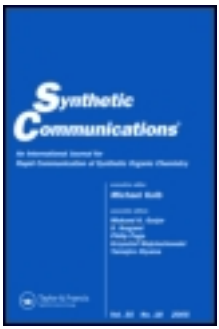

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Synthesis of Iodoalkylacylates and Their Use in the preparation of S-Alkyl Phosphorothiolates

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SYNTHESIS OF IODOALKYLACYLATES AND THEIR USE IN THE PREPARATION OF *S*-ALKYL PHOSPHOROTHIOLATES

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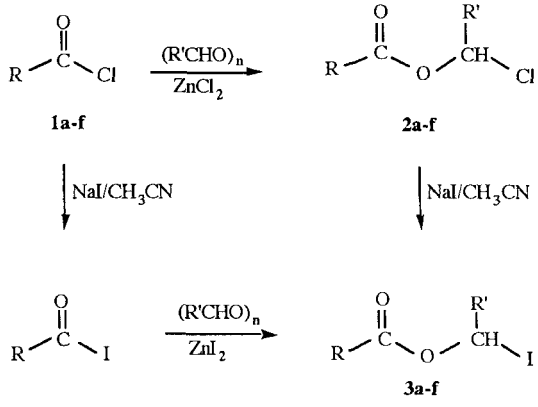
Abstract: Synthesis of iodoalkylacylates **3a-f** and use of **3c** in the preparation of an oligonucleoside phosphorothiate prodrug analog **5** is described.

Structure modifications of drug candidates to produce prodrugs for site-specific targeting and for improving their pharmacophoric features are current areas of research. Such chemical derivatizations of functional groups in drug entities are carried out with an intent to generate bio-reversible analogs.[1] *In vivo*, this drug design entails the enzyme-mediated conversion of the derivatized drug to the parent drug molecule. Increasing application of this generalized design concept is evident in recent publications among which acyloxyalkyl carboxylates or N-[acyloxyalkyl]ester derivatizations of drugs and bio-active molecules (which have carboxylic or amino groups) have been quite prominent.[2-12] In most instances, esterase-mediated regeneration of the parent drug from the prodrug has also been documented *in vitro* and *in vivo*. Recently, we used this design concept in the synthesis of S-acyloxyalkyl phosphorothiolates as bio-reversible analogs of dinucleotides,[13] in model studies, to develop oligonucleotide prodrugs[14] as "antisense" therapeutic agents.[15]

To synthesize the acyloxyalkyl ester type prodrugs, the drug and the chloro- or bromoalkylacylates are usually reacted in a dipolar aprotic solvent with or without NaI as a catalyst, over several hours, to produce the corresponding acyloxyalkylester respectively. However, in case of molecules with multiple nucleophilic sites e.g., oligonucleoside phosphorothioates and peptides, their chemoselective functionalization (e.g., *S*-acyloxyalkylation) with bioreversible groups, require the exclusive use of iodoalkylacylates under mild conditions (aqueous medium, pH 6-7, ambient temperature) for achieving *fast reactions* with complete chemo- and regioselectivity. We have reported the derivatization of oligonucleoside phosphorothioates[13,14] by their chemoselective alkylation with iodoalkylacylates. During the course of our work we needed ready access to a number of iodoalkyl acylates. These iodoalkylacylates **3a-f** are not commercially available and to the best of our knowledge, details of their synthesis and characterization by spectroscopy are documented only for iodomethyl pivalate **3d**.[11,12,16] We describe here the preparation and characterization of a number of iodoalkyl acylates and also provide an example of their use in the synthesis of a new bioreversible analog **5** of an antisense oligonucleoside phosphorothioate **4**, an anti-HIV agent.

The general scheme for the preparation of iodoalkylacylates (iodoalkyl esters) **3a-f** is as shown in Scheme 1. The requisite chloroalkylacylates (chloroalkyl esters)[17,18] **2a-f** were prepared from acid chlorides **1a-f** and paraformaldehyde in an addition condensation reaction catalyzed by anhydrous zinc chloride. The chloroalkylacylates were converted to the corresponding iodoalkylacylates **3a-f** by treatment with sodium iodide in acetonitrile. The reaction could be monitored by ^{1}H-NMR which clearly distinguished between the -$\underline{CH_2}$Cl (δ = 5.7 - 6.4 *ppm*) and -$\underline{CH_2}$I (δ = 5.9 - 6.8 *ppm*) signals. An alternate direct route (Scheme I) to iodoalkylacylates (Scheme I) from the corresponding acid iodides was briefly explored. The acid

Scheme 1



1-3	R	R'
a	$-CH_3$	H
b	$-C_2H_5$	H
c	$-CH(Me)_2$	H
d	$-C(Me)_3$	H
e	$-Ph$	H
f	$-OC_2H_5$	CH_3

iodides was prepared by reaction of the acid chlorides with sodium iodide. The acid iodide was difficult to handle and store as it became highly colored. No further attempts were made to pursue this synthetic approach.

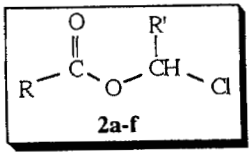
The iodoalkylacylates were purified by twice vacuum distillation and the distillate was continuously maintained at -78 °C. Two distillations became necessary because a small amount of unreacted chloroalkyl acylates (< 5%, as evaluated by ^{1}H-NMR) always contaminated the iodoalkyl acylates when the latter was isolated by only one distillation. When freshly distilled, the compounds **3a-f** were colorless to pale yellow liquids and were free from any other impurities as examined by ^{1}H and ^{13}C-NMR (Table 2). Storage of the iodocompounds at room temperature for more than one hour resulted in the development of a red color presumably resulting from their decomposition to unidentified materials. However the freshly distilled iodocompounds obtained as above could be stored at -80 °C for a few weeks with no apparent decomposition (as evaluated by ^{1}H and ^{13}C-NMR). Materials which had developed color could be decolorized by treatment of its solution in toluene with 5%

aqueous sodium bisulfite solution (1:1 v/v) and the resultant toluene solution could be used in reactions subsequently.

The chloro compounds **2a-f** and their iodo analogs **3a-f** were characterized by spectroscopy (Tables 1, 2). In both ^{1}H-NMR and ^{13}C-NMR the $\underline{CH_2}$-Cl (δ, ca. 5.7 - 6.4; 65 - 80 *ppm*) and $\underline{CH_2}$-I (δ, ca. 5.9 - 6.8, 30 - 50 *ppm*) signals were diagnostic for distinguishing between **2a-f** and **3a-f**. In the EI mass spectrum of the iodocompounds, **3a-f**, the molecular ion peak was not detected probably because of their high thermal lability. However the ion fragments (Scheme 2) at m/e127, m/e 141 for **3a-e** (m/e 155 for **3f**) and the acylium ion $[R\text{-}C{=}O]^{+}$ (base peak), resulting from the fragmentation of the corresponding unstable molecular ion, were indicative of the iodoalkylacylate structure.

To obtain the prodrug **5**, the oligonucleotide **4** was synthesized (as a mixture of diastereomers) on a 10 μmol scale by solid-phase synthesis in automated DNA synthesizer. The phosphoric diester linkages were incorporated using standard oxidation and the phosphorothioate linkages were introduced using 3*H*-1,2-benzodithiole-3-one-1,1-dioxide.[19] The chemoselective S-alkylation[20] of the oligonucleoside phosphorothioate **4** (an active anti-HIV agent) with **3c** in acetonitrile:tris buffer (0.5 M, pH 7.0) (1 : 1) at 37 °C for 1-3 hr gave the prodrug **5**. The product was isolated by ethanol precipitation at -78 °C and characterized as the *S*-acyloxyalkyl phosphorothiolate **5** by virtue of its characteristic upfield shift in ^{31}P-NMR (**4**, δ 51, - 4 *ppm*; **5**, δ, 25, - 4 *ppm*) and slower mobility in polyacrylamide gel electrophoresis (PAGE) (Fig. 1). Additionally, upon incubation with porcine liver esterase, (pH 7.0, 37 °C) the conversion of the prodrug **5** to the parent **4** could be demonstrated by ^{31}P-NMR (gradual disappearance of peak at δ 25 *ppm* and simultaneous appearance of peak at δ 51 ppm) and mobility shift as ascertained by PAGE. Fig. 1 illustrates the hydrolytic profile of **5** obtained at $t_{1/2}$ of hydrolysis.

Table 1
% Yield and Spectral Data for 2a-f

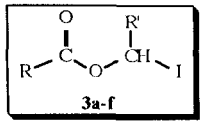


2a-f

Compd. No.	Yield (%)[a]	B.p. /mm Hg	^{1}H-NMR ($CDCl_3$)	^{13}C-NMR ($CDCl_3$)
2a (R = Me, R' = H)	45	109-12 °C/760	δ 5.71 (2H, s, CH_2-Cl), 2.09 (3H, s)	δ 168.7, 68.5, 20.4
2b (R = Et, R' = H)	44	35-39 °C/12	δ 5.71 (2H, s, CH_2-Cl), 2.4 (2H, q, J = 7 Hz), 1.18 (3H, t, J = 7 Hz)	δ 172.2, 68.5, 27.2, 8.4
2c (R = iPr, R' = H)	45	70-72 °C/15	δ 5.70 (2H, s, CH_2-Cl), 2.54 (1H, septet, J = 7 Hz) 1.14 (6H, d, J = 7 Hz)	δ 175.6, 79.2, 33.3, 18.1
2d (R = t-Bu, R' = H)	70	80-81 °C/15	δ 5.68 (2H, s, CH_2-Cl), 1.18 (s, 9H)	δ 176.1, 68.9, 38.6, 26.4
2e (R = Ph, R' = H)	53	75-78 °C/1.5	δ 8.02 (2H, m), 7.6 (1H, m), 7.45 (2H, m), 5.95 (2H, s, CH_2-Cl)	δ 164.4, 133.8, 131.2, 129.9, 128.5, 69.2
2f (R = OC_2H_5, R' = CH_3)	48	45-47 °C/20	δ 6.45 (1H, q, J = 5.9 Hz), 4.28 (2H, q, 7.1 Hz), 1.82 (3H, d, J = 5.9 Hz), 1.35 (3H, t, J = 7.1 Hz)	δ 152.6, 84.3 64.7, 25.0,13.9

[a]Yields (unoptimized) of product purified by two distillations.

Table 2
% Yield and Spectral Data for 3a-f

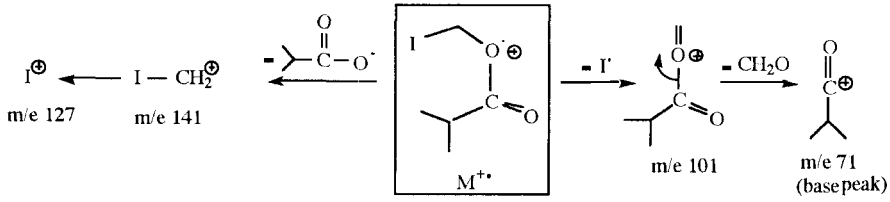


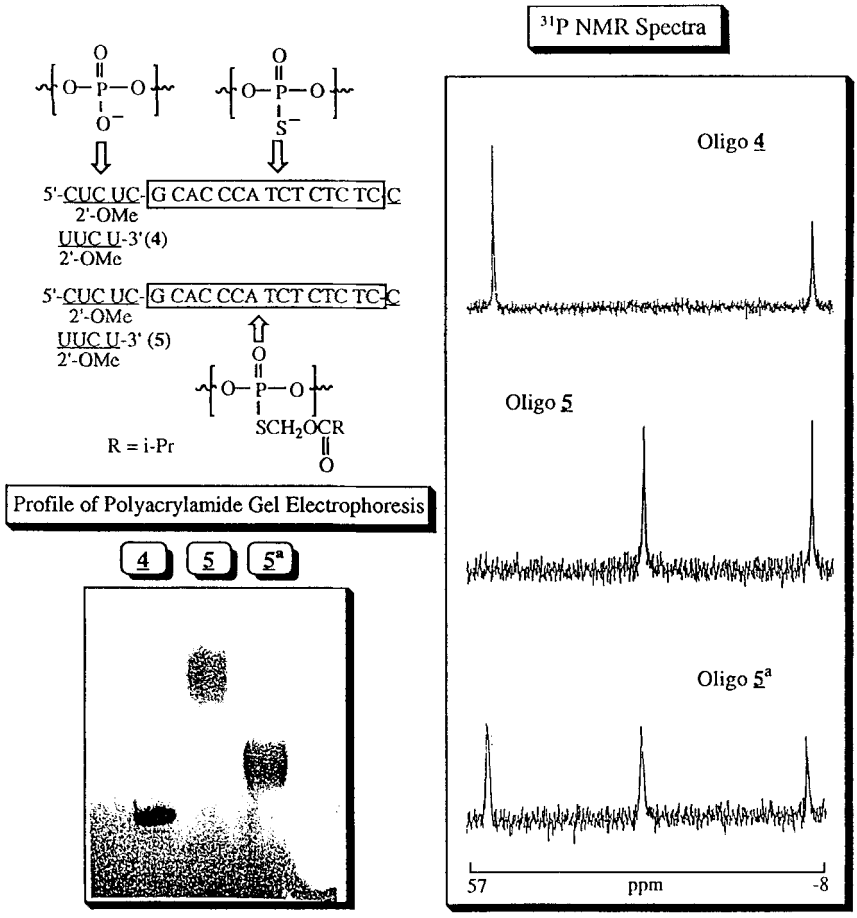
3a-f

Compd. No.	Yield (%)[a]	B.p. /mm Hg	^{1}H-NMR ($CDCl_3$)	^{13}C-NMR ($CDCl_3$)	MS (EI) m/z, (% abund.)
3a (R = Me, R' = H)	48	48-50 °C/10	δ 5.9 (2H, s, CH_2-I), 2.09 (3H, s)	δ 168.7, 30.6, 20.6	n.d.[b]
3b (R = Et, R' = H)	45	61-62 °C/10 (50 °C/4)[c]	δ 5.96 (2H, s, CH_2-I), 2.37 (2H, q, J = 7 Hz), 1.15 (3H, t, J = 7 Hz)	δ 171.8, 30.8, 27.3, 8.3	141 (14), 127 (43), 87 (29), 57 (100)
3c (R = iPr, R' = H)	50	70-71 °C/7	δ 5.94 (2H, s, CH_2-I), 2.60 (1H, septet, J = 7 Hz) 1.18 (6H, d, J = 7 Hz)	δ 174.3, 33.6, 30.9, 17.9	141 (16), 127 (37), 101 (14), 84 (9), 71 (100)
3d (R = t-Bu, R' = H)	70	80-81 °C/7 (68 °C/4)[c]	δ 5.91 (2H, s, CH_2-I), 1.19 (s, 9H)	δ 176, 38.7, 31.4, 26.4	169 (6), 141 (12), 127 (23), 115 (4), 85 (53), 57 (100)
3e (R = Ph, R' = H)	48	86-90 °C/1.5 (m.p. 26-27 °C)	δ 8.02 (2H, m), 7.6 (1H, m), 7.45 (2H, m), 6.16 (2H, s, CH_2-I))	δ 164.5, 133.8, 129.6, 128.7, 128.5, 31.2	141 (3), 135 (4), 127 (16), 105 (100), 77 (56), 51 (28)
3f (R = OC_2H_5, R' = CH_3)	50	75-77 °C/10	δ 6.78 (1H, q, J = 6.1 Hz), 4.26 (2H, q, 7.2 Hz), 2.23 (3H, d, J = 6.1 Hz), 1.35 (3H, t, J = 7.2 Hz)	δ 153.1, 65, 51.2, 30, 14	155 (59), 127 (100), 117 (28), 84 (30), 73 (5), 51 (20)

[a]Yields (unoptimized) of product purified by two distillations. [b]not determined. [c] ref. 12

Scheme 2
Mass spectral fragmentation pattern for iodomethyl isobutyrate





a. Hydrolysis of Prodrug oligonucleotide (5) by *Porcine Liver Esterase* at 37 °C for 42 hr.

Figure 1

In conclusion, we have carried out the syntheses and characterization of various iodoalkylacylates and demonstrated their use in the preparation of the bioreversible analog **5**, an oligonucleoside S-alkyl phosphorothiolate, from the corresponding oligonucleoside phosphorothioate **4**. Additional studies with **5** are in progress and will be reported in due course.

Experimental Section

All reagents and chemicals were purchased from manufacturers and used as such. Normal Fourier transform (F.T.) ^{1}H- and ^{1}H-heteronuclear spin-decoupled nuclear magnetic resonance (NMR) spectra were acquired using a Bruker AM-300 spectrometer. ^{13}C-NMR spectra were acquired in the presence of broad-band decoupling at 7.05 T. Samples were dissolved in $CDCl_3$ (ca. 50-100 mg in 0.6 mL) containing 1% tetramethylsilane (TMS). ^{31}P-NMR spectra are reported relative to trimethyl phosphate as an external standard (δ = 0 ppm).

Electron Ionization (EI) mass spectra were recorded with HP 5971A MSD gas chromatograph/mass spectrometer. The ionization potential was 70 eV.

All chemical operations were conducted in a well-ventilated hood with appropriate safety precautions and procedures.

General procedure for preparation of chloroalkylacylates 2a-f:

These compounds were prepared by adaptation of the reported procedures.[17,18] To a cooled (0-5 °C) mixture of paraformaldehyde (15 g, 0.5 mol equivalent of formaldehyde) or acetaldehyde (22 g, 0.5 mol) and (0.01 moles of anhydrous zinc chloride) was added dropwise the appropriate acid chloride (0.5 mol) over a period of 1 hr. After complete addition, the bath was removed, the contents were allowed to warm to room temperature. The temperature was raised to 50-55 °C and heating continued for 8-10 hours. Vacuum distillation of the reaction mixture gave the corresponding chloroalkylacylates in isolated yields of 45-70% and were used for conversion to iodoalkyl acylates **3a-f**.

General procedure for preparation of iodoalkylacylates 3a-f:

To a solution of sodium iodide (15 g, 0.1 mol), in anhydrous acetonitrile (125 mL), the chloroalkyl acylate (0.095 mol) was added dropwise over a period of 30 min at ambient temperature in the dark. A white precipitate/turbidity of sodium chloride began to appear during the addition. The contents were stirred for 12-18 hr. The

precipitate of NaCl was filtered and acetonitrile removed from the filtrate *in vacuo*. The filtrate was taken up in toluene (70-100 mL), washed with sodium bisulfite (5%, 2 X 40 mL) and then water (1 X 40 mL). The toluene layer was dried over anhydrous sodium sulfate. Toluene was removed *in vacuo* and distillation of the yellowish-orange liquid gave the iodoalkyl acylates **3a-f**. During distillation, the distillate was collected at -78 °C with minimal exposure to light and kept stored at -80 °C.

S-alkylation of oligonucleoside phosphorothioate 4:

The "hybrid" oligonucleotide **4**, with mixed phosphoric diester (PO)-phosphorothioate (PS) internucleotidic linkages, was synthesized and purified by previously described procedures,[14] wherein PO linkages were introduced using standard iodine oxidation and PS linkages were introduced using 3*H*-1,2-benzodithiole-3-one-1,1-dioxide.[19] The purified material was characterized by ^{31}P-NMR and polyacrylamide gel electrophoresis (Fig. 1)

To a solution of **4** (30 A_{260}, 0.5 mL, 500 mM tris buffer, pH 7.0) was added **3c** (20 µL), in 0.5 mL of acetonitrile. The reaction mixture was kept at 37 °C for 1-3 hr. The pH of the solution was maintained by addition of triethylamine. Solvent was removed *in vacuo* and water (200 µL) and NaCl solution (30 µL, 1M) were then added. After addition of cold ethanol (1 mL), the solution was kept at -80 °C for 1-2 hr. A pellet of **5** was obtained by centrifugation at 10000 g for 15 minutes. Repeat purification using 1M NaCl and ethanol gave 25 A_{260} units of the prodrug **5**.

^{31}P-NMR (D_2O): δ 25 (PS), - 4 (PO) *ppm* (see Fig. 1).

Bioreversibility studies on **5** were carried out as described.[14]

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21. This paper is dedicated to Prof. T. N. Krishnan.

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