14 (essentially one epimer), respectively. However, further complicating the mechanistic picture, small quantities of the corresponding cyclobutane derivatives 13 and 15[9] were also isolated. Cyclobutane formation from allenes is well precedented.[10]

This new reaction, which produces highly functionalized cyclopentene derivatives, has unraveled a hitherto unknown facet of the chemistry of *S*-propargyl xanthates with intriguing synthetic and mechanistic implications. Its scope and selectivity as well as the possibility of an intramolecular variant are currently under study.

Acknowledgment. We thank Prof. J.-Y. Lallemand for friendly discussions and the Société Nationale des Poudres et Explosifs (SNPE) for generous financial support.

(9) Only one geometrical isomer of 13 and 15 was observed, to which the configuration shown has been tentatively assigned by analogy (see ref 10d).

(10) See inter alia: (a) Schuster, H. F.; Coppola, G. M. *Allenes in Organic Synthesis*; Wiley-Interscience: New York, 1984. (b) Pasto, D. J.; Yang, S. H. *J. Org. Chem.* **1986**, *51*, 1676; *J. Am. Chem. Soc.* **1984**, *106*, 152. (c) Pasto, D. J.; Warren, S. E. *J. Am. Chem. Soc.* **1982**, *104*, 3670. (d) Pasto, D. J.; Heid, P. F.; Warren, S. E. *J. Am. Chem. Soc.* **1982**, *104*, 3676 and references therein.

Synthesis of Oligonucleotides via Monomers with Unprotected Bases

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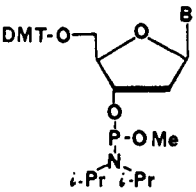
The need to protect the amino groups of nucleoside bases was recognized early in the development of chemical methods for synthesizing oligonucleotides.[1,2] Since then, N-protecting groups have been universally employed in oligonucleotide synthesis. Ideally, however, one would like to avoid protecting groups. At best they entail two additional steps, introduction and removal, and the reagents required in these steps limit the range of functional groups that can be tolerated in the synthesis.

We report here a procedure, utilizing phosphoramidite reagents,[3] that enables one to synthesize oligonucleotides of short to moderate length without resort to N-protecting groups. Selectivity with respect to OH and NH_2 groups is achieved not by blocking reaction at nitrogen but by selective group transfer from nitrogen.[4] The procedure appears promising for solid-phase synthesis of new oligonucleotide analogues containing substituents sensitive to N-deblocking reagents. Such compounds can have potential as selective inhibitors of viral replication and gene expression.[5]

Nucleoside phosphoramidites **1a**,**c**,**g** were obtained in ~90% yield by phosphitilating DMT-dA,[6] DMT-dC,[7] and DMT-dG[6] with $MeOP[N(iPr_2)]_2$.[8] Attempts to prepare these compounds with $MeOP(Cl)[N(iPr_2)]$ were unsatisfactory since extensive reaction occurred at the amino as well as hydroxyl groups of the nucleosides.



1a B = adenyl
1c B = cytosyl
1g B = guanyl

Condensation of **1a** (Figure 1) or **1c** under standard conditions with thymidine anchored to a solid support (dT-succinyl-CPG), followed by oxidation, gave a complex mixture of products. HPLC profiles and dimethoxytrityl cation assays indicated extensive phosphitilation at the unprotected amino groups as well as the 5'-hydroxyl group of the support-bound thymidine.[9] On the other hand, good quality d(GT) was obtained from the reaction with **1g**, showing that the amino group of dG is relatively resistant to the phosphitilating agent (Figure 1). Indeed, as we found by preparing d(GGGGGT), d(GAGGTCAGGT), and d(CCATTTTCAGAATTGGGTGT), one can obtain oligonucleotides of moderate length using conventional phosphoramidite methodology without protecting the amino group of guanine.[10]

For syntheses utilizing **1a** and **1c** we exploited the fact that products of phosphitilation at the amino groups of cytosine and adenine derivatives are themselves phosphoramidites and can serve as phosphitilating agents. A step was added to the synthetic protocol to cleave these amidites prior to oxidation. Of several cleavage systems examined, a mixture of pyridine hydrochloride (an acid to activate P(III)-N derivatives) and aniline (a nucleophile to accept the P(III) fragment) proved most effective.[11] As shown in Figure 1, good quality d(AT) and d(CT) were readily obtained when treatment with this acid/nucleophile combination was included in the synthetic procedure. Neither pyridine hydrochloride nor aniline alone was suitable.

The overall scheme is represented by the synthesis of d(GAGGTCAGGT), starting from dT-succinyl-CPG (0.5 μmol of dT). Each cycle consisted of detritylation (3% DCA in CH_2Cl_2), washing (MeCN), coupling (15 mg of **1a**, **1c**, **1g**, or standard dT phosphoramidite reagent, 8.8 mg of tetrazole, 0.25 mL of MeCN; 3 min), washing (MeCN), phosphityl transfer (0.1 M pyridine hydrochloride, 0.1 M aniline, MeCN; 5 min), washing (MeCN), oxidation (I_2/H_2O; 2 min), and washing (CH_2Cl_2). Finally, conventional demethylation, cleavage, and purification by reversed-phase and ion-exchange HPLC afforded the decamer, 8 A_{260} units (16%), >99% homogeneous by both ion-exchange (OmniPac Na100 column) and reversed-phase HPLC (conditions in Figure 1). For comparison, an oligomer with the same sequence was prepared (15 A_{260} units, 30%)[12] under standard conditions with N-protected nucleoside reagents. The two oligomers were the same, as shown by the HPLC elution time, PAGE (0.88 relative to bromophenol blue), and thermal dissociation (T_m 42 °C; 0.1 M NaCl) of the complexes formed with a complementary oligomer, d(ACCTGACCTC). In addition, hydrolysis (snake venom phosphodiesterase and alkaline phosphatase) of the oligomer derived from unprotected bases afforded dA, dC, dG, and dT in the predicted amounts.[13]

This methodology, in conjunction with use of a support with an oxalyl anchor,[14] should provide access to mixed-base oligonucleotide derivatives containing functional groups sensitive to

(1) Michelson, A. M. *J. Chem. Soc.* **1959**, 3655-3669.

(2) (a) Khorana, H. G.; Turner, A. F.; Vizsolyi, J. P. *J. Am. Chem. Soc.* **1961**, *83*, 686-698. (b) Ralph, R. K.; Khorana, H. G. *J. Am. Chem. Soc.* **1961**, *83*, 2926-2934. (c) Rammler, D. H.; Khorana, H. G. *J. Am. Chem. Soc.* **1962**, *84*, 3112-3122.

(3) Caruthers, M. H. *Science* **1985**, *230*, 281-285.

(4) Attempts to achieve selective phosphitilation without protecting amino groups have had only very limited success: (a) Letsinger, R. L.; Finnan, J. L.; Jacobs, S. A.; Juodka, B. A.; Varshney, A. K. *Synthesis, Structure and Chemistry of t-RNA*, International Conference, Poznan, Poland, 1976; pp 147-159. (b) Ogilvie, K. K.; Theriault, N.; Sadana, K. L. *J. Am. Chem. Soc.* **1977**, *99*, 7741-7743. (c) Ogilvie, K. K.; Schifman, A. L.; Penney, C. L. *Can. J. Chem.* **1979**, *57*, 2230-2238. (d) Fourrey, J.-L.; Varenne, J. *Tetrahedron Lett.* **1985**, *26*, 2663-2666.

(5) Cohen, J., Ed. *Oligodeoxynucleotides*; CRC Press, Inc.: boca Raton, FL, 1989.

(6) Sigma Chemical Co.

(7) Michelson, A. M.; Todd, A. R. *J. Chem. Soc.* **1954**, 34-40.

(8) The phosphitilating procedure was that used for N-protected derivatives by Barone et al. (Barone, A. D.; Tang, J.-Y.; Caruthers, M. H. *Nucleic Acids Res.* **1984**, *12*, 4051-4061), except that a larger excess (1.8/1) of amidite and tetrazolium salt was employed for DMT-dC. **1a**,**c**,**g** each showed only a double peak near 152 ppm in the ^{31}P NMR spectrum and exhibited the expected molecular ion in the FAB^- mass spectrum (713, 689, 729, respectively; MS by Doris Hung).

(9) DMT^+ released in the DCA step was ~2-fold and 4-fold greater, for reactions of **1a** and **1c**, respectively, than calculated, assuming no reaction at NH_2.

(10) Standard protocol with a Milligen Cyclone DNA synthesizer.

(11) Tetrazole (0.4 M) in methanol/acetonitrile (5 min) served as a transfer agent for work with dC derivatives but did not remove all P(III) fragments from nitrogen of dA derivatives. With 4 M tetrazole, P-O cleavage accompanied P-N cleavage.

(12) The difference in yields of the decamer reflects some additional side products in reactions involving unprotected bases.

(13) For methods, see ref 14.

(14) Alul, R.; Singman, C. N.; Zhang, G.; Letsinger, R. L. *Nucleic Acids Res.* **1991**, *19*, 1527-1532.

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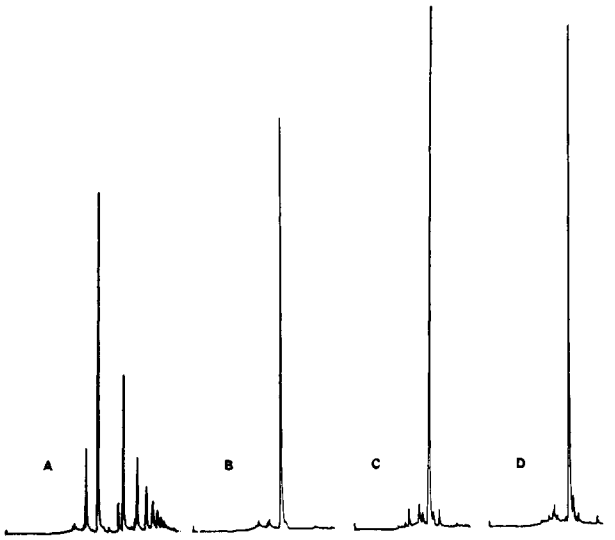

Figure 1. HPLC of crude products from syntheses with unprotected bases: A and D show products from preparation of d(AT) and d(GT), respectively, using a standard reaction cycle; B and C show products from preparation of d(AT) and d(CT) using a $C_5H_5N{\cdot}HCl/C_6H_5NH_2$ transfer step. A C18 ODS column (4.6 × 200 mm) was used, with 0.03 M $Et_3N{\cdot}HOAc$ (pH 7.0) and a CH_3CN gradient increasing from 0% at 1%/min; flow rate 1 mL/min. Elution times for major peaks in A-D are 15.2, 15.2, 13.0, and 13.9 min, respectively.

concentrated NH_4OH, the reagent used in standard protocols. As a preliminary example, dimers dC(OMe)T, dA(OMe)T, and dG(OMe)T were readily obtained by utilizing an oxalyl-CPG support, a transfer step (pyridine hydrochloride/aniline), and cleavage with 5% NH_4OH in MeOH (3 min).[15]

Acknowledgment. This work was supported by Grant 5R37 GM10265 from the National Institute of General Medical Sciences.

(15) For dA(OMe)T, dC(OMe)T, and dG(OMe)T, respectively: HPLC elution time (conditions in Figure 1) 23.0 and 23.3 min (stereoisomers), 18.5 min, 21.4, and 21.7 min; M + H$^+$ (FAB MS) 570, 546, 586; λ_{max} 262, 268, 256 nm. These compounds were further characterized by conversion to the dinucleoside phosphates by treatment with NH_4OH.

Stereochemical Analysis of a Quasisymmetrical Dialkyl Sulfoxide Obtained by a Diverted Biodehydrogenation Reaction†

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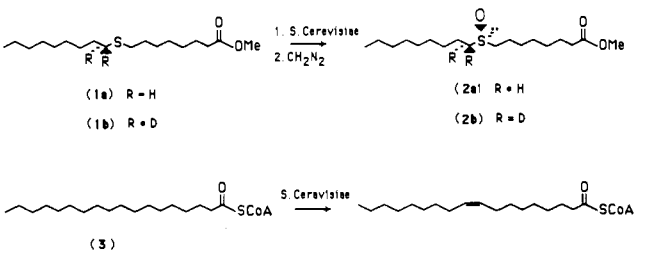
In a previous communication, we have reported that methyl 9-thiastearate (**1a**) is converted to the corresponding sulfoxy acid by cultures of bakers' yeast.[1] It appears that this sulfoxide is the product of a diverted fatty acid desaturase reaction which normally introduces a cis double bond into the hydrocarbon chain of stearoyl-CoA (**3**).[2] We now report that the quasisymmetrical sulfoxide **2a** is produced with very high enantioselectivity and has the *R* configuration.

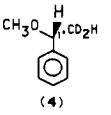
The first step in our stereochemical analysis was to label the C-10 position of the sulfide (**1a**) with deuterium. Thus the dianion of 8-mercaptooctanoic acid was S-alkylated with the tosylate of nonan-1-ol-*1,1-d*$_2$[3] in the manner previously described.[4] The



sample of 9-thiastearic-*10,10-d*$_2$ acid so prepared was methylated by using BF_3/MeOH and the resultant ester (**1b**) purified by flash chromatography (silica gel, 4% EtOAc/hexane).

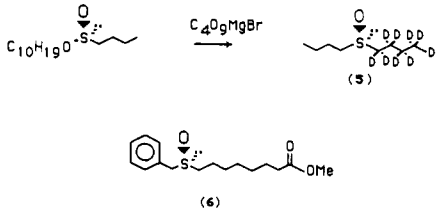
A sample of methyl 9-thiastearate-*10,10-d*$_2$ *S*-oxide (**2b**, 22 mg) was obtained essentially as previously reported[1] by administering **1b** (253 mg) to growing cultures of *Saccharomyces cerevisiae* ATCC 12341.

The optical purity of our biologically produced sulfoxide was assessed by taking advantage of the known ability of carboxylic acids such as trifluoroacetic and acetic acids to shift the ^{1}H NMR signals of diastereotopic protons adjacent to the sulfinyl group.[5] We reasoned that a chiral carboxylic acid might discriminate between the protons at C-8 of (*R*)- or (*S*)-**2b**. Thus addition of 3 equiv of (*S*)-(+)-α-methoxyphenylacetic acid (**4**) to a 20 mM solution of racemic **2b**[6] in $CDCl_3$ caused the ^{1}H NMR signals of one of the diastereotopic protons at C-8 to shift downfield by 0.15 ppm as shown in Figure 1A. That chiral discrimination had



occurred was apparent from the fact that all signals in the resultant ABXY pattern were doubled. When the chiral shift experiment was repeated with biologically produced **2b**, it became clear that the *downfield* half of each doublet had disappeared. (See Figure 1B.) We were thus able to estimate the enantiomeric excess of this material to be >96%.[7]

Our method for assessing optical purity is sufficiently general to allow us to correlate the absolute configuration of **2b** with that of a simpler chiral dialkyl sulfoxide. We thus synthesized a mixture of enantiomeric deuterated dibutyl sulfoxides in which the *R* enantiomer **5** was in excess. This material was prepared



via Grignard attack of (perdeuteriobutyl)magnesium bromide on a diastereomeric mixture of (−)-menthyl 1-butanesulfinates where the major diastereomer is known to bear the *R* configuration at sulfur.[8] The Grignard reaction is known to proceed with inversion of configuration.[8] Combination of the mixture of enantiomeric deuterated dibutyl sulfoxides with our chiral shift reagent (**4**) resulted in a set of NMR signals similar to that obtained for the

† This paper is dedicated to Karl Diedrich.

(1) Buist, P. H.; Dallmann, H. G.; Rymerson, R. R.; Seigel, P. M.; Skala, P. *Tetrahedron Lett.* **1988**, *29*, 435. For a general review of biosulfoxidation, see: Holland, H. L. *Chem. Rev.* **1988**, *88*, 473.

(2) Buist, P. H.; Marecak, D. *Tetrahedron Lett.* **1991**, *32*, 891.

(3) This material was produced by standard $LiAlD_4$ reduction of nonanoic acid.

(4) Buist, P. H.; Dallmann, H. G.; Rymerson, R. R.; Seigel, P. M. *Tetrahedron Lett.* **1987**, *28*, 857.

(5) Nishio, M. *Chem. Pharm. Bull.* **1969**, *17*, 262.

(6) Racemic **2b** was prepared by oxidation of **1b** with MCPBA as previously described.[1]

(7) We have found that DCl-free $CDCl_3$ must be used to determine % ee. Significant racemization occurs when these precautions are not taken as indicated by appearance of the low field set of signals. Interestingly, we observe substantial broadening of the upfield sulfinyl multiplet when very dry solvent is used.

(8) Mislow, K.; Green, M.; Laur, P.; Melillo, J. T.; Simmons, T.; Ternay, A. L., Jr. *J. Am. Chem. Soc.* **1965**, *87*, 1958.

0002-7863/91/1513-5877$02.50/0