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Reham A. I. Abou-Elkhair

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**SYNTHESIS OF ANTHRAQUINONE DERIVATIVES AND THEIR CONJUGATES
WITH 2'-DEOXYNUCLEOSIDES AS NEW PROBES FOR ELECTRON TRANSFER
STUDIES IN DNA**

by

REHAM A. I. ABOU-ELKHAIR

Under the Direction of Dr. Thomas L. Netzel

ABSTRACT

Anthraquinone (AQ) has been used in electron transfer studies in DNA. The focus of this dissertation is the synthesis of conjugates between AQ derivatives and 2'-deoxyadenosine (dA), which can be used to induce adenine oxidation in DNA. Different AQ derivatives were attached to dA via ethynyl or ethanyl linkers. If incorporated into DNA, these short linkers should enable regiocontrol for electron transfer from adenine within the DNA duplex structure. The challenge in working with anthraquinone-2'-deoxynucleosides conjugates is that they and their intermediates are insoluble in water and only sparingly soluble in most organic solvents. A strategy used to overcome this problem was the use of either *tert*-butyldiphenylsilyl (TBDPS) or 4,4'-dimethoxytrityl (DMTr) 5'-protected deoxynucleosides as starting materials. A water-soluble, ethynyl-linked AQ-dA conjugate with a 3'-benzyl hydrogen phosphate was synthesized

using DMTr protection. The DMTr group was not stable to the hydrogenation required to make the ethanyl-linked AQ-dA conjugate with 3'-benzyl hydrogen phosphate. Hence the latter was synthesized starting with the TBDPS protecting group. Both of these syntheses were based on the Pd coupling between ethynylanthraquinone and 8-bromodeoxyadenosine derivatives. New conjugates between AQ and A, in which the AQ moieties have been modified with formyl, trifluoroacetyl and methyl ester groups as electron withdrawing substituents were also synthesized. The synthesis of these AQ-dA conjugates was based on Pd coupling between bromoanthraquinone and 8-ethynyldeoxyadenosine derivatives. This route avoided the use of ethynylanthraquinone derivatives that had extremely low solubility and photoinstability. Other anthraquinones with electron withdrawing groups (which should provide enhanced driving force to enable respective AQ derivative to oxidize adenine) were synthesized as models. Cyclic voltammetry showed that the conjugate with the two ester groups and ethynyl linker was the most easily reduced of the derivatives synthesized. Conjugates between AQ and dU were also synthesized. Those conjugates can potentially be used to oxidize guanine or adenine or they can be used as a deep trap for an electron in reduced DNA.

INDEX WORDS: Anthraquinone, Electron transfer, Adenine oxidation, 2'-Deoxyadenosine, 2'-Deoxyuridine, Short linkers, *tert*-Butyldiphenylsilyl, 4,4'-Dimethoxytrityl, 3'-Benzyl hydrogen phosphate, Ethynylanthraquinone, 8-Ethynyldeoxyadenosine.

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REHAM A. I. ABOU-ELKHAIR

**A Dissertation Submitted in Partial Fulfillment of the Requirements for the Degree of
Doctor of Philosophy
in the College of Arts and Sciences
Georgia State University**

2008

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Reham A. I. Abou-Elkair
2008

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Electronic Version Approved by:

Office of Graduate Studies
College of Arts and Sciences
Georgia State University
August 2008

**All praise be to the Almighty God, the Cherisher and Sustainer of the worlds, Who granted
me success to achieve this work.**

All what we owe is indeed from Him and belongs to Him.

DEDICATION

To my father

Abdelaziem Ibrahiem Abou-Elkhair

and my children

Shadi, Abdelrahman and Yusuf

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LIST OF ABBREVIATIONS

δ	Chemical shift
(BnO) ₂ PN(<i>i</i> Pr) ₂	Dibenzyl <i>N,N</i> -diisopropylphosphoramidite
(BnO) ₂ POCl	Dibenzyl phosphorochloridate
(EtO) ₂ POCl	Diethyl phosphorochloridate
μ M	Micromolar
μ L	Microliter
4-DMAP	4-(Dimethylamino)pyridine
A	Adenine
A ^{•+}	Adenine radical cation
ACN	Acetonitrile
amu	Atomic mass unit
AQ	Anthraquinone
AQ ^{•-}	Anthraquinone anion radical
AQ*	Excited ³ (π,π^*) state of anthraquinone
AQ2S ⁻	2-Anthraquinone sulfonic acid sodium salt
AQ-8-dU(DMTr)	(<i>E</i>)- <i>N</i> -{2-[3-(5'- <i>O</i> -Dimethoxytrityl-2'-deoxyuridine-5-yl)acrylamido]ethyl}-anthraquinone-2-carboxamide
AQAc	2-Acetylanthraquinone
AQAmBu ^t	<i>N</i> -(<i>tert</i> -Butyl)anthraquinone-2-carboxamide
AQAmMe	<i>N</i> -Methylanthraquinone-2-carboxamide
AQAmPr	<i>N</i> -(<i>n</i> -Propyl)anthraquinone-2-carboxamide
AQBz	2-Benzoylanthraquinone
AQCN	2-Cyanoanthraquinone
AQ-dA	Anthraquinone-2'-deoxyadenosine conjugate
AQ-E-dA	8-[2-(Anthraquinone-2-yl)ethyl]-2'-deoxyadenine
AQ-E-dU(DMTr)	5'- <i>O</i> -Dimethoxytrityl-5-[2-(anthraquinone-2-yl)ethyl]-2'-deoxyuridine
AQNMe ₃ PF ₆	(2-Anthraquinonyl)trimethylammonium hexafluorophosphate
AQNMe ₃ Tf	(2-Anthraquinonyl)trimethylammonium trifluoromethanesulfonate
AQS ⁻	2-Anthraquinone sulfonic acid sodium salt
AQ-Y-dA	8-[(Anthraquinone-2-yl)ethynyl]-2'-deoxyadenine
AQ-Y-dU	5-[(Anthraquinone-2-yl)ethynyl]-2'-deoxyuridine
AQ-Y-dU(DMTr)	5'- <i>O</i> -Dimethoxytrityl-5-[(anthraquinone-2-yl)ethynyl]-2'-deoxyuridine
br	Broad
Bu	Butyl
calcd	Calculated
cm	centimeters
CS	Charge separated
CShf	Charge shift
CT	Charge transfer

cv	Cyclic voltammogram
d	doublet
dA	2'-Deoxyadenosine
DMF	<i>N,N</i> -Dimethylformamide
DMSO	Dimethyl sulfoxide
DMTr	4,4'-dimethoxytrityl
DMTrCl	4,4'-dimethoxytrityl chloride
DNA	Deoxyribonucleic acid
$E_{1/2}$	half-wave potential
EI	Electron impact
equiv	Equivalent(s)
ET	Electron transfer
Et-AQdiester	Dimethyl 6-ethylanthraquinone-2,3-dicarboxylate
EtOAc	Ethyl acetate
g	Gram
G	Guanine
$G^{\bullet+}$	Guanine radical cation
h	Hour(s)
HPLC	High performance liquid chromatography
HRMS	High resolution mass spectrometry
H-Y-AQdiester	Dimethyl 6-ethynylanthraquinone-2,3-dicarboxylate
Hz	Hertz
Im	Imidazole
L	Liter
LR	Low resolution
M	molar
m/z	Mass-to-charge ratio
<i>m</i> -CPBA	<i>meta</i> -Chloroperbenzoic acid
MeCN	Acetonitrile
MeOH	Methanol
Me-tetrazol	5-Methyl-1H-tetrazol
MeTf	Methyl trifluoromethanesulfonate
mg	Milligram
MHz	Megahertz
min	Minute(s)
mL	Milliliter(s)
mmol	Millimole(s)
mol	Mole(s)
MS	Mass spectroscopy
mV	Millivolt(s)
nm	Nanometer(s)
NMR	Nuclear magnetic resonance
ns	Nanosecond(s)
°C	Degree(s) Celsius
$\text{Pd}(\text{Ph}_3\text{P})_2\text{Cl}_2$	Dichlorobis(triphenylphosphine) palladium(II)
$\text{Pd}(\text{Ph}_3\text{P})_4$	Tetrakis(triphenylphosphine) palladium(0)

Pd/C	Palladium on activated carbon catalyst
PhCOCl	Benzoyl chloride
pK _a	The negative logarithm of an acid dissociation constant
ppm	Part per million
Ps	Picosecond(s)
psi	Pound per square inch
Py	Pyridine
q	Quartet
R _f	Retention factor
rt	Room temperature
s	Singlet
SCE	Saturated calomel electrode
SCS	Secondary charge separated
T	Thymine
t	Triplet
TA	Transient absorbance
TBDPS	<i>tert</i> -Butyldiphenylsilyl
TBDPSCI	<i>tert</i> -Butyldiphenylsilyl chloride
TEA	Triethylamine
<i>tert</i> -BuMgCl	<i>tert</i> -Butylmagnesium chloride
Tf	Trifluoromethanesulfonate
TFA	Trifluoroacetic acid
THF	Tetrahydrofuran
TLC	Thin layer chromatography
TMSA	Trimethylsilylacetylene
TMSBr	Trimethylsilyl bromide
TMSCl	Trimethylsilyl chloride
TMS-Y-AQdiester	Dimethyl 6-(trimethylsilylethynyl)anthraquinone-2,3-dicarboxylate
UV-vis	Ultraviolet and visible
V	Volt(s)
v	Volume

Chapter 1

General Introduction

1.1. Biological Significance of Electron Transfer in Oxidized DNA

Deoxyribonucleic acid (DNA) stores the genetic information of cells and controls the production of proteins and is thus responsible for the biochemistry of an organism. Interaction of DNA with various oxidants could lead to its damage.¹⁻³ Because guanine (G) is the most easily oxidized DNA base,^{4,5} 8-oxoguanine (8oxoG) that results from the oxidation of guanine (G) is the most abundant DNA oxidative damage.^{6,7} Accumulation of 8oxoG in the DNA could contribute to aging, apoptosis and mutations that can lead to unregulated cell division and cancer.^{3,8-11}

Experiments have shown that charges can migrate over long distances along the DNA double helix.¹²⁻¹⁷ This process is facilitated by the π -stacked array of its aromatic heterocyclic base pairs that are only 3.4 Å apart. This phenomenon has a role in DNA oxidative damage. For example, a radical cation (hole) formed initially on other nucleobases may undergo migration through the bases until it is trapped on G, which has the lowest oxidation potential of four DNA bases. GG or GGG sequences have even lower oxidation potentials than single Gs.¹⁸ This promotes the hole transfer to those G clusters by one electron migration from G clusters to the single G^{•+} (or hole migration from the single G to the G cluster). This process is referred to as electron transfer (ET) or hole transfer. The resulting G^{•+} in the G cluster is relatively long lived (millisecond to second timescale) in the absence of reducing agents,¹⁹ which further promotes formation of 8oxoG at G clusters.

1.2. Mechanisms for Hole Migration in DNA

Many studies have been conducted in order to investigate the mechanistic details of ET in DNA.^{14,15,18,20-27} Depending on the coupling strength between different base pairs and the energetics of the system one of the two following mechanisms will prevail.

1. *The Tunneling Mechanism:* also described as *charge transfer*, is a single step that is strongly distance dependent and favored in case of endergonic DNA bridge oxidation. This occurs when the energy of the DNA orbitals are higher than the donor and the acceptor, where the charge tunnels through the DNA bases that acts as a bridge without occupying it. A typical case of this process is the hole transfer between G's in duplex oligomers containing small stretches of A•T base pairs.
2. *The Hopping Mechanism:* also described as *charge transport*, is a multistep process that can occur over long distances (up to 200 Å). In this case the process is weakly distance dependent and is favored in the case of exergonic bridge oxidation. This occurs when the donor and acceptor orbitals are close in energy from that of the bridge, and the charge transiently occupies the bridge orbitals. In this case DNA bases themselves can act as the charge carriers.

1.3. General Background on Hole Transfer Studies in DNA

The migration of both positive charges (hole) and negative charges (excess electron) in DNA has been studied.²⁸ Many studies were focused on hole transfer because of its biological relevance in DNA oxidative damage. In those studies DNA was modified with electron accepting moieties in different ways. One class of those modifications involved incorporation of photosensitizers.^{17,29} In this case an electron is transferred directly from the DNA to the excited

photosensitizer, depending on the redox potential of both of the sensitizer and the interacting DNA base. This creates a hole that can migrate to distant sites along the DNA. The ease of oxidation of DNA bases is in the order of $G > A > C > T$.^{4,5} Even though in oxidized DNA both G or A can act as the charge carriers,³⁰ many studies of this type were focused on monitoring the process of hole transfer by the detection of DNA damage at G residues rather than A residues. This is attributed to the higher reactivity of the G radical cation ($G^{\bullet+}$)³¹ in addition to its stability. When $G^{\bullet+}$ is formed in duplex DNA, it can be easily trapped by water to form 8-oxoG in the steps shown in Figure 1.1.

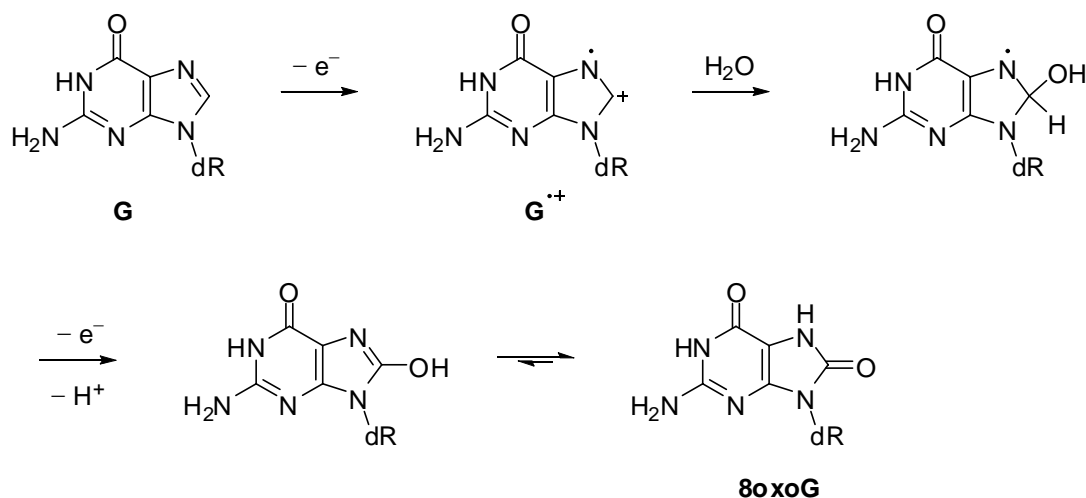


Figure 1.1. Steps involved in the formation of 8oxoG.

1.4. Oxidation of Adenine and its Role in Hole Migration in DNA

1.4.1. Reactivity of Adenine Radical Cation

Among the four DNA bases, A has the second lowest ionization potential after G, and experiments have shown that both G and A can be charge carriers as discussed above. However, in contrast to $G^{\bullet+}$, trapping of $A^{\bullet+}$ by water is insignificant, because it occurs through a transition

state that is much higher in energy than that in case of G^{*+} .³² Recent experiments have studied the oxidation of DNA duplexes containing only adenine and thymine (T).³³ One of these duplexes contained a sequence of five consecutive As and was modified with an anthraquinone (AQ) covalently attached to the sugar at one end (AQ-End, Figure 1.2) and a ^{32}P label at the 5' end of the complementary strand (3'-AATTAATTAAAAATTAATTATAT*-5'). It was expected that photolysis of this duplex would result in cleavage at the consecutive A sequence. However, cleavage was observed primarily at the four TT sequences, with cleavage decreasing with increasing distance between the AQ and the TT site. Introduction of a GG sequence in a related duplex that contained four TTT separated by AA sequences stopped the cleavage at T and lead to reaction at GG even though this sequence was beyond the farthest TTT farthest from the AQ. Replacing T with uridine (whose structure differs from T by only the absence of the methyl group) in a duplex with no Gs showed no cleavage. The authors proposed that trapping of the radical cation of T happens much more readily than trapping of A^{*+} . Thus, even though A^{*+} should be more readily formed by oxidation, products from oxidation of T are observed.

In another study on photocleavage of DNA duplexes comprised of all four DNA bases, AQ was linked to the backbone of one strand either at its 5'-end or in the middle.³⁴ The AQ moiety was either adjacent to A or flanked between two As; the complementary strand contained GG sequences and Ts complementary to As. Photocleavage resulted in products due to reaction at the GG sequences and new products that were obtained in a comparable yield, depending on the position of AQ in the duplex. These new products were identified as due to interstrand cross-links between AQ and T residues (mainly the 3'-T), which arise from the reaction between the semi-quinone radicals resulting from AQ reduction and the methyl radical in T^{*+} . This study and the one above clearly demonstrated that G^{*+} is much more reactive than A^{*+} and that it is the

reactivity of the radical cation of the DNA base that determines the cleavage site, but not its stability.

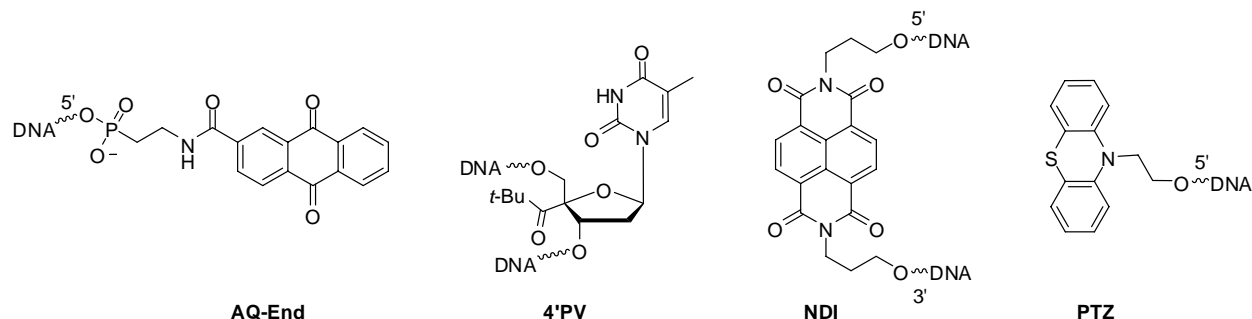


Figure 1.2. Structural drawings of residues used to study hole transfer in DNA.

1.4.2. Hole Hopping Among Adenines

Giese and co-workers have studied the mechanism for hole transfer from G^{++} to a GGG sequence through a number of A•T base pairs in DNA modified with 4'-pivaloyl (4'PV, Figure 1.2).^{30,35} When the A•T bridge was short (up to three A•T base pairs), then an increase in the length of the bridge caused a dramatic decrease in the hole transfer efficiency, ascribed to a tunneling process. When G^{++} and GGG were separated by four or more A•T base pairs the hole transfer rate became independent of bridge length. This was explained as a situation where the rate of hole transfer from G^{++} to A exceeded the rate of tunneling-mediated hole transfer to GGG. Nearest neighbor hole transfer among A's was even faster than G^{++} to A transfer. These studies provided evidence that a hole can migrate in oxidized DNA by A-hopping.

1.4.3. Importance of Adenine Oxidation in Creating Long Lived Charge Separated State and Improving the Yield of Hole Transfer

Majima and co-workers have investigated the effect of A-hopping through consecutive adenine sequences using laser flash photolysis experiments.³⁶ In their system both naphthaldiimide (NDI) as an electron acceptor and phenothiazine (PTZ) as a donor were conjugated to DNA (Figure 1.2). Photoexcitation of NDI at 355-nm lead to the reduction of NDI and formation of a hole on DNA that migrated rapidly to PTZ. The charge separation (CS) and charge recombination (CR) between NDI and PTZ were observed by monitoring the formation and decay of the transient absorption of $\text{NDI}^{\cdot-}$. The yields of the CS via hole transfer through the consecutive A-hopping were slightly dependent on the number of A bases between the NDI and PTZ, while the CR rate was strongly dependent on the distance. This suggested that CS occurred by hopping while CR occurred by tunneling. Increasing the number of consecutive As slowed down CR but did not greatly affect CS. The charge-separated state persisted over 300 μs when NDI was separated from PTZ by eight A bases. In this experiment, long stretches of As provided a spatial separation for the charges. However, the yield of this transfer was low ($\sim 2\%$), due to the rapid initial CR between $\text{NDI}^{\cdot-}$ and adjacent $\text{A}^{\cdot+}$. The same study also found that the charge separation yields were dramatically decreased by interruption of the consecutive A sequence with a G, presumably because the hole was trapped on G preventing it from further migration along the DNA. A similar decrease in charge separation yield was observed when the consecutive As were changed to AT repeats, interpreted as less efficient interstrand A-hopping compared to the intrastrand process. This idea was also supported by looking at the effect of flanking sequences on the redox active drugs in the DNA CT reaction; charge transport was more efficient through $(\text{AA})_n$ than $(\text{AT})_n$ series of the same length.³⁷

Majima and co-workers also studied the role of A oxidation by comparing the yields of hole transfer using four different imide derivatives as photosensitizers (Sens) in the presence and absence of a consecutive A stretch in DNA.³⁸ The consumption of G in this DNA was quantified as a measure of the yield; the farther away the $G^{•+}$ from the $Sens^{*-}$, the longer the lifetime of the radical cation and the greater the chance of its reaction to give products. The Sens used were anionic naphthalindiimide phosphate derivative (NDIP) that can oxidize A while unbound to DNA, two cationic Sens that bind to DNA and can oxidize A and one anionic Sens that does not bind to DNA and can oxidize only G. Bound Sens oxidize DNA from their triplet excited state and unbound Sens react from their singlet excited state. In the case of derivatives that could oxidize A from the singlet state, DNA damage yield increased as the length of the consecutive A stretch increased. This was consistent with hole hopping between adjacent As, with final formation of the $G^{•+}$. With the derivative that oxidizes only G, the yield was moderate and there was no preference for a consecutive A stretch. Interestingly, the most efficient hole transfer occurred from the anionic NDIP even though it was not bound to DNA. It appears that using a photo-oxidant that is capable of oxidizing A from the triplet state allows the formation of an initial triplet charge separated state ($NDIP^{*-}/A^{•+}$), which is relatively long lived because charge recombination is spin forbidden.³⁹

1.5. Anthraquinone (AQ) as DNA Photo-oxidant via Adenine Oxidation

To study hole transfer DNA in a high yield it may be essential to start the oxidation at A using a strategy that will produce a sufficiently long lived initial $A^{•+}$ photoproduct. One way to achieve this goal is to obtain this photoproduct in the triplet excited state. Linking the photo-oxidant to A will provide regiocontrol that is expected to diminish direct oxidation of other DNA bases by the photo-oxidant in the duplex under study. The shorter and the more rigid the linker,

the better this regiocontrol should be. AQ derivatives with the proper redox properties would provide an excellent tool to form a primary $AQ^{\bullet-}/A^{++}$ charge transfer product in the triplet state. The AQ moiety is a particularly good choice because when excited it intersystem crosses very rapidly (< 600 fs)³⁹ to give the triplet state. Hence, even when it is covalently attached to A, it is expected to react from the triplet state rather than the singlet state to form $AQ^{\bullet-}/A^{++}$ in the triplet state. Therefore, the research in this dissertation was focused on the synthesis of conjugates between AQ and 2'-deoxyadenosine (dA) with ethynyl and ethanyl linkers where AQ was attached to the C-8 of dA. Linking AQ to the C-8 of dA would allow AQ to be pointing into the major groove if the conjugate is to be incorporated into DNA.

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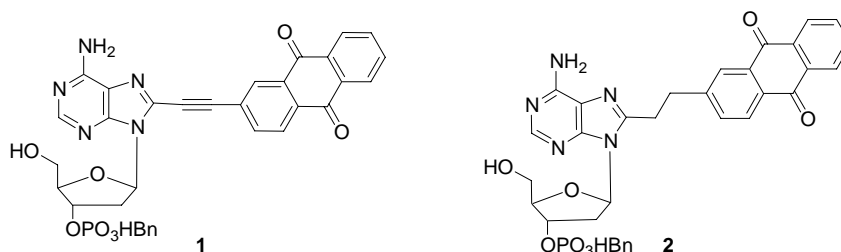
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Chapter 2

Synthesis of Two 8-[(Anthraquinone-2-yl)-linked]-2'-Deoxyadenosine 3'-Benzyl Hydrogen Phosphates for Studies of Photoinduced Hole Transport in DNA

This chapter is a reprint of a paper published in *Nucleosides, Nucleotides & Nucleic Acids* © 2005 Taylor & Francis, Inc. I did all of the experimental work, as well as most of the writing.

(Reference: R. A. I. Abou-Elkhair and T. L. Netzel, *Nucleosides, Nucleotides & Nucleic Acids*, **2005**, *24*, 85-110).



2.1. Abstract

The challenge in working with anthraquinone-2'-deoxyadenosine (AQ-dA) conjugates is that they are insoluble in water and only sparingly soluble in most organic solvents. However, water-soluble AQ-dA conjugates with short linkers are required for study of their electrochemical and intramolecular electron transfer properties in this solvent prior to their use in laser kinetics investigations of photoinduced hole (cation) transport in DNA. This paper first describes the synthesis of a water-soluble, ethynyl-linked AQ-dA conjugate, 8-[(anthraquinone-2-yl)ethynyl]-2'-deoxyadenosine 3'-benzyl hydrogen phosphate, based on initial formation of a 5'-O-(4,4'-dimethoxytrityl) (5'-O-DMTr) intermediate. Because intended H₂ over Pd/C

reduction of the ethynyl linker in 5'-*O*-DMTr protected 2'-deoxyadenosines cleaves the DMTr protecting group and precipitates multiple side products, this work also describes the synthesis of an ethanyl-linked AQ-dA conjugate, 8-[2-(anthraquinone-2-yl)ethyl]-2'-deoxyadenosine 3'-benzyl hydrogen phosphate, starting with a 5'-*O*-*tert*-butyldiphenylsilyl protecting group.

2.2. Introduction

The DNA double helix presents a unique medium for electron transfer (ET) due to the π -stacked array of its aromatic heterocyclic base pairs. Several approaches have been developed to study ET in DNA.¹⁻²³ In most of these approaches, either photoexcitation or ionizing irradiation is employed to inject a hole (cation radical) or an excess electron (anion radical) into the DNA base stack depending, respectively, upon whether a DNA base is initially oxidized or reduced.^{24,25} Long range ET is then monitored, generally indirectly, by detecting effects due to the oxidation or reduction of a nucleobase remote from the injection site.²⁶⁻³³ In these experiments yields of the detected oxidized or reduced bases or their chemical products are low ($\leq 5 \times 10^{-3}$).³⁴⁻³⁸ Our approach is to develop modified nucleosides that will permit direct study of the short- and long-range dynamics of charge transport in modified DNA duplexes using real-time transient absorbance (TA) spectroscopy. Key to reaching this goal is the construction of DNA containing covalently modified nucleosides that can yield long-lived charge separated (CS) products upon photoexcitation. CS products can be comprised of either holes or excess electrons on DNA bases depending on the nature of the conjugate group joined to a particular base.

From a hole transfer perspective, conjugates between electron-deficient derivatives of anthraquinone (AQ) and 2'-deoxyadenosine (dA) are interesting as sources of holes in DNA.^{12,13,15,16,39-47} In AQ-dA conjugates photoexcited AQ is expected to be the electron

acceptor, and adenine is expected to be the electron donor; thus photoexcitation of such conjugates is expected to form the $AQ^{\bullet-}/dA^{\bullet+}$ CS product. Photoexcited AQ is unique in that it intersystem crosses very rapidly (≤ 600 fs) from its lowest energy $^1(n,\pi^*)$ state to its lowest energy $^3(\pi,\pi^*)$ state.⁴⁸ This rapid intersystem crossing rate makes AQ a non-fluorescent molecule. If AQ in its triplet state were able to oxidize dA, the CS product would also be a triplet. Such a $^3(AQ^{\bullet-}/dA^{\bullet+})$ product should be relatively long-lived, because its back electron transfer reaction is spin forbidden. The formation and lifetime of such a CS product could be monitored by TA detection of the anthraquinone anion radical ($AQ^{\bullet-}$) in the 500-600 nm region.⁴⁸ If AQ-dA conjugates would produce the $^3(AQ^{\bullet-}/dA^{\bullet+})$ product when inserted into DNA, the $dA^{\bullet+}$ hole could be created at a specific site in a DNA duplex. The expected long lifetime of the initial triplet CS product may then allow the initial $dA^{\bullet+}$ hole to hop to nearby purine bases (Gs or other As). If so, a reversibly hopping purine cation might be able to be observed as it is trapped on a distant base of lower $D^{\bullet+}/D$ reduction potential such as guanine or 7,8-dihydro-8-oxoguanine. Separating $AQ^{\bullet-}$ from its associated hole in the $^3(AQ^{\bullet-}/dA^{\bullet+})$ photoproduct should prolong the lifetime of the resulting $AQ^{\bullet-}/dA/dN^{\bullet+}$ secondary charge separated (SCS) product, where dN is any purine base. Key questions concern how efficiently can SCS products be produced and how long can they be made to live.

Upon photoexcitation of the ethynyl-linked AQ-dA conjugate, **1a**, and the ethanyl-linked AQ-dA conjugate, **2a**, (see Figure 1) at 341 nm in methanol (MeOH), we observed formation of the $^3(\pi,\pi^*)$ state of AQ (AQ^*) but no formation of the hoped for $^3(AQ^{\bullet-}/dA^{\bullet+})$ photoproduct.⁴⁸ Additionally, photoexcitation of a bimolecular solution of dA and 2-anthraquinone sulfonic acid sodium salt (AQS^-) in MeOH also produced only the AQ-localized $^3(\pi,\pi^*)$ state of AQS^- .⁴⁸ The

above results showed that in MeOH the energy of initial triplet state of AQ in **1a**, **2a**, and AQS^- was below the energy of the $\text{AQ}^{\bullet-}/\text{dA}^{\bullet+}$ CS state. Surprisingly, the $\text{AQS}^{\bullet 2-}/\text{dA}^{\bullet+}$ CS product was found after changing the medium of the last experiment from MeOH to water.⁴⁸ Thus in the latter experiment, interactions with water stabilized the CS product more than in MeOH, as changing the solvent from MeOH to water does not appreciably change the energy of the $^3(\pi,\pi^*)$ state of AQS^- .

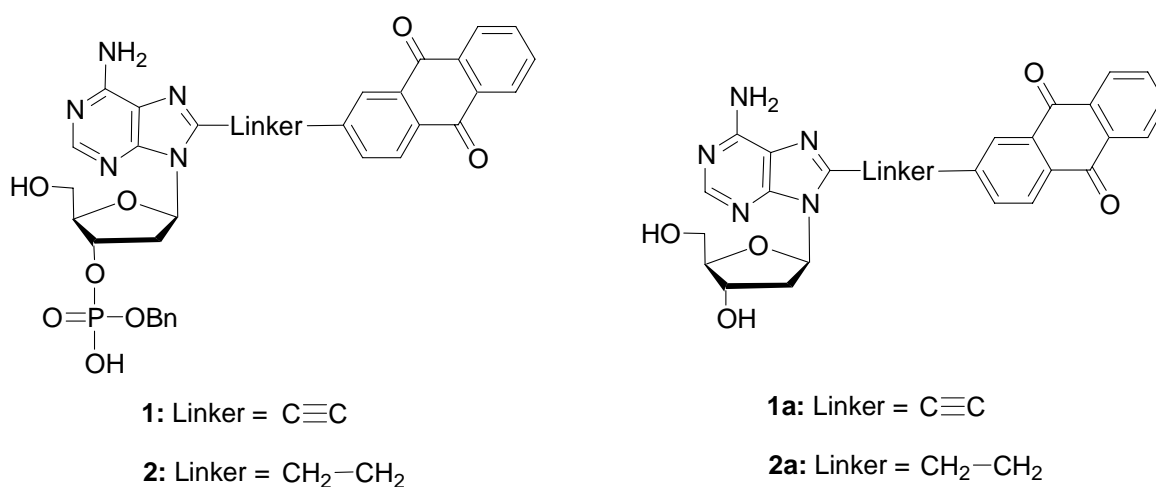


Figure 2.1. Structures of four AQ-dA conjugates: the 3'-benzyl phosphate nucleotides **1** and **2** are soluble in both water and MeOH, while the nucleosides **1a** and **2a** are insoluble in water but sparingly soluble in MeOH.

Water can lower the energy of an $\text{AQ}^{\bullet-}/\text{dA}^{\bullet+}$ CS product compared to MeOH in at least four ways. First, the high dielectric constant of water ($\epsilon = 78$) can lower the free energy of the CS state compared to MeOH ($\epsilon = 38$). However, dielectric stabilization is likely to be small in this case, because it scales as $1/\epsilon$. Second, hydrogen bonding at the AQ-carbonyls can increase the stability of $\text{AQ}^{\bullet-}$. However, again there is not likely to be much difference in this effect

between MeOH and water. Third, deprotonation of $\text{dA}^{\bullet+}$ at N^6 in water to form $\text{dA}^{\bullet}(-\text{H})$ could provide nearly 500 meV of extra stabilization energy. Thus, in water a new, much more stable CS product, $\text{AQ}^{\bullet-}/\text{dA}^{\bullet}(-\text{H})$, could be formed. However, for this free energy change to drive reductive quenching of AQ^* by dA , a proton would have to move from dA first so that ET could follow. Energetic considerations make this seem unlikely. Fourth, our electrochemical studies show that AQS^- is nearly 300 mV easier to reduce in water at pH 13 than in acetonitrile (MeCN).⁴⁹ This surprising “hydroelectrochemical” effect on AQS^- reduction is independent of protonation of AQS^{2-} , which does not occur at or above pH 7.⁴⁹ The last of these four modes of $\text{AQ}^{\bullet-}/\text{dA}^{\bullet+}$ CS product stabilization appears likely to be the reason that reductive quenching of the AQ-localized $^3(\pi,\pi^*)$ state of AQS^- by dA occurs in water but not in MeOH. Our goal here is to synthesize the water-soluble AQ-dA 3'-phosphates, **1** and **2**, so that their electrochemistry and intramolecular ET photochemistry can be studied in water. If they can be shown to undergo reductive quenching of their $^3(\pi,\pi^*)$ excited states to form $^3(\text{AQ}^{\bullet-}/\text{dA}^{\bullet+})$ photoproducts in water, it will then be desirable to transform them into the corresponding phosphoramidite reagents for incorporation into DNA oligomers for studies of hole transport in DNA.

Synthesis of the 3'-phosphate AQ-dA conjugates **1** and **2** appears appealing for study of the chemistry of water soluble AQ-dA conjugates, because the phosphate group is attached to the sugar and, therefore, is not likely to affect the redox potentials of either the AQ or dA subunits. From the perspective of substitution of **1** and **2** into DNA duplexes, the rigid ethynyl linker in **1** appears advantageous over the more flexible ethanyl linker in **2**, because it will likely permit fewer nonbonded interactions between the anthraquinonyl group and neighboring DNA bases. However, at this point the electrochemistry and ET photochemistry of neither compound is

known in water and both need to be made and studied. The challenge in working with AQ-dA conjugates is that in general they are insoluble in water and only sparingly soluble in most organic solvents. Thus the goal of producing moderate amounts of the 3'-phosphates **1** and **2** requires development of protecting group strategies to increase the solubility of synthetic intermediates. Since the 4,4'-dimethoxytrityl (DMTr) group is commonly used to make phosphoramidite reagents for use on automated solid-phase DNA synthesizers, we begin this work with a description of the synthesis of the ethynyl-linked AQ-dA 3'-phosphate conjugate **1** based on initial formation of a 5'-*O*-DMTr intermediate as shown in Scheme 1. Unfortunately, attempted reduction of the ethynyl linker in 5'-*O*-DMTr protected 2'-deoxyadenosines with H₂ over Pd/C cleaves the DMTr protecting group and precipitates multiple side products. Thus this work also describes in Scheme 2 a synthetic strategy based on initial 5'-*O*-*tert*-butyldiphenylsilyl (5'-*O*-TBDPS) protection of 8-bromo-2'-deoxyadenosine to make the ethanyl-linked AQ-dA conjugate **2**. Finally, useful syntheses of two substituted-anthraquinones are described in Scheme 3.

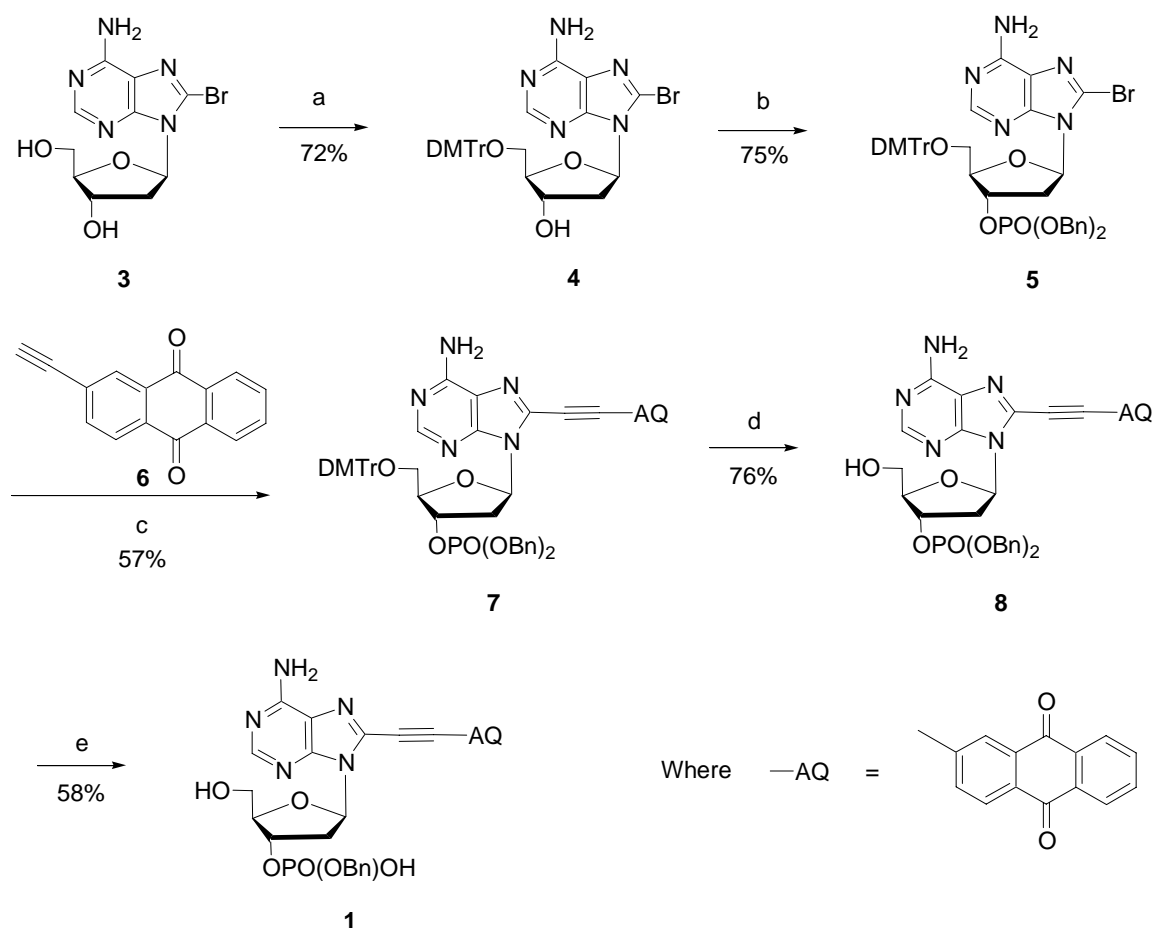
2.3. Results and Discussion

2.3.1. Synthesis of an Ethynyl Linked AQ-dA Conjugate (1)

The water insoluble AQ-dA nucleosides **1a** and **2a** were made on a 100-mg scale without protecting groups, but with silica gel column purification difficulties. However, their extremely poor solubility in organic solvents precluded phosphorylating them directly with POCl₃ in trimethyl phosphate⁵⁰⁻⁵² or β-cyanoethyl phosphate.⁵³ To allow chemical transformations of AQ-dA intermediates in organic solvents, 8-bromo-2'-deoxyadenosine must first be protected at the 5'-*O* position to ensure that its AQ-adducts remain soluble. With this in mind, we attempted to

use $(\text{BnO})_2\text{POCl}$ and *tert*-BuMgCl to produce a 5'-O protected 3'-phosphate. Unfortunately, this modified version of a reported reaction⁵⁴ did not succeed. Either phosphite triester^{55,56} or phosphoramidite coupling⁵⁷⁻⁶⁰ followed by oxidation also appeared reasonable as alternate ways of producing our desired 5'-O protected 3'-phosphates of AQ-dA. We chose the latter procedure as dibenzyl *N,N*-diisopropylphosphoramidite was commercially available, while tribenzyl phosphite was not.

The synthetic strategy to form **1** described in Scheme 1 was developed based on trial phosphorylation reactions of 2'-deoxyadenosine rather than the more expensive 8-bromo-2'-deoxy adenosine reagent. The goal of these trial reactions was three-fold: 1) to test phosphoramidite chemistry without N⁶-protection, 2) to determine the stability of phosphate protection toward DMTr deprotection, and 3) to establish the stability of both the N-glycosidic bond and the phosphate group itself toward the conditions needed to produce the 2'-deoxyadenosine 3'-benzyl phosphate products in Scheme 1. While unprotected, ethynyl and ethanyl AQ-dA conjugates are only sparingly soluble in most solvents, all three 3'-dibenzyl phosphates in Scheme 1 were readily soluble in a variety of standard organic solvents. However, purification of **7** was sometimes a problem as will be discussed.



Scheme 2.1. Reagents and conditions: (a) DMTrCl, 4-DMAP, TEA, Py, rt, 4 h; (b) *i*- $(\text{BnO})_2\text{PN}(\text{iPr})_2$, Me-tetrazol, THF, rt, 1 h; *ii*- *m*-CPBA in CH_2Cl_2 , -78°C , 15 min; (c) $\text{Pd}(\text{Ph}_3\text{P})_4$, CuI, TEA, DMF, 65°C , 6 h; (d) 30% DCA in CH_2Cl_2 , rt, 30 min; (e) DABCO, 1,4-dioxane, reflux, 2 h.

2.3.2. Synthesis of 5'-O-Dimethoxytrityl-8-bromo-2'-deoxyadenosine 3'-Dibenzyl

Phosphate (5) from 2'-Deoxyadenosine

Treatment of 2'-deoxyadenosine with Br_2 in a 1 M solution of acetate buffer at pH 4.0 in the dark for 4 h produced **3** in 79% yield. Adjusting the pH to 4.0 rather than 5.0⁶¹ or 5.4⁶² and using a buffer concentration of 1 M was essential to dissolve the starting materials. Additionally,

pure **3** was obtained by extracting it from the buffer with chloroform (CHCl_3);⁶³ extraction was aided by CHCl_3 mixing with the large amount of acetic acid used required to drop the pH of the buffer to 4.0. Evaporation of CHCl_3 resulted in a solution of **3** in acetic acid; acetic acid in turn was removed by co-evaporation with toluene. ^1H and ^{13}C NMR data for **3** were identical to those previously published, and the product was used without further purification. Alternately, separation of **3** from the acetate buffer was also done by precipitation following neutralization with sodium hydroxide.⁶⁴ However, in this case recrystallization from boiling water was required to separate a trace amount of unreacted 2'-deoxyadenosine. The yield for this latter product collection method was only 50%.

5'-*O*-Dimethoxytrityl-8-bromo-2'-deoxyadenosine (**4**) was prepared in 72% yield by tritylation of **3** using DMTrCl, TEA, and 4-DMAP (as a catalyst) in pyridine.⁶⁵ DMTrCl was added dropwise as a solution in pyridine at 0 °C. This procedure was intended to increase the selectivity of tritylation at 5'-OH. Phosphorylation of **4** by coupling with $(\text{BnO})_2\text{PN}(i\text{Pr})_2$ followed by oxidation produced **5** as shown in Scheme 1. Based on our trial phosphorylation of 2'-deoxyadenosine, we avoided using less than 1.5 equivalents of $(\text{BnO})_2\text{PN}(i\text{Pr})_2$ in order to completely react **4**, and hoped that any N^6 -phosphorylated side-product would be easily removable by silica gel chromatography as in the trial chemistry. However, the amount of this side product here, as shown by TLC, was larger than the amount seen previously when phosphorylating 5'-*O*-DMTr-2'-deoxyadenosine. This could have been due to additional activation of N^6 in **4** by the inductive effect of Br at C8. Worse, complete separation of the 3', N^6 -bis(dibenzyl phosphate) side product from the 3'-dibenzyl phosphate **5** was required by biotage chromatography. Therefore, to eliminate N^6 -phosphorylation, it was worth re-optimizing the number of equivalents of $(\text{BnO})_2\text{PN}(i\text{Pr})_2$ used. Some published procedures used lower

equivalents of similar phosphoramidite reagents but an excess of the required base.^{66,67} Based on this reasoning, 2.2 equivalents of Me-tetrazol were used with 1.2 equivalents of (BnO)₂PN(*i*Pr)₂ keeping all other conditions unchanged. This modified procedure yielded a single spot of **5** on TLC with complete consumption of **4**.

2.3.3. Coupling Anthraquinone to **5** Followed by Two Deprotections to Yield **1**

5'-*O*-Dimethoxytrityl-8-[(anthraquinone-2-yl)ethynyl]-2'-deoxyadenosine 3'-dibenzyl phosphate (**7**) was synthesized by Pd(0) catalyzed cross-coupling of **5** and **6**. The coupling was achieved by stirring the reactants in DMF at 65 °C for 5-6 h under anhydrous conditions with two equivalents of TEA (as base) and catalytic amounts of tetrakis(triphenylphosphine) palladium(0) (Pd(Ph₃P)₄) and CuI. Monitoring the progress of this reaction by TLC was not possible, because both the starting material **5** and the product **7** had the same R_f in most commonly used eluting solvent mixtures. The time of the reaction was suggested by comparison with similar Pd(0) catalyzed cross-coupling reactions in the literature (2.5-6 h).⁶⁷⁻⁶⁹ Complete consumption of **5** by reaction with **6** was essential to obtain **7** pure after silica gel chromatography; purity of **7** was checked by ¹H NMR. Frequently, however, **5** reacted incompletely even when fresh Pd(Ph₃P)₄ catalyst and 30% excess of **6** were used. Unfortunately, in these cases increasing the reaction time did not lead to a complete reaction. Because unreacted **5** and **7** underwent the same kinds of reactions in Scheme 1, proceeding to the next step also yielded products with same R_f values. Thus, obtaining **8** pure was also a problem unless all of **5** reacted when forming **7**. However, obtaining **1** pure was not a problem as HPLC separated it cleanly from all other reaction products.

The DMTr group of **7** in Scheme 1 was cleaved by stirring with 40 equivalents of 30% DCA in dichloromethane (CH₂Cl₂) at room temperature for 30 min. (Lower concentrations or equivalents of the acid caused incomplete detritylation of trial compound 5'-*O*-DMTr-2'-deoxyadenosine 3'-dibenzyl phosphate.) After silica gel chromatographic purification, 8-[(anthraquinone-2-yl)ethynyl]-2'-deoxyadenosine 3'-dibenzyl phosphate (**8**) was obtained in 76% yield. No depurination of **8** was observed during the acidic cleavage of DMTr.

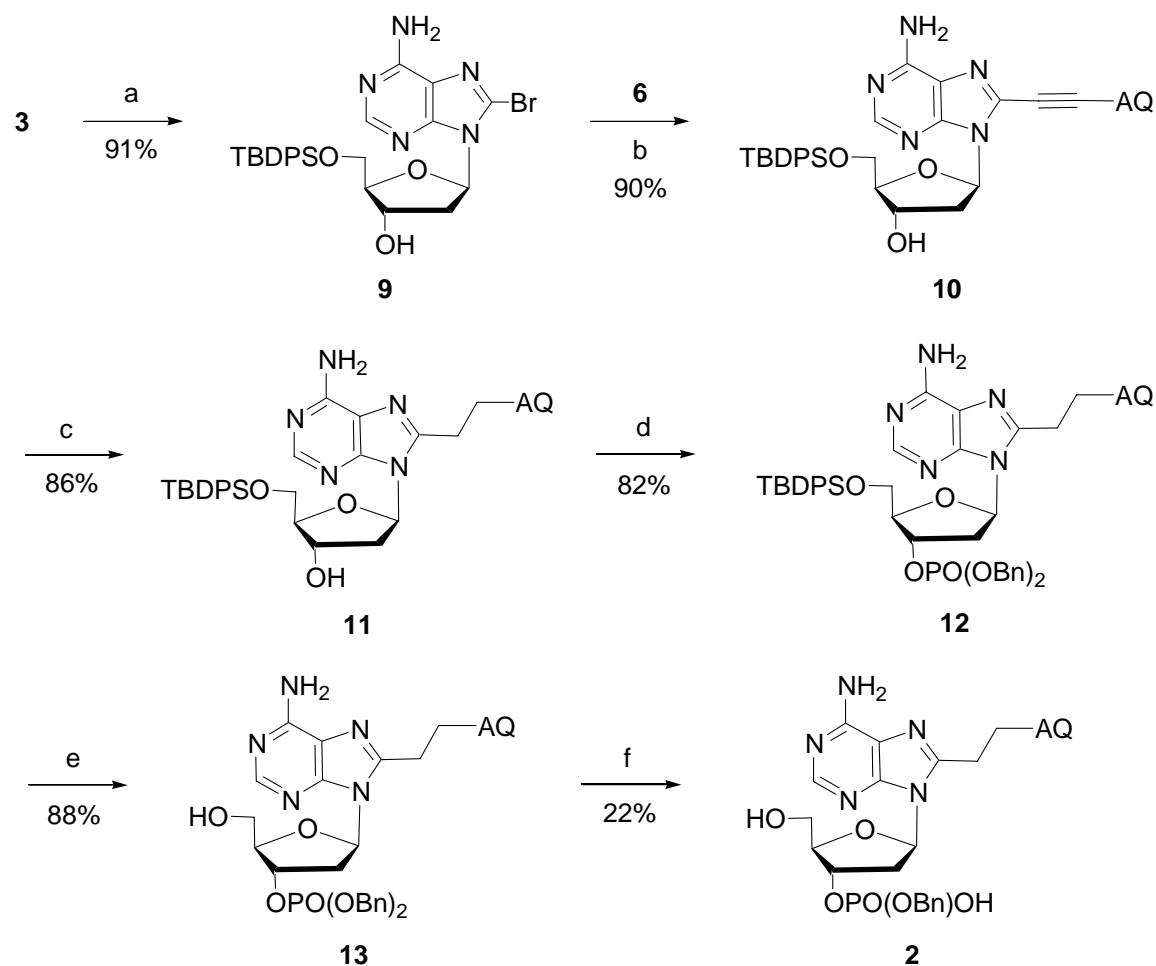
Mono-deprotection of the dibenzyl phosphate group in **8** to produce **1** was achieved by treatment with 2.7 equivalents of DABCO under anhydrous conditions in refluxing 1,4-dioxane. During purification of **1** by preparative HPLC eluting with MeCN/water, the Bn-quaternarized DABCO counter cation was replaced by hydrogen as judged by ¹H NMR. Hydrogenolysis was not used for benzyl deprotection as this would have also reduced the alkynyl linker. Additionally, benzyl deprotection of trial 2'-adenosine 3'-dibenzyl phosphate with 5 equivalents of TMSBr in anhydrous CH₂Cl₂ followed by quenching with 1 M aqueous ammonium bicarbonate⁷⁰ produced a mixture of unidentified products as judged by ¹H NMR. Thus this benzyl deprotection method was not used either. Note also that 2.7 equivalents of DABCO produced only monobenzyl deprotected **1** without evidence of any dibenzyl deprotected product.

2.3.4. Synthesis of an Ethanyl Linked AQ-dA Conjugate (2)

The initial strategy to synthesize **2** was based on using Pd/C catalyzed hydrogenation of **8** both to reduce the ethynyl linker and also to remove the benzyl groups on the 3'-phosphate.⁷¹ Surprisingly, however, this strategy was not successful due to precipitation of multiple side products following benzyl cleavage prior to complete reduction of the triple bond. (In our experience unprotected ethynyl-linked AQ-dA conjugates had poorer solubility in most organic

solvents than the corresponding ethanyl-linked conjugates). Therefore, we decided to reduce the triple bond independently from 3'-dibenzyl phosphate deprotection. Additionally, since protected phosphates were more stable and easier to handle than unprotected ones, we also decided to form a 3'-dibenzyl-protected phosphate intermediate immediately after reduction of the ethynyl linker. The last step in our synthesis of the water-soluble AQ-dA nucleotide **2** (as shown in Scheme 2) was then a second hydrogenation to monobenzyl-deprotect the 3'-dibenzyl phosphate intermediate **13**.

To improve the solubility of AQ-dA intermediates prior to hydrogenation of the alkynyl linker, we tried to use DMTrCl to protect the 5'-OH of **3** before coupling it to **6** (2-ethynylanthraquinone). Accordingly, we reacted 5'-O-DMTr-protected **4** (see Scheme 1) with **6** using Pd(0) catalyzed cross-coupling chemistry. However, hydrogenation of the resulting product, 5'-O-DMTr-8-[anthraquinone-2-yl]ethynyl-2'-deoxyadenosine, using 10% Pd/C and H₂ (40 psi) at room temperature for 24 h cleaved the DMTr group and precipitated multiple side products as determined by TLC. This was surprising, because DMTr protection of 5'-OH on 2'-deoxyuridine was stable to the same hydrogenation conditions.^{72,73} Perhaps the 5'-O DMTr group in 2'-deoxypurine nucleosides was more labile than in 2'-deoxypyrimidine nucleosides.⁷⁴ However, it was also true that trityl groups on other compounds were cleaved by hydrogenation under similar conditions.⁷⁵ Clearly, a different 5'-OH protecting group was needed to solubilize intermediates during our synthesis of **2**. We selected the *tert*-butyldiphenylsilyl (TBDPS) group, because it could selectively protect 5'-OH in 2'-deoxyribonucleosides,⁷⁶⁻⁷⁹ and it could be cleaved under mild conditions using NH₄F in MeOH.⁸⁰ Finally, our second water-soluble AQ-dA target compound **2** was successfully prepared as described in Scheme 2 following initial 5'-OH protection of **3** with TBDPSCl.



Scheme 2.2. Reagents and conditions: (a) TBDPSCl, Im, Py, rt, 3h; (b) Pd(Ph₃P)₄, CuI, TEA, DMF, 65 °C, 6 h; (c) 10% Pd/C, H₂, 40 psi, EtOAc/MeOH, rt, 24 h; (d) *i*- (BnO)₂PN(*i*Pr)₂, Me-tetrazol, THF, rt, 1h; *ii*- *m*-CPBA in CH₂Cl₂, -78 °C, 15 min; (e) NH₄F, MeOH/THF, 60 °C, 6 h; (f) 10% Pd/C, H₂, 40-45 psi, EtOAc/MeOH, rt, 31 h.

2.3.5. Synthesis of 5'-*O*-*tert*-Butyldiphenylsilyl-8-[2-(anthraquinone-2-yl)ethyl]-2'-deoxyadenosine (**11**)

Compound **11** was synthesized in three steps starting with **3** as described in Scheme 2. To produce a 5'-*O* protected form of **3**, TBDPSCl (1.05 equivalents) was added dropwise at 0 °C to a mixture of **3** and imidazol (1 equivalent each) in anhydrous pyridine; stirring at room

temperature for 3 h afforded **9** in 91% yield after silica gel chromatographic purification. When more equivalents of imidazol and TBDPSCI were used, disilylation of **3** occurred readily. Importantly for future work, both the purity and yield of 5'-*O*-TBDPS protected **9** were better than those of 5'-*O*-DMTr protected **4** (see Scheme 1).

5'-*O*-*tert*-Butyldiphenylsilyl-8-[(anthraquinone-2-yl)ethynyl]-2'-deoxyadenosine (**10**) was synthesized in 90% yield via Pd(0) catalyzed cross-coupling between **9** and **6**. The coupling was achieved by stirring the reactants in DMF at 65 °C for 5-6 h under anhydrous conditions with two equivalents of TEA (as base) and catalytic amounts of Pd(Ph₃P)₄ and CuI. Filtration of the reaction mixture over Celite separated insoluble materials from the reaction products. Yellow colored **10** was frequently purified with two silica gel columns to remove dark colored impurities that developed during the reaction. However, many times even after two chromatographic purifications, **10** was still mixed with a trace amount of unreacted **9**. This was the case because nucleosides **9** and **10** had nearly the same R_f in most commonly used eluting solvent mixtures. A similar problem was encountered previously when synthesizing **1** also starting with **3**; in fact both 8-bromo-2'-deoxyadenosine nucleosides **9** and **4** protected, respectively, with TBDPS and DMTr groups had the same R_f values. Even using fresh Pd(Ph₃P)₄ catalyst, 30% excess of **6**, and increased reaction time did not always eliminate **9**. The latter contaminant was easily eliminated, however, after ethynyl reduction during purification of **11**.

5'-*O*-*tert*-Butyldiphenylsilyl-8-[2-(anthraquinone-2-yl)ethyl]-2'-deoxyadenosine (**11**) was produced in 86% yield by hydrogenation of **10** using 10% Pd/C and H₂ (40 psi) at room temperature for 24 h in MeOH/ethyl acetate (EtOAc) (3:2). The yellow color of **11** was less

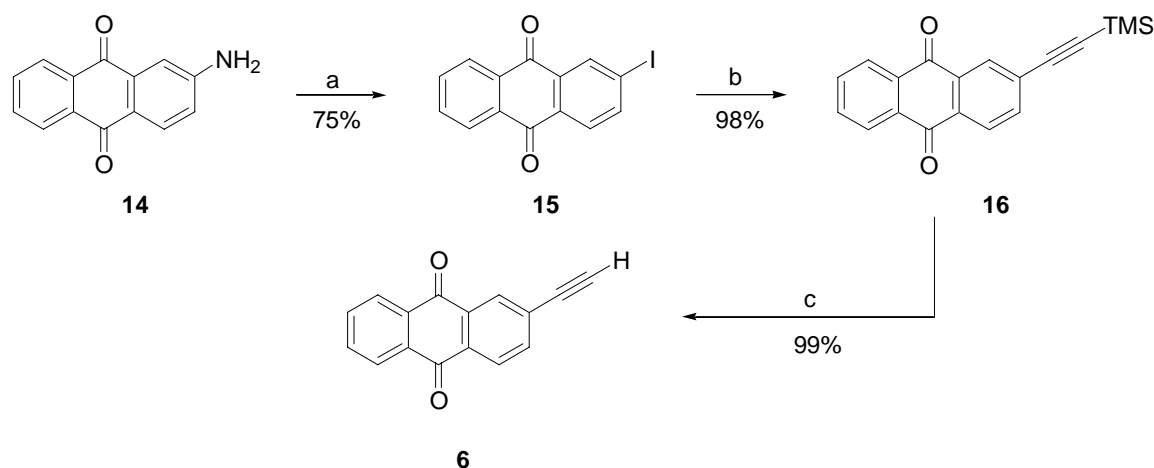
intense than that of **10** and it was more soluble in organic solvents. Importantly, **11** had a more polar R_f value on TLC than **10**. Since unreacted **9** from the Pd(0) catalyzed cross-coupling step did not undergo hydrogenation, traces of **9** were easily separated from **11** using Biotage silica gel chromatography. Importantly for the synthesis of **2**, the 5'-*O*-TBDPS group in **10** was stable with respect to hydrogenation, and thus the ethanyl-linked product **11** remained soluble in MeOH/EtOAc solution.

2.3.6. Phosphorylation of **11** and Two Subsequent Deprotections to Form **2**

5'-*O*-*tert*-Butyldiphenylsilyl-8-[2-(anthraquinone-2-yl)ethyl]-2'-deoxyadenosine 3'-dibenzyl phosphate (**12**) was prepared by phosphorylation of **11** using 1.2 equivalents of (BnO)₂PN(iPr)₂ and 2.2 equivalents of Me-tetrazol in THF, followed by oxidation with *m*-CPBA. Phosphorylation of the ethanyl-linked AQ-dA conjugate **11** showed five product spots in addition to a minor amount of unreacted **11** on TLC compared to a single spot found earlier when phosphorylating **4**. The major TLC spot corresponded to **12**, while the side products were not identified. Nevertheless, silica gel column chromatography easily purified **12** in 82% yield.

The desilylated product 8-[2-(anthraquinone-2-yl)ethyl]-2'-deoxyadenosine 3'-dibenzyl phosphate (**13**) was produced in 88% yield by treatment of **12** with NH₄F in THF/MeOH (1:6.7) at 60 °C. The amount of NH₄F added to this reaction was 11.5 equivalents with respect to **12**, but this amount of NH₄F was not fully soluble in the reaction solvent. Nevertheless, after reaction completion the solvent was reduced in volume, and the residue was purified via silica gel chromatography on a Biotage column (without prior separation NH₄F by filtration or water workup). Finally, the monobenzyl-protected product **2** was formed from the 3'-dibenzyl phosphate intermediate **13** by catalytic hydrogenation using 10% Pd/C and H₂ (40 psi) at room

temperature for 31 h in MeOH/EtOAc (8:1). Although, Pd/C hydrogenolysis has been reported many times to cleavage both benzyls on a phosphate group,^{55-57,59,71,81} hydrogenolysis of **13** produced only monobenzyl deprotection. Analytical reverse phase HPLC of the crude product mixture showed 71% yield of **2**. Purification by preparative reverse phase HPLC, however, gave **2** in 22% yield.



Scheme 2.3. Reagents and conditions: (a) *i.* HCl, NaNO₂, THF/H₂O, 0 °C; *ii.* KI, 0 °C, 75 min; (b) Pd(Ph₃P)₂Cl₂, CuI, Et₃N, THF, TMSA, rt, 10 min; (c) KF, THF/MeOH, rt, 1h.

2.3.7. Syntheses of Two Substituted-anthraquinones: 2-Iodoanthraquinone (**15**) and 2-Ethynylantraquinone (**6**)

Early steps in the syntheses of both **1** and **2** involve Pd(0) catalyzed cross-coupling between a 5'-O protected 2'-deoxyadenosine (**5** or **9**) and 2-ethynylantraquinone (**6**). Compound **6** in turn is prepared in two steps beginning with **15** as shown in Scheme 3. In the first step, Pd(Ph₃P)₂Cl₂ catalyzed cross-coupling of trimethylsilylacetylene (TMSA) with **15** gives 2-(trimethylsilylethynyl)anthraquinone (**16**); then the terminal alkyne of **16** is deprotected with KF to give **6**. Although 2-chloroanthraquinone is commercially available, its Pd(0)

catalyzed cross-coupling reaction is likely to occur less readily than those for either 2-bromo or 2-iodoanthraquinone. Also, direct bromination of commercially available anthraquinone yields a mixture of polybromoanthraquinones that is difficult to separate.⁸² Other reported syntheses of 2-bromoanthraquinone either have low yields⁸³ or require harsh conditions.⁸⁴ Thus, for these reasons as well as the fact that iodide is a better leaving group than bromide, 2-iodoanthraquinone (**15**) appears to be a good precursor for forming first **16** and finally **6**.

2.3.8. Synthesis of 2-Iodoanthraquinone (**15**)

To form **15**, 2-aminoanthraquinone (**14**) was diazotised with nitrous acid that was prepared *in situ* from hydrochloric acid and sodium nitrite, and then substituted with iodide in a manner similar to the Sandmeyer reaction. First, compound **14** was suspended in THF, and HCl was added while stirring until the color of the suspension changed from reddish brown to rosy beige. Addition of water and more THF reduced the thickness of the suspension and returned the suspension's color to reddish brown. Next, addition of more HCl restored the rosey beige color. The mixture was then stirred at 40 °C for 24 h to produce a thick suspension. An aqueous solution of NaNO₂ was added dropwise at 0 °C, followed by addition of an aqueous solution of KI also at 0 °C. Addition of KI caused gas evolution. The reaction mixture was then heated gradually to 60 °C until the gas evolution stopped. Neutralization of the reaction mixture with Na₂CO₃ was avoided due to the large amount of base that would have been required. Instead, THF was evaporated from the mixture, and HCl was removed by filtering the residue and washing it with water first, and then with a saturated solution of aqueous Na₂CO₃. TLC of the product mixture, however, showed a less polar, minor product very close to **15**. Thus, although

15 was difficult to purify by silica gel column chromatography, three separations using 30% CH₂Cl₂ in hexane as the mobile phase produced 3.4 g of **15** in 75% yield.

2.3.9. Synthesis of 2-Ethynylanthraquinone (**6**)

Following a general procedure for Pd catalyzed cross-coupling between an aryl halide and trimethylsilylacetylene (TMSA),⁸⁵ **15** was readily converted to **16** via cross-coupling with TMSA using dichlorobis(triphenylphosphine)palladium(II) (Pd(Ph₃P)₂Cl₂) as the catalyst. This reaction was complete within 10 minutes in tetrahydrofuran (THF) at room temperature. However, the above difficulty in purifying **15** made using commercially available 2-chloroanthraquinone to produce **16** attractive. Unfortunately, both of our attempts to do so by substituting 2-chloroanthraquinone for **15** a) under the same reaction conditions and also b) by heating the reaction mixture at 60 °C for 24 hrs failed. Thus, at present, the best route for producing **6** appears to be via precursors **14** and **15**.

All of the anthraquinones in Scheme 3 had low solubility in organic solvents. For example, 26 mL of THF/MeOH (1:1 v:v) along with 30 minutes of stirring were required to dissolve 200 mg of **16** for deprotection by potassium fluoride to produce **6**, and it was harder to dissolve **6** than **16** in CH₂Cl₂, CHCl₃, THF, MeOH, or EtOAc. Additionally, the low solubility of **6** made difficult purification of its crude reaction mixture by silica gel chromatography. Thus initially the crude product was extracted with CHCl₃, and the extract's dark color was reduced with active charcoal. Then **6** was recrystallized from CHCl₃ as a brown solid, and the recrystallized product was used for Pd catalyzed cross-coupling reactions with the 8-bromo-2'-deoxyadenosines **5** and **9**. TLC analysis of the precipitated crystals showed very faint spots with higher polarity than **6**, and ¹H NMR of the crystals showed extra proton resonances. To

eliminate the extra resonances in NMR, 100 mg of **6** was purified further on a 20 cm long by 5 cm dia. silica gel column. Late in our work, we found that **6** was sensitive to light below 370 nm. In particular, **6** changed its color from pale yellow to brown soon after silica gel purification when exposed to fluorescent laboratory light.

2.4. Conclusions

Working with **6** as described in Schemes 1 and 2 to make, respectively, the 3'-benzyl phosphates **1** and **2** imposes synthetic constraints due to the poor solubility and photo-instability of **6**. Nevertheless, careful control of reaction conditions, including avoidance of short wavelength light (<370 nm) when using **6**, allows synthesis of **1** and **2** and their precursors on a few-gram scale. Based on our experience with the chemistry in Schemes 1-3, a common modification to Schemes 1 and 2 appears worth investigating in the future as a means of possibly making the 3'-phosphate conjugates **1** and **2** and related AQ-dA nucleosides with less effort and in larger quantities. This alternate chemistry would switch the order of the alkylation steps in each scheme. Schemes 1 and 2 alkylation 2-iodoanthraquinone (**15**) with TMSA first and then Pd cross-couple the resulting **6** with the 5'-protected-8-bromo-2'-deoxyadenosines, respectively, **5** and **9**. An interesting modification of these two schemes would alkylation their 5'-protected-8-bromo-2'-deoxyadenosines with TMSA first and then Pd cross-couple the resulting 5'-protected-8-ethynyl-2'-deoxyadenosines with **15**. One clear advantage of such a switched reaction order would be that use of photo-chemically unstable **6** would be avoided. A second and also important advantage would be that 5'-protected-8-ethynyl-2'-deoxyadenosine intermediates would replace the 5'-protected-8-bromo-2'-deoxyadenosines **5** and **9**. Thus, in these two cases the vexing coincidence of R_f values for intermediates **5** and **7** and **9** and **10** would also be eliminated. In short, the work presented here provides a sound basis for synthesizing the 3'-benzyl

phosphates **1** and **2** for investigation of their electrochemical reduction and photoinduced intramolecular ET properties in water. Lacking the solubilizing effect of the 3'-benzyl phosphate group, the nucleosides **1a** and **2a** are insoluble in water, and thus these physical chemical experiments cannot be done on them. Finally, future synthesis of AQ-dA substituted DNA oligomers based on standard solid-phase synthetic protocols appears achievable based either on the chemistry of Schemes 1-3 themselves or on variations of Schemes 1 and 2 that use a switched order of alkynylation.

2.5. Experimental

2.5.1. Materials and General Synthetic Methods

2'-Deoxyadenosine monohydrate was purchased from TCI America as white a powder. TMSA obtained from GFS Chemicals was used after vacuum transfer from CaH₂. DMTrCl and Br₂ were obtained from Alfa-Aesar. 10% Pd on activated carbon catalyst (Pd/C) and Pd(Ph₃P)₂Cl₂ were obtained from Strem Chemicals and used as received. 2-Amino-anthraquinone was purchased from Aldrich as a brown powder (93% pure) and was used as received. Other remaining reagents used were purchased from Aldrich or VWR. 2-Ethynylantraquinone (**6**) was synthesized as described in Scheme 3. Pd(Ph₃P)₄ was prepared according to the literature⁸⁶ and stored between uses in our glove-box's freezer at -20 °C. Anhydrous DMF was purchased from Aldrich and stored in our Vacuum Atmospheres M040-2 glove-box that was pressurized with nitrogen boil-off gas from a liquid nitrogen tank. All starting materials for anhydrous reactions were dried prior to use on a vacuum line (1-4 × 10⁻⁴ torr) for at least 12 h. Two to three co-evaporation cycles were also used for drying compounds as indicated below. Solvents for synthesis were dried and redistilled in continuous circulation

distillation apparatus: THF was dried with benzophenone /Na⁰ and stored over activated molecular sieves in our glove-box. Pyridine was always freshly distilled over CaH₂ before use. Water was deionized by a Millipore (Milli-Q Plus) ultrapure water system (18.0 MΩ). All manipulations of DMTrCl, TBDPSCl, Pd(Ph₃P)₄, Pd(Ph₃P)₂Cl₂, and (BnO)₂PN(*i*Pr)₂ were performed in our glove-box. Most of the reactions were monitored with glass-backed TLC Plates precoated with silica gel 60 F₂₅₄ (EMD Chemicals). TLC was run using 5-7 % MeOH in CH₂Cl₂ as the eluent. HPLC grade solvents were used for chromatographic purifications. Flash column chromatography was carried out on either a Biotage Flash-40™ system using prepackaged KP-Sil™ cartridges, or on Whatman™ flash silica (60Å pore, 230-400 mesh) that was packed in glass columns and pressurized with boil-off nitrogen. NMR Spectra were recorded at GSU on three spectrometers, a Varian Unity +300, a Varian Unity Inova 500, and a Bruker Avance 400, using either CDCl₃ or DMSO-d₆ as solvents. Chemical shifts for ¹H NMR in these solvents were referenced, respectively, relative to tetramethylsilane (0.00 ppm) and DMSO (2.49 ppm). ³¹P NMR spectra were recorded using orthophosphoric acid (85%) as an external standard in a capillary tube inside of the NMR tube. Low resolution (LR) MS were obtained at GSU: for substituted anthraquinones EI (electron impact) MS were recorded in positive ion mode on a Shimadzu 5050A MSD single quadrupole spectrometer with one amu resolution, and for substituted 2'-deoxyadenosines ESI (electrospray ionization) MS were recorded in either positive or negative ion modes on a Waters Micromass Q-TOFTM with 50 ppm error.

2.5.2. 8-Bromo-2'-deoxyadenosine (3)

2'-Deoxyadenosine monohydrate (6 g, 22.28 mmol) was dissolved in 800 mL of 1 M acetate buffer at pH 4.0 (prepared by adding glacial acetic acid to 700 mL of 1 M sodium acetate and adjusting the pH). Br₂ (2.3 mL, 44.43 mmol, 2 equiv) was added, and the reaction mixture was stirred at room temperature for 4 h in the dark. The reaction was quenched by gradual addition of a saturated aqueous solution of NaHSO₃ until the red color of Br₂ disappeared. The product was extracted by shaking with CHCl₃ (8 × 500 mL) in a separatory funnel; the combined extracts were dried over anhydrous MgSO₄ and evaporated to yield a solution of the product in acetic acid. Co-evaporation with toluene (2 × 200 mL) provided **3** as a pale yellow powder that was used without further purification (5.96 g, 79% yield). ¹H and ¹³C NMR spectra for **3** were identical to ones published in the literature.

2.5.3. 5'-O-Dimethoxytrityl-8-bromo-2'-deoxyadenosine (4)

Compound **3** (640 mg, 1.94 mmol) was dried 3 times by co-evaporation with dry pyridine and suspended in dry pyridine (5 mL). To the suspension was added TEA (0.37 mL, 2.72 mmol, 1.4 equiv) and 4-DMAP (13 mg, 0.1 mmol, 0.05 equiv) in our glove-box. The mixture was next removed from our glove-box, and DMTrCl (789 mg, 2.33 mmol, 1.2 equiv) was dissolved in dry pyridine and added dropwise at 0 °C to the reaction mixture with **3** under a nitrogen atmosphere using a cannula. The orange reaction mixture was stirred at room temperature under a nitrogen atmosphere for 4 h after which time the mixture was homogeneous, and TLC showed complete consumption of **3**. The reaction was quenched with a saturated aqueous solution of NaHCO₃, and the solvent was evaporated. The residue was dissolved in CH₂Cl₂ (50 mL) and water (20 mL); the organic layer was separated, washed with water (2 × 20 mL), and dried over MgSO₄.

The crude product was purified by silica gel chromatography on a column that had been pre-equilibrated with 1% TEA in CH₂Cl₂ and eluted with MeOH/CH₂Cl₂ (0:100-3:97). Evaporation of the eluting solvent afforded **4** as pale yellow foam (940 mg, 72% yield). The ¹H NMR spectrum given here is very similar to ones previously reported in CD₂Cl₂⁶⁸ and CDCl₃.⁶⁷ ¹H NMR (300 MHz, CDCl₃): δ (ppm) 2.31-2.4 (1H, m, H-2'), 3.39 (2H, d, *J* = 5.7 Hz, H-5', H-5'), 3.51-3.60 (1H, m, H-2'), 3.77 [6H, s, OCH₃ (DMTr)], 4.12 (1H, dd, *J* = 5.7 and 10.2 Hz, H-4'), 4.95 (1H, dd, *J* = 4.8 and 10.2 Hz, H-3'), 5.78 (2H, br s, NH₂), 6.4 (1H, t, *J* = 6.7 Hz, H-1'), 7.66 [4H, dd, *J* = 4.2 and 8.7 Hz, OPh-*meta* (DMTr)], 7.16-7.28 [7H, m, OPh-*ortho* (DMTr, 4H) and Ph-*meta* and *para* (DMTr, 3H)], 7.37 [2H, d, *J* = 7.5 Hz, Ph-*ortho* (DMTr)], and 8.06 [1H, s, H-2 (dA)].

2.5.4. 5'-*O*-Dimethoxytrityl-8-bromo-2'-deoxyadenosine 3'-Dibenzyl Phosphate (**5**)

To **4** (440 mg, 0.70 mmol), previously dried 2 times by co-evaporation with anhydrous THF, was added Me-tetrazole (130 mg, 1.54 mmol, 2.2 equiv) and anhydrous THF (5 mL) in our glove-box. The mixture was removed from our glove-box and cooled to 0 °C using an ice-water bath, and (BnO)₂PN(*i*Pr)₂ (0.28 mL, 0.84 mmol, 1.2 equiv) was added dropwise under nitrogen with a syringe. The reaction mixture was stirred under a nitrogen atmosphere for 10 minutes at 0 °C; then it was warmed to room temperature, and the reaction progress was monitored by TLC. A white precipitate began to form after 20 min, and TLC showed complete consumption of **4** after a total reaction time of 1.5 h. The suspension obtained was cooled to -78 °C using a dry ice-acetone bath, and a solution of *m*-CPBA (250 mg) in CH₂Cl₂ (5 mL) was added gradually to the chilled mixture. After 15 minutes of stirring at -78 °C, the mixture became homogeneous, and TLC showed conversion of an intermediate spot to a more polar one. The mixture was next

warmed to room temperature, and saturated aqueous NaHCO₃ was added. More CH₂Cl₂ (30 mL) and water (15 mL) were added; the organic layer was separated, washed with water, dried with anhydrous MgSO₄, and evaporated to dryness. The syrup obtained was purified using silica gel chromatography on a Biotage column that had been pre-equilibrated with 1% TEA and eluted with MeOH/CHCl₃ (0:100-3:97). Evaporation of the eluting solvent afforded **5** as white foam (469 mg, 75% yield). ¹H NMR (500 MHz, CDCl₃): δ (ppm) 2.39 (1H, ddd, *J* = 2, 6.5 and 13.5 Hz, H-2'), 3.30 (1H, dd, *J* = 5.5 and 10 Hz, H-5'), 3.39 (1H, dd, *J* = 6.5 and 10 Hz, H-5'), 3.75-3.81 [7H, m, H-2' and OCH₃ (DMTr)], 4.33-4.35 (1H, m, H-4'), 5.05 [4H, m, CH₂ (Bn)], 5.35-5.37 (1H, m, H-3'), 5.65 (2H, br s, NH₂), 6.4 (1H, t, *J* = 7 Hz, H-1'), 6.72 [4H, dd, *J* = 7 and 9 Hz, OPh-*meta* (DMTr)], 7.16-7.25 [7H, m, OPh-*ortho* (DMTr, 4H) and Ph-*meta* and *para* (DMTr, 3H)], 7.31 (10 H, s, Ph (Bn)), 7.36 [2H, d, *J* = 6.5 Hz, Ph-*ortho* (DMTr)], and 7.94 [1H, s, H-2 (dA)]. ¹³C NMR (75 MHz, CDCl₃): δ (ppm) 43.26 (C-2'), 55.15 [OCH₃ (DMTr)], 62.93 (C-5'), 69.55 [d, *J*_{C-P} = 5.7 Hz, CH₂ (Bn)], 84.68, 86.19, 88.75 [C-1', C-4' and CAr₃ (DMTr)], 112.94, 120.41 [OPh-*meta* (DMTr), and C-5 (dA)], 126.67 [C-8 (dA)], 127.66, 128, 128.11, 128.62, 130, 135.56, 135.81, 144.61 [Ar (DMTr and Bn)], 150.82 [C-4 (dA)], 152.49 [C-2 (dA)], 154.07 [C-6 (dA)] and 158.35 [OPh-C-4 (DMTr)]. ³¹P NMR (121 MHz, CDCl₃): δ (ppm) -1.19. Low resolution ESI MS *m/z* (M+H)⁺: calc'd. 892.21, found 892.18.

2.5.5. 5'-O-Dimethoxytrityl -8-[(anthraquinone-2-yl)ethynyl]-2'-deoxyadenosine 3'-

Dibenzyl Phosphate (7)

To **5** (410 mg, 0.46 mmol) was added in our glove-box Pd(Ph₃P)₄ (24 mg, 0.02 mmol, 0.05 equiv), CuI (9 mg, 0.05 mmol, 0.1 equiv), TEA (0.13 mL, 0.92 mmol, 2 equiv), compound **6** (130 mg, 0.56 mmol, 1.3 equiv), and anhydrous DMF (5 mL). The mixture was removed from

our glove-box and stirred under a nitrogen atmosphere for 6 h at 65 °C in an oil bath, after which time DMF was removed under reduced pressure. The residue was dissolved in CH₂Cl₂ and filtered over Celite to remove insoluble materials. After evaporation of solvent, the crude product was applied to a silica gel column that had been pre-equilibrated with 1% TEA in CHCl₃, and eluted with MeOH/CHCl₃ (0:100-3:97). Evaporation of the eluting solvent afforded **6** as a yellow foam (469 mg, 57% yield). ¹H NMR (300 MHz, CDCl₃): δ (ppm) 2.53 (1H, ddd, *J* = 2.7, 6.6 and 14.1 Hz, H-2'), 3.36-3.49 (2H, m, H-5' and H-5'), 3.66-3.75 [7H, m, H-2' and OCH₃ (DMTr)], 4.39-4.43 (1H, m, H-4'), 5.06 [4H, d, *J*_{H-P} = 8.4 Hz, CH₂ (Bn)], 5.39-5.43 (1H, m, H-3'), 5.73 (2H, br s, NH₂), 6.57 (1H, t, *J* = 6.9 Hz, H-1'), 6.71 [4H, dd, *J* = 4.2 and 8.7 Hz, OPh-*meta* (DMT)], 7.15-7.39 [19H, m, OPh-*ortho* (DMT, 4H), Ph (DMT, 5H), and Ph (Bn, 10H)], 7.82-7.85 [2H, m, H-6 (AQ) and H-7 (AQ)], 7.94 [1H, dd, *J* = 1.5 and 8.1 Hz, H-3 (AQ)], 8.04 [1H, s, H-2 (dA)], 8.30-8.36 [3H, m, H-4 (AQ), H-5 (AQ) and H-8 (AQ)] and 8.53 [1H, d, *J* = 1.5 Hz, H-1 (AQ)].

2.5.6. 8-[(Anthraquinone-2-yl)ethynyl]-2'-deoxyadenosine 3'-Dibenzyl Phosphate (**8**)

Compound **7** (250 mg, 0.24 mmol) was dissolved in a 30% DCA in CH₂Cl₂ mixture (7.5 mL), and the resulting red solution was stirred at room temperature for 30 min. (Note that many times it was convenient to perform this detritylation step directly on the crude product **7** without silica gel purification.) The reaction was quenched by addition of saturated aqueous NaHCO₃ until the red color of the DMTr cation disappeared. More CH₂Cl₂ (25 mL) and water (10 mL) were added, and the organic layer was separated, washed with water, dried with anhydrous MgSO₄, and evaporated to dryness. The syrup obtained was purified using flash silica gel chromatography on a biotage column with MeOH/CHCl₃ (0:100-3:97) as the eluting system to

yield yellow foam after evaporating the eluting solvent. This foam was dissolved in a minimum amount of CH_2Cl_2 and precipitated by adding hexane. Evaporation of CH_2Cl_2 /hexane from the precipitate afforded **8** as a bright yellow solid (135 mg, 76% yield). ^1H NMR (300 MHz, CDCl_3): δ (ppm) 2.46 (1H, dd, $J = 5.7$ and 14.1 Hz, H-2'), 3.08-3.15 (1H, m, H-2'), 3.37-3.65 (1H, m, H-5') 3.86-3.91 (1H, m, H-5'), 4.33 (1H, s, H-4'), 5.04-5.18 [4H, m, CH_2 (Bn)], 5.20-5.24 (1H, m, H-3'), 6.17 (2H, br s, NH_2), 6.56-6.62 (2H, m, H-1' and OH-5'), 7.29-7.37 [10H, m, Ph (Bn)], 7.72-7.83 [2H, m, H-6 (AQ) and H-7 (AQ)], 7.92 [1H, d, $J = 8.1$ Hz, H-3 (AQ)], 8.23-8.26 [3H, m, H-2 (dA), H-5 (AQ) and H-8 (AQ)], 8.32 [1H, d, $J = 8.1$ Hz, H-4 (AQ)], and 8.56 [1H, s, H-1 (AQ)]. ^1H NMR (500 MHz, $\text{CDCl}_3 + \text{D}_2\text{O}$): δ (ppm) 2.45 (1H, dd, $J = 5$ and 14 Hz, H-2'), 3-3.14 (1H, m, H-2'), 3.59 (1H, d, $J = 12.5$ Hz, H-5'), 3.87 (1H, d, $J = 12.5$ Hz, H-5'), 4.33 (1H, s, H-4'), 5.05-5.16 [4H, m, CH_2 (Bn)], 5.21-5.23 (1H, m, H-3'), 6.59 (1H, dd, $J = 5$ and 9 Hz, H-1'), 7.31-7.38 [10H, m, Ph (Bn)], 7.76-7.82 [2H, m, H-6 (AQ) and H-7 (AQ)], 7.93 [1H, d, $J = 8$ Hz, H-3 (AQ)], 8.23-8.25 [3H, m, H-2 (dA), H-5 (AQ) and H-8 (AQ)], 8.32 [1H, d, $J = 8$ Hz, H-4 (AQ)], and 8.56 [1H, s, H-1 (AQ)]. ^{31}P NMR (121 MHz, CDCl_3): δ (ppm) -1.55 . ^{13}C NMR (100 MHz, CDCl_3): δ (ppm) 38.67 (C-2'), 63.10 (C-5'), 69.79 [d, $J_{P-C} = 6$ Hz, CH_2 (Bn)], 80.05 (d, $J_{P-C} = 6$ Hz, C-3'), 80.86 [dA-C (ethynyl)], 87.19, 88.04 (C-1' and C-4'), 94.48 [AQ-C (ethynyl)], 120.85 [C-5 (dA)], 126.11 [C-8 (dA)], 127.40, 127.60, 128.19, 128.71, 128.87, 131.08 [AQ and Ph (Bn)], 132.05, 133.25, 133.40, 133.54, 133.63 (AQ), 134.44 (d, $J_{P-C} = 3.1$ Hz, Ph-C-1 (Bn)], 135.50, 136.78 [AQ and Ph (Bn)], 148.40 (AQ), 152.20 [C-4 (dA)], 153.36 [C-2 (dA)], 155.67 [C-6 (dA)], 181.89 (CO), and 182.15 (CO). Low resolution ESI MS m/z ($\text{M}+\text{H}$) $^+$: calc'd 742.21, found 742.18.

2.5.7. 8-[(Anthraquinone-2-yl)ethynyl]-2'-deoxyadenosine 3'-Benzyl Hydrogen Phosphate**(1)**

Compound **8** (59 mg, 0.08 mmol) was dissolved in anhydrous 1,4-dioxane (8 mL) by heating to 60 °C. DABCO (24 mg, 0.22 mmol, 2.7 equiv) was added to this solution in our glove-box, and the homogeneous mixture was refluxed on the bench top for 2 h under a nitrogen atmosphere. The solvent was evaporated, and the crude product was separated by analytical HPLC to give *ca.* 95% yield of **1**. The crude product was then purified by preparative HPLC using a Varian Microsorb C-18 reverse phase column (250 mm × 4.14 mm dia.). The column was eluted with a flow rate of 40 mL/min, and the fractions were monitored by UV detection at 260 nm. The mobile phase consisted of a programmed gradient of solution A (70% MeCN in water) and solution B (water): from 0% A/100% B to 50% A/50% B over 15 min; then to 100% A/0% B over 5 min, and finally at 100% A/0% B for another 10 min. Evaporation of the eluent was facilitated by co-evaporation with MeOH to afford **1** as yellow plates (30 mg, 58% yield). ¹H NMR (500 MHz, DMSO-*d*₆): δ (ppm) 2.52-2.56 (1H, m, H-2'), 3.08-3.14 (1H, m, H-2'), 3.35 (1H, br s, OH-5'), 3.52-3.54 (1H, m, H-5'), 3.64-3.66 (1H, m, H-5'), 4.14 (1H, s, H-4'), 4.80 [2H, d, *J*_{H-P} = 5.5 Hz, CH₂ (Bn)], 5.03 (1H, s, H-3'), 6.55 (1H, t, *J* = 7 Hz, H-1'), 7.17 [1H, t, *J* = 7.5 Hz, Ph-*para* (Bn)], 7.24 [2H, t, *J* = 7.5 Hz, Ph-*meta* (Bn)], 7.33 [2H, d, *J* = 7.5 Hz, Ph-*ortho* (Bn)], 7.68 (2H, br s, NH₂), 7.94 [2H, dd, *J* = 5.5 and 3.5 Hz, H-6 (AQ) and H-7 (AQ)], 8.13 [1H, s, H-2 (dA)], 8.17 [1H, d, *J* = 8 Hz, H-3 (AQ)], 8.20-8.22 [3H, m, H-4 (AQ), H-5 (AQ) and H-8 (AQ)], and 8.32 [1H, s, H-1 (AQ)]. ¹H NMR (500 MHz, DMSO-*d*₆ + D₂O): δ (ppm) 2.52-2.56 (1H, m, H-2'), 3.11 (1H, s, H-2'), 3.50-3.53 (1H, m, H-5'), 3.65-3.67 (1H, m, H-5'), 4.15 (1H, s, H-4'), 4.79 [2H, s, CH₂ (Bn)], 5.0 (1H, s, H-3'), 6.55 (1H, s, H-1'), 7.18 [1H, d, *J* = 6 Hz, Ph-*para* (Bn)], 7.24 [2H, t, *J* = 6 Hz, Ph-*meta* (Bn)], 7.32 [2H, d, *J* = 6 Hz, Ph-*ortho* (Bn)],

7.94 [2H, d, $J = 2.5$ Hz, H-6 (AQ) and H-7 (AQ)], 8.11 [1H, s, H-2 (dA)], 8.14 [1H, d, $J = 8.5$ Hz, H-3 (dA)], 8.19-8.21 [3H, m, H-4 (AQ), H-5 (AQ) and H-8 (AQ)] and 8.30 [1H, s, H-1 (AQ)]. ^{13}C NMR (125 MHz, DMSO- d_6): δ (ppm) 45.22(C-2'), 62.38 (C-5'), 66.07 [d, $J_{C-P} = 4.6$ Hz, CH₂ (Bn)], 75.31 (d, $J = 20$ Hz, C-3'), 82.22 [dA-C (ethynyl)], 84.92, 86.96 (C-1' and C-4'), 92.73 [AQ-C (ethynyl)], 119.85 [C-5 (dA)], 125.57 [C-8 (dA)], 126.87, 127.07, 127.30, 128.07, 129.65, 132.07 [AQ and Ph (Bn)], 132.83, 132.97, 133.05, 133.16, 136.96, 134.75 (AQ) 139.28 [d, $J_{C-P} = 8.3$ Hz, Ph-C-1 (Bn)], 148.48 [C-4 (dA)], 153.75 [C-2 (dA)], 156.16 [C-6 (dA)], 181.55 (CO), and 181.66 (CO). ^{31}P NMR (202 MHz, DMSO- d_6): δ (ppm) -0.35. Low resolution ESI MS m/z (M-H)⁻: calc'd 650.14, found 650.11.

2.5.8. 5'-*O*-*tert*-Butyldiphenylsilyl-8-bromo-2'-deoxyadenosine (9)

Compound **3** (5 g, 15 mmol) was dried two times by co-evaporation with anhydrous pyridine, and then suspended in anhydrous pyridine (40 mL); imidazol (1.02 g, 15 mmol, 1 equiv) was added to this suspension in our glove-box. The mixture was removed from the glove-box and cooled to 0 °C using an ice-water bath. TBDPSCl (4.09 mL, 15.7 mmol, 1.05 equiv) was added dropwise (over 27 min) under nitrogen with a syringe. The pale yellow reaction mixture was warmed to room temperature, at which time the mixture became homogeneous. Stirring continued at room temperature under a nitrogen atmosphere, and the reaction progress was monitored with TLC. The starting materials were completely consumed after a total reaction time of 3 h. The reaction was quenched with MeOH (5 mL), and the solvent was evaporated. The residue was dissolved in EtOAc (500 mL) and washed with water (2 × 200 mL); the organic phase was dried over MgSO₄. The crude product was purified on a silica gel column that was eluted with MeOH/CH₂Cl₂ (0:100-3:97). Evaporation of the eluting solvent

afforded **9** as white foam (7.755 g, 91% yield). ^1H NMR (300 MHz, CDCl_3): δ (ppm) 1.02 [9H, s, *t*-Bu (TBDPS)], 2.3-2.41 (2H, m, H-2' and OH-3'), 3.53-3.62 (1H, m, H-2'), 3.84 (1H, dd, $J = 8.1$ and 10.2 Hz, H-5'), 3.98 (1H, dd, $J = 7.5$ and 10.2 Hz, H-5'), 4.04-4.1 (1H, m, H-4'), 4.99-5.08 (1H, m, H-3'), 5.6 (2H, br s, NH_2), 6.38 (1H, dd, $J = 6.3$ and 7.2 Hz, H-1'), 7.29-7.43 [6H, m, Ph-*ortho* and *para* (TBDPS)], 7.62 [4H, dt, $J = 1.5$ and 8.1 Hz, Ph-*meta* (TBDPS)], and 8.09 [1H, s, H-2 (dA)]. ^{13}C NMR (75 MHz, CDCl_3): δ (ppm) 19 [CMe_3 (TBDPS)], 26.67 [CH_3 (TBDPS)], 36.37 (C-2'), 63.68 (C-5'), 72.09 (C-3'), 86.04, 87.05 (C-4' and C-1'), 120.15 [C-5 (dA)], 127.51 [Ph-*meta* (TBDPS)], 127.66 [C-8 (dA)], 129.63 [Ph-*para* (TBDPS)], 133 [Ph-C-1 (TBDPS)], 135.38 [Ph-*ortho* (TBDPS)], 150.55 [C-4 (dA)], 152.38 [C-2 (dA)], and 154.28 [C-6 (dA)]. Low resolution ESI MS m/z (M+H) $^+$: Calc'd 568.14, found 568.13.

2.5.9. 5'-*O*-*tert*-Butyldiphenylsilyl-8-[(anthraquinone-2-yl)ethynyl]-2'-deoxyadenosine (**10**)

To **9** (1.914 g, 3.37 mmol) was added in our glove-box $\text{Pd}(\text{Ph}_3\text{P})_4$ (194 mg, 0.17 mmol, 0.05 equiv), CuI (65 mg, 0.34 mmol, 0.1 equiv), TEA (0.94 mL, 6.7 mmol, 2 equiv), compound **6** (1.016 g, 4.38 mmol, 1.3 equiv), and anhydrous DMF (29 mL). The mixture was removed from our glove-box and stirred under a nitrogen atmosphere in the dark for 6 h at 65 °C in an oil bath. DMF was removed under reduced pressure, and the residue was dissolved in 10% MeOH in CH_2Cl_2 ; insoluble materials were removed by filtration over Celite/Celite. After evaporation the crude product was purified with two silica gel columns using MeOH/ CH_2Cl_2 (0:100-3:97) as the eluting system to yield yellow foam after evaporation of the eluting solvent. This foam was dissolved in a minimum amount of CH_2Cl_2 and precipitated by adding hexane. Evaporation of CH_2Cl_2 /hexane from the precipitate afforded **10** as a bright yellow solid (2.19 g, 90%). ^1H NMR (300 MHz, CDCl_3): δ (ppm) 1.03 [9H, s, *t*-Bu (TBDPS)], 2.45-2.53 (1H, m, H-2'), 2.63 (1H, d,

$J = 3$ Hz, OH-3'), 3.5-3.59 (1H, m, H-2'), 3.89 (1H, dd, $J = 5.1$ and 10.2 Hz, H-5'), 4.03 (1H, dd, $J = 7.8$ and 10.2 Hz, H-5'), 4.13-4.19 (1H, m, H-4'), 5-5.08 (1H, m, H-3'), 5.96 (2H, br s, NH₂), 6.32 (1H, t, $J = 6.9$ Hz, H-1'), 7.29-7.42 [6H, m, Ph-*ortho* and *para* (TBDPS)], 7.63 [4H, t, $J = 6.6$ Hz, Ph-*meta* (TBDPS)], 7.79 [2H, t, $J = 3.6$ Hz, H-6 (AQ) and H-7 (AQ)], 7.88 [1H, d, $J = 8.1$ Hz, H-3 (AQ)], 8.12 [1H, s, H-2 (dA)], 8.25 [1H, d, $J = 8.1$ Hz, H-4 (AQ)], 8.27-8.32 [2H, m, H-5 (AQ) and H-8 (AQ)], and 8.48 [1H, s, H-1 (AQ)]. ¹³C NMR (100 MHz, CDCl₃): δ (ppm) 19.17 [CMe₃ (TBDPS)], 26.80 [CH₃ (TBDPS)], 37.02 (C-2'), 64.12 (C-5'), 73.05 (C-3'), 81.78 [dA-C (ethynyl)], 85.19, 87.02 (C-4' and C-1'), 93.74 [AQ-C (ethynyl)], 120.01 [C-5 (dA)], 126.32 [C-8 (dA)], 127.30 and 127.67 [Ph-*meta* (TBDPS), and AQ], 129.74 [Ph-*para* (TBDPS)], 130.80 (AQ), 133.10, 133.30, 134.02, 134.27, 134.32 [Ph-C-1 (TBDPS) and AQ], 135.48 [Ph-*ortho* (TBDPS)], 136.47 (AQ), 149.12 [C-4 (dA)], 153.69 [C-2 (dA)], 155.24 [C-6 (dA)], 181.89 (CO), and 182.06 (CO). ESI MS m/z for C₄₂H₄₂N₅O₅Si (M+1): calc'd 724.90, found 724.20. Low resolution ESI MS m/z (M+H)⁺: Calc'd 720.26, found 720.25.

2.5.10. 5'-*O*-*tert*-Butyldiphenylsilyl-8-[2-(anthraquinone-2-yl)ethyl]-2'-deoxyadenosine (11)

Compound **10** (940 mg, 1.3 mmol) was dissolved in EtOAc (60 mL) by slight heating while stirring; MeOH (90 mL) was then added to the solution. The resulting solution was transferred to a hydrogenation vessel containing 10% Pd/C (500 mg) that had been activated by stirring under H₂ (40 psi) in MeOH (20 mL) for 30 min at room temperature. The vessel was next charged with H₂ gas and then degassed using an aspirator in a cycle that was repeated 5-6 times. The vessel was finally charged with hydrogen gas at 40 psi and stirred at room temperature for 24 h, by which time TLC showed complete conversion of an initial spot to a more polar spot. The Pd/C catalyst was then removed by filtration over Celite, and adsorbed nucleoside residue was extracted from the catalyst by washing with boiling 10% MeOH in

CHCl₃. The crude product was applied to a silica gel column and eluted with MeOH/CH₂Cl₂ (0:100-3:97). Evaporation of the eluting solvent afforded **11** as a pale yellow foam (810 mg, 86% yield). ¹H NMR (300 MHz, CDCl₃): δ (ppm) 0.98 [9H, s, *t*-Bu (TBDPS)], 2.28 (1H, ddd, *J* = 3.9, 6.9 and 13.2 Hz, H-2'), 3.03 (1H, br s, OH-3'), 3.19-3.4 (4H, m, ethylene), 3.58-3.68 (1H, m, H-2'), 3.8 (1H, dd, *J* = 4.8 and 10.5 Hz, H-5'), 3.96 (1H, dd, *J* = 6.9 and 10.5 Hz, H-5'), 4.05-4.1 (1H, m, H-4'), 4.92-4.98 (1H, m, H-3'), 5.67 (2H, br s, NH₂), 6.25 (1H, t, *J* = 6.9 Hz, H-1'), 7.26-7.38 [6H, m, Ph-*ortho* and *para* (TBDPS)], 7.52-7.66 [5H, m, Ph-*meta* (TBDPS) and H-3 (AQ)], 7.79 [2H, t, *J* = 4.5 Hz, H-6 (AQ) and H-7 (AQ)], 8.09 [1H, s, H-2 (dA)], 8.16 [1H, d, *J* = 7.8 Hz, H-4 (AQ)], 8.2 [1H, d, *J* = 1.8 Hz, H-1 (AQ)], and 8.24-8.3 [2H, m, H-5 (AQ) and H-8 (AQ)]. ¹³C NMR (125 MHz, CDCl₃): δ (ppm) 19.17 [CMe₃ (TBDPS)], 26.79 [CH₃ (TBDPS)], 29.16 [dA-CH₂ (ethyl)], 33.35 [AQ-CH₂ (ethyl)], 36.86 (C-2'), 64.02 (C-5'), 72.69 (C-3'), 84.18, 86.76 (C-4' and C-1'), 118.96 [C-5 (dA)], 127.17 [C-8 (dA)], 127.65 [Ph-*meta* (TBDPS)], 129.73 [Ph-*para* (TBDPS)], 131 (AQ), 133.2, 133.52, 133.63 [Ph-C-1 (TBDPS) and AQ], 135.49 [Ph-*ortho* (TBDPS)], 147.52 (AQ), 150.84 [C-4 (dA)], 151.92 [C-2 (dA)], 154.62 [C-6 (dA)], 182.75 (CO), and 183.14 (CO). Low resolution ESI MS *m/z* (M+H)⁺: Calc'd 724.30, found 724.25.

2.5.11. 5'-*O*-*tert*-Butyldiphenylsilyl-8-[2-(anthraquinone-2-yl)ethyl]-2'-deoxyadenosine 3'-Dibenzyl Phosphate (12)

To **11** (750 mg, 1.036 mmol), previously dried two times by co-evaporation with anhydrous THF, was added Me-tetrazole (193 mg, 2.28 mmol, 2.2 equiv) and anhydrous THF (7 mL) in our glove-box. The mixture was removed from our glove-box and cooled to 0 °C using an ice-water bath, and (BnO)₂PN(*i*Pr)₂ (0.41 mL, 1.24 mmol, 1.2 equiv) was added dropwise

under nitrogen with a syringe. The reaction mixture was stirred under a nitrogen atmosphere for 10 minutes at 0 °C and then allowed to warm to room temperature with continued stirring for a total reaction time of 1 h. A white precipitate began to form after 20 min of stirring, and TLC showed formation of a spot that was less polar than the starting material. This new TLC spot did not increase with additional stirring. The suspension was cooled to -78 °C using a dry ice-acetone bath, and a solution of *m*-CPBA (375 mg) in CH₂Cl₂ (7 mL) was added gradually to the chilled mixture. After 15 minutes of stirring at -78 °C, the mixture became homogeneous, and TLC showed conversion of an initial spot to a more polar one. The mixture was next warmed to room temperature, and saturated aqueous NaHCO₃ (8 mL) was added. More CH₂Cl₂ (50 mL) and water (20 mL) were then added. Finally, the organic layer was separated, washed with water, dried with anhydrous MgSO₄, and evaporated to dryness. The syrup obtained was purified on silica gel column (16 cm x 2.5 cm dia.) that was eluted with MeOH/CHCl₃ (0:100-3:97). Evaporation of the eluting solvent afforded **12** as pale yellow foam (833 mg, 82% yield). ¹H NMR (300 MHz, CDCl₃): δ (ppm) 0.99 [9H, s, t-Bu (TBDPS)], 2.4 [1H, ddd, *J* = 2.1, 6 and 13.8 Hz, H-2''], 3.18-3.37 (4H, m, ethylene), 3.70 (1H, dd, *J* = 5.4 and 11.1 Hz, H-5'), 3.82-3.90 (1H, m, H-2'), 3.96 (1H, dd, *J* = 6.6 and 11.1 Hz, H-5'), 4.23-4.28 (1H, m, H-4'), 5-5.12 [4H, m, CH₂ (Bn)], 5.35-5.39 (1H, m, H-3'), 5.54 (2H, br s, NH₂), 6.4 (1H, dd, *J* = 6 and 7.8 Hz, H-1'), 7.23-7.4 [16H, m, Ph-*ortho* and *para* (TBDPS, 6H) and Ph (Bn, 10H)], 7.54-7.59 [4H, m, Ph-*meta* (TBDPS)], 7.62 [1H, dd, *J* = 1.8 and 8.1 Hz, H-3 (AQ)], 7.76-7.82 [2H, m, H-6 (AQ) and H-7 (AQ)], 7.98 [1H, s, H-2 (dA)], 8.21 [1H, d, *J* = 8.1 Hz, H-4 (AQ)], 8.2 [1H, d, *J* = 1.8 Hz, H-1 (AQ)], and 8.23-8.32 [2H, m, H-5 (AQ) and H-8 (AQ)]. ¹³C NMR (125 MHz, CDCl₃): δ (ppm) 19.13 [CMe₃ (TBDPS)], 26.69 [CH₃ (TBDPS)], 29.05 [dA-CH₂ (ethyl)], 33.07 [AQ-CH₂ (ethyl)], 34.68 (C-2'), 63.04 (C-5'), 69.31-69.68 [m, CH₂ (Bn)], 78.66 (C-3'), 84.18, 86.76 (C-4'

and C-1'), 118.85 [C-5 (dA)], 127.13 [C-8 (dA)], 127.58 [Ph-*meta* (TBDPS)], 128.02, 128.6 [Ph (Bn)], 129.68 [Ph-*para* (TBDPS)], 131.88 (AQ), 132.95 [d, $J_{P-C} = 6.1$, Ph-C-1 (Bn)], 133.43, 133.44, 133.52 [Ph-C-1 (TBDPS) and AQ], 134.04, 134.3 [Ph (Bn)], 135.48 [Ph-*ortho* (TBDPS)], 147.42 (AQ), 150.86 [C-4 (dA)], 151.80 [C-2 (dA)], 154.48 [C-6 (dA)], 182.82 (CO), and 183.16 (CO). ^{31}P NMR (121 MHz, CDCl_3): δ (ppm) -1.19. Low resolution ESI MS m/z (M+H) $^+$: Calc'd 984.36, found 984.31.

2.5.12. 8-[2-(Anthraquinone-2-yl)ethyl]-2'-deoxyadenosine 3'-Dibenzyl Phosphate (13)

Compound **12** (375 mg, 0.38 mmol) was dissolved in THF (1.5 mL) by stirring at 60 °C for 1 min, and then MeOH (10 mL) was added. To this solution was added NH_4F (162 mg, 4.38, 11.5 equiv), and the reaction mixture was stirred at 60 °C for 6 h by which time TLC showed nearly complete consumption of the starting material. The solvent was reduced in volume, and the residue was adsorbed on silica gel. The adsorbed residue was applied to flash silica gel on a biotage column that was eluted with MeOH/ CHCl_3 (0:100-5:95). Evaporation of the eluting solvent yielded pale yellow foam. This foam was dissolved in a minimum amount of CH_2Cl_2 and precipitated by adding hexane. Evaporation of CH_2Cl_2 /hexane from the precipitate afforded **13** as pale yellow powder (250 mg, 88% yield). ^1H NMR (300 MHz, CDCl_3): δ (ppm) 2.22 (1H, dd, $J = 5.4$ and 14.1 Hz, H-2'), 3.02-3.38 (5H, m, ethylene, and H-2'), 3.55 (1H, d, $J = 12.9$ Hz, H-5'), 3.8 (1H, d, $J = 12.9$ Hz, H-5'), 4.21 (1H, s, H-4'), 5.0-5.17 [5H, m, CH_2 (Bn), and H-3'], 5.77 (2H, br s, NH_2), 6.13 (1H, dd, $J = 5.1$ and 9.9 Hz, H-1'), 6.73 (1H, br s, OH-5'), 7.31-7.38 [10H, m, Ph (Bn)], 7.65 [1H, dd, $J = 1.5$ and 7.8 Hz, H-3 (AQ)], 7.78-7.82 [2H, m, H-6 (AQ) and H-7 (AQ)], 8.22-8.33 [5H, m, H-2 (dA), H-4 (AQ), H-1 (AQ), H-5 (AQ), and H-8 (AQ)]. ^{13}C NMR (125 MHz, CDCl_3): δ (ppm) 29.01 [dA- CH_2 (ethyl)], 33.01 [AQ- CH_2 (ethyl)], 38.16 (C-

2'), 63.01 (C-5'), 69.71 [d, $J_{P-C} = 1$ Hz, CH₂ (Bn)], 80.21 (C-3'), 85.67, 87.60 (C-1' and C-4'), 119.38 [C-5 (dA)], 127.22 [C-8 (dA)], 127.83, 128.20, 128.7, 128.83 [AQ and Ph (Bn)], 132.05 (AQ), 133.45 [d, $J_{P-C} = 3.1$, Ph-C-1 (Bn)], 133.63 (AQ), 134.09, 134.20, 134.32, 135.41, 135.46 [AQ and Ph (Bn)], 147.01, 149.78 (AQ), 150.62 [C-4 (dA)], 151.75 [C-2 (dA)], 155.08 [C-6 (dA)], 182.82 (CO), and 183.17 (CO). ³¹P NMR (162 MHz, CDCl₃): δ (ppm) -1.23. Low resolution ESI MS m/z (M+H)⁺: Calc'd 746.24, found 746.24.

2.5.13. 8-[2-(Anthraquinone-2-yl)ethyl]-2'-deoxyadenosine 3'-Benzyl Hydrogen Phosphate (2)

Compound **13** (210 mg, 0.28 mmol) was dissolved in a mixture of EtOAc (10 mL) and MeOH (80 mL). The resulting solution was transferred to a hydrogenation vessel containing 10% Pd/C (30 mg) that had been activated by stirring under H₂ (40 psi) in MeOH (20 mL) for 30 min at room temperature. The vessel was next charged with H₂ gas and then degassed using an aspirator in a cycle that was repeated 5-6 times. The vessel was finally charged with hydrogen gas at 40 psi and stirred at room temperature for 24 h at which time TLC showed that some **13** was still present. More 10% Pd/C (20 mg) was added, and the hydrogenation vessel was recharged with H₂ as described above. The mixture was stirred at room temperature for an additional 7 h under H₂ gas (45 psi) by which time TLC showed complete consumption of **13**. The Pd/C catalyst was then removed by filtration over Celite, and adsorbed nucleotide residue was extracted from the catalyst by washing with boiling MeOH. The crude product was then purified by preparative HPLC using a Varian Microsorb C-18 reverse phase column (250 mm × 41.4 mm dia.). The column was eluted with a flow rate of 40 mL/min, and the fractions were monitored with UV detection at 260 nm. The mobile phase consisted of a programmed gradient

of solution A (70% MeCN in water) and solution B (water): from 0% A/100% B to 50% A/50% B over 15 min; then to 100% A/0% B over 5 min, and finally at 100%A/0% B for another 10 min. Evaporation of the eluent was facilitated by co-evaporation with MeOH and afforded **2** as yellow plates (30 mg, 22% yield). ^1H NMR (500 MHz, DMSO- d_6): δ (ppm) 2.33 (1H, ddd, $J = 3.5, 6.5$ and 13 Hz, H-2'), 2.98-3.04 (1H, m, H-2'), 3.25-3.4 (4H, m, ethylene), 3.45-3.49 (1H, m, H-5'), 3.5-3.58 (1H, m, H-5'), 4.01 (1H, d, $J = 3$ Hz, H-4'), 4.71 [2H, d, $J_{H-P} = 5$ Hz, CH₂ (Bn)], 4.87-4.96 (1H, m, H-3'), 6.12 (1H, br d, $J = 7$ Hz, OH-5'), 6.28 (1H, t, $J = 7$ Hz, H-1'), 7.15 (2H, br s, NH₂), 7.18 [1H, t, $J = 7.5$ Hz, Ph-*para* (Bn)], 7.25 [2H, t, $J = 7.5$, Ph-*meta* (Bn)], 7.33 [2H, d, $J = 7.5$ Hz, Ph-*ortho* (Bn)], 7.87 [1H, d, $J = 8$ Hz, H-3 (AQ)], 7.91-7.93 [2H, m, H-6 (AQ) and H-7 (AQ)], 8.05 [1H, s, H-2 (dA)], 8.12-8.14 [2H, m, H-1 (AQ) and H-4 (AQ)], and 8.18-8.22 [2H, m, H-5 (AQ) and H-8 (AQ)]. ^1H NMR (500 MHz, DMSO- d_6 + D₂O): δ (ppm) 2.3-2.38 (1H, m, H-2'), 2.98-3.04 (1H, m, H-2'), 3.25-3.4 (4H, m, ethylene), 3.52-3.6 (2H, m, H-5'), 4.05 (1H, s, H-4'), 4.71 [2H, d, $J_{H-P} = 5$ Hz, CH₂ (Bn)], 4.86 (1H, s, H-3'), 6.27 (1H, t, $J = 7$ Hz, H-1'), 7.18 [1H, t, $J = 7.5$ Hz, Ph-*para* (Bn)], 7.25 [2H, t, $J = 7.5$, Ph-*meta* (Bn)], 7.32 [2H, d, $J = 7.5$ Hz, Ph-*ortho* (Bn)], 7.87 [1H, d, $J = 8$ Hz, H-3 (AQ)], 7.91 [2H, s, H-6 (AQ) and H-7 (AQ)], 8.04 [1H, s, H-2 (dA)], 8.1-8.13 [2H, m, H-1 (AQ) and H-4 (AQ)], and 8.19 [2H, m, H-5 (AQ) and H-8 (AQ)]. ^{13}C NMR (125 MHz, DMSO- d_6): δ (ppm) 28.34 [dA-CH₂ (ethyl)], 32.72 [AQ-CH₂ (ethyl)], 37.15 (C-2'), 62.42 (C-5'), 65.96 [d, $J_{C-P} = 1$ Hz, CH₂ (Bn)], 75.4 (d, $J = 20.3$ Hz, C-3'), 84.16 (C-4'), 86.79 (C-1'), 118.18 [C-5 (dA)], 126.71 [C-8 (dA)], 127.03, 128.05, 131.28 [AQ and Ph (Bn)], 132.98, 133, 134.52, 134.92 (AQ), 139.5 [d, $J_{C-P} = 7.4$, Ph-C-1 (Bn)], 148.15, 149.79 (AQ), 150.82 [C-4 (dA)], 151.63 [C-2 (dA)], 155.49 [C-6 (dA)], 182.22 (CO), and 182.53 (CO). ^{31}P NMR (202 MHz, DMSO- d_6): δ (ppm) 0.77. Low resolution ESI MS m/z (M-H)⁻: Calc'd 654.18, found 654.20.

2.5.14. 2-Iodoanthraquinone (**15**)

To a suspension of **14** (3.06 g, 0.137 mol) in THF (21 mL) was added HCl (28 mL) and water (7 mL). The mixture was stirred at 40 °C for 24 h to give a thick, rosey beige suspension. This suspension was cooled to 0 °C using an ice-water bath, and a solution of NaNO₂ (1.90 g, 0.027 mol, 2 equiv) in H₂O (6.5 mL) was added dropwise with a syringe. A solution of KI (5.69 g, 0.034 mol, 2.5 equiv) in H₂O (12.5 mL) was added gradually to the reaction mixture while stirring at 0 °C. The reaction mixture was then stirred for an additional 15 min at 0 °C, for 30 min at room temperature, and finally for 30 min at 60 °C. THF was evaporated from the mixture, and the residue was filtered. The precipitate was collected and washed first with water and then with a saturated solution of aqueous Na₂CO₃. The residue was dissolved in CHCl₃ (54 mL), and the solution was shaken with an aqueous solution of Na₂S₂O₃. The CHCl₃ layer was separated, dried with anhydrous MgSO₄, and evaporated to dryness. Three consecutive silica gel columns eluted with CH₂Cl₂/hexane (10:90-30:70) afforded **15** as yellow powder (3.42 g, 75% yield). ¹H NMR (300 MHz, CDCl₃): δ (ppm) 7.83 (2H, m, H-7), 8.01 (1H, d, *J*= 8.4 Hz, H-4), 8.16 (1H, dd, *J*= 8.4 and 1.5 Hz, H-3), 8.30-8.33, (2H, m, H-5, and H-8), and 8.66 (1H, s, H-1). ¹³C NMR (75 MHz, CDCl₃): δ (ppm) 127.30, 128.6, 132.5, 133, 133.27, 134, 134.34, 136.23, 143.06, 181.87, and 182.58. Low resolution EI MS *m/z* (M⁺): 334.

2.5.15. 2-(Trimethylsilanylethynyl)anthraquinone (**16**)

Pd(Ph₃P)₂Cl₂ (24 mg, 0.03 mmol, 0.05 equiv), CuI (13.1 mg, 0.07 mmol, 0.1 equiv), TEA (0.19 mL, 1.38 mmol, 2 equiv), and **15** (229.8 mg, 0.69 mmol) were combined and stirred in dry THF (4.6 mL) under a nitrogen atmosphere until dissolved; then TMSA (0.23 mL, 2.064 mmol, 3 equiv) was added. After 10 minutes of stirring at room temperature, TLC (CH₂Cl₂/hexanes,

1:1 v:v) showed complete consumption of **15**. The solvent was evaporated *in vacuo*, and the crude material was purified by flash silica gel chromatography (CH₂Cl₂/hexanes, 0:100-20:80 v:v) to give **16** as pale yellow solid (210 mg, 96% yield). ¹H NMR (300 MHz, CDCl₃): δ (ppm) 0.3 (9H, s, 3 × CH₃), 7.84-7.77 (3H, m, H-4, H-6, and H-7), 8.234 (1H, dd, *J*= 8.1 and 0.3 Hz, H-3), 8.319-8.268 (2H, m, H-5, and H-8), and 8.35 (1H, dd, *J*= 1.5 and 0.3 Hz, H-1). ¹³C NMR (75 MHz, CDCl₃): δ (ppm) -0.27, 100.11, 103.14, 126.21, 127.26, 127.29, 129.30, 130.62, 132.44, 133.26, 133.33, 133.45, 134.17, 134.26, 136.80, 182.43, and 182.47. Low resolution EI MS *m/z* (M⁺): 304.

2.5.16. 2-Ethynylantraquinone (**6**)

To a solution of **16** (200 mg, 0.657 mmol) in THF/ MeOH (1:1 v:v, 26 mL) was added potassium fluoride (57.26 mg, 0.985 mmol, 1.5 equiv) at room temperature. After 1 hour the solvent was evaporated; the product was extracted with CHCl₃, shaken with charcoal, and recrystallized from CHCl₃ to afford **6** as brown solid (150 mg, 99% yield). NMR spectra were run on a pale yellow, silica-gel purified portion of this product. ¹H NMR (300 MHz, CDCl₃): δ (ppm) 3.37 (1H, s, ≡C-H), 7.82 (2H, dd, *J*= 5.7 and 3.3 Hz, H-6, H-7), 7.67 (1H, dd, *J*= 8.1 and 1.8 Hz, H-3), 8.34-8.27 (3H, m, H-4, H-5 and H-8), and 8.41 (1H, d, *J*= 1.5 Hz, H-1). ¹³C NMR (75 MHz, CDCl₃): δ (ppm) 81.88, 127.33, 130.85, 134.3, 134.36, 137.1, and 182.42. EI MS *m/z* (M⁺): 232.

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2.7. References.

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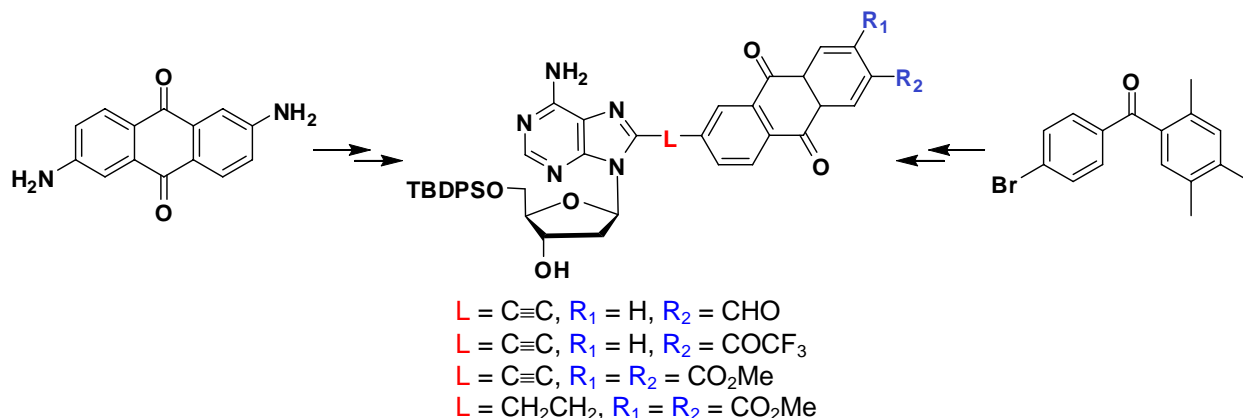
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Chapter 3

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



3.1. Abstract

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 (A) 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 A via two carbon atom linkers should enable the intended regiocontrol within the DNA duplex structure. Herein we report the synthesis of new conjugates between AQ and A, 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.

3.2. Introduction

Oxidation of guanine (G) to 8-oxoguanine is the major oxidative damage in DNA that is implicated in mutagenesis, carcinogenesis and ageing.^{1,2} This DNA lesion occurs predominantly at G clusters,³ because initial cation radical (hole) on G migrates to GG and GGG sequences. Many studies have been conducted in order to investigate the details of this important phenomenon.³⁻¹² In many instances anthraquinone (AQ) has been used as an electron acceptor chromophore to initiate DNA oxidation, when photoexcited by UV light.¹³⁻¹⁵ In these systems, because the singlet excited state of AQ lives for less than 1 ps,¹⁶ it is the triplet excited state of AQ that gives 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.¹⁶

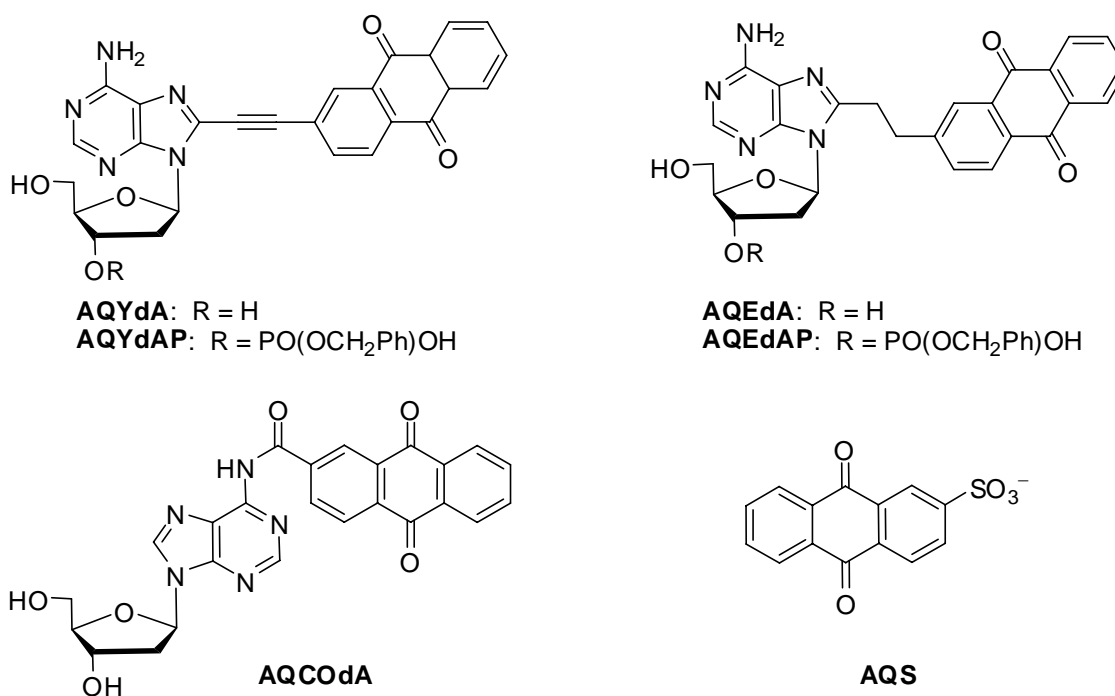
If the position of the initial radical on DNA is to be pinpointed, it is necessary to have the AQ covalently bound to the DNA. This should minimize side reactions such as oxidation of distant bases and charge recombination during hole migration along the DNA duplex. A variety

of AQ derivatives linked to DNA have been synthesized, including those attached to the 5'-end phosphate group,^{17,18} the C-2' of a sugar,^{19,20} a phosphate of the DNA backbone,²¹ and individual bases in the DNA strand.^{22,23} 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 in the DNA.^{22,24,25}

In general, oxidized DNA gives final products due to reactions at a G radical cation ($G^{\bullet+}$) site. However, a recent study on one-electron oxidation of DNA linked to AQ showed not only the expected reaction at GG doublets, but also products from thymine (T) residues.²¹ Another study also on DNA linked to AQ, in which the DNA had only adenine (A) and thymine residues, found that T formed oxidation products in preference to A.^{26,27} Since A is easier to oxidize than T,^{28,29} this finding was unexpected. These observations were attributed to the significantly higher reactivity of the T radical cation ($T^{\bullet+}$) compared to that of the A radical cation ($A^{\bullet+}$). On the other hand, it has been shown that oxidation of A in a consecutive A stretch, rather than oxidation of G, results in a higher yield of DNA damage.³⁰

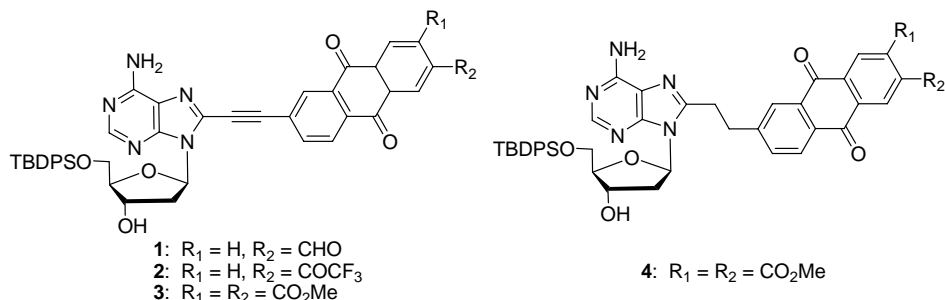
Our previous work showed that $A^{\bullet+}$ can be formed by photolysis of a system containing both A and AQ.¹⁶ Whether or not an AQ-A conjugate forms the $AQ^{\bullet-}/A^{\bullet+}$ CT photoproduct depends on the details of the system studied. For example, irradiation of the AQ-A conjugates AQYdA or AQEdA in methanol³¹ or AQYdAP or AQEdAP³² in water³³ 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.¹⁶ In addition, photoexcitation of a bimolecular solution of 2'-deoxyadenosine (dA) and anthraquinone-2-sulfonate (AQS) in water produced the $AQ^{\bullet-}/A^{\bullet+}$ CT

product.³¹ 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. Indeed, most literature examples of DNA oxidation with AQ have employed derivatives with an amide carbonyl^{17,18,21,22,34} or sulfonyl^{35,36} attached to the AQ.



Future studies in the area of A oxidation would be greatly aided by access to a series of AQ-dA derivatives with different driving forces for CT. Our work and literature studies indicate that these AQ derivatives should have electron withdrawing substituents on the AQ ring. In such systems, the AQ can oxidize its covalently attached A, giving a precise point of initial DNA oxidation. 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. Electrochemical potentials of these conjugates were measured in acetonitrile versus saturated calomel electrode (SCE).



3.3. Results and Discussion

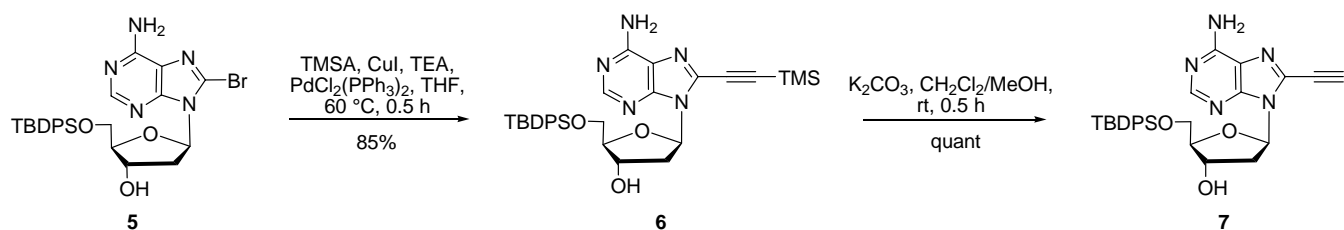
Our choice of the substituents to modify AQ was not based only on the feasibility of their preparations, but also on the compatibility of such substituents toward a variety of conditions used in the conventional DNA synthesis protocols. The final target conjugates required protection of at least one 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.

3.3.1. Synthesis of deoxyadenosine substrate

In our previous work³² 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-ethynylantraquinone permitted a second Pd coupling with 5'-protected 8-bromo-dA to form the desired AQ-dA conjugates. However, working with the ethynylantraquinone imposed synthetic

constraints due to its poor solubility, photoinstability, and the chromatographic similarity of the protected bromo-dA starting material and AQ-dA products.

SCHEME 1



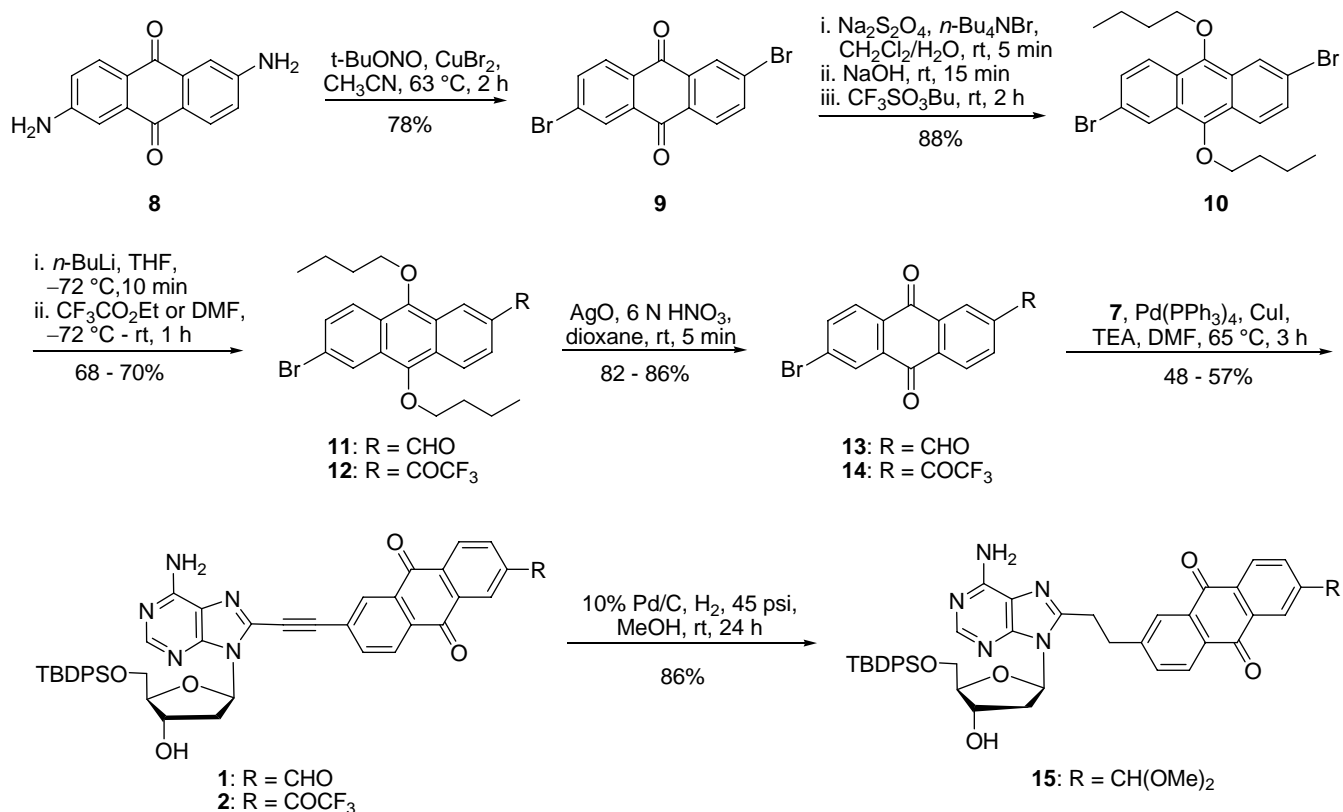
Therefore, in our present work, we switched the order of the alkylation steps. Protected 8-bromo-2'-deoxyadenosine **5**³² was first coupled with TMSA using Pd-based chemistry as illustrated in Scheme 1 to give the protected alkyne **6**. The yield of this step was greatly affected by the interval between 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 K₂CO₃ as an oxide³⁷ 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.

3.3.2. Modification of AQ moiety with a formyl group

We chose to introduce the formyl group via lithiation of a bromide on AQ using butyl lithium. 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 substitutions³⁸⁻⁴⁰ or Diels-Alder reactions⁴¹ were avoided in order to circumvent obtaining regioisomers. The feasibility of monolithiation of 2,6-dihaloanthraquinone was key in this synthesis.

SCHEME 2



As illustrated in Scheme 2, the initial step was transformation of 2,6-diaminoanthraquinone (**8**) to the dibromo derivative **9** as described in the literature⁴² 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.⁴²⁻⁴⁵ 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. Even though the literature⁴² 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 Na₂S₂O₄ in phase transfer catalysis gave the dibutylanthracene derivatives **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 $-72\text{ }^{\circ}\text{C}$; 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 $-72\text{ }^{\circ}\text{C}$, resulting in reduced yields of monosubstitution with the formyl group.

Oxidative dealkylation of **11** to the anthraquinone aldehyde **13** was effected using AgO/HNO₃ in dioxane⁴⁶ for 5 min or PhI(OCOFCF₃)₂ in water⁴⁷ 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 a 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 unprotected formyl.⁴⁸ The formyl group has also been introduced into DNA

using the phosphorimidite of 3-formylindole 2'-deoxynucleoside directly without protection,⁴⁹ indicating that this group can survive DNA synthesis conditions.

3.3.3. Modification of 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/HNO₃ gave the desired product **14**, and was preferable to PhI(OCOCF₃)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 ¹H NMR resonances for H-5 at both 8.92 ppm (ketone) and 8.56 ppm (gem diol), and ¹⁹F NMR resonances at both -72.34 (ketone) and -83.90 (gem diol) ppm. COCF₃ moieties are known to hydrate.⁵⁰⁻⁵³ Following a protocol in the literature,⁵⁴ shaking of **14** in ethyl acetate with a solution of NaHCO₃ in water, followed by removal of the ethyl acetate, gave the ketone exclusively as shown by NMR.

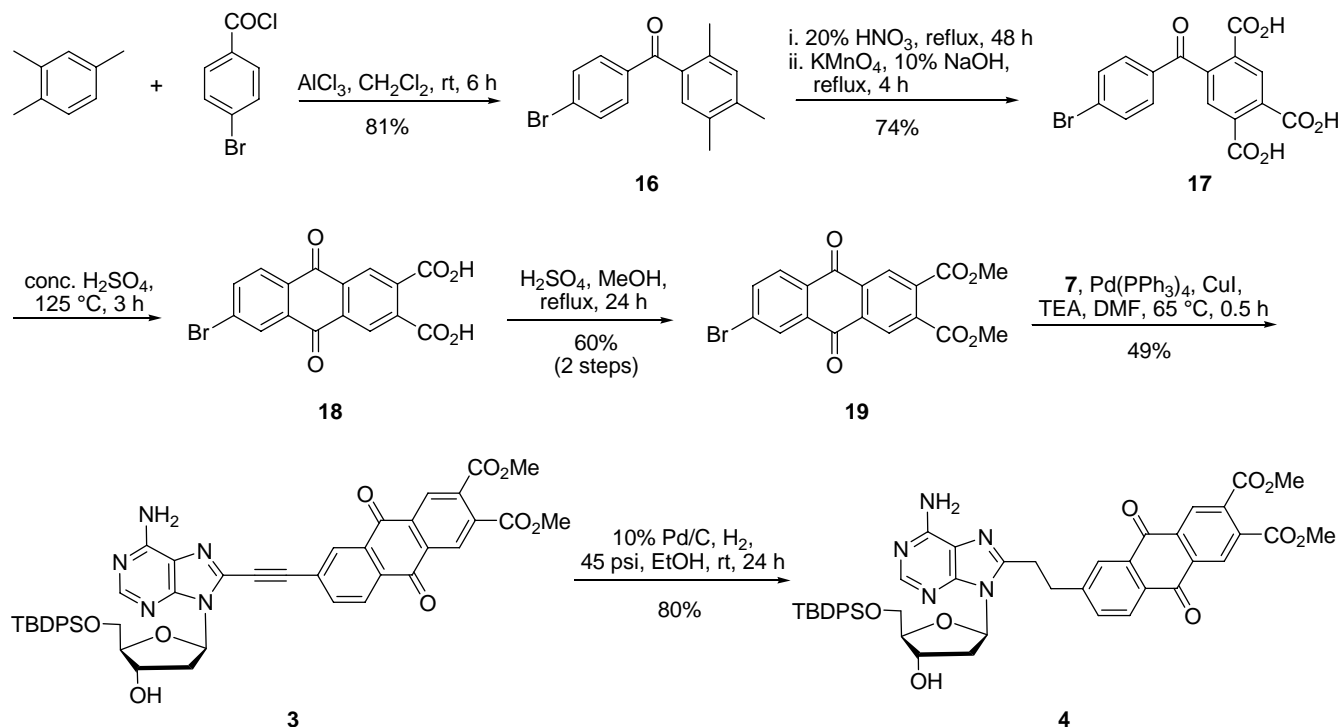
Conjugate **2** was synthesized by Pd coupling of anthraquinone **14** to **7** as described above. The product was isolated via silica gel purification using a MeOH/EtOAc/hexane solvent mixture. ¹⁹F NMR in CDCl₃/CD₃OD showed a single resonance at -83.57 ppm. The mass spectrum indicated that the majority of the product was the geminal diol (rather than the hemiketal⁵⁵ or ketal). Treatment of the diol form of **2** with NaHCO₃ as described for **14** did not result in reversion to the ketone form. However, when conjugate **2** was dissolved in a saturated solution of K₂CO₃ in methanol and stirred overnight, treated with water, and extracted with chloroform, the ketone form was obtained. This was confirmed by the ¹⁹F 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 .

3.3.4. 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.^{56,57} 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 by-product that was difficult to remove by either fractional distillation or chromatography. This by-product 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^{58,59}). 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 $-20\text{ }^\circ\text{C}$. Fractional crystallization of the crude product from ethyl acetate/methanol allowed separation of pure **16**.

SCHEME 3



The tricarboxylic acid **17** was obtained by refluxing **16** in 20% aqueous HNO₃ to give an alkali-soluble mixture of monocarboxylic acids⁶⁰ that was further oxidized with KMnO₄ in 4% NaOH. To prevent any possible displacement of the bromide on the product by hydroxide, the crude product mixture was taken to pH \approx 9 before concentration. Cyclodehydration of **17** was effected by conc. H₂SO₄ to give the bromo anthraquinone **18**. Removal of all H₂SO₄ 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 H₂SO₄ 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**.

Solid phase DNA synthesis generally employs ammonia in the final deprotection step. However, ammonia can react with methyl esters to form amides⁶¹ which can further hydrolyze under basic conditions to the corresponding carboxylic acids.⁶² The ammonia step can be replaced by treatment with 0.05 M K₂CO₃ in methanol; the AQ dimethyl ester was stable to these milder conditions.

3.3.5. Electrochemical results

Table 3.1. Reduction potentials of AQ and AQdA conjugates in CH₃CN.

Compound	Linker with dA	substituent	E _{1/2} (SCE)
AQ	none	none	-0.939
AQYdA-5'-O-TBDPS	C≡C	none	-0.810
AQEdA-5'-O-TBDPS	CH ₂ CH ₂	none	-0.935
AQCOdA-3',5'-di-O-Ac	amide	amide	-0.734
1	C≡C	CHO	-0.639
3	C≡C	di(CO ₂ Me)	-0.570
4	CH ₂ CH ₂	di(CO ₂ Me)	-0.703

Reduction potentials for selected AQ-dA conjugates were measured in acetonitrile versus SCE (Table 1). For these conjugates, the cyclic voltammogram (CV) is reported only for the first one electron reduction of the AQ, because the second was not reversible. The conjugates synthesized herein were compared with previous conjugates AQYdA, AQEdA and AQCOdA, for which photophysical studies have been performed.^{16,31,33} To increase the solubility in

acetonitrile for the electrochemistry measurements, the AQYdA and AQEdA conjugates were studied as the 5'-*O*-TBDPS derivatives³² and AQCOdA conjugate was studied as the 3',5'-di-*O*-Ac derivative.¹⁶ AQCOdA-3',5'-di-*O*-Ac had a reduction potential of -0.735 V, making it easier to reduce than AQ itself by 200 mV. This ease of reduction is consistent with the observation that this conjugate undergoes charge transfer upon photoexcitation.¹⁶

Two conjugates with ethanyl linkers were investigated. The reduction potential of AQEdA-5'-*O*-TBDPS conjugate 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. With the same linker, diester substitution (**4**) gave a conjugate easier to reduce by 240 mV. The conjugate bearing a CH(OMe)₂ group on the anthraquinone (**15**) did not show reversible electrochemistry.

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 and **3** was easier to reduce than **4** by approximately 130 mV. The comparative ease of reduction of AQ in the conjugates with the ethynyl linker is consistent with the extended conjugation in these systems as well as the electron withdrawing nature of the ethynyl group.

Two additional molecules with ethynyl linkers were evaluated. Efforts to obtain reproducible reduction potential values for the conjugate **2** with the C(OH)₂CF₃ 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.^{63,64} We also looked at the formyl

conjugate **1**. This conjugate was more difficult to reduce than the diester conjugate **3** by approximately 70 mV. Formyl conjugate **1** also showed some degradation on repeated cycles of reduction and reoxidation. Thus, although the formyl group has been reported to be stable on DNA in aqueous solution,^{48,49,65} the somewhat more favorable reduction potential and the enhanced stability of the anthraquinone diester make **3** the molecule of choice for future photophysical studies.

3.4. Conclusion

The syntheses of novel 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 240 mV compared with the corresponding unmodified conjugates. In addition, the ethynyl linkers further enhances the reduction potential of the AQ by 130 mV relative to ethanyl linkers. Conjugates of AQ-dA with 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.

3.5. Experimental

3.5.1. Materials and general synthetic methods

n-Butyl triflate was purchased from TCI America. Anhydrous and air-sensitive solvents and reagents were used and stored in between uses in a Vacuum Atmospheres Company (VAC)

M040-2 glove box that was pressurized with nitrogen boil-off gas from a liquid nitrogen tank or in a VAC CS-40 glove box freezer at $-20\text{ }^{\circ}\text{C}$. Solvents for synthesis were dried and redistilled using standard methods. Ethyl trifluoroacetate was distilled over CaH_2 prior to use. Distilled solvents and reagents were transferred under nitrogen gas to the glove box immediately after distillation using an evacuated Schlenk tube or flask containing activated molecular sieves. Dichloromethane and methanol were freshly distilled over CaH_2 and Mg^0 , respectively, before use. All glassware used for anhydrous reactions was treated with hexamethyldisilazane and/or baked in an oven at $150\text{ }^{\circ}\text{C}$ for at least 2 h. All starting materials for anhydrous reactions were dried prior to use on a vacuum line ($1 - 4 \times 10^{-4}$ torr). All reaction vessels were sealed with rubber septa and pressure change (due reagent addition or temperature change) inside the vessels was stabilized using a balloon filled with nitrogen gas. The temperatures of the oil baths were controlled using a programmable Dataplate® 721P digital hotplate/stirrer. Reactions were monitored with glass-backed TLC plates pre-coated with silica gel 60 F₂₅₄ (EMD Chemicals). Flash column chromatography was carried out on either a Biotage Flash-40™ system using prepackaged KP-Sil™ cartridges, or on Whatman™ flash silica (60Å pore, 230-400 mesh) that was packed in glass columns and pressurized with nitrogen. Melting points were corrected. NMR Spectra were recorded on a Varian Unity +300, Varian Unity Inova 500, or Bruker Avance 400 spectrometer, using either CDCl_3 or $\text{DMSO}-d_6$ as solvents. Chemical shifts for ^1H NMR in these solvents were referenced relative to tetramethylsilane (0.00 ppm), CDCl_3 (7.24 ppm) or DMSO (2.50 ppm). Chemical shifts for ^{13}C NMR were referenced relative to CDCl_3 (77.23 ppm) or DMSO (39.50 ppm). ^{13}C NMR signals were assigned using ^{13}C -APT technique. ^{19}F NMR spectra were recorded using trifluoroacetic acid (-76.55 ppm) as an external standard.

High resolution (HR) MS were either obtained with electrospray ionization (ESI) on a Q-TOFTM Waters Micromass or with electron impact (EI) on a 70SE VG instrument.

3.5.2. 5'-*O*-*tert*-Butyldiphenylsilyl-8-[(trimethylsilyl)ethynyl]-2'-deoxyadenosine (6)

Compound **5**³² (2.5 g, 3.4 mmol), Pd(PPh₃)₂Cl₂ (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 glove box. 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 glove box 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/CH₂Cl₂ (0:100-3:97) and the second was eluted with EtOAc/CH₂Cl₂ (1:9-1:1). Evaporation of the eluting solvent afforded **6** as a light brown foam (2.20 g, 85% yield). ¹H NMR (300 MHz, CDCl₃): δ (ppm) 0.03 [9H, s, CH₃ (TMS)], 1.05 [9H, s, *t*-Bu (TBDPS)], 2.27 [1H, d, *J* = 3.3 Hz, OH-3' (dA)], 2.41 [1H, ddd, *J* = 4.5, 7.5 and 13.5 Hz, H-2' (dA)], 3.35-3.44 [1H, m, H-2' (dA)], 3.89 [1H, dd, *J* = 4.8 and 9.6 Hz, H-5' (dA)], 3.99-4.10 [2H, m, H-5' and H-4' (dA)], 4.97-5.14 [1H, m, H-3' (dA)], 5.66 (2H, br s, NH₂), 6.52 [1H, dd, *J* = 3.3, 4.5 Hz, H-1' (dA)], 7.26-7.45 [6H, m, Ph-*ortho* and *para* (TBDPS)], 7.61-7.64 [4H, m, Ph-*meta* (TBDPS)], and 8.14 [1H, s, H-2 (dA)]. ¹³C NMR (125 MHz, CDCl₃): δ (ppm) -0.6 [CH₃ (TMS)], 19.2, 26.9, 37.3, 64.4, 73.4, 84.8, 86.7, 92.5 and 103.7 (C≡C), 120.0, 127.7, 129.8, 133.2, 134.3, 135.6, 149.2, 153.6 and 155.1. HRMS (ESI) calcd for C₃₁H₄₀N₅O₃Si₂ [M + H]⁺ 586.2670, found 586.2646.

3.5.3. 5'-*O*-*tert*-Butyldiphenylsilyl-8-ethynyl-2'-deoxyadenosine (**7**)

To a solution of **6** (0.29 g, 0.50 mmol) in CH₂Cl₂/ MeOH (1:5 v:v, 6 mL) was added K₂CO₃ (89 mg, 0.64 mmol, 1.5 equiv). The mixture was stirred at rt for 30 min. Water and more CH₂Cl₂ were added, and the mixture was transferred to a separatory funnel. The organic layer was separated, dried with anhyd MgSO₄ and evaporated to dryness. The residue was purified by silica gel column eluted with MeOH/CH₂Cl₂ (0:100-4:96). Evaporation of the eluting solvent afforded **7** as a white foam (252 mg). ¹H NMR (400 MHz, CDCl₃): δ (ppm) 0.93 [9H, s, *t*-Bu (TBDPS)], 2.29 [1H, ddd, *J* = 4, 7.2 and 13.6 Hz, H-2' (dA)], 3.29 (1H, s, ≡C-H), 3.34-3.42 [1H, m, H-2' (dA)], 3.53 [1H, br s, OH-3' (dA)], 3.79 [1H, dd, *J* = 5.2 and 10.4 Hz, H-5' (dA)], 3.93 [1H, dd, *J* = 7.6 and 10.4 Hz, H-5' (dA)], 4.03-4.09 [1H, m, H-4' (dA)], 4.86-4.89 [1H, m, H-3' (dA)], 6.29 (2H, br s, NH₂), 6.49 [1H, t, *J* = 7.2 Hz, H-1' (dA)], 7.18-7.34 [6H, m, Ph-*ortho* and *para* (TBDPS)], 7.51-7.57 [4H, m, Ph-*meta* (TBDPS)], and 8.07 [1H, s, H-2 (dA)]. ¹³C NMR (100 MHz, CDCl₃): δ (ppm) 19.4, 27.0, 37.1, 64.2, 72.8 and 72.9, 84.7 (C≡C), 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 C₂₈H₃₂N₅O₃Si [M + H]⁺ 514.2274, found 514.2271.

3.5.4. 2,6-dibromoanthraquinone (**9**)⁴²

To 2,6-diaminoanthraquinone (10.0 g, 41.4 mmol) was added CuBr₂ (20.8 g, 93.2 mmol, 2.25 equiv) and anhyd acetonitrile (175 mL). To the resulting suspension was added *t*-BuONO (12.35 mL, 93.20 mmol, 2.250 equiv) under nitrogen gas. The mixture was heated to 63 °C, which led to evolution of a colorless gas that continued for 15 min. The mixture was stirred at the same temperature for an additional 2 h, and then quenched by adding 20% HCl (98 mL) and stirring for 5 min. Water (150 mL) was added and the light brown precipitate was separated by

filtration, washed with water and air dried. The crude product was dissolved in boiling dioxane and then active charcoal was added. Recrystallization from dioxane (800 mL) afforded **9** as light yellow crystals (11.7 g, 78% yield), mp 285-287 °C. ¹H NMR (400 MHz, CDCl₃): δ (ppm) 7.94 (2H, dd, *J* = 2 and 8.4 Hz, H-3 and H-7), 8.17 (2H, d, *J* = 8.4 Hz, H-4 and H-8) and 8.44 (2H, d, *J* = 2 Hz, H-1 and H-5). ¹³C NMR (100 MHz, CDCl₃): δ (ppm) 129.4, 130.4, 130.6, 132.0, 134.6, 137.7 and 181.6 (C-9 and C-10). Low resolution EI MS *m/z* (*M*⁺): 366.

3.5.5. 2,6-Dibromo-9,10-di-*n*-butoxyanthracene (**10**)

A suspension of **9** (3.0 g, 8.1 mmol) and *n*-Bu₄NBr (2.37 g, 7.30 mmol, 0.900 equiv) in CH₂Cl₂ (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 was ≤ 5 millitorr. 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 stirring 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 stirring 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 NaHCO₃ was added until the mixture became basic. The organic layer was separated, and the aqueous layer was extracted two times with CH₂Cl₂. The combined organic layers were dried with anhyd MgSO₄ 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 CH₂Cl₂/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. ¹H NMR (400 MHz, CDCl₃): δ (ppm) 1.06 [6H, t, *J* = 7.2 Hz, CH₃], 1.62-1.72 [4H, m, H-3 (Bu)], 1.94-2.02 [4H, m, H-2 (Bu)], 4.09 [4H, t, *J* = 6.4 Hz, H-1 (Bu)], 7.49 (2H, dd, *J* = 2 and 9.2 Hz, H-3 and H-7), 8.09 (2H, d, *J* = 9.2 Hz, H-4 and H-8) and 8.36 (2H, d, *J* = 2 Hz, H-1 and H-5). ¹³C NMR (100 MHz, CDCl₃): 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 C₂₂H₂₄Br₂O₂ [M]⁺ 478.0143, found 478.0195.

3.5.6. 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 NH₄Cl was added; this was followed by addition of water and CH₂Cl₂. The organic layer was separated, dried over anhyd MgSO₄ and evaporated to

dryness. The crude product was applied to a silica gel column and eluted with CH₂Cl₂/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. ¹H NMR (400 MHz, CDCl₃): δ (ppm) 1.06 [3H, t, *J* = 7.2 Hz, CH₃], 1.07 [3H, t, *J* = 7.2 Hz, CH₃], 1.63-1.72 [4H, m, H-3 (Bu)], 1.95-2.05 [4H, m, H-2 (Bu)], 4.09 [2H, t, *J* = 6.4 Hz, H-1 (Bu)], 4.15 [2H, t, *J* = 6.4 Hz, H-1 (Bu)], 7.52 (1H, dd, *J* = 2 and 9.2 Hz, H-3), 7.86 (1H, dd, *J* = 1.6 and 9.2 Hz, H-7), 8.11 (1H, d, *J* = 9.2 Hz, H-4), 8.24 (1H, d, *J* = 9.2 Hz, H-8), 8.39 (1H, d, *J* = 2 Hz, H-1), 8.67 (1H, d, *J* = 1.6 Hz, H-5) and 10.15 (1H, s, CHO). ¹³C NMR (100 MHz, CDCl₃): 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, 125.2 and 125.2, 127.4, 128.5, 129.6, 132.1, 134.0, 147.0, 150.4 and 192.1 (CHO). HRMS (EI) calcd for C₂₃H₂₅BrO₃ [M]⁺ 428.0987, found 428.0984.

3.5.7. 2-Bromo-6-trifluoroacetyl-9,10-di-*n*-butoxyanthracene (**12**)

Compound **10** (0.24 g, 0.50 mmol) was dissolved in anhyd THF (20 mL) and cooled to –72 °C (dry ice-ethanol bath). *n*-BuLi (0.330 mL, 0.530 mmol, 1.05 equiv) was added dropwise (over 5 min), and the homogeneous brown reaction mixture was stirred for an additional 10 min at the same temperature. CF₃COOEt (0.10 mL, 0.75 mmol, 1.5 equiv) was added, which led to the change of the brown color to orange. The reaction mixture was stirred for 30 min at –72 °C and for 30 min at rt. A saturated aqueous solution of NH₄Cl was added; this was followed by addition of water and CH₂Cl₂. The organic layer was separated, dried over anhyd MgSO₄ and evaporated to dryness. The crude product was purified using silica gel chromatography on a Biotage column (40+S cartridge) eluted with CH₂Cl₂/hexane (1:4 v:v). Evaporation of the eluting solvent afforded **12** as an orange powder (169 mg, 68% yield), mp 105-107 °C. ¹H NMR (400 MHz, CDCl₃): δ (ppm) 1.07 (3H, t, *J* = 7.2 Hz, CH₃), 1.08 (3H, t, *J* = 7.2 Hz, CH₃), 1.63-

1.74 [4H, m, H-3 (Bu)], 1.96-2.04 [4H, m, H-2 (Bu)], 4.10 [2H, t, $J = 6.4$ Hz, H-1 (Bu)], 4.16 [2H, t, $J = 6.4$ Hz, H-1 (Bu)], 7.55 (1H, dd, $J = 2$ and 9.2 Hz, H-3), 7.97 (1H, dd, $J = 1.6$ and 9.2 Hz, H-7), 8.13 (1H, d, $J = 9.2$ Hz, H-4), 8.27 (1H, d, $J = 9.2$ Hz, H-8), 8.41 (1H, d, $J = 2$ Hz, H-1) and 9.07 (1H, d, $J = 1.6$ Hz, H-5). ^{13}C NMR (100 MHz, CDCl_3): δ (ppm) 14.2, 14.2, 19.6, 19.6, 32.8, 32.8, 76.7, 77.9, 117.2 (q, $J = 290$ Hz, $\underline{\text{C}}\text{F}_3$), 122.6, 122.1 and 123.9, 124.4, 124.5, 125.2 and 125.3, 126.8, 126.9 and 129.2, 129.9, 130.3 (q, $J = 3$ Hz, C-5), 146.9, 151.4 and 180.4 (q, $J = 34$ Hz, $\underline{\text{C}}\text{OCF}_3$). ^{19}F NMR (282 MHz, CDCl_3): δ (ppm) -70.53. High resolution ESI MS m/z $[\text{M}+\text{H}]^+$: Calc'd for $\text{C}_{24}\text{H}_{25}\text{BrF}_3\text{O}_3$ 497.0939, found 478.0959.

3.5.8. 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. Anhyd 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 co-evaporated with toluene to afford **13** as a pale yellow powder (54 mg, 82% yield), mp 276-278 °C dec. ^1H NMR (400 MHz, CDCl_3): δ (ppm) 7.95 (1H, dd, $J = 2$ and 8 Hz, H-3), 8.21 (1H, d, $J = 8$ Hz, H-4), 8.28 (1H, dd, $J = 1.6$ and 8 Hz, H-7), 8.46 (1H, d, $J = 2$ Hz, H-1), 8.45 (1H, d, $J = 8$ Hz, H-8), 8.77 (1H, d, $J = 1.6$ Hz, H-5) and 10.22 (1H, s, $\underline{\text{C}}\text{HO}$). ^{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 (C-9 and C-10) and 192.0 (CHO). HRMS (EI) calcd for $C_{15}H_7BrO_3$ $[M]^+$ 313.9579, found 313.9603.

3.5.9. 2-Bromo-6-trifluoroacetylanthraquinone (**14**)

Solid **12** (95 mg, 0.19 mmol) and AgO (36 mg, 0.29 mmol, 1.5 equiv) were combined in a 25 mL flask and dried *in vacuo* for 30 min. Anhyd dioxane (4 mL) was added under nitrogen gas, and the mixture was stirred until **12** was completely soluble (AgO was in suspension). When viewed under long wavelength light, the solution was fluorescent. HNO_3 (6.0 N, 0.38 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 fluorescence. EtOAc (10 mL) and saturated aqueous solution of $NaHCO_3$ (4 mL) were added and the mixture was stirred at rt for 30 min. More EtOAc and water were added, and the mixture was filtered over a layer of Celite. The organic layer was separated, dried with anhyd $MgSO_4$ and evaporated. The residue was co-evaporated with toluene to afford **14** as a pale yellow powder (63 mg, 86% yield), mp 173-174 °C. 1H NMR (400 MHz, $CDCl_3$): δ (ppm) 7.97 (1H, dd, $J = 2$ and 7.6 Hz, H-3), 8.21 (1H, d, $J = 7.6$ Hz, H-4), 8.43-8.46 (3H, m, H-1, H-7 and H-8) and 8.95 (1H, d, $J = 1.2$ Hz, H-5). ^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) 116.5 (q, $J = 289$ Hz, C F_3), 128.5, 129.5 (q, $J = 3$ Hz, C-5), 129.6, 130.8, 130.8, 132.1, 134.0, 134.4, 134.8 (q, $J = 1$ Hz, C-7), 137.2, 138.1, 179.9 (q, $J = 36$ Hz, CO C F_3), 181.1 and 181.2 (C-9 and C-10). ^{19}F NMR (282 MHz, $CDCl_3$): δ (ppm) -72.4. HRMS (EI) calcd for $C_{16}H_6BrF_3O_3$ $[M]^+$ 381.9452, found 381.9453.

3.5.10. 5'-*O*-*tert*-Butyldiphenylsilyl-8-[(6-formylanthraquinone-2-yl)ethynyl]-2'-deoxyadenosine (1)

To **13** (43 mg, 0.11 mmol) was added Pd(PPh₃)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/CH₂Cl₂ (0:100-3:97) to yield yellow glass after evaporation of the eluting solvent. This glass was dissolved in a minimum amount of CH₂Cl₂ and precipitated by adding hexane. Evaporation of CH₂Cl₂/hexane from the precipitate afforded **1** as a bright yellow solid (55 mg, 57% yield). ε₃₅₅ (THF): 11730 ± 100 Lmol⁻¹cm⁻¹, ε₃₅₅ (acetonitrile): 7133 ± 100 Lmol⁻¹cm⁻¹, ε₃₅₅ (MeOH): 6907 ± 100 Lmol⁻¹cm⁻¹. ¹H NMR (400 MHz, CDCl₃): δ (ppm) 1.01 [9H, s, *t*-Bu (TBDPS)], 1.90 [1H, br s, OH-3' (dA)], 2.43-2.49 [1H, m, H-2' (dA)], 3.47-3.54 [1H, m, H-2' (dA)], 3.90 [1H, dd, *J* = 5.2 and 10.4 Hz, H-5' (dA)], 4.03 [1H, dd, *J* = 8 and 10.4 Hz, H-5' (dA)], 4.12-4.17 [1H, m, H-4' (dA)], 4.99-5.02 [1H, m, H-3' (dA)], 6.33 (2H, br s, NH₂), 6.57 [1H, t, *J* = 6.8 Hz, H-1' (dA)], 7.27-7.39 [6H, m, Ph-*ortho* and *para* (TBDPS)], 7.59-7.64 [4H, m, Ph-*meta* (TBDPS)], 7.81 [1H, d, *J* = 7.2 Hz, H-3 (AQ)], 8.03 [1H, s, H-2 (dA)], 8.13 [1H, d, *J* = 7.2 Hz, H-4 (AQ)], 8.17 [1H, d, *J* = 8 Hz, H-7 (AQ)], 8.32 [1H, d, *J* = 8 Hz, H-8 (AQ)], 8.46 [1H, s, H-1 (AQ)], 8.71 [1H, s, H-5 (AQ)] and 10.12 (1H, s, CHO). ¹³C NMR (100 MHz, CDCl₃): δ (ppm) 19.4, 27.1, 37.1, 64.5, 73.3, 82.0 (C≡C), 85.5, 87.4, 94.1 (C≡C), 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 and 181.6 [C-9 and C-10 (AQ)] and 190.6 (CHO). HRMS (ESI) calcd for C₄₃H₃₈N₅O₆Si [M + H]⁺ 748.2591, found 748.2627.

3.5.11. 5'-*O*-*tert*-Butyldiphenylsilyl-8-[[6-(2,2,2-trifluoro-1,1-dihydroxyethyl)anthraquinone-2-yl]ethynyl]-2'-deoxyadenosine (2)

To **14** (44 mg, 0.11 mmol) was added Pd(PPh₃)₄ (7.0 mg, 0.01 mmol, 0.05 equiv), CuI (2.0 mg, 0.01 mmol, 0.10 equiv), and TEA (30 μL, 0.23 mmol, 2.0 equiv). A solution of **7** (53 mg, 0.10 mmol, 0.90 equiv) in anhyd DMF (3.5 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/EtOAc/hexane (0:1:1- 0.02:1:1) to yield yellow glass after evaporation of the eluting solvent. This glass was dissolved in a minimum amount of CH₂Cl₂ and precipitated by adding hexane. Evaporation of CH₂Cl₂/hexane from the precipitate afforded **2** as a bright yellow solid (40 mg, 48% yield). ε₃₅₅ (THF): 4502 ± 100 Lmol⁻¹cm⁻¹, ε₃₅₅ (acetonitrile): 4588 ± 100 Lmol⁻¹cm⁻¹, ε₃₅₅ (MeOH): 4597 ± 100 Lmol⁻¹cm⁻¹. ¹H NMR (400 MHz, CDCl₃/CD₃OD): δ (ppm) 0.93 [9H, s, *t*-Bu (TBDPS)], 2.37 [1H, ddd, *J* = 4, 6.8 and 13.6 Hz, H-2' (dA)], 3.41-3.49 [1H, m, H-2' (dA)], 3.79 [1H, dd, *J* = 5.6 and 10.8 Hz, H-5' (dA)], 3.96 [1H, dd, *J* = 6.8 and 10.8 Hz, H-5' (dA)], 4.09-4.11 [1H, m, H-4' (dA)], 4.80-4.85 [1H, m, H-3' (dA)], 6.56 [1H, t, *J* = 6.8 Hz, H-1' (dA)], 7.19-7.32 [6H, m, Ph-*ortho* and *para* (TBDPS)], 7.51-7.56 [4H, m, Ph-*meta* (TBDPS)], 7.90 [1H, dd, *J* = 2 and 8 Hz, H-3 (AQ)], 7.99 [1H, s, H-2 (dA)], 8.05 [1H, dd, *J* = 1.6 and 8 Hz, H-7 (AQ)], 8.24 [1H, d, *J* = 8 Hz, H-4 (AQ)], 8.28 [1H, d, *J* = 8 Hz, H-8 (AQ)], 8.41 [1H, d, *J* = 2 Hz, H-1 (AQ)] and 8.54 [1H, d, *J* = 1.6 Hz, H-5 (AQ)]. ¹³C NMR (100 MHz, CDCl₃/CD₃OD): δ (ppm) 19.3, 26.9, 37.1, 64.2, 72.4, 82.0 (C≡), 85.5, 87.6, 93.9 (C≡), 126.8, 127.6, 127.7, 127.8, 127.9, 129.8, 129.8, 131.0, 133.3, 133.4, 133.5, 133.6, 133.7, 134.0, 134.3, 135.6, 135.7, 137.2, 141.7, 149.3, 153.7, 155.2 and 182.04 [C-9 and

C-10 (AQ)]. ^{19}F NMR (282 MHz, $\text{CDCl}_3/\text{CD}_3\text{OD}$): δ (ppm) -83.57 . HRMS (ESI) calcd for $\text{C}_{44}\text{H}_{39}\text{F}_3\text{N}_5\text{O}_7\text{Si}$ $[\text{M} + \text{H}]^+$ 834.2574, found 834.2609.

3.5.12. 5'-*O*-*tert*-Butyldiphenylsilyl-8-[2-(6-dimethoxymethylanthraquinone-2-yl)ethyl]-2'-deoxyadenosine (15)

Compound **1** (33 mg, 0.04 mmol) was dissolved in MeOH (20 mL) by slight heating while stirring. The resulting solution was transferred to a hydrogenation vessel containing 10% Pd/C (17 mg) that had been activated by stirring under H_2 gas (45 psi) in MeOH (5 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 5-6 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) to yield a pale yellow glass after evaporation of the eluting solvent. This glass was dissolved in a minimum amount of CH_2Cl_2 and precipitated by adding hexane. Evaporation of CH_2Cl_2 /hexane from the precipitate afforded **4** as a pale yellow solid (29 mg, 82% yield). ^1H NMR (400 MHz, CDCl_3): δ (ppm) 0.97 [9H, s, *t*-Bu (TBDPS)], 2.30 [1H, ddd, $J = 4, 6.8$ and 13.2 Hz, H-2' (dA)], 3.23-3.33 (4H, m, $\text{CH}_2\text{-CH}_2$), 3.35 (6H, s, OCH_3), 3.56-3.65 [1H, m, H-2' (dA)], 3.78 [1H, dd, $J = 5.2$ and 10.4 Hz, H-5' (dA)], 3.92 [1H, dd, $J = 6.8$ and 10.4 Hz, H-5' (dA)], 4.01-4.06 [1H, m, H-4' (dA)], 4.90-4.97 [1H, m, H-3' (dA)], 5.50 [1H, s, $\text{CH}(\text{OMe})_2$], 5.63 (2H, br s, NH_2), 6.22 [1H, t, $J = 6.8$ Hz, H-1' (dA)], 7.25-7.39 [6H, m, *Ph-ortho* and *para* (TBDPS)], 7.54-7.62 [5H, m, *Ph-meta* (TBDPS) and H-3 (AQ)], 7.86 [1H, dd, J

= 1.2, 8 Hz, H-7 (AQ)], 8.08 [1H, s, H-2 (dA)], 8.17 [1H, d, $J = 8$ Hz, H-4 (AQ)], 8.20 [1H, d, $J = 1.6$ Hz, H-1 (AQ)], 8.27 [1H, d, $J = 8$ Hz, H-8 (AQ)] and 8.36 [1H, d, $J = 1.2$ Hz, H-5 (AQ)]. LRMS (ESI) calcd for $C_{46}H_{48}N_5O_7Si$ [M + H]⁺ 798.3, found 798.6.

3.5.13. 4'-Bromo-2,4,5-trimethylbenzophenone (16)

A mixture of 4-bromobenzoyl chloride (6.09 g, 27.7 mmol) and AlCl₃ (3.88 g, 29.1 mmol, 1.05 equiv) were dried *in vacuo* for 1h. The flask was filled with nitrogen gas and placed in a -20 °C bath (ethanol-ice-salt). Anhyd CH₂Cl₂ (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 conc. HCl (9 mL), which led to discharge of the dark color. CH₂Cl₂ and water were added, and the mixture was transferred to a separatory funnel and shaken. The organic layer was separated, washed with saturated aqueous NaHCO₃, dried with anhyd MgSO₄, 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. ¹H NMR (400 MHz, CDCl₃): δ (ppm) 2.21 (3H, s, CH₃), 2.25 (3H, s, CH₃), 2.27 (3H, s, CH₃), 7.04 (1H, s, H-3), 7.05 (1H, s, H-6), 7.55-7.58 (2H, m, H-3' and H-5') and 7.62-7.65 (2H, m, H-2' and H-6'). ¹³C NMR (100 MHz, CDCl₃): δ (ppm) 19.4, 19.8, 20.0, 128.2, 130.4, 131.8, 131.9, 132.8, 133.7, 134.8, 135.7 and 137.3, 139.9 and 197.8 (C=O). HRMS (ESI) calcd for C₁₆H₁₆BrO [M + H]⁺ 303.0385, found 303.0374; and for C₁₆H₁₅BrNaO [M + Na]⁺ 325.0204, found 325.0157.

3.5.14. 4'-Bromobenzophenone-2,4,5-tricarboxylic acid (17)

To a two-neck flask containing **16** (6.619 g, 21.83 mmol) was added 20% HNO₃ (44 mL), and then a condenser was attached. The contents of the flask was 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 HNO₃ solution was removed with a pipette. The remaining semisolid was triturated with water (3 × 30 mL) that was also removed with a pipette. Addition of 10% NaOH (50 mL) followed by H₂O (75 mL) dissolved the semisolid. The resulting dark colored solution was then heated to reflux (110 °C oil bath), and solid KMnO₄ (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 NaHCO₃ (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. ¹H NMR (400 MHz, DMSO-*d*₆): δ (ppm) 7.58 (2H, d, *J* = 8.4 Hz, H-3' and H-5'), 7.70 (1H, s, H-3), 7.73 (2H, d, *J* = 8.4 Hz, H-2' and H-6'), and 8.27 (1H, s, H-6). ¹³C NMR (100 MHz, DMSO-*d*₆): δ (ppm) 127.3, 127.7, 130.4, 130.9, 131.3, 132.0, 133.0, 135.4 and 137.2, 143.2, 165.5 (COOH), 167.0 (COOH), 167.7 (COOH) and 194.3 (CO). HRMS (ESI) calcd for C₁₆H₈BrO₇ [M-H]⁻ 390.9453, found 390.9468.

3.5.15. 6-Bromoanthraquinone-2,3-dicarboxylic acid (18)⁶⁶

To dry **17** (0.800 g, 2.04 mmol) was added conc. H₂SO₄ (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 cooling 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. The resulting oily brown suspension was transferred (with methanol) to a 100-mL recovery flask. The methanol was removed under reduced pressure and vacuum was applied to the remaining residue overnight. The anhydrous crude obtained (mixed with H₂SO₄) was used in the next step without further purification. A pure sample of **18** (3 mg), for NMR analysis, was obtained from a small amount of this crude by further washing it with water. ¹H NMR (400 MHz, DMSO-*d*₆): δ (ppm) 8.13-8.18 (2H, m, H-5 and H-6), 8.31 (1H, d, *J* = 1.6 Hz, H-8) and 8.37 (2H, s, H-1 and H-4). ¹³C NMR (100 MHz, DMSO-*d*₆): δ (ppm) 126.66 and 126.69, 128.89, 129.1, 129.2, 132.1, 134.1, 134.2, 134.6, 137.3, 137.4, 167.31 (COOH), 167.34 (COOH), 180.5 and 180.9 (C-9 and C-10). HRMS (ESI) calcd for C₁₆H₆BrO₆ [M-H]⁻ 372.9348, found 372.9335.

3.5.16. Dimethyl 6-bromoanthraquinone-2,3-dicarboxylate (19)

The flask containing crude mixture was flushed with nitrogen gas and a condenser was attached. Anhyd methanol (25 mL) was added under nitrogen gas, and the yellow suspension obtained was refluxed (80 °C) for 24 h until TLC (CH₂Cl₂) showed no further progress in the reaction. The mixture was cooled to rt and treated with saturated aqueous solution of NaHCO₃ 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.⁶⁶ 178-179 °C). ¹H NMR (400 MHz, CDCl₃): δ (ppm) 3.97 (6H, s, CH₃), 7.95 (1H, dd, *J* = 2 and 8 Hz, H-7), 8.18 (1H, d, *J* = 8 Hz, H-8), 8.44 (1H, d, *J* = 2 Hz, H-5) and 8.60 (2H, s, H₁ and H₄). ¹³C NMR (100 MHz, CDCl₃): δ (ppm) 53.4 (CO₂CH₃), 128.4, 128.4, 129.5, 1130.6, 130.7, 132.0, 134.3, 134.5, 134.7, 136.8, 137.0, 137.9, 166.5 (CO₂Me), 166.5 (CO₂Me), 180.7 and 181.0 (C-9 and C-10). HRMS (ESI) calcd for C₁₈H₁₂BrO₆ [M + H]⁺ 402.9817, found 402.9802.

3.5.16. 5'-*O*-*tert*-Butyldiphenylsilyl-8-[(2,3-dicarboxyanthraquinone-6-yl)ethynyl]-2'-deoxyadenosine dimethyl ester (**3**)

Compound **19** (86 mg, 0.21 mmol) was reacted with Pd(PPh₃)₄ (13 mg, 0.01 mmol, 0.05 equiv), CuI (4.0 mg, 0.02 mmol, 0.10 equiv), TEA (0.06 mL, 0.43 mmol, 2.0 equiv) and **7** (104 mg, 0.200 mmol, 0.950 equiv) in anhyd DMF (5 mL) for 30 min under the same conditions used to prepare **2** above. Similar purification procedure to the one described above afforded **3** as a bright yellow solid (82 mg, 49% yield). ε₃₅₅ (THF): 14898 ± 100 Lmol⁻¹cm⁻¹, ε₃₅₅ (acetonitrile): 12013 ± 100 Lmol⁻¹cm⁻¹, ε₃₅₅ (MeOH): 12292 ± 100 Lmol⁻¹cm⁻¹. ¹H NMR (400 MHz, CDCl₃): δ (ppm) 0.96 [9H, s, *t*-Bu (TBDPS)], 2.31 [1H, br s, OH-3' (dA)], 2.41 [1H, ddd, *J* = 4.9, 6.8 and 13.6 Hz, H-2' (dA)], 3.42-3.49 [1H, m, H-2' (dA)], 3.82 [1H, dd, *J* = 5.2 and 10.4 Hz, H-5' (dA)], 3.92 (6H, s, CO₂CH₃), 3.92-3.97 [1H, m, H-5' (dA)], 4.05-4.10 [1H, m, H-4' (dA)], 4.96-5.01 [1H, m, H-3' (dA)], 5.84 (2H, br s, NH₂), 6.53 [1H, t, *J* = 6.8 Hz, H-1' (dA)], 7.22-7.34 [6H, m, Ph-*ortho* and *para* (TBDPS)], 7.55 [4H, t, *J* = 8 Hz, Ph-*meta* (TBDPS)], 7.88 [1H, dd, *J* = 1.2 and 8 Hz, H-7 (AQ)], 8.10 [1H, s, H-2 (dA)], 8.23 [1H, d, *J* = 8 Hz, H-8 (AQ)], 8.42 [1H, d, *J* = 1.2 Hz, H-5 (AQ)] and 8.60 [2H, s, H-1 and H-4 (AQ)]. ¹³C NMR (100 MHz, CDCl₃): δ

(ppm) 19.4, 27.0, 37.3, 53.4 (CO₂CH₃), 64.4, 73.2, 82.5 (C≡), 85.4, 87.3, 93.91 (C≡), 120.3, 126.8, 127.7, 127.9, 127.9, 128.25, 128.5, 130.0, 131.1, 132.9, 133.1, 133.3, 133.4, 133.9, 134.5, 134.7, 135.7, 135.7, 136.56 and 136.8, 136.9, 149.1, 154.0, 155.5, 166.4 (CO₂Me), 166.5 (CO₂Me), 180.5 and 180.6 [C-9 and C-10 (AQ)]. HRMS (ESI) calcd for C₄₆H₄₂N₅O₉Si [M + H]⁺ 836.2752, found 836.2731.

3.5.18. 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 H₂ gas (45 psi) in EtOH (10 mL) for 30 min at rt. The vessel was next charged with H₂ gas and then degassed using an aspirator in a cycle that was repeated 5-6 times. The vessel was finally charged with H₂ gas at 40 psi and stirred at rt for 24 h, by which time TLC (MeOH/CH₂Cl₂, 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 CHCl₃. The crude product was applied to a silica gel column and eluted with MeOH/CH₂Cl₂ (0:100-3:97) to yield a pale yellow glass after evaporation of the eluting solvent. This glass was dissolved in a minimum amount of CH₂Cl₂ and precipitated by adding hexane. Evaporation of CH₂Cl₂/hexane from the precipitate afforded **4** as a pale yellow solid (43 mg, 80% yield). ε₃₅₅ (THF): 2553 ± 100 Lmol⁻¹cm⁻¹, ε₃₅₅ (acetonitrile): 2907 ± 100 Lmol⁻¹cm⁻¹, ε₃₅₅ (MeOH): 2598 ± 100 Lmol⁻¹cm⁻¹. ¹H NMR (400 MHz, CDCl₃): δ (ppm) 0.97 [9H, s, *t*-Bu (TBDPS)], 2.30 [1H, ddd, *J* = 2.4, 6.8 and 13.2 Hz, H-2' (dA)], 3.23-3.38 (4H, m, CH₂-CH₂), 3.60-3.67 [1H, m, H-2' (dA)], 3.80 [1H, dd, *J* = 5.2 and 10.4 Hz, H-5' (dA)], 3.92-3.97 [1H, m,

H-5' (dA)], 3.98 (6H, s, CO₂CH₃), 4.08-4.12 [1H, m, H-4' (dA)], 4.92-4.95 [1H, m, H-3' (dA)], 5.91 (2H, br s, NH₂), 6.26 [1H, t, $J = 6.8$ Hz, H-1' (dA)], 7.24-7.38 [6H, m, Ph-*ortho* and *para* (TBDPS)], 7.55-7.65 [5H, m, Ph-*meta* (TBDPS) and H-7 (AQ)], 8.06 [1H, s, H-2 (dA)], 8.13 [1H, d, $J = 8$ Hz, H-8 (AQ)], 8.19 [1H, d, $J = 1.2$ Hz, H-5 (AQ)], 8.54 [1H, s, H-1 (AQ)] and 8.56 [1H, s, H-4 (AQ)]. ¹³C NMR (100 MHz, CDCl₃): δ (ppm) 19.9, 27.0, 29.2 (CH₂), 33.4 (CH₂), 37.0, 53.4 (CO₂CH₃), 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 (CO₂Me), 166.5 (CO₂Me), 181.3 and 181.7 [C-9 and C-10 (AQ)]. HRMS (ESI) calcd for C₄₆H₄₆N₅O₉Si [M + H]⁺ 840.3065, found 840.3062.

3.5.19. Electrochemical measurements

Cyclic voltammograms were recorded using a CHI750C electrochemical workstation with Faraday cage using an Ag wire reference electrode and platinum auxiliary and working electrodes (BAS Inc.). The reference electrode solution [Ag/AgNO₃ (0.01 M) + 0.1 M TBAH in CH₃CN] was prepared and the electrochemical apparatus was assembled in the glove box. The reference electrode was calibrated against ferrocene potential, taken as $E_{1/2} = 0.087$ V. The working electrode was mechanically polished using 0.05 μ m alumina powder (Buhler) and sonicated in 1:1 methanol:water (18.6 M Ω -cm from a Milli-Q water purification system, Millipore) for 5 min before taking data on each compound. Compounds were dried for 30 min to 24 h on a vacuum line (4×10^{-4} torr) or in the antechamber of the glove box (25 millitorr) before measurements were taken. All AQ derivatives solutions were freshly prepared in acetonitrile with 0.10 M TBAH as the electrolyte in a glove box under nitrogen. They were then loaded into the CV cell which had the electrodes in place and transferred to the electrochemical workstation

under nitrogen. The Faraday cage was filled with argon prior to taking the measurement, which was done at room temperature (22 °C). The scan rate was 0.100 V s⁻¹ unless indicated otherwise. Each measurement was repeated with 3 - 5 scans at least twice on separate solutions. All electrode potentials are quoted relative to the saturated calomel electrode (SCE).

3.6. Acknowledgment

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Chapter 4.

Synthesis of Anthraquinone Derivatives for Electrochemical Studies.

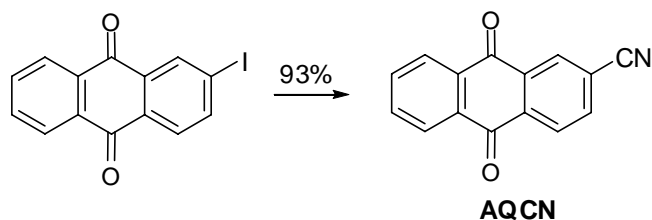
4.1. Introduction

In the course of our work on designing and synthesizing novel anthraquinone conjugates for incorporation into DNA, a variety of other anthraquinone model compounds and conjugates were made. In general, the syntheses of those compounds followed established literature procedures. Below I discuss molecules that were new or have been made by routes that are improvements over those in the literature. These molecules include anthraquinones modified with electron withdrawing groups and anthraquinone linked to deoxyadenosine or deoxyuridine.

4.2. Results and Discussion

4.2.1. 2-Cyanoanthraquinone (AQC�)

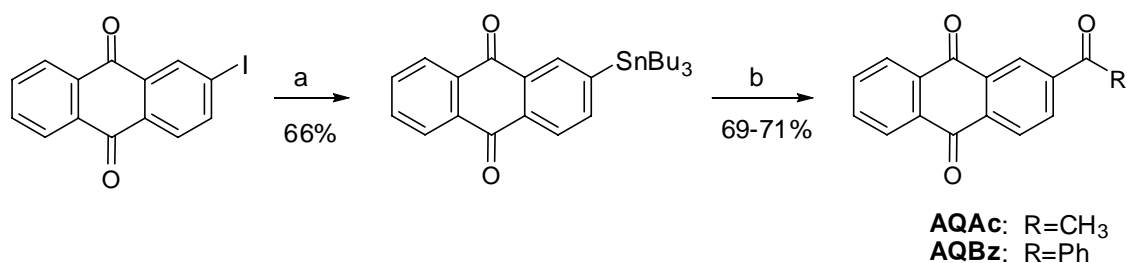
Of note was the use of Pd(0) coupling¹ to make the cyano derivative (AQC�) from the iodo precursor, which had a very high yield of 93% under mild conditions. The time required for this conversion depended on the solvent used. The completion of the reaction required 11 h when neat THF was used, but only 30 min when acetonitrile was added to THF. A mixture of these two solvents was used because of the limited solubility of the iodo starting material in acetonitrile alone. This approach may be useful in addition of the cyano group to other, more substituted, anthraquinones because it has a much higher yield than the reported yield of 43% when the same compound was prepared from the chloro precursor using CuCN/pyridine.²



Scheme 4.1. Reagents and conditions: $\text{Pd}(\text{Ph}_3\text{P})_4$, CuI , KCN , $\text{THF}/\text{CH}_3\text{CN}$, 70°C , 30 min.

4.2.2. Carbonyl modified AQ derivatives: Ketones, amides and esters

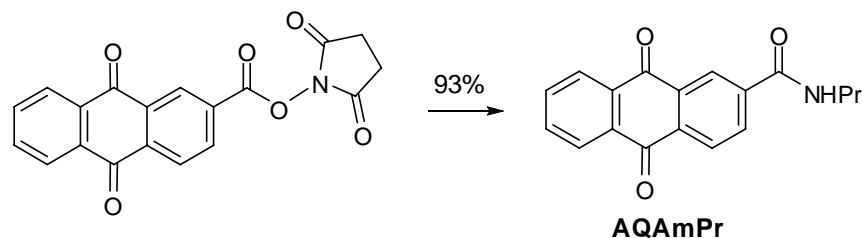
Following a reported procedure of preparing aromatic ketones,³ the acetyl- and benzoyl- derivatives AQAc and AQBz, respectively, were synthesized from iodoanthraquinone via tributylstannylanthraquinone, which in turn was reacted with the corresponding acyl chloride in a Pd(II) catalyzed reaction as shown in Scheme 4.2. Use of acyl anhydrides, reported to transform aromatic tributylstannyl derivatives to trifluoroketones,⁴ instead of acyl chlorides did not yield the desired products.



Scheme 4.2. Reagents and conditions: (a) $\text{Pd}(\text{Ph}_3\text{P})_2\text{Cl}_2$, $n\text{-Bu}_6\text{Sn}_2$, toluene, 80°C , 96 h; (b)

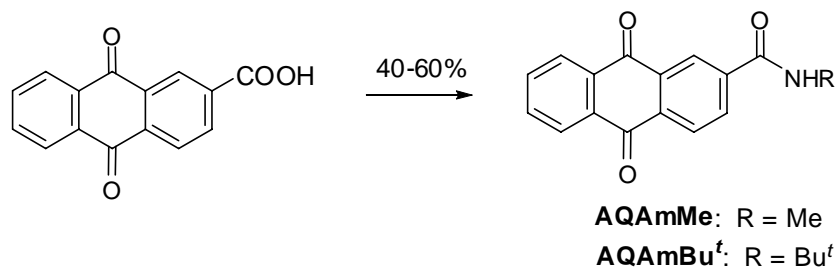
$\text{Pd}(\text{Ph}_3\text{P})_2\text{Cl}_2$, CH_3COCl or PhCOCl , toluene, $50 - 90^\circ\text{C}$, 3 h - overnight.

As shown in Scheme 4.3 the *n*-propyl amide derivative (AQAmPr) was synthesized in 93% yield from the *N*-hydroxysuccinimide ester⁵ by reaction with *n*-propyl amine.



Scheme 4.3. Reagents and conditions: *n*-propylamine, DMF/dioxane, rt, 30 min.

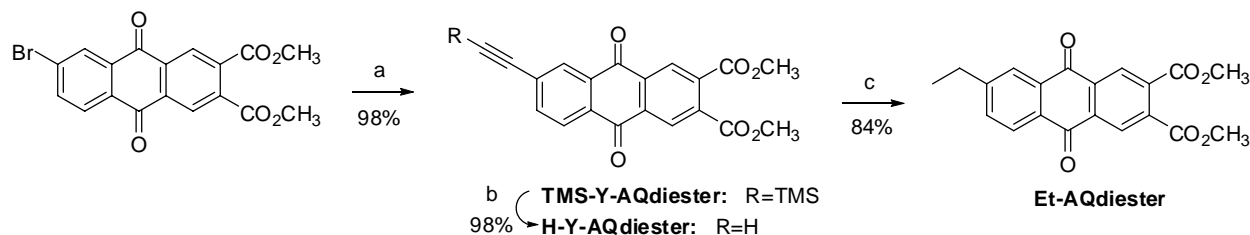
The corresponding methyl and *tert*-butyl amides, AQAmMe and AQAmBu^t respectively were synthesized by I. M. Abdou in the laboratory of Dr. L. Strekowski from the carboxylic acid in 40-60% by activation with carbonyldiimidazole⁶ followed by treatment with the corresponding amine (Scheme 4.4). Spectra are reported herein as they have not been previously published.



Scheme 4.4. Reagents and conditions: *i.* 1,1'-carbonyldiimidazole, DMF, rt, 2 h. *ii.* MeNH₂ or *tert*-BuNH₂, rt - 40 °C, 12 h.

The TMS protected ethynyl diester (TMS-Y-AQdiester) was synthesized starting with the bromo diester derivative (Chapter 3) via palladium coupling with trimethylsilylacetylene (TMSA) under the conditions shown in Scheme 4.5. Treatment with fluoride gave the free

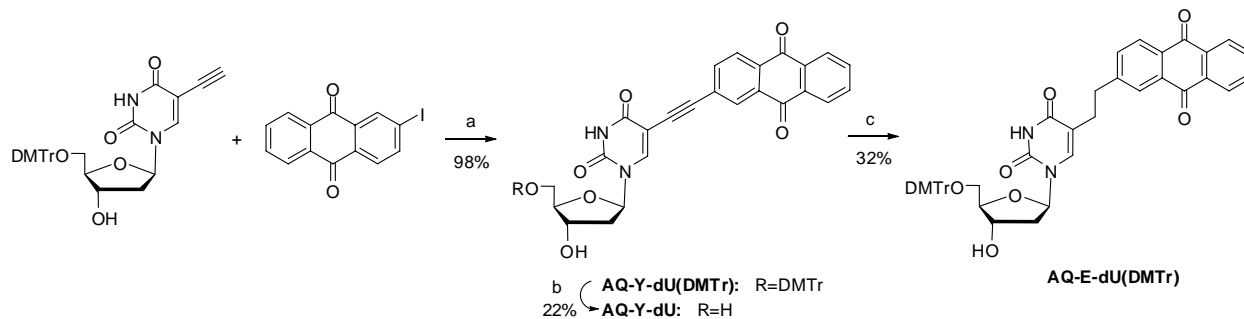
alkyne (H-Y-AQdiester) that was reduced with H₂ gas over 10% Pd/C turn to give the ethyl derivative (Et-AQdiester).



Scheme 4.5. Reagents and conditions: (a) Pd(PPh₃)₂Cl₂, CuI, Et₃N, THF, TMSA, 60 °C, 45 min; (b) KF, THF/MeOH, rt, 1h; (c) 10% Pd/C, H₂, 40 psi, EtOAc, rt, 24 h.

4.2.3. AQ-dU conjugates

Four steps were employed to synthesize the conjugate AQ-Y-dU(DMTr) from the commercially available 5-iodo-2'-deoxyuridine. First, the 5'-OH was protected using 4,4'-dimethoxytrityl chloride (DMTrCl), 4-(dimethylamino)pyridine (4-DMAP) and triethylamine (TEA) as described in the literature.⁷ Second, the resulting 5'-O-DMT nucleoside was coupled to TMSA using Pd(II) catalyzed chemistry, which was followed, after purification, by removal of the TMS group with KF. Finally, as shown in Scheme 4.6, the obtained 5-ethynyl derivative of dU was coupled to 2-iodoanthraquinone via Pd(0) coupling to give AQ-Y-dU(DMTr). Treatment of AQ-Y-dU(DMTr) with dichloroacetic acid (DCA) gave the free 5'-OH derivative, AQ-Y-dU in 22% yield. The low yield of this deprotection reaction was due to the extremely low solubility of AQ-Y-dU, which hindered its complete extraction during workup and chromatography. The solubility of AQ-Y-dU was also unsuitable for CV measurements, and hence, the AQ-Y-dU(DMTr) was used.



Scheme 4.6. Reagents and conditions: (a) $\text{Pd}(\text{Ph}_3\text{P})_4$, CuI , TEA, DMF, 65 °C, 30 min; (b) DCA in CH_2Cl_2 , rt, 45 min; (c) 10% Pd/C, H_2 , 40 psi, EtOAc/MeOH, rt, 7 h.

Catalytic hydrogenation of AQ-Y-dU(DMTr) over 10% Pd/C for 24 h resulted in the desired product AQ-E-dU(DMTr) with the ethanyl linker along with a hydrogenation side product. HPLC showed that the ratio between AQ-E-dU(DMTr) and this side product was ca 2:1. This ratio did not change if the hydrogenation reaction was stopped once the alkyne starting material was consumed, which was done by UV-vis monitoring of the starting material absorbance at 377 nm (See Figure 4.1). The R_f of AQ-E-dU(DMTr) and the side product on silica gel TLC were similar in most common eluting systems. The use of MeOH/THF/hexane (1:40:60 v:v:v) as the eluent allowed separation by silica gel. The side product showed two C-13 signals at 184.85 and 184.89 ppm (similar to the shifts of AQ carbonyls) and a shift in the UV-vis spectrum (blue spectrum in Figure 4.1). It is reported that some AQ derivatives degrade on carbon supported palladium catalysts to give different products including reduction of a double bonds in the AQ ring.⁸

AQ-E-dU(DMTr) had limited stability in solution. Over a few hours, it darkened and gave decomposition products, the major of which were of higher polarity than AQ-E-dU(DMTr)

itself as observed on TLC eluted with MeOH/THF/hexane (1:40:60 v:v:v) or MeOH/CH₂Cl₂ (5:95 v:v).

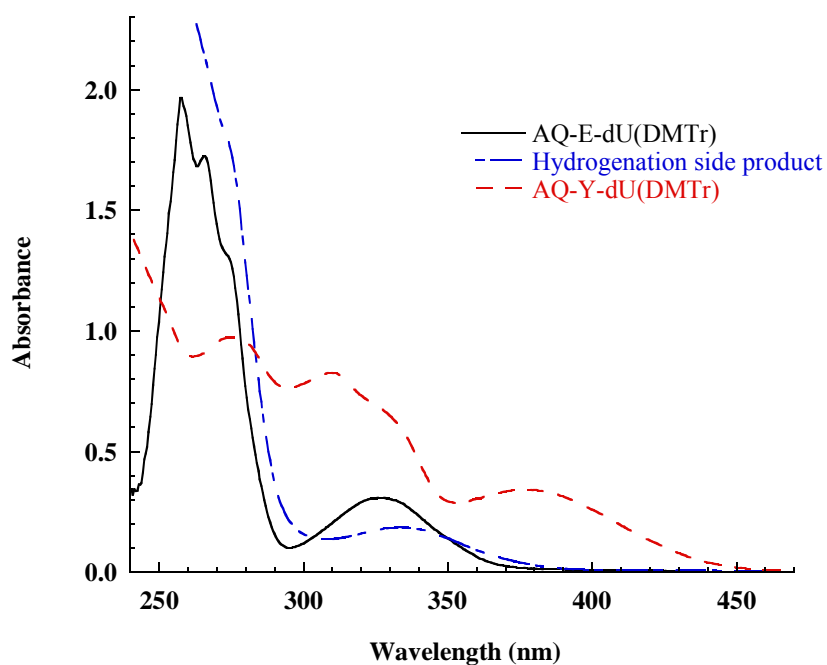
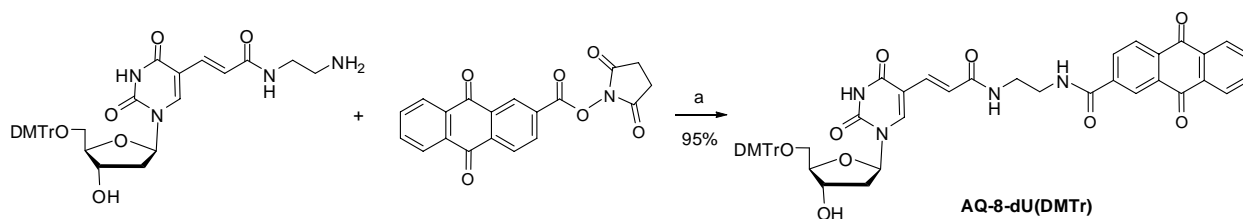


Figure 4.1. UV-vis absorbance of AQ-Y-dA, AQ-E-dA and AQ-Y-dA hydrogenation side product.

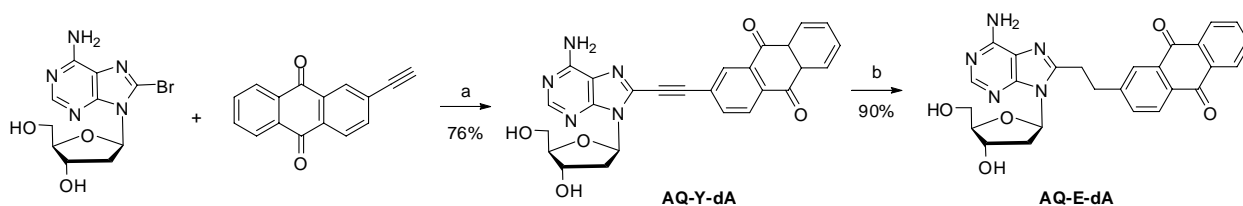
AQ-8-dU(DMTTr) was synthesized by coupling between 2-[3-(deoxyuridine-5-yl)acrylamido]ethylamine and *N*-succinimidyl 2-anthraquinonecarboxylate as shown in Scheme 4.7.⁵



Scheme 4.7. Reagents and conditions: TEA, DMF, rt, overnight.

4.2.4. Unprotected AQ-dA conjugates

AQ-Y-dA was synthesized by a palladium catalyzed cross-coupling between 8-bromo-2'-deoxyadenosine and 2-ethynylantraquinone as shown in Scheme 4.8. The low solubility of AQ-Y-dA in organic solvents made loading the crude reaction product for column purification problematic. The following solvents and many of their combinations were tried to dissolve AQ-Y-dA: dichloromethane, chloroform, THF, methanol, ethyl acetate, acetone, isopropanol, benzene, and water. The best solvent combination was 15% (by volume) methanol in chloroform. However, 10 mL of this solvent mixture dissolved only 60 mg of AQ-Y-dA with slight heating and 30 min of stirring. This solubility problem meant that it was not practical to purify more than 100 mg of AQ-Y-dA at a time. To do this, a solution of the crude product AQ-Y-dA in methanol/chloroform (15:85 v:v) was divided into three parts to load silica gel for column chromatography. Each third was successively loaded onto the same gel, and the excess solvent was removed *in vacuo*. After all three parts of the solution of AQ-Y-dA were loaded onto the gel, normal column purification was carried out.



Scheme 4.8. Reagents and conditions: (a) $\text{Pd}(\text{Ph}_3\text{P})_4$, CuI , Et_3N , DMF , 8-bromo-2'-deoxyadenosine, 60°C , 3h; (b) 10% Pd/C , H_2 , 40 psi, MeOH , rt, 24 h.

The conjugate with reduced linker (AQ-E-dA) was prepared from AQ-Y-dA by catalytic hydrogenation over 10% Pd/C for 24 h

4.3. Experimental

4.3.1. Materials and general synthetic considerations

Anhydrous DMF was purchased from Aldrich and stored in a Vacuum Atmospheres M040-2 glove-box that was pressurized with nitrogen gas from a liquid nitrogen tank. All starting materials for anhydrous reactions were dried prior to use on a vacuum line (5×10^{-4} torr) for at least 12 h. Solvents for synthesis were dried and redistilled in a continuous circulation distillation apparatus. THF was dried with benzophenone /Na⁰ and stored over activated molecular sieves in the glove-box. Water was provided by a Millipore (Milli-Q Plus) ultrapure water system. All manipulations of Pd(PPh₃)₂Cl₂ and Pd(PPh₃)₄ were performed in the glove-box. Most of the reactions were monitored with glass-backed TLC Plates precoated with silica gel 60 F₂₅₄ (EMD Chemicals). HPLC grade solvents were used for chromatographic purifications. Flash column chromatography was carried out on either a Biotage Flash-40™ system using prepackaged KP-Sil™ cartridges or on Whatman™ flash silica (60Å pore, 230 – 400 mesh) that was packed in glass columns and pressurized with nitrogen. Melting points were corrected. Molar absorption values were determined using Beer's law. NMR spectra were obtained on either a Varian Unity +300 NMR spectrometer or on a Bruker Avance 400 MHz NMR spectrometer. High resolution MS (HRMS) were obtained with a Q-TOF QSTAR mass spectrometer. Negative ion ESI (electrospray ionization) MS were recorded for all compounds by loop injections into a flow of 50/50 water/ACN containing 0.5% triethylamine (TEA). The samples were dissolved in about 100 µL of CHCl₃, diluted 1/100 with the water/ACN/TEA solvent, and injected into the mass spectrometer. Low-resolution (LR) electron impact (EI) MS were recorded in positive ion mode on a Shimadzu 5050A MSD single quadrupole spectrometer with one amu resolution.

4.3.2. 2-Cyanoanthraquinone (AQCN)

To a solution of 2-iodoanthraquinone (70 mg, 0.21 mmol) in THF/CH₃CN (1:1 v:v, 6 mL) was added in the glove box Pd(Ph₃P)₄ (12 mg, 0.01 mmol, 0.05 equiv), CuI (4 mg, 0.02 mmol, 0.1 equiv) and finely ground, anhydrous KCN (27 mg, 0.42 mmol, 2.0 equiv). The mixture was removed from the glove-box and stirred under a nitrogen atmosphere for 30 min at 70 °C in an oil bath, at which time TLC (CH₂Cl₂/hexane, 1:1 v:v) showed complete reaction. The solvent was removed under reduced pressure, and the residue was purified by silica gel column eluted with CH₂Cl₂/hexane (0:10-7:10). Evaporation of the eluting solvent afforded the title product as a pale yellow solid (46 mg, 93% yield), mp 213-215 °C (lit.⁹ 217 °C). ¹H NMR (400 MHz, CDCl₃): δ (ppm) 7.85-7.90 (2H, m, H-6 and H-7), 8.07 (1H, dd, *J* = 1.6 and 8 Hz, H-3), 8.32-8.36 (2H, m, H-5 and H-8), 8.43 (1H, d, *J* = 8.0 Hz, H-4) and 8.60 (1H, d, *J* = 1.6 Hz, H-1). The ¹³C NMR¹⁰ (100 MHz, CDCl₃): δ (ppm) 117.17 and 117.73 (C_N and C-2), 127.58, 127.59, 128.02, 131.28, 132.96, 133.08, 133.81, 134.83, 134.84, 135.62, 136.62, 181.18 and 181.66 (C-9 and C-10). EI MS *m/z* (M⁺): 233.

4.3.3. 2-tri-*n*-Butylstannylanthraquinone

In a round bottom flask was placed dry 2-iodoanthraquinone (300 mg, 0.9 mmol), Pd(Ph₃P)₂Cl₂ (33 mg, 0.050 mmol, 0.050 equiv), hexabutylstannane (0.70 mL, 1.3 mmol, 1.5 equiv) and anhydrous toluene (10 mL), and the mixture was stirred at 80 °C for 96 h under a nitrogen atmosphere. The reaction was quenched by adding a solution of KF (105 mg) in water (1 mL) and stirring overnight. The mixture was filtered over a layer of Celite, and the filtrate was diluted with ethyl acetate and water. The organic layer was separated, dried with MgSO₄ and evaporated to dryness. The residue was loaded on silica gel and purified using silica gel

chromatography on a Biotage column (40+S cartridge) eluted with CH₂Cl₂/hexane (3:8 v:v). Evaporation of the eluting solvent afforded the title product as a yellow oil (295 mg, 66% yield). ¹H NMR (400 MHz, CDCl₃): δ (ppm) 0.87 (9H, t, *J* = 7.2 Hz, CH₃), 1.12-1.16 (6H, m, CH₂CH₃), 1.27-1.37 (6H, m, CH₂CH₂CH₃), 1.50-1.62 [6H, m, CH₂(CH₂)₂CH₃], 7.74-7.78 (2H, m, H-6 and H-7), 7.91 (1H, dd, *J* = 0.8 and 7.6 Hz, H-3), 8.18 (1H, d, *J* = 7.6 Hz, H-4), 8.26-8.30 (2H, m, H-5 and H-8) and 8.40 (1H, d, *J* = 0.8 Hz, H-1). ¹³C NMR (100 MHz, CDCl₃): δ (ppm) 9.87 (CH₂), 13.65 (CH₃), 27.31 (CH₂), 29.03 (CH₂), 125.50, 127.06, 127.11, 131.55, 132.98, 133.49, 133.61, 133.97, 134.03, 134.78, 142.36, 152.68, 183.77 and 183.96 (C-9 and C-10).

4.3.4. 2-Acetylanthraquinone (AQAc)

To 2-tri-*n*-butylstannylantraquinone (113 mg, 0.230 mmol) was added Pd(Ph₃P)₂Cl₂ (8.0 mg, 0.01 mmol, 0.05 equiv), anhydrous toluene (4 mL) and CH₃COCl (0.05 mL, 0.70 mmol, 3.0 equiv). The dark brown reaction mixture was stirred under nitrogen gas at 50 °C overnight. The solvent was evaporated and the residue was purified by a silica gel column eluted with CH₂Cl₂/hexane (2:3-4:1). Evaporation of the eluting solvent afforded the title product as a pale yellow solid (39 mg, 69% yield), mp 142-144 °C (lit.¹¹ 143-144 °C). ¹H NMR¹² (400 MHz, CDCl₃): δ (ppm) 2.72 (3H, s, CH₃), 7.80-7.84 (2H, m, H-6 and H-7), 8.31-8.36 (3H, m, H-3, H-5 and H-8), 8.39 (1H, dd, *J* = 0.4 and 8.0 Hz, H-4) and 8.81 (1H, dd, *J* = 0.4 and 1.6 Hz, H-1). ¹³C NMR (100 MHz, CDCl₃): δ (ppm) 27.28 (CH₃), 127.59, 127.68, 127.70, 128.08, 133.14, 133.62, 133.66, 133.97, 134.73, 136.27, 141.27, 182.68 and 182.74 (C-9 and C-10) and 197.01 (COCH₃). HRMS *m/z* (M+H)⁺: calcd for C₁₆H₁₁O₃ 251.0708, found 251.0720.

4.3.5. 2-Benzoylanthraquinone (AQBz).¹³

To 2-tri-*n*-butylstannylantraquinone (102 mg, 0.210 mmol) was added Pd(Ph₃P)₂Cl₂ (7.0 mg, 0.01 mmol, 0.05 equiv), anhydrous toluene (4 mL) and PhCOCl (0.07 mL, 0.60 mmol, 3.0 equiv). The yellow reaction mixture was stirred under nitrogen gas at 90 °C for 3 h. The solvent was evaporated and the residue was purified by a silica gel column eluted with CH₂Cl₂/hexane (2:3-4:1). Evaporation of the eluting solvent afforded the title product as a pale yellow solid (45 mg, 71% yield), mp 217-218 °C (lit.¹³ 217 °C). ¹H NMR (400 MHz, CDCl₃): δ (ppm) 7.49-7.54 [2H, m, H-*meta* (Ph)], 7.61-7.66 [1H, m, H-*para* (Ph)], 7.79-7.84 [4H, m, H-*ortho* (Ph), H-6 and H-7], 8.18 (1H, dd, *J* = 1.6 and 8.0 Hz, H-3), 8.29-8.35 (2H, m, H-5 and H-8), 8.42 (1H, d, *J* = 8.0 Hz, H-4) and 8.62 (1H, d, *J* = 1.6 Hz, H-1). ¹³C NMR (100 MHz, CDCl₃): δ (ppm) 127.45, 127.46, 127.63, 128.58, 128.73, 130.15, 133.33, 133.40, 133.47, 134.50, 134.61, 135.44, 136.40, 142.50, 182.42 and 182.57 (C-9 and C-10) and 195.12 (COPh). EI MS *m/z* (M⁺): 312.

4.3.6. *N*-(*n*-Propyl)anthraquinone-2-carboxamide (AQAmPr)

To a solution of *N*-succinimidyl 2-anthraquinonecarboxylate⁵ (51 mg, 0.11 mmol) in DMF/dioxane (1:1 v:v, 4 mL) was added *n*-propylamine (0.090 mL, 1.1 mmol). The reaction mixture was stirred at rt for 30 min, by which time a white precipitate had formed. The solvent was evaporated to dryness and the residue was purified using preparative TLC (10 cm long x 10 cm wide x 0.2 cm thick) eluted with 2% methanol in dichloromethane. The product band was scratched off the plate and eluted with 5% MeOH in CH₂Cl₂. Evaporation of the solvent afforded the title compound as a white solid (30 mg, 93% yield), mp 201-202 °C (lit.¹⁴ 300 °C). ¹H NMR (300 MHz, CDCl₃): δ (ppm) 1.03 (3H, t, *J* = 7.5 Hz, CH₃), 1.65-1.78 (2H, m, CH₂CH₃), 3.50 (2H, q, *J* = 7.2 Hz, CH₂CH₂), 6.54 (1H, br s, NH), 7.81-7.84 (2H, m, H-6 and H-

7), 8.26-8.37 (4H, m, H-3, H-5, H-4 and H-8) and 8.54 [1H, d, $J = 1.8$ Hz, H-1]. ^{13}C NMR (100 MHz, CDCl_3): δ (ppm) 11.43 ($\underline{\text{C}}\text{H}_3$), 22.83 ($\underline{\text{C}}\text{H}_2\text{CH}_3$), 42.10 ($\underline{\text{C}}\text{H}_2\text{CH}_2$), 124.68, 127.36, 127.38, 127.89, 133.14, 133.35, 133.41, 134.34, 134.42, 135.04, 139.76, 165.61 ($\underline{\text{C}}\text{ONH}$), 182.38 and 182.53 (C-9 and C-10). HRMS m/z (M+H) $^+$: calcd for $\text{C}_{18}\text{H}_{16}\text{NO}_3$ 294.1130, found 294.1121.

4.3.7. *N*-Methylantraquinone-2-carboxamide (AQAmMe, reaction run by I. M. Abdou)¹⁵

To a suspension of anthraquinone 2-carboxylic acid (0.25 g, 1.0 mmol) in dry DMF (3 mL) was gradually added a solution of 1,1'-carbonyldiimidazole (0.20 g, 1.1 mmol) in dry DMF (1 mL) and the mixture was stirred at rt for 2 h, by which time the reaction mixture became homogeneous. Methylamine (2 M solution in THF, 2 mL, 2 mmol) was added, and the reaction mixture was stirred under nitrogen gas at rt for 12 h. The solvent was evaporated to dryness and the residue was purified using silica gel column eluted with MeOH/ CH_2Cl_2 (0:100-3:97). Evaporation of the solvent afforded the title compound as a white solid (0.16 g, 60% yield), mp 228-230 °C (lit.⁶ 227-229 °C). ^1H NMR (300 MHz, CDCl_3): δ (ppm) 3.1 (3H, d, $J = 4.8$ Hz, CH_3), 6.50 (1H, br s, NH), 7.81-7.85 (2H, m, H-6 and H-7), 8.27-8.34 (3H, m, H-3, H-5 and H-8), 8.35 (1H, dd, $J = 8.1$ Hz, H-4) and 8.50 [1H, d, $J = 1.8$ Hz, H-1]. ^{13}C NMR (75 MHz, CDCl_3): δ (ppm) 27.09 ($\underline{\text{C}}\text{H}_3$), 124.78, 127.42, 127.45, 127.99, 133.13, 133.40, 133.45, 133.49, 134.42, 134.50, 135.15, 139.53, 166.30 ($\underline{\text{C}}\text{ONH}$), 182.45 and 182.56 (C-9 and C-10). Anal calcd for $\text{C}_{16}\text{H}_{11}\text{NO}_3$: C, 72.45; H 4.18; N 5.28. Found: C, 72.39; H, 4.28; N, 5.31.

4.3.8. *N*-(*tert*-Butyl)anthraquinone-2-carboxamide (AQAmBu^t, reaction run by I. M. Abdou)

To a suspension of anthraquinone 2-carboxylic acid (0.25 g, 1.0 mmol) in dry DMF (3 mL) was gradually added a solution of 1,1'-carbonyldiimidazole (0.20 g, 1.1 mmol) in dry DMF

(1 mL) and the mixture was stirred at rt for 2 h, by which time the reaction mixture became homogeneous. *tert*-Butylamine (0.21 mL, 2.0 mmol) was added, and the reaction mixture was stirred under nitrogen gas at 40 °C for 12 h. The solvent was evaporated to dryness and the residue was purified using silica gel column eluted with MeOH/CH₂Cl₂ (0:100-3:97). Evaporation of the solvent afforded the title compound as a white solid (0.12 g, 40% yield), mp 235-237 °C. ¹H NMR (300 MHz, CDCl₃): δ (ppm) 1.53 (9H, s, *t*-Bu), 6.16 (1H, br s, NH), 7.82-7.88 (2H, m, H-6 and H-7), 8.25 (1H, dd, *J* = 1.8 and 8.1 Hz, H-3), 8.32-8.39 (3H, m, H-4, H-5, H-8) and 8.5 [1H, d, *J* = 1.8 Hz, H-1]. ¹³C NMR (75 MHz, CDCl₃): δ (ppm) 28.82 (C-CH₃), 52.31 (C-Me₃), 124.56, 127.43, 127.94, 133.12, 133.38, 133.41, 133.45, 134.39, 134.49, 134.93, 140.88, 164.98 (C=NH), 182.50 and 182.73 (C-9 and C-10). Anal calcd for C₁₉H₁₇NO₃: C, 74.27; H, 5.58; N, 4.56. Found: C, 74.24; H, 5.62; N, 4.50.

4.3.9. Dimethyl 6-(trimethylsilylethynyl)anthraquinone-2,3-dicarboxylate (TMS-Y-AQdiester)

Dimethyl 6-bromoanthraquinone-2,3-dicarboxylate (73 mg, 0.18 mmol), Pd(PPh₃)₂Cl₂ (7.0 mg, 0.010 mmol, 0.05 equiv), CuI (4.0 mg, 0.020 mmol, 0.10 equiv), TEA (50 μL, 0.40 mmol, 2.0 equiv) and TMSA (60 μL, 0.50 mmol, 3.0 equiv) were combined and stirred in dry THF (3.5 mL) under a nitrogen atmosphere at 60 °C for 45 min. TLC (eluting three times in CH₂Cl₂) showed complete consumption of the starting material. The solvent was evaporated *in vacuo*, and the crude material was purified by flash silica gel column chromatography eluted with CH₂Cl₂/hexanes (1:1-4:1). Evaporation of the eluting solvent afforded the title compound as a yellow solid (74 mg, 98%), mp 185-188 °C. ¹H NMR (400 MHz, CDCl₃): δ (ppm) 0.27 [9H, s, Si(CH₃)₃], 3.97 (6H, s, CO₂CH₃), 7.84 (1H, d, *J* = 8 Hz, H-7), 8.25 (1H, d, *J* = 8 Hz, H-

8), 8.36 (1H, s, H-5), 8.59 (1H, s) and 8.60 (1H, s) (H-1 and H-4). ^{13}C NMR (100 MHz, CDCl_3): δ (ppm) -0.06 [$\text{Si}(\underline{\text{C}}\text{H}_3)_3$], 53.43 ($\text{CO}_2\underline{\text{C}}\text{H}_3$), 101.33, 103.07, 127.83, 128.42, 128.48, 130.29, 131.16, 132.39, 133.30, 134.84, 134.99, 136.81, 136.95, 137.57, 166.64 ($\underline{\text{C}}\text{O}_2\text{Me}$), 166.68 ($\underline{\text{C}}\text{O}_2\text{Me}$), 181.18 and 182.29 (C-9 and C-10). HRMS m/z ($\text{M}+\text{H}$) $^+$: calcd for $\text{C}_{23}\text{H}_{21}\text{O}_6\text{Si}$ 421.1107, found 421.1089.

4.3.10. Dimethyl 6-ethynylantraquinone-2,3-dicarboxylate (H-Y-AQdiester)

To a solution of dimethyl 6-(trimethylsilylethynyl)anthraquinone-2,3-dicarboxylate (63 mg, 0.15 mmol) in THF/MeOH (1:1 v:v, 6 mL) was added KF (13 mg, 0.23 mmol, 1.5 equiv) and the mixture was stirred at rt for 1 h. The solvent was evaporated, and the crude product was purified by flash silica gel column chromatography eluted with CH_2Cl_2 . Evaporation of the eluting solvent afforded the title compound as a pale yellow powder (51 mg, 98%), mp 187-190 $^\circ\text{C}$. ^1H NMR (400 MHz, CDCl_3): δ (ppm) 3.37 (1H, s, $\equiv\text{C}-\text{H}$), 3.96 (6H, s, CH_3), 7.84 (1H, dd, $J = 1.6$ and 8 Hz, H-7), 8.24 (1H, d, $J = 8$ Hz, H-8), 8.35 (1H, d, $J = 1.6$ Hz, H-5), 8.55 (1H, s) and 8.57 (1H, s) (H-1 and H-4). ^{13}C NMR (100 MHz, CDCl_3): δ (ppm) 53.29 ($\underline{\text{C}}\text{H}_3$), 82.02 and 82.74 ($\text{C}\equiv\text{C}$), 127.83, 128.40, 128.44, 129.23, 131.29, 132.86, 134.82, 134.93, 136.95, 137.06, 137.75, 166.50 ($\underline{\text{C}}\text{O}_2\text{Me}$), 166.54 ($\underline{\text{C}}\text{O}_2\text{Me}$) and 180.05 (C-9 and C-10).

4.3.11. Dimethyl 6-ethynylantraquinone-2,3-dicarboxylate (Et-AQdiester)

Dimethyl 6-ethynylantraquinone-2,3-dicarboxylate (0.040 g, 0.12 mmol) was dissolved in EtOAc (30 mL). The resulting solution was transferred to a hydrogenation vessel containing 10% Pd/C (20 mg) that had been activated by stirring under H_2 gas (45 psi) in EtOAc (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 5-6 times. The vessel was finally charged with H_2 gas at 40 psi and

stirred at rt for 24 h, by which time TLC (alumina, eluted with CH₂Cl₂) showed a 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 CHCl₃. The crude product was loaded on silica gel and purified using silica gel column eluted with CH₂Cl₂/hexane (4:1-4:0). Evaporation of the eluting solvent afforded the title product as a pale yellow solid (34 mg, 84% yield), mp 152-155 °C. ¹H NMR (400 MHz, CDCl₃): δ (ppm) 1.29 (3H, t, *J* = 7.6 Hz, CH₃CH₂), 2.79 (2H, q, *J* = 7.6 Hz, CH₃CH₂), 3.95 (6H, s, CO₂CH₃), (1H, dd, *J* = 8 and 2 Hz, H-7), 8.09 (1H, d, *J* = 2 Hz, H-5), 8.17 (1H, d, *J* = 8 Hz, H-8), 8.547 (1H, s) and 8.550 (1H, s) (H-1 and H-4). ¹³C NMR (100 MHz, CDCl₃): δ (ppm) 15.11 (CH₃CH₂), 29.39 (CH₃CH₂), 53.33 (CO₂CH₃), 126.88, 128.07, 128.21, 128.24, 131.39, 133.45, 134.62, 134.99, 135.04, 136.51, 136.62, 152.34, 166.74 (CO₂Me), 181.55 and 182.05 (C-9 and C-10). HRMS *m/z* (M+Na)⁺: calcd for C₂₀H₁₆O₆Na 375.0845, found 375.0839.

4.3.12. 5'-*O*-Dimethoxytrityl-5-[(trimethylsilyl)ethynyl]-2'-deoxyuridine

To 5-iodo-2'-deoxyuridine (810 mg, 1.23 mmol), previously dried 2 times by co-evaporation with anhydrous THF, was added in the glove-box Pd(Ph₃P)₄ (72 mg, 0.060 mmol, 0.050 equiv), CuI (24 mg, 0.12 mmol, 0.10 equiv), TEA (0.34 mL, 2.5 mmol, 2.0 equiv), trimethylsilylacetylene (0.42 mL, 3.7 mmol, 3.0 equiv) and anhydrous DMF (6 mL). The homogeneous mixture was removed from the glove-box and stirred overnight under a nitrogen atmosphere at 50 °C in an oil bath; after which time DMF was removed under reduced pressure. The residue was applied to a silica gel column that had been pre-equilibrated with 1% TEA in CH₂Cl₂, and eluted with MeOH/CH₂Cl₂ (0:100-2:98). Evaporation of the eluting solvent

afforded the title compound as a white foam (768 mg, 99% yield). ^1H NMR (300 MHz, CDCl_3): δ (ppm) 0.00 [9H, s, CH_3 (TMS)], 2.16-2.25 (1H, m, H-2'), 2.49 (1H, ddd, $J = 2.1, 5.4$ and 13.5 Hz, H-2'), 3.31 (1H, dd, $J = 3.3$ and 10.8 Hz, H-5'), 3.41 (1H, dd, $J = 3$ and 10.8 Hz, H-5'), 3.79 [6H, s, OCH_3 (DMTr)], 4.04-4.16 (1H, m, H-4'), 4.42-4.51 (1H, m, H-3'), 6.28 (1H, dd, $J = 6$ and 7.2 Hz, H-1'), 6.84 [4H, d, $J = 8.4$ Hz, OPh-*meta* (DMT)], 7.18-7.35 [7H, m, OPh-*ortho* (DMTr, 4H) and Ph-*meta* and *para* (DMTr, 3H)], 7.44 [2H, d, $J = 8.1$ Hz, Ph-*ortho* (DMTr)], and 8.00 [1H, s, H-6 (dU)]. ^{13}C NMR (75 MHz, CDCl_3): δ (ppm) -0.41 [CH_3 (TMS)], 41.37 (C-2'), 55.17 [OCH_3 (DMTr)], 63.44 (C-5'), 72.15 (C-3'), 85.59, 86.33, 86.87 [C-1', C-4' and $\text{C}_{\text{Ar}3}$ (DMTr)], 94.96, 99.38 (C \equiv C), 100.44 [C-5 (dU)], 113.29 [OPh-*meta* (DMTr)], 126.88, 127.87, 128.00, 129.94, 135.50, 142.64, 144.39 [DMTr and C-6 (dU)], 149.36 [C-2 (dU)], 158.52 [OPh-C-4 (DMTr)], and 161.56 [C-4 (dU)]. HRMS m/z (M-H) $^-$: calcd 625.2370, found 625.2329.

4.3.13. 5'-*O*-Dimethoxytrityl-5-ethynyl-2'-deoxyuridine.^{16,17}

To a solution of 5'-*O*-Dimethoxytrityl-5-[(trimethylsilyl)ethynyl]-2'-deoxyuridine (587 mg, 0.940 mmol) in THF/MeOH (1:1 v:v, 10 mL) was added KF (0.070 g, 1.2 mmol, 1.2 equiv) and the mixture was stirred overnight at room temperature. The solvent was removed under reduced pressure and the residue was purified on a silica gel column that had been pre-equilibrated with 1% TEA in CH_2Cl_2 , and eluted with MeOH/ CH_2Cl_2 (0:100-3:97). Evaporation of the eluting solvent afforded the title compound as a white foam (500 mg, 96% yield). ^1H NMR (300 MHz, CDCl_3): δ (ppm) 2.21-2.33 (1H, m, H-2'), 2.52 (1H, ddd, $J = 3, 5.7$ and 13.5 Hz, H-2'), 2.90 (1H, s, $\equiv\text{C-H}$), 3.32-3.43 (2H, m, H-5'), 3.78 [6H, s, OCH_3 (DMTr)], 4.06-4.13 (1H, m, H-4'), 4.52-4.56 (1H, m, H-3'), 6.30 (1H, dd, $J = 6$ and 7.5 Hz, H-1'), 6.82-6.87 [4H, m,

OPh-*meta* (DMT)], 7.18-7.35 [7H, m, OPh-*ortho* (DMTr, 4H) and Ph-*meta* and *para* (DMTr, 3H)], 7.40-7.45 [2H, m, Ph-*ortho* (DMTr)], and 8.07 [1H, s, H-6 (dU)]. ^{13}C NMR (100 MHz, CDCl_3): δ (ppm) 41.53 (C-2'), 55.30 [OCH₃ (DMTr)], 63.45 (C-5'), 72.18 (C-3'), 74.38 ($\equiv\text{C-H}$), 81.85 ($\equiv\text{C-dU}$), 85.71, 86.45, 87.10 [C-1', C-4' and CAr₃ (DMTr)], 99.22 (C-5 (dU)), 113.40 [OPh-*meta* (DMTr)], 127.01, 127.95, 128.07, 130.03, 135.36, 135.53, 143.58, 144.42 [DMTr and C-6 (dU)], 149.53 [C-2 (dU)], 158.65 [OPh-C-4 (DMTr)], and 161.96 [C-4 (dU)]. HRMS m/z (M-H)⁻: calc'd 553.1975, found 553.2001.

4.3.14. 5'-O-Dimethoxytrityl-5-[(anthraquinone-2-yl)ethynyl]-2'-deoxyuridine [AQ-Y-dU(DMTr)]

To 2-iodoanthraquinone (0.40 g, 1.2 mmol) was added in the glove box Pd(Ph₃P)₄ (0.07 g, 0.06 mmol, 0.05 equiv), CuI (23 mg, 0.12 mmol, 0.10 equiv), TEA (0.33 mL, 2.4 mmol, 2.0 equiv), 5'-O-dimethoxytrityl-5-ethynyl-2'-deoxyuridine, previously dried three times by co-evaporation with anhydrous THF, (0.48 g, 0.86 mmol, 0.72 equiv) and anhydrous DMF (10 mL). The mixture was removed from the glove box and stirred under a nitrogen atmosphere for 30 min at 60 °C in an oil bath, after which time the mixture became homogeneous. DMF was removed under reduced pressure, and the residue was purified on a Biotage silica gel column that had been pre-equilibrated with 1% TEA in CHCl₃, and eluted with MeOH/CHCl₃ (0:100-5:95). Evaporation of the eluting solvent afforded AQ-Y-dU(DMTr) as a yellow foam (640 mg, 98% yield). $\epsilon_{378} = 15,817 \pm 100 \text{ Lmol}^{-1}\text{cm}^{-1}$ in MeOH. ^1H NMR (300 MHz, CDCl_3): δ (ppm) 2.36-2.46 (1H, m, H-2'), 2.51 (1H, ddd, $J = 2.4, 5.4$ and 13.5 Hz, H-2'), 3.34 (1H, dd, $J = 3$ and 10.8 Hz, H-5'), 3.52 (1H, dd, $J = 2.7$ and 10.8 Hz, H-5'), 3.69 [3H, s, OCH₃ (DMTr)], 3.70 [3H, s, OCH₃ (DMTr)], 4.14-4.20 (1H, m, H-4'), 4.62-4.68 (1H, m, H-3'), 6.40 (1H, dd, $J = 6$ and 7.2

Hz, H-1'), 6.79 [4H, dd, $J = 1.8$ and 9 Hz, OPh-*meta* (DMTr)], 7.04 [1H, dd, $J = 1.5$ and 8.1 Hz, H-3 (AQ)], 7.14 [1H, t, $J = 7.2$ Hz, Ph-*para* (DMTr)], 7.27 [2H, dd, $J = 7.2$ and 7.8 Hz, Ph-*meta* (DMTr)], 7.36 [4H, dd, $J = 0.9$ and 9 Hz, OPh-*ortho* (DMTr)], 7.46 [2H, d, $J = 7.8$ Hz, Ph-*ortho* (DMTr)], 7.76-7.84 [2H, m, H-6 (AQ) and H-7 (AQ)], 8.00 [1H, d, $J = 8.1$ Hz, H-4 (AQ)], 8.11 [1H, d, $J = 1.5$ Hz, H-1 (AQ)], 8.25-8.32 [2H, m, H-5 (AQ) and H-8 (AQ)], and 8.40 [1H, s, H-6 (dU)]. ^{13}C NMR (75 MHz, CDCl_3): δ (ppm) 41.85 (C-2'), 55.17 [OCH₃ (DMTr)], 63.41 (C-5'), 72.26 (C-3'), 84.85 ($\equiv\text{C}$ -dU), 85.99, 86.78, 87.18 [C-1', C-4' and CAr₃ (DMTr)], 92.31 ($\equiv\text{C}$ -AQ), 99.68 [C-5 (dU)], 113.35 [OPh-*meta* (DMTr)], 126.81, 127.08, 127.25, 127.87, 128.08, 128.66, 129.89, 132.20, 133.10, 133.39, 133.47, 134.16, 135.44, 136.62, 143.41, 144.41 [AQ, DMTr and C-6 (dU)], 148.92 [C-2 (dU)], 158.64 [OPh-C-4 (DMTr)], 160.74 [C-4 (dU)], 182.26 and 182.52 [C-9 and C-10 (AQ)]. HRMS m/z (M-H)⁻: calcd for C₄₆H₃₅N₂O₉ 759.2343, found 759.2320.

4.3.15. 5-[(Anthraquinone-2-yl)ethynyl]-2'-deoxyuridine (AQ-Y-dU)

5'-*O*-Dimethoxytrityl-5-[(anthraquinone-2-yl)ethynyl]-2'-deoxyuridine (130 mg, 0.17 mmol) was dissolved in CH_2Cl_2 (1.31 mL), and then dichloroacetic acid (DCA) (560 μL , 6.80 mmol, 40.0 equiv) was added. The resulting red slurry was stirred at room temperature for 45 min. The reaction mixture was then diluted by adding CHCl_3 (20 mL), and quenched by addition of triethylamine until the red color of the DMTr cation disappeared. More CHCl_3 (200 mL) and water (10 mL) were added, and the organic layer was separated, washed with water dried with anhydrous MgSO_4 , and evaporated to dryness. The solid was purified using flash silica gel chromatography on a Biotage column with MeOH/ CH_2Cl_2 (0:100-7:93) as the eluting system to yield AQ-Y-dU as an orange solid after evaporation of the eluting solvent (17 mg, 22% yield). ^1H NMR (300 MHz, $\text{DMSO}-d_6$): δ (ppm) 2.12-2.27 (2H, m, H-2' and H-2'), 3.57-3.73 (2H, m,

H-5' and H-5'), 3.80-3.84 (1H, m, H-4'), 4.24-4.31 (1H, m, H-3'), 5.24 (1H, t, $J = 4.8$ Hz, 5'-OH), 5.28 (1H, d, $J = 4.2$ Hz, 3'-OH), 6.13 (1H, t, $J = 6.6$ Hz, H-1'), 7.88-7.95 [3H, m, H-3 (AQ), H-6 (AQ), and H-7 (AQ)], 8.12 (1H, d, $J = 1.8$ Hz, H-1 (AQ)), 8.14-8.27 [3H, m, H-4 (AQ), H-5 (AQ), and H-8 (AQ)], and 8.53 [1H, s, H-6 (dU)]. ^1H NMR (300 MHz, DMSO- d_6 + D $_2$ O): δ (ppm) 2.04-2.26 (2H, m, H-2' and H-2'), 3.57 (1H, dd, $J = 3$ and 12 Hz, H-5'), 3.67 (1H, dd, $J = 3$ and 12 Hz, H-5'), 3.79-3.86 (1H, m, H-4'), 4.24-4.28 (1H, m, H-3'), 6.11 (1H, t, $J = 6.6$ Hz, H-1'), 7.87-7.94 [3H, m, H-3 (AQ), H-6 (AQ), and H-7 (AQ)], 8.10 [1H, d, $J = 1.8$ Hz, H-1 (AQ)], 8.14-8.20 [3H, m, H-4 (AQ), H-5 (AQ), and H-8 (AQ)], and 8.50 [1H, s, H-6 (dU)]. ^{13}C NMR (100 MHz, DMSO- d_6): δ (ppm) 39.74 (C-2'), 60.23 (C-5'), 69.29 (C-3'), 84.56 ($\equiv\text{C-dU}$), 87.14, 87.21 (C-1' and C-4'), 90.19 ($\equiv\text{C-AQ}$), 96.79 [(C-5 (dU)], 126.27, 126.80, 127.83, 128.15, 131.43, 132.38, 132.55, 132.73, 134.04, 134.18, 135.51 (AQ), 144.66 [C-6 (dU)], 148.85 [C-2 (dU)], 160.72 [C-4 (dU)], 181.20 and 181.36 [C-9 and C-10 (AQ)]. HRMS m/z (M-H) $^-$: calcd for C $_{25}$ H $_{17}$ N $_2$ O $_7$ 457.1036, found 457.1021.

4.3.16. 5'-*O*-Dimethoxytrityl-5-[2-(anthraquinone-2-yl)ethyl]-2'-deoxyuridine [AQ-E-dU(DMTr)]

5'-*O*-Dimethoxytrityl-5-[(anthraquinone-2-yl)ethynyl]-2'-deoxyuridine (1.0 g, 1.3 mmol) was dissolved in a mixture of ethyl acetate (50 mL) and MeOH (80 mL). The resulting solution was transferred to a hydrogenation vessel containing 10% Pd/C (500 mg) that had been activated by stirring under H $_2$ gas (45 psi) in MeOH (30 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 5-6 times. The vessel was finally charged with H $_2$ gas at 40 psi and stirred at rt. The reaction progress was monitored by UV-vis absorbance at 377 nm until the reaction mixture showed no absorbance (7

h). 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 CHCl₃. The crude product was loaded on silica gel and purified using silica gel chromatography on a Biotage column (40+M cartridge) that had been pre-equilibrated with 1% TEA in THF/hexane (2:3 v:v), eluting with MeOH/THF/hexane (1:40:60 v:v:v). Evaporation of the eluting solvent afforded AQ-E-dU(DMTr) as a pale yellow foam (323 mg, 32% yield). ¹H NMR (300 MHz, CDCl₃): δ (ppm) 1.82-1.97 (1H, m, H-2'), 2.23-2.41 (2H, m, CH₂CH₂), 2.46-2.63 (2H, m, CH₂CH₂), 2.73-2.87 (1H, m, H-2'), 3.35 (1H, dd, *J* = 2.5 and 8.4 Hz, H-5'), 3.56 (1H, dd, *J* = 2.7 and 8.4 Hz, H-5'), 3.68 [6H, s, OCH₃ (DMTr)], 4.10-4.18 (1H, m, H-4'), 4.59-4.67 (1H, m, H-3'), 6.52 (1H, dd, *J* = 6 and 7.2 Hz, H-1'), 6.72-6.85 [4H, m, OPh-*meta* (DMT)], 7.08 [1H, dd, *J* = 1.5 and 8.1 Hz, H-3 (AQ)], 7.14-7.34 [7H, m, Ph-*para*, Ph-*meta* and OPh-*ortho* (DMTr)], 7.42 [2H, d, *J* = 7.2 Hz, Ph-*ortho* (DMTr)], 7.70-7.79 [3H, m, H-6 (AQ), H-7 (AQ) and H-6 (dU)], 7.84 [1H, d, *J* = 1.5 Hz, H-1 (AQ)], 8.11 [1H, d, *J* = 8.1 Hz, H-4 (AQ)] and 8.22-8.27 [2H, m, H-5 (AQ) and H-8 (AQ)]. ¹³C NMR (75 MHz, CDCl₃): δ (ppm) 28.03 (CH₂CH₂), 34.74 (CH₂CH₂), 40.99 (C-2'), 55.08 [OCH₃ (DMTr)], 63.51 (C-5'), 72.39 (C-3'), 84.80, 86.39, 86.68 [C-1', C-4' and CAr₃ (DMTr)], 113.14 and 114.16 [OPh-*meta* (DMTr) and C-5 (dU)], 126.69, 126.96, 127.01, 127.14, 127.89, 128.14, 129.96, 130.07, 131.34, 133.16, 133.36, 133.40, 133.84, 134.16, 135.15, 135.26, 136.43, 144.14 [AQ, DMTr and C-6 (dU)], 148.40 [C-2 (dU)], 150.57 [C-2 (AQ)], 158.55 [OPh-C-4 (DMTr)], 163.46 [C-4 (dU)], 182.92 and 182.95 [C-9 and C-10 (AQ)]. HRMS *m/z* (M-H)⁻: calcd for C₄₆H₃₉N₂O₉ 763.2661, found 763.2648.

4.3.17. (*E*)-*N*-{2-[3-(5'-*O*-Dimethoxytrityl-2'-deoxyuridine-5-yl)acrylamido]ethyl}-anthraquinone-2-carboxamide [AQ-8-dU(DMTr)]

To a solution of dry (*E*)-2-[3-(5'-*O*-Dimethoxytrityl-2'-deoxyuridine-5-yl)acrylamido]ethylamine (Berry & Associates, Dexter, MI, 0.02 g, 31 μmol) in anhydrous DMF (1.5 mL) was added *N*-succinimidyl 2-anthraquinone-carboxylate⁵ (22 mg, 62 μmol), and triethylamine (13 μL , 93 μmol). The yellow solution was stirred overnight at room temperature. The solvent was evaporated and the residue was purified using preparative TLC (20 cm long x 10 cm wide x 0.2 cm thick) eluted with 6.5% methanol in dichloromethane. The product band was scratched off the plate and eluted with 10% methanol in dichloromethane. Evaporation of the solvent afforded AQ-8-dU(DMTr) as a pale yellow solid (26 mg, 95% yield). ϵ_{304} (MeOH): $19157 \pm 100 \text{ Lmol}^{-1}\text{cm}^{-1}$. ^1H NMR (400 MHz, DMSO-*d*₆): δ (ppm) 2.15-2.21 (1H, m, H-2'), 2.26-2.34 (1H, m, H-2'), 3.13-3.20 (2H, m, H-5'), 3.37-3.42 (4H, m, ethylene), 3.70 (1H, s, OCH₃), 3.71 (1H, s, OCH₃), 3.87-3.90 (1H, m, H-4'), 4.22-4.26 (1H, m, H-3'), 5.26 (1H, d, 6.55, $J = 4.7$ Hz, OH₃'), 6.15 (1H, t, $J = 6.5$ Hz, H-1'), 6.83-6.87 (4H, m, OPh-*meta* (DMTr)), 7.11 (2H, d, $J = 7.7$, vinyl), 7.17 (1H, t, $J = 8$ Hz, Ph-*para* (DMTr)), 7.22-7.29 (6H, m, OPh-*ortho* (DMTr, 4H) and Ph-*meta* (DMTr, 2H)), 7.35 (2 H, d, $J = 8$ Hz, Ph-*ortho* (DMTr)), 7.92-7.96 (3H, m, NH (amide), H-6 (AQ), and H-7 (AQ)), 8.21-8.25 (3H, m, H-3 (AQ), H-5 (AQ) and H-8 (AQ)), 8.27 (1H, s, H-6 (dU)), 8.32 (1H, dd, $J = 1.6$ and 8 Hz, H-4 (AQ)), 8.65 (1H, d, $J = 1.6$ Hz, H-1 (AQ)), 8.99 (1H, t, $J = 5$ Hz, NH (amide)), and 11.58 (1H, s, H-3 (dU)). ^{13}C NMR (100 MHz, DMSO-*d*₆): δ (ppm) 55.44, 55.45, 64.39, 70.74, 85.16, 86.07, 109.76, 113.66, 122.29, 126.06, 127.14, 127.29, 127.34, 127.52, 128.15, 128.28, 130.11, 132.98, 133.30, 133.54, 133.57, 133.60, 135.01, 135.14, 135.97, 136.06, 139.93, 143.38, 145.27, 149.75, 158.54, 162.27, 165.28,

166.47, and 182.62 (CO (AQ)). HRMS m/z (M-H)⁻: calcd for C₅₀H₄₃N₄O₁₁ 875.2928, found 875.2907.

4.3.18. 8-[(Anthraquinone-2-yl)ethynyl]-2'-deoxyadenine (AQ-Y-dA)

Pd(Ph₃P)₄ (32 mg, 0.028 mmol), CuI (11 mg, 0.055 mmol), and TEA (0.40 mL, 2.9 mmol) and 2-ethynylantraquinone (32 mg, 0.55 mmol) were combined and stirred in dry DMF (4.6 mL) under a nitrogen atmosphere. To this mixture was transferred a suspension of 8-bromo-2'-deoxyadenosine (140 mg, 0.61 mmol) in DMF (8 mL). After stirring the reaction mixture at 60 °C for 3 h, the solvent was evaporated *in vacuo*, and the crude material was purified three times by silica gel column chromatography (once by CH₂Cl₂/methanol, 0:100-7:93 v:v, and then twice by EtOAc/methanol, 0:100-2:98 v:v) to afford AQ-Y-dA (200 mg, 76%), mp 239-241 °C. ¹H NMR (300 MHz, DMSO-*d*₆): δ (ppm) 2.17 (1H, ddd, *J* = 12.9, 6.6, 2.7 Hz, H₂'), 3.22-3.13 (1H, m, H-2'), 3.56-3.47 (1H, m, H-5'), 3.72-3.65 (1H, m, H-5'), 3.93 (1H, dd, *J* = 13.5, 4.5 Hz, H-4'), 5.29 (1H, dd, *J* = 7.2, 4.5 Hz, H-3'), 5.40 (1H, d, *J* = 4.2 Hz, OH), 6.90 (1H, t, *J* = 6.59 Hz, H-1'), 7.68 (2H, br s, NH₂), 7.98-7.93 [2H, m, H-6, and H-7 (AQ)], 8.24-8.15 [4H, m, H-3, H-5 and H-8 (AQ), and H-2 (dA)], 8.27 [1H, d, *J* = 7.8 Hz, H-4 (AQ)], and 8.37 [1H, d, *J* = 1.5 Hz, H-1 (AQ)]. ¹³C NMR (75 MHz, DMSO-*d*₆): δ (ppm) 62.18, 71.21, 82.30, 85.23, 88.38, 92.61, 119.92, 125.72, 126.91, 127.48, 129.74, 132.22, 132.95, 133.09, 133.34, 133.45, 134.79, 134.87, 136.98, 148.62, 153.80, 156.26, 181.70, and 181.80. HRMS (FAB) m/z for C₂₆H₁₉N₅O₅ (M + H)⁺: calc'd. 482.47, found 482.10.

4.3.19. 8-[2-(Anthraquinone-2-yl)ethyl]-2'-deoxyadenine (AQ-E-dA)

Compound AQ-Y-dA (100 mg, 0.2 mmol) was dissolved in MeOH (150 mL) by heating while stirring. The resulting solution was transferred to a hydrogenation vessel containing 10%

Pd/C (50 mg) that had been activated by stirring under H₂ (40 psi) in MeOH (20 mL) for 30 min at room temperature. The vessel was next charged with H₂ gas and then degassed using an aspirator in a cycle that was repeated 5-6 times. The vessel was finally charged with hydrogen gas at 40 psi and stirred at room temperature for 24 h, by which time TLC showed a complete conversion of an initial spot to a more polar spot. The Pd/C catalyst was then removed by filtration over Celite, and adsorbed nucleoside residue was extracted from the catalyst by washing with boiling 10% MeOH in CHCl₃. The crude product was applied to a silica gel chromatography column eluted with MeOH/EtOAc (0:100-5:95). Evaporation of the eluting solvent afforded AQ-E-dA as a yellow powder (91 mg, 90% yield), mp 207-208 °C. ¹H NMR (400 MHz, DMSO-*d*₆): δ (ppm) 2.13 (1H, ddd, *J* = 8.4, 6.0, 2.0 Hz, H₂'), 3.05-3.10 (1H, m, H₂'), 3.27-3.41 (4H, m, CH₂CH₂), 3.47-3.52 (1H, m, H-5'), 3.63-3.66 (1H, m, H-5'), 3.86-3.89 (1H, m, H-4'), 4.45 (1H, br s, OH), 5.27 (1H, br s, OH), 5.44-5.53 (1H, m, H-3'), 6.32 (1H, t, *J* = 6.8 Hz, H-1'), 7.21 (2H, s, NH₂), 7.82-7.95 [3H, m, H-3, H-6, and H-7 (AQ)] and 8.06-8.18 [5H, m, H-1, H-4, H-5, and H-8 (AQ)]. ¹³C NMR (100 MHz, DMSO-*d*₆): δ (ppm) 27.91, 32.27, 37.37, 61.66, 70.74, 83.86, 87.59, 117.70, 126.06, 126.15, 126.21, 126.51, 130.79, 132.50, 133.92, 133.99, 134.32, 147.64, 149.30, 150.35, 150.95, 154.95, 181.68 and 181.99. HRMS *m/z* (M+H)⁺: calcd for C₂₆H₂₄N₅O₅ 486.1777, found 486.1769.

4.4. References

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Chapter 5

Synthesis, Electrochemistry and Spectroscopic Studies of (2-Anthraquinonyl)trimethylammonium Trifluoromethanesulfonate

5.1. Introduction

Previous work in our research group¹ showed that photoexcitation of an AQ-dA conjugate (AQEdA, Figure 5.1) at 341 nm in methanol did not produce the expected $AQ^{\bullet-}/dA^{\bullet+}$ product. On the other hand, photoexcitation at the same wavelength of an aqueous solution of dA and 2-anthraquinone sulfonic acid sodium salt (AQ2S⁻) did produce the $AQ2S^{\bullet 2-}/dA^{\bullet+}$ product. This implies that in the second case, the CS product may have been stabilized by proton transfer reactions with water or by the presence of the electron-withdrawing sulfonate group on AQ, or by both processes together. This suggested to us that introduction of a quaternary ammonium group on AQ in an AQ-dA conjugate (for example, AQqN⁺-dA, Figure 5.1) might both stabilize an electron transfer (ET) product and render the conjugate soluble in aqueous solutions. Furthermore, the quaternary ammonium group on the AQ subunit of the conjugate would allow formation of an AQqN[•]/dA⁺ charge shift (CShf) product upon photoexcitation. A CShf product might live longer than a CS product due to the lack of Coulombic attraction between the oxidized and reduced subunits. Herein I report the synthesis of AQqN⁺ (both as CF₃SO₃⁻ and PF₆⁻ salts), measurements of the UV-vis spectra of its CF₃SO₃⁻ salt in acetonitrile (MeCN), methanol (MeOH), and water, and results of electrochemical measurements.

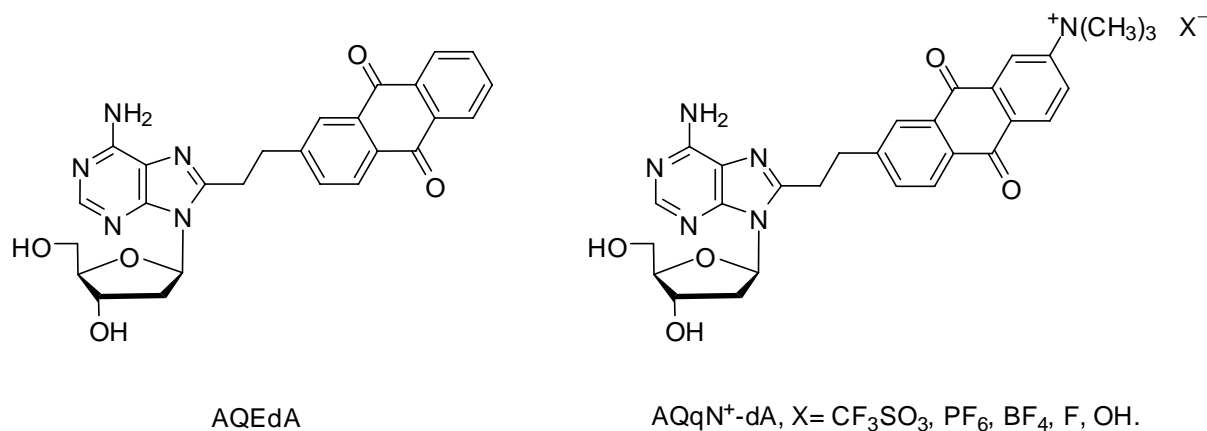


Figure 5.1. Structural drawings of AQEdA and AQN⁺-dA.

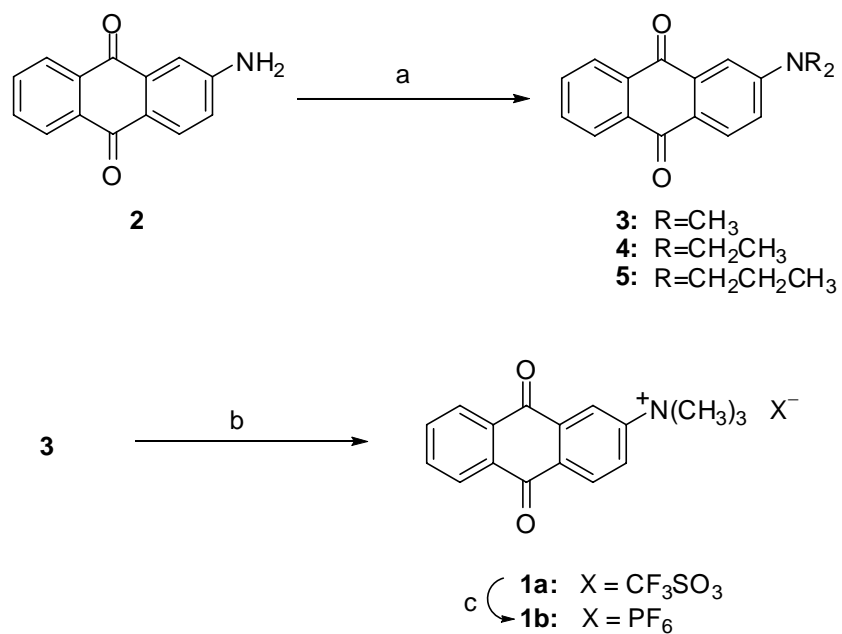
5.2. Results and Discussion

5.2.1. Synthesis of AQNMe₃Tf (1a)

As shown in Scheme 5.1, the starting material 2-dimethylaminoanthraquinone (**3**) was prepared from 2-aminoanthraquinone (**2**) and iodomethane in the presence of sodium hydride in *N,N*-dimethylformamide (DMF).² Treatment of **3** with 10 equivalents of iodomethane³ in refluxing dichloromethane for 24 hours did not result in quaternization of the amine of **3**. Instead, **3** was completely recovered. The observed lack of reactivity of this dialkylamine derivative toward quaternization might be attributed to the presence of the dialkylamine moiety *para* to one of the carbonyl groups of AQ. This *para* substitution pattern could delocalize the lone pair of electrons of the amino group of **3** into the ring and consequently deactivate it with respect to nucleophilic reactions. Trifluoromethanesulfonate (Tf) is a better leaving group than iodide and indeed methyl trifluoromethanesulfonate (MeTf) has been reported to quaternize tertiary aromatic amines having electron-withdrawing groups on their ring.^{4,5} Treatment of **3** with 1.1 equivalent of MeTf in CH₂Cl₂ and stirring overnight at room temperature resulted in the

formation of AQNMe₃Tf (**1a**) in a quantitative yield. Interestingly, a change in the color characteristics of the dialkylamine derivatives upon quaternization was observed. The colors of **3**, **4**, and **5** were bright reddish, while **1a** and **1b** had a light pink color. This color change upon quaternization indicated loss of a low energy amine-to-AQ charge transfer state in the ammonium salt.

We envisioned that the presence of groups with more lipophilic character than methyl on the ammonium group of AQqN⁺ would enhance its solubility in the organic solvents. This enhanced solubility would be needed for multistep synthesis of the nucleoside conjugate and for spectroscopic studies. Thus, 2-diethylaminoanthraquinone (**4**) and 2-dipropylaminoanthraquinone (**5**) were synthesized using the corresponding iodoalkanes under the same conditions used for the synthesis of **3**. Compound **1a** was found to be soluble in dimethylsulfoxide (DMSO), MeOH, MeCN, and also water. Thus **1a** meets our solubility requirements for spectroscopic studies. The triflate counter ion in **1a** was exchanged for hexafluorophosphate by addition of a saturated aqueous solution of NH₄PF₆ to a saturated aqueous solution of **1a**. The mixing resulted in the immediate precipitation of AQNMe₃PF₆ (**1b**). The identity of **1b** was confirmed by IR as shown in Figure 5.2. An attempt to exchange the triflate with chloride using 10 equivalents of HCl in methanol⁶ failed due to the much lower pK_a of triflic acid compared to HCl. The stability of **1a** toward basic conditions, such as concentrated ammonium hydroxide (which is used during solid phase oligonucleotide synthesis) was also tested. Anthraquinone **1a** was dissolved in concentrated aqueous ammonium hydroxide for 5 hours while stirring at 60 °C. No decomposition of **1a** occurred as judged by ¹H NMR.



Scheme 5.1. Reagents and conditions. (a) *i.* NaH, DMF. *ii.* RI. (b) CF₃SO₃CH₃, CH₂Cl₂. (c) H₂O, NH₄PF₆

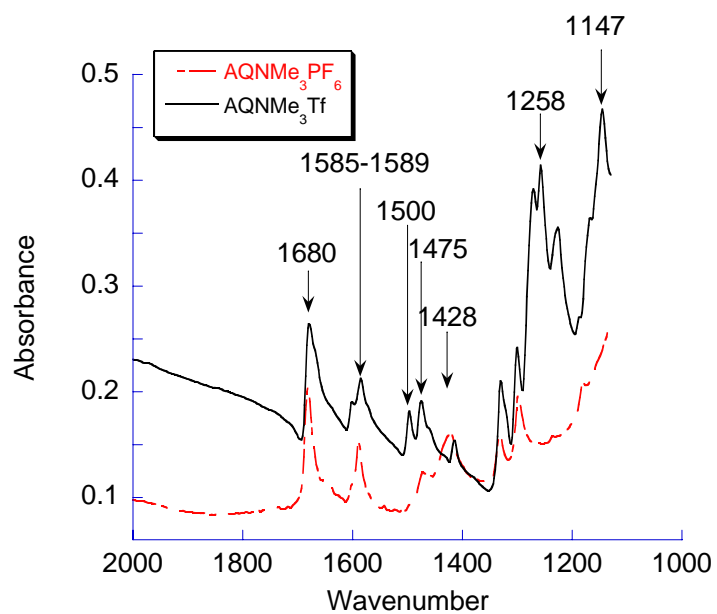


Figure 5.2. IR spectra of AQNMe₃Tf (**1a**) and AQNMe₃PF₆ (**1b**).

5.2.2. UV-vis Studies of (2-Anthraquinonyl)trimethylammonium

Trifluoromethanesulfonate (**1a**)

Figure 5.3 shows the UV-Vis absorbance spectrum of a 2.89×10^{-5} M solution of **1a** in MeOH. The two characteristic (π , π^*) bands of anthraquinone¹ have absorbance maxima (λ_{\max}) at 251 and 316 nm. The forbidden (n , π^*) state occurs at 424 nm, but is not seen in this spectrum due to its very low molar absorbance.

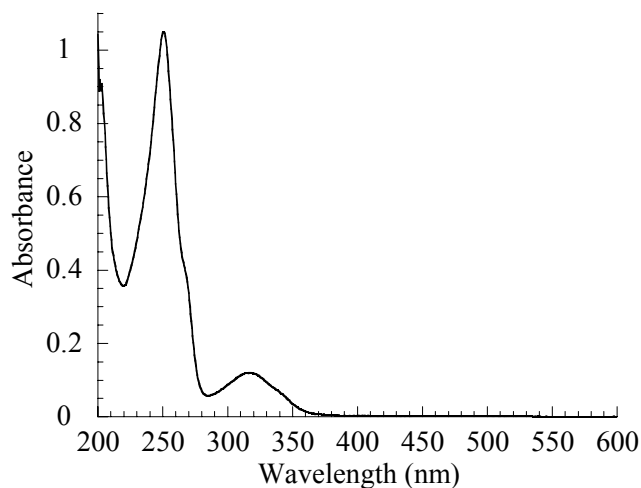


Figure 5.3. UV-Vis absorbance spectrum of a 2.89×10^{-5} M solution of AQNMe₃Tf in MeOH.

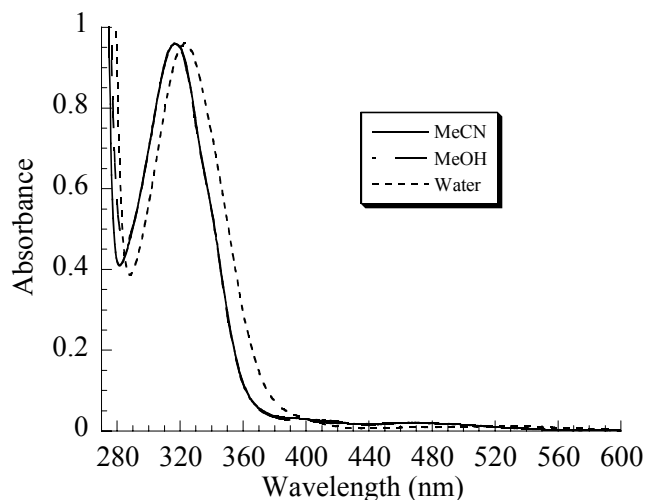


Figure 5.4. Normalized UV-Vis absorption spectra of AQNMe₃Tf in MeCN, MeOH, and water. Solution concentrations were 1.84×10^{-4} , 2.31×10^{-4} , and 2.02×10^{-4} M respectively. (Note that the spectra in MeOH and MeCN are nearly identical.)

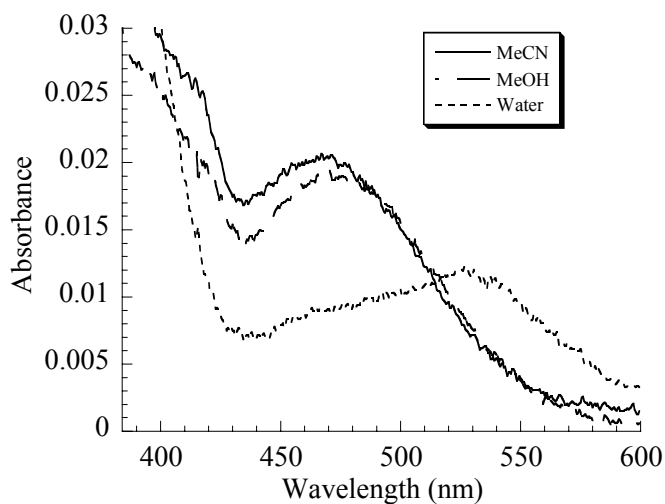


Figure 5.5. UV-Vis absorption spectra of AQNMe₃Tf in MeCN, MeOH, and water at the same concentrations in Figure 5.4.

Figure 5.4 shows the UV-vis normalized absorbance spectra of **1a** in MeCN, MeOH, and water. The 316 nm band occurs at the same wavelength in both MeCN and MeOH, but is red-

shifted 7 nm to 323 nm in water. Molar absorbances (ϵ) for this band are, respectively, 5210, 4140, and 4750 (± 100) $M^{-1}cm^{-1}$ in MeCN, MeOH, and water. A new absorbance band not present in anthraquinone itself also occurs for **1a** in all three solvents: 468 nm in MeCN, 476 nm in MeOH and 531 nm in water (Figure 5.5). Compound **1b** (the PF_6^- salt) shows the same new band in water also at the 531 nm. The small molar absorbance (108 $M^{-1}cm^{-1}$ in both MeCN and MeOH, and 54 $M^{-1}cm^{-1}$ in water) of this new band and its wavelength shift with change in solvent dielectric properties strongly suggest that it is due to a counter ion-to-AQqN⁺ charge transfer state.

5.2.3. Electrochemical and Laser Transient Absorbance results

Electrochemical measurements were performed by Y. Hussein for compound **1b** in both anhydrous acetonitrile and aqueous media and reduction potential values were reported versus saturated calomel electrode (SCE) (Table 5.1). Experiments in acetonitrile with 0.10 M TBAH as the supporting electrolyte and aqueous electrochemistry was run using 0.10 M KCl in phosphate buffer at different pH values.

Table 5.1. Reduction potential values of AQ and AQNMe₃PF₆.

Compound	Solvent	$E_{1/2}$ (V) vs. SCE
AQ	MeCN	-0.939
AQNMe ₃ PF ₆	MeCN	-0.713
AQNMe ₃ PF ₆	H ₂ O (pH = 13)	-0.560
AQNMe ₃ PF ₆	H ₂ O (pH = 7)	-0.439
AQNMe ₃ PF ₆	H ₂ O (pH = 1)	-0.128

Modification of AQ with the ammonium group made AQNMe₃PF₆ easier to reduce than AQ itself by 226 mV in acetonitrile. In water the reduction potential values were in general lower than in acetonitrile. The reduction potential at pH 7 is lower than that at pH 13 by 121 mV. An additional decrease by 311 mV in the reduction potential occurred when the pH was further lowered to 1. This decrease in potential at low pH is due to the stabilization of the resulting AQ anion radical by proton transfer from the buffer.

Transient absorbance measurements of AQNMe₃PF₆ was also done by Y. Hussein and showed that excitation at 341 nm at pH 1 and pH 5 resulted in the formation of electron transfer product with the fully protonated anion formed [AQNMe₃(H)^{•+}]. Excitation at pH 7-12 resulted in the formation of the non-protonated anion product (AQNMe₃^{•-}), while excitation at pH 6 the spectra for both the protonated and non-protonated anion product. On the other hand, excitation at pH 13 did not show any electron transfer product. In conclusion, excitation of AQNMe₃PF₆ at pH < 13 can result in electron transfer and reduction of AQ by anions from the buffer. It has been previously reported that some excited AQ derivatives undergo electron transfer reactions with simple inorganic anions.⁷

5.3. Experimental

5.3.1. Reagents and General Procedures

2-Aminoanthraquinone was purchased from Lancaster as a yellowish brown powder (96% purity) and used as received. Solvents were dried and redistilled in continuous circulation distillation apparatus. THF was dried with benzophenone /Na⁰, and both DMF and methylene chloride were redistilled over CaH₂. Water was supplied from a Millipore ultrapure water

system (model Milli-Q plus). All manipulations of methyl triflate were performed in a Vacuum Atmospheres M040-2 glove box pressurized with dry nitrogen gas. Additions of alkyl iodide and methylene chloride were carried out on the benchtop under a dry nitrogen atmosphere using cannula techniques. Most of the reactions were monitored with glass-backed TLC Plates precoated with silica gel 60 F₂₅₄ (EMD Chemicals). HPLC grade solvents were used for chromatographic purifications. Flash column chromatography was carried out on either a Biotage Flash-40™ system using prepackaged KP-Sil™ cartridges or on Whatman™ flash silica (60Å pore, 230 – 400 mesh) that was packed in glass columns and pressurized with nitrogen. Melting points were corrected.

5.3.2. UV-vis Spectroscopy and NMR

UV-vis absorbance spectra were recorded with a Shimadzu UV-2501 spectrophotometer. THF, MeCN, and MeOH solvents used in these measurements were HPLC grade and used without further purification. Molar absorption values were determined using Beer's law. NMR Spectra were obtained at Georgia State University from a Varian Unity+300 NMR spectrometer operating at either 75 or 300 MHz frequencies.

5.3.3. 2-(Dimethylamino)anthraquinone (3)

Dry 2-aminoanthraquinone **2** (300 mg, 1.34 mmol) was dissolved in neat DMF (7 mL), followed by addition of NaH (71 mg, 3.0 mmol). The dark green mixture was stirred under a nitrogen atmosphere for one hour, and turned reddish upon addition of iodomethane (0.20 mL, 3.2 mmol). The reaction was quenched with ammonium chloride when TLC showed complete consumption of the starting material after stirring for 2 hours. The solvent was evaporated under reduced pressure, the crude product was extracted with methylene chloride, washed with water,

dried over MgSO₄, and purified by nitrogen pressurized silica gel column chromatography (CH₂Cl₂/ ethyl acetate, 9:1-1:1 v:v), to give **3** (329 mg, 97% yield) as red-orange solid, mp 183-185 °C (lit.⁸ 186 °C). ¹H and ¹³C NMR spectra for **3** were identical to those published in the literature.²

5.3.4. 2-(Diethylamino)anthraquinone (**4**)⁹

Dry 2-aminoanthraquinone **2** (3 g, 13.4 mmol) was dissolved in neat DMF (20 mL), followed by addition of NaH (0.71 g, 29.6 mmol). The dark green mixture was stirred under nitrogen atmosphere for one hour, and formed a reddish paste upon addition of iodoethane (2.68 mL, 33.5 mmol). The reaction was quenched with ammonium chloride after stirring for 2 hours. The solvent was evaporated under reduced pressure; the crude product was extracted with methylene chloride, washed with water, dried over MgSO₄, and purified by unpressurized silica gel column chromatography using 20% hexanes in CH₂Cl₂ as the eluting system. A fluorescent by-product was still present after chromatography and was removed by extraction with hexane. Crystallization of the remaining solid from ethanol gave **4** as bright red needles (1.07 g, 29% yield), mp 161-163 °C (lit.¹⁰ 164-165 °C). ¹H NMR (300 MHz, CDCl₃): δ (ppm) 8.29-8.22 (m, 2H), 8.14 (d, 1H, *J* = 9 Hz) 7.80-7.66 (m, 2H), 7.42 (d, 1H, *J* = 3 Hz), 6.90 (dd, 1H, *J* = 9 and 3 Hz), 3.51 (q, 4H, *J* = 7.2 Hz), and 1.25 (t, 6H, *J* = 7.2 Hz). ¹³C NMR (75 MHz, CDCl₃): δ (ppm) 184.31, 181.22, 151.63, 135.13, 134.38, 133.83, 133.62, 132.78, 129.95, 126.76, 126.75, 121.28, 115.29, 107.92, 44.72, and 12.52.

5.3.5. 2-(Dipropylamino)anthraquinone (**5**)

Dry 2-aminoanthraquinone **2** (3.00 g, 13.4 mmol) was dissolved in neat DMF (20 mL), followed by addition of NaH (0.710 g, 29.6 mmol). The dark green mixture was stirred under

nitrogen atmosphere for one hour. It formed a reddish paste upon addition of 1-iodopropane (3.27 mL, 33.5 mmol). The reaction was quenched with ammonium chloride after stirring for 2 hours. The solvent was evaporated under reduced pressure; the crude product was extracted with methylene chloride, washed with water, dried over MgSO₄, and purified by unpressurized silica gel column chromatography using 30% hexanes in CH₂Cl₂ as the eluting system. A fluorescent by-product was still present after chromatography and was removed by extraction with hexane. Crystallization of the remaining solid from ethanol gave **5** as dark red plates (0.83 g, 20% yield), mp 142-144 °C. ¹H NMR (300 MHz, CDCl₃): δ (ppm) 8.29-8.22 (m, 2H), 8.14 (d, 1H, *J* = 9 Hz) 7.77-7.67 (m, 2H), 7.42 (d, 1H, *J* = 3 Hz), 6.90 (dd, 1H, *J* = 9 and 3 Hz), 3.4 (t, 4H, *J* = 7.5 Hz), 1.69 (s, 4H, *J* = 7.5 Hz), and 0.99 (t, 6H, *J* = 7.5 Hz). ¹³C NMR (75 MHz, CDCl₃): δ (ppm) 184.43, 181.31, 152.18, 135.12, 134.47, 133.91, 133.70, 132.87, 129.93, 126.82, 121.30, 115.51, 108.19, 52.85, 20.47 and 11.37.

5.3.6. (2-Anthraquinonyl)trimethylammonium Trifluoromethanesulfonate (**1a**)

To a solution of **3** (125 mg, 0.497 mmol) in dry dichloromethane, methyl trifluoromethanesulfonate (0.055 mL, 0.55 mmol) was added in a glove box. The mixture was stirred for 48 hr until the red-orange color of the mixture disappeared, and TLC (10% hexanes in CH₂Cl₂) showed complete consumption of the starting material. The resulting precipitate was filtered, washed with CH₂Cl₂, and dried in vacuo to afford **1a** as a pink solid (202 mg, 98% yield), mp 276-278 °C. ε₃₂₃ (H₂O): 4747 ± 100 Lmol⁻¹cm⁻¹, ε₃₁₆ (MeCN): 5191 ± 100 Lmol⁻¹cm⁻¹, ε₃₁₆ (MeOH): 4139 ± 100 Lmol⁻¹cm⁻¹, ε₃₁₇ (THF): 4697 ± 100 Lmol⁻¹cm⁻¹. ¹H NMR (300 MHz, CDCl₃): δ (ppm) 8.63 (d, 1H, *J* = 2.7 Hz), 8.49 (dd, 1H, *J* = 9 and 2.7 Hz), 8.39 (d, 1H, *J* = 9 Hz), 8.26-8.21 (m, 2H), 7.97-7.94 (m, 2H), and 3.70 (s, 9H) ¹³C NMR (75 MHz, CDCl₃): δ

(ppm) 181.33, 181.29, 151.10, 135.00, 134.90, 134.32, 133.70, 132.99, 132.87 129.01, 127.00, 126.94, 126.59, 119.05, and 56.37. IR (cm^{-1} , solid film) 1680, 1586, 1500, 1475, 1413, 1331, 1300, 1258, and 1147.

5.3.7. (2-Anthraquinonyl)trimethylammonium hexafluorophosphate (**1b**)

To a saturated solution of **1a** (100 mg) in water, was added an excess amount of saturated aqueous solution of ammonium hexafluorophosphate while stirring. A pink precipitate formed immediately and was filtered, washed with water, and dried in vacuo to afford **1b** as a pink solid in a quantitative yield, mp 267-269 °C. ϵ_{324} (H_2O): $6435 \pm 100 \text{ Lmol}^{-1}\text{cm}^{-1}$, ϵ_{341} (H_2O): $4803 \pm 100 \text{ Lmol}^{-1}\text{cm}^{-1}$. ^1H NMR (300 MHz, CDCl_3): δ (ppm) 8.63 (d, 1H, $J = 2.7$ Hz), 8.49 (dd, 1H, $J = 9$ and 2.7 Hz), 8.39 (d, 1H, $J = 9$ Hz), 8.25-8.20 (m, 2H), 7.96-7.93 (m, 2H), and 3.71 (s, 9H). ^{13}C NMR (75 MHz, CDCl_3): δ (ppm) 181.31, 181.27, 151.10, 134.99, 134.90, 134.32, 133.70, 132.97, 132.85 129.03, 127.00, 126.94, 126.57, 119.05, and 56.37. IR (cm^{-1} , solid film) 1685, 1589, 1428, 1331, and 1300.

5.4. References

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Chapter 6

Synthesis of Two Intermediates Useful for Substituting DNA with AQ-dA Conjugates and Optimization of 3'-Phosphorylation and Other Reaction Conditions using 2'-Deoxyadenosine as a Substrate.

6.1 Introduction

Herein is reported the synthesis of two intermediates useful for substituting DNA with AQ-dA conjugates via solid phase DNA synthesis. Also reported is the optimization of reaction conditions starting with 2'-deoxyadenosine (dA) to obtain the corresponding 3'-benzyl hydrogen phosphate. The optimized reaction conditions were used to prepare the 3'-benzyl hydrogen phosphate of AQ-dA conjugates described in Chapter 2. dA was used for this purpose for three reasons: 1) to test phosphoramidite chemistry without N⁶-protection, 2) to determine stability of phosphate protection toward DMTr deprotection, and 3) to establish the stability of both the N-glycosidic bond and the phosphate group itself toward the variety of reaction conditions needed to produce 2'-deoxyadenosine 3'-benzyl phosphate (**20**).

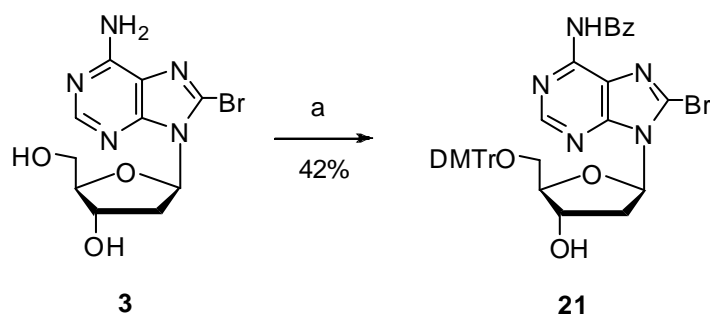
6.2. Results and Discussion

6.2.1. Synthesis of Two Intermediates Useful for Substituting DNA With AQ-dA Conjugates

6.2.1.1. N⁶-Benzoyl-5'-O-(4,4'-dimethoxytrityl)-8-bromodeoxyadenosine (21)

Compound **21** (shown in Scheme 6.1) was prepared from **3** (8-bromo-2'-deoxyadenosine) by protection of 6-NH₂ with benzoyl and 5'-OH with DMTr, respectively, using BzCl and

DMTrCl. Both of these protecting groups were specifically required for dA incorporation into DNA by standard solid-phase synthetic protocols. Literature methods to produce brominated **21** employed separate steps of benzylation and tritylation of **3** in arbitrary in either order.¹⁻³ On the other hand, a one-pot reaction procedure was reported for small scale preparation of non-brominated *N*⁶-benzoyl-5'-*O*-dimethoxytrityl-2'-deoxyadenosine from 2'-deoxyadenosine. In this method 2'-deoxyadenosine is successively tritylated and then benzyolated in one step without isolation of an intermediate.⁴ Here, we applied this same one-pot procedure to produce brominated **21** from **3** (see Scheme 6.1). Thus, the 5'-OH of **3** was first protected with DMTr by treatment with 1.1 equivalents of DMTrCl in anhydrous pyridine while monitoring the reaction with TLC. Next, 3'-OH was transiently protected with TMS,⁵ and lastly, 6-NH₂ was protected with Bz. The reaction was kept anhydrous, and 5 equivalents of TMSCl were added to the reaction mixture to silylate 3'-OH. This was followed by addition of 5 equivalents of BzCl to the same mixture to dibenzoylate 6-NH₂. Successive stirring with 2 M NH₄OH for 1 h cleaved both the trimethylsilyl group at 3'-OH and one of the benzoyl groups at 6-NH₂ to give **21** after chromatography (42% unoptimized yield).

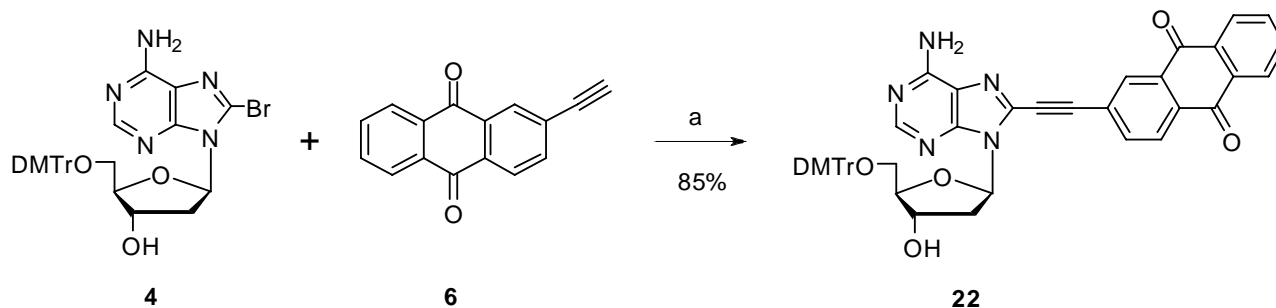


Scheme 6.1. Reagents and conditions: (a) *i*- DMTrCl, 4-DMAP, TEA, Py, rt, 4h; *ii*- TMSCl, rt, 1h, *iii*- BzCl, rt, overnight, *iv*- NH₄OH, MeOH, rt, 2h.

To incorporate an AQ-dA conjugate into DNA, **21** could be joined to AQ via palladium catalyzed cross-coupling and then treated with 2-cyanoethyl *N,N*-diisopropylchlorophosphoramidite to produce the 3'- β -cyanoethylphosphoramidite of the AQ-dA or **21** itself could be turned into a phosphoramidite. In the latter case ethynyl-AQ could be Pd cross-coupled to 8-bromo-dA on the DNA strand during solid-phase synthesis, but prior to detritylation.^{6,7} To date neither of these reactions has been run on **21**, so we do not know their efficiencies.

6.2.1.2. 5'-*O*-(4,4'-Dimethoxytrityl)-8-[(anthraquinone-2-yl)ethynyl]-2'-deoxyadenosine (**22**)

In case benzylation of 6-NH₂ leads to problems during alkylation of **21**, it is also possible to form **22** first (see Scheme 6.2) via palladium catalyzed cross-coupling between 5'-*O*-(4,4-dimethoxytrityl)-8-bromo-2'-deoxyadenosine (**4**) and 2-ethynylantraquinone (**6**) (Syntheses of **4** and **6** are shown in Chapter 2). Alkylation of **4** was achieved by stirring the reactants for 6 h with catalytic amounts of Pd(Ph₃P)₄ and CuI, and 2 equivalents of TEA (as base) in DMF at 65 °C under anhydrous conditions. Filtration of the reaction mixture over Celite removed unwanted insoluble materials. Evaporation of DMF and silica gel purification afforded **22** in 85% yield.

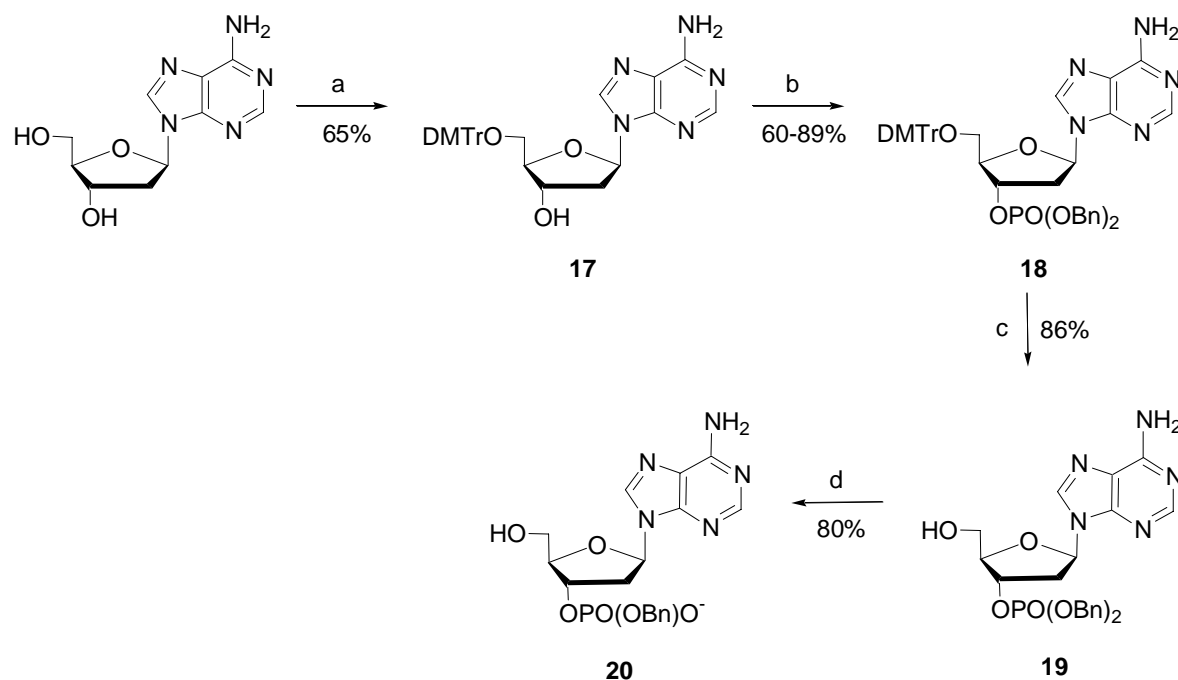


Scheme 6.2. Reagents and conditions: (a) Pd(Ph₃P)₄, CuI, TEA, DMF, 65 °C, 6 h.

Compound **22** is a possible intermediate for the synthesis of the 3'- β -cyanoethylphosphoramidite of an ethynyl linked AQ-dA conjugate for incorporation into DNA. Prior to phosphitylation, **22** would have to be protected at N⁶ with benzoyl. This reaction on **22** has not been run yet. Additionally, in Chapter 2, **4** was phosphorylated to produce **5** prior to alkylation via Pd cross-coupling to produce **7**. Thus, Pd catalyzed cross-coupling of either **4** or **5** proceeds smoothly. However, the yield of **22** (85%) exceeds the yield of **7** (57%).

6.2.2. Optimization of Phosphorylation and Other Reaction Conditions using 2'-Deoxyadenosine as a Substrate

Compound **17** in Scheme 6.3 was prepared in 65% yield by tritylation of 2'-deoxyadenosine using DMTrCl, TEA and 4-DMAP (as a catalyst) in pyridine.⁴ This relatively low yield was due to the conversion of the remainder of 2'-deoxyadenosine to a ditritylated product as identified by ¹H NMR. This latter product was separated first during a silica gel column using 3% MeOH in CH₂Cl₂ as eluent; then **17** was collected by increasing the polarity of the eluent to 5% MeOH in CH₂Cl₂.



Scheme 6.3. Compounds used to optimize reaction conditions. Reagents and conditions: (a) DMTrCl, 4-DMAP, TEA, Py, rt, 2 h; (b) *i*- (BnO)₂PN(*i*Pr)₂, Me-tetrazol, THF, rt, 1 h; *ii*- *m*-CPBA in CH₂Cl₂, -78 °C, 15 min; (c) 30% DCA in CH₂Cl₂, rt, 30 min; (d) DABCO, 1,4-dioxane, reflux, 2 h.

5'-*O*-Dimethoxytrityl-2'-deoxyadenosine 3'-dibenzyl phosphate (**18**) was prepared from **17** using the earlier described phosphoramidite chemistry. Similar phosphitylation reactions in the literature used a range of equivalents of the phosphoramidite reagent (2.5-1.1).⁸⁻¹⁰ To test reacting the 3'-OH of **17** while avoiding N⁶-phosphorylation and to optimize the yield of **18**, the reaction was run three times in THF using 2.5, 1.5, and 1.1 equivalents of (BnO)₂PN(*i*Pr)₂ (see Table 6.1). The amount of Me-tetrazol was equimolar to (BnO)₂PN(*i*Pr)₂, and (BnO)₂PN(*i*Pr)₂ was added dropwise at 0 °C to increase the reaction selectivity; finally, the reaction mixture was slowly warmed to room temperature as stirred for 1 h. Reacting 2.5 equivalents of the reagent led to complete consumption of **17** and showed a single spot on TLC. Later, ¹H NMR of the

final oxidized phosphorylation products showed that this single TLC spot was actually due to phosphorylation at 3'-OH as well as at 6-NH₂. Subsequent treatment with *m*-CPBA at -78 °C for 1 h produced 2 spots, one from each phosphorylation product. Table 6.1 shows that the yield of **18** in this case was 72% after chromatographic purification. Reacting 1.5 equivalents of (BnO)₂PN(*i*Pr)₂ with **17** using the same procedures produced **18** in 89% yield after chromatographic separation also, less N⁶-phosphorylated product was produced than with 2.5 equivalents of reagent. Decreasing the equivalents of (BnO)₂PN(*i*Pr)₂ to 1.1, however, dropped the yield of **18** to about 60% (as observed via TLC before oxidation) and 40% of **17** remained unreacted.

Table 6.1. Yield of **18** Versus the Number of Equivalents of (BnO)₂PN(*i*Pr)₂.

Entry	No. of equiv.	%Yield
1	2.5	72 ^a
2	1.5	89 ^a
3	1.1	60 ^b

^a Yield after separation of final oxidized product **18**.

^b Yield estimated based on TLC spot size before the *m*-CBPA oxidation step.

As shown in Scheme 6.3, detritylation of **18** produced 2'-deoxyadenosine 3'-dibenzyl phosphate (**19**). This was accomplished by treatment of **18** with 40 equivalents of 30% DCA in dichloromethane at room temperature for 30 min. Lower concentrations or equivalents of the acid caused incomplete detritylation. This result was surprising as DMTr was readily cleaved at 5'-O in less than 1 min either using 3% DCA,¹¹ or 1% TFA¹² during solid-phase oligodeoxynucleotide synthesis. Here use of TFA was avoided, because it might lead to

depurination.¹¹ After the treatment of **18** with DCA, the acidic reaction mixture was neutralized with TEA or saturated aqueous sodium bicarbonate, and **19** was extracted from the aqueous phase with dichloromethane. Purification of the crude product by silica gel chromatography gave **19** in 85% yield. This result showed that dibenzyl protection of the phosphate group was stable under DMTr-deprotection conditions; additionally, no depurination occurred for either the nucleotides **18** or **19**.

Monodeprotection of the dibenzyl phosphate **19** formed **20** in 80% yield. Deprotection of benzyl groups by hydrogenolysis could not be used in this work, because it would reduce the ethynyl linker in the synthesis of nucleotide **1**. Therefore, we tested two other literature procedures for deprotection of dibenzyl phosphates. The first procedure used 5 equivalents of TMSBr in anhydrous CH₂Cl₂, with quenching of the reaction under slightly basic conditions using a 1 M aqueous solution of ammonium bicarbonate to yield a dideprotected product.¹³ However, in our reaction with **19** a dark color was produced after evaporation of the reaction solvent, and ¹H NMR showed a mixture of unidentified products. These products could have been due to side reactions with HBr generated by silylation of 5'-OH, as a red color developed immediately upon addition of TMSBr. A second reported procedure used stoichiometric amounts of DABCO in refluxing toluene for 2 h to monodeprotect benzylphosphonic acid dibenzyl ester.¹⁴ In an attempt to cleave both benzyl groups, **19** was treated with 2.2 equivalents of DABCO in refluxing 1,4-dioxane/toluene (1:1). 1,4-Dioxane was used because **19** was not sufficiently soluble in toluene. ¹H NMR for the crude product showed that only monodeprotection of **19** occurred to give **20** as an anion with Bn-quaternarized DABCO as the counter cation. A remaining trace amount of **19** was removed by dissolving the crude product in water and washing it with dichloromethane. This did not remove, however, the excess DABCO.

No side products were observed. Thus, the phosphate group of **20** was shown to be stable to reflux at 101-112 °C. Since **20** was water soluble nucleotide, we applied the reaction steps summarized in Scheme 6.3 to the synthesis of the AQ-dA nucleotide conjugate **1** (in Chapter 2). The conjugate **1** was also water soluble.

6.3. Experimental

6.3.1. Materials and General Synthetic Considerations

2'-Deoxyadenosine monohydrate was purchased from TCI America as white a powder. All reagents used were purchased from Aldrich, except DMTrCl which was obtained from Alfa-Aesar. 2-Ethynylantraquinone (**6**) was synthesized as described in Chapter 2. Anhydrous DMF was purchased from Aldrich and stored in a Vacuum Atmospheres M040-2 glove-box that was pressurized with nitrogen gas from a liquid nitrogen tank. All starting materials for anhydrous reactions were dried prior to use on a vacuum line (2×10^{-5} torr) for at least 12 h. Two to three co-evaporation cycles were also used for drying compounds as indicated below. THF was dried with benzophenone /Na⁰ and stored over activated molecular sieves in the glove-box. Water was deionized by a Millipore (Milli-Q Plus) ultrapure water system. All manipulations of Pd(Ph₃P)₄, and (BnO)₂PN(*i*Pr)₂ were performed in the glove-box. Most of the reactions were monitored with glass-backed TLC Plates precoated with silica gel 60 F₂₅₄ (EMD Chemicals). TLC was run using 5-7% MeOH in CH₂Cl₂ as the eluent. HPLC grade solvents were used for chromatographic purifications. Flash column chromatography was carried out on either a Biotage Flash-40™ system using prepackaged KP-Sil™ cartridges, or on Whatman™ flash silica (60Å pore, 230 – 400 mesh) that was packed in glass columns and pressurized with nitrogen. NMR spectra were obtained on either a Varian Unity +300 NMR spectrometer or on a Bruker

Avance 400 MHz NMR spectrometer. Low-resolution (LR) electron impact (EI) MS were recorded in positive ion mode on a Shimadzu 5050A MSD single quadrupole spectrometer with one amu resolution.

6.3.2. 5'-O-(4,4'-Dimethoxytrityl)-N⁶-benzoyl-8-bromodeoxyadenosine (21)

Compound **3** (140 mg, 0.42 mmol) was dried three times by co-evaporation with anhydrous pyridine, and then suspended in anhydrous pyridine. To this suspension was added 4-DMAP (7.0 mg, 0.055 mmol, 0.12 equiv) in the glove box. Outside the glove box, DMTrCl (156 mg, 0.460 mmol, 1.1 equiv) was dissolved in pyridine and transferred dropwise using cannula to the reaction mixture with **3** under nitrogen at 0 °C (ice-water bath). After the reaction mixture was stirred under a nitrogen atmosphere for 4 h, it was cooled to -4 °C using an ice-water-salt bath, and TMSCl (0.27 mL, 2.1 mmol, 5 equiv) was added dropwise (over 15 min) using a syringe. This reaction mixture was then stirred at room temperature for 1 h under nitrogen. Next, it was cooled to 0 °C using an ice-water bath, and benzoyl chloride (0.25 mL, 2.1 mmol, 5.0 equiv) was added dropwise (over 15 min) using a syringe. Finally, the reaction mixture was stirred at room temperature overnight under nitrogen; at this point a white precipitate had formed. The reaction solvent was evaporated under reduced pressure, and the yellow residue obtained was dissolved in a mixture of MeOH (24 mL) and aqueous ammonia (2.73 mL) by stirring at room temperature for 45 min. The resulting solution was stirred for an additional 30 min, and then the solvent was evaporated. The residue was dissolved in EtOAc (25 mL) and water (10 mL). The EtOAc layer was collected and washed twice with water, dried over anhydrous MgSO₄, and evaporated to dryness. A silica gel column eluted with EtOAc/CH₂Cl₂

(0:100-60:40) afforded **21** as a pale yellow foam (130 mg, 42% yield). ^1H and ^{13}C NMR spectra for **21** were identical to ones published in the literature.²

6.3.3. 8-[(Anthraquinone-2-yl)ethynyl]-5'-O-(4,4'-dimethoxytrityl)-2'-deoxyadenosine (**22**)

To **4** (0.50 g, 0.79 mmol) was added in the glove-box Pd(Ph₃P)₄ (46 mg, 0.040 mmol, 0.050 equiv), CuI (16 mg, 0.080 mmol, 0.10 equiv), TEA (220 μL , 1.58 mmol, 2.00 equiv), compound **6** (221 mg, 0.950 mmol, 1.20 equiv) and anhydrous DMF (7 mL). The mixture was removed from the glove-box and stirred under a nitrogen atmosphere for 6 h at 65 °C in an oil bath; then DMF was removed under reduced pressure. The residue was dissolved in dichloromethane and filtered over Celite to remove insoluble materials. After evaporation the crude product was applied to a silica gel column (16 \times 3 cm) that had been pre-equilibrated with 1% TEA in CHCl₃. MeOH/CHCl₃ (0:100-3:97) was used as the eluting system to afford **22** (521 mg, 85%) as a yellow foam after evaporation of the eluting solvent. ^1H NMR (300 MHz, CDCl₃): δ (ppm) 2.46-2.57 (1H, m, H_{2' α}), 3.49 (2H, d, $J = 5.4$, H_{5' α} and H_{5' β}), 3.52-3.52 (1H, m, H_{2' β}), 3.71 (6H, d, $J = 1.2$, OCH₃ (DMTr)), 4.26-4.36 (1H, m, H_{4'}), 4.89-4.98 (1H, m, H_{3'}), 6.63 (1H, t, $J = 6.9$ Hz, H_{1'}), 6.74 [4H, dd, $J = 3.6$ and 9 Hz, OPh-*meta* (DMT)], 7.05 (2H, br s, NH₂), 7.10-7.31 [7H, m, OPh-*ortho* (DMTr, 4H) and Ph-*meta* and *para* (DMTr, 3H)], 7.37-7.61 [5H, m, Ph-*ortho* (DMTr, 2H), H₃ (AQ), H₆ (AQ) and H₇ (AQ)], 7.85 [1H, s, H₂ (dA)], 7.96-8.03 [2H, m, H₅ (AQ) and H₈ (AQ)], 8.17 [1H, d, $J = 7.8$ Hz, H₄ (AQ)] and 8.46 [1H, s, H₁ (AQ)].

6.3.4. 5'-O-Dimethoxytrityl-2'-deoxyadenosine 3'-Dibenzyl Phosphate (**18**)

To **17** (0.10 g, 0.18 mmol), previously dried 2 times by co-evaporation with anhydrous THF, was added 5-Me-tetrazole (23 mg, 0.27 mmol, 1.5 equiv) and anhydrous THF (3 mL) in

the glove-box. The mixture was removed from the glove-box and cooled to 0 °C using an ice-water bath; (BnO)₂PN(*i*Pr)₂ (0.090 mL, 0.27 mmol, 1.5 equiv) was added dropwise under a nitrogen atmosphere with a syringe. The reaction mixture was stirred under a nitrogen atmosphere for 10 minutes at 0 °C; then it was warmed to room temperature, and the reaction progress was monitored by TLC. A white precipitate began to form after 20 min, and TLC showed complete consumption of **17** after a total reaction time of 1 h. The mixture was cooled to -78 °C using a dry ice-acetone bath, and a solution of *m*-CPBA (70 mg) in CH₂Cl₂ (3 mL) was added gradually to the chilled reaction mixture. After 15 minutes of stirring at -78 °C, the mixture became homogeneous. The reaction mixture was warmed to room temperature and stirred for an additional 30 min; then the reaction was quenched by adding a saturated aqueous solution of NaHCO₃. More CH₂Cl₂ (10 mL) and water (5 mL) were added; the organic layer was separated, washed with water, dried with anhydrous MgSO₄, and the solvent was evaporated. The syrup obtained was purified using silica gel chromatography on a column pre-equilibrated with 1% TEA in CH₂Cl₂ and eluted with MeOH/CH₂Cl₂ (0:100-2:98). Evaporation of the eluting solvent afforded **18** as a white foam (131 mg, 89% yield). ¹H NMR (300 MHz, CDCl₃): δ (ppm) 2.59 (1H, dd, *J* = 5.7 and 13.8 Hz, H_{2'α}), 2.86-2.95 (1H, m, H_{2'β}), 3.27-3.38 (2H, m, H_{5'α}, H_{5'β}), 3.75 [H, s, OCH₃ (DMTr)], 4.26-4.30 (1H, m, H_{4'}), 5.01-5.06 [4H, m, CH₂ (Bn)], 5.16-5.19 (1H, m, H_{3'}), 6.08 (2H, br s, NH₂), 6.33 (1H, dd, *J* = 5.7 and 8.4 Hz, H_{1'}), 6.76 [4H, d, *J* = 9 Hz, OPh-*meta* (DMTr)], 7.18-7.38 [19H, m, OPh-*ortho* (DMTr, 4H), Ph (DMTr, 5H), and Ph (Bn, 10H)], 7.87 [1H, s, H₈ (dA)] and 8.24 [1H, s, H₂ (dA)]. ³¹P NMR (121 MHz, CDCl₃): δ (ppm) -0.72.

6.3.5. 2'-Doxyadenosine 3'-Dibenzyl Phosphate (19)

Compound **18** (65 mg, 0.080 mmol) was dissolved in a 30% DCA in CH₂Cl₂ mixture (1.5 mL), and the resulting red solution was stirred at room temperature for 30 min. The reaction was quenched by adding TEA until the red color of the DMTr cation disappeared. More CH₂Cl₂ (10 mL) and water (5 mL) were added; then the organic layer was separated, washed with saturated aqueous solution of NaHCO₃ and then with water, dried with anhydrous MgSO₄, and evaporated to dryness. The syrup obtained was purified using a silica gel column for which MeOH/CH₂Cl₂ (0:100-3:97) was used as the eluting system. Evaporation of the eluting solvent afforded **19** as a white foam (35 mg, 86% yield). ¹H NMR (300 MHz, CDCl₃): δ (ppm) 2.46 (1H, dd, *J* = 5.1 and 13.8 Hz, H_{2'α}), 2.97-3.06 (1H, m, H_{2'β}), 3.56-3.65 (1H, m, H_{5'α}) 3.83 (1H, d, *J* = 12.6 Hz, H_{5'β}), 4.25 (1H, s, H_{4'}), 5.02-5.19 [5H, m, CH₂ (Bn), and H_{3'}], 6.07 (1H, dd, *J* = 5.1 and 9.9 Hz, H_{1'}), 6.23 (2H, br s, NH₂), 6.62 (1H, br s, OH_{5'}), 7.38 [10H, s, Ph (Bn)], 7.75 [1H, s, H₈ (dA)], and 8.26 [1H, s, H₂ (dA)]. ³¹P NMR (121 MHz, CDCl₃): δ (ppm) -1.38.

6.3.6. 2'-Deoxyadenosine 3'-Benzyl Phosphate Benzyl-quaternarized DABCO Salt (20)

To **19** (61 mg, 0.12 mmol) was added DABCO (31 mg, 0.27 mmol, 2.3 equiv) and anhydrous toluene/1,4-dioxane (1:1, 10 mL). The mixture was refluxed for 3 h under a nitrogen atmosphere. The solvent was evaporated, and the product was dissolved in water and washed once with CH₂Cl₂. Water was evaporated to afford **20** (60 mg, 80%) as a colorless solid. ¹H NMR (300 MHz, D₂O): δ (ppm) 2.18-2.44 (2H, m, H_{2'α} and H_{2'β}), 2.8-2.91 [6H, m, CH₂N (DABCO)], 3.02-3.15 [6H, m, CH₂N⁺ (DABCO)], 3.38-3.53 (2H, m, H_{5'α} and H_{5'β}), 3.92-4.01 (1H, m, H_{4'}), 4.09 [2H, s, DABCO-CH₂ (Bn)], 4.52-4.62 (1H, m, H_{3'}), 4.7 [2H, d, *J* = 8.1, PO₄-

CH₂ (Bn)], 6.07 (1H, dd, $J = 6$ and 7.8 Hz, H_{1'}), 6.95-7.19 [10H, m, Ph (Bn)], 7.73 [1H, s, H₈ (dA)], and 7.82 [1H, s, H₂ (dA)]. ³¹P NMR (121 MHz, CDCl₃): δ (ppm) -0.34.

6.4. References

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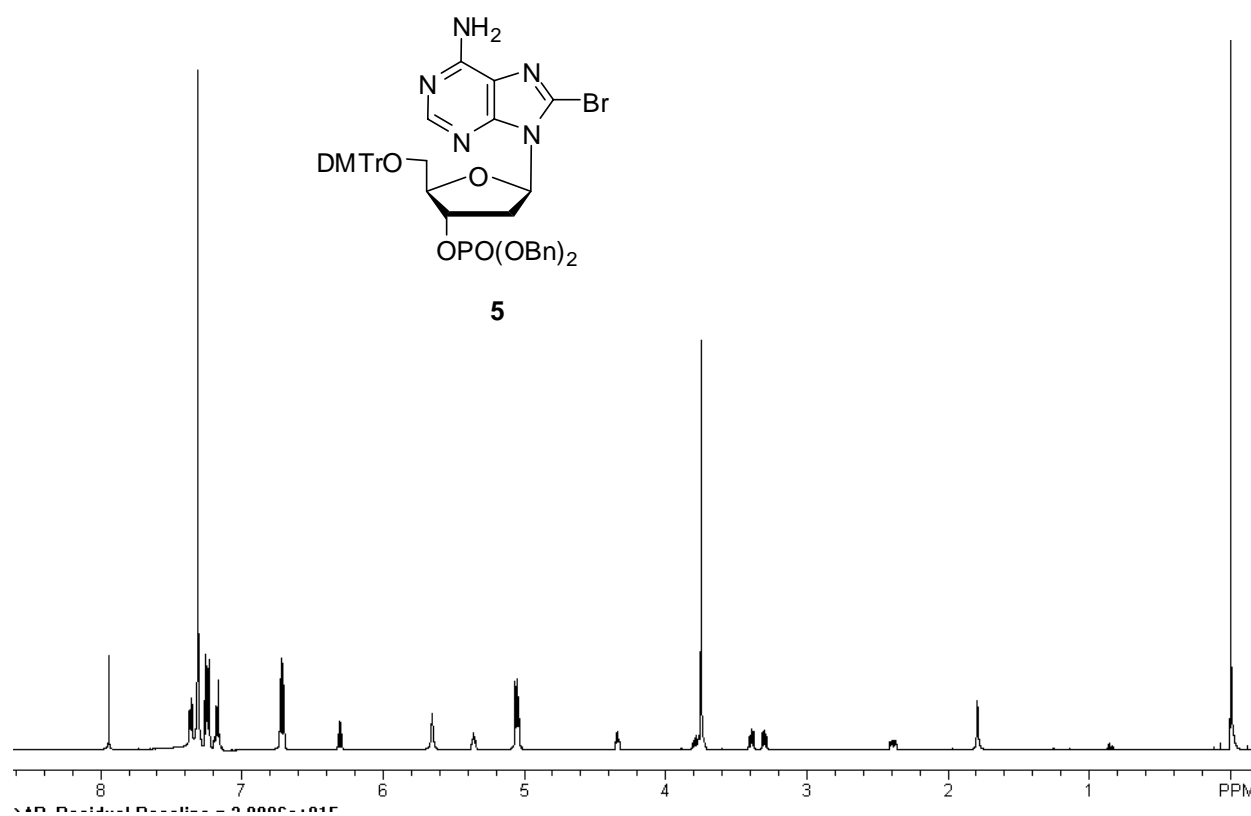
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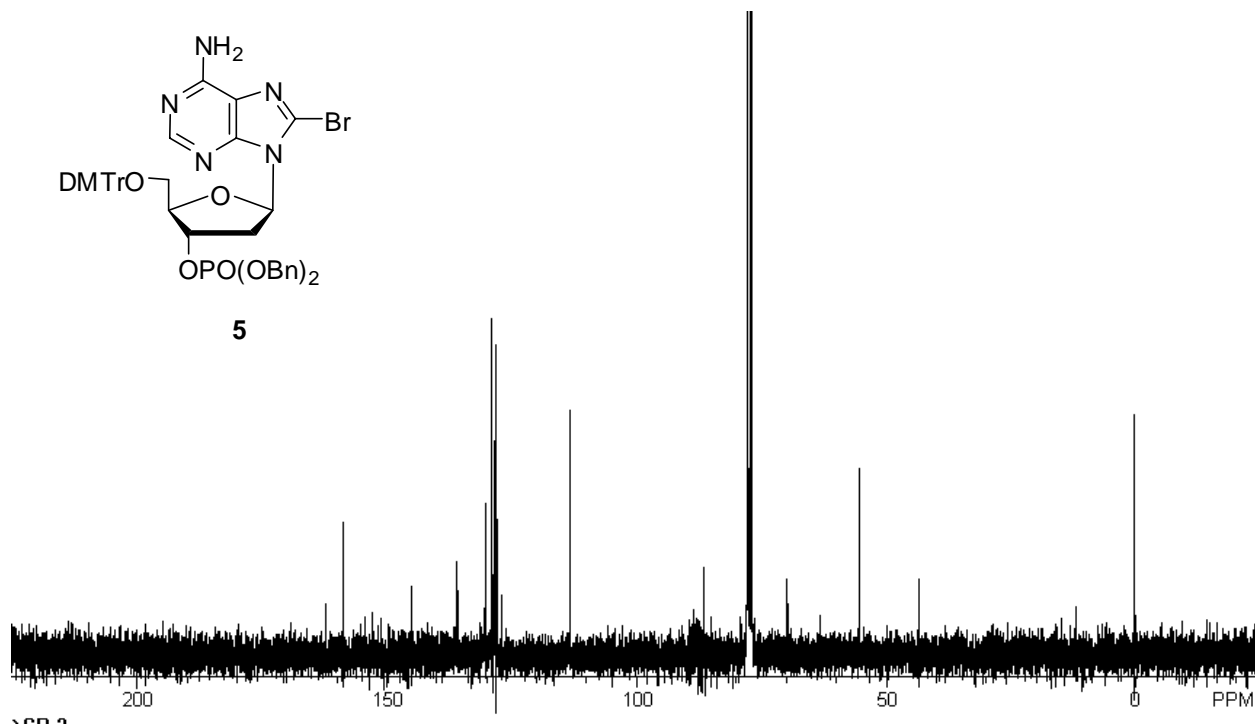
APPENDIX A: ^1H , ^{13}C , ^{32}P and ^{19}F NMR spectra

NMR spectra for compounds in Chapter 2:

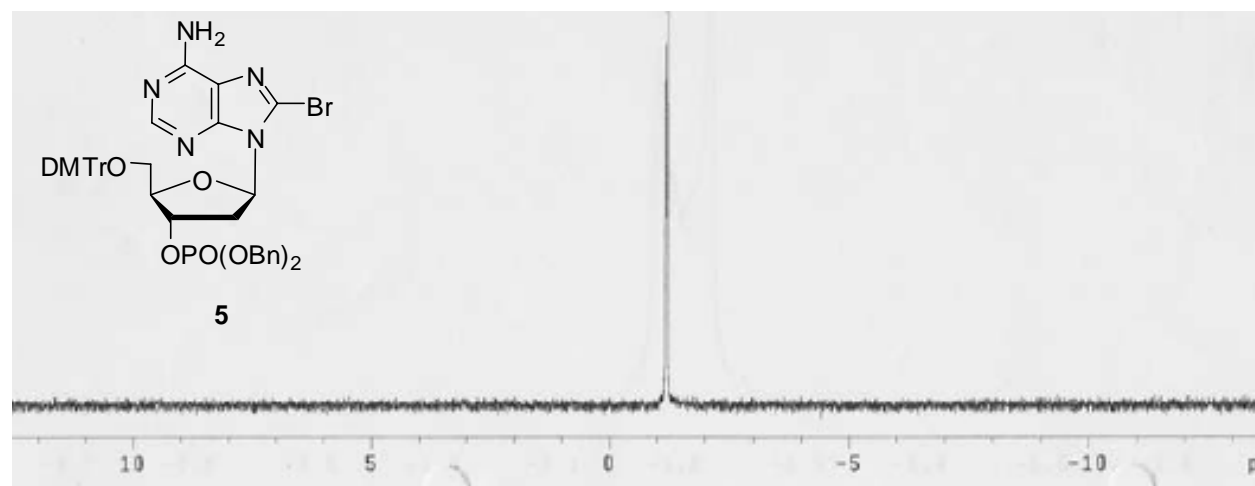
^1H NMR (500 MHz, CDCl_3)



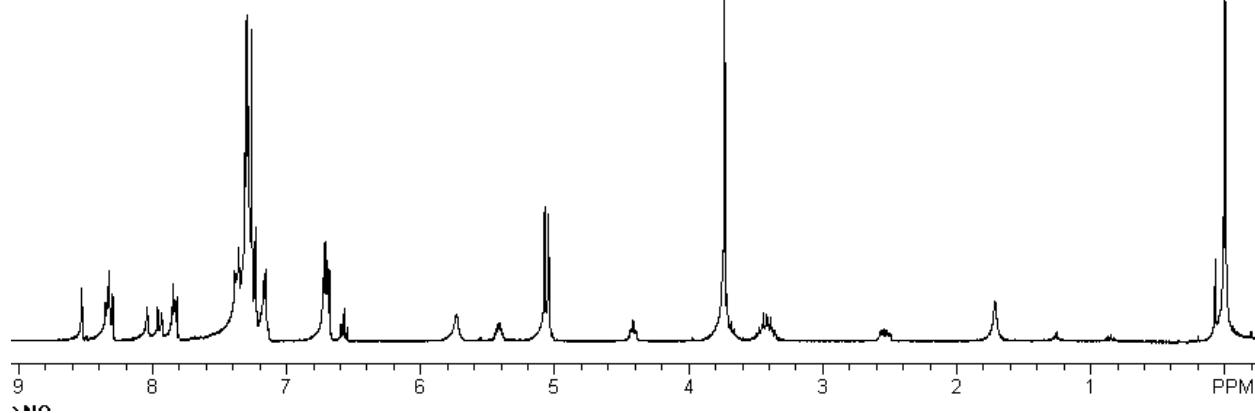
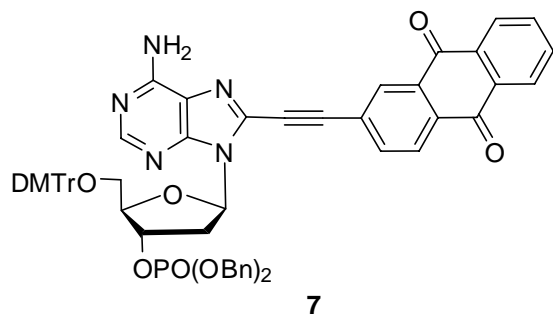
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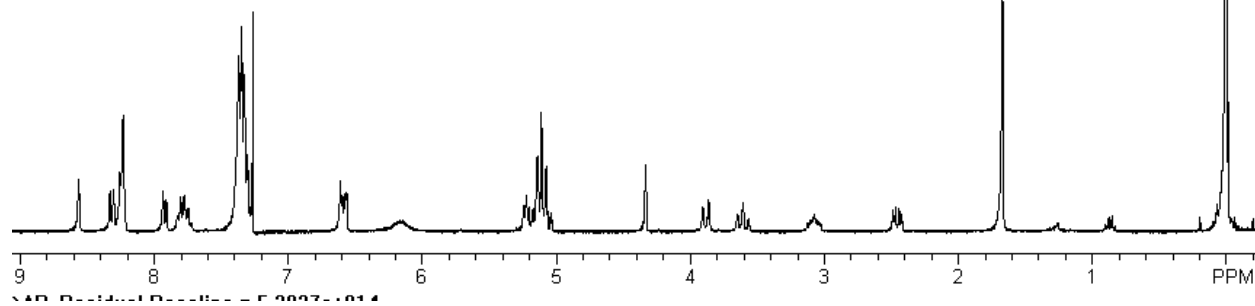
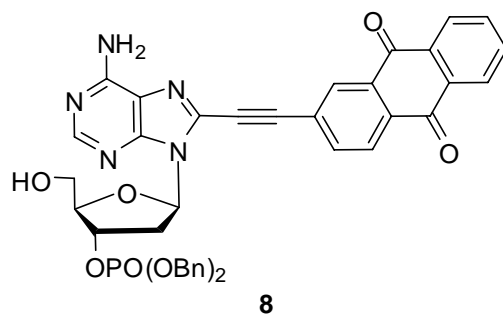
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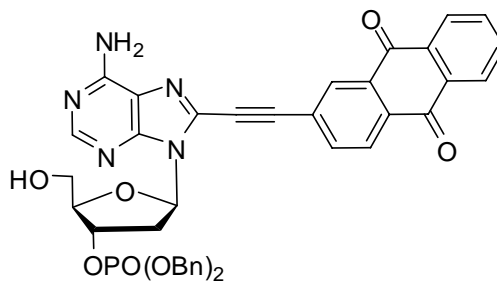
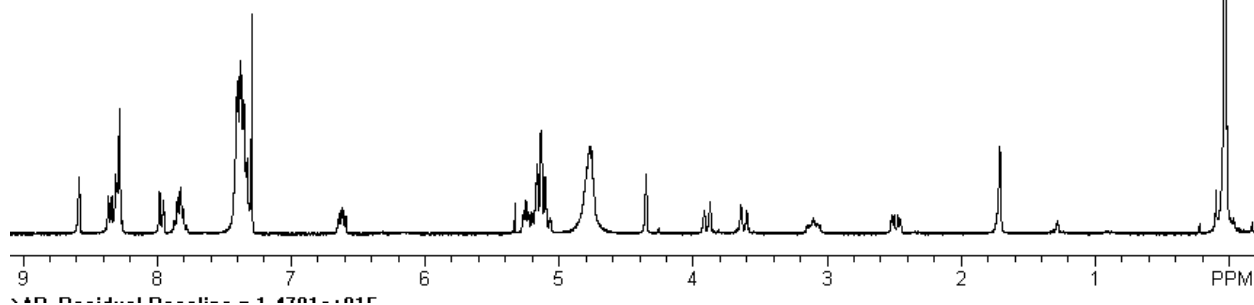


^1H NMR (300 MHz, CDCl_3)



^1H NMR (300 MHz, CDCl_3)

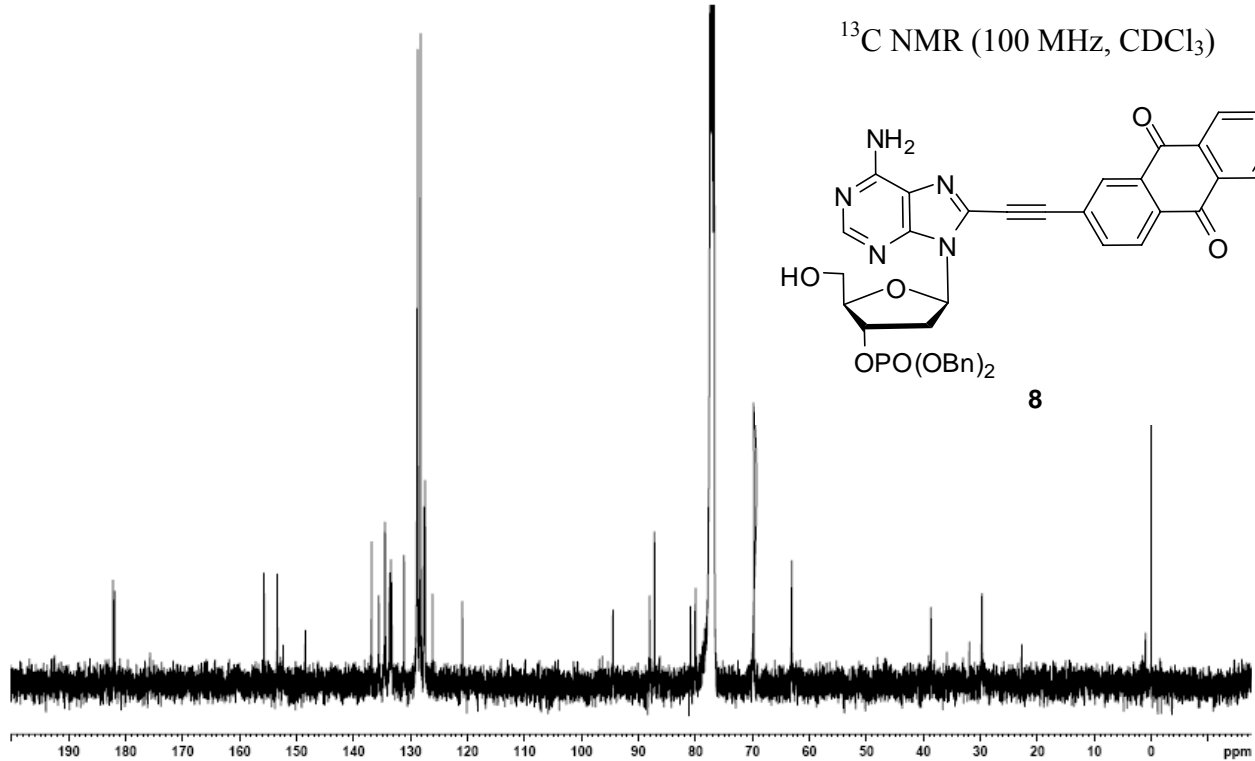


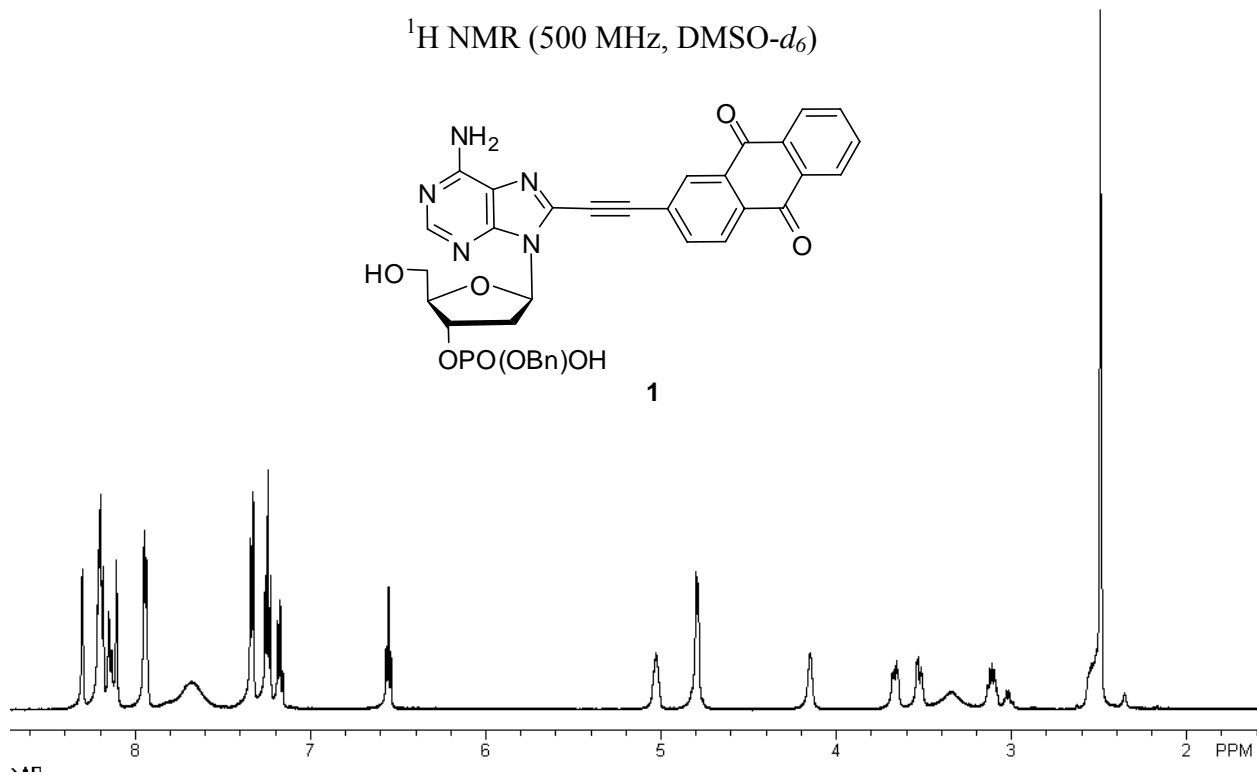
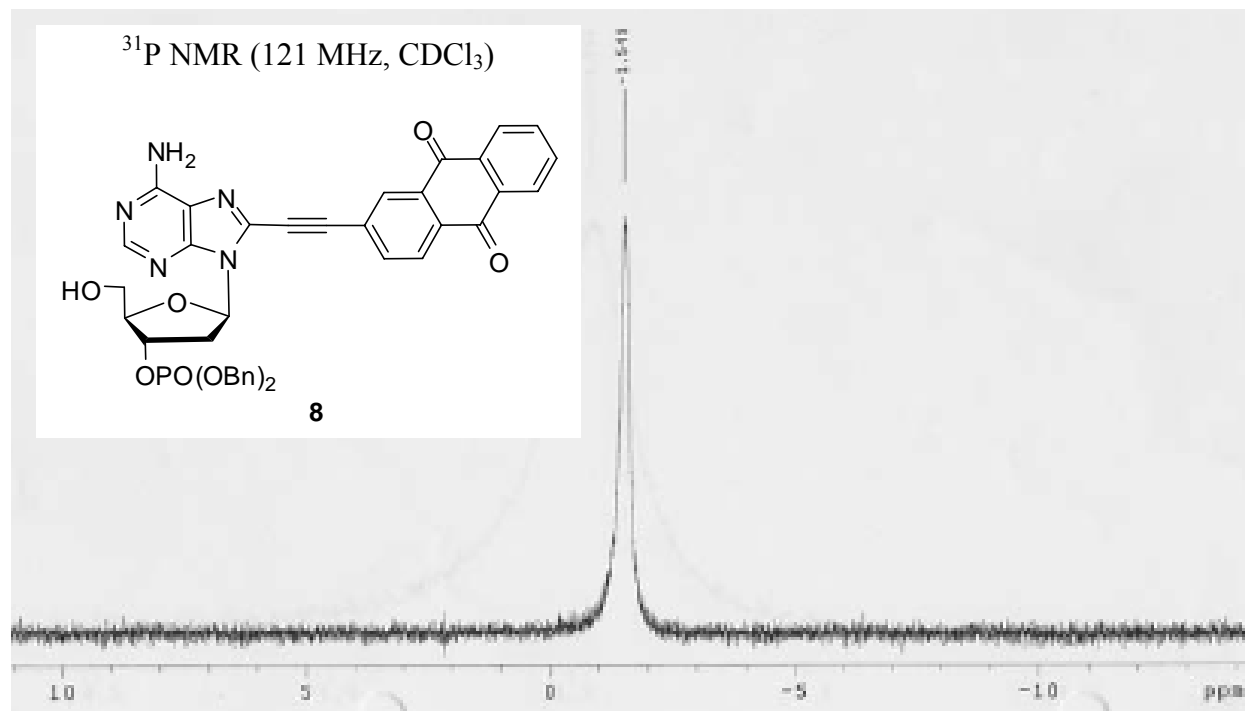
^1H NMR (300 MHz, $\text{CDCl}_3 + \text{D}_2\text{O}$)**8**

SAR Residual Data: 1.4701-0.015

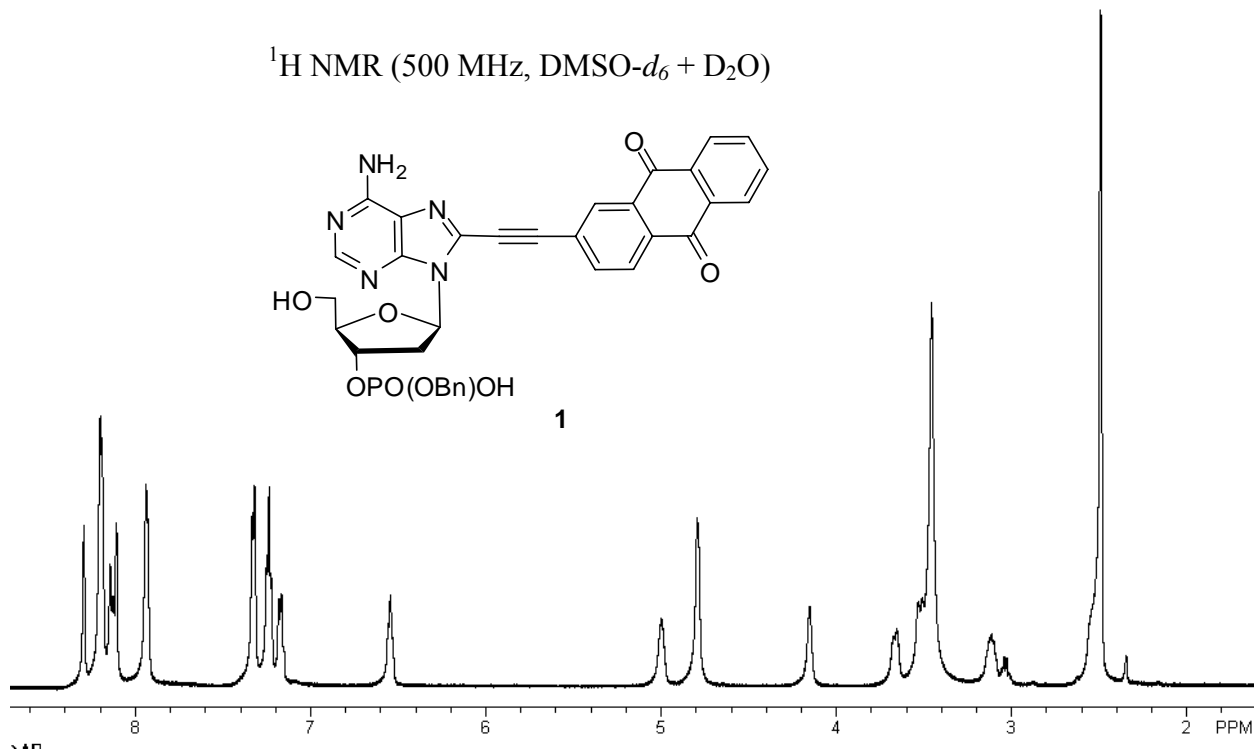
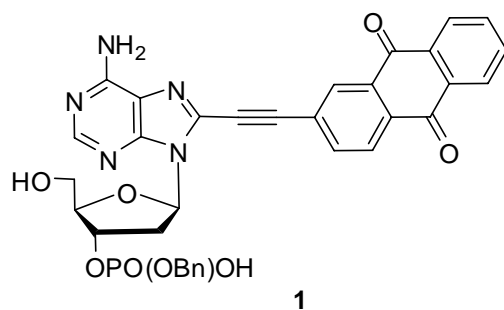
^{13}C NMR (100 MHz, CDCl_3)

Chemical structure of compound **8** is shown, featuring a nucleoside core with a 2-aminopyrimidin-5-yl group, a hydroxyl group, and a diethyl phosphonate group, linked via an ethynyl group to a 1,2-diphenyl-1,2-dione moiety.

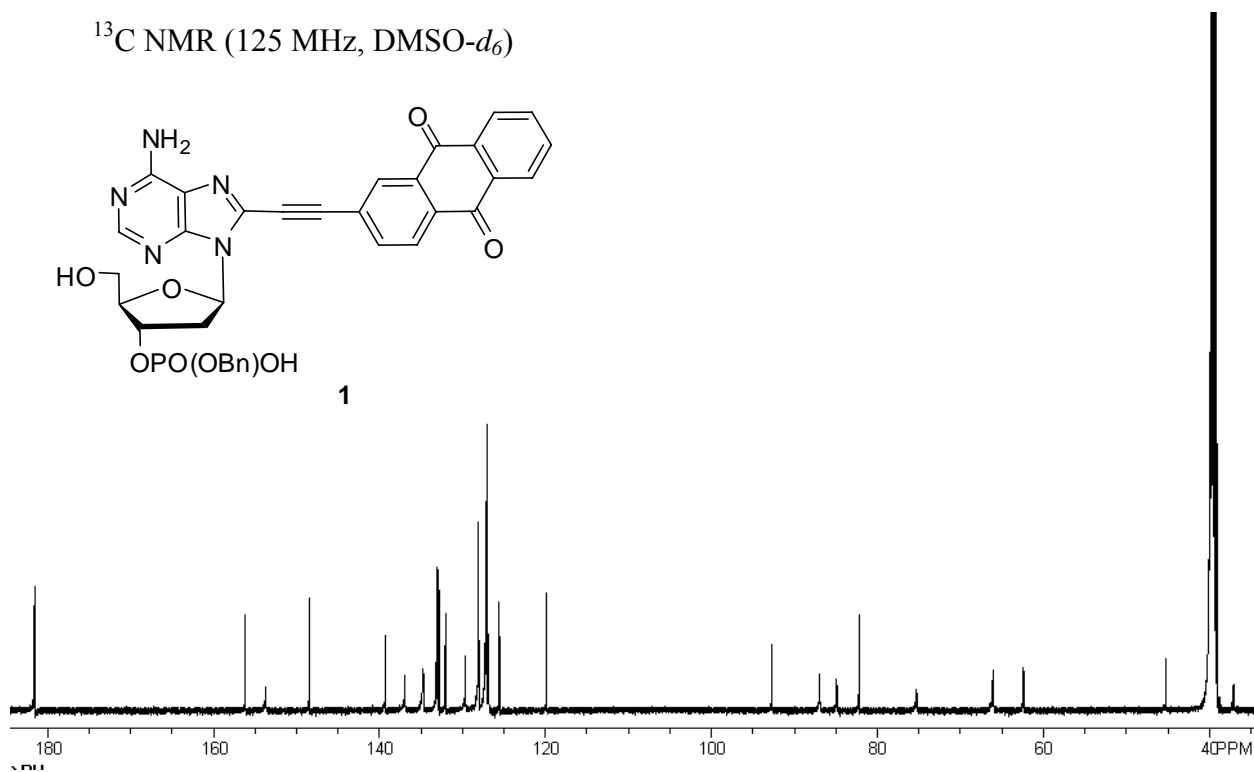
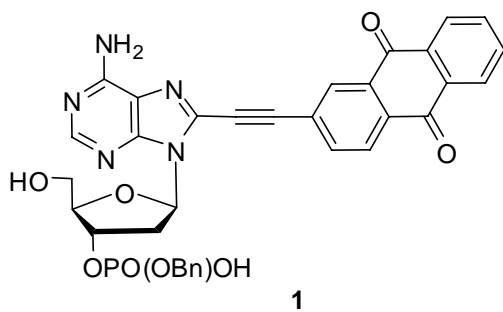
8



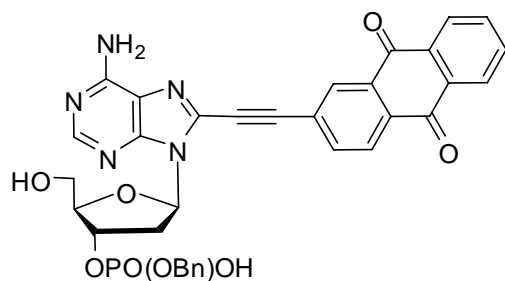
^1H NMR (500 MHz, $\text{DMSO-}d_6 + \text{D}_2\text{O}$)



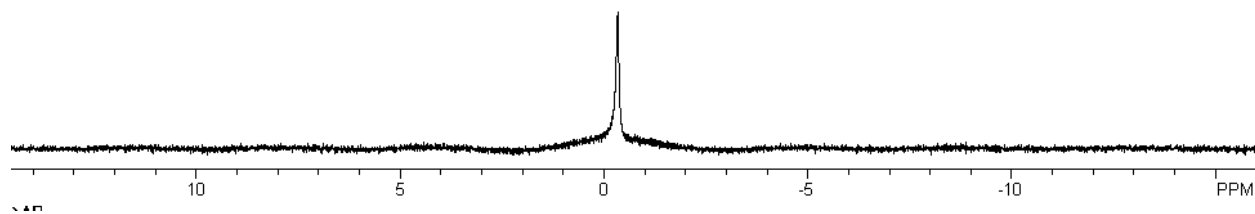
^{13}C NMR (125 MHz, $\text{DMSO-}d_6$)



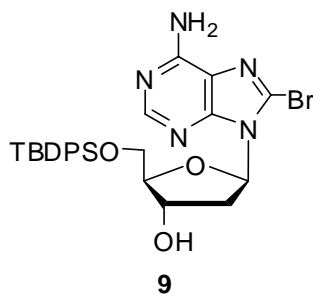
^{31}P NMR (202 MHz, $\text{DMSO-}d_6$)



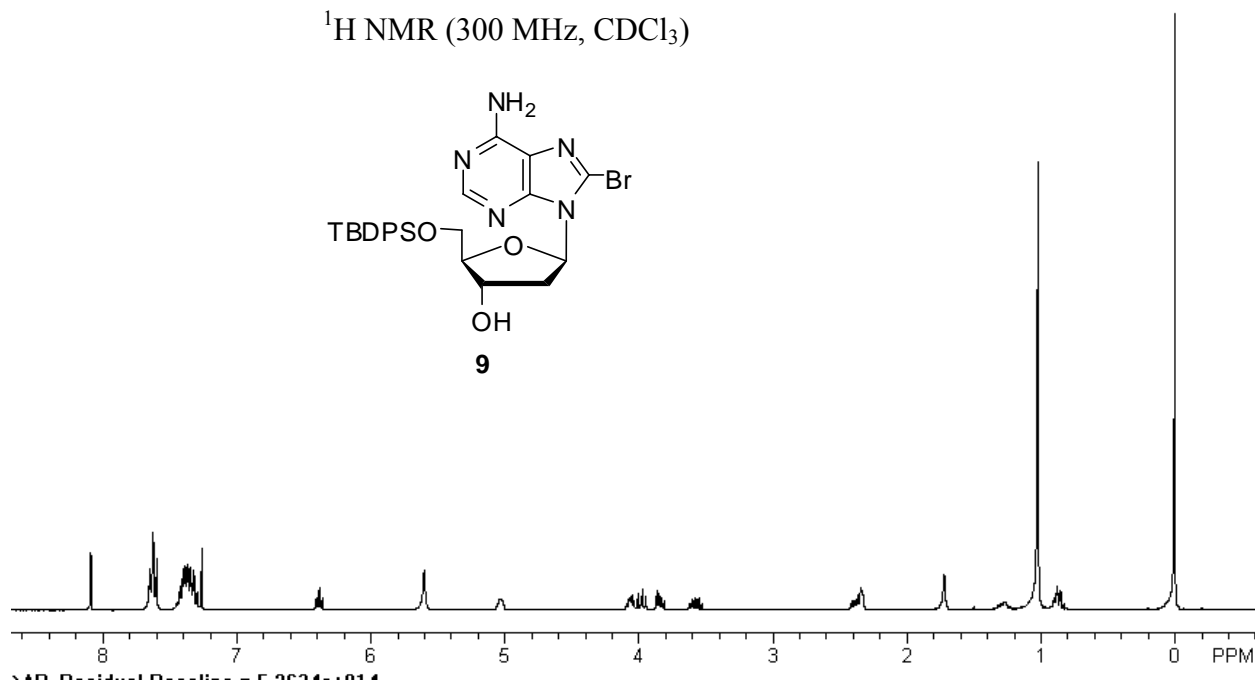
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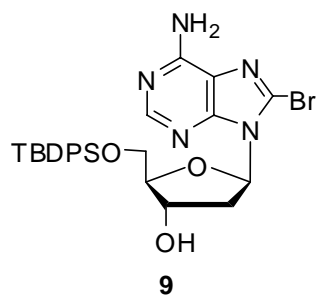
^1H NMR (300 MHz, CDCl_3)



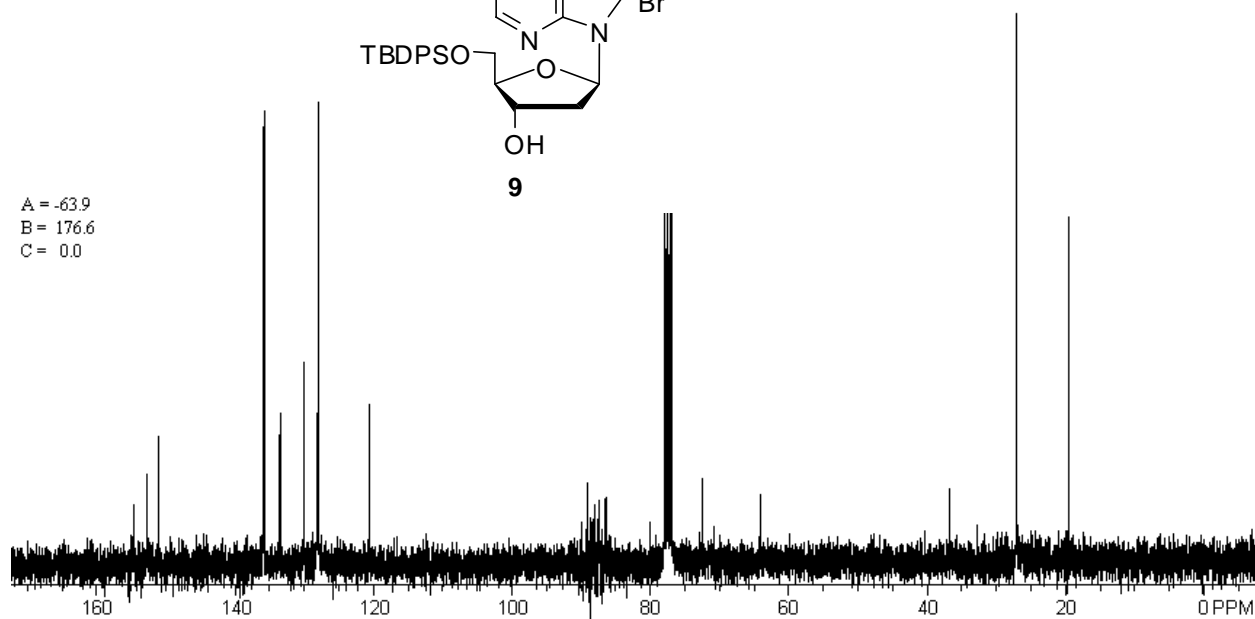
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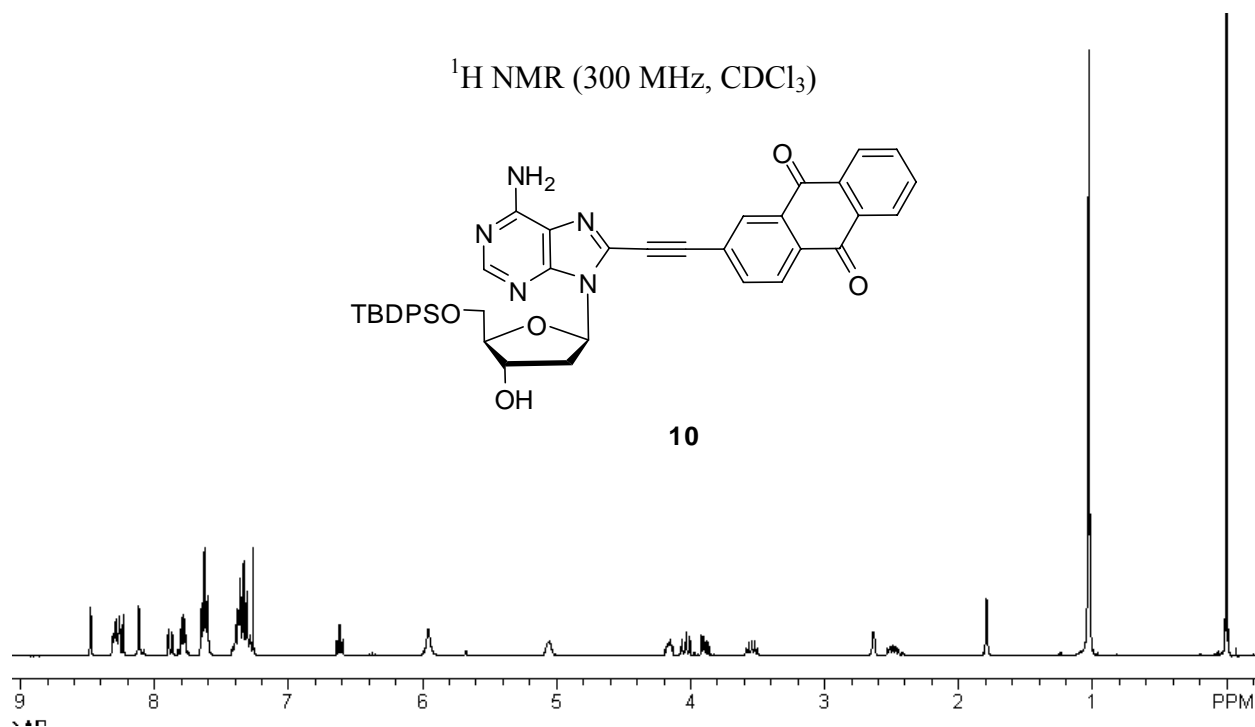
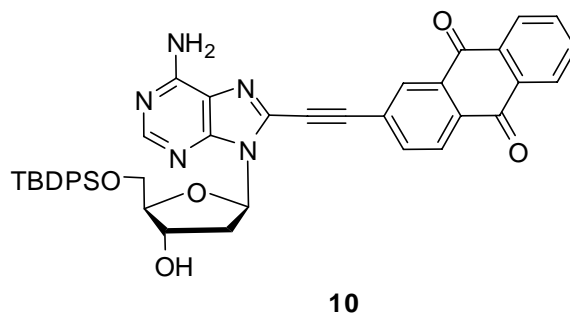
^{13}C NMR (75 MHz, CDCl_3)

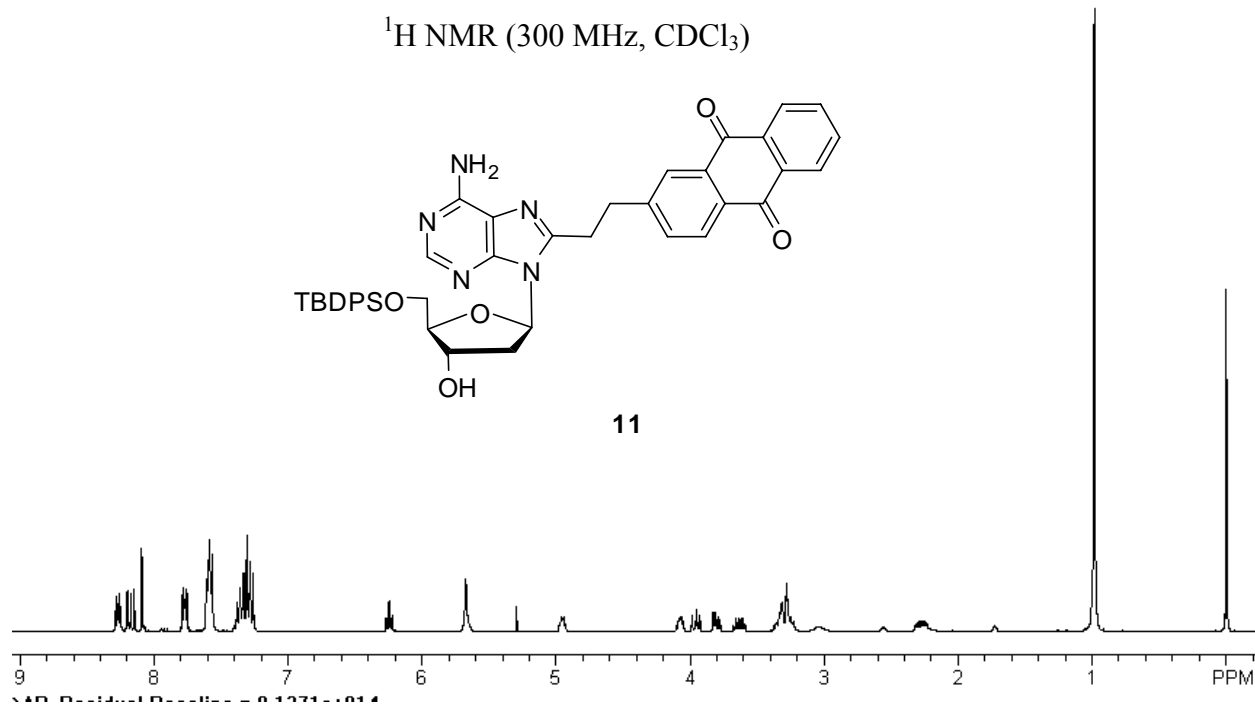
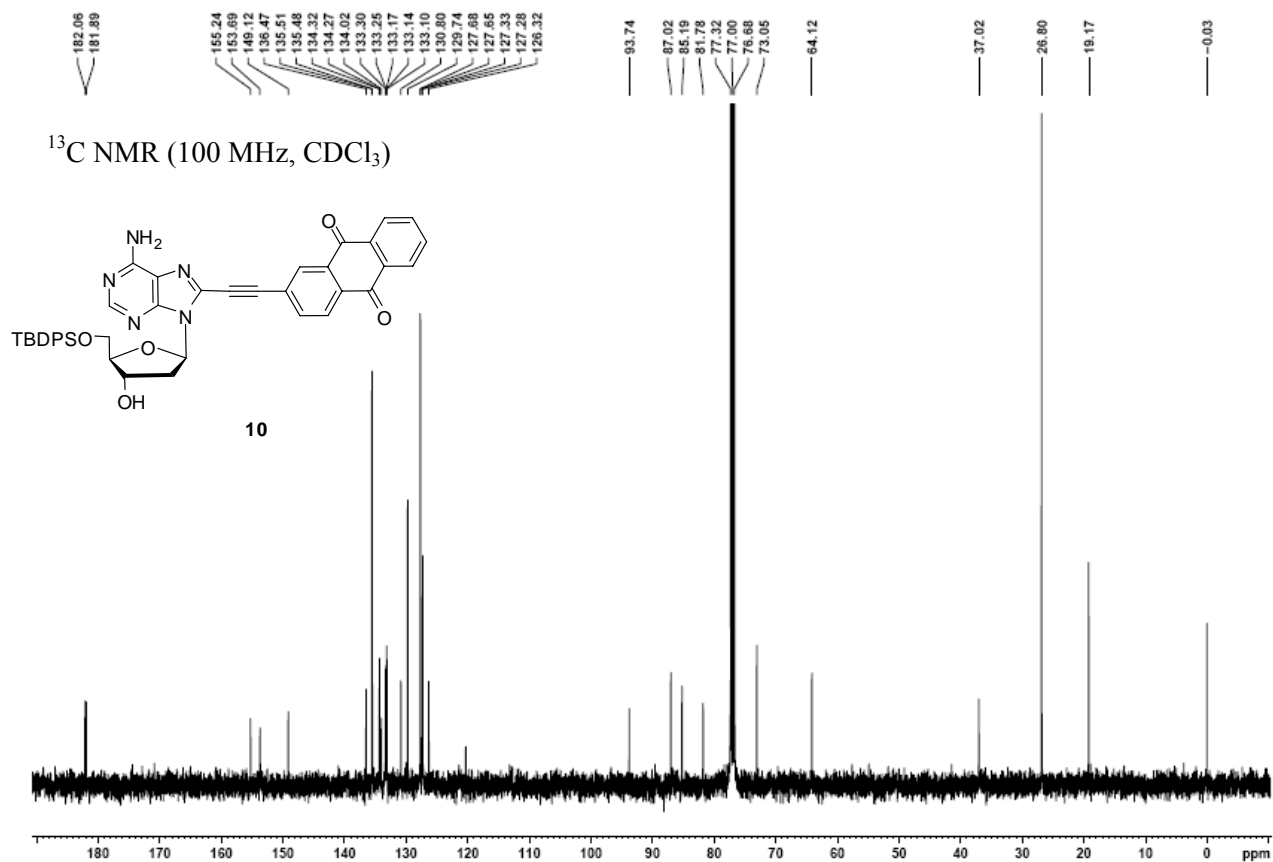


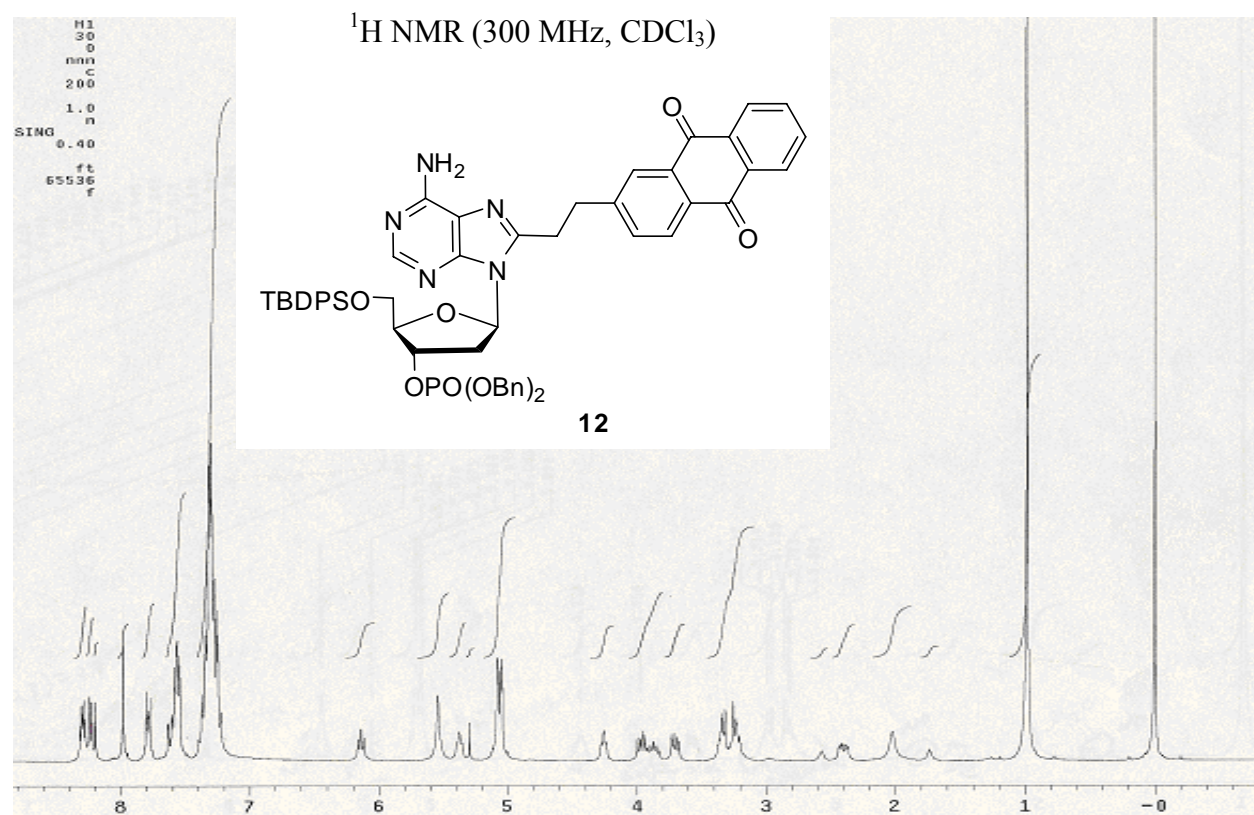
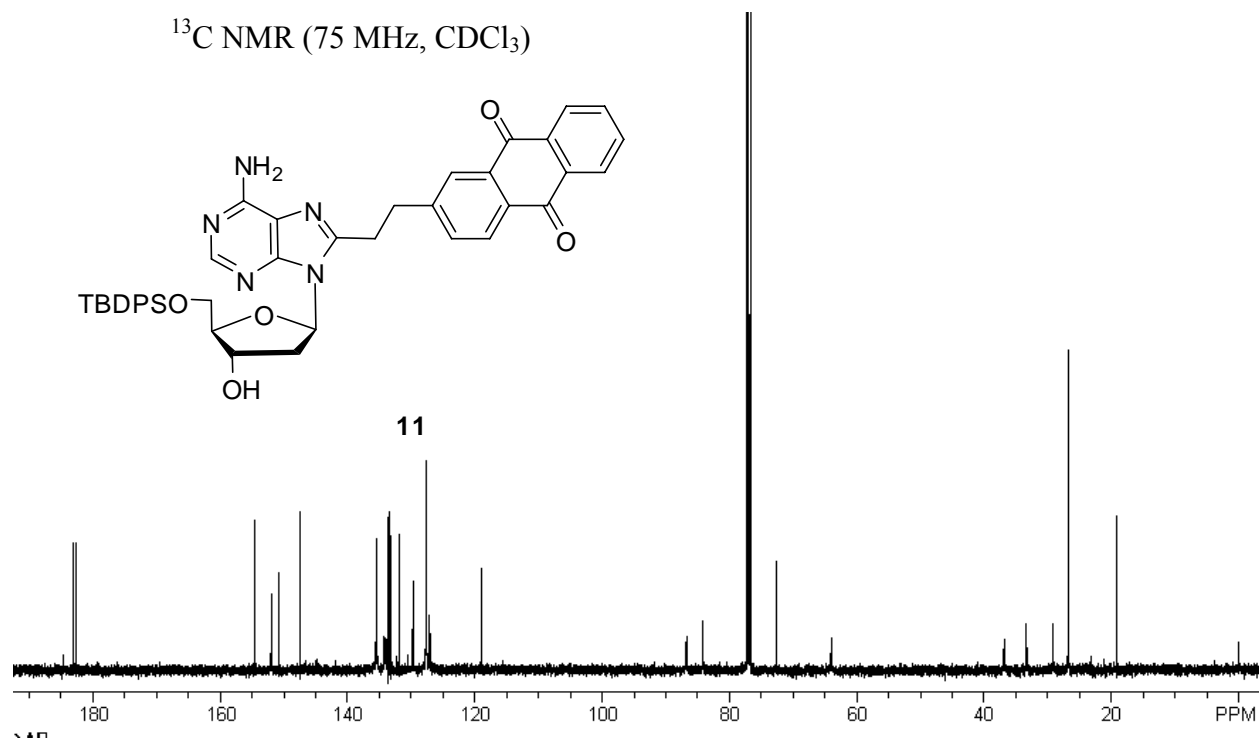
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C = 0.0



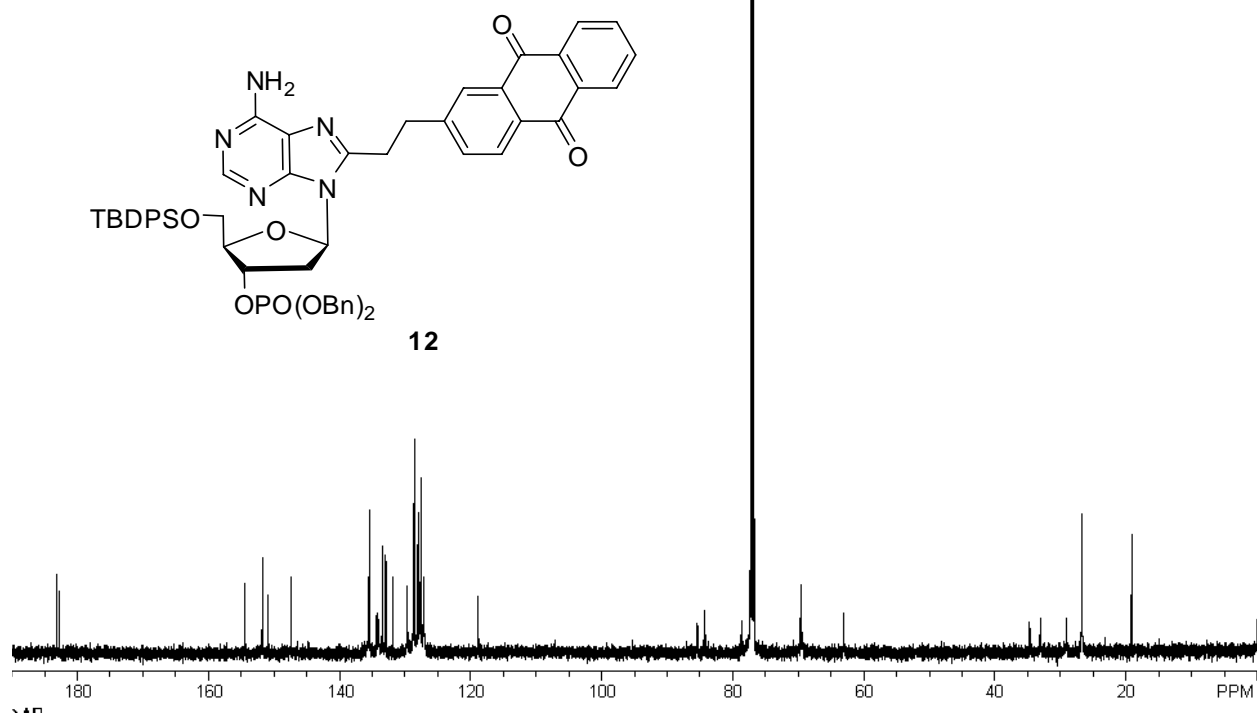
^1H NMR (300 MHz, CDCl_3)



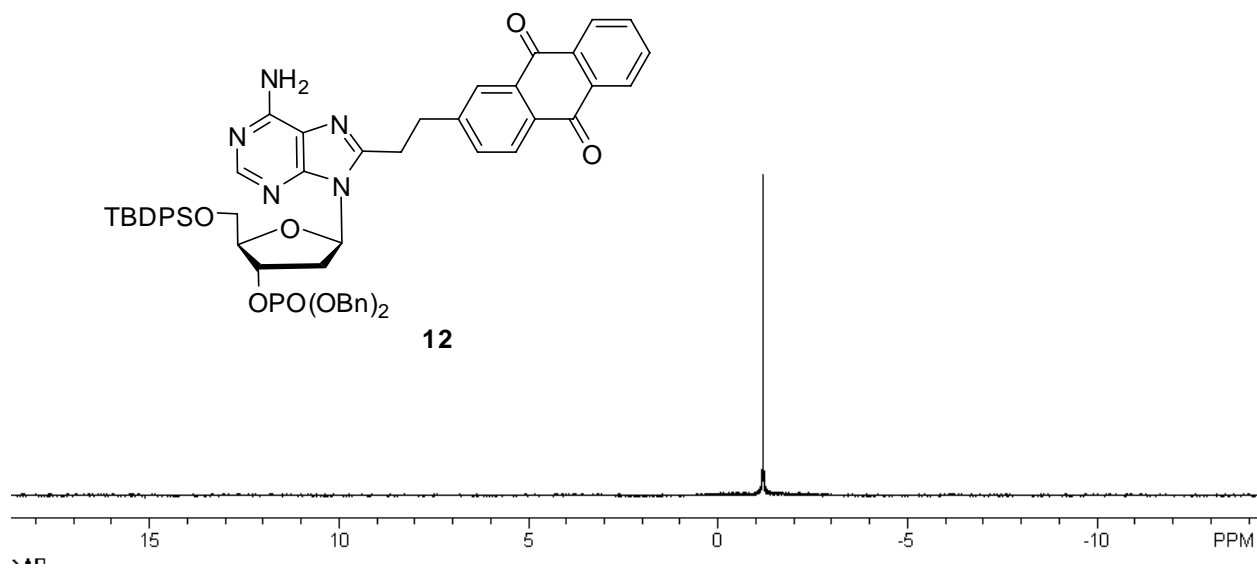




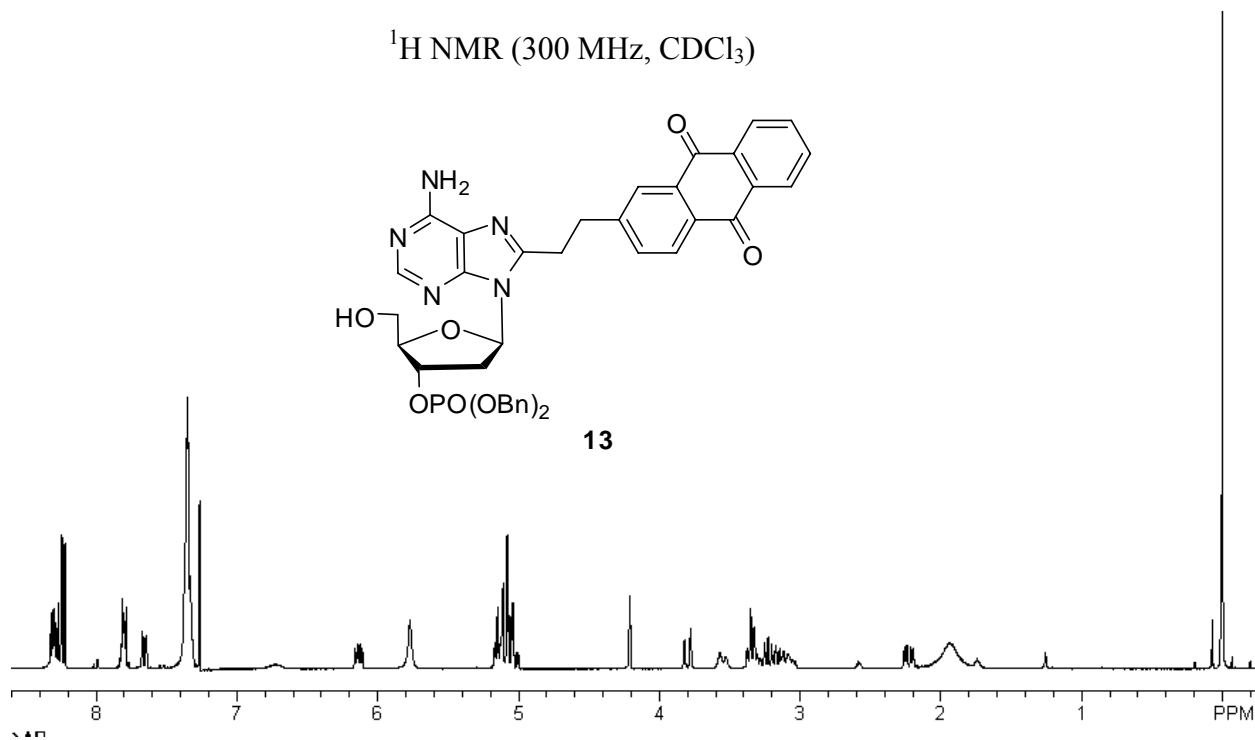
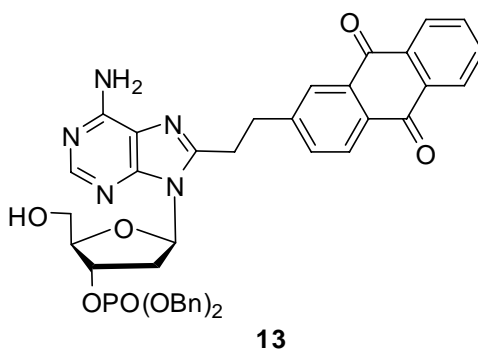
^{13}C NMR (125 MHz, CDCl_3)



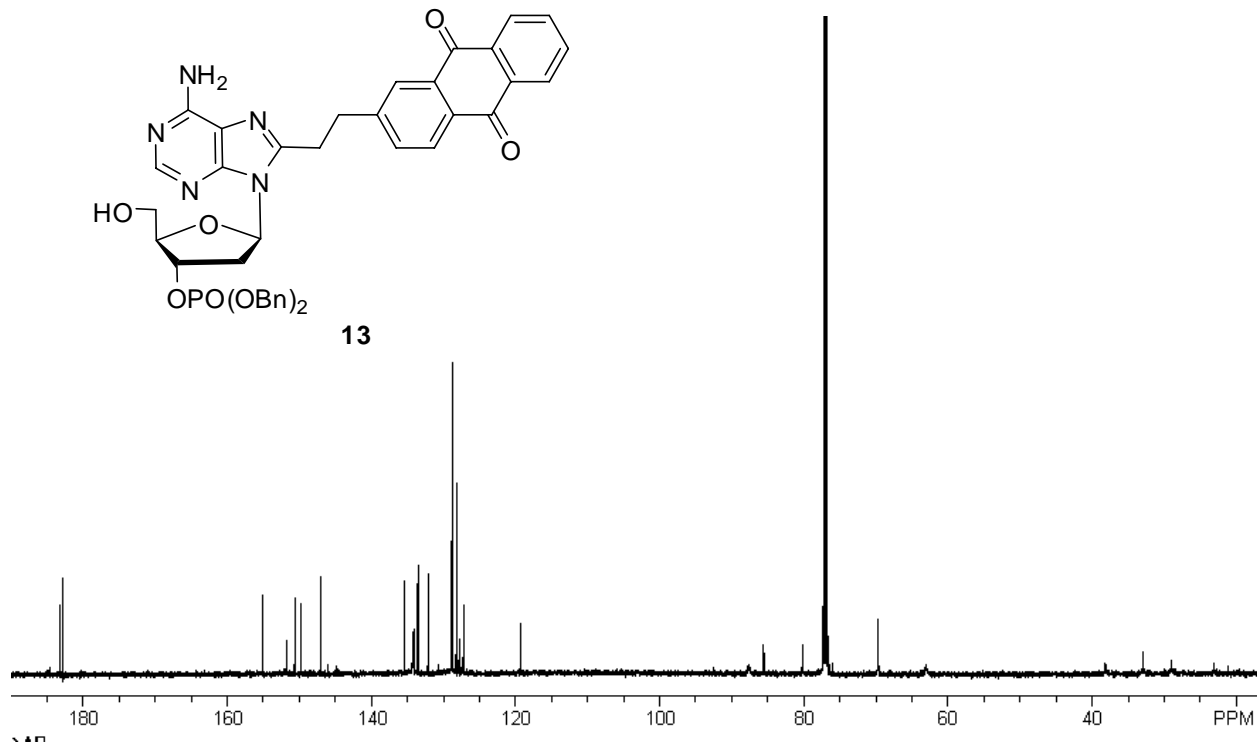
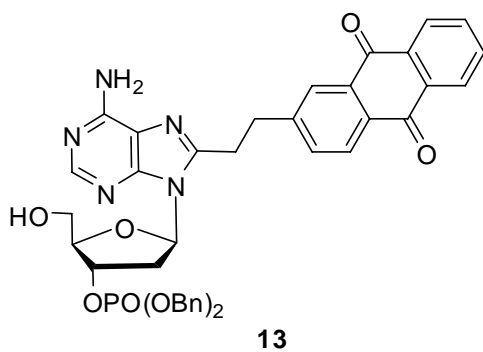
^{31}P NMR (121 MHz, CDCl_3)



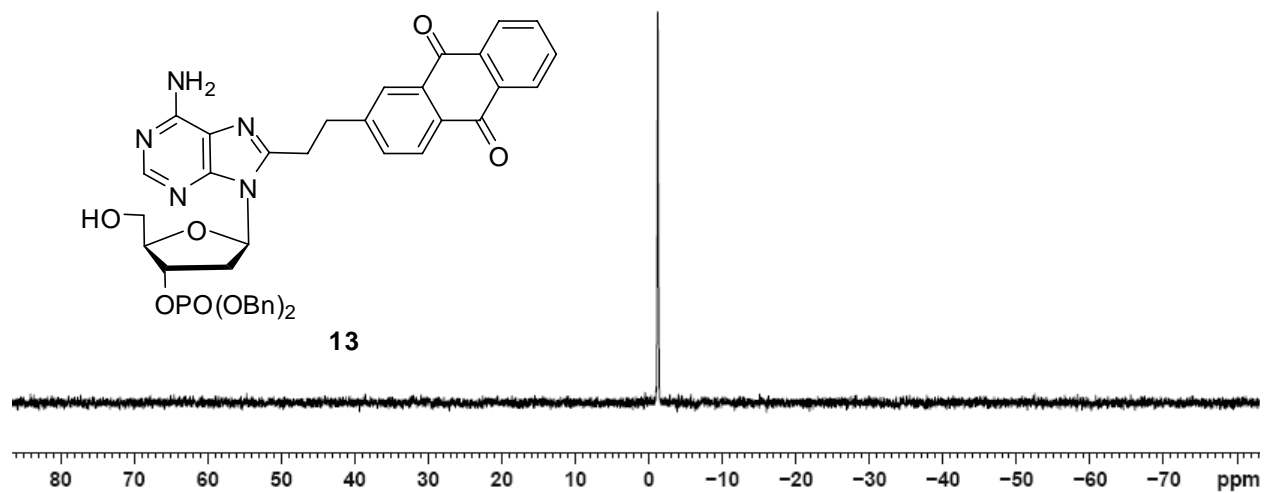
^1H NMR (300 MHz, CDCl_3)



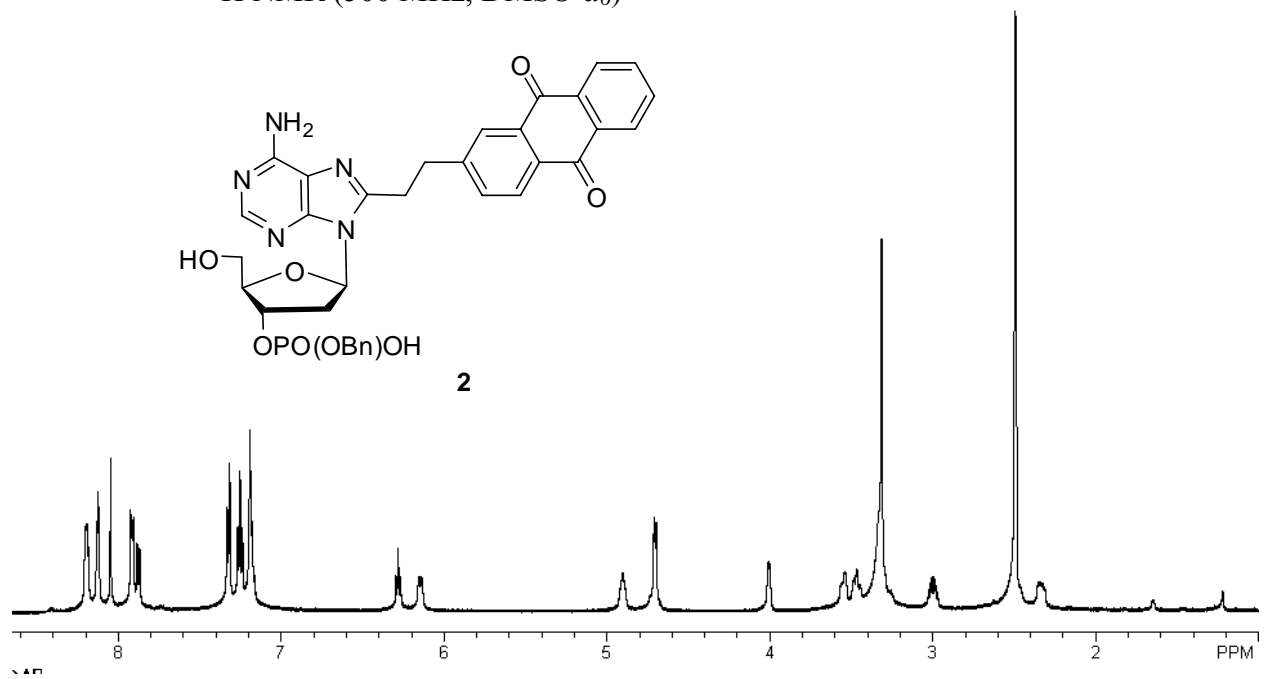
^{13}C NMR (75 MHz, CDCl_3)



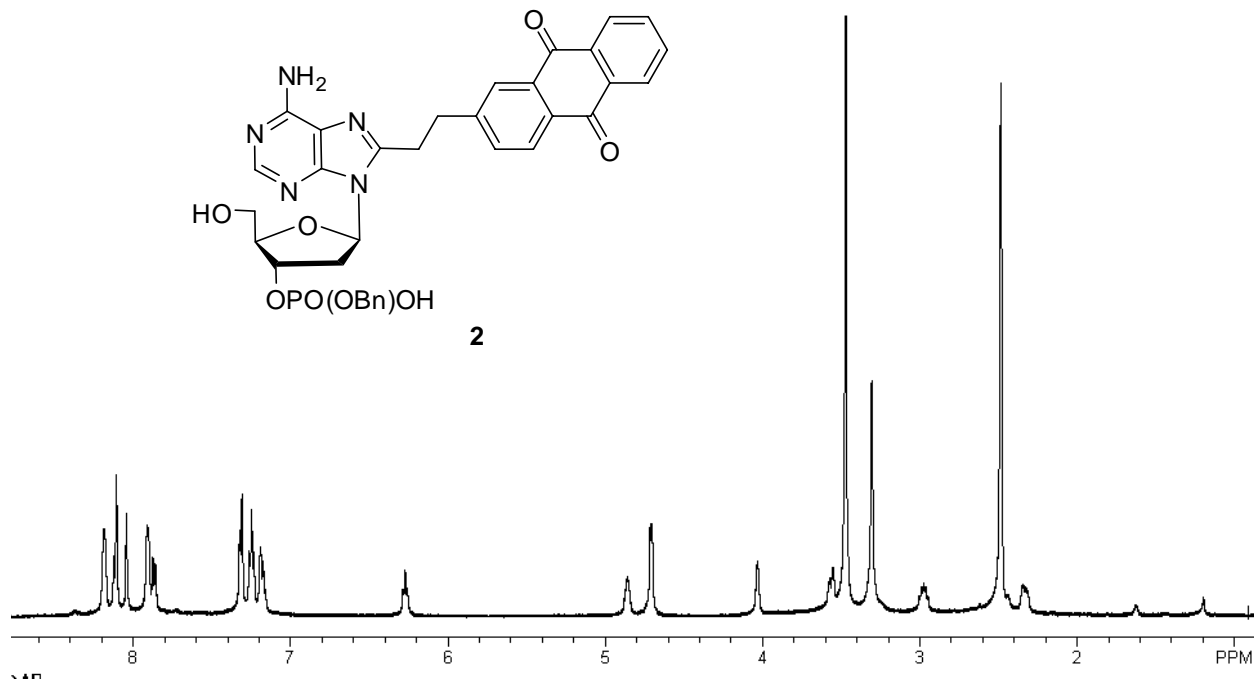
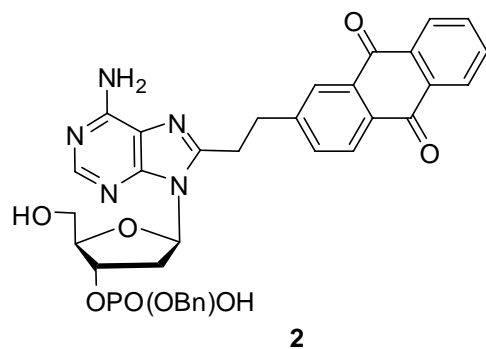
^{31}P NMR (162 MHz, CDCl_3)



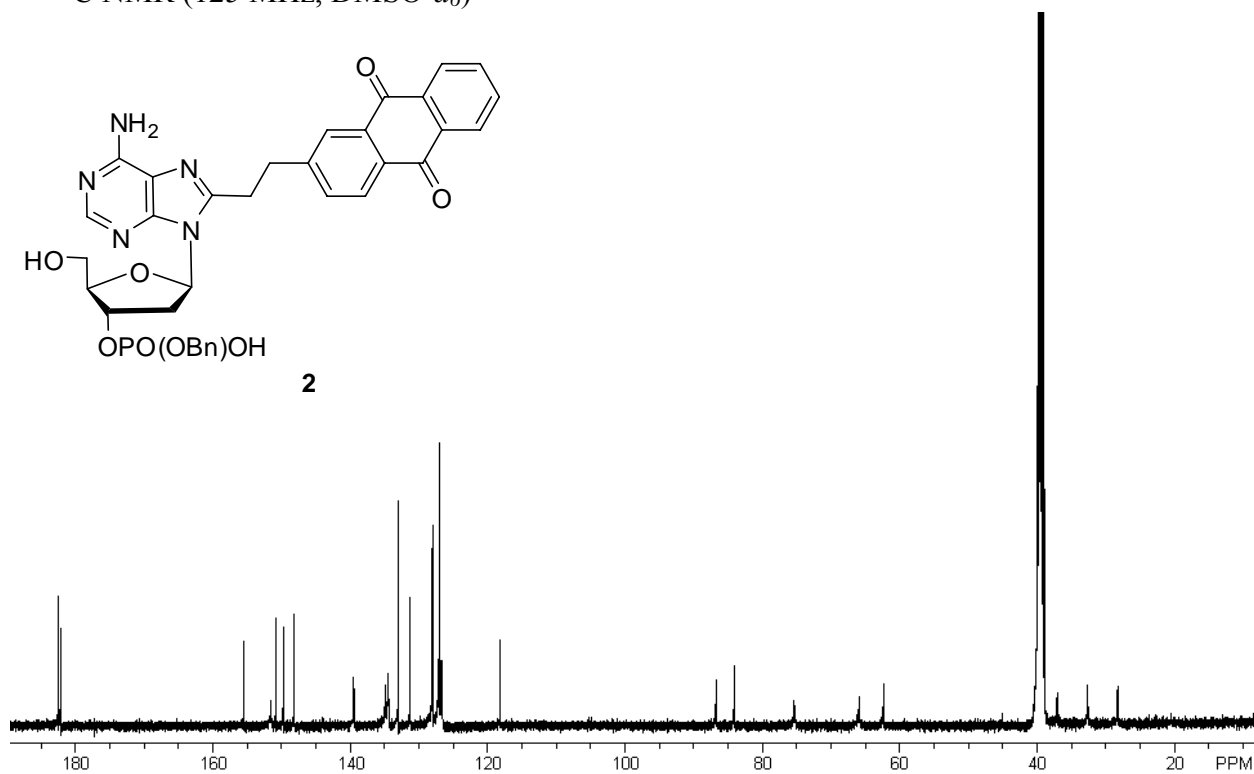
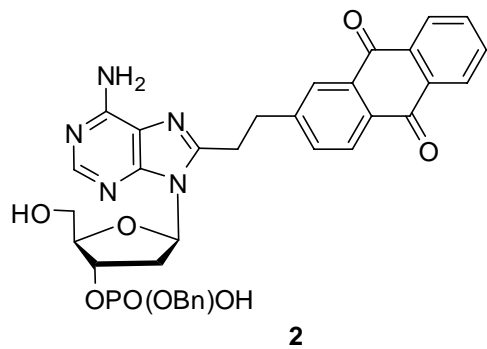
^1H NMR (500 MHz, $\text{DMSO}-d_6$)

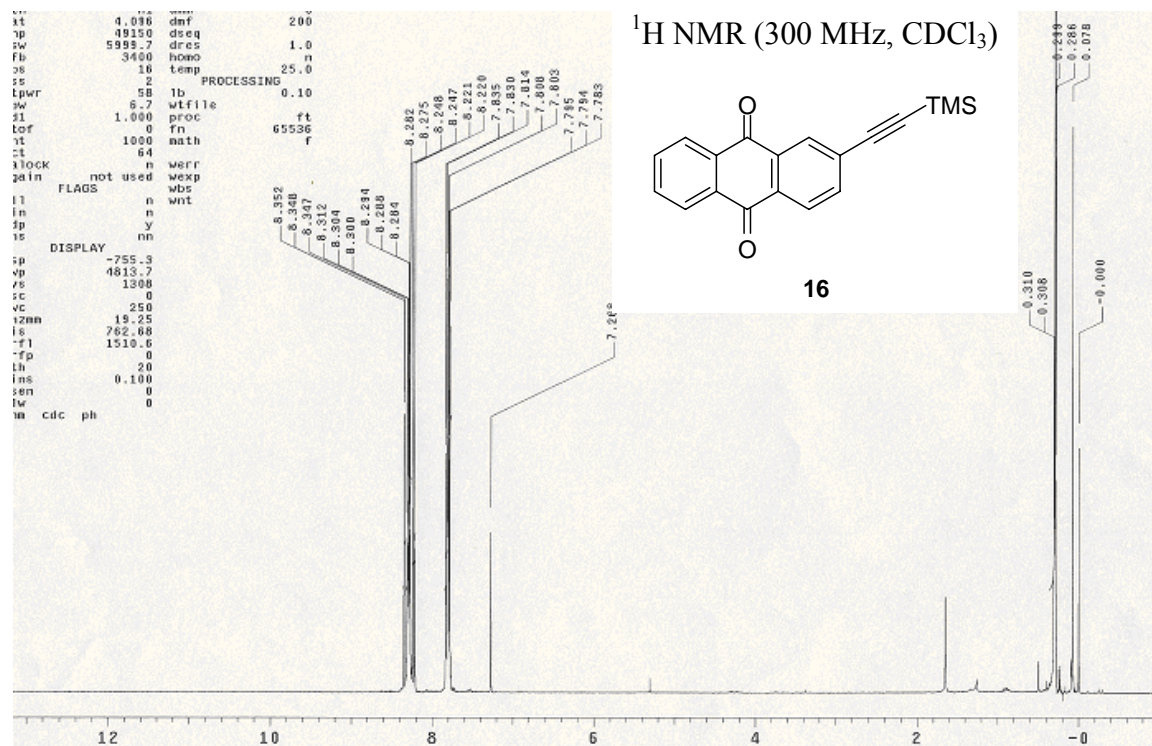
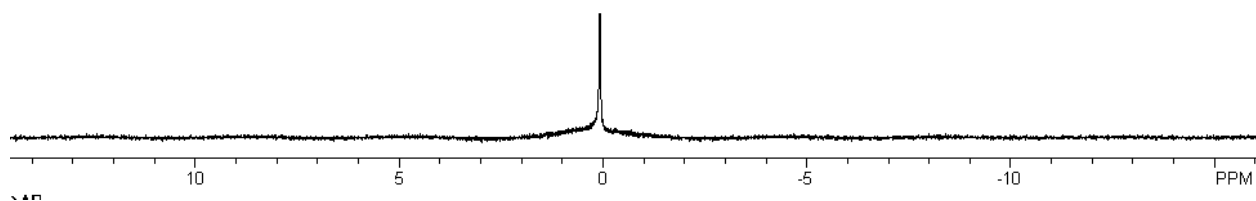
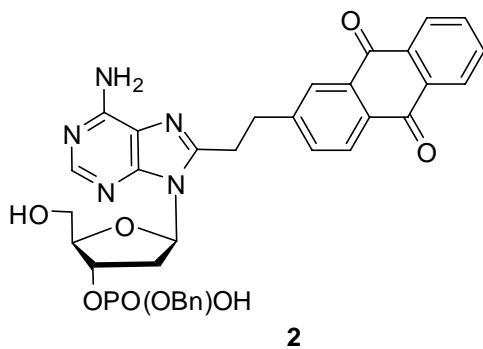


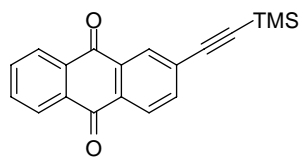
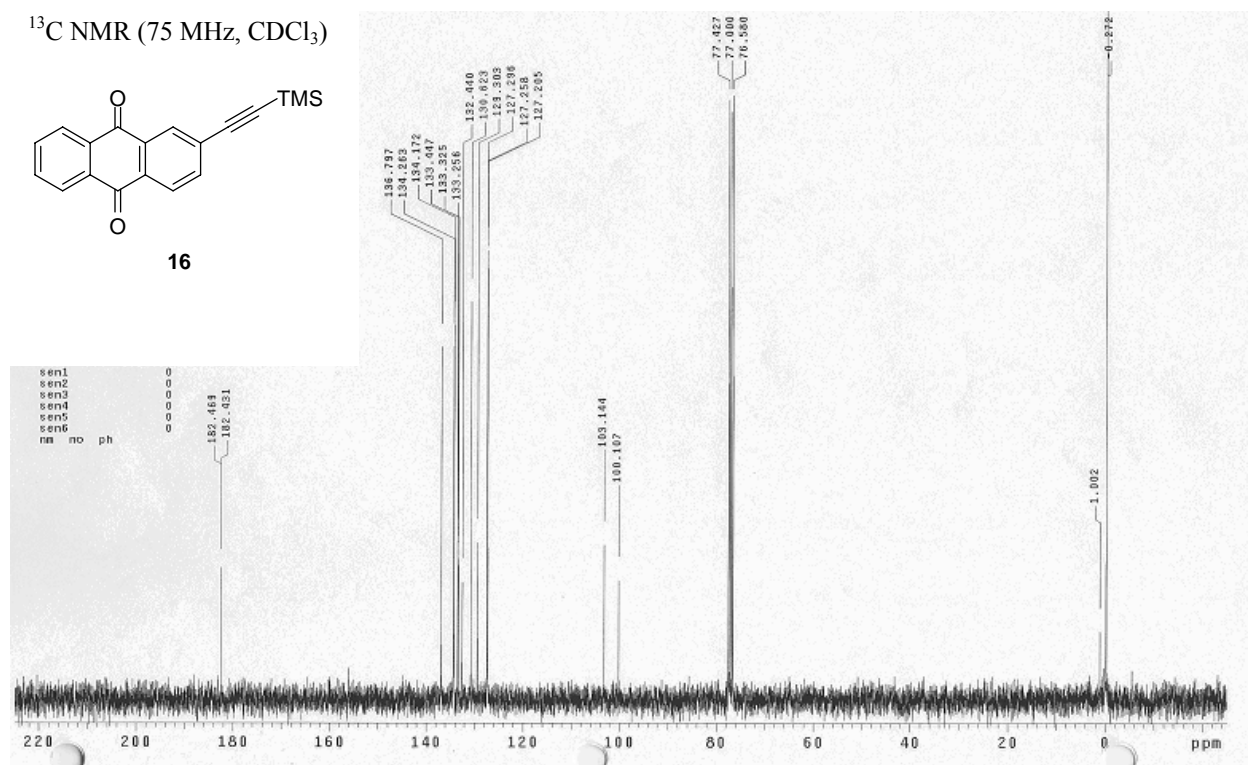
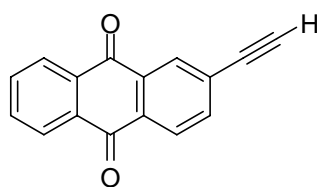
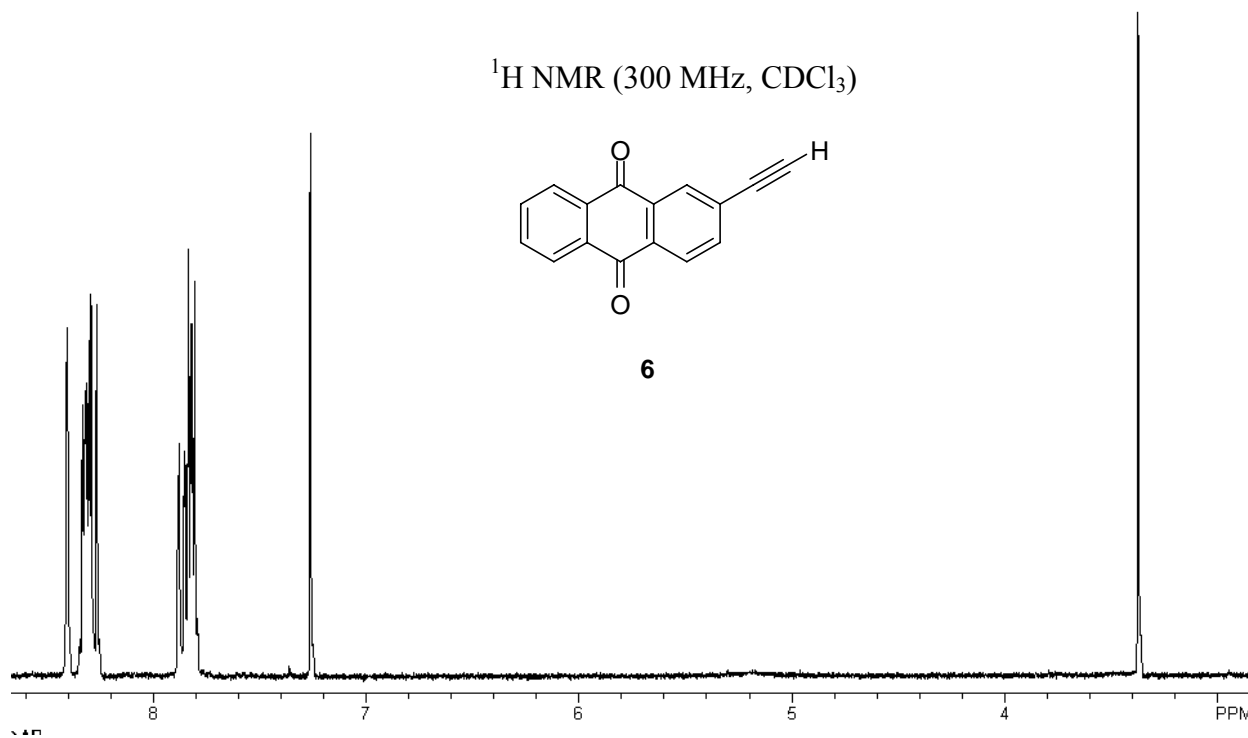
^1H NMR (500 MHz, $\text{DMSO-}d_6 + \text{D}_2\text{O}$)

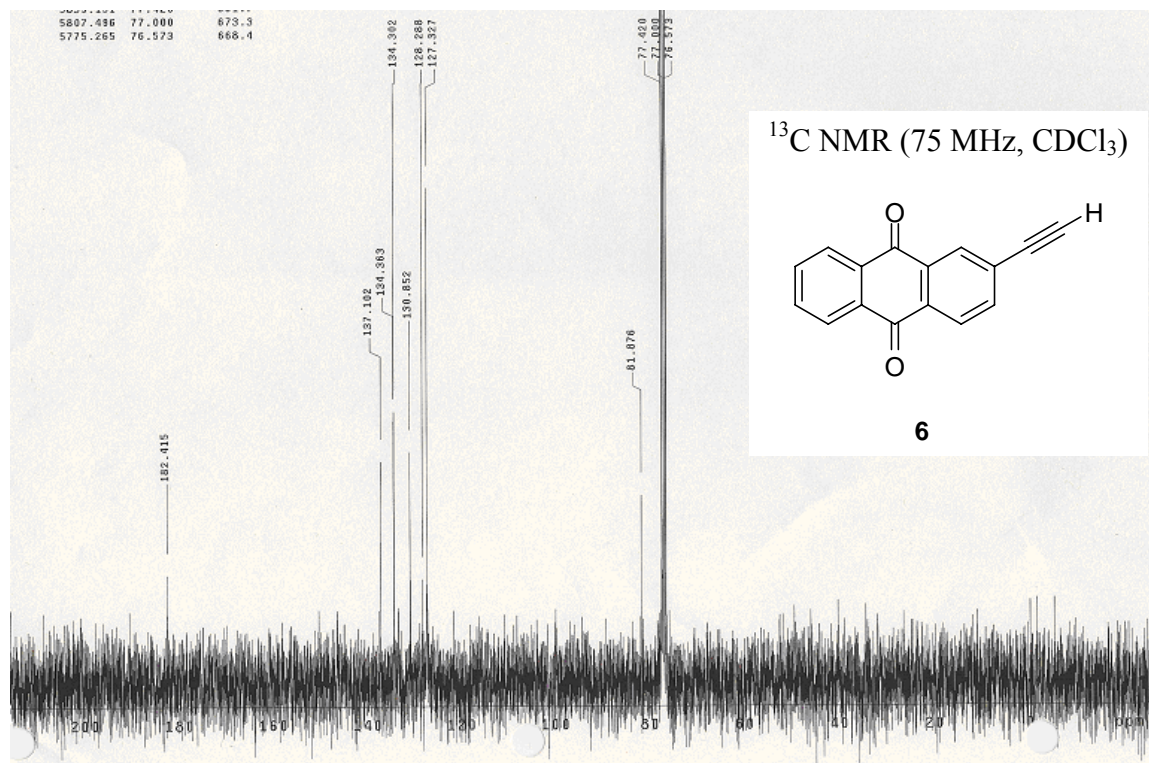


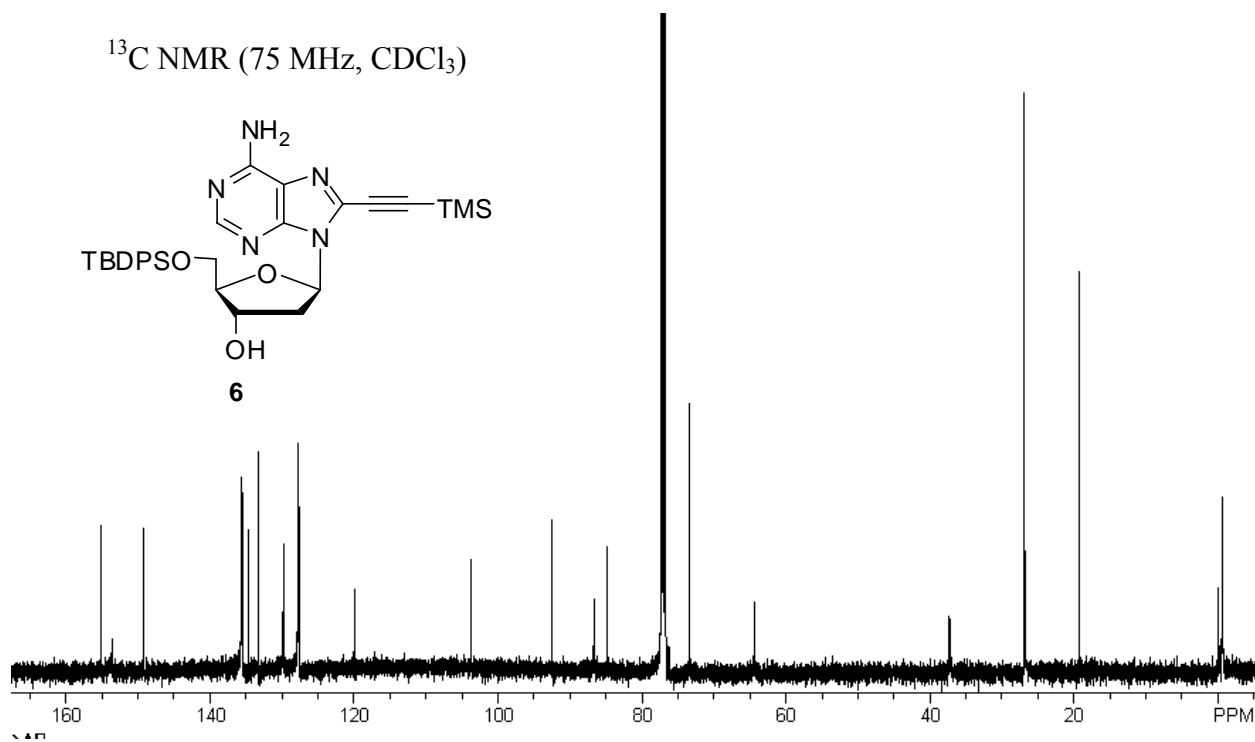
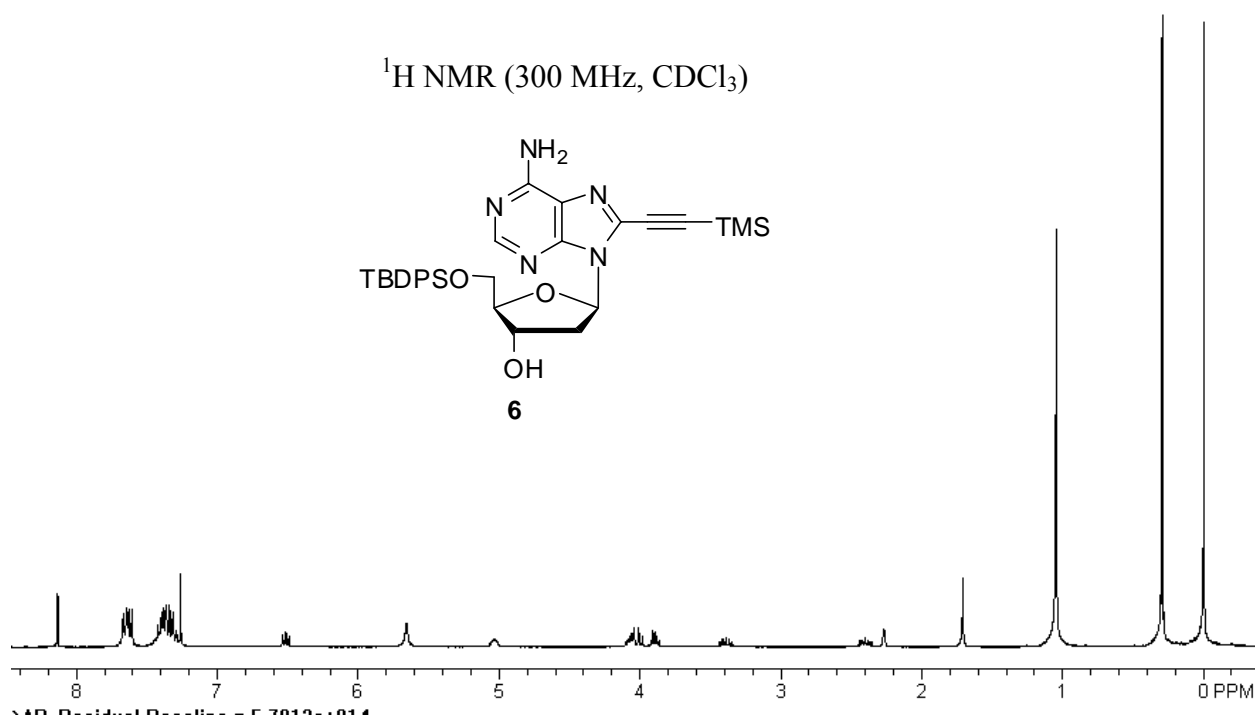
^{13}C NMR (125 MHz, $\text{DMSO-}d_6$)

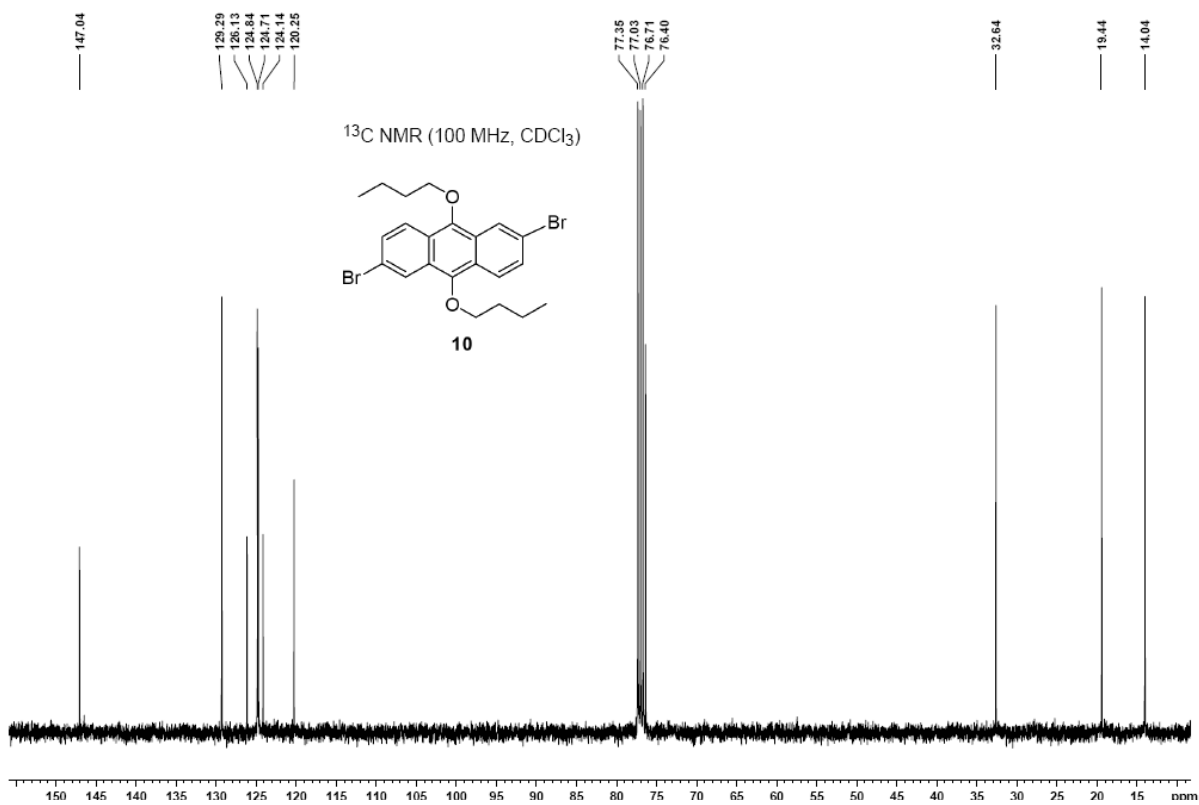
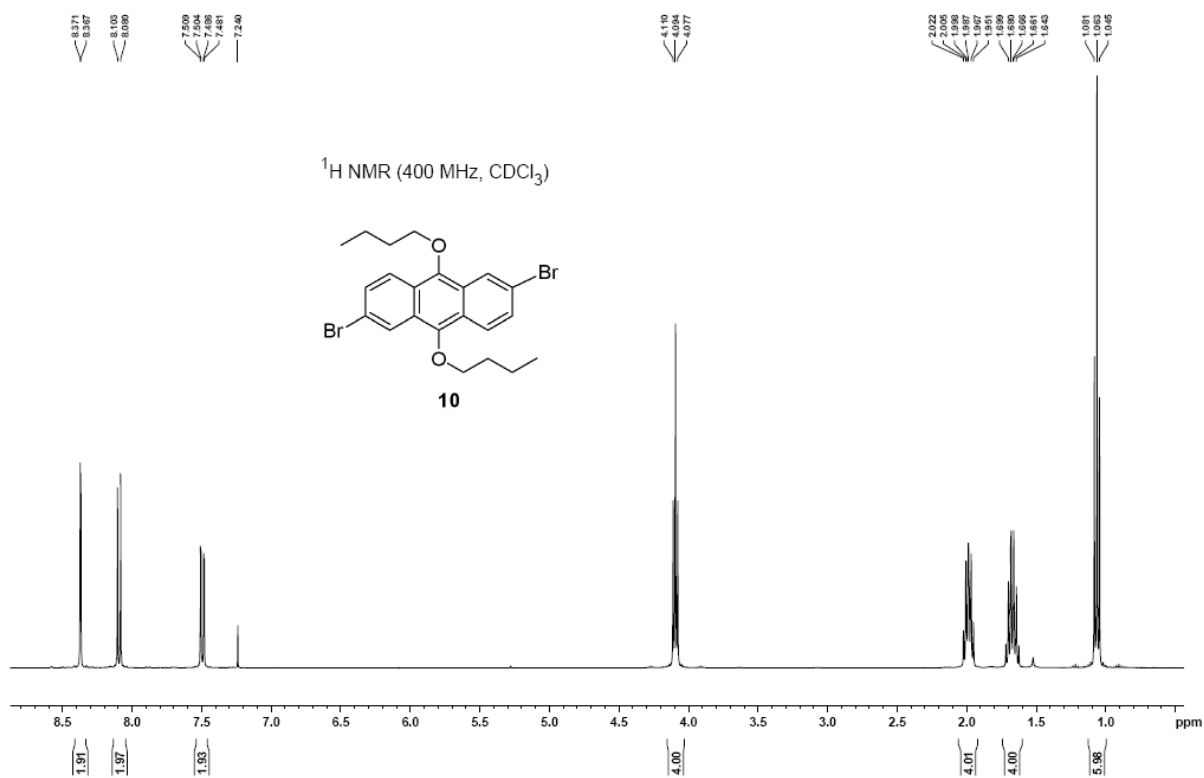


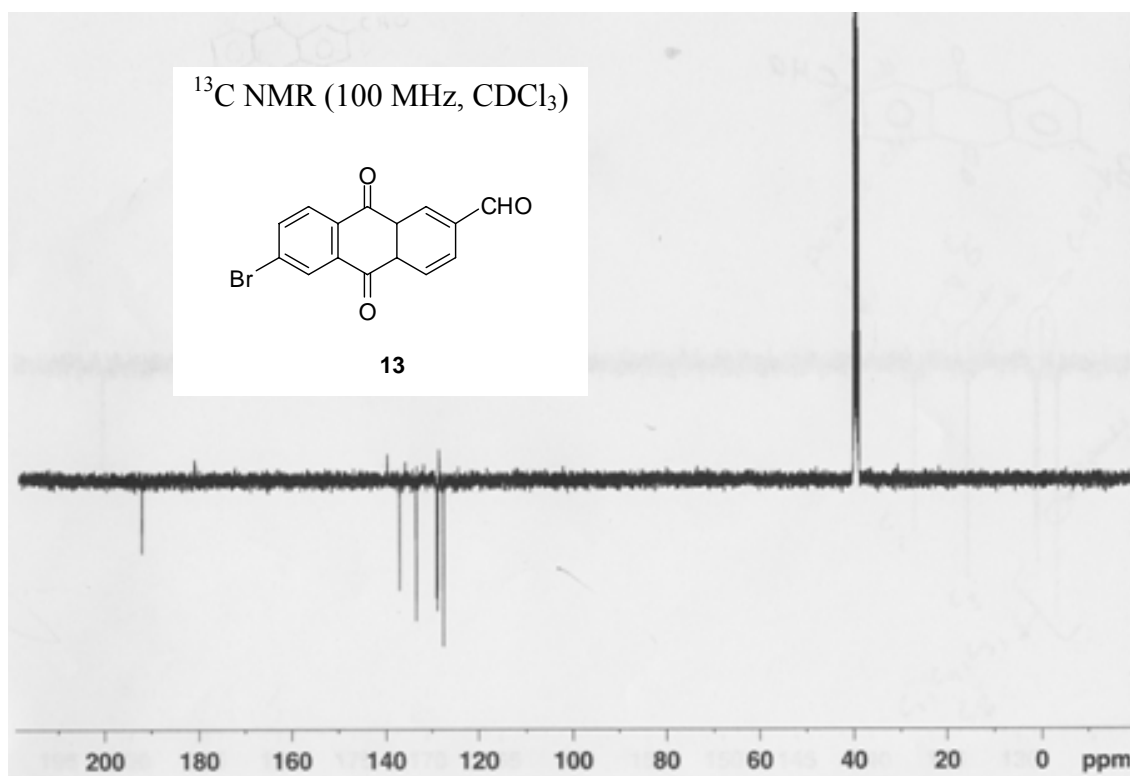
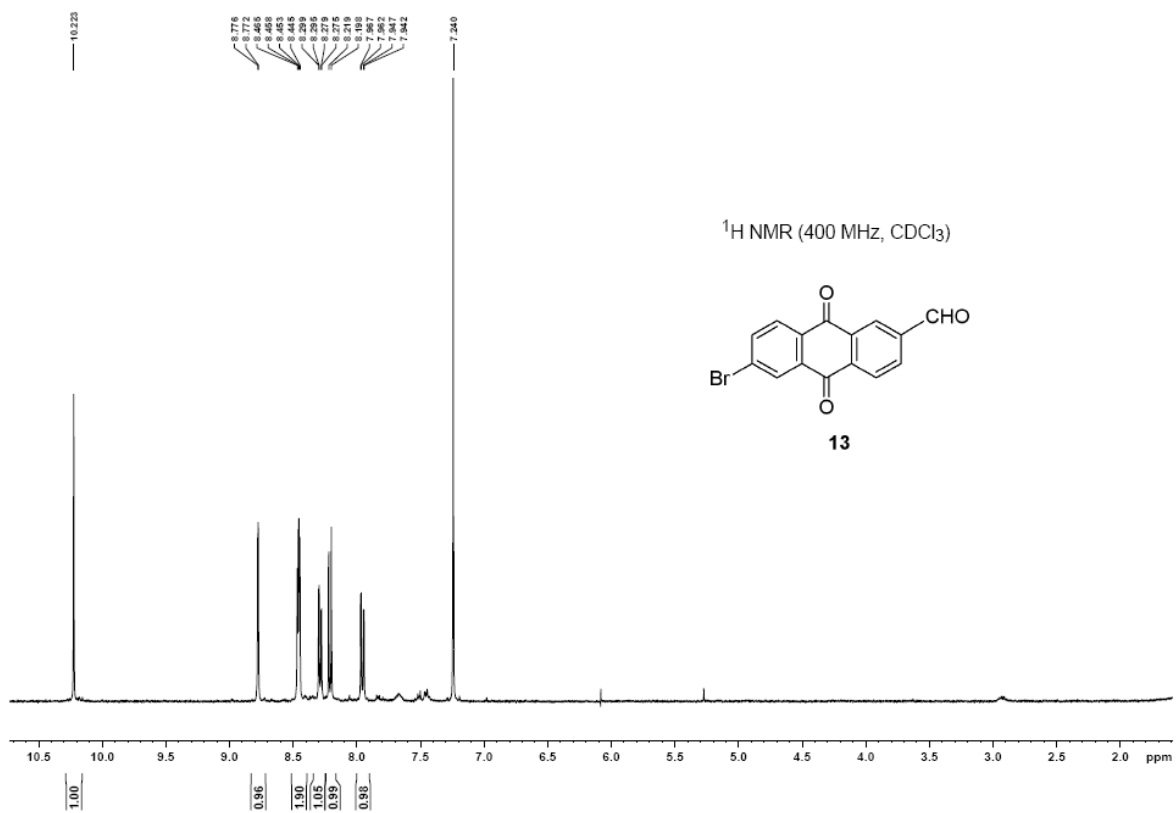
^{31}P NMR (202 MHz, $\text{DMSO-}d_6$)

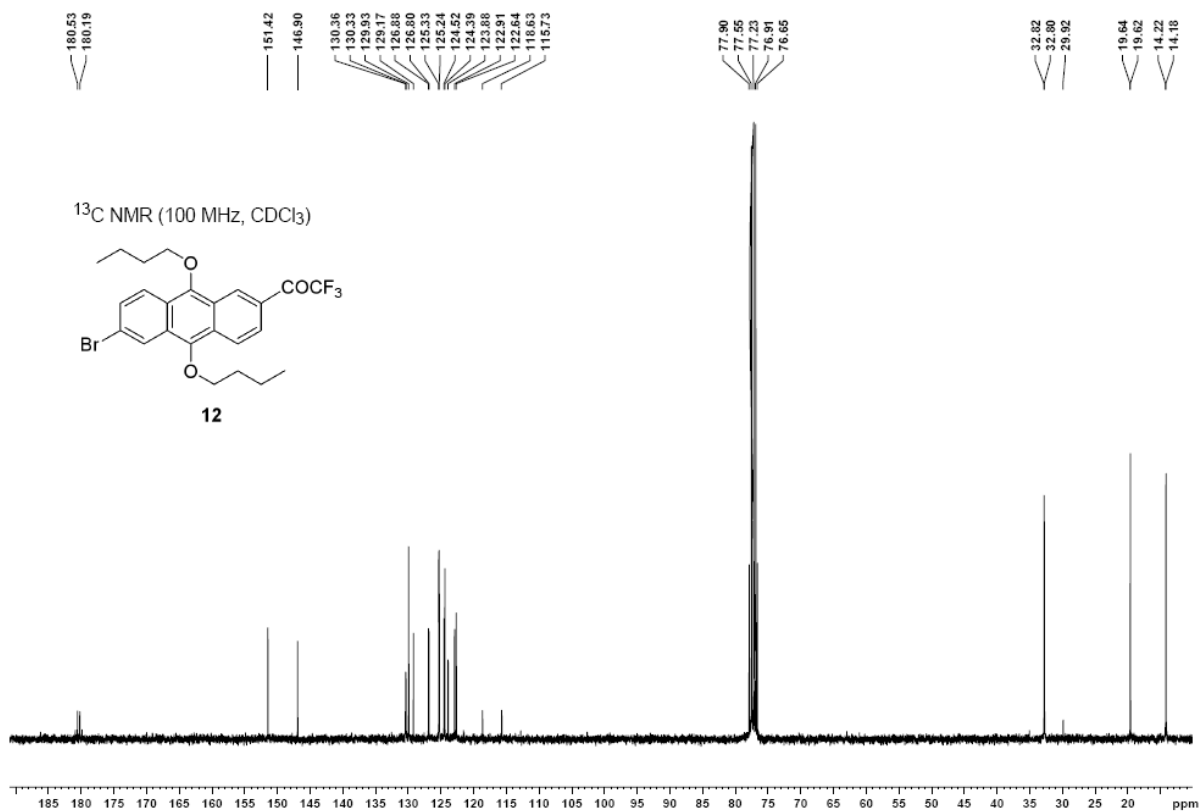
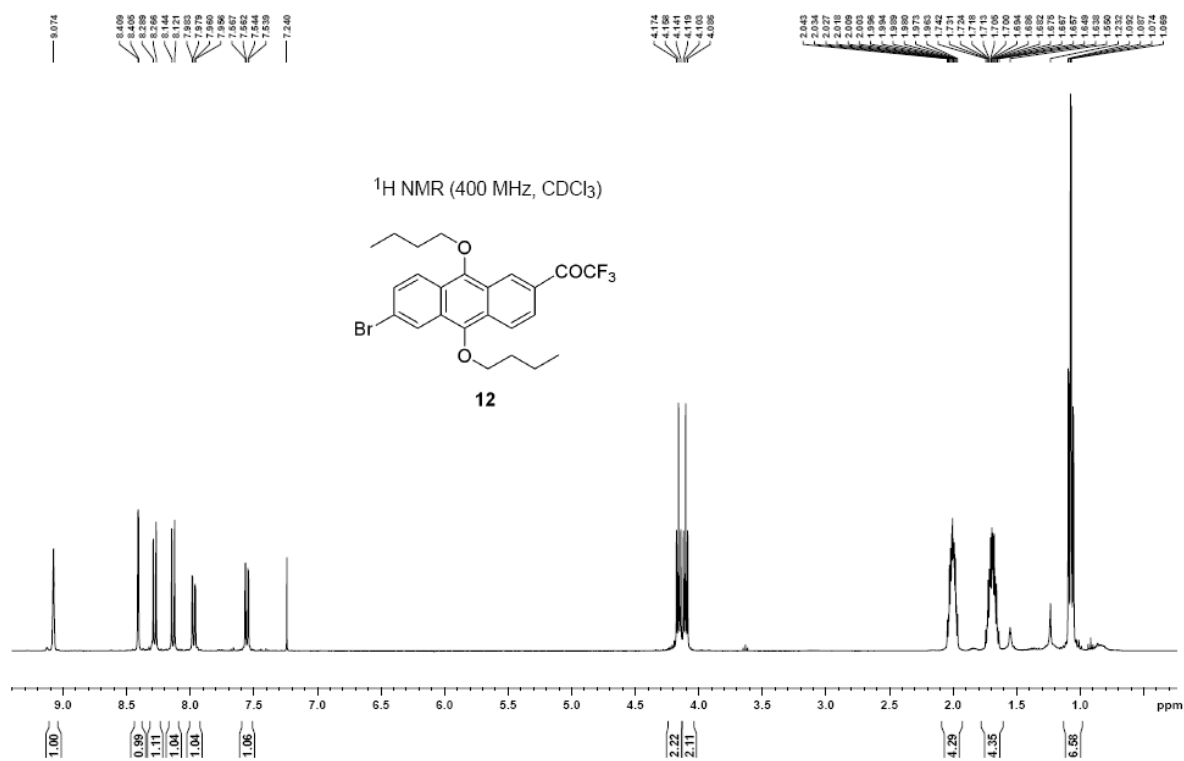
^{13}C NMR (75 MHz, CDCl_3)**16** ^1H NMR (300 MHz, CDCl_3)**6**

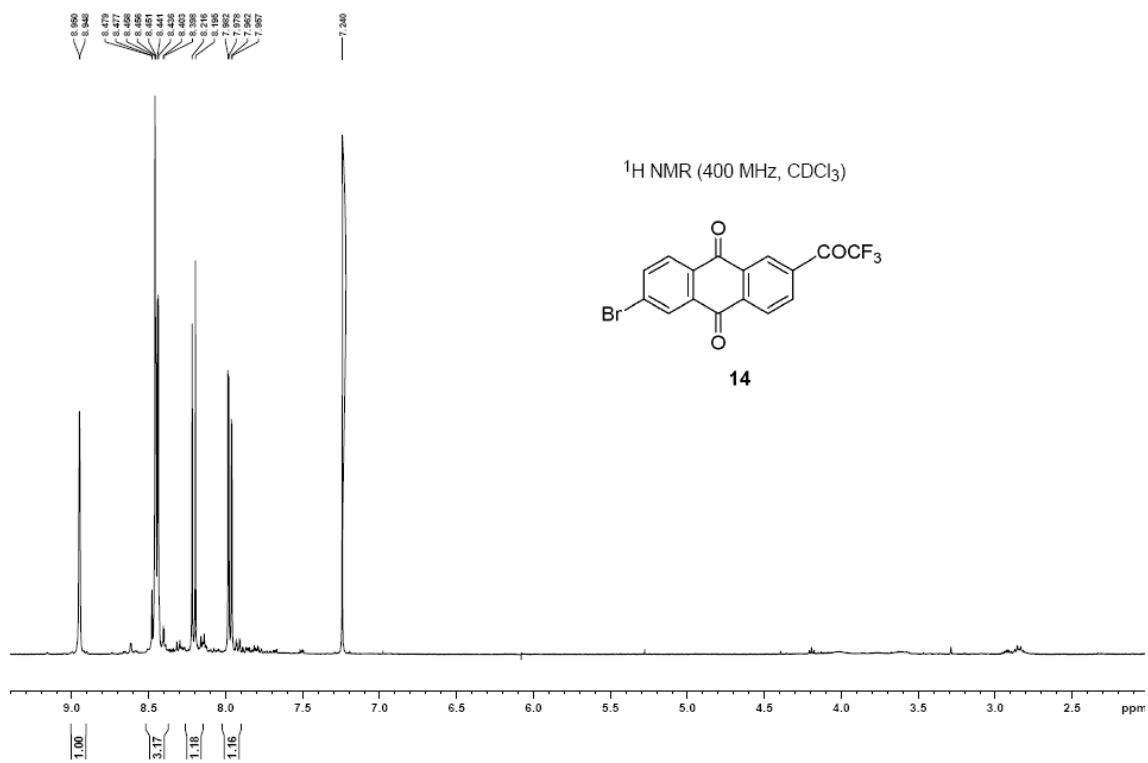
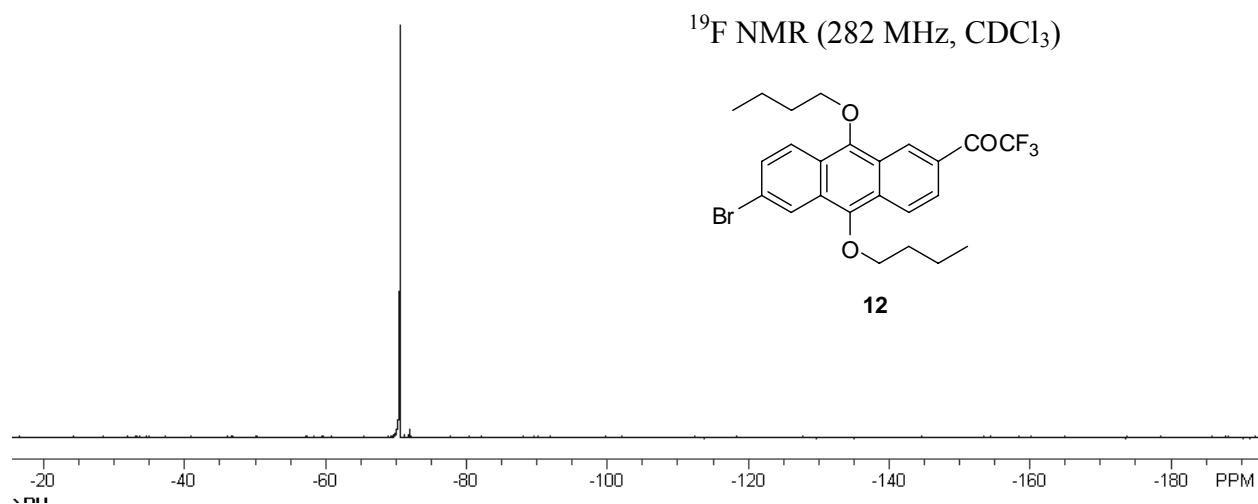


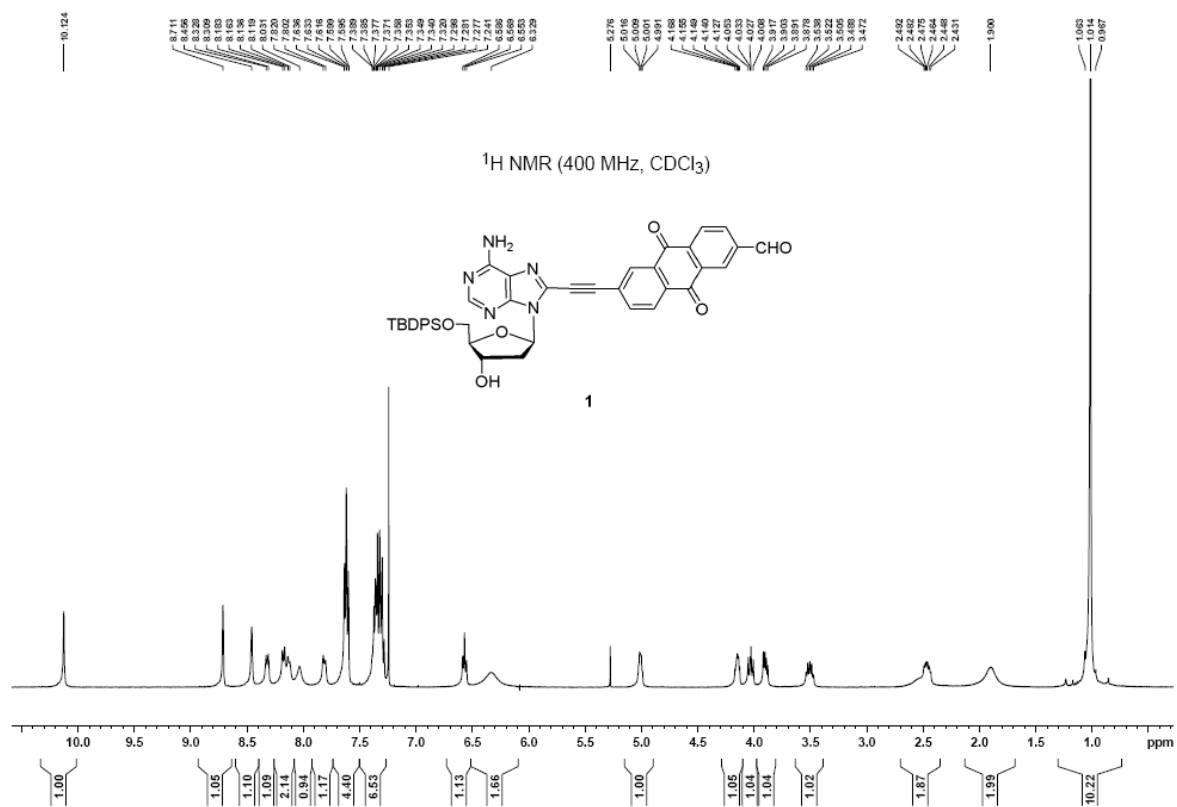
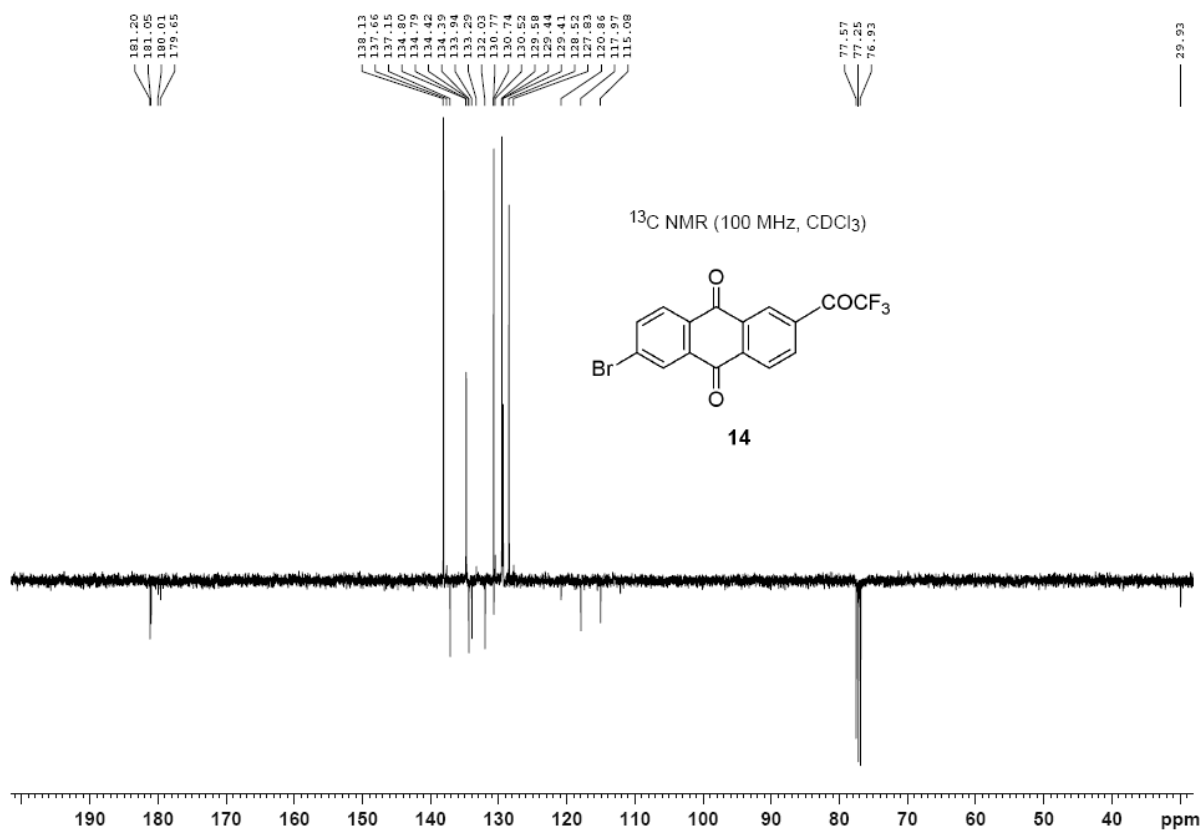
NMR spectra for compounds in Chapter 3:

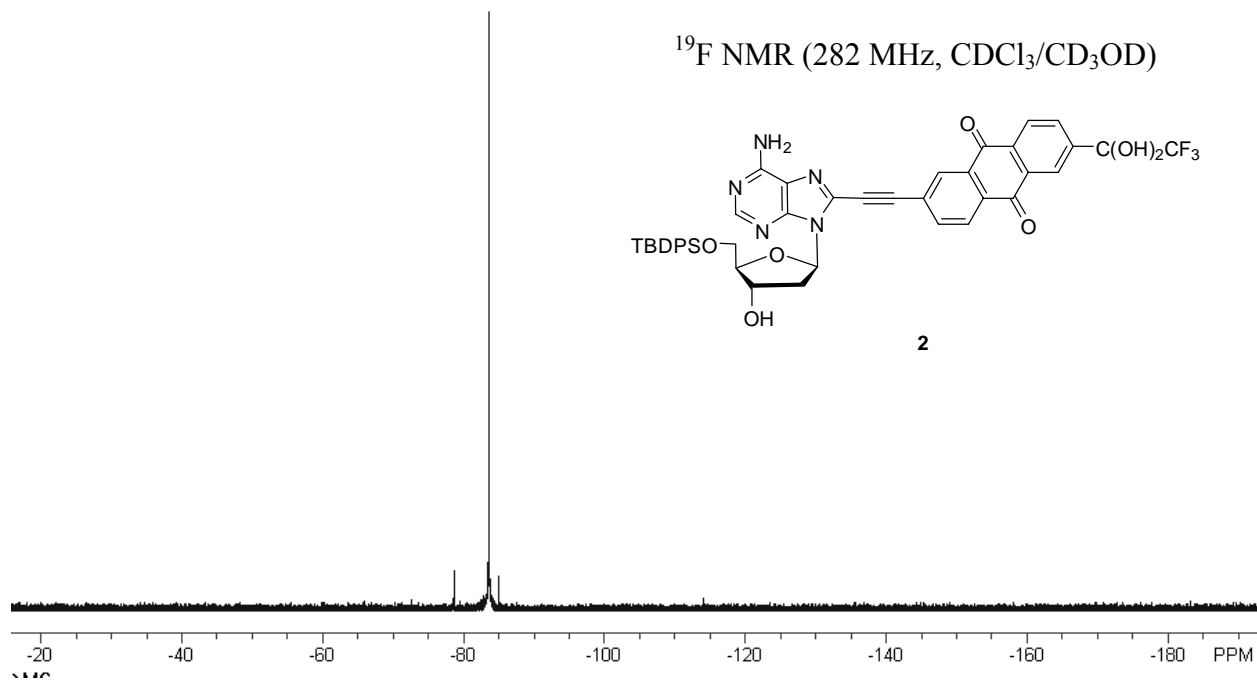
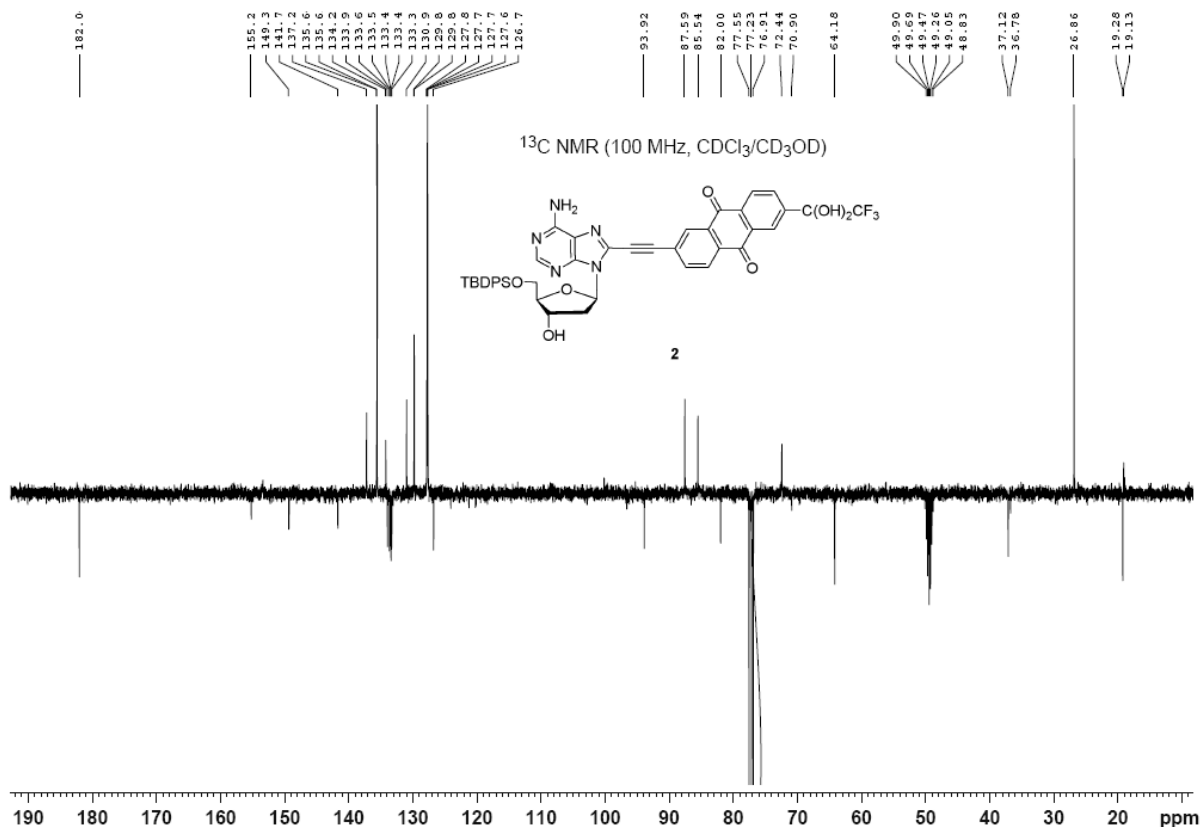


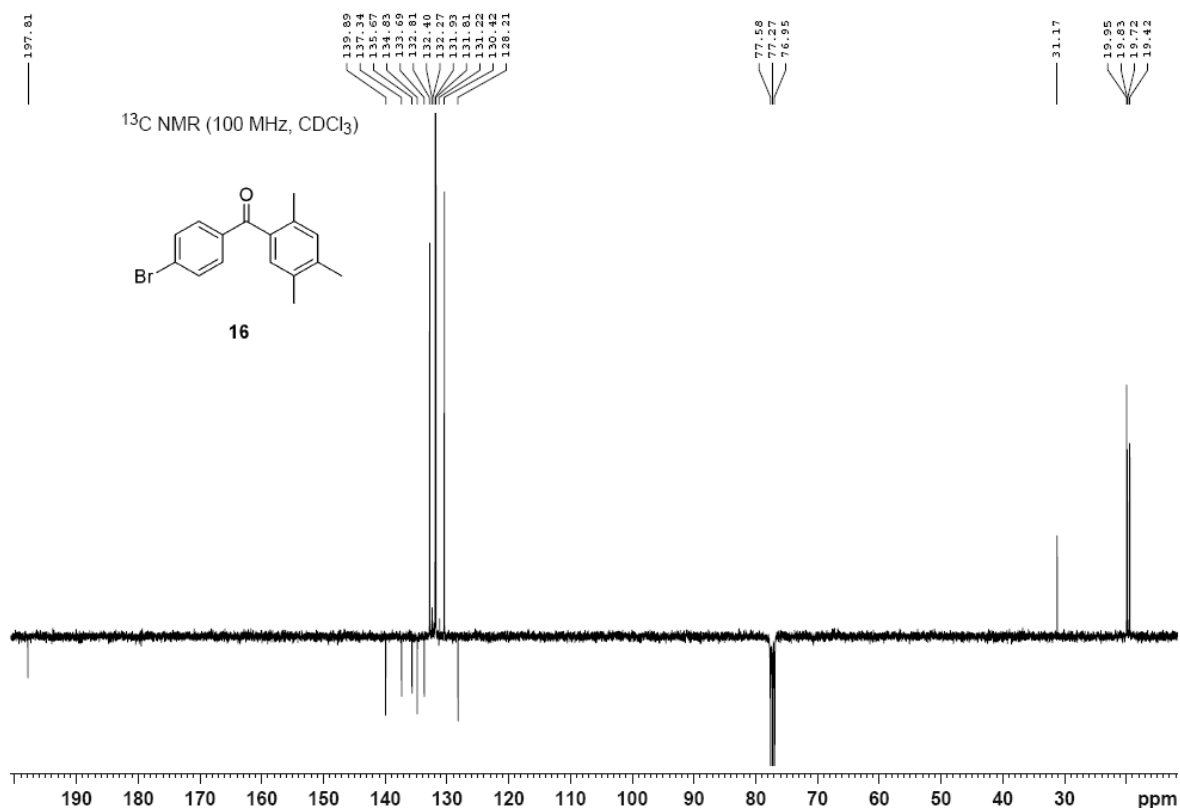
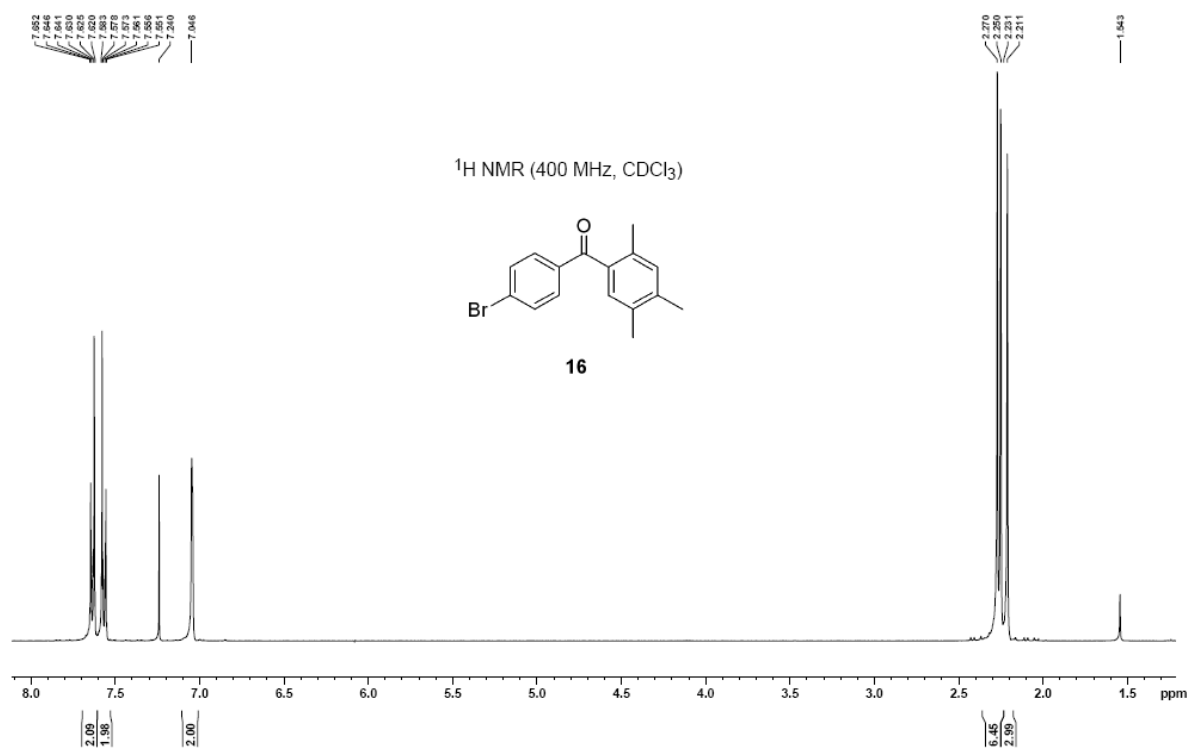


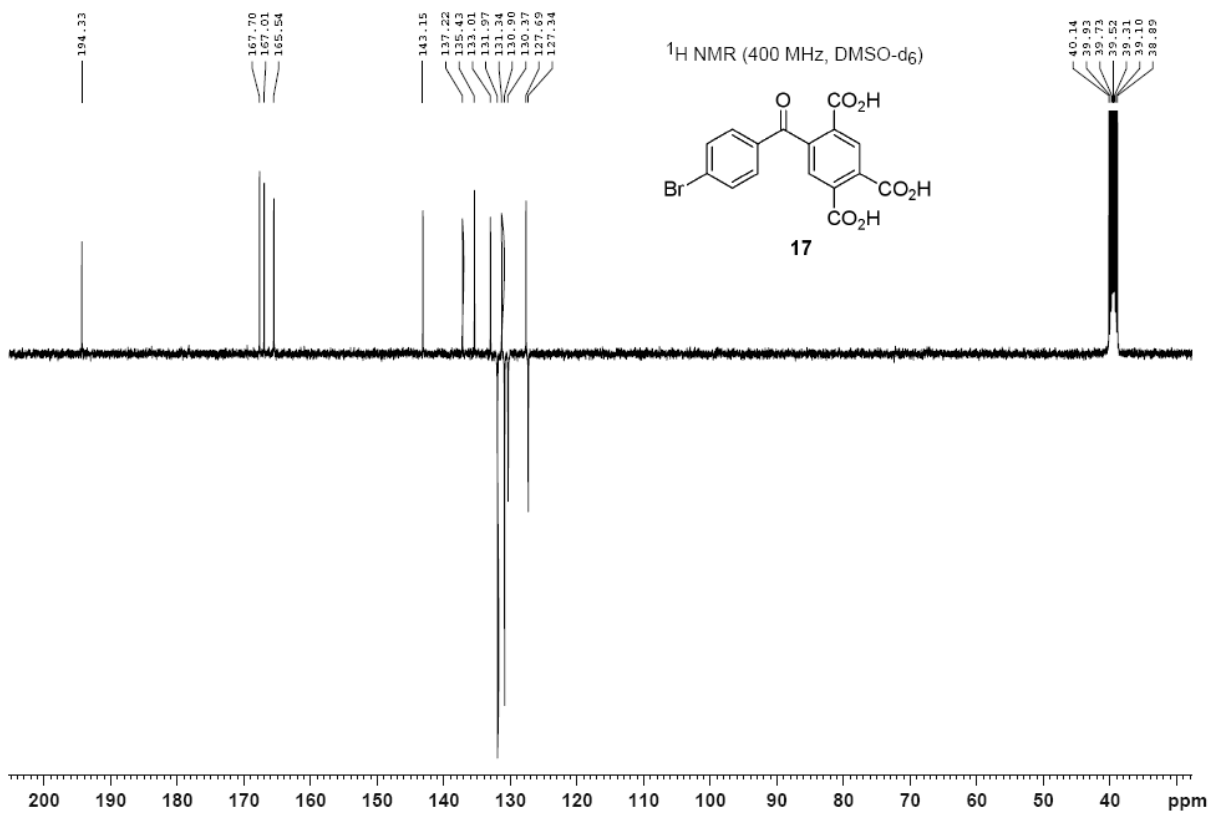
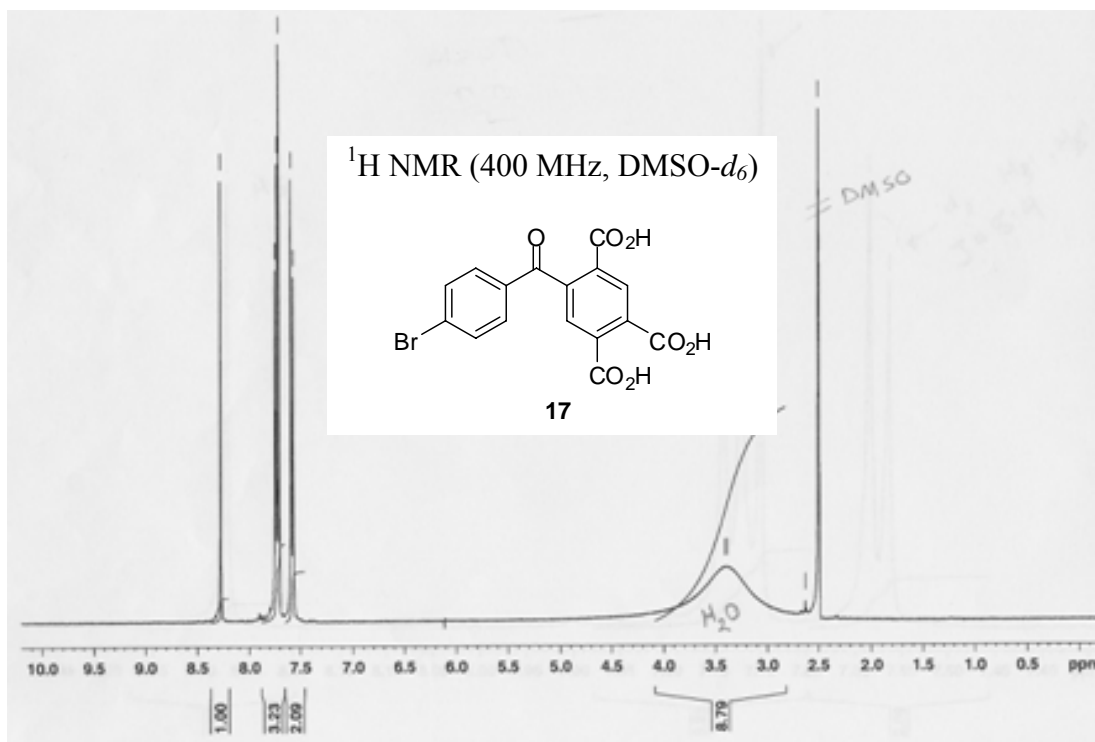


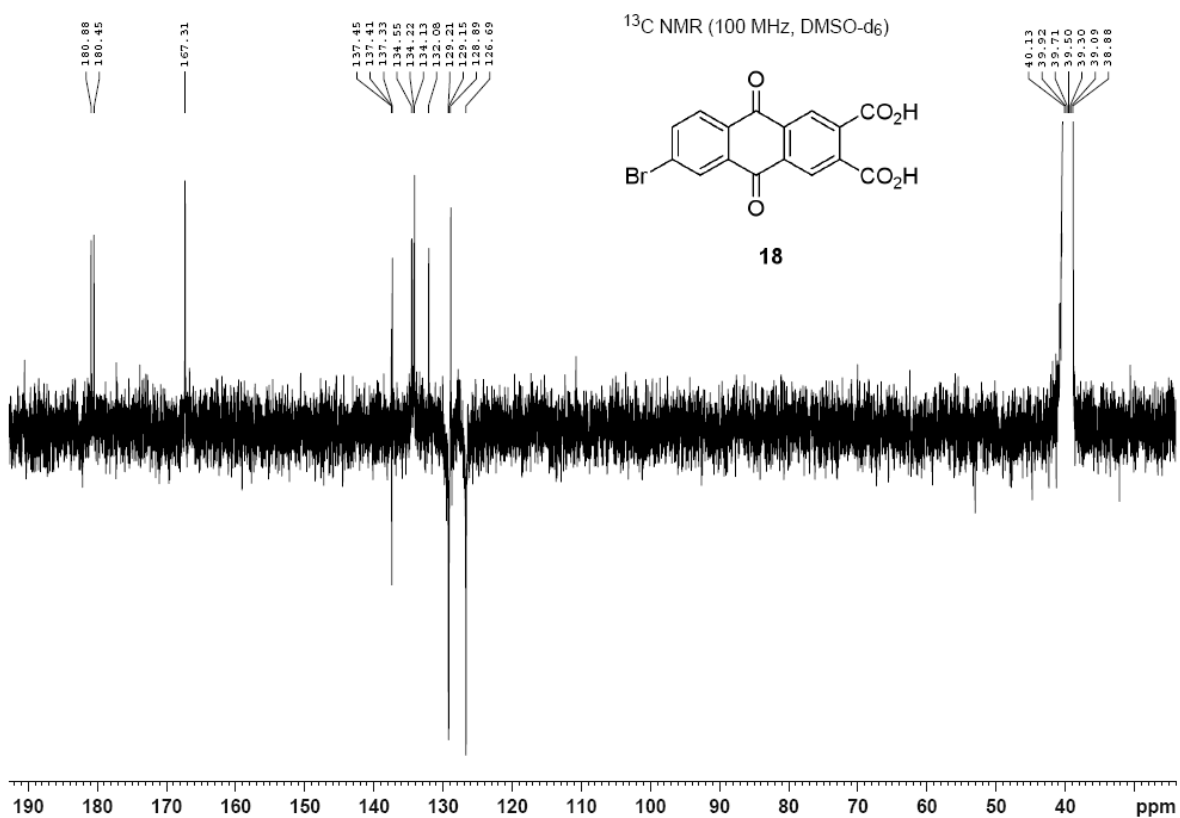
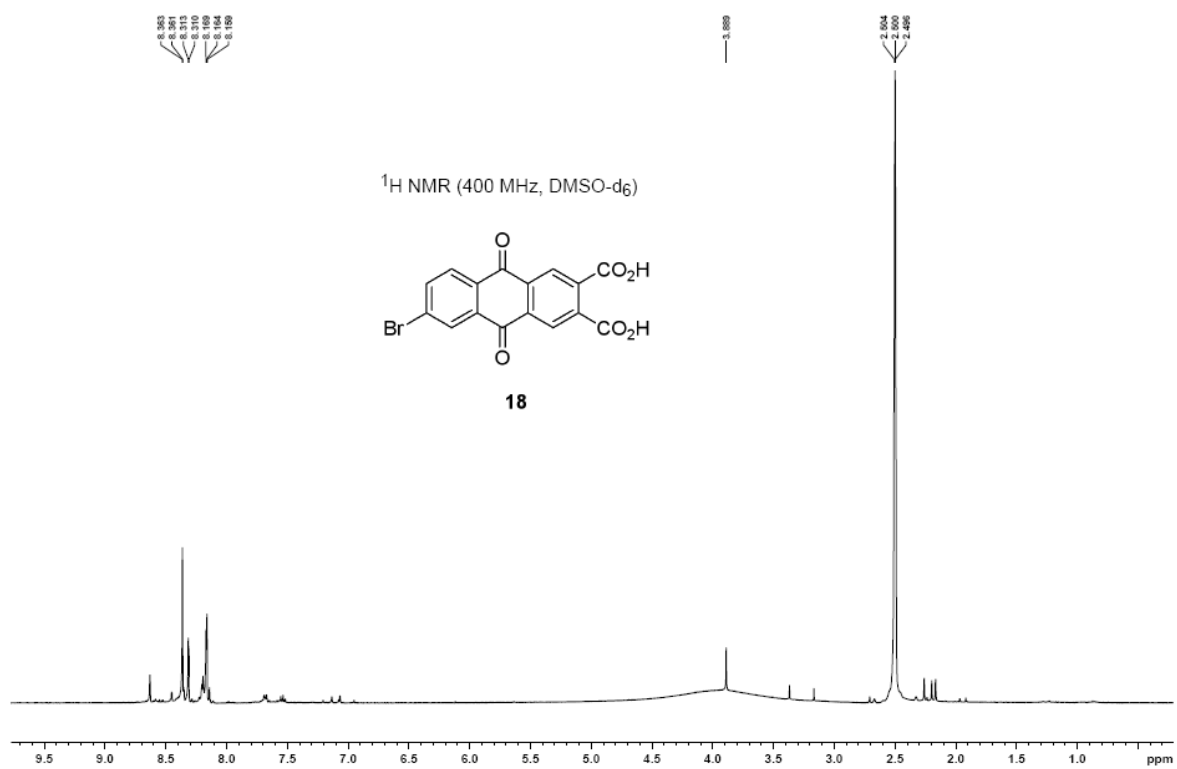


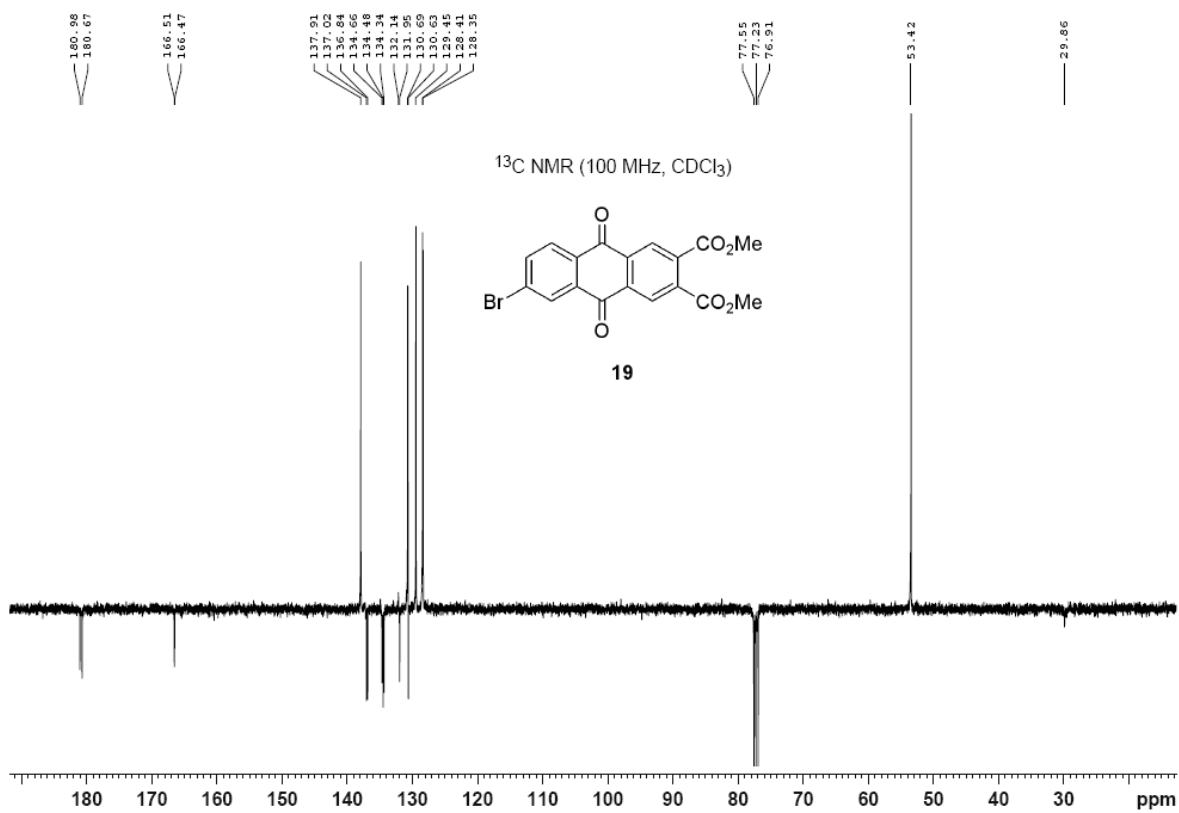
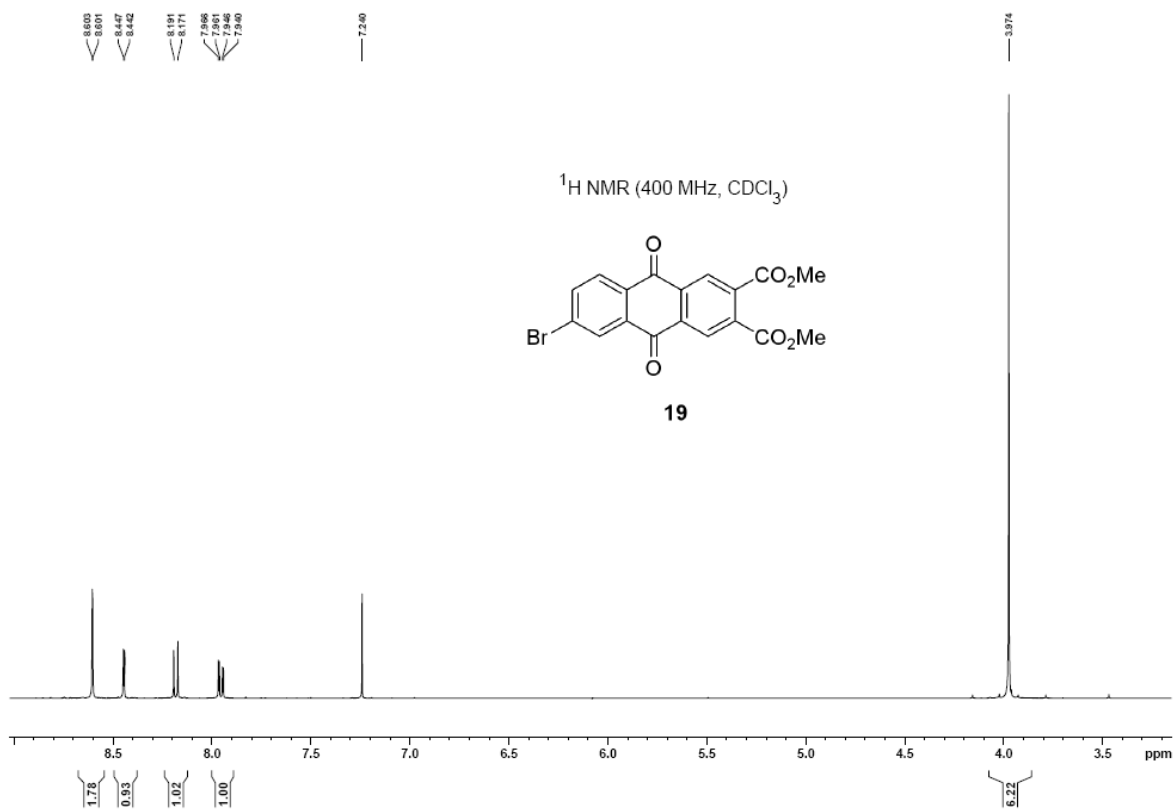


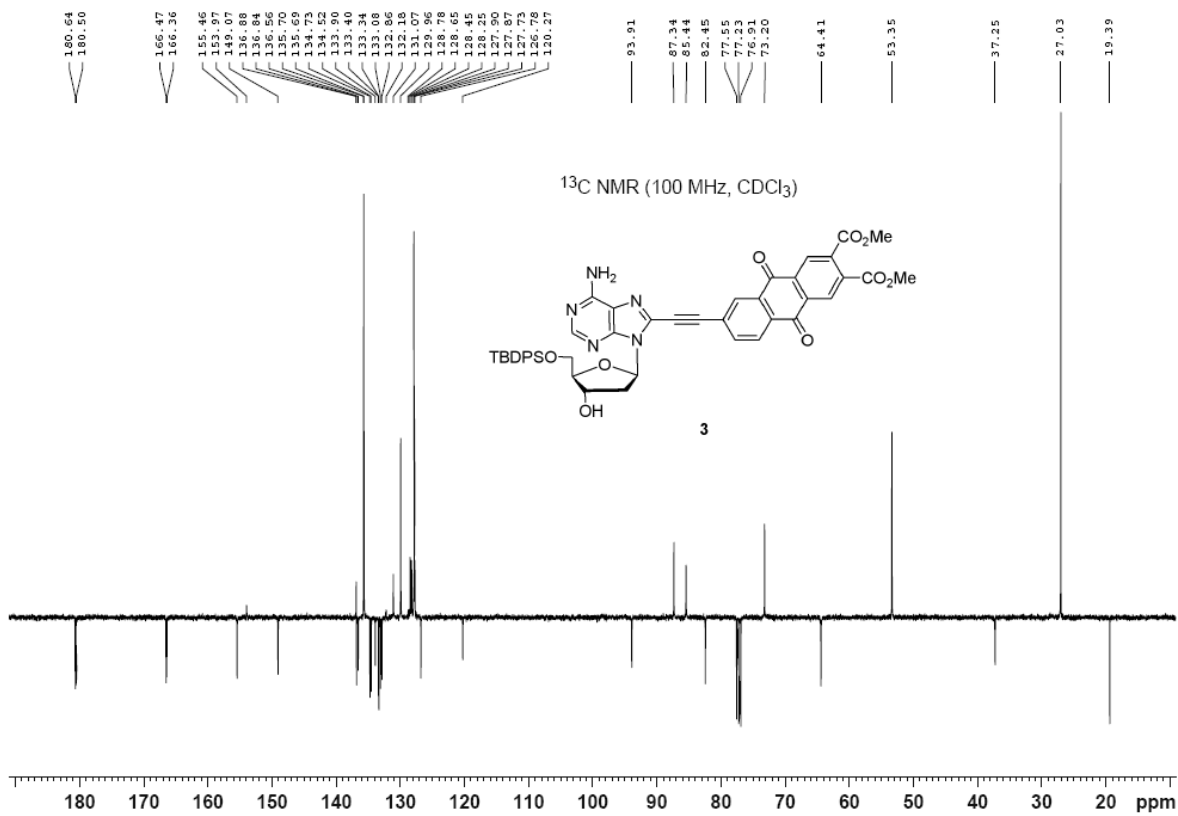
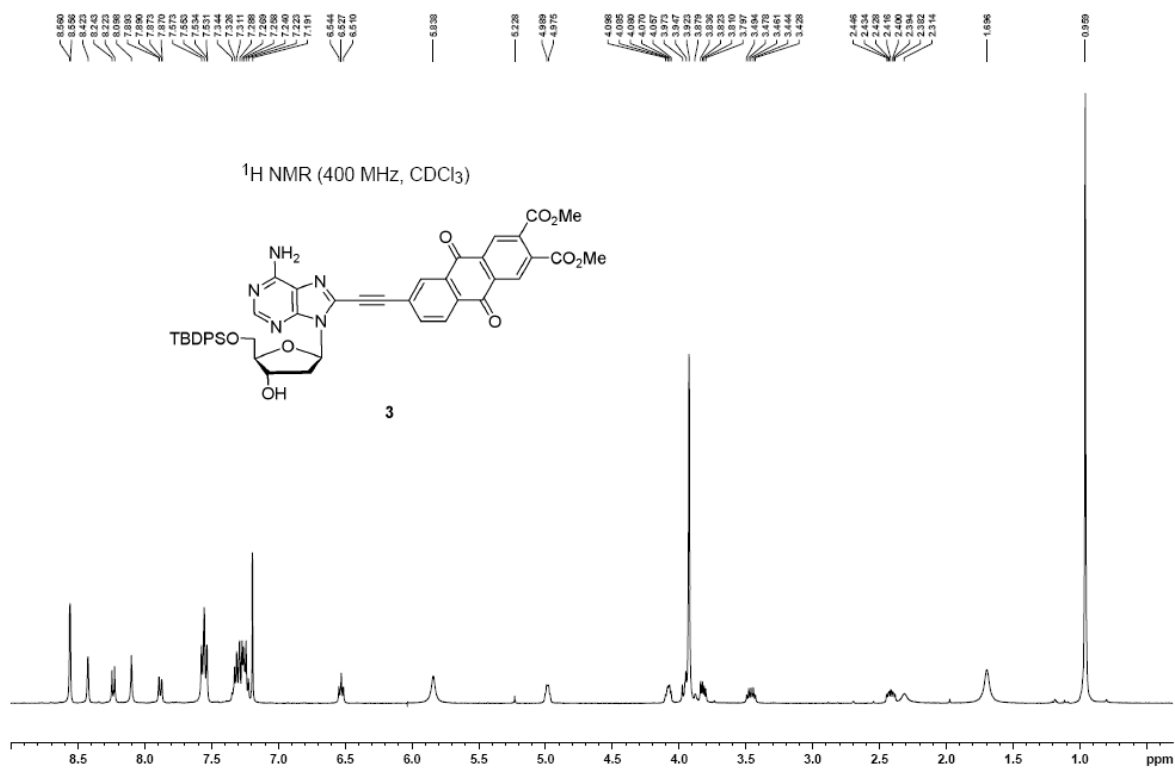


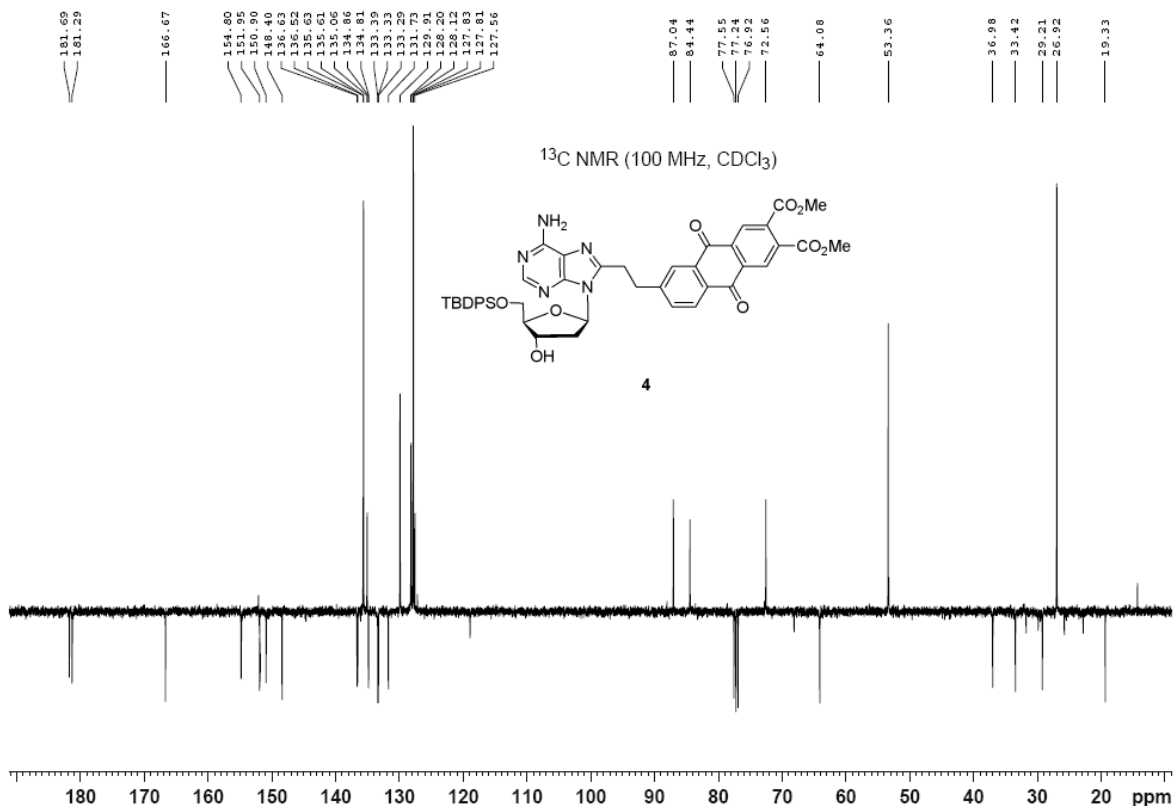
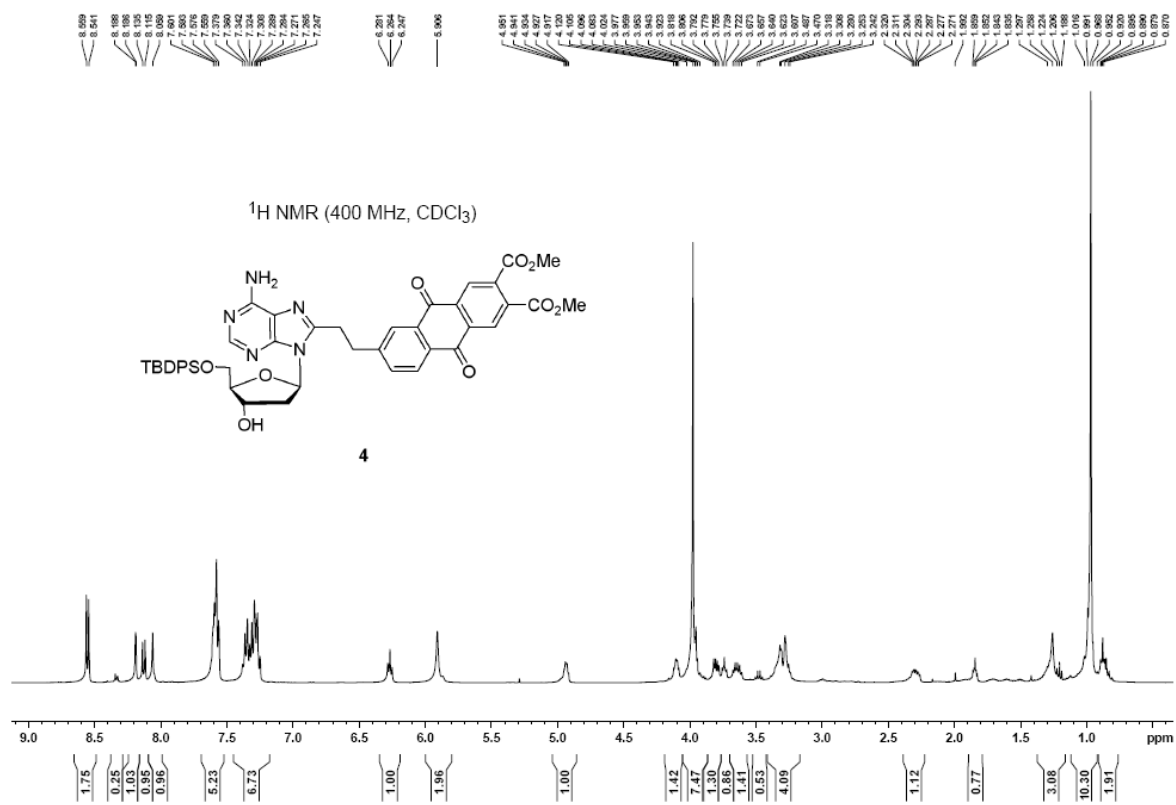




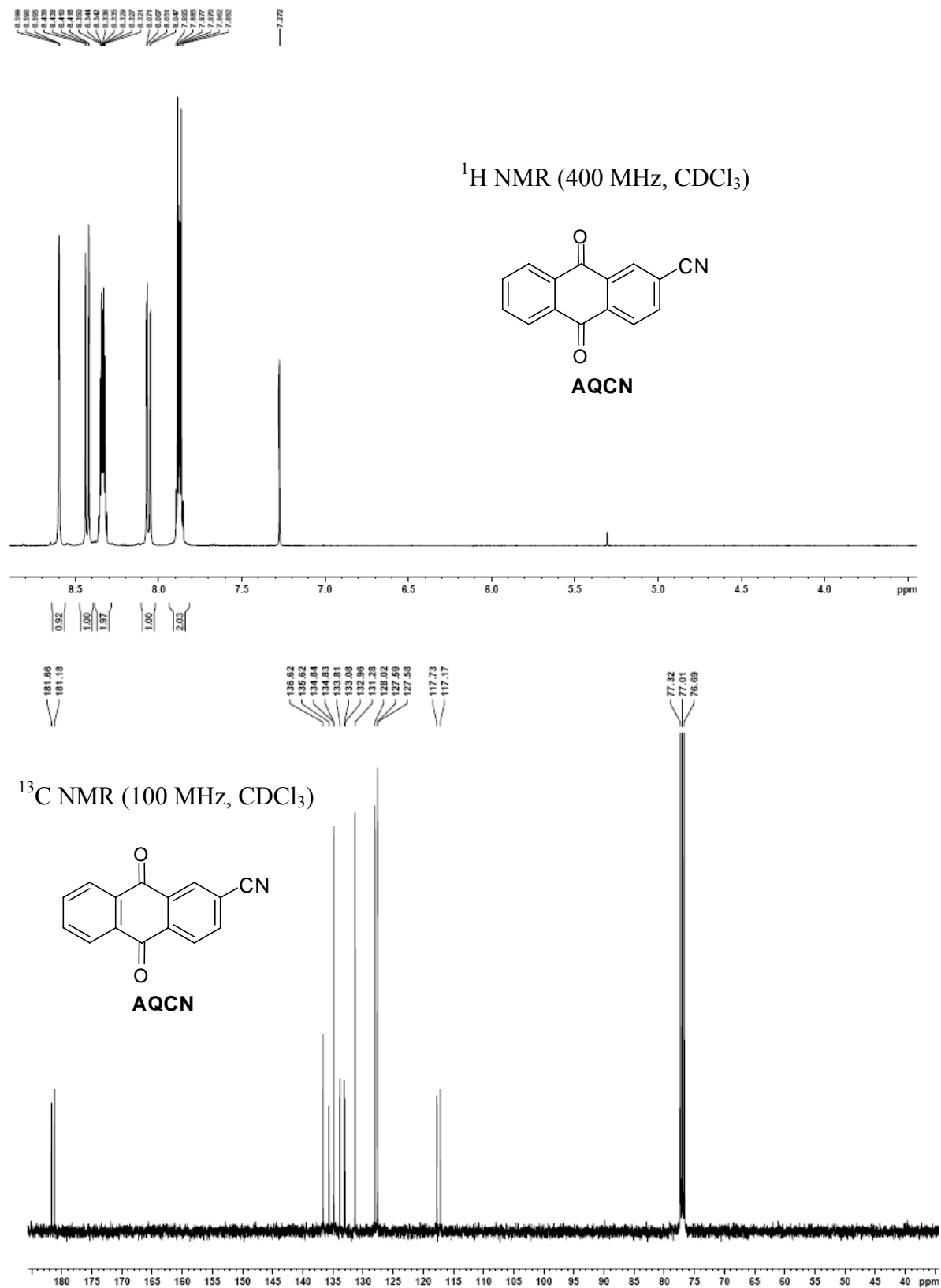


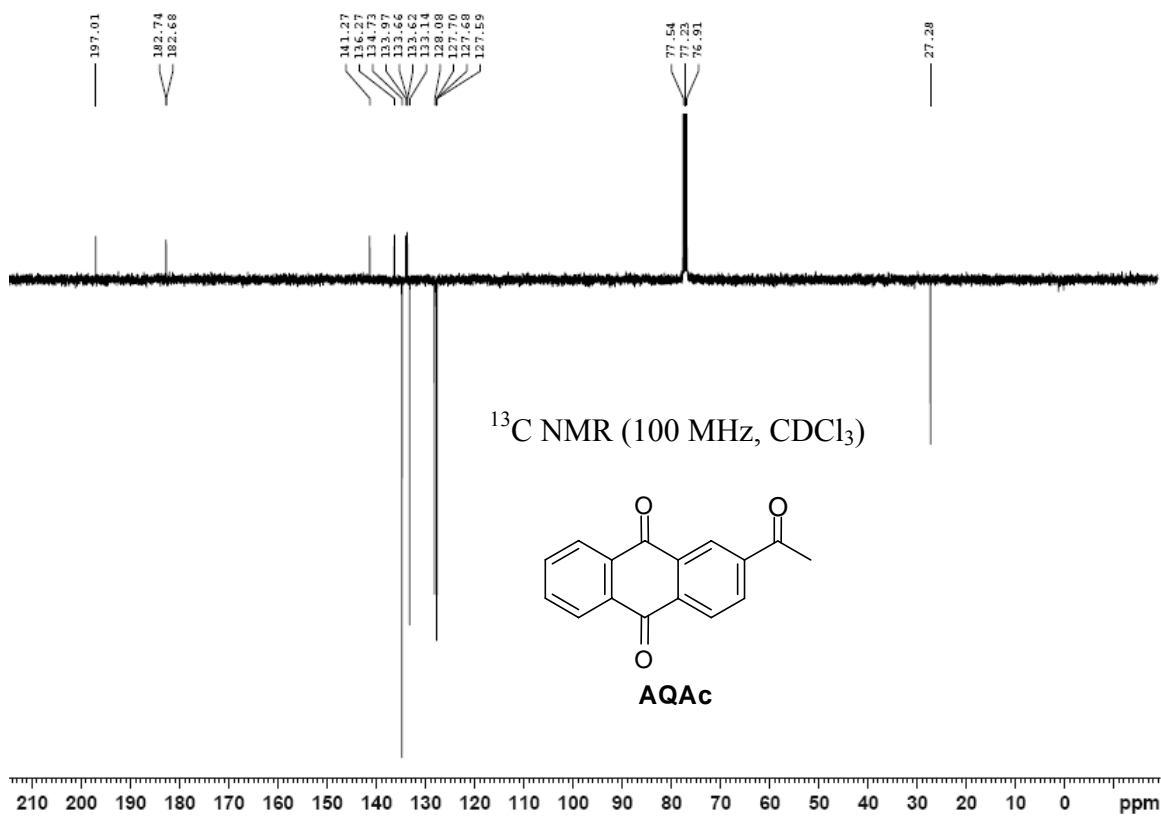
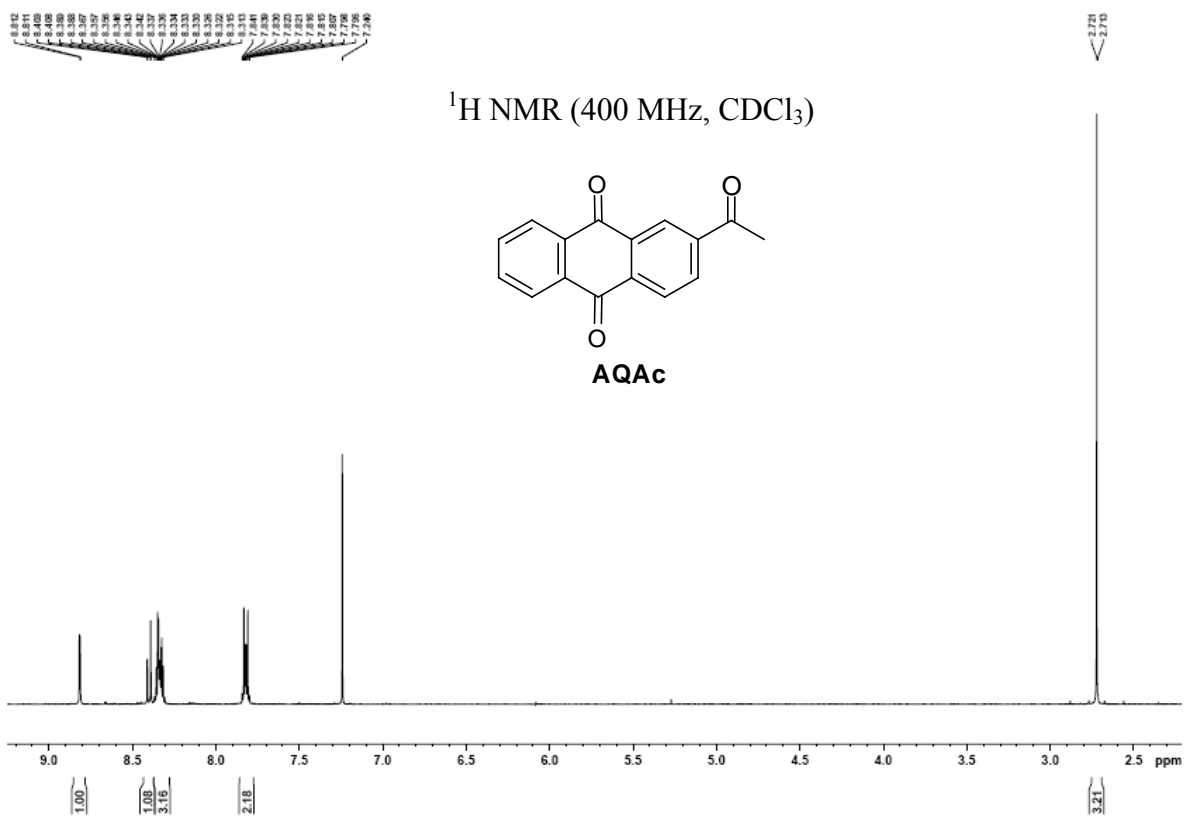




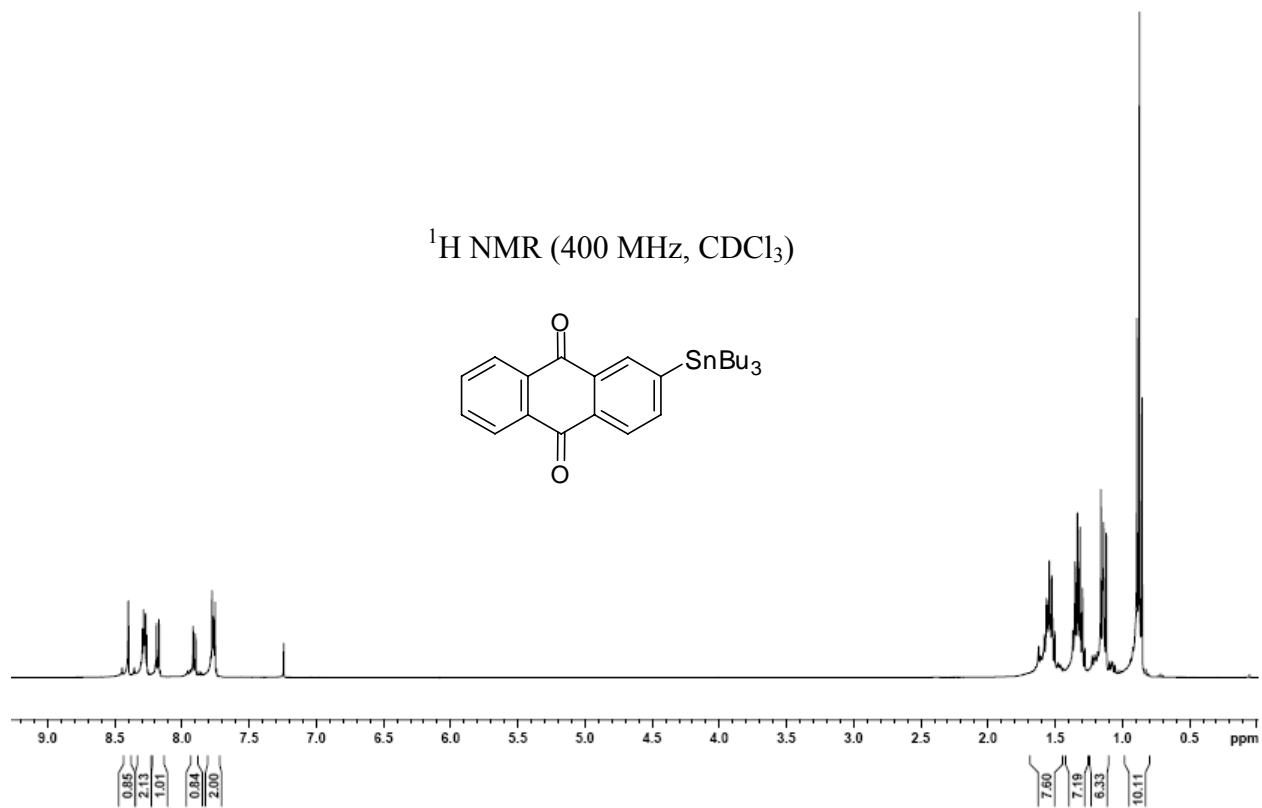
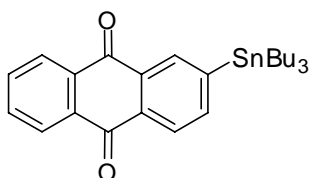


NMR spectra for compounds in Chapter 4:

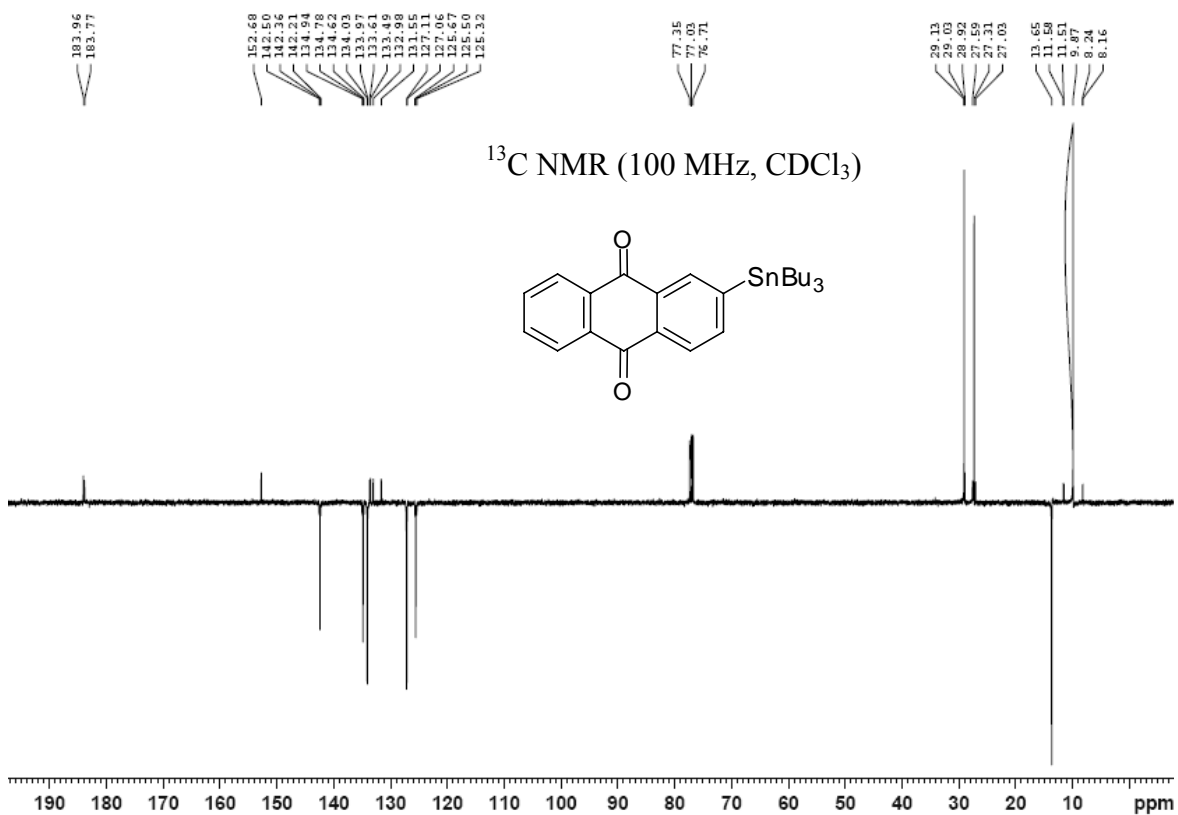
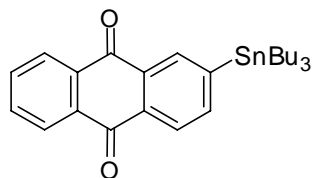


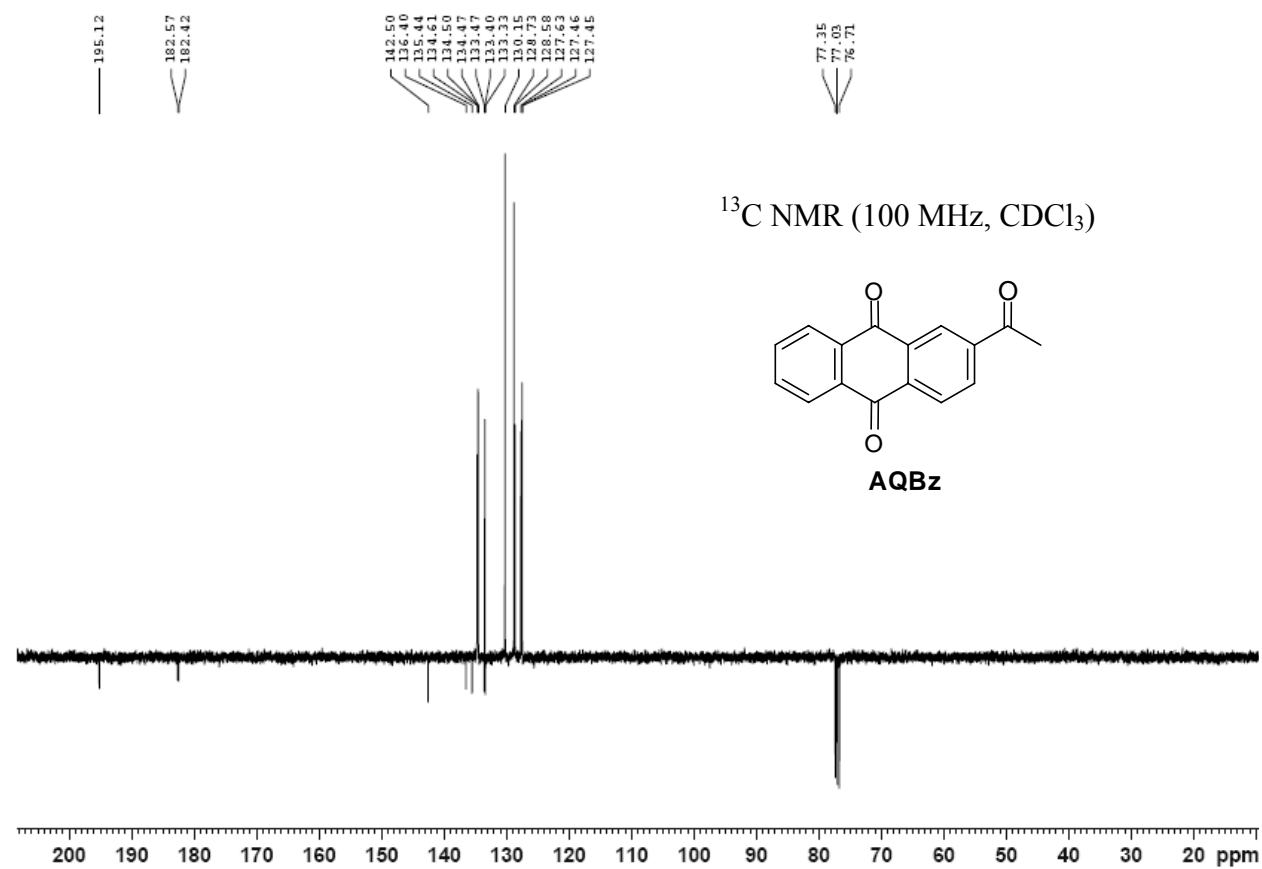
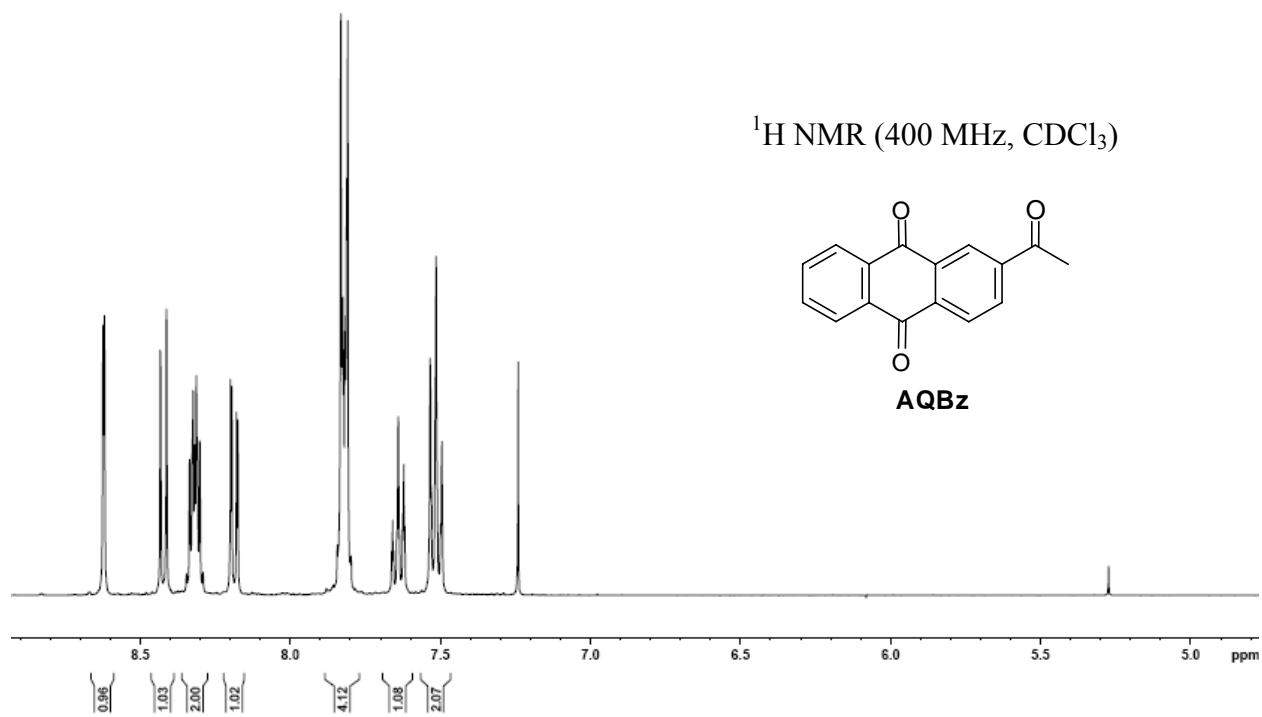


^1H NMR (400 MHz, CDCl_3)

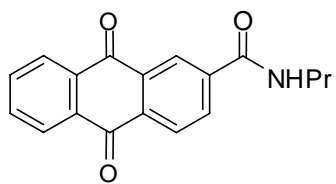


^{13}C NMR (100 MHz, CDCl_3)

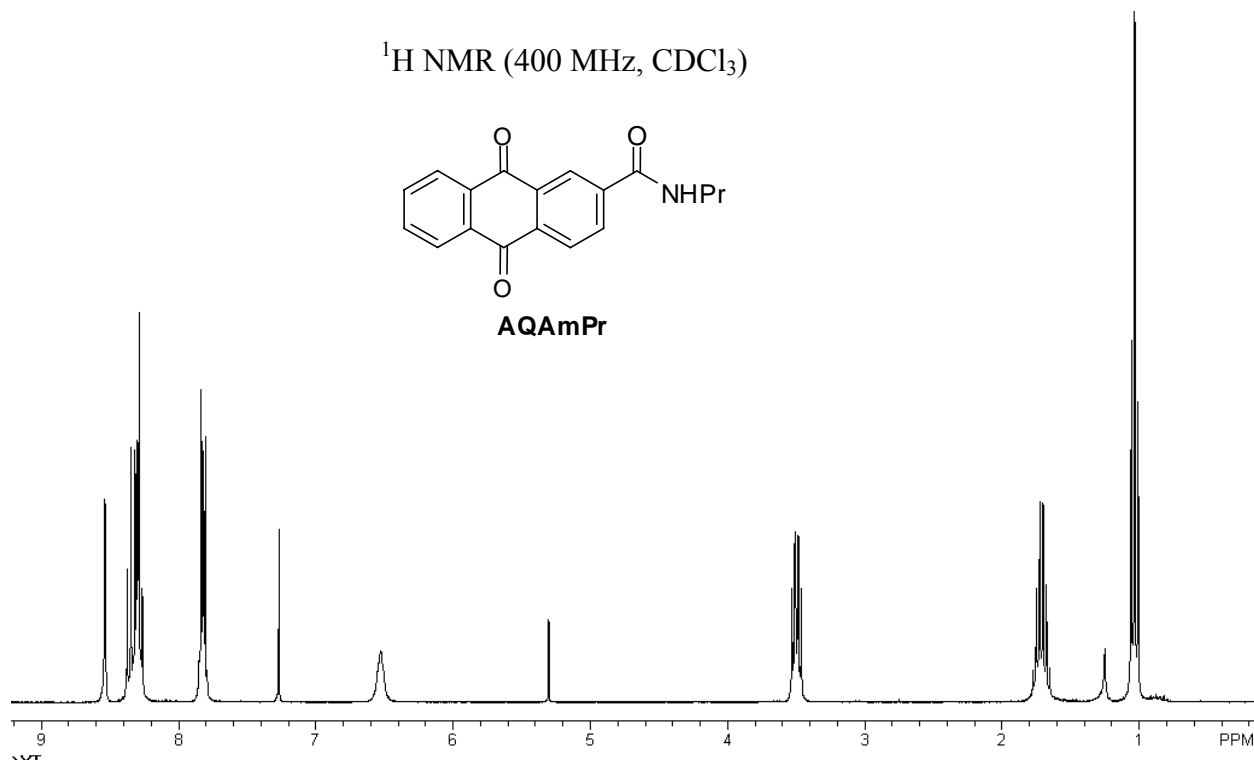




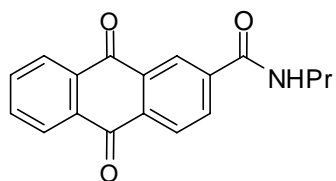
^1H NMR (400 MHz, CDCl_3)



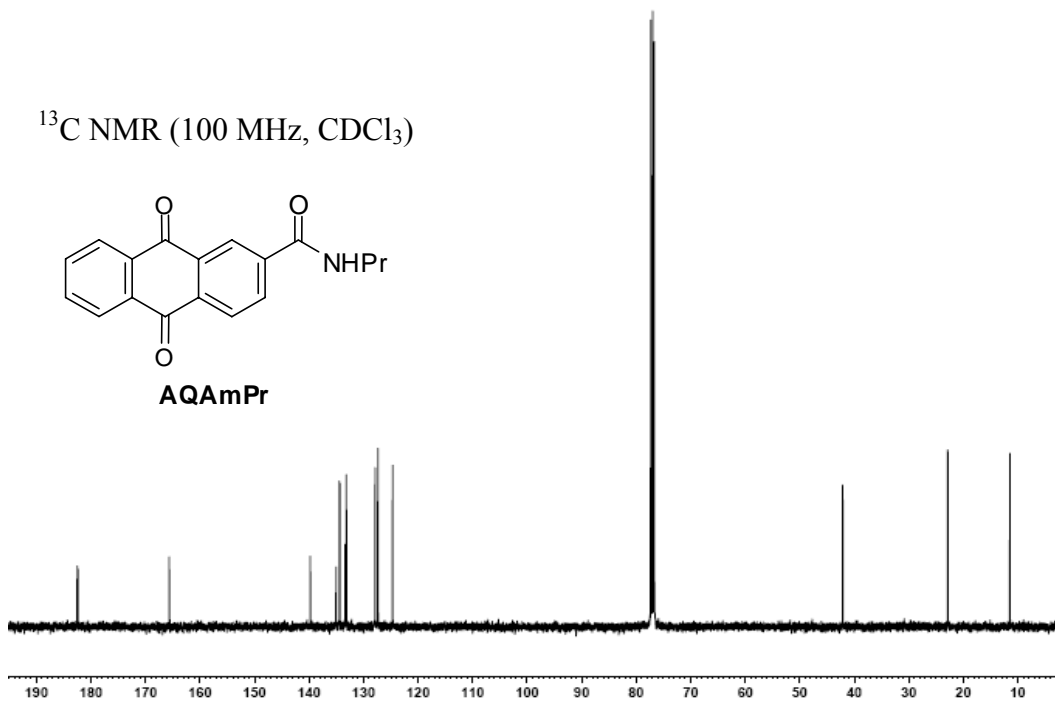
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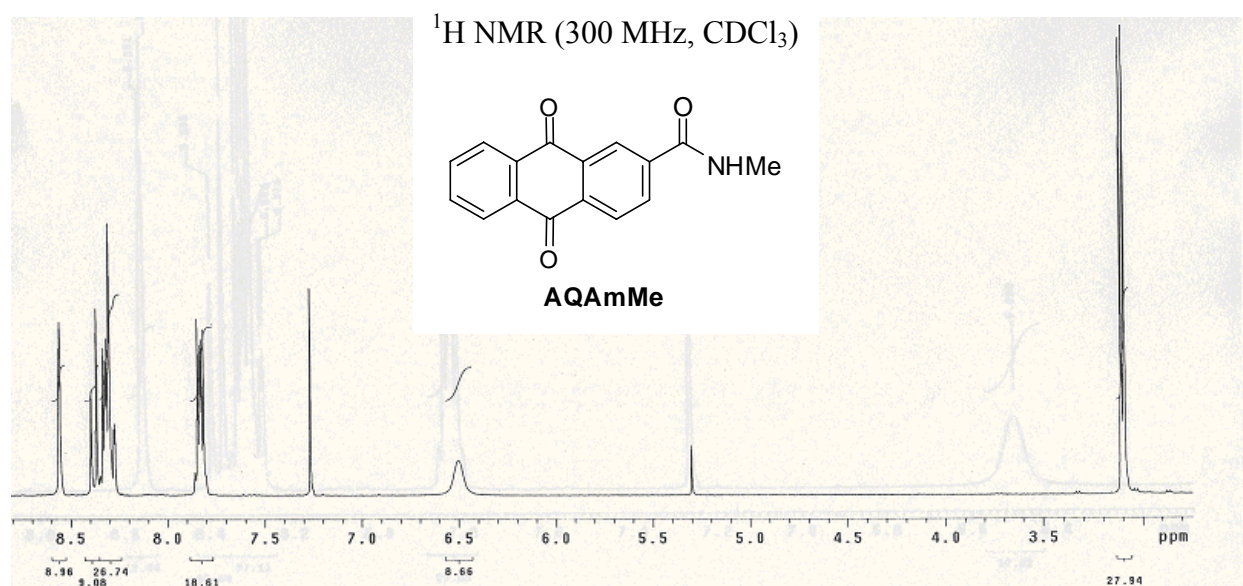


^{13}C NMR (100 MHz, CDCl_3)

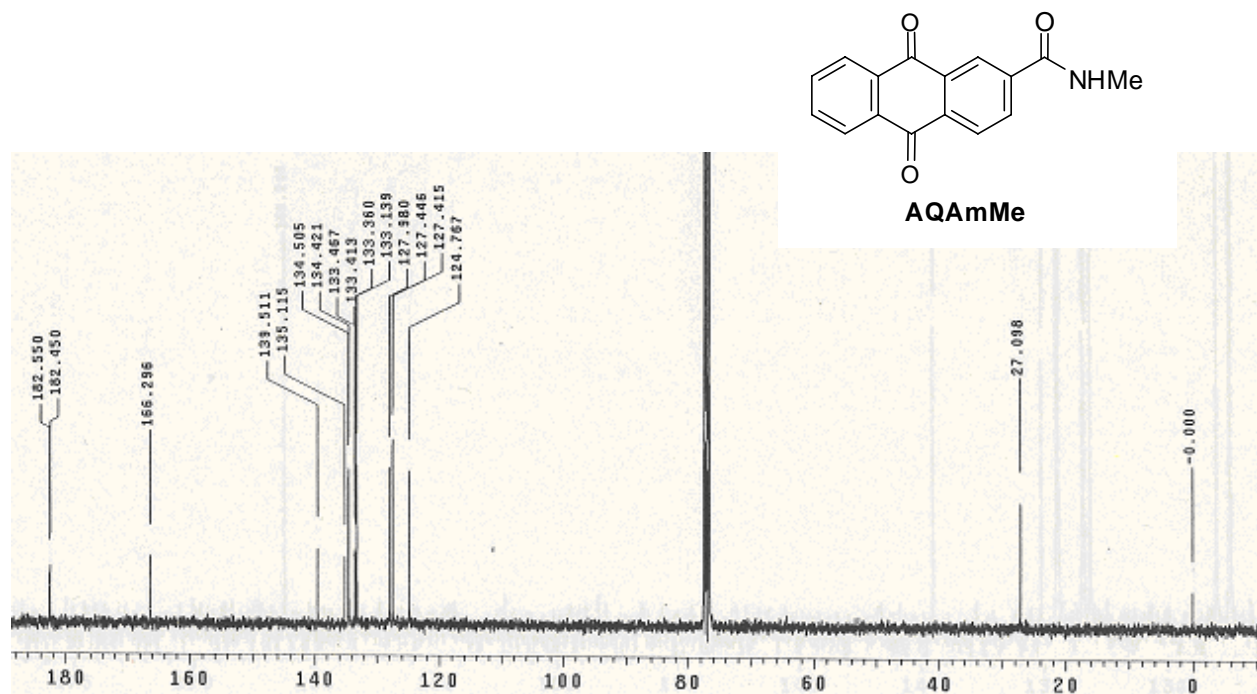


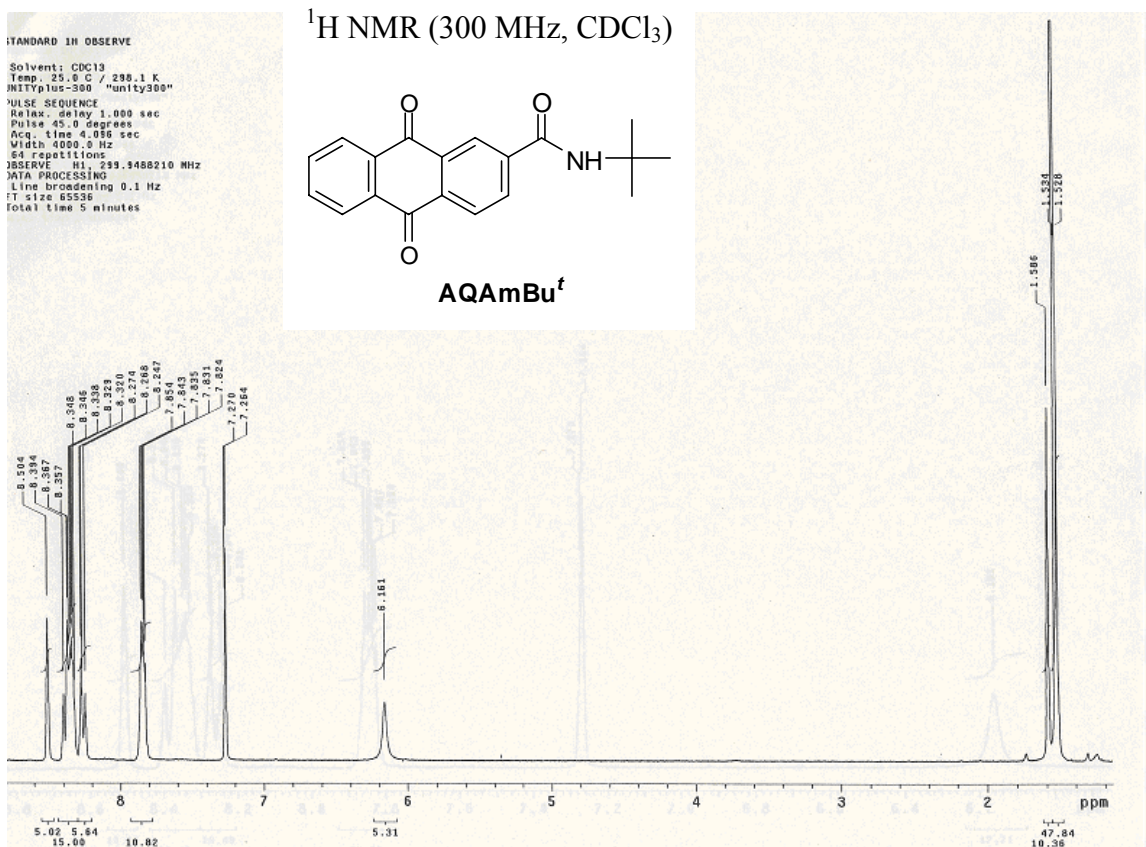
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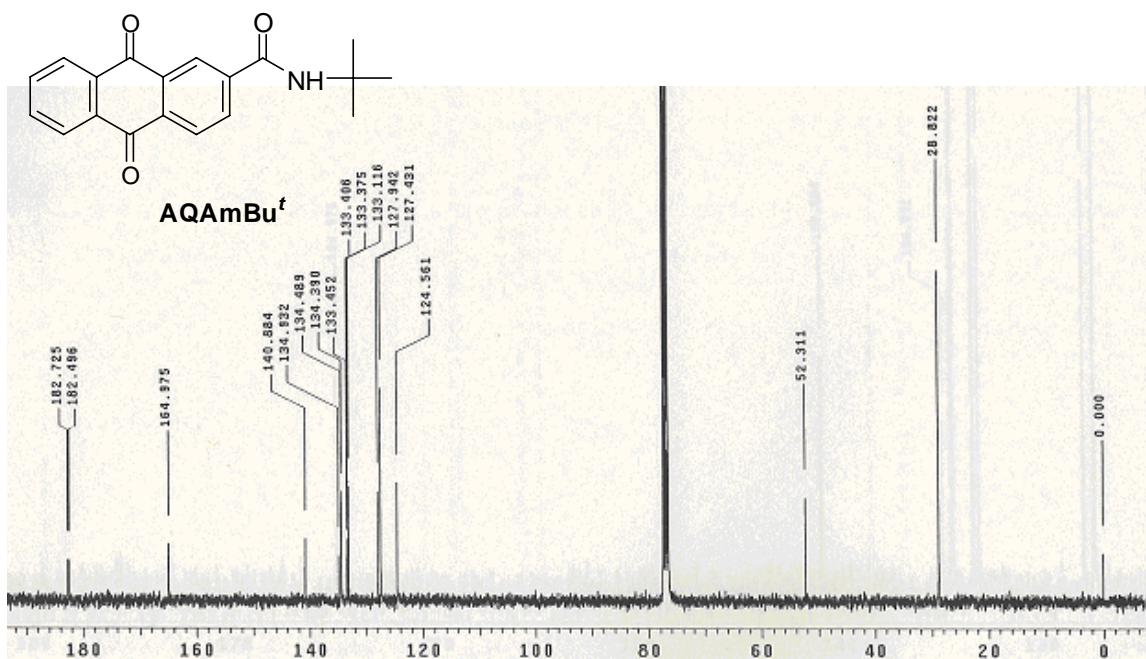


^{13}C NMR (75 MHz, CDCl_3)

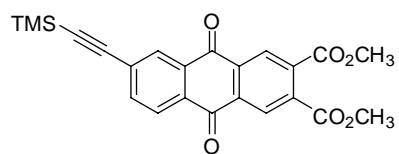




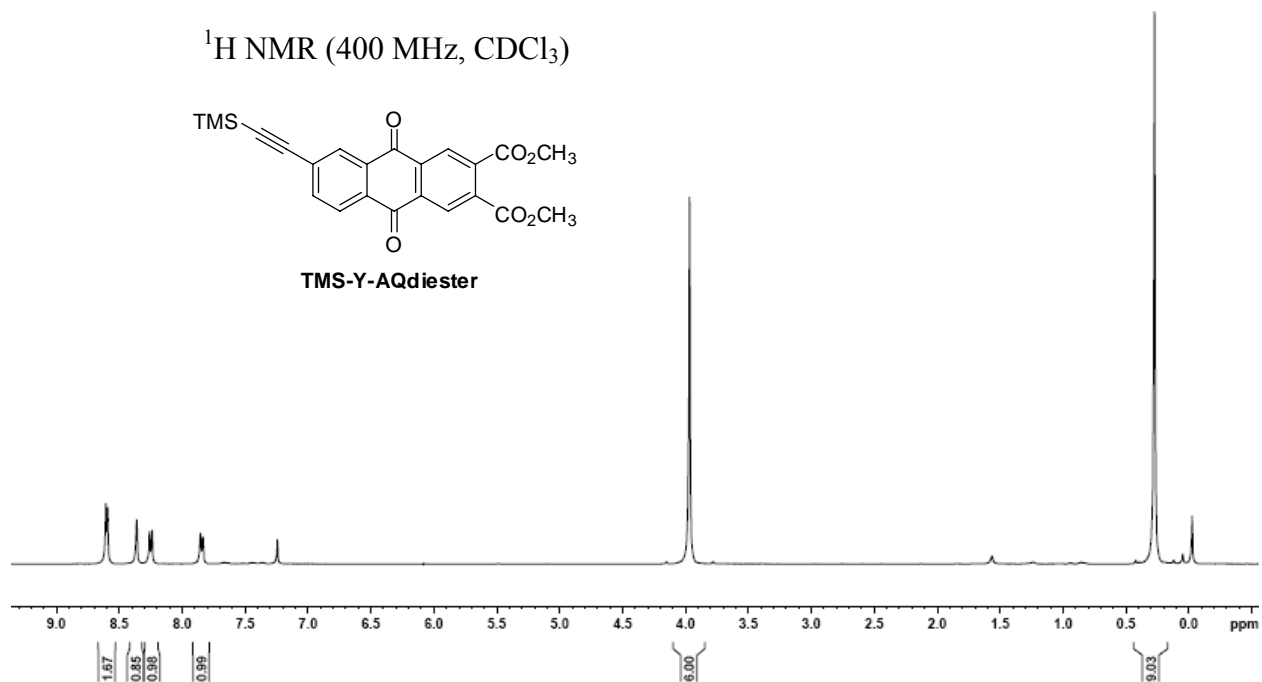
¹³C NMR (75 MHz, CDCl₃)



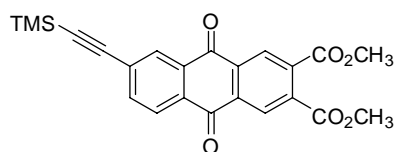
^1H NMR (400 MHz, CDCl_3)



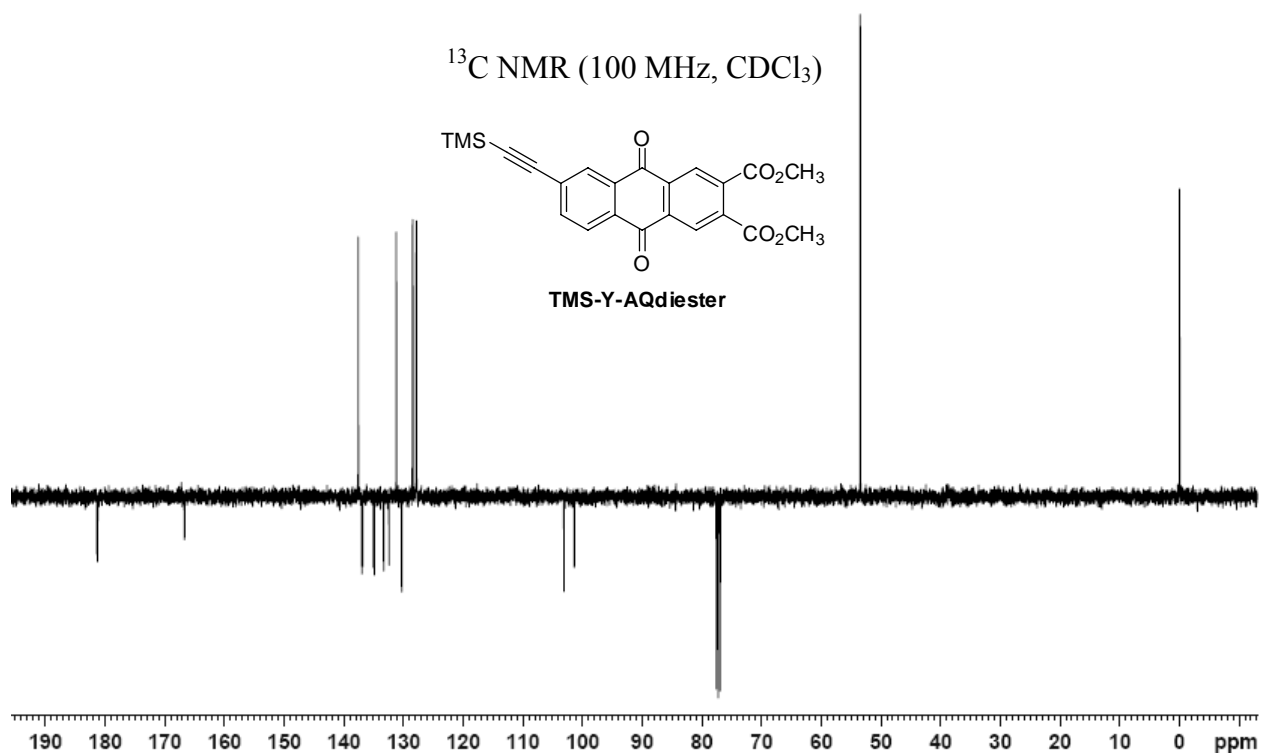
TMS-Y-AQdiester



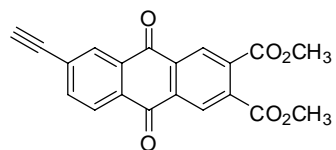
^{13}C NMR (100 MHz, CDCl_3)



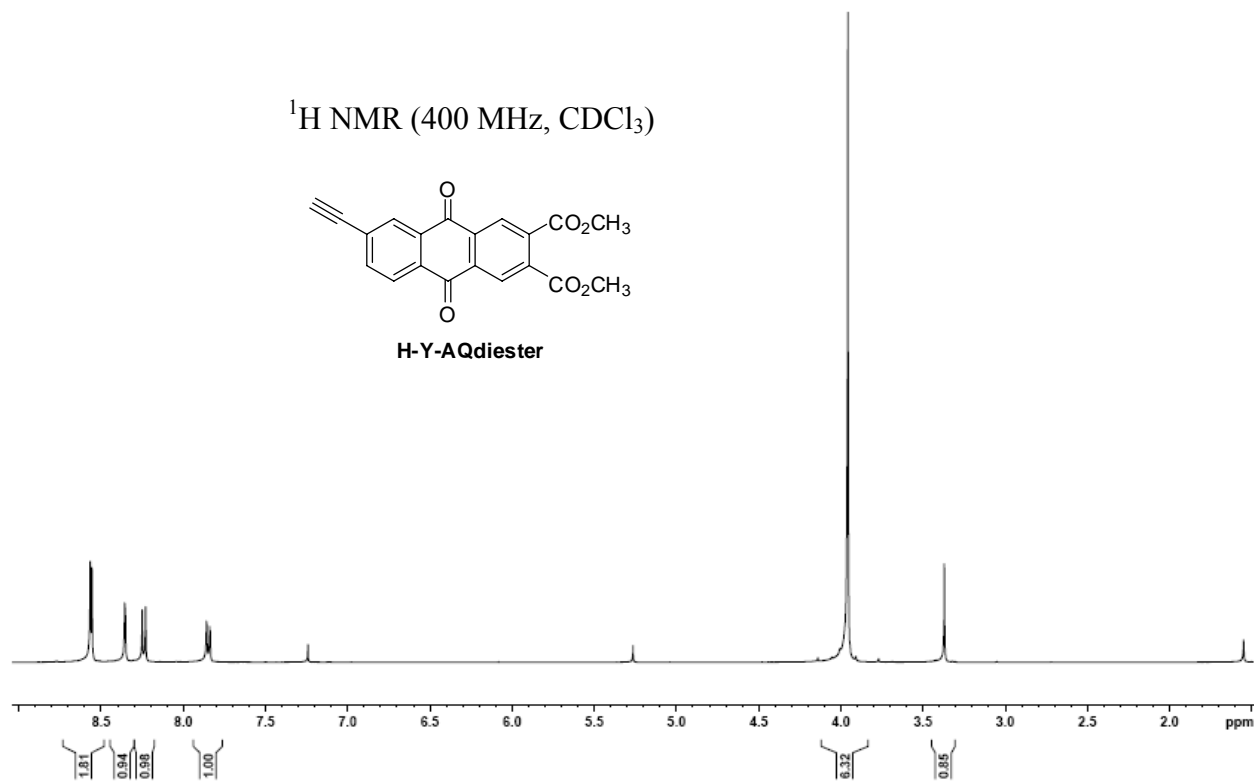
TMS-Y-AQdiester



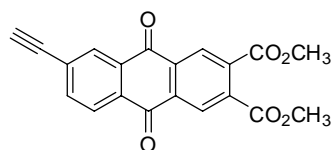
^1H NMR (400 MHz, CDCl_3)



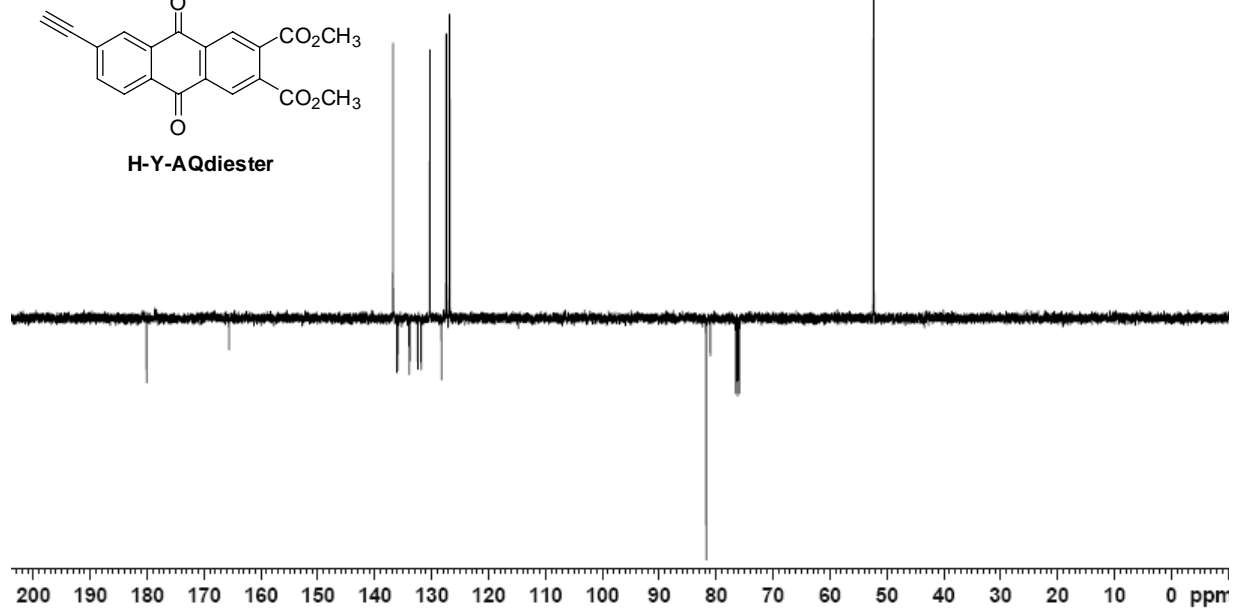
H-Y-AQdiester

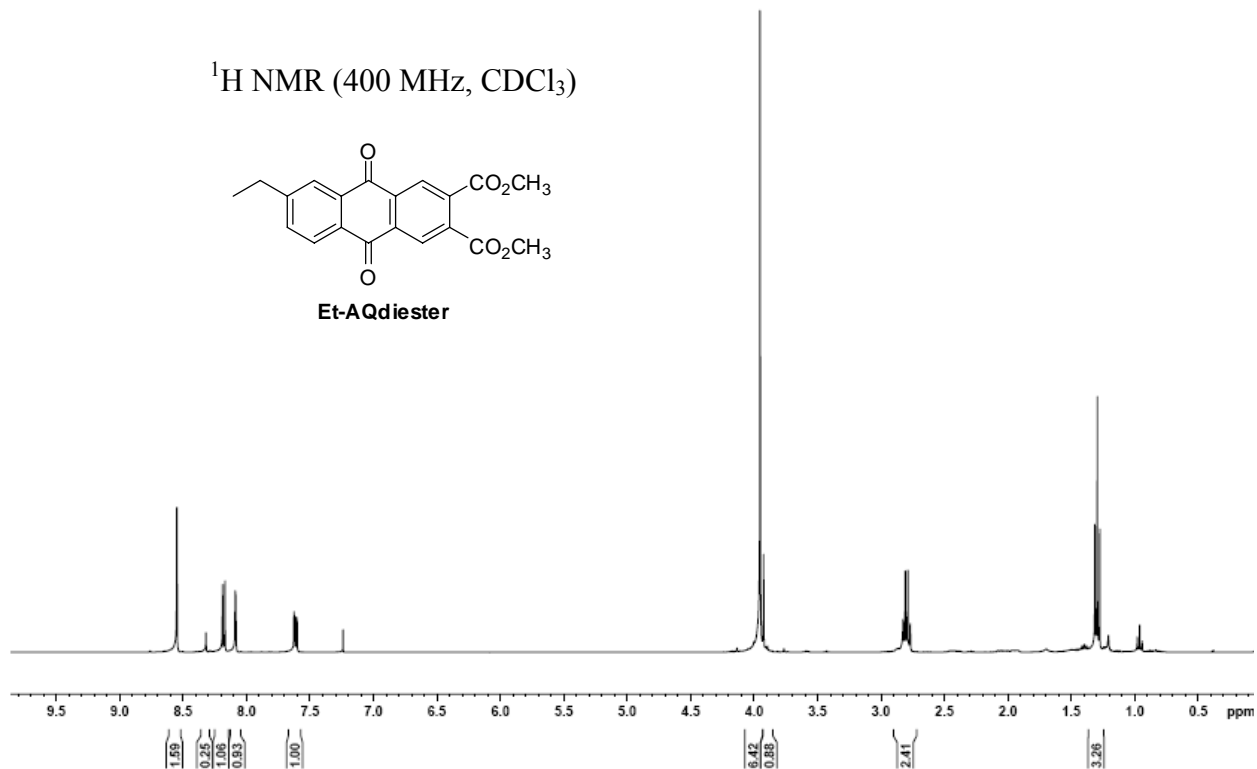
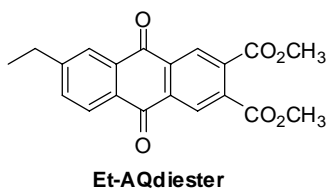
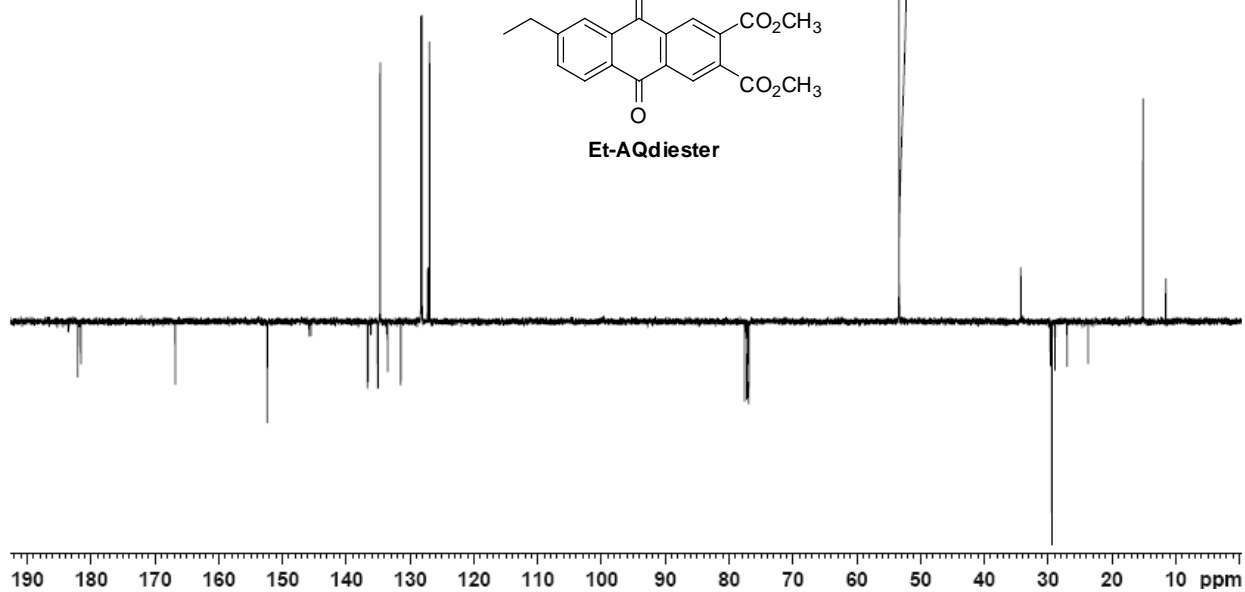
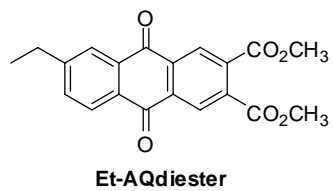


^{13}C NMR (100 MHz, CDCl_3)

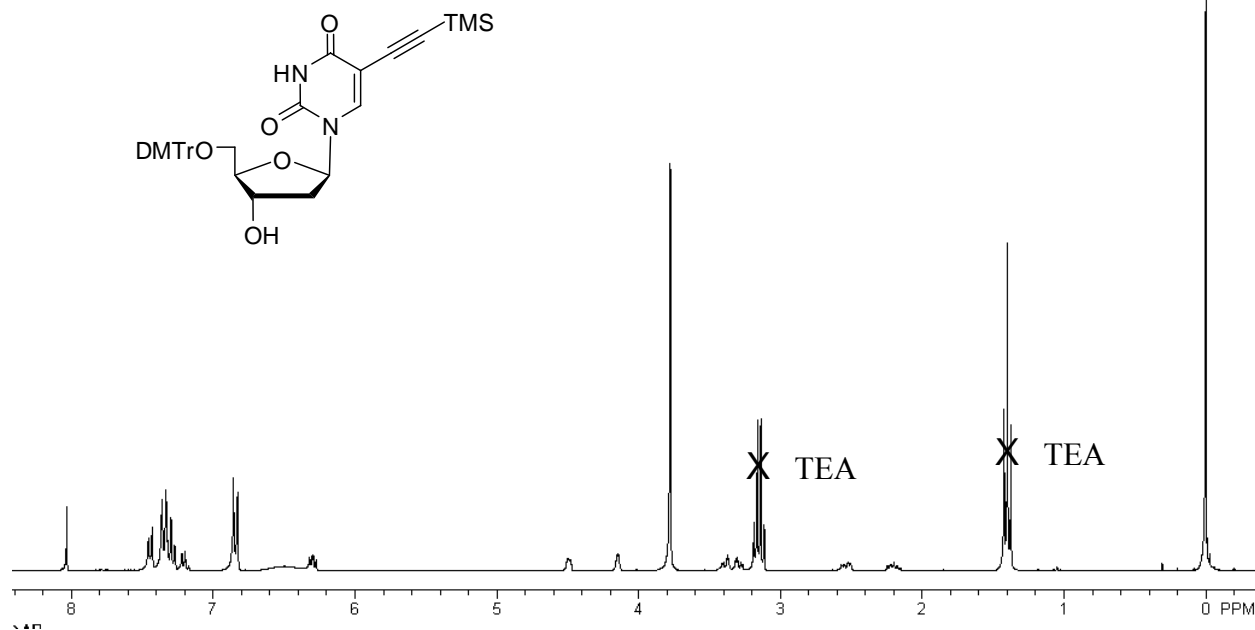


H-Y-AQdiester

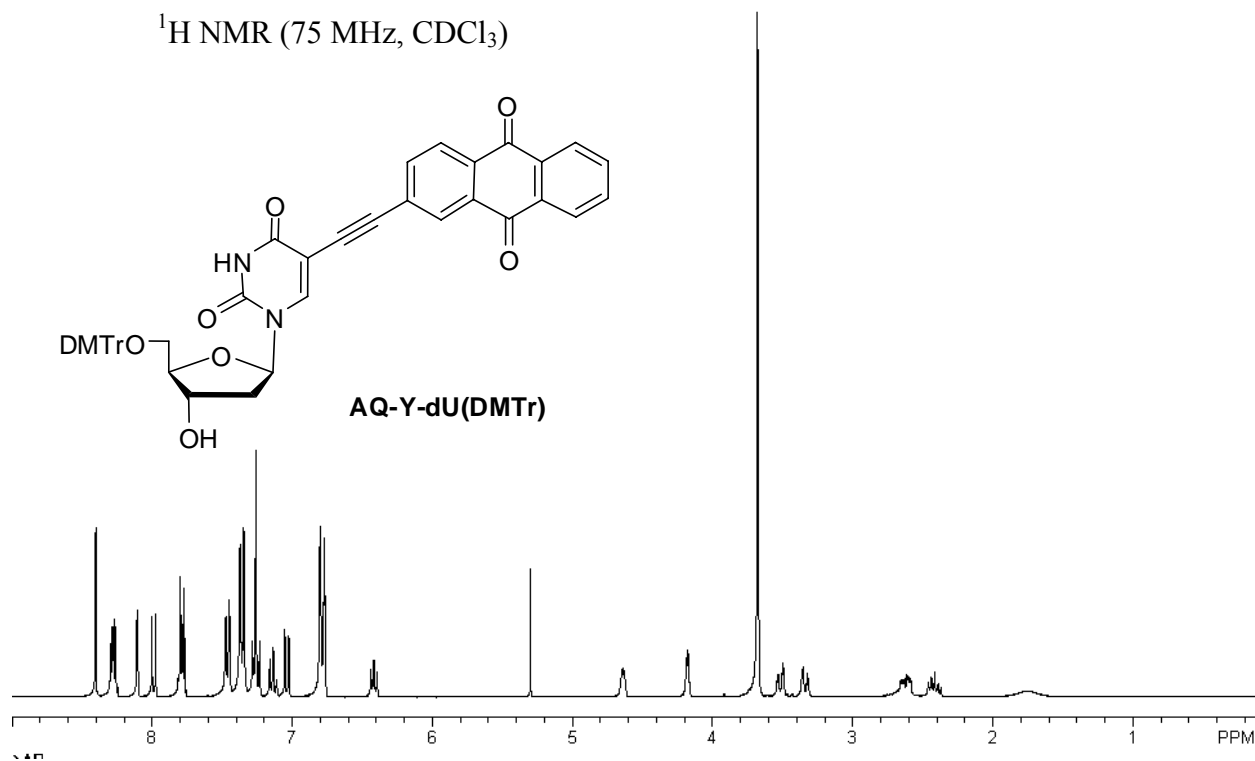


^1H NMR (400 MHz, CDCl_3) ^{13}C NMR (100 MHz, CDCl_3)

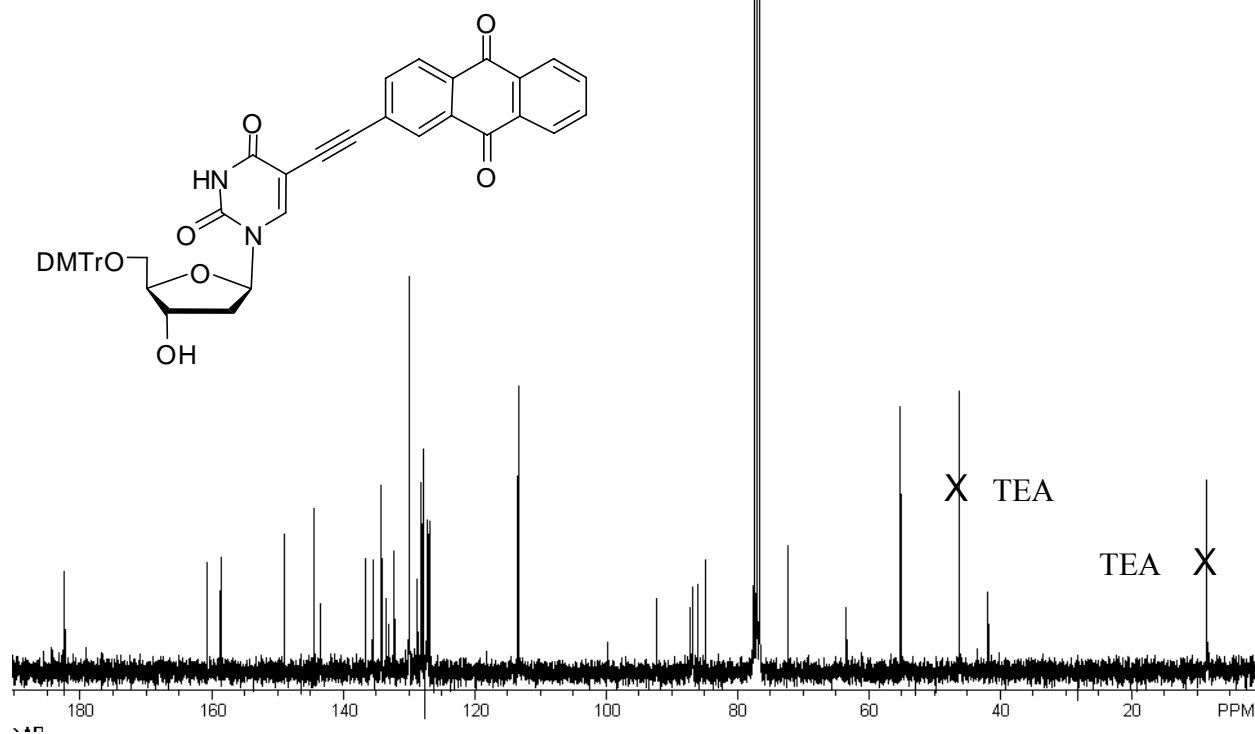
^1H NMR (300 MHz, CDCl_3)



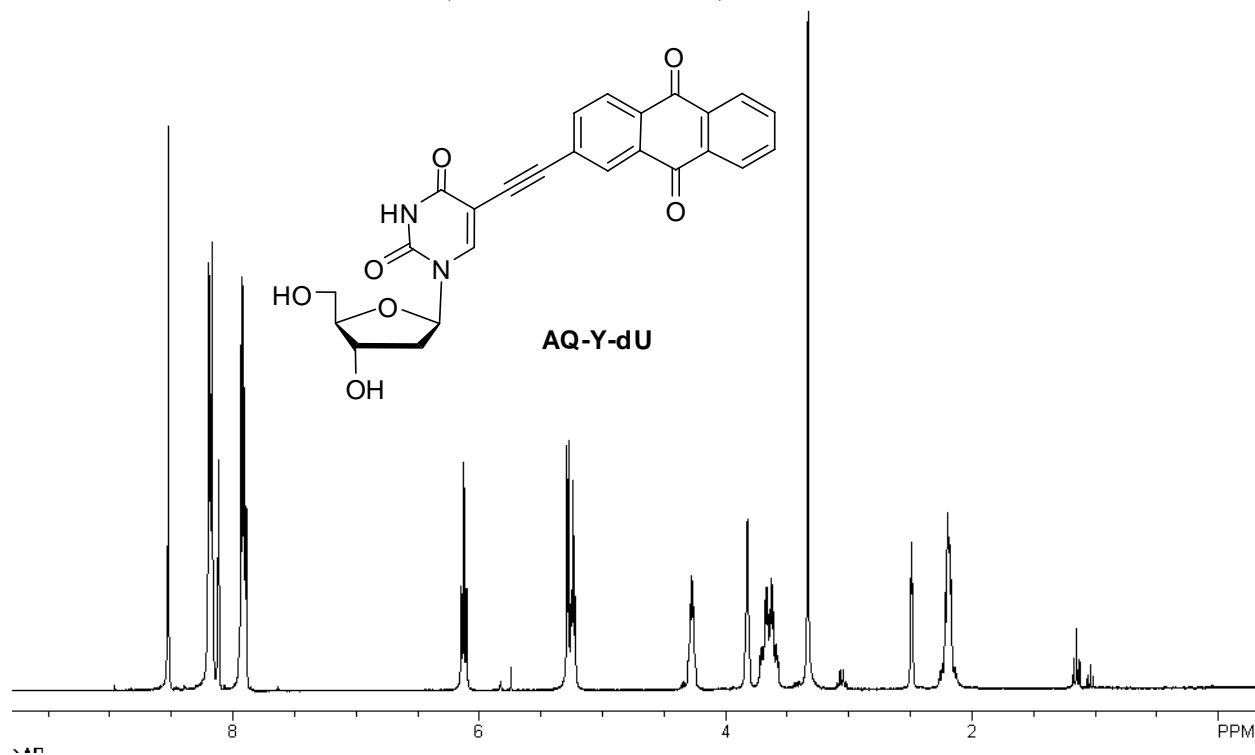
^1H NMR (75 MHz, CDCl_3)

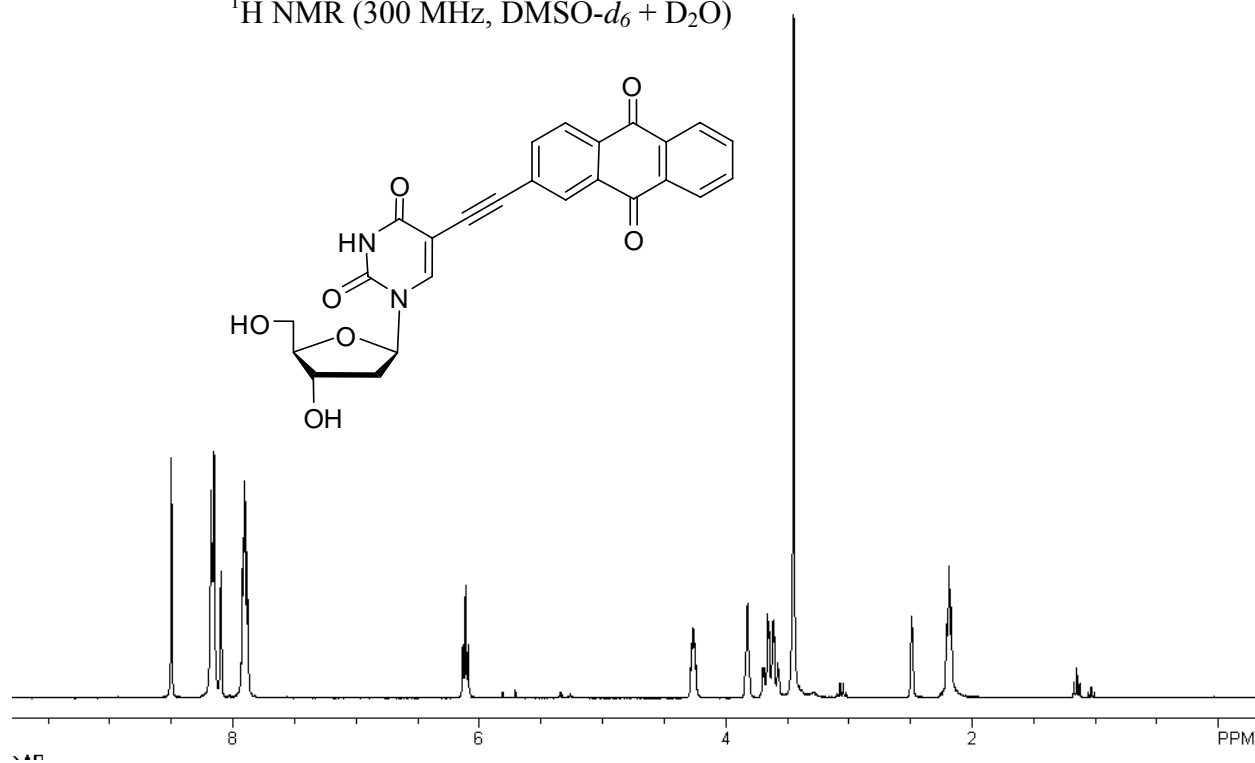
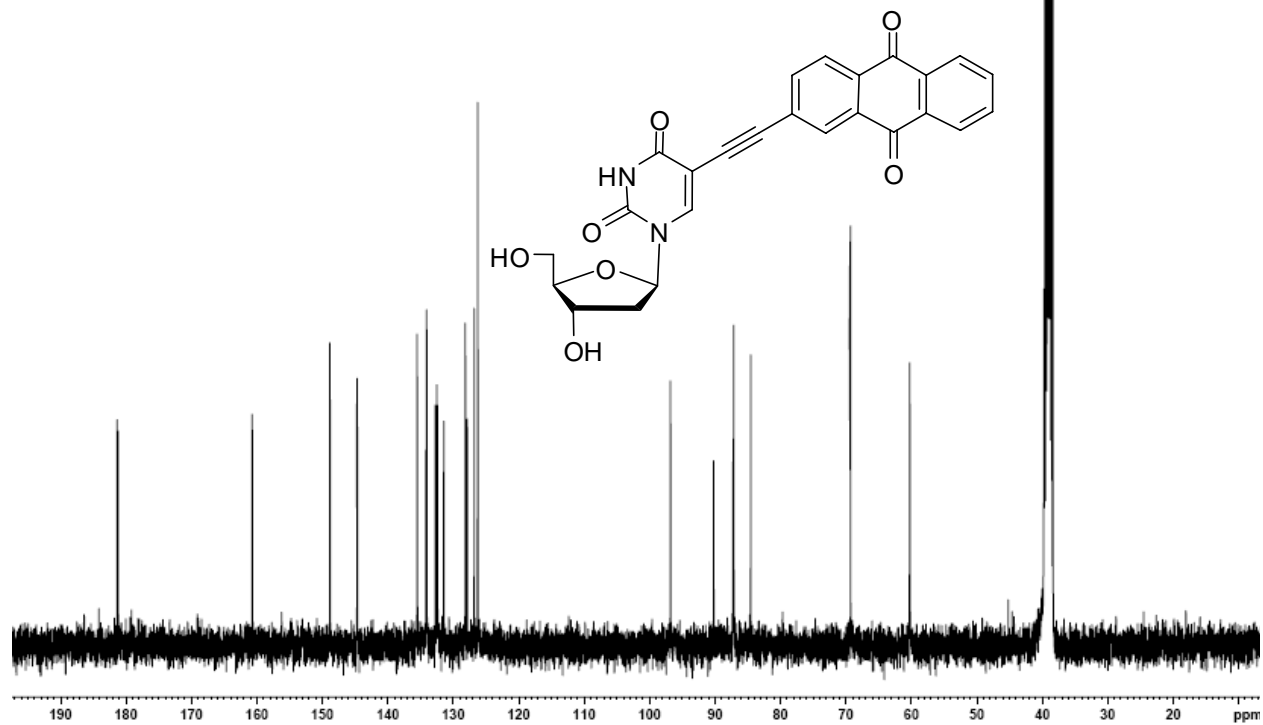


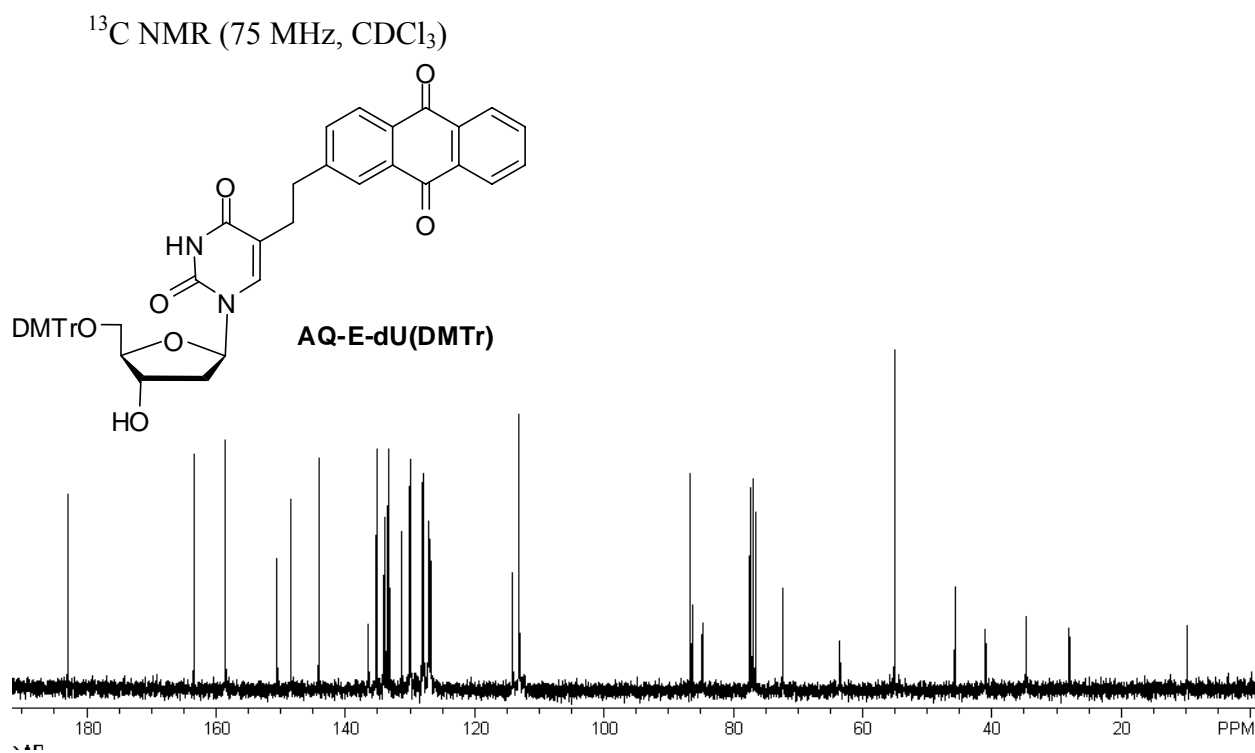
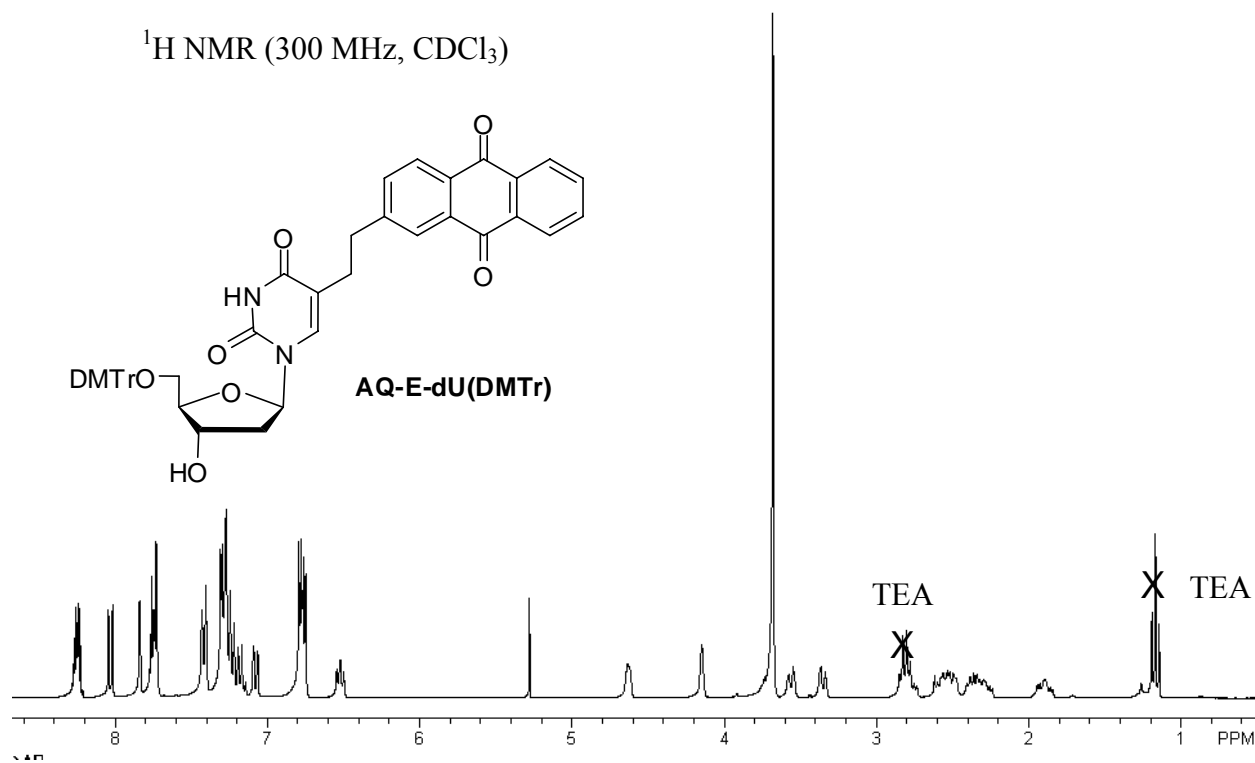
^{13}C NMR (75 MHz, CDCl_3)

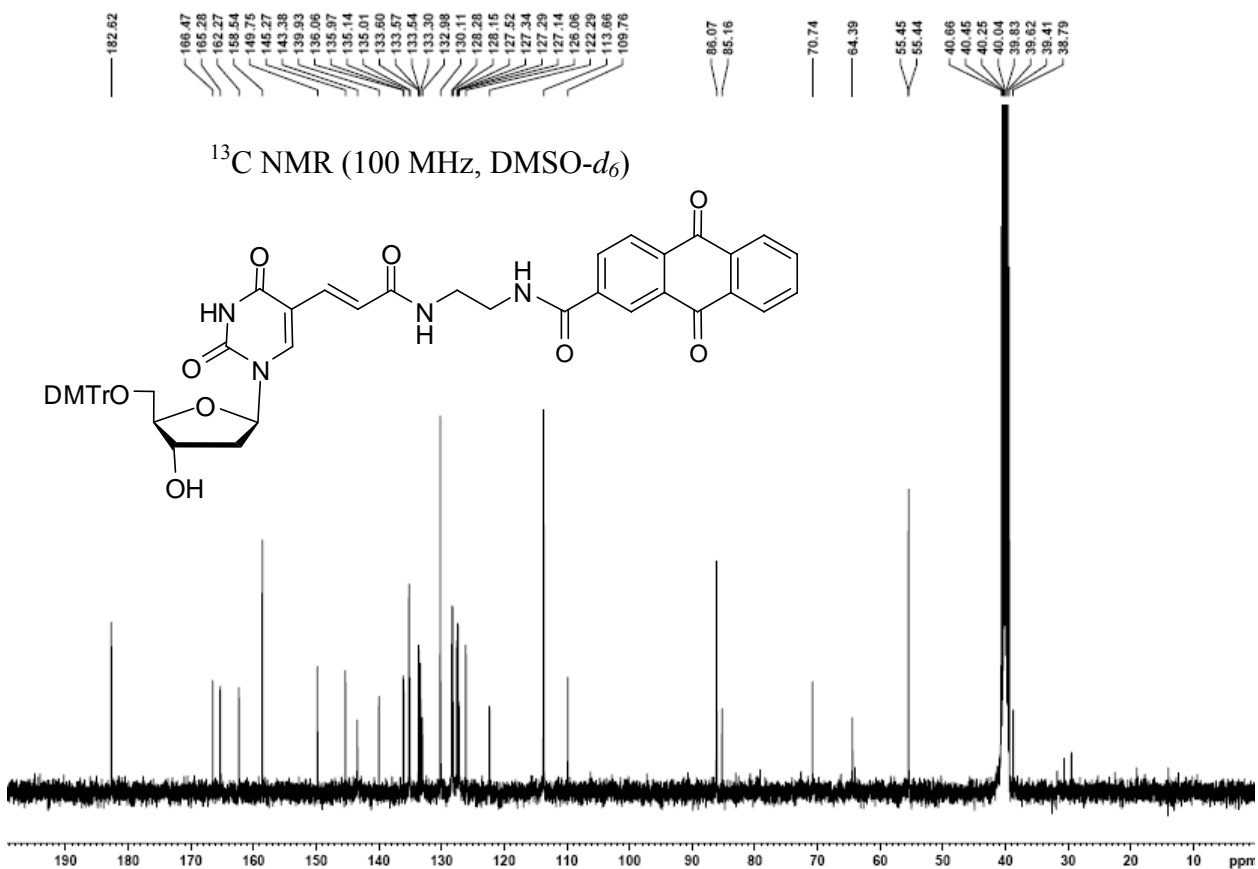
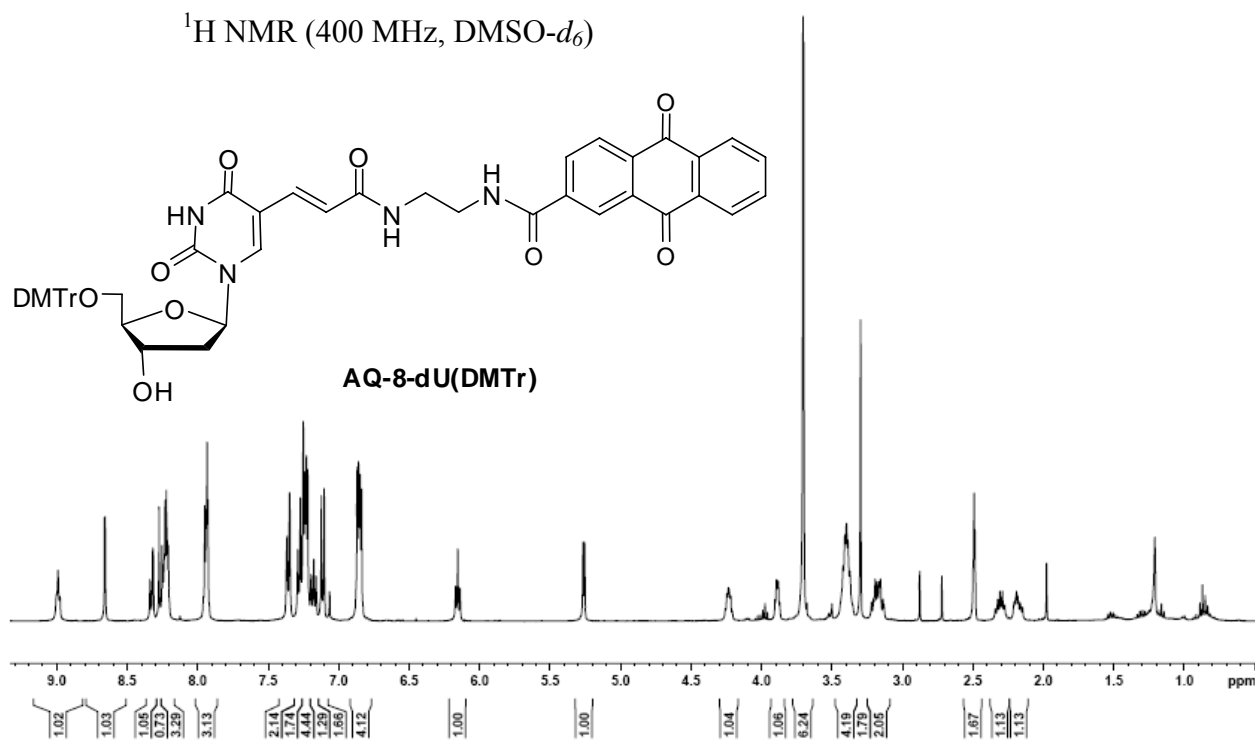


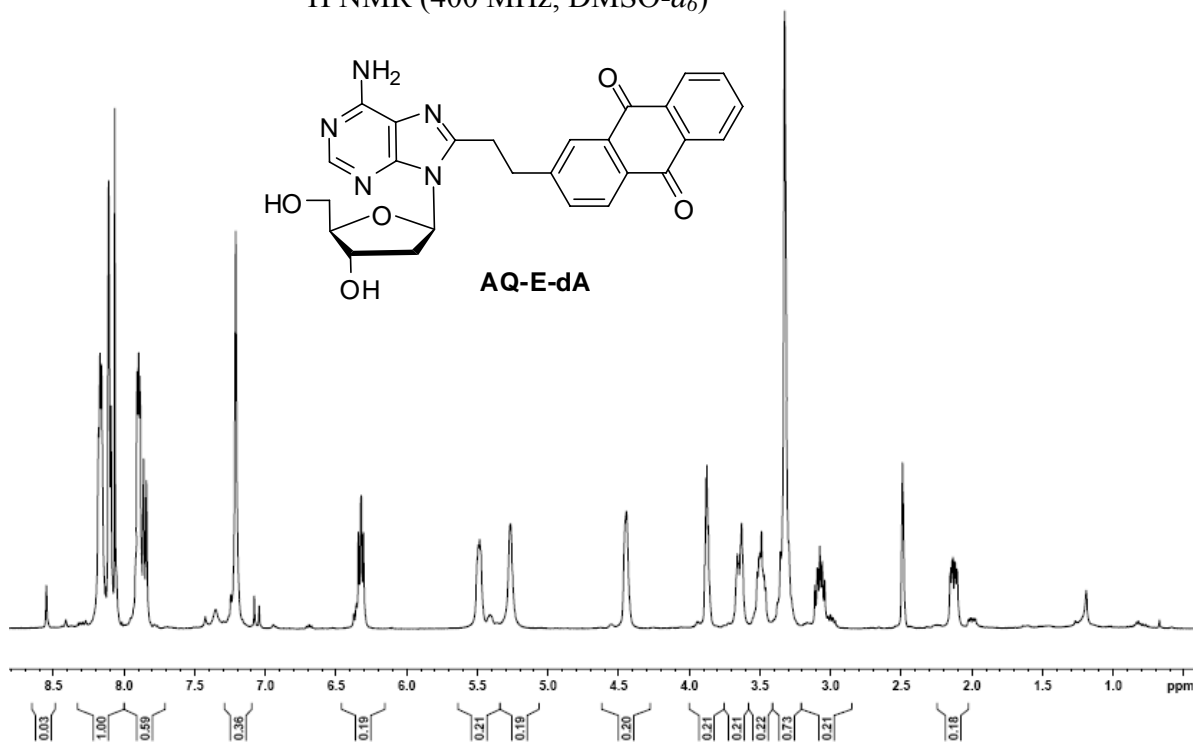
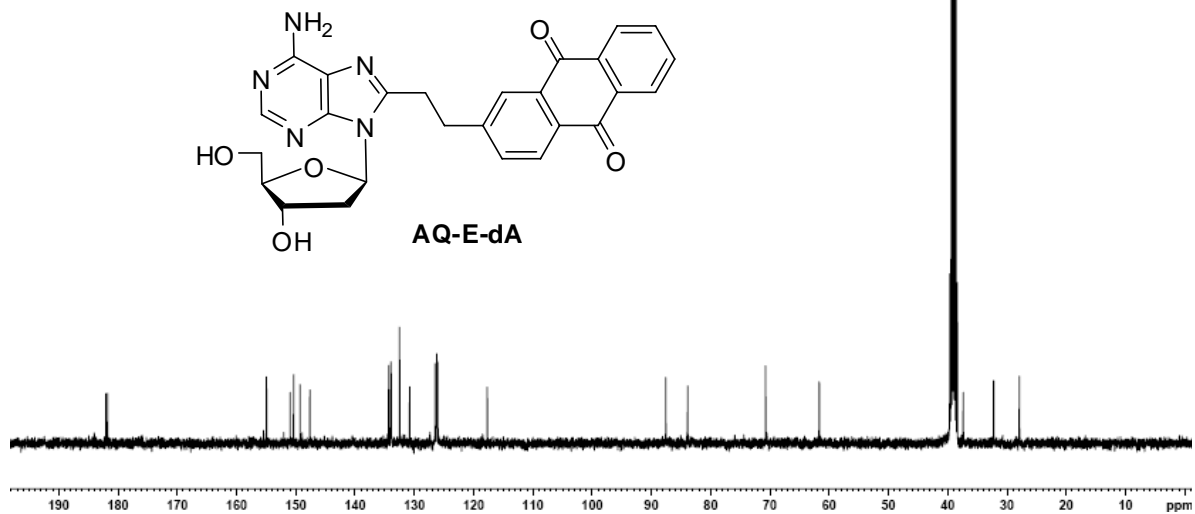
^1H NMR (300 MHz, $\text{DMSO}-d_6$)

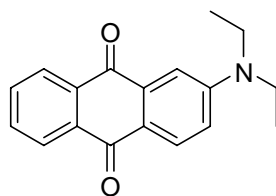
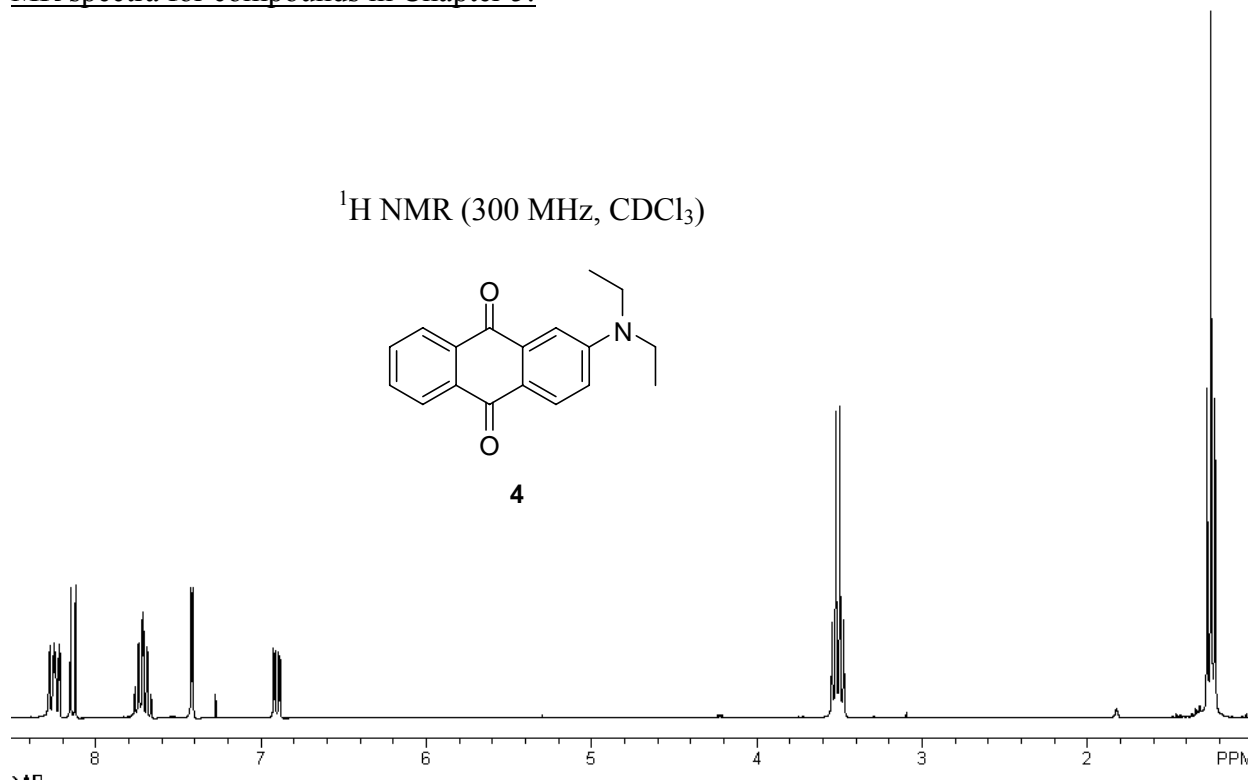
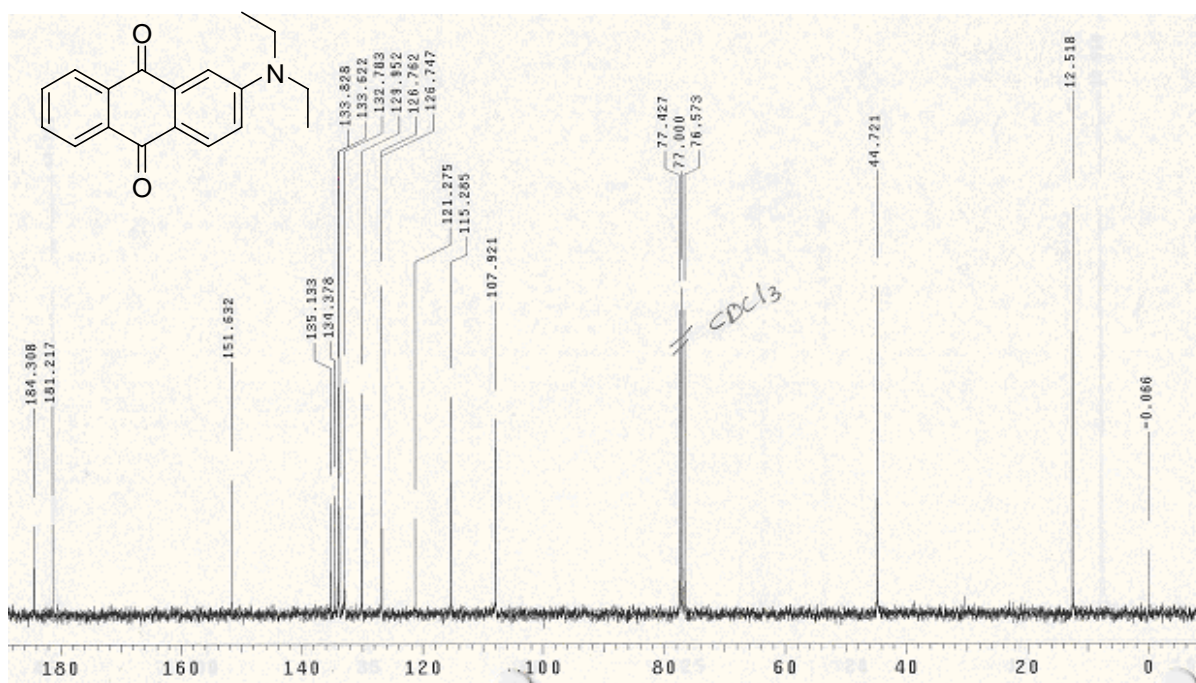


^1H NMR (300 MHz, $\text{DMSO-}d_6 + \text{D}_2\text{O}$) ^{13}C NMR (100 MHz, $\text{DMSO-}d_6$)

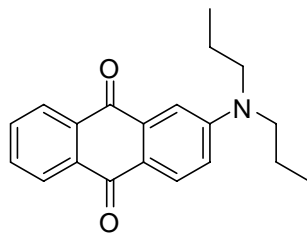




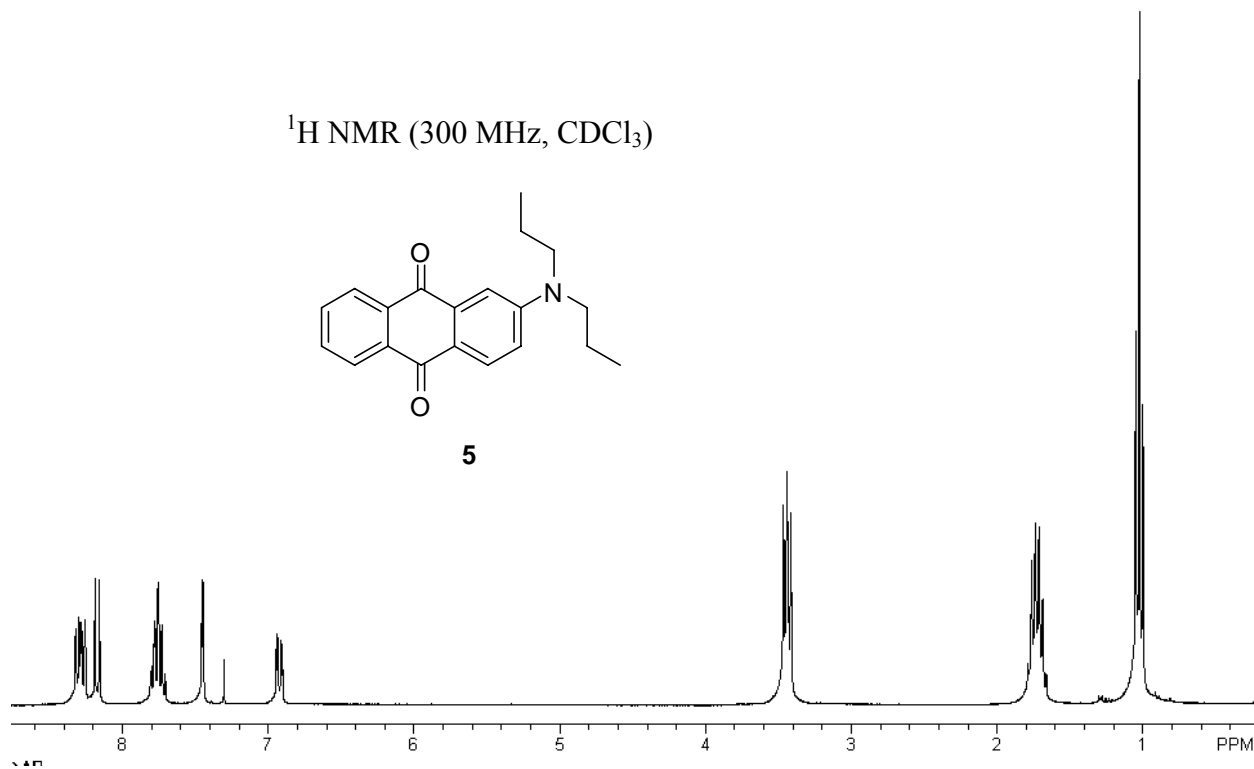
^1H NMR (400 MHz, $\text{DMSO-}d_6$) ^{13}C NMR (100 MHz, $\text{DMSO-}d_6$)

MR spectra for compounds in Chapter 5: ^1H NMR (300 MHz, CDCl_3)**4** ^{13}C NMR (75 MHz, CDCl_3)

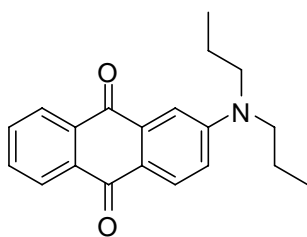
^1H NMR (300 MHz, CDCl_3)



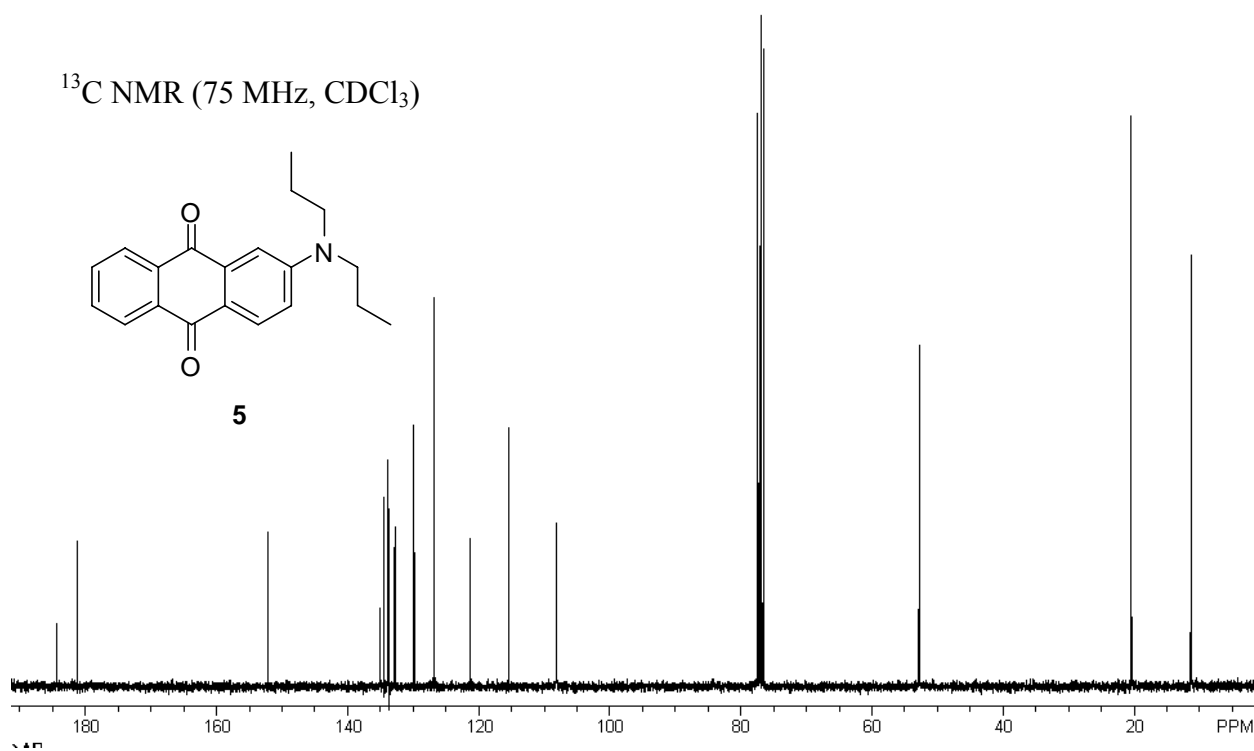
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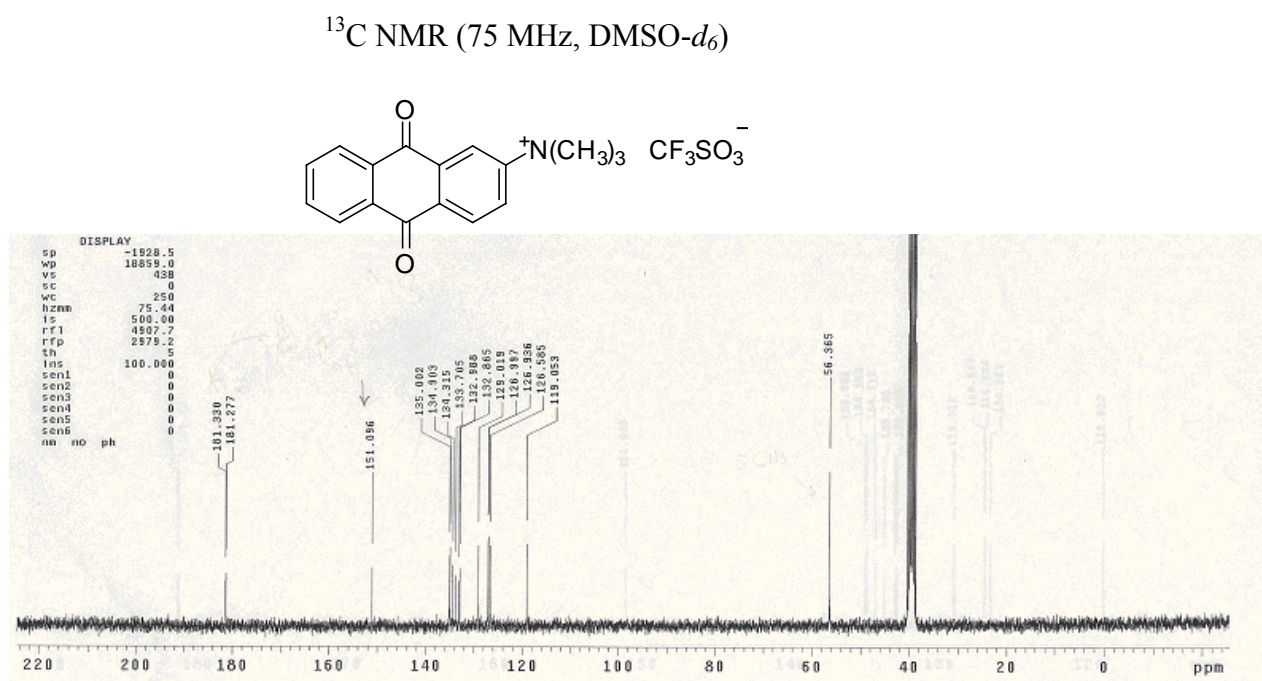
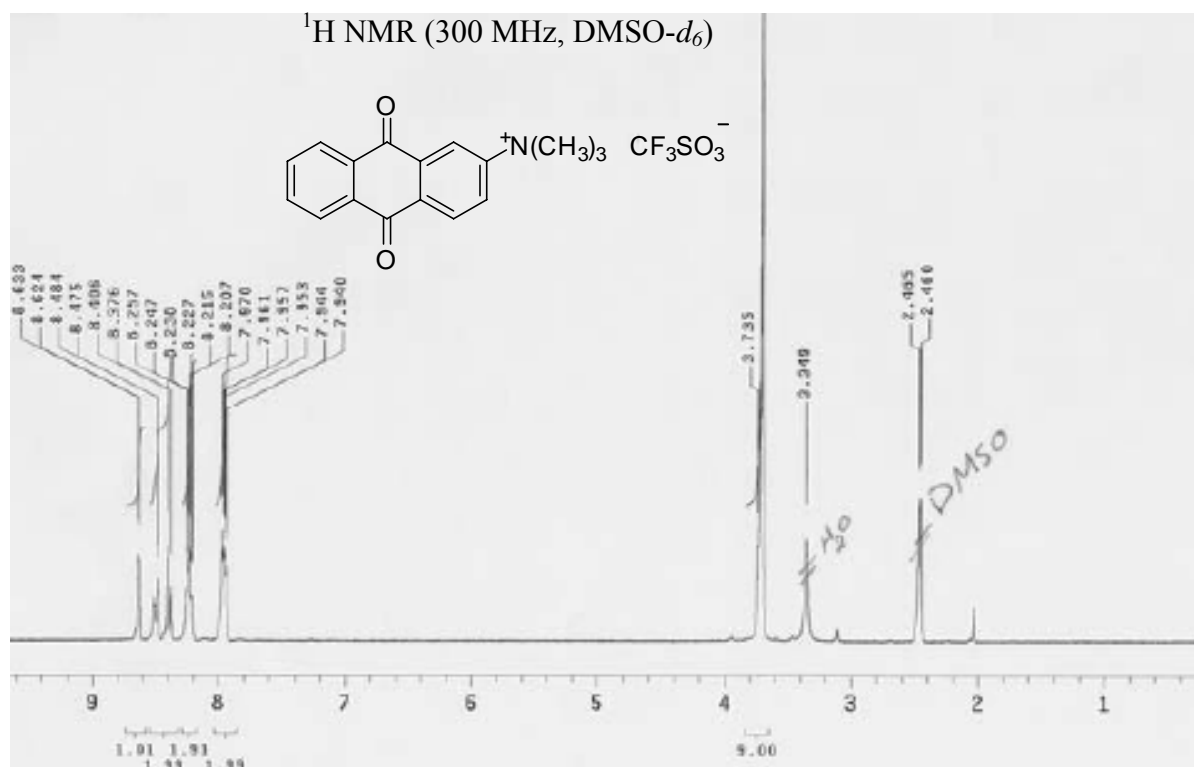


^{13}C NMR (75 MHz, CDCl_3)

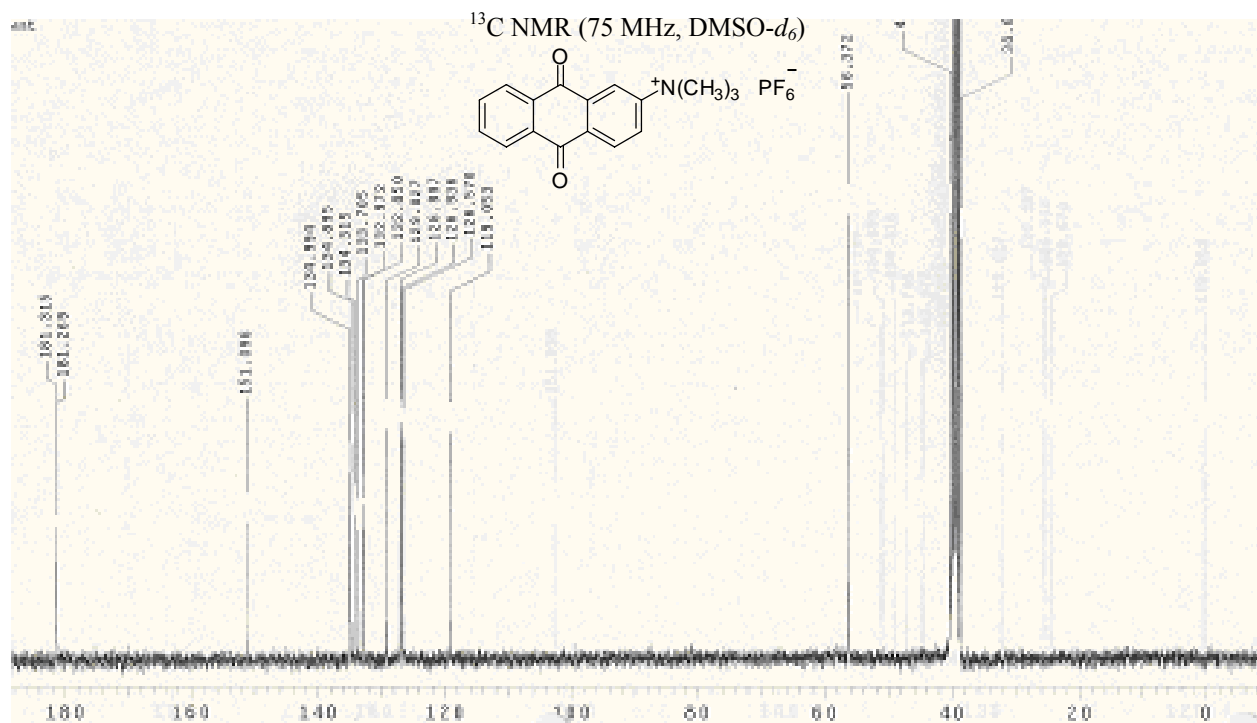
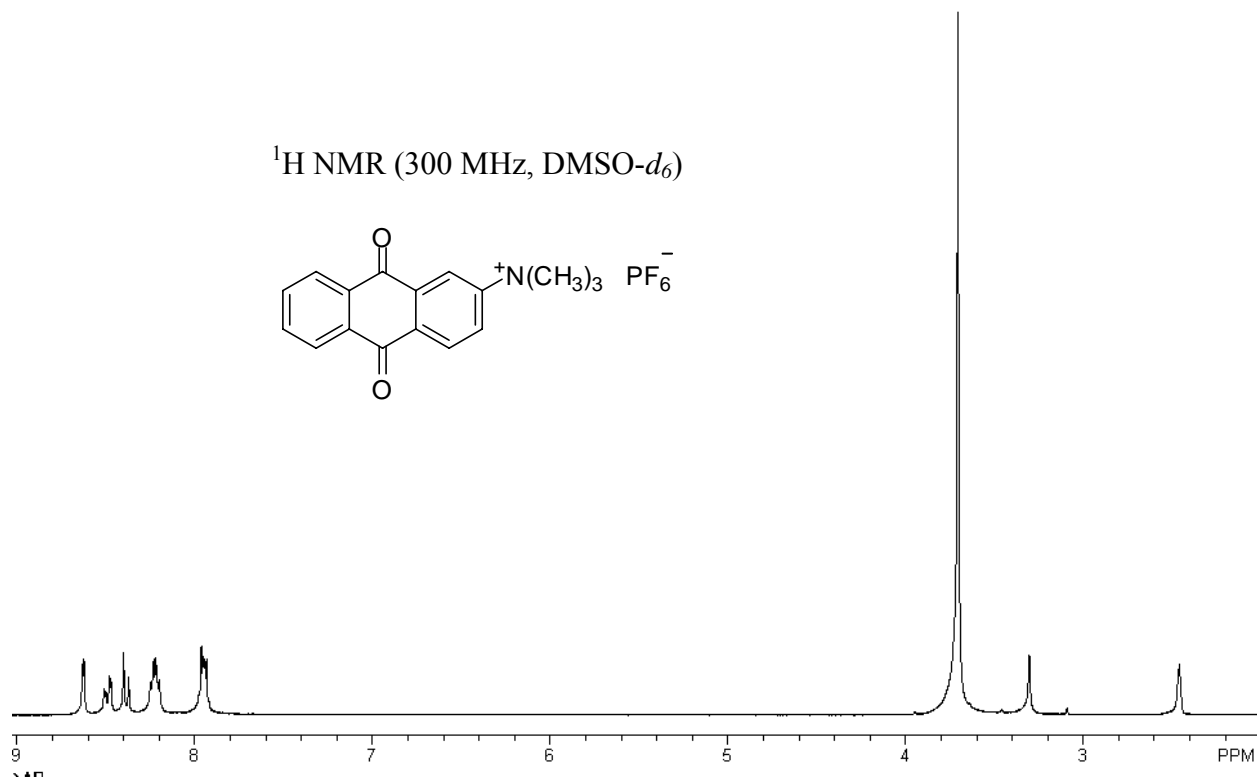
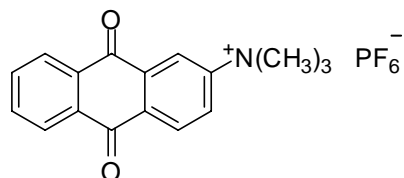


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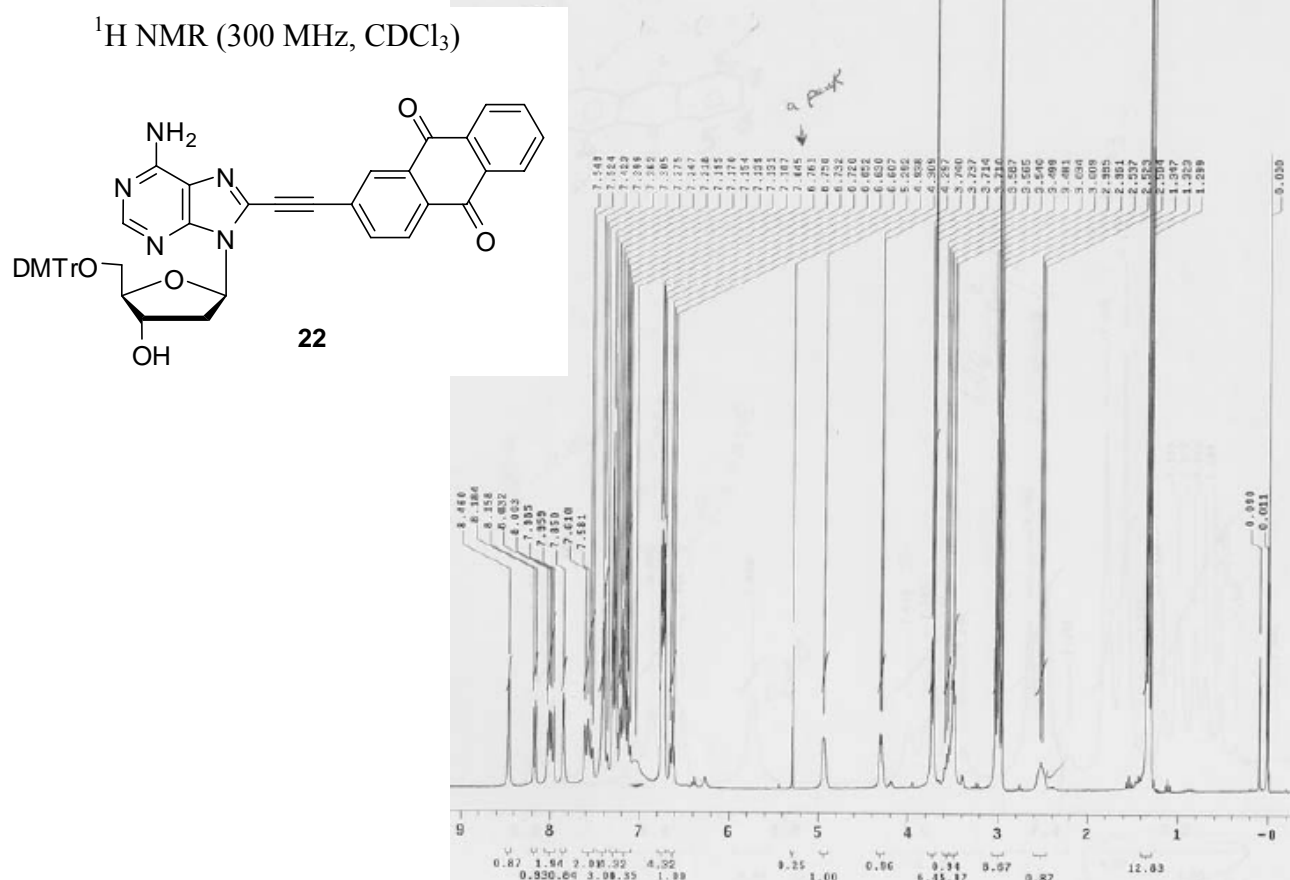
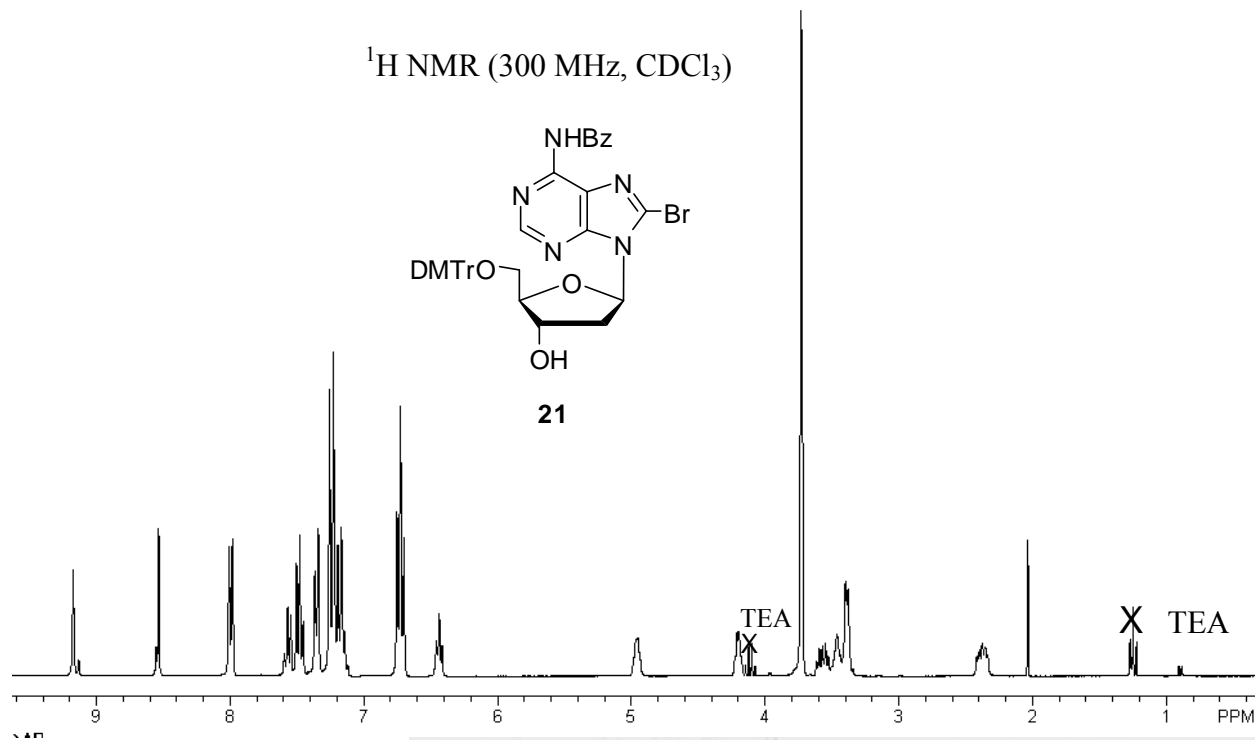


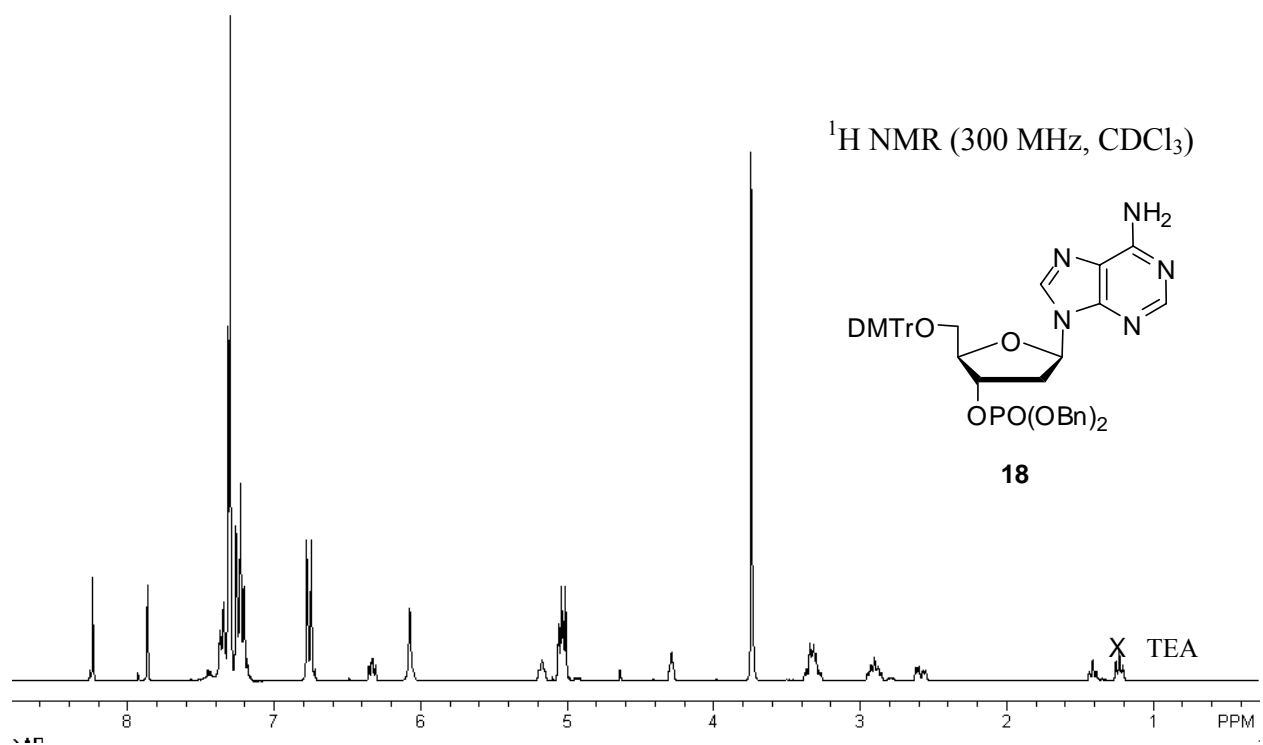
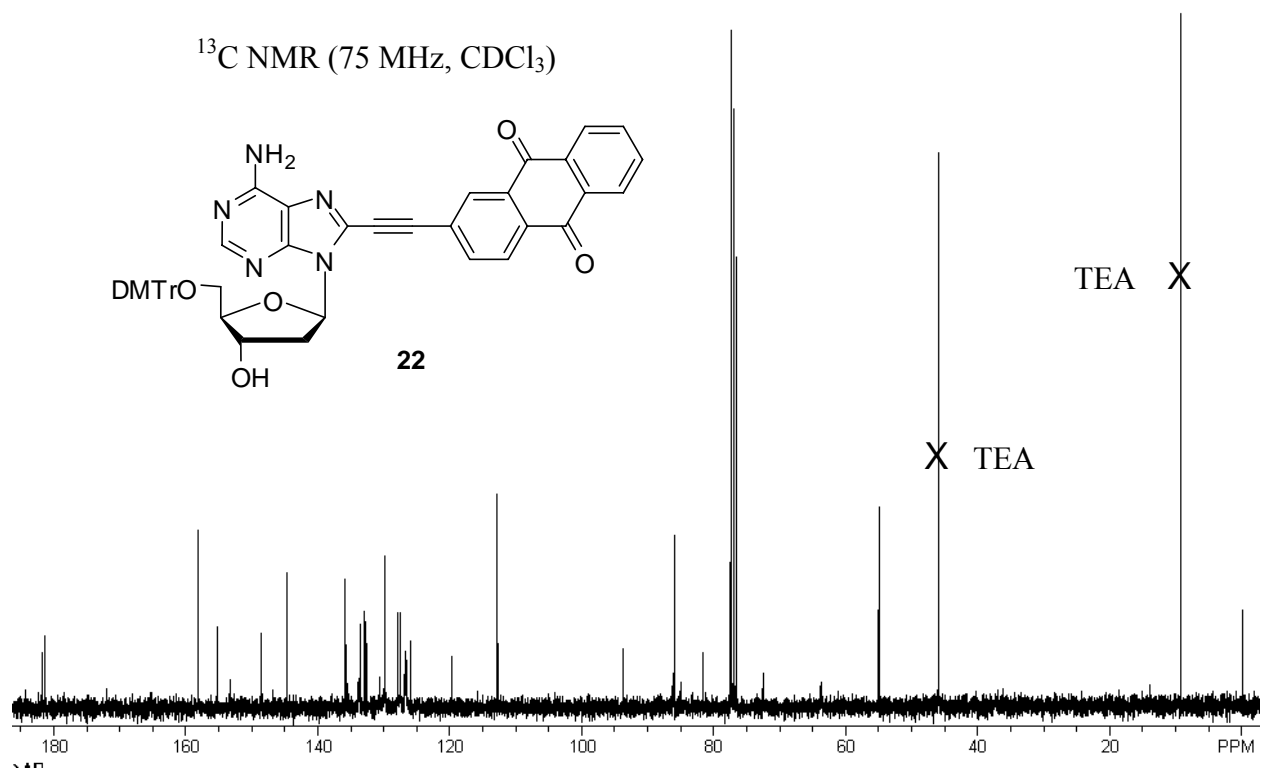


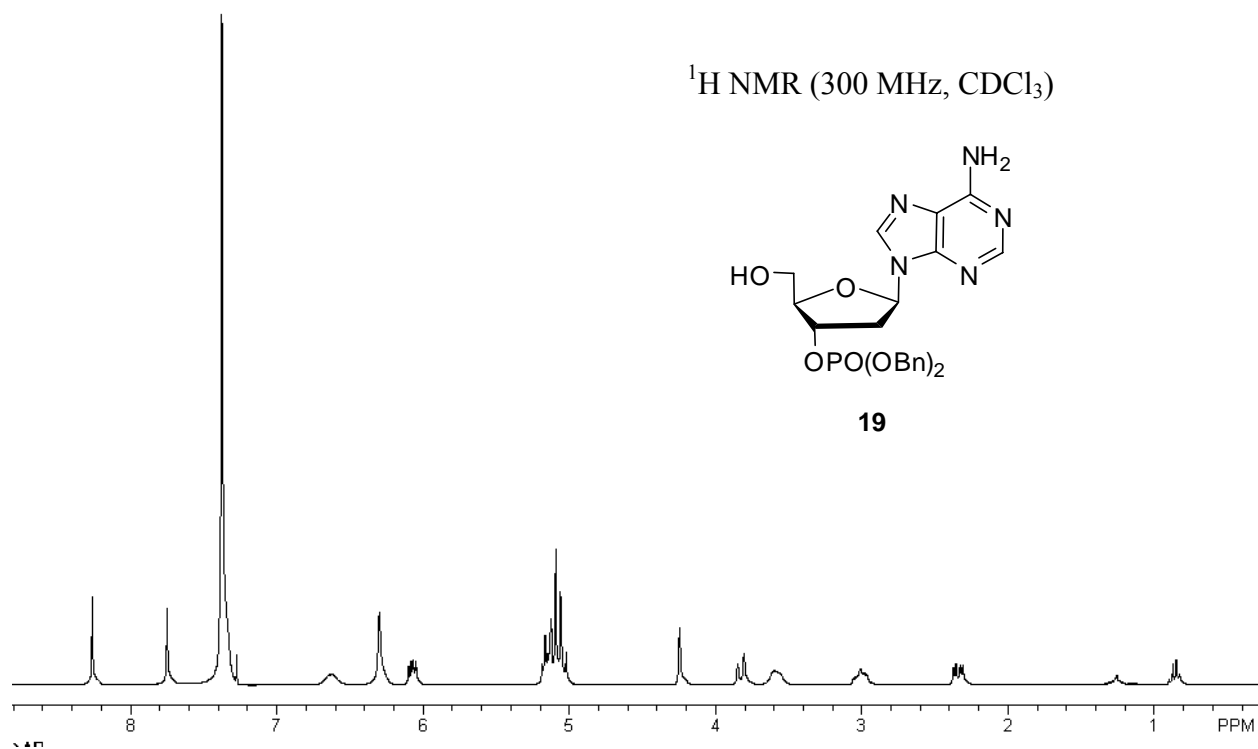
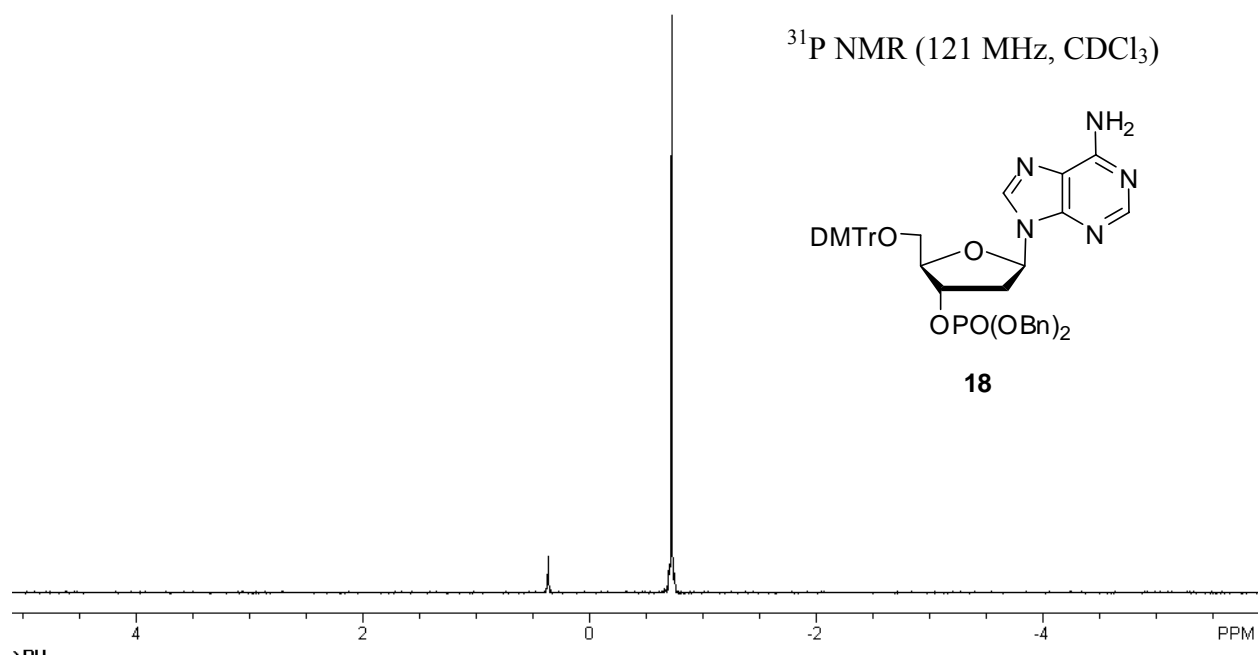
^1H NMR (300 MHz, $\text{DMSO-}d_6$)



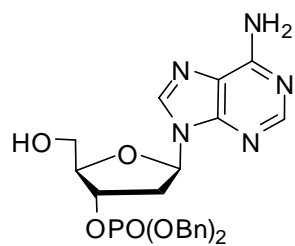
MR spectra for compounds in Chapter 6:



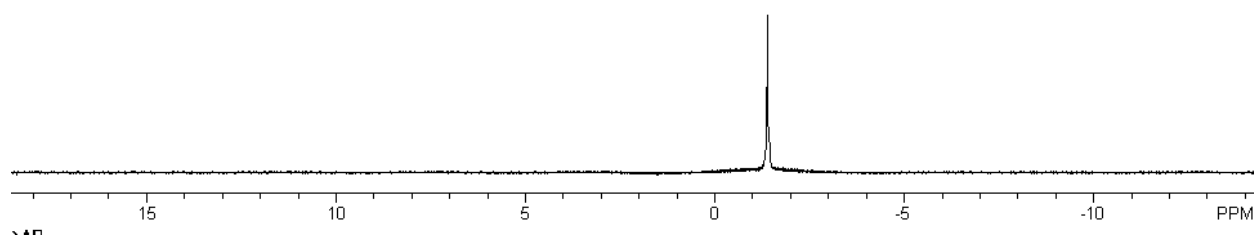




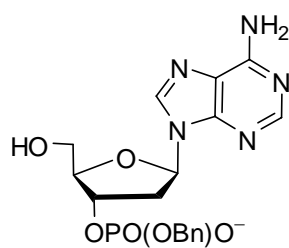
^{31}P NMR (121 MHz, CDCl_3)



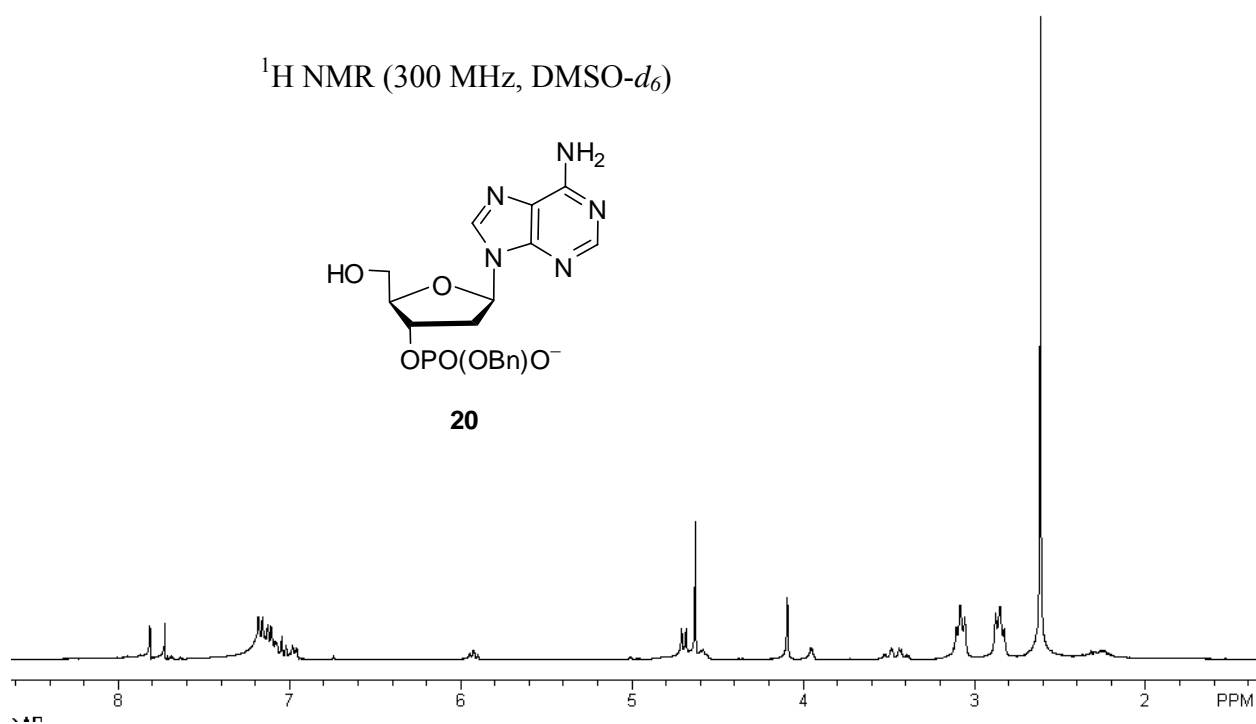
19



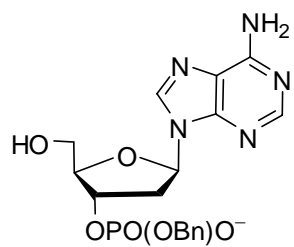
^1H NMR (300 MHz, $\text{DMSO}-d_6$)



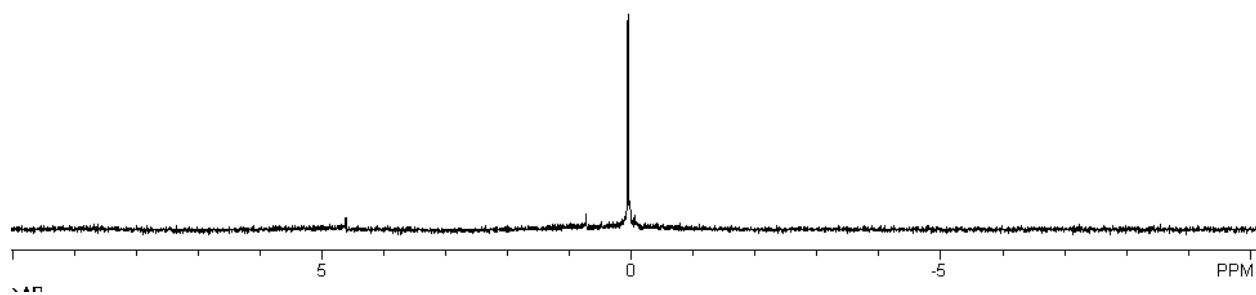
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^{31}P NMR (121 MHz, $\text{DMSO-}d_6$)

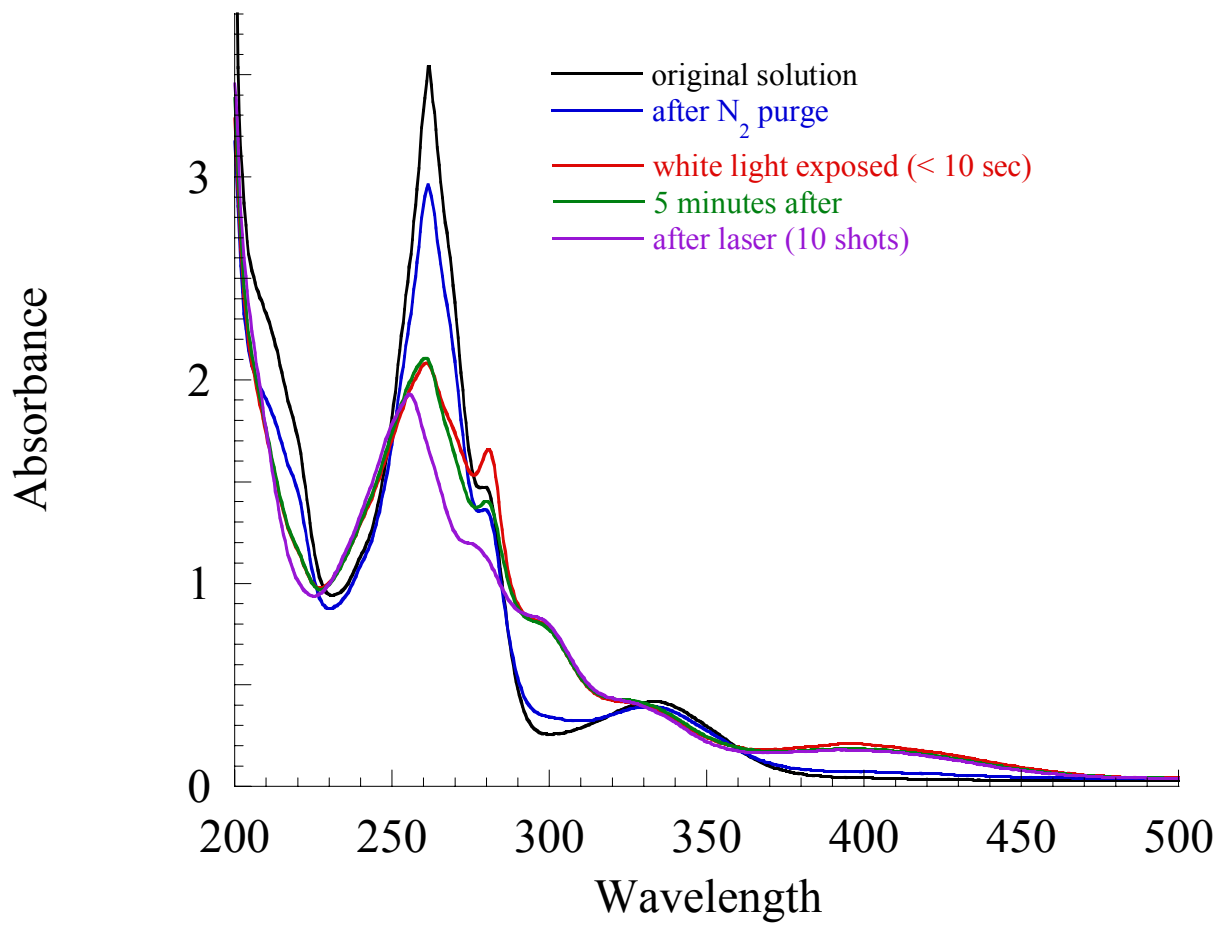


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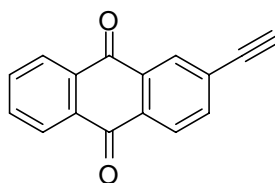


APPENDIX B: UV-vis spectroscopic measurements

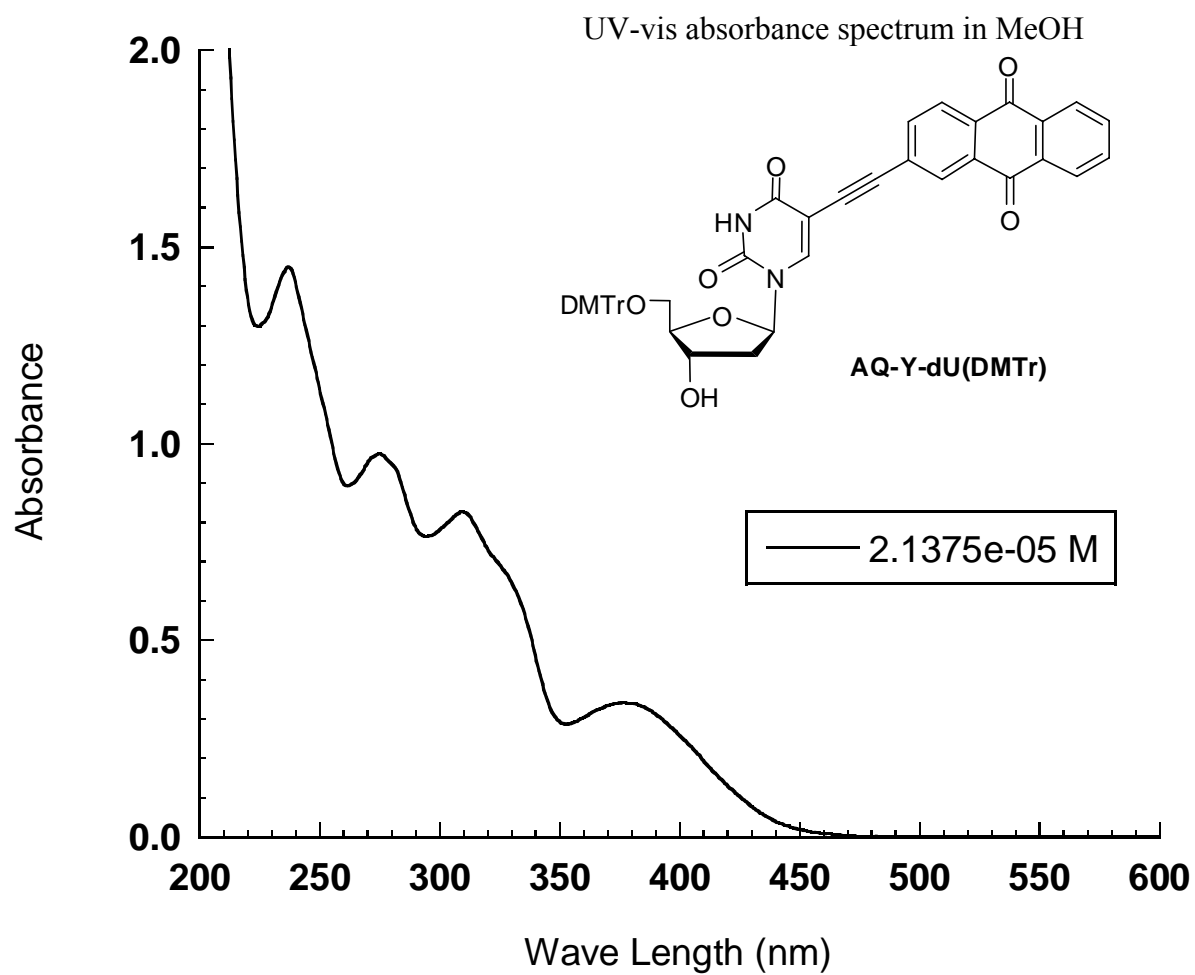
UV-vis and molar absorption spectra:

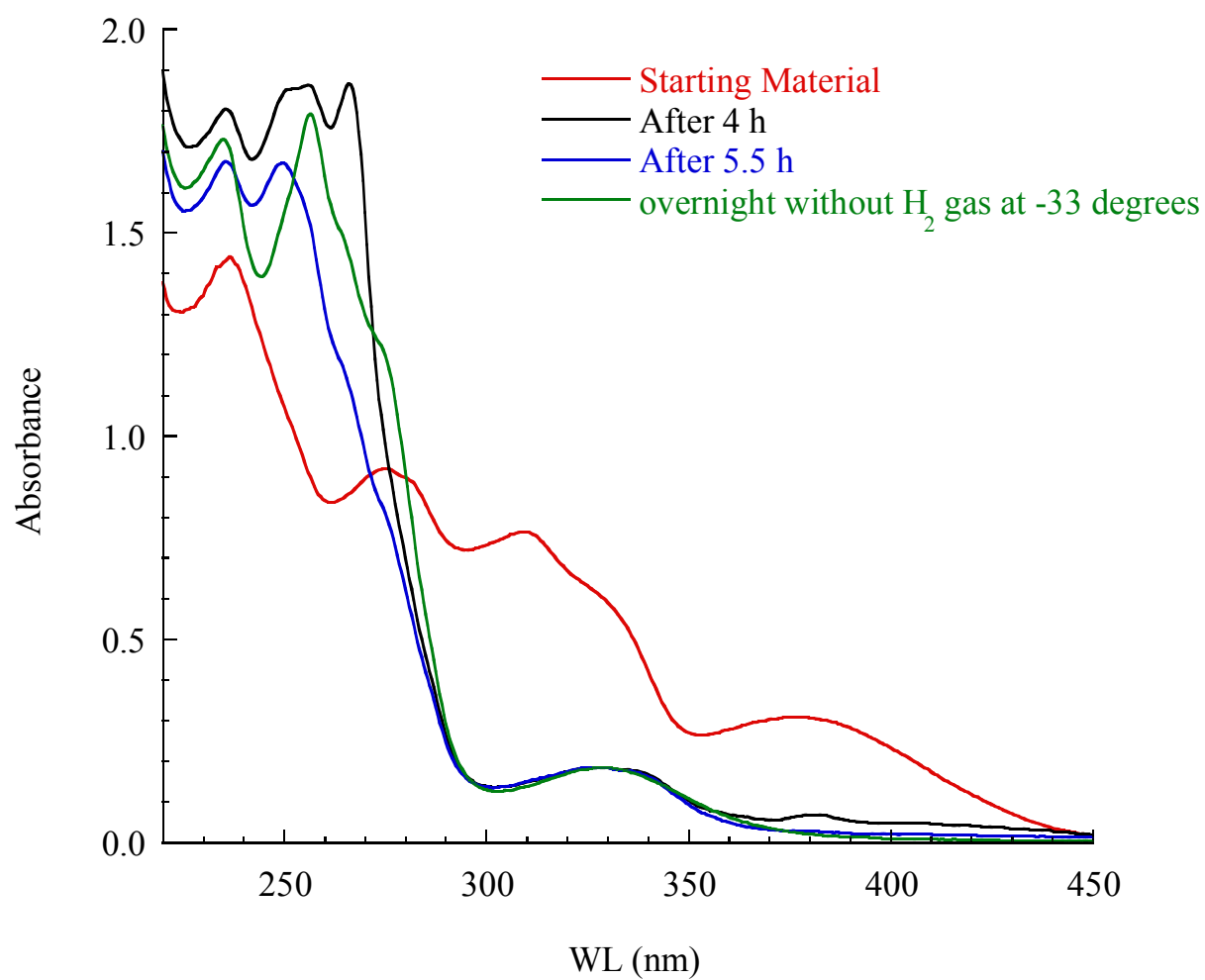


Change in UV-vis absorbance of 2-ethynyl anthraquinone upon exposure to light.



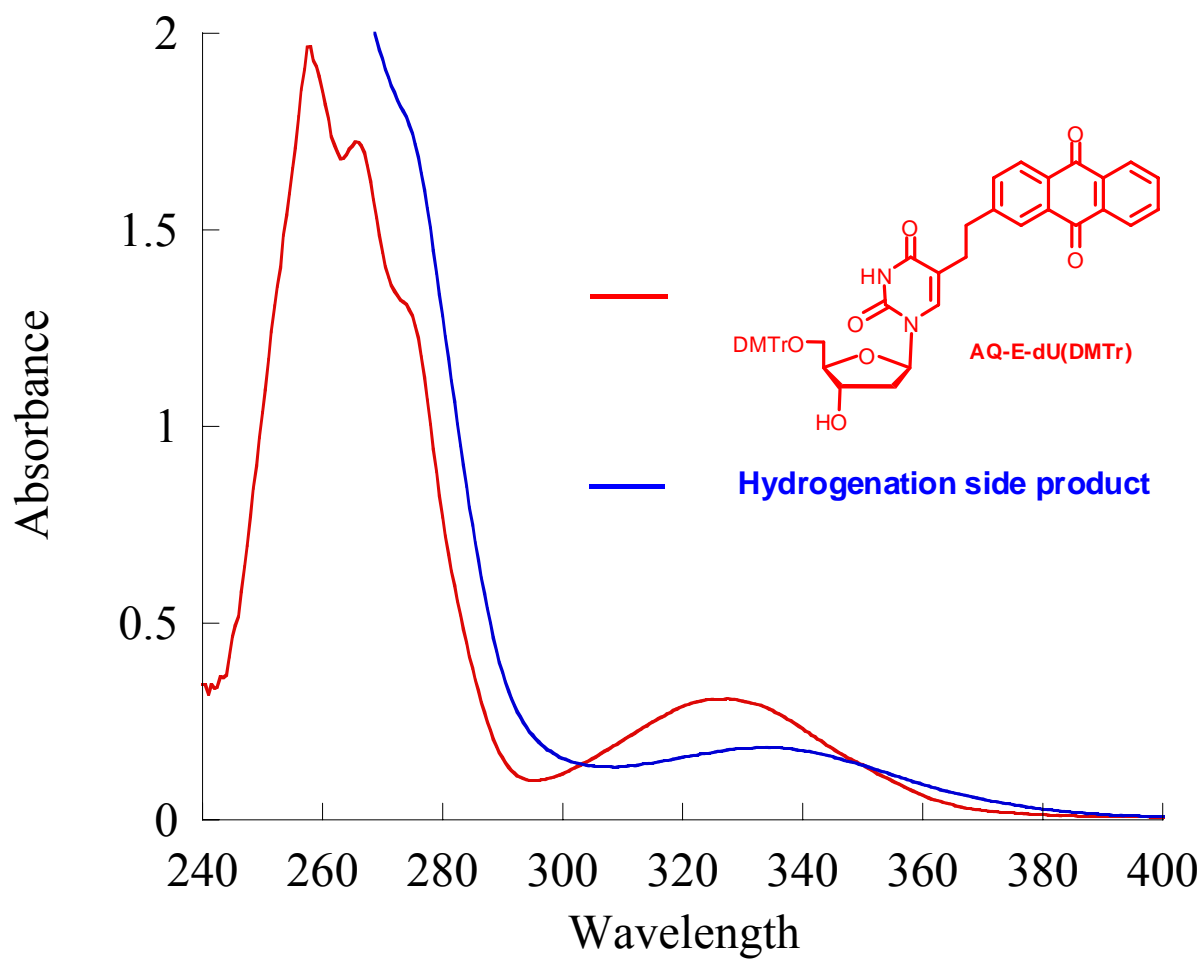
2-Ethynyl anthraquinone



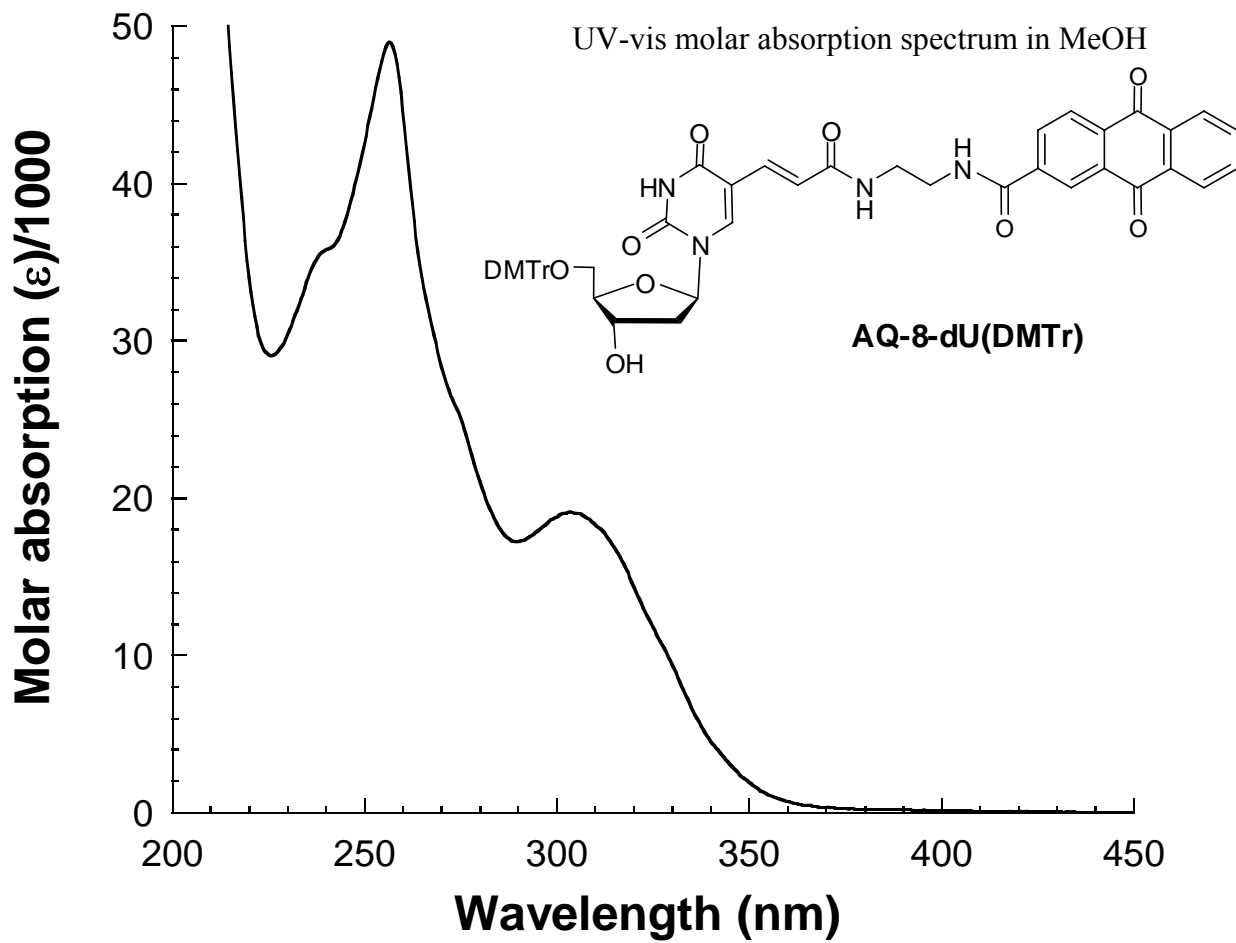


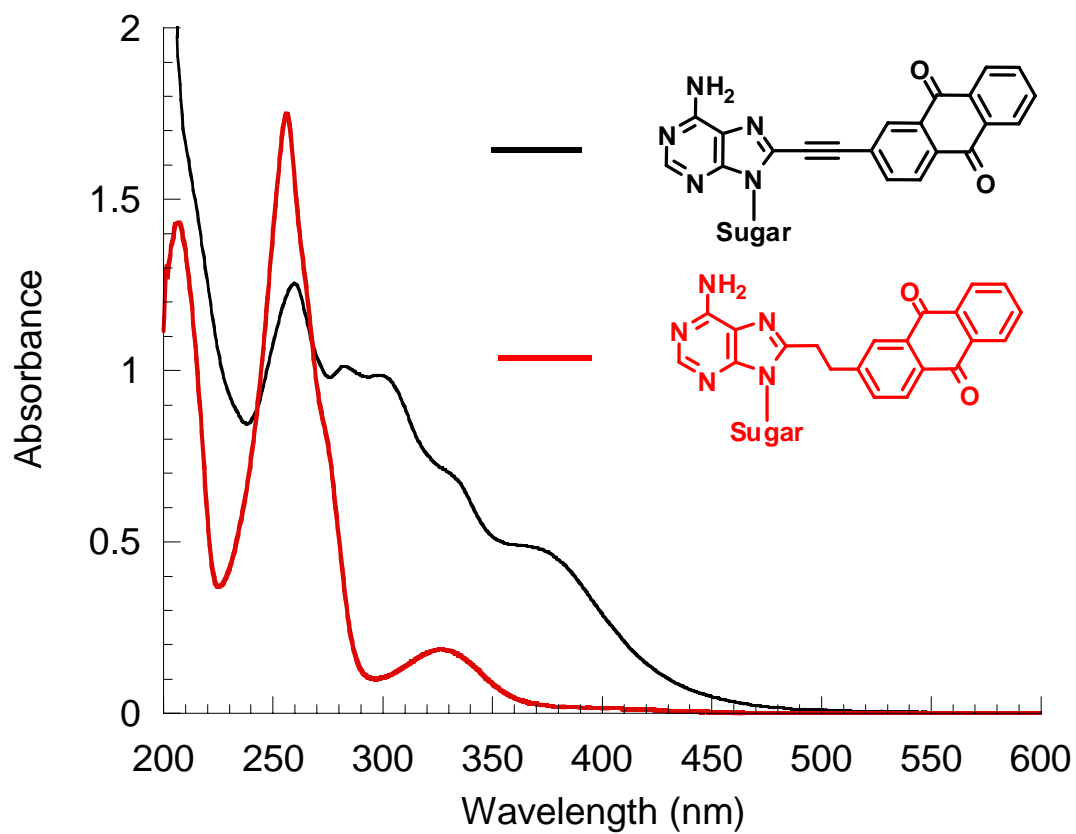
UV-vis monitoring of the catalytic hydrogenation of AQ-Y-dU(DMTr) to AQ-E-dU(DMTr)

(Chapter 4)

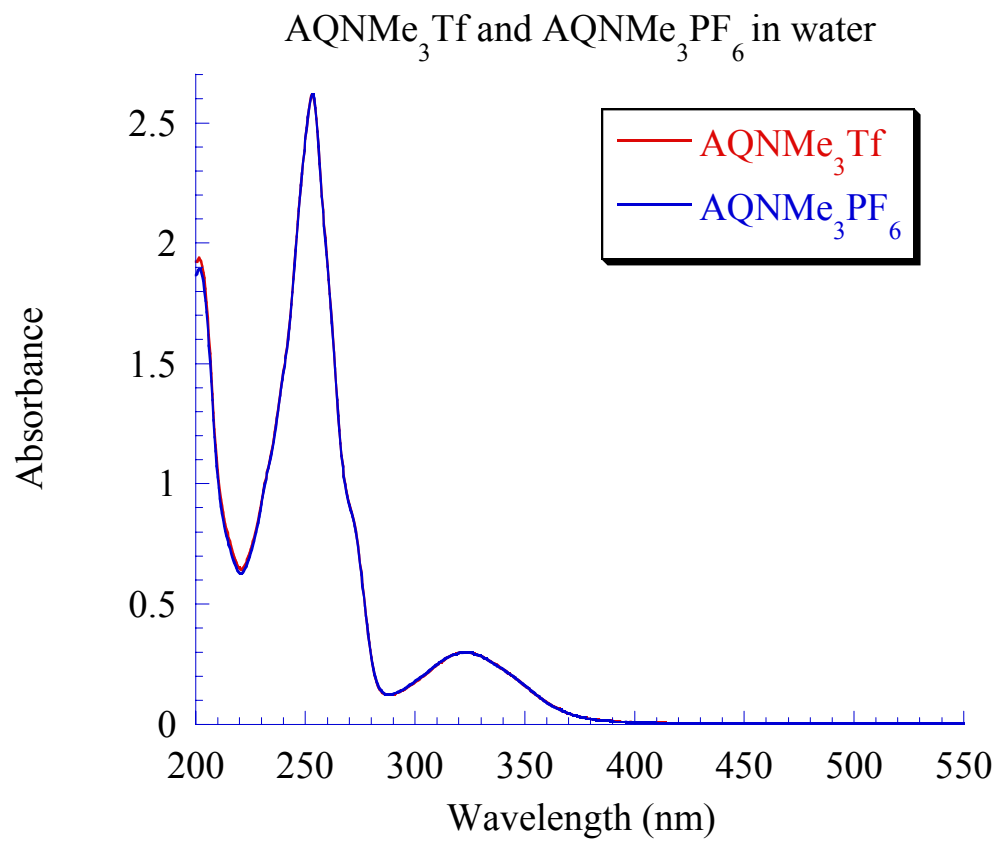


UV-vis absorbance spectra of AQ-E-dU(DMTr) and the major hydrogenation side product of AQ-Y-dU(DMTr) (Chapter 4)





UV-vis absorbance spectrum for AQ-dA conjugates in MeOH



Determination of molar absorption using Beer's law:

