B

Anticancer Agents, 15[1]

Squaric Acid Diethyl Ester: A New Coupling Reagent for the Formation of Drug Biopolymer Conjugates. Synthesis of Squaric Acid Ester Amides and Diamides

Lutz F. Tietze*[a], Michael Arlt[a], Matthias Beller[a], Karl-Heinz Glüsenkamp[b], Eckhard Jähde[b], and Manfred F. Rajewsky[b]

Institut für Organische Chemie der Universität Göttingen[a], Tammannstraße 2, W-3400 Göttingen (F.R.G.)

Institut für Zellbiologie (Tumorforschung), Universität Essen[b], Hufelandstraße 55, W-4300 Essen 1 (F.R.G.)

Received October 26, 1990

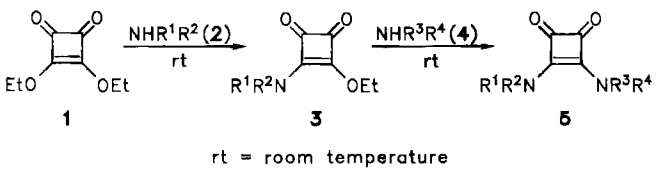
Key Words: Squaric acid, ester amides of, diamides of / Coupling reagents / Biopolymers / Anticancer agents / Conjugates

Reaction of squaric acid diethyl ester (**1**) with a slight excess of a primary or secondary amine **2** in ethanol, dichloromethane or aqueous buffer (pH 7) at 20°C for 0.3–12 h gives the squaric acid amide esters **3** in mostly excellent yields. Treatment of **3** with amines **2** or **4** in organic solvents in the presence of triethylamine or in aqueous buffer (pH 9) leads to the corresponding symmetrical and unsymmetrical squaric acid diamides **5**, respectively. The reaction can be followed by UV spectroscopy.

There is a great need for the development of new mild coupling procedures to immobilize enzymes or to bind pharmacologically active molecules to polymers[2]. Thus, the use of biopolymer drug conjugates is a promising approach to the treatment and diagnosis of many deseases[3]. Conjugates of radioactive compounds or dyes with monoclonal antibodies which bind with some specifity to tumor-associated surface antigens are successfully used in the diagnosis of cancer[4]; indeed, a number of monoclonal antibodies directed against cell surface antigens on human tumors such as breast and colon carcinomas, melanomas and gliomas have already been prepared using the hybridoma technique.

The advantage of applying conjugates lies in the selective delivery to the target site and also in the possible protection of drugs against fast enzymatic degration and excretion, thereby leading to a higher drug concentration in the tumor. However, treatment of cancer with monoclonal antibody-drug conjugates has not been successful so far, in part due to the low accessibility of malignant cells in the interior parts of tumors; thus, the drug antibody complex is cytotoxic only to those cells that bind the antibody. In our concept for the development of new anticancer drugs with increased tumor selectivity and lower general toxicity we also use monoclonal antibodies for targeting, but in contrast to known approaches our procedure aims at the liberation of the anticancer drug from the monoclonal antibody within the tumor tissue via tumor-selective, proton-mediated activation of a prodrug[5].

Several methods have been developed for the coupling of small molecules to proteins and other biopolymers[3,6]. Many of these procedures require harsh conditions, which may cause a loss of the affinity and specificity of the monoclonal antibody employed. Moreover, most of these methods do not allow an effective determination of the coupling rate. Finally, within the development of our more selective anticancer agents slightly basic conditions are required for coupling because of the acid lability of these compounds.



In this paper we describe the coupling of two amines using squaric acid diethyl ester[7] by sequential formation of the squaric acid amide ester and squaric acid diamides. The reaction can be performed in organic solvents and in water with primary and secondary amines under mild conditions.

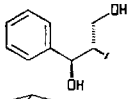
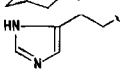
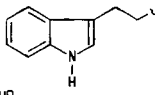
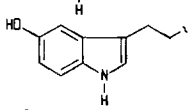
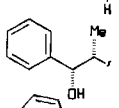
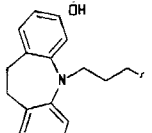
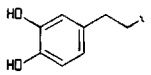
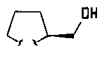
Treatment of squaric acid diethyl ester (**1**) with a slight excess of a primary or a secondary amine **2** in ethanol or dichloromethane at room temperature for 0.3–12 h gave the corresponding squaric acid amide esters **3** in mostly excellent yield (Table 1). Alcoholic and phenolic hydroxy groups did not interfere in this process. The reaction could also be performed in a buffered aqueous solution (pH 7) which was especially appropriate for biopolymers; the yields may be slightly lower because of difficulties arising in the workup.

It is most important that under the conditions mentioned above only the monoamides **3**, but not the diamides **5**, are formed. Under more basic conditions, however, applying either a large excess of the amine **2** or by adding triethylamine, we obtained the symmetrical diamides **5** ($R^1 = R^3$, $R^2 = R^4$). The synthesis of some symmetrical squaric acid diamides in a one-pot procedure has already been described[8]. The preferred formation of the monoamides **3** using only a slight excess of the amine **2** (<2 mol) is due to the much higher rate of the reaction of **1** and **2** as compared to that of **3** and **2**[9].

  0009–2940/91/0505–1215 $ 3.50+.25/0

When squaric acid ester is used as a coupling agent, the synthesis of unsymmetrical squaric acid diamides **5** (R^1, $R^2 \neq R^3$, R^4) must be feasible. This could easily be achieved by reaction of the squaric acid amide esters **3** in ethanol with a slight excess of a second amine **4** in the presence of triethylamine at room temperature (Table 2). Again, the reaction can also be performed in an aqueous buffer (pH 9). Of course, also the symmetrical diamides **5** ($R^1 = R^3$, $R^2 = R^4$) may be synthesized by this method by using the corresponding amine **2** instead of **4**. The whole sequence can also be run without isolation of the squaric acid amide ester **3**.

Table 1. Synthesis of 4-amino-3-ethoxy-3-cyclobutene-1,2-diones **3** from **1** and amines **2**

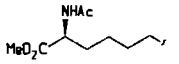
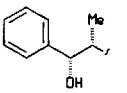
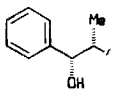
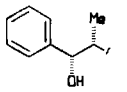
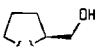
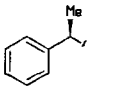
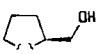
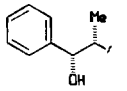
Substrates	R^1	R^2	Products	Yield (%)
1 + **2a**	$HOCH_2CH_2$-	H	**3a**	90(69)[a]
1 + **2b**	$HOCH_2CH_2CH_2$-	H	**3b**	84
1 + **2c**	$HOCH_2C(CH_3)_2$	H	**3c**	79
1 + **2d**	(2R)-$HOCH_2CHC_2H_5$	H	**3d**	92
1 + **2e**	p-HO-$C_6H_4CH_2CH_2$-	H	**3e**	87
1 + **2f**		H	**3f**	76
1 + **2g**		H	**3g**	76
1 + **2h**		H	**3h**	87
1 + **2i**		H	**3i**	74
1 + **2j**		H	**3j**	48
1 + **2k**		CH_3	**3k**	97
1 + **2l**		CH_3	**3l**	100
1 + **2m**		H	**3m**	69
1 + **2n**			**3n**	85

[a] Yield in aqueous buffer, pH = 7.

Purification of the vinylogous amide esters **3** and especially of the vinylogous diamides **5** is sometimes difficult, since these compounds are often poorly soluble in organic solvents. However, all products described here were purified by either column chromatography or crystallization. It must be mentioned, that caution has to be taken during the preparation of some of the amide esters **3** and the diamides **5** as contact of squaric acid esters with the skin may cause allergic reactions[11].

The structures of **3a**–**n** and **5a**–**f** were determined by ^{1}H- and by ^{13}C-NMR spectroscopy. The signals for the CH_2 and CH_3 groups of the vinylogous ethyl esters in **3a**–**m** are typically found at $\delta = 4.2-4.8$ and $\delta = 1.1-1.5$, respectively. In the ^{13}C-NMR spectra the corresponding signals appear at $\delta = 69-70$ and 15–16. The carbon atoms of the cyclobutene moiety resonate at $\delta = 170$ and 190. A characteristic feature in the NMR spectra of the squaric acid amide esters **3** is the splitting of some signals. This is due to the double bond nature of the vinylogous amide group.

Table 2. Synthesis of 3,4-diamino-3-cyclobutene-1,2-diones **5** from **1** or **3** and amines **2** or **4**

Substrates	R^1	R^2	R^3	R^4	Products	Yield (%)
3b + **2b**	$HO[CH_2]_3$-	H	$HO[CH_2]_3$-	H	**5a**	77
3b + **4p**	$HO[CH_2]_3$-	H		H	**5b**	78
1 + **2k**		CH_3		CH_3	**5c**	81
3b + **2k**	$HO[CH_2]_3$-	H		CH_3	**5d**	49
3n + **2o**				H	**5e**	80
3n + **2k**				CH_3	**5f**	81

Based on the NMR experiments the coalescence temperature was determined as 80 ± 5°C, and the free energy of activation for the rotation about the C–N bond was calculated as 75 kJ/mol[10d]. This is in good agreement with the results obtained for other vinylogous amide esters[8b]. In the squaric acid diamides **5a**–**d** the signals for the carbons of the cyclobutene moiety appear at $\delta = 167-183$, i.e. at slightly higher field than those of **3**. The UV spectra of squaric acid amide esters **3a**–**h** show two maxima of absorption at $\lambda_{max} = 260$ and 271–283 nm ($\lg\varepsilon = 4.34-4.45$), whereas for the squaric acid diamides **5** only one maximum is observed, which, due to the stronger π conjugation, appears at longer wave length ($\lambda_{max} = 289-300$ nm, $\lg\varepsilon = 3.9-4.5$). Since the UV spectra allow a clear differentiation between the squaric acid derivatives **1**, **3** and **5**, the kinetics of the reaction **1** → **3** → **5** can easily be followed by UV spectroscopy. In addition, the magnitude of the coupling at the biopolymer may be determined by this spectroscopic method.

We thank the *Bundesminister für Forschung und Technologie* (Förderkennzeichen 03189-5219) and the *Fonds der Chemischen Industrie* for their generous support.

Experimental

^1H and ^{13}C NMR: Varian FT-80 A, XL 200, VXR 200, VXR 500 (internal TMS); multiplicities were determined with the APT pulse sequence. – MS: Varian MAT 311 A; high resolution: Varian MAT 731. – Optical rotation: Perkin-Elmer polarimeter 241; all measurements were done by using the Na-D line. – IR: Bruker IFS 25. – UV: Varian Cary 219, Perkin-Elmer Lambda 2, Perkin-Elmer Lambda 9. – Melting points: Mettler FP 61 (uncorrected values). – Elemental analyses were carried out at the analytical laboratory of Göttingen University. – The progress of all reactions was monitored by TLC (Macherey-Nagel & Co., DC-Fertigfolien SIL G/UV_{254}, 0.25 mm). Flash chromatography and column chromatography were performed with Kieselgel of Woelm Pharma (0.032–0.063 mm). – Solvents used for TLC and flash chromatography: A, *tert*-butyl methyl ether/hexane (1:1); B, *tert*-butyl methyl ether/hexane (3:1); C, dichloromethane/petroleum ether/ethanol (10:1:1); D, dichloromethane/petroleum ether/ethanol (3:1:1); E, chloroform/methanol/hexane (6:2:1); F, dichloromethane/petroleum ether/ethanol (8:1:1).

Synthesis of Squaric Acid Ester Amides and Diamides

General Procedure I. – Synthesis of 4-Amino-3-ethoxy-3-cyclobutene-1,2-diones (Squaric Acid Ester Amides) (**3**) *from 3,4-Diethoxy-3-cyclobutene-1,2-dione (Squaric Acid Diethyl Ester)* (**1**) *and Amines* **2**: To a solution of **2** (3.20 mmol) in 10 ml of ethanol was added **1** (500 mg, 2.94 mmol) at room temperature with stirring, and stirring was continued until the reaction was completed (0.3–12 h, TLC, silica gel, solvent B). For workup the solvent was removed either by evaporation or, if the product precipitated, by filtration. Further purification was achieved by either crystallization or column chromatography on silica gel.

General Procedure II. – Synthesis of **3** *from* **1** *and Ammonium Salts of* **2**: To a solution of **2** (3.20 mmol) and one equivalent of triethylamine in 10 ml of ethanol was added **1** (500 mg, 2.94 mmol) at room temperature with stirring. Stirring was continued until the reaction was completed. Workup as in general procedure I.

General Procedure III. – Synthesis of **3** *from* **1** *in Buffer Solution:* To a solution of **2** (3.20 mmol) in 10 ml of buffer (pH 7) was added **1** (500 mg, 2.94 mmol) with stirring at room temperature and occasional control of the pH. Workup as in general procedure I.

General Procedure IV. – Synthesis of Squaric Acid Diamides (**5**) *from* **3** *and Amines* **2** *or* **4**: A solution or suspension of **3** (2.00 mmol), **4** (2.40 mmol) and triethylamine (1.39 ml, 10.0 mmol) in 10 ml of ethanol was stirred at room temperature until the reaction was completed (TLC, silica gel, solvent C). The solvent was evaporated in vacuo and the crude product was purified by either column chromatography or crystallization.

General Procedure V. – Synthesis of Squaric Acid Diamides (**5**) *from* **3** *and Amines* **2** *or* **4** *in Buffer Solution:* To a solution or suspension of **3** (2.00 mmol) in 10 ml of borax buffer solution (pH 9) was added 2.40 mmol of **4** at room temperature with stirring. Workup as in general procedure IV.

General Procedure VI. – Synthesis of Squaric Acid Diamides (**5**) *from* **1** *and Amines* **2**: To a solution of **2** (6.2 mmol) and triethylamine (2 ml, 14.4 mmol) in 10 ml of ethanol was added **1** (500 mg, 2.94 mmol) at room temperature with stirring. Workup as in general procedure IV.

3-Ethoxy-4-[(2-hydroxyethyl)amino]-3-cyclobutene-1,2-dione (**3a**). – A: Reaction of 2-aminoethanol (**2a**) (195 mg, 3.20 mmol) with **1** (500 mg, 2.94 mmol) for 2 h, general procedure I; purification was achieved by flash chromatography (60 g silica gel, solvent C) to afford 490 mg (90%) of **3a** as colorless cubes.

B: Reaction of **2a** (195 mg, 3.20 mmol) with **1** (500 mg, 2.94 mmol) for 3 h, general procedure III; workup as in A. – R_f = 0.33 (solvent C). – M.p. 112°C (ethanol/dichloromethane/petroleum ether). – UV (methanol): λ_{max} (lg ε) = 257 nm (4.406), 271 (4.418). – IR (KBr): $\tilde{\nu}$ = 3470 cm^{-1} (OH), 3260 (NH), 2990, 2945 (CH_3, CH_2), 1817, 1708, 1690, 1630 (C=O), 1583, 1533 (C=C, NH), 1045 (C–O). – ^1H NMR ($CDCl_3$): δ = 1.46 (t, *J* = 7 Hz, 3H, CH_3), 1.90–3.00 (m, br., 1H, OH), 3.48–3.90 (m, 4H, CH_2N, CH_2OH), 4.77 (q, *J* = 7 Hz, 2H, CH_2O), 6.95–7.36 (m, 1H, NH). – ^{13}C NMR ($CDCl_3$): δ = 15.68 (CH_3), 46.36, 46.43, 46.70 (CH_2N), 60.36, 60.64 (CH_2OH), 68.86 (CH_2O), 172.9, 173.2, 176.6, 176.8, 176.9, 182.3, 189.3 (4 cyclobutene C). – MS (70 eV): *m/z* (%) = 185 (38) $[M]^+$, 129 (22) $[M - 2\,CO]^+$, 102 (8) $[M - 2\,CO - HCN]^+$, 73 (19), 45 (100) $[CH_3CHO]^+$.

$C_8H_{11}NO_4$ (185.2)
Calcd. C 51.89 H 5.99 N 7.56
Found C 51.99 H 6.08 N 7.62

3-Ethoxy-4-[(3-hydroxypropyl)amino]-3-cyclobutene-1,2-dione (**3b**): Reaction of 3-amino-1-propanol (**2b**) (240 mg, 3.20 mmol) with **1** (500 mg, 2.94 mmol) for 2 h, general procedure I; the crude product was purified by flash chromatography (60 g of silica gel, solvent C) to afford 492 mg (84%) of **3b** as colorless needles. – R_f = 0.28 (solvent C). – M.p. 101°C (dichloromethane/petroleum ether). – UV (methanol): λ_{max} (lg ε) = 259 nm (4.445), 271 (4.446). – IR (KBr): $\tilde{\nu}$ = 3416 cm^{-1} (OH), 3260 (NH), 2990, 2950 (CH_3, CH_2), 1808, 1690, 1620, 1562 (C=O, C=C), 1536 (NH), 1090, 1056, 1006 (C–O). – ^1H NMR ([D_6]DMSO): δ = 1.34 (t, *J* = 7 Hz, 3H, CH_3), 1.63 (quint, *J* = 6 Hz, 2H, CH_2), 3.10–3.63 (m, 4H, CH_2N, CH_2OH), 4.44 (t, *J* = 5 Hz, 1H, OH), 4.61 (q, *J* = 7 Hz, 2H, CH_2O), 8.30–8.76 (m, 1H, NH). – ^{13}C NMR ($CDCl_3$): δ = 15.85 (CH_3), 32.50, 32.89, 33.03 (CH_2), 42.29 (CH_2N), 59.66 (CH_2OH), 69.81 (CH_2O), 172.7, 172.8, 172.9, 177.3, 182.8, 189.3, 189.5 (4 cyclobutene C). – MS (70 eV): *m/z* (%) = 199 (96) $[M]^+$, 143 (41) $[M - 2\,CO]^+$, 126 (6) $[M - 2\,CO - HCN]^+$, 59 (70) $[CH_2CH_2CH_2OH]^+$, 43 (100). – MS (high resolution): Calcd. 199.0844, found 199.0844.

$C_9H_{13}NO_4$ (199.2)
Calcd. C 54.27 H 6.58 N 7.03
Found C 54.37 H 6.58 N 7.17

3-Ethoxy-4-[(2-hydroxy-1,1-dimethylethyl)amino]-3-cyclobutene-1,2-dione (**3c**): Reaction of 2-amino-2-methyl-1-propanol (**2c**) (285 mg, 3.20 mmol) with **1** (500 mg, 2.94 mmol) for 2 h, general procedure I; the solvent was removed in vacuo and the crude product was crystallized to give 495 mg (79%) of **3c** as slightly yellow crystals. – R_f = 0.43 (solvent C). – M.p. 63°C (dichloromethane/*tert*-butyl methyl ether/petroleum ether). – UV (methanol): λ_{max} (lg ε) = 259 nm (4.401), 272 (4.425). – IR (KBr): $\tilde{\nu}$ = 3450 cm^{-1} (OH), 3290, 3214 (NH), 2980, 2938 (CH_3, CH_2), 1800, 1712, 1700, 1608 (C=O, C=C), 1530, 1500 (NH), 1058 (C–O). – ^1H NMR ([D_6]DMSO): δ = 1.25 (s, 6H, CH_3), 1.39 (t, *J* = 7 Hz, 3H, CH_3CH_2), 3.41 (d, *J* = 6 Hz, 1H, CH_2OH), 4.73 (q, *J* = 7 Hz, 2H, CH_2O), 4.89 (t, *J* = 6 Hz, 1H, OH), 8.35 (s, br., 1H, NH). – ^{13}C NMR ([D_6]DMSO, 100°C): δ = 15.55 (CH_3), 24.88 (CH_3CH_2), 56.86 (CN), 68.82 (CH_2O), 172.9, 176.8, 182.6, 188.9 (4 cyclobutene C). – MS (70 eV): *m/z* (%) = 213 (66) $[M]^+$, 182 (100) $[M - CH_3O]^+$, 157 (6) $[M - 2\,CO]^+$, 154 (21), 127 (3) $[M - 2\,CO - HCN]^+$, 126 (24), 98 (34), 73 (65) $[(CH_3)_2CCH_2OH]^+$, 55 (45).

$C_{10}H_{15}NO_4$ (213.2)
Calcd. C 56.33 H 7.09 N 6.57
Found C 56.52 H 7.19 N 6.52

3-Ethoxy-4-[(1R)-(1-ethyl-2-hydroxyethyl)amino]-3-cyclobutene-1,2-dione (**3d**): Reaction of (*R*)-2-amino-1-butanol (**2d**) (285 mg, 3.20 mmol) with **1** (500 mg, 2.94 mmol) for 2 h, general procedure I; the solvent was removed in vacuo and the residue purified by column chromatography (60 g silica gel, solvent C) to afford 577 mg (92%) of **3d** as colorless fine crystals. – R_f = 0.69 (solvent D). – $[\alpha]_D^{20}$ = +58.6 (*c* = 1 in chloroform). – M.p. 173°C (dichloromethane/petrolcum ether). – UV (methanol): λ_{max} (lgε) = 257 nm (4.393), 272 (4.388). – IR (film): $\tilde{\nu}$ = 3390 cm^{-1}, 3250 (NH, OH), 2970, 2930 (CH_3, CH_2), 1805, 1700 (C=O), 1600 (C=C), 1530 (NH), 1050 (C–O). – ^1H NMR ([D$_6$]DMSO): δ = 0.86 (t, *J* = 7 Hz, 3H, CH_3), 1.05–1.75 (m, 2H, CH_2), 1.39 (t, *J* = 7 Hz, 3H, CH_3), 3.44 (s, br., 2H, CH_2OH), 3.60–4.85 (m, 2H, CH, OH), 4.67 (q, *J* = 7 Hz, 2H, CH_2O), 8.22–8.73 (m, 1H, NH). – ^{13}C NMR ($CDCl_3$): δ = 10.36 (CH_3), 15.81 (CH_3CH_2O), 24.72 (CH_2), 59.41 (CH), 64.18, 64.48 (CH_2OH), 69.75 (CH_2O), 173.2, 173.4, 176.6, 177.0, 183.0, 188.9, 189.4 (4 cyclobutene C). – MS (70 eV): *m/z* (%) = 213 (38) $[M]^+$, 182 (12) $[M - CH_3O]^+$, 157 (14) $[M - 2\,CO]^+$, 98 (16), 70 (60), 55 (100).

$C_{10}H_{15}NO_4$ (213.2)
Calcd. C 56.33 H 7.09 N 6.57
Found C 56.15 H 7.07 N 6.50

3-Ethoxy-4-[2-(4-hydroxyphenyl)ethylamino]-3-cyclobutene-1,2-dione (**3e**): Reaction of tyramine (**2e**) (464 mg, 3.38 mmol) with **1** (0.50 ml, 3.38 mmol) for 12 h, general procedure I; yield after chromatography (70 g, silica gel, ethanol/dichloromethane/petroleum ether, 10:3:1) and crystallization 768 mg (87%) of **3e** as colorless crystals. – R_f = 0.49 (solvent C). – M.p. 162°C (ethanol/dichloromethane/petroleum ether). – UV (methanol): λ_{max} (lgε) = 225 nm (4.021), 260 (4.414), 274 (4.435). – IR (KBr): $\tilde{\nu}$ = 3250 cm^{-1} (OH, NH), 3058 (Ph-H), 2992, 2964, 2946 (CH_3, CH_2), 1812, 1688, 1604, 1586 (C=O, C=C), 1074, 1026 (C–O), 836 (Ph-H). – ^1H NMR ([D$_6$]DMSO): δ = 1.36 (t, *J* = 7 Hz, 3H, CH_3), 2.73 (t, *J* = 5.5 Hz, $PhCH_2$), 3.36–3.76 (m, 2H, CH_2N), 4.52–4.74 (m, 2H, CH_2O), 6.88 (2 d, AA′BB′ system, δ_A = 6.73, δ_B = 7.03, 4H, Ph-H). – ^{13}C NMR ($CDCl_3$): δ = 15.56 (CH_3), 35.53, 35.89 (Ph-CH_2), 45.19, 45.39, 45.43, 45.62 (CH_2N), 68.71 (CH_2O), 115.2 (*m*-Ph C), 128.3 (*p*-Ph C), 129.7 (*o*-Ph C), 155.9 (*i*-Ph C), 172.4, 172.5, 172.6, 176.5, 176.8, 182.1, 189.2 (4 cyclobutene C). – MS (70 eV): *m/z* (%) = 261 (37) $[M]^+$, 232 (9) $[M - C_2H_5]^+$, 205 (9) $[M - 2\,CO]^+$, 204 (24), 148 (72), 120 (100) $[C_8H_8O]^+$, 107 (46) $[C_7H_7O]^+$, 77 (52) [phenyl]$^+$. – MS (high resolution): Calcd. 261.1001, found 261.1001.

$C_{14}H_{15}NO_4$ (261.28)
Calcd. C 64.36 H 5.79 N 5.36
Found C 64.49 H 5.86 N 5.44

3-Ethoxy-4-[(1S,2S)-(2-hydroxy-1-hydroxymethyl-2-phenylethyl)amino]-3-cyclobutene-1,2-dione (**3f**): Reaction of (1*S*,2*S*)-2-amino-1-phenyl-1,3-propanediol (**2f**) (535 mg, 3.20 mmol) with **1** (500 mg, 2.94 mmol) for 2 h, general procedure I. The solvent was removed in vacuo and the residue purified by column chromatography (75 g of silica gel, solvent C) and crystallization to afford 651 mg (76%) of **3f** as colorless crystals. – R_f = 0.30 (solvent C). – $[\alpha]_D^{20}$ = +14.5 (*c* = 1 in chloroform). – M.p. 53°C (dichloromethane/petroleum ether). – UV (methanol): λ_{max} (lgε) = 259 nm (4.338), 272 (4.354). – IR (KBr): $\tilde{\nu}$ = 3382 cm^{-1} (NH, OH), 3088, 3060 (Ph-H), 2984, 2940 (CH_3, CH_2), 1808, 1696, 1598 (C=O, C=C), 1528 (NH), 1090, 1050 (C–O), 736, 702 (Ph-H). – ^1H NMR ($CDCl_3$): δ = 1.31 (t, *J* = 7 Hz, 3H, CH_3), 3.90 (s, br., 3H, CH_2CH, $CHCH_2$), 4.21 (s, br., 1H, OH), 4.32–4.70 (m, 3H, CH_2O, OH), 5.11 (s, br., 1H, CHN), 7.25–7.48 (m, 5H, Ph-H), 7.66–7.83 (m, 1H, NH). – ^{13}C NMR ($CDCl_3$): δ = 15.46, 15.60 (CH_3), 60.80, 60.95 (CH_2OH), 62.37, 63.37 (CHN), 68.38 (CH_2O), 71.42, 71.50 (Ph-*C*H), 126.3, 126.4 (*o*-Ph C), 127.1 (*p*-Ph C), 127.8, 127.9 (*m*-Ph C), 142.7 (*i*-Ph C), 173.3, 173.5, 175.8, 176.0, 182.0, 182.1, 188.8, 188.8 (4 cyclobutene C). – MS (70 eV): *m/z* (%) = 291 (2) $[M]^+$, 273 (1) $[M - H_2O]^+$, 185 (100), 167 (40) $[C_9H_{14}NO_5]^+$, 156 (44), 110 (57), 77 (32) [phenyl]$^+$.

$C_{15}H_{17}NO_5$ (291.3)
Calcd. C 61.85 H 5.88 N 4.81
Found C 61.73 H 5.99 N 4.67

4-(1-Adamantylamino)-3-ethoxy-3-cyclobutene-1,2-dione (**3g**): Reaction of 1-aminoadamantane (**2g**) (484 mg, 3.20 mmol) with **1** (500 mg, 2.94 mmol) for 2 h, general procedure I; purification by filtration on 3 g of silica gel (ethanol) removal of ethanol in vacuo and subsequent washing of the remainder with 30 ml of diethyl ether; yield 615 mg (76%) of **3g** as colorless fine crystals. – R_f = 0.25 (solvent B). – M.p. 161°C. – UV (EtOH/H_2O, 7:3): λ_{max} (lgε) = 260 nm (4.425), 273 (4.427). – IR (KBr): $\tilde{\nu}$ = 1798 cm^{-1}, 1692, 1600 (2 C=O), 1584. – ^1H NMR ($CDCl_3$): δ = 1.48 (t, *J* = 7.5 Hz, 3H, CH_3), 1.58 (s, br., 6H, adam. 4-H), 1.91 (s, br., 6H, adam. 2-H), 2.14 (s, br., 3H, adam. 3-H), 4.82 (s, br., 2H, OCH_2), 5.50, 6.16 (2 s, br., 1H, NH). – ^{13}C NMR ($CDCl_3$): δ = 15.86 (CH_3), 29.48 (adam. C-3), 35.69 (adam. C-4), 42.75 (adam. C-2), 53.52 (N–C), 69.40 (OCH_2), 170.9, 175.8, 175.9, 183.2, 190.1, 190.2 (4 cyclobutene C). – MS (70 eV): *m/z* (%) = 275 (30) $[M]^+$, 219 (25) $[M - C_2O_2]^+$, 197 (27), 155 (61), 136 (13) $[C_{10}H_{16}]^+$, 135 (85) $[C_{10}H_{15}]^+$, 98 (70), 68 (25), 57 (23), 55 (31), 44 (100) $[CH_3CHO]^+$, 42 (61).

$C_{16}H_{21}NO_3$ (275.3)
Calcd. C 69.79 H 7.69 N 5.09
Found C 69.85 H 7.74 N 5.12

3-Ethoxy-4-[2-(5-imidazolyl)ethylamino]-3-cyclobutene-1,2-dione (**3h**): Reaction of histamine dihydrochloride (**2h** · 2 HCl) (590 mg, 3.20 mmol) and triethylamine (890 µl, 6.40 mmol) with **1** (500 mg, 2.94 mmol) for 5 h; general procedure II; the white precipitate was filtered and washed with 30 ml of ethanol to yield 603 mg (87%) of **3h** as colorless fine crystals. – R_f = 0.38 (solvent E). – M.p. 204°C. – UV (EtOH/H_2O, 7:3): λ_{max} (lgε) = 259 nm (4.410), 272 (4.410). – IR (KBr): $\tilde{\nu}$ = 1806 cm^{-1}, 1700, 1590 br. (2 C=O). – ^1H NMR ([D$_6$]DMSO, 60°C): δ = 1.08, 1.38 (2 t, *J* = 7.0 Hz, 3H, CH_3), 2.80 (t, *J* = 7.0 Hz, 2H, 2′-H), 3.64 (m, 2H, 1′-H), 3.49, 4.67 (2 q, *J* = 7.0 Hz, 2H, OCH_2), 5.40–8.00 (s, br., 1H, aromatic NH), 6.84 (s, 1H, 4″-H), 7.57 (s, 1H, 2″-H), 8.65 (s, br., 1H, N*H*CH_2). – ^{13}C NMR ([D$_6$]DMSO, 90°C): δ = 15.46 (CH_3), 28.19 (C-2′), 43.89 (C-1′), 68.69 (OCH_2), 116.4 (C-4″), 134.2 (C-5″), 134.7 (C-2″), 172.8, 176.8, 182.4, 189.3 (4 cyclobutene C). – MS (70 eV): *m/z* (%) = 235 (4) $[M]^+$, 189 (15), 178 (8) $[M - C_2O_2H]^+$, 123 (15), 110 (5) [NHCH$_2$CH$_2$–imidazole]$^+$, 95 (19), 81 (18) [CH_2–imidazole]$^+$, 45 (100) $[C_2H_5O]^+$.

$C_{11}H_{13}N_3O_3$ (235.4)
Calcd. C 56.13 H 5.57 N 17.93
Found C 56.33 H 5.77 N 17.76

3-Ethoxy-4-[2′-(3″-indolyl)ethylamino]-3-cyclobutene-1,2-dione (**3i**): Reaction of tryptamine (**2i**) (520 mg, 3.20 mmol) with **1** (500 mg, 2.94 mmol) for 0.3 h, general procedure I. The white precipitate was filtered off and recrystallized from ethanol to give 618 mg (74%) of **3i** as fine colorless crystals. – R_f = 0.10 (solvent B). – M.p. 167°C (ethanol). – UV (EtOH/H_2O, 7:3): λ_{max} (lgε) = 222 nm (4.054), 270 (3.939). – IR (KBr): $\tilde{\nu}$ = 3254 cm^{-1} (indole NH), 1812, 1690, 1606 (2 C=O). – ^1H NMR ([D$_6$]DMSO): δ = 1.24, 1.37 (2 t, *J* = 7.0 Hz, 3H, CH_3), 2.98 (t, *J* = 7.5 Hz, 2H, 2′-H), 3.42–3.92 (m, 2H, 1′-H), 4.55, 4.66 (2 q, *J* = 7.0 Hz, 2H, OCH_2), 6.96–7.26, 7.34–7.48, 7.56–7.70 (3 m, 6H, Ph-H), 7.74, 7.91 (2 s, br., 1H, alkyl-NH). – ^{13}C NMR ([D$_6$]DMSO): δ = 15.38, 15.49 (CH_3), 26.40, 26.83 (C-2′), 44.44, 44.79 (C-1′), 68.61 (OCH_2),

110.6 (C-1″), 111.4 (C-5″), 118.1, 118.3 (C-8″, C-6″), 120.9 (C-7″), 123.0, 123.1 (C-2″), 127.2 (C-9″), 136.3 (C-4″), 172.4, 172.6, 176.4, 182.0, 189.2 (4 cyclobutene-C). – MS (70 eV): *m/z* (%) = 284 (27) $[M]^+$, 255 (18) $[M - CH_3CH_2]^+$, 227 (4) $[M - C_2O_2H]^+$, 171 (23), 143 (23), [3″-indolylethen]$^+$, 130 (100) [3″-indolyl-CH_2]$^+$, 44 (41) $[CH_3CHO]^+$.

$C_{16}H_{16}N_2O_3$ (284.3)
Calcd. C 67.59 H 5.69 N 9.85
Found C 67.54 H 5.77 N 9.76

3-Ethoxy-4-[2′-(5″-hydroxy-3″-indolyl)ethylamino]-3-cyclobutene-1,2-dione (**3j**): Reaction of serotonine dihydrogen oxalate (**2j** · $H_2C_2O_4$) (780 mg, 3.20 mmol) with triethylamine (890 µl, 6.40 mmol) and **1** (500 mg, 2.94 mmol) for 1 h, general procedure II; the precipitate was filtered off and recrystallized from ethanol to afford 414 mg (48%) of **3j** as fine colorless crystals. – R_f = 0.66 (solvent E). – M.p. 198°C (ethanol). – UV (EtOH/H_2O, 7:3): λ_{max} (lgε) = 222 nm (3.392), 271 (4.460). – IR (KBr): $\tilde{\nu}$ = 1808 cm^{-1}, 1690, 1598 (2 C=O). – ^{1}H NMR ([D$_6$]DMSO): δ = 1.20–1.30 (m, 3H, CH_3), 2.87 (t, *J* = 7.0 Hz, 2H, 2′-H), 3.54, 3.75 (2 s, br., 2H, 1′-H), 4.24–4.78 (m, 2H, OCH_2), 6.45 (dd, J^{ortho} = 9.0 Hz, J^{meta} = 3.0 Hz, 1H, 5″-H), 6.88, 7.07 (2 s, 2H, 7″-H, 2″-H), 7.17 (d, J^{ortho} = 9.0 Hz, 1H, 4″-H), 8.58 (s, 1H, OH), 8.70, 8.88 (2 s, br., 1H, N*H*CH_2). – ^{13}C NMR ([D$_6$]DMSO): δ = 15.44 (CH_3), 26.47, 26.84 (C-2′), 44.35, 44.59 (C-1′), 68.67 (OCH_2), 102.3 (C-4″), 109.7 (C-1″), 111.4, 111.5, 111.6 (C-5″, C-7″), 123.4, 123.5 (C-2″), 127.9, 130.8 (C-8″, C-9″), 150.3 (C-6″), 172.4, 172.6, 176.4, 176.9, 182.0, 189.2, 189.3 (4 cyclobutene C). – MS (70 eV): *m/z* (%) = 301 (5) $[M + H]^+$, 300 (30) $[M]^+$, 272 (2) $[M + H - C_2H_5]^+$, 243 (3) $[M - C_2O_2H]^+$, 146 (100) [(5″-hydroxyindolyl)-3″-CH_2]$^+$, 45 (34) $[C_2H_5O]^+$.

$C_{16}H_{16}N_2O_4$ (300.3)
Calcd. C 63.99 H 5.37 N 9.37
Found C 63.94 H 5.46 N 9.35

3-Ethoxy-4-[(1′S,2′R)-(2′-hydroxy-1′-methyl-2′-phenylethyl)-(methyl)amino]-3-cyclobutene-1,2-dione (**3k**): Reaction of (–)-ephedrine (**2k**) (530 mg, 3.20 mmol) with **1** (500 mg, 2.94 mmol) for 1 h, general procedure I; the mixture was filtered through silica gel (1 g, ethanol) and the eluent was removed in vacuo. The crude product was purified by flash chromatography (60 g silica gel, solvent B) to afford 838 mg (97%) of **3k** as colorless fine crystals. – R_f = 0.22 (solvent B). – M.p. 125°C. – $[\alpha]_D^{20}$ = –2.6 (*c* = 1 in ethanol). – UV (EtOH/H_2O, 7:3): λ_{max} (lgε) = 282 nm (4.400). – IR (KBr): $\tilde{\nu}$ = 1796 cm^{-1}, 1696, 1590 br. (2 C=O), 1490. – ^{1}H NMR ($CDCl_3$): δ = 1.29, 1.35 (2 d, *J* = 7.0 Hz, 3H, NCHC*H*$_3$), 1.42, 1.48 (2 t, *J* = 7.0 Hz, 3H, OCH_2CH_3), 2.75, 2.93 (2 d, *J* = 4.0 Hz, 1H, OH), 3.06, 3.32 (2 s, 3H, NCH_3), 4.17, 4.77 (2 dq, *J* = 7.0 Hz, *J* = 7.0 Hz, 1H, 1′-H), 4.73 (q, *J* = 7.0 Hz, 2H, OCH_2), 4.90–4.98 (m, 1H, 2′-H), 7.28–7.60 (m, 5H, Ph-H). – ^{13}C NMR ($CDCl_3$): δ = 11.80, 11.90 (NCHCH_3), 15.74 (OCH_2CH_3), 32.18, 33.03 (NCH_3), 59.91, 60.92 (C-1′), 69.57 (OCH_2), 75.51, 76.55 (C-2′), 126.1, 126.2 (*m*-Ph C), 127.8, 127.9 (*o*-Ph C), 128.3, 128.3 (*p*-Ph C), 141.1, 141.4 (*i*-Ph C), 171.8, 172.6, 175.7, 176.2, 182.2, 188.7, 188.7 (4 cyclobutene C). – MS (70 eV): *m/z* (%) = 289 (5) $[M]^+$, 183 (58), 182 (100), $[M - C_7H_7O]^+$, 154 (26) $[M - C_7H_7O - CO]^+$, 126 (14) $[M - C_7H_7O - C_2O_2]^+$, 98 (16), 69 (18), 58 (27), 42 (21).

$C_{16}H_{19}NO_4$ (289.3) Calcd. C 66.42 H 6.62
Found C 66.34 H 6.76

4-⟨[3′-(10″,11″-Dihydro-5H-dibenz[b,f]azepin-5″-yl)propyl]-(methyl)amino⟩-3-ethoxy-3-cyclobutene-1,2-dione (**3l**): Reaction of desipramine hydrochloride (**2l** · HCl) (980 mg, 3.20 mmol) with triethylamine (445 µl, 3.20 mmol) and **1** (500 mg, 2.94 mmol) for 5 h, general procedure II; the crude product was concentrated by removing the solvent in vacuo; flash chromatography (60 g silica gel, solvent B) afforded 1.16 g (100%) of **3l** as a yellow oil. – R_f = 0.29 (solvent B). – UV (EtOH/H_2O, 7:3): λ_{max} (lgε) = 210 nm (4.409), 267 (4.471). – IR (KBr): $\tilde{\nu}$ = 1800 cm^{-1}, 1712, 1612 (2 C=O). – ^{1}H NMR ([D$_6$]DMSO): δ = 1.06, 1.26, 1.35 (3 t, *J* = 7.0 Hz, 3H, OCH_2CH_3), 1.79 (tt, $J^{1'}$ = $J^{3'}$ = 8.0 Hz, 2H, 2′-H), 2.99, 3.16 (2 s, 3H, NCH_3), 3.10 (s, 4H, Ph-CH_2), 3.41, 3.67 (2 t, *J* = 8.0 Hz, 2H, 1′-H), 3.70–3.82 (m, 2H, 3′-H), 4.55, 4.65 (2 q, *J* = 7.0 Hz, 2H, OCH_2), 6.88–7.02, 7.08–7.24 (2 m, 8H, Ph-H). – ^{13}C NMR ([D$_6$]DMSO): δ = 15.54 (OCH_2CH_3), 25.53, 26.09 (C-2′), 31.49 (Ph-CH_2), 35.82, 36.22 (NCH_3), 47.08, 47.19 (3′-C), 48.91, 49.98 (C-1′), 68.82 (OCH_2), 119.7 (C-6″), 122.5 (C-4″), 126.3, 129.7 (C-3″, C-5″), 133.7 (C-2″), 148.0 (C-1″), 171.4, 171.7, 175.8, 176.1, 181.2, 188.6, 188.8 (4 cyclobutene C). – MS (70 eV): *m/z* (%) = 391 (15) $[M + H]^+$, 390 (55) $[M]^+$, 208 (100) [CH_2–dibenz-azepane]$^+$, 195 (70) [dibenz-azepane]$^+$, 193 (31) [dibenz-azepine]$^+$, 84 (15).

$C_{24}H_{26}N_2O_3$ (390.6)
Calcd. C 73.80 H 6.71 N 7.20
Found C 73.73 H 6.88 N 7.17

4-[2′-(3″,4″-Dihydroxyphenyl)ethylamino]-3-ethoxy-3-cyclobutene-1,2-dione (**3m**): Reaction of dopamine hydrochloride (**2m** · HCl) (607 mg, 3.20 mmol) with triethylamine (445 µl, 3.20 mmol) and **1** (500 mg, 2.94 mmol) for 1 h, general procedure II; filtration over 2 g of silica gel (ethanol) and crystallisation from ethanol and hexane afforded 570 mg (69%) of **3m** as fine colorless crystals. – R_f = 0.13 (solvent B, 1% acetic acid). – M.p. 196°C (ethanol/hexane). – UV (EtOH/H_2O, 7:3): λ_{max} (lgε) = 260 nm (4.354), 275 (4.376). – IR (KBr): $\tilde{\nu}$ = 1814 cm^{-1}, 1690, 1596 (C=O). – ^{1}H NMR ([D$_6$]DMSO): δ = 1.08, 1.37 (2 t, *J* = 7.0 Hz, 3H, CH_3), 2.86 (t, *J* = 8.0 Hz, 2H, 2′-H), 3.37–3.74 (m, 1H, 1′-H), 4.64 (q, *J* = 7.0 Hz, 2H, OCH_2), 6.42–6.78 (m, 3H, Ph-H), 8.57–9.04 (m, 3H, 2 OH, NH). – ^{13}C NMR ([D$_6$]DMSO): δ = 15.54 (CH_3), 35.74, 35.84 (C-2′), 45.17, 45.57 (C-1′), 68.69 (OCH_2), 115.5 (C-2″), 116.2, 116.3 (C-5″), 119.4 (C-6″), 129.0 (C-1″), 143.7 (C-4″), 145.1 (C-3″), 172.3, 172.6, 176.5, 176.6, 176.8, 182.0, 189.1, 189.2, 189.3 (4 cyclobutene C). – MS (70 eV): *m/z* (%) = 277 (90) $[M]^+$, 249 (5) $[M - CO]^+$, 248 (13) $[M - C_2H_5]^+$, 221 (6) $[M - C_2O_2]^+$, 220 (36) $[M - C_2O_2H]^+$, 192 (43), 164 (38), 137 (24) [dihydroxystyrene + H]$^+$, 136 (100) [dihydroxystyrene]$^+$, 124 (23), 123 (42) [dihydroxybenzyl]$^+$, 91 (43), 70 (48).

$C_{14}H_{15}NO_5$ (277.3) Calcd. C 60.63 H 5.45
Found C 60.75 H 5.53

3-Ethoxy-4-[(2S)-2-(hydroxymethyl)pyrrolidino]-3-cyclobutene-1,2-dione (**3n**): Reaction of **2n** (0.33 ml, 3.38 mmol) with **1** (0.50 ml, 3.38 mmol) for 8 h, general procedure I; yield after chromatography (70 g of silica gel, dichloromethane/petroleum ether/ethanol, 20:5:1) and crystallization 647 mg (85%) of **3n** as colorless fine crystals. – R_f = 0.56 (solvent D). – $[\alpha]_D^{20}$ = –109.8 (*c* = 1 in chloroform). – M.p. 105°C (ethanol/*tert*-butyl methyl ether). – UV (methanol): λ_{max} (lgε) = 283 nm (4.438). – IR (KBr): $\tilde{\nu}$ = 3410 cm^{-1} (OH), 2980, 2940 (CH_3, CH_2), 1795, 1700, 1610 (C=O, C=C), 1085, 1055 (C–O). – ^{1}H NMR ($CDCl_3$): δ = 1.46 (t, *J* = 7 Hz, 3H, CH_3), 1.89–2.12 (m, 4H, CH_2), 3.18–3.38 (m, 1H, OH), 3.48–4.01 (m, 6H, CH_2N, C*H*$_2$OH, CH_2O), 4.03–4.16, 4.32–4.47 (2 m, 1H, CHN). – ^{13}C NMR ($CDCl_3$): δ = 15.61 (CH_3), 23.02, 23.18, 23.29, 23.40 (CH_2), 26.53, 26.69 (CH_2), 48.78, 49.00 (CH_2N), 60.99, 61.11, 61.32 (CH), 62.74, 62.87, 62.91 (CH_2OH), 68.74 (CH_2O), 169.8, 170.2, 176.1, 176.9, 181.6, 181.6, 181.6 (4 cyclobutene C). – MS (70 eV): *m/z* (%) = 225 (100) $[M]^+$, 194 (92) $[M - CH_2OH]^+$, 169 (9) $[M - 2\,CO]^+$, 110 (17), 69 (38) $[C_4H_7N]^+$.

$C_{11}H_{15}NO_4$ (225.24)
Calcd. C 58.66 H 6.71 N 6.22
Found C 58.49 H 6.58 N 6.22

3,4-Bis[(3-hydroxypropyl)amino]-3-cyclobutene-1,2-dione (**5a**): Reaction of **3b** (398 mg, 2.00 mmol) with **2b** (0.18 ml, 2.40 mmol) for 8 h, general procedure IV; yield after column chromatography (40 g of silica gel, dichloromethane/methanol/petroleum ether, 5:1:1) 352 mg (77%) of **5a** as slightly yellow fine crystals. – R_f = 0.11 (solvent C). – M.p. 215°C. – UV (methanol): λ_{max} (lgε) = 291 nm (4.493). – IR (KBr): $\tilde{\nu}$ = 3374 cm^{-1}, 3296, 3162, 3080 (OH, NH), 2998, 2954 (CH_2), 1800, 1638, 1578 (C=O, C=C), 1038 (C–O). – ^1H NMR ([D_6]DMSO): δ = 1.68 (p, *J* = 6.5 Hz, 4H, CH_2), 3.48 (dt, *J* = 5, 6.5 Hz, 4H, CH_2O), 3.56, 3.59 (2 t, *J* = 6.5 Hz, 4H, CH_2N), 4.53 (t, *J* = 5 Hz, 2H, OH), 7.43 (s, br., 2H, NH). – ^{13}C NMR ($CDCl_3$): δ = 33.77 (CH_2), 40.62 (CH_2N), 57.89 (CH_2O), 167.9, 182.3 (4 cyclobutene C). – MS (70 eV): *m/z* (%) = 228 (11) $[M]^+$, 195 (3) $[M - CH_2OH]^+$, 172 (7) $[M - 2\,CO]^+$, 109 (38), 91 (66), 44 (100) $[C_2H_4O]^+$.

$C_{10}H_{16}N_2O_4$ (228.3)
Calcd. C 52.62 H 7.07 N 12.27
Found C 52.68 H 7.21 N 12.24

3-[(5″S)-5″-Acetamido-5″-(methoxycarbonyl)pentylamino]-4-[(3′-hydroxypropyl)amino]-3-cyclobutene-1,2-dione (**5b**): Reaction of **3b** (398 mg, 2.00 mmol) and N^1-acetyl-L-lysine methyl ester (**4p**) (444 mg, 2.40 mmol) for 8 h, general procedure V; yield after column chromatography (50 g of silica gel, solvent C) 554 mg (78%) of **5b** as colorless fine crystals. – R_f = 0.19 (solvent C). – M.p. 154°C (methanol/dichloromethane). – UV (methanol): λ_{max} (lgε) = 289 nm (3.877). – $[\alpha]_{20}^{D}$ = –3.0 (*c* = 1 in ethanol). – IR (KBr): $\tilde{\nu}$ = 3384 cm^{-1}, 3294, 3276, 3074 (OH, NH), 2950 (CH_3, CH_2), 1800, 1742, 1652, 1590 (C=O, C=C), 1548 (NH), 1372 (CH_3CO), 1068 (C–O). – ^1H NMR ([D_6]DMSO): δ = 1.11–1.78 (m, 8H, 2′-H_2, 3′-H_2, 4′-H_2, 2-H_2), 1.85 (s, 3H, CH_3CON), 3.43–3.66 (m, 6H, CH_2O, 2 CH_2N), 3.67 (s, 3H, CH_3O), 4.25 (q, *J* = 5 Hz, 1H, CH), 4.55 (t, *J* = 4 Hz, 1H, OH), 7.46 (s, br., 2H, NH), 8.26 (d, *J* = 5 Hz, 1H, NHAc). – ^{13}C NMR ([D_6]DMSO): δ = 22.19 (CH_3), 22.38 (C-3′), 30.36, 30.61 (C-2′, C-4′), 33.84 (C-2), 40.64 (C-1), 43.13 (C-1′), 51.68 (CH_3O), 51.93 (C-5′), 57.93 (CH_2OH), 167.9, 169.6, 172.7, 182.3, 182.4 (4 cyclobutene C). – MS (70 eV): *m/z* (%) = 355 (60) $[M]^+$, 337 (3) $[M - H_2O]^+$, 323 (3) $[M - CH_3O]^+$, 299 (4) $[M - 2\,CO]^+$, 237 (30), 144 (52), 84 (100), 44 (53) $[C_2H_4O]^+$. – MS (high resolution): Calcd. 355.1743, found 355.1743.

$C_{16}H_{25}N_3O_6$ (355.4) Calcd. C 54.08 H 7.09
Found C 54.39 H 6.44

3,4-Bis[(1′S,2′R)-(2′-hydroxy-1′-methyl-2′-phenylethyl)(methyl)amino]-3-cyclobutene-1,2-dione (**5c**): Reaction of **1** (500 mg, 2.94 mmol) with **2k** (1.07 g, 6.47 mmol) for 2 d; general procedure VI; the white precipitate was removed and crystallized from ethanol to yield 972 mg (81%) of **5c** as colorless needles. – R_f = 0.62 (solvent F). – M.p. 216–220°C. – UV (MeOH): λ_{max} (lgε) = 293 nm (4.290). – $[\alpha]_D^{20}$ = +136.7 (*c* = 1 in EtOH). – IR (KBr): $\tilde{\nu}$ = 1782 cm^{-1}, 1654, 1566, 1516 (C=O). – ^1H NMR ($CDCl_3$): δ = 1.34 (d, *J* = 6.9 Hz, 6H, 2 C-CH_3), 2.86 (s, 6H, 2 N-CH_3), 4.58 (dq, J_1 = 6.9 Hz, J_2 = 6.9 Hz, 1H, 1′-H), 4.69 (d, *J* = 4.5 Hz, 2H, 2 OH), 4.82 (dd, J_1 = 6.9 Hz, J_2 = 4.5 Hz, 2H, 2′-H), 7.20–7.46 (m, 10H, Ph-H). – ^{13}C NMR ([D_6]acetone): δ = 13.54 (C-CH_3), 34.91 (NCH_3), 60.97 (C-1′), 76.61 (C-2′), 127.3 (*o*-Ph C), 128.6 (*p*-Ph C), 128.9 (*m*-Ph C), 144 (*i*-Ph C), 170.6, 184.8 (4 cyclobutene C). – MS (70 eV): *m/z* (%) = 301 (100) $[M - C_6H_5CHOH]^+$, 196 (23), 195 (27), 167 (37), 146 (51), 105 (35) $[C_6H_5CO]^+$, 82 (34).

$C_{24}H_{28}N_2O_4$ (408.5) Calcd. C 70.57 H 6.91
Found C 70.73 H 7.00

3-[(1′S,2′R)-(2′-Hydroxy-1′-methyl-2′-phenylethyl(methyl)amino]-4-[(3″-hydroxypropyl)amino]-3-cyclobutene-1,2-dione (**5d**): Reaction of **3b** (219 mg, 1.10 mmol) with **2k** (218 mg, 1.32 mmol) for 5 d, general procedure IV; the white precipitate was removed and washed with ethanol to yield 170 mg (49%) of **5d** as colorless needles. – R_f = 0.26 (solvent F). – M.p. 150–152°C. – $[\alpha]_D^{20}$ = –18.7 (*c* = 0.6 in EtOH). – UV (EtOH): λ_{max} (lgε) = 302 nm (4.329). – IR (KBr): $\tilde{\nu}$ = 1794 cm^{-1}, 1638, 1550 (br.) (C=O). – ^1H NMR (CD_3OD): δ = 1.39 (d, *J* = 7 Hz, 3H, C-CH_3), 1.77 (tt, J_1 = J_2 = 6 Hz, C-CH_2-C), 3.12 (s, 3H, NCH_3), 3.52–3.70 (m, 5H, NCH_2, OCH_2, $CHCH_3$), 5.76 (d, *J* = 8 Hz, 1H, CHOH), 7.22–7.45 (m, 5H, Ph-H). – ^{13}C NMR (CD_3OD): δ = 13.93 (C-CH_3), 32.84 (NCH_3), 35.02 (C-CH_2-C), 42.61 (NCH_2), 60.23 (OCH_2), 61.97 (C-CH_3), 76.76 (CHOH), 127.7 (*o*-Ph C), 128.9 (*p*-Ph C), 129.2 (*m*-Ph C), 143.3 (*i*-Ph C), 168.9, 170.0, 183.4, 183.5 (4 cyclobutene C). – MS (70 eV): *m/z* (%) = 318 (1) $[M^+]$, 211 (96) $[M - C_6H_5CHOH]^+$, 193 (21), 181 (35), 96 (21), 68 (40), 58 (100).

$C_{17}H_{22}N_2O_4$ (318.5) Calcd. C 64.11 H 6.96
Found C 64.17 H 6.92

3-[(2′S)-2′-(Hydroxymethyl)pyrrolidino]-4-[(1″R)-(1″-phenylethyl)amino]-3-cyclobutene-1,2-dione (**5e**): Reaction of **3n** (294 mg, 1.31 mmol) and (+)-(*R*)-1-phenylethylamine (**2o**) (174 mg, 1.44 mmol) for 3 d; general procedure V; yield after flash chromatography (20 g of silica gel, petroleum ether/ethanol, 4:1) 314 mg (1.04 mmol, 80%) of **5e** as colorless fine crystals. – R_f = 0.20 (solvent F). – M.p. 129°C. – $[\alpha]_D^{20}$ = –112 (*c* = 0.6 in EtOH). – UV (EtOH): λ_{max} (lgε) = 304 nm (4.193). – IR (film): $\tilde{\nu}$ = 1790 cm^{-1}, 1656, 1572, 1516 (C=O). – ^1H NMR ($CDCl_3$): δ = 1.64 (d, *J* = 8 Hz, 3H, CH_3), 1.80–2.12 (m, 4H, 2 CH_2), 3.40–3.84 (m, 4H, NCH_2, OCH_2), 5.46 (quint, *J* = 8 Hz, 1H, C*H*-CH_3), 7.20–7.60 (m, 6H, Ph-H, NH). – ^{13}C NMR ($CDCl_3$): δ = 22.91, 25.12 (C-CH_3), 23.90, 27.51 (2 CH_2), 48.95 (NCH_2), 51.33, 53.41, 62.47 (2 NCH), 63.94 (CH_2OH), 125.7, 126.2 (*o*-Ph C), 127.1, 127.4 (*p*-Ph C), 128.6, 128.7 (*m*-Ph C), 143.5 (*i*-Ph C), 167.3, 182.4, 183.3 (4 cyclobutene C). – MS (70 eV): *m/z* (%) = 300 (13) $[M^+]$, 269 (16), 244 (2) $[M - C_2O_2]^+$, 165 (22), 139 (30), 106 (100) $[C_6H_5CH_2CH_3^+]$, 105 (77) $[C_6H_5CHCH_3^+]$.

$C_{17}H_{20}N_2O_3$ (300.5) Calcd. C 67.95 H 6.71
Found C 67.91 H 6.80

3-[(1″S,2″R)-(2″-Hydroxy-1″-methyl-2″-phenylethyl)(methyl)amino]-4-[(2′S)-2′-(hydroxymethyl)pyrrolidino]-3-cyclobutene-1,2-dione (**5f**): Reaction of **3n** (230 mg, 1.02 mmol) with **2k** (185 mg, 1.12 mmol) for 3 d, general procedure V. Yield after flash chromatography (20 g of silica gel, petroleum ether/ethanol, 4:1) 286 mg (81%) of **5f** as a colorless oil. – R_f = 0.43 (solvent F). – $[\alpha]_D^{20}$ = –14.15 (*c* = 1 in EtOH). – UV (EtOH): λ_{max} (lgε) = 310 nm (4.322). – IR (KBr): $\tilde{\nu}$ = 1782 cm^{-1}, 1662, 1564, 1504 (C=O). – ^1H NMR ([D_6]DMSO, 35°C): δ = 1.30 (d, *J* = 7 Hz, 3H, CH_3), 1.60–2.00 (m, 4H, 2 CH_2), 2.96 (s, 3H, NCH_3), 2.98–3.14 (m, 1H, OH), 3.30–3.52 (m, 3H, NCH_2, C*H*-CH_3), 4.35–4.50 (quint, *J* = 5 Hz, 1H, C*H*-CH_2OH), 4.56–4.84 (m, 3H, C*H*-OH, CH_2-OH), 5.87 (d, *J* = 4 Hz, 1H, OH). – ^{13}C NMR ([D_6]DMSO): δ = 13.47, 18.46 (C-CH_3), 24.10, 26.52 (2 CH_2), 33.24 (NCH_3), 52.41, 56.00 (NCH_2), 59.31 (*C*H-CH_2OH), 60.82 (*C*H-CH_3), 63.31 (OCH_2), 74.79 (CHOH), 126.5 (*o*-Ph C), 127.2 (*p*-Ph C), 127.8 (*m*-Ph C), 143.1 (*i*-Ph C), 166.6, 168.8, 182.7, 182.9 (4 cyclobutene C). – MS (70 eV): *m/z* (%) = 344 (5) $[M]^+$, 237 (100) $[M - C_6H_5CHOH]^+$, 181 (9), 149 (19), 146 (24), 110 (21), 105 (16).

$C_{19}H_{24}N_2O_4$ (344.5) Calcd. C 66.24 H 7.02
Found C 66.09 H 7.16

CAS Registry Numbers

1: 5231-87-8 / **2a**: 141-43-5 / **2b**: 156-87-6 / **2c**: 124-68-5 / **2d**: 5856-63-3 / **2e**: 51-67-2 / **2f**: 28143-91-1 / **2g**: 768-94-5 / **2h** · 2 HCl: 56-92-8 / **2i**: 61-54-1 / **2j** · $H_2C_2O_4$: 3036-16-6 / **2k**: 299-42-3 / **2l** ·

HCl: 58-28-6 / **2m** · HCl: 62-31-7 / **2n**: 23356-96-9 / **2o**: 3886-69-9 / **3a**: 131588-93-7 / **3b**: 131588-94-8 / **3c**: 131588-95-9 / **3d**: 131588-96-0 / **3e**: 131588-97-1 / **3f**: 131588-98-2 / **3g**: 131588-99-3 / **3h**: 131589-00-9 / **3i**: 131589-01-0 / **3j**: 131589-02-1 / **3k**: 131589-03-2 / **3l**: 131589-04-3 / **3m**: 131589-05-4 / **3n**: 131589-06-5 / **4p**: 6072-02-2 / **5a**: 131589-07-6 / **5b**: 131589-08-7 / **5c**: 131589-09-8 / **5d**: 131588-92-6 / **5e**: 131589-10-1 / **5f**: 131589-11-2

---

[1] Anticancer Agents, 14: H.-J. Gabius, C. Schröter, S. Gabius, U. Brinck, L. F. Tietze, *J. Histochem. and Cytochem.* **38** (1990) 1625. – Anticancer Agents, 13: E. Jähde, K.-H. Glüsenkamp, I. Klünder, D. F. Hülser, L. F. Tietze, M. F. Rajewsky, *Cancer Res.* **49** (1989) 2965. – Anticancer Agents, 12: L. F. Tietze, M. Beller, R. Fischer, M. Lögers, K.-H. Glüsenkamp, E. Jähde, M. F. Rajewsky, *Angew. Chem.* **102** (1990) 812; *Angew. Chem. Int. Ed. Engl.* **29** (1990) 782.

[2] [2a] R. Arnon, M. Sela, *Imm. Rev.* **62** (1982) 5. – [2b] G. F. Rowland, *Eur. J. Cancer* **13** (1977) 593. – [2c] E. Haber, L. B. Page, G. A. Jacoby, *Biochem.* **4** (1965) 693. – [2d] L. Gros, H. Ringsdorf, H. Schupp, *Angew. Chem.* **93** (1981) 311; *Angew. Chem. Int. Ed. Engl.* **20** (1981) 305.

[3] [3a] *Handbook of Experimental Immunology* (D. M. Weis, Ed.), Blackwell Scientific Publications, Oxford 1986. – [3b] F. R. Seiler, P. Gronski, R. Kurrle, G. Lüben, H.-P. Harthus, W. Ax, K. Bosslet, H.-G. Schwick, *Angew. Chem.* **97** (1985) 141; *Angew. Chem. Int. Ed. Engl.* **24** (1985) 139. – [3c] B. F. Erlanger, *Methods Enzymol.* **70** (1980) 85. – [3d] H. Kiefer, *Immunol. Methods* **1979**, 137. – [3e] F. K. Jansen, H. E. Blythman, D. Carrière, P. Casellas, O. Gros, P. Gros, J. C. Laurent, F. Paolucci, B. Pau, P. Pocelel, G. Richter, H. Vidal, G. A. Voissin, *Immunol. Rev.* **62** (1982) 185. – [3f] S. Bauminger, M. Wilcheck, *Methods Enzymol.* **70** (1980) 151. – [3g] L. F. Tietze, A. Goerlach, M. Beller, *Liebigs Ann. Chem.* **1988**, 559, 565.

[4] [4a] D. E. Yelton, M. D. Scharff, *Ann. Rev. Biochem.* **50** (1981) 657. – [4b] W. F. Bale, M. A. Contreras, E. D. Grady, *Cancer Res.* **40** (1980) 2965. – [4c] D. Pressmann, *Cancer Res.* **40** (1980) 2960. – [4d] Z. Steplewski et al. *Cancer Res.* **42** (1982) 150. – [4e] G. A. Pietersz, J. Kanellos, M. J. Smyth, J. Zalcberg, I. F. C. McKenzie, *Immun. Cell. Bio.* **65** (1987) 111.

[5] [5a] L. F. Tietze, *Nachr. Chem. Techn. Lab.* **36** (1988) 728. – [5b] L. F. Tietze, in *Molecular Aspects of Chemotherapy* (E. Borowski, D. Shugar, Eds.), Pergamon Press, Oxford 1990.

[6] [6a] H. D. Jakubke, H. Jeschkeit, *Aminosäuren Peptide, Proteine*, Verlag Chemie, Weinheim 1982. – [6b] W. König, R. Geiger, *Chem. Ber.* **103** (1970) 788. – [6c] M. Reichlin, *Methods Enzym.* **70** (1980) 159. – [6d] H. Rosemeyer, F. Seela, *Angew. Chem.* **96** (1984) 365; *Angew. Chem. Int. Ed. Engl.* **23** (1984) 385.

[7] Recently, squaric acid esters have become useful substrates in organic syntheses: [7a] L. S. Liebeskind, R. W. Fengl, K. R. Wirtz, T. T. Shawe, *J. Org. Chem.* **53** (1988) 2482. – [7b] J. L. Kraus, *Tetrahedron Lett.* **1985**, 1867. – [7c] S. T. Perri, H. W. Moore, *Tetrahedron Lett.* **1987**, 4507.

[8] [8a] S. Cohen, S. G. Cohen, *J. Am. Chem. Soc.* **88** (1966) 1533. – [8b] J. E. Thorpe, *J. Chem. Soc. (B)* **1968**, 435. – [8c] E. Neuse, B. Green, *Liebigs Ann. Chem.* **1973**, 619. – [8d] F. Vögtle, P. Dix, *Liebigs Ann. Chem.* **1977**, 1698. – [8e] H. Ehrhardt, S. Hünig, H. Pütter, *Chem. Ber.* **110** (1977) 2506. – [8f] For the synthesis of unsymmetrical diamides see also: A. H. Schmidt, *Synthesis* **1980**, 961.

[9] K. H. Glüsenkamp, E. Jähde, W. Drosdziok, M. F. Rajewsky, M. Arlt, M. Beller, L. F. Tietze, unpublished results.

[10] [10a] W. Weis, J. H. Brown, S. Cusak, J. C. Paulson, J. J. Skehel, D. C. Wiley, *Nature* **333** (1988) 426. – [10b] M. G. Rossmann, *Nature* **333** (1988) 392. – [10c] G. Maahs, P. Hegenberg, *Angew. Chem.* **78** (1966) 927; *Angew. Chem. Int. Ed. Engl.* **5** (1966) 888. – [10d] J. A. Pople, W. G. Schneider, H. J. Bernstein, *High Resolution Nuclear Magnetic Resonance*, McGraw-Hill, New York 1959.

[11] Personal communication, Dr. M. Bratz.

[357/90]