chlorobenzyl) derivatives of O-[5'-O-(4,4'-methoxytrityl)thymidin-3'-yl], O-(β-cyanoethyl) thiophosphite were prepared and kept at room temperature in a 1 M solution of tetrazole for several hours (Figure 6). When monitored by ^{31}P NMR, Arbuzov rearrangement of the 4-chlorobenzyl group of **18a** to yield **19a** occurred with a $t_{1/2}$ = 20 min whereas the 2,4-dichlorobenzyl derivative rearranged with $t_{1/2}$ = 150 min. During these prolonged exposures to tetrazole, a small fraction (see Experimental Section) of **18a** or **18b** was oxidized to the deoxynucleoside phosphorothioate (**20a** and **20b**, respectively). The amount of oxidation was dependent upon the oxygen content of solvents and the atmosphere over the reaction mixture. Surprisingly, when **7a** was tested under these same conditions, it did not rearrange detectably to the (4-chlorobenzyl) thiophosphonate in 3 h. These results demonstrate that Arbuzov rearrangements are not significant side reactions during the time (5 min) required to synthesize a thiophosphite linkage. They also are in agreement with earlier observations that dinucleotide methylphosphites do not undergo Arbuzov reactions with iodine or iodomethane[3,9,44] even though phosphites bearing simple alkyl groups readily rearrange with these reagents.

Conclusions

Two methods were developed for synthesizing deoxydinucleoside phosphorodithioates. With deoxynucleoside 3'-phosphordiamidites, the intermediate H-phosphonothioate could be used to synthesize deoxydinucleoside phosphorothioamidates, phosphorodithioates, and phosphorothioate triesters in addition to phosphorodithioate triesters. Thus, this approach can be used for introducing several unique analogues bearing specific reporter groups into DNA. This may well be the long-term utility of the method. The second approach, whereby deoxynucleoside 3'-(*N*,*N*-dimethylphosphorothioamidites) or deoxynucleoside 3'-(*N*,*N*-tetramethylenephosphorothioamidites) are used as synthons, appears to be more useful for the synthesis of dithioate oligonucleotides as the intermediates are readily activated with tetrazole, are stable toward storage, and yield deoxydinucleoside phosphorodithioates rapidly without forming detectable side products. Deoxydinucleoside phosphorodithioate triesters were found to be stable to all the current conditions used for the repetitive synthesis of DNA on polymeric supports (acid removal of the dimethoxytrityl group, acylation with acetic anhydride and (dimethylamino)-pyridine, and iodine oxidation) and could be freed of protecting groups by using reagents (triethylammoniumthiophenolate and concentrated ammonium hydroxide) compatible with the synthesis of natural internucleotide linkages.

Recently deoxynucleoside 3'-phosphorothioamidites have been used to synthesize several oligonucleotides with both phosphorodithioate and natural internucleotide linkages.[32] Biochemical and biological reports, which are now beginning to appear in abbreviated form,[52,53] demonstrate that DNA containing phosphorodithioate linkages mimic natural DNA in certain important properties such as forming duplexes with complementary deoxyoligonucleotides, stimulating RNase H activity in Hela cell nuclear extracts and the binding of *lac* repressor and *cro* repressor to their respective operators. Phosphorodithioate DNA has certain unique and very useful properties as well. These include complete resistance to cellular nucleases, ease of derivatization with various reporter groups such as fluoresceine and biotin, and as an extremely potent inhibitor of HIV reverse transcriptase. This combination of observations suggests a large number of potential applications for phosphorodithioate-linked DNA in biology and biochemistry. Undoubtedly the value of these compounds as research tools will continue to grow in the years to come.

Acknowledgment. We thank Mr. Ron Sadecky and Dr. Robert Barkley for recording the FAB mass spectra.

Supplementary Material Available: Spectral data for the synthesis of **14a-c** and **8a** (10 pages). Ordering information is given on any current masthead page.

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(+)-CC-1065 DNA Alkylation: Key Studies Demonstrating a Noncovalent Binding Selectivity Contribution to the Alkylation Selectivity

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Abstract: A comparative study of the selectivity and relative intensity of DNA alkylation with a series of (+)-CC-1065 analogues is detailed. The results of the study (1) reveal a nonselectivity of the simple alkylation event in the absence of noncovalent binding selectivity, (2) highlight the enhanced selectivity of the alkylation in the event of noncovalent binding selectivity, (3) demonstrate that a DNA autocatalytic phosphate activation of the alkylation reaction (Lewis acid complexation/protonation) may not be uniquely responsible for the nonselective or selective alkylations, and (4) address the assumption that stereoelectronic effects associated with the (+)-CC-1065 cyclopropane alkylation contribute uniquely to the alkylation selectivity.

(+)-CC-1065 (**1**), a potent antitumor antibiotic and the subject of extensive investigations,[2-4] has been shown to exert its effects through the sequence selective alkylation of DNA. In initial and continued investigations, the Upjohn group in collaboration with

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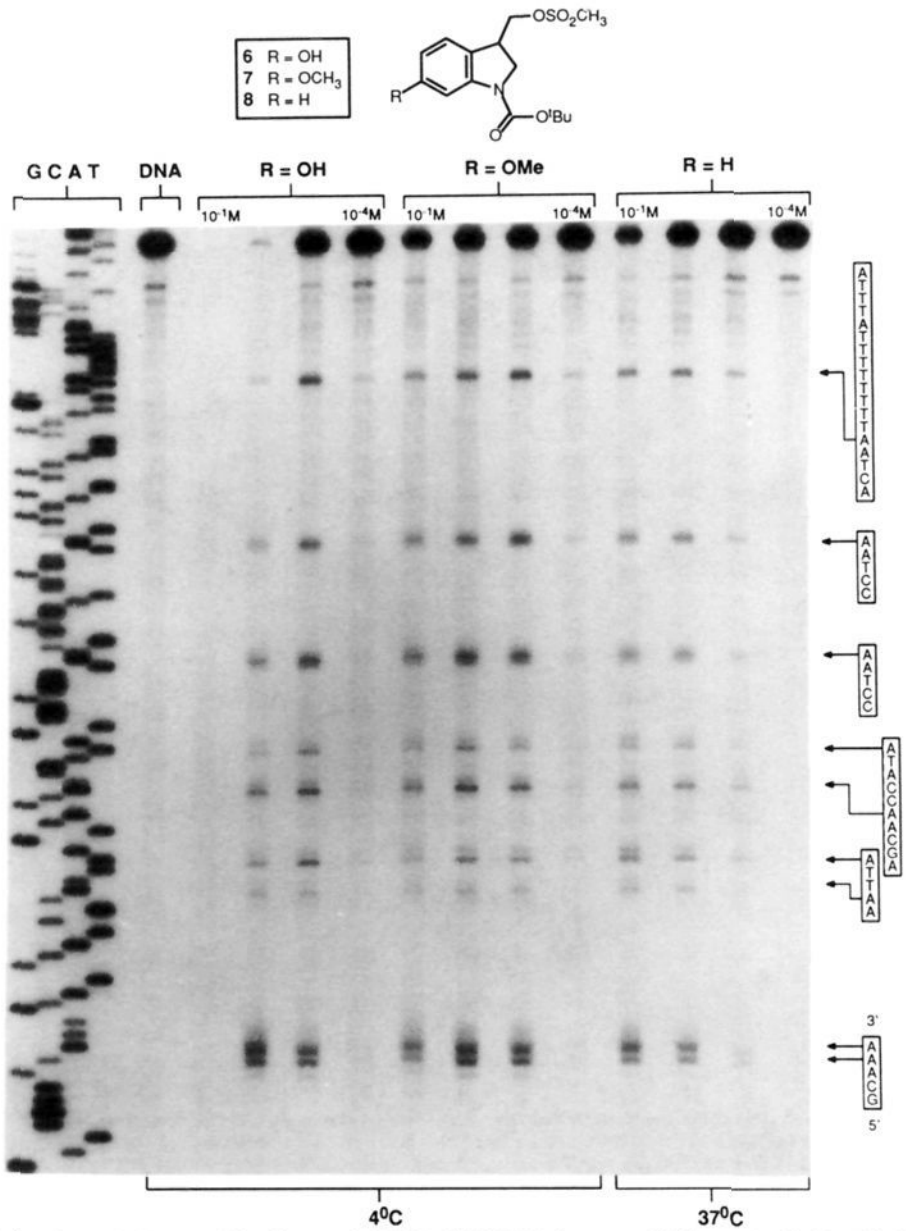

Figure 1. Thermally induced strand cleavage of double-stranded DNA (SV40 DNA fragment, 144 bp; nucleotide No. 138–5238, clone w794) after a 24-h incubation of agent:DNA at 4 or 37 °C followed by removal of unbound agent and a 30-min incubation at 100 °C; 8% denaturing poly(acrylamide) gel and autoradiography. Lanes 1–4, Sanger G, C, A, and T reactions; lane 5, control DNA; lanes 6–9, (±)-**6** (R = OH, 2.5×10^{-1} to 2.5×10^{-4} M, 4 °C); lanes 10–13, (±)-**7** (R = OMe, 2.5×10^{-1} to 2.5×10^{-4} M, 4 °C); lanes 14–17, (±)-**8** (R ≈ H, 2.5×10^{-1} to 2.5×10^{-4} M, 37 °C).

Hurley and co-workers have defined the nature of the (+)-CC-1065 DNA alkylation and have shown that the reaction proceeds by 3′-adenine $N-3$ alkylation of the electrophilic cyclopropane present in the CC-1065 left-hand subunit within A-T rich minor groove regions of DNA.[2,4] In the course of these efforts and our own complementary studies to define the structural origin of the (+)-CC-1065 DNA alkylation selectivity, distinctions in the extent of the selectivity and its potential origin have been detailed.[4–7] Most prominent among the distinctions has been the extent[2,4,5–7] to which the (+)-CC-1065 noncovalent binding selectivity may contribute to the DNA alkylation selectivity. We have suggested a prominent[3,5–7,10] role for the noncovalent binding selectivity that may be expected to occur preferentially within the narrower, sterically more accessible A-T rich minor groove versus the wider, sterically less accessible G-C rich minor groove. Alternatively, the Upjohn and Hurley groups have suggested that the (+)-CC-1065 DNA alkylation constitutes a sequence dependent reaction independent of noncovalent binding selectivity. This has been suggested to be the result of a sequence dependent (Lewis) acid-catalyzed DNA alkylation requiring autocatalytic activation of the alkylation step through carbonyl complexation or protonation by a strategically located phosphate in the DNA backbone two base pairs removed from the alkylation site in the 5′-direction.[2,4]

In efforts to clarify the relative importance of the alkylation reaction versus the agent noncovalent binding selectivity, we have prepared agents possessing modified alkylation subunits[8–14] in-

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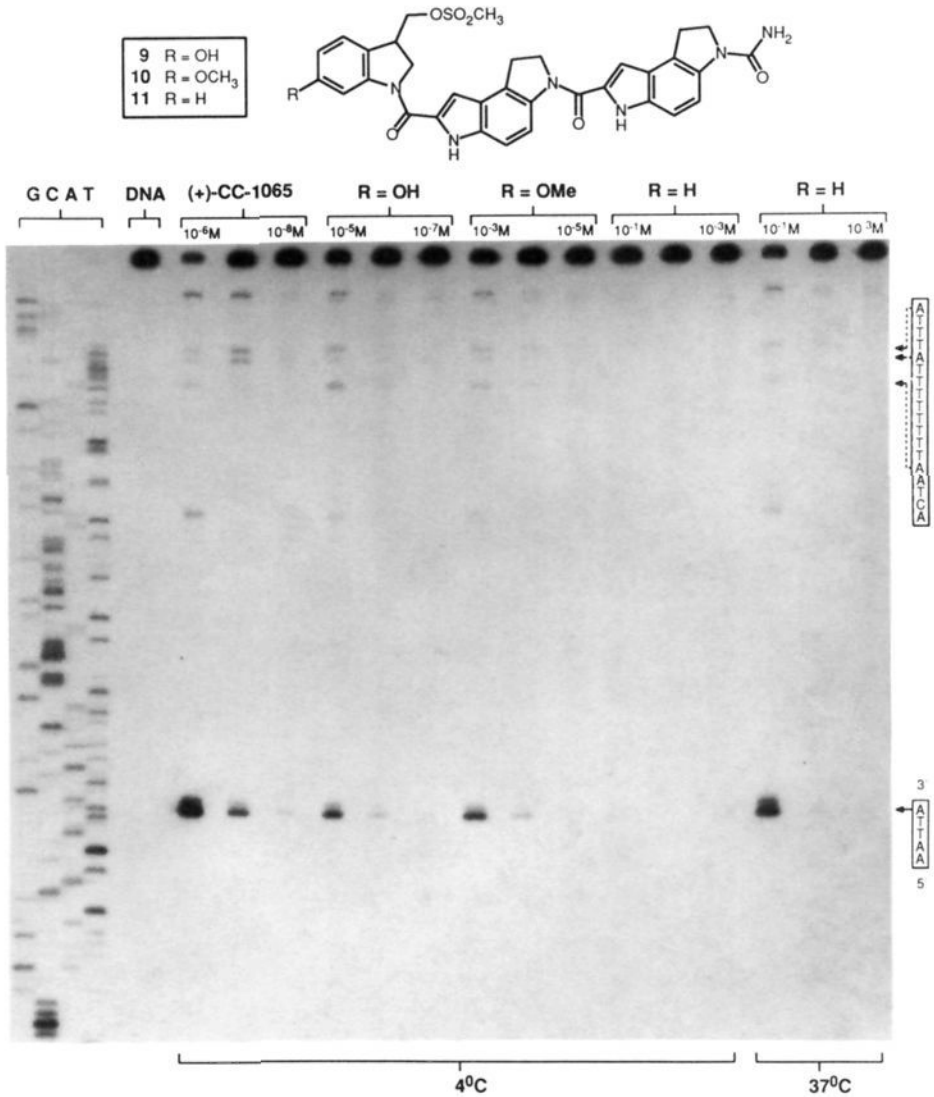

Figure 2. Thermally induced strand cleavage of double-stranded DNA (SV40 DNA fragment, 144 bp; nucleotide No. 138-5238, clone w794) after a 24-h incubation of agent:DNA at 4 or 37 °C followed by removal of unbound agent and 30-min incubation at 100 °C; 8% denaturing poly(acrylamide) gel and autoradiography. Lanes 1–4, Sanger G, C, A, and T reactions; lane 5, control DNA; lanes 6–8, (+)-CC-1065 (**1**, 2.5×10^{-6} to 2.5×10^{-8} M, 4 °C); lanes 9–11, (±)-**9** (R = OH, 2.5×10^{-5} to 2.5×10^{-7} M, 4 °C); lanes 12–14, (±)-**10** (R = OMe, 2.5×10^{-3} to 2.5×10^{-5} M, 4 °C); lanes 15–17, (±)-**11** (R = H, 2.5×10^{-1} to 2.5×10^{-3} M, 4 °C); lanes 18–20, (±)-**11** (R = H, 2.5×10^{-1} to 2.5×10^{-3} M, 37 °C).

cluding the exceptionally reactive CI subunit.[8-10] Herein, we present the unexpected results of studies conducted in the course of these investigations that serve to highlight the nonselectivity of the simple alkylation event in the absence of noncovalent binding and that illustrate a prominent role for the noncovalent binding selectivity of agents with such capabilities. Further, the studies demonstrate that the proposed autocatalytic phosphate activation of the alkylation reaction may not be uniquely responsible for the nonselective or selective DNA alkylations and that stereoelectronic effects associated with the CC-1065 cyclopropane alkylation may not contribute uniquely to the alkylation selectivity. Through the use of a protocol detailed elsewhere,[15] the profiles of DNA alkylation for the agents **1**–**11** were examined within ^{32}P singly 5′ end-labeled w794 double-stranded DNA.[16] Thus, singly 5′ ^{32}P end-labeled double-stranded DNA constituting SV40 DNA nucleotides No. 5238-138 (144 base-pairs) cloned into the *Sma* I site of M13mp10 was prepared by treatment of single-stranded templates (clone w794)[16] with end-labeled universal primer [5′-d(GTAAAACGACGGCCAGT)-3′], extension of the primer–template duplex with the Klenow fragment of DNA polymerase I, and subsequent *Eco*R I cleavage of the double-stranded DNA immediately following the inserted DNA.[15] The resultant DNA was treated with the agents **1**–**11** at 4 or 37 °C (24 h) at a range of concentrations. Removal of the unreacted agent through ethanol precipitation of the DNA, redissolution of the alkylated DNA in aqueous buffer, thermally induced cleavage of the DNA at the sites of alkylation (100 °C, 30 min),[2] gel electrophoresis of the resultant DNA adjacent to Sanger sequencing reactions, and subsequent autoradiography revealed the agent sites of alkylation. The results for agents **6**–**11**[17] are shown in Figures 1

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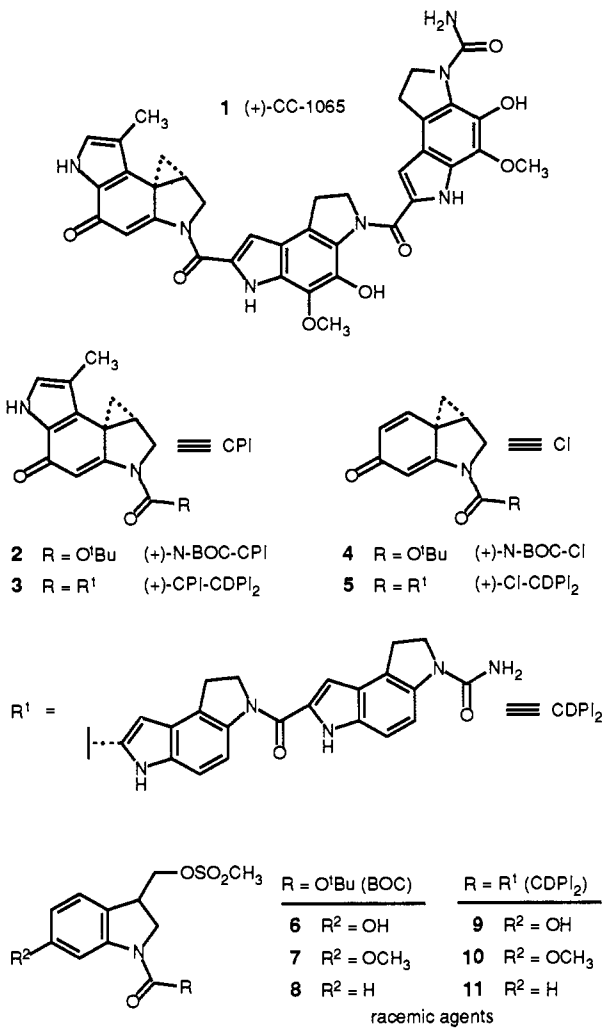
Chart I



and 2.

A number of important conclusions may be drawn from the comparative study. The DNA alkylation profiles of **6–8** are identical (Figure 1), indistinguishable from that of (±)-*N*-BOC-CI (**4**, supplementary material),[15] and independent of the absolute configuration of the agent ((+)-**6** = (−)-**6** = (±)-**6**, supplementary material).[10,12,15] Consistent with the reactivity of **4** ($t_{1/2}$ = 5.2 h, pH = 7; $t_{1/2}$ = 35 s, pH = 3),[9] we have interpreted this profile of DNA alkylation as a relatively nondiscriminant alkylation event.[15,18] Nonetheless, the alkylation profile of **4** and **6–8** has proven comparable to that of (+)-*N*-BOC-CPI (**2**), the authentic alkylation subunit of (+)-CC-1065 (supplementary material).[10,12] The methyl ether **7** and the agent **8** cannot close to the putative CI agent **4** illustrating that the cyclopropane is not obligatory[12] for observation of the CI characteristic DNA alkylation. Moreover, the DNA alkylation by **7** and **8** is not subject to the proposed autocatalytic phosphate activation of a sequence dependent alkylation event suggesting that it does not play a role in establishing the selectivity of DNA alkylation by **4**.[4]

Similarly, the DNA alkylation profiles of **9–11** are identical (Figure 2), indistinguishable from that of (+)-CI-CDPI$_2$ (**5**, supplementary material),[10] and independent of the absolute configuration of the agent ((+)-**5** = (−)-**5** = (±)-**5**, supplementary material).[10] The DNA alkylation selectivity of **5** and **9–11** was found to be strikingly similar to that of (+)-CC-1065, confined to A-T rich regions of DNA, and substantially more selective than that of **4** and **6–8**. The methyl ether **10** and the agent **11** cannot close to the putative CI agent **5** illustrating that the cyclopropane is not required for observation of the selective DNA alkylation reaction characteristic of (+)-CC-1065 and that the selective reaction by **10** and **11** may not be the result of a sequence dependent phosphate activation of the alkylation event. The results are, however, consistent with the assignment of a prominent role for the (+)-CC-1065 noncovalent binding selectivity within the narrower, sterically more accessible A-T rich minor groove effectively restricting the number of available alkylation sites (accessible hydrophobic binding).[3,5,6,10]

Experimental Section

Agents **4–7**, **9–10** were prepared as detailed.[9] The preparation and characterization of **8** and **11** are provided in the supplementary material. The samples of **7** and **10** were purified by HPLC (Alltech 10 × 250 mm, 10-μm econosil column, flow rate = 3.0 mL/min; for **7**, 30% EtOAc–hexane, R_T = 17 min (**7**), R_T = 25 min (**6**); for **10**, 10% DMF in CH_2Cl_2, R_T = 22 min (**10**), R_T = 25 min (**9**)) and determined to be free of contaminant phenol **6** or **9** (**7**, ≥99.4% pure, ≤0.05% **6**; **10**, ≥99.9% pure, ≤0.1% **9**).[17]

DNA binding and alkylation studies were conducted following the procedure detailed elsewhere.[15]

Acknowledgment. This work was supported financially by the National Institutes of Health (CA 41986) and the American Cancer Society (PF-3311, S.A.M.). We gratefully acknowledge Professor M. Bina and her group for assistance and the gift of M13mp10 clones (w794).

Supplementary Material Available: Full details of the preparation and characterization of **8** and **11** and supporting DNA alkylation studies (4 figures) are provided (6 pages). Ordering information is given on any current masthead page.

(17) The relative intensity of the alkylation of **7** and **9** cannot be attributed to a phenol contamination of the samples (requires ca. 10–100% **6** in **7** and ca. 1% **9** in **10**).

(18) 40–45% of the adenines within w794 DNA are alkylated by **4** and **6–8**.

(19) The cytotoxic potency of the agents follow trends established in the relative intensity of the agent DNA covalent alkylation: L1210, IC_{50} (μg/mL) (+)-CC-1065 (**1**), 1 × 10^{-5}; (±)-**4**, 10; (±)-**5**, 0.002; (±)-**6**, 0.2; (±)-**7**, 1; (±)-**8**, > 10; (±)-**9**, 0.001; (±)-**10**, 0.004; (±)-**11**, 0.5.