Pergamon

Tetrahedron Letters 40 (1999) 8971–8974

TETRAHEDRON LETTERS

Nucleoside recovery in DNA and RNA synthesis

Weimin Wang, Quanlai Song and Roger A. Jones *

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

Received 24 August 1999; accepted 27 September 1999

Abstract

Nucleoside phosphoramidites and H-phosphonate diesters can be converted to nucleosides under mild conditions and in high yields by reaction with polyhydroxy alcohols.

H-Phosphonate diesters are known to be subject to transesterification reactions,[1] that may result in chain cleavage during oligonucleotide synthesis,[2] and that can be accelerated by the vicinal hydroxyl of a ribonucleoside.[3] The use of an imidazole catalyzed transesterification in methanol to effect dephosphonylation of nucleoside 3′-cyanoethyl-H-phosphonates has been applied to the problem of recycling the excess phosphoramidites used in automated oligonucleotide synthesis.[4] Recently, we found that ethylene glycol or glycerol can be used to cleave a nucleoside 3′-H-phosphonate moiety by means of a facile intramolecular transesterification reaction,[5] which Reese has also found.[6] We now report that a similar reaction can be used with 3′-cyanoethyl-H-phosphonates and with phosphoramidites.

Transesterification of 3′-cyanoethyl-H-phosphonates with ethylene glycol or glycerol gives a much faster dephosphonylation reaction than does methanol, such that catalysis by imidazole is not required. The absence of imidazole allows this approach to be applied to recycle ribonucleoside phosphoramidites having a 2′-*O*-*tert*-butyldimethylsilyl (BDMS) protecting group without concomitant isomerization of this group. Alternatively, reaction of an activated nucleoside phosphoramidite with ethylene glycol or glycerol directly, without prior hydrolysis to the H-phosphonate diester, leads to dephosphonylation in a one-flask procedure.

Dephosphonylation after hydrolysis of the phosphoramidite, **1**, to the nucleoside 3′-cyanoethyl-H-phosphonate, **2**, is a two-step procedure in which the first step is to isolate **2** free from tetrazole and water (by extraction and concentration, respectively).[7] The presence of water in the reaction mixture during dephosphonylation leads to competing hydrolysis to the 3′-H-phosphonate monoester, while tetrazole catalyzes isomerization of the 2′-BDMS group in ribonucleosides. As shown in Fig. 1, HPLC monitoring of the dephosphonylation step detects only the formation of **4**, along with the disappearance of **2**. The intermediate **3** is not seen. This is consistent with the transformation shown in Scheme 1, where the slow step is the transesterification of **2** to give either the free 3′-OH nucleoside **4**, or the diester **3**. We previously have shown that coupling of a nucleoside 3′-H-phosphonate with

* Corresponding author.

0040-4039/99/$ - see front matter
PII: S0040-4039(99)01914-0

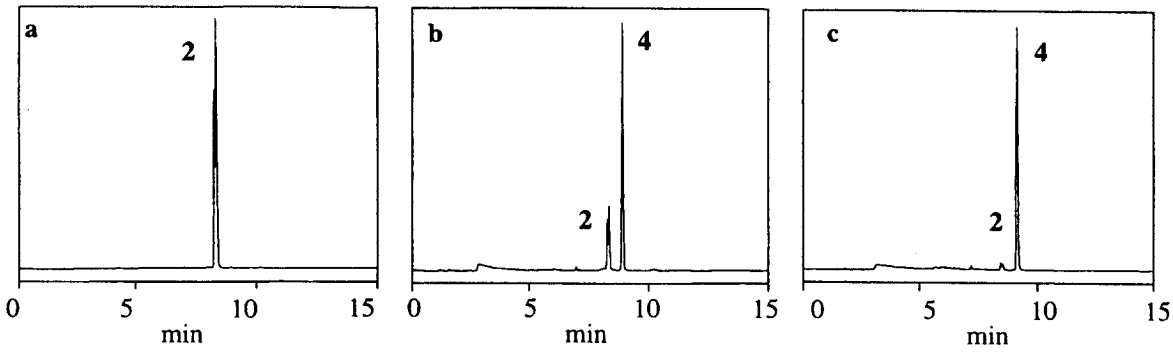
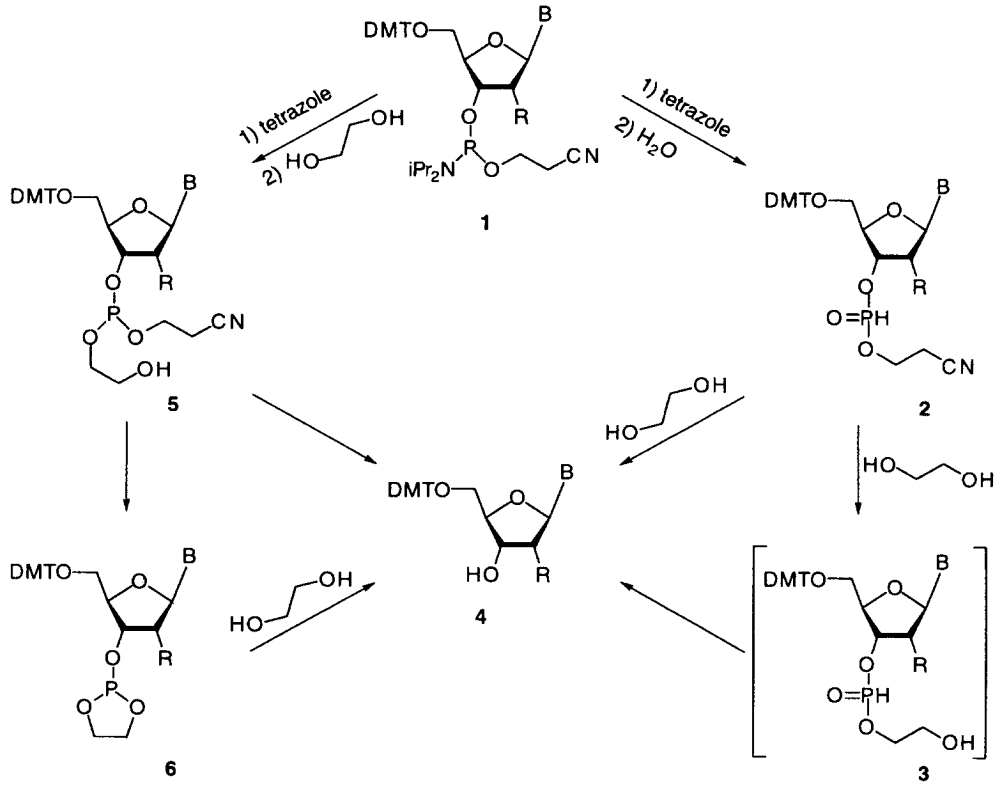


Figure 1. HPLC profiles (2–80% acetonitrile: 0.1 M triethylammonium acetate in 5 min at 2 mL/min on a Waters 3.9×150 mm Nova–Pak® C18 column) of; (a) the cytidine 3′-cyanoethyl-H-phosphonate diastereomers, **2**, before treatment with glycerol; (b) after 12 h with glycerol; and (c) after 2 days[7]



Scheme 1. R=H; B=thymine, 4-*N*-benzolycytosine, 2-*N*-isobutyrylguanine, 6-*N*-benzoyladenine. R=*O*-BDMS; B=uracil, 4-*N*-acetylcytosine, 2-*N*-isopropylphenoxyacetylguanine, 6-*N*-phenoxyacetyladenine

ethylene glycol or glycerol, which presumably gives the diester corresponding to **3**, results in rapid dephosphonylation, presumably by intramolecular transesterification involving the vicinal hydroxyl group of **3**.[5] The complete conversion of nucleoside 3′-cyanoethyl-H-phosphonates, **2**, to free 3′-OH nucleosides, **4**, takes 1 day for deoxynucleosides and 2 days for ribonucleosides.[7] There is no isomerization of the BDMS group under these conditions. Fig. 2c and Fig. 3c show additional examples of the crude reaction mixtures from the conversion of **2** to **4** for, respectively, the deoxyguanosine and adenosine derivatives.

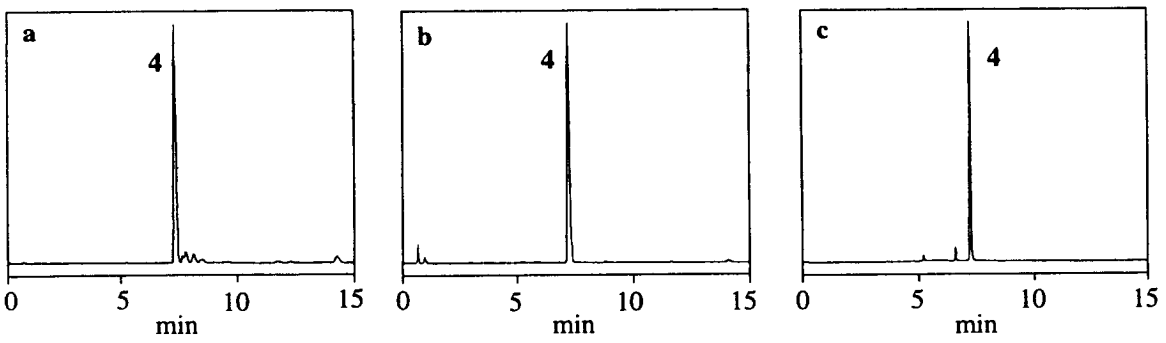


Figure 2. HPLC profiles (under the same conditions as Fig. 1) for reaction of the deoxyguanosine phosphoramidite **1** with ethylene glycol (a) after 5 min, and (b) 8 h after addition of pyridine.[8] Panel (c) shows the final reaction mixture obtained after treatment of the deoxyguanosine 3′-cyanoethyl-H-phosphonate **2** with glycerol[7]

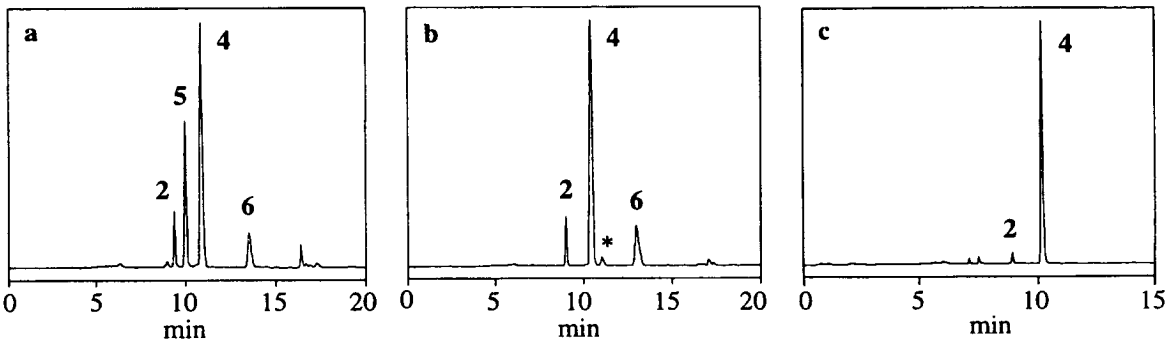


Figure 3. HPLC profiles (conditions as Fig. 1) for reaction of the adenosine phosphoramidite **1** with ethylene glycol after (a) 5 min, and (b) 50 min. Panel (c) shows the final reaction mixture obtained after treatment of the adenosine 3′-cyanoethyl-H-phosphonate **2** with glycerol[7]

One-flask dephosphonylation by direct coupling of a phosphoramidite, **1**, with ethylene glycol or glycerol gives initially the phosphite triester, **5**, which then can undergo cyclization by the vicinal hydroxyl group to give either the free 3′-OH nucleoside **4**, or phosphite triester **6**. The latter is then more slowly converted to **4** by reaction with another molecule of alcohol or water, if present. Fig. 2a shows a typical reaction mixture of a deoxynucleoside 5 min after addition of the activated phosphoramidite to a solution of ethylene glycol in acetonitrile.[8] The diastereomers of the 3′-cyanoethyl-H-phosphonate, **2**, are seen at a slightly longer retention time than **4**, and the other minor peaks nearby presumably correspond to **5** and **6** (see below). Some amount of **2** is normally present in material recovered from the synthesizer due to hydrolysis of **1** by trace amounts of water. The addition of pyridine then completes conversion to **4** to give the final reaction mixture shown in Fig. 2b.[8] Fig. 2c shows, for comparison, the final mixture obtained by the two-step procedure.

The minor impurity in Fig. 2c co-migrates with the corresponding 3′-H-phosphonate monoester, while the minor impurity visible in Fig. 2a and b near 15 min co-migrates with the dimer produced by coupling of two molecules of the activated phosphoramidite to one molecule of ethylene glycol. When the coupling reaction is carried out by adding ethylene glycol to a solution of the activated phosphoramidite, the dimer can be the major product. The deoxyadenosine dimer has been isolated and identified by LCMS at (m+1)/z 1575.7. The conversion of phosphoramidites **1** to the free 3′-OH nucleosides **4** is complete within minutes for deoxynucleosides, while several hours are required for ribonucleosides. In the latter

case, however, the tetrazole that is present causes detectable isomerization of the BDMS group in this time (Fig. 3b).

In the one-flask dephosphonylation, the coupling of the activated phosphoramidite with the alcohol is the fastest step, so that intermediates **5** and **6** are detectable by HPLC, especially for ribonucleosides, and we have made use of the slow reaction of 2′-BDMS protected ribonucleoside to isolate the ribonucleoside derivatives **5** and **6**. Fig. 3 shows HPLC of the reaction of the adenosine phosphoramidite **1** with ethylene glycol after 5 min and again after 50 min. The adenosine derivatives of **5** and **6** were isolated and identified by LCMS at (m+1)/z 979.7 and 908.7, respectively. The peak identified with an asterisk in Fig. 3b co-migrates with the 3′-BDMS isomer of **4**. Fig. 3c shows, for comparison, the crude mixture obtained for the adenosine derivative from the two-step procedure, where there is no detectable isomerization.

Nucleoside recovery is particularly important with valuable modified or labeled monomers. Both of the procedures described above efficiently convert phosphoramidites or cyanoethyl-H-phosphonates to free nucleosides. The one-flask procedure is the easiest to carry out, but because of isomerization of the BDMS group, the two-step route is best for BDMS protected ribonucleosides.

Acknowledgements

This work was supported by the National Institutes of Health (GM48802). We thank Provid Pharmaceuticals, in particular George Elgar, and Waters Assoc., for the LCMS analyses.

References

1. Troev, K.; Borisov, G. *Phosphorus and Sulfur* **1987**, *29*, 129–145.
2. Froehler, B. C. Oligodeoxynucleotide Synthesis: H-Phosphonate Approach. In *Protocols for Oligonucleotides and Analogs*; Agrawal, S., Ed.; Humana Press: Totowa, NJ, 1993; Vol. 20; pp. 63–80.
3. Huss, S.; Gosselin, G.; Stawinski, J.; Strömberg, R.; Imbach, J.-L. *Nucleosides and Nucleotides* **1988**, *7*, 321–337.
4. Brill, W. K.-D. *Tetrahedron Lett.* **1994**, *35*, 3041–3044.
5. Song, Q.; Wang, W.; Fischer, A.; Zhang, X.; Gaffney, B. L.; Jones, R. A. *Tetrahedron Lett.* **1999**, *40*, 4153–4156.
6. Reese, C. B.; Visintin, C. *Tetrahedron Lett.* **1999**, *40*, 6477–6480.
7. The general procedure was: To 1.0 mmol of **1** in 9 mL of acetonitrile was added 10 mmol of tetrazole and 1.0 mL of water. After 5 min, toluene (50 mL) was added, and the mixture was washed with phosphate buffer (25 mL×3, 0.5 M, pH 7.0). To the organic layer was added glycerol (1.0 mL). The mixture was evaporated to a gum, pyridine (20 mL) was added, and the reaction mixture was maintained at room temperature for 1 day (DNA) or 2 days (RNA). Toluene (50 mL) was added and the mixture was washed with phosphate buffer (25 mL×2, 0.5 M, pH 7.0). The organic layer was dried (Na_2SO_4) and evaporated. The residue was purified by flash chromatography on silica gel (ethyl acetate:dichloromethane (0:100–40:60 v/v) for RNA products, methanol:dichloromethane (0:100–5:95 v/v) for DNA products), to give the nucleosides **4** as pure colorless solids in yields of 85 to 95%.
8. The general procedure for deoxynucleosides was: To 0.5 mmol of **1** in 6 mL dry acetonitrile was added 5 mmol of tetrazole. The mixture was shaken for 5 min, and then injected by syringe into a solution of 0.5 mL glycerol or ethylene glycol in 4 mL of dry acetonitrile with stirring, making a final 5% alcohol solution (v/v). After 5 min, 0.5 mL of pyridine was added, and the reaction solution was stirred for 8 h. Dichloromethane (25 mL) was added, and the mixture was washed twice with 25 mL of phosphate buffer (0.5 M, pH 7.0). The organic layer was evaporated, and the residue was purified by flash chromatography on silica gel using methanol:dichloromethane (gradient: 0:100 to 5:95), to give the deoxynucleosides **4** as pure colorless solids in the same high yields as the two-step procedure.