A Highly Efficient Method for the N-Alkylation of Aminopyrazines: Synthesis of Hydrophilic Red Fluorescent Dyes

Amruta R. Poreddy,* Bethel Asmelash, William L. Neumann,¹ Richard B. Dorshow

Covidien Pharmaceuticals, 675 McDonnell Boulevard, Hazelwood, MO 63042, USA Fax +1(314)6548900; E-mail: amruta.poreddy@covidien.com *Received 12 February 2010; revised 5 April 2010*

Abstract: A robust and scalable method was developed for the synthesis of N,N'-dialkylated aminopyrazines by a reductive amination route. These new fluorescent pyrazine analogues were shown to absorb and emit light at relatively long wavelengths (~50 nm) compared to the corresponding diaminopyrazines. The utility of these compounds was demonstrated by the synthesis of hydrophilic fluorescent probes with potential diagnostic applications.

Key words: GFR, renal function, pyrazine, fluorescence, reductive amination

Current medical guidelines are gravitating toward the use of glomerular filtration rate (GFR) as the most appropriate measure of renal function in the determination of kidney health or illness. Interventions based on GFR values are then applied to patients. Typically, GFR is not measured directly; the most common method of assessing renal function in the clinic involves determination of serum concentration of an endogenous blood marker such as creatinine. Serum creatinine has an inverse relationship to GFR,² and is highly dependent on age, gender, muscle mass, and many other anthropometric variables, thus making it a poor surrogate for a renal function assay.³ The best methodology for measurement of GFR is by clearance of exogeneous tracer agents. Several such agents have been employed such as inulin,⁴ iothalamate,⁵ Gd-DTPA,⁶ and ^{99m}Tc-DTPA.⁷ However, all suffer from drawbacks such as the use and disposal of radioactivity, and the laborious ex-vivo handling of blood and urine samples. A rapid, dynamic (i.e., continuous, real-time), and accurate procedure for measuring renal excretion rate is long overdue and, in fact, considerable effort is now being directed towards developing exogenous GFR agents for rapid real-time assessment of renal function using nonradioactive methods.8

In continuation of our efforts to develop exogenous GFR markers that absorb and emit light in the visible region, we have identified simple derivatives of 2,5-diaminopyrazine **1** as low molecular weight scaffolds with surprisingly bright emission in the yellow-to-red region of the electromagnetic spectrum (Figure 1).⁹ A wide variety of hydrophilic pyrazine dyes, which include amino acid and poly(ethylene glycol) (PEG) functionalized analogues of the type **2**, have been developed as new optical tracers that

function as GFR agents. These new conjugates retain the photophysical properties of the parent pyrazine **1b**, with typical absorption and emission maxima around 450 and 560 nm, and Stokes' shifts generally greater than 100 nm.¹⁰ Dyes that absorb and emit at longer wavelengths should further enhance the optical detection method due to their increased tissue penetration resulting from the minimal absorption of hemoglobin, water, and lipids.¹¹ Consequently, an efficient method was developed for the synthesis of *N*,*N*'-dialkylaminopyrazines via a reductive amination route, the details of which are presented in this paper.¹²



Figure 1 Diaminopyrazine scaffold and its dicarboxamide derivatives

Alkyl substitution on the amino groups of pyrazine 1 was shown to significantly increase the wavelength of both absorption and emission maxima.^{9,13} Whereas alkylation of nitrile 1a with alkyl halides in the presence of sodium hydride or powdered sodium hydroxide in N,N-dimethylacetamide (DMA) produced N,N,N',N'-tetraalkyl products (6-28%), alkylation of esters of 1b resulted only in the formation of the corresponding N,N'-dialkyl compounds. For example, esterification of 1b with alkyl/arylalkyl halides in the presence of 1,8-diazabicyclo[5.4.0]undec-7ene in N,N-dimethylformamide or N,N-dimethylacetamide at ambient temperature has been reported to give variable yields (50% average) of the diester 3 (Scheme 1).^{9,13a} Further treatment of 3 with alkyl/arylalkyl halides at elevated temperatures (70-120 °C) gave rather low yields of N,N'-dialkylaminopyrazines 4 (3-14%). Exhaustive alkylation of **1b** with alkyl or arylalkyl halides in 1,8-diazabicyclo[5.4.0]undec-7-ene and N,Ndimethylacetamide at 70-120 °C was also found to be poor, with 7–28% overall yield of 4.^{13a} Even with simple 2-aminopyrazines, base-induced alkylations with alkyl halides were found to give only moderate yields ($\sim 50\%$) of N-alkyl-2-aminopyrazine derivatives.¹⁴ Thus, alkylation of aminopyrazines in the presence of a strong base is not a synthetically useful reaction because it often leads to

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complex mixtures. The reaction is further complicated by over-alkylation and decomposition of the pyrazine core under harsh reaction conditions employed in the procedure.^{13a} Thus, there is a need to develop a simple and efficient process for alkylating aminopyrazines. In an effort to improve the N,N'-dialkylation of pyrazine amines, a reductive amination route was explored.¹⁵ Though a myriad of reagents are available for direct and indirect reductive amination, the former approach, which involves sodium triacetoxyborohydride as the reducing agent, proved to be the most convenient method.¹⁶ Consequently, diethyl ester **3a** ($R^1 = Et$), which was prepared in 68% yield from 1b, was reacted with benzaldehyde in the presence of galcial acetic acid and sodium triacetoxyborohydride in 1,2dichloroethane overnight at room temperature, to yield the corresponding bisbenzyl derivative 5 in 80% yield. The overall yield of 54% from 1b is clearly superior to ~3% realized earlier using base-induced alkylation.^{13a}



Scheme 1 Alkylation of aminopyrazines



Scheme 2 Synthesis of N,N'-dialkylaminopyrazines 7a-k by reductive alkylation of aminopyrazines

^a Isolated yield.

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Encouraged by this early success, we examined the scope of the reductive amination reaction with the key intermediate 6a, which was prepared in 87% yield from 1b by standard coupling reaction with N-Boc-ethylenediamine using N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide and 1-hydroxybenzotriazole (Scheme 2).¹⁷ Thus, compound 6a was reductively alkylated with propionaldehyde in the presence of acetic acid and sodium triacetoxyborohydride in 1,2-dichloroethane to give 7a in 80% yield. No trace of the over-alkylated tetrapropyl derivatives were formed in the reaction. Since the reaction was found to be very clean and robust, the methodology was extended to a variety of aldehydes, leading to the corresponding products **7b-h** in excellent yields (Table 1). The sole exception was 7f (29%), wherein the reaction was not very clean and never went to completion because the highly reactive methoxy acetaldehyde was prone to undergo aldol condensation reactions. Similarly, compound 6b, which was prepared in 79% yield from 1b and N-carbobenzoxy-1,2-diaminoethane using N-(3-dimethylaminopropyl)-N'ethylcarbodiimide/1-hydroxybenzotriazole coupling, was smoothly transformed into 7i (70%) and 7i (77%) by reaction with the appropriate aldehyde under reductive amination conditions. The orthogonal protecting groups on the side chains of 7g and 7i introduce the flexibility to conjugate different moieties as desired later on. Compound 7k, a four carbon variant of 7h, was synthesized in 51% overall yield by initial coupling of 1b with N-1-Boc-1,4-diaminobutane, followed by reductive alkylation of the bisamide intermediate 6c with 4-tert-butoxycarbonylaminobutanal.¹⁸ Towards this end, reductive alkylation of with propionaldehyde under Borch conditions **6a** (NaBH₃CN, MeOH)¹⁹ was attempted, however, the reaction could not be driven to completion even with a large excess of reagent over a prolonged period of time (5 d). This could be partially due to relative insolubility of **6a** in the methanol solvent, and it seems that sodium triacetoxy-

Table 1	Synthesis	of Compound	ds 7a–k
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Product	R ¹	\mathbb{R}^2	Yield (%) ^a
7a	(CH ₂) ₂ NHBoc	Et	80
7b	(CH ₂) ₂ NHBoc	<i>n</i> -Pr	62
7c	(CH ₂) ₂ NHBoc	Ph	72
7d	(CH ₂) ₂ NHBoc	4-MeOC ₆ H ₄	85
7e	(CH ₂) ₂ NHBoc	$4-O_2NC_6H_4$	82
7f	(CH ₂) ₂ NHBoc	CH ₂ OMe	29
7g	(CH ₂) ₂ NHBoc	(CH ₂) ₂ CO ₂ Me	95
7h	(CH ₂) ₂ NHBoc	CH ₂ NHBoc	92
7i	(CH ₂) ₂ NHCbz	CH ₂ NHBoc	70
7j	(CH ₂) ₂ NHCbz	(CH ₂) ₂ NHCbz	77
7k	(CH ₂) ₄ NHBoc	(CH ₂) ₃ NHBoc	75

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borohydride in an aprotic solvent such as 1,2-dichloroethane seems to be the reagent of choice. It should be mentioned here that, even under optimized conditions, the nitrile **1a** failed to undergo reductive amination with propionaldehyde due to its extreme insolubility in common organic solvents that were suitable for the reaction, such as 1,2-dichloroethane and tetrahydrofuran.



Scheme 3 Reductive alkylation of 6a with cyclohexanone

Reductive alkylation of **6a** with ketones such as cyclohexanone was found to be very sluggish, and multiple additions of reagents over a period of time were needed to complete the reaction. The product **10a** was isolated in only moderate yield (46%) due to undesired N-ethylation that led to both **10b** and **10c** as byproducts (34% combined; Scheme 3). The formation of these byproducts can be rationalized by generation of acetaldehyde in situ, and subsequent competing reductive amination under the prolonged reaction conditions. In fact, N-ethylation was shown to be a major process in reductions with sodium borohydride in neat acetic acid, and is believed to proceed through acetaldehdye formation.²⁰

As mentioned above, the base-induced alkylation of the weakly basic and poorly nucleophilic 2-aminopyrazines is hampered by low yields and also by the formation of quaternary salts at the ring nitrogen atoms. Moreover, depending on the base strength, elimination from alkyl halides could occur. The reductive amination reported herein employs acid catalysis to: (i) enhance the electrophilicity of the carbonyl component and to compensate for the poor nucleophilicity of the amino groups; (ii) drive formation of the intermediate imine by enhancing the rate of the dehydration step, and (iii) increase the electrophilicity of the imino group by reduction through the iminum ion intermediate. These factors provide an alkylation methodology with far greater synthetic utility than the base-mediated alkylation. This chemistry has become a 'work-horse' technology for the systematic synthesis of mono-N-functionalized pyrazine dyes.

The small molecule pyrazines described above are all lipophilic and possess handles for further chemical modification. A few examples highlighting the utility of these compounds as intermediates in the synthesis of long wavelength hydrophilic conjugates are shown in the Scheme 4. Thus, initial deprotection of Boc groups from the intermediate 7a with trifluoroacetic acid, followed by acylation of the resulting amine-trifluoroacetate salt 11 with N-hydroxysuccinimide ester of the 12-mer m-dPEGacid 12^{21} in the presence of 4-methylmorpholine and purification by reverse phase preparative HPLC, furnished pyrazine-PEG conjugate 13 in 42% yield. Similar Bocdeprotection of 7h gave the corresponding trifluoroacetic acid salt 14, which, upon acylation with 12 and 15, afforded the tetra-PEG conjugates 16a (29%) and 16b (10%), respectively. Compound 16c, which contains differentsized PEG moieties on the amino and carboxyl groups, was prepared from orthogonally bis-protected intermediate 7i in a stepwise manner. Initial Boc-deprotection with trifluoroacetic acid, followed by reaction of the intermediate 17 with N-hydroxysuccinimide ester 12, gave the compound 18. Subsequent transfer hydrogenation with ammonium formate in the presence of 10% Pd/C to remove Cbz groups furnished 19, which, upon treatment with *N*-hydroxysuccinimide ester **15**, afforded **16c** in 20% overall yield.

The photophysical properties of some of the representative small molecule pyrazines and the PEG-conjugates are summarized in Table 2. All the *N*-alkyl compounds **7b**, **7d**, and **7h** exhibited very similar photophysical patterns, with significant (35–45 nm) enhancements in both absorption and emission maxima over those of aminopyrazine **6a**. The PEG conjugate **13** exhibited a bathochromic shift of about 50 nm for both absorption and emission maxima compared to the corresponding des-propylpyrazine analogue ($\lambda_{abs} = 449$ nm, $\lambda_{em} = 559$ nm).¹⁰ The absorption and emission maxima for the relatively hydrophilic tetra-PEG conjugates **16a–c** were found to be very similar, with slightly lower emission maxima compared to **13**, probably due to the dimished ability to donate

 Table 2
 Absorption and Fluorescence Spectra of Small Molecule

 Pyrazines^a and Pyrazine-PEG Conjugates^b

Entry	Compound	Absorption λ_{max} (nm) Emission λ_{max} (nm)
1	6a	458	548
2	7b	504	593
3	7d	497	587
4	7h	497	584
5	13	498	613
6	16a	497	595
7	16b	496	590
8	16c	495	590

⁴ Measured as DMSO solutions.

^b Measured as phosphate-buffered saline (PBS) solutions.



Scheme 4 Synthesis of hydrophilic pyrazine-PEG conjugates 13 and 16

electrons to the pyrazine core. This is our entry into red fluorescent hydrophilic pyrazines and the detailed photophysical, plasma-protein binding, and renal clearance properties of this class of compounds will be described elsewhere.

In summary, an efficient and improved method has been developed for the synthesis of N,N'-dialkylated aminopyrazines of the type 7 by a general reductive amination route. The utility of these new pyrazine analogues, which absorb and emit at longer wavelengths, was demonstrated by the synthesis of new hydrophilic compounds of the type 13 and 16.

Unless otherwise noted, all reagents were used as supplied. Organic extracts were dried over anhyd Na2SO4 and filtered using a fluted filter paper (P8). Solvents were removed on a rotary evaporator under reduced pressure. Analytical TLC was performed on Analtech silica gel GF plates (250 µm) and flash chromatography was carried out using silica gel 60 (40-63 µm). RP-LC/MS (ESI, positive ion mode) analyses were carried out on a Waters Micromass ZQ system equipped with a PDA detector using either a BDS Hypersil C18 (3 μ m, 50 × 4.6 mm) or a ThermoElectron Hypersil Gold C18 (3 μ m, 4.6×50 mm) column. Compounds were injected under gradient conditions (5 to 50-95% B/6 min) with a flow rate of 1 mL/min (mobile phase A: 0.05% TFA in H₂O; mobile phase B: 0.05% TFA in MeCN). Preparative RP-HPLC was carried out with a Waters Dual Pump system equipped with a Liquid Handler using a Waters XBridge[™] Prep C18 5 µm OBD[™] 30 × 150 mm or a 19 × 250 mm column [λ_{max} : PDA (200–800 nm); flow: 50 mL/min; gradient: 20– 30% to 50–95% B/10–17 min; mobile phase A: 0.1% TFA in H₂O; mobile phase B: 0.1% TFA in MeCN]. RP-HPLC analyses were carried out on an Agilent 1200 series system using a Phenomenex Luna 5 μ m C18(2) 100 Å 250 × 4.6 mm column [detection: UV; flow: 1 mL/min; gradient: 10/20% B to 80/90% B/20 min; mobile phase A: 0.1% TFA in H₂O; mobile phase B: 0.1% TFA in MeCN]. UV/Vis spectra were measured either with an Agilent 8453 or a Shimadzu UV-3101 PC spectrophotometer. Fluorescence spectra were measured either with a PTI Quantamaster or a Jobin Yvon Fluorolog®-3 spectrofluorimeter. NMR spectra were recorded using either a Varian Gemini-300 or a VNMRS-500 spectrometer. ¹H chemical shifts are expressed in parts per million (δ) relative to TMS ($\delta = 0$ ppm) as an internal standard. ¹³C chemical shifts are referenced to either TMS ($\delta = 0$ ppm) or to the residual solvent peaks in the spectra. Coupling constants (J) are reported in Hz. HRMS (ESI) data was obtained with a ThermoFisher LTQ-Orbitrap mass spectrometer equipped with an IonMax electrospray ionization source operating in the FTMS mode with resolution 30 K. Elemental analyses were carried out by Atlantic Microlab, Inc., Norcross, GA.

Diethyl 3,6-Diaminopyrazine-2,5-dicarboxylate (3a)

Compound **3a** was prepared from 3,6-diaminopyrazine-2,5-dicarboxylic acid (**1b**)²² in 68% yield according to a reported procedure.⁹

Diethyl 3,6-Bis(benzylamino)pyrazine-2,5-dicarboxylate (5)

To a well-stirred, red suspension of diester **3a** (0.127 g, 0.500 mmol) in anhyd DCE (20 mL), benzaldehyde (0.202 mL, 2.00 mmol) was added, and the reaction flask was immersed in an ice bath. AcOH (0.115 mL, 2.00 mmol) was added, followed by the addition of Na(OAc)₃BH (0.424 g, 2.00 mmol) in small portions over a 15 min period. The resulting suspension was slowly allowed to warm to r.t. and stirred overnight (ca. 16 h; RP-LC/MS analysis indicated the presence of some substrate) in an atmosphere of argon. At this stage, the reaction mixture was treated with more benzaldehyde (0.202 mL, 2.00 mmol), AcOH (0.115 mL, 2.00 mmol), and

 $Na(OAc)_3BH$ (0.424 g, 2.00 mmol) as described above, and the reaction was allowed to continue overnight (ca. 24 h). The reaction was quenched by slow addition of sat. $NaHCO_3$ (20 mL) while stirring at 0 °C. The biphasic mixture was stirred for 30 min and extracted with CHCl₃ (3 × 25 mL). The combined organic extracts were successively washed with sat. $NaHCO_3$, H_2O , and brine (30 mL each). Removal of the solvent gave a red solid (0.200 g), which upon flash chromatography over silica gel (CHCl₃) afforded **5**.^{13a}

Yield: 0.174 g (80%); dark-red powder; $R_f = 0.49$ (CHCl₃).

¹H NMR (DMSO- d_6): δ = 7.60 (t, J = 5.9 Hz, 2 H), 7.42 (dd, J = 7.7, 1.7 Hz, 4 H), 7.28–7.18 (m, 6 H), 4.51 (d, J = 5.9 Hz, 4 H), 4.32 (q, J = 7.1 Hz, 4 H), 1.30 (t, J = 7.1 Hz, 6 H).

¹³C NMR (DMSO- d_6): δ = 165.4, 146.3, 140.1, 128.1, 128.0, 126.7, 124.8, 61.4, 44.4, 44.3, 14.1.

RP-LC/MS (ESI): $m/z = 435.3 \text{ [M+H]}^+$, 457.2 [M + Na]^+ ($t_R = 5.53 \text{ min}$, 5-95% B).

Anal. Calcd for $C_{24}H_{26}N_4O_4$: C, 66.34; H, 6.03; N, 12.89. Found: C, 66.10; H, 5.98; N, 12.67.

3,6-Diamino- N^2 , N^5 -bis[2-(*tert*-butoxycarbonyl)aminoethyl]pyrazine-2,5-dicarboxamide (6a)

To a well-stirred suspension of diacid **1b** (0.991 g, 5.00 mmol) in anhyd DMF (50 mL), *N*-(2-aminoethyl)carbamic acid *tert*-butyl ester (1.76 g, 11.0 mmol) and HOBt·H₂O (1.84 g, 12.0 mmol) were added and the mixture was stirred for 10 min in an atmosphere of Ar. The reaction flask was cooled in an ice bath, EDC·HCl (2.30 g, 12.0 mmol) was introduced, and the reaction mixture was slowly warmed to r.t. and then stirred overnight (ca. 17 h). Most of the DMF was removed under high vacuum and the residue was taken up in CHCl₃ (700 mL) and transferred to a separating funnel. The solution was successively washed with H₂O, sat. NaHCO₃, H₂O, and brine (250 mL portions). The solvents were removed in vacuo and the residue was dried under high vacuum to give spectroscopically pure bisamide **6a**.

Yield: 2.10 g (87%); yellow powder.

¹H NMR (DMSO- d_6): $\delta = 8.46$ (t, J = 5.9 Hz, 2 H), 6.92 (t, J = 5.5 Hz, 2 H), 6.49 (s, 4 H), 3.34–3.28 (q, 4 H, overlaps with H₂O in solvent), 3.10 (q, J = 6.0 Hz, 4 H), 1.36 (s, 18 H).

¹³C NMR (DMSO- d_6): $\delta = 165.8, 156.2, 146.6, 126.8, 78.2, 28.7.$

RP-LC/MS (ESI): $m/z = 483.3 \text{ [M + H]}^+$, 505.4 [M + Na]⁺ ($t_R = 3.62 \text{ min}$, 25–95% B).

Anal. Calcd for $C_{20}H_{34}N_8O_6$: C, 49.78; H, 7.10; N, 23.22. Found: C, 49.66; H, 7.25; N, 22.98.²³

3,6-Diamino-*N*²,*N*⁵-bis[2-(benzyloxycarbonyl)aminoethyl]pyrazine-2,5-dicarboxamide (6b)

A suspension of *N*-carbobenzoxy-1,2-diaminoethane hydrochloride (4.61 g, 20.0 mmol) in anhyd DMF (100 mL) was stirred with *N*,*N*-disopropylethylamine (DIPEA; 3.50 mL, 20.1 mmol) for 30 min in an atmosphere of N₂. Compound **1b** (1.98 g, 10.0 mmol) was added and, after 15 min, HOBt·H₂O (3.37 g, 22.0 mmol) and EDC·HCl (4.22 g, 22.0 mmol) were added. The resulting dark suspension was stirred at r.t. overnight (ca. 16 h). Most of the DMF was removed under high vacuum and the slurry was stirred with anhyd Et₂O–MeOH (1:3, v/v; 200 mL) for about 30 min. The precipitate was collected by filtration, thoroughly washed with MeOH and anhyd Et₂O and dried under high vacuum to give the bisamide **6b**.

Yield: 4.37 g (79%); yellow powder.

¹H NMR (DMSO- d_6): δ = 8.50 (t, *J* = 5.5 Hz, 2 H), 7.39–7.31 (m, 10 H), 6.50 (br s, 4 H), 5.02 (s, 4 H), 3.37–3.34 (br q, 4 H), 3.20–3.17 (br q, 4 H).

¹³C NMR (DMSO-*d*₆): δ = 165.8, 156.7, 146.7, 137.6, 128.8, 128.22, 128.20, 126.8, 65.7, 40.4 (overlaps with solvent), 39.2 (overlaps with solvent).

RP-LC/MS (ESI): $m/z = 551.2 [M + H]^+$, 573.2 $[M + Na]^+$ ($t_R = 3.86$ min, 25–95% B).

Anal. Calcd for $C_{26}H_{30}N_8O_6$: C, 56.72; H, 5.49; N, 20.35. Found: C, 56.79; H, 5.49; N, 20.30.

3,6-Diamino- N^2 , N^5 -bis[4-(*tert*-butoxycarbonyl)aminobutyl]pyrazine-2,5-dicarboxamide (6c)

The reaction of **1b** (1.85 g, 11.8 mmol) and *N*-Boc-1,4-diaminobutane hydrochloride (2.72 g, 12.1 mmol) in the presence of DIPEA (10.3 mL, 59.2 mmol), HOBt·H₂O (1.85 g, 11.8 mmol), and EDC·HCl (2.56 g, 13.4 mmol) in anhyd DMF (40 mL) was carried out overnight as described for the preparation of **6b**. Most of the DMF was removed under high vacuum and the viscous residue was dissolved in CHCl₃ (200 mL), and successively washed with 0.50 M KHSO₄, sat. NaHCO₃, and brine (60 mL portions). Solvent was removed and the crude product was subjected to flash chromatography over silica gel (CHCl₃–MeOH, 19:1, v/v) to afford the bisamide **6c**.

Yield: 1.82 g (68%); yellow powder; $R_f = 0.48$ (CHCl₃–MeOH, 9:1, v/v).

¹H NMR (CDCl₃): δ = 7.83 (t, *J* = 5.8 Hz, 2 H), 6.03 (s, 4 H), 4.61 (br s, 2 H), 3.42 (q, *J* = 6.7 Hz, 4 H), 3.18 (br q, 4 H), 1.68–1.54 (m, 8 H), 1.44 (s, 18 H).

¹³C NMR (CDCl₃): δ = 165.2, 156.0, 146.5, 127.0, 79.2, 40.2, 38.9, 28.4, 27.6, 26.9.

RP-LC/MS (ESI): $m/z = 539.3 [M + H]^+$, 561.3 $[M + Na]^+$ ($t_R = 4.47$ min, 5–95% B).

Anal. Calcd for $C_{24}H_{42}N_8O_6$: C, 53.52; H, 7.86; N, 20.80. Found: C, 53.39; H, 8.01; N, 20.66.

N^2 , N^5 -Bis[2-(*tert*-butoxycarbonyl)aminoethyl]-3,6-bis(propyl-amino)pyrazine-2,5-dicarboxamide (7a)

To a partially dissolved, yellow suspension of bisamide **6a** (0.483 g, 1.00 mmol) in anhyd DCE (20 mL), propionaldehyde (0.290 mL, 4.02 mmol) and AcOH (0.290 mL, 5.03 mmol) were added with stirring at 0 °C under an argon atmosphere. The resulting, somewhat lighter suspension was allowed to stir for 5 min before the addition of Na(OAc)₃BH (0.848 g, 4.00 mmol) in small portions over a 10 min period. The reddish suspension was slowly allowed to warm to r.t. and stirred overnight (ca. 19 h) in an atmosphere of argon. The reaction was quenched by the slow addition of sat. NaHCO₃ (20 mL) at 0 °C. The biphasic mixture was stirred for 30 min and extracted with CHCl₃ (3 × 25 mL). The combined organic extracts were successively washed with H₂O and brine (50 mL each). Removal of the solvent gave a red solid (0.680 g), which upon flash chromatography over silica gel (CH₂Cl₂–EtOAc, 17:3 to 3:1, v/v) afforded **7a**.

Yield: 0.454 g (80%); crimson red solid; $R_f = 0.44$ (CH₂Cl₂-EtOAc, 7:3, v/v).

¹H NMR (CDCl₃): $\delta = 8.13$ (br s, 2 H), 7.78 (t, J = 5.4 Hz, 2 H), 4.87 (br s, 2 H), 3.53 (q, J = 5.9 Hz, 4 H), 3.39–3.34 (quint, J = 7.8 Hz, 8 H), 1.70–1.63 (sext, J = 7.2 Hz, 4 H), 1.42 (s, 18 H), 1.01 (t, J = 7.4 Hz, 6 H).

¹³C NMR (CDCl₃): δ = 166.8, 156.3, 146.0, 126.1, 79.6, 42.9, 40.4, 39.8, 28.3, 22.8, 11.8.

RP-LC/MS (ESI): $m/z = 567.4 [M + H]^+$, 589.4 [M + Na]⁺ ($t_R = 5.17$ min, 5–95% B).

Anal. Calcd for $C_{26}H_{46}N_8O_6$: C, 55.11; H, 8.18; N, 19.77. Found: C, 55.17; H, 8.31; N, 19.53.

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N²,N⁵-Bis[2-(*tert*-butoxycarbonyl)aminoethyl]-3,6-bis(butylamino)pyrazine-2,5-dicarboxamide (7b)

The reaction of 6a (0.483 g, 1.00 mmol) with butyraldehyde (0.358 mL, 4.00 mmol) in the presence of AcOH (0.230 mL, 4.00 mmol) and Na(OAc)₃BH (0.848 g, 4.00 mmol) in DCE (25 mL) was carried out overnight (ca. 18 h) as described for the preparation of 7a. After a similar workup, the brick-red crude product (0.610 g) was subjected to flash chromatography over silica gel (CHCl₃-EtOAc, 3:1, v/v), and the residue was triturated with anhyd Et_2O to give **7b**.

Yield: 0.366 g (62%); red powder; $R_f = 0.48$ (CHCl₃-EtOAc, 7:3, v/v).

¹H NMR (CDCl₃): δ = 8.13 (br s, 2 H), 7.76 (t, J = 5.1 Hz, 2 H), 4.86 (br s, 2 H), 3.53 (q, J = 6.0 H, 4 H), 3.43-3.34 (2 × q, overlapping, 8 H), 1.66-1.60 (m, 4 H), 1.49-1.38 (m, 22 H, includes Boc singlet at $\delta = 1.42$ ppm), 0.96 (t, J = 7.3 Hz, 6 H).

¹³C NMR (CDCl₃): δ = 166.9, 156.3, 146.0, 126.1, 79.6, 40.7, 40.5, 39.8, 31.7, 28.3, 20.4, 14.0.

RP-LC/MS (ESI): $m/z = 595.4 [M + H]^+$, 617.3 $[M + Na]^+$ ($t_R = 4.45$ min, 50-95% B).

Anal. Calcd for C₂₈H₅₀N₈O₆: C, 56.55; H, 8.47; N, 18.84. Found: C, 56.39; H, 8.57; N, 18.73.

3,6-Bis(benzylamino)-N²,N⁵-bis[2-(tert-butoxycarbonyl)aminoethyl]pyrazine-2,5-dicarboxamide (7c)

The reaction of 6a (0.121 g, 0.250 mmol) with benzaldehyde (0.101 mL, 1.00 mmol) in the presence of AcOH (0.058 mL, 1.00 mmol) and Na(OAc)₃BH (0.212 g, 1.00 mmol) in DCE (10 mL) was carried out overnight (ca. 16 h) as described for the preparation of **7a**. After a similar workup, the brick-red crude product (0.240 g) was subjected to flash chromatography over silica gel (CHCl₃-EtOAc, 4:1, v/v), and the residue triturated with anhyd Et_2O to give 7c.

Yield: 0.119 g (72%); orange powder; $R_f = 0.40$ (CHCl₃-EtOAc, 7:3, v/v).

¹H NMR (CDCl₃): $\delta = 8.20$ (br t, J = 5.0 Hz, 2 H), 7.76 (br t, 2 H), 7.37-7.30 (m, 8 H), 7.25-7.21 (m, 2 H), 4.77 (br s, 2 H), 4.58 (d, *J* = 5.4 Hz, 4 H), 3.44–3.40 (br q, 4 H), 3.31–3.25 (br q, 4 H), 1.43 (s, 18 H).

¹³C NMR (CDCl₃): δ = 166.5, 156.2, 145.6, 140.3, 128.5, 127.0, 126.8, 126.4, 79.6, 45.6, 40.4, 39.8, 28.4.

RP-LC/MS (ESI): $m/z = 663.2 [M + H]^+$, $685.2 [M + Na]^+$ ($t_R = 4.30$ min, 50-95% B).

Anal. Calcd for C₃₄H₄₆N₈O₆: C, 61.61; H, 7.00; N, 16.91. Found: C, 61.72; H, 7.07; N, 16.89.

N²,N⁵-Bis[2-(tert-butoxycarbonyl)aminoethyl]-3,6-bis(4-methoxybenzylamino)pyrazine-2,5-dicarboxamide (7d)

The reaction of 6a (0.483 g, 1.00 mmol) with 4-methoxybenzaldehyde (0.485 mL, 4.00 mmol) in the presence of AcOH (0.230 mL, 4.00 mmol) and Na(OAc)₃BH (0.848 g, 4.00 mmol) in DCE (25 mL) was carried out overnight as described for the preparation of 7a. After a similar workup, the brick-red crude product (1.14 g) was subjected to flash chromatography over silica gel (CHCl3-EtOAc, 3:1, v/v), and the material was recrystallized from EtOAc-Et₂O to give 7d.

Yield: 0.615 g (85%); orange-red microcrystalline solid; $R_f = 0.30$ (CHCl₃-EtOAc, 7:3, v/v).

¹H NMR (CDCl₃): δ = 8.14 (br t, *J* = 5.0 Hz, 2 H), 7.90 (br t, 2 H), 7.28 (d, J = 8.5 Hz, 4 H), 6.86 (d, J = 8.5 Hz, 4 H), 4.82 (br t, 2 H), 4.52 (d, J = 5.4 Hz, 4 H), 3.78 (s, 6 H), 3.46–3.43 (br q, 4 H), 3.33– 3.28 (br q, 4 H), 1.42 (s, 18 H).

¹³C NMR (CDCl₃): δ = 166.6, 158.6, 156.3, 145.6, 132.2, 128.3, 126.4, 113.9, 79.6, 55.3, 45.0, 40.5, 39.8, 28.4.

RP-LC/MS (ESI): $m/z = 723.3 [M + H]^+$, 745.3 [M + Na]⁺ ($t_R = 4.08$ min, 50-95% B).

Anal. Calcd for C₃₆H₅₀N₈O₈: C, 59.82; H, 6.97; N, 15.50. Found: C, 60.01; H, 7.05; N, 15.43.

N²,N⁵-Bis[2-(tert-butoxycarbonyl)aminoethyl]-3,6-bis(4-nitrobenzylamino)pyrazine-2,5-dicarboxamide (7e)

The reaction of 6a (0.121 g, 0.250 mmol) with 4-nitrobenzaldehyde (0.151 mL, 1.00 mmol) in the presence of AcOH (0.058 mL, 1.00 mmol) and Na(OAc)₃BH (0.212 g, 1.00 mmol) in DCE (10 mL) was carried out overnight (ca. 18 h) as described for the preparation of 7a. After a similar workup, the brick-red crude product (0.260 g) was subjected to flash chromatography over silica gel (CHCl₃-EtOAc, 7:3, v/v), and the residue was recrystallized from EtOAc- Et_2O to give **7e**.

Yield: 0.155 g (82%); orange microcrystalline solid; $R_f = 0.33$ (CHCl₃-EtOAc, 1:1, v/v).

¹H NMR (CDCl₃): δ = 8.44 (br t, 2 H), 8.18 (d, *J* = 8.7 Hz, 4 H), 8.03 (br s, 2 H), 7.57 (d, J = 8.5 Hz, 4 H), 4.78 (br m, 6 H), 3.46-3.42 (br q, 4 H), 3.36-3.30 (br m, 4 H), 1.39 (s, 18 H).

¹³C NMR (CDCl₃): δ = 166.2, 156.7, 148.2, 147.0, 145.6, 127.8, 126.5, 123.8, 79.8, 44.6, 40.7, 40.0, 28.3.

RP-LC/MS (ESI): $m/z = 753.2 [M + H]^+$, 775.1 [M + Na]⁺ ($t_R = 4.02$ min, 50-95% B).

Anal. Calcd for C₃₄H₄₄N₁₀O₁₀: C, 54.25; H, 5.89; N, 18.61. Found: C, 54.20; H, 5.97; N, 18.32.

N²,N⁵-Bis[2-(tert-butoxycarbonyl)aminoethyl]-3,6-bis(2-methoxyethylamino)pyrazine-2,5-dicarboxamide (7f)

The reaction of 6a (0.121 g, 0.25 mmol) with methoxyacetaldehyde (0.074 g, 1.00 mmol) in the presence of AcOH (0.058 mL, 1.00 mmol) and Na(OAc)₃BH (0.212 g, 1.00 mmol) in DCE (10 mL) was carried out overnight (ca. 15 h) as described for the preparation of 7a. After a similar workup, the red crude product (0.146 g) was subjected to flash chromatography over silica gel [CHCl₃-EtOAc, 1:1 (v/v) to 2% MeOH in CHCl₃-EtOAc, 1:1 (v/v)] to afford 7f.

Yield: 0.043 g (29%); orange-red solid; $R_f = 0.34$ [2% MeOH in CHCl₃-EtOAc, 1:1 (v/v)].

¹H NMR (CDCl₃): $\delta = 8.20$ (br, 2 H), 7.96 (t, J = 4.9 Hz, 2 H), 4.93 (br, 2 H), 3.66-3.59 (m, 8 H), 3.52 (q, J = 5.9 Hz, 4 H), 3.41 (s, 6 H), 3.36 (br q, J = 5.3 Hz, 4 H), 1.42 (s, 18 H).

¹³C NMR (CDCl₃): δ = 166.6, 156.5, 145.9, 126.4, 79.6, 71.6, 59.0, 41.0, 40.4, 40.1, 28.4.

RP-LC/MS (ESI): $m/z = 599.4 [M + H]^+$, $621.4 [M + Na]^+$ ($t_R = 4.47$ min, 5-95% B).

HRMS (ESI): m/z [M + H]⁺ calcd for C₂₆H₄₇N₈O₈: 599.3511; found: 599.3511; $[M + Na]^+$ calcd for $C_{26}H_{46}N_8O_8Na$: 621.3331; found: 621.3329.

Dimethyl 4,4'-[3,6-Bis{2-(tert-butoxycarbonylamino)ethylcarbamoyl}pyrazine-2,5-diyl]bis(azanediyl)dibutanoate (7g)

The reaction of 6a (0.965 g, 2.00 mmol) with methyl 4-oxobutanoate (0.838 mL, 8.00 mmol) in the presence of AcOH (0.460 mL, 7.98 mmol) and Na(OAc)₃BH (1.70 g, 8.00 mmol) in DCE (40 mL) was carried out overnight (ca. 20 h) as described for the preparation of 7a. After a similar workup, the orange crude product (1.74 g) was subjected to flash chromatography over silica gel (CHCl₃-EtOAc, 7:3, v/v) to give 7g.

Yield: 1.30 g (95%); orange-red powder; $R_f = 0.33$ (CHCl₃-EtOAc, 1:1, v/v).

¹H NMR (CDCl₃): δ = 8.66 (t, *J* = 5.9 Hz, 2 H), 7.93 (t, *J* = 6.0 Hz, 2 H), 5.21 (br t, 2 H), 3.67 (s, 6 H), 3.56 (q, J = 5.8 Hz, 4 H), 3.463.30 (m, 8 H), 2.42 (t, *J* = 6.5 Hz, 4 H), 1.99–1.89 (quint, *J* = 6.9 Hz, 4 H), 1.41 (s, 18 H).

 ^{13}C NMR (CDCl_3): δ = 174.4, 166.7, 156.0, 145.6, 126.1, 79.2, 51.8, 40.8, 40.4, 39.5, 30.9, 28.4, 24.4.

RP-LC/MS (ESI): $m/z = 683.3 \text{ [M + H]}^+$, 705.3 [M + Na]^+ ($t_R = 4.75 \text{ min}$, 15–95% B).

HRMS (ESI): m/z [M + H]⁺ calcd for $C_{30}H_{51}N_8O_{10}$: 683.3723; found: 683.3719.

Anal. Calcd for $C_{30}H_{50}N_8O_{10}$ ·1/6CHCl₃: C, 51.56; H, 7.20; N, 15.95. Found: C, 51.97; H, 7.25; N, 15.59.

N^2 , N^5 -Bis[2-(*tert*-butoxycarbonyl)aminoethyl]-3,6-bis[2-(*tert*-butoxycarbonylamino)ethylamino]pyrazine-2,5-dicarbox-amide (7h)

The reaction of **6a** (2.00 g, 4.15 mmol) with *N*-Boc-2-aminoacetaldehyde (2.60 g, 16.3 mmol) in the presence of AcOH (0.960 mL, 16.6 mmol) and Na(OAc)₃BH (3.52 g, 16.6 mmol) in DCE (25 mL) was carried out overnight (ca. 20 h) as described for the preparation of **7a**. After a similar workup, the red crude product obtained was subjected to flash chromatography over silica gel (CHCl₃–EtOAc, 1:1, v/v) to afford **7h**.

Yield: 2.94 g (92%); brick-red solid; $R_f = 0.27$.

¹H NMR (DMSO-*d*₆): δ = 8.81 (t, *J* = 5.9 Hz, 2 H), 7.95 (t, *J* = 5.9 Hz, 2 H), 6.96 (t, *J* = 5.6 Hz, 2 H), 6.86 (br t, *J* = 5.1 Hz, 2 H), 3.41 (q, *J* = 6.4 Hz, 4 H), 3.35 (q, *J* = 6.2 Hz, 4 H), 3.15–3.08 (quint, *J* = 6.3 Hz, 8 H), 1.38 (s, 18 H), 1.35 (s, 18 H).

¹³C NMR (DMSO- d_6): δ = 165.4, 155.8, 155.5, 145.0, 125.7, 77.7, 77.5, 40.2 (overlaps with solvent), 39.1 (overlaps with solvent).

RP-LC/MS (ESI): $m/z = 769.3 [M + H]^+$, 791.3 [M + Na]⁺ ($t_R = 5.10$ min, 15–95% B).

HRMS (ESI): m/z [M + H]⁺ calcd for $C_{34}H_{61}N_{10}O_{10}$: 769.4567; found: 769.4567.

Anal. Calcd for $C_{34}H_{60}N_{10}O_{10}$ ·2/3EtOAc: C, 53.21; H, 7.96; N, 16.92. Found: C, 53.01; H, 8.03; N, 16.58.

N^2, N^5 -Bis[2-(benzyloxycarbonyl)aminoethyl]-3,6-bis[2-(tert-butoxycarbonyl)aminoethylamino]pyrazine-2,5-dicarbox-amide (7i)

The reaction of **6b** (0.500 g, 0.908 mmol) with *N*-Boc-2-aminoacetaldehyde (0.578 g, 3.63 mmol) in the presence of AcOH (0.208 mL, 3.61 mmol) and Na(OAc)₃BH (0.770 g, 3.63 mmol) in anhyd DCE (40 mL) was carried out overnight (ca. 19 h) as described above for the preparation of **7a**. After a similar workup, the crude product (0.900 g) was subjected to flash chromatography over silica gel (CH₂Cl₂–MeOH, 97:3, v/v) to afford **7i**.

Yield: 0.530 g (70%); brick-red powder; $R_f = 0.77$ (CH₂Cl₂–MeOH, 19:1, v/v).

¹H NMR (CDCl₃): δ = 9.03 (br t, 2 H), 7.93 (t, *J* = 5.8 Hz, 2 H), 7.33–7.26 (m, 10 H), 5.73 (br t, 2 H), 5.07 (s, 4 H), 4.85 (t, *J* = 6.2 Hz, 2 H), 3.61 (br q, 4 H), 3.48–3.25 (m, 8 H), 3.30–3.25 (m, 4 H), 1.42 (s, 18 H).

¹³C NMR (CDCl₃): δ = 167.1, 156.7, 156.5, 145.8, 136.8, 128.4, 128.0, 127.9, 126.4, 79.5, 66.5, 41.7, 41.1, 39.3, 39.2, 28.5.

RP-LC/MS (ESI): $m/z = 837.4 \text{ [M + H]}^+$, 859.3 [M + Na]⁺ ($t_R = 5.15 \text{ min}$, 5–95% B).

Anal. Calcd for $C_{40}H_{56}N_{10}O_{10}$: C, 57.40; H, 6.74; N, 16.74. Found: C, 57.33; H, 6.78; N, 16.48.

N^2 , N^5 -Bis[2-(benzyloxycarbonyl)aminoethyl]-3,6-bis[3-(benzyl-oxycarbonylamino)propylamino]pyrazine-2,5-dicarboxamide (7j)

The reaction of **6b** (1.10 g, 2.00 mmol) with 3-[(benzyloxycarbonyl)amino]propionaldehyde (1.24 g, 6.00 mmol) in the presence of AcOH (0.340 mL, 5.90 mmol) and sodium triacetoxyborohydride (1.27 g, 6.00 mmol) in anhyd DCE (50 mL) was carried out overnight (ca. 40 h) as described above for the preparation of **7a**. After a similar workup, the crude product was suspended in MeCN– anhyd Et₂O (1:1, v/v; 100 mL) and stirred at r.t. for 30 min. The precipitate was collected by filtration, washed with MeCN–anhyd Et₂O, and dried under high vacuum to give **7j** (1.35 g). The filtrate was concentrated and subjected to flash chromatography over silica gel (CHCl₃–MeOH, 49:1, v/v)] to obtain an additional 0.09 g of the product.

Yield: 1.44 g (77%); orange powder; $R_f = 0.42$ (CHCl₃–MeOH, 19:1, v/v).

¹H NMR (DMSO- d_6): $\delta = 8.53$ (t, J = 5.5 Hz, 2 H), 7.86 (br t, 2 H), 7.42 (t, J = 5.5 Hz, 2 H), 7.36–7.21 (m, 20 H), 4.99 (s, 4 H), 4.98 (s, 4 H), 3.50–3.30 (m, 10 H), 3.18 (q, J = 6.1 Hz, 4 H), 3.07 (q, J = 6.4 Hz, 4 H), 1.66 (quint, J = 6.6 Hz, 4 H).

¹³C NMR (DMSO- d_6): δ = 165.7, 156.4, 156.1, 145.3, 137.2, 137.0, 128.22, 128.18, 127.64, 127.60, 125.7, 65.2, 65.1, 38.1, 37.6, 29.6.

RP-LC/MS (ESI): $m/z = 933.4 [M + H]^+ (t_R = 4.96 \text{ min}, 15-95\% \text{ B}).$

Anal. Calcd for $C_{48}H_{56}N_{10}O_{10}$: C, 61.79; H, 6.05; N, 15.01. Found: C, 61.53; H, 5.92; N, 14.96.

N^2 , N^5 -Bis[4-(*tert*-butoxycarbonyl)aminobutyl]-3,6-bis[4-(*tert*-butoxycarbonylamino)butylamino]pyrazine-2,5-dicarbox-amide (7k)

The reaction of **6c** (0.250 g, 0.464 mmol) with *N*-Boc-4aminobutyraldehyde¹⁸ (0.695, 3.71 mmol) in the presence of AcOH (0.212 mL, 3.68 mmol) and sodium triacetoxyborohydride (0.787 g, 3.71 mmol) in DCE (15 mL) was carried out overnight as described for the preparation of **7a**. After a similar workup, the crude product (0.678 g) was subjected to flash chromatography over silica gel (CHCl₃–EtOAc, 3:2, v/v) to give **7k**.

Yield: 0.307 g (75%); brick-red powder; $R_f = 0.45$.

¹H NMR (CDCl₃): δ = 7.92 (br s, 2 H), 7.85 (t, *J* = 5.5 Hz, 2 H), 4.73 (br s, 2 H), 4.61 (br s, 2 H), 3.44–3.39 (m, 8 H), 3.17 (br q, 8 H), 1.68–1.54 (m, 16 H), 1.43 (s, 36 H).

¹³C NMR (CDCl₃): δ = 166.1, 156.02, 156.0, 145.9, 126.2, 79.1, 40.4, 40.2, 38.9, 28.4, 27.7, 27.6, 27.1, 26.8.

RP-LC/MS (ESI): $m/z = 881.4 [M + H]^+$, 903.4 $[M + Na]^+$ ($t_R = 5.32 min, 5-95\%$ B).

Anal. Calcd for $C_{42}H_{76}N_{10}O_{10}$: C, 57.25; H, 8.69; N, 15.90. Found: C, 57.53; H, 8.83; N, 15.70.

N^2 , N^5 -Bis[2-(*tert*-butoxycarbonyl)aminoethyl]-3,6-bis(cyclohexylamino)pyrazine-2,5-dicarboxamide (10a)

To a partially dissolved yellow suspension of **6a** (0.121 g, 0.250 mmol) in anhyd DCE (10 mL), cyclohexanone (0.104 mL, 1.00 mmol) was added, and the reaction flask was immersed in an ice bath. AcOH (0.058 mL, 1.00 mmol) was added followed by the addition of sodium triacetoxyborohydride (0.212 g, 1.00 mmol) in small portions over a 10 min period. The resulting suspension was slowly allowed to warm to r.t. and stirred overnight (ca. 17 h; RP-LC/MS analysis indicated intact substrate) in an atmosphere of N₂. At this stage, the reaction mixture was treated with further cyclohexanone (0.104 mL, 1.00 mmol), AcOH (0.058 mL, 1.00 mmol), and sodium triacetoxyborohydride (0.212 g, 1.00 mmol) as described above, and the reaction was continued for 48 h (RP-LC/MS analysis indicated some substrate remaining). Similar quantities

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of the reagents were added once again and the reaction was continued over the weekend. After the usual workup described for the preparation of **7a**, the crude red product (0.456 g) was subjected to flash chromatography over silica gel (CHCl₃ to CHCl₃–EtOAc, 17:3, v/v) to afford the desired product **10a** along with the byproducts **10b** and **10c**.

Yield: 0.075 g (46%); crimson red powder; $R_f = 0.58$ (CHCl₃- EtOAc, 7:3, v/v).

¹H NMR (CDCl₃): δ = 8.02 (br t, 2 H), 7.75 (d, *J* = 7.7 Hz, 2 H), 4.83 (br t, 2 H), 3.90–3.76 (br m, 2 H), 3.52 (q, *J* = 5.9 Hz, 4 H), 3.34 (q, *J* = 5.9 Hz, 4 H), 2.02–1.20 (m, 38 H, includes Boc singlet at δ = 1.42 ppm).

¹³C NMR (CDCl₃): δ = 166.5, 156.4, 144.8, 125.8, 79.4, 48.9, 40.4, 39.5, 32.8, 28.3, 25.9, 24.6.

RP-LC/MS (ESI): $m/z = 647.5 [M + H]^+ (t_R = 5.36 min, 30-95\% B).$

HRMS (ESI): $m/z [M + H]^+$ calcd for $C_{32}H_{55}N_8O_6$: 647.4239; found: 647.4238.

Anal. Calcd for $C_{32}H_{54}N_8O_6\cdot 1/3EtOAc\cdot 1/3H_2O$: C, 58.69; H, 8.47; N, 16.43. Found: C, 58.31; H, 8.39; N, 16.08.

*N*²,*N*⁵-Bis[2-(*tert*-butoxycarbonyl)aminoethyl]-3-(cyclohexylamino)-6-(ethylamino)pyrazine-2,5-dicarboxamide (10b)

Yield: 0.040 g (27%); red solid; $R_f = 0.38$ (CHCl₃-EtOAc, 7:3, v/v).

¹H NMR (CDCl₃): $\delta = 8.16$ (br t, 1 H), 8.01 (br t, 1 H), 7.79 (d, J = 7.7 Hz, 1 H), 7.63 (t, J = 5.1 Hz, 1 H), 4.83 (br s, 2 H), 3.83 (br m, 1 H), 3.55–3.34 (m, 10 H), 1.99–1.21 (m, 31 H, include Boc singlet at $\delta = 1.42$ and Me triplet at $\delta = 1.27$ ppm).

¹³C NMR (CDCl₃): δ = 166.9, 166.8, 156.4, 156.3, 145.7, 145.3, 126.1, 79.6, 49.0, 40.5, 40.4, 39.9, 39.6, 35.8, 32.9, 28.3, 28.2, 26.0, 24.6, 14.9.

RP-LC/MS (ESI): $m/z = 593.4 [M + H]^+ (t_R = 4.88 \text{ min}, 30-95\% \text{ B}).$

HRMS (ESI): $m/z \,[M + H]^+$ calcd for $C_{28}H_{49}N_8O_6$: 593.3770; found: 593.3760.

N^2 , N^5 -Bis[2-(*tert*-butoxycarbonyl)aminoethyl]-3,6-bis(ethyl-amino)pyrazine-2,5-dicarboxamide (10c)

Yield: 0.010 g (7%); orange solid; $R_f = 0.25$ (CHCl₃-EtOAc, 7:3, v/v).

¹H NMR (CDCl₃): δ = 8.17 (br t, 2 H), 7.67 (t, *J* = 5.0 Hz, 2 H), 4.86 (br t, 2 H), 3.55–3.33 (m, 12 H), 1.42 (s, 18 H), 1.27 (t, *J* = 7.2 Hz, 6 H).

¹³C NMR (CDCl₃): δ = 166.8, 156.4, 145.9, 126.1, 79.6, 40.4, 39.9, 35.9, 28.3, 14.9.

RP-LC/MS (ESI): $m/z = 539.3 [M + H]^+$, 561.5 $[M + Na]^+$ ($t_R = 4.34$ min, 30–95% B).

HRMS (ESI): m/z [M + Na]⁺ calcd for C₂₄H₄₂N₈O₆Na: 561.3120; found: 561.3117.

N^2 , N^5 -Bis(2-aminoethyl)-3,6-bis(propylamino)pyrazine-2,5-dicarboxamide TFA Salt (11)

To a solution of **7a** (0.430 g, 0.759 mmol) in anhyd CH_2Cl_2 (5 mL), was carefully added TFA (5 mL) while stirring at ice-bath temperature. After a few minutes, the reaction mixture was slowly allowed to warm to r.t. and stirred for 1 h in an atmosphere of argon. The reaction mixture was concentrated in vacuo, and the viscous residue was co-evaporated with CH_2Cl_2 (4 × 20 mL), and then dried overnight under high vacuum to give TFA salt **11**, which was used as such in the next reaction.

¹H NMR (DMSO- d_6): $\delta = 8.68$ (t, J = 6.2 Hz, 2 H), 7.90 (br s, 6 H), 3.54 (q, J = 6.2 Hz, 4 H), 3.44 (t, J = 6.7 Hz, 4 H), 3.03–2.97 (sext, J = 5.8 Hz, 4 H), 1.61–1.55 (sext, J = 7.2 Hz, 4 H), 0.96 (t, J = 7.2 Hz, 6 H).

RP-LC/MS (ESI): $m/z = 367.3 [M + H]^+ (t_R = 3.36 min).$

N^2 , N^5 -Bis(38-oxo-2,5,8,11,14,17,20,23,26,29,32,35-dodecaoxa-39-azahentetracontan-41-yl)-3,6-bis(propylamino)pyrazine-2,5-dicarboxamide (13)

To a red solution of the above TFA salt **11** (0.759 mmol) in anhyd DMF (7 mL), 4-methylmorpholine (NMM; 0.835 mL, 7.59 mmol) was added at 0 °C, and the reaction was stirred for 30 min in an atmosphere of argon. A solution of m-dPEG₁₂-NHS (**12**; 1.25 g, 1.82 mmol) in anhyd CH₂Cl₂ (3 mL) was added and the reaction mixture was stirred overnight at ambient temperature. Most of the solvent was removed under high vacuum and the crude product (1.82 g) was subjected to purification by preparative RP-HPLC (30×150 mm, 20–50% B/10 min). The pure fractions were concentrated in vacuo, the residue was partitioned between CHCl₃ and sat. NaHCO₃, and the CHCl₃ layer was washed with H₂O and brine. After removal of the solvent, the residue was dried under high vacuum to give **13**.

Yield: 0.481 g (42%); brick-red solid.

¹H NMR (DMSO-*d*₆): δ = 8.58 (t, *J* = 5.7 Hz, 2 H), 8.02 (t, *J* = 5.6 Hz, 2 H), 7.87 (t, *J* = 5.6 Hz, 2 H), 3.60–3.24 (m, 110 H, includes characteristic m-dPEG signals at δ = 3.50 and 3.24 ppm), 2.32 (t, *J* = 6.5 Hz, 4 H), 1.60–1.52 (sext, *J* = 7.2 Hz, 4 H), 0.95 (t, *J* = 7.5 Hz, 6 H).

¹³C NMR (DMSO-*d*₆): δ = 171.2, 166.4, 146.0, 126.2, 71.7, 70.3, 70.09, 70.05, 70.0, 67.2, 58.5, 42.4, 38.5, 36.6, 23.0, 12.1.

RP-HPLC (264 nm): 90.3% (*t*_R = 17.63 min, 20–80% B).

RP-LC/MS (ESI): $m/z = 1507.9 [M + H]^+ (t_R = 3.44 \text{ min}, 25-95\% \text{ B}).$

HRMS (ESI): $m/z \ [M + 2H]^{2+}$ calcd for $C_{68}H_{132}N_8O_{28}$: 754.4570; found: 754.4568; $[M + H]^+$ calcd for $C_{68}H_{131}N_8O_{28}$: 1507.9067; found: 1507.9055.

N^2 , N^5 -Bis(2-aminoethyl)-3,6-bis[2-(amino)ethylamino]pyrazine-2,5-dicarboxamide TFA Salt (14)

The reaction of **7h** (0.93 g, 1.21 mmol) with TFA (15 mL) in anhyd CH_2Cl_2 (15 mL) was carried out as described above for the preparation of **11** to give TFA salt **14** as a reddish brown solid that was used as such in the next reaction.

¹H NMR (DMSO-*d*₆): δ =8.75 (t, *J* = 6.1 Hz, 2 H), 8.06 (br t, 2 H), 7.97 (br s, 4 H), 7.86 (br s, 4 H), 3.73 (br q, 4 H), 3.55 (q, *J* = 6.3 Hz, 4 H), 3.04–2.95 (m, 8 H).

RP-LC/MS (ESI): $m/z = 369.3 [M + H]^+ (t_R = 1.22 \text{ min}, 5-95\% \text{ B}).$

3,6-Bis(38-oxo-2,5,8,11,14,17,20,23,26,29,32,35-dodecaoxa-39azahentetracontan-41-ylamino)-*N*²,*N*⁵-bis(38-oxo-

2,5,8,11,14,17,20,23,26,29,32,35-dodecaoxa-39-azahentetracontan-41-yl)pyrazine-2,5-dicarboxamide (16a)

The reaction of the above TFA salt **14** (1.21 mmol) with NHS ester **12** (3.89 g, 5.67 mmol) in the presence of NMM (2.66 mL, 24.2 mmol) in anhyd DMF (20 mL) and anhyd CH_2Cl_2 (5 mL) was carried out overnight (ca. 15 h) as described above for the preparation of **13**. A similar purification of the crude product by preparative RP-HPLC (30 × 150 mm, 20–50% B/15 min) afforded **16a**.

Yield: 0.94 g (29%); brick-red solid.

¹H NMR (DMSO-*d*₆): δ = 9.03 (t, *J* = 5.9 Hz, 2 H), 8.12 (t, *J* = 6.0 Hz, 2 H), 7.96, 7.95 (2 × t, 4 H), 3.62 (t, *J* = 6.5 Hz, 4 H), 3.58 (t, *J* = 6.6 Hz, 4 H), 3.51–3.22 (m, 204 H, includes characteristic mdPEG signals at δ = 3.50 and 3.24 ppm), 2.33 (t, *J* = 6.5 Hz, 4 H), 2.30 (t, *J* = 6.6 Hz, 4 H).

¹³C NMR (DMSO- d_6): $\delta = 171.3, 170.8, 166.1, 145.7, 126.4, 71.7, 70.3, 70.1, 70.05, 70.0, 69.98, 67.3, 67.2, 58.5, 39.3, 38.7, 38.6, 36.6.$

RP-HPLC (264 nm): 95.5% ($t_{\rm R}$ = 16.35 min, 20–80% B).

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RP-LC/MS (ESI): $m/z = 884.3 [M + 3H]^{3+}$, 1325.4 $[M + 2H]^{2+}$ ($t_R = 3.81 \text{ min}$, 5–95% B).

HRMS (ESI): $m/z \ [M + 3H]^{3+}$ calcd for $C_{118}H_{231}N_{10}O_{54}$: 884.1874; found: 884.1872; $[M + 2H]^{2+}$ calcd for $C_{118}H_{230}N_{10}O_{54}$: 1325.7774; found: 1325.7769.

$\begin{array}{l} 3,6\text{-Bis}(74\text{-}oxo\text{-}2,5,8,11,14,17,20,23,26,29,32,35,38,41,44,47,50,\\ 53,56,59,62,65,68,71\text{-}tetracosaoxa\text{-}75\text{-}azaheptaheptacontan\text{-}77\text{-}ylamino)-N^2,N^5\text{-}bis(74\text{-}oxo\text{-}2,5,8,11,14,17,20,23,26,29,32,35,38,\\ 41,44,47,50,53,56,59,62,65,68,71\text{-}tetracosaoxa\text{-}75\text{-}azaheptaheptacontan\text{-}77\text{-}yl)pyrazine\text{-}2,5\text{-}dicarboxamide (16b) \end{array}$

The reaction of the above TFA salt **14** (0.221 g, 107% mass balance, 0.250 mmol) with NHS ester **15** (1.40 g, 1.15 mmol) in the presence of NMM (0.55 mL, 5.00 mmol) in anhyd DMF (9 mL) and anhyd CH₂Cl₂ (1 mL) was carried out overnight (ca. 24 h) as described above for the preparation of **13**. A similar purification of the crude product by preparative RP-HPLC (19×250 mm, 25–95% B/17 min) afforded **16b**.

Yield: 0.118 g (10%); brick-red semi-solid.

¹H NMR (DMSO-*d*₆): δ = 9.04 (t, *J* = 5.2 Hz, 2 H), 8.12 (t, *J* = 6.0 Hz, 2 H), 7.95 (t, *J* = 5.2 Hz, 4 H), 3.74–3.22 (m, 404 H, includes characteristic m-dPEG signals at δ = 3.50 and 3.23 ppm), 2.36–2.26 (m, 8 H).

RP-HPLC (264 nm): 91.5% ($t_{\rm R}$ = 17.31 min, 10–90% B).

RP-LC/MS (ESI): $m/z = 1192.9 [M + 4H]^{4+} (t_R = 3.87 \text{ min}, 5-95\% B).$

HRMS (ESI): $m/z [M + 6H]^{6+}$ calcd for $C_{214}H_{426}N_{10}O_{102}$: 794.8070; found: 794.8063; $[M + 4H]^{4+}$ calcd for $C_{214}H_{424}N_{10}O_{102}$: 1191.7069; found: 1191.7022.

3,6-Bis(2-aminoethylamino)-N²,N⁵-bis[2-(benzyloxycarbonyl)aminoethyl]pyrazine-2,5-dicarboxamide TFA Salt (17)

The reaction of **7i** (0.418 g, 0.500 mmol) with TFA (3.85 mL) in anhyd CH_2Cl_2 (10 mL) was carried out as described above for the preparation of **11** to give TFA salt **17** as a reddish brown solid that was used as such in the next reaction.

¹H NMR (DMSO- d_6): $\delta = 8.60$ (t, J = 6.1 Hz, 2 H), 8.12 (br, 2 H), 7.71 (br, 6 H), 7.46 (t, J = 5.8 Hz, 2 H), 7.34–7.29 (m, 8 H), 5.01 (s, 4 H), 3.70 (br, 4 H), 3.37 (q, J = 6.3 Hz, 4 H), 3.21 (q, J = 6.3 Hz, 4 H), 3.01–2.95 (m, 4 H).

RP-LC/MS (ESI): $m/z = 637.2 [M + H]^+ (t_R = 3.72 min, 5-95\% B).$

N^2 , N^5 -Bis[2-(benzyloxycarbonyl)aminoethyl]-3,6-bis(38-oxo-2,5,8,11,14,17,20,23,26,29,32,35-dodecaoxa-39-azahentetra-contan-41-ylamino)pyrazine-2,5-dicarboxamide (18)

The reaction of the above TFA salt **17** (0.50 mmol) with NHS ester **12** (1.00 g, 1.46 mmol) in the presence of NMM (0.55 mL, 5.00 mmol) in anhyd DMF (25 mL) and anhyd CH_2Cl_2 (5 mL) was carried out overnight as described above for the preparation of **13**. Most of the solvent was evaporated under high vacuum, and the residue was dissolved in CHCl₃ (150 mL) and then successively washed with 0.5 M KHSO₄ in brine (1:4, v/v), H₂O, and brine (50 mL each). Removal of the solvent gave a reddish gum (1.21 g), which was subjected to flash chromatography over silica gel (CHCl₃–MeOH, 19:1, v/v) to give bis-PEG derivative **18**.

Yield: 0.714 g (80%); reddish gum.

¹H NMR (CDCl₃): $\delta = 9.22$ (t, J = 5.9 Hz, 2 H), 8.02 (t, J = 6.1 Hz, 2 H), 7.34–7.28 (m, 10 H), 7.20 (br t, 2 H), 7.08 (t, J = 6.1 Hz, 2 H), 5.06 (s, 4 H), 3.86–3.35 (m, 108 H, includes characteristic m-dPEG signals at $\delta = 3.65$ and 3.38 ppm), 2.90 (t, J = 6.5 Hz, 2 H), 2.62 (br, 4 H), 2.35 (t, J = 5.6 Hz, 4 H).

¹³C NMR (CDCl₃): δ = 173.2, 168.9, 166.7, 166.6, 156.9, 146.0, 137.0, 128.4, 128.3, 128.0, 126.5, 72.0, 70.7, 70.65, 70.64, 70.62,

70.58, 70.55, 70.5, 70.2, 70.1, 66.9, 66.4, 65.8, 59.0, 41.9, 41.0, 40.0, 39.1, 38.8, 36.5, 32.2, 25.6.

RP-LC/MS (ESI): $m/z = 1778.3 [M + H]^+$, 889.9 $[M + 2H]^{2+}$ ($t_R = 4.24 \text{ min}, 5-95\% \text{ B}$).

HRMS (ESI): $m/z [M + 2Na]^{2+}$ calcd for $C_{82}H_{140}N_{10}O_{32}Na_2$: 911.4710; found: 911.4713; $[M + Na]^+$ calcd for $C_{82}H_{140}N_{10}O_{32}Na$: 1799.9527; found: 1799.9534.

N²,N⁵-Bis(2-aminoethyl)-3,6-bis(38-oxo-

2,5,8,11,14,17,20,23,26,29,32,35-dodecaoxa-39-azahentetracontan-41-ylamino)pyrazine-2,5-dicarboxamide (19)

To a suspension of **18** (0.714 g, 0.400 mmol) in MeOH (25 mL), a slurry of 10% Pd/C (0.143 g) in H₂O (5 mL) was added, followed by ammonium formate (0.103 g, 1.64 mmol). The resulting mixture was heated at 70 °C for 1 h, allowed to cool to r.t., and filtered through a pad of Celite to remove the catalyst. The filtrate was concentrated and the residue was dissolved in CHCl₃ (100 mL), and washed with brine–H₂O (4:1, v/v). Removal of the solvent afforded **19**, which was used as such in the next reaction.

Yield: 0.548 (91%).

¹H NMR (CDCl₃): δ = 9.08 (br, 2 H), 8.03 (br, 2 H), 6.90 (br, 2 H), 3.78–3.42 (m, 104 H, includes broad peak at δ = 3.50 ppm for PEG), 3.38 (s, 6 H), 2.96 (br, 4 H), 2.45 (br, 4 H), 1.76 (br, 4 H).

RP-LC/MS (ESI): $m/z = 755.6 \text{ [M + 2H]}^{2+}$ ($t_{\text{R}} = 3.57 \text{ min}$, 5–95% B).

3,6-Bis(38-oxo-2,5,8,11,14,17,20,23,26,29,32,35-dodecaoxa-39-azahentetracontan-41-ylamino)- N^2 , N^5 -bis(74-oxo-2,5,8,11,14, 17,20,23,26,29,32,35,38,41,44,47,50,53,56,59,62,65,68,71-tetra-cosaoxa-75-azaheptaheptacontan-77-yl)pyrazine-2,5-dicarbox-amide (16c)

The reaction of the diamine **19** (0.54 g, 0.36 mmol) with NHS ester **15** (1.00 g, 0.82 mmol) in the presence of NMM (0.20 mL, 1.82 mmol) in anhyd DMF (20 mL) and anhyd CH_2Cl_2 (4 mL) was carried out over the weekend as described above for the preparation of **13**. A similar purification of the crude product by preparative RP-HPLC (19 × 250 mm, 30–70% B/14 min) afforded **16c**.

Yield: 0.379 g (28%); reddish gum.

¹H NMR (DMSO-*d*₆): δ = 9.03 (t, *J* = 5.8 Hz, 2 H), 8.11 (t, *J* = 5.6 Hz, 2 H), 7.94 (t, *J* = 5.7 Hz, 4 H), 3.62–3.36 (m, 296 H, includes broad peak at δ = 3.50 ppm for PEG), 3.233, 3.230 (2 × s, 12 H), 2.34–2.29 (2 × t, 8 H).

¹³C NMR (DMSO- d_6): δ = 171.3, 170.8, 166.2, 145.7, 126.4, 71.7, 70.3, 70.1, 70.05, 70.01, 69.98, 67.3, 67.2, 58.5, 40.4, 40.3, 40.1, 36.6.

RP-HPLC (264 nm): 96.2% ($t_{\rm R}$ = 16.99 min, 10–90% B).

RP-LC/MS (ESI): $m/z = 928.2 [M + 4H]^{4+}$, 1237.7 $[M + 3H]^{3+}$ ($t_R = 3.87 \text{ min}$, 5–95% B).

HRMS (ESI): $m/z [M + 4H]^{4+}$ calcd for $C_{166}H_{328}N_{10}O_{78}$: 927.5496; found: 927.5543; $[M + 3H]^{3+}$ calcd for $C_{166}H_{327}N_{10}O_{78}$: 1236.3971; found: 1236.4004; $[M + 2H]^{2+}$ calcd for $C_{166}H_{326}N_{10}O_{78}$: 1854.0920; found: 1854.0960.

Supporting Information for this article is available online at http://www.thieme-connect.com/ejournals/toc/synthesis.

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