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O-ARYL PHOSPHORAMIDITES: SYNTHESIS, REACTIVITY AND EVALUATION OF THEIR USE FOR SOLID-PHASE SYNTHESIS OF OLIGONUCLEOTIDES

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Abstract --Deoxynucleoside 3′-O-arylphosphoramidites were studied as synthons for preparing natural and modified internucleotide linkages. Of the various aryl derivatives examined, the 3′-O-phenylphosphoramidite was found to be the most attractive. It reacted with high overall yield to form deoxydinucleoside O-phenyl-phosphite triesters which were converted to phosphoramidates, methylphosphonates, and H-phosphonates.

The recent development of deoxynucleoside tervalent phosphorus derivatives as synthons for rapidly and efficiently preparing natural internucleotide linkages[1] has also stimulated the adaptation of these procedures to the synthesis of DNA analogs having modifications at phosphorus.[2] Moreover the many unique applications of these analogs to a large variety of biochemical and biological research areas[1,2,3] have further encouraged the development of new methodologies for synthesizing these compounds. One objective of our research has been to formulate a methodology whereby one synthon can be used for introducing not only the natural internucleotide linkage but several additional analogs at phosphorus as well.

Two basic approaches have been explored. In one methodology, certain dinucleoside phosphite triesters are oxidized in the presence of various nucleophiles to yield internucleotide linkages containing the natural dinucleoside phosphate, dinucleoside thiophosphate, various dinucleoside phosphoramidates, and dinucleoside phosphate triesters.[4] The second method, as outlined in this report, is based upon an early observation that the

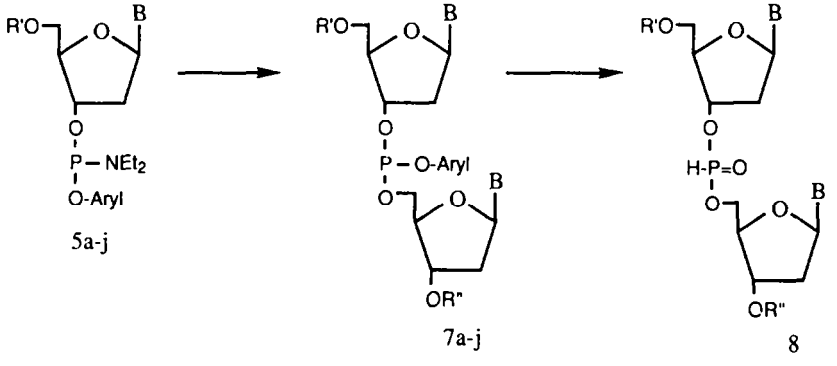

Figure 1. Synthesis of Deoxydinucleoside H-Phosphonates From Deoxynucleoside 3′-O-Arylphosphoramidites. Abbreviations: R′, *t*-butyldimethylsilyl; R", dimethoxytrityl; B, thymine, N-benzoyladenine, N-benzoylcytosine, or N-isobutyrylguanine; Et, ethyl; aryl, 2,4-dinitrophenyl (a), pentafluorophenyl (b), pentachlorophenyl (c), 2,4,5-trichlorophenyl (d), 4-nitrophenyl (e), 2-bromophenyl (f), 4-bromophenyl (g), phenyl (h), 2-methylphenyl (i), 2,6-dimethylphenyl (j). These aryl substituents, **a-j**, correspond to the aryl groups found in compounds **1a-j**, **3a-j**, **5a-j**, and **7a-j**.

o-chlorophenoxy group can be selectively eliminated from a dinucleoside O-arylphosphite triester.[5] The approach consists of two steps (Figure 1). The first is condensation of a deoxynucleoside (**6**) with a deoxynucleoside 3′-O-arylphosphoramidite (**5a-j**) to yield a dinucleoside arylphosphite triester (**7a-j**). In the second step of this sequence, the triester is hydrolyzed to the H-phosphonate (**8**). By oxidation with various regents, the dinucleoside arylphosphite triester or H-phosphonate is then converted to dinucleoside phosphoramidates, thiophosphates, alkylphosphonates, arylphosphate triesters, and the natural internucleotide linkage. Our initial investigations with both approaches appear promising and encourage us to proceed further with this concept.

RESULTS AND DISCUSSION

Preparation of Deoxynucleoside 3′-O-Arylphosphoramidites

O-arylphosphordiamidites (**3a-j**) were synthesized in high yield (70-95%) and purity (Table 1, >90% as measured by ^{31}P NMR) by reacting bis(N,N-diethylamino)chlorophosphine (**1**) with the appropriate sodium phenolate (**2a-j**, see the legend to Figure 1 for a definition of the aryl groups **a-j**). These salts proved to be especially attractive as they were easily rendered anhydrous and reacted with chlorophosphines to yield inert sodium chloride as well as the appropriate product. Attempts to use the corresponding phenols in the presence of tertiary amines (diisopropylethylamine or triethylamine) failed because the resulting amine hydrochloride was difficult to remove completely and, as a consequence, further activated the phosphorodiamide to yield an undesirable side-product, the bis(O-aryl)-N,N-diethylphosphoramidite. However even the use of sodium phenolates failed to yield exclusively **3a-j** with phenols having bulky substituents at positions 2 and 6 (see Table 1, compounds **3f**, **3i**, and **3j**).

Table 1. Yields and ^{31}P NMR Data on O-Arylphosphordiamidites

Compound[a]	Yield (%)[b]	31P NMR (ppm)[c]		
		O-Arylphosphordiamidite	Bis(O-aryl)phosphoramidite	Other
3a	75	133.6 (90%)		
3b	79	149.8 (t, J = 43 Hz 75%)	154.4 (t, J = 43 Hz 75%)	18.4 (s, 15%)
3c	90	142.0 (90%)	-	14.0 (10%)
3d	95	132.1 (95%)	-	-
3e	89	131.0 (95%)	-	-
3f	40	131.5 (60%)	144.7 (40%)	-
3g	95	129.1 (90%)	-	-
3h	95	131.0 (95%)	-	-
3i	74	127.8 (80%)	143.4 (20%)	-
3j	70	129.2 (85%)	143.4 (15%)	-

[a]Compounds **3a-j** correspond to various aryl derivatives of O-aryl-N,N,N′,N′-tetraethylphosphordiamidite. The aryl groups **a-j** are defined in the legend to Figure 1.
[b]Isolated yields.
[c]The numbers within parentheses represent the relative amount of phosphorus containing product in each NMR signal.

Deoxynucleoside 3′-O-arylphosphoramidites (**5a-j**) were synthesized from 5′-protected deoxynucleosides (**4**), the appropriate O-aryl-N,N,N′,N′-tetraethylphosphordiamidite (**3a-j**), and one equivalent of tetrazole as

activating agent.[6] Benzotriazole was also a satisfactory activating agent when the aryl moiety did not contain electron withdrawing substituents. The resulting deoxynucleoside 3′-O-arylphosphoramidites were purified in good yield (36-68%). The lower yielding derivatives all had electron withdrawing substituents on the aromatic rings and were partially unstable to chromatography on silica.

Kinetic Studies on Deoxydinucleotide Synthesis

The reactivity of various aryl derivatives towards synthesis of internucleotide phosphite triesters (**7a-j**) was evaluated by condensing **5a-j** with a 3′-dimethoxytrityl protected deoxynucleoside (**6**) in the presence of tetrazole and monitoring the reaction progress by analytical tlc. Using this system, quantitation was simplified as only products due to condensation of the deoxynucleoside O-aryl phosphoramidite with (**6**) developed the orange color indicative of the dimethoxytrityl cation. With the O-methylphosphoramidite as a control, the reaction was complete in less than 20 seconds and only one new dimethoxytrityl positive spot corresponding to the dinucleoside phosphite triester was observed. In contrast, the O-arylphosphoramidites reacted more slowly with the rates being dependent upon the phenyl ring substituents. (**5i>5j>5h>5e>5d>5f**) and whether electron withdrawing or bulky groups were located at positions 2 and 6 (Figure 2). For example the 4-bromophenyl derivative reacted more

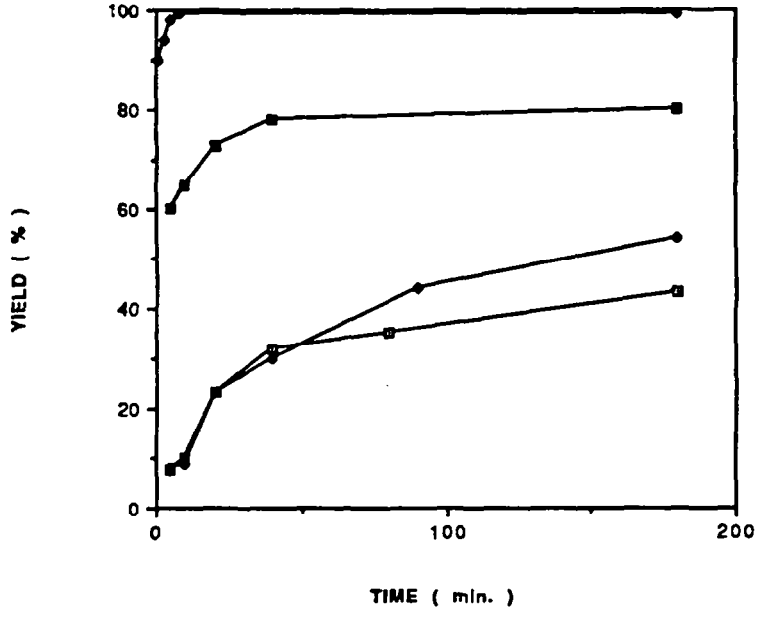

Figure 2. The Influence of Different Aryl Groups on the Synthesis of Dinucleoside Arylphosphites. Reactions were completed with tetrazole as catalyst. 5h, -◊-◊-◊-; 5g, -■-■-■-; 5e, -◆-◆-◆-; 5f, -❑-❑-❑-.

rapidly than the 2-bromophenyl and both were considerably slower in coupling rates than the corresponding phenyl compound. Moreover the kinetics changed as the reactions progressed. This was undoubtedly due to accumulation of the tetrazolide salt. This salt buffered the reactivity of tetrazole toward protonation of the electron withdrawing O-arylphosphoramidites which was not the case for the more reactive O-alkyl derivatives.[7] This

activation process was further studied using catalysts having different acidities. Typical results showed that activation toward condensation was dependent upon the acidity of the catalyst. Thus of the catalysts tested, N-methylanilinium trifluoroacetate was found to be the most efficient followed by 1-hydroxy benzotriazole, 5-(4′-nitrophenyl) tetrazole, 5-(2′-nitrophenyl)tetrazole and finally tetrazole (data not shown).

Hydrolysis of Deoxynucleoside Arylphosphite Triesters

Preliminary experiments demonstrated that dinucleoside arylphosphites decomposed in the presence of aqueous triethylamine to a mixture of deoxynucleoside and deoxynucleoside phosphite but that these same compounds were not degraded in the presence of water or even tetrazole-water. These results suggested that perhaps dinucleoside arylphosphites could be hydrolyzed selectively, without degradation of the internucleotide linkage, to dinucleoside H-phosphonates under mild acidic conditions. This concept was tested by first synthesizing dinucleoside arylphosphites using various acid catalysts, adding water, and measuring the progress of the reaction by observing the disappearance of the ^{31}P-NMR phosphite triester signal and the appearance of a chemical shift identical to that of the dinucleoside H-phosphonate (**8**). The results are shown in Table 2.

Table 2. Hydrolysis of Deoxydinucleoside Arylphosphite Triesters

Aryl Substituent	Hydrolysis Conditions[a,b]					
	I	II	III	IV	V	VI
Pentachlorophenyl	15 min	<10 min				
4-nitrophenyl	1 hr	25 min				
2-bromophenyl	10 hr	1.5 hr	6 hr			
4-bromophenyl	3 hr	30 min	30 min			20 min
phenyl	2 hr	<10 min	20 hr	*c*	<10 min	1 hr
2,4,5-trichlorophenyl	30 min	1 hr				

[a]The results show the time required for complete disappearance, via ^{31}P NMR, of the signal corresponding to the phosphite triester.

[b]Hydrolysis conditions were as follows:

I 5-(2′-nitrophenyl)tetrazole:H_2O (1:9, w/v)
II 5-(2′-nitrophenyl)tetrazole:H_2O:$ZnBr_2$ (1:9, w/v with 1 eq. $ZnBr_2$)
III N-methylanilinium trifluoroacetate (0.4 M) in H_2O
IV tetrazole:H_2O (1:9, w/v)
V tetrazole:H_2O:$ZnBr_2$ (1:9, w/v with 1 eq $ZnBr_2$)
VI 1-hydroxybenzotriazole:H_2O (1:9, w/v)

[c]Less than 10% in 20 hr.

The most clearly recognizable trend was a correlation of the hydrolysis rate with increased acidity of the acid catalyst. For example, the phenyl derivative was hydrolyzed completely to the H-phosphonate in two hours with aqueous 5-(4′-nitrophenyl)tetrazole whereas tetrazole yields only 10% conversion in 20 hours. Addition of one equivalent zinc bromide to reaction mixtures increased the hydrolysis rate even further with the dinucleoside phenyl phosphite derivative being the most significantly affected by the Lewis acid. Such a dramatic enhancement

in hydrolysis rate seems reasonable as previous studies have shown that dinucleoside 2-bromophenyl phosphate triesters were hydrolyzed in the presence of zinc bromide.[8] Other trends were enhanced reaction rates with phenyl derivatives having electron withdrawing groups and bulky substituents at position two of the phenyl ring leading to a substantial decrease in rates.

Based upon these results, dinucleoside H-phosphonates were synthesized from the dinucleoside phenylphosphite triester. Typically, reactions were completed by condensing the deoxynucleoside 3′-diethylaminophenylphosphoramidite with a 3′-protected deoxynucleoside using 5-(4′-nitrophenyl)tetrazole as catalyst. The resulting phosphite triester was then treated with either water or 1 M aqueous zinc bromide (2 hr or 10 min, respectively, for complete conversion). Isolation was by column chromatography on Sephadex LH20 and silica gel with yields of fully characterized product (^{31}P- and ^{1}H-NMR, mass spectrum) being 30-35%. When 3′-O-acetyl was substituted for dimethoxytrityl, the dinucleoside H-phosphonate was more easily isolated free of side-products. In order to check the acid stability of H-phosphonate deoxydinucleosides, samples were kept in NMR tubes for three days under various hydrolysis conditions (Table 2). Only samples containing N-methylanilinium trifluoroacetate decomposed to mixtures of H-phosphonate monoesters (16-20 hr for complete hydrolysis). The remaining solutions were stable which suggested that these acids can be used to hydrolyze aromatic dinucleoside phosphite triesters to the corresponding H-phosphonates.

Reaction of Phenylphosphites with Phenyl Azide and Methyl Iodide

One objective of this work was to determine if nucleoside phenylphosphites could be converted directly to phosphoramidates and methyl phosphonates. When a dinucleoside phenylphosphite (**7h**) was treated with phenyl azide, the ^{31}P-NMR signal corresponding to a phosphite triester disappeared within one hour and three phosphoramidates form. The major product was isolated and characterized as the dinucleoside phenylphosphoramidate. Treatment of 5′-O-dimethoxytritylthymidylyl-3′-ethylphenylphosphite with methyl iodide to yield the nucleoside methylphosphonate required two days and resulted in one major ^{31}P-NMR peak (67% of the total phosphorus) and one secondary peak corresponding to the hydrolysis product. Isolation and characterization of the major product indicated that it corresponded to the nucleoside methylphosphonate.

Solid Phase Synthesis with Deoxynucleoside Phenylphosphoramidites

Both styrene and silica were tested as insoluble supports for deoxyoligonucleotide synthesis. 5′-O-Dimethoxytrityldeoxythymidine was attached to these supports through a standard linkage and detritylated to yield a support bound deoxynucleoside having a free 5′-hydroxyl group.[1] Coupling reactions were then studied using 5′-O-dimethoxytrityldeoxythymidine-3′-O-(N,N-diethylamino)phenylphosphoramidite as a synthon and either 5-(2′-nitrophenyl)tetrazole or N-methylanilinium trifluoroacetate as catalysts. After ten minutes, conversion of the dinucleoside phenylphosphite triester to the dinucleoside H-phosphonate on silica supports was completed by adding 1 M aqueous zinc bromide to the coupling solution. For polystyrene where the support must be maintained in an open, swollen form, the reaction solution was removed by filtration and a fresh hydrolysis mixture consisting of THF:water (1:1), 0.5 M zinc bromide, and 0.1 M 5-(2′-nitrophenyl)tetrazole was added. After the usual capping, oxidation and detritylation steps, analysis indicated that the coupling yields were low for both silica (40-60%) and polystyrene (50-80%). These low yields appear to be caused by hydrolysis of the deoxynucleoside phenylphosphoramidite during the coupling step. This was shown by ^{31}P-NMR analysis of reaction mixtures following ten minutes contact with the resin. The only products were hydrolysates of the phenylphosphoramidite.

None of the peaks corresponded to the tetrazolide or phosphoramidite. Although similar results were observed with deoxynucleoside N,N-diisopropylamino-O-β-cyanoethyl phosphoramidite and tetrazole, quantitative coupling was obtained. These results suggested that there was sufficient residual water present either in the reaction vessel or tightly bonded to the support so as to totally hydrolyze the activated phosphoramidite. However because the standard methyl or β-cyanoethyl phosphoramidites were so reactive, they effectively phosphitylated the support linked nucleoside even in the presence of water. Conversely, the less reactive phenylphosphoramidites were hydrolyzed before complete coupling with the deoxynucleoside. In an attempt to eliminate this residual water, the polymeric carriers were first treated with 2,2-dimethoxypropane and then dried under high vacuum. Following this procedure, the coupling yields on polystyrene resins increased to 80-99% but no significant change was observed on silica. Presumably the water bonded to silica was retained in a form that was unreactive toward dimethoxypropane but still available for reaction with phosphoramidites.

Conclusions

These results demonstrate that a large variety of deoxynucleoside 3′-O-arylphosphoramidites can be synthesized and used for preparing internucleotide arylphosphite triester linkages. As expected from the electron withdrawing characteristics of aryl groups, acids stronger than tetrazole are required in order to achieve efficient coupling. These aryl triesters can be converted directly to amidates and phosphonates in less than quantitative yield. By hydrolysis to the dinucleoside H-phosphonates, a new route is available to dinucleoside phosphate diesters, phosphate triesters, phosphoramidates, phosphorothioates, and alkylphosphonates. Of particular interest were the results directed toward synthesis of deoxyoligonucleotides on polymeric supports. Although promising results were achieved, the presence of water tightly adsorbed to the supports appeared to reduce the coupling efficiency. This problem was partially overcome by using mild dehydrating reagents but will require further study before arylphosphoramidite synthons can be used for preparing DNA analogs on polymeric supports. Two attractive features of this approach encourage us to continue these investigations. (1.) The approach is quite flexible as normal and modified internucleotide linkages can be generated in any designated combination and sequence. (2.) The chemistry is compatible with the highly successful phosphoramidite approach for DNA synthesis. Thus if the challenges can be overcome, this high yielding chemistry can be used to introduce normal and modified DNA via the same synthon.

EXPERIMENTAL

General

5′-O-Dimethoxytritylthymidine,[9] N-benzoyl-5′-O-dimethoxytrityl-2′-deoxyadenosine,[10] N-isobutyryl-5′-O-dimethoxytrityl-2′-deoxyguanosine,[11] 5′-O-*t*-butyldimethylsilylthymidine,[12] 5′-O-*t*-butyldimethylsilylthymidine 3′-O-hydrogen phosphonate,[13] bis(N,N-diethylamino)chlorophosphine,[14] 5′-(2′-nitrophenyl)tetrazole and 5-(4′-nitrophenyl)tetrazole[15] were prepared following published procedures. 3′-O-Dimethoxytritylthymidine and 3′-O-acetylthymidine were prepared by reacting 5′-O-*t*-butyldimethylsilylthymidine with dimethoxytritylchloride or acetic anhydride followed by removal of the *t*-butyldimethylsilyl group with tetrabutylammonium fluoride. ^{1}H and ^{31}P NMR were recorded on a Brucker WM-250 and JOEL FX-90Q, respectively. Trimethylsilane and 10% H_3PO_4 in acetonitrile were external standards.

Preparation of O-Arylphosphordiamidites

Sodium ethoxide was first synthesized by adding sodium (0.26 g, 11.3 mmol) to absolute ethanol (100 ml), concentrating the resulting product *in vacuo* to remove excess ethanol, re-evaporation with toluene to eliminate residual ethanol, and dissolving the salt in dry tetrahydrofuran (25 ml). Phenol (1.06 g, 11.2 mmol) was added to this solution to yield sodium phenolate which was isolated by repeated evaporation with toluene. The resulting sodium phenolate was dissolved in dry tetrahydrofuran and added dropwise to a solution of bis(N,N-diethyl-amino)chlorophosphine in tetrahydrofuran. After standing overnight at room temperature, sodium chloride was removed by centrifugation and washed twice with dry tetrahydrofuran. The combined solutions were concentrated to dryness to yield O-phenyl-N,N,N′,N′-tetraethylphosphordiamidite (2.85 g, 10.6 mmol, 95%). The remaining O-arylphosphordiamidites were synthesized using the same procedure. The results are summarized in Table 1.

Preparation of 5′-O-*t*-butyldimethylsilylthymidine-3′-O-aryl-N,N-diethylaminophosphoramidites

In a typical experiment, 5′-O-*t*-butyldimethylsilylthymidine (0.2 g, 0.56 mmol) and benzotriazole (0.067 g, 0.56 mmol) were dried by coevaporation with dry acetonitrile. The residue was dissolved in dry dichloro-methane (5 ml) and phenyl-N,N,N′,N′-tetraethylphosphordiamidite (0.195 g, 0.70 mmol) was added. After one hour, the reaction appeared complete (^{31}P NMR and tlc analysis) and was quenched by addition of triethylamine (0.5 ml) and hexane (2 ml). The reaction mixture was charged directly on to a silica gel column (15 g) and the product eluted with 5% triethylamine in dichloromethane:hexane (1:1). The yield was 0.211 g (0.38 mmol, 68%). ^{31}P NMR (dichloromethane) δ 145.2, 144.6 (diastereomers). Similar results were obtained with tetrazole in acetonitrile as catalyst.

Several additional arylphosphoramidites were synthesized using this procedure. Characterization data for these compounds follows: compound **5d** (44% yield), ^{31}P NMR (dichloromethane) δ 147.2; compound **5e** (56% yield), ^{31}P NMR (tetrahydrofuran), δ 145.0; compound **5f** (47% yield), ^{31}P NMR (dichloromethane) δ 144.8; compound **5g** (36% yield), ^{31}P NMR (dichloromethane) δ 145.1; compound **5i** (45% yield), ^{31}P NMR (dichloromethane) δ 144.1; compound **5j** (40% yield), ^{31}P NMR (dichloromethane) δ 147.6.

Kinetic Experiments on the Synthesis of Deoxydinucleoside Phosphite Triesters

Typically 3′-O-dimethoxytritylthymidine (4 μmole) and an appropriate catalyst (8 μmol) were mixed, rendered anhydrous by coevaporation with dry acetonitrile, and the residue dissolved in dry acetonitrile (0.4 ml). 5′-O-*t*-butyldimethylsilylthymidine-3′-O-(N,N-diethylamino)arylphosphoramidite (5 μmol) was dissolved in dry dichloromethane (0.1 ml) and the reaction solution, containing the protected deoxynucleoside and catalyst, was added to this amidite. At certain times, two aliquots (0.02 ml) were removed. One was added to triethylamine: tetrahydrofuran (1:4, 0.02 ml) and the other to water:tetrahydrofuran (1:4). These samples were layered on a pre-run tlc plate (5 X 20 cm, aluminum-silica gel 60-F254, Merck) and developed with dichloromethane:triethylamine: methanol (93:2:5, v/v/v). Upon analysis of these developed plates by UV light and exposure to HCl vapors, three dimethoxytrityl containing and UV absorbing spots were observed. Compounds having the highest mobility in this solvent (R_f = 0.75) were the deoxydinucleoside phosphite (**7a-j**) and the corresponding H-phosphonate (**8**). Compound **6** had the next highest mobility (R_f = 0.65). The slowest migrating compound (R_f = 0.06) was the 3′-O-dimethoxytritylthymidine 5′-phosphite. This compound was present because the dinucleoside arylphosphite triesters were unstable to the triethylamine-water stop solution and gave the dinucleoside H-phosphonate derivative upon hydrolysis. Further hydrolysis of this H-phosphonate dimer yields thymidine and H-phosphonate

monoesters. The silica gel containing these compounds was treated with 3% trichloroacetic acid in dichloromethane (2.0 ml), and removed by filtration. The absorbance of each filtrate was then read at 500 nm. The yield of deoxydinucleoside phosphite triester (**7a-j**) was calculated by adding the absorbance of solutions from the samples having mobilities of 0.75 and 0.06 and comparing this number to the sum of the absorbances for all three samples. Typical results are reported in Figure 2.

Studies on the Hydrolysis of Aryl Phosphite Triesters

3′-O-Dimethoxytritylthymidine (0.1 mmol) and catalyst (0.4 mmol) were mixed, rendered anhydrous by coevaporation with dry acetonitrile, dissolved in acetonitrile (0.5 ml), and the arylphosphoramidite (**5a-j**, 0.11 mmol) added. The resulting reaction solution was transferred to a NMR tube and the spectra of **7a-j** recorded (132-136 ppm). Water or water containing zinc bromide was then added and ^{31}P NMR used to measure conversion to the H-phosphonate (compound **8**, 8.7 and 7.7 ppm, J_{P-H} = 720 and 732 Hz, respectively). Minor signals corresponding to thymidine O-aryl H-phosphonate diester (2.5 ppm, J_{P-H} = 630 Hz), thymidine H-phosphonate monoester (1.5 ppm), and phosphorus acid (0.3 ppm) were also observed. These minor products were obtained by hydrolysis of excess phosphoramidite (**5a-j**) rather than from **7a-j** or **8**. The only exception was with N-methylanilinium trifluoroacetate as catalyst which slowly hydrolyzed compound **8** to these side products (16-20 hr for complete hydrolysis) under conditions where the remaining catalysts were unreactive (3 days at r.t.). The results of these studies are presented in Table 2.

Synthesis of Deoxydinucleoside H-Phosphonate

3′-O-Acetylthymidine (0.051 g, 0.18 mmol) and 5-(2′-nitrophenyl)tetrazole (0.145 g, 0.66 mmol) were added to dry acetonitrile (2 ml), concentrated to a gum, and dissolved in dry acetonitrile (2 ml). Compound **5h** (0.120 g, 0.22 mmol) was added and the resulting solution divided into two equal parts. Water (0.2 ml) was added to one reaction mixture and 1 M aqueous zinc bromide (0.2 ml) to the other. Using ^{31}P NMR to monitor the reactions, the signal corresponding to the phenylphosphite triester (133.8 and 133.5 ppm) disappeared in less than ten minutes with aqueous zinc bromide and in two hours with water. The reaction mixture containing zinc bromide was diluted with dichloromethane, extracted sequentially with brine and 5% aqueous sodium bicarbonate, dried over magnesium sulfate, concentrated to dryness, and fractionated on Sephadex LH-20 using tetrahydrofuran as eluant. The product was further purified by chromatography on a silica gel column using a gradient of methanol (2-10%) in dichloromethane as eluant. The reaction mixture containing only water was applied directly to a Sephadex LH-20 column and further purified on silica gel as described above. After precipitation from hexane: dichloromethane, compound **8** was obtained in 21 mg (0.03 mmol) and 18 mg (0.026 mmol) from the water and zinc bromide reactions, respectively (total yield 31%). Both samples had the same physical and spectroscopic properties. FAB+ mass spectrum: 687 (M + 1), 465, 339 (100%). FAB- mass spectrum: 686 (M), 535, 463, 419 (100%), 347. ^{31}P NMR (acetonitrile) δ 8.9 and 8.0 (2 diastereoisomers).

Conversion of Deoxydinucleoside Phenylphosphite to the Corresponding Phenyl Azide

3′-O-Acetylthymidine (0.033 g, 0.12 mmol) and 5-(2′-nitrophenyl)tetrazole (0.067 g, 0.36 mmol) were added to dry acetonitrile (2 ml), concentrated to a gum, and dissolved in dry acetonitrile (0.8 ml). Compound **5h** (0.079 g, 0.14 mmol) was added. After 10 min at room temperature, phenylazide (0.10 g, 0.84 mmol) was added and the reaction monitored by ^{31}P NMR. Signals corresponding to the deoxydinucleoside phenylphosphite triester

(134.6, 133.4 ppm) disappeared after one hour and new signals corresponding to phosphoramidate derivatives appeared at -15.6 ppm (67%), -14.4 ppm (12%) and -7.8 ppm (21%). Other minor signals due to the hydrolysis of excess phosphoramidite were also observed (3.6, 1.4 and 2.5 ppm). The reaction mixture was loaded directly on to a Sephadex LH-20 column and fractionated using tetrahydrofuran as eluant. Fractions containing the desired product, the dinucleoside phenyl azide, were pooled, concentrated and then further purified on a silica gel column eluted with a gradient of methanol (0-10%) in dichloromethane. Product fractions were pooled and concentrated to yield 20 mg (0.026 mmol, 21%). FAB+ mass spectrum: 779 (M + 1), 440, 339 (100%), 254. FAB- mass spectrum: 777 (M - 1), 510, 438 (100%), 384, 342, 252. ^{31}P NMR (acetonitrile) δ -15.6.

Conversion of a Phenylphosphite Triester to the Methylphosphonate

5′-O-Dimethoxytritylthymidine 3′-O-(N,N-diethylamino)phenylphosphoramidite (0.150 g, 0.27 mmol) and tetrazole (0.030 g, 0.43 mmol) were added to dry acetonitrile. Absolute ethanol (0.2 ml) was added. After ten min at room temperature, the reaction solution was concentrated *in vacuo* to a gum, dissolved in dichloromethane, extracted with 5% aqueous sodium bicarbonate, dried over magnesium sulfate, and concentrated to a gum. The residue was dissolve in acetonitrile (1 ml) and methyl iodide added (0.4 ml). After two days at room temperature, the ^{31}P NMR signal corresponding to thymidylyl-3′-ethylphenylphosphite (133.0 ppm) completely disappeared and four new peaks appeared in the spectrum. The major signal (61%, 27.8 ppm) was the methylphosphonate derivative. Unassigned peaks were observed at 6.8 ppm (26%), 30.0 ppm (4%) and 24.0 ppm (9%). The reaction mixture was concentrated to dryness, dissolved in dichloromethane, applied to a silica gel column, and fractionated using a gradient of methanol (0-10%) in dichloromethane. The major product, which was isolated in 20% yield (0.035 g, 0.054 mmole), corresponded to the methylphosphonate. ^{31}P NMR (acetonitrile) δ 27.8.

Synthesis of 5′-O-Dimethoxytrityl-N-protected Deoxynucleoside 3′-O-(N,N-diethylamino)phenylphosphoramidites (Compounds **5h** Where R′ is Dimethoxytrityl)

Each 5′-O-dimethoxytrityl and N-protected deoxynucleoside (1.8 mmol) was reacted with phenyl-N,N,N′,N′-tetraethylphosphordiamidite (0.54 g, 2 mmol) in the presence of benzotriazole (0.21 g, 1.8 mmol) as described previously for the synthesis of 5′-O-*t*-butyldimethylsilylthymidine-3′-O-phenyl-N,N-diethylamino-phosphoramidite. The thymidine derivative was purified on silica gel by isocratic elution with dichloromethane: hexane (1:1) containing 5% triethylamine. For the deoxyadenosine, deoxycytidine, and deoxyguanosine derivatives, purification was achieved on a silica gel column using a gradient elution procedure (dichloromethane: hexane, 1:1 to dichloromethane with both solvents containing triethylamine). The following ^{31}P NMR data were obtained for each derivative: 5′-O-dimethoxytritylthymidine 3′-O-(N,N-diethylamino)phenylphosphoramidite (68% yield), ^{31}P NMR (tetrahydrofuran) δ 144.8 and 144.0; 5′-O-dimethoxytrityl-N-benzoyldeoxyadenosine 3′-O-(N,N-diethylamino)phenylphosphoramidite (65% yield), ^{31}P NMR (dichloromethane) δ 144.2; 5′-O-dimethoxytrityl-N-benzoyldeoxycytidine 3′-O-(N,N-diethylamino)phenylphosphoramidite (65% yield), ^{31}P NMR (dichloromethane) δ 144.3; 5′-O-dimethoxytrityl-N-isobutyryldeoxyguanosine 3′-O-(N,N-diethylamino)phenyl-phosphoramidite (75% yield), ^{31}P NMR (dichloromethane) δ 143.9.

Solid Phase Synthesis

The following general procedure was used for studies on the synthesis of deoxyoligonucleotides on either polystyrene or silica gel supports. A deoxynucleoside was linked to the support through the 3′-hydroxyl using

standard procedures.[1] The standard synthesis protocol, including variations for either polystyrene or silica, were as follows. (1.) Wash the support with dichloromethane and then acetonitrile. (2.) Treat the support for three minutes with 10% dimethoxypropane and 0.1 M tetrazole in dry tetrahydrofuran (polystyrene) or dry acetonitrile (silica gel). (3.) Wash with dry acetonitrile. (4.) Dry *in vacuo* for five minutes. (5.) Synthesize a dinucleotide on the support. For polystyrene supports, compound **5h** (0.1 M) in dry dichloromethane (0.2 ml) and 5-(2′-nitrophenyl)tetrazole (0.4 M) or N-methylanilinium trifluoroacetate (0.4 M) in dry tetrahydrofuran (0.2 ml) were added to the resin and the reaction allowed to proceed for ten minutes. For silica supports, the same protocol was used except that all reagents were dissolved in dry acetonitrile. (6.) Wash the support with either dichloromethane (polystyrene) or acetonitrile (silica). (7.) Treat the support with capping solution as outlined previously.[1] (8.) Wash the support with acetonitrile. (9.) Hydrolyze the phosphite triester to the H-phosphonate. For polystyrene supports, the resin was treated for five minutes with tetrahydrofuran:water (1:1) containing 5-(2′-nitrophenyl)-tetrazole (0.1 M) and zinc bromide (0.5 M). For silica supports, the hydrolysis reaction was completed by adding 0.4 ml of 1 M zinc bromide in water directly to the coupling reagents (step 5) and then following with steps 6 and 7. (10.) Wash the support with N,N-dimethylformamide. (11.) Wash the support with dichloromethane (polystyrene) or acetonitrile (silica). (12.) Oxidize the H-phosphonate to phosphate and remove the dimethoxytrityl ether using procedures outlined previously.[1]

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