Inhibition of Protein Kinase Cα by Dequalinium Analogues: Dependence on Linker Length and Geometry

Donghui Qin, Regina Sullivan, William F. Berkowitz, Robert Bittman, and Susan A. Rotenberg*

Department of Chemistry and Biochemistry, Graduate School and University Center, and Queens College of The City University of New York, Flushing, New York 11367

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Analogues of a bipartite compound, dequalinium (DECA) (quinolinium, 1,1′-(1,10-decanediyl)-bis(4-amino-2-methyl diiodide)), were tested for inhibition of protein kinase Cα (PKCα). In vitro assays of monomeric and dimeric analogues support a model in which DECA inhibits PKCα by an obligatory two-point contact, a unique mechanism among PKC inhibitors. The presence of unsaturation in the center of the C_{10}-alkyl linker produced geometric isomers with different inhibitory potencies: *cis* $IC_{50} = 52 \pm 12$ μM and *trans* $IC_{50} = 12 \pm 3$ μM, where the *trans* isomer was equipotent to that of the saturated C_{10}-DECA. DECA analogues with longer, saturated linkers (C_{12}, C_{14}, or C_{16}) exhibited enhanced inhibitory potencies which reached a plateau with the C_{14}-linker ($IC_{50} = 2.6 \pm 0.2$ μM). Metastatic melanoma cells treated with 250 nM C_{12}-, C_{14}-, or C_{16}-DECA and irradiated with long-wave UV light (which causes irreversible inhibition of PKCα by DECA) confirmed the linker-dependent inhibition of intracellular PKCα activity.

Introduction

Dequalinium (DECA) (quinolinium, 1,1′-(1,10-decanediyl)bis(4-amino-2-methyl diiodide)) (**1c** in Table 1) was sold for over 30 years as an antimicrobial agent and was the active ingredient in mouthwash and other topical formulations. Since 1987, DECA has been studied as a potent antitumor agent that recognizes several isoforms (e.g., α, β1) of protein kinase C (PKC) in vitro (IC_{50} = 10−14 μM) and in cells.[1,2] This monomeric serine/threonine protein kinase consists of a family of 11 structurally related isoforms and is well-known for its role in cellular signaling pathways that govern normal cell growth and differentiation.[3] Because of its function in tumor formation and metastasis, PKC continues to attract interest as a target for new chemotherapeutic agents. A potent antitumor agent in several animal models,[4] C_{10}-DECA is accumulated by transformed cells in culture to a greater extent than by nontransformed cells.[5,6] C_{10}-DECA inhibits PKC activity in vitro and in cells at low micromolar concentrations[1,2] by interfering with binding sites in both the regulatory[1,2] and catalytic domains.[2,7] Inhibition of catalytic activity involves a site or sites located in the catalytic domain of PKCα,[2,7] but an additional C_{10}-DECA recognition site in the regulatory domain involving the RACK-1 (*R*eceptor for *A*ctivated *C*-*K*inase) binding domain has also been demonstrated.[1]

A series of PKCα mutants that represented progressive truncation from the amino-terminus (regulatory domain) were tested for sensitivity to inhibition to C_{10}-DECA in order to examine the importance of the regulatory domain to C_{10}-DECA-mediated inhibition of catalytic activity. That analysis revealed that the sites of interaction in the regulatory and catalytic domains are independent of each other and that the site at which C_{10}-DECA produces inhibition of catalytic activity lies exclusively in the catalytic domain.[7] Other structurally homologous protein kinases that were tested for inhibition and found to be insensitive to micromolar DECA concentrations are the cAMP-dependent protein kinase (PKA), the calmodulin-dependent myosin light chain kinase, and the $pp60^{src}$ tyrosine protein kinase (S. A. Rotenberg, unpublished data). The finding that PKA is insensitive to DECA at high micromolar concentrations is compelling in view of the close sequence homology of the PKC and PKA catalytic domains. Additional cellular targets of C_{10}-DECA have been reported, namely the mitochondrial F1-ATPase,[8] the calmodulin-dependent phosphodiesterase,[9] and the calcium-activated K^+-channel.[10]

An unusual property of DECA is that it can be photolyzed with long-wave UV light which converts it into an irreversible inhibitor of PKC activity.[1] Photoreactivity was previously employed to demonstrate inactivation of recombinant PKCα activity in vitro and PKCα translocation in cells.[1] It is used in the present work to demonstrate the improved PKC-inhibitory action of DECA analogues in intact cells.

C_{10}-DECA consists of two quinolinium moieties linked by a 10-carbon methylene bridge (Figure 1A). An earlier examination of structure−activity relationships with DECA analogues revealed certain structural attributes of this compound that were critical for inhibition of PKC activity.[2] For example, decreasing the alkyl linker to 8 or 6 carbons proportionately weakened the PKC inhibitory potency of the resulting DECA analogue. In the present work, results obtained with DECA analogues with longer alkyl linkers were tested for enhanced inhibition of PKCα activity both in vitro and in metastatic melanoma cells. To account for the dependence of inhibition on the presence of the linker and its precise length, a two-site binding model is presented.

Results

Inhibition of PKCα by Monomeric and Dimeric Analogues of DECA. The parent DECA molecule

* Corresponding author. Tel: (718) 997-4133. Fax: (718) 997-5531. E-mail: susan_rotenberg@qc.edu.

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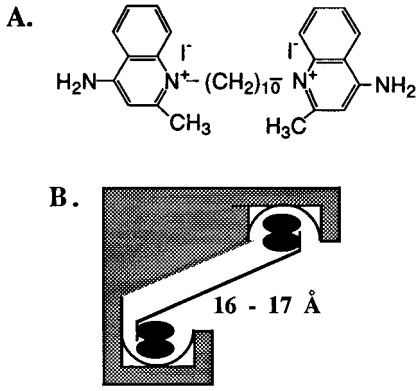
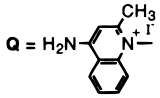
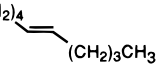
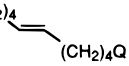
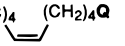


Figure 1. (A) Chemical structure of DECA diiodide (C_{10}-DECA). (B) Scheme depicting a two-point binding model for C_{14}-DECA that binds with a *trans*-oid geometry to the PKC catalytic domain.

Table 1. Linker Distance and Geometry of DECA Analogues Determine Inhibitory Potency with PKCα[a]



Compound		IC50 (μM)[b]	Spacer (Å)[c]	
1a	$Q(CH_2)_6Q$	54 ± 8	6.4	
1b	$Q(CH_2)_8Q$	25 ± 9	8.8	
1c	$Q(CH_2)_{10}Q$	11 ± 5	11.5	parent compound
1d	$Q(CH_2)_{12}Q$	5 ± 2	14.0	
1e	$Q(CH_2)_{14}Q$	2.6 ± 0.2	16.6	
1f	$Q(CH_2)_{16}Q$	2.8 ± 0.2	19.1	
2	$Q(CH_2)_9CH_3$	117 ± 8	---	monomer
3	QCH_3	3590 ± 510	---	monomer
4	$Q(CH_2)_4$  $(CH_2)_3CH_3$	137 ± 6	---	monomer
5	$Q(CH_2)_4$  $(CH_2)_4Q$	12 ± 3	11.1	
6	$Q(H_2C)_4$  $(CH_2)_4Q$	52 ±12	10.5	

[a] Assays were performed under conditions of reversible binding as described in the Experimental Section. [b] IC_{50} represents the analogue concentration producing 50% inhibition. Each IC_{50} value is the average of two or more independent experiments (±SD), where each experiment consisted of triplicate measurements of PKCα activity. [c] For each alkyl linker consisting of *n* carbons, the distance (*d*) between the terminal alkane carbons was measured from energy-minimized structures with PCModel v6.0 and using standard bond length values where $d = (n - 1)1.25$ Å.

contains a 10-carbon alkyl linker that bridges two aromatic moieties (Figure 1A; C_{10}-DECA, **1c** in Table 1). Based on previous observations[2] with C_{10}-DECA, it was hypothesized that the two aromatic moieties inhibit PKCα activity by coincident contact with two nonoverlapping target sites in the catalytic domain. A two-site binding model[11] predicts that if binding by both aromatic moieties (A, B) in the dimer is critical to enzyme inhibition, then the presence of the linker should produce a lower IC_{50} value (equivalent to a lower K_d) than would be measured for a monomer that binds coincidently to two sites. Experimental results given in Table 1 indicate that the $IC_{50} = 11 \pm 5$ μM for the C_{10}-dimer (**1c**) is in fact >300-fold lower than that observed for the C_1-monomer (**2**) for which $IC_{50} = 3590 \pm 510$ μM. The higher potency of the C_{10}-dimer signified that the C_{10}-linker contributes a significant entropic advantage to the dimer for coincident binding of the aromatic moieties at their respective target sites (see Discussion). A noteworthy secondary observation is that the linker itself contributes somewhat to the binding of the individual aromatic moieties, as shown by the improved potency of the C_{10}-monomer when compared with that of the C_1-monomer, compounds **2** and **3**, respectively.

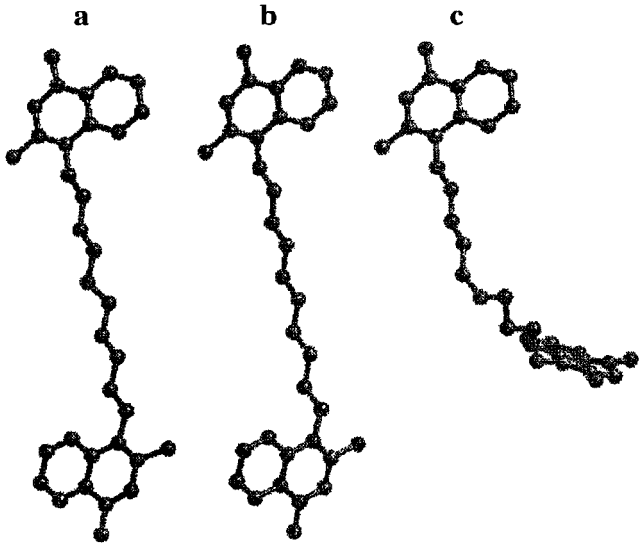


Figure 2. Conformations of geometric isomers of C_{10}-DECA. Energy-minimized conformations of (a) saturated C_{10}-DECA ($H_f = 258$ kcal/mol), (b) the *trans* isomer ($H_f = 288$ kcal/mol), and (c) the *cis* isomer ($H_f = 291$ kcal/mol). Structures were generated with PCModel v6.0 (Serena software).

The nature of the linker contribution to PKCα inhibition by C_{10}-DECA was next investigated in terms of its geometry and length.

Effect of Linker Geometry on PKCα Inhibition. To explore the spatial requirements required for potent interaction of the C_{10}-dimer (**1c**) with its proposed tandem target sites in PKCα, analogues (**5** and **6**) were synthesized that bear a single site of unsaturation in the center of a C_{10}-linker (Table 1) and were assayed for concentration-dependent inhibition of PKCα. The *trans* isomer **5** was the more potent ($IC_{50} = 12$ μM) and was indistinguishable from the saturated parent compound **1c**, whereas the IC_{50} value for the *cis* isomer **6** was 52 μM. It was noted that the *trans* geometry of the linker probably does not itself contribute to potency since the *trans*-5-decene C_{10}-monomer (**4**) was no more potent than the saturated C_{10}-monomer (**2**) (Table 1).

The disparate inhibitory potencies observed for the *trans* and *cis* unsaturated dimers (**5** and **6**) suggest that the geometry of the linker may affect correct positioning of the aromatic rings with the enzyme. Comparison of energy-minimized structures of the saturated C_{10}-DECA with the *cis* and *trans* isomers (Figure 3) indicates that the *cis* conformation is significantly different from the similar conformations obtained for the saturated and *trans* compounds. Because the *cis* isomer exhibits a pyridinium−pyridinium distance (12.7 Å) that is only 1 Å shorter than the analogous distance in the *trans* isomer (13.7 Å) and saturated C_{10}-DECA (14 Å), the lower inhibitory potency observed for the *cis* isomer could be the outcome of an altered spatial relationship of the quinolinium ring moieties.

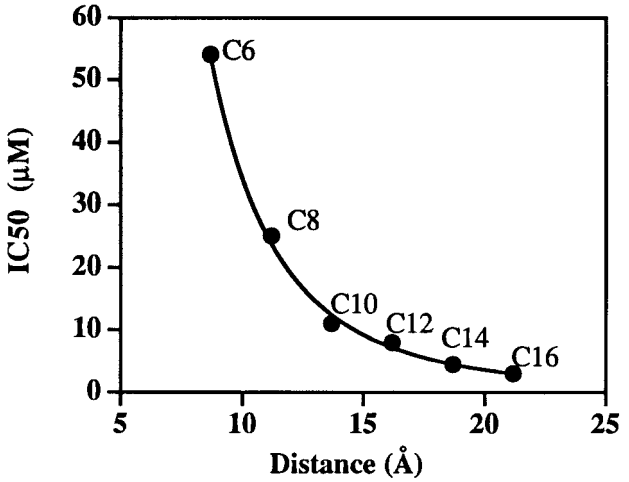


Figure 3. Correlation of linker length with PKCα inhibition. Inhibitory potency (IC_{50}) was measured for each analogue with PKCα from the dose−response curves reported in Table 1.

Effect of Linker Length on PKCα Inhibition. Both PKCα and β1 isoforms (which have 96% sequence homology) are identical in their sensitivity to **1c**.[1] Previously described structure−activity relationships of DECA analogues that had been synthesized with alkyl linkers having lengths of 6, 8, or 10 carbons[2] indicated that inhibition of PKCβ1 was most potent with a C_{10}-linker ($IC_{50} = 14 \pm 4$ μM). In the present study, PKCα inhibition was tested with DECA analogues having C_6-, C_8-, C_{10}-, C_{12}-, C_{14}-, and C_{16}-alkyl linkers (compounds **1a**−**1f**, Table 1). The strictest dependence between linker length and inhibitory potency was observed for analogues **1a**−**1f** representing C_6- to C_{14}-linkers, whereby each stepwise elongation by two carbons (2.5 Å) was accompanied by a 2-fold enhancement of potency (Figure 3). Maximal inhibitory potency ($IC_{50} = 2.6 \pm 0.2$ μM) was achieved with a linker length of C_{14} (**1e**) and C_{16} (**1f**). This value represented a 4-fold stronger inhibitory potency than that of **1c** ($IC_{50} = 11$ μM) and was 20-fold stronger when compared with the value of C_6-DECA (**1a**) ($IC_{50} = 54$ μM; Table 1). These findings underscore the likelihood of coincident contact by both aromatic moieties with distinct target sites on the enzyme that are separated by a distance given by at least 14 carbons. As judged by standard bond lengths, this distance is estimated to be 16−17 Å (Table 1), which is approximately 5 Å longer than the linker present in the parent compound C_{10}-DECA (11−12 Å).

DECA Analogues Inactivate PKC in Murine Melanoma Cells. UV irradiation was successfully employed to inactivate intracellular PKC with DECA in metastatic B16 F10 melanoma cells. PKCα is the only Ca^{2+}- and phospholipid-dependent isoform expressed by these cells, and it is present in high abundance. B16 F10 cells were treated with 250 nM C_{12}-, C_{14}-, or C_{16}-DECA, followed by treatment with long-wave light to photolyze the analogue in situ. Cell lysates were prepared that were partially purified by DEAE-Sephacel chromatography and then assayed for Ca^{2+}/phospholipid-dependent PKC activity. The results (Figure 4) demonstrated that linker-length-dependent inhibition of PKC activity was evident with 250 nM of each analogue, where the highest extent of PKC inhibition was typically 40−60% of the total PKC activity. The pattern of inactivation of intracellular PKC activity

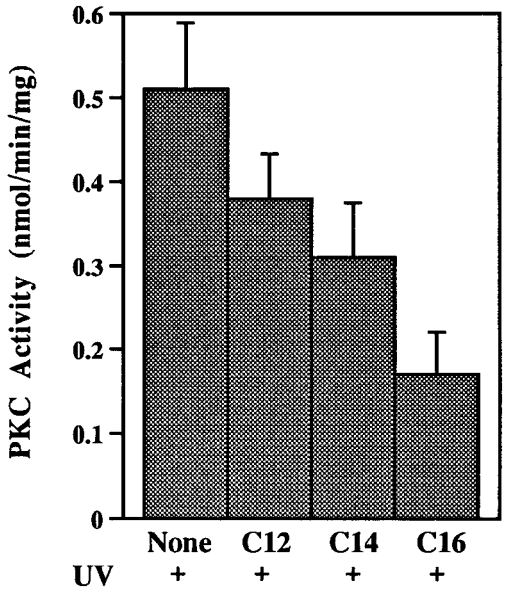


Figure 4. Photoinduced inactivation of intracellular PKCα activity by DECA analogues. PKC activity was isolated from mouse melanoma cells that had been treated for 1 h with DMSO as a control (0.1% v/v), 250 nM C_{12}-, C_{14}-, or C_{16}-DECA analogues, and irradiated for 5 min with long-wave UV light. Each value is the average of triplicate measurements.

reflected the same rank order of inhibitory potencies observed in vitro for these analogues (Table 1). Importantly, this pattern of enhanced inactivation as a function of linker length was shown by others to be absent in in vitro assays of two other potential cellular targets of DECA, namely the calcium-activated K^+ channel[10] and the mitochondrial F1-ATPase.[12] Inhibition of PKC activity by nanomolar concentrations of DECA analogues can be attributed to both the high levels of accumulation of dicationic compounds previously found for B16 F10 cells and other cancer cells[5,6] and the potentiation of inhibition by UV light, as previously described.[1]

Discussion

Within the class of small molecule PKC inhibitors, the bipartite nature of DECA is unique. The proposed two-point interaction by DECA is believed to occur within the catalytic domain[2] and is independent of its interaction with the regulatory domain.[1,7] A working model for the inhibitory interaction of DECA with PKCα depicts the heterocycle moieties of a single DECA molecule bound coincidently to two target sites in the catalytic domain (Figure 1B).

On the basis of a two-site model described by Shuker et al.,[11] the binding affinity of the dimer A−B is defined in terms of the dissociation constant (K_d) of the individual ligands A and B and a term L that represents the contribution of the linker to the binding affinity: $K_d(AB) = K_d(A) \cdot K_d(B) \cdot L$. The value for $K_d(AB)$ can be calculated from the millimolar potency of the C_1-monomer ($IC_{50} = 3.6 \pm 0.5$ mM) as: $[3.6 \times 10^{-3}\ M] \cdot [3.6 \times 10^{-3}\ M] = 12.7 \times 10^{-6}$ M. For $L = 1$, the value for $K_d(AB)$ is within reasonable error of the potency observed for the C_{10}-dimer ($IC_{50} = 11 \pm 5$ μM). In view of the observed dependence on linker length in the present study, L is a variable.Overall, these results indicate that the performance of the dimeric analogues with PKCα

shows dramatic improvement over that of the monomers and is consistent with the predictions of a two-site binding model.

The linker distance between the two aromatic moieties proved to be a critical variable. The potency of inhibition of PKCα with analogues containing C_6- to C_{16}-linkers revealed a length dependency that reached a plateau at $IC_{50} = 2.5\ \mu M$ with C_{14}-DECA (Figure 3), or representing a linker distance of 16−17 Å. This finding suggests that the additional linker distance between the aromatic moieties in C_{14}-DECA facilitates binding to their tandem sites with greater efficiency than the parent compound C_{10}-DECA, which has a linker distance of 11−12 Å. The notion of a two-point contact by DECA compounds may explain the weak or absent inhibition by DECA with other protein kinases such as PKA that have closely related catalytic domains but apparently lack one or both binding sites. The additional observation[2] that C_{10}-DECA is not competitive with ATP underscores the possibility that compared to other protein kinases, DECA binds to a combination of sites that is unique to the PKCα protein.

The divergent inhibitory potencies observed for the *cis* and *trans* C_{10}-linker analogues were not simply the outcome of a slight difference in linker distance. Based on a series of DECA analogues for which the relationship of saturated alkyl linker length (from standard bond lengths) and potency had been established (Table 1), the distance for each unsaturated linker was used to predict its potency with PKCα. If linker distance were the sole determinant of inhibitory potency, then the *cis* compound (**6**), whose linker length (10.5 Å) is close to that of a saturated C_9-analogue (10.2 Å), should have exhibited a potency of approximately 25 μM rather than the observed 52 μM. In contrast, the *trans* isomer (**5**) exhibited both a linker distance (11.1 Å) and an inhibitory potency ($IC_{50} = 12\ \mu M$) that were closely aligned with that of the saturated parent compound **1c** (11.5 Å and $IC_{50} = 11-12\ \mu M$, respectively). The substantial departure between the predicted and observed potencies for the *cis* analogue (**6**) but not for the *trans* analogue argues that the geometry of the linker itself determines the productive binding of the analogue and consequently the extent of enzyme inhibition. Energy-minimized structures of the saturated, *cis* and *trans* analogues (Figure 2) are consistent with the experimental findings and support a model in which the most effective binding mode requires the aromatic moieties to bind the enzyme in a *trans*-oid manner (Figure 1B).

The efficacy of DECA analogues with PKCα in vitro was reproduced in metastatic murine B16 melanoma cells with UV light to render the drug photoreactive which in turn causes irreversible inhibition of PKCα, as previously described.[1] Photoactivation of the drug potentiated the action of DECA in melanoma cells such that intracellular PKC inhibition could be detected with nanomolar concentrations of the drug. As judged by assay of PKC activity partially purified from cell lysates, irreversible inhibition of intracellular PKCα provided the means by which to compare the effects of 250 nM C_{12}-, C_{14}-, and C_{16}-DECA analogues. The improved response to analogues as a result of a longer linker is consistent with the improved sensitivity of PKCα observed in vitro (Table 1). It is notable that other potential cellular targets of C_{10}-DECA such as Ca^{2+}-activated K^+-channels and mitochondrial F1-ATPase have not been found to exhibit inhibition that is dependent on the linker length.[10,12] Moreover, the observed patterned response produced by 250 nM DECA analogues with intracellular PKC is similarly reflected in the inhibition of motility of melanoma cells in vitro.[13] This correlation strengthens a role for PKC as the critical target of DECA and supports a mechanistic role for PKC in cellular metastasis. Our findings further suggest that UV-assisted treatment could offer a therapeutic approach to inhibit metastatic behavior of melanoma cells.

In summary, these studies support a binding model (Figure 1B) in which DECA makes coincident contacts with the enzyme at two discrete sites and in a *trans*-oid conformation. Maximal inhibition of PKC is achieved in vitro with an alkyl linker that is approximately 16−17 Å in length (C_{14}-DECA) which suggests that the two key binding sites in the catalytic domain are separated by this distance.

Experimental Section

Stock solutions for DECA analogues were prepared in DMSO and standardized spectrophotometrically (333 nm) on a Perkin-Elmer Lambda II spectrophotometer using a molar extinction coefficient of 13 500 for monomeric analogues and 27 000 for dimeric analogues.

Assay of PKCα Catalytic Activity in Vitro. Recombinant human PKCα (>95% pure) (PanVera Corp., Madison, WI) was used for testing DECA analogues in vitro. Total PKCα catalytic activity (36 ng protein) and the indicated concentration of DECA analogue were measured in triplicate in the presence of activating cofactors (10 μg of phosphatidylserine, 0.5 mM Ca^{2+}) by the transfer of ^{32}P from [γ-^{32}P]ATP to the modified pseudosubstrate peptide (RFARKGSLRQKNV), as described elsewhere.[1] For assay of partially purified B16 F10 cell lysates (25 μg/assay), PKC activity was taken as the difference in phosphotransferase activity measured in triplicate in the presence and absence of activating cofactors (10 μg of phosphatidylserine, 0.5 mM Ca^{2+}) as previously described.[2] Graphical representations were carried out with Cricketgraph software.

Photoactivation of Cells Treated with DECA Analogues and Isolation of PKC. Murine melanoma B16 F10 cells were cultured in 15-cm^2 dishes to 75−80% confluence in RPMI medium containing 10% fetal bovine serum, 1% penicillin/streptomycin, and 0.125 μg/mL fungizone. Prior to drug application, cells were washed twice with phosphate-buffered saline (PBS) and were restored in serum-free medium containing either DMSO (0.1% v/v) or 250 nM C_{12}-, C_{14}-, or C_{16}-DECA analogue. Monolayers of cells were treated with or without the indicated DECA analogue for 1 h and irradiated for 5 min (1200 μW/cm^2) with a UV lamp (American Ultraviolet Co., Murray Hill, NJ). Following this treatment, the medium was removed and the cells were washed twice with PBS. Preparation of cell lysates was followed by partial purification by DEAE-Sephacel chromatography, as previously described.[2]

Synthetic Methods. Materials. All solvents were distilled before use and dried over sodium benzophenone ketal. Hexamethylphosphoramide (HMPA) was distilled from CaH_2 prior to use. With the exception of 5-hexyn-1-ol (Farchan Laboratories), all reagents were purchased from Aldrich. Silica gel 60 (230−400 ASTM) was used for flash chromatography. TLC was carried out using Merck silica gel 60 aluminum-backed plates. ^1H and ^{13}C NMR were recorded on a Bruker NMR spectrometer at 400 and 100 MHz, respectively. Electrospray ionization-mass spectrometry was carried out by Dr. Clifford Soll at the Hunter College Mass Spectrometry Facility. Elemental analysis was performed by Desert Analytics (Tucson, AZ) and Quantitative Technologies, Inc. (Whitehouse, NJ).

General Synthesis of DECA Analogues. Synthesis of unsaturated linkers for the compounds was carried out by reduction (Lindlar catalyst and Birch reaction, respectively) of the 1,10-alkynediol, which had been prepared by a previously described method.[14] Saturated linkers were commercially available as diols and were converted to diiodides.[15] Alkylation of each alkyl linker by 4-amino-2-methylquinoline (present in 3-fold stoichiometric excess) was carried out in 2−10 mL of 2-butanone at 95 °C for 48 h. The product, which precipitates out of solution as a diiodide salt, was filtered and washed three times with 5−10 mL of 2-butanone.

Characterization of DECA Analogues.[15] Product compounds were analyzed for purity by HPLC on a Waters Delta-Pak C_{18} reverse-phase column (2 × 150 mm, 300 Å), using an acetonitrile gradient (0−60% acetonitrile) in water for 1 h. By this analysis, each product was judged to be >90% pure. As demonstrated by HPLC, the isomeric purity of the *trans* compound **6** was nearly 100%. The *cis* isomer **5** however contained 17% of **6** and was therefore composed of a 5:1 *cis:trans* ratio. Compounds were analyzed by ^{1}H NMR using a Bruker NMR spectrometer at 400 MHz and by low-resolution electrospray ionization-mass spectrometry.

1,1′-[(*E*)-5-Decene-1,10-diyl]bis[4-amino-2-methylquinolinium], diiodide (5): ^{1}H NMR (DMSO-d_6) δ 1.52 (m, 4 H), 1.74 (m, 4 H), 2.10 (m, 4 H), 2.72 (br s, 6 H), 4.46 (m, 4 H), 5.45 (m, 2 H), 6.72 (br s, 2 H), 7.72 (m, 2 H), 8.00 (m, 2 H), 8.14 (m, 2 H), 8.45 (m, 2 H), 8.84 (br s, 4 H). Anal. Calcd for $C_{30}H_{38}N_4I_2$: C, 50.86%; H, 5.41%; N, 7.91%. Found: C, 50.82%; H, 5.30%; N, 6.97%. MS: [M − 2I$^-$] = 227.1 (*m*/*z*) (calcd 227.32 for $C_{30}H_{38}N_4$).

1,1′-[(*Z*)-5-Decene-1,10-diyl]bis[4-amino-2-methylquinolinium], diiodide (6): ^{1}H NMR (DMSO-d_6) δ 1.52 (m, 4 H), 1.74 (m, 4 H), 2.10 (m, 4 H), 2.72 (br s, 6 H), 4.46 (m, 4 H), 5.40 (m, 2 H), 6.72 (br s, 2 H), 7.72 (m, 2 H), 8.00 (m, 2 H), 8.14 (m, 2 H), 8.45 (m, 2 H), 8.84 (br s, 4 H). Anal. Calcd for $C_{30}H_{38}N_4I_2$: C, 50.86%; H, 5.41%; N, 7.91%. Found: C, 50.88%; H, 5.28%; N, 7.76%. MS: [M − 2I$^-$] = 227.1 (*m*/*z*) (calcd 227.32 for $C_{30}H_{38}N_4$).

1,1′-(1,12-Dodecanediyl)bis[4-amino-2-methylquinolinium], diiodide (1d): ^{1}H NMR (DMSO-d_6) δ 1.35 (br s, 8 H), 1.40 (m, 4 H), 1.48 (m, 4 H), 1.73 (m, 4 H), 2.75 (s, 6 H), 3.35 (br s, 4 H), 4.45 (t, J = 7.7 Hz, 4 H), 6.75 (s, 2 H), 7.72 (t, J = 7.7 Hz, 2 H), 8.05 (t, J = 7.7 Hz, 2 H), 8.15 (d, J = 8.0 Hz, 2 H), 8.45 (d, J = 8.0 Hz, 2 H), 8.85 (br s, 4 H). Anal. Calcd for $C_{32}H_{44}N_4I_2$: C, 52.09%; H, 6.01%; N, 7.59%. Found: C, 51.52%; H, 6.08%; N, 7.13%. MS: [M − 2I$^-$] = 242.1 (*m*/*z*) (calcd 242.35 for $C_{32}H_{44}N_4$).

1,1′-(1,14-Tetradecanediyl)bis[4-amino-2-methylquinolinium], diiodide (1e): ^{1}H NMR (DMSO-d_6) δ 1.30 (br s, 8 H), 1.35 (m, 4 H), 1.45 (m, 4 H), 1.75 (m, 4 H), 2.75 (s, 6 H), 3.35 (m, 4 H), 4.47 (m, 4 H), 6.75 (s, 2 H), 7.72 (t, J = 7.7 Hz, 2 H), 8.05 (t, J = 7.7 Hz, 2 H), 8.15 (d, J = 8.0 Hz, 2 H), 8.45 (d, J = 8.0 Hz, 2 H), 8.85 (br s, 4 H); ^{13}C NMR (DMSO-d_6) δ 24.31, 28.57, 30.75, 31.53, 31.71, 31.75, 50.65, 106.61, 106.68, 119.25, 119.31, 121.14, 127.01, 128.61, 136.56, 141.72, 157.72, 159.37, 159.43. Anal. Calcd for $C_{34}H_{48}N_4I_2$: C, 53.27%; H, 6.31%; N, 7.31%. Found: C, 52.93%; H, 6.30%; N, 6.93%. MS: [M − 2I$^-$] = 256.2 (*m*/*z*) (calcd 256.38 for $C_{34}H_{48}N_4$).

1,1′-(1,16-Hexadecanediyl)bis[4-amino-2-methylquinolinium], diiodide (1f): ^{1}H NMR (DMSO-d_6) δ 1.30 (m, 16 H), 1.45 (br s, 4 H), 1.73 (br s, 4 H), 2.74 (s, 6 H), 3.34 (br s, 4 H), 4.46 (m, 4 H), 6.74 (s, 2 H), 7.73 (t, J = 7.5 Hz, 2 H), 8.02 (t, J = 8.2 Hz, 2 H), 8.16 (d, J = 8.9 Hz, 2 H), 8.44 (d, J = 8.2 Hz, 2 H), 8.83 (br s, 4 H); ^{13}C NMR (DMSO-d_6) δ 26.53, 30.80, 32.98, 33.57, 33.93, 34.00, 52.88, 108.85, 108.91, 121.49, 121.55, 123.37, 129.25, 130.84, 139.36, 143.96, 159.96, 161.68. Anal. Calcd for $C_{36}H_{52}N_4I_2$: C, 54.41%; H, 6.59%; N, 7.05%. Found: C, 53.94%; H, 6.68%; N, 6.65%. MS: [M − 2I$^-$] = 270.2 (*m*/*z*) (calcd 270.41 for $C_{36}H_{52}N_4$).

***N*-Decyl-4-aminoquinaldinium Iodide (2).** This compound was prepared by mixing commercially available iododecane and 4-aminoquinaldine in methyl ethyl ketone and refluxing for 72 h. The solid product was collected by filtration and recrystallized twice in absolute ethanol: ^{1}H NMR (in DMSO-d_6) δ 0.85 (t, J = 5.1 Hz, 3H), 1.24 (br, 12H), 1.43 (m, 2H), 1.70 (m, 2H), 2.73 (s, 3H), 4.45 (t, J = 8.09 Hz, 2H), 6.73 (s, 1H), 7.72 (dd, 1H), 8.02 (dd, 1H), 8.15 (d, J = 8.94 Hz, 1H), 8.43 (d, J = 8.37 Hz), 8.81 (br, 2H). Anal. Calcd for $C_{20}H_{31}N_2I$: C, 56.33%; H, 7.33%; N, 6.57%. Found: C, 56.23%; H, 7.41%; N, 6.42%. MS: [M − I$^-$] = 299 (*m*/*z*) (calcd 299.47 for $C_{20}H_{31}N_2$).

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Supporting Information Available: Synthetic methods are detailed along with a listing of NMR spectra and HPLC chromatograms of key new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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(15) See Supporting Information: synthetic methods along with a listing of NMR spectra and HPLC chromatograms of key new compounds.

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