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Pachychalines A–C: Novel 3-Alkylpyridinium Salts from the Marine Sponge *Pachychalina* sp.

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Three 3-alkylpyridinium salts, pachychalines A (**1**), B (**2**) and C (**3**), were isolated from the Caribbean marine sponge *Pachychalina* sp. (order Haplosclerida). They are the first examples of 3-(aminoalkyl)pyridinium salts. Their chemical structures were elucidated by NMR spectroscopy and detailed ESI HRMS–MS analysis. The total synthesis of **1** allowed the confirmation of the unusual C–C connection between both pyridinium moieties.

Introduction

3-Alkylpyridinium salts form a large class of natural products widely distributed in marine sponges of the order Haplosclerida.[1] Monomeric, dimeric, trimeric as well as polymeric 3-alkylpyridinium derivatives are known to exhibit a wide range of biological activities including cytotoxicity and ichthyotoxicity.[2] Common representatives of this family are the cyclostellettamines,[3] the viscosamine[4] or the cyclohaliclonamines.[5] The Caribbean marine sponge *Pachychalina* sp. (order Haplosclerida) has been the subject of only few recent chemical studies by Berlinck and coworkers.[6] From the brazilian species *P. alcaloidifera* they described various alkaloids, derived biogenetically from 3-alkylpyridinium salts.[7] We report herein our results on a specimen of *Pachychalina* sp. collected off the north-west coast of Martinique Island, which led to the isolation of a new family of 3-alkylpyridinium salts named pachychalines. The structural characterization of compounds **1**, **2** and **3** was achieved through extensive NMR spectroscopic and HRMS–MS studies. The total synthesis of **1** confirmed the rather unusual C–C connection between both pyridinium rings, which was previously found in niphatoxins A,B.[8]

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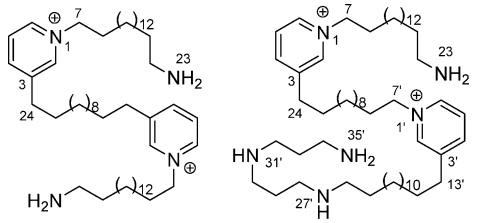
Results and Discussion

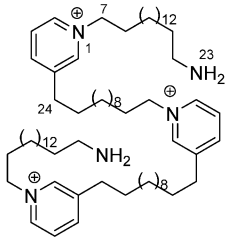
Pachychalina sp. was collected off the north-west coast of Martinique Island by scuba diving. The specimen was immediately frozen and kept at –18 °C until bioguided fractionation. The wet specimen was extracted with MeOH/CH_2Cl_2 (1:1), and the crude oil was partitioned between H_2O and CH_2Cl_2. The aqueous layer was subjected to C_{18}-reverse-phase flash chromatography (eluted with a decreasing polarity gradient of H_2O/MeOH, 1:0 to 0:1, then MeOH/CH_2Cl_2, 1:0 to 0:1). The subsequent MeOH and MeOH/CH_2Cl_2 (1:1) fractions were further purified by C_{18}-reverse-phase semipreparative HPLC to afford pure compounds **1**, **2** and **3**.

Compound **1** was obtained as a colourless oil and the molecular formula, $C_{54}H_{100}N_4^{2+}$, was established by ESI HRMS (m/z = 402.4006 $[M]^{2+}$, Δ = 9.3 ppm; Figure 1). The UV spectrum (λ_{max} = 267 nm, ε = 6600 $M^{-1}cm^{-1}$) and the ^1H NMR spectrum of **1** [δ_H = 8.99 (s, 2 H, 2-H), 8.92 (d, J = 6.0 Hz, 2 H, 6-H), 8.46 (d, J = 7.8 Hz, 2 H, 4-H), 8.06 (dd, J = 7.8, 6.0 Hz, 2 H, 5-H) ppm] suggested the presence of 3-substituted pyridinium moieties (Table 1). Relative to those of the known cyclostellettamines, the [D_6]DMSO ^1H and ^{13}C NMR spectra of **1** exhibited new signals at δ_H = 1.51 (m, 4 H, 21-H), 2.77 (m, 4 H, 22-H), 7.64 (br. s, 6 H, H–N) ppm and at δ_C = 27.1 (CH_2, C-21), 38.9 (CH_2, C-22) ppm.[3] Combined with the molecular formula, these observations suggested the presence of two terminal primary amines. This assumption was confirmed by the strong 22-H_2/N-23 ^1H–^{15}N HMBC correlation. The length of the alkyl chains was further deduced from ESI HRMS–MS data (Table 2). The usual Hofmann-type fragmentation of pyridinium moieties gave only two characteristic monocharged fragments for **1** at m/z = 325.2651 ($C_{22}H_{33}N_2^+$),

240.2719 ($C_{16}H_{34}N^+$), which is in accordance with two C16 aminoalkyl chains branched on the nitrogen atom of both pyridinium units connected by a third C12 alkyl chain at their C-3 position (C–C connection).[9]



Pachychaline A (**1**) Pachychaline B (**2**)



Pachychaline C (**3**)

Figure 1. Structures of pachychalines A (**1**), B (**2**), and C (**3**).

Table 1. ^1H and ^{13}C NMR spectroscopic data for compounds **1**, **2** and **3** in [D_6]DMSO.

	1 and **3**		**2**	
No.	δ_C and N	δ_H m (*J* in Hz)	δ_C and N	δ_H m (*J* in Hz)
1	(214.0)[a]		(214.0)[a]	
2	144.0	8.99 s	144.1	9.00 s
3	143.2		143.3	
4	145.2	8.46 d (7.8)	145.2	8.46 d (7.8)
5	127.7	8.06 dd (7.8, 6.0)	127.7	8.06 dd (7.8, 6.0)
6	142.3	8.92 d (6.0)	142.3	8.92 d (6.0)
7	60.8	4.54 t (7.2)	60.8	4.53 t (7.2)
8	30.8	1.90 m	30.8	1.89 m
9–20	29.0	1.20–1.40 m	29.0	1.20–1.40 m
21	27.1	1.51 m	27.1	1.50 m
22	38.9	2.77 m	38.9	2.77 m
23	(32.4)[a]	(7.64 br. s)[b]	(32.4)[a]	(7.67 br. s)[b]
24	31.8	2.78 m	31.8	2.77 m
25	29.9	1.63 m	29.9	1.62 m
26–29	29.0	1.20–1.40 m	29.0	1.20–1.40 m
25′			25.6	1.56 m
26′			47.0	2.87 m
27′				(8.58 br. s)[b]
28′			44.3	2.97 m
29′			22.6	1.89 m
30′			44.2	2.97 m
31′				(8.76 br. s)[b]
32′			44.0	2.97 m
33′			24.0	1.89 m
34′			36.3	2.87 m
35′			(32.4)[a]	(7.85 br. s)[b]

[a] ^{15}N NMR spectroscopic data. [b] Observed after addition of one drop of TFA in [D_6]DMSO.

Compound **2** was obtained as a colourless oil and the molecular formula, $C_{58}H_{110}N_6^{2+}$, was deduced from ESI HRMS (*m*/*z* = 445.4400 $[M]^{2+}$, Δ = 2.1 ppm; Figure 1). The

Table 2. ESI HRMS–MS of compounds **1**, **2** and **3**.

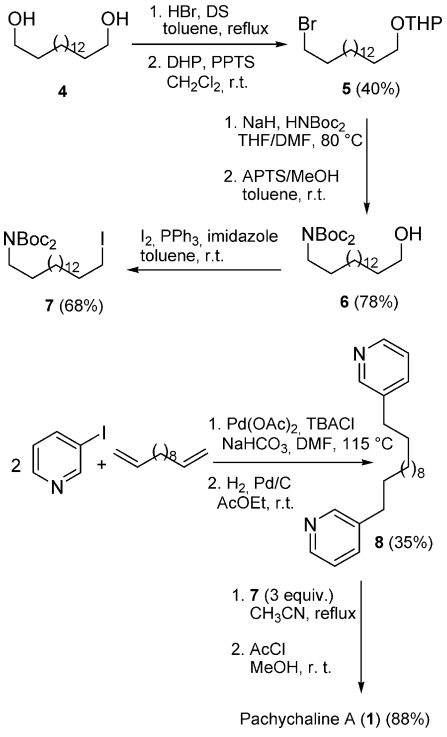
	HRMS *m*/*z*	Molecular formula (deviation in ppm)	HRMS–MS (ESI+) Fragment ion *m*/*z*	Molecular formula (deviation in ppm)
1	402.4006	$C_{54}H_{100}N_4^{2+}$ (9.3)	240.2719	$C_{16}H_{34}N^+$, (14.1)
			325.2651	$C_{22}H_{33}N_2^+$, (3.9)
2	445.4400	$C_{58}H_{110}N_6^{2+}$ (2.1)	240.2700	$C_{16}H_{34}N^+$, (6.2)
			246.2218	$C_{17}H_{28}N^+$, (2.7)
			485.4846	$C_{33}H_{61}N_2^+$ (3.4)
			650.6151	$C_{42}H_{76}N_5^+$ (8.6)
3	350.3402	$C_{71}H_{128}N_5^{3+}$ (5.1)	240.2690	$C_{16}H_{34}N^+$, (2.1)
			246.2224	$C_{17}H_{28}N^+$, (3.2)
			285.7441	$C_{39}H_{61}N_3^{2+}$, (4.7)
			325.2631	$C_{22}H_{33}N_2^+$, (–2.1)

^1H NMR spectrum of **2** in [D_6]DMSO showed four groups with exchangeable protons at δ_H = 7.67 (23-H), 7.85 (35′-H), 8.58 (27′-H), 8.76 (31′-H) ppm. Relative to **1** and even if an amino terminal 3-alkyl chain was still present, NMR spectroscopic data clearly indicated a loss of symmetry in compound **2**. The structure of the other terminal part of the molecule was easily deduced from the key COSY sequence 25′-H_2 to 35′-H_2 and supported by the characteristic loss of a fragment with a mass of 115 in the ESI MS–MS, which evidenced the presence of a norspermidine moiety. Finally, the length of the three alkyl chains was determined by ESI HRMS–MS data analysis (Table 2). The C16 fragment at *m*/*z* = 240.2700 ($C_{16}H_{34}N^+$) was still present but the fragment at *m*/*z* = 325 was replaced by both fragments at *m*/*z* = 246.2218 ($C_{17}H_{28}N^+$) and 485.4846 ($C_{33}H_{61}N_2^+$), which were consistent with a C12 alkyl chain linked to both pyridinium moieties through a C–N connection. Finally, the fragment at *m*/*z* = 650.6151 ($C_{42}H_{76}N_5^+$) proved the norspermidine moiety to be linked to a pyridinium ring through a C14 alkyl chain.

Compound **3** was obtained as a colourless oil and the molecular formula, $C_{71}H_{128}N_5^{3+}$, was established by ESI HRMS (*m*/*z* = 350.3402 $[M]^{3+}$, Δ = 5.1 ppm). The ^1H and ^{13}C NMR spectra of **3** were identical to those of pachychaline A (**1**), except for the integration pattern, which corresponded to three pyridinium moieties (Figure 1). ESI HRMS–MS analysis was also required to establish the length of the alkyl chains (Table 2). The characteristic fragment at *m*/*z* = 240.2690 ($C_{16}H_{34}N^+$) was reminiscent of an amino terminal C16 alkyl chain. The fragments at *m*/*z* = 246.2224 ($C_{17}H_{28}N^+$) and 325.2631 ($C_{22}H_{33}N_2^+$) suggested one C–N and one C–C connection between the three pyridinium rings, through C12 alkyl chains. Confirmation was obtained by the observation of the fragment at *m*/*z* = 285.7441 ($C_{39}H_{61}N_3^{2+}$), which includes three pyridine units and two C12 alkyl chains.

From a biosynthetic point of view, the C–C connection between both pyridinium rings was very intriguing. We then decided to undertake the total synthesis of pachychaline A (**1**), inspired by previous work on the synthesis of 3-alkylpyridinium salts (Scheme 1).[10] The synthesis began with the monobromation of commercially available hexadecane-

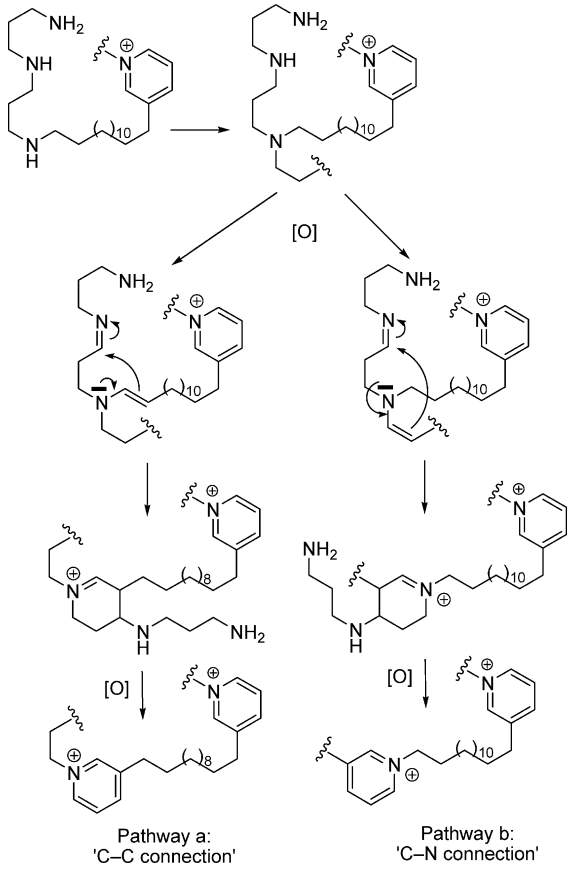
1,16-diol (**4**) followed by the protection of the remaining hydroxy group to yield bromo–THP ketal **5**. The amine functionality was introduced through its diBoc protected form by nucleophilic substitution of the bromo substituent of **5**. Deprotection of the alcohol yielded pure compound **6** and subsequent iodination was achieved under PPh_3, I_2 and imidazole conditions to give iodo compound **7** in a good overall yield. Bis(pyridine) compound **8** was straightforwardly obtained by double Heck coupling between 3-iodopyridine and commercially available dodeca-1,11-diene followed by dihydrogenation of the resulting mixture with Pd/C. The total synthesis was completed by nucleophilic displacement of the iodo substituent of **7** in excess by bis(pyridine) **8** and deprotection of the terminal primary amines by acetyl chloride in MeOH. The exact matching of both NMR and ESI HRMS–MS spectra from natural and synthetic pachychalines A (**1**) confirmed the structure of this new compound, especially the unusual C–C connection between the pyridinium moieties.



Scheme 1. Total synthesis of pachychaline A (**1**).

Pachychaline A (**1**) and C (**3**) are the first examples of dimeric and trimeric amino terminal 3-alkylpyridinium salts, respectively. Another important feature of these molecules is the C–C connection between the two pyridinium moieties, which is only present in niphatoxins A and B.[8] Pachychaline B (**2**) appears as a closely related derivative characterized by an unusual norspermidine entity. Even if polyamines have found large applications in medicinal chemistry, they are seldom found in marine organisms.[11] Squalamine isolated from the dogfish shark, *Squalus acanthias*, is a famous example of an aminosterol with a spermine used in anticancer therapy.[12] To the best of our knowledge, pachychaline B (**2**) is the third occurrence of a norspermidine moiety in marine natural products after the motuporamines[13] and the penaramides.[14]

The presence of pachychalines B (**2**) and C (**3**) in the same organism prompted us to postulate a biosynthetic pathway by considering the norspermidine moiety as a precursor of the pyridinium ring (Scheme 2).



Scheme 2. Biosynthetic hypothesis.

Indeed, given the length of the alkyl chains, the third pyridinium moiety of **3** could be built from the norspermidine of **2** through successive steps of condensation/oxidation including a key enamine/imine cyclization. According to the site of the tertiary amine oxidation, two enamine units could be obtained (pathways a and b) that could cyclize with an imine, which would lead to the C–C or C–N connections. The existing hypotheses on the formation of the pyridinium ring are based on the condensation of C3 aldehydes on terminal amines (acrolein for the Baldwin group,[15] malonaldehyde for the Marazano group[16]). The structures of pachychalines would confirm the role of terminal amines but the C3 units may originate from a norspermidine. Work is currently in progress to confirm this new hypothesis by a biomimetic approach.

The three new compounds showed moderate antitumoural activity against three tumoural cell lines; trimer **3** was the most active (Table 3).

Table 3. Antitumoural activities of compounds **1**, **2** and **3**.

Cell line	IC_{50} [μM]		
	1	**2**	**3**
MDA-MB-231	8.1	6.4	4.0
A549	8.1	3.9	2.6
HT29	7.9	4.5	3.7

Experimental Section

General: Tetrahydrofuran (THF) was distilled from sodium/benzophenone ketyl. Dichloromethane (CH_2Cl_2) was distilled from calcium hydride. All other solvents and reagents were used as received unless otherwise noted. ^1H and ^{13}C NMR spectra were recorded with either a Bruker Avance 500 MHz or Avance 200 MHz NMR spectrometer. Chemical shifts (δ) are recorded in ppm with $CDCl_3$ (δ_H = 7.26 ppm and δ_C = 77.16 ppm) or CD_3OD (δ_H = 3.31 ppm and δ_C =49.0 ppm) as internal standards with multiplicity (s singlet, d doublet, t triplet, q quartet, quint quintuplet, m multiplet, br. broad). Electrospray ionisation (ESI) mass spectra were obtained with a Bruker Esquire 3000 Plus spectrometer in the positive or negative mode. High-resolution mass spectra were obtained from a LCT Waters-Micromass mass spectrometer (ESI TOF) or a QStar Elite Applied Biosystems (API TOF) for HRMS–MS. IR spectra were obtained with a Perkin–Elmer Paragon 1000 FT–IR spectrometer.

Extraction and Isolation: *Pachychalina* sp. was collected off the north-west coast of Martinique Island (14°N; 61°W) by scuba diving. The specimen was immediately frozen and kept at –18 °C until bioguided fractionation. The wet specimen (150 g) was extracted with MeOH/CH_2Cl_2 (1:1; 2 × 1000 mL), and the crude oil (6.0 g) was partitioned between H_2O (2 × 500 mL) and CH_2Cl_2 (400 mL). The aqueous layer was subjected to C_{18}-reverse-phase flash chromatography (eluted with a decreasing polarity gradient of H_2O/MeOH, 1:0 to 0:1, then MeOH/CH_2Cl_2, 1:0 to 0:1). The subsequent MeOH and MeOH/CH_2Cl_2 (1:1) fractions were further purified by semipreparative reverse-phase HPLC (Phenomenex, Luna C_{18}, 250 × 10 mm, 5 μm, MeOH/H_2O/TFA, 60:40:0.1 to 80:20:0.1) to afford pure compounds **1** (6.2 mg, 0.004% wet weight), **2** (3.1 mg, 0.002% wet weight), and **3** (1.5 mg, 0.001% wet weight).

Pachychaline A (1): Colourless oil. ^1H NMR (500 MHz, [D_6]-DMSO) and ^{13}C NMR (125 MHz, [D_6]DMSO) see Table 1. UV (MeOH): λ_{max} (ε, $M^{-1}cm^{-1}$) = 267 (6600) nm. HRMS (ESI+): *m*/*z* = 402.4006 $[M]^{2+}$ (Δ = 9.3 ppm with $C_{54}H_{100}N_4^{2+}$).

Pachychaline B (2): Colourless oil. ^1H NMR (500 MHz, [D_6]-DMSO) and ^{13}C NMR (125 MHz, [D_6]DMSO) see Table 1. UV (MeOH): λ_{max} (ε, $M^{-1}cm^{-1}$) = 267 (6800) nm. HRMS (ESI+): *m*/*z* = 445.4400 $[M]^{2+}$ (Δ = 2.1 ppm with $C_{58}H_{110}N_6^{2+}$).

Pachychaline C (3): Colourless oil. ^1H NMR (500 MHz, [D_6]-DMSO) and ^{13}C NMR (125 MHz, [D_6]DMSO) see Table 1. UV (MeOH) λ_{max} (ε, $M^{-1}cm^{-1}$) = 267 (9700) nm. HRMS (ESI+): *m*/*z* = 350.3402 $[M]^{3+}$ (Δ = 5.1 ppm with $C_{71}H_{128}N_5^{3+}$).

16-Bromohexadecan-1-ol: To a suspension of hexane-1,16-diol (500 mg, 1.93 mmol, 1 equiv.) in toluene (20 mL) in a round-bottomed flask equipped with a Dean–Stark trap was added hydrobromic acid (48% aqueous solution, 360 μL, 2.13 mmol, 1.1 equiv.). The resulting solution was stirred at reflux for 24 h. After cooling to room temperature, the mixture was diluted in toluene (50 mL) and washed with HCl (1 M, 75 mL), NaOH (2 M, 75 mL) and brine (75 mL). The organic layer was dried with $MgSO_4$, filtered and concentrated. Purification of the residue by silica gel flash chromatography (MeOH/CH_2Cl_2, 5:95) led to the monobrominated alcohol (285 mg, 46%) as a white solid. ^1H NMR (200 MHz, $CDCl_3$): δ = 1.20–1.45 (m, 24 H), 1.56 (quint, *J* = 6.4 Hz, 2 H), 1.85 (quint, *J* = 7.9 Hz, 2 H), 3.39 (t, *J* = 6.8 Hz, 2 H), 3.63 (t, *J* = 6.8 Hz, 2 H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 63.1, 34.1, 32.9, 32.9, 29.8, 29.7, 29.6, 29.5, 28.9, 28.3, 25.9 ppm. CAS: 59101–28–9

2-(16-Bromohexadecyloxy)tetrahydro-2*H*-pyran (5): Pyridinium *p*-toluenesulfonate (126 mg, 0.5 mmol, 0.55 equiv.) and 3,4-dihydro-2*H*-pyran (100 μL, 1 mmol, 1.1 equiv.) were added to a solution of the above bromoalcohol (285 mg, 0.89 mmol, 1 equiv.) in CH_2Cl_2 (10 mL), and the mixture was stirred at room temperature for 24 h. The reaction was then quenched with an aqueous K_2CO_3 solution (2 M, 2 × 25 mL), and the resulting aqueous layer was extracted with CH_2Cl_2 (2 × 50 mL). The combined organic layer was dried with $MgSO_4$, filtered and concentrated. Purification of the residue by silica gel flash chromatography (hexane/EtOAc, 9:1) yielded 310 mg of **5** (87%) as a colourless oil. ^1H NMR (200 MHz, $CDCl_3$): δ = 1.20–1.76 (m, 34 H), 1.84 (quint, *J* = 7.3 Hz, 2 H), 3.34 (m, 1 H), 3.39 (t, *J* = 6.9 Hz, 2 H), 3.51 (m, 1 H), 3.70 (dt, *J* = 9.7, 6.9 Hz, 1 H), 3.86 (m, 1 H), 4.56 (br. t, *J* = 3.0 Hz, 1 H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 98.9, 67.8, 62.4, 34.1, 33.0, 30.9, 29.9, 29.8, 29.7, 29.6, 29.5, 28.9, 28.3, 26.4, 25.7, 19.8 ppm. CAS: 82741–59–1

Di-*tert*-butyl 16-(Tetrahydro-2*H*-pyran-2-yloxy)hexadecyliminodicarbonate: To a solution of di-*tert*-butyliminodicarboxylate (42.5 mg, 0.195 mmol, 1.3 equiv.) in a dry mixture of THF/DMF (50:50, 5 mL) at 0 °C was added NaH (60% in oil suspension, 15 mg, 0.21 mmol, 1.4 equiv.) under an atmosphere of argon. The mixture was stirred at 80 °C for 2 h under an atmosphere of argon. Compound **5** (60.5 mg, 1 equiv.) was then added and stirring was continued for 42 h. The reaction mixture was quenched with water (25 mL) and extracted with Et_2O (2 × 50 mL). The combined organic layer was then dried with $MgSO_4$, filtered and concentrated. Purification by silica gel flash chromatography (hexane/EtOAc, 8:2) afforded the pure diBoc-protected amine (66 mg, 82%). ^1H NMR (200 MHz, $CDCl_3$): δ = 1.20–1.76 (m, 52 H), 1.84 (quint, *J* = 7.3 Hz, 2 H), 3.36 (dt, *J* = 9.5, 6.8 Hz, 1 H), 3.52 (t, *J* = 7.4 Hz, 2 H), 3.51 (m, 1 H), 3.70 (dt, *J* = 9.7, 6.9 Hz, 1 H), 3.86 (m, 1 H), 4.56 (br. t, *J* = 3.0 Hz, 1 H) ppm. ^{13}C NMR (200 MHz, $CDCl_3$): δ = 99.0, 82.0, 77.2, 67.8, 62.4, 46.6, 30.9, 29.9, 29.8, 29.7, 29.6, 29.4, 29.2, 26.9, 26.4, 25.6, 19.8 ppm. IR (neat): $\tilde{\nu}$ = 2925, 2853, 1792, 1746, 1718, 1697 cm^{-1}. MS (ESI+): *m*/*z* = 564.4 $[M + Na]^+$, 464.4 $[M – Boc + Na]^+$, 408.3 $[M – Boc – tBu + Na]^+$. HRMS (ESI+): calcd. for $C_{31}H_{59}NNaO_6^+$ 564.4240; found 564.4253.

Di-*tert*-butyl 16-Hydroxyhexadecyliminodicarbonate (6): To a solution of the above protected alcohol (100 mg, 0.18 mmol, 1 equiv.) in toluene/MeOH (2:1, 6 mL) was added *p*-toluenesulfonic acid (7 mg, 0.04 mmol, 0.2 equiv.). The solution was stirred at room temperature for 14 h. The reaction mixture was then concentrated and subjected to silica gel flash chromatography (MeOH/CH_2Cl_2, 5:95) to yield 80 mg (95%) of pure compound **6**. ^1H NMR (200 MHz, $CDCl_3$): δ = 1.20–1.76 (m, 52 H), 3.53 (t, *J* = 7.6 Hz, 2 H), 3.64 (dd, *J* = 6.5, 6.4 Hz, 2 H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 82.1, 63.2, 46.7, 33.0, 29.8, 29.7, 29.6, 29.5, 29.2, 28.2, 27.0, 25.9 ppm. IR (neat): $\tilde{\nu}$ = 3444, 2978, 2924, 2852, 1782, 1731, 1693, 1633 cm^{-1}. MS (ESI+): *m*/*z* = 480.4 $[M + Na]^+$, 324.3 $[M – Boc – tBu + Na]^+$. HRMS (ESI+): calcd. for $C_{26}H_{51}NNaO_5^+$ 480.3665; found 480.3642.

Di-*tert*-Butyl 16-Iodohexadecyliminodicarbonate (7): To a solution of **6** (80 mg, 0.18 mmol, 1 equiv.) and triphenylphosphane (117 mg,

0.45 mmol, 2.5 equiv.) in a dry mixture of toluene/Et_2O (1:2, 10 mL) was added imidazole (48 mg, 0.72 mmol, 4 equiv.). The mixture was stirred under an inert atmosphere until total dissolution of the reactants. Iodine (87 mg, 0.34 mmol, 2 equiv.) was then added, and the reaction mixture was stirred at room temperature for 2 h. After the addition of few drops of triethylamine, the solution was diluted with CH_2Cl_2 (30 mL), and the organic layer was washed with a saturated aqueous solution of $NaHCO_3$ (2 × 50 mL). The organic layer was then dried with $MgSO_4$, filtered and concentrated. The resulting residue was purified by silica gel flash chromatography using a solvent system gradient (50 mL of each solvent: cyclohexane, cyclohexane/CH_2Cl_2, 50:50 and CH_2Cl_2) to yield 67 mg of the pure compound **7** (68%). ^{1}H NMR (200 MHz, $CDCl_3$): δ = 1.20–1.76 (m, 44 H), 1.74 (quint, J = 7.2 Hz, 2 H), 3.17 (t, J = 7.0 Hz, 2 H), 3.53 (t, J = 7.5 Hz, 2H 2 rotamers) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 82.0, 46.7, 33.7, 30.6, 29.8, 29.7, 29.6, 29.5, 29.2, 28.7, 28.2, 26.9, 7.5 ppm. IR (neat): $\tilde{\nu}$ = 2978, 2920, 2851, 1788, 1753, 1720, 1695, 1675, 1630 cm^{-1}.

3,3′-(Dodecan-1,12-diyl)bis(pyridine) (8): To a solution of 3-iodopyridine (205 mg, 1 mmol, 2 equiv.), palladium acetate (22.4 mg, 0.1 mmol, 0.2 equiv.), tetrabutylamonium chloride (556 mg, 2 mmol, 4 equiv.) and $NaHCO_3$ (84.0 mg, 0.1 mmol, 2 equiv.) in dry DMF (5 mL) was added dodeca-1,11-diene (83 mg, 0.5 mmol, 1 equiv.). The solution was stirred at 115 °C for 17 h. The reaction mixture was diluted in Et_2O (25 mL) and washed with brine (2 × 30 mL). The combined organic layer was dried with $MgSO_4$, filtered and concentrated. Purification by silica gel flash chromatography (MeOH/CH_2Cl_2, 5:95) afforded 50 mg of a mixture of saturated and unsaturated analogues of **8** (by NMR analysis). The residue was then dissolved in EtOAc (5 mL) and Pd/C (8 mg) was added. The reaction mixture was vigorously stirred under H_2 at atmospheric pressure for 6 h. Filtration through a thick pad of Celite (EtOAc) yielded 50 mg of pure bispyridine **8** (overall yield 35%). ^{1}H NMR (200 MHz, $CDCl_3$): δ = 1.10–1.40 (m, 16 H), 1.56 (quint, J = 6.9 Hz, 4 H), 2.55 (t, J = 7.9 Hz, 4 H), 7.18 (dd, J = 7.5, 4.6 Hz, 2 H), 7.46 (dt, J = 7.7, 1.5 Hz, 2 H), 8.34 (m, 4 H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 158.3, 150.0, 147.2, 135.9, 123.3, 33.1, 31.2, 29.7, 29.6, 29.5, 29.3 ppm. IR (neat): $\tilde{\nu}$ = 2925, 2853, 1654 cm^{-1}. UV (MeOH): λ_{max} (ε, M^{-1} cm^{-1}) = 269 (3500), 263 (4900), 257 (4400) nm. MS (ESI+): m/z = 347.3 [M + Na]$^+$, 325.3 [M + H]$^+$. HRMS (ESI+): calcd. for $C_{22}H_{33}N_2^+$ 325.2644; found 325.2655.

3,3′-(Dodecane-1,12-diyl)bis(1-{16-[bis(*tert*-butoxycarbonyl)amino]-hexadecyl}pyridinium) Diiodide: A solution of **7** (52 mg, 0.09 mmol, 3 equiv.) and **8** (10 mg, 0.03 mmol, 1 equiv.) in CH_3CN (5 mL) was stirred under reflux for 72 h. After concentration of the reaction mixture, the residue was purified by reverse-phase HPLC (C_{18}, Luna, 150 × 4.6 mm, 5 µm, 100% MeOH) to give 32 mg of tetraBoc protected pachychaline A (**1**) (88%). ^{1}H NMR (200 MHz, CD_3OD): δ = 1.25–1.44 (m, 36 H), 1.50 (s, 36 H), 1.74 (m, 4 H), 2.00 (m, 4 H), 2.89 (t, J = 7.8 Hz, 4 H), 3.56 (t, J = 7.5 Hz, 4 H), 4.62 (t, J = 7.5 Hz, 4 H), 8.02 (dd, J = 7.7, 6.0 Hz, 2 H), 8.46 (d, J = 7.9 Hz, 2 H), 8.86 (d, J = 5.8 Hz, 2 H), 8.98 (s, 2 H) ppm. ^{13}C NMR (200 MHz, CD_3OD): δ = 154.1, 146.7, 145.7, 128.9, 83.5, 62.9, 33.5, 32.5, 31.6, 30.7, 30.6, 30.5, 30.4, 30.3, 30.1, 30.0, 28.8, 28.3, 27.7, 27.1 ppm. IR (neat): $\tilde{\nu}$ = 2977, 2921, 2854, 1771, 1732, 1698, 1682, 1633 cm^{-1}. UV (MeOH): λ_{max} (ε, M^{-1} cm^{-1}) = 267 (8800) nm. MS (ESI+): m/z = 602.5 [M]$^{2+}$, 552.5 [M – Boc]$^{2+}$, 502.4 [M – 2Boc]$^{2+}$.

Synthetic Pachychaline A (1): To a solution of the tetraBoc protected pachychaline A (25 mg, 0.02 mmol, 1 equiv.) in MeOH (5 mL) was added acetyl chloride (50 µL, 0.4 mmol, 20 equiv.), and the solution was stirred at room temperature for 24 h. The solvent removal gave 10 mg of pure pachychaline A (**1**) (100%)

Supporting Information (see footnote on the first page of this article): NMR and MS spectra of pachychalines A (**1**), B (**2**) and C (**3**). ^{1}H and ^{13}C NMR spectroscopic data for compounds **4** to **8**.

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[1] For reviews on 3-alkylpyridinium salts, see: a) K. Sepcik, *J. Toxicol., Toxin Rev.* **2000**, *19*, 139–160; b) R. J. Andersen, R. W. van Soest, F. Kong, "Alkaloids" in *Chemical and Biological Perspectives* (Eds.: S. W. Pelletier), Pergamon, Elsevier Science, Oxford, **1996**, vol. 10, pp. 301–355.
[2] K. Sepcik, T. Turk, *Prog. Mol. Subcell. Biol.* **2006**, *42*, 105–124.
[3] a) N. Fusetani, N. Asai, S. Matsunaga, *Tetrahedron Lett.* **1994**, *23*, 3967–3970; b) J. H. H. L. De Oliveira, A. Grube, M. Köck, R. G. S. Berlinck, M. L. Macedo, A. G. Ferreira, E. Hajdu, *J. Nat. Prod.* **2004**, *67*, 1685–1689.
[4] C. A. Volk, M. Köck, *Org. Lett.* **2003**, *5*, 3567–3569.
[5] T. Teruya, K. Kobayashi, K. Suenaga, H. Kigoshi, *J. Nat. Prod.* **2006**, *69*, 135–137.
[6] J. H. H. L. Oliveira, M. H. R. Seleghim, C. Timm, A. Grube, M. Köck, G. G. F. Nascimento, A. C. T. Martins, E. G. O. Silva, A. O. Souza, P. R. R. Minarini, F. C. S. Galetti, C. L. Silva, E. Hajdu, R. G. S. Berlinck, *Mar. Drugs* **2006**, *4*, 1–8.
[7] J. H. H. L. Oliveira, A. M. Nascimento, M. H. Kossuga, B. C. Cavalcanti, C. O. Pessoa, M. O. Moraes, M. L. Macedo, A. G. Ferreira, E. Hajdu, U. S. Pinheiro, R. G. S. Berlinck, *J. Nat. Prod.* **2007**, *70*, 538–543.
[8] R. Talpir, A. Rudi, M. Ilan, Y. Kashman, *Tetrahedron Lett.* **1992**, *21*, 3033–3034.
[9] A. Grube, C. Timm, M. Köck, *Eur. J. Org. Chem.* **2006**, 1285–1295.
[10] C. Timm, M. Köck, *Synthesis* **2006**, *15*, 2580–2584 and references cited therein; A. Kaiser, C. Marazano, M. Maier, *J. Org. Chem.* **1999**, *64*, 3778–3782.
[11] F. Liang, S. Wan, Z. Li, X. Xiong, L. Yang, X. Zhou, C. Wu, *Curr. Med. Chem.* **2006**, *13*, 711–727.
[12] K. S. Moore, S. Wherli, H. Roder, M. Rogers, J. N. Forrest Jr, D. McCrimmon, M. Zasloff, *Proc. Natl. Acad. Sc. USA* **1993**, *90*, 1354–1358.
[13] D. E. Williams, P. Lassota, R. J. Andersen, *J. Org. Chem.* **1998**, *63*, 4838–4841; D. E. Williams, K. S. Craig, B. Patrick, L. M. McHardy, R. van Soest, M. Roberge, R. J. Andersen, *J. Org. Chem.* **2002**, *67*, 245–258.
[14] N. Ushio-Sata, S. Matsunaga, N. Fusetani, K. Honda, K. Yasumuro, *Tetrahedron Lett.* **1996**, *37*, 225–228.
[15] J. E. Baldwin, R. C. Whitehead, *Tetrahedron Lett.* **1992**, *33*, 2059–2062.
[16] A. Kaiser, X. Billot, A. Gateau-Olesker, C. Marazano, B. C. Das, *J. Am. Chem. Soc.* **1998**, *120*, 8026–8034.

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