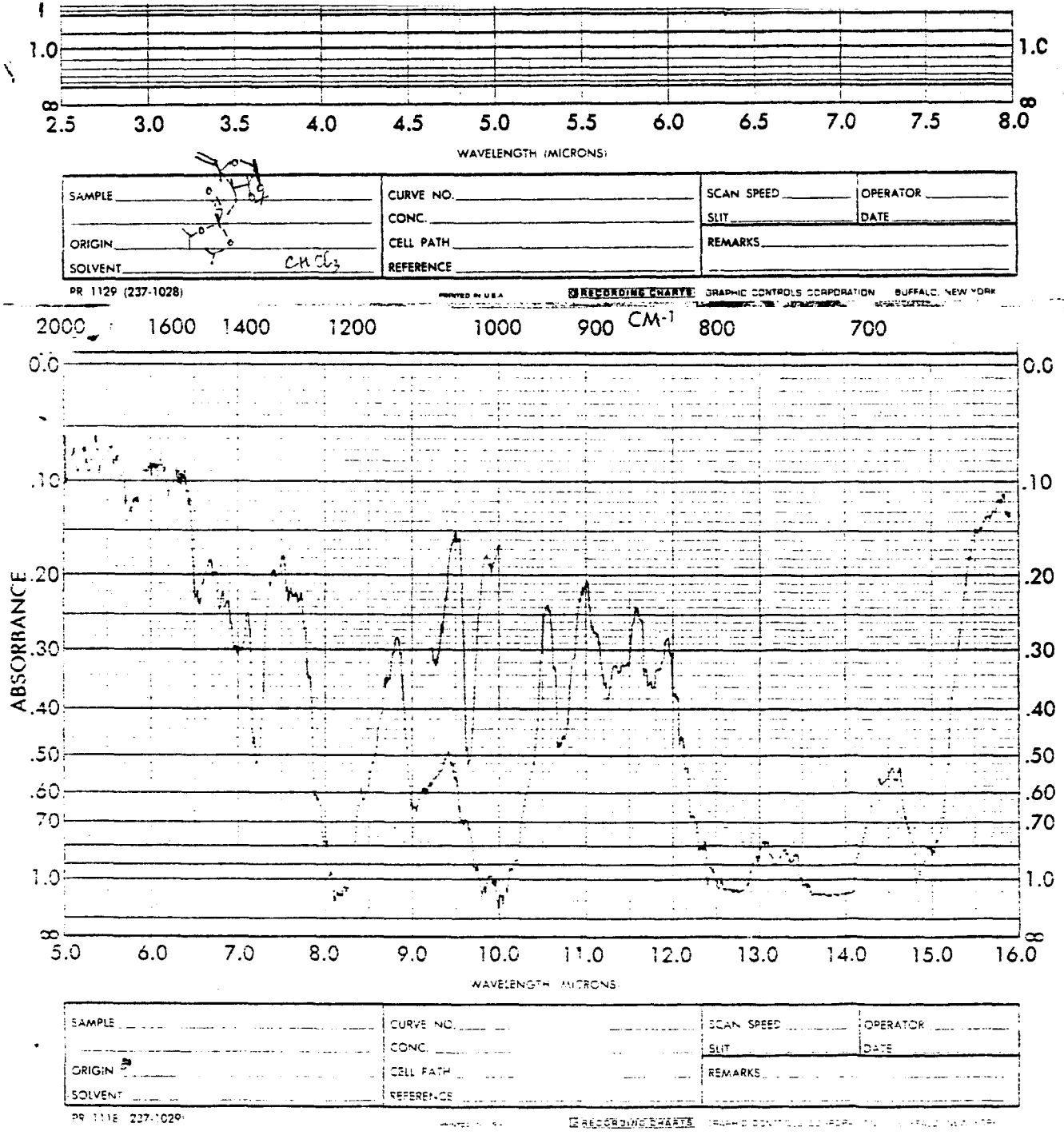

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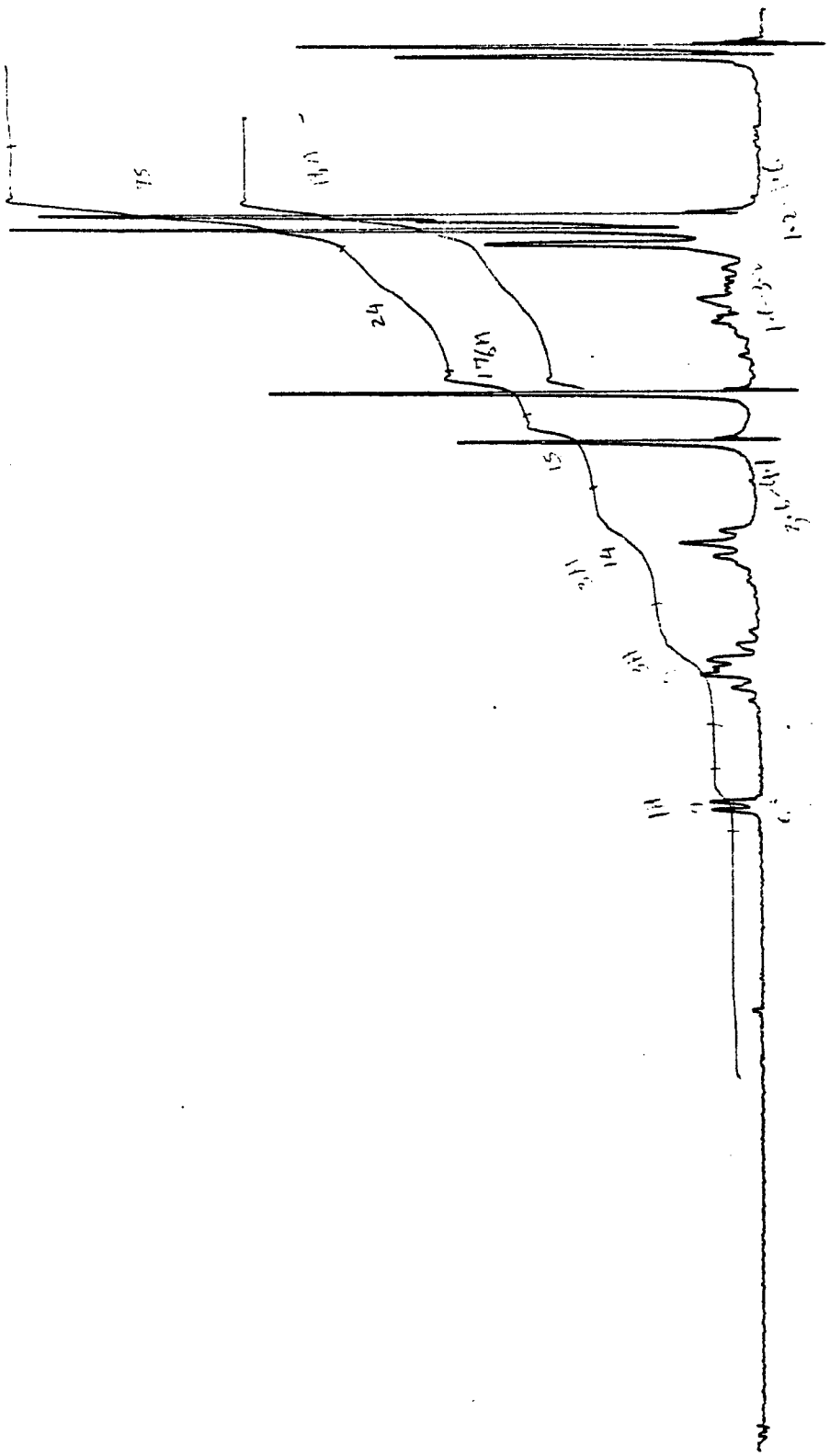
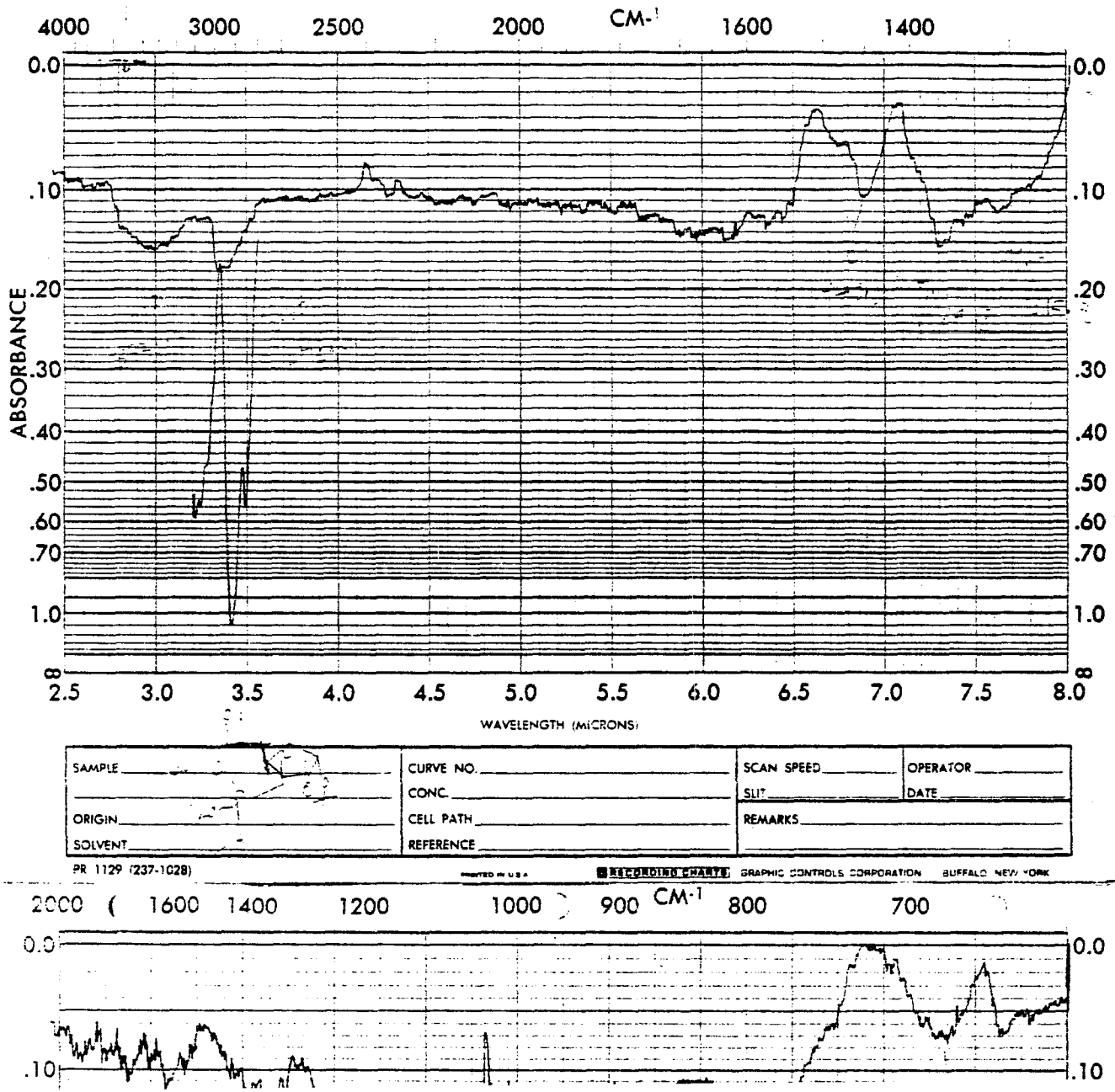


NMR OF XC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CM-1
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1.0
ABSORBANCE
2.5
3.0
3.5
4.0
4.5
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5.5
6.0
6.5
7.0
7.5
8.0
WAVELENGTH (MICRONS)
SAMPLE
CURVE NO.
SCAN SPEED
OPERATOR
CONC.
SLIT
DATE
ORIGIN
CELL PATH
REMARKS
SOLVENT
REFERENCE
PR 1129 (237-1028)
RECORDING CHARTS
GRAPHIC CONTROLS CORPORATION
BUFFALO, NEW YORK
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1600
1400
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CM-1
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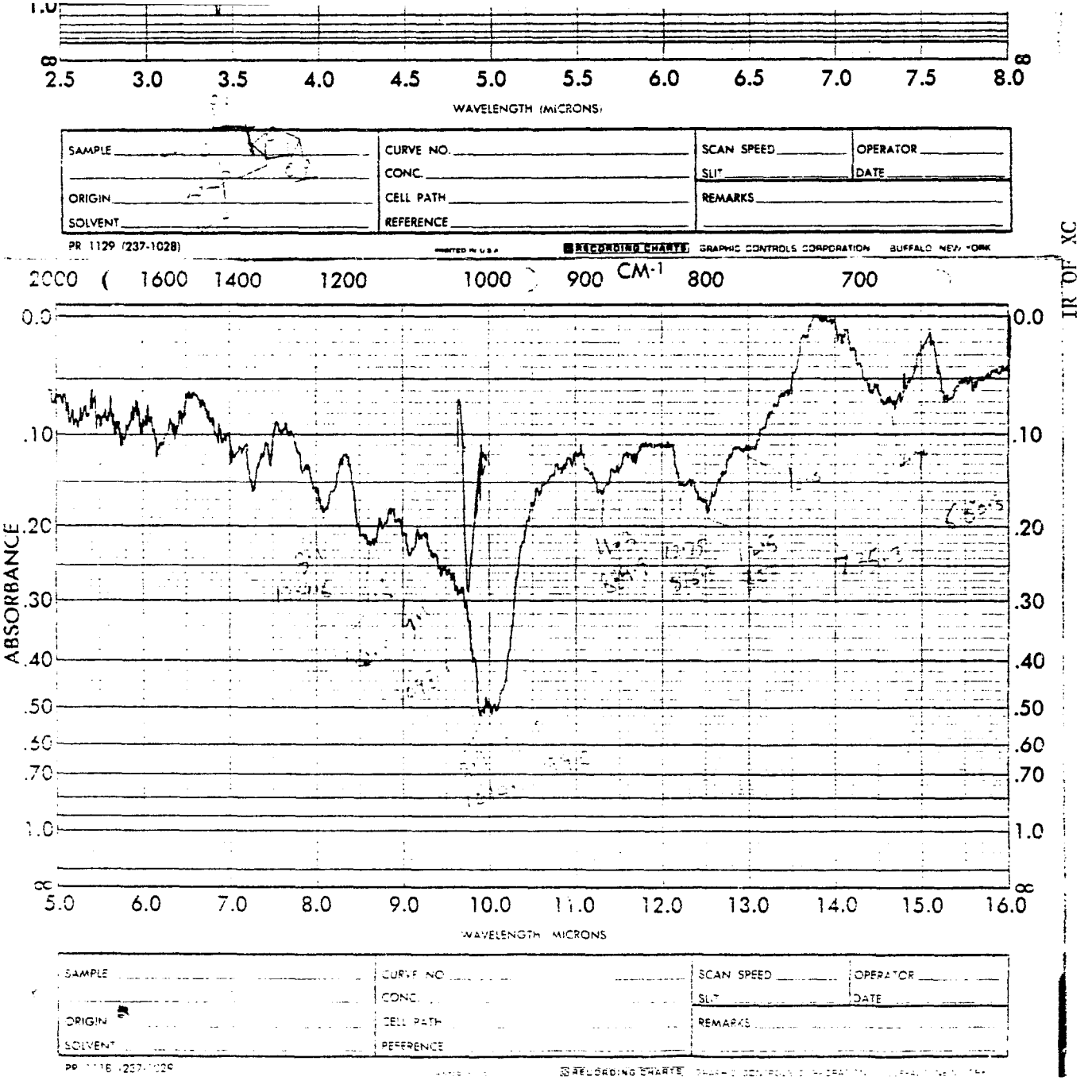
700
IR OF XC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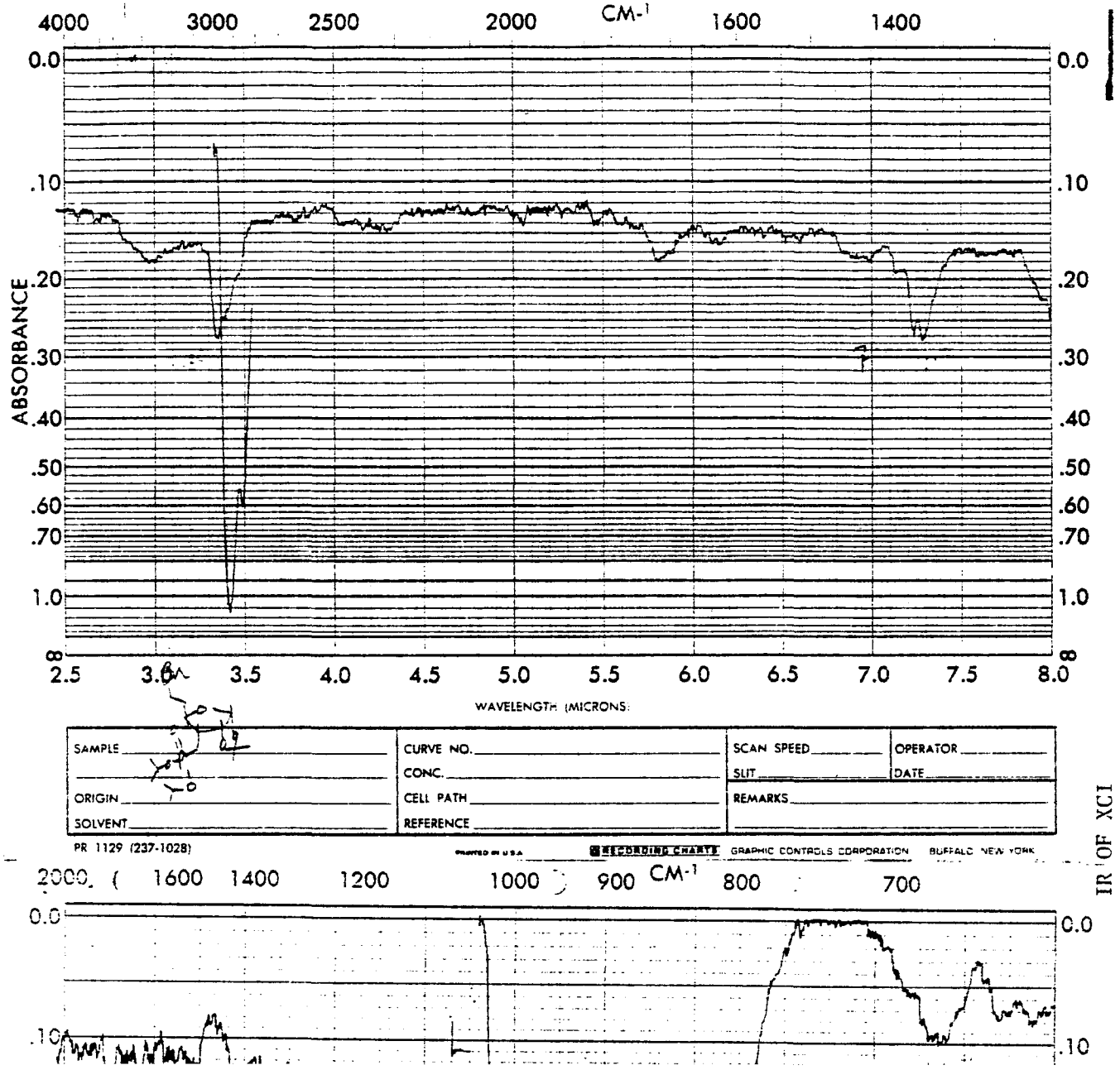
WAVELENGTH (MICRONS)
SAMPLE
CURVE NO.
SCAN SPEED
OPERATOR
CONC.
SLIT
DATE
ORIGIN
CELL PATH
REMARKS
SOLVENT
REFERENCE
PR 1129 (237-1028)
RECORDING CHARTS GRAPHIC CONTROLS CORPORATION BUFFALO NEW YORK
2000 1600 1400 1200 1000 900 CM-1 800 700
ABSORBANCE
0.0 .10 .20 .30 .40 .50 .60 .70 1.0 ∞
5.0 6.0 7.0 8.0 9.0 10.0 11.0 12.0 13.0 14.0 15.0 16.0
WAVELENGTH MICRONS
SAMPLE
CURVE NO.
SCAN SPEED
OPERATOR
CONC.
SLIT
DATE
ORIGIN
CELL PATH
REMARKS
SOLVENT
REFERENCE
RECORDING CHARTS

NMR OF XCI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.50
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.70
1.0
ABSORBANCE
2.5
3.0
3.5
4.0
4.5
5.0
5.5
6.0
6.5
7.0
7.5
8.0
WAVELENGTH (MICRONS)
SAMPLE
ORIGIN
SOLVENT
CURVE NO.
CONC.
CELL PATH
REFERENCE
SCAN SPEED
SLIT
OPERATOR
DATE
REMARKS
PR 1129 (237-1028)
RECORDING CHARTS
GRAPHIC CONTROLS CORPORATION
BUFFALO NEW YORK
2000
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CM-1
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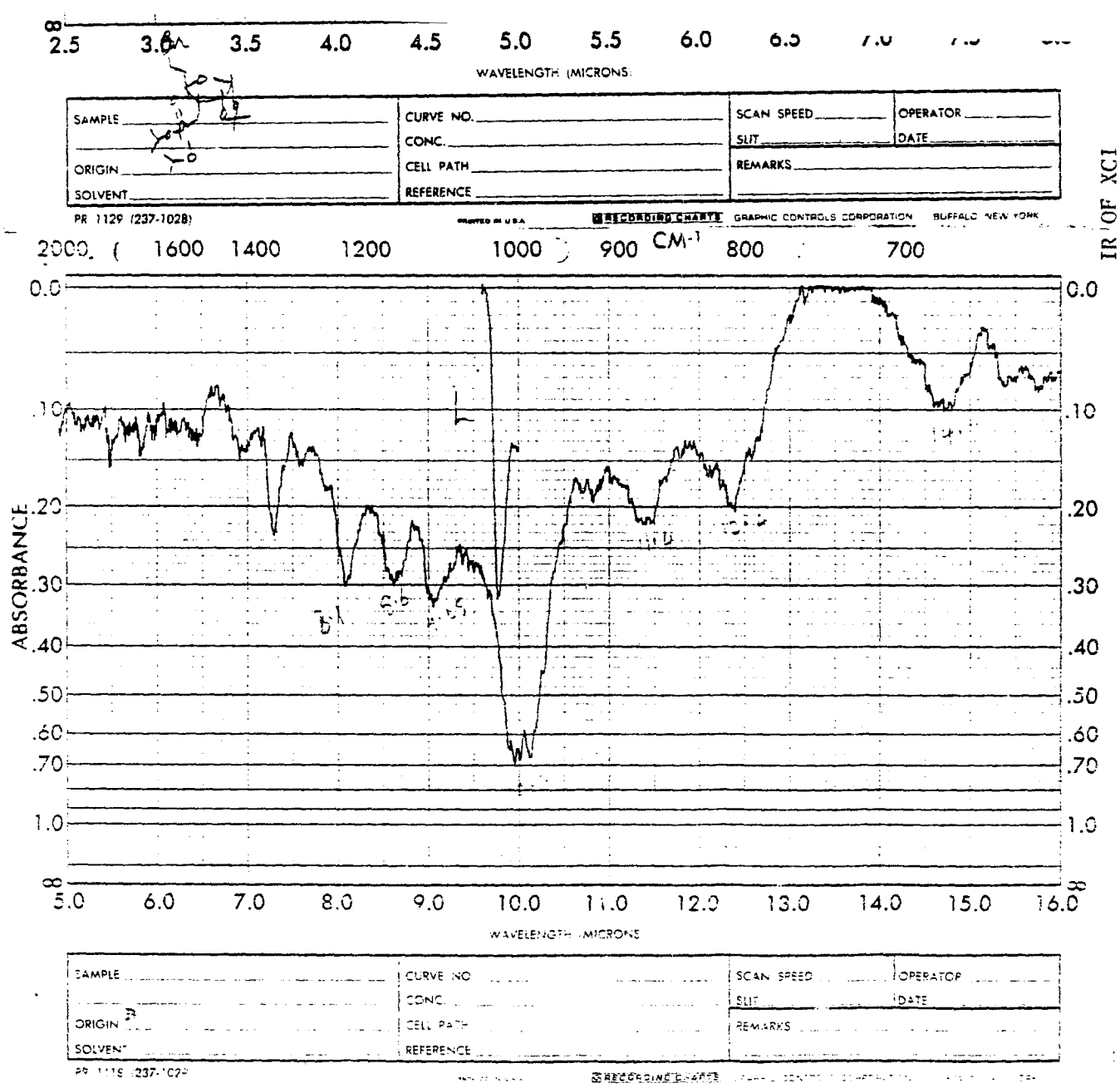
IR OF XCI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WAVELENGTH (MICRONS)
SAMPLE
ORIGIN
SOLVENT
CURVE NO.
CONC.
CELL PATH
REFERENCE
SCAN SPEED
SLIT
OPERATOR
DATE
REMARKS
PR 1129 (237-1028)
RECORDING CHARTS GRAPHIC CONTROLS CORPORATION BUFFALO NEW YORK
2000 1600 1400 1200 1000 900 CM-1 800 700
IR OF XC1
ABSORBANCE
0.0
.10
.20
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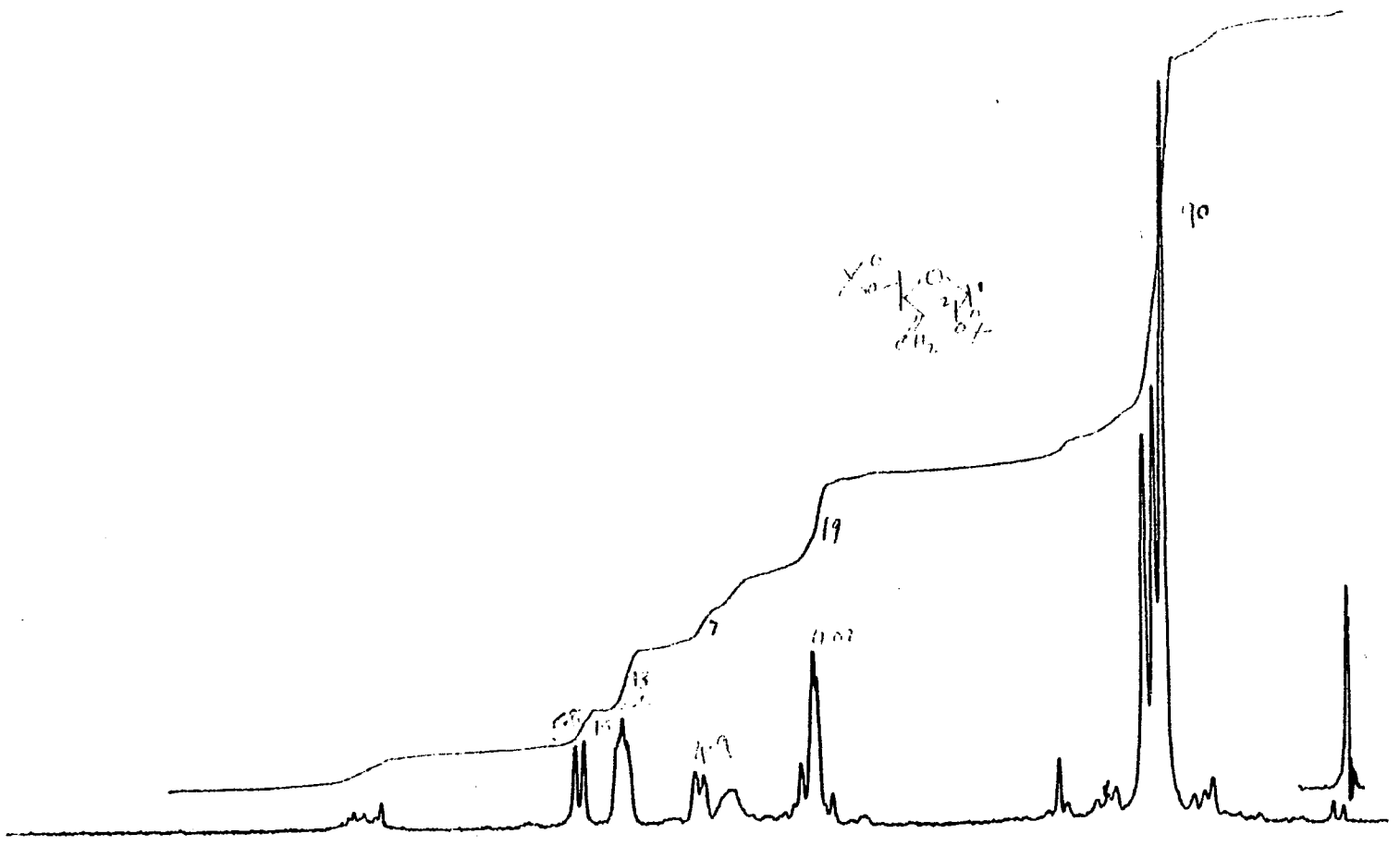
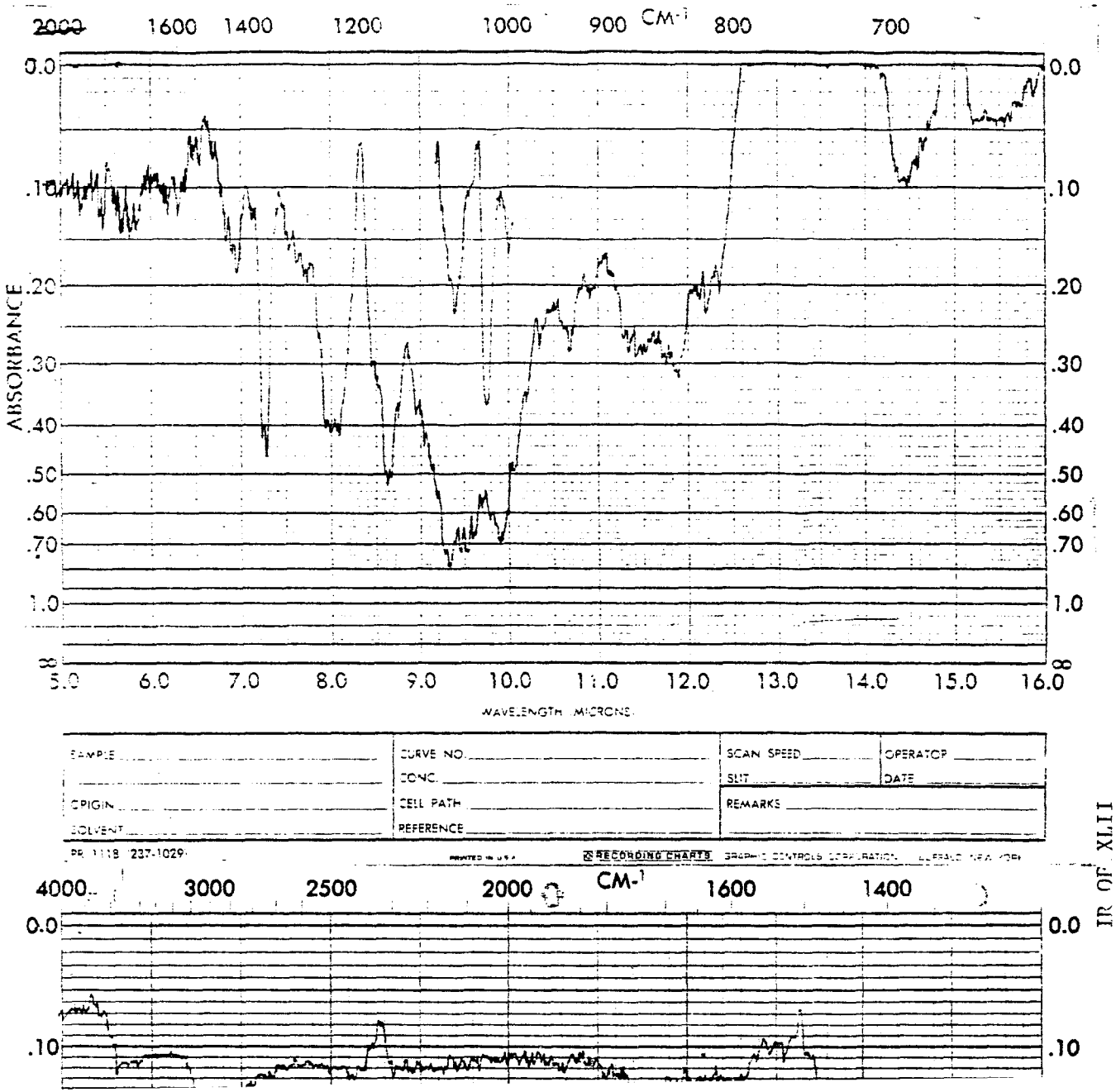
1.0
5.0 6.0 7.0 8.0 9.0 10.0 11.0 12.0 13.0 14.0 15.0 16.0
WAVELENGTH (MICRONS)
SAMPLE
ORIGIN
SOLVENT
CURVE NO.
CONC.
CELL PATH
REFERENCE
SCAN SPEED
SLIT
OPERATOR
DATE
REMARKS



NMR OF XLII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∞
ABSORBANCE
5.0
6.0
7.0
8.0
9.0
10.0
11.0
12.0
13.0
14.0
15.0
16.0
WAVELENGTH MICRONS
SAMPLE
CURVE NO.
SCAN SPEED
OPERATOR
CONC.
SLIT
DATE
ORIGIN
CELL PATH
REMARKS
SOLVENT
REFERENCE
RECORDING CHARTS
4000
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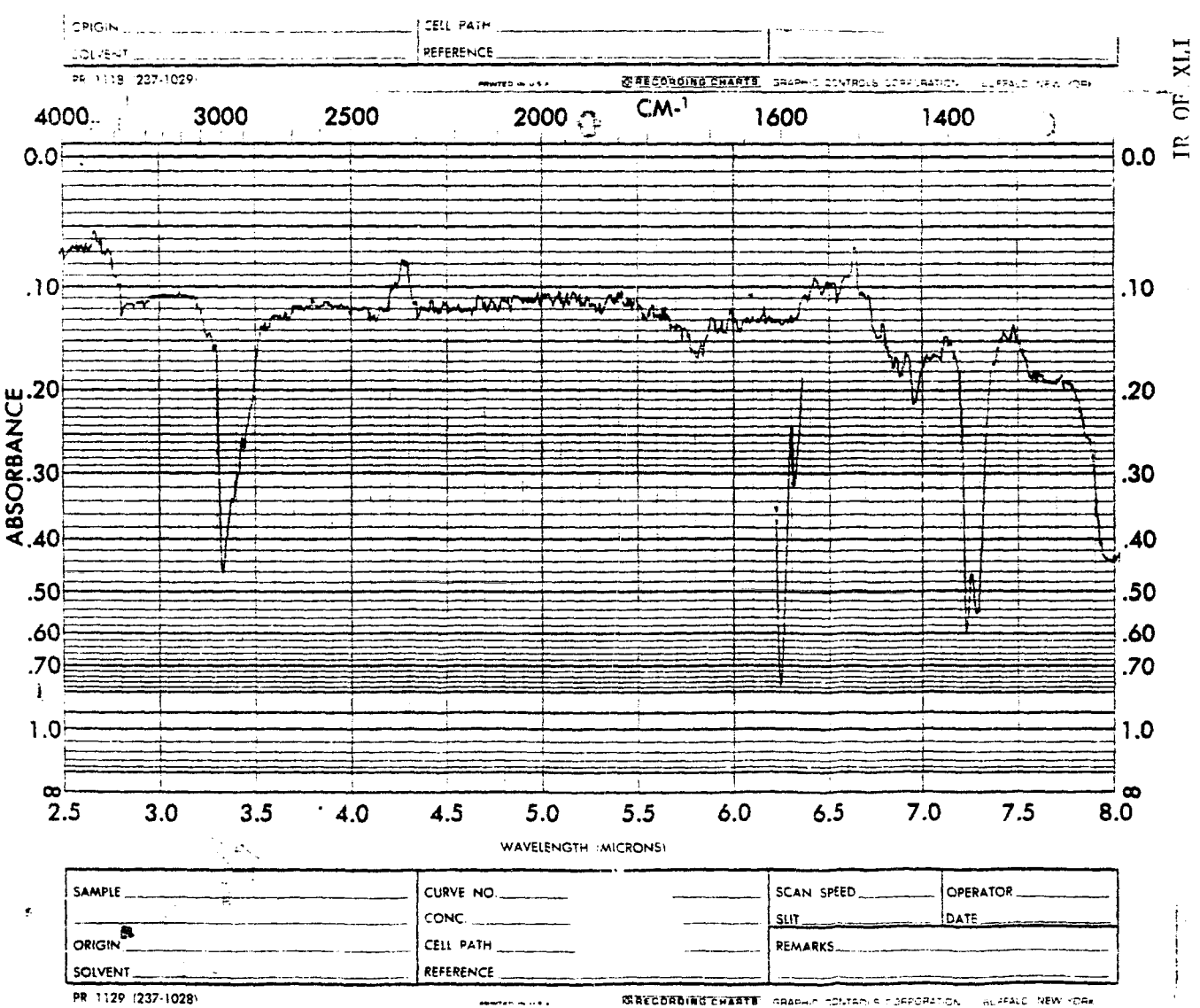
2500
2000
CM-1
1600
1400

IR OF XLII


ORIGIN
SOLVENT
CELL PATH
REFERENCE
RECORDING CHARTS
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CM-1
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1.0
ABSORBANCE
2.5
3.0
3.5
4.0
4.5
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5.5
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7.0
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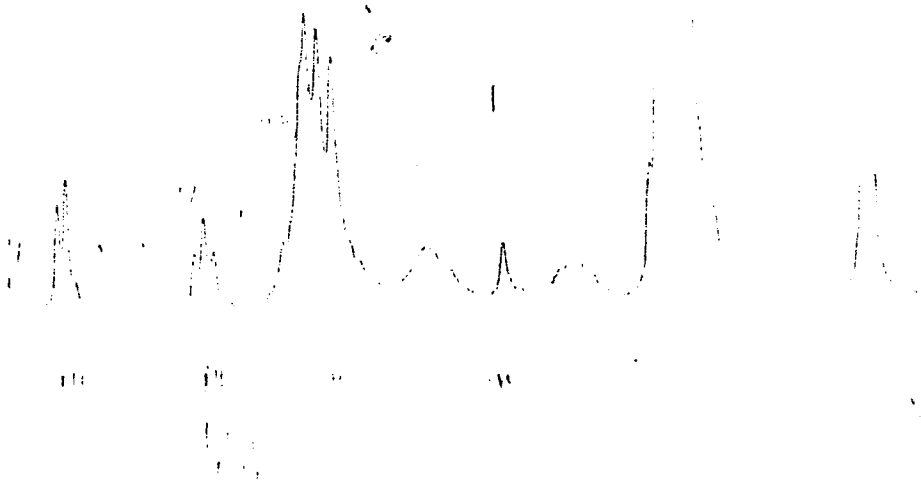
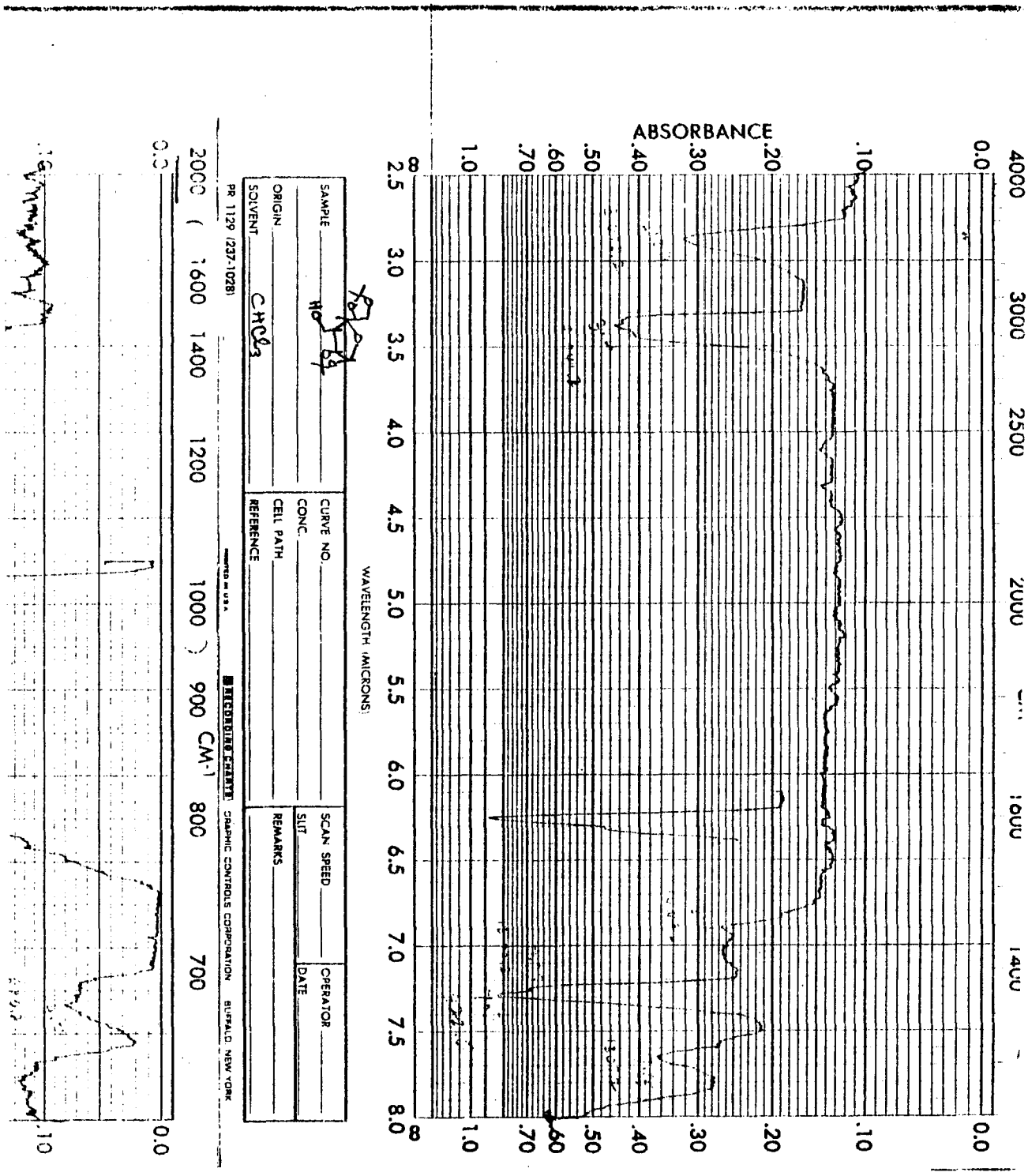
8.0
WAVELENGTH (MICRONS)
SAMPLE
CURVE NO.
CONC.
CELL PATH
REFERENCE
SCAN SPEED
SLIT
OPERATOR
DATE
REMARKS
PR 1129 (237-1028)

IR OF XLI



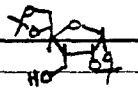
NMR OF XCIII


ABSORBANCE
WAVELENGTH (MICRONS)
SAMPLE
ORIGIN
SOLVENT
CHCl3
CURVE NO.
CONC.
CELL PATH
REFERENCE
SCAN SPEED
SLIT
REMARKS
OPERATOR
DATE
PR 1129 (237-1028)
GRAPHIC CONTROLS CORPORATION BUFFALO, NEW YORK
CM-1

IR OF XCIII

2.5 3.0 3.5 4.0 4.5 5.0 5.5 6.0 6.5 7.0 7.5 8.0

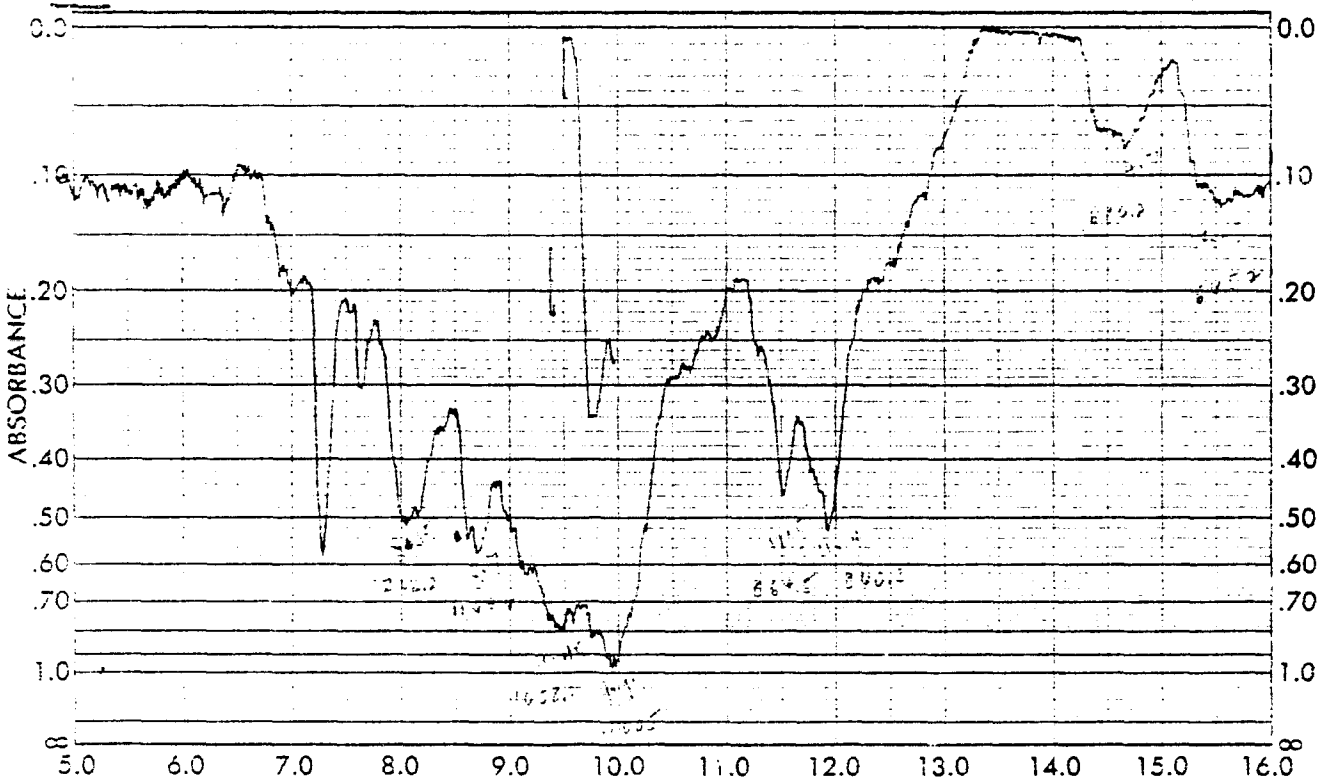
WAVELENGTH (MICRONS)



SAMPLE	CURVE NO.	SCAN SPEED	OPERATOR
	CONC.	SLIT	DATE
ORIGIN	CELL PATH	REMARKS	
SOLVENT $CHCl_3$	REFERENCE		

PR 1129 (237-1028) RECORDING CHARTS GRAPHIC CONTROLS CORPORATION BUFFALO NEW YORK

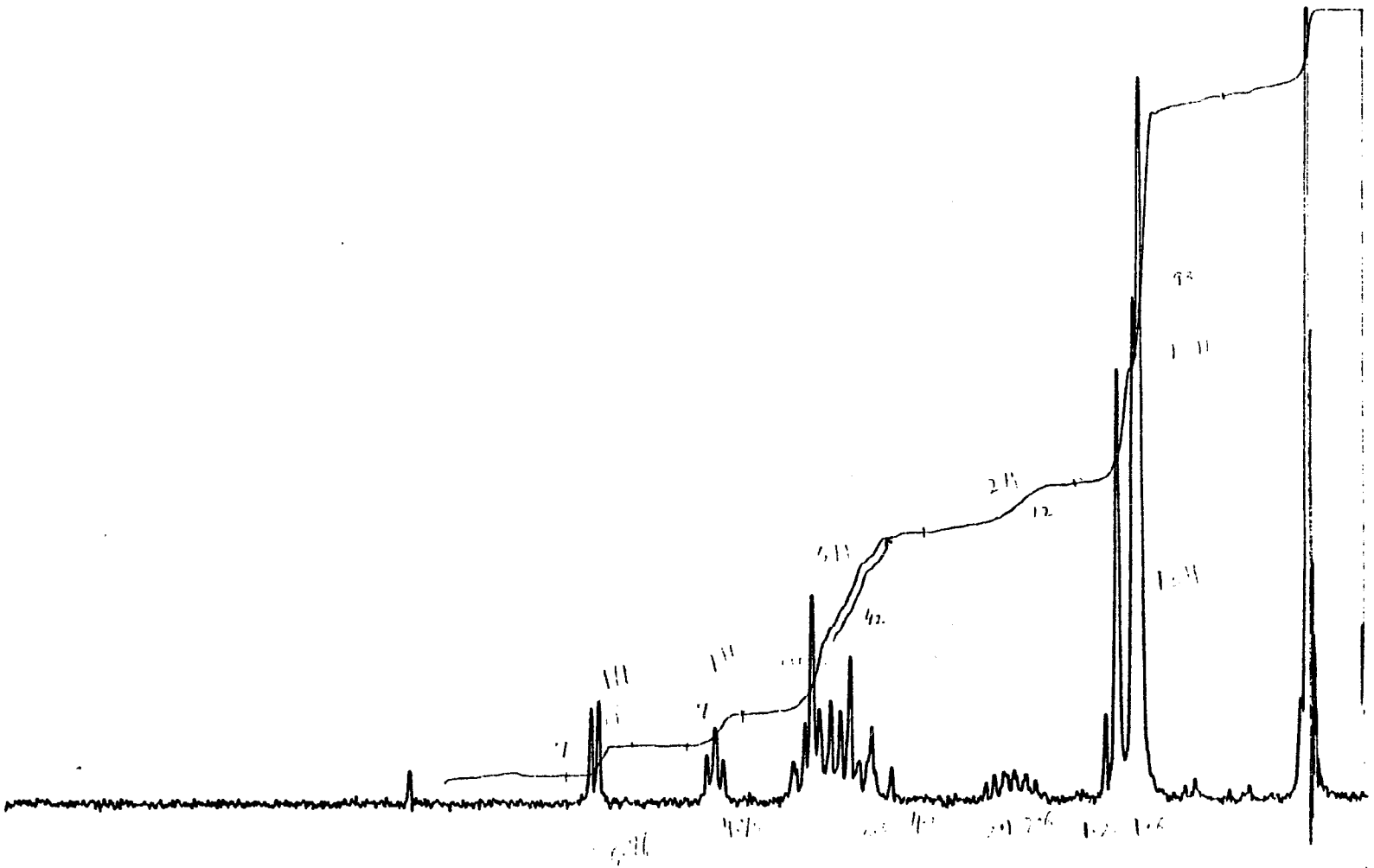
2000 1600 1400 1200 1000 900 CM-1 800 700



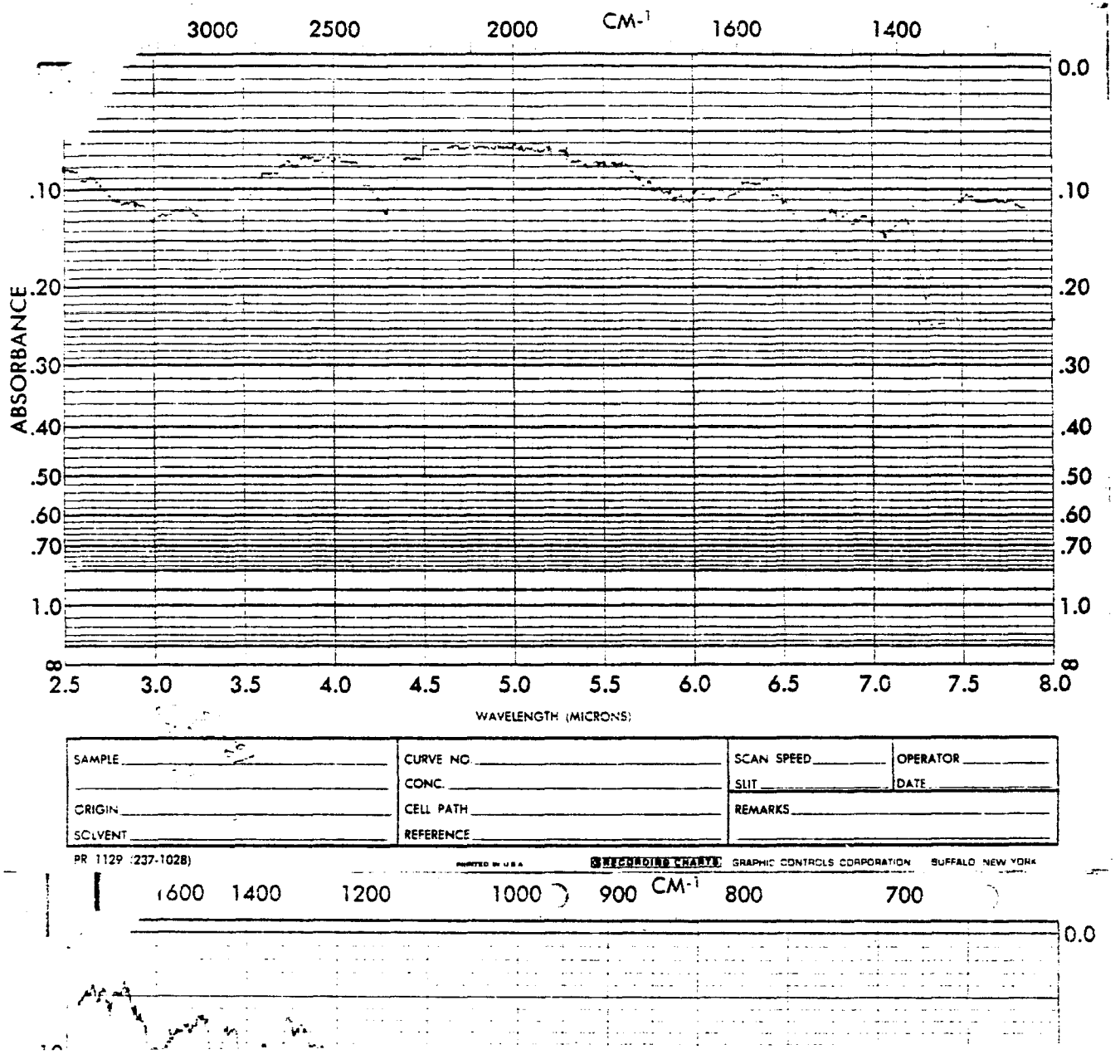
WAVELENGTH (MICRONS)

SAMPLE	CURVE NO.	SCAN SPEED	OPERATOR
	CONC.	SLIT	DATE
ORIGIN	CELL PATH	REMARKS	
SOLVENT	REFERENCE		

PR 1129 (237-1029) RECORDING CHARTS



NMR OF XCIV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1.0
ABSORBANCE
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8.0
WAVELENGTH (MICRONS)
SAMPLE
CURVE NO.
SCAN SPEED
OPERATOR
CONC.
SLIT
DATE
ORIGIN
CELL PATH
REMARKS
SOLVENT
REFERENCE
PR 1129 (237-1028)
RECORDING CHARTS
GRAPHIC CONTROLS CORPORATION
BUFFALO NEW YORK
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IR OF XCIV

IR OF XCIV

ORIGIN
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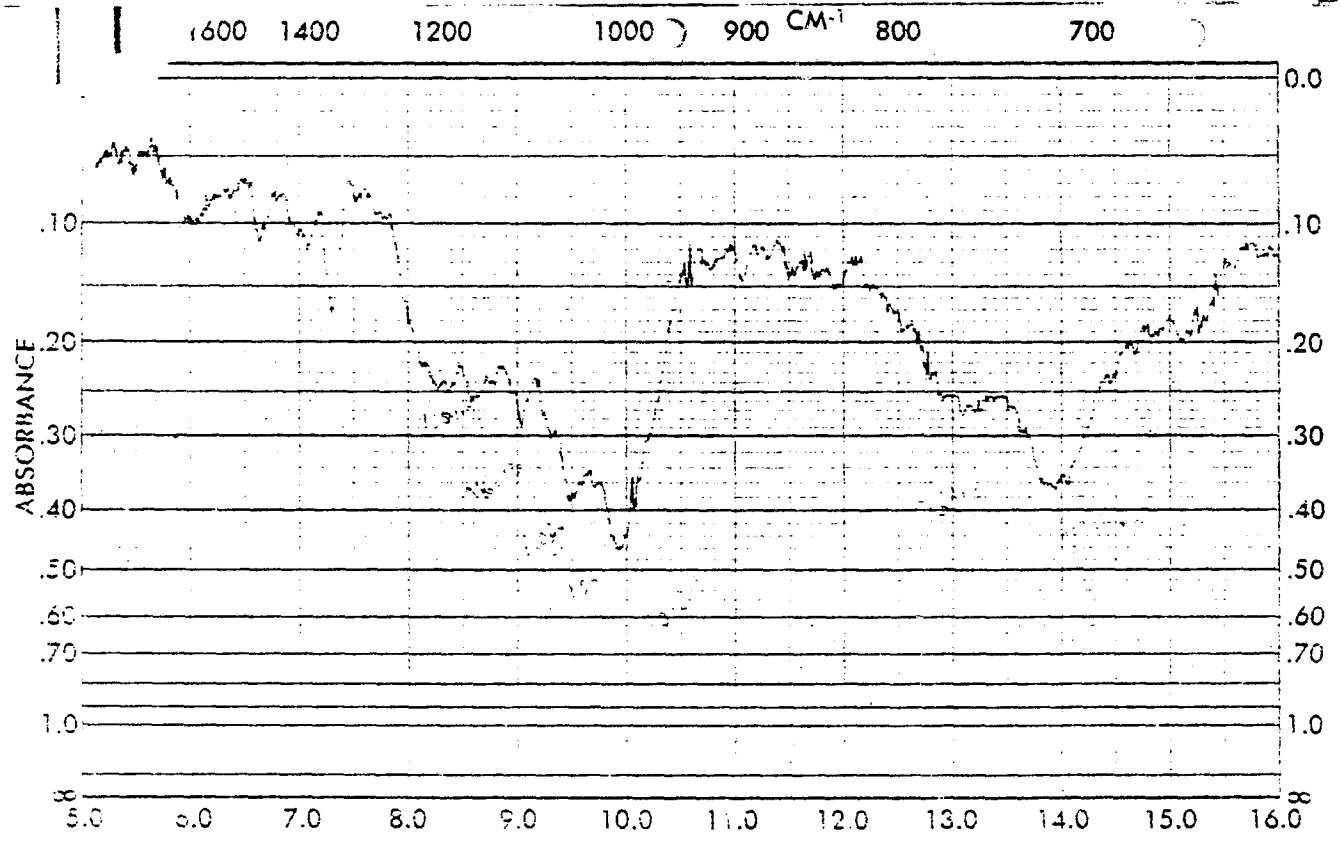
CELL PATH
REFERENCE
REMARKS

PR 1129 (237-1028) PRINTED IN U.S.A. RECORDING CHARTS GRAPHIC CONTROLS CORPORATION BUFFALO NEW YORK



SAMPLE
ORIGIN
SOLVENT
CURVE NO.
CONC.
CELL PATH
REFERENCE
SCAN SPEED
SLIT
OPERATOR
DATE
REMARKS