This article was downloaded by: [University of Chicago Library] On: 25 April 2013, At: 02:13 Publisher: Taylor & Francis Informa Ltd Registered in England and Wales Registered Number: 1072954 Registered office: Mortimer House, 37-41 Mortimer Street, London W1T 3JH, UK



### Nucleosides, Nucleotides and Nucleic Acids

Publication details, including instructions for authors and subscription information: <u>http://www.tandfonline.com/loi/lncn20</u>

## Second Generation of cycloSal-Pronucleotides with Esterase-Cleavable Sites: The "Lock-In"-Concept

Chris Meier<sup>a</sup>, Manuel F.H. Ruppel<sup>a</sup>, Dalibor Vukadinović<sup>a</sup> & Jan Balzarini<sup>b</sup> <sup>a</sup> Institute of Organic Chemistry, University of Hamburg, Martin-Luther-King-Platz 6, D-20146, Hamburg, Germany

<sup>b</sup> Rega-Institute for Medical Research, K.U. Leuven, Minderbroedersstraat 10, B-3000, Leuven, Belgium

Version of record first published: 01 Jun 2007.

To cite this article: Chris Meier , Manuel F.H. Ruppel , Dalibor Vukadinović & Jan Balzarini (2004): Second Generation of cycloSal-Pronucleotides with Esterase-Cleavable Sites: The "Lock-In"-Concept , Nucleosides, Nucleotides and Nucleic Acids, 23:1-2, 89-115

To link to this article: http://dx.doi.org/10.1081/NCN-120027820

### PLEASE SCROLL DOWN FOR ARTICLE

Full terms and conditions of use: http://www.tandfonline.com/page/terms-and-conditions

This article may be used for research, teaching, and private study purposes. Any substantial or systematic reproduction, redistribution, reselling, loan, sub-licensing, systematic supply, or distribution in any form to anyone is expressly forbidden.

The publisher does not give any warranty express or implied or make any representation that the contents will be complete or accurate or up to date. The accuracy of any instructions, formulae, and drug doses should be independently verified with primary sources. The publisher shall not be liable for any loss, actions, claims, proceedings, demand, or costs or damages whatsoever or howsoever caused arising directly or indirectly in connection with or arising out of the use of this material.

NUCLEOSIDES, NUCLEOTIDES & NUCLEIC ACIDS Vol. 23, Nos. 1 & 2, pp. 89–115, 2004

### Second Generation of *cyclo*Sal-Pronucleotides with Esterase-Cleavable Sites: The "Lock-In"-Concept<sup>†</sup>

### Chris Meier,<sup>1,\*</sup> Manuel F. H. Ruppel,<sup>1</sup> Dalibor Vukadinović,<sup>1</sup> and Jan Balzarini<sup>2</sup>

<sup>1</sup>Institute of Organic Chemistry, University of Hamburg, Hamburg, Germany <sup>2</sup>Rega-Institute for Medical Research, K.U. Leuven, Leuven, Belgium

#### ABSTRACT

A conceptual extension of the *cyclo*Sal-pronucleotide approach is presented. The characteristic feature of the new *cyclo*Sal-derivatives of the anti-HIV active nucleoside analogue d4T **1** is the incorporation of an enzymatically cleavable carboxylic ester moiety with the intention to trap the triesters inside cells ('lock-in''-concept). *Cyclo*Sal-triesters bearing different ester groups in the 3-or 5-position of the *cyclo*Sal-moiety are described. Surprisingly, only acetyl-and levulinyl esters are cleaved readily in CEM cell extracts while alkyl esters were found to be stable. Nevertheless, in in-vitro anti-HIV assays most of the compounds achieve the thymidine–kinase bypass, thus proving that they act at least as nucleotide delivery systems.

Key Words: Pronucleotides; Nucleoside analogue; Anti-HIV; CycloSal-triesters.

89

DOI: 10.1081/NCN-120027820 Copyright © 2004 by Marcel Dekker, Inc. 1525-7770 (Print); 1532-2335 (Online) www.dekker.com

<sup>&</sup>lt;sup>†</sup>In honor and celebration of the 70th birthday of Professor Leroy B. Townsend.

<sup>\*</sup>Correspondence: Chris Meier, Institute of Organic Chemistry, University of Hamburg, Martin-Luther-King-Platz 6, D-20146 Hamburg, Germany; Fax: + 49-40-42838-2495; E-mail: chris. meier@chemie.uni-hamburg.de.

ORDER		REPRINTS
-------	--	----------

#### **INTRODUCTION**

Recently we developed a new class of chemically cleavable prodrugs of the corresponding nucleotide analogues of antivirally active nucleosides like 2',3'-dideoxy-2',3'-didehydrothymidine (d4T) 1.<sup>[1]</sup> It was unambiguously shown that the so-called cycloSal-pronucleotides (prototype A, Figure 1) deliver d4T monophosphate and other nucleotides inside human cells.<sup>[2,3]</sup> Thymidine kinase (TK) is a salvage pathway enzyme that is responsible for the conversion of a nucleoside analogue into its nucleoside monophosphate (nucleotide).<sup>[4]</sup> This conversion is the first and often most critical step in the bioactivation of nucleoside analogues into their ultimately bioactive triphosphates.<sup>[5]</sup> This problem cannot be circumvented by the direct use of the nucleotide because of its high polarity and its efficient catabolism in the blood stream by unspecific nucleotidases. In contrast, nucleotide releasing lipophilic systems like the cycloSal-pronucleotide approach can bypass these limitations by achieving the thymidine-kinase bypass (TK-bypass).<sup>[6,7]</sup> The nucleotide delivery from cyclo-Sal-triesters is a result of a chemically triggered releasing process that liberates the nucleotide by a cascade reaction.<sup>[8]</sup> The cycloSal-approach has been applied successfully to various nucleoside analogues, e.g. d4T  $\mathbf{1}$ ,<sup>[9,10]</sup> 5-(*E*)-bromovinyl-2'-deoxyuridine,<sup>[11,12]</sup> acyclovir,<sup>[13,14]</sup> 2',3'-dideoxyadenosine<sup>[15]</sup> and 2'-*ribo*-fluoro-2',3'dideoxyadenosine.<sup>[16]</sup> However, it cannot be excluded that a concentration equilibrium through the membrane is formed due to the lipophilic nature of the cycloSal-triesters (Scheme 1).

For an effective expression of the antiviral activity it is a prerequisite that high concentrations of the pronucleotide must accumulate inside cells. This would then lead also to high concentrations of the mononucleotide and consequently also of the bioactive triphosphate. Therefore, we report here on an extension of the *cyclo*Sal-phosphate triester approach. In order to trap the *cyclo*Sal-triester intracellularly, an enzyme-cleavable site has been attached to the *cyclo*Sal-moiety. An enzymatic reaction, that should occur predominately inside the cells, releases a highly or at least a more polar *cyclo*Sal-derivative.<sup>[17]</sup> For this reason we introduced an ester group that should be cleaved by (carboxy)esterases or lipases (''lock-in'' triesters **B**; Figure 1).

The intracellular concentrations of esterases are known to be higher as compared to the extracellular medium. The use of esters/esterases in prodrug development has been



prototype *cyclo*Sal-d4TMP **A** with unfunctionalized substituents X

"lock-in"-*cyclo*Sal-d4TMP **B** with esterase-cleavable substituents (X' = ROC(0)- or R'C(0)0-)

*Figure 1.* General structures of the prototype *cyclo*Sal-phosphate triesters **A** of 2',3'-dideoxy-2',3'-didehydrothymidine (d4T 1) and the second generation derivatives **B**.

Copyright © Marcel Dekker, Inc. All rights reserved

90





X = ROC(O)O- or R'C(O)O-Y = HOC(O)- or HO-

*Scheme 1.* Formation of an equilibrium through the membrane and the general idea of the ''lock-in''-concept.



*Figure 2.* Structures of the target new *cyclo*Sal-phosphate triesters of d4TMP bearing different ester modifications.

91

Marcel Dekker, Inc. 270 Madison Avenue, New York, New York 10016



ORDER		REPRINTS
-------	--	----------

widely applied.<sup>[6,7,18,19]</sup> However, in the case of the *cyclo*Sal-pronucleotides the ester group attached to the *cyclo*Sal-moiety, represents an electron-withdrawing group that should also decrease the stability of the phosphate ester. In order to avoid this, an ethylene linker was introduced as a spacer unit between the ester group and the *cyclo*Sal-moiety. Two types of ester groups can be attached that differ in the resulting *cyclo*Sal-triesters. After enzymatic cleavage, a *cyclo*Sal-d4TMP acid **2** can be liberated from *cyclo*Sal-d4TMP acid ester **3** or a *cyclo*Sal-d4TMP alcohol **4** can be released from *cyclo*Sal-d4TMP alcohol ester **5** (Figure 2). In both cases, a more polar *cyclo*Sal-derivative (Y-prodrug; Scheme 1) as compared to the parent triester (X-prodrug, Scheme 1) is released that should accumulate inside the cell (''lock-in''-concept).

Here we report on the synthesis and the properties of these new *cyclo*Sal-d4TMP derivatives 2-5 having alkyl ester groups attached via a C2-linker in the *cyclo*Sal-residue. In this report, we focused our interest on the nucleoside analog d4T **1**. The ester-linker group has been attached either to the 3-or to the 5-position of the *cyclo*Sal-aromatic ring. Previous *cyclo*Sal-derivatives bearing substituents in these positions showed appropriate chemical stability, selective d4TMP delivery and strong bioactivity in previous work.<sup>[1,9,10,15,16]</sup>

#### CHEMISTRY, RESULTS AND DISCUSSION

In order to use our previously reported synthetic approach towards the title triesters,<sup>[1,9,10]</sup> the different salicyl alcohols 6 were prepared first from the corresponding phenols 7 (Figure 3). The methyl, ethyl and *i*-propyl 3-(2-hydroxyphenyl)propionates were prepared by transesterification of dihydrocoumarin 8 with methanol, ethanol and 2-propanol in the presence of  $H_2SO_4$  in 73%-96%. The benzyl ester was prepared by alkylation of 3-(2-hydroxyphenyl)propionic acid 9 using benzyl bromide in the presence of DBU in toluene (97% yield)<sup>[20]</sup> while both t-butyl ester were obtained by esterification of the same starting material or 3-(4-hydroxyphenyl)propionic acid 10 with N,N-dimethylformamide-dineopentylacetale in toluene in 85% and 73% yield, respectively.<sup>[21]</sup> The methyl- and the benzyl esters of 3-(4-hydroxyphenyl)propionic acid 10 were prepared in 93% and 73% yield by acid catalyzed esterification (H<sub>2</sub>SO<sub>4</sub> in the former and H<sub>3</sub>PO<sub>4</sub><sup>[22]</sup> in the latter case). The 2-propyl ester was obtained in 62% using 2-propanol and dry HCl-gas.<sup>[23]</sup> The acetyl esters of 2-(2hydroxyphenyl)ethanol 11 and 2-(4-hydroxyphenyl)ethanol 12 were also obtained by a transesterification reaction. However, here the transesterification of the acetyl group of ethylacetate was achieved in the presence of SiO<sub>2</sub>·NaHSO<sub>4</sub> as a catalyst in 87% yield.<sup>[24]</sup> This procedure has also been used for the formation of the levulinyl ester of 2-(2-hydroxyphenyl)ethanol 11 from ethyllevulinate. Finally, the pivaloyl esters of 2-(2-hydroxyphenyl)ethanol 11 and 2-(4-hydroxyphenyl)ethanol 12 were synthesized using the "twisted amides" as an activated pivaloyl-donor<sup>[25]</sup> in 80% and 51% yield, respectively.

All phenols **7** were then transformed into the corresponding salicyl alcohols **6** by reaction of p-formaldehyde and phenylboronic acid in the presence of a trace amount of propionic acid.<sup>[26]</sup> The intermediate 2-phenyl-4*H*-benzo[1.3.2]-dioxaborinanes were purified and subsequently treated with a 30% aqueous solution of  $H_2O_2$  to give the salicylalcohols. Yields for this two step procedure were found to be between 50–85%.







*Figure 3.* Synthesis pathways towards the title compounds. Method A: alcohol,  $H_2SO_4$ , reflux, 5–8 h; method B: benzyl bromide, DBU, toluene, reflux, 7 h; method C:  $(CH_3)_2NCH(OCH_2tBu)_2$ , toluene, reflux, 5 h; method D: methanol,  $CH_2Cl_2$ ,  $H_2SO_4$ , reflux, 5 h; method E: benzyl alcohol, toluene,  $H_3PO_4$ , reflux, 10 h; method F: 2-propanol, HCl-gas, rt, 16 h; method G: ethylacylate, *n*-hexane, SiO<sub>2</sub>·NaHSO<sub>4</sub>, 67°C, 6-18 h; method H: 3-pivaloyl-1,3-thiazolidine-2-thion, toluene, 65°C, 48 h; method I: i. phenylboronic acid, propionic acid (cat.), *p*-formaldehyde, toluene, reflux, 6–8 h; ii)  $H_2O_2$ , THF, 0°C, 30 min; method J: PCl<sub>3</sub>, pyridine, diethylether, 0–21°C, 12 h; method K: i. d4T 1, AcCN, DIPEA, 0–20°C; ii. *t*BuOOH, AcCN, rt, 30 min; method L: hydrazine-hydrate, pyridine/acetic acid 3:2, pyridine, 0°C, 10 min; method M: TFA (10 equiv.), CH<sub>2</sub>Cl<sub>2</sub>, rt, 1h.

These diols were then reacted with PCl<sub>3</sub> in the presence of pyridine to yield cyclic chlorophosphanes **13** as described previously.<sup>[27]</sup> The chlorophosphanes **13** were used as crude products (85–95% pure as judged from the <sup>31</sup>P-NMR) for the next reactions. Typically, the chlorophosphanes showed resonance signals at ~ 140.5 ppm in the <sup>31</sup>P-NMR. These were then reacted with the nucleoside analog d4T **1**, and the intermediately formed phosphite triesters were oxidized by *t*-butyl hydroperoxide in a



Table 1. Lipophilicity, half-lives and antiviral data of the "lock-in" -cycloSal-d4TMP triesters.

94

						$EC_{50} [\mu M]^{e}$		
			t <sub>1/2</sub> []	1] <sup>b</sup>	CEN	O/V	CEM/TK <sup>-</sup>	$CC_{50}^{f}$
	R	$\log P_{calc}{}^{a}$	$PBS^{c}$	$\operatorname{CE}^{\operatorname{d}}$	HIV-1	HIV-2	HIV-2	[μΜ]
2a	Н	-3.96	22.9	20.4	$0.19 \pm 0.08$	$1.4 \pm 0.60$	$20.0 \pm 0.00$	100
2b	Н	-3.96	12.5	11.4	$0.14 \pm 0.10$	$0.80 \pm 0.20$	$50 \pm 30$	76
<b>3a</b>	Me	0.46	7.3	7.2	$0.09 \pm 0.05$	$0.25 \pm 0.15$	$0.40 \pm 0.42$	57
3b	Et	0.94	8.5	8.0	$0.19 \pm 0.09$	$0.65 \pm 0.07$	$0.36 \pm 0.00$	50
3с	<i>i</i> Pr	1.25	12.5	10.1	$0.14 \pm 0.06$	$1.00 \pm 0.28$	$0.08 \pm 0.13$	54
3d	Bn	2.19	9.1	6.0	$0.15 \pm 0.09$	$1.00 \pm 0.28$	$2.89 \pm 1.96$	44
3e	<i>t</i> Bu	1.65	13.5	9.0	$0.33 \pm 0.11$	$0.50 \pm 0.14$	$1.14 \pm 0.96$	43
3f	Me	0.46	7.0	5.7	$0.33 \pm 0.11$	$1.05 \pm 0.35$	$1.20 \pm 1.1$	58
3g	<i>i</i> Pr	1.25	7.3	9.9	$0.17 \pm 0.08$	$0.90 \pm 0.42$	$3.00 \pm 0.00$	59
3h	Bn	2.19	6.5	4.3	$0.08 \pm 0.00$	$1.00 \pm 0.28$	$2.00 \pm 0.00$	61
3i	<i>t</i> Bu	1.65	7.1	6.3	$0.18 \pm 0.08$	$2.40 \pm 2.30$	$4.00 \pm 0.00$	42
4a	Н	-0.53	12.6	15.0	$0.24 \pm 0.01$	$0.33 \pm 0.11$	$0.49 \pm 0.3$	96
5a	Ac	0.42	13.6	1.9	$0.16 \pm 0.09$	$0.33 \pm 0.24$	$0.15 \pm 0.0$	40
5b	Piv	1.65	13.1	9.9	$0.16 \pm 0.09$	$0.70 \pm 0.42$	$0.40 \pm 0.96$	55
5c	Lev	0.37	12.5	2.0	$0.13 \pm 0.04$	$0.33 \pm 0.11$	$0.15 \pm 0.1$	58
5d	Ac	0.42	6.3	2.6	$0.15 \pm 0.06$	$0.80 \pm 0.57$	$0.55 \pm 0.21$	44
5e	Piv	1.65	5.4	5.5	$0.23 \pm 0.16$	$0.90 \pm 0.42$	$0.60 \pm 0.28$	22
1	I	-0.48	n.a.	n.a.	$0.23 \pm 0.04$	$0.24 \pm 0.02$	$15.0 \pm 7.1$	60
<sup>a</sup> Calculatec <sup>b</sup> Hydrolysis <sup>c</sup> 25 mM ph <sup>d</sup> CEM cell <sup>e</sup> Antiviral <i>z</i> <sup>f</sup> Cytostatic	l partition coef s half-lives. nosphate buffer extracts, pH 6 activity in T-ly activity: 50%	ficients (log <i>P</i> ). , pH 7.3. .9. mphocytes: 50% e	effective concer ration.	ntration.				

Meier et al.

Marcel Dekker, Inc. 270 Madison Avenue, New York, New York 10016





one-pot reaction to give the title triesters **3,5** in 30-50% yield.<sup>[9,10]</sup> Moreover, *cyclo*Sal-d4TMP acids **2** were prepared by cleavage of the *t*-butyl ester in **3e** and **3i**, respectively, by TFA treatment (87% yield). Finally, *cyclo*Sal-d4TMP alcohol **4a** was obtained after delevulinylation of **5b** with hydrazine-hydrate (25% yield).<sup>[28]</sup>

All new cycloSal-d4TMP derivatives 2-5 were studied regarding their stability in aqueous phosphate buffer, pH 7.3. The half-lives are shown in Table 1.

First, all cycloSal-d4TMP esters bearing the modification in the 3-position will be discussed. It was interesting to note that the stability of cycloSal-d4TMP acid esters 3a-e varied between 7.3 h and 13.5 h. However, this variation cannot be attributed to changing lipophilicities because no correlation was found between the  $t_{1/2}$  and the  $\log P_{calc}$  values (Table 1). As expected, *cyclo*Sal-d4TMP alcohol esters **5a**-**c** showed a stability of 13-13.5 hours. These results prove that the ethylene-spacer is long enough to separate the electron-withdrawing ester group from the cycloSal-ring system. This can be concluded from the half-lives of two cycloSal-triesters published before: the prototype cycloSal derivatives without any substituent showed a  $t_{1/2}$  of 4.4 h and for the donor substituted 3-methyl-cycloSal-d4TMP a t<sub>1/2</sub> value of 17.5 h was measured.<sup>[8]</sup> Interestingly, the chemical stability of the cycloSal-d4TMP acid 2a was 1.8-3-fold higher as those found for of the corresponding esters 3a - e. This increase in stability may be attributed to the charged carboxylate at pH 7.3 of the buffer, with the result of an overall negative charge on the triester molecule. Thus, a nucleophilic attack at the phosphate group is less efficient. In contrast, cycloSal-d4TMP alcohol 4a showed the same chemical stability as triesters 5a-c as expected.

Within the series of *cyclo*Sal-triesters **3f**-**i**,**5d**,**5e** bearing the ester modification in the 5-position, the half-lives were found to be between 6.1 and 7.3 h. Again, no correlation with the lipophilicity was observed. As for the 3-acid derivative **2a**, the 5-acid *cyclo*Sal-derivative **2b** showed an 1.8-fold increase in stability. As compared to the prototype 5-methyl-*cyclo*Sal-d4TMP ( $t_{1/2} = 8$  h)<sup>8</sup>, triesters **3f**-**i**,**5d**,**5e** showed comparable chemical stability thus, proving the efficiency of the ethylene linker again.

Finally, in all cases the product formed after the hydrolysis in aqueous phosphate buffer, pH 7.3 was d4TMP and the corresponding diol without cleaving the different ester groups.

In contrast, the cleavage of the ester groups attached to the 3-position of the cycloSal-moiety was clearly observed in the case triesters 5a (acetyl-ester;  $t_{1/2} = 1.9$  h instead of 13.6 in the chemical hydrolysis) and 5c (Lev-ester;  $t_{1/2} = 2.0$  h instead of 12.5 in the chemical hydrolysis) in hydrolysis studies using T-lymphocyte (CEM) cell extracts (CE; Table 1). In HPLC-co-elution experiments, it was unambiguously shown that triester alcohol 4a was formed which then hydrolyzed to yield d4TMP. As expected, the Piv-triester 5c was more stable because (carboxy)esterases are not readily "working" on branched alkyl esters. Nevertheless, the stability decreased still 3-fold. Thus, for the first time, a fast enzymatic cleavage led to a product that is considerably more polar (log $P_{calc} = -0.53$  (4a) vs. 0.42 (5a) and 0.37 (5b)) as the precursor. However, the 3-modified *cyclo*Sal-d4TMP acid esters 3a - e that should release the even more polar carboxylate group were found to be inert against enzymatic cleavage: no formation of the cycloSal-d4TMP acid 2a (log $P_{calc} = -3.96$ ) has been observed in the HPLC-chromatograms. This selectivity of the involved esterases is surprising because often carboxyl groups are bioreversibly protected by esterification. Moreover, the inability to observe ester cleavage by the (carboxy)esterases in the extracts was

ORDER		REPRINTS
-------	--	----------

surprising because prior to the *cyclo*Sal-triesters we incubated 3-propionylsalicylalcohol methyl ester with pig liver esterase (PLE; E.C. 3.1.1.1.) in phosphate buffer and an extremely rapid deesterification was observed. In contrast, 3-or 5-modified *cyclo*Sal-d4TMP triesters were not cleaved by PLE! Thus, the attached nucleoside significantly impacts the ability of these compounds to serve as PLE substrates.

In the series of the 5-modified *cyclo*Sal-d4TMP triesters 3f-i,5d,5e the situation was comparable: only the acetyl ester 5d was cleaved although the half-life dropped only 2.4-fold and the appearance of a reaction intermediate was observed in the HPLC. Chemical hydrolysis was only observed for all other derivatives. Again, a selective delivery of d4TMP was observed.

Finally, triesters 2-5 were tested for their anti-HIV activity in CEM/O and CEM/ TK<sup>-</sup> cells. All triesters showed the TK-bypass effect (antiviral activity in the wild-type CEM cells and in the CEM/TK<sup>-</sup> cells) except triesters 2. The non-TK-bypass of cycloSal-d4TMP acids 2 is most probably due to an inability of membrane penetration due to the charged carboxylate moiety. In contrast, cycloSal-d4TMP alcohol 4a showed antiviral activity in the TK-competent and the TK-deficient cells. Although the lipophilicity is considerably different among the esters, this result is suggestive of a membrane penetration even if a free hydroxy group is present in the molecule. It is interesting to note that the triesters 5a,b that were enzymatically cleaved in the cell extracts showed the best antiviral activity in the CEM/TK<sup>-</sup> cells.

The promising results of the enzymatic cleavage of the *cyclo*Sal-d4TMP alcohol esters **5a**,**b**,**d** at the introduced ester site provides supporting evidence for the feasibility of the concept. Further experiments will be conducted in our laboratories in order to assess the role of ester lability on antiviral activity and enzymatic stability.

#### **EXPERIMENTAL SECTION**

All experiments involving water-sensitive compounds were conducted under scrupulously dry conditions (argon or nitrogen atmosphere). Solvents: Anhydrous methylene chloride  $(CH_2Cl_2)$  and anhydrous acetonitrile  $(CH_3CN)$  were obtained in a Sure/Seal bottle from Fluka and stored over 4 Å molecular sieves. Ethyldiisopropylamine (DIPEA) was distilled from Na prior to use. The solvents for HPLC were obtained from Merck (acetonitrile, HPLC grade). Ion pairing buffer solution was prepared by mixing 6.6 mL tetrabutylammonium hydroxide with 1000 mL water. The pH-value was adjusted to 3.8 by adding concentrated phosphoric acid (buffer I). To 60 mL of buffer I-solution, 1000 mL water were added (buffer II). Chromatography: Chromatotron (Harrison Research 7924), silica gel 60<sub>Pf</sub> (Merck, "gipshaltig"); UV detection at 254 nm. TLC: analytical thin layer chromatography was performed on Merck precoated aluminium plates 60 F<sub>254</sub> with a 0.2-mm layer of silica gel containing a fluorescence indicator; sugar-containing compounds were visualized with the sugar spray reagent (0.5 mL of 4-methoxybenzaldehyde, 9 mL of ethanol, 0.5 mL of concentrated sulfuric acid, and 0.1 mL of glacial acectic acid) by heating with a fan or a hot plate. HPLC: (Merck-Hitachi) analytical HPLC, LiChroCART 250-3 with LiChrospher 100 RP-18 endcapped (5 µm), gradient I 5-100% CH<sub>3</sub>CN (0-20 min), 5% CH<sub>3</sub>CN (20–35 min), flow 0.5 mL, UV detection at 265 nm; gradient II 8–100% CH<sub>3</sub>CN (0-22 min), 100% CH<sub>3</sub>CN (22-27 min), 8% CH<sub>3</sub>CN (27-33 min), flow 0.6 mL, UV detection at 256 nm (in gradient II tetrabutylammonium phosphate buffer





was used instead of water as in gradient I). NMR spectra were recorded using (<sup>1</sup>H NMR) Bruker AC 250 at 250 MHz, Bruker AMX 400 at 400 MHz or Bruker DMX 500 at 500 MHz; (13C NMR) Bruker WM 400 at 101 MHz, Bruker AMX 400 at 101 MHz or Bruker DMX 500 at 123 MHz (Calibration was done in both cases with the solvent);  $(^{31}P \text{ NMR})$  Bruker AMX 400 at 162 MHz or Bruker DMX 500 at 202 MHz (H<sub>3</sub>PO<sub>4</sub> as external standard). All <sup>1</sup>H and <sup>13</sup>C NMR chemical shifts ( $\delta$ ) are quoted in parts per million (ppm) downfield from tetramethylsilane, (CD<sub>3</sub>)(CD<sub>2</sub>H)SO being set at  $\delta_{\rm H}$  2.49 as a reference. UV spectra were taken with a Varian Cary 1E UV/Vis spectrometer. Infrared spectra were recorded with a Perkin Elmer 1600 Series FT-IR or a ATI Mattson Genesis Series FT-IR spectrometer in KBr pellets. Mass spectra were obtained with a Finnigan electrospray MAT 95 Trap XL (ESI) or a VG Analytical VG/70-250 F spectrometer (FAB, matrix was *m*-nitrobenzylalcohol). The test compounds were isolated as mixtures of diastereomers arising from the mixed stereochemistry at the phosphate center. From our experience, elemental analyses are difficult to obtain from phosphorus and nucleoside containing compounds. However, the obtained lyophilized triesters were found to be pure by HPLC analysis, high-field multinuclear NMR spectroscopy and mass spectrometry.

#### **Preparation of the Phenols 7**

**Methyl-3-(2-hydroxyphenyl)propionate 7a:** 10.4 g (70.0 mmol) Dihydrocumarin 45 have been added to 60 ml dry methanol. 100 μl H<sub>2</sub>SO<sub>2</sub> were added and the mixture was heated under reflux for 5 h. Then, the methanol was distilled off and the residue was dissolved in diethylether and the organic phase was extracted with sodium bicarbonate solution. The organic phase was dried with sodium sulfate, the solvent was evaporated and the residue was subjected to a chromatography on silica gel (CH<sub>2</sub>Cl<sub>2</sub>-hexane gradient 50 to 0 %). The product was isolated as colorless needles. Yield: 96%; m.p.: 41.5°C; TLC: R<sub>f</sub> (CH<sub>2</sub>Cl<sub>2</sub>): 0.56; <sup>1</sup>H-NMR (250 MHz, CDCl<sub>3</sub>) δ: 7.33 (s, 1H, aryl-OH); 7.16–7.10 (m, 1H, H5-aryl); 7.12 (d, <sup>3</sup>J<sub>H-H</sub> = 7.3 Hz, 1H, H3-aryl); 6.91–6.85 (m, 2H, H4-aryl + H6-aryl); 3.71 (s, 3H, H10); 2.96 (t, <sup>3</sup>J<sub>H-H</sub> = 6.7 Hz, 2H, H8); 2.75 (t, <sup>3</sup>J<sub>H-H</sub> = 6.7 Hz, 2H, H7); <sup>13</sup>C-NMR (63 MHz, CDCl<sub>3</sub>) δ: 175.7 (C9); 154.1 (C1); 130.3 (C3); 127.8 (C2); 127.0 (C5); 120.6 (C4); 116.5 (C6); 52.0 (C10); 34.6 (C7); 24.9 (C8); UV (CH<sub>3</sub>CN) λ<sub>max</sub>: 298, 272 nm; IR (KBr) ν<sup>-1</sup>: 3457 (OH), 3038, 1719 (CO), 1594, 1506, 1426, 1421, 1102, 989, 845, 786, 751, 707.

**Ethyl-3-(2-hydroxyphenyl)propionate 7b:** Phenol **7b** was prepared as **7a**. The product was isolated as colorless needles. Yield: 81%; m.p.  $34-35^{\circ}$ C; TLC: R<sub>f</sub> (CH<sub>2</sub>Cl<sub>2</sub>/MeOH 9:1 v/v): 0.66; <sup>1</sup>H-NMR (250 MHz, CDCl<sub>3</sub>)  $\delta$ : 7.42 (s, 1H, aryl-OH); 7.13 (ddd,  $2^{\cdot3}J_{H-H} = 6.7$  Hz, <sup>4</sup> $J_{H-H} = 1.5$  Hz, 1H, H5-aryl); 7.11 (d, <sup>3</sup> $J_{H-H} = 7.3$  Hz, 1H, H3-aryl); 6.90 (d, <sup>3</sup> $J_{H-H} = 7.9$  Hz, 1H, H6-aryl); 6.88 (ddd,  $2^{\cdot3}J_{H-H} = 7.6$  Hz, <sup>4</sup> $J_{H-H} = 1.2$  Hz, 1H, H4-aryl); 4.17 (q, <sup>3</sup> $J_{H-H} = 7.0$  Hz, 2H, H10); 2.93 (t, <sup>3</sup> $J_{H-H} = 6.7$  Hz, 2H, H8); 2.74 (t, <sup>3</sup> $J_{H-H} = 6.7$  Hz, 2H, H7); 1.26 (t, <sup>3</sup> $J_{H-H} = 7.0$  Hz, 3H, H11); <sup>13</sup>C-NMR (63 MHz, CDCl<sub>3</sub>)  $\delta$ : 175.4 (C9); 154.2 (C1); 130.3 (C3); 127.7 (C5); 127.1 (C2); 120.5 (C4); 116.6 (C6); 61.1 (C10); 34.9 (C8); 24.9 (C7); 14.0 (C11); IR (KBr) v<sup>-1</sup>: 3500 (OH), 1705 (CO).

2-Propyl-3-(2-hydroxyphenyl)propionate 7c: Phenol 7c was prepared as 7a. The product was isolated as a colorless oil. Yield: 73%; TLC:  $R_f$  (CH<sub>2</sub>Cl<sub>2</sub>/MeOH 9:1

97

ORDER		REPRINTS
-------	--	----------

v/v): 0.68; <sup>1</sup>H-NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$ : 7.40 (s(br), 1H, aryl-OH); 7.13 (ddd, 2·<sup>3</sup>J<sub>H-H</sub> = 7.6 Hz, <sup>4</sup>J<sub>H-H</sub> = 1.8 Hz, 1H, H5-aryl); 7.09 (dd, <sup>3</sup>J<sub>H-H</sub> = 7.5 Hz, <sup>4</sup>J<sub>H-H</sub> = 1.7 Hz, 1H, H3-aryl); 6.92–6.85 (m, 2H, H4-aryl + H6-aryl); 5.02 (sept, <sup>3</sup>J<sub>H-H</sub> = 6.2 Hz, 1H, H10); 2.90 (t, <sup>3</sup>J<sub>H-H</sub> = 6.2 Hz, 2H, H7); 2.69 (dd, <sup>3</sup>J<sub>H-H</sub> = 6.5 Hz, <sup>3</sup>J<sub>H-H</sub> = 6.2 Hz, 2H, H8); 1.21 (d, <sup>3</sup>J<sub>H-H</sub> = 6.2 Hz, 6H, H11a,b); <sup>13</sup>C-NMR (101 MHz, CDCl<sub>3</sub>)  $\delta$ : 175.0 (C9); 154.3 (C1); 130.4 (C3); 127.7 (C5); 127.2 (C2); 120.5 (C4); 116.6 (C6); 68.7 (C10); 35.2 (C8); 24.9 (C7); 21.6 (C11a,b); UV (CH<sub>3</sub>CN)  $\lambda_{max}$ : 272, 214, 202 nm; IR (film): v<sup>-1</sup>: 3398 (OH), 3038, 1702 (CO), 1609, 1595, 1506, 1491, 1458, 1375, 1236, 1105, 754.

Benzyl-3-(2-hydroxyphenyl)propionate 7d: To a solution of 2.08 g (12.5 mmol) 3-(2-hydroxyphenyl)propionic acid 47 in 30 ml dry toluene, 1.87 ml DBU and 1.48 ml (12.5 mmol) benzylbromide was added. The mixture was heated for 7 h under reflux. The organic phase was washed with water, sodium hydrogencarbonate and sodium chloride solutions. After drying, the solvent was evaporated and the residue was purified by chromatography on silica gel (*n*-hexane - ethylacetate-gradient 10-50 %). The product was isolated as a yellowish oil. Yield: 97%; TLC: R<sub>f</sub> (n-hexan/ethylacetate 3:2 v/v): 0.48; R<sub>f</sub> (CH<sub>2</sub>Cl<sub>2</sub>/MeOH 9:1 v/v): 0.71; <sup>1</sup>H-NMR (400 MHz, DMSO-d<sub>6</sub>) δ: 9.36 (s, 1H, aryl-OH); 7.37–7.29 (m, 5H, Ph); 7.02 (dd,  ${}^{3}J_{H-H} = 7.3$  Hz, 1H, H3aryl); 7.00 (ddd,  $2 \cdot {}^{3}J_{H-H} = 7.6$  Hz,  ${}^{4}J_{H-H} = 1.8$  Hz, 1H, H5-aryl); 6.77 (dd,  ${}^{3}J_{H-H} = 7.9$  Hz,  ${}^{4}J_{H-H} = 1.1$  Hz, 1H, H6-aryl); 6.67 (ddd,  $2 \cdot {}^{3}J_{H-H} = 7.4$  Hz,  ${}^{4}J_{H-H} = 1.2$  Hz, 1H, H4-aryl); 5.06 (s, 2H, H10); 2.79 (t,  ${}^{3}J_{H-H} = 7.7$  Hz, 2H, H7); 2.61 (dd,  ${}^{3}J_{H-H} = 8.1$  Hz,  ${}^{3}J_{H-H} = 7.2$  Hz, 2H, H8);  ${}^{13}C$ -NMR (101 MHz, DMSO-d<sub>6</sub>) δ: 172.6 (C9); 155.3 (C1); 136.4 (C11); 129.9 (C3); 128.6 (2 × Cm-Ph); 128.1 (Cp-Ph); 128.1 (2 × Co-Ph); 127.4 (C5); 126.5 (C2); 119.1 (C4); 115.0 (C6); 65.5 (C10); 33.7 (C8); 25.7 (C7).

*tert*-Butyl-3-(2-hydroxyphenyl)propionate 7e: A solution of 1.50 g (9.03 mmol) 3-(2-hydroxyphenyl)propionic acid 47 and 8.70 g (117 mmol) *tert*-butanol was refluxed in 22 ml toluene. To this solution, 6.27 g (27.1 mmol) *N*,*N*-DMF-dineopentylacetal was added dropwise during 30 min and stirring was continued for further 5 h. The organic phase was washed with sodium bicarbonate solution and water. After drying the organic phase, the solvent was removed and the residue was purified by chromatography (CH<sub>2</sub>Cl<sub>2</sub>- methanol-gradient 0–20 %). The product was isolated as a colorless oil. Yield: 81%; TLC: R<sub>f</sub> (CH<sub>2</sub>Cl<sub>2</sub>/MeOH 9:1 v/v): 0.61; <sup>1</sup>H-NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$ : 8.06 (s, 1H, aryl-OH); 7.13 (ddd,  $2 \times^{3}J_{H-H} = 7.6$  Hz, <sup>4</sup> $J_{H-H} = 1.5$  Hz, 1H, H5-aryl); 7.08 (dd, <sup>3</sup> $J_{H-H} = 7.5$  Hz, <sup>4</sup> $J_{H-H} = 1.6$  Hz, 1H, H3-aryl); 6.90 (dd, <sup>3</sup> $J_{H-H} = 8.1$  Hz, <sup>4</sup> $J_{H-H} = 1.0$  Hz, 1H, H6-aryl); 6.86 (ddd,  $2 \times^{3}J_{H-H} = 7.4$  Hz, <sup>4</sup> $J_{H-H} = 1.1$  Hz, 1H, H4-aryl); 2.86 (t, <sup>3</sup> $J_{H-H} = 6.2$  Hz, 2H, H7); 2.65 (t, <sup>3</sup> $J_{H-H} = 6.2$  Hz, 2H, H8); 1.43 (s, 9H, H11a-c); <sup>13</sup>C-NMR (101 MHz, CDCl<sub>3</sub>)  $\delta$ : 174.5 (C9); 153.8 (C1); 129.9 (C3); 127.3 (C5); 126.7 (C2); 120.0 (C4); 116.2 (C6); 73.5 (C10); 35.8 (C8); 26.8 (C11); 25.6 (C7).

**Methyl-3-(4-hydroxyphenyl)propionate 7f:** Phenol **7f** was prepared as **7a**. The product was isolated as a colorless solid. Yield: 93%; TLC:  $R_f$  (CH<sub>2</sub>Cl<sub>2</sub>/MeOH 9:1 v/v): 0.70; <sup>1</sup>H-NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$ : 7.07 (d, <sup>3</sup>J<sub>H-H</sub> = 8.3 Hz, 2H, H3-aryl + H5-aryl); 6.76 (d, <sup>3</sup>J<sub>H-H</sub> = 8.4 Hz, 2H, H4-aryl + H6-aryl); 3.68 (s, 3H, H10);

98





2.89 (t,  ${}^{3}J_{H-H} = 7.7$  Hz, 2H, H7); 2.61 (t,  ${}^{3}J_{H-H} = 7.7$  Hz, 2H, H8);  ${}^{13}C$ -NMR (101 MHz, CDCl<sub>3</sub>)  $\delta$ : 173.6 (C9); 154.0 (C1); 132.6 (C4); 129.4 (C5 + C3); 115.3 (C2 + C6); 51.6 (C10); 36.0 (C8); 30.1 (C7).

**2-Propyl-3-(4-hydroxyphenyl)propionate 7g:** To a stirred solution of 3.34 g (20.1 mmol) 3-(4-hydroxyphenyl)propionic acid **51** in 150 ml (1.95 mol) 2-propanol HCl-gas was blubbed through. The methanol solution started to boil. After 1 h the addition of HCl was stopped and the reaction was kept at room temperature was further 16 h. The solvent was removed and the remaining residue was chromatographed (CH<sub>2</sub>Cl<sub>2</sub>/MeOH-gradient 0 to 20 %). The product was isolated as a colorless solid. Yield: 62%; TLC: R<sub>f</sub> (CH<sub>2</sub>Cl<sub>2</sub>/MeOH 9:1 v/v): 0.71; <sup>1</sup>H-NMR (400 MHz, DMSO-d<sub>6</sub>)  $\delta$ : 9.12 (s, 1H, aryl-OH); 6.98 (d, <sup>3</sup>J<sub>H-H</sub> = 8.5 Hz, 2H, H3-aryl + H5-aryl); 6.64 (d, <sup>3</sup>J<sub>H-H</sub> = 8.5 Hz, 2H, H4-aryl + H6-aryl); 4.84 (sept, <sup>3</sup>J<sub>H-H</sub> = 6.3 Hz, 1H, H10); 2.70 (t, <sup>3</sup>J<sub>H-H</sub> = 7.5 Hz, 2H, H7); 2.47 (t, <sup>3</sup>J<sub>H-H</sub> = 7.5 Hz, 2H, H8); 1.12 (d, <sup>3</sup>J<sub>H-H</sub> = 6.3 Hz, 6H, H11a,b); <sup>13</sup>C-NMR (101 MHz, CDCl<sub>3</sub>)  $\delta$ : 171.9 (C9); 155.7 (C1); 132.6 (C4); 129.2 (C5 + C3); 115.2 (C2 + C6); 67.1 (C10); 36.0 (C8); 29.7 (C7); 21.7 (C11a,b); IR (NaCl) v<sup>-1</sup>: 3412, 3024, 1712 (CO), 1613, 1595, 1519, 1449, 1377, 1298, 1266, 1149, 1108, 904, 837, 820, 609.

**Benzyl-3-(4-hydroxyphenyl)propionate 7h:** 7.00 g (42.1 mmol) 3-(4-Hydroxyphenyl)propionic acid **51**, 13 ml benzyl alcohol and 3 drops of 85 % phosphoric acid in 80 ml toluene were heated under reflux. The formed water during the esterification was continuously destilled off in a Dean-Stark-apparatus at 145–150°C for 10h. Then the excess of benzyl alcohol was evaporated in a vacuo. The residue was taken up in diethylether and washed with a saturated sodium bicarbonate solution and water and was subsequently dried with sodium sulfate. The crude reaction product was purified by distillation in a vacuo at 185–190°C. The product was isolated as a yellowish oil. Yield: 73%; TLC: R<sub>f</sub> (CH<sub>2</sub>Cl<sub>2</sub>/MeOH 9:1 v/v): 0.85; <sup>1</sup>H-NMR (400 MHz, DMSO-d<sub>6</sub>)  $\delta$ : 9.15 (s, 1H, aryl-OH); 7.36–7.27 (m, 5H, Ph); 6.98 (d, <sup>3</sup>J<sub>H-H</sub> = 8.4 Hz, 2H, H3aryl + H5-aryl); 6.64 (d, <sup>3</sup>J<sub>H-H</sub> = 8.5 Hz, 1H, H4-aryl + H6-aryl); 5.06 (s, 2H, H10); 2.74 (t, <sup>3</sup>J<sub>H-H</sub> = 7.5 Hz, 2H, H7); 2.60 (t, <sup>3</sup>J<sub>H-H</sub> = 7.4 Hz, 2H, H8); <sup>13</sup>C-NMR (101 MHz, DMSO-d<sub>6</sub>)  $\delta$ : 172.4 (C9); 155.8 (C1); 136.4 (C11); 130.6 (C4); 129.3 (C5 + C3); 128.6 (2 · Cm-Ph); 128.1 (Cp-Ph); 128.0 (2 · Co-Ph); 115.3 (C2 + C6); 65.5 (C10); 35.7 (C8); 29.7 (C7).

*t*-Butyl-3-(4-hydroxyphenyl)propionat 7i: Phenol 7i was prepared as 7e. The product was isolated as a colorless solid. Yield: 73%; TLC:  $R_f$  (CH<sub>2</sub>Cl<sub>2</sub>/MeOH 9:1 v/v): 0.79; <sup>1</sup>H-NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$ : 7.05 (d, <sup>3</sup>J<sub>H-H</sub> = 8.5 Hz, 2H, H3-aryl + H5-aryl); 6.75 (d, <sup>3</sup>J<sub>H-H</sub> = 8.5 Hz, 2H, H4-aryl + H6-aryl); 2.84 (t, <sup>3</sup>J<sub>H-H</sub> = 7.7 Hz, 2H, H7); 2.51 (t, <sup>3</sup>J<sub>H-H</sub> = 7.7 Hz, 2H, H8); 1.42 (s, 9H; H-tBu); <sup>13</sup>C-NMR (101 MHz, CDCl<sub>3</sub>)  $\delta$ : 172.7 (C9); 154.3 (C1); 132.4 (C4); 129.3 (C5 + C3); 115.2 (C2 + C6); 73.5 (C10); 37.4 (C8); 30.3 (C7); 28.0 (C11).

**2-(2-Hydroxyphenyl)ethylacetate 7j:** To 1 mmol of the phenol **11** in 15 ml of a 30 % solution of ethylacetate in *n*-hexane 100 mg of the NaHSO<sub>4</sub>· SiO<sub>2</sub>-catalyst was added. The reaction temperature was increased to  $67^{\circ}$ C. When the starting material was comsumed (18 h) completely the reaction was stopped. The catalyst was filtered of and

ORDER		REPRINTS
-------	--	----------

washed with CH<sub>2</sub>Cl<sub>2</sub>. The organic phases were evaporated and the oily crude product was chromatographed (CH<sub>2</sub>Cl<sub>2</sub>-methanol-gradient 0–20 %). The product was isolated as a colorless, crystalline solid. Yield: 87%; TLC: R<sub>f</sub> (*n*-hexane/ethylacetate 3:2 v/v): 0.44; <sup>1</sup>H-NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$ : 8.08 (s, 1H, aryl-OH); 7.13 (ddd,  $2 \times {}^{3}J_{H-H} = 7.7$  Hz,  ${}^{4}J_{H-H} = 1.6$  Hz, 1H, H5-aryl); 7.12 (dd,  ${}^{3}J_{H-H} = 7.5$  Hz,  ${}^{4}J_{H-H} = 1.6$  Hz, 1H, H3-aryl); 6.88 (dd,  ${}^{3}J_{H-H} = 7.7$  Hz,  ${}^{4}J_{H-H} = 1.0$  Hz, 1H, H6-aryl); 4.29 (t,  ${}^{3}J_{H-H} = 7.0$  Hz, 2H, H8); 2.97 (t,  ${}^{3}J_{H-H} = 7.0$  Hz, 2H, H7); 2.09 (s, 3H, H10);  ${}^{13}$ C-NMR (101 MHz, CDCl<sub>3</sub>)  $\delta$ : 172.0 (C9); 154.8 (C1); 130.9 (C3); 128.3 (C5); 123.6 (C2); 120.7 (C4); 115.9 (C6); 64.5 (C8); 30.1 (C7); 21.0 (C10).

**2-(2-Hydroxyphenyl)ethyllevulinate 7k:** Phenol **7k** was prepared as described for **7j**. The reaction time here was 72 h. The product was isolated as a colorless oil. Yield: 82%; TLC:  $R_f$  (CH<sub>2</sub>Cl<sub>2</sub>/MeOH 9:1 v/v): 0.63; <sup>1</sup>H-NMR (400 MHz, DMSO-d<sub>6</sub>)  $\delta$ : 2.08 (s, 3H, H13), 2.43 (t, 2H, <sup>3</sup>J<sub>HH</sub> = 6.5 Hz, H10), 2.68 (t, 2H, <sup>3</sup>J<sub>HH</sub> = 6.5 Hz, H11) 2.81 (t, 2H, <sup>3</sup>J<sub>HH</sub> = 7.1 Hz, H7), 4.15 (t, 2H, <sup>3</sup>J<sub>HH</sub> = 7.1 Hz, H8), 6.72 (ddd, 1H, <sup>3</sup>J<sub>HH</sub> = 7.8 Hz, <sup>4</sup>J<sub>HH</sub> = 1.2 Hz, H4), 6.79 (dd, 1H, <sup>3</sup>J<sub>HH</sub> = 7.8 Hz, <sup>4</sup>J<sub>HH</sub> = 1.2 Hz, H6), 7.03 (ddd, 1H, <sup>3</sup>J<sub>HH</sub> = 7.8 Hz, <sup>4</sup>J<sub>HH</sub> = 1.7 Hz, H3), 9.38 (s, 1H, aryl-OH); <sup>13</sup>C-NMR (100 MHz, DMSO-d<sub>6</sub>)  $\delta$ : 28.02 (C10), 29.61 (C7), 29.88 (C13), 37.75 (C11), 63.57 (C8), 115.28 (C6), 119.27 (C4), 124.07 (C2), 127.92 (C5), 130.90 (C3), 155.71. (C1), 172.54 (C9), 207.14 (C12).

**2-(2-Hydroxyphenyl)ethylpivalate 71:** A mixture of 1.0 mmol of the phenol and 3-pivaloyl-1,3-thiazolidine-2-thion (''twisted-amide'') was stirred for 48 h at 65°C in 20 ml of dry toluene. After completion of the reaction, the solvent was evaporated and the residue was chromatographed twice on a chromatotron on silica gel (1. eluent: *n*-hexane/ethylacetate 3:1 v/v; 2. eluent: *n*-hexane/diethylether 7:3 v/v). The product was isolated as a colorless oil. Yield: 80%; TLC: R<sub>f</sub> (*n*-hexane/Et<sub>2</sub>O 7:3 v/v): 0.32; <sup>1</sup>H-NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$ : 8.01 (s, 1H, aryl-OH); 7.12 (ddd,  $2 \times^{3} J_{H-H} = 7.7$  Hz, <sup>4</sup>J<sub>H-H</sub> = 1.8 Hz, 1H, H5-aryl); 7.10 (dd, <sup>3</sup>J<sub>H-H</sub> = 7.5 Hz, <sup>4</sup>J<sub>H-H</sub> = 1.8 Hz, 1H, H3-aryl); 6.85 (ddd,  $2 \times^{3} J_{H-H} = 7.4$  Hz, <sup>4</sup>J<sub>H-H</sub> = 1.2 Hz, 1H, H4-aryl); 6.83 (dd, <sup>3</sup>J<sub>H-H</sub> = 7.9 Hz, <sup>4</sup>J<sub>H-H</sub> = 1.1 Hz, 1H, H6-aryl); 4.27 (t, <sup>3</sup>J<sub>H-H</sub> = 7.1 Hz, 2H, H8); 2.95 (t, <sup>3</sup>J<sub>H-H</sub> = 7.1 Hz, 2H, H7); 1.19 (s, 9H, H-tBu); <sup>13</sup>C-NMR (101 MHz, CDCl<sub>3</sub>)  $\delta$ : 176.8 (C9); 154.5 (C1); 130.9 (C3); 128.2 (C5); 123.5 (C2); 120.5 (C4); 115.8 (C6); 64.3 (C8); 38.8 (C10); 30.1 (C7); 27.1 (C11a-c); IR (film) v<sup>-1</sup>: 3418, 1704 (CO), 1481, 1458, 1292, 1233, 1170, 753.

**2-(4-Hydroxyphenyl)ethylacetate 7m:** Phenol **7m** was prepared as **7j**. The reaction time here was 18 h. The product was isolated as a colorless, crystalline solid. Yield: 87%; TLC:  $R_f$  (*n*-hexane/ethylacetate 3:2 v/v): 0.44; <sup>1</sup>H-NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$ : 8.08 (s, 1H, aryl-OH); 7.13 (ddd,  $2 \times {}^{3}J_{H-H} = 7.7$  Hz,  ${}^{4}J_{H-H} = 1.6$  Hz, 1H, H5-aryl); 7.12 (dd,  ${}^{3}J_{H-H} = 7.5$  Hz,  ${}^{4}J_{H-H} = 1.6$  Hz, 1H, H3-aryl); 6.88 (dd,  ${}^{3}J_{H-H} = 7.6$  Hz,  ${}^{4}J_{H-H} = 1.3$  Hz, 1H, H4-aryl); 6.84 (dd,  ${}^{3}J_{H-H} = 7.7$  Hz,  ${}^{4}J_{H-H} = 1.0$  Hz, 1H, H6-aryl); 4.29 (t,  ${}^{3}J_{H-H} = 7.0$  Hz, 2H, H8); 2.97 (t,  ${}^{3}J_{H-H} = 7.0$  Hz, 2H, H7); 2.09 (s, 3H, H10);  ${}^{13}$ C-NMR (101 MHz, CDCl<sub>3</sub>)  $\delta$ : 172.0 (C9); 154.8 (C1); 130.9 (C3); 128.3 (C5); 123.6 (C2); 120.7 (C4); 115.9 (C6); 64.5 (C8); 30.1 (C7); 21.0 (C10).

Downloaded by [University of Chicago Library] at 02:13 25 April 2013



**2-(4-Hydroxyphenyl)ethylpivalate 7n:** Phenol **7n** was prepared as **7l**. The product was isolated as a colorless oil. Yield: 51%; TLC:  $R_f$  (*n*-hexane/ethylacetate 3:1 v/v): 0.81; <sup>1</sup>H-NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$ : 7.09 (d, <sup>3</sup>J<sub>H-H</sub> = 8.6 Hz, 2H, H3-aryl + H5-aryl); 6.78 (d, <sup>3</sup>J<sub>H-H</sub> = 8.4 Hz, 2H, H4-aryl + H6-aryl); 4.24 (t, <sup>3</sup>J<sub>H-H</sub> = 6.9 Hz, 2H, H8); 2.87 (t, <sup>3</sup>J<sub>H-H</sub> = 6.9 Hz, 2H, H7); 1.17 (s, 9H, H-tBu); <sup>13</sup>C-NMR (101 MHz, CDCl<sub>3</sub>)  $\delta$ : 177.2 (C9); 154.4 (C1); 130.0 (C3 + C5); 129.8 (C4); 115.3 (C2 + C6); 65.2 (C8); 38.7 (C10); 34.2 (C7); 27.1 (C-tBu); IR (film) v<sup>-1</sup>: 3403, 2973, 1704, 1517, 1292, 1225, 1169.

General Procedure for the Preparation of the Salicylalcolhol 6: A mixture of the phenol 7 (1 equiv.), phenylboronic acid (1.2 equiv.), p-formaldehyde (2 equiv.) and propionic acid (0.5 equiv.) was heated in dry toluene under reflux. The formed water was destilled of in a Dean-Stark-apparatus. After 2 h further 2 equiv. of p-formaldehyde were added in portions. Often this procedure was repeated two to three times. After complete comsumption of the phenol, the solvent was removed by evaporation and the remaining material was dissolved in CH<sub>2</sub>Cl<sub>2</sub>. The organic phase was washed with 10% sodium carbonate solution and with water. After evaporation of the organic solvent, the product was obtained as yellowish oil that crystallizes upon cooling. This material was generally pure enough to continue immediately with the oxidation/ hydrolysis procedure. Then, the 2-phenyl-4H-benzo[1.3.2]-dioxaborine derivative was stirred with 30% H<sub>2</sub>O<sub>2</sub>-solution in THF for 30 min. The reaction mixture was added to water and this mixture was extracted several times with diethylether. The collected ether phases were washed with a sodium hydrogensulfite solution and then dried by addition of sodium sulfate. The ether was removed and the residue was chromatographed using *n*-hexane-diethylether gradients of 10-60%.

**Methyl-3-(2-hydroxy-3-hydroxymethyl-phenyl)propionate 6a:** The product was isolated as a colorless oil. Yield: 77%; TLC: R<sub>f</sub> (CH<sub>2</sub>Cl<sub>2</sub>/MeOH 9:1 v/v): 0.67; <sup>1</sup>H-NMR (250 MHz, CDCl<sub>3</sub>) δ: 8.11 (s, 1H, aryl-OH); 7.05 (d, <sup>3</sup>J<sub>H-H</sub> = 7.6 Hz, 1H, H4-aryl); 6.95 (d, <sup>3</sup>J<sub>H-H</sub> = 7.6 Hz, 1H, H6-aryl); 6.79 (dd,  $2 \times {}^{3}J_{H-H} = 7.3$  Hz, 1H, H5-aryl); 4.79 (s, 2H, H7); 3.68 (s, 3H, H11); 2.96 (t,  ${}^{3}J_{H-H} = 7.0$  Hz, 2H, H8); 2.63 (t,  ${}^{3}J_{H-H} = 7.0$  Hz; 2H, H9); <sup>13</sup>C-NMR (63 MHz, CDCl<sub>3</sub>) δ: 175.2 (C10); 153.9 (C2); 130.0 (C4); 127.7 (C1); 126.4 (C6); 125.8 (C3); 119.9 (C5); 64.2 (C7); 51.9 (C11); 34.4 (C9); 25.0 (C8); IR (film) v<sup>-1</sup>: 3358 (OH), 1712 (C = O), 1595, 1465, 1369, 1227, 1083, 1006, 845, 779, 750; UV (CH<sub>3</sub>CN): λ<sub>max</sub>: 275, 208 nm; MS(ESI<sup>-</sup>, m/z): calc.: 210.2 (M), found: 209.4 (M-H<sup>+</sup>).

**Ethyl-3-(2-hydroxy-3-hydroxymethyl-phenyl)propionate 6b:** The product was isolated as a colorless oil. Yield: 88%; TLC:  $R_f$  (CH<sub>2</sub>Cl<sub>2</sub>/MeOH 9:1 v/v): 0.63; <sup>1</sup>H-NMR (400 MHz, DMSO-d<sub>6</sub>) δ: 8.51 (s(br), 1H, aryl-OH); 7.06 (dd, <sup>3</sup>J<sub>H-H</sub> = 7.5 Hz, <sup>4</sup>J<sub>H-H</sub> = 1.6 Hz, 1H, H4-aryl); 6.96 (dd, <sup>3</sup>J<sub>H-H</sub> = 7.5 Hz, <sup>4</sup>J<sub>H-H</sub> = 1.6 Hz, 1H, H4-aryl); 6.96 (dd, <sup>3</sup>J<sub>H-H</sub> = 7.5 Hz, <sup>4</sup>J<sub>H-H</sub> = 1.6 Hz, 1H, H6-aryl); 6.72 (dd,  $2\times^3 J_{H-H} = 7.6$  Hz, 1H, H5-aryl); 5.38 (s(br), 1H, Bn-OH); 4.56 (s, 2H, H7); 4.03 (q, <sup>3</sup>J<sub>H-H</sub> = 7.1 Hz, 2H, H11); 2.80 (t, <sup>3</sup>J<sub>H-H</sub> = 7.7 Hz; 2H, H8); 2.52 (t, <sup>3</sup>J<sub>H-H</sub> = 7.7 Hz, 2H, H9); 1.15 (t, <sup>3</sup>J<sub>H-H</sub> = 7.1 Hz, 3H, H12); <sup>13</sup>C-NMR (101 MHz, CDCl<sub>3</sub>) δ: 172.7 (C10); 152.6 (C2); 128.4 (C4); 128.3 (C1); 127.4 (C3); 125.8 (C6); 119.4 (C5); 60.2 (C7); 60.0 (C11); 33.9 (C9); 25.5 (C8); 14.3 (C12).

ORDER		REPRINTS
-------	--	----------

**Isopropyl-3-(2-hydroxy-3-hydroxymethyl-phenyl)propionate 6c:** The product was isolated as a yellow oil. Yield: 49%; TLC: R<sub>f</sub> (CH<sub>2</sub>Cl<sub>2</sub>/MeOH 9:1 v/v): 0.72; <sup>1</sup>H-NMR (400 MHz, DMSO-d<sub>6</sub>) δ: 8.50 (s(br), 1H, aryl-OH); 7.06 (dd, <sup>3</sup>J<sub>H-H</sub> = 7.5 Hz, <sup>4</sup>J<sub>H-H</sub> = 1.7 Hz, 1H, H4-aryl); 6.96 (dd, <sup>3</sup>J<sub>H-H</sub> = 7.5 Hz, <sup>4</sup>J<sub>H-H</sub> = 1.7 Hz, 1H, H4-aryl); 6.96 (dd, <sup>3</sup>J<sub>H-H</sub> = 7.5 Hz, <sup>4</sup>J<sub>H-H</sub> = 1.7 Hz, 1H, H6-aryl); 6.72 (dd, <sup>3</sup>J<sub>H-H</sub> = 7.5 Hz, 1H, H5-aryl); 5.38 (s(br), 1H, Bn-OH); 4.86 (sept, <sup>3</sup>J<sub>H-H</sub> = 6.3 Hz, 1H, H11); 4.56 (s, 2H, H7); 2.79 (t, <sup>3</sup>J<sub>H-H</sub> = 7.7 Hz, 2H, H8); 2.49 (t, <sup>3</sup>J<sub>H-H</sub> = 7.7 Hz, 2H, H9); 1.14 (d, <sup>3</sup>J<sub>H-H</sub> = 6.3 Hz, 6H, H12a,b); <sup>13</sup>C-NMR (125 MHz, DMSO-d<sub>6</sub>) δ: 172.2 (C10); 152.6 (C2); 128.4 (C4); 128.2 (C1); 127.3 (C3); 125.8 (C6); 119.3 (C5); 67.2 (C11); 60.2 (C7); 34.2 (C9); 25.5 (C8); 21.8 (C12a,b); UV (CH<sub>3</sub>CN) λ<sub>max</sub>: 275, 211 nm; IR (film) ν<sup>-1</sup> [cm<sup>-1</sup>]: 3352 (OH), 1705 (C = O), 1596, 1467, 1375, 1268, 1228, 1107, 1083, 1009, 940, 836, 778, 749.

**Benzyl-3-(2-hydroxy-3-hydroxymethyl-phenyl)propionate 6d:** The product was isolated as a yellow solid. Yield: 86%; TLC:  $R_f (CH_2Cl_2/MeOH 9:1 v/v)$ : 0.56;  $R_f (n-hexan/diethylether 1:1 v/v)$ : 0.31; <sup>1</sup>H-NMR (400 MHz, DMSO-d<sub>6</sub>)  $\delta$ : 8.49 (s(br), 1H, aryl-OH); 7.05 (dd, <sup>3</sup>J<sub>H-H</sub> = 7.8 Hz, <sup>4</sup>J<sub>H-H</sub> = 2.2 Hz, 1H, H4-aryl); 6.96 (dd, <sup>3</sup>J<sub>H-H</sub> = 7.6 Hz, <sup>4</sup>J<sub>H-H</sub> = 2.0 Hz, 1H, H6-aryl); 6.72 (dd, <sup>3</sup>J<sub>H-H</sub> = 7.5 Hz, 1H, H5-aryl); 5.37 (s(br), 1H, Bn-OH); 4.55 (s, 2H, H7); 2.76 (t, <sup>3</sup>J<sub>H-H</sub> = 7.8 Hz, 2H, H8); 2.43 (t, <sup>3</sup>J<sub>H-H</sub> = 7.7 Hz, 2H, H9); <sup>13</sup>C-NMR (101 MHz, DMSO-d<sub>6</sub>)  $\delta$ : 172.1 (C10); 152.6 (C2); 128.4 (C4); 128.2 (C1); 127.5 (C3); 125.8 (C6); 119.3 (C5); 79.8 (C11); 60.3 (C7); 35.1 (C9); 28.0 (C12a-c); 25.6 (C8).

*t*-Butyl-3-(2-hydroxy-3-hydroxymethyl-phenyl)propionate 6e: The product was isolated as a yellowish oil. Yield: 33%; TLC:  $R_f$  (CH<sub>2</sub>Cl<sub>2</sub>/MeOH 9:1 v/v): 0.77; <sup>1</sup>H-NMR (400 MHz, DMSO-d<sub>6</sub>)  $\delta$ : 8.52 (s(br), 1H, aryl-OH); 7.38–7.29 (m, 5H, H-pharyl); 7.07 (dd, <sup>3</sup>J<sub>H-H</sub> = 7.5 Hz, <sup>4</sup>J<sub>H-H</sub> = 1.8 Hz, 1H, H4-aryl); 6.96 (dd, <sup>3</sup>J<sub>H-H</sub> = 7.5 Hz, <sup>4</sup>J<sub>H-H</sub> = 1.9 Hz, 1H, H6-aryl); 6.72 (dd, <sup>3</sup>J<sub>H-H</sub> = 7.5 Hz, 1H, H5-aryl); 5.37 (t(br), <sup>3</sup>J<sub>H-H</sub> = 4.2 Hz, 1H, Bn-OH); 5.07 (s, 2H, H11); 4.55 (d, <sup>3</sup>J<sub>H-H</sub> = 4.2 Hz, 2H, H7); 2.83 (t, <sup>3</sup>J<sub>H-H</sub> = 7.7 Hz, 2H, H8); 2.61 (dd, <sup>3</sup>J<sub>H-H</sub> = 8.1 Hz, <sup>3</sup>J<sub>H-H</sub> = 7.1 Hz, 2H, H9); <sup>13</sup>C-NMR (101 MHz, DMSO-d<sub>6</sub>)  $\delta$ : 172.5 (C10); 152.6 (C2); 136.4 (C12); 128.6 (2 × C*m*-Ph); 128.4 (C4); 128.3 (C1); 128.1 (2 × C*o*-Ph); 127.2 (C3); 125.8 (C6); 119.4 (C5); 65.5 (C11); 60.2 (C7); 33.8 (C9); 25.4 (C8).

**Methyl-3-(3-hydroxymethyl-4-hydroxyphenyl)propionate 6f:** The product was isolated as a colorless solid. Yield: 80%; TLC:  $R_f$  (CH<sub>2</sub>Cl<sub>2</sub>/MeOH 9:1 v/v): 0.53; <sup>1</sup>H-NMR (400 MHz, DMSO-d<sub>6</sub>) δ: 9.09 (s(br), 1H, aryl-OH); 7.10 (d, <sup>4</sup>J<sub>H-H</sub> = 2.4 Hz, 1H, H6-aryl); 6.85 (dd, <sup>3</sup>J<sub>H-H</sub> = 8.1 Hz, <sup>4</sup>J<sub>H-H</sub> = 2.4 Hz, 1H, H4-aryl); 6.64 (d, <sup>3</sup>J<sub>H-H</sub> = 8.3 Hz, 1H, H3-aryl); 4.88 (t, <sup>3</sup>J<sub>H-H</sub> = 5.0 Hz, 1H, Bn-OH); 4.43 (d, <sup>3</sup>J<sub>H-H</sub> = 4.8 Hz, 2H, H7); 3.56 (s, 3H, H11); 2.73 (t, <sup>3</sup>J<sub>H-H</sub> = 7.7 Hz, 2H, H8); 2.53 (t, <sup>3</sup>J<sub>H-H</sub> = 7.6 Hz, 2H, H9); <sup>13</sup>C-NMR (101 MHz, DMSO-d<sub>6</sub>) δ: 172.9 (C10); 152.6 (C2); 130.5 (C5); 128.2 (C1); 127.3 (C4); 127.0 (C6); 114.6 (C3); 58.4 (C7); 51.4 (C11); 35.6 (C9); 29.9 (C8).

**2-Propyl-3-(3-hydroxymethyl-4-hydroxyphenyl)propionate 6g:** The product was isolated as a colorless oil. Yield: 93%; TLC:  $R_f$  (CH<sub>2</sub>Cl<sub>2</sub>/MeOH 9:1 v/v): 0.61; <sup>1</sup>H-NMR (400 MHz, DMSO-d<sub>6</sub>)  $\delta$ : 9.08 (s(br), 1H, aryl-OH); 7.10 (d, <sup>4</sup>J<sub>H-H</sub> = 2.3 Hz, 1H, H6-aryl); 6.85 (dd, <sup>3</sup>J<sub>H-H</sub> = 8.1 Hz, <sup>4</sup>J<sub>H-H</sub> = 2.4 Hz, 1H, H4-aryl); 6.64 (d, <sup>3</sup>J<sub>H-H</sub> = 8.1 Hz, 1H, H3-aryl); 4.85 (sept, <sup>3</sup>J<sub>H-H</sub> = 6.3 Hz, 2H, H11 + Bn-OH); 4.43 (d,

Downloaded by [University of Chicago Library] at 02:13 25 April 2013



 ${}^{3}J_{H-H} = 3.4$  Hz, 2H, H7); 2.72 (t,  ${}^{3}J_{H-H} = 7.5$  Hz, 2H, H8); 2.47 (t,  ${}^{3}J_{H-H} = 7.5$  Hz, 2H, H9); 1.14 (d,  ${}^{3}J_{H-H} = 6.3$  Hz, 6H, H-i-Pr);  ${}^{13}C$ -NMR (101 MHz, DMSO-d<sub>6</sub>)  $\delta$ : 172.0 (C10); 152.6 (C2); 130.5 (C5); 128.4 (C1); 127.4 (C4); 127.1 (C6); 114.6 (C3); 67.1 (C11); 58.4 (C7); 36.2 (C9); 30.0 (C8); 21.8 (C-i-Pr).

**Benzyl-3-(3-hydroxymethyl-4-hydroxyphenyl)propionate 6h:** The product was isolated as a yellowish solid. Yield: 87%; TLC:  $R_f (CH_2Cl_2/MeOH 9:1 v/v)$ : 0.60; <sup>1</sup>H-NMR (400 MHz, DMSO-d<sub>6</sub>)  $\delta$ : 9.10 (s(br), 1H, aryl-OH); 7.10 (d, <sup>4</sup>J<sub>H-H</sub> = 2.3 Hz, 1H, H6-aryl); 6.85 (dd, <sup>3</sup>J<sub>H-H</sub> = 8.2 Hz, <sup>4</sup>J<sub>H-H</sub> = 2.3 Hz, 1H, H4-aryl); 6.63 (d, <sup>3</sup>J<sub>H-H</sub> = 8.1 Hz, 1H, H3-aryl); 4.88 (t, <sup>3</sup>J<sub>H-H</sub> = 4.9 Hz, 1H, Bn-OH); 4.42 (d, <sup>3</sup>J<sub>H-H</sub> = 4.9 Hz, 2H, H7); 2.68 (t, <sup>3</sup>J<sub>H-H</sub> = 7.5 Hz, 2H, H8); 2.41 (t, <sup>3</sup>J<sub>H-H</sub> = 7.5 Hz, 2H, H9); 1.35 (s, 9H, H12a-c); <sup>13</sup>C-NMR (101 MHz, DMSO-d<sub>6</sub>)  $\delta$ : 173.7 (C10); 152.5 (C2); 130.6 (C5); 128.4 (C1); 127.4 (C4); 127.1 (C6); 114.5 (C3); 79.7 (C11); 58.4 (C7); 37.1 (C9); 30.2 (C8); 27.9 (C12a-c).

*t*-Butyl-3-(3-hydroxymethyl-4-hydroxyphenyl)propionate 6i: The product was isolated as a yellowish solid. Yield: 87%; TLC:  $R_f (CH_2Cl_2/MeOH 9:1 v/v)$ : 0.60; <sup>1</sup>H-NMR (400 MHz, DMSO-d<sub>6</sub>)  $\delta$ : 9.10 (s(br), 1H, aryl-OH); 7.10 (d, <sup>4</sup>J<sub>H-H</sub> = 2.3 Hz, 1H, H6-aryl); 6.85 (dd, <sup>3</sup>J<sub>H-H</sub> = 8.2 Hz, <sup>4</sup>J<sub>H-H</sub> = 2.3 Hz, 1H, H4-aryl); 6.63 (d, <sup>3</sup>J<sub>H-H</sub> = 8.1 Hz, 1H, H3-aryl); 4.88 (t, <sup>3</sup>J<sub>H-H</sub> = 4.9 Hz, 1H, Bn-OH); 4.42 (d, <sup>3</sup>J<sub>H-H</sub> = 4.9 Hz, 2H, H7); 2.68 (t, <sup>3</sup>J<sub>H-H</sub> = 7.5 Hz, 2H, H8); 2.41 (t, <sup>3</sup>J<sub>H-H</sub> = 7.5 Hz, 2H, H9); 1.35 (s, 9H, H-tBu); <sup>13</sup>C-NMR (101 MHz, DMSO-d<sub>6</sub>)  $\delta$ : 173.7 (C10); 152.5 (C2); 130.6 (C5); 128.4 (C1); 127.4 (C4); 127.1 (C6); 114.5 (C3); 79.7 (C11); 58.4 (C7); 37.1 (C9); 30.2 (C8); 27.9 (C-tBu).

**2-(2-Hydroxy-3-hydroxymethylphenyl)ethylacetate 6j:** The product was isolated as a colorless oil. Yield: 81%; TLC:  $R_f$  (CH<sub>2</sub>Cl<sub>2</sub>/MeOH 9:1 v/v): 0.51; <sup>1</sup>H-NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$ : 7.73 (s(br), 1H, aryl-OH); 7.09 (dd, <sup>3</sup>J<sub>H-H</sub> = 7.5 Hz, <sup>4</sup>J<sub>H-H</sub> = 1.4 Hz, 1H, H4-aryl); 6.95 (dd, <sup>3</sup>J<sub>H-H</sub> = 7.5 Hz, <sup>4</sup>J<sub>H-H</sub> = 1.4 Hz, 1H, H6-aryl); 6.80 (dd,  $2\cdot^{3}J_{H-H} = 7.5$  Hz, 1H, H5-aryl); 4.86 (s, 2H, H7); 4.31 (t, <sup>3</sup>J<sub>H-H</sub> = 7.1 Hz, 2H, H9); 2.98 (t, <sup>3</sup>J<sub>H-H</sub> = 7.1 Hz, 2H, H8); 2.05 (s, 3H, H11); <sup>13</sup>C-NMR (101 MHz, DMSO-d<sub>6</sub>)  $\delta$ : 170.6 (C10); 153.0 (C2); 129.8 (C4); 128.0 (C6); 125.6 (C1); 125.3 (C3); 119.7 (C5); 63.5 (C9); 60.3 (C7); 29.6 (C8); 21.1 (C11).

**2-(2-Hydroxy-3-hydroxymethylphenyl)ethyllevulinate 6k:** The product was isolated as a yellowish oil. Yield: 14%; TLC:  $R_f (CH_2Cl_2/MeOH 9:1 v/v): 0.53; {}^{1}H-NMR (400 MHz, DMSO-d_6) \delta: 2.03 (s, 3H, H14), 2.43 (t, 2H, {}^{3}J_{HH} = 6.5 Hz, H12), 2.68 (t, 2H, {}^{3}J_{HH} = 6.5 Hz, H11), 2.86 (t, 2H, {}^{3}J_{HH} = 7.0 Hz, H8), 4.15 (t, 2H, {}^{3}J_{HH} = 7.0 Hz, H9), 4.59 (s, 2H, H7), 5.30-5.70 (br, 1H, benzyl-OH), 6.76 (dd, 1H, {}^{3}J_{HH} = 7.4 Hz, {}^{4}J_{HH} = 1.3 Hz, H4), 6.77 (dd, 1H, {}^{3}J_{HH} = 7.5 Hz, {}^{4}J_{HH} = 1.3 Hz, H6), 6.99-7.11 (m, 2H, H4, H6), 8.40-8.80 (br, 1H, aryl-OH); {}^{13}C-NMR (100 MHz, DMSO-d_6) \delta: 28.01 (C11), 29.47 (C8), 29.89 (C14), 37.75 (C12), 60.37 (C7), 63.72 (C9), 119.58 (C5), 122.54 (C1)124.77 (C3), 126.31(C6), 129.26 (C4), 153.02. (C2), 172.55 (C10), 207.14 (C13); MS (ESI<sup>+</sup>, m/z): calc.: 266.12 (M), found: 289.15 (M + Na<sup>+</sup>).$ 

**2-(2-Hydroxy-3-hydroxymethylphenyl)ethylpivalate 61:** The product was isolated as a yellowish oil. Yield: 86%; TLC:  $R_f$  (CH<sub>2</sub>Cl<sub>2</sub>/MeOH 9:1 v/v): 0.70; <sup>1</sup>H-NMR

Marcel Dekker, Inc.

270 Madison Avenue, New York, New York 10016



(400 MHz, DMSO-d<sub>6</sub>)  $\delta$ : 7.08 (dd,  ${}^{3}J_{H-H} = 7.5$  Hz,  ${}^{4}J_{H-H} = 1.8$  Hz, 1H, H4-aryl); 6.98 (dd,  ${}^{3}J_{H-H} = 7.5$  Hz,  ${}^{4}J_{H-H} = 1.8$  Hz, 1H, H6-aryl); 6.74 (dd,  ${}^{2}{}^{3}J_{H-H} = 7.5$  Hz, 1H, H5-aryl); 4.56 (s, 2H, H7); 4.15 (t,  ${}^{3}J_{H-H} = 6.9$  Hz, 2H, H9); 2.86 (t,  ${}^{3}J_{H-H} = 6.9$  Hz, 2H, H8); 1.08 (s, 9H, H12a-c);  ${}^{13}$ C-NMR (101 MHz, DMSO-d<sub>6</sub>)  $\delta$ : 177.6 (C10); 152.8 (C2); 129.4 (C4); 128.3 (C1); 126.1 (C6); 124.7 (C3); 119.3 (C5); 63.5 (C9); 60.2 (C7); 38.3 (C11); 29.3 (C8); 27.1 (C12a-c).

**2-(3-Hydroxymethyl-4-hydroxyphenyl)ethylacetate 6m:** The product was isolated as a colorless solid. Yield: 95%; TLC:  $R_f$  (CH<sub>2</sub>Cl<sub>2</sub>/MeOH 9:1 v/v): 0.51; <sup>1</sup>H-NMR (400 MHz, DMSO-d<sub>6</sub>)  $\delta$ : 9.16 (s, 1H, aryl-OH); 7.13 (d, <sup>4</sup>J<sub>H-H</sub> = 2.1 Hz, 1H, H6-aryl); 6.89 (dd, <sup>3</sup>J<sub>H-H</sub> = 8.1 Hz, <sup>4</sup>J<sub>H-H</sub> = 2.3 Hz, 1H, H4-aryl); 6.67 (d, <sup>3</sup>J<sub>H-H</sub> = 8.1 Hz, <sup>1</sup>H, H3-aryl); 4.92 (t, <sup>3</sup>J<sub>H-H</sub> = 5.5 Hz, 1H, Bn-OH); 4.44 (d, <sup>3</sup>J<sub>H-H</sub> = 5.4 Hz, 2H, H7); 4.11 (t, <sup>3</sup>J<sub>H-H</sub> = 7.1 Hz, 2H, H9); 2.75 (t, <sup>3</sup>J<sub>H-H</sub> = 7.1 Hz, 2H, H8); 1.97 (s, 3H, H11); <sup>13</sup>C-NMR (101 MHz, DMSO-d<sub>6</sub>)  $\delta$ : 170.5 (C10); 152.8 (C2); 128.6 (C5); 127.9 (C1); 127.8 (C4); 127.7 (C6); 114.7 (C3); 65.0 (C9); 58.4 (C7); 34.0 (C8); 20.9 (C11).

**2-(3-Hydroxymethyl-4-hydroxyphenyl)ethylacetate 6n:** The product was isolated as a yellowish solid. Yield: 72%; TLC:  $R_f$  (CH<sub>2</sub>Cl<sub>2</sub>/MeOH 9:1 v/v): 0.61; <sup>1</sup>H-NMR (400 MHz, DMSO-d<sub>6</sub>)  $\delta$ : 9.14 (s, 1H, aryl-OH); 7.14 (d, <sup>4</sup>J<sub>H-H</sub> = 2.1 Hz, 1H, H6-aryl); 6.88 (dd, <sup>3</sup>J<sub>H-H</sub> = 8.0 Hz, <sup>4</sup>J<sub>H-H</sub> = 2.3 Hz, 1H, H4-aryl); 6.66 (d, <sup>3</sup>J<sub>H-H</sub> = 8.1 Hz, 1H, H3-aryl); 4.90 (t, <sup>3</sup>J<sub>H-H</sub> = 5.5 Hz, 1H, Bn-OH); 4.43 (d, <sup>3</sup>J<sub>H-H</sub> = 5.5 Hz, 2H, H7); 4.12 (t, <sup>3</sup>J<sub>H-H</sub> = 6.9 Hz, 2H, H9); 2.75 (t, <sup>3</sup>J<sub>H-H</sub> = 6.7 Hz, 2H, H8); 1.09 (s, 9H, H12a-c); <sup>13</sup>C-NMR (101 MHz, DMSO-d<sub>6</sub>)  $\delta$ : 177.5 (C10); 152.8 (C2); 130.6 (C5); 128.0 (C4); 128.0 (C1); 127.8 (C6); 114.5 (C3); 65.1 (C9); 58.4 (C7); 34.0 (C8); 30.9 (C11); 27.1 (C12a-c).

General procedure for the synthesis of the cyclic chlorophosphanes 13: The reactions have been carried out as described previously in references 7 and 23. In all cases, diethylether has been used as solvent. After evaporation of the solvent, the chlorophosphanes were obtained as slightly yellow oils. <sup>31</sup>P-NMR charcterisation showed in all cases a resonance signal at ~140 ppm. The crude reaction products have been use directly for the synthesis of the triesters.

General procedure for the synthesis of the *cycloSal*-triesters 2–5: The reactions have been carried out as described previously in reference 7. In all cases, acetonitrile has been used as solvent. All reactions have been carried out in an inert atmosphere at  $-20^{\circ}$ C. Two equivalents of the chlorophosphites 13 have been used. Prior to the reaction, d4T 1 has been coevaporated twice with pyridine. Purification has been done by chromatography on a Chromatotron on silica gel with EtOAc/MeOH and CH<sub>2</sub>Cl<sub>2</sub>/MeOH gradients.

**3-Methylpropionyl-***cyclo***Sal-d4TMP 3a:** The product was isolated as a colorless foam. Yield: 32%; TLC:  $R_f$  (CH<sub>2</sub>Cl<sub>2</sub>/MeOH) 9 : 1 v/v) 0.57; <sup>1</sup>H-NMR (400 MHz, DMSO-d<sub>6</sub>)  $\delta$ : 11.33, 11.32 (2s, 1H, NH), 7.26 (ddd, 1H, H4-aryl), 7.20 (q 1H<sub>1.D</sub>, H6), 7.18 (q, 1H<sub>2.D</sub>, H6), 7.16–7.08 (m, 2H, H6-aryl + H5-aryl), 6.79 (ddd, 1H<sub>1.D</sub>, H1'), 6.79 (ddd, 1H<sub>2.D</sub>, H1'), 6.40, 6.36 (2 × ddd 1H, H3'), 6.02 (ddd, 1H<sub>1.D</sub>, H2'), 6.00 (ddd, 1H<sub>2.D</sub>, H2'), 5.48 (dd, 1H<sub>1.D</sub>, H7-benzyl), 5.44 (dd, 1H<sub>1.D</sub>, H7-benzyl), 5.38 (dd





Downloaded by [University of Chicago Library] at 02:13 25 April 2013

1H<sub>2.D</sub>, H7-benzyl), 5.35 (dd, 1H<sub>2.D</sub>, H7-benzyl), 4.98–4.93 (m, 1H, H4'), 4.34–4.24 (m, 2H, H5'), 3.57, 3.56 (2s, 3H, H11), 2.90–2.75 (m, 2H, H8), 2.59 (dd, 2H<sub>1.D</sub>, H9), 2.57 (dd, 2H<sub>2.D</sub>, H9), 1.63 (d, 3H<sub>1.D</sub>, H7), 1.59 (d, 3H<sub>2.D</sub>, H7); <sup>13</sup>C-NMR (101 MHz, DMSO-d<sub>6</sub>)  $\delta$  172.5 (2 × C10), 163.9 (C4), 150.9, 150.8 (2 × C2), 148.1 (d, C2<sub>1.D</sub>-aryl), 147.9 (d, C2<sub>2.D</sub>-aryl), 135.8, 135.7 (2 × C6), 133.0, 132.9 (2 × C3'), 130.3, 130.2 (2 × C4-aryl), 129.6, 129.5 (2 × C1-aryl), 127.6, 127.5 (2 × C2'), 124.5 (2 × C6-aryl), 124.3 (C5-aryl), 121.7, 121.6 (2 × C3-aryl), 109.9, 109.8 (2 × C5), 89.4, 89.3 (2 × C1'), 84.3 (2 × C4'), 68.8 (d, C5'\_{1.D}), 68.8 (d, C5'\_{2.D}), 68.4 (2d C7-benzyl), 51.5 (2 × C11), 33.3, 33.2 (2 × C9), 24.3, 24.2 (2 × C8), 12.0, 11.9 (2 × C7); <sup>31</sup>P-NMR (202 MHz, DMSO-d<sub>6</sub>)  $\delta$  – 7.53, –7.60; HPLC: t<sub>R</sub> = 11.3, 11.5 min (method I); t<sub>R</sub> = 10.4 min (method II); MS (ESI<sup>+</sup>, m/z): calc. 478.2 (M), found 478.9 (M + H<sup>+</sup>).

3-EtPr-cycloSal-d4TMP 3b: The product was isolated as a colorless foam. Yield: 35%; TLC: R<sub>f</sub> (CH<sub>2</sub>Cl<sub>2</sub>/MeOH 9:1 v/v): 0.58; <sup>1</sup>H-NMR: (500 MHz, DMSO-d<sub>6</sub>) δ: 11.32, 11.31 (2s, 1H, NH); 7.26 (dddd,  ${}^{3}J_{H-H} = 7.1$  Hz,  $3 \times {}^{4}J_{H-H} = 2.0$  Hz, 1H, H4aryl); 7.20 (q,  ${}^{4}J_{H-H}$  = 1.2 Hz, 1H<sub>1.D</sub>, H6); 7.18 (q,  ${}^{4}J_{H-H}$  = 1.3 Hz, 1H<sub>2.D</sub>, H6); 7.15– 7.08 (m, 2H, H6-aryl + H5-aryl); 6.80-6.78 (m, 1H, H1'); 6.40, 6.36 (2ddd,  ${}^{3}J_{H-H} = 6.0$  Hz,  ${}^{3}J_{H-H} = 1.8$  Hz,  ${}^{4}J_{H-H} = 1.8$  Hz, 1H, H3'); 6.02 (ddd,  ${}^{3}J_{H-H} = 6.0$ Hz,  ${}^{3}J_{H-H} = 2.5$  Hz,  ${}^{4}J_{H-H} = 1.3$  Hz,  $1H_{1,D}$ , H2'); 6.00 (ddd,  ${}^{3}J_{H-H} = 5.9$  Hz,  ${}^{3}J_{H-H} = 5.9$  $_{\rm H}$  = 2.4 Hz,  ${}^{4}J_{\rm H-H}$  = 1.3 Hz, 1H<sub>2.D</sub>, H2'); 5.48 (dd,  ${}^{2}J_{\rm H-H}$  = 14.2 Hz,  ${}^{3}J_{\rm H-P}$  = 5.6 Hz,  $1H_{1,D}$ , H7-benzyl); 5.44 (dd,  ${}^{2}J_{H-H} = 14.2$  Hz,  ${}^{3}J_{H-P} = 5.3$  Hz,  $1H_{1,D}$ , H7-benzyl); 5.38  ${}^{3}J_{H-P} = 5.2 \text{ Hz}, 1H_{2.D}, H7\text{-benzyl}; 4.97-4.93 (m, 1H, H4'); 4.33-4.25 (m, 2H, H5');$ 4.03, 4.02 (2q,  ${}^{3}J_{H-H} = 7.1$  Hz, 2H, H11); 2.88–2.77 (m, 2H, H8); 2.57 (dd,  $2 \times {}^{3}J_{H-H} = 7.4$  Hz, 2H, H9); 1.63, 1.58 (2d,  ${}^{4}J_{H-H} = 1.3$  Hz, 3H, H7); 1.14, 1.13 (2dd,  ${}^{3}J_{H-H}$  = 7.1 Hz, 3H, H12);  ${}^{13}C$ -NMR (101 MHz, DMSO-d<sub>6</sub>)  $\delta$ : 173.1 (2 × C10); 163.9 (C4); 150.9, 150.8 (2 × C2); 148.1, 147.9 (2d,  ${}^{2}J_{C-P} = 7.1$  Hz, C2-aryl); 135.8, 135.7  $(2 \times C6)$ ; 133.0, 132.9  $(2 \times C3')$ ; 130.3  $(2 \times C4$ -aryl); 129.7, 129.6  $(2 \times C1$ -aryl); 127.6, 127.5 (2 × C2'); 124.5 (2 × C6-Aryl); 124.3 (C5-aryl); 121.7, 121.6 (2 × C3aryl); 109.9, 109.8 (2 × C5); 89.4, 89.3 (2 × C1'); 84.3 (2 × C4'); 68.8 (2d,  ${}^{2}J_{C-P} = 6.1$  Hz, C5'); 68.4 (2d,  ${}^{2}J_{C-P} = 6.6$  Hz, C7-benzyl); 60.1 (C11); 33.5, 33.4  $(2 \times C9)$ ; 24.3, 24.2  $(2 \times C8)$ ; 14.2 (C12); 12.0, 11.9  $(2 \times C7)$ ; <sup>31</sup>P-NMR (202 MHz, DMSO-d<sub>6</sub>)  $\delta$ : - 7.52, -7.57; HPLC: t<sub>R</sub> = 12.8 min (method I); t<sub>R</sub> = 11.3 min (method II); ); MS (ESI<sup>+</sup>, m/z): calc. 492.1298 (M), found 493.3 (M + H<sup>+</sup>).

**3-i-PrPr-***cyclo***Sal-d4TMP 3c:** The product was isolated as a colorless foam. Yield: 54%; TLC:  $R_f (CH_2Cl_2/MeOH 9:1 v/v): 0.60; {}^{1}H-NMR (500 MHz, DMSO-d_6) \delta: 11.35, 11.34 (2s, 1H, NH); 7.27-7.23 (m, 1H, H4-aryl); 7.20, 7.18 (2q, {}^{4}J_{H-H} = 1.1 Hz, 1H, H6); 7.15-7.08 (m, 2H, H5-, H6-aryl); 6.80-6.78 (m, 1H, H1'); 6.40 (ddd, {}^{3}J_{H-H} = 6.0 Hz, {}^{3}J_{H-H} = 1.8 Hz, {}^{4}J_{H-H} = 1.8 Hz, 1H_{1.D}, H3'); 6.36 (ddd, {}^{3}J_{H-H} = 5.9 Hz, {}^{3}J_{H-H} = 1.8 Hz, {}^{4}J_{H-H} = 1.8 Hz, 1H_{2.D}, H3'); 6.02 (ddd, {}^{3}J_{H-H} = 6.1 Hz, {}^{3}J_{H-H} = 2.3 Hz, {}^{4}J_{H-H} = 1.3 Hz, 1H_{1.D}, H2'); 6.00 (ddd, {}^{3}J_{H-H} = 6.0 Hz, {}^{3}J_{H-H} = 2.5 Hz, {}^{4}J_{H-H} = 1.3 Hz, 1H_{2.D}, H2'); 5.48 (dd, {}^{2}J_{H-H} = 14.1 Hz, {}^{3}J_{H-P} = 4.8 Hz, 1H_{1.D}, H7-benzyl); 5.38 (dd, {}^{2}J_{H-H} = 12.4 Hz, {}^{3}J_{H-P} = 4.8 Hz, 1H_{2.D}, H7-benzyl); 5.34 (dd, {}^{2}J_{H-H} = 12.6 Hz, {}^{3}J_{H-P} = 4.5 Hz, 1H_{2.D}, H7-benzyl); 4.97-4.93 (m, 1H, H4'); 4.85, 4.84 (2sept, 1)$ 

ORDER		REPRINTS
-------	--	----------

<sup>3</sup>J<sub>H-H</sub> = 6.3 Hz, 1H, H11); 4.32–4.25 (m, 2H, H5'); 2.87–2.77 (m, 2H, H8); 2.54 (2dd, <sup>3</sup>J<sub>H-H</sub> = 7.3 Hz, 2H, H9); 1.63, 1.57 (2d, <sup>4</sup>J<sub>H-H</sub> = 1.1 Hz, 3H, H7); 1.14–1.11 (m, 6H, H12a,b); <sup>13</sup>C-NMR (101 MHz, DMSO-d<sub>6</sub>) δ: 171.6, 171.5 (2 × C10); 163.9 (C4); 150.9, 150.8 (2 × C2); 148.0 (d, <sup>2</sup>J<sub>C-P</sub> = 7.6 Hz, C2-aryl); 147.9 (d, <sup>2</sup>J<sub>C-P</sub> = 8.1 Hz, C2-aryl); 135.8 (2 × C6); 133.0 132.9 (2 × C3'); 130.3 (2 × C4-aryl); 129.6 (2 × C1-aryl); 127.6, 127.5 (2 × C2'); 124.5 (2 × C6-aryl); 124.3 (C5-aryl); 121.7 (C3-aryl); 109.9, 109.8 (2 × C5); 89.4, 89.3 (2 × C1'); 84.4, 84.3 (2 × C4'); 68.8, 68.7 (2 × C5'); 68.4 (d, <sup>2</sup>J<sub>C-P</sub> = 7.6 Hz, C7-benzyl); 68.4 (d, <sup>2</sup>J<sub>C-P</sub> = 6.6 Hz, C7-benzyl); 67.5 (C11); 33.7 (2 × C9); 24.3 (2 × C8); 21.7 (C12a,b); 12.0, 11.9 (2 × C7); <sup>31</sup>P-NMR (202 MHz, DMSO-d<sub>6</sub>) δ: -7.50, -7.53; HPLC: t<sub>R</sub> = 13.7 min (method I) t<sub>R</sub> = 12.3 min (method II); ); MS (ESI<sup>+</sup>, m/z): calc. 506.1454 (M), found 507.2 (M + H<sup>+</sup>).

3-Benzylpropionyl-cycloSal-d4TMP 3d: The product was isolated as a colorless foam. Yield 43%; <sup>1</sup>H-NMR (500 MHz, DMSO-d<sub>6</sub>) δ 11.33, 11.31 (2s, 1H, NH), 7.36-7.28 (m, 5H, Ph) 7.26-7.23 (m, 1H, H4-aryl), 7.18 (q, 1H<sub>1.D</sub>, H6), 7.17 (q, 1H<sub>2.D</sub>, H6), 7.15–7.12 (m, 2H, H6-aryl), 7.09, 7.08 (2dd 1H, H5-aryl) 6.79–6.77 (m, 1H, H1'), 6.37 (ddd, 1H<sub>1.D</sub>, H3'), 6.32 (ddd, 1H<sub>2.D</sub>, H3'), 5.99 (ddd, 1H<sub>1.D</sub>, H2'), 5.97 (ddd, 1H<sub>2.D</sub>, H2'), 5.47 (dd, 1H<sub>1.D</sub>, H7-benzyl), 5.44 (dd, 1H<sub>1.D</sub>, H7-benzyl), 5.37 (dd, 1H<sub>2.D</sub>, H7-benzyl), 5.34 (dd, 1H<sub>2.D</sub>, H7-benzyl), 5.07, 5.06 (2s, 2H, H11) 4.94-4.90 (m, 1H, H4'), 4.32-4.22 (m, 2H, H5'), 2.92-2.80 (m, 2H, H8), 2.68-2.62 (m, 2H, H9), 1.64, 1.58 (2 × d, 3H, H7);  $^{13}$ C-NMR (101 MHz, DMSO-d<sub>6</sub>)  $\delta$  172.4, 172,3  $(2 \times C10)$ , 163.9 (C4), 150.8, 150.7  $(2 \times C2)$ , 148.0 (2d, C2-aryl), 135.8, 135.7  $(2 \times C6), 132.9, (2 \times C3'), 131.2, (C12), 130.3, (2 \times C4-aryl), 129.5, (2 \times C1-aryl).$ 128.6 (2 × Cm-Ph), 128.1 (2 × Co-Ph), 128.1 (Cp-Ph), 127.5 (2 × C2'), 124.5 (2 × C6-aryl), 124.3 (C5-aryl), 121.7 (C3-aryl), 109.9, 109.8 (2 × C5), 89.4, 89.3  $(2 \times C1')$ , 84.3, 84.2  $(2 \times C4')$ , 68.7  $(2 \times C5')$ , 68.4 (d, C7-benzyl), 68.4 (d, C7-benzyl), 65.7 (C11), 33.4 (2 × C9), 24.3, 24.2 (2 × C8), 12.0, 11.9 (2 × C7); <sup>31</sup>P-NMR (202 MHz, DMSO-d<sub>6</sub>)  $\delta$  - 7.57 (only 1 peak); R<sub>f</sub> 0.64 (CH<sub>2</sub>Cl<sub>2</sub>/MeOH) 9 : 1 v/v); HPLC:  $t_{R} = 15.0$  min (method I);  $t_{R} = 13.6$  min (method II); MS (FAB, m/z): calc. 554.15 (M), found 555.2 (M + H<sup>+</sup>), 555.1532 (M + H<sup>+</sup>, FAB-HR).

3-t-Butylpropionyl-cycloSal-d4TMP 3e: The product was isolated as a colorless foam. Yield 73%; <sup>1</sup>H-NMR (500 MHz, DMSO-d<sub>6</sub>) δ 11.32, 11.31 (2s, 1H, NH), 7.27– 7.24 (m, 1H, H4-aryl), 7.20, 7.18 (2q, 1H, H6), 7.15–7.08 (m, 2H, H6-aryl + H5-aryl), 6.80–6.78 (m, 1H, H1'), 6.40, 6.36 (2ddd, 1H H3'), 6.02 (ddd, 1H<sub>1.D</sub>, H2'), 6.00 (ddd, 1H<sub>2.D</sub>, H2'), 5.47 (dd, 1H<sub>1.D</sub>, H7-benzyl), 5.44 (dd, 1H<sub>1.D</sub>, H7-benzyl), 5.37 (dd, 1H<sub>2.D</sub>, H7-benzyl), 5.35 (dd, 1H<sub>2.D</sub>, H7-benzyl), 4.97-4.93 (m, 1H, H4'), 4.34-4.25 (m, 2H, H5'), 2.87-2.73 (m, 2H, H8), 2.47 (dd, 2H<sub>1.D</sub>, H9), 2.46 (dd, 2H<sub>2.D</sub>, H9), 1.64, 1.57 (2d, 3H, H7), 1.35, 1.34 (2s, 9H, H12a-c); <sup>13</sup>C-NMR (101 MHz, DMSO-d<sub>6</sub>) δ 171.4, 171,3  $(2 \times C10)$ , 163.9 (C4), 150.8  $(2 \times C2)$ , 148.0 (d, C2-aryl), 148.0 (d, C2-aryl), 135.8, 135.7 (2 × C6), 133.0 132.9 (2 × C3'), 130.3 (2 × C4-aryl), 129.7, 129.6 (2 × C1aryl), 127.5 (2 × C2'), 124.4 (2 × C6-aryl), 124.2 (C5-aryl), 121.7, 121.6 (C3-aryl), 109.8 (2 × C5), 89.3 (2 × C1'), 84.3 (2 × C4'), 80.0 (C11), 68.8 (d, C5'), 68.7 (d, C5'), 68.4 (d, C7-benzyl), 68.4 (d, C7-benzyl), 34.6, 34.5 (2 × C9), 27.9 (12a-c), 24.4  $(2 \times C8)$ , 12.0, 11.9  $(2 \times C7)$ ; <sup>31</sup>P-NMR (202 MHz, DMSO-d<sub>6</sub>)  $\delta$  -7.50, -7.53; R<sub>f</sub> 0.46 (CH<sub>2</sub>Cl<sub>2</sub>/MeOH) 9 : 1 v/v); HPLC:  $t_{R} = 14.7$  min (method I);  $t_{R} = 13.2$  min (method II); MS (FAB, m/z): calc. 520.1611 (M), found 521.2 (M + H<sup>+</sup>).





**5-Methylpropionyl-***cyclo***Sal-d4TMP 3f:** The product was isolated as a colorless foam. Yield: 39%; TLC: R<sub>f</sub> (CH<sub>2</sub>Cl<sub>2</sub>/MeOH 9 : 1 v/v) 0.52; <sup>1</sup>H-NMR (500 MHz, DMSO-d<sub>6</sub>) δ 11.33, 11.32 (2s, 1H, NH), 7.23-7.18 (m, 1H, H4-aryl), 7.18, 7.15 (2q, 1H, H6), 7.13-7.11 (m, 1H, H6-aryl), 7.03, 7.00 (2d, 1H, H3-aryl), 6.79, 6.78 (2ddd, 1H, H1'), 6.40 (ddd, 1H<sub>1.D</sub>, H3'), 6.34 (ddd, 1H<sub>2.D</sub>, H3'), 6.01 (ddd, 1H<sub>1.D</sub>, H2'), 5.99 (ddd, 1H<sub>2.D</sub>, H2'), 5.45 (dd, 1H<sub>1.D</sub>, H7-benzyl), 5.41 (dd, 1H<sub>1.D</sub>, H7-benzyl), 5.35 (d(br), 1H<sub>2.D</sub>, H7-benzyl), 5.33 (d(br), 1H<sub>2.D</sub>, H7-benzyl), 4.96-4.91 (m, 1H, H4'), 4.34-4.22 (m, 2H, H5'), 3.57 (s, 3H, H11), 2.81 (dd, 2H, H8), 2.60 (dd, 2H, H9), 1.66, 1.59 (2d, 3H, H7); <sup>13</sup>C-NMR (101 MHz, DMSO-d<sub>6</sub>)  $\delta$  172.6 (2 × C10), 163.9 (2 × C4), 150.8 (C2), 148.0 (2d, C2-aryl), 137.0 (C5-aryl), 135.8 (2 × C6), 133.0, 132.9 (2 × C3'), 129.8, 129.7 (2 × C4-aryl), 129.0, 128.9 (2 × C1-aryl), 127.5, 127.4  $(2 \times C2')$ , 125.9  $(2 \times C6$ -aryl), 118.2, 118.1  $(2 \times C3$ -aryl), 109.8  $(2 \times C5)$ , 89.3 (C1'), 84.3, 84.2 (2 × C4'), 68.5 (d, C5'<sub>1.D</sub>), 68.5 (d, C5'<sub>2.D</sub>), 68.4 (2d, C7-benzyl), 51.5 (C11), 34.8 (C9), 29.5 (C8), 12.1, 12.0 (2  $\times$  C7); <sup>31</sup>P-NMR (202 MHz, DMSO-d<sub>6</sub>)  $\delta$ -7.98, -8.04; HPLC:  $t_R = 11.9$ , 12.0 min (method I);  $t_R = 10.6$  min (method II); MS (FAB, m/z): calc. 478.1141 (M), found 479.4 (M +  $H^+$ ).

5-i-PrPr-cycloSal-d4TMP 3g: The product was isolated as a colorless foam. Yield: 33%; TLC R<sub>f</sub> (CH<sub>2</sub>Cl<sub>2</sub>/MeOH 9:1 v/v): 0.63; <sup>1</sup>H-NMR (500 MHz, DMSO-d<sub>6</sub>)  $\delta$ : 11.32, 11.31 (2s, 1H, NH); 7.24–7.18 (m, 1H, H4-Aryl); 7.18, 7.15 (2q,  ${}^{4}J_{H-H} = 1.1$  Hz, 1H, H6); 7.13–7.11 (m, 1H, H6-aryl); 7.03 (d,  ${}^{3}J_{H-H} = 8.6$  Hz,  $1H_{1.D}$ , H3-aryl); 7.00 (d,  ${}^{3}J_{H-H} = 8.4 \text{ Hz}, 1H_{2.D}, \text{H3-aryl}); 6.79 \text{ (ddd, } {}^{3}J_{H-H} = 3.6 \text{ Hz}, 2 \times {}^{4}J_{H-H} = 1.8 \text{ Hz}, 1H_{1.D},$ H1'); 6.77 (ddd,  ${}^{3}J_{H-H} = 3.7$  Hz,  $2 \times {}^{4}J_{H-H} = 1.9$  Hz,  $1H_{2,D}$ , H1'); 6.40 (ddd,  ${}^{3}J_{H-H} = 6.0$ Hz,  ${}^{3}J_{H-H} = 1.8$  Hz,  ${}^{4}J_{H-H} = 1.8$  Hz,  $1H_{1,D}$ , H3'); 6.33 (ddd,  ${}^{3}J_{H-H} = 5.9$  Hz,  ${}^{3}J_{H-H} = 5.9$ 1.7 Hz,  ${}^{4}J_{H-H} = 1.7$  Hz,  ${}^{1}H_{2.D}$ , H3'); 6.00 (ddd,  ${}^{3}J_{H-H} = 5.6$  Hz,  ${}^{3}J_{H-H} = 2.0$  Hz,  ${}^{4}J_{H-H} = 2.0$  Hz,  ${}^{4}J_{H-H} = 2.0$  Hz,  ${}^{1}H_{1.D}$ , H2'); 5.99 (ddd,  ${}^{3}J_{H-H} = 6.0$  Hz,  ${}^{3}J_{H-H} = 2.3$  Hz,  ${}^{4}J_{H-H} = 1.5$  Hz,  ${}^{1}H_{2.D}$ , H2'); 5.45 (dd,  ${}^{2}J_{H-H} = 14.4$  Hz,  ${}^{3}J_{H-P} = 4.6$  Hz, 1H<sub>1.D</sub>, H7-benzyl); 5.40 (dd,  ${}^{2}J_{H-H} = 14.4$  Hz,  ${}^{3}J_{H-P} = 4.6$  Hz, 1H<sub>1.D</sub>, H7-benzyl); 5.40 (dd,  ${}^{2}J_{H-H} = 14.4$  Hz,  ${}^{3}J_{H-P} = 4.6$  Hz, 1H<sub>1.D</sub>, H7-benzyl); 5.40 (dd,  ${}^{2}J_{H-H} = 14.4$  Hz,  ${}^{3}J_{H-P} = 4.6$  Hz, 1H<sub>1.D</sub>, H7-benzyl); 5.40 (dd,  ${}^{2}J_{H-H} = 14.4$  Hz,  ${}^{3}J_{H-P} = 4.6$  Hz, 1H<sub>1.D</sub>, H7-benzyl); 5.40 (dd,  ${}^{2}J_{H-H} = 14.4$  Hz,  ${}^{3}J_{H-P} = 4.6$  Hz, 1H<sub>1.D</sub>, H7-benzyl); 5.40 (dd,  ${}^{2}J_{H-H} = 14.4$  Hz,  ${}^{3}J_{H-P} = 4.6$  Hz, 1H<sub>1.D</sub>, H7-benzyl); 5.40 (dd,  ${}^{2}J_{H-H} = 14.4$  Hz,  ${}^{3}J_{H-P} = 4.6$  Hz, 1H<sub>1.D</sub>, H7-benzyl); 5.40 (dd,  ${}^{2}J_{H-H} = 14.4$  Hz,  ${}^{3}J_{H-P} = 4.6$  Hz, 1H<sub>1.D</sub>, H7-benzyl); 5.40 (dd,  ${}^{2}J_{H-H} = 14.4$  Hz,  ${}^{3}J_{H-P} = 4.6$  Hz, 1H<sub>1.D</sub>, H7-benzyl); 5.40 (dd,  ${}^{2}J_{H-H} = 14.4$  Hz,  ${}^{3}J_{H-P} = 4.6$  Hz, 1H<sub>1.D</sub>, H7-benzyl); 5.40 (dd,  ${}^{2}J_{H-H} = 14.4$  Hz,  ${}^{3}J_{H-P} = 4.6$  Hz, 1H<sub>1.D</sub>, H7-benzyl); 5.40 (dd, {}^{2}J\_{H-H} = 14.4 Hz,  ${}^{3}J_{H-P} = 4.6$  Hz, 1H<sub>1.D</sub>, H7-benzyl); 5.40 (dd, {}^{2}J\_{H-H} = 14.4 Hz,  ${}^{3}J_{H-P} = 4.6$  Hz, 1H<sub>1.D</sub>, H7-benzyl); 5.40 (dd, {}^{2}J\_{H-H} = 14.4 Hz,  ${}^{3}J_{H-P} = 4.6$  Hz, 1H<sub>1.D</sub>, H7-benzyl); 5.40 (dd, {}^{3}J\_{H-P} = 14.4 Hz,  ${}^{3}J_{H-P} = 14.4$  Hz,  ${}^{3}J_{H-P} =$ 14.2 Hz,  ${}^{3}J_{H-P} = 4.1$  Hz, 1H<sub>1.D</sub>, H7-benzyl); 5.35 (d(br),  ${}^{2}J_{H-H} = 14.5$  Hz, 1H<sub>2.D</sub>, H7benzyl); 5.32 (d(br),  ${}^{2}J_{H-H} = 14.1$  Hz, 1H<sub>2,D</sub>, H7-benzyl); 4.96–4.92 (m, 1H, H4'); 4.85  $(2\text{sept}, {}^{3}\text{J}_{\text{H}-\text{H}} = 6.2 \text{ Hz}, 1\text{H}, \text{H}11); 4.34-4.21 \text{ (m, 2H, H5')}; 2.80 \text{ (dd, } 2 \times {}^{3}\text{J}_{\text{H}-\text{H}} = 7.4 \text{ Hz},$ 2H, H8); 2.55 (dd,  $2 \times {}^{3}J_{H-H} = 7.5$  Hz, 2H, H9); 1.66, 1.60 (2d,  ${}^{4}J_{H-H} = 1.1$  Hz, 3H, H7); 1.13, 1.12 (2d,  ${}^{3}J_{H-H} = 6.2$  Hz, 6H, H12a,b);  ${}^{13}C$ -NMR (101 MHz, DMSO-d<sub>6</sub>)  $\delta$  172.6  $(2 \times C10)$ ; 163.9 (2 × C4); 150.8 (C2); 148.0 (2d, <sup>2</sup>J<sub>C-P</sub> = 7.1 Hz, C2-aryl); 137.0 (C5aryl); 135.8 (2 × C6); 133.0, 132.9 (2 × C3'); 129.8, 129.7 (2 × C4-aryl); 129.0, 128.9  $(2 \times C1$ -aryl); 127.5, 127.4  $(2 \times C2')$ ; 125.9  $(2 \times C6$ -aryl); 118.2, 118.1  $(2 \times C3$ -aryl); 109.8 (2 × C5); 89.3 (C1'); 84.3, 84.2 (2 × C4'); 68.5 (d,  ${}^{2}J_{C-P}$  = 7.6 Hz, C5'<sub>1.D</sub>); 68.5 (d,  ${}^{2}J_{C-P} = 6.1$  Hz, C5'<sub>2.D</sub>); 68.4 (2d,  ${}^{2}J_{C-P} = 6.6$  Hz, C7-benzyl); 51.5 (C11); 34.8 (C9); 29.5 (C8); 12.1, 12.0 (2 × C7); <sup>31</sup>P-NMR (202 MHz, DMSO-d<sub>6</sub>)  $\delta$ : - 8.00, - 8.02; HPLC  $t_R = 13.9$  min (method I)  $t_R = 12.4$  min (method II); MS (FAB, m/z): calc. 506.1454 (M), found 507.4 (M +  $H^+$ ).

**5-Benzylpropionyl-***cyclo***Sal-d4TMP 3h:** The product was isolated as a colorless foam. Yield: 59%; TLC:  $R_f$  (CH<sub>2</sub>Cl<sub>2</sub>/MeOH 9 : 1 v/v) 0.63; <sup>1</sup>H-NMR (500 MHz, DMSO-d<sub>6</sub>)  $\delta$  11.33, 11.32 (2s, 1H, NH), 7.36–7.26 (m, 5H, Ph), 7.23–7.18 (m, 1H, H4-aryl), 7.18, 7.15 (2q, 1H, H6), 7.11–7.09 (m, 1H, H6-aryl), 7.01, 6.99 (2d, 1H, H3-aryl), 6.79 (ddd, 1H<sub>1.D</sub>, H1'), 6.77 (ddd, 1H<sub>2.D</sub>, H1'), 6.40, 6.34 (2ddd, 1H, H3'), 6.01 (ddd, 1H<sub>1.D</sub>, H2'), 5.99 (ddd, 1H<sub>2.D</sub>, H2'), 5.41 (dd, 1H<sub>1.D</sub>, H7-benzyl), 5.38 (dd, 1H<sub>1.D</sub>,

Marcel Dekker, Inc.

270 Madison Avenue, New York, New York 10016

ORDER		REPRINTS
-------	--	----------

H7-benzyl), 5.33 (d(br), 1H<sub>2.D</sub>, H7-benzyl), 5.30 (d(br), 1H<sub>2.D</sub>, H7-benzyl), 5.06 (s, 2H, H11), 4.96–4.92 (m, 1H, H4'), 4.34–4.22 (m, 2H, H5'), 2.83 (dd, 2H, H8), 2.67 (dd, 2H, H9), 1.66, 1.60 (2d, 3H, H7); <sup>13</sup>C-NMR (101 MHz, DMSO-d<sub>6</sub>) δ 172.1, 171.9 (2 × C10), 163.9 (2 × C4), 150.9 (2 × C2), 148.1 (C2-aryl), 136.9 (C12), 136.3 (C5-aryl), 135.9, 135.8 (2 × C6), 133.0 (2 × C3'), 129.9, 129.8 (2 × C4-aryl), 128.6 (2 × C*m*-Ph), 128.5 (C1-aryl), 128.2 (C*p*-Ph), 128.1 (2 × C*o*-Ph), 127.5 (2 × C2'), 126.0, 125.9 (2 × C6-aryl), 118.2, 118.1 (2 × C3-aryl), 109.8 (2 × C5), 89.3 (C1'), 84.3, 84.2 (2 × C4'), 68.5, 68.4 (2d, C5'), 68.4 (d, C7<sub>1.D</sub>-benzyl), 68.3 (d, C7<sub>2.D</sub>-benzyl), 65.6 (C11), 35.0 (C9), 29.6 (C8), 12.1, 12.0 (2 × C7); <sup>31</sup>P-NMR (202 MHz, DMSO-d<sub>6</sub>) δ –7.98, –8.05; HPLC t<sub>R</sub> = 15.3 min (method I) t<sub>R</sub> = 13.7 min (method II); MS (FAB, m/z): calc. 554.1454 (M), found 555.2 (M + H<sup>+</sup>).

5-t-Butylpropionyl-cycloSal-d4TMP 3i: The product was isolated as a colorless foam. Yield: 44%; TLC: R<sub>f</sub> (CH<sub>2</sub>Cl<sub>2</sub>/MeOH 9 : 1 v/v) 0.49; <sup>1</sup>H-NMR (500 MHz, DMSO-d<sub>6</sub>) δ 11.33, 11.32 (2s, 1H, NH), 7.23-7.18 (m, 1H, H4-aryl), 7.18, 7.15 (2q, 1H, H6), 7.12 (d, 1H, H6-aryl), 7.03 (d, 1H<sub>1.D</sub>, H3-aryl), 7.01 (d, 1H<sub>2.D</sub>, H3-aryl), 6.79 (ddd, 1H<sub>1,D</sub>, H1'), 6.77 (ddd, 1H<sub>2,D</sub>, H1'), 6.40 (ddd, 1H<sub>1,D</sub>, H3'), 6.34 (ddd, 1H<sub>2,D</sub>, H3'), 6.00 (ddd,  $1H_{1.D}$ , H2'), 5.99 (ddd,  $1H_{2.D}$ , H2'), 5.44, 5.41 (2dd,  $1H_{1.D}$ , H7benzyl), 5.35 (dd 1H<sub>2.D</sub>, H7-benzyl), 5.32 (dd, 1H<sub>2.D</sub>, H7-benzyl), 4.96-4.92 (m, 1H, H4'), 4.33-4.22 (m, 2H, H5'), 2.77 (dd, 2H, H8), 2.48 (dd, 2H, H9), 1.67, 1.60 (2d, 3H, H7), 1.34, 1.33 (2s, 9H, H-tBu); <sup>13</sup>C-NMR (101 MHz, DMSO-d<sub>6</sub>) δ 171.5 (C10), 163.9, 163.8  $(2 \times C4)$ , 150.8  $(2 \times C2)$ , 148.0 (C2-aryl), 137.1 (C5-aryl), 135.8  $(2 \times C6)$ , 133.0, 132.9 (2 × C3'), 132.2 (C1-aryl), 129.8 (C4-aryl), 127.5, 127.4 (2 × C2'), 126.0, 125.9 (2 × C6-aryl), 118.1, 118.0 (2 × C3-aryl), 109.8 (2 × C5), 89.3 (C1'), 84.3, 84.2 (2 × C4'), 79.9 (C11), 68.5-68.3 (C5' + C7-benzyl), 36.2 (C9), 29.8 (C8), 27.9 (2 × C-tBu), 12.1, 12.0 (2 × C7); <sup>31</sup>P-NMR (202 MHz, DMSO-d<sub>6</sub>)  $\delta$ : -8.02 (only 1 Peak); HPLC  $t_R = 14.6$  min (method I)  $t_R = 13.4$  min (method II); MS (FAB, m/z): calc. 520.1611 (M), found 521.3 (M + H<sup>+</sup>).

3-AcEt-cycloSal-d4TMP 5a: The product was isolated as a colorless foam. Yield 31%; TLC R<sub>f</sub> (CH<sub>2</sub>Cl<sub>2</sub>/MeOH 9:1 v/v): 0.61; <sup>1</sup>H-NMR (500 MHz, DMSO-d<sub>6</sub>)  $\delta$ : 11.33, 11.31 (2s, 1H, NH); 7.29 (dddd,  ${}^{3}J_{H-H} = 7.3$  Hz,  $3 \times {}^{4}J_{H-H} = 1.7$  Hz, 1H, H4aryl); 7.19, 7.18 (2q,  ${}^{4}J_{H-H} = 1.3$  Hz, 1H, H6); 7.16–7.10 (m, 2H, H5-aryl + H6-aryl); 6.80-6.78 (m, 1H, H1'); 6.40 (ddd,  ${}^{3}J_{H-H} = 6.1$  Hz,  ${}^{3}J_{H-H} = 1.7$  Hz,  ${}^{4}J_{H-H} = 1.7$  Hz,  $1H_{1,D}$ , H3'); 6.36 (ddd,  ${}^{3}J_{H-H} = 6.0$  Hz,  ${}^{3}J_{H-H} = 1.8$  Hz,  ${}^{4}J_{H-H} = 1.8$  Hz,  $1H_{2,D}$ , H3'); 6.02 (ddd,  ${}^{3}J_{H-H} = 6.0$  Hz,  ${}^{3}J_{H-H} = 2.3$  Hz,  ${}^{4}J_{H-H} = 1.5$  Hz, 1H<sub>1.D</sub>, H2'); 6.00 (ddd,  ${}^{3}J_{H-H} = 6.0 \text{ Hz}, {}^{3}J_{H-H} = 2.2 \text{ Hz}, {}^{4}J_{H-H} = 1.7 \text{ Hz}, 11_{2.D}, 12'); 5.48 \text{ (dd, } {}^{2}J_{H-H} = 14.3 \text{ Hz}, 14_{2.D}, 14'); 5.48 \text{ (dd, } {}^{2}J_{H-H} = 14.3 \text{ Hz}, 14_{2.D}, 14'); 5.48 \text{ (dd, } {}^{2}J_{H-H} = 14.3 \text{ Hz}, 14'); 5.48 \text{ (dd, } {}^{2}J_{H-H} = 14.3 \text{ Hz}, 14'); 5.48 \text{ (dd, } {}^{2}J_{H-H} = 14.3 \text{ Hz}, 14'); 5', 14''', 14'', 14'', 1$ Hz,  ${}^{3}J_{H-P} = 7.7$  Hz, 1H<sub>1.D</sub>, H7-benzyl); 5.45 (dd,  ${}^{2}J_{H-H} = 14.3$  Hz,  ${}^{3}J_{H-P} = 7.3$  Hz,  $1H_{1.D}$ , H7-benzyl); 5.38 (dd,  $^{2}J_{H-H} = 14.0$  Hz,  $^{3}J_{H-P} = 7.4$  Hz,  $1H_{2.D}$ , H7-benzyl); 5.35  $(dd, {}^{2}J_{H-H} = 14.2 \text{ Hz}, {}^{3}J_{H-P} = 8.0 \text{ Hz}, 1H_{2,D}, H7\text{-benzyl}); 4.96-4.93 (m, 1H, H4');$ 4.32-4.26 (m, 2H, H5'); 4.22-4.12 (m, 2H, H9); 2.97-2.82 (m, 2H, H8); 1.96, 1.95  $(2s, 3H, H11); 1.63, 1.59 (2d, {}^{4}J_{H-H} = 1.3 Hz, 3H, H7); {}^{13}C-NMR (101 MHz, DMSO$ d<sub>6</sub>)  $\delta$ : 173.3, 173.0 (2 × C10); 163.9 (C4); 150.9, 150.8 (2 × C2); 148.3, 148.2  $(2 \times C2$ -aryl); 135.8  $(2 \times C6)$ ; 133.0 132.9  $(2 \times C3')$ ; 131.1, 131.0  $(2 \times C4$ -aryl); 130.3, 130.2 (2 × C1-aryl); 127.6, 127.5 (2 × C2'); 124.8 (2 × C6-aryl); 124.3 (C5aryl); 121.7, 121.6 (2 × C3-aryl); 109.9, 109.8 (2 × C5); 89.4, 89.3 (2 × C1'); 84.3, 84.2 (2 × C4'); 68.8 (d,  ${}^{2}J_{C-P}$  = 5.1 Hz, C5'<sub>1.D</sub>); 68.8 (d,  ${}^{2}J_{C-P}$  = 6.1 Hz, C5'<sub>2.D</sub>); 68.4

Downloaded by [University of Chicago Library] at 02:13 25 April 2013





(d,  ${}^{2}J_{C-P} = 6.6$  Hz, C7-benzyl); 68.4 (d,  ${}^{2}J_{C-P} = 7.6$  Hz, C7-benzyl); 63.0, 62.9 (2 × C9); 28.3 (2 × C8); 20.8 (2 × C11); 12.0, 11.9 (2 × C7);  ${}^{31}P$ -NMR (202 MHz, DMSO-d<sub>6</sub>)  $\delta$ : -7.50, -7.64; HPLC: t<sub>R</sub> = 10.9 min (method I); t<sub>R</sub> = 10.3 min (method II); MS (FAB, m/z): calc. 478.1141 (M), found 479.2 (M + H<sup>+</sup>).

**3-LevEt-***cyclo***Sal d4TMP 5b:** The product was isolated as a colorless foam. Yield: 30%; TLC:  $R_f$  (CH<sub>2</sub>Cl<sub>2</sub>/MeOH 9:1 v/v) 0.42; <sup>1</sup>H-NMR (500 MHz, DMSO-d<sub>6</sub>)  $\delta$ : 1.61 (d, 3H,  ${}^{4}J_{HH} = 1.3$  Hz, thymine-H7), 1.66 (d, 3H,  ${}^{4}J_{HH} = 1.1$  Hz, thymine-H7), 2.08 (s, 2 × 3H, 2 × H14), 2.42 (t, 2H,  ${}^{3}J_{HH}$  = 6.5 Hz, H12), 2.43 (t, 2H,  ${}^{3}J_{HH}$  = 6.7 Hz, H12), 2.67 (t, 2H,  ${}^{3}J_{HH} = 6.7$  Hz, H11), 2.68 (t, 2H,  ${}^{3}J_{HH} = 6.5$  Hz, H11), 2.83– 2.96 (m,  $2 \times 2H$ ,  $2 \times H8$ ), 4.13–4.23 (m, $2 \times 2H$ ,  $2 \times H9$ ) 4.26–4.35 (m,  $2 \times 2H$ ,  $2 \times \text{H5'}$ , 4.95–4.99 (m,  $2 \times 1\text{H}$ ,  $2 \times \text{H4'}$ ) 5.37 (dd, 1H,  ${}^{2}J_{\text{HH}} = 12.5$  Hz,  ${}^{3}J_{\text{HP}} = 7.0$ Hz, H7), 5.40 (dd, 1H,  ${}^{2}J_{HH}$  = 12.5 Hz,  ${}^{3}J_{HP}$  = 7.0 Hz, H7), 5.47 (dd, 1H,  ${}^{2}J_{HH}$  = 14.2 Hz,  ${}^{3}J_{\text{HP}} = 7.2$  Hz, H7), 5.51 (dd, 1H,  ${}^{2}J_{\text{HH}} = 14.2$  Hz,  ${}^{3}J_{\text{HP}} = 7.2$  Hz, H7), 6.02 (ddd, 1H,  ${}^{3}J_{HH} = 6.0$  Hz,  ${}^{3}J_{HH} = 1.6$  Hz,  ${}^{4}J_{HH} = 2.4$  Hz, H2'), 6.04 (ddd, 1H,  ${}^{3}J_{HH} = 6.0$  Hz,  ${}^{3}J_{\rm HH} = 1.6$  Hz,  ${}^{4}J_{\rm HH} = 2.4$  Hz, H2'), 6.38 (ddd, 1H,  ${}^{3}J_{\rm HH} = 6.0$  Hz,  ${}^{3}J_{\rm HH} = 1.6$  Hz,  ${}^{4}J_{\rm HH} = 1.8$  Hz, H3'), 6.41 (ddd, 1H,  ${}^{3}J_{\rm HH} = 6.0$  Hz,  ${}^{3}J_{\rm HH} = 1.6$  Hz,  ${}^{4}J_{\rm HH} = 1.8$  Hz, H3'), 6.80-6.83(m, 2 × 1H, 2 × H1'), 7.13-7.21 (m, 2 × 3H, thymine-H6, H4, H5), 7.29-7.33 (m, 2 × 1H, H6), 11.33 (s, 1 × 1H, 1xNH), 11.35 (s, 1 × 1H, 1 × NH); <sup>13</sup>C-NMR (100 MHz, DMSO-d<sub>6</sub>) δ: 11.71, 11.82 (2x thymine-C7), 27.53, 27.55  $(2 \times C12)$ , 28.07  $(2 \times C8)$ , 29.47  $(2 \times C14)$ , 37.34  $(2 \times C11)$ , 62.85, 62.89  $(2 \times C9)$ , 68.17 (d,  ${}^{3}J_{CP}$  = 4.4 Hz, 1 × C7), 68.23 (d,  ${}^{3}J_{CP}$  = 4.6 Hz, 1 × C7), 68.57 (d,  ${}^{3}J_{CP} = 2.0$  Hz, 1 × C5'), 68.63 (d,  ${}^{3}J_{CP} = 2.5$  Hz, 1 × C5') 84.07, 84.13 (2 × C4'), 89.12, 89.22 (2 × C1'), 109.65, 109.70 (2 × thymine-C5), 120.36 (2 × C1) 124.15  $(2 \times C4)$ , 124.62, 124.65  $(2 \times C5)$ , 126.98, 127.05  $(2 \times C3)$ , 127.33, 127.36  $(2 \times C2')$ , 130.84, 130.93  $(2 \times C6)$  132.71, 132.78  $(2 \times C3')$ , 135.60, 135.66 (2 × thymine-C6) 150.67 (2 × thymine-C2), 155.71 (2 × C2), 163.69 (2 × thymine-C4), 172.54 (2 × C10), 207.14 (2 × C13); <sup>31</sup>P-NMR (202 MHz, DMSO-d<sub>6</sub>)  $\delta$ : -7.60, -7.52; HPLC: t<sub>R</sub> = 12.22 min (method I); t<sub>R</sub> = 11.23, 11.41 min (method II); MS  $(ESI^+, m/z)$ : calc. 534.14 (M), found 557.16 (M + Na<sup>+</sup>).

**3-PivEt-***cyclo***Sal-d4TMP 5c:** The product was isolated as a colorless foam. Yield: 52%; TLC:  $R_f (CH_2Cl_2/MeOH 9:1 v/v): 0.60; {}^{1}H-NMR (400 MHz, DMSO-d_6) \delta:$  11.33, 11.31 (2s, 1H, NH); 7.30–7.26 (m, 1H, H4-aryl); 7.19, 7.18 (2q,  ${}^{4}J_{H-H} = 1.2$  Hz, 1H, H6); 7.17–7.10 (m, 2H, H5-aryl + H6-aryl); 6.81–6.78 (m, 1H, H1'); 6.40 (ddd, {}^{3}J\_{H-H} = 6.0 Hz, {}^{3}J\_{H-H} = 1.7 Hz, {}^{4}J\_{H-H} = 1.7 Hz, 1H\_{1.D}, H3'); 6.36 (ddd, {}^{3}J\_{H-H} = 6.0 Hz, {}^{3}J\_{H-H} = 1.8 Hz, {}^{4}J\_{H-H} = 1.8 Hz, 1H\_{2.D}, H3'); 6.02 (ddd, {}^{3}J\_{H-H} = 6.2 Hz, {}^{3}J\_{H-H} = 2.4 Hz, {}^{4}J\_{H-H} = 1.2 Hz, 1H\_{1.D}, H2'); 6.00 (ddd, {}^{3}J\_{H-H} = 5.9 Hz, {}^{3}J\_{H-H} = 2.4 Hz, {}^{4}J\_{H-H} = 1.4 Hz, 1H\_{2.D}, H2'); 5.48 (dd, {}^{2}J\_{H-H} = 14.3 Hz, {}^{3}J\_{H-P} = 5.1 Hz, 1H\_{1.D}, H7-benzyl); 5.44 (dd, {}^{2}J\_{H-H} = 14.3 Hz, {}^{3}J\_{H-P} = 4.8 Hz, 1H\_{1.D}, H7-benzyl); 5.38 (dd, {}^{2}J\_{H-H} = 14.2 Hz, {}^{3}J\_{H-P} = 4.8 Hz, 1H\_{1.D}, H7-benzyl); 5.38 (dd, {}^{2}J\_{H-H} = 14.2 Hz, {}^{3}J\_{H-P} = 4.8 Hz, 1H\_{1.D}, H7-benzyl); 5.38 (dd, {}^{2}J\_{H-H} = 14.2 Hz, {}^{3}J\_{H-P} = 4.8 Hz, 1H\_{1.D}, H7-benzyl); 5.38 (dd, {}^{2}J\_{H-H} = 14.2 Hz, {}^{3}J\_{H-P} = 4.8 Hz, 1H\_{1.D}, H7-benzyl); 5.38 (dd, {}^{2}J\_{H-H} = 14.2 Hz, {}^{3}J\_{H-P} = 4.8 Hz, 1H\_{1.D}, H7-benzyl); 5.38 (dd, {}^{2}J\_{H-H} = 14.2 Hz, {}^{3}J\_{H-P} = 4.8 Hz, 1H\_{1.D}, H7-benzyl); 5.38 (dd, {}^{2}J\_{H-H} = 14.2 Hz, {}^{3}J\_{H-P} = 4.8 Hz, 1H\_{2.D}, H7-benzyl); 5.35 (dd, {}^{2}J\_{H-H} = 14.2 Hz, {}^{3}J\_{H-P} = 5.3 Hz, 1H\_{2.D}, H7-benzyl); 4.97-4.92 (m, 1H, H4'); 4.35-4.24 (m, 2H, H5'); 4.22-4.16 (m, 2H, H9); 2.99-2.82 (m, 2H, H8); 1.64, 1.59 (2d, {}^{4}J\_{H-H} = 1.2 Hz, 3H, H7); 1.05 (2s, 9H, H12a-c); {}^{13}C-NMR (101 MHz, DMSO-d\_6) \delta: 177.4 (2 \times C10); 163.9 (C4); 150.8 (2 \times C2); 148.2 (d, {}^{2}J\_{H-H} = 7.1 Hz, C2\_{1.D}-aryl); 148.1 (d, {}^{2}J\_{H-H} = 7.6 Hz, C2\_{2.D}-aryl); 135.8 (2 \times C6); 132.9 (2 \times C3'); 131.2 (2 \times C4-aryl); 127.5 (2 \times C2'); 127.3, 127.2 (2 \times C1-aryl); 124.8 (2 \times C6-aryl); 124.2, 124.1 (2 \times C5-aryl); 121.7, 1



ORDER		REPRINTS
-------	--	----------

121.6 (2 × C3-aryl); 109.9, 109.8 (2 × C5); 89.4, 89.3 (2 × C1'); 84.3 (2 × C4'); 68.8 (d,  ${}^{2}J_{C-P} = 5.1$  Hz, C5'<sub>1.D</sub>); 68.7 (d,  ${}^{2}J_{C-P} = 6.1$  Hz, C5'<sub>2.D</sub>); 68.4 (d,  ${}^{2}J_{C-P} = 7.1$  Hz, C7-benzyl); 68.4 (d,  ${}^{2}J_{C-P} = 6.1$  Hz, C7-benzyl); 62.9 (2 × C9); 30.8 (C11); 28.3 (2 × C8); 26.9 (C12a-c); 12.0, 11.9 (2 × C7);  ${}^{31}$ P-NMR (202 MHz, DMSO-d<sub>6</sub>)  $\delta$ : -7.51, -7.58; HPLC: t<sub>R</sub> = 13.9 min (method I), t<sub>R</sub> = 13.3 min (method II); MS (FAB, m/z): calc. 520.1611 (M), found 521.2 (M + H<sup>+</sup>), 521.1689 (M + H<sup>+</sup>, FAB-HR).

**5-AcEt-***cyclo***Sal-d4TMP 5d:** The product was isolated as a colorless foam. Yield: 41%; TLC R<sub>f</sub> (CH<sub>2</sub>Cl<sub>2</sub>/MeOH 9:1 v/v): 0.65; <sup>1</sup>H-NMR (500 MHz, DMSO-d<sub>6</sub>) δ: 11.33, 11.32 (2s, 1H, NH); 7.26–7.21 (m, 1H, H4-aryl); 7.18 (q, <sup>4</sup>J<sub>H-H</sub> = 1.3 Hz, 1H<sub>1.D</sub>, H6); 7.15 (q,  ${}^{4}J_{H-H} = 1.1$  Hz, 1H<sub>2.D</sub>, H6); 7.16–7.14 (m, 1H, H6-aryl); 7.05, 7.03 (2d,  ${}^{3}J_{H-H} = 8.4$  Hz, 1H, H3-aryl); 6.79 (ddd,  ${}^{3}J_{H-H} = 3.5$  Hz,  $2 \times {}^{4}J_{H-H} = 1.7$  Hz, 1H<sub>1.D</sub>, H1'); 6.77 (ddd,  ${}^{3}J_{H-H} = 3.7$  Hz,  $2 \times {}^{4}J_{H-H} = 1.8$  Hz,  $1H_{2,D}$ , H1'); 6.41 (ddd,  ${}^{3}J_{H-H} = 6.0$ Hz,  ${}^{3}J_{H-H} = 1.8$  Hz,  ${}^{4}J_{H-H} = 1.8$  Hz,  $1H_{1.D}$ , H3'); 6.34 (ddd,  ${}^{3}J_{H-H} = 6.0$  Hz,  ${}^{3}J_{H-H} = 1.7 \text{ Hz}, {}^{4}J_{H-H} = 1.7 \text{ Hz}, 1\text{H}_{2.D}, \text{H3'}$ ; 6.01 (ddd,  ${}^{3}J_{H-H} = 6.1 \text{ Hz}, {}^{3}J_{H-H} = 2.4$ Hz,  ${}^{4}J_{H-H} = 1.3$  Hz,  $1H_{1.D}$ , H2'); 5.99 (ddd,  ${}^{3}J_{H-H} = 6.0$  Hz,  ${}^{3}J_{H-H} = 2.4$  Hz,  ${}^{4}J_{H-H} = 1.4$  Hz,  $1H_{2,D}$ , H2'); 5.47 (dd,  ${}^{2}J_{H-H} = 14.5$  Hz,  ${}^{3}J_{H-P} = 6.1$  Hz,  $1H_{1,D}$ , H7benzyl); 5.43 (dd,  ${}^{2}J_{H-H} = 14.4$  Hz,  ${}^{3}J_{H-P} = 5.9$  Hz,  $1H_{1.D}$ , H7-benzyl); 5.37 (d(br),  ${}^{2}J_{H-H} = 14.3$  Hz, 1H<sub>2.D</sub>, H7-benzyl); 5.35 (d(br),  ${}^{2}J_{H-H} = 14.2$  Hz, 1H<sub>2.D</sub>, H7-benzyl);  $4.96-4.92 \text{ (m, 1H, H4')}; 4.34-4.23 \text{ (m, 2H, H5')}; 4.17 \text{ (dd, } 2 \times^{3} J_{H-H} = 6.9 \text{ Hz}, 2H, H9);$ 2.85 (dd,  $2 \times {}^{3}J_{H-H} = 6.8$  Hz, 2H, H8); 1.97 (s, 3H, H11); 1.66 (d,  ${}^{4}J_{H-H} = 1.1$  Hz,  $3H_{1,D}$ , H7); 1.59 (d,  ${}^{4}J_{H-H} = 1.3$  Hz,  $3H_{2,D}$ , H7);  ${}^{13}C$ -NMR (101 MHz, DMSO-d<sub>6</sub>)  $\delta$ : 170.4 (C10); 163.9 (2 × C4); 150.9, 150.8 (2 × C2); 148.3, 148.2 (2d,  ${}^{2}J_{C-P} = 7.1$  Hz, C2aryl); 135.8 (2 × C6); 134.5 (C5-aryl); 133.0, 132.9 (2 × C3'); 130.4, 130.3 (2 × C4aryl); 129.2 (C1-aryl); 127.5 ( $2 \times C2'$ ); 126.5, 126.4 ( $2 \times C6$ -aryl); 118.2, 118.1  $(2 \times C3$ -aryl); 109.8  $(2 \times C5)$ ; 89.3 (C1'); 84.3, 84.2  $(2 \times C4')$ ; 68.5  $(d, {}^{2}J_{C-P} = 8.1)$ Hz,  $C5'_{1,D}$ ); 68.4 (d,  ${}^{2}J_{C-P} = 6.1$  Hz,  $C5'_{2,D}$ ); 68.3 (d,  ${}^{2}J_{C-P} = 7.1$  Hz, C7-benzyl); 64.2 (C9); 33.6 (C11); 20.8 (C8); 12.1, 12.0 (2  $\times$  C7); <sup>31</sup>P-NMR (202 MHz, DMSO-d<sub>6</sub>)  $\delta$ : - 7.99, - 8.04; HPLC t<sub>R</sub> = 11.6 min (method I), t<sub>R</sub> = 10.4 min (method II); MS (FAB, m/z): calc. 478.1141 (M), found 479.4 (M + H<sup>+</sup>), 479.1232 (M + H<sup>+</sup>, FAB-HR).

**5-PivEt-***cyclo***Sal-d4TMP 5e:** The product was isolated as a colorless foam. Yield: 39%; TLC:  $R_f (CH_2Cl_2/MeOH 9:1 v/v): 0.56; {}^{1}H-NMR (500 MHz, DMSO-d_6) \delta: 11.33, 11.32 (2s, 1H, NH); 7.26-7.20 (m, 1H, H4-aryl); 7.17, 7.15 (2q, {}^{4}J_{H-H} = 1.1 Hz, 1H, H6); 7.15-7.13 (m, 1H, H6-aryl); 7.06, 7.04 (2d, {}^{3}J_{H-H} = 8.4 Hz, 1H, H3-aryl); 6.79 (ddd, {}^{3}J_{H-H} = 3.5 Hz, 2 × {}^{4}J_{H-H} = 1.7 Hz, 1H_{1.D}, H1'); 6.77 (ddd, {}^{3}J_{H-H} = 3.7 Hz, 2 × {}^{4}J_{H-H} = 1.8 Hz, 1H_{2.D}, H1'); 6.40 (ddd, {}^{3}J_{H-H} = 6.0 Hz, {}^{3}J_{H-H} = 1.7 Hz, {}^{4}J_{H-H} = 1.8 Hz, 1H_{1.D}, H3'); 6.34 (ddd, {}^{3}J_{H-H} = 6.0 Hz, {}^{3}J_{H-H} = 1.7 Hz, {}^{4}J_{H-H} = 1.8 Hz, 1H_{1.D}, H3'); 6.34 (ddd, {}^{3}J_{H-H} = 2.3 Hz, {}^{4}J_{H-H} = 1.3 Hz, 1H_{1.D}, H2'); 5.99 (ddd, {}^{3}J_{H-H} = 5.9 Hz, {}^{3}J_{H-H} = 2.5 Hz, {}^{4}J_{H-H} = 1.3 Hz, 1H_{1.D}, H2'); 5.99 (ddd, {}^{3}J_{H-H} = 5.8 Hz, {}^{3}J_{H-H} = 2.5 Hz, {}^{4}J_{H-H} = 2.5 Hz, {}^{4}J_{H-H} = 1.4 Hz, {}^{3}J_{H-H} = 5.9 Hz, {}^{3}J_{H-H} = 2.5 Hz, {}^{4}J_{H-H} = 1.4 Hz, {}^{3}J_{H-H} = 5.9 Hz, {}^{3}J_{H-H} = 2.5 Hz, {}^{4}J_{H-H} = 1.4 Hz, {}^{3}J_{H-H} = 5.9 Hz, {}^{3}J_{H-H} = 2.5 Hz, {}^{4}J_{H-H} = 1.4 Hz, {}^{3}J_{H-H} = 5.9 Hz, {}^{3}J_{H-H} = 2.5 Hz, {}^{4}J_{H-H} = 1.4 Hz, {}^{3}J_{H-P} = 5.9 Hz, {}^{3}J_{H-H} = 1.4 Hz, {}^{3}J_{H-H} = 14.4 Hz, {}^{3}J_{H-P} = 5.9 Hz, {}^{1}H_{1.D}, H7$ -benzyl); 5.45 (dd, {}^{2}J\_{H-H} = 14.5 Hz, {}^{3}J\_{H-P} = 6.0 Hz, {}^{1}H\_{1.D}, H7-benzyl); 5.42 (dd, {}^{2}J\_{H-H} = 14.4 Hz, {}^{3}J\_{H-P} = 5.9 Hz, {}^{1}H\_{1.D}, H7-benzyl); 5.33 (dd, {}^{2}J\_{H-H} = 14.4 Hz, {}^{3}J\_{H-P} = 3.7 Hz, {}^{1}H\_{2.D}, H7-benzyl); 4.95–4.92 (m, 1H, H4'); 4.34–4.22 (m, 2H, H5'); 4.18 (dd,  $2 \times {}^{3}J_{H-H} = 6.6 Hz, 2H, H9); 2.85 (dd(br), <math>2 \times {}^{3}J_{H-H} = 6.5 Hz, 2H, H8); 1.66, 1.60 (2d, {}^{4}J_{H-H} = 1.1 Hz, 3H, H7); 1.06 (2s, 9H, H12a-c); {}^{1}C-NMR (101 MHz, DMSO-d_6) \delta: 177.4 (C10); 163.9, 163.8 (2 \times C4);$ 

110

Downloaded by [University of Chicago Library] at 02:13 25 April 2013



150.9, 150.8 (2 × C2); 148.0 (2d,  ${}^{2}J_{C-P} = 7.1$  Hz, C2-aryl); 135.8 (2 × C6); 134.6 (C5-aryl); 133.0, 132.9 (2 × C3'); 130.5, 130.4 (C4-aryl); 129.7, 129.6 (2 × C1-aryl); 127.5 (2 × C2'); 126.6, 126.5 (2 × C6-aryl); 118.1 (2 × C3-aryl); 109.8 (2 × C5); 89.3 (C1'); 84.3, 84.2 (2 × C4'); 68.5 (d,  ${}^{2}J_{C-P} = 6.6$  Hz, C5'<sub>1.D</sub>); 68.4 (d,  ${}^{2}J_{C-P} = 8.1$  Hz, C5'<sub>2.D</sub>); 68.4 (d,  ${}^{2}J_{C-P} = 6.1$  Hz, C7<sub>1.D</sub>-benzyl); 68.3 (d,  ${}^{2}J_{C-P} = 7.1$  Hz, C7<sub>2.D</sub>-benzyl); 64.3 (C9); 38.3 (C11); 33.6 (C8); 27.0 (C12a-c); 12.1, 12.0 (2 × C7);  ${}^{31}$ P-NMR (202 MHz, DMSO-d<sub>6</sub>)  $\delta$ : -8.03, -8.06; HPLC: t<sub>R</sub> = 15.0 min (method I), t<sub>R</sub> = 13.4 min (method II); MS (FAB, m/z): calc. 520.1611 (M), found 521.2 (M + H<sup>+</sup>), 520.1532 (M + H<sup>+</sup>, FAB-HR).

3-(2-Carboxyethyl)-cycloSal-d4TMP 2a: The product was isolated as a colorless foam. Yield: 71%; TLC: R<sub>f</sub> (CH<sub>2</sub>Cl<sub>2</sub>/MeOH 9 : 1 v/v) 0.16; <sup>1</sup>H-NMR (500 MHz, DMSO-d<sub>6</sub>) δ 12.17 (s(br), 1H, COOH), 11.31 (2s, 1H, NH), 7.27-7.25 (m, 1H, H4-aryl), 7.19 (2q, 1H, H6), 7.14-7.09 (m, 2H, H5-aryl + H6-aryl), 6.80-6.78 (m, 1H, H1'), 6.40 (ddd,  $1H_{1,D}$ , H3'), 6.35 (ddd,  $1H_{2,D}$ , H3'), 6.02, 6.00 (2ddd, 1H, H2'), 5.47 (dd, 1H<sub>1,D</sub>, H7-benzyl), 5.44 (dd, 1H<sub>1,D</sub>, H7-benzyl), 5.37 (dd, 1H<sub>2,D</sub>, H7-benzyl), 5.34 (dd, 1H2,D, H7-benzyl), 4.96-4.92 (m, 1H, H4'), 4.34-4.24 (m, 2H, H5'), 2.85-2.75 (m, 2H, H8), 2.51, 2.50 (2dd, 2H, H9), 1.65, 1.58 (2d, 3H, H7); <sup>13</sup>C-NMR (101 MHz, DMSO-d<sub>6</sub>)  $\delta$  173.6 (2 × C10), 163.9 (C4), 150.9, 150.8 (2 × C2), 147.9 (d, C2-aryl), 147.9 (d, C2-aryl), 135.8, 135.7 (2  $\times$  C6), 133.0 132.9 (2  $\times$  C3'), 130.2 (2  $\times$  C4-aryl), 130.0, 129.9 (2 × C1-aryl), 127.5 (2 × C2'), 124.4 (2 × C6-aryl), 124.3 (C5-aryl), 121.7, 121.6 (C3-aryl), 109.9, 109.8 (2 × C5), 89.4, 89.3 (2 × C1'), 84.3 (2 × C4'), 68.7 (d, C5'), 68.7 (d, C5'), 68.4 (d, C7-benzyl), 68.4 (d, C7-benzyl), 33.5 (2 × C9), 24.2 (2 × C8), 12.0, 11.9 (2 × C7); <sup>31</sup>P-NMR (202 MHz, DMSO-d<sub>6</sub>)  $\delta$  -7.51, -7.56; HPLC:  $t_R = 9.2$ , 9.5 min (method I),  $t_R = 9.7$  min (method II); MS (FAB, m/z): calc. 464.0985 (M + H<sup>+</sup>), found 464.2 (M + H<sup>+</sup>).

5-(2-Carboxyethyl)-cycloSal-d4TMP 2b: The product was isolated as a colorless foam. Yield: 93%; TLC: R<sub>f</sub> (CH<sub>2</sub>Cl<sub>2</sub>/MeOH 9 : 1 v/v) 0.16; <sup>1</sup>H-NMR (500 MHz, DMSO-d<sub>6</sub>) δ 12.08 (s(br), 1H, COOH), 11.33, 11.32 (2s, 1H, NH), 7.24–7.19 (m, 1H, H4-aryl), 7.18, 7.15 (2q, 1H, H6), 7.13–7.11 (m, 2H, H6-aryl), 7.03 (d, 1H<sub>1.D</sub>, H3-aryl), 7.01 (d, 1H<sub>2.D</sub>, H3-aryl), 6.79, 6.77 (2ddd, 1H, H1'), 6.40, 6.33 (2ddd, 1H, H3'), 6.01 (ddd, 1H<sub>1.D</sub>, H2'), 5.99 (ddd, 1H<sub>2.D</sub>, H2'), 5.45 (dd, 1H<sub>1.D</sub>, H7-benzyl), 5.41 (dd, 1H<sub>1,D</sub>, H7-benzyl), 5.36 (d(br), 1H<sub>2,D</sub>, H7-benzyl), 5.33 (dd, 1H<sub>2,D</sub>, H7-benzyl), 4.96–4.92 (m, 1H, H4'), 4.33–4.22 (m, 2H, H5'), 2.78 (dd, 2H, H8), 2.51 (dd, 2H, H9), 1.67, 1.61 (2d, 3H, H7);  $^{13}$ C-NMR (101 MHz, DMSO-d<sub>6</sub>)  $\delta$  173.7 (2 × C10), 163.9 (C4), 150.9, 150.8  $(2 \times C2)$ , 146.5, 146.2 (d, C2-aryl), 137.4 (C5-aryl), 135.8 (2 × C6), 133.0 132.9 (2 × C3'), 129.8, 129.7 (2 × C4-aryl), 128.7 (C1-aryl), 127.5, 127.4  $(2 \times C2')$ , 125.9, 125.8  $(2 \times C6\text{-aryl})$ , 118.1  $(2 \times C3\text{-aryl})$ , 109.8  $(2 \times C5)$ , 89.3 (C1'), 84.3, 84.2 (2 × C4'), 68.5 (d, C5'), 68.5 (d, C5'), 68.4 (2d, C7-benzyl), 35.1 (C9), 29.6 (C8), 12.1, 12.0 (2 × C7); <sup>31</sup>P-NMR (202 MHz, DMSO-d<sub>6</sub>)  $\delta$ -8.01, -8.06; HPLC:  $t_R = 9.5$  min (method I),  $t_R = 9.6$  min (method II)MS (FAB, m/z): calc. 464.0985 (M + H<sup>+</sup>), found 464.3 (M + H<sup>+</sup>).

**3-(2-Hydroxyethyl)**-cycloSal-d4TMP 4a: 65 mg (0.12 mmol) 3-LevEt cycloSal-d4TMP 5b were dissolved in 5 ml pyridine. To this solution, a mixture of

ORDER		REPRINTS
-------	--	----------

1 ml (0.24 mmol) hydrazine-hydrate, 3.1 ml (0.48 mmol) pyridine and 2 ml (0.36 mmol) acetic acid was added at 0°C. After 10 min water and ethylacetate was added and the separated organic phase was washed with a 5% sodium bicarbonate solution. After drying with sodium sulfate, the organic solvent was removed and the crude product was purified by chromatography ( $CH_2Cl_2$ -MeOH gradient 0–4%). The product was isolated as a colorless foam. Yield: 25 %; TLC: Rf (CH2Cl2/MeOH 9:1 v/v) 0.32; <sup>1</sup>H-NMR (500 MHz, DMSO-d<sub>6</sub>)  $\delta$ : 1.62 (d, 3H, <sup>4</sup>J<sub>HH</sub> = 1.3 Hz, thymine-H7), 1.66 (d, 3H,  ${}^{4}J_{\rm HH} = 1.3$  Hz, thymine-H7), 2.69–2.80 (m, 2 × 2H, 2 × H8), 4.26–4.35 (m,  $2 \times 2H$ ,  $2 \times H5'$ ), 4.68 (t, 1H,  ${}^{3}J_{HH} = 5.2$  Hz, -OH), 4.72 (t, 1H,  ${}^{3}J_{HH} = 5.2$  Hz, -OH), 4.92–4.98 (m, 2 × 1H, 2 × H4') 5.36 (dd, 1H,  ${}^{2}J_{HH}$  = 12.5 Hz,  ${}^{3}J_{HP}$  = 5.4 Hz, H7), 5.38 (dd, 1H,  ${}^{2}J_{HH}$  = 12.5 Hz,  ${}^{3}J_{HP}$  = 5.4 Hz, H7), 5.45 (dd, 1H,  ${}^{2}J_{HH}$  = 14.5 Hz,  ${}^{3}J_{\rm HP} = 8.0$  Hz, H7), 5.54 (dd, 1H,  ${}^{2}J_{\rm HH} = 14.5$  Hz,  ${}^{3}J_{\rm HP} = 8.0$  Hz, H7), 6.02 (ddd, 1H,  ${}^{3}J_{\rm HH} = 6.0$  Hz,  ${}^{3}J_{\rm HH} = 1.5$  Hz,  ${}^{4}J_{\rm HH} = 2.5$  Hz, H2'), 6.04 (ddd, 1H,  ${}^{3}J_{\rm HH} = 6.0$  Hz,  ${}^{3}J_{\text{HH}} = 1.3 \text{ Hz}, {}^{4}J_{\text{HH}} = 2.4 \text{ Hz}, \text{H2'}), 6.36 (ddd, 1H, {}^{3}J_{\text{HH}} = 6.0 \text{ Hz}, {}^{3}J_{\text{HH}} = 1.3 \text{ Hz}, {}^{4}J_{\text{HH}} = 1.8 \text{ Hz}, \text{H3'}), 6.42 (ddd, 1H, {}^{3}J_{\text{HH}} = 6.0 \text{ Hz}, {}^{3}J_{\text{HH}} = 1.5 \text{ Hz}, {}^{4}J_{\text{HH}} = 1.8 \text{ Hz},$ H3'),  $6.78-6.83(m, 2 \times 1H, 2 \times H1')$ , 7.13-7.21 (m,  $2 \times 3H$ , thymine-H6, H4, H5), 7.29–7.33 (m,  $2 \times 1$ H, $2 \times$ H6), 11.32–11.41 (br,  $2 \times 1$ H,  $2 \times$ NH); <sup>13</sup>C-NMR (100 MHz, DMSO,  $d_6$ )  $\delta$ : 11.74, 11.83 (2 × thymine-C7), 32.41 (2 × C8), 60.38 (2 × C9), 68.62-68.91 (m,  $2 \times C5'$ ,  $2 \times C7$ ) 84.04,  $(2 \times C4')$ , 89.11, 89.19 ( $2 \times C1'$ ), 120.20 $(2 \times \text{thymine-C5}), 120.36 (2 \times C1) 124.01 (2 \times C4), 124.62, 124.65 (2 \times C5),$ 126.98, 127.05  $(2 \times C3)$ , 127.33, 127.36  $(2 \times C2')$ , 130.84, 130.93  $(2 \times C6)$ 132.71, 132.78 (2 × C3'), 135.60, 135.66 (2 × thymine-C6) 150.67 (2 × thymine-C2), 155.71 (2 × C2), 163.69 (2 × thymine-C4), 172.54 (2 × C10), 207.14  $(2 \times C13)$ ; <sup>31</sup>P-NMR (202 MHz, DMSO-d<sub>6</sub>)  $\delta$ : -7.48, -7.43; HPLC: t<sub>R</sub> = 10.27 min (method I);  $t_R = 9.07$ , 9.36 min (method II); MS (ESI<sup>+</sup>, m/z): calc.: 436.10 (M), found:  $459.13 (M + Na^{+})$ .

**Kinetic Data.** (a) Aqueous Buffers: 12  $\mu$ L of DMSO stock solutions (50 mM) of the triesters were diluted in 300  $\mu$ L water or water/DMSO (c = 2.0 mM). 0.3 mL of this solution were added to 0.3 mL of aqueous buffer (50 mM phosphate buffer, pH 7.3 or 50 mM phosphate buffer, pH 6.8) containing 5  $\mu$ L of an aqueous AZT solution (AZT as internal standard) at 37°C. The final concentrations were 0.96 mM for the triesters and 25 mM for the aqueous buffer. Aliquots of 60  $\mu$ L of the hydrolysis mixture were taken and the hydrolysis was stopped by addition of 5  $\mu$ L glacial acetic acid and frozen in liquid air. After thawing, samples were analyzed by analytical HPLC (Merck LiChroCART column, LiChrospher 100 reversed-phase silica gel RP-18 endcapped (5  $\mu$ m); UV detection at 250 nm). The hydrolysis of the compounds 2–4 was followed by integration of the peak areas in the HPLC chromatograms. The rate constants *k* were calculated by using the rate constants *k*.

(b) CEM Cell Extract: 3.0 mM stock solution of the triesters in DMSO were prepared. 20  $\mu$ L of this stock solution was mixed with 100  $\mu$ L cell extract and 20  $\mu$ L of a 70 mM magnesium chloride solution. The hydrolysis process was stopped after 8 hours by addition of 300  $\mu$ L acidic methanol and storage for 5 min at 0°C. The mixtures were centrifuged by 13000 rpm for 10 min and the supernatant was analyzed as mentioned above.





113

**HIV-Assay:** The anti-HIV evaluation has been carried out as described previously.<sup>[8]</sup>

#### ACKNOWLEDGMENTS

Financial support by the Deutsche Forschungsgemeinschaft, Germany, the Fonds der Chemischen Industrie, Germany and the René Descartes Prize 2001 of the European Commission is gratefully acknowledged.

#### REFERENCES

- Meier, C. *Cyclo*Sal-pronucleotides—design of chemical trojan horses. Mini Rev. Med. Chem. 2002, 2, 219–234.
- Balzarini, J.; Aquaro, S.; Knispel, T.; Rampazzo, C.; Bianchi, V.; Perno, C.-F.; De Clercq, E.; Meier, C. Cyclosaligenyl-2',3'-didehydro-2',3'-dideoxythymidine monophosphate: Efficient intracellular delivery of d4TMP. Mol. Pharmacol. 2000, 58, 928–935.
- Balzarini, J.; Naesens, L.; Aquaro, S.; Knispel, T.; Perno, C.-F.; De Clercq, E.; Meier, C. Intracellular Metabolism of cyclosaligenyl-3'-azido-2',3'-dideoxythymidine monophosphate, a prodrug of 3'-azido-2',3'-dideoxythymidine (zidovudine). Mol. Pharmacol. **1999**, *56*, 1354–1361.
- 4. Balzarini, J.; Baba, M.; Pauwels, R.; Herdewijn, P.; De Clercq, E. Anti-Retrovirus activity of 3'-fluoro-and 3'-azido-substituted pyrimidine 2',3'-dideoxynucleoside analogues. Biochem. Pharmacol. **1988**, *37*, 2847–2856.
- Balzarini, J.; De Clercq, E. Biochemical pharmacology of nucleoside analogs active against HIV. Biochem. Pharmacol. 1994, 49, 751–772.
- Meier, C. Pronucleotides—recent advances in the design of efficient tools for the delivery of biologically active nucleoside monophosphates. Synlett 1998, 233–242.
- Wagner, C.R.; Iyer, V.V.; McIntee, E.J. Pronucleotides: towards the in vivo delivery of antiviral and anticancer nucleotides. Med. Res. Rev. 2000, 20, 417–451.
- Meier, C.; Renze, J.; Ducho, C.; Balzarini, J. *CycloSal-d4TMP* pronucleotides structural variations, mechanistic insights and antiviral activity. Curr. Top. Med. Chem. 2002, 2, 1111–1121.
- Meier, C.; Lorey, M.; De Clercq, E.; Balzarini, J. *CycloSal-2',3'-didehydrothymidine monophosphate (cycloSal-d4TMP): synthesis and antiviral evaluation of a new d4TMP delivery system. J. Med. Chem.* **1998**, *41*, 1417–1427.
- Meier, C.; Lorey, M.; De Clercq, E.; Balzarini, J. Cyclic saligenyl phosphotriesters of 2',3'-didehydrothymidine (d4T)—a new pro-nucleotide approach. Bioorg. Med. Chem. Lett. 1997, 7, 99–104.
- Meier, C.; Lomp, A.; Meerbach, A.; Wutzler, P. *CycloSal-BVdUMP* pronucleotides: How to convert an antiviral-inactive nucleoside analogue into a bioactive compound against EBV. J. Med. Chem. **2002**, *45*, 5157–5172.
- 12. Meier, C.; Lomp, A.; Meerbach, A.; Wutzler, P. *Cyclo*Saligenyl-5-[(E)-2-bromovinyl]-2'-deoxyuridine monophosphate (*cyclo*Sal-BVDUMP) pronucleotides active against epstein-barr virus. ChemBioChem. **2001**, *4*, 283–285.



	PRINTS
--	--------

- Balzarini, J.; Haller-Meier, F.; De Clercq, E.; Meier, C. Antiviral activity of 13. cyclosaligenyl prodrugs of acyclovir, carbovir and abacavir. Antivir. Chem. Chemother. 2002, 12, 301-306.
- 14. Meier, C.; Habel, L.; Haller-Meier, F.; Lomp, A.; Herderich, M.; Klöcking, R.; Meerbach, A.; Wutzler, P. Chemistry and anti-herpes simplex virus type 1 evaluation of cycloSal-nucleotides of acyclic nucleoside analogues. Antivir. Chem. Chemother. 1998, 9, 389-402.
- Meier, C.; Knispel, T.; De Clercq, E.; Balzarini, J. CycloSal-pro-nucleotides 15. (cycloSal-NMP) of 2',3'-dideoxyadenosine (ddA) and 2',3'-dideoxy-2',3'-didehydroadenosine (d4A): synthesis and antiviral evaluation of a highly efficient nucleotide delivery system. J. Med. Chem. 1999, 42, 1604-1614.
- 16. Meier, C.; Knispel, T.; Marquez, V.E.; De Clercq, E.; Balzarini, J. CycloSal-pronucleotides of 2'-fluoro-ara-and 2'-fluoro-ribo-2',3'-dideoxyadenosine (F-ara- and F-ribo-ddA) as a strategy to bypass a metabolic blockade. J. Med. Chem. 1999, 42, 1615-1624.
- 17. Part of these data have been presented at the 15th International Conference on Antiviral Research, April 2003, Savannah, USA. Antivir. Res. 2003, 57, A39.
- McGuigan, C.; Cahard, D.; Sheeka, H.M.; De Clercq, E.; Balzarini, J. Aryl 18. phosphoramidate derivatives of d4T have improved anti-HIV efficacy in tissue culture and may act by the generation of a novel intracellular metabolite. J. Med. Chem. 1996, 39, 1748-1753.
- 19. Périgaud, C.; Gosselin, G.; Imbach, J.-L. Anti-HIV phosphotriester pronucleotides: basis for the rational design of biolabile phosphate protecting groups. In *Biomedical* Chemistry: Applying Chemical Principles to the Understanding and Treatment of Disease; Torrence, P.F., Ed.; John Wiley & Sons, Inc., 2000; 115–141. Chapter 5.
- Pearson, A.J.; Zhang, P.; Lee, K. Application of arene-ruthenium chemistry to a 20. formal totsl synthesis of OF 4949III. J. Org. Chem. 1996, 61, 6581-6586.
- Brechbühler, H.; Büchi, H.; Hatz, E.; Schreiber, J.; Eschenmoser, A. Die reaktion 21. von carbonsäuren mit acetalen des N,N-dimethylformamids: eine veresterungsmethode. Helv. Chim. Acta 1965, 48, 1746-1771.
- Wengatz, I.; Stoutamire, D.W.; Gee, S.J.; Hammock, B.D. Development of an 22. enzyme-linked immunosorbent assay for the detection of the pyrethroid insecticide fenpropathrin. J. Agric. Food Chem. 1998, 46, 2211-2221.
- Garber, S.B.; Kingsbury, J.S.; Gray, B.L.; Hoveyda, A.H. Efficient and recyclable 23. monomeric and dentritic Ru-based metathesis catalysts. J. Am. Chem. Soc. 2000. 122, 8168-8179.
- 24. Breton, G.W. Selective monoacetylation of unsymmetric diols catalyzed by silica gel-supported sodium hydrogen sulfate. J. Org. Chem. 1997, 62, 8952-8954.
- 25. Yamada, S.; Sugaki, T.; Matsuzaki, K. Twisted amides as selective acylating agents for hydroxyl groups under neutral conditions: models for activated peptides during enzymatic acyl transfer reaction. J. Org. Chem. 1996, 61, 5932-5938.
- 26. Nagata, W.; Okada, K.; Aoki, T. Ortho-Specific  $\alpha$ -hydroxylation of phenols with aldehydes: An efficient synthesis of saligenol derivatives. Synthesis 1979, 365-368.
- 27. Meier, C.; De Clercq, E.; Balzarini, J. CycloSal-3'-azido-2',3'-dideoxythymidine monophosphate (cycloSal-AZTMP) – An unexpected failure of nucleotide delivery from a proven pro-nucleotide system. Eur. J. Org. 1998, 837-846.

114

ORDER		REPRINTS
-------	--	----------

28. van Boom, J.H.; Burgers, P.M.J. Use of levulinic acid in the protection of oligonucleotides via the modified phosphotriester method: Synthesis of decaribonucleotide U-A-U-A-U-A. Tetrahedron Lett. **1976**, *52*, 4875–4878.

Received July 31, 2003 Accepted September 19, 2003



## **Request Permission or Order Reprints Instantly!**

Interested in copying and sharing this article? In most cases, U.S. Copyright Law requires that you get permission from the article's rightsholder before using copyrighted content.

All information and materials found in this article, including but not limited to text, trademarks, patents, logos, graphics and images (the "Materials"), are the copyrighted works and other forms of intellectual property of Marcel Dekker, Inc., or its licensors. All rights not expressly granted are reserved.

Get permission to lawfully reproduce and distribute the Materials or order reprints quickly and painlessly. Simply click on the "Request Permission/ Order Reprints" link below and follow the instructions. Visit the <u>U.S. Copyright Office</u> for information on Fair Use limitations of U.S. copyright law. Please refer to The Association of American Publishers' (AAP) website for guidelines on <u>Fair Use in the Classroom</u>.

The Materials are for your personal use only and cannot be reformatted, reposted, resold or distributed by electronic means or otherwise without permission from Marcel Dekker, Inc. Marcel Dekker, Inc. grants you the limited right to display the Materials only on your personal computer or personal wireless device, and to copy and download single copies of such Materials provided that any copyright, trademark or other notice appearing on such Materials is also retained by, displayed, copied or downloaded as part of the Materials and is not removed or obscured, and provided you do not edit, modify, alter or enhance the Materials. Please refer to our <u>Website</u> User Agreement for more details.

# **Request Permission/Order Reprints**

Reprints of this article can also be ordered at http://www.dekker.com/servlet/product/DOI/101081NCN120027820