Isolation, Structure Elucidation, and Synthesis of Novel Hydroxylamine-Containing Polyamines from the Venom of the *Agelenopsis aperta* Spider

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Abstract: Novel hydroxylamine-containing polyamines have been isolated from the venom of *Agelenopsis aperta*, a funnel-web spider found throughout the western United States. ^1H and ^{13}C NMR in concert with UV spectroscopy provided important structural inputs particularly for determining the aromatic chromophores. Fast-atom bombardment (FAB) mass spectrometry, however, provided the key data for establishing the polyamine functionality of Agel 489 (**1**), 505 (**2**), 452 (**3**), 468 (**4**), and 448 (**5**). These polyamine structures were confirmed by total synthesis. The recurring propylamine portions of the polyamine chains were assembled by amine cyanoethylation followed by nitrile reduction. Hydroxylamine incorporation was best achieved via a two-step sequence involving amine oxidation with 2-(phenylsulfonyl)-3-phenyloxaziridine followed by sodium cyanoborohydride reduction of the nitrone products. TFA and/or dioxane/HCl treatment of the *tert*-butoxycarbonyl (BOC) and methoxymethoxy (MOM) protected intermediates provided the natural Agelenopsis hydroxylamine products **1**-**5** as their acid salts. In the HCl-mediated removal of the protecting groups to generate 4-hydroxyindoleacetamides **2** (Agel 505) and **5** (Agel 448), intermediate 1-carboxyl-3-indoleacetamides were isolated.

Introduction

The venom of *Agelenopsis aperta* (family Agelenidae), a common funnel-web spider found throughout the western United States, contains both peptide and polyamine constituents.[1] Agelenopsis peptides, which are highly disulfide bridged, are potent irreversible blockers of synaptic transmission and have been extensively examined in insect and animal models.[2] The low molecular weight Agelenopsis acylpolyamines affect excitatory amino acid neurotransmission[3] and cause immediate but reversible paralysis of insects.[1] The structures of these novel hydroxylamine-containing Agelenopsis polyamines which are potent *N*-methyl-D-aspartate (NMDA) antagonists, have recently been disclosed.[4] The structure elucidation and synthesis of some of the more abundant polyamines in this venom are the subject of this report.

Results and Discussion

Fractionation. Crude venom containing acylpolyamine and peptide components was fractionated by reverse-phase HPLC, yielding first the smaller and more hydrophilic polyamines followed by the larger and highly disulfide-bridged peptides (Figure 1). In most cases, multiple HPLC fractionations were required for purification of individual constituents. A more detailed HPLC examination of the acylpolyamine constituents is shown in Figure 2. UV spectra were obtained on the individual components resolved by HPLC. Providing sufficient sample was isolated, NMR spectra were obtained. All purified isolates were analyzed by low-resolution FAB mass spectrometry. Protonated molecular and selected fragment ions were analyzed by using high-resolution FAB mass spectrometry.

Structural Elucidation. The structures of known polyamine toxins isolated from orb weaving spiders contain the general structural sequence of arylacetamide–amino acid$_1$–polyamine–amino acid$_2$.[5] While UV analysis of purified constituents obtained with a diode array detector indicated indole (λ_{max} = 218, 279, 287 nm) and 4-hydroxyindole (λ_{max} = 219, 267, 282, 292 nm) chromophores for the major Agelenopsis polyamines, amino acid analysis suggested the absence of amino acid residues in the polyamine structures. The FAB mass spectra of the purified components of polyamine spider toxins yielded intense $[M + H]^+$ ions (or, in the case of quaternary ammonium salts, $[M]^+$ ions) along with many structurally useful fragment ions. FAB mass spectra indicated molecular weights of 416, 448, 452, 464, 468, 489, 489, 505, 505, and 521 amu for purified toxins. Agel 489 (**1**) was the most abundant polyamine extracted from this spider and as a result was analyzed in greatest detail. High-resolution FABMS analysis (Table I) was obtained on six fragment ions of Agel 489 (**1**).

Table I. High-Resolution Data for Agel 489

elemental composition	exact mass observed, amu	error, ppm	comments
$C_{26}H_{48}N_7O_2$	490.3863	−1.3	$[M + H]^+$
$C_{29}H_{32}N_5O_2$	362.2554	−0.6	
$C_{16}H_{41}N_6O$	333.3340	−0.6	
$C_{13}H_{34}N_5$	260.2809	−0.5	
$C_{13}H_{15}N_2O$	215.1189	+2.1	215 A fragment
$C_{11}H_{27}N_4$	215.2236	+0.1	215 B fragment
$C_{10}H_{27}N_4$	203.2232	−1.8	

The low-resolution FAB mass spectrum of Agel 489 (**1**) produced an intense $[M + H]^+$ ion at m/z = 490 (Figure 3). High resolution of this ion yielded the composition $C_{26}H_{48}N_7O_2$ which indicated the presence of seven degrees of unsaturation. A ^{13}C NMR spectra of this compound suggested the presence of a 3-indolylacetamide (136.10, 127.17, 123.75, 120.92, 118.67, 118.26, 111.33, and 108.97 ppm). A 3-indolylacetamide contains seven degrees of unsaturation indicating that the rest of the molecule is saturated. Subtracting out the elemental composition of the indolylacetamide from the intact molecule leaves a unit with the composition of $C_{16}H_{39}N_6O$, suggesting the presence of a polyamine substructure due to the high nitrogen content. Also, the presence of an oxygen in this substructure implies either a hydroxy or an ether linkage. An ion was observed in the low-resolution FAB mass spectrum 18 amu lower than the molecular

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(2) Bindokas, V. P.; Adams, M. E. *J. Neurobiol.* **1989**, *20*, 171. Adams, M. E.; Bindokas, V. P.; Hasegawa, L.; Venema, V. J. *Soc. Neurosci. Abstr.* **1988**, *14*, 30. Artman, L. D.; Saccomano, N. A.; Volkmann, R. A.; Nemeth, E. F.; Parks, T. N. *Ibid.* **1989**, *15*, 356. Adams, M. E.; Bindokas, V. P.; Dolphia, A. C.; Imperial, J. S.; Olivera, B. M.; Scott, R. H.; Venema, V. J. *Ibid.* **1989**, *15*, 652. Adams, M. E.; Bindokas, V. P., Hasegawa, L.; Venema, V. J. *J. Biol. Chem.* **1990**, *265*, 861.
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(5) Saccomano, N. A.; Volkmann, R. A.; Jackson, H.; Parks, T. N. *Annu. Rep. Med. Chem.* **1989**, *24*, 287.

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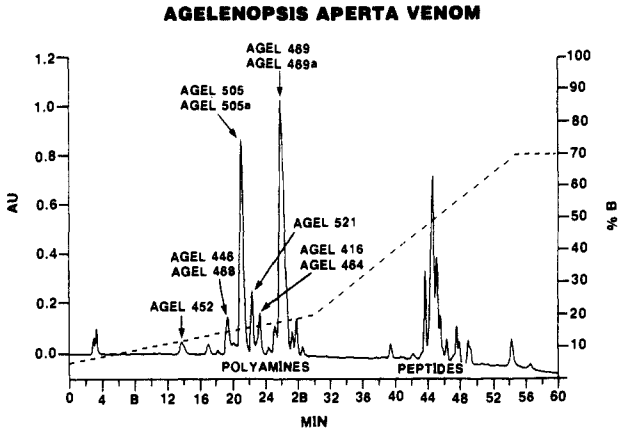

Figure 1. Reverse-phase HPLC (VYDAC C-18) column (22 × 250 mm, 10 μm, 300 Å) separation using a 0–20% linear gradient of CH_3CN/H_2O containing 0.1% CF_3COOH for 30 min followed by a 20–70% gradient for 25 min at a flow rate of 15 mL/min.

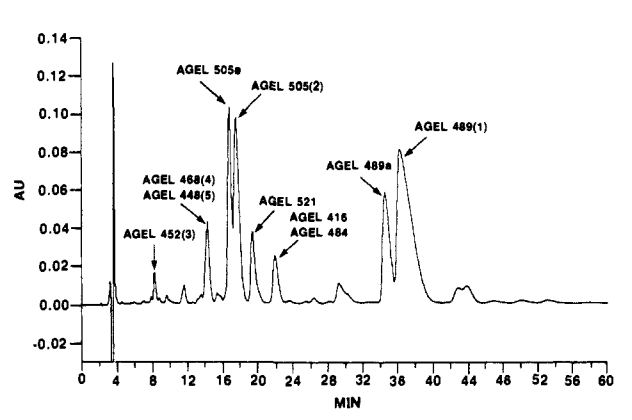

Figure 2. Reverse-phase HPLC (Baker C-18) column (4.6 × 250 mm, 5 μm, 300 Å) separation using isocratic (8% CH_3CN/H_2O containing 0.1% CF_3CO_2H) conditions at a flow rate of 1 mL/min.

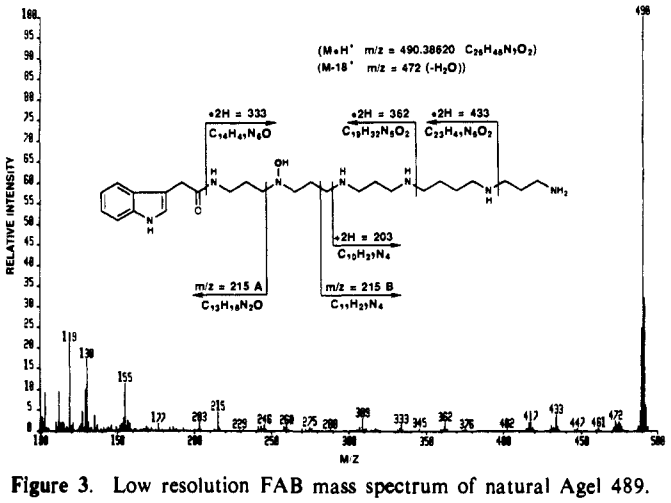

Figure 3. Low resolution FAB mass spectrum of natural Agel 489.

ion at m/z = 472 due to loss of water from the intact molecule, indicating that the oxygen is bound as a hydroxyl group. Many low intensity fragment ions (Figure 3) are observed in this mass spectrum at m/z = 130, 203, 215, 260, 333, 362, and 433 with matrix ions (see General Methods) appearing at m/z = 103, 119, 135, 155, 177, 309, and 461. The fragment ion at m/z = 130 is assumed to arise from the 3-indolylacetamide moiety (which was suggested by UV analysis) and was not analyzed by high-resolution FAB mass spectrometry.

Secondary amines and secondary amides often fragment under FAB conditions to produce a two proton rearrangement[6] which

Table II. High-Resolution Data of $[M + H]^+$ Ions for *Agelenopsis aperta*

elemental composition	exact mass observed, amu	error, ppm	comments
$C_{26}H_{48}N_7O_2$	490.3863	+1.2	Agel 489
$C_{23}H_{45}N_6O_4$	469.3501	+0.2	Agel 468
$C_{23}H_{45}N_6O_3$	453.3535	+4.0	Agel 452
$C_{26}H_{48}N_7O_3$	506.3810	−1.7	Agel 505

is the case with many of the ions observed in Agel 489 (**1**). The ions at m/z = 203, 333, 362, and 433 were all cleavages at amine bonds with two proton rearrangements (Figure 3). The identities of these ions were confirmed by high-resolution FAB mass spectrometry (see Table I). From these fragmentations, a general structure, which lacked the hydroxyl group, could be proposed. The fragmentation did, however, suggest that the hydroxyl group must be located somewhere on the first or second propylamine units. An important clue to the location of the hydroxyl group in Agel 489 (**1**) was provided by a DEPT ^{13}C NMR experiment which suggested that no methine carbons were present in this molecule. We were, of course, aware of the fact that while the apparent absence of an appropriate signal in the DEPT ^{13}C NMR was enlightening, it was not rigorous in excluding the presence of a methine carbon. Additional high-resolution FABMS revealed that the ion at m/z = 215 was actually a doublet resulting from two separate fragmentations as shown in Figure 3 (and Table I). Realizing that the hydroxyl group had to be on the first or second propylamine nitrogen atoms (and not on any of the carbons atoms from the DEPT experiment) left only one possibility as shown in Figure 4 for the structure of Agel 489 (**1**).

An ion was observed in the low-resolution mass spectrum of natural Agel 489 (**1**) at m/z = 260 and was initially believed to be a fragment ion from the intact molecule. However, the synthetic sample of Agel 489 (**1**) did not show the presence of an ion at m/z = 260. Upon reisolating natural Agel 489 (**1**) and analyzing immediately, no ion at m/z = 260 was observed. We believe this material is $H_2N(CH_2)_3NH(CH_2)_3NH(CH_2)_4NH(CH_2)_3NH_2$, a possible decomposition product of (or precursor to) Agel 489 (**1**).

The structures assigned to Agel 489 (**1**), 505 (**2**), 452 (**3**), 468 (**4**), and 448 (**5**) are shown in Figure 4. Four (Agel 489 (**1**), 505 (**2**), 452 (**3**) and 468 (**4**)) of the five compounds exhibited similar FABMS fragmentation. As a result, we concluded that these compounds contain the same polyamine side chain and differ only in the aromatic residue. The elemental compositions of the four compounds are given in Table II. By subtracting the polyamine sidechain from each compound we obtained an elemental composition for the chromophore. The presence and the position of the hydroxyl groups in the chromophores were determined by UV spectrophotometry[7] and NMR analysis.

The fifth compound illustrated in Figure 4, Agel 448 (**5**), closely resembles Agel 505 (**2**) by virtue of its hydroxyindoleacetamide moiety. The FAB mass spectrum suggested that Agel 448 (**5**) contains a shorter 3,3,4,3 chain (with the numerals denoting the number of methylene groups between amino groups) as opposed to the ubiquitous Agelenopsis 3,3,3,4,3 chain.

Agelenopsis Synthesis. Extensive methodology has been developed for the synthesis of polyamines in recent years.[5,8] An iterative approach patterned after the work of Israel et al.,[9] involving amine cyanoethylation followed by nitrile reduction, which was recently utilized by Shih et al.[10] for the synthesis of Argiotoxin 636, was selected for polyamine construction. The presence of a hydroxylamine functionality proximal to the aromatic chro-

(6) The two proton rearrangement is analogous to the Y″ cleavage as described by Roepstorff and Fohlman for peptides. See: Roepstorff, P.; Fohlman, J. *Biomed. Mass. Spectrom.* **1984**, *11*, 601.

(7) Kamath, B. V.; Mehta, J. D.; Bafna, S. L. *J. Appl. Chem. Biotechnol.* **1975**, *25*, 743.

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(9) Israel, M.; Rosenfield, J. S.; Modest, E. J. *J. Med. Chem.* **1964**, *7*, 710.

(10) Shih, T. L.; Ruiz-Sanchez, J.; Mrozik, H. *Tetrahedron Lett.* **1987**, *28*, 6015.

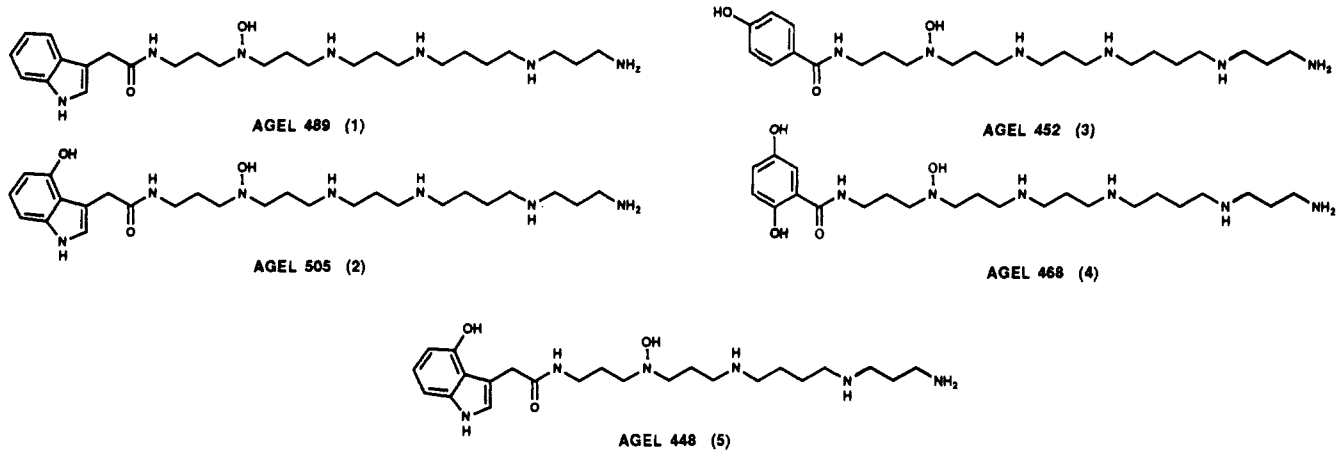
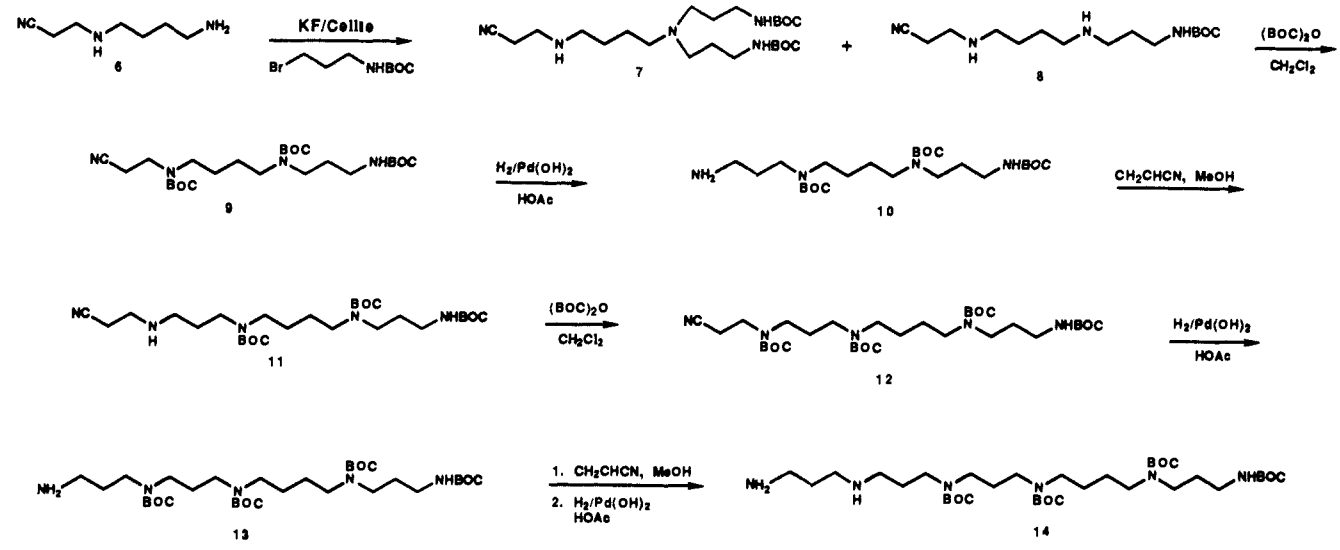


Figure 4.

Scheme I



mophore required a synthetic strategy for regiospecific hydroxylamine incorporation. With the inherent instability of secondary hydroxylamines, we opted to generate this functionality late in our synthetic scheme. Of the methods commonly employed for hydroxylamine synthesis[11] (such as amine oxidation, nitrone reduction, organometallic addition to nitrones, and manipulations involving N,O-protected hydroxylamines) selective amine oxidation appeared best suited to our approach for the assembly of the Agelenopsis 3,3,3,4,3 polyamine chain present in Agel 489 (**1**), 505 (**2**), 452 (**3**), and 468 (**4**). Such a selective amine oxidation would require protection of the resident amine functionalities in the polyamine chains. The *tert*-butoxycarbonyl (BOC) group appeared ideal for this purpose.

Readily accessible *N*-(cyanoethyl)-1,4-butyldiamine (**6**)[12] provided a convenient source for incorporating the internal 3,4-spermidine portion of the molecule. Alkylation of **6** with *N*-BOC-3-bromopropylamine[13] in the presence of neutral potassium fluoride/Celite as originally described by Ando and Yamawaki[14] and later utilized by Samejima[15] and us[13,16] provided desired nitrile **8** in 60% yield and bisalkylated diamine **7** (Scheme I). BOC protection (di-*tert*-butyl dicarbonate (2 equiv)) of **8** followed by a catalytic hydrogenation (Pearlman's catalyst ($Pd(OH)_2$), HOAc) cleanly afforded amine **10** containing the polyamine's 3,4,3 spermine terminus. Acrylonitrile addition to **10** followed by BOC protection of the resultant amine **11** provided nitrile **12**. Biscyanoethylated product in this and in similar acrylonitrile reactions are minor (<5%), a result of the reduced nucleophilicity of cyanoethylated amines. Increased amounts of biscyanoethylated products are produced, however, with excess acrylonitrile and longer reaction periods. Standard nitrile reduction of **12** generated amine **13** which was converted via standard protocol (acrylonitrile addition followed by catalytic hydrogenation) to polyamine **14** having the 3,3,3,4,3 Agelenopsis polyamine backbone.

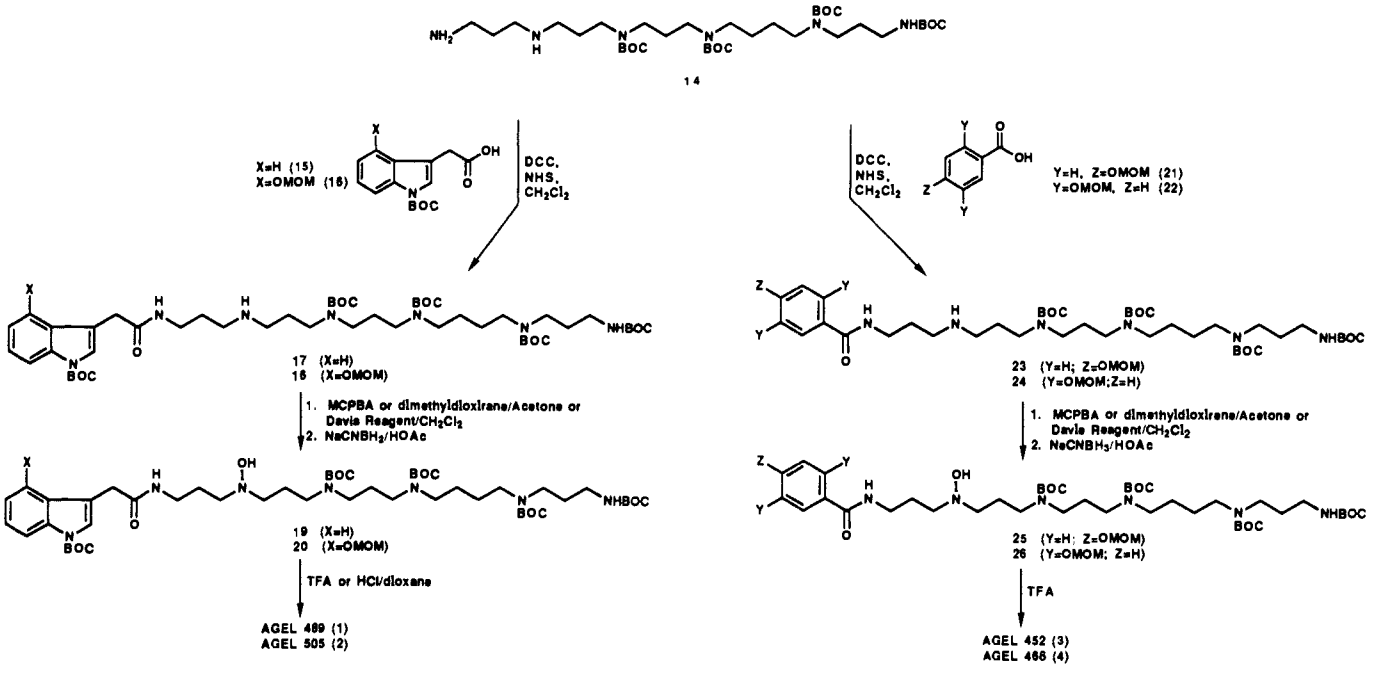
Bisamine 14 was coupled with indoleacetic acids **15** and **16** to access Agel 489 (**1**) and Agel 505 (**2**) and with methoxymethoxy (MOM) protected hydroxybenzoic acids **21** and **22** to access Agel 452 (**3**) and 468 (**4**) (Scheme II). We were concerned about the possibility of secondary amine acylation of **14**, resulting in the formation of undesired branched acylpolyamines. Standard dicyclohexylcarbodiimide (DCC)/*N*-hydroxysuccinimide coupling provided the desired amides **17**, **18**, **23**, and **24** in ca. 50–70% yield in addition to minor amounts of less polar impurities (bisacylated polyamines). Coupling yields could be substantially improved if the internal amine functionality were protected as its benzylamine.[17]

MCPBA oxidation of amine **17** in dichloromethane produced a mixture of products. Tollen's reagent, which has been used as

(11) Sandler, S. R.; Karo, W. In *Organic Functional Group Preparations*; Academic Press, Inc.: San Diego, CA 1986; Vol. III, Chapter 10.
(12) Yamamoto, H.; Maruoka, K. *J. Am. Chem. Soc.* **1981**, *103*, 6133.
(13) Jasys, V. J.; Kelbaugh, P. R.; Nason, D. M.; Phillips, D.; Saccomano, N. A.; Volkmann, R. A. *Tetrahedron Lett.* **1988**, *29*, 6223.
(14) Ando, T.; Yamawaki, J. *Chem. Lett.* **1979**, 45.
(15) Samejima, K.; Takeda, Y.; Kawase, M.; Okada, M.; Kyogoku, Y. *Chem. Pharm. Bull.* **1984**, *32*, 3428.
(16) Nason, D. M.; Jasys, V. J.; Kelbaugh, P. R.; Phillips, D.; Saccomano, N. A.; Volkmann, R. A. *Tetrahedron Lett.* **1989**, *30*, 2337.

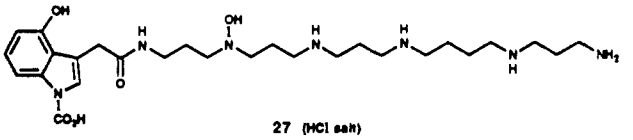
(17) While making of the internal amine with a benzyl group improves the coupling yields (>90%), removal via catalytic hydrogenation ($Pd(OH)_2$/HOAc) is unfortunately accompanied, in the case of indoleacetamides, with some indole reduction, generating indolines.

Scheme II



a TLC spray for hydroxylamines, confirmed the presence of hydroxylamine product in the crude reaction mixture. Excess oxidant was required for consumption of starting material. On the basis of literature precedent,[18] we anticipated and observed substantial amounts of nitrone side products. For this reason, we typically subjected the crude oxidation product mixture to sodium cyanoborohydride reduction in acetic acid to afford crude hydroxylamines. In our hands, the best oxidants for amine oxidation are the neutral 2-(phenylsulfonyl)-3-aryloxaziridines developed by Davis[19] which have been examined by Zajac, et al.[20] on a variety of secondary amines. Oxidation using dimethyldioxirane/acetone solutions described by Murray, et al.[21] also yielded hydroxylamine products in somewhat lower yield. Conditions, however, for these oxidations have not been optimized and as a result, a two-step sequence involving oxidations with the Davis oxaziridines followed sodium cyanoborohydride reduction was routinely employed to generate hydroxylamines **19**, **20**, **25**, and **26**.

Clean removal of the BOC (and MOM) protecting groups proved to be quite sensitive to reaction conditions. As an added complication, the hydroxylamine products **1**–**5** were prone to degradation. Agel 452 (**3**) and Agel 468 (**4**) were generated by treatment of benzamides **25** and **26** with trifluoroacetic acid. Agel 489 (**1**) was produced in high yield with the inverse addition of a dichloromethane solution of indoleacetamide **19** to trifluoroacetic acid which was continuously purged with argon to remove isobutylene and to prevent the air oxidation of the hydroxylamine products. These conditions were unfortunately not satisfactory for alkoxyindoleacetamides **20** and **29** as substantial amounts of alkylated indoles (resulting from isobutylene and formaldehyde addition) were obtained. The addition of isobutylene scavengers failed to provide a solution to this problem. In the course of our investigation we observed that, in the absence of water, dioxane/HCl treatment of **20** and **29** removed both the indole MOM group and the polyamine side chain BOC groups while *retaining the protective indole-N-carboxyl substituent*. The resultant indole acids, which were also intermediates in the trifluoroacetic acid deprotections, could be fully characterized and were best isolated from dioxane/HCl treatment of the BOC-protected indoles. The presence of a *N*-carboxylic acid moiety in indole **27** was supported by ^{13}C NMR (carbamoyl carbon at 153.95 ppm), FABMS ((M + H) m/z = 550.3712), IR (carbonyl absorption at 1726 cm^{-1}), and UV ((H_2O) λ_{max} = 222, 250, 293, and 302 nm) spectroscopy. The stability of these indole acids is compound specific as they slowly decarboxylate (over a period of days) in the solid state at 23 °C, are stable at −78 °C, and rapidly lose carbon dioxide (over a period of hours) in aqueous solution. The conversion of a 2.73 mM solution of indole **27** to Agel 505 (**2**) obeyed first-order kinetics ($t_{1/2}$ = 80 min). Because of the sensitivity of the hydroxylamine products to oxidation, the indole acids were typically added to argon-saturated aqueous solutions. The reactions were monitored by reverse-phase HPLC.



1-Indolecarboxylic acids have recently been generated by Katritzky et al.[22] and Boger and Patel[23] by carboxylation of *N*-lithioindole. To the best of our knowledge, no one has reported the isolation of these acids in the removal of an indole BOC group. Certainly the ability to retain the deactivating indole *N*-carboxyl substituent retards electrophilic addition to the hydroxyindole and allows us to cleanly generate **2** and **5**. We are in the process of examining the scope of this result which could increase the utility of the BOC protecting group particularly with electron rich heterocycles.

Agel 448 (**5**), having a shorter 3,3,4,3 sidechain, was obtained in an identical fashion with Agel 505 (**2**), i.e. conversion of nitrile **11** to hydroxylamine **29** via bisamine **28** and conversion of **29** to Agel 448 (**5**) using the above mentioned HCl/dioxane protocol (Scheme III).

These synthetic materials were found to be identical with the natural products in all respects (^1H NMR, FABMS, HPLC, UV, and biological activity). ^{13}C NMR of the trifluoroacetate salt of the most abundant isolated polyamine, Agel 489 (**1**) was identical with that of the synthetic material. The ^{13}C NMR spectrum of a mixture of natural and synthetic Agel 489 (**1**) is

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(19) For a review of oxaziridines in organic synthesis, see: Davis, F. A.; Sheppard, A. C. *Tetrahedron* **1989**, *45*, 5703.
(20) Zajac, W. W., Jr.; Walters, T. R.; Darcy, M. G. *J. Org. Chem.* **1988**, *53*, 5856.
(21) Murray, R. W.; Jeyaraman, R. *J. Org. Chem.* **1985**, *50*, 2847. Murray, R. W. *Chem. Rev.* **1989**, *89*, 1187. Murray, R. W.; Singh, M. *Syn. Commun.* **1989**, *19*, 3509.
(22) Katritzky, A. R.; Akutagawa, K. *Tetrahedron Lett.* **1985**, *26*, 5935. Katritzky, A. R.; Akutagawa, K.; Jones; R. A. *Syn. Commun.* **1988**, *18*, 1151.
(23) Boger, D. L.; Patel, M. *J. Org. Chem.* **1987**, *52*, 3934.

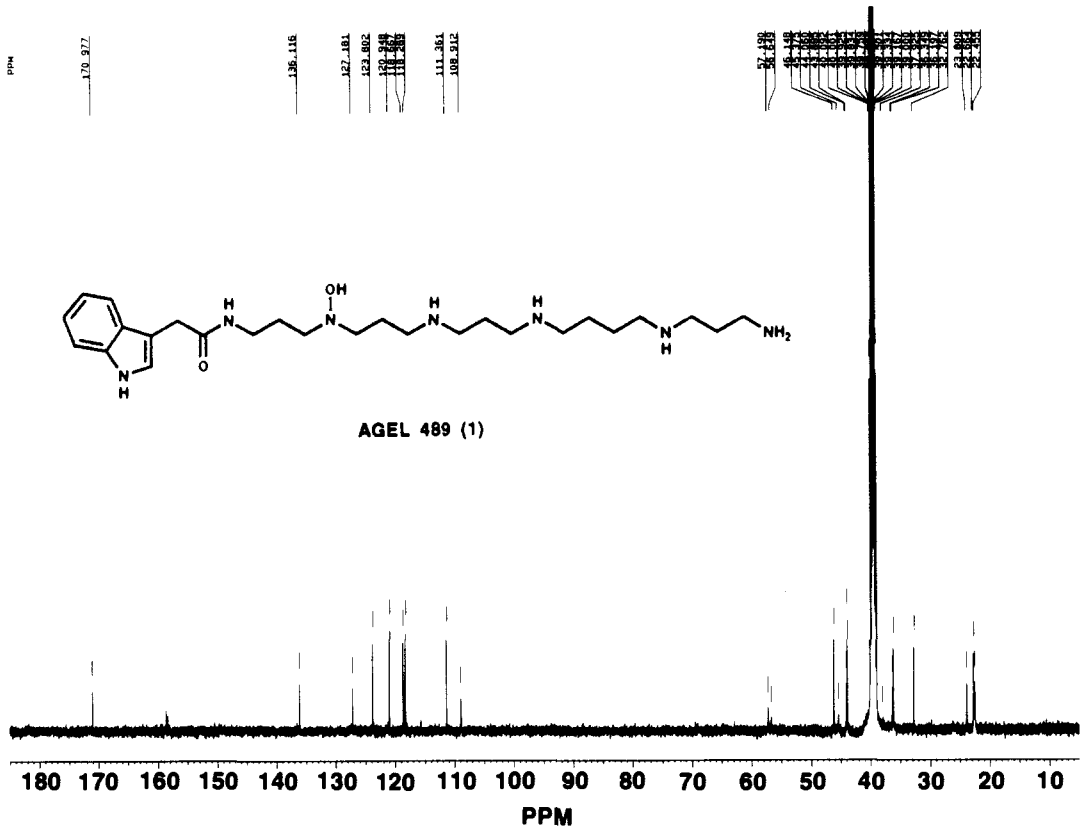
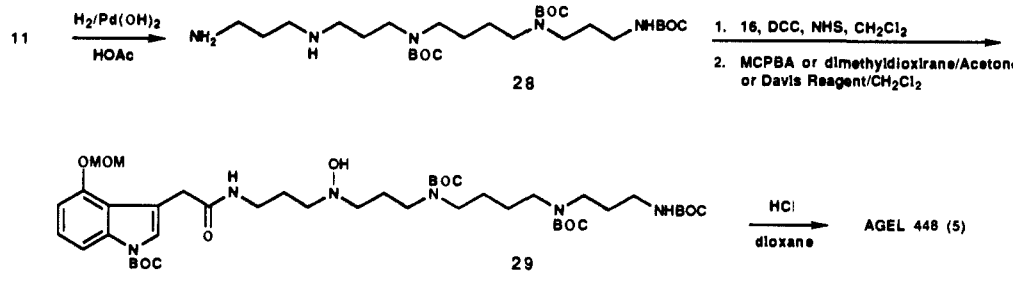


Figure 5. 125.76 MHz ^{13}C NMR spectrum of natural and synthetic Agel 489 (TFA Salt in DMSO-d_6).

Scheme III



shown in Figure 5. As acid salts, the hydroxylamine-containing products are stable in aqueous solution provided the solutions are purged with argon. In the presence of air these products have poor solution stability.

The *Agelenopsis aperta* acylpolyamines are structurally distinct from the polyamine toxins isolated to date as they are devoid of amino acids and reveal an unprecedented hydroxylamine substituent at the arylacylamide chain terminus.[5] Further details of the biological activity of these Agelenopsis hydroxylamine-containing polyamines will be published soon.

Experimental Section

Venom Extraction. *Agelenopsis 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.[24] 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 use. 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 448 (**5**), 452 (**3**), and 468 (**4**) required additional purification, which was obtained on the same column with use of a 5–10% linear gradient for 30 min at a flow rate of 15 mL/min. Agel 505 (**2**) and structurally distinct 505a were further purified on a VYDAC C-4 column (22 × 250 mm, 10 μm, 300 Å) with use of a 0–10% linear gradient for 20 min followed by a 10–20 linear gradient of CH_3CN/H_2O containing 0.1% CF_3COOH at a flow rate of 15 mL/min. Agel 416 and Agel 464 were resolved via reverse-phase HPLC (Baker C-18 column (4.6 × 250 mm, 5 μm, 300 Å)) with use of isocratic (8% CH_3CN/H_2O containing 0.1% CH_3COOH) conditions at a flow rate of 1 mL/min. These conditions were employed for the purification of Agel 521. Agel 489a and the major Agelenopsis polyamine, Agel 489 (**1**) were resolved by using a Dynamax 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. Venom constituents were isolated following lyophilization of HPLC fractions and were stored at −80 °C under an argon atmosphere.

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 was determined. Accordingly 1 μL of whole venom contains approximately 2.86 μg of Agel 452, 3.54 μg of Agel 448, 1.32 μg of Agel 468, 4.37 μg of Agel 505a, 5.59 μg of Agel 505, 3.38 μg of Agel 521, 0.96 μg of Agel 464, 1.52 μg of Agel

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

416, 9.47 μg of Agel 489a, and 33.02 μg of Agel 489. These weights correspond to the trifluoroacetate salts of each polyamine. On the basis of elemental analysis data, we assume each amino(hydroxylamino) moiety forms an amine salt with TFA (vide infra).

General Methods. 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 on 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 or on a Bruker WM-250 equipped with an Apect 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.

16-Cyano-2,2-dimethyl-3-oxa-4-oxo-5,9,14-triazahexadecane (8). To a solution of *N*-cyanoethyl-1,4-diaminobutane (**6**) (6.44 g, 0.0457 mol) in acetonitrile (200 mL) under a nitrogen atmosphere was added KF/Celite (11 g) followed by the dropwise addition over a 7-h period of *N*-(*tert*-butoxycarbonyl)-3-bromopropylamine (10.87 g, 0.0457 mol). The reaction was allowed to stir for 16 h at ambient temperature and then heated to 70 °C for 24 h. The reaction was allowed to cool and was filtered and concentrated in vacuo. The residue was dissolved in CH_2Cl_2 (200 mL), washed with 1 N NaOH (100 mL), dried, and concentrated in vacuo to afford crude product which was chromatographed on silica gel (using 9:1 CH_2Cl_2/MeOH) to afford 3.32 g or branched amine **7**: ^1H NMR ($CDCl_3$) δ 1.31–1.65 (m, 27 H), 2.36–2.44 (m, 6 H), 2.49 (t, *J* = 6.6 Hz, 2 H), 2.61 (m, 2 H), 2.90 (t, *J* = 6.6 Hz, 2 H), 3.09–3.16 (m, 4 H), 5.28 (br s, 2 H). ^{13}C NMR ($CDCl_3$) δ 18.74, 24.60, 27.05, 27.96, 28.50, 39.52, 45.14, 49.07, 52.21, 53.76, 78.90, 118.79, 156.09; HR FABMS observed (M + H) *m/z* = 456.3549. $C_{23}H_{46}N_5O_4$ (Req 456.3550). The column was then eluted with 9:1:0.5 (CH_2Cl_2/MeOH/diisopropylamine) to give 8.2 g (60%) of desired product **8**: ^1H NMR ($CDCl_3$) δ 1.19–1.59 (m, 17 H), 2.42 (t, *J* = 6.6 Hz, 2 H), 2.44–2.58 (m, 6 H), 2.82 (*t*, *J* = 6.6 Hz, 2 H), 3.08 (m, 2 H), 5.22 (br s, 1 H); ^{13}C NMR ($CDCl_3$) δ 18.68, 27.70, 27.74, 28.42, 29.94, 39.16, 45.03, 47.68, 48.99, 49.65, 78.78, 118.75, 156.11; HR FABMS observed (M + H) m/z = 299.2434, $C_{15}H_{31}N_4O_2$ (req 299.2447).

2-(2-Cyanoethyl)-7-[(1,1-dimethylethoxy)carbonyl]-2,7,11-triazadodecanedioic Acid Bis(1,1-dimethylethyl) Ester (9). To a dichloromethane (150 mL) solution under a nitrogen atmosphere containing diamine **8** (3.50 g, 0.0118 mol) was added di-*tert*-butyl dicarbonate (5.20 g, 0.0238 mol). The mixture was stirred for 68 h at ambient temperature. The mixture was concentrated in vacuo and chromatographed on silica gel (400 g) with use of 3:2 hexane/ethyl acetate to afford 5.62 g (96%) of nitrile **9**: ^1H NMR ($CDCl_3$) δ 1.20–1.59 (m, 33 H), 2.55 (m, 2 H), 3.01–3.37 (m, 8 H), 3.39 (t, *J* = 6.6 Hz, 2 H), 5.25 (br s, 1 H); ^{13}C NMR δ 17.21, 25.73, 25.94, 28.22, 28.24, 28.27, 37.91, 43.78, 44.24, 46.60, 47.95, 78.96, 79.57, 80.44, 155.01, 155.75, 155.98, HR FABMS observed (M + H) *m/z* = 499.3501, $C_{25}H_{47}N_4O_6$ (req 499.3496).

2-(3-Aminopropyl)-7-[(1,1-dimethylethoxy)carbonyl]-2,7,11-triazadodecanedioic Acid Bis(1,1-dimethylethyl) Ester (10). A solution of nitrile **9** (3.00 g, 0.0060 mol) in 30 mL of glacial acetic acid was hydrogenated over $Pd(OH)_2/C$ (3 g) at 50 psi hydrogen pressure for 2 h. The catalyst was removed by filtration through a Millipore (0.45 μM, 47 mm) filter. Evaporation of the solvent gave crude product which was dissolved in 75 mL of CH_2Cl_2 washed with 1 N NaOH (2×) and dried over K_2CO_3. The solution was concentrated in vacuo to afford 2.86 g (95%) of amine **10**: ^1H NMR ($CDCl_3$) δ 1.24–1.59 (m, 35 H), 2.14 (s, 2 H), 2.61 (t, *J* = 6.7 Hz, 2 H), 2.98–3.14 (m, 10 H), 5.22 (br s, 1 H); ^{13}C NMR ($CDCl_3$) δ 25.89, 28.42, 31.38, 32.36, 37.55, 38.95, 43.95, 46.65, 79.34, 79.48, 155.65, 156.03; HR FABMS observed (M + H) *m/z* = 503.3804, $C_{25}H_{51}N_4O_6$ (req *m/z* = 503.3809).

20-Cyano-2,2-dimethyl-4-oxo-3-oxa-5,9,14,18-tetraazaeicosane-9,14-dicarboxylic Acid Bis(1,1-dimethylethyl) Ester (11). To a solution of amine **10** (2.86 g, 0.0057 mol) in 75 mL of methanol was added acrylonitrile (0.41 mL, 0.0057 mol). The reaction was stirred at ambient temperature for 12 h and concentrated in vacuo. The concentrate was dissolved in 30 mL of dichloromethane and concentrated in vacuo (3×) to afford 3.18 g (ca. 100%) of crude nitrile **11**: ^1H NMR ($CDCl_3$) δ 1.26–1.73 (m, 36 H), 2.44 (t, *J* = 6.7 Hz, 2 H), 2.54 (t, *J* = 6.7 Hz, 2 H), 2.83 (t, *J* = 6.7 Hz, 2 H), 3.00–3.16 (m, 10 H), 5.24 (br s, 1 H); ^{13}C NMR ($CDCl_3$) δ 18.64, 25.84, 28.09, 28.43, 28.74, 37.84, 44.18, 44.68, 45.14, 46.29, 46.73, 46.85, 49.70, 78.90, 79.29, 79.46, 118.52, 155.84, 155.98; HR FABMS observed (M + H) *m/z* = 556.4064, $C_{28}H_{54}N_5O_6$ (req *m/z* = 556.4074).

2-(2-Cyanoethyl)-6,11-bis[(1,1-dimethylethoxy)carbonyl]-2,6,11,15-tetraazahexadecanedioic Acid Bis(1,1-dimethylethyl) Ester (12). To a solution of crude nitrile **11** (3.18 g, 0.0057 mol) in 100 mL of CH_2Cl_2 was added di-*tert*-butyl dicarbonate (1.37 g, 0.0063 mol). The reaction was stirred for 90 min at ambient temperature, concentrated in vacuo, and chromatographed on silica gel (300 g) with use of 3:2 hexane/ethyl acetate to afford 3.12 g (85%) of product **12**: ^1H NMR ($CDCl_3$) δ 1.26–1.73 (m, 44 H), 3.03–3.24 (m, 14 H), 3.42 (t, *J* = 6.6 Hz, 2 H), 5.25 (br s, 1 H); ^{13}C NMR ($CDCl_3$) δ 17.20, 25.88, 27.83, 28.12, 28.35, 28.45, 28.77, 37.87, 43.91, 44.20, 44.77, 46.27, 46.77, 46.88, 78.94, 79.42, 79.50, 80.54, 117.91, 154.96, 155.44, 155.74, 155.99; HR FABMS observed (M + H) *m/z* = 656.4579, $C_{33}H_{62}N_5O_8$ (req *m/z* = 656.4598).

2-(3-Aminopropyl)-6,11-bis[(1,1-dimethylethoxy)carbonyl]-2,6,11,15-tetraazahexadecanedioic Acid Bis(1,1-dimethylethyl) Ester (13). Nitrile **12** (3.12 g, 0.0048 mol) was hydrogenated as described above for **9** to yield 3.02 g (95%) of crude amine **13**: ^1H NMR ($CDCl_3$) δ 1.28–1.71 (m, 46 H), 2.16 (br s, 2 H), 2.65 (t, *J* = 6.7 Hz, 2 H), 3.01–3.18 (m, 14 H), 5.24 (br s, 1 H); ^{13}C NMR ($CDCl_3$) δ 25.85, 27.66, 28.45, 28.76, 39.10, 44.21, 44.91, 46.80, 79.27, 79.46, 155.45, 155.67, 155.99; HR FABMS observed (M + H) *m/z* = 660.4914, $C_{33}H_{66}N_5O_8$ (req *m/z* = 660.4911).

25-Amino-2,2-dimethyl-4-oxo-3-oxa-5,9,14,18,22-pentaazapentacosane-9,14,18-tricarboxylic Acid Tris(1,1-dimethylethyl) Ester (14). Acrylonitrile (100 μL, 0.00167 mol) treatment of amine **13** (1.00 g, 0.0015 mol) using the protocol described above for **10** gave 1.00 g (93%) of crude nitrile: ^1H NMR ($CDCl_3$) δ 1.26–1.66 (m, 47 H), 2.45 (t, *J* = 6.6 Hz, 2 H), 2.56 (t, *J* = 6.7 Hz, 2 H), 2.85 (t, *J* = 6.6 Hz, 2 H), 3.01–3.30 (m, 14 H), 5.25 (br s, 1 H); ^{13}C NMR ($CDCl_3$) δ 18.68, 25.92, 28.46, 28.48, 37.49, 44.19, 44.88, 45.16, 46.73, 78.93, 79.32, 79.44, 118.70, 155.46, 155.61, 156.04; HR FABMS observed (M + H) *m/z* = 713.5191, $C_{36}H_{69}N_6O_8$ (req *m/z* = 713.5177). Crude nitrile (1.00 g, 0.0014 mol) was hydrogenated as described above for **9** to give 0.850 g (85%) of bisamine **14**: ^1H NMR ($CDCl_3$) δ 1.26–1.65 (m, 49 H), 2.16 (s, 2 H), 2.51 (t, *J* = 6.8 Hz, 2 H), 2.58 (t, *J* = 6.8 Hz, 2 H), 2.70 (t, *J* = 6.7 Hz, 2 H), 3.00–3.15 (m, 14 H), 5.22 (br s, 1 H); ^{13}C NMR ($CDCl_3$) δ 25.79, 27.10, 27.61, 28.45, 30.90, 33.28, 37.51, 40.52, 44.04, 44.77, 46.69, 47.91, 48.35, 79.27, 79.33, 79.49, 155.43, 155.55, 156.02; HR FABMS observed (M + H) *m/z* = 717.5495, $C_{36}H_{73}N_6O_8$ (req *m/z* = 717.5490).

***N*-(*tert*-Butoxycarbonyl)-1*H*-indole-3-acetic Acid (15).** To a solution of tetrabutylammonium hydrogen sulfate (6.78 g, 0.020 mol) in 75 mL of H_2O was slowly added $NaHCO_3$ (3.36 g, 0.040 mol) followed by 1*H*-indole-3-acetic acid (3.50 g, 0.020 mol). Chloroform (75 mL) was added, and the solution was allowed to stir for 10 min. The layers were separated. The aqueous layer was saturated with Na_2SO_4 and extracted with chloroform. The organic extracts were combined, dried over Na_2SO_4, concentrated in vacuo, and dissolved in 75 mL of acetone. Allyl bromide (1.9 mL, 0.022 mol) was added, and the mixture was stirred for 30 min. The mixture was concentrated in vacuo and chromatographed on silica gel with use of 3:1 hexane/ethyl acetate to afford 3.57 g (83%) of allyl 1*H*-indole-3-acetate. To this ester (2.15 g, 0.010 mol) dissolved in 20 mL of acetonitrile was added di-*tert*-butyl dicarbonate (2.61 g, 0.012 mol) followed by 4-(*N*,*N*-dimethylamino)pyridine (0.122 g, 0.001 mol). The reaction was allowed to proceed for 15 min and then diluted with 125 mL of ethyl acetate and washed with 0.1 N HCl (2×), H_2O (3×), and brine (1×), dried over Na_2SO_4, and concentrated in vacuo to afford after silica gel chromatography using 14.5:1 hexane/ethyl acetate 2.87 g (91%) of allyl *N*-(*tert*-butoxycarbonyl)-1*H*-indole-3-acetate. Treatment of this indole allyl ester (3.27 g, 0.0104 mol) in 20 mL of dichloromethane with 7.4 mL of an ethyl acetate solution containing sodium 2-ethylhexanoate (0.231 g/mL, 1.71 g, 0.0103 mol) followed by triphenylphosphine (0.5 g, 0.0019 mol) and tetrakis(triphenylphosphine)palladium (0.5 g, 0.0004 mol) provided after 60 min of stirring a solution containing the desired product as its sodium salt. The mixture

was concentrated in vacuo, taken up in 150 mL of ethyl acetate, washed with H_2O (5×), and back-extracted with ethyl acetate (1×) and diethyl ether (1×). The aqueous extracts were combined with 75 mL of ethyl acetate and was acidified with 0.1 N HCl to pH 3. The aqueous layer was extracted with ethyl acetate (3×), and the combined organic extracts were washed with H_2O (2×) and brine (1×), dried, and concentrated in vacuo to afford, after hexane trituration, 1.37 g (48%) of **15** as a white solid: ^1H NMR ($CDCl_3$) δ 1.68 (s, 9 H), 3.77 (s, 2 H), 7.27 (m, 1 H), 7.35 (m, 1 H), 7.54 (d, J = 8 Hz, 1 H), 7.59 (s, 1 H), 8.5 (d, 1 H); ^{13}C NMR (125.76 MHz, $CDCl_3$) 28.11, 30.74, 83.73, 112.31, 115.30, 118.93, 122.66, 124.62, 124.65, 129.84, 135.33, 149.53, 177.01.

***N*-(*tert*-Butoxycarbonyl)-4-(methoxymethoxy)-1*H*-indole-3-acetic Acid (16).** 4-(Methoxymethoxy)-1*H*-indole was converted to 4-(methoxymethoxy)-1*H*-indole-3-acetic acid according to a known procedure.[25] To an aqueous solution (25 mL) containing tetrabutylammonium hydrogen sulfate (1.59 g, 0.0047 mol) was slowly added $NaHCO_3$ (0.79 g, 0.0094 mol) followed by 4-(methoxymethoxy)-1*H*-indole-3-acetic acid (1.10 g, 0.0047 mol). Dichloromethane (50 mL) was added, and the solution was allowed to stir for 10 min. The layers were separated, and the aqueous layer was saturated with Na_2SO_4 and extracted with dichloromethane. The organic extracts were combined, dried over Na_2SO_4, concentrated in vacuo, and dissolved in 40 mL of acetone. Iodomethane (0.50 mL, 0.008 mol) was added, and the mixture was stirred for 1.5 h. The mixture was concentrated in vacuo and chromatographed on silica gel with use of ethyl acetate to afford the desired ester which was dissolved in dichloromethane (30 mL) and treated with di-*tert*-butyl dicarbonate (1.05 g, 0.0047 mol) and 4-(*N*,*N*-dimethylamino)pyridine (0.015 g, 0.00012 mol). The reaction was allowed to proceed for 12 h and then diluted with 100 mL of ethyl acetate and washed with 0.1 N HCl (2×), H_2O (3×) and brine (1×), dried over Na_2SO_4, and concentrated in vacuo to afford, after silica gel chromatography using 2:1 hexane/ethyl acetate, 1.41 g (87%) of methyl *N*-(*tert*-butoxycarbonyl)-4-(methoxymethoxy)-1*H*-indole-3-acetate. To a methanol (25 mL) solution of ester (1.40 g, 0.004 mol) was added H_2O (20 mL) and enough THF to obtain a one-phase solution. Potassium hydroxide (0.325 g, 0.005 mol) was added. The reaction proceeded slowly as monitored by TLC. Additional potassium hydroxide (0.100 g, 0.0015 mol) was added, and the reaction was allowed to proceed for 4 h. The methanol/THF was then removed under reduced pressure, H_2O (70 mL) was added, and the mixture was extracted with ethyl acetate (3×). The aqueous solution was overlayed with ethyl acetate, and the pH was adjusted with 6 N HCl to 2. The aqueous solution was extracted with ethyl acetate (2×). The combined extracts were washed with H_2O (3×) and brine, dried over Na_2SO_4, and concentrated in vacuo to afford 1.28 g of crude product. Silica gel chromatography (1:1 hexane/acetone) afforded 0.95 g (86%, based on the recovery of 0.135 g of starting acid) of **16**: ^1H NMR ($CDCl_3$) 1.65 (s, 9 H), 3.45 (s, 3 H), 3.89 (s, 2 H), 5.23 (s, 2 H), 6.86 (d, J = 8 Hz, 1 H), 7.19 (dd, J = 8 Hz, J = 8 Hz, 1 H), 7.42 (s, 1 H), 7.78 (d, J = 8 Hz, 1 H); ^{13}C NMR ($CDCl_3$) δ 28.20, 32.33, 56.09, 83.74, 94.53, 106.69, 109.53, 112.34, 120.02, 123.95, 125.56, 137.29, 149.50, 151.33, 175.87, HR FABMS observed (M + H) m/z = 335.1365, $C_{17}H_{21}NO_6$ (req 335.1369).

28-[1-[(1,1-Dimethylethoxy)carbonyl]-1*H*-indol-3-yl]-2,2-dimethyl-4,27-dioxo-3-oxa-5,9,14,18,22,26-hexaazaoctacosane-9,14,18-tricarboxylic Acid Tris(1,1-dimethylethyl) Ester (17). To a CH_2Cl_2 solution (25 mL) under nitrogen atmosphere containing *N*-(*tert*-butoxycarbonyl)-3-indoleacetic acid (**15**) (200 mg, 0.00073 mol) was added *N*-hydroxysuccinimide (84 mg, 0.00073 mol) and dicyclohexylcarbodiimide (DCC) (150 mg, 0.00073 mol). The reaction was allowed to stir for 3 h. Dicyclohexylurea was filtered, and the filtrate was added dropwise to a CH_2Cl_2 solution (40 mL) of amine **14** (524 mg, 0.00073 mol). The mixture was stirred for 12 h at ambient temperature and was washed with 0.1 N NaOH, 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 400 mg (56%) of product **17**: ^1H NMR ($CDCl_3$) δ 1.23–1.73 (m, 58 H), 2.45–2.60 (m, 4 H) 2.80–3.31 (m, 16 H), 3.61 (s, 2 H), 5.30 (br s, 1 H), 6.70 (bs, 1 H), 7.21 (m, 1 H), 7.26 (m, 1 H), 7.49 (d, J = 8 Hz, 1 H), 7.52 (s, 1 H), 8.11 (d, J = 8 Hz, 1 H); ^{13}C NMR ($CDCl_3$) δ 25.89, 27.70, 27.96, 28.16, 28.23, 28.47, 28.68, 28.77, 29.65, 33.29, 38.03, 44.22, 44.89, 45.05, 46.56, 46.82, 47.18, 79.35, 79.52, 79.74, 83.76, 114.25, 115.37, 119.09, 122.77, 124.71, 124.75, 130.09, 135.71, 149.53, 155.46, 156.01, 170.41; HR FABMS observed (M + H) m/z = 974.6520, $C_{51}H_{88}N_7O_{11}$ (req 974.6542).

28-[1-[(1,1-Dimethylethoxy)carbonyl]-1*H*-indol-3-yl]-22-hydroxy-2,2-dimethyl-4,27-dioxo-3-oxa-5,9,14,18,22,26-hexaazaoctacosane-9,14,18-tricarboxylic Acid Tris(1,1-dimethylethyl) Ester (19). Under a nitrogen atmosphere, indoleacetamide **17** (340 mg, 0.00035 mol) was dissolved in 30 mL of acetone and cooled to 0 °C. A solution of 85% 3-chloroperoxybenzoic acid (212 mg, 0.00105 mol) in 20 mL of acetone was added dropwise over a period of 10 min. The reaction was stirred for 30 min whereupon 80 mL of diethylether was added. The organic solution was washed with 10% K_2CO_3, H_2O, 10% K_2CO_3 and H_2O, dried over K_2CO_3, and concentrated in vacuo to afford crude intermediate nitrone (330 mg) which was dissolved in 3 mL of acetic acid and allowed to react with $NaBH_3CN$ (20 mg, 0.00032 mol). The mixture was stirred for 12 h, concentrated in vacuo, dissolved in 50 mL of CH_2Cl_2, and was combined with pH 7 buffer (2 mL), and the pH was adjusted with 1 N NaOH to 7. The organic extract was washed with pH 7 buffer (1×) and brine (1×), dried over K_2CO_3, and concentrated in vacuo to afford crude product. Silica gel chromatography (100 g) using 95:5 ethyl acetate/methanol provided 108 mg (31%) of **19**: ^1H NMR ($CDCl_3$) δ 1.16–1.73 (m, 57 H), 2.44–2.56 (m, 4 H), 2.61 (s, 1 H), 3.06–3.32 (m, 16 H), 3.64 (s, 2 H), 5.28 (s, 1 H), 6.45 (br s, 1 H), 7.23 (m, 1 H), 7.33 (m, 1 H), 7.51 (d, J = 8 Hz, 1 H), 7.54, (s, 1 H), 8.13 (d, J = 8.0 Hz, 1 H); ^{13}C NMR ($CDCl_3$) δ 25.92, 26.23, 26.69, 27.86, 28.24, 28.49, 28.51, 28.78, 29.33, 29.66, 33.33, 37.88, 38.45, 44.25, 45.09, 45.23, 46.82, 57.98, 58.44, 79.41, 79.58, 83.90, 114.32, 115.48, 119.05, 122.87, 124.78, 124.87, 130.03, 135.74, 155.57, 155.61, 170.06; HR FABMS observed (M + H) m/z = 990.6461, $C_{51}H_{88}N_7O_2$ (req 990.6491).

***N*-(20-Amino-4-hydroxy-4,8,12,17-tetraazaeicos-1-yl)-1*H*-indole-3-acetamide (1).** A CH_2Cl_2 solution (1.5 mL) containing hydroxylamine **19** (108 mg, 0.000109 mol) was added under an argon atmosphere (with use of argon to purge isobutylene) to 50 mL of trifluoroacetic acid. The mixture was stirred for 1 h at ambient temperature and then concentrated in vacuo. Diethyl ether (15 mL) was added, and the resultant mixture was stirred for 30 min and filtered. The powder was washed well with diethyl ether and dried under a nitrogen atmosphere followed, by high vacuum, to give 109 mg (95%) of Agel 489 (**1**) as its trifluoroacetate salt: UV (H_2O) λ_{max} nm (log ϵ) 218 (4.41), 279 (3.64), 287 (3.57); IR (KBr) ν_{max} 722, 798, 835, 1135, 1165, 1198, 1670, 2875, 3075 cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.62–1.68 (m, 4 H), 1.78 (m, 2 H), 1.88 (m, 6 H), 2.82–3.03 (m, 19 H), 3.13 (m, 2 H), 3.50 (s, 2 H), 6.98 (dd, J = 8 Hz, J = 8 Hz, 1 H), 7.08 (dd, J = 8 Hz, J = 8 Hz, 1 H) 7.19 (s, 1 H), 7.34 (d, J = 8 Hz, 1 H), 7.55 (d, J = 8 Hz, 1 H), 7.85–8.90 (m, 5 H), 10.89 (s, 1 H); ^{13}C NMR (DMSO-d_6) δ 22.45, 22.65, 23.06, 23.79, 26.71, 32.78, 36.22, 36.52, 43.89, 43.89, 44.06, 46.12, 46.12, 57.07, 57.39, 108.97, 111.33, 118.26, 118.67, 120.92, 123.75, 127.17, 136.10, 170.94; HR FABMS observed (M + H) m/z = 490.3887, $C_{26}H_{48}N_7O_2$ (req 490.3869).

28-[1-[(1,1-Dimethylethoxy)carbonyl]-4-(methoxymethoxy)-1*H*-indol-3-yl]-2,2-dimethyl-4,27-dioxo-3-oxa-5,9,14,18,22,26-hexaazaoctacosane-9,14,18-tricarboxylic Acid Tris(1,1-dimethylethyl) Ester (18). *N*-*tert*-Butoxycarbonyl-4-(methoxymethoxy)-3-indoleacetic acid (**16**) (0.480 g, 1.43 mmol) in dichloromethane (50 mL) was treated with *N*-hydroxysuccinimide (0.165 g, 1.43 mmol) followed by DCC (0.294 g, 1.43 mmol) and allowed to stir for 5 h. The reaction mixture was filtered, and the filtrate containing crude hydroxysuccinimide ester was added dropwise over a 20-min period to a dichloromethane solution (100 mL) containing amine **14** (1.03 g, 1.43 mmol). The reaction was stirred for 72 h. The crude reaction mixture was washed with 1 N NaOH (2 × 20 mL) and dried over potassium carbonate, filtered, and concentrated to afford crude product which was chromatographed on silica gel with 4:1 CH_2Cl_2/MeOH followed by 9:1:1 CH_2Cl_2/MeOH/diisopropylamine to afford 912 mg (62%) of **18**: ^1H NMR ($CDCl_3$) δ 1.34–1.70 (m, 58 H), 2.32–2.52 (m, 4 H), 3.00–3.25 (m, 16 H), 3.45 (s, 3 H), 3.72 (s, 2 H), 5.23 (s, 2 H), 6.35 (br s, 1 H), 6.84 (d, J = 8 Hz, 1 H), 7.16 (dd, J = 8 Hz, J = 8 Hz, 1 H), 7.38 (s, 1 H), 7.77 (d, J = 8 Hz, 1 H); ^{13}C NMR ($CDCl_3$) δ 25.86, 27.65, 27.74, 27.94, 28.18, 28.47, 28.61, 28.67, 28.73, 29.54, 35.06, 37.84, 38.18, 44.19, 44.86, 44.92, 46.80, 46.89, 47.51, 56.30, 79.28, 79.32, 79.51, 83.85, 94.77, 106.90, 109.51, 113.81, 124.17, 125.68, 137.50, 151.41, 155.45, 155.53, 156.01, 171.05; HR FABMS observed (M + H) m/z = 1034.6759, $C_{53}H_{92}N_7O_{13}$ (req 1034.6753).

28-[1-[(1,1-Dimethylethoxy)carbonyl]-4-(methoxymethoxy)-1*H*-indol-3-yl]-22-hydroxy-2,2-dimethyl-4,27-dioxo-3-oxa-5,9,14,18,22,26-hexaazaoctacosane-9,14,18-tricarboxylic Acid Tris(1,1-dimethylethyl) Ester (20). Under a nitrogen atmosphere, indoleacetamide **18** (1.10 g, 1.065 mmol) was dissolved in 40 mL of dichloromethane. To this solution was added 2-(phenylsulfonyl)-3-phenyloxaziridine (611 mg, 2.34 mmol). Progress of the reaction was monitored by TLC. After 20 min additional oxaziridine reagent (61 mg, 0.234 mmol) was added, and 10 min later the reaction mixture was concentrated in vacuo to give crude nitrone which was dissolved in 45 mL of acetic acid. A large excess of sodium cyanoborohydride (750 mg, 11.9 mmol) was added and the reaction was allowed to stir for ca. 3 h. The reaction was concentrated in vacuo, taken up in dichloromethane (75 mL), washed with pH 7 buffer (1 × 50 mL) adjusting the pH to 7 with 1 N NaOH. The organic layer was washed

(25) Poon, G.; Chui, Y.-C.; Law, F. C. P. *J. Labelled Compd. Radiopharm.* **1986**, *23*, 167.

again with pH 7 buffer (1 × 50 mL), dried over potassium carbonate, and concentrated in vacuo to generate crude product which was chromatographed on silica gel with use of ethyl acetate to remove the less polar impurities followed by 1:1 hexane/acetone to afford 840 mg (75%) of **20** as a white foam: ^{1}H NMR ($CDCl_3$) δ 1.21–1.69 (m, 57 H), 2.38–2.50 (m, 4 H), 3.00–3.39 (m, 16 H), 3.46 (s, 3 H), 3.72 (s, 2 H), 5.23 (s, 2 H), 6.25 (br s, 1 H), 6.85 (d, J = 8 Hz, 1 H), 7.19 (dd, J = 8 Hz, J = 8 Hz, 1 H), 7.39 (s, 1 H), 7.78 (d, J = 8 Hz, 1 H); ^{13}C NMR ($CDCl_3$) δ 25.90, 26.27, 26.98, 28.19, 28.46, 28.49, 35.07, 37.86, 38.17, 44.20, 45.05, 46.79, 56.36, 58.02, 58.33, 79.36, 79.55, 83.93, 94.82, 106.95, 109.55, 113.82, 119.84, 124.23, 125.76, 137.48, 149.40, 151.41, 155.54, 156.03, 171.04; HR FABMS observed (M + H) m/z = 1050.6693, $C_{53}H_{92}N_7O_{14}$ (req 1050.6702).

4-(Methoxymethoxy)benzoic Acid (21),[26] **21**: ^{1}H NMR ($CDCl_3$) δ 3.47 (s, 3 H), 5.23 (s, 2 H), 7.07 (d, J = 8.9 Hz, 2 H), 8.04 (d, J = 8.9 Hz, 2 H); ^{13}C NMR ($CDCl_3$) δ 56.24, 94.03, 115.70, 122.65, 132.25, 161.64, 171.85.

2,5-Bis(methoxymethoxy)benzoic Acid (22). A two-step procedure using methodology described by Dunn and Bruice[25] involving methoxymethylation followed ester hydrolysis was employed: ^{1}H NMR ($CDCl_3$) 3.44 (s, 3 H), 3.52 (s, 3 H), 5.13 (s, 2 H), 5.33 (s, 2 H), 7.19 (m, 2 H), 7.78 (m, 1 H); ^{13}C NMR ($CDCl_3$) 56.03, 56.91, 95.07, 95.54, 117.14, 119.89, 120.29, 123.28, 151.27, 152.57, 165.94.

1-[4-(Methoxymethoxy)phenyl]-26,26-dimethyl-1,24-dioxo-25-oxa-2,6,10,14,19,23-hexaazaheptacosane-10,14,19-tricarboxylic Acid Tris-(1,1-dimethylethyl) Ester (23). Treatment of a dichloromethane solution (20 mL) containing acid **21** (0.127 g, 0.7 mmol), DCC (0.144 g, 0.7 mmol), and *N*-hydroxysuccinimide (0.081 g, 0.7 mmol) with diamine **14** (0.500 g, 0.7 mmol), as described for the preparation of **18**, afforded, after silica gel chromatography (9:1 CH_2Cl_2/MeOH followed by 9:1:0.25 CH_2Cl_2/MeOH/diisopropylamine), 370 mg (56%) of **23**: ^{1}H NMR ($CDCl_3$) δ 1.25–1.75 (m, 49 H), 2.57 (t, J = 6.6 Hz, 2 H), 2.74 (t, J = 5.6 Hz, 2 H), 3.05–3.21 (m, 14 H), 3.44 (s, 3 H), 3.45–3.54 (m, 2 H), 5.17 (s, 2 H), 7.00 (d, J = 8.8 Hz, 2 H), 7.72 (d, J = 8.8 Hz, 2 H); ^{13}C NMR ($CDCl_3$) δ 25.87, 27.68, 28.48, 28.78, 39.78, 44.21, 44.92, 46.83, 48.72, 56.04, 79.32, 79.43, 79.52, 94.38, 115.85, 128.57, 128.64, 155.47, 155.59, 159.66, 166.72, HR FABMS observed (M + H) m/z = 881.5981, $C_{45}H_{81}N_6O_{11}$ (req m/z = 881.5963).

1-[2,5-Bis(methoxymethoxy)phenyl]-26,26-dimethyl-1,24-dioxo-25-oxa-2,6,10,14,19,23-hexaazaheptacosane-10,14,19-tricarboxylic Acid Tris(1,1-dimethylethyl) Ester (24). Treatment of a dichloromethane solution (20 mL) containing acid **22** (0.170 g, 0.0007 mol), DCC (0.144 g, 0.0007 mol), and *N*-hydroxysuccinimide (0.081 g, 0.0007 mol) with diamine **14** (0.500 g, 0.0007 mol), as described for the preparation of **18**, afforded, after silica gel chromatography (9:1 CH_2Cl_2/MeOH followed by 9:1:0.25 CH_2Cl_2/MeOH/diisopropylamine), 0.370 g (56%) of **24**: ^{1}H NMR ($CDCl_3$) δ 1.21–1.91 (m, 49 H), 2.50–2.65 (m, 4 H), 3.03–3.34 (m, 14 H), 3.42 (s, 3 H), 3.46 (s, 3 H), 3.42–3.52 (m, 2 H), 5.11 (s, 2 H), 5.21 (s, 2 H), 5.30 (br s, 1 H), 7.04 (m, 2 H), 7.76 (m, 1 H), 7.92 (m, 1 H); ^{13}C NMR ($CDCl_3$) δ 25.86, 27.69, 27.97, 28.17, 28.47, 28.76, 30.03, 38.00, 44.20, 44.92, 46.81, 47.15, 47.49, 55.91, 56.57, 78.95, 79.33, 79.51, 95.13, 96.11, 116.83, 118.92, 119.46, 120.65, 150.06, 152.43, 155.47, 155.57, 156.01, 164.90; HR FABMS observed (M + H) m/z = 941.6191, $C_{47}H_{85}N_6O_{13}$ (req m/z = 941.6175).

1-[4-(Methoxymethoxy)phenyl]-6-hydroxy-26,26-dimethyl-1,24-dioxo-25-oxa-2,6,10,14,19,23-hexaazaheptacosane-10,14,19-tricarboxylic Acid Tris(1,1-dimethylethyl) Ester (25). Treatment of amide **23** (0.335 g, 0.38 mmol) with 2-(phenylsulfonyl)-3-phenyloxaziridine (0.230 g, 0.88 mmol), as described for the preparation of **20**, afforded, after silica gel chromatography (50:50 acetone/hexane), 253 mg (74%) of hydroxylamine **25**: ^{1}H NMR ($CDCl_3$) δ 1.14–1.89 (m, 49 H), 2.60–2.74 (m, 4 H), 3.04–3.53 (m, 16 H), 3.43 (s, 3 H), 5.16 (s, 2 H), 5.30 (br s, 1 H), 6.98 (d, J = 8.7 Hz, 2 H), 7.20 (br s, 1 H), 7.72 (d, J = 8.7 Hz, 2 H); ^{13}C NMR ($CDCl_3$) δ 25.92, 26.54, 26.69, 27.83, 28.20, 28.46, 28.49, 28.76, 29.32, 37.86, 39.04, 44.25, 45.12, 45.32, 46.78, 46.95, 56.04, 58.17, 59.01, 79.43, 79.58, 94.37, 115.87, 128.44, 128.69, 155.58, 155.65, 156.05, 159.70, 166.99; HR FABMS observed (M + H) m/z = 897.5931, $C_{45}H_{81}N_6O_{12}$ (req m/z = 897.5912).

***N*-(20-Amino-4-hydroxy-4,8,12,17-tetraazaeicos-1-yl)-4-hydroxybenzamide (3)**. Under an argon atmosphere at room temperature was combined hydroxylamine **25** (0.200 g, 0.223 mmol) and trifluoroacetic acid (5 mL). The reaction was stirred for 1.5 h. Additional trifluoroacetic acid (2 mL) was added to wash the walls of the flask. The reaction was allowed to proceed for an additional 1.5 h and was concentrated in vacuo, triturated with ethyl ether, filtered, and dried under a nitrogen atmosphere to afford 218 mg (96%) of ARG 452 (**3**) as its trifluoroacetate salt: UV (H_2O) λ_{max} nm (log ϵ) 251 (4.12). IR (KBr) ν_{max} 722, 798, 835, 1134, 1167, 1199, 1671, 2875, 3083 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 1.63 (m, 4 H), 1.81–1.97 (m, 8 H), 2.80–2.99 (m, 18 H), 3.38 (m, 2 H), 6.79 (d, J = 8.6 Hz, 2 H), 7.71 (d, J = 8.6 Hz, 2 H), 7.96 (br s), 8.30 (m, 1 H), 8.84 (m), 10.00 (m); ^{13}C NMR (DMSO-d_6) δ 22.45, 22.65, 23.80, 36.20, 36.81, 43.90, 44.06, 45.20, 46.14, 56.41, 57.41, 114.75, 125.11, 129.06, 160.13, 166.15; HR FABMS observed (M + H) m/z = 453.3571 $C_{23}H_{45}N_6O_3$ (req m/z = 453.3553).

1-[2,5-Bis(methoxymethoxy)phenyl]-6-hydroxy-26,26-dimethyl-1,24-dioxo-25-oxa-2,6,10,14,19,23-hexaazaheptacosane-10,14,19-tricarboxylic Acid Tris(1,1-dimethylethyl) Ester (26). To an acetone solution (8 mL) containing amide **24** (0.100 g, 0.114 mmol) at 0 °C under a nitrogen atmosphere was added 2 mL of a 0.071 M acetone dimethyldioxirane solution (0.142 mmol). Thin-layer chromatography (1:1 acetone/hexane) showed a mixture of **24**, hydroxylamine product **26**, and nitrone byproduct. Additional oxidant (4 mL, 0.284 mmol) was added. After 15 min, the reaction was concentrated in vacuo, and acetic acid (15 mL) was added followed by a large excess of sodium cyanoborohydride (50 mg, 0.80 mmol). The reaction was allowed to stir for 2 h. The reaction was then concentrated in vacuo and worked up in the normal manner (as described in the preparation of **20**) to afford, after silica gel chromatography (1:1 acetone/hexane), 34 mg (33%) of **26**. Alternatively, treatment of amide **24** (0.308 g, 0.00033 mol) with 2-(phenylsulfonyl)-3-phenyloxaziridine (0.250 g, 0.00096 mol), as described for **20**, afforded, after silica gel chromatography (ethyl acetate followed by 50:50 acetone/hexane), 210 mg (66%) of hydroxylamine **26**: ^{1}H NMR ($CDCl_3$) δ 1.23–1.90 (m, 49 H), 2.59–2.74 (m, 4 H), 3.05–3.39 (m, 14 H), 3.44 (s, 3 H), 3.48 (s, 3 H), 3.48–3.57 (m, 2 H), 5.12 (s, 2 H), 5.22 (s, 2 H), 5.25 (br s, 1 H), 7.05 (m, 2 H), 7.76 (m, 1 H), 7.99 (t, 1 H); ^{13}C NMR ($CDCl_3$) δ 25.92, 26.49, 27.29, 28.50, 28.78, 29.32, 38.19, 44.24, 45.08, 45.29, 46.82, 55.93, 56.71, 58.19, 58.42, 79.38, 79.56, 95.13, 96.26, 116.88, 119.46, 120.67, 150.04, 152.46, 155.57, 155.60, 156.03, 156.31, 164.91; HR FABMS observed (M + H) m/z = 957.6145, $C_{47}H_{85}N_6O_{14}$ (req m/z = 957.6124).

***N*-(20-Amino-4-hydroxy-4,8,12,17-tetraazaeicos-1-yl)-2,5-dihydroxybenzamide (4)**. Trifluoroacetic acid (5 mL) treatment of hydroxylamine **26** (0.100 g, 0.104 mmol) as described in the preparation of **3** afforded 108 mg (100%) of Agel 468 (**4**) as its trifluoroacetate salt: UV (H_2O) λ_{max} nm (log ϵ) 202 (4.29), 321 (3.50); IR (KBr) ν_{max} 722, 798, 835, 1135, 1198, 1671, 2877, 3051 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 1.63 (m, 4 H), 1.83–2.09 (m, 8 H), 2.80–2.99 (m, 18 H), 3.33–3.40 (m, 2 H), 6.73 (d, J = 8.8 Hz, 1 H), 6.85 (dd, J = 8.8 Hz, J = 2.8 Hz, 1 H), 7.22 (d, J = 2.8 Hz, 1 H), 7.95 (br s), 8.72–8.83 (m), 11.71 (br s); ^{13}C NMR (DMSO-d_6) δ 22.44, 22.65, 23.80, 36.20, 36.75, 43.90, 44.02, 45.09, 46.14, 56.32, 57.36, 113.37, 115.74, 117.75, 121.18, 149.26, 152.23, 168.55; HR FABMS observed (M + H) m/z = 469.3517. $C_{23}H_{45}N_6O_4$ (Req m/z = 469.3502).

***N*-(20-Amino-4-hydroxy-4,8,12,17-tetraazaeicos-1-yl)-4-hydroxy-1-(hydroxycarbonyl)indole-3-acetamide (27)**. To a saturated dioxane/HCl solution (100 mL) purged with argon was added over 1.5 min hydroxylamine **20** (520 mg, 0.495 mmol) in dioxane (10 mL). A precipitate formed immediately. The reaction was allowed to stir for 1 h. Diethyl ether (100 mL) was added, and after 5 min, the solution was filtered. The resultant solids were washed with diethyl ether, dried under a nitrogen atmosphere, and then under reduced pressure to afford 345 mg of acid **27** which could be stored at −78 °C: UV (H_2O) λ_{max} nm (log ϵ) 222 (4.40), 258 (3.77), 293 (3.79), 302, (3.78); IR (KBr) ν_{max} 755, 1267, 1447, 1573, 1639, 1726, 2803, 2955 cm^{-1}; ^{1}H NMR (D_2O) δ 1.68–2.18 (m, 12 H), 2.92–3.38 (m, 20 H), 3.80 (s, 2 H), 6.73 (d, J = 8 Hz, 1 H), 7.25 (m, 1 H), 7.51 (s, 1 H), 7.72 (d, J = 8 Hz, 1 H); ^{13}C NMR (D_2O) δ 21.14, 23.44, 23.54, 24.03, 24.52, 34.33, 36.60, 37.33, 45.16, 45.31, 45.40, 47.78, 56.51, 57.11, 108.27, 109.09, 113.77, 119.13, 125.08, 126.67, 138.06, 150.81, 153.95, 176.33; HR FABMS observed (M + H) m/z = 550.3712. $C_{27}H_{48}N_7O_5$ (req m/z = 550.3717).

***N*-(20-Amino-4-hydroxy-4,8,12,17-tetraazaeicos-1-yl)-4-hydroxy-1*H*-indole-3-acetamide (2)**. To an aqueous solution (75 mL) which was purged with argon was added acid **27** (150 mg, 0.205 mmol). The conversion of **27** to **2** was monitored by HPLC and took ca. ~8 h. The reaction mixture was freeze-dried to afford 103 mg (73%)[27] of Agel 505 (**2**) as its hydrochloride salt: UV (H_2O) λ_{max} nm (log ϵ) 219 (4.53), 267 (3.68), 282 (3.60), 292 (3.53); IR (KBr) ν_{max} 745, 1038, 1257, 1454, 1505, 1552, 1588, 1638, 2800, 2954 cm^{-1}; ^{1}H NMR (D_2O) δ 1.74–2.10 (m, 12 H), 3.00 (d, J = 8 Hz, 2 H), 3.05–3.24 (m, 18 H), 3.74 (s, 2 H), 6.51 (m, 1 H), 7.04 (m, 2 H), 7.13 (s, 1 H); ^{13}C NMR (D_2O) δ 21.38, 23.46, 23.55, 24.23, 24.53, 34.59, 36.65, 37.33, 45.19, 45.32, 45.57, 47.78, 56.56, 57.14, 104.81, 105.47, 107.67, 117.00, 124.01, 125.00, 139.50, 150.72, 177.64; HR FABMS observed (M + H) m/z = 506.3831, C_{26}-

(26) Dunn, B. M.; Bruice, T. C. *J. Am. Chem. Soc.* **1970**, *92*, 2410.

(27) We assume for the purpose of establishing a percent yield for the preparation of final products that each polyamine nitrogen atom forms a TFA or HCl salt. This assumption, for example, is consistent with the elemental analysis obtained with **2** which suggests the addition of 5 equiv of HCl.

$H_{48}N_7O_3$ (req m/z = 506.3819). Anal. Calcd for $C_{26}H_{48}N_7O_3 \cdot 5HCl \cdot 2H_2O$: C, 43.07; H, 7.92; N, 13.52; Cl, 24.44. Found C, 44.22; H, 7.57; N, 13.40; Cl, 24.32.

21-Amino-2,2-dimethyl-4-oxo-3-oxa-5,9,14,18-tetraazaheneicosane-9,14-dicarboxylic Acid Bis(1,1-dimethylethyl) Ester (28). Nitrile **11** (1.15 g, 2.07 mmol) was hydrogenated as described above for **9** to give 1.10 g (95%) of crude amine **28**: ^{1}H NMR ($CDCl_3$) 1.29-1.64 (m, 40 H), 2.48-2.71 (m, 6 H), 3.01-3.15 (m, 10 H), 5.24 (s, 1 H); ^{13}C NMR ($CDCl_3$) 25.85, 28.14, 28.43, 28.73, 29.01, 33.93, 37.89, 40.55, 44.17, 44.96, 46.74, 47.26, 47.87, 79.16, 155.59, 155.98; HR FABMS observed (M + H) m/z = 560.4373. $C_{28}H_{58}N_5O_6$ (req 560.4387).

24-[1-[(1,1-Dimethylethoxy)carbonyl]-4-(methoxymethoxy)-1*H*-indol-3-yl]-18-hydroxy-2,2-dimethyl-4,23-dioxo-3-oxa-5,9,14,18,22-pentaazatetracosane-9,14-dicarboxylic Acid Bis(1,1-dimethylethyl) Ester (29). Condensation of amine **28** (0.380 g, 0.0068 mol) with a dichloromethane solution (15 mL) containing acid **16** (0.228 g, 0.0068 mol), *N*-hydroxysuccinimide (0.078 g, 0.0068 mol), and DCC (0.140 g, 0.0068 mol) as described in the preparation of **18** afforded 0.407 g (68%) of desired amide intermediate after silica gel chromatography (9:1 CH_2Cl_2/MeOH followed by 9:1:0.5 CH_2Cl_2/MeOH/diisopropylamine): ^{1}H NMR ($CDCl_3$) δ 1.28-1.71 (m, 47 H), 2.33-2.52 (m, 4 H), 3.06-3.27 (m, 12 H), 3.47 (s, 3 H), 3.73 (s, 2 H), 5.24 (s, 2 H), 6.40 (br s, 1 H), 6.84 (d, J = 8 Hz, 1 H), 7.19 (dd, J = 8 Hz, J = 8 Hz, 1 H), 7.39 (s, 1 H), 7.78 (d, J = 8 Hz, 1 H); ^{13}C NMR ($CDCl_3$) δ 25.95, 28.20, 28.48, 29.39, 35.09, 37.56, 38.17, 44.09, 46.75, 47.52, 56.35, 79.34, 79.59, 83.94, 94.61, 106.78, 109.46, 113.70, 119.80, 124.22, 125.78, 137.44, 149.44, 151.36, 156.06, 171.21; HR FABMS observed (M + H) m/z = 877.5626, $C_{45}H_{77}N_6O_{11}$ (req 877.5650). Treatment of this intermediate (0.350 g, 0.0004 mol) with 2-(phenylsulfonyl)-3-phenyloxaziridine (0.230 g, 0.00088 mol) as described in the preparation of hydroxylamine **20** gave 0.274 g (77%) of desired hydroxylamine **29** following silica gel chromatography (50:50 acetone/hexane). This sample contained minor amounts (<5%) of phenyl sulfonamide which was further purified on silica gel (ethyl acetate followed by 50:50 acetone/hexane): ^{1}H NMR ($CDCl_3$) δ 1.17-1.72 (m, 47 H), 2.41 (t, J = 6.7 Hz, 2 H), 2.48 (t, J = 6.3 Hz, 2 H), 3.05-3.29 (m, 12 H), 3.46 (s, 3 H), 3.73 (s, 2 H), 5.25 (s, 2 H), 5.30 (br s, 1 H), 6.29 (br s, 1 H), 6.85 (d, J = 8 Hz, 1 H), 7.20 (dd, J = 8 Hz, 8 Hz, 1 H), 7.39 (s, 1 H), 7.79 (d, J = 8 Hz, 1 H); ^{13}C NMR ($CDCl_3$) δ 25.90, 26.30, 26.95, 28.19, 28.47, 28.77, 29.32, 35.07, 38.23, 44.25, 45.01, 46.96, 54.12, 56.36, 58.04, 58.39, 79.25, 79.57, 83.96, 94.84, 106.97, 109.56, 113.82, 124.24, 125.77, 137.49, 149.40, 151.42, 155.61, 156.04, 171.05; HR FABMS observed (M + H) m/z = 893.5618, $C_{45}H_{77}N_6O_{12}$ (req m/z = 893.5599).

***N*-(16-Amino-4-hydroxy-4,8,13-triazahexadec-1-yl)-4-hydroxy-1*H*-indole-3-acetamide (5).** Treatment of hydroxylamine **29** (166 mg, 0.186 mmol) with a saturated dioxane/HCl solution as described in the preparation of **27** provided 130 mg of crude acid. Crude acid (114 mg) was dissolved in water under an argon purge for 8 h and then freeze-dried as described above for the preparation of **2** to give 77 mg (73%) of Agel 448 (**5**) as its hydrochloride salt: ^{1}H NMR (D_2O) of 1.72-2.09 (m, 10 H), 2.93-3.28 (m, 16 H), 3.72 (q, J_{AB} = 4 Hz, 2 H), 6.51 (m, 1 H), 7.03 (m, 2 H), 7.11 (s, 1 H); ^{13}C NMR (D_2O) δ 21.18, 23.54, 24.05, 24.53, 34.55, 36.53, 37.32, 45.22, 45.32, 47.77, 56.49, 57.08, 104.77, 105.46, 107.64, 116.98, 123.98, 125.00, 139.50, 150.71, 177.72; HR FABMS observed (M + H) m/z = 449.3252, $C_{23}H_{41}N_6O_3$ (req m/z = 449.3240).

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Intramolecular Trapping of the Quinone Methide from Reductive Cleavage of Daunomycin with Oxygen and Nitrogen Nucleophiles

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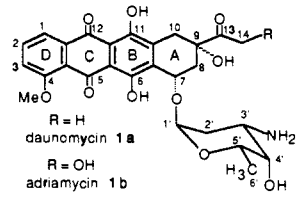
Abstract: Intramolecular trapping with oxygen and nitrogen nucleophilic sites of the quinone methide from reductive cleavage of daunomycin (**1a**) is described. The oxygen and nitrogen nucleophilic sites were located at the 13-position of daunomycin in oxime, semicarbazone, and benzoylhydrazone derivatives. Reduction of daunomycin oxime (**2a**) and semicarbazone (**2b**) in methanol and water with the one-electron reducing agents bi(3,5,5-trimethyl-2-oxomorpholin-3-yl) (TM-3 dimer, **3a**) and bi[3,5-dimethyl-5-(hydroxymethyl)-2-oxomorpholin-3-yl] (DHM-3 dimer, **3b**) yielded cyclooxime **8a** and cyclosemicarbazone **8b** as well as 7-deoxydaunomycinone oxime (**6a**) and 7-deoxydaunomycinone semicarbazone (**6b**), respectively. Product ratios were pH dependent. Cyclooxime but not cyclosemicarbazone was reductively cleaved to the respective 7-deoxy aglycon. Reduction of daunomycin benzoylhydrazone (**2c**) yielded only 7-deoxydaunomycinone benzoylhydrazone (**6c**). Quinone methide intermediates, **5b** and **5c**, were observed by UV-visible spectroscopy. Cyclomer formation is discussed in terms of intramolecular nucleophilic attack at the 7-position of the quinone methide. The lack of cyclomer formation during the reduction of **2c** resulted from the configuration of the benzoylhydrazone functionality, syn to the methyl at the 14-position.

Introduction

Daunomycin (**1a**) and adriamycin (**1b**) are antitumor antibiotics of the anthracycline class,[1] which have been proposed to be bioreductively activated.[2,3] Under aerobic conditions, chemical or enzymatic reduction leads to the catalytic production of toxic reactive oxygen species.[4] Under anaerobic conditions, reduction in one-electron steps leads to formation of a quinone methide via semiquinone and hydroquinone states.[5,6] The quinone methide



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