A mechanistic investigation of the synthesis of thyroxine

A Mechanistic Investigation of the Synthesis of Thyroxine

by

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A Doctoral Thesis

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of
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To Granny, Grandad, Grandma and Grandpa,
With Love

"We are romanced in fascination.....
Look what you can do with a little imagination"

(The Wonderstuff)

"It is not possible to explore deeper oceans
without first having the courage to lose sight
of the shore"

(Anon.)

Abstract

Chapter One reviews the literature concerning oxidative phenolic coupling and summarises the biological aspects of thyroxine and early syntheses of the compound. The aims of the research are also discussed.

Chapter two discusses the *ortho-ortho* coupling of diiodinated phenols comparing a radical mechanism *versus* a polar mechanism. The conditions under which this coupling occurs are carefully examined. The evidence towards the reaction going through a polar mechanism gives rise to methods of blocking this *ortho-ortho* coupling during the reaction to form thyroxine.

Chapter three discusses the results obtained from an electrochemical study of the oxidation potentials of various phenols, with particular interest in the effects of various substituents on the *ortho* and *para* positions on the oxidation potential of the phenols and phenoxide ions. The implications of these results to the conditions necessary for thyroxine synthesis and their relevance to the oxygen-*para* coupling mechanism in the formation of thyroxine derivatives are discussed.

Chapter four summarises the literature precedent for the oxidation of the tyrosine amino acid side chain prior to coupling in the formation of thyroxine, and other similar oxidations. The experiments carried out using a variety of known oxidants and oxidative conditions and their failure to perform the desired oxidation are discussed. The results from the coupling of ethyl *N*-acetyl 3,5-diiodotyrosinate with various oxidised derivatives provides further evidence for the lack of oxidation of the amino acid prior to coupling.

Chapter five investigates the various requirements of the industrial synthesis of thyroxine, and their function. A mechanism is proposed for the coupling to form thyroxine, and is discussed with reference to the products formed as a result of exposing other diiodinated phenols to the 'thyroxine coupling' conditions.

Acknowledgements

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Contents

Abbreviations

BMR	Basal metabolic rate
DCM	Dichloromethane
DIHPPA	3,5-Diiodo-4-hydroxyphenylpyruvic acid
DMF	Dimethylformamide
DMSO	Dimethylsulfoxide
Ep	Peak potential
Ethyl *N*-acetylDIT	*N*-acetyl-3,5-diiodotyrosine ethyl ester
HPLC	High pressure liquid chromatography
LDA	Lithium diisopropylamide
NMR	Nuclear magnetic resonance
PTC	Phase transfer catalyst
TBAF	Tetrabutylammonium fluoride
TBDMS	*tert*-Butyldimethylsilyl
TEMPO	2,2,6,6-tetramethylpiperidinyloxy, free radical
THF	Tetrahydrofuran
TLC	Thin layer chromatography
T_3	3,5,3'-Triiodothyronine
T_4	3,5,3',5',-tetraiodothyronine

Chapter One

Phenolic coupling, the formation of thyroxine and its biological importance

1.1 Introduction

The oxidation of phenols leading to the formation of complicated mixtures of dimeric, polymeric and quinonoid compounds has been known for many years. A suggestion that phenolic oxidation is a key step in the formation of many naturally occurring compounds stimulated intensive research into the biosynthesis of these compounds and the proof that phenolic oxidation occurs *in vivo* and *in vitro*.[1] The syntheses of these compounds through their proposed biogenetic pathway was also of interest. Through this research and the consequent increased knowledge of the mechanism through which the phenolic oxidation proceeds, it is now easier to use phenolic oxidation as a synthetic procedure in the formation of target compounds.

The following chapter provides a brief summary of the mechanisms of phenolic coupling and a few examples of the products arising from phenolic oxidations. It describes the importance of thyroxine as a natural compound, the various chemical syntheses used for its production, and the research which has gone into establishing its formation *in vivo* and *in vitro.*

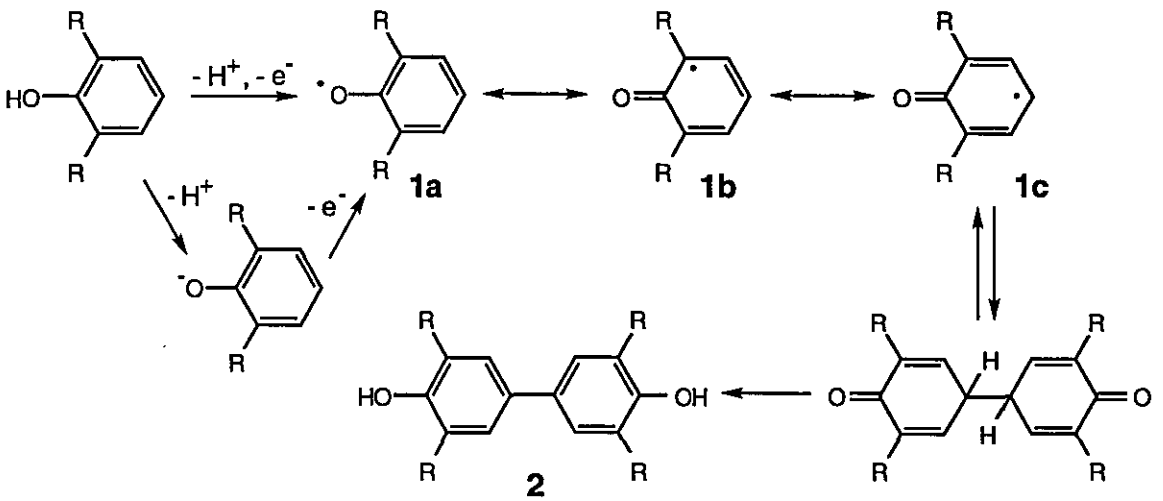
1.2 Mechanistic aspects of phenolic coupling

There are several possible pathways to the coupling of phenols,[2] which will be summarised below. The possibilities involve either two one electron steps, or one two electron step, to give either a radical or polar process. In the case of the oxidant being a metal ion then either a metal-carbon, or a metal-oxygen bond may form a part of the mechanism. It is also possible that one phenolic nucleus undergoes two electron oxidation to an intermediate quinone or quinone methide prior to coupling with a non-oxidised phenolic group.

1.2.1 Coupling of phenoxyl radicals

This mechanism, shown in **scheme 1**, is one of the first mechanisms of phenolic coupling investigated by Pummerer, who postulated a free phenoxyl radical as the first intermediate in the coupling, formed either by the removal of an electron from the phenoxide ion, or by the removal of a proton and electron simultaneously. Evidence supporting the formation of these radicals has been obtained by EPR spectroscopic studies.[3] Coupling was found to occur on the oxygen, the *ortho* and the *para* carbon atoms due to the resonance structures (**1a,b,c**) giving rise to many

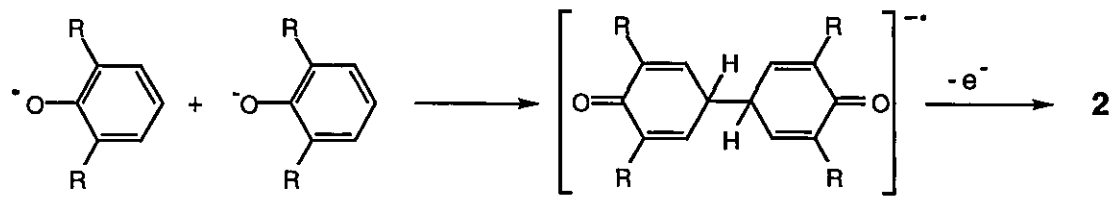
possible products. For simplicity, in **scheme 1** the ortho positions are shown to be substituted and therefore a *para-para* coupled product is obtained.



Scheme 1

1.2.2 Coupling of phenoxyl radicals with phenoxide ions

The above mechanism requires an efficient oxidation system and that the radical only reacts with another radical. However in many reactions the concentration of unoxidised phenol or phenoxide ions will be greater than the concentration of the radical, and so it is likely that the radical will react through substitution mechanism. The mechanism (**scheme 2**) involves radical insertion into a phenoxide ion to form a radical anion which on single electron transfer yields the coupled product.

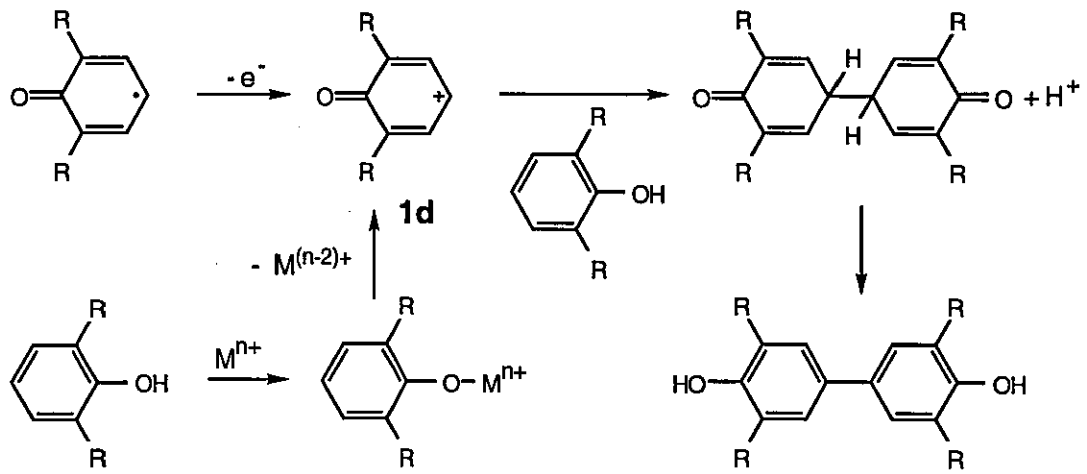


Scheme 2

1.2.3 Oxidation to a cation and subsequent polar coupling

It is possible that the first oxidation to a phenoxyl radical is followed by a second electron oxidation to an aryloxonium species (**1d**), which would react rapidly with a phenol or phenoxide ion through electrophilic substitution to afford a dimer (**scheme 3**). The energy requirement for the removal of the second electron will be

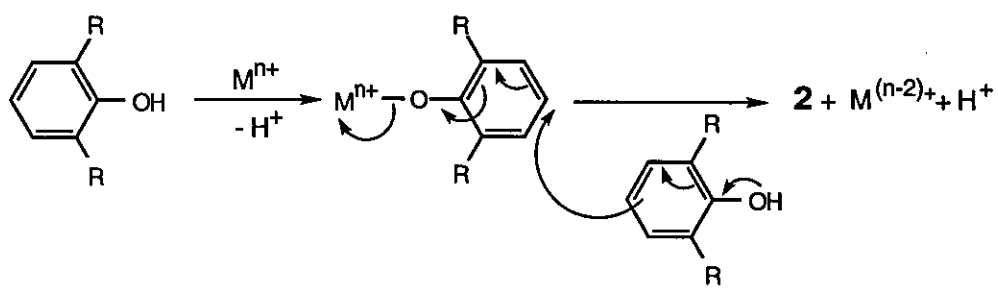
higher than for the formation of the phenoxyl radical and so the chance of this mechanism occurring is reduced. However, the oxidation of a phenoxyl radical is possible, and the potential can be measured by electrochemical methods (Chapter 3).



Scheme 3

1.2.4 Concerted coupling and electron transfer

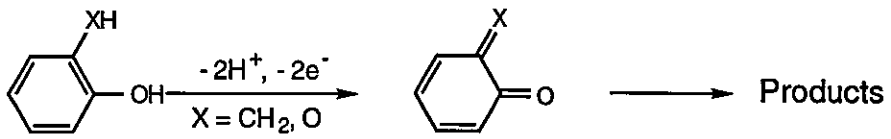
Scheme 4 shows that by forming a metal phenoxide, which can react with a phenol, the formation of a cation, as in **scheme 3** is avoided. The energetics of this mechanism will be favourable over the energetics of the mechanism shown in **scheme 4** since the oxidation and coupling are concerted.



Scheme 4

1.2.5 Post-oxidative coupling

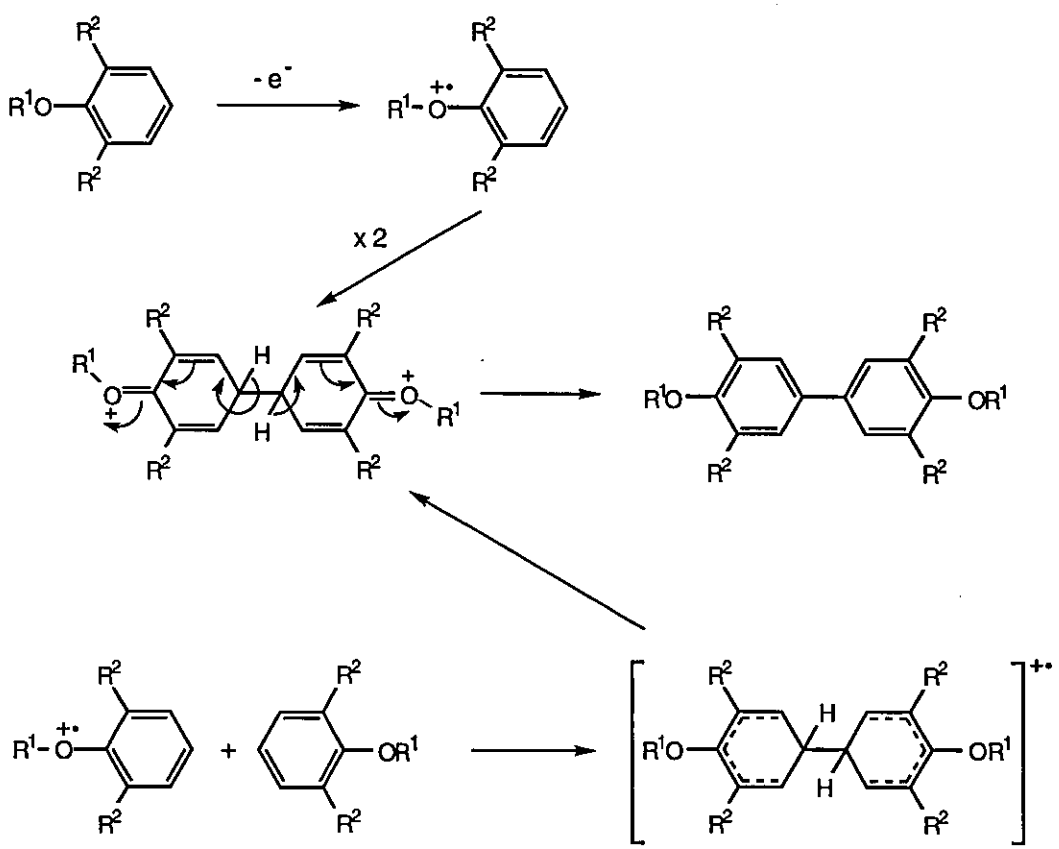
In some cases, as shown in **scheme 5**, oxidation will occur to yield quinones or quinone methides as intermediates followed by nucleophilic addition to afford coupled compounds.



Scheme 5

1.2.6 Radical cation reactions

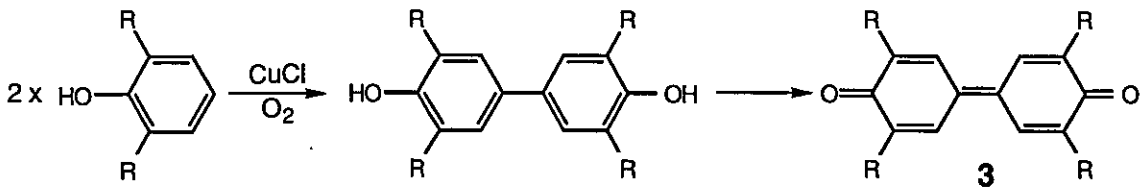
Radical cations can be formed by the one electron oxidation of an aryl ether, the fate of which may be radical coupling or coupling with a neutral phenol ether as shown in **scheme 6**. Both paths are shown to form a biaryl product, which is only one of a number of possible products. It is possible that in certain cases phenols also couple through this mechanism, however, the radical cation of most phenols can be expected to be more acidic than the phenol itself and would therefore be likely to lose a proton to give the phenoxyl radical.



Scheme 6

1.3 Phenolic carbon-carbon coupling

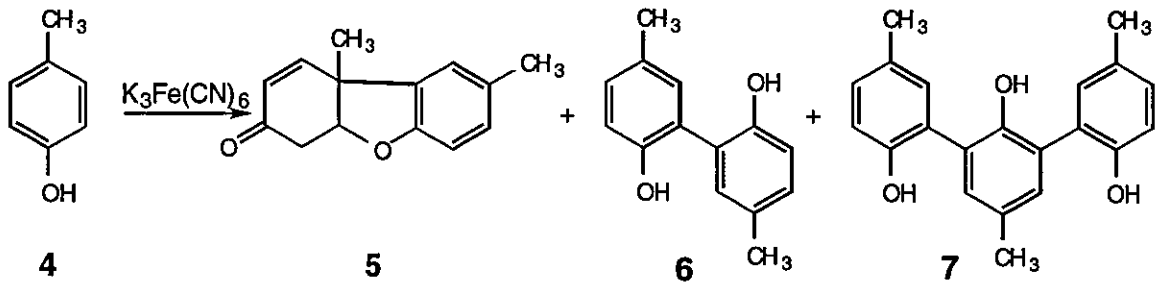
On the formation of a phenoxyl radical there are several possibilities through which it can couple to form either a carbon-carbon, or carbon-oxygen coupled product. The resonance forms of the phenoxyl radical show that coupling can occur *ortho-ortho*, *ortho-para*, *para-para*, *oxygen-ortho*, or *oxygen-para*. Since the highest spin density is in general at the *para* position it would be expected that in the case of this position being unsubstituted coupling would mainly occur at the para positions. However, the products and their ratios are also dependent on pH, temperature, concentration, solvent choice and oxidant. In the case of two different phenolic compounds being present in a reaction, the amount of possible products is further increased. Evidence of the *para* position being the favoured position for coupling is shown by the reaction of *p*-cresol (**4**) on treatment with silver carbonate. Despite the *para* position being substituted and the *ortho* positions unsubstituted, *ortho-para* coupling occurs to yield Pummerer's ketone (**5**) in good yield.[4] If both ortho positions are substituted *para-para* coupling occurs to yield the dimer which with strong oxidants can be oxidised to the diphenoquinone (**3**) (**scheme 7**),[5] i.e. no coupling occurs on the *ortho* substituted carbon as is seen with the *para* substituted phenol in the case of Pummerer's ketone.



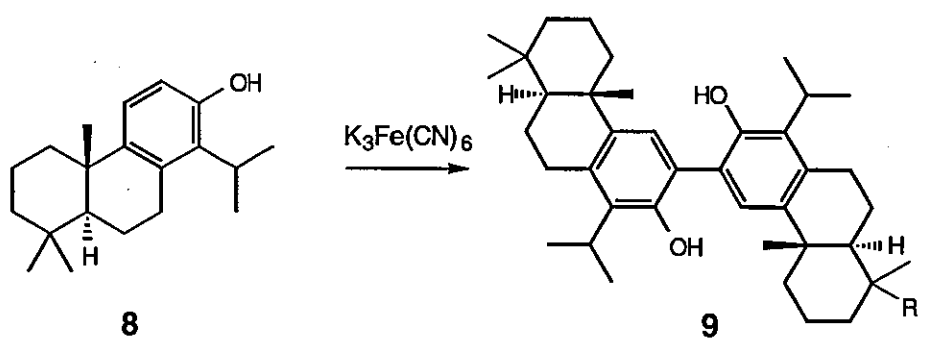
Scheme 7

The effect of different oxidants on product ratios is exemplified by the reaction of *p*-cresol (**4**) in the presence of iron(III) chloride which, in contrast to the reaction with silver carbonate, yields Pummerer's ketone **5** and the *ortho-ortho* dimer **6**, trimer **7** and polymers.

Potassium ferricyanide using an alkali-organic two phase solvent system has been widely used in the oxidation of phenols, since the two phase system may protect the product from further oxidation. For example the potassium ferricyanide oxidation of totarol (**8**) through *o-o* coupling gave podototarin (**9**) in 43 % yield.[6]

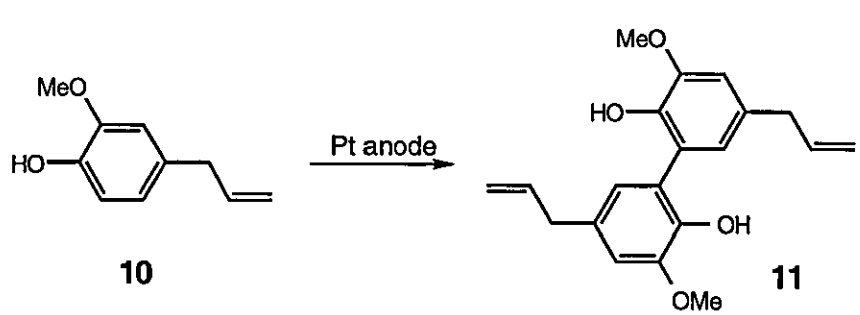


Scheme 8

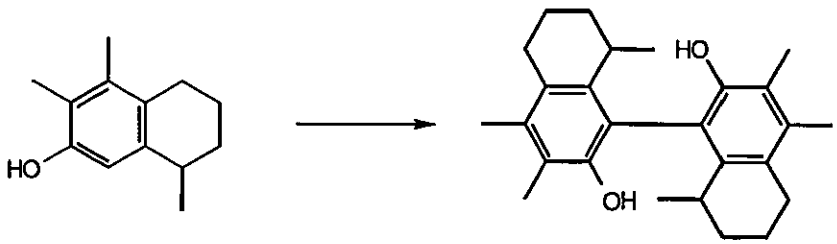


Scheme 9

The quantitative oxidation of allylguaiacol (**10**) at a platinum anode in alkaline electrolyte gave the biphenol **11**.[7] A copper(II)-amine complex was employed for the oxidation of the trimethyltetralol **12** to give the dimer **13**.[8]



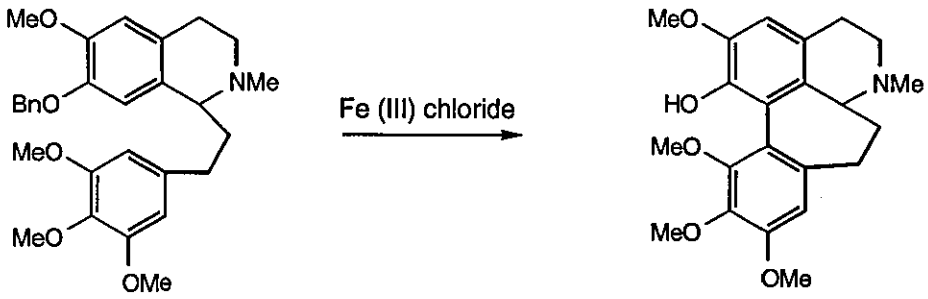
Scheme 10



12 **13**

Scheme 11

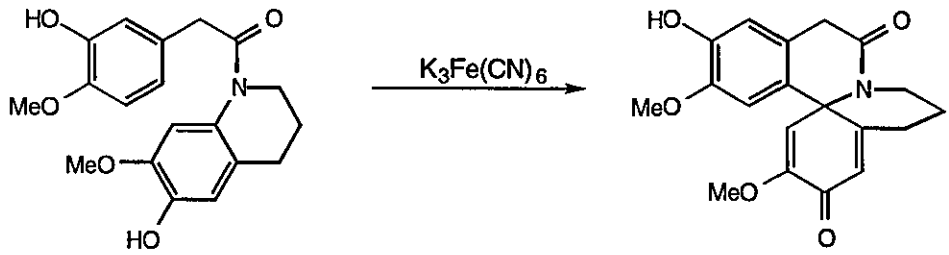
An example of the coupling of phenol ethers is shown in the coupling of isoquinoline (**14**) with iron(III) chloride to form the alkaloid kreysigine (**15**).[9]



14 **15**

Scheme 12

In the case of intramolecular phenolic coupling, when the preferred coupling site is already substituted, the formation of a spirodienone may be observed if such a coupling is favoured over the coupling at an unsubstituted site. The oxidation of the phenacyltetrahydroquinoline **16** with ferricyanide led to the formation of the dienone **17** (67 %).[10]

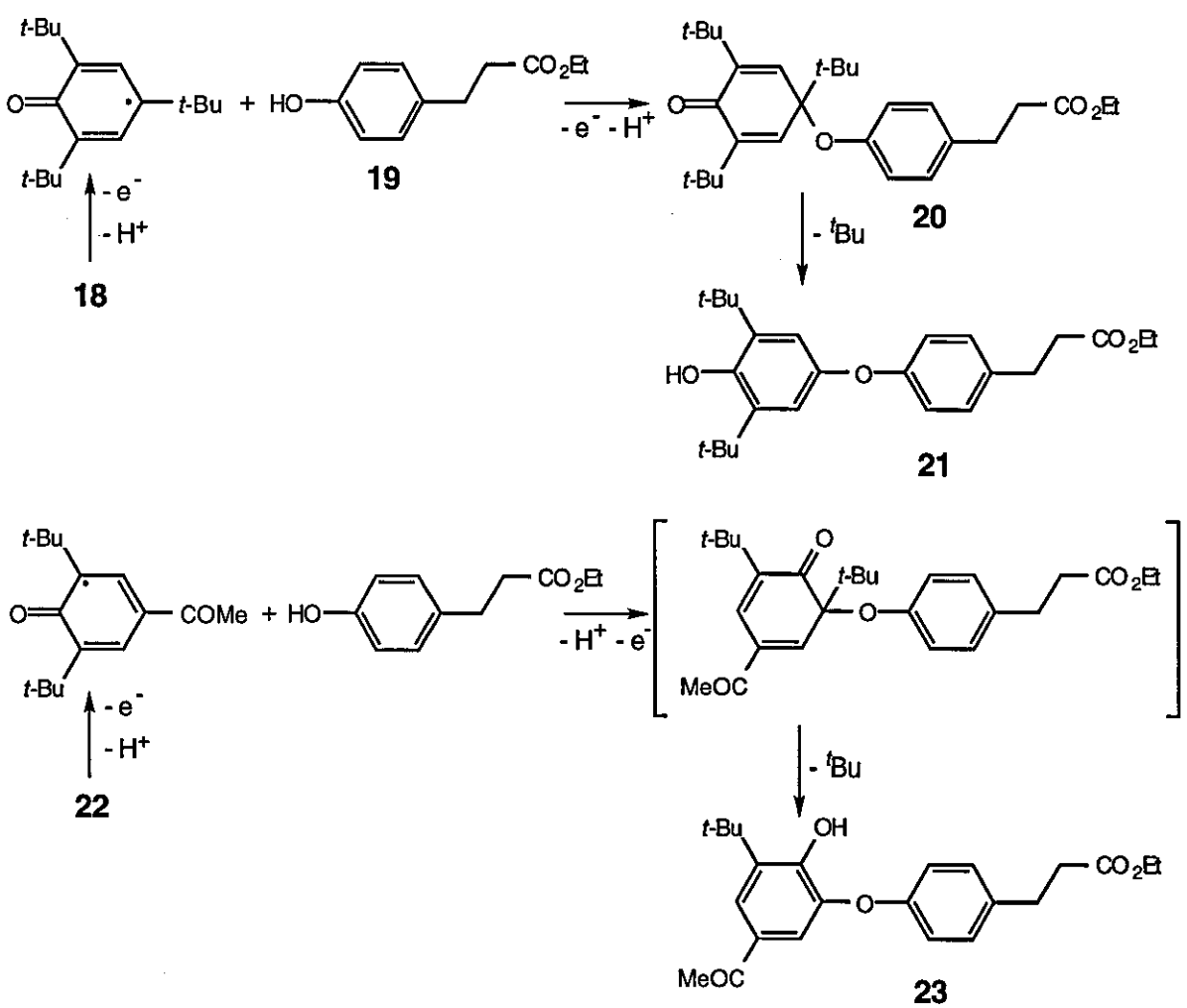


16 **17**

Scheme 13

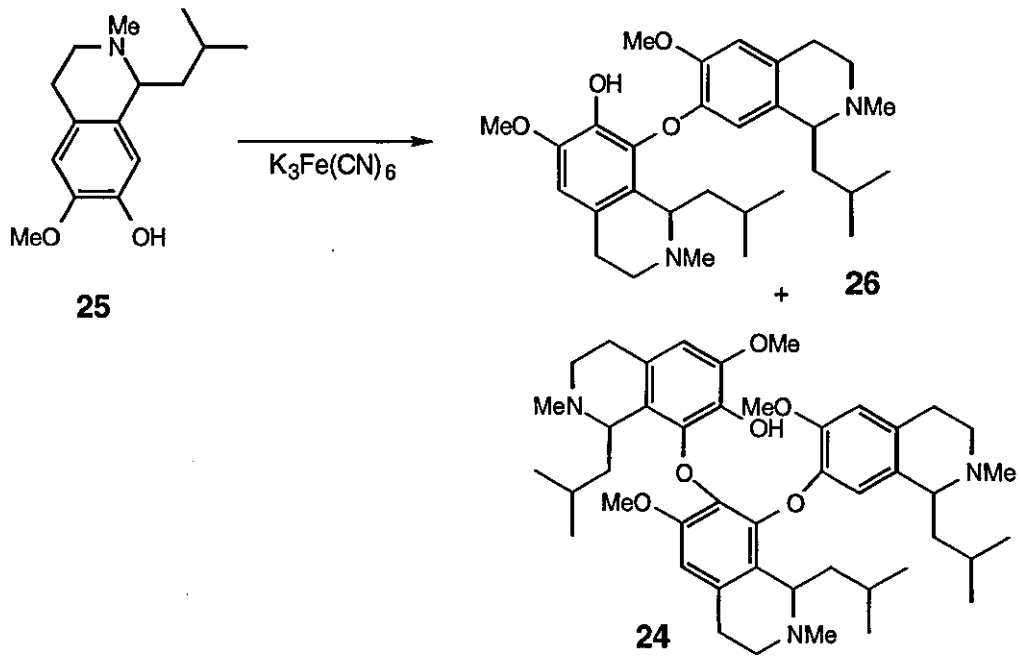
1.4 Phenolic carbon-oxygen coupling

The coupling of phenolics through carbon-oxygen atoms has been found to occur in many natural compounds, thyroxine being the obvious example in this text. A discussion of the literature concerning the formation of thyroxine and similar derivatives through oxidative phenolic coupling can be found later in this chapter. The work of Matsuura and Cahnmann in a model study of the oxidative coupling to form thyroxine gives an example of the effect of the substituents on the phenol to the major reaction product (**scheme 14**). They found that when 2,4,6-tri-*tert*-butylphenol (**18**) was oxidised with potassium ferricyanide, followed by the addition of a solution of ethyl 3-(4-hydroxyphenyl)-propionate (**19**), the reaction gave the quinol ether **20** which on heating gave the thyroxine analogue **21**. In contrast the oxidation of 3,5-di-*tert*-butyl-4-hydroxyacetophenone (**22**) followed by addition of ethyl 3-(4-hydroxyphenyl)-propionate (**19**) gave the *ortho*-oxygen coupled compound **23** *via* the unstable quinol intermediate.[11]



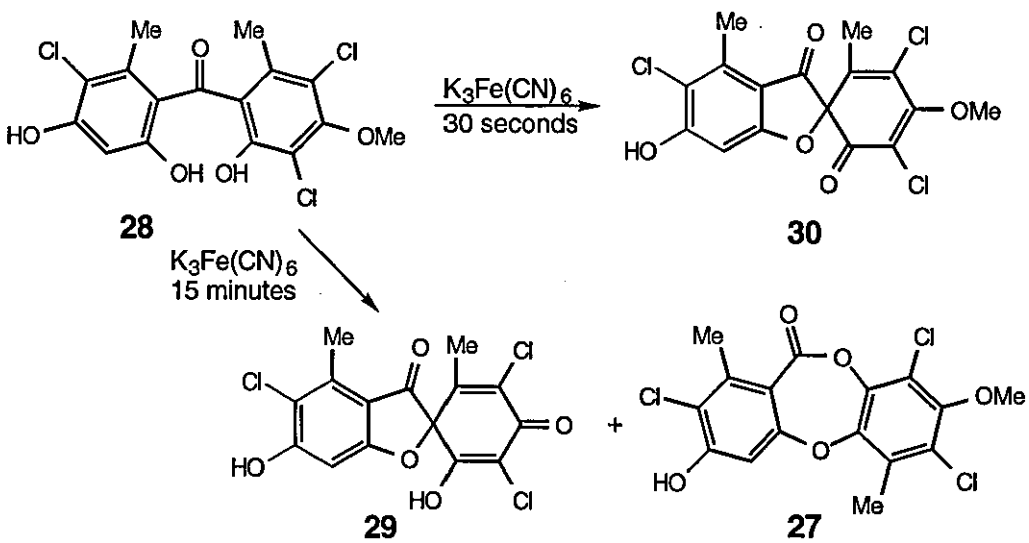
Scheme 14

The formation of the isoquinoline alkaloid pilocereine (**24**) is postulated to occur through an oxidative coupling of lophocerine (**25**), but also produces a greater yield of the dimer **26**. The chemical synthesis of **24** has been achieved using potassium ferricyanide (**scheme 15**).[12]



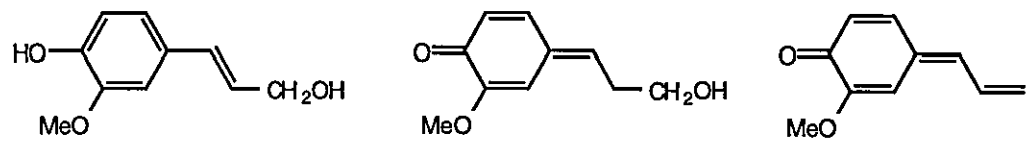
Scheme 15

Scheme 16 shows the synthesis of the depsidone dechlorodiploicin (**27**) *via* intramolecular oxidative coupling of **28** with potassium ferricyanide, which also gave the grisadienedione **29**. If the reaction was quenched after only 30 seconds the grisadienedione **30** was produced in high yield.[13]



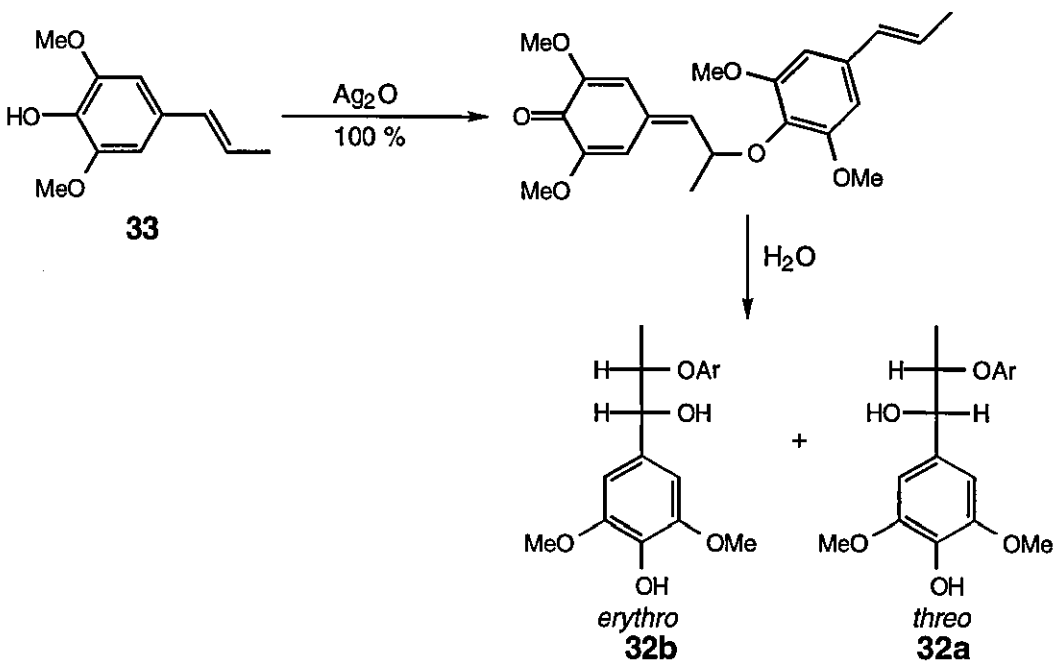
Scheme 16

The biogenesis of lignin has been postulated to occur through the oxidative coupling of coniferyl alcohol (**31**) *via* a quinone methide intermediate, such as **31a**, which is susceptible to nucleophilic addition of hydroxy compounds. Vinyl quinone methide compounds **31b** can be derived from the dehydration of alcohols or from the corresponding allyl and propenyl phenols by oxidation, and have been found to be stable in neutral or slightly basic aqueous conditions.[14]



31 **31a** **31b**

These vinyl quinone methides can be used to synthesise some naturally occurring compounds which are coupled through the oxygen-side chain such as the antileukaemia neolignan, *threo* (±)-virolin (**32a**). This compound was afforded by the oxidation of (*E*)-isoeugenol (**33**) using silver oxide and addition of water after 7 minutes, and gave an *erythro* : *threo* ratio of 1 : 1.[15]



33

erythro **32b** *threo* **32a**

Scheme 17

1.5 Summary

The account given of the mechanisms of, and examples of products resulting from, phenolic oxidation serves only to introduce the chemistry which will be discussed in the coupling of tyrosine derivatives to form thyroxine derivatives, and show the variety of possible products resulting from phenolic oxidation reactions. It is a very brief account in comparison with the extent of research which has been carried out on this subject. Further and more detailed accounts of phenolic oxidation may be found in other texts.[16] The most recent review is by D. A. Whiting.[17]

1.6 Biological background of thyroxine

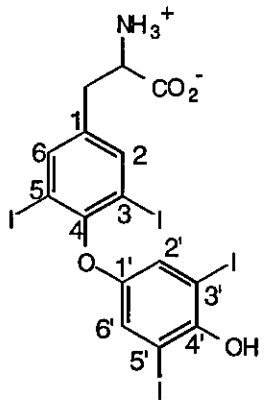
Thyroxine (T_4) is a naturally occurring hormone produced by the thyroid gland found in all vertebrate animals. It plays a vital role in the regulation of metabolism in the body, the principle target tissues being the skeletal muscle, cardiac muscle, liver and kidney. The basal metabolic rate (BMR) of an organism is a measure of the energy expended after completion of the intestinal absorption of food. The BMR for an adult euthyroid male is 35-40 kcal/m^2 body surface/hr, and for a woman is 6-10 % lower. In thyroid deficiency (hypothyroid state) the BMR can be reduced by up to 45 %, and in excess (hyperthyroid state) can be increased by up to 85 %.

1.7 The thyroid gland

In humans the thyroid gland consists of two lobes on either side of the trachea which are connected by a band of similar tissue called the isthmus. In an adult man the gland weighs about 20 g and is slightly larger in women. The gland is divided into lobules which are composed of follicles. The follicles consist of a single layer of epithelial cells lining the follicle lumen, which is filled with a colloidal substance, and it is here that the thyroid hormones are stored. In the resting state colloid accumulates in the follicle lumen and the epithelial cells take up a flattened form. In conditions of over-activity the colloid is partly or wholly absorbed and the cells become elongated (hyperplastic).

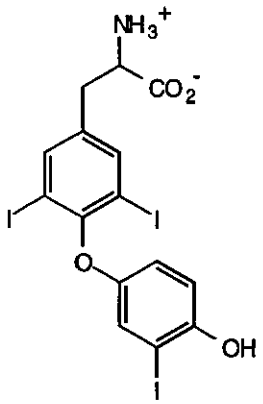
1.8 Thyroxine and the iodine requirement

The thyroid gland biosynthesises, stores and secretes two molecular species of thyroid hormone - thyroxine (T_4, **34**) or *L*-3,5,3',5'-tetraiodothyronine and triiodothyronine (T_3, **35**) or *L*-3,5,3'-triiodothyronine. The numbering system of the carbon atoms is shown below.



Thyroxine (T_4)

34



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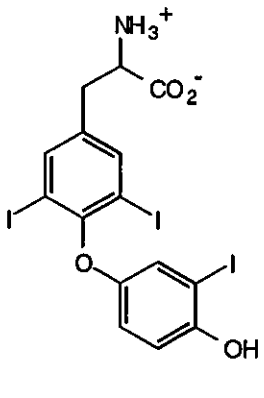


proximal conformer

Triiodothyronine (T_3)

35

Since the hormones contain three or four organically bound iodine atoms, for the thyroid to function normally an adult human should intake 150 μg/day of iodine. The main source of iodine is from iodised salts and seafood. A lack of iodine in the diet will cause endemic goiter, a largening of the thyroid gland resulting in hypothyrodism. This condition can occur by eating crops grown on soil which has sub-optimal levels of iodine.

1.9 Biological effects of thyroxine

The biological effects produced by the thyroid hormones include increased oxygen consumption and concomitant heat production. The thyroid hormones have been also been found to be necessary in ensuring normal growth of the organism and differentiation of the cells. At the cellular level the thyroid hormones are responsible for the stimulation of oxygen consumption, glycolysis and succinate oxidation. Associated with these effects is the increased activity of many of the enzymes involved in the metabolic pathways, including the respiratory enzymes NADPH-cytochrome-*c* reductase and cytochrome oxidase. The thyroid hormones also show an increase in RNA and protein synthesis.

1.10 Clinical aspects

1.10.1 Thyroxine deficiency (hypothyroidism)

Goiter was the first thyroid disease to be recognised, the symptoms being swelling in the neck which can be very slight, or so severe that the large swelling hangs down to the waist. In regions where goiter is common the disease is termed *endemic* goiter. The reason for this goiter is generally a lack of iodine in the diet.

The classical disease caused by hypothyroidism is myxedema and causes an accumulation of a mucilaginous layer under the skin, giving the patient a "puffy" appearance and causing the eyelids to droop. The patient is sensitive to cold and the body temperature may drop by up to three degrees. The heart rate is reduced giving rise to poor circulation and loss of feeling in the extremities. Physically the patient will be lethargic and mental activity will also be slower causing forgetfulness. The appetite will be decreased, but since the BMR is also much reduced the patient may become obese.

Hashimoto's disease shows the signs of myxedema, but rather than being caused by an underactive thyroid it is due to circulating thyroid antibodies.

As reported earlier, the thyroid hormones are necessary for normal growth and cell differentiation, and as a result hypothyroidism in infancy can lead to physical underdevelopment and dwarfism. The disease is known as cretinism and causes severe mental retardation and an impairment of the central nervous system. Cretinism can also develop in adults.

In some cases hypothyroidism is due to hereditary defects affecting the biosynthesis of the thyroid hormones, e.g. iodide organification, iodide concentration and thyroid gland insensitivity to thyroid stimulating hormone (TSH). This hormone is part of a feed back system which regulates the amount of thyroid hormone in circulation, stimulating the release of the thyroid hormone whenever necessary.

1.10.2 Thyroxine excess (hyperthyroidism)

General symptoms of hyperthyroidism are increased nervous tension, accelerated pulse rate, loss of body weight, increased oxygen consumption, restlessness, insomnia and sensitivity to heat. The BMR is greatly increased causing flushes and excessive perspiration. The increased tissue activity is generally compensated for by increased food intake, but usually it still does not meet the demands and weight

loss occurs. The central nervous system is, in general, over-sensitive causing over-reaction.

Over-activity of the thyroid gland is generally due to stress, and can be effectively treated by counselling.

There are however two diseases due to over-activity of the thyroid gland which lead to thyrotoxicosis, these are Grave's disease and adenoma. Grave's disease is characterised by bulging of the eyeballs resulting from the accumulation of fluid and the connective tissue ground substance. It is an autoimmune disease resulting from the presence of a long acting thyroid stimulator (LATS). It is suggested that the disorder is inherited. Adenoma is generally the over-production of thyroxine, due to a gradual transition to autonomous function from the over-sensitivity to TSH.

1.11 Thyroxine as a drug

Thyroxine is not only of great importance in the treatment of hypothyroid patients, but it is also used to treat obesity in patients where no thyroid deficiency is detected. It is also administered occasionally along with other gland products, and although no satisfactory rationale of this procedure is known, it often appears effective. Possibly the influence that thyroxine has on the "specific dynamic action" of foodstuffs is involved.

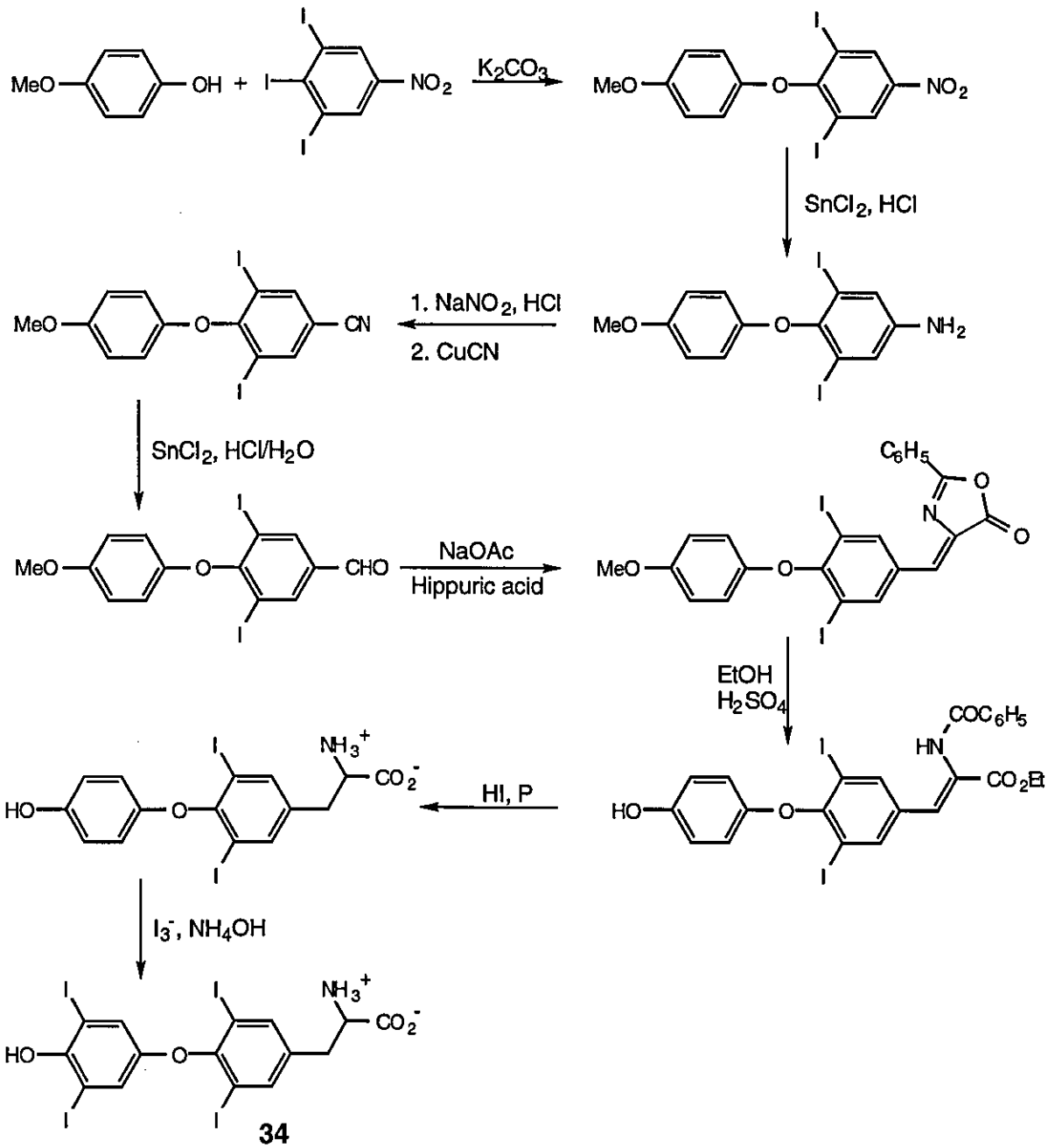
Thyroxine used in small doses has a building up (anabolic) effect, however in the higher dosage range it has a stimulating (catabolic) effect for which the drug is more commonly prescribed. Correspondingly, it may have a sedative or exciting influence depending on the dosage used.[18]

1.12 Isolation of thyroxine and the early chemical syntheses

The diphenyl ether nature of the structure of thyroxine was established by Harington in 1926.[19] He isolated thyroxine by refluxing thyroid tissue with 10 % barium hydroxide solution to give crude crystals of the barium salt of thyroxine which were purified by refluxing with sodium hydroxide solution containing sodium sulfate followed be filtration and acidification with sulfuric acid. The resulting precipitate was dissolved in alkali treated with alcohol, filtered, and acidified with acetic acid. Iodine could be removed quantitatively by catalytic hydrogenation in potassium

hydroxide solution with palladium oxide-calcium carbonate, the resultant compound was named desiodothyroxine by Harington, and later thyronine.

The first synthesis of thyronine was through the condensation of *p*-bromoanisole with phenol to give *p*-methoxybiphenyl ether which was converted to the corresponding *p*-aldehyde via the Gatterman reaction. From the aldehyde, thyronine was prepared by hydantoin condensation, and found to be identical with that obtained by de-iodination of the thyroxine isolated from the thyroid tissue after alkaline hydrolysis.[20]



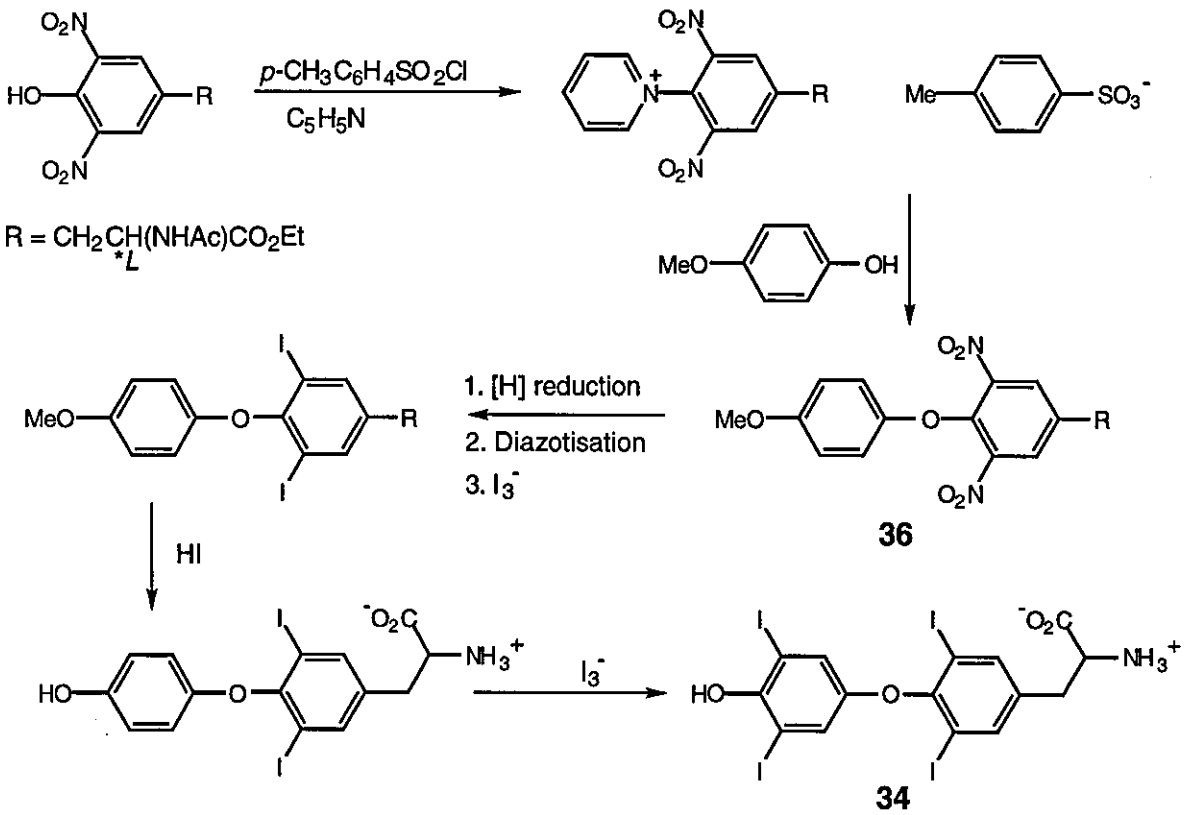
34

Scheme 18

Since iodination of thyronine only led to the introduction of iodines in the 3' and 5' positions it appeared essential that the 3 and 5 positions should be iodinated prior to phenyl ether synthesis. Harington's first successful synthesis of thyroxine (**scheme 18**) involved the reaction of 3,4,5-triiodonitrobenzene with *p*-methoxyphenol to yield 3,5-diiodo-4-(4'-methoxyphenoxy)nitrobenzene. The nitro group was then reduced with stannous chloride, which was converted to the nitrile by diazotization and treatment with cuprous cyanide. Conversion to the aldehyde with anhydrous stannous chloride led to 3,5-diiodo-4-(4'-methoxyphenoxy) benzaldehyde. This aldehyde was condensed with hippuric acid followed by the ethanolysis of the oxazolone ring. The resulting cinnamic ester was treated with hydriodic acid and red phosphorus to yield 3,5-diiodothyronine. *DL*-Thyroxine (**34**) was achieved by the reaction of the 3,5-diiodothyronine in a solution of ammonium hydroxide, iodine and potassium iodide, and was identical to the material isolated from the alkaline treatment of the thyroid tissue.[21] This procedure for the synthesis of *DL*-thyroxine has been used with adaptations to synthesise various analogues of thyroxine.[22] Gemmill and co-workers[23] followed the same reaction path using 3,4-diiodonitrobenzene with *p*-methoxyphenol and a milder reduction method, a brief treatment with red phosphorus and hydriodic acid in acetic acid, followed by heating under reflux conditions with hydrobromic acid to remove protective groups to give 3-iodo-*DL*-thyronine prior to iodination to yield 3,3'-diiodo-*DL*-thyronine.

Between 1949 and the mid 1950s, Hems and his colleagues at the Glaxo research laboratories developed an eight step synthesis of *L*-thyroxine from *L*-tyrosine leading to an overall yield of 26 % (**scheme 19**).[24] The procedure involved the initial protection of 3,5-dinitro-*L*-tyrosine to the *N*-acetyl ethyl ester, followed by condensation with *p*-methoxyphenol under the action of *p*-toluenesulfonyl chloride in pyridine to give **36**, which was reduced using hydrogen and poisoned palladium. The diazotisation and Sandmeyer iodination of **36**, followed by deprotection and iodination of the phenolic ring using iodine in aqueous ethylamine yielded *L*-thyroxine.

Further investigations by Harington[25] of the extracts from the alkaline treatment of the thyroid tissue led to the isolation of 3,5-diiodotyrosine. This gave further evidence to Harington's hypothesis that thyroxine was formed by the coupling of two diiodotyrosine residues, with the subsequent loss of one amino acid side chain. This led to the research of a biomimetic synthesis of thyroxine, which was of interest to several research groups whose work will be discussed in the following section.



Scheme 19

1.13 Biosynthesis of thyroxine

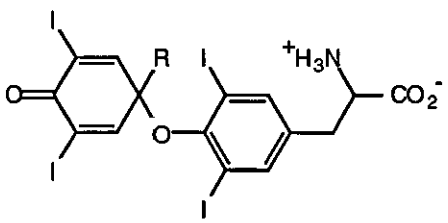
The two general theories proposed for the formation of thyroxine within the thyroid gland are intramolecular coupling and intermolecular coupling. Intramolecular coupling involves the formation of protein bound 3,5-diiodotyrosine (DIT) radicals by the action of thyroid peroxidase, then two DIT radicals (which are in close proximity on the thyroglobulin chain) couple to form a quinol ether intermediate which then splits off by a rearrangement process leaving dehydroalanine residue in the position of the transferred DIT. The intermolecular theory involves the coupling of free DIT with a radical of 3,5-diiodo-4-hydroxyphenylpyruvic acid (DIHPPA) in thyroglobulin. Much time has been spent in the research of both of these mechanisms which will be summarised below.

1.13.1 Intramolecular coupling

Since the discovery that thyroxine could be formed by the incubation of DIT under mild alkaline conditions,[26] research has been devoted to the requirements of the reaction in order to improve the low yields obtained from this method of synthesis

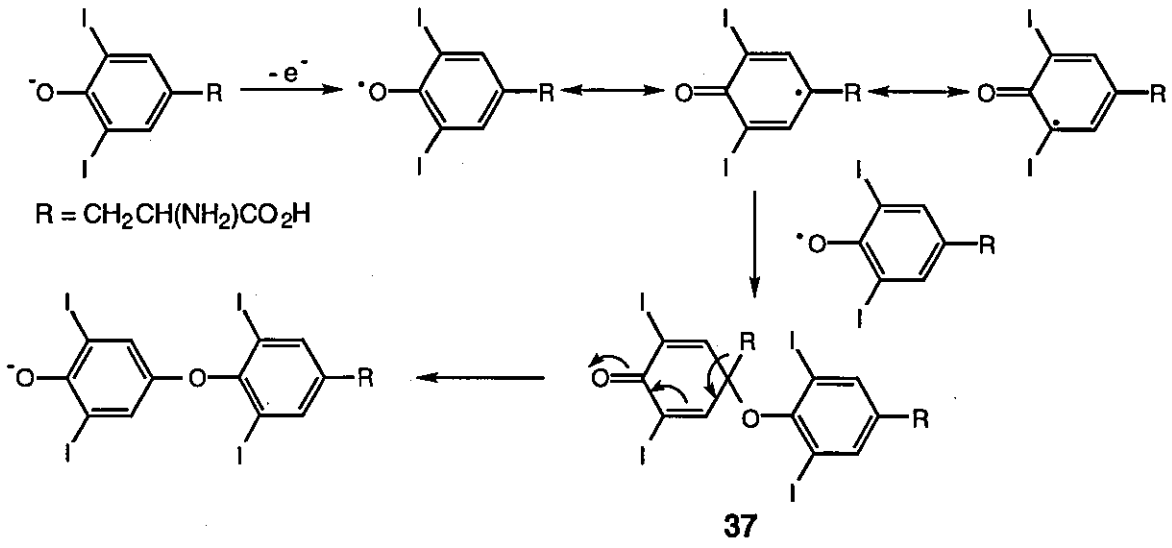
and to the understanding of the reaction mechanism. From these early studies it was apparent that the reaction was aerobic and would only occur in the presence of oxygen. In 1945 Pitt-Rivers reported results which clearly showed that the pH of the reaction had an effect on the extent to which 3,5-diiodotyrosine was converted to thyroxine. At high pH (13-14) the only product of the reaction was 3,5-diiodo-4-hydroxybenzaldehyde, whereas at pH 10 the benzaldehyde and thyroxine are produced simultaneously, and at pH 7-9 thyroxine was the only isolated product of the reaction. These results led to the hypothesis that 3,5-diiodo-4-benzaldehyde was actually an intermediate in the synthesis of the of thyroxine. However, on incubation of the benzaldehyde with diiodotyrosine thyroxine was not obtained.[26c] Further work by Pitt-Rivers and James[27] investigated the requirements of the side chain for successful coupling. They succeeded in coupling derivatives of 3,5-diiodotyrosine to give corresponding thyroxine analogues, including the coupling of 2-(3,5-diiodo-4-hydroxyphenyl)acetic acid. The lack of reaction shown by 3,5-diiodo-4-hydroxybenzoic acid led to the conclusion that the benzylic methylene group was a necessity for the reaction, and that oxidation occurred in this position giving rise to the elimination of the side chain. Saul and Trikojus[28] had also synthesised the thyroxine analogue of 3-(3,5-diiodo-4-hydroxyphenyl)lactic acid. These results prompted Cahnmann and Matsuura[29] to carry out further investigations of the side chain requirement with a variety of 3,5-diiodotyrosine analogues. They used acids of varying length side chains, and obtained the best yield with 3-(3,5-diiodo-4-hydroxyphenyl)propionic acid. The 2-(3,5-diiodo-4-hydroxyphenyl)acetic acid derivative led to some thyroxine analogue, but also to oxidative degradation and the formation of the benzaldehyde derivative. Lengthening and branching of the side chain gave rise to little or no thyroxine analogue being formed, the 3,5-diiodo-4-hydroxycinnamic acid and 3,5-diiodo-4-hydroxybenzoic acid also did not couple. Cahnmann and Matsuura[30] then turned their attention to the fate of the lost side chain, the isolation of which would give vital evidence to the form of the coupled intermediate. It had been postulated[32] that the conversion of 3,5-diiodotyrosine to thyroxine occurs through a quinol ether intermediate **37** which by the loss of the alanine side chain, in the form of serine or dehydroalanine allows rearomatisation to give thyroxine (**34**).

$R = CH_2CH(NH_3^+)CO_2^-$



37

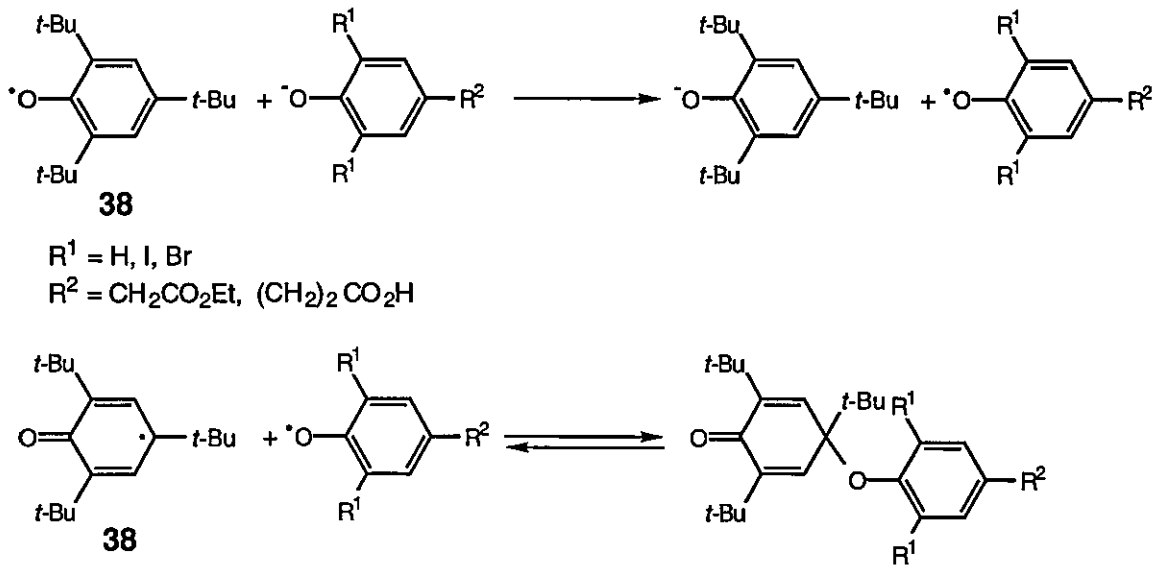
Cahnmann and Matsuura reported the isolation of 3-hydroxypropionic acid and acetic acid after the incubation of 3-(3,5-diiodo-4-hydroxyphenyl)propionic acid. They suggested that the formation of the 3-hydroxypropionic acid was from the hydration of eliminated acrylic acid, however, their incubation of acrylic acid under the reaction conditions left the acrylic acid unchanged. The same researchers also investigated the initial oxidation step postulated by Johnson and Tewkesbury[31] and elaborated by Harington[32] based on the extensive research carried out by Pummerer and co-workers that many oxidations of phenols take place through a free radical mechanism. The first step proposed was the oxidation of 3,5-diiodotyrosine in its phenoxide form to the free radical (**scheme 20**), the second step being the dimerisation of two molecules of the free radical to form the quinol ether **37**, and the final step being the elimination of the side chain.



Scheme 20

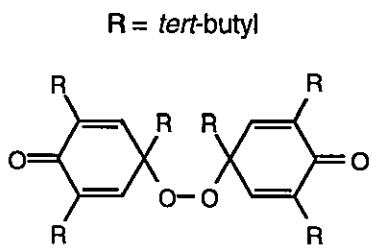
Cahnmann and Matsuura[33] used 2,4,6-tri-*tert*-butylphenol as a model compound, since it forms a stable free radical **38**. The oxidation was carried out using potassium ferricyanide to give the blue coloured radical **38**, then tyrosine or an analogue of tyrosine was added and the disappearance of the blue colour was observed. This was reported to be due to electron transfer between the stable free radical **38** and the tyrosine analogue. Further oxidations by the additions of fresh potassium ferricyanide were carried out before isolation of the coupled products. It was reported that the quinol ethers dissociate at their ether linkage to the corresponding free radicals **38** and the phenoxyl radical of the tyrosine analogue. This was particularly apparent at elevated temperatures when the yellow-greenish solutions of the quinol ether turned blue (the colour of the original radical **38**) then assumed their yellow-greenish colour on cooling. The bulkier the substituents on

the tyrosine analogue the lower the temperature at which the dissociation occurs; the quinol ethers of iodinated derivatives had to be cooled in dry ice-acetone baths. The authors explain this by the steric interactions of the *tert*-butyl and iodine groups causing a strong barrier to rotation. Once isolated, the quinol ethers if heated formed the thyroxine analogue, and the same conversion could be achieved in solution with acid catalysis.



Scheme 21

In a later publication[34] Cahnmann and Matsuura reported that in the case of coupling dibromo and diiodotyrosines with 2,4,6-tri-*tert*-butylphenol, the products were the starting material and the peroxide **39**, formed by the reaction of the radical **38** with oxygen, and the corresponding thyroxine analogue. They explained this to be due to the dissociation of the unstable quinol ether or the elimination of the side chain to form the more stable thyroxine derivative.



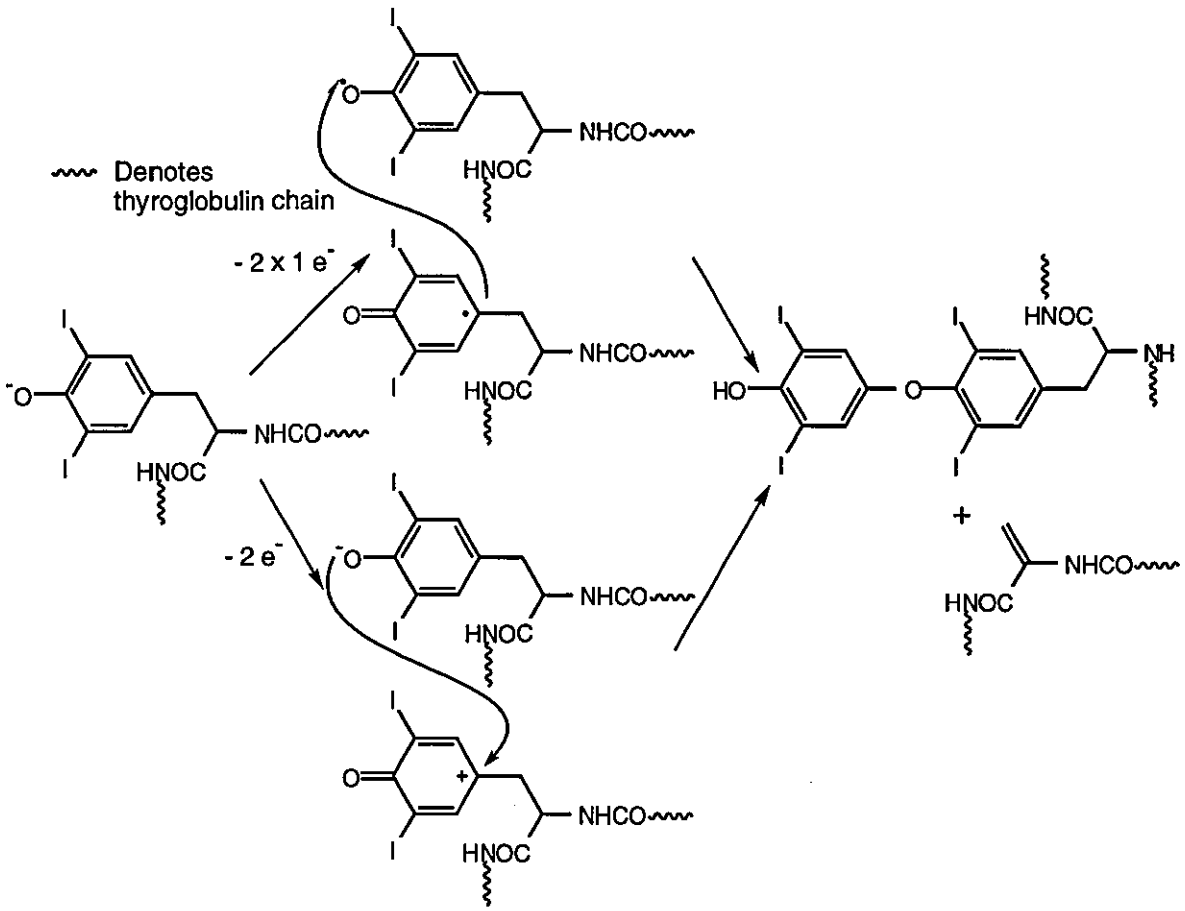
39

Biological studies of this intramolecular coupling involved the incubation of thyroglobulin, thyroid peroxidase, glucose and glucose peroxidase to yield 3,3',5-

triiodothyronine (**35**) and thyroxine (**34**). Thyroglobulin is a large glycoprotein having a molecular mass of 660,000, and is the principal iodoprotein of the thyroid gland. Thyroglobulin contains about 130 tyrosine residues, and both *in vivo* and *in vitro* studies have indicated that there are different categories of tyrosines, some having the potential to be iodinated to 3-iodotyrosine others to 3,5-diiodotyrosine, and only some of the iodinated residues have potential for coupling.[35] Thyroid peroxidase is required in the coupling both to catalyse the iodination of the tyrosine residues on thyroglobulin and the coupling to form thyroxine. Thyroid peroxidase is not unique in its ability to catalyse the coupling reaction, lactoperoxidase was found to have very similar activity in the formation of thyroxine, however, the enzymes show superior activity to the reaction with hydrogen peroxide.[36]

The proposed mechanisms for the coupling of two 3,5-diiodotyrosine residues in the presence of thyroglobulin are shown in **scheme 22.** In the originally postulated mechanism two radicals react with each other. However, Cahnmann *et al* pointed out that a two electron oxidation to form a cation could occur, with the formation of a charge transfer complex to line up the 3,5-diiodotyrosine residues prior to coupling.[37] This possibility was opposed by Taurog and co-workers who observed the inhibition of the coupling in the presence of nitrosobenzene. They obtained evidence that nitrosobenzene was not a substrate for thyroid peroxidase, and that at concentrations at which nitrosobenzene would inhibit the coupling reaction it did not inhibit the thyroid peroxidase or lactoperoxidase catalysed iodination or oxidation of guaiacol.[38]

The location of the "lost side chain" has been a matter addressed by most of the researchers concerned with the intermolecular or intramolecular biosynthesis of thyroxine. The isolation of the side chain which would eliminate in the formation of thyroxine would give evidence to the form of the side chain prior to coupling. In the case of intramolecular coupling, the location of the "lost side chain" would provide information concerning the location of the so-called "donor" 3,5-diiodotyrosine residues (i.e. the 3,5-diiodotyrosine residues which lose their side chain on coupling) within the thyroglobulin molecule prior to coupling. The side chain has not as yet been reported to have been isolated from the reaction to form thyroxine in the absence of thyroglobulin, although Pitt-Rivers and James[27] detected pyruvic acid and acetamide in their post-coupling reaction mixtures and suggested that these compounds were derived from dehydroalanine.

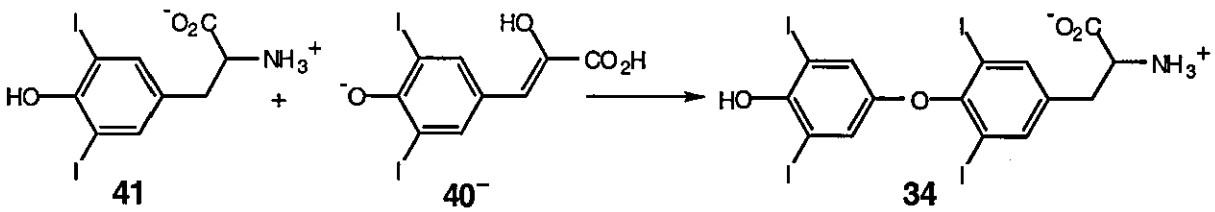


Scheme 22

In 1979 Gavaret and co-workers[39] reported that after the formation of ^{14}C labelled thyroxine, ^{14}C labelled pyruvic acid was detected, which they also suggested was derived from dehydroalanine. A later report by Palumbo[40] described the treatment of thyroglobulin with ^{3}H sodium borohydride and ^{14}C sodium cyanide after coupling, followed by cleavage with cyanobromide and analysis. Evidence of the detection of the labelled alanine and its prior derivation from the coupling to form thyroxine was lacking, the interest of the research was mainly in the location of the so-called "donor" sites which hold the "donor" tyrosine residues prior to their coupling. Kondo *et al* were also interested in locating these "donor" tyrosines and used 4-aminothiophenol after the coupling to react with the dehydroalanine to give *S*-(4-aminophenyl)cysteine (APC) residues. Digestion of the thyroglobulin and separation of the resulting peptides by size exclusion HPLC, followed by Edman degradation allowed the sequence of the peptide to be established. The presence of APC residues was confirmed by colorimetry experiments in which the APC was first treated with sodium nitrite, followed by the addition of *N*-(1-naphthyl)ethenediamine which gave a purple colour.[41]

1.13.2 Intermolecular coupling

The prevailing view of the mechanism of the biosynthesis is that it proceeds by intramolecular coupling on the thyroglobulin protein as described in the preceding section. However, much of the early research indicated that the biosynthesis of thyroxine went *via* an intermolecular mechanism involving 3,5-diiodo-4-hydroxyphenylpyruvic acid (DIHPPA). The research into this intermolecular theory was initiated by the work of Taurog and co-workers[42] who reported the presence of DIHPPA and 3,5-diiodo-4-hydroxyphenyllactic acid as products of the metabolism of the 3,5-diiodotyrosine during studies of the metabolism of 3,5-diiodotyrosine by liver and kidney slices. In 1960, Meltzer and Stanaback[43] carried out research into the possibility of 3,5-diiodo-4-hydroxyphenylpyruvic acid being formed as an intermediate from 3,5-diiodotyrosine during the synthesis of thyroxine. They studied the reaction of DIHPPA (**40**) with 3,5-diiodotyrosine (**41**) in the formation of thyroxine (**34**, **scheme 23**) using various oxidants and found that oxygen gave the best results. Other successful oxidants included hydrogen peroxide, *tert*-butyl peroxide and iodic acid. Surprisingly the reaction with potassium ferricyanide, a commonly used oxidant in phenolic coupling, yielded no thyroxine. They also experimented with the presence of various salts and found that manganese sulfate had a beneficial effect on the yield which was not seen with sodium sulfate or magnesium sulfate.



41 **40**$^-$ **34**

Scheme 23

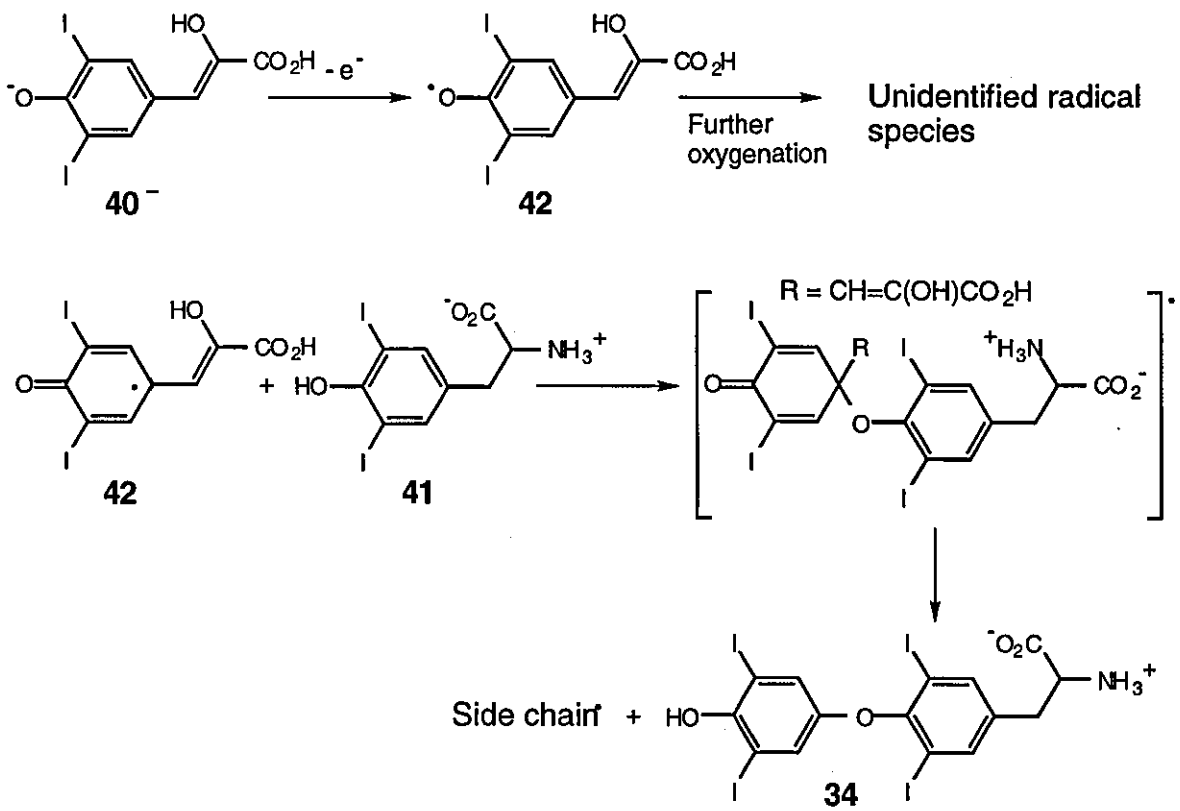
The reactions had been carried out between pH 7.4-7.8 due to the insolubility of the 3,5-diiodotyrosine (**41**) at lower pH and the alkaline instability of DIHPPA (**40**) at higher pH. The coupling of 4-hydroxy-3-iodophenylpyruvic acid or 4-hydroxyphenylpyruvic acid with 3,5-diiodotyrosine (**41**) was attempted, but did not produce any coupled compounds. It is possible that the DIHPPA (**40**) was simply an oxidation catalyst in the reaction of the coupling of two 3,5-diiodotyrosine molecules, however, by using ^{131}I labelling Cahnmann and Shiba[44] showed that when either [^{131}I]-3,5-diiodotyrosine or [^{131}I]-DIHPPA was used the thyroxine produced showed the same radioactivity as the starting material, no matter which compound was in excess. Substitution of 3,5-diiodotyrosine with 3-iodotyrosine or tyrosine in its coupling with DIHPPA was attempted by Cahnmann and Shiba[45] who

found that DIHPPA (**40**) would couple with 3,5-diiodotyrosine (**41**) and 3-iodotyrosine (to produce a so-called 'reverse' triiodothyronine) but not with tyrosine. These reactions were carried out around pH 6.7-7.7 due to the instability of DIHPPA (**40**) at higher pH, however, it was noted in the report that at this pH the 3-iodotyrosine and tyrosine are largely in their phenolic form, and that at higher pH with ionisation of the phenol the reaction may have occurred. Further studies[46] on the requirements of the DIHPPA (**40**) for the coupling showed that the iodines could be replaced by bromines and coupling would occur with 3,5-diiodotyrosine (**41**) and with 3,5-dibromotyrosine, however a change in the length or form of the side chain would not be tolerated by the reaction. Replacement of an iodine on DIHPPA (**40**) by a methoxy group would yield 4 % of the thyroxine analogue in comparison with a 20 % yield from the standard 3,5-diiodotyrosine (**41**) and DIHPPA (**40**) reaction. The same authors had previously discovered an enzymic method for the formation of thyroxine from 3,5-diiodotyrosine.[47] By the use of rattlesnake venom, a source of *L*-amino acid oxidase, and in the presence of oxygen and catalase a 16 % yield was reported after only a few hours of reaction. The authors suggested that conversion of 3,5-diiodotyrosine (**41**) to DIHPPA (**40**), by oxidative deamination, prior to coupling may occur but have no evidence that this oxidative deamination takes place. The enzymes may simply be catalysing the coupling of two 3,5-diiodotyrosine residues rather than converting the 3,5-diiodotyrosine (**41**) to DIHPPA (**40**). The procedure did however, provide a simple and relatively fast method for the synthesis of thyroxine.

Investigation of the initial step in the coupling of DIHPPA (**40**) with 3,5-diiodotyrosine (**41**), involving the oxidation of the DIHPPA to a radical was investigated by electron spin resonance spectroscopy.[48] Oxygenation of a solution of DIHPPA (**40**) gave rise to a radical which was detected as a triplet signal by EPR spectroscopy. After a further 5-10 minutes oxygenation this signal was replaced by a doublet which was stable for several hours. The triplet signal was interpreted by Blasi as corresponding to the phenoxyl radical of DIHPPA (**42**). The interpretation of the second signal was more difficult, but from the data and shape of the signal it appeared that the radical was still iodinated and that the unpaired electron was interacting with a proton on the side chain. Further evidence in support of the formation of the phenoxyl radical **42** was obtained by comparing the reactivity of **42** to 3,5-diiodotyrosine (**41**), propylgallate and *p*-nitrophenol in terms of the minimum concentrations for the disappearance of the phenoxyl radical signal. It was found that propylgallate and 3,5-diiodotyrosine show the same activity and *p*-nitrophenol shows only half the activity of the other two compounds. The radical resulting from the further oxidation of **42** reacted with propylgallate and *p*-nitrophenol, but not

with 3,5-diiodotyrosine (**41**). These results, along with the blocking of the coupling of DIHPPA (**40**) with 3,5-diiodotyrosine (**41**) in the presence of propylgallate, led the author to suggest that **42** couples with 3,5-diiodotyrosine (**41**) to produce a quinol ether radical which dissociates, the side chain coming off as a radical leading to the formation of thyroxine (**scheme 24**).

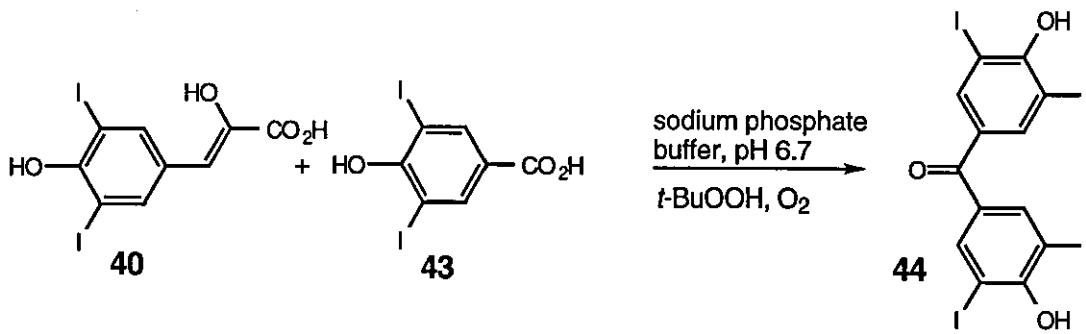
Later, the second radical observed on continuing oxygenation of **42** was reported to be the radical of 2,6-diiodobenzosemiquinone (DISQ)[49] resulting from the autoxidation of the radical **42**. The possibility of the DISQ radical being an intermediate in the coupling of DIHPPA with 3,5-diiodotyrosine was again disproven by the absence of thyroxine (**34**) formation when reacting equimolar amounts of 2,6-diiodobenzoquinone and 2,6-diiodohydroquinone (which give rise to the EPR signal for DISQ) with an excess of 3,5-diiodotyrosine (**41**) under the conditions that DIHPPA (**40**) reacts with 3,5-diidotyrosine (**41**) to form thyroxine in good yield.



Scheme 24

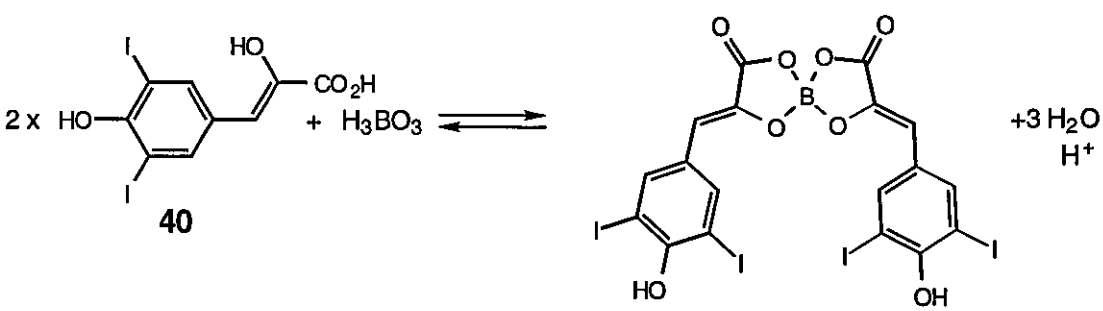
The reaction of DIHPPA (**40**) with 3,5-diiodo-4-hydroxybenzoic acid (**43**) to produce 4,4'-dihydroxy-3,3',5,5'-tetraiodobenzophenone (**44**) was investigated by Matsuura and co-workers.[50] The results they obtained from carrying out radiolabeling with both ^{131}I and ^{14}C showed that the DIHPPA (**40**) was reacting

with the 3,5-diiodo-4-hydroxybenzoic acid (**43**) to produce **44** in which the carbonyl carbon originated from the DIHPPA (**40**). In order for this to occur it was proposed that the methylene carbon of DIHPPA (**40**) was oxidised prior to the coupling.



Scheme 25

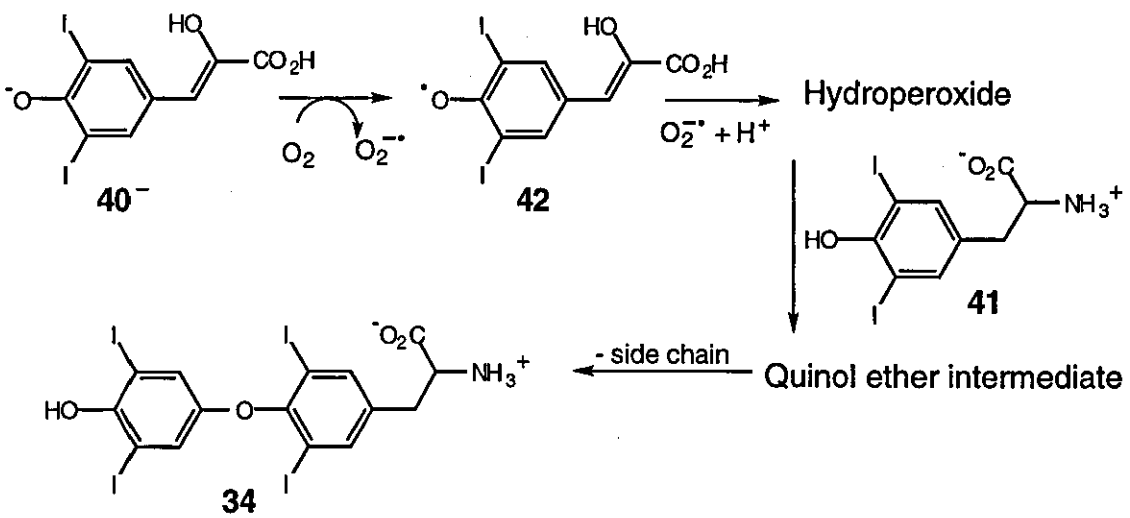
Further studies by the same researchers provided further evidence for the oxidation of the methylene carbon.[51] They carried out an investigation of the oxygen uptake of DIHPPA (**40**) and found that at 24 °C and pH 7.2-7.6 just under a mole equivalent of oxygen was consumed by the DIHPPA (**40**), and upon the addition of 3,5-diiodotyrosine (**41**), thyroxine (**34**) formation was observed. Although the oxygen consumption was slower at 2 °C a full mole equivalent was consumed, indicating that at the higher temperature some degradation of the DIHPPA (**40**) was occurring. At higher pH the oxygen consumption was lower, except in strong alkali in which it was very fast, however, the addition of 3,5-diiodotyrosine (**41**) did not give rise to thyroxine (**34**), but 3,5-diiodo-4-hydroxybenzaldehyde and the recovery of 3,5-diiodotyrosine (**41**). These experiments were all carried out in the presence of borate ions, and the substitution of borate for phosphate ions gave rise to little or no oxygen consumption. Research by Knox and Pitt[52] had shown that the enol form of DIHPPA (**40**) was favoured in the presence of borate ions which they suggested to be due to the formation of an enol-borate complex, the structure which they proposed for this complex is shown in **scheme 26**.



Scheme 26

They concluded that only that enol form of DIHPPA (**40**) was capable of consuming oxygen to form the precursor in the formation of thyroxine (**34**) which they proposed to be a hydroperoxide, for which a structure was not suggested. The authors also proposed a mechanism for the coupling of this precursor with 3,5-diiodotyrosine (**41**) in the formation of thyroxine (**scheme 27**) in which the first step is the oxidation of DIHPPA (**40**) to a phenoxyl radical (**42**), followed by the formation of the hydroperoxide which couples with 3,5-diiodotyrosine (**41**) to form an intermediate quinol ether, the elimination and rearomatisation of which yields thyroxine.

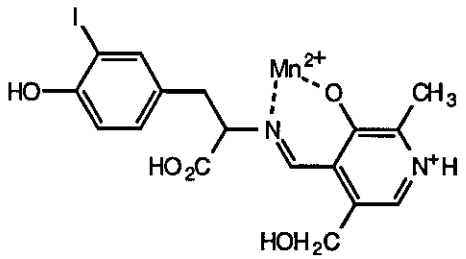
Work reported by Blasi *et al*[53] indicated that oxygen was not required throughout the coupling reaction, and that once the thyroxine precursor was formed (from DIHPPA during the 'lag phase') the rest of the reaction could proceed in the absence of oxygen.



Scheme 27

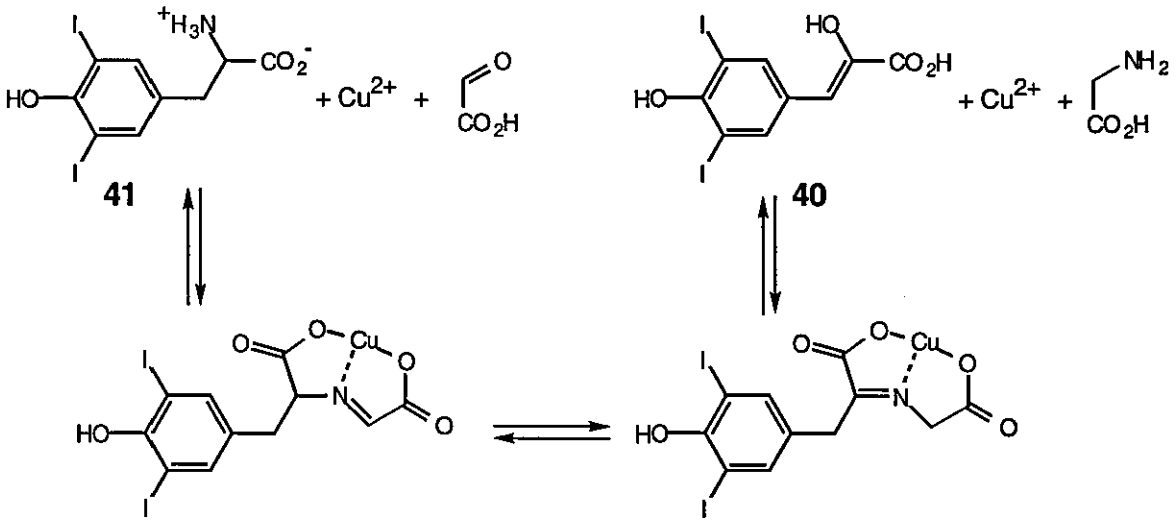
They also showed that by the addition of free radical scavengers such as propylgallate the lag phase was increased and the yield of thyroxine decreased. This gave further evidence that the first step in the reaction sequence is the formation of the phenoxyl radical of DIHPPA (**42**) which subsequently consumes oxygen to produce the hydroperoxide which then couples with 3,5-diiodotyrosine (**41**) to produce thyroxine (**34**), i.e. this last step is anaerobic. Studies of the non-enzymatic conversion of 3-iodotyrosine to 4-hydroxy-3-iodophenylpyruvic acid (MIHPPA) were performed using pyridoxal phosphate.[54] The conversion of 3-iodotyrosine to both MIHPPA and 3,3'-diiodothryronine by the incubation of 3-iodotyrosine with pyridoxal phosphate and various metal ions was reported. Stanbury *et al*[54b] experimented using manganese(II), iron(II), magnesium(II) and calcium(II) in their studies and showed manganese to be the favourable ion for the conversion and

postulated the structure of the complex **45**. From their studies, Ito *et al*[54a] concluded that copper(II) and cobalt(II) were the favourable ions above manganese(II) for the conversion.



45

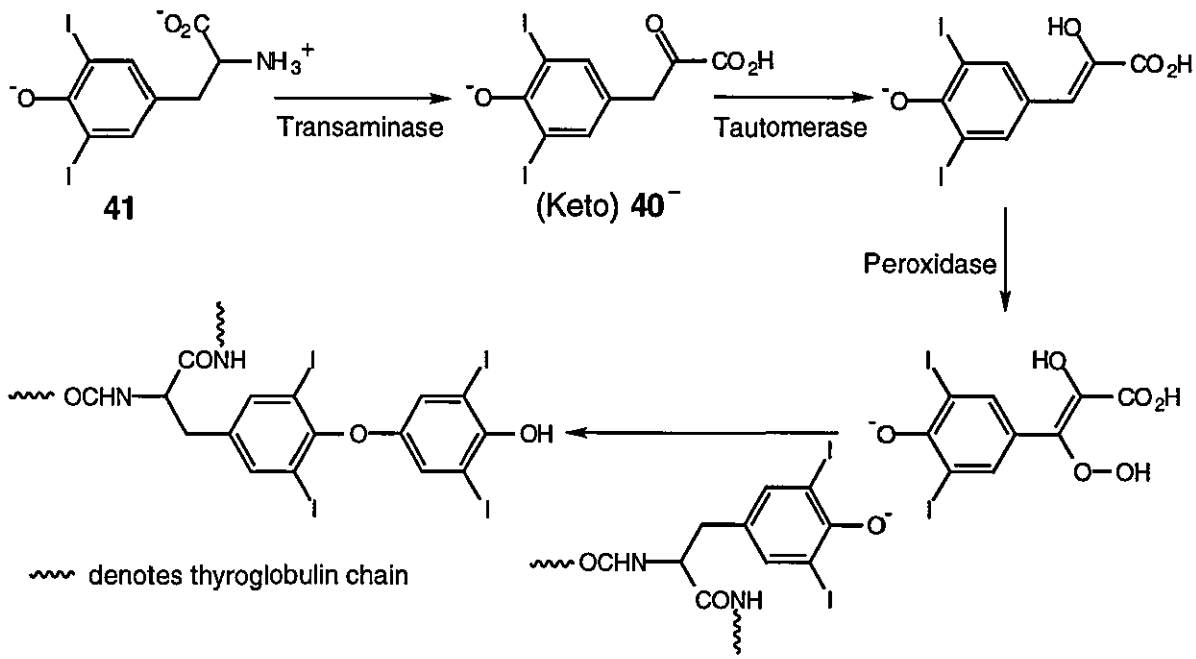
Shiba *et al*[55] continued these non-enzymatic models using sodium glyoxylate to effect the transamination and found that when cupric acetate was used the reaction gave a higher yield of thyroxine than when the reaction was performed with other metal salts ($FeSO_4$, $MnCl_2$, $Zn(OAc)_2$, $Pb(OAc)_2$, $CdCl_2$, $HgSO_4$, $NiSO_4$, $MgSO_4$, $CoSO_4$). The authors suggested the formation of the complex **46** and that the transamination was the slow step after which coupling with 3,5-diiodotyrosine (**41**) would occur rapidly to form thyroxine.



Scheme 28

These non-enzymatic reactions were followed by attempts to show that the conversion of 3,5-diiodotyrosine (**41**) to DIHPPA (**40**) could take place in the presence of enzymes from thyroid homogenate.[56] They compared the conversion of 3,5-diiodotyrosine (**41**) to DIHPPA (**40**) using pyridoxal phosphate and α-ketoglutarate and the same conversion using the post-mitochondrial supernatant of

rat thyroid homogenate. The rate of the reaction was doubled in the presence of post-mitochondrial supernatant of rat thyroid homogenate which indicated the presence of a transamines enzyme in the thyroid homogenate. They also showed the presence of a keto-enol tautomerase enzyme in beef, rat and human thyroid homogenate. Oxidation to the hydroperoxide was performed using horseradish peroxidase in place of thyroid peroxidase. The peroxidase treated DIHPPA (**40**) was successfully coupled with 3,5-diiodotyrosine (**41**) under a nitrogen atmosphere. It was concluded that the thyroid gland contains enzymes able to convert 3,5-diiodotyrosine (**41**) to thyroxine (**34**) through the pathway shown in **scheme 29**. They therefore suggested that a transamination of 3,5-diiodotyrosine (**41**) is more likely to be the conversion occurring in the thyroid gland rather than the oxidative deamination suggested by Cahnmann and Shiba in their experiments with rattlesnake venom.[47]



Scheme 29

1.14 Summary and aims of the project

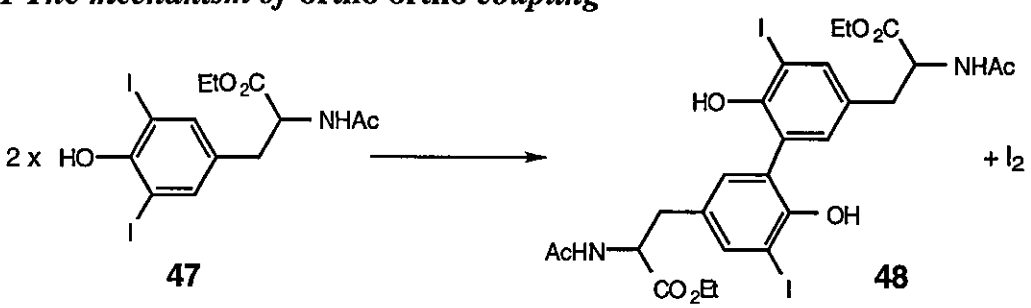
From the overview of the research carried out concerning the intermolecular and intramolecular coupling it is apparent that either or both of the mechanisms could actually occur in the biosynthesis of thyroxine, since neither mechanism has as yet been fully proven. In the intermolecular coupling, the presence of the necessary enzymes for the formation of DIHPPA (**40**), its tautomerism and oxidation in the thyroid gland indicate that the DIHPPA (**40**) is a precursor in the biosynthesis of

thyroxine. However, the actual mechanism of the coupling of DIHPPA (**40**) and loss of the side chain is still not completely clear since neither the postulated hydroperoxide or the "lost side chain" have been isolated or detected.

For the intramolecular coupling of the tyrosine residues the actual location of the donor and acceptor tyrosine residues is still of interest to biochemical researchers. On the chemical side the interest lies in the necessity of the various functional groups and substituents of 3,5-diiodotyrosine (**41**) and their rôle in the coupling to form thyroxine (**34**).

Our investigation has involved synthesis of *N*-acetylthyroxine ethyl ester (**46**) by the reaction of *N*-acetyl-3,5-diiodotyrosine ethyl ester (**47**) in an ethanol/sodium hydroxide solution at pH 9.5 in the presence of catalytic quantities of manganese(II) sulfate and boric acid at 60-70 °C and 5 bar pressure of oxygen. During this reaction the pH gradually drops, and in some cases the pH drops quickly and results in the formation of an *ortho-ortho* coupled compound **48** (**scheme 30**) with concomitant loss of iodine. The aim of the investigation was twofold, firstly to elucidate the mechanisms in all the steps of the reactions (i.e. the *ortho-ortho* coupling and the oxygen-*para* coupling), and secondly to modify the conditions of the reaction in order to direct the coupling to give essentially only the *N*-acetyl thyroxine ethyl ester (**46**). The key mechanistic features which were investigated are outlined below.

1.14.1 The mechanism of ortho-ortho *coupling*

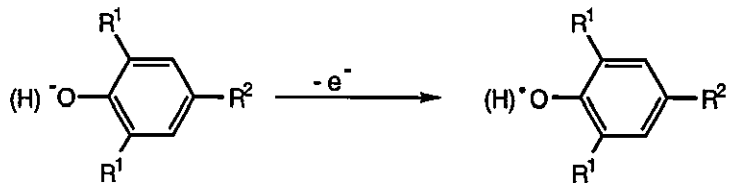


Scheme 30

If the conditions for the reaction to form *N*-acetylthyroxine ethyl ester (**46**) are to be modified in order to eliminate the possibility of this side reaction occurring, then first the mechanism of this *ortho-ortho* coupling must be understood and the specific conditions leading to the *ortho-ortho* coupled by-product **48** known. Once the conditions for the *ortho-ortho* coupling have been optimised then further

investigations can be carried out into this reaction. The rôle of the substituents on the phenol in directing this coupling is of interest in ascertaining how useful this method of *ortho-ortho* coupling may be as a synthetic tool.

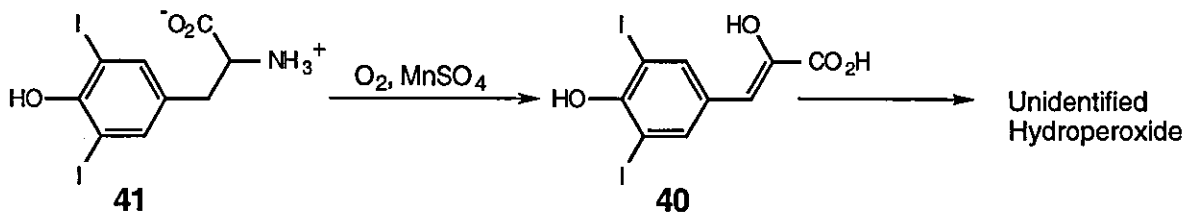
1.14.2 Oxidation of the phenol to the phenoxyl radical



Scheme 31

The oxygen-*para* coupling in the formation of thyroxine has been suggested to occur through either a single or two electron oxidation of the phenol, whether the oxidised phenol is DIHPPA or 3,5-diiodotyrosine. It is therefore of interest to study these oxidations electrochemically, and by varying the substituents on the phenol more information may be gained about the purpose of iodines and the amino acid side chain of 3,5-diiodotyrosine (**41**), and the α-keto acid of DIHPPA (**40**). The active form of the thyroid hormone is thought to be 3,3',5-triiodothyronine (**35**) which begs the question of why both compounds involved in the coupling are diiodinated prior to coupling.

1.14.3 Oxidation of the amino acid side chain prior to coupling

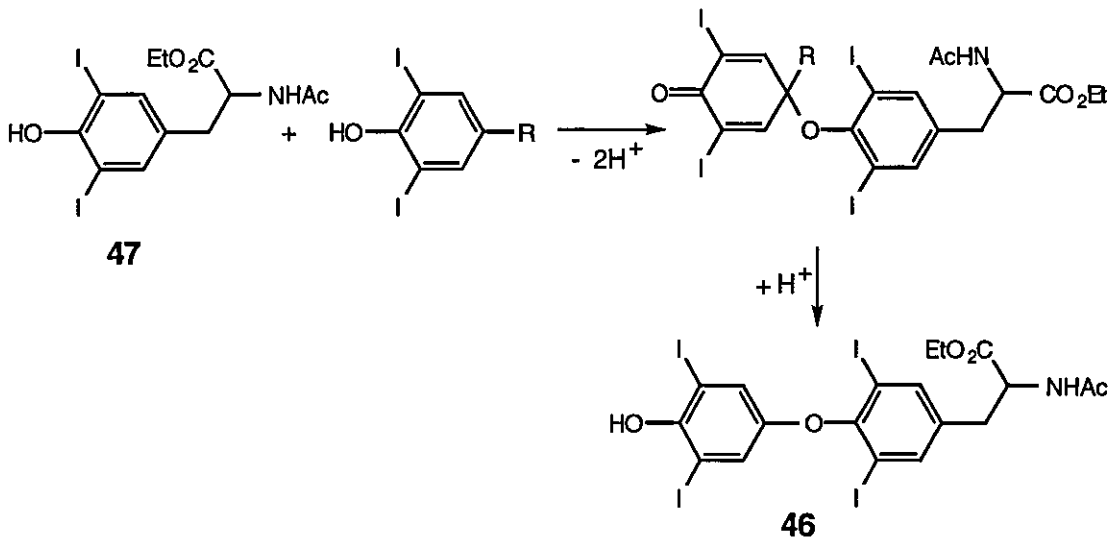


Scheme 32

Research carried out into the intermolecular formation of thyroxine suggests that the first step in the synthesis is the conversion of 3,5-diiodotyrosine (**41**) to DIHPPA (**40**) either by transamination or oxidative deamination, followed by oxidation of the DIHPPA to a hydroperoxide. This conversion is thought to be the slow step of the reaction and that once the oxidised form of DIHPPA (**40**) is in the presence of 3,5-diiodotyrosine (**41**) rapid coupling occurs. Since the synthesis of *N*-acetylthyroxine

ethyl ester (**46**) under investigation only yields 30-40 % product then by understanding the mechanism of the conversion and its requirements it may be possible to modify conditions to favour the conversion and so increase the yield of *N*-acetylthyroxine ethyl ester (**46**). From the research described earlier it can be postulated that the presence of the manganese(II) sulfate is for catalysis of the conversion and that boric acid may help in stabilising the DIHPPA as the enol once formed.

1.14.4 Coupling to form a quinol ether and the subsequent loss of the side chain to yield thyroxine



Scheme 33

It is possible that the synthesis of *N*-acetylthyroxine ethyl ester (**46**) under investigation may go through an initial oxidation of the side chain. As this is believed to be rate limiting step in the reaction, by studying the reaction of *N*-acetyl-3,5-diiodotyrosine ethyl ester (**47**) with DIHPPA (**40**) it is possible to establish whether DIHPPA (**40**) is an intermediate in this synthesis, and if so, whether an improved synthesis may be possible by synthesising the DIHPPA (**40**) then reacting it with the *N*-acetyl-3,5-diiodotyrosine ethyl ester (**47**).

By studying the reaction of *N*-acetyl-3,5-diiodotyrosine ethyl ester (**47**) with other side chain oxidised derivatives, which may be intermediates in the synthesis, information concerning the side chain properties necessary for the oxidation, and the extent of oxidation of the side chain prior to coupling, may be obtained. Isolation of the "lost side chain" has, so far, only been successfully carried out following the thyroglobulin bound synthesis of thyroxine. The isolation or detection using HPLC

of the side chain from this synthesis may provide further evidence of the form of the side chain prior to coupling and its mechanism of elimination. Once information is gained concerning the possible form of the side chain prior to coupling it may be possible to synthesise and couple a tyrosine derivative with a non-scissionable side chain with the aim of trapping the quinol ether intermediate.

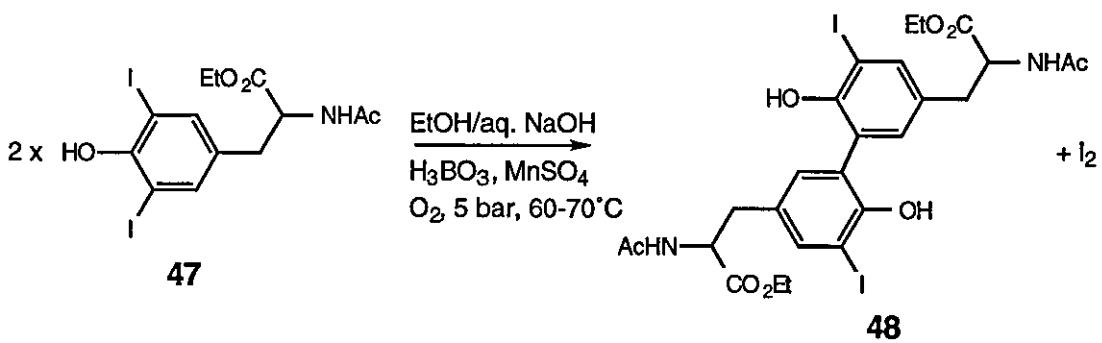
The reaction of other *ortho-ortho* diiodinated, *para* substituted phenols to form their corresponding thyroxine analogues or react otherwise may give further information of the necessary properties of the *para* substituent, and may also provide evidence for the possible mechanisms in the elimination of the side chain.

Chapter Two

Ortho-Ortho coupling of 2,6-diiodinated phenols

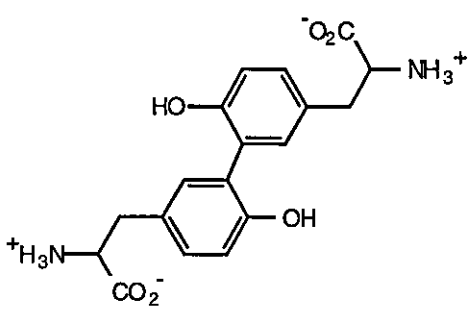
2.1 Introduction

This chapter reports the investigation into the coupling of two *N*-acetyl-3,5-diiodotyrosine ethyl ester molecules (ethyl *N*-acetylDIT, **47**) to form 2,2'-dihydroxy-3,3'-diiodo-5,5'-di-[(2-acetamido-2-ethyloxycarbonyl)ethyl]-1,1'-biphenyl (**48**). The reaction was discovered during studies of the synthesis of *N*-acetylthyroxine ethyl ester (**46**) from ethyl *N*-acetylDIT (**47**) in the Knoll Pharmaceuticals research laboratories. The conditions used were a solution of ethyl *N*-acetylDIT in ethanol/sodium hydroxide solution with catalytic quantities of boric acid and manganese(II) sulfate at 60-70 °C, and under 5 bar pressure of oxygen. The formation of **48** was found to occur simultaneously with a drop in pH of the solution, and without knowledge of the mechanism it was unclear whether the formation of **48** was the cause of the drop in pH, or whether the drop in pH caused the formation of **48**.

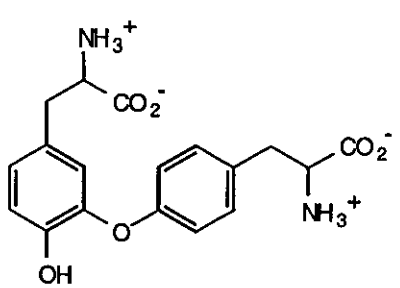


Scheme 34

The essential structure of this compound is commonly referred to as dityrosine, **49**. The formation of this compound (**49**) has been reported to occur from the potassium ferricyanide oxidation of tyrosine derivatives also producing the isodityrosine derivative (the essential isodityrosine structure **50** is shown),[57] and from peroxidase and hydrogen peroxide oxidation of tyrosine to form exclusively dityrosine (**49**).[58]



Dityrosine
49



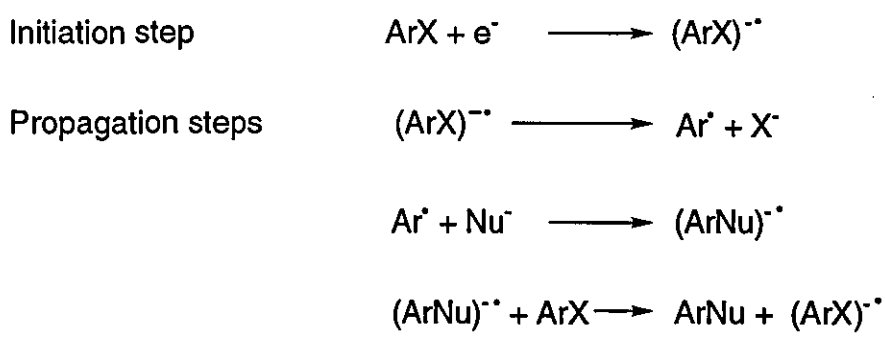
Isodityrosine
50

Isodityrosine derivatives have also been synthesised utilising the Ullmann condensation for the formation of the diaryl ether unit in the total syntheses of vancomycins and piperazinomycins.[59] Isodityrosine and dityrosine derivatives have been reported to result from the electrolysis of the dibromotyrosine derivative and diiodotyrosine derivative in methanol and in the presence of lithium perchlorate. The first of these yields the isodityrosine derivative exclusively, whereas the latter yields the dityrosine derivative exclusively.[60] These reported syntheses of dityrosine derivatives indicate that the formation of the product goes through a phenoxyl radical mechanism. It is worth noting that no thyronine derivatives are detected, which may be expected if phenoxyl radicals of tyrosine derivatives are being formed.

As literature reports indicate that the coupling of halobenzenes with aryloxides can proceed through the $S_{RN}1$ mechanism, and that the carbanion coupling of aryloxides give *ortho* or *para* coupled products, our investigations of the *ortho-ortho* coupling started with the investigation of the $S_{RN}1$ mechanism theory.

2.1.1 The aromatic $S_{RN}1$ mechanism

The $S_{RN}1$ mechanism[61] involves a chain reaction and therefore requires initiation, propagation and termination steps. The schematic reaction is shown in **scheme 35**.



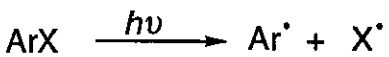
Scheme 35

Any reaction which produces one of the reactive intermediates will initiate the propagation cycle. Conversely, any reaction which interrupts the sequence by destroying one of the reactive intermediates will terminate the cycle. The termination step has been omitted from **scheme 35** since this step can be dependent on the method of initiation or specific reaction environment, e.g. termination can involve the abstraction of hydrogen from the solvent by the intermediate aryl radical.[62] Termination steps which are independent of the reaction environment or

method of initiation can involve the coupling of two radicals or disproportionation of a radical anion and a radical to form an anion and a neutral species.

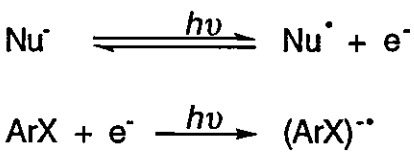
2.1.2 Methods of initiation

Some $S_{RN}1$ reactions can occur spontaneously without stimulation other than temperature. However, most require the reaction to be promoted by other means and there are several possible initiation methods. The easiest method to use is the photostimulation by near UV light. This initiation can occur through various mechanisms such as homolytic bond dissociation of an aryl derivative where the substrate absorbs a photon then dissociates to produce an aryl radical. **Scheme 36** shows the reaction thought to occur in the case of aryl iodides.



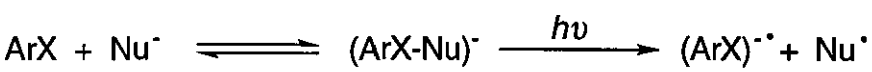
Scheme 36

Photoejection of electrons from an anion which are then captured by the substrate forming a radical anion reactive intermediate and so initiating the chain propagation is shown in **scheme 37**.



Scheme 37

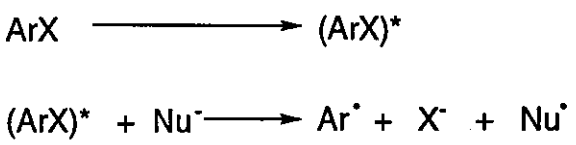
Photoassisted electron transfer occurs by the formation of a charge-transfer complex between the nucleophile and substrate followed by an electron transfer as shown in **scheme 38**.



Scheme 38

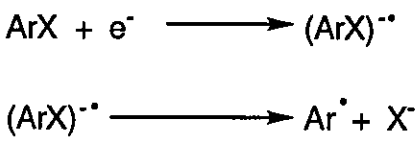
The transfer of electrons to excited states is another possible mechanism for photostimulation. Either the substrate can absorb a photon to become excited then react with the nucleophile to afford aryl radicals, as shown in **scheme 39**, or

alternatively, the nucleophile can react with the substrate's excited state to give a radical anion that breaks down to give an aryl radical and halide anion.



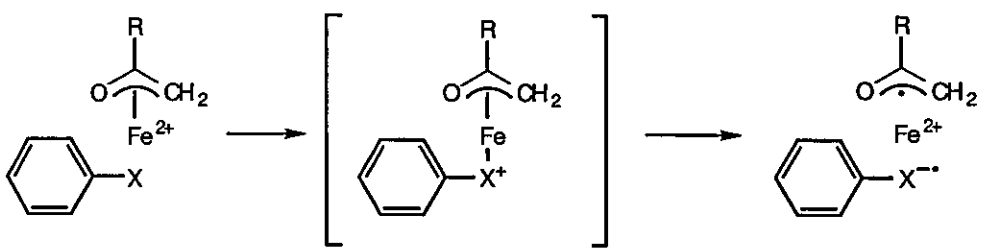
Scheme 39

Solvated electrons can also be used to initiate the reaction. In this case, the substrate reacts with an electron to form a radical anion and then the radical anion dissociates to form a halide anion and aryl radical (**scheme 40**). These reactions are usually performed in liquid ammonia with either sodium amalgam or potassium.



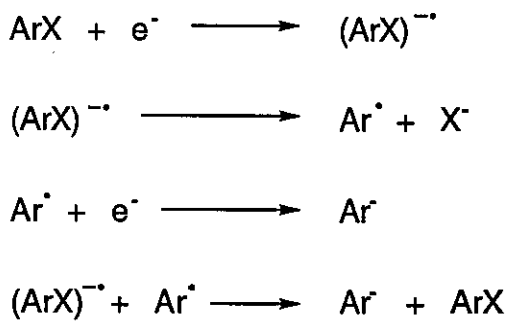
Scheme 40

The same initiation process, but using iron(II) sulfate, was reported by Galli and Bunnett[63] who found that they could initiate a reaction between bromobenzene and the enolate anion of pinacolone in the dark. In the absence of the iron(II) salt no reaction occurred. However, on further investigation of this initiation process[64] they proposed that the iron(II) associates with the enolate and acts as an electron relay between the enolate and aryl halide facilitating the electron transfer (**scheme 41**). There is a possibility that in the process conditions which we are investigating manganese(II) may facilitate the same reaction.



Scheme 41

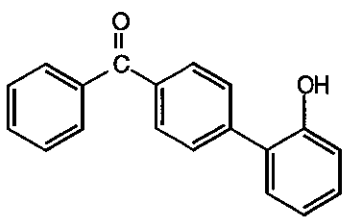
Initiation can also be carried out electrochemically at the cathode surface to form the radical anion of the aryl halide which dissociates and can then either react with a nucleophile or radical in solution, or undergo further reduction at the cathode surface (**scheme 42**). If an aryl anion is formed it will quickly protonate due to its basicity.



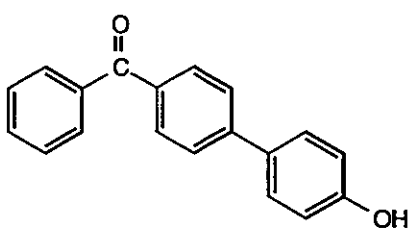
Scheme 42

2.1.3 The reaction of aryloxides under $S_{RN}1$ conditions

The $S_{RN}1$ mechanism was used to explain the coupling between halobenzenes and a variety of keto enolates and carbanions, although early attempts of the reaction using aryloxide nucleophiles were unsuccessful.[65] However, it was later found that phenoxide ions can react under the $S_{RN}1$ conditions, acting exclusively as a carbon rather than an oxygen nucleophile. Pinson *et al* studied the electrochemical initiation of the reaction between 4-bromobenzophenone and phenoxide ions. They found that the phenoxide coupled through the *ortho* and *para* carbon-centre in a ratio of 2:3 to produce the compounds **51** and **52** respectively.[66]



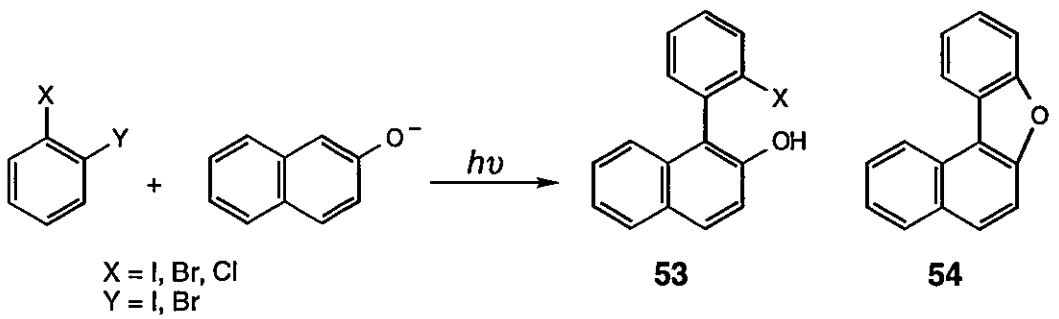
51



52

Having established that it was possible to couple phenoxides with haloaryls under $S_{RN}1$ conditions, the reaction between iodonaphthalenes and phenoxides or naphthoxides was tested and found to be a useful method for the synthesis of unsymmetrically substituted 1,1', 1,2' and 2,2' binaphthyl or phenylnaphthyl derivatives.[67] Rossi *et al* used dihaloarenes to achieve the coupled halosubstituted

product **53** and the cyclized product **54**, which in the case of *o*-diiodobenzene, were produced in a ratio of approximately 2:1 respectively.[68]

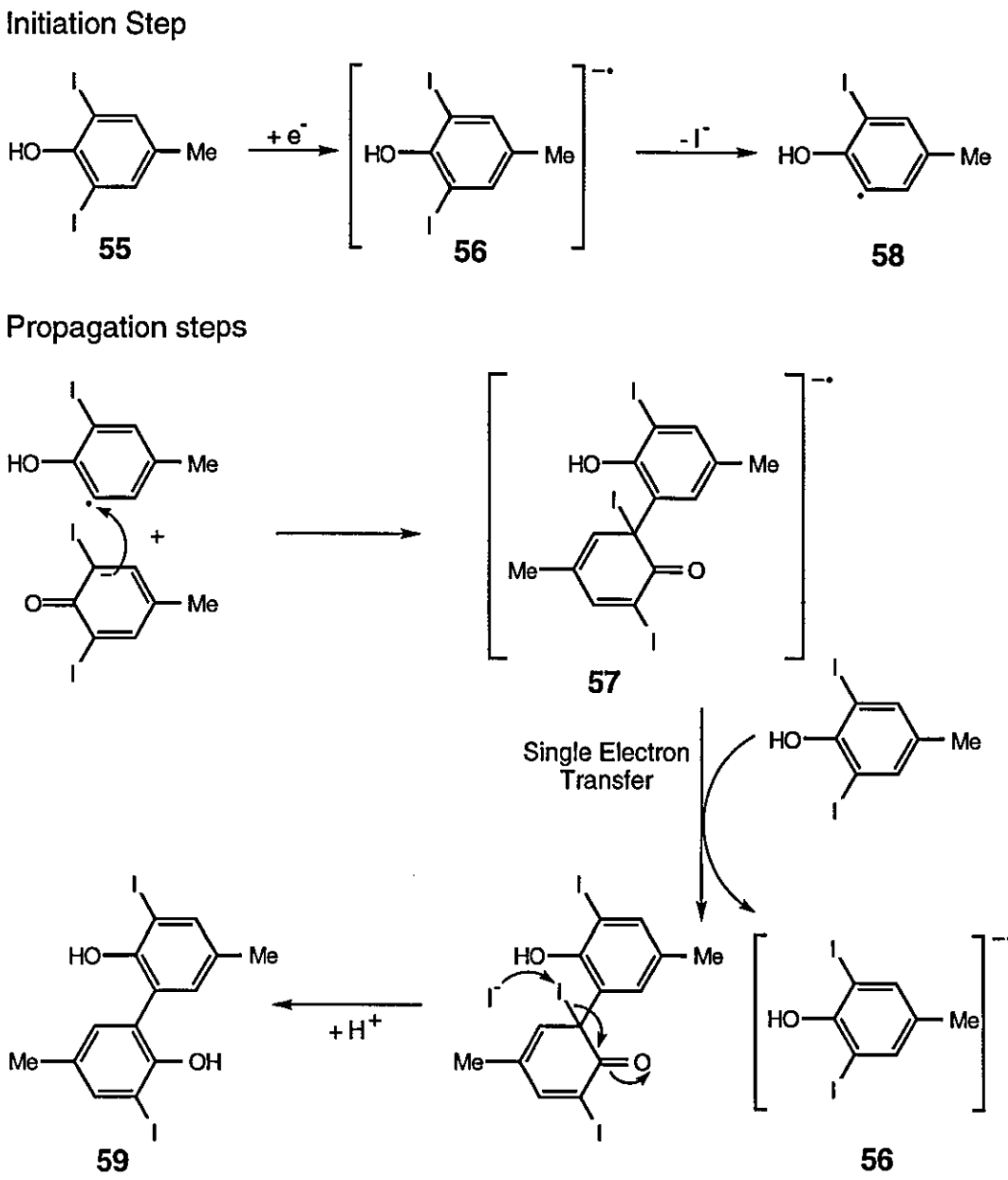


Scheme 43

2.2 Attempted coupling of diiodophenol derivatives under $S_{RN}1$ conditions

Our reasons for initially postulating that the dityrosine derivative formation occurred through an $S_{RN}1$ mechanism were due to the reaction conditions under which it was afforded. Since some $S_{RN}1$ reactions occur spontaneously it was possible that this could be the case with the dityrosine derivative formation, the reaction conditions being fairly vigorous (60-70 °C and 5 bar pressure) and the carbon-iodine being relatively weak. Alternatively, light initiation could have been the cause of the reaction. The presence of manganese(II) sulfate in the reaction also gave the possibility of radical initiation similar to that known to be caused by iron(II) sulfate.[64]

The studies were carried out mainly using the simpler analogue 2,6-diiodo-4-methylphenol (**55**) since it appeared unlikely that changing the *para* substituent from alanine to methyl would affect the reaction. The specific reaction mechanism under investigation is shown in **scheme 44**. Initiation occurs to form a radical anion which dissociates to give a radical and iodide, the radical being an sp^2 hybridised σ radical. The radical is subsequently attacked by the phenoxide ion through its *ortho* carbanion form, yielding a second radical anion which on single electron transfer with starting material and loss of an iodonium ion, gives rise to the product and the radical anion **56** which continues the propagation cycle.

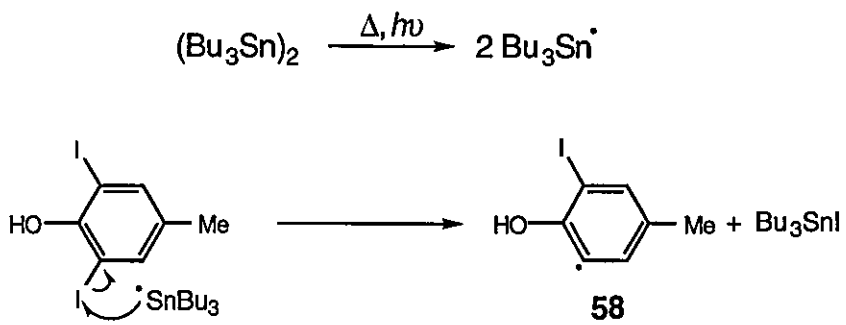


Scheme 44

The reaction requires that half of the phenol is deprotonated, therefore a half equivalent of organic base was used (sodium methoxide or potassium *tert*-butoxide) in the reactions and all of the reactions were carried out under an inert atmosphere. The first attempts to achieve the *ortho-ortho* coupling were carried out by simply heating the phenol and base in DMF at 60-70 °C. This attempt was followed by irradiating the reaction with fluorescent lamps. In both cases the starting material was recovered quantitatively. Reports by Petrillo *et al*[69] indicated the apparent ability of DMSO to trigger electron transfer processes and so the reaction was carried out by refluxing in this solvent. This reaction failed and so it was repeated and irradiated using a photolysis apparatus with light of wavelength 350 nm. This reaction also returned the starting material quantitatively.

We therefore turned to the possible donation of an electron from the manganese(II) sulfate in the reaction and carried out reactions in refluxing methanol with varying quantities of manganese(II) acetate dihydrate or manganese(II) sulfate (from 0.1 equivalents to 2 equivalents). These reactions also failed to produce any detectable amount of coupled compound. Since the conditions for the synthesis of thyroxine include oxygen, it is possible that the manganese(II) is oxidised to manganese(III) by the oxygen with the concomitant production of superoxide. The reaction of manganese(III) triacetylacetonate with 2,6-diiodo-4-methylphenol (**55**) in methanol was therefore attempted and reactions using varying quantities of manganese(II) and manganese(III) were tried in an attempt to initiate an oxidation-reduction cycle. However, all of the reactions were unsuccessful.

The $S_{RN}1$ reaction is a chain reaction, and it was clear that the problem with our reactions was in their initiation, since even if termination steps were occurring other compounds should have been recovered along with the starting material, e.g. recovery of the 2-iodo-4-methylphenol from the termination by hydrogen abstraction from the solvent. We therefore employed hexabutylditin as a chemical initiator in the reaction and DMSO as the solvent and heated the reaction to 150 °C for 5 hours. Again though only starting material was recovered. The reaction was then repeated in refluxing toluene and was irradiated with tungsten lamps. The hexabutylditin was added in small portions over the first hour of the reaction. The initiation was successful and 2-iodo-4-methylphenol in 28 % yield along with recovered starting material was produced. The reaction of hexabutylditin is shown in **scheme 45**.



Scheme 45

The reaction was repeated several times and a similar yield of the mono-iodo compound was obtained each time. When the reaction was repeated using *N*-acetyl-3,5-diiodotyrosine ethyl ester (**47**) as the phenol derivative, the only reaction detected was the hydrolysis of the ethyl ester. Formation of 2-iodo-4-methylphenol in the reaction through reaction with hexabutylditin indicates that the aryl radical was formed and that hydrogen abstraction could occur, possibly from the solvent.

This also indicates that although the radical may have been formed, it did not react through an $S_{RN}1$ mechanism with the carbanion of the aryloxide to form the desired *ortho-ortho* coupled product. This evidence suggests that the $S_{RN}1$ mechanism is not the mechanism by which the dityrosine derivative is formed and that an alternative mechanism needed to be investigated. Had the attempts to synthesise the dityrosine derivative under $S_{RN}1$ mechanism conditions been successful, it would have been the first $S_{RN}1$ reaction reported where the halobenzene had been a phenol and the phenoxide iodinated. However, it is still difficult to understand the reason for the failure of this reaction since the aryl radical was successfully formed, and the iodine substituent on the *ortho* carbon through which the coupling is desired should not affect the ability of the phenoxide to react through its carbanion. In fact, the presence of the two *ortho* iodines reduces the pK_a of the phenol to approximately 6.5,[45] and being electron withdrawing should stabilise the desired resonance form. The steric hindrance due to the iodine substituent should be taken into consideration. Models indicate that the 100 ° approach of the aryl σ radicals to the planar anion is sterically feasible even with the large *ortho*-iodine in the anion. The reason for the lack of dityrosine derivative formation (**59**) in these reactions is unclear, however, it was necessary to explore other mechanisms and our attention was turned to the possibility that coupling proceeds through a polar mechanism.

Had time allowed, further investigations of this $S_{RN}1$ mechanism may have been carried out to discover the requirements of the reaction which our system did not fit, i.e. could a non-iodinated aryloxide react with the σ radical formed from an iodophenol, and conversely could an *ortho* substituted iodoaryloxide react with an iodobenzene derived aryl radical?

2.3 Investigatigation of the formation of dityrosine derivatives through a polar mechanism

During the study of the reaction conditions required for the formation of *N*-acetyl thyroxine ethyl ester (**46**) from *N*-acetyl-3,5-diiodotyrosine ethyl ester (ethyl *N*-acetylDIT, **47**), a pink colour appeared during the course of the reaction. Ethyl *N*-acetylDIT (**47**) was stirred in dichloromethane and phosphate buffer (0.003 M, pH 7.1), at room temperature and under an atmosphere of nitrogen. The pink colour was attributed to the production of iodine, and a small amount of the dityrosine derivative **48** was detected in the ^1H NMR spectrum of the reaction mixture.

On repetition of this reaction it became quite apparent that the pH of the reaction was crucial for the formation of dityrosine derivative, **48**, with the reaction failing to occur if the pH was above 7.5. On the other hand, the actual ions in the buffer did not affect the reaction. An alternative aqueous phase using boric acid solution (0.2 M) and sodium hydroxide (0.05M), to adjust the pH of the reaction to pH 6.5-7.0, was found to be successful in producing the dityrosine derivative **48** in similar quantities to the reaction using a phosphate buffer. Over a one day period, at pH 6.4 and under an inert atmosphere, the reactions using either a phosphate buffer or the boric acid/sodium hydroxide solution system both produced 50 % of the dityrosine derivative **48**. The boric acid/sodium hydroxide solution system was therefore used to study the effect of pH on the quantity of dityrosine derivative production since pH adjustment is easier in this system. Due to the concomitant production of iodine, resulting in the pink/purple colouration of the solution, with the formation of the dityrosine derivative it was always clear whether the reaction was producing any dityrosine derivative **48**. The results are shown in **table 1**. The yield of product can vary for reactions carried out over the same number of days since the pH drops and requires addition of sodium hydroxide solution. Thus the yield is dependent upon the frequency of pH checks. However, the reactions shown in the table for each time period are comparable since they were run simultaneously and therefore were checked at the same frequency. The reactions are clean, with the product mixture containing only starting material (**47**) and product (**48**) with quantitative recovery. For this reason the compounds were not routinely separated by chromatography and instead the yields were calculated from the integration of the signals arising from the ^{1}H NMR spectroscopy of the product mixture, and are therefore relative.

Reaction No.	pH range	Time	% yield **48**	% **47** recovered
1	6.1 - 4.8	1 day	50	50
2	5.2 - 4.1	1 day	50	50
3	5.7 - 4.4	1 day	50	50
4	7.9 - 7.4	1 day	0	100
5	6.4 - 5.9	2 days	72	28
6	4.4 - 4.3	2 days	60	40
7	4.7 - 4.6	2 days	60	40
8	6.4 - 5.9	4 days	66	34
9	7.2 - 7.1	4 days	66	34

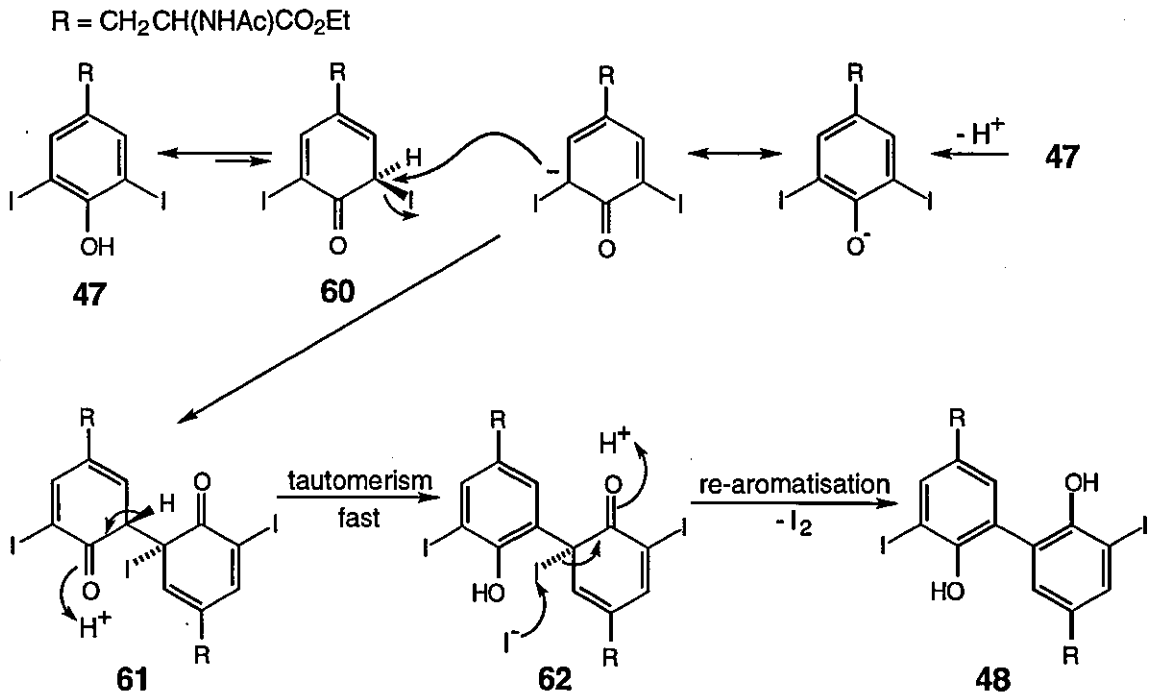
All reactions were carried out using *N*-acetyl-3,5-diiodotyrosine ethyl ester

Table 1

All the reactions reported in **table 1** were carried out under an inert atmosphere. From **table 1** it can be concluded that the production of **48** is greatest below pH 7.2 and that above pH 7.4 it is unlikely that any reaction will occur. It also appears that below pH 5, although product formation does occur, the yield starts to drop. The optimum pH for the reaction is therefore between pH 7.2 and 5, and it clear that raising the pH above 7.2 has a much more drastic affect on the reaction than lowering the pH below 5.

The results reported in **table 1** are for reactions in which the solutions had been degassed and kept under a nitrogen atmosphere throughout. To investigate the effect of an oxygen atmosphere, the reaction was conducted both in air, and with oxygen bubbling through the reaction for the first seven hours. A three day reaction with the pH maintained between 6.5 and 2.4 produced the desired product (**48**) in 75 % yield. Although this was not carried out alongside a control reaction (i.e. under a nitrogen atmosphere) the result shows that the reaction is not blocked by the presence of oxygen. The fact that the reaction works under an atmosphere of nitrogen indicates that it is not aerobic as is thought to be the case with the reaction to form *N*-acetylthyroxine ethyl ester (**46**).

These results led us to propose a polar mechanism for the formation of the dityrosine derivative **48** as shown in **scheme 46**. The mechanism shows that the formation of a small amount of the α-iodoketo tautomer **60** can then undergo S_N2 substitution with the ambident phenoxide ion of **47** through its *ortho* carbanion affording the intermediate **61**. It is worth noting that the α-iodoketo tautomer **60** is an intermediate in the diiodination of the ethyl ester of *N*-acetyltyrosine. The diketo intermediate **61** will undergo rapid tautomerism to the more stable phenol form **62**, which in turn will lose iodide with re-aromatisation as the driving force. This mechanism accounts for the iodine which is clearly seen by the colour change occurring during the course of the reaction, and also provides an explanation for the lack of reaction when the reaction pH is raised by approximately more than one pH unit above the pK_a of the phenol. If the phenol is totally ionised to the phenoxide ion then the tautomeric form **60** cannot be formed and the reaction is therefore blocked. This observation is consistent with the lack of formation of the dityrosine derivative **48** when the synthesis of *N*-acetylthyroxine ethyl ester (**46**) is carried out at high pH (ca. 9.5).



Scheme 46

In order to establish whether our proposal of a polar mechanism was viable, reactions were carried out in the presence of TEMPO, a radical inhibitor. In all cases a full equivalent of TEMPO was used with the ethyl *N*-acetylDIT (**47**), and the reactions were carried out wrapped in foil to exclude light. The affect of the phase transfer catalyst (PTC), benzyltriethylammonium chloride was also of interest, since the reaction is carried out in a two-phase system. To investigate this a set of reactions were run simultaneously in order to compare the yields, the results of which are shown in **table 2**. Standard conditions refers to the reaction of ethyl *N*-acetylDIT (**47**) in dichloromethane and boric acid/sodium hydroxide solution kept at pH 6-6.5. All of the reactions were carried out under a nitrogen atmosphere and at room temperature.

Reaction No.	Addition to standard conditions	% yield **48**	% recovered **47**
10	standard reaction	66	34
11	TEMPO	62	38
12	PTC	66	34
13	Light excluded	50	50

All reactions were carried out using *N*-acetyl-3,5-diiodotyrosine ethyl ester

Table 2

Reaction 11 shows only a slight decrease in yield of the reaction in the presence of the radical inhibitor and is within the variation of the yield of the standard reaction, i.e. within experimental error. However, reaction 13 shows a 16 % drop in yield between the reactions with and without light. Repetitions of this reaction alongside a standard reaction gave similar results, although all of the light excluded reactions did produce the dityrosine derivative **48** which suggests that light is not initiating the reaction. The drop in yield caused by excluding light from the reactions appears to be inexplicable. We considered that the difference in yield caused by excluding light from the reaction was not significant enough to warrant detailed repetition in an attempt to understand this effect.

The fact that the reaction occurs in the presence of a radical inhibitor, in the exclusion of light, in the presence and absence of oxygen and only below a certain pH suggests a polar rather than radical mechanism.

In a standard reaction the amount of iodine present was determined by titration with sodium thiosulfate solution. The titration was not very accurate since it was carried out in the two-phase system and the end point was determined literally by the disappearance of the pink iodine colour rather than using starch indicator as is the standard practice in the analytical procedure. The reason the titration was not carried out more accurately was that in attempting to remove the dichloromethane layer of the reaction some iodine would be lost and therefore by attempting to make the titration more accurate we would actually lose some of the accuracy. The reason for not adding the starch indicator was that one of the objects of the exercise was to ascertain whether the removal of iodine from the reaction mixture would help to push the reaction to completion. The reaction was to be continued after the initial titration, although it was unlikely that the product could re-iodinate and the reaction be reversed. It was found that once the sodium thiosulfate solution had been added no further iodine production was observed over a further two days. The calculated quantity of iodine produced by the reaction corresponded to a 12 % yield, and the relative yield calculated from the ^{1}H NMR spectrum was 16 %. The result indicates that the stoichiometry of the reaction is one molecule of iodine produced to each molecule of coupled compound produced and is consistent with the ratio required by the S_N2 mechanism.

In order to test the tolerance of the reaction, it was attempted using solvents other than dichloromethane. The reactions were all carried out at room temperature and with boric acid/sodium hydroxide as the aqueous system at pH 5.8-6.5. The solvents tested were, THF, ethyl acetate, ethanol, DMSO and diethyl ether, none of which

gave rise to the production of the dityrosine derivative either in the presence or absence of oxygen. However, upon leaving a flask of ethyl *N*-acetylDIT (**47**) in ethyl acetate overnight and open to the atmosphere, coupling to form the dityrosine derivative **48** was found to have occurred. In an attempt to understand the specific requirements of the reaction conditions, e.g. the necessity of the aqueous system, some reactions were carried out in dichloromethane alone and ethyl acetate alone. The results are shown in **table 3**.

Reaction No.	Solvent	Air/N_2	Time	Other conditions	% yield **48**	% **47**
14	EtOAc	air	4 days		0	100
15	EtOAc	air	10 days		33	67
16	EtOAc	air	10 days	Et_3N (1 equivalent)	0	100
17	EtOAc	air	10 days	Et_3N (0.5 equivalent)	0	100
18	EtOAc	air	10 days	light excluded	22	78
19	EtOAc	N_2	5 days	10 % H_2O	20	80
20	EtOAc	N_2	5 days	1 % AcOH	0	100
21	EtOAc	N_2	5 days	10 % H_2O, 1 % AcOH	0	100
22	EtOAc	N_2	5 days	TEMPO (1 equivalent)	0	100
23	EtOAc	N_2	5 days	10 % TEMPO	4	96
24	DCM	N_2	4 days		16	84
25	DCM	air	4 days		16	84
26	DCM	N_2	4 days	TEMPO (1 equivalent)	28	72
27	DCM	N_2	4 days	10 % TEMPO	< 1	99

All reactions were carried using *N*-acetyl-3,5-diiodotyrosine ethyl ester

Table 3

From the comparison of the results of reactions 14 and 15 and the fact that the reactions were first seen to occur overnight (i.e. over approximately 16 hours) it is clear that the reaction is inconsistent in the yield of dityrosine derivative **48** produced. For this reason reactions 19, 20 and 21 with additions of water and acetic acid were conducted to elucidate whether the grade, or dryness, of the ethyl acetate had an affect on the reaction. Increasing the acidity of the reaction proved to be of no benefit, whereas the addition of a little water was found to increase the yield slightly in comparison with using dry ethyl acetate. No reaction occurred in the presence of triethylamine (reactions 16 and 17). However, this could not be used as a conclusive result due the apparent inconsistency of the result of reaction 14. It is

consistent again with the lack of formation of the dityrosine derivative **48** when the synthesis of *N*-acetylthyroxine ethyl ester (**46**) is carried out at high pH (ca. 9.5).

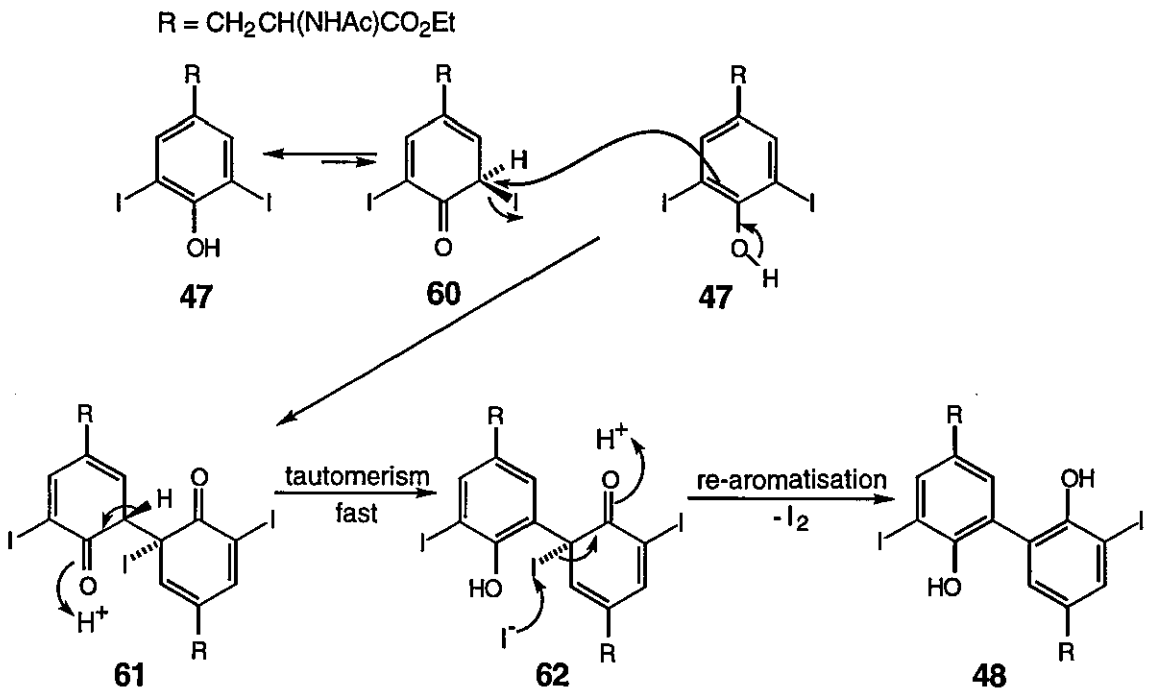
Although reaction 18 indicates that light is not required in order for the reaction to occur, reactions 22 and 23 are inconsistent with each other in providing evidence for or against a radical mechanism. The inconsistency of these results is also seen from reactions 26 and 27 carried out in dichloromethane, although the affect of the higher concentration is reverse to that obtained in reactions 22 and 23. These yields could be due to the inconsistency of the reaction rather than the affect of the TEMPO, although repeat reactions did produce similar results and in all cases a small amount of the dityrosine derivative was formed. There is a possibility that the reaction in ethyl acetate is occurring through a different mechanism than the reaction in the dichloromethane two-phase system. The fact that the coupling occurred at all in the presence of the radical inhibitor could be interpreted as evidence towards a polar mechanism.

The reactions 24 and 25 indicate that the reaction in dichloromethane is independent of the atmosphere.

With the exception of the erratic TEMPO results, it appears that the reactions reported in **table 3** may occur through the same mechanism as the reactions occurring in the dichloromethane/aqueous system. The fact that the reaction is unaffected by the presence or absence of oxygen, and that the reaction does not occur in the presence of base, strongly indicates a polar mechanism. However, further studies of the reactions in dichloromethane or ethyl acetate alone would be required before any conclusions can be made concerning the mechanism occurring in these solvent systems.

The S_N2 mechanism shown in **scheme 46** indicates that half of the phenol derivative should be in the form of the phenoxide ion in order for the reaction to work and the presence of the phenoxide ion is unlikely to occur in the ethyl acetate and dichloromethane reactions in the absence of a small amount of base or an aqueous phase in which the phenol can be deprotonated, however this should not block the reaction. **Scheme 47** shows that the reaction could still occur when the compound is totally in the phenol form. However, this reaction may be expected to be slower than if the phenoxide ion is present for immediate reaction and could provide an explanation for the affect of a little water in reaction 19. The inconsistency of reactions carried out over different time spans could be due to the variation of room

temperature which may have a marked affect on the reaction rate if the initial steps of the mechanism are as shown in **scheme 47**.



Scheme 47

These results prompted us to test for the formation of the dityrosine derivative **48** under the conditions in which it had first been seen to be synthesised, and to obtain evidence as to whether the reaction in the dichloromethane/aqueous system was going through the same mechanism as the ethanol/aqueous system of the thyroxine derivative **46** synthesis. The results of these reactions are shown in **table 4**.

Reaction No.	Organic solvent	pH Range	Time	N_2/ O_2	Temp °C/ P bar	% yield **48**	% **47**	% yield **46**
28	EtOH	6.5-6.1	4 days	air	60 °C/atm	12	84	4
29	EtOH	6.3-6.1	1 day	N_2	60 °C/5bar	5	93	2
30	EtOH	9.3-9.1	3 days	N2	60 °C/5bar	3	97	0
31	EtOH	6.1-5.9	3 days	O_2	60 °C/5bar	11	78	11
32	DCM	6.5-3.4	6.5 h	N_2	60 °C/5bar	40	60	0
33	DCM	8.8-7.6	6.5 h	N_2	60 °C/5bar	0	100	0

All reactions were carried out using *N*-acetyl-3,5-diiodotyrosine ethyl ester

Table 4

Reactions 28, 29 and 31 show that at a pH < 7 the reactions forming *N*-acetylthyroxine ethyl ester (**46**) and the dityrosine derivative **48** are competing. However the pH effect is exemplified by the comparison of the yield of **48** produced from conditions as shown in the reaction 30 over three days in comparison with the yield of **48** produced over three days as is shown in reaction 31, and over one day in reaction 29. The higher pH suppresses the extent of the *ortho-ortho* coupling reaction. The production of *N*-acetylthyroxine ethyl ester (**46**) in reaction 29 is a little unusual since the reaction to form **46** is thought to be aerobic. It is possible though that the reaction vessel was not completely flushed with nitrogen prior to pressurisation, or that although the solution was degassed prior to being charged to the pressure vessel, it was impossible to avoid contact with air during the transfer of the solution to the pressure vessel. Hence oxygen could have been present in the solution during the reaction. The fact that reaction 30 produced no *N*-acetylthyroxine ethyl ester (**46**) indicates that oxygen was not present during this reaction. Since no other oxidant was present it appears that in the ethanol/aqueous system the formation of the dityrosine derivative occurs without oxidation and that the reduced yield at high pH indicates that the reaction mechanism occurring in this solvent system is the same as that which occurs in the dichloromethane/aqueous system at room temperature.

Reaction 32 produced a reasonable yield (40 %) of the dityrosine derivative **48**, and the affect of pH on this coupling reaction is clearly demonstrated by the total lack of dityrosine derivative **48** production in reaction 33 when the pH was raised to 8.8.

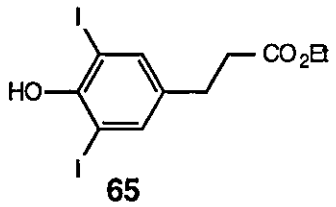
Comparing the results from the coupling reactions in the ethanol/aqueous solvent system with those in dichloromethane/aqueous solvent system it is clearly seen that the ethanol/aqueous system does not favour the reaction. This provides further evidence in the favour of a S_N2 mechanism since reactions which are believed to occur through the S_N2 mechanism have been found to favour non-hydroxylic solvents due to the hydrogen bonding affects in protic solvents which stabilise the nucleophile, rendering it less reactive. In our reaction it is possible that the aqueous phase enables the deprotonation of the phenol to occur, the resulting phenoxide ion will predominate in the aqueous layer although during the stirring of the reaction some of the phenoxide ion will pass into the dichloromethane solution where it will be less stabilised and will react with the tautomerised phenol fairly rapidly. In the ethanol/aqueous solvent system the phenoxide ion is highly solvated and therefore more stable. This reduces the nucleophilic ability of the phenoxide ion thereby slowing the reaction rate, and as we have observed the reaction will only produce the dityrosine derivative **48** under higher pressure and high temperature conditions.

It is possible that some of the other solvents tested at room temperature might produce the dityrosine derivative **48** at higher temperature and pressure. Since it appeared that we could reproduce the coupling in the conditions from which the dityrosine derivative was initially isolated [the reaction occurring independently of oxygen or manganese(II)], and that the reaction appeared to follow the same mechanistic pathway as for the coupling in the dichloromethane/aqueous solvent system which could be studied at room temperature, time was not given to the further study of the reaction in these other solvents.

2.3.1 Coupling of other 2,6-diiodophenols in the two-phase system

The S_N2 mechanism proposed for the coupling of *N*-acetyl-3,5-diiodotyrosine ethyl ester (**47**) to form the dityrosine derivative **48** shown in **scheme 46** does not involve any participation of the side chain, the coupling should therefore be general to all 2,6-diiodophenol derivatives. For this reason several *para*-substituted phenols were iodinated and where necessary protected and then subjected to the coupling conditions (shown below). Of interest in this study was the reaction of *N*-acetyl-3,5-dibromotyrosine ethyl ester (**63**) and *N*-acetyl-3-iodotyrosine ethyl ester (**64**) since the brominated compound may undergo oxygen-*ortho* coupling to give the isodityrosine derivative as was reported by Nishiyama *et al.*[60] The bromines cause less steric hindrance to the approach of the oxygen-centred anion which allows carbon-oxygen coupling. In addition to this, the mono-iodo compound could possibly lead to a monoiodo, diiodo, or noniodinated dityrosine derivative.

The diiodinated phenols were reacted at pHs between 5 and 6.8, with the esters **65** and **66** appearing to favour a slightly lower pH than the pH at which *N*-acetyl-3,5-diiodotyrosine ethyl ester (**47**) reacts best. The tyramine derivative **67** reacted at around the same pH as **47**, the slight variation in optimum pH possibly being due to the slight difference in pK_a of the phenol owing to their differing *para* substituents. The products of the two-phase coupling system are shown below and the results were as recorded in **table 5**. The products were purified by flash column chromatography on silica gel and were fully characterised. Most of the reactions were not optimised, but in the case of the esters **65** and **66**, the reaction pH was varied between pH 4 and 7 until the reaction started to produce iodine in the pH range as shown in the table. This is another example of the pH dependence of the reaction.



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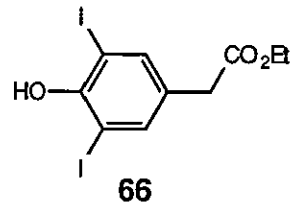

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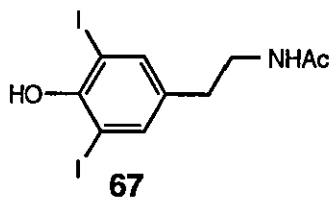

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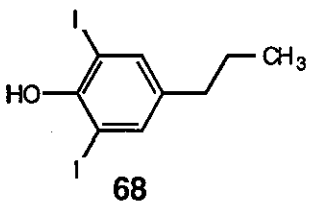

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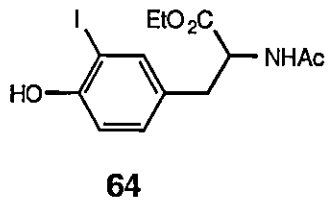

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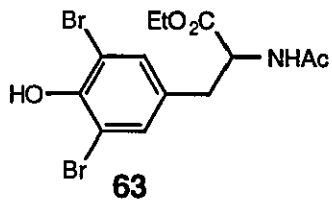

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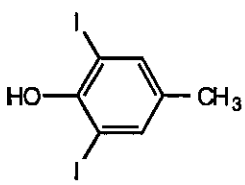

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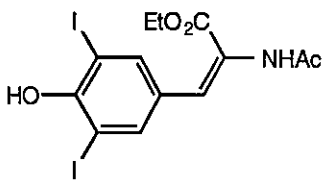

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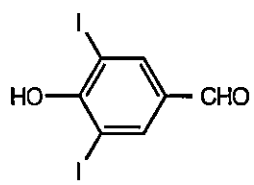

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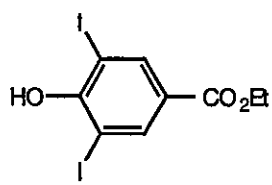

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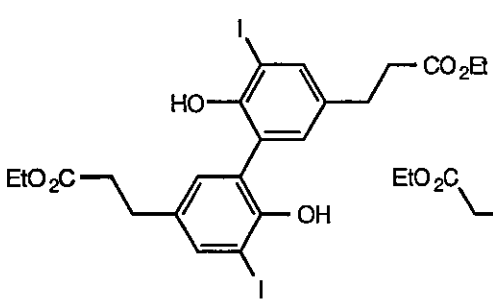

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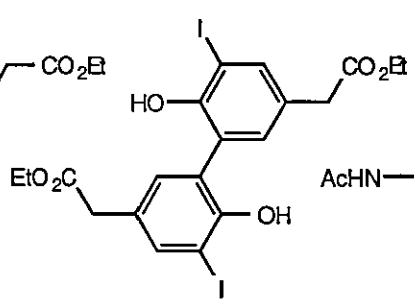

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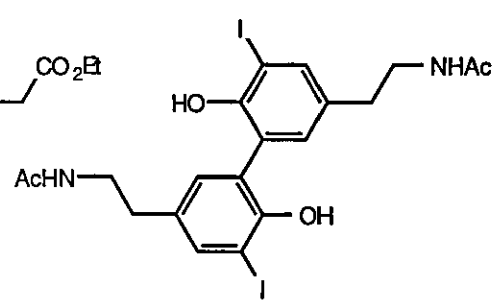


74

Reaction No.	4-substituent (reactant no.)	pH range	Time	% product (product no.)	% recovered s.m.†
34	$(CH_2)_2CO_2Et$ (**65**)	5.5-6.0	3 days	33 (**72**)	67
35	CH_2CO_2Et (**66**)	5.2-5.8	4 days	22 (**73**)	78
36*	$(CH_2)_2NHAc$ (**67**)	6.0-6.6	4 days	62 (**74**)	38

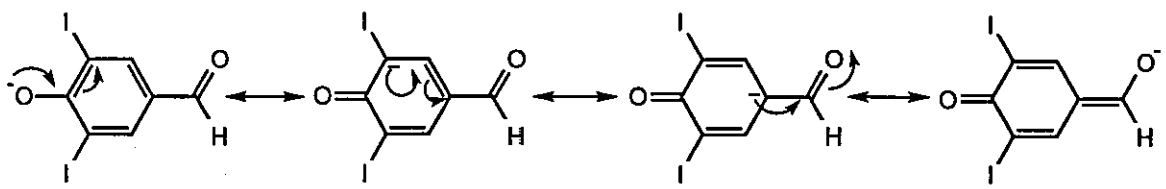
* Ethyl acetate (ca. $1cm^3$) was added to the reaction to aid the solubility of the compound.

† s.m. = starting material

Table 5

The reaction of the tyramine derivative **67** indicated *ortho-ortho* coupling almost immediately at the same pH as for the production of **48** from *N*-acetyl-3,5-diiodotyrosine ethyl ester (**47**), and produced the best yield despite the fact that it is of limited solubility in the reaction mixture. The same compound also underwent *ortho-ortho* coupling in ethyl acetate alone producing a 40 % yield of **74** over 10 days.

The compounds **55**, **63**, **64**, **68**, **69**, **70** and **71** did not react despite attempts at various pHs. For the compounds with a conjugated *para*-substituent, both tautomerism and resonance will continue through to the side chain making reaction at the *ortho* position less likely due to the electron withdrawing effect of the conjugated 4-substituent. This makes the anions less nucleophilic and possibly gives tautomers other than the phenol and α-iodoketone. This is clearly seen in the comparison of the rate of iodination of 4-methyl phenol *versus* 4-hydroxybenzaldehyde, the iodination reaction also proceeding *via* nucleophilic attack by the *ortho* carbanion. Under comparable iodination conditions the 4-methylphenol will diiodinate in only 12 hours as compared with the 8-9 days in the case of the 4-hydroxybenzaldehyde. **Scheme 48** shows the resonance structures of 3,5-diiodo-4-hydroxybenzaldehyde.



Scheme 48

The 4-alkyl-2,6-diiodophenols **55** and **68** are very soluble in the dichloromethane and it is possible that if the compound does not go into the aqueous phase at all then the phenoxide anion may not be formed. A few reactions were therefore carried out using predominantly aqueous phase with only 5 % v/v dichloromethane to try to force the phenols into the aqueous phase. These reactions were carried out at various pHs, however, no coupling products were observed.

The dibromotyrosine derivative **63** and monoiodotyrosine derivative **64** also failed to produce any coupled compound under the reaction conditions. The pK_a of the monoiodo derivative is 8.7[45] and the dibromo derivative 6.45.[21] The reactions were therefore initially attempted around these pH values. As these reactions failed to

produce any coupled compound, further reactions were carried out over a wide range of pH but again were unsuccessful.

2.3.2 Coupling of 2,6-diiodophenols in toluene

During a repetition of a reaction under radical conditions, using hexabutylditin and tungsten lamps to initiate the reaction with half an equivalent of potassium *tert*-butoxide in refluxing toluene under a nitrogen atmosphere, both the 2,6-diiodo-4-methylphenol (**55**) and *N*-acetyl-3,5-diiodotyrosine ethyl ester (**47**) produced *ortho-ortho* coupled compounds. Since the hexabutylditin that had been used in these reactions was over two years old and could have been oxidised, both reactions were repeated in the absence of the hexabutylditin and without shining light on the reactions, again they produced *ortho-ortho* coupled compounds indicating that the hexabutylditin was not causing the coupling. The results of these reactions and the reaction of 2,6-diiodo-4-propylphenol in refluxing toluene and half an equivalent of potassium *tert*-butoxide are shown in **table 6.**

Reaction 37 and 38 showed little difference in yield between reaction in the presence or absence of hexabutylditin, indicating that it is independent of the radical initiator. Also in the previous investigations of the $S_{RN}1$ mechanism this reaction produced 2-iodo-4-methylphenol, i.e. the radical had been formed but had not reacted with any phenoxide ion to produce the coupled compound **59**. The fact that the 2-iodo-4-methylphenol was not produced indicates that the hexabutylditin was no longer effective as an initiator.

Reaction No. (Reactant no.)	4-substituent	Other conditions	% product	% recovered s.m.†
37 (**55**)	CH_3	$(Bu_3Sn)_2 + h\upsilon$	47	53
38 (**55**)	CH_3		40	60
39 (**47**)	$CH_2CH(NHAc)CO_2Et$	$(Bu_3Sn)_2 + h\upsilon$	50	50
40 (**47**)	$CH_2CH(NHAc)CO_2Et$		16	84*
41 (**68**)	$(CH_2)_2CH_3$		16	84

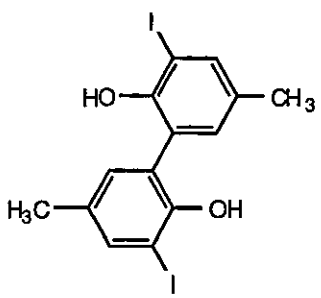
* Some of the recovered compound was as the acid, i.e. ester hydrolysis had occurred.

† s.m. = starting material

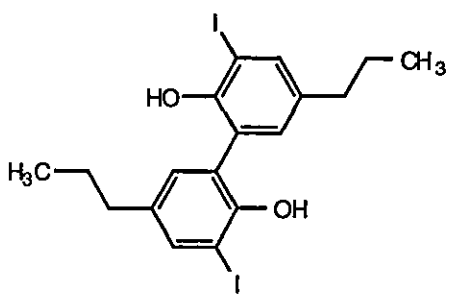
Table 6

All of the reactions were refluxed in toluene for 18 hours. The difference in yield between reactions 39 and 40 may be explained by the hydrolysis of the ethyl ester, which took place in reaction 40 which was also seen in an earlier experiment in the investigation of the $S_{RN}1$ mechanism. Quite why the same hydrolysis did not occur in reaction 39 is not clear, although having checked the equivalents of potassium *tert*-butoxide used in the reaction 39, 0.4 equivalents were used whereas 0.5 equivalents were used in reaction 40 and in the reaction reported with the attempted $S_{RN}1$ reactions 0.58 equivalents were used. These differences may have influenced the results of the reactions.

The products of the reactions of the 4-alkyl-diiodophenols **55** and **68** are shown below. Even though the product **75** was only obtained in a 16 % yield, no repetitions of this reaction were carried out and so the optimum conditions for this derivative remain undetermined.



59



75

This method uses a system in which 2,6-diiodophenols are in solution and can be partially deprotonated by the action of an organic base without the need of an inorganic base in an aqueous media. It has been shown to be an effective method for the coupling of the 4-alkyl substituted phenols which have very limited solubility in water. This again suggests that the coupling in the two-phase system requires an aqueous phase for the formation of the phenoxide ion which will remain predominantly in the aqueous phase but may react at the interface with a tautomerised phenol.

The coupling of the other *para*-substituted 2,6-diiodophenols **63**, **64** and **69** was not studied using the toluene system since it had been shown that the reaction causes some ester hydrolysis. It therefore appeared that their reaction in a two-phase system would be cleaner and possibly higher yielding than their reaction in toluene with potassium *tert*-butoxide.

2.4 Summary of the evidence supporting a polar mechanism

From the results of our studies into a possible $S_{RN}1$ mechanism it has been shown that despite employing vigorous conditions and investigating various methods of initiation, no *ortho-ortho* coupled compounds were obtained as a result of the reactions. By the use of a chemical radical initiator, de-iodination was effected although no coupled product was detected in the reaction mixture. These results indicate that it is possible to form a σ-radical by the homolysis/reduction of a carbon-iodine bond *ortho* to a phenol, but reaction of this radical with a diiodinated phenoxide is not favoured. The failure of these conditions to produce any coupled compound in the reaction mixture gave evidence that the $S_{RN}1$ mechanism was not the mechanism through which the *ortho-ortho* coupling was occurring.

An alternative radical mechanism which involves the formation of a phenoxyl radical has been suggested by Nishiyama *et al*[60] in their electrosynthesis of the dityrosine derivative from the corresponding 2,6-diiodinated tyrosine derivative. However, our investigations using a two-phase system have shown that this reaction can be blocked by raising the pH by more than one pH unit above the pK_a of the phenol, i.e. by the deprotonation of all the phenol in the mixture. This deprotonation of the phenol should not affect the ability to remove an electron in the oxidation to the phenoxyl radical, in fact it should aid the oxidation (evidence of this can be found in chapter 3). We also found that the presence of radical inhibitors and exclusion of light from the reaction did not inhibit the reaction and in the case of reactions carried out in the presence of TEMPO, a radical trapping reagent, there was no affect on the yield of the coupled product. The *ortho-ortho* coupling is unaffected by an atmosphere of either nitrogen or oxygen. Under nitrogen, no oxidant is present, therefore clearly eliminating phenoxyl radical as intermediates. Reaction 30 (**table 4**) shows the formation of dityrosine derivative **48** in conditions in which thyroxine is not produced, indicating that the phenoxyl radical was not formed (thyroxine formation is thought to occur *via* phenoxyl radicals). Coupling via phenoxyl radicals is usually regioselective giving predominantly oxygen-*para* coupling in the reaction of a phenoxyl radical and a phenoxide ion, or *para-para* coupling in the case of the reaction of two phenoxyl radicals. Although these products may not be formed exclusively, the reaction conditions as well as the substituents on the *ortho* and *para* positions may also have an affect on the products obtained, we may not expect to obtain the *ortho-ortho* coupled product alone if the reaction proceeds through phenoxyl radical intermediates.

Taking into account the steric implications involved in the possible mechanisms of *ortho-ortho* coupling, the $S_{RN}1$ mechanism requires the loss of the iodine at the site of coupling prior to the approach by an anion affording little steric hindrance to the coupling. However, in the phenoxyl radical mechanism the phenoxyl radical can be delocalized and is therefore likely to be planar causing steric hindrance to the approach of either another phenoxyl radical or to a phenoxide carbanion. The S_N2 mechanism, however, allows for the approach of an anion since the phenol can undergo tautomerisation affording an sp^3 hybridised carbon on which the iodine is directed away from the approach of the carbanion. Below are the Chem 3D models of the possible intermediates and also the *ortho-ortho* coupled intermediate. All of the intermediates have been shown for 2,6-diiodo-4-methylphenol for simplicity. **Figure 1** has been atom labelled as a key for the other structures and shows the diiodophenol as its α-iodoketo tautomer required by the S_N2 mechanism. **Figure 2** shows the intermediate phenoxyl radical viewed along the plane of the molecule from the oxygen, and gives an indication of the steric hindrance caused by the iodines if the reaction proceeds by an phenolic oxidation coupling mechanism. **Figures 3** and **4** show the *ortho-ortho* coupled intermediate viewed from different angles to exemplify the lack of steric hindrance around the iodine on the sp^3 hybridised carbon.

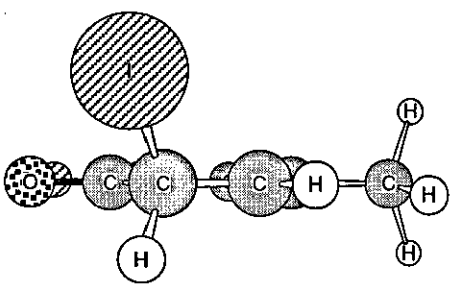


Figure 1

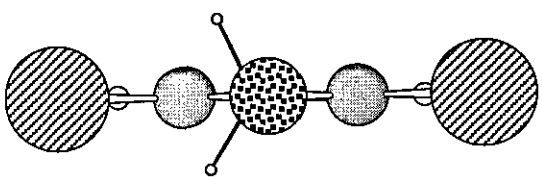


Figure 2

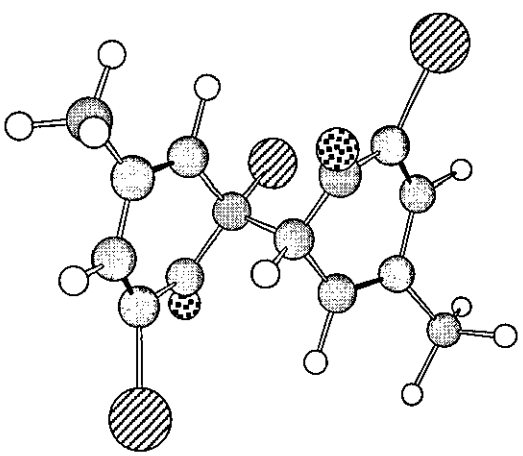


Figure 3

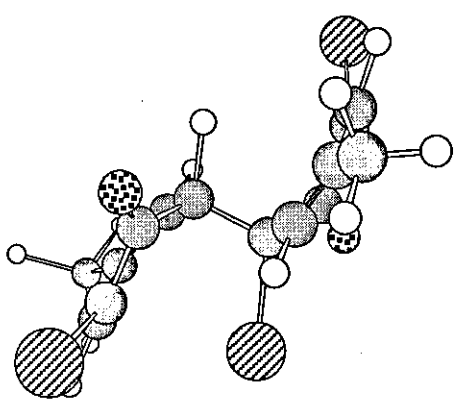


Figure 4

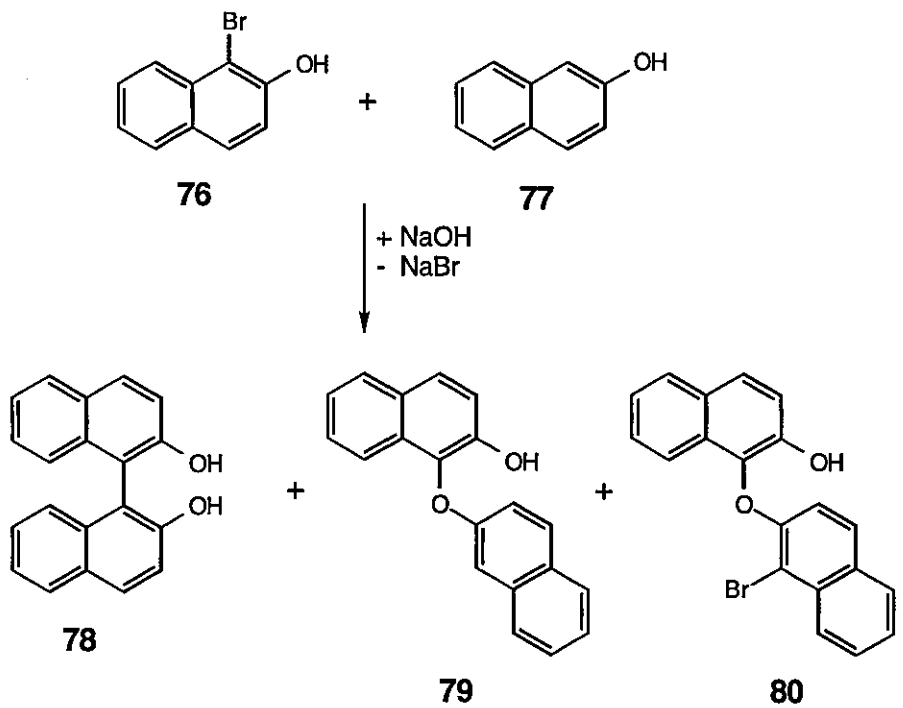
If the *ortho-ortho* coupling were to take place through a radical mechanism it would be difficult to explain the lack of further reaction after dimerisation. In a radical reaction further oxidation of the phenoxide ion to the phenoxyl radical of the dityrosine derivative would be possible, as would further reduction/homolysis of the carbon-iodine bond in the case of the $S_{RN}1$ mechanism. However, our reactions produced only the dimer, starting material and iodine. A polar mechanism may explain the lack of further reaction. The coupling has been shown to be pH sensitive occurring within a 2-3 pH unit range. On coupling the phenol loses an iodine for an aryl group and therefore the pK_a of the coupled mono-iodophenol is higher than that of the starting diiodophenol. Attempts to measure the pK_a of the dityrosine derivative **48** were abandoned due to its insolubility in water, but it is reasonable to expect it to have a pK_a in the region of 3-iodotyrosine, i.e. around 8.7. The reaction pH would therefore be around 2 pH units lower than the pK_a of the phenol, making further coupling less likely to occur than if it was afforded by radical means.

The discussion so far indicates that the conditions under which the dityrosine derivative **48** is formed, support a polar rather than radical mechanism is occurring. We have proposed the S_N2 mechanism shown in **scheme 46** as the coupling process. This mechanism affords an explanation for the reduction of the reaction rate in the use of an ethanol/aqueous system rather than a dichloromethane/aqueous system. Although in both cases water is present, in the dichloromethane system some phenoxide ion may pass back into the organic phase in which it will be a more reactive nucleophile, or the reaction could occur at the interface. In the ethanol/aqueous system the phenoxide ion will be highly solvated and therefore more stable and hence less reactive. As a result, the reaction requires heat and pressure to proceed at a rate still slower than that occurring at room temperature and atmospheric pressure in the dichloromethane/aqueous system.

The evidence presented towards an S_N2 mechanism is not conclusive, but our evidence does appear to strongly disfavour a radical mechanism. There is literature precedent in similar studies involving the reaction between halogenonaphthols and nucleophiles, which have been suggested to go *via* polar S_N2 mechanisms.[70] Bélohradsky *et al*[71] carried out a study of the reaction between 1-bromo-2-naphthol (**76**) and 2-naphthol (**77**) using base to form the binaphthol **78**, the ethers **79** and **80** and polymeric materials. They also reported that the reaction was confined to narrow limits (**scheme 49**).

They showed that the stoichiometry of the base to starting materials strongly affected the reaction, the maximum rate occurring when the molar ratio of **76**:**77**:base is 1:1:1

and that an increase in quantity of base caused a reduction in the yield of the coupled product. A solvent effect was also observed, with toluene giving better yields than water, ethylene glycol or DMSO. Comment was also made that binaphthol formation was unaffected by the use of an argon atmosphere as opposed to being reacted in air. However, under argon the quantity of by-products (polymers were reported) was substantially reduced (indicating that these by-products resulted from the formation of naphthoxyl radicals). These findings have a strong similarity to the results of our investigations using 2,6-diiodophenols.



Scheme 49

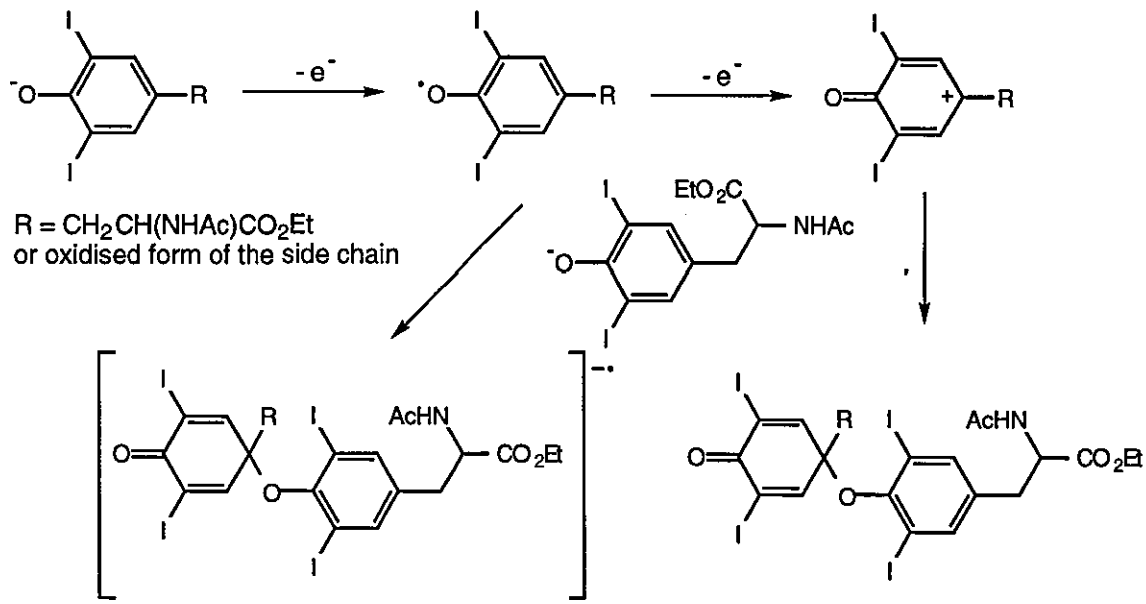
In conclusion; although the coupling of halogenated phenols through a S_N2 mechanisms has not been previously reported, there does not appear to be a reason why this coupling should not follow the same mechanism as proposed for the coupling of halogenonaphthols.

Chapter Three

Electrochemical oxidation of phenolic compounds

3.1 Introduction

In chapter one the literature concerning the biosynthesis of thyroxine was summarised. Most of the reports in the literature suggest that in order for the coupling of 3,5-diiodotyrosine, either with another molecule of 3,5-diiodotyrosine or with 3,5-diiodo-4-hydroxyphenylpyruvic acid (DIHPPA), to form thyroxine either a one electron oxidation to form a phenoxyl radical or a two electron oxidation yielding the phenoxonium ion should take place.[33,34,37,48,53] The process of oxidation and coupling to form the dienone intermediate is shown in **scheme 50** with the possibility of one or two single electron oxidations. The phenoxide ion, phenoxyl radical and phenolic cation are each shown in only one of the three possible resonance representations. Although it is not shown in the scheme it is possible that a one step two electron oxidation may be occurring.



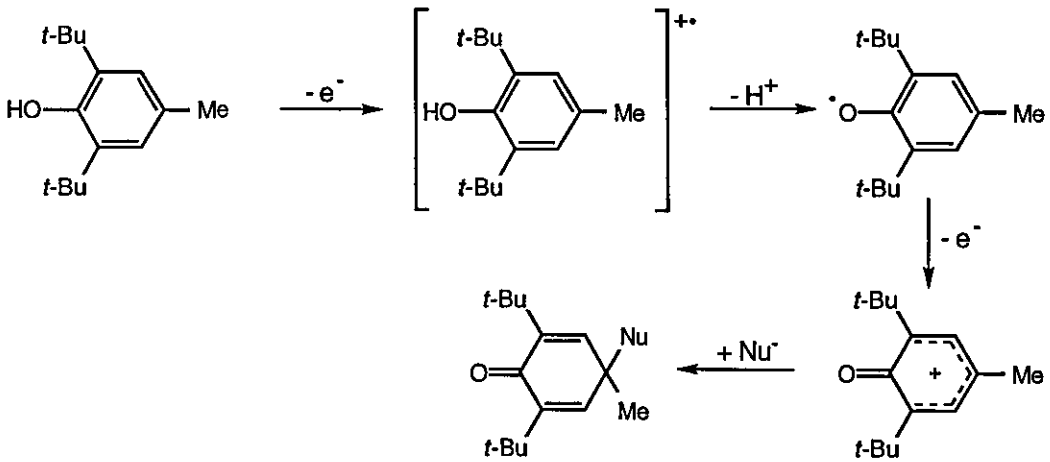
Scheme 50

The synthesis of *N*-acetylthyroxine ethyl ester (**46**), of interest in this investigation, is thought to be biomimetic, involving the initial loss of one electron in the formation of the phenoxyl radical. The electrochemical study of this oxidation process of the phenol or phenoxide may therefore provide valuable information concerning the actual oxidation step occurring in the (bio)synthesis. Investigations of the coupling of 3,5-diiodotyrosine alone and with DIHPPA to form thyroxine have shown that the process is aerobic.[26,43,49,53] From the results of electrochemically oxidising the phenols and phenoxides it may be possible to deduce how the oxidation is actually taking place in the chemical synthesis, e.g. are oxygen

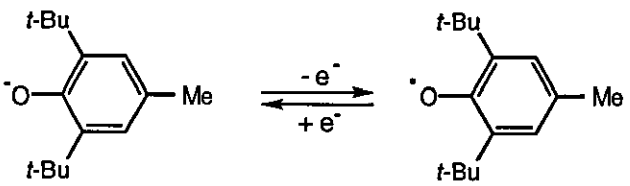
and manganese(II) sulfate forming a successful redox couple according to the oxidation potentials of the phenols/phenoxide ions.

It is known that the oxidation potentials of phenols are dependent on the substituents, particularly those on the *ortho* and *para* positions due to the resonance stabilisation of the radical resulting from the oxidation. Electron-donating substituents will effect a more negative oxidation potential (i.e. the phenol is more readily oxidised), conversely electron-withdrawing substituents will give a more positive oxidation potential.[72]

The pH of the medium in which the oxidation is carried out is also crucially important to the observed oxidation potential. At a pH below the pK_a of the phenol the oxidation potential will be more positive and generally involves a two electron oxidation and loss of a proton yielding a phenoxonium ion. When the phenol is deprotonated the oxidation potential will be more negative and generally undergoes one single electron oxidation. This is often irreversible due to the reactivity of the phenoxyl radical, giving rise to the formation of passivating films on the electrode surface. However, if the phenol is 2,4,6-trisubstituted with either bulky or electron-donating substituents the oxidation can be reversible.[72]



Scheme 51



Scheme 52

This is exemplified by the oxidation of 2,6-di-*tert*-butyl-4-methylphenol in acetonitrile in which the phenol is protonated and undergoes two single electron oxidations and the loss of a proton yielding the phenoxonium ion. This couples on addition of a nucleophile e.g. MeOH, AcO^- to give a dienone product shown in **scheme 51**. In basic solution when the same phenol is completely deprotonated, the phenoxide undergoes a reversible single electron oxidation, shown in **scheme 52**.[72,73]

3.2. Oxidation of iodinated phenols

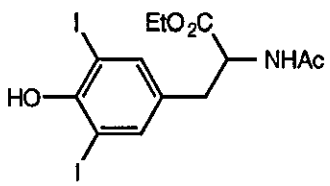
Since the form of the *para*-substituent of the diiodinated phenol prior to coupling was unclear at the time the study was undertaken, and in order to obtain general information of the effect of the substituents on the oxidation potentials of phenol/phenoxides, our study was carried out using compounds with a variety of *para*-substituents. Previous work by Hapiot *et al,*[74] carried out using *tert*-butyl, phenyl, methyl and methoxy substituents in the *ortho* positions and a variety of *para*-substituents, has shown that a change in the *para*-substituent can affect the oxidation potential and the reactivity of the radical produced.

The compounds tested are shown below. The oxidation potentials of the tyrosine derivatives **47**, **64** and **81** were measured in order to determine the affect of the one and two *ortho* iodine substituents. The study of the diiodinated compounds was predicted to show the affect of varying the *para*-substituent.

The thyroxine derivative **46** is of interest in order to ascertain whether it is easier to oxidise than *N*-acetyl-3,5-diiodotyrosine ethyl ester (ethyl *N*-acetylDIT, **47**). If this is the case then we could expect to see the formation of trimers and further polymers during the reaction of ethyl *N*-acetylDIT (**47**).

The electrochemical studies were carried out using cyclic voltammetry. In order to measure the electrochemical potentials, the phenols were dissolved in acetonitrile, and the phenoxide ions of the phenols were dissolved in water, in both cases sodium perchlorate was used as the background electrolyte. The oxidation potentials could not be measured in ethanol/water (i.e. a direct comparison with the synthetic procedure under investigation) since when background runs were attempted it was found that the oxidation of ethanol would mask the oxidation of the phenols. The oxidation potentials of the phenols and phenoxide ions were measured from the resulting cyclic voltammograms by measuring the distance of the apex of the peak

from zero potential in centimetres, then the potential calculated from the X scale (cm/V) used on the chart recorder. The values quoted in the tables found later in this chapter are *versus* the saturated calomel electrode.



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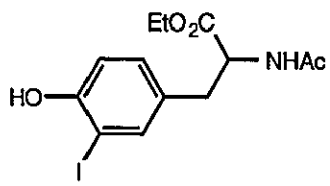
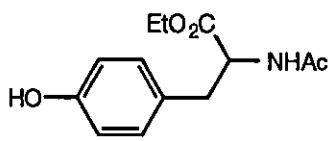
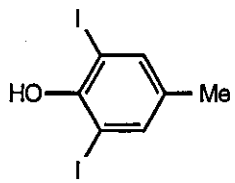


64



81



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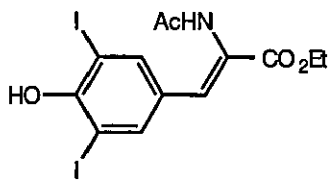

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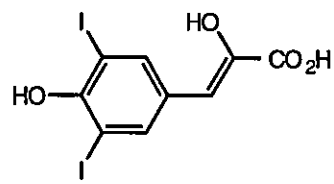

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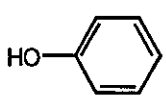

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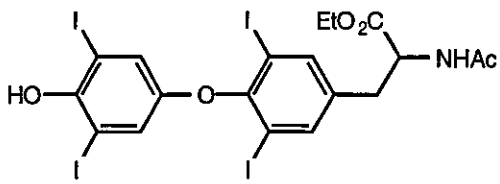


46

3.2.1 Oxidation of phenols in acetonitrile

Table 7 shows the measured oxidation potentials of the phenols in acetonitrile. The cyclic voltammograms from which these results were obtained are attached in appendix I.

Phenol itself was the only phenol found to be soluble in water so the oxidation potentials were therefore measured in water as well as acetonitrile to provide an indication of the difference between the oxidation potentials in the different solvents. This could aid the comparison of the oxidation potentials of the phenols and phenoxides since the oxidation potentials of the phenoxides were measured in water due to their insolubility in acetonitrile.

From the oxidation potentials of phenol measured in water and acetonitrile it can be seen that the oxidation occurs more readily in water. The oxidation potential of the loss of the second electron from phenol in acetonitrile was not observed in the scan since it fell outside the voltammetric range scanned.

Compound	Concentration (mmol)	Ep (V)
47	5.12	1.20 1.56
64	4.73	1.30 1.62
81	4.95	1.35 1.59
46	1.30	1.25 1.60
55	4.99	1.33 1.68
40	4.81	0.86 1.58
69	5.04	1.06 1.78
82	5.42/5.68*	1.52/0.83* 1.12

* In water

Table 7

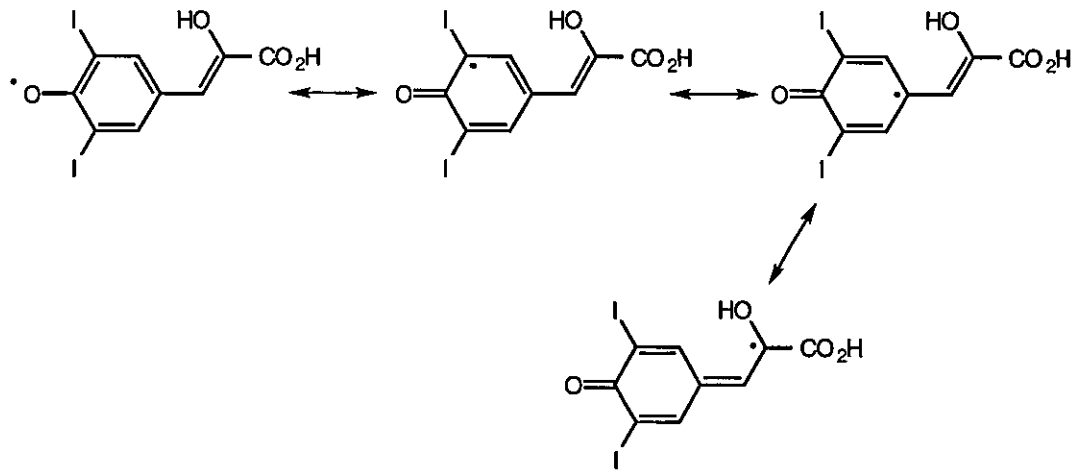
Oxidation potentials of phenols in acetonitrile

The phenols (other than phenol) all showed two oxidation steps, both corresponding to the removal of one electron. It is observed that the difference between the first and second oxidation potentials show a pattern according to the substituents, irrespective of the potential of the first oxidation. For the diiodinated compounds with saturated *para*-substituents **47**, **55** and **46** the differences are 0.36, 0.35 and 0.35 V respectively. The monoiodo compound **64** has a difference of potentials of 0.32 V, and the noniodinated compounds **82** (phenol in water) and **81**, 0.29 and 0.24 V respectively, the phenol values, however, were measured in water. The electron-withdrawing affect of the iodines may stabilise the phenoxyl radical making the removal of the first electron a more favourable process than in the absence of the iodine substituents. Due to the stability of the radical produced in the iodinated compounds the removal of the second electron requires more energy.

The diiodinated compounds with unsaturated side chains **40** and **69** both have a difference of 0.72 V. This affect may be due to the relative stability of the phenoxyl radical afforded by the resonance possibilities of the conjugated *para*-substituent

shown in **scheme 53**, thereby making the removal of the first electron easier, and the removal of the second electron more difficult.

From the results of the tyrosine derivatives **47**, **64** and **81**, and of **55** and **82**, all in acetonitrile, it can be seen that *ortho*-iodine substituents effect a decrease in the oxidation potential of the phenol. This is unexpected since electron-withdrawing substituents generally cause a more positive oxidation potential.



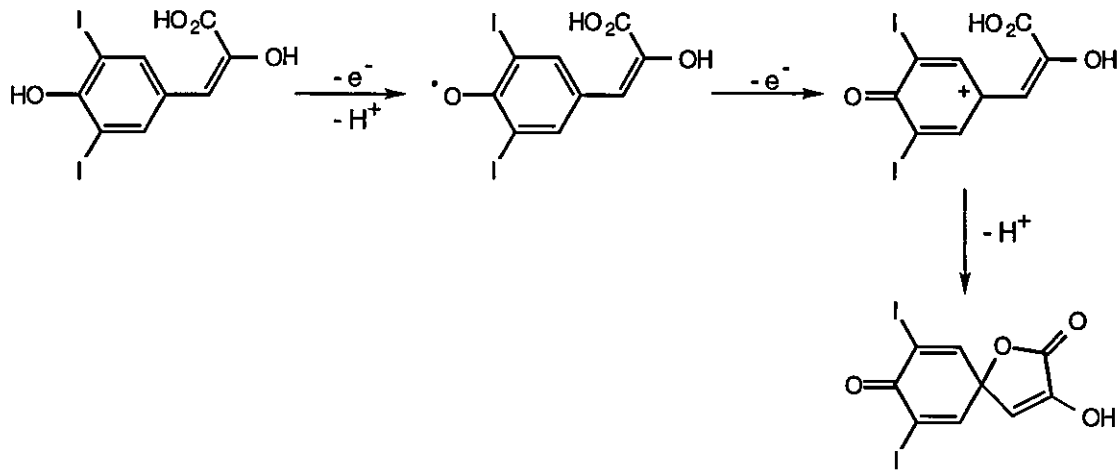
Scheme 53

However as the pK_a of the phenol decreases from 10.0 (for **81**), 8.7 (for **64**) to 6.5 (for **47**)[45] it is likely that the radical cation is a very short lived species and the proton may even be lost simultaneously with the first oxidation. This first oxidation would therefore be of similar nature to the oxidation of the phenoxide, which has a more negative value. These compounds all give irreversible cyclic voltammograms which are, with the exception of phenol, reproducible since the product of oxidation does not coat the electrode surface thereby passivating it, as is seen in the case of phenol.

The effect of the unsaturated and conjugated *para*-substituent, **40** and **69**, is seen to reduce the oxidation potential of the phenols. This could be due to the increased resonance and therefore stability of the newly formed radical as discussed above and shown in **scheme 53**.

The oxidation of these conjugated compounds (**40** and **69**) is irreversible and is not reproducible although the oxidation product does not coat the electrode since further voltammetric waves are observed, i.e. the electrode is not passivated. There is a possibility that the side chain of may cyclise forming a spiro compound although

this would require the carboxylic acid or ester group to be *cis* to the aromatic ring, as shown in **scheme 54**, which is the less likely conformation.



Scheme 54

3.2.2 Oxidation of the phenoxide ions in water

phenoxide ion of	Concentration (mmol)	Ep (V)
47	5.06	0.44 1.10
64	5.06	0.42 1.19
81	4.50	0.39 0.99
46	2.43	0.51 1.04
55	3.82	0.29
40	5.26	0.51 0.76, 1.05
69	3.63	0.39 0.61, 1.09
82	6.36	0.41 1.04

Table 8

Oxidation potentials of phenoxide ions in water

Table 8 shows the measured oxidation potentials (*versus* the saturated calomel electrode) for the phenoxide ions in water. The cyclic voltammograms from which these results are derived are attached in appendix I.

From the oxidation potentials of the tyrosine derivatives **47**, **64** and **81** it can be seen that the reverse trend is seen with the phenoxide ions than with the phenols. A possible explanation of this trend is that the lower the pK_a of the phenol and therefore, the more stable the phenoxide ion, the harder it is to remove the electron, whereas the less stable the phenoxide ion, the easier it is to remove the electron since the energy gap between the anion and the radical will be smaller. Although the values of oxidation potentials are close together, the trend was reproducible. Also taking the values for the first oxidation of the phenoxide ion of *N*-acetylthyroxine ethyl ester (**46**) *versus* that of the phenoxide ion of phenol (**82**) the trend is consistent even though **46** is *para*-substituted. However, in the comparison between the phenoxide ion of 2,6-diiodo-4-methylphenol (**55**) and the phenoxide ion of phenol (**82**) the trend is reversed, which may be due to the slightly electron-donating affect of the *para*-methyl substituent, although such a marked difference may not be expected from a methyl group alone.

The second values seen in the case of the oxidation of the phenoxyl radicals of **47**, **64**, **82**, **46**, and **82** could be due to the removal of a second electron in the formation of the corresponding phenoxonium ion. There appears to be no apparent trend in this oxidation, or in the difference between the first and second electron oxidation. In all the cyclic voltammograms the first scan is only reproducible for the next scan, after which the potential either shifts, or no potential is measured possibly due to the electrodes being passivated. This passivation is seen in the case of phenol (**82**) and 2,6-diiodo-4-methylphenol (**55**). It is possible that the oxidation product of ethyl *N*-acetylDIT (**47**) may react with the phenoxide ions in the bulk solution producing *N*-acetylthyroxine ethyl ester (**46**), however on comparing the second to fifth scans of the phenoxide ions of ethyl *N*-acetylDIT (**47**) with the first scan from the phenoxide of *N*-acetylthyroxine ethyl ester (**46**) the voltammetric wave for the phenoxide ion of *N*-acetylthyroxine ethyl ester (**46**) is not apparent in the second to fifth scans of the phenoxide ion of ethyl *N*-acetylDIT (**47**). This does not necessarily indicate that the thyroxine derivative is not formed, but that if it is present in the solution after oxidation it is not of high enough concentration to contribute to the voltammetric wave.

The oxidation of the compounds **40** and **69** with conjugated *para*-substituents show three potentials. The first is the one electron oxidation of the phenoxide ion, the

second could be due to the reaction of the side chain with the radical, or the reaction of the radical with another radical or a nucleophile in the bulk solution, the product of which is oxidised to a radical. The third potential is probably the removal of a further electron to form the phenoxonium ion. Again these measurements were not reproducible on further scans.

From the oxidation potential of phenol in water compared with the oxidation potential of the phenoxide ion of phenol in water it can be seen that the deprotonated phenol is more readily oxidised than the phenol, and it may be postulated that this would be true of the other phenols tested. The lower oxidation potentials of the phenoxide ions compared to the oxidation potentials of the phenols indicates that by carrying the reaction out at pH 9.5 less energy may be required in the oxidation to the corresponding phenoxyl radical than if the reaction were carried out at neutral or acidic pH.

3.2.3 Reduction potentials of oxygen and manganese(II), and their implications to the oxidation of the phenoxide ions

Having measured the oxidation potentials of the phenoxide ions it is now possible to establish, from the oxidation potential of manganese(II) and reduction potential of oxygen, whether the oxidation of the manganese(II) to manganese(III) is thermodynamically feasible, and whether manganese(III) or oxygen is the primary oxidant, for the formation of the phenoxyl radical, in the synthetic process.

Manganese(II) is the most stable oxidation state of manganese due to the *d* orbitals being half filled ($3d^5$) and is for this reason unlikely to oxidise the phenoxide ions to phenoxyl radicals. A possibility is that the manganese(II) sulfate, which in alkaline solution forms a precipitate of manganese(II) hydroxide, may be oxidised to manganese(III) by the reduction of oxygen. Attempts were made to measure the potentials of the manganese(II) hydroxide oxidation and oxygen reduction. However, neither gave a voltammetric wave despite several attempts. The manganese(II) hydroxide forms an insoluble precipitate in alkaline solution and appeared to simply coat the cell and electrodes. Hence we used literature values to calculate whether our proposed system could oxidise the phenoxides.[75a]

The equations and electrode potentials for oxygen and manganese(II) in alkaline solution are shown in **equations 1a** and **1b**, the standard potentials are reported *versus* the saturated calomel electrode.[75,76]

1a $Mn(OH)_3 + e^- \rightleftharpoons Mn(OH)_2 + OH^-$ E° -0.09 V

1b $O_2 + 2\,H_2O + 4\,e^- \rightleftharpoons 4\,OH^-$ E° +0.161 V

Equation 1

To calculate the potential of the reaction required we used the equation;

$$Ecell = Ered - Eox$$

The cell potential must be positive if it is to occur spontaneously, and from this equation we can calculate that the reduction potential of oxygen is sufficient to oxidise the manganese(II) hydroxide to manganese(III) hydroxide (**equation 2**).

$$O_2 + Mn(II) \rightleftharpoons O_2^{-\bullet} + Mn(III)$$

$$+0.161 - 0.09 = +0.071\ V$$

Equation 2

However, on calculating the potential of the oxidation of the phenoxides by the reduction of oxygen (**equation 3**), or manganese(III) to manganese(II) (**equation 4**), the reaction potential is negative indicating that the reaction is not spontaneous at standard conditions (the Ep for the oxidation of the phenoxide ion of ethyl *N*-acetylDIT (**47**) has been used in the equations shown). This indicates that the high temperature and pressure conditions used in the industrial synthesis of *N*-acetylthyroxine ethyl ester are required for the oxidation of the phenoxide ion of ethyl *N*-acetylDIT (**47**) to be favourable. The pH will also affect the reaction potential.

$$ArO^- + O_2 \rightleftharpoons ArO^\bullet + O_2^{-\bullet}$$

$$+0.161 - 0.44 = -0.279\ V$$

Equation 3

$$ArO^- + Mn(III) \rightleftharpoons ArO^\bullet + Mn(II)$$

$$-0.09 - 0.44 = -0.53\ V$$

Equation 4

3.3 Conclusions

The results of the anodic oxidation of phenols in acetonitrile show that *ortho* iodine substituents will decrease the oxidation potential of the phenol. Conversely with the oxidation of the phenoxide ions, *ortho* iodine substituents increase the oxidation potential. This can possibly be attributed to the larger energy difference between the anion and radical due to the stability of the of the anion from the electron-withdrawing affect of the iodines.

The oxidation potentials of manganese(II) and oxygen in alkaline solution have shown that oxygen can afford the oxidation of manganese(II) to manganese(III) at standard temperature and pressure. However, the reduction potentials of manganese(III) and oxygen indicate that the oxidation of the phenoxide ions may only occur if the reaction conditions provide the necessary energy. It is possible that both oxygen and manganese(III) play a part in the oxidation of the phenoxide ion of ethyl *N*-acetylDIT (**47**) to the phenoxyl radical, although oxygen may be the primary oxidant since the manganese(III) hydroxide is precipitated at the pH of the reaction.

Acknowledgement

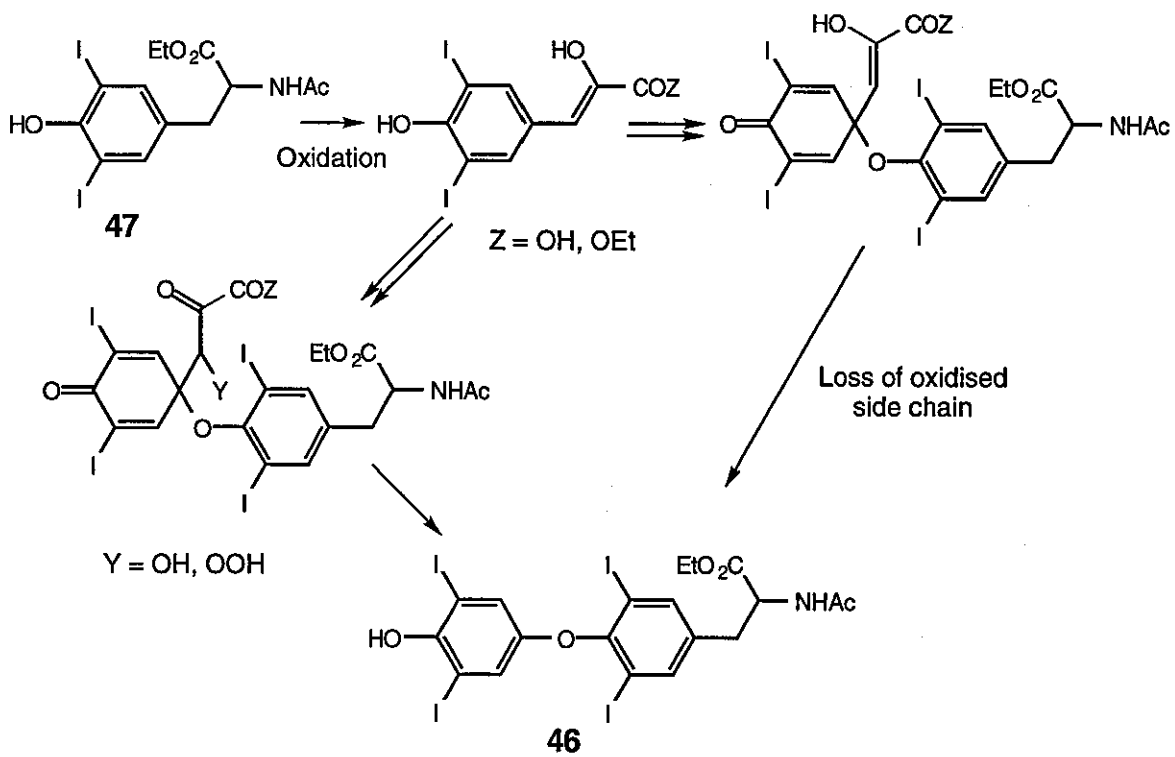
The research described in this chapter was carried out in collaboration with Dr. Roger Mortimer and Deborah Hall who also had interests in the electrochemical oxidation of phenolic compounds. I am grateful to them for their work, help and advice.

Chapter Four

Oxidation of the amino acid and the subsequent coupling to form the ethyl ester of *N*-acetylthyroxine

4.1 Introduction

As described in chapter one, various theories have been proposed for the biosynthesis of thyroxine. Biomimetic studies have been carried out to gain evidence in support of the proposals. Our aim was to establish whether the chemical synthesis which we were studying was biomimetic and occurred through one of the proposed mechanisms. The central argument for these proposals was the oxidation of the diiodotyrosine side chain to the corresponding phenylpyruvic acid prior to coupling, as shown in **scheme 55**.

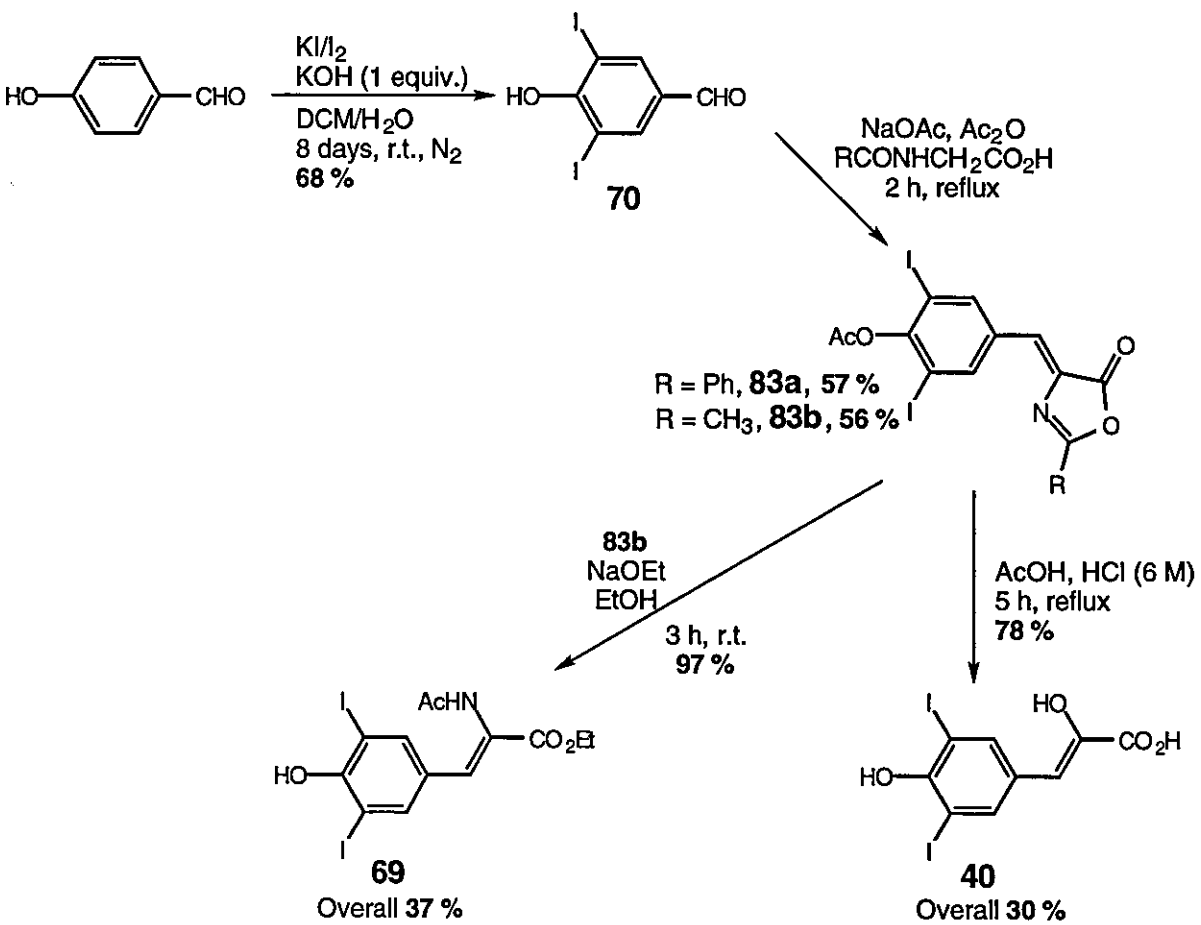


Scheme 55

It is possible that the chemical process under investigation could involve the oxidation of the amino acid side chain of *N*-acetyl-3,5-diiodotyrosine ethyl ester (ethyl *N*-acetylDIT, **47**) to afford the corresponding α-keto acid (or ester). In biological systems these oxidations are catalysed by oxidase enzymes or facilitated by transaminases with pyridoxal as a coenzyme. In our chemical synthesis the ester of the resulting α-keto ester could be hydrolysed under the conditions of the reaction. It is therefore possible that the coupling could occur between the ethyl *N*-acetylDIT (**47**) and either the acid or ethyl ester of the phenylpyruvate derivative.

4.2 Coupling between *N*-acetyl-3,5-diiodotyrosine ethyl ester and DIHPPA in the formation of *N*-acetylthyroxine ethyl ester

Our initial studies centred on the assumption that in the chemical process, the ethyl *N*-acetylDIT (**47**) was oxidised to the α-keto ester prior to coupling and that the oxidative elimination of the side chain could proceed equally efficiently for the acid or ester. The putative reaction sequence is shown in **scheme 55**. The synthesis of DIHPPA (**40**) is shown in **scheme 56**.[43]



Scheme 56

4-Hydroxybenzaldehyde was iodinated, the reaction requiring eight days to afford a 68 % yield due to the mesomeric effect of the aldehyde. 4-Hydroxy-3-iodobenzaldehyde and unaltered 4-hydroxybenzaldehyde were also isolated. The 3,5-diiodo-4-hydroxybenzaldehyde (**70**) was then refluxed with *N*-benzoylglycine or *N*-acetylglycine and sodium acetate in acetic anhydride over two hours to yield the desired oxazolones (**83a** or **83b**, respectively) which were recrystallised from toluene to give 57 % and 56 % yield respectively. Increasing the reaction time had

little affect on the yield. DIHPPA (**40**) was obtained (78 %) from the refluxing of either of the oxazolones (**83a/83b**) in acetic and hydrochloric acids, followed by filtration and washing of the resulting solid which gave clean compound.[43] The moderate yield obtained in this reaction may be due to the DIHPPA oxidising to the benzaldehyde (**70**), this compound may have been dissolved in the filtrate and therefore not recovered with the DIHPPA. The dehydrotyrosine derivative (**69**) was afforded by treating the oxazolone (**83b**) with sodium ethoxide in ethanol and afforded a 97 % yield of pure **69**. The reactions carried out using the dehydrotyrosine derivative (**69**) will be reported later in this chapter.

Ideally the studies of the coupling with ethyl *N*-acetylDIT (**47**) should have been conducted using the ethyl ester of 3,5-diiodo-4-hydroxyphenylpyruvic acid (DIHPPA). However, difficulties were encountered in converting the DIHPPA (**40**) to the methyl or ethyl ester, due to the instability of the compound to the reaction conditions; therefore the studies were centred on the DIHPPA (**40**).

The coupling reactions were carried out using a method reported by Meltzer and Stanaback.[43] They added small amounts of DIHPPA (**40**) to a solution of *L*-3,5-diiodotyrosine dihydrate in a borate buffer and chloroform at pH 7.6 over approximately two hours, with a molar ratio of DIHPPA (**40**) to amino acid of 3:2, oxygen was vigorously bubbled through the solution. The removal of solvent gave rise to thyroxine obtained in a 36 % yield based on the quantity of amino acid used. The reaction has been reported to be aerobic,[48] the oxygen being required for the formation of a hydroperoxide intermediate, the radical of which has been detected by EPR spectroscopic studies, although the actual structure of the hydroperoxide has not been assigned.[51,52]

Whereas the reported protocol used DIHPPA (**40**) with 3,5-diiodotyrosine (**41**) we used ethyl *N*-acetylDIT (**47**) because this is likely to be the reactant in the coupling in the chemical synthesis under investigation. DIHPPA (**40**) was reacted with ethyl *N*-acetylDIT (**47**) in dichloromethane and borate buffer at pH 8.3, however, the reaction were unsuccessful in the production of *N*-acetylthyroxine ethyl ester (**46**). However, on changing the organic solvent to ethanol small amounts of *N*-acetylthyroxine ethyl ester (**46**) were produced. The reaction was carried out at different pHs according to the nature of the buffer used (i.e. borate or phosphate buffer), the results of which are shown in **table 9**. The reactions were carried out using an excess of DIHPPA (approximately 1.5 equivalents to the tyrosine derivative **47**). The yields are calculated from the integration of the signals arising from the ^{1}H NMR spectroscopy of the product mixture, since the separation of the

tyrosine (**47**) and thyroxine (**46**) derivatives cannot be carried out by column chromatography (since a solvent system could not be found in which they had different rf values) and are based on the assumption of a 1:1 ethyl *N*-acetylDIT:DIHPPA reaction to produce one mole *N*-acetylthyroxine ethyl ester (**46**).

Reaction No.	Buffer	Reaction pH	N_2/O_2	% Yield **46**
42	Phosphate	7.5	O_2	6 %
43	Borate	8.5	O_2	2 %
44	Phosphate	7.5	N_2	12%
45	Borate	8.5	N_2	5 %

Table 9

From the results it is apparent that in reactions 42 and 44 the use of a phosphate buffer at pH 7.5 affords a higher yield of the desired product than reactions 43 and 45 which use a borate buffer at pH 8.5. This could be due to the pH effect rather than the chemical nature of the buffer, although the investigations by Knox *et al* suggest that the enol form of the DIHPPA is stabilised by borate ions and that the presence of these ions can have a beneficial effect on the coupling reaction aiding the oxygen consumption of the DIHPPA in the formation of the hydroperoxide.[51,52,53] This theory is not consistent with our results of the coupling carried out in the absence of oxygen where *N*-acetylthyroxine ethyl ester (**46**) was still seen to be produced, and in comparably high yield. Despite deoxygenating the solution prior to reaction it is possible that a small amount of oxygen remained dissolved in the reaction solution and took part in the reaction. 3,5-Diiodo-4-hydroxybenzaldehyde (**70**) and 3,5-diiodo-4-hydroxybenzoic acid (**43**) were present in the ^1H NMR spectra of all the reactions due to the breakdown of DIHPPA (**40**).

Blank reactions using ethyl *N*-acetylDIT alone were carried out under the same reaction conditions, and failed to produce any thyroxine derivative giving a quantitative return of the starting material **47**. This result suggests that the *N*-acetylthyroxine ethyl ester (**46**) is formed by the coupling of ethyl *N*-acetylDIT (**47**) with DIHPPA (**40**) rather than the coupling of ethyl *N*-acetylDIT (**47**) alone. It was during a blank reaction in dichloromethane and phosphate buffer that the formation of the dityrosine derivative **48** was observed, leading to the research of this reaction as described in chapter two.

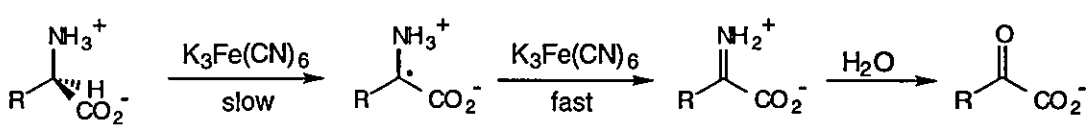
The experiments from our studies using ethyl *N*-acetylDIT (**47**) in place of 3,5-diiodotyrosine (**41**) failed to give the higher yields reported in the literature for the same coupling. This prompted us to study the oxidation of ethyl *N*-acetylDIT to DIHPPA before investigating this coupling any further.

4.3 Oxidation of the amido ester side chain of *N*-acetyl-3,5-diiodotyrosine ethyl ester

The research of Stanbury *et al*[54b] showed that the conversion of 3,5-diiodotyrosine to DIHPPA could be effected non-enzymically using pyridoxal and manganese(II). Ito and co-workers[54a] carried out a similar study and showed that copper(II) was superior to manganese(II) in the oxidation of the amino acid in the presence of pyridoxal. More recently the same oxidative deamination using 2-phenylglycine and phenylalanine was, again, reported to be more efficient in the presence of copper(II) rather than manganese(II), and it was also pointed out that the reaction will only proceed at pH > 9.[77]

Our process contains no pyridoxal or pyridoxal derivatives which may effect this oxidative deamination process, although it is carried out at pH 9.5, and we therefore postulated that manganese and oxygen may be involved in the oxidation and the boric acid may be present to complex with the DIHPPA thus stabilising it. The side chain is, however, significantly different in ethyl *N*-acetylDIT (**47**) and 3,5-diiodotyrosine (**41**). Pyridoxal catalysed reactions require a free amino group, which in our case is blocked as the *N*-acetylamine. However, redox reactions *via* the α-amino radical would have a similar reactivity.

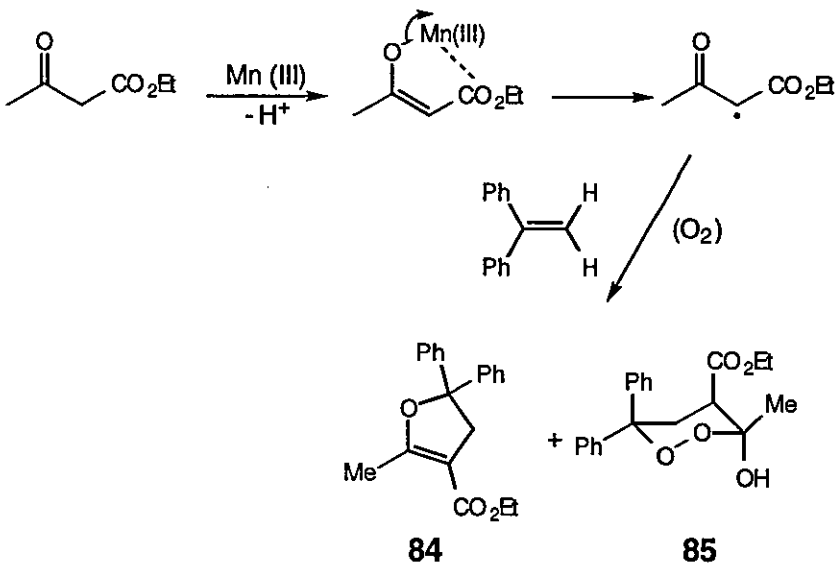
Oxidation of amino acids by the action of potassium ferricyanide has been suggested to occur through the mechanism shown in **scheme 57**. The captodative radical intermediate is formed by the loss of a hydrogen atom from the methine carbon, and was detected by EPR spectroscopy. This radical is then thought to react rapidly with further oxidant in the formation of the imine which is subsequently hydrolysed.[78]



Scheme 57

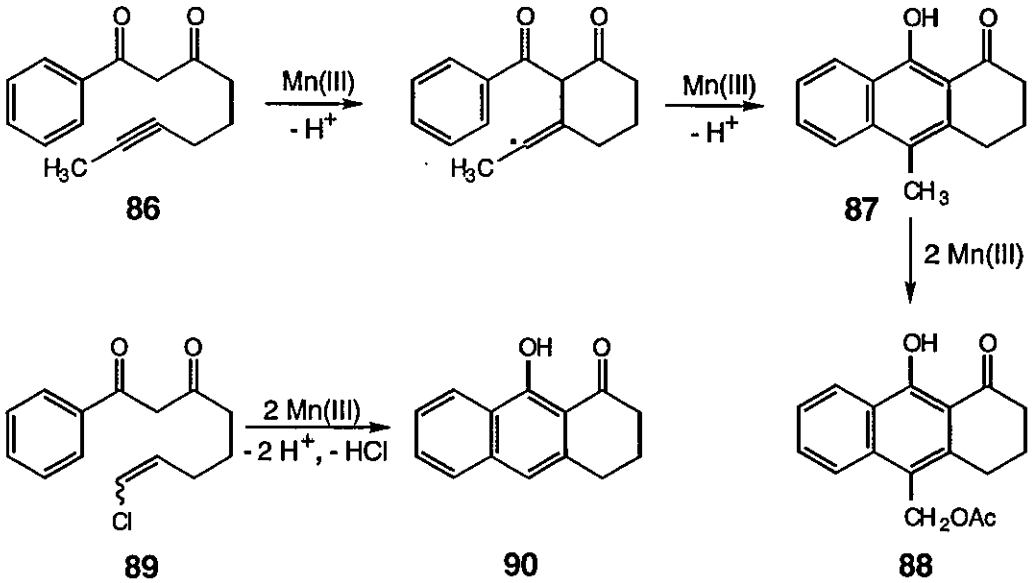
The oxidation of phenylalanine and 2-phenylglycine has also been shown to be effected by the reaction of vanadium(V) in ethanol, however, the reaction also yielded other products, such as the ethyl benzoate.[79] Oxidation of phenylalanine to phenylpyruvic acid via the α-amino radical has also been afforded through Fremy's salt oxidation. The reaction was reported to be pH dependent and only occurred above pH 9.[80]

These examples show how various salts can be used in the conversion of amino acids to α-keto acids, although there do not appear to be reports in the literature concerning the use of manganese salts for this conversion. However, manganese(II) and (III) mediated reactions are well known and have been used to afford the inter and intramolecular cyclisations of unsaturated systems *via* the α-ester radicals.[81] An example of the use of manganese(II) diacetate and manganese(III) triacetate is illustrated in the reaction between alkenes and 1,3-diketones or β-ketoesters to form either the dihydrofuran **84** or the cyclic peroxide **85, scheme 58**.[82]



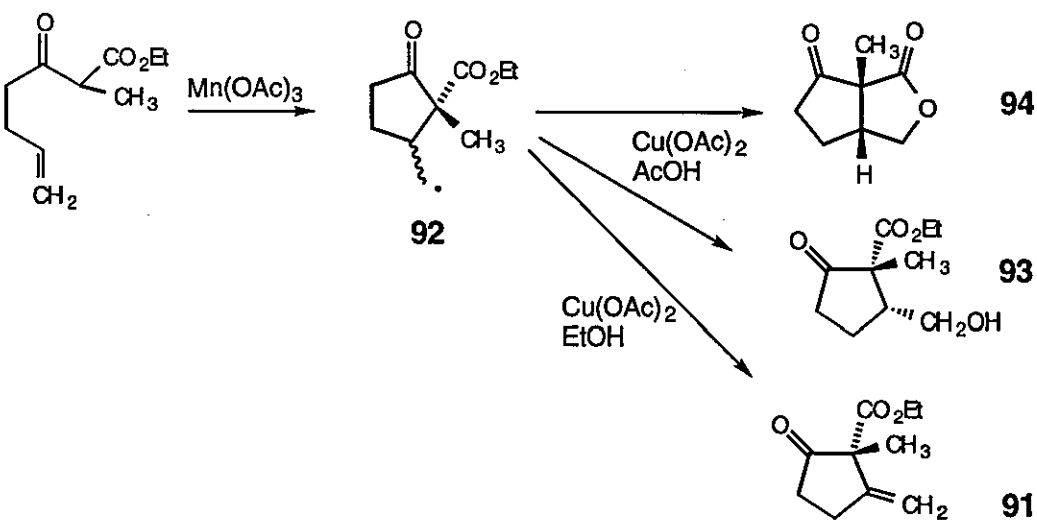
Scheme 58

Snider *et al*[83] have carried out extensive research into the use of Mn(III) triacetate to mediate radical reactions and have reported mono, tandem and triple cyclisations. They established that the radical formed from 1,3-dicarbonyl compounds is manganese free. They carried out the synthesis of dihydroanthracenes using the terminal alkyne **86**. When **86** was used the cyclised compound **87** is further oxidised to a quinone methide which reacts with the acetic acid to give **88**. However the chloroalkene **89** gave the desired compound **90, scheme 59**.



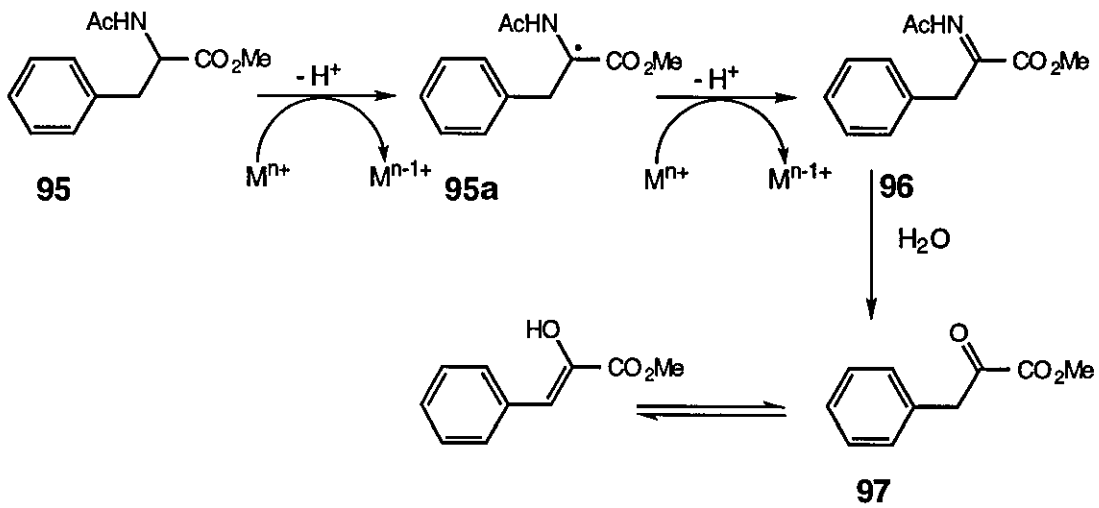
Scheme 59

In another study[84] the same research group show that the Mn(III) and Cu(II) induced free radical cyclisations which had previously been carried out in acetic acid were also successfully carried out in ethanol. In fact some cyclisations would only occur when ethanol was used as the solvent, and in some cases the solvent used was found to affect the regioselectivity of the reaction as shown in **scheme 60.** In ethanol the methylene cyclopentane **91** is almost exclusively derived from the oxidation of the radical **92.** However, in acetic acid the major reaction product is **93** with the production of a small amount of **94.**



Scheme 60

The manganese(II) oxidations require an enolisable hydrogen as found in β-lactones. We considered that the α-hydrogen in ethyl *N*-acetylDIT may be enolisable enough to allow manganese(II) oxidation to take place. Therefore we commenced our investigations using *N*-acetylphenylalanine methyl ester (**95**) as a model compound in order to avoid the complications of possible phenolic oxidation and subsequent coupling. The possible mechanism which we proposed for our conversion of the amino acid derivative to an α-keto acid derivative is shown in **scheme 61**. The initial oxidation and loss of a proton yields the captodative radical **95a**, which on further oxidation and loss of a second proton gives rise to the imine **96** which is then hydrolysed to the α-keto ester **97**.



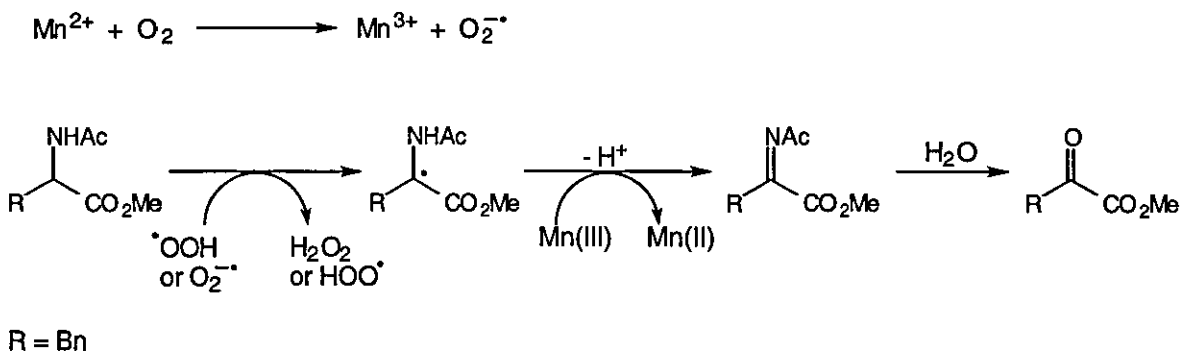
Scheme 61

Our initial attempt to effect this conversion involved the reported oxidation of amino acids using potassium ferricyanide (2 equivalents) in sodium hydroxide solution and dichloromethane.[78a] This reaction was unsuccessful, possibly due to the poor solubility of the protected amino acid in the aqueous solution.

We moved on to the investigation of manganese(III) oxidation and carried out reactions using manganese(III) diacetate (2 equivalents) in acetic acid, and in methanol, under oxygen and nitrogen atmospheres, at room temperature and at reflux, and in the presence and absence of sodium hydroxide (3 equivalents). Unfortunately these reactions were all unsuccessful giving a quantitative recovery of the amido ester. The reactions at reflux (with oxygen and nitrogen atmospheres, in acetic acid and methanol, and in the presence and absence of sodium hydroxide) were repeated using manganese(III) triacetylacetonate and again only gave quantitative recovery of the starting material. The lack of reaction in these studies,

and evidence from the work reported by Snider *et al*,[83,84] indicates that the manganese(III) oxidations have the requirement of a carbonyl group with a readily enolised hydrogen and that the proton at the α-centre of the *N*-acetylphenylalanine methyl ester is not acidic enough. However, the resulting captodative radical would be stable and favoured.

The oxidation reactions using manganese(II) and oxygen were then investigated. We postulated that the reaction could occur through the oxidation of manganese(II) to manganese(III) *via* the reduction of oxygen to superoxide (or peroxide in aqueous solution), which could then react with the amido ester giving the desired radical and either a peroxide radical or hydrogen peroxide. The second oxidation being afforded by the reduction of manganese(III) is shown in **scheme 62**.



Scheme 62

Reactions were carried out with manganese(II) diacetate dihydrate and with manganese(II) sulfate and with oxygen bubbling through the reaction mixture. The reactions were attempted in methanol and in acetic acid at reflux. These reactions were again unsuccessful and gave a quantitative recovery of the starting material.

The reason for the failure of these reactions to produce the desired oxidised compounds was unclear, although it was possible that the oxidation of the side chain required more energetic conditions than we had used. The synthesis of *N*-acetylthyroxine ethyl ester (**46**) is carried out at 60-70 °C and 5 bar pressure and this may provide the necessary energy for the oxidation of the side chain to occur. For this reason reactions were carried out in a pressure reactor reproducing these energetic conditions. Initially *N*-acetylphenylalanine ethyl ester (**98**) was used in ethanol solution with manganese(II) sulfate (0.5 equivalent) and the reaction was pressurised to 6 bar with oxygen and the temperature taken up to 62 °C. The reaction gave a quantitative recovery of starting material, so it was repeated using *N*-

acetyltyrosine ethyl ester (**81**) in ethanol, and with *N*-acetyltyrosine ethyl ester (**81**) in ethanol, borate buffer and sodium hydroxide at pH 9. Neither of these reactions produced the desired α-ketoester. Interestingly, they did not produce any coupled compounds either, though this may have been due to the phenol not being deprotonated in the reaction. This observation suggests that one of the purposes of the iodines in the reaction of ethyl *N*-acetylDIT (**47**) to produce *N*-acetylthyroxine ethyl ester (**46**) is to reduce the pK_a of the phenol, allowing deprotonation and hence the reaction to occur at a lower pH.

These results indicate that the oxidation of amido ester cannot be achieved by the use of manganese(III) or manganese(II) salts or complexes under a variety of conditions. The fact that the reactions using manganese(II) sulfate under pressure did not yield the desired α-ketoester indicates that perhaps this compound is possibly not an intermediate in the synthesis of *N*-acetylthyroxine ethyl ester (**46**) from *N*-acetyl-3,5-diiodotyrosine ethyl ester (**47**). This possibility, and the attempt to discover an alternative intermediate was further investigated and discussed in the next section. It is appreciated that a more extensive study could possibly find a protocol for oxidation under these conditions, but because time was limited, this avenue of research was abandoned.

4.4 Coupling of the side chain oxidised *ortho,ortho*-diiodinated phenols with *N*-acetyl-3,5-diiodotyrosine ethyl ester

The failure of the amido ester oxidation attempts to produce any of the desired α-ketoester suggested that this compound may not be involved in our synthesis of *N*-acetylthyroxine ethyl ester (**46**) and that perhaps an alternative intermediate may be formed during the high pressure synthesis of *N*-acetylthyroxine ethyl ester (**46**). For this reason we studied the coupling of other side chain oxidised intermediates with *N*-acetyl-3,5-diiodotyrosine ethyl ester (**47**) both at atmospheric temperature and pressure, and at 60-70 °C and 5 bar pressure.

The syntheses of the various compounds (**40**, **69**, **70**, **83b**) used for this study were discussed in section 4.2 and are shown in **scheme 56**.[43] The 3,5-diiodo-4-hydroxybenzoic acid (**43**) was commercially available and used as purchased.

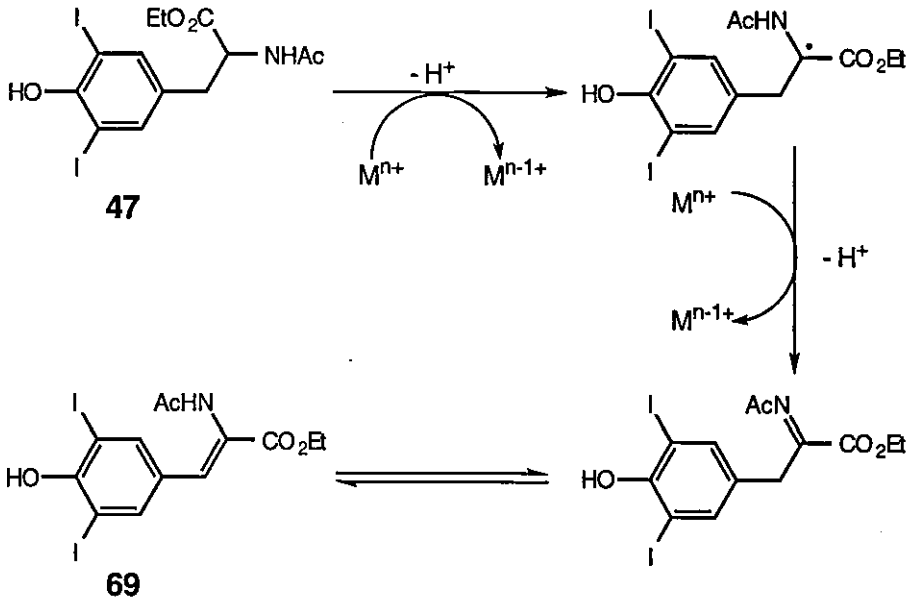
Since it has been found possible to synthesise *N*-acetylthyroxine ethyl ester (**46**) by reacting ethyl *N*-acetylDIT (**47**) with DIHPPA (**40**) at atmospheric temperature and pressure, whereas the reaction of ethyl *N*-acetylDIT (**47**) alone requires temperature

and pressure if the thyroxine derivative **46** is to be obtained over a similar time period, it was thought appropriate to investigate the coupling of other side chain conjugated 2,6-diiodophenols to establish whether the side chain conjugation was the element of DIHPPA (**40**) which increased its reactive capacity for the coupling to form the thyroxine derivative **46**. This investigation also provided an opportunity to investigate whether the use of pressure in the commercial synthesis could be eliminated by the use of 'already oxidised' derivatives with ethyl *N*-acetylDIT (**47**). It was also possible that this investigation may have allowed us to deduce the nature of the side chain required in order for coupling to occur, and hence propose a mechanism for its elimination. We therefore used 3,5-diiodo-4-hydroxybenzoic acid (**43**), 3,5-diiodo-4-hydroxybenzaldehyde (**70**), ethyl 2-acetamido-3-(3,5-diiodo-4-hydroxyphenyl)propenoate(**69**), 4-(4-acetoxy-3,5-diiodobenzylidene)-2-methyl-5-oxazolone (**83b**) and DIHPPA (**40**).

The oxazolone derivative **83b** was also a readily available intermediate for the synthesis of DIHPPA (**40**) and the dehydro amino acid **69**. It was used to provide evidence that the reaction required an unprotected phenol in order for the oxidation of the phenol to occur. Attempts were made to hydrolyse the phenol acetate group, but the hydrolysis of the oxazolone was found to occur prior to the hydrolysis of the acetate. When the oxazolone derivative **83b** was reacted under coupling conditions it was found that the oxazolone hydrolysed to give the *O,N*-diacetyl-3,5-diiodotyrosine ethyl ester and the *O,N*-diacetyl-3,5-diiodotyrosine. This indicated that if the phenol was deprotected the resulting oxazolone derivative may react in the same manner as **69** during the coupling reaction. For this reason no further attempts were made to synthesise the oxazolone derivative with an unprotected phenol group.

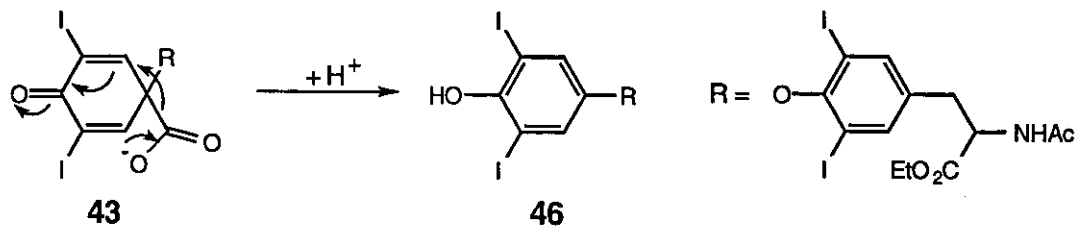
The reaction between DIHPPA (**40**) and ethyl *N*-acetylDIT (**47**) under pressure was investigated in order to compare this reaction with the reaction of ethyl *N*-acetylDIT (**47**) alone, the result from which may allow us to eliminate the DIHPPA (**40**) as a possible intermediate in the synthesis of *N*-acetylthyroxine ethyl ester (**46**).

The ethyl 2-acetamido-3-(3,5-diiodo-4-hydroxyphenyl)propenoate (**69**) was considered as a possible intermediate in the synthesis of *N*-acetylthyroxine ethyl ester (**46**), if the oxidation could take place forming the imine followed by a tautomerism shown in **scheme 63**, rather than hydrolysis to form the α-keto ester as shown in **scheme 62**.



Scheme 63

3,5-Diiodo-4-hydroxybenzaldehyde (**70**) and 3,5-diiodo-4-hydroxybenzoic acid (**43**) were also used in the investigation since they were available and it was possible that after phenolic coupling the elimination of the side chain could be achieved by decarboxylation, as shown in **scheme 64**.



Scheme 64

The reactions were carried out under the same conditions as the general synthesis of *N*-acetylthyroxine ethyl ester (**46**) from ethyl *N*-acetylDIT (**47**), i.e. with catalytic boric acid and manganese(II) sulfate, in ethanol and water, adjusted to pH 9.5 using sodium hydroxide solution, and pressurised to 5 bar with oxygen and at a temperature of 60-70 °C for 20 hours. The reactions carried out at high temperature and pressure were also carried out at atmospheric pressure and room temperature and were started and finished at the same time as the pressure reactions. The oxidised side chain derivative was added in excess (approximately 1.5 equivalents) to the ethyl *N*-acetylDIT. The results from the reactions are shown in **table 10**. The results of the atmospheric pressure reactions are only shown if product was obtained.

Therefore, when the reactions produced *N*-acetylthyroxine ethyl ester (**46**) successfully at high pressure and 60-70 °C but were unsuccessful at atmospheric pressure and room temperature it can be concluded that the *N*-acetylthyroxine ethyl ester (**46**) production required the energetic reaction conditions.

Reaction No.	Oxidised derivative	Temp. and Pressure	% Yield **46**
46	none	60-70 °C, 5 bar	23
47	**69**	69-70 °C, 5 bar	22
48	**83b**	20-70 °C, 5 bar	2
49	**43**	60-70 °C, 5 bar	18
50	**70**	60-70 °C, 5 bar	22
51	**40**	60-70 °C, 5 bar	11
52	**40**	atmos. T and P	7

Table 10

The yields of *N*-acetylthyroxine ethyl ester (**46**) include any *N*-acetylthyroxine since occasionally the ethyl ester was partially hydrolysed, and was calculated on the reaction of ethyl *N*-acetylDIT alone in a mole ratio of 2:1 to produce *N*-acetylthyroxine ethyl ester (**46**). In this way if an enhancement of yield was observed in the reaction of ethyl *N*-acetylDIT (**47**) with the oxidised compounds above the reaction of ethyl *N*-acetylDIT (**47**) alone, the reaction could be further investigated to establish whether the ethyl *N*-acetylDIT (**47**) was coupling with the oxidised derivative or with itself.

The yields were calculated from the HPLC analysis of the resulting reaction mixtures and calibrated against standard HPLC analysis of the pure compounds at known concentrations. The results obtained from the HPLC analyses were compared with the results obtained by calculating the yield from the integration of the signals resulting from the ^{1}H NMR spectroscopy of the reaction mixtures, and were found to give yields within 2 % difference. Since the reaction mixtures were recovered quantitatively, the yields of *N*-acetylthyroxine ethyl ester (**46**) are relative to the recovered ethyl *N*-acetylDIT (**47**).

Reaction 46 is that of ethyl *N*-acetylDIT (**47**) alone in the absence of other compounds and is therefore the standard reaction to which we can compare the affect of the use of the side chain oxidised derivatives. It can be seen that reactions 47, 49, and 50 gave similar yields of *N*-acetylthyroxine ethyl ester (**46**)to the yield in

the standard reaction. This suggests that the coupling was probably only occurring between two molecules of ethyl *N*-acetylDIT (**47**), and that the side chain oxidised compounds were not playing a part in the reaction. This was confirmed by the ratio of 3,5-diiodo-4-hydroxybenzaldehyde (**70**), or 3,5-diiodo-4-hydroxybenzoic acid (**43**) to ethyl *N*-acetylDIT (**47**) found in the reaction mixtures of reaction 49 and 50. In reaction 49 the ratio of 3,5-diiodo-4-hydroxybenzoic acid (**43**) to ethyl *N*-acetylDIT (**47**) prior to the reaction was 1.55:1 exactly, whereas the post reaction ratio was found to increase to 1.75:1 indicating that the ethyl *N*-acetylDIT (**47**) is used up in the reaction to produce *N*-acetylthyroxine ethyl ester (**46**) and that the benzoic acid derivative **43** is not taking part in the reaction. The same is seen to occur in reaction 50, although some of the benzaldehyde derivative is, as can be expected, oxidised to the benzoic acid derivative. The starting ratio of 3,5-diiodo-4-hydroxybenzaldehyde (**70**) to ethyl *N*-acetylDIT (**47**) was 2.2:1 exactly and the post reaction ratio of 3,5-diiodo-4-hydroxybenzaldehyde (**70**) to 3,5-diiodo-4-hydroxybenzoic acid (**43**) to ethyl *N*-acetylDIT (**47**) in this case was 3:0.5:1. In the reactions with the other oxidised derivatives **40**, **69**, and **83b**, a small amount of the compound survived, although several breakdown products were also recovered along with the ethyl *N*-acetylDIT (**47**) and *N*-acetylthyroxine ethyl ester (**46**). These breakdown products were the 3,5-diiodo-4-hydroxybenzaldehyde (**70**) and 3,5-diiodo-4-hydroxybenzoic acid (**43**).

Reaction 48 produces an unusually low yield of *N*-acetylthyroxine ethyl ester (**46**) which could have been due to the reaction losing heat overnight when the thermostat on the water bath developed a fault allowing the water jacket to cool. It would have been expected that the oxazolone derivative **83b** should not hinder the reaction since the oxazolone and acetate on the phenol are hydrolysed yielding the unsaturated amido ester **69**, and a small amount of the corresponding acid derivative, which did not show any hindrance to the *N*-acetylthyroxine ethyl ester (**46**) formation in reaction 47.

Only in the case of the reaction of ethyl *N*-acetylDIT (**47**) with DIHPPA (**40**) is the reaction, again, shown to produce *N*-acetylthyroxine ethyl ester (**46**) at room temperature (reaction 52) indicating that the DIHPPA (**40**) is coupling with the ethyl *N*-acetylDIT (**47**). In reaction 51 at higher temperature and pressure the DIHPPA (**40**) does not give an enhancement of yield as may be expected since the reaction will occur without the energetic conditions. In comparison, the reaction of ethyl *N*-acetylDIT (**47**) alone at room temperature and pressure will only produce a very small amount of *N*-acetylthyroxine ethyl ester (**46**) over about 10 days, whereas under the energetic conditions used in this study around 20 % of the product is

produced in 20 hours. It is clear that the DIHPPA (**40**) is unstable at high pH, under high pressure and at high temperature. The atmospheric pressure and room temperature, and energetic conditions all gave rise to breakdown products as described above. However, the reason for reaction 51 being so low yielding remains unclear, since the reaction of ethyl *N*-acetylDIT (**47**) in the formation of the product (**46**) should not be blocked.

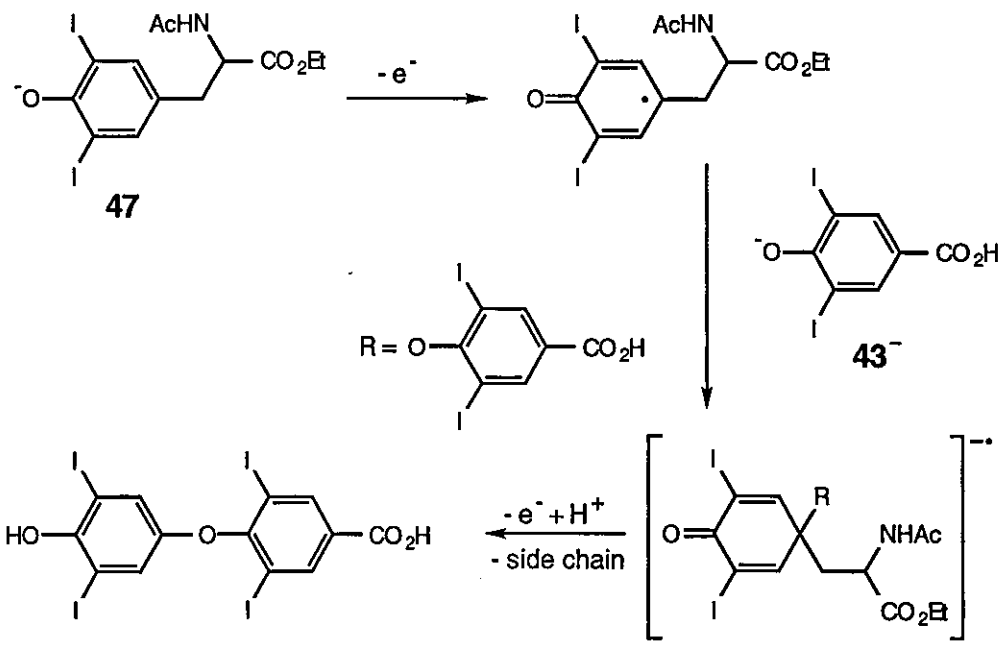
4.5 Conclusions

From the reactions of DIHPPA (**40**) with ethyl *N*-acetylDIT (**47**) at atmospheric pressure in buffered solution described in section 4.2, it appeared that DIHPPA (**40**) was a plausible intermediate for the synthesis of *N*-acetylthyroxine ethyl ester (**46**) under investigation. The reaction of ethyl *N*-acetylDIT (**47**) under the same conditions gave only quantitative recovery of the starting material, thus providing evidence that the DIHPPA (**40**) was taking part in the coupling reaction rather than merely playing the rôle of a catalyst.

Attempts to oxidise the protected phenylalanine derivative **95** and **98**, initially at atmospheric temperature and pressure, then at higher temperatures and pressure, did not produce the phenylpyruvic acid or its methyl ester derivative (**97**). The reactions were repeated with the protected tyrosine (**81**) but again no DIHPPA (**40**) or its ethyl ester was yielded. Thus the possibility that this oxidation may not occur in the process had to be considered. The elimination of the oxidation of the amido ester side chain as a step in the mechanism of the synthesis of *N*-acetylthyroxine ethyl ester (**46**), and hence the possibility that ethyl ester of DIHPPA is not an intermediate in the process, requires further investigation. This was carried out in the form of a study of the coupling of the side chain oxidised derivatives with ethyl *N*-acetylDIT (**47**).

The reactions of ethyl *N*-acetylDIT (**47**) in the presence of excess, **43**, **69**, **70** and **83b** showed no enhancement in the yield of *N*-acetylthyroxine ethyl ester (**46**) over the reaction of ethyl *N*-acetylDIT (**47**) alone. This suggests that the side chain oxidised derivatives are not actually taking part in the coupling, and instead in the case of **40**, **69**, and **83b** the HPLC analyses and ^{1}H NMR spectroscopy of the reaction mixtures clearly show the breakdown products of the DIHPPA (**40**) and the unsaturated amido ester (**69**). Some of the very minor peaks in the HPLC analyses and signals arising in the ^{1}H NMR spectroscopy of these reactions could be due to the reaction between the radical of ethyl *N*-acetylDIT (**47**) and the phenoxide ion of

the side chain oxidised derivative giving the corresponding thyroxine analogue as shown for the 3,5-diiodo-4-hydroxybenzoic acid in **scheme 65**. The product of this reaction has been reported by Matsuura *et al*[50] from the reaction of DIHPPA (**40**) with the 3,5-diiodo-4-hydroxybenzoic acid (**43**).



Scheme 65

The fact that the HPLC analyses and ^{1}H NMR spectroscopy of the reaction of ethyl *N*-acetylDIT (**47**) alone are clean showing only the starting material, *N*-acetylthyroxine ethyl ester (**46**), and occasionally a little *N*-acetylthyroxine or *N*-acetyl-3,5-diiodotyrosine, provides evidence that an oxidation step yielding a compound with a conjugated side chain does not occur during the course of the coupling reaction. If an oxidation did occur we would expect to see some of the breakdown products of the oxidised compound in the analytical data of the product mixtures. Also the fact that the DIHPPA (**40**) has been shown to be unstable under the reaction conditions suggests that the formation of this compound is unfavourable in these conditions. The instability of DIHPPA (**40**) to high pH conditions has been reported by various authors.[44,48b,85]

These reactions do show that although the DIHPPA may be a part of the biosynthetic intermolecular coupling process since it is stable at physiological pH, it is very unlikely that DIHPPA (**40**) is an intermediate in the alkaline, high pressure and high temperature process. These results also show that the addition of a 'ready

oxidised' derivative to the reaction would not offer a higher yielding alternative synthesis of *N*-acetylthyroxine ethyl ester (**46**).

From the results of the investigations in this chapter it appears that the inclusion of oxygen and manganese in the synthesis of *N*-acetylthyroxine ethyl ester (**46**) from ethyl *N*-acetylDIT (**47**) may only be necessary for the oxidation of the phenoxide ion to a phenoxyl radical.

The inclusion of the boric acid in the reaction had been thought to be for the stabilisation of the DIHPPA (**40**), however since it now appears unlikely that this compound is an intermediate in the reaction this can no longer be regarded as an explanation for the inclusion of the boric acid in the reaction mixture.

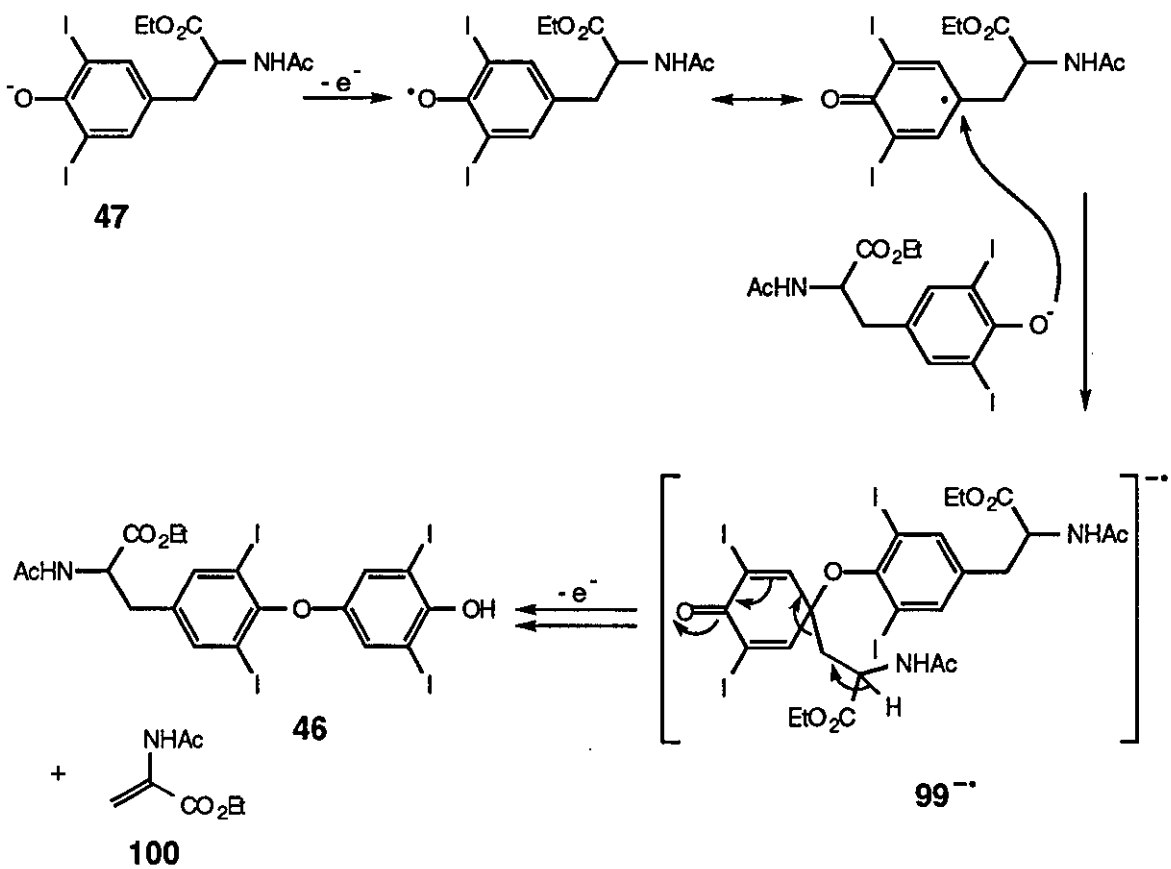
The actual necessity of the manganese(II) sulfate and boric acid in the reaction will therefore be investigated in the next chapter when the coupling of ethyl *N*-acetylDIT (**47**) alone is discussed.

Chapter Five

Investigation of the requirements, and mechanism, of coupling in the formation of thyroxine derivatives

5.1 Introduction

The results reported and discussed in chapter four indicate that the reaction in the formation of *N*-acetylthyroxine ethyl ester (**46**) from *N*-acetyl-3,5-diiodotyrosine ethyl ester (ethyl *N*-acetylDIT, **47**) in ethanol/sodium hydroxide system in the presence of catalytic quantities of boric acid and manganese(II) sulfate, and oxygen at 5 bar pressure at a temperature of 60-70 °C, does not involve an oxidation of the amido ester side chain as a part of the mechanism prior to the coupling. We therefore propose that the coupling mechanism may be similar to that outlined in the biosynthetic 'intramolecular' coupling theory despite the absence of the thyroglobulin molecule on which this biosynthetic coupling is believed to occur in the thyroid gland.[18-21] The proposed mechanism which will be discussed is shown in **scheme 66**. The literature precedent for this 'intramolecular' theory is outlined in chapter one.



Scheme 66

This chapter initially discusses the necessity for the various reaction conditions. The attempts to trap the coupled dienone intermediate and the eliminated side chain both

of which would provide evidence for a mechanism of elimination of the side chain are then discussed.

The results of the oxygen-*para* coupling of diiodinated phenols with varying unconjugated *para* substituents will then be reported and discussed, some of which we would not, from the postulated elimination mechanism shown in **scheme 66**, expect to allow side chain elimination.

5.2 Investigation of the requirements of the reaction in terms of the reaction conditions

The standard reaction conditions used in the synthesis of *N*-acetylthyroxine ethyl ester (**46**) from ethyl *N*-acetylDIT (**47**) are a solvent system of ethanol/sodium hydroxide solution (pH 9.5), catalytic quantities of boric acid and manganese(II) sulfate, oxygen at 5 bar pressure, and a temperature between 60-70 °C. In order to establish the necessity of these reaction 'ingredients' and their function in the reaction, a variety of reactions were carried out each in the absence of one of the reaction 'ingredients'. The results of these reactions are shown in **table 11**. The yields of *N*-acetylthyroxine ethyl ester (**46**) are relative to the recovered ethyl *N*-acetylDIT (**47**), the recovery of material being quantitative, and are calculated from the HPLC analyses of the reaction mixture and the integration of the signals arising from the ^1H NMR spectroscopy of the product mixtures.

Reaction No.	O_2/N_2	Pressure	Temp. °C	Other alterations	% Yield **46**
53	O_2	5 bar	60-70		19-24
54	O_2	atmos.	reflux		19-22
55	O_2	5 bar	r.t.		< 1
56	O_2	5 bar	60-70	no H_3BO_3	11
57	O_2	atmos.	reflux	no $MnSO_4$	10-16
58	N_2	5 bar	60-70		0
59	N_2	5 bar	60-70	MnO_2 instead of $MnSO_4$	0
60	N_2	5 bar	60-70	MnO_2 and $MnSO_4$	0

All reactions were carried out using *N*-acetyl-3,5-diiodotyrosine ethyl ester

Table 11

From the results of reactions 53, 54, and 55 it can be seen that by increasing the temperature without an increase in pressure the yield of *N*-acetylthyroxine ethyl ester (**46**) produced is unaffected (reactions 53 and 55). Whereas reaction at room temperature with an increase in pressure produces less than 1 % of the *N*-acetylthyroxine ethyl ester (**46**). We can therefore conclude that the pressure has little affect on the yield of the reaction.

Reaction 56, carried out in the absence of boric acid shows a reduced yield compared with reaction 53, and in the HPLC analyses and ^{1}H NMR spectrum of the product mixture, the presence of *N*-acetyl-3,5-diiodotyrosine was detected. When the reaction mixture was prepared it was observed that adjusting the pH to 9.5 by the addition of the sodium hydroxide solution was much more problematic in the absence of boric acid. In the absence of boric acid the reaction pH rose rapidly over 10, at which pH the hydrolysis of the ethyl ester occurs, and addition of a small amount of dilute hydrochloric acid to lower the pH was necessary. Although the coupling of *N*-acetyl-3,5-diiodotyrosine was not specifically studied, it was observed that whenever ester hydrolysis occurred in the case of the coupling of ethyl *N*-acetylDIT (**47**) the yield of *N*-acetylthyroxine ethyl ester (**46**) was reduced, indicating that the reaction is unsuccessful with the acid. From this experimental observation it may be concluded that the rôle of boric acid is to aid the stabilisation of the reaction at the optimum pH for the commencement of the reaction, i.e. acting as a buffer. The pK_a of boric acid which is 9.24[86] supports this theory.

The range of yields reported for reaction 54 and 57 may be compared to show the effect of manganese(II) sulfate on the yield of coupled products. It is clear on repetition of these reactions that in the presence of manganese(II) sulfate the yield of *N*-acetylthyroxine ethyl ester (**46**) is higher than the reaction in the absence of this salt and the difference cannot simply be attributed to experimental difference. The actual increase in yield for the reaction containing manganese(II) sulfate over a simultaneously run reaction in the absence of manganese(II) sulfate was ca. 50 %. It therefore appears that although the inclusion of manganese(II) sulfate in the reaction mixture is not crucial for the coupling reaction to occur, the manganese(III) hydroxide formed in the reaction solution, despite being a precipitate, does play a part in the oxidation of the phenoxide ions in the reaction solution.

Reaction 58, which was repeated several times, shows the necessity for oxygen in the reaction, but this contrasts the result of reaction 29 in chapter two, where *N*-acetylthyroxine ethyl ester (**46**) was seen to be produced (2 %) in the absence of oxygen. However, having already carried out reaction 29, the solutions for reactions

58, 59 and 60 were deoxygenated for longer and the pressure reactor flushed with nitrogen once before charging the solution and flushed with nitrogen five times after charging the vessel prior to pressurisation.

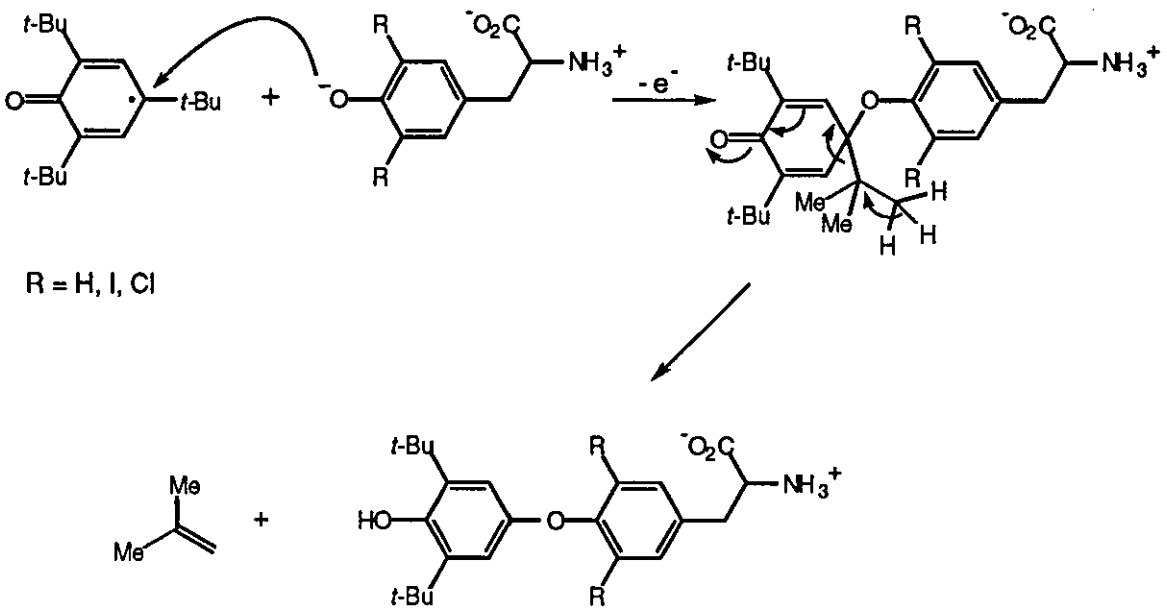
Reaction 59 was carried out to investigate whether manganese(IV), a stronger oxidant, than manganese(III) was capable of carrying out the oxidation of the phenoxide to the phenoxyl radical in the absence of oxygen. An alternative oxidant to replace oxygen in the synthetic procedure is desirable since the reduction of oxygen in the reaction produces superoxide and hence the formation of peroxyl radicals in the solution is likely, which at high concentrations is potentially explosive. The reaction using manganese(IV) was unsuccessful, therefore an attempt was made to utilise the oxidising power of manganese(IV) oxide in the oxidation of manganese(II) hydroxide to manganese(III) hydroxide. For this reason in reaction 60 equimolar quantities of the two manganese salts were used, but unfortunately the reaction was unsuccessful. If the reaction potential for both the oxidation of the phenoxide by manganese(IV) or the oxidation of manganese(II) hydroxide to manganese(III) hydroxide is calculated [the reduction potential of manganese(IV) oxide to manganese(II) is +0.984 V *versus* the saturated calomel electrode] the values for both reactions are positive indicating that the reaction should be spontaneous. A possible explanation for the lack of reaction may that the manganese(IV) oxide is insoluble in the reaction solution and therefore may not be able to afford the desired oxidations.

5.3 Study of the mechanism of the elimination of the side chain from the intermediate dienone

The mechanism shown in **scheme 66** shows the formation of the radical anion of intermediate (**99**) from phenoxide anion attack onto the phenoxyl radical of **47**, followed by the loss of an electron and the subsequent elimination of a dehydroalanine derivative (**100**) *via* an E2 type mechanism. The driving force for this elimination is the re-aromatisation of the phenol. It is possible that the dienone intermediate is afforded through the reaction of two radicals, although the concentration of the phenoxide ion of **47** in the reaction solution is high in comparison with the concentration of phenoxyl radical of **47**, and it is therefore more likely that the phenoxide ion, will be the attacking species.

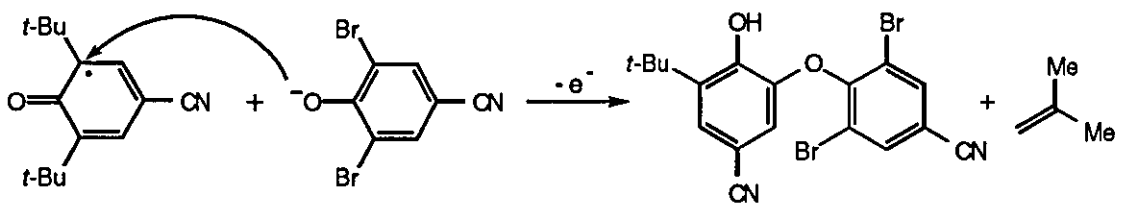
The E2 elimination mechanism has been used to explain the loss of the side chain from the aryloxydienone intermediate (e.g. **99**) in phenolic coupling by several

research groups. Firstly the loss of a dehydroalanine derivative in the formation of thyroxine, and secondly the loss of 2-methylpropene in the phenolic coupling of the radical prepared from the oxidation of 2,4,6-tri-*tert*-butylphenol in the presence of tyrosine derivatives as shown in **scheme 67**.[30,33,34]



Scheme 67

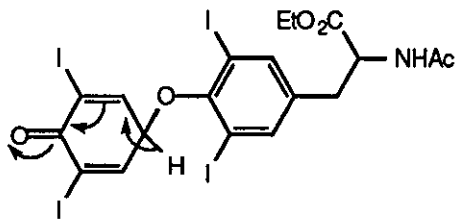
A similar elimination reaction has been reported in the reaction, shown in **scheme 68**, between the 2,6-di-*tert*-butyl-4-cyanophenoxyl radical and 2,6-bromo-4-cyanophenoxide ion to yield the the oxygen-*ortho* coupled product. The direction of coupling will be discussed later in the chapter.[87]



Scheme 68

For an E2 elimination mechanism to occur at a speed at which the aryloxydienone intermediate will not accumulate the reaction requires a good leaving group. Consideration of the acidity of the conjugated acid provides an assessment of

leaving group ability. The conjugate acid of the leaving group in the reaction of ethyl *N*-acetylDIT to form *N*-acetylthyroxine ethyl ester, **101**, is acidic due to the driving force of aromatisation and the effect of the electron withdrawing groups (aryloxy, iodine substituents and the α,β-unsaturated ketone). It is therefore reasonable to propose that this E2 mechanism may occur at a rate which will avoid the accumulation of the intermediate.



101

The E2 elimination mechanism requires that the acetamido ester α-hydrogen lines up at 180 ° to the aryloxydienone leaving group. The Chem3D model of this conformation is shown in **figure 5** and does not appear to be sterically hindered.

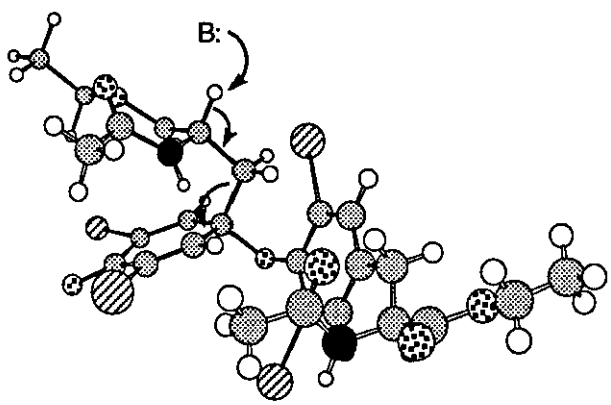
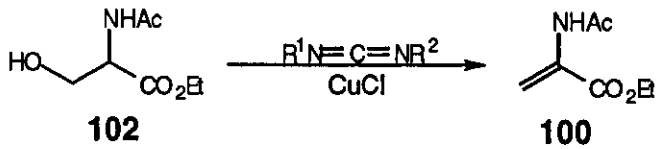


Figure 5

5.3.1 The detection of the eliminated side chain

Evidence to support the formation of thyroglobulin bound dehydroalanine residues, resulting from the thyroglobulin bound synthesis of thyroxine, has been reported, These studies have enabled the assignment of the location of some of the 'donor' tyrosines on the thyroglobulin chain.[39-41] However, there are no reports of the definite identification or the isolation of this compound from the non-thyroglobulin bound synthesis of thyroxine. Pitt-Rivers reported the detection of alanine in the reaction solutions, although attempts to isolate the compound were unsuccessful.[85]

In an attempt to collect evidence for the mechanism of elimination, occurring in the synthesis under investigation, ethyl 2-acetamidopropenoate (**100**) was synthesised by the dehydration of *N*-acetylserine ethyl ester (**102**) using a carbodiimide (shown in **scheme 69**).[88] The carbodiimide used was water soluble allowing easy separation of the resulting urea compound from the product (**100**). This compound was subsequently tested for its stability to the reaction conditions, and used as a standard in the HPLC analysis of the product mixtures from the coupling reactions. *N*-Acetylserine ethyl ester (**102**) from which the ethyl 2-acetamidopropenoate had been synthesised, was also used as an HPLC standard in the analysis of the product mixtures from the coupling reactions.



102 **100**

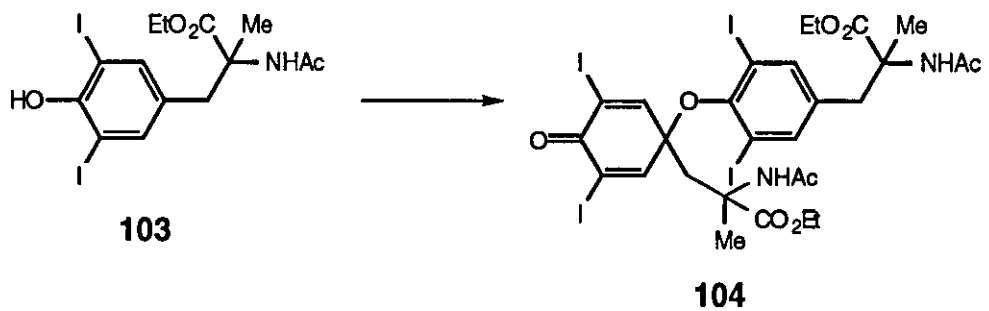
Scheme 69

Neither of the compounds were detected in any of the product mixtures from the coupling reactions by HPLC or ^{1}H NMR spectral analysis. When they were subjected to the conditions of the standard coupling reaction (i.e. ethanol/sodium hydroxide solution at pH 9.5, catalytic quantities of boric acid and manganese(II) sulfate, oxygen being bubbled through the reaction at reflux temperature ca. 65-72 °C) the *N*-acetylserine ethyl ester (**102**) survived the reaction 67 % of the material being recovered. However, only 5 % of the unsaturated compound **100** was recovered following its reaction under the same reaction conditions. The low recovery of ethyl 2-acetamidopropenoate (**100**) may be due to the presence of peroxide in the reaction medium which initiates the polymerisation of the compound. This polymerisation was reported by Marvin[88] during the syntheses of these unsaturated amino acids. The poor recovery of the ethyl 2-acetamidopropenoate (**100**) from the oxidative coupling reaction conditions, along with the fact that expected yield of the eliminated side chain would be in the region of 0.2 mmol (equimolar quantities to the thyroxine derivative), for a reaction commencing with 2 mmol of the ethyl N-acetylDIT (**47**) considering that the coupled product is only obtained yields in the region of 20 %, indicates that the detection of **100** as the eliminated side chain is likely to be difficult. The *N*-acetylserine ethyl ester (**102**), is very water soluble and may not have been isolatable in the work up of the coupling reactions. During the work up following the ethylation of *N*-acetylserine, difficulty was encountered during the extraction of the compound **102** from the aqueous phase into the organic phase. It was necessary to

extract the aqueous (a saturated brine solution) layer ten times with ethyl acetate and five times with dichloromethane to obtain a 70 % yield, despite the fact that TLC of the reaction mixture indicated that the reaction had gone to completion. It is therefore likely that during the work up following the coupling reactions any serine derivative present may not actually have been isolated. These observations suggest that neither of the two compounds should be discounted as possible products of the eliminated side chain. Further studies are required to isolate the eliminated side chain or an adduct thereof.

5.3.2 Attempts to trap the dienone ether intermediate

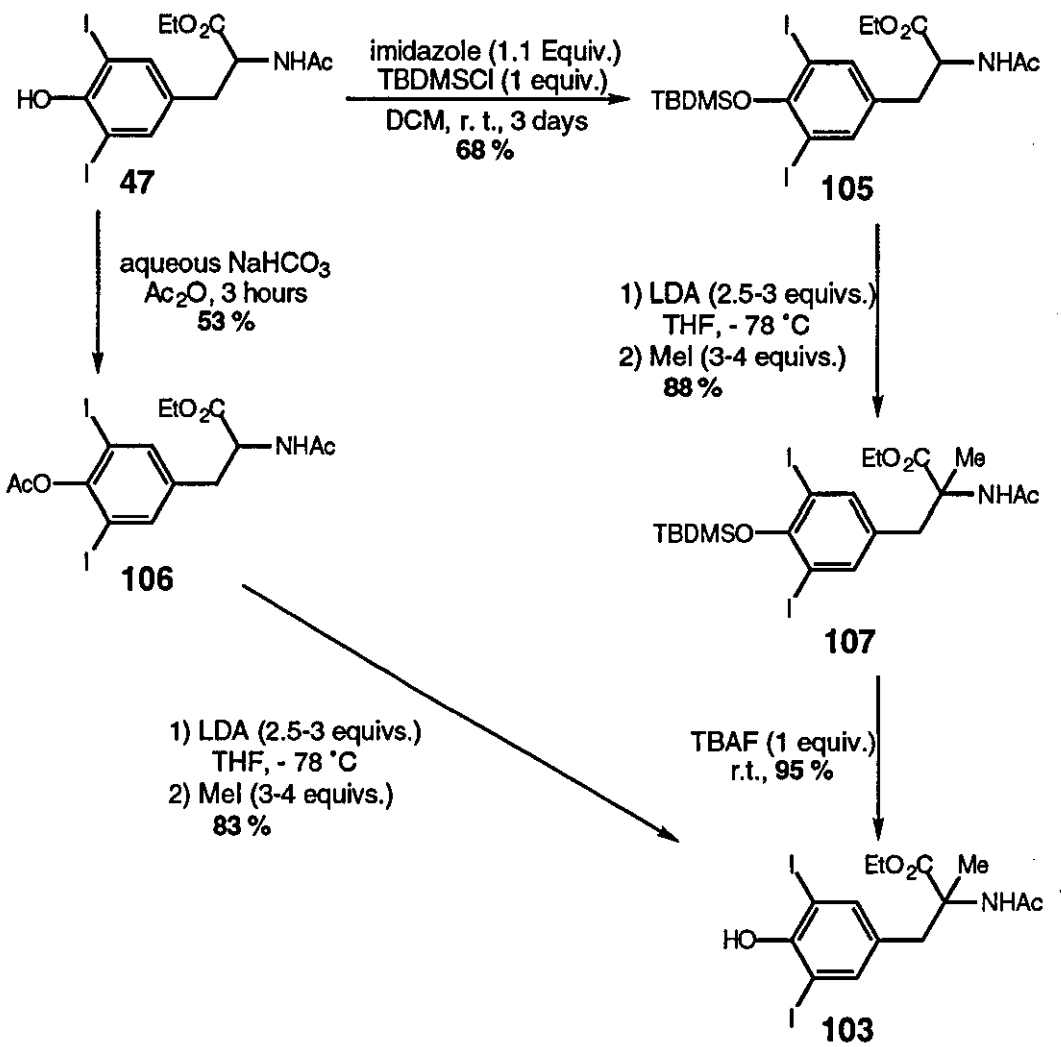
The attempts to isolate the eliminated side chain from the coupling of ethyl *N*-acetylDIT (**47**) having failed, alternative methods of obtaining information concerning the mechanism were considered. The E2 elimination mechanism proposed in **schemes 66** and **67** requires a hydrogen to be present on the carbon β to the dienone. We therefore postulated that by replacing the hydrogen with group which would not allow the E2 elimination, e.g. a methyl group, it should be possible to trap the aryloxydienone intermediate **104** as shown in **scheme 68**. If the aryloxydienone intermediate **104** were trapped then this would also indicate the lack of oxidation of the alanine side chain prior to coupling.



Scheme 68

The α-methyl derivative of ethyl *N*-acetylDIT [ethyl 2-acetamido-3-(3,5-diiodo-4-hydroxyphenyl)-2-methylpropionate **103**] was therefore synthesised in order to study its reaction to the coupling conditions, **scheme 69**. The synthesis of compound **103** first required the protection of the phenolic group using either *tert*-butyldimethylsilyl chloride (TBDMS chloride) to afford **105**, or acetic anhydride affording **106**. The fully protected compounds **105** and **106** were treated with 2.5-3 equivalents of lithium diisopropylamide (LDA) at -78 °C (made *in situ* from diisopropylamine and n-butyllithium in THF) to ensure the deprotonation of the amide followed by the deprotonation of the less acidic α-centre.[89] The formation of

the dianion was indicated by its deep red colour. The dianion was treated with methyl iodide (3-4 equivalents) to afford **103** in the case of the *O*-acetylated ethyl *N*-acetylDIT derivative **106** (the oxygen protecting acetate group was possibly hydrolysed during the work up), or the *O-tert*-butyldimethylsilyl protected compound **107** in the case of the TBDMS protected ethyl *N*-acetylDIT derivative **105**. The TBDMS protective group was removed by stirring compound **107** in a solution of tetra-butylammonium fluoride (1 equivalent) in THF for two hours, the syntheses are shown in **scheme 69**.

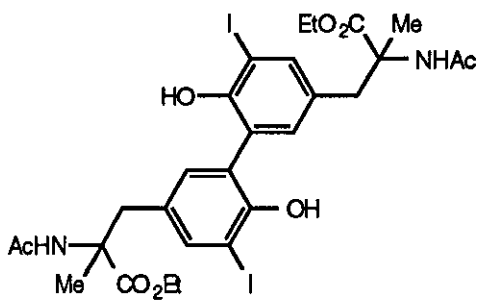


Scheme 69

The overall yield produced by using the three step synthesis with TBDMS protection was 56 %, whereas the two step synthesis using *O*-acetate protection gave a 44 % overall yield. However, the lower yielding reaction can be carried out much faster, since reaction of the bulky TBDMS chloride with the diiodinated phenol was slow due to the steric hindrance to the reaction caused by the two *ortho* iodines.

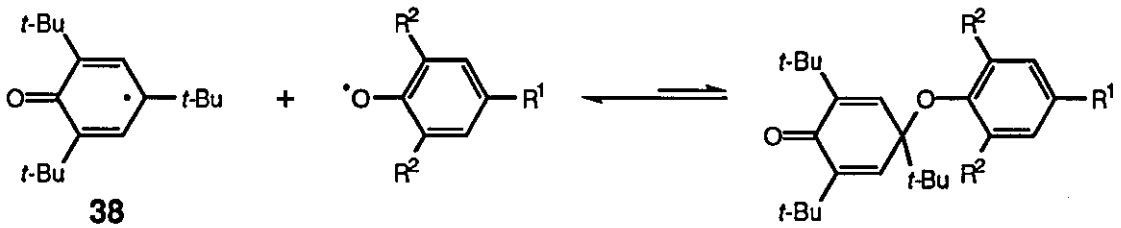
The α-methylated compound **103** was oxidised under the same reaction conditions as for the synthesis of *N*-acetylthyroxine ethyl ester (**46**) from ethyl *N*-acetylDIT (**47**) at atmospheric pressure. At the commencement of the reaction the pH was 9.5, and the final pH was measured to be 6.4.

Upon isolation and characterisation of the product it was found that *ortho-ortho* coupling had occurred to yield the dimeric compound **108**. On repetitions of the reaction keeping a careful check on the reaction pH, maintaining it above 8.5 by the addition of small amounts of triethylamine and hydroxide solution whenever necessary, the reaction gave only quantitative recovery of the starting material. Some reactions were carried out over 4 day periods, however, only starting material was recovered, unless the pH was allowed to drop, and again the *ortho-ortho* coupled dimer **108** was formed.



108

The absence of the aryloxydienone **104** in the reaction product could be due to the inability of the compound to eliminate its side chain. By comparison with the oxidation of ethyl *N*-acetylDIT under the same conditions, there is no reason why the formation of the dienone ether radical anion intermediate should not occur. It is possible that the coupling reaction is reversible, and the equilibrium may only be shifted towards the products by the ability of the intermediate aryloxydienone to eliminate its side chain affording the more stable, re-aromatised, thyroxine derivative. In the reaction of the α-methyl derivative **103**, the E2 elimination mechanism is not possible, and the starting phenoxide ion is likely to be thermodynamically more stable under the reaction conditions than the aryloxydienone. The dissociation of the aryloxydienone intermediate was also suggested by Cahnmann and Matsuura[33] when they carried out the coupling of the phenoxyl radical from 2,4,6-tri-*tert*-butylphenol (**38**) with 3-(4-hydroxyphenyl)propionic acid and 2-(4-hydroxyphenyl)acetic acid, and the corresponding *ortho,ortho* dibromo and diiodo derivatives, as shown in **scheme 70**.



$R^1 = (CH_2)_2CO_2H, CH_2CO_2H$
$R^2 = H, Br, I$

Scheme 70

In their study they found that when the *ortho* non-halogenated phenols were used the resulting aryloxydienone was stable and could be isolated. They also observed colour changes of the solutions during the coupling indicating that dissociation was taking place. The initial colour of the reaction solution was blue due to the 2,4,6-tri-*tert*-butylphenoxyl radical (**38**), the solution then turning yellow-green on formation of the aryloxydienone. On heating, the solution resumed its blue colour indicating the dissociation of the aryloxydienone into its starting radicals. The temperature at which this dissociation occurred decreased in going from H>Br>I. In fact, in the case of the iodinated phenols, the aryloxydienone intermediate could only be isolated at temperatures below 0 °C. They attributed this behaviour to the steric hindrance occurring between the *tert*-butyl group and the iodines or bromines causing a barrier to rotation, thereby decreasing the stability of the aryloxydienone. The equilibrium shown in **scheme 70** is as proposed by the authors, but the reaction is also likely to proceed *via* a phenoxyl-phenoxide coupling as proposed for the coupling of two molecules of ethyl *N*-acetylDIT in **scheme 66**.

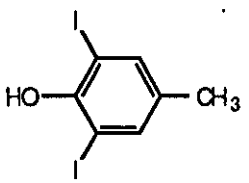
In our reaction the coupling of two ethyl *N*-acetylDIT molecules (**47**) will not occur at room temperature. The radical of **47** is not as stable as that of the 2,4,6-tri-*tert*-butylphenol which can be easily formed and reacted at lower temperatures. This indicates that in the formation of *N*-acetylthyroxine ethyl ester (**46**) from the coupling of two ethyl *N*-acetylDIT (**47**) molecules, the loss of the electron and elimination of the side chain from the aryloxyldienone radical anion must occur faster than dissociation, if the coupled product is to be formed, due to the high temperature instability of the intermediate. The instability of the aryloxydienone intermediate may be the explanation for the low yield of *N*-acetylthyroxine ethyl ester (**46**) from the reaction, i.e. at the high temperature required for the oxidation of the phenoxide ion to the phenoxyl radical, and possibly also for the elimination of the side chain, the aryloxydienone is unstable.

The reversibility of the aryloxydienone formation indicated in the report by Cahnmann and Matsuura[33] provides an explanation for the lack of aryloxydienone formation by the α-methylated compound **103**. Therefore although the aryloxydienone **104** was not isolated, and therefore its formation was not proven, this result does not detract from the proposed coupling and E2 elimination mechanism for the formation of the *N*-acetylthyroxine ethyl ester (**46**) from ethyl *N*-acetylDIT (**47**). Our results indicate that, in the reaction of ethyl *N*-acetylDIT derivatives, when the E2 elimination pathway is blocked an oxygen-*para* coupled compound cannot be isolated.

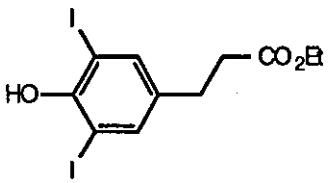
The reaction giving rise to the formation of the *ortho-ortho* coupled product when the pH dropped, gives further evidence of the affect of pH on the *ortho-ortho* coupling as was described in chapter two, i.e. the *ortho-ortho* coupling occurs when the reaction is carried out at a pH around the pK_a of the phenol. The reaction can therefore be eliminated by keeping the reaction pH over one pH unit above the pK_a of the phenol.

5.3.3 Study of the oxidative coupling of 2,6-diiodo-4-substituted phenols

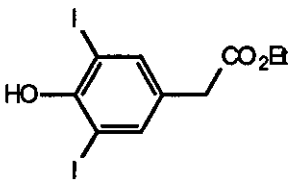
In order to gain further evidence for the mechanism the 2,6-diiodo-4-substituted phenols **55**, **65**, **66**, **67** and **68** were subjected to the reaction conditions in which ethyl *N*-acetylDIT (**47**) forms *N*-acetylthyroxine ethyl ester (**46**). We anticipated that these reactions may provide evidence for mechanism of elimination of the side chain from the aryloxydienone intermediate **99**, in the coupling of ethyl *N*-acetylDIT (**47**) to form *N*-acetylthyroxine ethyl ester (**46**), and for the possible dissociation of the aryloxydienone intermediate when the side chain cannot be eliminated *via* the E2 elimination mechanism.



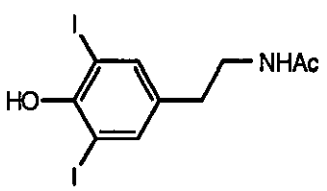
55



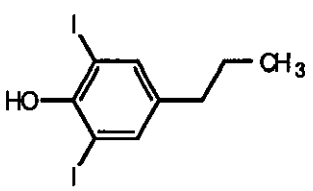
65



66



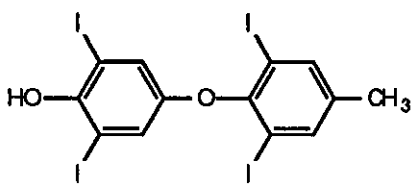
67



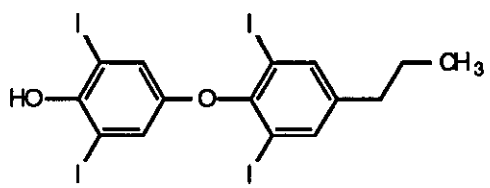
68

If the proposed mechanism shown in **scheme 66** is that which is involved in the *para*-oxygen coupling and that the formation of the aryloxydienone intermediate is reversible then we would expect to form thyroxine analogues from the ethyl propionate derivative **65** and the tyramine derivative **67**. The compounds **55** and **66** do not have a proton bearing carbon β to the aromatic ring and therefore the intermediates formed by these compounds cannot eliminate their 4-substituent through an E2 elimination mechanism. Therefore either recovery of the starting material, or isolation of some of the aryloxydienone was expected. In the case of the coupling of **68** it was expected that in the intermediate the proton on the carbon β to the carbon at which the ether bond is formed would not be acidic enough for the E2 elimination to occur.

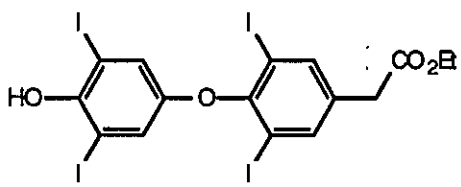
Compounds **55**, **66**, **67**, and **68** gave rise to the formation of thyroxine analogues. Their products and percentage isolated yields are shown below, though the reactions were not optimised due to shortage of time. In all cases the reactions were clean, showing only recovered starting material and thyroxine analogue. The results of the reactions were not as predicted and forced us to consider alternative mechanisms for the elimination of the side chain in the reaction of the compounds.



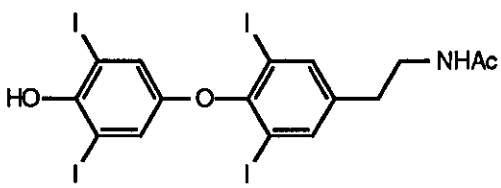
58 %
109



7 %
110



54 %
111

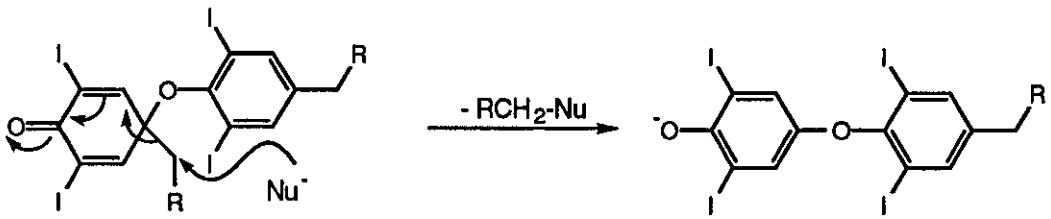


37 %
112

It should be possible for the coupling of the tyramine derivative **67** and 3,5-diiodo-4-propylphenol **68** to undergo reaction *via* the same E2 elimination mechanism as the ethyl *N*-acetylDIT (**47**), since they have the necessary proton on the carbon β to the phenolic ring. If this is the case, this indicates that re-aromatisation is a very strong driving force, since in the case of the propyl group a non-acidic proton, relative to the protons α to an ester or amide, is lost in order for the elimination to occur. (An

ester α to an acid will increase the pK_a of the acid by ca. 1.32 pK_a units; an amide α to an acid will increase the pK_a of the acid by ca. 0.94 pK_a units.[90])

Scheme 71 shows an alternative mechanism which could explain the elimination occurring in the formation of **109** and **111**, and which may also be taking place in the formation of all the thyroxine analogues. This involves the possibility of nucleophilic attack on the methylene carbon (which was previously benzylic) of the aryloxydienone intermediate, affording the elimination of the side chain. The most likely attacking nucleophile would be hydroxide, with the diiodinated aryloxydienone providing a good leaving for elimination.



R = H, CO_2Et, CH_2NHAc, $CH(NHAc)CO_2Et$, CH_2CH_3

Scheme 71

This mechanism was suggested by Cahnmann and Matsuura in their studies of the reaction of the incubation of 3-(3,5-diiodo-4-hydroxyphenyl)propionic acid and 2-(3,5-diiodo-4-hydroxphenyl)acetic acid.[30] They recovered 3-hydroxypropionic acid as the eliminated side chain from the reaction of the propionic acid derivative, and suggested that the hydroxide attack and elimination was concerted.

In the putative S_N2 mechanism the aryloxydiiododienone acts as a good leaving group for the same reasons as the proposed for the E2 elimination mechanism. S_N2 reactions on 'neopentyl' systems are extremely slow due to the quaternary centre blocking the approach of the nucleophile. The aryloxydienone intermediates have a quaternary centre at the carbon at which the ether bond has been formed, however this carbon bears the leaving group and is therefore at 180 ° to the incoming nucleophile causing minimal steric problems (**figure 6**).

If the S_N2 mechanism is the predominating mechanism in the elimination of the side chain to form thyroxine, then the presence of a methyl group on the α-carbon of the amino acid should not block the substitution.

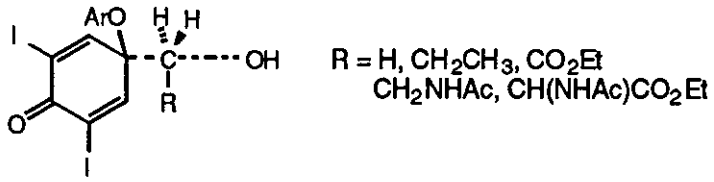
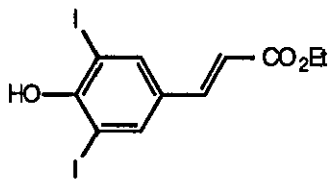


Figure 6

Models of the aryloxydienone intermediates indicate that the approach of the nucleophile is not sterically hindered in the case of the coupling of **55**, **66**, **67**, and **68**. In the case of the aryloxydienone intermediate of ethyl *N*-acetylDIT (**47**), the approach of the nucleophile may be slightly sterically hindered by the protected amino acid, and in this case an E2 elimination may be favoured over the possibly more hindered S_N2 reaction. However, the model of the aryloxydienone intermediate **104** shows that the steric hindrance caused by the three substituents α to the methylene undergoing S_N2 attack is similar to the affect of a neopentyl carbon in S_N2 reactions, e.g. the rate of reaction of ethoxide with 1-bromo-2-methylpropane is 3×10^{-2} sec^{-1} compared to 4.2×10^{-6} sec^{-1} for the reaction with 1-bromo-2,2-dimethylpropane.[91] As discussed ealier, the aryloxydienone intermediate is probably a very short lived species particularly at the high temperatures of our reaction. The formation of the coupled product is therefore dependent on a fast and favoured E2 and/or S_N2 elimination step. The S_N2 elimination for the aryloxydienone intermediate of the α-methyl derivative **104** is likely to be slower than the dissociation of the coupled intermediate to the more stable starting materials.

It is possible that in the case of **47**, **67**, and **68** both mechanisms are occurring simultaneously, although in the case of **68** it would appear that without an electron withdrawing group α to the proton which is lost in the E2 elimination, the S_N2 mechanism is likely to be more favourable for this derivative. However, in the studies of Cahnmann and Matsuura described earlier (**scheme 67**) a *tert*-butyl group was eliminated from the aryloxydienone intermediate arising from the coupling of the phenoxyl radical of 2,4,6-*tert*-butylphenol with tyrosine derivatives. In this case the E2 elimination, despite requiring the loss of a non-acidic proton, is possibly more likely than the nucleophilic attack to the tertiary carbon and has, in its favour, a strong driving force for re-aromatisation.

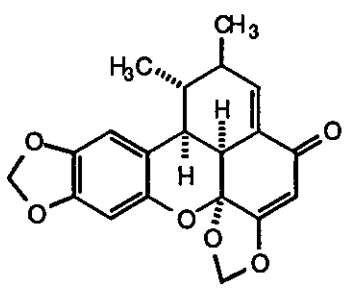
The reaction of ethyl 3-(3,5-diiodo-4-hydroxyphenyl)propionate (**65**) under the same oxygen-*para* coupling conditions gave a completely different product, ethyl 3-(3,5-diido-4-hydroxyphenyl)propenoate (**113**), in 53 % yield.



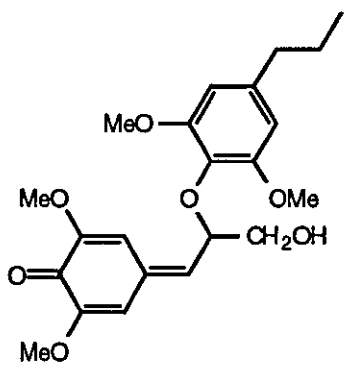
53 %
113

Matsuura and Cahmann[29] carried out the incubation of the propionic acid derivative at pH 7.5 and produced the corresponding thyroxine analogue. However, at this pH the carboxylic acid would be deprotonated as the carboxylate ion and the loss of the a proton from the α-centre would be unfavourable. The side chain elimination possibly going *via* the S_N2 mechanism.

Several alternative mechanisms have been considered to explain this anomaly. Oxidation to a quinone methide intermediate was the first considered. Quinone methides have been used as intermediates in the syntheses of natural compounds such as carpanone **114**[92] and are believed to be intermediates in the biosynthesis of lignin, e.g. **115**.[93]

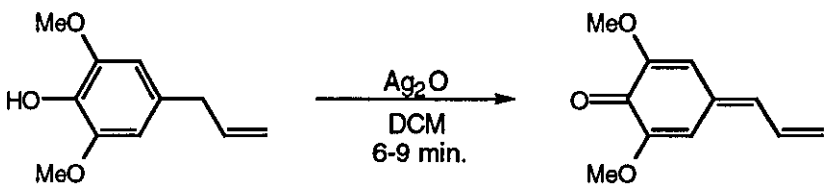


114



115

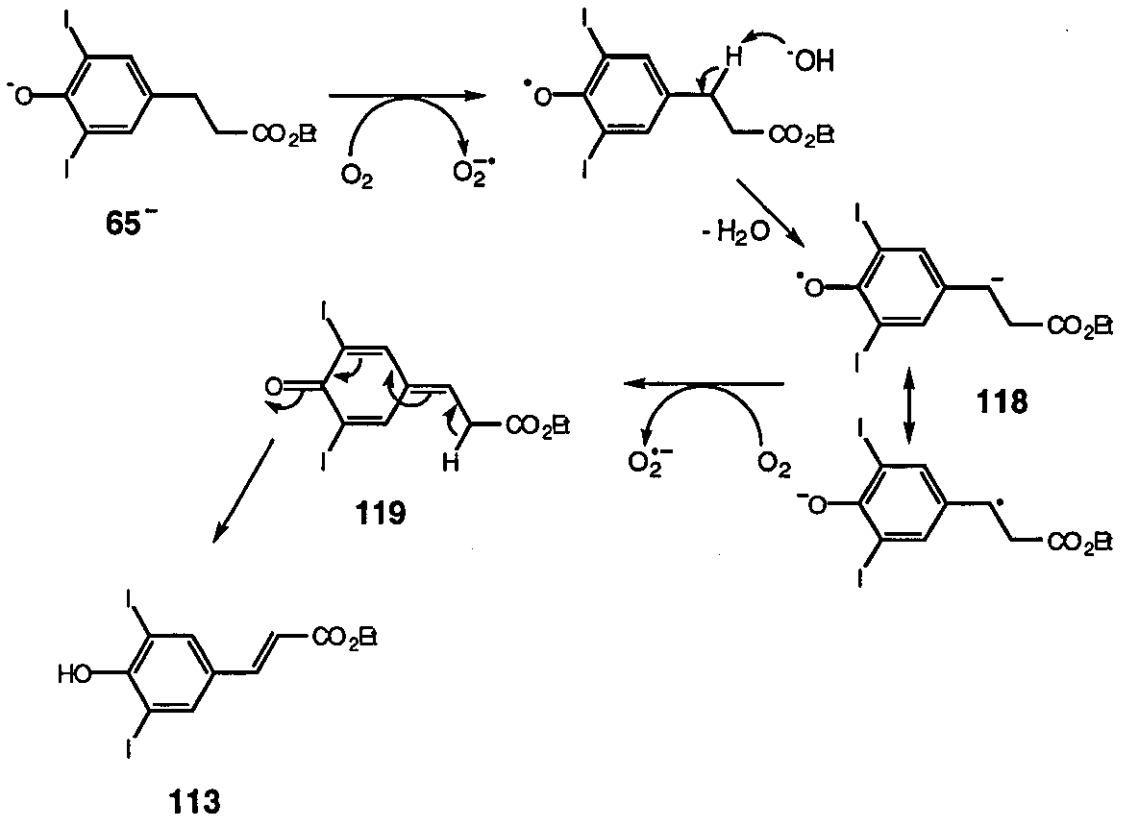
The formation of the vinyl quinone methide **116** from **117, scheme 72,** has been achieved through silver oxide oxidation. The vinyl quinone methide has been found to be stable to acid and alkaline solutions.[14]



117 **116**

Scheme 72

The possible mechanism for the formation of **113** through a quinone methide intermediate is shown in **scheme 73**. The initial oxidation occurs to yield the phenoxyl radical, followed by the loss of a hydrogen from the carbon β to the ester to form the radical anion **118** followed by the loss of an electron and the formation of the quinone methide **119**. The proton on the carbon α to the ester is then lost to effect re-aromatisation.

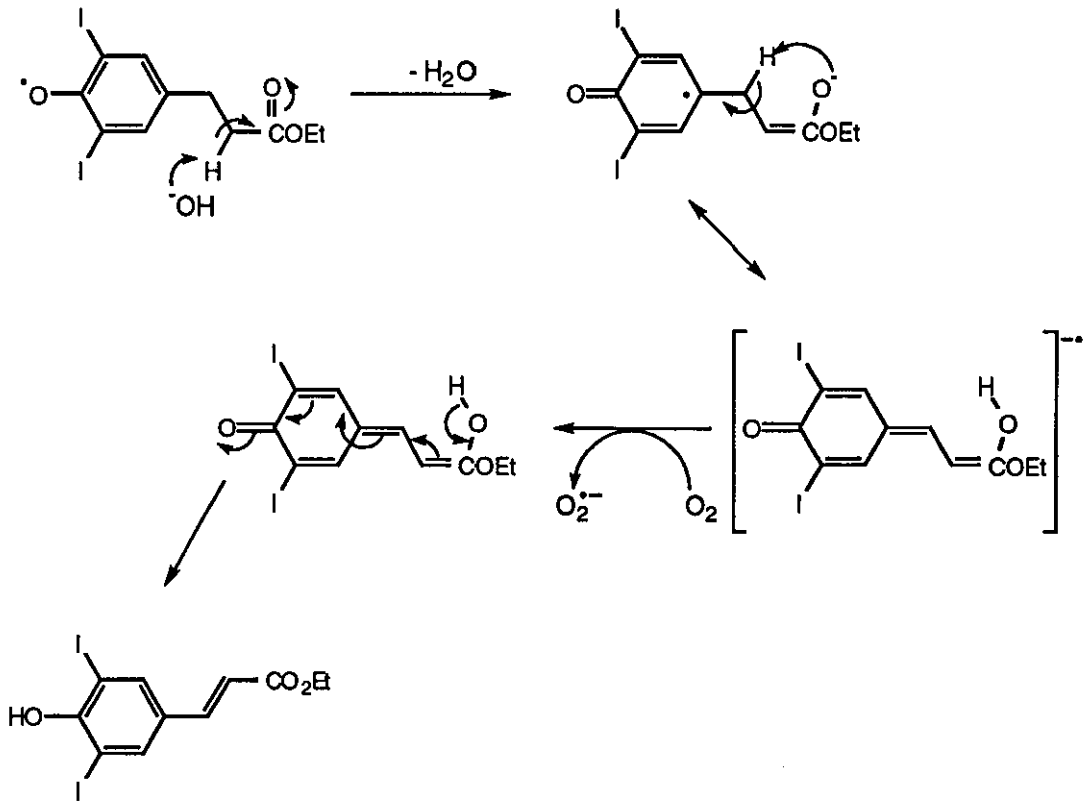


Scheme 73

It may appear unlikely that hydroxide is a strong enough base to remove the benzylic proton. However, on formation of the phenoxyl radical the acidity of these hydrogens will be enhanced because the acidity of radicals is considerably higher than the corresponding non-radical compound, e.g. $PhCH_2OH$, $pK_a = 15$ and $PhCH(\bullet)OH$, $pK_a = 10.5$ and hydroquinone, $pK_a = 9.9$, 4-hydroxyphenoxyl, $pK_a = 4.1$.[94]

If the relative acidity of the two sets of methylene protons of **65** in the intermediate phenoxyl radical are considered it is possible that the protons α to the ester may be the relatively more acidic. Therefore an alternative mechanism *via* enolisation of the ester may be considered. The enolate may effect a deprotonation at the benzylic

carbon followed by quinone methide formation and subsequent re-aromatisation of the phenolic ring yielding the conjugated compound as shown in **scheme 74**.



113

Scheme 74

An explanation must be sought for the lack of quinone methide formation by any of the other derivatives and in particular with the ethyl 2-(3,5-diiodo-4-hydroxyphenyl)ethanoate (**66**) which can be expected to have the most relatively acidic methylene hydrogens of the compounds reacted under oxidative coupling conditions, being benzylic and α to an ester. Isolation of the quinone methides formed is unlikely, since in the presence of hydroxide, superoxide and possibly peroxide, further oxidised derivatives may be expected, e.g. benzyl alcohol derivatives. If the quinone methide mechanism was operative, the same rearrangement would be expected for ethyl *N*-acetylDIT (**47**) we would expect to isolate some of the congugated side chain derivative i.e. **69**. However, these oxidised derivatives have not been detected in the reactions of the other diiodinated phenols the reactions yielding only the thyroxine analogue and recovered starting material. Therefore, it appears that the amide present in ethyl *N*-acetylDIT (**47**) has some affect on the reaction making the coupling reaction more favourable than the formation of the conjugated derivative **69**.

The apparent lack of quinone methide formation in the reaction mixture, under oxidative coupling conditions of **55**, **67**, and **68** could be explained by a faster rate of attack of the phenoxyl radical by the phenoxide ion than the loss of a proton to form the quinone methide shown in **schemes 73** and **74**. This may be explained by the absence of the electron-withdrawing ester group to enhance the acidity of the side chain protons (although the tyramine derivative **67** does have an amide group to enhance the acidity of the side chain protons). However, formation of a quinone methide in the reaction of the ethyl ethanoate derivative (**66**) would be expected to be fast based on the relative acidity of the benzylic protons. A possible explanation for the lack of quinone methide formation during the reaction of **66** and **103** under oxidative coupling conditions may be due to the allylic strain between the bulkier groups on the side chain and the protons of the aromatic ring, shown for the quinone methide of **66** in **figure 7**. In the case of **66** the quinone methide requires the compound to be planar, and this allylic strain may cause the formation of the quinone methide to be unfavourable.

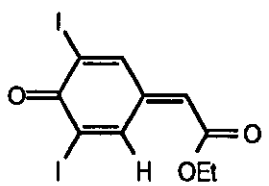
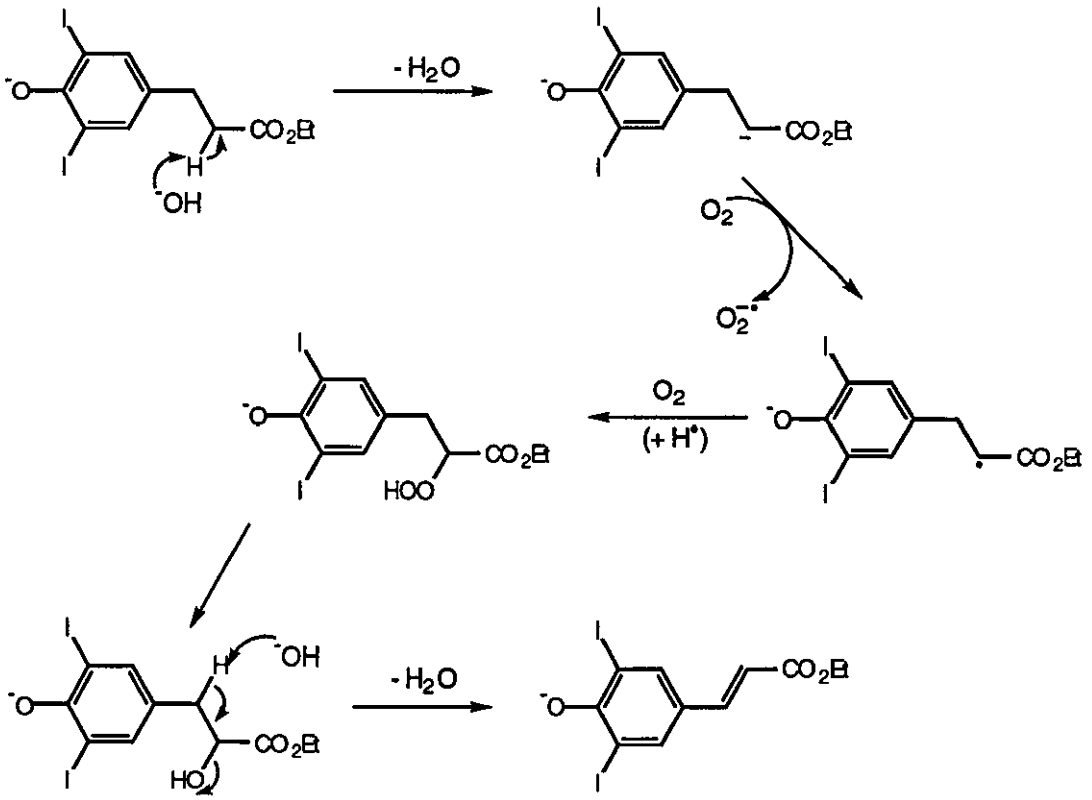


Figure 7

Another possible mechanism for the formation of **113** from the ethyl propionate derivative **65** is the *via* a hydroperoxide as shown in **scheme 75**.[95] This reaction would again require the deprotonation to be effected by hydroxide and is therefore questionable.

It would be incorrect to make any conclusions about the mechanism occurring in the formation of **113** from **65** without carrying out further research. It can therefore only be commented on that in the case of the synthesis of *N*-acetylthyroxine ethyl ester (**46**) from ethyl *N*-acetylDIT (**47**) the coupling reaction is faster than the reaction leading to double bond formation, and therefore that the amide group must play a part in this affect, since this is the only obvious difference between **65** and **47**.



Scheme 75

5.4 Summary of the results from the investigations of the coupling mechanism and elimination of the side chain moiety

In summary we propose that the phenoxide ion of ethyl *N*-acetylDIT (**47**) is oxidised to the phenoxyl radical through the reduction of oxygen or manganese(III), a process which requires energetic reaction conditions. The phenoxyl radical of ethyl *N*-acetylDIT (**47**) undergoes attack by the phenoxide ion of another molecule of ethyl *N*-acetyl DIT (**47**), faster than the loss of a proton and subsequent formation of a quinone methide. On formation of the aryloxydienone intermediate the side chain may be eliminated through an E2 and/or S_N2 elimination mechanism. The E2 elimination mechanism is possibly favoured due to the slight steric hindrance in the approach of the nucleophile in the S_N2 reaction around the α-acetamido ester. Both the E2 and S_N2 elimination mechanisms have a strong re-aromatisation driving force.

The α-methylated analogue of ethyl *N*-acetylDIT **103** was expected to react in the same manner as ethyl *N*-acetylDIT (**47**) and may undergo coupling to form the aryloxydienone intermediate faster than the loss of a benzylic proton in the

formation of the quinone methide. From our results though, it appears likely that the aryloxydienone intermediate is unstable to the high temperature of the reaction and therefore cannot be isolated. The reactions resulted only in the recovery of starting material, or if the pH dropped the *ortho-ortho* coupled derivative.

The tyramine derivative **67** produces the corresponding thyroxine analogue under oxidative coupling conditions. The reaction is likely occur through the same mechanism as ethyl *N*-acetylDIT (**47**). Elimination of the side chain of the aryloxydienone intermediate could occur through either an E2 elimination mechanism or a S_N2 mechanism in which the approach of the attacking hydroxide is less sterically hindered than in the case of ethyl *N*-acetylDIT (**47**).

The oxidative coupling of the 4-alkylated compounds **55** and **68** also give rise to thyroxine analogues. This indicates that the coupling reaction takes place faster than the mechanism occurring in the formation of the ethyl propenoate derivative **113** from the ethyl propionate derivative **65**. In the methyl derivative **55** only the proposed S_N2 mechanism may occur for loss of the side chain. In the case of the propyl derivative **68**, the propyl side chain of the aryloxyldienone may undergo S_N2 and/or E2 elimination.

The reaction of ethyl propionate derivative **65** under oxidative coupling conditions to form the ethyl propenoate derivative **113** indicates that either the benzylic protons or the protons α to the ester may be acidic enough to be removed faster than the coupling to form the aryloxydienone intermediate. The mechanism of this reaction is unclear and requires further investigation.

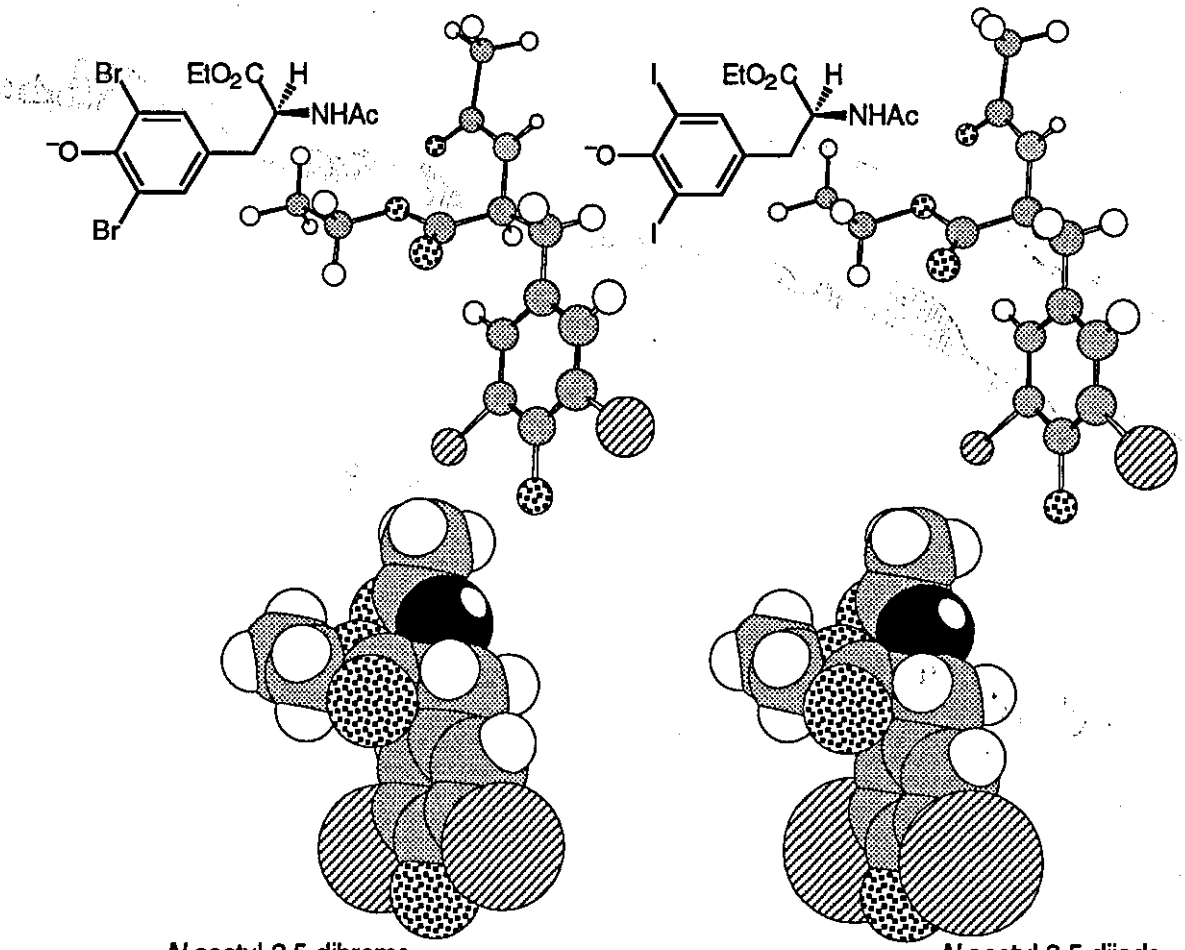
The formation of the thyroxine analogue of **66** under oxidative coupling conditions indicates that the coupling to form the aryloxydienone occurs faster than the loss of a benzylic proton. The aryloxydienone intermediate of **66** may undergo S_N2 elimination to yield the thyroxine analogue.

5.5 The direction of the phenolic coupling

The reactions producing only oxygen-*para* coupled compounds despite the many other possible products (as described in chapter one) shows the directing affect of the *ortho* iodines. Conversely the *ortho,ortho* dibrominated phenols have been reported to react under oxidative coupling conditions to produce predominantly oxygen-*ortho* coupled compounds due to the electron withdrawing effect of the

bromines stabilising the phenoxyl radical at the *ortho* carbon.[60,87] It is likely that in the coupling under study, the coupling occurs through the reaction of a phenoxide ion with a phenoxyl radical since there will be a higher concentration of the phenoxide ion than of the phenoxyl radical in the reaction solution.

The anion could either attack the radical on the oxygen-centre forming a peroxide, or on the *ortho* or *para* carbons. In the case of *ortho,ortho* dibrominated phenols oxygen-*ortho* coupling is often reported to predominate.[60,87] Since the mesomeric effect of the bromines increases the stability of the radical at the *ortho* position and we would expect the same to occur in the case of iodine.



N-acetyl-3,5-dibromo tyrosine ethyl ester
63

N-acetyl-3,5-diiodo tyrosine ethyl ester
47

Figure 8

However, the iodines are larger than the bromines and provide greater steric hindrance around the oxygen anion as indicated by the Chem3D model of the phenoxide ion of **47** compared with the dibrominated phenoxide ion **63**, **figure 8**. The models shown are elementary and serve only to illustrate the discussion.

The steric interactions of the iodines *ortho* to the oxygen-centred phenoxide anion with the groups on the phenoxyl radical during the approach for coupling, and the steric hindrance of the oxygen-centred phenoxide ion due to the *ortho* iodines, as indicated in **figure 8** may be the factors affecting the direction of the phenolic coupling. In the oxygen-*ortho* coupling of the *ortho,ortho* diiodophenols the phenoxyl radical is conjugated and planar until the oxygen-carbon bond starts to be formed, at which point the carbon will become sp^3 hybridised.

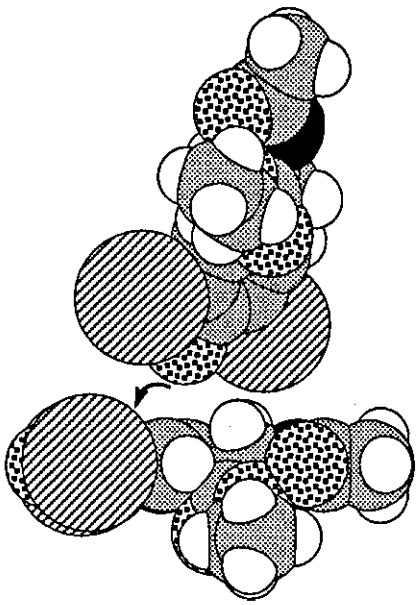


Figure 9a

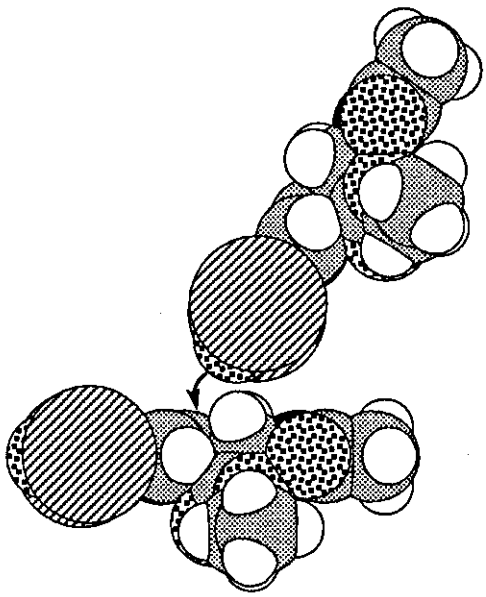


Figure 9b

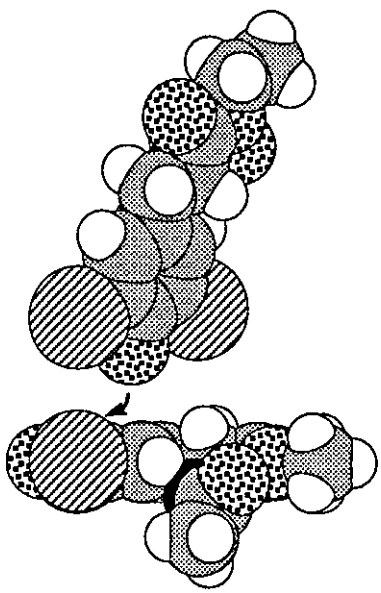


Figure 9c

The iodine of the phenoxyl radical can therefore be expected to cause steric hindrance to the approach of the nucleophile and the *ortho* iodines of the phenoxide

ion also cause steric hindrance, particularly between the iodine of the phenoxide ion and the oxygen of the phenoxyl radical, **figure 9a.**

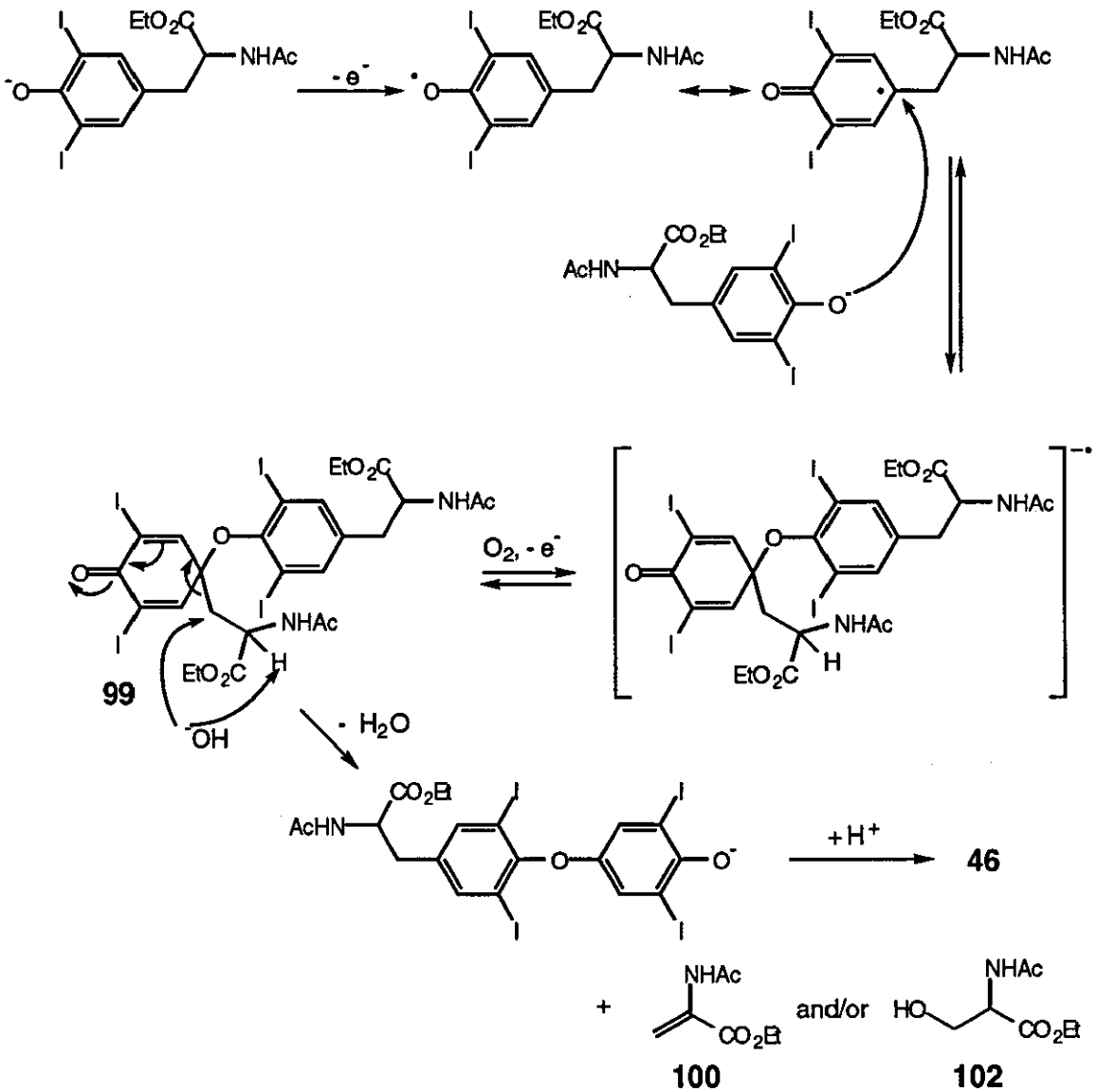
Coupling of *ortho,ortho* diiodinated phenols between the oxygen-centred phenoxide ion and the *para* carbon of the phenoxyl radical is shown in **figure 9b.** The oxygen of the approaching phenoxide ion may be sterically hindered by the *ortho* iodines, however, the *para* side chain of the phenoxyl radical should cause less steric hindrance than an iodine. Also the positions *ortho* to the point of coupling in the phenoxyl radical are unsubstituted, therefore less steric interaction is involved than in the case of the oxygen-*ortho* coupling where the iodine of the phenoxide ion and the oxygen of the phenoxyl radical would be in very close proximity. **Figure 9c** shows the oxygen-*ortho* coupling of *ortho,ortho* dibrominated phenols. As was mentioned earlier and shown in **figure 8**, the bromines on the phenoxide ion do not cause as much steric hindrance to the oxygen-centred phenoxide ion as in the case of the *ortho,ortho* diiodinated phenoxide ion. Also the bromines of the incoming phenoxide ion will not effect the same extent of steric interaction to the oxygen of the phenoxyl radical, *ortho* to the point of coupling, as would occur in the oxygen-*ortho* coupling of *ortho,ortho* diiodinated phenols. The bromines of the phenoxyl radical also provide less steric hindrance to the incoming nucleophile than in the case of the iodinated phenoxyl radical.

5.6 Conclusions

Our investigations of the coupling stage in the industrial synthesis of thyroxine have firstly given us valuable information concerning the mechanism of *ortho-ortho* coupling allowing the adaptation of the process to eliminate the possible formation of this unwanted byproduct; and secondly information concerning the mechanism of oxygen-*para* coupling of the ethyl *N*-acetylDIT molecules in the formation of *N*-acetylthyroxine ethyl ester.

The results obtained in chapter four suggest that, under the conditions used in our investigations, the reaction proceeds without the necessity of side chain oxidation prior to coupling. It therefore appears that oxygen and manganese are required in the oxidation of the phenoxide ion to the phenoxyl radical. The results and electrochemical data reported in chapter three indicate that these reactions require temperature and pressure.

Results from the investigations of the oxygen-*para* coupling suggest that the phenoxyl radical of ethyl *N*-acetylDIT (**47**) undergoes a reversible attack by the phenoxide ion of ethyl *N*-acetylDIT (**47**) to form the radical anion of the aryloxydienone intermediate (**99**) shown in **scheme 76**. This radical anion rapidly loses an electron to oxygen followed by the elimination of the side chain which must also occur rapidly to avoid the dissociation of the aryloxydienone intermediate. The elimination of the side chain may go *via* either an E2 or a S_N2 mechanism with the aryloxydienone intermediate having a strong driving force for re-aromatisation and acting as a good leaving group.



Scheme 76

The two reactions giving side chain elimination possibly occur simultaneously to produce either serine or acrylate derivatives. The serine derivative is very water soluble and was possibly not extracted after the reaction and therefore not observed

in the ^1H NMR spectra or HPLC analyses of the reaction mixtures, and the acrylate derivative is likely to undergo polymerisation under the reaction conditions.

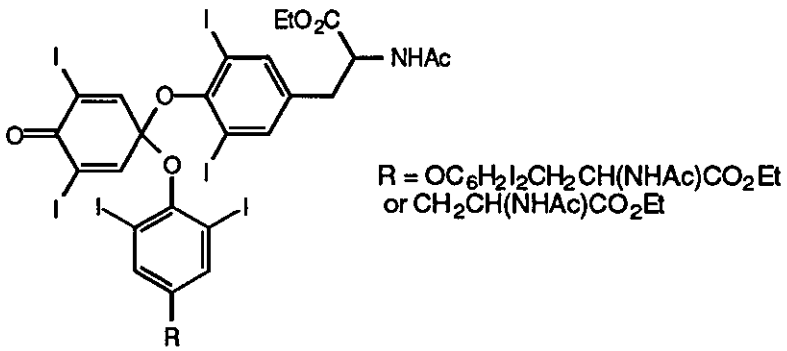
The purpose of the boric acid is to aid the buffering of the reaction. In the absence of boric acid the pH of the reaction mixture was less controlable and ester hydrolysis was observed. The oxidative coupling reaction using the *N*-acetylDIT appears to be less successful than when the acid is protected.

The steric affect of the iodines on the direction of the coupling was discussed in section 5.5. The reduction of the phenolic pK_a due to the *ortho* iodines, compared with the pK_a of tyrosine, allows the phenol to be deprotonated at physiological pH during the biosynthesis. It was demonstrated in chapter three that the oxidation of the phenoxide ion of phenol requires less energy than the oxidation of the protonated phenol group of phenol, and it is likely that this will be the case in the oxidation of DIT. The deprotonation of the DIT and thyroxine at physiological pH, due to the reduction of pK_a by the affect of the *ortho* iodines, makes these compound soluble at physiological pH, and being in the phenoxide form at physiological pH aids the oxidation of the DIT to the phenoxyl radical.

In chapter two the reaction of 2,6-diiodo-4-substituted phenols to form the corresponding dityrosine analogues was discussed and an S_N2 mechanism proposed for these reactions. It was shown that the formation of the dityrosine derivative in aqueous ethanol near the pH of the phenol is very slow and requires heat and pressure. At the lower pH range (5-7) in the presence of oxygen, the *ortho-ortho* coupling reaction will compete with the coupling reaction to form the thyroxine analogue. The formation of thyroxine analogues under these conditions, over a wide pH range, can be blocked by the efficient removal of oxygen from the reaction solution. In contrast, the formation of the dityrosine derivative **48** will occur irrespective of the atmosphere, although it can be blocked by buffering the reaction solution effectively at a pH above ca. 8.5. This can be difficult since the pH has a tendency to drop during the reaction, possibly due to the increase in proton concentration resulting from the E2 elimination of the side chain.

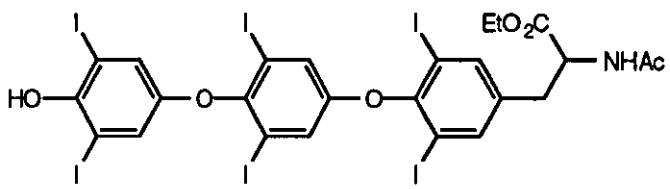
One argument used in the radical *versus* polar mechanism discussion for the formation of the dityrosine is that if the reaction occurs through a radical mechanism then we could expect to observe the formation of trimers etc.. We should therefore consider the further reaction of the thyroxine analogues. The results of the electrochemical studies show that the oxidation of *N*-acetylthyroxine ethyl ester (**46**) requires more energy than the oxidation of ethyl *N*-acetylDIT (**47**). The resulting

phenoxyl radical may react with a phenoxide ion of ethyl *N*-acetylDIT (**47**) or *N*-acetylthyroxine ethyl ester (**46**) with the formation of the intermediate **120** which will dissociate at one of the ether bonds since elimination and re-aromatisation cannot occur. Alternatively the phenoxide ion of *N*-acetylthyroxine ethyl ester (**46**) may attack the phenoxyl radical of ethyl *N*-acetylDIT (**47**), the side chain in this case can eliminate.



120

The reason that this trimer formation is not seen to occur in our synthesis could be due to the observed precipitation of the *N*-acetylthyroxine ethyl ester (**46**) during the reaction. The formation of the trimer **121**, the 'so-called' T_6 compound, has been observed during reactions at the Knoll Pharmaceutical laboratories.



121

The results of the oxygen-*para* coupling experiments indicate that the reaction is biomimetic, occurring through a mechanism similar to that postulated for the thyroglobulin bound reaction to form thyroxine. It has also been shown that the reaction conditions used in the synthesis of *N*-acetylthyroxine ethyl ester (**46**) may be useful synthetic protocol for the formation of thyroxine analogues of 2,6-diiodo-4-substituted phenols.

Chapter Six

Experimental

6.1 General Information

Commercially available solvents and reagents were used throughout without further purification, except for those outlined below which were purified as described. Light petroleum (refers to that fraction boiling between 40 °C and 60 °C) and ethyl acetate were distilled from calcium chloride; dichloromethane and toluene from phosphorus pentoxide; methanol and ethanol from magnesium and iodine.

Analytical thin layer chromatography was carried out using aluminium backed plates coated with Merck Kieselgel 60 GF_{254}. Plates were visualised under UV light (at 254 and/or 360 nm), or by staining with potassium permanganate dip followed by heating. Flash column chromatography was carried out using Merck Kieselgel 60 H silica. Pressure was applied at the column head with hand bellows. Samples were applied pre-absorbed on silica or as a saturated solution in an appropriate solvent. HPLC was carried out using a Pye Unicam PU4015 pump and Pye Unicam PU4025 UV detector used fitted with a Zorbax SB-C8 4.6 mm x 150 mm column.

Infra-red spectra were recorded using a Nicolet 205 FT-IR Spectrometer, with internal calibration. ^{1}H and ^{13}C NMR spectra were recorded using Bruker AC250 and/or DPX400 Spectrometer. Electron impact, chemical ionisation and fast atom bombardment mass spectra were recorded on a Kratos MS80 instrument or on a VG Analytical ZAB-E instrument. (SERC mass spectroscopy service, Swansea). Elemental analyses were carried out on a Perkin Elmer 2400 CHN Elemental Analyser.

Melting points were measured on an Electrothermal digital melting point apparatus and are uncorrected.

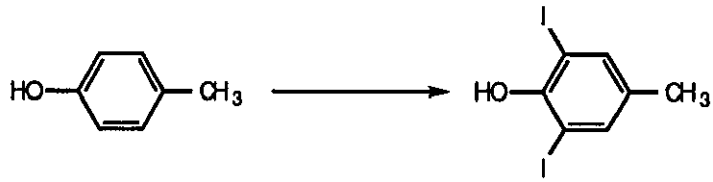
All of the following experimental reactions were carried out using dry glassware and under an atmosphere of nitrogen except where otherwise stated or in cases where it was obviously unnecessary. Reactions carried out at high pressures were carried out using a Buchi autoclave.

6.2 Experimental to Chapter Two

Iodination of phenols

General procedure

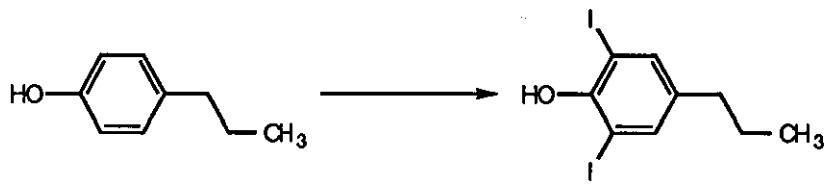
2,6-Diiodo-4-methylphenol **55**



55

p-Cresol (1.023 g, 9.25 mmol) was dissolved in dichloromethane (75 cm^3). A solution of potassium carbonate (1.412 g, 10.08 mmol) in water (100 cm^3) was added to give a two-phase reaction mixture. A solution of iodine (4.713 g, 18.55 mmol) and potassium iodide (12.521 g, 75.42 mmol) in water (75 cm^3) was added to the *p*-cresol mixture. The reaction was stirred for 14 h and acidified using hydrochloric acid solution (2 M). The product was extracted into dichloromethane (2 x 25 cm^3) and washed with dilute hydrochloric acid (0.5 M, 25 cm^3) and sodium thiosulfate solution (0.2 M, 25 cm^3). The organic extracts were dried ($MgSO_4$) and evaporated to dryness. Flash column chromatography of the crude product (light petroleum : dichloromethane, 8:1) yielded the 2,6-diiodo-4-methylphenol as a colourless solid (2.332 g, 70 %); m.p. 55-58 °C (lit.[96] 62 °C); ν_{max} (nujol) 3575, 1545, 1250 and 852 cm^{-1}; δ_H (250 MHz, $CDCl_3$) 7.57 (2 H, s, ArH), 5.60 (1 H, s, OH) and 2.25 (3 H, s, CH_3); δ_C (62.50 MHz, $CDCl_3$) 152.08 (ArCOH), 139.52 (ArCH), 133.79 (Ar*C*-CH_3), 82.12 (ArCI) and 19.52 (CH_3).

2,6-Diiodo-4-propylphenol **68**

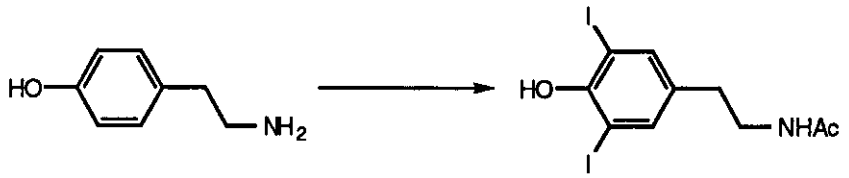


68

4-Propylphenol (11.689g, 8.60 mmol) was iodinated as described in the general procedure. Flash column chromatography of the crude product (light petroleum : dichloromethane, 9:1) yielded the *2,6-diiodo-4-propylphenol* as a pale yellow oil (27.693 g, 82 %); (Found M^+, 387.8824. $C_9H_{10}I_2O$ requires M^+, 387.8821); ν_{max}

(film) 3475, 2956, 1545, 1312, 1154 and 884 cm^{-1}; δ_H (400 MHz, $CDCl_3$) 7.51 (2 H, s, ArH), 5.60 (1 H, s, OH), 2.48 (2 H, t, *J* 7.2 Hz, Ar-CH_2), 1.66-1.56 (2 H, m, C*H*$_2CH_3$) and 0.95 (3 H, t, *J* 7.2 Hz, CH_3); δ_C (100.62 MHz, $CDCl_3$) 151.91 (ArCOH), 189.46 (ArCH), 139.22 (Ar*C*-CH_2), 82.45 (ArCI), 36.45 (Ar-CH_2), 24.83 (CH_2CH_3) and 13.99 (CH_3); *m/z* (E.I.) 388 (82, M^+), 359 (100, M^+ -CH_2CH_3) and 232 (13, M^+ -I).

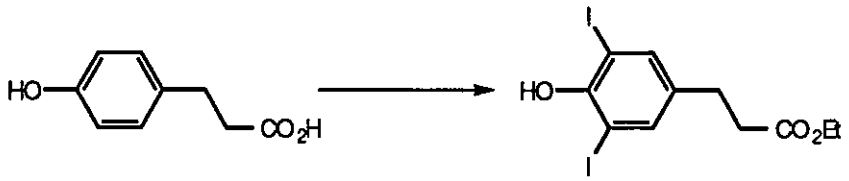
N-*[2-(4-hydroxy-3,5-diiodophenyl)-ethyl]-acetamide* **67**



67

Acetic anhydride (5.291 g, 52.87 mmol) was added slowly to a stirred solution of tyramine (6.597 g, 48.15 mmol) in sodium hydrogen carbonate solution (0.5 M, 150 cm^3). The resulting solution was stirred for 2 h. Sodium hydroxide solution was added (0.2 M, 70 cm^3) and any *O*-acetylated compound was washed out with ethyl acetate (2 x 100 cm^3). The aqueous layer was acidified with hydrochloric acid solution (2 M) and extracted with ethyl acetate (3 x 75 cm^3). The organic extracts were dried ($MgSO_4$) and evaporated to dryness. The resulting compound was iodinated following the general iodination procedure except that the reaction was stirred for 2 days. Flash column chromatography of the crude product (ethyl acetate : dichloromethane, 10:1) yielded N-*[2-(4-hydroxy-3,5-diiodophenyl)-ethyl]-acetamide* as yellow crystals (10.892 g, 52 %); m.p. 143-145 °C; (Found C, 28.04; H, 2.20; N, 3.28. $C_{10}H_{11}I_2NO_2$ requires C, 27.87; H, 2.57; N, 3.25); (Found M^+, 430.8874. $C_{10}H_{11}I_2NO_2$ requires M^+, 430.8882); ν_{max} (dichloromethane) 3298, 2932, 1633, 1556, 1402 and 869 cm^{-1}; δ_H (400 MHz, D_6-acetone) 8.12 (1 H, s, OH/NH), 7.63 (2 H, s, ArH), 7.26 (1 H, s, NH/OH), 3.37 (2 H, q, *J* 7.0 Hz, C*H*$_2$NHAc), 2.68 (2 H, t, *J* 7.0 Hz, Ar-CH_2) and 1.86 (3 H, s, $COCH_3$); δ_C (100.62 MHz, D_6-acetone) 169.68 (*C*OCH_3), 153.95 (ArCOH), 140.17 (ArCH), 136.38 (Ar*C*-CH_2), 84.17 (ArCI), 40.77 (CH_2NHAc), 32.84 (Ar-CH_2) and 22.40 (CO*C*H_3); *m/z* (E.I.) 431 (100, M^+) and 372 (11, M^+ -$COCH_3$).

Ethyl 3-(3,5-diiodo-4-hydroxyphenyl)propionate **65**



65

3-(4-Hydroxyphenyl)propionic acid (4.382 g, 26.39 mmol) was dissolved in a two-phase mixture of dichloromethane (150 cm^3) and potassium carbonate (7.184 g, 52.05 mmol) in water (200 cm^3). A solution of iodine (20.371 g, 80.20 mmol) and potassium iodide (18.562 g, 111.15 mmol) in water (150 cm^3) was added. The reaction was stirred for 2 days, acidified with hydrochloric acid (2 M) and the product filtered and washed with hydrochloric acid (0.05 M, 200 cm^3) and dried. The resulting solid was dissolved in ethanol (200 cm^3). Concentrated sulfuric acid (1 cm^3) was added and the reaction was heated to reflux for 3 h, the reaction was cooled and evaporated to dryness. The resulting solid was dissolved in dichloromethane (200 cm^3) and worked up as described in the general procedure for iodination of phenols. Flash column chromatography of the crude product (dichloromethane : ethyl acetate, 10:1) yielded the *ethyl 3-(3,5-diiodo-4-hydroxyphenyl)propionate* as a colourless solid (7.658 g, 65 %); m.p. 82-85 °C; (Found C, 29.62; H 2.711. $C_{11}H_{12}I_2O_3$ requires C, 29.51; H, 2.61); (Found M^+, 445.8878. $C_{11}H_{12}I_2O_3$ requires M^+, 445.8879); ν_{max} (dichloromethane) 3405, 2977, 1715, 1540, 1414 and 872 cm^{-1}; δ_H (360 MHz, $CDCl_3$) 7.52 (2 H, s, ArH), 5.65 (1 H, s, OH), 4.13 (2 H, q, *J* 7.1 Hz, CO_2CH_2), 2.81 (2 H, t, *J* 7.5 Hz, CH_2CO_2), 2.55 (2 H, t, *J* 7.6 Hz, Ar-CH_2) and 1.24 (3 H, t, *J* 7.1 Hz, CH_3); δ_C (100.62 MHz, $CDCl_3$) 172.73 (CO_2Et), 152.49 (ArCOH), 139.47 (ArCH), 137.07 (Ar*C*-CH_2), 82.66 (ArCI), 61.03 (CO_2CH_2), 36.16 (CH_2CO_2), 29.37 (Ar-CH_2) and 14.69 (CH_3); *m/z* (E.I.) 446 (100, M^+), 372 (85, M^+ -CO_2Et) and 246 (20, M^+ -CO_2Et, I).

Ethyl 2-(3,5-Diiodo-4-hydroxyphenyl)ethanoate **66**

66

2-(4-Hydroxyphenyl)ethanoic acid (2.822 g, 18.56 mmol) was added to a two-phase mixture of potassium carbonate (5.813 g, 42.12 mmol) in water (100 cm^3) and dichloromethane (80 cm^3). A solution of iodine (15.342 g, 60.40 mmol) and potassium iodide in water (200 cm^3) was added and the reaction stirred for 2 days. The mixture was acidified with hydrochloric acid (2 M), filtered and the product washed with hydrochloric acid (0.05 M, 200 cm^3) and dried. The resulting solid was dissolved in ethanol (200 cm^3). Concentrated sulfuric acid (1cm^3) was added and the reaction was heated to reflux for 3 h. The reaction was cooled and evaporated to dryness. The resulting solid was dissolved in dichloromethane (120 cm^3) and worked up as described in the general procedure for iodination of phenols. Flash column chromatography of the crude product (dichloromethane : ethyl acetate, 10:1) yielded the ethyl 2-(3,5-diiodo-4-hydroxyphenyl)ethanoate as a colourless solid (5.523 g, 69 %); m.p. 119-121 °C (lit.[33] 121-122 °C); (Found C, 27.80; H, 2.33. $C_{10}H_{10}I_2O_3$ requires C, 27.63; H, 2.08); (Found M^+, 431.8722. $C_{10}H_{10}I_2O_3$ requires M^+, 431.8723); ν_{max} (dichloromethane) 3453, 2982, 1722, 1545, 888 cm^{-1}; δ_H (400 MHz, $CDCl_3$) 7.60 (2 H, s, ArH), 5.72 (1 H, s, OH), 4.16 (2 H, q, *J* 7.0 Hz, CO_2CH_2), 3.46 (2 H, s, CH_2CO_2), 1.26 (3 H, t, *J* 7.0 Hz, CH_3); δ_C (100.62 MHz, $CDCl_3$) 171.23 (CO_2Et), 153.18 (ArCOH), 140.32 (ArCH), 130.37 (Ar*C*-CH_2), 82.51 (ArCI), 61.58 (CO_2CH_2), 39.50 (Ar-CH_2), 14.57 (CH_3); *m/z* (E.I.) 432 (76, M^+), 359 (99, M^+ -CO_2Et) and 232 (21, M^+ -CO_2Et, I).

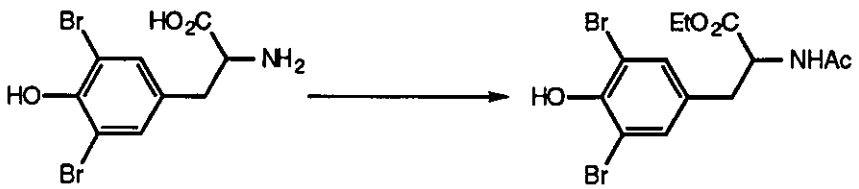
N-*Acetyl-3-iodotyrosine ethyl ester* **64**

64

3-Iodotyrosine (4.122 g, 13.05 mmol) was dissolved in acetic anhydride (100 cm^3) and acetic acid (100 cm^3) and was heated under reflux for 2 h. After cooling, water (100 cm^3) was added and the reaction evaporated to dryness. The product was dissolved in ethanol (350 cm^3) and concentrated sulfuric acid (1 cm^3) added. The reaction was heated to reflux for 3 h, cooled, water (50 cm^3) added and the reaction evaporated to dryness. The remaining solid was dissolved in ethyl acetate (75 cm^3) and washed with hydrochloric acid (0.05 M, 50 cm^3). The aqueous layer was extracted with ethyl acetate (2 x 75 cm^3) and the organic extracts were washed with water (2 x 75 cm^3), dried and evaporated to dryness. Flash column chromatography of the crude product (dichloromethane : ethyl acetate, 4:1) yielded the *N*-acetyl-3-

iodotyrosine ethyl ester as yellow crystals (3.493 g, 71 %); m.p. 135-137 °C (lit.[22] 138-140 °C); (Found: C, 41.10; H, 3.96; N, 3.74. $C_{13}H_{16}INO_4$ requires C, 41.39; H, 4.27; N, 3.71); (Found M^+, 377.0128. $C_{13}H_{16}INO_4$ requires M^+, 377.0126); ν_{max} (liquid paraffin) 3359, 1731, 1651, 1505, 1137 and 859 cm^{-1}; δ_H (250 MHz, D_6-acetone) 9.41 (1 H, s, OH), 7.79 (1 H, d, *J* 7.9 Hz, NH), 7.58 (1 H, d, *J* 1.9 Hz, ArH), 7.10 (1 H, dd, *J* 1.9, 8.2 Hz, ArH), 6.85 (1 H, d, *J* 8.2 Hz, ArH), 4.66 [1 H, dt, *J* 6.0, 7.8 Hz, C*H*(NHAc)CO_2Et], 4.17-4.06 (2 H, m, CO_2CH_2), 3.06 (1 H, dd, *J* 5.9, 13.9 Hz, Ar-CH_2), 2.90 (1 H, dd, *J* 8.0, 13.9 Hz, Ar-CH_2), 1.98 (3 H, s, $COCH_3$) and 1.19 (3 H, t, *J* 7.0 Hz, CH_2CH_3); δ_C (62.50 MHz, D_6-acetone) 176.43, 175.94 (CO_2Et, $COCH_3$), 160.75 (ArCOH), 144.93, 135.61 (ArCH), 134.98 (Ar*C*-CH_2), 120.03 (ArCH), 88.75 (ArCI), 66.08 (Ar-CH_2), 59.24 [*C*H(NHAc)CO_2Et], 41.19 (CO_2CH_2), 27.11 ($COCH_3$) and 18.97 (CH_2CH_3); *m/z* (E.I.) 377 (99, M^+), 318 (99, M^+ -NH_2COCH_3) and 233 [55, M^+ -$CH(NHCOCH_3)CO_2CH_2CH_3$].

N-*Acetyl-3,5-dibromotyrosine ethyl ester* **63**

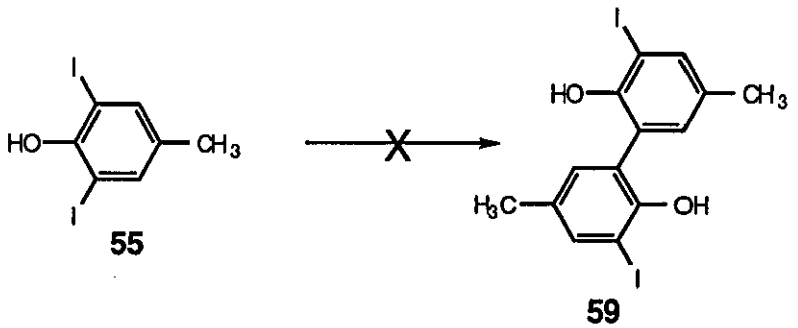


63

3,5-Dibromotyrosine (10.551 g, 31.12 mmol) was added to a solution of sodium hydrogen carbonate (0.2 M, 250 cm^3). Acetic anhydride (3.876 g, 38.00 mmol) was added slowly to the solution and the reaction stirred for 3 h. The reaction was acidified with hydrochloric acid (2 M) and extracted with ethyl acetate (5 x 75 cm^3). The organic extracts were washed with hydrochloric acid (0.5 M, 100 cm^3), dried ($MgSO_4$), filtered and evaporated to dryness. The resulting solid was dissolved in ethanol (300 cm^3) and concentrated sulfuric acid (1.5 cm^3) and heated under reflux for 3 h. The reaction was cooled and evaporated to dryness. The remaining solid was dissolved in dichloromethane (150 cm^3) and washed with hydrochloric acid solution (0.05 M, 3 x 100 cm^3). The organic extract was dried ($MgSO_4$) and evaporated to dryness. Flash column chromatography of the crude product (dichloromethane : ethyl acetate, 5:1) yielded the *N*-acetyl-3,5-dibromotyrosine ethyl ester as a colourless solid (9.291 g, 73 %); m.p. 135-137 °C (lit.[97] 140-141 °C); (Found M^+, 408.9331. $C_{13}H_{15}Br_2NO_4$ requires M^+, 408.9349); ν_{max} (liquid paraffin) 3298, 1730, 1654, 1550, 1135, 878 and 737 cm^{-1}; δ_H (400 MHz, $CDCl_3$) 7.24 (2 H, s, ArH), 6.08 (1 H, d, *J* 7.05 Hz, NH), 4.81 [1 H, dt, *J* 5.5, 7.5 Hz, C*H*(NHAc)CO_2Et], 4.27-4.19 (2 H, m, CO_2CH_2), 3.07 (1 H, dd, *J* 6.0, 14.0 Hz, Ar-

CH_2), 3.02 (1 H, dd, *J* 5.3, 14.0 Hz, Ar-CH_2), 2.05 (3 H, s, $COCH_3$) and 1.31 (3 H, t, *J* 7.3 Hz, CH_2CH_3); δ_C (100.62 MHz, $CDCl_3$) 171.63, 170.06 (CO_2Et, $COCH_3$), 148.98 (ArCOH), 133.23 (ArCH), 131.09 (Ar*C*-CH_2), 110.17 (ArCBr), 62.27 (CO_2CH_2), 53.62 [*C*H(NHAc)CO_2Et], 36.92 (Ar-CH_2), 23.52 ($CO\,CH_3$) and 14.56 (CH_2CH_3); *m/z* (E.I.) 409 (49, M^+) and 350 (63, M^+ -NHAc).

Attempted coupling of diiodophenols under $S_{RN}1$ radical conditions



2,6-Diiodo-4-methylphenol (0.751 g, 2.32 mmol) was added to a stirred solution of sodium methoxide (0.059 g, 1.12 mmol) in DMF (50 cm^3) and the reaction heated at 60-70 °C for 15 h. After cooling the reaction was evaporated to dryness to yield a crude oil which was taken up in diethyl ether (30 cm^3) and water (40 cm^3). The aqueous layer was extracted with diethyl ether (2 x 20 cm^3) and washed with water (7 x 20 cm^3), and sodium thiosulphate solution (20 cm^3). The organic extracts were dried ($MgSO_4$) and evaporated to dryness to give a colourless solid of 2,6-diiodo-4-methylphenol (0.581 g, 78 %). Only starting material was recovered which was identified by TLC and characterised by ^1H NMR spectroscopy and was found to be consistent with authentic material.

This reaction was repeated with alterations in the reaction conditions which are described in chapter 2.

Attempted coupling of 2,6-diiodophenols using manganese salts

2,6-Diiodo-4-methylphenol (0.609 g, 1.69 mmol) was dissolved in ethanol (30 cm^3). Manganese(II) diacetate dihydrate (0.481 g, 2.31 mmol), and manganese(III) triacetate (0.038g, 0.16 mmol) were added and the reaction heated to reflux. After 15 h the reaction was cooled and evaporated to dryness, the resulting oil was taken up in dichloromethane (20 cm^3) and water (50 cm^3), the aqueous layer was extracted

with dichloromethane (2 x 30 cm^3), and the organic extracts were washed with water (2 x 30 cm^3), dried ($MgSO_4$) and evaporated to dryness. Only starting material was recovered which was identified by TLC and characterised using ^1H NMR spectroscopy and was found to be consistent with authentic material.

The reaction was also repeated using manganese(III) triacetylacetonate under the same conditions, and with varying quantities of manganese(II) diacetate and manganese(III) triacetate as described in chapter 2.

Attempted coupling of diiodophenols using radical initiators

General procedure

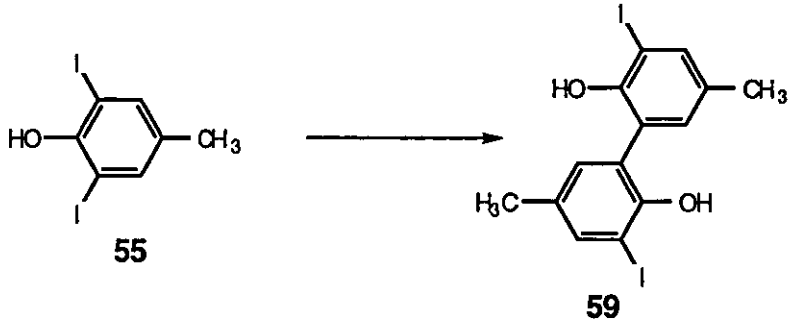
2,6-Diiodo-4-methylphenol (1.142 g, 3.17 mmol) was dissolved in DMSO (150 cm^3) and the solution was degassed with nitrogen. Hexabutylditin (2.442 g, 4.22 mmol) was added and the reaction heated to reflux for 5 h. The reaction mixture was cooled and evaporated to dryness. The remaining oil dissolved in dichloromethane (200 cm^3), the phenolic compounds were extracted into sodium hydroxide solution (0.01 M, 4 x 100 cm^3). The alkaline extracts were washed with light petroleum (7 x 100 cm^3). The alkaline extracts were acidified to pH 2 with hydrochloric acid solution (6 M) and the product extracted into dichloromethane (3 x 150 cm^3). The dichloromethane solution was dried ($MgSO_4$) and evaporated to dryness. 2,6-Diiodo-4-methylphenol was recovered (1.050 g, 92 %) and identified by TLC and characterised by ^1H NMR spectroscopy and was found to be consistent with authentic material.

The general procedure for coupling reactions using radical initiators was repeated using *N*-acetyl-3,5-diiodotyrosine ethyl ester (1.105 g, 2.19 mmol) and potassium *tert*-butoxide (0.156 g, 1.28 mmol) in toluene (150 cm^3). The reaction was heated under reflux and hexabutylditin (0.421 g, 0.715 mmol) was added three times at 40 minute intervals. The reaction was irradiated using tungsten lamp, and refluxed for a further 2 h after the additions were complete.[98] *N*-Acetyl-3,5-diiodotyrosine ethyl ester was recovered as a colourless powder (0.735 g, 66 %); m.p. 155-156 °C; δ_H (250 MHz, $CDCl_3$) 7.43 (2 H, s, ArH), 6.03 (1 H, d, *J* 7.2 Hz, NH), 5.74 (1 H, s, OH), 4.75 [1 H, dt, *J* 5.6, 7.5 Hz, CH(NHAc)CO_2Et], 4.21-4.10 (2 H, m, CO_2CH_2), 3.05 (1 H, dd, *J* 6.0, 14.0 Hz, Ar-CH_2), 2.99 (1 H, dd, *J* 5.3, 14.0 Hz, Ar-CH_2), 2.03 (3 H, s, $COCH_3$) and 1.27 (3 H, t, *J* 7.12 Hz, CH_2CH_3); and *N*-acetyl-3,5-diiodotyrosine as a yellow powder (0.218 g, 20 %), m.p. 132-135 °C (lit.[85] 125 °C); δ_H [250 MHz, D_6-DMSO:$CDCl_3$, 10:1] 8.15 (1 H, s, OH), 7.12 (2 H, s, ArH), 6.03

(1 H, d, *J* 7.1 Hz, NH), 4.13 [1 H, dt, *J* 5.7, 7.1 Hz, $CH_2CH(NHAc)CO_2H$], 2.99 (1 H, dd, *J* 6.1, 14.0 Hz, Ar-CH_2), 2.87 (1 H, dd, *J* 5.7, 14.0 Hz, Ar-CH_2) and 1.90 (3 H, s, $COCH_3$).

The general procedure for coupling reactions using radical initiators was repeated using 2,6-diiodo-4-methylphenol (0.561 g, 1.55 mmol) and potassium *tert*-butoxide (0.179 g, 1.59 mmol) in toluene (100 cm^3). The reaction was heated under reflux, hexabutylditin (0.298 g, 0.51 mmol) was added three times at 40 minute intervals, and the reaction irradiated using a tungsten lamp. On completion of addition the reaction was heated under reflux for a further 18 h. Purification by flash column chromatography of the crude product (light petroleum : dichloromethane, 9:1) yielded 2-iodo-4-methylphenol as a yellow oil (0.101 g, 28 %); (Found M^+, 233.9542, C_7H_7IO requires M^+, 233.9541); ν_{max} (neat) 3477, 2919, 1601, 1486, 1281, 1179 and 858 cm^{-1}; δ_H (250 MHz, $CDCl_3$) 7.48 (1 H, d, *J* 1.4 Hz, ArH), 7.04 (1 H, dd, *J* 1.5, 8.3 Hz, ArH), 6.8 (1 H, d, *J* 8.3 Hz, ArH), 5.23 (1 H, s, OH) and 2.26 (3 H, s, CH_3); δ_C (62.50 MHz, $CDCl_3$) 152.55 (ArCOH), 138.27 (ArCH), 131.91 (Ar*C*-CH_3), 130.78 (ArCH), 114.69 (ArCH), 85.38 (ArCI) and 19.93 (CH_3); *m/z* (E.I.) 233 (5, M^+) and 107 (81, M^+-I).

2,2'-Dihydroxy-3,3'diiodo-5,5'-dimethyl-1,1'-biphenyl **59**

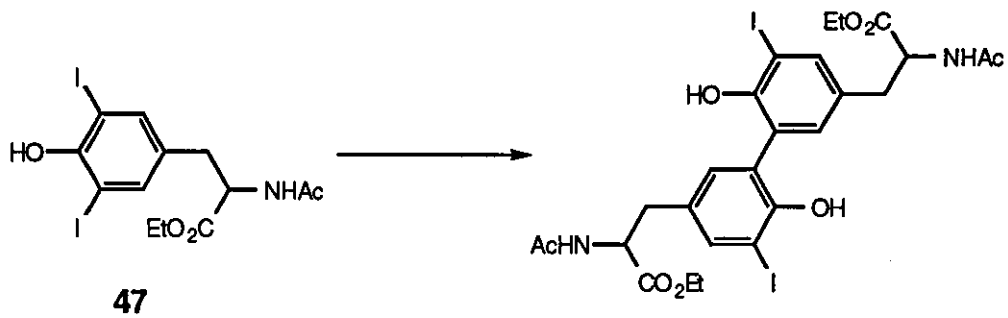


The general procedure for radical initiation reactions was repeated using 2,6-diiodo-4-methylphenol (1.730 g, 4.81 mmol) and potassium *tert*-butoxide (0.258 g, 2.34 mmol) in toluene (120 cm^3). The reaction was heated at 80 °C, hexabutylditin (0.780 g, 1.34 mmol) added and the reaction irradiated using a tungsten lamp. The reaction was maintained at 80 °C for a further 22 h. Flash column chromatography of the crude product (light petroleum : dichloromethane, 7:3) yielded the *2,2'-dihydroxy-3,3'diiodo-5,5'-dimethyl-1,1'-biphenyl* in a relative yield of 47 % compared to the 2,6-diiodo-4-methylphenol, isolated as a colourless solid (0.459 g, 41 %); m.p. 150-151 °C; (Found M^+, 465.8927. $C_{14}H_{12}I_2O_2$ requires M^+, 465.8926); ν_{max} (solid) 3475, 2957, 1545, 1401 and 1239 cm^{-1}; δ_H (400 MHz,

$CDCl_3$) 7.60 (2 H, d, *J* 1.7 Hz, ArH), 7.05 (2 H, d, *J* 1.8 Hz, ArH), 5.77 (2 H, s, OH) and 2.32 (6 H, s, CH_3); δ_C (100.62 MHz, $CDCl_3$) 149.91 (ArCOH), 139.48 (ArCH), 132.89 (Ar*C*-CH_3), 132.87 (ArCH), 124.72 (ArCCAr), 86.64 (ArCI) and 20.40 (CH_3); *m/z* (E.I.) 466 (92, M^+) and 340 (50, M^+ -I).

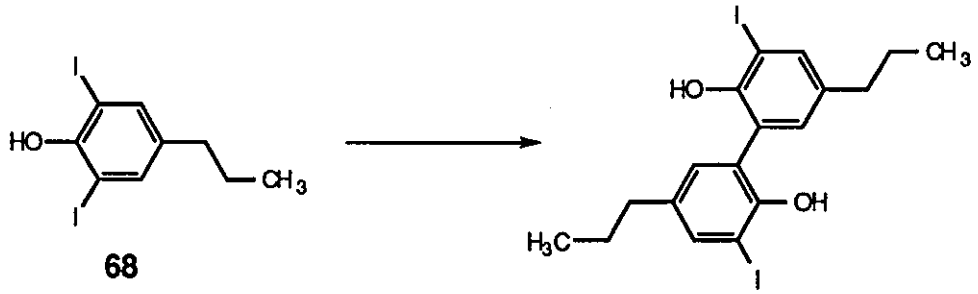
The above experiment was repeated omitting the addition of hexabutylditin to give a relative yield of 40 % of the dimer compared to the recovered 2,6-diiodo-4-methylphenol.

2,2'-Dihydroxy-3,3'-diiodo-5,5'-di-[(2-acetamido-2-ethyloxycarbonyl)ethyl]-1,1'-biphenyl **48**



The general procedure for coupling reactions using radical initiators was repeated using *N*-acetyl-3,5-diiodotyrosine ethyl ester (1.353 g, 2.69 mmol) and potassium *tert*-butoxide (0.121 g, 1.08 mmol) in toluene (120 cm^3). The reaction was heated at 80 °C for 22 h without addition of hexabutylditin. The *title compound* was obtained as a yellow solid (relative yield 16 % compared to the recovered *N*-acetyl-3,5-diiodotyrosine ethyl ester, 84 %; recovered mass 1.253 g); m.p. 125-127 °C; (Found M^+, 752.0087. $C_{26}H_{30}I_2NO_8$ requires M^+, 752.0091); ν_{max} (solid) 3432, 3301, 1718, 1643, 1109 and 882 cm^{-1}; δ_H (250 MHz, $CDCl_3$) 7.48 (2 H, d, *J* 1.9 Hz, ArH), 7.08 (2 H, d, *J* 1.9 Hz, ArH), 6.20 (1 H, d, *J* 8.0 Hz, NH), 4.86 [1 H, dt, *J* 5.1, 7.9 Hz, CH_2CH(NHAc)CO_2Et], 4.21 (2 H, q, *J* 7.5 Hz, OCH_2CH_3), 3.14 (1 H, dd, *J* 4.9, 13.7 Hz, Ar-CH_2), 2.76 (1 H, dd, *J* 7.9, 13.7 Hz, Ar-CH_2), 1.91 (3 H, s, $COCH_3$) and 1.28 (3 H, t, *J* 7.5 Hz, CH_2CH_3); δ_C (62.50 MHz, $CDCl_3$) 171.39 (CO_2Et), 169.8 ($COCH_3$), 151.85 (ArCOH), 140.27 (ArCH), 132.23 (ArCH), 129.86 (Ar*C*-CH_2), 122.95 (ArCCAr), 85.25 (ArCI), 61.86 (CO_2CH_2), 53.21 [*C*H(NHAc)CO_2Et], 37.79 (Ar-CH_2), 22.98 ($COCH_3$) and 14.13 (CH_2CH_3); *m/z* (F.A.B.) 753 (83, MH^+), 711 (19, M^+ -$COCH_3$), 637 (46, M^+ -$COCH_3$, $CO_2CH_2CH_3$) and 521 (53, M^+-$COCH_3$, $CO_2CH_2CH_3$, I).

2,2'-Dihydroxy-3,3'-diiodo-5,5'-dipropyl-1,1'-biphenyl **75**



The general procedure for coupling reactions using radical initiators was repeated using 2,6-diiodo-4-propylphenol (7.637 g, 19.68 mmol) and potassium *tert*-butoxide (1.023 g, 9.13 mmol) in toluene (200 cm^3). The reaction was heated at 80 °C for 24 h without the addition of hexabutylditin. Flash column chromatography of the crude product (light petroleum : dichloromethane, 4:1) yielded the *2,2'-dihydroxy-3,3'-diiodo-5,5'-dipropyl-1,1'-biphenyl* as a colourless oil (0.822 g, 16 %); (Found M^+, 521.9557. $C_{18}H_{20}I_2O_2$ requires M^+, 521.9552); ν_{max} (neat) 3475, 2957, 1545, 1401, 1153 and 884 cm^{-1}; δ_H (250 MHz, $CDCl_3$) 7.57 (2 H, d, *J* 1.9 Hz, ArH), 7.02 (2 H, d, *J* 1.9 Hz, ArH), 5.77 (2 H, s, OH), 2.53 (2 H, t, *J* 7.3 Hz, Ar-CH_2), 1.67-1.54 (2 H, m, CH_2CH_3) and 0.95 (3 H, t, *J* 7.2, CH_3); δ_C (100.62 MHz, $CDCl_3$) 148.86 (ArCOH), 137.76 (ArCH), 136.69 (Ar*C*-CH_2), 131.16 (ArCH), 85.53 (ArCI), 35.87 (CH_2), 23.80 (CH_2CH_3) and 12.96 (CH_3); *m/z* (C.I.) 540 (99, $M^+ + NH_4^+$), 522 (7, M^+) and 396 (12, M^+ -I).

Ortho-ortho *coupling using an aqueous buffer system*

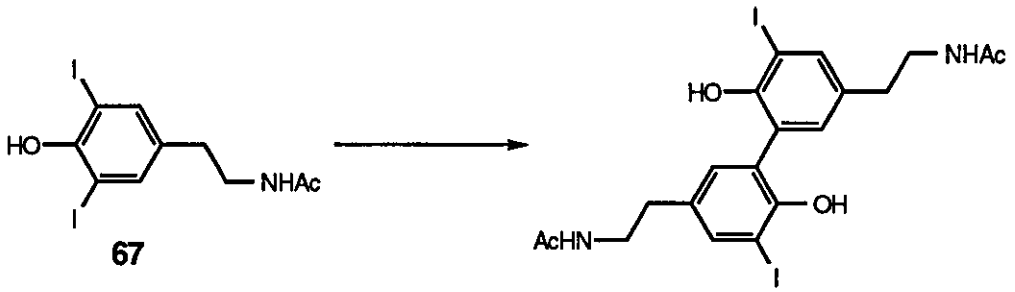
General procedure

2,2'-Dihydroxy-3,3'-diiodo-5,5'-di-[(2-acetamido-2-ethyloxycarbonyl)ethyl]-1,1'-biphenyl **48**

Dichloromethane (100 cm^3) and either phosphate buffer (0.003 M, pH 6.5, 100 cm^3) or boric acid solution (0.2 M, 100 cm^3) were stirred together and degassed using nitrogen for 30 minutes. *N*-Acetyl-3,5-diiodotyrosine ethyl ester (0.756 g, 1.50 mmol) was added and the pH measured and adjusted to 6.5 by the addition of sodium hydroxide solution (0.05 M). The reaction was stirred at room temperature and the pH carefully monitored for 24 h and further additions of sodium hydroxide solution (0.05 M) were made whenever necessary to keep the pH close to 6.5. After 24 h the colour of the reaction mixture was pink/purple colour due to the iodine produced. The solution was acidified with hydrochloric acid solution (0.1 M, a few

drops). The layers separated and the aqueous layer extracted with dichloromethane (3 x 50 cm^3). The dichloromethane extracts were washed with sodium thiosulfate solution (0.5 M, 100 cm^3) and with hydrochloric acid solution (0.05 M, 2 x 80 cm^3). The dichloromethane layer was dried ($MgSO_4$) and evaporated to dryness. 2,2'-Dihydroxy-3,3'-diiodo-5,5'-di-[(2-acetamido-2-ethyloxycarbonyl)ethyl]-1,1'-biphenyl was obtained along with recovered *N*-acetyl-3,5-diiodotyrosine ethyl ester. Both of the compounds were identified by ^1H NMR. The relative yield was calculated from integration of the signals arising from the ^1H NMR spectroscopy of a representative sample of the mixture, to give 33-50 % yield of the product.

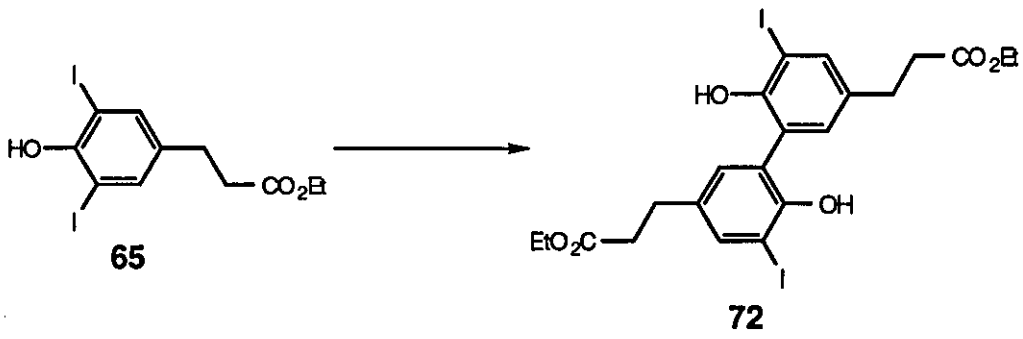
5,5'-Di-(2-acetamidoethyl)-2,2'-dihydroxy-3,3-diiodo-1,1'-biphenyl **74**



The general procedure for *ortho-ortho* coupling in an aqueous buffer system was followed without degassing the reaction mixture, and the reaction was left in open to air. *N*-[2-(4-Hydroxy-3,5-diiodophenyl)-ethyl]acetamide (1.031 g, 2.39 mmol) was dissolved in dichloromethane (90 cm^3), ethyl acetate (10 cm^3) and phosphate buffer (0.003 M, 70 cm^3) and the pH was adjusted to 6.5 with sodium hydroxide solution (0.05 M). The reaction was stirred at room temperature for 4 days. The pH was checked and adjusted to 6.5 whenever necessary. Flash column chromatography of the crude product (ethyl acetate : ethanol, 4:1) yielded the *5,5'-di-(2-acetamidoethyl)-2,2'-dihydroxy-3,3-diiodo-1,1'-biphenyl* as yellow crystals (0.413 g, 57 %); m.p. 112-116 °C; (Found M^+, 607.9665. $C_{20}H_{22}I_2N_2O_4$ requires M^+, 607.9668); ν_{max} (dichloromethane) 3363, 2929, 1628, 1558, 1363 and 1135 cm^{-1}; δ_H (400 MHz, D_6-acetone) 7.58 (2 H, d, *J* 2.0 Hz, ArH), 7.13 (2 H, s, NH/OH), 7.13 (2 H, d, *J* 2.0 Hz, ArH), 5.62 (2 H, s, NH/OH), 3.43-3.38 (2 H, m, CH_2NHAc), 2.71-2.68 (2 H, m, Ar-CH_2) and 1.84 (6 H, s, CH_3); δ_C (100.62 MHz, D_6-acetone) 168.95 ($COCH_3$), 152.15 (ArCOH), 138.92 (ArCH), 132.78 (Ar*C*-CH_2), 131.86 (ArCH), 124.68 (ArCCAr), 85.68 (ArCI), 40.07 (CH_2NHAc), 33.92 (Ar-CH_2) and 21.68

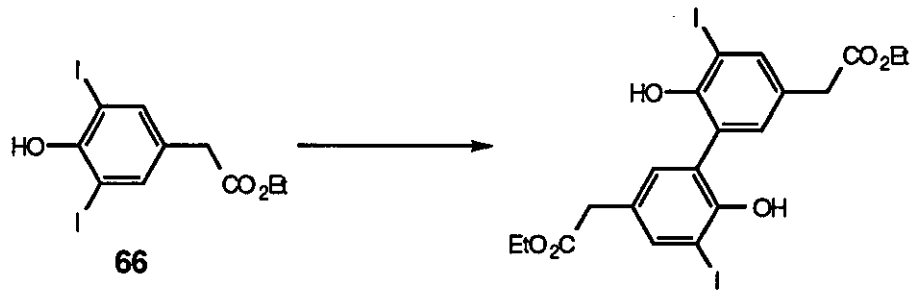
(CH_3); *m/z* (F.A.B.) 609 (62, M^+ +H), 549 (8, M^+ -NHAc) and 490 (13, M^+ -2 x NHAc).

5,5'-Di-[(2-ethoxycarbonyl)ethyl]-2,2'-dihydroxy-3,3'-diiodo-1,1'-biphenyl **72**



The general procedure for *ortho-ortho* coupling in an aqueous buffer system was followed using ethyl 3-(3,5-diiodo-4-hydroxyphenyl)propionate (1.023g, 2.30 mmol) in dichloromethane (100 cm^3) and phosphate buffer (0.003 M, pH 6.1, 90 cm^3). The reaction was maintained between pH 6.0-6.5, by the addition of sodium hydroxide solution (0.05 M) whenever necessary. The reaction was stirred at room temperature for 3 days. Flash column chromatography of the crude product (dichloromethane) yielded the *5,5'-di-[(2-ethoxycarbonyl)ethyl]-2,2'-dihydroxy-3,3'-diiodo-1,1'-biphenyl* as a colourless oil (0.198 g, 27 %); (Found M^+, 637.9660. $C_{22}H_{24}I_2O_6$ requires M^+, 637.9662); ν_{max} (film) 3416, 2980, 1726, 1559, 1235, 1185 cm^{-1}; δ_H (250 MHz, $CDCl_3$) 7.60 (2 H, d, *J* 2.1 Hz, ArH), 7.04 (2 H, d, *J* 1.9 Hz, ArH), 5.86 (2 H, s, OH), 4.12 (4 H, q, *J* 7.1 Hz, CO_2CH_2), 2.88 (4 H, t, *J* 7.5 Hz, CH_2CO_2), 2.59 (4 H, t, *J* 7.5 Hz, $ArCCH_2$) and 1.23 (6 H, t, *J* 7.2 Hz, CH_3); δ_C (100.62 MHz, $CDCl_3$) 172.96 (CO_2Et), 150.61 (ArCOH), 138.95 (ArCH), 135.69 (Ar*C*-CH_2), 132.28 (ArCH), 124.90 (ArCCAr), 86.95 (ArCI), 61.06 (CO_2CH_2), 36.27 (CH_2CO_2), 29.95 (Ar-CH_2) and 14.63 (CH_3); *m/z* (E.I.) 638 (100, M^+), 564 (8, M^+ -CO_2Et), 512 (23, M^+ -I) and 438 (4, M^+ -CO_2Et, I).

5,5'-Di-[(ethoxycarbonyl)methyl]-2,2'-dihydroxy-3,3'-diiodo-1,1'-biphenyl **73**



The general procedure for *ortho-ortho* coupling in an aqueous buffer was followed using ethyl 2-(3,5-diiodo-4-hydroxyphenyl)ethanoate (1.306 g, 3.03 mmol) in dichloromethane (85 cm^3) and phosphate buffer (0.003 M, pH 6.0, 90 cm^3). The reaction was stirred at room temperature for 4 days maintaining the pH between 5.8-6.2 with additions of sodium hydroxide solution (0.05 M) whenever necessary. Flash column chromatography of the crude product (dichloromethane) yielded the *5,5'-di-[(ethoxycarbonyl)methyl]-2,2'-dihydroxy-3,3'-diiodo-1,1'-biphenyl* as a colourless solid (0.175 g, 19 %); m.p. 98-100 °C; (Found M^+, 609.9350. $C_{20}H_{20}I_2O_6$ requires M^+, 609.9349); ν_{max} (dichloromethane) 3539, 2987, 1727, 1564, 1232 and 1062 cm^{-1}; δ_H (250 MHz, $CDCl_3$) 7.67 (2 H, d, *J* 2.0 Hz, ArH), 7.13 (2 H, d, *J* 2.0 Hz, ArH), 5.69 (2 H, s, OH), 4.14 (4 H, q, *J* 7.2 Hz, CH_2CH_3), 3.53 (4 H, s, Ar-CH_2) and 1.25 (6 H, t, *J* 7.2 Hz, CH_3); δ_C (62.50 MHz, $CDCl_3$) 171.21 (CO_2Et), 150.95 (ArCOH), 139.46 (ArCH), 132.86 (ArCH), 128.86 (Ar*C*-CH_2), 124.19 (ArCCAr), 86.44 (ArCI), 61.08 (CH_2CH_3), 39.68 (Ar-CH_2) and 14.14 (CH_3); *m/z* (E.I.) 610 (73, M^+), 537 (17, M^+ -CO_2Et), 507 (29, M^+ -CO_2Et, Et), 463 (49, M^+ -2 x CO_2Et), 335 (25, M^+ -2 x CO_2Et, I) and 209 (27, M^+ -CO_2Et, 2 x I).

Ortho-ortho coupling of diiodophenols in organic solvent alone

General procedure

The diiodophenol (ca. 3 mmol) was dissolved in either ethyl acetate or dichloromethane (ca. 80 cm^3) and stirred at room temperature under a variety of conditions and for varying times. The reaction mixture was washed with sodium thiosulfate solution (0.2 M, 50 cm^3), hydrochloric acid solution (0.05 M, 2 x 50 cm^3), dried ($MgSO_4$) and evaporated to dryness to give a mixture of the *ortho-ortho* coupled product and starting material. The relative yield was calculated from integration of the signals arising from the ^1H NMR spectroscopy of a representative sample of the mixture.

6.3 Experimental to Chapter Three

Instrumentation

The oxidation potentials of a range of phenols were measured using a Sycopel Scientific Scanning ministat and Goerz chart recorder. The electrodes used were as follows;

Working Electrode (WE) : Platinum wire
Counter Electrode (CE) : Platinum mesh
Reference Electrode (RE) : Saturated Calomel Electrode

The WE and CE were cleaned prior to use with the aid of a galvanostat. The two electrodes were placed in a sulfuric acid solution (1 M), the scale was set at 100 mA/V, limit A was set at -1.00 V, and limit B at +1.00 V. The electrodes were held at -100 mA for one minute and then at +100 mA for a further minute. This first stage oxidises any organic impurities off and converts the platinum to platinum oxide, and the second stage is necessary to change the platinum oxide back to its original form. The electrodes were cleaned daily and when it was necessary between scans to remove any coating that had been deposited. The oxidation potential measurements were carried out using a three compartment electrochemical cell.

All solutions were degassed for approximately 10 minutes prior to use with nitrogen, except where stated.

The phenols were either commercially available and used as such, or were prepared by the methods described in section 6.2. The phenoxides were either obtained by adding sodium hydroxide solution (0.2 M) to the test solution, adjusting the pH to 8-9; or by addition of sodium hydride (1 equivalent) to a stirred solution of the phenol in THF, with further stirring for 30 minutes followed by the removal of the solvent.

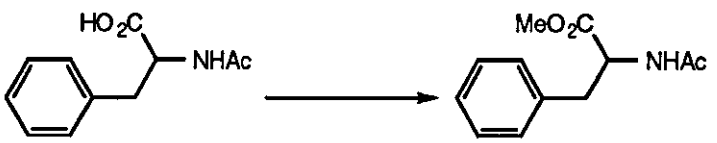
The cyclic voltammograms were measured at a scan rate of 0.150 Vs^{-1} with the solution concentration being approximately 5 mM other than in the case of *N*-acetylthyroxine ethyl ester where it was approximately 1.5 mM. The oxidation potentials of the phenols were measured in acetonitrile, only phenol itself was measured in water. The oxidation potentials of the phenoxide ions were measured in water and acetonitrile. The background electrolyte in all the solutions was sodium perchlorate (0.2 M). In acetonitrile the oxidation potential was scanned

between approximately 0.0 V and +1.8 V, in water the limits were approximately 0.0 V and +1.3 V. All the oxidation potentials were measured as Ep values at standard temperature and pressure.

6.4 Experimental to Chapter Four

Preparation of starting materials

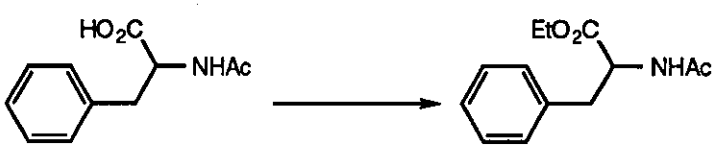
N-*Acetylphenylalanine methyl ester* **95**



95

N-Acetylphenylalanine (2.502 g, 12.10 mmol) was dissolved in methanol. A solution of diazomethane in diethyl ether was added until the effervescence on addition stopped and the solution remained a pale yellow colour. Nitrogen was passed over the solution until the yellow colour was no longer evident. The solution was evaporated to dryness yielding the *N*-acetylphenylalanine methyl ester as a colourless solid (2.581 g, 97 %); m.p. 86-88 °C (lit.[99] 89-90 °C); δ_H (250 MHz, $CDCl_3$) 7.34-7.19 (3 H, m, ArH), 7.08-6.99 (2 H, m, ArH), 6.06 (1 H, d, *J* 7.3 Hz, NH), 4.88 [1 H, dt, *J* 5.8, 7.3 Hz, $CH_2CH(NHAc)CO_2Me$], 3.71 (3 H, s, OCH_3), 3.12 (1 H, dd, *J* 5.8, 13.8 Hz, Ar-CH_2), 3.05 (1 H, dd, *J* 5.9, 13.8 Hz, Ar-CH_2) and 1.91 (3 H, s, $COCH_3$); δ_C (62.50 MHz, $CDCl_3$) 172.04 (CO_2CH_3), 169.56 ($COCH_3$), 135.77 (Ar*C*-CH_2), 129.20, 128.50, 127.05 (ArCH), 53.04 [$CH(NHCOCH_3)CO_2CH_3$], 52.23 (CO_2CH_3), 37.76 (Ar-CH_2) and 23.03 ($COCH_3$).

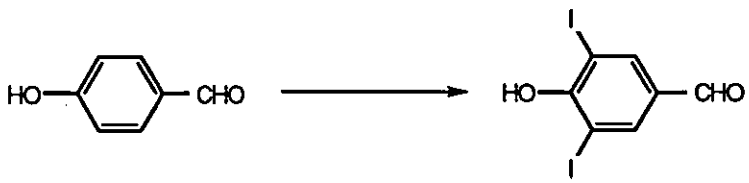
N-*Acetylphenylalanine ethyl ester* **98**



98

N-Acetylphenylalanine (10.013 g, 48.31 mmol) was dissolved in ethanol (200 cm^3) and sulfuric acid (1.5 cm^3) was added, and the reaction was heated under reflux for 2 h. After cooling the reaction mixture was evaporated to dryness, taken up in ethyl acetate (120 cm^3) and water (100 cm^3), separated and the aqueous layer extracted with ethyl acetate (80 cm^3). The organic extracts were washed with water (2 x 100 cm^3), dried ($MgSO_4$) and evaporated to dryness. The *N*-acetylphenylalanine ethyl ester was obtained as a colourless solid (10.781 g, 95 %); (Found MH^+, 236.1287. $C_{13}H_{17}NO_3$ requires MH^+, 236.1286); m.p. 55-58 °C (lit.[100] 68 °C); ν_{max} (liquid paraffin) 3274, 1733, 1642 and 1554 cm^{-1}; δ_H (250 MHz, $CDCl_3$) 7.30-7.11 (3 H, m, ArH), 7.00-6.97 (2 H, m, ArH), 6.02 (1 H, d, *J* 7.7 Hz, NH), 4.86 [1 H, dt, *J* 6.2, 7.7 Hz, C*H*(NHAc)CO_2Et], 4.15 (2 H, q, *J* 7.1 Hz, CO_2CH_2), 3.12 (1 H, dd, *J* 6.1,13.8 Hz, Ar-CH_2), 3.04 (1 H, dd, *J* 6.3, 13.8 Hz, Ar-CH_2), 1.98 (3 H, s, $COCH_3$) and 1.20 (3 H, t, *J* 7.1 Hz, CH_2CH_3); δ_C (90.55 MHz, $CDCl_3$) 171.81, 169.93 (CO_2Et, *C*OCH_3), 136.14 (Ar*C*-CH_2), 129.27, 128.45, 126.98 (ArCH), 61.37 (CO_2CH_2), 53.33 [*C*H(NHAc)CO_2Et], 37.89 (Ar-CH_2), 22.91 (CO*C*H_3) and 14.07 (CH_2CH_3); *m/z* (C. I.) 236 (100, MH^+), 190 (33, M^+ -NHAc), 162 (24, M+ -NHAc, CH_2CH_2) and 120 (28, M+ -NHAc, CO_2Et).

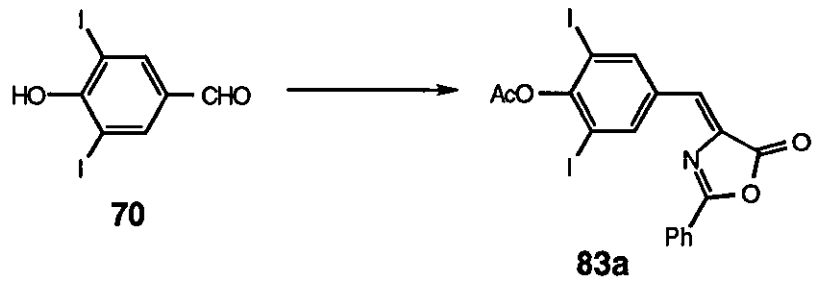
3,5-Diiodo-4-hydroxybenzaldehyde **70**



70

4-Hydroxybenzaldehyde (1.204 g, 9.85 mmol) was dissolved in two-phase mixture of dichloromethane (200 cm^3) and a solution of potassium carbonate (1.437 g, 10.03 mmol) in water (100 cm^3). A solution of iodine (8.24 g, 32.44 mmol) and potassium iodide (20.567 g, 123.89 mmol) in water (350 cm^3) was added to the mixture over 30 min. The reaction was stirred for 8 days and acidified to pH 3 with hydrochloric acid solution (4 M). The aqueous layer was extracted with dichloromethane (3 x 150 cm^3). The organic extracts were washed with water (3 x 100 cm^3) and sodium thiosulfate solution (0.2 M, 80 cm^3). The solution was dried ($MgSO_4$) and evaporated to dryness to give 3,5-diiodo-4-hydroxybenzaldehyde as colourless crystals (2.478 g, 68 %); m.p. 195-197 °C (lit.[43] 199-200 °C); ν_{max} (nujol) 3194, 1710, 1675, 1400 and 864 cm^{-1}; δ_H (250 MHz, $CDCl_3$) 9.73 (1 H, s, CHO) and 8.21 (2 H, s, ArH); δ_C (62.50 MHz, $CDCl_3$) 187.56 (CHO), 175.57 (ArCOH), 140.95 (ArCH), 132.85 (Ar*C*-CHO) and 82.58 (ArCI).

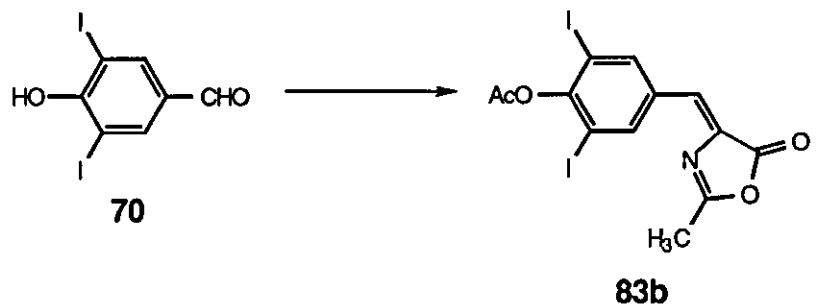
4-(4-Acetoxy-3,5-diiodobenzylidene)-2-phenyl-5-oxazolone **83a**



70 **83a**

A mixture of *N*-benzoylglycine (0.359 g, 2.17 mmol), 3,5-diiodo-4-hydroxybenzaldehyde (0.733 g, 1.96 mmol) and sodium acetate (8.432 g, 102.82 mmol) was dissolved in acetic anhydride (150 cm^3) and heated to reflux for 2 h. The reaction was cooled, filtered, and washed with water to remove the sodium acetate. The crude product was recrystallised from toluene to give 4-(4-acetoxy-3,5-diiodobenzylidene)-2-phenyl-5-oxazolone as yellow crystals (0.410g, 56 %); (Found: C, 38.59; H, 1.90; N, 2.57. $C_{18}H_{11}I_2NO_4$ requires C, 38.67; H, 1.98; N, 2.51 %); m.p. 238-241 °C (lit.[101] 242-242.5 °C); ν_{max} (nujol) 1805, 1765, 1660 and 1590 cm^{-1}; δ_H (250 MHz, $CDCl_3$) 8.59 (2 H, s, ArH), 8.10 (2 H, dd, *J* 1.6, 8.4 Hz, ArH), 7.60-7.47 (3 H, m, ArH), 6.96 (1 H, s, =CH) and 2.38 (3 H, s, CH_3); δ_C (62.50 MHz, $CDCl_3$) 167.07, 166.76, 164.83 (C-5 oxazolone, CH_3CO, ArCOAc), 153.08 (C-2 oxazolone), 142.63 (ArCH), 134.90, 134.76 (ArC, C-4 oxazolone), 133.81 (ArCH), 129.00 (ArCH), 128.55 (ArCH), 126.43 (alkene CH), 124.93 (ArC-oxazolone), 90.49 (ArCI) and 21.24 (CH_3CO).

4-(4-Acetoxy-3,5-diiodobenzylidene)-2-methyl-5-oxazolone **83b**

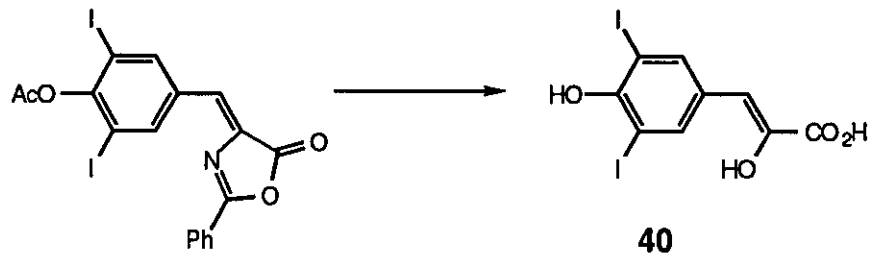


70 **83b**

The oxazolone 83b was prepared using the same method as described above using *N*-acetylglycine (1.932 g, 16.53 mmol), 3,5-diiodo-4-hydroxybenzaldehyde (5.983 g, 15.92 mmol), sodium acetate (16.326 g, 199.09 mmol) and acetic anhydride (200 cm^3). Recrystallisation of the reaction mixture from toluene gave

the 4-(4-acetoxy-3,5-diiodobenzylidene)-2-methyl-5-oxazolone as yellow crystals (4.568 g, 57 %); m.p. 225-227 °C (lit.[43] 231-233 °C); (Found M^+, 496.8625. $C_{13}H_9I_2NO_4$ requires M^+, 496.8625); ν_{max} (liquid paraffin) 1756, 1661, 1558, 1161 and 894 cm^{-1}; δ_H (250 MHz, $CDCl_3$) 8.52 (2 H, s, ArH), 6.90 (1 H, s, =CH), 2.44 (3 H, s, CH_3), 2.43 (3 H, s, CH_3); δ_C (62.50 MHz, $CDCl_3$) 167.99, 167.42, 167.33 (C-5 oxazolone, CH_3CO, Ar*C*OAc), 153.47 (C-2 oxazolone), 142.86 (ArCH), 134.72, 134.60 (ArC, C-4 oxazolone), 126.69 (=CH), 91.11 (ArCI), 21.76 ($COCH_3$) and 16.18 (CH_3-oxazolone); *m/z* (E.I.) 496 (5, M^+), 456 (8, M^+ -$NCCH_3$) and 385 (43, M^+ -$NCCH_3$, CH_2CCO_2H).

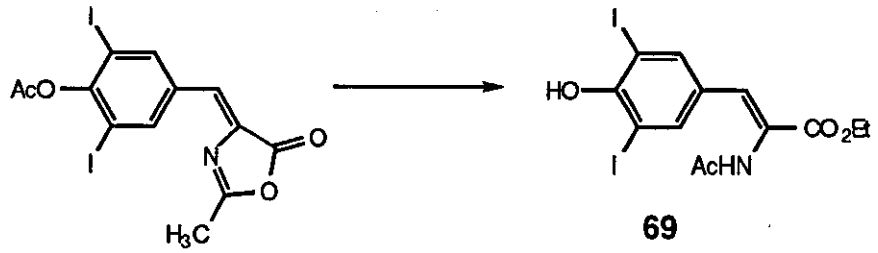
3,5-Diiodo-4-hydroxyphenylpyruvic acid **40**



83a **40**

4-(4-Acetoxy-3,5-benzylidene)-2-phenyl-5-oxazolone (0.223 g, 0.42 mmol) was dissolved in glacial acetic acid (160 cm^3) and hydrochloric acid (6 M, 40 cm^3) was added and the reaction heated to reflux for 5 h. The reaction was cooled and placed in an ice bath. The resulting crystals were filtered, washed with water, then dried to give the 3,5-diiodo-4-hydroxyphenylpyruvic acid as a colourless powder (0.135 g, 78 %); m.p. 222-228 °C (decomposition) (lit.[43] 215-220 °C); (Found M^+ 431.8401. $C_9H_6I_2O_4$ requires M^+, 431.8359); ν_{max} (nujol) 3525, 3050, 1720, 1675, 1550, 1460 and 1260 cm^{-1}; δ_H (250 MHz, D_4-methanol) 8.16 (2 H, s, ArH) and 6.27 (1 H, s, =CH); δ_C (62.50 MHz, D_4-methanol) 168.34 (CO_2H), 143.05 (ArCOH), 142.73 ($CHC(OH)CO_2H$), 142.10 (ArCH), 133.24 (Ar*C*-CH), 108.76 (=CH) and 85.33 (ArCI); *m/z* (E.I.) 432 (63, M^+) and 306 (30,M^+ -I).

Ethyl 3-(3,5-diiodo-4-hydroxyphenyl)-2-acetamidopropenoate **69**

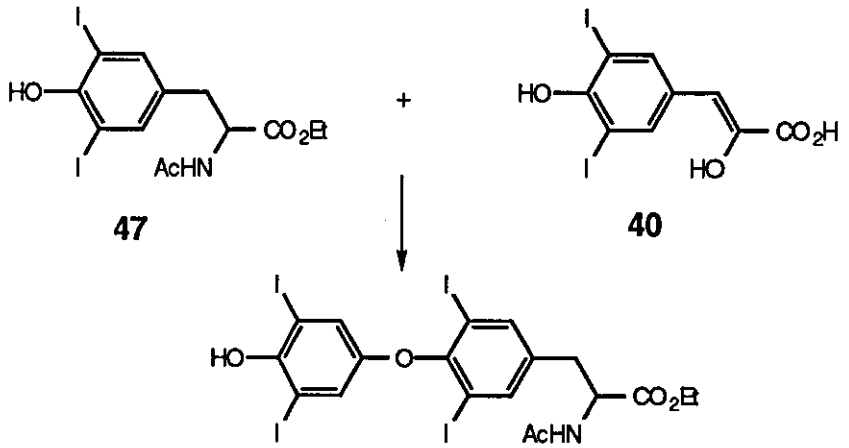


83b **69**

4-(4-Acetoxy-3,5-diiodobenzylidene)-2-methyl-5-oxazolone (0.305 g, 0.61 mmol) was dissolved in anhydrous ethanol (150 cm^3) and sodium hydride added (0.034 g, 1.41 mmol). The reaction was stirred for 3 h, water (30 cm^3) added and the reaction evaporated to dryness. The remaining solid was dissolved in ethyl acetate (50 cm^3) and hydrochloric acid solution (0.1 M, 30 cm^3). The aqueous layer was extracted with ethyl acetate (2 x 50 cm^3). The organic extracts were washed with water (2 x 50 cm^3), dried ($MgSO_4$) and evaporated to dryness. Flash column chromatography of the crude product (dichloromethane : ethyl acetate, 8:1) gave the *ethyl 3-(3,5-diiodo-4-hydroxyphenyl)-2-acetamidopropenoate* as a colourless solid (0.297 g, 97 %); m.p. 190-194 °C; (Found: C, 31.04; H, 2.48; N, 2.87. $C_{13}H_{13}I_2NO_4$ requires C, 31.16; H, 2.61; N, 2.79), (Found M^+, 500.8941. $C_{13}H_{13}I_2NO_4$ requires M^+, 500.8938); ν_{max}(nujol) 3442, 3234, 1664, 1653, 1522, 1310 and 1208 cm^{-1}; δ_H (250 MHz, D_6-acetone) 8.73 (1 H, s, NH), 8.05 (2 H, s, ArH), 7.14 (1 H, s, =CH), 4.21 (2 H, q, *J* 7.1 Hz, CO_2CH_2), 2.09 (3 H, s, $COCH_3$) and 1.28 (3 H, t, *J* 7.1 Hz, CH_2CH_3); δ_C (100.62 MHz, D_6-acetone) 169.49 (CO_2Et), 165.60 ($NHCOCH_3$), 156.64 (ArCOH), 141.82 (ArCH), 131.29 (Ar*C*-CH), 128.89 (=CH), 127.44 (=C), 83.99 (ArCI), 61.77 (CO_2CH_2), 22.83 ($COCH_3$) and 14.49 (CH_2CH_3); *m/z* (E.I.) 500 (11, M^+), 459 (32, M^+ - $COCH_3$) and 385 (16, M^+-$COCH_3$, $CO_2C_2H_5$).

Coupling of* N-*acetyl-3,5-diiodotyrosine ethyl ester with 3,5-diiodo-4-hydroxyphenylpyruvic acid at ambient temperature and pressure

General procedure

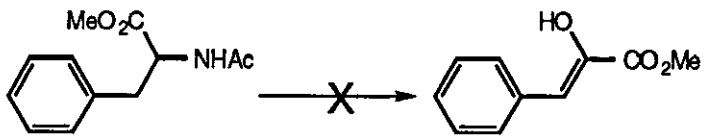


N-Acetyl-3,5-diiodotyrosine ethyl ester (1.006 g, 2.00 mmol) was dissolved in a mixture of dichloromethane (50 cm^3) and phosphate buffer (0.003 M, pH 7.5, 20 cm^3). The pH was adjusted to pH 7.7 with sodium hydroxide solution (1 M). 3,5-Diiodo-4-hydroxyphenylpyruvic acid (1.290 g, 3.00 mmol) was added in small portions every 5-10 mins over 1 h. The pH was checked regularly during the additions and when necessary sodium hydroxide solution (1 M) was added to keep the pH between 7.6 and 7.8. Oxygen was bubbled through the mixture until the additions were complete, after this time the oxygen was bubbled through the reaction mixture until the organic solvent had evaporated and the reaction was left stirring in air for a further 18 h. The reaction was acidified to pH 4 with hydrochloric acid solution (4 M). The aqueous layer was extracted with ethyl acetate (3 x 50 cm^3), washed with saturated ammonium chloride solution (2 x 50 cm^3), dried ($MgSO_4$) and evaporated to dryness. The products and recovered starting material were identified by TLC and characterised by ^1H NMR spectroscopy. The ratios and therefore relative yields were calculated from the integration of the signals arising from the ^1H NMR spectrum and the HPLC traces.

The general procedure for the coupling of *N*-acetyl-3,5-diiodotyrosine ethyl ester with 3,5-diiodo-4-hydroxyphenylpyruvic acid at ambient temperature and pressure was followed, however, in some reactions ethanol was used in place of dichloromethane. In these reactions the reaction mixture was evaporated to dryness prior to being taken up in ethyl acetate and hydrochloric acid solution (0.1 M) for the extraction. In some reactions a borate buffer (0.1 M, pH 8.3) was used in place

of the phosphate buffer and the pH in these reactions was kept between 7.6 and 8.3 throughout the reaction.

Attempted oxidation of the alanine side chain of* N-*acetylphenylalanine methyl ester using potassium ferricyanide



95 **97**

N-Acetylphenylalanine methyl ester (0.393 g, 1.70 mmol) in dichloromethane (100 cm^3) was added to a solution of potassium ferricyanide (1.172 g, 3.52 mmol) and sodium hydroxide (0.242 g, 6.05 mmol) in water (100 cm^3). The reaction mixture was stirred for 2 h, after which the organic and aqueous layers were separated. The aqueous layer was acidified to pH 4 with hydrochloric acid solution (2 M), and extracted with dichloromethane (30 cm^3). The organic extracts were washed with water (4 x 30 cm^3), dried ($MgSO_4$) and evaporated to dryness to give colourless crystals of *N*-acetylphenylalanine methyl ester (0.349 g, 89 %). The product was identified by TLC and characterised by ^1H NMR spectroscopy and was found to be consistent with authentic material.

Attempted oxidation of the side chain of* N-*acetylphenylalanine methyl ester using manganese(III) triacetate and manganese(III) triacetylacetonate

General procedure

N-Acetylphenylalanine methyl ester (0.331 g, 1.50 mmol) was dissolved in methanol (100 cm^3) under an atmosphere of nitrogen. To this solution was added the manganese(III) oxidant (3.00 mmol) and the reaction was stirred for 24 h. The solution was filtered to remove the manganese(III) oxidant and evaporated to dryness. The remaining solid was taken up in dichloromethane (30 cm^3) and water (30 cm^3), the aqueous layer was extracted with dichloromethane (2 x 30 cm^3) and the organic extracts were washed with water (5 x 30 cm^3), dried ($MgSO_4$) and evaporated to dryness. *N*-Acetylphenylalanine methyl ester was recovered as colourless crystals (0.293 g, 89 %). The product was identified by TLC and characterised by ^1H NMR spectroscopy and was found to be consistent with authentic material.

The general procedure for attempted oxidation using manganese(III) oxidants was repeated under varying conditions. Some reactions were carried out using glacial acetic acid as the solvent, and in some cases the reactions were refluxed and either carried out open to the atmosphere, or with compressed air or oxygen bubbling through the reaction.

Attempted oxidation of the side chain of* N-*acetylphenylalanine methyl ester using manganese(II) diacetate dihydrate and manganese(II) sulfate

General procedure

N-Acetylphenylalanine methyl ester (0.221 g, 1.00 mmol) was added to a solution of manganese(II) diacetate dihydrate (0.352 g, 2.00 mmol) in glacial acetic acid (150 cm^3). The reaction was heated under reflux for 24 h with oxygen bubbling through the reaction solution. The reaction was then cooled and evaporated to dryness. The remaining solid was taken up in dichloromethane (30 cm^3) and water (30 cm^3). The aqueous layer was extracted with dichloromethane (3 x 30 cm^3), and the resulting organic extracts washed with water (4 x 40 cm^3), dried ($MgSO_4$) and evaporated to dryness. *N*-Acetylphenylalanine methyl ester was recovered as colourless crystals (0.198 g, 90 %). The product was identified by TLC and characterised by ^1H NMR spectroscopy and was found to be identical to authentic material.

The general procedure for the oxidation using manganese(II) diacetate dihydrate was followed using *N*-acetylphenylalanine methyl ester (0.221 g, 1.00 mmol) and manganese(II) sulfate (0.302 g, 2.00 mmol), in methanol (150 cm^3). Oxygen was bubbled through the solution and the reaction heated under reflux for 24 h. In all the reactions attempted *N*-acetylphenylalanine methyl ester was recovered as colourless crystals (0.198 g, 90 %). The product was identified by TLC and characterised by ^1H NMR spectroscopy and was found to be consistent with authentic material.

Attempted oxidation of the side chain of* N-*acetylphenylalanine ethyl ester using manganese(II) sulfate at high pressure

General procedure

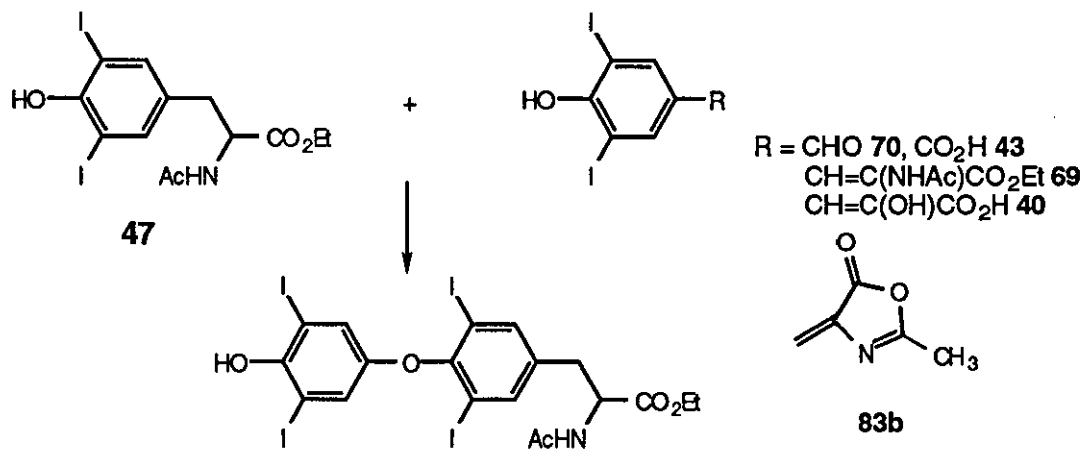
N-Acetylphenylalanine ethyl ester (1.880 g, 8.00 mmol) and manganese(II) sulfate (0.604 g, 4.00 mmol) were dissolved in ethanol (100 cm^3) and water (100 cm^3) and the mixture stirred. The solution was charged to a pressure vessel and pressurised with oxygen to 5 bar and heated at 60-65 °C for 20 h. The reaction was cooled, the pressure released and the solution evaporated to dryness. The remaining residue

was taken up in ethyl acetate (100 cm^3) and water (100 cm^3). The aqueous layer was extracted with ethyl acetate (2 x 50 cm^3) and the organic extracts washed with water (2 x 80 cm^3), dried ($MgSO_4$) and evaporated to dryness. *N*-Acetylphenylalanine ethyl ester was recovered as a colourless solid (1.729 g, 92 %), identified by TLC and characterised by ^1H NMR spectroscopy and was found to be constistent with authentic material.

The general procedure for the attempted oxidation using manganese(II) sulfate at high pressure was repeated using *N*-acetyltyrosine ethyl ester both at neutral pH and at pH 9. The reaction at pH 9 was carried out using borate buffer (0.1 M, pH 8.3) in place of water, sodium hydroxide solution (0.05 M) was used to adjusted the pH to 9.5 and the reaction was neutralised prior to isolation of the products. The reaction did not yield any oxidised compounds. The starting material was recovered (88-95 %) and identified by TLC and characterised by ^1H NMR spectroscopy and was found to be consistent with authentic material.

Synthesis of* N-*acetylthyroxine ethyl ester: Coupling studies

General procedure



N-Acetyl-3,5-diiodotyrosine ethyl ester (1.006 g, 2.00 mmol) and a 4-substituted 2,6-diiodophenol (40, 43, 69 or 70) (3.00 mmol) were dissolved in ethanol (150 cm^3) and water (100 cm^3) and a catalytic quantity of boric acid and manganese(II) sulfate added with stirring. Sodium hydroxide solution (2 M) was used to adjust the pH to 9.5. When the pH had stabilised at 9.5 for 10 minutes the solution was charged to a pressure vessel, heated to between 55-65 °C and pressurised to 5 bar with oxygen. The reaction was kept under these conditions for 20 h, cooled and the

pressure released. The final reaction pH was measured and the reaction solution was evaporated to dryness. The remaining solid was taken up in ethyl acetate (100 cm^3) and hydrochloric acid solution (0.01 M, 100 cm^3), and the aqueous layer was washed with ethyl acetate (3 x 50 cm^3). The organic extracts were washed with hydrochloric acid solution (0.01 M, 2 x 100 cm^3), dried ($MgSO_4$) and evaporated to dryness to give a mixture of the starting materials and *N*-acetylthyroxine ethyl ester. The yield was determined by HPLC, and the product was identified by ^1H NMR spectroscopy in comparison with the following data from authentic material. M.p. 206-211 °C; ν_{max} (dichloromethane) 3401, 3277, 1708, 1636, 1545, 1180 and 911 cm^{-1}; δ_H (250 MHz, $CDCl_3$: D_6-DMSO, 20:1) 9.08 (1 H, s, OH), 8.29 (1 H, d, *J* 7.5 Hz, NH), 7.76 (2 H, s, ArH), 7.06 (2 H, s, ArH), 4.56 [1 H, dt, *J* 7.5, 8.2 Hz, $CH_2CH(NHAc)CO_2Et$], 4.14-4.03 (2 H, m, CO_2CH_2), 3.00 (1 H, dd, *J* 6.0, 13.8 Hz, Ar-CH_2), 2.89 (1 H, dd, *J* 8.1, 13.8 Hz, Ar-CH_2), 1.89 (3 H, s, $COCH_3$) and 1.21 (3 H, t, *J* 7.1 Hz, CH_2CH_3); δ_C (100.62 MHz, $CDCl_3$) 171.08, 169.58 (CO_2Et, $COCH_3$), 152.57, 150.12, 149.48 (ArCOCAr, ArCOH), 141.22 (ArCH), 137.73 (Ar*C*-CH_2), 126.01 (ArCH), 90.56, 81.51 (ArCI), 62.00 (CO_2CH_2), 53.20 [*C*H(NHAc)CO_2Et), 36.57 (Ar-CH_2), 23.18 ($COCH_3$) and 14.35 (CH_2CH_3).

Atmospheric pressure and room temperature reactions were carried out alongside each pressure reaction and chemically were treated identically to the pressure experiment.

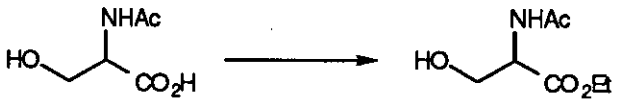
HPLC determination of the extent of reaction

Standards of the pure starting materials and *N*-acetylthyroxine ethyl ester in acetonitrile : water (9:1) were analysed by HPLC to obtain peak areas at known concentrations. The mobile phase was acetonitrile : water : orthophosphoric acid (520:479.5:0.5 cm^3) and compounds were detected by UV at 225 nm.. Samples of the reaction mixtures were dissolved in the sample solution (acetonitrile : water, 9:1) and analysed by HPLC. From the peak areas the ratios of compounds and hence relative yield of *N*-acetylthyroxine ethyl ester could be calculated.

6.5 Experimental to Chapter Five

Preparation of starting materials

N-*Acetylserine ethyl ester* **102**



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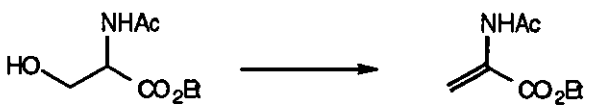
N-Acetylserine (2.327 g, 15.83 mmol) was dissolved in ethanol (150 cm^3) and sulfuric acid (catalytic quantity) was added. The reaction was heated under reflux for 6 h, cooled and evaporated to dryness. The resulting oil was taken up in ethyl acetate (100 cm^3) and concentrated brine solution (100 cm^3). The aqueous layer was washed with ethyl acetate (10 x 50 cm^3) and dichloromethane (5 x 50 cm^3). The organic extracts were dried ($MgSO_4$) and evaporated to dryness. Flash column chromatography of the crude product (dichloromethane : ethyl acetate, 1:1) yielded the *N*-acetylserine ethyl ester as a colourless oil (1.936 g, 69%); (Found MH^+, 176.0925. $C_7H_{13}NO_4$ requires M^+, 736.0923); ν_{max} (film) 3361, 2925, 1738, 1659, 1377 and 1148 cm^{-1}; δ_H (250 MHz, $CDCl_3$) 6.83 (1 H, d, *J* 7.1 Hz, NH), 4.62-4.58 (1 H, m, CH_2CH), 4.20 (2 H, q, *J* 7.1 Hz, CO_2CH_2), 3.95-3.84 (2 H, m, CH_2CH), 2.03 (3 H, s, $COCH_3$) and 1.27 (3 H, t, *J* 7.1 Hz, CH_2CH_3); δ_C (100.62 MHz, $CDCl_3$) 171.37, 171.02 ($COCH_3$, CO_2Et), 63.43, 62.22 (CO_2CH_2, CH_2OH), 55.23 (CH_2CH), 23.32 ($COCH_3$) and 14.46 (CH_2CH_3); *m/z* (E.I.) 176 (100, MH^+), 158 (15, $M^+ - H_2O$), 145 (20, $M^+ - C_2H_6$), 134 (12, $COCH_3$), 102 (50, $M^+ - CO_2$, C_2H_6) and 99 (22, $M^+ - H_2O$, $NHCOCH_3$)

Ethyl 2-acetamidopropenoate **100**



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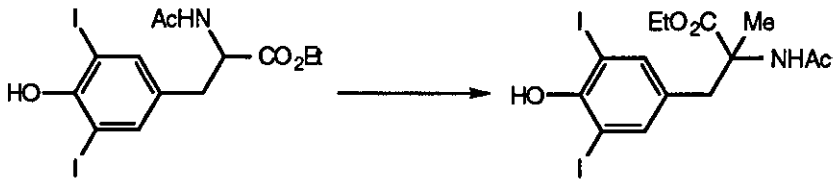
N-Acetylserine ethyl ester (0.105 g, 0.60 mmol) was dissolved in acetonitrile (60 cm^3), and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (0.135 g, 7.06 mmol) and copper(I) chloride (0.017g, 0.17 mmol) were added. The was reaction stirred at 40 °C for 24 h and evaporated to dryness. The remaining oil was taken up in ethyl acetate (40 cm^3) and washed with sodium hydrogen carbonate solution (0.2 M, 2 x 50 cm^3) and water (2 x 50 cm^3), dried ($MgSO_4$) and evaporated

to dryness. Flash column chromatography (dichloromethane : ethyl acetate, 5:1) yielded the ethyl 2-acetamidopropenoate as a colourless oil (0.073 g, 78 %); (Found M^+, 157.0739. $C_7H_{11}NO_3$ requires M^+, 157.0739); ν_{max} (film) 3365, 3024, 1695, 1630 and 1039 cm^{-1}; δ_H (250 MHz, $CDCl_3$) 7.74 (1 H, s, NH), 6.58 (1 H, s, =CH), 5.88 (1 H, s, =CH), 4.29 (2 H,q, *J* 7.1Hz, CO_2CH_2), 2.12 (3 H, s, $COCH_3$) and 1.34 (3 H, t, *J* 7.1 Hz, CH_2CH_3); δ_C (100.62 MHz, $CDCl_3$) 167.95, 163.30 ($COCH_3$, CO_2Et), 130.20 (CH_2C), 107.51 (CH_2C), 61.37 (CO_2CH_2), 23.82 ($COCH_3$) and 13.23 (CH_2CH_3); *m/z* (E.I.) 157 (61, M^+), 115 (100, M^+ -$COCH_3$) and 71 (98, M^+ -$NHCOCH_3$, C_2H_4).

Ethyl 2-acetamido-3-(3,5-diiodo-4-hydroxyphenyl)-2-methylpropionate **103**



N,O-*Diacetyl-3,5-diiodotyrosine ethyl ester* **106**

N-Acetyl-3,5-diiodotyrosine ethyl ester (2.697 g, 5.36 mmol) was added to sodium hydrogen carbonate solution (2 M, 300 cm^3) and stirred for 30 mins. Acetic anhydride (0.764 g, 7.49 mmol) was added and the reaction stirred for a further 3 h. The reaction was neutralised with hydrochloric acid solution (0.1 M) and extracted with dichloromethane (3 x 100 cm^3). The dichloromethane extracts were washed with sodium hydroxide solution (1 M, 2 x 100 cm^3) to remove any remaining free phenol, and with water (3 x 100 cm^3). The organic extracts were dried ($MgSO_4$) and evaporated to dryness to yield the N,O-*diacetyl-3,5-diiodotyrosine ethyl ester* as a colourless solid (1.573 g, 53 %); m.p. 114-116 °C; (Found M^+, 544.9192. $C_{15}H_{17}I_2NO_5$ requires M^+, 544.9196); ν_{max} (solid) 3405, 2988, 1720, 1652 and 1548 cm^{-1}; δ_H (250 MHz, $CDCl_3$) 7.49 (2 H, s, ArH), 6.04 (1 H, d, *J* 7.5 Hz, NH), 4.73-4.70 [1 H, m, CH(NHAc)CO_2Et], 4.14-4.08 (2 H, m, CO_2CH_2), 2.96 (2 H, d, *J* 5.8 Hz, Ar-CH_2), 2.32 (3 H, s, $OCOCH_3$), 1.96 (3 H, s, $NHCOCH_3$) and 1.19 (3 H, t, *J* 7.1 Hz, CH_3); δ_C (100.62 MHz, $CDCl_3$) 171.46, 170.16 ($NHCOCH_3$, CO_2Et) 167.16 ($OCOCH_3$), 151.04 (ArCOH), 140.68 (ArCH), 138.14 (Ar*C*-CH_2), 90.65 (ArCI), 62.37 (CO_2CH_2), 53.49 [*C*H(NHAc)CO_2Et], 36.64 (Ar-CH_2), 23.54 ($NHCOCH_3$), 21.72 ($OCOCH_3$) and 14.59 (CH_2CH_3); *m/z* (C.I.) 546 (100, MH^+), 419 (38, M^+ -I), 375 (28, M^+ -I, $COCH_3$), 292 (42, M^+ -2 x I) and 250 (26, M^+ -2 x I, $COCH_3$).

N-*Acetyl*-O-(tert-*butyldimethylsilyl)-3,5-diiodotyrosine ethyl ester* **105**

N-Acetyl-3,5-diiodotyrosine ethyl ester (2.066 g, 4.10 mmol) was dissolved in dichloromethane (120 cm^3) under an atmosphere of nitrogen. Imidazole (0.323 g, 4.74 mmol) was added and the reaction stirred and cooled to 0 °C. *tert*-Butyldimethylsilylchloride (0.617 g, 4.11 mmol) was added and the reaction stirred for 72 h and allowed to warm to room temperature. The reaction was washed with water (80 cm^3) and sodium hydroxide solution (1 M, 2 x 80 cm^3) to remove any unreacted phenol. The organic extract was washed with water (2 x 80 cm^3), dried ($MgSO_4$) and evaporated to dryness. Flash column chromatography (dichloromethane) yielded the N-*acetyl*-O-(tert-*butyldimethylsilyl)-3,5-diiodotyrosine ethyl ester* as a pale yellow solid (1.72 g, 68 %); m.p. 58-61 °C; (Found M^+, 616.9954. $C_{19}H_{29}I_2NO_4Si$ requires M^+, 616.9954); ν_{max} (solid) 3405, 2930, 2120, 1733, 1652, 1548, 1252, 1117 and 885 cm^{-1}; δ_H (250 MHz, $CDCl_3$) 7.52 (2 H, s, ArH), 6.23 (1 H, d, *J* 7.6 Hz, NH), 4.75-4.70 [1 H, m, C*H*(NHAc)CO_2Et], 4.22-4.12 (2 H, m, CO_2CH_2), 2.94-2.91 (2 H, m, Ar-CH_2), 2.00 (3 H, s, $COCH_3$), 1.25 (3 H, t, *J* 7.1 Hz, CH_2CH_3), 1.04 [9 H, s, $C(CH_3)_3$] and 0.44 (6 H, s, CH_3Si); δ_C (100.62 MHz, $CDCl_3$) 171.29, 169.71 (CO_2Et, $COCH_3$), 154.34 (ArCO), 141.11 (ArCH), 132.85 (Ar*C*-CH_2), 88.32 (ArCI), 61.79 (CO_2CH_2), 53.19 [*C*H(NHAc)CO_2Et], 35.83 (Ar-CH_2), 26.52 [$C(CH_3)_3$], 23.08 ($COCH_3$), 18.58 [$C(CH_3)_3$], 14.26 (CH_2CH_3) and 0.00 (CH_3Si); *m/z* (C.I.) 618 (44, M^+), 492 (33, M^+ -I), 451 (43, M^+ -I, $COCH_3$) and 364 (100, M^+ -2 x I).

α-Methylation of* O-*protected* N-*acetyl-3,5-diiodotyrosine ethyl ester

General procedure

*Ethyl 2-acetamido-3-[4-*O-(tert-*butyldimethylsilyl)-3,5-diiodophenyl]-2-methylpropionate* **107**

Diisopropylamide (2.260 g, 22.43 mmol) was stirred in THF (4 cm^3) and cooled to -78 °C. Butyl lithium (1.436 g, 22.43 mmol) was added and the reaction stirred for 30 minutes, until the solution was opaque in appearance. A solution of *N*-acetyl-*O*-(*tert*-butyldimethylsilyl)-3,5-diiodotyrosine ethyl ester (4.621 g, 7.48 mmol) in THF (20 cm^3) was added slowly and the reaction allowed to warm to -30 °C, cooled to -78 °C and stirred for a further 10 minutes. The nitrogen flow was stopped during the addition of methyl iodide (2.818 g, 19.98 mmol) and the reaction stirred for 30 minutes and warmed to room temperature. The reaction was quenched by the addition of a few drops of saturated ammonium chloride solution and allowed to stir 5 minutes. Further saturated ammonium chloride solution (50 cm^3) and diethyl ether (40 cm^3) were added. The aqueous layer was extracted with diethyl ether (2 x 50 cm^3). The resulting organic extracts were washed with water (2 x 70 cm^3), dried

($MgSO_4$) and evaporated to dryness to yield the *title compound* as a colourless solid (4.186 g, 88 %); m.p. 135-137 °C; (Found C, 37.75; H, 4.84; N, 2.36. $C_{20}H_{31}I_2NO_4Si$ requires C, 38.05; H, 4.95; N, 2.12); (Found M^+, 631.0110. $C_{20}H_{31}I_2NO_4Si$ requires M^+, 631.0110); ν_{max} (solid) 3405, 2930, 2102, 1733, 1652, 1548, 1252, 1117 and 885 cm^{-1}; δ_H (250 MHz, $CDCl_3$) 7.46 (2 H, s, ArH), 6.18 (1 H, s, NH), 4.24 (2 H, q, *J* 6.9 Hz, CO_2CH_2), 3.51 (1 H, d, *J* 13.6 Hz, Ar-CH_2), 3.00 (1 H, d, *J* 13.5 Hz, Ar-CH_2), 2.00 (3 H, s, $COCH_3$), 1.65 (3 H, s, CCH_3), 1.35 (3 H, t, *J* 7.1 Hz, CH_2CH_3), 1.06 [9 H, s, $C(CH_3)_3$] and 0.46 (6 H, s, CH_3Si); δ_C (100.62 MHz, $CDCl_3$) 173.56, 169.67 (CO_2Et, $COCH_3$), 154.09 (ArCO), 141.52 (ArCH), 133.45 (Ar*C*-CH_2), 88.27 (ArCI), 62.16 (CO_2CH_2), 61.34 (CH_2CCH_3), 38.29 (Ar-CH_2), 26.47 [$C(CH_3)_3$], 24.01 (CCH_3), 23.35 ($COCH_3$), 18.89 [$C(CH_3)_3$], 14.23 (CH_2CH_3) and 0.00 (CH_3Si); *m/z* (C.I.) 632 (100, MH^+), 506 (25, M^+ -I), 465 (22, M^+ -I, $COCH_3$) and 378 (66, M^+ -2 x I).

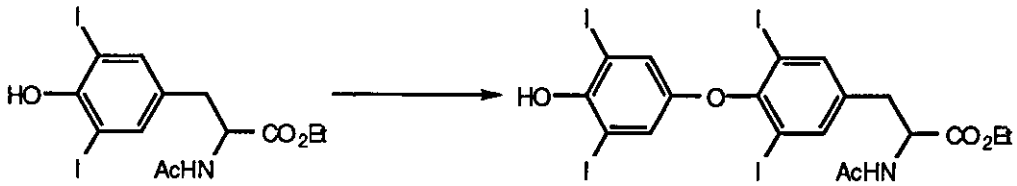
Ethyl 2-acetamido-3-(3,5-diiodo-4-hydroxyphenyl)-2-methylpropionate **103**
The ethyl 2-acetamido-3-[4-*O*-(*tert*-butyldimethylsilyl)-3,5-diiodophenyl]-2-methylpropionate (4.186 g, 6.63 mmol) was dissolved in THF (80 cm^3) and tetrabutylammonium fluoride (0.190 g, 6.63 mmol) was added as a solution in THF. The solution was stirred for 2 h , a small amount of water added, and the reaction evaporated to dryness. The resulting solid was dissolved in dichloromethane (80 cm^3) and hydrochloric acid solution (0.01 M, 100 cm^3). The aqueous layer was extracted with dichloromethane (2 x 70 cm^3) and the organic extracts washed with hydrochloric acid (2 x 100 cm^3), dried ($MgSO_4$) and evaporated to dryness. Flash column chromatography of the crude product (dichloromethane : ethyl acetate, 4:1) yielded the *title compound* as a pale yellow solid (3.263 g, 95 %); m.p. 66-68 °C; (Found M^+, 516.9246. $C_{14}H_{17}I_2NO_4$ requires M^+, 516.9253); ν_{max} (solid) 3381, 2982, 1737, 1657, 1544, 1181 and 883 cm^{-1}; δ_H (250 MHz, $CDCl_3$) 7.37 (2 H, s, ArH), 6.18 (1 H, s, NH/OH), 5.69 (1 H, s, NH/OH), 4.25 (2 H, q, *J* 7.1 Hz, CH_2CH_3), 3.55 (1 H, d, *J* 13.7 Hz, $ArCCH_2$), 3.03 (1 H, d, *J* 13.7 Hz, $ArCCH_2$), 2.00 (3 H, s, $COCH_3$), 1.65 (3 H, s, CCH_3) and 1.36 (3 H, t, *J* 7.0 Hz, CH_2CH_3); δ_C (100.62 MHz, $CDCl_3$) 174.00, 170.14 (CO_2Et, $COCH_3$), 152.93 (ArCOH), 140.82 (ArCH), 133.26 (Ar*C*-CH_2), 82.30 (ArCI), 62.64 (CO_2CH_2), 61.86 (CH_2CCH_3), 38.86 (Ar-CH_2), 24.46 , 23.77 (CCH_3, $COCH_3$) and 14.68 (CH_2CH_3); *m/z* (C.I.) 518 (22, MH^+), 392 (32, M^+ -I) and 266 (100, M^+ -2 x I).

The general procedure for α-methylation of *O*-protected *N*-acetyl-3,5-diiodotyrosine ethyl ester was followed using diisopropylamine (0.062 g, 0.62 mmol) and n-butyl lithium (0.048 g, 0.60 mmol) in THF (2 cm^3), followed by the addition of *N,O*-diacetyl-3,5-diiodotyrosine ethyl ester (0.113 g, 0.207 mmol) in THF (8 cm^3).

Methyl iodide (0.127 g, 0.90 mmol) was added to yield ethyl 2-acetamido-3-(3,5-diiodo-4-hydroxyphenyl)-2-methylpropionate as yellow crystals (0.089 g, 83 %) which was characterised using ^{1}H NMR spectroscopy and m.p. to give data consistent with that reported above.

Pressurised coupling of* N-*acetyl-3,5-diiodotyrosine ethyl ester to form* N-*acetylthyroxine ethyl ester

General procedure



47 **46**

N-Acetyl-3,5-diiodotyrosine ethyl ester (1.006 g, 2 mmol) was dissolved in ethanol (150 cm^3) and water (100 cm^3) and a catalytic quantity of boric acid and manganese(II) sulfate was added with stirring. Sodium hydroxide solution (2 M) was used to adjust the pH to 9.5. When the pH had stabilised at 9.5 for 10 minutes the solution was charged to a pressure vessel. The reaction was heated to between 55-65 °C and pressurised to 5 bar with oxygen, and kept under these conditions for 20 h, cooled and the pressure released. The final reaction pH was measured and the reaction solution evaporated to dryness. The remaining solid was taken up in ethyl acetate (100 cm^3) and hydrochloric acid solution (0.01 M, 100 cm^3) and the aqueous layer washed with ethyl acetate (3 x 50 cm^3). The organic extracts were washed with hydrochloric acid solution (0.01 M, 2 x 100 cm^3), dried ($MgSO_4$) and evaporated to dryness to give a mixture of the starting materials and *N*-acetylthyroxine ethyl ester. The products were characterised, and the yield was determined, using HPLC and ^{1}H NMR spectroscopy as described in section 6.4.

Synthesis of oxygen-*para *coupled derivatives at atmospheric pressure

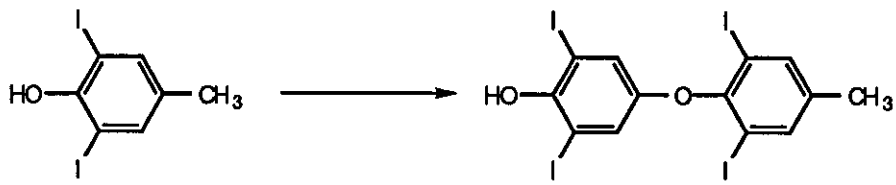
General procedure

N-*acetylthyroxine ethyl ester* **46**

N-Acetyl-3,5-diiodotyrosine ethyl ester (0.754 g, 1.5 mmol) was dissolved in ethanol (90 cm^3) and water (75 cm^3) and catalytic quantities of boric acid and manganese(II) sulfate added. Sodium hydroxide solution (0.5 M) was used to adjust the pH to 9.5. The reaction was heated under reflux for 48 h with oxygen bubbling through the solution throughout the reaction. The pH was checked regularly and

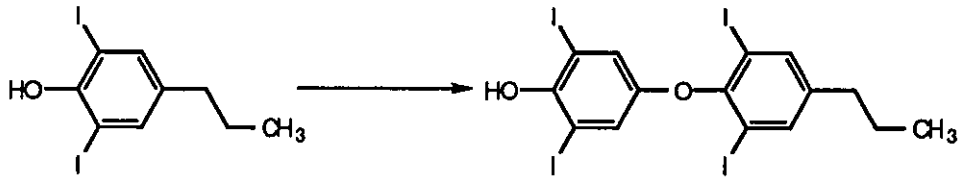
kept close to 9.5 by the addition of a little triethylamine or sodium hydroxide solution (0.5 M) whenever necessary. The reaction was cooled, evaporated to dryness and the remaining solid was taken up in ethyl acetate (100 cm^3) and hydrochloric acid solution (0.01 M, 100 cm^3) the aqueous layer washed with ethyl acetate (3 x 50 cm^3). The organic extracts were washed with hydrochloric acid solution (0.01 M, 2 x 100 cm^3), dried ($MgSO_4$) and evaporated to dryness to give a mixture of the starting materials and *N*-acetylthyroxine ethyl ester. The product was identified, and the yield determined, by HPLC and ^1H NMR spectroscopy in comparison with data from authentic material.

2,6-Diiodo-1-(3,5-diiodo-4-hydroxyphenoxy)-4-methylbenzene **109**



The general procedure for the synthesis of oxygen-*para* coupled derivatives was followed using 2,6-diiodo-4-methylphenol (1.338 g, 3.71 mmol) in ethanol (80 cm^3) and water (40 cm^3). Boric acid and manganese(II) sulfate (catalytic quantities) were added and sodium hydroxide solution (0.5 M) was used to adjust the reaction pH to 9.5. The reaction was heated under reflux with oxygen bubbling through the reaction solution for 48 h. Flash column chromatography of the crude product (light petroleum : dichloromethane, 7:3) yielded the *2,6-diiodo-1-(3,5-diiodo-4-hydroxyphenoxy)-4-methylbenzene* as a colourless solid (0.756 g, 58 %); m.p. 176-178 °C; (Found M^+, 703.6703. $C_{13}H_8I_4O_2$ requires M^+, 703.6694); ν_{max} (dichloromethane) 3476, 3058, 1586, 1435, 1183 and 853 cm^1; δ_H (250 MHz, $CDCl_3$) 7.66 (2 H, s, ArH), 7.12 (2 H, s, ArH), 5.46 (1 H, s, OH) and 2.31 (3 H, s, CH_3); δ_C (100.62 MHz, $CDCl_3$) 151.57, 150.73, 149.67 (ArCOCAr, ArCOH), 141.35 (ArCH), 139.78 (Ar*C*CH_3), 126.34 (ArCH), 90.82, 81.97 (ArCI) and 20.27 (CH_3); *m/z* (C.I.) 703 (21, M^+), 578 (10, M^+ -I), 450 (15, M^+ - 2 x I) and 324 (4, M^+ - 3 x I).

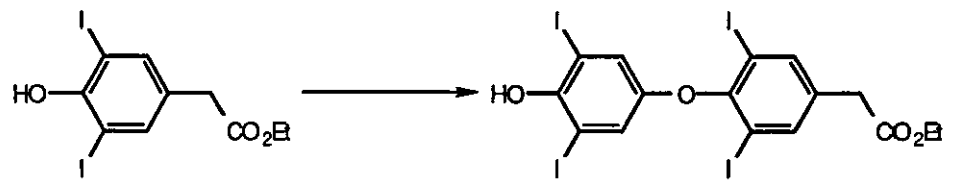
2,6-Diiodo-1-(3,5-diiodo-4-hydroxyphenoxy)-4-propylbenzene **110**



68 **110**

The general procedure for the synthesis of oxygen-*para* coupled derivatives was followed using 2,6-diiodo-4-propylphenol (1.123 g, 2.89 mmol) in ethanol (80 cm^3), water (40 cm^3) and a catalytic quantity of boric acid and manganese(II) sulfate added. Sodium hydroxide solution (0.5 M) was used to was adjust the reaction pH to 9.5. The reaction was heated under reflux with oxygen bubbling through the reaction solution for 48 h. Flash column chromatography of the crude product (light petroleum : dichloromethane, 4:1) yielded the *2,6-diiodo-1-(3,5-diiodo-4-hydroxyphenoxy)-4-propylbenzene* as a colourless oil (0.074 g, 7 %); (Found M^+, 731.7016. $C_{15}H_{12}I_4O_2$ requires M^+, 731.7016); ν_{max} (film) 3486, 2960, 2930, 1585, 1422, 1183 and 851 cm^{-1}; δ_H (400 MHz, $CDCl_3$) 7.58 (2 H, s, ArH), 7.04 (2 H, s, ArH), 5.38 (1 H, s, OH), 2.46 (2 H, t, *J* 7.3 Hz, $ArCCH_2$), 1.61-1.51 (2 H, m, CH_2CH_3) and 0.90 (3 H, t, *J* 7.3 Hz, CH_3); δ_C (100.62 MHz, $CDCl_3$) 151.65, 150.73, 149.66 (ArCOCAr, ArCOH), 144.55 ($ArC\text{-}CH_2$), 140.64(ArCH), 126.35 (ArCH), 90.85, 81.91 (ArCI), 36.78 ($Ar\text{-}CH_2$), 24.60 (CH_2CH_3) and 14.14 (CH_3); *m/z* (C.I.) 732 (7, M^+), 606 (4, M^+ -I) and 478 (2, M^+ - 2 x I).

2,6-Diiodo-1-(3,5-diiodo-4-hydroxyphenoxy)-4-(ethoxycarbonylmethyl)benzene **111**

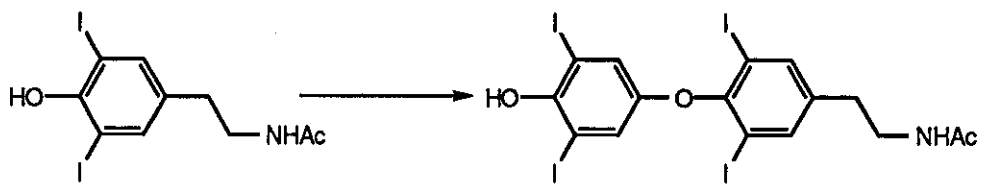


66 **111**

The general procedure for the synthesis of oxygen-*para* coupled derivatives was followed using ethyl 2-(3,5-diiodo-4-hydroxyphenyl)ethanoate (1.761 g, 4.08 mmol), ethanol (80 cm^3), water (40 cm^3) and catalytic quantities of boric acid and manganese(II) sulfate. Sodium hydroxide solution (0.5 M) was used to adjust the reaction pH to 9.5. The reaction was heated under reflux with oxygen bubbling through the reaction solution for 48 h. Flash column chromatography (dichloromethane : ethyl acetate, 9:1) yielded the *2,6-diiodo-1-(3,5-diiodo-4-*

hydroxyphenoxy)-4-(ethoxycarbonylmethyl)benzene as a colourless solid (0.853 g, 54 %); m.p. 72-75 °C; (Found M^+, 775.6914. $C_{16}H_{12}I_4O_4$ requires M^+, 775.6917); ν_{max} (dichloromethane) 3471, 3065, 1726, 1585, 1423, 1182 and 850 cm^{-1}; δ_H (250 MHz, $CDCl_3$) 7.81 (2 H, s, ArH), 7.11 (2 H, s, ArH), 5.47 (1 H, s, OH), 4.20 (2 H, q, *J* 7.1 Hz, CO_2CH_2), 3.57 (2 H, s, CH_2CO_2) and 1.29 (3 H, t, *J* 7.1 Hz, CH_2CH_3); δ_C (100.62 MHz, $CDCl_3$) 170.05 (CO_2Et), 153.52, 150.13, 149.14 (ArCOCAr, ArCOH), 140.97 (ArCH), 134.98 (Ar*C*-CH_2), 125.70 (ArCH), 90.11, 81.32 (ArCI), 61.17 (CO_2CH_2), 39.08 (Ar-CH_2) and 13.92 (CH_2CH_3); *m/z* (C.I.) 793 (100, MNH_4^+), 776 (6, M^+) and 668 (61, MNH_4^+-I).

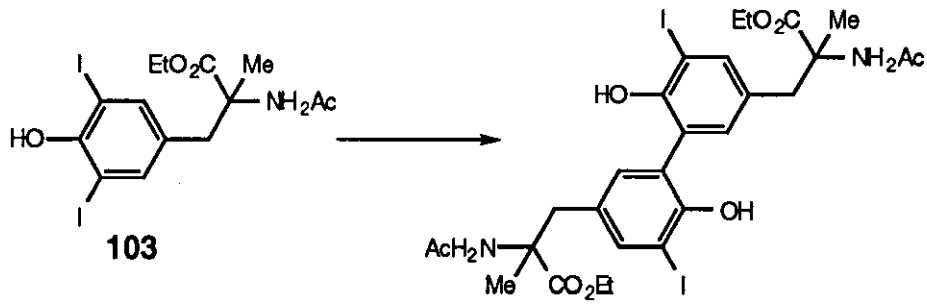
4-(2-Acetamidoethyl)-2,6-diiodo-1-(3,5-diiodo-4-hydroxyphenoxy)benzene **112**



67 **112**

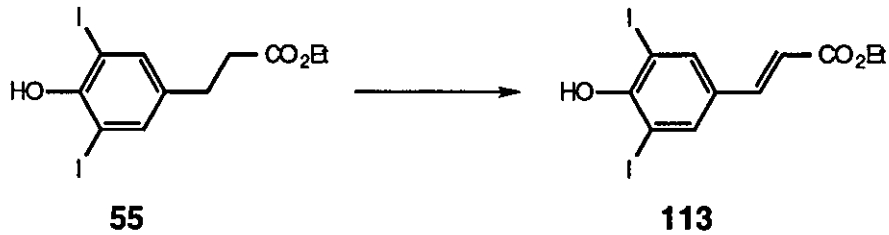
The general procedure for the synthesis of oxygen-*para* coupled derivatives was followed using *N*-acetyl-3,5-diiodotyramine (1.034 g, 2.40 mmol), ethanol (80 cm^3), water (40 cm^3) and catalytic quantities of manganese(II) sulfate and boric acid. Sodium hydroxide solution (0.5 M) was used to achieve and maintain pH 9.5 throughout the reaction. The reaction was heated under reflux with oxygen bubbling through the reaction solution for 48 h. Trituration of the crude product with ethyl acetate yielded the *4-(2-acetamidoethyl)-2,6-diiodo-1-(3,5-diiodo-4-hydroxyphenoxy)benzene* as a colourless solid (0.327 g, 37 %); m.p. 233-235 °C; (Found M^+, 774.6956. $C_{16}H_{13}I_4NO_3$ requires M^+, 774.7074); ν_{max} (liquid paraffin) 3358, 1637, 1534 and 1186 cm^{-1}; δ_H (400 MHz, D_6-acetone) 7.84 (2 H, s, ArH), 7.20 (2 H, s, ArH), 7.18 (1 H, s, NH/OH), 3.46-3.41 (2 H, m, CH_2NHAc), 2.80 (2 H, t, *J* 7.0 Hz, Ar-CH_2) and 1.84 (3 H, s, $COCH_3$); δ_C (100.62 MHz, D_6-acetone) 168.59 ($COCH_3$), 150.49, 150.34, 149.62 (ArCOCAr, ArCOH), 140.86 (Ar*C*-CH_2), 139.63, 124.54 (ArCH), 91.01, 86.99 (ArCI), 38.83 (Ar-CH_2), 32.71 (CH_2NHAc) and 21.93 ($CO\mathit{C}H_3$); *m/z* (C.I.) 776 (30, MH^+), 647 (6, M^+ -I) and 432 (10, M^+ -2 x I, CH_2CH_2NHAc).

Diethyl 2,2'-diacetamido-3,3'-(3,3'-biphenyl-4,4'-dihydroxy-5,5'-diiodo)-2,2'dimethyldipropionate **108**



The general procedure for the synthesis of oxygen-*para* coupled derivatives was followed using ethyl 3-(3,5-diiodo-4-hydroxyphenyl)-2-methylpropionate (0.848 g, 1.64 mmol), ethanol (50 cm^3), water (30 cm^3) and catalytic quantities of manganese(II) sulfate and boric acid. Sodium hydroxide solution (0.5 M) was used to adjust the reaction pH to 9.5. The reaction was heated under reflux with oxygen bubbling through the reaction solution for 48 h. Flash column chromatography of the crude product (dichloromethane : ethyl acetate, 4:1) yielded the *title compound* as yellow crystals (0.287 g, 45 %); m.p. 115-117 °C; (Found M^+, 780.0404. $C_{28}H_{34}I_2N_2O_8$ requires M^+, 780.0404); ν_{max} (dichloromethane) 3381, 2983, 1729, 1652, 1548, 1462, 1119 and 883 cm^{-1}; δ_H (250 MHz, $CDCl_3$) 7.43 (2 H, d, *J* 1.7 Hz, ArH), 6.85 (2 H, d, *J* 1.9 Hz, ArH), 6.21 (2 H, s, NH/OH), 4.23 (4 H, q, *J* 7.0 Hz, CO_2CH_2), 3.58 (2 H, d, *J* 13.7 Hz, $ArCCH_2$), 3.10 (2 H, d, *J* 13.7 Hz, Ar-CH_2), 1.97 (6 H, s, $COCH_3$), 1.63, 1.62 (2 x 3 H, s, CCH_3) and 1.30 (3 H, t, *J* 7.1 Hz, CH_2CH_3); δ_C (100.62 MHz, $CDCl_3$) 174.14, 170.34 ($COCH_3$, CO_2Et), 151.31 (ArCOH), 140.56 (ArCH), 133.50 (ArCH), 131.62 (Ar*C*-CH_2), 124.34 (ArCCAr), 86.66 (ArCI), 62.54 (CO_2CH_2), 61.61 (CCH_3), 39.67 (Ar-CH_2), 24.38, 23.74 (CCH_3, $COCH_3$) and 14.58 (CH_2CH_3); *m/z* (C.I.) 781 (29, M^+), 655 (72, M^+ -I) and 529 (29, M^+ -2 x I).

Ethyl 3-(3,5-diiodo-4-hydroxyphenyl)propenoate **113**



The general procedure for the synthesis of oxygen-*para* coupled derivatives was followed using ethyl 3-(3,5-diiodo-4-hydroxyphenyl)propionate (1.456 g, 3.27 mmol), ethanol (80 cm^3), water (40 cm^3) and catalytic quantities of boric acid and manganese(II) sulfate. Sodium hydroxide solution (0.5 M) was used to adjust the pH to 9.5. The reaction was heated under reflux with oxygen bubbling through the reaction solution for 48 h. Flash column chromatography (dichloromethane : ethyl acetate, 9:1) yielded the *ethyl 3-(3,5-diiodo-4-hydroxyphenyl)propenoate* as colourless crystals (0.769 g, 53 %); m.p. 104-106 °C; (Found M^+, 443.8719. $C_{11}H_{10}I_2O_3$ requires M^+, 443.8719); ν_{max} (liquid paraffin) 3460, 1687, 1624, 1577, 1199 and 1114, 856 cm^{-1}; δ_H (250 MHz, $CDCl_3$) 7.85 (2 H, s, ArH), 7.45 (1 H, d, *J* 15.9 Hz, $CHCO_2Et$), 6.29 (1 H, d, *J* 15,9 Hz, Ar-CH), 5.94 (1 H, s, OH), 4.25 (2 H, q, *J* 7.1 Hz, CO_2CH_2) and 1.32 (3 H, t, *J* 7.1 Hz, CH_3); δ_C (100.62 MHz, $CDCl_3$) 166.86 (CO_2Et), 155.35 (ArCOH), 141.12 (Ar-*C*H), 139.17 (ArCH), 131.48 (Ar*C*-CH), 118.79 (*C*HCO_2Et), 82.94 (ArCI), 61.03 (*C*H_2CH_3) and 14.68 (CH_3); *m/z* (C.I.) 445 (5, MH^+), 336 (16, MNH_4^+ -I), 319 (17, MH^+ -I) and 193 (100, MH^+ -2 x I).

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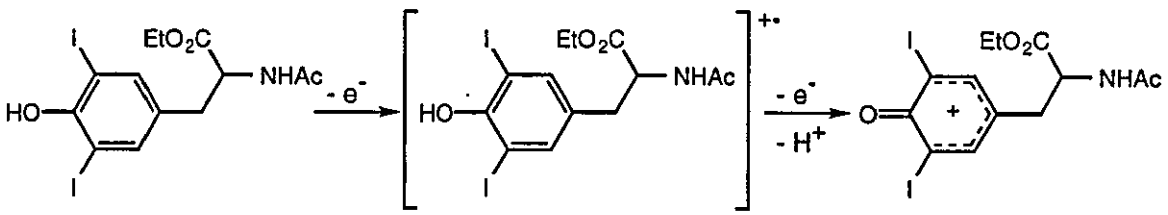
Appendix I

I.1 Cyclic Voltammograms of Phenols in acetonitrile

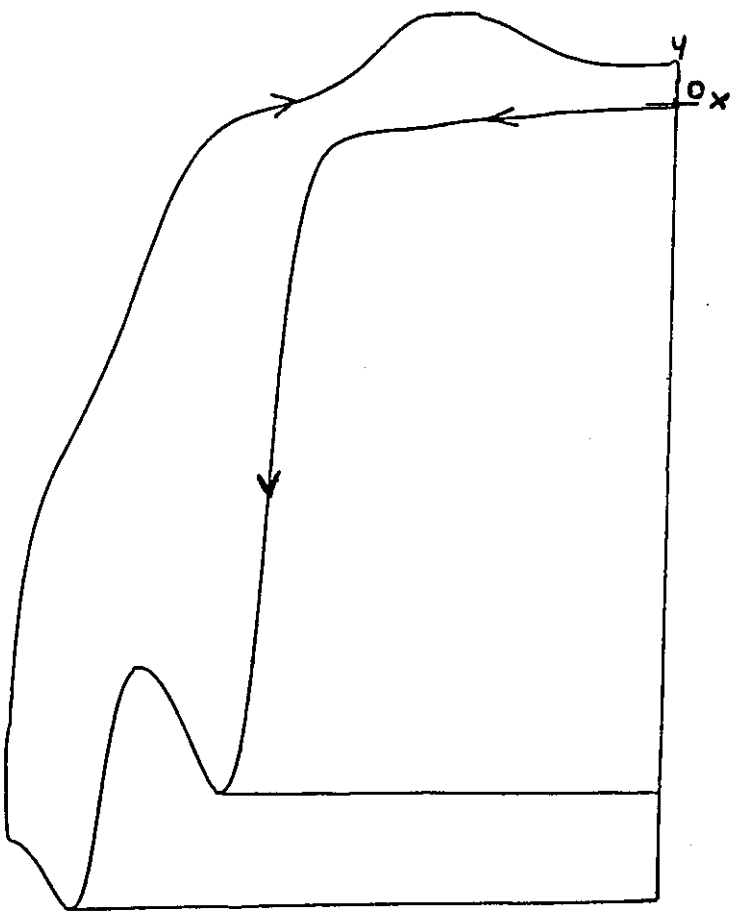
The cyclic voltammograms found in this section were scanned between approximately 0.00 and +1.80 V except where indicated. The Y scale setting was 10 mA/V, and scan speed was 0.150 V/s, and the setting of the chart recorder was X = 0.2 V/cm and Y = 5 mV/cm. Zero on the cyclic voltammograms indicates zero potential *versus* the saturated calomel electrode and zero current, and the arrow indicates the direction of the sweep.

I.1.1 *N*-Acetyl-3,5-diiodotyrosine ethyl ester 47



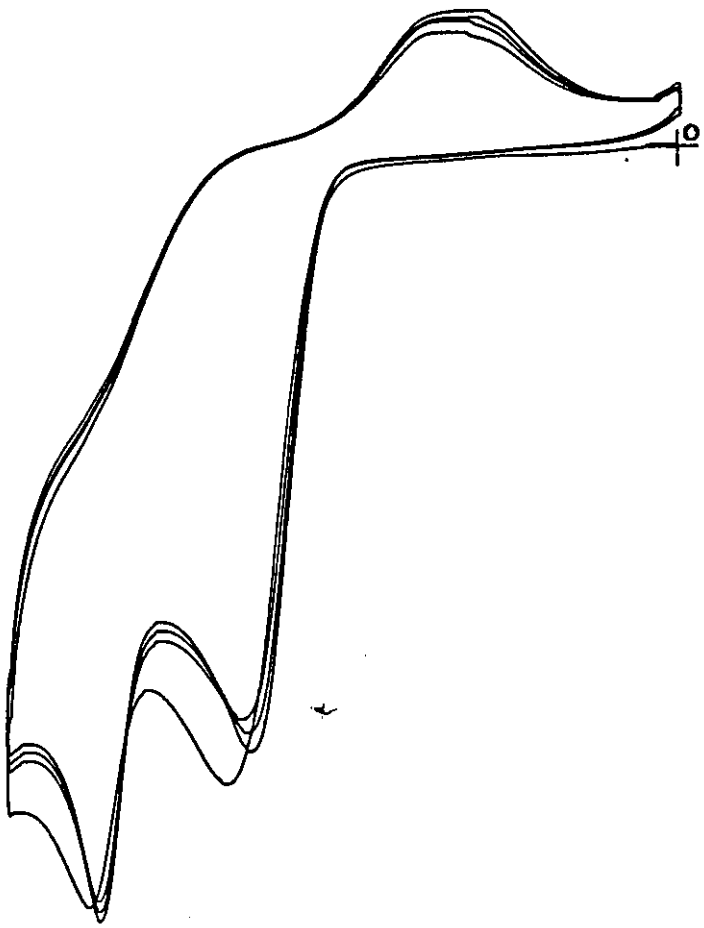
First scan

Ep = 1.20, 1.56 V

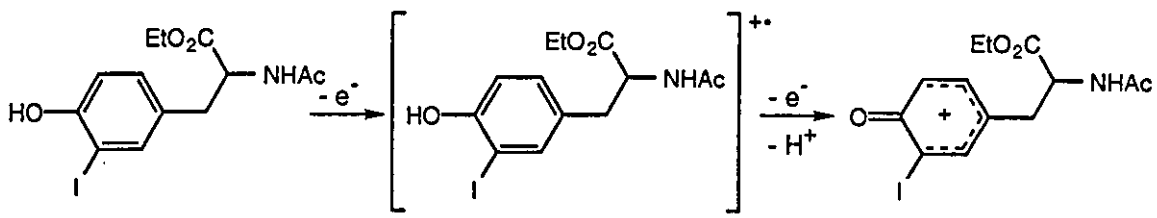


I.1.2 *N*-Acetyl-3,5-diiodotyrosine ethyl ester 47

Scans 2,3,4 and 5

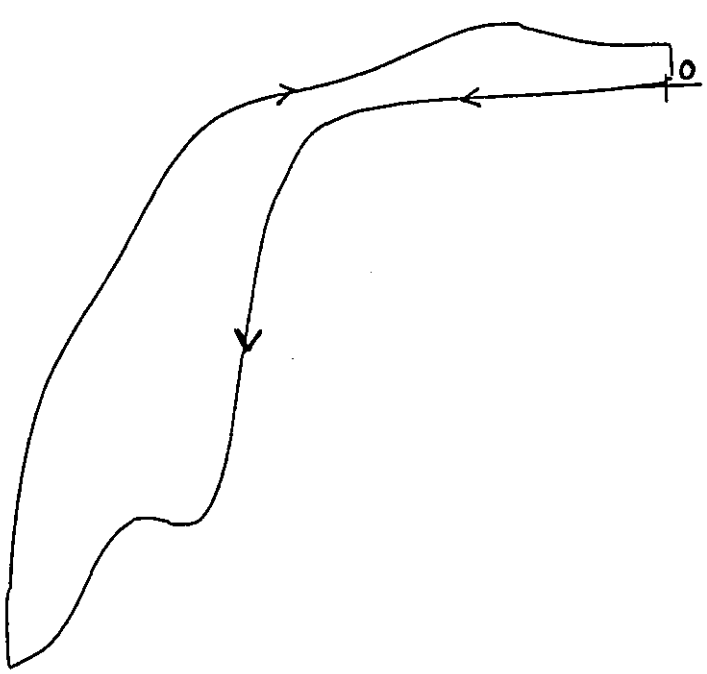


I.1.3 *N*-acetyl-3-iodotyrosine ethyl ester 64



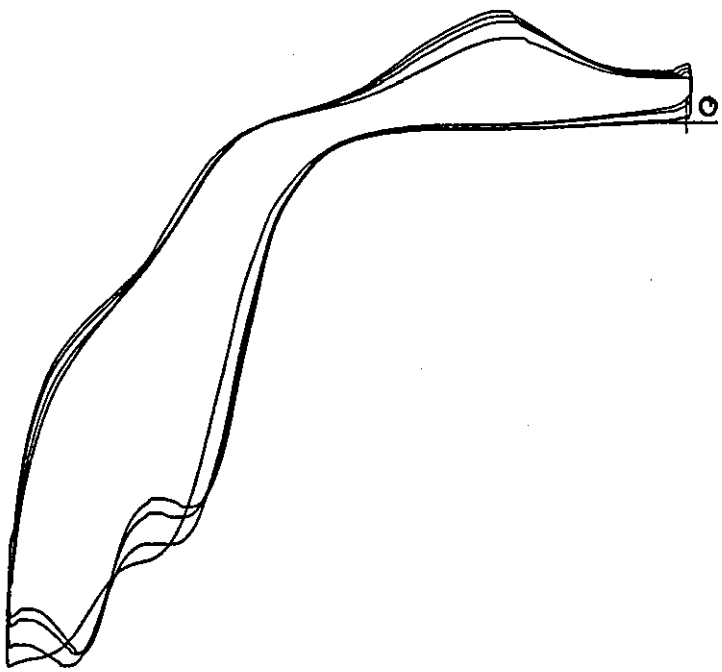
First scan

Ep = 1.30, 1.62 V

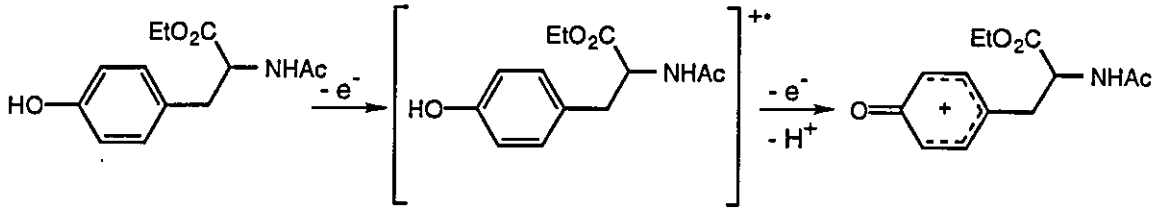


I.1.4 *N*-Acetyl-3-iodotyrosine ethyl ester 64

Scans 2,3,4 and 5

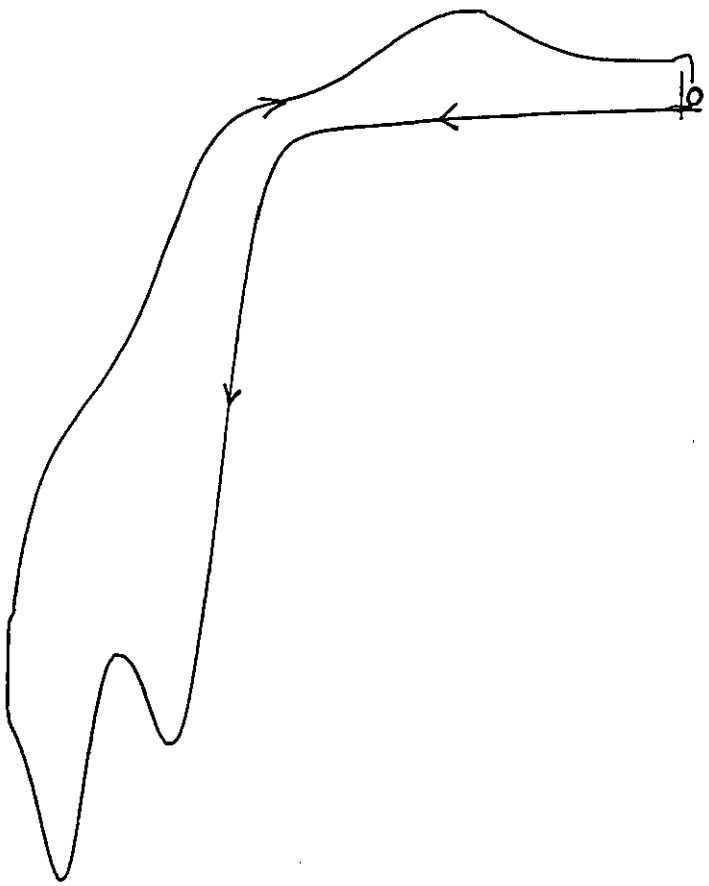


I.1.5 *N*-Acetyltyrosine ethyl ester 81



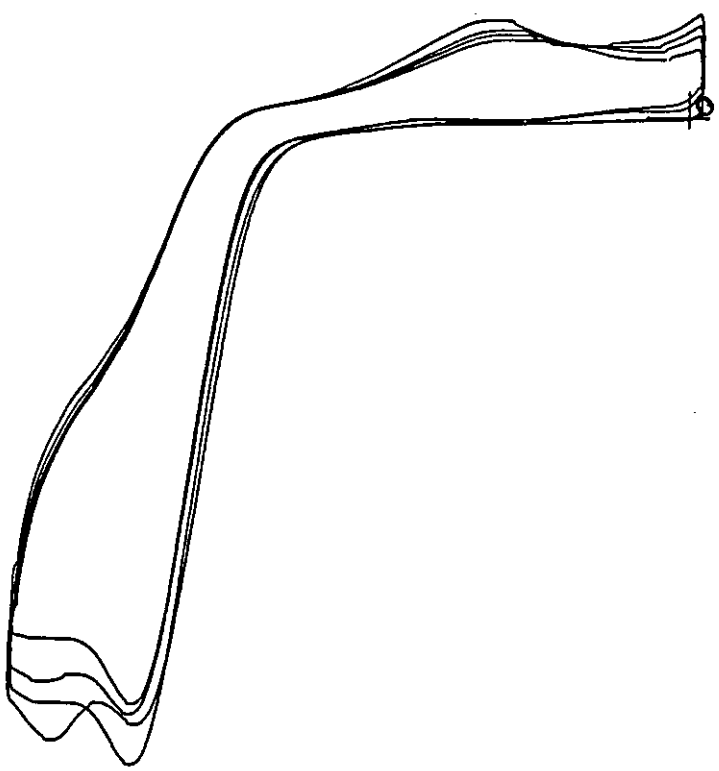
First scan

Ep = 1.35, 1.59 V

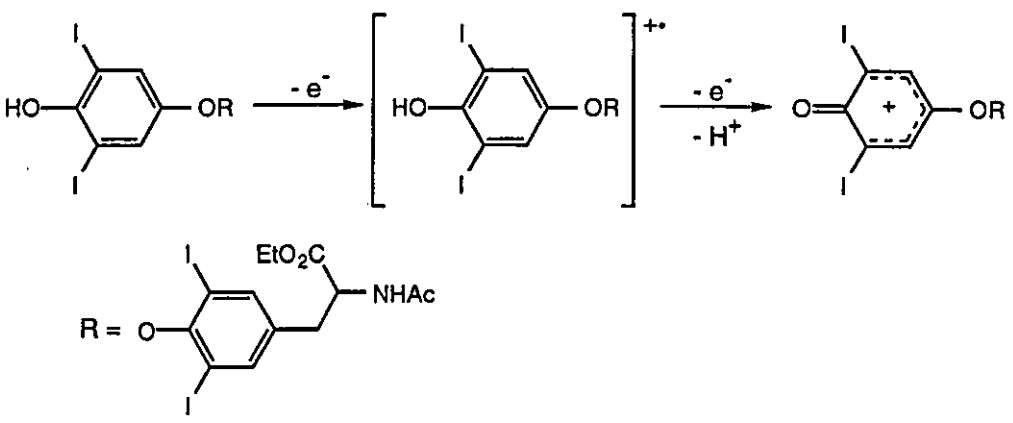


I.1.6. *N*-Acetyltyrosine ethyl ester 81

Scans 2,3,4 and 5

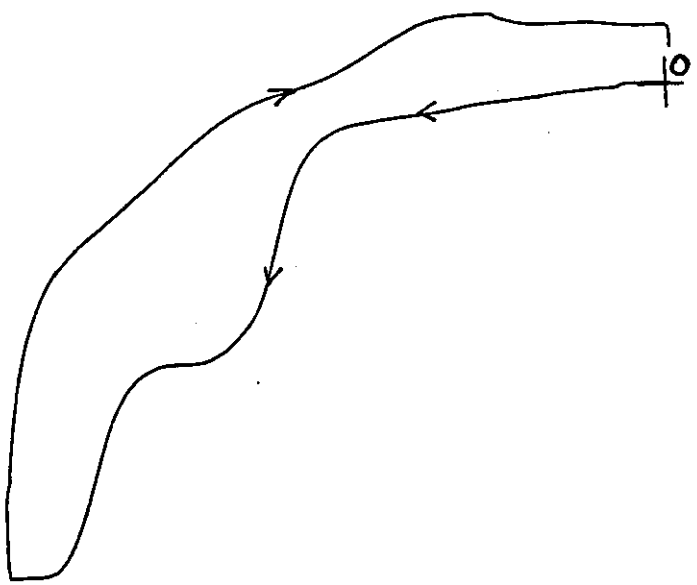


I.1.7 *N*-Acetylthyroxine ethyl ester 46



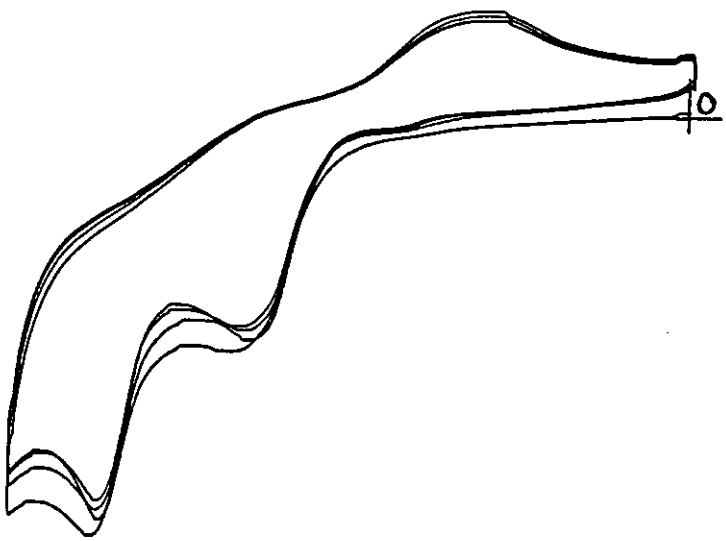
First scan

Ep = 1.25, 1.60 V

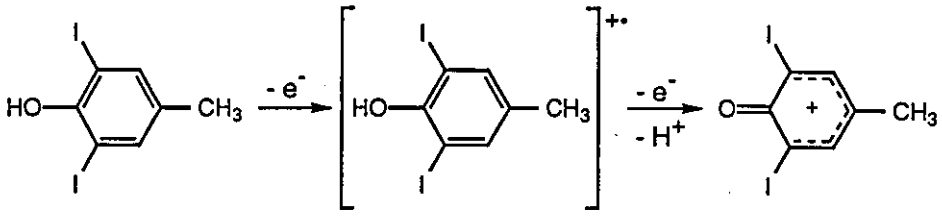


I.1.8 *N*-Acetylthyroxine ethyl ester 46

Scans 2,3,4 and 5

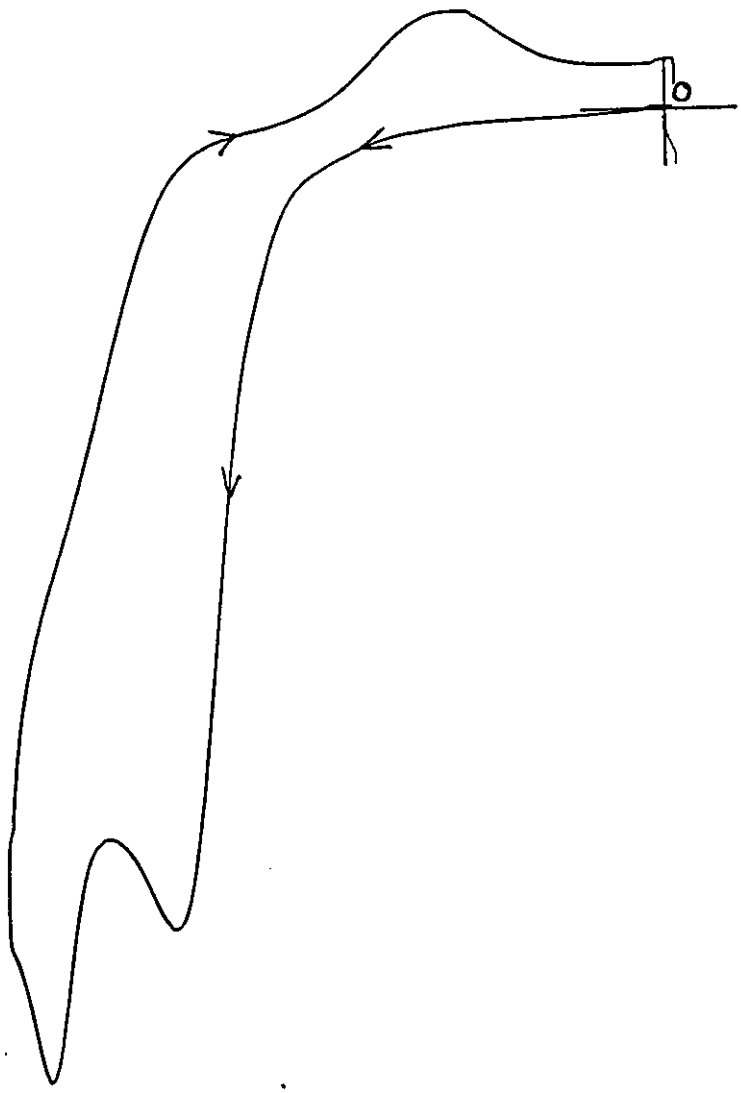


I.1.9 2,6-Diiodo-4-methylphenol 55



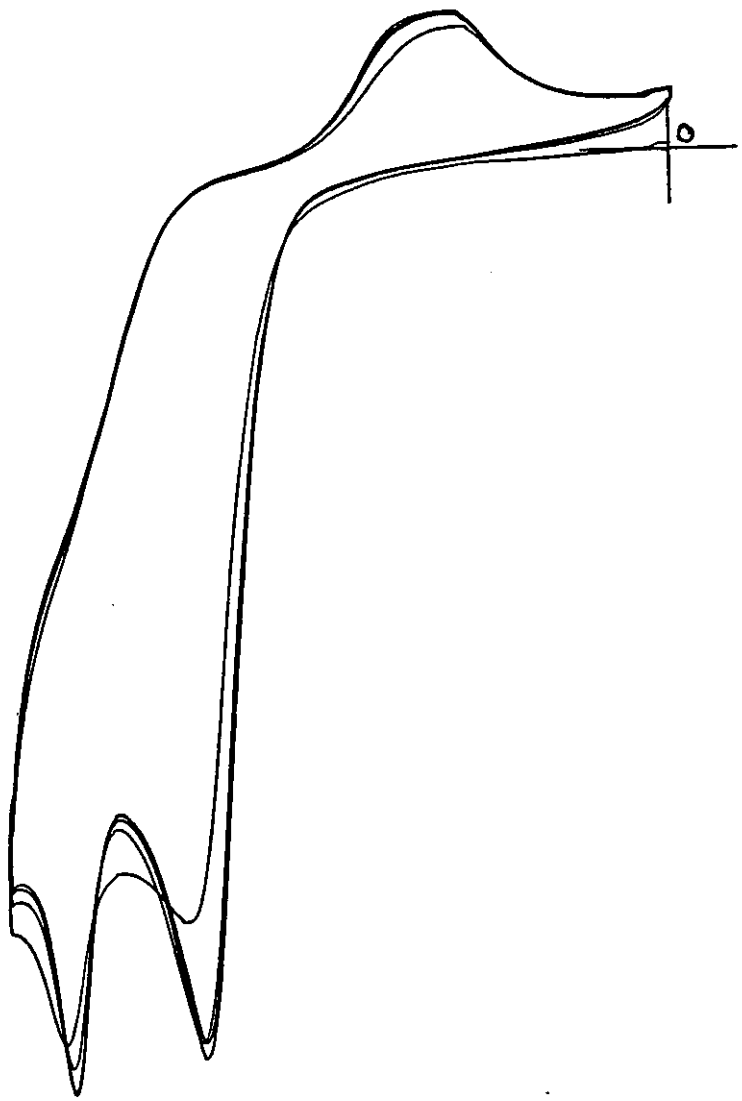
First scan

Ep = 1.33, 1.68 V

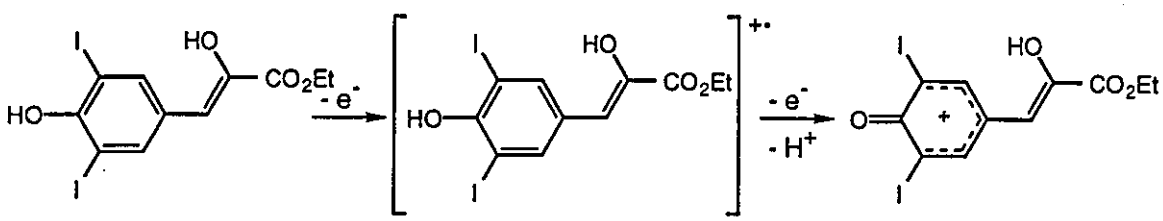


I.1.10 2,6-Diiodo-4-methylphenol 55

Scans 2,3,4 and 5

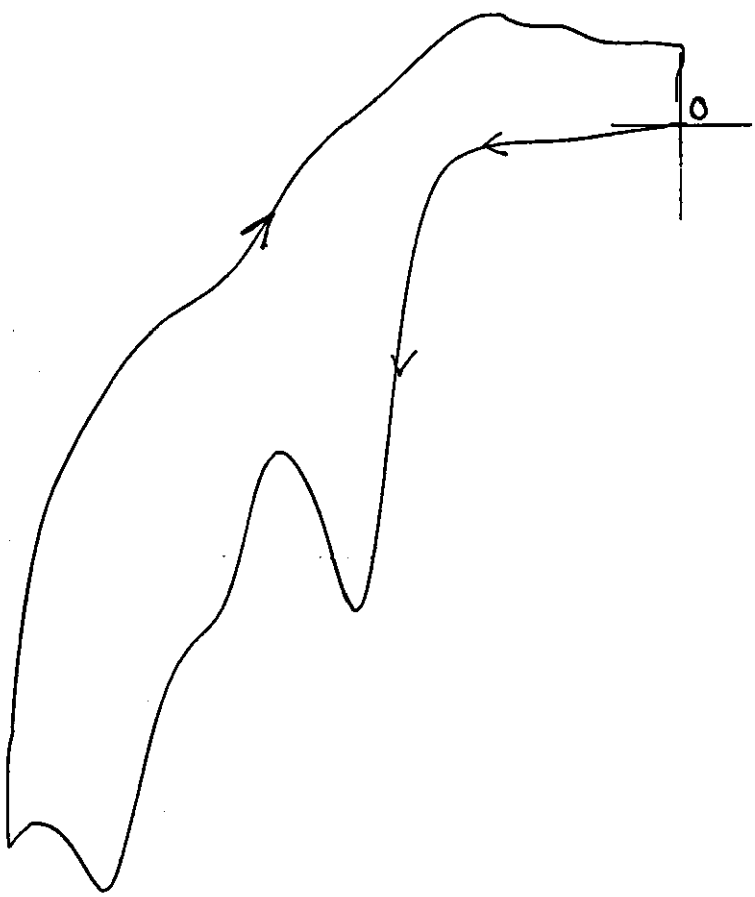


I.1.11 3,5-Diiodo-4-hydroxyphenylpyruvic acid 40



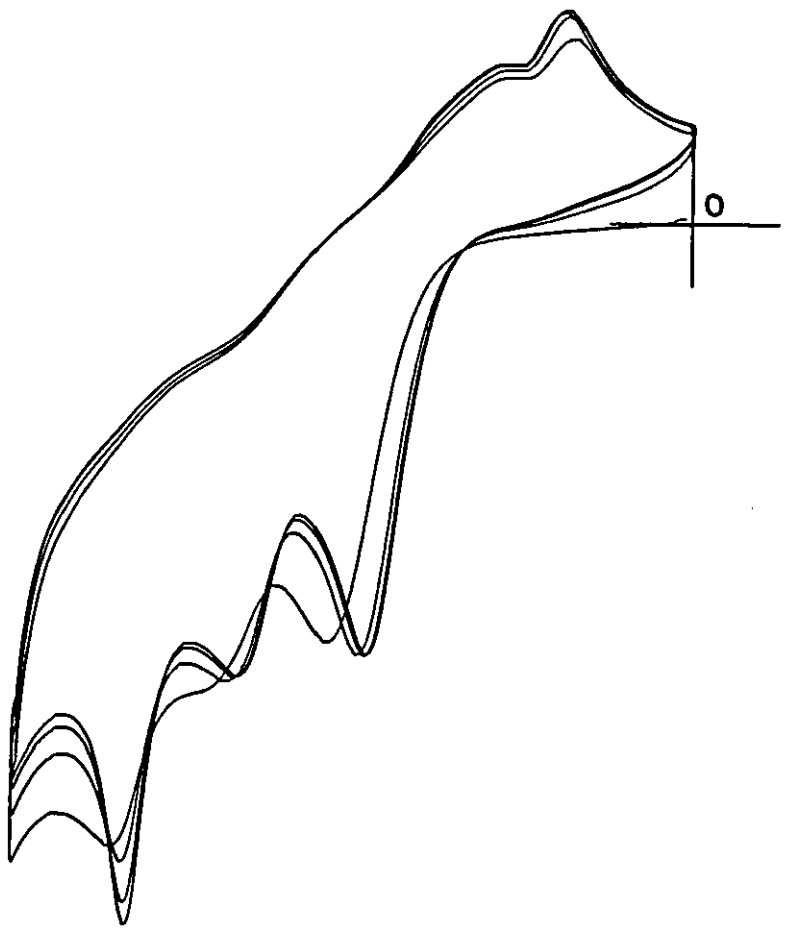
First scan

Ep = 0.86, 1.58 V

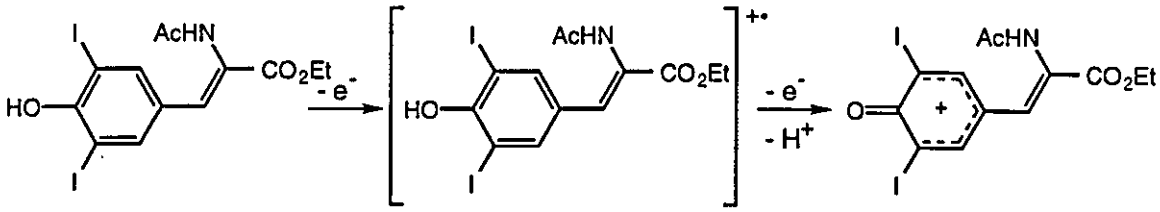


I.1.12 3,5-Diiodo-4-hydroxyphenylpyruvic acid 40

Scans 2,3,4 and 5

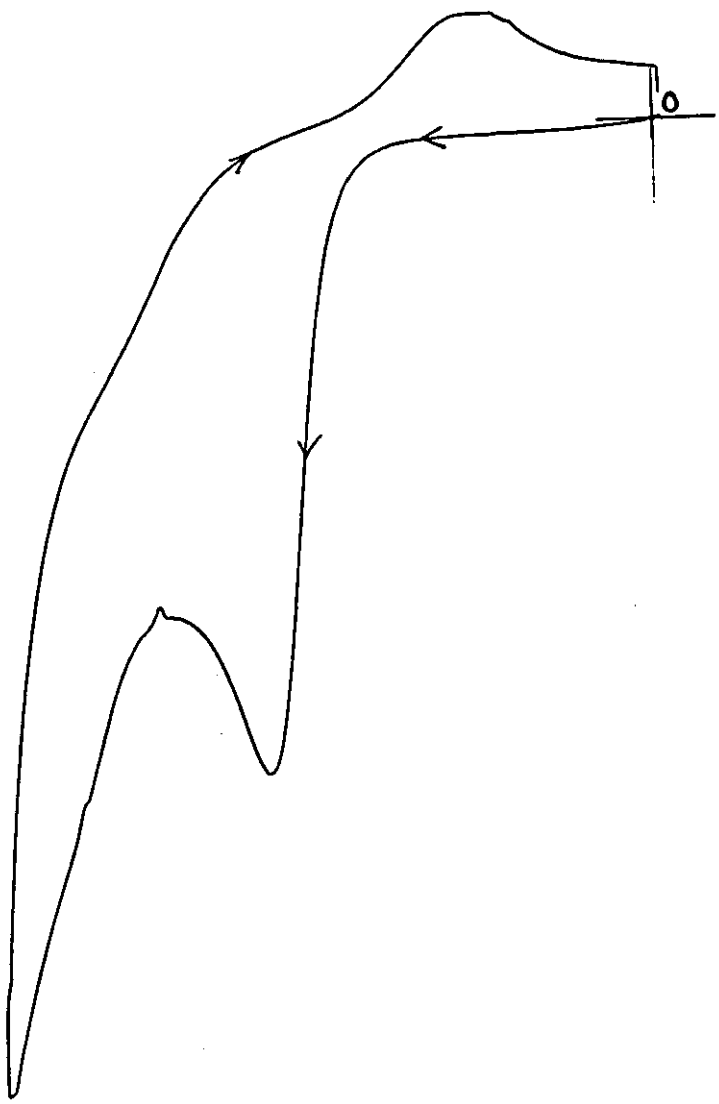


I.1.13 Ethyl 2-acetamido-3-(3,5-diiodo-4-hydroxyphenyl)-propenoate 69



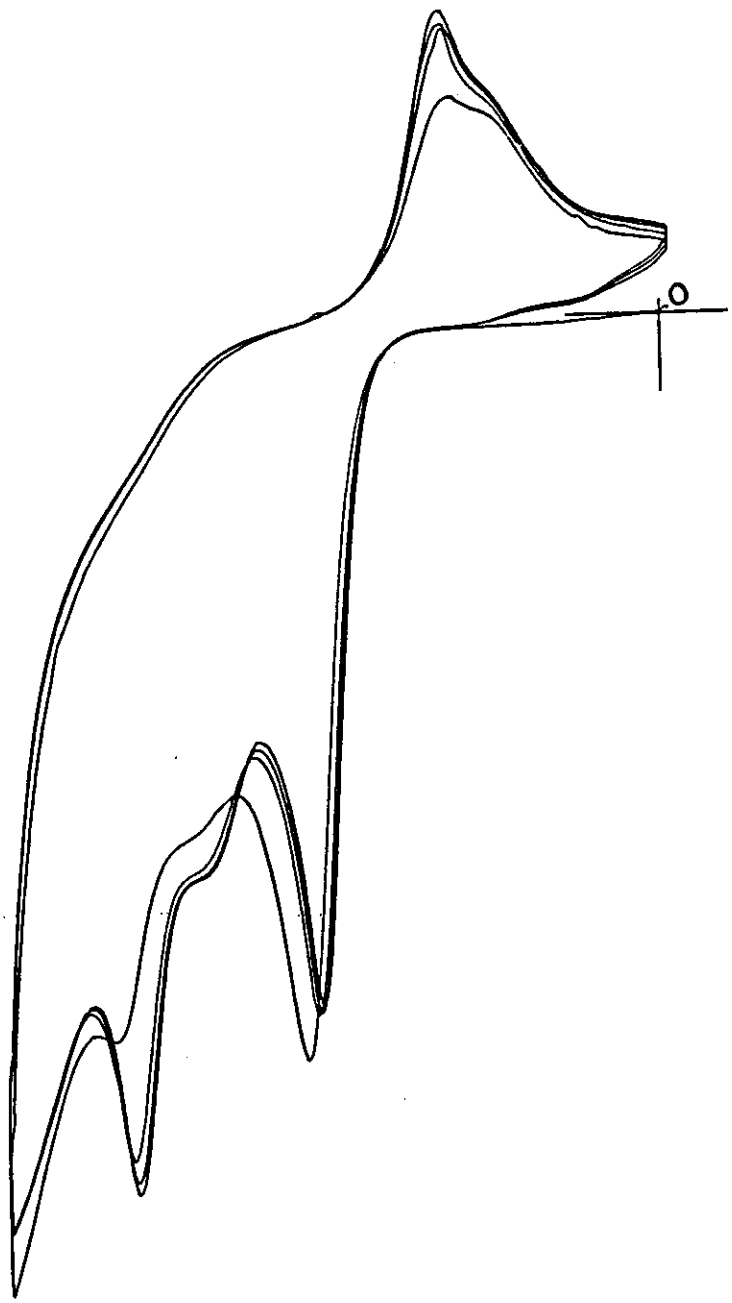
First scan

Ep = 1.06, 1.78 V

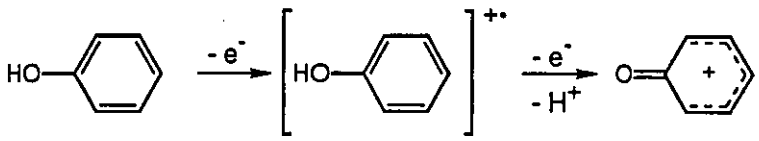


I.1.14 Ethyl 2-acetamido-3-(3,5-diiodo-4-hydroxyphenyl)-propenoate 69

Scans 2,3,4 and 5

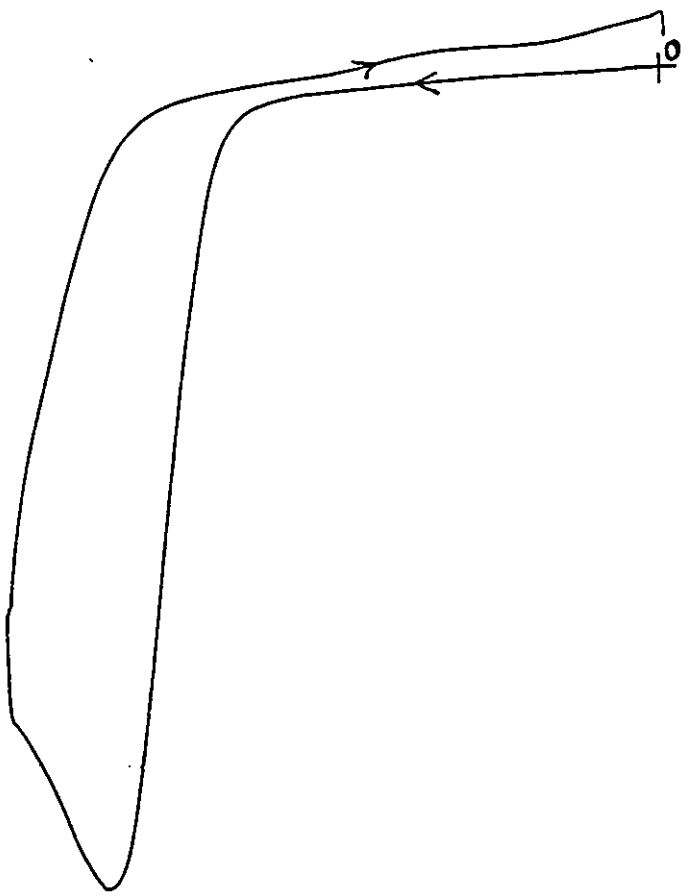


I.1.15 Phenol 82



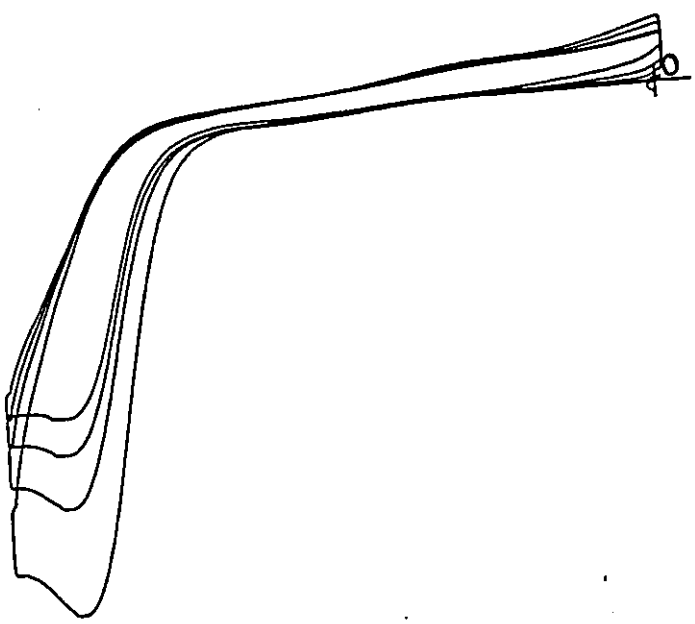
First scan

Ep = 1.52 V

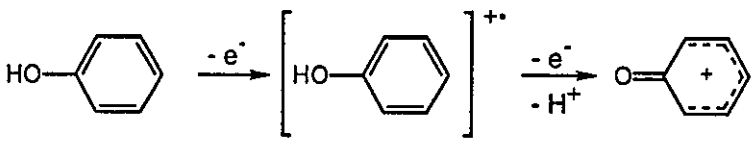


I.1.16 Phenol 82

Scans 2,3,4 and 5

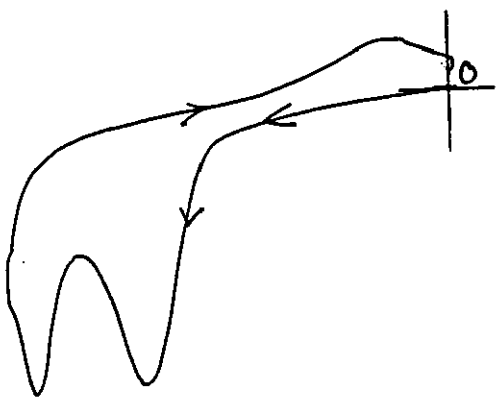


I.1.17 Phenol 82 in water



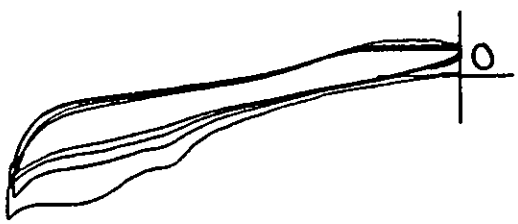
In this case the limits were 0.00 and +1.30 V.
First scan

Ep = 0.83, 1.12 V



I.1.18 Phenol 82 in water

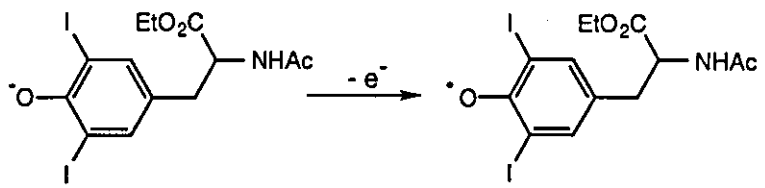
Scans 2,3,4 and 5



I.2 Oxidation of phenoxide ions in water

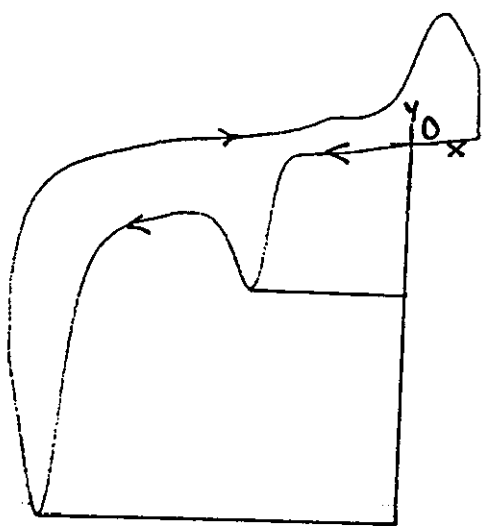
The cyclic voltammograms found in this section were recorded between the approximate limits of -0.225 and + 1.225 V except where indicated. The Y scale setting was 10 mA/V and the scan speed was 0.150 V/s, and the chart recorder settings were X = 0.23 V/cm and Y = 5 mV/cm. Zero on the cyclic voltammograms indicates zero potential *versus* the saturated calomel electrode and zero current, and the arrow indicates the direction of the sweep.

I.2.1 *N*-Acetyl-3,5-diiodotyrosine ethyl ester 47



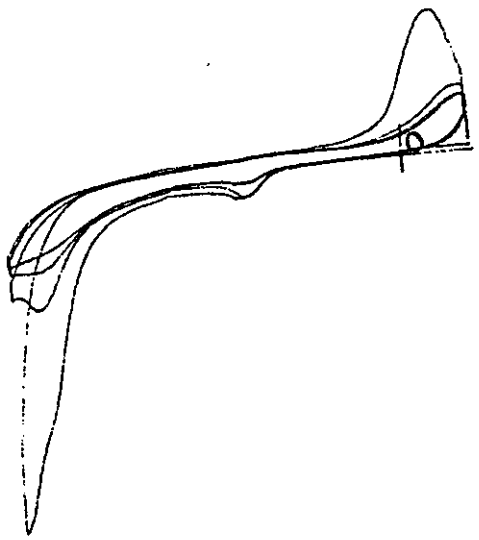
First scan

Ep = 0.44, 1.10 V

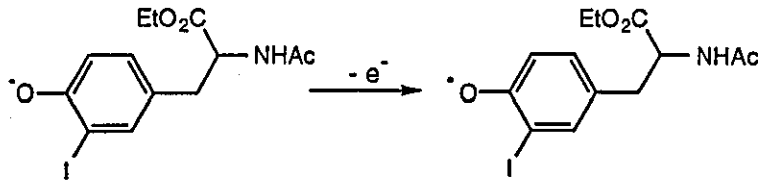


I.2.2 *N*-Acetyl-3,5-diiodotyrosine ethyl ester 47

Scans 2,3,4 and 5

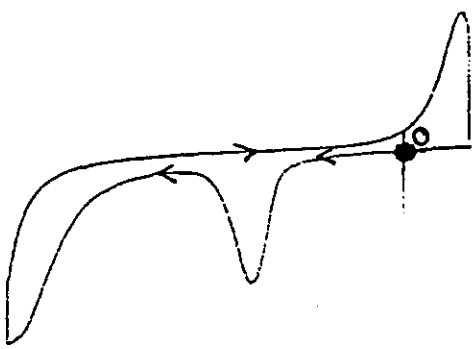


I.2.3 *N*-Acetyl-3-iodotyrosine ethyl ester 64



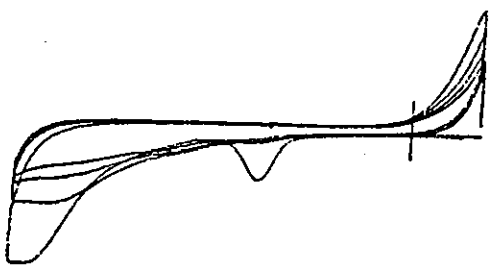
First scan

Ep = 0.42, 1.19 V

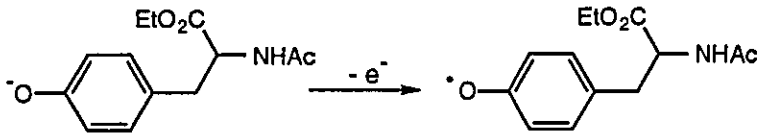


I.2.4 *N*-Acetyl-3-iodotyrosine ethyl ester 64

Scans 2,3,4 and 5

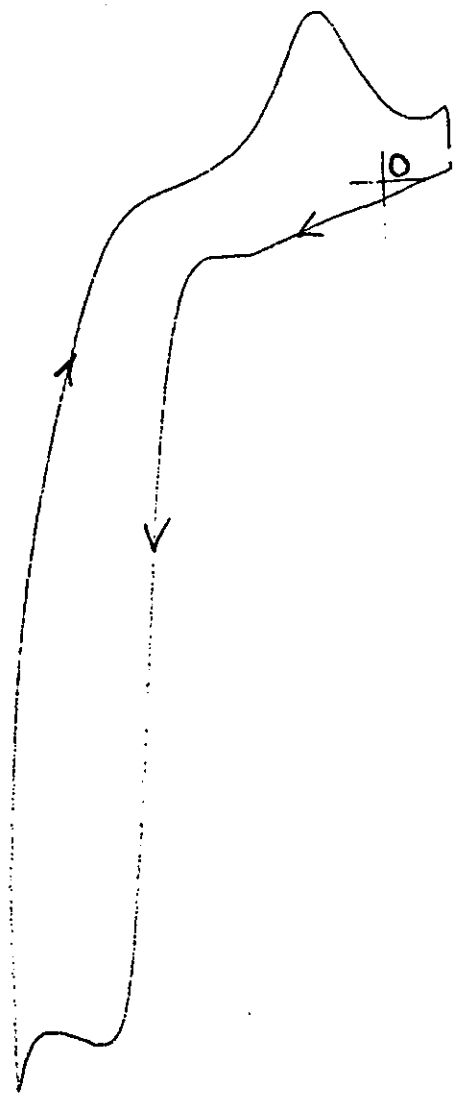


I.2.5 *N*-Acetyltyrosine ethyl ester 81



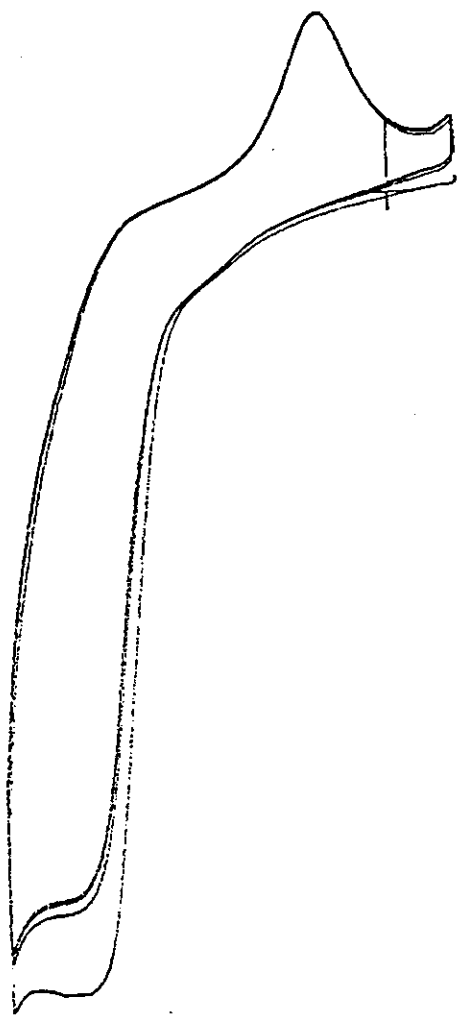
First Scan

Ep = 0.39, 0.99 V

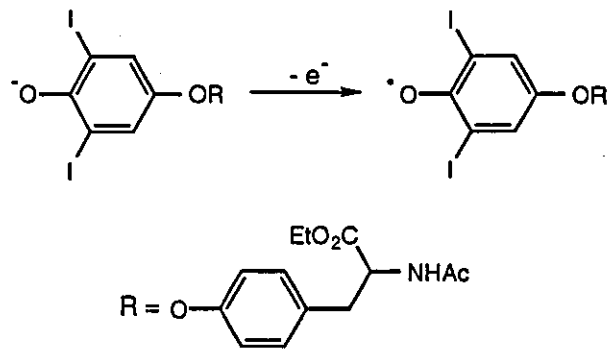


I.2.6 *N*-Acetyltyrosine ethyl ester 81

Scans 2,3,4 and 5

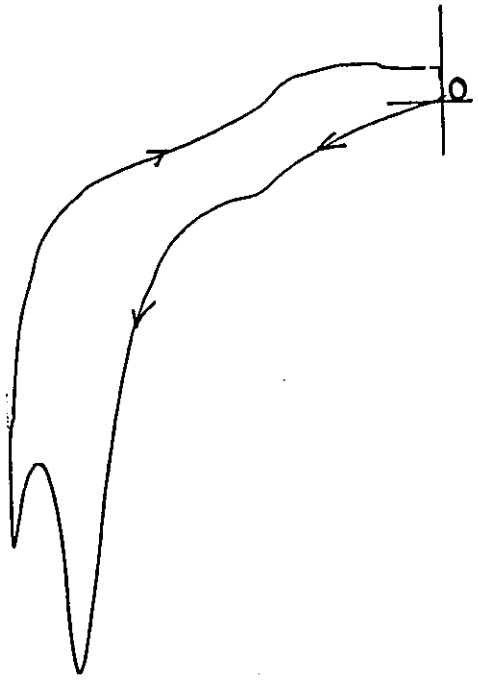


I.2.7 *N*-Acetylthyroxine ethyl ester 46



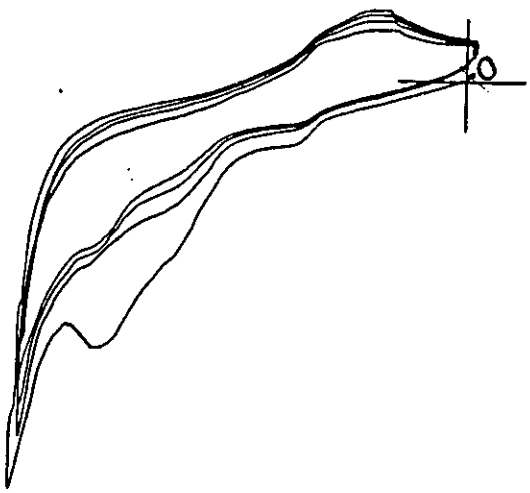
Scanning limits 0.000 to 1.301 V
First scan

Ep = 0.51, 1.04 V

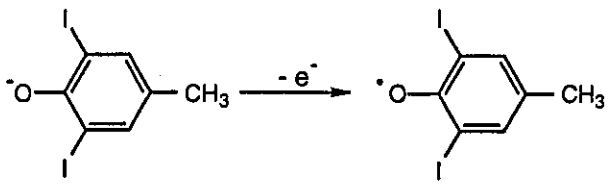


I.2.8 *N*-Acetylthyroxine ethyl ester 46

Scans 2,3,4 and 5



I.2.9 2,6-Diiodo-4-methylphenol 55



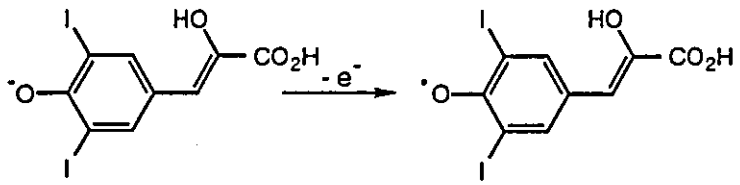
First scan

Ep = 0.29 V

I.2.10 2,6-Diiodo-4-methylphenol 55

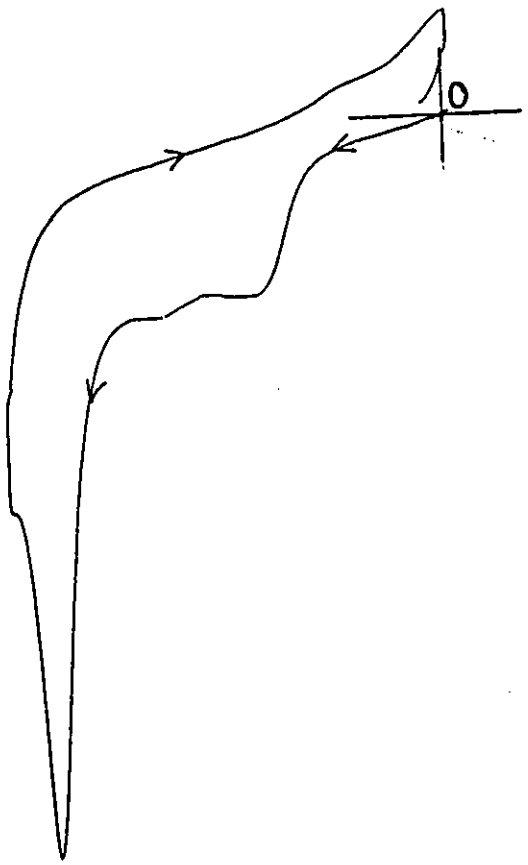
Scans 2,3,4 and 5

I.2.11 3,5-Diiodo-4-hydroxyphenylpyruvic acid 40



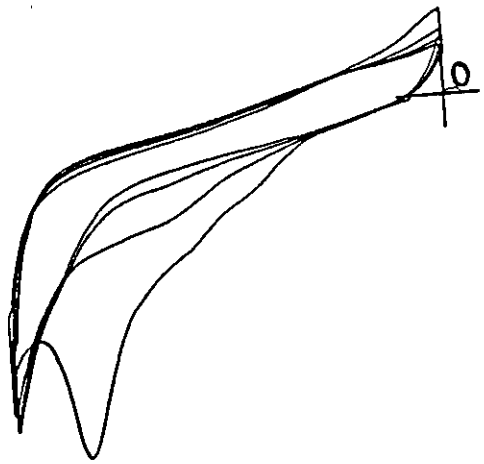
Scanning limits 0.002 to 1.303 V
First scan

Ep = 0.51, 0.76, 1.05 V

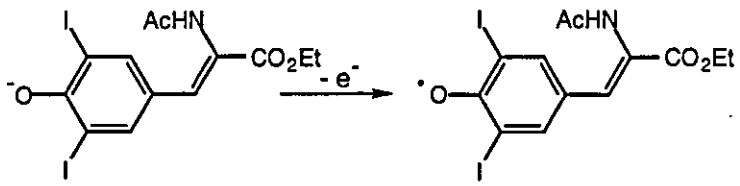


I.2.12 3,5-Diiodo-4-hydroxyphenylpyruvic acid 40

Scans 2,3,4 and 5

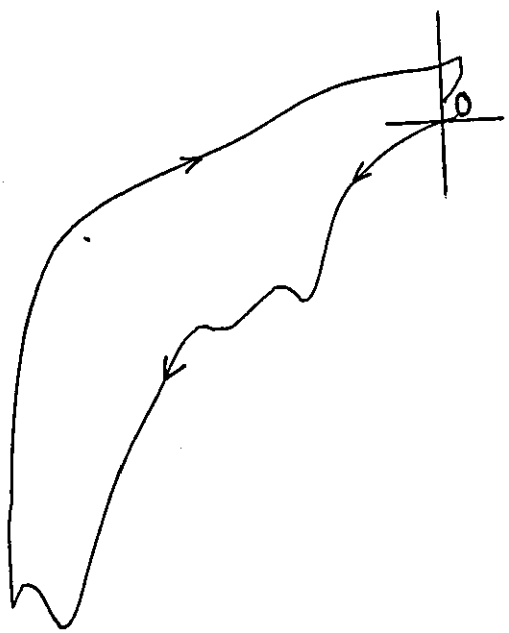


I.2.13 Ethyl 2-acetamido-3-(3,5-diiodo-4-hydroxyphenyl)-propenoate 69



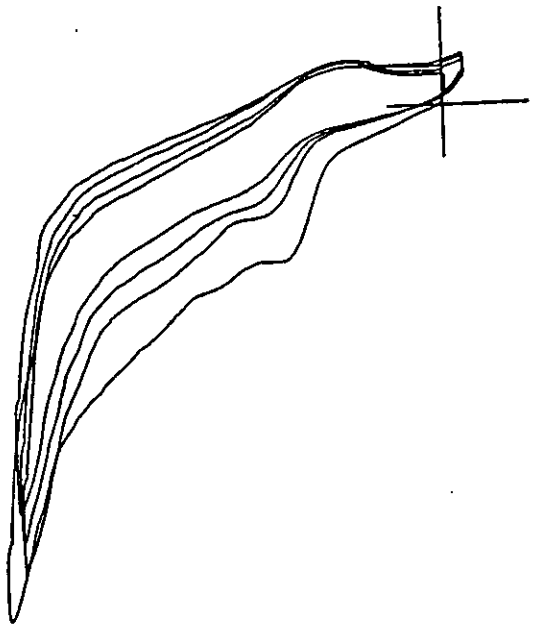
Scanning limits 0.001 to 1.303 V
First scan

Ep = 0.39, 0.61, 1.09 V

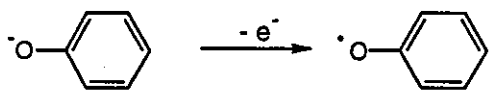


I.2.14 Ethyl 2-acetamido-3-(3,5-diiodo-4-hydroxyphenyl)-propenoate 69

Scans 2,3,4 and 5

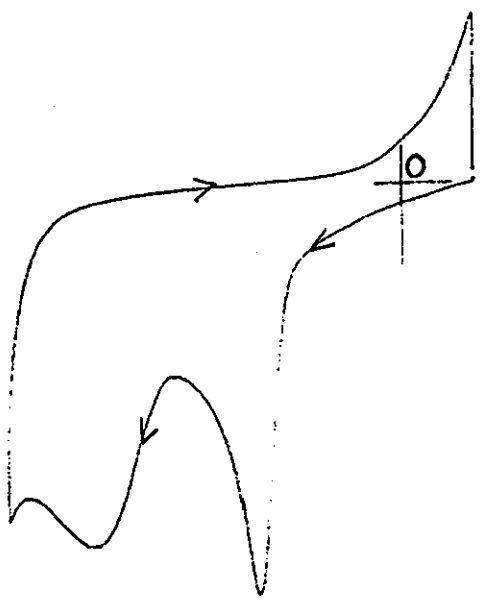


I.2.15 Phenol 82



First scan

Ep = 0.41, 1.04 V



I.2.16 Phenol 82

Scans 2,3,4 and 5