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SYNTHESIS OF A NUCLEOSIDE HAPTEN WITH A [P(O)-O-N] LINKAGE TO ELICIT CATALYTIC ANTIBODIES WITH PHOSPHODIESTERASE ACTIVITY

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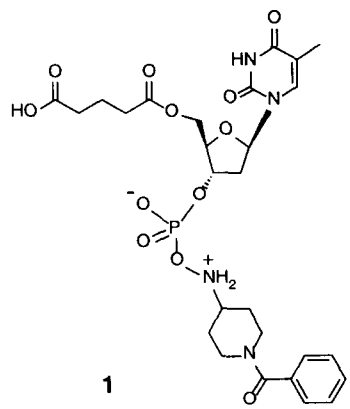
Abstract: The nucleoside analogue **1** incorporating a [P(O)-O-N] linkage was synthesized as a hapten to obtain catalytic antibodies that may cleave a phosphodiester bond. Secondary alcohol **15** was successfully phosphorylated with the phosphodiester derived from hydroxylamine derivative **7b** to give a protected form of the hapten. Deprotection was accomplished using Pd-catalyzed deallylation that afforded hapten **1** after HPLC purification. Copyright © 1996 Elsevier Science Ltd

The phosphodiester bond in DNA and RNA is one of the most stable chemical linkages with regard to hydrolysis. For example, the half-lives of DNA and RNA at pH 7 and 25 °C were estimated to be 200 million and 800 years, respectively.[1] Clearly, cleavage of a phosphodiester bond is a challenging target for catalytic antibody studies. An approach to this problem has already been investigated in our laboratory through the design and synthesis of a metal-chelated transition-state analogue for RNA hydrolysis.[2] We used a technetium-99 complex that was representative of the 2',3'-pentacordinate, trigonal-bipyramidal phosphorane.[3]



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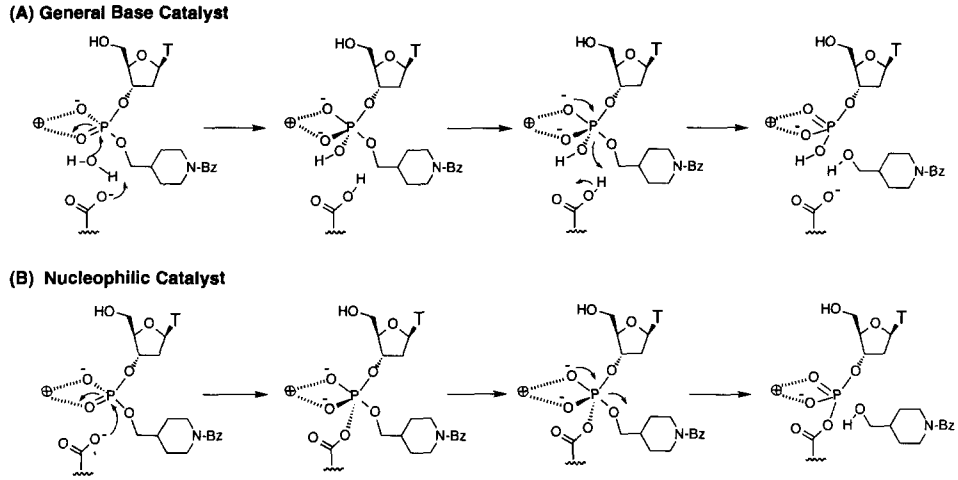
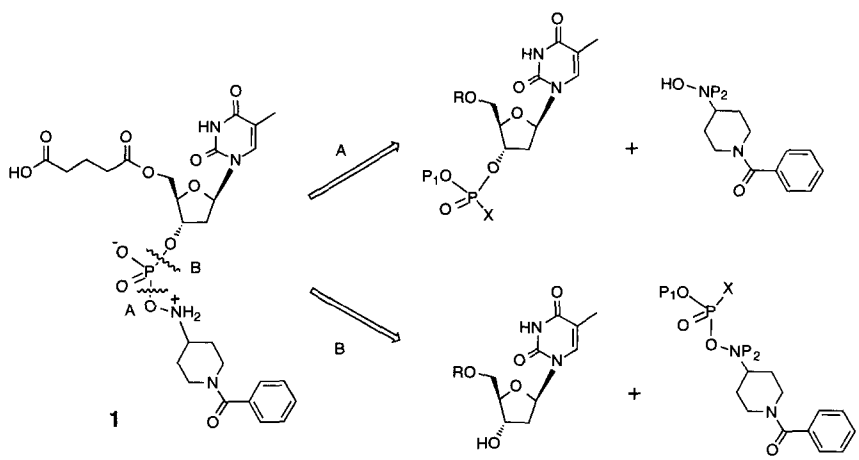
Here, we attempt to model a mechanism that would not be metal-dependent and would be applicable to both DNA and RNA hydrolyses. To this end, a nucleoside analogue was designed containing an *O*-phosphorylated hydroxylamine moiety [P(O)-O-N] as a new 3',5'-phosphodiester-bond replacement. This mimetic possesses several important features: (1) The geometry and spatial constraints are similar to the natural phosphate linkage; (2) The stereoelectronics of the phosphorus atom are retained; (3) A nitrogen atom is substituted for the 5'-methylene carbon. This amine is expected to exist predominantly as the positive ammonium species under physiological conditions. The [P(O)-O-N] functionality was incorporated into the hapten structure **1** as a simplified version of a dinucleotide in our initial work. This would allow a more unequivocal assessment of low antibody activities that might otherwise be ascribed to contaminating nucleases.

Scheme 1



The mechanisms of antibody-catalyzed phosphodiester bond cleavage that might be anticipated are shown in Scheme 1. The retention of a phosphate backbone in the hapten will allow formation of an oxyanion hole to increase the electrophilicity of the phosphorus center in corresponding substrates. Furthermore, a negatively charged amino acid residue, most likely aspartate or glutamate, should be induced at the antibody combining site complementary to the positive charge of the hapten.[4] This group should be in close proximity to the site of reaction and might participate either as a general base to activate attack of water (A) or as a nucleophile in the direct addition to phosphorus (B). The phosphocarboxy anhydride intermediate would then undergo hydrolytic breakdown.

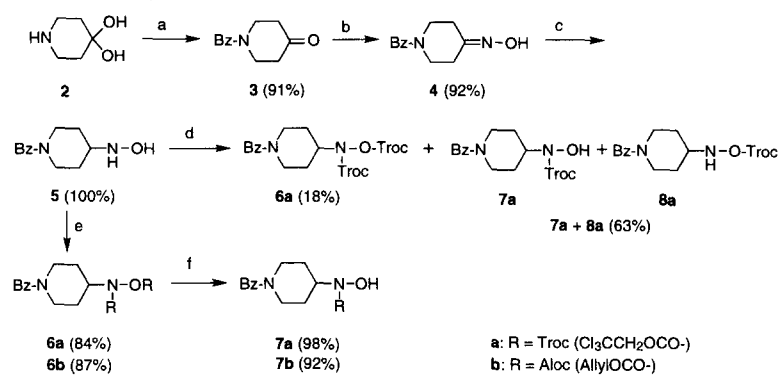
Scheme 2



No previous report of the [P(O)-O-N] linkage in nucleoside chemistry was found. Its description has been limited to a study of phosphotriesters as a radical precursor,[5a] an intermediate in the oxidative reaction of hexamethylphosphoramide (HMPA),[5b] and as a substrate for the lithium-ammonia reduction of the N-O bond.[5c] Our retrosynthetic analyses for the preparation of **1** are shown in Scheme 2. Pathway A utilizes an *N*-protected hydroxylamine that is phosphorylated with a 3'-phosphoryl-5'-protected thymidine. In pathway B, a 5'-protected thymidine is reacted with an *N*-protected-*O*-phosphoryl hydroxylamine derivative. In either case, post-coupling deprotection would afford the desired hapten.

Two protected hydroxylamines were prepared (Scheme 3). The protecting groups 2,2,2-trichloroethoxycarbonyl (Troc)[6] and allyloxycarbonyl (Aloc)[7] were expected to be good candidates when considering the reactivity and stability of [P(O)-O-N]. For instance, benzyl protecting groups used in model compounds like **7a** and **7b** were inappropriate for nitrogen protection because N-O bond cleavage occurred upon hydrogenation (Pd/C, H_2). Hence, 4-piperidinone monohydrate **2** was benzoylated to give compound **3** and transformed into oxime **4** in good yield. The oxime was reduced to **5** with $NaBH_3CN$ in AcOH.[8] The hydroxylamine **5** was reacted with 1.1 equiv of Troc-Cl in an aqueous solvent. This resulted in the nonselective formation of *N,O*-diprotected compound **6a**, along with an inseparable mixture of the desired *N*-protected hydroxylamine **7a** and *O*-protected compound **8a**. Consequently, an alternative approach that used 2.2 equiv of Troc-Cl provided **6a** in good yield and this compound was then selectively hydrolyzed and gave **7a**. In the same way, compound **7b** was obtained with Aloc-Cl instead of Troc-Cl.

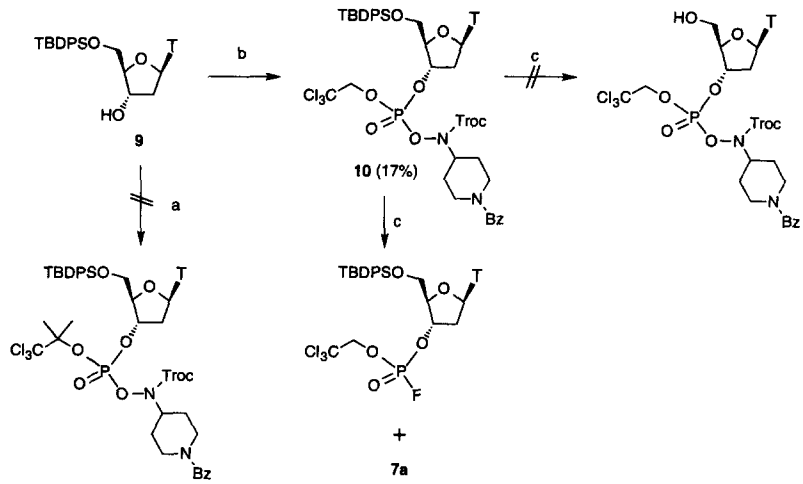
Scheme 3



Reagents and Conditiions: (a) Bz-Cl, Et_3N, THF; (b) $HCl{\bullet}NH_2OH$, Et_3N, Mol. Sieves, THF; (c) $NaBH_3CN$, AcOH; (d) 1.1 equiv Troc-Cl, Et_3N, dioxane-H_2O; (e) 2.2 equiv R-Cl, Et_3N, THF; (f) LiOH, MeOH.

Pathway A (Scheme 2) was investigated using commercially available phosphorus reagents to couple compounds **9** and **7a** (Scheme 4). First, phosphitylation of **9** with 2,2,2-trichloro-1,1-dimethyl dichlorophosphite[9] at -78 °C, reaction with **7a**, and oxidation with aqueous I_2 did not afford the desired compound. The major product was the phosphodiester derived from alcohol **9** perhaps because of the decreased reactivity of **7a** at the low reaction temperature necessary to avoid the formation of the symmetric phosphate. Next, reaction of **9** with 2.5 equiv of 2,2,2-trichloroethylphosphorodichloridate at -20 °C gave

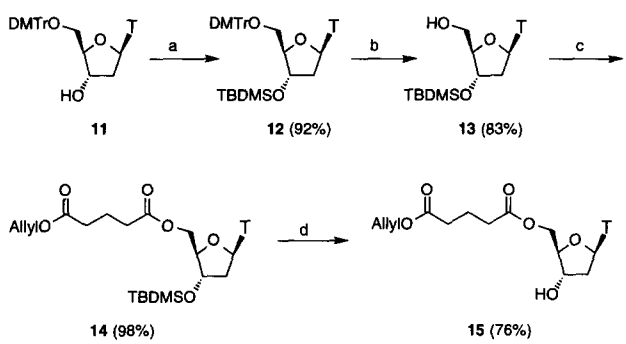
Scheme 4



Reagents and Conditions: (a) i. 2,2,2-Trichloro-1,1-dimethyl dichlorophosphite, Pyridine, ii. **7a**, Et_3N, iii. aq. I_2, THF; (b) i. 2,2,2-Trichloroethyl phophorodichloridate, Pyridine, ii. **7a**, Et_3N; (c) Tetrabutylammonium fluoride, THF.

compound **10**, but in poor yield (17%). This compound was stable at room temperature and could be purified with silica-gel column chromatography. However, the next transformation forced us to abandon this route. It was found that fluoride ion intended for the deprotection of the *tert*-butyldiphenylsilyl (TBDPS) group at the 5'-alcohol attacked the phosphorus atom to give a mixture of **7a** and a phosphorylfluoride that ultimately hydrolyzed. While this might seem unexpected, in retrospect it could be rationalized in view of hard-soft acid-base theory.[10] Apparently, the hard fluoride ion prefers bonding to the harder phosphorus that also has the hydroxylamine moiety as a good leaving group.

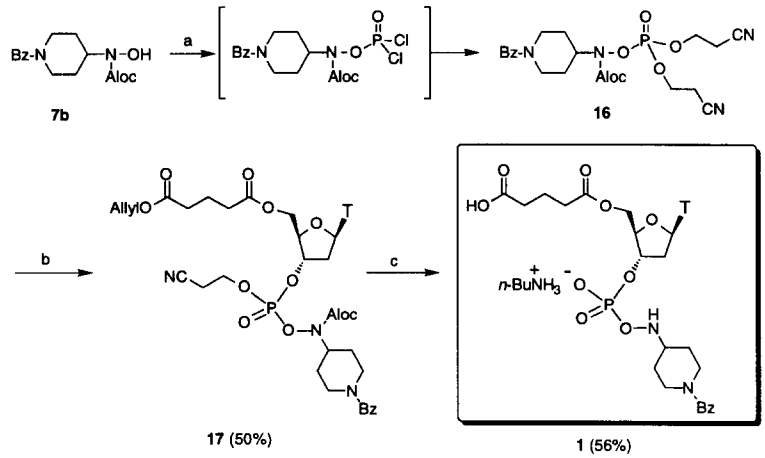
Scheme 5



Reagents and Conditions: (a) TBDMS-Cl, Imidazole, DMF; (b) 3% TFA in CH_2Cl_2; (c) Glutaric acid monoallylester, EDC, DMAP, CH_2Cl_2; (d) TBAF, THF.

To avoid using fluoride for deprotection after the coupling step, the requisite linker group protected as an allyl ester was installed (Scheme 5). After the 3'-hydroxyl group of commercially available 5'-*O*-(4,4'-dimethoxytrityl)thymidine **11** was protected with *tert*-butyldimethylsilyl chloride (TBDMS-Cl), glutaric acid monoallylester was coupled with alcohol **13** using 1-(3'-dimethylaminopropyl)-3-ethylcarbodiimide (EDC) and 4-dimethylaminopyridine (DMAP). Subsequently, the TBDMS group of compound **14** was removed and gave the nucleoside **15** for entry into pathway B (Scheme 2).

Scheme 6



Reagents and Conditions: (a) i. $POCl_3$, Pyridine, THF, ii. Cyanoethanol, THF; (b) i . Et_3N, Pyridine, ii. HCl, iii. **15**, TIPBSNT, Pyridine; (c) i. Et_3N, Pyridine, ii. HCl, iii. $Pd(PPh_3)_4$, PPh_3, HCO_2H, *n*-$BuNH_2$, CH_2Cl_2.

After treatment of compound **7b** with 4 equiv of $POCl_3$ at -30 °C, a large excess of cyanoethyl alcohol was added into the reaction mixture that resulted in compound **16** contaminated with a small amount of tricyanoethylphosphate (Scheme 6). Notably, compound **16** could not be formed if the reaction with $POCl_3$ was conducted at 0 °C. Although this was not fully explored, an intramolecular reaction might occur between the phosphoryl dichloride and the oxygen of the Aloc group. One of the cyanoethyl groups of compound **16** and the tricyanoethylphosphate by-product was deprotected with triethylamine in pyridine at room temperature.[11] The undesired dicyanoethylphosphate was eliminated after the aqueous workup. The secondary alcohol **15** was then phosphorylated with the phosphodiester obtained from **16** in the presence of 1-(2',4',6'-triisopropylbenzenesulfonyl)-3-nitro-1,2,4-triazole (TIPBSNT)[12] at room temperature. The yield of **17** was 50% from compound **7a**. Finally, deprotection of the cyanoethyl group (Et_3N in pyridine) followed by removal of the two allyl-based protecting groups ($Pd(PPh_3)_4$, PPh_3, *n*-$BuNH_2$, and HCO_2H in CH_2Cl_2) gave the hapten **1** as the butylamine salt after HPLC purification (ODS column (ϕ 4.6 x 250 mm); CH_3CN-H_2O mobile phase; gradient, 5% CH_3CN to 41% CH_3CN, 12 min; flow rate, 1 mL/min; t_R = 8.9 min). The order of the deprotections was very important in that initial cleavage of the allyl groups from **17** gave an unidentified mixture. Also, an HPLC mobile phase component of 0.1% TFA in H_2O was not used since this resulted in slow decomposition of **1**. The hapten was very stable in 10 mM phosphate buffered

saline, pH 7.4, 25 °C with less than 2% decomposition detected after two days. Consequently, the hapten was conjugated with keyhole limpet hemocyanin (KLH) for the immunization of mice and production of antibodies.

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References

1 (a) Chin, J.; Banaszczyk, M.; Jubian, V.; Zou, X. *J. Am. Chem. Soc.* **1989**, *111*, 186. (b) Komiyama, M.; Matsumura, K.; Matsumoto, Y. *J. Chem. Soc. Chem. Commun.* **1992**, 640.
2 Chen, Y.-C. J.; Janda, K. D. *J. Am. Chem. Soc.* **1992**, *114*, 1488.
3 (a) Saenger, W.; Suck, D.; Eckstein, F. *Eur. J. Biochem.* **1974**, *46*, 559. (b) Usher, D.A.; Erenrich, E. S.; Eckstein, F. *Proc. Natl. Acad. Sci. U.S.A.* **1972**, *69*, 115.
4 Lavey, B. J.; Janda, K. D. *Antibody Expression and Engineering*, ACS Symposium Series 604, Wang, H. Y.; Imanaka, T., eds.; , chap. 10, 1995.
5 (a) Negareche, M.; Boyer, M.; Tordo, P. *Tetrahedron Lett.* **1981**, *22*, 2879. (b) Holden, I.; Segall, Y.; Kimmel, E. C.; Casida, J. E. *Tetrahedron Lett.* **1982**, *23*, 5107. (c) Chang, Z.-Y.; Coates, R. M. *J. Org. Chem.* **1990**, *55*, 3464.
6 Windholz, T. B.; Johnston, B. R. *Tetrahedron Lett.* **1967**, 2555.
7 (a) Corey, E. J.; Suggs, J.W. *J. Org. Chem.* **1973**, *38*, 3223. (b) Hayakawa, Y.; Uchiyama, M.; Kato, H.; Noyori, R. *Tetrahedron Lett.* **1985**, *26*, 6505. (c) Hayakawa, Y.; Kato, H.; Uchiyama, M.; Kajino, H.; Noyori, R. *J. Org. Chem.* **1986**, *51*, 2400.
8 Gribble, G. W.; Leuby, R. W.; Sheehan, M. N. *Synthesis* **1977**, 856.
9 Letsinger, R. L.; Groody, E. P.; Tanaka, T. *J. Am. Chem. Soc.* **1982**, *104*, 6805.
10 (a) Pearson, R. G. In *Hard and Soft Acids and Bases*; Dowden, Hutchinson and Ross: Stroudsberg, 1973. (b) T.-L., Ho. *Chem. Rev.* **1975**, *75*, 1.
11 Adamiak, R. W.; Barciszewska, M .Z.; Biala, E.; Grzéskowiak, K.; Kierzek, R.; Kraszewski, A.; Markiewicz, W. T.; Wiewiórowski, M. *Nucleic Acid Res.* **1976**, *3*, 3379.
12 Uklman, E.; Pfleiderer, W. *Tetrahedron Lett.* **1980**, *21*, 1181.

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