of lead tetraacetate and allowed to stand at −90° for 1.5 hr, while the lead diacetate precipitated without any oxygen evolution. The solution was then brought to constant temperatures over the range from −82 to −68° and the oxygen evolution was followed kinetically. Good *first-order* lines were obtained under these conditions according to the equations

$$\frac{d(O_2)}{dt} = \frac{-d(R_2O_4)}{dt} =$$

$$k_{O_2}(R_2O_4) = k_{O_2}([O_2]_\infty - [O_2]_t) \quad (7)$$

$$\log k_{O_2} = \left[9.27 - \frac{2440}{T}\right] \text{sec}^{-1};\ E_a = 11 \text{ kcal/mole} \quad (8)$$

The concentrated solutions prepared at −90° could be frozen and cooled to temperatures as low as −160° before they ceased to exhibit an esr signal. The fact that these signal levels were rapidly established and reasonably constant at each temperature below the freezing point (−96°) of methylene chloride indicates considerable mobility of the tetroxide and peroxy radicals, either in a polycrystalline mixture or in pockets of concentrated solution.[10]

An estimate of the dissociation equilibrium constant $K = k_{-1}/k_1$ could be made by assuming that at 0.25 M, where k_{O_2} was measured, the tetroxide was essentially undissociated, so that k_{O_2} equals the k_2 of eq 2, and that at concentrations below 10^{-4} M, as in the measurement of k_{esr}, the dissociation is nearly complete. These assumptions, together with $k_{-1} \gg k_2$, lead to the equation

$$K = \frac{2k_{O_2}}{k_{esr}} \quad (9)$$

Combination of eq 6 and 8 then yields

$$\log K = 3.55 - \frac{1340}{T};\ \Delta H = 6 \text{ kcal/mole} \quad (10)$$

The two temperatures requiring the least extrapolation, −55 and −68°, then give values for K of 2.57×10^{-3} and 1.05×10^{-3}, respectively. The assumption that the $RO_2\cdot$ at 6.0×10^{-5} M at −75° was totally unassociated affords values of $K^{-85°} = 1.8 \times 10^{-4}$ and $K^{-95°} = 4.9 \times 10^{-5}$. These values are shown plotted in Figure 4, along with the estimate made in methanol by Thomas[3] by fitting his rate measurements at 22° to the equation for decomposition of the partly dissociated t-Bu_2O_4 under nonlimiting conditions. Although we might have expected the dissociation in methanol to be higher, not lower, than in methylene chloride, this value may be compatible with ours within the uncertainties of both methods.[10a]

From these values of K we calculate that in the measurement of k_{O_2} at −68° and 0.25 M the dissociation was about 4.5%, while in the measurement of k_{esr} at −55° and 10^{-5} M the dissociation was 99%. Thus the approximations used are adequate and yield self-consistent results.

A compound formerly thought[11] to be di-t-butyl tetroxide must be, according to its decomposition temperature, the trioxide instead.[6] A stable substance reported[12] as the trioxide has been shown to be 2,2-bis-(t-butylperoxy)propane.[13,14]

Acknowledgment. This work was supported by the B. F. Goodrich Co.

(10) R. E. Pincock and T. E. Iovsky, *J. Am. Chem. Soc.*, **87**, 2072, 4100 (1965).

(10a) NOTE ADDED IN PROOF. Dr. J. R. Thomas has kindly informed us that, on the basis of new evidence, his value of K shown in Figure 4 should be regarded only as a lower limit.

(11) N. A. Milas and S. M. Djokic, *Chem. Ind.* (London), 405 (1962).

(12) N. A. Milas and G. G. Arzoumanidis, *ibid.*, 66 (1966).

(13) Bartlett and Günther, ref 5, footnote 9a.

(14) R. D. Youssefyeh and R. W. Murray, *Chem. Ind.* (London), 1531 (1966).

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A Convenient Method for Stepwise Synthesis of Oligothymidylate Derivatives in Large-Scale Quantities[1,2]

Sir:

For the synthesis of high molecular weight polydeoxyribonucleotides with arbitrary, defined sequences of the four nucleotide units, methods are needed which enable one (a) to prepare and purify readily on a relatively large scale oligonucleotides or suitable oligonucleotide derivatives and (b) to utilize efficiently the oligonucleotides (or derivatives) as building blocks for construction of high molecular weight material.[3] In this communication we describe a synthetic method which satisfies stipulation a for oligothymidylate derivatives. Work is in progress to adapt the procedure to the synthesis of mixed oligonucleotide derivatives and to the construction of polynucleotides from these units.

The basic feature of the method is that chains are built with phosphotriester rather than phosphodiester links. In a final step blocking groups are removed hydrolytically to give the desired phosphodiester chains. Initial work on this approach was carried out in experiments with polymer support reactions.[4] We now find that the method can be adapted readily to work in solution, in which case the phosphotriesters can be isolated, characterized, and utilized as intermediates in subsequent synthetic steps. There are two principal advantages to the new procedure, both of which stem from the fact that the phosphotriesters obtained are uncharged, neutral molecules. (1) The products can be handled by conventional organic techniques; for example, they can be separated by chromatography with organic solvents on silica gel, which has a much higher capacity and greater flow rate than DEAE cellulose. (2) Yields do not fall off significantly as the chain length of the oligonucleotide derivative increases. As a consequence it is not necessary to employ increasingly large excesses of nucleoside reagent as the stepwise synthesis progresses.[5]

The chemistry is illustrated by the synthesis of the β-cyanoethyl-TpT derivative I, indicated in Scheme I.

(1) Part VIII in our series on nucleotide chemistry. Part VII: K. K. Ogilvie and R. L. Letsinger, *J. Org. Chem.*, **32**, 2365 (1967).

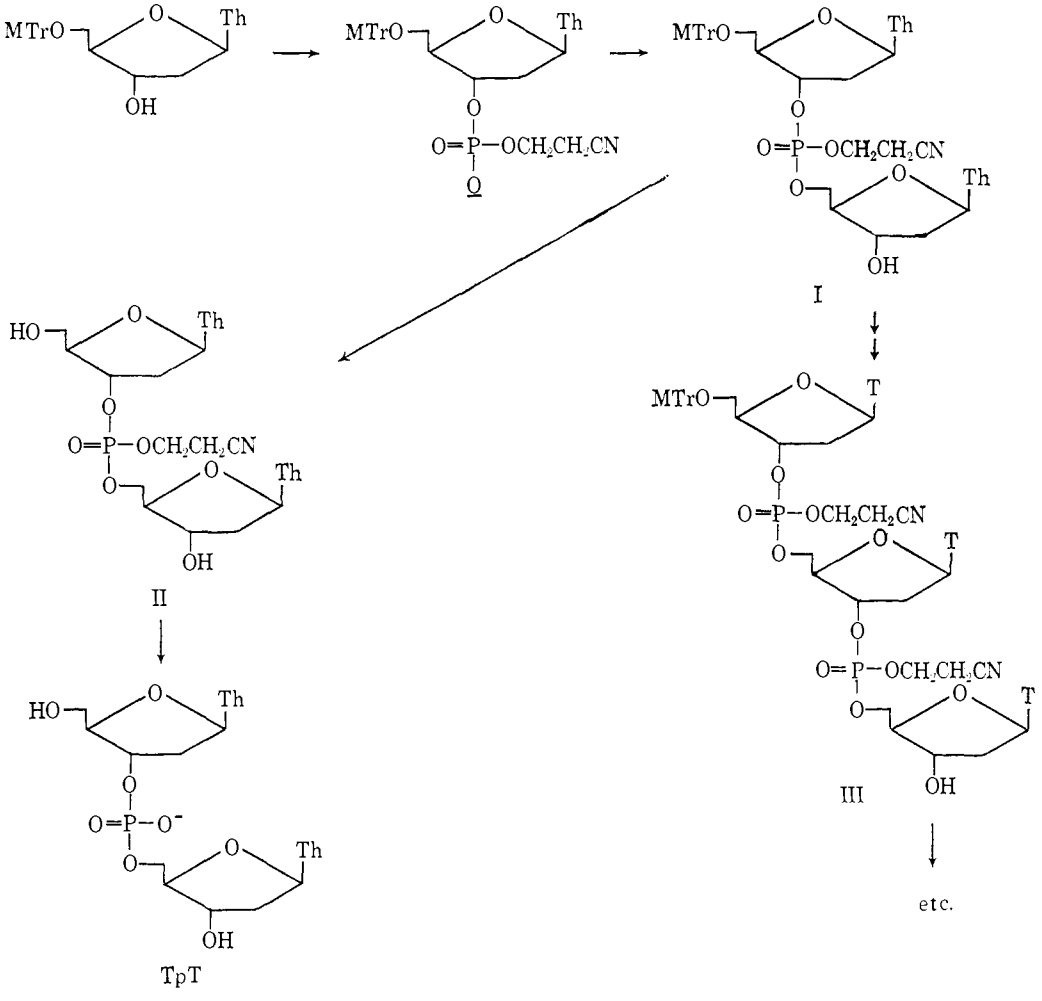
(2) This research was supported by the Division of General Medical Sciences, National Institutes of Health, Grant GM-10265.

(3) For a study of the condensation of small blocks of oligonucleotides see E. Ohtsuka and H. G. Khorana, *J. Am. Chem. Soc.*, **89**, 2195 (1967).

(4) R. L. Letsinger and V. Mahadevan, *ibid.*, **87**, 3526 (1965); **88**, 5319 (1966).

(5) See T. M. Jacob and H. G. Khorana, *ibid.*, **87**, 368 (1965); S. A. Narang and H. G. Khorana, *ibid.*, **87**, 2981 (1965).

Scheme I



MTr = *p*-monomethoxytrityl; Th = thymine ring

In this case 36.0 g (70 mmoles) of 5′-O-monomethoxytritylthymidine was stirred with 70 mmoles of pyridinium β-cyanoethyl phosphate and 30.5 g (140 mmoles) of mesitylenesulfonyl chloride in 140 ml of pyridine for 6 hr. Following treatment with water to hydrolyze pyrophosphates present, the phosphorylated product was extracted into chloroform, transferred to 140 ml of pyridine, and stirred with 42.5 g (140 mmoles) of triisopropylbenzenesulfonyl chloride[6] and 33.9 g (140 mmoles) of thymidine for 24 hr. The mixture was then treated with water and the solution extracted with chloroform. Two-thirds of the chloroform solution was evaporated, rediluted with chloroform, and applied to a column (100 × 3 cm) of silica gel in ethyl acetate. The column was eluted successively with 8.5 l. of ethyl acetate and 4 l. of tetrahydrofuran. Most of compound I was eluted in the first liter of tetrahydrofuran. Concentration of the tetrahydrofuran solution and addition of hexane gave I as a white solid, mp[7] 126–128°. With the material obtained from the other third of the solution, which was purified in the same manner, the yield was 39.3 g (64% based on monomethoxytritylthymidine). On warming for a few minutes with 80% aqueous acetic acid this compound was converted to the cyanoethyl derivative of thymidylylthymidine (II), mp[7] 113–115°, which in turn afforded thymidylylthymidine (TpT) cleanly on brief treatment with ammonium hydroxide at room temperature. Assay of the TpT thus obtained by hydrolysis with snake venom phosphodiesterase indicated that 96% was the desired 3′–5′ isomer (hydrolysis to thymidine 5′-phosphate and thymidine) and 4% was the 3′–3′ isomer (undegraded by the venom enzyme). For isolation of the pure 3′–5′ isomer 7.0 g (8.0 mmoles) of I was stirred with 1.23 g (4.0 mmoles) of monomethoxytrityl chloride in 40 ml of pyridine for 20 hr. The solvent was stripped off and the products chromatographed on silica gel with ethyl acetate and tetrahydrofuran to give 6.2 g (88% recovery) of the cyanoethyl ester of 5′-O-monomethoxytritylthymidylyl-(3′–5′)-thymidine, mp[7] 126–130°. The TpT obtained on removal of the methoxytrityl and cyanoethyl groups was completely hydrolyzed by an aqueous solution of the snake venom enzyme. This separation of the 3′–5′ and 3′–3′ isomers is based on the fact that monomethoxytrityl chloride reacts much faster with 5′-OH groups than with 3′-OH groups.

That satisfactory yields are maintained in the condensation steps as the chain is extended was demonstrated by converting I to the tetranucleoside triphosphate derivative. Thus, by essentially the same procedure used to make I, the bis(cyanoethyl) derivative of 5′-O-monomethoxytritylthymidylylthymidylylthymi-

(6) R. Lohrmann and H. G. Khorana, *J. Am. Chem. Soc.*, **88**, 829 (1966).

(7) The melting points are not sharp for this series of compounds. After some preliminary softening the solid gradually becomes a glassy liquid.

dine (III, mp[7] 133–136°) was obtained in 49% yield from I, and the tris(cyanoethyl) derivative of 5′-O-monomethoxytritylthymidylylthymidylylthymidylylthymidine (mp[7] 144–146°) was obtained in 57% yield from III. These compounds were characterized by elemental analysis, by isolation of the de(methoxytrityl) derivatives, and by conversion to TpTpT and TpTpTpT, respectively.

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Bimolecular Substitution–Fragmentation. The Reaction of Phenylmethanesulfonyl Halides with Halide Ion

Sir:

While studying the action of nucleophilic reagents on sulfonyl halides,[1] we have encountered a new reaction of alkanesulfonyl halides exemplified by eq 1. We wish

$$PhCH_2SO_2Br \xrightarrow{Br^-} PhCH_2Br + SO_2 \qquad (1)$$

to present evidence which indicates that the reaction is simultaneously a bimolecular nucleophilic displacement on carbon and a concerted fragmentation reaction, and thereby displays mechanistic features which have not hitherto been demonstrated to exist in the same process.

Reaction 1 proceeds readily and quantitatively at room temperature in inert solvents such as methylene chloride or acetonitrile, using alkylammonium bromides as the source of bromide ion. Methanesulfonyl bromide reacts similarly but roughly $^1/_{1000}$ as fast. The analogous reaction of phenylmethanesulfonyl chloride with chloride ion also occurs, but again more slowly (see Table I).

Table I. Reaction of Sulfonyl Halides with Alkylammonium Halides in Dichloromethane at 25.0 ± 0.1°

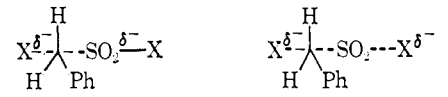
Sulfonyl halide	Salt (concn, *M*)	*k*, l. mole^{-1} sec^{-1}
$PhCH_2SO_2Br$	$Et_4N^+Br^-$ (0.00012)	3.10×10^{-2}
$PhCH_2SO_2Br$	$Et_4N^+Br^-$ (0.001)	2.45×10^{-2}
$PhCH_2SO_2Br$	$Et_4N^+Br^-$ (0.0123)	2.35×10^{-2}
$PhCH_2SO_2Br$	$Bu_4N^+Br^-$ (0.00082)	2.6×10^{-2}
$PhCH_2SO_2Br$	$Et_3NH^+Br^-$ (0.0098)	1.53×10^{-4}
p-$NO_2C_6H_4CH_2SO_2Br$	$Et_4N^+Br^-$ (0.001)	3.85×10^{-2}
m-$NO_2C_6H_4CH_2SO_2Br$	$Et_4N^+Br^-$ (0.001)	3.50×10^{-2}
p-$ClC_6H_4CH_2SO_2Br$	$Et_4N^+Br^-$ (0.001)	3.15×10^{-2}
m-$ClC_6H_4CH_2SO_2Br$	$Et_4N^+Br^-$ (0.001)	1.72×10^{-2}
p-$MeC_6H_4CH_2SO_2Br$	$Et_4N^+Br^-$ (0.001)	5.26×10^{-2}
$PhCH_2SO_2Cl$	$Et_4N^+Cl^-$ (0.01)	1.14×10^{-4}

Reaction 1 is cleanly first order in the sulfonyl halide but only roughly so in the bromide salt, the deviation from simple second-order kinetics probably deriving to a substantial extent from ionic association.[2] *meta* or *para* substitution has a relatively small influence on the rate of reaction. The nonlinear Hammett plot (see Table I) is akin to those that have been observed in the reaction of nucleophilic reagents with benzyl halides, thereby pointing to a SN2 mechanism.[3,4]

Consistent with this interpretation were the results from ^{13}C kinetic isotope effect studies in which it was found that the presence of ^{13}C at the benzylic carbon slowed the reaction by more than 3% at 25° (k_{12}/k_{13} = 1.035 ± 0.0016).[5] A kinetic isotope effect of such a magnitude in α-phenyl-substituted systems has previously been found in bimolecular processes,[6] whereas unimolecular solvolysis of such species shows a much smaller isotope effect.[7]

Stereochemical evidence for the SN2 mechanism was obtained using (*R*)-phenylmethanesulfonyl-1-*d* bromide[8] (PhC^*HDSO_2Br), which gave mainly the *inverted* product, (*S*)-benzyl-1-*d* bromide.[9] It is estimated that at least 60% of the product is formed by inversion, but it is not yet possible to assign a more precise value owing to uncertainty in the optical purity of the sulfonyl bromide.

There remains the question of whether the leaving anion is simply SO_2X^- (which subsequently decomposes into SO_2 and X^-), or whether the reaction is a SN2–fragmentation process in which the S–X bond is partially broken in the transition state. The distinction between the two possible mechanisms may be summarized by structures I and II, which represent the respective transition states for the two pathways. Evidence suggesting the second mechanism is as follows. The reac-



I II

tion of $PhCH_2SO_2Cl$ with $Et_4N^+Cl^-$ in methylene chloride is more than 200 times slower than that of $PhCH_2SO_2Br$ with $Et_4N^+Br^-$. Since tetraethylammonium bromide and chloride were found to be almost identical in their rate of reaction with benzyl tosylate in methylene chloride under conditions comparable to those used in the desulfonation reaction, this difference probably does not derive from a difference in nucleophilicity toward benzylic carbon between the chloride and bro-

(1) Previous work: J. F. King and T. Durst, *J. Am. Chem. Soc.*, **87**, 5684 (1965); *Can. J. Chem.*, **44**, 819 (1966).

(2) *Cf.* S. Winstein, L. G. Savedoff, S. Smith, I. D. R. Stevens, and J. S. Gall, *Tetrahedron Letters*, 24 (1960), and references cited.

(3) C. G. Swain and W. P. Langsdorf, Jr., *J. Am. Chem. Soc.*, **73**, 2813 (1951); R. F. Hudson and G. Klopman, *J. Chem. Soc.*, 1062 (1962).

(4) The possibility that the benzyl halide, under favorable circumstances, might be formed *via* a sulfene was excluded by the finding that the reaction of *p*-nitrophenylmethanesulfonyl-1,1-d_2 bromide in the presence of triethylamine and excess $Et_3NH^+Br^-$ gave *p*-nitrobenzyl-1,1-d_2 bromide with no significant loss of deuterium detected.

(5) These experiments were carried out in collaboration with Dr. J. B. Stothers and Mr. A. J. McNamara, and will be described in detail at a later date.

(6) J. B. Stothers and A. N. Bourns, *Can. J. Chem.*, **40**, 2007 (1962); *cf.* also A. Fry, *Pure Appl. Chem.*, **8**, 409 (1964). Recent reinvestigation (J. B. Stothers and J. Bron, private communication) has shown that in the reaction of α-phenylethyl bromide with ethoxide ion (at 25°) k_{12}/k_{13} = 1.030, a higher value than that previously reported.

(7) J. B. Stothers and A. N. Bourns, *Can. J. Chem.*, **38**, 923 (1960).

(8) The sulfonyl bromide was prepared from optically active benzyl-1-*d* bromide by a procedure adapted from the preparation of the sulfonyl chloride described by J. L. Kice, R. H. Engebrecht, and N. E. Pawlowski, *J. Am. Chem. Soc.*, **87**, 4131 (1965).

(9) This finding, especially when taken in conjunction with our observation that the reaction is unaffected by the presence of oxygen or styrene, clearly distinguishes the halide-catalyzed reaction from the pyrolytic decomposition of alkanesulfonyl halides, which has been found to show the properties of a free-radical chain process (H. F. Herbrandson, W. S. Kelly, and J. Versnel, *ibid.*, **80**, 3301 (1958); G. Geiseler, *et al.*, *Z. Physik. Chem.* (Frankfurt), **28**, 24, 33 (1961); **33**, 264 (1962); **36**, 23 (1963)).