preference. Most importantly, we have shown that the thermodynamic cycle-perturbation approach is a feasible route for the analysis and prediction of affinity in large biomolecular systems.

Acknowledgment. Dr. W. van Gunsteren generously provided the GROMOS programs. This work was supported in part by the National Science Foundation, the Robert A. Welch Foundation, and the Texas Advanced Technology Research Program. J.A.M. is a Camille and Henry Dreyfus Teacher-Scholar.

Registry No. Benzamidine, 618-39-3; *p*-fluorobenzamidine, 2339-59-5; trypsin, 9002-07-7.

Phosphorothioite Method. 1. A New Type of Internucleotidic Bond Formations Involving Simultaneous Oxidation Process by Use of Deoxyoligonucleoside Phosphorothioites as New Key Intermediates for Deoxyoligonucleotide Synthesis†

Mitsuo Fujii, Hajime Nagai, Mitsuo Sekine, and Tsujiaki Hata*

Department of Life Chemistry
Tokyo Institute of Technology
Nagatsuta, Midoriku, Yokohama 227, Japan
Received February 11, 1986

In recent years, oligonucleotide synthesis has been markedly facilitated by developments of the phosphite and phosphoramidite approaches introduced by Letsinger[1] and Caruthers.[2] These methods have also been extended to the synthesis of nucleic acid analogues involving P—CH_3 and P=S bonds.[3]

Especially the phosphoramidite approach has proved to be practically useful for molecular biology.[4] However, the whole process for the phosphoramidite approach involves an additional oxidation step of the tervalent phosphite intermediates after condensation compared with the phosphotriester approach.

In this paper, we wish to report a more straightforward approach to the oligodeoxyribonucleotide synthesis involving a one-step reaction for both condensation and oxidation.

It is known the *S*-alkyl nucleoside phosphorothioates of the diester type were activated by iodine to give highly reactive metaphosphate intermediates.[5] Contrary to this fact, *S,S'*-diaryl nucleoside phosphorodithioates of the triester type did not react with iodine.[6] On the other hand, phosphite derivatives *S,S'*-diethyl alkyl phosphorodithioites were accessible to oxidative hydrolysis by means of aqueous iodine giving rise to monoalkyl phosphates.[7] The facile activation of tervalent P–S bonds with iodine suggested the possibility that if iodine was added to $(R'O)_2P$–SR″ in the presence of ROH under anhydrous conditions the SR″ group could be oxidatively replaced by the OR group to give the corresponding phosphotriesters, $(R'O)_2P(O)OR$.

† This paper is dedicated to Professor Morio Ikehara on the occasion of his retirement from Osaka University on March 1986.

(1) Letsinger, R. L.; Finnan, J. L.; Heavner, G. A.; Lunsford, W. B. *J. Am. Chem. Soc.* **1975**, *97*, 3278. Letsinger, R. L.; Lunsford, W. B. *J. Am. Chem. Soc.* **1976**, *98*, 3655.

(2) Beaucage, S. L.; Caruthers, M. H. *Tetrahedron Lett.* **1981**, *22*, 1859. McBride, L. J.; Caruthers, M. H. *Tetrahedron Lett.* **1983**, *24*, 245. McBride, L. J.; Caruthers, M. H. *Tetrahedron Lett.* **1983**, *24*, 2953.

(3) Stec, W. J.; Zon, G.; Egan, W.; Byrd, R. A.; Phillips, L. R.; Gallo, K. A. *J. Org. Chem.* **1985**, *50*, 3908. Stec, W. J.; Zon, G.; Egan, W.; Stec, B. *J. Am. Chem. Soc.* **1984**, *18*, 6077. Nobel, S. A.; Fisher, E. F.; Caruthers, M. H. *Nucleic Acids Res.* **1984**, *12*, 3387. Nemer, M. J.; Ogilvie, K. K. *Tetrahedron Lett.* **1980**, *21*, 4149. Nemer, M. J.; Ogilvie, K. K. *Tetrahedron Lett.* **1980**, *21*, 4153.

(4) Caruthers, M. H. *Science (Washington, D.C.)* **1985**, *230*, 281. Eckstein, F. *Angew. Chem., Int. Ed. Engl.* **1983**, *22*, 423. Connolly, B. A.; Potter, B. V. L.; Eckstein, F.; Pingoud, A.; Grotjahn, L. *Biochemistry* **1984**, *23*, 3443.

(5) Cock, A. F.; Holman, M. J.; Nussbaum, A. L. *J. Am. Chem. Soc.* **1969**, *91*, 1522. Cock, A. F.; Holman, M. J.; Nussbaum, A. L. *J. Am. Chem. Soc.* **1969**, *91*, 6479.

(6) Sekine, M.; Hamaoki, K.; Hata, T. *Bull. Chem. Soc. Jpn.* **1981**, *54*, 3815.

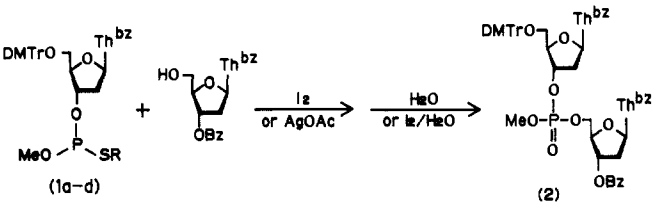
(7) Takaku, H.; Shimada, Y. *Tetrahedron Lett.* **1972**, *5*, 411.

Table I. Isolated Yields and ^{31}P NMR[a] Data of the Reported Compounds (**1**, **2**)

compd	yield/%	^{31}P NMR/ppm	yield of **2**/% I_2	yield of **2**/% AgOAc
1a	55	−139.26	42	66
1b	70	−138.63	72	88
1c	72	−139.12	78	78
1d	73	−139.12	80	81

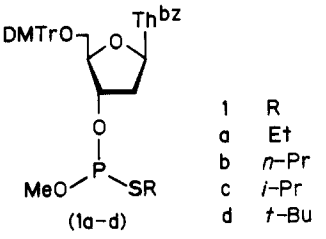
[a] Chemical shifts ($CDCl_3$/Py, 3:1, v/v) of compounds **1a–d** with respect to an external standard of 85% H_3PO_4 (aqueous). The lower field than the reference peak of 85% H_3PO_4 is described as the minus region.

Scheme I



In order to examine whether *S*-alkyl nucleoside phosphorothioites can be used as the starting units for oligodeoxyribonucleotide synthesis, several experiments have been conducted.

First, *S*-alkyl nucleoside phosphorothioites (**1a–d**) were synthesized by reaction of 5′-O-(dimethoxytrityl)-3-benzoylthymidine ($DMTrT^{bz}$) with an (alkylthio)methoxychlorophosphine.[8] A typical procedure is shown as follows: [$CH_3OP(Cl)S$-*t*-Bu] was added dropwise to a solution of $DMTrT^{bz}$ in dry pyridine at room temperature. Aftr 10 min the solution was transferred with CH_2Cl_2 to a separatory funnel. The solution was washed 3 times with water. The organic phase was dried over anhydrous Na_2SO_4, filtrated, and evaporated under reduced pressure. The residue was separated by silica gel chromatography with CH_2Cl_2/hexane/pyridine (6:3:1, v/v/v). The purified product was dissolved in benzene and the solution was added dropwise to hexane while the mixture was stirred vigorously. The precipitate was collected and dried over P_4O_{10} under reduced pressure. The isolated yields of **1a–d** were 56–73% (Table I). These powders are found to be stable on storage at −30 °C for several months except for **1a**.



It was found that iodine or silver acetate could activate the P–S bond of **1a–d**. A mixture of compound **1** and 3,3′-O-dibenzoylthymidine was dissolved in a small amount of pyridine, coevaporated 3 times with dry pyridine, and dissolved in CH_2Cl_2/lutidine/NEt_3 (8:1:1, v/v/v). Iodine or AgOAc was added to the solution, and the mixture was vigorously stirred for 2 min. In the case of iodine, the 3′–5′ internucleotidic phosphate linkage was directly formed by addition of a small amount of water after the coupling reaction was completed. When AgOAc was used, dinucleoside methyl phosphite was initially formed and it required in situ treatment of the mixture with a solution (THF/2,6-lutidine/H_2O, 2:1:1, v/v/v) containing 0.2 M iodine for oxidation. After the usual workup followed by silica gel column chromatography, the coupling product (**2**) was isolated as shown in Table I (Scheme I).

Application of the present phosphorothioite approach to the synthesis of oligothymidylate on polymer support was also tested. Polystyrene (1% DVB cross-linking, 46 μmol/g) which was derived

(8) Schrader, G. Ger. Pat. 1112752, 1960; *Chem. Abstr.* **1962**, *56*, 2331c.

0002-7863/86/1508-3832$01.50/0

with a thymidine unit was used as support. Aftr removal of the DMTr group in the usual manner, a mixture of compound **1d** (10 equiv) and iodine (50 equiv) in CH_2Cl_2/lutidine/NEt_3 (5:4:1, v/v/v) was added to the polymer support. After being gently shaken for 10 min, the mixture was quenched with lutidine containing a small amount of water. Subsequent filtration followed by deprotection of the DMTr group gave the dimer loaded on the resin. The same cycle was repeated 3 times. The average coupling yield was 94% based on assay of the DMTr cation. After the usual deprotection, tetrathymidylate $(Tp)_3T$ was obtained and analyzed with snake venom phosphodiesterase. The enzyme assay showed complete degradation to T and pT in a 1:3 ratio.

In conclusion, compounds **1b–d** are easily prepared and stable under normal laboratory conditions. They are readily activated by iodine or silver acetate. It is noteworthy that the former reagent does not require the oxidation step, because this could be simultaneously performed during condensation. The application of the present phosphorothioite method is now in progress.

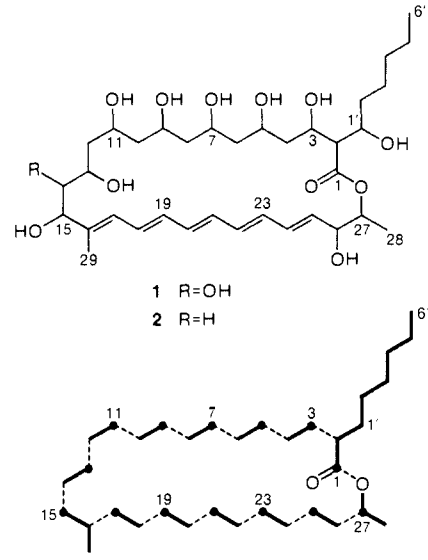


Figure 1.

Biosynthesis of Polyene Antibiotics: Intact Incorporation of ^{13}C-Labeled Octanoate into Fungichromin by *Streptomyces cellulosae*

Paul H. Harrison, Hiroshi Noguchi, and John C. Vederas*

Department of Chemistry, University of Alberta Edmonton, Alberta, Canada T6G 2G2

Received March 6, 1986

Fungichromin (**1**)[1] and filipin (**2**)[2] from *Streptomyces cellulosae* are typical members of the family of polyene antibiotics,[3] some of which (e.g., amphotericin B) are widely used in treatment of fungal infections.[4] Such steroid-binding antibiotics[5] usually possess a macrocyclic lactone ring which consists of a conjugated polyene chain of three to eight double bonds and has a complementary alkyl chain adorned with hydroxyl groups at alternating carbons.[3] A number of biosynthetic studies of this family have employed incorporations of radioactive precursors and subsequent chemical degradations to locate labeled sites.[6] These experiments suggest that the macrolide rings are formed from acetate and propionate by a polyketide pathway similar to fatty acid biogenesis. On this basis Martin proposed[6b] an expected[7] arrangement of biosynthetic units for filipin (**2**) with the exception of an implied introduction of a preformed eight-carbon fatty acid chain (Figure 1). Since intact incorporation of carboxylic acids longer than four carbons into microbial polyketides is extremely rare,[8] current interest in polyketide biosynthesis[7,9] led us to examine this hypothesis. The present work describes the use of ^{13}C-labeled precursors to determine the origin of the carbon skeleton of fungichromin (**1**). The results show that an unusual intact incorporation of [^{13}C]octanoate occurs.

Table I

carbon	δ	precursor	enhancement[b]
1	172.98	*c*	2.9
16	138.55		
19	135.36	*d*	2.7
21	134.81	*d*	2.4
25	134.28	*d*	2.9
23	134.21	*d*	2.5
20	134.13	*e*	2.2
22	133.66	*e*	2.5
24	131.97	*e*	2.6
17	129.91	*d*	2.5
18	129.06	*e*	2.2
15	80.43	*f*	5.6
14	78.31	*e*	2.8
27	75.25	*d*	2.5
h	74.20	*d*	2.5
h	74.08	*d*	2.6
h	73.92	*d*	2.7
3	73.41	*d*	2.3
26	73.25	*e*	2.7
1′	72.59	*g*	2.5
h	71.45	*d*	2.3
13	70.34	*d*	2.5
2	60.35		
i	45.33	*e*	2.9
i	45.17	*e*	2.8
i	44.34	*e*	2.5
4	41.38	*e*	2.9
i	39.58	*e*	2.5
2′	36.22		
4′	32.88		
3′	26.01		
5′	23.65		
28	17.96	*e*	3.0
6′	14.38		
29	11.74		

[a] 100.6-MHz ^{13}C NMR spectra in methanol-d_4 with solvent reference at 49.00 ppm. [b] Ratio of carbon signal intensities for enriched and natural abundance sample measured under identical conditions. [c] Sodium [1-^{13}C]octanoate. [d] Sodium [1-^{13}C]acetate. [e] Sodium [2-^{13}C]acetate. [f] Sodium [1-^{13}C]propionate. [g] Sodium [3-^{13}C]octanoate. [h] C-5, C-7, C-9, or C-11; see ref 17. [i] C-6, C-8, C-10, or C-12; see ref 17.

Addition of the sodium salts of [1-^{13}C]acetate, [2-^{13}C]acetate, or [1-^{13}C]propionate to cultures of *S. cellulosae* ATCC 12625 produced samples of fungichromin (**1**)[10] whose ^{13}C NMR spectra[11]

(1) (a) Fungichromin, cogomycin, and lagosin are identical compounds: Pandey, R. C.; Guenther, E. C.; Aszalos, A. A. *J. Antibiot.* **1982**, *35*, 988–996 and references therein. (b) Tytell, A. A.; McCarthy, F. J.; Fisher, W. P.; Bolhofer, W. A.; Charney, J. *Antibiot. Annu.* **1955**, 716–718.

(2) Kelly, P. M.; Nabinger, K. A. *J. Antibiot.* **1985**, *38*, 485–489 and references therein.

(3) For recent reviews, see: (a) Omura, S.; Tanaka, H. In *Macrolide Antibiotics—Chemistry, Biology and Practice*; Omura, S., Ed.; Academic Press: New York, 1984; pp 351–404. (b) Boggs, S.; Schlessinger, D.; Pandey, R. C.; Rinehart, K. L., Jr. *Antimicrob. Agents Chemother.* **1979**, *15*, 716–722.

(4) Schaffner, C. P. In ref 3a, pp 457–507.

(5) (a) Clejan, S.; Bittman, R. *J. Biol. Chem.* **1985**, *260*, 2884–2889. (b) Gale, E. F. In ref 3a, pp 425–455. (c) Norman, A. W.; Spielvogel, A. M.; Wong, R. G. *Adv. Lipid Res.* **1976**, *14*, 127–171.

(6) For reviews, see: (a) Martin, J. F. In ref 3a, pp 405–424. (b) Martin, J. F. *Annu. Rev. Microbiol.* **1977**, *31*, 13–38.

(7) Simpson, T. J. *Nat. Prod. Rep.* **1985**, *2*, 321–347.

(8) Hexanoic acid has been incorporated intact as a polyketide starter unit in one instance: Townsend, C. A.; Christensen, S. B.; Trautwein, K. *J. Am. Chem. Soc.* **1984**, *106*, 3868–3869.

(9) Moore, R. N.; Bigam, G.; Chan, J. K.; Hogg, A. M.; Nakashima, T. T.; Vederas, J. C. *J. Am. Chem. Soc.* **1985**, *107*, 3694–3701 and references therein.

0002-7863/86/1508-3833$01.50/0