The first cyclic monomeric 3-alkylpyridinium alkaloid from natural sources: identification, synthesis, and biological activity†

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3-Alkylpyridine alkaloids are very common secondary metabolites from marine sponges of the order Haplosclerida. Here, we report on the identification and synthesis of the first cyclic monomeric 3-alkylpyridinium alkaloid from natural sources. Due to the lack of a pure sample of the new compound, structure elucidation had to rely on HPLC and MS^n.

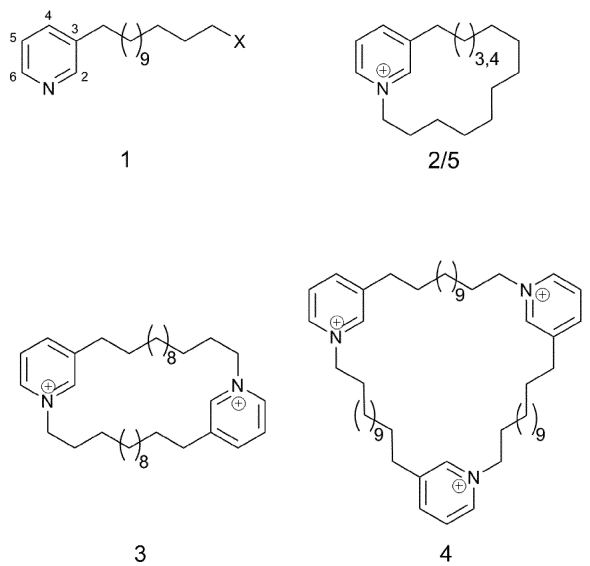
Introduction

In our search for bioactive secondary metabolites of marine sponges from Polar waters, a new 3-alkylpyridinium alkaloid has been isolated from the sponge *Haliclona viscosa* from Svalbard, Norway. 3-Alkylpyridine alkaloids (3-APA)[1] are common in marine sponges of the order Haplosclerida.[2] Two major groups of 3-APA metabolites with fully saturated alkyl chains are known: linear and (macro)cyclic (Scheme 1). Each group can be further divided by the number of pyridine/pyridinium or tetrahydropyridine rings occurring in one molecule.

Most of the linear compounds contain only one pyridine unit (**1**), *e.g.* theonelladin C (X = NH_2) and D (X = NHMe),[3] ikimine C (X = NHOMe),[4] xestamine D (X = CH_2NHOMe) and E (X = CH_2CH_2NHOMe),[5] pyrinodemin precursors (X = NOH)[6] and untenine B (X = NO_2);[7] exceptions are viscosaline[8] and the polymeric halitoxins.[9] Monomeric 3-APAs without a terminal functionality (*e.g.* **1**, X = H) are yet unknown as natural products.[10]

The group of cyclic 3-APAs is dominated by dimeric compounds with two pyridine moieties, *e.g.* cyclostellettamines (**3**)[11] and haliclamines.[2c,12] So far, only one cyclic trimer, viscosamine (**4**),[13] is known as a natural product. Cyclic compounds carrying one pyridinium unit (cyclic monomeric 3-APAs, *e.g.* **2**/**5**) are yet unknown as natural products, but they were obtained as undesired side product (**2**) in the synthesis of cyclostellettamines.[11b,d]

Despite their rather simple structure, 3-alkylpyridine alkaloids display a broad variety of biological properties, *e.g.* cytotoxic, antibacterial, and antifouling activities.[3c,7,11a,g] Interestingly, the antimicrobial and cytotoxic activites were very sensitive to the methylation of the pyridine nitrogen and the alkyl chain lenghts of the 3-APAs.[6,11i] Stierle and Faulkner reported a 100-fold increase in the antimicrobial activity upon methylation of the pyridine nitrogen in xestamines and a 100-fold decrease in cytotoxic activity in the brine shrimp assay.[5a] Kobayashi *et al.* observed a decrease of MIC towards *Staphylococcus aureus* from 16 to 2 μg mL^{-1} by extending the alkyl chain from 12 to 13 methylene groups.[6] To deepen the understanding of the relationship between structure and biological properties, the new compound and related compounds synthesised in our laboratory were tested against *E. coli* tolC, *Staphylococcus aureus* and cells of mouse fibroblasts L929.



Scheme 1 Structural formulae of different 3-alkylpyridinium alkaloids.

Results and discussion

During the investigation of the *n*-BuOH fraction of the sponge *Haliclona viscosa*, we have identified a compound with a molecular mass of *m*/*z* 274.2506 by HPLC-HRMS. Unfortunately, it is only a minor component of the extract (see Fig. 1A) and a pure sample of the natural product could not be obtained from the limited amount of sponge material. Therefore, the structure elucidation had to rely on MS/MS and HPLC data. HRMS data correspond to a monomeric 3-APA with the molecular formula $C_{19}H_{32}N$. According to the degrees of unsaturation, the compound is a cyclized monomeric 3-APA (**5**) or a linear monomeric 3-APA with a double bond in the alkyl chain (**6**). From the MS/MS spectra of **5**/**6**, it can be concluded that if it is the linear molecule, the double bond has to be a terminal. Since **6** is the major fragment

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† Electronic supplementary information (ESI) available: MS/MS spectra of **5**, **6** and **8**; HPLC chromatogram of **5** and **6**; HPLC comparison of the crude extract and synthetic **5**; MS/MS spectra of the natural product and synthetic **5**. See DOI: 10.1039/b808647h

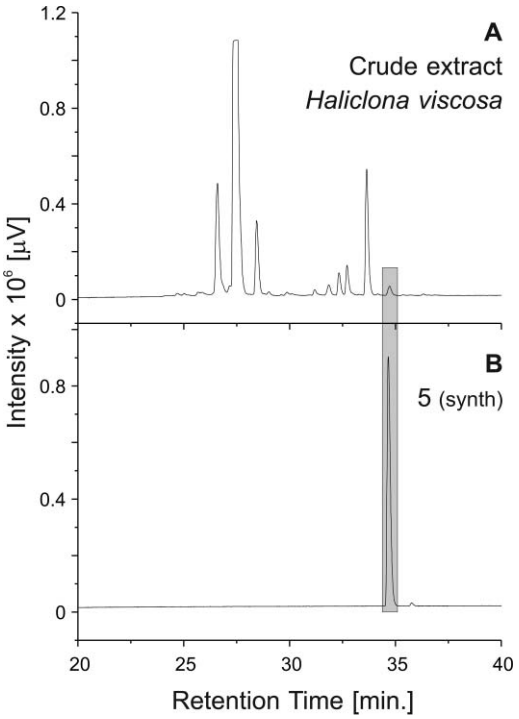
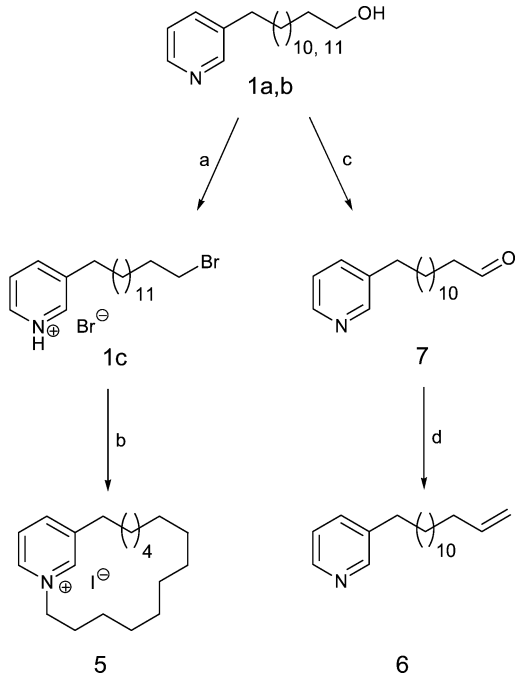


Fig. 1 Comparison of the HPLC chromatograms of the crude extract of *Haliclona viscosa* (A) and the cyclic monomeric 3-APA **5** obtained by synthesis (B).



Scheme 2 Synthesis of compounds **5** and **6**: a: HBr aq., 110 °C, 12 h; 93%; b: (i) Na_2CO_3, (ii) NaI, butan-2-one, Δ, 4 d, 53%; c: oxalyl chloride, DMSO, Et_3N, Ar, CH_2Cl_2, –70 °C, 45 min, 82%; d: Ph_3PMeBr, LDA, Ar, THF, –60 °C–rt, 2 h; 43%.

of **5**, the differentiation of the two constitutional proposals is not possible by MS/MS analysis (see ESI, Fig. S1†). Fragmentation of 3-APAs usually occurs *via* the Onium reaction or Hofmann fragmentation.[11j] The retention time of the new compound in RP_{18}-HPLC lies close to those observed for compounds as **3** and **4** (see ESI, Fig. S2†), which makes a linear molecule as **6** rather unlikely and led us to propose **5** as the potential structure.[14] To verify these arguments, we have decided to synthesise compounds **5** and **6** and compare both to the natural product by HPLC.

Compounds **5** and **6** were each synthesised in a two-step procedure, starting from alcohols **1a/b** (see Scheme 2), which were prepared according to literature procedures.[11c,d,15] Oxidation of **1a** under Swern conditions gave **7** in 82% yield. Wittig olefination of the aldehyde **7** with methyltriphenyl phosphonium bromide yielded alkene **6** in 43%. Cyclic 3-APA **5** was obtained by bromination of **1b** and subsequent cyclisation under high dilution in 49% yield over two steps. With the two possible constitutional isomers in hand, we could clearly identify **5** as the natural product by comparing the retention times of **5** with the natural compound in the *n*-BuOH fraction of the sponge sample (see Fig. 1).

The cyclic structure of compound **5** was proven by the application of an $^{1}H,^{13}C$-HMBC NMR experiment. The following correlations are indicative: (a) methylene protons adjacent to the ring nitrogen (4.61 ppm) to C-2 (144.2 ppm) and C-6 (142.4 ppm) of the pyridinium ring, (b) H-2 (9.08 ppm) of the pyridinium ring to the methylene carbons adjacent to position 1 (60.9 ppm) and 3 (31.4 ppm) of the pyridinium ring, and (c) protons of the methylene groups next to the pyridinium ring (4.61 and 2.84 ppm) to the methylene carbon atoms of the alkyl chain (24.0–30.0 ppm). This finally proves the proposed structure of **5** as the first cyclic monomeric 3-APA from natural sources.

Several related 3-APAs previously synthesised in our laboratory and the new compounds were tested against *E. coli* tolC, *Staphylococcus aureus* and cells of mouse fibroblasts L929. For a direct comparison of the biological activities to cyclostellettamine C (**3**) and viscosamine (**4**), a monomeric cyclic 3-APA with a C_{13} alkyl chain (**2**) was also synthesised. So far, **5** shows the highest cytotoxicity observed for 3-APAs tested in our group. Whereas, the monomeric cyclic 3-APA (**2**) with a C_{13} alkyl chain exhibits considerably lower antimicrobial and cytotoxic activities compared to **5** (see Fig. 2 and Table 1). This is in accordance with results obtained by Kobayashi *et al*.[6] Comparison of **2** with cyclostellettamine C (**3**) and viscosamine (**4**) reveals a decrease

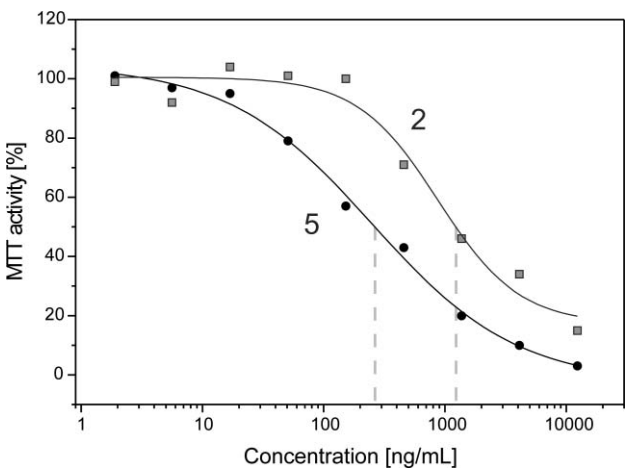


Fig. 2 Comparison of the cytotoxic activity of monomeric 3-APA macrocycles (**2** and **5**). The two compounds just differ in the length of the alkyl chain, which is C_{13} in **2** and C_{14} in **5**.

Table 1 Results of the biological assays[a]

	E. coli tolC[b]	*Staphylococcus aureus*[b]	Cytotoxicity[c]
Monomeric 3-APA **1a** (**1**, X=OH)	10	7	1.5
PyrNMe-**1a**	15	11	5.0
Theonelladin C (**1**, X=NH_2)	12	8	6.0
PyrNMe-theonelladin C	15	11	6.0
Cyclic monomeric 3-APA (**2**)	19	16	1.2
Cyclostellettamine C (**3**)	12	11	1.0
Viscosamine (**4**)	8	9	1.2
Cyclic monomeric 3-APA (**5**)	23	22	0.3
Monomeric 3-APA **6**	<7	<7	13
Monomeric 3-APA **8** (**1**, X=CH_3)	<7	<7	12

[a] For details, see experimental section. [b] Results were obtained from an agar diffusion test with a sample amount of 20 µg. The diameter of growth inhibition is given in [mm]. [c] Tested against cells of mouse fibroblasts L929; activity is given as IC_{50} value in µg mL^{-1}.

of the antimicrobial activity with increasing size and/or total charges (see Table 1 and Fig. 3). Both antimicrobial activity and cytotoxicity have a relationship with the size and/or total charges, and the trends are opposite (**4** is most potent in cytotoxicity but weakest in antimicrobial). The activities obtained for the cyclic compounds are shown in Fig. 3.

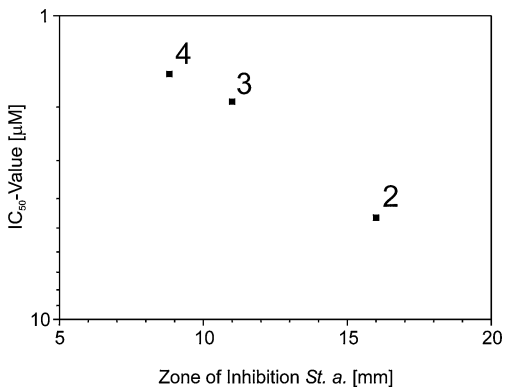


Fig. 3 Cytotoxic (mouse fibrolasts L929) *vs.* antibacterial (*Staphylococcus aureus*) activity of mono- (**2**), di- (**3**) and trimeric (**4**) 3-alkylpyridinium macrocyles.

Linear 3-APAs with fully saturated alkyl chains (**8**) or a terminal double bond (**6**) show virtually no antimicrobial and low cytotoxic activities. Replacing the terminal methylene/methyl group with an amino (theonelladin) or hydroxyl (**1a**) functionality results in moderate antimicrobial activities comparable to those of cyclostellettamine C (**3**). The methylation of the pyridine nitrogen introducing a permanent charge leads to a slight increase in the antimicrobial activity. For the amino compound, the cyctoxicity remains constant, whereas the hydroxy compound shows a decrease by a factor of 3. The very strong effects reported by Stierle and Faulkner[5a] were not observed here.

Conclusions

In conclusion, we have demonstrated the identification of the first monomeric cyclic 3-APA from natural sources by comparsion of the synthetic compound with the natural product. The analysis of the potential natural products was based on HPLC and MS^n analysis.

Starting from MS data, we suggested (and synthesised) two structural proposals for the new natural product. The structure was elucidated by comparison of the two possible synthetic compounds with the natural product in the *n*-BuOH fraction by RP-HPLC. In this case, the lack of pure substance could be compensated by the knowledge of the properties of 3-APAs in MS and HPLC experiments. The synthesis finally proved the proposed cyclic structure of the new natural product.

This first cyclic monomeric 3-APA shows the highest activities observed so far in our investigations. From the comparison of the natural products with other 3-APAs, the following general statements are possible: (a) linear 3-APAs with an alkyl chain without heteroatoms have no activity, (b) adding or removing methylene groups in the alkyl chains alters the activity, (c) a permanent charge increases the antibacterial activity, and (d) the ratio (molecular mass/charge) of the molecule is the key to bioactivity. Although the effects were weaker, statements (b) and (c) are in accordance with the results obtained by Kobajashi *et al.*[6] and Stierle and Faulkner.[5a] Thus, our experiments confirm previous results and also deepen the understanding of the biological activity of 3-APAs.

We would like to thank Prof. Michael Göbel (Universität Frankfurt/Main, Germany) for his support of this project, especially for providing the laboratory space, Ellen Lichte for performing the HPLC analysis, Achim Grube for the HPLC-MS measurements and Bettina Hinkelmann for performing the biological assays.

Experimental

General experimental methods

Haliclona viscosa was collected off Blomstrandhalvøya by SCUBA diving in Kongsfjorden, Svalbard (June 2001). Voucher specimens are deposited at the Zoölogisch Museum, Amsterdam, The Netherlands (ZMA No. 17009). Samples of *H. viscosa* were divided into portions, immediately frozen after collection and kept at –20 °C until extraction. Freeze-dried sponge tissue was extracted at room temperature with a 1 : 1 mixture of MeOH and CH_2Cl_2. The resulting crude extract was partitioned between *n*-hexane and MeOH. The MeOH extract was concentrated and further partitioned between EtOAc and H_2O. Finally, the aqueous layer was extracted with *n*-butanol. The extracted portions were investigated by analytical HPLC.

Analytical HPLC was carried out on a Kromasil RP_{18} column (4.6 × 250 mm, 5 μm, Knauer) with a Sedex 75 (EDC) light scattering detector. Separation was achieved by applying a gradient from 0 to 80% MeCN (0.1% TFA)–H_2O (0.1% TFA) in 40 minutes, 100% MeCN (0.1% TFA) from 40 to 45 minutes, 50 : 50 MeOH–MeCN (0.1% TFA) from 45 to 50 minutes and 100% MeOH for 5 minutes. Total analysis time was 55 minutes with a flow rate of 1.0 mL min^{-1} and an oven temperature of 40 °C.

Mass spectra were acquired with a micrOTOF$_{LC}$ mass spectrometer equipped with an ESI source (Bruker Daltonik). The following ESI inlet conditions were applied: dry gas temperature: 180 °C; dry gas flow: 10 L min^{-1}; nebulizer pressure: 1.5 bar; capillary voltage: 4500 V. The system was calibrated in positive mode by external calibration with sodium formiate clusters. MS^n spectra were acquired with an Esquire 3000plus ion trap in the positive mode equipped with an ESI source (Bruker Daltonik).

NMR spectra were recorded with a Bruker AM 250 (250 MHz) spectrometer. Chemical shifts are quoted in ppm and are referenced to the appropriate solvent signal. FT-IR spectra were recorded on a Perkin–Elmer 1600 series spectrometer. Absorption maxima are reported in wavenumbers. Elemental analysis was performed with a Heraeus CHN Rapid. Melting points were obtained with a Kofler melting point apparatus and are uncorrected. Column chromatography was performed on silica gel 60 (Merck, particle size 0.04–0.063 mm). TLC was performed on aluminium plates precoated with Merck silica 60. Compounds were visualised by UV irradiation (254 nm) or dyeing with $KMnO_4$ solution (1 g $KMnO_4$, 6.6 g K_2CO_3, 2 mL 5% NaOH solution in 100 mL H_2O). All solvents were purified by simple distillation, except for THF, which was distilled from sodium–benzophenone under argon.

Biological assays

Antimicrobial assay: antimicrobial activities were determined by agar diffusion tests using paper discs of 6 mm diameter soaked with 20 μL of the test compound in MeOH (1 mg mL^{-1}). The microorganisms were received from the GBF collection, grown on standard media and seeded into liquid agar medium to a final O.D. of 0.01. Plates were incubated at 30 °C and the diameter of resulting inhibition zones were measured after 1 day.

Cell proliferation assay: L929 mouse fibroblasts were obtained from the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ) and cultivated at 37 °C and 10% CO_2 in DME medium (high glucose) supplemented with 10% fetal calf serum. Cell culture reagents were purchased from Life Technologies Inc. (GIBCO BRL). Growth inhibition was measured in microtiter plates. Aliquots of 120 μL of the suspended cells (50 000/mL) were added to 60 μL of serial dilutions of the test compounds. After 5 days, the growth was determined using the MTT assay.

Syntheses

3-(14-Bromo-tetradecyl)-pyridinium bromide (1c). A mixture of 14-(pyridine-3-yl)tetradecanol (**1b**) (0.94 g, 3.2 mmol) and 15 mL of hydrobromic acid (48%) was heated to 110 °C for 12 h. After cooling, the mixture was poured onto 50 mL of ice water, the precipitate was filtered off and washed with cool H_2O. Drying *in vacuo* gave **1c** (1.31 g, 93%) as a grey solid. R_f: 0.4 (free base) hexane–EtOAc 1 : 1, mp 121 °C, (Found: C, 52.5; H, 7.6; N, 3.2. $C_{19}H_{33}Br_2N$ requires C, 52.4; H, 7.6; N, 3.2%); ν_{max}(KBr)/cm^{-1} 2919, 2850, 2611, 1549, 1466, 1120, 809, 690; δ_H(250 MHz; $CDCl_3$) 1.19–1.47 (20 H, m, 10 × CH_2), 1.62–1.75 (2 H, m, 3-CH_2CH_2), 1.76–1.90 (2 H, m, CH_2CH_2Br), 2.84 (2 H, t, *J* 7.8, 3-CH_2), 3.38 (2 H, t, *J* 6.8, CH_2Br), 7.91 (1 H, dd, *J* 8.0 and *J* 5.7, 5-H), 8.26 (1 H, d, *J* 8.1, 4-H), 8.67 (1 H, s, 2-H), 8.72 (1 H, d, *J* 5.4, 6-H); δ_C(62.5 MHz; $CDCl_3$) 28.1, 28.6, 28.9, 29.1, 29.3, 29.5 (10 × CH_2), 30.3 (3-CH_2CH_2), 32.7 (CH_2CH_2Br), 32.8 (CH_2Br), 34.1 (3-CH_2), 126.6 (C-5), 138.1 (C-3), 140.0 (C-6), 143.3 (C-2), 145.8 (C-4).

Cyclic monomer 5. 3-(14-Bromo-tetradecyl)-pyridinium bromide (**1c**) (0.5 g, 1.15 mmol) was dissolved in 25 mL of CH_2Cl_2 and neutralised by washing with 2 M Na_2CO_3 (3 × 10 mL). The organic layer was dried over $MgSO_4$ and most of the solvent removed, leaving about 1 mL. It was diluted with 10 mL of butan-2-one, and the resulting mixture was added to a refluxing solution of NaI (0.21 g, 1.38 mmol) in 200 mL of butan-2-one *via* syringe pump at a rate of 0.5 mL/h. After 4 d of refluxing, it was allowed to cool down, the solvent removed and the residue triturated with Et_2O to give **5** as yellow solid. Recrystallisation from water yielded pure **5** (0.244 g, 53%) as slightly yellow powder. R_f: 0.27 CH_2Cl_2: MeOH 9:1; mp 147–149 °C; (Found: C, 56.6; H, 8.0; N, 3.3. $C_{19}H_{32}IN$ requires C, 56.9; H, 8.0; N, 3.5%); ν_{max}(KBr)/cm^{-1} 3013, 2925, 2854, 1627, 1501, 1459, 1156, 829, 698; δ_H(250 MHz; [d_6]DMSO) 1.02–1.34 (20 H, m, 10 × CH_2), 1.61–1.74 (2 H, m, 3-CH_2CH_2), 1.84–1.98 (2 H, m, N-CH_2CH_2), 2.84 (2 H, t, *J* 6.5, 3-CH_2), 4.61 (2 H, t, *J* 6.1, N-CH_2), 8.12 (1 H, dd, *J* 6.2, *J* 7.7, H-5), 8.52 (1 H, d, *J* 8.0, H-4), 8.97 (1 H, d, *J* 6.0, H-6), 9.09 (1 H, s, H-2); δ_C(62.5 MHz; [d_6]DMSO) 24.0, 25.3, 25.4, 26.3, 26.4, 27.1, 27.3, 27.6 (10 × CH_2), 29.1 (3-CH_2CH_2), 30.0 (NCH_2CH_2), 31.4 (3-CH_2), 60.9 (NCH_2), 127.9 (C-5), 142.4 (C-6), 142.6 (C-3), 144.2 (C-2), 145.5 (C-4).

3-(Tetradec-13-enyl)pyridine (6). A suspension of Ph_3PMeBr (0.68 g, 1.9 mmol) in 30 mL of THF at –30 °C under an atmosphere of argon was treated with 2 M LDA solution (0.9 mL, 1.8 mmol) and the resulting mixture was stirred for 30 min. It was cooled to –60 °C and a solution of **7** in 5 mL of THF was added dropwise. After 30 min, the cooling was stopped and it was stirred for another hour at r.t. The reaction mixture was then poured on 50 mL of water, acidified (pH 2) with HCl and extracted with Et_2O (3 × 20 mL). The aqueous phase was basified with Na_2CO_3 and extracted with Et_2O (3 × 20 mL). The combined organic layers were dried over $MgSO_4$ and the solvent removed. The raw product was purified by column chromatography on silica (hexane–EtOAc 4 : 1), which yielded **6** (0.21 g; 43%) as yellow oil. R_f: 0.57 hexane–EtOAc 2 : 1; (Found: C, 83.2; H, 11.5; N, 5.2. $C_{19}H_{31}N$ requires C, 83.5; H, 11.4; N, 5.1%); ν_{max}(neat)/cm^{-1} 2925, 2854, 1575, 1421, 1026, 909, 713; δ_H(250 MHz; $CDCl_3$) 1.19–1.42 (18 H, m, 9 × CH_2), 1.52–1.69 (2 H, m, 3-CH_2CH_2), 1.98–2.10 (2 H, m, CH_2CHCH_2), 2.60 (2 H, t, *J* 7.7, 3-CH_2), 4.88–5.04 (2 H, m, $CHCH_2$), 5.73–5.90 (1 H, m, CH), 7.19 (1 H, dd, *J* 4.8, *J* 7.7, 5-H), 7.48 (1 H, d, *J* 7.8, 6-H), 8.40–8.47 (2 H, m, 3-H and 2-H); δ_C(62.5 MHz; $CDCl_3$) 28.9, 29.1, 29.4, 29.5, 29.6 (9 × CH_2), 31.1 (3-CH_2CH_2), 33.0 (CH_2CHCH_2), 33.8 (3-CH_2), 114.0 ($CHCH_2$), 123.2 (C-5), 135.7 (C-4), 138.0 (C-3), 139.2 (CH), 147.2 (C-6), 150.0 (C-2).

13-(Pyridin-3-yl)tridecanal (7). To a solution of oxalyl chloride (0.61 mL, 7.2 mmol) in 30 mL of CH_2Cl_2 at –70 °C under an atmosphere of argon successively dimethylsulfoxide (0.64 mL, 9.0 mmol) and alcohol **1a** (1.0 g, 3.2 mmol) in 5 mL of CH_2Cl_2 were

slowly added. The mixture was stirred at −70 °C for 45 min. After the addition of Et_3N (1 mL, 7.2 mmol), it was allowed to reach r.t. and was diluted with 30 mL of Et_2O. The precipitate was filtered off and washed with Et_2O (3 × 10 mL) The combined solvents were evaporated and the residue purified by column chromatography on silica (hexane–EtOAc 1 : 1) to yield **7** (0.81 g, 82%) as slightly yellow oil. R_f: 0.5 hexane–EtOAc 2 : 1; (Found: C, 78.6; H, 10.7; N, 5.2. $C_{18}H_{29}NO$ requires C, 78.5; H, 10.6; N, 5.1%); ν_{max}(KBr)/cm^{-1} 2926, 2854, 1725, 1466, 1422, 1026, 794, 714; δ_H(250 MHz; $CDCl_3$) 1.20–1.37 (18 H, br s, 9 × CH_2), 1.53–1.68 (4 H, m, C$H_2$$CH_2$CHO and 3-$CH_2CH_2$), 2.41 (2 H, td, *J* 7.3 and *J* 1.8, CH_2CHO), 2.59 (2 H, t, *J* 7.7, 3-CH_2), 7.19 (1 H, dt, *J* 4.8 and *J* 7.7, 5-H), 7.48 (1 H, dt, *J* 7.8 and *J* 1.8, 4-H), 8.40–8.45 (2 H, m, 2-H and 6-H), 9.76 (1 H, t, *J* 1.8, CHO); δ_C(62.5 MHz; $CDCl_3$) 22.1 (C$H_2$$CH_2$CHO), 29.1, 29.3, 29.4, 29.5 (10 × CH_2), 31.1 (3-CH_2CH_2), 33.0 (3-CH_2), 43.9 (CH_2CHO), 123.2 (C-5), 135.8 (C-4), 138.0 (C-3), 147.0 (C-6), 149.8 (C-2), 202.9 (CHO).

Notes and references

1 3-APA (3-alkylpyridine alkaloids) is a synonym for pyridinium, pyridine, tetrahydropyridine or piperidine alkaloids.

2 (*a*) R. J. Andersen, R. W. M. van Soest and F. Kong, *Alkaloids: Chemical and Biological Perspectives*, ed. S. W. Pelletier, Pergamon Press, Oxford, 1996, vol. 10, p. 301; (*b*) G. Cimino, S. de Stefano, G. Scognamiglio, G. Sodano and E. Trivellone, *Bull. Soc. Chim. Belg.*, 1986, **95**, 783; (*c*) N. Fusetani, K. Yasumuro, S. Matsunaga and H. Hirota, *Tetrahedron Lett.*, 1989, **30**, 6891; (*d*) N. Fusetani, N. Asai, S. Matsunaga, K. Honda and K. Yasumuro, *Tetrahedron Lett.*, 1994, **35**, 3967; (*e*) R. Sakai, T. Higa, C. W. Jefford and G. Bernardinelli, *J. Am. Chem. Soc.*, 1986, **108**, 6404; (*f*) R. Sakai, S. Komoto, T. Higa, C. W. Jefford and G. Bernardinelli, *Tetrahedron Lett.*, 1987, **28**, 5493.

3 (*a*) A. Teubner and H. Gerlach, *Liebigs Ann. Chem.*, 1993, 161; (*b*) A. V. R. Rao, G. R. Reddy and B. V. Rao, *J. Org. Chem.*, 1991, **56**, 4545; (*c*) J. Kobayashi, T. Murayama, Y. Ohizumi, T. Sasaki, T. Ohta and S. Nozoe, *Tetrahedron Lett.*, 1989, **30**, 4833.

4 A. R. Carroll and P. J. Scheuer, *Tetrahedron*, 1990, **46**, 6637.

5 (*a*) D. B. Stierle and D. J. Faulkner, *J. Nat. Prod.*, 1991, **54**, 1134; (*b*) R. C. Larock and Y. Wang, *Tetrahedron Lett.*, 2001, **43**, 21; (*c*) Y. Wang, X. Dong and R. C. Larock, *J. Org. Chem.*, 2003, **68**, 3090.

6 K. Hirano, T. Kubota, M. Tsuda, Y. Mikami and J. Kobayashi, *Chem. Pharm. Bull.*, 2000, **48**, 974.

7 G.-Y.-S. Wang, M. Kuramoto and D. Uemura, *Tetrahedron Lett.*, 1996, **37**, 1813.

8 C. A. Volk and M. Köck, *Org. Biomol. Chem.*, 2004, **2**, 1827.

9 (*a*) M. H. Baslow and P. Turlapaty, *Proc. West. Pharmcol. Soc.*, 1969, **12**, 6; (*b*) F. J. Schmitz, K. H. Hollenbeak and D. C. Campbell, *J. Org. Chem.*, 1978, **43**, 3916; (*c*) R. G. S. Berlinck, C. A. Ogawa, A. M. P. Almeida, M. A. A. Sanchez, E. L. A. Malpezzi, L. V. Costa, E. Hajdu and J. C. de Freitas, *Comp. Biochem. Physiol., C*, 1996, **115C**, 155; (*d*) R. H. Scott, A. D. Whyment, A. Foster, K. H. Gordon, B. F. Milne and M. Jaspars, *J. Membr. Biol.*, 2000, **176**, 119; (*e*) S. J. Tucker, D. McClelland, M. Jaspars, K. Sepčić, D. J. MacEwan and R. H. Scott, *Biochim. Biophys. Acta, Biomembr.*, 2003, **1614**, 171.

10 3-APAs with alkyl chain lengths of C_{13} and C_{14} are known as synthetic products. See ref. 5*c* and R. Hulst, I. Muizebelt, P. Oosting, C. v. d. Pol, A. Wagenaar, J. Smisterova, E. Bulten, C. Driessen, D. Hoekstra and J. B. F. N. Engberts, *Eur. J. Org. Chem.*, 2004, 835.

11 (*a*) N. Fusetani, N. Asai, S. Matsunaga, K. Honda and K. Yasumuro, *Tetrahedron Lett.*, 1994, **35**, 3967; (*b*) H. Anan, N. Seki, O. Noshiro, K. Honda, K. Yasumuro, T. Ozasa and N. Fusetani, *Tetrahedron*, 1996, **52**, 10849; (*c*) J. E. Baldwin, D. R. Spring, C. E. Atkinson and V. Lee, *Tetrahedron*, 1998, **54**, 13655; (*d*) A. Kaiser, X. Billot, A. Gateau-Olesker, C. Marazano and B. C. Das, *J. Am. Chem. Soc.*, 1998, **120**, 8026; (*e*) M. J. Wanner and G.-J. Koomen, *Eur. J. Org. Chem.*, 1998, 889; (*f*) K. Jakubowicz, K. Ben Abdeljelil, M. Herdemann, M.-T. Martin, A. Gateau-Olesker, A. Al Mourabit, C. Marazano and B. C. Das, *J. Org. Chem.*, 1999, **64**, 7381; (*g*) J. H. H. L. de Oliveira, A. Grube, M. Köck, R. G. S. Berlinck, M. L. Macedo, A. G. Ferreira and E. Hajdu, *J. Nat. Prod.*, 2004, **67**, 1685; (*h*) N. Oku, K. Nagai, N. Shindoh, Y. Terada, R. W. M. van Soest, S. Matsunaga and N. Fusetani, *Bioorg. Med. Chem. Lett.*, 2004, **14**, 2617; (*i*) J. H. H. L. de 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. de Souza, P. R. R. Minarini, F. C. S. Galetti, C. L. Silva, E. Hajdu and R. G. S. Berlinck, *Marine Drugs*, 2006, **4**, 1; (*j*) A. Grube, C. Timm and M. Köck, *Eur. J. Org. Chem.*, 2006, 1285.

12 (*a*) Y. Morimoto, C. Yokoe, H. Kurihara and T. Kinoshita, *Tetrahedron*, 1998, **54**, 12197; (*b*) J. E. Baldwin, D. A. James and V. Lee, *Tetrahedron Lett.*, 2000, **41**, 733; (*c*) S. Michelliza, A. Al-Mourabit, A. Gateau-Olesker and C. Marazano, *J. Org. Chem.*, 2002, **67**, 6474; (*d*) C. A. Volk, H. Lippert, E. Lichte and M. Köck, *Eur. J. Org. Chem.*, 2004, 3154.

13 (*a*) C. A. Volk and M. Köck, *Org. Lett.*, 2003, **5**, 3567; (*b*) C. Timm and M. Köck, *Synthesis*, 2006, **15**, 2580.

14 The major difference of the two proposals is the heterocyclic nitrogen atom (pyridine *vs.* pyridinium). The chemical shift of the nitrogen and the methylene group attached to the nitrogen would allow to differentiate between **5** and **6**. Furthermore, this could be confirmed by analysis of the ^{1}H,^{13}C-HMBC spectrum..

15 S.-K. Kang, W.-S. Kim and B.-H. Moon, *Synthesis*, 1985, **12**, 1161.