A New EGF-Active Polymeric Pyridinium Alkaloid from the Sponge *Callyspongia fibrosa*

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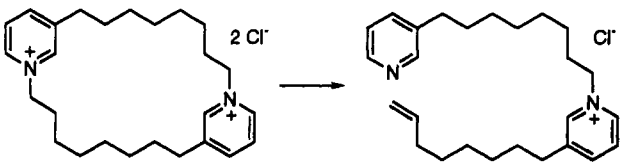
An inhibitor of the epidermal growth factor (EGF) receptor was isolated from an extract of the Micronesian sponge *Callyspongia fibrosa*. The structure of the EGF-active compound was first proposed, on the basis of ion-spray mass spectrometry, to be the cyclic dimer 1 (n = 2) but comparison of a sample produced by an efficient synthetic route revealed that hypothesis to be incorrect. The EGF-active constituent must therefore be a large oligomer or a polymer of the same repeating subunit 1.

Activation of the EGF receptor, a 170-kDa transmembrane protein, by EGF (epimermal growth factor) results in DNA synthesis and cell proliferation. Tumors such as squamous cell carcinomas of the lung and some advanced gastric tumors have amplified or overexpressed EGF receptors.[1] This provides a rationale for the use of EGF antagonists as possible antitumor agents. Screening of extracts from marine invertebrates in an EGF mitogenic bioassay[2] revealed that an aqueous extract of the Micronesian sponge *Callyspongia fibrosa* inhibited an EGF-stimulated mitogenic response in cells in culture. A bioassay-guided fractionation of the extract yielded a novel EGF-active consituent for which a polymeric structure 1 is proposed.

A methanolic extract of the lyophilized sponge was concentrated and partitioned between ethyl acetate and water. The aqueous extract was lyophilized and triturated with methanol to separate the EGF-active organic material from inorganic salts. Bioassay-guided chromatography on LH-20 using methanol as eluant, followed by centrifugal countercurrent chromatography gave a mixture of pyridinium salts. Final purification by HPLC on an amino column gave a single EGF-active compound as a pale brown oil.

The spectral data of the EGF-active compound was consistent with a 3-alkylpyridinium salt devoid of any other functional groups. Both the UV (267, 273 nm) and IR (1630 cm^{-1}) spectra are in accordance with the published values for pyridinium salts.[3,4] There was a misleading band at 3400 cm^{-1} in the IR spectrum but this was due to water of hydration, which had also been observed in the IR spectrum of the halitoxins.[3] The ^1H NMR spectrum contained signals at δ 8.95 (s, 1 H, H-2), 8.84 (d, 1 H, J = 5 Hz, H-6), 8.45 (d, 1 H, J = 8 Hz, H-4), and 8.01 (dd, 1 H, J = 8, 5 Hz, H-5) that are typical of a 3-substituted pyridinium salt. A signal at δ 4.60 (t, 2 H, J = 7.5 Hz), assigned to a methylene group attached to the quaternary nitrogen, was coupled to a methylene signal at 2.20 (m, 2 H) that was in turn coupled to a methylene envelope at 1.41 (br s, 8 H). A signal at δ 2.87 (t, 2 H, J = 7.5 Hz), assigned to a methylene group attached to the C-3 position of the pyridinium ring, was coupled to a methylene signal at 1.73 (br s, 2 H) that was also coupled to the methylene envelope. The alkyl chain of eight methylene groups joining the 3-position of the pyridinium ring to a quaternary nitrogen gave rise to eight methylene signals between δ 27.3 and 62.9 in the ^{13}C NMR spectrum. *It is significant that there was no signal that could be assigned to terminal methyl or pyridine groups.* These data are consistent with a cyclic structure or with a linear head-to tail polymer that is so large that ^1H NMR signals due to the terminal units are not observed.

Scheme I. Proposed Mass Spectral Cleavage of a Dimeric Pyridinium Salt To Give m/z = 379



Elucidation of the structure of the EGF-active metabolite required determination of the molecular weight of a charged polymer of molecular formula $(C_{13}H_{20}N^+X^-)_n$, where X^- is predominantly chloride in the natural product, in which the mass to charge ratio is the same for all values of n. It is therefore not surprising that the dominant ion in the high-resolution mass spectrum was at m/z = 190.1596, corresponding to the $C_{13}H_{20}N$ subunit, with a smaller peak at m/z = 226.1363 due to the addition of HCl. These mass spectral data effectively eliminate alternate head-to-head and/or tail-to-tail arrangements within the polymer since these substructures would require different major molecular ions arising from cleavages of

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(1) Aaronson, S. A. *Science* 1991, *254*, 1146–1153.

(2) The mitogenic response due to EGF was quantitated by measuring the incorporation of [^3H]thymidine into NIH3T3 cells transfected with the human EGF receptor gene. These cells were kindly provided by Dr. I. Pastan, National Cancer Institute (Velu *et al. Science* 1987, *238*, 1408–1410). Cells were grown in low serum (0.1%) for 4 h and then test samples were added and preincubated with the cells for 1 h, before the addition of the mitogen EGF (0.5 ng/mL) or insulin (0.1 mg/mL). Stimulation of these cells with insulin was used as a "nonspecific" mitogen control. After 16 h, mitogenic stimulation was assessed by a 1-h incorporation of [^3H]-thymidine (2.5 nCi/μL). Background controls which received no mitogenic stimulation were used to determine baseline proliferation. The concentration at which control EGF stimulation was inhibited 50% by the test sample was calculated (EGF IC_{50}) as was the same value for insulin (Insulin IC_{50}). The values were used to calculate a selectivity ratio which is (Insulin IC_{50})/(EGF IC_{50}).

(3) Schmitz, F. J.; Hollenbeak, K. H.; Campbell, D. C. *J. Org. Chem.* 1978, *34*, 3916–3922.

(4) Talpir, R.; Rudi, A.; Ilan, M.; Kashman, Y. *Tetrahedron Lett.* 1992, *33*, 3033–3034.

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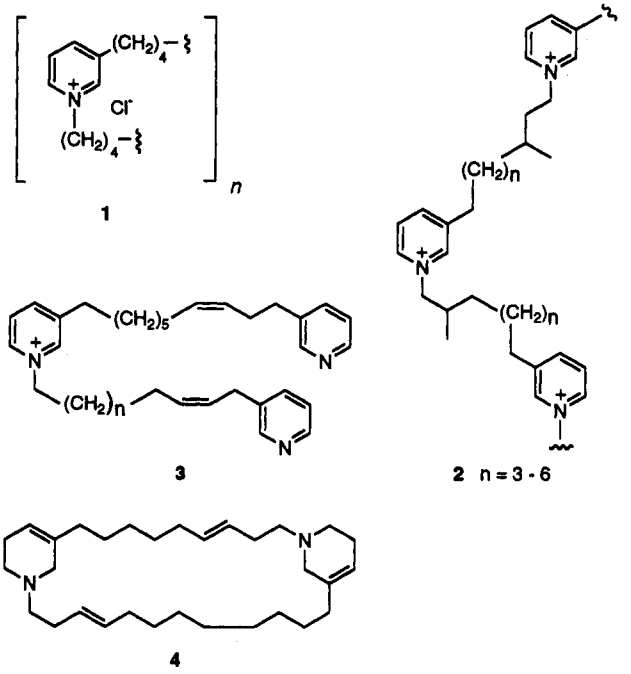
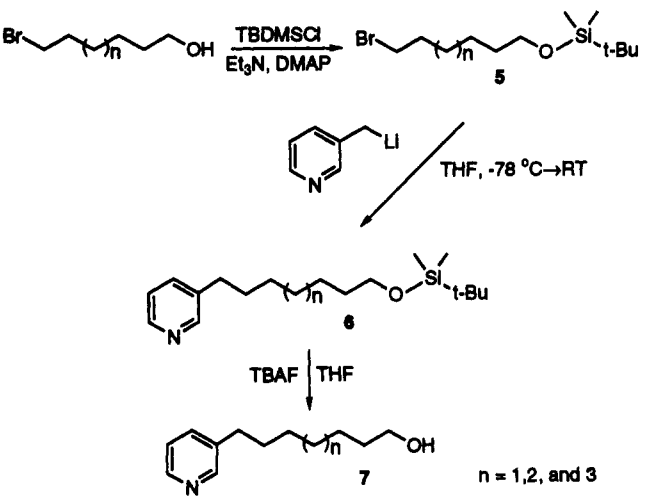


the bond between the pyridinium nitrogen and the alkyl chain as illustrated in Scheme I. Similar mass spectral data had been obtained by Schmitz *et al.*,[3] who in 1978 reported the structure of halitoxin (**2**) as an oligomeric/polymeric toxin of molecular weight >5000 Da. Since that time, although several investigators have encountered these pyridinium salts, there has been little progress in defining the nature of these materials. The structure of niphatoxins A (**3**, n = 2) and B (**3**, n = 3) are of interest because they illustrate that the ^{1}H NMR signals of the terminal pyridine groups are easily differentiated from those of the pyridinium ring.[4] In addition, there are an increasing number of metabolites from related sponges, such as haliclamines A (**4**) and B from *Haliclona* sp.,[5] the manzamines from *Haliclona* sp.,[6,7] and possibly the petrosins from *Petrosia seriata*,[8] all of which might be regarded as being derived biosynthetically from dimeric 3-substituted pyridinium cyclophanes.

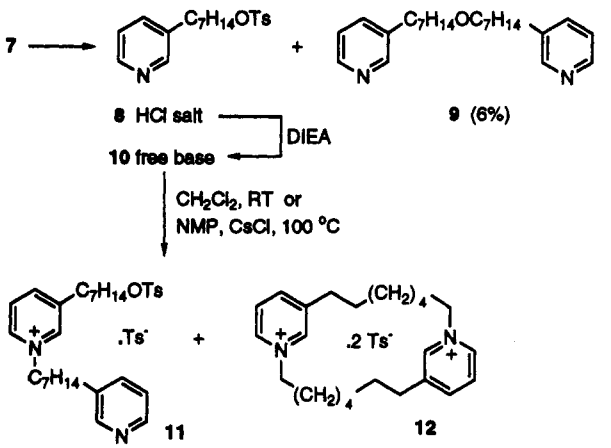
Our initial approach to the determination of the molecular weight of the pyridinium salt was directed toward the production of an uncharged derivative for mass spectrometry. Despite many literature precedents,[9,10] attempts to hydrogenate (50 psi) a methanolic solution of the pyridinium salt over platinum oxide were unsuccessful. Likewise, the well precedented[11] reduction of pyridinium salts with sodium borohydride resulted in the conversion of EGF-active material to an intractable dark brown gum. All attempts to crystallize the tetraphenylborate salt for X-ray studies were also unsuccessful although this derivative readily forms a solid precipitate from ethanol.

The problem of determining the molecular weight appeared to be solved by the application of ion-spray mass spectrometry but, as will be shown later, this result was not correct. The mass spectrum from ion-spray mass spectrometry showed a prominent peak at m/z 379, which was interpreted as arising from a dimeric structure **1** (n = 2) [≡ **12** (n = 2)] that has undergone the cleavage illustrated in Scheme I. The molecular formula of the EGF-active pyridinium salt was therefore thought to be $C_{26}H_{40}N_2Cl_2$.

Scheme II



Scheme III

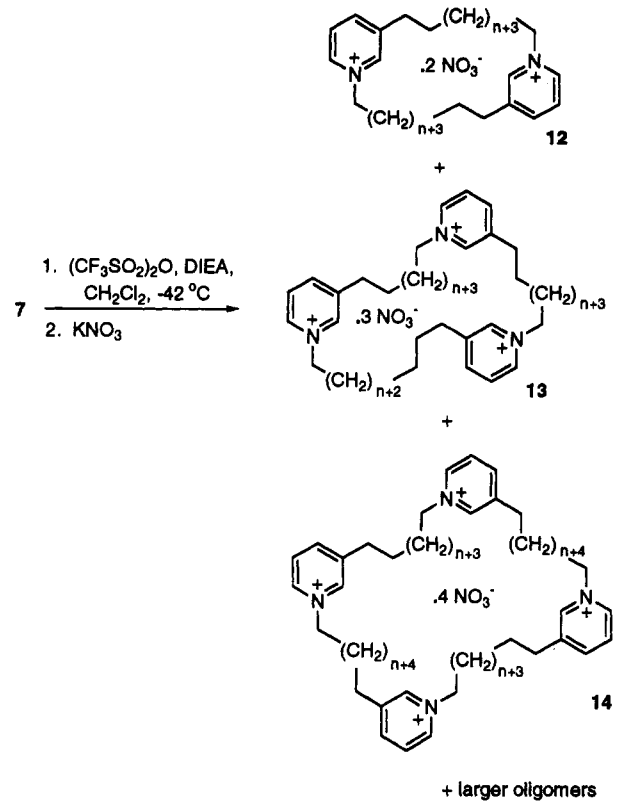


It is fortunate that a synthetic program was undertaken to prepare a large quantity of material for *in vivo* testing and to prepare analogues to try to optimize activity. The pyridine alcohols **7** (n = 1–3) were synthesized as shown in Scheme II. Treatment of 3-picoline with LDA in THF at −78 °C gave the lithiated adduct, which was cleanly monoalkylated with 0.4 equiv of TBDMS-protected bromo alcohols **5**, with yields ranging from 62–83%. The amounts of dialkylated product depended on the distance of the bulky TBDMS group from the site of attack. No dialkylation was observed when n = 1 while there was 26% dialkylation when n = 3. Treatment with TBAF gave the alcohols **7** quantitatively.

The induction of a head-to-tail cyclic oligomerization required conversion of the terminal hydroxyl group in **7** to a leaving group. We first made the tosylate **10** (Scheme III). This could be formed as the stable hydrochloride **8**, along with a small amount of the ether **9**, without any external base, although the reaction stalled at ca. 85% conversion. Addition of diisopropylethylamine forced the reaction to completion, and **10** could be purified on silica.

(5) Fusetani, N.; Yasumuro, K.; Matsunaga, S.; Hirota, H. *Tetrahedron Lett.* **1989**, *30*, 6891–6894.
(6) Sakai, R.; Higa, T.; Jefford, C. W.; Bernardinelli, G. *J. Am. Chem. Soc.* **1986**, *108*, 6404–6405.
(7) Baldwin, J. E.; Whitehead, R. C. *Tetrahedron Lett.* **1992**, *33*, 2059–2062.
(8) Braekman, J. C.; Daloze, D.; Macedo de Abreu, P.; Piccinni-Leopardi, C.; Germain, G.; van Meersche, M. *Tetrahedron Lett.* **1982**, *23*, 4277–4280.
(9) Freifelder, M. *J. Pharm. Sci.* **1966**, *55*, 535.
(10) Lee, J.; Freudenberg, W. *J. Org. Chem.* **1944**, *9*, 537–546.
(11) Eisner, U.; Kuthan, J. *Chem. Rev.* **1972**, *72*, 1–42.

Scheme IV



After chromatography, **10** showed a trace of monopyridinium tosylate **11** by ^1H NMR. At room temperature in CH_2Cl_2 overnight, 0.9% of the cyclized dimer **12** was observed by HPLC, along with 33% **11** and 66% **10**. After a further week the fraction of **12** had risen to only 2.5%. A portion of **10** was heated in *N*-methylpyrrolidinone at 100 °C overnight in the presence of CsCl to encourage cyclization; HPLC showed the reaction to have reached the following proportions: 14% **12**, 23% **11**, and 63% **10**.

In order to speed up the reaction, alcohol **7** (n = 1) was treated with triflic anhydride and diisopropylethylamine at −42 °C and allowed to warm to rt (Scheme IV). HPLC showed mostly one component, formed in ca. 67% yield, along with some other material that, by HPLC and TLC, behaved like higher order oligomers. Dimer **12** could be purified somewhat on a neutral alumina column, eluting with very low polarity CH_2Cl_2/MeOH mixtures. However, the best separations were achieved on silica, eluting with 7:3 20% saturated KNO_3/CH_3CN.[12] In this case the dimer **12**, trimer **13**, and tetramer **14**, could be isolated as their nitrate salts by evaporation of the fractions, trituration with 10:1 CH_2Cl_2/MeOH, filtration, and evaporation of the filtrate. This reaction was repeated for alcohols **7** (n = 2 and 3), giving essentially the same results. In order for the reaction to go to completion, i.e. for the starting material to be completely converted into cyclic oligomeric products, it must be carried out at high concentrations (≥0.4 M). For the (n = 2) and (n = 3) series, the reaction was performed at various temperatures in order to determine whether mixtures enriched in the higher order oligomers might show greater activity in EGF assay (see below). There was no clear difference, as judged by TLC, between the room temperature reactions and those run at low temperatures. This was reflected in the biological results (Table II).

(12) Elliott, C. M.; Freitag, R. A.; Blaney, D. D. *J. Am. Chem. Soc.* **1985**, *107*, 4647–4655.

Table I. Ion-Spray Mass Spectral Data for Pyridinium Compounds 12–15 (n = 1)

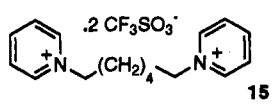
compound	peak	assignment
15·2Tf⁻	391.2	$(M^{2+} + Tf^-)^+$
	162.0	hexenyl pyridinium
	121.2	$M^{2+}/2$
12·2Tf⁻	501.2	$(M^{2+} + Tf^-)^+$
	176.4	$M^{2+}/2$
13·3Tf⁻	976.8	$(M^{3+} + 3Tf^- + H^+)^+$
	826.4	$(M^{3+} + 2Tf)^+$
	338.8	$(M^{3+} + Tf^-)^{2+}/2$
12·2NO_3^-	414.4	$(M^{2+} + NO_3^-)^+$
	351.2	**16**
	176.0	$M^{2+}/2$
13·3NO_3^-	652.4	$(M^{3+} + 2NO_3^-)^+$
	351.2	**16**
	296.0	$(M^{3+} + NO_3^-)^{2+}/2$
	263.6	single elimination$^{2+}/2$
14·4NO_3^-	414.4	$(M^{3+} + 2NO_3^-)^+$
	351.2	**16**
	255.6	$(M^{3+} + NO_3^-)^{2+}/2$
	234.4	single elimination$^{2+}/2$

Table II. Inhibition of EGF and Insulin Receptor-Mediated Mitogenesis (μg/mL)[a]

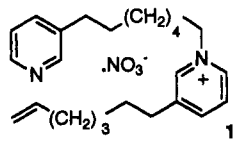
compound(s)	IC_{50} EGF	IC_{50} insulin	selectivity ratio
natural product (**1**)	<2.0	20.5	>10.5
dimer **15**·2Tf⁻	>250	>250	–
dimer **12**·2NO_3^- (n = 1)	129.2	89.2	0.7
trimer **13**·3NO_3^- (n = 1)	>200	>200	–
tetramer **14**·4NO_3^- (n = 1)	>200	>200	–
reaction mixture·NO_3^- (n = 1)	29.3	30.5	1.0
dimer **12**·2NO_3^- (n = 2)	34.5	126.1	3.6
trimer **13**·3NO_3^- (n = 2) and tetramer **14**·4NO_3^-	90.9	129.1	1.4
higher order oligomers·NO_3^- (n = 2)	7.1	11.9	1.6
crude reaction mixture (n = 2), −78 °C	41.9	86.1	2.0
crude reaction mixture (n = 2), 0 °C[a]	9.5	15.3	1.6
crude reaction mixture (n = 2), 24 °C[a]	128.8	144.3	1.1
higher order oligomers·Tf⁻ (n = 3)	1.3	2.6	2.0
crude reaction mixture (n = 3), −78 °C[a]	3.9	7.6	1.9
crude reaction mixture (n = 3), −42 °C[a]	4.4	4.6	1.1
crude reaction mixture (n = 3), 24 °C	7.7	15.8	2.0

[a] These contain 1 equiv of diisopropylammonium triflate.

A comparison of the authentic natural product by mass spectroscopy, HPLC, and TLC with **12** (n = 1 and 2), and with a simple dipyridinium model compound **15** whose structure is unequivocal, clearly showed that the natural product was not a cyclic dimer. On TLC, for example, the dimers **12** and **15** run close to one another with the trimer **13**, tetramer **14**, and higher oligomers having successively smaller R_f values. In this system the natural product stays at the origin, suggesting that it is a large oligomer or polymer containing at least eight subunits.



15



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Ion-spray mass spectral data (Table I) for **12**–**15** (n = 1) also support a large oligomeric structure for the natural product. All of the purified oligomeric species, including the dimer model **15**, show mass peaks corresponding to parent ion-anion adducts which can be confidently assigned. In addition, the larger the molecule, the less abundant are the parent ion peaks and the more significant is elimination product **16**. This is the largest discrete singly-charged species and can be derived from an oligomer

of any size.[13] If the natural product had been the dimer 12, then the ion-spray mass spectrum should have included the $(M^{2+} + A^-)^+$ peak as observed for authentic material.

Since the natural product is a high molecular weight oligomer or polymer, we tested not only the purified components from the (n = 2) and (n = 3) series in the EGF assay but also mixtures enriched in higher order oligomers and crude reaction mixtures, including the (n = 3) series (Table II). The natural product showed very potent activity in the EGF assay (IC_{50} 2 μg/mL). The IC_{50} for insulin stimulation was 20.5 μg/mL, which is greater than a 10-fold difference and suggests that the natural product is more specific for the EGF-mediated mitogenic response than the insulin-mediated response. None of the purified oligomers 12–14 (n = 1 or 2) showed any appreciable activity or selectivity for the EGF or the insulin receptor. The greatest selectivity (3.6) was shown by C-8 dimer 12 (n = 2) but this was only one third of the selectivity exhibited by the natural product and it was 17-fold less potent. The greatest potency (1.3 μg/mL) was shown by a (n = 3) fraction which contained only oligomers greater than the pentamer, but this fraction was only marginally selective. It is possible that a component representing the natural product is present in the mixture of higher order (n = 2) oligomers, but its purification, at least in useful quantities, looks unlikely at this point.

Experimental Section

General Methods. All reagents were purchased from Aldrich Chemical Co. and were used as received. Butyllithium concentration was determined by titration with diphenylacetic acid. Tetrahydrofuran was distilled from sodium benzophenone ketyl, and methylene chloride from calcium hydride. All other solvents were reagent grade and used as received. UV-vis spectra were recorded on a Shimadzu UV-2101PC spectrophotometer. HPLC analysis of synthetic materials was done on a Varian system using a Model 9010 solvent delivery system, a 9050 variable wavelength UV-vis detector, and a Micropak C-18 column. The aqueous buffer in all cases was 50 mM triethylammonium formate (pH 2.8). Centrifugal countercurrent chromatography was accomplished using an Itoh multilayered planet coil centrifuge from P. C. Inc. NMR spectra were obtained using Varian Gemini 300 (Bristol-Myers Squibb) or Unity 500 (UCSD) spectrometers. Microanalyses were carried out at Oneida Research Services.

Collection, Extraction, and Isolation Procedures. The sponge *Callyspongia fibrosa* (162 g dry wt, collection no. 89–079, SIO Benthic Invertebrate Collection no. P1139) was collected by hand using SCUBA (−12 m) from a coral reef at Pohnpei, Micronesia. The sponge was frozen and stored at −20 °C for 2 years. A portion (70 g) was extracted with MeOH (1450 mL) for 3 days and the extract was filtered, concentrated in vacuo, and partitioned between EtOAc (150 mL) and water (2 × 100 mL). The EtOAc layer was dried and the solvent evaporated to give a brown gum (1.3 g) that was inactive in the EGF assay. The aqueous extract was lyophilized to obtain an off-white solid (8 g) that was mainly salt. The solid was triturated with MeOH to obtain an EGF-active yellow residue (2 g) that was chromatographed on Sephadex LH-20 using methanol as eluant to obtain several EGF-active fractions that had similar ^1H NMR spectra. A portion (860 mg) of these combined fractions (1.8 g) was subjected to centrifugal countercurrent chromatography (7:13:8 $CHCl_3$–MeOH–H_2O) to obtain fractions (545 mg total) that contained pyridinium compounds as indicated by their ^1H NMR spectra. Chromatography of a portion (500 mg) of the EGF-active material by HPLC on a Selectosil 10 amino column with 1:1 EtOAc–MeOH as eluant gave six fractions, three of which contained different pyridinium salts as judged by ^1H NMR. Only one of these fractions was active in the EGF assay and this was further purified under the same HPLC conditions to obtain the pyridinium salt 1 (14.6 mg, 0.05% dry wt.): oil; IR (neat) 3400, 2920, 2845, 1630, 1505, 1460, 1220, 1040 cm^{-1}; UV (MeOH) 273 (ε 1800/unit), 267 (ε 2150), 204 (ε 5150); ^1H NMR (500 Mz, CD_3OD) δ 1.41 (br s, 8 H), 1.73 (br s, 2 H), 2.20 (br s, 2 H), 2.87 (t, 2 H, J = 7.5 Hz), 4.60 (t, 2 H, J = 7.5 Hz), 8.01 (dd, 1 H, J = 8, 5 Hz), 8.45 (d, 1 H, J = 8 Hz), 8.84 (d, 1 H, J = 5 Hz), 8.95 (s, 1 H); ^{13}C NMR (50 MHz, CD_3OD) δ 27.3 (t), 30.0 (t), 30.2 (t, 2 C), 31.6 (t), 32.6 (t), 33.6 (t), 62.9 (t), 128.9 (d), 143.3 (d), 145.3 (d), 145.7 (s), 146.6 (d); HRCIMS, obsd m/z = 190.1601, $C_{13}H_{20}N$ requires 190.1596, obsd m/z = 226.1362, $C_{13}H_{21}ClN$ $(M + HCl)^+$ requires 226.1363.

6-Bromohexyl *tert*-Butyldimethylsilyl Ether (5, n = 1). A stirred mixture of 6-bromohexanol (4.930 g, 27.23 mmol) and *tert*-butyldimethylsilyl chloride (6.764 g, 1.65 equiv) in ether (25 mL) at 0 °C were treated with triethylamine (6.27 mL, 1.65 equiv), dropwise over 2 min, and DMAP (100 mg). After 20 min at 0 °C the mixture was allowed to warm at rt for 36 h. More ether was added and the mixture was washed with 10% citric acid (2×), water, and brine, dried, and evaporated. The residue was eluted through 100 g of silica gel with hexane giving the product as a thick, colorless liquid (6.81 g, 85%): ^1H NMR ($CDCl_3$) δ 0.03 (s, 6 H, $SiCH_3$), 0.89 (s, 9 H, *t*-Bu), 1.23–1.58 (m, 6 H, CH_2), 1.88 (quint., 2 H, $BrCH_2CH_2$), 3.41 (t, 2 H, OCH_2), 3.61 (t, 2 H, $BrCH_2$); MS (DCI) 295 $(MH)^+$, 215 $(M - Br)^+$. Anal. Calcd for $C_{12}H_{27}OSiBr$: C, 48.80, H, 9.21. Found: C, 48.92, H, 8.87.

7-Bromoheptyl *tert*-Butyldimethylsilyl Ether (5, n = 2). This was prepared as described above for 5 (n = 1) in 85% yield: ^1H NMR ($CDCl_3$) δ 0.05 (s, 6 H, $SiCH_3$), 0.88 (s, 9 H, *t*-Bu), 1.22–1.57 (m, 8 H, CH_2), 1.88 (m, 2 H, $BrCH_2CH_2$), 3.41 (t, 2 H, OCH_2), 3.60 (t, 2 H, $BrCH_2$); MS (DCI) 309 $(MH)^+$, 229 $(M - Br)^+$.

8-Bromooctyl *tert*-Butyldimethylsilyl Ether (5, n = 3). This was prepared as described above for 5 (n = 1) in 87% yield: ^1H NMR ($CDCl_3$) δ 0.03 (s, 6 H, $SiCH_3$), 0.90 (s, 9 H, *t*-Bu), 1.22–1.57 (m, 10 H, CH_2), 1.88 (m, 2 H, $BrCH_2CH_2$), 3.40 (t, 2 H, OCH_2), 3.60 (t, 2 H, $BrCH_2$); MS (DCI) 323 $(MH)^+$, 243 $(M - Br)^+$.

7-(3-Pyridyl)heptyl *tert*-Butyldimethylsilyl Ether (6, n = 1). A stirred solution of 3-picoline (3.315 mL, 34.06 mmol) in dry THF (10 mL) under argon at −78 °C was treated with a solution of LDA [from diisopropylamine (5.01 mL, 1.05 equiv) in THF (10 mL) at 0 °C treated with butyllithium in hexanes (1.47 M, 24.3 mL, 1.05 equiv) and left to stand at 0 °C for 15 min)]. After 15 min at −78 °C the bromo TBDMS ether 5 (n = 1) (4.024 g, 13.63 mmol) was added via syringe The mixture was allowed to gradually warm to rt and was stirred for 14 h. It was then washed with saturated NH_4Cl, water, and brine, dried, and evaporated. The residue was chromatographed on silica, eluting with 25% ethyl acetate/hexane, to give the product as a thick, colorless oil (3.973 g, 95%): ^1H NMR ($CDCl_3$) δ 0.06 (s, 6 H, $SiCH_3$), 0.91 (s, 9 H, *t*-Bu), 1.34 (m, 6 H, CH_2), 1.50 (m, 2 H, pyr-CH_2CH_2), 1.62 (m, 2 H, OCH_2CH_2), 2.61 (t, 2 H, pyr-CH_2), 3.60 (t, 2 H, OCH_2), 7.20 (dd, 1 H, pyr H-5), 7.49 (d, 1 H, pyr H-4), 8.43 (m, 2 H, pyr H-2 and H-6); UV (MeOH) 269 (ε 3190), 263 (ε 4190), 257 (ε 3800), 199 (ε 26850); MS (DCI) 308 $(MH)^+$, 250 $(M - C_4H_9)^+$; HRMS, obsd m/z = 330.2222, $C_{18}H_{33}NOSiNa$ requires m/z = 330.2229. Anal. Calcd for $C_{18}H_{33}NOSi$: C, 70.30; H, 10.81; N, 4.55. Found: C, 69.98; H, 10.76; N, 4.42.

8-(3-Pyridyl)octyl *tert*-Butyldimethylsilyl Ether (6, n = 2). This was prepared as described above for 6 (n = 1) in 96% yield: ^1H NMR ($CDCl_3$) δ 0.04 (s, 6 H, $SiCH_3$), 0.89 (9H, s, *t*-Bu), 1.30 (br s, 8 H, CH_2), 1.50 (m, 2 H, pyr-CH_2CH_2), 1.60 (m, 2 H, OCH_2CH_2), 2.59 (t, 2 H, pyr-CH_2), 3.59 (t, 2 H, OCH_2), 7.19 (m, 2 H, pyr H-5), 7.47 (d, 1 H, pyr H-4), 8.42 (d, 1 H, pyr H-6), 8.44 (s, 1 H, pyr H-2); UV (MeOH) 270 (ε 3150), 264 (ε 4080), 258 (ε 3700), 199 (ε 25990); MS (DCI) 324 $(MH)^+$, 266 $(M - C_4H_9)^+$; HRMS, obsd m/z = 322.2748, $C_{20}H_{36}NOSi$ requires m/z = 322.2755. Anal. Calcd for $C_{19}H_{35}NOSi$: C, 79.96; H, 10.97; N, 4.35. Found: C, 79.90; H, 11.01; N, 4.32.

9-(3-Pyridyl)nonyl *tert*-Butyldimethylsilyl Ether (6, n = 3). This was prepared as described above for 6 (n = 1) in 62% yield: ^1H NMR ($CDCl_3$) δ 0.02 (2, 6 H, $SiCH_3$), 0.86 (9H, s, *t*-Bu), 1.28 (br s, 10 H, CH_2), 1.46 (m, 2 H, pyr-CH_2CH_2), 1.54 (m, 2 H,

(13) There is a large anion effect in the mass spectrum with these pyridinium salts. With the triflate counterion, the present mass ions are very abundant and no elimination products are observed. With the nitrate counterion, elimination products (e.g. 20) are prominent.

OCH_2CH_2), 2.53 (t, 2 H, pyr-CH_2), 3.54 (t, 2 H, OCH_2), 7.12 (m, 2 H, pyr H-5), 7.41 (d, 1 H, pyr H-4), 8.39 (d, 2 H, pyr H-2 and H-6); UV (MeOH) 270 (ε 3080), 263 (ε 4040), 258 (ε 3670), 200 (ε 25930); MS (DCI) 336 $(MH)^+$, 278 $(M - C_4H_9)^+$; HRMS, obsd m/z = 336.2712, $C_{20}H_{38}NOSi$ requires m/z 336.2723. Anal. Calcd for $C_{20}H_{37}NOSi$: C, 71.58; H, 11.11; N, 4.17. Found: C, 71.44; H, 10.92; N, 4.06.

7-(3-Pyridyl)heptanol (7, n = 1). The pyridyl TBDMS ether **6** (n = 1) (1.007 g, 3.275 mmol) in THF (10 mL) at rt was treated with tetrabutylammonium fluoride (1 M in THF, 6.6 mL, 2 equiv). After 2 h TLC indicated completion, and the solution was poured into 10% isopropyl alcohol/ethyl acetate (30 mL). The mixture was washed with water and the aqueous layer was extracted with 10% isopropyl alcohol/ethyl acetate (2×). The combined organic layers were washed with water and brine, dried, and evaporated. The residue was chromatographed on silica, eluting with (1) ethyl acetate and (2) 20:1 ethyl acetate/methanol, to give the product as a thick, colorless oil (633.1 mg, 100%): ^1H NMR ($CDCl_3$) δ 1.32 (br s, 6 H, CH_2), 1.58 (m, 4 H, CH_2), 2.59 (t, 2 H, pyr-CH_2), 2.77 (br s, 1 H, OH), 3.62 (t, 2 H, OCH_2), 7.19 (dd, 1 H, pyr H-5), 7.48 (d, 1 H, pyr H-4), 8.41 (m, 2 H, pyr H-2 and H-6); UV (MeOH) 270 (ε 3220), 263 (ε 4230), 256 (ε 3840), 200 (ε 27100); MS (DCI) 194 $(MH)^+$; HPLC (C-18, 75:25 methanol/buffer, 1 mL/min, 270 nm) single peak at 2.4 min; HRMS, obsd m/z = 194.1539, $C_{12}H_{20}NO$ requires 194.1545.

8-(3-Pyridyl)octanol (7, n = 2). This was prepared as described above for **7** (n = 1) in 92% yield: ^1H NMR ($CDCl_3$) δ 1.35 (br s, 8 H, CH_2), 1.60 (m, 4 H, CH_2), 2.59 (t, 2 H, pyr-CH_2), 3.64 (t, 2 H, OCH_2), 7.18 (dd, 1 H, pyr H-5), 7.46 (d, 1 H, pyr H-4), 8.43 (m, 2 H, pyr H-2 and H-6); UV (MeOH) 270 (ε 3210), 264 (ε 4210), 257 (ε 3780), 200 (ε 25990); MS (DCI) 208 $(MH)^+$; HPLC (C-18, 75:25 methanol/buffer, 1 mL/min, 270 nm) single peak at 3.1 min; HRMS, obsd m/z = 207.1631, $C_{13}H_{21}NO$ requires 207.1623.

9-(3-Pyridyl)nonyl Alcohol (7, n = 3). This was prepared as described above for **7** (n = 1) in quantitative yield: ^1H NMR ($CDCl_3$) δ 1.24 (br s, 10 H, CH_2), 1.53 (m 4 H, CH_2), 2.52 (t, 2 H, pyr-CH_2), 3.41 (br, 1 H, OH), 3.58 (t, 2 H, OCH_2), 7.13 (dd, 1 H, pyr H-5), 7.42 (d, 1 H, d, pyr H-4), 8.32 (m, 2 H, pyr H-2 and H-6); UV (MeOH) 270 (ε 3050), 263 (ε 4000), 257 (ε 3620), 203 (ε 25580); MS (DCI) 222 $(MH)^+$; HPLC (C-18, 75:25 methanol/buffer, 1 mL/min, 270 nm) single peak at 4.7 min; HRMS, obsd m/z = 222.1866, $C_{14}H_{24}NO$ requires m/z = 222.1858.

7-(3-Pyridyl)heptyl *p*-Toluenesulfonate (10). 7-(3-Pyridyl)heptanol (**7**) (n = 1) (148.8 mg, 770 mmol) in CH_2Cl_2 (2 mL) at 0 °C was treated with *p*-toluenesulfonyl chloride (161.4 mg, 1.1 equiv) and the stirred mixture was allowed to warm to rt. After 14 h TLC (silica, ethyl acetate) indicated ca. 80% conversion. Diisopropylethylamine (134 mL, 1 equiv) was added and stirring was continued for 4 h, at which time no starting material was present. The mixture was washed with water (3×) and brine, dried, and evaporated. The residue was chromatographed on silica, eluting with ethyl acetate, to give the product as a waxy solid (208.7 mg, 78%): ^1H NMR ($CDCl_3$) δ 1.29 (br s, 6 H, CH_2), 1.61 (m, 4 H, CH_2), 2.42 (s, 3 H, $ArCH_3$), 2.57 (t, 2 H, pyr-CH_2), 4.00 (t, 2 H, OCH_2), 7.19 (dd, 1 H, pyr H-5), 7.32 (d, 2 H, Ar-H), 7.78 (d, 2 H, Ar-H), 7.47 (d, 1 H, pyr H-4), 8.42 (m, 2 H, pyr H-2 and H-6); MS (FAB) 348 $(MH)^+$; HPLC (C-18, 6:4 acetonitrile/buffer, 1 mL/min, 270 nm) 6.1 min.

1-[7-(3′-Pyridyl)heptyl]-3-[7-[(*p*-tolylsulfonyl)oxy]heptyl]-pyridinium *p*-Toluenesulfonate (11). A solution of the pyridyl tosylate **10** (78.1 mg, 0.225 mmol) in CH_2Cl_2 (3 mL) was allowed to stand at rt for 24 h. The solvent was evaporated. NMR and HPLC of the residue showed ca. 33% conversion of starting material to the title product. ^1H NMR ($CDCl_3$) δ 1.05–2.02 (m, CH_2), 2.29 (s, 3 H), 2.42 (s, 3 H, $ArCH_3$), 2.52 (t, 2 H, pyr-CH_2), 2.77 (t, 2 H, pyr$^+$-CH_2), 3.96 (m, 2 H, OCH_2), 4.73 (t, 2 H, N^+CH_2), 7.08 (d, 2 H, ArH), 7.80–8.22 (m, pyr$^+$ H-4 and H-5), 8.82–9.41 (m, pyr$^+$ H-4 and H-6); HPLC (C-18, 6:4 acetonitrile/buffer, 1 mL/min, 270 nm) 20.0 min; MS (FAB) 523 $(M)^+$.

C-7 Cyclic Pyridinium Oligomers 12–14 (n = 1). A stirred solution of pyridine alcohol **7** (n = 1) (319.1 mg, 1.650 mmol) in dry CH_2Cl_2 (5 mL) under argon at −42 °C was treated with trifluoromethanesulfonic anhydride (0.305 mL, 1.1 equiv) followed by diisopropylethylamine (0.316 mL, 1.1 equiv). The cooling bath was allowed to warm to rt (ca. 2 h) and the mixture was stirred at rt for 14 h. The solvents were then evaporated and the maroon-colored residue chromatographed on silica eluting with a solvent gradient from 7:3 to 9:1 20% saturated KNO_3/acetonitrile. The fractions were evaporated under reduced pressure (bath temp 35 °C), and the resulting solids dried in vacuo (ca. 0.01 mmHg) for several hours and then triturated and sonicated with 10:1 CH_2Cl_2/methanol. The solid was removed by filtration and the filtrate evaporated. Trituration was repeated to ensure removal of all inorganics. The separated cyclic oligomers were isolated as colorless glasses and were shown by mass spectroscopy to be the corresponding nitrate salts. Dimer **12** (203.2 mg, 52%): ^1H NMR (CD_3OD) δ 1.03 (m, 4 H, CH_2), 1.27 (m, 8 H, CH_2), 1.63 (m, 4 H, pyr-CH_2CH_2), 1.89 (m, 4 H, N^+-CH_2CH_2), 2.80 (t, 4 H, pyr-CH_2), 4.54 (t, 4 H, N^+CH_2), 7.90 (dd, 2 H, pyr H-5), 8.32 (d, 2 H, pyr H-4), 8.71 (d, 2 H, pyr H-6), 8.79 (s, 2 H, pyr H-2); UV (MeOH) 274 (ε 6460), 267 (ε 7430), 199 (ε 49800); MS (ion-spray) 176.0 $(M^{+2}/2)$, 351.2 (**16** single elimination$^+$), 414.4 $(M^{+2} + NO_3^-)^+$; HPLC (C-18, 6:4 acetonitrile/buffer, 1 mL/min, 270 nm) 3.30 min. Trimer **13** (95.9 mg, 24%): ^1H NMR (CD_3OD) δ 1.34 (m, 18 H, CH_2), 1.62 (m, 6 H, pyr-CH_2CH_2), 1.91 (m, 6 H, $N^+CH_2CH_2$), 2.80 (t, 6 H, pyr-CH_2), 4.53 (t, 6 H, N^+CH_2), 7.90 (dd, 3 H, pyr H-5), 8.34 (d, 3 H, pyr H-4), 8.72 (d, 3 H, pyr H-6), 8.97 (s, 3 H, pyr H-2); UV (MeOH) 273 (ε 8970), 266 (ε 10450), 199 (ε 72160); MS (ion-spray) 263.6 (single elimination^{+2}/2), 296.0 $(M^{+3} + NO_3^-)^{+2}/2$, 351.2 (**16**)$^+$, 652.4 $(M^{+3} + 2NO_3^-)^+$; HPLC (C-18, 6:4 acetonitrile/buffer, 1 mL/min, 270 nm) 8.9 min (broad, tailing peak). Tetramer **14** (45.5 mg, 12%): ^1H NMR (CD_3OD) δ 1.30 (br s, 24 H, CH_2), 1.62 (m, 8 H, pyr-CH_2CH_2), 1.92 (m, 8 H, $N^+CH_2CH_2$), 2.79 (t, 8 H, pyr-CH_2), 4.53 (t, 8 H, N^+CH_2), 7.91 (dd, 4 H, pyr H-5), 8.83 (d, 4 H, pyr H-4), 8.74 (d, 4 H, pyr H-6), 8.91 (s, 4 H, pyr H-2); UV (MeOH) 273 (ε 11960), 267 (ε 13750), 200 (ε 98200); MS (ion-spray) 234.4 (single elimination^{+3}/3), 255.6 $(M^{+4} + NO_3^-)^{+3}/3$, 351.2 (**16**)$^+$, 414.4 $(M^{+4} + 2NO_3^-)^{+2}/2$; HPLC (C-18, 6:4 acetonitrile/buffer, 1 mL/min, 270 nm): 16.0 min (very broad).

C-8 Cyclic Pyridinium Oligomers 12–14 (n = 2). These were prepared as described above for the (n = 1) series: ^1H NMR (CD_3OD) δ (per pyridyl C-8 unit) 1.38 (br s, 8 H, CH_2), 1.75 (m, 2 H, pyr-CH_2CH_2), 2.01 (m, 2 H, $N^+CH_2CH_2$), 2.83 (t, 2 H, pyr-CH_2), 4.59 (t, 2 H, N^+CH_2), 8.01 (dd, 1 H, pyr H-5), 8.43 (d, 1 H, pyr H-4), 8.80 (d, 1 H, pyr H-6), 8.92 (s, 1 H, pyr H-2); UV (MeOH) 273 (ε/unit 3180), 267 (ε 3730), 200 (ε 24800). Dimer **12**: MS (ion-spray) 379.2 (single elimination$^+$), 529.2 $(M^{+2} + Tf^-)^+$; HPLC (C-18, 6:4 acetonitrile/buffer, 1 mL/min, 270 nm): 9.6 min (broad, tailing peak). Trimer **13**: MS (ion-spray) 284.8 (single elimination^{+2}/2), 359.6 $(M^{+3} + Tf^-)^{+2}/2$, 868.4 $(M^{+3} + 2Tf^-)^+$; HPLC (C-18, 6:4 acetonitrile/buffer, 1 mL/min, 270 nm) 24.8 min (very broad). Tetramer **14**: MS (ion-spray) 253.2 (single elimination^{+3}/3).

C-9 Cyclic Pyridinium Oligomers 12–14 (n = 3). These were prepared as described above for the (n = 1) series but were only subjected to crude separation on neutral alumina, eluting with CH_2Cl_2/methanol (a gradient from 35:1 to 2:1). With 2:1 CH_2Cl_2/methanol higher order oligomers were eluted which did not move above the origin on TLC (3:7 acetonitrile/20% saturated KNO_3): ^1H NMR (CD_3OD) δ (per pyridyl-C-9 unit) 1.27 (br s, 10 H, CH_2), 1.63 (m, 2 H, pyr-CH_2CH_2), 1.92 (m, 2 H, $N^+CH_2CH_2$), 2.80 (t, 2 H, pyr-CH_2), 4.51 (t, 2 H, N^+CH_2), 7.91 (dd, 1 H, pyr H-5), 8.38 (d, 1 H, pyr H-4), 8.72 (d, 1 H, pyr H-6), 8.82 (s, 1 H, pyr H-2); UV (MeOH) 274 (ε/unit 3310), 268 (ε 3870), 200 (ε 26310); MS (ion-spray): dimer **12** 204.1 $M^{+2}/2$, 557.2 $(M^{+2} + Tf^-)^+$; trimer **13** 380.8 $(M^{+3} + Tf^-)^{+2}/2$, 911.6 $(M^{+3} + 2Tf^-)^+$; tetramer **14** 272.0 (single elimination^{+3}/3).

1,6-Bis(1-pyridyl)hexane Bis(trifluoromethanesulfonate) (15). A stirred mixture of 1,6-hexanediol (250.0 mg, 2.115 mmol) and pyridine (0.36 mL, 2.1 equiv) in dry CH_2Cl_2 (3 mL) under argon at −41 °C were treated with diisopropylethylamine (0.737 mL, 2 equiv) and then triflic anhydride (0.712 mL, 2 equiv), dropwise over 1 min. The cooling bath was removed after 30 min and the mixture was stirred at rt for 5 h. The solvent was evaporated and the residue dried in vacuo. The resulting gummy solid was chromatographed on neutral alumina, eluting with (1) CH_2Cl_2 and (2) 25:1 CH_2Cl_2/methanol, to give the product as an off-white solid (826.2 mg, 82%): mp 112–113 °C; ^1H NMR (CD_3OD) δ 1.42 (m, 4 H, CH_2), 1.96 (m, 4 H, CH_2), 4.57 (t, 4 H, N^+CH_2), 8.03 (t, 4 H), 8.51 (t, 2 H), 8.91 (d, 4 H); UV (MeOH) 266

(ε 7600), 260 (ε 9800), 198 (ε 51400); MS (ion-spray), 391.2 (M^{+2} + $CF_3SO_3^-$)$^+$; HPLC (C-18, 6:4 acetonitrile/buffer, 1 mL/min, 270 nm) single peak at 1.5 min. Anal. Calcd for $C_{18}H_{22}N_2O_6S_2F_6$: C, 40.00; H, 4.10; N, 5.18. Found: C, 40.24; H, 4.08; N, 5.16.

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Supplementary Material Available: Copies of the ^1H NMR spectra of **1**, **5** (n = 1, 2, and 3), **6** (n = 1, 2, and 3), **7** (n = 1, 2, and 3), **10**, **11**, **12** (n = 1), **13** (n = 1), **14** (n = 1), [**12**–**14** (n = 2)], [**12**–**14** (n = 3)], and **15** and the ^{13}C NMR spectrum of **1** (20 pages). This material is contained in libraries at microfiche, immediately following this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.