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Synthesis of analogues of anthraquinones linked to tuftsin or retro-tuftsin residues as potential topoisomerase inhibitors

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Abstract: A novel group of [(4-, 5- or 8)-hydroxy-9,10-anthraquinone-1-yl]-(tuftsin or retro-tuftsin) acids and methyl esters has been synthesized as potential anticancer compounds. The corresponding protected tuftsin or retro-tuftsin derivatives were also synthesized. We hope that combining compounds of different mechanisms of action will improve their clinical properties, and that our new analogues will be much more effective against multidrug-resistant tumour cell lines. Copyright © 2006 European Peptide Society and John Wiley & Sons, Ltd.

Keywords: anthraquinone peptide derivatives; tuftsin; retro-tuftsin; synthesis

INTRODUCTION

Hundreds of anthraquinone derivatives have been synthesized to date [1]. Some of them exhibit very interesting and promising anticancer properties, e.g. mitoxantrone or ametantrone [2]. These compounds enable the interaction with DNA-metabolizing enzymes and the perturbation of the replication and transcription of genetic information process using enzymes topoisomerase I and II (topo I and II) as inhibitors [3–5]. Topoisomerase inhibition represents a key therapeutic target in chemotherapy. Doxorubicin and mitoxantrone have been shown to form cleavable complexes with topo II and camptothecin and its analogues with topo I [5,6]. Till now, several anthraquinone compounds exhibiting very interesting and promising anticancer properties have been synthesized, e.g. derivatives with unsymmetrical side chains [7], aza analogues containing pyridine [8] or pyridazine [9] rings and analogues possessing a five- or six-membered heterocyclic ring. Among them anthrapyrazoles [10] and their aza analoges [11], anthrapyridones, anthrapyridazones and benzo[*e*]perimidines have been designed and obtained [12]. Also, anthraquinone derivatives have been synthesized, which contained amino acid or peptide chains as topoisomerase inhibitors [5,13–16]. The clinical usefulness of anthracyclines is limited because of some drawbacks, such as cardiac toxicity and tumour resistance [1]. These drawbacks have stimulated scientists and clinicians to seek anticancer agents devoid of the mentioned shortcomings.

On the other hand, the tetrapeptide tuftsin (H-Thr-Lys-Pro-Arg-OH) [17] possesses significant immunomodulatory properties. It is capable of stimulating granulocyte and macrophage functions such as phagocytosis, motility, somnogenic response, as well as bactericidal and tumoricidal activity. Moreover, tuftsin has a number of other interesting biological properties, such as anti-infective, anticancer, anti-AIDS and growth factor activities [18,19]. It is possible that combining compounds showing different mechanisms of action may improve clinical properties of both components, so we hope that new analogues of anthraquinones and tuftsin will be much more effective against multidrug resistance (MDR) of tumour cell lines. MDR is one of the main obstacles in chemotherapy of cancer. The idea of combining these two biologically active compounds was based on the synergistic activity of muramyl dipeptide (MDP) [20,21]. This paper reports novel anthraquinone peptide analogues and the method of their syntheses. The results on the biological tests of these compounds will be reported in due course.

RESULTS AND DISCUSSION

Continuing our search for potential anticancer agents [20,22–25], we synthesized new anthraquinone analogues containing covalently bonded tuftsin or retro-tuftsin derivatives as potential topoisomerase inhibitors. We hope that combining these compounds (tuftsin – a well-known immunostimulator – and anthraquinone derivatives) with different mechanisms of action will improve their clinical properties and that these new analogues will be much more effective against MDR of cancer cells. The synthesis of the compounds was carried out according to Scheme 1. During the first step of the synthesis, 1,4-, 1,5- or 1,8-bis(tosyloxy)anthraquinone from 1,4-, 1,5- or 1,8-dihydroxy-anthraquinone and *para*-toluenesulfonyl chloride was obtained [26]. These

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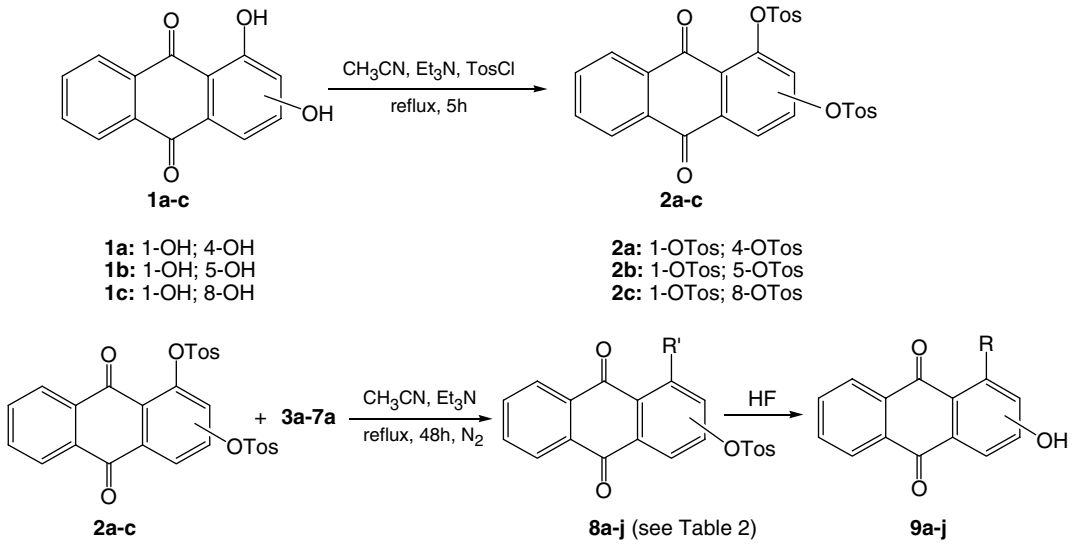
compounds with an excess of the corresponding tuftsin derivatives **3a–7a** in the presence of triethylamine (TEA) gave the monosubstituted derivatives **8a–j**. The yields of compounds **8a–j** depended on the excess of tuftsin or retro-tuftsin derivatives: TFA·Thr-Lys(Z)-Pro-Arg(NO_2)-OBzl, TFA·Thr-Lys(ZAla)-Pro-Arg(NO_2)-OMe, TFA·Arg(NO_2)-Pro-Lys(Z)-Thr-OMe, TFA·Arg(NO_2)-Pro-Lys(ZAla)-Thr-OMe and TFA·Arg(NO_2)-Pro-Lys(ZVal)-Thr-OMe. When a fourfold excess of tuftsin derivatives **3a–7a** was used, it was possible to obtain the protected compounds **8a–j** with 35–44% yield (Table 2). Removal of the tosyl protection was necessary for carrying out the next step of the synthesis. The susceptibility of the tosyl group for splitting was checked in various solvents and temperatures. We selected acetonitrile as the solvent of choice and reflux heating. The reaction was monitored by means of thin layer chromatography (TLC) with the following solvent systems: (A) $CHCl_3$–MeOH (4 : 1, v/v), (B) $CHCl_3$–MeOH (9 : 1, v/v), (C) $CHCl_3$–MeOH (20 : 1, v/v), and (D) hexane–ethyl acetate (70 : 30, v/v).

We synthesized also the following protected tuftsin and derivatives: Boc-Thr-Lys(Z)-Pro-Arg(NO_2)-OBzl **3** [20], Boc-Thr-Lys(ZAla)-Pro-Arg(NO_2)-OMe **4**, Boc-Arg(NO_2)-Pro-Lys(Z)-Thr-OMe **5** [21], Boc-Arg(NO_2)-Pro-Lys(ZAla)-Thr-OMe **6** and Boc-Arg(NO_2)-Pro-Lys(ZVal)-Thr-OMe **7** using the mixed anhydride method with isobutyl chloroformate and *N*-methylmorpholine (NMM) in anhydrous DMF [21] (Scheme 2). The applied methodology allowed the diminished formation of impurities, and ensured a high chiral purity of the product and a good yield of about 75–80% at each step. The protected tetrapeptides **3–7** were isolated by column chromatography and purified with preparative TLC. The preparation of tuftsin and its analogue in high purity was always difficult. The identities of the protected products were confirmed by high-resolution ^{1}H-NMR (500 MHz, COSY, TOCSY, ROESY) spectroscopy, TLC qualitative amino acid analysis, and elemental analyses. Yields and melting points of the protected tetrapeptide are collected in Table 1. The *tert*-butoxycarbonyl (Boc) group was removed with trifluoroacetic acid to give protected tuftsin derivatives **3–7**. The final protected products **8a–j** were purified with preparative TLC, and their identities were confirmed by high-resolution ^{1}H-NMR (500 MHz, COSY, ROESY, TOCSY) spectroscopy and qualitative TLC amino acid analysis. After the removal of the protected group with liquid HF, the expected products **9a–j** were obtained (Scheme 1).



1a: 1-OH; 4-OH
1b: 1-OH; 5-OH
1c: 1-OH; 8-OH

2a: 1-OTos; 4-OTos
2b: 1-OTos; 5-OTos
2c: 1-OTos; 8-OTos

3a: TFA.Thr-Lys(Z)-Pro-Arg(NO_2)-OBzl;
4a: TFA.Thr-Lys(ZAla)-Pro-Arg(NO_2)-OCH_3;
5a: TFA.Arg(NO_2)-Pro-Lys(Z)-Thr-OCH_3;
6a: TFA.Arg(NO_2)-Pro-Lys(ZAla)-Thr-OCH_3;
7a: TFA.Arg(NO_2)-Pro-Lys(ZVal)-Thr-OCH_3

R'=Thr-Lys(Z)-Pro-Arg(NO_2)-OBzl;
Thr-Lys(ZAla)-Pro-Arg(NO_2)-OCH_3;
Arg(NO_2)-Pro-Lys(Z)-Thr-OCH_3;
Arg(NO_2)-Pro-Lys(ZAla)-Thr-OCH_3;
Arg(NO_2)-Pro-Lys(ZVal)-Thr-OCH_3

4-OTos; 5-OTos or 8-OTos

9a: R=Thr-Lys-Pro-Arg-OH; 4-OH
9b: R=Thr-Lys-Pro-Arg-OH; 5-OH
9c: R=Thr-Lys-Pro-Arg-OH; 8-OH
9d: R=Thr-Lys(Ala)-Pro-Arg-OMe; 4-OH
9e: R=Arg-Pro-Lys-Thr-OCH_3; 4-OH
9f: R=Arg-Pro-Lys-Thr-OCH_3; 5-OH
9g: R=Arg-Pro-Lys-Thr-OCH_3; 8-OH
9h: R=Arg-Pro-Lys(Ala)-Thr-OCH_3; 4-OH
9i: R=Arg-Pro-Lys(Val)-Thr-OCH_3; 4-OH
9j: R=Arg-Pro-Lys(Val)-Thr-OCH_3; 5-OH

Scheme 1 Synthesis of the anthraquinone derivatives.

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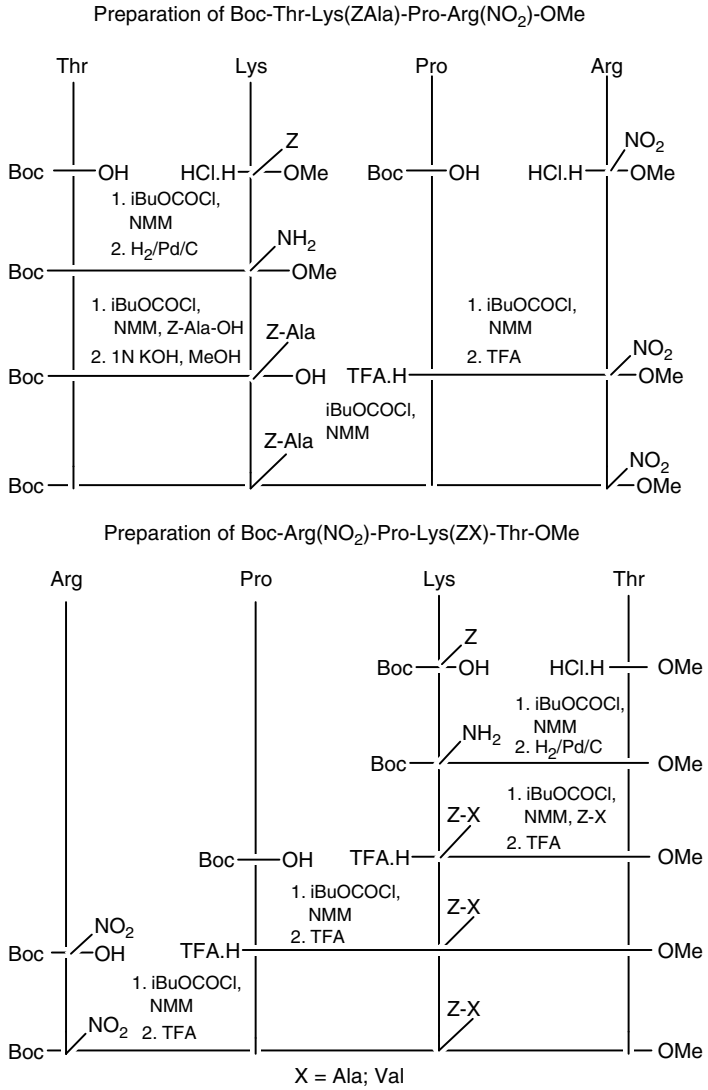

Scheme 2 Preparation of Boc-Thr-Lys(ZAla)-Pro-Arg(NO_2)-OMe (a) and Boc-Arg(NO_2)-Pro-Lys(ZX)-Thr-OMe (b).

Their identities were confirmed by mass spectra and ^{1}H-NMR (500 MHz) spectroscopy. These compounds were prepared and sent to assay their biological activity as topoisomerase inhibitors.

EXPERIMENTAL SECTION

Melting points (m.p., uncorrected) were determined on the Kofler-block apparatus. ^{1}H-NMR spectra were measured in DMSO or $CDCl_3$ solutions with a Varian 500 and 200 NMR spectrometers. Preparative column chromatography and radial chromatography were performed on silica gel (Kieselgel 60, 100–200 mesh) in solvent systems specified below. All chemicals and solvents were of reagent grade and were used without further purification. The reactions were monitored by TLC on Merck F_{254} silica gel precoated plates. The following solvent systems (by vol.) were used for TLC and radial chromatography development: (A) *n*-BuOH–pyridine–AcOH–H_2O (60 : 45 : 4 : 30, v/v), (B) *n*-BuOH–AcOH–H_2O (4 : 2 : 2, v/v), (C) $CHCl_3$–MeOH (4 : 1, v/v), (D) $CHCl_3$–MeOH (9 : 1, v/v), (E) $CHCl_3$–MeOH (2 : 1, v/v), (F) $CHCl_3$–MeOH (20 : 1, v/v), (G) hexane–ethyl acetate (70 : 30, v/v). All synthesized protected peptides were homogeneous on TLC in solvent A, C, D or E. Mass spectra were recorded on matrix-assisted laser desorption/ionization-time of flight mass spectrometry (MALDI-TOF MS, Biflex III Bruker). Qualitative amino acid analyses of the hydrolyzates of the compounds were accomplished on TLC in solvent A. The detection was by UV and ninhydrin.

In the following AQ stands for anthraquinone.

The procedure for the synthesis of compounds **3a, 5a** has been published previously [20,21]. The compounds **4a, 6a** and **7a** were prepared according to the same procedure as described for **3a, 5a**. Removal of the Z-protecting groups in Boc-Lys(Z)-Thr-OMe or Boc-Thr-Lys(Z)-OMe by catalytic hydrogenation over 10%

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Table 1 Tuftsin Derivatives **4**, **6** and **7**

Compounds[a]	Molecular formula	Yield (%)	m.p. (°C)	$[\alpha]_{589}$ MeOH (*c* 1.0)
Boc-Thr-Lys(ZAla)-Pro-Arg(NO_2)-OMe; **4**	$C_{38}H_{60}N_{10}O_{13}$ (864.94)	78	93–97	−36°
H-Thr-Lys(ZAla)-Pro-Arg(NO_2)-OMe trifluoroacetate; **4a**	$C_{35}H_{53}N_{10}O_{13}F_3$ (878.85)	85	Oil	—
Boc-Arg(NO_2)-Pro-Lys(ZAla)-Thr-OMe; **6**	$C_{38}H_{60}N_{10}O_{13}$ (864.94)	69	71–75	−44°
H-Arg(NO_2)-Pro-Lys(ZAla)-Thr-OMe trifluoroacetate; **6a**	$C_{35}H_{53}N_{10}O_{13}F_3$ (878.85)	80	Oil	—
Boc-Arg(NO_2)-Pro-Lys(ZVal)-Thr-OMe; **7**	$C_{40}H_{64}N_{10}O_{13}$ (893.00)	72	98–102	−30°
H-Arg(NO_2)-Pro-Lys(ZVal)-Thr-OMe trifluoroacetate; **7a**	$C_{37}H_{57}N_{10}O_{13}F_3$ (906.90)	94	Oil	—

[a] ^{1}H NMR spectra of all compounds were in compliance with the expected ones.

Table 2 Protected Compounds **8a–j**

Comp[a]	R	Position of—OTos	Molecular formula	Yield (%)
8a	Thr-Lys(Z)-Pro-Arg(NO_2)-OBzl	4	$C_{57}H_{63}N_9O_{15}S$ (1145.42)	40
8b	Thr-Lys(Z)-Pro-Arg(NO_2)-OBzl	5	$C_{57}H_{63}N_9O_{15}S$ (1145.42)	36
8c	Thr-Lys(Z)-Pro-Arg(NO_2)-OBzl	8	$C_{57}H_{63}N_9O_{15}S$ (1145.42)	35
8d	Thr-Lys(ZAla)-Pro-Arg(NO_2)-OMe	4	$C_{54}H_{64}N_{10}O_{16}S$ (1140.42)	38
8e	Arg(NO_2)-Pro-Lys(Z)-Thr-OMe	4	$C_{51}H_{59}N_9O_{15}S$ (1069.39)	44
8f	Arg(NO_2)-Pro-Lys(Z)-Thr-OMe	5	$C_{51}H_{59}N_9O_{15}S$ (1069.39)	42
8g	Arg(NO_2)-Pro-Lys(Z)-Thr-OMe	8	$C_{51}H_{59}N_9O_{15}S$ (1069.39)	38
8h	Arg(NO_2)-Pro-Lys(ZAla)-Thr-OMe	4	$C_{54}H_{64}N_{10}O_{16}S$ (1140.42)	42
8i	Arg(NO_2)-Pro-Lys(ZVal)-Thr-OMe	4	$C_{56}H_{68}N_{10}O_{16}S$ (1168.45)	40
8j	Arg(NO_2)-Pro-Lys(ZVal)-Thr-OMe	5	$C_{56}H_{68}N_{10}O_{16}S$ (1168.45)	35

[a] ^{1}H NMR spectra of all compounds were in compliance with the expected ones.

palladium on charcoal and then coupling with Z-Ala-OH or Z-Val-OH gave compounds: Boc-Lys(ZAla)-Thr-OMe, Boc-Lys(ZVal)-Thr-OMe or Boc-Thr-Lys(ZAla)-OMe. Next the *tert*-butoxy-carbonyl group was removed by treatment with TFA or -OMe group with basic hydrolysis and the obtained peptides were coupled to Boc-Pro-OH and Boc-Arg(NO_2)-OH or TFA·Pro-Arg(NO_2)-OMe. Pentapeptide Boc-Thr-Lys(ZAla)-Pro-Arg(NO_2)-OMe **4** was obtained by coupling of Boc-Thr-Lys(ZAla)-OH with dipeptide TFA·Pro-Arg(NO_2)-OMe. Pentapeptides Boc-Arg(NO_2)-Pro-Lys(ZAla)-Thr-OMe **6** and Boc-Arg(NO_2)-Pro-Lys(ZVal)-Thr-OMe **7** were obtained in the reaction of TFA·Lys(ZAla)-Thr-OMe or TFA·Lys(ZVal)-Thr-OMe with Boc-Pro-OH followed by coupling with Boc-Arg(NO_2)-OH. Finally from peptides **4, 6**, and **7,** the Boc group was removed by treatment with TFA to give trifluoroacetates **4a, 6a,** and **7a** (Table 1). The NMR spectra of new compounds **4, 6**, and **7** are presented below.

Boc-Thr-Lys(ZAla)-Pro-Arg(NO_2)-OMe (4)

^{1}H-NMR (500 MHz, DMSO-d_6) δ: 1.02 (d, $J = 5.4$ Hz, 3H, T-CH_3), 1.17 (d, $J = 7.1$ Hz, 3H, A-CH_3), 1.31 (m, 2H, K-γCH_2), 1.38 (m, 2H, K-δCH_2), 1.38 (s, 9H, *t*-Bu), 1.59 (m, 1H, R-βCH), 1.48 (m, 1H, K-βCH), 1.55 (m, 2H, R-γCH_2), 1.62 (m, 1H, R-βCH), 1.63 (m, 1H, K-βCH), 1.78 (m, 1H, P-βCH), 1.87 (m, 2H, P-γCH), 1.75 (m, 1H, R-βCH), 2.05 (m, 1H, P-βCH), 3.02 (m, 2H, K-εCH_2), 3.16 (m, 2H, R-δCH_2), 3.53 (m, 1H, P-δCH), 3.67 (m, 1H, P-δCH), 3.61 (s, 3H, $COOCH_3$), 3.98 (q, $J = 7.2$ Hz, 1H, A-αCH), 4.25 (m, 1H, R-αCH), 3.88 (m, 2H, T-αCH, T-βCH), 4.52 (m, 1H, K-αCH), 4.35 (dd, $J = 4.5$ Hz, $J = 8.3$ Hz, 1H, P-αCH), 4.70 (d, $J = 5.4$ Hz, 1H, T-OH), 5.01 (m, 2H, Z-CH_2), 6.96 and 8.54 (bs, R-NH_2), 7.39 (m, 1H, A-αNH), 7.82 (t, $J = 5.3$ Hz, 1H, K-εNH), 7.28–7.38 (m, 5H, Ph), 8.31 (d, $J = 7.4$ Hz, 1H, R-αNH), 6.43 (d, $J = 8.3$ Hz, 1H, T-αNH), 7.89 (d, $J = 7.7$ Hz, 1H, K-αNH), 8.54 (bs, 1H, R-δNH). ^{13}C NMR (125 MHz, DMSO-d_6) δ: 20.0 (T-CH_3), 28.2 (Boc-CH_3), 40.8 (R-δCH_2), 40.8 (K-εCH_2), 46.7 (P-δCH_2), 51.7 (T-OCH_3), 51.8 (K-αCH), 52.4 (R-αCH), 57.6 (T-αCH), 59.1 (P-αCH), 65.1 (Z-CH_2), 66.3 (T-βCH), 127.8, 128.4, 137.3 (Ph), 156.1–155.4 (Z-C1), 170.5, 171.0, 171.5, 172.2 (four signals of carbonyl group). Anal. Calcd for $C_{38}H_{60}N_{10}O_{13}$: C, 52.77; H, 6.99; N, 16.19. Found: C, 52.92; H, 7.12; N, 16.25.

Boc-Arg(NO_2)-Pro-Lys(ZAla)-Thr-OMe (6)

^{1}H-NMR (500 MHz, DMSO-d_6) δ: 1.04 (d, $J = 6.5$ Hz, 3H, T-CH_3), 1.18 (d, $J = 7.1$ Hz, 3H, A-CH_3), 1.31 (m,

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2H, K-γCH_2), 1.40 (m, 2H, K-δCH_2), 1.36 (s, 9H, *t*-Bu), 1.46 (m, 1H, R-βCH), 1.51 (m, 1H, K-βCH), 1.56 (m, 2H, R-γCH_2), 1.62 (m, 1H, R-βCH), 1.68 (m, 1H, K-βCH), 1.81 (m, 1H, P-βCH), 1.88 (m, 2H, P-γCH_2), 2.03 (m, 1H, P-βCH), 3.02 (m, 2H, K-εCH_2), 3.11 (m, 2H, R-δCH_2), 3.54 (m, 1H, P-δCH), 3.62 (m, 1H, P-δCH), 3.61 (s, 3H, $COOCH_3$), 3.99 (q, 1H, A-αCH), 4.12 (m, 1H, T-βCH), 4.15 (m, 1H, R-αCH), 4.28 (m, 1H, T-αCH), 4.28 (m, 1H, K-αCH), 4.38 (dd, $J = 3.5$ Hz, $J = 7.9$ Hz, 1H, P-αCH), 4.99 (m, 1H, T-OH), 5.02 (m, 2H, Z-CH_2), 7.83 (t, $J = 5.4$ Hz, 1H, K-εNH), 7.28–7.38 (m, 5H, Ph), 6.94 (d, $J = 7.6$ Hz, 1H, R-NH), 6.9 (bs, 2H, R-NH_2), 7.38 (m, 1H, A-αNH), 7.79 (d, $J = 8.1$ Hz, 1H, T-αNH), 8.01 (d, $J = 7.6$ Hz, 1H, K-αNH), 8.47 (bs, 1H, R-δNH). ^{13}C NMR (125 MHz, DMSO-d_6) δ: 20.0 (T-CH_3), 28.2 (Boc-CH_3), 40.8 (R-δCH_2), 40.8 (K-εCH_2), 46.7 (P-δCH_2), 51.7 (T-OCH_3), 51.8 (K-αCH), 52.4 (R-αCH), 57.6 (T-αCH), 59.1 (P-αCH), 65.1 (Z-CH_2), 66.3 (T-βCH), 127.8, 128.4, 137.3 (Ph), 156.1–155.4 (Z-C1), 170.5, 171.0, 171.5, 172.2 (four signals of carbonyl group). Anal. Calcd for $C_{38}H_{60}N_{10}O_{13}$: C, 52.77; H, 6.99; N, 16.19. Found: C, 52.99; H, 7.04; N, 15.98.

Boc-Arg(NO_2)-Pro-Lys(ZVal)-Thr-OMe (7)

^{1}H-NMR (500 MHz, DMSO-d_6) δ: 0.83 and 0.84 (d, $J = 6.6$ Hz, 6H, V-CH_3), 1.04 (d, $J = 6.3$ Hz, 3H, T-CH_3), 1.30 (m, 2H, K-γCH_2), 1.37 (m, 2H, K-δCH_2), 1.36 (s, 9H, *t*-Bu), 1.52 (m, 1H, R-βCH), 1.52 (m, 1H, K-βCH), 1.56 (m, 2H, R-γCH_2), 1.67 (m, 1H, R-βCH), 1.67 (m, 1H, K-βCH), 1.82 (m, 1H, P-βCH), 1.89 (m, 2H, P-γCH_2), 1.91 (m, 1H, V-βCH), 2.01 (m, 1H, P-βCH), 2.97 and 3.08 (m, 2H, K-εCH_2), 3.11 (m, 2H, R-δCH_2), 3.54 (m, 1H, P-δCH), 3.62 (m, 1H, P-δCH), 3.62 (s, 3H, $COOCH_3$), 3.77 (q, 1H, V-αCH), 4.11 (m, 1H, T-βCH), 4.38 (m, 1H, R-αCH), 4.28 (m, 2H, T-αCH, K-αCH), 4.38 (dd, $J = 3.7$ Hz, $J = 8.3$ Hz, 1H, P-αCH), 4.99 (m, 1H, T-OH), 5.02 (m, 2H, Z-CH_2), 6.62 and 6.94 (bs, R-NH_2), 7.92 (t, $J = 5.4$ Hz, 1H, K-εNH), 7.28–7.38 (m, 5H, Ph), 6.95 (d, $J = 7.8$ Hz, 1H, R-αNH), 7.24 (d, $J = 8.8$ Hz, 1H, V-αNH), 7.80 (d, $J = 8.3$ Hz, 1H, T-αNH), 8.02 (d, $J = 7.8$ Hz, 1H, K-αNH), 8.49 (bs, 1H, R-δNH). ^{13}C NMR (125 MHz, DMSO-d_6) δ: 20.0 (T-CH_3), 28.2 (Boc-CH_3), 40.8 (R-δCH_2), 40.8 (K-εCH_2), 46.7 (P-δCH_2), 51.7 (T-OCH_3), 51.8 (K-αCH), 52.4 (R-αCH), 57.6 (T-αCH), 59.1 (P-αCH), 65.1 (Z-CH_2), 66.3 (T-βCH), 127.8, 128.4, 137.3 (Ph), 156.1–155.4 (Z-C1), 170.5, 171.0, 171.5, 172.2 (four signals of carbonyl group). Anal. Calcd for $C_{40}H_{64}N_{10}O_{13}$: C, 53.80; H, 7.22; N, 15.69. Found: C, 53.92; H, 7.12; N, 15.38.

Preparation of the 1,4-, 1,5- or 1,8-bis(tosyloxy)anthraquinones (2a–c)

The general procedure for the synthesis of compound **2a** has been published previously [26]. The new compounds **2b,c** were prepared according to the same procedure as described for **2a**.

1,4-Bis(tosyloxy)anthraquinone (2a)

Yield 85%; m.p. 224–226 °C (lit. [26] 225–226 °C); ^{1}H-NMR (500 MHz, DMSO-d_6) δ: 2.26 (s, 6H), 7.35–7.37 (d, $J = 8.2$ Hz, 4H), 7.64 (s, 2H), 7.62–7.64 (d, $J = 6.3$ Hz, 4H), 7.67–7.70 (m, 2H), 7.80–7.83 (m, 2H).

1,5-Bis(tosyloxy)anthraquinone (2b)

Yield 83%; m.p. 208–211 °C; ^{1}H-NMR (500 MHz, DMSO-d_6) δ: 2.29 (s, 6H), 7.36–7.38 (d, $J = 8.0$ Hz, 4H), 7.53–7.54 (d, $J = 8.2$ Hz, 2H), 7.61–7.63 (d, $J = 8.0$ Hz, 4H), 7.81–7.82 (d, $J = 7.7$ Hz, 2H), 7.86–7.92 (t, $J = 8.0$ Hz, 2H). Anal. Calcd for $C_{28}H_{20}S_2O_8$: C, 61.30; H, 3.67; S, 11.69. Found: C, 61.12; H, 3.82; S, 11.56.

1,8-Bis(tosyloxy)anthraquinone (2c)

Yield 82%; m.p. 175–177 °C; ^{1}H-NMR (500 MHz, DMSO-d_6) δ: 2.37 (s, 6H), 7.42–7.43 (d, $J = 7.8$ Hz, 4H), 7.57–7.58 (d, $J = 8.3$ Hz, 2H), 7.81–7.83 (d, $J = 7.8$ Hz, 4H), 7.87–7.89 (t, $J = 8.0$ Hz, 2H), 8.10–8.12 (d, $J = 7.8$ Hz, 2H). Anal Calcd. for $C_{28}H_{20}S_2O_8$: C, 61.30; H, 3.67; S, 11.69. Found: C, 61.13; H, 3.79; S, 11.55.

General procedures for the syntheses of compounds (8a–j)

To a solution of 1,4-, 1,5- or 1,8-bis(tosyloxy)anthraquinones **2a–c** (0.035 mmol) in MeCN (4 ml), tuftsin derivatives **3a–7a** (0.14 mmol) and Et_3N (0.04 ml, 0.28 mmol) were added. The solution was heated at reflux for 48 h with stirring under nitrogen. After evaporation of the solvent the reaction mixture was purified using column chromatography and preparative TLC in solvent D or E to obtain compounds **8a–j**.

4-Tosyloxy-9,10-anthraquinone-1-yl-Thr-Lys(Z)-Pro-Arg(NO_2)-OBzl (8a)

^{1}H-NMR (500 MHz, DMSO-d_6) δ: 1.30 (m, 1H, R-γCH_2), 1.33 (d, $J = 6.3$ Hz, 3H, T-CH_3), 1.45 (m, 2H, K-γCH_2), 1.45 (m, 2H, K-δCH_2), 1.57 (m, 1H, R-βCH), 1.60 (m, 1H, R-γCH_2), 1.71 (m, 1H, K-βCH), 1.82 (m, 1H, K-βCH), 1.96 (m, 1H, R-βCH), 1.98 (m, 2H, P-βCH, P-γCH), 2.14 (m, 1H, P-γCH), 2.18 (m, 1H, P-βCH), 2.38 (s, 3H, Tos-CH_3), 2.85 (m, 2H, R-δCH_2), 3.14 (m, 2H, K-εCH_2), 3.60 (m, 1H, P-δCH), 3.92 (m, 1H, P-δCH), 4.05 (m, 1H, T-αCH), 4.12 (m, 1H, P-αCH), 4.39 (m, 1H, T-βCH), 4.69 (m, 1H, R-αCH), 4.70 (m, 1H, K-αCH), 5.07 (bd, 1H, T-OH), 5.10 and 5.19 (s, m, 2H, Z-CH_2), 5.40 (bs, 1H, K-εNH), 6.57 (m, 1H, R-αNH), 6.86 (d, $J = 9.5$ Hz, 1H, AQ3), 7.2–7.4 (m, 10H, Ph), 7.29 (m, 1H, AQ2), 7.30 (d, $J = 8.3$ Hz, 2H, Tos-H3), 7.56 (bs, 1H, R-δNH), 7.67 (t, $J = 7.1$ Hz, 1H, AQ7), 7.72 (t, $J = 7.1$ Hz, 1H, AQ6), 7.86 (d, $J = 7.8$ Hz, 2H, Tos-H2), 7.97 (d, $J = 7.1$ Hz, 1H, AQ8), 8.25 (d, $J = 7.1$ Hz, 1H, AQ5), 8.55 (m, 1H, K-αNH), 10.43 (bs, 1H, T-αNH). Anal. Calcd for

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$C_{57}H_{63}N_9O_{15}S$: C, 59.73; H, 5.54; N, 11.00; S 2.80. Found: C, 59.24; H, 5.46; N, 11.05; S 2.76.

5-Tosyloxy-9,10-anthraquinone-1-yl-Thr-Lys(Z)-Pro-Arg(NO_2)-OBzl (8b)

^{1}H-NMR (500 MHz, DMSO-d_6) δ: 1.28 (m, 1H, R-γCH_2), 1.30 (d, $J = 6.3$ Hz, 3H, T-CH_3), 1.44 (m, 2H, K-γCH_2), 1.43 (m, 2H, K-δCH_2), 1.55 (m, 1H, R-βCH), 1.62 (m, 1H, R-γCH_2), 1.70 (m, 1H, K-βCH), 1.80 (m, 1H, K-βCH), 1.94 (m, 1H, R-βCH), 1.97 (m, 2H, P-βCH, P-γCH), 2.14 (m, 1H, P-γCH), 2.18 (m, 1H, P-βCH), 2.35 (s, 3H, Tos-CH_3), 2.84 (m, 2H, R-δCH_2), 3.12 (m, 2H, K-εCH_2), 3.60 (m, 1H, P-δCH), 3.92 (m, 1H, P-δCH), 4.05 (m, 1H, T-αCH), 4.12 (m, 1H, P-αCH), 4.39 (m, 1H, T-βCH), 4.69 (m, 1H, R-αCH), 4.70 (m, 1H, K-αCH), 5.08 (bd, 1H, T-OH), 5.01 (s, 2H, Z-CH_2), 5.42 (bs, 1H, K-εNH), 6.55 (m, 1H, R-αNH), 7.16 (d, $J = 7.3$ Hz, 1H, AQ4), 7.19–7.20 (m, 10H, Ph), 7.28 (m, 1H, AQ2), 7.30 (d, $J = 8.3$ Hz, 2H, Tos-H3), 7.56 (bs, 1H, R-δNH), 7.60 (d, $J = 9.0$ Hz, 1H, AQ3), 7.83 (t, $J = 8.0$ Hz, 1H, AQ6), 7.84 (d, $J = 7.8$ Hz, 2H, Tos-H2), 7.42 (d, $J = 8.7$ Hz, 1H, AQ8), 7.87 (t, $J = 8$ Hz, 1H, AQ7), 8.53 (m, 1H, K-αNH), 10.40 (bs, 1H, T-αNH).

8-Tosyloxy-9,10-anthraquinone-1-yl-Thr-Lys(Z)-Pro-Arg(NO_2)-OBzl (8c)

^{1}H-NMR (500 MHz, DMSO-d_6) δ: 1.32 (m, 1H, R-γCH_2), 1.34 (d, $J = 6.3$ Hz, 3H, T-CH_3), 1.46 (m, 2H, K-γCH_2), 1.47 (m, 2H, K-δCH_2), 1.57 (m, 1H, R-βCH), 1.59 (m, 1H, R-γCH_2), 1.72 (m, 1H, K-βCH), 1.81 (m, 1H, K-βCH), 1.97 (m, 1H, R-βCH), 1.99 (m, 2H, P-βCH, P-γCH), 2.12 (m, 1H, P-γCH), 2.16 (m, 1H, P-βCH), 2.36 (s, 3H, Tos-CH_3), 2.85 (m, 2H, R-δCH_2), 3.14 (m, 2H, K-εCH_2), 3.60 (m, 1H, P-δCH), 3.92 (m, 1H, P-δCH), 4.05 (m, 1H, T-αCH), 4.12 (m, 1H, P-αCH), 4.39 (m, 1H, T-βCH), 4.69 (m, 1H, R-αCH), 4.70 (m, 1H, K-αCH), 5.0 (s, 2H, Z-CH_2), 5.40 (bs, 1H, K-εNH), 5.08 (bd, 1H, T-OH), 6.57 (m, 1H, R-αNH), 7.30 (m, 10H, Ph), 7.32 (m, 1H, AQ2), 7.32 (d, $J = 8.3$ Hz, 2H, Tos-H3), 7.43 (m, 1H, AQ4), 7.55 (bs, 1H, R-δNH), 7.60 (t, $J = 8.2$ Hz, 1H, AQ3), 7.60 (m, 1H, AQ5), 7.86 (d, $J = 7.8$ Hz, 2H, Tos-H2), 7.88 (t, $J = 7.7$ Hz, 1H, AQ6), 8.10 (d, $J = 7.6$ Hz, 1H, AQ7), 8.46 (m, 1H, K-αNH), 10.12 (bs, 1H, T-αNH). Anal. Calcd for $C_{57}H_{63}N_9O_{15}S$: C, 59.73; H, 5.54; N, 11.00; S 2.80. Found: C, 59.31; H, 5.44; N, 10.95; S 2.83.

4-Tosyloxy-9,10-anthraquinone-1-yl-Thr-Lys(ZAla)-Pro-Arg(NO_2)-OMe (8d)

^{1}H-NMR (500 MHz, DMSO-d_6) δ: 1.19 (d, $J = 6.9$ Hz, 3H, A-CH_3), 1.32 (d, $J = 6.4$ Hz, 3H, T-CH_3), 1.45 (m, 2H, K-γCH_2), 1.46 (m, 2H, K-δCH_2), 1.59 (m, 1H, R-βCH), 1.70 (m, 1H, K-βCH), 1.30 (m, 2H, R-γCH_2), 1.96 (m, 1H, R-βCH), 1.82 (m, 1H, K-βCH), 1.98 (m, 2H, P-βCH, P-γCH), 1.61 (m, 2H, R-γCH_2), 2.14 (m, 1H, P-γCH), 2.18 (m, 1H, P-βCH), 2.36 (s, 3H, Tos-CH_3), 3.14 (m, 2H, K-εCH_2), 2.85 (m, 2H, R-δCH_2), 3.60 (m, 1H, P-δCH), 3.92 (m, 1H, P-δCH), 3.60 (s, 3H, $COOCH_3$), 3.96 (m, 1H, A-αCH), 4.40 (m, 1H, T-βCH), 4.69 (m, 1H, R-αCH), 4.05 (m, 1H, T-αCH), 4.70 (m, 1H, K-αCH), 4.12 (m, 1H, P-αCH), 5.07 (bd, 1H, T-OH), 5.18 (s, m, 2H, Z-CH_2), 7.72 (t, $J = 7.1$ Hz, 1H, AQ6), 5.40 (bs, 1H, K-εNH), 7.29 (m, 1H, AQ2), 6.56 (m, 1H, R-αNH), 6.86 (d, $J = 9.5$ Hz, 1H, AQ3), 7.2–7.39 (m, 5H, Ph), 7.30 (d, $J = 8.3$ Hz, 2H, Tos-H3), 7.34 (m, 1H, A-αNH), 7.97 (d, $J = 7.0$ Hz, 1H, AQ8), 7.67 (t, $J = 7.0$ Hz, 1H, AQ7), 7.86 (d, $J = 7.8$ Hz, 2H, Tos-H2), 8.26 (d, $J = 7.1$ Hz, 1H, AQ5), 8.55 (m, 1H, K-αNH), 7.82 (t, $J = 7.8$ Hz, 1H, AQ6), 7.56 (bs, 1H, R-δNH), 10.40 (bs, 1H, T-αNH). Anal. Calcd for $C_{54}H_{64}N_{10}O_{16}S$: C, 56.83; H, 5.65; N, 12.27; S 2.81. Found: C, 57.86; H, 5.76; N, 12.09; S 2.86.

4-Tosyloxy-9,10-anthraquinone-1-yl-Arg(NO_2)-Pro-Lys(Z)-Thr-OMe (8e)

^{1}H-NMR (500 MHz, DMSO-d_6) δ: 1.05 (d, $J = 6.3$ Hz, 3H, T-CH_3), 1.36 (m, 2H, K-γCH_2), 1.42 (m, 2H, K-δCH_2), 1.76 (m, 1H, R-βCH), 1.55 (m, 1H, K-βCH), 1.49 (m, 2H, R-γCH_2), 1.89 (m, 1H, R-βCH), 1.68 (m, 1H, K-βCH), 1.84 (m, 1H, P-βCH), 1.61 (m, 2H, R-γCH_2), 2.12 (m, 1H, P-βCH), 2.32 (s, 3H, Tos-CH_3), 3.01 (m, 2H, K-εCH_2), 3.18 (m, 2H, R-δCH_2), 3.60 (m, 1H, P-δCH), 3.84 (m, 1H, P-δCH), 3.61 (s, 3H, $COOCH_3$), 4.11 (m, 1H, T-βCH), 4.78 (m, 1H, R-αCH), 4.28 (m, 1H, T-αCH), 4.31 (m, 1H, K-αCH), 4.43 (dd, $J = 4.9$ Hz, $J = 8.1$ Hz, 1H, P-αCH), 5.07 (bd, 1H, T-OH), 4.99 (s, 2H, Z-CH_2), 7.15 (d, $J = 7.3$ Hz, 1H, AQ4), 7.25 (t, $J = 5.5$ Hz, 1H, K-εNH), 7.29 (m, 2H, AQ2, AQ3), 7.32 (m, 5H, Ph), 7.35 (d, $J = 8.3$ Hz, 2H, Tos-H3), 7.64 (d, $J = 8.3$ Hz, 2H, Tos-H2), 7.74 (d, $J = 7.3$ Hz, 1H, AQ8), 7.79 (t, $J = 7.3$ Hz, 1H, AQ7), 7.83 (t, $J = 7.1$ Hz, 1H, AQ6), 8.63 (bs, 1H, R-δNH), 7.95 (d, $J = 8.3$ Hz, 1H, T-αNH), 8.10 (d, $J = 7.3$ Hz, 1H, AQ5), 8.20 (d, $J = 7.7$ Hz, 1H, K-αNH), 10.34 (d, $J = 7.3$ Hz, 1H, R-αNH). Anal. Calcd for $C_{51}H_{59}N_9O_{15}S$: C, 57.24; H, 5.56; N, 11.78; S 3.00. Found: C, 57.03; H, 5.37; N, 11.62; S 2.93.

5-Tosyloxy-9,10-anthraquinone-1-yl-Arg(NO_2)-Pro-Lys(Z)-Thr-OMe (8f)

^{1}H-NMR (500 MHz, DMSO-d_6) δ: 1.04 (d, $J = 6.4$ Hz, 3H, T-CH_3), 1.38 (m, 2H, K-γCH_2), 1.42 (m, 2H, K-δCH_2), 1.76 (m, 1H, R-βCH), 1.52 (m, 1H, K-βCH), 1.49 (m, 2H, R-γCH_2), 1.90 (m, 1H, R-βCH), 1.67 (m, 1H, K-βCH), 1.82 (m, 1H, P-βCH), 1.60 (m, 2H, R-γCH_2), 2.11 (m, 1H, P-βCH), 2.32 (s, 3H, Tos-CH_3), 3.02 (m, 2H, K-εCH_2), 3.15 (m, 2H, R-δCH_2), 3.60 (m, 1H, P-δCH), 3.84 (m, 1H, P-δCH), 3.60 (s, 3H, $COOCH_3$), 4.11 (m, 1H, T-βCH), 4.43 (q, $J = 6.5$ Hz, 1H, R-αCH), 4.28 (m, 1H, T-αCH), 4.33 (m,1H, K-αCH), 4.43 (dd, $J = 4.9$ Hz, $J = 8.3$ Hz, 1H, P-αCH), 5.07 (bd, 1H, T-OH), 4.99 (s, 2H, Z-CH_2), 7.15 (d, $J = 7.3$ Hz, 1H, AQ4), 7.23 (t, $J = 5.1$ Hz, 1H, K-εNH), 7.28 (m, 1H, AQ2), 7.32 (m,

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5H, Ph), 7.39 (d, $J = 8.3$ Hz, 2H, Tos-H3), 7.42 (d, $J = 8.7$ Hz, 1H, AQ8), 7.63 (t, $J = 8.0$ Hz, 1H, AQ3), 7.68 (d, $J = 8.3$ Hz, 2H, Tos-H2), 7.89 (t, $J = 8.0$ Hz, 1H, AQ7), 7.92 (d, $J = 7.3$ Hz, 1H, T-αNH), 8.16 (d, $J = 7.8$ Hz, 1H, K-αNH), 8.23 (d, $J = 7.3$ Hz, 1H, AQ6), 8.60 (bs, 1H, R-δNH), 9.97 (d, $J = 7.3$ Hz, 1H, R-αNH). Anal. Calcd for $C_{51}H_{59}N_9O_{15}S$: C, 57.24; H, 5.56; N, 11.78; S 3.00. Found: C, 57.01; H, 5.40; N, 11.65; S 2.94.

8-Tosyloxy-9,10-anthraquinone-1-yl-Arg(NO_2)-Pro-Lys(Z)-Thr-OMe (8g)

^{1}H-NMR (500 MHz, DMSO-d_6) δ: 1.04 (d, $J = 6.0$ Hz, 3H, T-CH_3), 1.35 (m, 2H, K-γCH_2), 1.41 (m, 2H, K-δCH_2), 1.79 (m, 1H, R-βCH), 1.57 (m, 1H, K-βCH), 1.66 (m, 2H, R-γCH_2), 1.94 (m, 1H, R-βCH), 1.67 (m, 1H, K-βCH), 1.88 (m, 1H, P-βCH), 1.60 (m, 2H, R-γCH_2), 1.92 (m, 2H, P4), 2.14 (m, 1H, P-βCH), 2.31 (s, 3H, Tos-CH_3), 2.97 (m, 2H, K-εCH_2), 3.20 (m, 2H, R-δCH_2), 3.66 (m, 1H, P-δCH), 3.85 (m, 1H, P-δCH), 3.61 (s, 3H, $COOCH_3$), 4.14 (m, 1H, T-βCH), 4.80 (q, $J = 6.8$ Hz, 1H, R-αCH), 4.28 (m, 1H, T-αCH), 4.34 (m, 1H, K-αCH), 4.52 (dd, $J = 4.4$ Hz, $J = 8.4$ Hz, 1H, P-αCH), 5.11 (bd, 1H, T-OH), 5.0 (s, 2H, Z-CH_2), 7.40 (m, 1H, AQ4), 7.22 (t, $J = 5.2$ Hz, 1H, K-εNH), 7.32 (m, 1H, AQ2), 7.33 (m, 5H, Ph), 7.38 (d, $J = 8.0$ Hz, 2H, Tos-H3), 7.62 (dd, $J = 7.5$ Hz, $J = 3.3$ Hz, 1H, AQ5), 7.63 (t, $J = 8.4$ Hz, 1H, AQ3), 7.68 (d, $J = 8.0$ Hz, 2H, Tos-H2), 8.16 (d, $J = 7.7$ Hz, 1H, AQ7), 8.0 (d, $J = 8.4$ Hz, 1H, T-αNH), 8.27 (d, $J = 7.0$ Hz, 1H, K-αNH), 7.89 (t, $J = 7.7$ Hz, 1H, AQ6), 8.62 (bs, 1H, R-δNH), 9.85 (d, $J = 7.3$ Hz, 1H, R-αNH). Anal. Calcd for $C_{51}H_{59}N_9O_{15}S$: C, 57.24; H, 5.56; N, 11.78; S 3.00. Found: C, 57.09; H, 5.48; N, 11.67; S 2.91.

4-Tosyloxy-9,10-anthraquinone-1-yl-Arg(NO_2)-Pro-Lys(ZAla)-Thr-OMe (8h)

^{1}H-NMR (500 MHz, DMSO-d_6) δ: 1.05 (d, $J = 6.3$ Hz, 3H, T-CH_3), 1.18 (d, $J = 7$ Hz, 3H, A-CH_3), 1.36 (m, 2H, K-γCH_2), 1.41 (m, 2H, K-δCH_2), 1.76 (m, 1H, R-βCH), 1.55 (m, 1H, K-βCH), 1.88 (m, 1H, R-βCH), 1.68 (m, 1H, K-βCH), 1.84 (m, 1H, P-βCH), 1.61 (m, 2H, R-γCH_2), 1.90 (m, 2H, P4), 2.11 (m, 1H, P-βCH), 2.24 (s, 3H, Tos-CH_3), 3.07 (m, 2H, K-εCH_2), 3.16 (m, 2H, R-δCH_2), 3.59 (m, 1H, P-δCH), 3.98 (m, 1H, A-αCH), 3.84 (m, 1H, P-δCH), 3.62 (s, 3H, $COOCH_3$), 4.11 (m, 1H, T-βCH), 4.79 (q, $J = 6.2$ Hz, 1H, R-αCH), 4.29 (m, 1H, T-αCH), 4.33 (m, 1H, K-αCH), 4.44 (dd, $J = 4.2$ Hz, $J = 7.5$ Hz, 1H, P-αCH), 5.18 (m, 1H, T-OH), 5.0 (s, 2H, Z-CH_2), 7.75 (d, $J = 7.5$ Hz, 1H, AQ5), 7.36 (m, 1H, A-αNH), 7.88 (t, $J = 5.3$ Hz, 1H, K-εNH), 7.26–7.38 (m, 7H, AQ2, AQ3 and Ph), 7.33 (d, $J = 8.4$ Hz, 2H, Tos-H3), 8.12 (d, $J = 7.5$ Hz, 1H, AQ8), 7.85 (t, $J = 7.5$ Hz, 1H, AQ7), 7.63 (d, $J = 8.4$ Hz, 2H, Tos-H2), 7.80 (t, $J = 7.5$ Hz, 1H, AQ6), 8.02 (d, $J = 8.4$ Hz, 1H, T-αNH), 8.29 (d, $J = 6.8$ Hz, 1H, K-αNH), 8.59 (bs, 1H, R-δNH), 10.31 (d, $J = 7.1$ Hz, 1H, R-αNH). Anal. Calcd for $C_{54}H_{64}N_{10}O_{16}S$: C, 56.83; H, 5.65; N, 12.27; S 2.81. Found: C, 57.04; H, 5.49; N, 11.97; S 2.92.

4-Tosyloxy-9,10-anthraquinone-1-yl-Arg(NO_2)-Pro-Lys(ZVal)-Thr-OMe (8i)

^{1}H-NMR (500 MHz, DMSO-d_6) δ: 0.81 and 0.83 (2d, $J = 8.4$ Hz, 6H. V-CH_3), 1.05 (d, $J = 6.2$ Hz, 3H, T-CH_3), 1.34 and 1.40 (m, 2H, K-γCH_2), 1.42 (m, 2H, K-δCH_2), 1.77 (m, 1H, R-βCH), 1.57 (m, 1H, K-βCH), 1.91 (m, 1H, R-βCH), 1.67 (m, 1H, K-βCH), 1.86 (m, 1H, P-βCH), 1.61 (m, 2H, R-γCH_2), 1.92 (m, 1H, V-βCH), 1.93 (m, 2H, P4), 2.13 (m, 1H, P-βCH), 2.24 (s, 3H, Tos-CH_3), 3.0 and 3.12 (m, 2H, K-εCH_2), 3.15 (m, 2H, R-δCH_2), 3.59 (m, 1H, P-δCH), 3.78 (dd, $J = 7.2$ Hz, $J = 8.9$ Hz, 1H, V-αCH), 3.86 (m, 1H, P-δCH), 3.63 (s, 3H, $COOCH_3$), 4.12 (m, 1H, T-βCH), 4.80 (q, $J = 5.9$ Hz, 1H, R-αCH), 4.28 (m, 1H, T-αCH), 4.33 (m, 1H, K-αCH), 4.43 (dd, $J = 4.8$ Hz, $J = 7.9$ Hz, 1H, P-αCH), 5.06 (bd, 1H, T-OH), 5.03 (s, 2H, Z-CH_2), 7.75 (d, $J = 7.5$ Hz, 1H, AQ5), 7.25 (d, $J = 8.9$ Hz, 1H, V-αNH), 7.98 (t, $J = 5.3$ Hz, 1H, K-εNH), 7.34 (m, 2H, AQ2, AQ3), 7.31 (m, 5H, Ph), 7.33 (d, $J = 8.4$ Hz, 2H, Tos-H3), 8.11 (d, $J = 7.5$ Hz, 1H, AQ8), 7.84 (t, $J = 7.5$ Hz, 1H, AQ7), 7.62 (d, $J = 8.4$ Hz, 2H, Tos-H2), 7.80 (t, $J = 7.5$ Hz, 1H, AQ6), 7.94 (d, $J = 8.4$ Hz, 1H, T-αNH), 8.22 (d, $J = 7.7$ Hz, 1H, K-αNH), 8.64 (bs, 1H, R-δNH), 10.36 (d, $J = 7.5$ Hz, 1H, R-αNH). Anal. Calcd for $C_{56}H_{68}N_{10}O_{16}S$: C, 57.52; H, 5.86; N, 11.98; S 2.74. Found: C, 57.51; H, 5.79; N, 11.83; S 2.79.

5-Tosyloxy-9,10-anthraquinone-1-yl-Arg(NO_2)-Pro-Lys(ZVal)-Thr-OMe (8j)

^{1}H-NMR (500 MHz, DMSO-d_6) δ: 0.80 and 0.82 (2d, $J = 8.2$ Hz, 6H, V-CH_3), 1.02 (d, $J = 6.2$ Hz, 3H, T-CH_3), 1.32 and 1.38 (m, 2H, K-γCH_2), 1.40 (m, 2H, K-δCH_2), 1.76 (m, 1H, R-βCH), 1.56 (m, 1H, K-βCH), 1.90 (m, 1H, R-βCH), 1.68 (m, 1H, K-βCH), 1.86 (m, 1H, P-βCH), 1.60 (m, 2H, R-γCH_2), 1.92 (m, 1H, V-βCH), 2.13 (m, 1H, P-βCH), 2.26 (s, 3H, Tos-CH_3), 3.0 and 3.12 (m, 2H, K-εCH_2), 3.16 (m, 2H, R-δCH_2), 3.60 (m, 1H, P-δCH), 3.78 (dd, $J = 7.2$ Hz, $J = 8.9$ Hz, 1H, V-αCH), 3.86 (m, 1H, P-δCH), 3.64 (s, 3H, $COOCH_3$), 4.12 (m, 1H, T-βCH), 4.80 (q, $J = 6.0$ Hz, 1H, R-αCH), 4.26 (m, 1H, T-αCH), 4.32 (m, 1H, K-αCH), 4.44 (dd, $J = 4.8$ Hz, $J = 7.8$ Hz, 1H, P-αCH), 5.06 (bd, 1H, T-OH), 5.03 (s, 2H, Z-CH_2), 7.16 (d, $J = 7.2$ Hz, 1H, AQ4), 7.24 (d, $J = 8.8$ Hz, 1H, V-αNH), 7.30 (m, 5H, Ph), 7.27 (m, 1H, AQ2), 7.33 (d, $J = 8.4$ Hz, 2H, Tos-H3), 7.40 (d, $J = 8.6$ Hz, 1H, AQ8), 7.62 (m, 1H, AQ3), 7.84 (t, $J = 8.0$ Hz, 1H, AQ7), 7.63 (d, $J = 8.2$ Hz, 2H, Tos-H2), 7.96 (d, $J = 8.2$ Hz, 1H, T-αNH), 7.98 (t, $J = 5.3$ Hz, 1H, K-εNH), 8.18 (d, $J = 7.7$ Hz, 1H, K-αNH), 8.61 (bs, 1H, R-δNH), 8.24 (t, $J = 7.3$ Hz, 1H, AQ6), 10.02 (d, $J = 7.3$ Hz, 1H, R-αNH). Anal. Calcd for $C_{56}H_{68}N_{10}O_{16}S$:

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C, 57.52; H, 5.86; N, 11.98; S 2.74. Found: C, 57.49; H, 5.78; N, 11.87; S 2.81.

After completion of the synthesis, the protected compounds **8a–j** were treated with 5 ml of liquid hydrogen fluoride (HF) containing 1 ml of anisole at −70 °C and stirred for 60 min at 0 °C. After removal of HF and anisole *in vacuo*, the mixture was diluted with acetic acid. The solvent was evaporated under reduced pressure, and the residue was dissolved in water and lyophilized. New compounds **9a–j** gave satisfactory MS and ^{1}H-NMR. We present below the ^{1}H-NMR spectra of the two compounds **9a** and **9e**.

4-Hydroxy-9,10-anthraquinone-1-yl-Thr-Lys-Pro-Arg-OH (9a)

^{1}H-NMR (500 MHz, DMSO-d_6) δ: 1.15 (d, $J = 6.1$ Hz, 3H, T-CH_3), 1.35 (m, 2H, K-γCH_2), 1.52 (m, 2H, K-δCH_2), 1.53 (m, 1H, R-γCH_2), 1.54 (m, 1H, K-βCH), 1.60 (m, 1H, R-βCH), 1.66 (m, 1H, K-βCH), 1.74 (m, 1H, R-γCH_2), 1.74 (m, 1H, R-βCH), 1.83 (m, 1H, P-βCH), 1.88 (m, 2H, P-γCH_2), 2.07 (m, 1H, P-βCH), 2.72 (m, 2H, K-εCH_2), 3.10 (m, 2H, R-δCH_2), 3.53 (m, 1H, P-δCH), 3.66 (m, 1H, P-δCH), 4.02 (m, 1H, T-βCH), 4.12 (m, 1H, R-αCH), 4.31 (t, $J = 6.4$ Hz, 1H, T-αCH), 4.36 (m, 1H, P-αCH), 4.53 (m, 1H, K-αCH), 7.39 (s, 2H, AQ2, AQ3), 7.56 (t, $J = 5.4$ Hz, 1H, R-δNH), 7.67 (bs, 2H, K-εNH_2), 7.88 (t, $J = 7.5$ Hz, 1H, AQ6), 7.95 (t, $J = 7.5$ Hz, 1H, AQ7), 8.18 (d, $J = 7.6$ Hz, 1H, R-αNH), 8.28 (d, $J = 7.5$ Hz, 2H, AQ5, AQ8), 8.28 (m, 1H, K-αNH), 10.67 (d, $J = 7.9$ Hz, 1H, T-αNH), 13.65 (s, 1H, AQ OH). MS $[M + H]^+$ calcd 723.35, found 723.2.

5-Hydroxy-9,10-anthraquinone-1-yl-Thr-Lys-Pro-Arg-OH (9b)

MS $[M + H]^+$ calcd 723.35, found 723.3.

8-Hydroxy-9,10-anthraquinone-1-yl-Thr-Lys-Pro-Arg-OH (9c)

MS $[M + H]^+$ calcd 723.35, found 723.2.

4-Hydroxy-9,10-anthraquinone-1-yl-Thr-Lys(Ala)-Pro-Arg-OMe (9d)

MS $[M + H]^+$ calcd 808.4, found 808.3.

4-Hydroxy-9,10-anthraquinone-1-yl-Arg-Pro-Lys-Thr-OMe (9e)

^{1}H-NMR (500 MHz, DMSO-d_6) δ: 1.04 (d, $J = 6.3$ Hz, 3H, T-CH_3), 1.41 (m, 2H, K-γCH_2), 1.57 (m, 2H, K-δCH_2), 1.54 (m, 1H, K-βCH), 1.60 (m, 2H, R-γCH_2), 1.61 (m, 1H, R-γCH_2), 1.72 (m, 1H, K-βCH), 1.79 (m, 1H, R-βCH), 1.82 (m, 1H, P-βCH), 1.88 (m, 1H, R-βCH), 1.91 (m, 2H, P-γCH), 2.12 (m, 1H, P-βCH), 2.81 (m, 2H, K-εCH_2), 3.15 (m, 2H, R-δCH_2), 3.58 (m, 1H, P-δCH), 3.64 (s, 3H, $COOCH_3$), 3.85 (m, 1H, P-δCH), 4.11 (dq, $J = 2.9$ Hz, $J = 6.3$ Hz, 1H, T-βCH), 4.29 (dd, $J = 2.9$ Hz, $J = 8.4$ Hz, 1H, T-αCH), 4.38 (m, 1H, K-αCH), 4.42 (dd, $J = 5.6$ Hz, $J = 8.0$ Hz, 1H, P-αCH), 4.81 (q, $J = 7.1$ Hz, 1H, R-αCH), 5.07 (bd, 1H, T-OH), 7.33 (d, $J = 7.6$ Hz, 1H, AQ3), 7.63 (d, $J = 7.6$ Hz, 1H, AQ2), 7.71 (bs, 1H, K-εNH), 7.87 (t, $J = 7.7$ Hz, 1H, AQ6), 7.91 (d, $J = 8.3$ Hz, 1H, T-αNH), 7.94 (t, $J = 7.7$ Hz, 1H, AQ7), 8.24 (d, $J = 8.0$ Hz, 1H, K-αNH), 8.27 (d, $J = 7.7$ Hz, 1H, AQ5), 8.30 (d, $J = 7.7$ Hz, 1H, AQ8), 8.64 (bs, 1H, R-δNH), 10.35 (d, $J = 7.1$ Hz, 1H, R-αNH), 13.57 (s, 1H, AQ OH). MS $[M + H]^+$ calcd 737.35, found 737.4.

5-Hydroxy-9,10-anthraquinone-1-yl-Arg-Pro-Lys-Thr-OMe (9f)

MS $[M + H]^+$ calcd 737.35, found 737.3.

8-Hydroxy-9,10-anthraquinone-1-yl-Arg-Pro-Lys-Thr-OMe (9g)

MS $[M + H]^+$ calcd 737.35, found 737.2.

4-Hydroxy-9,10-anthraquinone-1-yl-Arg-Pro-Lys(Ala)-Thr-OMe (9h)

MS $[M + H]^+$ calcd 808.4, found 808.3.

4-Hydroxy-9,10-anthraquinone-1-yl-Arg-Pro-Lys(Val)-Thr-OMe (9i)

MS $[M + H]^+$ calcd 836.43, found 836.3.

5-Hydroxy-9,10-anthraquinone-1-yl-Arg-Pro-Lys(Val)-Thr-OMe (9j)

MS $[M + H]^+$ calcd 836.43, found 836.4.

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