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Increase of the Adenallene Anti-HIV Activity in Cell Culture Using Its Bis(*t*BuSATE) Phosphotriester Derivative

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Abstract—The bis(*S*-pivaloyl-2-thioethyl) phosphotriester derivative of 9-(4′-hydroxy-1′,2′-butadienyl)adenine (adenallene) was synthesized. This mononucleotide prodrug proved to be more effective than the parent nucleoside in inhibiting HIV-1 replication in several human T4 lymphoblastoid cell lines.

Allenic nucleoside analogues[1] represent a class of unsaturated acyclic nucleoside derivatives where the ribofuranose part is replaced by a four-carbon chain with two cumulated double bonds at the 1′ and 2′ positions. In this class of nucleoside analogues, adenallene [9-(4′-hydroxy-1′,2′-butadienyl)adenine] (Fig. 1) was found to inhibit in vitro replication and cytopathic effect of human immunodeficiency viruses HIV-1 and HIV-2 with an antiretroviral effect similar to the corresponding 2′,3′-dideoxyribonucleoside[2] (i.e., 2′,3′-dideoxyadenosine, ddA). One strategy to enhance the antiviral potency of a nucleoside analogue is to increase the intracellular availability of its 5′-monophosphate,[3–5] a metabolite required for subsequent phosphorylation to the bioactive triphosphate derivative. Thus, we previously demonstrated that the use of mononucleoside phosphotriesters bearing *S*-acyl-2-thioethyl (SATE) groups as biolabile phosphate protections leads to the selective intracellular release of the corresponding 5′-mononucleotide in infected cells.[6,7] Herein we would like to report the synthesis and the in vitro anti-HIV evaluation of the phosphotriester derivative of the racemic (±)-adenallene incorporating the *S*-pivaloyl-2-thioethyl (*t*BuSATE, Fig. 1) as protecting group.

The bis(*t*BuSATE) phosphotriester of (±)-adenallene was prepared according to a published procedure[8] involving the reaction of adenallene[9] with the appropriate phosphoramidite reagent followed by in situ oxidation. The target compound was obtained in 70% yield and was characterised by high-field multinuclear NMR spectroscopy, FAB mass spectrometry, UV and HPLC analysis, all these data being consistent with its structure and purity.[10]

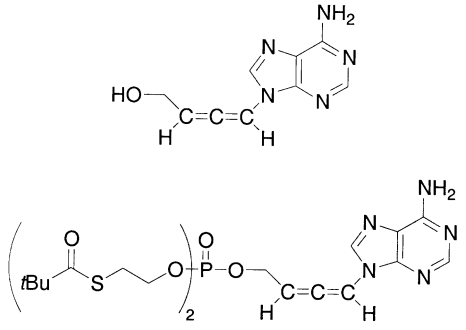

Figure 1. Adenallene and its bis(*t*BuSATE) phosphotriester derivative.

The bis(*t*BuSATE) phosphotriester of (±)-adenallene was evaluated, in comparison to the parent nucleoside, for its inhibitory effects on the replication of HIV-1 in several human T4 lymphoblastoid cell lines (Table 1).

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Table 1. Anti-HIV activity of the bis(*t*BuSATE) phosphotriester of (±)-adenallene compared to the parent nucleoside

Compd	CEM-SS		MT-4		MT-2	
	EC_{50} (μM)[a]	CC_{50} (μM)[b]	EC_{50} (μM)[a]	CC_{50} (μM)[b]	EC_{50} (μM)[a]	CC_{50} (μM)[b]
bis(*t*BuSATE) Phosphotriester of (±) Adenallene	1.2	>10	8.9	>10	6.4	28
(±) Adenallene	56	>100	>100	>100	>100	>100

[a]EC_{50}, effective concentration.
[b]CC_{50}, cytotoxic concentration.

This mononucleotide prodrug significantly inhibits the multiplication of HIV-1 with micromolar EC_{50} values lower to those observed for (±)-adenallene. This result confirms data obtained with the use of another series of mononucleotide prodrug[11] and clearly supports the hypothesis that phosphorylated forms of adenallene should be involved in its antiviral mode of action. Finally, the increased anti-HIV activity of the bis (SATE) phosphotriester derivative is also associated with concomitant cytotoxicity. This toxicity may be related to intracellular accumulation of the phosphorylated forms of adenallene which could also interact with cellular enzymes.[11]

In conclusion, the present results confirm that the first metabolisation step of adenallene plays a pivotal role in its antiviral effect. The use of the corresponding bis(-SATE) prodrug leads in vitro to enhance the anti-HIV activity as well as the cytotoxicity raising the importance of the selectivity of the resulting 5′-triphosphate for the HIV reverse transcriptase versus human host polymerases.

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10. Selected data for bis(*t*BuSATE) phosphotriester of (±)-adenallene: δ_P (DMSO-d_6) −0.6, δ_H (DMSO-d_6) 8.21, 8.16 (2H, 2s, H_2 and H_8), 7.58 (1H, m, $H_{1'}$), 7.4 (2H, bs, NH_2), 6.33 (1H, q, $H_{3'}$), 4.69 (2H, m, $H_{4'}$), 4.02 (4H, m, CH_2O), 3.07 (4H, m, CH_2S), 1.13, 1.12 (18H, 2s, *t*Bu), δ_C (DMSO-d_6) 205.9, 205.8 (2s, CO), 197.8 ($C_{2'}$), 156.9 (C_6), 153.9 (C_2), 149.2 (C_4), 139.2 (C_8), 119.7 (C_5), 101.7 (d, $J_{P\text{-}C}=6.1$ Hz, $C_{3'}$), 95.8 ($C_{1'}$), 66.6–66.5 (m, CH_2O), 65.0 (d, $J_{P\text{-}C}=5.4$ Hz, $C_{4'}$), 46.8 ($C(CH_3)_3$), 29.0–28.9 (m, CH_2S), 27.7 ($C(CH_3)_3$), MS (glycerol–thioglycerol matrix) FAB>0 *m/e* 572 $(M+H)^+$, 136 $(adenine+H)^+$, FAB<0 *m/e* 426 $(M-t\text{BuSATE})^-$, 385 $(M-adenallene)^-$, 134 $(adenine\text{-}H)^-$, UV (EtOH) λ_{max} 260 nm (ε 14,000), 232 nm (ε 20,000), HPLC retention time 29.0 min. The column was a reverse-phase analytical column (Nucleosil, C_{18}, 150×4.6 mm, 5 μm) protected by a prefilter and a precolumn (Nucleosil, C_{18}, 5 μm). To be analysed, the compound was eluted using a linear gradient from 0 to 80% acetonitrile in 50 mM triethylammonium acetate buffer (pH 6.9) over a 40 min period at a flow rate of 1.00 mL/min.
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