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Synthesis of a Tetracyclic 2'-Deoxyadenosine Analog

Chris A. Buhr*, Mark D. Matteucci, and Brian C. Froehler

Gilead Sciences Inc.
333 Lakeside Drive, Foster City, CA 94404

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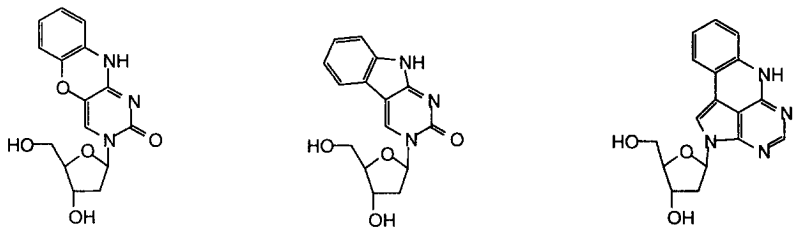
Abstract

A tetracyclic 2'-deoxyadenosine has been synthesized via a Stille biaryl coupling on a protected 6-chloro-7-deaza-7-iodopurine-2'-deoxyribose followed by cyclization. The nucleoside was incorporated into an oligodeoxynucleotide (ODN) and shown to pair with thymine.

Keywords: tetracyclic 2'-deoxyadenosine, 7-deaza-2'-deoxyadenosine

Replacement of a single 2'-deoxycytidine in an oligodeoxynucleotide (ODN) with either of the tricyclic 2'-deoxycytidine analogs **1** or **2** (Figure 1) has been shown to increase the affinity of the ODN towards its complementary RNA.[1] The tetracyclic adenosine analog **3** could be expected to enhance hybridization in an analogous fashion upon incorporation into an ODN. We report the synthesis of the novel adenosine analog **3**, as well as the protected hydrogen phosphonate **7**.



1 **2** **3**

Figure 1.

The synthesis of **3** begins with 6-chloro-7-deaza7-iodopurine-2'-deoxyribose **4**,[2,3] and is depicted in Scheme 1. Stille coupling[4] of the iodonucleoside **4**[2] and N-(tert-butoxycarbonyl)-2-(trimethylstannyl)aniline[5] with bis(triphenylphosphine)palladium(II) chloride afforded biaryl nucleoside **5**. Treatment of **5** with 1,4-diazabicyclo[2.2.2]octane (Dabco)[6] and 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) formed the tetracyclic skeleton, presumably via activation of the chloro group of **5** followed by cyclization. Some of the Boc protecting group was removed during this step. Therefore the mixture of products was treated with trifluoroacetic acid (TFA) affording **6** in 96% yield from **5**. Removal of the toluoyl groups with NaOMe afforded the parent nucleoside **3**.[7] Standard dimethoxy-tritylation and phosphitylation[8] of **3** afforded the hydrogen phosphonate **7**.[9]

Incorporation of the tricyclic phenoxazine **1** has been shown to enhance cellular permeation of the oligonucleotides.[10] Similarly, incorporation of the lipophilic tetracycle **3** in combination with other lipophilic enhancements may render such ODN's permeable to cellular membranes. Thus, these ODN's may be useful as antisense reagents for in vitro and in vivo applications.[11]

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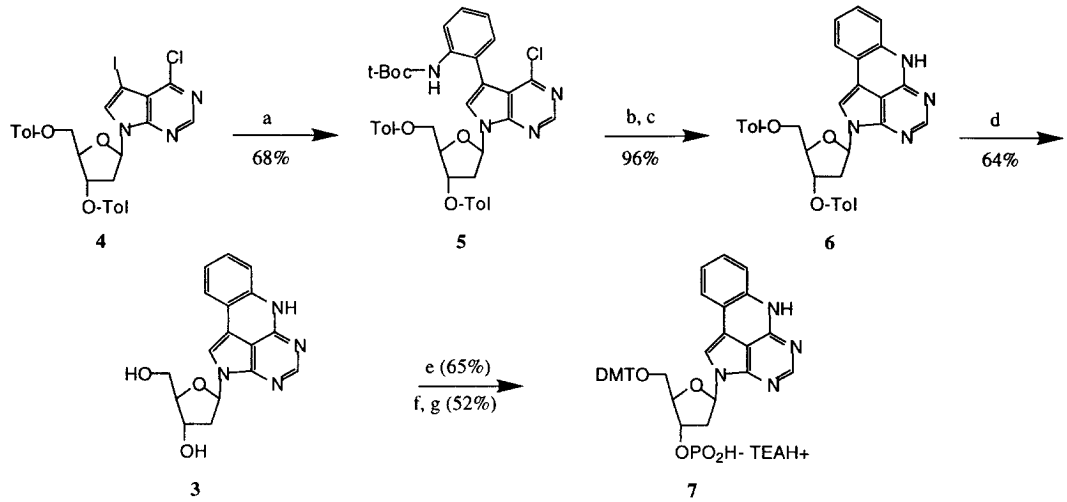
Scheme 1. Synthesis of Tetracyclic Nucleoside



4 5 6 3 7

Reaction conditions: (a) N-(tert-butoxycarbonyl)-2-(trimethylstannyl)aniline (4 eq.), $(Ph_3P)_2PdCl_2$ (0.1 eq.), DMF, 60 °C, 24 h; (b) Dabco (2 eq.), DBU (2 eq.), DMF, 75 °C, 21 h; (c) 25% TFA in CH_2Cl_2, 3 h, RT; (d) NaOMe, MeOH, RT; (e) DMT-Cl, pyridine; (f) 2-chloro-4H-1,3,2-benzodioxaphosphorin-4-one (10 eq.), pyridine, CH_2Cl_2, 0 °C to RT, 30 min.; (g) aq. triethylammonium bicarbonate.

References and Notes:

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6. A 1.5 M solution of Dabco in DMF was stored over activated 3Å molecular sieves. Aliquots of this solution containing the appropriate amounts of Dabco were added to the reaction.
7. Compound **3**: ^{1}H NMR (300 MHz, dmso-d_6) δ 10.69 (1H, s, NH), 8.13 (1H, s, H_2), 7.62 (1H, d, J = 7.4 Hz, aryl CH), 7.48 (1H, s, H_8), 7.25-7.15 (2H, m, aryl CH), 7.15-7.00 (1H, m, aryl CH), 6.40 (1H, t, J = 6.8 Hz, 1'-H), 5.33 (1H, d, J = 3.6 Hz, 3'-OH), 5.22 (1H, t, J = 5.5 Hz, 5'-OH), 4.43 (1H, s, 3'-H), 3.90 (1H, s, 4'-H), 3.72-3.51 (2H, m, 5'-CH_2), 2.76-2.63 (1H, m, 2'-H_a), 2.34-2.22 (1H, s, 2'-H_b). The UV properties of compound **3** in MeOH are: ε_{260} = 15,550 M^{-1} cm^{-1}; λ_{max} = 228 nm, ε_{228} = 23,540 $M^{-1}cm^{-1}$. FAB MS: m/z calculated for $C_{17}H_{17}N_4O_3$ (MH^+) 325.1300, found 325.1295.
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9. A single incorporation of **7** into a propyne containing phosphorothioate ODN was performed using the H-phosphonate method followed by S_8 oxidation.[8] All ODN's, except complementary ODN's, were 2'-deoxyphosphorothioates. Complementary and mismatch complementary ODN's were diesters. Thermal melting measurements showed that the tetracyclic adenosine **3** paired with thymidine. One incorporation of tetracyclic adenosine **3** did not significantly change the T_m relative to a control in this context. Incorporation of multiple adjacent tetracyclic adenosine **3** may have a positive cumulative effect. Similar observations have been made in the tricyclic cytidine series.[1a,c]
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