SYNTHESIS OF A NOVEL DEAZAAMINOPTERIN ANALOGUE

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ISBN 0-315-55009-0

Synthesis of a Novel Deazaaminopterin Analogue

by

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A thesis submitted to the School of Graduate

Studies in partial fulfillment of the

requirements for the degree

Master of Science

Department of Chemistry

Memorial University of Newfoundland

September 1989

St. John's Newfoundland

ABSTRACT

This thesis describes the synthesis of a novel deazaaminopterin analogue, which has the glutamate-derived NH group replaced by methylene. This involved the preparation of an aminobenzoyl glutarate by a troublesome decarboxylation of an aroylmalonate, which was studied in some detail. Antibacterial screening revealed activity at 100 μg/mL against *Staphylococcus aureus* in the case of the aminopterin analogue, but the aminobenzoylmethylglutarate was inactive against a variety of organisms.

During synthetic work directed towards the corresponding methotrexate analogue it was found that N-methylation of N-arylbenzenesulfonamides causes enhanced loss of SO_2 in the mass spectrum, as well as interesting shielding effects in the proton and carbon magnetic resonance spectra. This was studied through synthesis and spectroscopic examination of a series of sulfonamides having electron-releasing and/or electron withdrawing substituents.

ACKNOWLEDGEMENTS

I would like to give sincere thanks to Dr. Brian Gregory, who provided the support and advice which helped carry this project to completion, and to my wife Margaret who provided encouragement and moral support when it was needed most. I acknowledge Dr. Charles Loader who provided invaluable assistance in the word processing of the manuscript. For technical support I thank Miss Marion Baggs for mass spectral data, and Mr. Avery Earle, Mr. Ramaswami Sammynaiken, and Ms. Nathalie Brunet for NMR spectra. I would also like to thank Dr. Peter Golding and Dr. Allan Stein for encouragement over the course of the project. I acknowledge Memorial University for financial support.

I would like to thank my parents for the support and encouragement which has helped me to get this far.

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INTRODUCTION

1.1 Folic Acid

1.1A Structure and Isolation

The vast knowledge of biochemistry at the molecular level which has been accumulated over the past century, has had a dramatic effect upon synthetic research aimed at producing new "biologically active" compounds. An expanded knowledge of biochemical pathways has provided the ability to "design" molecules which may selectively and predictably affect a metabolic sequence. This, then, causes a desired alteration in the biochemistry of the organism.

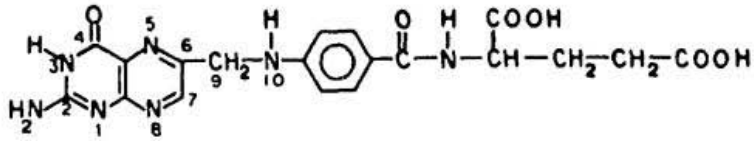
The central components of biochemistry are enzymes, which are macromolecules, that with the aid of cofactors, are capable of catalysing and controlling chemical reactions. These enzymes manipulate compounds referred to as substrates and control the chemistry which constitutes metabolism. Since this manipulation requires the enzyme to associate with both substrate and cofactor, these molecules provide the simplest means of affecting the enzyme.

An inhibitor is a substrate analogue which, by binding to an enzyme and inhibiting its catalytic ability, provides control over the enzyme. These substrate analogues therefore become drugs which can be used to influence or change the metabolism of an organism.

One biologically important substrate which has instigated a tremendous amount of experimental research is folic acid (Fa). Its isolation in 1946 was quickly followed by structural determination and synthesis (1, 2, 3).

Folic acid (I) consists of a pteridine ring bound via a *p*-aminobenzoyl group to an L-glutamic acid residue and its crystal structure has been determined by X-ray methods (4).

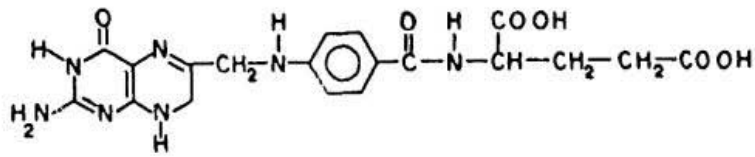
(I)



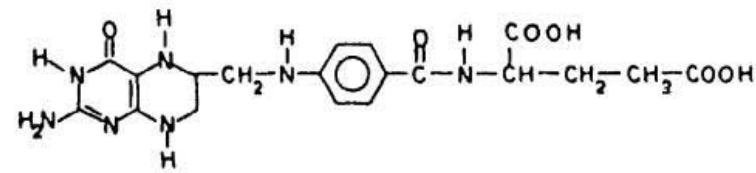
1.1B Biochemistry of Folic Acid

Folic acid is a necessary vitamin in nearly all organisms. It primarily occurs in one of two reduced forms in the cell, either 7,8-dihydrofolic acid (FH_2) (**II**) or 5,6,7,8- tetrahydrofolic acid (FH_4) (**III**).

(II)



(III)



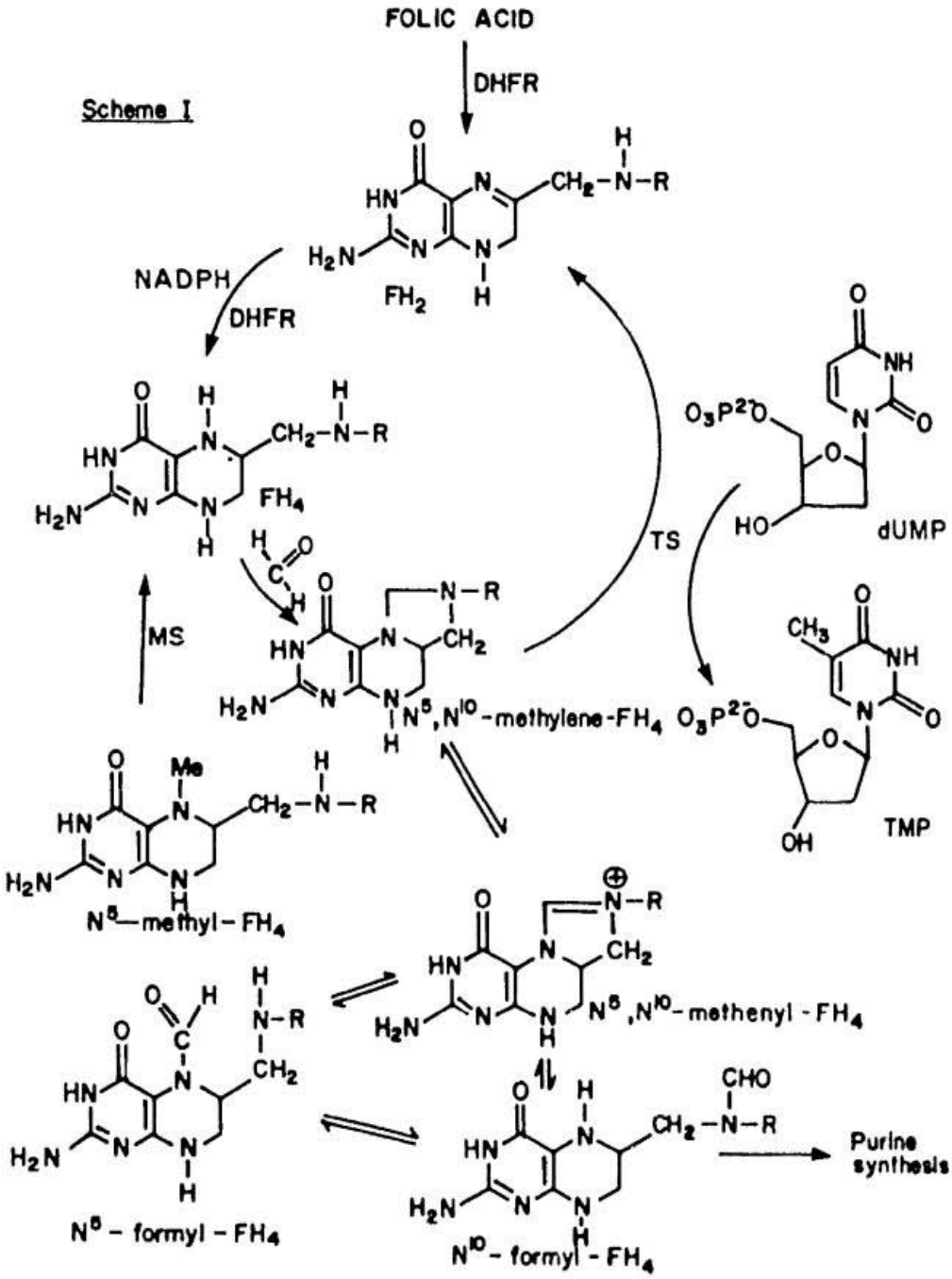
The active form of folic acid is tetrahydrofolate which is a cofactor in the transfer of one carbon units in metabolism. In this role tetrahydrofolate is present in several forms, including N^5-methyl, N^{10}-methyl, N^5-formyl, N^{10}-formyl, N^5-formimino and N^5, N^{10}-methylene FH_4. As such it acts as a carrier of methyl, formyl, formimino, and methylene groups respectively. The biosynthetic pathways which utilize FH_4 and the ones which regenerate it are closely related (**Scheme I**), and several reviews and books have summarized this information in many forms (5, 6, 7, 8). One of the more important roles of FH_4 is in the thymidylate synthase cycle

where N^5, N^{10}-methylene-FH_4 provides the methyl group which is inserted into the 5-position of the pyrimidine ring of deoxyuridine monophosphate. Subsequent hydride transfer yields thymidine monophosphate and FH_2. N^{10}-Formyl-FH_4 is used directly in purine synthesis and N^5-methyl-FH_4 plays a role in serine and methionine metabolism (8). The thymidylate synthase reaction is unique as it is the only reaction which utilizes FH_4 as both a carbon donor and a reducing agent, producing FH_2 which is reduced back to FH_4 by DHFR (8). This is the same enzyme which reduces folic acid to FH_2 and is the primary source of reduced folate in the cell.

In most mammals folic acid is obtained through dietary sources, and is supplied to the cell via active transport systems (9). Several reduced forms of folate including N^5-formyl and N^5-methyl-FH_4 are also actively transported into the cell. In contrast, most bacteria are impermeable to folic acid (9) and therefore have to synthesize it from smaller molecules. One of the more important of these smaller molecules is *p*-aminobenzoic acid (PABA) and this is the reason for prontosil's antibacterial activity. In the body prontosil is converted to sulfanilamide (10, 11) which competes with PABA in the bacterial production of folic acid to produce a non-active folate analogue. It was this antibacterial activity which first generated interest in the folate area. However, most mammalian antifolates fail to show antibacterial activity as they do not penetrate the bacterial membrane.

It was quickly realized that rapidly multiplying cells have very active folate biochemistry and interference with this metabolic pathway could have a profound effect on cell growth. The potential use of this methodology against fast growing tumor cells, and the resulting search for antifolate compounds initiated the biochemical approach to cancer chemotherapy. With the recognition that dihydrofolate reductase (DHFR) was essential to maintain cellular levels of FH_4, it became apparent that selective inhibition of this enzyme would provide a means of controlling the biosynthetic pathways which utilize FH_4.

Scheme I


FOLIC ACID
DHFR
NADPH
DHFR
FH2
FH4
MS
TS
dUMP
TMP
N5,N10-methylene-FH4
N5-methyl-FH4
N5,N10-methenyl-FH4
N5-formyl-FH4
N10-formyl-FH4
Purine synthesis

Inhibition of DHFR quickly results in a depletion of FH_4 levels as it is converted to FH_2 by the thymidylate synthase cycle. This depletion of FH_4 then affects not only thymidine production but also the other cycles which utilize FH_4 as a cofactor. The resulting halt in nucleotide biosynthesis stops cell replication. The result of this nucleotide shortage on rapidly replicating cells is especially dramatic as this type of cell would normally have a very active nucleotide biosynthesis.

In many forms of malignant growth, cell replication is quite fast, and this provides one way of achieving some specificity against tumor populations. In early days it was hoped that the DHFR of malignant cells might differ from the DHFR of normal cells, but this did not prove to be the case (12,13). As a result, the accelerated rate of growth was chosen as a means of attaining specificity and patients were treated with large doses of a DHFR inhibitor, followed after a specified length of time, by a rescue drug. This quickly showed positive results against certain types of malignant growth and greatly increased interest in obtaining DHFR inhibitors.

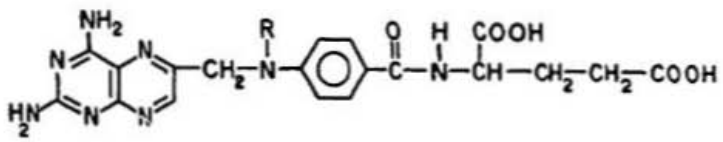
This same kind of rationale also applies to bacterial growth; the major difference being that many bacteria manufacture their folic acid from sub-units, and as a result are unaffected by many DHFR inhibitors, as they fail to penetrate the bacterial membrane. This is beneficial, however, as small molecules which are analogues of the folic acid building blocks can be used to inhibit bacterial FA production. This makes selective inhibition of bacterial DHFR quite easy and has lead to the synthesis of some very successful antibacterial compounds.

1.2 Dihydrofolate Reductase

1.2A Inhibitors

The first DHFR inhibitor, a crude x-methyl folic analogue of folic acid (14), was found to produce "folate deficiency" symptoms when given to animals (14,15). This was quickly followed by the synthesis of aminopterin (AMT) (16), a more potent inhibitor of DHFR which was not reversed by addition of folate. The successful use of this compound to induce remission against childhood leukemia (17), and the discovery of citrovorum factor(18), which was found to competitively reverse the effects of aminopterin (19,20), led to a more developed understanding of folate chemistry as well as generating much interest in anti-folates. Subsequent synthesis of methotrexate (MTX), the N-10 methylated version of aminopterin, (IV) gave what has become one of the most useful of anti-cancer drugs.

(IV)



R=H Aminopterin, R=CH_3 MTX.

MTX is an inhibitor of DHFR (22) which binds tightly, but reversibly and stoichiometrically at the DHFR active site (23, 24), a feature which has facilitated X-ray diffraction studies of the MTX:enzyme complex. This has given much information about the binding mechanism and requirements for successful DHFR inhibitors and has contributed greatly to the current understanding of this enzyme. MTX has been used successfully against several forms of cancer, including childhood leukemia and choriocarcinoma, but is toxic at therapeutic levels. The drug must penetrate the cell before it can successfully inhibit the

DHFR and it does this by both active transport and passive diffusion where active transport is carried out by the reduced folate transport systems (25,26,27). The most efficient method of achieving high cellular MTX levels is short internal high dose treatment, but this results in high toxicity to normal cells. This toxicity is offset by low doses of a rescue agent first known as citrovorum factor or leucovorin, which is actually folinic acid or 5-formyl-FH_4. The low doses result in active transport of this folate (28) selectively into normal cells, where it or its metabolite, 5-methyl-FH_4, compete with MTX for the binding site of DHFR (27). This type of treatment has success against fast growing malignant cells as their thymidylate synthase cycle is very actively producing thymine nucleotides for DNA synthesis, and blockage of this production results in selective stoppage of DNA synthesis without RNA stoppage. This leads to "thymineless death" of the cell, probably as a result of irreversible DNA damage (30) leading to faulty transcription.

While MTX is still the most widely used anti-tumor drug, it has major limitations in its clinical usefulness. It suffers from poor transport properties pertaining to solid tumors, and has a limited ability to penetrate the blood-brain barrier (31). There is also a problem of acquired and/or natural resistance of some mammalian cell lines to MTX treatment (29).

Intrinsic resistance may arise from (32):

(i) poor MTX transport,

(ii) elevated dihydrofolate reductase levels,

(iii) rapid synthesis of DHFR,

(iv) utilization of salvage pathways (thymidine, hypoxanthine) and,

(v) high and competitive levels of intracellular folates.

Other factors which may contribute to this intrinsic resistance includes cell kinetics, insufficient MTX polyglutamylation, and low NADPH levels (29).

Acquired resistance occurs from incomplete destruction of a tumor cell population by MTX treatment, which selectively leaves those cells which are inherently most suited to MTX resistance. This acquired resistance appears to be the result of three modes of response to MTX treatment including (8):

(i) decreased uptake of the drug, resulting from an alteration of the active transport system responsible for MTX uptake (33-39),

(ii) reduced affinity of the DHFR enzyme for MTX (33,40-44) as a result of a missense mutation of the DHFR gene causing a critical amino acid substitution in the enzyme (45) and,

(iii) increased levels of DHFR in the cell (33,46-61) with up to several hundred times the normal amount of enzyme being present.

Indeed, cells which demonstrate resistance to high levels of MTX treatment may make use of a combination of these mechanisms (33,62-64), but thus far always utilize an overproduction of DHFR (8).

As well as this limitation in its usefulness, MTX also has problems with associated toxicity. This toxicity takes two forms, the obvious acute toxicity of a non-selective antifolate which is intrinsically of equal toxicity to all cells which it can enter, and long-term toxicity associated with prolonged or repeated treatment with the drug.

The initial toxicity, which arises from MTX not being selective to tumor cells, is easily dealt with by incorporating a rescue agent such as leucovorin or carboxypeptidase G_1 in the treatment regiment. Carboxypeptidase G_1 cleaves the amide linkage of MTX (65) giving 2,4-diamino-N^{10}-methylpteroate, and thereby inactivating it.

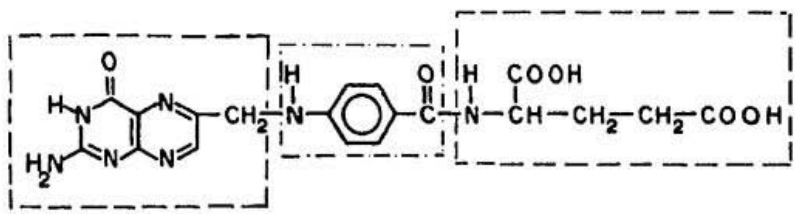
However, folates in the cell are polyglutamylated enzymatically which involves the condensing of glutamate residues onto the terminal glutamate carboxyl. This polyglutamylation, which is catalyzed by folylpolyglutamate synthase (FPGS), occurs in all organisms (66) and

appears to be vital for cell life (67). While this process is a necessity for maintaining cellular folate (68), the enzyme also polyglutamylates MTX, giving a substrate with retained affinity for DHFR (69,70,71,72) and potent inhibitory properties for thymidylate synthase (TS) (73) and aminoimidazolecarboxamide ribonucleotide transformylase (74) the latter of which is involved in *de-novo* purine synthesis. The reduced transport properties of MTX polyglutamates can contribute to selectivity in tumor cells, but also contributes to cytotoxicity in normal cells. The liver is particularly susceptible (75-82) to accumulation of these polyglutamates contributing to MTX hepatotoxicity.

1.2B Binding Requirements of DHFR

The structure of folic acid can be subdivided into three sections (V) each of which contribute to the overall binding of the substrate to DHFR.

(V)



These three regions are referred to as:

(i) the pteridine ring,

(ii) the *p*-aminobenzoyl (bridge region) and

(iii) the glutamic acid residue.

Each of these regions have been varied in attempts to synthesize inhibitors of DHFR and the resulting inhibition studies have given much information about the binding requirements of the enzyme. This has been supplemented by X-ray studies of complexes of MTX

with DHFR from *Escherichia coli* (*E. coli*) (83), MTX plus NADPH with DHFR from *Lactobacillus casei* (*L. casei*) (84,85,86,87), 2,4-diamino-5-(3,4,5-trimethoxybenzyl)pyrimidine (trimethoprim) with DHFR from *E. coli* (88,89), trimethoprim plus NADPH with DHFR from chicken liver (89,90) and DHFR from chicken liver with 2,4-diamino-5,6-dihydro-6,6-dimethyl-5-(x-phenyl)-S-triazines, and with 2,4-diamino-5-(1-adamantyl)-6-methylpyrimidine (DAMP) (89,90,91), which has given more detailed information about the active site enzyme:substrate interactions. The tremendous volume of work done in this area makes it impossible to relate all the observations here, but some of the main points which are important to substrate/inhibitor binding will be covered. An excellent compilation of the literature work in this area by R. L. Blakely is available in Ref. 8, Chapter 5.

The active site of DHFR is a cavity which is 15Å deep cutting across one face of the enzyme. It is lined with hydrophobic side chains but contains one polar side chain, which is Asp-27 (*E. coli* numbering) in bacterial DHFR or Glu-30 in vertebrate DHFR. The important hydrophobic interactions between substrate and enzyme are further aided by NADPH, as it binds with the nicotinamide moiety inserted into the hydrophobic cavity.

The desolvation of the pteridine ring due to NADPH binding is believed to be at least partially responsible for the cooperative binding of MTX and NADPH with DHFR. The polar side chain present in the hydrophobic pocket also plays an important role in inhibitor and, presumably, substrate binding. The carboxylate of this group is adjacent to the N^1 and 2-amino nitrogens of bound 2,4-diaminopyrimidines, triazines, quinazolines and pteridines, which are protonated and carry a delocalized positive charge. The result is an electrostatic interaction between the carboxylate and the two nitrogens, as well as hydrogen bonding between the two nitrogens and the carbonyl which is nearly coplanar with the ring system. In addition to these interactions the *L. casei* DHFR:MTX complex has a hydrogen bond between the 2-amino group and a fixed water molecule which is also H-bonded to the

side chain hydroxyl at Thr-116. Since this Thr-116 is conserved in all DHFR species, this bridging water molecule is probably a common feature of MTX binding to DHFR. It also has hydrogen bonding of the 4-amino group to the backbone carbonyls of Leu-4 and Ala-97 and this is presumed to occur with analogous carbonyls in *E. coli* and chicken liver DHFRs as well.

While the pteridine ring of bound folate is buried in the active site cavity, the *p*-aminobenzoylglutamate side chain extends out of the cavity and is draped across the surface of the enzyme. X-ray data for MTX bound bacterial DHFR show the benzoyl ring of the side chain to be nearly perpendicular to the pteridine ring with the α-carboxylate of the glutamate moiety interacting with the invariant Arg-57. The γ-carbonyl, by contrast, has different interactions depending upon the specific structure of the enzyme. Studies of the binding of 2,4-diamino-5-(3,4,5-trimethoxybenzyl)pyrimidine (trimethoprim), a selective inhibitor of bacterial DHFRs has given more information about the binding requirements of the enzyme pertaining to the aromatic side chain portion of folate. While the diaminopyrimidine portion of this drug is located in a position analogous to bound MTX (88,89), and the trimethoxybenzyl group makes hydrophobic contact, which are analogous with those made by the aminobenzoyl portion of bound MTX for bacterial DHFRs, the binding to chicken liver DHFR shows some differences. Work on the ternary DHFR,NADPH,trimethoprim complex (90) of chicken liver DHFR has shown that, while the 2,4-diaminopyrimidine moiety is located in a position analogous to that of trimethoprim bound to bacterial DHFR, the aromatic side chain is positioned in a different region of the cavity than that occupied in the bacterial complex. Using this knowledge and x-ray crystallographic data for the complexes of chicken liver DHFR with 2,4-diamino-5,6-dihydro-6,6-dimethyl-5-(x-phenyl)-s-triazines and with 2,4-diamino-5-(1-adamantyl)-6-methylpyridine(DAMP) Matthews and his colleagues have generalized (89) that each DHFR has two potential binding sites, an upper and a lower for the

inhibitor side chains, which are distinct from the region occupied by the 2,4-diamino-heterocycle which directs the inhibitor to the active site. These upper and lower sites in bacterial DHFRs show considerable geometrical differences from those found in vertebrate DHFRs, and these differences are responsible for the selectivity of some inhibitors to bacterial *versus* vertebrate DHRF.

1.3 Analogues of Folic Acid

Over the years a tremendous number of compounds have been synthesized as potential inhibitors of firstly DHFR and later TS. These variations first concentrated on achieving tight binding to the enzyme active site and the variations in structure, compared to the activity of the compound, gave clues to the shape and nature of the enzyme active site. Then modifications were made in hopes of affecting the transport properties of the compounds, as it was realized that this provided more potential for selective action than differences between the DHFR of "normal" vs "tumorous" cells. As the modes of metabolism of these drugs became known, the causes of toxicity became better understood, and compound design began to reflect alterations which would change the potential for metabolism of the compounds, in order to alleviate some of the harmful side effects. Today the search for new compounds with slightly different structures which will fulfill the role of an ideal inhibitor of folate is still very active. Much of the work on antifolate compounds have been summarized extensively in many excellent reviews, including recent ones by Palmer, Skotnicki and Taylor (92a), Rosousky (92b) and a large review by Blaney et al. (92c) gives activity data on over 1700 compounds. For this reason only highlights of recent work will be given here to indicate the direction of current work.

While current compounds are still tested as DHFR inhibitors as well as TS inhibitors, recent work also includes tests for activity against folylpolyglutamate synthase (FPGS). As previously mentioned polyglutamylation of folates is necessary for cellular retention of quantities of folate, and elimination of this polyglutamylation (67) has resulted in a cell line which requires adenine, thymidine and glycine for cell growth (67). Therefore, inhibition of this enzyme could produce the same cellular effect as DHFR inhibition without the problem of retained folate analogues.

Structural analogues of FA fall into three categories:

(i) pteridine modified compounds,

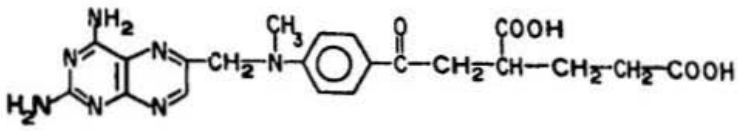
(ii) bridge region altered analogues, and

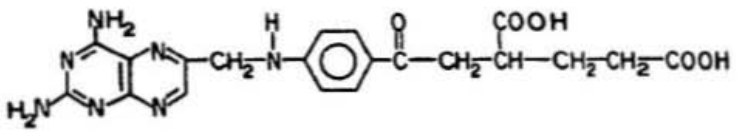
(iii) glutamic acid residue altered compounds.

Presented in Table 1 are some recently synthesized compounds with comparisons of structural modification to activity. As can be seen, all regions of the molecule are still receiving attention, as modifications are made to evaluate the requirements of different areas of folate biochemistry. There is such a variety of factors involved in inhibition of DHFR, TS and FPGS that simultaneous breakthroughs are being made with widely varying modifications of the classical folic acid structure.

It is interesting to note that very little work has been done on the amide linkage of the glutamate residue other than replacement of the carbonyl by a CH_2 (108) and the recent manipulation of shifting the CH_2CH_2COOH residue from the α-carbon to the amide nitrogen (105). The former indicated the necessity of the carbonyl group for good biological activity, but did little to evaluate the role of the nitrogen. Polyglutamylation plays an important role in both the inhibitory activity and chronic toxicity of MTX and, since the nitrogen of the amide must have some role to play in the binding of FPGS, it was decided to synthesize side chain analogues of MTX, AMT and FA which have the amide nitrogen replaced with a CH_2 group,

giving the following structures:

(VI) 

(VII) 

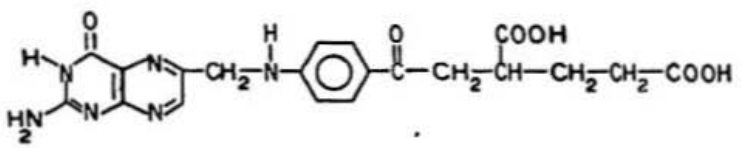
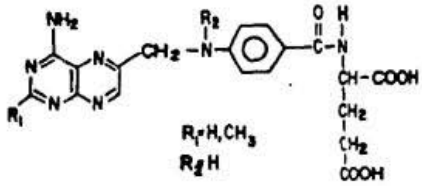
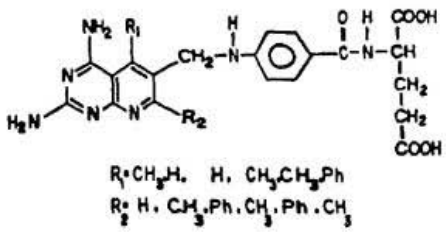
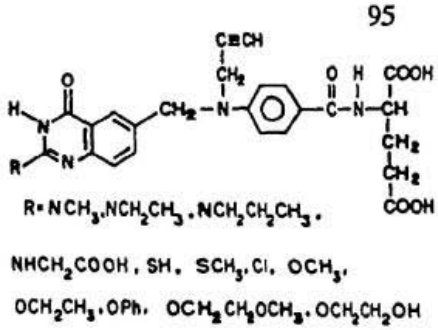
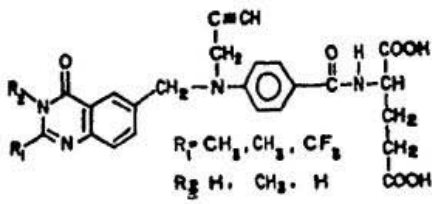
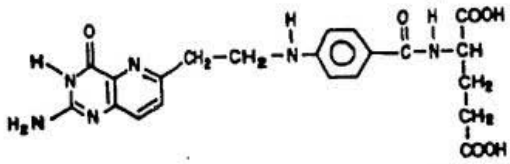
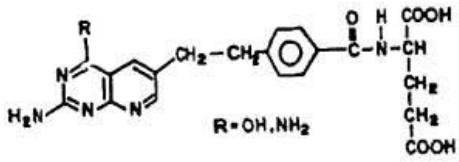
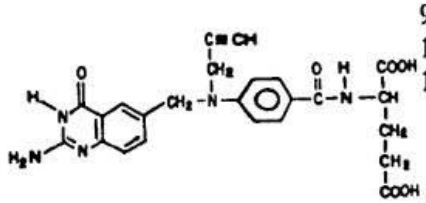
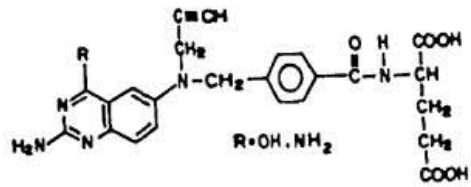
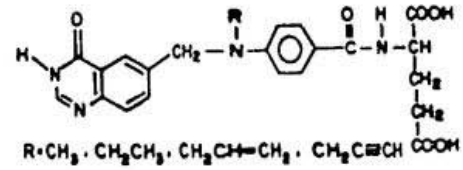
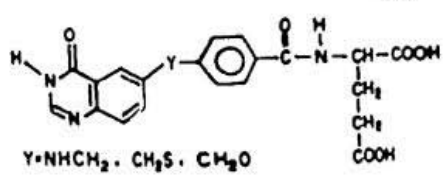
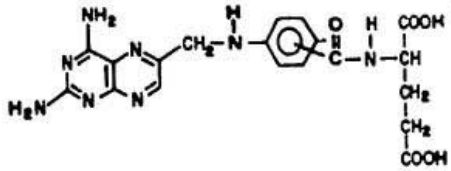
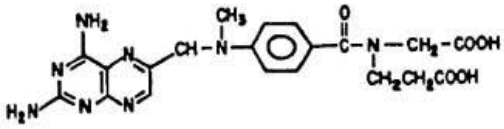
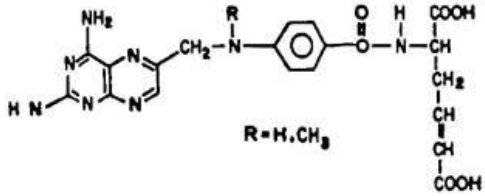
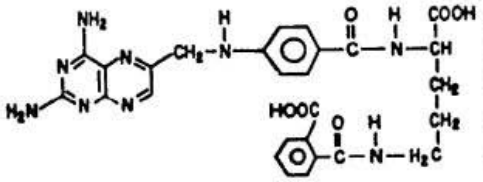
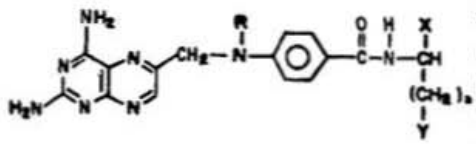
(VIII) 

Table I. Structure Activity relationships

Structure	Ref.	Change	Activity
	93	Replace the 2-amino group with hydrogen and methyl to give the 2-desamino and 2-desamino-2-methyl analogues of AMT.	Gives a 1000-fold decrease in activity against DHFR from human leukemic lymphoblasts (W1-L2 cells) relative to MTX, but with growth inhibitory activity against W1-L2 cells only 2 to 6 fold decreased for 2-desamino AMT and 2-desamino-2-methyl AMT respectively. Both compounds show good substrate activity for FPGS.
	94	C-5 and C-7 substituted analogues of 5-deaza AMT.	The 5-methyl compound is a potent inhibitor of growth and DHFR from L1210 but moving the methyl to C-7 greatly reduces its activity. All phenyl substituents reduce activity, but 7-methyl & 5,7-dimethyl analogues exhibit cell growth inhibition of L1210 while being extremely weak DHFR inhibitors.
	95	Replacement of the 2-amino group of N-propargyl-5,8-dideazafolic acid (CB3717) to evaluate C-2 analogues of this highly active compound.	All substituents other than OCH_3 show greatly decreased activity with the methoxy analogue showing slightly reduced activity against TS but slightly increased activity against L1210 cells in culture. The latter is probably due to increased aqueous solubility.
	96	C-2 & N-3 analogues of N-propargyl-5,8-dideazafolate CB3717 to improve the lipophilicity of this compound.	While 6 times less active then CB3717 against TS, the C-2 methyl compound was 40 times more potent against MANCA human lymphoma cells & 64 times more potent against wild type hepatoma cells (H35N). It enters the cell via the MTX/reduced folate transport system and its activity is

		reversed by thymidine administration. The N-H is vital for activity.
97 	The homofolic analogue of 8-deaza folic acid and its tetrahydro derivative were made.	Both show considerably less activity then their 8-deaza folate counterparts.
98 	The N deaza analogue of 5-methyl-5-deaza folic acid & AMT were made along with their tetrahydro derivatives.	All compounds are poor inhibitors of L1210 cell growth or isolated DHFR. The AMT analogue, which shows the best activity, is a potent inhibitor of bacterial DHFR and a moderate inhibitor of TS.
99 100 101 	The N-propargyl analogue of 5,8-dideaza folic acid was made.	Showed good activity against L1210 in mice & went through phase I & II clinical trials. Clinical use was stopped because of renal toxicity and hepatotoxicity.
102 	The bridge reversed isomers of CB3717 and N-propargyl-5,8-deaza AMT were prepared.	The N-propargyl-5,8-dideaza isofolic acid showed greatly reduced activity against TS & DHFR compared to CB3717. The N-propargyl-2,4-diamino compound also showed inferior activity vs TS compared to CB3717 but showed DHFR activity only 2 fold less than MTX.
103 	N-analogues of 2-desamino-5,8-dideaza folic acid were prepared.	Of this series the N-propargyl derivative shows the best activity against isolated L1210 TS and against cell growth. The cytotoxicity to L1210 cells is 8.5 times that of CB3717 despite being 8 fold less active against L1210 TS. It is also >340 fold more soluble than CB3717 at pH 7.4.
104 	Two bridge modified versions of 2-desamino-5,8-dideaza	These compounds show higher activity than their 2-amino counterparts but inferior activity

	isofolic acid were prepared.	when compared with N-propargyl-2-desamino-5,8-dideaza folic acid.
	105 The ortho & meta isomers of aminopterin were prepared.	The positional isomers show enormously reduced activity against isolated L1210 DHFR compared to AMT. They also failed to show either substrate or inhibitor activity vs mouse liver FPGS.
	105 The CH_2CH_2COOH side chain has been moved from the α-carbon to the amide nitrogen.	These compounds show weak inhibition of isolated DHFR as well as a lack of activity as a FPGS substrate. May be due to the inability of the amide to act as a proton donor.
	105 The glutamate side chain has been replaced with an unsaturated amino adipate residue.	These compounds were found to be equipotent with MTX & AMT as inhibitors of isolated DHFR but were 10-100 fold less effective against L1210 cell cultures, probably due to their inability to be polyglutamylated. This was confirmed when they failed to show substrate activity with FPGS.
	106 The Nδ-hemiphthaloyl derivative of Nα-(4-amino-4-deoxypteroyl)-L-ornithine (APA-L-ORN) was synthesised along with other Nδ-acyl derivatives.	This compound showed 2 fold less activity than MTX against isolated DHFR but a 6 fold increase against L1210 cell growth. Against human squamous cell carcinoma lines with moderate MTX resistance, this compound was 6-10 times more potent than MTX against the parent cell line. Against the parent cell lines its activity was 10-30 times that of MTX. Presumably the Nδ-acyl derivative is efficiently taken up into the cell then cleaved to APA-L-ORN which is a simultaneous inhibitor of DHFR & FPGS.

	107	A series of AMT & MTX analogues with varying chain lengths containing terminal phosphonic and sulfonic acid groups with and without an α-carboxyl were synthesised.	The activity of terminal sulfonic & phosphonic acid analogues against DHFR & FPGS requires an α-carboxyl group and when the terminal group is acidic, the optimal number of CH_2 groups is 2.

DISCUSSION

2.1 Synthesis of the Target Compounds

The first step in outlining a synthetic route for this project was a retroanalysis of the target compound **Scheme II** . There are always considerations to be made when developing a synthetic plan as these will influence the retrosynthetic analysis. In this case the following requirements were set:

(i) If the biological activity of the products warranted their use as drugs, then the synthesis should be based on inexpensive starting materials.

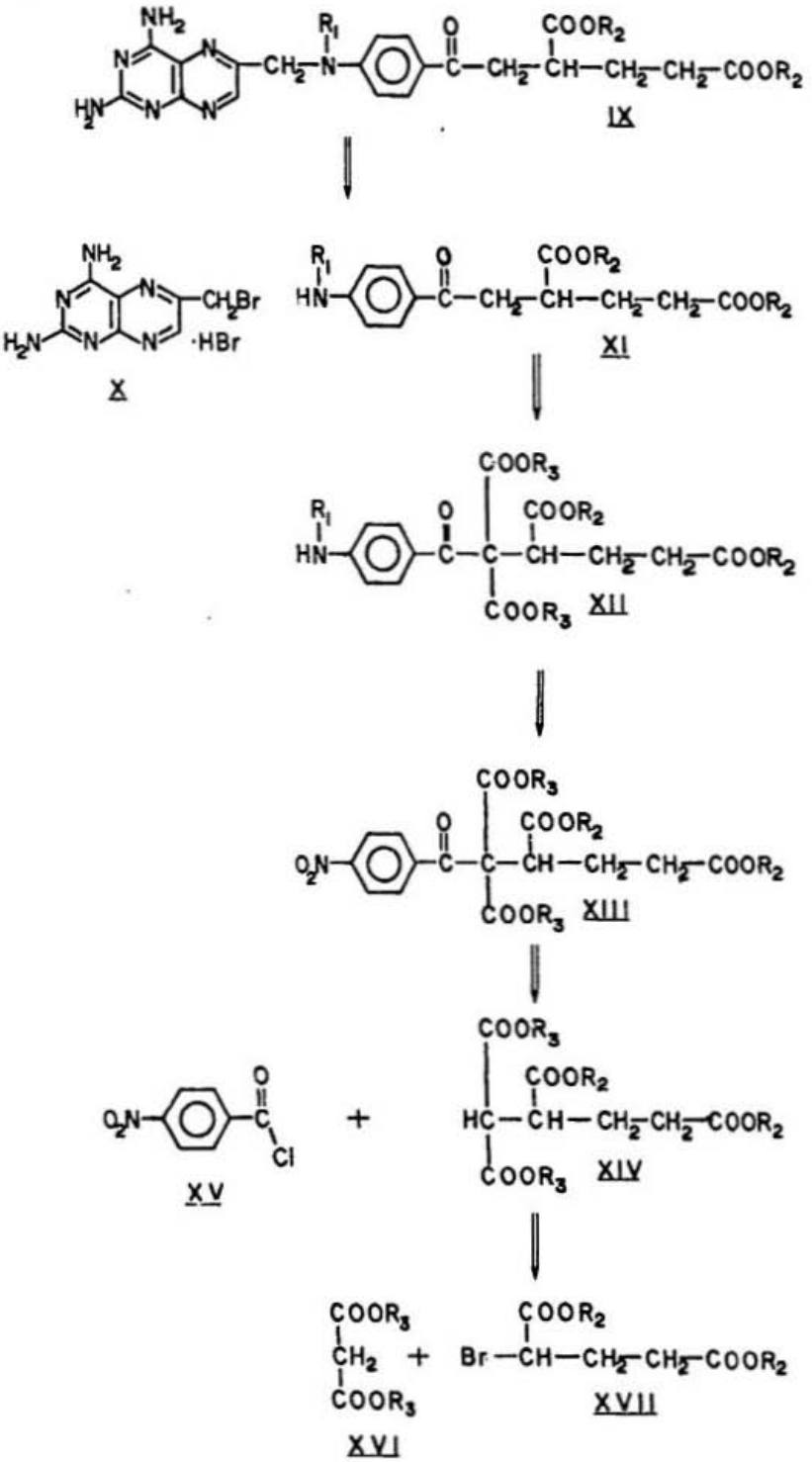
(ii) Due to the current interest in lipophilic MTX analogues, it was felt that some of the esterified intermediates might be useful to test for biological activity. For this reason the synthetic Scheme should be sufficiently flexible to allow the esters to be removed at either an early, or late stage in the synthesis.

(iii) The N^{10} nitrogen was envisaged as arising from a nitro group, as this should allow the flexibility of having the reduction and alkylation occur at more than one point in the synthesis.

(iv) The overall Scheme should be flexible to allow changes at as many steps as possible in the sequence

Current work on MTX analogues, makes use of a convergent synthesis involving the condensation of a pteridine (**X**) and a compound to provide an appropriate side chain (**XI**). 2,4-Diamino-6-bromomethylpteridine.hydrobromide (**X**) has previously been prepared by Baugh and Shaw (109) and this method, with some modification, was found acceptable to produce the ring system.

Scheme II

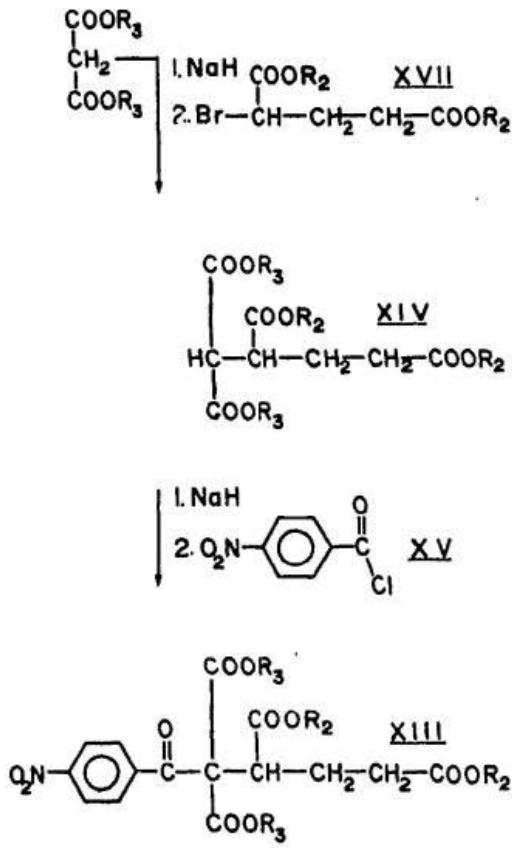


The synthetic route to the side chain (**XI**) could be kept flexible if the esters (R_2) were such that their removal could be achieved in non-basic media. This is a necessary condition if their removal was to be accomplished after condensation with the ring system, as the 4-amino group of the pteridine has been found to be easily converted to hydroxyl in mildly basic conditions, giving the folic acid analogue. Compound (**XII**) provides a very flexible route to (**XI**) via deesterification of the esters (R_3) and subsequent decarboxylation. Indeed if $R_2=R_3=t$-Bu, acid catalyzed deesterification of (**XII**) would lead directly to (**XI**), where R_2=H. Also the condensation of (**XII**) to the pteridine ring system (**X**), would give a compound which, upon treatment with dilute acid, could give the target compound (**IX**) where R_2=H. Indeed, with the current interest in esterified analogues of MTX, compound (**XII**) coupled to the pteridine ring system might make a good candidate for biological evaluation.

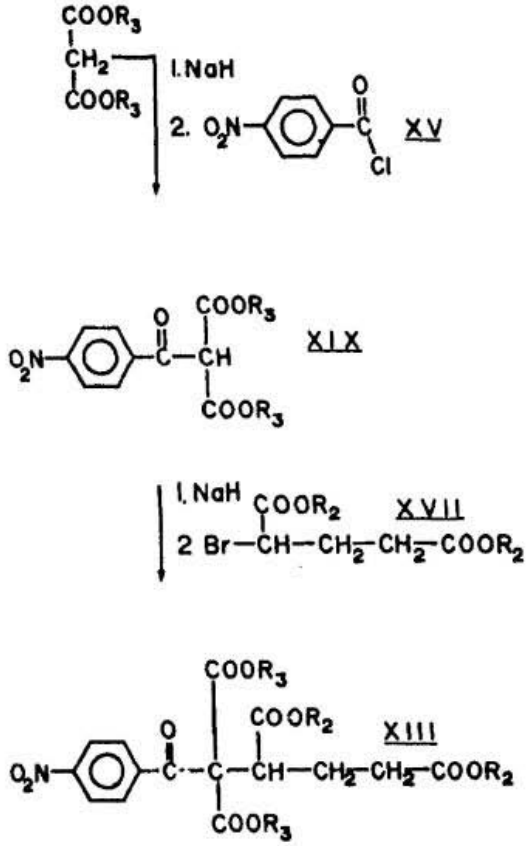
Compound (**XII**) can now be subdivided into three distinct sections for synthetic means, (**Scheme III**) a *p*-aminobenzoyl group and a glutaric acid derivative, which are coupled to a malonate moiety. Synthesis of this unit can be approached in several ways utilizing nucleophilic attack by the anion of di-*t*-butyl malonate.

The malonate anion could be alkylated with di-*t*-butyl 2-bromo-1,5-pentanedioate (**XVII**) **Scheme III**, giving di-*t*-butyl 2,3-dicarbo-*t*-butoxy-1,6-hexanedioate (**XIV**) which after treatment with sodium hydride could be acylated with *p*-nitrobenzoyl chloride (**XV**) to give di-*t*-butyl 2-*p*-nitrobenzoyl-2,3-dicarbo-*t*-butoxy-1,6-hexanedioate (**XIII**). Reduction of the nitro group of (**XIII**) would then give (**XII**), Alternatively the malonate anion could first be acylated with (**XV**) (**Scheme IV**) to give di-*t*-butyl 2-*p*-nitrobenzoylmalonate (**XIX**), which could then be alkylate with (**XVII**) to give (**XII**). In theory this latter route is less appealing as the anion of (**XIX**), being resonance stabilized, would be less nucleophilic than the anion of (**XIV**), and alkylation using (**XVII**) would be more difficult than acylation using (**XV**). This three segment approach to (**XII**) gives another possible route if the *p*-nitrobenzoyl chloride is

Scheme III



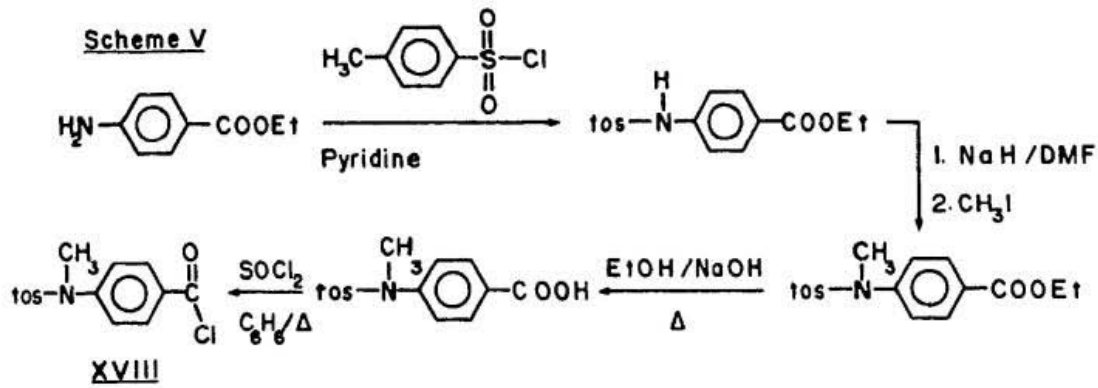
Scheme IV



replaced with Santi's compound (110) N-tosyl-N-methyl-*p*-aminobenzoyl chloride (**XVIII**): this provides a route to (**XII**) with $R_1=CH_3$ directly from (**XIV**), after the tosyl protecting group is removed.

Compound (**XVIII**) can be obtained via **Scheme V (110, 111)** which utilizes benzocaine as a starting material, and this could be obtained in large quantities as it is a product in the undergraduate laboratories.

Scheme V



XVIII

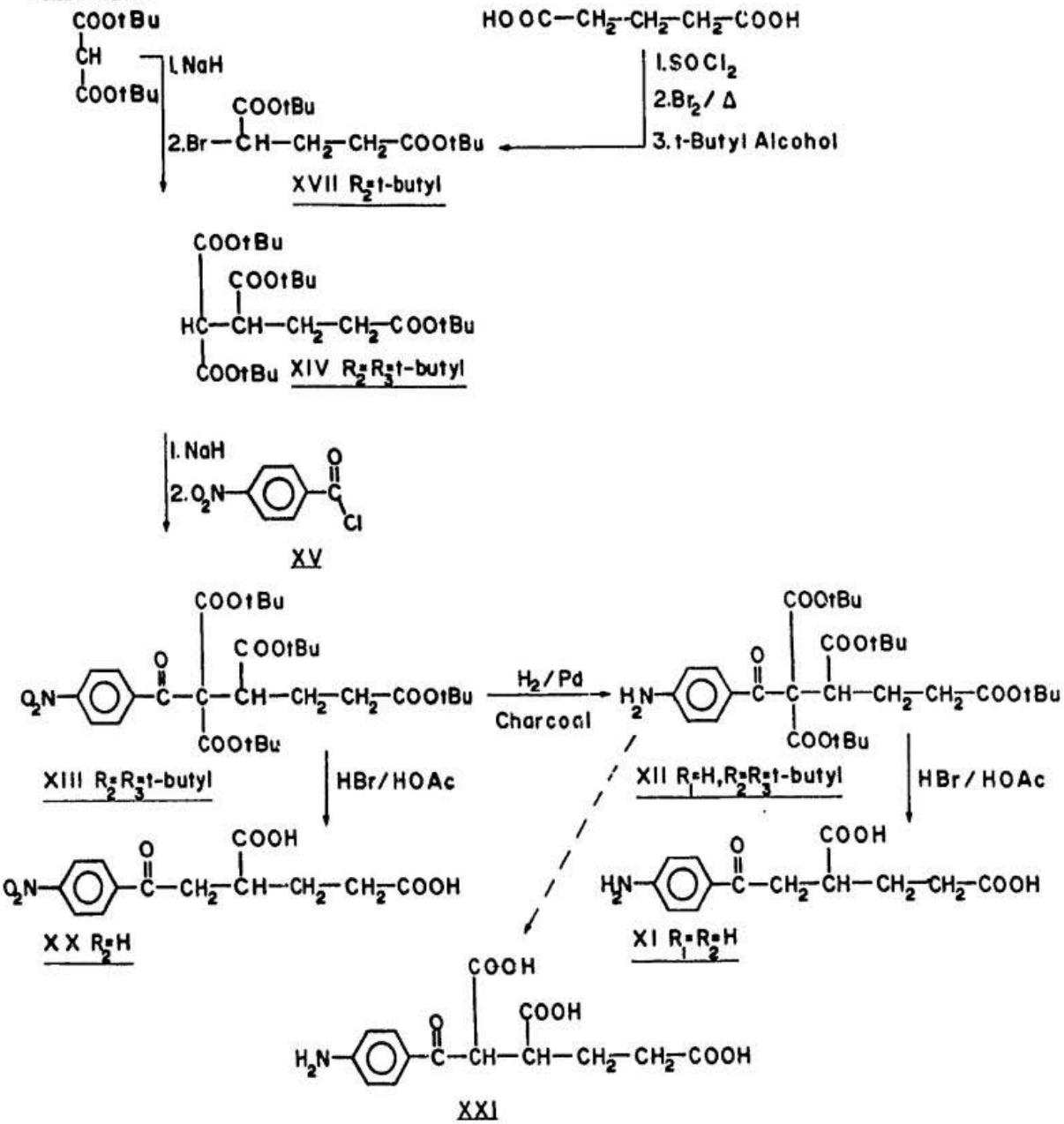
The first compound needed for the synthesis (**Scheme VI**) was (**XVII**) and this was obtained by a modification of Ingold's route to the diethyl ester(**112**). Glutaric acid was converted to the acid chloride by treatment with thionyl chloride, selectively α-brominated using the Hell-Volhard-Zelinsky method (**113**) and then converted to the di-*t*-butyl ester using *t*-butyl alcohol in the presence of dimethylaniline(**114**). Vacuum distillation gave (**XVII**, R_2=*t*-butyl) as a colourless oil. The yield however was very variable and always low, with a maximum overall yield of 24%. This was primarily due to the esterification procedure, as the bromoacid chloride could be isolated by vacuum distillation giving a corrected yield of 78%. The esterification was then tried using 10 molar equivalents of *t*-butyl alcohol with an equal volume of anhydrous ethyl ether. The reaction mixture was kept cold during the addition of the acid chloride and no acid scavenger was used. The yield by this method was consistently around 65% which was a vast improvement. An analytical sample was obtained by vacuum distillation giving colourless oil B.P.=108-111°C 1.5 mmHg. The ^{1}H nmr contained two singlets at δ=1.46 & δ=1.50 for the *t*-butyl esters, a multiplet at δ=2.13-2.55 for the -CHBrCH_2CH_2COOtBu protons and a multiplet at δ=4.15-4.47 for the -CH BrCH_2- proton. Di-*t*-butyl malonate was then treated with sodium hydride in THF to give the monoanion and this was alkylated with (**XVII**) to give the tetraester (**XIV**, where R_2=R_3=*t*-butyl) as a white

crystalline solid. While this compound, as isolated, was slightly impure, it proved most expedient to use the crude product in the next step. When required (**XIV**) was easily purified by chromatography to give an analytical sample for spectrographic analysis. The ^1H nmr spectrum of this compound showed singlets for the *t*-butyl esters with the malonate derived esters being equivalent and the malonate derived proton gave the expected doublet at $\delta = 3.49$ ($J = 10.0Hz$). The $CH(COOtBu)_2CH$ $(COOtBu)CH_2$- proton gave a multiplet at $\delta = 2.98$ and the glutarate derived CH_2 groups each gave complex multiplets, indicating that the bulkiness of the ester groups were preventing completely free rotation about the C-C bonds of the carbon chain.

Treatment of the tetraester (**XIV**) with sodium hydride in THF followed by acylation with (**XV**) gave the *p*-nitrobenzoyl tetraester (**XIII**, $R_2 = R_3 =$ *t*-butyl) as a white solid. Since this compound could be easily detected on UV fluorescent thin layer chromatography plates, it was decided to purify at this stage. The ^1H nmr for the purified (**XIII**) thus obtained showed the expected *t*-butyl ester singlets along with a broad multiplet at $\delta 1.75$-2.53 for the glutarate derived CH_2 groups. There was also a double doublet at $\delta = 3.12$ ($J = 2.99Hz$ $Jgem = 10.03Hz$) for the -CH $(COOtBu)CH_2$- proton and two doublets at $\delta = 8.06$ and $\delta = 8.25$ for the aromatic protons *meta* and *ortho* respectively to the NO_2 group.

Catalytic hydrogenation of (**XIII**) afforded the *p*-aminotetraester (**XII**, $R_1 = H$, $R_2 = R_3 =$ *t*-butyl). The reduction went smoothly with a linear H_2 uptake until the theoretical volume was reached. Workup gave an off-white solid which was pure by TLC. The ^1H nmr showed the expected singlets for the *t*-butyl esters and a broad multiplet at $\delta = 1$ 80-2.50 for the glutarate derived CH_2 groups, with a double doublet at $\delta = 3.08$ ($J = 3.44Hz$, $Jgem = 8.96Hz$) for the -CH $(COOtBu)CH_2$- group and a broad singlet at $\delta = 4.09$ for the amino protons. There were also two doublets at $\delta = 6.57$ and $\delta = 7.77$ ($J = 8.80$) for the aromatic protons *ortho* and *meta* respectively to the amino group.

Scheme VI



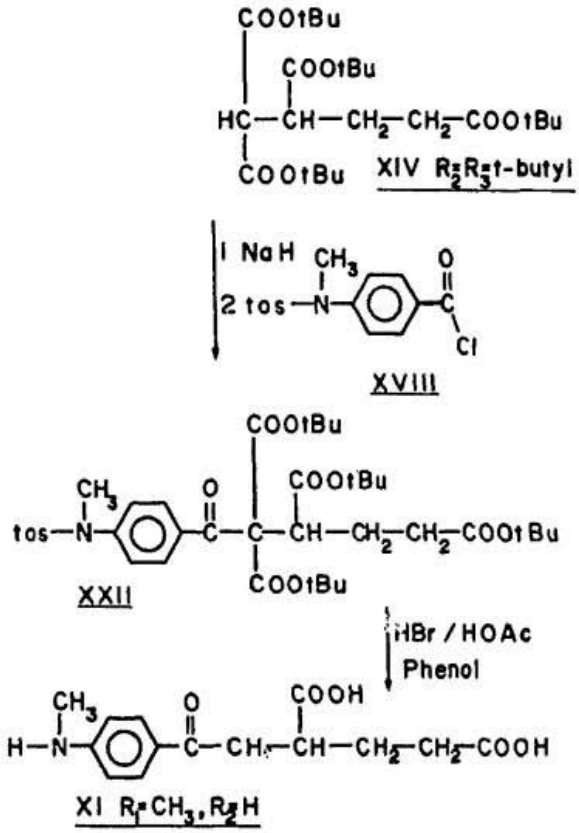
At this point de-*t*-butoxycarbonylation of the malonate derived *t*-butoxycarbonyls on the side chain compound(**XII**), to give the *p*-aminobenzoyl diester (**XI**, R_1=H, R_2=*t*-butyl),

prior to condensation with the pteridine ring system was attempted. Treatment of (**XII**) with sodium cyanide in wet DMSO (**115**) with heating to 140 °C. gave evolution of a gas which decolourized Br_2/CCl_4, but workup gave a brown oil which showed a decrease in the number and intensity of the *t*-butyl peaks in the ^1H nmr, but also showed loss of the aromatic protons. Another attempt using DMF instead of DMSO gave an insoluble yellow solid which also gave a very discouraging NMR. A second set of conditions using trifluoroacetic acid (TFA) in toluene at 100 °C. was tried with no noticeable reaction occuring. *p*-Toluene sulfonic acid (PTSA) was then added to this reaction mixture and a slow evolution of isobutene was noted at 100 °C.. Workup, after gas evolution had ceased, produced a brown solid which gave a ^1H nmr showing neither starting material nor recognizable product.

At this point deesterification using more classical reagents was tried. Compound (**XII**) was dissolved in glacial acetic acid (HOAc) and dilute HCl was added, with the mixture being warmed to 100°C. This gave upon workup a yellow solid with no *t*-butyl peaks in the ^1H nmr, but there were no signals assignable in the spectra.

After this discouraging halt in progress it was felt that perhaps the unprotected amino function may have in some way been causing the total decomposition that seemed to occur in the above attempts at deesterification and decarboxylation. It was decided that utilizing the N-tosyl-N-methylaminobenzoyl compound (**XVIII**) to acylate (**XIV**) might give a compound which would be more stable to the conditions necessary to achieve the deesterification (**Scheme VII**). Treatment of the tetraester (**XIV**) with NaH followed by acylation with (**XVIII**) gave di-*t*-butyl 2-(N-tosyl-N-methyl-*p*-aminobenzoyl)-2,3-dicarbo-*t*-butoxy-1,6-hexanedioate (**XXII**) as a white solid. The ^1H nmr of a sample purified by chromatography showed singlets at δ1.27, δ1.44, δ1.46 and δ1.52 for the *t*-butyl esters with a double doublet at δ3.11 (J = 2.58, Jgem = 10.33) for the -C*H*(COO*t*-Bu)CH_2- proton and multiplets at δ1.84-1.96, δ2.20-2.37 and δ2.45-2.53 for the glutaric derived protons. There were also

Scheme VII



doublets at δ=7.17 and δ7.85 (J=8.87) for the protons *ortho* and *meta* respectively to the amine, and doublets at δ7.20 and δ=7.39 (J=8.18 Hz) for the aromatic protons *ortho* and *meta* respectively to the methyl group, with singlets at δ2.40 and δ3.18 for the CH_3-Ar and CH_3-N protons respectively. Removal of the tosyl group from (**XXII**) could then be facilitated using a solution of 30% HBr in HOAc, with phenol present to remove any Br_2 liberated during the reaction (**110, 116**). Obviously these conditions should also remove the *t*-butyl esters, so it was felt that this treatment would yield the N-methylamino compound (**XI**, $R_1=CH_3$, $R_2=H$). However upon treatment with 30% HBr in HOAc for a variety of time intervals and at vari-

ous temperatures, (**XXII**) gave only a beige solid. This solid seemed quite consistent by ^1H nmr for each set of conditions, and showed loss of the carbon chain beyond the carbonyl. Since a quantity of the 30% HBr in HOAc had been prepared it was tried as a means of deesterifying the amino tetraester (**XII**). Reaction at room temperature for 3 hours gave a precipitate which was isolated as a light beige solid in good yield. The ^1H nmr spectrum of this compound in pyridine-d_5 showed it to be 2-*p*-aminobenzoyl-3-carboxy-1,6-hexanedioic acid (**XXI**), as it contained a multiplet at δ= 3.93-4.19 for the CH(COOH)C*H*(COOH)CH_2- proton and a doublet at δ=4.69 (J=10.26) for the -COC*H*(COOH)CH(COOH)- proton along with a multiplet at δ=2.63-2.85 for the CH(COOH)CH_2CH_2COOH protons and a multiplet at δ=2.85-3.19 for the -CH_2CH_2COOH group. There were also doublets at δ=6.97 & δ=8.34 (J=8.72) for the aromatic protons *ortho* and *meta* respectively to the amino group. This was very surprising as this compound is a β-keto-carboxylic acid. Apparently loss of the *t*-butyl esters had occurred and subsequently one of the malonate derived carboxylic acid groups had decarboxylated. However the second malonate carboxyl had remained attached to the carbon chain. It was felt that warming might be required but subsequent warming of the isolated product, while causing CO_2 evolution, resulted in decomposition of the compound into unrecognizable material. Also it was found that warming the initial reaction mixture resulted in a progressively lower yield of (**XXI**) until vigorous heating yielded no characterizable product at all. This proved that thermal decarboxylation was not going to work so chemical decarboxylation was tried. Treatment of (**XXI**) with 1,4-diazabicyclo-2,2,2-octane (**117**) in xylene gave no encouraging results. It was now decided that something was destabilizing the carbonyl to α-carbon bond of the triacid (**XXI**) causing it to break under conditions necessary to effect decarboxylation of the second malonate derived carboxyl. There is some literature precedent for this as described by Fonken and Johnson (**118** and references therein), where acylalkylmalonates preferentially undergo hydrolysis of the acyl-carbon bond *versus* decarboxylation,

but they report that the use of *t*-butyl malonate esters circumvents this problem. Since 30% HBr in HOAc had shown the best results in deesterifying these compounds, it was tried on the *p*-nitrobenzoyl tetraester compound (**XIII**). This, however, resulted in a *p*-nitrobenzoyl triacid compound similar to (**XXI**), which was less easily isolated. Again warming the reaction resulted in decomposition to a mixture with no recognizable isolatable material. Apparently the synthetic scheme was running into a problem at this point as all routes were designed on the premise that the malonate derived carboxyls could be decarboxylated. Since all routes based on this synthetic scheme must pass through this decarboxylation step it was essential that it work. As the triacid compound (**XXI**) could be isolated and all attempts at decarboxylating it resulted in decomposition, it was felt that this was the point where the previous attempts (see page 26) had also decomposed. The first step in overcoming this problem would seem to be identifying the cause of the β-keto acid stability. Several possible explanations for this were considered:

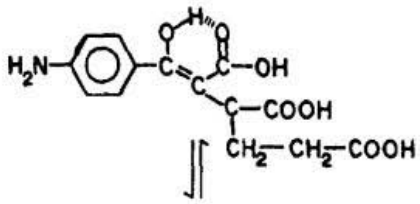
(i) if the initial decarboxylation compound was an enol with the wrong geometrical configuration and stabilized by intramolecular H-bonding as shown (**XXIII**), then the second CO_2 loss (XXIV) could be impeded. However the ^1H nmr of (**XXI**) shows no evidence of enol formation and gives a very clean doublet of doublets for the proton on the α-carbon.

(ii) formation of a unsaturated γ-lactone (**XXV**) would also explain the inability to achieve decarboxylation but as above the presence of the α-proton precludes this as a possibility.

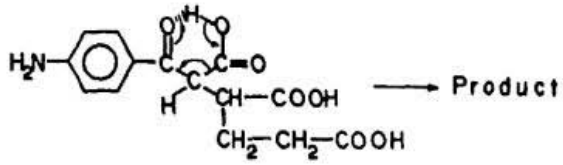
(iii) a third possible explanation would be the formation of an anhydride (**XXVI**). This structure could effectively prevent decarboxylation while satisfying the signals found in the ^1H nmr. However one would expect such a structure to give rise to an anhydride band in the I.R. spectrum of the compound, and no such band was found for (**XXI**).

At this point it seemed that only conformational restrictions remained as an explanation for the β-keto acid stability. Perhaps one of the other carboxylic acid groups could

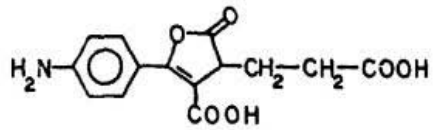
(XXIII)



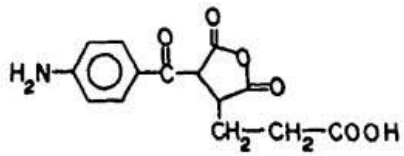
(XXIV)



(XXV)

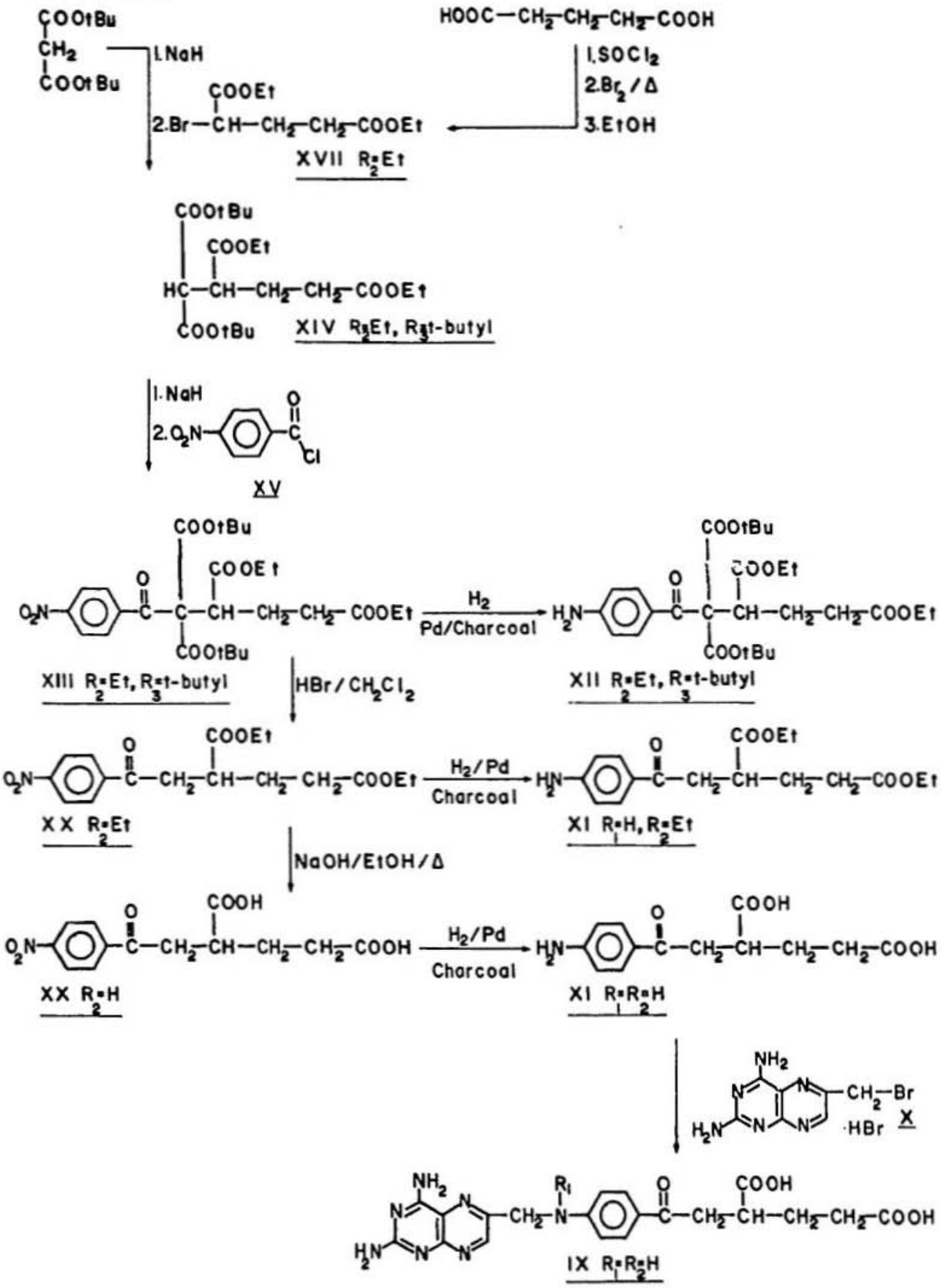


(XXVI)



be interacting with the C-3 carbonyl preventing it from achieving the correct spatial orientation with the keto-carbonyl to facilitate decarboxylation. If hydrogen bonding by one of these glutaric acid derived carboxyl groups was causing the interaction, then it might be prevented by keeping these esterified during the decarboxylation step. This could be done by switching from *t*-butyl to ethyl esters on compound (**XVII**, R_2=Et) in **Scheme VIII** . This would give a tetraester compound (**XIII**, R_2=Et,R_3=*t*-butyl) which would allow the malonate derived esters to be removed selectively to give the diethyl ester (**XX**, R_2=Et).

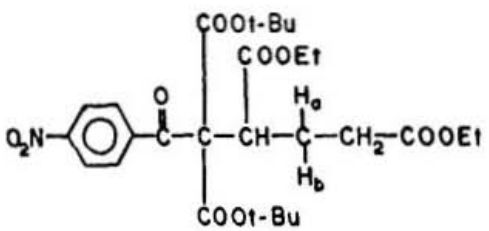
Scheme VIII


COOtBu
CH2
COOtBu
1.NaH
2.Br—CH—CH2—CH2—COOEt
COOEt
XVII R2=Et
HOOC—CH2—CH2—CH2—COOH
1.SOCl2
2.Br2/Δ
3.EtOH
COOtBu
COOEt
HC—CH—CH2—CH2—COOEt
COOtBu
XIV R2=Et, R3=t-butyl
1.NaH
2.O2N
O
C
Cl
XV
O2N
H2
Pd/Charcoal
H2N
XIII R2=Et, R3=t-butyl
XII R2=Et, R3=t-butyl
HBr/CH2Cl2
XX R2=Et
H2/Pd
Charcoal
XI R1=H, R2=Et
NaOH/EtOH/Δ
XX R2=H
XI R1=R2=H
NH2
N
CH2—Br
HBr
X
R1
CH2—N
IX R1=R2=H

The glutaric acid derived compound (**XVII**, R_2=Et) was prepared similarly to (**XVII**, R_2=*t*-butyl) by using ethanol instead of *t*-butyl alcohol. The di-*t*-butyl malonate anion was then alkylated with (**XVII**, R_2=Et) to give *t*-butyl ethyl 2-carbo-*t*-butoxy-3-carboethoxy-1,6-hexanedioate (**XIV**, R_2=Et,R_3=*t*-butyl) as a pale yellow oil. This compound was slightly impure by TLC but could be used as such in the next step. Purification by chromatography gave a colourless oil which was further purified by distillation. Along with the singlets from the *t*-butyl esters and the triplet-quartet patterns from the ethyl esters the ^{1}H nmr of (**XIV**, R_2=Et,R_3=*t*-butyl) showed a doublet from the malonate proton at δ3.55 with J=10.58Hz and a doublet of triplets at δ3.03 for the -CH(COOtBu)$_2$C*H* (COOEt)CH$_2$- proton along with a multiplet for each CH_2 group of glutarate protons. Clearly the protons of the CH_2 groups of the glutarate moiety are non-equivalent, resulting in much complication of the splitting patterns. This indicates that steric interaction is affecting free rotation of the carbon chain.

Compound (**XIV**, R_2=Et,R_3=*t*-butyl) was then treated with sodium hydride followed by *p*-nitrobenzoyl chloride to give *t*-butyl ethyl 2-*p*-nitrobenzoyl-2-carbo-*t*-butoxy-3-carboethoxy-1,6-hexanedioate, as a pale yellow oil (**XIII**, R_2=Et,R_3=*t*-butyl). This compound was slightly impure but could be purified easily by chromatography. However for synthetic ease it was best used without purification; careful purification was carried out at the next step. The ^{1}H nmr of purified (**XIII**, R_2=Et,R_3=*t*-butyl), a viscous colourless

(**XIII**)

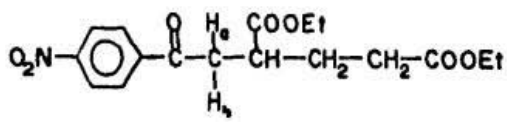


oil, showed a doublet of doublets at δ3.29 for the -C*H* (COOEt)CH$_2$- proton and a multiplet at δ2.30-2.45 for the -CH$_2$C*H* $_2$COOEt protons, along with the *t*-butyl ester singlets and the

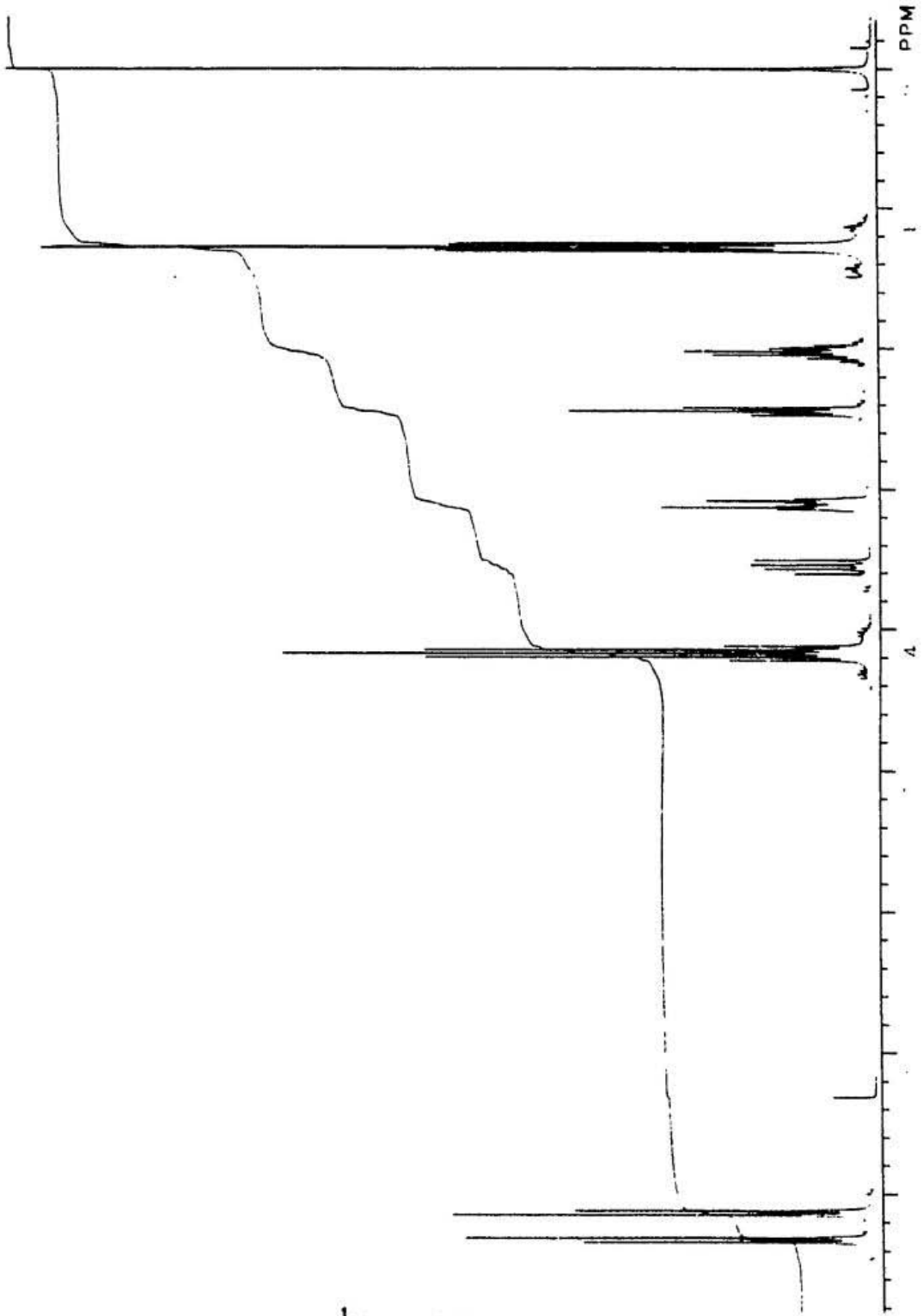
ethyl ester triplet-quartets. The -CH(COOEt)CH_2CH$_2$- protons gave two distinct signals, Hb at δ1.95-2.08 ppm and Ha at δ2.49-2.62 ppm, (these assignments were made on the basis of two models compounds which were subsequently prepared) and the aromatic protons gave two doublets at δ8.05 and δ8.26 with J=8.77Hz for the protons *meta* and *ortho* respectively to the nitro group.

The malonate derived *t*-butyl esters were then removed selectively by treatment with 30% HBr in HOAc to give the *p*-nitrobenzoyl diethyl ester compound (**XX**, R_2=Et). It was found that the isolation of this compound could be simplified by doing the deesterification in refluxing methylene chloride, while passing a stream of HBr(g) through the solution and monitoring the reaction by TLC. This gave the deesterified and decarboxylated compound (XX,R_2=Et), diethyl (2-(*p*-nitrobenzoyl)methyl)-pentane-1,5-dioate as a yellow oil in good yield. This compound was then purified on a large scale by chromatography using a Chromatotron, to give a pale yellow oil. The purification was done at this point as it was felt that the remaining steps could be done cleanly and purification after removal of the ethyl esters would be complicated by reduced solubility. The ^{1}H nmr of (**XX**, R_2=Et) showed the expected

(XX, R_2=Et)



triplet-quartet for the ethyl esters and two signals for the -COCH_2CH(COOEt)- protons. The Ha proton gave 4 peaks at δ3.55 (J=10.04Hz and Jgem=18.8Hz) while the Hb signal from this CH_2 was buried under the -CHC*H*(COOH)CH_2- signal as a multiplet at δ3.07-3.15. These assignments were confirmed by a ^{1}H-^{13}C correlation (**119**) which shows both these proton signals correlated to one carbon signal. The -CH_2CH_2COOEt- signal was a slightly distorted triplet δ2.44 (J=7.24Hz), and the -CH(COOH)CH_2CH$_2$- protons gave a multiplet

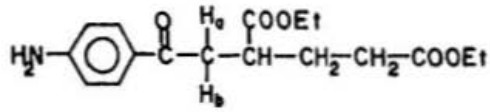


^{1}H nmr of **XX**, R_2 = Et

δ2.01 with the J=7.24Hz splitting from the terminal CH_2 visible in the pattern. The aromatic region contained two doublets one at δ8.13 (J=8.72Hz) for the protons *meta* to the nitro group and the other at δ8.74 (J=8.72Hz) for the protons *ortho* to the nitro group.

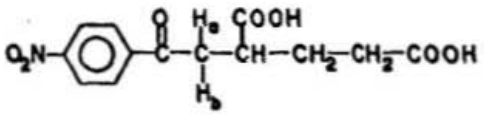
Catalytic reduction of (**XX**, R_2=H) afforded the amino ester (**XI**, R_1=H,R_2=Et). Hydrogen up-take was smooth to the theoretical amount and subsequent drying and evaporation of the solvent gave the amino compound as a pale yellow oil. An analytical sample was obtained by chromatography for spectral data and accurate mass analysis. The ^{1}H nmr showed the expected triplet-quartet for the ethyl esters and multiplets at δ=2.95-3.08 & δ=3.31-3.40 for Hb and Ha respectively. There was also a triplet for the -CH_2CH_2COOEt protons at δ=2.40 (J=7.44) and multiplets for the other glutarate derived protons, with doublets at δ=6.63 & δ=7.79 (J=8.70) for the aromatic protons *ortho* and *meta* respectively to the amino group and a broad singlet for the amine protons.

(**XI**, R_1=H,R_2=Et)



Hydrolysis of the esters at the nitrobenzoyl stage (**XX**, R_2=Et) could be achieved by using a solution of ethanol and 1.0 M NaOH with warming to 45° C to give, upon isolation, 2-(*p*-nitrobenzoylmethyl)-1,5-pentanedioic acid (**XX**, R_2=H) as an off-white soiid which was quite pure. An analytical sample was obtained by recrystalization from ethanol to give an off-white solid. The ^{1}H nmr showed a doublet of doublets at δ3.63 (J=9.38Hz,Jgem=18.26Hz) for Ha, and a doublet of doublets at δ=3.33 (J=3.94Hz,Jgem=18.26Hz) for Hb with a multiplet at δ=3.13 for the -CHaCHbC*H*(COOH)CH_2- proton. It also contained multiplets at, δ=2.45-2.57 for the -CH_2CH_2COOH protons and δ=1.94-2.12 for the -CH(COOH)C*H*$_2CH_2$- protons, along with doublets at δ=8.37 & δ=8.30 (J=8.80Hz) for the aromatic protons

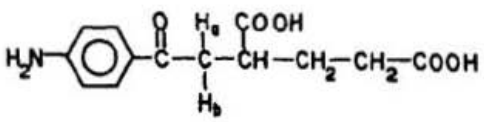
(XX, R_2=H)



ortho and *meta* respectively to the nitro group.

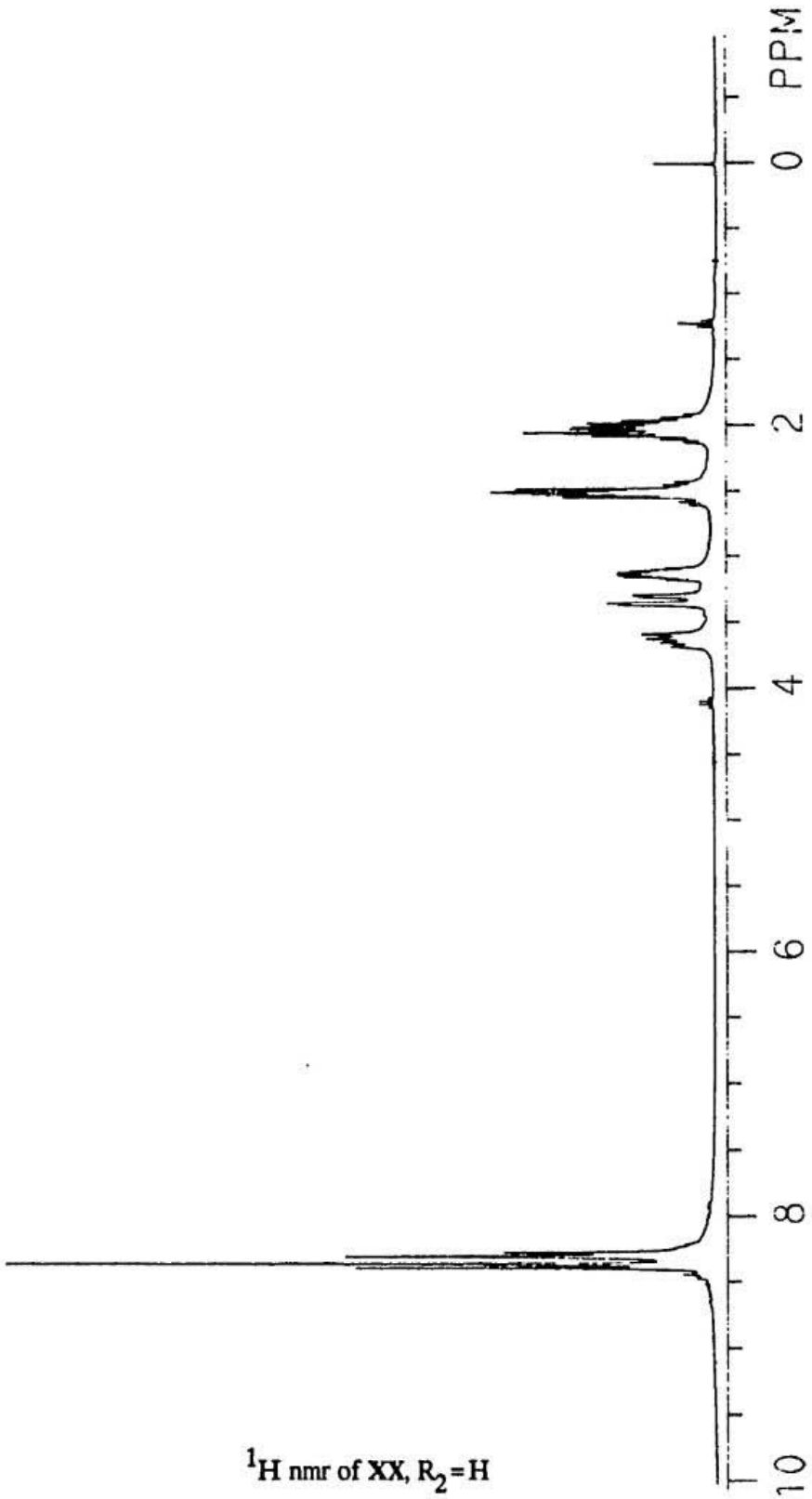
Catalytic reduction of (**XX**, R_2=H) gave 2-(*p*-aminobenzoylmethyl)-1,5-pentanedioic acid (**XI**, R_1=R_2=H) as a

(XI, R_1=R_2=H).



beige solid which was pure by TLC. The ^{1}H nmr contained a double doublet at δ3.34 (J=8.91Hz, Jgem=17.13Hz) for Ha and a multiplet at δ2.92-3.05 for Hb along with multiplets at δ1.87-1.97 and δ2.36-2.47 for the -CH_2CH_2COOH and -CH_2CH_2COOH protons respectively. It also contained two doublets at δ6.63 and δ7.75 (J=8.65Hz) for the aromatic protons *ortho* and *meta* to the amino group respectively.

The amino acid (**XI**, R_1=R_2=H) was then condensed with the pteridine ring system (**X**) by stirring them together in dimethylacetamide (DMA) under N_2 at room temperature while protected from light for 72 hours. The DMA was removed under reduced pressure and the beige solid isolated from water by centrifuge. Washing with methylene chloride yielded (**IX**, R_1=R_2=H) as a yellow/beige solid. The ^{1}H nmr showed the expected multiplets for the glutarate derived methylenes, a multiplet for the -C*H* (COOH)- proton, double doublets for the Ha and Hb protons, AA' BB'doublets for the side chain ring system and a singlet at δ=8.81 for the C-7 proton, with a doublet at δ4.63 (J=4.72Hz) for the C_9-CH_2 group split by the N^{10} amino proton which was a triplet at δ7.23 (J=4.62).



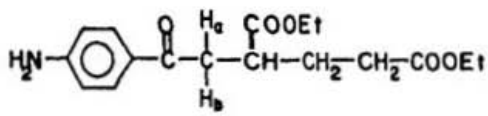
^{1}H nmr of XX, R_2 = H

These latter peaks indicated that the coupling reaction joining the ring system to the side chain had proceeded to give the expected product and this was confirmed by a FAB mass spectrum which gave a strong M+1 molecular ion at 440.

2.2 Alternative Routes to the Side Chain Target Compound

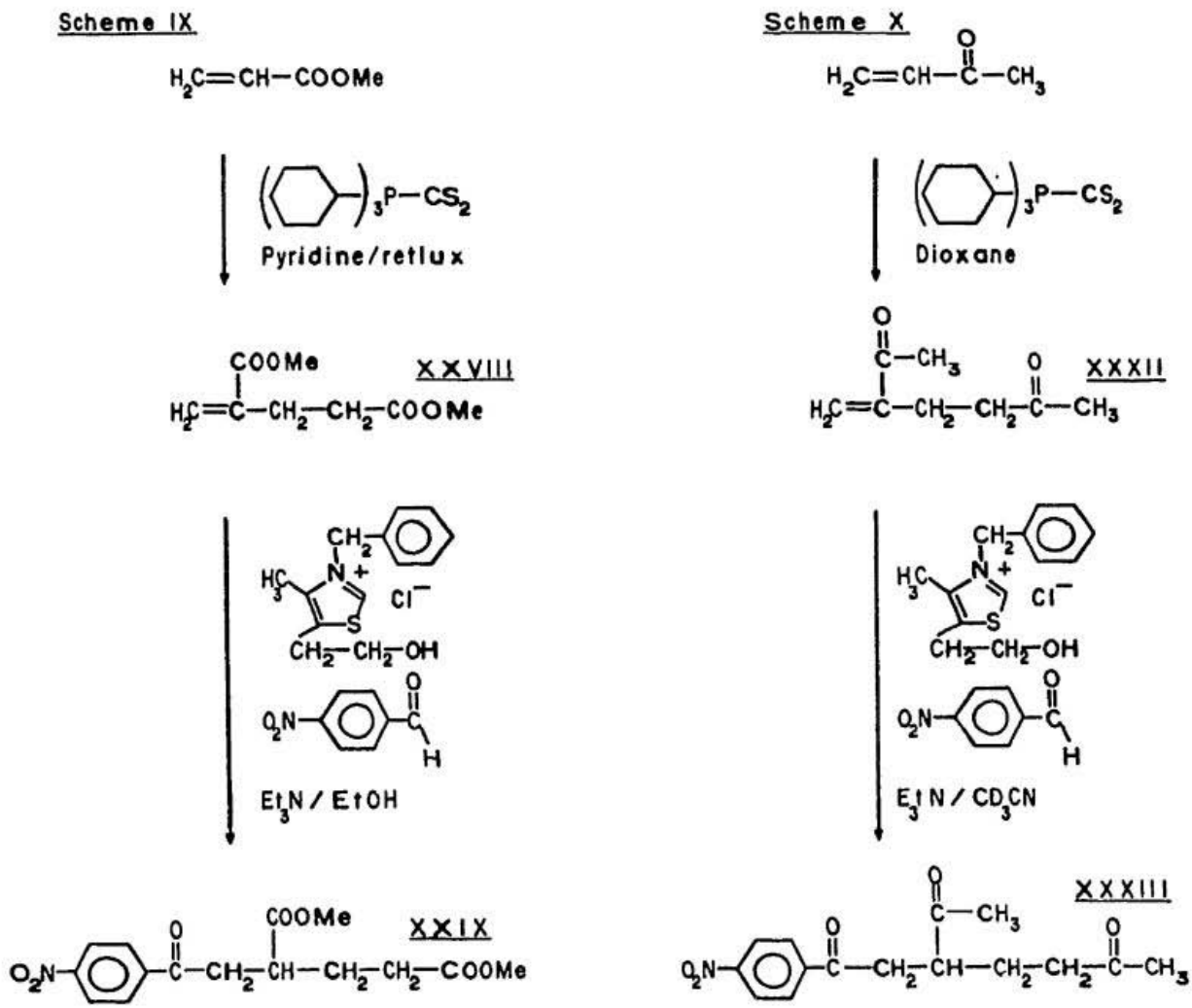
The main objective was still the synthesis of the *p*-aminobenzoyl diacid compound (**XI**, $R_1 = R_2 = H$), but without going via the decarboxylation step which was causing so much trouble (see page 21) in the main route. Since the principle objective in

(XI, $R_1 = R_2 = H$)



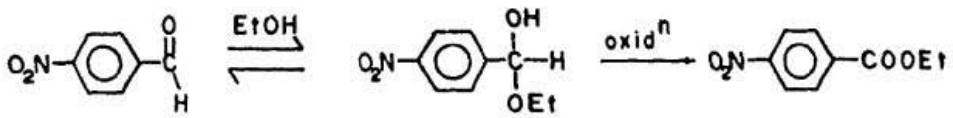
these attempts was to obtain the desired side chain compound (**XI**, $R_1 = R_2 = H$), less emphasis was placed on cheap reagents, easy to scale-up methods or flexibility of route.

The first of these alternative routes was based on a retroanalytical bond disconnection between the aryl carbonyl and the rest of the side chain, which suggests an approach based on an "umpolung" Michael addition of the type described by Steller (**120, 121**) **Scheme IX**. Methyl acrylate was dimerized (**122**) by refluxing in freshly distilled pyridine in the presence of a tris-cyclohexylphosphine carbon disulfide complex under argon. Work-up and vacuum distillation gave dimethyl 2-methylene-1,5-pentanedioate (**XXVIII**, $R = CH_3$) as a colourless oil. A thiazolium salt catalysed Michael addition was then tried in order to condense this compound to *p*-nitrobenzaldehyde. The unsaturated ester (**XXVIII**, $R = CH_3$) and thiazolium salt were stirred in ethanol, and a mixture of *p*-nitrobenzaldehyde plus triethylamine in ethanol was added under argon at room temperature (**121**). The mixture rapidly turned deep



purple and remained this colour although a little darkening occurred overnight. Work-up yielded a discouraging mixture which contained neither the starting material nor required product, but instead contained ethyl *p*-nitrobenzoate (**XXX**). Small scale test tube tests showed that the aldehyde plus the thiazolium salt and triethylamine in ethanol would generate the deep purple colour and this was believed to indicate formation of the anion. However, if these three reagents were mixed overnight under argon, work-up afforded no aldehyde starting

material, but rather gave ethyl *p*-nitrobenzoate (**XXX**) in 50-60% yield. Since there was no chance of atmospheric oxidation and only a catalytic quantity of the thiazolium salt was used, this may have been a Cannizzaro reaction. However isolation of the *p*-nitrobenzyl alcohol which should also be present proved impossible, probably due to the work-up needed to remove the thiazolium salt. This product could also be the result of a reaction involving a hemiacetal (**XXXI**) but again it is unclear what was responsible for the oxidation of (**XXXI**)



XXXI

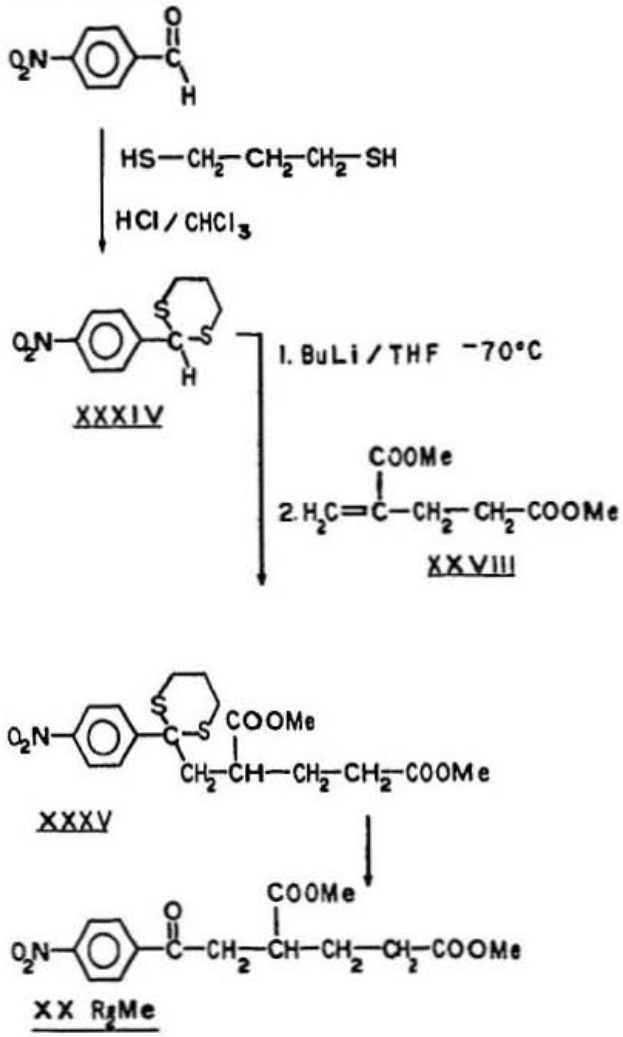
in the last step. The reaction of the *p*-nitrobenzaldehyde was repeated using deuterated acetonitrile as solvent instead of ethanol, with the mixture being monitored by ^1H nmr for 48 hours, but no reaction was noted. Work then switched to **Scheme X** which involved the same sort of thiazolium salt catalysed reaction but used 2-methylene-2,6-heptanedione (**XXXII**) in place of (**XXVIII**). Since acetyl is a better electron withdrawing group than ester, this compound should be a better Michael accepter then (**XXVIII**), and it could be converted to the diacid by treatment with $NaOH/I_2$. Compound (**XXXII**) was prepared from methyl vinyl ketone (**123**) using tris cyclohexylphosphine carbon disulfide complex and adipic acid in dioxane, in the presence of hydroquinone. This produced (**XXXII**) as a colourless oil after vacuum distillation.

The Michael condensation of (**XXXII**) to *p*-nitrobenzaldehyde using triethylamine and the thiazolium salt was then tried in deuteroacetonitrile but gave no evidence of success by nmr. At this point it was decided that further work utilizing the thiazolium salt was not warranted.

Efforts then switched to the alternative "umpolung" Michael addition reaction **Scheme XI** which utilized the dithiane anion, by using butyl lithium to generate an anion on the

dithiane protected *p*-nitrobenzaldehyde.

Scheme XI

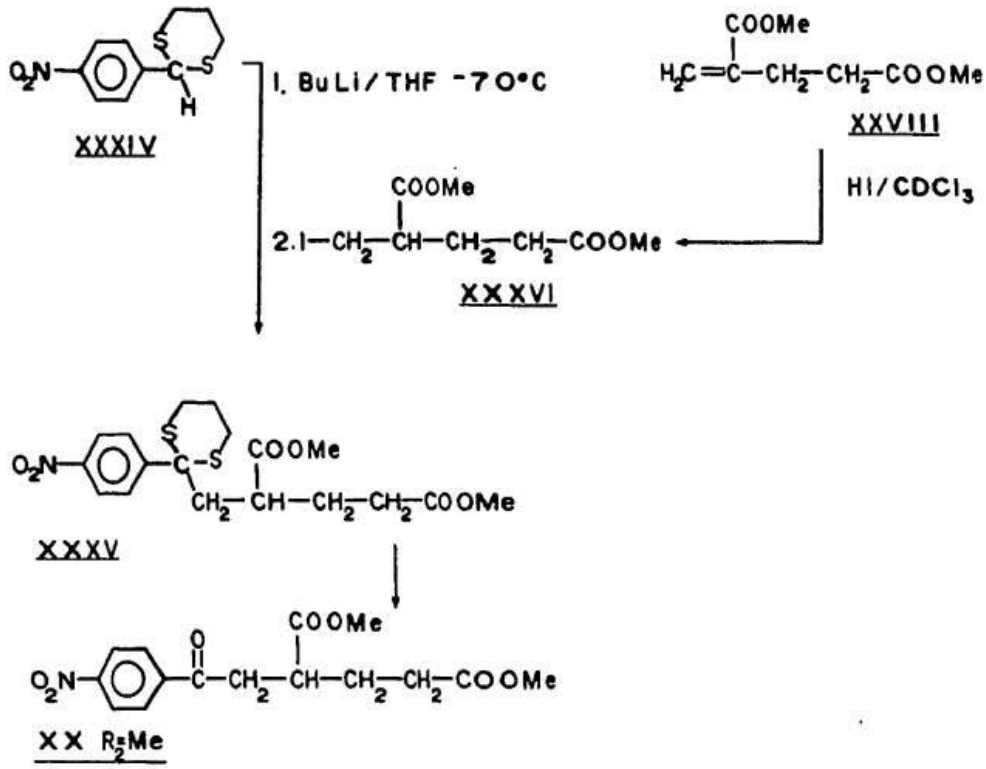


The dithiane of *p*-nitrobenzaldehyde (**XXXIV**) was prepared by stirring *p*-nitrobenzaldehyde and 1,3-propanedithiol in $CHCl_3$ while passing a HCl(g) stream through the mixture (**124 -126**). This gave, after work-up, the dithiane as a pale yellow solid. Compound (**XXXIV**) was then dissolved in THF under argon and cooled to -78°C in a dry ice/acetone bath. Butyllithium in hexane was then added (**124**) to give a reddish/orange solution and this was treated with (**XXVIII**) and stirred over night with warming to room

temperature. Work-up produced an orange oil which crystallized on standing. The ^1H nmr spectrum of this solid showed it to be a mixture of the two unreacted starting materials. Since it was felt that the anion was being formed, this indicated that the α,β unsaturated ester (**XXVIII**) was not sufficiently susceptible to nucleophilic attack for this reaction to proceed. This problem was probably complicated by the stability of the anion of (**XXXIV**). Since the nitro group would be electron withdrawing, it would help delocalize the negative charge of the anion, thereby making it less reactive as a nucleophile. Perhaps the best way around this problem would be either to make (**XXVIII**) a better alkylating agent or to make the anion less stable and therefore more reactive. An approach to the former, **Scheme XII**, was achieved by reacting the unsaturated ester (**XXVIII**) with HI(g) in $CHCl_3$ (**127**) to give dimethyl 2-iodomethyl-1,5-pentanedioate (**XXXVI**) as a colourless oil. The anion of (**XXXIV**) was then reacted with (**XXXVI**) at -78°C with warming to 0°C over 4.5 hours. Work-up gave an orange oil which proved to contain at least 9 bands by prep. TLC. Separation gave two major fractions which proved to be the unreacted dithiane (**XXXIV**) and dimethyl 2-methylene-1,5-pentanedioate (**XXVIII**), which must have been regenerated from (**XXXVI**) by elimination of HI. Catalytic reduction of the nitro group to amino would have presumably generated a more reactive anion but this was not tried since by this time success had been achieved by the initial route. As a second approach to a more reactive alkylating agent, **Scheme XIII**, a Michael addition using the anion of dithiane (**XXXIV**) and 2-methylene-2,6-heptanedione (**XXXII**) (**Scheme XIII**) was also tried for completeness sake, but gave a mixture of unreacted starting materials and decomposition products. At this point further work along this line was terminated and the methodology switched to using dithiane as a linking group (**128**) between a *p*-nitrobenzoyl group and a glutarate moiety **Schemes XIV, & XV**.

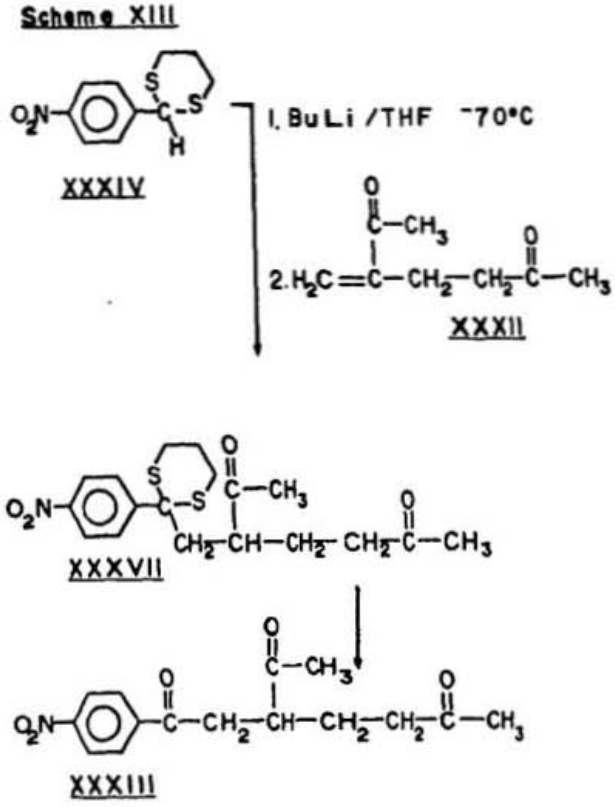
In this scheme 1,3-dithiane would play the same part as the malonate moiety had played in the initial reaction scheme (**Scheme III**). However, since the dithiane protons would be

Scheme XII



less acidic than the malonate protons, butyl lithium would need to be used instead of sodium hydride. In Scheme XIV the dithiane was stirred in THF at -78°C under argon and butyl lithium was added to give a colourless solution. Addition of the *p*-nitrobenzoyl chloride in THF gave an orange/red colour within seconds, and the mixture was kept stirring at -78°C for 7 hours. It was then allowed to warm to room temperature over night and following work-up gave an orange oil. Separation of this oil by preparative TLC gave 5 bands, none of which showed any characteristics of (XXXVIII) in the ^{1}H nmr. Scheme XV was then tried by treating the dithiane anion with the bromo di-*t*-butylester compound (XVII, R_2=*t*-butyl) using the

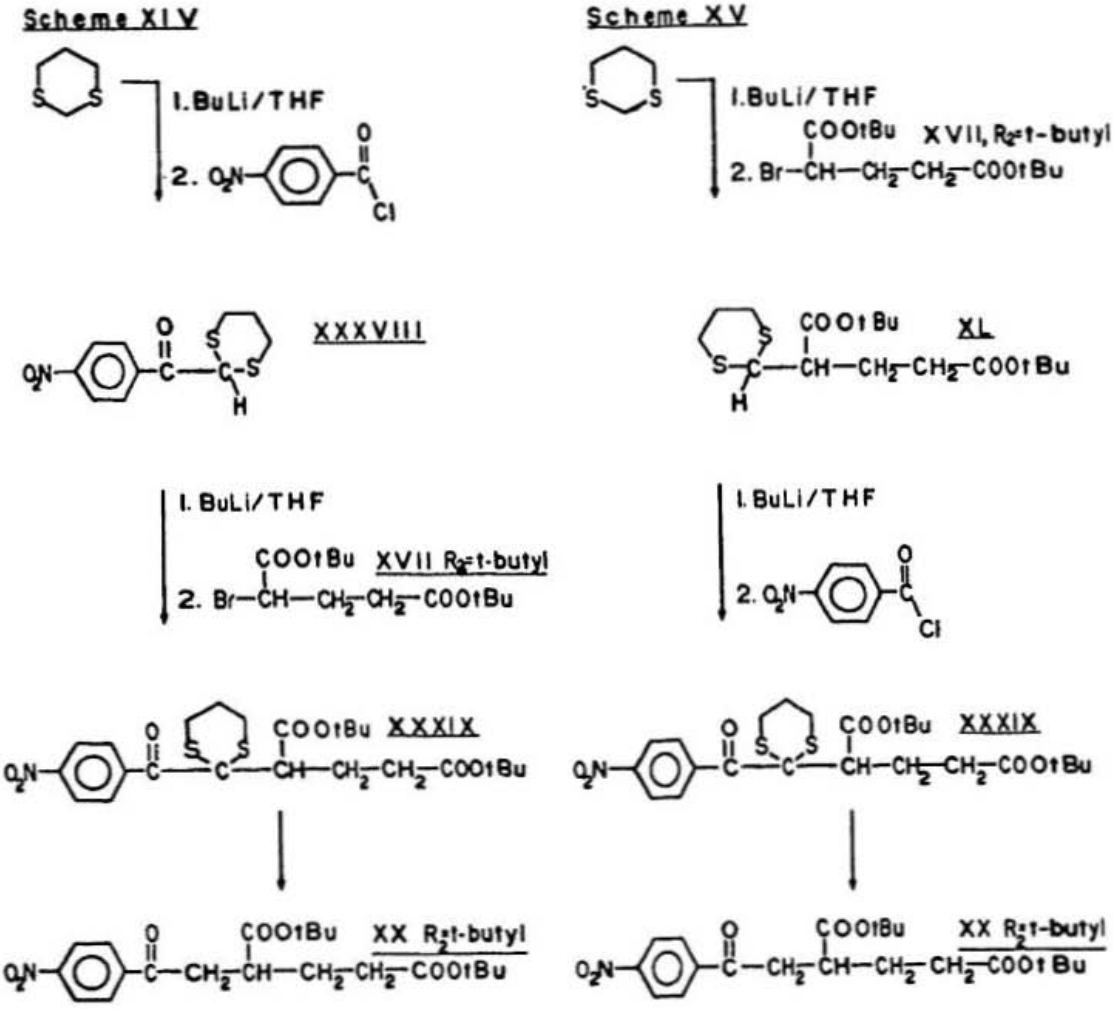
Scheme XIII



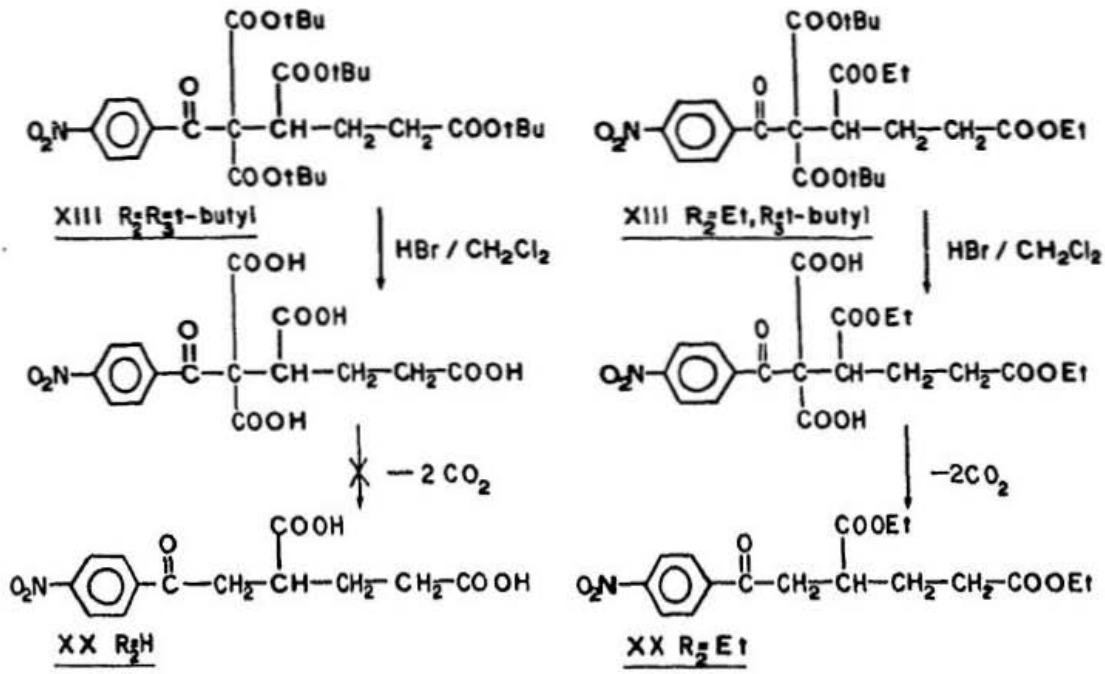
same conditions as above. Work-up gave a yellow oil which was mostly unreacted starting material but contained a small amount of two unidentifiable products, which showed none of the expected characteristics for (XL). At this point further work on alternative routes to the target compounds was terminated.

2.3 Preparation of Models to Investigate the Decarboxylation Problems Encountered in Schemes VI & VII.

It has been seen that deesterification and decarboxylation of the malonate derived carbonyls of (**XIII**, $R_2 = R_3 = t$-Bu) could not be achieved when the glutarate-derived esters were



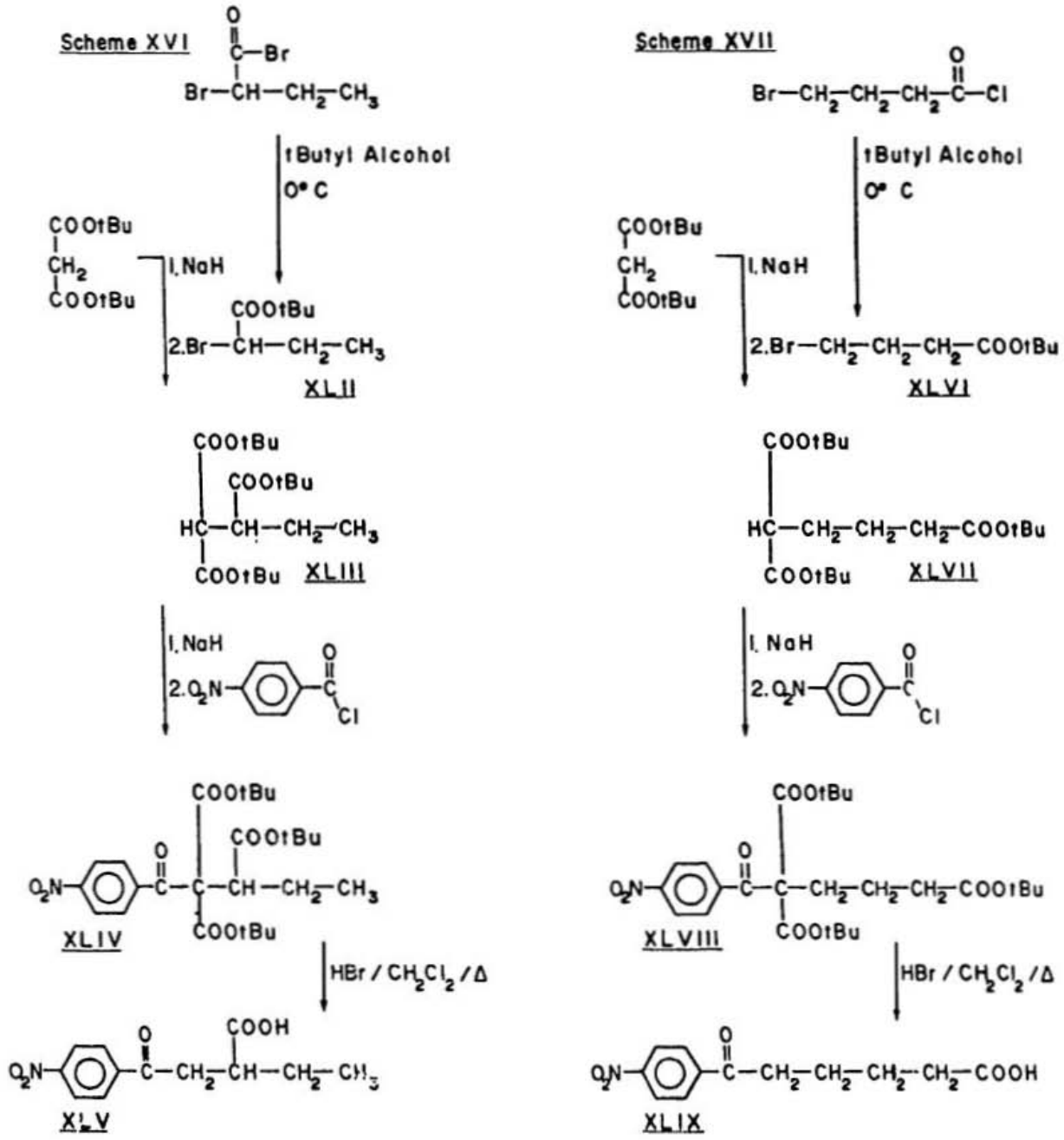
simultaneously removed, but this process could be performed on compound (**XIII**, R_2=Et,R_3=*t*-Bu) where the glutarate derived ethyl esters remained intact during the deesterification and decarboxylation. This suggests that the presence of the glutarate carboxyls as acids somehow causes some intramolecular steric interaction which prevents loss of the second malonate-derived carboxyl, or some alteration in the bond strength of the carbonyl to α-carbon bond which causes hydrolysis of this acyl-carbon bond to be the energetically



preferred process. However, why the presence of a carboxylic acid would have a different effect on this bond strength than the presence of an esterified carboxyl is not clear. The latter case is supported by the chemistry of the β-keto acid (**XXI**) which was isolated upon deesterification of the aminotetraester (**XII**, R_1=H, R_2=R_3=*t*-Bu). As has been seen, all efforts to decarboxylate this compound resulted in failure and an effort to esterify it, to obtain a crystallizable compound, resulted in the formation of a mixture whose ^{1}H nmr spectrum was consistent with the presence of methyl *p*-aminobenzoate and the trimethyl ester of the remainder of the side chain, although these compounds were not isolated. This appears to confirm the hydrolysis of the acyl-carbon bond. In the case of steric interaction, it would have to be an interaction which prevents the malonate from attaining the correct spatial orentation with the β-keto carbonyl to facilitate its decarboxylation. It was felt that the first step towards

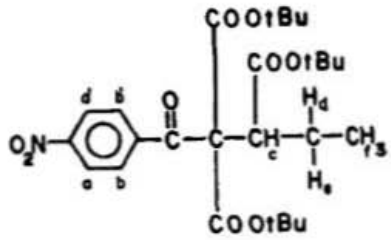
understanding this observed anomaly would be to decide which one of the glutarate derived carboxylic acid groups was causing the interaction, if in fact only one was. Synthesis of two analogues, **Schemes XVI & XVII**, of (**XIII**, $R_2=R_3=t$-Bu), each containing only one of the glutarate derived esters, would be the most straight foward way of getting this information.

Compound (**XLII**) was obtained from 2-bromobutyryl bromide by treatment with *t*-butyl alcohol in diethyl ether at 0°C. Work-up followed by vacuum distillation gave *t*-butyl 2-bromobutanoate (**XLII**) as a colourless oil. The ^{1}H nmr spectrum showed the expected triplets at δ4.06 and δ1.02 for the -C*H* $BrCH_2$- proton and C*H* $_3CH_2$- protons respectively. The *t*-butyl ester protons gave a singlet at δ1.48 and the -CHBrC*H*$_2CH_3$ protons gave a multiplet containing 13 lines from δ1.88-2.17. Treatment of di-*t*-butyl malonate with sodium hydride in THF followed by (**XLII**) gave after work-up *t*-butyl 2,3-dicarbo-*t*-butoxypentanoate (**XLIII**) as a pale yellow oil, which was sufficiently pure to be used in the next step. An analytical sample was purified by vacuum distillation to give a colourless oil. The ^{1}H nmr showed three singlets at δ1.447*, δ1.454* and δ1.46 for the *t*-butyl ester protons, a doublet at δ3.49 for the malonate derived proton, and a triplet at δ0.94 for the terminal methyl group. It also showed a multiplet containing 5 lines which was assigned to the -CH(COOtBu)C*H*$_2CH_3$ protons and a multiplet containing 8 lines at δ2.86 for the -C*H* (COOtBu)CH_2- protons. The triethyl ester compound (**XLIII**) was then treated with sodium hydride in THF followed by acylation with *p*-nitrobenzoyl chloride to give crude *t*-butyl 2-*p*-nitrobenzoyl-2,3-dicarbo-*t*-butoxypentanoate (**XLIV**), which was purified by chromatography to give a viscous colourless oil. (* reported to 3 decimal places to show nonequivalence) The ^{1}H nmr spectrum of (**XLIV**) gave two separate multiplets for protons d and e, one at δ1.91-2.10 containing 13 lines and one at δ1.60-1.74 also containing 13 lines. These assignments were confirmed by a ^{13}C-^{1}H correlation (**119**). This matched these two signals to the same carbon signal, which by ^{13}C APT. spectrum contained 2 protons. The ^{1}H spectrum also showed a triplet at δ1.09 for Hf and a double doublet at



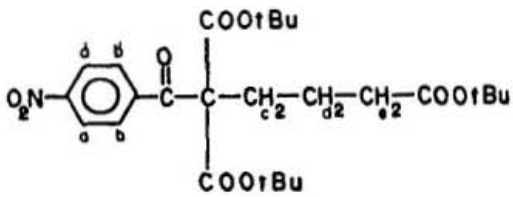
δ3.04 (J = 2.40Hz & J = 10.48Hz) for Hc, as well as two slightly distorted doublets at δ8.25 & δ8.04 for the aromatic protons Ha,Ha' and Hb,Hb' respectively.

(XLVI)



Compound **(XLVI)**, **Scheme XVII**, was obtained from 4-bromobutyryl chloride by treatment with *t*-butyl alcohol in diethyl ether at 0°C. Work-up followed by vacuum distillation gave *t*-butyl 4-bromobutanoate as a colourless oil. The ^{1}H nmr showed the expected singlet for the *t*-butyl ester at δ1.44, and two triplets at δ3.45 & δ2.39 for the CH_2 $BrCH_2$- and -CH_2CH_2COOtBu protons respectively. There was also a multiplet containing 5 peaks at δ2.11 for the -CH_2CH_2COOtBu protons. Di-*t*-butyl 2-carbo-*t*-butoxy-1,6-hexanedioate **(XLVII)** was obtained by treating di-*t*-butyl malonate with sodium hydride in THF followed by **(XLVI)** to give a pale yellow oil. The ^{1}H nmr gave singlets at δ1.46 & δ1.44 for the *t*-butyl esters, a triplet at δ2.24 for the -CH_2COOtBu protons, and a triplet at δ3.13 for the malonate derived proton. It also showed complex multiplets at δ1.82 & δ1.63 which correspond to the $CH(COOtBu)_2CH_2CH_2$- and -$CH_2CH_2CH_2COOtBu$ protons respectively. Treatment of **(XLVII)** with sodium hydride in THF followed by *p*-nitrobenzoyl chloride gave upon work-up a yellow oil. This was purified by chromatography to give di-*t*-butyl 2-*p*-nitrobenzoyl-2-carbo-*t*-butoxy-1,6-hexanedioate **(XLVIII)**, a colourless oil which crystallized upon standing.

(XLVIII)



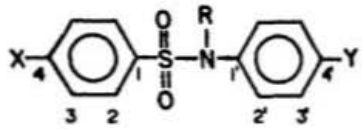
The ^{1}H nmr spectrum showed two doublets at δ8.27 & δ8.06 for the aromatic protons Ha,Ha'

and Hb,Hb' respectively, singlets at δ1.38 & δ1.44 for the *t*-butyl ester peaks, a triplet at δ2.28 for the terminal CH_2 protons (e) and two complex multiplets at δ1.70-1.80 & δ2.20-2.50 for the CH_2 protons (d) and (e) respectively.

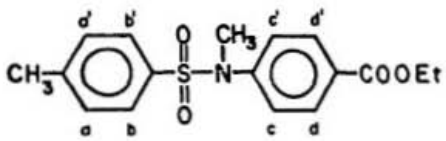
Treatment of the tri-*t*-butylester (**XLIV**) with HBr(g) in refluxing methylene chloride failed to produce the decarboxylated acid (**XLV**) but gave a mixture which, when treated with thionyl chloride followed by methanol, produced a mixture of three compounds, which were subsequently separated by TLC. The ^1H nmr of the major product showed it to be methyl *p*-nitrobenzoate, while a second component of the mixture gave an nmr somewhat consistent with that expected for (**L**). Treatment of the other triester (**XLVIII**) with HBr(g) in refluxing methylene chloride followed by evaporation of the solvent gave a white solid whose ^1H nmr was consistent with the structure (**XLIX**) but contained two unexpected triplets which have a combined integration of one proton. Also an analysis for C,H and N indicated that there may be some diacid present along with the monoacid. Clearly hydrolysis of the acyl-carbon bond does not seem to be a factor in the deesterification of this compound, but more work is necessary to completely evaluate the role played by the presence of the terminal carboxyl as an acid during the decarboxylation.

2.4 Effect of N-Methylation on the NMR and Mass Spectra of 4,4'-Disubstituted Benzenesulfonamides.

The synthesis of (**XXII**) involved the preparation of N-tosyl-N-methylbenzocaine by the method of Santi (**110**). Upon N-methylation of the tosylated amine there was noticed a pronounced shift in the aromatic proton resonances in the nmr. Addition of the methyl group seemed to shift the B, B' protons of the tosyl ring to higher field, while the C, C' protons of the benzocaine ring seemed to remain the same. This was curious as the protons affected by



the methyl group are quite far removed from the amino nitrogen. When routine MS was done on the N-tosyl-N-methylbenzocaine there was a large peak showing loss of SO_2 from the molecular ion. This further stimulated interest about these shifts, and whether or not they were related to the loss of SO_2 in the mass spectrum. It was decided to prepare a series of compounds with varying *p*-substituents on both the rings, in an effort to rationalize the requirements for these observations. For simplicity in the following discussion, the ring derived from the sulfonyl compound will be known as the "A" ring and



the amine-derived ring as the "B" ring, with the Chemical Abstracts numbering system being applied to the ring carbons.

All possible compounds having X= NO_2, Br, CH_3, CH_3O, and Y= COOEt, $COCH_3$, Br, CH_3, and CH_3O and R_3= H, CH_3 were prepared, (**Table II**), allowing comparison of electron withdrawal and electron donation at each *para* position.

NMR on this series confirmed the generality of the observed *ortho* proton shifts, as well as showing other trends. The ^1H data is presented in tabular form in **table (X)**, with the ^{13}C data in **tables (X,XI)**, located in the experimental section, while the shifts on methylation for proton and carbon spectra are presented in **tables (III)** and **(IV)** respectively in the discussion. In **tables (III & IV)** a negative value represents a shift to higher field.

Beyond confirming the generality of the upfield shift of the C-2 protons, upon N-methylation, **table III** shows that the magnitude of this shift depends upon the nature of the substituent at C-4'. For Y=$COCH_3$ or COOEt the C-2 protons are shifted upfield by 0.30-0.37 ppm, while for Y=Br the shift is 0.26-0.29ppm with the exception of X=CH_3O, and for Y=CH_3O the shift is 0.16-0.19ppm with the exception of X=CH_3. For Y=CH_3 the shift in the C-2 protons is more variable. There seems to be no obvious correlation between the magnitude of this shift with the nature of the X substituent.

The effect of N-methylation on the aromatic carbon resonances, **table IV**, is also quite pronounced for carbons 1, 1', 2 'and 4', and is related to the Y substituent. In **table V** it can be seen that the magnitude of the shift for carbons 4', 2', and 1' are strictly related to the substituent at 4', while the magnitude of the shift of carbon 1 is constant for all combinations of X and Y.

The effects of varying substituents on the loss of SO_2 in the mass spectrum is not as straightforward as is the case for the nmr shift values. Since the stability of a fragment, and the energy of the fragmentation pathway leading to its formation, *versus* the energy of fragmentation leading to other fragments, are the overwhelming criteria for the presence of a fragment in the mass spectrum, one will not always see analogous compounds fragment in the same way. Therefore one cannot directly relate substituent effects to the behavior of the compounds' molecular ion or large fragments without also considering the effect the substituent might have on the stability of ions formed by alternate fragmentation pathways. The lack of a predicted fragment ion may be the result of an alternative lower energy pathway, rather than the breakdown of a trend noted in a group of similar compounds.

Despite this complication several trends were noticed in the mass spectra (data in **table XIII** in the experimental section) of this series of compounds, with the effect of N-methylation on loss of SO_2 being quite consistent. **Table VI** lists the intensities of the M^+, M-SO_2 and

$M\text{-}SO_2H$ ions along with a ratio of $M\text{-}SO_2/M^+$ for each compound, before and after N-methylation, as well as a ratio of $M\text{-}SO_2/M^+$ before *versus* after N-methylation (last column of table).

As can be seen from the table, there is a dramatic increase of 5 to 33 times the intensity of the $M\text{-}SO_2$ peaks upon N-methylation, for the compounds that show $M\text{-}SO_2$ ions. The infinity values arise when a compound that does not show loss of SO_2 in the N-H form, gives $M\text{-}SO_2$ ions after N-methylation.

The introduction of a NO_2 group at the C-4 position decreases the ease of loss of SO_2 as can be seen for compounds 7,15,23,31,and 39. The N-H compounds show no loss of SO_2 while their N-methyl counterparts, compounds 8,16,24,32,and 40, do show $M\text{-}SO_2$ ions, but have lower values of D/C than other N-methyl analogues.

Occurrence of MeO at C-4' decreases the loss of SO_2 relative to other fragmentation pathways as only one such substituted compound, 27, shows a $M\text{-}SO_2$ ion; and this is very weak. The presence of MeO at C-4 increases the loss of SO_2 as can be seen by the high values of B/A for the N-H compounds 5,13,21, and 37, and the high values of D/C for compounds 6,14,22,and 38.

Table VII compares the effect on SO_2 loss of varying the X substituent for a given Y substituent before and after N-methylation.

It is apparent that loss of SO_2 is enhanced by having a electron donating group for substituent X and an electron withdrawing group for substituent Y. The highest ratio of $M\text{-}SO_2/M^+$ occurs with the mesityl A ring and Y=COOEt which agrees with this statement as there are now three methyl groups donating electron density to the A ring. However the magnitude of the increase of B/A for this may be larger then can be rationalized on the bases of electronic properties alone. There may be a steric factor involved in this case which enhances loss of SO_2 but a few more analogues are required to substantiate this theory.

Loss of SO_2H is significant, by comparison with SO_2 loss, only when Y=Ac,COOEt,and CH_3, and is increased upon N-methylation. However in the N-H compounds SO_2H loss exceeds SO_2 loss only when $Y=CH_3$, and $X=Br,CH_3$, and CH_3O, with N-methylation of these compounds enhancing loss of SO_2, more so than loss of SO_2H.

2.5 Antibacterial Activity of Synthesised Compounds.

As mentioned in the introduction, the target side chain compound (**XI**, $R_1=R_2=H$) could have antibacterial activity if it was absorbed by the bacteria and then incorporated into folic acid. Alternately this compound might bind to one or more of the enzymes responsible for assimilating such FA building blocks as *p*-aminobenzoic acid, and inhibition could result in antibacterial activity. For this reason it was decided that preliminary antibacterial screening on several of the compounds synthesised in this work would be done. The initial screening involved checking for inhibition zones on agar plates done by the standard method using *Pseudomonas aeruginosa, Streptococcus faecalis, Staphylococcus aureus, Escherichia coli, Mycobacterium smegmatis, Lactobacillus casei, Bacillus subtilis, Saccharomyces cerevisiae and Sarcina lutea.*

For each compound 10mgs was dissolved in 1mL of ethanol (95%) and 100μL aliquots were placed on a 1.3 cm disc. The ethanol was allowed to evaporate and the discs were then dried in a pistol at 35°C 0.1 mmHg. for 4 hours, then overnight at room temperature. Blank discs were made by putting on 100μL of ethanol then drying in a pistol at 35°C 0.1 mmHg. for 2 hours. Each disc was placed on the agar surface after the latter had been streaked with the appropriate organism and each disc was "wetted" with 100μL of sterile water to aid diffusion through the medium. Penicillin G discs were made by weighing out 10.3 mg of its sodium salt and dissolving this in 5μL of sterile water, then placing 100μL samples of this solution onto the

disc. The Penicillin G discs then contained 103 μg of Penicillin G, while the compounds under examination were applied at 1mg per disc.

A second screening was performed using broth cultures as the disk method is only effective if the compounds in question diffuse away from the disks. In this testing 500μg of compound was placed in 5mL of broth and the culture grown for 18 hours.

From **table VIII** compounds **XI** $R_1=R_2=H$, **XXI** and **XX** $R_2=H$ appear to show some activity against *L casei* but only after 42 hours incubation. All compounds also seem to have some effect on the growth of *Pseudomonas aeraginosa* which does not inhibit growth but rather affects the pigmentation of the growth. Compound **XXI** showed the greatest activity in this respect. In the broth cultures, **table IX**, compound **IX** $R_1=R_2=H$ showed definite growth inhibition against *Staph. aureus*, and compounds **XXI** and **XI** $R_1=R_2=H$ showed some activity against *Strept. faecalis*.

2.6 Further Work

Further work to be done on the synthetic component of this project would involve coupling the side chain compound (**IX** $R_1=R_2=H$) to an appropriate 2-amino-4-hydroxypteridine to give the folic acid analogue (**VII**), and N-10 methylation of the aminopterin analogue (**VII**) to give the MTX analogue (**VI**), as well as synthesis of various deaza analogues. These compounds then need to be screened for anti-tumor activity and also for FPGS activity.

The decarboxylation problem needs further work to understand how the α-carboxylic acid is preventing the second decarboxylation, and an x-ray structure of the triacid compound (**XXI**) may help in this regard. As it has been seen that the presence of an amino group rather than a nitro group stabilizes the triacid, then perhaps reduction of the model com-

pounds (XLIV) and (XLVIII) prior to deesterification may simplify the task of obtaining "pure" products from these reactions.

Finally work on the sulfonamides is ongoing and x-ray crystal work could give more information about the steric contribution of methylation to SO_2 loss. Synthesis of a 2,2' tetrasubstituted compound might give information about the structure of the $[M\text{-}SO_2]^+$ ion.

Table II. Identity Table for Sulfonamides.

number	X	Y	Z	number	X	Y	Z
1	Br	Ac	H	2	Br	Ac	CH_3
3	CH_3	Ac	H	4	CH_3	Ac	CH_3
5	CH_3O	Ac	H	6	CH_3O	Ac	CH_3
7	NO_2	Ac	H	8	NO_2	Ac	CH_3
9	Br	COOEt	H	10	Br	COOEt	CH_3
11	CH_3	COOEt	H	12	CH_3	COOEt	CH_3
13	CH_3O	COOEt	H	14	CH_3O	COOEt	CH_3
15	NO_2	COOEt	H	16	NO_2	COOEt	CH_3
17	Br	Br	H	18	Br	Br	CH_3
19	CH_3	Br	H	20	CH_3	Br	CH_3
21	CH_3O	Br	H	22	CH_3O	Br	CH_3
23	NO_2	Br	H	24	NO_2	Br	CH_3
25	Br	CH_3O	H	26	Br	CH_3O	CH_3
27	CH_3	CH_3O	H	28	CH_3	CH_3O	CH_3
29	CH_3O	CH_3O	H	30	CH_3O	CH_3O	CH_3
31	NO_2	CH_3O	H	32	NO_2	CH_3O	CH_3
33	Br	CH_3	H	34	Br	CH_3	CH_3
35	CH_3	CH_3	H	36	CH_3	CH_3	CH_3
37	CH_3O	CH_3	H	38	CH_3O	CH_3	CH_3
39	NO_2	CH_3	H	40	NO_2	CH_3	CH_3
41	mesityl A ring	COOEt	H	42	mesityl A ring	CH_3	COOEt

Table III. ^{1}H Shift differences which occur after N-Methylation

compounds		C-3	C-2	C-2'	C-3'
1	2	0.00	-0.36	+0.04	+0.05
3	4	+0.01	-0.34	+0.04	+0.07
5	6	0.00	-0.36	+0.04	+0.05
7	8	0.00	-0.30	+0.06	+0.06
9	10	-0.01	-0.37	+0.03	+0.05
11	12	+0.01	-0.34	+0.05	+0.08
13	14	0.00	-0.33	-0.07	+0.08
15	16	-0.02	-0.30	+0.05	+0.06
17	18	+0.02	-0.26	+0.03	+0.10
19	20	+0.06	-0.29	-0.02	+0.11
21	22	+0.01	-0.18	+0.02	+0.11
23	24	0.00	-0.26	-0.01	+0.09
25	26	+0.07	-0.16	+0.03	+0.08
27	28	-0.08	-0.36	-0.18	-0.08
29	30	+0.06	-0.19	-0.02	+0.08
31	32	+0.05	-0.18	+0.03	0.00
33	34	+0.04	-0.23	0.00	+0.10
35	36	+0.10	-0.19	0.00	+0.17
37	38	+0.02	-0.28	-0.05	+0.08
39	40	+0.03	-0.19	-0.01	+0.10
41	42	+0.04	--	+0.27	+0.09

Table IV. ^{13}C Shift differences which occur after N-Methylation

Compounds		^{13}C Shifts							
		4	*3*	*2*	*1*	*1'*	*2'*	*3'*	*4'*
1	2	+0.35	-0.08	+0.55	-3.58	+2.93	+7.23	-0.87	+2.70
3	4	+0.27	-0.05	+0.54	-3.47	+2.84	+7.21	-0.93	+2.69
5	6	+0.22	-0.08	+0.52	-3.49	+2.86	+7.31	-0.92	+2.73
7	8	+0.10	-0.16	+0.67	-3.46	+3.06	+7.27	-0.82	+2.71
9	10	+0.49	+0.04	+0.66	-3.57	+3.09	+7.15	-0.67	+3.17
11	12	+0.27	-0.20	+0.42	-3.65	+2.75	+6.93	-0.94	+2.97
13	14	+0.25	-0.06	+0.57	-3.56	+2.93	+7.19	-0.81	+3.18
15	16	+0.14	-0.13	+0.68	-3.59	+3.09	+7.09	-0.72	+3.08
17	18	+0.48	-0.03	+0.74	-3.66	+3.37	+6.14	-0.24	+3.67
19	20	+0.41	0.00	+0.74	-3.67	+3.20	+6.35	-0.24	+3.80
21	22	+0.32	-0.02	+0.76	-3.69	+3.25	+6.44	-0.24	+3.85
23	24	+0.19	-0.07	+0.79	-3.57	+3.42	+6.06	-0.17	+3.59
25	26	+0.59	+0.04	+0.73	-3.50	+3.65	+4.09	-0.25	+1.57
27	28	+0.52	+0.03	+0.78	-3.55	+3.48	+4.43	-0.28	+1.71
29	30	+0.50	+0.14	+0.91	-3.54	+3.56	+4.58	-0.16	+1.81
31	32	+0.23	-0.02	+0.76	-3.43	+3.76	+3.96	-0.21	+1.52
33	34	+0.56	0.00	+0.72	-3.51	+3.61	+5.29	-0.20	+3.15
35	36	+0.58	+0.10	+0.82	-3.51	+3.51	+5.64	-0.15	+3.44
37	38	+0.38	-0.02	+0.78	-3.61	+3.49	+5.65	-0.23	+3.25
39	40	+0.25	-0.04	+0.80	-3.38	+3.74	+5.19	-0.09	+3.17
41	42	+0.51	+0.07	+0.73	-1.65	+3.06	+7.51	-0.73	+3.13

Table V. Range of N-methylation $\Delta \delta_c$ values for Carbons C-1, C-1', C-2', and C-4'.

Y substituent with upper and lower values shown.	carbon $\Delta \delta_c$, for X=NO_2, Br, CH_3 and OCH_3			
	C-1	C-1'	C-2'	C-4'
Ac	-3.46, -3.58	2.84, 3.06	7.21, 7.31	2.69, 2.73
COOEt	-3.56, -3.65	2.75, 3.05	6.93, 7.19	2.97, 3.18
Br	-3.57, -3.69	3.20, 3.42	6.06, 6.44	3.59, 3.85
CH_3O	-3.43, -3.55	3.48, 3.76	3.96, 4.58	1.52, 1.81
CH_3	-3.38, -3.61	3.49, 3.74	5.19, 5.65	3.15, 3.44

Table VI. Relative intensities of M^+, $[M-SO_2]^+$ and $[M-SO_2H]^+$ ions before and after N-methylation.

NH compounds					N-methyl compounds					
Comp	M^+ A	$M-SO_2$ B	$M-SO_2H$	B/A	Comp	M^+	$M-SO_2$ C	$M-SO_2H$ D	D/C	$\frac{D/C}{B/A}$
1	41.3	0.7	0.4	0.017	2	10.8	6.1	1.7	0.56	32.9
3	33.3	0.7	0.7	0.021	4	36.6	13.5	5.4	0.37	17.6
5	34.8	3.0	0.9	0.086	6	21.4	27.0	3.8	1.26	14.7
7	31.3	0	0	0	8	25.4	4.0	1.0	0.16	∞
9	48.1	1.5	0.4	0.031	10	11.4	9.2	1.3	0.81	26.1
11	43.5	1.5	0	0.034	12	41.8	39.3	9.7	0.94	27.6
13	20.8	2.7	0	0.130	14	11.1	30.9	4.3	2.78	21.4
15	63.1	0	0	0	16	19.5	8.2	0	0.42	∞
17	11.1	0	0	0	18	6.2	1.3	0	0.21	∞
19	34.1	0	0	0	20	29.9	5.1	1.9	0.17	∞
21	25.6	1.2	0.5	0.043	22	24.8	10.0	0	0.40	9.3
23	23.2	0	0	0	24	15.3	1.1	0	0.072	∞
25	7.1	0	0	0	26	4.5	0	0	0	0
27	14.5	0.1	0.1	0.007	28	6.5	0	0	0	0
29	13.9	0	0	0	30	13.0	0	0	0	0
31	6.1	0	0	0	32	9.6	0	0	0	0
33	14.0	0.2	0.5	0.014	34	5.6	0.8	0	0.143	10.2
35	26.7	0.6	3.0	0.022	36	20.6	2.8	2.0	0.136	6.2
37	31.3	1.1	1.9	0.035	38	20.1	3.7	0.6	0.18	5.1
39	32.5	0	0	0	40	12.4	0.6	0	0.048	∞
41	6.6	2.8	0.1	0.424	42	2.8	16.0	0	5.71	13.5

[1] Normalized intensities are given; in bromine-containing compounds, values shown are for species containing the ^{79}Br isotope.

Table VII. Comparison of $[M\text{-}SO_2]^+$ and $[M\text{-}SO_2H]^+$ Intensity* Variations, versus X for a Given Y Substituent.

	NH		NMe		
Y	$\frac{M\text{-}SO_2}{M^+}$	$M\text{-}SO_2H$	$\frac{M\text{-}SO_2}{M^+}$	$M\text{-}SO_2H$	$\frac{D/C}{B/A}$
Ac	MeO>Me>Br>NO_2	++ exc.NO_2	MeO>Br>Me>NO_2	++	large, Br largest
COOEt exc. mesityl	MeO>Me>Br>NO_2	+++ exc.NO_2	MeO>Me>Br>NO_2	++ exc.NO_2	large, CH_3 largest
Br	only MeO loses SO_2	only MeO	MeO>Br>Me>NO_2	only Me	9.3 for CH_3O
MeO	only CH_3 loses SO_2	---	no SO_2 lost	---	0
Me	MeO>Me>Br>NO_2	++ exc.NO_2	MeO>Br>Me>NO_2	only Me & MeO	medium, Br largest

* ∞ values ignored here.
++ is relatively large ratio.
+++ is very large ratio.
exc. means excluding.

Table VIII. Diameters of Inhibition Zones

°C	*organism*	zone diameters in mm after 42 hours Pen G	XI $R_1=R_2=H$	XXI	XXII	XX $R_2=H$	blank
37°C	*L. Casei*	40,37	17.5*	20*	---	17*	---
37°C	*Strept. Faecalis*	42,43	---	---	---	---	---
37°C	*Pseud. aeruginosa*	†18,--	18	35	--	21	16
		†24,45	24	42	45	35	27
37°C	*E. coli*	18,18	---	---	---	---	---
37°C	*Staph. aureus*	50,52	---	---	---	---	---
37°C	*Mycobact. Smegmatis*	---	---	---	---	---	---
25°C	*B. Subtilis*	---	---	---	---	---	---
25°C	*Sacch. cerevisiae*	---	---	---	---	---	---
25°C	*Sarcina lutea*	---	---	---	---	---	---

* zone became apparent only after 42 hours incubation.

† two coloured zones were observed, the inside one was green, the outer one blue. eg. for Pen G 18 &: 24 mm diam. respectively. Looking at the plate surface however, suggested that the organism had grown but the pigmentation was affected by the disk/compound.

Table IX. Antibacterial Activity in Broth Cultures

Organism	blank	IX $R_1=R_2=H$	XXI	XI $R_1=R_2=H$
E. coli	+	+	+	+
Staph. aureus	+	a	+	+
Strept. faecalis	+	+	b	b

a broth was yellow due to the compound, but quite clear with no signs of growth.

b broth was turbid indicating growth, but the turbidity was noticeably less than in the blank.

EXPERIMENTAL

3.1 Preamble

Melting points were determined on a Thomas Hoover Capillary melting point apparatus and are uncorrected. Evaporation under reduced pressure was carried out on a Büchi rotary evaporator. Chemicals were reagent grade; solvents were reagent grade and distilled prior to use. Thin layer separations (TLC) were carried out on a Chromatotron Model 7924T (Harrison Research, Palo Alto, CA) and Merck silica gel 60 PF containing gypsum. Baker silica gel for flash chromatography (40-μm) was used in column chromatographic separations. Mass spectra were determined on a VG 7070HS mass spectrometer equipped with a VG2035 data system. Electron impact spectra were obtained at 70 eV with a source temperature of 200°C; samples were introduced via a direct probe inlet. ^{1}H and ^{13}C NMR (proton-decoupled) spectra were determined on a General Electric GN300NB 300-MHz NMR spectrometer or a Bruker WP80 80-MHz NMR spectrometer. Chemical shifts are expressed in ppm (δ) downfield from internal tetramethylsilane. ^{13}C Chemical shift assignments were aided by APT and ^{13}C-^{1}H shift correlation experiments (119). In asymmetrically *p*-disubstituted benzene derivatives, the AB approximation method of Sohar is used to estimate the ortho coupling constant and proton chemical shif ts for the aromatic protons (129). Compounds were analyzed for C, H, N, Br,and S by Guelph Chemical Laboratories, Ontario, Canada.

3.2 Target Compound Synthesis

Di-t-butyl 2-Bromopentanedioate (**XVII**, R_2=*t*-Bu)

2-Bromopentanoyl dichloride (10 g,0.040 mol.) in anhydrous ethyl ether (20 mL) was added slowly with stirring to a solution of *t*-butyl alcohol (37.7 mL) in dry ether (20 mL) at 0°C. This mixture was allowed to warm to room temperature over 2 hours and, after an additional 2.5 hours stirring, was diluted with ethyl ether (400 mL). It was washed with 1x125 mL 10% sodium carbonate, then 1x50 mL H_2O, dried over $MgSO_4$ and evaporated under reduced pressure to give a clear oil 8.24 g. (65% crude yield). This crude product was sufficiently pure to use in the next step. A sample was purified by distillation under reduced pressure; B.P. 108-111°C. 1.5 mm Hg. Spectral data: ^{1}H nmr ($CDCl_3$) 1.45 & 1.48 (s, 9H, *t*-butyl esters) 2.10-2.45 (m, 4H, -CHBrCH_2CH_2COO*t*Bu) 4.14-4.32 (m, 1H, CHBr(COO*t*Bu)CH_2-). Analysis calculated for $C_{13}H_{23}BrO_4$: C48.31, H7.17, Br24.72; found: C47.97, H7.32, Br24.33

Di-t-butyl 2,3-dicarbo-t-butoxy-1,6-hexanedioate (XIV, $R_2 = R_3$=*t*-Bu)

A solution of di-*t*-butyl malonate (5.09 g, 0.024 mol.) in dry THF (15 mL) was added with stirring to sodium hydride 50% disp. in paraffin (1.13 g, 0.024 mol.) in dry tetrahydrofuran (THF) (15 mL). After 15 minutes the di-*t*-butyl 2-bromoglutarate (7.5 g, 0.024 mol.) in dry THF (20 mL) was added with stirring. The mixture was warmed to 60°C for 5 hours then left at room temperature for 18 hours. The THF was then removed under reduced pressure to give a white solid, 7.13 g.(67%), which was used in the next step. A sample was purified by sublimation at 85°C. 0.5 mm Hg. to give hard clear crystals: M.P. 96.5-97.5°C. Spectral data: ^{1}H nmr ($CDCl_3$) 1.44 (s, 18H, *t*-butyl esters of malonate) 1.46 (s, 9H, *t*-butyl esters of glutarate) 1.47 (s, 9H, *t*-butyl esters of glutarate) 1.68-2.03 (m, 8 lines, 2H,

$CH(COOtBu)_2CH_2CH_2COOtBu$) 2.15-2.40 (m, 9 lines, 2H, $-CH_2CH_2COOtBu$)2.75-3.05 (m, 8 lines, 1H, $-CH(COOtBu)_2CH(COOtBu)CH_2-$) 3.49 (d, J=10.0 Hz, 1H, $-CH(COOtBu)_2CH(COOtBu)-$). Analysis calculated for $C_{24}H_{42}O_8$: C62.86, H9.23; found: C62.65, H9.23.

Di-t-butyl 2-(p-nitrobenzoyl)-2,3-dicarbo-t-butoxy-1,6-hexanedioate (**XIII**, $R_2 = R_3 = t\text{-Bu}$)

Di-*t*-butyl 2,3-dicarbo-t-butoxy-1,6-hexanedioate (2.5 g, 0.00546 mol.) in THF (10 mL) was added with stirring to sodium hydride 50% disp. (0.26 g, 0.00546 mol) in THF (10 mL) and warmed to reflux for 20 minutes. *p*-Nitrobenzoyl chloride (1.01 g 0.00546 mol) in THF (10 mL) was then added dropwise with stirring. The mixture was stirred at 60°C. for 1.5 hours, then at room temperature for 2 hours. It was then poured into 10% NH_4Cl(aq) (25 mL) and extracted with 2x30 mL ethyl acetate (EtOAc). The organic layer was washed with 1x20 mL 10% $NaHCO_3$(aq) and 1x5 mL H_2O, dried over $MgSO_4$ and evaporated under reduced pressure to give a pale yellow oil, which crystallized upon standing to give a white solid, 1.83 g (60%). This compound was then purified by chromatography on silica gel to give a white solid which was one spot by TLC; M.P. = 100-102°C Spectral data: ^{1}H nmr ($CDCl_3$) 1.26 (s, 9H, *t*-butyl esters) 1.44 (s, 9H, *t*-butyl esters) 1.47 (s, 9H, *t*-butyl esters) 1.52 (s, 9H, *t*-butyl esters) 1.75-2.53 (m containing at least 21 lines, 4H, $-CH(COOtBu)CH_2CH_2COOtBu$) 3.12 (dd, J=2.99, Jgem = 10.03, 1H, $-CH(COOtBu)CH_2-$) 8.06 (d, J=9.24, 2H, aromatic protons *meta* to NO_2) 8.25 (d, J=9.24, 2H, aromatic protons *ortho* to NO_2). Analysis calculated for $C_{31}H_{45}NO_{11}$: C61.27, H7.46, N2.31; found: C61.52, H7.70, N2.02.

Di-t-butyl 2-(p-aminobenzoyl)-2,3-dicarbo-t-butoxy-1,6-hexanedioate (**XII**, $R_1 = H$, $R_2 = R_3 = t\text{-Bu}$)

Di-*t*-butyl 2-*p*-nitrobenzoyl-2,3-dicarbo-t-butoxy-1,6-hexanedioate (1.06 g, 0.00175 mol) in EtOAc (50 mL) containing 9% Pd on charcoal (0.20 g) was stirred under a hydrogen atmo-

sphere, at room temperature. Hydrogen up-take was plotted vs time and after up-take slowed, excess H_2(g) was removed under reduced pressure, and the resulting mixture dried over $MgSO_4$. Filtration and removal of the solvent under reduced pressure, gave a white solid 0.803 g (80%) which was one spot by TLC. An analytical sample was prepared by preparative TLC; M.P.= 135-137°C. Spectral data: ^{1}H nmr ($CDCl_3$) 1.29 (s, 9H, *t*-butyl ester) 1.43 (s, 18H, *t*-butyl esters) 1.50 (s, 9H, *t*-butyl ester) 1.80-2.50 (m containing at least 17 peaks, 4H, CH(COO*t*Bu)CH_2CH_2COO*t*Bu) 3.08 (dd, J=3.44, Jgem=8.96, -C*H*(COO*t*Bu)CH_2-) 4.09 (s broad, 2H, NH_2) 6.57 (d, J=8.80, 2H, aromatic protons *ortho* to NH_2) 7.77 (d, J=8.80, 2H, aromatic protons *meta* to NH_2). Accurate mass calculated for $C_{31}H_{47}NO_9$: 577.3250; found: 577.3196.

2-p-Aminobenzoyl-3-carboxy-1,6-hexanedioic acid (**XXI**).

Di-*t*-butyl 2-(*p*-aminobenzoyl)-2,3-dicarbo-*t*-butoxy-1,6-hexanedioate (0.70 g, 1.18m mol) was dissolved in a solution of 30% HBr in glacial acetic acid, and the mixture stirred at room temperature. A precipitate began to form after 10 minutes and the mixture gradually darkened to a dark grey colour. After 3 hours the HBr/HOAc was removed under reduced pressure at 40°C to give a beige solid which was dried over $CaCl_2$ and silica gel. The solid was washed with 4X4 mL methylene chloride filtered to give a light beige solid 0.51 g which was the HBr salt. Spectral data: ^{1}H nmr (pyridine-d5) 2.55-3.20 (m containing more than 13 peaks, 4H, CH(COOH)CH_2CH_2COOH) 3.93-4.25 (m, 1H, -CH(COOH)C*H*(COOH)CH_2-) 4.69 (d, J=10.26, 1H, -COC*H* (COOH)CH(COOH)) 6.97 (d, J=8.72, 2H, aromatic protons *ortho* to NH_2) 8.34 (d, J=8.72, 2H, aromatic protons *meta* to NH_2); ^{13}C nmr 25.96 (-*C*H_2CH_2COOH) 32.11 (-CH_2*C* H_2COOH) 44.93 (-CH(COOH)*C*H(COOH)CH_2-) 55.61 (-COCH(COOH)CH(COOH)-) 113.28 (aromatic carbon *ortho* to NH_2) 119.27 (aromatic carbon *para* to NH_2) 131.83 (aromatic carbon *meta* to NH_2) 153.27 (aromatic carbon attached to NH_2) * 168.83, 171.73, 174.98, and 176.26 (acid carbonyls plus C_6H_4CO-) Analysis calculated

for $C_{14}H_{15}NO_7$.0.93HBr.2.1H_2O: C39.81, H4.80, N3.32, Br17.60; found: C39.55, H4.18, N2.82, Br17.14.

Di-t-butyl 2-(N-tosyl-N-methyl-p-aminobenzoyl)-2,3-dicarbo-t-butoxy-1,6-hexanedioate (**XXII**).

Di-*t*-butyl 2,3-dicarbo-*t*-butoxy-1,6-hexanedioate (7.1 g, 0.0155 mol) in THF (35 mL) was added with stirring to sodium hydride 60% disp. (0.75 g, 0.0155 mol) in THF (15 mL) and the mixture was stirred at room temperature for 30 minutes. N-Tosyl-N-methyl-*p*-aminobenzoyl chloride (5.0 g, 0.0155 mol) in THF (25 mL) was added, and the mixture stirred for 4.5 hours. It was then poured into 10% NH_4Cl(aq) (150 mL) and extracted with 2x200 mL EtOAc, washed with 1x150 mL 10% $NaHCO_3$(aq) and 1x100 mL H_2O, then dried over $MgSO_4$ and evaporated under reduced pressure to give a viscous, pale yellow oil which crystallized when stirred with petroleum ether bp 30-60 °C. This yielded 10.24g (88%) of a white solid (88%). An analytical sample was obtained by chromatographic purification. Spectral data: ^1H nmr ($CDCl_3$) 1.27 (s, 9H, *t*-butyl ester) 1.44 (s, 9H, *t*-butyl ester) 1.46 (s, 9H, *t*-butyl ester) 1.52 (s, 9H, *t*-butyl ester) 1.84-1.96 (m, 1H, -CH(COO*t*-Bu)CH*a*H*b*-) 2.20-2.37 (m, 2H, CH_2COO*t*-Bu) 2.40 (s, 3H, CH_3Ar) 2.45-2.53 (m, 1H, CH(COO*t*-Bu)C*H*aHb-) 3.11 (dd, J=2.58, Jgem=10.33, 1H, -C*H*(COO*t*-Bu-) 3.18 (s, 3H, CH_3-N-) 7.17 (d, J=8.87, 2H, aromatic protons *ortho* to amine) 7.20 (d, J=8.18, 2H, aromatic protons *ortho* to methyl group) 7.39 (d, J=8.18, 2H, aromatic protons *meta* to methyl group) 7.85 (d, J=8.87, 2H, aromatic protons *meta* to amine) ^{13}C nmr 21.53 (CH_3Ar) 25.16 (-CH(COO*t*-Bu)CH_2-) 27.41, 27.76, 28.04 and 28.08 (*t*-butyl methyls) 34.55 (-CH_2COO*t*-Bu) 37.60 (CH_3-N) 49.62 (-CH(COO*t*-Bu)-) 72.01 (COC(COO*t*-Bu)$_2$-) 80.09, 81.16, 83.29 and 83.69 (O-C$(CH_3)_3$) 124.75 (aromatic carbons *ortho* to amine) 127.61 (aromatic carbon *meta* to methyl group) 129.44 (aromatic carbon *meta* to amine) 129.88 (aromatic carbon *ortho* to methyl group) 133.19 (aromatic carbon *para* to methyl group) 134.89 (aromatic carbon *para* to amine) 143.87 (aromatic carbon attached to methyl group) 145.09 (aromatic carbon attached to amine) 165.25, 166.20, 171.48 and 171.79

(ester carbonyls) 191.41 (C_6H_4CO-). Analysis calculated for $C_{39}H_{55}NO_{11}S$: C62.80, H7.43, N1.88, S4.30; found: C62.35, H7.54, N1.91, S4.53.

t-Butyl ethyl 2-carbo-t-butoxy-3-carboethoxy-1,6-hexanedioate (XIV, R_2 = Et, R_3 = t-Bu)

Di-*t*-butyl malonate (30 g, 0.139 mol) in dry THF (100 mL) was added with stirring to sodium hydride 60% disp. (6.0 g, 0.15 mol) in THF (50 mL) and the mixture warmed to 45°C for one hour. Ethyl 2-bromoglutarate (39.67 g, 0.15 mol) in THF (100 mL) was added and the mixture stirred over night at 35°C. The THF was then removed under reduced pressure and the residue poured in ethyl ether (1000 mL). This was washed with 1X200 mL 10% NH_4Cl(aq), and 3X200 mL H_2O, dried over $MgSO_4$ and evaporated to give a yellow oil 46.55 g (77%). A sample was purified by distillation to give a viscous colourless oil, B.P. 153-156°C 0.5 mm Hg. Spectral data: ^1H nmr ($CDCl_3$) 1.25 (t, J = 6.93,3H, $CH_3$of ethyl ester) 1.27 (t, J = 6.97, 3H, CH_3 of ethyl esters) 1.44 & 1.48 (s, 9H, t-butyl esters) 1.90 (m containing 19 lines, 2H, -CH(COOEt)CH_2CH_2-) 2.37 (m containing 14 lines, 2H, CH_2CH_2COOEt) 3.03 (d of t's, 1H, CH(COO*t*Bu)$_2$C*H*(COOEt)CH_2-) 3.55 (d, J = 10.58, 1H, C*H*(COO*t*Bu)$_2$CH(COOEt)-). ^{13}C nmr 13.96 & 14.01 (CH_3 of ethyl ester) 24.78 (-CH(COOEt)CH_2CH_2-) 27.62 (*t*-butyl ester methyl) 31.08 (-CH_2CH_2COOEt) 43.42 (CH(COO*t*Bu)$_2$*C* H(COOEt)CH_2-) 55.50 (*C*H(COO*t*Bu)$_2$CH(COOEt)) 60.22 and 60.65 (CH_2 of ethyl ester) 81.77 & 81.87 ($COCMe_3$) 166.83 & 166.88 (*t*-butyl ester carbonyls) 172.22 & 172.89 (ethyl ester carbonyls). Analysis calculated for $C_{20}H_{34}O_8$: C59.68, H8.52;found: C59.37, H8.76.

t-Butyl ethyl 2-((p-nitrobenzoyl)methyl)-2-carbo-t-butoxy-3-carboethoxy-1,6-hexanedioate (XIII, R_2 = Et, R_3 = t-Bu)

Diethyl di-*t*-butyl tetraester (XIV, R_2 = Et, R_3 = *t*-Bu) (40 g, 0.0995 mol) in THF (100 mL) was added with stirring to sodium hydride 60% disp. (4.2 g, 0.105 mol) in THF (100 mL) and

warmed to reflux for 30 minutes. *p*-Nitrobenzoyl chloride (18.5 g, 0.0995 mol) in THF (100 mL) was added and the mixture stirred for 18 hours at 30°C. The THF was then removed under reduced pressure and the residue poured into ethyl ether (1000 mL). It was washed with 1X200 mL NH_4Cl(aq), 1X200 mL $NaHCO_3$(aq), and 2X200 mL H_2O, dried over $MgSO_4$ and evaporated to give a light brown oil 42.45g (77%) which was used in the next step. An analytical sample was obtained by chromatography giving a colourless oil. Spectral data: ^{1}H nmr ($CDCl_3$) 1.26 (t, J = 7.15, 3H, CH_3 of ethyl ester) 1.28 (s, 9H, *t*-butyl ester) 1.33 (t, J = 7.14, 3H, CH_3 of ethyl ester) 1.48 (s, 9H, *t*-butyl ester) 1.95-2.08 (m, 1H, *CH(COOEt)CHa*CH*bCH_2-) 2.30-2.45 (m, 2H, -CH_2*C*H_2COOEt) 2.49-2.62 (m, 1H, *CH(COOEt)*CH*aCHbCH_2-) 3.29 (dd, J = 3.00, Jgem = 10.80, 1H, *CH*(COOEt)CH_2-) 4.14 (q, J = 7.20, 2H, CH_2 of ethyl ester) 4.25 (q, J = 7.15, 2H, CH_2 of ethyl ester) 8.05 (d, J = 8.77, 2H, aromatic protons *meta* to NO_2) 8.26 (d, J = 8.77, 2H, aromatic protons *ortho* to NO_2) ^{13}C nmr 14.15 & 14.21 (CH_3's of ethyl esters) 24.52 (CH(COOEt)CH_2*C*H_2-) 27.40 & 27.74 (*t*-butyl methyls) 32.86 (CH_2*C*H_2COOEt) 49.00 (*C* H(COOEt)CH_2-) 60.40 & 61.07 (CH_2s of ethyl esters) 72.44 (*C*OC(COO*t*Bu)$_2$-) 84.31 &84.66 (*C* $(CH_3)_3$ of *t*-butyl esters) 123.09 (aromatic carbon *ortho* to NO_2) 129.99 (aromatic carbon *meta* to NO_2) 142.10 (aromatic carbon *para* to NO_2) 149.59 (aromatic carbon attached to NO_2) 164.99 & 165.77 (*t*-butyl ester carbonyls) 172.28 & 172.33 (ethyl ester carbonyls) 191.95 (C_6H_4*C*OC(COO*t*Bu)$_2$-) (*see discussion page 32) Analysis calculated for $C_{27}H_{37}NO_{11}$: C58.79, H6.76, N2.54; found,: C58.56, H7.14 N2.54.

Diethyl 2-((p-nitrobenzoyl)methyl)-1,5-pentanedioate (**XX**,R_2 = Et)

Diethyl di-*t*-butyl ester(**XIII**,R_2 = Et, R_3 = *t*-Bu) (42.45 g, 0.077 mol) was dissolved in CH_2Cl_2 (900 mL) and HBr(g) was bubbled through intermittently with stirring at reflux for 50 hours until TLC showed the reaction to be complete. The mixture was washed with 2X125 mL 10% $NaHCO_3$(aq) and 1X125 mL H_2O, dried over $MgSO_4$ and evaporated to give a

deep yellow oil. This was purified by chromatography to give a pale yellow oil 13.71g (51%). Spectral data: ^{1}H nmr ($CDCl_3$) 1.27 (t, J=7.22, 3H, CH_3 of ethyl ester) 1.28 (t, J=7.27, 3H, CH_3 of ethyl ester) 2.01 (m, J=7.24 from adjacent CH_2 is visible, 2H,CH(COOEt)C*H*$_2$CH$_2$-) 2.44 (t, J=7.24, 2H, CH_2CH_2COOEt) 3.07-3.15 (m containing at least 12 lines, 2H, CH_2CH(COOEt)CH_2-) 3.07-3.15 (m containing at least 12 lines, 2H, *COCHa*H*bCH(COOEt)-) 3.55 (m, J=10.04, Jgem=18.8, 1H, *COC*H*aHbCH(COOEt)-) 4.15 (q, J=7.23, 2H, CH_2 of ethyl ester) 4.17 (q, J=7.33, 2H, CH_2 of ethyl ester) 8.13 (d, J=8.70, 2H, aromatic protons *meta* to NO_2) 8.32 (d, J=8.74, 2H, aromatic protons *ortho* to NO_2). ^{13}C nmr 14.21 (CH_3 of ethyl esters) 26.95 (-CH(COOEt)C*H*$_2$CH$_2$-) 31.79 (-CH_2C*H*$_2$COOEt) 39.66 (-CH_2C H(COOEt)CH_2-) 40.88 (COC*H*$_2$CH(COOEt)-) 60.60 & 60.95 (CH_2 of ethyl esters) 123.88 (aromatic carbon *ortho* to NO_2) 129.11 (aromatic carbon *meta* to NO_2) 140.94 (aromatic carbon *para* to NO_2) 150.40 (aromatic carbon attached to NO_2) 172.65 & 174.43 (ethyl ester carbonyls) 196.48 (C_6H_4*C*O). (*see discussion page 33) Analysis calculated for $C_{17}H_{21}NO_7$: C58.11, H6.02, N3.99; found: C58.31, H6.17 N4.10.

Diethyl 2-((p-aminobenzoyl)methyl)-1,5-pentanedioate (XI,R_1=H,R_2=Et)

Diethyl 2-((*p*-nitrobenzoyl)methyl)-1,5-pentanedioate (XX,R_2=Et) (102mg, 0.291m mol) in EtOAc (10 mL) containing 9% Pd on charcoal (15mg) was stirred at room temperature under a hydrogen atmosphere. Hydrogen up-take was plotted vs time and after up-take ceased excess hydrogen was removed under reduced pressure and the mixture dried over $MgSO_4$. Evaporation of the solvent, from the filtered solution, under reduced pressure gave a yellow oil 68mg (73%). A sample was purified by preparative TLC using 40% EtOAc/hexane. Spectral data: ^{1}H nmr ($CDCl_3$) 1.25 (t, J=7.12, 6H, CH_3 of ethyl esters) 1.88-2.06 (m containing at least 10 lines, 2H, -CH(COOEt)C*H*$_2$CH$_2$-) 2.40 (t, J=7.44, 2H, -CH_2C*H*$_2$COOEt) 2.95-3.08 (m containing at least 10 lines, 1H, -CH_2C*H*(COOEt)CH_2-) 2.95-3.08 (m containing at least 10 lines, 1H, *-COCHaC*H*bCH(COOEt)-) 3.31-3.40 (m

containing at least 8 lines, 1H, *COC*HaHbCH(COOEt)-) 4.13 (q, J=7.12, 2H, CH_2 of ethyl ester) 4.15 (q, J=7.16, 2H, CH_2 of ethyl ester) 4.19 (broad s, 2H, NH_2) 6.63 (d, J=8.70, 2H, aromatic protons *ortho* to NH_2) 7.79 (d, J=8.70, 2H, aromatic protons *meta* to NH_2) ^{13}C nmr 14.15 (CH_3 of ethyl esters) 27.09 ($CH(COOEt)CH_2CH_2COOEt$) 31.94 (CH_2CH_2COOEt) 39.74 ($COCH_2CH(COOEt)$-) 39.86 ($CH_2CH(COOEt)CH_2$-) 60.44 (CH_2 of ethyl ester) 60.58 (CH_2 of ethyl ester) 113.64 (aromatic carbon *ortho* to NH_2) 126.98 (aromatic carbon *meta* to NH_2) 130.43 (aromatic carbon *para* to NH_2) 151.24 (aromatic carbon attached to NH_2) 172.83 & 175.04 (ethyl ester carbonyls) 195.65 ($C_6H_4COCH_2$-). (*see discussion page 35) Accurate mass calculated for $C_{17}H_{23}NO_5$: 321.1575; found: 321.1569.

2-((p-nitrobenzoyl)methyl)-1,5-pentanedioic acid (XX,R_2=H)

2-((*p*-Nitrobenzoyl)methyl)-1,5-pentanedioate (XX,R_2=Et) (2.0 g, 0.0057 mol) was dissolved in ethanol (EtOH) (30 mL) and then 1M NaOH (20 mL) was added with stirring. The mixture was heated to 40-45°C for 4 hours and cooled to room temperature. 6M HCl (5 mL) was added and the mixture extracted with CH_2Cl_2 (150 mL), the organic layer dried over $MgSO_4$ and evaporated under reduced pressure to give a beige solid 1.5 g (89%). A sample was recrystalized 3 times from EtOH to give an off white solid which was dried at 35°C under vacuum (0.5 mm Hg.). M.P.=155.5-156.5°C Spectral data: ^1H nmr (CD_3COCD_3) 1.94-2.12 (m containing at least 17 lines, 2H, -CH(COOH)C*H*$_2$CH$_2$-) 2.25-2.58 (m containing 13 lines, 2H, -CH_2CH_2COOH) 3.13 (m containing 9 lines, 1H, -CHaCHbC*H*(COOH)CH_2-) 3.33 (dd, J=3.94, Jgem=18.26, 1H, *-COCHaC*H*bCH(COOH)-) 3.63 (dd, J=9.38, Jgem=18.26, 1H, COC*H*aCHbCH(COOH)-) 8.29 (d, J=8.80, 2H, aromatic protons *meta* to NO_2) 8.37 (d, J=8.80, 2H, aromatic protons *ortho* to NO_2). ^{13}C nmr 27.56 (-CH_2CH_2COOH) 31.67 (-CH_2CH_2COOH) 40.03 (-$CH_2CH(COOH)CH_2$-) 41.22 (-COC H_2CH(COOH)-) 124.51 (aromatic carbon *ortho* to NO_2) 130.05 (aromatic carbon *meta* to NO_2) 142.00 (aromatic carbon *para* to NO_2) 151.20 (aromatic carbon attached to NO_2) 174.33 & 176.15 (acid carbonyls)

206.45 (-C_6H_4CC-). (* see discussion page 36) Analysis calculated for $C_{13}H_{13}NO_7$: C52.89, H4.44, N4.74; found: C52.89, H4.19, N4.82.

2-((p-Aminobenzoyl)methyl)-1,5-pentanedioic acid (**XI**, $R_1=R_2=H$)

To 2((*p*-nitrobenzoyl)methyl)-1,5-pentanedioic acid (**XX**, $R_2=H$) (1.25 g, 0.0042 mol) dissolved in freshly distilled absolute EtOH (200 mL) was added 9% Pd on charcoal (220mg). This mixture was then placed under a hydrogen atmosphere with stirring at room temperature. The hydrogen up-take was plotted vs time until up-take slowed. Excess hydrogen was removed under reduced pressure, the catalyst was filtered off, and the EtOH was removed under reduced pressure to give a viscous yellow oil. This oil was then crystallized using EtOAc (10 mL) and then hexane (10 mL) and the solid obtained by filtration. This gave an off white solid 0.95 g (85%), which was one spot by TLC. An analytical sample was prepared by recrystallization from MeOH, M.P.= 156-157°C. Spectral data: ^{1}H nmr (CD_3OD) 1.87-1.97 (m containing more than 5 lines, 2H, -CH_2CH_2COOH) 2.36-2.47 (m containing 2 distorted triplets, 2H, -CH_2CH_2COOH) 2.92-3.05 (m containing more than 9 lines, 1H, *CHaCHbCH(COOH)CH_2-) 2.92-3.05 (m containing more than 9 lines with an apparent doublet having a J value of 17.13 Hz, 1H, CHaCHbCH(COOH)-) 3.34 (dd, J=8.91, Jgem=17.13, 1H, *CHaCHbCH(COOH)-) 6.63 (d, J=8.65, 2H, aromatic protons *ortho* to NH_2) 7.75 (d, J=8.65, 2H, aromatic protons *meta* to NH_2) ^{13}C nmr 28.28 (CH_2CH_2COOH) 32.61 (CH_2CH_2COOH) 40.65 (COCH_2CH(COOH)-) 41.37 (COCH(COOH)CH_2-) 114.21 (aromatic carbon *ortho* to NH_2) 126.48 (aromatic carbon *para* to NH_2) 131.69 (aromatic carbon *meta* to NH_2) 155.17 (aromatic carbon attached to NH_2) 178.82 & 178.93 (acid carbonyls) 198.42 (-C_6H_4CO-). Analysis calculated for $C_{13}H_{15}NO_5$: C58.86, H5.70, N5.28; found: C58.91, H5.97 N5.68.

2[[p-[[(2,4-diamino-6-pteridinyl)methyl]amino]benzoyl]methyl]pentanedioic acid (IX $R_1 = R_2 = HP$)

The amino acid compound (XI, $R_1 = R_2 = H$) (560mg, 2.1mmol) and the pteridine ring system (X) (533mg, 2.1mmol) were stirred together at room temperature in DMA (25 mL) under a nitrogen atmosphere and protected from light. After 0.5 hours all material had dissolved and the mixture was stirred for a further 72 hours. The DMA was removed under reduced pressure at 50-55°C and H_2O (10 mL) was then added. The resulting solid was isolated by centrifuge, washed with 2X10 mL H_2O, isolated by centrifuge again and dried over silica gel in vacuo. The solid was then ground to a yellow/beige powder and washed with 3X4 mL $CHCl_3$, filtered and dried in a drying pistol at 30°C. 0.5mm Hg. to give a yellow solid 0.313 g (52%). Spectral data: ^1H nmr (d6-DMSO) 1.67-1.86 (m containing 10 lines, 2H, -$CH_2$$CH_2$COOH) 2.24-2.35 (m containing at least 6 peaks, 2H, -$CH_2$$CH_2$COOH) 2.74-2.84 (m, 1H, CHaCHbC*H*(COOH)CH_2-) 2.96 (dd, J=3.92, Jgem=17.55, 1H, *-CHaC*H*bCH(COOH)-) 3.24 (dd, J=9.31, Jgem=17.55, 1H, *-C*H*aCHbCH(COOH)-) 4.63 (d, J=4.72, 2H, C-9 protons) 6.78 (d, J=8.59, 2H, aromatic protons *ortho* to amino group) 7.23 (t, J approx.4.5, 1H, N-10 amino proton) 7.80 (d, J=8.59, 2H, aromatic protons *meta* to amino group) 8.81 (s, 2H, C-7 proton coincident with broad singlet of pteridine ring NH_2 group); 8.81 & 8.90 (s, approx. 1H, C-2 & C-4 NH_2 groups) 12.13 (s broad, approx. 2H, acid protons). ^{13}C nmr 26.83 (-$CH_2$$CH_2$COOH-) 31.41 (-$CH_2$$CH_2$COOH) 45.47 (-$CH_2$NH-) 111.55 (aromatic carbon *ortho* to amino group) 125.25 (aromatic carbon *para* to amino group) 130.21 (aromatic carbon *meta* to amino group) 152.12 (aromatic carbon attached to amino group) 162.36 (C-2) 158.07 (C-4) 149.39 (C-6) 148.35 & 148.61 (C-7 & carbon between N-1 and N-8) 121.89 (carbon between C-4 and N-5) with the -COCH_2C H(COOH)CH_2- carbons hidden under the DMSO carbon signal.

3.3 Model Compounds

t-Butyl 2-bromobutanoate (**XLII**)

The 2-bromobutanoyl bromide (50 g 0.20 mol) dissolved in anhydrous ethyl ether (100 mL) was added slowly with stirring to a solution of *t*-butyl alcohol (250 mL) in anhydrous ethyl ether (750 mL) at 0°C. The mixture was allowed to warm to room temperature overnight and was stirred for 48 hours. The mixture was then diluted further with ethyl ether (500 mL) and washed with 2X250 mL 10% $NaHCO_3$(aq) followed by 1X250 mL H_2O, dried over $MgSO_4$ and evaporated to give a clear yellow oil. This was distilled under reduced pressure to give a colourless oil 42.6 g (89%). B.P.=48.5-49.0°C. 4.0mm Hg. Spectral data: ^1H nmr ($CDCl_3$) 1.02 (t, J=7.32, 3H, -CH_2CH_3) 1.48 (s, 9H, *t*-butyl ester) 1.88-2.17 (m containing 13 lines, 2H, CHBrC*H*$_2$$CH_3$) 4.06 (t, J=7.24, COO*t*BuC*H*BrCH$_2$-) ^{13}C nmr 11.62 (-CH_2CH$_3$) 27.49 (*t*-butyl ester methyl) 28.19 (CHBr*C*H_2CH_3) 49.15 (COO*t*Bu*C* HBrCH$_2$-) 81.81 (O*C*$(CH_3)_3$) 168.44 (ester carbonyl). Analysis calculated for $C_8H_{15}BrO_2$: C43.07, H6.78, Br35.81; found: C43.15, H6.77, Br35.90.

t-Butyl 2,3-dicarbo-t-butoxypentanoate (**XLIII**)

A solution of di-*t*-butyl malonate (10.0 g, 0.046 mol) in dry THF (50 mL) was added over 10 minutes to sodium hydride 60% disp. (2.02 g, 0.051 mol) in dry THF (100 mL) with stirring and warming to 45°C. The mixture was then stirred at 45°C for 1 hour. The *t*-butyl 2-bromobutanoate (10.28 g, 0.046 mol) in THF (50 mL) was added and the mixture stirred at 35°C over night. It was then poured into ethyl ether (400 mL) and washed with 1X200 mL 10% NH_4Cl(aq) and 3X50 mL H_2O, then dried over $MgSO_4$. Evaporation under reduced pressure gave a pale yellow oil. This sample was purified by vacuum distillation to give a colourless oil 13.4 g (81%), B.P.=120-121°C. 0.5mm Hg. Spectral data: ^1H nmr ($CDCl_3$) 0.94

(t, J=7.47, 3H, -CH_2CH_3) *1.447 (s, 9H,*t*-butyl ester), 1.454 (s, 9H, *t*-butyl ester), 1.464 (s, 9H, *t*-butyl ester) 1.65 (m, 2H, CH(COO*t*Bu)C*H* $_2CH_3$) 2.86 (m containing 8 lines, 1H, $CH(COOtBu)_2CH(COOtBu)CH_2$-) 3.49 (, J=11.18, 1H, $CH(COOtBu)_2CH(COOtBu)$-); ^{13}C nmr 10.36 (-CH_2C H_3) 22.88 (-CH_2CH_3) 27.57, 27.69 (*t*-butyl methyls) 46.05 ($CH(COOtBu)_2CH(COOtBu)CH_2$-) 55.31 ($CH(COOtBu)_2CH(COOtBu)$-) 80.30, 81.15 81.29 (OC (CH_3) of *t*-butyl esters) 167.14, 172.39 (ester carbonyls). *Shifts recorded to 3 decimal places to show non-equivalence, otherwise numbers rounded to 2 decimal places. Analysis calculated for $C_{19}H_{34}O_6$: C63.66, H9.56; found: C63.73, H9.82.

t-Butyl 2-(p-nitrobenzoyl)-2,3-dicarbo-t-butoxypentanoate (**XLIV**)

t-Butyl 2,3-dicarbo-*t*-butoxypentanoate (20.0 g, 0.056 mol) in dry THF (50 mL) was added with stirring to sodium hydride 60% disp. (2.5 g, 0.062 mol) in THF (100 mL) and the mixture warmed to reflux for 30 minutes. *p*-Nitrobenzoyl chloride (10.344 g, 0.056 mol) in THF (50 mL) was then added and the mixture kept at 45°C for 4 hours. It was then allowed to cool to room temperature and was stirred over night. The mixture was poured into 10% NH_4Cl(aq) (250 mL) and diluted with ethyl ether (500 mL), separated, and the organic layer washed with 2X250 mL 10% $NaHCO_3$(aq) and 1X250 mL H_2O. It was then dried over $MgSO_4$ and evaporated under reduced pressure to give a crude oil which contained some unreacted starting material. Purification using a Chromatotron gave a viscous oil 4.53 g (16%). Spectral data: ^1H nmr ($CDCl_3$) 1.09 (t, J=7.43, 3H, -CH_2CH_3) 1.24 (s, 9H, *t*-butyl ester) 1.47 (s, 9H, *t*-butyl ester) 1.52 (s, 9H, *t*-butyl ester) 1.60-1.74 (m containing 13 lines, 1H, *CH(COO*t*Bu)CHaC*H*bCH$_3$) 1.91-2.10 (m containing 13 lines, 1H, *CH(COO*t*Bu)C*H*aCHbCH$_3$) 3.04 (dd, J=10.47, 1H, -C*H*(COO*t*Bu)CH_2-) 8.04 (d, J=8.88, 2H, aromatic protons *meta* to NO_2) 8.25 (d, J=8.88, 2H, aromatic protons *ortho* to NO_2); ^{13}C nmr 13.77 (-CH_2CH_3) 23.26 (-CH_2CH_3) 27.44, 27.79, 28.01 (-OC(CH_3)$_3$) 52.43 (-*C*H(COO*t*Bu)CH_2-) 81.05, 83.71, 83.94 (-*C*(CH_3)$_3$) 122.97 (aromatic carbons *ortho* to NO_2)

129.90 (aromatic carbons *meta* to NO_2) 142.57 (aromatic carbon *para* to NO_2) 149.38 (aromatic carbon attached to NO_2) 165.17, 166.09, 171.94 (ester carbonyls) 192.07 (C_6H_4CO). (*See discussion on page 49) Analysis calculated for $C_{26}H_{37}NO_9$: C61.52, H7.35, N2.76; found: C61.65, H7.23 N2.83.

t-Butyl 4-bromobutanoate (XLVI)

The 4-bromobutanoyl chloride (50 g, 0.20 mol) dissolved in anhydrous ethyl ether (100 mL) was added slowly with stirring to a solution of *t*-butyl alcohol (250 mL) in anhydrous ethyl ether (750 mL) at 0.0°C. The mixture was allowed to warm to room temperature overnight and was stirred for 48 hours. The mixture was then diluted with a further 750 mL of ether and washed with 2X250 mL H_2O, dried over $MgSO_4$ and evaporated to give a pale yellow oil. This was distilled under reduced pressure to give a colourless oil, 42.8g (90%), B.P. = 64.5-65.0°C. 4.0mm Hg.. Spectral data: ^1H nmr ($CDCl_3$) 1.44 (s, 9H, *t*-butyl ester) 2.11 (m containing 5 peaks, 2H, $-CH_2CH_2CH_2-$) 2.39 (t, J=7.14, 2H, $-CH_2CH_2COOtBu$) 3.45 (t, J=6.52, 2H, CH_2BrCH_2-); ^{13}C nmr 27.69 ($C(CH_3)_3$) 32.40 ($CH_2BrCH_2CH_2COOtBu$) 33.40 (CH_2Br-) 79.93 ($OC(CH_3)_3$) 171.21 (ester carbonyl). Analysis calculated for $C_8H_{15}BrO_2$: C43.07, H6.78, Br35.81; found: C43.13, H6.77, Br35.64.

Di-t-butyl 2-carbo-t-butoxy-1,6-hexanedioate (XLVII)

A solution of di-*t*-butyl malonate (10.0g, 0.046mol) in dry THF (50 mL) was added over 10 minutes to sodium hydride 60% disp. (2.0 g, 0.050 mol) in dry THF (100 mL) with stirring and warming to 35°C. The mixture was then stirred at this temperature for 30 minutes. The *t*-butyl 4-bromobutanoate (10.26g, 0.046mol) in dry THF (50 mL) was added and the stirred mixture warmed to 45°C for 5 hours. It was then stirred overnight at room temperature. The mixture was poured into ethyl ether (500 mL) and washed with 1X250 mL 10% NH_4Cl followed by 2X250 mL H_2O, dried over $MgSO_4$ and evaporated under reduced pressure to give

a yellow oil 14.7 g (89%), which was used in the next step. A sample was purified by distillation under reduced pressure, B.P. 126-129°C 0.5mm Hg., to give a colourless oil. Spectral data: ^{1}H nmr ($CDCl_3$) 1.44 (s, 9H, *t*-butyl ester) 1.46 (s, 18H, *t*-butyl ester from malonate) 1.63 (m containing 7 lines, 2H, -$CH_2CH_2CH_2$COO*t*Bu) 1.82 (m containing 6 lines, 2H, CH(COO*t*Bu)$_2CH_2CH_2$-) 2.24 (t, J=7.41, 2H, -CH_2CH_2COO*t*Bu) 3.15 (dd, J=7.76, Jgem=15.70, 1H, CH(COO*t*Bu)$_2CH_2$-) ^{13}C nmr 22.67 (-CH(COO*t*Bu)$_2C$ $H_2CH_2CH_2$COO*t*Bu) 27.79 (C(CH_3)$_3$ from malonate) 27.96 ((C(CH_3)$_3$) 35.03 (-CH_2C H_2COO*t*Bu) 53.51 (CH(COO*t*Bu)$_2$-) 80.00 (OC(CH_3)$_3$) 81.20 (OC (CH_3)$_3$ from malonate) 168.55 & 172.33 (ester carbonyls). Analysis calculated for $C_{19}H_{34}O_6$: C63.66, H9.66; found: C63.76, H9.58.

Di-t-butyl 2-((p-nitrobenzoyl)methyl)-2-carbo-t-butoxy-1,6 -hexanedioate (**XLVIII**)

Di-*t*-butyl 2-carbo-*t*-butoxy-1,6-hexanedioate (13 g, 0.036 mol) in THF (50 mL) was added with stirring to sodium hydride 60% disp. (1.60 g, 0.040 mol) in THF (100 mL) and the mixture warmed to 65°C for 3 hours. *p*-Nitrobenzoyl chloride (6.66 g, 0.036 mol) in THF (50 mL) was then added and the mixture kept at 45°C for 4 hours. It was then stirred at room temperature for 18 hours. The mixture was poured into ethyl ether (500 mL) and washed with 1X250 mL 10% NH_4Cl(aq), 2X250 mL 10% $NaHCO_3$(aq) and 2X250 mL H_2O. It was then dried over $MgSO_4$ and evaporated under reduced pressure to give a crude yellow oil. This was purified by chromatography to give a colourless oil 15.2 g (83%), which crystallized on standing. Spectral data: ^{1}H nmr ($CDCl_3$) 1.38 (s, 18H, *t*-butyl esters from malonate) 1.44 (s, 9H, *t*-butyl ester) 1.70-1.85 (m containing at least 11 peaks, 2H, -$CH_2CH_2CH_2$-) 2.20-2.25 (m containing at least 5 peaks, 2H, -C(COO*t*Bu)$_2CH_2CH_2$-) 2.28 (t, J=7.27, 2H, -CH_2CH_2COO*t*Bu) 8.06 (d, J=8.95, aromatic protons *meta* to NO_2) 8.27 (d, 2H, aromatic protons *ortho* to NO_2) ^{13}C nmr 20.59 (-$CH_2CH_2CH_2$-) 27.65, 27.90, 28.06 (C(C H_3)$_3$ from esters) 33.89 (C(COO*t*Bu)$_2CH_2CH_2$-) 35.68 (-CH_2C H_2COO*t*Bu) 69.52 (-COC(COO*t*Bu)$_2$-) 80.23,

83.75 ($C(CH_3)_3$) 123.20 (aromatic carbon *ortho* to NO_2) 129.79 (aromatic carbon *meta* to NO_2) 141.59 (aromatic carbon *para* to NO_2) 149.69 (aromatic carbon attached to NO_2) 166.34, 172.14 (ester carbonyls) 191.80 (-C_6H_4CO-). Analysis calculated for $C_{26}H_{37}NO_9$: C60.95, H7.35, N2.76; found: C61.23, H7.49, N2.94.

3.4 General Preparations for Sulfonamides

General procedure for the preparation of sulfonamides.

To an ice-cooled, solution of the sulfonyl chloride (0.04 mol) in dry pyridine (15 mL) was added the amine (0.04 mol) and the solution refluxed for 0.5 hours. After cooling, the reaction mixture was added to water (150 mL) and extracted with EtOAc (100 mL). The EtOAc layer was washed with 3X30ml (3M) hydrochloric acid, 1X30 mL water, dried over $MgSO_4$, filtered and evaporated. The sulfonamide was recrystalized from EtOAc.

General procedure for N-methylation of the sulfonamides.

To the sulfonamide (0.02 mol) in dry dimethylformamide (DMF) (25 mL) was added, with ice cooling and stirring, sodium hydride 50% dispersion (0.02 mol). After hydrogen evolution ceased, dry iodomethane (2.0 mL, 0.032 mol) and the ice bath removed. After stirring at room temperature for 2 hours, the reaction mixture was poured into EtOAc (250 mL), washed with 1X150 mL (0.5 M) NaOH, 1X100 mL water, dried over $MgSO_4$, filtered and evaporated. The product was recrystalized from hexane /EtOAc.

Table X. ^{1}H Chemical Shifts for Ring Protons with ^{1}H and ^{13}C Chemical Shifts for Substituents.

COMP	x	y	z	3	2	2'	3'	$^{1}H(^{13}C)$
1	Br	Ac	H	7.82	7.82	7.29	7.90	CH_3 2.51(26.40) C=O (196.38)
2			CH_3	7.82	7.46	7.33	7.95	CH_3 2.58(26.71) C=O (196.98) NCH_3 3.21(37.49)
3	CH_3	Ac	H	7.37	7.77	7.27	7.86	CH_3 2.48(26.36) C=O (196.40) CH_3Ar 2.32(20.94)
4			CH_3	7.38	7.43	7.31	7.93	CH_3 2.57(26.67) C=O (196.96) CH_3Ar 2.38(21.00) CH_3N 3.18(37.30)
5	CH_3O	Ac	H	7.10	7.84	7.28	7.88	CH_3 2.49(26.36) C=O (196.42) CH_3O 3.81(55.62)
6			CH_3	7.10	7.48	7.32	7.93	CH_3 2.57(26.68) C=O (196.96) CH_3O 3.84(55.70) CH_3N 3.17(37.26)
7	NO_2	Ac	H	8.41	8.11	7.28	7.89	CH_3 2.50(26.43) C=O (196.47)
8			CH_3	8.41	7.81	7.34	7.95	CH_3 2.58(26.72) C=O (197.01) N 3.26(37.75)
9	Br	COOEt	H	7.82	7.82	7.30	7.89	CH_3 1.30(14.12) CH_2 4.28(60.50) C=O (165.06)
10			CH_3	7.81	7.45	7.33	7.94	CH_3 1.32(14.21) CH_2 4.32(60.96) C=O (165.12) CH_3N 3.20(37.54)
11	CH_3	COOEt	H	7.37	7.75	7.26	7.84	CH_3 1.28(14.22) CH_2 4.26(60.58) C=O (165.24) CH_3Ar 2.33(21.02)
12			CH_3	7.38	7.41	7.31	7.92	CH_3 1.32(14.07) CH_2 4.31(60.74) C=O (165.00) CH_3Ar 2.38(20.95) CH_3N 3.17(37.20)

13	CH_3O	COOEt	H	7.09	7.79	7.25	7.84	CH_3 1.27(14.16)
								CH_2 4.25(60.47) C=O (165.15) CH_3O 3.80(55.63)
14			CH_3	7.09	7.46	7.32	7.92	CH_3 1.32(14.13) CH_2 4.31(60.80)
								C=O (165.08) CH_3N 3.16(37.21) CH_3O 3.83(55.69)
15	NO_2	COOEt	H	8.42	8.10	7.29	7.88	CH_3 1.29(14.14) CH_2 4.27(60.58)
16			CH_3	8.40	7.80	7.34	7.94	CH_3 1.32(14.11) CH_2 4.32(60.91)
								CH_3N 3.25(37.71)
17	Br	Br	H	7.80	7.70	7.08	7.46	
18			CH_3	7.82	7.44	7.11	7.56	CH_3N 3.13(37.69)
19	CH_3	Br	H	7.34	7.69	7.10	7.43	CH_3Ar 2.32(20.93)
20			CH_3	7.40	7.40	7.08	7.54	CH_3Ar 2.39(21.02)
21	CH_3O	Br	H	7.07	7.72	7.08	7.43	CH_3O 3.80(55.61)
22			CH_3	7.08	7.44	7.10	7.54	CH_3O 3.84(55.69) CH_3N 3.09(37.48)
23	NO_2	Br	H	8.42	8.06	7.13	7.48	
24			CH_3	8.42	7.80	7.12	7.57	CH_3N 3.19(37.95)
25	Br	CH_3O	H	7.75	7.60	6.99	6.82	CH_3O 3.67(55.13)
26			CH_3	7.82	7.44	7.02	6.90	CH_3O 3.75 (55.29) CH_3N 3.12(38.26)
27	CH_3	CH_3O	H	7.47	7,75	7.16	6.96	CH_3O 3.81(55.10)
								CH_3Ar 2.47(20.94)
28			CH_3	7.39	7.39	6.98	6.88	CH_3O 3.74(55.25) CH_3Ar 2.39(21.01)
								CH_3N 3.08(38.15)

29	CH_3O	CH_3O	H	7.04	7.63	7.00	6.80	CH_3O at C-4 3.78(55.53)
								CH_3O at C-4' 3.66(55.07)
30			CH_3	7.10	7.44	6.98	6.88	CH_3O at C-4 3.84(55.73)
								CH_3O at C-4' 3.74(55.33) CH_3N 3.06(38.17)
31	NO_2	CH_3O	H	8.13	7.69	6.77	6.59	CH_3O 3.43(55.14)
32			CH_3	8.18	7.55	6.80	6.59	CH_3O 3.52(55.30) CH_3N 2.93(38.50)
33	Br	CH_3	H	7.76	7.66	6.99	7.05	CH_3Ar 2.19(20.30)
34			CH_3	7.80	7.43	6.99	7.15	CH_3Ar 2.29(20.55) CH_3N 3.12(38.04)
35	CH_3	CH_3	H	7.27	7.59	6.96	6.96	CH_3 at C-4 2.26(20.92)
								CH_3 at C-4' 2.12(20.28)
36			CH_3	7.73	7.40	6.96	7.13	CH_3 at C-4 2.38(21.06)
								CH_3 at C-4' 2.27(20.56) CH_3N 3.09(37.97)
37	CH_3O	CH_3	H	7.07	7.71	7.02	7.05	CH_3Ar 2.21 (20.29)
								CH_3O 3.81(55.57)
38			CH_3	7.09	7.44	6.97	7.13	CH_3Ar 2.28(20.52) CH_3O 3.83(55.65)
								CH_3N 3.07(37.84)
39	NO_2	CH_3	H	8.38	7.99	7.02	7.07	CH_3Ar 2.20(20.31)
40			CH_3	8.41	7.80	7.01	7.17	CH_3Ar 2.30(20.59) CH_3N(38.34)
41	CH_3	COOEt	H	7.02	--	7.09	7.82	CH_3 at C-4 2.21(20.41)
								CH_3b at C-2, C-6 2.62(22.43) CH_3t 1.26(14.21)
								CH_2q 4.23(60.49) C=O (165.19)
42			CH_3	7.06	--	7.36	7.91	CH_3 at C-4 2.26(20.42) CH_3b
								at C-2, C-6 2.39(22.42) CH_3t 1.31(14.13)
								CH_2q 4.29(60.78) C=O (165.04) CH_3N 3.21(36.51)

Table XI. ^{13}C Chemical Shifts for Ring Carbons

Cmpd	x	y	z	4	3	2	1	1'	2'	3'	4'
1	Br	Ac	H	127.24	132.56	128.70	138.50	141.91	118.23	129.87	132.22
2			CH_3	127.59	132.48	129.25	134.92	144.84	125.46	129.00	134.9
3	CH_3	Ac	H	143.69	129.84	126.77	136.43	142.39	117.83	129.84	131.88
4			CH_3	143.96	129.79	127.31	132.96	145.23	125.04	128.91	134.57
5	CH_3O	Ac	H	162.70	114.55	129.02	130.84	142.51	117.76	129.82	131.82
6			CH_3	162.92	114.47	129.54	127.35	145.37	125.07	128.90	134.55
7	NO_2	Ac	H	150.03	124.84	128.31	144.55	141.42	118.56	129.92	132.54
8			CH_3	150.13	124.68	128.98	124.09	144.48	125.83	129.10	135.25
9	Br	COOEt	H	127.20	132.51	128.68	138.42	141.93	118.47	130.63	125.02
10			CH_3	127.69	132.55	129.34	134.85	145.02	125.62	129.96	128.19
11	CH_3	COOEt	H	143.78	129.90	126.85	136.39	142.49	118.15	130.64	124.68
12			CH_3	143.91	129.70	127.27	132.74	145.24	125.08	129.70	127.65
13	CH_3O	COOEt	H	162.67	114.52	129.00	130.72	142.51	117.99	130.56	124.4
14			CH_3	162.92	114.46	129.57	**127.16**	145.44	125.18	129.75	**127.67**
15	NO_2	COOEt	H	150.01	124.80	128.32	144.47	141.50	118.82	130.70	125.39
16			CH_3	150.15	124.67	129.01	140.88	144.59	125.91	129.98	128.47
17	Br	Br	H	127.03	132.46	128.63	138.35	136.76	122.16	132.14	116.61
18			CH_3	127.51	132.43	129.37	134.69	140.13	128.30	131.90	120.28
19	CH_3	Br	H	143.43	129.73	126.70	136.32	137.28	121.67	132.00	116.07
20			CH_3	143.84	129.73	127.44	132.65	140.48	128.02	131.76	119.87
21	CH_3O	Br	H	162.52	114.44	128.90	130.72	137.36	121.63	132.00	115.98
22			CH_3	162.84	114.42	129.66	127.03	140.61	128.07	131.74	119.83
23	NO_2	Br	H	149.90	124.71	128.29	144.50	136.35	122.48	132.23	117.05
24			CH_3	150.09	124.64	129.08	140.93	139.77	128.54	132.06	120.64

25	Br	CH_3O	H	126.59	132.23	128.71	138.66	129.69	123.77	114.35	156.74
26			CH_3	127.18	132.27	129.42	135.16	133.34	127.86	114.10	158.31
27	CH_3	CH_3O	H	142.96	129.54	126.73	136.65	130.27	123.27	114.25	156.44
28			CH_3	143.48	129.57	127.51	**133.10**	*133.75*	127.70	113.97	158.15
29	CH_3O	CH_3O	H	162.23	114.19	128.85	131.12	130.38	123.23	114.19	156.38
30			CH_3	162.73	114.33	129.76	127.58	133.94	127.81	114.03	158.19
31	NO_2	CH_3O	H	149.71	124.51	128.31	144.90	129.17	124.11	114.45	156.99
32			CH_3	149.94	124.49	129.07	141.47	132.93	128.07	114.24	158.51
33	Br	CH_3	H	126.67	132.28	128.67	138.71	134.63	120.91	129.65	133.75
34			CH_3	127.23	132.28	129.39	135.20	138.24	126.20	129.45	136.90
35	CH_3	CH_3	H	143.05	129.55	126.71	136.70	135.16	120.45	129.55	133.22
36			CH_3	143.63	129.65	127.53	133.19	138.67	126.09	129.40	136.66
37	CH_3O	CH_3	H	162.30	114.28	128.87	131.16	135.26	120.41	129.53	133.26
38			CH_3	162.68	114.26	129.65	127.55	138.75	126.06	129.30	136.51
39	NO_2	CH_3	H	149.75	124.57	128.28	144.91	134.19	121.26	129.76	134.19
40			CH_3	150.00	124.53	129.08	141.53	**137.93**	126.45	129.67	**137.36**
41	CH_3	COOEt	H	142.43	131.96	138.80	133.42	142.51	117.00	130.66	124.02
42			CH_3	142.94	132.03	139.53	131.77	145.57	124.51	129.93	127.15

Bold assignments can be interchanged.

Table XII. Physical Data for Sulfonamides.

Compound Reference, Melting Point and Accurate Mass Data.

Compound	MP °C	Ref	Literature MP/°C and/or Accurate Mass Data
1	186.5-187.5	a	M.P.= 143; M^+ = 352.9735; $C_{14}H_{12}BrNO_3S$ requires 352
2	133.5-135		M^+ = 366.9873 $C_{15}H_{14}BrNO_3S$ requires 366.9878
3	203.5-204.5	b,c	M.P.= 203(b), 204-205(c)
4	101.5-102	c	M.P.= 103-103.5
5	178-178.5		M^+ = 305.0704; $C_{15}H_{15}NO_4S$ requires 305.0722
6	81-82		M^+ = 319.0890; $C_{16}H_{17}NO_4S$ requires 319.0878
7	184-185	d	M.P.= 192-194
8	171.5-172		M^+ = 334.0611; $C_{15}H_{14}N_2O_5S$ requires 334.0623
9	189.5-190.5		M^+ = 382.9838; $C_{15}H_{14}BrNO_4S$ requires 382.9827
10	102.5-104.5		M^+ = 396.9964; $C_{16}H_{16}BrNO_4S$ requires 396.9983
11	205-207	e,f	M.P.= 206-207(e), 175-177(f)
12	84.5-85.5	g	M.P.= 88-90
13	165-165.5	f	M.P.= 170 M^+ = 335.0861; $C_{16}H_{17}NO_5S$ requires 335.08
14	114-115		M^+ =349.1015; $C_{17}H_{19}NO_5S$ requires 349.0984
15	194-194.5	h	M.P.= 187-189
16	136-136.5		M^+ = 364.0753; $C_{16}H_{16}N_2O_6S$ requires 364.0729
17	140.5-141	i,j	M.P. = 141(i), 145(j)
18	97.5-99	k	M^+ = 402.8909; $C_{13}H_{11}BrNO_2S$ requires 402.8877
19	148-148.5	l,m,n,o,p	M.P.= 147.5(l), 148(m), 143-146(n), 146(o,p)
20		q,p	M.P.= 87-88(q), 87(p)

21	99.5-101	r,s	M.P.= 100(r), 99-102(s)
22	114.5-115.5		M^+ = 354.9904; $C_{14}H_{14}BrNO_3S$ requires 354.9878
23	206.5-207	o	M.P.= 209
24	124.5-126.5	k	
25	143-143.5	j,t	M.P.= 142(j,t)
26	95-96	t	M^+ = 354.9901; $C_{14}H_{14}BrNO_3S$ requires 354.9878
27	112.5-113.5	u,v	M.P.= 114(u), 113-114(v)
28	63-64	v,w	M.P.= 68-69(v), 89-90(w)
29	93-94	x,ss,y	M.P.= 100-101(x), 98-100(s), 92-93(y)
30	72.5-73.5	k,y	M.P.= 77(y)
31	184-184.5	o,z	M.P.=182-183(z), 187(o)
32	138.5-140.5		M^+ = 322.0609; $C_{14}H_{14}N_2O_5S$ requires 322.0623
33	93-94	i,j	M.P.= 99(i), 98(j)
34	118.5-120.5	k	M^+ = 338.9898; $C_{14}H_{14}BrNO_2S$ requires 338.9928
35	118-118.5	aa,bb,cc,dd	M.P.= 118(aa), 116-117(bb,cc), 117.5-119.5(dd)
36	57-58	cc	M.P.= 60
37	96-96.5		M^+ = 277.0775; $C_{14}H_{15}NO_3S$ requirc3 277.0773
38	81-82.5		M^+ = 291.0951; $C_{15}H_{17}NO_3S$ requires 291.0929
39	179-180	dd,ee,o	M.P.= 179-180(ee), 180.5-183(dd) 178(o)
40	130-130.5	k	M^+ = 306.0655; $C_{14}H_{14}N_2O_4S$ requires 306.0674
41	205.5-206		M^+ = 347.1190; $C_{18}H_{21}NO_4S$ requires 347.1191
42	97-98		M^+ = 361.1359 0236; $C_{19}H_{23}NO_4S$ requires 361.1348

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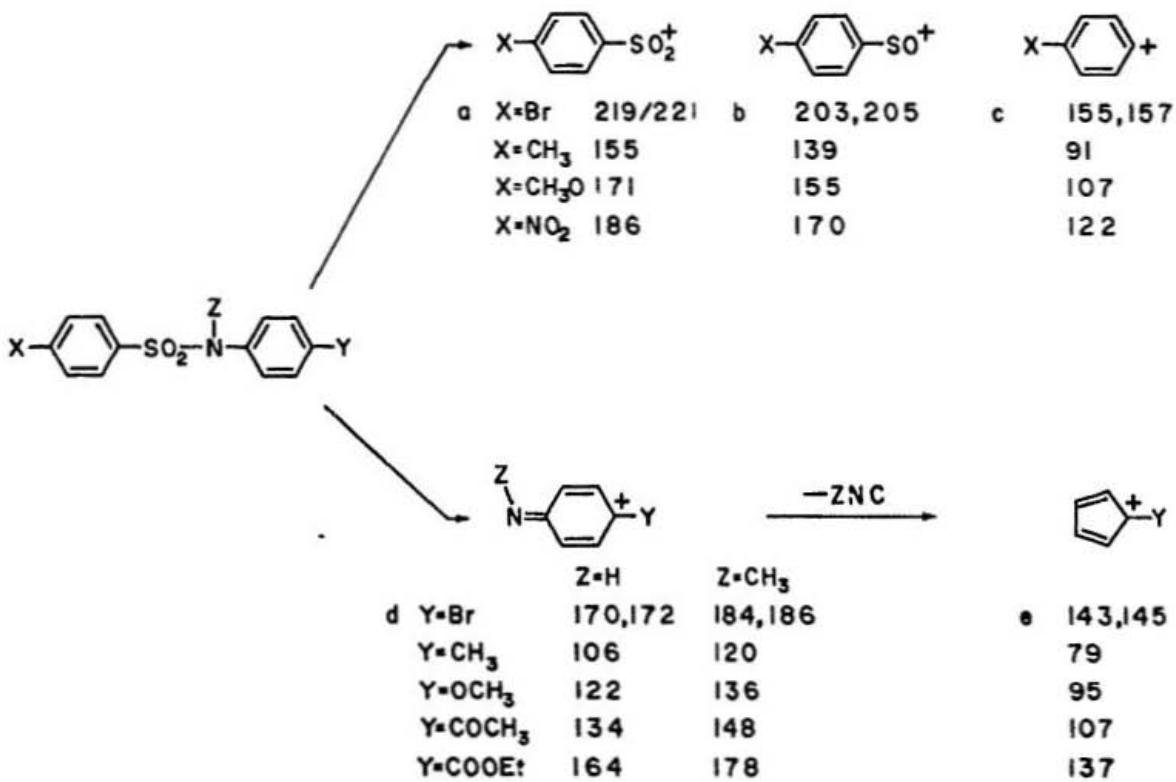
Sulfonamide fragmentation pathways



a	X=Br	219/221	b	203,205	c	155,157
	$X=CH_3$	155		139		91
	$X=CH_3O$	171		155		107
	$X=NO_2$	186		170		122

		Z=H	$Z=CH_3$		
d	Y=Br	170,172	184,186	e	143,145
	$Y=CH_3$	106	120		79
	$Y=OCH_3$	122	136		95
	$Y=COCH_3$	134	148		107
	Y=COOEt	164	178		137

Table XIII. Mass Spectrum Data for Sulfonamides

Compound	Mass spectrum; m/z (assignment, normalized intensity)
1	355(M^+, ^{81}Br, 46.6), 353(M^+, ^{79}Br, 41.3), 340(M-CH_3, 100), 339(14), 338(M-CH_3, 96), 291(M-SO_2, 0.4), 290(M-SO_2H, 0.4), 289(M-SO_2, 0.7), 288(M-SO_2, 0.4), 221(a, 16) 219(a, 15), 205(b, 4), 203(b, 4), 167(6), 157(c, 39), 155(c, 41), 134(d, 15), 120(9), 119(d-CH_3, 48), 106(49), 92(8), 91($C_7H_7^+$, 27), 79(23), 77(16), 76(21), 75(20), 64(18), 63(13), 51(6), 50(16), 43(32).
2	369(M^+, ^{81}Br, 11.5), 367(M^+, ^{79}Br, 10.8), 354(M-CH_3, 7), 352(M-CH_3, 7), 305(M-SO_2, 5.7), 304(M-SO_2H, 2.4), 303(M-SO_2, 6.1), 302(M-SO_2H, 1.7), 290(M-SO_2-CH_3, 2), 288(M-SO_2-CH_3, 2), 262(3), 221(a, 3), 219(a, 2), 182(4), 157(c, 7), 155(c, 8), 149(11), 148(d, 100), 133(d-CH_3, 10), 132(d-CH_4, 17), 106(e-H, 21), 105(24), 104(7), 91(7), 90(5), 78(5), 77(14), 76(11), 75(8), 63(5), 43(40).
3	289(M^+, 33.3), 275(7), 274(M-CH_3, 42), 225(M-SO_2, 0.7), 224(M-SO_2H, 0.7), 182(2), 155(a, 27), 139(b, 4), 120(4), 119(d-CH_3, 7), 106(d, 11), 92(10), 91(c, 100), 79(7), 77(6), 65(21), 64(6), 63(7), 43(10).
4	303(M^+, 36.6), 288(M-CH_3, 15), 239(M-SO_2, 13.5), 238(M-SO_2H, 5.4), 224(M-SO_2-CH_3, 6), 196(M-SO_2-CH_3CO, 12), 155(a, 23), 149(11), 148(d, 100), 133(d-CH_3, 8), 132(d-CH_4, 20), 106(e-H, 16), 105(20), 104(6), 92(7), 91(c, 74), 90(5), 77(11), 65(14), 63(6), 43(22).
5	305(M^+, 34.8), 290(M-CH_3, 12), 241(M-SO_2, 3.0), 240(M-SO_2H, 0.9), 173(5), 172(9), 171(a, 100), 155(b, 5), 123(25), 108(6), 107(c, 76), 106(8), 92(26), 91(8), 79(10), 78(5), 77(45), 65(6), 64(19), 63(11).
6	319(M^+, 21.4), 304(M-CH_3, 5), 256(5), 255(M-SO_2, 27.0), 254(M-SO_2H, 3.8), 240(M-SO_2-CH_3, 4), 212(M-SO_2-CH_3CO, 7), 173(5), 172(8), 171(a, 100), 155(b, 4), 149(5), 148(d, 42), 132(d-CH_4, 12), 123(19), 108(5), 107(c, e, 55), 105(14), 104(5), 92(12), 91(5), 78(5), 77(28), 64(7), 63(6), 43(20).
7	320(M^+, 31.3), 306(16), 305(M-CH_3, 100), 259(11), 156(4), 134(d, 5), 122(c, 4), 120(6), 119(d-CH_3, 39), 106(25), 92(9), 91(19), 79(15), 77(10), 76(9), 75(5), 65(7), 64(11), 63(7), 50(8), 43(19).

8 334(M^+, 25.4), 319(M-CH_3, 26), 270(M-SO_2, 4.0), 269(M-SO_2H, 1.0), 254(3), 149(10), 148(d, 100), 134(4), 133(d-CH_3)132(d-CH_4, 16), 106(19), 105(23), 104(7), 91(6), 78(5), 77(11), 76(6), 50(5), 43(33).

9 385(M^+, ^{81}Br, 50.4), 383(M^+, ^{79}Br, 48.1), 357(M-C_2H_4, 4), 355(M-C_2H_4, 3.5), 341(5), 340(M-OEt, 28), 339(5), 338(M-OEt, 26), 321(M-SO_2, 1.5), 320(M-SO_2H), 319(M-SO_2, 1.5), 318(M-SO_2H, 0.4), 293(M-$SO_2C_2H_4$, 5), 292(M-SO_2H-C_2H_4, 3), 291(M-$SO_2C_2H_4$, 5), 290(M-SO_2H-C_2H_4, 2), 222(2), 221(a, 29), 220(2), 219(a, 28), 205(b, 4), 203(b, 4), 167(11), 166(4), 165(12), 164(d, 100), 157(c, 44), 155(c, 44), 136(12), 120(12), 119(d-OEt, 49), 109(5), 108(70), 92(37), 91(39), 90(14), 81(10), 77(5), 76(26), 75(24), 65(20), 64(27), 63(19), 51(6), 50(16).

10 399(M^+, ^{81}Br, 11.8), 397(M^+, ^{79}Br, 11.4), 354(M-OEt, 6), 352(M-SO_2, 92), 334(M-SO_2H, 3.1), 333(M-SO_2, 9.2), 332(M-SO_2H, 1.3), 307(M-SO_2-C_2H_4, 1.3), 306(M-SO_2H-C_2H_4, 1.3), 305(M-SO_2-C_2H_4, 1.3), 304(M-SO_2-C_2H_4, 1.3), 294(2), 292(2), 262(2), 260(2), 225(3), 221(a, 3), 219(a, 3), 182(3), 181(12), 179(9), 178(d, 74), 157(c, 10), 155(c, 10), 151(9), 150(d-C_2H_4, 100), 134(2), 133(d-OEt, 11), 132(d-EtOH, 24), 106(5), 105(14), 104(12), 90(10), 79(7), 78(9), 77(19), 76(13), 75(11), 65(11), 63(7), 51(6), 50(8).

11 319(M^+, 43.5), 275(3), 274(14), 255(M-SO_2, 1.5), 227(M-SO_2-C_2H_4, 2), 226(M-SO_2H-C_2H_4, 2), 182(M-SO_2-COOEt, 5), 165(2), 164(d, 11), 157(2), 156(4), 155(a, 47), 139(b, 4), 136(2), 120(5), 119(d-OET, 16), 108(13), 92(14), 91(100), 65(16), 64(5), 63(5).

12 333(M^+, 41.8), 289(3), 288(M-OEt, 18), 270(7), 269(M-SO_2, 39.3), 268(M-SO_2H, 9.7), 254(3), 241(M-SO_2-C_2H_4, 6.1), 240(M-SO_2H-C_2H_4, 11.7), 228(5), 197(7), 196(M-SO_2-COOEt, 40), 179(10), 178(d, 72), 155(a, 27), 151(11), 150(d-C_2H_4, 100), 139(b, 3), 134(7), 133(d-OEt, 20), 132(d-EtOH, 33), 106(7), 105(18), 104(14), 92(8.4), 91(83), 90(9), 79(9), 78(10), 77(20), 65(27), 63(8), 51(6).

13 335(M^+, 20.8), 290(M-OEt, 8), 271(M-SO_2, 2.7), 173(5), 172(8), 171(a, 100), 164(d, 2), 155(b, 4), 123(20), 119(8), 108(13), 107(c, 60), 92(20), 91(7), 77(27), 65(5), 64(13), 63(7).

14 349(M^+, 11.1), 304(M-OEt, 9), 286(6), 285(M-SO_2, 30.9), 284(M-SO_2H, 43), 270(2), 256(6), 244(2), 212(M-SO_2-COOEt, 11), 178(d, 22), 173(5), 172(8), 171(a, 100), 155(b, 5), 151(4), 150(d-C_2H_4, 48), 134(2), 133(d-OEt, 14), 132(d-EtOH, 19), 123(22), 108(6), 107(c, 65), 105(d-COOEt, 10), 104(9), 92(15), 79(6), 78(8), 77(38), 65(9), 64(10), 63(7).

15 350(M^+, 63.1), 323(4), 322(M-C_2H_4, 21), 307(5), 306(15), 305(m-OEt, 77), 289(6), 270(5), 259(12), 186(a, 3), 170(b, 2), 165(12), 164(d, 100), 163(5), 136(d-C_2H_4, 26), 122(c, 13), 120(13), 119(d-OEt, 53), 109(6), 108(82), 92(49), 91(45), 90(15), 81(12), 76(21), 75(13), 65(23), 64(30), 63(19), 51(5), 50(15).

16 364(M^+, 19.5), 319(M-OEt, 10), 300(M-SO_2, 8.2), 284(6), 227(M-SO_2-COOEt, 4), 181(5), 179(13), 178(d, 65), 156(5), 151(11), 150(d-C_2H_4, 100), 134(12), 133(d-OEt, 11), 132(22), 106(7), 105(d-COOEt, 13), 104(12), 92(5), 90(8), 79(8), 78(9), 77(19), 76(8), 75(5), 65(13), 63(6), 51(5), 50(6).

17 393(M^+, $^{81}Br^{81}Br$, 11.6), 391(M^+, $^{81}Br^{79}Br$, 21.5), 389(M^+, $^{79}Br^{79}Br$, 11.1), 221(a, 8), 219(a, 7), 205(b, 1), 203(b, 1), 173(8), 172(d, 100), 171(10), 170(d, 96), 167(6), 157(15), 155(17), 145(e, 9), 143(e, 9), 92(5), 91(49), 90(10), 76(18), 75(17), 64(15), 63(18), 50(14).

18 407(M^+, $^{81}Br^{81}Br$, 6.2), 405(M^+, $^{81}Br^{79}Br$, 1.3), 403(M^+, $^{79}Br^{79}Br$, 6.2), 343(M-SO_2, 1.3), 342(M-SO_2H, 0.4), 341(M-SO_2, 3.1), 339(M-SO_2, 1.3), 221(a, 1), 219(a, 1), 187(8), 186(d, 90), 185(10), 184(d, 100), 157(c, 19), 155(c, 19), 105(d-Br, 47), 104(d-BrH, 22), 90(9), 78(8), 77(14), 76(21), 75(20), 63(12), 50(14).

19 327(M^+, ^{81}Br, 34.5), 325(M^+, ^{79}Br, 34.1), 182(4), 181(2), 180(2), 173(5), 172(d, 40), 170(d, 44), 156(4), 155(a, 47), 145(e, 5), 143(e, 5), 139(b, 3), 92(10), 91(c, 100), 90(7), 65(21), 64(8), 63(12).

20 341(M^+, ^{81}B31.6), 339(M^+, ^{79}Br, 29.9), 277(M-SO_2, 5.1), 276(M-SO_2H, 1.9), 275(M-SO_2, 5.8), 274(M-SO_2H, 0.7), 196(6), 187(8), 186(d, 92), 185(12), 184(d, 100), 183(4), 182(5), 181(3), 155(a, 12), 139(b, 2), 105(d-Br, 37), 104(d-BrH, 13), 91(c, 28), 90(6), 77(7), 65(9), 63(6).

21 343(M^+, ^{81}Br, 25.0), 341(M^+, ^{79}Br, 25.6), 279(M-SO_2, 1.1), 278(M-SO_2H, 0.8), 277(M-SO_2, 1.2), 276(M-SO_2H, 0.5), 198(M-SO_2-Br, 3), 173(7), 172(d, 12), 171(a, 100), 170(d, 10), 155(b, 11), 145(e, 2), 143(e, 2), 123(23), 107(34),

92(13), 91(12), 77(23), 64(11), 63(13).

22 357(M^+, ^{81}Br, 26.0), 355(M^+, ^{79}Br, 24.8), 293(M-SO_2, 10.5), 292(M-SO_2H, 2.1), 291(M-SO_2, 10.0), 212(M-SO_2Br, 7.4), 187(7), 186(d, 75), 185(11), 184(d, 81), 183(5), 182(5), 173(5), 172(8), 171(a, 100), 157(11), 155(b, 11), 145(e, 0.8), 143(e, 0.8), 123(16), 108(5), 107(c, 50), 105(38), 104(21), 92(19), 90(7), 78(10), 77(40), 76(9), 75(7), 64(16), 63(15), 51(7), 50(6).

23 358(M^+, ^{81}Br, 24.9), 356(M^+, ^{79}Br, 23.2), 342(M-O, 0.8), 340(M-o, 0.8), 186(a, 0.3), 173(8), 172(d, 100), 171(9), 170(d, 100), 145(e, 10), 122(c, 2), 92(6), 91(48), 90(9), 76(9), 75(6), 64(13), 63(14), 50(10).

24 372(M^+, ^{81}Br, 16.0), 370(M^+, ^{79}Br, 15.3), 342(M-NO), 340(M-NO), (M-SO_2, 1.0), 306(M-SO_2, 1.1), 227(M-SO_2Br)187(9), 186(d, 89), 185(11), 184(d, 100), 157(11), 155(11), 145(e, 1), 105(d-Br, 49), 104(d-BrH, 23), 92(6), 90(8), 78(8), 77(15), 76(16), 75(11), 64(6), 63(12), 51(6), 50(12).

25 343(M^+, ^{81}Br, 7.3), 341(M^+, ^{79}Br, 7.1), 221(a, 0.4), 219(a, 0.4), 205(b, 0.5), 203(b, 0.5), 157(c, 5), 155(c, 5), 123(20), 122(d, 100), 95(e, 21), 80(5), 79(8), 76(8), 75(8), 65(5), 53(7), 52(13), 51(5), 50(8).

26 357(M^+, ^{81}Br, 4.7), 355(M^+, ^{79}Br, 4.5), 221(a, 0.5), 219(a, 0.5), 157(c, 5), 155(c, 5), 137(19), 136(d, 100), 122(10), 121(19), 120(9), 108(17), 93(9), 92(7), 78(5), 77(9), 76(8), 75(8), 67(8), 66(7), 65(5), 50(6).

27 277(M^+, 14.5), 213(M-SO_2, 0.1), 212(M-SO_2H, 0.1), 155(a, 0.7), 139(b, 0.9), 124(4), 123(14), 122(100), 108(4), 95(e, 11), 91(c, 13), 65(8), 52(5).

28 291(M^+, 6.5), 137(9), 136(d, 100), 121(8), 120(3), 108(7), 91(c, 3).

29 293(M^+, 13.9), 171(a, 1), 155(b, 0.5), 123(9), 122(d, 100), 107(c, 3), 95(e, 7), 92(4), 77(5).

30 307(M^+, 13.0), 171(a, 1), 155(b, 0.4), 137(16), 136(d, 100), 121(d-CH_3, 14), 120(5), 108(13), 93(5), 92(7), 77(9).

31 308(M^+, 6.1), 123(8), 122(d, 100), 95(e, 10), 52(5).

32 322(M^+, 9.6), 306(M-O, 0.7), 186(a, 0.4), 137(23), 136(d, 100), 122(12), 121(d-CH_3, 26), 120(10), 109(5), 108(22), 93(10), 92(10), 78(6), 77(12), 76(6), 67(9), 66(7), 65(6), 64(5), 50(6).

33 327(M^+, ^{81}Br, 14.0), 325(M^+, ^{79}Br, 14.0), 263(M-SO_2, 0.1), 262(M-SO_2H, 0.4), 261(M-SO_2, 0.2), 260(M-SO_2H, 0.5), 221(a, 0.7), 219(a, 0.7), 205(b, 0.7), 203(b, 0.7), 157(c, 5), 155(c, 5), 107(16), 106(d, 100), 79(e, 37), 78(9), 77(28), 76(8), 75(8), 51(6), 50(7).

34 341(M^+, ^{81}Br, 5.8), 339(M^+, ^{79}Br, 5.6), 277(M-SO_2, 0.5), 275(M-SO_2, 0.8), 196(M-SO_2Br, 1.1), 157(c, 3), 155(c, 3), 121(10), 122(d, 100), 118(5), 106(5), 93(5), 92(7), 91(25), 77(10), 76(6), 75(5), 65(10), 50(5).

35 261(M^+, 26.7), 197(M-SO_2, 0.6), M-SO_2H, 3.0), 155(a, 3), 107(10), 106(d, 100), 91(18), 79(e, 20), 78(5), 77(15), 65(8).

36 275(M^+, 20.6), 211(M-SO_2, 2.8), 210(M-SO_2H, 2.0), 196(3), 155(a, 1), 121(10), 120(d, 100), 118(6), 93(5), 92(10), 91(46), 77(12), 65(25), 63(5), 51(6).

37 277(M^+, 31.3), 213(M-SO_2, 1.1), 212(M-SO_2H, 1.9), 198(M-SO_2-CH_3, 3), 171(a, 30), 155(b, 2), 123(6.3), 107(26), 106(d, 100), 92(8), 79(e, 22), 78(6), 77(27), 64(6).

38 291(M^+, 21.1), 227(M-SO_2, 3.7), 226(M-SO_2H, 0.6), 212(M-SO_2-CH_3, 1.7), 171(a, 6), 121(12), 120(d, 100), 107(c, 6), 92(8), 91(21), 77(13), 65(7).

39 292(M^+, 32.5), 276(M-O, 1), 262(M-NO, 0.5), 246(M-NO_2, 0.6), 186(a, 0.5), 122(c, 2), 107(21), 106(d, 100), 80(5), 79(e, 61), 78(13), 77(46), 76(11), 73(7), 53(7), 52(7), 51(9), 50(11).

40 306(M^+, 12.4), 290(M-O, 0.5), 242(M-SO_2, 0.6), 121(13), 120(D, 100), 118(5), 93(5), 92(9), 91(28), 77(9), 65(9).

41	347(M^+, 6.6), 302(M-OEt, 3), 283(M-SO_2, 2.8), 282(M-SO_2H, 0.1), 268(M-SO_2-CH_3, 1), 255(M-SO_2-C_2H_4, 0.3), 254(M-SO_2H-C_2H_4, 0.8), 238(M-SO_2-OEt, 6), 210(4), 183($(CH_3)_3C_6H_2SO_2^+$, 5), 165(5), 120(11), 119($C_9H_{11}^+$, 100), 91(12), 77(5).
42	361(M^+, 2.8), 316(M-OEt, 5), 297(M-SO_2, 16.0), 282(M-SO_2-CH_3, 10), 252(M-SO_2-OEt, 4), 224(M-SO_2-COOEt, 6), 183($(CH_3)_3C_6H_2SO_2^+$, 3), 179(32), 178(d, 19), 151(5), 150(28), 134(8), 133(8), 132(13), 120(11), 119($C_9H_{11}^+$, 100), 117(5), 105(9), 104(8), 103(5), 91(16), 79(6), 78(5), 77(14), 65(7).

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