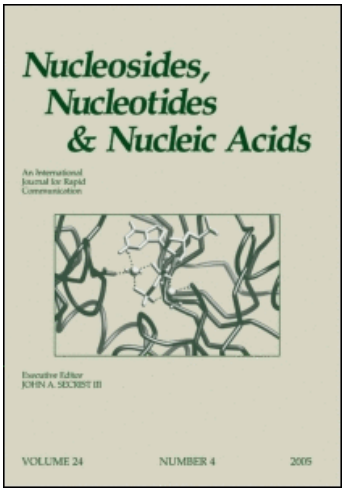

Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

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To cite this Article Ducho, C. , Jessel, S. , Gisch, N. , Balzarini, J. and Meier, C.(2005) 'Novel *Cyclo*sal Nucleotides with Reduced Inhibitory Potency Toward Human Butyrylcholinesterase', Nucleosides, Nucleotides and Nucleic Acids, 24: 5, 519 — 522
To link to this Article: DOI: 10.1081/NCN-200061791
URL: http://dx.doi.org/10.1081/NCN-200061791

Nucleosides, Nucleotides, and Nucleic Acids, 24 (5–7):519–522, (2005)

ISSN: 1525-7770 print/ 1532-2335 online
DOI: 10.1081/NCN-200061791

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NOVEL *CYCLO*SAL NUCLEOTIDES WITH REDUCED INHIBITORY POTENCY TOWARD HUMAN BUTYRYLCHOLINESTERASE

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▫ *Two novel cycloSal-d4T monophosphates (d4TMPs) with increased steric demand have been synthesized via a new synthetic route. While 3-cyclohexyl-cycloSal d4TMP did not show a significantly reduced inhibitory potency toward human butyrylcholinesterase, the opposite was the case for the second novel pronucleotide, bis-(cycloSal-d4TMP).*

Keywords Pronucleotides, *cyclo*Sal, Butyrylcholinesterase, Antiviral Activity

INTRODUCTION

The *cyclo*Sal pronucleotide system has been developed for an intracellular delivery of therapeutically active nucleoside monophosphates (NMPs) and has already been applied to different nucleoside analogues successfully, e.g., the anti-HIV active 3′-deoxy-2′,3′-didehydrothymidine (d4T) **1**.[1] Recently, the interaction of cholinesterases with *cyclo*Sal nucleotides has been reported. While no inhibition of the physiologically essential acetylcholinesterase (AChE, E.C. 3.1.1.7) has been observed,[2] a structure-activity relationship has been obtained for butyrylcholinesterase (BChE, E.C. 3.1.1.8).[3] As the inhibition of BChE is an unwanted effect and could become a hurdle in the application of *cyclo*Sal derivatives in antiviral chemotherapy, ways to overcome this effect are needed. One promising approach is based on the thought that bulky substituents in the aryl moiety of the *cyclo*Sal system could prevent the pronucleotides from binding in the active site of BChE due to steric repulsion. Hence, novel *cyclo*Sal nucleotides with increased steric demand were prepared. We already reported on 3,5-bis-*tert*butyl-6-fluoro-*cyclo*Sal-d4TMP **2**,

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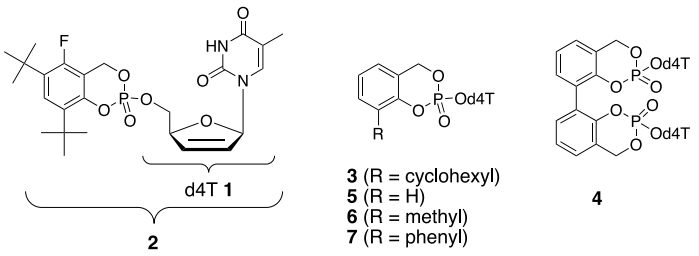
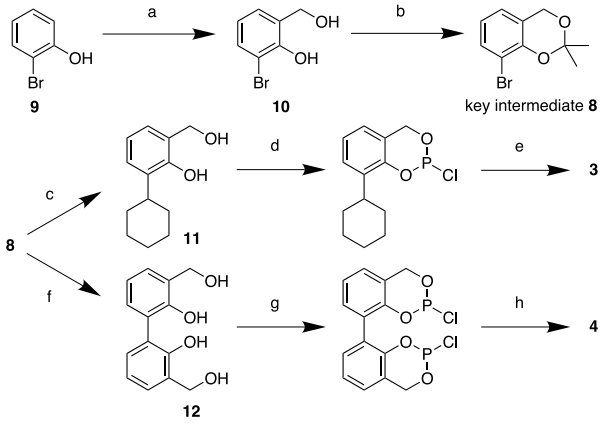


FIGURE 1 Compound **2**, a derivative of d4T **1**, the two target structures **3** and **4**, and the reference compounds ***5*–7**.

a pronucleotide with strongly reduced inhibitor activity toward human BChE.[4] Here, we present two novel *cyclo*Sal nucleotides, 3-cyclohexyl-*cyclo*Sal-d4TMP **3** and bis-(*cyclo*Sal-d4TMP) **4** and compare them to the known reference compounds **5**–**7**[1,3] (Figure 1).

RESULTS

For the synthesis of **3** and **4**, a new synthetic route *via* 3-bromo salicyl alcohol isopropylidene acetal **8** as the key intermediate has been established (Figure 2). Compound **8** was synthesized starting from 2-bromophenol **9**, which was converted to 3-bromo salicyl alcohol **10** in a two-step procedure with an overall yield of 53%. Alcohol **10** was protected as isopropylidene acetal to afford **8** in 99% yield. For the synthesis of **3**, intermediate **8** was reacted in a Grignard cross-



a i) HCHO, $PhB(OH)_2$, C_2H_5COOH, toluene, reflux, 18 h ii) H_2O_2/H_2O, THF, 0°C, 45 min., 53% (2 steps) **b** 2,2-dimethoxypropane, *p*-TsOH, Na_2SO_4, acetone, 40°C, 3 d, 99% **c** i) $C_6H_{11}MgBr$, $Pd(dppf)Cl_2$, Et_2O/THF, r.t., 16 h ii) TFA, DCM/MeOH 1:1, r.t., 11 d, 34% (2 steps) **d** PCl_3, pyridine, Et_2O, -20°C to r.t., 5 h, no purification **e** i) d4T, DIPEA, CH_3CN, -20°C to r.t., 1 h ii) *t*BuOOH, -20°C to r.t., 1 h, 30% (one pot) **f** i) *n*-BuLi, THF, -80°C, 1 h ii) $Fe(acac)_3$, -80°C to r.t., 17 h iii) Dowex 50 X 8, DCM/MeOH 1:1, r.t., 2 d, 24% (2 steps) **g** like d, but -40°C to r.t., no purification **h** like e, 8% (one pot).

FIGURE 2 Synthesis of target compounds **3** and **4**.

TABLE 1 Properties of Target Compounds **3**, **4** Compared to Reference *cyclo*Sal-d4TMPs

Compound	Hydrolysis half-life[a] (h)	BChE inhibition[b] (IC_{50}, μM)	Anti-HIV activity[c] (EC_{50}, μM)	
			CEM/O	CEM/TK$^-$
5	4.4	0.77	0.13	0.30
7	5.1	0.35	0.27	0.15
4 *mix*	8.2	40	0.27	2.3
4 *fast*	3.2	14	n.d.	n.d.
4 *slow*	8.5	>50	n.d.	n.d.
6	17	1.2	0.08	0.08
3	25	3.1	n.d.	n.d.
d4T **1**	–	–	0.19	22

[a]Hydrolytic stability in phosphate buffer, pH 7.3, 37°C.

[b]Cholinesterase assay using human serum as source of BChE activity, procedure as published before. (From Ref. [3].)

[c]Effective concentration to protect CEM wild-type or CEM thymidine kinase (TK)-deficient cells against the cytopathogenicity of HIV-2 by 50%; n.d. = not determined yet.

coupling reaction to yield, after deprotection, 3-cyclohexyl salicyl alcohol **11** (34% over two steps). Compound **11** was subsequently converted to target compound **3** (mixture of two diasteromers) using chlorophosphite chemistry as described before[1,3,4] (30% yield). For the synthesis of **4**, intermediate **8** was converted in a homo-coupling reaction. After deprotection, alcohol **12** was isolated in 24% yield. Again, target compound **4** could be obtained from **12** using the chlorophosphite approach. Due to difficult purification, **4** (mixture of three diastereomers) could only be isolated in 8% yield. Partial separation of the diastereomeric mixture of **4** was possible using preparative RP-HPLC to afford a *fast* fraction (one diastereomer) and a *slow* fraction (mixture of two diastereomers).

The *fast* and the *slow* fraction of **4** displayed significantly different hydrolytic stabilities (Table 1). While **4** *fast* ($t_{1/2}$ = 3.2 h) was less stable than the unsubstituted *cyclo*Sal-d4TMP **5** ($t_{1/2}$ = 4.4 h), **4** *slow* was about 2-fold more stable ($t_{1/2}$ = 8.5 h). Both the *fast* and the *slow* fraction showed a significantly reduced inhibitory potency toward human BChE as compared to phosphate triester **5**, *fast* about 18-fold, and *slow* more than 65-fold lower. In comparison to 3-phenyl-*cyclo*Sal-d4TMP **7**, this effect was even more pronounced with IC_{50} values about 40-fold lower for **4** *fast* and more than 140-fold for **4** *slow*. Consequently, **4** *slow* actually is a non-inhibitor of BChE. The diastereomeric mixture **4** *mix* displayed an anti-HIV activity in wild-type CEM cells comparable to that of d4T and the unsubstituted prototype, but in thymidine kinase (TK)-deficient CEM cells it turned out to lose activity about 9-fold in comparison to the data obtained in the wild-type cells. This points to an insufficient TK bypass, possibly a result of bad membrane penetration due to the highly increased size of the molecule and/or its polarity. On the other hand, the antiviral activity of dimer **4** in the CEM/TK$^-$ cells still was about 10-fold better than that of d4T **1**, which proves a partial intracellular delivery of d4TMP.

In contrast to **4**, derivative **3** only showed a 4-fold reduction of inhibitor activity toward BChE as compared to the unsubstituted prototype **5** and 2.5-fold as compared to the 3-methyl derivative **6**, while its hydrolysis half-life ($t_{1/2}$ = 25 h) is in the same range than that of 3-methyl-*cyclo*Sal-d4TMP **6** ($t_{1/2}$ = 17 h). On the other hand, **3** turned out to be about 9-fold less inhibitory to BChE than the structural similar 3-phenyl derivative **7**.

In conclusion, *bis-cyclo*Sal nucleotides like **4** may become an interesting new class of *cyclo*Sal prodrugs as they display significantly reduced inhibitory activity toward human BChE and have a mask–drug ratio of 1:2. Further work has to be done in order to optimize the antiviral properties of **4**, eventually by increasing its hydrolytic stability.

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