reaction of **1** with cysteamine is shown here to mimic the transglutaminase enzymes, at least phenomenologically, by proceeding through an *S*-acyl intermediate and completing the S → N acyl transfer to form subsequently an amide.

Acknowledgment. We gratefully acknowledge the financial support of the University of Alberta and the Natural Sciences and Engineering Research Council of Canada (operating grant for R.S.B. and postgraduate scholarship for J.W.K.).

Supplementary Material Available: Table of k_{2obsd} values vs pH for the reaction of **2a-c** with **1** (2 pages). Ordering information is given on any current masthead page.

Sequence-Specific Alkylation of DNA Activated by an Enzymatic Signal

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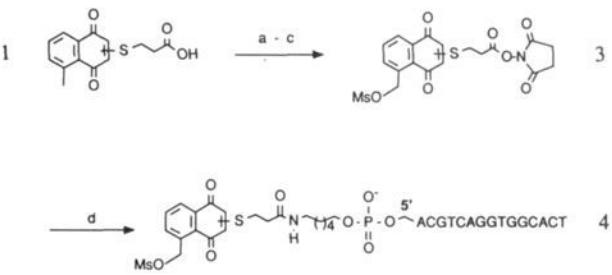
Received February 4, 1991

Enzymatic reduction of such drugs as mitomycin C, adriamycin, and daunomycin is thought to play a crucial role in the antitumor activity of these compounds. In each case, a highly electrophilic quinone methide or related semiquinone is likely generated under reducing conditions for the ultimate alkylation of DNA.[1,2] Numerous model studies have continued on this topic in order that bioactivation may become an integral step in the activity of newly developed therapeutics.[3] This communication now reports the successful production of a sequence-selective alkylating agent that is controlled by chemical and biological reduction. In this study, a naphthoquinone derivative[4] serves as the latent quinone methide while an attached oligonucleotide provides the site specificity for reaction.

Photolytic bromination of β-[(5-methyl-1,4-naphthoquinonyl)thio]propionic acid[5] (**1**) (Scheme I), followed by transformation of the 5-bromomethyl moiety to its corresponding (methylsulfonyl)oxy derivative by treatment with silver mesylate,[6] yielded the acid **2**. Treatment of the acid **2** with *N*-hydroxysuccinimide in the presence of 1-ethyl-3-[3-(dimethylamino)propyl]carbodiimide yielded the activated ester **3**. This in turn was used to acylate a hexamethyleneamino linking arm that was coupled to the 5′ terminus of an oligonucleotide 15 bases in length. The final product **4** was then purified by reverse-phase chromatography. A target strand of DNA, 5′-d(AGTGCCACCTGACGTGAG) (**5**), was also prepared and labeled at the 5′ terminus with ^{32}P.[7] The 5′ amino linker derivative (Clontech) and other oligonucleotides were synthesized via the standard procedures of solid-phase phosphoramidite chemistry.

Scheme I[a]



[a] (a) *N*-Bromosuccinimide, dibenzoyl peroxide, 2,2′-azobis(2-methylpropionitrile), *hν*, 90 °C, CCl_4/Ac_2O; (b) silver mesylate, CH_3CN; (c) *N*-hydroxysuccinimide, 1-ethyl-3-[3-(dimethylamino)propyl]carbodiimide, DMF; (d) oligo-$(CH_2)_6NH_2$, 3-(*N*-morpholino)propanesulfonic acid pH 7.5, 66% DMF.

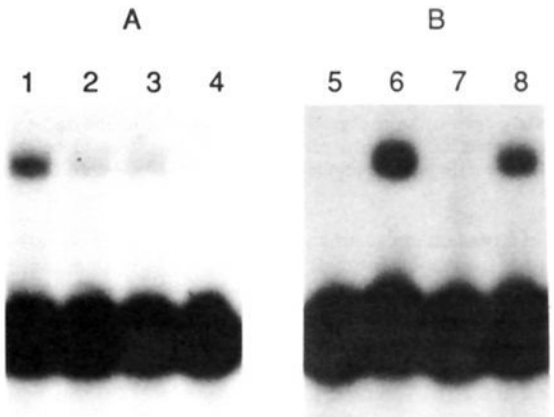


Figure 1. Autoradiograms of denaturing polyacrylamide gels (20%) used to identify the cross-link form of duplex DNA generated upon reduction. Oligonucleotides **4** and **5** (2.2 μM in each strand) were incubated in 100 mM potassium phosphate (pH 7) at room temperature (30 min) and then treated with the following reducing agents for an additional 30 min. (A) Lane 1: sodium borohydride (100 mM). Lane 2: sodium dithionite (100 mM). Lane 3: nicotinamide mononucleotide (10 mM). Lane 4: glutathione (10 mM). (B) Lane 5: cytochrome *c* reductase (1 mg/mL). Lane 6: cytochrome *c* reductase (1 mg/mL) and NADH (100 μM). Lane 7: NADH (100 μM). Lane 8: NADH (10 mM). The reaction mixtures were diluted 10-fold, dialyzed, lyophilized, and resuspended in 80% formamide for electrophoresis.

Oligonucleotide **4** was first annealed to its target strand **5** and then treated with a variety of reducing agents prior to analysis by polyacrylamide gel electrophoresis (20%, 7 M urea) and autoradiography (Figure 1). Treatment of the annealed duplex with sodium borohydride led to formation of a high molecular weight species consistent with the covalent attachment of the fully hybridized strands (lane 1, Figure 1A).[5] The yield of cross-linked material (12%, by densitometry) did not change appreciably by increasing the time of reaction from 10 min to 2 h or by changing the reaction temperature from 4 °C to 37 °C. The use of sodium dithionite (lane 2, Figure 1A) and NMNH[8] (lane 3, Figure 1A) as reductants also resulted in the formation of the cross-linked species, but these reductants appeared much less efficient at generating the desired product. Glutathione (lane 4, Figure 1A) did not induce the cross-linking reaction, perhaps because of its

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(8) Abbreviations used: β-nicotinamide adenine dinucleotide reduced form (NADH) and β-nicotinamide mononucleotide reduced form (NMNH).

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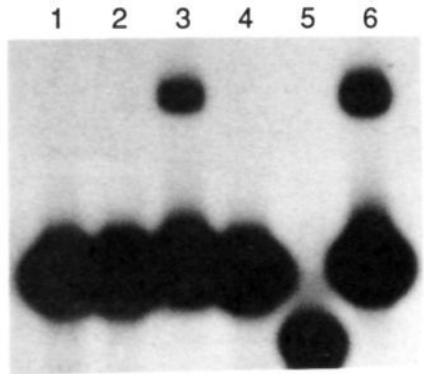
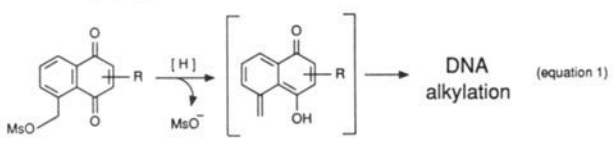


Figure 2. Enzyme dependence and sequence specificity of an oligonucleotide-based alkylating agent. All incubations were maintained at room temperature and buffered at pH 7 with 100 mM potassium phosphate. Product analysis was carried out in parallel with that described in Figure 1. Lane 1: **4** + **5** (2.2 μM each) were incubated in the absence of reducing agent for 30 min. Lane 2: **4** (2.2 μM) was pretreated with cytochrome *c* reductase (1 mg/mL) and NADH (100 μM) for 2 h before **5** was added and further incubated for 30 min. Lanes 3 and 4: **4** and **5** (2.2 μM each) were incubated for 30 min and then treated with cytochrome *c* reductase (1 mg/mL) and NADH (100 μM) in the absence (lane 3) and presence of 10 mM glutathione (lane 4) for an additional 30 min. Lane 5: **4** and a noncomplementary oligonucleotide 5′-[^{32}P]-d(CATGCGCTACCCGTG) were incubated for 30 min and then treated with cytochrome *c* reductase (1 mg/mL) and NADH (100 μM) for an additional 30 min. Lane 6: the 5-methylnaphthoquinone derivatives of **4** and **5** (2.2 μM each) were incubated for 30 min and photolyzed for 2 min.[5]

ability to react with the electrophilic intermediate of alkylation (see below).

Most important to our goal of future study in vivo, the annealed duplex **4** + **5** was also reduced enzymatically with cytochrome *c* reductase in the presence of a low concentration of NADH (100 μM). This yielded (14%) a cross-linked product equivalent to that above (lane 1 vs lane 6, Figure 1). No cross-linking was observed in the presence of either enzyme or 100 μM NADH alone (lanes 5 and 7, Figure 1B); however, a high concentration of NADH did allow for reaction (8%) (lane 8, Figure 1B) with an efficiency much greater than that of NMNH (2%). The extent of cross-linking was unaffected by the addition of 10 mM methyl viologen. If reduction of the quinone had occurred at some distance from the enzyme, then addition of methyl viologen might have been expected to facilitate electron transfer and thus to stimulate cross-linking of DNA. Since no effect was detected, cytochrome *c* reductase likely reduced the quinone directly even though this moiety was held adjacent to the duplex.

The reactivity and selectivity of our model reagent **4** suggests that target alkylation proceeds through the rapid formation and depletion of an electrophilic intermediate such as a quinone methide (eq 1).[4] No reaction was detected in the absence of a



R = site directing appendage

reductant or when **4** was reduced prior to the addition of its target sequence **5** (lanes 1 and 2, Figure 2). The transient intermediate generated upon reduction was then likely trapped by solvent to produce a derivative that was incapable of later alkylation.[9] Furthermore, reaction between **4** + **5** was suppressed in the presence of 10 mM glutathione, a competing nucleophile (lane 4, Figure 2). Cross-linking was also specific for a complementary sequence of DNA; enzymatic reduction of a mixture of **4** and a noncomplementary sequence, 5′-[^{32}P]-d(CATGCGCTACCCGTG), did not produce any detectable covalent complexation (lane 5, Figure 2). Finally, the entire nucleotide sequence of **4** + **5** was likely present in the high molecular weight product of alkylation since its electrophoretic mobility was equivalent to that generated and characterized in a related system[5] in which **5** and the 5-methyl derivative of **4** were labeled alternatively (lane 3 vs 6, Figure 2).

Enzymatic control of target alkylation has now been demonstrated by integrating the principles of bioactivation and antisense technologies.[10] In this manner, the equivalent of an extremely reactive intermediate can be delivered to a chosen site without loss of target selectivity. Logical extension of this work should enhance both the cell and gene specificity of drugs that act upon nucleic acids.

Acknowledgment. This research was supported in part by the University Exploratory Research Program of The Procter and Gamble Company.

Supplementary Material Available: Preparation of oligonucleotides, syntheses of **1**–**4**, and a model reductive alkylation (3 pages). Ordering information is given on any current masthead page.

(9) A preliminary description of a model reaction is included in the supplementary material.

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Cyclization Protocols for Controlling the Glycosidic Stereochemistry of Nucleosides. Application to the Synthesis of the Antiviral Agent 3′-Azido-3′-deoxythymidine (AZT)

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Received March 6, 1991

As a class, nucleosides have proven to be particularly effective against a variety of viral infections. For example, 3′-azido-3′-deoxythymidine (AZT, **2**) is a potent inhibitor of HIV reverse transcription and is presently the only drug that has been approved by the FDA for the treatment of AIDS. The synthesis of nucleosides, such as AZT, can be categorized under two broad classifications: those that modify intact nucleosides[1,2] and those that couple a modified carbohydrate to a nucleoside base.[3] The former suffers from the high cost associated with using nucleosides as starting materials, while the latter is complicated by the general inability to control the glycosidic stereochemistry during base coupling.[4] Both approaches are schematically illustrated in Scheme I.

Given the problems cited above, we set the goal of developing a synthetic protocol that permits the stereoselective construction

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