With compliments of the Author

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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 diamino pyrazines. 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, 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. The best methodology for measurement of GFR is by clearance of exogenous tracer agents. Several such agents have been employed such as inulin, iothalamate, Gd-DTPA, and 99mTc-DTPA. However, all suffer from poor, with 7–28% overall yield of N,N′-dialkyl products. Even with simple N,N′-dialkylamines, the details of which are presented in this paper.

Alkyl substitution on the amino groups of pyrazine 1 was shown to significantly increase the wavelength of both absorption and emission maxima. 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-7-ene gave lower 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,N-dimethylacetamide at 70–120 °C was also found to be poor, with 7–28% overall yield of 4. Even with simple 2-aminopyrazines, base-induced alkylation with alkyl halides were found to give only moderate yields (~50%) of N-alkyl-2-aminopyrazine derivatives. Thus, alkylation of aminopyrazines in the presence of a strong base is not a synthetically useful reaction because it often leads to 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. 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. 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.

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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.\textsuperscript{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.\textsuperscript{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.\textsuperscript{16} Consequently, diethyl ester triacetoxyborohydride was employed as the reducing agent, providing the corresponding bisbenzyl derivative 5 in 80% yield. The overall yield of 54% from 1\textsubscript{b} is clearly superior to ~3% realized earlier using base-induced alkylation.\textsuperscript{13a}

Encouraged by this early success, we examined the scope of the reductive amination reaction with the key intermediate 6\textsubscript{a}, which was prepared in 87% yield from 1\textsubscript{b} by standard coupling reaction with N-Boc-ethylcarbenediimine using N-(3-dimethylaminopropyl)-N′-ethylcarboxydimide and 1-hydroxybenzotriazole (Scheme 2).\textsuperscript{17} Thus, compound 6\textsubscript{a} was reductively alkylated with propionaldehyde in the presence of acetic acid and sodium triacetoxyborohydride in 1,2-dichloroethane overnight at room temperature, to yield the corresponding bisbenzyl derivative 7\textsubscript{a} 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 7\textsubscript{b–h} in excellent yields (Table 1). The sole exception was 7\textsubscript{f} (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 6\textsubscript{b}, which was prepared in 79% yield from 1\textsubscript{b} and N-carbobenzoxy-1,2-diaminoethane using N-(3-dimethylaminopropyl)-N′-ethylcarboxydimide/1-hydroxybenzotriazole coupling, was smoothly transformed into 7\textsubscript{i} (70%) and 7\textsubscript{j} (77%) by re-action with the appropriate aldehyde under reductive amination conditions. The orthogonal protecting groups on the side chains of 7\textsubscript{g} and 7\textsubscript{i} introduce the flexibility to conjugate different moieties as desired later on. Compound 7\textsubscript{k}, a four carbon variant of 7\textsubscript{h}, was synthesized in 51% overall yield by initial coupling of 1\textsubscript{b} with N-1-Boc-1,4-diaminobutane, followed by reductive alkylation of the bisamide intermediate 6\textsubscript{c} with 4-tert-butoxycarbonylaminobutanal.\textsuperscript{18} Towards this end, reductive alkylation of 6\textsubscript{a} with propionaldehyde under Borch conditions (NaBH\textsubscript{3}CN, MeOH)\textsuperscript{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 6\textsubscript{a} in the methanol solvent, and it seems that sodium triacetoxy-

![Scheme 1 Alkylation of aminopyrazines](image1)

![Scheme 2 Synthesis of N,N′-dialkylaminopyrazines 7a–k by reductive alkylation of aminopyrazines](image2)

**Table 1 Synthesis of Compounds 7a–k**

<table>
<thead>
<tr>
<th>Product</th>
<th>R\textsuperscript{1}</th>
<th>R\textsuperscript{2}</th>
<th>Yield (%)\textsuperscript{a}</th>
</tr>
</thead>
<tbody>
<tr>
<td>7a</td>
<td>(CH\textsubscript{2})\textsubscript{3}NHBOc</td>
<td>Et</td>
<td>80</td>
</tr>
<tr>
<td>7b</td>
<td>(CH\textsubscript{2})\textsubscript{3}NHBOc</td>
<td>n-Pr</td>
<td>62</td>
</tr>
<tr>
<td>7c</td>
<td>(CH\textsubscript{2})\textsubscript{3}NHBOc</td>
<td>Ph</td>
<td>72</td>
</tr>
<tr>
<td>7d</td>
<td>(CH\textsubscript{2})\textsubscript{3}NHBOc</td>
<td>4-MeOC\textsubscript{6}H\textsubscript{4}</td>
<td>85</td>
</tr>
<tr>
<td>7e</td>
<td>(CH\textsubscript{2})\textsubscript{3}NHBOc</td>
<td>4-O\textsubscript{2}NC\textsubscript{6}H\textsubscript{4}</td>
<td>82</td>
</tr>
<tr>
<td>7f</td>
<td>(CH\textsubscript{2})\textsubscript{3}NHBOc</td>
<td>CH\textsubscript{3}OMe</td>
<td>29</td>
</tr>
<tr>
<td>7g</td>
<td>(CH\textsubscript{2})\textsubscript{3}NHBOc</td>
<td>(CH\textsubscript{2})\textsubscript{3}CO\textsubscript{2}Me</td>
<td>95</td>
</tr>
<tr>
<td>7h</td>
<td>(CH\textsubscript{2})\textsubscript{3}NHBOc</td>
<td>CH\textsubscript{3}NHBOc</td>
<td>92</td>
</tr>
<tr>
<td>7i</td>
<td>(CH\textsubscript{2})\textsubscript{3}NHBOc</td>
<td>CH\textsubscript{2}NHBOc</td>
<td>70</td>
</tr>
<tr>
<td>7j</td>
<td>(CH\textsubscript{2})\textsubscript{3}NHBOc</td>
<td>(CH\textsubscript{2})\textsubscript{2}NHBoc</td>
<td>77</td>
</tr>
<tr>
<td>7k</td>
<td>(CH\textsubscript{2})\textsubscript{3}NHBOc</td>
<td>(CH\textsubscript{2})\textsubscript{3}NHBoc</td>
<td>75</td>
</tr>
</tbody>
</table>

\textsuperscript{a} Isolated yield.
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 acetaldehyde 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 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 iminium intermediate. These factors provide an alkylation methodology with far greater synthetic utility than the base-mediated alkylation. This chemistry has become a ‘workhorse’ 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-dPEG-acid 12 in the presence of 4-methylmorpholine and purification by reverse phase preparative HPLC, furnished pyrazine–PEG conjugate 13 in 42% yield. Similar Boc-deprotection 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 different-sized 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 \( \lambda_{abs} = 449 \text{ nm}, \lambda_{em} = 559 \text{ nm} \). 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 diminished ability to donate...
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 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 Varian Gemini-300 or a VNMRS-500 spectrometer. 1H chemical shifts are referenced to either TMS (δ = 0 ppm) as an internal standard. 13C chemical shifts are referenced to either TMS (δ = 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)
The compound 3a was prepared from 3,6-diaminopyrazine-2,5-dicarboxylic acid (1b) in 68% yield according to a reported procedure.9

Diethyl 3,6-Bis(phenylimino)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)BH (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. NaHCO₃ (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. NaHCO₃, H₂O, 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. ¹³C NMR (DMSO-d₆): 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⁺] (tᵣ = 3.86 min, 25–95% B).

Analytical data: C₂₀H₁₆N₄O₄; C, 56.79; H, 5.49; N, 20.30.

3.6-Diamino-N₂,N⁴-bis[(4-tert-butoxycarbonyl)aminoethyl]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 KH₂PO₄, sat. NH₄Cl, 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; Rᵣ = 0.48 (CHCl₃–MeOH, 9:1, v/v).

1H NMR (DMSO-d₆): δ = 7.83 (t, J = 5.8 Hz, 2 H), 6.03 (s, 4 H), 4.61 (br s, 2 H), 3.40 (q, J = 6.7 Hz, 4 H), 3.18 (br q, 4 H), 1.68–1.54 (m, 4 H), 1.44 (s, 18 H).
13C NMR (DMSO-d₆): 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⁺] (tᵣ = 4.47 min, 25–95% B).

Analytical data: C₂₀H₂₂N₈O₆; C, 53.39; H, 8.01; N, 20.66.

N₂,N⁴-Bis[2-(benzoxycarbonyl)aminoethyl]-3,6-bis(propylamino)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)BH (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 with extraction 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; Rᵣ = 0.44 (CHCl₃–EtOAc, 7:3, v/v).

1H NMR (CDCl₃): δ = 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⁺] (tᵣ = 5.17 min, 5–95% B).

Analytical data: C₂₇H₂₄N₈O₆; C, 55.11; H, 8.18; N, 19.77.

The reaction of 6a (0.121 g, 0.250 mmol) with 4-nitrobenzaldehyde (0.074 g, 1.00 mmol) in the presence of AcOH (0.058 mL, 1.00 mmol) and Na(OAc)2BH (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, 1:1, v/v). Yield: 0.155 g (82%); orange microcrystalline solid; Rf = 0.47 (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), 7.82 (d, J = 5.8 Hz, 4 H), 7.48 (br m, 6 H), 3.46–3.32 (br q, 4 H), 3.36–3.30 (br m, 4 H), 1.43 (s, 18 H).

13C NMR (CDCl3): δc = 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]+ (tR = 4.02 min, 50–95% B).

Anal. Calcd for C26H47N8O8: C, 56.38; H, 8.57; N, 18.73.

3.6-Bis(benzamino)-N2,N2′-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)2BH (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, 1:1, v/v), and the residue was triturated with anhyd Et2O to give 7c. Yield: 0.119 g (72%); orange powder; Rf = 0.40 (CHCl3–EtOAc, 7:3, v/v).

1H NMR (CDCl3): δ = 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), 3.44–3.34 (br q, 4 H), 3.31–3.25 (br q, 4 H), 1.43 (s, 18 H).

13C NMR (CDCl3): δc = 165.5, 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]+ (tR = 4.30 min, 50–95% B).

Anal. Calcd for C19H20N4O2: C, 61.61; H, 7.00; N, 16.91. Found: C, 61.72; H, 7.07; N, 16.89.

3.6-Bis(benzamino)-N2,N2′-bis[2-(tert-butoxycarbonyl)aminoethyl]-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)2BH (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; Rf = 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), 3.33–3.32 (br q, 4 H), 1.42 (s, 18 H).

13C NMR (CDCl3): δc = 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]+ (tR = 4.08 min, 50–95% B).

Anal. Calcd for C31H26N6O6: C, 59.82; H, 6.97; N, 15.50. Found: C, 60.01; H, 7.05; N, 15.43.

3.6′,3′-Bis[2-(tert-butoxycarbonyl)aminoethyl]-3,6-bis(4-nitrobenzamino)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)2BH (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–Et2O to give 7e. Yield: 0.155 g (82%); orange microcrystalline solid; Rf = 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), 3.46–3.34 (br q, 4 H), 3.36–3.30 (br m, 4 H), 1.39 (s, 18 H).

13C NMR (CDCl3): δc = 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 = 735.2 [M + H]+, 757.1 [M + Na]+ (tR = 4.02 min, 50–95% B).

The reaction of 6a (2.00 g, 4.15 mmol) with N-Boc-2-aminacetaldehyde (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 above for the preparation of 7a. After a similar workup, the crude product was subjected to flash chromatography over silica gel (CHCl3–MeOH, 1:1, v/v) to afford 7i.

Yield: 2.94 g (92%); brick-red solid; Rf = 0.27

1H NMR (DMSO-d6): δ = 8.85 (t, J = 5.5 Hz, 2 H), 7.10 (J = 5.9 Hz, 2 H), 6.96 (t, J = 5.6 Hz, 2 H), 6.86 (br s, 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, 3 H), 1.35 (s, 1 H), 1.89 (s, 9 H), 13.5 (s, 1 H), 13.5 (s, 1 H), 13.5 (s, 1 H).

13C NMR (DMSO-d6): δ = 163.4, 155.3, 155.4, 145.1, 125.7, 77.7, 77.8, 40.2 (overlaps with solvent), 39.1 (overlaps with solvent).


Found: C, 53.01; H, 8.03; N, 16.58.

N2,N3-Bis[2-(tert-butoxycarbonylamino)ethylamino]-3,6-bis[2-(tert-butoxycarbonylamino)pyrazine-2,5-dicarboxamide (7k) (7k) The reaction of 6c (0.250 g, 0.464 mmol) with N-Boc-4-aminoacetaldehyde (0.695, 3.71 mmol) in the presence of AcOH (0.212 mL, 3.68 mmol) and sodium triacetoxoborohydride (0.787 g, 3.71 mmol) in DCE (15 mL) was carried out overnight as described above 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; Rf = 0.45.

1H NMR (CDCl3): δ = 7.92 (brs, 2 H), 7.85 (t, J = 5.5 Hz, 2 H), 4.73 (brs, 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, 156.0, 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): mlz = 881.4 [M + H]+, 903.4 [M + Na]+ (tR = 5.32 min, 5–95% B).

Anal. Calcd for C34H49N10O10: C, 57.25; H, 8.69; N, 15.90.

Found: C, 53.73; H, 8.83; N, 15.70.

N2,N3-Bis[2-(tert-butoxycarbonylamino)ethylamino]-3,6-bis(cyclohexylamino)pyrazine-2,5-dicarboxamide (10a) (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 triacetoxoborohydride (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 triacetoxoborohydride (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

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; Rf = 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]+ (tR = 5.36 min, 30–95% B).


Anal. Calcd for C32H54N8O6·1/3EtOAc·1/3H2O: C, 58.69; H, 8.47; N, 16.43. Found: C, 58.31; H, 8.39; N, 16.08.

N2,N3-Bis[2-(tert-butoxycarbonylamino)ethyl]-3-(cyclohexylamino)-6-(ethylpyrazine-2,5-dicarboxamide (10b)

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

1H NMR (CDCl3): δ = 8.16 (br t, 1 H), 8.01 (br t, 1 H), 7.79 (d, J = 7.7 Hz, 2 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 δ = 1.42 and Me triplet at δ = 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]+ (tR = 4.88 min, 30–95% B).


N2,N3-Bis[2-(tert-butoxycarbonylaminoethyl)-3,6-bis(ethyl)-pyrazine-2,5-dicarboxamide (10c)

Yield: 0.010 g (7%); orange solid; Rf = 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]+ (tR = 4.34 min, 30–95% B).


N2,N3-Bis(2-aminoethyl)-3,6-bis(ethyl)pyrazine-2,5-dicarboxamide TFA Salt (11)

To a solution of 7a (0.430 g, 0.759 mmol) in anhyd CH2Cl2 (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 CH2Cl2 (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-d6): δ = 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-LCMS (ESI): m/z = 884.3 [M + 3H]+, 1325.4 [M + 2H]+ (t_R = 3.81 min, 5–95% B).
HRMS (ESI): m/z [M + 3H]+ calcd for C_{11}H_{23}N_{10}O_{25}: 884.1874; found: 884.1872; [M + 2H]+ calcd for C_{11}H_{22}N_{10}O_{25}: 1325.7774; found: 1325.7769.

The reaction of the above TFA salt 14 afforded a redish gum (1.21 g), which was used as such in the next reaction.

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), 2.90 (t, J = 5.8 Hz, 2 H), 7.34–7.29 (m, 8 H), 5.01 (s, 12 H), 3.27 (q, J = 6.3 Hz, 4 H), 3.21 (q, J = 6.3 Hz, 4 H), 3.01–2.95 (m, 4 H).

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

HRMS (ESI): m/z [M + 6H]^{6+} calcd for C_{11}H_{24}N_{10}O_{25}: 794.8070; found: 794.8067; [M + 4H]^{4+} calcd for C_{11}H_{22}N_{10}O_{25}: 1191.7106; found: 1191.7102.

3.6-Bis-(benzyloxycarbonyl)-3,6-bis-[74]-oxo-2,5,8,11,14,17,20,23,26,29,32,35-dodecaoxa-39-azahentetracontan-41-ylamino)pyrazine-2,5-dicarboxamide (19)

1H NMR (DMSO-d_6): 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), 3.83 (s, 6 H), 2.96 (br, 4 H), 2.45 (br, 4 H), 1.76 (br, 4 H).

RP-LCMS (ESI): m/z = 755.6 [M + 2H]^{2+} (t_R = 3.57 min, 5–95% B).

The reaction of the diamine 19 with N,N,N',N'-tetramethylmethylenediamine (0.35 mol) and NHS ester 15 (1.00 g, 0.82 mmol) in the presence of NMM (0.20 mL, 1.82 mmol) in anhyd DMF (25 mL) and anhyd CHCl_3 (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, 25–95% B/17 min) afforded 16c.

Yield: 0.548 (91%).

1H NMR (CDCl_3): δ = 9.05 (t, J = 5.9 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 characteristic m-dPEG signals at δ = 3.50 and 3.23 ppm), 3.27 (q, J = 6.3 Hz, 4 H), 3.22 (q, J = 6.3 Hz, 4 H), 3.01–2.95 (m, 4 H).

RP-LCMS (ESI): m/z = 1236.4004 [M + 2H]^{2+} calcd for C_{166}H_{326}N_{10}O_{78}: 1236.3971; found: 1236.4004; [M + 3H]^{3+} calcd for C_{166}H_{327}N_{10}O_{78}: 1325.7740; found: 1325.7769; [M + 4H]^{4+} calcd for C_{166}H_{328}N_{10}O_{78}: 1854.0920; found: 1854.0960.

HRMS (ESI): m/z [M + 2Na]^{2+} calcd for C_{166}H_{328}N_{10}O_{78}Na_2: 911.4710; found: 911.4713; [M + Na]^{+} calcd for C_{166}H_{328}N_{10}O_{78}Na: 1799.9527; found: 1799.9534.

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

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References

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(21) PEG-reagent is discrete, i.e., monodisperse and was purchased from Quanta BioDesign, Ltd., Powell, Ohio.


(23) Analytical sample was prepared by filtering through a plug of silica gel using CHCl₃–MeOH (19:1, v/v) as eluent.