necticut Department of Chemistry by Marvin R. Thompson. FAB-MS and -HRMS data were measured by Catherine E. Costello and Chen-hui Zeng at the Massachusetts Institute of Technology Mass Spectrometry Facility, which is supported by NIH Grant No. RR00317 (to K. Biemann). This work was supported in part by a grant from Glaxo, Inc.

Registry No. (*R*)-1, 143680-83-5; 2, 143680-84-6; 2′, 143730-66-9; 3, 143730-65-8; 3′, 143730-67-0; (±)-4, 13071-59-5; *cis*-(±)-5, 143669-37-8; *trans*-(+)-5, 143669-36-7; *cis*-(±)-6, 143669-39-0; *trans*-(±)-6, 143669-38-9; (±)-7, 143669-40-3; (±)-8, 143669-41-4; 9, 1731-94-8; 10, 1454-84-8; 11, 17352-32-8; (*E*)-12, 143669-34-5; (*Z*)-12, 143669-33-4; 13, 143669-35-6; 14, 14347-78-5; 14′, 22323-82-6; 16, 17325-85-8; 16′, 56552-80-8; (2*R*,4*R*,1′*R*)-17, 143669-19-6; (2*R*,4*R*,1′*S*)-17, 143669-20-9; (2*S*,4*R*,1′*R*)-17, 143669-17-4; (2*S*,4*R*,1′*S*)-17, 143669-18-5; (2*R*,4*S*,1′*R*)-17′, 143669-28-7; (2*R*,4*S*,1′*S*)-17′, 143669-29-8; (2*S*,4*S*,1′*R*)-17′, 143680-76-6; (2*S*,4*S*,1′*S*)-17′, 143669-30-1; (2*R*,4*R*,1′*R*)-18, 143669-23-2; (2*R*,4*R*,1′*S*)-18, 143669-24-3; (2*S*,4*R*,1′*R*)-18, 143669-21-0; (2*S*,4*R*,1′*S*)-18, 143669-22-1; (2*R*,4*S*,1′*R*)-18′, 143669-31-2; (2*R*,4*S*,1′*S*)-18′, 143705-56-0; (2*S*,4*S*,1′*R*)-18′, 143680-75-5; (2*S*,4*S*,1′*S*)-18′, 143669-32-3; 19, 143669-25-4; 19′, 143730-09-0; 20, 143730-06-7; 20′, 143730-10-3; 21, 143669-26-5; 21′, 143730-11-4; 22, 143669-27-6; 22′, 143730-13-6; 23, 143730-07-8; 23′, 143730-12-5; 24, 143730-08-9; 24′, 143730-14-7; isopropenyl acetate, 108-22-5; β-bromoethyl dichlorophosphate, 4167-02-6; bromoacetaldehyde dimethyl acetal, 7252-83-7.

Synthesis of Diribonucleoside Phosphorothioates via Stereospecific Sulfurization of H-Phosphonate Diesters

H. Almer, J. Stawinski,* R. Strömberg,* and M. Thelin

Department of Organic Chemistry, Arrhenius Laboratory, Stockholm University, S-10691 Stockholm, Sweden

Received May 8, 1992

Sulfurization of diribonucleoside H-phosphonates with elemental sulfur was found to be a stereospecific reaction. With this finding as the basis, an efficient method for the preparation of stereochemically homogeneous diribonucleoside phosphorothioates has been developed. The procedure consists of the synthesis and separation of the diastereomeric (R_p and S_p) pairs of the corresponding H-phosphonate diesters, followed by their stereospecific sulfurization and a single deprotection step using fluoride ion. The methodology has been used in synthesis of eight diribonucleoside phosphorothioates (four pairs of R_p and S_p diastereomers).

Introduction

Dinucleoside phosphorothioates are chiral analogues of phosphodiesters in which one of the nonbridging oxygens has been replaced by sulfur. These compounds are important research tools in stereochemical investigations, in mechanistic studies of various enzymatic reactions, and in other biochemical studies.[1-3] Different affinities of enzymes for compounds with opposite stereochemistry at the phosphorus center usually necessitate the use of optically pure phosphorothioates in biological experiments. Several synthetic methods, based on the phosphodiester,[4] phosphotriester,[5] and phosphite[6] approaches have been designed for the preparation of deoxyribonucleoside phosphorothioates. They involve either separation of phosphorothioate diesters after a final deprotection step or separation of chiral precursors followed by their stereospecific conversion into phosphorothioate diesters. A new promising approach to the chemical synthesis of phosphorothioate oligodeoxyribonucleotides has been reported by Stec et al.[7] It involves separation of suitably protected 2-(deoxyribonucleosid-3′-yloxy)-2-thio-1,3,2-oxathiaphospholanes into diastereomers, followed by their stereospecific reactions with another nucleoside.

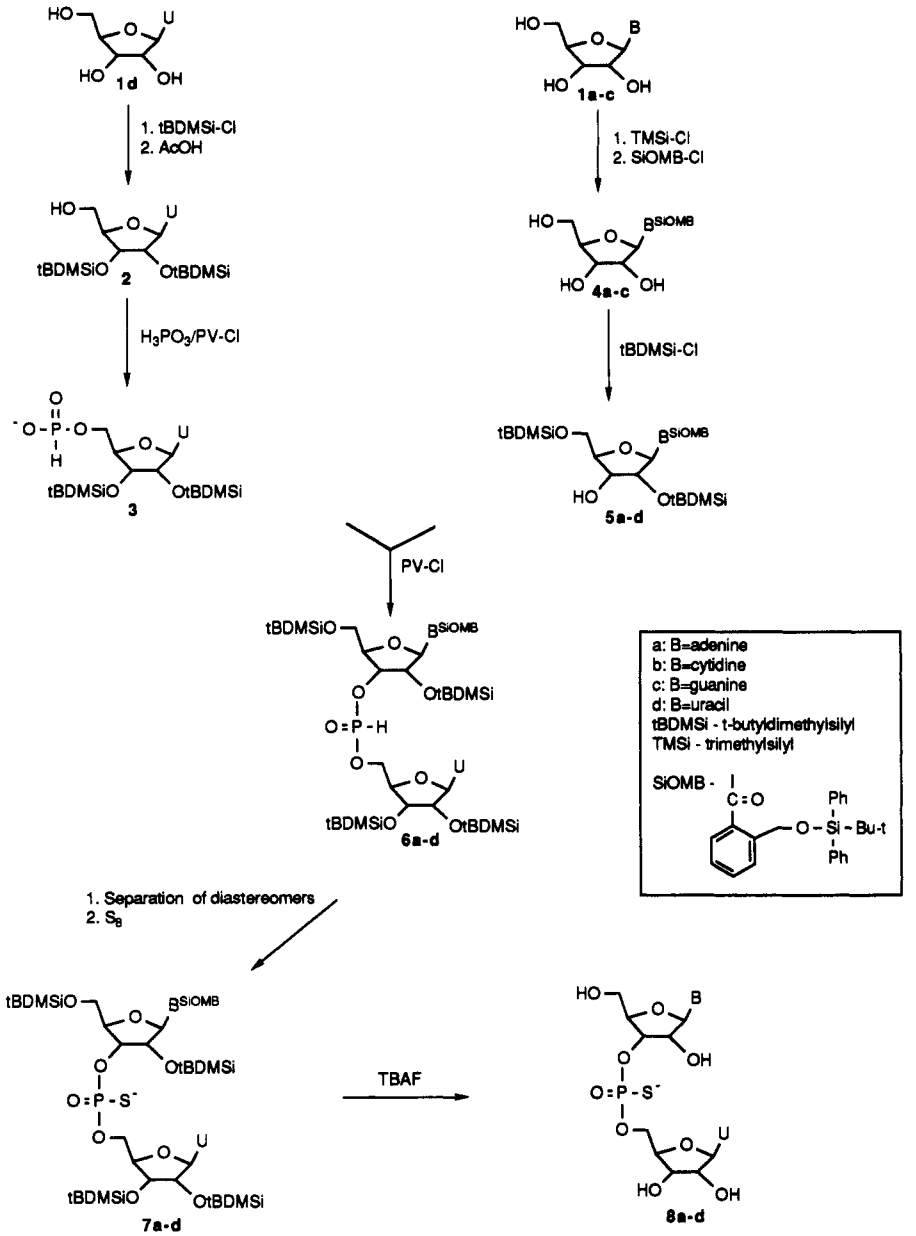
There have, however, only been a few methods reported for the preparation of ribonucleoside phosphorothioates,[8-11] and these are quite laborious and inefficient by comparison to methods available for deoxyribonucleoside phosphorothioate synthesis. Only lately have oligoribonucleotides containing stereochemically defined phosphorothioate functions been produced by chemical means.[12,13]

Recent studies[14-16] have shown that the H-phosphonate approach can be a method of choice for the preparation of phosphorothioate diesters. In this paper we describe an efficient procedure for the preparation of stereochem-

(1) Eckstein, F. *Angew. Chem., Int. Ed. Engl.* **1983**, *22*, 423.
(2) Eckstein, F. *Annu. Rev. Biochem.* **1985**, *54*, 367.
(3) Eckstein, F.; Gish, G. *TIBS* **1989**, 97.
(4) Eckstein, F. *Tetrahedron Lett.* **1967**, 1157.
(5) Malkiewicz, A.; Smrt, J. *Collect. Czech. Chem. Commun.* **1973**, *38*, 2957.
(6) Uznanski, B.; Niewiarowski, W.; Stec, W. J. *Tetrahedron Lett.* **1982**, *23*, 4289.
(7) Stec, W. J.; Grajkowski, A.; Koziolkiewicz, M.; Uznanski, B. *Nucl. Acids Res.* **1991**, *19*, 5883.
(8) Eckstein, F. *Tetrahedron Lett.* **1967**, 3495.
(9) Burgers, P. M. J.; Eckstein, F. *Tetrahedron Lett.* **1978**, 3835.
(10) Burgers, P. M. J.; Eckstein, F. *Biochemistry* **1979**, *18*, 592.
(11) Marlier, J. F.; Benkovic, S. J. *Tetrahedron Lett.* **1980**, *21*, 1121.
(12) Slim, G.; Gait, M. J. *Nucl. Acids Res.* **1991**, *19*, 1183.
(13) Koizumi, M.; Ohtsuka, E. *Biochemistry* **1991**, *30*, 5145.
(14) The first preliminary reports concerning stereochemical aspects in the synthesis of thiophosphates via H-phosphonates were presented at the 9th International Round Table on Nucleosides, Nucleotides & their Biological Application in Uppsala, Sweden, 29/7–3/8, 1990. Results on stereospecific sulfurization (a–c) and stereoselectivity in condensation (a and d) have been published in the proceedings of the conference (*Nucleosides Nucleotides* **1991**, *10*): (a) Stawinski, J.; Strömberg, R.; Thelin, M., p 511. (b) Almer, H.; Strömberg, R., p 653 (later published, ref 13). (c) Seela, F.; Kretschmer, U., p 711 (later published, ref 14). (d) Battistini, C.; Brasca, M. G.; Fustioni, S., p 723 (full paper just published: *Tetrahedron* **1992**, *48*, 3209).
(15) Almer, H.; Strömberg, R. *Tetrahedron Lett.* **1991**, *30*, 3723.
(16) (a) Seela, F.; Kretschmer, U. *J. Chem. Soc. Chem. Commun.* **1990**, 1154; (b) *J. Org. Chem.* **1991**, *56*, 3861.

0022-3263/92/1957-6163$03.00/0

Scheme I



ically homogeneous diribonucleoside phosphorothioates via H-phosphonate intermediates. The method is based on the findings that sulfurization of diribonucleoside H-phosphonates is completely stereospecific and that ribonucleoside H-phosphonate diesters can be conveniently separated into the R_p and S_p diastereomers using ordinary silica gel chromatography.

To minimize the number of synthetic steps on the way to diribonucleoside phosphorothioates we decided to use a set of protecting groups, for both the hydroxyl functions and the heterocyclic bases, that can be removed at the end of the synthesis in a single deprotection step. One suitable choice is to use silyl-containing groups for all position so that mild fluoride treatment can be used for their removal (an approach that has been firmly established to be a safe procedure for ribonucleotides[17,18]). Thus, *tert*-butyldimethylsilyl (t-BDMSi) protection was chosen for alcoholic functions and the 2-[(*tert*-butyldiphenylsiloxy)methyl]-benzoyl (SiOMB) group[19] for exo-amino functions in adenosine, cytidine, and guanosine.

Results and Discussion

A crucial point in the synthesis of optically pure dinucleoside phosphorothioates is separation of diastereomers of precursors or final products that differ only in their configuration around phosphorus. We have previously observed that column chromatography on silica gel is an efficient and convenient way to separate the diastereomers of H-phosphonate diesters such as **6**.[14a,b,15] Since these compounds can be considerably more easily separated than the charged compounds **7** or **8**, we decided to make use of this in a synthetic procedure (see Scheme I). Obviously, separation of diastereomers at the early stages of synthesis would be pointless if one of the subsequent reactions caused epimerization at the phosphorus center.

Sulfurization of the separate diastereomers of 5′-*O*-(*tert*-butyldimethylsilyl)uridin-3′-yl 2′,3′-bis-*O*-(*tert*-butyldimethylsilyl)uridin-5′-yl H-phosphonate (**6d**) with el-

(17) Stawinski, J.; Strömberg, R.; Thelin, M.; Westman, E. *Nucl. Acids Res.* **1988**, *16*, 9285.
(18) Wu, T.; Ogilvie, K. K.; Pon, R. T. *Nucl. Acids Res.* **1989**, *17*, 3501.
(19) (a) Dreef-Tromp, C. M.; Hoogerhout, P.; van der Marel, G. A.; van Boom, J. H. *Tetrahedron Lett.* **1990**, *31*, 427; (b) *Nucl. Acids Res.* **1990**, *18*, 6491.

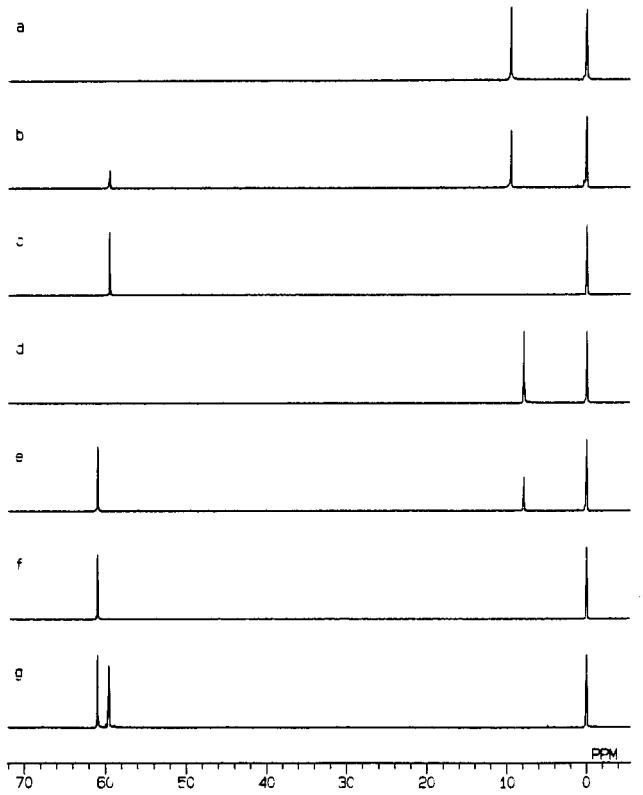


Figure 1. Sulfurization of uridin-3′-yl uridin-5′-yl H-phosphonate (**6d**) with S_8 (ca. 0.15 M, 6 equiv) in pyridine as monitored by ^{31}P NMR spectroscopy. (a) ^{31}P NMR spectrum of **6d**(S_p) (diastereomer migrating faster during silica gel chromatography); (b) ^{31}P NMR spectrum ca. 10 min after addition of sulfur to **6d**(S_p); (c) ^{31}P NMR spectrum after completion of sulfurization of **6d**(S_p) [thus, a spectrum of **7d**(R_p)]; (d) ^{31}P NMR spectrum of the **6d**(R_p) (diastereomer migrating slower during silica gel chromatography); (e) ^{31}P NMR spectrum ca. 40 min after addition of sulfur to **6d**(R_p); (f) ^{31}P NMR spectrum after completion of sulfurization of **6d**(R_p) [thus, a spectrum of **7d**(S_p)]; (g) ^{31}P NMR spectrum after mixing of the two isomers of **7d** [(c) + (f)].

emental sulfur was found to be completely stereospecific (monitored with ^{31}P NMR, see Figure 1) and afforded the corresponding pure isomers of **7d**. Sulfurizations of other investigated dinucleoside H-phosphonates were similarly monitored using ^{31}P NMR, and all reactions were found to proceed with complete stereospecificity. Each isomer of **6a–d** thus produces a pure isomer of the corresponding phosphorothioate diester (**7a–d**). This is contrary to the results obtained when acylphosphonates were treated with butylamine, DBU, and sulfur.[20] An interesting result from that work was that virtually complete stereoselectivity was observed, although only in one particular case.

The stereochemistry of the produced phosphorothioates was unaffected by the deprotection procedure of our synthetic scheme. This was established by subjecting the separate diastereomers of **6d** to sulfurization and subsequent deprotection with tetrabutylammonium fluoride (TBAF) and then analyzing the crude reaction mixtures of **7d** using ^{31}P NMR spectroscopy. These studies confirmed that deprotection proceeded without any epimerization at phosphorus.

Two diastereomers with opposite configurations around phosphorus are formed in almost equal amounts when condensation is carried out between a nucleoside and a deoxynucleoside 3′-H-phosphonate. However, with 2′-*O*-(*tert*)-butyldimethylsilyl (2′-*O*-t-BDMSi) protected ribonucleoside 3′-H-phosphonates[14a,b] (or similarly with 3′-*O*-t-BDMSi protected ribonucleoside 2′-H-phosphonates[14d]), formation of one diastereomer is always favored. This stereoselectivity during condensation is probably due to steric influence from the *tert*-butyldimethylsilyl group in the nucleoside H-phosphonate unit. In initial studies 5′-*O*-(monomethoxytrityl)-2′-*O*-(*tert*-butyldimethylsilyl)uridine 3′-H-phosphonate (25 mM) was condensed with 2′,3′-dibenzoyluridine (1.1 equiv) to produce one diastereomer of the H-phosphonate diester in excess [ca. 80–85% of the isomer resonating at lower field in ^{31}P NMR, see Figure 2 (major isomer δ = 9.64 ppm, minor isomer δ = 8.67 ppm)]. This is a most interesting find that can be of importance for stereoselective synthesis of oligonucleotide analogues. We found that the ratio of diastereomers is insensitive to the choice of coupling agent, within the selection used (i.e., 2–3 equiv of pivaloyl chloride, 1-adamantanecarbonyl chloride, diphenyl phosphorochloridate, or 2,2-dimethylpropane-1,3-diyl phosphorochloridate). Whether neat pyridine or acetonitrile–pyridine (1:1) was used also did not affect the selectivity of the condensation reaction. On the other hand, when a ribonucleoside 5′-H-phosphonate (e.g., 2′,3′-bis-*O*-(*tert*-butyldimethylsilyl)uridin-5′-yl H-phosphonate, **3**) was condensed with a nucleoside having a free 3′-hydroxy group (eg., 3′,5′-bis-*O*-(*tert*-butyldimethylsilyl)uridine, **5d**) the amount of the minor diastereomer was increased from 15–20% to around 40%. These conditions are less interesting from the point of view of stereoselectivity of condensation but may be preferable when comparable amounts of both diastereomers are wanted. Thus, the procedure for synthesis of diribonucleoside H-phosphonates (see Scheme I) was designed to involve condensation of a suitably protected ribonucleoside 5′-H-phosphonate (i.e., **3**) with ribonucleosides having free 3′-hydroxyl functions (i.e., **5a–d**).

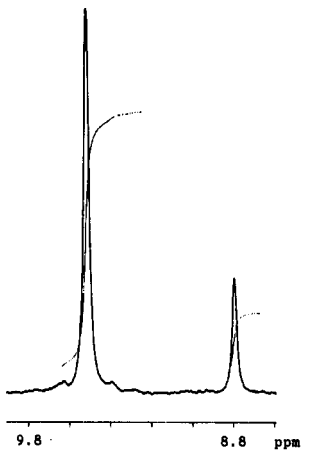


Figure 2. ^{31}P NMR spectrum of a reaction mixture from condensation of 5′-*O*-(monomethoxytrityl)-2′-*O*-(*tert*-butyldimethylsilyl)uridine 3′-H-phosphonate (25 mM) with 2′,3′-dibenzoyluridine (1.1 equiv) using pivaloyl chloride (3 equiv) in pyridine [major isomer (ca. 80–85%) δ = 9.64 ppm, minor isomer δ = 8.67 ppm].

To demonstrate that our approach can be considered as a general method for synthesis of diribonucleoside phosphorothioates we have synthesized eight diribonucleoside phosphorothioates (**8**) containing the four common bases. These are the pairs of R_p and S_p diastereomers of adenosin-3′-yl uridin-5′-yl phosphorothioate [**8a**, Ap(S)U], cytidin-3′-yl uridin-5′-yl phosphorothioate [**8b**, Cp(S)U], guanosin-3′-yl uridin-5′-yl phosphorothioate [**8c**, Gp(S)U], and uridin-3′-yl uridin-5′-yl phosphorothioate [**8d**, Up(S)U].

(20) Fujii, M.; Ozaki, K.; Sekine, M.; Hata, T. *Tetrahedron* **1987**, *43*, 3395.

The route to the key precursors, 2',3'-bis-*O*-(*tert*-butyldimethylsilyl)uridin-5'-yl H-phosphonate (**3**) and the protected ribonucleosides **5a–d**, commences with preparation of the N-protected nucleosides **4a–c** and the nucleoside **2** with a free 5'-hydroxy function. One-pot reactions, involving presilylation of ribonucleosides **1a–c** with trimethylsilyl chloride and treatment with 2-[(*tert*-butyldiphenylsiloxy)methyl]benzoyl chloride[19] (SiOMB-Cl) followed by removal of the trimethylsilyl groups under basic conditions, gave, after silica gel chromatography, the N-protected ribonucleosides **4a–c** in ca. 90% yield.

Conversion of **4a–c** into 2',5'-di-*tert*-butyldimethylsilylated nucleosides **5a–d** and preparation of the 2',3'-disilylated nucleoside **2** were carried out according to Ogilvie et al.[21] That the t-BDMSi groups were attached to the 2' and 5' positions in **5a–d** was confirmed by ^{1}H NMR[22] and by analysis of changes in chemical shifts of the ribose protons after conversion of **5a–d** into the corresponding (trichloroacetyl)carbamate derivatives.[23] The 5'-H-phosphonate **3** was prepared using a phosphonylation procedure recently developed for the synthesis of deoxyribonucleoside 3'-H-phosphonates.[24] This involves condensation of the nucleoside **2** with phosphonic acid in the presence of pivaloyl chloride, followed by aqueous workup and silica gel chromatography (yield ~80%).

Formation of the H-phosphonate internucleotidic bond was accomplished via condensation of **3** with each of the four different 2',5'-bis(*tert*-butyldimethylsilyl) N-protected nucleosides **5a–d**. All reactions were carried out in pyridine using pivaloyl chloride as coupling agent. The diastereomeric pairs of H-phosphonates **6a–d** were separated into individual isomers by silica gel chromatography. Good separation was achieved using a toluene–ethyl acetate system as eluent, except for **6b**, where chromatography with a chloroform–acetone system was more successful in separating the diastereomers. The isomerically pure compounds **6a–d** were sulfurized with S_8 in pyridine to produce the corresponding phosphorothioate diesters **7a–d**.

With a 0.05 M solution of sulfur (2 equiv relative to **6**) the reaction was quite slow and, although otherwise clean, accompanied by slight (less than 1–2%) cleavage of the H-phosphonate diesters. An increase in concentration of sulfur to 0.1–0.15 M (4–6 equiv relative to **6**) was sufficient to bring down the level of competing cleavage so that it was no longer detectable (^{31}P NMR, TLC), and the sulfurization was also complete in about 2–3 h under such reaction conditions.

Deprotection of phosphorothioates **7a–d** was accomplished by treatment with tetrabutylammonium fluoride (TBAF) or triethylammonium hydrofluoride (TEAHF) in THF. This was followed by exchange of the counterions in **8a–d** [Dowex 50X (Na^+) or SP Sephadex G25 (Na^+)] and their final desalting (Sephadex G10 column).

All pairs of diastereomers of **8a–d** were subjected to enzymatic hydrolysis using snake venom phosphodiesterase (SVPD). Only compounds obtained from sulfurization of **6a–d** diastereomers resonating at a lower field in ^{31}P NMR were substrates for the enzyme. This identifies the stereochemistry around phosphorus in these compounds as R_p.[10,25] Since sulfurization of **6** is likely to take place with retention, it follows that the diastereomers of ribonucleoside H-phosphonates **6a–d** resonating at a lower field in ^{31}P NMR (and migrating faster during silica gel chromatography) should have the S_p configuration at the phosphorus center. By the same token, the absolute configuration of the diastereomers of H-phosphonates **6a–d** resonating at higher field in ^{31}P NMR (and migrating slower during silica gel chromatography) should be R_p.

Table I. ^{31}P NMR Shifts[a] of Diribonucleoside H-Phosphonates 6 and Diribonucleoside Phosphorothioates 7 and 8

base sequence in 6, 7, or 8	H-phosphonate 6[b]	phosphorothioate 7[b]	phosphorothioate 8[c]
AU	9.46 (S_p) $^1J_{PH}$ = 728 Hz	60.1 (R_p)	56.7 (R_p)
AU	8.17 (R_p) $^1J_{PH}$ = 715 Hz	60.9 (S_p)	55.7 (S_p)
CU	9.60 (S_p) $^1J_{PH}$ = 724 Hz	59.9 (R_p)	57.0 (R_p)
CU	8.18 (R_p) $^1J_{PH}$ = 715 Hz	61.1 (S_p)	56.0 (S_p)
GU	9.09 (S_p) $^1J_{PH}$ = 728 Hz	60.0 (R_p)	56.5 (R_p)
GU	8.64 (R_p) $^1J_{PH}$ = 715 Hz	61.0 (S_p)	55.8 (S_p)
UU	9.50 (S_p) $^1J_{PH}$ = 728 Hz	59.7 (R_p)	56.7 (R_p)
UU	7.88 (R_p) $^1J_{PH}$ = 719 Hz	60.9 (S_p)	56.1 (S_p)

[a] 2% H_3PO_4 in D_2O as an external reference. [b] Chemical shifts in ppm (pyridine). [c] Chemical shifts in ppm (D_2O).

The ^{31}P NMR chemical shifts of diastereomeric pairs of **6a–d**, **7a–d**, and **8a–d** are collected in Table I. It is apparent that all R_p isomers of the dinucleoside phosphorothioates **8a–d** resonate at a lower field than the corresponding S_p isomers. This is consistent with the postulation[1] that correlates absolute configurations of dinucleoside phosphorothioates with their chemical shifts in ^{31}P NMR. For protected phosphorothioates **7a–d** the opposite tendency is observed, but this may not be a particularly general phenomenon since the nature of protecting groups and their position in the molecule can affect the ^{31}P NMR shift of R_p and S_p diastereomers in different ways.

Also for the parent H-phosphonates **6a–d**, the *Sp* isomers resonate at a lower field compared to the R_p ones. We are not aware of any exception to this correlation, so far, but the same reservations as those mentioned for protected thiophosphates are probably valid. However, taking into account that the difference in chemical shifts for the H-phosphonate diastereomers is much larger than for the corresponding phosphorothioates, the correlation of absolute stereochemistry with ^{31}P chemical shifts of protected compounds may hold stronger in the former case.[26]

As pointed out by one reviewer, $^1J_{PH}$ coupling constants may also be considered as a parameter of diagnostic value for assigning absolute configurations of H-phosphonate diesters.[27] However, in light of available data this correlation seems less general than that involving ^{31}P NMR chemical shifts.[28]

(21) Hakimelahi, G. H.; Proba, Z. A.; Ogilvie, K. K. *Can. J. Chem.* **1982**, *60*, 1106.
(22) Ogilvie, K. K. In *Nucleosides, Nucleotides and their Biological Applications*; Rideout, J. L., Henry, D. W., Beacham, L. M., III, Eds.; Academic Press, Inc.: New York, 1983, p 209.
(23) Goodlett, V. W. *Anal. Chem.* **1965**, *37*, 431.
(24) Stawinski, J.; Thelin, M. *Nucleosides Nucleotides* **1990**, *9*, 129.
(25) Bryant, F. R.; Benkovic, S. J. *Biochemistry* **1979**, *18*, 2825.
(26) Indeed, the observed correlation seems to be valid also for deoxyribonucleoside H-phosphonate diesters as well as for ribo- and deoxyribonucleoside H-phosphonothioates [see ref 14 and also Stawinski, J.; Strömberg, R.; Zain, R. *Tetrahedron Lett.* **1992**, *33*, 3185–3188. Stawinski, J.; Thelin, M. *Tetrahedron Lett.* **1992**, *33*, 3189–3192].
(27) The usefulness of this kind of parameter in structural studies of some cyclic phosphorus compounds has already been reported. See: Gorenstein, D. G. In *Phosphorus-31 NMR. Principles and Applications*; Gorenstein, D. G., Ed.; Academic Press, Inc.: London, 1984; pp 37–53.

In conclusion, sulfurization of suitably protected diribonucleoside H-phosphonates with elemental sulfur was found to be a stereospecific reaction, which is most likely proceeding with retention of configuration at the phosphorus center. This stereospecific sulfurization of diribonucleoside H-phosphonate diesters, followed by deprotection in a single step, presents a simple and efficient way for the preparation of optically pure diribonucleoside phosphorothioate diesters. The method is experimentally simple, efficient, and can be considered as a general procedure for synthesis of dinucleoside phosphorothioates.

Optically pure precursors of type 6 also have a large potential for use (after proper synchronization of protecting groups) in the preparation of stereochemically defined synthons for incorporation of chiral internucleotidic linkages (e.g. phosphorothioates, phosphoroselenoates, phosphoroamidates, etc.) into oligonucleotides. This is currently investigated in this laboratory.

Experimental Section

Materials and Methods. Pyridine, acetonitrile, and triethylamine (TEA) were refluxed with CaH_2 and then distilled and stored over molecular sieves (4Å) or CaH_2 (TEA). Elemental sulfur, *tert*-butyldimethylsilyl chloride (t-BDMSi-Cl), and phosphonic acid were all commercial grade (Aldrich). Pivaloyl chloride was distilled under atmospheric pressure and stored at −20 °C in a sealed flask. 2-[(*tert*-Butyldiphenylsiloxy)methyl]-benzoyl chloride was freshly prepared prior to use from the appropriate acid according to van Boom et al.[19] Compounds **2** and **5d** were synthesized according to published procedures.[18] Nucleosides and snake venom phosphodiesterase (SVPD, *Crotalus atrox*) were purchased from Sigma. Reactions monitored by ^{31}P NMR were carried out in 10-mm NMR tubes. All nucleosides, nucleoside 3′-H-phosphonates, and phosphonic acid were rendered anhydrous by evaporation of added pyridine before being used for reactions.

2′,3′-Bis-*O*-(*tert*-butyldimethylsilyl)uridin-5′-yl Hydrogen Phosphonate, Triethylammonium Salt (3). To a solution of phosphonic acid (3.28 g, 40 mmol) and 2′,3′-bis-*O*-(*tert*-butyldimethylsilyl)uridine (3.78 g, 8 mmol) in anhydrous pyridine (50 mL) was added pivaloyl chloride (2.46 mL, 20 mmol) with stirring, and the progress of the reaction was followed by TLC. The reaction was quenched after 3 h by careful addition of 2 M triethylammonium bicarbonate buffer (TEAB, 20 mL). After evolution of carbon dioxide ceased the reaction mixture was evaporated to dryness and the residue partitioned between chloroform and 0.5 M TEAB. The organic layer was evaporated and the residue purified on a silica gel column (flash mode) using a stepwise gradient of methanol in chloroform (2–20%) as eluent. Yield: 4.12 g (80%). Anal. Calcd for $C_{27}H_{56}N_3O_8PSi_2 \cdot {}^1/_2H_2O$: C, 50.1; H, 8.9; P, 4.8. Found: C, 50.0; H, 8.9; P, 4.8. ^{31}P NMR (CH_3CN/MeOH, 4:1; δ in ppm): 3.38 ($^1J_{PH}$ = 611 Hz, $^3J_{PH}$ = 6.1 Hz). ^1H NMR ($CDCl_3$; δ in ppm): 8.14 (d, ^3J = 8.06 Hz, 1 H, 6-H), 6.90 (d, ^1J = 618 Hz, 1 H, P-H), 5.89 (d, ^3J = 4.03 Hz, 1 H, 1′-H), 5.72 (d, ^3J = 8.06 Hz, 1 H, 5-H), 4.2–3.9 (m, 5 H, 2′-H, 3′-H, 4′-H, 5′-H), 2.96 (q, ^3J = 7.3 Hz, 6 H, CH_2-N), 1.27 (t, ^3J = 7.3 Hz, 9 H, CH_3CH_2N), 0.90 and 0.88 (2 s, 18 H, t-Bu), 0.099, 0.087. 0.079, and 0.066 (4 s, 12 H, Me-Si).

6-*N*-[2-[(*tert*-Butyldiphenylsiloxy)methyl]benzoyl]-adenosine (4a). A solution of adenosine (1.87 g, 7 mmol) in anhydrous pyridine (35 mL) was chilled on an ice bath, and trimethylsilyl chloride (6.22 mL, 49 mmol) was added with stirring. The ice bath was then removed, and after 1 h 2-[(*tert*-butyldiphenylsiloxy)methyl]benzoyl chloride (prepared from 15 mmol of the corresponding acid) in a small amount of toluene (≈5 mL) was added. After standing overnight the reaction mixture was cooled down on an ice bath and water (7 mL) was added, followed by 32% aqueous ammonia (14 mL) and enough ethanol to obtain a homogeneous solution. After 2 h the mixture was evaporated, the residue was partitioned between equal volumes of water and a mixture of dichloromethane/methanol (8:2, v/v). The organic layer was dried with Na_2SO_4, evaporated to dryness, and purified on a silica gel column using a stepwise gradient of methanol in chloroform (5–20%) as eluent. Yield: 3.86 g (86%). Anal. Calcd for $C_{34}H_{37}N_5O_6Si \cdot {}^1/_2H_2O$: C, 62.9; H, 5.9. Found: C, 63.4; H, 5.9. ^1H NMR ($CDCl_3$/CD_3OD, 4:1; δ in ppm): 8.68 (s, 1 H, 8), 8.21 (s, 1 H, 2), 7.8–7.3 (m, 14 H, aromatic protons), 5.92 (d, ^3J = 6.6 Hz, 1 H, 1′-H), 5.13 (s, 2 H, CH_2), 4.81 (dd, ^3J = 6.7 Hz and ^3J = 5.2, 1 H, 2′-H), 4.30 (dd, ^3J = 1.7 Hz and ^3J = 5.0, 1 H, 3′-H), 4.24 (m, 1 H, 4′-H), 3.97 (dd, ^2J = 12.7 Hz, ^3J = 1.9 Hz, 1 H, 5′-H), 3.77 (dd, ^2J = 12.7 Hz, ^3J = 1.8 Hz, 1 H, 5′-H), 1.06 (s, 9 H, t-Bu).

4-*N*-[2-[(*tert*-Butyldiphenylsiloxy)methyl]benzoyl]cytidine (4b). A solution of cytidine (2.43 g, 10 mmol) in anhydrous pyridine (75 mL) was chilled on an ice bath, and trimethylsilyl chloride (7.6 mL, 60 mmol) was added with stirring. The ice bath was then removed, and after 1 h 2-[(*tert*-butyldiphenylsiloxy)-methyl]benzoyl chloride (prepared from 15 mmol of the corresponding acid) in a small amount of toluene (≈ 5 mL) was added. After the mixture was allowed to stand overnight, water (20 mL) was added and the resulting suspension was then coevaporated twice with pyridine–water (1:1, v/v) in order to hydrolyze the trimethylsilyl esters (TLC analysis). The residue was then partitioned between ethyl acetate (containing 1% ethanol) and saturated aqueous $NaHCO_3$ solution, and the organic layer was dired with Na_2SO_4, evaporated to dryness, and purified on a silica gel column using a stepwise gradient of methanol in chloroform (5–10%) as eluent. Yield: 5.49 g (89%). Anal. Calcd for $C_{33}H_{37}N_3O_7Si \cdot {}^1/_2H_2O$: C, 63.4; H, 6.1. Found: C, 63.6; H, 6.1. ^1H NMR ($CDCl_3$/CD_3OD, 4:1; δ in ppm): 8.30 (d, ^3J = 7.3 Hz, 1 H, 6), 7.7–7.3 (m, 15 H, aromatic protons and 5-H), 5.75 (d, ^3J = 3.2 Hz, 1 H, 1′-H), 5.05 (s, 2 H, CH_2), 4.20 (dd, ^3J = 3.7 Hz, and ^3J = 5.2 Hz, 1 H, 2′-H), 4.15 (t, ^3J = 5.1 Hz, 1 H, 3′-H), 4.06 (m, 1 H, 4′-H), 3.84 (dd, ^2J = 12.6 Hz, ^3J = 2.1 Hz, 1 H, 5′-H), 3.69 (dd, ^2J = 12.6 Hz, ^3J = 2.3 Hz, 1 H, 5′-H), 1.01 (s, 9 H, t-Bu).

2-*N*-[2-[(*tert*-Butyldiphenylsiloxy)methyl]benzoyl]-guanosine (4c). A solution of guanosine (4.25 g, 15 mmol) in anhydrous pyridine (100 mL) was cooled down on an ice bath, and trimethylsilyl chloride (13.3 mL, 105 mmol) was added with stirring. The ice bath was removed, and after 1 h 2-[(*tert*-butyldiphenylsiloxy)methyl]benzoyl chloride (prepared from 22.5 mmol of the corresponding acid) in a small amount of toluene (≈ 5 mL) was added. After the mixture was allowed to stand overnight methanol (2 mL) was added in order to consume excess of SiOMB-Cl, and the reaction mixture was evaporated to dryness. The residue was dissolved in ethyl acetate (300 mL), washed with saturated aqueous NaCl (2 × 75 mL), dried with Na_2SO_4, and evaporated. The trimethylsilyl groups were removed by dissolving the residue in cold dioxane (75 mL) and then adding 1 M sodium methoxide in methanol (60 mL). After 15 min the deprotection was over (TLC), and the pH of the reaction mixture was adjusted to 7 by addition of acetic acid. Methylene chloride was added, and the solution was washed with saturated brine (2 × 50 mL), dried, and evaporated to dryness. The crude **4b** was then purified on a silica gel column using chloroform–methanol (9:1, v/v) as eluent. Yield: 8.69 g (88%). Anal. Calcd for $C_{34}H_{37}N_5O_7Si$: C, 62.3; H, 5.7. Found: C, 62.2; H, 5.7. ^1H NMR ($CDCl_3$/CD_3OD, 4:1; δ in ppm): 8.08 (s, 1 H, 8-H), 7.7–7.3 (m, 14 H, aromatic protons), 5.87 (d, ^3J = 5.5 Hz, 1 H, 1′-H), 4.57 (s, 2 H, CH_2), 4.55 (t, ^3J = 5.3 Hz, 1 H, 2′-H), 4.39 (dd, ^3J = 5.3 and 3.7 Hz, 1 H, 3′-H), 4.11 (m, 1 H, 4′-H), 3.88 (dd, ^2J = 12.3 Hz, ^3J = 2.6 Hz, 1 H, 5′-H), 3.75 (dd, ^2J = 12.3 Hz, ^3J = 2.4 Hz, 1 H, 5′-H), 1.07 (s, 9 H, t-Bu).

2′,5′-Bis-*O*-(*tert*-butyldimethylsilyl)-6-*N*-[2-[(*tert*-butyldiphenylsiloxy)methyl]benzoyl]adenosine (5a). To a solution of **4a** (1.92 g, 3 mmol) in freshly distilled THF (70 mL) were added pyridine (1.45 mL, 18 mmol), silver nitrate (1.53 g, 9 mmol), and *tert*-butyldimethylsilyl chloride (1.36 g, 9 mmol), and the mixture was then left overnight with vigorous stirring. The reaction mixture was filtered through Celite, the solvent was removed by evaporation, and the residue was partitioned between toluene and $NaHCO_3$(aq). The organic layer was then dried with Na_2SO_4 and evaporated, and the crude **5a** was purified on a silica gel column using a stepwise gradient of ethyl acetate in toluene (20–35%) as eluent. Yield: 1.41 g (38%). The 3′,5′ isomer: 0.51 g (20%). Anal. Calcd for $C_{46}H_{65}N_5O_6Si_3 \cdot {}^1/_2H_2O$: C, 63.0; H, 7.6.

(28) $|^1J_{P-H}(S_p)| > |^1J_{P-H}(R_p)|$ for ribonucleoside H-phosphonate and H-phosphonothioate diesters with 2′-*O*-*t*-BDMSi protection, but seems to be opposite ($|^1J_{P-H}(S_p)| < |^1J_{P-H}(R_p)|$) for deoxyribonucleoside H-phosphonates and H-phosphonothioates (J. Stawinski, R. Strömberg, M. Thelin, R. Zain, unpublished results).

Found: C, 62.9; H, 7.6. ^{1}H NMR ($CDCl_3$; δ in ppm): 8.8 (s, 1 H, 8), 8.4 (s, 1 H, 2), 7.8–7.3 (m, 14 H, aromatic protons), 6.2 (d, ^3J = 5.1 Hz, 1 H, 1′-H), 5.1 (s, 2 H, CH_2), 4.6 (t, ^3J = 5.1 Hz, 1 H, 2′-H), 4.28 (m, 1 H, 3′-H), 4.24 (m, 1 H, 4′-H), 4.0 (dd, ^2J = 11.7 Hz, ^3J = 2.6 Hz, 1 H, 5′-H), 3.84 (dd, ^2J = 11.7 Hz, ^3J = 2.6 Hz, 1 H, 5″-H), 1.1, 0.9, and 0.8 (3 s, 27 H, t-Bu), 0.1–0.0 (4 s, 12 H, CH_3-Si). After addition of trichloroacetyl isocyanate (TAI), ^{1}H NMR (only data for ribose protons shown): 6.22 (d, ^3J = 7.0 Hz, 1 H, 1′-H), 5.45 (d, ^3J = 4.8 Hz, 1 H, 3′-H), 4.85 (dd, ^3J = 7.0 and 5.1 Hz, 1 H, 2′-H), 4.39 (m, 1 H, 4′-H), 4.00 (dd, ^2J = 11.4 Hz, ^3J = 2.6 Hz, 1 H, 5′-H), 3.91 (dd, ^2J = 11.4 Hz, ^3J = 2.6 Hz, 1 H, 5″-H).

2′,5′-Bis-*O*-(*tert*-butyldimethylsilyl)-4-*N*-[2-[(*tert*-butyldiphenylsiloxy)methyl]benzoyl]cytidine (5b). To a stirred solution of **4b** (3.08 g, 5 mmol) in THF (100 mL) were added silver nitrate (1.87 g, 11 mmol), pyridine (2 mL, 25 mmol), and *tert*-butyldimethylsilyl chloride (1.87 g, 12 mmol). After ca. 5 h additional amounts of $AgNO_3$ (0.5 g, 3 mmol) and t-BDMSi-Cl (0.5 g, 3 mmol) were added. The reaction mixture was left stirring overnight and subsequently filtered through Celite. The solvent was then removed by evaporation and the residue partitioned between toluene and aqueous $NaHCO_3$. The organic layer was dried with Na_2SO_4 and evaporated, and the crude **5b** was then purified on a silica gel column using toluene–ethyl acetate (4:1, v/v) as eluent. Yield: 2.21 g (52%). The 3′,5′ isomer: 0.87 g (21%). Anal. Calcd for $C_{45}H_{65}N_3O_7Si_3 \cdot H_2O$: C, 62.7; H, 7.8. Found: C, 62.7; H, 7.8. ^{1}H NMR ($CDCl_3$; δ in ppm): 8.46 (d, ^3J = 7.3 Hz, 1 H, 6), 7.7–7.3 (m, 15 H, aromatic protons and H-6), 5.98 (d, ^3J = 2.2 Hz, 1 H, 1′-H), 4.99 (s, 2 H, CH_2), 4.2–4.0 (m, 4 H, 2′-H, 3′-H, 4′-H, and 5′-H), 3.88 (dd, ^2J = 11.7 Hz, ^3J = 1.5 Hz, 1 H, 5′), 1.06, 0.95, and 0.94 (3 s, 27 H, t-Bu), 0.24, 0.16, 0.15, and 0.14 (4 s, 12 H, CH_3-Si). After addition of trichloroacetyl isocyanate (TAI), ^{1}H NMR (only data for ribose protons shown): 6.19 (d, ^3J = 4.8 Hz, 1 H, 1′-H), 5.20 (dd, ^3J = 4.4 and 4.7 Hz, 1 H, 3′-H), 4.44 (dd, ^3J = 5.1 and 4.8 Hz, 1 H, 2′-H), 4.37 (m, 1 H, 4′-H), 4.04 (dd, ^2J = 11.7 Hz, ^3J = 1.8 Hz, 1 H, 5′-H), 3.86 (dd, ^2J = 11.7 Hz, ^3J = 1.6 Hz, 1 H, 5′-H).

2′,5′-Bis-*O*-(*tert*-butyldimethylsilyl)-2-*N*-[2-[(*tert*-butyldiphenylsiloxy)methyl]benzoyl]guanosine (5c). To a vigorously stirred solution of **4c** (1.0 g, 1.52 mmol) in freshly distilled THF (40 mL) were added silver nitrate (0.646 g, 3.8 mmol), pyridine (610 mL, 7.6 mmol), and *tert*-butyldimethylsilyl chloride (573 mg, 3.8 mmol). After 4 h additional amounts of $AgNO_3$ (0.130 g, 0.75 mmol) and t-BDMSi-Cl (0.115 g, 0.75 mmol) were added. After standing overnight the reaction mixture was filtered through Celite, the solvent was evaporated, and the residue was partitioned between toluene and staturated aqueous $NaHCO_3$. The organic layer was collected, dried with Na_2SO_4, and evaporated, and the crude material purified on a silica gel column using a stepwise gradient of acetone in methylene chloride (2–10%) as eluent. Yield: 0.530 g (39%). The 3′,5′ isomer: 0.280 g (21%). Anal. Calcd for $C_{46}H_{65}N_5O_7Si_3$: C, 62.5; H, 7.4. Found: C, 62.3; H, 7.5. ^{1}H NMR ($CDCl_3$; δ in ppm): 8.09 (s, 1 H, 8), 7.8–6.8 (m, 14 H, aromatic protons), 5.93 (d, ^3J = 6.7 Hz, 1 H, 1′-H), 4.93 (2 d, 2 H, CH_2), 4.50 (t, ^3J = 6.1 Hz, 1 H, 2′-H), 4.2–4.1 (m, 2 H, 3′-H and 4′-H), 3.89 (dd, ^2J = 11.4 Hz, ^3J = 2.0 Hz, 1 H, 5′-H), 3.80 (dd, ^2J = 11.4 Hz, ^3J = 2.0 Hz, 1 H, 5″-H), 1.11, 0.96, and 0.86 (3 s, 27 H, t-Bu), 0.15 to –0.1 (4 s, 12 H, CH_3Si). After addition of trichloroacetyl isocyanate (TAI), ^{1}H NMR (only data from ribose protons shown): 5.90 (d, ^3J = 7.5 Hz, 1 H, 1′-H), 5.38 (d, ^3J = 5.5 Hz, 1 H, 3′-H), 4.64 (dd, ^3J = 7.5 and 5.5 Hz, 1 H, 2′-H), 4.26 (m, 1 H, 4′-H), 3.88 (m, 2 H, 5′- and 5″-H).

A General Procedure for the Synthesis of Diribonucleoside H-Phosphonates 6. To a stirred solution of **3** (1.1 mmol) and **5** (1 mmol) in anhydrous pyridine (10 mL) was added pivaloyl chloride (3 mmol). When TLC analysis indicated that the reaction was over, 2 M TEAB (1 mL) was added. The reaction mixture was concentrated and the residue partitioned between chloroform and 0.5 M TEAB. The organic layer was collected, dried with Na_2SO_4, and evaporated. The crude diastereomeric mixture of **6** was then purified on a silica gel column using a stepwise gradient of ethyl acetate in toluene (30–50%) (**6a,c–d**) or chloroform–acetone (18:3, v/v) (**6b**) as eluent.

2′,5′-Bis-*O*-(*tert*-butyldimethylsilyl)-6-*N*-[2-[(*tert*-butyldiphenylsiloxy)methyl]benzoyl]adenosin-3′-yl 2′,3′-Bis-*O*-(*tert*-butyldimethylsilyl)uridin-5′-yl Hydrogen Phosphonate (S_p Isomer) [6a(S_p)]: faster moving isomer (from **3** + **5a**). Yield: 1.07 g (52%). ^{31}P NMR data, see Table I.

2′,5′-Bis-*O*-(*tert*-butyldimethylsilyl)-6-*N*-[2-[(*tert*-butyldiphenylsiloxy)methyl]benzoyl]adenosin-3′-yl 2′,3′-Bis-*O*-(*tert*-butyldimethylsilyl)uridin-5′-yl Hydrogen Phosphonate (R_p Isomer) [6a(R_p)]: slower moving isomer (from **3** + **5a**). Yield: 0.61 g (29%). ^{31}P NMR data, see Table I.

2′,5′-Bis-*O*-(*tert*-butyldimethylsilyl)-4-*N*-[2-[(*tert*-butyldiphenylsiloxy)methyl]benzoyl]cytidin-3′-yl 2′,3′-Bis-*O*-(*tert*-butyldimethylsilyl)uridin-5′-yl Hydrogen Phosphonate (S_p Isomer) [6b(S_p)]: faster moving isomer (from **3** + **5b**). Yield: 0.852 g (63%). ^{31}P NMR data, see Table I.

2′,5′-Bis-*O*-(*tert*-butyldimethylsilyl)-4-*N*-[2-[(*tert*-butyldiphenylsiloxy)methyl]benzoyl]cytidin-3′-yl 2′,3′-Bis-*O*-(*tert*-butyldimethylsilyl)uridin-5′-yl Hydrogen Phosphonate (R_p Isomer) [6b(R_p)]: slower moving isomer (from **3** + **5b**). Yield: 0.410 g (30%). ^{31}P NMR data, see Table I.

2′,5′-Bis-*O*-(*tert*-butyldimethylsilyl)-2-*N*-[2-[(*tert*-butyldiphenylsiloxy)methyl]benzoyl]guanosin-3′-yl 2′,3′-Bis-*O*-(*tert*-butyldimethylsilyl)uridin-5′-yl Hydrogen Phosphonate (S_p Isomer) [6c(S_p)]: faster moving isomer (from **3** + **5c**). Yield: 0.885 g (42%). ^{31}P NMR data, see Table I.

2′,5′-Bis-*O*-(*tert*-butyldimethylsilyl)-2-*N*-[2-[(*tert*-butyldiphenylsiloxy)methyl]benzoyl]guanosin-3′-yl 2′,3′-Bis-*O*-(*tert*-butyldimethylsilyl)uridin-5′-yl Hydrogen Phosphonate (R_p Isomer), [6c(R_p)]: slower moving isomer (from **3** + **5c**). Yield: 0.552 g (26%). ^{31}P NMR data, see Table I.

2′,5′-Bis-*O*-(*tert*-butyldimethylsilyl)uridin-3′-yl 2′,3′-Bis-*O*-(*tert*-butyldimethylsilyl)uridin-5′-yl Hydrogen Phosphonate (S_p Isomer) [6d(S_p)]: faster moving isomer (from **3** + **5d**). Yield: 0.572 g (58%). ^{31}P NMR data, see Table I.

2′,5′-Bis-*O*-(*tert*-butyldimethylsilyl)uridin-3′-yl 2′,3′-Bis-*O*-(*tert*-butyldimethylsilyl)uridin-5′-yl Hydrogen Phosphonate (R_p Isomer) [6d(R_p)]: slower moving isomer (from **3** + **5d**). Yield: 0.353 g (35%). ^{31}P NMR data, see Table I.

A General Procedure for the Synthesis of Diribonucleoside Phosphorothioates 8. Each R_p or S_p diastereomer of **6a–d** (50 mmol) was dissolved in pyridine (2 mL), and elemental sulfur (6.5 mg, 4 equiv) was added. The reaction mixture was stirred for 2–3 h (TLC), pyridine was evaporated, and the residue was passed through a short silica gel column using a stepwise gradient of methanol in chloroform (2–20%) as eluent. The isolated diastereomers of the phosphorothioates **7a–d** were then deprotected according to method a or b.

Method a. Each separate diastereomer of **7a–d** was dissolved in 1 M triethylammonium hydrofluoride (TEAHF) in THF (4 mL) and left with stirring for 3 days. The reaction mixture was then partitioned between water and diethyl ether. The aqueous layer was collected, evaporated briefly to remove traces of organic solvent, and lyophilized. The sample was redissolved in water, passed through a Sephadex C-25 Na^+ column, desalted on a Sephadex G 10 column, and finally lyophilized.

Method b. Each separate diastereomer of **7a–d** was dissolved in 1 M tetrabutylammonium fluoride (TBAF) in THF (0.5 mL) and left with stirring over night. Further workup as in method a with the exception that Dowex 50-X2 Na^+ (100–200 mesh) was used instead of Sephadex C-25.

Adenosin-3′-yl Uridin-5′-yl phosphorothioate (R_p Isomer) [8a(R_p), Ap(S)U(R_p)] [from 6a(S_p)]. Yield: 25 mg (81%). ^{31}P NMR data, see Table I. ^{1}H NMR (D_2O; δ in ppm): 8.35 (s, 1 H, 8-H_A), 8.23 (s, 1 H, 2-H_A), 7.88 (d, ^3J = 8.1 Hz, 1 H, 6-H_U), 6.09 (d, ^3J = 6.7 Hz, 1 H, 1′-H_A), 5.77 (d, ^3J = 3.7 Hz, 1 H, 1′-H_U), 5.71 (d, ^3J = 8.1 Hz, 1 H, 5-H_U), 4.9 (m, 2 H, 2′-H_A, 3′-H_A), 4.3–4.1 (m, 6 H, 2′-H_U, 3′-H_U, 4′-H_U, 5′-H_U, 4′-H_A), 4.04 (dd, ^2J = 13.2 Hz, ^3J = 2.6 Hz, 1 H, 5′-H_A), 3.94 (dd, ^2J = 13.2 Hz, ^3J = 3.7 Hz, 1 H, 5′-H_A). FAB-mass (negative) 588.2 (M – Na).

Adenosin-3′-yl Uridin-5′-yl Phosphorothioate (S_p Isomer) [8a(S_p), Ap(S)U(S_p)] [from 6a(R_p)]. Yield: 27 mg (88%). ^{31}P NMR data, see Table I. ^{1}H NMR (D_2O; δ in ppm): 8.34 (s, 1 H, 8-H_A), 8.24 (s, 1 H, 2-H_A), 7.9 (d, ^3J = 8.1 Hz, 1 H, 6-H_U), 6.1 (d, ^3J = 4.8 Hz, 1 H, 1′-H_A), 5.9 (d, ^3J = 3.7 Hz, 1 H, 1′-H_U), 5.8 (d, ^3J = 8.1 Hz, 1 H, 5-H_U), 4.9 (m, 2 H, 2′-H_A, 3′-H_A), 4.3–4.1 (m, 6 H, 2′-H_U, 3′-H_U, 4′-H_U, 5′-H_U, 5′-H_A), 4.0 (dd, ^2J = 13.0 Hz, ^3J = 2.7 Hz, 1 H, 5′-H_A), 3.9 (dd, ^2J = 13.0 Hz, ^3J = 3.5 Hz, 1 H, 5′-H_A). FAB-mass (negative) 588.2 (M – Na).

Cytidin-3′-yl Uridin-5′-yl Phosphorothioate (R_p Isomer) [8b(R_p), Cp(S)U(R_p)] [from 6b(S_p)]. Yield: 26 mg (88%). ^{31}P NMR data, see Table I. ^{1}H NMR (D_2O; ∂ in ppm): 8.04 (d, ^3J = 8.2 Hz, 1 H, 6-H_U), 7.91 (d, ^3J = 7.5 Hz, 1 H, 6-H_C), 5.92 (d, ^3J = 7.5 Hz, 1 H, 5-H_C), 5.86 (d, ^3J = 2.7 Hz, 1 H, 1′-H_C), 5.82 (d, ^3J = 8.1 Hz, 1 H, 5-H_U), 5.75 (d, ^3J = 2.4 Hz, 1 H, 1′-H_U), 4.61 (ddd, $^3J_{PH}$ = 12.6 Hz, ^3J = 4.9 and 7.7 Hz, 1 H, 3′-H_C), 4.47 (dd, ^3J = 2.5 and 4.7 Hz, 1 H, 2′-H_C), 4.3–4.1 (m, 6 H, 2′-H_U, 3′-H_U, 4′-H_U, 5′-H_U, 4′-H_C), 4.03 (dd, ^2J = 13.2 Hz, ^3J = 2.5 Hz, 1 H, 5′-H_C), 3.90 (dd, ^2J = 13.2 Hz, ^3J = 3.8 Hz, 1 H, 5′-H_C). FAB-mass (negative) 564.1 (M – Na).

Cytidin-3′-yl Uridin-5′-yl Phosphorothioate (S_p Isomer) [8b(S_p), Cp(S)U(S_p)] [from 6b(R_p)]. Yield: 27 mg (92%). ^{31}P NMR data, see Table I. ^{1}H NMR (D_2O; ∂ in ppm): 8.00 (d, ^3J = 8.1 Hz, 1 H, 6-H_C), 7.94 (d, ^3J = 8.0 Hz, 1 H, 6-H_U), 6.12 (d, ^3J = 7.8 Hz, 1 H, 5-H_C), 5.93 (d, ^3J = 3.3 Hz, 1 H, 1′-H_U), 5.89 (d, ^3J = 8.0 Hz, 1 H, 5-H_U), 5.88 (d, ^3J = 3.8 Hz, 1 H, 1′-H_C), 4.71 (dt, $^3J_{PH}$ = 11.0 Hz, ^3J = 5.5 Hz, 1 H, 3′-H_C), 4.47 (t, ^3J = 4.0 Hz, 1 H, 2′-H_C), 4.3–4.1 (m, 6 H, 2′-H_U, 3′-H_U, 4′-H_U, 5′-H_U, 4′-H_C), 3.96 (dd, ^2J = 12.8 Hz, ^3J = 2.6 Hz, 1 H, 5′-H_C), 3.85 (dd, ^2J = 12.8 Hz, ^3J = 4.0 Hz, 1 H, 5′-H_C). FAB-mass (negative) 564.1 (M – Na).

Guanosin-3′-yl Uridin-5′-yl Phosphorothioate (R_p Isomer) [8c(R_p), Gp(S)U(R_p)] [from 6c(S_p)]. Yield: 25 mg (81%). ^{31}P NMR data, see Table I. ^{1}H NMR (D_2O; ∂ in ppm): 8.0 (s, 1 H, 8-H_G), 7.9 (d, ^3J = 8.2 Hz, 1 H, 6-H_U), 5.91 (d, ^3J = 4.7 Hz, 1 H, 1′-H_G), 5.90 (d, ^3J = 2.6 Hz, 1 H, 1′-H_U), 5.80 (d, ^3J = 8.1 Hz, 1 H, 5-H_U), 4.46 (m, 1 H, 3′-H_G), 4.8 (t, ^3J = 4.8, 1 H, 2′-H_G), 4.46 (t, ^3J = 3.3, 1 H, 2′-H_U), 4.2–4.1 (m, 5 H, 3′-H_U, 4′-H_U, 5′-H_U, 4′-H_G), 3.98 (dd, ^2J = 12.8 Hz, ^3J = 2.9 Hz, 1 H, 5′-H_G), 3.89 (dd, ^2J = 12.8 Hz, ^3J = 4.0 Hz, 1 H, 5′-H_G). FAB-mass (negative) 604.2 (M – Na).

Guanosin-3′-yl Uridin-5′-yl Phosphorothioate (S_p Isomer) [8c(S_p), Gp(S)U(S_p)] [from 6c(R_p)]. Yield: 28 mg (89%). ^{31}P NMR data, see Table I. ^{1}H NMR (D_2O; ∂ in ppm): 8.0 (s, 1 H, 8-H_G), 7.9 (d, ^3J = 8.1 Hz, 1 H, 6-H_U), 5.93 (d, ^3J = 4.0 Hz, 1 H, 1′-H_U), 5.91 (d, ^3J = 5.5 Hz, 1 H, 1′-H_G), 5.85 (d, ^3J = 8.1 Hz, 1 H, 5-H_U), 5.0 (m, 1 H, 3′-H_G), 4.90 (t, ^3J = 5.5 Hz, 1 H, 2′-H_G), 4.4–4.1 (m, 6 H, 2′-H_U, 3′-H_U, 4′-H_U, 5′-H_U, 4′-H_G), 3.95 (dd, ^2J = 12.8 Hz, ^3J = 2.9 Hz, 1 H, 5′-H_G), 3.85 (dd, ^2J = 12.8 Hz, ^3J = 4.0 Hz, 1 H, 5′-H_G). FAB-mass (negative), 604.2 (M – Na).

Uridin-3′-yl Uridin-5′-yl Phosphorothioate (R_p Isomer) [8d(R_p), Up(S)U(R_p)] [from 6d(S_p)]. Yield: 25 mg (85%). ^{31}P NMR data, see Table I. ^{1}H NMR (D_2O; ∂ in ppm, symbol * indicates protons in the uridin-5′-yl unit of the dimer): 7.97 (d, ^3J = 8.1 Hz, 1 H, 6-H_{U*}), 7.86 (d, ^3J = 8.2 Hz, 1 H, 6-H_U), 5.91 (d, ^3J = 3.7 Hz, 1 H, 1′-H_{U*}), 5.88 (d, ^3J = 8.2 Hz, 1 H, 5-H_U), 5.85 (d, ^3J = 4.3 Hz, 1 H, 1′-H_U), 5.84 (d, ^3J = 8.2 Hz, 1 H, 5-H_{U*}), 4.72 (dt, $^3J_{PH}$ = 11.4 Hz, ^3J = 5.5 Hz, 1 H, 3′-H_U), 4.44 (t, ^3J = 4.8 Hz, 1 H, 2′-H_U), 4.3–4.2 (m, 6 H, 2′-H_{U*}, 3′-H_{U*}, 4′-H_{U*}, 5′-H_{U*}, 4′-H_U), 3.94 (dd, ^2J = 13.0 Hz, ^3J = 2.7 Hz, 1 H, 5′-H_U), 3.84 (dd, ^2J = 13.0 Hz, ^3J = 4.2 Hz, 1 H, 5′-H_U). FAB-mass (negative), 565.0 (M – Na).

Uridin-3′-yl Uridin-5′-yl Phosphorothioate (S_p Isomer) [8d(S_p), Up(S)U(S_p)] [from 6d(R_p)]. Yield: 27 mg (92%). ^{31}P NMR data, see Table I. ^{1}H NMR (D_2O; ∂ in ppm, symbol * indicates protons in the uridin-5′-yl unit of the dimer): 7.93 (d, ^3J = 8.2 Hz, 1 H, 6-H_{U*}), 7.84 (d, ^3J = 8.2 Hz, 1 H, 6-H_U), 5.92 (d, ^3J = 3.8 Hz, 1 H, 1′-H_{U*}), 5.89 (d, ^3J = 8.5 Hz, 1 H, 5-H_{U*}), 5.87 (d, ^3J = 4.7 Hz, 1 H, 1′-H_U), 5.86 (d, ^3J = 8.4 Hz, 1 H, 5-H_U), 4.74 (dt, $^3J_{PH}$ = 10.6 Hz, ^3J = 5.1 Hz, 1 H, 3′-H_U), 4.45 (t, ^3J = 5.1 Hz, 1 H, 2′-H_U), 4.3–4.2 (m, 6 H, 2′-H_{U*}, 3′-H_{U*}, 4′-H_{U*}, 5′-H_{U*}, 4′-H_U), 3.91 (dd, ^2J = 12.8 Hz, ^3J = 2.7 Hz, 1 H, 5′-H_U), 3.81 (dd, ^2J = 12.8 Hz, ^3J = 4.0 Hz, 1 H, 5′-H_U). FAB-mass (negative), 565.0 (M – Na).

Enzymatic Hydrolysis of 8a–d Using Snake Venom Phosphodiesterase (SVPD). One milligram of each diastereomer of the phosphorothioate 8 was dissolved in a buffer solution (100 μL; 2 mM $MgCl_2$ and 50 mM Tris-HCl, pH = 8.9) and snake venom phosphodiesterase (SVPD) from *C. atrox* (1 mg of the enzyme dissolved in the same buffer; 100 μL) was added. The samples were kept on a water bath at 37 °C overnight. TLC analysis (2-propanol–ammonia–water, 7:1:2, v/v/v) revealed that all samples of **8a–d** obtained from the diastereomers of **6** that had more downfield ^{31}P NMR signals were cleaved to a substantial degree (hence, identified as the R_p isomers of 8), whereas the phosphorothioates **8a–d** deriving from the diastereomers of H-phosphonates **6a–d** with more upfield ^{31}P NMR shifts were completely resistant toward enzymatic hydrolysis (the S_p diastereomers of 8).

Acknowledgment. We are indebted to Prof. Per J. Garegg for his interest and to the Swedish Natural Science Research Council and the Swedish Research Council for Engineering Sciences for financial support.

Registry No. **1a**, 58-61-7; **1b**, 65-46-3; **1c**, 118-00-3; **1d**, 58-96-8; **3**, 143294-18-2; **4a**, 143294-14-8; **4b**, 143294-15-9; **4c**, 143294-16-0; **5a**, 143294-19-3; **5b**, 143294-20-6; **5c**, 143294-21-7; (R_p)-**6a**, 143294-22-8; (S_p)-**6a**, 143344-21-2; (R_p)-**6b**, 143294-23-9; (S_p)-**6b**, 143344-22-3; (R_p)-**6c**, 143294-24-0; (S_p)-**6c**, 143344-23-4; (R_p)-**6d**, 143294-25-1; (S_p)-**6d**, 143344-24-5; (R_p)-**7a**, 143294-26-2; (S_p)-**7a**, 143344-25-6; (R_p)-**7b**, 143294-27-3; (S_p)-**7b**, 143344-26-7; (R_p)-**7c**, 143294-28-4; (S_p)-**7c**, 143344-27-8; (R_p)-**7d**, 143294-29-5; (S_p)-**7d**, 143344-28-9; (R_p)-**8a**, 143344-29-0; (S_p)-**8a**, 143344-30-3; (R_p)-**8b**, 143059-90-9; (S_p)-**8b**, 143059-89-6; (R_p)-**8c**, 143294-30-8; (S_p)-**8c**, 143344-31-4; (R_p)-**8d**, 143344-32-5; (S_p)-**8d**, 143344-33-6; SiOMb-Cl, 129452-86-4; phosphodiesterase, 9025-82-5.

Asymmetric Synthesis of (*S*)-4-Aminohex-5-enoic Acid: A Potent Inhibitor of 4-Aminobutyrate-2-oxoglutarate Aminotransferase[1a]

Tae Woo Kwon,[1b] Paul F. Keusenkothen, and Michael B. Smith*

Department of Chemistry, Room 151, 215 Glenbrook Road, University of Connecticut, Storrs, Connecticut 06269-3060

Received February 10, 1992

The potent inhibitor of 4-aminobutyrate-2-oxoglutarate aminotransferase (GABA-T), 4-aminohex-5-enoic acid (vinyl GABA), has been synthesized with excellent enantioselectivity in six steps from L-glutamic acid in an overall yield of 33%. This is the most efficient synthesis of this important compound and illustrates the use of a novel alkenyl protecting group for pyroglutamate. This allowed the preparation and manipulation of the key (*S*)-2-oxopyrrolidine-5-carboxaldehyde intermediate.

4-Aminobutanoic acid (γ-aminobutyric acid, GABA, 1) is an important neurotransmitter in mammalian systems.[2] GABA deficiency has been associated with a variety of neurological disorders including Parkinson's disease,[3] ep-

0022-3263/92/1957-6169$03.00/0