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Synthesis and Electrochemical Evaluation of Conjugates between 2′-Deoxyadenosine and Modified Anthraquinone: Probes for Hole-Transfer Studies in DNA

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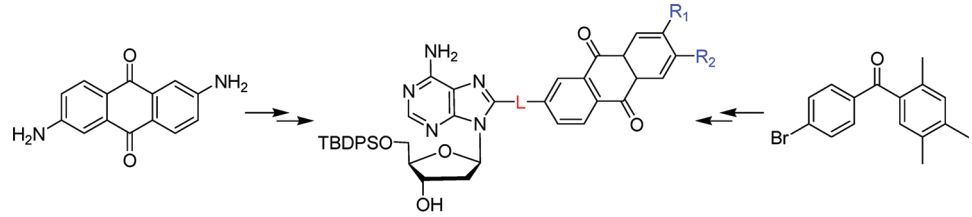

Photoexcitation of anthraquinones (AQ) in association with DNA results in DNA damage mainly at guanine residues, with products from thymine oxidation also observed. Studies of adenine oxidation will be aided by systems with an increased driving force for charge transfer, achieved by adding electron-withdrawing groups to the AQ ring. Attaching AQ derivatives to adenine via a bridge with two carbon atoms should enable the intended regiocontrol within the DNA duplex structure. Herein we report the synthesis of conjugates between AQ and adenine in which the AQ moieties have been modified with a formyl, a trifluoroacetyl, and two methyl ester groups. These have been synthesized by palladium coupling of *tert*-butyldiphenylsilyl 5′-protected 8-ethynyl-2′-deoxyadenosine with the corresponding bromoanthraquinone intermediates. Bromo intermediates bearing formyl or trifluoroacetyl were prepared by monolithiation of 2,6-dibromoanthraquinone, a step that required protection of the anthraquinone carbonyls. A bromo intermediate bearing two methyl ester groups was obtained from 1,2,4-trimethylbenzene by Friedel−Crafts acylation with 4-bromobenzoyl chloride followed by oxidation to the tricarboxylic acid, cyclization to form the anthraquinone ring, and finally esterification. Hydrogenation of the ethynyl linker gave the ethanyl linker. Cyclic voltammetry showed that the conjugate with the two ester groups and ethynyl linker was the most easily reduced of the derivatives synthesized. These derivatives, with reduction potentials favorable for electron transfer, can be used in studies of adenine oxidation in DNA.

Introduction

Electron transfer through the DNA duplex continues to be a field of intense study.[1−6] In many instances, anthraquinone (AQ) has been used as an electron acceptor chromophore to initiate DNA oxidation upon photoexcitation with UV light. In these systems, because the singlet excited state of AQ lives for less than 1 ps,[7] it is the triplet excited state of AQ that gives the charge transfer (CT) product: a radical anion on AQ and a hole on a DNA base. In this aspect, AQ is advantageous over other chromophores in that it can give an initial triplet CT product. All things being equal, a triplet CT product will live considerably longer than a singlet CT product. This would facilitate a higher yield of secondary hole transfer along DNA.[7]

Although adenine can serve as a hole carrier,[8−14] oxidized DNA in general gives final products due to reactions at a G radical cation ($G^{\bullet+}$) site. Recent work on DNA linked to AQ in which the DNA had only adenine (A) and thymine residues (T) found that T formed oxidation products in preference to A.[15,16] Since A is easier to oxidize than T,[17,18] this finding was unexpected. These observations were attributed to the signifi-

[†] Deceased September 4, 2008. We remember him as a careful scholar, dedicated mentor, caring teacher, and close friend.

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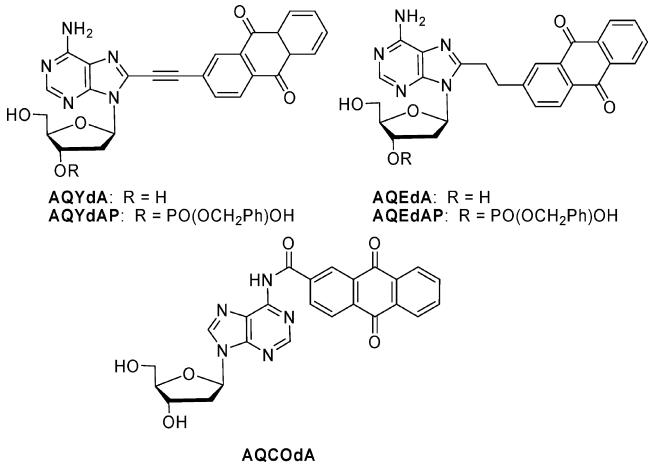
cantly higher reactivity of the T radical cation ($T^{\bullet+}$) compared to that of the A radical cation ($A^{\bullet+}$). Upon photolysis, conjugates with the anthraquinone covalently linked to DNA have also been shown to give products arising from T radical cations.[19]

Our previous work showed that $A^{\bullet+}$ can be formed by photolysis of a system containing both A and AQ.[7] Whether or not an AQ−A conjugate forms the $AQ^{\bullet-}/A^{\bullet+}$ CT photoproduct depends on the details of the system studied. For example, irradiation of the AQ−A conjugates AQYdA or AQEdA in methanol[20] or AQYdAP or AQEdAP[21] in water[22] did not give the $AQ^{\bullet-}/A^{\bullet+}$ photoproduct. On the other hand, photoexcitation of AQCOdA did give the $AQ^{\bullet-}/A^{\bullet+}$ in MeOH and DMSO.[7] In addition, photoexcitation of a bimolecular solution of 2′-deoxyadenosine (dA) and anthraquinone-2-sulfonate in water produced the $AQ^{\bullet-}/A^{\bullet+}$ CT product.[20] One possibility for these observations is that $AQ^{\bullet-}/A^{\bullet+}$ is formed only when the anthraquinone is substituted with an electron-withdrawing group, which would be expected to result in a greater driving force for the CT reaction.



If the position of the initial radical on DNA is to be pinpointed, it is necessary to have the AQ covalently bound to specific positions along the DNA strand. This should minimize side reactions such as oxidation of distant bases and charge recombination during hole migration along the DNA duplex. A variety of AQ derivatives linked to positions along the DNA duplex have been reported in the literature. For example, AQ has been linked to both the C-1′ and the C-2′ of a sugar.[23−26] It has also replaced a base[19,27] or been incorporated into the backbone.[27] AQ has also been covalently attached to both adenine[28] and uridine.[29−33] This last class of derivatives has the greatest potential for creation of a hole at a well-defined position on the DNA duplex provided that the linker between the base and AQ is very short. Long linkers permit a variety of options for the position of AQ with respect to the DNA, including intercalation between adjacent base pairs.[30,34,35]

Future studies in the area of A oxidation would be greatly aided by access to a series of AQ−dA derivatives with short linkers between the AQ and DNA and higher driving forces for CT. Our work and literature studies indicate that these AQ derivatives should have electron-withdrawing substituents on the AQ ring.[19,27,34,36−39] In such systems, the AQ can oxidize its covalently attached A, giving a precise point of initial DNA oxidation. This approach complements chemical production of the $A^{\bullet+}$.[8,9]

Herein, we report the synthesis of AQ−dA conjugates (**1**−**4**) bearing formyl, trifluoroacetyl, or methyl ester groups as carbonyl-containing substituents. In view of the probable importance of the linker in studies of AQ−A conjugates, the diester has been prepared with both ethanyl and ethynyl linkers. To place the anthraquinone in the major groove, we attached it to the 8-position of the adenine. Literature studies indicate that attachment of groups in this position may lead to slight destabilization of the DNA duplex.[28,40−44] Photolyses of DNA

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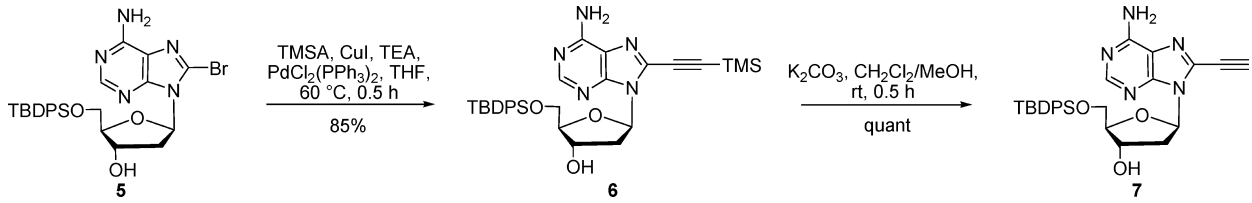
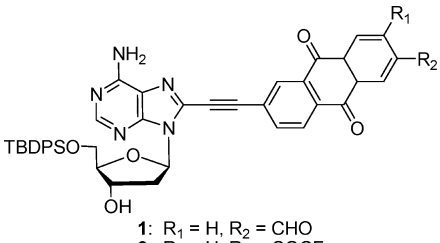
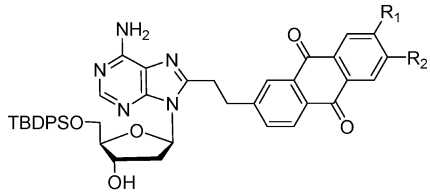
SCHEME 1



duplexes with donors conjugated to the bases through an alkyne group have been shown to give charge transfer products.[45] Electrochemical potentials of these conjugates were measured in acetonitrile.



1: R_1 = H, R_2 = CHO
2: R_1 = H, R_2 = $COCF_3$
3: R_1 = R_2 = CO_2Me



4: R_1 = R_2 = CO_2Me

Results and Discussion

Our choice of the substituents to modify AQ was based not only on the feasibility of their preparations, but also on the compatibility of such substituents toward a variety of conditions used in the conventional DNA synthesis protocols. The final target conjugates also required protection of at least one hydroxyl on dA to improve solubility in acetonitrile to the necessary concentrations for electrochemical measurements. Hence, dA 5′-protected with a *tert*-butyldiphenylsilyl (TBDPS) group was used as the starting material. This protection was also important to increase the solubility of the synthetic intermediates.

Synthesis of Deoxyadenosine Substrate. In our previous work,[21] we joined AQ to dA via an alkyne linker by first allowing 2-iodoanthraquinone to react with trimethylsilylacetylene (TMSA) using Pd-catalyzed cross-coupling chemistry. Deprotection of the trimethylsilyl group on the resulting 2-ethynylanthraquinone permitted a second Pd coupling with 5′-protected 8-bromo-dA to form the desired AQ−dA conjugates. However, working with the ethynylanthraquinone imposed synthetic constraints due to its poor solubility, photoinstability, and the chromatographic similarity of the protected bromo-dA starting material and AQ−dA products.

Therefore, in our present work, we switched the order of the alkynylation steps. 8-Bromo-2′-deoxyadenosine **5**[21] was first coupled with TMSA using Pd-based chemistry as illustrated in Scheme 1 to give the protected alkyne **6**. The yield of this step was greatly affected by the interval between the addition of the reagents. For example, when TMSA was added 2 min after triethylamine (TEA) addition, the yield was 57%. On the other hand, immediate addition of TMSA after TEA improved the yield to 85%. The silyl group on the ethynyl of **6** was removed quantitatively by using K_2CO_3 as an oxide[46] leaving the silyl protection on 5′-O intact to yield the free alkyne **7**. This in turn was Pd-coupled with bromoanthraquinone derivatives bearing a formyl, a trifluoroacetyl, or two methyl ester groups.

Modification of AQ Moiety with a Formyl Group. We chose to introduce the formyl group via lithiation of a bromide on AQ using butyllithium. Because the AQ carbonyls are susceptible to addition of such a strongly nucleophilic reagent, protection of the AQ quinone function was necessary. A second bromide on AQ was needed to couple AQ with the terminal alkyne on **7** using Pd-based coupling. Thus, our synthetic strategy started with 2,6-dibromoanthraquinone. Other strategies based on electrophilic aromatic substitutions[47,48] or Diels−Alder reactions[49] were avoided in order to circumvent obtaining regioisomers. The feasibility of monolithiation of 2,6-dihaloanthraquinone was key in this synthesis.

As illustrated in Scheme 2, the initial step was transformation of 2,6-diaminoanthraquinone (**8**) to the dibromo derivative **9** as described in the literature[41] with minor modifications in the procedure. In general, protection of the quinone functionality is most commonly done by reductive methylation to form the dimethoxyanthracene derivatives.[50−53] However, a number of different modifications of this reaction all gave mixtures with low solubility including a substantial amount of unreacted **9**, which made chromatographic purification troublesome. To solve this problem, use of a larger alkyl group was necessary. Although the literature[53] has reported good yields of reductive propylation of **9** using *n*-propyl bromide, only trace amounts of the propylated product were obtained in our hands. However, use of butyl triflate as a more reactive alkylating agent in the one pot reductive alkylation employing $Na_2S_2O_4$ in phase-

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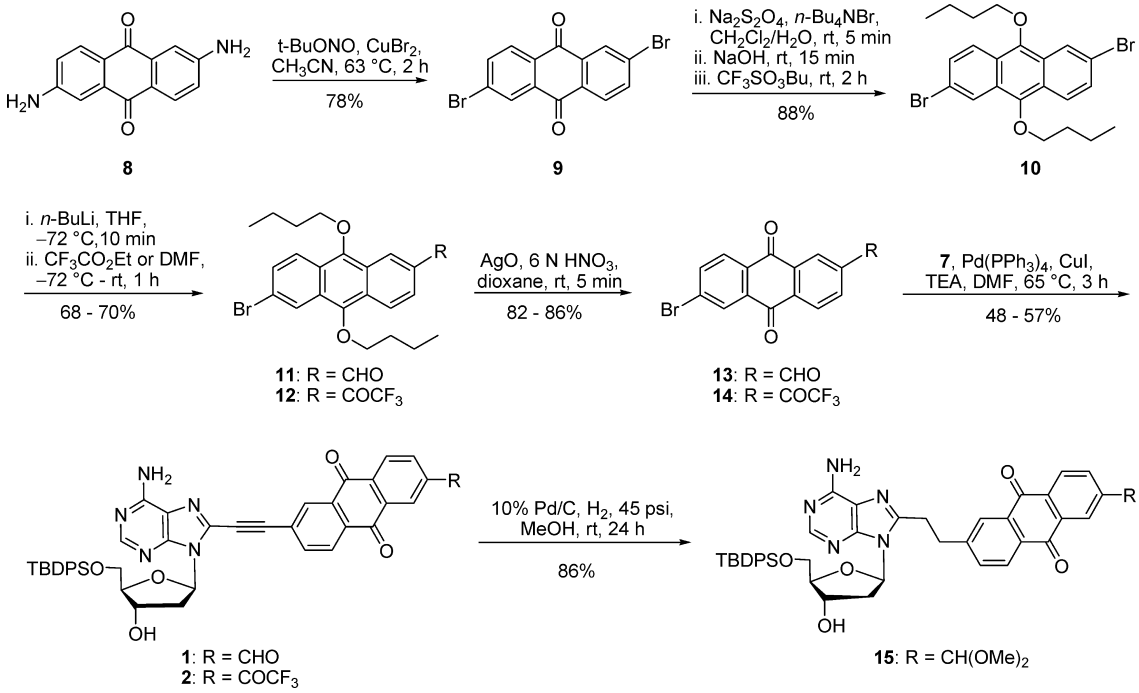
SCHEME 2



transfer catalysis gave the dibutylanthracene derivative **10** in 88% yield. Strict anaerobic conditions were essential to obtain the product in good yield. A solution of the protected **10** in tetrahydrofuran (THF) was successfully monolithiated with 1.05 equiv of *n*-BuLi at −72 °C; subsequent quenching with DMF afforded the desired bromo intermediate **11**. The optimal yield was obtained when 15 mg/mL as a concentration of **10** in THF was used; higher concentrations led to precipitation of **10** when the solution was cooled to −72 °C, resulting in reduced yields of monosubstitution with the formyl group.

Oxidative dealkylation of **11** to the anthraquinone aldehyde **13** was effected using AgO/HNO_3 in dioxane[54] for 5 min or $PhI(OCOCF_3)_2$ in water[55] and THF for 7 h. The former reagent was preferred because filtration and water workup removed all side products and chromatography was not required. The AQ−dA conjugate **1** was obtained in moderate yield via Pd-catalyzed coupling between **13** and **7**, with **13** used in a higher molar ratio than **7** to ease purification. Hydrogenation of **1** using 10% Pd/C in methanol resulted in the reduction of the alkyne linker, with the product isolated as the corresponding dimethyl acetal **15** in 86% yield.

Literature studies indicate that the formyl group can be incorporated into DNA. For instance, the formyl group has been protected before incorporation into DNA, with later acidic hydrolysis to afford an unprotected formyl moiety.[56] The formyl group has also been formed by postsynthetic oxidation of DNA containing 1,2-dihydroxyethyl group with sodium periodate.[57,58] In a third strategy, the formyl group has been introduced into DNA using the phosphorimidite of 3-formylindole 2′-deoxynucleoside directly without protection,[59] indicating that this group can survive DNA synthesis conditions.

Modification of the AQ Moiety with a Trifluoroacetyl Group. The bromo trifluoroacetate **12** was synthesized as described above for **11**, except that ethyl trifluoroacetate was used instead of DMF as the quenching electrophile after lithiation. Oxidative dealkylation of **12** with AgO/HNO_3 gave the desired product **14**, and was preferable to $PhI(OCOCF_3)_2$, which gave substantial amounts of a side product. Compound **14** was obtained as a mixture of the ketone and its water adduct, the geminal diol, in a ratio of 2:1. This was judged by ^{1}H NMR resonances for H-5 at both 8.92 (ketone) and 8.56 (*gem*-diol) ppm, and ^{19}F NMR resonances at both −72.34 (ketone) and −83.90 (*gem*-diol) ppm. $COCF_3$ moieties are known to hydrate.[60−63] Following a protocol in the literature,[64] shaking of **14** in ethyl acetate with a solution of $NaHCO_3$ in water, followed by removal of the ethyl acetate, gave the ketone exclusively as shown by NMR.

Conjugate **2** was synthesized by Pd coupling of anthraquinone **14** to **7** as described above. The product was isolated via silica gel purification using a MeOH/EtOAc/hexane solvent mixture. ^{19}F NMR in $CDCl_3/CD_3OD$ showed a single resonance at −83.57 ppm. The mass spectrum indicated that the majority of the product was the geminal diol (rather than the hemiketal[65] or ketal). Treatment of the diol form of **2** with $NaHCO_3$ as described for **14** did not result in reversion to the ketone form.

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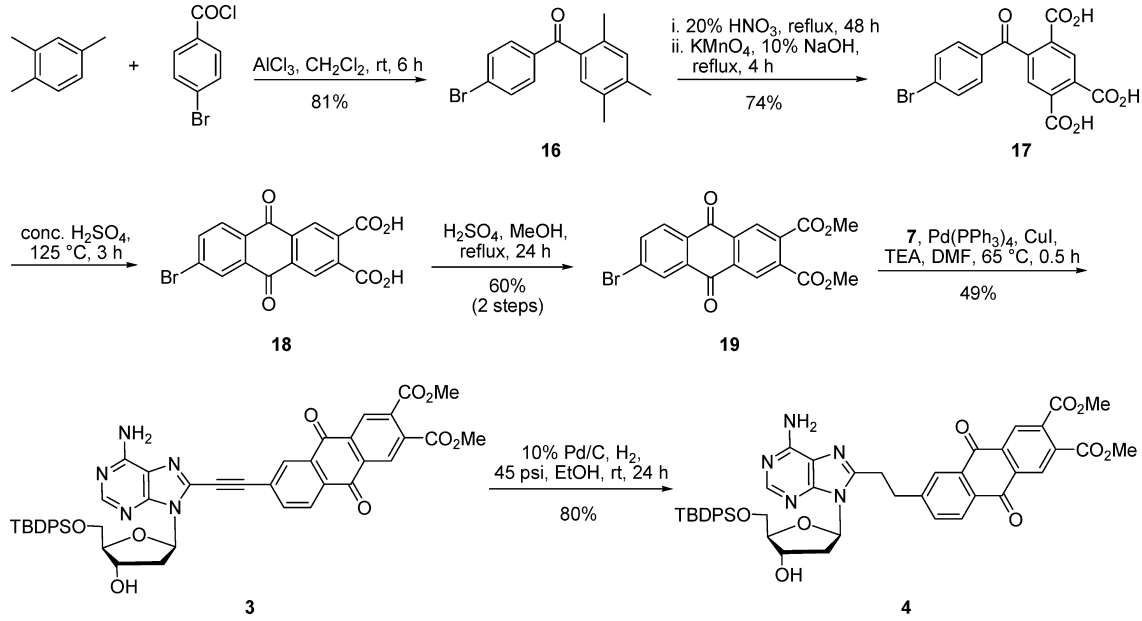
SCHEME 3



However, when conjugate **2** was dissolved in a saturated solution of K_2CO_3 in methanol and stirred overnight, treated with water, and extracted with chloroform, the ketone form was obtained. This was confirmed by the ^{19}F NMR peak at −72.54 ppm and ^1H NMR ($CDCl_3$) peaks at 9.02, 8.53, and 8.49 ppm corresponding to the three protons (H-5, H-8, and H-7, respectively) of the AQ ring bearing the $COCF_3$.

Modification of the AQ Moiety with Two Methyl Ester Groups. 6-Bromoanthraquinone-2,3-dicarboxylic acid (**18**) was the key intermediate for making AQ−dA conjugates modified with two methyl ester groups. As shown in Scheme 3, **18** was prepared analogously to the literature procedure for anthraquinone-2,3-dicarboxylic acid.[66,67] Friedel−Crafts acylation of 1,2,4-trimethylbenzene with 4-bromobenzoyl chloride afforded benzophenone derivative **16**. Following the original procedure, the desired **16** was often contaminated with significant amounts of a byproduct that was difficult to remove by either fractional distillation or chromatography. This byproduct was isolated by crystallization from ethyl acetate and identified by NMR as a methyl homologue of **16** (methyl homologues result from a side reaction common under Friedel−Crafts conditions[68,69]). To increase the yield of **16**, we minimized this side reaction by preliminary mixing of $AlCl_3$ and 4-bromobenzoyl chloride and dropwise addition of 1,2,4-trimethylbenzene at −20 °C. Fractional crystallization of the crude product from ethyl acetate/methanol allowed separation of pure **16**.

The tricarboxylic acid **17** was obtained by refluxing **16** in 20% aqueous HNO_3 to give an alkali-soluble mixture of monocarboxylic acids[70] that was further oxidized with $KMnO_4$ in 4% NaOH. To prevent any possible displacement of the bromide on the product by hydroxide, the crude product mixture was taken to pH ≈ 9 before concentration. Cyclodehydration of **17** was effected by concd H_2SO_4 to give the bromoanthraquinone **18**. Removal of all H_2SO_4 from the product **18** by washing with water was difficult due to its solubility in water. Hence, the crude mixture of **18** was esterified in refluxing methanol using the H_2SO_4 present in this crude material as the catalyst to give the dimethyl ester **19**. Compound **19** in turn was coupled to **7** using Pd-catalyzed chemistry to give conjugate **3** and then hydrogenated in a manner similar to that described above to give conjugate **4**. The conjugates studied are stable to ambient light, air, and water.

TABLE 1. Reduction Potentials of AQ and AQdA Cconjugates in CH_3CN

compd	linker with dA	substituent	$E_{1/2}$ (SCE)
AQ	none	none	−0.939
AQYdA-5′-O-TBDPS	C≡C	none	−0.782
AQEdA-5′-O-TBDPS	CH_2CH_2	none	−0.935
AQCOdA-3′,5′-di-*O*-Ac	amide	amide	−0.734
1	C≡C	CHO	−0.626
3	C≡C	di(CO_2Me)	−0.574
4	CH_2CH_2	di(CO_2Me)	−0.700

Solid-phase DNA synthesis generally employs ammonia in the final deprotection step. Ammonia can react with methyl esters to form amides[71] which can further hydrolyze under basic conditions to the corresponding carboxylic acids.[72] The ammonia step can be replaced by treatment with 0.05 M K_2CO_3 in methanol; the AQ dimethyl ester was stable to these milder conditions.

Electrochemical Results. Our first goal was to examine the effect of electron-withdrawing groups on the reduction potential of the AQ in the conjugate. Diester substitution (**4**) gave a reduction potential of −0.700 V; this disubstitution makes the conjugate easier to reduce than AQEdA-5′-*O*-TBDPS by 235 mV (both compounds with ethanyl linkers, Table 1). Formyl conjugate **1** was more difficult to reduce than the diester

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conjugate **3** by approximately 50 mV (both compounds with ethynyl linkers). Although the formyl group has been reported to be stable on DNA in aqueous solution,[56,57,59,73,74] formyl conjugate **1** showed some degradation on repeated cycles of reduction and reoxidation, indicating that photophysical studies of this derivative might be problematic. Efforts to obtain reproducible reduction potential values for the conjugate **2** with the $C(OH)_2CF_3$ substituent were thwarted by the ease with which this compound converted between the ketone and geminal diol forms under the conditions of the electrochemistry experiments. This has literature precedence in the electrochemistry of trifluoroacetylbenzophenone[75,76] and makes its use in DNA conjugates problematic.

Our second goal was to understand the effect of the linker on the reduction potential of the AQ. The reduction potential of the AQEdA-5′-*O*-TBDPS conjugate, with an ethanyl linker, was −0.935 V, which was the same as that of AQ itself at −0.939 V. Thus, the ethanyl linker seems to isolate the AQ group effectively from the rest of the molecule. Comparison of the ethanyl and the ethynyl linkers showed that the conjugates with the ethynyl linker were more easily reduced. Thus, AQYdA-5′-*O*-TBDPS was easier to reduce than AQEdA-5′-*O*-TBDPS by approximately 150 mV, and **3** was easier to reduce than **4** by approximately 125 mV. The comparative ease of reduction of AQ in the conjugates with the ethynyl linker is consistent with the electron-withdrawing nature of the ethynyl group as well as the extended conjugation in these systems. The extended conjugation is seen spectroscopically in the significantly larger extinction coefficient, and the red shift of the absorbance, for the ethynyl derivative **3** in comparison with the ethanyl derivative **4**. This is also observed for AQYdA in comparison with AQEdA (Supporting Information). Overall, the molecule of choice for future photophysical studies is anthraquinone diester **3**, both because it has the most favorable reduction potential of the molecules studied and because it can undergo cycles of reduction and reoxidation without degradation.

The redox potentials of both the adenine and anthraquinone may alter when studied in DNA in aqueous solution. The reduction potentials of anthraquinones in water are pH dependent, with more negative potentials as the pH increases.[77] Oxidation of adenine is significantly affected not only by pH and medium effects, but also by base pairing and base stacking.[78−81] Thus, the driving force for electron transfer in aqueous solution of these conjugates incorporated into duplex DNA is expected to depend on a variety of factors, including the base sequence studied.

Conclusions

The syntheses of AQ−dA conjugates with the AQ bearing electron-withdrawing formyl, trifluoroacetyl and dimethyl ester groups have been achieved. In these conjugates, AQ was linked to adenine via ethanyl or ethynyl groups. Electrochemical measurements of these conjugates revealed that the diester modification facilitates the reduction of AQ by 235 mV compared with the corresponding unmodified conjugates. In addition, the ethynyl linkers further enhance the reduction potential of the AQ by 125 −150 mV relative to ethanyl linkers. Conjugates of AQ−dA with an ethynyl linker and diester substituents on AQ would satisfy two advances required for achieving better yields for hole transfer in DNA. The first is favorable oxidation of A by virtue of the electron withdrawing nature of the substituent groups. The second is regiocontrol of the DNA secondary structure by the short and rigid linker between AQ and A.

Experimental Section

5′-*O*-*tert*-Butyldiphenylsilyl-8-[(trimethylsilyl)ethynyl]-2′-deoxyadenosine, 6. Compound **5**[21] (2.5 g, 3.4 mmol), $Pd(PPh_3)_2Cl_2$ (154 mg, 0.220 mmol, 0.0500 equiv), and CuI (84 mg, 0.44 mmol, 0.10 equiv) were combined and stirred in dry THF (25 mL) in a glovebox. Triethylamine (TEA) (1.23 mL, 8.80 mmol, 2.00 equiv) was added followed immediately by trimethylsilylacetylene (TMSA) (1.50 mL, 13.2 mmol, 3.00 equiv). The dark brown mixture was removed from the glovebox and stirred under a nitrogen atmosphere at 60 °C in an oil bath for 30 min. The solvent was removed under reduced pressure, and the black foam residue was dried in vacuo at 45 °C for 2 h. The dry crude material was purified two times with silica gel column chromatography. The first column was eluted with $MeOH/CH_2Cl_2$ (0:100−3:97), and the second was eluted with $EtOAc/CH_2Cl_2$ (1:9−1:1). Evaporation of the eluting solvent afforded **6** as a light brown foam (2.20 g, 85% yield). ^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm) 0.03 (9H, s), 1.05 (9H, s), 2.27 (1H, d, $J = 3.3$ Hz), 2.41 (1H, ddd, $J = 4.5$, 7.5, and 13.5 Hz), 3.35−3.44 (1H, m), 3.89 (1H, dd, $J = 4.8$ and 9.6 Hz), 3.99−4.10 (2H, m), 4.97−5.14 (1H, m), 5.66 (2H, br s), 6.52 (1H, dd, $J = 3.3$, 4.5 Hz), 7.26−7.45 (6H, m), 7.61−7.64 (4H, m), and 8.14 (1H, s). ^{13}C NMR (125 MHz, $CDCl_3$): δ (ppm) −0.6, 19.2, 26.9, 37.3, 64.4, 73.4, 84.8, 86.7, 92.5, 103.7, 120.0, 127.7, 129.8, 133.2, 134.3, 135.6, 149.2, 153.6, and 155.1. HRMS (ESI): calcd for $C_{31}H_{40}N_5O_3Si_2$ $[M + H]^+$ 586.2670, found 586.2646.

5′-*O*-*tert*-Butyldiphenylsilyl-8-ethynyl-2′-deoxyadenosine, 7. To a solution of **6** (0.29 g, 0.50 mmol) in CH_2Cl_2/MeOH (1:5 v/v, 6 mL) was added K_2CO_3 (89 mg, 0.64 mmol, 1.5 equiv). The mixture was stirred at rt for 30 min. Water and more CH_2Cl_2 were added, and the mixture was transferred to a separatory funnel. The organic layer was separated, dried with anhyd $MgSO_4$, and evaporated to dryness. The residue was purified by silica gel column eluted with $MeOH/CH_2Cl_2$ (0:100−4:96). Evaporation of the eluting solvent afforded **7** as a white foam (252 mg). ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 0.93 (9H, s), 2.29 (1H, ddd, $J = 4$, 7.2, and 13.6 Hz), 3.29 (1H, s), 3.34−3.42 (1H, m), 3.53 (1H, br s), 3.79 (1H, dd, $J = 5.2$ and 10.4 Hz), 3.93 (1H, dd, $J = 7.6$ and 10.4 Hz), 4.03−4.09 (1H, m), 4.86−4.89 (1H, m), 6.29 (2H, br s), 6.49 (1H, t, $J = 7.2$ Hz), 7.18−7.34 (6H, m), 7.51−7.57 (4H, m), and 8.07 (1H, s). ^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) 19.4, 27.0, 37.1, 64.2, 72.8, 72.9, 84.7, 85.2, 87.3, 127.8, 127.9, 129.9, 129.9, 133.2, 133.5, 133.8, 135.7, 135.7, 149.3, 153.9, and 155.6. HRMS (ESI): calcd for $C_{28}H_{32}N_5O_3Si$ $[M + H]^+$ 514.2274, found 514.2271.

2,6-Dibromo-9,10-di-*n*-butoxyanthracene, 10. A suspension of **9** (3.0 g, 8.1 mmol) and *n*-Bu_4NBr (2.37 g, 7.30 mmol, 0.900 equiv) in CH_2Cl_2 (90 mL) was placed in a Synthware Glass 250-mL round-bottom Schlenk flask with a side arm inlet controlled with a glass stopcock. This Schlenk flask was equipped with a stirring bar and connected to the vacuum line through an adapter with a valve. The contents of the flask were degassed by freezing the suspension and applying vacuum through the adapter until the pressure in the flask

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was ≤5 mTorr. The vacuum was then disconnected, and the mixture was allowed to thaw. The freeze−vacuum−thaw cycle was repeated until the pressure remained constant in the flask when the mixture was frozen. The vacuum adapter was closed and the space in the flask was filled with nitrogen gas through the side arm. Sodium dithionite (4.92 g, 24.3 mmol, 3.00 equiv) was added to frozen degassed water (23 mL) in a separate flask, and then the water−dithionite mixture was degassed again. The resulting solution was added to the suspension of **9** under nitrogen gas via a cannula. After the mixture was stirred for 1 min, a dark green color developed; the mixture was stirred for 5 min at rt. A degassed solution of NaOH (1.65 g, 40.5 mmol, 5.00 equiv) in water (15 mL) was added under nitrogen gas via a cannula. After being stirred for 15 min at rt, the dark red mixture was cooled to 0 °C using an ice−water bath, and *n*-butyl triflate (13.2 mL, 81.0 mmol, 10.0 equiv) was added gradually (over 10 min). The ice bath was removed, and the mixture was stirred at rt for 2 h to give two clear layers with a yellow fluorescent organic layer. Saturated aqueous $NaHCO_3$ was added until the mixture became basic. The organic layer was separated, and the aqueous layer was extracted two times with CH_2Cl_2. The combined organic layers were dried with anhyd $MgSO_4$ and evaporated to dryness. The crude product was dissolved in a minimal amount of hot chloroform, loaded on silica gel and purified using silica gel chromatography on four Biotage columns (40+M cartridge) eluted with CH_2Cl_2/hexane (5:95 v/v). Evaporation of the eluting solvent afforded **10** as bright yellow crystals (3.43 g, 88% yield). Mp: 125−127 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 1.06 (6H, t, J = 7.2 Hz), 1.62−1.72 (4H, m), 1.94−2.02 (4H, m), 4.09 (4H, t, J = 6.4 Hz), 7.49 (2H, dd, J = 2 and 9.2 Hz), 8.09 (2H, d, J = 9.2 Hz) and 8.36 (2H, d, J = 2 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) 14.0, 19.4, 32.6, 76.4, 120.3, 124.1, 124.7, 124.8, 126.1, 129.3, and 147.0. HRMS (ESI): calcd for $C_{22}H_{24}Br_2O_2$ $[M]^+$ 478.0143, found 478.0195.

2-Bromo-6-formyl-9,10-di-*n*-butoxyanthracene, 11. Compound **10** (0.12 g, 0.25 mmol) was dissolved in anhyd THF (8 mL) and cooled to −72 °C (dry ice−ethanol bath). *n*-BuLi (160 μL, 0.27 mmol, 1.1 equiv) was added dropwise (over 5 min), and the homogeneous brown reaction mixture was stirred for an additional 10 min at the same temperature. DMF (0.10 mL, 0.75 mmol, 1.5 equiv) was added, which led to the change of the brown color to yellow. The reaction mixture was stirred for 30 min at −72 °C and for 30 min at rt. A saturated aqueous solution of NH_4Cl was added; this was followed by addition of water and CH_2Cl_2. The organic layer was separated, dried over anhyd $MgSO_4$, and evaporated to dryness. The crude product was applied to a silica gel column and eluted with CH_2Cl_2/hexane (0:100−35:65). Evaporation of the eluting solvent afforded **11** as a bright intense yellow powder (65 mg, 70% yield). Mp: 116−118 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 1.06 (3H, t, J = 7.2 Hz), 1.07 (3H, t, J = 7.2 Hz), 1.63−1.72 (4H, m), 1.95−2.05 (4H, m), 4.09 (2H, t, J = 6.4 Hz), 4.15 (2H, t, J = 6.4 Hz), 7.52 (1H, dd, J = 2 and 9.2 Hz), 7.86 (1H, dd, J = 1.6 and 9.2 Hz), 8.11 (1H, d, J = 9.2 Hz), 8.24 (1H, d, J = 9.2 Hz), 8.39 (1H, d, J = 2 Hz), 8.67 (1H, d, J = 1.6 Hz) and 10.15 (1H, s). ^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) 14.2, 19.6, 19.6, 32.8, 32.9, 76.4, 77.4, 121.5, 122.0, 124.3, 124.3, 124.5, 125.16, 125.18, 127.4, 128.5, 129.6, 132.1, 134.0, 147.0, 150.4, and 192.1. HRMS (EI): calcd for $C_{23}H_{25}BrO_3$ $[M]^+$ 428.0987, found 428.0984.

2-Bromo-6-formylanthraquinone, 13. Solid **11** (74 mg, 0.18 mmol) and AgO (34 mg, 0.27 mmol, 1.5 equiv) were combined and dried in vacuo for 30 min. Anhydrous dioxane (4 mL) was added under nitrogen gas, and the mixture was stirred until **11** was completely soluble (AgO was in suspension). When viewed under long wavelength UV light, the solution was fluorescent. HNO_3 (6 N, 0.36 mL) was added via a syringe and the mixture was stirred at rt for 5 min, by which time all AgO was soluble and the mixture lost its fluorescence. A saturated aqueous solution of $NaHCO_3$ (4 mL) was added and the mixture was stirred for 1 min. Hot EtOAc (120 mL) and water (50 mL) were added, the insoluble material in the mixture was removed by filtration over a layer of Celite and washed with hot EtOAc. The organic layer was separated, dried with anhyd $MgSO_4$, and evaporated. The residue was coevaporated with toluene to afford **13** as a pale yellow powder (54 mg, 82% yield). Mp: 276−278 °C dec. ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 7.95 (1H, dd, J = 2 and 8 Hz), 8.21 (1H, d, J = 8 Hz), 8.28 (1H, dd, J = 1.6 and 8 Hz), 8.46 (1H, d, J = 2 Hz), 8.45 (1H, d, J = 8 Hz), 8.77 (1H, d, J = 1.6 Hz) and 10.22 (1H, s). ^{13}C NMR (100 MHz, DMSO-d_6): δ (ppm) 127.50, 127.53, 128.8, 129.0, 128.6, 131.8, 133.4, 133.5, 136.1, 137.1, 139.8, 180.7, 181.0, and 192.0. HRMS (EI): calcd for $C_{15}H_7BrO_3$ $[M]^+$ 313.9579, found 313.9603.

5′-*O*-*tert*-Butyldiphenylsilyl-8-[(6-formylanthraquinone-2-yl)-ethynyl]-2′-deoxyadenosine, 1. To **13** (43 mg, 0.11 mmol) were added $Pd(PPh_3)_4$ (8.0 mg, 0.01 mmol, 0.05 equiv), CuI (3 mg, 10 μmol, 0.1 equiv), and TEA (0.04 mL, 0.27 mmol, 2.0 equiv). A solution of **7** (67 mg, 0.13 mmol, 0.95 equiv) in anhyd DMF (5 mL) was immediately added to the reaction mixture. The mixture was stirred under a nitrogen atmosphere in the dark at 65 °C in an oil bath for 3 h. DMF was removed under reduced pressure, and the brown residue was purified on a silica gel column eluted with MeOH/CH_2Cl_2 (0:100−3:97). Evaporation of the eluting solvent afforded **1** as a bright yellow glass (55 mg, 57% yield). ε_{355} (THF): 11730 ± 100 L mol^{-1} cm^{-1}. ε_{355} (acetonitrile): 7133 ± 100 L mol^{-1} cm^{-1}. ε_{355} (MeOH): 6907 ± 100 L mol^{-1} cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 1.01 (9H, s), 1.90 (1H, br s), 2.43−2.49 (1H, m), 3.47−3.54 (1H, m), 3.90 (1H, dd, J = 5.2 and 10.4 Hz), 4.03 (1H, dd, J = 8 and 10.4 Hz), 4.12−4.17 [1H, m), 4.99−5.02 (1H, m), 6.33 (2H, br s), 6.57 (1H, t, J = 6.8 Hz), 7.27−7.39 (6H, m), 7.59−7.64 (4H, m), 7.81 (1H, d, J = 7.2 Hz), 8.03 (1H, s), 8.13 (1H, d, J = 7.2 Hz), 8.17 (1H, d, J = 8 Hz), 8.32 (1H, d, J = 8 Hz), 8.46 (1H, s), 8.71 (1H, s) and 10.12 (1H, s). ^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) 19.4, 27.1, 37.1, 64.5, 73.3, 82.0, 85.5, 87.4, 94.1, 119.9, 126.2, 127.4, 128.0, 128.2, 130.1, 131.2, 132.7, 133.0, 133.4, 133.4, 133.7, 134.0, 135.7, 136.2, 136.4, 139.8, 148.9, 155.4, 181.0, 181.6, and 190.6. HRMS (ESI): calcd for $C_{43}H_{38}N_5O_6Si$ $[M + H]^+$ 748.2591, found 748.2627.

4′-Bromo-2,4,5-trimethylbenzophenone, 16. A mixture of 4-bromobenzoyl chloride (6.09 g, 27.7 mmol) and $AlCl_3$ (3.88 g, 29.1 mmol, 1.05 equiv) were dried in vacuo for 1 h. The flask was filled with nitrogen gas and placed in a −20 °C bath (ethanol−ice−salt). Anhydrous CH_2Cl_2 (30 mL) was added, and the mixture was stirred at the same temperature for 10 min. To the resulting homogeneous mixture was added 1,2,4-trimethylbenzene (3.78 mL, 27.8 mmol, 1.00 equiv) dropwise (over a period of 30 min) via a syringe. The ice bath was removed, and the obtained light brown mixture was stirred overnight. The reaction mixture was then poured into a mixture of ice (20 g) and concd HCl (9 mL), which led to discharge of the dark color. CH_2Cl_2 and water were added, and the mixture was transferred to a separatory funnel and shaken. The organic layer was separated, washed with saturated aqueous $NaHCO_3$, dried with anhyd $MgSO_4$, and evaporated to dryness. The white foam obtained was purified by crystallization from EtOAc/MeOH to afford **16** as white needle-like crystals (6.79 g, 81% yield). Mp: 69−71 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 2.21 (3H, s), 2.25 (3H, s), 2.27 (3H, s), 7.04 (1H, s), 7.05 (1H, s), 7.55−7.58 (2H, m) and 7.62−7.65 (2H, m). ^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) 19.4, 19.8, 20.0, 128.2, 130.4, 131.8, 131.9, 132.8, 133.7, 134.8, 135.7, 137.3, 139.9, and 197.8. HRMS (ESI): calcd for $C_{16}H_{16}BrO$ $[M + H]^+$ 303.0385, found 303.0374; for $C_{16}H_{15}BrNaO$ $[M + Na]^+$ 325.0204, found 325.0157.

4′-Bromobenzophenone-2,4,5-tricarboxylic Acid, 17. To a two-neck flask containing **16** (6.619 g, 21.83 mmol) was added 20% HNO_3 (44 mL), and then a condenser was attached. The contents of the flask were heated to 120 °C using oil bath, and the mixture was refluxed for 48 h, by which time a pale yellow semisolid was formed on the bottom. The mixture was cooled to 0 °C for 1 h, and the HNO_3 solution was removed with a pipet. The remaining semisolid was triturated with water (3 × 30 mL) that was also removed with a pipet. Addition of 10% NaOH (50 mL) followed

by H_2O (75 mL) dissolved the semisolid. The resulting dark colored solution was then heated to reflux (110 °C oil bath), and solid $KMnO_4$ (13.8 g, 87.5 mmol, 4.00 equiv) was added in small portions over 1 h (caution: frothing occurs with rapid addition). The reaction mixture was refluxed for additional 3 h, and then the resulting brown solid was removed by filtration while still hot. The collected solid was mixed with water (50 mL) and refluxed overnight, then removed once again by filtration and washed with hot water. The filtrates were combined, treated with solid $NaHCO_3$ (5 g), and reduced in volume to 100 mL by evaporation under reduced pressure. The resulting solution was heated (~ 70 °C) and filtered once more over a layer of Celite while still hot and then treated immediately with conc. HCl until bubbling stopped and pH was about 2 (pH paper). The resulting white precipitate was separated by filtration using a Büchner funnel, washed with water, air-dried, and finally further dried in vacuo to afford **17** as a white solid (6.314 g, 74% yield). Mp: 224−227 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ (ppm) 7.58 (2H, d, J = 8.4 Hz), 7.70 (1H, s), 7.73 (2H, d, J = 8.4 Hz), and 8.27 (1H, s). ^{13}C NMR (100 MHz, DMSO-d_6): δ (ppm) 127.3, 127.7, 130.4, 130.9, 131.3, 132.0, 133.0, 135.4, 137.2, 143.2, 165.5, 167.0, 167.7, and 194.3. HRMS (ESI): calcd for $C_{16}H_8BrO_7$ $[M - H]^-$ 390.9453, found 390.9468.

Dimethyl 6-Bromoanthraquinone-2,3-dicarboxylate, 19. To dry **17** (0.800 g, 2.04 mmol) was added concd H_2SO_4 (3.5 mL) under a nitrogen atmosphere. The resulting homogeneous brown reaction mixture was stirred at 125 °C for 3 h, at which time a precipitate formed in the mixture. After being cooled to rt, the mixture was poured over ice (10 g), and the resulting beige suspension was transferred to two 20-mL centrifuge tubes. Additional water (3.5 mL) was used to transfer all of the crude material. After centrifugation, the supernatant was removed with a pipette, and the remaining paste was lyophilized to give **18**[82] as an oily brown suspension. This suspension was transferred (with methanol) to a 100-mL recovery flask. The methanol was removed under reduced pressure and vacuum was applied overnight. The flask containing crude **18** was flushed with nitrogen gas and a condenser was attached.

The flask containing the crude mixture was flushed with nitrogen gas, and a condenser was attached. Anhydrous methanol (25 mL) was added under nitrogen gas, and the yellow suspension obtained was refluxed (80 °C) for 24 h until TLC (CH_2Cl_2) showed no further progress in the reaction. The mixture was cooled to rt and treated with a saturated aqueous solution of $NaHCO_3$ until the reaction mixture became basic (pH paper); water (20 mL) was then added. The precipitate in the reaction mixture was separated by filtration, washed with water followed by cold methanol, and finally air-dried to afford **19** as a fine yellow powder (487 mg, 60% yield from **17**). Mp: 174−175 °C (lit.[82] mp 178−179 °C). The ^{1}H and ^{13}C NMR matched those in the literature.[82] HRMS (ESI): calcd for $C_{18}H_{12}BrO_6$ $[M + H]^+$ 402.9817, found 402.9802.

5′-*O*-*tert*-Butyldiphenylsilyl-8-[2-(2,3-dicarboxyanthraquinone-6-yl)ethyl]-2′-deoxyadenosine Dimethyl Ester, 4. Compound **3** (54 mg, 0.06 mmol) was dissolved in EtOH (20 mL) by slight heating while stirring. The resulting solution was transferred to a hydrogenation vessel containing 10% Pd/C (25 mg) that had been activated by stirring under H_2 gas (45 psi) in EtOH (10 mL) for 30 min at rt. The vessel was next charged with H_2 gas and then degassed using an aspirator in a cycle that was repeated five to six times. The vessel was finally charged with H_2 gas at 40 psi and stirred at rt for 24 h, by which time TLC ($MeOH/CH_2Cl_2$, 7:93 v/v) showed complete conversion of an initial spot to a more polar spot. The Pd/C catalyst was then removed by filtration over Celite, and the adsorbed nucleoside residue was extracted from the catalyst by washing with boiling 10% MeOH in $CHCl_3$. The crude product was applied to a silica gel column and eluted with $MeOH/CH_2Cl_2$ (0:100−3:97). Evaporation of the eluting solvent afforded **4** as a pale yellow glass (43 mg, 80% yield). ε_{355} (THF): 2553 ± 100 L mol^{-1} cm^{-1}. ε_{355} (acetonitrile): 2907 ± 100 L mol^{-1} cm^{-1}. ε_{355} (MeOH): 2598 ± 100 L mol^{-1} cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 0.97 (9H, s), 2.30 (1H, ddd, J = 2.4, 6.8, and 13.2 Hz), 3.23−3.38 (4H, m), 3.60−3.67 (1H, m), 3.80 (1H, dd, J = 5.2 and 10.4 Hz), 3.92−3.97 (1H, m), 3.98 (6H, s), 4.08−4.12 (1H, m), 4.92−4.95 (1H, m), 5.91 (2H, br s), 6.26 (1H, t, J = 6.8 Hz), 7.24−7.38 (6H, m), 7.55−7.65 (5H, m), 8.06 (1H, s), 8.13 (1H, d, J = 8 Hz), 8.19 (1H, d, J = 1.2 Hz), 8.54 (1H, s) and 8.56 (1H, s). ^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) 19.9, 27.0, 29.2, 33.4, 37.0, 53.4, 64.1, 72.6, 84.4, 87.0, 118.9, 127.6, 127.8, 127.8, 128.1, 128.2, 129.9, 131.7, 133.3, 133.3, 133.4, 134.8, 133.9, 135.1, 135.6, 135.6, 136.5, 136.6, 148.4, 150.9, 152.0, 154.8, 166.7, 166.5, 181.3, and 181.7. HRMS (ESI): calcd for $C_{46}H_{46}N_5O_9Si$ $[M + H]^+$ 840.3065, found 840.3062.

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Supporting Information Available: Experimental procedures for the preparation of **2**, **3**, **9**, **12**, **14**, and **15**; copies of ^{1}H, ^{13}C, and ^{19}F NMR spectra for compounds prepared by the methods described; electrochemical measurement conditions and cyclic voltammetry plots; and UV−vis spectra of conjugates **3**, **4**, AQYdA and AQEdA. This material is available free of charge via the Internet at http://pubs.acs.org.

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