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Non-natural phenolic amino acids
Synthesis and application in peptide chemistry

Review Article

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Summary. Several non-natural phenolic amino acids have been synthesized. *t*-Butylated tyrosine and thyroxine derivatives, on one-electron oxidation, give persistent radicals which can be used as positional and/or spin labels for amino acids. Two-electron oxidation of *N*-protected tyrosines leads to spirolactones, useful active esters for peptide coupling.

Keywords: Amino acids – Tyrosine – Thyroxine – Spin labels – Active esters – Radicals – Spirolactones

1 Introduction

Non-natural amino acids have become more and more important in the last few years. They have proven to be of considerable experimental value in probing amino acid and peptide chemistry and functionality (Holtzer, 1992). They are especially valuable as a tool for the profound understanding of the roles and functions of natural amino acids, for example as analogues and/or competitive inhibitors of natural amino acids in biochemical studies.

2 Non-natural phenolic amino acids as positional or spin labels

Positional labeling is the replacement of an amino acid in a peptide by a non-natural amino acid, preferentially with a similiar structure, in order to test the reactivity or function of the original amino acid.

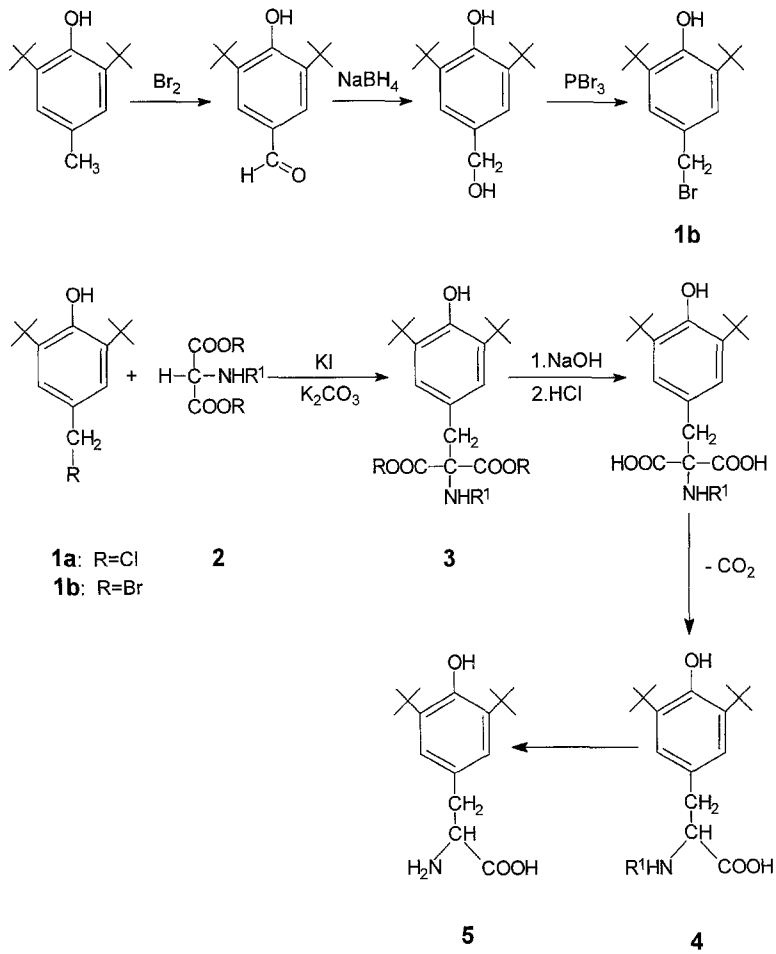
The marking of proteins, cofactors, nucleic acids, polymers, membranes and other compounds with radicals, which have an unpaired electron and are therefore paramagnetic, is called *spin labeling*. These systems can be used to investigate the molecular surrounding of the spin by EPR-spectroscopy. In this way, information about the mobility of the label and the influence of

neighbouring dipoles can be obtained. This allows to draw conclusions on the configuration and conformation as well as the tertiary structure of the labeled compound, e.g. a peptide, and to enlighten the biochemical structure-activity relationship.

Radicals used as spin labels generally belong to the Rozantzev nitroxyl-type, but radicals of the *tert*-butylphenoxy-type (Müller radicals) (Müller and Ley, 1954; Müller et al., 1958) can also be used for this purpose. There may be an advantage in incorporating a spin label, in which the radical function (i.e. the *tert*-butylphenoxy-group) is present in a shielded form (i.e. as phenol-moiety), as an integral part of a polypeptide chain. The radical can then be generated by oxidation, immediately before the amino acid or peptide is used for EPR-measurements.

2.1 Synthesis of t-*butyl-substituted tyrosine derivatives*

The synthesis of 3,5-di-*tert*-butyltyrosine (**5**, Scheme 1) *via* the acylated amino malonic acid ester **3** was first described by Teuber and his co-workers



Scheme 1. Synthesis of 3,5-di-*tert*-butyl tyrosine derivates

(Teuber et al., 1978). As starting phenol they used 2,6-di-*tert*-butyl-4-chloromethylphenol **1a** which was coupled with diethyl formamidomalonate **2** to give the phenolic malonate **3**. After hydrolysis and decarboxylation the *N*-protected di-*tert*-butyl-tyrosine **4** was obtained.

We used the brominated phenol **1b** instead since **1a** could not be prepared in good yields. The synthesis of **1b** could be performed in 3 steps from 2,6-di-*tert*-butyl-4-methylphenol (ionol) in an overall yield of 80% (Scheme 1).

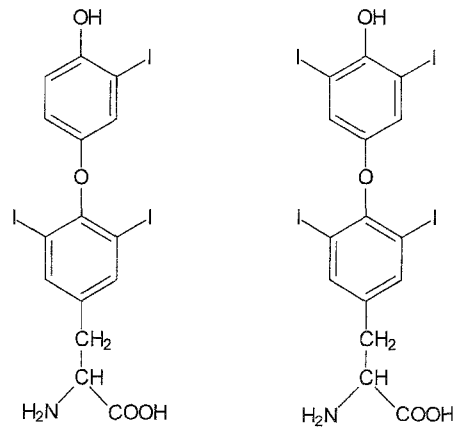
The cleavage of the *N*-protecting group (R^1 = For; For = formyl) was performed by heating **4** in aniline. The synthesis of **5** was optimized, and the yields of the respective steps could be raised as follows: the coupling step with diethyl formamidomalonate from 81% to 92%, the hydrolysis from 68% to 89%, the decarboxylation from 87% to 93% and the cleavage of the formyl group from 83% to 96%. So, the overall yield could be increased from 40% to 73%.

The protection of the functional groups of **5** was achieved in the same way as with tyrosine itself. Fmoc-Tyr(3,5-*t*Bu_2)-OH, for example, could be synthesized in 72–92% yield with Fmoc-ONSu (Ten Kotrenaar et al., 1986).

2.2 Synthesis of thyronine derivatives

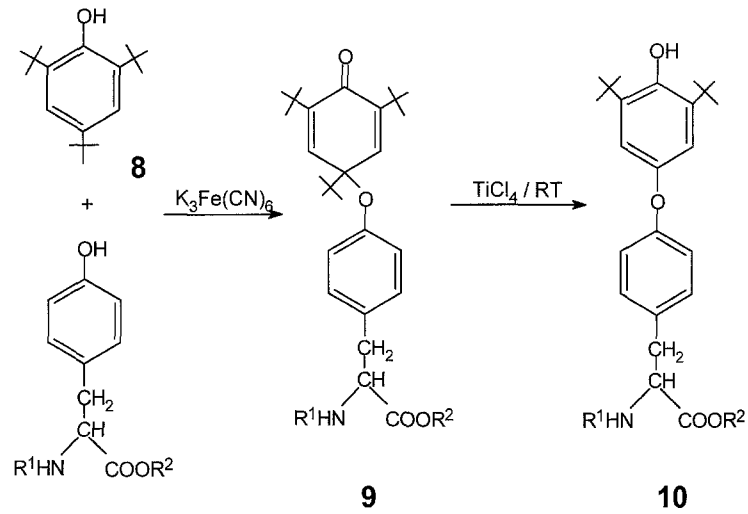
The thyroid hormones L-3,3′,5-triiodothyronine (T_3) **6** and L-thyroxine **7** (Fig. 1) reduce the circulating cholesterol level in animals of hypercholesterolaemia and in humans (Boyd and Oliver, 1960a; Hansson et al., 1983). The adverse cardiac effects of these hormones have precluded their therapeutic use in the treatment of hyperlipidaemia (Boyd and Oliver, 1960b). In 1986 it was demonstrated that a novel class of 3′-arylmethyl analogues of T_3 are highly effective in reducing plasma cholesterol levels but have little or no effect on cardiac function (Benson et al., 1986).

Already 30 years ago a model for the biosynthesis of thyroxine was proposed (Matsuura and Cahnmann, 1960). This reaction sequence represents a



6 **7**

Fig. 1. T_3 (**6**) and L-thyroxine (**7**)



Scheme 2. Synthesis of *tert*-butyl substituted thyronine derivatives

nonenzymatic route for the formation of thyroxine from diiodotyrosine *in vivo*. Corresponding analogues of thyroxine have been synthesized *via* quinol ethers **9** obtained by oxidative coupling of the respective tyrosine and a sterically hindered auxiliary phenol **8** with $K_3Fe(CN)_6$ in a basic medium.

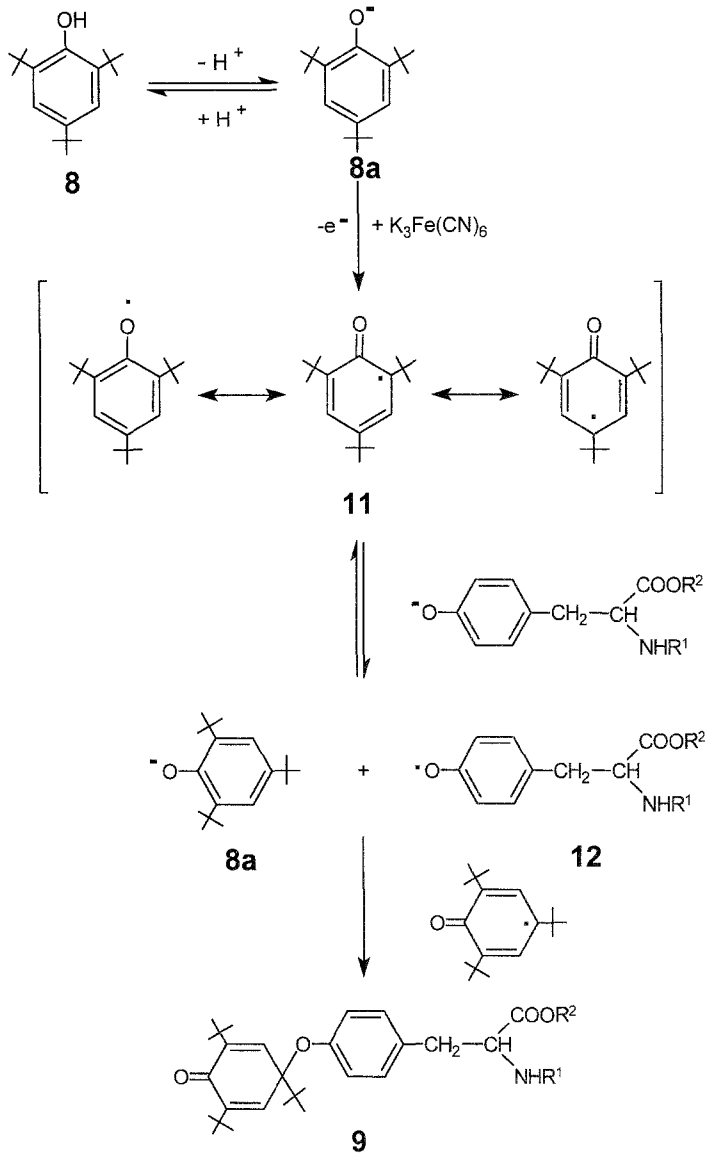
The free 2,4,6-tri-*tert*-butylphenoxy radical **11** (Scheme 3) is generated as an intermediate. The quinol ethers **9** gave the desired thyroxine derivatives **10** on treatment with acids or on pyrolysis (Scheme 2).

The proposed reaction mechanism is shown in Scheme 3. The first step in this synthesis is the removal of an electron from the 2,4,6-tri-*tert*-butylphenolate **8a**, present in basic solution, leading to the corresponding free radical **11**.

Next, an electron transfer takes place, resulting in the oxidation of the tyrosine anion to the free radical **12**. As a result of this step, the 2,4,6-tri-*tert*-butylphenoxy radical **11** is reduced to 2,4,6-tri-*tert*-butylphenolate **8a**. Alternatively, tyrosine anion may be oxidized to **12** also by $K_3Fe(CN)_6$. The intermediary tyrosine radical **12** reacts with unreduced excessive radical **11** to form the quinol ether **9**.

Thus, two molecules of the 2,4,6-tri-*tert*-butylphenoxy radical **11** and one molecule of tyrosine are needed for the formation of one molecule quinol ether **9** and one of the starting 2,4,6-tri-*tert*-butylphenolate **8a**.

The last step of this reaction is the de-*tert*-butylation of the quinol ether **9** to give the diaryl ether **10** (Scheme 2), which can be performed in the presence of a Lewis acid. For this purpose, several Lewis acid catalysts were investigated (Hickey et al., 1988) but only titanium tetrachloride, tin tetrachloride or aluminium chloride-nitromethane (Tashiro et al., 1978) gave the desired products. Yields up to 90% can be achieved by de-*tert*-butylation of **9** with titanium tetrachloride (Table 1). This step was originally performed using *p*-toluenesulfonic acid (Matsuura and Nishinaga, 1962) whereby a simultaneous ester hydrolysis occurred.



Scheme 3. The reaction mechanism for the formation of **9**

Table 1. Thyronine derivatives synthesized according to Scheme 2

R^1	R^2	Yield of **9** [%]	Yield of **10** [%]
Z	Me	66	73
Z	H	64	5
H	Me	64	90

2.3 EPR- and ENDOR-measurements

2.3.1 Tyrosine derivatives

The oxidation of 3,5-di-*tert*-butyl-substituted tyrosine derivatives with PbO_2 in methanol or toluene generates the corresponding phenoxy radicals (e.g. **13**),

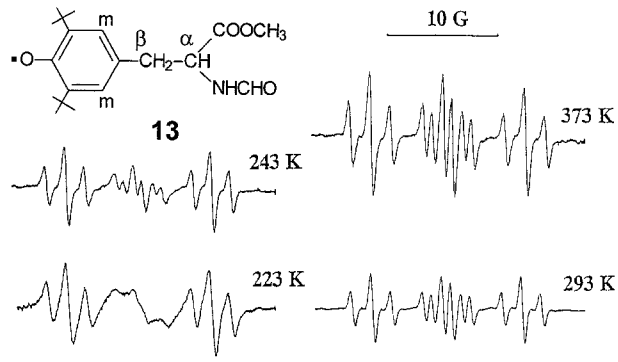


Fig. 2. Temperature dependent EPR-spectra of **13**

which are stable on exclusion of O_2. Their electron paramagnetic resonance spectra (EPR) reveal a coupling of the free electron with the two equivalent aromatic *m*-protons (1.7–1.8 G) and the two non-equivalent β-hydrogen atoms (6.4–8.2 G; 5.2–7.5 G) (Hutinec et al., 1994). The spectra show temperature dependent dynamic effects due to the hindered rotation around the bonds C_α-C_β and probably C_{arom}-C_β (Fig. 2). At low temperature one, two or three of the three conformers are frozen, resulting in an EPR spectrum consisting of a quartet (non-equivalent H_β-protons) of triplets (H_{meta}). At high temperatures the two main conformers are interchanging rapidly by rotational vibration of 120° around C_α-C_β, exchanging the positions of NHCHO and $COOCH_3$, leading again to a quartet of triplets with different a-values of the H_β-protons, which are still magnetically non-equivalent (Ziogas, 1993).

2.3.2 Thyronine derivatives

The oxidation of thyronine derivatives **10** with PbO_2 or PIFA [phenyliodine(III) bis(trifluoroacetate)] in toluene or CH_2Cl_2 gives very persistent radicals, which is essential for an application as amino acid spin labels.

EPR-measurements have been performed to see how long radical **10a**, generated in solution, can survive in different atmospheres (under argon and under air):

1. The radical stored under argon could be detected by EPR-measurements even after 8 weeks. During this period the signal intensity declined only to one quarter of that at the beginning.
2. The radical stored under air could be detected for 5 to 6 weeks. After that time the EPR signal was no longer observable.

The EPR-spectrum (Fig. 3-a) of **10a** shows a triplet that is caused by coupling of the free electron with the two equivalent aromatic *meta* protons. With good resolution, this triplet reveals further hyperfine splitting due to additional protons.

The ENDOR-spectrum of this radical at 253 K (Fig. 3-c) reveals *3* splittings with a_{Hm} = 1.16 G, a_{HtBu} = 0.068 G and a_{Hx} = 0.141 G. The first and

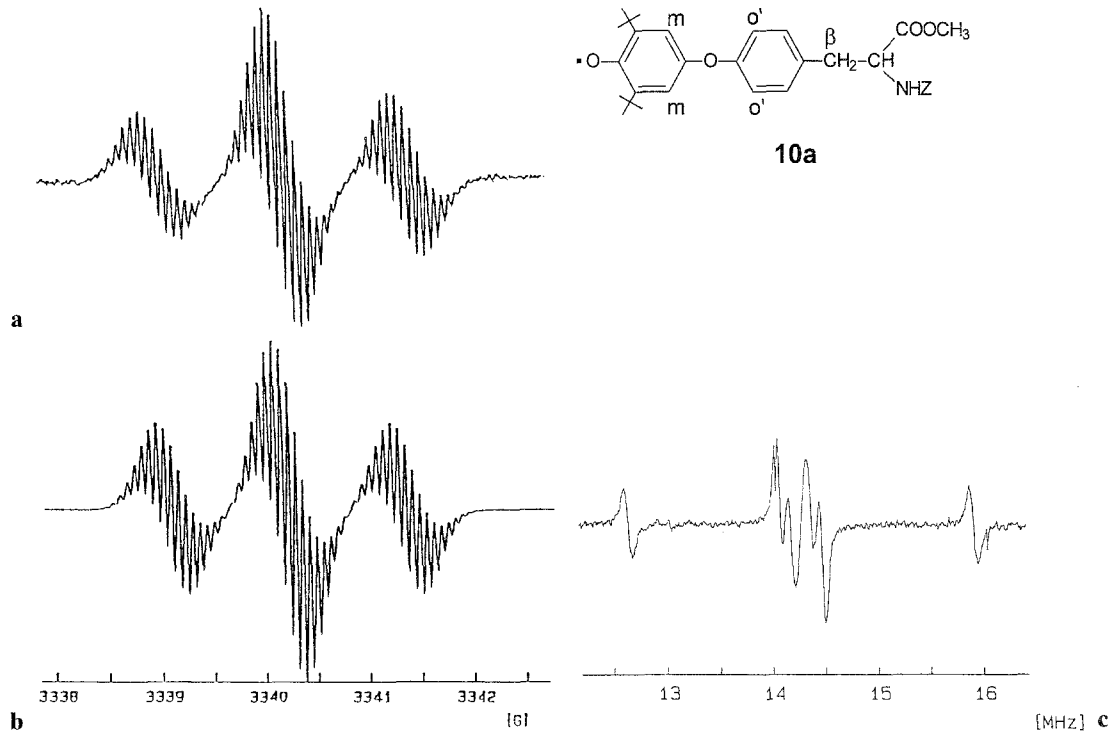


Fig. 3. EPR- and ENDOR-spectra of **10a**

second value correspond well to those determined for the *meta*-ring protons and *ortho-tert*-butyl protons of other phenoxy radicals (Petránek and Pilar, 1970; Stegmann et al., 1984). The third probably arises from the *ortho'* protons in the second aromatic ring and/or from the β-protons of the amino acid moiety. Indeed a computer simulation of the EPR spectrum (Fig. 3-b) with a = 1.13G (2 *meta*-H), a = 0.063G (18 *t*Bu-H), a = 0.146G (2-*ortho'*-H) and a = 0.146G (2 β-H) corresponds almost perfectly to the experimental one. To prove this assignment further, EPR- and ENDOR-spectra of similar compounds have been measured.

Thus the spectra of **14**, shown in Fig. 4a, are very similar to those of **10a**. The ENDOR-spectrum (at 253K) Fig. 4c also consists of three splittings, and the values are a_{Hm} = 1.11G, $a_{Ht\text{-}Bu}$ = 0.075G, $a_{Hp'}$ = 0.16G. The simulated EPR-spectrum Fig. 4b of this compound [a = 1.12G (2 *meta*-H), a = 0.068G (18 *t*-Bu-H), a = 0.166G (2-*ortho'*-H) and a = 0.156G (1 *para'*-H)] corresponds well to the experimental one.

Radical **15** shows also a triplet in the EPR spectrum Fig. 5a but has only two signals in the ENDOR-spectrum with the splitting constants of a_{Hm} = 1.31G and a_{HtBu} = 0.069G (Fig. 5b). The value of a_{Hm} is larger than that of compounds **10a** and **14**, and no splittings are observed from the second ring because the *ortho'*- and *para'*-positions are occupied by chlorine atoms, which give couplings too small to be resolvable.

The above measurements cannot confirm with certainty whether the third splitting of **10a** belongs to the *ortho'*-and/or to the β-protons.

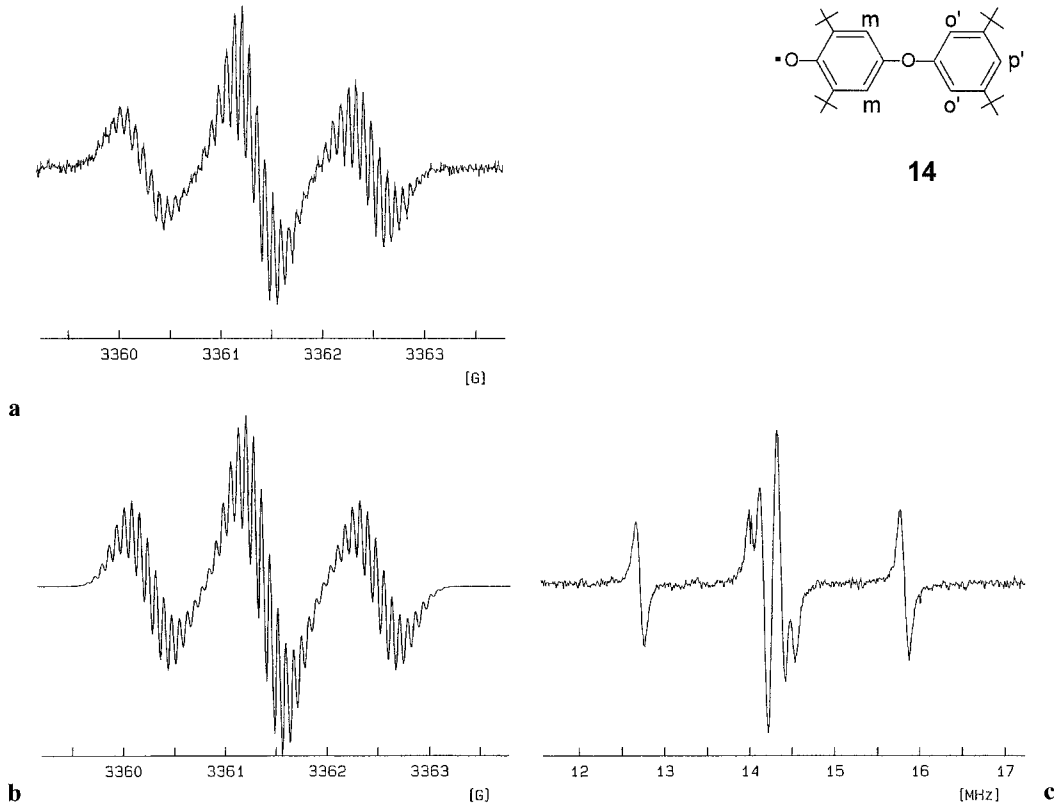


Fig. 4. EPR- and ENDOR spectra of **14**

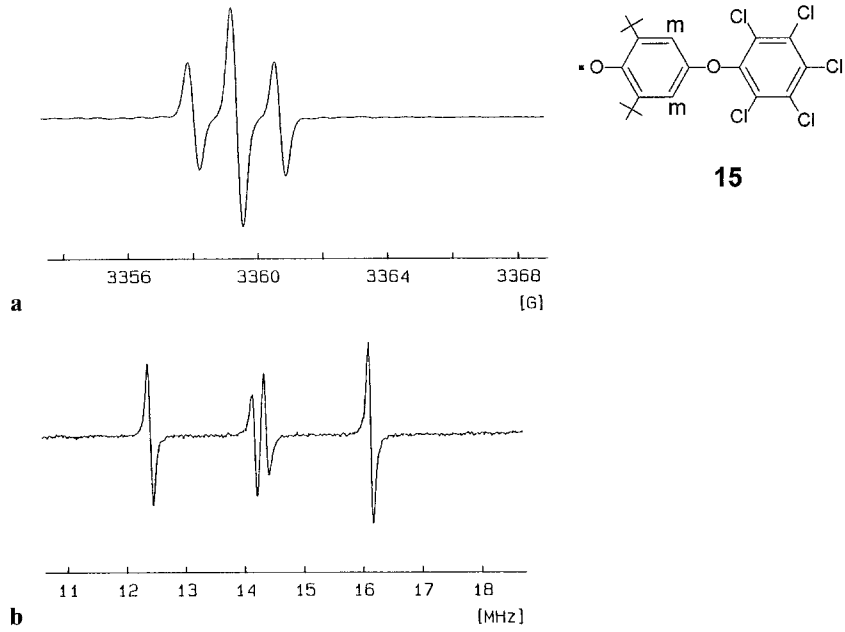


Fig. 5. EPR- and ENDOR spectra of **15**

2.4 Applications

In 1977 the non-natural amino acid 3,5-di-*tert*-butyl-4-hydroxyphenylglycine was synthesized by Hewgill and Webb (Hewgill and Webb, 1977). Mild oxidation of this compound produces a relatively stable aryloxy radical. Due to EPR-measurements of this compound and its derivatives they proposed that this amino acid, when incorporated into a polypeptide, would provide a spin label sensitive to changes in conformation.

The 3,5-di-*tert*-butyl-substituted tyrosine and thyronine derivatives **5** and **10** can be used in the same way: in the oxidized form, they may be applied as amino acid spin labels in peptide chemistry. The species **5** will be especially valuable for tyrosyl peptides, because they are still tyrosines, whereas the radicals **11** derived from the thyronines **10** are very persistent because they have no CH-fragment in 4-position relative to the oxyl. A substrate for the potential application of the tyrosine derivatives **5** and the corresponding phenoxy radicals is the neuropeptide Y (NPY), Fig. 6.

Tyrosines in the NPY have been specifically substituted by di-*tert*-butyl tyrosines **5** to see how this change affects the hormone-receptor interaction. It turned out that the binding of the NPY containing di-*tert*-butyl tyrosine in position 36 to the human Y_1-receptor is about 1.000-times weaker than that of the normal NPY. The binding to the human Y_2-receptor is 10 times weaker.

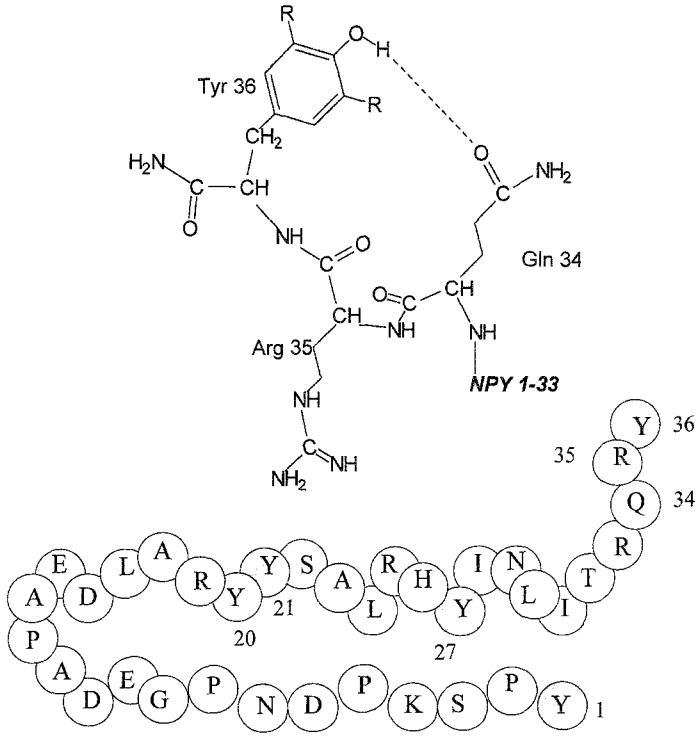


Fig. 6. Neuropeptide Y

The molecular dynamics simulation (Beck, 1989) of the neuropeptide Y shows that at the C-terminus several hydrogen-bonds can be active. Thus, a hydrogen-bond between the hydroxy-group of the tyrosine 36 and the carbonyl-group of the glutamine 34 might be of importance. Indeed, if the tyrosine 36 in the NPY is substituted with the *tert*-butyl-tyrosine, the hydrogen-bond between this tyrosine and glutamine 34 cannot be established anymore. Later, it was found that glutamine 34 does not play a role in the Y_1-receptor because it can be replaced by other amino acids without any consequence for the activity. Steric effects or a relevant hydrogen bond of tyrosine 36 seem to play a more significant role (Beck-Sickinger, 1995).

EPR-measurements of oxidized NPY-derivatives, where the tyrosine is replaced by the di-*tert*-butylated tyrosine **5** in positions 1, 20, 21, 27, 36 or twice in positions 20 and 21, are currently performed.

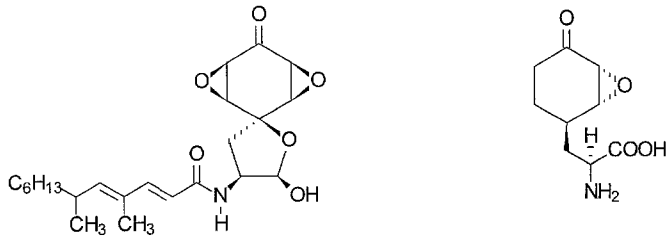
3 Spirolactones

Spirolactones of tyrosine are playing a significant role in the last few years because they are intermediates in the synthesis of some antibiotics and alkaloids. However, these compounds are not only precursors for the synthesis of antibiotics such as aranorosine **16** (Wipf and Kim 1993, 1993; Rama Rao et al., 1991; McKillop et al., 1992) or anticapsine **17** (Hara et al., 1992) (Fig. 7) but also synthons useful in peptide synthesis.

The key-step in the synthesis of these compounds is the oxidative spirocyclization of L-tyrosine. Tyrosine derivatives are redox-active phenols and can therefore be oxidized chemically or electrochemically not only to phenoxy radicals but even to phenoxy cations (Rieker and Speiser, 1977, 1979), depending on the applied oxidation potential and the proton concentration. The intermediate cationic species would react intramoleculary with nucleophilic functions to give synthons useful in peptide chemistry, as will be shown below.

3.1 Electrochemical oxidation

By means of cyclic voltammetry it is possible to get important information about the redox behaviour of each functional group of **5**, using selective



16 **17**

Fig. 7. Aranorosine (**16**) and anticapsine (**17**)

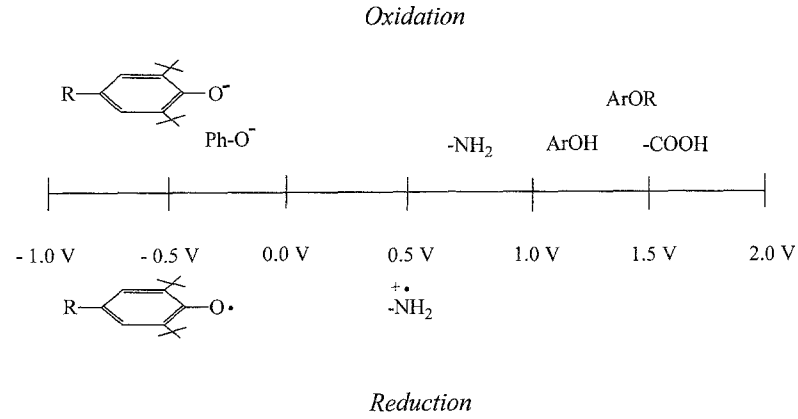


Fig. 8. Oxidation and reduction areas of tyrosine derivates (potentials *vs.* Ag/0.01 N Ag^+)

masking of all other functions. The redox scale of functions in Fig. 8 is taken from the thesis of A. Ziogas (1993).

The cyclic voltammograms of *N*-protected tyrosines reveal two oxidation peaks at about 1.150 and 1.560 mV. The first peak (1,150 mV) corresponds to the oxidation of the phenolic OH-group to the radical cation. This potential is characteristic of 2,6-di-*tert*-butyl-4-alkyl-phenols (Rieker and Speiser, 1979). The second peak belongs to the oxidation of the carboxylic group.

The oxidation peaks of alkyl and benzyl tyrosine-ethers are located about 200 mV higher than that of the free phenolic OH group at about 1.370 mV. From CV-measurements of H-Tyr-OMe × HCl we get information about the oxidation peak of the amino group, which is observed at 828 mV. This CV experiment reveals also a reduction peak at 566 mV which belongs to the reduction of the radical cation of the amino group, produced by oxidation at 828 mV.

In the presence of an excess of a base (NH_4OH or potassium-*tert*-butylate), the phenolic and the carboxylic part of the *N*-protected amino acid exist as phenolate and carboxylate, respectively. CV-measurements in this medium give different oxidation and reduction peaks. Thus, the oxidation peak of the phenolate can be located at about −630 mV and the corresponding reduction peak at about −750 mV, whereas the oxidation of the carboxylate occurs nearly at the same potentials as the carboxy group itself.

With all these information we can conclude:

1. The redox raction of phenols or phenol ethers, in neutral acetonitrile, is irreversible. A two-electron transfer takes place which can be described as an ECE-mechanism (Rieker and Speiser, 1979; Rieker et al., 1991).
2. The oxidation of the carboxylate group presumably leads to the carboxy radical by means of a one-electron transfer and is also irreversible.
3. The redox processes in basic acetonitrile of all unsubstituted tyrosines are irreversible, whereas those of the sterically hindered species are reversible or quasireversible.

The preparative anodic oxidation was performed in an undivided cell using a cylindrical glass vessel with a lateral tube to take up the reference

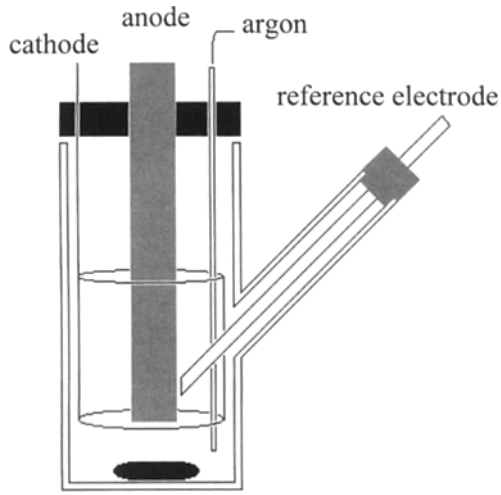


Fig. 9. Cell for the preparative oxidation. The reference electrode is connected to the Haber-Luggin capillary *via* two glass frits

electrode (Fig. 9). The anode was made of graphite, and the cathode was a cylindrical platinum-net. For the potential controlled electrolysis the reference electrode was Ag/Ag^+ (Ag/0.01 N Ag^+ in acetonitrile) with a potential of 0.35 V *vs* SCE (Regier, 1989).

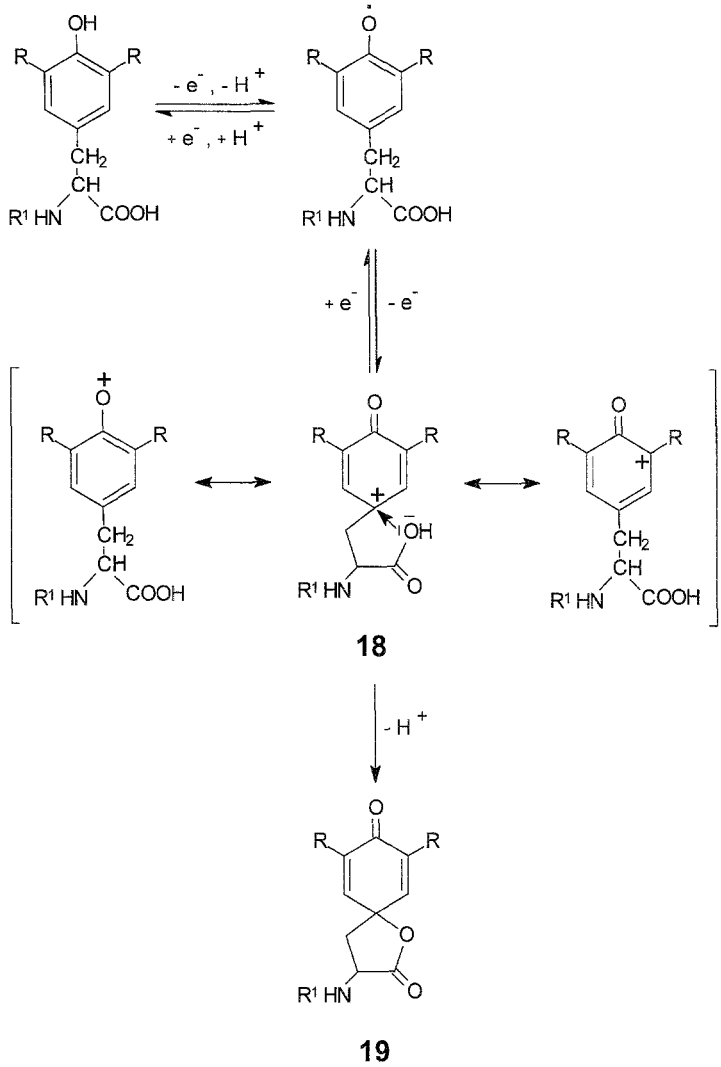
Exclusively $NaClO_4$ was used as electrolyte, and the produced protons were neutralized by an excess of 2,6-lutidine (2,6-dimethylpyridine). The oxidation potential was set between 1.300–1.400 mV (*vs* Ag/0.01 Ag^+), to oxidize the phenolic OH-group, and the concentration of the solution amounted to about 0.01 mol/l.

3.1.1 Intramolecular cyclization

Spirolactones can be synthesized with different oxidants, either electrochemically or chemically. In all cases discussed here, however, phenoxenium ions are the intermediates, which yield spirolactones **19** by intramolecular addition of the nucleophilic carboxy group. As a result of this cyclization $2e^-$ are transferred to the anode with loss of $2H^+$ (Scheme 4).

The electrochemical oxidation is affected by strong adsorption effects. Very often a fast decrease of the current is observed, caused by the covering of the electrode and responsible for a poor conversion of the tyrosines. Acetonitrile was used as solvent for all reactions because of the good solubility of the unsubstituted tyrosine derivatives in this solvent.

Spirolactones of *N*-protected 3,5-di-*tert*-butyl-tyrosine derivatives (R = *t*Bu and R^1 = Ac, Bz, Fmoc, For, Z) can be synthesized anodically in 28 to 85% yield. The oxidation of the unsubstituted tyrosines (R = H) gives only modest yields (10–15%) of the pure product (El-Mobayed, 1981), although the yield of the crude product was about 70–80% (NMR). Thus, the conver-



Scheme 4. Anodic oxidation of *N*-protected tyrosine derivatives

Table 2. Electrochemical oxidation of tyrosine derivatives

R	R^1	Yield of **19** [%]
*t*Bu	Ac	44
*t*Bu	Bz	28
*t*Bu	Fmoc	64
*t*Bu	For	40
*t*Bu	Z	85
Br	Boc	54
Br	Z	72
I	Boc	39
I	Z	66
H	Boc	18
H	Z	17

sion of Z-tyrosine in 0.1N $NaClO_4$/MeCN with a graphite electrode is nearly quantitative (analytical TLC) but after the purification only 17% of the desired product can be isolated.

3.2 Chemical oxidation

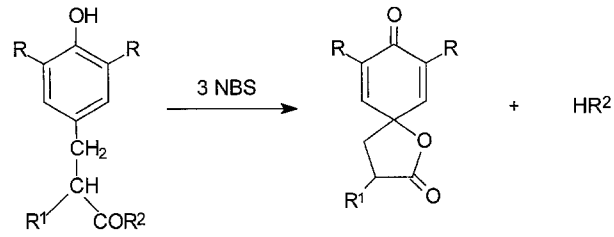
The intramolecular cyclization of tyrosine derivatives can also be achieved by chemical oxidants, provided they are able to produce phenoxenium ions in a two-electron oxidation. Thus, the choice of the oxidant is very important in this reaction, because it determines the yield of the spirolactones.

3.2.1 Oxidation with NBS

In 1959 Schmir, Cohen and Witkop (1959) reported a study on the action of bromine and NBS on derivatives of tyrosine and simpler analogs. *N*-protected tyrosines were converted in high yields into their respective lactones by the action of NBS in acetate buffer at pH 4.6.

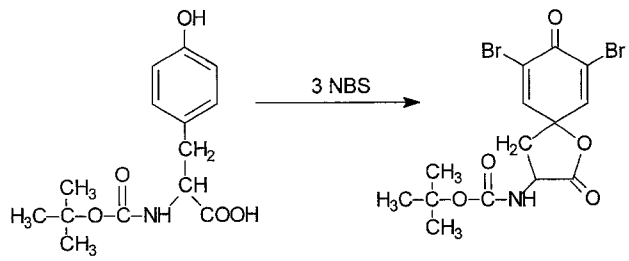
The spirolactone formation occurs even with *N*-carbobenzyloxy-S-benzyl-L-cysteinyl-L-tyrosyl-L-isoleucine leading however to a cleavage of the peptide bond and the release of isoleucine in 40% yield (Scheme 5).

The *N*-protected tyrosine derivative **20** reacts with 3 equivalents of NBS in 20% acetonitrile/acetate buffer (pH 4.6). Within a few minutes the lactone



R = Br, H
R^1 = H, Z-Cys(S-Bzl)-NH
R^2 = Ile-OH

Scheme 5. Oxidative cleavage of tyrosyl-peptides



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Scheme 6. Oxidation of Boc-Tyr-OH with NBS

Table 3. Chemical oxidation of tyrosine derivatives

R	R^1	Oxidant	Yield of **19** [%]
*t*Bu	Ac	NBS	100
*t*Bu	Bz	NBS	100
*t*Bu	Fmoc	NBS	100
*t*Bu	For	NBS	100
*t*Bu	Z	NBS	92
Br	Boc	NBS	93
Br	Z	NBS	97
I	Boc	NBS	70
I	Z	NBS	98
H	Boc	PIFA	23/76[a]
H	Z	PIFA	38/85[b]

[a]Pure product: 23%; crude product (NMR): 76%. [b]Pure product: 38%; crude product (NMR): 85%.

ring is formed almost quantitatively, however, simultaneously, the phenolic side chain is dibrominated (Scheme 6).

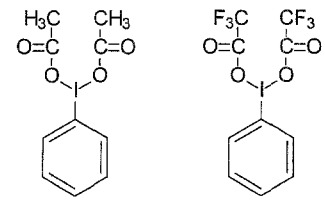
On the other hand, the oxidation of 3,5-di-*tert*-butyl-tyrosine derivatives with NBS does not produce the dibrominated lactone but gives the desired 3,5-di-*tert*-butylated species in excellent yields (Table 3).

3.2.2 Iodosobenzene bisacetates as oxidants

Despite of the very good two-electron oxidative property of NBS, the brominating side effect of this oxidant led to the investigation of new oxidants for the transformation of *N*-protected L-tyrosines into the corresponding dienone lactones.

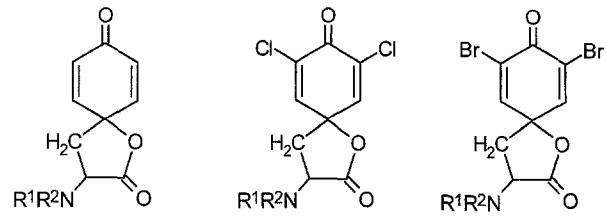
Among the oxidants, phenyliodine(III) diacetate (PIDA) and phenyliodine(III) bis(trifluoroacetate) (PIFA) (Fig. 10) proved to be the most effective ones (Scott et al., 1963; Inoue et al., 1983; Tamura et al., 1987).

On closer examination of the reaction conditions suitable for the oxidation of tyrosine derivatives, Hara and his co-workers (1992) found unexpected reaction products. The PIDA oxidation of *N*-benzyl-*N*-benzyloxycarbonyl tyrosine in one pot followed by quenching with aqueous sodium chloride or bromide solution gave the corresponding dihalodienone lactones **22** and **23** (Scheme 7).



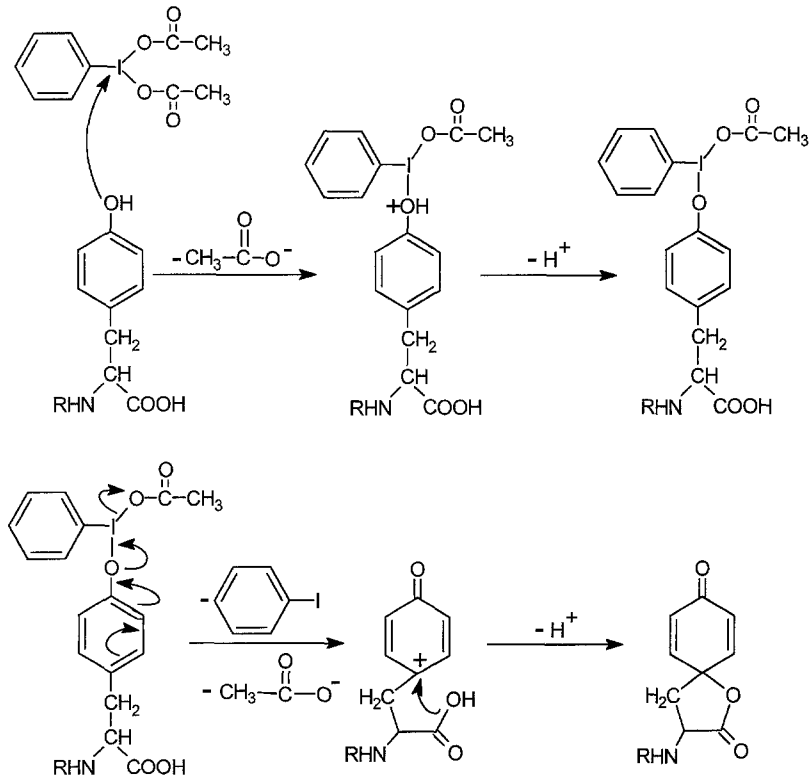
PIDA PIFA

Fig. 10. Oxidants PIDA and PIFA



21 **22** **23**

Scheme 7. Products of PIDA oxidation



Scheme 8. Oxidation of tyrosine derivatives with iodosobenzene bisacetates

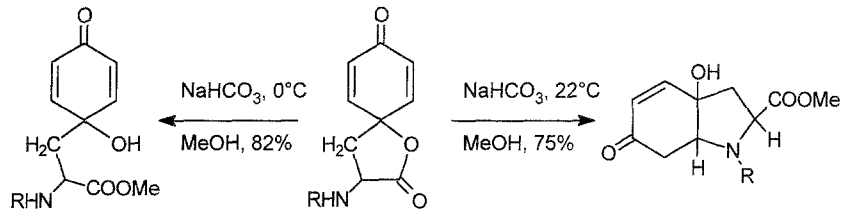
The unexpected formation of **22** and **23** was found to be attributable to the different work-up, in which a saturated aqueous solution of sodium chloride or bromide was added to the reaction mixture before quenching with citric acid. Work-up in reversed order gave the normal product **21**.

We found that *N*-protected tyrosines can be converted to the corresponding lactones by oxidation with a small excess of the oxidant under argon. The reaction conditions are depending on the oxidant.

1. Using PIDA as oxidant, the reaction in carried out in methanol at −5°C for 30 minutes with an excess of 10–15% of the oxidant.
2. Using PIFA as oxidant, a 10% excess of the oxidant is used in acetonitrile, with pyridine as catalyst.

The yields of the chemical oxidation in the case of 3,5-di-R-substituted tyrosines were in most cases higher than 90% (Table 3) but the isolated yields of the unsubstituted spirocyclic tyrosines were very modest. Analytical TLC showed that only one product was formed. NMR spectra of the crude mixture indicated that 85% of the desired product was formed. The purification on SiO_2 was ineffective because only 38% of the pure product could be isolated due to decomposition. The mechanism of this oxidation can be described as shown in Scheme 8.

The spirocyclus is resistent towards weak acids, but methanolysis at 22°C in the presence of $NaHCO_3$ leads to the hydroindolenone **25**. In contrast, methanolysis at 0°C provides exclusively dienone **24** (Wipf and Kim, 1992).



24 **19** **25**

Scheme 9. Behaviour of spirolactone **19** in weakly basic media

3.3 Spirolactones as active esters

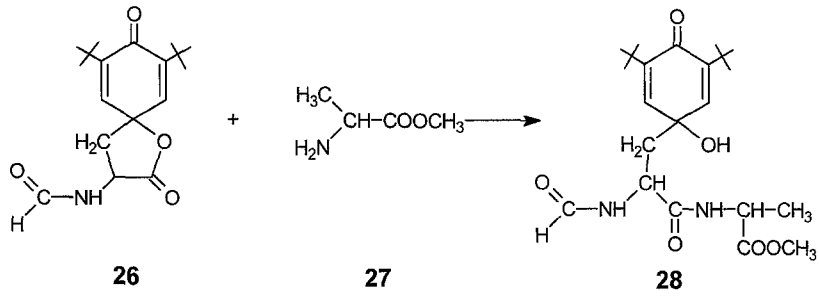
Although there is a large number of protective groups suitable for peptide synthesis, new groups and novel reagents for their preparation were developed.

The electrochemical and the chemical oxidation of *N*-protected tyrosine derivatives leads to intramolecular cyclization. The resulting spirolactone **19** represents a simultaneous protection of the *C*-terminus and the phenolic side chain, needing no external protective reagent. Unfortunately, it was not possible up to now to remove the *N*-protective groups without cleavage of the lactone ring.

3.3.1 Coupling step

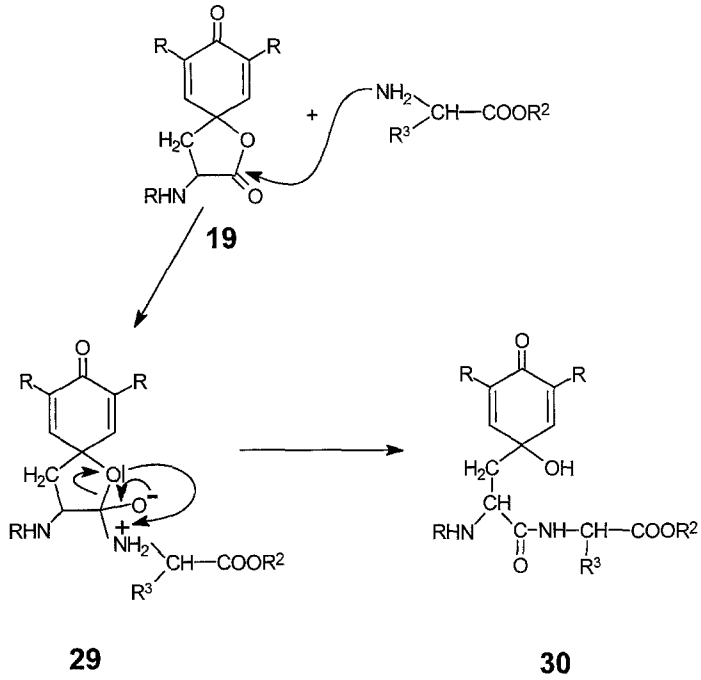
On the other hand, these spirocyclic lactones behave as active esters which can react with amino acid esters to give tyrosyl dipeptides, in which the tyrosine side chain is present as quinol. The lactone ring is opened during this reaction.

Thus, the oxidative esterification finally leading to the lactone can be looked at as an activation step of the carboxy group. Consequently, mixing equimolar amounts of 3-(*N*-formamido)-7,9-di-*tert*-butyl-1-oxaspiro[4,5]-deca-6,9-dien-2,8-dione **26**, as example, and an amino acid ester e.g. H-Ala-OMe **27** in CH_2Cl_2 for 60 h, gave the dipeptide **28** (Ziogas, 1993) (Scheme 10).



26 **27** **28**

Scheme 10. Reaction of a spirolactone with an amino acid ester



19

29 **30**

Scheme 11. Proposed coupling mechanism

A nucleophilic attack of the amino group on the carboxy group takes place leading to the coupling of the amino acids. It is assumed that an unstable amino hemiketal **29** is formed as an intermediate which passes over to the stable dipeptide **30** with a simultaneous ring opening (Scheme 11).

It can be observed that sterically unhindered amino acid esters on the one hand react faster with spirolactones but on the other hand they also polymerize faster. Therefore, an excess of the amino acid ester is required for the reaction. Higher temperatures reduce the reaction time but increase the polymerization of the ester. The reaction conditions and yields of this coupling can be seen in Table 4.

To increase the yield of the dipeptide suitable catalysts were needed. The polymeric acid Amberlyst 15, a macroporous sulfonic acid resin on a polystyrol basis has proved to be unsuitable for this reaction. Even after 100 h no dipeptide could be detected. It was noticed that the amino acid ester

Table 4. Coupling conditions and yields of **30**

19 amino acid ester				Reaction conditions	Yield of **30** [%]
R	R^1	R^2	R^3		
*t*Bu	For	Me	Me	CH_2Cl_2, 96h, 20°C	77
*t*Bu	For	H	Me	CH_2Cl_2, 72h, 20°C	66
*t*Bu	Z	Et	*i*-Bu	$CHCl_3$, 80h, 60°C Kat.:*p*-Tos-OH	67
*t*Bu	Z	Et	*i*-Bu	$CHCl_3$, 120h, 60°C	37
*t*Bu	Fmoc	Et	*i*-Bu	$CHCl_3$, 80h, 60°C Kat.:*p*-Tos-OH	51
*t*Bu	Fmoc	Et	*i*-Bu	$CHCl_3$, 96h, 60°C	15
Br	Z	Et	*i*-Bu	$CHCl_3$, 80h, 60°C Kat.:*p*-Tos-OH	14
H	Z	Et	*i*-Bu	$CHCl_3$, 80h, 60°C Kat.:*p*-Tos-OH	7

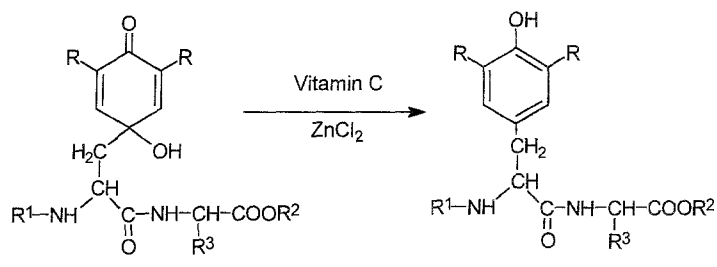
disappears during the reaction without reacting with the spirolactone. Presumably, the ester is adsorbed on the porous catalyst. Presently, *p*-toluenesulfonic acid is the best catalyst for this reaction, the yields of the dipeptides can be increased for 2–3 times with this catalyst.

3.3.2 Reduction of the tyrosyldipeptide

The reaction between spirolactones and amino acid esters lead to dipeptides in which the phenolic part of the tyrosine has a quinoidal form, which may hamper the practical application, although these compounds can be regarded as peptides containing a non-natural amino acid.

However, the rearomatization can be also achieved by reducing the quinoidal dipeptide. As possible reduction methods electrochemical (cathodic reduction) or chemical methods (catalytic hydrogenation) can be envisaged.

As a chemical rearomatization step we suggest reduction of **30** to the tyrosyldipeptides **31** with the acetonide of ascorbic acid in $CHCl_3$ using $ZnCl_2$ as catalyst, where yields of **31** of up to 75% can be reached (Scheme 12).



30 **31**

Scheme 12. Reduction to the tyrosyl dipeptide

4 Conclusions

Di-*tert*-butyl-substituted tyrosine and thyronine derivatives are potential amino acid spin labels. Because of the stability of their phenoxy radicals, they can be used in peptide chemistry to determine the tertiary structure of proteines. These derivatives may be of considerable experimental value in investigation of the hormone-receptor interaction (e.g. NPY).

Moreover, tyrosine derivatives can be oxidized chemically or electrochemically in a two-electron transfer to form spirolactones. These compounds are not only precursors for the synthesis of some antibiotics such as aranorosine or anticapsine but also synthons useful in peptide synthesis. They react as active esters with amino acid esters to give quinonoid dipeptides that can be reduced to tyrosyl dipeptides. This procedure constitutes a new method to introduce a tyrosine at the *N*-terminus of amino acids or oligopeptides.

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