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

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(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).


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 NH2 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 nucleoside derivatives containing substituents sensitive to N-deblocking reagents. Such compounds can have potential as selective inhibitors of viral replication and gene expression.5

Nucleoside phosphoramidates 1a-e,g were obtained in ~90% yield by phosphitilating DMT-dA,6 DMT-dC,7 and DMT-dG6 with MeOPCl[N(iPr3)].1 Attempts to prepare these compounds with MeOPCl[N(tert-Pr)] were unsatisfactory since extensive reaction occurred at the amino as well as hydroxyl groups of the nucleosides.

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 S-hydroxyl group of the support-bound thymidine.8 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(GGGGTT), d(GAGTCGTT), and d(CAATTTCAAGTTGTTG), 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 amides 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-GAGTTCAGGT, starting from dT-succinyl-CPG (0.5 µmol of dT). Each cycle consisted of detritylation (3% DCA in CH2Cl2, washing (MeCN), coupling (15 mg of 1a, 1c, 1g, or standard dT phosphoramidite reagent, 0.25 mL of MeCN; 3 min), washing (MeCN), phosphityl transfer (0.1 M pyridine hydrochloride, 0.02 M aniline, MeCN; 5 min), washing (MeCN), oxidation (I2/H2O; 2 min), and washing (CH3Cl). Finally, conventional demethylation, cleavage, and purification by reversed-phase and ion-exchange HPLC afforded the decamer, 8 A260 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 A260 units, 30%) 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 (Tm = 42 °C; 0.1 M NaCl) of the complexes formed with a complementary oligomer, d(ACGTGACCTC). 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.12

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

(11) 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 NH2.

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

(13) For methods, see ref 14.

cleavage with dG(0Me)T were readily obtained by utilizing an oxalyl-CPG 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 CSHSN.HCl/C6HSNH2 transfer step. A C18 ODS column (4.6 x 200 mm) was used, with 0.03 M Et3N-HOAc (pH 7.0) and a CH3CN 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 NH4OH, 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% NH4OH in MeOH (3 min).

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Stereochromatic Analysis of a Quasisymmetrical Diallyl 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 sulfoxide by cultures of bakers' yeast.1 It appears that this sulfoxide is produced with very high enantioselectivity and has the R configuration.

The first step in our stereochromatic analysis was to label the C-10 position of the sulfide (1a) with deuterium. Thus the dianion of 8-mercaptoctanoic acid was S-alkylated with the tosylate of nonan-1-ol-1-d6 in the manner previously described.4 The sample of 9-thiastearic-10,10-d2 acid so prepared was methylated by using BF3/MeOH and the resultant ester (1b) purified by flash chromatography (silica gel, 4% EtOAc/hexane).

A sample of methyl 9-thiastearate-10,10-d2, S-oxide (2b, 22 mg) was obtained essentially as previously reported5 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 1H NMR signals of diastereotopic protons adjacent to the sulfinyl group.6 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 2b6 in CDCl3 caused the 1H 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.)

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 (perdeuteriobuty1)magnesium bromide on a diastereomeric mixture of (−)-menthyl 1-butanesulfinates where the major diastereomer is known to bear the R configuration at sulfur.6 The Grignard reaction is known to proceed with inversion of configuration.6 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.