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# Synthesis and Antiretroviral Evaluation of 3-Alkyl 2-Piperazinone Nucleoside Analogs

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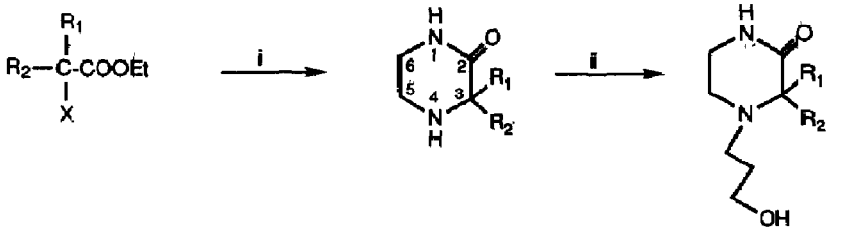
**Abstract:** Glycosylation of 3-alkyl $N^4$-(3-hydroxypropyl) 2-piperazinones by protected 1-*O*-acetyl ribofuranoses produces nucleoside analogs in which the base is separated from the sugar by a hydrocarbon spacer arm. The preliminary *in vitro* test results against retroviruses seem promising for compounds bearing a long alkyl chain.

As part of our research program on the synthesis of nucleoside analogs[1-3], we became interested in the preparation of such analogs derived from 2-piperazinone in which the "base" is linked to the ribose by an aliphatic chain. It is now known that an aliphatic chain not only serves as a neutral linkage but also affects the coiling of DNA. Increasing the length of the spacer arm linkage would thus enhance bending of DNA, leading to base pair opening where the bases then become susceptible to attack by reactive groups.[4] Spacer arms have also been used in oligomer nucleoside antisense synthesis for preventing the enzymatic degradation by nucleases.[5] Also, a hydrophobic tail was introduced on the base to improve cellular uptake.[6] We wish to report herein the synthesis and characterisation of a new class of nucleoside analogs, and preliminary results of their *in vitro* activity against sheep retroviruses. One of the representative compounds of this new series shows moderate activity. The synthetic route used in this work is illustrated in Schemes I and II.

Starting piperazinones **2a,b,c** were prepared by a modification of known methods.[7-9] Syntheses were carried out by the action of ethylenediamine (6 eq) on the corresponding ethyl α-halogenoalkanoate **1a,b,c** followed by neutralization and then cyclisation. After the usual work-up, the expected compounds were obtained with 50, 62 and 80% yields, respectively. By refluxing **2a,b,c** in dry ethanol with 3-bromopropanol (1 eq) and a catalytic amount of KI for 24 h, **3a,b,c** were obtained after recrystallization in EtOH in acceptable yields.[10] The N-4 linkage between *n*-propanol and piperazinone in compounds **3a,b,c** was shown in the corresponding $^1$H NMR spectra by both the disappearance of the amine proton previously recorded at δ 1.82 for **2a**, δ 1.68 for **2b** and δ 1.61 for **2c** as well as the continuous presence of the amide proton at δ 6.10 for **3a**, δ 6.18 for **3b** and δ 6.21 for **3c**.

Glycosylation of **3a,b,c** was performed according to the method of Hannessian.[11] Use of dichloroethane as solvent even in the presence of a large excess of $SnCl_4$ resulted in poor yields (15%) probably due to the precipitation of a complex formed from the base and $SnCl_4$. Inversely, in acetonitrile , higher yields were obtained, as no precipitation occurred in the presence of excess $SnCl_4$. In a typical experiment, under argon, 2 mmol of $SnCl_4$ were added to a solution of 1-*O*-acetyl 2,3,5-tri-*O*-benzoyl-β-D ribofuranose (1 mmol) in dry acetonitrile. After 15 min, 1 eq of each aglycon **3a,b,c** was introduced in the medium and the solution was stirred for 3h at room temperature. The resulting solution was then neutralized by $NaHCO_3$ and extracted with

chloroform. After separation by column chromatography on silica gel (eluent $CHCl_3$-EtOH = 99:1) compounds **4a,b,c** were obtained with 50, 45 and 41% overall yields respectively.[12] Compound **4c** gave a chromatographically unseparable mixture of diastereoismers due to the asymmetrical C-3 atom. Subsequent attempts to separate the diastereoisomers by various chromatographic means remain presently unsuccessful.

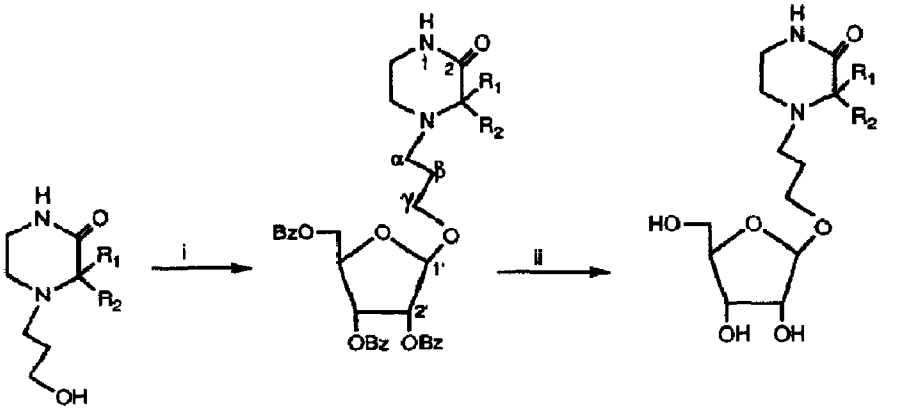


**1a,b,c** **2a,b,c** **3a,b,c**

**a** X = Cl, $R_1 = R_2 = H$
**b** X = Br, $R_1 = R_2 = CH_3$
**c** X = Br, $R_1 = H$, $R_2 = n\text{-}C_{10}H_{21}$

i) $H_2N(CH_2)_2NH_2$ ; KOH ; in refluxing EtOH for **2a** and **2c**, toluene for **2b**.
ii) $Br(CH_2)_3OH$ ; KI ; in refluxing EtOH.

**Scheme I**



**3a,b,c** **4a,b,c** **5a,b,c**

**a** $R_1 = R_2 = H$
**b** $R_1 = R_2 = CH_3$
**c** $R_1 = H$, $R_2 = n\text{-}C_{10}H_{21}$

i) 1-*O*-acetyl 2,3,5-tri-*O*-benzoyl β-D ribofuranose ; $SnCl_4$ ; MeCN ; r.t.
ii) $NH_3$ / MeOH ; r.t.

**Scheme II**

For all new compounds **4a,b,c** satisfactory $^{1}H$, $^{13}C$ NMR and mass spectra data were obtained on chromatographically homogeneous samples. As an example we have included the $^{1}H$ and $^{13}C$ NMR data for compound **4a**.[13] In all glycosides, the small coupling constant value ($J_{1',2'} < 1$ Hz) strongly indicates a β configuration of the ribofuranose moiety; this is also confirmed by the $^{13}C$ NMR data relative to the anomeric carbon (δ 105.5-105.7).

Treatment of **4a,b,c** with ammonia in methanol at room temperature for one week gave deprotected products **5a,b,c** in good yields.[14,15]

Compounds **2b, 3b, 5b** and **5c** were evaluated for their antiviral activities against a mammalian retrovirus, the Visna virus, strain K796, in sheep choroïd plexus cells (SCP cells). AZT, the most potent inhibitor of this virus was also tested in order to compare the effectiveness of the newly synthesized compounds. Antiviral activities were measured by a modified MTT[16] method applied to adherent cells.[17] SCP cells were infected with Visna virus at a MOI of 0.5 and incubated in the presence of the tested compound. After 5 days incubation, the number of viable cells was determined. The results are shown in Table 1.

| Compounds | EC50 (μM)[a] | CC50 (μM)[b] |
|---|---|---|
| **2b** | - | >700 |
| **3b** | - | >400 |
| **5a** | - | >500 |
| **5b** | - | >800 |
| **5c** | 80 | 170 |
| AZT | 5 | >500 |

[a]EC50: 50% antiviral effective concentration.

[b]CC50: 50% cytotoxic concentration.

**TABLE 1**

As shown in Table 1, among the compounds tested, piperazinone **2b** and the intermediate **3b** showed no antiviral activities. Final piperazinone nucleosides modified with a spacer arm exhibited different results according to $R_1$ and $R_2$. Only compound **5c** with an aliphatic long chain had moderate antiviral activity; however, this molecule was cytotoxic at a concentration greater than 170 μM. No comparable inhibition such as that observed with AZT was obtained. An extensive evaluation is in progress and complete results will be published in due course.

In summary, we have successfully synthesized and tested new nucleosides with a spacer arm linking the sugar and the base. New compounds in this series are currently under investigation in our laboratory.

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10. **3a :** yield 81%; syrup; IEMS *m/z :* 159 $[M+H]^+$, 113 $[M-(CH_2)_2OH]^+$; **3b:** yield 60%; mp 96-98°C; IEMS *m/z :* 186 $[M]^+$, 171 $[M-CH_3]^+$; **3c:** yield 52%; syrup; IEMS *m/z :* 298 $[M]^+$, 157 $[M-(C_{10}H_{21})]^+$.
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12. **4a :** syrup; $R_f$ = 0.32 ($CHCl_3$-EtOH = 95:5); $[\alpha]^{20}_D$ = +23.9° (c 1.96, $CHCl_3$); **4b :** syrup; $R_f$ = 0.41 ($CHCl_3$-EtOH = 95:5); $[\alpha]^{20}_D$ = +18.2° (c 1.24, $CHCl_3$); **4c :** syrup; $R_f$ = 0.48 ($CHCl_3$-EtOH = 95:5).
13. **4a :** $^1H$ NMR (200 MHz, $CDCl_3$, δ ppm) *base*: 3.09 (2H, s, H-3), 2.61 (2H, t, *J* = 6.1 Hz, H-5), 3.33 (2H, dt, *J* = 6.2, 2.2 Hz, H-6), 5.68 (1H, br s, NH); *arm:* 2.47 (2H, t, *J* = 6.9 Hz, α-H), 1.73 (2H, quintuplet, *J* = 6.6 Hz, β-H), 3.55 (1H, dt, *J* = 9.4, 6.2 Hz, $\gamma_a$-H), 3.84 (1H, dt, *J* = 9.6, 6.1 Hz, $\gamma_b$-H); *ose:* 5.25 (1H, br s, H-1'), 5.66 (1H, dd, *J* = 4.9, 0.6 Hz, H-2'), 5.85 (1H, dd, *J* = 6.5, 4.9 Hz, H-3'), 4.71 (2H, m, H-4', H-5'$_a$), 4.54 (1H, dd, *J* = 12.7, 6.7 Hz, H-5'$_b$); *benzoyl groups:* 7.90, 8.04, 8.08 (2H each, dd, *J* = 8.5, 1.4 Hz, H-2,6), 7.35 (6H, m, H-3,5), 7.53 (6H, m, H-4). $^{13}C$ NMR (50 MHz, $CDCl_3$, δ ppm) *base*: 169.4 (C-2), 49.1 (C-3), 54.0 (C-5), 56.7 (C-6); *arm:* 41.4 (C-α), 26.7 (C-β), 66.2 (C-γ); *ose:* 105.6 (C-1'), 75.5 (C-2'), 72.6 (C-3'), 78.9 (C-4'), 65.0 (C-5'); *benzoyl groups :* 129.0 (3C, C-1), 129.7 (6C, C-2,6), 128.4 (4C, C-3,5), 128.5 (2C, C-3,5), 133.2, 133.4 and 133.5 (1C each, C-4), 165.3, 165.4 and 166.1 (1C each, C-7).
14. **5a** : yield 85%; syrup; $R_f$ = 0.52 (MeOH - 30% $NH_4OH$ = 99 : 1); $[\alpha]^{20}_D$ = -30.2° (c 0.55, MeOH); **5b** : yield 75%; syrup; $R_f$ = 0.62 (MeOH - 30% $NH_4OH$ = 99 : 1); $[\alpha]^{20}_D$ = -21.1° (c 0.40, MeOH); **5c** : yield 72%; syrup; $R_f$ = 0.68 (MeOH - 30% $NH_4OH$ = 99 : 1).
15. Each reaction was followed by TLC on silica gel.
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