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## Synthesis of a Tetracyclic 2'-Deoxyadenosine Analog

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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. © 1999 Elsevier Science Ltd. All rights reserved.

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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.<sup>1</sup> 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.



The synthesis of 3 begins with 6-chloro-7-deaza7-iodopurine-2'-deoxyribose 4,<sup>2,3</sup> and is depicted in Scheme 1. Stille coupling<sup>4</sup> of the iodonucleoside  $4^2$  and N-(tert-butoxycarbonyl)-2-(trimethylstannyl)aniline<sup>5</sup> with bis(triphenylphosphine)palladium(II) chloride afforded biaryl nucleoside 5. Treatment of 5 with 1,4diazabicyclo[2.2.2]octane (Dabco)<sup>6</sup> 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.<sup>7</sup> Standard dimethoxy-tritylation and phosphitylation<sup>8</sup> of 3 afforded the hydrogen phosphonate 7.<sup>9</sup>

Incorporation of the tricyclic phenoxazine 1 has been shown to enhance cellular permeation of the oligonucleotides.<sup>10</sup> 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.<sup>11</sup>





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<sub>2</sub>Cl<sub>2</sub>, 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<sub>2</sub>Cl<sub>2</sub>, 0 °C to RT, 30 min.; (g) aq. triethylammonium bicarbonate.

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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: <sup>1</sup>H NMR (300 MHz, dmso-d<sub>6</sub>)  $\delta$  10.69 (1H, s, NH), 8.13 (1H, s, H<sub>2</sub>), 7.62 (1H, d, J = 7.4 Hz, aryl CH), 7.48 (1H, s, H<sub>8</sub>), 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<sub>2</sub>), 2.76-2.63 (1H, m, 2'-H<sub>8</sub>), 2.34-2.22 (1H, s, 2'-H<sub>6</sub>). The UV properties of compound 3 in MeOH are:  $\epsilon_{260}$  = 15,550 M<sup>-1</sup> cm<sup>-1</sup>;  $\lambda_{max}$  = 228 nm,  $\epsilon_{228}$  = 23,540 M<sup>-1</sup> cm<sup>-1</sup>. FAB MS: m/z calculated for C<sub>17</sub>H<sub>17</sub>N<sub>4</sub>O<sub>3</sub> (MH<sup>+</sup>) 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.<sup>8</sup> 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.<sup>1ac</sup>
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