Amino Acids (2002) 22: 71–93

Amino
Acids

Synthesis, electrochemical and spectral properties of some ω-N-quinonyl amino acids

S. Bittner, S. Gorohovsky, O. Paz-Tal (Levi), and **J. Y. Becker**

Department of Chemistry, Ben-Gurion University of the Negev, Beer-Sheva, Israel

Accepted March 28, 2001

Summary. Four series of ω-N-quinonyl amino acids were synthesized by Michael-like additions. The quinones include 2-phenylthio-1,4-benzoquinone, 1,4-naphthoquinone, 2-methyl-1,4-naphthoquinone and 2,3-dichloro-1,4-naphthoquinone. These modified amino acids can be used for post chain assembly modifications of biologically active peptides, which target the quinonic drug to a cancer damaged area. The electron-transfer capabilities of the modified amino acids were probed by cyclic voltammetry measurements. The results described in this paper show that the novel N-quinonyl amino acids are effective in producing semiquinone radicals similarly to the unconjugated quinones themselves. A direct relation was found between the first reduction potentials of the quinones and their reactivity towards the ω-amino acids. The successful generation of stable semiquinone radicals by the novel quinone derivatives is a prerequisite for the manifestation of site-directed antitumor activity of corresponding quinone-peptide conjugates.

Keywords: Amino acids – Quinones – Cyclic voltammetry – Redox potentials

Introduction

Quinonic compounds are ubiquitous in nature (Thompson, 1997). They are implicated in numerous cellular functions and are involved in mechanisms of electron and hydrogen transfers (Powis, 1989). Some quinonic compounds are used as antibacterial drugs (Theriault et al., 1986; Brewer et al., 1984), while other quinonic compounds are used in fungal diseases (Grosvenor and Gray, 1998; Hirosawa et al., 1997). Several quinones have long been reported to exhibit antimalarial capacity (Lopez-Shirley et al., 1994; Martin et al., 1973; Prescott, 1969), and a whole group of quinones are used in cancer chemotherapy (Gutierrez, 1989). To date, quinones form the second large class of antitumor agents approved for clinical use and many other antitumor quinones are in different stages of clinical and preclinical development. From the more efficient agents we will mention the simple menadione (2-methyl-

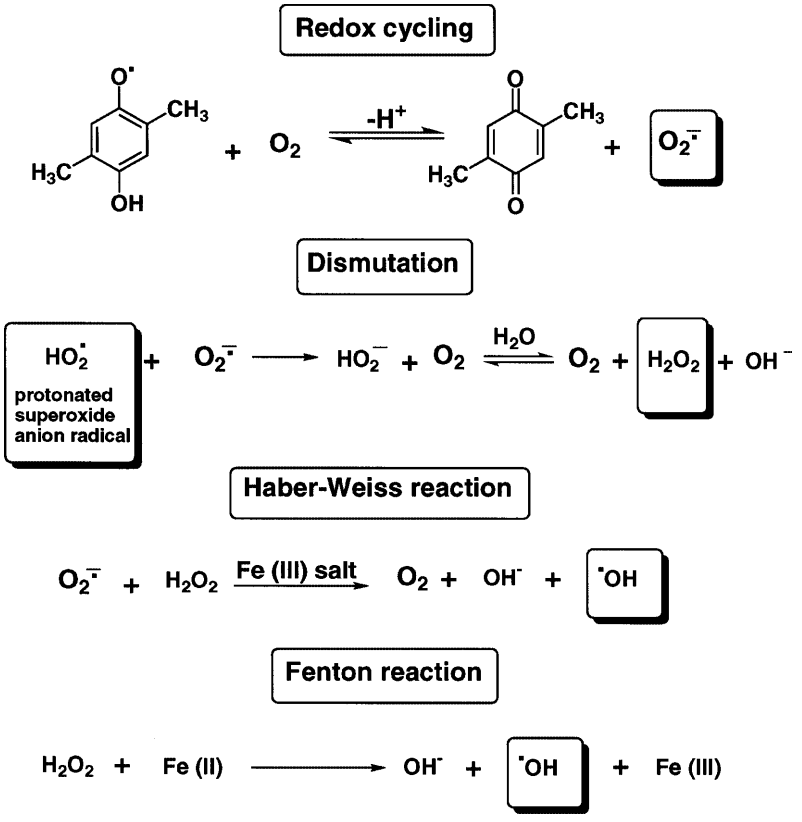
1,4-naphthoquinone), the antracycline-glycosides, *e.g.* daunorubicin, doxorubicin, aclacinomycin A, the benzoquinone derivatives, *e.g.* mitomycin C, carbazilquinone and diaziquone and the more complex quinones, *e.g.* mitoxantrone, streptonigrin and actinomycin D.

The efficiency of the quinonic compounds in inhibiting cancer cells growth is believed to stem from (i) their ability to associate and intercalate with DNA duplexes, thus impairing appropriate template function and nucleic acid synthesis (Crooke et al., 1981), (ii) their participation in key cellular redox mechanisms with consequent generation of highly reactive oxygen species (ROS), which in turn modify and degrade nucleic acids and proteins within the cancer cells (Pacifici and Davies, 1990; Burkit et al., 1989; Stadtman, 1993).

Under physiological conditions quinones can undergo non-enzymatic one-electron reduction to give the moderately toxic species semiquinone anion radical. This can occur either by reacting with reduced pyridine nucleotide or with glutathione or *via* electron transfer from an appropriate radical. Quinones can also undergo enzymatic one-electron reduction, usually by reaction with flavoenzymes, *e.g.* NADPH-cytochrome P450 reductase. This enzymatic reduction results in the formation of the toxic semiquinone radicals as well.

Under aerobic conditions the semiquinone radicals auto-oxidize, *via* the redox cycling process, forming superoxide anion radicals. Protonated superoxide anion radicals dismutate and form hydrogen peroxide. Neither superoxide anion radical, nor hydrogen peroxide are particularly toxic to cells. It is the product of their reaction, the hydroxyl radical (·OH), which is thought to be responsible for most oxygen radical cytotoxicity. This last reaction, which is catalyzed by trace amounts of a transition metal ion (usually iron salt) is referred to as the "iron catalyzed Haber-Weiss reaction" (Scheme 1). The hydroxyl radical can also be obtained from the reaction of Fe(II) with hydrogen peroxide (Fenton reaction).

One of the major problems in this field is how to target the active species (*e.g.* quinones) and their cytotoxicity in such a way that they will differentiate between normal healthy cells and afflicted cells. Indeed, target chemotherapy has gained considerable attention in the last few years (Morier-Teissier et al., 1990; 1993) and one of the ways by which it might be achieved is through conjugation of the active component to a vectors having unique affinities to specific binding sites. It is already well established that human tumors are hormone dependent and contain the corresponding hormone receptors. Receptors for peptide hormones, such as the neurodecapeptide luteinizing hormone-releasing hormone (LH-RH) (Bajusz et al., 1989), somatostatic hormones (Koppan et al., 1998), growth hormone-releasing hormone (GH-RH) (Schally, 1997) and bombesine/gastrine releasing peptide (Kiaris et al., 1999) have been detected in different kinds of human cancers. Different cytotoxic compounds were conjugated to LH-RH, its agonists and antagonists and their specific binding affinities to LH-RH receptors studied. Among others, two quinonic compounds, *e.g.* doxorubicin and 2-hydroxymethyl-9,10-anthraquinone were chemically attached to the neurodecapeptide *via* the ε-amino side chain of D-lysine residue (Janaky et al., 1992). It was found that



Scheme 1

the cytotoxicity of those peptide-drug hybrids was markedly augmented *in vitro*, far beyond that of the drug components itself. This was the first time that quinonyl amino acids incorporated into biological active peptides proved to have cytotoxic anticancer activity.

In view of the potential clinical significance of cytotoxic quinone-bearing peptides, it became very important to increase the arsenal of related natural and unnatural quinonyl amino acids and study their chemical, spectral and electrochemical properties (Gorohovsky and Bittner, 2001). In this paper we describe the preparation of a large variety of quinonyl amino acids via the 1,4-Michael-like addition and present some of their spectroscopic and electrochemical properties.

Materials and methods

IR spectra were recorded on a Nicolet 5ZDX FT-IR spectrometer. ^{1}H-NMR were run on a Bruker WP 200 or 500 SY spectrometer and mass spectra (CI in methane) were obtained on a Finnigan 4020 quadrupole spectrometer. The correct elemental composition was proved by high resolution mass spectrometry, which was performed on High Resolution Magnetic Sector Mass Spectrometer VG Fison's AutoSpec. Melting points were determined using a Thomas-Hoover capillary apparatus and are uncorrected. All starting materials, reagents and solvents were of commercial quality. 2-phenylthio-

1,4-benzoquinone was prepared according to procedure of Dimroth (Dimroth et al., 1940). Cyclic voltammetry measurements were done on a Princeton Applied Research Model 173 potentiostat/galvanostat combined with model 175 Universal Programmer and Yokohawa X-Y recorder type 3086. Solutions (10 ml) containing 0.1 M tetrabutylammonium perchlorate (TBAP) in DMF and 1–2 mM of the samples were used in a one compartment cell. Potentials were recorded under argon atmosphere on glassy carbon working electrode *vs.* Ag/AgCl reference electrode.

Synthesis

N-(1,4-naphthoquinon-2-yl)-glycine (**3a**)

A solution of glycine (10 mmol) in water (20 ml) was added to a hot solution of 1,4-naphthoquinone (20 mmol) in EtOH (150 ml) and the mixture was stirred at rt for 24 h. Precipitated black impurities were removed by filtration and the filtrate was evaporated under reduced pressure. The crude deep brown product was purified twice on a silica gel column (70–200 mesh) eluting with a mixture of CH_2Cl_2/MeOH (9:1) to afford a brown solid decomposing at 197–199°C; yield 0.92 g (40%).
^{1}H-NMR (d_6-DMSO) δ (ppm) (J (Hz)): 7.95 (d, 1H, 7.6), 7.91 (d, 1H, 7.5), 7.80 (t, 1H, 7.1), 7.70 (t, 1H, 7.1), 7.17 (br s, 1H), 5.50 (s, 1H), 3.50 (d, 2H).
^{13}C-NMR (d_6-DMSO) δ (ppm): 181.4 (C = O), 181.2 (C = O), 171.2 (CO_2H), 147.3, 134.9, 133.3, 132.1, 130.2, 125.9, 125.4, 99.8, 45.8.
IR (KBr) ν (cm^{-1}): 3455, 3382, 1725, 1679, 1611.
UV (MeOH) λ_{max} (nm) (log (ε)): 222 (4.00), 270 (4.13), 328 (3.23), 446 (3.33).
MS (m/z): 188 $[MH—CO_2]^+$

N-(1,4-naphthoquinon-2-yl)-β-alanine (**3b**)

A solution of β-alanine (10 mmol) in water (20 ml) was added to a hot solution of 1,4-naphthoquinone (20 mmol) in EtOH (150 ml) and the mixture was stirred at rt for 48 h. The precipitated product was filtered. Recrystallization from a mixture of EtOH/H_2O (1:1) afforded orange fine needles melting at 206–207°C; yield 1.93 g (79%).
^{1}H-NMR (d_6-DMSO) δ (ppm) (J (Hz)): 12.03 (br s, 1H), 7.97 (dd, 1H, 7.6, 1.3), 7.93 (dd, 1H, 7.6, 1.3), 7.82 (td, 1H, 7.6, 1.3), 7.71 (td, 1H, 7.6, 1.3), 7.47 (t, 1H, 5.9), 5.70 (s, 1H), 3.37 (m, 2H), 2.58 (t, 2H, 6.9).
IR (KBr) ν (cm^{-1}): 3455, 3341, 1723, 1669, 1615.
UV (MeOH) λ_{max} (nm) (log (ε)): 242 (4.05), 270 (4.41), 322 (3.43), 450 (3.30).
MS (m/z): 246 $[MH]^+$.

N-(1,4-naphthoquinon-2-yl)-ω-amino acids (**3c–e**)

A solution of the appropriate ω-amino acid (10 mmol) in water (20 ml) was added to a hot solution of 1,4-naphthoquinone (3.16 g, 20 mmol) in EtOH (150 ml) and the mixture was stirred at rt for 24 h. The precipitated product was filtered and recrystallized from EtOH to afford fine orange needles.

N-(1,4-naphthoquinon-2-yl)-4-aminobutanoic acid (**3c**)

Yield 2.07 g (80%); m.p. = 210–211°C.

^{1}H-NMR (d_6-DMSO) δ (ppm) (J (Hz): 12.12 (br s, 1H), 7.96 (dd, 1H, 7.6, 1.3), 7.92 (dd, 1H, 7.6, 1.3), 7.81 (td, 1H, 7.6, 1.3), 7.71 (td, 1H, 7.5, 1.3), 7.63 (t, 1H, 6.1), 5.70 (s, 1H), 3.18 (m, 2H), 2.58 (t, 2H, 7.2), 1.74–1.80 (m, 2H).
IR (KBr) ν (cm^{-1}): 3516, 3341, 1702, 1669, 1621, 1608.
UV (MeOH) λ_{max} (nm) (log (ε)): 234 (4.26), 270 (4.43), 328 (3.43), 450 (3.63).
MS (m/z): 260 $[MH]^+$.

N-(1,4-naphthoquinon-2-yl)-5-aminopentanoic acid (**3d**)

Yield 2.34 g (86%); m.p. = 191–192°C, lit: 175–178°C (Fokin and Dezina, 1969).
^{1}H-NMR (d_6-DMSO) δ (ppm) (J (Hz)): 12.02 (br s, 1H), 7.96 (dd, 1H, 7.7, 1.2), 7.92 (dd, 1H, 7.7, 1.2), 7.81 (td, 1H, 7.5, 1.2), 7.70 (td, 1H, 7.5, 1.2), 7.58 (t, 1H, 6.1), 5.66 (s, 1H), 3.18 (m, 2H), 2.24 (t, 2H, 7.0), 1.53–1.57 (m, 4H).
IR (KBr) ν (cm^{-1}): 3454, 3337, 1702, 1674, 1624, 1602.
UV (MeOH) λ_{max} (nm) (log (ε)): 234 (4.36), 270 (4.52), 328 (3.50), 452 (3.73).
MS (m/z): 274 $[MH]^+$.

N-(1,4-naphthoquinon-2-yl)-6-aminohexanoic acid (**3e**)

Yield 2.67 g (93%); m.p. = 150–151°C.
^{1}H-NMR (d_6-DMSO) δ (ppm) (J (Hz)): 11.95 (br s, 1H), 7.96 (dd, 1H, 7.7, 1.4), 7.92 (dd, 1H, 7.7, 1.4), 7.81 (td, 1H, 7.5, 1.4), 7.70 (td, 1H, 7.5, 1.4), 7.56 (t, 1H, 5.7), 5.65 (s, 1H), 3.16 (m, 2H), 3.19 (t, 2H, 7.3), 1.48–1.59 (m, 4H), 1.27–1.33 (m, 2H).
IR (KBr) ν (cm^{-1}): 3479, 3318, 1721, 1686, 1630, 1611.
UV (MeOH) λ_{max} (nm) (log (ε)): 234 (4.17), 272 (4.33), 328 (3.35), 450 (3.56).
MS (m/z): 288 $[MH]^+$.

N-(3-chloro-1,4-naphthoquinon-2-yl)-ω-amino acids (**4a–e**)

A solution of the appropriate ω-amino acid (20 mmol) in water (20 ml) was added to a suspension of 2,3-dichloro-1,4-naphthoquinone (10 mmol) in EtOH (150 ml) and the mixture was refluxed for 24 h. The solvents were evaporated under reduced pressure and the residue purified using a silica gel column (70–230 mesh) and eluting with a mixture of CH_2Cl_2/MeOH (9:1). The products were recrystallized from EtOH/H_2O (1:1) to afford red needles.

N-(3-chloro-1,4-naphthoquinon-2-yl)-glycine (**4a**)

Yield 2.25 g (85%); m.p. = 170–172°C, lit: 170–171°C (Okamoto and Ohta, 1980).
^{1}H-NMR (d_6-DMSO) δ (ppm) (J (Hz)): 12.80 (br s, 1H), 7.98 (dd, 2H, 7.4, 1.5), 7.80 (td, 2H, 7.6, 1.7), 7.52 (br s, 1H), 4.39 (d, 2H, 6.6).
IR (KBr) ν (cm^{-1}): 3489, 3353, 1739, 1698, 1610.
UV (MeOH) λ_{max} (nm) (log (ε)): 236 (4.29), 274 (4.65), 332 (3.59), 462 (3.80).
MS (m/z): 266 $[MH]^+$, 232, 208.

N-(3-chloro-1,4-naphthoquinon-2-yl)-β-alanine (**4b**)

Yield 2.40 g (86%); m.p. = 158–160°C, lit: 160–161°C (Okamoto and Ohta, 1980).
^{1}H-NMR (d_6-DMSO) δ (ppm) (J (Hz)): 12.30 (br s, 1H), 7.90 (d, 2H, 7.4), 7.63–7.79 (m,

2H), 7.28 (br s, 1H), 3.90 (m, 2H), 2.55 (t, 2H, 7.0).
IR (KBr) ν (cm^{-1}): 3462, 3288, 1743, 1696, 1642, 1611.
UV (MeOH) λ_{max} (nm) (log (ε)): 236 (4.23), 274 (4.31), 330 (3.30), 464 (3.45).
MS (m/z): 282 $[M + 3H]^+$, 280 $[MH]^+$, 246, 202, 174.

N-(3-chloro-1,4-naphthoquinon-2-yl)-4-aminobutanoic acid (**4c**)

Yield 2.52 g (86%); m.p. = 174–176°C, lit: 175°C (Okamoto and Ohta, 1980).
^{1}H-NMR (d_6-DMSO) δ (ppm) (J (Hz)): 7.95 (d, 2H, 7.4), 7.80 (t, 1H, 7.3), 7.72 (t, 1H, 7.4), 7.54 (br s, 1H), 3.72 (m, 2H), 2.36 (t, 2H, 7.5), 1.85 (m, 2H).
IR (KBr) ν (cm^{-1}): 3490, 3290, 1741, 1690, 1640, 1611.
UV (MeOH) λ_{max} (nm) (log (ε)): 236 (4.40), 276 (4.48), 334 (3.44), 468 (3.60).
MS (m/z): 296 $[M + 3H]^+$, 294 $[MH]^+$, 260, 244, 174.

N-(3-chloro-1,4-naphthoquinon-2-yl)-5-aminopentanoic acid (**4d**)

Yield 2.58 g (84%); m.p. = 124–126°C, lit: 133°C (Ikeda and Zasshi, 1955).
^{1}H-NMR ($CDCl_3$) δ (ppm) (J (Hz)): 8.15 (dd, 1H, 7.5, 1.0), 8.03 (dd, 1H, 7.4, 1.0), 7.74 (td, 1H, 7.5, 1.5), 7.62 (td, 1H, 7.5, 1.5), 6.13 (br s, 1H), 3.86 (m, 2H), 2.45 (t, 2H, 6.5), 1.74–1.81 (m, 4H).
IR (KBr) ν (cm^{-1}): 3525, 3325, 1714, 1672, 1644, 1616.
UV (MeOH) λ_{max} (nm) (log (ε)): 238 (4.23), 276 (4.49), 330 (3.35), 468 (3.65).
MS (m/z): 310 $[M + 3H]^+$, 308 $[MH]^+$, 274, 175.

N-(3-chloro-1,4-naphthoquinon-2-yl)-6-aminohexanoic acid (**4e**)

Yield 2.76 g (86%); m.p. = 130–132°C, lit: 130–132°C (Okamoto and Ohta, 1980).
^{1}H-NMR ($CDCl_3$) δ (ppm) (J (Hz)): 8.15 (dd, 1H, 7.2, 1.1), 8.03 (dd, 1H, 7.2, 1.0), 7.74 (td, 1H, 7.5, 1.5), 7.62 (td, 1H, 7.5, 1.5), 6.08 (br s, 1H), 3.86 (m, 2H), 2.40 (t, 2H, 7.2), 1.64–1.81 (m, 4H), 1.43–1.54 (m, 2H).
IR (KBr) ν (cm^{-1}): 3521, 3261, 1714, 1686, 1644, 1616.
UV (MeOH) λ_{max} (nm) (log (ε)): 242 (4.20), 276 (4.39), 330 (3.41), 472 (3.55).
MS (m/z): 332 $[MH]^+$, 288, 243, 174.

N-(3-methyl-1,4-naphthoquinon-2-yl)-ω-amino acids (**3f–j**)

A solution of the appropriate ω-amino acid (10 mmol) in water (20 ml) was added to a hot solution of 2-methyl-1,4-naphthoquinone (20 mmol) and the mixture was stirred at rt for 24 h. The solvents were evaporated under reduced pressure to give the crude deep red product. It was purified on a silica gel column (70–230 mesh) eluting with a mixture of CH_2Cl_2/MeOH (4:1) to afford a deep red solid.

N-(3-methyl-1,4-naphthoquinon-2-yl)-glycine (**3f**)

Yield 0.25 g (10%), m.p. = 119–122°C.
^{1}H-NMR (d_6-DMSO) δ (ppm) (J (Hz)): 7.90 (d, 2H, 7.4), 7.76 (td, 1H, 7.3, 1.4), 7.69 (td, 1H, 7.4, 1.6), 7.04 (br s, 1H), 3.94 (d, 2H, 3.7), 2.12 (s, 3H).
IR (KBr) ν (cm^{-1}): 3462, 3341, 1723, 1669, 1635, 1608.
UV (MeOH) λ_{max} (nm) (log (ε)): 234 (3.95), 276 (4.03), 470 (3.18).
MS (m/z): 200 $[M—CO_2H]^+$, 175.

N-(3-methyl-1,4-naphthoquinon-2-yl)-β-alanine (**3g**)

Yield 0.33 g (13%), m.p. = 140–142°C.
^{1}H-NMR (d_6-DMSO) δ (ppm) (J (Hz)): 7.90 (dd, 2H, 7.3, 1.3), 7.78 (td, 1H, 7.3, 1.4), 7.65 (td, 1H, 7.5, 1.6), 6.79 (br s, 1H), 3.70–3.73 (m, 2H), 2.47 (t, 2H, 6.6), 2.07 (s, 3H).
IR (KBr) ν (cm^{-1}): 3469, 3354, 1748, 1683, 1619.
UV (MeOH) λ_{max} (nm) (log (ε)): 236 (4.16), 276 (4.27), 476 (3.40).
MS (m/z): 260 $[MH]^+$, 258 $[M—H]^+$, 181, 175.

N-(3-methyl-1,4-naphthoquinon-2-yl)-4-aminobutanoic acid (**3h**)

Yield 0.19 g (7%), m.p. = 124–128°C.
^{1}H-NMR ($CDCl_3$) δ (ppm) (J (Hz)): 8.10 (d, 1H, 7.6), 8.00 (d, 1H, 7.6), 7.68 (t, 1H, 7.5), 7.50 (t, 1H, 7.5), 5.70 (br s, 1H), 3.60 (m, 2H), 2.50 (t, 2H, 7.2), 2.20 (s, 3H), 2.00 (m, 2H).
IR (KBr) ν (cm^{-1}): 3423, 3289, 1714, 1665, 1616.
UV (MeOH) λ_{max} (nm) (log (ε)): 236 (4.10), 276 (4.22), 476 (3.36).
MS (m/z): 274 $[MH]^+$.

N-(3-methyl-1,4-naphthoquinon-2-yl)-5-aminopentanoic acid (**3i**)

Yield 0.29 g (10%), m.p. = 65–68°C.
^{1}H-NMR ($CDCl_3$) δ (ppm) (J (Hz)): 8.05 (d, 1H, 7.6), 7.96 (d, 1H, 7.6), 7.65 (t, 1H, 7.3), 7.55 (t, 1H, 7.4), 5.76 (br s, 1H), 3.55 (m, 2H), 2.38 (m, 2H), 2.20 (s, 3H), 1.75–1.82 (m, 4H).
IR (KBr) ν (cm^{-1}): 3483, 3290, 1733, 1683, 1611.
UV (MeOH) λ_{max} (nm) (log (ε)): 236 (4.04), 276 (4.14), 476 (3.28).
MS (m/z): 288 $[MH]^+$, 230, 186.

N-(3-methyl-1,4-naphthoquinon-2-yl)-6-aminohexanoic acid (**3j**)

Yield 0.30 g (10%), m.p. = 107–109°C.
^{1}H-NMR (d_6-DMSO) δ (ppm) (J (Hz)): 12.00 (br, s, 1H), 7.87 (d, 1H, 7.7), 7.86 (d, 1H, 7.7), 7.74 (td, 1H, 7.5, 1.1), 7.63 (td, 1H, 7.5, 1.1), 6.55 (t, 1H, 6.4), 3.49 (m, 2H), 2.17 (t, 2H, 7.3), 2.04 (s, 3H), 1.45–1.55 (m, 4H), 1.25–1.31 (m, 2H).
IR (KBr) ν (cm^{-1}): 3476, 3336, 1710, 1655, 1632, 1607.
UV (MeOH) λ_{max} (nm) (log (ε)): 236 (4.25), 276 (4.55), 476 (3.49).
MS (m/z): 304 $[M + 3H]^+$, 302 $[MH]^+$, 132.

N-(5-phenylthio-1,4-benzoquinon-2-yl)-ω-amino acids (**3k–o**)

A solution of the appropriate ω-amino acid (5 mmol) in water (10 ml) was added dropwise to a hot solution of 2-phenylthio-1,4-benzoquinone (2.5 mmol) in EtOH (50 ml) and the mixture was stirred for 24 h. The precipitated crude product was filtered and recrystallized from EtOH to afford red micro-crystals.

N-(5-phenylthio-1,4-benzoquinon-2-yl)-glycine (**3k**)

Yield 0.25 g (35%), m.p. = 191–192°C.
^{1}H-NMR (d_6-DMSO) δ (ppm) (J (Hz)): 7.57 (m, 5H), 5.53 (s, 1H), 5.48 (s, 1H), 3.26 (m, 2H).
IR (KBr) ν (cm^{-1}): 3348, 1724, 1642, 1608.

UV (MeOH) λ_{max} (nm) (log (ε)): 226 (3.87), 354 (3.78), 520 (2.36).
MS (m/z): 290 $[MH]^+$.

N-(5-phenylthio-1,4-benzoquinon-2-yl)-β-alanine (**3l**)

Yield 0.33 g (44%), m.p. = 194–195°C.
^{1}H-NMR (d_6-DMSO) δ (ppm) (J (Hz)): 7.52 (m, 5H), 5.46 (s, 1H), 5.39 (s, 1H), 3.25 (m, 2H), 2.34 (t, 2H, 6.9).
IR (KBr) ν (cm^{-1}): 3344, 1717, 1646, 1605.
UV (MeOH) λ_{max} (nm) (log (ε)): 226 (3.98), 354 (3.95), 514 (2.58).
MS (m/z): 304 $[MH]^+$.

N-(5-phenylthio-1,4-benzoquinon-2-yl)-4-aminobutanoic acid (**3m**)

Yield 0.28 g (35%), m.p. = 143–145°C.
^{1}H-NMR (d_6-DMSO) δ (ppm) (J (Hz)): 7.56 (m, 5H), 5.52 (s, 1H), 5.51 (s, 1H), 3.27 (m, 2H), 2.80 (m, 2H), 2.37 (t, 2H, 6.9).
IR (KBr) ν (cm^{-1}): 3308, 1726, 1645, 1608.
UV (MeOH) λ_{max} (nm) (log (ε)): 226 (3.95), 354 (3.87), 514 (2.53).
MS (m/z): 318 $[MH]^+$.

N-(5-phenylthio-1,4-benzoquinon-2-yl)-5-aminopentanoic acid (**3n**)

Yield 0.45 g (54%), m.p. = 184–185°C.
^{1}H-NMR (d_6-DMSO) δ (ppm) (J (Hz)): 7.54 (m, 5H), 5.49 (s, 1H), 5.49 (s, 1H), 3.22 (m, 2H), 2.30 (m, 2H), 1.65 (m, 4H).
IR (KBr) ν (cm^{-1}): 3345, 1722, 1646, 1611.
UV (MeOH) λ_{max} (nm) (log (ε)): 224 (3.95), 352 (3.87), 508 (2.82).
MS (m/z): 332 $[MH]^+$.

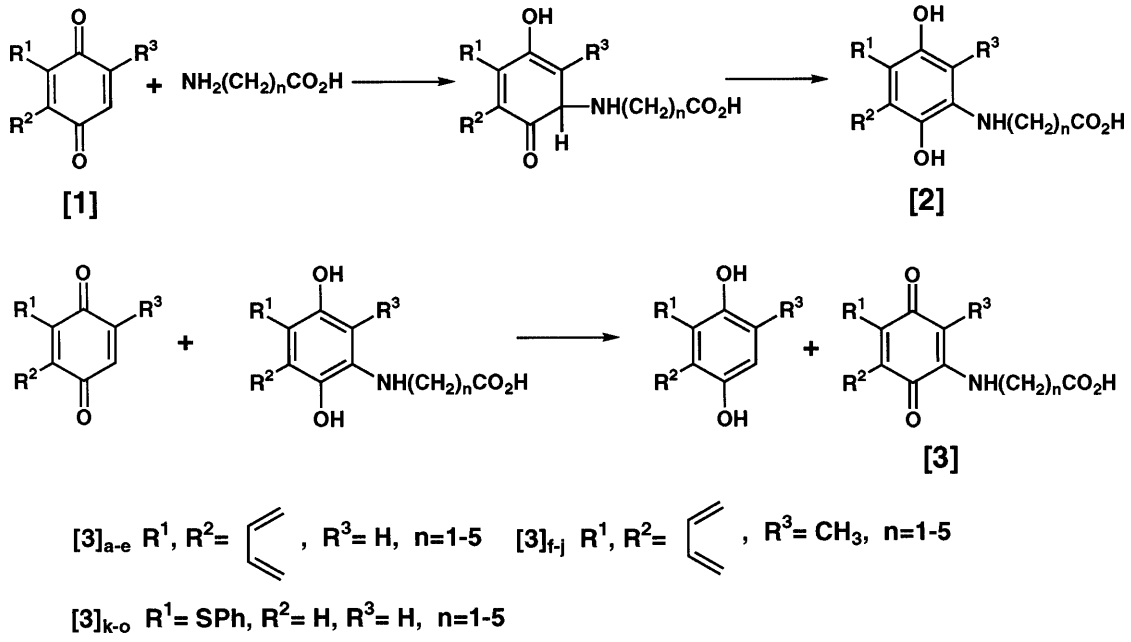
N-(5-phenylthio-1,4-benzoquinon-2-yl)-6-aminohexanoic acid (**3o**)

Yield 0.34 g (39%), m.p. = 186–187°C.
^{1}H-NMR (d_6-DMSO) δ (ppm) (J (Hz)): 7.53 (m, 5H), 5.48 (s, 1H), 5.43 (s, 1H), 3.21 (m, 2H), 2.25 (m, 2H), 1.64 (m, 4H), 1.34 (m, 2H).
IR (KBr) ν (cm^{-1}): 3355, 1703, 1640, 1604.
UV (MeOH) λ_{max} (nm) (log (ε)): 224 (3.92), 354 (3.79), 516 (2.57).
MS (m/z): 346 $[MH]^+$.

Results and discussion

a. Synthesis of ω-N-quinonyl amino acids

Four series of modified quinones were prepared in which ω-amino carboxylic acids are attached to the quinonic moiety *via* their ω-amino group. The first series (**3a–e**) involves derivatives of 1,4-naphthoquinone and the second series (**3f–j**) are all derivatives of 2-methyl-1,4-naphthoquinone (menadione, or vitamin K_3). The third series (**4a–e**) contains a much stronger oxidizing quinone, namely the 2-chloro-1,4-naphthoquinone, while the fourth series

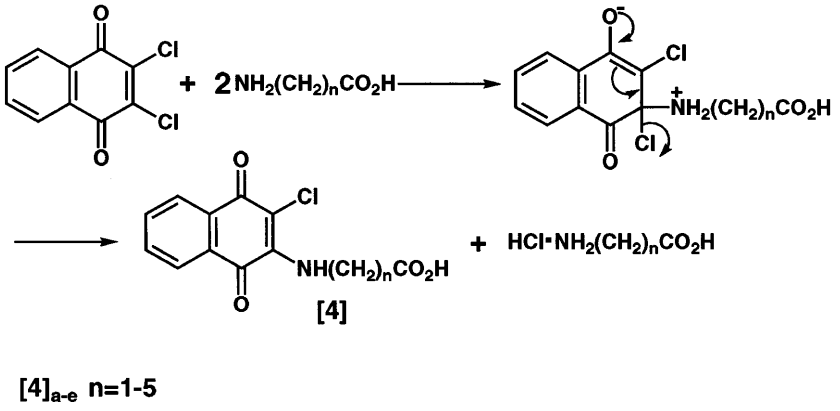


Scheme 2. 1,4-Michael-like addition of ω-amino carboxylic acids to quinones

(**3k–o**) was composed with a weaker oxidizing quinone, namely the 2-phenylthio-1,4-benzoquinone. All these vinylogous amides have a free carboxylic end group, which could be easily used for post-chain modifications of cytotoxic peptides. The N-quinonyl amino acids (**3a–o**) were obtained *via* a reductive 1,4-Michael type addition of the ω-amino acids to the appropriate quinone (Scheme 2). Two equivalents of the starting quinone were needed for the facile completion of the reaction. In the first stage the product is obtained in its reduced (hydroquinonic) form. Reaction of the hydroquinone with a second equivalent of the starting quinone gave the oxidized product, namely the N-quinonyl amino acid, together with the reduced (hydroquinonic) form of the starting material (Scheme 2). This redox process takes advantage of the fact that amino-substituted quinones have higher oxidation potentials than their parent quinones.

Products **4a–e** were obtained from the reaction of ω-amino carboxylic acids with 2,3-dichloro-1,4-naphthoquinone. The reaction resembles a substitution process by displacing one of the chlorine atoms. It involves a spontaneous elimination of HCl and, consequently, requires two equivalents of amino acid. In this case no redox process is needed and the reaction proceeds using only one equivalent of the starting quinone (Scheme 3). Also, under these conditions, no displacement of the second chlorine atom take place.

The four series of N-quinonyl amino acids prepared differ from each other by the type and oxidation potential of the quinonic building block. Within



Scheme 3. Substitution of 2,3-dichloro-1,4-naphthoquinone by ω-amino carboxylic acid

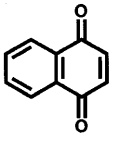
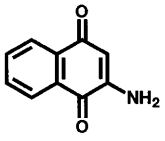
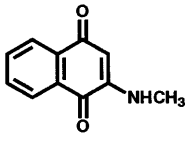
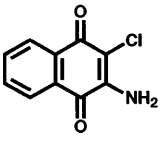
each series there is a gradual change in the distance between the quinonic moiety and the carboxylic group. It is interesting to note that the length of the alkyl chain has a profound effect on both the yields and the requisite reaction time in the 1,4-naphthoquinone series. The longer is the distance between the amino and the carboxylic groups the higher are the yields of the products, and the shorter is the reaction time. For example, the reaction of 6-aminohexanoic acid with 1,4-naphthoquinone needs only 3 hours at room temperature for its completion (TLC) and the yield is 93%, while the same reaction involving glycine requires 24 hours for completion and the yield is only 40%. By comparing the pKa_2 values of the ω-amino acids it is obvious that their basicity is a function of the distance between the amino and carboxylic groups. The longer is the chain the higher is the basicity and, consequently, the higher is the nucleophilicity. For example, the pKa_2 of glycine is 9.60, while the pKa_2 value of β-alanine is 10.19, the one of 4-aminobutanoic acid is 10.56, and the one of 6-aminohexanoic acid is 10.75 (Merck Index, 1990). Interestingly, in the reaction with the more reactive 2,3-dichloro-1,4-naphthoquinone, similar influence on yields or on the time was not observed. The two chlorine atoms increase the electrophilicity of the quinonic ring considerably and, subsequently, lower the selectivity of the reaction.

While a facile reaction take place with 1,4-naphthoquinone and with 2,3-dichloro-1,4-naphthoquinone, affording the quinonyl amino acids in average to high yields, the reaction with menadione are sluggish and the yields obtained are low. Similar behavior of menadione in various addition reactions are well recorded in the literature. For example, menadione react with 3-aminophenol to yield only 11% of the addition product (Kallmayer, 1979). Both steric hindrance and electronic effects might be responsible for this inhibition in the addition reactions. 2-Phenylthio-1,4-benzoquinone are more reactive than menadione and gives moderate yields (35–55%) of the corresponding ω-quinonyl amino acids.

b. Reduction potentials of ω-N-quinonyl amino acids

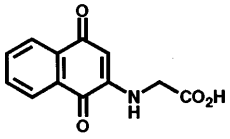
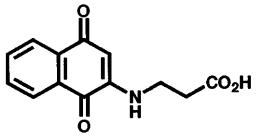
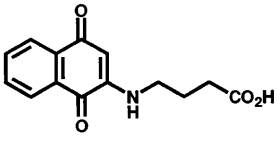
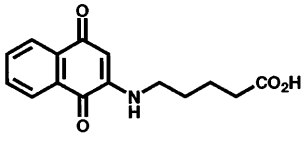
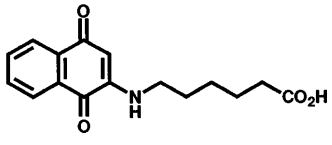
In order to characterize the *ω*-N-quinonyl amino acids and evaluate their ability to form stable semiquinone anion-radicals, reduction potentials were measured by using the cyclic voltammetry technique. All measurements were produced in HPLC grade DMF with 0.1 M TBAP electrolyte on glassy carbon electrode under Ar atmosphere. Table 1 contains simple naphthoquinone derivatives, for comparison with the *ω*-N-naphthoquinonyl amino acids studied (Tables 2 and 3). Each of the four compounds in the Table 1 exhibits a first reversible reduction wave and a second irreversible one. The first wave corresponds to the reduction of the naphthoquinone moiety to the semiquinone anion-radical ($Q^{\cdot-}$). The second wave is due to the reduction of the semiquinone anion-radical to hydroquinone dianion (Q^{2-}) (Clambers, 1988), As expected (Klopman and Doddapaneni, 1974), the donor amino group increases the first reduction potential by over 300 mV with respect to the unsubstituted naphthoquinone (entries 2 and 3 vs. 1). However, the naphthoquinone derivative in entry 4, which also contains a chlorine atom as an electron-withdrawing group, exhibits two contradicting electronic effects. Apparently, the mesomeric effect (by NH_2) is stronger than the inductive effect (by Cl) and as a consequence, it is more difficult to reduce it than the

Table 1. Reduction potentials of simple 1,4-naphthoquinones (in V vs. Ag/AgCl)[a]

Entry No.	*Compound*	$E^1_{1/2}(rev.)$	$E^2_{p_{red}}(irrev.)$
1		−0.48	−1.22
2		−0.79	−1.45
3		−0.81	−1.45
4		−0.64	−1.30

[a] Measured in DMF with-0.1 M TBAP on glassy carbon electrode, under Ar atmosphere.

Table 2. Reduction potentials of N-(1,4-naphthoquinon-2-yl)-ω-amino acids (in V vs. Ag/AgCl)

Compound	$E^{1}_{p_{red}}$(*irrev.*)	$E^{2}_{1/2}$(*rev.*)
	−0.72	−0.83
	−0.69	−0.83
	−0.72	−0.82
	−0.72	−0.79
	−0.71	−0.77

[a] Measured in DMF-0.1 M TBAP on glassy carbon electrode, under Ar atmosphere. Each derivative exhibits a third irreversible (or quasi-reversible) wave in the region of −1.2 to −1.6 V.

parent naphthoquinone by 160 mV, but easier than the amino-substituted derivatives.

Under the same experimental conditions all of the N-(1,4-naphthoquinon-2-yl)-*ω*-amino acids gave three reduction waves (Table 2). Surprisingly, the first reduction wave is irreversible while the second one is reversible (see a representative example in Fig. 1). Also, the $E^{1}_{p_{red}}$ values of the 1,4-naphthoquinonyl series are more negative (*e.g.* ~−0.70 V) compared to the first reduction wave of 1,4-naphthtoquinone itself (−0.48 V), but more positive than 2-amino-1,4-naphthoquinone (−0.79 V). The latter observation could be attributed to the presence of the carboxylic groups, which could form inter and/or intramolecular hydrogen bonding with the quinonic oxygens.

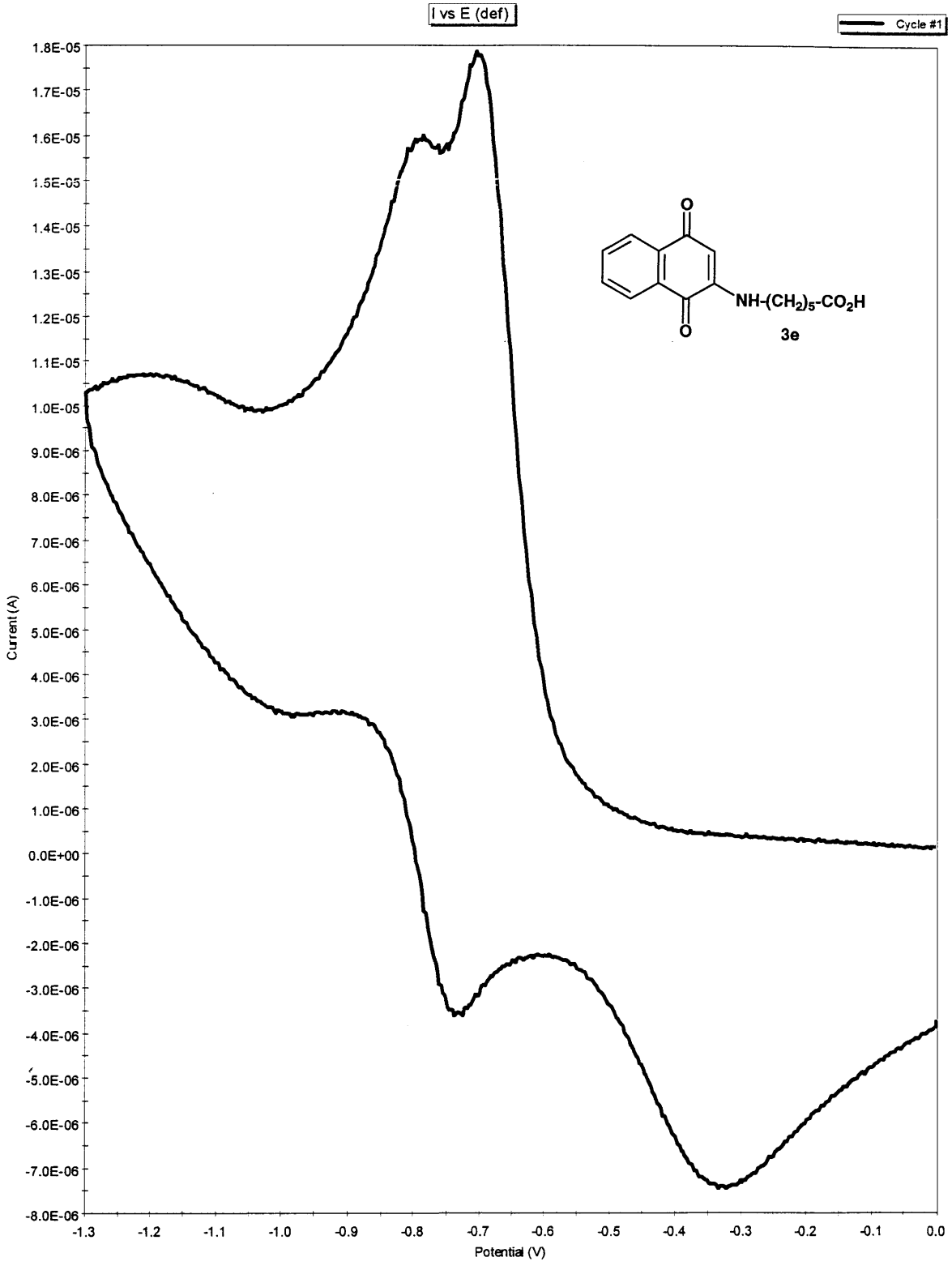
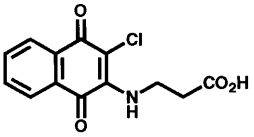
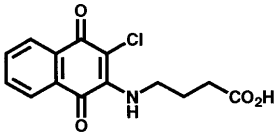
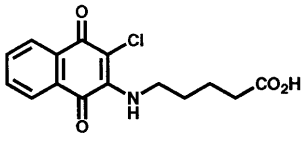
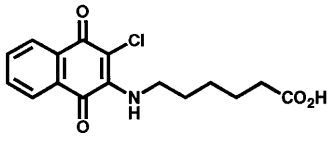
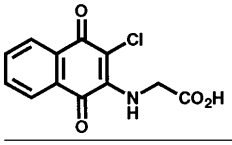


Fig. 1. Cyclic voltammogram of N-(1,4-naphthoquinon-2-yl)-6-aminohexanoic acid **3e** as a representative example

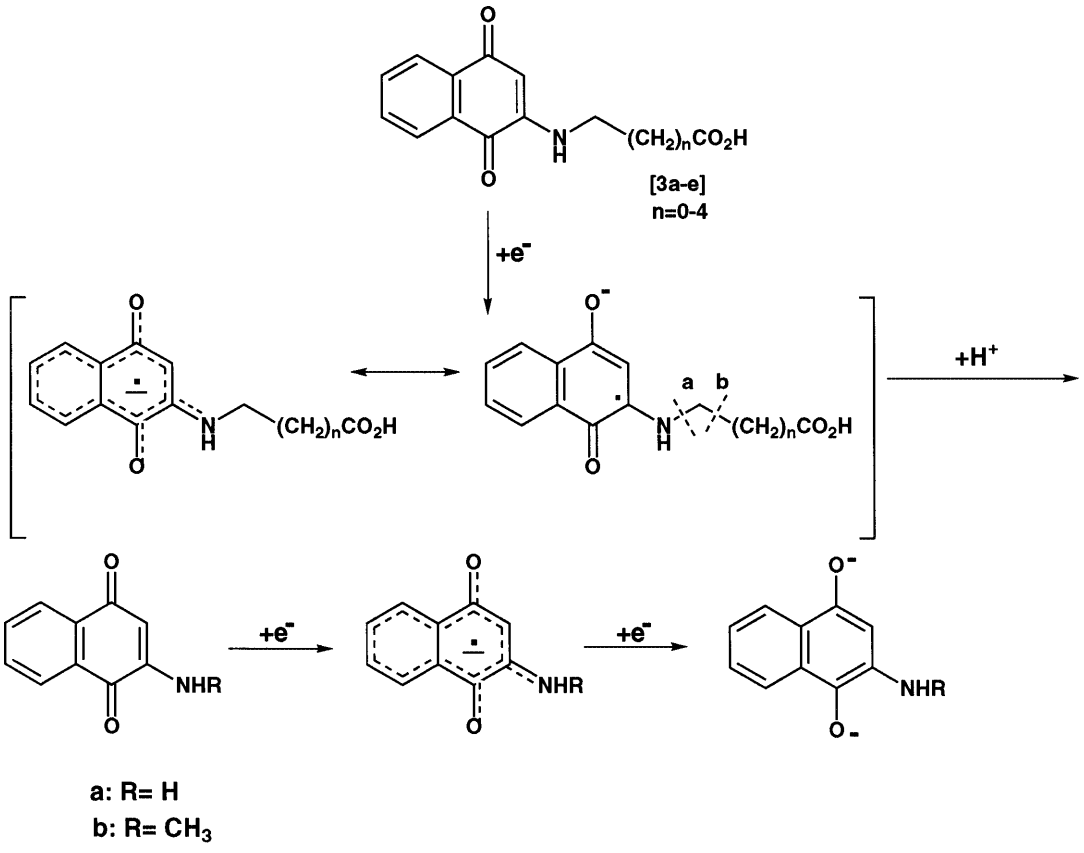
Table 3. Reduction potentials of N-(3-chloro-1,4-naphthoquinon-2-yl)-ω-amino acids (in V vs. Ag/AgCl)

Compound	$E^{1}_{1/2}$(*rev.*)	$E^{2}_{p_{red}}$(*irr.*)
	−0.59	−1.26
	−0.60	−1.27
	−0.61	−1.20
	−0.60	−1.25
	$E^{1}_{P_{red}}$(irrev.) = −0.52	$E^{2}_{1/2}$(rev.) = −0.70[b]

[a] Measured in DMF-0.1 M TBAP on glassy carbon electrode, under Ar atmosphere. [B] This derivative shows a third irreversible reduction wave at −1.23 V.

Such a phenomenon could lead to a decrease of charge density in the quinonic moiety, resulting in lower reduction potentials compared to the compounds listed in entries 2 and 3 (Table 1). The observation that all members of the 1,4-naphthoquinone series have very similar $E^{1}_{p_{red}}$, indicates that the length of the side chain of the amino acid has no effect on the reduction potential values in this series.

The irreversibility of the first reduction wave suggests that the primary semiquinone anion-radicals formed are unstable and undergo fast decomposition. We suggest that the follow-up chemical reaction may involve NH—CH_2 or $NHCH_2$—CH_2 bond cleavage at the side chain (Scheme 4). Thus, the primary semiquinone radical formed decomposes to 2-amino-1,4-naphthoquinone and/or 2-methylamino-1,4-naphthoquinone. In such a case,



Scheme 4

the second reversible reduction wave ($E^2_{1/2}$) obtained is due to the reduction of the new product, 2-amino-1,4-naphthoquinone or 2-methylamino-1,4-naphthoquinone, to its corresponding semiquinone anion-radical. The third irreversible wave ($E^3_{p_{red}}$(irr.)) (see footnote in Table 2) corresponds to the reduction of the new semiquinone to the hydroquinone dianion.

In order to support the above notion, we ran cyclic voltammetry of 2-amino-1,4-naphthoquinone and of 2-methylamino-1,4-naphthoquinone under the same experimental conditions and obtained two waves, the first is reversible and the second irreversible (Table 1). As can be seen, the $E^1_{1/2}$ values of 2-amino-1,4-naphthoquinone and of 2-methylamino-1,4-naphthoquinone (−0.79 V and −0.81 V, respectively) are very close to the $E^2_{1/2}$ values obtained for 1,4-naphthoquinonyl *ω*-amino acids (~−0.80 V). These almost identical $E^1_{1/2}$ values points to the possibility of the above mentioned cleavage as described in Scheme 4.

In contrast to the N-(1,4-naphthoquinon-2-yl)-*ω*-amino acids, four of the five N-(3-chloro-1,4-naphthoquinon-2-yl)-*ω*-amino acids **4b–e** gave two reduction waves (Table 3). The first wave is reversible, which shows that the semiquinone anion-radical produced from the chloronaphthoquinonyl amino acids is stable compared to the semiquinone anion radicals produced from the

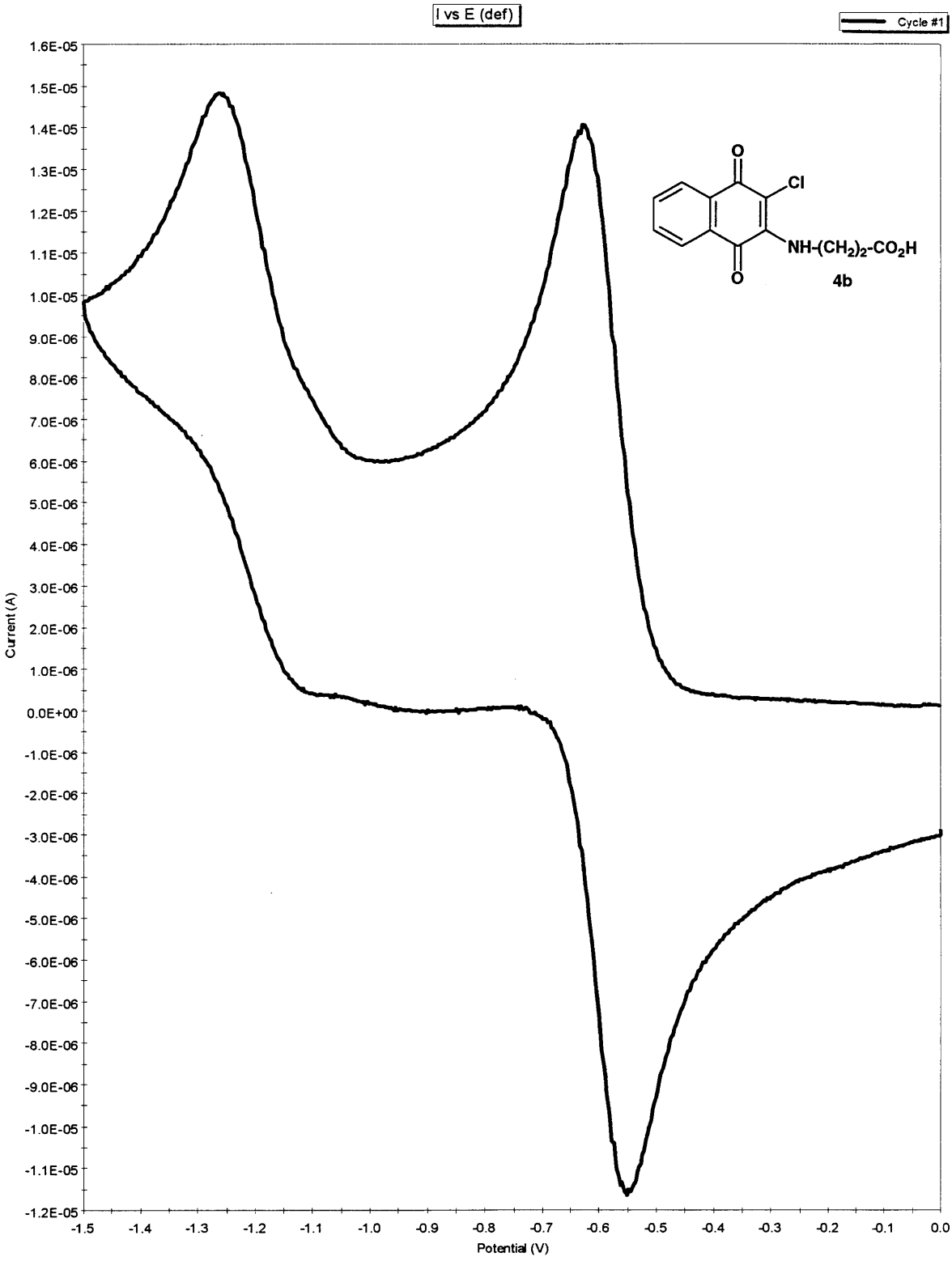


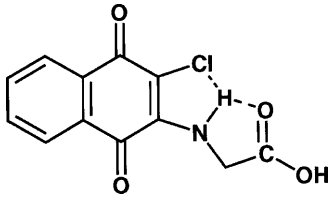
Fig. 2. Cyclic voltammogram of N-(3-chloro-1,4-naphthoquinon-2-yl)-β-alanine **4b** as a representative example

naphthoquinonyl amino acids (see Fig. 2). Evidently, the higher stability is due to the electron withdrawing nature of the chlorine atom at position 3. This is also expressed by the observation that the first reduction potential values of the chloronaphthoquinonyl amino acids are considerably lower compared to their 1,4-naphthoquinone analogs (Tables 2 and 3).

Surprisingly, the smallest member in this series, namely N-(3-chloro-1,4-naphthoquinon-2-yl)-glycine **4a** produced three reduction waves in the voltammogram, the first being irreversible (last entry in Table 3). This behavior, which is similar to that found for the 1,4-naphthoquinonyl amino acids series, suggests that the glycine derivative forms also an unstable semiquinone anion-radical, which undergoes NH—CH_2 or CH_2—CO_2H bond cleavage, thus forming the appropriate 2-amino-1,4-naphthoquinone. EPR studies of semiquinone anion radicals of different quinonyl amino acids (Bittner et al., 2000; Rahimipour et al., 1996) proved that while the unpaired electron is equally distributed on the naphthalenic ring, its density is mainly localized on the nitrogen atom. The unstable semiquinone radical leads to spontaneous decomposition of the semiquinone *via* the side chain cleavage. It is noteworthy that the first reduction potential of quinonyl glycine **4a** (−0.52 V) is lower than those of the other members of this series (~−0.60 V). Such a positive shift can be explained by assuming the formation of fused bicyclic ring involving two five-membered rings formed by hydrogen bonding between the secondary amine hydrogen and the spatially adjacent chlorine atom and the carboxylic carbonyl group (Scheme 5). In such a bicyclic ring system, both the donor ability of the nitrogen and the acceptor ability of the chlorine atoms decrease, causing the quinonyl moiety to be more electron deficient, and therefore, to be reduced at a less negative potential.

c. ^{1}H-NMR spectra

All the *ω*-N-naphthoquinonyl derivatives showed four different aromatic protons with different shifts, as expected from the NMR of such non-symmetrical systems. Ha and Hd appear as double doublets with two different coupling constants. Ortho splitting of these protons results in large splitting constants (J around 7.6 Hz), while the meta splittings are much smaller (J between 1.1–1.4 Hz). Hb and Hc appear as triplets, which are equivalents of double doublets with coupling constants of 7.5–7.6 Hz and 1.2–1.4 Hz. In the $CDCl_3$ ^{1}H-NMR spectra of the menadione derivatives **3h** and **3i**, the meta splitting is not observed. In the 2-phenylthio-1,4-benzoquinone series, the two



Scheme 5

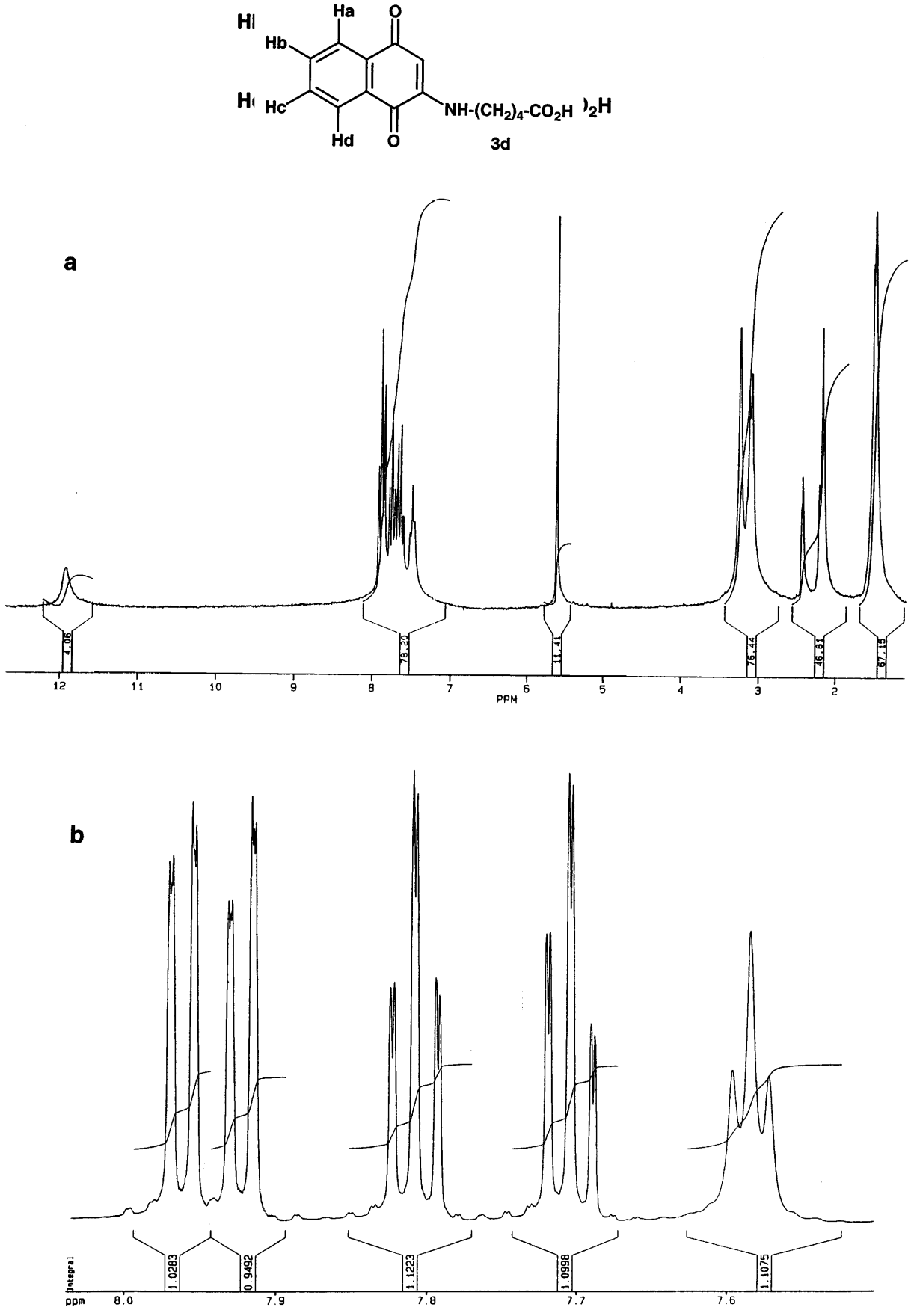


Fig. 3. a ^{1}H-NMR spectrum of N-(1,4-naphthoquinon-2-yl)-5-aminopentanoic acid **3d** recorded in d_6-DMSO at 300 K; **b** Expansion of the aromatic region

quinonic hydrogens appear as singlets between $\delta = 5.4$–5.5 ppm and the five aromatic hydrogens resonate as a multiplet around 7.5 ppm. The NH protons of all the compounds resonate between 6.7–7.7 ppm. This unusual down shift of the NH protons stems from its proximity to the quinonic nucleus and illustrates its amidic nature. The methylenic group attached to the NH resonate as a multiplet between 3.1–3.8 ppm, the α-CH_2 protons appear as a triplet around 2.2 ppm and the rest methylenic hydrogens appear as a mixed multiplet between 1.2–1.8 ppm. In most N-ω-quinonyl amino acids, the acidic proton resonate around $\delta = 12$ ppm (see Fig. 3).

d. Infrared and UV-Vis spectra

In the IR spectra the typical NH absorptions are observed between 3,326–3,382 cm^{-1}. In addition, two quinonic carbonyl absorptions appear in the region of 1,630–1,690 cm^{-1}, and the carboxylic carbonyl can be seen around 1,700–1,730 cm^{-1} (see Fig. 4).

In the UV-Vis two benzenoid π-π^* transitions are typical for all compounds. One appears at the range 222–242 nm and the other around 330 nm. Two quinonic π-π^* transitions are also observed and appear in the 1,4-naphthoquinonyl series at 270 nm and 450 nm (see Fig. 3). In the chlorinated series these transitions are shifted by the chlorine atom at the 2-position and appear at 274–276 nm and 468 nm. In the menadionyl series these two transitions are observed at 276 nm and 476 nm. The 2-phenylthio-1,4-benzoquinone series show these transitions at 224–226 nm and at 352–362 nm but, in addition, a weak of quinonic n-π^* absorption is observed at 500–515 nm (see Fig. 5).

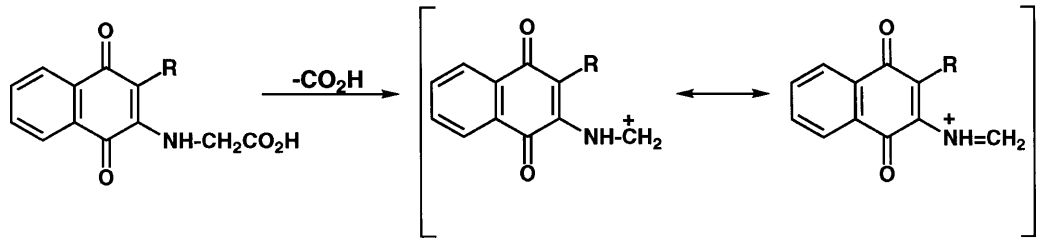
e. Mass spectra

Almost all the amino acids-quinonic conjugates gave the typical MH^+ parent ions. Few gave as the parent peak the decarboxylated ion, *e.g.* $[MH—CO_2]^+$ for **3a** and $[M\text{-}CO_2H]^+$ for **3f**.

The ease of decarboxylation is attributed to the formation of a stable carbonium – iminium ion, which is stabilized by the neighboring NH group (Silverstein, 1992) (see Scheme 6). Indeed, the phenomenon is observed only in the MS of the glycine derivatives **3a** and **3f**, since such stabilization can only be achieved with α-amino carboxylic acid derivatives. Other main fragments that could be detected, stem from fission of the C-Cl, CH_2-COOH and NH—CH_2 bonds.

Conclusions

We have synthesized four series of ω-N-quinonyl amino acids, which can be used for post-chain-assembly modifications of biologically active peptides. A direct relation was observed between the reduction potentials of the quinones and their reactivity towards ω-amino acids. The highly reactive 2,3-dichloro-



Scheme 6

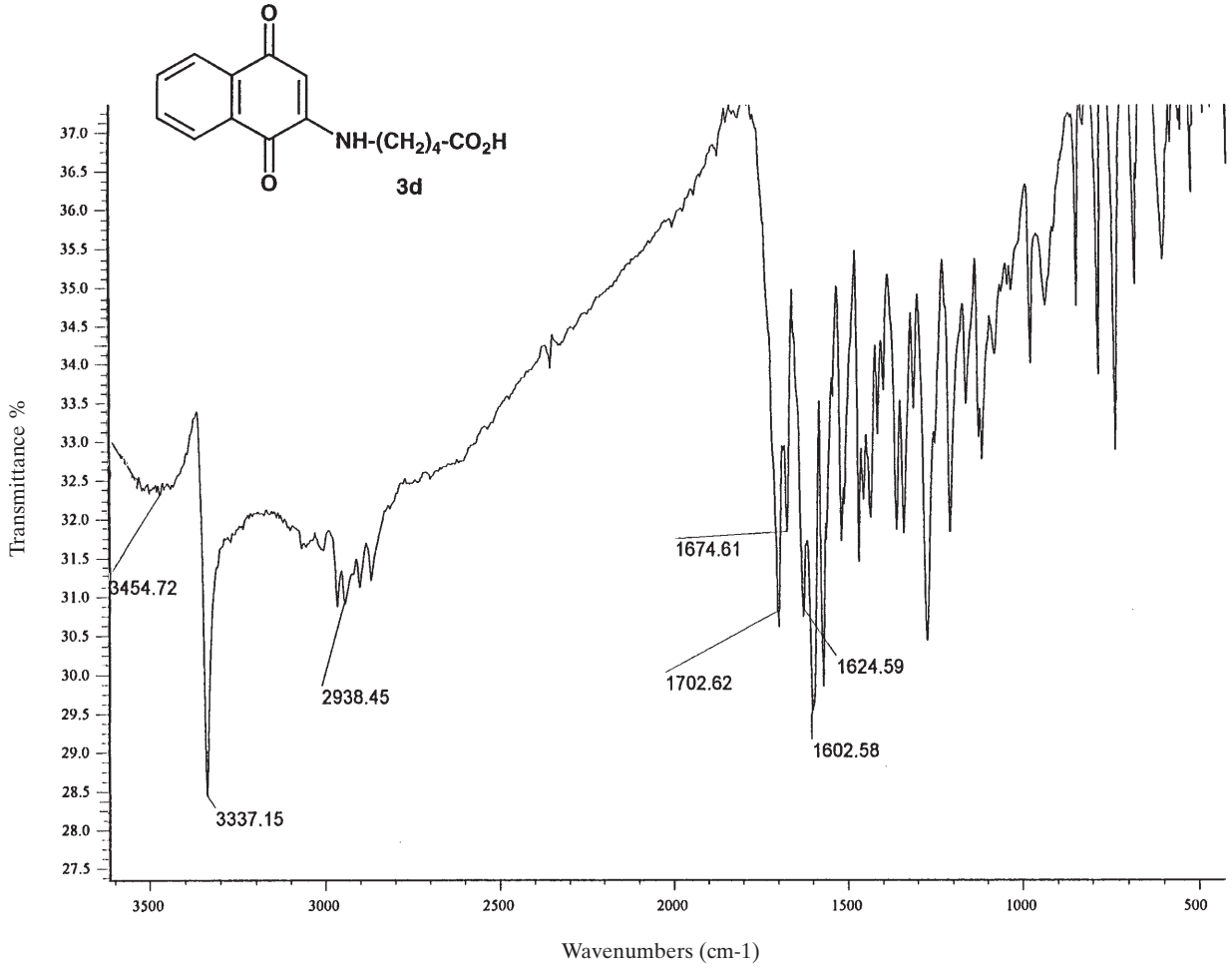


Fig. 4. IR spectrum of N-(1,4-naphthoquinon-2-yl)-5-aminopentanoic acid **3d** recorded with KBr at r.t.

1,4-naphthoquinone gives high yields of conjugates with all amino acids. The yields with 1,4-naphthoquinone which exhibits moderate reduction potential was found to depend on the length of the amino acid chain, the longer is the chain, the higher is the yield. The less electrophilic and hindered 2-methyl-1,4-naphthoquinone reacts sluggishly and gives low yields. The electron-transfer

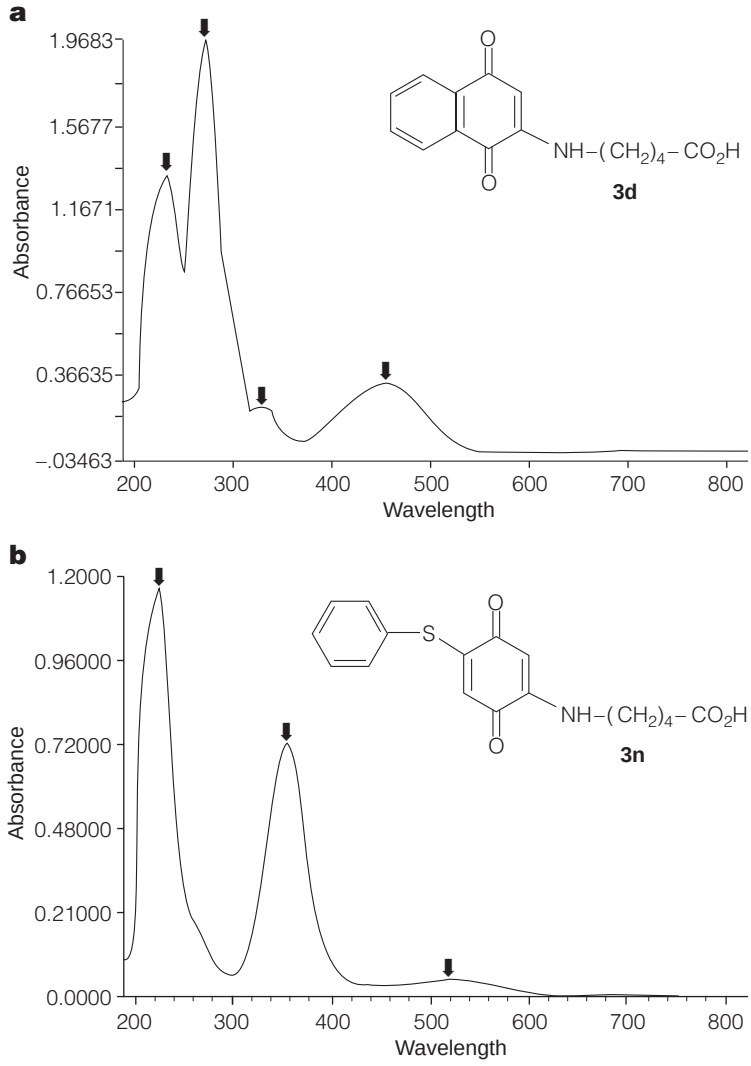


Fig. 5. **a** UV-Vis spectrum of N-(1,4-naphthoquinon-2-yl)-5-aminopentanoic acid **3d** recorded in MeOH at r.t.; **b** UV-Vis spectrum of N-(5-phenylthio-1,4-benzoquinon-2-yl)-5-aminopentanoic acid **3n** recorded in MeOH at r.t.

capabilities of the modified amino acids was probed by cyclic voltammetry measurements. The values for the first one-electron reduction potentials show a distinct pattern of changing towards more positive potentials with electron-withdrawing groups (*e.g.* chlorine), and to less positive potentials upon substitution with electron donating groups (*e.g.* amine). From the preliminary results described in this paper one could conclude that N-quinonyl amino acids are effective in producing stable semiquinone radicals, only when the quinonyl ring is substituted with chlorine atom and when the side chain is not glycine. The successful generation of these reactive species might be prerequisite for the manifestation of site-directed antitumor activity of corresponding quinone-peptide conjugates.

Acknowledgements

This research was supported by the Israel Science Foundation founded by the Academy of Sciences and Humanities. We wish to thank Mrs. Ethel Solomon for her skilled technical assistance.

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Authors' address: Prof. Shmuel Bittner, Department of Chemistry, Ben-Gurion University of the Negev, P.O. Box 653, Beer-Sheva 84105, Israel, Fax: (972)-8-6472943, E-mail: bittner@bgumail.bgu.ac.il

Received January 3, 2001