7.12–7.59 (m, 6 H). Anal. Calcd for $C_{14}H_{11}ClO_3$: C, 64.01; H, 4.22. Found: C, 64.05; H, 4.36.

2,9-Dichloro-1-(chloromethyl)-9*H*-xanthene (2). To 60 mL of thionyl chloride was added 20 g (76 mmol) of **7** in small portions, with stirring under an argon sweep, taking as much care as possible to avoid contact of **7** with HCl vapors. The mixture was heated at reflux for 2.5 h. Excess thionyl chloride was removed under reduced pressure, and the residue was treated with cold (0 °C) hexane. The resulting solid was collected and recrystallized from methylene chloride to give 17 g (75%) of **11**: mp 145–147 °C; IR (KBr) 3088, 1597, 1456, 1268 cm^{-1}; ^1H NMR ($CDCl_3$) δ 5.09 (d, 2 H, J = 1.0 Hz), 6.81 (s, 1 H), 7.6–7.15 (m, 6 H). Anal. Calcd for $C_{14}H_9Cl_3$: C, 56.13; H, 3.03; Cl, 35.50 Found: C, 56.20; H, 3.06; Cl, 35.45.

5-Chloro-3a,4,12b,12c-tetrahydro-2-methyl-1*H*-xantheno-[9,1-*ef*]isoindole-1,3(2*H*)-dione (10a). To a mixture of 2.25 g (15 mmol) of sodium iodide and 1.11 g (10 mmol) of *N*-methylmaleimide in 5 mL of anhydrous DMF at 73 °C under argon was added 1.50 g (5.0 mmol) of **2** in small portions with stirring. After addition, the mixture was diluted with 10 mL of DMF and stirred at 73 °C for 5.5 h. The reaction was poured onto a mixture of ice and sodium bisulfite, and the resulting yellow precipitate was collected and washed thoroughly with water. Recrystallization from acetone–ethyl acetate afforded 0.77 g (45%) of **2**: mp 260–261 °C; IR ($CHCl_3$) 1782, 1705 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.65 (s, 3 H), 2.71 (dd, 1 H, J = 15.1, 6.8 Hz), 3.48 (ddd, 1 H, J = 6.6, 1.7, 8.8 Hz), 3.71 (dd, 1 H, J = 5.7, 9.1 Hz), 3.90 (dd, 1 H, J = 15.2, 1.4 Hz), 4.33 (d, 1 H, J = 5.7 Hz), 6.80–7.42 (6 H, m). Anal. Calcd for $C_{19}H_{14}ClNO_3$: C, 67.16; H, 4.15; N, 4.12; Cl, 10.43. Found: C, 66.99; H, 4.32; N, 4.19; Cl, 10.24.

5-Chloro-2-methyl-1*H*-xantheno[9,1-*ef*]isoindole-1,3-(2*H*)-dione (11). A solution of **10a** (1.5 g, 4.5 mmol) and 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (3.0 g, 13.5 mmol) in 80 mL of dioxane containing several drops of acetic acid was heated at reflux for 20 h. The reaction was cooled to room temperature, and the resulting precipitate was collected by filtration and washed with methanol to give 1.4 g (91%) of the title compound. An analytical sample was prepared by recrystallization from acetone: mp > 295 °C dec; IR (KBr) 1757, 1703 cm^{-1}; ^1H NMR (DMSO-d_6) δ 3.28 (s, 3 H), 7.17–7.32 (overlapping m, 3 H), 7.50–7.53 (m, 1 H), 7.69 (d, 1 H, J = 8.6 Hz), 8.47 (s, 1 H), 9.65 (d, 1 H, J = 9.0 Hz); HRMS calcd for $C_{19}H_{10}NClO_3$ 335.0349, found 335.0328.

Dimethyl 4-Chlorobenzo[*kl*]xanthene-1,2-dicarboxylate (12). To a slurry of NaI (0.45 g, 3.0 mmol), dimethyl acetylenedicarboxylate (0.31 mL, 2.5 mmol), and 5 mL of DMF was added **2** (0.30 mmol, 1 mmol) portionwise and with stirring under argon. After 4.5 h at 70 °C, the reaction was cooled to room temperature and partitioned between ether and water. The aqueous layer was extracted three times with ether, the combined extracts were washed with water, dried (Na_2SO_4), and filtered, and the solvent was removed. The residue was purified by column chromatography (silica gel, 4:1 hexane–ethyl acetate) to afford 37 mg (10%) of the title compound as a bright yellow solid. An analytical sample was obtained by crystallization from ethyl acetate–hexane: mp 195–196 °C; IR (KBr) 3431, 3007, 1725, 1504 cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.92 (s, 3 H), 4.00 (s, 3 H), 7.00–7.68 (m, 6 H), 8.68 (s, 1 H); mass spectrum (CI) 369 (MH^+, 35), 338 (100); HRMS calcd for $C_{20}H_{13}ClO_5$ 368.04515, found 368.04391.

Dimethyl 4-Chloro-1(*SR*),2(*SR*),3,11b-tetrahydrobenzo-[*kl*]xanthene-1,2-dicarboxylate (13). To a slurry of 1.4 g (9.9 mmol) of dimethyl fumarate, 0.65 g (9.9 mmol) of zinc powder, and 10 mL of DMF at 50–55 °C was added portionwise and with stirring 1.0 g (3.3 mmol) of **2**. After addition, the mixture was heated at 100–105 °C for 3 h. The dark brown slurry was filtered hot through filter aid, and the filter pad was washed with THF. The filtrate was concentrated, and the residue was purified via flash column chromatography (silica gel, 4:1 hexane–ethyl acetate) to give 245 mg (20%) of a mixture of **13a** and **13b** (1.0:1.5 by 300-MHz ^1H NMR); mp 124–131 °C. Fractional crystallization of the mixture from ethyl acetate gave **13b**: mp 146–148 °C; IR (KBr) 2955, 1727, 1450 cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.04 (dd, 1 H, J = 3.6, 5.5 Hz), 6.87 (d, 1 H, J = 8.7 Hz), 7.01–7.08 (m, 2 H), 7.19–7.29 (m, 3 H); HRMS calcd for $C_{20}H_{17}ClO_5$ 372.0765, found 372.0748. The mother liquors were concentrated, and the residue was recrystallized from methanol–ether to give **13a**: mp 143–145 °C; IR (KBr) 2953, 1743, 1445 cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.04–3.39 (overlapping m, 4 H), 3.75 (s, 3 H), 3.84 (s, 3 H), 4.34 (d, 1 H, J = 10.3 Hz), 6.97 (d, 1 H, J = 8.6 Hz), 7.06–7.26 (m, 5 H); HRMS calcd for $C_{20}H_{17}ClO_5$ 372.0765, found 372.0746.

5-Chloro-2,3,3a,4,12b,12c-hexahydro-2-methyl-1*H*-xantheno[9,1-*ef*]isoindole Hydrochloride (14). To a slurry of 3.00 g (79 mmole) of lithium aluminum hydride in 0.50 L of ether was added 2.97 g (8.74 mmol) of **10a** as a solid. The mixture was stirred at reflux overnight, cooled to 0 °C, and then treated successively with 3.0 mL of water, 3.0 mL of 3 N NaOH (aq), and 12.0 mL of water. After being stirred for 0.5 h, the ether layer was filtered and the filter cake was washed thoroughly with ether. The filtrate was dried over potassium carbonate, filtered, and concentrated. The resulting solid was dissolved in methanol and acidified with etheral HCl. The solvents were partially evaporated on a steam bath and replaced with ethyl acetate. Upon cooling, 1.16 g (43%) of **14** was collected by filtration: mp > 240 °C dec; IR ($CHCl_3$) 3402, 2972, 2346 (br), 1624, 1608, 1577, 1492 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.96 (1 H, q, J = 10.6 Hz), 2.14 (1 H, ddd, J = 10.8, 10.9, 10.8, Hz), 2.53 (d, 3 H, J = 4.8 Hz), 2.58 (1 H, dd, J = 15.6, 6.1 Hz). Anal. Calcd for $C_{19}H_{18}ClNO{\cdot}HCl$: C, 65.53; H, 5.50; Cl, 20.36; N, 3.98. Found: C, 65.45; H, 5.54; Cl, 20.36; N, 4.02.

Acknowledgment. We thank Dr. Harold Almond for energy minimization (MAXIMIN) calculations and for the SYBYL-generated X-ray drawing of **14**·HCl. We are grateful to Dr. Bruce E. Maryanoff for valuable suggestions and advice.

Supplementary Material Available: General X-ray data for **14**·HCl, tables of atomic coordinates, bond lengths, thermal parameters, and bond angles for **14**·HCl, numbered ORTEP drawing of **14**·HCl, and NMR spectra of **11–13** (12 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

Synthesis and Utility of a DNA Phosphorylating Agent Based on 2-(Triphenylsilyl)ethanol

Joseph E. Celebuski* and Clifford Chan

Diagnostics Division, Abbott Laboratories, Abbott Park, Illinois 60064

Roger A. Jones

Department of Chemistry, Rutgers, The State University of New Jersey, Piscataway, New Jersey 08854

Received April 8, 1992

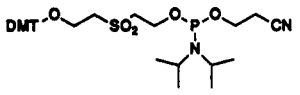
Introduction

The enzyme DNA ligase catalyzes the template-driven condensation of the 3′-hydroxyl group of one DNA strand with the 5′-monophosphate group of a second DNA strand. As part of a study of DNA ligase, we required a method for 5′-phosphorylation of an oligonucleotide during phosphoramidite-based, automated DNA synthesis.[1] Furthermore, this phosphorylated oligonucleotide needed to be separable, by reversed-phase HPLC, from the "failure sequences" generated during DNA synthesis. Work with the commercial phosphorylating agent **1** of Urdea[2] showed that, while the phosphorylated DNA could be separated from failure sequences by polyacrylamide gel electrophoresis or ion-exchange chromatography, satisfactory conditions for separation by HPLC could not be established. Uhlmann[3] showed that (*p*-nitrophenyl)ethyl phosphor-

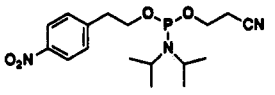
(1) McBride, L. J.; Caruthers, M. H. *Tetrahedron Lett.* **1983**, *24*, 245.
(2) Urdea, M. S.; Horn, T. *Tetrahedron Lett.* **1986**, *27*, 4705.
(3) Uhlmann, E.; Engels, J. *Tetrahedron Lett.* **1986**, *27*, 1023.

0022-3263/92/1957-5535$03.00/0

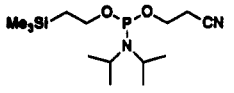
amidite 2 enabled the reversed-phase HPLC separation of full-length products from failure sequences for DNA hexamers. The (*p*-nitrophenyl)ethyl group is removed from the oligonucleotide with DBU to afford 5′-phosphorylated DNA. When this technique of automated DNA phosphorylation is attempted with a 20-mer, however, the reversed-phase HPLC separation is minimal between failure and full-length molecules.[4] With the above results in mind, we set out to design and synthesize a phosphorylating agent which would be compatible with the conditions of DNA automated synthesis, would be sufficiently hydrophobic for reversed-phase HPLC separation, and would be easily converted to a 5′ terminal phosphate group.



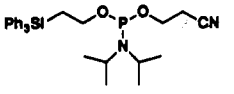
1



2



3



4

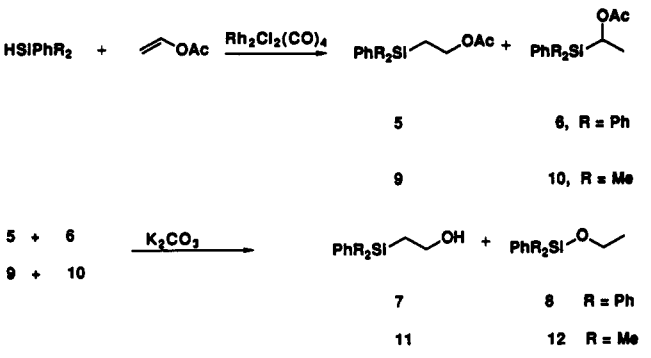
Results and Discussion

One moderately hydrophobic protecting group which could be used under oligonucleotide synthesis conditions is the 2-(trimethylsilyl)ethyl moiety, which can be fragmented with fluoride ion. The phosphoramidite **3**, prepared from 2-(trimethylsilyl)ethanol and 2-cyanoethyl *N,N*-diisopropylchlorophosphoramidite, was coupled successfully to the 5′-terminus of a 20-mer oligonucleotide. However, reversed-phase HPLC analysis showed little difference between full-length product and failure sequences. Our attention then turned to the preparation of a more hydrophobic silyl phosphoramidite. Honda[5] has prepared (methyldiphenylsilyl)ethanol. While the phosphoramidite derived from this substituted silylethanol would probably confer hydrophobic properties to a product oligonucleotide, we wanted the reversed-phase HPLC profile of the crude, silylated oligonucleotide to mimic the profile of the analogous 5′-dimethoxytritylated compound as closely as possible. Currently, reversed-phase HPLC purification of oligonucleotides relies on the presence of a dimethoxytrityl group at the 5′-terminus to enable separation of failure sequences from full-length product. Extension of this concept suggests the preparation of phosphoramidite **4**, derived from 2-(triphenylsilyl)ethanol. A search of the literature revealed no references to 2-(triphenylsilyl)ethanol, possibly due to facile Peterson elimination[6] to give triphenylsilanol and ethylene.

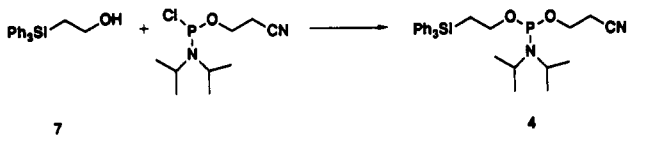
One possible method for preparation of 2-silylethanols with bulky silicon substituents is hydrosilylation[7] of vinyl acetate followed by mild base hydrolysis, shown in Scheme I.

The hydrosilylation of vinyl acetate with triphenylsilane (R = Ph) using catalytic dichlorodirhodium tetracarbonyl gave a 1.6:1.0 ratio of **5**:**6**, as judged by proton NMR integration. Potassium carbonate hydrolysis in methanol of the mixture afforded **7** and **8**. Ether **8** is the result of a Brook rearrangement[8] of the hydrolysis product of **6**. The hydrolysis reaction was monitored closely, since the Peterson elimination cited above was a major side reaction. Chromatographic separation of alcohol **7** from ether **8** was straightforward, giving pure 2-(triphenylsilyl)ethanol. In contrast to the case of triphenylsilane above, hydrosilylation of vinyl acetate with the sterically less hindered phenyldimethylsilane (R = Me) under dichlorodirhodium tetracarbonyl catalysis gave a 1.0:1.4 ratio of **9**:**10**, as judged by NMR integration. The hydrosilylation reaction with phenyldimethylsilane was complete in less than 1 h at ambient temperature. In contrast, the triphenylsilane reaction required 3 days at room temperature to go to completion. Potassium carbonate mediated hydrolysis of the mixture of **9** and **10** gave (phenyldimethylsilyl)ethanol (**11**).

Scheme I



Scheme II



With alcohol **7** in hand, phosphoramidite **4** was prepared from condensation with commercial 2-cyanoethyl *N,N*-diisopropylchlorophosphoramidite, shown in Scheme II. Phosphoramidite **4** was used in automated DNA synthesis as follows.

A 25-mer oligonucleotide was prepared via standard automated phosphoramidite coupling techniques, and the 5′-terminus was detritylated. The oligonucleotide was treated with a 100 mM acetonitrile solution of **4** for 52 s. After iodine oxidation, the base protecting groups were cleaved with concentrated ammonia at 55 °C overnight. The desired product oligo was separated from the failure sequences by reversed-phase HPLC using a 100 mM triethylammonium acetate/acetonitrile gradient (Figure 1). As is evident from the chromatogram, the hydrophobic (triphenylsilyl)ethyl group provides the desired separation between the product and failure sequences. Desilylation of the 25-mer oligonucleotide with 2 M *n*-Bu_4NF in DMSO at 70 °C gave the 5′-phosphorylated oligonucleotide. The reversed-phase HPLC chromatogram of this desilylation, run under the same gradient conditions, is shown in Figure 2. We have found that reaction at 70 °C in DMSO is the method of choice for 20–25-mers. At that temperature, any secondary structure in the oligonucleotide is destroyed, precluding the "burial" of the hydrophobic triphenylsilyl group in an inaccessible matrix. The 2 M solution of *n*-Bu_4NF in DMSO is prepared fresh every 3 months, due

(4) Zon, G. in *HPLC in Biotechnology*; Hancock, W. S., Ed.; John Wiley & Sons: New York, 1990; pp 359–363.
(5) Honda, S.; Hata, T. *Tetrahedron Lett.* **1981**, *22*, 2093.
(6) Peterson, D. J. *J. Org. Chem.* **1968**, *33*, 780.
(7) Collman, J. P.; Hegedus, L. S. In *Principles and Applications of Organotransition Metal Chemistry*; University Science Books: Mill Valley, CA, 1980; pp 384–389.
(8) Brook, A. G. *Acc. Chem. Res.* **1974**, *7*, 77.

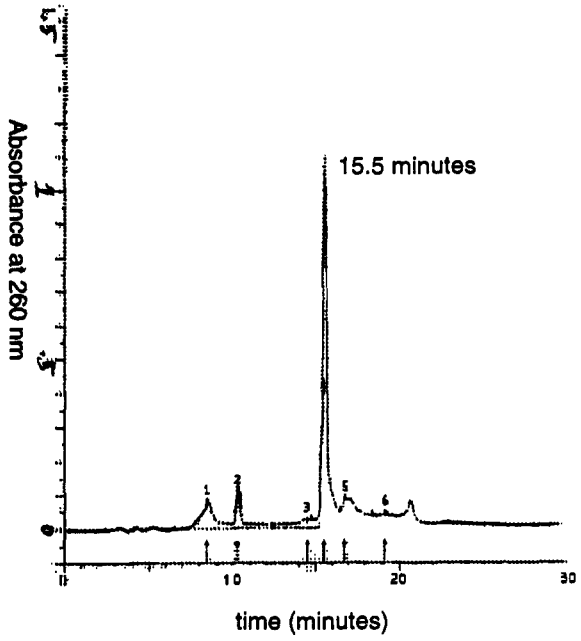


Figure 1. Preparative injection of crude, 5′-silyl phosphate 25-mer. The triphenylsilylated peak elutes at 15.5 min. Column: Waters μBondapak C18, 7.8 × 300 mm. Flow rate: 3 mL/min. Gradient table: initial, 90% A, 10% B; 15 min, 60% A, 40% B; 25 min, 60% A, 40% B. Solvent A is 100 mM triethylammonium acetate, solvent B is acetonitrile.

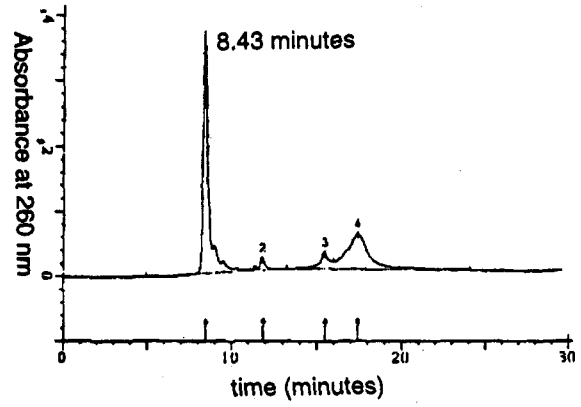


Figure 2. Preparative injection of desilylated 25-mer. The 5′-terminal phosphate peak elutes at 8.5 min. Conditions as in Figure 1.

to the instability of the tetraalkylammonium fluoride.[9] The overall DNA silylation/desilylation process is shown in Scheme III.

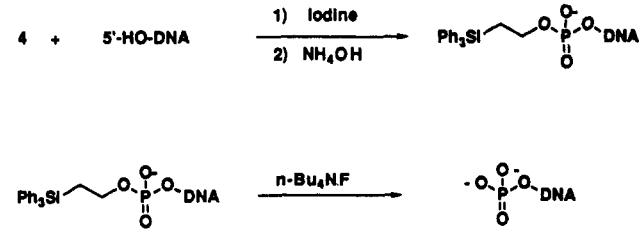
Finally, the oligonucleotides which are phosphorylated with 4 are biologically active when used as DNA ligase substrates.

Conclusions

The preparation of 2-(triphenylsilyl)ethanol was achieved by hydrosilylation of vinyl acetate by triphenylsilane, followed by acetate hydrolysis. This methodology represents a new route to 2-silylethanol compounds. The DNA 5′-terminal phosphorylating agent 4, derived from 2-(triphenylsilyl)ethanol, enables the separation of DNA failure sequences from full-length oligonucleotides by virtue of its hydrophobic character. The triphenylsilylethyl group was removed with *n*-Bu_4NF to give 5′-phosphorylated DNA which can be used as a DNA ligase substrate.

(9) Sharma, R. K.; Fry, J. L. *J. Org. Chem.* **1983**, *48*, 2112.

Scheme III



Experimental Section

General Methods. TLC analyses were carried out using 20% EtOAc in cyclohexane on 250-μm silica gel plates. Flash chromatographies were performed with 230–400 mesh silica gel. Reactions were run in flame-dried flasks under a dry nitrogen atmosphere unless otherwise noted. Silanes were obtained from Aldrich Chemical or Petrarch Chemical. Solid *n*-Bu_4NF was obtained from Aldrich. Melting points are uncorrected. Infrared values are quoted in cm^{-1}. Proton NMR spectra were run at 300 MHz, and carbon-13 NMR spectra were run at 75 MHz. Combustion analyses were performed by Galbraith Laboratories, Knoxville, TN.

2-(Trimethylsilyl)ethyl 2-Cyanoethyl *N,N*-Diisopropylphosphoramidite (3). To a solution of 573 μL (4 mmol) of 2-(trimethylsilyl)ethanol and 1.39 mL (8 mmol) of *i*-Pr_2NEt in 8 mL of THF at 0 °C was added 892 μL (4 mmol) of 2-cyanoethyl *N,N*-diisopropylchlorophosphoramidite all at once. The reaction became very cloudy almost immediately. The ice bath was removed, and the reaction stirred to room temperature overnight, for a total of 19 h. After filtration to remove *i*-Pr_2NEt–HCl, the THF was evaporated. The residue was partitioned between 50/50 mL EtOAc/0.1 M Na_2CO_3, pH 12. After phase separation and solvent removal of organic phase, the residue was vacuum dried. Flash chromatography using 12% EtOAc in cyclohexane on a 150-mm × 25-mm i.d. column afforded 574 mg (78%) of **3** as a water-white viscous oil, R_f = 0.65 in 15% EtOAc in cyclohexane. MS: (DCI, NH_3) 319 (M + H), 291 (M − HCN). NMR: (CD_2Cl_2) δ 3.9–3.62 (m, 4 H), 3.56 (dsept, 2 H, J_{CH} = 7.0 Hz, J_{PH} = 10.0 Hz, NH), 2.59 (t, 2 H, J = 6.2 Hz, CH_2CN), 1.15 (dd, 12 H, J_{CH} = 7.0 Hz, J_{PH} = 2.2 Hz, Me), 0.97 (tq, 2 H, J = 8.0, 0.7 Hz, CH_2Si), 0.03 (s, 9 H, SiMe).

1,1,1-Triphenyl-3-acetoxy-1-silapropane (5). A solution of 3.7 mL (40 mmol) of vinyl acetate, 10.4 g (40 mmol) of triphenylsilane, and 78 mg (0.25 mmol) of $Rh_2Cl_2(CO)_4$ in 40 mL of toluene was stirred at room temperature under N_2 for a total of 63 h. **CAUTION**: Several runs of the reaction at this scale had unpredictable induction periods, followed by rapid heat evolution. Scaleup of this reaction should be done with a cooling bath close at hand. The very dark reaction mixture was treated with 5 g of decolorizing carbon, and the mixture boiled briefly. After being cooled, the mixture was filtered through a 1-cm pad of Celite. After concentration of the filtrate and washings, the residue was vacuum dried. NMR analysis showed a 5:6 ratio of 1.6:1. A 100-mg sample of crude material was flash chromatographed using 4% EtOAc in cyclohexane on a 25-mm i.d. × 150-mm long silica gel column. This afforded 29 mg of **5** after recrystallization from MeOH, mp 67–68 °C. IR: ($CDCl_3$) 3070 (m), 1728 (vs), 1425 (vs), 1249 (vs). MS: (DCI/NH_3) *m/e* 364 (M + NH_4). NMR: (CD_2Cl_2) δ 7.6–7.3 (m, 15 H, phenyl), 4.22 (B_2 of A_2B_2, 2 H, CH_2O), 1.87 (s, 3 H, CH_3), 1.86 (A_2 of A_2B_2, 2 H, CH_2Si). ^{13}C NMR: ($CDCl_3$) δ 171.1 (C=O), 135.5 (meta), 134 (ipso), 129.7 (para), 128 (ortho), 62.1 (CH_2O), 21 (Me), 14.4 (CH_2Si). Calcd for $C_{22}H_{22}O_2Si$: C, 76.26; H, 6.40. Found: C, 76.45; H, 63.7.

1,1,1-Triphenyl-1-silapropan-3-ol (7). The above mixture of **5** and **6** was dissolved in 100 mL of MeOH, and 10 g of K_2CO_3 was added all at once. The reaction was complete after 1 h of stirring at room temperature. The mixture was filtered, and the filtrate was concentrated. The residue was partitioned with 200 mL of 1:1 water–EtOAc. After concentration of the organic layer, the residue was vacuum dried. Flash chromatography (18% EtOAc in cyclohexane, R_f = 0.32) using a 41-mm i.d. × 150-mm long silica gel column afforded 3.4 g (28%) of **7**. Recrystallization from cyclohexane gave the analytical sample as a snow-white solid,

mp 96–97 °C. IR: ($CDCl_3$) 3616 (m), 2970 (m), 1429 (vs). MS: (FAB/DMF-KI) *m/e* 343 (M + K). NMR: (CD_3OD) δ 7.55–7.3 (m, 15 H, phenyl), 3.73 (B_2 of A_2B_2, 2 H, CH_2O), 1.78 (A_2 of A_2B_2, 2 H, CH_2Si). ^{13}C NMR: ($CDCl_3$) δ 135.5 (meta), 134.4 (ipso), 129.6 (para), 128 (ortho), 59.8 (CH_2O), 18.7 (CH_2Si). Calcd for $C_{20}H_{20}OSi·0.2\ H_2O$: C, 77.98; H, 6.67. Found: C, 77.92; H, 6.62.

1,1-Dimethyl-1-phenyl-3-acetoxy-1-silapropane (9). To a solution of 6.1 mL (40 mmol) of $PhMe_2SiH$ and 3.7 mL of vinyl acetate in 40 mL of toluene was added 61 mg (0.16 mmol) of $Rh_2Cl_2(CO)_4$. Immediately, the reaction evolved heat and gas. Within 5 min, the golden yellow reaction had turned dark brown in color. After 1 h, the reaction was complete. The reaction was worked up as in **5** to give 8.4 g of crude adduct. Proton NMR analysis showed a 9:10 addition ratio of 1.0:1.4. A 100-mg sample was purified by flash chromatography as in **5** to give 28 mg of **9** as a colorless oil. IR: ($CDCl_3$) 2960 (m), 1724 (vs), 1426 (m), 1255 (vs). MS: (DCI/NH_3) *m/e* 240 (M + NH_4). NMR: ($CDCl_3$) δ 7.6–7.3 (m, 5 H, phenyl), 4.18 (B_2 of A_2B_2, 2 H, CH_2O), 1.99 (s, 3 H, Me), 1.25 (A_2 of A_2B_2, 2 H, CH_2Si), 0.35 (s, 6 H, SiMe). ^{13}C NMR: ($CDCl_3$) δ 171.1 (CO), 138 (ipso), 133.4 (meta), 129.2 (para), 127.9 (ortho), 62.3 (CH_2O), 21.1 (Me), 16.5 (CH_2Si), –2.9 (SiMe). Anal. Calcd for $C_{12}H_{18}O_2Si$: C, 64.82; H, 8.16. Found: C, 65.02; H, 8.07.

1,1-Dimethyl-1-phenyl-1-silapropan-3-ol (11). The remaining 8.3 g of crude **9** was worked up as in the case of the (triphenylsilyl)ethanol **7** to give 1.6 g of 1,1-dimethyl-1-phenyl-1-silapropan-3-ol as a colorless oil, 23% overall. IR: ($CDCl_3$) 3616 (m), 2960 (m), 1425 (m), 1251 (s). MS: (DCI/NH_3) *m/e* 198 (M + NH_4). NMR: ($CDCl_3$) δ 7.6–7.3 (m, 5 H, phenyl), 3.75 (B_2 of A_2B_2, 2 H, CH_2O), 1.49 (s, 1.2 H, OH), 1.22 (A_2 of A_2B_2, 2 H, CH_2Si), 0.33 (s 6 H, SiMe). ^{13}C NMR: ($CDCl_3$) δ 138.5 (ipso), 133.4 (meta), 129 (para), 127.8 (ortho), 59.9 (CH_2O), 21.1 (CH_2Si), –2.8 (SiMe). Anal. Calcd for $C_{10}H_{16}OSi·0.1H_2O$: C, 65.92; H, 8.99. Found: C, 65.95; H, 8.97.

2-(Triphenylsilyl)ethyl 2-Cyanoethyl *N,N*-Diisopropylphosphoramidite (4). To a solution of 3.0 g (10 mmol) of **7**, 4.2 mL (24 mmol) of *i*-Pr_2NEt, and 5 mg of 4,4-(dimethylamino)-pyridine in 15 mL of THF at 0 °C was added 2.7 mL (12 mmol) of 2-cyanoethyl *N,N*-diisopropylchlorophosphoramidite all at once. A white precipitate formed almost immediately. Reaction was complete after 30 min at 0 °C. After solvent removal, the residue was partitioned with 200 mL of 1:1 0.1 M Na_2CO_3–EtOAc, and the phases were separated. The aqueous phase was reextracted with 50 mL of EtOAc, and the combined organic phases were concentrated and vacuum dried. Flash chromatography (10% EtOAc in cyclohexane) using a 41-mm i.d. × 150-mm long silica gel column gave 3.4 g of **4** (66%) after vacuum drying overnight as a viscous colorless oil. This material gradually crystallized in a –20 °C freezer over the course of several weeks. During the chromatography, it was necessary to add 100 μL of NEt_3 to each fraction, in order to minimize the effects of adventitious acid in the fraction tubes or in the silica gel used for flash chromatography. IR: (film) 2962 (m), 1426 (m). MS: (DCI/NH_3) *m/e* 505 (M + H). NMR: (CD_3CN) δ 7.6–7.3 (m, 15 H, phenyl), 3.9–3.7 (m, 2 H, CH_2O), 3.66 (dt, 2 H, J_{CH} = 5.9 Hz, J_{PH} = 7.7 Hz, CH_2O), 3.51 (dsept, 2 H, J_{CH} = 6.6 Hz, J_{PH} = 9.9 Hz, NH), 2.54 (t, 2 H, *J* = 5.5 Hz, CH_2CN), 1.87 (br t, 2 H, *J* = 6.3 Hz, CH_2Si), 1.07 (dd, 12 H, J_{CH} = 6.6 Hz, J_{PH} = 29.4 Hz, Me). ^{13}C NMR: (CD_3CN) δ 136.3 (meta), 135.5 (ipso), 130.7 (para), 129 (ortho), 117.7 (CN), 61.1 (d, J_{PC} = 18.3 Hz, CH_2O), 59.3 (d, J_{PC} = 18.3 Hz, CH_2O), 43.6 (d, J_{PC} = 12.2 Hz, NCH), 24.8 (virtual t, J_{PC} = 7.3 Hz, Me), 21 (d, J_{PC} = 7.3 Hz, CH_2CN), 17.2 (d, J_{PC} = 7.3 Hz, CH_2Si). ^{31}P NMR: (202 MHz, CD_3CN) δ 145.6 (s). Anal. Calcd for $C_{29}H_{37}N_2O_2PSi$: C, 69.02; H, 7.39; N, 5.55. Found: C, 69.02; H, 7.36; N, 5.42.

Use of 4 in Automated Phosphorylation of DNA. The phosphoramidite **4** was used to phosphorylate a 25-mer oligonucleotide at 1 μmol CPG loading using an ABI (Foster City, CA) 380A DNA synthesizer. The phosphoramidite couplings were run using a modified synthesis program from the manufacturer, wherein the contact time of 100 mM phosphoramidite **4** in MeCN to the column was increased to 52 s. The preparative HPLC run, showing separation of the failure sequences from full-length oligo, is shown in Figure 1. The collected material was dried in vacuo. The purified, silylated DNA was then desilylated using 200 μL of 2 M TBAF in DMSO. The reaction was performed in a 70 °C heating block for 2 h. The reaction was diluted to 500 μL with 300 μL of 1 M ammonium acetate, and the reaction mixture was desalted using a NAP-5 column (Pharmacia), following the manufacturer's instructions. The 1.0 mL eluate was dried in vacuo, and then ethanol precipitated from 100 μL of 0.2 M NaOAc to give purified, terminally phosphorylated DNA. HPLC analysis of this material is shown in Figure 2.

Acknowledgment. We gratefully acknowledge the Analytical Research department of Abbott Laboratories for all spectral measurements.

Regioselective Lithiation and Reaction of [1,2,4]Triazolo[1,5-*a*]pyridine and Pyrazolo[1,5-*a*]pyridine

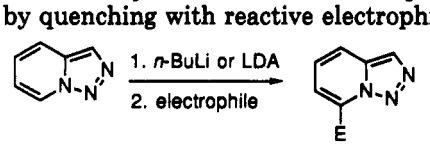
Bruce L. Finkelstein

E. I. Dupont de Nemours Agricultural Products, Stine-Haskell Research Center, P.O. Box 30, Newark, Delaware 19714

Received March 17, 1992 (Revised Manuscript Received July 2, 1992)

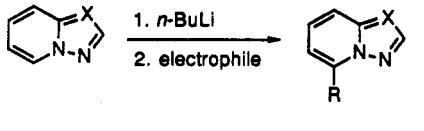
In order to probe the structure–activity relationships in a series of herbicidal 6,5-fused nitrogen heterocycles,[1] I required access to 5-substituted [1,2,4]triazolo[1,5-*a*]-pyridines. A search of the literature[2] failed to reveal methodology which would be flexible enough to allow the rapid introduction of a variety of substituents in the 5-position of this ring system. This Note reports a highly regioselective solution to this synthetic problem.

At the outset I was aware of the reports of Jones et al.[3,4] on the preparation of 7-substituted [1,2,3]triazolo[1,5-*a*]-pyridines by metalation of the parent ring system with either *n*-butyllithium or lithium diisopropylamide followed by quenching with reactive electrophiles[5] (eq 1).



Equation 1

It seemed reasonable that a similar strategy might operate in the [1,2,4]triazolo[1,5-*a*]pyridine ring system. Indeed treatment of a THF solution of [1,2,4]triazolo-[1,5-*a*]pyridine (**1**) with *n*-butyllithium at –78 °C followed by introduction of a variety of electrophiles affords 5-substituted products (**3–8**) in good yields (eq 2, X = N and



Equation 2

1 (X=N)
2 (X=CH)

3-8 (X=N)
10-13 (X=CH)

Table I). The reaction is highly regioselective; no other regioisomers were isolated. Entry 8 of Table I is worth

(1) (a) Selby, T. P. EP-A-353,902. (b) Selby, T. P.; Andrea, T. A.; Denes, L. R.; Finkelstein, B. L.; Fuesler, T. P.; Smith, B. K. In *Synthesis and Chemistry of Agrichemicals*; Baker, D. R., Feynes, J. G., Steffens, J. J., Eds.; ACS Symposium Series, in press.

(2) For a review of triazolopyridine chemistry, see: Jones, Gurnos; Sliskovic, D. R. *Adv. Heterocycl. Chem.* **1983**, *34*, 79–143.

(3) (a) Jones, Gurnos; Sliskovic, D. R. *J. Chem. Soc., Perkin Trans. 1* **1982**, 967. (b) Abaraca, B.; Ballesteros, R.; Mojarred, F.; Jones, Gurnos; Mouat, D. J. *J. Chem. Soc., Perkin Trans. 1* **1987**, 1865.

(4) For more recent work involving lithiation of [1,2,3]triazolo[5,1-*b*]thiazoles, see: Jones, Gurnos; Ollivierre, H.; Fuller, L. S.; Young, J. H. *Tetrahedron* **1991**, *47*, 2861.

(5) Only very reactive electrophiles work in this reaction (e.g. aldehydes, chlorotrimethylsilane, but not iodomethane) since [1,2,3]triazolo-[1,5-*a*]pyridines are prone to ring opening.[2,3]

0022-3263/92/1957-5538$03.00/0