# Spirolactones of tyrosine: synthesis and reaction with nucleophiles

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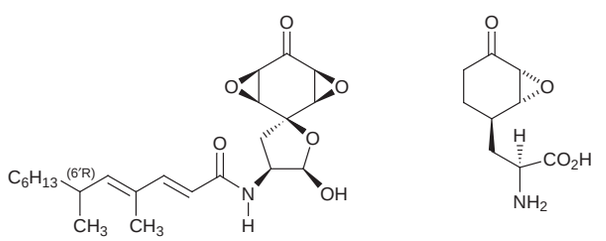
**Several quinonoidal spirolactones (1-oxaspiro[4,5]deca-6,9-diene-2,8-diones) 12 have been synthesized chemically or electrochemically by oxidation of the corresponding tyrosine phenols 3. The spirolactones are of considerable value in peptide chemistry, because they react as active esters with amino acid esters to give dipeptides. In the latter, the side chain of the tyrosine moiety is present as a quinol (4-hydroxycyclohexa-2,5-dien-1-one) system, which can be reduced to the genuine tyrosyl dipeptide. Unsubstituted spirolactones also react as active esters but give dipeptides only in modest yields.**

## Introduction

Spirolactones of tyrosines have played a significant role in organic chemistry for several years. They are intermediates in the synthesis of some alkaloids and antibiotics such as aranorosin **A**[1–4] or anticapsin **B**.[5] However, they may also serve as




**A** **B**

synthons useful in peptide chemistry, as is shown in this paper.

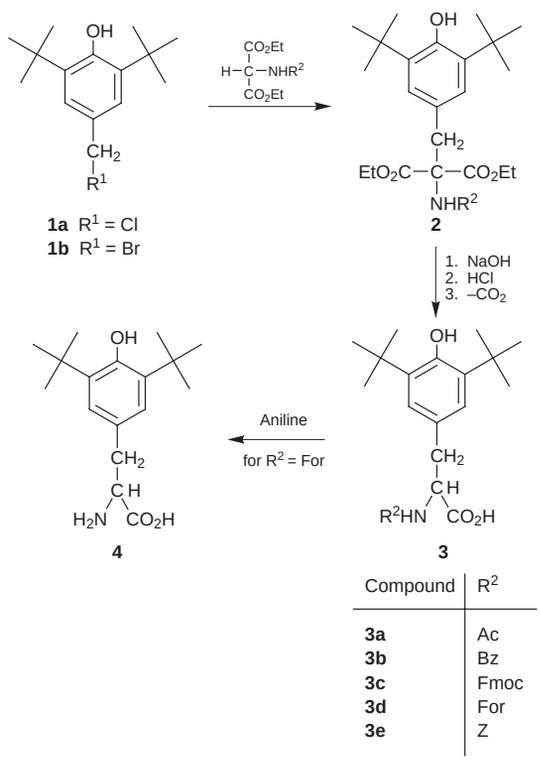
The key-step in their synthesis is the oxidative cyclization of L-tyrosine and its derivatives **3** (see Scheme 1 for synthesis). Since tyrosines are redox-active phenols, they can be oxidized chemically or electrochemically, to phenoxy radicals or phenoxenium cations,[6] depending on the applied oxidation potential and the extant proton concentration. The relevant oxidation potentials can be determined from cyclic voltammograms (CVs) of **3**. In this context, the phenoxenium ions are the desired species, because they should react intramolecularly with the nucleophilic carboxy group of the side chain to give the spirolactones **12** (Scheme 2) with an autoprotected C-terminus *and* side-chain of the original tyrosine. These spirolactones were indeed formed in very high yields when the intermediate phenoxenium ions were stabilized by *tert*-butyl groups in both *ortho*-positions of the oxylium oxygen. Other reasons to use *tert*-butyl substituted tyrosines were the higher stability of the *tert*-butylated spirolactones **12** themselves, as well as the less pronounced adsorption of the corresponding tyrosines at the anode in the case of the electrochemical synthesis. The unsubstituted spirolactones could be synthesized only in modest yields (up to 40%), even in the chemical oxidation with phenyliodine(III) bis(trifluoroacetate) (PIFA), where no adsorption effects were interfering. The oxidation of the ring-unsubstituted tyrosine derivatives with *N*-bromosuccinimide (NBS) gave the 7,9-dibrominated spirolactones,[7] whereas the *tert*-butylated species furnished **12** without bromination.

The spirolactones **12**, as expected, proved to be active esters capable of coupling with nucleophiles like amines or amino acid esters to form tyrosine amides or dipeptides.

## Results and discussion

### Synthesis of *tert*-butyl-substituted tyrosine derivatives

Two different methods have been used to synthesize *tert*-butyl-substituted tyrosine derivatives. The first one proceeds *via* a formylated amino malonic acid ester and was first described by Teuber *et al.*[8] (Scheme 1). As starting phenol they used 2,6-di-




| Compound | $R^2$ |
|---|---|
| **3a** | Ac |
| **3b** | Bz |
| **3c** | Fmoc |
| **3d** | For |
| **3e** | Z |

**Scheme 1**

*tert*-butyl-4-chloromethylphenol **1a** which was coupled with diethyl 2-(formylamino)malonate to give the phenolic malonate **2**. After hydrolysis and decarboxylation the *N*-protected di-*tert*-butyltyrosine **3d** ($R^2$ = For, formyl) was obtained. The formyl group was cleaved by heating **3d** in aniline to give **4**. We used the brominated phenol **1b** instead of **1a**, since **1a** could not be prepared in good yields.

The synthesis of **4** was optimized, and the yields of the respective steps could be raised as follows: the coupling with diethyl 2-(formylamino)malonate to give **2** ($R^2$ = For) from 81 to 92%, the hydrolysis of the ester groups in **2** from 68 to 89%,

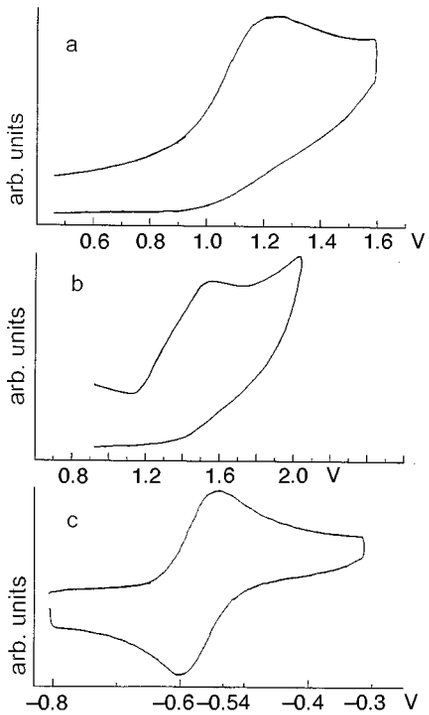



**Fig. 1** Cyclic voltammograms in 0.1 M $(Et)_4NBF_4$–MeCN. a: **5**, $v = 500$ mV $s^{-1}$, $c = 1 \times 10^{-3}$ mol $l^{-1}$; b: **7**, $v = 200$ mV $s^{-1}$, $c = 1 \times 10^{-3}$ mol $l^{-1}$; c: **5**, $v = 200$ mV $s^{-1}$, $c = 2 \times 10^{-4}$ mol $l^{-1}$, $c$ (KOBu$^t$) $= 4 \times 10^{-4}$ mol $l^{-1}$.

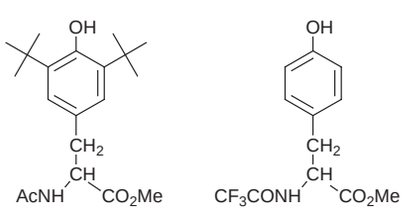
the decarboxylation to give **3** from 87 to 93% and the cleavage of the formyl group in **3d** from 83 to 96%. The overall yield of **4** could therefore be increased from 40 to 73%.

With the second method described by Cohen *et al.*[9] we synthesized *N*-benzoyltyrosine **3b** ($R^2$ = Bz) in adequate yields.

### Electrochemical oxidation

**Cyclic voltammetry.** By means of cyclic voltammetry it is possible to get important information on the redox behaviour of individual parts of the tyrosine molecule with simultaneous masking of all other functions.

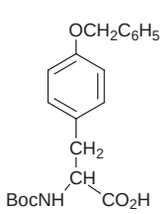
The CVs of *N*-protected tyrosine esters, like **5**[10] (Fig. 1a),




**5**




**6**

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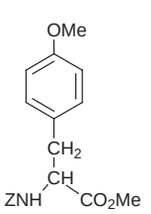



**8**

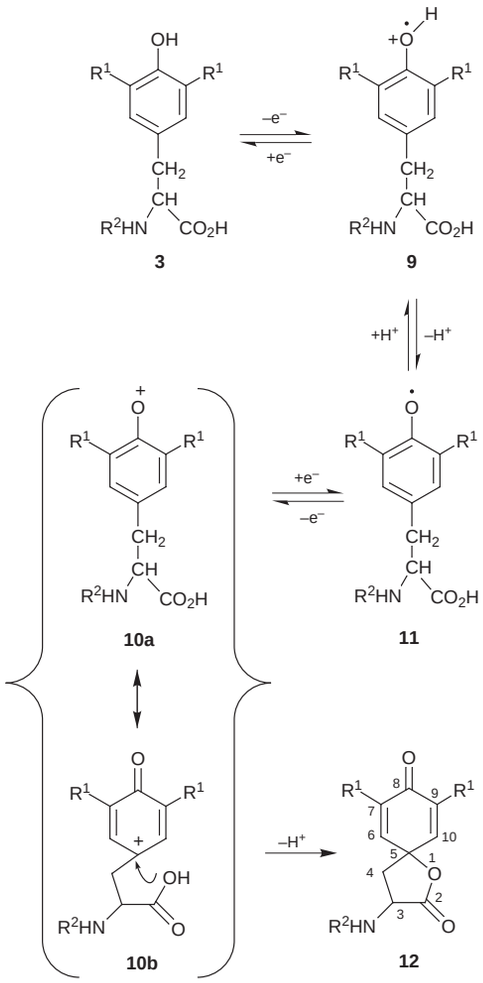
show completely irreversible oxidation peaks at 1200–1250 mV (*vs.* Ag/$10^{-2}$ M $Ag^+$/MeCN). These peaks correspond to the oxidation of the phenolic OH-group, and their potentials are characteristic of 2,6-di-*tert*-butyl-4-alkylphenols.[11] The corresponding oxidation peak of a non-*tert*-butylated tyrosine, like **6**[10] is shifted to more positive potentials (1330 mV), which is reasonable because the *tert*-butyl groups exert a weak electron-donating effect. Similarly, the tyrosines **3** with a free phenolic OH and unprotected carboxy group, as in **3a** and **3d**, give oxidation peaks at 1100–1200 mV; a separate peak for the oxidation of the carboxy group cannot be found up to 1600 mV. On the other hand, compound **3c** reveals two oxidation peaks, one at 1210 mV due to phenolic oxidation, and one at 1560 mV which might be attributed to the oxidation of the carboxy group. The *N*- and side-chain-protected tyrosine **7**[10] also reveals a peak at 1540 mV (Fig. 1b), however, this may be due to phenol ether oxidation, and does not give unequivocal proof of the oxidation of the carboxy group at this potential; the totally protected tyrosine **8**[10] shows a phenol ether oxidation at 1380 mV. Hence, we measured the CV of acetic acid under the same conditions and found an oxidation peak at 1580 mV in good agreement with that of **3c**. The fact that **3a** and **3d** do not show an oxidation peak in this area is not well understood. The peak may be shifted to higher potentials; for **3a** a shoulder was observed at 1.9 V in the end-rise of the current of the supporting electrolyte oxidation. These results are in agreement with those obtained with other phenols,[11] also showing that the tyrosines **3** are first oxidized in one-electron transfers to the cation radicals **9** (Scheme 2), which deprotonate to the neutral phenoxyl radicals **11**. These are further oxidized at the given potential to form the phenoxenium ions **10**. Thus, the oxidation peaks at 1100–1200 mV correspond to overall ECE reactions of **3** to **10**.

In the presence of an excess of a base ($NH_4OH$ or potassium *tert*-butoxide), the phenolic and carboxylic functions of the *N*-protected amino acid exist as anions, *i.e.* phenolate and carboxylate, respectively. CV Measurements in this medium give results different from those in neutral solutions. The oxidation peak is now shifted to −460 to −640 mV and is accompanied by a re-reduction peak (−560 to −740 mV). Thus, for example, the oxidation peak of the phenolate of **5** can be located at −537 mV and the corresponding re-reduction peak at −597 mV (details given in Fig. 1c). There is no dependence of the peak potential on the scan rate $v$, $\Delta E = E_p^{ox} - E_p^{red} = 60$ mV, and $i_{ox}/i_{red} \sim 1$, which means that a reversible one-electron transfer occurs with $E^0 = (E_p^{ox} + E_p^{red})/2 = -567$ mV between phenolate $\mathbf{5}^-$, the electrode, and phenoxyl radical $\mathbf{5}^{\bullet}$. Similarly, the phenolates $\mathbf{3}^-$ are oxidized to the phenoxyl radicals in the presence of a base, but here the one-electron transfer is quasi-reversible, as can be seen from the increasing $\Delta E$ with increasing scan rate $v$. For the case of **3a** the relevant data are given in Table S1 of the Supplementary Material.† The $E^0$ of $\mathbf{3a}^-$ is −691 mV and therefore more negative than that of $\mathbf{5}^-$. This seems to be a result of the presence of the carboxylate anion in the latter.

The oxidation of the carboxylate, on the other hand, occurs nearly at the same potential as that of the carboxy group itself (*i.e.* at about 1500 to 1600 mV), which is due to the strong acidity of the latter producing the carboxylate in equilibrium ($pK_a$ of the unsubstituted tyrosine = 2.2). This is also demonstrated by the fact that the $E_p^{ox}$ of acetic acid (see above) in MeCN does not change by adding KOBu$^t$ to the solution.

**Preparative electrochemistry.** As a consequence of the above potential considerations, for a preparative-scale electrolysis, *N*-protected tyrosines **3** were anodically oxidized to the phenoxenium ions **10** at potentials of 1300–1400 mV under neutral conditions or in the presence of weak bases such as lutidine. Then, the nucleophilic carboxy group of the side chain indeed added to the *para*-position of the ions **10** to give the spirolactones **12** (Scheme 2). Unfortunately, the electrochemical oxidation was affected by strong adsorption effects; very often, a fast decrease in the current was observed, caused by coating of the electrode leading to a poor conversion of the tyrosines.

† This data is available as supplementary material (SUPPL. NO. 57389, 1 p.). For details of the Supplementary Publications Scheme see 'Instructions for Authors', *J. Chem. Soc., Perkin Trans. 1*, available *via* the RSC Web page (http://www.rsc.org/authors).



Scheme 2

Better yields could be achieved using graphite instead of platinum as the working electrode.[12]

Spirolactones of *N*-protected 3,5-di-*tert*-butyltyrosine derivatives **3**‡ ($R^1$ = Bu$^t$, $R^2$ = Ac, Bz, Fmoc, For, Z) could thus be synthesized anodically in acetonitrile in 28 to 85% yields (Table 1). In the case of ring-unsubstituted tyrosines **3**‡ ($R^1$ = H), however, the yields of isolable spirolactones **12** were relatively modest (17–18%).[13,14] This is presumably due to the instability of the species under the work-up conditions. Thus, the conversion of Z-tyrosine in 0.1 M $NaClO_4$–MeCN at a graphite electrode into the spirolactone was nearly quantitative (analytical TLC) but after purification only 17% of the desired product could be isolated.

## Chemical oxidation

Spirolactones **12** can also be synthesized using chemical oxidants. These must be able to produce phenoxenium ions **10** in a formal two-electron oxidation process. Thus, the choice of the oxidant is very important, because its oxidizing strength indirectly determines the yield of the spirolactones.

Schmir, Cohen and Witkop[7] reported a study on the action of bromine or *N*-bromosuccinimide (NBS) on derivatives of tyrosine and simpler analogues. In contrast to the anodic oxidation, the formation of a spirolactone with NBS was almost quantitative within a few minutes, however, the phenolic side chain was brominated too. On the other hand, the oxidation of 3,5-di-*tert*-butylated tyrosine derivatives with NBS did *not* produce a dibrominated lactone but gave the desired 3,5-di-*tert*-butylated species **12** in excellent yields (Table 1). The brominating side reactions made it desirable to test new oxidants for the transformation of *N*-protected-L-tyrosines **3** ($R^1$ = H) into the corresponding dienone lactones **12**. Among those oxidants, phenyliodine(III) diacetate (PIDA) and phenyliodine(III) bis-(trifluoroacetate) (PIFA) proved to be the most effective ones.[1,2,15,16] These high valent iodoso derivatives, in a small excess under argon, indeed converted *N*-protected tyrosines into the corresponding lactones.[1] The yields of the isolated ring-unsubstituted spirocyclic tyrosines were again very modest. Analytical TLC showed that originally only one product was formed and the NMR spectra of the crude product indicated that up to 85% of the desired compound was present. Chromatography on $SiO_2$, however, was ineffective for purification, and only 23–38% of pure **12** could be isolated.

‡ For the numbering see Scheme 2, for the type of substituents Table 1.

**Table 1** Oxidation of tyrosine derivatives **3**[a]

| 12 | $R^1$ | $R^2$ | Oxidant | Yield (%) |
|---|---|---|---|---|
| **a** | Bu$^t$ | Ac | NBS | 97 |
| | | | An[a] | 44 |
| **b** | Bu$^t$ | Bz | NBS | 100 |
| | | | An | 28 |
| **c** | Bu$^t$ | Fmoc | NBS | 100 |
| | | | An | 64 |
| **d** | Bu$^t$ | For | NBS | 93 |
| | | | An | 40 |
| **e** | Bu$^t$ | Z | NBS | 92 |
| | | | An | 85 |
| **f** | Br | Boc | NBS | 97 |
| | | | An | 54 |
| **g** | Br | Z | NBS | 97 |
| | | | An | 72 |
| **h** | I | Z | NBS | 98 |
| | | | An | 66 |
| **i** | H | Boc | PIFA | 76[b] |
| | | | An | 18 |
| **j** | H | Z | PIFA | 85[c] |
| | | | An | 17 |

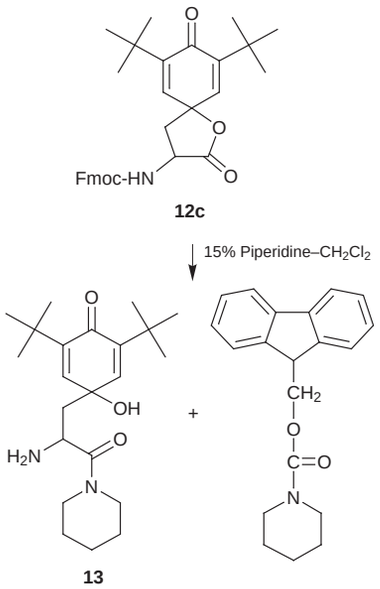
[a] Anodic oxidation. [b] Determined by NMR spectroscopy; after chromatography 23%. [c] Determined by NMR spectroscopy; after chromatography 38%.

## Spirolactones as active esters

As has been shown above, the oxidation of *N*-protected tyrosine derivatives leads to intramolecular cyclization. The resulting spirolactone **12** may be regarded as a tyrosine in which the C-terminus *and* the phenolic side chain are intramolecularly protected, needing no external protective reagent. One could think of cleaving the *N*-protecting group in **12** and using the free amino group for peptide coupling. While trying to remove the *N*-protecting group from 3-(*N*-Fmoc)-7,9-di-*tert*-butyl-1-oxaspiro[4,5]deca-6,9-diene-2,8-dione **12c** with 15% piperidine–$CH_2Cl_2$, we obtained after a few minutes the piperidine amide **13** (Scheme 3), the result of a simultaneous cleavage of the Fmoc group and ring opening of the spirocycle.

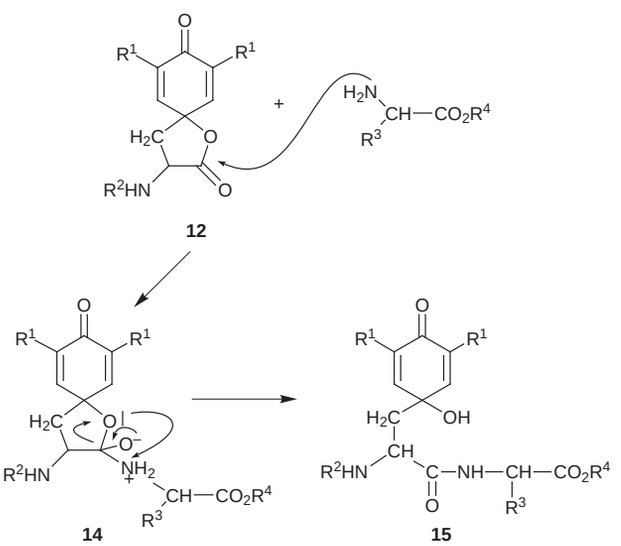
Up to now it was not possible to remove the *N*-protecting groups in **12** without cleavage of the lactone ring. On the other hand, the above mentioned reaction of **12c** to give **13** led us to the idea to react these spirocyclic lactones with amino acid esters to form a peptide bond. In the resulting dipeptides, the original tyrosine side chain would be present as a quinol. This strategy was indeed found to be successful, and treating equimolar amounts of 3-(*N*-formylamino)-7,9-di-*tert*-butyl-1-oxaspiro[4,5]deca-6,9-diene-2,8-dione **12d** with the amino acid ester $NH_2$-Ala-OMe in $CH_2Cl_2$, for example, gave the dipeptide **15a** ($R^1$ = Bu$^t$, $R^2$ = For, $R^3$ = Me, $R^4$ = Me) after 60 h.

The oxidative intramolecular esterification to form the lactone **12** can therefore be regarded as an activation process of the carboxy group. Apparently, nucleophilic attack of the amino group of the ester at the carbonyl group of the lactone takes place, finally leading to coupling of both amino acids.




**Scheme 3**

Presumably, an unstable amino hemiketal **14** is formed as an intermediate which is converted to the stable dipeptide **15** with simultaneous ring opening (Scheme 4).




**Scheme 4**

It was observed that sterically unhindered amino acid esters react faster with the spirolactones **12** 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 also increase the polymerization rate of the ester. The reaction conditions and coupling yields are collected in Table 2.

It was expected that proton donors would further activate the spiroester carbonyl group and thus increase the yield of the dipeptide. However, only a catalytic amount of acid might be useful, in order not to block the amino group of the amino ester. The polymeric acid Amberlyst 15, a macroporous sulfonic acid resin on a polystyrene base, was first tested, because it should provide non-hydrated protons. Unfortunately, it proved to be unsuitable for this reaction: even after 100 h no dipeptide could be detected. The amino acid ester, however, disappears during the reaction without reacting with the spirolactone. Presumably, it is adsorbed on the porous catalyst. Presently, *p*-toluenesulfonic acid is the best catalyst for this reaction, and yields of the dipeptides could be increased 2–3 times with this

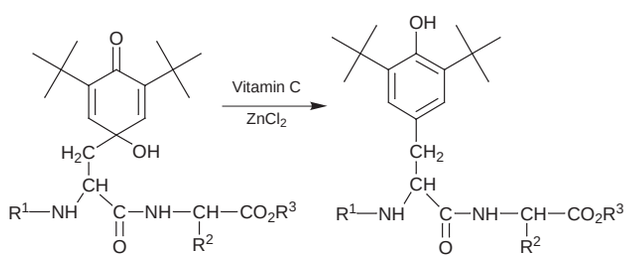
**Table 2** Coupling conditions and yields of **15**

| Compd. **15** | **12** $R^1$ | **12** $R^2$ | Amino acid ester $R^3$ | Amino acid ester $R^4$ | Reaction conditions | Yield (%) |
|---|---|---|---|---|---|---|
| **a** | Bu$^t$ | For | Me | Me | $CH_2Cl_2$, 96 h, 20 °C | 60 |
| **b** | Bu$^t$ | For | H | Et | $CH_2Cl_2$, 72 h, 20 °C | 63 |
| **c** | Bu$^t$ | Z | Bu$^i$ | Et | $CHCl_3$, 80 h, 60 °C cat. TsOH | 67 |
| **c** | Bu$^t$ | Z | Bu$^i$ | Et | $CHCl_3$, 120 h, 60 °C | 37 |
| **d** | Bu$^t$ | Fmoc | Bu$^i$ | Et | $CHCl_3$, 80 h, 60 °C cat. TsOH | 51 |
| **d** | Bu$^t$ | Fmoc | Bu$^i$ | Et | $CHCl_3$, 96 h, 60 °C | 15 |
| **e** | Br | Z | Bu$^i$ | Et | $CHCl_3$, 80 h, 60 °C cat. TsOH | 14 |
| **f** | H | Z | Bu$^i$ | Et | $CHCl_3$, 80 h, 60 °C cat. TsOH | 7 |

catalyst. Basic catalysts, such as *N*-methyl morpholine, were not effective.

As already mentioned, the reaction of the spirolactones with amino acid esters leads to a dipeptide in which the phenolic part of the tyrosine has a quinonoidal structure. This may hamper the application, although such non-natural amino acids might be of interest for screening tests. The rearomatization to the normal tyrosine form should, however, also be achievable by reducing the quinonoidal dipeptide.

We did not work out such procedures in detail. However, we found that reduction of **15** to the tyrosyl dipeptides **16** can be achieved in 52–75% yield by the acetonide of ascorbic acid in $CHCl_3$ using $ZnCl_2$ as catalyst (Scheme 5). De-*tert*-butylation




| Compound | $R^1$ | $R^2$ | $R^3$ | Yield of **16** (%) |
|---|---|---|---|---|
| **a** | For | Me | Me | 75 |
| **c** | Z | $CH_2CH(Me)_2$ | Et | 52 |

**Scheme 5**

of **16c** with $AlCl_3$ in toluene (3–22 h at 25 °C) or in nitromethane–benzene (4–5 h, 25–50 °C) led only to decomposition.

**Structure determination**

The structure of the synthesized compounds has been determined by $^1$H NMR, $^{13}$C NMR, IR and mass spectroscopy. NMR Spectroscopy is a particularly good tool for characterizing the present reaction products, which are quinolide compounds on the one hand and amino acids or dipeptides on the other. A series of compounds with these structural units has been measured earlier,[17,18,19] and serve as basic molecules for chemical shift considerations with the help of increment systems. Thus, for instance, it was possible to assign the fragments -Ala-OMe, -Gly-OEt and -Leu-OEt in the spectra. Because of the presence of the asymmetric α-carbon atom of the amino acid moiety, the signals of equivalent nuclei may split due to magnetic non-equivalence (hereafter abbreviated as MNE). Thus, the $^1$H and $^{13}$C NMR signals of the *tert*-butyl groups of the quinolide ring, as well as the $^{13}$C signals of the olefinic

carbons of the rings may be doubled. The protons at the positions *meta* to the carbonyl group generally cause an AB splitting pattern, because, in addition to MNE, they couple with $^4J \approx 2$–3 Hz. The same is true for the geminal protons of the $CH_2$ moiety in the Z group or in the tyrosine side chain ($^1J \approx 12$–14 Hz).

The synthesis of the *tert*-butyl-substituted tyrosine derivatives according to Scheme 1 leads to compounds with D,L-configuration. As a consequence, the corresponding spirolactones obtained on electrochemical or chemical oxidation are also in the D,L-configuration. Since the reaction between the spirolactones and L-amino acid esters does not lead to racemization of the latter, two diastereomeric quinolide dipeptides are present after the reaction causing a doubling or broadening of most of the NMR signals. The interpretation of the $^1$H NMR spectra is therefore difficult because many signals overlap and/or coincide. Thus, in the Experimental section, only the positions of the multiplets are presented.

## Conclusions

Tyrosine derivatives can be oxidized chemically or electrochemically in a two-electron oxidation *via* a free or incipient phenoxenium ion to form spirolactones. These compounds are not only precursors for the synthesis of some antibiotics, such as aranorosin or anticapsin, but also synthons useful in peptide synthesis. They react as active esters with amines and amino acid esters to give quinonoidal amides or dipeptides that can be reduced to tyrosyl amides or tyrosyl dipeptides. This procedure constitutes a new method to introduce a tyrosine, preferentially with a non-natural side chain, at the *N*-terminus of amino acids or oligopeptides. The *tert*-butyl substituted derivatives can then be used as amino acid spin labels to draw conclusions on the configuration and conformation as well as the tertiary structure of the labelled compound, *e.g.* a peptide.[20]

## Experimental

Melting points were determined on a Mettler FP61 melting point apparatus and are uncorrected. IR Spectra were recorded on a Perkin-Elmer IR-281 (KBr disks). Low resolution mass spectra were obtained on a Varian Matt 711 (200 °C, 70 eV or 50 °C/FD). NMR Spectra were recorded on a Bruker AC 250 (250 MHz for $^1$H and 62.9 MHz for $^{13}$C) spectrometer in $CDCl_3$ unless otherwise noted. Chemical shifts ($\delta$) are given in ppm and the coupling constants ($J$) in Hz. Anhydrous solvents were freshly distilled from either $P_2O_5$ or magnesium. For analytical TLC, Merck silica gel 60 F-254 plates were used. Cyclovoltammetric experiments were conducted on a Bruker polarograph E 350 with a Wenking voltage scanner and a MVS 87 Houston Instrument Omnigraphic 2000 xy-printer in MeCN *vs*. Ag/$10^{-2}$ M $Ag^+$/MeCN with $(Et)_4NBF_4$ or $NaClO_4$ as electrolytes. *Although no problems were encountered, it should be noted that perchlorates in general may be explosive and should be handled with due care*.

### Oxidation with PIFA. General procedure 1

To a stirred solution of 1.80–2.20 mmol of *N*-protected tyrosine **3** in 30 ml of absolute acetonitrile at −5 °C a few drops of pyridine and 2.0–2.45 mmol of phenyliodoso bis(trifluoroacetate) (PIFA) were added under argon. The reaction mixture was stirred for 20 min at −5 °C. Afterwards, the mixture was diluted with 20 ml of water and extracted with $CH_2Cl_2$. The combined organic layers were dried over $MgSO_4$ and concentrated to give a greenish oil which was chromatographed on $SiO_2$ (50% EtOAc–*n*-hexane) to give the spirocyclic product as a white solid.

### Oxidation with NBS. General procedure 2

To a solution of *N*-protected tyrosine derivatives **3** (1.50 mmol) in 192 ml of 20% acetonitrile–acetate buffer (38.4 ml of acetonitrile and 153.6 ml of acetate buffer, pH = 4.6) a solution of NBS (800 mg, 4.5 mmol) in 54 ml of 20% acetonitrile–acetate buffer was added. A crystalline substance separated within a few minutes. The reaction mixture was chilled for 2 h and the product was collected, washed with *n*-hexane and dried. If necessary, the product was recrystallized from acetonitrile–water or *n*-hexane–EtOAc. For exceptions see the detailed procedures.

### Electrochemical oxidation. General procedure 3

The preparative anodic oxidation was performed in an undivided cell, *i.e.* a cylindrical glass vessel with a lateral tube to take up the reference electrode. The anode was made of graphite and the cathode was a cylindrical platinum-net (90% Pt and 10% Ir). For the potential-controlled electrolysis the reference electrode was $Ag/Ag^+$ (Ag/0.01 M $Ag^+$ in acetonitrile) with a potential of 0.35 V *vs*. SCE.[21] A 0.1 M $NaClO_4$ solution in acetonitrile was used as the sole supporting electrolyte (SE), and the produced protons were neutralized by an excess of 2,6-lutidine (2,6-dimethylpyridine).

The oxidation potential was set to 1300–1400 mV to oxidize the phenolic OH-group, and the concentration of the substrate in the solution amounted to about 0.01 mol $l^{-1}$. At the end of the reaction, the mixture was evaporated to one half its original volume and diluted with 400 ml of water. The product was extracted with diethyl ether (3 × 100 ml) and washed with water. The organic layer was dried over $MgSO_4$, and evaporated to yield the crude products which were recrystallized or purified by silica gel chromatography.

### Quinoidal tyrosyldipeptides 15 from spirolactones 12. General procedure 4

To a stirred solution of the spirolactone **12** (0.69 mmol) in $CH_2Cl_2$ or $CHCl_3$ (30 ml) the amino acid ester (1.16 mmol) was added. The mixture was stirred for several hours under reflux (see Table 2). The solvent was removed, the residue washed with *n*-hexane and dried. The product was purified by column chromatography on silica gel with $CH_2Cl_2$–methanol to yield the desired quinolide dipeptides. Sometimes, catalytic amounts (5–10 mg) of *p*-toluenesulfonic acid were used to catalyse the reaction. For exceptions see the detailed procedures.

### 3-Acetylamino-7,9-*tert*-butyl-1-oxaspiro[4,5]deca-6,9-diene-2,8-dione 12a

General procedure 2: the reaction of Ac-Tyr(3,5-Bu$^t_2$)-OH **3a** (510 mg, 1.50 mmol) and NBS (800 mg, 4.5 mmol) gave the product **12a** (485 mg, 97%) as a white solid, mp 179 °C (Found: C, 68.5; H, 8.3; N, 4.2. $C_{19}H_{27}NO_4$ requires C, 68.4; H, 8.2; N, 4.2%); $R_f$ 0.23 (*n*-hexane–EtOAc, 1:1); $\delta_H$(250 MHz; $CDCl_3$) 1.20 (18H, s, Bu$^t$), 2.06 (3H, s, Ac), 2.34 (1H, dd, $J \approx 12$, $J \approx 13$, CC$H_2$CH), 2.68 (1H, dd, $J \approx 9$, $J \approx 13$, CC$H_2$CH), 4.85 (1H, ddd, $J \approx 6.5$, $J \approx 9$, $J \approx 12$, $CH_2$C$H$NHR), 6.46 (1H, d, $J \approx 6.5$, N$H$), 6.50 (1H, d, $J \approx 3$, $H_{quin}$), 6.52 (1H, d, $J \approx 3$, $H_{quin}$); $\delta_C$(62.9 MHz; $CDCl_3$) 22.84, 29.25; 34.95, 35.04 (MNE); 40.09, 49.70, 78.25; 135.23, 137.61 (MNE); 148.09, 148.63 (MNE); 170.60, 174.33, 185.32; *m*/*z* (EI) 333 ($M^+$).

General procedure 3: the reaction of Ac-Tyr(3,5-Bu$^t_2$)-OH **3a** (2.0 g, 6.0 mmol) in 200 ml SE at a potential of $E^{ox}$ = 1300 mV yielded **12a** (875 mg, 44%) after 10 h.

### 3-Benzoylamino-7,9-di-*tert*-butyl-1-oxaspiro[4,5]deca-6,9-diene-2,8-dione 12b

General procedure 2: the reaction of Bz-Tyr(3,5-Bu$^t_2$)-OH **3b** (200 mg, 0.5 mmol) and NBS (237 mg, 1.33 mmol) gave the product **12b** (197 mg, 100%) as a white solid, mp 215–245 °C (decomp.) (Found: C, 72.69; H, 7.52; N, 3.46. $C_{24}H_{29}NO_4$ requires C, 72.89; H, 7.39; N, 3.54%); $R_f$ 0.46 (*n*-hexane–$CH_2Cl_2$, 1:1); $\delta_H$(250 MHz; $CDCl_3$) 1.22 (9H, s, Bu$^t$), 1.24 (9H, s, Bu$^t$), 2.48 (1H, t, $J \approx 13$, CC$H_2$CH), 2.85 (1H, dd, $J \approx 9$, $J \approx 13$, CC$H_2$CH), 5.00–5.12 (1H, m, $CH_2$C$H$NHR), 6.55 (1H,

d, $J \approx 3$, $H_{quin}$), 6.58 (1H, d, $J \approx 3$, $H_{quin}$), 6.97 (1H, d, $J \approx 8$, N*H*), 7.39–7.93 (5H, m, Bz); $\delta_C$(62.9 MHz; $CDCl_3$) 29.2; 35.1, 35.2 (MNE); 40.5, 50.1, 78.4, 127.1, 128.7, 132.4, 132.7; 135.1, 137.6 (MNE); 146.7, 148.1 (MNE); 167.6, 174.4, 185.3; *m*/*z* (EI) 395 ($M^+$).

General procedure 3: the reaction of Bz-Tyr(3,5-Bu$^t_2$)-OH **3b** (600 mg, 1.51 mmol) in 200 ml SE at a potential of $E^{ox}$ = 1400 mV gave **12b** (166 mg, 28%) after 5 h.

### 3-(Fluoren-9-ylmethoxycarbonylamino)-7,9-di-*tert*-butyl-1-oxaspiro[4,5]deca-6,9-diene-2,8-dione 12c

General procedure 2: the reaction of Fmoc-Tyr(3,5-Bu$^t_2$)-OH **3c** (3.0 g, 5.8 mmol) in buffer (745 ml) and NBS (2.70 g, 15.2 mmol) in buffer (210 ml) yielded white crystals (2.98 g, 100%), mp 115–117 °C; $\delta_C$(250 MHz, $CD_3COCD_3$) 1.24 (18H, s, Bu$^t$), 2.62 (2H, dd, $J \approx 10$, $J \approx 3$, CC$H_2$CH), 4.25 [1H, t, $J \approx 7$, $CH_2CH(C_6H_4)_2$], 4.40 (2H, d, $J \approx 7$, OC$H_2$CH), 4.86–4.95 (1H, m, $CH_2CH$NHR), 6.70 (1H, d, $J \approx 3$, $H_{quin}$), 7.00 (1H, d, $J \approx 3$, $H_{quin}$), 7.19 (1H, d, $J \approx 8$, N*H*), 7.32–7.38 (4H, m, $H_{arom}$-Fmoc), 7.69 (2H, d, $J \approx 7$, $H_{arom}$-Fmoc), 7.86 (2H, d, $J \approx 7$, $H_{arom}$-Fmoc); $\delta_C$(62.9 MHz, $CD_3COCD_3$) 29.5; 35.3, 35.5 (MNE); 38.6, 47.9, 51.2, 67.3, 77.3, 120.7, 125.9, 127.8, 128.5; 138.9, 140.2 (MNE); 142.0, 144.8; 147.2, 147.9 (MNE); 156.7, 174.3, 186.3; *m*/*z* (EI) 513 ($M^+$).

General procedure 3: the reaction of Fmoc-Tyr(3,5-Bu$^t_2$)-OH **3c** (600 mg, 1.17 mmol) in 100 ml SE at a potential of $E^{ox}$ = 1400 mV gave after 4 h the desired product **12c** (382 mg, 64%).

### 3-Formylamino-7,9-di-*tert*-butyl-1-oxaspiro[4,5]deca-6,9-diene-2,8-dione 12d

General procedure 2: the reaction of For-Tyr(3,5-Bu$^t_2$)-OH **3d** (500 mg, 1.56 mmol) and NBS (833 mg, 4.68 mmol) gave **12d** (460 mg, 93%), mp 121–123 °C (Found: C, 67.62; H, 7.45; N, 4.32. $C_{18}H_{25}NO_4$ requires C, 67.69; H, 7.89; N, 4.39%); $R_f$ 0.34 ($CH_2Cl_2$–MeOH, 20:1); $\delta_H$(250 MHz; $CDCl_3$) 1.22 (18H, s, Bu$^t$), 2.36 (1H, dd, $J \approx 12$, $J \approx 13$, CC$H_2$CH), 2.78 (1H, dd, $J \approx 9$, $J \approx 13$, CC$H_2$CH), 4.87 (1H, ddd, $J \approx 6$, $J \approx 9$, $J \approx 13$, $CH_2CH$NHR), 6.33 (1H, d, $J \approx 6$, N*H*), 6.49 (1H, d, $J \approx 3$, $H_{quin}$), 6.51 (1H, d, $J \approx 3$, $H_{quin}$), 8.29 (1H, s, CHO); $\delta_C$(62.9 MHz; $CDCl_3$) 29.26; 35.38, 35.48 (MNE); 39.56, 48.69, 78.75; 134.96, 137.63 (MNE); 148.41, 148.99 (MNE); 162.05, 174.65, 186.32; *m*/*z* (EI) 319 ($M^+$).

General procedure 3: the reaction of For-Tyr(3,5-Bu$^t_2$)-OH **3d** (1.0 g, 3.1 mmol) in 160 ml SE at a potential of $E^{ox}$ = 1400 mV gave **12d** (400 mg, 40%) after 6 h.

### 3-Benzyloxycarbonylamino-7,9-di-*tert*-butyl-1-oxaspiro[4,5]-deca-6,9-diene-2,8-dione 12e

General procedure 2: the reaction of Z-Tyr(3,5-Bu$^t_2$)-OH **3e** (1.0 g, 2.33 mmol) and NBS (1.248 g, 7.01 mmol) yielded **12e** (910 mg, 92%), mp 177–179 °C (Found: C, 70.19; H, 7.55; N, 3.47. $C_{25}H_{31}NO_5$ requires C, 70.56; H, 7.34; N, 3.29%); $R_f$ 0.46 (*n*-hexane–EtOAc, 2:1); $\delta_H$(250 MHz; $CDCl_3$) 1.14 (18H, s, Bu$^t$), 2.32 (1H, t, $J \approx 13$, CC$H_2$CH), 2.56 (1H, dd, $J \approx 9$, $J \approx 13$, CC$H_2$CH), 4.63 (1H, m, $J \approx 9$, $J \approx 13$, $CH_2CH$NHR), 5.12 (2H, d, $J \approx 12$, $C_6H_5CH_2O$), 5.55 (1H, d, $J \approx 7$, N*H*), 6.45 (2H, s, $H_{quin}$), 7.26 (5H, s, $H_{quin}$-Z); $\delta_C$(62.9 MHz; $CDCl_3$) 29.26; 34.95, 35.03 (MNE); 40.14, 50.98, 67.58, 77.87, 128.20, 128.42, 128.62, 135.78; 135.28, 137.69 (MNE); 148.11, 148.62 (MNE); 155.99, 173.76, 185.38; *m*/*z* (EI) 426 ($M^+ + H$).

General procedure 3: the reaction of Z-Tyr(3,5-Bu$^t_2$)-OH **3e** (900 mg, 2.1 mmol) in 200 ml SE at a potential of $E^{ox}$ = 1400 mV gave **12e** (761 mg, 85%) after 6 h.

### 3-*tert*-Butoxycarbonylamino-7,9-dibromo-1-oxaspiro[4,5]deca-6,9-diene-2,8-dione 12f

General procedure 2: the reaction of Boc-Tyr-OH **3i** (1.0 g, 3.55 mmol) and NBS (1.896 g, 10.65 mmol) gave a white solid **12f** (1.38 g, 89%), mp 188–189 °C; $\delta_H$(250 MHz; $CDCl_3$) 1.46 (9H, s, Bu$^t$), 2.53 (1H, dd, $J \approx 10$, $J \approx 13$, CC$H_2$CH), 2.72 (1H, dd, $J \approx 10$, $J \approx 13$, CC$H_2$CH), 4.62 (1H, t, $J \approx 10$, $CH_2CH$NHR), 7.59 (1H, d, $J \approx 3$, $H_{quin}$), 7.76 (1H, d, $J \approx 3$, $H_{quin}$); $\delta_C$(62.9 MHz; $CDCl_3$) 28.36, 36.50, 50.08, 79.37, 80.44; 123.13, 123.98 (MNE); 148.13, 148.95 (MNE); 156.13, 173.51, 183.99; *m*/*z* (FD) 437 ($M^+$).

General procedure 2: the reaction of Boc-Tyr(3,5-$Br_2$)-OH **3f** (100 mg, 0.22 mmol) and NBS (42.7 mg, 0.27 mmol) yielded **12f** (93.3 mg, 97%), mp 187–189 °C.

General procedure 3: the reaction of Boc-Tyr(3,5-$Br_2$)-OH **3f** (600 mg, 1.37 mmol) in 150 ml SE at a potential of $E^{ox}$ = 1300 mV yielded **12f** (323 mg, 54%) after 6 h.

### 3-Benzyloxycarbonylamino-7,9-dibromo-1-oxaspiro[4,5]deca-6,9-diene-2,8-dione 12g[7]

General procedure 2: the reaction of Z-Tyr-OH **3j** (5.0 g, 15.8 mmol) in buffer (2.0 l) and NBS (8.4 g, 47.2 mmol) in buffer (580 ml) yielded **12g** (7.44 g, 97%) as a white solid, mp 215 °C (Found: C, 43.41; H, 2.72; N, 2.63; Br, 33.98. $C_{17}H_{13}Br_2NO_5$ requires C, 43.34; H, 2.78; N, 2.97; Br, 33.92%); $R_f$ 0.64 ($CH_2Cl_2$–EtOAc, 10:3); $\delta_H$(250 MHz; $CD_3COCD_3$) 2.76 (1H, dd, $J \approx 11$, $J \approx 14$, CC$H_2$CH), 2.99 (1H, dd, $J \approx 11$, $J \approx 14$, CC$H_2$CH), 4.90 (1H, q, $J \approx 10.5$, $CH_2CH$NHR), 5.13 (2H, s, $C_6H_5CH_2O$), 7.33–7.37 (5H, m, $H_{arom}$-Z), 7.65 (1H, d, $J \approx 3$, $H_{quin}$), 7.95 (1H, d, $J \approx 3$, $H_{quin}$); $\delta_C$(62.9 MHz; $CD_3COCD_3$) 36.4, 50.5, 67.3, 79.5; 122.5, 123.3 (MNE); 128.6, 128.7, 129.2, 137.5; 147.9, 148.7 (MNE); 156.7, 171.9, 173.3; *m*/*z* (FD) 473 ($M^+ + 2H$).

General procedure 3: the reaction of Z-Tyr(3,5-$Br_2$)-OH **3g** (700 mg, 1.48 mmol) in 150 ml SE at a potential of $E^{ox}$ = 1400 mV gave **12g** (504 mg, 72%) after 6 h.

### 3-Benzyloxycarbonylamino-7,9-diiodo-1-oxaspiro[4,5]deca-6,9-diene-2,8-dione 12h

General procedure 2: Z-Tyr(3,5-$I_2$)-OH **3h** (1.0 g, 1.76 mmol) and NBS (1.0 g, 5.62 mmol) yielded white solid **12h** (975 mg, 98%), mp 155–157 °C; $\delta_H$(250 MHz; $CDCl_3$) 2.72 (1H, dd, $J \approx 10$, $J \approx 13.5$, CC$H_2$CH), 2.97 (1H, dd, $J \approx 10$, $J \approx 13.5$, CC$H_2$CH), 4.84–4.95 (1H, m, $CH_2CH$NHR), 5.13 (2H, s, $CH_2$-Z), 7.37 (5H, br s, $H_{arom}$-Z), 7.95 (1H, d, $J \approx 3$, $H_{quin}$), 8.24 (1H, d, $J \approx 3$, $H_{quin}$); *m*/*z* (FD) 565 ($M^+$).

General procedure 3: the reaction of Z-Tyr(3,5-$I_2$)-OH **3h** (300 mg, 0.53 mmol) in 175 ml SE at a potential of $E^{ox}$ = 1400 mV gave **12h** (197 mg, 66%) after 3 h. Compound **3h** was synthesized in 68% yield by mixing a solution of iodine and sodium iodide (in water) with a solution of Z-Tyr-OH in 20% aqueous ethylamine.[22]

### 3-*tert*-Butoxycarbonylamino-1-oxaspiro[4,5]deca-6,9-diene-2,8-dione 12i[4]

General procedure 1: the reaction of Boc-Tyr-OH **3i** (618 mg, 2.19 mmol) and PIFA (1.05 g, 2.45 mmol) yielded **12i** (140 mg, 23%) [76% as the crude product (by NMR spectroscopy)], mp 184–185 °C (Found: C, 59.89; H, 5.91; N, 5.14. $C_{14}H_{17}NO_5$ requires C, 60.20; H, 6.14; N, 5.02%); $R_f$ 0.51 (*n*-hexane–EtOAc, 1:1); $\delta_H$(250 MHz; $CDCl_3$) 1.39 (9H, s, Bu$^t$), 2.43 (1H, dd, $J \approx 12$, $J \approx 13$, CC$H_2$CH), 2.65 (1H, dd, $J \approx 9$, $J \approx 13$, CC$H_2$CH), 4.48–4.58 (1H, m, $CH_2CH$NHR), 5.36 (1H, d, $J \approx 7$, N*H*), 6.19–6.26 (2H, m, MNE, $H_{quin}$), 6.83 (2H, d, $J \approx 9$, $H_{quin}$); $\delta_C$(62.9 MHz; $CDCl_3$) 28.25, 38.68, 50.24, 76.08, 81.20; 129.12, 129.26 (MNE); 144.23, 146.18 (MNE); 155.98, 173.51, 183.99; *m*/*z* (FD) 280 ($M^+ + H$).

General procedure 3: the reaction of Boc-Tyr-OH **3i** (600 mg, 2.13 mmol) in 250 ml SE at a potential of $E^{ox}$ = 1400 mV yielded **12i** (107 mg, 18%) after 4 h.

### 3-Benzyloxycarbonylamino-1-oxaspiro[4,5]deca-6,9-diene-2,8-dione 12j[1,2,4]

General procedure 1: the reaction of Z-Tyr-OH **3j** (567 mg, 1.80 mmol) and PIFA (857 mg, 2.0 mmol) yielded white solid **12j** (216 mg, 38%) [85% as the crude product (by NMR spec-

troscopy)], mp 46 °C (Found: C, 65.28; H, 4.89; N, 4.33. $C_{17}H_{15}NO_5$ requires C, 65.17; H, 4.82; N, 4.47%); $R_f$ 0.45 (*n*-hexane–EtOAc, 1:1); $\delta_H$(250 MHz; $CDCl_3$) 2.38 (1H, t, $J \approx 12$, CC$H_2$CH), 2.56 (1H, dd, $J \approx 9$, $J \approx 13$, CC$H_2$CH), 4.51–4.61 (1H, m, $CH_2CH$NHR), 5.03 (2H, br s, $C_6H_5CH_2O$), 5.79 (1H, d, $J \approx 7$, N$H$), 6.14–6.18 (2H, m, $H_{quin}$, MNE), 6.69–6.78 (2H, m, $H_{quin}$, MNE), 7.25 (5H, s, $H_{arom}$); $\delta_C$(67.8 MHz; $CDCl_3$) 38.20, 50.50, 67.65, 76.16, 128.22, 128.50, 128.67, 129.13, 129.73, 135.69; 144.21, 146.11 (MNE); 155.99, 173.44, 184.06; *m*/*z* (FD) 313 ($M^+$).

General procedure 3: the reaction of Z-Tyr-OH **3j** (700 mg, 2.22 mmol) in 250 ml SE at a potential of $E^{ox}$ = 1400 mV gave **12j** (116 mg, 17%) after 3 h.

**4-(2-Amino-3-oxo-3-piperidinopropyl)-2,6-di-*tert*-butyl-4-hydroxycyclohexa-2,5-dienone 13**

The spirolactone **12c** (810 mg, 1.58 mmol) was dissolved in 30 ml of 15% piperidine–$CH_2Cl_2$. After 5 min the reaction was completed and the solvent removed. The crude product was chromatographed (silica gel, $CH_2Cl_2$–methanol, 20:1) to give **13** (487 mg, 1.29 mmol); $\nu_{max}/cm^{-1}$ 3500–3300, 3060, 2950, 1700, 1635, 1450, 1360; $\delta_H$(250 MHz; $CDCl_3$) 1.17 (9H, s, Bu$^t$), 1.20 (9H, s, Bu$^t$) MNE; 1.43–1.50 (8H, m, piperid.), 1.83–1.90 (1H, m, CC$H_2$CH), 2.10–2.17 (1H, m, CC$H_2$CH), 4.04 (1H, d, $J \approx 10$, $CH_2CH$N$H_2$), 5.27 (1H, s, O$H$), 6.44–6.51 (1H, d, $J \approx 3$, $H_{quin}$), 6.84–6.92 (1H, d, $J \approx 3$, $H_{quin}$); $\delta_C$(62.9 MHz; $CDCl_3$) 24.3, 26.1; 29.4, 29.5 (MNE); 34.5, 34.7 (MNE); 44.5, 45.9, 49.8, 69.6; 142.2, 144.1 (MNE); 145.5, 146.1 (MNE); 171.1, 186.7.

***N*-[2-Formylamino-3-(3,5-di-*tert*-butyl-1-hydroxy-4-oxocyclohexa-2,5-dien-1-yl)propionyl] alanine methyl ester 15a**

General procedure 4: **12d** (220 mg, 0.69 mmol) and H-Ala-OMe (120 mg, 1.16 mmol) in 30 ml of $CH_2Cl_2$ gave **15a** (176 mg, 60%) as a white solid at 20 °C after 96 h. No catalyst was used. Mp 145 °C (Found: C, 62.48; H, 8.29; N, 6.74. $C_{22}H_{34}N_2O_6$ requires C, 62.54; H, 8.11; N, 6.63%); $R_f$ 0.37 ($CH_2Cl_2$–MeOH, 15:1); $\delta_H$(250 MHz; $CD_3OD$) 1.21 (9H, s, Bu$^t$), 1.22 (9H, s, Bu$^t$) MNE; 1.30 (3H, d, $J \approx 7$, MeOCOCHC$H_3$), 1.90 (1H, dd, $J \approx 8$, $J \approx 14$, CC$H_2$CH), 2.23–2.37 (1H, m, CC$H_2$CH), 3.70 (3H, s, $CO_2CH_3$), 4.37 (1H, q, $J \approx 7$, $CH_2CH$NHR), 4.51–4.64 (1H, m, NHC$H$CO$_2$Me), 6.53–6.61 (2H, m, $H_{quin}$, MNE), 8.10 (1H, s, CHO); $\delta_C$(62.9 MHz; $CD_3OD$) 14.0, 27.5, 33.2, 39.8, 42.0, 48.2, 50.2, 66.7; 141.1, 141.7 (MNE); 144.5, 144.9 (MNE); 170.7, 172.0, 175.0, 185.2; *m*/*z* (FD) 422 ($M^+$).

***N*-[2-Formylamino-3-(3,5-di-*tert*-butyl-1-hydroxy-4-oxocyclohexa-2,5-dien-1-yl)propionyl] glycine ethyl ester 15b**

General procedure 4: **12d** (50 mg, 0.15 mmol) and H-Gly-OEt (20 mg, 0.19 mmol) in 30 ml of $CH_2Cl_2$ gave **15b** (40 mg, 63%) at 20 °C after 72 h as a colourless oil. No catalyst was used. (Found: C, 62.48; H, 8.29; N, 6.74. $C_{22}H_{34}N_2O_6$ requires C, 62.54; H, 8.11; N, 6.63%); $R_f$ 0.37 ($CH_2Cl_2$–MeOH, 15:1); $\delta_H$(250 MHz; $CDCl_3$) 0.93 (3H, t, $J \approx 4$, $CO_2CH_2CH_3$); 1.15 (9H, s, Bu$^t$), 1.16 (9H, s, Bu$^t$) MNE; 1.82–1.95 (1H, m, CC$H_2$CH), 2.31–2.41 (1H, m, CC$H_2$CH), 3.90 (2H, d, $J \approx 3$, NHC$H_2CO_2$), 3.99 (1H, s, O$H$), 4.11–4.28 [4H, m, N$H$(Tyr), N$H$(Gly), $CO_2CH_2CH_3$], 4.50–4.70 (1H, m, $CH_2CH$NHR), 6.56 (2H, s, $H_{quin}$), 8.03 (1H, s, CHO); $\delta_C$(62.9 MHz; $CD_3OD$) 14.4, 29.8; 34.6, 34.7 (MNE); 41.4, 44.6, 48.6, 61.4, 68.7; 143.6, 144.3 (MNE); 145.7, 145.8 (MNE); 161.5, 169.6, 170.2, 183.6; *m*/*z* (FD) 422 ($M^+$).

***N*-[2-Benzyloxycarbonylamino-3-(3,5-di-*tert*-butyl-1-hydroxy-4-oxocyclohexa-2,5-dien-1-yl)propionyl] leucine ethyl ester 15c**

General procedure 4: **12e** (300 mg, 0.704 mmol) and H-Leu-OEt (156 mg, 0.980 mmol) in 50 ml of $CHCl_3$ in the presence of 12 mg (0.704 mmol) *p*-toluenesulfonic acid as catalyst at 60 °C yielded **15c** (275 mg, 67%) after 80 h as a colourless oil; $R_f$ 0.31 ($CH_2Cl_2$–MeOH, 50:1); $\delta_H$(250 MHz; $CD_3OD$) 0.84 [6H, d, $J \approx 4$, $CH_2CH(CH_3)_2$]; 1.11 (9H, s, Bu$^t$), 1.12 (9H, s, Bu$^t$), (MNE); 1.15–1.21 (3H, m, $CO_2CH_2CH_3$), 1.45–1.62 [3H, m, $CHCH_2CH(CH_3)_2$, $CH_2CH(CH_3)_2$], 1.76 (1H, t, $J \approx 8$, $J \approx 14$, CC$H_2$CH), 2.25 (1H, ddd, $J \approx 3$, $J \approx 4$, $J \approx 14$, CC$H_2$CH), 3.52–3.69 (1H, m, NHC$H$CO$_2$Et), 4.06–4.12 (2H, m, $CO_2CH_2CH_3$), 4.41–4.50 (1H, m, $CH_2CH$NHR), 4.47 (1H, s, O$H$), 5.02–5.04 (2H, m, $C_6H_5CH_2O$), 5.61 [1H, d, $J \approx 8$, N$H$(Tyr)], 5.72–5.82 [1H, m, N$H$(Tyr)];§ 6.46 (1H, d, $J \approx 3$, $H_{quin}$), 6.58 (1H, d, $J \approx 3$, $H_{quin}$) MNE; 6.92 [1H, d, $J \approx 9$, N$H$(Leu)], 7.24–7.26 (5H, m, $H_{arom}$ Z); $\delta_C$(62.9 MHz; $CD_3OD$) 14.10; 21.91, 22.74 (MNE); 24.87; 29.32, 29.35 (MNE); 34.64, 34.71 (MNE); 41.23, 44.34, 51.07, 61.45, 67.37, 68.55, 72.28, 128.16, 128.29, 128.54; 135.87, 140.99 (MNE); 141.76; 146.13, 146.51 (MNE); 155.92, 171.70, 172.79, 186.14; *m*/*z* (FD) 584 ($M^+$).

***N*-[2-(Fluoren-9-ylmethoxycarbonylamino)-3-(3,5-di-*tert*-butyl-1-hydroxy-4-oxocyclohexa-2,5-dien-1-yl)propionyl] leucine ethyl ester 15d**

General procedure 4: **12c** (100.6 mg, 0.2 mmol) and H-Leu-OEt (50 mg, 0.31 mmol) in 20 ml of $CHCl_3$ in the presence of 3.5 mg (0.2 mmol) *p*-toluenesulfonic acid as catalyst at 60 °C yielded colourless oily **15d** (68 mg, 51%) after 80 h; $R_f$ 0.23 ($CH_2Cl_2$–MeOH, 50:1); $\delta_H$(250 MHz; $CD_3OD$) 0.91–0.95 [6H, m, $CH_2CH(CH_3)_2$]; 1.19 (9H, s, Bu$^t$), 1.20 (9H, s, Bu$^t$) (MNE); 1.24–1.32 (3H, m, $CO_2CH_2CH_3$); 1.55–1.79 [3H, m, $CHCH_2$-$CH(CH_3)_2$, $CH_2CH(CH_3)_2$], 1.73–1.90 (1H, m, CC$H_2$CH), 2.25–2.36 (1H, m, CC$H_2$CH); 3.54–3.55 (1H, m, NHC$H$-$CO_2$Et), 4.11–4.20 [3H, m, $CO_2CH_2CH_3$, $(C_6H_4)_2CHCH_2$], 4.36–4.45 (2H, m, $C_6H_5CH_2O$), 4.46–4.59 (1H, m, $CH_2CH$-NHR), 5.67 [1H, d, $J \approx 9$, N$H$(Tyr)]; 6.53 (1H, d, $J \approx 3$, $H_{quin}$), 6.63–6.74 (1H, m, $H_{quin}$) MNE, 6.78–6.87 [1H, m, N$H$(Leu)], 7.25–7.41 (4H, m, $H_{arom}$-Fmoc), 7.57 (2H, d, $J \approx 5$, $H_{arom}$-Fmoc), 7.75 (2H, d, $J \approx 7$, $H_{arom}$-Fmoc); *m*/*z* (FD) 672 ($M^+$).

***N*-[2-Benzyloxycarbonylamino-3-(3,5-dibromo-1-hydroxy-4-oxocyclohexa-2,5-dien-1-yl)propionyl] leucine ethyl ester 15e**

General procedure 4: **12g** (100 mg, 0.212 mmol) and H-Leu-OEt (40.5 mg, 0.255 mmol) in 8 ml of $CHCl_3$ in the presence of 3.65 mg (0.212 mmol) *p*-toluenesulfonic acid as catalyst at 60 °C yielded **15e** (19 mg, 14%) after 68 h; $R_f$ 0.49 ($CH_2Cl_2$–MeOH, 20:1); $\delta_H$(250 MHz; $CD_3OD$) 0.78–1.00 [6H, m, $CH_2CH(CH_3)_2$], 1.21–1.28 (3H, m, $CO_2CH_2CH_3$), 1.47–1.57 [2H, m, $CHCH_2CH(CH_3)_2$], 1.58–1.70 [1H, m, $CH_2CH(CH_3)_2$], 1.72–2.26 (2H, m, CC$H_2$CH), 4.09–4.18 (2H, m, $CO_2CH_2CH_3$), 4.39–4.51 (1H, m, NHC$H$CO$_2$Et), 4.64–4.74 (1H, m, $CH_2CH$-NHR), 5.11–5.15 (2H, m, $C_6H_5CH_2O$), 7.32–7.34 (5H, m, $H_{arom}$-Z), 7.36–7.39 (2H, m, $H_{quin}$); *m*/*z* (FD) 630 ($M^+$).

***N*-[2-Benzyloxycarbonyl-3-(1-hydroxy-4-oxocyclohexa-2,5-dien-1-yl)propionyl] leucine ethyl ester 15f**

General procedure 4: **12j** (100 mg, 0.319 mmol) and L-Leu-OEt (66 mg, 0.415 mmol) in 50 ml of $CHCl_3$ in the presence of 5.5 mg (0.319 mmol) *p*-toluenesulfonic acid as catalyst at 60 °C gave **15f** (11 mg, 7%) after 80 h; $R_f$ 0.39 ($CH_2Cl_2$–MeOH, 30:1); $\delta_H$(250 MHz; $CDCl_3$) 0.87–0.97 [6H, m, $CH_2CH(CH_3)_2$], 1.20–1.31 (3H, m, $CO_2CH_2CH_3$), 1.45–1.72 [3H, m, $CHCH_2$-$CH(CH_3)_2$, $CH_2CH(CH_3)_2$], 1.90–2.08 (1H, m, CC$H_2$CH), 2.37–2.43 (1H, m, CC$H_2$CH), 3.85 (1H, s, O$H$), 4.09–4.22 (2H, m, $CO_2CH_2CH_3$), 4.48–4.52 (1H, m, NHC$H$CO$_2$Et), 4.63–4.74 (1H, m, $CH_2CH$NHR), 5.10 (2H, s, $C_6H_5CH_2O$), 6.61–6.62 (2H, m, $H_{quin}$), 7.04–7.05 (2H, m, $H_{quin}$), 7.35 (5H, s, $H_{arom}$-Z); *m*/*z* (FD) 454 ($M^+ - 2 - OH$).

**Methyl 2-[3-(3,5-di-*tert*-butyl-4-hydroxyphenyl)-2-(formylamino)propionylamino]propanoate 16a by reduction of 15a**

Dipeptide **15a** (20.0 mg, 0.047 mmol), (+)-5,6-*O*-isopropyl-

§ Doubling of the NMR signals due to the presence of two diastereomers.

idene-L-ascorbic acid (21.0 mg, 0.095 mmol), $CHCl_3$ (15 ml) and anhydrous $ZnCl_2$ (*ca*. 5 mg) were refluxed for *ca*. 30 h. Separation by TLC (silica gel, $CH_2Cl_2$–MeOH, 20:1) yielded **16a** (14 mg, 75%), identical to an authentic sample prepared by classical coupling of **3d** and Ala-OMe·HCl with dicyclohexylcarbodiimide in the presence of *N*-methylmorpholine and hydroxybenzotriazole, mp 93–95 °C; $R_f$ 0.52; $\nu_{max}/cm^{-1}$ 3630, 3300, 3260, 3060, 2960, 2910, 2870, 1740, 1690, 1665, 1540, 1500, 1455, 1380, 1365; *m*/*z* (FD) 406 ($M^+$). The by-product was the methyl ether of **15a** {*N*-[2-formylamino-3-(3,5-di-*tert*-butyl-1-methoxy-4-oxocyclohexa-2,5-dien-1-yl)propionyl] alanine methyl ester} (3 mg, 16%); $R_f$ 0.46; *m*/*z* (FD) 435 ($M^+ - 1$).

**Ethyl 2-[2-benzyloxycarbonylamino-3-(3,5-di-*tert*-butyl-4-hydroxyphenyl)propionylamino]-4-methylpentanoate 16c by reduction of 15c**

A solution of **15c** (60.0 mg, 0.102 mmol) in 50 ml of $CHCl_3$ was treated with (+)-5,6-*O*-isopropylidene-L-ascorbic acid (44.5 mg, 0.205 mmol) and anhydrous $ZnCl_2$ (10 mg). This mixture was heated to 60 °C for 50 h, filtered and the solvent was removed under reduced pressure. The crude product was chromatographed on silica gel with *n*-hexane–EtOAc to give **16c** (30 mg, 52%); $R_f$ 0.36 (*n*-hexane–EtOAc, 8:2) 0.36; $\delta_H$(250 MHz; $CDCl_3$) 0.77–0.79 [6H, m, $CH_2CH(CH_3)_2$], 1.14–1.22 (3H, m, $CO_2CH_2CH_3$), 1.33 (18H, s, Bu$^t$), 1.32–1.53 [3H, m, $CHCH_2$-$CH(CH_3)_2$, $CH_2CH(CH_3)_2$], 2.87–2.95 (2H, m, $CCH_2CH$), 4.00–4.12 (2H, m, $CO_2CH_2CH_3$), 4.32–4.46 (2H, m, $NHCH$-$CO_2Et$, $CH_2CHNHR$); 5.01 (1H, s, $C_6H_5CH_2O$), 5.02 (1H, s, $C_6H_5CH_2O$);¶ 5.06 (1H, s, O*H*), 5.21–5.31 [1H, m, N*H*(Leu)]; 6.12 [1H, d, $J \approx 8$, N*H*(Tyr)], 6.22 [1H, d, $J \approx 8$, N*H*(Tyr)];¶ 6.89 (1H, s, $H_{arom}$), 6.91 (1H, s, $H_{arom}$);¶ 7.21–7.29 (5H, m, $H_{arom}$-Z); $\delta_C$(62.9 MHz; $CD_3OD$) 14.11, 22.10, 22.59, 24.82, 30.29, 34.28, 38.14, 41.61, 51.00, 56.16, 61.28, 67.02; 125.66, 125.86 (MNE); 126.86, 128.14; 127.94, 128.53 (MNE); 136.23, 152.82, 155.94, 170.81, 172.44; *m*/*z* (FD) 568 ($M^+$).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¶ Doubling of the NMR signals due to the presence of 2 diastereomers.

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