

- [13] Analysis by HPLC was performed with an Adsorbosphere SAX column (5 μ , 4.6 $\times$ 250 mm) with a gradient from 140 mM to 320 mM potassium phosphate buffer, pH 3.5, over 20 min, followed by a 5-min wash with 500 mM potassium phosphate buffer, pH 3.5.
- [14] Substrate **5** was prepared as described previously: H. Chen, G. Agnihotri, Z. Guo, N. L. S. Que, X. H. Chen, H.-w. Liu, *J. Am. Chem. Soc.* **1999**, *121*, 8124–8125.
- [15] **6**: ^1H NMR (500 MHz, $^2\text{H}_2\text{O}$): δ = 1.11 (d, $^3J(\text{H,H})$ = 6.0 Hz, 3H; 5-Me hydrated form), 1.15 (d, $^3J(\text{H,H})$ = 6.0 Hz, 3H; 5-Me keto form), 1.37 (s, 3H; 3-Me hydrated form), 1.45 (s, 3H; 3-Me keto form), 1.82 (s, 3H; 5'-Me), 1.92 (m, 2H; 2-H hydrated form), 2.23–2.32 (m, 2H; 2'-H), 2.40 (m, 2H; 2-H keto form), 4.01–4.10 (m, 3H; 5-H hydrated form, 4'-H, 5'-H), 4.47–4.53 (m, 1H; 3'-H), 4.72 (q, $^3J(\text{H,H})$ = 6.0 Hz, 1H; 5-H keto form), 5.49 (m, 1H; 1-H hydrated form), 5.64 (m, 1H; 1-H keto form), 6.24 (t, $^3J(\text{H,H})$ = 4.2 Hz, 1H; 1'-H), 7.61 (s, 1H; 6''-H); ^{13}C NMR (75 MHz, $^2\text{H}_2\text{O}$, hydrated form): δ = 11.6, 12.0, 23.5, 38.3, 40.9 (d, $^3J(\text{C,P})$ = 7.5 Hz; C-2), 65.3 (d, $^2J(\text{C,P})$ = 5.6 Hz; C-5'), 68.1, 70.7, 71.8, 84.7, 85.0 (d, $^3J(\text{C,P})$ = 7.5 Hz; C-4'), 94.0 (d, $^2J(\text{C,P})$ = 5.3 Hz; C-1), 94.3, 111.6, 137.2, 151.6, 166.5. The ratio of the hydrated form to the keto form is approximately 3:1.
- [16] The HPLC assay used for determining the kinetic parameters was performed on an Adsorbosphere SAX column (5 μ , 4.6 $\times$ 250 mm), which was eluted isocratically with 50 mM potassium phosphate buffer, pH 3.5. The peak integrations of (S)-adenosylmethionine and (S)-adenosylhomocysteine were used to determine the product conversion.
- [17] Inductively coupled plasma (ICP) analysis for metal ions indicated the presence of approximately 0.4 mol of Zn^{II} per mole of TylC3. However, the zinc does not appear to be important for activity, since dialysis of the enzyme against 5 mM 1,10-phenanthroline for 4 d did not reduce the activity, although ICP analysis indicated that approximately half of the zinc was removed. Attempts to reconstitute the enzyme with zinc also failed to increase the activity. Likewise, the addition of Mg^{II} did not increase the activity of TylC3.
- [18] Eneidiolates are common intermediates in many biotransformations. For a few examples, see: a) R. V. J. Chari, J. W. Kozarich, *J. Am. Chem. Soc.* **1983**, *105*, 7169–7171; b) A. E. Johnson, M. E. Tanner, *Biochemistry* **1998**, *37*, 5746–5754; c) C. J. Jeffery, B. J. Bohnson, W. Chien, D. Ringe, G. A. Petsko, *Biochemistry* **2000**, *39*, 955–964.
- [19] H. H. Bear, H. R. Hanna, *Carbohydr. Res.* **1982**, *110*, 19–41.
- [20] S. Hanessian, N. R. Plessas, *J. Org. Chem.* **1969**, *34*, 1035–1044.
- [21] **8**: ^1H NMR (300 MHz, $^2\text{H}_2\text{O}$): δ = 1.10 (d, $^3J(\text{H,H})$ = 6.6 Hz, 3H; 5-Me), 1.73 (s, 3H; 5'-Me), 1.86 (m, 1H; 2-H_{ax}), 2.08 (m, 1H; 2-H_{eq}), 2.18 (m, 2H; 2'-H), 3.98 (m, 4H; 3-, 3'- and 5'-H), 4.06 (dq, $^3J(\text{H,F})$ = 21.6, $^3J(\text{H,H})$ = 6.6 Hz, 1H; 5-H), 4.44 (m, 1H; 4'-H), 5.40 (m, 1H; 1-H), 6.16 (m, 1H; 1'-H), 7.57 (s, 1H; 6''-H); ^{13}C NMR (75 MHz, $^2\text{H}_2\text{O}$): δ = 11.6, 12.0, 23.5, 38.3, 40.9 (d, $^3J(\text{C,P})$ = 7.5 Hz; C-2), 65.3 (d, $^2J(\text{C,P})$ = 5.6 Hz; C-5'), 68.6 (dd, $^1J(\text{C,F})$ = 31.7, $^1J(\text{C,F})$ = 24.1 Hz; C-4), 70.9, 84.8, 85.2 (d, $^3J(\text{C,P})$ = 9.0 Hz; C-4'), 93.6 (d, $^2J(\text{C,P})$ = 4.6 Hz; C-1), 111.6, 137.3, 151.6, 166.5; ^{19}F NMR (282 MHz, $^2\text{H}_2\text{O}$): δ = -125.9 (d, $^2J(\text{F,F})$ = 253 Hz), -128.3 (dd, $^2J(\text{F,F})$ = 253, $^3J(\text{H,F})$ = 21.2 Hz); ^{31}P NMR (121 MHz, $^2\text{H}_2\text{O}$): δ = -11.2 (d, $^2J(\text{P,P})$ = 20.7 Hz), -12.1 (d, $^2J(\text{P,P})$ = 20.7 Hz); HRMS (ESI) calcd for $\text{C}_{16}\text{H}_{23}\text{F}_2\text{N}_2\text{O}_{13}\text{P}_2$ [$M - \text{H}$] $^-$: 551.0649; found: 551.0669.
- [22] The difluoromethylene moiety is a strongly electron-withdrawing group which can stabilize the corresponding β -anion both by induction and by negative hyperconjugation: B. E. Smart in *Chemistry of Organic Fluorine Compounds II* (Eds.: M. Hudlicky, A. E. Pavlath), American Chemical Society, Washington, **1995**, pp. 979–1010. Although the $\text{p}K_{\text{a}}$ data of protons adjacent to the difluoromethylene group are not available, examples of α -anion-induced β -fluoride elimination in similar structures are well known: A. M. Kornilov, I. B. Kulik, A. E. Sorochinsky, V. P. Kukhar, *Tetrahedron Asymmetry* **1995**, *6*, 199–206; D. Schirlin, S. Baltzer, J. M. Altenburger, C. Tarnus, J. M. Remy, *Tetrahedron* **1996**, *52*, 305–318.
- [23] D. A. Johnson, H.-w. Liu in *Comprehensive Natural Products Chemistry*, Vol. 3 (Eds.: D. Barton, K. Nakanishi, O. Metho-Cohn), Elsevier, Oxford, **1999**, pp. 311–366.

Oxathiaphospholane Approach to the Synthesis of P-Chiral, Isotopomeric Deoxy(ribonucleoside phosphorothioate)s and Phosphates Labeled with an Oxygen Isotope**

Piotr Guga, Krzysztof Domański, and Wojciech J. Stec*

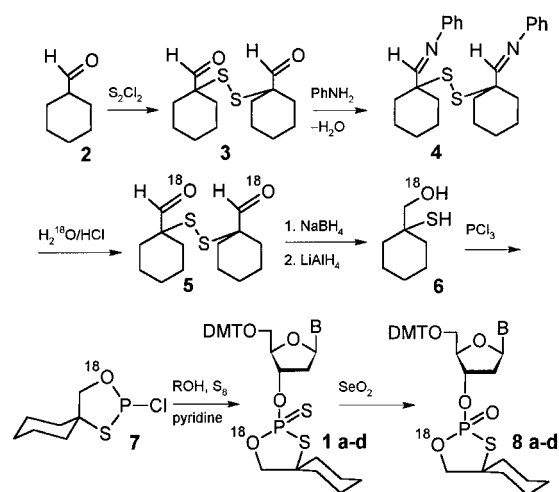
Introduced by Eckstein, phosphorothioate analogues of nucleotides have become an indispensable tool for studying the metabolism of nucleic acids.^[1] Standard chemical methods for the synthesis of oligo(deoxyribonucleoside phosphorothioate)s (PS-Oligos) provide a mixture of 2ⁿ diastereoisomers, where n is the number of phosphorothioate linkages.^[2] The enzymatic synthesis of stereodefined PS-Oligos is limited to the preparation of (all- R_{P})-oligomers because of the stereoselectivity of available DNA and RNA polymerases. The first method for stereocontrolled chemical synthesis of PS-Oligos was elaborated in our group,^[3] and several alternative methods were recently reported.^[4, 5] Stereodefined PS-Oligos were used for studying the mode of action of several bacterial and human enzymes^[6–8] and the stereodependent avidity of PS-Oligos toward complementary DNA or RNA.^[9] However, the presence of a sulfur atom affects the properties of internucleotide bonds, mostly due to the different steric requirements of sulfur atoms (P–S vs P–O bond length), different affinity towards metal ions, and changes in the distribution of the negative charge in the phosphorothioate anion.^[10] Therefore, the hydration pattern of PS-Oligos is different from that of natural oligonucleotides,^[11] and this obstructs the evaluation of kinetic data of “rescue effects” of thiophilic metal ions, and makes analysis of direct or water-mediated contacts between metal ions and phosphate groups much more difficult. These inconveniences could be avoided by using P-chiral isotopomeric phosphates.^[12] Here we describe the synthesis of stereodefined oligo(deoxyribonucleoside [^{18}O]phosphorothioate)s (PS ^{18}O -Oligos) and oligo(deoxyribonucleoside [^{18}O]phosphate)s (P ^{18}O -Oligos), in which both of the nonbridging oxygen atoms of the internucleotide bond were replaced by S and ^{18}O , or one of them was replaced by ^{18}O , respectively. Oligonucleotides containing a single P-chiral [^{16}O , ^{18}O] internucleotide bond were first used by Eckstein^[13] in studies on Eco RI endonuclease. Stereodefined P ^{18}O -Oligos can be used to investigate the interaction of particular oxygen atoms with other molecules or metal ions, given analytical methods that allow the isotopic effect to be measured with satisfactory accuracy.^[14]

To obtain stereodefined PS ^{18}O -Oligos, we synthesized 5'-O-DMT-nucleoside-3'-O-(2-thio-“spiro”-4,4-pentamethylene-

[*] Prof. Dr. W. J. Stec, Dr. P. Guga, K. Domański
Centre of Molecular and Macromolecular Studies
Polish Academy of Sciences
Department of Bioorganic Chemistry
Sienkiewicza 112, 90-363 Łódź (Poland)
Fax: (+48)42-6815483
E-mail: wjstec@bio.cbmm.lodz.pl

[**] This work was financially supported by the State Committee for Scientific Research (KBN, Poland, Grant 4P05F00617, to W.J.S.), and, in part, by the Human Science Promotion Foundation (Japan, to H. Takaku and W.J.S.).

1,3,2-[^{18}O]oxathiaphospholane)s (**1**), and separated them into diastereomerically pure forms (Scheme 1). Commercially available cyclohexanecarbaldehyde (**2**) was converted into the 2,2'-dithiobis(cyclohexanecarbaldehyde) **3** in 70% yield;^[15] this compound was subsequently transformed into



Scheme 1. Synthesis of **1a–d** and **8a–d**. DMT = 4,4'-dimethoxytrityl, ROH = 5'-O-DMT-3'-OH-nucleoside.

the bis(imine) **4** by treatment with aniline in benzene with azeotropic removal of water. A solution of crude **4** in THF was cooled to 0–5 °C and saturated with anhydrous HCl in the presence of 1.1 mole equivalents of [^{18}O]water (95 atom % ^{18}O) to form the 2,2'-dithiobis([^{18}O]cyclohexanecarbaldehyde) **5**. Its analysis by EI-MS [m/z 286 ($2 \times ^{16}\text{O}$, 18.3%), m/z 288 (^{16}O , ^{18}O , 78.1%), m/z 290 ($2 \times ^{18}\text{O}$, 100%)] showed an isotopic enrichment of 71 atom % ^{18}O . A similar method was formerly applied for almost quantitative incorporation of an ^{18}O label into benzaldehyde,^[16] but in these exploratory studies no special precautions with respect to the dryness of THF were taken. Treatment of **5** with NaBH_4 followed by LiAlH_4 gave (1-sulfanylcyclohexyl)methan[^{18}O]ol (**6**) with retention of the isotopic enrichment. At this stage, **6** was diluted with unlabeled sulfanylalcohol, and the final isotope enrichment was about 45%. Reaction of **6** with PCl_3 provided the [^{18}O]oxathiaphospholane **7**. The ^{31}P NMR spectrum (Figure 1A) contained two peaks at $\delta = 218.88$ and 218.79 corresponding to unlabeled and ^{18}O -labeled compounds. Phosphitylation of 5'-O-DMT-nucleosides with **7** followed by sulfurization gave monomers **1a–d**, which subsequently were separated by chromatography into pure fast- and slow-eluting P-diastereomers, with R_p and S_p absolute configuration, respectively (Table 1). Their ^{31}P NMR spectra consisted of a downfield and an upfield signal (Figure 1B), corresponding to unlabeled and isotopically labeled monomers, respectively. The chemical shift difference of $\Delta\delta = 0.025$ –0.027 indicates that the ^{18}O atom is attached to the phosphorus atom by a single bond.^[17]

The monomers *fast-1a* and *slow-1a* were used for the synthesis of dithymidyl [^{18}O]phosphorothioates. The condensation was performed in CH_3CN on a 40-mg scale with 15 mg of 3'-O-Ac-thymidine in the presence of 1,8-diazabicy-

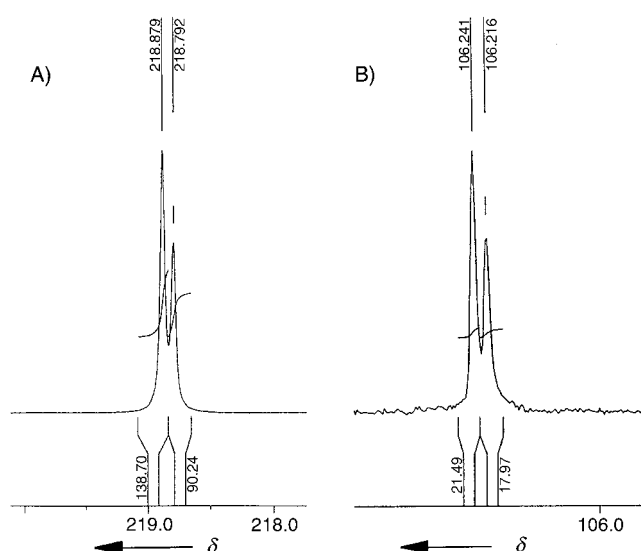


Figure 1. ^{31}P NMR spectra (CD_3CN) of A) **7**, B) *fast-1b*. Spectral parameters: time domain 16 k, size 16 k, sweep width 800 Hz.

Table 1. ^{31}P NMR and FAB-MS characteristics of isotopically labeled monomers **1a–d** and **8a–d**.^[a]

Nucleobase, isomer	$\delta(^{31}\text{P})$ ^[b]			m/z (FAB-MS) ^[c]		Isotope enrichment ^[d] [%]
	^{16}O	^{18}O	$\Delta\delta$	^{16}O	^{18}O	
Thy, <i>fast-1a</i>	106.284	106.258	0.026	749.0	751.0	47
Thy, <i>slow-1a</i>	106.646	106.619	0.027			
Cyt ^{Bz} , <i>fast-1b</i>	106.241	106.216	0.025	838.3	840.2	48
Cyt ^{Bz} , <i>slow-1b</i>	106.621	106.595	0.026			
Ade ^{Bz} , <i>fast-1c</i>	105.910	105.885	0.025	862.4	864.4	46
Ade ^{Bz} , <i>slow-1c</i>	106.402	106.376	0.026			
Gua ^{Bu} , <i>fast-1d</i>	106.353	106.328	0.025	844.3	846.3	46
Gua ^{Bu} , <i>slow-1d</i>	106.838	106.813	0.025			
Thy, <i>fast-8a</i>	44.078	44.060	0.018	733.1	735.0	44
Thy, <i>slow-8a</i>	44.590	44.572	0.018			
Cyt ^{Bz} , <i>fast-8b</i>	44.111	44.091	0.020	822.2	823.9	46
Cyt ^{Bz} , <i>slow-8b</i>	44.551	44.534	0.017			
Ade ^{Bz} , <i>fast-8c</i>	43.799	43.780	0.019	846.0	848.0	45
Ade ^{Bz} , <i>slow-8c</i>	44.204	44.186	0.018			
Gua ^{Bu} , <i>fast-8d</i>	44.749	44.730	0.019	828.2	830.0	44
Gua ^{Bu} , <i>slow-8d</i>	45.426	45.407	0.019			

[a] For compounds **8a–d** the terms *fast* and *slow* refer to the chromatographic mobility of their precursors **1a–d**. [b] ^{31}P NMR spectra were recorded on a Bruker AC 200 spectrometer in CD_3CN . [c] The spectra were recorded for unseparated mixtures of both diastereoisomers. The observed m/z values are in accord with calculated ones. [d] Calculation based on molecular ion intensities for ten averaged FAB-MS scans.

clo[5.4.0]undec-7-ene (DBU). Reaction was complete in 5 min, and the dinucleotides were obtained almost quantitatively (^{31}P NMR). The observed chemical shift difference of $\Delta\delta = 0.04$ indicated a bond order of greater than 1.5 between the ^{18}O and P atoms, while the matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrum recorded for the unprotected dinucleotide confirmed the isotope enrichment [R_p -T_{PS}T, m/z 560.8 (56%), 562.8 (44%); S_p -T_{PS}T, m/z 561.1 (54%), 563.1 (46%)]. Configurational assignments based on the known stereochemistry of the condensation process^[9] allowed us to conclude that *slow*- and *fast-1a–d* provide [^{18}O]phosphorothioate dinucleotides with S_p and R_p absolute configurations, respectively. According to

the Cahn–Ingold–Prelog rules, the isotopic replacement $^{16}\text{O} \rightarrow ^{18}\text{O}$ in dinucleoside phosphorothioates changes neither the sense of chirality nor the absolute configuration of the phosphorus atom, as the atomic numbers of the two oxygen isotopes are the same.

Pure *fast*- and *slow*-**1a** were used for the solid-phase synthesis of octa(thymidine [^{18}O]phosphorothioate)s. The purified oligomers were treated with snake venom phosphodiesterase (svPDE). As expected, the (all- S_P)-octamer was not digested by R_P -selective svPDE. Its (all- R_P)-counterpart was digested by the enzyme, and the reacting mixture containing consecutively shortened, partially digested oligomers (down to a pentamer) was analyzed by MALDI-TOF mass spectrometry (Figure 2). The observed relative intensities of the mass peaks were in accord with calculated values.

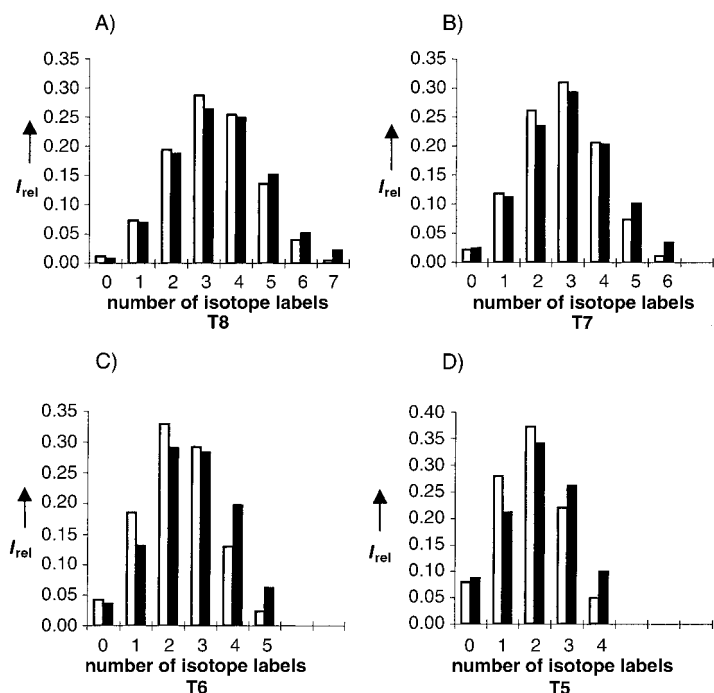
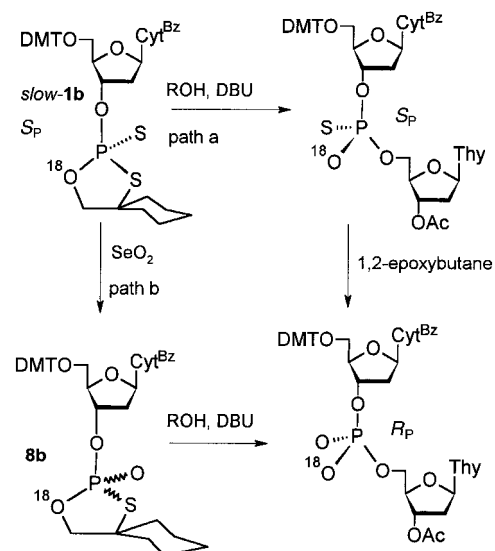


Figure 2. Comparison of calculated (open bars) and measured (full bars) relative intensities I_{rel} of molecular ions and corresponding isotopic bands from MALDI-TOF analysis for oligomers resulting from svPDE digestion of isotopically labeled (All- R_P -PS ^{18}O)-T $_8$. An isotopic enrichment of 47% was used for calculation. A) [starting octamer] m/z = 2482. B) [heptamer] m/z = 2162. C) [hexamer+Na $^+$ +K $^+$] m/z = 1904. D) [pentamer+Na $^+$ +K $^+$] m/z = 1584.

To obtain monomers for direct synthesis of stereodefined P ^{18}O -Oligos, diastereomerically pure monomers **1a–d** (ca. 400 mg) were stereospecifically (as judged by ^{31}P NMR spectroscopy) oxidized with SeO $_2$ (twofold molar excess) in CH $_3$ CN (3 mL) at room temperature with stirring (15 min) to give 5'-O-DMT-nucleoside-3'-O-(2-oxo-“spiro”-4,4-pentamethylene-1,3,2-[^{18}O]oxathiaphospholane)s (**8a–d**, see Scheme 1). The reaction mixture was passed through a dry silica gel column (ca. 1 g), with exclusion of moisture, to absorb traces of dissolved SeO $_2$. The monomers were recovered in amounts of about 350–360 mg and characterized by FAB-MS and ^{31}P NMR spectroscopy (see Table 1).

The applicability of the unlabeled monomer **8a** for synthesis of oligonucleotides possessing phosphate internucleotide bonds has already been reported.^[18] However, the stereochemistry of the isotopomeric P ^{18}O -Oligos had to be determined. For that purpose, the model dinucleotide [PS ^{18}O]-DMTC Bz T $_{\text{OAc}}$ obtained from *slow*-**1b** was converted with 1,2-epoxybutane in stereoretentive manner^[19] into [P ^{18}O]^{DMT}C Bz T $_{\text{OAc}}$ (Scheme 2, path a). The same monomer



Scheme 2. Sequence of reactions used for determining the absolute configuration at the P atom in P ^{18}O -Oligos. ROH = 3'-O-Acetylthymidine.

1b was oxidized with SeO $_2$, and resulting **8b** was used for synthesis of [P ^{18}O]^{DMT}C Bz T $_{\text{OAc}}$ (path b). The K $^+$ salt of each [P ^{18}O]dinucleotide obtained via path a and path b, without deprotection, was converted into a methyl ester with MeI/DMSO/[18]crown-6^[20] and analyzed by ^{31}P NMR spectroscopy. The spectra showed identical patterns with isotope chemical shifts of $\Delta\delta$ = 0.04 and 0.02 for the downfield and upfield signals, respectively. These results indicate that a sequence of reactions: *slow*-**1b** $\rightarrow$ **8b** $\rightarrow$ P ^{18}O -Oligo yields the [^{18}O]phosphate internucleotide with R_P absolute configuration. Most probably, the oxidation of **1b** to **8b**, as well as the condensation of **8b** with 3'-O-Ac-thymidine, occurred with retention of configuration.

The two sets of ^{18}O -labeled monomers **1a–d** and **8a–d** and their unlabeled counterparts allow medium-size oligonucleotides to be synthesized with any combination of internucleotide bond with respect to its type (P–S vs P–O) or absolute configuration (R_P vs S_P), as well as to the position and the number of isotope labels along the chain. This flexibility is exemplified by the solid-phase synthesis of (R_P ,Mix)-d(A $_{\text{PS}}$ C $_{\text{PO}}$ T $_{\text{PS}^{18}\text{O}}$ C) (**9**), which was obtained on a 1- μmol scale by using 20 mg of each monomer per coupling followed by two-step HPLC purification. The product was analyzed by polyacrylamide gel electrophoresis (PAGE) and MALDI-TOF mass spectrometry (Figure 3). Both techniques documented its homogeneity and the presence of the isotopic label.

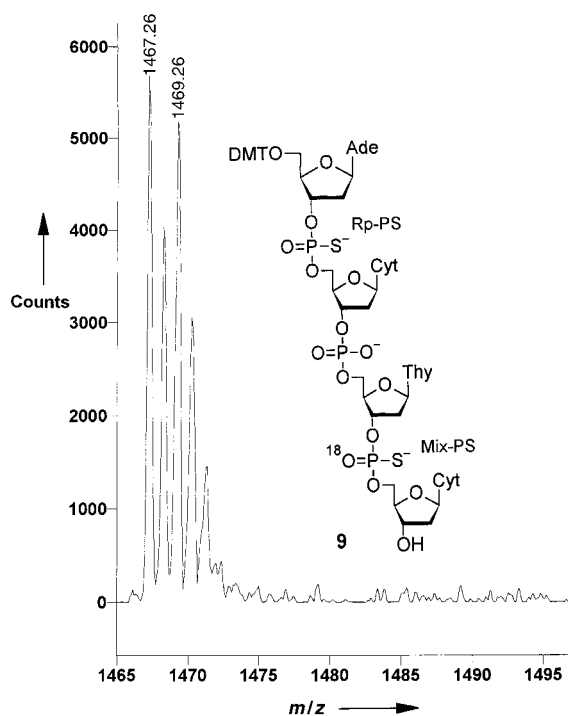


Figure 3. MALDI-TOF mass spectrum for **9** with a 5'-O-DMT protecting group. Spectral parameters: negative-ion spectrometry, reflector mode, accelerating voltage 20 kV, delay 100 ns, 60 scans averaged.

Experimental Section

The detailed reaction conditions for synthesis of unlabeled compounds **1**, **3**, **5**, **6**, **7** and **8**, their full characteristics and chromatographic conditions for separation of **1a–d** into pure diastereomers were published elsewhere.^[9, 18] The ¹⁸O-labeled water (95 atom % ¹⁸O) was supplied by ICON Services, Inc. (Summit, NJ). The FAB mass spectra were recorded on a Finnigan MAT 95 spectrometer (Bremen, Germany) in negative-ion mode. High-resolution MALDI-TOF mass spectrometry (negative ion spectrometry) was performed with a Voyager-Elite mass spectrometer (PerSeptive Biosystems Inc., Framingham, MA) operating in the reflector mode.

Received: September 26, 2000 [Z15859]

- [1] F. Eckstein, *Antisense Nucleic Acid Drug Dev.* **2000**, *10*, 117–121, and references therein.
- [2] W. J. Stec, A. Wilk, *Angew. Chem.* **1994**, *106*, 747–761; *Angew. Chem. Int. Ed. Engl.* **1994**, *33*, 709–722.
- [3] P. Guga, M. Koziolkiewicz, A. Okruszek, W. J. Stec in *Applied Antisense Oligonucleotide Technology* (Eds.: C. Stein, A. Krieg), Wiley, New York, **1998**, pp. 23–50.
- [4] R. P. Iyer, D. Yu, N.-H. Ho, W. Tan, S. Agrawal, *Tetrahedron: Asymmetry* **1995**, *6*, 1051–1054; R. P. Iyer, D. Yu, T. Devlin, N.-H. Ho, W. Tan, S. Agrawal, *J. Org. Chem.* **1995**, *60*, 5388–5389; R. P. Iyer, M.-J. Guo, D. Yu, S. Agrawal, *Tetrahedron Lett.* **1998**, *39*, 2491–2494.
- [5] A. Wilk, A. Grajkowski, L. R. Phillips, S. L. Beaucage, *J. Am. Chem. Soc.* **2000**, *122*, 2149–2156.
- [6] C. A. Brautigam, T. A. Steitz, *J. Mol. Biol.* **1998**, *277*, 363–377.
- [7] M. Koziolkiewicz, A. Krakowiak, M. Kwinkowski, M. Boczkowska, W. J. Stec, *Nucleic Acids Res.* **1995**, *23*, 5000–5005.
- [8] M. Koziolkiewicz, A. Krakowiak, A. Owczarek, M. Boczkowska in *RNA Biochemistry and Biotechnology* (Eds.: J. Barciszewski, B. Clark), Kluwer, Dordrecht, **1999**, pp. 315–324.
- [9] W. J. Stec, B. Karwowski, M. Boczkowska, P. Guga, M. Koziolkiewicz, M. Sochacki, M. Wiczorek, J. Błaszczyk, *J. Am. Chem. Soc.* **1998**, *120*, 7156–7167.
- [10] P. A. Frey, R. D. Sammons, *Science* **1985**, *228*, 541–545.
- [11] T. James, UCSF, CA, personal communication.

- [12] S. L. Buchwald, D. E. Hansen, A. Hassett, J. R. Knowles in *Methods in Enzymology* (Ed.: D. L. Purich), Academic Press, New York, **1982**, pp. 279–301.
- [13] B. A. Connolly, F. Eckstein, A. Pingoud, *J. Biol. Chem.* **1984**, *259*, 10760–10763.
- [14] V. Cepus, A. J. Scheidig, R. S. Goody, K. Gerwert, *Biochemistry* **1998**, *37*, 10263–10271.
- [15] K. Hayashi, *Macromolecules* **1970**, *3*, 5–9.
- [16] J. Baraniak, K. Lesiak, M. Sochacki, W. J. Stec, *J. Am. Chem. Soc.* **1980**, *102*, 4533.
- [17] M. Cohn, A. Hu, *J. Am. Chem. Soc.* **1980**, *102*, 913.
- [18] B. Karwowski, P. Guga, A. Kobylańska, W. J. Stec, *Nucleosides Nucleotides* **1998**, *17*, 1747–1759.
- [19] A. Okruszek, P. Guga, W. J. Stec, *Nucleic Acids Symp. Ser.* **1984**, *14*, 267.
- [20] R. L. Jarvest, G. Lowe, B. V. L. Potter, *J. Chem. Soc. Perkin Trans. 1* **1981**, 3186–3195.

Chiral Recognition in Bis-Urea-Based Aggregates and Organogels through Cooperative Interactions**

Maaïke de Loos, Jan van Esch,* Richard M. Kellogg, and Ben L. Feringa*

Chiral recognition is indisputably one of the most intriguing phenomena in nature. In chemistry, it is a key feature in applications such as enantioselective catalysis,^[1] separation techniques,^[2] and in the formation of supramolecular systems, both in solid assemblies^[2, 3] and in solution.^[2, 4–6]

Intermediate to solid- and solution-phase systems one might place organogels.^[7, 8] These consist of molecules, self-assembled into large fiberlike aggregates which intertwine to form a reversible gel in organic solvents. Especially the rationally designed 1,2-bis(ureido)cyclohexane derivatives were found to be highly effective gelators for organic solvents.^[9, 10] Furthermore, these compounds contain two stereogenic centers, which for **1** are expressed in the formation of twisted fibers in ethanol with a helicity that depends on the handedness of **1**.^[10b] Related chiral phenomena are found for other gel systems.^[3, 7, 8a,d]

Here we report on chiral recognition through cooperative interactions between aggregates and gels of **1** and a co-aggregating guest **2**.^[11] Guest **2** exhibits the same bis(ureido)-cyclohexane skeleton as **1**, but azobenzene chromophores have been attached to the side chains to differentiate **2** from host **1** and to probe the microenvironment by spectroscopic methods.

[*] Dr. J. van Esch, Prof. Dr. B. L. Feringa, M. de Loos, Prof. Dr. R. M. Kellogg
Department of Organic and Molecular Inorganic Chemistry
Stratingh Institute, University of Groningen
Nijenborgh 4, 9747 AG Groningen (The Netherlands)
Fax: (+31) 50-3634296
E-mail: J.v.Esch@chem.rug.nl, Feringa@chem.rug.nl

[**] This work was supported by the Dutch Foundation for Scientific Research (NWO). The Royal Netherlands Academy of Sciences is gratefully acknowledged for a fellowship to J.v.E.