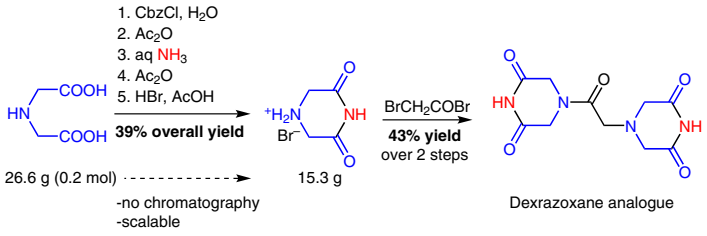
Synthesis J. Roh et al. Paper

Large-Scale Synthesis of Piperazine-2,6-dione and Its Use in the Synthesis of Dexrazoxane Analogues

Jaroslav Roh*
Galina Karabanovich
Veronika Novakova
Tomáš Šimůnek
Kateřina Vávrová

Charles University in Prague, Faculty of Pharmacy in Hradec Králové, Heyrovského 1203, 50005 Hradec Králové, Czech Republic
jaroslav.roh@faf.cuni.cz



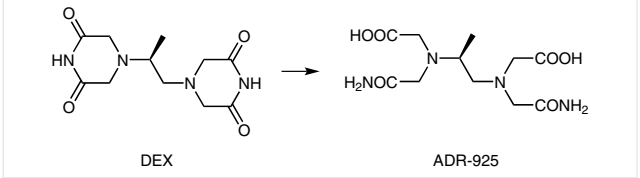
Received: 07.06.2016
Accepted after revision: 22.07.2016
Published online: 31.08.2016
DOI: 10.1055/s-0035-1562618; Art ID: ss-2016-z0415-op

Abstract An efficient, large-scale synthesis of piperazine-2,6-dione was developed. The advantages of this procedure include the use of inexpensive starting materials, satisfactory yields, and a convenient work-up without the need for chromatographic techniques. Furthermore, this procedure can be easily modified for the preparation of 1-substituted piperazine-2,6-dione hydrobromides. The utility of the prepared piperazine-2,6-dione was demonstrated in the synthesis of a novel analogue of the only drug used in clinical practice to prevent anthracycline-induced cardiotoxicity, dexrazoxane.

Key words piperazine-2,6-dione, large-scale synthesis, dexrazoxane, cyclization, synthesis, formamide

Dexrazoxane (DEX, ICRF-187, ADR-529, (+)-(*S*)-4,4′-(propane-1,2-diyl)bis(piperazine-2,6-dione), Scheme 1) is the only drug used in clinical practice to prevent anthracycline (ANT)-induced cardiotoxicity. DEX can be used to effectively protect the heart against the chronic type of ANT cardiotoxicity, which represents the most serious limitation of the use of ANT in anticancer treatment.[1] However, the mechanism(s) of ANT cardiotoxicity and DEX-induced cardioprotection are still not fully understood.[2–4] There are two main hypotheses. The first (traditional) hypothesis focuses on the iron chelation properties of the DEX metabolite ADR-925, which may prevent ANT-induced oxidative stress.[5] The second (and more recent) hypothesis involves the specific interaction of DEX with topoisomerase II, an enzyme that regulates the topology of DNA and manages its tangles and supercoils.[6,7]

Hence, new analogues of DEX are needed as potential novel cardioprotective agents and as tools to dissect the mechanism(s) of ANT-induced cardiotoxicity as well as to provide effective and cardio-specific protection.



Scheme 1 Structures of the cardioprotective drug dexrazoxane (DEX) and its metabolite ADR-925

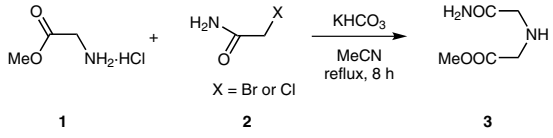
To date, few structure–cardioprotective activity relationship studies of DEX analogues have been published, mostly with negative results.[5,8–10] Such studies have been hampered by the unmet need for large amounts of DEX analogues because no in vitro model of chronic ANT cardiotoxicity has been established to date. Thus, each DEX analogue must be repeatedly administered to experimental animals together with selected ANT for several weeks or months; that is, a period of time necessary for the development of chronic ANT cardiotoxicity in an appropriate in vivo model.

DEX is prepared on a large scale through the cyclization of (*S*)-1,2-diaminopropane-*N*,*N*,*N*′,*N*′-tetraacetic acid in formamide by heating at 100–110 °C under reduced pressure for 1–2 h, followed by heating at 150–160 °C for 4–5 h. The majority of DEX analogues that have previously been described were prepared by using this or a similar methodology starting from diaminoalkane-*N*,*N*,*N*′,*N*′-tetraacetic acids.[11,12] We successfully applied this method for the synthesis of racemic razoxane, 4,4′-(ethylene)bis(piperazine-2,6-dione) or 4,4′-(propane-1,3-diyl)bis(piperazine-2,6-dione). However, only DEX analogues with linkers that are stable under the harsh cyclization conditions, for example 4,4′-(alkanediyl)bis(piperazine-2,6-diones), can be prepared by using this method. To overcome the limitations connected with the cyclization in the last step of the DEX analogue

synthesis, piperazine-2,6-dione would be a valuable intermediate for the preparations of DEX analogues with less stable linkers between the two rings.

Hence, the aim of this study was to develop a reliable, scalable, and inexpensive synthesis of piperazine-2,6-dione and to demonstrate its use in the synthesis of DEX analogues.

We first attempted to prepare piperazine-2,6-dione by using known methods.[13–17] Piperazine-2,6-dione can be prepared by hydrolysis of 2,6-bishydroxyiminopiperazine. However, the reported preparation of 2,6-bishydroxyiminopiperazine starting from iminodiacetonitrile and hydroxylamine suffers from very low yield (14%).[13] The reaction between methyl glycinate hydrochloride (**1**) with haloacetamide (**2**) in boiling acetonitrile was reported to give reasonable yields of piperazine-2,6-dione (40 and 33%) without the need for column chromatography.[14,15] Unfortunately, we did not obtain any piperazine-2,6-dione by using either of those two methods; only methyl *N*-(carbamoylmethyl)glycinate (**3**) was isolated from the reaction mixtures (Scheme 2).

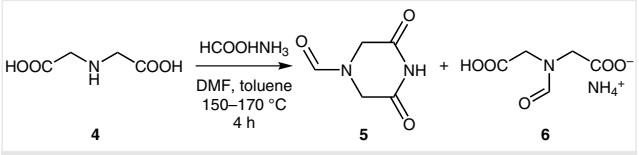


1 2 3

Scheme 2 Unsuccessful preparation of piperazine-2,6-dione through the reaction of methyl glycinate hydrochloride with bromo/chloroacetamide according to previous procedures[14,15]

Another potentially scalable procedure is the preparation of 4-formylpiperazine-2,6-dione (**5**) from iminodiacetic acid (**4**), followed by hydrolysis of the formyl group.[16] Although the reaction using a Dean–Stark trap proceeded well, we were not able to isolate the product **5** by using the described procedure (by evaporation of the reaction mixture and dilution with methanol). No solids were formed, even after one month of crystallization at 4 °C. The addition of acetone to the methanol solution enabled us to obtain a beige solid after one week of crystallization at 4 °C. However, NMR and HRMS experiments showed that the product was *N*-formyliminodiacetic acid (**6**). When the reaction was carried out without a Dean–Stark trap and the water and volatiles were azeotropically distilled off during the reaction, crude **5** was obtained in 8% yield (Scheme 3). However, the low yield meant that this method was not suitable for the large-scale synthesis of piperazine-2,6-dione.

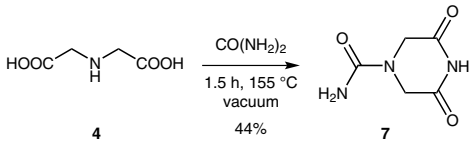
The reaction of **4** with formamide according to the cyclization protocol used in the DEX synthesis[12] expectedly gave no product; only black tar was obtained after evaporation of the reaction mixture, from which no solids crystallized after dilution with various organic solvents.



4 5 6

Scheme 3 Synthesis of 4-formylpiperazine-2,6-dione (**5**) from iminodiacetic acid (**4**)

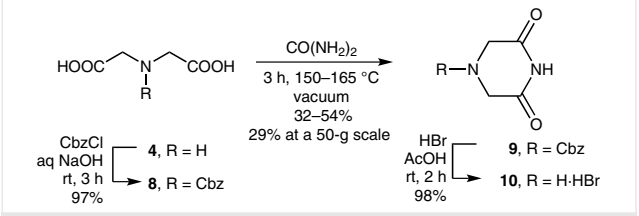
Melting acid **4** with urea gave 4-carbamoylpiperazine-2,6-dione (**7**) in 44% yield (Scheme 4). However, the isolation was complicated by the residues of urea and its decomposition products; thus, column chromatography was needed, which is a serious drawback in large-scale procedures.



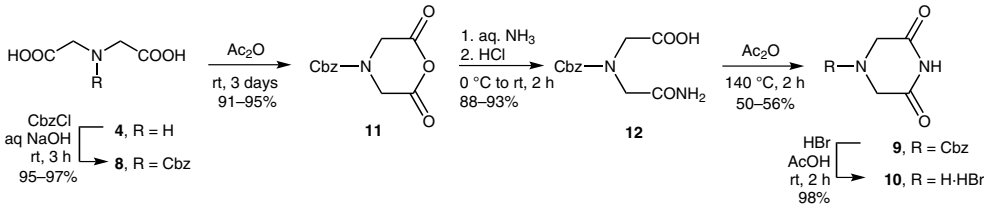
4 7

Scheme 4 Synthesis of 4-carbamoylpiperazine-2,6-dione (**7**) through melting of iminodiacetic acid (**4**) with urea

Therefore, we decided to protect iminodiacetic acid (**4**) with a Cbz group before the cyclization reactions. The Cbz-protected acid **8** was cyclized to Cbz-protected piperazine-2,6-dione (**9**) by using three different approaches involving formamide,[12] ammonium formate in *N*,*N*-dimethylformamide (DMF),[16] or urea.[18] The only successful procedure was the latter: melting protected acid **8** with urea under vacuum gave 32–54% yield of product **9** at a gram scale, but only 29% yield on a 50-gram scale. The crucial step of this process, the urea-assisted cyclization, was hampered by the inconvenient work-up that resulted in variable yields of Cbz-protected piperazine-2,6-dione **9**. Upon cooling the melt, a hard, glass-like material was obtained that had to be mechanically removed from the reaction vessel, ground and further purified. This method is problematic especially at larger scales. Final deprotection with HBr in acetic acid gave the final piperazine-2,6-dione hydrobromide (**10**; Scheme 5).



Scheme 5 Large-scale synthesis of piperazine-2,6-dione hydrobromide **10** through urea-assisted cyclization

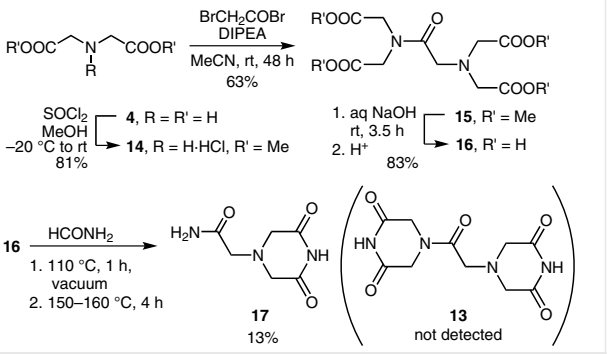


Scheme 6 Large-scale synthesis of piperazine-2,6-dione hydrobromide (**9**) through acetic anhydride assisted cyclization

To optimize the above method for additional convenience, we attempted to replace the urea-assisted cyclization with a three-step imide synthesis.[19,20] In the first step, the Cbz-protected acid **8** was dehydrated to Cbz-protected morpholine-2,6-dione **11** in acetic anhydride.[21] Simple evaporation of the reaction mixture afforded product **11**, which was pure enough to be used in the next step. The obtained Cbz-protected morpholine-2,6-dione **11** was added to ice-cold aqueous ammonia. After complete dissolution, the reaction mixture was acidified to obtain crystalline *N*-Cbz-*N*-(carbamoylmethyl)glycine (**12**).

Product **12** was then heated in acetic anhydride to 140 °C for 2 h. Cooling of the reaction mixture and addition of diethyl ether provided Cbz-protected piperazine-2,6-dione (**9**) in good yields and with high purity. Final deprotection was accomplished by using HBr in acetic acid. The overall yield of this method was 39% at the 0.2-mol scale (15.3 g of **10** was obtained from 26.6 g of **4**). This method repeatedly gave similar yields and was scalable, robust, and convenient; the whole reaction sequence involved only evaporations, crystallizations, and filtrations, with no chromatographic methods being employed (Scheme 6).

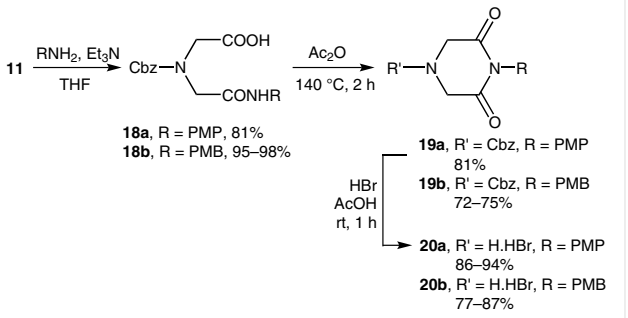
To demonstrate the usefulness of **10** in the synthesis of DEX analogues, we report here the synthesis of a new DEX analogue 4,4′-(1-oxoethane-1,2-diyl)bis(piperazine-2,6-dione) (**13**). It should be noted that attempts to prepare **13** by using the method described previously,[22] starting from tetramethyl glycinamide-*N*,*N*,*N*′,*N*′-tetraacetate (**15**) and through urea-assisted cyclization or standard DEX synthesis[12] starting from glycinamide-*N*,*N*,*N*′,*N*′-tetraacetic acid (**16**), failed. Only 4-(carbamoylmethyl)piperazine-2,6-dione (**17**) was isolated in the second reaction (Scheme 7).



Scheme 7 Unsuccessful preparation of **13** according to standard DEX synthesis

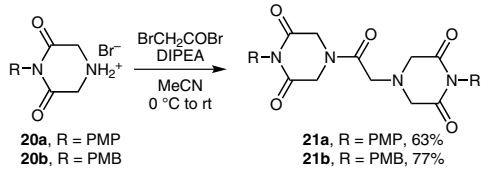
Therefore, **10** was used in the synthesis of DEX analogue **13**. First, we attempted a one-step reaction between two equivalents of **10** and one equivalent of bromoacetyl bromide in acetonitrile or DMF by using various bases. However, these methods gave very low yields (less than 10%).

In an effort to increase the yields of DEX analogue **13**, we attempted to use an imide protection/deprotection strategy, which would increase the solubility of the piperazine-2,6-dione moiety and also protect the imide nitrogen against unwanted side reactions. Thus, we modified the large-scale procedure described in Scheme 6 to be generally applicable for the preparation of 1-substituted piperazine-2,6-diones and used it for the preparation of 1-(4-methoxyphenyl)piperazine-2,6-dione (**20a**) and 1-(4-methoxybenzyl)piperazine-2,6-dione hydrobromides (**20b**, Scheme 8). 4-Methoxyphenyl (PMP) and 4-methoxybenzyl (PMB) substituents were chosen because of their use as protective groups in similar compounds – hydantoins[23] and phthalimides.[24]



Scheme 8 Synthesis of 1-PMP- and 1-PMB-protected piperazine-2,6-dione hydrobromides **20a** and **20b**

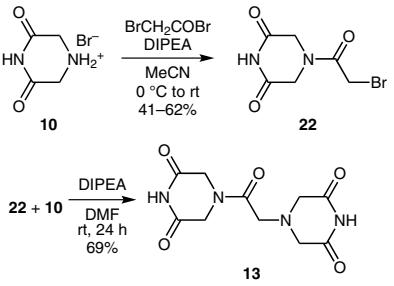
Compounds **20a** and **20b** were used in the synthesis of PMP- and PMB-protected DEX analogues **21a** and **21b**, respectively (Scheme 9). Cleavage of the PMP and PMB groups with ceric ammonium nitrate[23,25–27] and cleavage of PMB through palladium-catalyzed hydrogenolysis,[28] or with trifluoroacetic acid[29,30] or $AlCl_3$[31] failed; the target DEX analogue **13** was not detected in any of these reactions. Unfor-



Scheme 9 Synthesis of PMP- and PMB-protected DEX analogues **21a** and **21b**

tunately, this imide protection/deprotection strategy was not successful in this application.

Hence, we proceeded with unprotected piperazine-2,6-dione hydrobromide (**10**) and developed a two-step procedure that consists of the synthesis and isolation of 4-(2-bromoacetyl)piperazine-2,6-dione (**22**) and its conversion into the target DEX analogue **13**. Gratifyingly, this procedure led to 43% overall yield of the product (Scheme 10).



Scheme 10 Piperazine-2,6-dione-based synthesis of DEX analogue 4,4′-(1-oxoethane-1,2-diyl)bis(piperazine-2,6-dione) (**13**)

To conclude, a novel large-scale synthesis of piperazine-2,6-dione hydrobromide was developed and optimized. The advantage of this procedure is the use of inexpensive materials, satisfactory yields, and a convenient workup without the need for column chromatography. We also demonstrated that 1-substituted piperazine-2,6-dione hydrobromides can be easily prepared by using this method after small modifications.

The synthetic value of the obtained piperazine-2,6-dione hydrobromide was demonstrated by the synthesis of a new DEX analogue 4,4′-(1-oxoethane-1,2-diyl)bis(piperazine-2,6-dione) (**13**), which was not possible to prepare by using standard DEX synthetic approaches. This robust and convenient synthetic methodology opens up possibilities to further vary the DEX structure to explore the importance of the individual structural fragments in the cardioprotective activity of DEX and its mechanism of action.

The structural identities of the prepared compounds were confirmed by ^{1}H NMR and ^{13}C NMR spectroscopic analysis. All chemicals used for synthesis were obtained from Sigma–Aldrich (Schnelldorf, Germany) and used as received. TLC was performed on Merck aluminum plates with silica gel 60 F_{254}. Merck silica gel 60 (0.040–0.063 mm) was used for column chromatography. Melting points were recorded with a Büchi B-545 apparatus and are uncorrected. ^{1}H and ^{13}C NMR spectra were recorded with Varian Mercury Vx BB 300 or VNMR S500 NMR spectrometers (Varian Inc., Palo Alto, CA). Chemical shifts are reported as δ values in parts per million (ppm) and are indirectly referenced to tetramethylsilane (TMS) via the solvent signal. The elemental analysis was carried out with an Automatic Microanalyser EA1110CE (Fisons Instruments S.p.A., Milano, Italy). Atmospheric pressure chemical ionization (APCI) was performed with an Agilent 500 Ion Trap LC/MS (Agilent Technologies, Santa Clara, California, USA) by direct infusion into the detector of the sample dissolved in methanol. HRMS (ESI+, ESI–) experiments were performed with a Q-Exactive Plus Mass Spectrometer (Thermo Scientific, Bremen, Germany) by direct infusion into the detector of the sample dissolved in acetonitrile–water (1:1). IR spectra were measured with a Nicolet 6700 Spectrophotometer in the ATR mode (Thermo Scientific, Waltham, MA, USA).

Synthesis of 4-Formylpiperazine-2,6-dione (5)[16]

Method A: Iminodiacetic acid (13.1 g, 0.1 mol) and ammonium formate (18.9 g, 0.3 mol) were heated to reflux in a mixture of DMF (100 mL) and toluene (40 mL) for 4 h. A reaction vessel equipped with a Dean–Stark trap was used to remove the water formed as an azeotropic mixture. The reaction mixture was then evaporated under vacuum, and the residue was dissolved in MeOH (20 mL). Acetone (20 mL) was added, and the mixture was cooled to 4 °C. After one week, the crystalline solid was collected by filtration.

Yield: 6.9 g (39%) of crude *N*-formyliminodiacetic acid monoammonium salt (**6**) as a beige solid.

^{1}H NMR (500 MHz, DMSO-d_6): δ = 8.01 (s, 1 H), 3.91 (s, 2 H), 3.76 (s, 2 H).

^{1}H NMR (500 MHz, D_2O): δ = 8.10 (s, 1 H), 4.15 (s, 2 H), 4.06 (s, 2 H).

^{13}C NMR (126 MHz, DMSO-d_6): δ = 173.05, 171.63, 164.21, 53.08, 50.16.

^{13}C NMR (126 MHz, D_2O): δ = 175.70, 174.30, 167.02, 51.93, 47.49.

HRMS (ESI–): *m*/*z* [M – H]$^-$ calcd for $C_5H_6NO_5$: 160.02515; found: 160.02271.

Anal. Calcd for $C_5H_{10}N_2O_5$: C, 33.71; H, 5.66; N, 15.73. Found: C, 33.61; H, 5.65; N, 16.39.

Method B: Iminodiacetic acid (13.1 g, 0.1 mol) and ammonium formate (18.9 g, 0.3 mol) were heated in DMF (100 mL) in distillation apparatus at a bath temperature of 150–160 °C for 1 h. Toluene (40 mL) was added and the mixture was heated for 3 h at a bath temperature of 160–170 °C, distilling off the toluene with water together with a part of other volatiles. The reaction mixture was then evaporated under vacuum, the residue was dissolved in MeOH (30 mL), and the mixture was cooled to 4 °C overnight. The crystalline solid was collected by filtration and washed with MeOH (20 mL).

Yield: 1.2 g (8%) of crude 4-formylpiperazine-2,6-dione (**5**);[16] beige solid.

^{1}H NMR (500 MHz, DMSO-d_6): δ = 11.33 (s, 1 H), 8.08 (s, 1 H), 4.29 (s, 2 H), 4.20 (s, 2 H).

^{13}C NMR (126 MHz, DMSO-d_6): δ = 169.54, 169.25, 161.98, 47.32, 42.42.

HRMS (ESI+): *m*/*z* [M + H]$^+$ calcd for $C_5H_7N_2O_3$: 143.04512; found: 143.04457.

4-Carbamoylpiperazine-2,6-dione (7)

Iminodiacetic acid (1 g, 0.0075 mol) and urea (0.9 g, 0.015 mol) were melted together at 155 °C under vacuum (30 mbar) for 1.5 h. The melt was cooled to r.t. and stirred with MeOH (10 mL) at r.t. overnight. The resulting suspension was evaporated, and the product was purified by column chromatography ($CHCl_3$–MeOH, 3:1).

Yield: 0.52 g (44%); white solid; mp 187–188 °C.

IR (ATR): 3350, 3234, 3182, 1726, 1681, 1657, 1469, 1397, 1329, 1293, 1251, 1185, 1119, 1104, 995, 963, 910, 759 cm^{-1}.

^1H NMR (300 MHz, DMSO-d_6): δ = 10.67 (s, 1 H), 7.53 (s, 1 H), 7.17 (s, 1 H), 3.90 (s, 2 H), 3.79 (s, 2 H).

^{13}C NMR (75 MHz, DMSO-d_6): δ = 172.15, 169.97, 157.71, 51.91, 44.53.

Anal. Calcd for $C_5H_7N_3O_3$: C, 38.22; H, 4.49; N, 26.74. Found: C, 37.82; H, 4.4; N, 26.75.

***N*-(Benzyloxycarbonyl)iminodiacetic Acid (8)**[32]

Iminodiacetic acid (26.6 g, 0.2 mol) was dissolved in 2 M NaOH (200 mL) and the resulting solution was cooled in an ice bath. Benzyloxycarbonyl chloride (37.5 g, 31.4 mL, 0.22 mol) and 2 M NaOH (120 mL) were subsequently added to the stirred solution, the reaction mixture was stirred at r.t. for 6 h and then washed with Et_2O (2 × 100 mL). The aqueous phase was acidified with conc. HCl to pH 1–2 and extracted with Et_2O (3 × 200 mL). The organic extract was dried over Na_2SO_4 and evaporated. The product was used in the subsequent reactions without further purification.

Yield: 51 g (95%); colorless viscous oil.

^1H NMR (300 MHz, $CDCl_3$): δ = 10.37 (s, 1 H), 8.53 (s, 1 H), 7.38–7.23 (m, 5 H), 5.14 (s, 2 H), 4.16 (s, 2 H), 4.11 (s, 2 H).

^{13}C NMR (75 MHz, $CDCl_3$): δ = 174.10, 173.39, 156.09, 135.45, 128.55, 128.31, 127.86, 68.50, 50.06, 49.94.

***N*-Benzyloxycarbonyl-*N*-(carbamoylmethyl)glycine (12)**

N-(Benzyloxycarbonyl)iminodiacetic acid (**8**; 51 g, 0.19 mol) was dissolved in acetic anhydride (100 mL) and stirred at r.t. for 3 days. The reaction mixture was then evaporated under high vacuum and the resulting oil crystallized in one day at r.t. under an Ar atmosphere to give *N*-(benzyloxycarbonyl)morpholine-2,6-dione (**11**)[21] as a white solid, which was used immediately without further purification.

N-(Benzyloxycarbonyl)morpholine-2,6-dione (**11**; 44 g, 0.177 mol) was added in several portions into ice cold 25% aqueous ammonia (240 mL) under vigorous stirring. The reaction mixture was stirred for 2 h in an ice bath. During this period, the entire solid dissolved. The reaction mixture was then acidified to pH 1 with 36% HCl, and the formed precipitate was filtered, washed with water and dried.

Yield: 42.7 g (91%; 93% and 88% when started from 5 g and 15 g of **11**, respectively); white crystalline solid; mp 173–174 °C.

IR (ATR): 3365, 3235, 1706, 1646, 1558, 1541, 1472, 1450, 1428, 1400, 1363, 1338, 1258, 1200, 1142, 1017, 974, 916, 773 cm^{-1}.

^1H NMR (500 MHz, DMSO-d_6): δ = 13.02 (s, 1 H), 7.62 (d, *J* = 5.5 Hz, 1 H), 7.39–7.26 (m, 5 H), 7.21 (d, *J* = 7.2 Hz, 1 H), 5.07 (d, *J* = 4.1 Hz, 2 H), 4.01 (d, *J* = 15.4 Hz, 2 H), 3.90 (d, *J* = 13.1 Hz, 2 H).

^{13}C NMR (126 MHz, DMSO-d_6): δ = 171.44 (d, *J* = 10.2 Hz), 171.30, 155.68 (d, *J* = 10.8 Hz), 136.73 (d, *J* = 4.0 Hz), 128.46, 127.88 (d, *J* = 1.6 Hz), 127.24 (d, *J* = 2.2 Hz), 66.74 (d, *J* = 6.8 Hz), 51.30 (d, *J* = 45.4 Hz), 50.60 (d, *J* = 60.1 Hz).

MS (APCI+): *m*/*z* (%) = 223.1 (100) $[M - CO_2 + H]^+$, 267.0 (30) $[M + H]^+$.

Anal. Calcd for $C_{12}H_{14}N_2O_5$: C, 54.13; H, 5.30; N, 10.52. Found: C, 54.01; H, 5.14; N, 10.33.

4-(Benzyloxycarbonyl)piperazine-2,6-dione (9)

*Through urea-catalyzed cyclization of **8***: *N*-(Benzyloxycarbonyl)iminodiacetic acid (**8**; 1.6 g, 0.006 mol) and urea (0.8 g, 0.013 mol) were melted together at 150–165 °C under vacuum (30 mbar) for 3 h. MeOH (15 mL) was added to the cooled melt, and the mixture was stirred for 2 days until none of the melt remained in the mixture. MeOH was then evaporated and the residue was partitioned between $CHCl_3$ (30 mL) and sat. aq $NaHCO_3$ (30 mL). The aqueous phase was then extracted with $CHCl_3$ (2 × 30 mL) and the combined chloroform extracts were dried over Na_2SO_4 and evaporated.

Yield: 0.8 g (54%; 29% when started from 67 g of **8**); white solid.

*Through cyclization of **12***: *N*-Benzyloxycarbonyl-*N*-(carbamoylmethyl)glycine (**12**; 42.7 g, 0.160 mol) and acetic anhydride (160 mL) were heated with stirring at 140 °C for 2 h. The reaction mixture was then cooled and Et_2O (160 mL) was added. After 3 days at 4 °C, the white crystals were filtered off, washed with Et_2O, and dried.

Yield: 19.9 g (50%; 54% and 56% when started from 12 g and 9 g of **12**, respectively); white crystalline solid; mp 170–172 °C.

IR (ATR): 3193, 3089, 2884, 1732, 1711, 1459, 1445, 1428, 1389, 1363, 1344, 1300, 1239, 1199, 1118, 977, 862, 761, 741 cm^{-1}.

^1H NMR (300 MHz, DMSO-d_6): δ = 11.41 (s, 1 H), 7.47–7.27 (m, 5 H), 5.11 (s, 2 H), 4.22 (s, 4 H).

^{13}C NMR (75 MHz, DMSO-d_6): δ = 169.58, 154.10, 136.41, 128.65, 128.27, 127.95, 67.32, 46.58.

Anal. Calcd for $C_{12}H_{12}N_2O_4$: C, 58.06; H, 4.87; N, 11.29. Found: C, 57.79; H, 4.73; N, 11.14.

Piperazine-2,6-dione Hydrobromide (10)

4-(Benzyloxycarbonyl)piperazine-2,6-dione (**9**; 19.9 g, 0.08 mol) was added in several portions to a stirring solution of HBr (33%) in acetic acid (60 mL). The resulting suspension was stirred vigorously for 2 h at r.t., then Et_2O (100 mL) was added and the solid was filtered off, washed with Et_2O and left under vacuum over NaOH in a dessicator for 24 h.

Yield: 15.3 g (98%); white solid; mp 290–300 °C (decomp.).

IR (ATR): 3068, 2946, 2783, 1709, 1674, 1541, 1525, 1427, 1398, 1272, 1224, 1180, 1058, 976, 923, 801, 766 cm^{-1}.

^1H NMR (300 MHz, DMSO-d_6): δ = 11.83 (s, 1 H), 9.84 (br s, 2 H), 4.01 (s, 4 H).

^{13}C NMR (75 MHz, DMSO-d_6): δ = 166.49, 44.21.

Anal. Calcd for $C_4H_7BrN_2O_2$: C, 24.64; H, 3.62; N, 14.36. Found: C, 24.91; H, 3.69; N, 13.99.

Dimethyl Iminodiacetate Hydrochloride (14)[14]

Methanol (500 mL) was cooled to –20 °C, and $SOCl_2$ (36 mL, 0.5 mol) was added dropwise under vigorous stirring while maintaining the temperature of the reaction mixture below –10 °C. Iminodiacetic acid (13.3 g, 0.1 mol) was then added in one portion. The reaction mixture was stirred for 24 h at r.t. and then left to stand at r.t. for two days. The volatiles were evaporated, and the residue were crystallized from methanol.

Yield: 16 g (81%); white crystalline solid.

^1H NMR (300 MHz, D_2O): δ = 4.13 (s, 4 H), 3.85 (s, 6 H).

^{13}C NMR (75 MHz, D_2O): δ = 168.08, 54.12, 47.66.

Anal. Calcd for $C_6H_{12}ClNO_4$: C, 36.47; H, 6.12; N, 7.09. Found: C, 36.52; H, 6.01; N, 6.98.

Tetramethyl Glycinamide-*N*,*N*,*N*′,*N*′-tetraacetate (15)

Dimethyl iminodiacetate hydrochloride (**14**; 16 g, 0.081 mol) and DIPEA (20.93 g, 28.2 mL, 0.162 mol) were added to acetonitrile (150 mL), and then bromoacetyl bromide (8.17 g, 3.55 mL, 0.04 mol) was added dropwise. The reaction mixture was stirred at r.t. for 48 h, then the acetonitrile was evaporated and the residue was dissolved in EtOAc (200 mL). The organic solution was washed with H_2O (3 × 150 mL), dried over Na_2SO_4 and evaporated. The pure product was obtained by column chromatography ($CHCl_3$–EtOAc, 5:1).

Yield: 9.2 g (63%); colorless solid; mp 57–59 °C.

IR (ATR): 2956, 1754, 1741, 1647, 1475, 1433, 1410, 1373, 1304, 1205, 1183, 1146, 1002, 892, 728 cm^{-1}.

^1H NMR (300 MHz, $CDCl_3$): δ = 4.60 (s, 2 H), 4.13 (s, 2 H), 3.74 (s, 3 H), 3.70 (s, 3 H), 3.68 (s, 6 H), 3.65 (s, 2 H), 3.53 (s, 4 H).

^{13}C NMR (75 MHz, $CDCl_3$): δ = 170.86, 170.04, 169.97, 169.40, 56.52, 54.41, 52.26, 52.12, 51.59, 49.83, 48.19.

MS (APCI+): *m*/*z* (%) = 363.3 (100) $[M + H]^+$.

Anal. Calcd for $C_{14}H_{22}N_2O_9$: C, 46.41; H, 6.12; N, 7.73. Found: C, 46.16; H, 6.03; N, 7.55.

Glycinamide-*N*,*N*,*N*′,*N*′-tetraacetic Acid (16)

Tetramethyl glycinamide-*N*,*N*,*N*′,*N*′-tetraacetate (**15**; 2.84 g, 0.0078 mol) was dissolved in 1 M NaOH (31.3 mL), and the mixture was stirred at r.t. for 3.5 h. The reaction mixture was then acidified to pH 3 by using Amberlyst 15. The reaction mixture was filtered and evaporated to dryness and the residue was further dried over P_2O_5. Product **16** was contaminated with inorganic residues, as detected by elemental analysis. Product **16** was used in the next step without further purification.

Yield: 2 g (83%); white solid.

^1H NMR (300 MHz, D_2O): δ = 4.35 (s, 2 H), 4.15 (s, 2 H), 4.06 (s, 2 H), 3.88 (s, 4 H).

^{13}C NMR (75 MHz, D_2O): δ = 173.84, 173.67, 169.80, 166.45, 57.27, 55.06, 51.19, 50.55.

MS (APCI+): *m*/*z* (%) = 307.0 (100), 308.0 (10) $[M + H]^+$.

HRMS (ESI+): *m*/*z* $[M + H]^+$ calcd for $C_{10}H_{15}N_2O_9$: 307.07721; found: 307.07673.

Anal. Calcd for $C_{10}H_{14}N_2O_9$: C, 39.22; H, 4.61; N, 9.15. Found: C, 34.65; H, 4.46; N, 7.76.

4-(Carbamoylmethyl)piperazine-2,6-dione (17)[33]

Crude glycinamide-*N*,*N*,*N*′,*N*′-tetraacetic acid (**16**; 2 g, 0.0065 mol) was heated in formamide (12 mL) under vacuum (30 mbar) at 110 °C for 1 h and then under an argon atmosphere at 155 °C for 3 h. The formamide was then distilled off, and the residue was purified by column chromatography (EtOAc–acetone, 6:1).

Yield: 145 mg (13%); beige solid; mp 210–215 °C (Lit.[33] 212–215 °C).

IR (ATR): 3420, 3286, 3144, 3065, 2837, 1689, 1670, 1615, 1592, 1435, 1397, 1362, 1330, 1296, 1277, 1205, 1163, 1148, 1028, 933, 874, 852 cm^{-1}.

^1H NMR (300 MHz, DMSO-d_6): δ = 11.12 (s, 1 H), 7.39 (s, 1 H), 7.11 (s, 1 H), 3.37 (s, 4 H), 3.06 (s, 2 H).

^{13}C NMR (75 MHz, DMSO-d_6): δ = 171.35, 170.77, 57.85, 55.16.

Anal. Calcd for $C_6H_9N_3O_3$: C, 42.11; H, 5.30; N, 24.55. Found: C, 41.83; H, 5.32; N, 24.52.

N-Benzyloxycarbonyl-*N*-{2-[(4-methoxyphenyl)amino]-2-oxoethyl}glycine (18a)

p-Toluidine (1 g, 0.0081 mol) and triethylamine (1.1 mL, 0.008 mol) were dissolved in anhydrous THF (25 mL), then *N*-(benzyloxycarbonyl)morpholine-2,6-dione (**11**; 2 g, 0.008 mol) was added to the stirred solution, and the mixture was stirred at r.t. for 1 h. The THF was evaporated and the residue was dissolved in 2.5% aq NaOH (40 mL). The aqueous solution was washed with EtOAc (2 × 20 mL) and acidified to pH 1 with HCl. The resulting solid was filtered off and dried.

Yield: 2.4 g (81%); white solid; mp 137–138 °C.

IR (ATR): 3260, 3099, 2834, 1705, 1607, 1562, 1510, 1445, 1401, 1365, 1345, 1323, 1244, 1134, 1031, 982, 833, 769, 748, 733, 716 cm^{-1}.

^1H NMR (500 MHz, DMSO-d_6): δ = 12.99 (s, 1 H), 10.02 (d, *J* = 12.5 Hz, 1 H), 7.52–7.44 (m, 2 H), 7.38–7.30 (m, 2 H), 7.30–7.21 (m, 2 H), 6.92–6.86 (m, 2 H), 5.09 (d, *J* = 4.3 Hz, 2 H), 4.11 (s, 2 H), 4.09 (d, *J* = 2.9 Hz, 2 H), 3.72 (d, *J* = 2.5 Hz, 3 H).

^{13}C NMR (126 MHz, DMSO-d_6): δ = 171.90 (d, *J* = 3.3 Hz), 167.13 (d, *J* = 15.5 Hz), 155.78 (d, *J* = 3.8 Hz), 155.47 (d, *J* = 5.1 Hz), 136.73 (d, *J* = 5.7 Hz), 131.99 (d, *J* = 7.3 Hz), 128.46 (d, *J* = 12.9 Hz), 127.90 (d, *J* = 10.9 Hz), 127.24 (d, *J* = 13.3 Hz), 120.82 (d, *J* = 10.2 Hz), 114.06, 66.81 (d, *J* = 10.4 Hz), 55.34, 52.06 (d, *J* = 31.1 Hz), 50.53 (d, *J* = 65.9 Hz).

Anal. Calcd for $C_{19}H_{20}N_2O_6$: C, 61.28; H, 5.41; N, 7.52. Found: C, 61.62; H, 5.75; N, 7.16.

N-Benzyloxycarbonyl-*N*-{2-[(4-methoxybenzyl)amino]-2-oxoethyl}glycine (18b)

4-Methoxybenzylamine (1.23 g, 0.009 mol) and triethylamine (1.1 mL, 0.008 mol) were dissolved in anhydrous THF (20 mL), then *N*-(benzyloxycarbonyl)morpholine-2,6-dione (**11**; 2 g, 0.008 mol) was added to the stirred solution and the mixture was stirred at r.t. for 2 h. The THF was then evaporated and the residue was dissolved in 2.5% aq NaOH (40 mL). The aqueous solution was washed with Et_2O (2 × 20 mL) and acidified to pH 1 with HCl. The aqueous layer was extracted with EtOAc (3 × 40 mL), and the combined organic extract was dried over Na_2SO_4 and evaporated.

Yield: 3.04 g (98%; 95% when started from 10 g of **11**); colorless oil that crystallized after several weeks; mp 149–150 °C.

IR (ATR): 3303, 1717, 1655, 1543, 1515, 1469, 1446, 1410, 1398, 1364, 1316, 1243, 1230, 1183, 1143, 1036, 970, 958, 915, 819, 774, 761, 695 cm^{-1}.

^1H NMR (500 MHz, DMSO-d_6): δ = 12.97 (s, 1 H), 8.66–8.50 (m, 1 H), 7.42–7.26 (m, 5 H), 7.15 (dd, *J* = 29.6, 8.6 Hz, 2 H), 6.84 (dd, *J* = 34.9, 8.6 Hz, 2 H), 5.08 (d, *J* = 7.1 Hz, 2 H), 4.23 (dd, *J* = 12.6, 5.8 Hz, 2 H), 4.04 (d, *J* = 12.5 Hz, 2 H), 3.99 (d, *J* = 9.3 Hz, 2 H), 3.72 (d, *J* = 9.1 Hz, 3 H).

^{13}C NMR (126 MHz, DMSO-d_6): δ = 171.57 (d, *J* = 4.2 Hz), 168.92 (d, *J* = 11.9 Hz), 158.41 (d, *J* = 4.1 Hz), 155.75 (d, *J* = 5.2 Hz), 136.72 (d, *J* = 7.2 Hz), 131.13 (d, *J* = 3.4 Hz), 128.70 (d, *J* = 2.8 Hz), 128.52, 127.94, 127.32 (d, *J* = 5.4 Hz), 113.85 (d, *J* = 5.9 Hz), 66.84, 55.24, 51.59 (d, *J* = 37.0 Hz), 50.61 (d, *J* = 68.2 Hz), 41.77 (d, *J* = 3.0 Hz).

Anal. Calcd for $C_{20}H_{22}N_2O_6$: C, 62.17; H, 5.74; N, 7.25. Found: C, 61.8; H, 5.34; N, 7.37.

4-Benzyloxycarbonyl-1-(4-methoxyphenyl)piperazine-2,6-dione (19a)

N-Benzyloxycarbonyl-*N*-{2-[(4-methoxyphenyl)amino]-2-oxoethyl}glycine (**18a**; 2 g, 0.0056 mol) and acetic anhydride (8 mL) were heated to 140 °C under an Ar atmosphere with stirring for 2 h. The reaction mixture was then evaporated under vacuum, the residue was dissolved in EtOAc (50 mL), and the organic solution was washed with sat. aq $NaHCO_3$ (2 × 25 mL) and H_2O (30 mL). The organic fraction was dried over Na_2SO_4 and evaporated. The pure product was obtained by column chromatography (hexane–EtOAc, 2:1).

Yield: 1.53 g (81%); colorless oil that solidified into an amorphous colorless solid; mp 118–119 °C.

IR (ATR): 1746, 1693, 1609, 1511, 1456, 1363, 1289, 1232, 1199, 1108, 1030, 970, 831, 759, 723 cm^{-1}.

^1H NMR (500 MHz, DMSO-d_6): δ = 7.45–7.32 (m, 5 H), 7.10 (d, *J* = 8.9 Hz, 2 H), 6.99 (d, *J* = 8.9 Hz, 2 H), 5.17 (s, 2 H), 4.48 (s, 4 H), 3.78 (s, 3 H).

^{13}C NMR (126 MHz, DMSO-d_6): δ = 168.59, 159.15, 153.94, 136.38, 129.83, 128.64, 128.26, 127.97, 126.70, 114.23, 67.34, 55.48 (d, *J* = 3.8 Hz), 47.44.

Anal. Calcd for $C_{19}H_{18}N_2O_5$: C, 64.40; H, 5.12; N, 7.91. Found: C, 64.05; H, 5.32; N, 7.55.

4-Benzyloxycarbonyl-1-(4-methoxybenzyl)piperazine-2,6-dione (19b)

N-Benzyloxycarbonyl-*N*-{2-[(4-methoxybenzyl)amino]-2-oxoethyl}glycine (**18b**; 5 g, 0.0129 mol) and acetic anhydride (20 mL) were heated to 140 °C under an Ar atmosphere with stirring for 2 h. The reaction mixture was then evaporated under vacuum, the residue was dissolved in EtOAc (70 mL), and the organic solution was washed with sat. aq $NaHCO_3$ (2 × 40 mL) and H_2O (100 mL). The organic fraction was dried over Na_2SO_4 and evaporated. The pure product was obtained by column chromatography (hexane–EtOAc, 2:1).

Yield: 3.43 g (72%; 75% when started from 15 g of **18b**); colorless oil.

IR (ATR): 2957, 1684, 1611, 1513, 1442, 1385, 1344, 1310, 1237, 1214, 1178, 1115, 1031, 970, 921, 892, 852, 822, 759 cm^{-1}.

^1H NMR (500 MHz, DMSO-d_6): δ = 7.40–7.30 (m, 5 H), 7.17 (d, *J* = 8.7 Hz, 2 H), 6.84 (d, *J* = 8.7 Hz, 2 H), 5.12 (s, 2 H), 4.75 (s, 2 H), 4.41 (s, 4 H), 3.71 (s, 3 H).

^{13}C NMR (126 MHz, DMSO-d_6): δ = 168.49, 158.62, 153.96, 136.31, 129.42, 128.82, 128.62, 128.24, 127.91, 113.87, 67.35, 55.22 (d, *J* = 7.1 Hz), 47.22, 41.54.

MS (ESI+): *m*/*z* (%) = 391.12 (100) $[M + Na]^+$.

1-(4-Methoxyphenyl)piperazine-2,6-dione Hydrobromide (20a)

4-Benzyloxycarbonyl-1-(4-methoxyphenyl)piperazine-2,6-dione (**19a**; 1.4 g, 0.004 mol) was added at r.t. in several portions to stirring 33% HBr in acetic acid (7 mL). The resulting suspension was stirred vigorously at r.t. for 1 h, then Et_2O (30 mL) was added and the solid was filtered off, washed with Et_2O, and left under vacuum over NaOH in a dessicator for 24 h.

Yield: 1.12 g (94%; 86% when started from 3 g of **19a**); white solid; mp 253–256 °C.

IR (ATR): 3057, 2748, 2617, 1757, 1688, 1610, 1575, 1513, 1469, 1391, 1359, 1307, 1275, 1259, 1241, 1182, 1068, 1027, 937, 862, 839, 792 cm^{-1}.

^1H NMR (500 MHz, DMSO-d_6): δ = 7.09 (d, *J* = 9.0 Hz, 2 H), 7.03 (d, *J* = 9.0 Hz, 2 H), 4.25 (s, 4 H), 3.78 (s, 3 H).

^{13}C NMR (126 MHz, DMSO-d_6): δ = 165.84, 159.41, 129.55, 125.91, 114.53, 55.56 (d, *J* = 4.0 Hz), 45.49.

Anal. Calcd for $C_{11}H_{13}BrN_2O_3$: C, 43.87; H, 4.35; N, 9.30. Found: C, 43.72; H, 4.28; N, 8.96.

1-(4-Methoxybenzyl)piperazine-2,6-dione Hydrobromide (20b)

HBr (33% in acetic acid, 3 mL) was added to 4-benzyloxycarbonyl-1-(4-methoxybenzyl)piperazine-2,6-dione (**19b**; 0.9 g, 0.0024 mol), and the resulting mixture was stirred vigorously at r.t. for 1 h. Et_2O (20 mL) was added and the solid was filtered off, washed with Et_2O and left under vacuum over NaOH in a dessicator for 24 h.

Yield: 0.67 g (87%; 77% when started from 7 g of **19b**); white solid; mp 185–188 °C (with decomp.).

IR (ATR): 3007, 2942, 2837, 1741, 1693, 1613, 1514, 1432, 1393, 1343, 1303, 1250, 1217, 1175, 1114, 1030, 938, 893, 769 cm^{-1}.

^1H NMR (500 MHz, DMSO-d_6): δ = 7.24 (d, *J* = 8.7 Hz, 2 H), 6.86 (d, *J* = 8.7 Hz, 2 H), 4.78 (s, 2 H), 4.21 (s, 4 H), 3.71 (s, 3 H).

^{13}C NMR (126 MHz, DMSO-d_6): δ = 165.67, 158.75, 129.78 (d, *J* = 7.8 Hz), 128.16, 113.81, 55.32 (d, *J* = 12.6 Hz), 44.87, 41.78.

Anal. Calcd for $C_{12}H_{15}BrN_2O_3$: C, 45.73; H, 4.80; N, 8.89. Found: C, 46.10; H, 4.72; N, 8.66.

4,4′-(1-Oxoethane-1,2-diyl)bis[1-(4-methoxyphenyl)piperazine-2,6-dione] (21a)

Bromoacetyl bromide (0.34 g, 0.15 mL, 0.00168 mol) was slowly added to a solution of 1-(4-methoxyphenyl)piperazine-2,6-dione hydrobromide (**20a**; 1 g, 0.0033 mmol) and DIPEA (0.87 g, 1.17 mL, 0.0067 mmol) in MeCN (30 mL) under an inert atmosphere at 0 °C. The reaction mixture was stirred at 0 °C for 15 min and then at r.t. for 7 days. The solvent was evaporated under reduced pressure, and the residue was dissolved in EtOAc (35 mL) and washed with 0.5% HCl (35 mL). The organic layer was separated, dried over anhydrous sodium sulfate, and evaporated under reduced pressure. The product was purified by column chromatography (EtOAc).

Yield: 0.5 g (63%); white solid; mp 262–265 °C.

IR (ATR): 3014, 2838, 1737, 1687, 1642, 1612, 1512, 1381, 1359, 1301, 1250, 1185, 1170, 1030, 979, 834, 724 cm^{-1}.

^1H NMR (500 MHz, DMSO-d_6): δ = 7.07 (d, *J* = 8.9 Hz, 2 H), 7.01 (d, *J* = 8.9 Hz, 2 H), 6.98–6.93 (m, 4 H), 4.61 (s, 2 H), 4.53 (s, 2 H), 3.77 (s, 6 H), 3.75 (s, 4 H), 3.69 (s, 2 H).

^{13}C NMR (126 MHz, DMSO-d_6): δ = 170.29, 167.93, 159.15, 159.05, 129.88, 129.80, 126.76, 126.65, 114.23, 114.18, 55.72, 55.48, 55.46, 48.86, 45.57.

MS (APCI+): *m*/*z* (%) = 481.1 (100) $[M + H]^+$.

Anal. Calcd for $C_{24}H_{24}N_4O_7$: C, 60.00; H, 5.03; N, 11.66. Found: C, 59.98; H, 5.06; N, 11.26.

4,4′-(1-Oxoethane-1,2-diyl)bis[1-(4-methoxybenzyl)piperazine-2,6-dione] (21b)

Bromoacetyl bromide (0.32 g, 0.14 mL, 0.00159 mol) was slowly added to a solution of 1-(4-methoxybenzyl)piperazine-2,6-dione hydrobromide (**20b**; 1 g, 0.0032 mol) and DIPEA (0.82 g, 1.08 mL, 0.00635 mol) in MeCN (30 mL) under an inert atmosphere at 0 °C. The reaction mixture was stirred at 0 °C for 15 min and then at r.t. for 24 h. The solvent was evaporated under reduced pressure and the residue was dissolved in EtOAc (35 mL) and washed with 0.5% HCl (35 mL). The organic layer was separated, dried over anhydrous sodium sul-

fate, and evaporated. The product was purified by column chromatography (EtOAc–hexane, 7:1).

Yield: 0.62 g (77%); white solid; mp 77–80 °C.

IR (ATR): 2838, 1737, 1680, 1612, 1514, 1430, 1389, 1344, 1302, 1247, 1178, 1112, 1030, 973, 894, 823, 764 cm^{-1}.

^1H NMR (500 MHz, $CDCl_3$): δ = 7.32 (d, *J* = 8.6 Hz, 4 H), 6.82 (d, *J* = 8.8 Hz, 4 H), 4.87 (s, 2 H), 4.86 (s, 2 H), 4.46 (s, 2 H), 4.30 (s, 2 H), 3.78 (s, 3 H), 3.77 (s, 3 H), 3.56 (s, 4 H), 3.38 (s, 2 H).

^{13}C NMR (126 MHz, $CDCl_3$): δ = 168.84, 166.29, 159.28, 159.05, 130.79, 130.43, 128.58, 127.99, 113.84, 113.76, 56.78, 55.79, 55.20, 48.48, 45.26, 42.46, 41.83.

MS (APCI+): *m*/*z* (%) = 509.2 (100) $[M + H]^+$.

Anal. Calcd for $C_{26}H_{28}N_4O_7$: C, 61.41; H, 5.55; N, 11.02. Found: C, 61.46; H, 5.63; N, 10.68.

4-(2-Bromoacetyl)piperazine-2,6-dione (22)

Bromoacetyl bromide (5.17 g, 2.24 mL, 0.0256 mol) was slowly added to a suspension of piperazine-2,6-dione hydrobromide (**10**; 5 g, 0.0256 mol) and DIPEA (6.63 g, 8.77 mL, 0.051 mol) in MeCN (50 mL) at 0 °C in an ice bath. The reaction mixture was stirred for 15 min at 0 °C and then at r.t. for 1 h. The solvent was then evaporated, and the product was purified by column chromatography ($CHCl_3$–EtOAc, 1:1).

Yield: 3.73 g (62%); white solid; mp 157–159 °C (with decomp.).

IR (ATR): 3092, 2831, 1713, 1698, 1628, 1472, 1376, 1310, 1276, 1240, 1187, 1118, 1046, 976, 857 cm^{-1}.

^1H NMR (500 MHz, acetone-d_6): δ = 10.20 (s, 1 H), 4.50 (s, 2 H), 4.42 (s, 2 H), 4.22 (s, 2 H).

^{13}C NMR (126 MHz, acetone-d_6): δ = 169.13, 168.73, 166.16, 49.63, 45.53, 27.15.

Anal. Calcd for $C_6H_7BrN_2O_3$: C, 30.66; H, 3.00; N, 11.92. Found: C, 30.31; H, 2.96; N, 11.62.

4,4′-(1-Oxoethane-1,2-diyl)bis(piperazine-2,6-dione) (13)

DIPEA (1.1 g, 1.48 mL, 0.0085 mol) was added to a suspension of piperazine-2,6-dione hydrobromide (**10**; 1.66 g, 0.0085 mol) in DMF (13 mL) under an argon atmosphere. The suspension became a solution in several seconds. Then, 4-(2-bromoacetyl)piperazine-2,6-dione (**22**; 2 g, 0.0085 mol) in DMF (7 mL) was added, followed by the addition of further DIPEA (1.1 g, 1.48 mL, 0.00851 mol, 1 equiv). The reaction mixture was stirred at r.t. for 24 h. Upon completion, as determined by TLC (EtOAc–acetone, 1:1), the solvent was evaporated under reduced pressure. The crude product was washed with warm MeCN (30 mL) and with H_2O (2 × 15 mL) to give the pure product (57%). An additional 12% of the pure product crystallized from MeCN upon cooling. The product was filtered, washed with water and dried.

Yield: 1.57 g (69%); white solid; mp 247–250 °C.

IR (ATR): 3016, 2834, 1715, 1617, 1488, 1410, 1325, 1299, 1248, 1224, 1181, 1147, 1027, 887, 837, 741, 722 cm^{-1}.

^1H NMR (500 MHz, DMSO-d_6): δ = 11.36 (s, 1 H), 11.10 (s, 1 H), 4.30 (s, 2 H), 4.24 (s, 2 H), 3.50 (s, 2 H), 3.42 (s, 4 H).

^{13}C NMR (126 MHz, DMSO-d_6): δ = 171.24, 169.56, 169.35, 167.80, 55.94, 54.58, 47.67, 44.40.

MS (APCI+): *m*/*z* (%) = 269.0 (100) $[M + H]^+$.

Anal. Calcd for $C_{10}H_{12}N_4O_5$: C, 44.78; H, 4.51; N, 20.89. Found: C, 44.39; H, 4.53; N, 20.55.

Acknowledgment

This work was supported by the Czech Science Foundation (project no. 13-15008S).

Supporting Information

Supporting information for this article is available online at http://dx.doi.org/10.1055/s-0035-1562618.

References

(1) Gianni, L.; Herman, E. H.; Lipshultz, S. E.; Minotti, G.; Sarvazyan, N.; Sawyer, D. B. *J. Clin. Oncol.* **2008**, *26*, 3777.
(2) Jirkovsky, E.; Lencova-Popelova, O.; Hroch, M.; Adamcova, M.; Mazurova, Y.; Vavrova, J.; Micuda, S.; Simunek, T.; Gersl, V.; Sterba, M. *Toxicology* **2013**, *311*, 191.
(3) Simunek, T.; Sterba, M.; Popelova, O.; Adamcova, M.; Hrdina, R.; Gersl, V. *Pharmacol. Rep.* **2009**, *61*, 154.
(4) Sterba, M.; Popelova, O.; Vavrova, A.; Jirkovsky, E.; Kovarikova, P.; Gersl, V.; Simunek, T. *Antioxid. Redox Signal.* **2013**, *18*, 899.
(5) Hasinoff, B. B.; Hellmann, K.; Herman, E. H.; Ferrans, V. J. *Curr. Med. Chem.* **1998**, *5*, 1.
(6) Classen, S.; Olland, S.; Berger, J. M. *Proc. Natl. Acad. Sci. U.S.A.* **2003**, *100*, 10629.
(7) Zhang, S.; Liu, X.; Bawa-Khalfe, T.; Lu, L.-S.; Lyu, Y. L.; Liu, L. F.; Yeh, E. T. H. *Nat. Med.* **2012**, *18*, 1639.
(8) Herman, E. H.; Elhage, A. N.; Creighton, A. M.; Witiak, D. T.; Ferrans, V. J. *Res. Commun. Mol. Pathol. Pharmacol.* **1985**, *48*, 39.
(9) Herman, E. H.; Zhang, J.; Hasinoff, B. B.; Chadwick, D. P.; Clark, J. R.; Ferrans, V. J. *Cancer Chemother. Pharmacol.* **1997**, *40*, 400.
(10) Jirkovska-Vavrova, A.; Roh, J.; Lencova-Popelova, O.; Jirkovsky, E.; Hruskova, K.; Potuckova-Mackova, E.; Jansova, H.; Haskova, P.; Martinkova, P.; Eisner, T.; Kratochvil, M.; Sus, J.; Machacek, M.; Vostatkova-Tichotova, L.; Gersl, V.; Kalinowski, D. S.; Muller, M. T.; Richardson, D. R.; Vavrova, K.; Sterba, M.; Simunek, T. *Toxicol. Res. (Cambridge, U. K.)* **2015**, *4*, 1098.
(11) Prevention and treatment of cardiac conditions: Cross, M. R.; Dilly, S. G.; Scaife, M. US2008/275036, **2008**.
(12) Bis diketopiperazines: Creighton, A. M. US3941790, **1976**.
(13) Barot, N. R.; Elvidge, J. A. *J. Chem. Soc., Perkin Trans. 1* **1972**, 1009.
(14) Mancilla, T.; Carrillo, L.; Zamudio-Rivera, L. S.; Beltran, H. I.; Farfan, N. *Org. Prep. Proced. Int.* **2002**, *34*, 87.
(15) Hou, X.; Ge, Z.; Wang, T.; Guo, W.; Wu, J.; Cui, J.; Lai, C.; Li, R. *Arch. Pharm.* **2011**, *344*, 320.
(16) Shvedaite, I. P. *Chem. Heterocycl. Compd.* **1995**, *31*, 63.
(17) Dinsmore, C. J.; Beshore, D. C. *Tetrahedron* **2002**, *58*, 3297.
(18) Čapek, K.; Čapková, J.; Jarý, J.; Trška, P. *Collect. Czech. Chem. Commun.* **1984**, *49*, 1914.
(19) Cignarella, G.; Nathanson, G. *J. Org. Chem.* **1961**, *26*, 1500.
(20) Henry, D. W. *J. Heterocycl. Chem.* **1966**, *3*, 503.
(21) Gregory, H.; Morley, J. S.; Smith, J. M.; Smithers, M. J. *J. Chem. Soc. C* **1968**, 715.
(22) Snapka, R. M.; Woo, S. H.; Blokhin, A. V.; Witiak, D. T. *Biochem. Pharmacol.* **1996**, *52*, 543.
(23) Pham, T. Q.; Pyne, S. G.; Skelton, B. W.; White, A. H. *Tetrahedron Lett.* **2002**, *43*, 5953.
(24) Hennessy, E. J.; Buchwald, S. L. *J. Org. Chem.* **2005**, *70*, 7371.
(25) Kronenthal, D. R.; Han, C. Y.; Taylor, M. K. *J. Org. Chem.* **1982**, *47*, 2765.

(26) Yamaura, M.; Suzuki, T.; Hashimoto, H.; Yoshimura, J.; Okamoto, T.; Shin, C. *Bull. Chem. Soc. Jpn.* **1985**, *58*, 1413.
(27) Morris, J.; Wishka, D. G. *J. Org. Chem.* **1991**, *56*, 3549.
(28) Rigby, J. H.; Gupta, V. *Synlett* **1995**, 547.
(29) Chida, N.; Ohtsuka, M.; Ogawa, S. *J. Org. Chem.* **1993**, *58*, 4441.
(30) Brooke, G. M.; Mohammed, S.; Whiting, M. C. *Chem. Commun.* **1997**, 1511.
(31) Akiyama, T.; Takesue, Y.; Kumegawa, M.; Nishimoto, H.; Ozaki, S. *Bull. Chem. Soc. Jpn.* **1991**, *64*, 2266.
(32) Abalain, C.; Langlois, M. *Eur. J. Med. Chem.* **1998**, *33*, 155.
(33) Shvedaite, I. P. *Chem. Heterocycl. Compd.* **1998**, *34*, 1185.