SYNTHESIS AND P-DIASTEREOMERIC RESOLUTION OF NUCLEOSIDE 3'-O(S-ALKYL) AND NUCLEOSIDE 3'-O(S-ARYL) METHYLPHOSPHONOTHIOATES

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Successive displacement of oxybenzotriazoyl moieties from methylphosphonicbisoxybenzotriazolide by a deoxynucleoside and a mercaptan yields the 0,S-dialkyl and 0-alkyl-S-arylmethylphosphonothioate diastereomers which can be resolved by flash column chromatography.

Nucleotide analogs bearing a chiral phosphate are of potential significance for many enzymological and medicinal uses.¹ Alkylphosphonothioate analogs appear attractive to us for these applications because they are chiral and do not racemize easily in aqueous solutions. Previously we described the synthesis of deoxynucleotide and deoxydinucleotide methylphosphonothioates possessing a P = S moiety.² Here we report the synthesis of deoxynucleotide 3'-O(Salkyl)- and 3'-O(S-aryl) methylphosphonothioates. Of particular interest was the observation that P-diastereomers from this class of compounds could be resolved on silica gel via flash column chromatography.³ For all other P-chiral phosphodiester analogs so far investigated, the separation of diastereomers is difficult and often requires HPLC.²,4

Initial attempts to synthesize deoxynucleoside 3'-0(S-alkyl)- and 3'-0(S-aryl) methylphosphonothioates involved preparation of the deoxynucleoside 3'-0-methylphosphonylimidazolide(10ab) from 8 according to Miller et al.^{5,6} followed by condensation with two molar equivalents of the appropriate mercaptan.⁹ Compounds 17ab-19ab were obtained via this pathway after 5 hr at 25°C, purified using a standard work-up procedure,¹⁰ and fractionated via HPLC which separates the diastereomers as well as the symmetrical dimer (29). The main disadvantage of this approach was the formation of 29, which was present in 35-40% isolated yield. As a consequence the yield of the desired diastereomers never exceeded 25%. Furthermore synthesis of 22ab required 30 hours following addition of tetrazole (1 eq.) and 23ab could not be prepared even after 7 days in the presence of tetrazole.

A more attractive route for synthesizing 17ab-28ab was to use the oxybenzotriazolides 11ab-16ab which are available by adding 5 ml of a 0.22 M solution of methylphosphonicbisoxybenzotriazolide (9) in dioxane¹¹ to 1 mmole of the appropriate deoxynucleoside (1-6). The resulting product mixtures exhibited ³¹P-NMR spectra expected for the oxybenzotriazolides (11ab: δ 37.92, 37.62; 12ab: δ 38.02, 37.65; 13ab: δ 38.06; 14ab: δ 38.5; 15ab: δ 37.82; 16ab: δ 38.66; reaction mixtures). To each crude reaction mixture was next added the appropriate thiol (2 eq.) and N-methylimidazole (4 eq.). After five hours at room temperature, the desired compounds were the only nucleotidic material found in the reaction mixture. Isolation of the diastereomers involved aqueous extraction as described previously.¹⁰ If mercaptides¹² were used as in the synthesis of 22ab and 23ab, the reaction mixtures were neutralized with Dowex 50W-X (pyridinium form) before the aqueous work-up. The significance of this approach lies in the absence of side reactions, especially the formation of 29. Thus compounds 18ab-28ab were prepared in 55-65% isolated yields after resolution of the diastereomers by flash chromatography and precipitation into n-pentane.¹³⁻²⁵ A somewhat lower yield of 17ab (40%) was probably due to irreversible reactions with the silica gel column. For compounds 19ab-22ab, deprotection of the S-alkyl group with thiophenol:triethylamine:dioxane (1:2:2, v/v/v) was studied. While 22ab could not be detectably deprotected with the thiophenol solution at 55°C after 36 hours, the other nucleotides gave the following deprotection half-times: 19a, 1000 min (rt), 285 min (55°C); 20a, 230 min (rt); 21a, 110 min (rt). In all cases, 30 was obtained as the only nucleotidic, phosphorus containing product.²⁶







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I (R₁,R₂): 1 (Tr, T); 2 (*t*-butyldimethylsilyl, T); 3 (*t*-butyldimethylsilyl, C^{Bz}); 4 (Bz, C^{Bz}); 5 (Tr, A^{Bz}); 6 (Tr, G^{Ib}). II (R₁,R₂,X): 10ab (Tr, T, imidazolyl); 11ab (Tr, T, oxybenzotriazolyl); 12ab (*t*-butyldimethylsilyl, T, oxybenzotriazolyl); 13ab (*t*-butyldimethylsilyl, C^{Bz}, oxybenzotriazolyl); 14ab (benzoyl, C^{Bz}, oxybenzotriazolyl); 15ab (Tr, A^{Bz}, oxybenzotriazolyl); 16ab (Tr, G^{Ib}, oxybenzotriazolyl); 29 (Tr, T, 5'-O-tritylthymidyl). III (R₁,R₂,R₃): 17ab (Tr, T, p-chlorophenyl); 18ab (Tr, T, Ph); 19ab (Tr, T, Bn); 20ab (Tr, T, p-chlorobenzyl); 21ab (Tr, T, 2,4 dichlorobenzyl); 22ab (Tr, T, Et); 23ab (Tr, T, iPr); 24ab (*t*-butyldimethylsilyl, T, Bn); 25ab (*t*-butyldimethylsilyl, C^{Bz}, Bn); 26ab (Bz, C^{Bz}, Bn); 27ab (Tr, A^{Bz}, Bn); 28ab (Tr, G^{IB}, Bn). i (imidazole, 5 hr, rt); ii (hydroxybenzotriazole, 2.2 eq., pyridine, 2.2 eq., 8 hr, rt); iii (8, 1 hr, rt or 9, 1 hr, rt); iv (R-SH, activation, see text); v (dioxane:NEt3:PhSH, 2:2:1, v/v/v). Bn, benzyl; Bz, benzoyl; C^{Bz}, N4-benzoyldeoxycytidine; A^{Bz}, N6-benzoyldeoxyadenosine; G^{Ib}, N2-isobutyrldeoxyguanosine; Tr, trityl; T, thymine. These results demonstrate that diastereomerically pure deoxynucleoside 3'-methylphosphonothioates can be prepared in relatively high yield. Of particular interest was the unique observation that diastereomeric deoxynucleoside 3'-O(S-alkyl) and 3'-O(S-aryl) derivatives can be separated easily using flash chromatography. Moreover as shown by the various examples, this observation appears to apply to a wide range of deoxynucleotides containing all four bases and possessing different 5' and phosphorus protecting groups. The results therefore outline a synthesis pathway leading to the general availability of such compounds for further studies involving chiral phosphonothioate analogs.

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1. A. D. Griffiths, B. V. L. Potter, and I. C. Eperon, Nucleic Acids Res. 15, 4145 (1987).

- W. K.-D. Brill and M. H. Caruthers, *Tetrahedron Lett.* 28, 3205 (1987). The spectrometric data for bis(N,N-diisopropylamino)methylphosphine were incorrectly reported. ³¹P-NMR 38.5 δ (CDCl₃); ¹H-NMR δ 3.3 (sec. H of isopropyl groups), 1.12 (m, CH₃); bp: 85-88°C (2.2 Torr).
- 3. W. C. Still, M. Kahn, and A. Mitra, J. Org. Chem. 43, 2923 (1978).
- 4. (a) K. A. Gallo, K.-L. Shao, L. R. Phillips, J. B. Regan, M. Koziolkiewicz, B. Vznanski, W. J. Stec, and G. Zon, *Nucleic Acids Res.* 14, 7406 (1986). (b) P. Guga, M. Koziolkiewicz, A. Okruszek, B. Vznanski, and W. J. Stec, *Nucleosides and Nucleotides* 6, 111 (1987).
- 5. P. S. Miller, M. P. Reddy, A. Murakami, K. R. Blake, S.-B. Lin, and C. H. Agris, Biochemistry 25, 5092 (1986).
- 6. Protected deoxynucleosides were N-acylated according to Ti et al.⁷ Further 5'-O-benzoylation to yield 4 was according to Mitsunobu et al.⁸ Silyl compounds 2 and 3 were obtained in 88.5% and 76.5% yields, respectively (crystalline from toluene/hexane) for thymidine and N-benzoyldeoxycytidine by treatment with t-butyldimethylsilyl chloride (1.01 eq.) in pyridine (2.5 ml/mmol) and imidazole (0.01 eq.).
- 7. G. S. Ti, B. L. Gaffney, and R. A. Jones, J. Am. Chem. Soc. 104, 1316 (1982).
- 8. O. Mitsunobu, J. Kimura and Y. Fujisawa, Bull. Chem. Soc. Japan 45, 245 (1972).
- 9. Dichlorobenzylmercaptan was prepared from 2,4-dichlorobenzylchloride via its isothiuronium salt. Organikum, VEB Deutscher Verlag der Wissenschaften, Berlin, 1981, p. 263.
- 10. Reaction mixtures were poured into ethylacetate to precipitate salts. Filtrates were extracted with sodium bicarbonate (twice) and brine, dried over MgSO4 and concentrated to glasses. Products were purified by column chromatography on 85 g of silica 60 (0.04-0.063 mm/230-400 mesh ASTM, Machery Nagel).
- J. E. Marugg, E. de Vroom, C. E. Dreef, M. Tromp, G. A. van der Marel, and J. H. van Boom, Nucleic Acids Res. 14, 2171 (1986).
- 12. Sodium ethylmercaptide and sodium isopropylmercaptide were prepared by treatment of the corresponding thiol with sodium ethoxide and precipitation with anhydrous ether. The lithium mercaptides of the same thiols were obtained *in situ* by addition of lithium bistetramethylsilylamide to the reaction mixtures containing **llab**. Comparable yields for both **22ab** and **23ab** were obtained with the lithium and sodium salts.
- 13. The ³¹P-NMR and ¹H-NMR data for compounds **17ab-28ab** were recorded in CDCl₃. For ³¹P-NMR, 85% H₃PO₄ was the external reference. ¹H-NMR data for compounds **17ab-28ab** were not included due to space limitations but can be provided upon request. UV spectra were recorded in ethanol. R_f values refer to separations on silica gel plates. The elution conditions listed with the characterization data for compounds **17ab-28ab** (ref. 14-25) were for compounds synthesized via the hydroxybenzotriazole method. Solvents were as follows. A, toluene:ethylacetate (2:3, v/v); B, toluene:ethylacetate (1:9, v/v); C, ethylacetate: acetonitrile:toluene (85:10:5, v/v/v); D, CH₃CCl₃:CH₃OH (4:1, v/v).
- 14. 5'-O-Tritylthymidine 3'-O(S-p-chlorophenyl)methylphosphonothioate. FAB⁺ mass spectrum, 712 (M + Na), 243 Tr⁺; FAB⁻ mass spectrum, 687 (M 2H)⁻, 446 (M Tr)⁻; UV λ_{max} = 264 nm. Isomer A (17a): ³¹P-NMR δ 52.3; R_f = 0.63 (C). Isomer B (17b): ³¹P NMR δ 51.8; R_f = 0.36 (C). Chromatographic separation: 1. Solvent A, elution of mercaptan and colored side products. 2. Solvent B, elution of 17a. 3. Solvent C, elution of 17b.
- 15. 5'-O-Tritylthymidine-3'-O(S-phenyl)methylphosphonothioate. FAB⁺ mass spectrum, 672 (M 1 + Na)+, 467 (M S-phenylmethylphosphonothioate)+, 243 Tr+; UV λ_{max} = 266 nm. Isomer A

(18a): ^{31}P -NMR & 53.61; R_f = 0.65 (C). Isomer B (18b): ^{31}P NMR & 53.07; R_f = 0.37 (C). Chromatographic separation as in ref. 14.

- 5'-O-Tritylthymidine-3'-O(S-benzyl)methylphosphonothioate. FAB⁺ mass spectrum, 691 (M H 16. + Na)⁺, 489 (M - S-benzylmethylphosphonothioate + Na)⁺, 467 (M -S-benzylmethylphosphono-thioate)⁺, 243 Tr⁺; UV $\lambda_{max} = 266$ nm. Isomer A (**19a**): ³¹P-NMR & 55.99; R_f = 0.66 (C). Isomer B (**19b**): ³¹P-NMR & 54.12; R_f = 0.32 (C). Chromatographic separation as in ref. 14.
- 5'-O-Tritylthymidine-3'0(S-p-chlorobenzyl)methylphosphonothioate. FAB+ mass spectrum, 725 17. $(M + Na - H)^+$, 467 (M - S-p-chlorobenzylmethylphosphonothioate + Na)⁺, 243 Tr⁺; UV $\lambda_{max} = 264$ nm. Isomer A (**20a**): ³¹P-NMR & 55.36; R_f = 0.74 (C). Isomer B (**20b**): ³¹P-NMR & 53.62; $R_f = 0.26$ (C). Chromatographic separation: 1. Solvent A, elution of mercaptan and colored side products. 2. Solvent C, elution of 20a. 3. Solvent D, elution of 20b.
- 18. 5'-O-Tritylthymidine-3'-O(S-2,4 dichlorobenzylmethyl)methylphosphonothioate. FAB⁺ mass spectrum, 759 (M + Na - H)⁺, 467 (M - S(2,4 dichlorobenzylmethyl)methylphosphonothioate), 243 Tr⁺; UV $\lambda_{max} = 266$ nm. Isomer A (21a): ³¹P-NMR & 53.74; R_f = 0.63 (C). Isomer B (21b): ³¹P-NMR & 53.52; R_f = 0.33 (C). Chromatographic separation: 1. Solvent A, elution of mercaptan and colored side products. 2. Solvent B, elution of 21a. 3. Solvent D, elution of 21b.
- 19. 5'-O-Tritylthymidine 3'-O(S-ethyl)methylphosphonothioate. FAB+ mass spectrum, 629 (M + Na - H)⁺, 470 (M - S-ethyl methylphosphonothioate + 3H)⁺, 243 Tr⁺; UV $\lambda_{max} = 266$ nm. Isomer A (22a): ³¹P-NMR & 54.75; R_f = 0.51 (C). Isomer B (22b): ³¹P-NMR & 54.53; R_f = 0.18 (C). Chromatographic separation: 1. Solvent A, elution of mercaptan and colored side products. 2. Solvent C, elution of 22a. 3. Solvent D, elution of 22b.
- 5'-O-Tritylthymidine 3'-O(S-isopropyl)methylphosphonothioate. FAB+ mass spectrum, 643 (M + 20. Na - H)⁺, 467 (M - S-isopropyl methylphosphonothioate)⁺, 243 Tr⁺; UV $\lambda_{max} = 266$ nm. Isomer A (23a): ³¹P-NMR & 55.8; R_f = 0.66 (C). Isomer B (23b): ³¹P-NMR & 53.92; R_f = 0.29 (C). Chromatographic separation as in ref. 14.
- 5'-O-t-butyldimethylsilylthymidine 3'-O(S-benzyl)methylphosphonothioate. FAB⁺ mass spec-21.
- D'-O-t-butyldimethylsilylthymidine 3'-O(S-benzyl)methylphosphonothioate. FAB⁺ mass spectrum, 563 (M + Na H)⁺, 541 (M)⁺, 471 (M + Na toluene)⁺, 339 (5'-O-t-butyldimethylsilyl-3'-O²-anhydrothymidine)⁺; UV $\lambda_{max} = 266$ nm. Isomer A (**24a**): ³¹P-NMR & 54.5; R_f = 0.6 (C). Isomer B (**24b**): ³¹P-NMR & 53.67; R_f = 0.34 (C). Chromatographic separation as in ref. 17. 5'-O-t-butyldimethylsilylthymidine N⁴-benzoyldeoxycytidine 3'-O(S-benzyl)methylphosphonothioate. FAB⁺ mass spectrum, 652 (M H + Na)⁺, 630 (M⁺), 540 (M benzyl + 2H)⁺, 428 (5'-t-butyldimethylsilyl N⁶-benzoyl 3'-O²-anhydrodeoxycytidine)⁺; UV $\lambda_{max} = 262$ nm. Isomer A (**25a**): ³¹P-NMR & 54.53; R_f = 0.69 (ethylacetate:acetonitrile, 7:3, v/v). Isomer B (**25b**): ³¹P-NMR & 53.67; R_f = 0.23 (ethylacetate:acetonitrile, 7:3, v/v). Chromatographic separation: 1. Solvent A, elution of mercaptan and colored side products. 2. Ethylacetate:acetonitrile, 7:5h 22. nitrile, 7:3, v/v, elution of 25a. 3. Solvent D, elution of 25b.
- 23. 5'-0-N⁴-dibenzoyldeoxycytidine 3'-0(S-benzyl)methylphosphonothioate. FAB⁺ mass spectrum, 642 (M - H + Na)⁺, 620 (M⁺), 418 (5'-O-N⁴-dibenzoyl 3'-O²-anhydrodeoxycytidine)⁺; UV $\lambda_{max} = 262$ nm. Isomer A (**26a**): ³¹P-NMR & 55.36; R_f = 0.53 (ethylacetate:acetonitrile, 1:1, v/v). Isomer B (**26b**): ³¹P-NMR & 54.49; R_f = 0.24 (ethylacetate: acetonitrile, 1:1, v/v). Chromatographic separation: 1. Solvent A, elution of mercaptan and colored side products. 2. Ethylacetate:acetonitrile, 1:1, v/v, elution of 26a. 3. Solvent D, elution of 26b.
- 5'-O-Trityl-N⁶-benzoyldeoxyadenosine 3'-O(S-benzyl)methylphosphonothioate. FAB⁺ mass 24. spectrum, 805 (M + Na)⁺, 783 (M + H)⁺, 692 (M - benzyl + 2H)⁺, 522 (M - TrOH)⁺, UV_{max} = 282 nm. Isomer A (27a): ³¹P-NMR & 53.63; R_f = 0.57 (Solvent C: CH₃OH 97.5:2.5, v/v). Isomer B (27b): ³¹P-NMR & 53.91; Rf = 0.26 (Solvent C: CH₃OH 97.5:2.5, v/v). Chromatographic separation: 1. Solvent C, elution of mercaptan and colored side products. 2. Solvent C: CH30H, 98:2, v/v, elution of 27a. 3. Solvent C, CH30H, 9:1, v/v, elution of 27b.
- 25. 5'-0-Trityl-N²-isobutyryldeoxyguanosine 3'-0(S-benzyl)methylphosphonothioate. FAB⁺ mass spectrum, 787 (M + Na)⁺, 764 (M)⁺, 696 (M - benzyl + H + Na)⁺, 674 (M - benzyl + 2H)⁺, 504 (M - TrOH)⁺; UV_{max} = 260. Isomer A (**28a**): ³¹P-NMR & 55.05; R_f = 0.63 (CHCl3:CH3OH, 95:5, v/v). Isomer B (**28b**): ³¹P-NMR & 54.74; R_f = 0.35 (CHCl3:CH3OH, 95:5, v/v). Chromatographic separation in CHCl3:CH3OH, 98:2, v/v.
- 26. Triethylammonium 5'-O-tritylthymidine 3'-O-methylphosphonothioate (30). FAB+ mass spectrum (M refers to nucleotidic component), 645 (M⁺ + Na + ethylamine)⁺, 623 (M⁺ + ethylamine), 608 (M + C_2H_5), 507 (5'-O-trityl 2'-O² anhydrothymidine + K)⁺, 489 (5'-O-trityl 2'-0² anhydrothymidine + Na⁺), 243 Tr⁺. FAB⁻ mass spectrum, 599 (M - 2H + Na)⁻, 577 $(M - H)^{-}$; $UV_{max} = 268$ nm; $3^{1}P-NMR \delta$ 74.4 (NEt3:dioxane:Ph-SH, 2:2:1, v/v/v), $R_{f} = 0.28$ (C).

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