Novel Quaternary Ammonium Salt-Containing Polyamines from the *Agelenopsis aperta* Funnel-Web Spider

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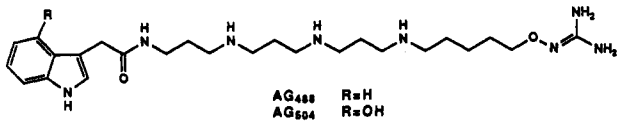
Two novel hydroxylamine-containing acylpolyamines, Agel 489a and Agel 505a, each bearing a terminal quaternary ammonium salt, were isolated from the venom of *Agelenopsis aperta*, a funnel-web spider found throughout the western United States. Published structures for these compounds were not consistent with our spectral data. On the basis of fast-atom bombardment (FAB) mass spectrometry and ^1H and ^{13}C NMR spectra, novel structures for Agel 489a and 505a are proposed. These structural assignments are confirmed by the total synthesis of Agel 489a.

Introduction

Earlier, we described the isolation, characterization and synthesis of five novel hydroxylamine-containing acylpolyamines (Agel 448, 452, 468, 489, and 505) from the venom of *Agelenopsis aperta*, a North American funnel-web spider (family *Agelenidae*).[1] These polyamine constituents (agelenotoxins) are of particular interest because of their ability to specifically and potently antagonize *N*-methyl-D-aspartate (NMDA) receptor function in mammalian tissue.[2] They also cause a reversible paralysis and block postsynaptic glutamate-sensitive receptor-operated ion channels in insect muscle.[3] Recently, the structures of two additional Agelenopsis polyamines, AG_{488} and AG_{504}, were proposed.[4] The structural assignments,



however, were not compatible with our own spectral data. Herein, we report alternate structures **1** and **2** for these acylpolyamines (Agel 489a and Agel 505a) which we have confirmed by total synthesis.

Results and Discussion

Fractionation. Crude venom-containing acylpolyamine and peptide components were fractionated by reverse-phase HPLC, yielding first the smaller and more hydrophilic polyamines followed by the larger highly disulfide-bridged peptides (Figure 1). The two major acylpolyamine HPLC fractions from the initial separation were subsequently resolved by an additional HPLC fractionation to afford in one instance Agel 489 and Agel 489a and from the other fraction Agel 505 and Agel 505a. UV spectra were obtained on the individual components resolved by HPLC. These purified isolates were analyzed by low- and high-resolution FAB mass spectrometry and by ^1H NMR. In the case of Agel 489a, enough compound was obtained to provide a ^{13}C NMR spectrum.

Structural Elucidation. UV spectrophotometry and FAB mass spectrometry (vide infra) suggested that the two compounds in question differ only in the aromatic chromophore with one having an indoleacetamide (λ_{max} = 218, 279, and 287 nm) and the other having a 4-hydroxyindoleacetamide (λ_{max} = 219, 267, 282, and 292 nm) moiety. We initially had no reason to doubt their molecular weights (488 and 504 amu) which were assigned on the basis of FAB-MS $[M + H]^+$ = 489 (Figure 2) and 505 (Figure 3). However, the appearance of a large methyl group singlet at ca. 53.6 ppm in the ^{13}C NMR of the lower molecular weight compound (Figure 4) and at 3.1 ppm in the ^1H NMR (Figure 5A) of both unknowns suggested the presence of a di- or possibly a trimethylammonium salt in each of these molecules. This assumption necessitated a change in our molecular weight assignments from 488 and 504 to 489 and 505 (since the parent ion of a quaternary ammonium salt in the FAB-MS would be an M^+ ion). These acylpolyamine constituents were labeled Agel 489a and 505a to distinguish them from Agel 489 and 505, the two major polyamine constituents in this venom, each of which coincidentally comigrated in our initial HPLC fractionation (Figure 1) with corresponding unknowns that appear to have the same molecular weight.

High resolution FAB-MS yielded the compositions $C_{27}H_{49}N_6O_2$ and $C_{27}H_{49}N_6O_3$ for Agel 489a and Agel 505a, respectively. The FAB mass spectrum of natural Agel 489a contains many fragment ions as indicated in Figure 2. The composition of most of the fragment ions were confirmed by high-resolution mass measurement. Interestingly, all the fragment ions that contain the quaternary ammonium ion result from a single-bond cleavage with loss of hydrogen. In contrast to the diagnostic FAB-MS fragmentations of nonquaternary acylpolyamines where bond cleavage occurs at secondary amine sites (via two proton rearrangements[1]), many fragmentations of Agel 489a produce fragment ions containing the terminal trimethylammonium group. The diagnostic fragmentation (m/z = 430) involving loss of trimethylamine from the chain terminus is consistent with the FAB mass spectra of other tetraalkylammonium salts.[5] Two signals between 57 and 58 ppm in the ^{13}C NMR of Agel 489a (Figure 4) reaffirmed our suspicion that Agel 489a, like the previously identified agelenotoxins, contains an internal hydroxylamine functionality. A 3,3,3,5 (with the numerals denoting the number of methylene groups between nitrogen atoms) poly-

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(4) Quistad, G. B.; Suwanrumpha, S.; Jarema, M. A.; Shapiro, M. J.; Skinner, W. S.; Jamieson, G. C.; Lui, A.; Fu, E. W. *Biochem. Biophys. Res. Commun.* **1990**, *169*, 51.

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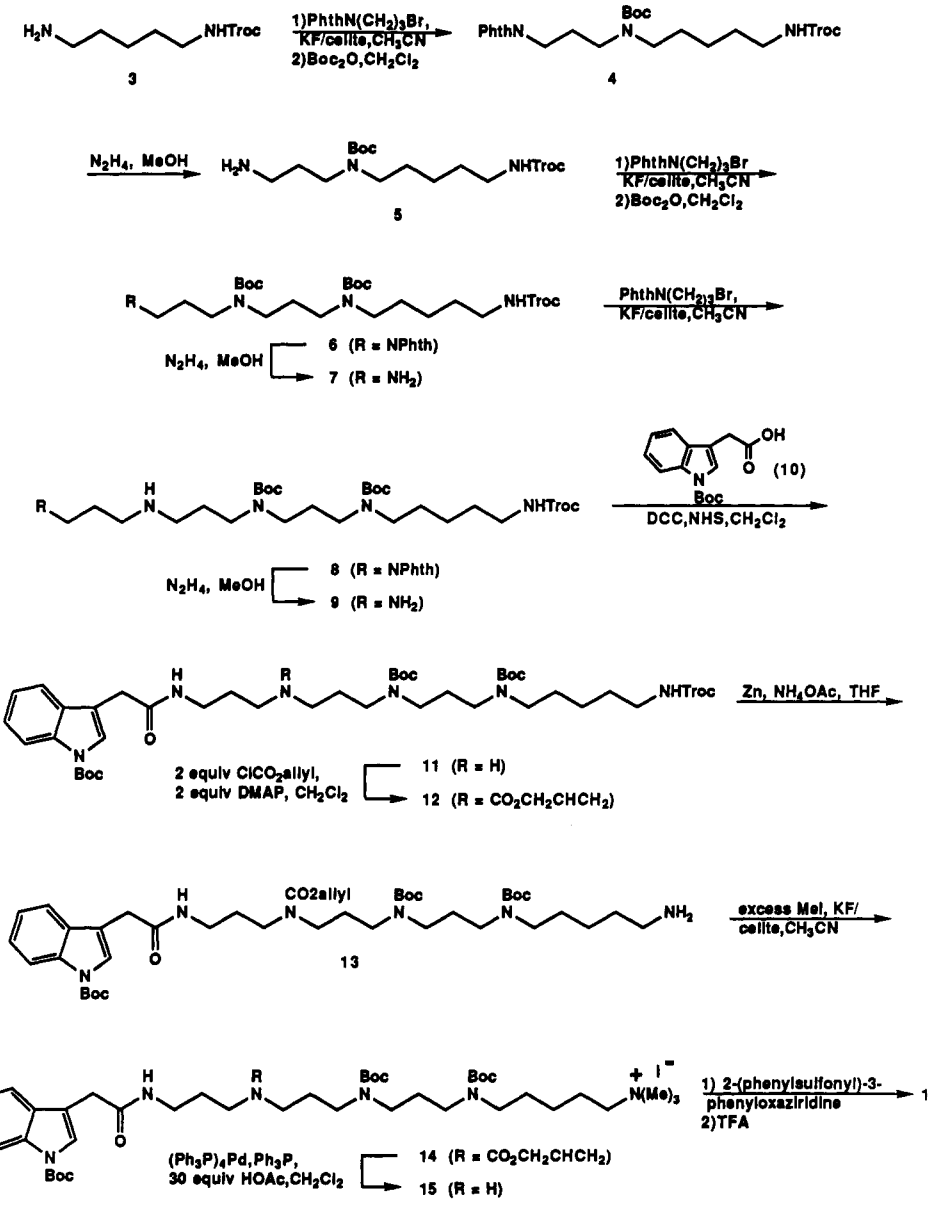
0022-3263/92/1957-1814$03.00/0

Scheme I



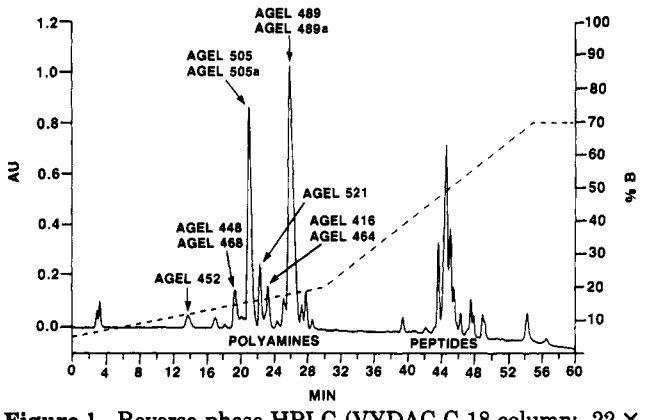
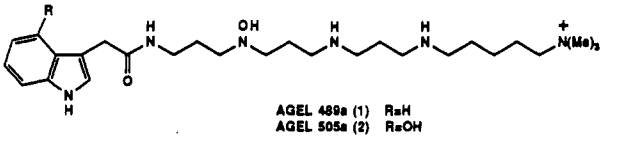


Figure 1. Reverse-phase HPLC (VYDAC C-18 column: 22 × 250 mm, 10 μm, 300 Å) separation using a 0–20% linear gradient of CH_3CN/H_2O containing 0.1% CF_3COOH for 30 min followed by a 20–70% gradient for 25 min at a flow rate of 15 mL/min.

amine chain was suggested by ^1H–^1H COSY NMR (Figure 5A) of Agel 489a, and structures 489a (1) and 505a (2) bearing a terminal quaternary ammonium salt were proposed.



Total Synthesis of Agel 489a. To confirm our assignment, a synthesis of Agel 489a (1) was initiated (Scheme I). Our strategy paralleled our early polyamine synthetic efforts[1,6] with the contiguous propylamine components being assembled by repetitive alkylation/amine protection processes. Concern over the stability of the terminal quaternary ammonium salt and the internal hydroxylamine moiety forced us to incorporate these functionalities late in our synthetic scheme. In addition, we would now need four different amine protecting groups and the Boc, Troc, phthalimido, and allyloxycarbonyl moieties

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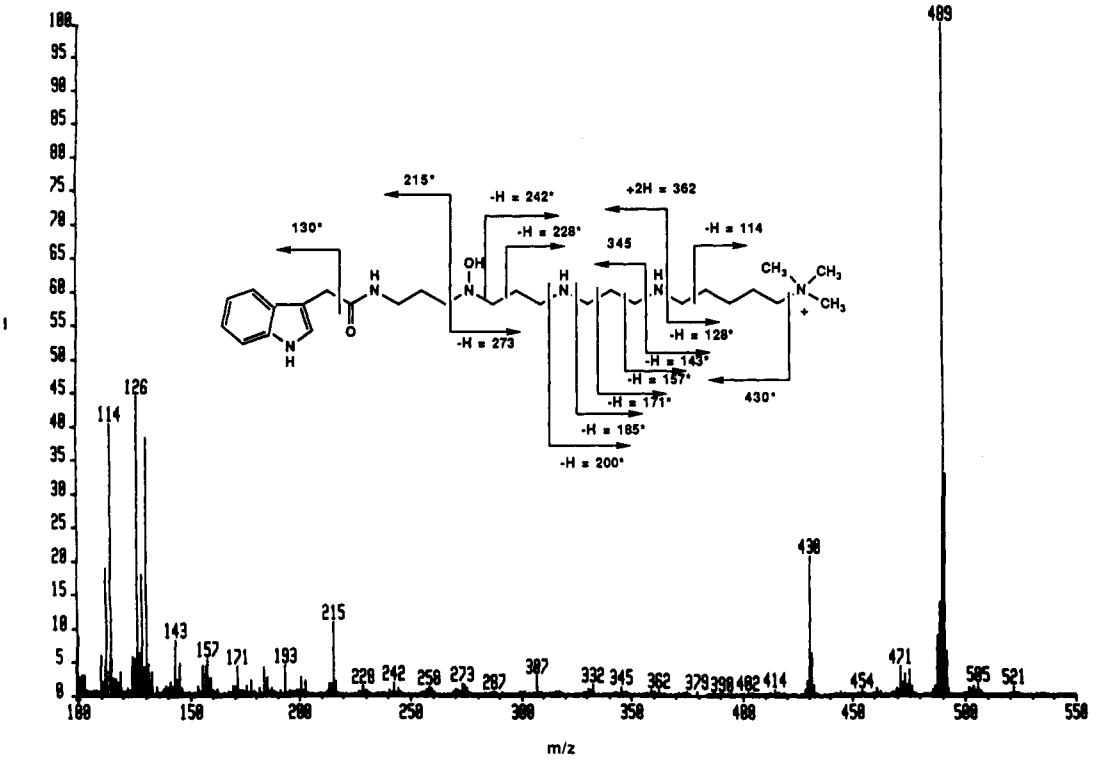

Figure 2. Low-resolution FAB mass spectrum of natural Agel 489a. Starred ions refer to those which have been confirmed by high resolution.

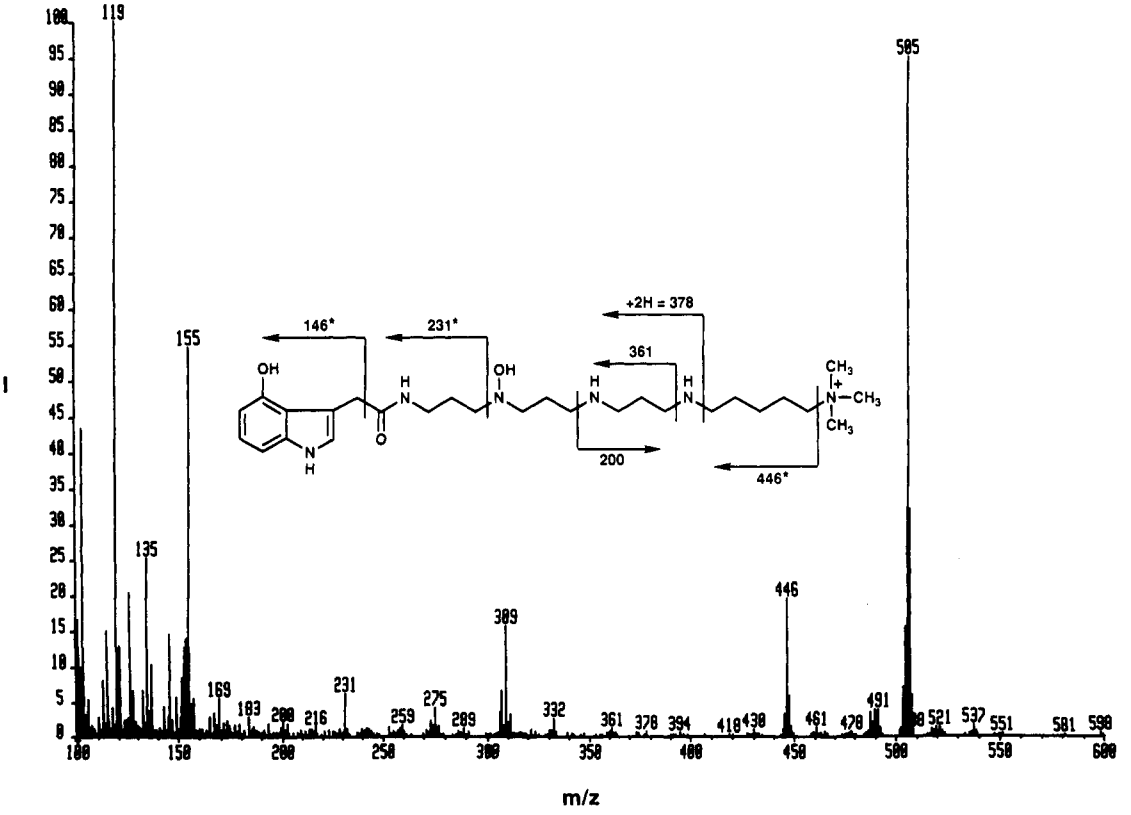

Figure 3. Low-resolution FAB mass spectrum of natural Agel 505a. Starred ions refer to those which have been confirmed by high resolution.

were selected to implement our strategy.

A KF/Celite[7]-mediated alkylation of *N*-Troc-1,5-pentanediamine (3) with *N*-(3-bromopropyl)phthalimide followed by Boc protection provided the 3,5 terminus 4 in 25% yield. Phthalimide removal (hydrazine/methanol) generated amine 5 which was subjected to another alkylation/protection sequence [(1) PhthN$(CH_2)_3$Br, KF/Celite, CH_3CN; (2) Boc_2O, CH_2Cl_2] to provide 6 in 45% yield. Phthalimide removal yielded amine 7 which was alkylated again with *N*-(3-bromopropyl)phthalimide to give

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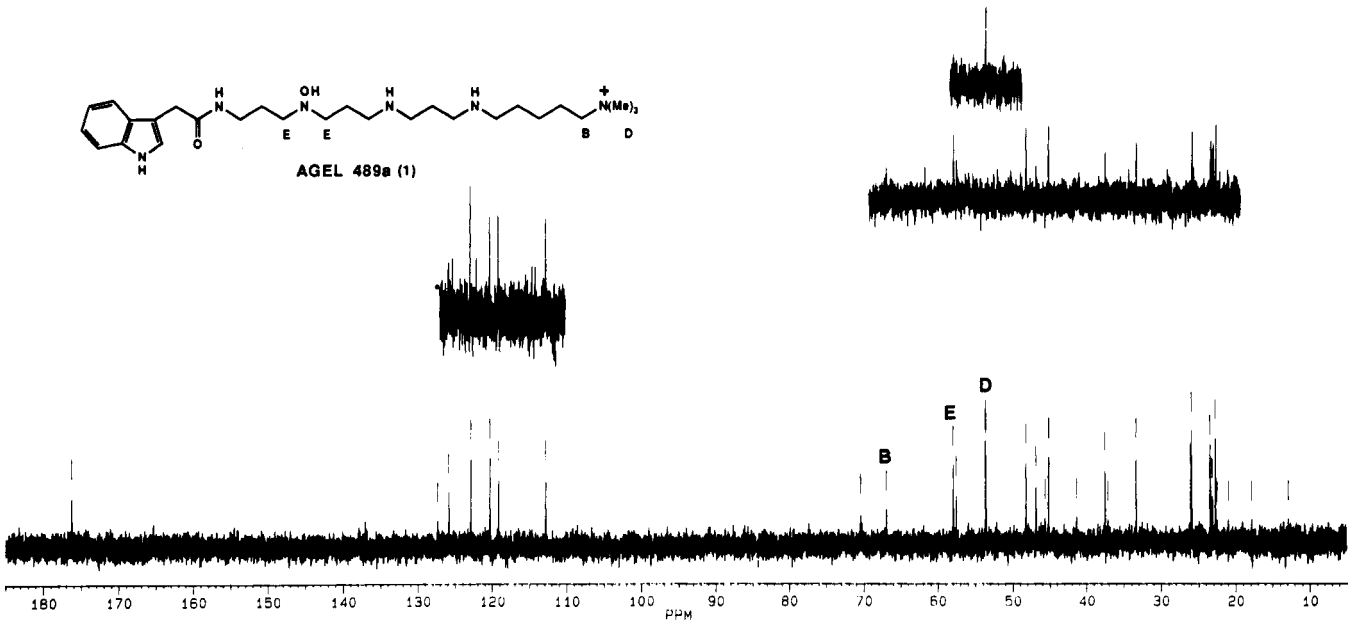

Figure 4. 125.76-MHz ^{13}C NMR (DEPT) of natural Agel 489a.

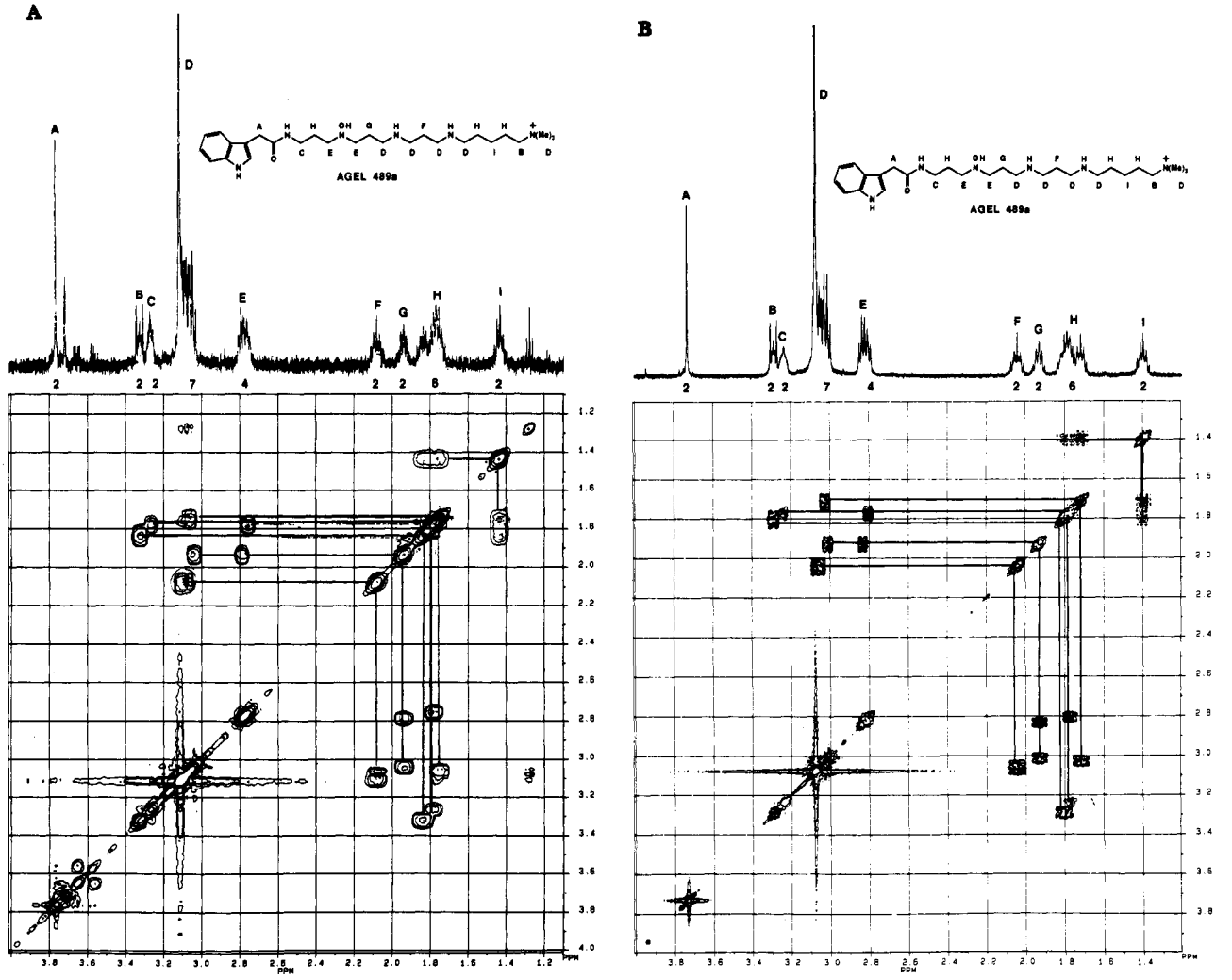

Figure 5. 500-MHz ^1H–^1H COSY NMRs of natural (A) and synthetic (B) Agel 489a.

phthalimide **8** having the requisite 3,3,3,5 Agel 489a polyamine chain. The remaining synthetic manipulations involving **8** to generate Agel 489a would require specific transformations at three of the five nitrogen atoms of this chain. Accordingly, **8** was subjected to standard phthalimide deprotection conditions to afford amine **9** in good yield (44% yield from **6**). Standard DCC coupling (DCC/NHS/CH_2Cl_2) with *N*-Boc-indoleacetic acid (**10**)[1] provided indoleacetamide **11** in 75% yield.

Finishing the synthesis would require generation of the terminal quaternary ammonium salt followed by selective amine oxidation. To this end, the internal basic nitrogen

atom of 11 was protected using standard conditions (allyl chloroformate, DMAP, CH_2Cl_2) to afford carbamate 12. Removal of the terminal Troc protecting group with Zn/NH_4OAc[8] generated terminal amine 13. Exhaustive methylation of the amine terminus with methyl iodide cleanly afforded ammonium salt 14 (47% from 11). Removal of the internal (allyloxycarbonyl)amine protecting group [$(Ph_3P)_4Pd$ with 30 equiv of HOAc to prevent N-alkylation] generated amine 15, which would require an oxidation to provide the hydroxylamine moiety present in Agel 489a. To carry out this reaction we decided to employ conditions which we had successfully utilized in previous Agelenopsis arylamine syntheses.[1] Accordingly, 15 was allowed to react with excess 2-(phenylsulfonyl)-3-phenyloxaziridine[9] to yield a mixture of hydroxylamine and nitrone-containing products. Subjection of the crude reaction mixture to $NaCNBH_3$ treatment to reduce overoxidized nitrone products provided the fully protected Agel 489a framework. Poor stability to silica gel precluded chromatographic purification of the crude hydroxylamine product at this stage. As a result, we opted to treat this crude product with TFA to afford crude Agel 489a (1) and to obtain an analytical sample of 1 by preparative reverse-phase HPLC.

The isolated yield of 1 following preparative reverse-phase HPLC was low (<20%). This synthetic material was found to be identical with the natural product in all aspects (^1H and ^{13}C NMR, FAB-MS, HPLC, and UV spectrophotometry). Particularly informative was the ^1H–^1H COSY NMR spectrum (Figure 5B) of Agel 489 (1) which was identical to the natural product (Figure 5A), thereby confirming the polyamine chain structure.

The identity of Agel 505a was easily discerned on the basis of its structural similarity to Agel 489a as the key difference in the ^1H NMRs of Agel 489a (1) and Agel 505a (2) was associated with replacement of the indole-3-acetamide moiety of 1 with a 4-hydroxyindole-3-acetamide functionality of 2. FAB mass spectroscopy was particularly informative in verifying our structural assignment of Agel 505a as mass spectral fragmentations of Agel 505a (Figure 3) were consistent with and paralleled those of Agel 489a (Figure 2). UV spectroscopy also lent support to the proposed Agel 505a structure.

In conclusion, structures of two novel quaternary ammonium salt-containing acylpolyamines, which we have designated Agel 489a and Agel 505a, have been determined. With this discovery, we have now confirmed via total synthesis the structures assigned to the major polyamine constituents from the venom of the *Agelenopsis aperta* spider. In due course, we will provide details on the structural and biological characterization of the larger highly disulfide-bridged peptide constituents of this venom which are also of great interest and are being extensively profiled in insect and animal models.

Experimental Section

General Methods. Venom Extraction. *A. aperta* (family *Agelenidae*) is a common funnel-web spider found throughout the western United States. Male and female spiders were collected in Utah, Arizona, and New Mexico or bred in captivity. The spiders were housed in individual containers at 27 °C and 60% relative humidity and given access to housefly larvae and water daily. After induction of anesthesia by exposure for 5 min to carbon dioxide, the spiders were placed in a restraining device under a microscope and an alternating current was passed across the spider's head.[10] A suction tube was placed in the mouth to prevent contamination of venom by digestive secretions. Small glass capillary tubes were used to suction the venom ejected from the fangs by the electrical stimulation. Since the average milking in this species yields only about 0.2 μL of venom, venom from many spiders was pooled and frozen at −80 °C until it was used. After milking, spiders were returned to their cages, where they appeared to recover fully and could be milked at regular intervals.

Venom Fractionation. Crude venom was passed through a reverse-phase HPLC column (VYDAC C-18, (22 × 250 mm, 10 μm, 300 Å)). A 0–20% linear gradient of CH_3CN/H_2O containing 0.1% CF_3COOH was used for 30 min to separate the polyamines, followed by a 20–70% gradient for 25 min at a flow rate of 15 mL/min to resolve the peptides. Agel 489a (1) and the major Agelenopsis polyamine, Agel 489, were resolved using a Dynamax-60A Phenyl column (4.6 × 250 mm, 8 μm, 60 Å) with use of isocratic (10% CH_3CN/H_2O containing 0.1% CF_3COOH) conditions at a flow rate of 1 mL/min. Agel 505 and structurally distinct 505a (2) were further purified on a VYDAC C-4 column (22 × 250 mm, 10 μm, 300 Å) with use of a 0–10% linear gradient of CH_3CN/H_2O containing 0.1% CF_3COOH at a flow rate of 15 mL/min over 20 min. Venom constituents were isolated following lyophilization of HPLC fractions and were stored at −80 °C under an argon atmosphere. In solution, the hydroxylamine-containing *A. aperta* polyamines are susceptible to a facile air oxidation. As a result, solutions containing these compounds are normally degassed with argon.

Concentration of Acylpolyamines in Whole Venom. By use of synthetic samples of the Agelenopsis polyamines as standards, the concentration of individual polyamine constituents in whole venom has been determined.[1] Isolated yields of individual constituents such as Agel 489a (1) or Agel 505a (2) vary and are considerably lower than our crude venom estimates. Crude venom (500 μL) for example, contains approximately 4.73 mg of Agel 489a and 2.18 mg of Agel 505a. Yet, the initial RP-HPLC fractionation (VYDAC C-18) of 500 μL of crude venom provided only 5.2 mg of a mixture of Agel 489 and Agel 489a. Subsequent RP-HPLC fractionation (Dynamax-60A-phenyl) yielded ca. 700 μg of Agel 489a. Isolated yields of Agel 505a were lower.

Laboratory Procedures. Reagents, starting materials, and solvents were purchased from common commercial suppliers and were used as received or distilled from the appropriate drying agent. Reactions requiring anhydrous conditions were performed under an atmosphere of nitrogen. Reaction products were purified, when necessary, by chromatography on silica gel (63–200 μm) with the solvent system indicated. ^1H spectra were recorded on a Varian VT-300 operating at 299.9 MHz, a Bruker WM-250 operating at 250 MHz, or a Bruker AM-500 operating at 500 MHz and are reported in δ units. ^{13}C NMR data was measured on a Varian VT-300 operating at 75.43 MHz, on a Bruker WM-250 equipped with an Aspect 3000 computer operating at 62.9 MHz, or on a Bruker AM-500 operating at 125.76 MHz. Spectra were recorded in $CDCl_3$ with $CHCl_3$ (7.26 ppm for ^1H) or $CDCl_3$ (77.0 ppm for ^{13}C) as an internal standard and in $(CD_3)_2SO$ and D_2O. All mass spectrometry experiments were performed on a VG 70/250 S mass spectrometer. The instrument was operated at an accelerating potential of 8 kV. Xenon was used as the FAB gas, and the atom gun was operated at 9 kV and 1 mA. The instrument was operated at a resolving power of 1000 for low-resolution measurements and at a resolving power of 10000 (10% valley definition) for high-resolution measurements. The instrument was scanned over the mass range 1000–100 at 5 s/dec for low-resolution data collection. High-resolution data was collected over a 110 amu mass range using an MCA. Poly(ethylene glycol) 200, 400, or 600 (average molecular weight) was used as a reference compound for high-resolution measurements. For all high-resolution measurements, three different poly(ethylene glycol) peaks were collected. The high and low mass peaks were used as references, and the center peak was treated as an unknown in order to confirm the accuracy of the measurements. Purified samples were dissolved in water. An aliquot of this solution was spotted onto a FAB target containing a mixture of dithiothreitol/dithioerythritol which was used as the matrix.

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(10) Bascur, L.; Yevenes, I.; Adrian, H. *Toxicon.* **1980**, *18*, 224.

***N*-Troc-1,5-pentanediamine (3).** To a solution of *N*-Boc-1,5-diaminopentane[11] (6.06 g, 0.03 mol) in CH_2Cl_2 (150 mL) under a nitrogen atmosphere cooled to 0 °C was added DMAP (3.66 g, 0.03 mol) followed by the dropwise addition of 2,2,2-trichloroethyl chloroformate (4.11 mL, 0.03 mol). The solution was allowed to stir for 45 min. H_2O (150 mL) was added, and the organic extract was washed with 2 N citric acid (50 mL), H_2O, and 1 N NaOH. The CH_2Cl_2 extract was dried over K_2CO_3, filtered, and concentrated in vacuo. Crude product was dissolved in dioxane (50 mL), a solution (150 mL) of saturated HCl/dioxane was added, and the reaction was allowed to stir until no starting material remained. The crude reaction mixture was concentrated in vacuo, and the product was taken up into CH_2Cl_2 (100 mL) and washed with 2 N NaOH (pH > 11). The organic layer was dried over K_2CO_3, filtered, and concentrated to afford 7.72 g of crude *N*-Troc-1,5-pentanediamine (3): ^1H NMR ($CDCl_3$) δ 1.31–1.55 (m, 6 H), 2.27 (bs, 2 H), 2.68 (m, 2 H), 3.19 (m, 2 H), 4.68 (s, 2 H), 5.62 (bs, 1 H); ^{13}C NMR ($CDCl_3$) δ 23.85, 29.73, 41.05, 41.70, 74.33, 95.70, 154.66; HR FABMS observed (M + H) m/z 277.0264, $C_8H_{16}N_2O_2Cl_3$ required m/z 277.0262.

Carbamic Acid, [3-(1,3-Dihydro-1,3-dioxo-2*H*-isoindol-2-yl)propyl][5-[[(2,2,2-trichloroethoxy)carbonyl]amino]pentyl]-, 1,1-Dimethylethyl Ester (4). Under a nitrogen atmosphere were combined *N*-Troc-1,5-pentanediamine (3) (7.72 g, 0.0278 mol), *N*-(3-bromopropyl)phthalimide (7.45 g, 0.0278 mol), and KF/Celite (15 g) in CH_3CN (150 mL). The reaction mixture was heated to 40 °C and allowed to stir for 18 h. The crude reaction was filtered, and the KF/Celite residue was washed with additional CH_3CN. The reaction mixture was concentrated in vacuo. The crude reaction mixture was dissolved in CH_2Cl_2 (200 mL), and di-*tert*-butyl dicarbonate (8.72 g, 0.04 mol) was added. The solution was allowed to stir for 3 h, concentrated in vacuo, and chromatographed on 900 g of silica gel using 3:1 hexane/ethyl acetate to afford 3.90 g (25%) of product 4 as a gum: ^1H NMR ($CDCl_3$) δ 1.26–1.56 (m, 15 H), 1.85–1.89 (m, 2 H), 3.16–3.19 (m, 6 H), 3.62–3.66 (m, 2 H), 4.66 (s, 2 H), 5.30–5.45 (m, 1 H), 7.68 (m, 2 H), 7.79 (m, 2 H); ^{13}C NMR ($CDCl_3$) δ 23.63, 27.88, 28.32, 29.11, 35.75, 41.07, 41.68, 44.97, 46.71, 74.31, 79.45, 95.68, 123.16, 131.96, 133.94, 154.56, 155.44, 168.24.

Carbamic Acid, [3-Aminopropyl][5-[[(2,2,2-trichloroethoxy)carbonyl]amino]pentyl]-, 1,1-Dimethylethyl Ester (5). To a methanol solution (50 mL) under a nitrogen atmosphere containing phthalimide 4 (3.90 g, 6.9 mmol) was added hydrazine (0.26 mL, 8.3 mmol). The solution was heated at 50 °C for 18 h and then concentrated in vacuo. The residue was dissolved in CH_2Cl_2 (75 mL), and this suspension was washed with concd NH_4OH (2 × 75 mL), dried over K_2CO_3, and concentrated in vacuo to afford 3.19 (100%) of crude amine (5): ^1H NMR ($CDCl_3$) δ 1.25–1.53 (m, 15 H), 1.58–1.64 (m, 2 H), 1.76 (bs, 2 H), 2.65 (m, 2 H), 3.09–3.23 (m, 6 H), 4.68 (s, 2 H), 5.40–5.62 (m, 1 H); HR FABMS observed (M + H) m/z 434.1410, $C_{16}H_{31}N_3O_4Cl_3$ required m/z 434.1407.

2,8,12-Triazapentadecanoic Acid, 15-(1,3-Dihydro-1,3-dioxo-2*H*-isoindol-2-yl)-8,12-bis[(1,1-dimethylethoxy)carbonyl]-, 2,2,2-Trichloroethyl Ester (6). The procedure described for the preparation of 4 was utilized. Treatment of amine 5 (3.0 g, 6.9 mmol) under a nitrogen atmosphere with *N*-(3-bromopropyl)phthalimide (1.85 g, 6.9 mmol) and KF/Celite (5 g) in CH_3CN (125 mL) at 65 °C for 6 h followed by filtration and concentration in vacuo afforded the crude product which was dissolved in CH_2Cl_2 (100 mL) and allowed to react with di-*tert*-butyl dicarbonate (2.18 g, 10 mmol) for 18 h. Concentration of the crude reaction mixture in vacuo followed by silica gel chromatography (65:35 hexane/ethyl acetate) yielded 2.25 g (45%) of phthalimide 6: ^1H NMR ($CDCl_3$) δ 1.29–1.57 (m, 24 H), 1.73 (m, 2 H), 1.90 (m, 2 H), 3.16–3.22 (m, 10 H), 3.68 (m, 2 H), 4.70 (s, 2 H), 5.20–5.40 (m, 1 H), 7.71 (m, 2 H), 7.83 (m, 2 H); HR FABMS observed (M + H) m/z 721.2546, $C_{32}H_{48}N_4O_8Cl_3$ required m/z 721.2538.

2,8,12-Triazapentadecanoic Acid, 15-Amino-8,12-bis[(1,1-dimethylethoxy)carbonyl]-, 2,2,2-Trichloroethyl Ester (7). Using the procedure for the preparation of 5, hydrazine (0.11 mL, 3.5 mmol) treatment of phthalimide 6 (2.15 g, 2.98 mmol) afforded 1.81 g (100%) of crude amine 7: ^1H NMR ($CDCl_3$) δ 1.27–1.32 (m, 2 H), 1.39–1.71 (m, 26 H), 1.93 (bs, 2 H), 2.67 (t, J = 6.7 Hz, 2 H), 3.13–3.42 (m, 10 H), 4.69 (s, 2 H), 5.28 (bs, 1 H); HR FABMS observed (M + H) m/z 591.2499, $C_{24}H_{46}N_4O_6Cl_3$ required m/z 591.2483.

2,8,12,16-Tetraazanonadecanoic Acid, 19-(1,3-Dihydro-1,3-dioxo-2*H*-isoindol-2-yl)-8,12-bis[(1,1-dimethylethoxy)carbonyl]-, 2,2,2-Trichloroethyl Ester (8). The alkylation procedure described for the preparation of 4 was employed. Treatment of amine 7 (1.76 g, 2.98 mmol) with *N*-(3-bromopropyl)phthalimide (0.80 g, 2.98 mmol) and KF/Celite (3 g) afforded after workup and silica gel chromatography (9:1 CH_2Cl_2/MeOH) 1.05 g (45%) of phthalimide 8: ^1H NMR ($CDCl_3$) δ 1.26–1.89 (m, 31 H), 2.51–2.72 (m, 4 H), 3.12–3.23 (m, 10 H), 3.73 (t, J = 6.8 Hz, 2 H), 4.67 (s, 2 H), 5.27 (m, 1 H), 7.69 (m, 2 H), 7.80 (m, 2 H); ^{13}C NMR ($CDCl_3$) δ 23.69, 27.73, 28.27, 28.38, 28.42, 29.11, 29.45, 35.69, 41.09, 44.23, 44.76, 46.46, 46.72, 74.32, 79.27, 95.67, 123.17, 131.99, 133.92, 154.57, 155.47, 168.36; HR FABMS observed (M + H) m/z 778.3120, $C_{35}H_{55}N_5O_8Cl_3$ required m/z 778.3116.

2,8,12,16-Tetraazanonadecanoic Acid, 19-Amino-8,12-bis[(1,1-dimethylethoxy)carbonyl]-, 2,2,2-Trichloroethyl Ester (9). The procedure employed for the preparation of 5 was utilized. Accordingly, hydrazine (0.050 mL, 1.6 mmol) treatment of phthalimide 8 (1.00 g, 1.28 mmol) afforded 0.81 g (98%) of crude amine 9: ^1H NMR ($CDCl_3$) δ 1.27–1.71 (m, 30 H), 2.24 (bs, 3 H), 2.58 (t, J = 6.9 Hz, 2 H), 2.67 (t, J = 6.9 Hz, 2 H), 2.78 (m, 2 H), 3.13–3.22 (m, 10 H), 4.70 (s, 2 H), 5.50 (bs, 1 H); ^{13}C NMR ($CDCl_3$) 23.83, 26.99, 28.11, 28.28, 28.42, 28.43, 29.11, 29.52, 40.46, 41.09, 41.70, 44.26, 44.74, 45.07, 46.53, 47.91, 74.36, 76.08, 79.29, 95.67, 154.61, 155.49; HR FABMS observed (M + H) m/z 648.3052, $C_{27}H_{53}N_5O_6Cl_3$ required m/z 648.3061.

2,8,12,16,20-Pentaazadocosanoic Acid, 8,12-Bis[(1,1-dimethylethoxy)carbonyl]-22-[1-[(1,1-dimethylethoxy)carbonyl]-1*H*-indol-3-yl]-21-oxo-, 2,2,2-Trichloroethyl Ester (11). To a CH_2Cl_2 solution (5 mL) under nitrogen atmosphere containing *N*-(*tert*-butoxycarbonyl)-3-indoleacetic acid (10) (322 mg, 1.17 mmol) was added *N*-hydroxysuccinimide (134 mg, 1.17 mmol) and dicyclohexylcarbodiimide (DCC) (240 mg, 1.17 mmol). The reaction was allowed to stir for 4 h. Dicyclohexylurea was filtered, and the filtrate was diluted to 125 mL with CH_2Cl_2 and was added dropwise to a CH_2Cl_2 solution (300 mL) of amine 9 (760 mg, 1.17 mmol). The mixture was stirred for 12 h at ambient temperature and was washed with saturated $NaHCO_3$ (2 × 25 mL), dried over K_2CO_3, concentrated in vacuo, and chromatographed on silica gel (150 g) with use of 9:1 CH_2Cl_2/methanol followed by 9:1:0.1 CH_2Cl_2/methanol/diisopropylamine to afford 800 mg (75%) of amine 11: ^1H NMR ($CDCl_3$) δ 1.30–1.75 (m, 39 H), 2.41 (bs, 1 H), 2.50–2.59 (m, 4 H), 3.13–3.31 (m, 12 H), 3.64 (s, 2 H), 4.71 (s, 2 H), 5.35 (m, 1 H), 6.65 (m, 1 H), 7.25 (m, 1 H), 7.33 (t, J = 7.5 Hz, 1 H), 7.50 (d, J = 7.5 Hz, 1 H), 7.54 (s, 1 H), 8.13 (m, 1 H); ^{13}C NMR ($CDCl_3$) 23.79, 27.74, 28.15, 28.42, 28.44, 28.82, 33.23, 38.38, 41.10, 44.10, 44.75, 46.59, 47.01, 47.50, 54.20, 74.37, 79.34, 83.82, 95.70, 114.04, 115.31, 119.01, 122.79, 124.79, 124.76, 129.90, 149.48, 154.59, 155.50, 170.22; HR FABMS observed (M + H) m/z 905.4108, $C_{42}H_{68}N_6O_9Cl_3$ required m/z 905.4113.

2,8,12,16,20-Pentaazadocosanoic Acid, 16-[(Allyloxy)carbonyl]-8,12-bis[(1,1-dimethylethoxy)carbonyl]-22-[1-[(1,1-dimethylethoxy)carbonyl]-1*H*-indol-3-yl]-21-oxo-, 2,2,2-Trichloroethyl Ester (12). To a CH_2Cl_2 solution (50 mL) containing amine 11 (775 mg, 0.855 mmol) cooled to 0 °C was added 4-(dimethylamino)pyridine (210 mg, 1.72 mmol) followed by dropwise addition of allyl chloroformate (0.180 mL, 1.70 mmol). The solution was allowed to stir for 45 min. H_2O (50 mL) was added, the pH of the aqueous solution was adjusted to 5 with citric acid, and the two layers were separated. The organic layer was washed with H_2O (1 × 10 mL), dried over K_2CO_3, and concentrated in vacuo. This crude product was chromatographed on silica gel using 9;1 ethyl acetate/hexane to afford 640 mg (71%) of product 12: ^1H NMR ($CDCl_3$) δ 1.26–1.78 (m, 39 H), 3.14–3.25 (m, 16 H), 3.64 (s, 2 H), 4.42–4.48 (m, 2 H), 4.71 (s, 2 H), 5.17–5.26 (m, 3 H), 5.85 (m, 1 H), 6.65 (m, 1 H), 7.24–7.32 (m, 2 H), 7.53–7.57 (m, 2 H), 8.15 (m, 1 H); ^{13}C NMR ($CDCl_3$) δ 23.72, 27.56, 28.16, 28.43, 28.44, 29.18, 29.49, 33.33, 35.88, 41.13, 43.98, 44.50, 44.86, 65.99, 74.38, 79.52, 115.24, 117.54, 118.96, 122.64, 124.57, 124.79, 132.82, 154.58, 155.33, 155.50, 170.28; HR FABMS observed (M

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+ H) m/z 989.4308, $C_{46}H_{72}N_6O_{11}Cl_3$ required m/z 989.4325.

3,7,11,15-Tetraazaeicosan-20-amine, 7-[(Allyloxy)carbonyl]-11,15-bis[(1,1-dimethylethoxy)carbonyl]-1-[1-[(1,1-dimethylethoxy)carbonyl]-1*H*-indol-3-yl]- (13). To a THF solution (4.5 mL) containing indole **12** (620 mg, 0.626 mmol) under a nitrogen atmosphere was added zinc (880 mg, 13.5 mmol) followed by 1 M ammonium acetate (0.88 mL). The suspension was allowed to stir for 12 h and was then filtered. The residue were washed well with THF. The combined filtrates were concentrated in vacuo and dissolved in CH_2Cl_2 (50 mL). The organic solution was washed with 1 N NaOH (1 × 25 mL), dried over K_2CO_3, filtered, and concentrated in vacuo to afford 510 mg (~100%) of crude amine **13**: ^{1}H NMR ($CDCl_3$) δ 1.30–1.85 (m, 41 H), 2.69 (t, J = 7.0 Hz, 2 H), 3.14–3.18 (m, 14 H), 3.64 (s, 2 H), 4.42–4.49 (m, 2 H), 5.17–5.30 (m, 3 H), 5.83 (m, 1 H), 7.24–7.33 (m, 2 H), 7.53–7.57 (m, 2 H), 8.15 (m, 1 H); ^{13}C NMR ($CDCl_3$) δ 24.06, 27.55, 28.15, 28.42, 28.44, 30.26, 33.32, 35.87, 36.73, 36.81, 41.98, 43.97, 44.51, 47.05, 48.99, 65.97, 79.22, 79.46, 114.02, 115.24, 117.50, 118.97, 122.63, 124.57, 124.77, 125.47, 132.82, 135.59, 155.31, 156.42, 170.26; HR FABMS observed (M + H) m/z 815.5298, $C_{43}H_{71}N_6O_9$ required m/z 815.5283.

3,7,11,15-Tetraazaeicosan-20-aminium, 7-[(Allyloxy)carbonyl]-11,15-bis[(1,1-dimethylethoxy)carbonyl]-1-[1-[(1,1-dimethylethoxy)carbonyl]-1*H*-indol-3-yl]-*N*,*N*,*N*-trimethyl-2-oxo-, Iodide (14). Amine **13** (460 mg, 5.64 mmol) in acetonitrile (40 mL) was treated with KF/Celite (5 g) followed by excess iodomethane (1.5 mL). The reaction mixture was allowed to stir for 2.5 h at ambient temperature and was filtered. The filtrate was concentrated in vacuo, and the crude product was chromatographed on silica gel (125 g) using 85:15 CH_2Cl_2/MeOH to afford 346 mg (70%) of quaternary ammonium salt **14**: ^{1}H NMR ($CDCl_3$) δ 1.30–1.82 (m, 39 H), 3.15–3.18 (m, 14 H), 3.39 (m, 9 H), 3.52–3.62 (m, 2 H), 3.65 (s, 2 H), 4.42–4.49 (m, 2 H), 5.16–5.25 (m, 3 H), 5.84 (m, 1 H), 7.23–7.31 (m, 2 H), 7.53–7.57 (m, 2 H), 8.13 (m, 1 H); ^{13}C NMR ($CDCl_3$) δ 22.86, 27.22, 27.62, 27.99, 28.16, 28.45, 28.48, 33.29, 36.07, 44.21, 44.60, 44.83, 53.69, 66.02, 66.89, 79.48, 115.23, 117.49, 119.06, 122.65, 124.58, 124.79, 132.80, 155.34, 170.42; HR FABMS observed (M^+) m/z 857.5726, $C_{46}H_{77}N_6O_9$ required m/z 857.5752.

3,7,11,15-Tetraazaeicosan-2-aminium, 11,15-Bis[(1,1-dimethylethoxy)carbonyl]-1-[1-[(1,1-dimethylethoxy)carbonyl]-1*H*-indol-3-yl]-*N*,*N*,*N*-trimethyl-2-oxo-, Iodide (15). To a dichloromethane solution (9 mL) under a nitrogen atmosphere containing quaternary ammonium salt **14** (310 mg, 0.361 mmol) was added acetic acid (650 mg, 10.8 mmol) followed by triphenylphosphine (200 mg, 0.763 mmol) and tetrakis(triphenylphosphine)palladium(0) (200 mg, 0.173 mmol). After 30 and 60 min additional charges (60 mg each) of triphenylphosphine and tetrakis(triphenylphosphine)palladium(0) were added. Additional dichloromethane (60 mL) was added, and the organic solution was washed with H_2O (2 × 20 mL). Acetic acid (650 mg, 10.8 mmol) was added to the organic extract, and this solution was again washed with H_2O (1 × 20 mL). The combined aqueous extracts were back-washed with dichloromethane (3 × 5 mL), and then the pH of the aqueous extract was adjusted to 7.0 with 1 N NaOH. The crude product was salted into dichloromethane (4 × 25 mL). The combined organic extracts were dried over K_2CO_3, filtered, and concentrated in vacuo to afford crude product which was dissolved in chloroform (35 mL). This solution was washed with H_2O (2 × 7 mL), dried over K_2CO_3, filtered, and concentrated in vacuo to afford crude product which still contained some catalyst related impurities. The chloroform extraction procedure utilized above was again repeated to afford 159 mg (57%) of amine **15**: ^{1}H NMR ($CDCl_3$) δ 1.31–1.89 (m, 40 H), 2.47 (m, 2 H), 2.56 (m, 2 H), 3.00–3.55 (m, 21 H), 3.63 (s, 2 H), 5.50 (m, 1 H), 7.21 (t, J = 7.4 Hz, 1 H), 7.30 (m, 1 H), 7.54 (m, 2 H), 8.11 (m, 1 H); ^{13}C NMR ($CDCl_3$) δ 22.29, 23.00, 27.43, 28.14, 28.41, 28.44, 33.09, 37.93, 44.75, 45.85, 46.32, 47.12, 53.20, 66.57, 79.41, 83.77, 114.29, 115.22, 119.19, 122.71, 124.66, 130.03, 135.45, 149.51, 155.46, 170.39; HR FABMS observed (M^+) m/z 773.5549, $C_{42}H_{73}N_6O_7$ required m/z 773.5541.

Agel 489a (1). Under a nitrogen atmosphere, amine **15** (29 mg, 0.0375 mmol) was dissolved in 4 mL of dichloromethane. To this solution was added 2-(phenylsulfonyl)-3-phenyloxaziridine (35 mg, 0.134 mmol) in 1.5 mL of dichloromethane. Progress of the reaction was monitored by FABMS. After 1.5 h, the reaction mixture was concentrated in vacuo to give crude nitrone which was dissolved in 4 mL of acetic acid. A large excess of sodium cyanoborohydride (40 mg, 0.640 mmol) was added, and the reaction mixture was allowed to stir for ca. 2 h. The reaction mixture was concentrated in vacuo, taken up in dichloromethane (5 mL), and washed with pH 7 buffer (1 × 50 mL), adjusting the pH to 7 with 1 N NaOH. The aqueous layer was saturated with NaCl and was extracted with dichloromethane (2 × 15 mL). The combined organic extracts were dried over K_2CO_3, filtered, and concentrated in vacuo to afford crude hydroxylamine which was combined with 2.5 mL of trifluoroacetic acid under a nitrogen atmosphere. The reaction mixture was allowed to stir at ambient temperature for 30 min and then concentrated in vacuo. The residue was dissolved in H_2O (5 mL), washed with diethyl ether (2 × 5 mL) and then ethyl acetate (1 × 5 mL), and subsequently lyophilized to afford 28 mg of crude Agel 489a (1). An analytical sample of Agel 489a (1) was obtained by preparative reverse-phase chromatography (Dynamax-60A-Phenyl column (21.4 × 250 mm, 8 μm), using isocratic conditions (10% CH_3CN/H_2O) containing 0.1% CF_3CO_2H) at 15 mL/min: ^{13}C NMR (125.76 MHz, D_2O) of 1 δ 22.73, 22.79, 23.37, 23.43, 25.69, 25.92, 33.29, 37.36, 45.11, 45.17, 46.59, 48.18, 53.56, 57.30, 57.80, 66.85, 108.48, 112.79, 115.96, 119.12, 120.30, 122.87, 125.85, 127.33, 176.35; ^{1}H NMR (500 MHz, D_2O) of 1 δ 1.38–1.42 (m, 2 H), 1.68–1.83 (m, 6 H), 1.91–1.95 (m, 2 H), 2.00–2.08 (m, 2 H), 2.79–2.85 (m, 4 H), 3.01–3.10 (m, 17 H), 3.21–3.25 (m, 2 H), 3.27–3.31 (m, 2 H), 3.73 (s, 2 H), 7.16 (dd, J = 8 Hz, J = 8 Hz, 1 H), 7.25 (dd, J = 8 Hz, J = 8 Hz, 1 H), 7.32 (s, 1 H), 7.51 (d, J = 8 Hz, 1 H), 7.60 (d, J = 8 Hz, 1 H); HR FABMS of 1 observed (M^+) m/z 489.3911, $C_{27}H_{49}N_6O_2$ required m/z 489.3917.

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Supplementary Material Available: ^{1}H NMR spectra for compounds **1–9** and **11–15**, ^{13}C NMR spectra for compounds **3–9** and **11–15**, reverse-phase HPLC chromatogram of the Agel 489a (1) and Agel 489 separation and of the Agel 505 and 505a (2) separation, UV spectra of natural 1 and 2, and the FAB mass spectrum and ^{13}C NMR spectrum of synthetic 1 (37 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.