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Radiolabeled Cyclosaligenyl Monophosphates of 5-Iodo-2′-deoxyuridine, 5-Iodo-3′-fluoro-2′,3′-dideoxyuridine, and 3′-Fluorothymidine for Molecular Radiotherapy of Cancer: Synthesis and Biological Evaluation

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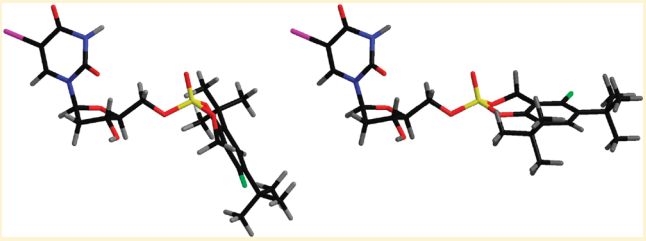
Supporting Information

ABSTRACT: Targeted molecular radiotherapy opens unprecedented opportunities to eradicate cancer cells with minimal irradiation of normal tissues. Described in this study are radioactive cyclosaligenyl monophosphates designed to deliver lethal doses of radiation to cancer cells. These compounds can be radiolabeled with SPECT- and PET-compatible radionuclides as well as radionuclides suitable for Auger electron therapies. This characteristic provides an avenue for the personalized and comprehensive treatment strategy that comprises diagnostic imaging to identify sites of disease, followed by the targeted molecular radiotherapy based on the imaging results. The developed radiosynthetic methods produce no-carrier-added products with high radiochemical yield and purity. The interaction of these compounds with their target, butyrylcholinesterase, depends on the stereochemistry around the P atom. IC_{50} values are in the nanomolar range. In vitro studies indicate that radiation doses delivered to the cell nucleus are sufficient to kill cells of several difficult to treat malignancies including glioblastoma and ovarian and colorectal cancers.



INTRODUCTION

Biological consequences of the Auger effect are confined to the proximity of the molecular site of the radionuclide decay. For this reason, the cytotoxicity of ^{125}I depends on the subcellular localization of this radionuclide.[1−3] Low energy electrons from ^{125}I decaying in DNA produce nonrepairable strand breaks and lesions[4,5] analogous to those produced by radiations with a high linear energy transfer (LET). Approximately 90% of these DNA breaks are located within 10 nm of the ^{125}I decay site. Each decay of the DNA-incorporated ^{125}I produces several single strand breaks and a double strand break with a probability of almost 1.[6−9] 5-[^{125}I]Iodo-2′-deoxyuridine (^{125}IUdR), a thymidine analogue, which is incorporated into DNA during the S phase of mitotic cells, is ∼1.6 times more effective in killing mammalian cells than 5.3 MeV α particles from intracellularly localized ^{210}Po citrate [10,11] Not surprisingly, significant research efforts are dedicated to the development of various carriers of ^{125}I with the goal of targeting ^{125}I to the cell nucleus. Investigated reagents include pyrimidines,[12−18] DNA intercalators,[19−21] antibodies,[22−27] steroids,[28−30] chemotherapeutic drugs,[31,32] as well as peptides[33] and other reagents.[34−37] Of these, ^{125}IUdR, which is incorporated into the DNA of proliferating cells in vitro[38] and in vivo,[39] is the most effective in the production of unrepairable DNA strand breaks, and for this reason, it is the most radiotoxic.[40−43]

An important factor limiting the effectiveness of ^{125}IUdR in the molecular radiotherapy of cancer is its short metabolic half-life in circulation. Intravenously administered ^{125}IUdR is destroyed within a few minutes after the injection. As a result, the tumor uptake is insufficient to kill cancer cells. Only cells that happen to make DNA when ^{125}IUdR is present can utilize this compound in place of thymidine. In view of proliferative heterogeneity of the cancer cell population, it is not surprising that even after intratumor injections only ∼0.02% of the injected ^{125}IUdR is associated with 1 g of tumor (range of 0.001−0.06% ID/g).[44] Various approaches to circumvent this problem were tested, for example, local or regional administration,[43,45,46] preparation of prodrugs,[47,48] and protein conjugates,[49,50] as well as the coadministration of various inhibitors and antagonists to block the dehalogenation pathways.[51−54] Recently, a targeted version of ^{125}IUdR and its monophosphate was synthesized and successfully evaluated as a molecular radiotherapeutic in experimental models of androgen receptor-expressing cancers such as ovarian and prostate.[55]

Compounds reported herein expand the range of targetable derivatives of ^{125}IUdR. In addition to binding butyrylcholinesterase (BChE), a tumor-associated target, these reagents have 5′-cyclosaligenyl monophosphate residues, which allow their

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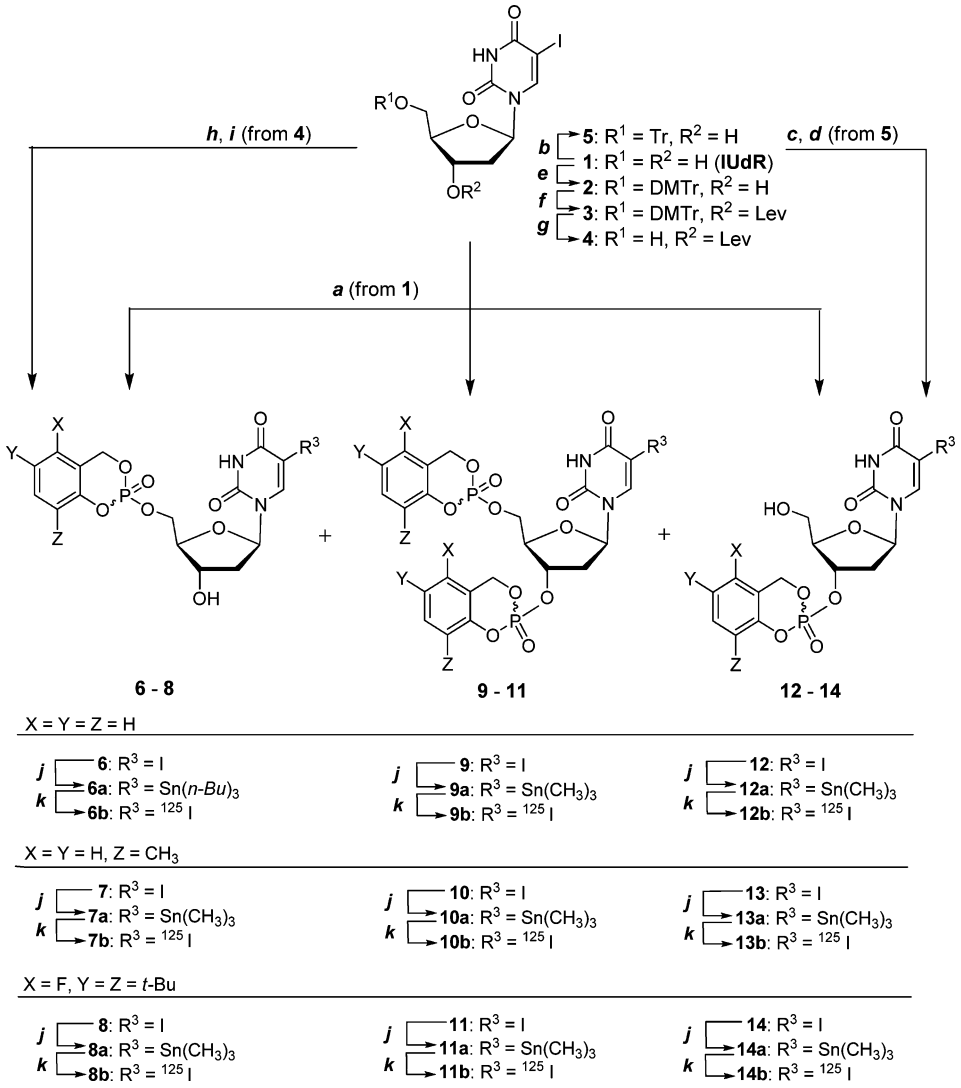
Scheme 1. Synthetic Pathways to Cyclosaligenyl Phosphotriesters 6b−14b[a]



[a]Reagents and conditions: (a) (i) crude **15** for X = Y = Z = H, 16 for X = Y = H, Z = CH_3, **17** for X = F, Y = Z = *t*-Bu, prepared using general procedure A, transferred in THF to IUdR (**1**) in DMF/THF, DIPEA, −60 °C → rt, 2 h; (ii) *t*-BuOOH, −40 °C → rt, 1 h; (iii) separation of 5′-, 3′-, and 5′,3′-regioisomers on SiO_2 column; (b) TrCl in pyridine, 0 °C → rt, 12 h; (c) (i) like (a) but chlorophosphites **15**−**17** transferred in diethyl ether into **5** in CH_3CN, DIPEA, −40 °C → rt and 3 h reaction period; (ii) *t*-BuOOH, −20 °C → rt, 1 h; (d) $ZrCl_4$ (2.5 equiv) in CH_3CN, 1 h, rt; or 1 N HCl/CH_3CN, 45 °C → rt, 10 min; (e) DMTrCl in pyridine, DMAP (0.05 equiv), 0 °C → rt, 12 h; (f) levulinic acid, DCC, DMAP (0.05 equiv), CH_2Cl_2/diethyl ether, rt; (g) $ZrCl_4$ (1 equiv), CH_3CN, 15 min, rt; (h) (i) like (c) but chlorophosphites were transferred in diethyl ether to **4** in CH_3CN, DIPEA, −40 °C → rt, 2 h reaction period; (ii) *t*-BuOOH, −20 °C → rt, 1 h; (i) $N_2H_4{\cdot}H_2O$, pyridine/AcOH, 2 min; (j) $Sn_2(CH_3)_6$ or $Sn_2(n\text{-}Bu)_6$ used in preparation of **6a**, (1.2−1.6 equiv) in ethyl acetate (preparation of **7a**, **8a**, **9a**, **10a**, **11a**) or in dioxane (preparation of **6a**, **7a**, **12a**, **13a**), $(Ph_3P)Pd(II)Cl_2$ (0.06 equiv), refluxed under nitrogen, 2−4 h; (k) (i) $Na^{125}I$ in NaOH (9.25−444 MBq), 30% H_2O_2, TFA/CH_3CN (0.1% v/v), 1 min sonication, 5−20 min reaction time; (ii) HPLC purification.

intracellular retention and sustained availability to participate in the DNA synthesis, resulting in high levels of ^{125}I incorporation into the DNA. The biological behavior of this series of targetable $^{125}IUdR$ derivatives was tested in cell lines derived from difficult to treat human cancers including glioblastoma, ovarian, and colorectal cancers, which express high levels of BChE.

RESULTS AND DISCUSSION

Synthesis. Evaluated in this study cyclosaligenyl (cycloSal) monophosphates of $^{125}IUdR$, and 5-[^{125}I]iodo-3′-fluoro-2′,3′-dideoxyuridine (**21b**) were synthesized in three consecutive steps as shown in Schemes 1 and 3. The nonradioactive iodo analogues **6**−**14**, **21**, and **24** were prepared first and subsequently reacted with hexamethylditin to afford the corresponding 5-trimethylstannyl cycloSal derivatives **6a**−**14a**, **21a**, and **24a**. These organotin precursors furnished the target ^{125}I-iodinated cycloSal phosphotriesters **6b**−**14b**, **21b**, and **24b** in the course of the electrophilic iododestannylation performed in a third phase of the synthesis. All ^{125}I radiolabelings were conducted at the noncarrier-added concentration level, i.e., using ^{125}I with specific activity of ∼80 475 GBq/mmol.

Most of the time, cycloSal monophosphates are attained through the phosphorus(III) route,[56] and a general course of the preparation was established by Meier's group.[57] We followed this strategy in the first part of the synthesis, introducing the cycloSal segment by means of chlorophosphites. Thus, unprotected IUdR (**1**), 5-iodo-3′-*O*-levulinyl-2′-

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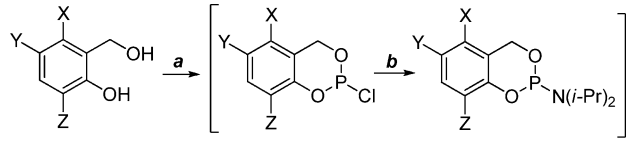
deoxyuridine (**4**), and 5-iodo-5′-*O*-trityl-2′-deoxyuridine (**5**) were coupled with cyclic chlorophosphites **15**−**17** in the presence of *N*,*N*-diisopropylethylamine[58] (Scheme 2), and generated corresponding phosphites were directly oxidized with *tert*-butyl hydroperoxide to give the expected diastereomeric mixtures of the cyclosaligenyl products. The same preparation scheme was used to introduce the cycloSal moiety into thymidine, 3′-fluoro-3′-deoxythymidine (**20**), and 5-iodo-3′-fluoro-2′,3′-dideoxyuridine (**21**) and to prepare cyclosaligenyl monophosphates **22**, **23**, and **24** (Scheme 3). Direct phosphorylation of **1**, performed without protecting the 3′-OH or 5′-OH group, gave access to the 5′-*O*- and 3′-*O*-cycloSal-5-^{125}IUdR monophosphates, permitting the concurrent biological evaluation of both groups of regioisomers. Typically, when phosphitylation of **1** by chlorophosphites **15**−**17** (1.05−1.25 mol equiv) was carried out at temperatures below −40 °C, the mixture of 5′-*O*- and 3′-*O*-cyclosaligenyl phosphotriesters was formed, with only marginal regioselectivity. The 3′,5′-*O*,*O*-diphosphorylation occurred in the range 16−22%. The separation of regioisomers, achieved without difficulty by flash column chromatography on a silica gel, furnished pure diastereomeric 5′-*O*-, 3′-*O*-, and 3′,5′-*O*,*O*-cycloSal products in a fair overall yield of 39−46%. The 5′-*O*-phosphitylation of **4**, along with the "one-pot" oxidation followed by the deprotection of 3′-levulinate group,[59] led to the corresponding phosphotriesters **6**−**8** in 52−62% yield. Phosphitylation of 5-iodo-5′-*O*-trityl-2′-deoxyuridine (**5**) provided the expected products **12**−**14** in the overall yield of ∼50%. A similar reaction applied to 5-iodo-5′-*O*-(4,4′-dimethoxytrityl)-2′-deoxyuridine (**2**) failed because of the apparent spontaneous cleavage of the 4,4′-dimethoxytrityl (DMTr) group during a very slow phosphitylation step, presumably caused by the induced large steric hindrance. Phosphitylation of IUdR protected at the 3′- or 5′-position did not improve overall efficiency of the preparation because of the necessary protection and deprotection steps, but the individual regioisomers synthesized in this way simplified the identification of the products originating from the unprotected **1**. The initial parameters used in the selection of compounds with 3-methylcycloSal, 3,5-di(*tert*-butyl)-6-fluorocycloSal, and unsubstituted cycloSal ring systems were based on previous reports of similarly substituted 5′-*O*-cycloSal derivatives of 2′,3′-dideoxy-2′,3′-didehydrothymidine,[57]

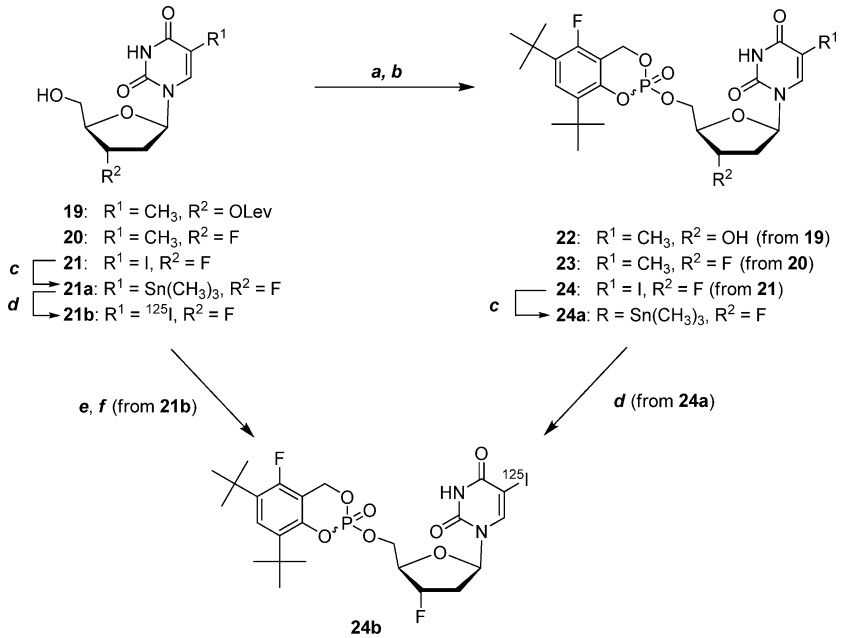
Scheme 2. Preparation of Chlorophosphites 15−17 and Phosphoramidite 18[a]



15: X = Y = Z = H
16: X = Y = H, Z = CH_3
17: X = F, Y = Z = *t*-Bu
18: X = F, Y = Z = *t*-Bu

[a]Reagents and conditions: (a) (i) PCl_3, in Et_2O, −40 °C, 15 min; (ii) Et_3N or pyridine, −40 °C → rt, 3 h; (iii) kept under nitrogen at −20 °C, 12 h; (b) (i) crude **17** prepared in the presence of Et_3N; (ii) $(i\text{-}Pr)_2NH$, −20 °C → rt, 2 h, no purification, used immediately.

Scheme 3. Synthesis of 5′-Sal-cyclosaligenyl Phosphotriesters 22 and 23 Derived from Thymidine and 3′-Fluoro-3′-deoxythymidine and Synthesis of 5-[^{125}I]Iodo-5′-*O*-[cyclo-3,5-di(*tert*-butyl)-6-fluorosaligenyl]-3′-fluoro-2′,3′-dideoxyuridine 24b via Iododestannylation of 24a or the Direct Coupling of Phosphoramidite 18 and 5-[^{125}I]Iodo-3′-fluoro-2′,3′-dideoxyuridine 21b[a]



[a]Reagents and conditions: (a) (i) **17** prepared by general procedure A, transferred in diethyl ether to a solution of **19** or **20** in CH_3CN, DIPEA, −40 °C → rt, 2 h; (ii) *t*-BuOOH, −20 °C → rt, 1 h; (b) for **22** from **19** $N_2H_4{\cdot}H_2O$, pyridine/AcOH, 2 min; (c) $Sn_2(CH_3)_6$, $(Ph_3P)Pd(II)Cl_2$, refluxed under nitrogen, 2 h (for **21**), 4 h (for **24**); (d) (i) $Na^{125}I$ in NaOH, 30% H_2O_2, 2 min; (ii) TFA/CH_3CN (0,1% v/v), 1 min sonication, 20 min reaction time; (iii) HPLC purification; (e) (i) **18** transferred in CH_3CN to **21b**, −40 °C; (ii) 1*H*-tetrazole (0.45 M solution in CH_3CN), −40 °C → rt, 10 min; (iii) *t*-BuOOH, −20 °C → rt; (iiii) a solution (5%, v/v, 1mL) of $NaHSO_3$/EtOAc; (f) HPLC purification.

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showing a broad range of lipophilicity and rates of hydrolysis in aqueous buffer at physiological pH 7.3.

The organotin precursors **6a**−**14a**, **21a**, and **24a** were prepared in reactions with hexamethylditin (except **6a**, which was a tri-*n*-butyl derivative) catalyzed by bis-(triphenylphosphine)palladium(II) dichloride. Stannylations were carried out under nitrogen in boiling dioxane or ethyl acetate, depending on the solubility of starting iodotriester. In ethyl acetate, the dehalogenation was reduced from ∼20% observed at higher temperatures, to less than 10% at 60 °C. Two major products were always present. The first product, with higher mobility on TLC and proven to be the trimethylstannyl derivative, was isolated in 52−77% yield. The second, with lower TLC mobility, was identified as a deiodinated starting phosphotriester. All synthesized cycloSal-5-trimethylstannyl-2′-deoxyuridine phosphotriesters were readily amenable to no-carrier-added radioiododestannylation, and excellent isolated yields of the ^{125}I-iodolabeled compounds were always achieved. Higher hydrophobicity of stannanes, compared to the corresponding iodo derivatives, allowed for a complete separation of the excess of trimethyltin precursors from the ^{125}I-iodinated products, even when a large volume of the crude reaction mixture (up to 1 mL) was loaded onto the HPLC column during the final purification.

We have also examined the possibility of the direct chlorophosphite coupling with radioiodinated **21b** (Scheme 3). This approach would permit a straightforward, one-step synthesis of radiolabeled cyclosaligenyl monophosphates derived from the already radiolabeled deoxyuridine or thymidine derivatives and could be particularly useful in the preparation of ^{18}F-containing cyclosaligenyl phosphotriesters. To evaluate this option, we first prepared 5-[^{125}I]iodo-3′-fluoro-2′,3′-dideoxyuridine (**21b**) and 5-[^{125}I]iodo-5′-*O*-[cyclo-3,5-di(*tert*-butyl)-6-fluorosaligenyl]-3′-fluoro-2′,3′-dideoxyuridine (**24b**) by ^{125}I-iododestannylation of the corresponding stannanes **21a** and **24a**. Subsequently, compound **21b**, which closely mimics physicochemical properties of 3′-[^{18}F]fluoro-3′-deoxythymidine (^{18}FLT), was used as a surrogate of ^{18}FLT in the coupling with **17**, whereas **24b** served as the analytical standard to monitor the reaction progress and to compare products appearing in both methods. The major product isolated after the coupling of **17** with **21b** was indeed **24b** and was identical (HPLC co-injections) with **24b** previously obtained by the iododestannylation of stannane **24a**. However, the acceptable radiochemical yields (40−47%, reproduced in five preparations) were achieved only if (1) the crude saligenyl *N*,*N*-diisopropylaminophosphoramidite (**18**) (Scheme 2) was initially generated by the treatment of **17** with *N*,*N*-diisopropylamine and (2) the phosphitylation was carried out in the presence of 1*H*-tetrazole. Even so, these results indicate that the coupling of cyclic chlorophosphites can be effectively performed at the noncarrier-added concentrations and applied to preparations of cyclosaligenyl-^{18}FLT derivatives and other radiolabeled cycloSal analogues.

All ^{125}I-iodinations were conducted within a range of 9.25−444 MBq, using ∼120 μg of the stannyl forerunner. The reaction mixtures were acidified with TFA in acetonitrile, and hydrogen peroxide was used to oxidize sodium [^{125}I]iodide. The modified procedure for radioiododestannylation, developed earlier in these laboratories for the synthesis of ^{125}IUdR,[60] gave consistently excellent radiochemical yields of 85−93% and a radiochemical purity of ≥95%, across the series of cycloSal-phosphotriesters. Proton destannylated byproduct (3−8%), found in all of the crude reaction mixtures, originated mostly from the frozen tin precursor samples, and the amount was slowly increasing after extended storage of stannanes (often exceeding 6 months).

To confirm the radiochemical purity and accurately measure the specific activity of labeled products, the reaction mixtures were purified on the HPLC system, with the concurrent monitoring of the radioactivity and absorbance (λ = 220/280 nm). Although the HPLC analysis performed within 24 h generally indicated products with ≥95% radiochemical purity, all radiolabeled compounds kept in a solution of aqueous acetonitrile overnight, usually at concentrations of ∼37 kBq/μL, were routinely purified one more time, shortly before the intended experiments. HPLC co-injections of the ^{125}I-radiolabeled cycloSal-products and the corresponding nonradioactive iodo analogues corroborated the identity of radioiodinated compounds. Characterization of all nonradioactive phosphotriesters was carried out by means of ^{1}H, ^{13}C, ^{119}Sn, and ^{31}P NMR and high-resolution mass spectrometry and thorough the HPLC analyses.

Resolution of Diastereomers. The synthesis of 5′-*O*- and 3′-*O*-cyclosaligenyl phosphotriesters was not stereoselective; therefore, all products were obtained as mixtures of diastereomers (S_P and R_P configurations at approximately 1:1 ratio) at each phosphorus center. To differentiate isomers in this study, diastereomers are labeled as the ***-fast*** and the ***-slow*** in correlation to the HPLC retention time (t_R) of each isomer. Only tentative assignment of the R_P/S_P stereochemistry at the phosphorus atom could be made, following the already known absolute stereochemical configuration of 5-formyl-3-*tert*-butyl-cyclosaligenyl-2′,3′-dideoxy-2′,3′-didehydrothymidine monophosphate as the reference.[61] Thus, it could be assumed that ***-fast*** diastereomers should have S_P configuration and that ***-slow*** isomers should have R_P configuration, although the unambiguous confirmation of this assignment requires further investigation.

Diastereomers of all synthesized 5′-*O*-cycloSal phosphotriesters (**6**−**8** and **24** (nonradioactive compounds) and also **6b**−**8b** and **24b** (^{125}I-radioiodolabeled products)) and diastereomers of all ^{125}I-radioiodolabeled 3′-*O*-cycloSal phosphotriesters (**12b**−**14b**) can be separated by reverse phase HPLC. Because of their close elution profiles, the separation of diastereomeric stannanes **6a**−**8a** and **24a** and particularly **12a**−**14a** was demanding and required numerous injections onto a tandem of two reverse phase HPLC columns. The amount of a single isolated diastereomer usually did not exceed a total of 30 mg. However, this amount was sufficient to complete all essential analyses and to prepare adequate quantities of each highly purified diastereomer of the tin precursors **6a**−**8a** (∼120 μg each) suitable for the immediate radioiododestannylation. ^{125}I-Radioiodinated diastereomers were separated in one of two ways: (1) by conducting ^{125}I-iododestannylation with a single diastereomer of the trimethylstannyl precursor (radioiodinated diastereomers of cycloSal triesters were repeatedly obtained in quantities of up to ∼450 MBq using this method) or (2) by using a diastereomeric mixture of stannane and the separation of isomers that followed the final purification of the ^{125}I-radioiodolabeled product. In this case, however, the separation often involved multiple injections and was limited by the largest amount of the diastereomeric mixture, which could be fully resolved in a single HPLC run. Therefore, when a larger batch of the ^{125}I-labeled 5′-*O*- or 3′-*O*-cycloSal triester diastereomer

Table 1. Rates of Hydrolysis and Half-Lives of Reagents Incubated in Phosphate Buffered Saline

	k, min^{-1}	std err for k, min^{-1}	half-life $t_{1/2}$, h	std err for $t_{1/2}$, h	P	R^2 unweighted
6b-*fast*	2.25×10^{-3}	7.0×10^{-5}	5.1	0.17	<0.0001	0.950
6b-*slow*	1.52×10^{-3}	4.9×10^{-5}	7.6	0.25		0.933
7b-*fast*	5.44×10^{-4}	1.0×10^{-5}	21.2	0.38	<0.0001	0.997
7b-*slow*	4.63×10^{-4}	1.0×10^{-5}	25.0	0.53		0.997
8-*fast*	2.41×10^{-4}	1.3×10^{-5}	47.9	2.58	0.0006	0.992
8-*slow*	1.88×10^{-4}	7.0×10^{-6}	61.5	2.28		0.998
12-*fast*	2.05×10^{-3}	5.8×10^{-5}	5.3	0.17	<0.0001	0.998
12-*slow*	1.64×10^{-3}	3.7×10^{-5}	7.1	0.16		0.998
13-*fast*	9.46×10^{-4}	4.5×10^{-5}	12.2	0.58	0.018	0.992
13-*slow*	8.13×10^{-4}	3.1×10^{-5}	14.2	0.55		0.993
14-*fast*	7.66×10^{-4}	1.9×10^{-5}	15.1	0.37	0.001	0.997
14-*slow*	6.67×10^{-4}	1.8×10^{-5}	17.3	0.50		0.996
24-*fast*	2.92×10^{-4}	2.0×10^{-6}	39.6	0.27	<0.0001	0.999
24-*slow*	2.22×10^{-4}	2.2×10^{-6}	52.0	0.52		0.999
24b-*fast*	3.01×10^{-4}	9.1×10^{-6}	38.5	1.17	<0.0001	0.999
24b-*slow*	2.24×10^{-4}	4.9×10^{-6}	51.5	1.13		0.999

was required (185−370 MBq), a method employing resolved diastereomers of the trimethyltin precursor was preferred.

All 3′,5′-*O*,*O*-di-cycloSal phosphotriesters **9**−**11** with two stereogenic centers formed during the synthesis were expected to exist as mixtures of four stereoisomers with configurations S_P/S_P, R_P/S_P, S_P/R_P, and R_P/R_P, all at a ratio of 1:1. Indeed, the HPLC and ^{31}PNMR analyses of 3′,5′-disubstituted iodides **9**−**11** and stannanes **9a**−**11a** indicated mixtures of four diastereomers. These, however, were inseparable, and all of the applied HPLC methods led only to a partial partition. Consequently, the ^{125}I-iodolabeled 3′,5′-*O*,*O*-di-cycloSal phosphotriesters **9b**−**11b** were isolated and purified only as mixtures of diastereomers.

Hydrolysis Pathways and Stability. In the experimentally established cycloSal pronucleotide concept,[62] the cycloSal moiety is cleaved because of nucleophilic attack of the hydroxide anion on the phosphorus atom and formation of the resonance stabilized phenolate. The lower stability of the phenyl compared to the benzyl phosphate ester moiety and activation of the phosphotriester molecule induce spontaneous cleavage of the remaining benzyl phosphate group and generate the nucleotide.[63] In this series of cycloSal monophosphates however, a second hydroxyl group is present in the glycone and the potential intramolecular attack at a phosphorus center could cause the formation of the undesired 3′,5′-cyclic products. Such a transformation has been observed for 3-methylcyclosaligenyl penciclovir monophosphate.[64] Therefore, it was essential to examine the hydrolysis of synthesized cycloSal triesters and establish whether the hydrolysis terminates with the desired monophosphates. Diastereomers of compounds **6**−**8**, **12**−**14**, and **24**, as well as their radiolabeled analogues **6b**−**8b**, **12b**−**14b**, and **24b**, were hydrolyzed in phosphate buffer (50 mM, pH 7.3 at 37 °C) to examine the chemical stability of triesters under conditions comparable to the physiological environment (Supporting Information, pp S93−S105). The release of 5-[$^{127/125}$I]iodo-2′-deoxyuridine monophosphate was monitored by the HPLC and verified in the ^{31}PNMR experiments. Half-lives ($t_{1/2}$) were determined by the integration of the decreasing phosphotriester peak areas in chromatograms (Table 1). The hydrolytic degradations followed pseudo-first-order kinetics and generated monophosphate of IUdR and salicyl alcohols, confirmed at the end of hydrolysis by means of co-injections of the independently prepared standard of 5-[$^{127/125}$I]iodo-2′-deoxyuridine monophosphate[55] and the corresponding salicyl alcohol. Diastereomers did not hydrolyze at the same rate. The ***fast*** diastereomers hydrolyzed more quickly with a ratio of $t_{1/2}$(***slow***) to $t_{1/2}$(***fast***) of 1.2−1.5, depending on the substitution of the cycloSal-phenyl ring (Table 1). Since IUdR was not detected among products of the hydrolysis, the dephosphorylation of the cycloSal triesters was excluded. The hydrolysis pathway of **8** was also confirmed by ^{31}P NMR experiments conducted in imidazole/hydrochloric acid buffer solution (pH 7.2) at ambient temperature as previously described.[65] Identification of the hydrolysis products was based on the observed chemical shifts and proton-coupled ^{31}P NMR experiments. The data obtained for **8-*fast*** and **8-*slow*** diastereomers show the intermediate formation of benzyl phosphate diester (a quintet in the proton-coupled and a singlet at 2.18 ppm in the decoupled mode) and, subsequently at the end of hydrolysis, IUdR monophosphate as a main product (a singlet at 0.08 ppm in the decoupled mode). Only traces of phenyl phosphate diester, secondary to the spontaneous benzyl C−O bond break, were detected during the hydrolysis of **7** and **13**, with no indication of the concurrent formation of 3′,5′-cyclic products.

BChE Inhibitory Activity. The inhibition potency toward human BChE of 5′-*O*- and 3′-*O*-cyclosaligenyl phosphotriesters was evaluated using a multiwell assay developed in our laboratories based on the previously described procedures,[66] using purified human enzyme. The significant influence of cycloSal phosphotriester stereochemistry on inhibitory potency has been identified. Only one diastereomer in each pair strongly inhibits BChE by irreversible binding to the active site of the enzyme. A similar observation was reported for other cycloSal triesters.[67] In this study, a comparison of the half-maximal inhibitory concentrations (IC_{50}) of the tested individual ***fast*** and ***slow*** diastereomers showed that only ***slow*** isomers are strong inhibitors of BChE (Table 2). In contrast, ***fast*** diastereomers have shown weak or no inhibition of BChE. The observed BChE inhibition by diastereomeric mixtures originates almost exclusively from the ***slow*** isomers. For example, when human BChE was inhibited with the unresolved **8**, IC_{50} was measured at 1135(43) nM, which is significantly lower compared to IC_{50} >60 mM estimated for **8b-*fast***. Under the same conditions, IC_{50} of **8b-*slow*** is 50.1(0.7) nM.

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Table 2. Inhibitory Activities of Cyclosaligenyl Phosphotriesters toward Human Butyrylcholinesterase

	IC_{50} [a]	
	slow	*fast*
6	9.2 (0.16) nM	1284 (256) nM
7	61.2 (1.81) nM	>7 mM[b]
8	50.1 (0.69) nM	>60 mM[b]
22	375.9 (8.43) nM	23.6 μM[b]
23	42.6 (0.17) nM	202.6 (0.95) nM
24	19.8 (0.12) nM	549.2 (10.98) nM
8 mix	1135 (43.4) nM	

[a]Shown are the average values with standard deviation in parentheses. [b]Estimated.

Diastereomeric 3′-*O*-cycloSal regioisomers do not inhibit BChE; e.g., IC_{50} values for **14** were estimated at >1 M and were about the same for **14-*fast*** and **14-*slow***. Only when human BChE was incubated with **14-*fast*** and **14-*slow*** for 24 h was some evidence observed of BChE inhibition for **14-*slow*** with an estimated IC_{50} > 3 mM.

Interactions of all radioactive compounds with human BChE were also evaluated using denaturing, nonreducing (Figure 1A

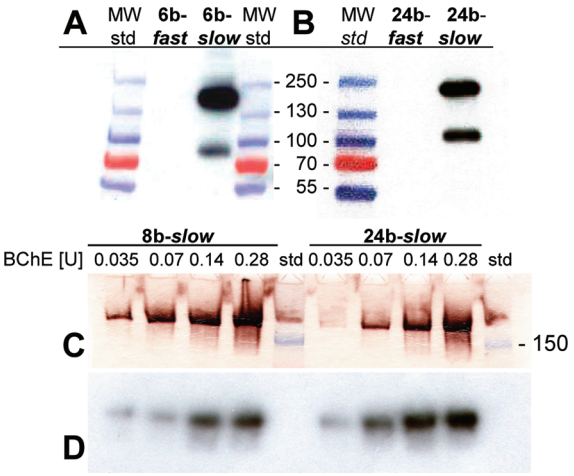

Figure 1. Electrophoretic analyses of interactions between radioactive compounds **6b**, **8b**, and **24b** and BChE. Autoradiograph of denaturing nonreducing 4–20% gradient SDS–PAGE gels of human BChE (0.02 U) reacted with (A) **6b-*fast*** and **6b-*slow*** and (B) **24b-*fast*** and **24b-*slow*** for 30 min. (C) Native gel stained with the Karnovsky and Roots stain for BChE activity. (D) Autoradiograph of the native gel shown in (C) illustrating activity-dependent interactions of **8b-*slow*** and **24b-*slow*** reacted for 30 min with increasing activities of BChE.

and Figure 1B), and native (Figure 1C and Figure 1D) gel electrophoresis as well as HPLC assays (Supporting Information pp S106–S110). Autoradiographs of human BChE interactions with diastereomers of **6b** and **24b** are shown in Figure 1A and Figure 1B. BChE (0.02 U) binds the ***slow*** diastereomers, while there is no detectable binding of ***fast*** diastereomers. The native gel stained for BChE activity and autoradiographs shown in Figure 1C and Figure 1D illustrate BChE-activity-dependent binding of **8b-*slow*** and **24b-*slow***.

Uptake Kinetics. Each diastereomer has a distinctive time-dependent uptake in LS 174T and OVCAR-3 cancer cell lines. Both cell lines retain more of the ***slow*** diastereomer over time (Figure 2). More radioactivity is retained in OVCAR-3 cells

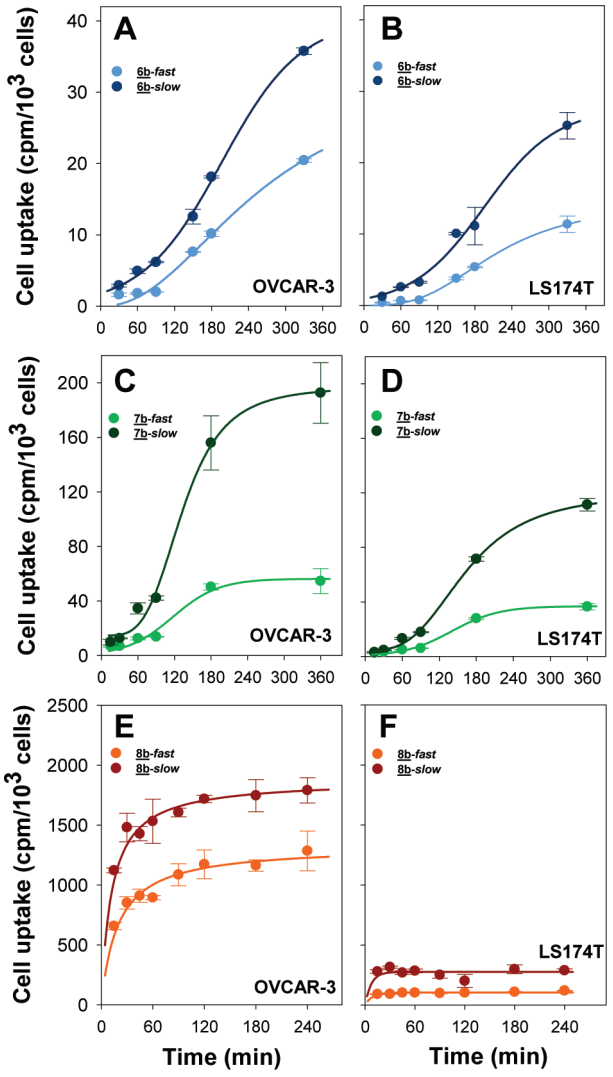

Figure 2. In vitro uptake kinetics in human ovarian OVCAR-3 adenocarcinoma and human colorectal LS 174T adenocarcinoma cells. (A) Uptake of **6b-*fast*** and **6b-*slow*** in OVCAR-3 cells. (B) Uptake of **6b-*fast*** and **6b-*slow*** in LS 174T cells. Radioactive concentration in (A) and (B) is 46.9 ± 1.1 kBq/mL. (C) Uptake of **7b-*fast*** and **7b-*slow*** in OVCAR-3 cells. (D) Uptake of **7b-*fast*** and **7b-*slow*** in LS 174T cells. Radioactive concentration in (C) and (D) is 35.1 ± 0.3 kBq/mL. (E) Uptake of **8b-*fast*** and **8b-*slow*** in OVCAR-3 cells. Radioactive concentration is 16.7 ± 1.2 kBq/mL. (F) Uptake of **8b-*fast*** and **8b-*slow*** in LS 174T cells. Radioactive concentration of **8b-*fast*** is 13.6 ± 0.4 kBq/mL. Radioactive concentration of **8b-*slow*** is 17.5 ± 0.3 kBq/mL. Shown are the average and standard deviation, n = 3 per time.

compared to LS 174T cells presumably because BChE levels are higher in OVCAR-3 cells. The activity of BChE in LS 174T cells grown in vitro is 10.6(0.3) U/mg total protein. OVCAR-3 cells have significantly higher levels of BChE activity 12.4(0.6) U/mg total protein (P = 0.017). In vitro grown LS 174T and OVCAR-3 cells have doubling times of 16 and ~40 h, respectively. The uptake profiles of **6b** and **7b** appear to have two stages. At earlier times, up to 90 min, the uptake of **6b-*slow*** is 6.5× and 4.6× faster than **6b-*fast*** in OVCAR-3 and LS 174T, respectively. In this initial phase, the radioactive content of the cells reflects the differences in BChE levels. After 90 min, the

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uptake ratios of **6b-*slow*** to **6b-*fast*** in both cell lines fall to ~1.7. The uptake in OVCAR-3 cells is still higher. However, the appearance of the hydrolysis products, which do not require BChE for the uptake and retention, cancels out a portion of the uptake differences. In the case of **7b-*slow*** and **7b-*fast***, which have longer $t_{1/2}$ of hydrolysis, the uptake is linear for at least 3 h and then levels off with the ratios of ***slow*** to ***fast*** uptake after 6 h of 3.5 and 3.0 in OVCAR-3 and LS 174T, respectively. The radioactivity associated with cells treated with **7b-*slow*** is 1.7× higher in OVCAR-3 compared to LS 174T, reflecting differences in the BChE expression. Within the time frame of the experiment, the hydrolysis is not a factor in the uptake of **8b-*slow*** and **8b-*fast***, which have $t_{1/2}$ of 2.5 and 2 days, respectively (Table 1). Therefore, the disparity of uptake between these diastereomers is likely in part a reflection of the active transport via the interactions with BChE. However, it appears that the hydrophobicity of **8b** facilitates passive diffusion through the cell membrane. The cell associated activity of **8b-*fast*** after 3 h of incubation was >20× and >110× higher compared to **7b-*fast*** and **6b-*fast***, respectively. Similarly, **8b-*slow*** accumulated at levels 35× and ~180× higher compared to **7b-*slow*** and **6b-*slow***. The evaluation of **24b** was somewhat complicated by the low solubility of this compound in aqueous media. For these reasons, all tests were conducted at 18.5 kBq/mL or lower. The intracellular processing of **24b** does not parallel the metabolic fate of **6b**, **7b**, and **8b**, and for this reason, the cellular uptake is not proportional to the time of the cell exposure to the radioactive compounds. The subcellular fractionation studies described below illustrate and explain these differences. The observed differences between the ***slow*** and ***fast*** diastereomer uptake can be related directly to the BChE's activity associated with the cancer cell. Additional factors contributing to the retention of each compound appear to be the rate of hydrolysis and intracellular processing. This multifactorial dependence of the intracellular uptake and retention may be useful as a selection criterion for in vivo studies; e.g., reagents with faster rates of hydrolysis can be used in the treatment of xenografts with high proliferative rates, whereas the treatment of xenografts with the lower turnover rates may benefit from reagents with prolonged intracellular retention.

Concentration-Dependent Uptake. The cell uptake is dependent on the extracellular concentration of the radioactive compound, and it is saturable (Figure 3A and Figure 3C). Cells were exposed to increasing concentrations of **6b-*slow*** and **6b-*fast*** for 3 h. ^{131}IUdR added to the growth medium was used as an internal standard to verify that the cell proliferation rates were not influenced by the presence of the radioactive drugs (Figure 3B and Figure 3D). The **6b-*fast*** uptake was lower compared to the **6b-*slow*** uptake in LS 174T and OVCAR-3 cells. B_{max} values were estimated in LS 174T cells at ~1300 molecules/cell and ~6800 molecules/cell for **6b-*fast*** and **6b-*slow***, respectively. OVCAR-3 cells have higher amounts of BChE. As a result, B_{max} values were also higher at ~7400 for **6b-*fast*** and >50000 molecules/cell for **6b-*slow***. The cell uptake of other compounds is also directly proportional to the extracellular concentration (Figure 4A) and time of exposure (Figure 4B).

Cell Survival and Subcellular Distribution Studies. U-87 MG glioblastoma and LS 174T colorectal adenocarcinoma cell lines, which produce colonies, were used to determine the effects of radioactive compound on the clonogenic survival, i.e., the reproductive integrity of treated cells. Surviving fractions

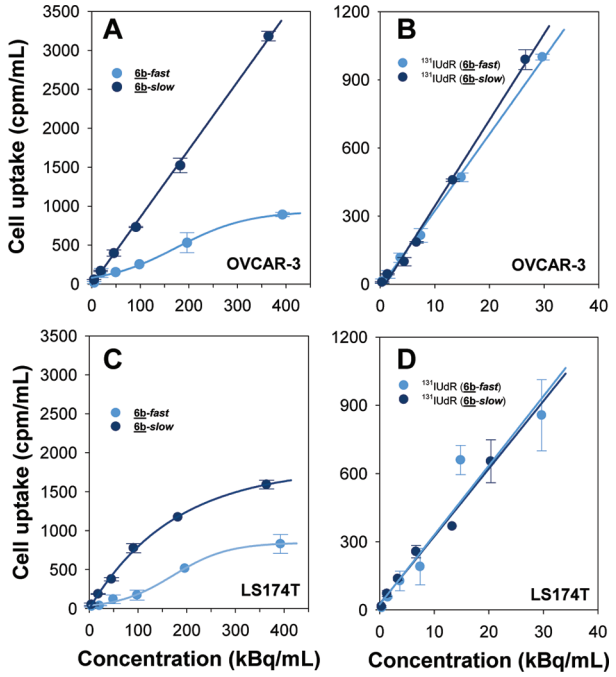

Figure 3. Concentration-dependent uptake of **6b-*fast*** and **6b-*slow*** in human adenocarcinoma cells. (A) Uptake of **6b** in OVCAR-3 cells. (B) Uptake of ^{131}IUdR in OVCAR-3 cells. (C) Uptake of **6b** in LS 174T cells. (D) Uptake of ^{131}IUdR in LS 174T cells.

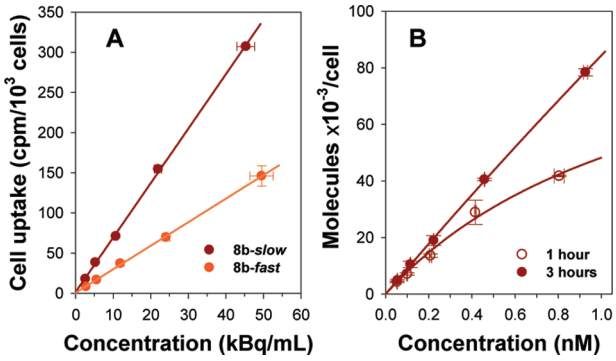

Figure 4. Concentration- and time-dependent uptake of **8b-*fast*** and **8b-*slow*** in human adenocarcinoma cells. (A) Uptake in LS 174T after 1 h of incubation with radioactive compounds. (B) Uptake of **8b-*slow*** expressed as molecules per 1000 cells in OVCAR-3 cells after 1 and 3 h of incubation with the radioactive compound.

were calculated as the ratio of the number of colonies derived from cells treated with the radioactive compound to the number of colonies derived from untreated control cells. In instances of zero or near zero clonogenic survival, the cell survival was also measured as the ratio of viable cells harvested at earlier times after treatment with radioactive drugs to the number of viable cells harvested from the control flasks treated with the vehicle only.

Glioblastoma is a good candidate for the clinical use of the described reagents because proliferating cancer cells are surrounded by cells of normal brain, which are mostly quiescent. U-87 MG is an epithelial cell line derived from grade IV glioblastoma resected from the brain of a 44-year-old Caucasian female.[68] U-87 cells grown in vitro express BChE activities at 11.1(0.3) U/mg total protein. Treatment with **6b** in vitro produces significant U-87 cell killing after 24 h of

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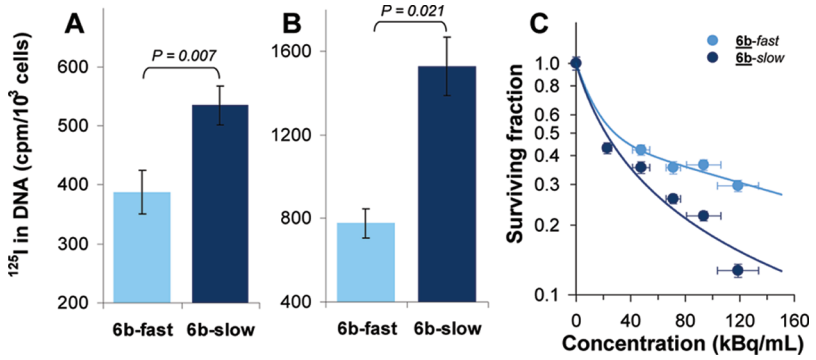

Figure 5. Evaluation of **6b** in U-87 MG human glioblastoma cells. (A) Retention of ^{125}I in DNA of U-87 MG cells treated with 27.3 kBq/mL **6b-*slow*** and **6b-*fast*** for 24 h and allowed to grow for an additional 144 h in fresh medium. (B) Retention of ^{125}I in DNA of U-87 MG cells treated with 185 kBq/mL of **6b-*slow*** and **6b-*fast*** for 120 h (bars represent average; capped lines are standard deviation). (C) Surviving fraction of U-87 MG cells treated for 24 h with increasing concentrations of **6b-*slow*** and **6b-*fast*** and allowed to grow in fresh medium for 144 h, at which time cells were counted and their viability was determined.

exposure to this compound (Figure 5C). As anticipated, **6b** is taken up by U-87 cells and ^{125}I is incorporated into the DNA, indicating that the intracellular processing of **6b** liberates the parent monophosphate, which then partakes in the DNA synthesis (Figure 5A and Figure 5B). The subcellular distribution study indicates that the ***slow*** diastereomer is retained within the cells and taken up into the DNA at levels of approximately 50−90% higher compared to the ***fast*** isomer (Figure 5A and Figure 5B). The cell killing is proportional to the amount of ^{125}I associated with DNA. The DNA retention of the radioactivity was also measured using the genomic tips method to confirm independently the presence of ^{125}I in DNA. The 40 h incubation of U-87 cells with 19.6 kBq/mL **6b-*slow*** accumulates 1.18(0.11) mBq ^{125}I/cell in DNA. This amount of ^{125}I incorporated into the DNA of U-87 cells is well within the range of D_{37} values reported for other mammalian cells.[69,70] For example, at 37% survival, the uptake of 0.13 mBq ^{125}I/cell and the cumulated mean lethal dose to the cell nucleus of ∼80 rad was reported.[3]

As shown above for other cancer cell lines, the uptake by U-87 cells is also concentration-dependent (compare data in Figure 5A and Figure 5B). The ***slow*** diastereomer is taken more avidly than the ***fast*** one. The number of cells surviving after the 24 h treatment with **6b-*fast*** and **6b-*slow*** is proportional to the extracellular concentration (Figure 5C and Figure 6). Both compounds cause clonogenic cell death at concentrations as low as 18.5 kBq/mL (0.23 nM). In the clonogenic assay studies, U-87 cells treated with **6b-*fast*** or **6b-*slow*** failed to produce any colonies even after 8 weeks in culture. Control cells treated with vehicle and cultured under identical conditions produced numerous colonies within 3 weeks of plating.

The radiotoxicity of the ***slow*** diastereomers in LS 174T cells is also greater compared to their ***fast*** counterparts (Figure 7).

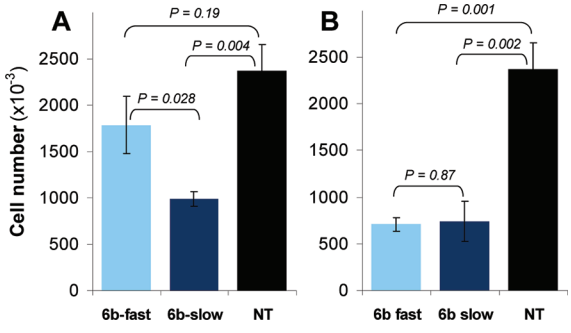

Figure 6. Viable U-87 MG cells recovered after 24 h of treatment with **6b-*slow*** and **6b-*fast*** at two concentrations: 37 kBq/mL (A) and 185 kBq/mL (B). Cells were harvested 96 h after treatment with ^{125}I-labeled compounds. The clonogenic death of these cells was confirmed by the lack of colony formation 8 weeks after plating.

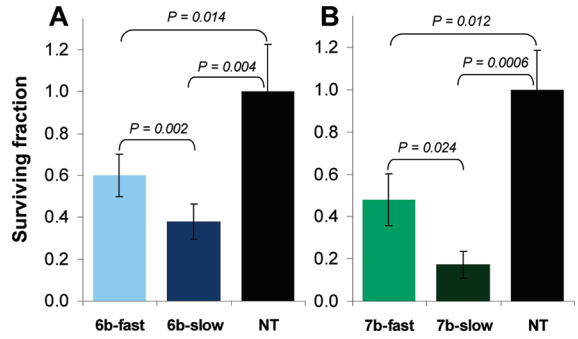

Figure 7. Surviving fraction of LS 174T colorectal adenocarcinoma cells treated with resolved diastereomers of **6b** and **7b**. (A) Clonogenic assay performed on cells exposed to **6b-*fast*** and **6b-*slow*** for 4 h at a drug concentration of 114.5 kBq/mL and followed by an additional 24 h culture in nonradioactive medium before the cell harvest and plating for clonogenic assay. (B) Surviving fraction of LS 174T cells treated with **7b-*fast*** and **7b-*slow*** for 4 h at a drug concentration of 185 kBq/mL. Cells were harvested immediately after the exposure to the drug and plated for the clonogenic assay.

The radiotoxicity depends on the concentration, duration of the exposure to the compound, and the retention of the radioactivity. Only 60% and 38% of LS 174T cells incubated for 4 h with 114.5 kBq/mL **6b-*fast*** and **6b-*slow***, respectively, produce colonies after 21 days in culture (Figure 7). The survival is further reduced when the extracellular concentration of the radioactive compound is increased. This is illustrated in Figure 7B for **7b-*fast*** and **7b-*slow***. At a concentration of 185 kBq/mL, the surviving fraction of LS 174T cells treated with **7b-*slow*** falls to 0.63(0.22) mBq. This clonogenic survival is proportional to the amount of ^{125}I retained within the nuclear DNA. LS 174T treated with **7b-*fast*** retained 0.196(0.06) mBq ^{125}I/cell compared to cells treated with **7b-*slow***, which retained 0.566(0.333) mBq ^{125}I/cell.

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Table 3. Subcellular Distribution of Mono-and Biscyclosalingenyl Derivatives in Human Ovarian Adenocarcinoma OVCAR-3 Cell Line

					^{125}I in cytoplasm cpm/10^3 cells (std dev)		^{125}I in nucleus cpm/10^3 cells (std dev)	
compd	cycloSal position	X	Y	Z	1 h	24 h	1 h	24 h
6b	5′	H	H	H	20.2 (3.2)	7.3 (1.0)	5.7 (1.6)	39.4 (5.8)
7b	5′	H	H	CH_3	11.7 (1.7)	1.6 (0.5)	4.5 (1.0)	25.9 (7.1)
8b	5′	F	*t*-Bu	*t*-Bu	88.3 (5.4)	3.2 (0.01)	7.9 (0.4)	92.2 (5.0)
9b	3′,5′	H	H	H	97.6 (10.0)	37.3 (10.0)	6.4 (0.5)	18.1 (3.9)
10b	3′,5′	H	H	CH_3	97.1 (14.6)	13.4 (3.3)	6.0 (0.9)	24.5 (5.4)
12b	3′	H	H	H	7.2 (0.7)	2.7 (0.3)	2.1 (0.2)	30.2 (5.3)
6b-*slow*	5′	H	H	H	14.8 (6.8)	6.0 (0.7)	4.9 (2.5)	31.3 (6.0)
7b-*slow*	5′	H	H	CH_3	13.0 (1.3)	1.8 (0.6)	5.4 (0.4)	29.3 (7.0)
8b-*slow*	5′	F	*t*-Bu	*t*-Bu	87.3 (7.5)	2.8 (0.7)	7.8 (0.6)	91.6 (5.7)
13b	3′	H	H	CH_3	6.2 (0.6)	1.4 (0.3)	2.0 (0.2)	15.7 (2.2)
14b	3′	F	*t*-Bu	*t*-Bu	62.5 (4.3)	2.7 (0.4)	4.3 (0.3)	66.7 (8.4)

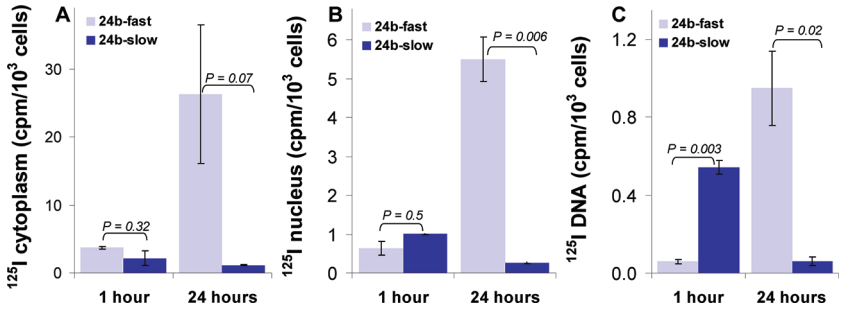

Figure 8. Subcellular distribution of ^{125}I in U-87 MG cells treated with **24b-*fast*** and **24b-*slow*** for 1 h and either processed immediately or after 24 h of additional culture in fresh nonradioactive medium: (A) ^{125}I recovered in cytoplasm; (B) soluble ^{125}I from the nuclear fraction; (C) ^{125}I associated with DNA.

The subcellular distribution of all ^{125}I-labeled reagents as a mixture of diastereomers was also analyzed in OVCAR-3 cells (Table 3). After 1 h of incubation with 55.5(0.04) kBq/mL ^{125}I-labeled reagents, the majority of the recovered radioactivity is associated with the cytoplasm. When these cells are given fresh nonradioactive medium and are allowed to continue to grow for an additional 24 h, nearly the entire radioactivity is recovered with the nuclear DNA. Compound **8b** retained the highest amount of radioactivity, >230% and ∼360% more than **6b** and **7b**, respectively.

The 3′,5′-disubstituted derivatives had an excellent initial uptake and retention in the cytoplasm; however, this uptake did not translate into the ^{125}I levels in the DNA comparable to the amounts observed for **8b** presumably because both 3′- and 5′-hydroxyl groups were not available. Nevertheless, the DNA-associated ^{125}I was at the level of ∼0.056 mBq/cell. The compound **14b**, a 3′-regioisomer of **8b**, had excellent cytoplasmic uptake at 1 h. Virtually the entire cytoplasmic content ended up in the DNA after 24 h of incubation, indicating that in cases where the targeted delivery may not be needed or the expression of BChE is low, the 3′-regioisomer can be as effective as the 5′-substituted derivative.

The subcellular distribution of **24b** (Figure 8) does not parallel any of the 3′-hydroxy-containing derivatives. Although IC_{50} values of **24** are comparable to IC_{50} values of **6**, the uptake and retention of both diastereomers of **24** are significantly lower. Only **24b-*fast*** after 1 h of incubation shows ^{125}I retention in the cytoplasm comparable to those of other reagents. The uptake and retention of **24b-*slow*** are ∼20× lower in the cytoplasm, nucleus, and DNA. Additionally, **24b-*slow*** appears to rapidly efflux from the intracellular compartments and at 24 h is practically undetectable in any of the intracellular compartments. This characteristic is clearly not compatible with therapeutic applications (Figure 9). However, a high affinity of **24b-*slow*** to its target, rapid clearance, and the ability to radiolabel **24b** with ^{18}F at the 3′-position are all promising features of a good imaging agent.

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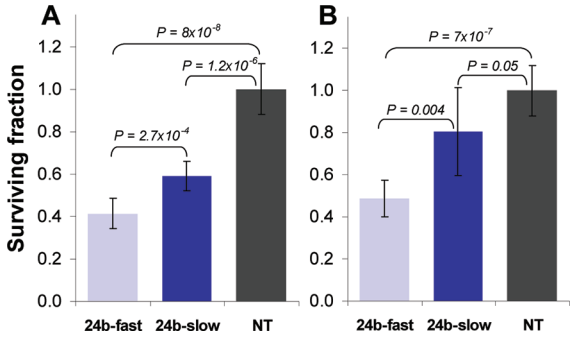

Figure 9. Clonogenic survival of U-87 MG cells treated with **24b-*fast*** and **24b-*slow*** for 1 h at a concentration of 4.76 kBq/mL. Cells were either processed immediately for the clonogenic assay (A) or allowed to continue to grow with fresh medium for an additional 24 h (B).

CONCLUSIONS

Methods developed in this study allow the synthesis of compounds with high specific activities at the no-carrier-added level. HPLC separation methods yield pure 5′-cycloSal and 3′-cycloSal diastereomers suitable for biological studies. Interactions of resolved diastereomers with BChE are distinct with the IC_{50} values in the nanomolar range for all tested ***slow*** diastereomers and in the micromolar range or higher for the corresponding ***fast*** diastereomers. 3′,5′-Disubstituted cycloSal derivatives cannot be resolved into the individual diastereomers with the available techniques. The wide-ranging hydrophobicity and rates of hydrolyses provide the opportunity to select reagents best suited to the proliferative rate, site of the disease, and the route of administration. Intracellular trapping and retention of the targeted cyclosalingenyl derivatives of ^{125}IUdR lead to high levels of ^{125}I in the nuclear DNA in the heterogeneous cancer cell population and produce extensive cell death. All developed reagents can be labeled with radionuclides suitable for imaging and molecular radiotherapy. Compounds **24b** are the exception; they have properties suited for ^{18}F or ^{124}I PET or ^{123}I SPECT imaging.

EXPERIMENTAL METHODS

Chemistry. Chemicals and reagents were purchased from commercial suppliers and used without further purification. Anhydrous diethyl ether was distilled from sodium wire with benzophenone as an indicator, and dichloromethane was distilled from CaH_2 under nitrogen. Na[^{125}I]I in 1×10^{-5} M NaOH (pH 8−11) was obtained from PerkinElmer (Waltham, MA). Radioactivity was measured with a Minaxi γ-counter (Packard, Waltham, MA) and a dose calibrator (Cap Intec Inc., Ramsey, NJ). Analytical TLC was carried out on precoated plastic plates, normal phase Merck 60 F_{254} with a 0.2 mm layer of silica, and spots were visualized with either short wave UV or iodine vapors. Radioactive drugs on TLC and ITLC plates were analyzed on a Vista-100 plate reader (Radiomatic VISTA model 100, Radiomatic Instruments & Chemical Co., Inc., Tampa, FL). Flash column chromatography was carried out using Merck silica gel 60 (40−60 μM) as stationary phase. Compounds were resolved and their purity confirmed by the HPLC analyses on Gilson (Middleton, WI) and ISCO (Lincoln, NE) systems using 5 μm, 250 mm × 4.6 mm, analytical columns, either Columbus C8 (Phenomenex, Torrance, CA) or ACE C18 (Advanced Chromatography Technologies, www.ace-hplc.com). Columns were protected by guard filters, and elution was at a rate of 0.8 mL/min with various gradients of CH_3CN (10−95%) in water with or without TFA (0.07%, w/v). Variable wavelength UV detectors UVIS-205 (Linear, Irvine, CA) and UV116 (Gilson) were used with the sodium iodide crystal Flow-Count detector (Bioscan, Washington, DC) connected in-line at the outlet of the UV detector. Both signals were monitored and analyzed simultaneously. NMR spectra were recorded at ambient temperature in $(CD_3)_2SO$ or $CDCl_3$ with a Varian INOVA 500 MHz NMR instrument spectrometer (Palo Alto, CA). Chemical shifts are given as δ (ppm) relative to TMS as internal standard, with J in hertz. Deuterium exchange and decoupling experiments were performed in order to confirm proton assignments. ^{31}P NMR and ^{119}Sn NMR spectra were recorded with proton decoupling. High resolution (ESI-HR) positive ion mass spectra were acquired on an LTQ-Orbitrap mass spectrometer with electrospray ionization (ESI). Samples were dissolved in 70% methanol. The 2 μL aliquots were loaded into a 10 μL loop and injected with a 5 μL/min flow of 70% acetonitrile, 0.1% formic acid. FAB high-resolution (FAB-HR) mass spectra analyses (positive ion mode, 3-nitrobenzyl alcohol matrix) were performed by the Washington University Mass Spectrometry Resource (St. Louis, MO) and at the University of Nebraska Mass Spectrometry Center (Lincoln, NE).

All target nonradioactive compounds were found to be ≥97% pure by rigorous HPLC analysis, with the integration of a peak area (detected at 220 and/or 280 nm). Radioiodinated products were identified and later evaluated by means of the independently prepared nonradioactive reference compounds. A comparison of UV signals of the nonradioactive standards with the radioactive signals using radio-TLC (R_f) and radio-HPLC (t_R) analyses was employed. The 5-iodo-3′-*O*-levulinyl-2′-deoxyuridine **4** and 3′-*O*-levulinylthymidine **19** were prepared starting from 5-iodo-5′-*O*-(4,4′-dimethoxytrityl)-2′-deoxyuridine **2** and 5′-*O*-(4,4′-dimethoxytrityl)thymidine with 4-oxopentanoic acid, using the DCC/DMAP esterification method. The subsequent cleavage[71] of the DMTr group with $ZrCl_4$ in CH_3CN led efficiently (~78% yield) to uridines **4** and **19**. Thymidine and 5-iodo-2′-deoxyuridine were also used as starting compounds in the preparations of 3′-fluorodeoxyuridines **20** and **21**. After the dimethoxytrityl protection of 5′-hydroxyl group, the configuration at the 3′-*O*-position was inverted by the mesylation/cyclization reaction sequence with methanesulfonyl chloride and 1,8-diazabicyclo[5.4.0]undecane (DBU), followed by the hydrolysis of the formed O^2,$O^{3'}$-anhydrouridines, to give [5′-*O*-(4,4′-dimethoxytrityl)-2′-deoxy-β-D-*threo*-pentofuranosyl]thymine and 5-iodo-[5′-*O*-(4,4′-dimethoxytrityl)-2′,3′-dideoxy-β-D-*threo*-pentofuranosyl]uracile, without separation of the crude mesylates.[72] Epimerized uridines were subsequently fluorinated with DAST (*N*,*N*′-diethylaminosulfur trifluoride) at the 3′-*O*-position, and the 5′-*O*-DMTr groups were removed with 1 N HCl in acetonitrile. Analytical HPLC traces, separations of diastereomers, and the related detailed HPLC analysis of all synthesized compounds **6**−**24** are provided in Supporting Information.

Statistical Analyses. The variables are expressed as average ± SD. Summary statistics were performed using a two-sided, unpaired Student's t test with a significance level of $P = 0.05$ using SigmaPlot/SigmaStat (Systat Software, Inc., Point Richmond, CA) and GraphPad InStat computer software (GraphPad Software, Inc., La Jolla, CA).

General procedure A (Synthesis of Saligenyl Chlorophosphites). To a solution of a dried salicyl alcohol derivative in Et_2O stirred at −16 °C under a nitrogen atmosphere, newly distilled PCl_3 was added. After 15 min, while the same temperature was maintained, a solution of pyridine in Et_2O was added dropwise over a period of 1 h. The reaction mixture was allowed to reach ambient temperature, and the stirring was continued for an additional 2 h. To facilitate a complete separation of pyridinium chloride, the mixture was stored in a tightly covered reaction flask at −20 °C overnight. After filtration under pressure of dry nitrogen, the solvent was evaporated in a vacuum and the resulting crude product was used in the next step of synthesis without delay. Initially crude products **15** and **16** were purified by short-path distillation under a high vacuum, giving both chlorophosphites as colorless unstable liquids, but no definite advantage has been noted in using the purified compound **15** or **16** during the synthesis of phosphotriesters with respect to achieved yields.

General Procedure B (Synthesis of Nonradioactive Cyclosaligenyl Phosphotriesters of 5-Iodo-2′-deoxyuridines 6−14, Using Unprotected IUdR 1 and Chlorophosphites). All reactions were performed under anhydrous conditions and a dry nitrogen or

argon atmosphere. To a stirred solution of IUdR **1** and DIPEA (~2.5 molar excess) in DMF/THF (2:1 mixture), cooled to or below −40 °C, the THF solution of the appropriate crude chlorophosphite (1.05−1.25 molar equiv) was added in small portions. Chlorophosphites (obtained using general procedure A) were transferred directly from the original reaction vessel by means of argon pressure and the syringe equipped with a long double needle. The reaction mixture was then slowly warmed to ambient temperature, and further stirring continued for 30 min to ensure completion of the reaction (TLC monitoring with DCM/MeOH, 10:(1.0−1.2) range). The reaction mixture was cooled to −40 °C once again, and a solution of *tert*-butyl hydroperoxide, 5−6 M (2.1−2.5 molar equiv) in *n*-decane was added. The resulting mixture was slowly warmed to room temperature, with continued stirring for about 1 h (the reaction progress was followed by TLC). The solvent was evaporated under reduced pressure, and the residue was treated with DCM. The precipitate of unreacted IUdR **1** was filtered off, washed with DCM, and dried under a high vacuum. The recovered IUdR was proven suitable for an immediate reuse. The filtrate was evaporated under reduced pressure and the residue purified by flash column chromatography on a silica gel, using a gradient of MeOH in DCM ((0.7−1.0):10) and/or a gradient of MeOH in EtOAc ((0.2−0.7):10), to yield each of three formed cyclosaligenyl regioisomers: 5′-*O*-, 3′-*O*-, and di-5′,3′-*O*,*O*-substituted, separated. Diastereomers of 5′-*O*-cyclosaligenyl phosphotriester and 3′-*O*-cyclosaligenyl phosphotriesters were later separated by means of the HPLC, giving small quantities (~30 mg) of each individual isomer.

General Procedure C (Synthesis of Nonradioactive Cyclosaligenyl Phosphotriesters of 5-Iodo-2′-deoxyuridines 6−14, Thymidines 22 and 23, and 5- Iodo-3′-dideoxy-3′-fluorouridine 24, Using Protected IUdRs 4 and 5 or Uridines 19−21, and Chlorophosphites). Under an argon or dry nitrogen atmosphere, DIPEA and the crude saligenyl chlorophosphite, dissolved in MeCN, were added to a stirred solution of protected IUdR **4** or **5** in MeCN at −40 °C. The reaction mixture was slowly warmed to ambient temperature and the reaction progress monitored by TLC (DCM/MeOH, 10:0.7). The reaction mixture cooled one more time to −40 °C was treated with a solution of *tert*-butyl hydroperoxide, 5−6 M (~2.5 molar equiv), in *n*-decane. The mixture was warmed slowly to room temperature and stirred for 1−2 h (the reaction progress was followed by TLC). The solvent was removed under reduced pressure and the residue treated with DCM (80 mL) or EtOAc (60 mL) and filtered. The filtrate was washed with 0.3% aqueous solution of $NaHSO_3$ (20 mL), brine (20 mL), dried over $MgSO_4$, and evaporated.

Deprotection Procedure 1. In reactions conducted with **4** the resulting solid was dissolved in pyridine (2 mL) and added to a stirred, cooled (on an ice−water bath) solution of hydrazine hydrate (1.5 mL) in pyridine (3 mL) containing acetic acid (2.2 mL). The stirring continued for 5 min, and then water (40 mL) and EtOAc (50 mL) were added. The organic layer was separated and washed with 10% aqueous solution of $NaHCO_3$ (20 mL), water (20 mL), dried over $MgSO_4$, and evaporated.

Deprotection Procedure 2. In reactions conducted with **5**, the crude solid was dissolved in MeCN (10−20 mL), and $ZrCl_4$ (1.2−1.6 molar equiv) was added. The mixture was stirred at room temperature for about 1 h (TLC monitoring). The solvent was evaporated in a vacuum, and the residue was treated with EtOAc (50 mL) and water (50 mL). The organic layer was washed with brine and dried over $MgSO_4$. The solvent was evaporated, and the residue was purified on a silica gel column.

General Procedure D (Synthesis of Trialkyltin Precursors 6a−14a, 21a, 24a). A solution of appropriate iodouridine **6−14**, **21**, or **24** (1.0 equiv), the hexamethylditin (hexa-*n*-butylditin was used in the preparation of **6a**) (1.25−1.50 equiv), and dichlorobis(triphenylphosphine)palladium(II) catalyst (0.10 equiv) in ethyl acetate or dioxane (for **6**, **7**, **12**, and **13**) was refluxed (2−6 h) under a nitrogen atmosphere (until the starting material was detected). The reaction progress was monitored by TLC. Two major products were formed in all the reactions. The first product, with a higher TLC mobility, isolated in 50−72% yield, was the trialkylstannyl derivative, and a second one (with a low TLC mobility) was a deiodinated starting compound. After cooling to ambient temperature, the mixture was freed from an excess of the catalyst and partially purified by the filtration through a thin pad of silica (EtOAc/hexanes, 2:1). The resulting crude product was purified by repeating the silica gel column chromatography procedure, using a gradient of EtOAc in hexanes ((2−5):10) and/or a gradient of MeOH in DCM or $CHCl_3$ ((0.4−0.7):10). Anhydrous samples of pure tin precursors (~120 μg) were stored up to 8 months with the exclusion of light under nitrogen at −20 °C, not showing excessive decomposition (≤7% by the HPLC analysis), and were suitable for the immediate radioiododestannylation. Diastereomers of 5′-*O*-cyclosaligenyl phosphotriesters and 3′-*O*-cyclosaligenyl-5-trimethyltin phosphotriesters were later separated by the HPLC using a reverse phase column, to give small quantities (~20 mg) of each individual diastereomer.

General Procedure E (Synthesis of [^{125}I]Radioiodinated Cyclosaligenyl Phosphotriesters (6b−14b, 21b, and 24b). Into a glass tube containing the appropriate tin precursor **6a−14a**, **21a**, or **24a** (100−120 μg, 130−150 μmol) dissolved in MeCN (50−100 μL), a solution of $Na^{125}I$/NaOH (10−100 μL, 40−370 MBq) was added, followed by a 30% water solution of H_2O_2 (5 μL) and then a TFA solution (50 μL, 0.1 N in MeCN) with 2 min delay. The mixture was briefly vortexed and left for 15 min at room temperature. The reaction was quenched with $Na_2S_2O_3$ (100 μg in 100 μL of H_2O) and the mixture taken up into a syringe. The reaction tube was washed twice with 50 μL of H_2O/MeCN (9:1) solution. The previously withdrawn reaction mixture and washes were injected onto the HPLC system and separated by means of a C8 or C18 reverse phase column. The eluate from a column (1 mL fractions collected) was monitored using a radioactivity detector connected to the outlet of UV detector (detection at 220 and 280 nm). Eluted fractions containing a product, combined and evaporated with a stream of dried nitrogen, were reconstituted in an appropriate solvent at concentrations required in subsequent experiments and were filtered through a sterile (Millipore 0.22 μm) filter into a sterile evacuated vial. Identity of radiolabeled products was confirmed by the evaluation of UV signals of nonradioactive iodo analogues (prepared independently, not through the iododestannylation reaction) with the radioactive signals and/or by comparing R_f obtained from the radio-TLC and t_R from the radio-HPLC analysis. The specific activities were determined by the UV absorbance of radioactive peaks, compared to the standard curves of unlabeled reference compounds. Radiolabeled products, if kept in a solution overnight at ambient temperature, were purified again before conducting further experiments, even though the performed HPLC analysis rarely indicated less than 95% of the radiochemical purity.

Nonradioactive Cyclosaligenyl Phosphotriesters. 5-Iodo-5′-*O*-cyclosaligenyl-2′-deoxyuridine Monophosphate (6). Method I. General procedure C with 3′-*O*-levulinyl IUdR **4** (1.22 g, 2.7 mmol), DIPEA (1.3 mL, 0.96 g, 7.44 mmol), and crude chlorophosphite **15** (950 mg, ~6 mmol), was conducted in 25 mL of MeCN and the oxidation carried out with a solution of *t*-BuOOH (0.86 mL, ≥4 mmol) after 40 min of phosphitylation. Before the deprotection of 3′-*O*-Lev group, a small amount of the crude product (19 mg) was purified by HPLC on a Columbus C18, 100 Å (5 μm, 10 mm × 250 mm) column, eluting at 2.5 mL/min with 37% MeCN in water. The HPLC analysis showed two diastereomers (47:53 ratio): t_R = 24.4 min, t_R = 24.8 min (≥98% pure, UV at 280 nm); ACE C18, 100 Å (5 μm, 4.6 mm × 250 mm) column; eluent, solvent A 10% MeCN in water, solvent B MeCN; elution at 1.0 mL/min with a linear gradient of B from 0% to 95% over 45 min and 95% B for 15 min. ^{1}H NMR (DMSO-d_6) δ = 11.39 (bs, 1H, NH), 8.11, 8.08 (2 s, 1H, uridine-H6), 7.26−7.19 (m, 1H, aryl-H4), 7.12−7.06 (m, 3H, aryl-H3, aryl-H5, aryl-H6), 6.08, 6.05 (2 d, 1H, H1′, $^2J_{H,H}$ = 7.2 Hz), 5.47 (dd, 1H, H_A-benzyl, $^2J_{H,H}$ = 14.0 Hz, $^3J_{H,H}$ = 7.3 Hz), 5.45 (dd, 1H, H_B-benzyl, $^2J_{H,H}$ = 14.0 Hz, $^3J_{H,H}$ = 7.1 Hz), 5.42 (dd, 1H, H_A-benzyl, $^2J_{H,H}$ = 14.0 Hz, $^3J_{H,H}$ = 7.6 Hz), 5.40 (dd, 1H, H_B-benzyl, $^2J_{H,H}$ = 14.0 Hz, $^3J_{H,H}$ = 7.3 Hz), 5.17−5.09 (m, 1H, H5″), 4.44−4.39 (m, 2H, H3′, H5′), 4.19−4.13 (m, 1H, H4′), 3.33−3.29 (m, 2H, Lev-C3-CH_2), 2.76−2.74 (m, 2H, Lev-C2-CH_2), 2.40−2.23 (m, 1H, H2″), 2.22, 2.20 (2 s, 3H, Lev-C5-CH_3), 2.21−2.16 (m, 1H, H2′) ppm. ^{31}P NMR (DMSO-d_6) δ = −8.29, −8.32 (2 s, diastereomeric mixture) ppm. The

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deprotection of the 3′-*O*-Lev group was completed in less than 10 min (a single band on TLC), and the crude product was purified on a silica gel column DCM/MeOH gradient, 10:(0.7−1.0)) to give **6** (735 mg, 52%) as a colorless foam: R_f = 0.42 (DCM/MeOH, 10:0.7). HPLC analysis has shown a diastereomeric mixture (4:5 ratio): t_R = 20.2 and t_R =22.2 min (∼98% pure, UV at 280 nm); conducted on Jupiter C18 300Å (5 μm, 4.6 mm × 250 mm) column; eluent, solvent A 20% MeCN in water, solvent B MeCN; elution at 0.8 mL/min with A for 25 min, then a linear gradient of B from 0% to 95% over 10 min, and finally 95% B for 10 min. ^{1}H NMR (DMSO-d_6) δ = 11.68, 11.63 (2 s, 1H, NH), 7.89, 7.78 (2 s, 1H, uridine-H6), 7.33 (tt, 1H, aryl-H4, J = 7.5 Hz), 7.25−7.19 (m, 2H, aryl-H6, aryl-H5), 7.17−7.12 (m, 1H, aryl-H3), 6.03, 5.98 (2 t, 1H, H1′, $^3J_{H,H}$ = 7.0 Hz), 5.22−5.19 (m, 2H, benzyl), 5.42 (d, 1H, C3′- OH, J = 4.6 Hz), 4.36−4.23 (m, 2H, H5″, H5′), 4.22−4.18 (m, 1H, H3′), 3.94−3.89 (m, 1H, H4′), 2.23−2.12 (m, 1H, H2″), 2.10−2.05 (m, 1H, H2′) ppm. ^{31}P NMR (DMSO-d_6) δ = −9.18, −9.33 (2 s, diastereomeric mixture) ppm. HR-FABMS (m/z): [M + Li]$^+$calcd for $C_{16}H_{16}N_2O_8PILi$, 528.9849; found, 528.9837. Diastereomers of **6** were separated by preparative HPLC on a Columbus C18, 100 Å (5 μm, 10 mm × 250 mm) column, eluting at 2.6 mL/min with 20% MeCN in water. The amount of 59 mg of the diastereomeric mixture used for the separation gave 19.1 mg of ***6-fast*** and 17.2 mg of ***6-slow***. Analytical data of diastereomer ***6-fast*** are as follows: ^{1}H NMR (DMSO-d_6) δ = 11.69 (s, 1H, NH), 7.98 (s, 1H, uridine-H6), 7.35 (tt, 1H, aryl-H4, $^3J_{HH}$ = 7.8 Hz, $^4J_{HH}$ = 1.0 Hz), 7.27, 7.25 (2 d, 1H, aryl-H6, J = 1.5 Hz), 7.18 (tt, 1H, aryl-H5, $^3J_{HH}$ = 7.5 Hz, $^4J_{HH}$ = 1.0 Hz), 7.14, 7.12 (2 d, 1H, aryl-H3, $^3J_{HH}$ = 7.6 Hz, $^4J_{HH}$ = 1,0 Hz), 6.07 (dd, 1H, H1′, $J_{1′-2′}$ = 7.4 Hz, $J_{1′-2″}$ = 6.5 Hz), 5.55−5.44 (m, 2H, benzyl), 5.41 (bd, 1H, C3′-OH, J = 4.4 Hz), 4.39 (ddd, 1H, H5″, $^2J_{HH}$ = 11.6 Hz, $^3J_{HH}$ = 7.1 Hz, $^3J_{HP}$ = 3.7 Hz), 4.29 (ddd, 1H, H5′, $^2J_{HH}$ = 11.6 Hz, $^3J_{HH}$ = 6.7 Hz, $^3J_{HP}$ = 4.6 Hz), 4.18−4.16 (m, 1H, H3′), 3.94−3.91 (m, 1H, H4′), 2.19−2.16 (m, 1H, H2″), 2.09−2.05 (m, 1H, H2′) ppm. ^{13}C NMR (DMSO-d_6) δ = 160.77 (C4), 150.38 (C2), 149.35 (C2-aryl), 144.33 (C6), 129.62 (C4-aryl), 126.0 (C6-aryl), 124.31 (C5-aryl), 120.93 (C1-aryl), 118.0 (C3-aryl), 109.42 (C5), 84.75 (C1′), 84.32 (C4′), 69.92 (C3′), 68.86 (C-benzyl), 67.64 (C5′), 38.72 (C2′) ppm. ^{31}P NMR (DMSO-d_6) δ = −9.19 ppm. Analytical data of diastereomer ***6-slow*** are as follows: ^{1}H NMR (DMSO-d_6) δ = 11.71 (s, 1H, NH), 7.99 (s, 1H, uridine-H6), 7.37 (tt, 1H, aryl-H4, $^3J_{HH}$ = 7.7 Hz, $^4J_{HH}$ = 1.1 Hz), 7.28 (dd, 1H, aryl-H6, $^3J_{HH}$ = 7.1 Hz, $^4J_{H,H}$ = 1.5 Hz), 7.20 (tt, 1H, aryl-H5, $^3J_{HH}$ = 7.6 Hz, $^4J_{HH}$ = 1.0 Hz), 7.15 (dd, 1H, aryl-H3, $^3J_{HH}$ = 7.6 Hz, $^4J_{HH}$ = 1,0 Hz), 6.10 (dd, 1H, H1′, $J_{1′-2′}$ = 7.5 Hz, $J_{1′-2″}$ = 6.4 Hz), 5.53−5.45 (m, 2H, benzyl), 5.43 (d, 1H, C3′-OH, J = 4.5 Hz), 4.35−4.27 (m, 2H, H5″ and H5′), 4.22−4.18 (m, 1H, H3′), 3.94−3.90 (m, 1H, H4′), 2.22−2.17 (m, 1H, H2″), 2.10−2.05 (m, 1H, H2′) ppm. ^{13}C NMR (DMSO-d_6) δ = 160.81 (C4), 150.42 (C2), 149.39 (C2-aryl), 144.41 (C6), 129.59 (C4-aryl), 126.12 (C6-aryl), 124.29 (C5-aryl), 120.87 (C1-aryl), 118.90 (C3-aryl), 110.02 (C5), 84.83 (C1′), 84.29 (C4′), 70.13 (C3′), 68.90 (C-benzyl), 67.76 (C5′), 38.59 (C2′) ppm. ^{31}P NMR (DMSO-d_6) δ = −9.32 ppm.

Method II. General procedure B was conducted with IUdR **1** (1.12 g, 3.16 mmol) dissolved in 15 mL of DMF, DIPEA (1.14 mL, 0.84 g, 6.5 mmol), and newly distilled chlorophosphite **15** (604 mg, 3.83 mmol) diluted with 6 mL of dry THF. The mixture was split into three (2 mL) portions. The oxidation was with a solution of *t*-BuOOH (0.86 mL, ≥ 4.3 mmol). The time of phosphitylation was 2 h, and the oxidation was carried out for 1 h. All three phosphotriesters, purified on a silica gel column (DCM/MeOH gradient, 10:(0.7−1.0)), were collected in the form of a colorless foam: 5′,3′-*O*,*O*′-dicycloSal-5-IUdRMP **9** (R_f = 0.72), 350 mg (16%); 3′-*O*-cycloSal-IUdRMP **12** (R_f = 0.58), 611 mg (37%); 5′-*O*-cycloSal-5-IUdRMP **6** (R_f = 0.42), 759 mg (46%). The analytical data of the product **6** were identical to those reported above for **6** obtained using method I.

5-Iodo-5′-*O*-cyclo(3-methylsaligenyl)-2′-deoxyuridine Monophosphate (7). Method I. General procedure C with 3′-*O*-levulinyl IUdR **4** (1.36 g, 3.0 mmol), DIPEA (1.5 mL, 1.11 g, 8.6 mmol), and the crude chlorophosphite **16** (1.04 g, ∼6 mmol) was conducted in 27 mL of dry MeCN. The reaction time was extended to 4 h (TLC monitoring). The oxidation was carried out with a solution of *t*-BuOOH (0.9 mL, ≥4.5 mmol). Before the deprotection of the 3′-*O*-Lev group, a small sample of the crude product (∼11 mg) was purified by HPLC on a Columbus C18, 100 Å (5 μm, 10 mm × 250 mm) column, eluting at 2.5 mL/min with 40% MeCN in water. The HPLC analysis showed two diastereomers (45:55 ratio): t_R = 26.3 min, t_R = 26.6 min (≥98% pure, UV at 280 nm); ACE C18, 100 Å (5 μm, 4.6 mm × 250 mm); eluent, solvent A 10% MeCN in water, solvent B MeCN; elution at 1.0 mL/min with a linear gradient of B from 0% to 95% over 45 min and 95% B for 15 min. Purified 3′-O-Lev derivative of **7** was further analyzed: ^{1}H NMR (DMSO-d_6) δ = 11.77 (bs, 1H, NH), 8.08, 8.05 (2 s, 1H, uridine-H6), 7.26−7.14 (m, 1H, aryl-H5), 7.10−7.08 (m, 2H, aryl-H6, aryl-H4), 6.09 (dd, 1H, H1′, $J_{1′-2′}$ = 7.3 Hz, $J_{1′-2″}$ = 6.4 Hz), 5.53−5.42 (m, 2H, benzyl), 5.15−5.09 (m, 1H, H3′), 4.41−4.36 (m, 2H, H4′, H5″), 4.15−4.11 (m, 1H, H5′), 3.35−3.33 (m, 2H, Lev-C3-CH_2), 2.74−2.72 (m, 2H, Lev-C2-CH_2), 2.41−2.24 (m, 4H, H2″, Lev-C5-CH_3), 2.23−2.18 (m, 1H, H2′), 2.11 (s, 3H, aryl-C3-CH3) ppm. ^{31}P NMR (DMSO-d_6) δ = −8.23, −8.27 (2 s, diastereomeric mixture) ppm. HR-FABMS (m/z): [M + H]$^+$calcd for $C_{22}H_{25}N_2O_{10}PI$, 635.0292; found, 635.0277. Subsequent cleavage of the 3′-*O*-Lev group was completed in <5 min and the crude product was purified on a silica gel column (DCM/MeOH, 10:0.7) to give **7** (933 mg, 58%) as a colorless foam: R_f = 0.46 (DCM/MeOH, 10:0.7). The HPLC analysis showed a mixture of diastereomers: t_R = 18.2 min, t_R = 18.8 min (≥98% pure, UV at 280 nm); ACE C18, 100 Å (5 μm, 4.6 mm × 250 mm) column; eluent, solvent A 10% MeCN in water, solvent B MeCN; elution at 1.0 mL/min with a linear gradient of B from 0% to 95% over 45 min and 95% B for 15 min. ^{1}H NMR (DMSO-d_6) δ = 11.73, 11.68 (2 s, 1H, NH), 7.99, 7.86 (2 s, 1H, uridine-H6), 7.25−7.21 (m, 1H, aryl-H5), 7.12−7.07 (m, 2H, aryl-H6, aryl-H4), 6.08 (dd, 1H, H1′, $^2J_{1′-2′}$ = 7.6 Hz, $^2J_{1′-2″}$ = 6.0 Hz), 6.05 (dd, 1H, H1′, $^2J_{1′-2′}$ = 7.8 Hz, $^2J_{1′-2″}$ = 5.8 Hz), 5.51−5.39 (m, 2H, benzyl), 5.41 (d, 1H, C3′-OH′, J = 3.7 Hz), 4.34−4.26 (m, 1H, H5″, H3′), 4.22−4.18 (m, 1H, H5′), 3.93−3.89 (m, 1H, H4′), 2.25−2.19 (m, 4H, H2″,), 2.23 (s, 3H, C3-aryl-CH_3), 2.10−2.05 (m, 1H, H2″) ppm. ^{13}C NMR (DMSO-d_6) δ = 162.87 (C4), 150.31 (C2), 148.55, 148.51 (C2-aryl), 141.23, 141.18 (C6), 132.86 (C5-aryl), 129.18 (C4-aryl), 126.33 (C6-aryl), 120.73, 120.66 (C1-aryl), 117.35 (C3-aryl), 109.67 (C5), 84.75, 84.65 (C1′), 84.32, 84.22 (C4′), 69.98 (C3′), 68.82 (C-benzyl), 67.64 (C5′), 38.72 (C2′), 21.11 (CH_3-aryl) ppm.^{31}P NMR (DMSO-d_6) δ = −8.89, −8.93 (2 s, diastereomeric mixture) ppm. HR-FABMS (m/z): [M + H]$^+$calcd for $C_{16}H_{19}N_2O_8PI$, 536.9924; found, 536.9899. Diastereomers were separated using preparative HPLC on a Columbus C18, 100 Å (5 μm, 10 mm × 250 mm) column; elution was with 22% MeCN in water at 2.5 mL/min flow rate. From a total of 66 mg of diastereomeric **7** used for separation, 14.1 mg of ***7-fast*** and 12.2 mg of ***7-slow*** were isolated. Diastereomer ***7-fast*** eluted within 72−76 min and ***7-slow*** within 79−81 min after the injection, and each isomer was collected in ∼9 mL of eluent. A solvent was evaporated to dryness in a high vacuum. The product residue was reconstituted in MeCN and analyzed one more time using analytical HPLC. Each diastereomer was found to be ≥97% pure (UV at 220 and 280 nm). Analytical data of ***7-fast*** are as follows: ^{1}H NMR (DMSO-d_6) δ = 11.70 (s, 1H, NH), 7.98 (s, 1H, uridine-H6), 7.23−7.20 (m, 1H, aryl-H5), 7.17 (dd, 1H, aryl-H4, $^3J_{HH}$ = 7.6 Hz, $^4J_{H,H}$ = 1.5 Hz), 7.12−7.06 (m, 1H, aryl-H6), 6.06 (dd, 1H, H1′, $J_{1′2″}$ = 6.6 Hz, $J_{1′-2″}$ = 5.8 Hz), 5.51−5.43 (m, 3H, benzyl, C3′-OH), 4.38−4.33 (m, 1H, H5″), 4.27−4.22 (m, 1H, H5′), 4.17−4.15 (m, 1H, H3′), 3.92−3.90 (m, 1H, H4′), 2.21−2.19 (m, 3H, C3-aryl-CH_3), 2.18−2.13 (m, 1H, H2″), 2.10−2.05 (m, 1H, H2′) ppm. ^{31}P NMR (DMSO-d_6) δ = −8.89 ppm. Analytical data of ***7-slow*** are as follows: ^{1}H NMR (DMSO-d_6) δ = 11.69 (s, 1H, NH), 7.88 (s, 1H, uridine-H6), 7.24 (dd, 1H, aryl-H5, $^3J_{HH}$ = 7.8 Hz, $^4J_{H,H}$ = 1.2 Hz),7.09−7.06 (m, 2H, aryl-H6, aryl-H4), 6.07 (dd, 1H, H1′, $J_{1′,2″}$ = 6.5 Hz, $J_{1′,2″}$ = 4.8 Hz), 5.49−5.38 (m, 3H, benzyl, C3′-OH), 4.33−4.25 (m, 2H, H5″, H5′), 4.21−4.17 (m, 1H, H3′), 3.92−3.89 (m, 1H, H4′), 2.24−2.18 (m, 4H, H2″, C3-aryl-CH_3), 2.11−2.06 (m, 1H, H2′) ppm. ^{31}P NMR (DMSO-d_6) δ = −8.94 ppm.

Method II. General procedure B was conducted with IUdR **1** (2.51 g, 7.09 mmol) dissolved in 18 mL of DMF and DIPEA (1.54 mL, 1.14 g, 8.8 mmol). The crude chlorophosphite **16** (1.39 g, ∼8 mmol) was dissolved in 6 mL of dry THF added in 3 × 2 mL portions. A solution

of *t*−BuOOH (1.65 mL, ≥ 8.25 mmol) was added after 3 h of phosphitylation. The oxidation was carried out for 2 h. Phosphotriesters were purified on a silica gel column (DCM/MeOH gradient, 10: 0.7−1.0) and followed by second purification (DCM/MeOH, 10: 0.4), to achieve a complete separation of the closely eluting 3′-*O*-isomer **13**. All three products: 5′,3′-*O*,*O*′-dicycloSal-5-IUdRMP **10** (R_f 0.77), 1.12 g (22%); 3′-*O*−*cyclo*Sal-IUdRMP **13** (R_f 0.64), 1.17 g (31%); 5′-*O*−*cyclo*Sal−5−IUdRMP **7** (R_f 0.47), 1.47 g (39%) were collected as colorless foams. The analytical data of product **7** were identical with those detailed above for **7** obtained according to Method I.

5-Iodo-5′-*O*-[cyclo-3,5-di(*tert*-butyl)-6-fluorosaligenyl]-2′-deoxyuridine Monophosphate (8). Method I. General procedure C was carried out with 3′-*O*-levulinyl IUdR **4** (1.04 g, 2.3 mmol), DIPEA (1.25 mL, 0.93 g, 7.15 mmol) and the crude chlorophosphite **17** (1.14 g, ∼ 3.6 mmol) in MeCN (20 mL). The oxidation with a solution of *t*-BuOOH (1.0 mL, ≥ 5 mmol) was started after 1 h of phosphitylation. A small portion (∼14 mg) of the crude product was purified by HPLC, on Columbus C18, 100 Å (5 μm, 10 mm × 250 mm) column, elution at 3.0 mL/min with 43% MeCN in water. The purified 3′-O-Lev derivative of **8** (∼7 mg), was further analyzed by HR-MS: HR-FABMS (*m*/*z*): $[M + Li]^+$calcd for $C_{29}H_{37}N_2O_{10}PIFLi$, 757.1375; found, 757.1355. The ^{13}C peak was observed at 758.1393, within −2.0 ppm of the expected value of 758.1409. The HPLC analysis showed a mixture of two diastereomers: t_R = 22.2 min, t_R = 22.7 min (≥98% pure, UV at 280 nm), performed on ACE C18, 100 Å (5 μm, 4.6 × 250 mm) column; eluent, solvent A 50% MeCN in water, solvent B MeCN; elution at 1.0 mL/min with a linear gradient of B from 0% to 55% over 25 min, then 55−95% B for the period of 20 min and 95% B for 15 min. Cleavage of 3′−*O*−Lev group was completed in <10 min (TLC monitoring) and the crude product was purified on a silica gel column (DCM/MeOH gradient, 10:0.7−0.9), to give **8** (930 mg, 62%) in a form of colorless foam; R_f = 0.52 (DCM/MeOH, 10:0.7). The HPLC analysis confirmed again the presence of two diastereomers: t_R = 20.9 min and t_R = 22.8 min (≥98% pure, UV at 220 and 280 nm). The analysis was conducted using Columbus C8, 100 Å (5 μm, 4.6 × 250 mm) column; eluent, solvent A 50% MeCN in water, solvent B MeCN; column elution at 1.0 mL/min with A for 25 min, then a linear gradient of B from 0% to 95% over 15 min, and 95% B for the period of 20 min.^1H NMR (DMSO-d_6) δ =11.11, 11.04 (2 s, 1H, NH), 7.94, 7.92 (2 s, 1H, uridine-H6), 7.28−7.25 (m, 1H, aryl-H4), 6.25−6.20 (m, 1H, H1′), 5.52−5.38 (m, 2H, benzyl), 4.64−4.57 (m, 2H, C3′- OH, H3′), 4.37−4.24 (m, 1H, H5″, H5′), 4.17−4.14 (m, 1H, H3′), 2.52−2.45 (m, 1H, H4′), 2.25−2.20 (m, 1H, H2″), 2.18−2.12 (m, 1H, H2′), 1.41 (s, 9H, 3 x CH_3−*t*-Bu), 1.37 (s, 9H, 3 x CH_3−*t*-Bu) ppm. ^{13}C NMR (DMSO-d_6) δ = 163.27 (C4), 155.13 (C6-aryl), 150.91 (C2), 148.51 (C2-aryl), 141.18 (C6), 133.46 (C5-aryl), 130.38 (d, C3-aryl, $^4J_{C,F}$ = 4.1 Hz), 127.15 (C4-aryl), 110.66 (C1-aryl), 109.77 (C5), 89.65 (C1′), 84.56, 84.45 (C4′), 71.06 (C3′), 69.22 (C-benzyl), 67.55 (C5′), 38.67 (C2′), 34.71 (1 x C-*t*Bu), 34.60 (1 x C-*t*Bu), 34.38 (d, 2 x C-*t*Bu, $^4J_{C,F}$ = 3.2 Hz), 29.91 (d, 2 x CH_3-*t*Bu, $^4J_{C,F}$ = 3.2 Hz), 29.86 (1 x CH_3-*t*Bu), 29.81 (1 x CH_3-*t*Bu) ppm. ^{31}P NMR (DMSO-d_6) δ = −8.67, −8.93 (2 s diastereomeric mixture) ppm. HR-FABMS (*m*/*z*): $[M + H]^+$calcd for $C_{24}H_{32}N_2O_8PFI$, 653.0925; found, 653.0930. The ^{13}C peak was measured at 654.0945, within −2.0 ppm of the expected value.

Diastereomers were isolated by preparative HPLC (≤6 mg of **8** per injection), on Columbus C18, 100 Å (5 μm, 10 mm × 250 mm) column; elution at 2.5 mL/min with 47% MeCN solution in water. The separation started with 96 mg of the diastereomeric **8** and 34.4 mg of **8** ***fast*** and 43.2 mg of **8** ***slow*** was isolated. Diastereomer **8** ***fast*** eluted within 26−27.5 min, and **8** ***slow*** within 28.5−30.5 min after the injection, and each isomer was collected in ∼ 5 mL of the eluate. Solvent was evaporated to dryness in a high vacuum; the product residue was reconstituted in MeCN and analyzed again on the analytical HPLC, showing the purity of ≥98% for each diastereomer. Analytical data of **8** ***fast*** are as follows: ^1H NMR (DMSO-d_6) δ = 11.70 (s, 1H, NH), 7.98 (s, 1H, uridine-H6), 7.22 (d, 1H, aryl-H4, $^4J_{HF}$ = 9.4 Hz), 6.06 (dd, 1H, H1′, $J_{1',2'}$ = 6.6 Hz, $J_{1',2''}$ = 4.3 Hz), 5.51−5.43 (m, 2H, benzyl), 5.42 (bd, 1H, C3′- OH, *J* = 3.6 Hz), 4.38−4.33 (m, 1H, H3′), 4.27−4.22 (m, 1H, H4′), 4.17−4.15 (m, 1H, H5′), 3.92−3.90 (m, 1H, H5″), 2.21 (s, 3H, aryl C5-CH_3), 2.18−2.13 (m, 1H, H2′), 2.10−2.05 (m, 1H, H2″), 1.43 (s, 9H, 3 x CH_3−*t*-Bu), 1.35 (s, 9H, 3 x CH_3−*t*-Bu) ppm. ^{31}P NMR (DMSO-d_6) δ = −8.89 ppm. Analytical data of **8** ***slow*** are as follows: ^1H NMR (DMSO-d_6) δ = 11.69 (s, 1H, NH), 7.99 (s, 1H, uridine-H6), 7.25−7.22 (m, 1H, aryl-H5), 7.09−7.06 (m, 2H, aryl-H3, aryl-H4), 6.07 (dd, 1H, H1′, $J_{1',2'}$ = 6.5 Hz, $J_{1',2''}$ = 4.3 Hz), 5.49−5.38 (m, 3H, benzyl, C3′-OH), 4.33−4.25 (m, 2H, H3′, H4′), 4.21−4.17 (m, 1H, H5′), 3.92−3.89 (m, 1H, H5″), 2.24−2.18 (m, 4H, H2′, aryl C5-CH_3), 2.18−2.13 (m, 1H, H2′), 2.10−2.06 (m, 1H, H2″), 1.37 (s, 9H, 3 x CH_3−*t*-Bu), 1.34 (s, 9H, 3 x CH_3−*t*-Bu) ppm. ^{31}P NMR (DMSO-d_6) δ = −8.93 ppm.

Method II. General procedure B was conducted with IUdR **1** (5.0 g, 14.1 mmol) in 30 mL of DMF and DIPEA (3.1 mL, 2.29 g, 17.7 mmol) and crude chlorophosphite **17** (5.4 g, ∼17 mmol, dissolved in 10 mL of dry THF) and transferred slowly in 2 × 5 mL portions. Oxidation with a solution of *t*-BuOOH (4 mL, ≥20 mmol) was carried out for 1 h. Phosphotriesters of IUdR were purified on a silica gel column (DCM/MeOH gradient, 10:(0.7−0.9)). All three products were obtained in the form of a colorless rigid foam: 5′,3′-*O*,*O*′-dicycloSal-5-IUdRMP **11** (R_f = 0.81), 2.81 g (21%); 3′-*O*-cycloSal-IUdRMP **14** (R_f = 0.66), 3.31 g (36%); 5′-*O*-cycloSal-5-IUdRMP **8** (R_f = 0.52), 3.95 g (43%). The analytical data of product **8** were identical to those reported above for **8** obtained using Method I.

5-Iodo-3′-*O*-cyclosaligenyl-2′-deoxyuridine Monophosphate (12). This compound was obtained in two ways: (1) as the side product (611 mg, 37% yield) in the preparation of **6** using method II; (2) by general procedure C conducted with 5′-*O*-trityl IUdR **5** (3.52 g, 5.90 mmol), DIPEA (1.8 mL, 1.33 g, 10.3 mmol), and the crude chlorophosphite **15** (1.3 g, ∼8 mmol). Both synthetic pathways furnished **12** with the identical analytical data. General procedure C was performed for 45 min in MeCN (30 mL), with the subsequent oxidation using a solution of *t*-BuOOH (2.2 mL, ≥11 mmol). After workup, the crude solid residue dissolved in MeCN (34 mL) was treated with $ZrCl_4$ (1.65 g, 7.0 mmol) and gave **12** (1.21 g, 39%) as a colorless rigid foam: R_f = 0.58 (DCM/MeOH, 10:0.7). HPLC analysis has shown a diastereomeric mixture (44:56 ratio): **12-*fast***, t_R = 37.9 min, and **12-*slow***, t_R = 38.6 min (≥98% pure, UV at 280 nm); column, ACE C18, 100 Å (5 μm, 4.6 mm × 250 mm); eluent, solvent A 10% MeCN in water, solvent B MeCN; elution at 0.8 mL/min with a linear gradient of B from 0% to 25% over 40 min and a gradient of B from 25% to 95% B for 20 min. ^1H NMR (DMSO-d_6) δ = 10.61 (s, 1H, NH), 10.58 (s, 1H NH), 8.36 (s, 1H, uridine-H6), 8.34 (s, 1H, uridine-H6), 7.13 (m, 1H, aryl-H4), 7.06−6.98 (m, 3H, aryl-H3, aryl-H5, aryl-H6), 6.21 (t, 1H, H1′, *J* = 7.0 Hz), 5.97 (t, 1H, H1′, *J* = 7.0 Hz), 5.34−5.28 (m, 2H, benzyl), 4.24−4.22 (m, 1H, H3′), 3.82−3.76 (m, 2H, H4′, H5′), 3.94−3.89 (m, 1H, H5″), 3.55 (bd, C5′-OH, *J* = 4.0 Hz), 2.56−2.44 (m, 1H, H2′), 2.34−2.26 (m, 1H, H2″) ppm. ^{31}P NMR (DMSO-d_6) δ = −9.74, −9.77 ppm. HR-FABMS (*m*/*z*): $[M + Li]^+$calcd for $C_{16}H_{16}N_2O_8PILi$, 528.9849; found, 528.9844. The ^{13}C isotope was observed at 529.9893 within 1.9 ppm of the expected 529.9883.

5-Iodo-3′-*O*-cyclo(3-methylsaligenyl)-2′-deoxyuridine Monophosphate (13). Compound **13** was obtained in two ways: (1) as the side product (1.17 g, 31%) during the preparation of **7** using method II; (2) by general procedure C conducted with 5′-*O*-trityl IUdR **5** (2.34 g, 3.93 mmol), DIPEA (1.5 mL, 1.11 g, 8.6 mmol), and crude chlorophosphite **16** (1.1 g, ∼6 mmol). Isolated product **13** in both synthetic pathways showed the identical analytical data. General procedure C was carried out in 20 mL of MeCN for 60 min, and the oxidation was with a solution of *t*-BuOOH (1.6 mL, ≥8 mmol) subsequent to the phosphitylation. After workup, the crude solid dissolved in MeCN (40 mL) was treated with $ZrCl_4$ (1.0 g, 4.3 mmol) and gave **13** (842 mg, 40%) as a colorless rigid foam: R_f = 0.64 (DCM/MeOH, 10:0.7). Further HPLC purification (23 mg of **13**, ∼4 mg per injection) was performed on a Columbus C18, 100 Å (5 μm, 10 mm × 250 mm) column; eluent, solvent A 20% MeCN in water and solvent B MeCN; elution at 2.5 mL/min with a linear gradient of B from 0% to 65% over 35 min. Product **13** (11.9 mg, ≥ 98% pure, UV at 280 nm) was collected within 28−30 min after the injection. The

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HPLC analysis showed a mixture of diastereomers: **13-*fast***, t_R = 21.8 min; **13-*slow***, t_R = 22.4 min (≥98% pure, UV at 280 nm); column, ACE C18, 100 Å (5 μm, 4.6 mm × 250 mm); eluent, solvent A 20% MeCN in water, solvent B MeCN; elution at 1 mL/min with a linear gradient of B from 0% to 60% over 45 min and using a linear gradient of B from 45% to 95% B for 15 min. ^{1}H NMR (DMSO-d_6) δ = 10.82 (s, 1H, NH), 10.65 (s, 1H NH), 8.31 (s, 1H, uridine-H6), 8.28 (s, 1H, uridine-H6), 7.23−7.06 (m, 3H, aryl-H4, aryl-H5, aryl-H6), 6.20−6.17 (m, 1H, H1′), 5.33−5.27 (m, 2H, benzyl), 4.30−4.23 (m, 1H, H3′), 3.83−3.76 (m, 2H, H4′, H5′), 3.94−3.89 (m, 1H, H5″), 3.61 (bd, C5′-OH, *J* = 4.0 Hz), 2.56−2.44 (m, 1H, H2′), 2.34−2.26 (m, 4H, H2″, aryl-C3-CH_3) ppm. ^{31}P NMR (DMSO-d_6) δ = −9.34, −9.38 ppm. HR-FABMS (*m*/*z*): [M + H]$^+$calcd for $C_{17}H_{19}N_2O_8PI$, 536.9924; found, 536.9940. The ^{13}C isotope peak was measured at 537.9981, 4.3 ppm of the expected value.

5-Iodo-3′-*O*-[cyclo-3,5-di(*tert*-butyl)-6-fluorosaligenyl]-2′-deoxyuridine Monophosphate (14). Compound **14** was obtained in two ways: (1) as the side product (3.31 g 36% yield) during the preparation of **8** using method II; (2) by conducting general procedure C with 5′-*O*-trityl IUdR **5** (3.52 g, 5.90 mmol), DIPEA (2 mL, 1.48 g, 11.5 mmol), and crude chlorophosphite **17** (2.6 g, ∼8 mmol). Isolated product **14** from both synthetic pathways showed the identical analytical data. General procedure C was carried on for 90 min in 30 mL of MeCN, and the oxidation with a solution of *t*-BuOOH (1.5 mL, ≥ 6 mmol) followed the phosphitylation. After the workup, a crude solid dissolved in MeCN (50 mL) was treated with $ZrCl_4$ (1.72 g, 7.38 mmol) and gave **14** (1.65 g, 43%) as a colorless rigid foam (93% pure by HPLC analysis): R_f = 0.66 (DCM/MeOH, 10:0.7). Further HPLC purification (31 mg of **14**, ∼5 mg per injection) was performed on a Columbus C18, 100 Å (5 μm, 10 mm × 250 mm) column; eluent, solvent A 50% MeCN in water, solvent B MeCN; elution at 2.5 mL/min with a linear gradient of B from 0% to 40% over 15 min and 40% B (isocratic) for 30 min. Product **14** (18.2 mg, ≥98% pure, UV at 280 nm) was collected within 25−27 min after the injection. The HPLC analysis showed a mixture of diastereomers: **14-*fast***, t_R = 31.1 min, and **14-*slow***, t_R = 33.3 min; column, Columbus C8, 100 Å (5 μm, 4.6 mm × 250 mm); eluent, solvent A 50% MeCN in water, solvent B MeCN; elution at 1 mL/min with A for 45 min (isocratic) and then a linear gradient of B from 0% to 95% B over 5 min and 95% B for 10 min. ^{1}H NMR (DMSO-d_6) δ = 10.89 (s, 1H, NH), 10.84 (s, 1H NH), 7.89 (s, 1H, uridine-H6), 7.84 (s, 1H, uridine-H6), 7.29−7.24 (m, 1H, aryl-H4), 6.27−6.22 (m, 1H, H1′), 5.56−5.44 (m, 2H, benzyl), 4.35−4.22 (m, 1H, H3′), 4.19−4.15 (m, 1H, H4′), 3.43 (bd, C5′-OH, *J* = 4.0 Hz), 2.54−2.46 (m, 2H, H5′, H5″), 2.27−2.23 (m, 1H, H2′), 2.15−2.11 (m, 1H, H2″), 1.39, 1.35, 1.32, 1.31 (overlapped s, 18H, aryl-C3, 3 × CH_3-*t*-Bu and aryl-C5, 3 × CH_3-*t*-Bu) ppm. ^{31}P NMR (DMSO-d_6) δ = −8.90, −8.77 ppm. HR-FABMS (*m*/*z*): [M + H]$^+$calcd for $C_{24}H_{32}N_2O_8PFI$, 653.0925; found, 653.0930. The ^{13}C isotope peak measured 654.0845, −2.0 ppm of the expected value.

5′-*O*-[Cyclo-3,5-di(*tert*-butyl)-6-fluorosaligenyl]thymidine Monophosphate (22). General Pprocedure C with 3′-*O*-levulinylthymidine **19** (2.43 g, 7.14 mmol), DIPEA (3.15 mL, 2.33 g, 18 mmol), and the crude chlorophosphite **17** (3.57 g, ∼ 11 mmol) was conducted in 30 mL of MeCN for 1 h, and the oxidation with a solution of *t*-BuOOH (2.5 mL, ≥12.5 mmol) followed the phosphitylation. Before deprotection, the crude product was purified on a silica gel column (DCM/MeOH, 10:0.4). Cleavage of the 3′-O-Lev group was completed in ≤5 min (a new single band on TLC) and the final product was separated on a silica gel column (DCM/MeOH gradient, 10:(0.7−0.9)) to give **22** (905 mg, 62%) as colorless rigid foam: R_f = 0.52 (DCM/MeOH, 10:0.7). The HPLC analysis showed a mixture of diastereomers: **22-*fast***, t_R = 19.3 min, and **22-*slow***, t_R = 20.2 min (≥96% pure, UV at 280 nm); column, ACE C18, 100 Å (5 μm, 4.6 mm × 250 mm); eluent, solvent A 50% MeCN in water, solvent B MeCN; elution at 0.8 mL/min with solvent A for 60 min (isocratic) and then a linear gradient of B from 0% to 95% B over 30 min. ^{1}H NMR (DMSO-d_6) δ = 11.40 (s, 1H, NH), 7.53 (s, 1H, uridine-H6), 7.49 (s, 1H, uridine-H6), 7.25 (s, 1H, aryl-H4), 7.23 (s, 1H, aryl-H4), 6.24−6.17 (m, 1H, H1′), 5.60−5.45 (m, 2H, benzyl), 5.41−5.29 (m, 1H, H3′), 4.60−4.53 (m, 1H, uridine-C3′-OH), 4.48−4.34 (m, 3H, H4′, H5′, H5″), 2.47−2.36 (m, 1H, H2′), 2.32−2.26 (m, 1H, H2″), 1.73 (s, 3H, uridine-C5-CH_3), 1.67 (s, 3H, uridine-C5-CH_3), 1.334, 1.325, 1.321, 1.317 (overlapped s, 18H, aryl-C3, 3 × CH_3-*t*-Bu; aryl-C5, 3 × CH_3-*t*-Bu) ppm. ^{31}P NMR (DMSO-d_6) δ = −9.73, −9.58 ppm. HR-FABMS (*m*/*z*): [M + H]$^+$calcd for $C_{25}H_{36}N_2O_8PF$, 542.2194; found, 542.2189. The ^{13}C isotope peak measured 543.2221, −2.0 ppm of the expected value.

5′-*O*-[Cyclo-3,5-di(*tert*-butyl)-6-fluorosaligenyl]-3′-deoxy-3′-fluorothymidine Monophosphate (23). General procedure C with 3′-deoxy-3′-fluorothymidine **20** (454 mg, 1.86 mmol) in DMF (3 mL), DIPEA (820 μL, 0.61 g, 4.7 mmol), and the crude chlorophosphite **17** (0.65 g, ∼2 mmol, 2 mL of THF solution) was conducted for 1 h, and the phosphitylated mixture was oxidized with a solution of *t*-BuOOH (400 μL, ≥2 mmol). The crude product was separated on a silica gel column (DCM/MeOH gradient, 10:(0.7−0.9)) to give **23** (806 mg, 80%) as a colorless rigid foam: R_f = 0.69 (DCM/MeOH, 10:0.7). The HPLC analysis showed a mixture of diastereomers: **23-*fast***, t_R = 45.4 min, and **23 *slow***, t_R = 47.0 min (≥97% pure, UV at 280 nm); column, ACE C18, 100 Å (5 μm, 4.6 mm × 250 mm); eluent, solvent A 50% MeCN in water, solvent B MeCN; elution at 0.8 mL/min with solvent A for 60 min (isocratic) and then a linear gradient of B from 0% to 95% B over 30 min. ^{1}H NMR (DMSO-d_6) δ = 11.31 (s, 1H, NH), 7.47 (s, 1H, uridine-H6), 7.43 (s, 1H, uridine-H6), 7.25 (s, 1H, aryl-H4), 7.23 (s, 1H, aryl-H4), 6.22−6.16 (m, 1H, H1′), 5.59−5.46 (m, 3H, benzyl, H3′), 4.43−4.39 (m, 2H, H5′, H5″), 3.95−3.91 (m, 1H, H4′), 2.17−2.10 (m, 2H, H2′, H2″), 1.71 (s, 3H, uridine-C5-CH_3), 1.66 (s, 3H, uridine-C5-CH_3), 1.34, 1.31 (overlapped s, 18H, aryl-C3, 3 × CH_3-*t*-Bu and aryl-C5, 3 × CH_3-*t*-Bu) ppm. ^{31}P NMR (DMSO-d_6) δ = −9.46, −9.62 ppm. HR-FABMS (*m*/*z*): [M + H]$^+$ calcd for $C_{25}H_{35}N_2O_7PF_2$, 544.2149; found, 544.2155. The ^{13}C isotope peak measured 545.2183, −2.0 ppm of the expected value.

5-Iodo-5′-*O*-[cyclo-3,5-di-(*tert*-butyl)-6-fluorosaligenyl]-3′-fluoro-2′,3′-dideoxyuridine Monophosphate (24). General procedure C with 5-iodo-3′-fluoro-2′,3′-dideoxyuridine **21** (390 mg, 1.1 mmol) in DMF (3 mL), DIPEA (480 μL, 355 mg, 2.75 mmol), and the crude chlorophosphite **17** (1.95 g, ∼6 mmol, 4.4 mL of THF solution) was conducted for 45 min. Phosphitylation was followed by the oxidation with a solution of *t*-BuOOH (650 μL, ≥3.25 mmol). The crude product was separated on a silica gel column (DCM/MeOH gradient, 10:(0.6−0.9)) to give **24** (181 mg, 76%) as colorless rigid foam: R_f = 0.73 (DCM/MeOH, 10:0.7). The HPLC analysis showed a mixture of diastereomers: **24-*fast***, t_R = 25.9 min, and **24-*slow***, t_R = 26.7 min (≥95% pure, UV at 280 nm); column, ACE C18, 100 Å (5 μm, 4.6 mm × 250 mm); eluent, solvent A 50% MeCN in water, solvent B MeCN. Elution was with a linear gradient of B from 0% to 95% B over 60 min and 95% B for 30 min at 1 mL/min. ^{1}H NMR (DMSO-d_6) δ = 11.78 (bs, 1H, NH), 8.12 (s, 1H, uridine-H6), 8.08 (s, 1H, uridine-H6), 7.25 (s, 1H, aryl-H4), 7.24 (m, 1H, aryl-H4), 6.14−6.10 (m, 1H, H1′), 5.57−5.46 (m, 2H, benzyl), 5.37−5.25 (m, 1H, H3′), 4.51−4.32 (m, 3H, H4′, H5′, H5″), 2.51−2.43 (m, 2H, H2′, H2″), 1.35, 1.34, 1.33, 1.32 (overlapped s, 18H, aryl-C3, 3 × CH_3-*t*-Bu and aryl-C5, 3 × CH_3-*t*-Bu) ppm. ^{31}P NMR (DMSO-d_6) δ = −9.35, −9.79 ppm. HR-FABMS (*m*/*z*): [M + Li]$^+$ calcd for $C_{24}H_{30}N_2O_7PF_2ILi$, 661.0964; found, 661.0953 The ^{13}C peak measured 662.0986, which was within −1.6 ppm of the expected value of 662.0998. Diastereomers were separated by the preparative HPLC (≤4 mg of **24** per injection) on a Columbus C18, 100 Å (5 μm, 10 mm × 250 mm) column; elution at 2.2 mL/min with 57% MeCN in water. Starting with 61 mg of diastereomeric **24**, 14.1 mg of **24-*fast*** and 16.0 mg of **24-*slow*** were isolated. Diastereomer **24-*fast*** eluted within 37−39 min and **24-*slow*** within 39.5−41.3 min after the injection. HPLC analysis results are as follows: **24-*fast***, t_R = 37.7 min, and **24-*slow***, t_R = 40.2 min (each isomer was ≥98% pure, UV at 280 nm); column, ACE C18, 100 Å (5 μm, 4.6 mm × 250 mm); eluent, solvent A 50% MeCN in water, solvent B MeCN; elution with a linear gradient of B from 0% to 10% B over 60 min at 0.8 mL/min. Analytical data of **24-*fast*** are as follows: ^{1}H NMR (DMSO-d_6) δ = 11.76 (s, 1H, NH), 8.14 (s, 1H, uridine-H6), 7.27 (s, 1H, aryl-H4), 6.14−6.10 (m, 1H, H1′), 5.57−5.49 (m, 2H, benzyl), 5.37−5.29 (m, 1H, H3′), 4.53−4.41 (m, 3H, H4′, H5′, H5″), 2.53−2.45 (m, 2H, H2′, H2″), 1.36, 1.32 (overlapped s, 18H, aryl-C3, 3 × CH_3-*t*-Bu and aryl-

C5, 3 × CH_3-*t*-Bu) ppm. ^{31}P NMR (DMSO-d_6) δ = −9.36 ppm. Analytical data of **24-*slow*** are as follows: ^{1}H NMR (DMSO-d_6) δ = 11.72 (s, 1H, NH), 8.08 (s, 1H, uridine-H6), 7.23 (m, 1H, aryl-H4), 6.12−6.09 (m, 1H, H1′), 5.53−5.41 (m, 2H, benzyl), 5.34−5.23 (m, 1H, H3′), 4.55−4.44 (m, 2H, H4′, H5′), 3.98−3.94 (m, 1H, H5″), 2.51−2.41 (m, 2H, H2′, H2″), 1.33, 1.31 (overlapped s, 18H, aryl-C3, 3 × CH_3-*t*-Bu and aryl-C5, 3 × CH_3-*t*-Bu) ppm. ^{31}P NMR (DMSO-d_6) δ = −9.77 ppm.

Trialkyltin Precursors. 5-Tri-*n*-butylstannyl-5′-*O*-cyclosaligenyl-2′-deoxyuridine Monophosphate (6a). General procedure D was performed with 5-iodo-5′-*O*-cyclosaligenyl-2′-deoxyuridine monophosphate **6** (520 mg, 1.0 mmol), hexa-*n*-butylditin (750 μL, 868 mg, 1.5 mmol), and the palladium(II) catalyst (77 mg, 0.11 mmol) in dioxane (35 mL) for 70 min. The crude product was purified by column chromatography on a silica gel, using a gradient of EtOAc in hexanes ((2−7):10). Further drying, by repeated evaporations with anhydrous MeCN and exposure to a high vacuum, gave **6a** (360 mg, 52%) as a rigid colorless foam: R_f = 0.69 (EtOAc/hexanes, 2:1). The HPLC analysis showed a mixture of diastereomers **6a-*fast***, t_R = 73.9 min, and **6a-*slow***, t_R = 74.4 min (≥97% pure, UV at 220 and 280 nm); column, ACE C18, 100 Å (5 μm, 4.6 mm × 250 mm); eluent, solvent A water, solvent B MeCN; elution at 0.8 mL/min with a linear gradient of B from 10−50% over 30 min, then 50% B (isocratic) for 30 min and a linear gradient of B from 50% to 95% for 30 min. Diastereomers were not separated on a preparative scale. ^{1}H NMR ($CDCl_3$) δ = 8.32 (s, 1H, NH), 8.27 (s, 1H NH), 7.33 (s, 1H, uridine-H6), 7.31 (s, 1H, uridine-H6), 7.15 (t, 1H, aryl-H5, J = 7.6 Hz), 7.13−7.06 (m, 3H, aryl-H3, aryl- H4, aryl- H6), 6.15−6.12 (m, 1H, H1′), 5.39−5.34 (m, 2H, benzyl), 4.58−4.54 (m, 1H, H3′), 4.45−4.37 (m, 2H, H5′, H5″), 4.12−4.09 (m, 1H, H4′), 3.23 (bd, 1H, C3′-OH, exchangeable with D_2O, J = 4.5 Hz), 2.46−2.40 (m, 1H, H2′), 2.35−2.25 (m, 1H, H2″), 1.56−1.42 (m, 3 × 2H, 3 × CH_2-*n*-Bu), 1.35−1.26 (m, 3 × 2H, 3 × CH_2-*n*-Bu), 1.02−0.98 (m, 3 × 2H, 3 × CH_2-*n*-Bu), 0.92−0.87 (m, 3 × 3H, 3 × 3 × CH_3-*n*-Bu) ppm. ^{31}P NMR ($CDCl_3$) δ = −8.23, −8.08 ppm. ^{119}Sn NMR ($CDCl_3$) δ = −1.97 ppm. HR-FABMS (*m*/*z*): [M + Li]$^+$ calcd for $C_{28}H_{43}N_2O_8PSnLi$, 693.1939; found, 693.1943.

5-Trimethylstannyl-5′-*O*-cyclo(3-methylsaligenyl)-2′-deoxyuridine Monophosphate (7a). General procedure D was conducted with 5-iodo-5′-*O*-cyclo(3-methylsaligenyl)-2′-deoxyuridine monophosphate **7** (710 mg, 1.32 mmol), hexamethylditin (0.64 g, 1.95 mmol), and the palladium(II) catalyst (90 mg, 0.13 mmol) in dioxane (40 mL) for 2 h. Purification of the crude product on a silica gel column ($CHCl_3$/MeOH, 10:0.8) and a twice repeated evaporation from dried MeCN gave pure stannane **7a** (374 mg, 49%) as a colorless foam: R_f = 0.67 (DCM/MeOH, 10:0.8). Purified **7a** was analyzed with HPLC, using ACE C18, 100 Å (5 μm, 4.6 mm × 250 mm) column; eluent, solvent A 10% MeCN in water, solvent B MeCN; elution at 1 mL/min with a linear gradient of B from 0% to 70% over 90 min. The analysis showed a mixture of diastereomers: **7a-*fast***, t_R = 55.2 min, **7a-*slow***, t_R = 55.4 min (≥98% pure, UV at 280 nm). Diastereomers were not separated preparatively. ^{1}H NMR ($CDCl_3$) δ = 8.13 (s, 1H, NH), 8.11 (s, 1H NH), 7.33 (s, 1H, uridine-H6, $^3J_{H,Sn}$ = 18.4 Hz), 7.29 (s, 1H, uridine-H6, $^3J_{H,Sn}$ = 18.4 Hz), 7.17 (m, 1H, aryl-H5), 7.12−7.04 (m, 3H, aryl-H3, aryl-H4, aryl-H6), 6.20−6.15 (m, 1H, H1′), 5.41−5.34 (m, 2H, benzyl), 4.55−4.52 (m, 1H, H3′), 4.47−4.39 (m, 2H, H4′, H5′), 4.16−4.11 (m, 1H, H5″), 3.57 (bd, 1H, C3′-OH, exchangeable with D_2O, J = 4.5 Hz), 2.45−2.36 (m, 1H, H2′), 2.34−2.27 (m, 1H, H2″), 2.21−2.19 (m, 3H, aryl-C3-CH_3), 0.49 (s, 9H, 3 × CH_3, $^2J_{Sn,H}$ = 29.2 Hz) ppm. ^{31}P NMR ($CDCl_3$) δ = −8.37, −8.18 ppm. ^{119}Sn NMR ($CDCl_3$) δ = −1.07 ppm. HR-FABMS (*m*/*z*): [M + H]$^+$ calcd for $C_{20}H_{28}N_2O_8PSn$, 575.0605; found, 575.0593. For the ^{13}C peak the measured value was 576.0640, −0.2 ppm.

5-Trimethylstannyl-5′-*O*-[cyclo-3,5-di(*tert*-butyl)-6-fluorosaligenyl]-2′-deoxyuridine Monophosphate (8a). General procedure D was conducted with 5-iodo-5′-*O*-[cyclo-3,5-di(*tert*-butyl)-6-fluorosaligenyl]-2′-deoxyuridine monophosphate **8** (1.51 g, 2.3 mmol), hexamethylditin (0.98 g, 3.0 mmol), and the palladium(II) catalyst (160 mg, 0.22 mmol) in EtOAc (80 mL) for 2 h. Purification of the crude product required two silica gel columns, DCM/MeOH, 10:0.7, and EtOAc/MeOH gradient, 10:(0−0.2), and final evaporation from dried MeCN to give pure stannane **8a** (1.22 g, 76%) as a colorless foam: R_f = 0.77 (DCM/MeOH, 10:0.7). The HPLC analysis showed a mixture of diastereomers: **8a-*fast***, t_R = 43.7 min, and **8a-*slow***, t_R = 44.1 min (≥98% pure, UV at 220 and 280 nm); column, ACE C18, 100 Å (5 μm, 4.6 mm × 250 mm); eluent, solvent A 50% MeCN in water, solvent B MeCN; elution at 0.8 mL/min with A for 25 min, then a linear gradient of B from 0% to 95% over 20 min and 95% B for 15 min. ^{1}H NMR ($CDCl_3$) δ = 8.21 (s, 1H, NH), 8.18 (s, 1H NH), 7.26 (m, 1H, aryl-H5), 7.20 (s, 1H, uridine-H6, $^3J_{H,Sn}$ = 18.5 Hz), 7.16 (s, 1H, uridine-H6, $^3J_{H,Sn}$ = 18.5 Hz), 6.20−6.17 (m, 1H, H1′), 5.49−5.32 (m, 2H, benzyl), 4.62−4.56 (m, 1H, H3′), 4.54−4.34 (m, 2H, H4′, H5′), 4.14−4.12 (m, 1H, H5″), 3.05 (bs, 1H, C3′-OH, exchangeable with D_2O), 2.47−2.41 (m, 1H, H2′), 2.34−2.27 (m, 1H, H2″), 1.39, 1.37, 1.35 (3*s*, 18H, aryl-C3, 3 × CH_3-*t*-Bu, aryl-C5, 3 × CH_3-*t*-Bu), 0.24 (t, 9H, 3 × CH_3Sn, $^2J_{Sn,H}$ = 29.0 Hz), 0.22 (t, 9H, 3 × CH_3Sn, $^2J_{Sn,H}$ = 29.0 Hz) ppm. ^{31}P NMR ($CDCl_3$) δ = −8.53, −8.38 ppm. ^{119}Sn NMR ($CDCl_3$) δ = −1.17 ppm. HR-FABMS (*m*/*z*): [M + H]$^+$calcd for $C_{27}H_{41}N_2O_8PFSn$, 691.1607; found, 691.1640. For ^{13}C peak the measured value was 692.1653, 1.8 ppm. Diastereomers were separated by preparative HPLC (≤440 μg of **8a** per injection), using a tandem of two ACE C18, 100 Å columns (5 μm, 4.6 mm × 250 mm); eluent, solvent A 50% MeCN in water, solvent B MeCN; elution at 0.7 mL/min with a linear gradient of B from 0% to 10% B over 110 min. After numerous HPLC injections, 16.4 mg of **8a-*fast*** and 23.2 mg of **8a-*slow*** was isolated. Diastereomer **8a-*fast*** eluted within 87.5−90 min and **8a-*slow*** within 90.5−93 min, after the injection. After an each separation run, the isolated isomer (120−200 μg) was collected in ∼2.5 mL of the eluate. The solvent was evaporated to dryness in a high vacuum. The combined product residue was reconstituted in MeCN and analyzed again using analytical HPLC (each diastereomer was ≥98% pure, UV at 220 and 280 nm). Analytical data of **8a-*fast*** are as follows: ^{1}H NMR ($CDCl_3$) δ = 7.84 (s, 1H, NH), 7.26 (m, 1H, aryl-H5), 7.20 (s, 1H, uridine-H6, $^3J_{H,Sn}$ = 18.5 Hz), 6.18 (t, 1H, H1′, $^2J_{H,H}$ = 6.5 Hz), 5.46 (d, 1H, 1 × H-benzyl, $^2J_{H,H}$ = 14.0 Hz), 5.34 (d, 1H, 1 × H-benzyl, $^2J_{H,H}$ = 14.0 Hz), 4.62−4.58 (m, 1H, H3′), 4.44−4.41 (m, 2H, H4′, H5′), 4.13−4.11 (m, 1H, H4′), 2.51 (bs, 1H, C3′-OH, exchangeable with D_2O), 2.45−2.41 (m, 1H, H2′), 2.31−2.25 (m, 1H, H2″), 1.37, 1.35 (2*s*, 18H, aryl-C3, 3 × CH_3-*t*-Bu, aryl-C5, 3 × CH_3-*t*-Bu), 0.23 (t, 9H, 3 × CH_3Sn, $^2J_{H,Sn}$ = 29.0 Hz) ppm. ^{31}P NMR ($CDCl_3$) δ = −8.52 ppm. Analytical data of **8a-*slow*** are as follows: ^{1}H NMR ($CDCl_3$) δ = 7.86 (s, 1H, NH), 7.27 (m, 1H, aryl-H5), 7.16 (s, 1H, uridine-H6, $^3J_{H,Sn}$ = 18.5 Hz), 6.16 (t, 1H, H1′, J = 6.5 Hz), 5.47−5.34 (m, 2H, benzyl), 4.63−4.58 (m, 1H, H3′), 4.55−4.50 (m, 2H, H5′), 4.38−4.33 (m, 2H, H5″), 4.07−4.04 (m, 1H, H4′), 2.80 (bs, 1H, C3′-OH, exchangeable with D_2O), 2.47−2.42 (m, 1H, H2′), 2.35−2.30 (m, 1H, H2″), 1.39, 1.36 (2*s*, 18H, aryl-C3, 3 × CH_3-*t*-Bu, aryl-C5, 3 × CH_3-*t*-Bu), 0.24 (t, 9H, 3 × CH_3Sn, $^2J_{H,Sn}$ = 29.0 Hz) ppm. ^{31}P NMR ($CDCl_3$) δ = −8.39 ppm.

5-Trimethylstannyl-3′-*O*-cyclosaligenyl-2′-deoxyuridine Monophosphate (12a). General procedure D was carried out with 5-iodo-3′-*O*-cyclosaligenyl-2′-deoxyuridine monophosphate **12** (193 mg, 0.37 mmol), hexamethylditin (157 mg, 0.48 mmol), and the palladium(II) catalyst (26 mg, 0.037 mmol) in EtOAc (30 mL) until starting **12** was detected on TLC (∼1.5 h). The crude product was initially separated and purified on a silica gel column (DCM/MeOH, 10:0.6). Further purification was done using the HPLC instrument equipped with a semipreparative Columbus C18, 100 Å (5 μm, 10 mm × 250 mm) column; eluent, solvent A 45% MeCN, solvent B MeCN; elution at 2.5 mL/min with a linear gradient of B for 60 min. Combined fractions, after repetitive evaporation from dried MeCN, gave pure stannane **12a** (119 mg, 57%) as a colorless amorphous solid: R_f = 0.64 (DCM/MeOH, 10:0.3). The HPLC analysis showed a mixture of diastereomers: **12a-*fast***, t_R = 47.0 min, and **12a-*slow***, t_R = 47.4 min (≥96% pure, UV at 280 nm); column, ACE C18, 100 Å (5 μm, 4.6 mm × 250 mm); eluent, solvent A 10% MeCN in water, solvent B MeCN; elution at 1 mL/min with a linear gradient of B from 0% to 95% over 45 min and then 95% B for 15 min. ^{1}H NMR ($CDCl_3$) δ = 8.79 (s, 1H, NH), 8.63 (s, 1H NH), 7.49 (s, 1H, uridine-H6, $^3J_{H,Sn}$ = 18.5 Hz), 7.37 (s, 1H, uridine-H6, $^3J_{H,Sn}$ = 18.5 Hz), 7.17

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(t, 1H, aryl-H4, J = 7.6 Hz), 7.14−7.07 (m, 3H, aryl-H3, H5, H6), 6.23−6.16 (m, 1H, H1′), 5.42−5.36 (m, 2H, benzyl), 4.31−4.28 (m, 1H, H3′), 4.25−4.21 (m, 1H, H4′), 3.97−3.85 (m, 2H, H5′, H5″, H4′), 3.58 (bd, 1H, C5′- OH, exchangeable with D_2O, J = 4.4 Hz), 2.61−2.45 (m, 2H, H2′, H2″), 0.32 (t, 3 × 3H, 3 × CH_3Sn, $^2J_{H,Sn}$ = 29.0 Hz) ppm. ^{31}P NMR ($CDCl_3$) δ = −9.80, −9.82 ppm. ^{119}Sn NMR ($CDCl_3$) δ = −1.47 ppm. HR-FABMS (m/z): $[M + Li]^+$ calcd for $C_{19}H_{25}N_2O_8PSnLi$, 567.0531; found, 567.0549. The ^{13}C isotope measured 568.0559, −0.9 ppm of the expected 568.0565 value.

5-Trimethylstannyl-3′-*O*-cyclo(3-methylsaligenyl)-2′-deoxyuridine Monophosphate (13a). General procedure D was carried out with iodo-3′-*O*-cyclo(3-methylsaligenyl)-2′-deoxyuridine monophosphate **13** (241 mg, 0.45 mmol), hexamethylditin (192 mg, 0.58 mmol), and the palladium(II) catalyst (32 mg, 0.046 mmol) in EtOAc (30 mL) until starting **13** disappeared on TLC (∼2 h). The crude product was separated and purified on a silica gel column (DCM/MeOH gradient, 10:(0.6−0.9)). Final purification proceeded on the HPLC equipped with a semipreparative Columbus C18, 100 Å (5 μm, 10 mm × 250 mm) column; eluent, solvent A 45% MeCN, solvent B MeCN; elution at 2.5 mL/min with a linear gradient of B for 60 min. Combined and dried fractions gave pure stannane **13a** (139 mg, 54%) as a colorless amorphous solid: R_f = 0.71 (DCM/MeOH, 10:0.8). The HPLC analysis showed a mixture of diastereomers: **13a-*fast***, t_R = 38.5 min, and **13a-*slow***, t_R = 39.7 min (≥98% pure, UV at 280 nm); column, ACE C18, 100 Å (5 μm, 4.6 × 250 mm); eluent, solvent A 15% MeCN in water, solvent B MeCN; column elution at 1 mL/min with a linear gradient of B from 0% to 25% over 50 min, then a gradient of B from 25% to 95% B over 5 min and 95% for 5 min. ^1H NMR ($CDCl_3$) δ = 8.73 (bs, 1H, NH), 7.52 (s, 1H, uridine-H6, $^3J_{H,Sn}$ = 18.6 Hz), 7.43 (s, 1H, uridine-H6, $^3J_{H,Sn}$ = 18.6 Hz), 7.18−7.03 (m, 3H, aryl-H4, H5, H6), 6.27−6.18 (m, 1H, H1′), 5.46−5.38 (m, 2H, benzyl), 4.30−4.25 (m, 1H, H3′), 4.23−4.21 (m, 1H, H4′), 3.97−3.85 (m, 2H, H5′, H5″), 3.63 (bd, 1H, C5′-OH, exchangeable with D_2O, J = 4.4 Hz), 2.64−2.43 (m, 5H, H2′, H2″, aryl-C3-CH_3), 0.37 (t, 3 × 3H, 3 × CH_3Sn, $^2J_{H,Sn}$ = 29.0 Hz) ppm. ^{31}P NMR ($CDCl_3$) δ = −9.84, −9.77 ppm. ^{119}Sn NMR ($CDCl_3$) δ = −1.41 ppm. HR-FABMS (m/z): $[M + H]^+$calcd for $C_{20}H_{28}N_2O_8PSn$, 575.0605; found, 575.0624. The ^{13}C isotope measured 576.0624, −2.5 ppm of the expected value.

5-Trimethylstannyl-3′-*O*-(cyclo-3,5-di(*tert*-butyl)-6-fluorosaligenyl)-2′-deoxyuridine Monophosphate (14a). General procedure D was carried out with 5-iodo-3′-*O*-(cyclo-3,5-di(*tert*-butyl)-6-fluorosaligenyl)-2′-deoxyuridine monophosphate **14** (306 mg, 0.47 mmol), hexamethylditin (205 mg, 0.62 mmol), and the palladium(II) catalyst (34 mg, 0.048 mmol) in EtOAc (40 mL) until starting **14** disappeared on TLC (∼3 h). The crude product was separated on a silica gel column (DCM/MeOH, 10:0.4) and the purification repeated (using EtOAc/hexanes, 10:5). Final purification was conducted using an HPLC instrument equipped with a semipreparative Columbus C18, 100 Å (5 μm, 10 mm × 250 mm) column; eluent, solvent A 45% MeCN, solvent B MeCN; elution at 2.5 mL/min with a linear gradient of B from 0% to 95% over 60 min. Combined fractions were evaporated under a vacuum and the residue was dried by repetitive evaporation from dry MeCN to give pure stannane **14a** (206 mg, 63%) as a colorless ridged foam: R_f = 0.79 (DCM/MeOH, 10:0.5). The HPLC analysis showed a mixture of diastereomers: **14a-*fast***, t_R = 26.4 min, and **14a-*slow***, t_R = 26.9 min (≥98% pure, UV at 220 and 280 nm); column, ACE C18, 100 Å (5 μm, 4.6 mm × 250 mm); eluent, solvent A 50% MeCN in water, solvent B MeCN; elution at 1 mL/min with a linear gradient of B from 0% to 95% over 40 min, then 95% B for 20 min. ^1H NMR ($CDCl_3$) δ = 8.63 (bs, 1H NH), 7.44 (s, 1H, uridine-H6, $^3J_{H,Sn}$ = 18.5 Hz), 7.40 (s, 1H, uridine-H6, $^3J_{H,Sn}$ = 18.5 Hz), 7.19 (t, 1H, aryl-H4, J = 7.6 Hz), 6.20−6.14 (m, 1H, H1′), 5.44−5.32 (m, 2H, benzyl), 4.33−4.29 (m, 1H, H3′), 4.27−4.24 (m, 1H, H4′), 3.94−3.82 (m, 2H, H5′, H5″), 3.66 (bd, 1H, C5′-OH, exchangeable with D_2O, J = 4.5 Hz), 2.64−2.47 (m, 2H, H2′, H2″), 1.325, 1.315, 1.312, 1.311 (overlapped s, 18H, aryl-C3, 3 × CH_3-*t*-Bu and aryl-C5, 3 × CH_3-*t*-Bu), 0.24 (t, 3 × 3H, 3 × CH_3Sn, $^2J_{H,Sn}$ = 29.0 Hz) ppm. ^{31}P NMR ($CDCl_3$) δ = −9.72, −9.62 ppm. ^{119}Sn NMR ($CDCl_3$) δ = −1.43 ppm. HR-FABMS (m/z): $[M + H]^+$ calcd for $C_{27}H_{41}N_2O_8PFSn$, 691.1607; found, 691.1635. The ^{13}C isotope measured 692.1648, 1.1 ppm of the expected value.

5-Trimethylstannyl-3′-fluoro-2′,3′-dideoxyuridine (21a). General procedure D was carried out with 5-iodo-3′-fluoro-2′,3′-dideoxyuridine **21** (203 mg, 0.57 mmol), hexamethylditin (250 mg, 0.76 mmol), and the palladium(II) catalyst (40 mg, 0.057 mmol) in EtOAc (25 mL) until starting **21** disappeared on TLC (∼90 min). Noticeable proton deiodination occurred. The crude product was separated and purified on a silica gel column (DCM/MeOH gradient, 10:(0.3−0.7)), and the purification was continued on an HPLC instrument equipped with a semipreparative Columbus C18, 100 Å (5 μm, 10 mm × 250 mm) column; eluent, solvent A H_2O, solvent B MeCN; elution at 2.7 mL/min flow rate with a linear gradient of B from 0% to 95% for 45 min and then 95% B for 15 min. Combined fractions (29−31) were evaporated and dried in a high vacuum to give the pure stannane **21a** (110 mg, 49%) as a colorless foam: R_f = 0.72 (DCM/MeOH, 10:0.7). The HPLC analysis showed a single peak at t_R = 27.1 min (≥95% pure, UV at 280 nm); column, ACE C18, 100 Å (5 μm, 4.6 × 250 mm); eluent, solvent A H_2O, solvent B MeCN; elution at 1 mL/min with a linear gradient of B from 0% to 95% over 45 min, then 95% B for 15 min. ^1H NMR (DMSO-d_6) δ = 11.19 (s, 1H, NH), 7.63 (t, 1H, uridine-H6, $^3J_{H,Sn}$ = 18.5 Hz), 6.24−6.21 (m, 1H, H1′), 5.34 (dd, 1H, H3′, $^2J_{H,H}$= 12.0 Hz, $^2J_{H,H}$ = 7.8 Hz), 5,15 (t, 1H, C5′-OH, $^2J_{H,H}$ = 5.5 HZ), 4.17 (d, 1H, H4′, $^2J_{H,H}$ = 10.6 Hz), 3.66−3.53 (m, 2H, H5′, H5″), 2.50−2.26 (m, 2H, H2′, H2″), 0.19 (t, 3 × 3H, 3 × CH_3Sn, $^2J_{H,Sn}$ = 28.5 Hz) ppm. ^{119}Sn NMR (DMSO-d_6) δ = −1.22 ppm. HR-FABMS (m/z): $[M + Li]^+$ calcd for $C_{12}H_{19}N_2O_4FSnLi$, 401.0511; found, 401.0516. The ^{13}C isotope peak measured 402.0555, which was within 2.6 ppm of the expected value of 402.0545.

5-Trimethylstannyl-5′-*O*-[cyclo-3,5-di-(*tert*-butyl)-6-fluorosaligenyl]-3′-fluoro-2′,3′-dideoxyuridine Monophosphate (24a). General procedure D was carried out with 5-iodo-5′-*O*-[cyclo-3,5-di(*tert*-butyl)-6-fluorosaligenyl]-3′-fluoro-2′,3′-dideoxyuridine **24** (166 mg, 0.25 mmol), hexamethylditin (112 mg, 0.34 mmol), and the palladium(II) catalyst (36 mg, 0.052 mmol) in EtOAc (14 mL) until starting **24** disappeared (∼45 min) on TLC (EtOAc/MeOH, 2:1). The crude product was initially separated and partially purified on a silica gel column (DCM/MeOH gradient, 10:(0.2−0.5)), and the purification was continued on an HPLC instrument equipped with a semipreparative Columbus C18, 100 Å (5 μm, 10 mm × 250 mm) column; eluent, solvent A 45% MeCN, solvent B MeCN; elution with a linear gradient of B from 0% to 95% over 60 min at 2.5 mL/min flow rate. Combined fractions after repetitive evaporation from dry MeCN gave pure stannane **24a** (87 mg, 49%) as a colorless foam: R_f = 0.84 (DCM/MeOH, 10:0.5). The HPLC analysis showed a mixture (47:53) of diastereomers: **24a-*fast***, t_R = 37.6 min, and **24a-*slow***, t_R = 38.2 min (≥95% pure, UV at 280 nm); column, ACE C18, 100 Å (5 μm, 4.6 mm × 250 mm); eluent, solvent A 50% MeCN in water, solvent B MeCN; elution with a linear gradient of B from 0% to 95% B over 60 min and then 95% B for 30 min at 1 mL/min. Diastereomers were not separated preparatively. ^1H NMR (DMSO-d_6) δ = 11.26 (bs, 1H, NH), 7.30 (s, 1H, uridine-H6, $^3J_{H,Sn}$ = 18.5 Hz), 7.29 (s, 1H, uridine-H6, $^3J_{H,Sn}$ = 18.5 Hz), 7.25 (s, 1H, aryl-H4), 7.23 (m, 1H, aryl-H4), 6.18−6.11 (m, 1H, H1′), 5.58−5.29 (m, 3H, benzyl, H3′), 4.70−4.32 (m, 3H, H4′, H5′, H5″), 2.54−2.42 (m, 2H, H2′, H2″), 1.331, 1.324, 1.319, 1.314 (overlapped s, 18H, aryl-C3, 3 × CH_3-*t*-Bu and aryl-C5, 3 × CH_3-*t*-Bu), 0.183 (t, 3 × 3H, 3 × CH_3Sn, $^2J_{H,Sn}$ = 29.0 Hz), 0.169 (t, 3 × 3H, 3 × CH_3Sn, $^2J_{H,Sn}$ = 29.0 Hz) ppm. ^{31}P NMR (DMSO-d_6) δ = −9.48, −9.90 ppm. ^{119}Sn NMR (DMSO-d_6) δ = −1.33 ppm. HR-FABMS (m/z): $[M + Li]^+$ calcd for $C_{27}H_{39}N_2O_7PF_2SnLi$, 699.1645; found, 699.1637. The ^{13}C isotope peak measured 700.1682, 0.4 ppm of the expected value of 700.1679.

[^{125}I]Radioiodinated Cyclosaligenyl Phosphotriesters. 5-[^{125}I]Iodo-5′-*O*-cyclosaligenyl-2′-deoxyuridine Monophosphate (6b). General procedure E was conducted within the 20−433 MBq range and repeated six times to give ∼1550 MBq **6b** in successive radioiodinations of the stannyl precursor **6a**. An average isolated yield of the product was 88%. The latest preparation was carried out with stannane **6a** (120 μg) and [^{125}I]NaI/NaOH (94 μL,

377.4 MBq). The HPLC purification of the product proceeded on a Jupiter C18, 300 Å (5 μm, 4.6 mm × 250 mm) column; eluent, solvent A 10% MeCN in water, solvent B MeCN; 0.8 mL/min elution rate of a linear gradient of B from 0% to 20% over 33 min, followed by a linear gradient of B from 20% to 95% for 5 min and finally 95% B for 15 min. The main radioactivity peak (344.1 MBq, 91% yield) was eluted and collected in four fractions of the eluate (total volume, ∼3.3 mL), within 28−32 min after the injection of 460 μL (∼373.7 MBq) of the reaction mixture. An excess of unreacted tin precursor **6a** was separated from the radioiodinated product without difficulty, eluting ∼20 min later (t_R = 50.6 min). If required, diastereomers of **6b** were separated using the same HPLC conditions. The diastereomer **6b-*fast*** eluted at t_R = 29.8 min and **6b-*slow*** at t_R = 30.8 min. When a solution of 20% MeCN in water was used as solvent A and elution was at the 0.8 mL/min flow rate with solvent A for 25 min, followed by a linear gradient of B from 0% to 95% over 10 min and finally with 95% B for 10 min, diastereomers were eluted more quickly and fully separated: **6b-*fast***, t_R = 20.6 min, and **6b-*slow***, t_R = 22.4 min. In a single HPLC run, complete separation (each diastereomer ≥98% pure, Bioscan NaI(T) detector) was achieved, if the total amount of injected **6b** was ≤8.51 MBq. Larger batches of the single diastereomer were acquired by repeating the HPLC injections or using a semipreparative column, Columbus C18, 100 Å (5 μm, 10 mm × 250 mm), with elution at a 2.6 mL/min flow rate with a 20% MeCN solution in water. Diastereomer **6b-*fast*** eluted within 75−77 min and **6b-*slow*** within 83−87 min, after the injection, and each was collected in ∼8 mL volume of the eluate. The solvent was evaporated to dryness in a high vacuum at 30 °C, using the SpeedVac system. The log *P* values were as follows: **6b-*fast***, 0.63 (±0.02), **6b-*slow***, 0.66 (±0.03). The HPLC co-injections of the purified **6b** with the nonradioactive analogue **6** and parallel monitoring of the radioactivity and UV signal confirmed the identical elution of both compounds.

5-[^{125}I]Iodo-5′-*O*-cyclo(3-methylsaligenyl)-2′-deoxyuridine Monophosphate (7b). General procedure E was conducted within the 19−374 MBq range to give ∼1220 MBq **7b** from six repeated radioiodinations of the stannyl precursor **7a**. An average isolated yield was 81%. The latest radioiodination was performed with stannane **7a** (∼110 μg) and [^{125}I]NaI/NaOH (90 μL, 344.1 MBq). The HPLC purification of the product continued on a Jupiter C18, 300 Å (5 μm, 4.6 mm × 250 mm) column; eluent, solvent A 18% MeCN in water, solvent B MeCN; 0.8 mL/min flow rate. Elution was with solvent A for 30 min, then with a linear gradient of B from 0% to 95% over 10 min and 95% B for 20 min. The product **7b** (299.7 MBq, 87%), which eluted within 21.5−24.7 min after the injection of 410 μL (∼336.7 MBq) of the reaction mixture, was collected in three fractions (2.5 mL volume of the eluate). An excess of unreacted tin precursor **7a** was fully separated, eluting between 27.3−27.7 min. The HPLC co-injections of the purified **7b** with nonradioactive analogue **7** and monitoring of the radioactivity (Bioscan NaI(T) detector) and UV signal at 280 nm verified the same HPLC mobility of both compounds. Diastereomers of **7b** were separated on a Jupiter C18, 300 Å (5 μm, 4.6 mm × 250 mm) column, eluting with 20% MeCN solution in water, for 45 min at the 0.8 mL/min flow rate. Single diastereomers, **7b-*fast*** (t_R = 25.3 min) and **7b-*slow*** (t_R = 26.8 min), were ≥98% pure (Bioscan NaI(T)). In the course of a single HPLC run, the full separation of diastereomers was limited to a total of ∼9.98 MBq **7b** loaded onto a column. Larger batches of resolved diastereomers were attainable through repetitive HPLC injections or using a larger column: Columbus C18, 100 Å (5 μm, 10 mm × 250 mm); eluent, a solution of 22% MeCN in water; flow rate of 2.5 mL/min. Diastereomer **7b-*fast*** eluted within 72−77 min and **7b-*slow*** within 79−81 min after the injection. The solvent was evaporated to dryness in a high vacuum, on the SpeedVac system. The log *P* values were as follows: **7b-*fast*** 0.85 (±0.04), **7b-*slow*** 0.86 (±0.04). The product residue was analyzed using analytical HPLC shortly before conducting scheduled experiments and was reconstituted in an appropriate solvent.

5-[^{125}I]Iodo-5′-*O*-[cyclo-3,5-di(*tert*-butyl)-6-fluorosaligenyl]-2′-deoxyuridine Monophosphate (8b). The overall ∼1925 MBq **8b** was acquired in 11 radioiodinations, using one of the purified tin precursors **8a**, **8a-*fast***, or **8a-*slow***, and conducting general procedure E within the 9.25−396 MBq range. An average isolated yield of the product was 88%. The latest radiolabeling was performed with diastereomeric stannane **8a** (∼115 μg) and [^{125}I]NaI/NaOH (40 μL, 144.7 MBq). The HPLC purification of the product proceeded on ACE C18, 100 Å (5 μm, 4.6 mm × 250 mm) column; eluent, solvent A 50% MeCN in water, solvent B MeCN; flow rate of 1.0 mL/min. Elution was with solvent A for 60 min, then with a linear gradient of B from 0% to 95% over 10 min and 95% B for 20 min. The product **8b** (128.76 MBq, 89%), which eluted within 25−29 min after the injection of 350 μL (∼139.12 MBq) of the reaction mixture, was collected in four fractions. An excess of unreacted stannane **8a** eluted ∼15 min later. A mixture of purified **8b** (∼444 kBq, 10 μL) and the corresponding nonradioactive analogue **8** (∼15 μg, 20 μL) was prepared in acetonitrile and injected onto the HPLC instrument, using the same column and conditions as during the separation of the product. Both compounds eluted together, showing identical retention times. Diastereomers of **8b** were separated on ACE C18, 100 Å (5 μm, 4.6 mm × 250 mm) column, with elution at the 0.8 mL/min flow rate with a 45% MeCN solution in water for 45 min. Each diastereomer, **8b-*fast*** (t_R = 30.3 min) and **8b-*slow*** (t_R = 32.8 min), was ≥98% pure (Bioscan NaI(T)). The complete separation of diastereomers in the single HPLC run was reached if the total amount of **8b** loaded onto the column was ≤8.14 MBq. Larger batches of the individual diastereomers were obtained by repetitive HPLC injections of purified **8b** or by conducting the radioiodination using a single diastereomer of the tin precursor **8a-*fast*** or **8a-*slow***. Fractions containing the product were combined, and the solvent was evaporated in a high vacuum on the SpeedVac system. The log *P* values were as follows: **8b-*fast*** 2.22 (±0.16), **8b-*slow*** 2.24 (±0.14). The product residue was reconstituted in MeCN and analyzed again on the analytical HPLC instrument before conducting planned experiments.

5-[^{125}I]Iodo-3′-*O*-cyclosaligenyl-2′-deoxyuridine Monophosphate (12b). Four consecutive radioiodinations was carried out according to general procedure E within the 9.25−262.7 MBq range, to give an overall 360.4 MBq **12b**. An average isolated yield of the product was 87%. The latest radiolabeling was performed with the diastereomeric stannane **12a** (∼100 μg, ∼97% pure, UV at 280 nm) and [^{125}I]NaI/NaOH (65 μL, 262.7 MBq). The HPLC purification of the product proceeded on ACE C18, 100 Å (5 μm, 4.6 mm × 250 mm) column; eluent, solvent A 10% MeCN in water, solvent B MeCN; elution at 1.0 mL/min with a linear gradient of B from 0% to 40% over 40 min, followed by a gradient of B from 40% to 95% for 20 min. The radioactivity peak of **12b** (233.84 MBq, 89%) was collected in four fractions within 21−25 min after the injection of the reaction mixture (∼500 μL, 256.1 MBq). An excess of the unreacted tin precursor **12a** was separated from the radioiodinated product, eluting ∼5 min later (t_R = 30.2 min). Combined fractions containing **12b** were evaporated and reconstituted in MeCN (∼66.7 MBq/mL), and 10 μL (∼666 kBq) was reinjected onto the HPLC column: ACE C18, 100 Å (5 μm, 4.6 mm × 250 mm) column; eluent, solvent A 10% MeCN in water, solvent B MeCN; elution at 0.8 mL/min with a linear gradient of B from 0% to 40% over 45 min, then a gradient of B from 40% to 95% for 15 min. Analysis showed a mixture (44:56 ratio) of diastereomers: **12b-*fast***, t_R = 26.4 min, and **12b-*slow***, t_R = 26.7 min (≥98% pure, Bioscan NaI(T)). Diastereomers of **12b** were not separated preparatively. The co-injected solutions (in 50% MeCN in water) of the purified **12b** and the corresponding nonradioactive analogue **12**, with parallel monitoring of the radioactivity and UV signal, have shown the identical elution mobility of both analogues. Diastereomer **12b-*fast*** eluted at t_R = 26.3 min and **12b-*slow*** at t_R = 26.7 min, together with **12-*fast*;**, t_R = 26.1 min, and **12-*slow***, t_R = 26.4 min.

5-[^{125}I]Iodo-3′-*O*-cyclo(3-methylsaligenyl)-2′-deoxyuridine Monophosphate (13b). The total amount of prepared **13b** was 462.5 MBq, obtained in five successive radioiodinations of **13a**, carried out within the 9.25−189 MBq range. Each reaction proceeded according to general procedure E. An average isolated yield of the product was 73%. The latest radiolabeling was performed with the diastereomeric stannane **13a** (∼100 μg) and [^{125}I]NaI/NaOH (45 μL,

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174.3 MBq). The HPLC purification was achieved on Jupiter C18, 300 Å (5 μm, 4.6 mm × 250 mm) column; eluent, solvent A 10% MeCN in water, solvent B MeCN. A column was eluted at 1.0 mL/min with a linear gradient of B from 0% to 95% over 45 min, then 95% B for 15 min. The radioactivity peak of **13b** (123.95 MBq, 71%) was collected within three fractions (24−27 min) after the injection of ∼300 μL (160.95 MBq) of the reaction mixture. An excess of the unreacted tin precursor **13a** was separated from the radioiodinated product, eluting ∼6 min later (t_R = 29.6 min). Combined fractions containing the purified **13b** were evaporated, reconstituted in MeCN (∼44.5 MBq/mL), and 10 μL (∼444 kBq) was reinjected onto the HPLC column: Jupiter C18, 300 Å (5 μm, 4.6 mm × 250 mm); eluent, solvent A 10% MeCN in water, solvent B MeCN; elution at 1.0 mL/min with a linear gradient of B from 0% to 95% over 45 min, then 95% B for 15 min. The analysis showed a mixture (47:53 ratio) of **13b-*fast***, t_R = 23.4 min, and **13b-*slow***, t_R = 23.7 min (≥98% pure, Bioscan NaI(T)). Diastereomers of **13b** were not separated preparatively. Co-injected solutions (in 50% MeCN) of the purified **13b** (∼660 kBq, 15 μL) and the nonradioactive analogue **13** (∼17 μg, 25 μL) were analyzed, with monitoring of the radioactivity (Bioscan NaI(T)) and UV at 280 nm: ACE C18, 100 Å (5 μm, 4.6 mm × 250 mm) column; eluent, solvent A 10% MeCN in water, solvent B MeCN; elution at 1.0 mL/min with a linear gradient of B from 0% to 95% over 60 min. The analysis confirmed the identical elution of both, the [^{125}I]- and [^{127}I]-iodoanalog.

5-[^{125}I]Iodo-3′-*O*-(cyclo-3,5-di(*tert*-butyl)-6-fluorosaligenyl)-2′-deoxyuridine Monophosphate (14b). General procedure E was carried out within the 18.5−211 MBq range to give, after four conducted radioiodinations, 466.2 MBq **14b** in an average yield of 86%. The latest radiolabeling proceeded with stannane **14a** (∼110 μg) and [^{125}I]NaI/NaOH (60 μL, 211 MBq). The HPLC purification of the crude product was achieved on a Jupiter C18, 300 Å (5 μm, 4.6 mm × 250 mm) column; eluent, solvent A 40% MeCN in water, solvent B MeCN. Elution was at 1.0 mL/min flow rate with a linear gradient of B from 0% to 95% over 35 min, followed by 95% B for 25 min. The product (179.1 MBq, 81%) collected within 21−23 min after the injection of 425 μL (∼203.5 MBq) of the reaction mixture was separated from an excess of the unreacted tin precursor **14a**, which eluted ∼10 min later (32.8−33.6 min). Appropriate fractions were combined, evaporated with a stream of nitrogen, and the residue was further dried in a high vacuum. A mixture of purified **14b** (∼440 kBq, 10 μL) and the corresponding nonradioactive analogue **14** (∼15 μg, 20 μL) in acetonitrile was reinjected onto the HPLC, using the same settings as during the separation of the crude product. The analysis has shown **14b-*fast***, t_R = 21.2 min, and **14b-*slow***, t_R = 21.7 min, coeluting with diastereomers of the [^{127}I]iodo analogue: **14-*fast***, t_R = 20.9 min, and **14-*slow***, t_R = 21.6 min. Diastereomers of **14b** were most efficiently separated on a ACE C18, 100 Å (5 μm, 4.6 mm × 250 mm) column, eluted with 50% MeCN in water at the 0.8 mL/min flow rate. Each isomer, **14b-*fast*** (t_R = 41.2 min) and **14b-*slow*** (t_R = 45.7 min), was ≥98% pure (Bioscan NaI(T) detector). The complete separations of diastereomers in the single HPLC run were attained if the total amount of **14b** loaded onto a column was ≤11.9 MBq. Larger lots of the single diastereomers were obtained in multiple injections.

5-[^{125}I]Iodo-3′-fluoro-2′,3′-dideoxyuridine (21b). General procedure E was performed in four consecutive radioiodinations carried out within the 19.2−148 MBq range to give 237 MBq **21b**. The average isolated yield of the product was 91%. The largest conducted radiolabeling proceeded with the stannane **21a** (∼120 μg) and [^{125}I]NaI/NaOH (35 μL, 148.4 MBq). The HPLC purification of the crude product was achieved on a ACE C18, 100 Å (5 μm, 4.6 mm × 250 mm) column; eluent, solvent A water, solvent B MeCN; 1.0 mL/min flow rate. Elution was with a linear gradient of B from 0% to 95% over 40 min, followed by 95% B for 20 min. The product **21b** (136.5 MBq, 92%) was collected in two fractions within 18−20 min after the injection of 420 μL (∼138.4 MBq) of the reaction mixture and was freed from an excess of the unreacted tin precursor **21a**, which eluted ∼7 min later (27.1−27.5 min). Appropriate fractions were combined. The solvent was evaporated with a stream of nitrogen and the residue dried in a high vacuum, using the SpeedVac system at 30 °C: log *P* = −0.030 (±0.0012). The combined solutions of purified **21b** (∼550 kBq, 15 μL) and the nonradioactive analogue **21** (∼14 μg, 25 μL) were prepared in acetonitrile and injected onto the HPLC column: ACE C18 100 Å (5 μm, 4.6 mm × 250 mm) column; eluent, solvent A water, solvent B, MeCN; elution at 0.8 mL/min with a linear gradient of B from 0% to 95% over 45 min. The synchronized monitoring of the radioactivity and UV signal at 280 nm showed the product (≥98% pure, Bioscan NaI(T)) **21b** eluting after t_R = 21.8 min, together with the [^{127}I]iodo analogue **21** (t_R = 21.6 min).

5-[^{125}I]Iodo-5′-*O*-[cyclo-3,5-di-(*tert*-butyl)-6-fluorosaligenyl]-3′-fluoro-2′,3′-dideoxyuridine (CycloSal[^{125}I]IUdRFMP) (24b). Method I: Destannylation of Trimethyltin Precursor. The overall quantity of prepared **24b** was 390 MBq, acquired in four consecutive radioiodinations. General procedure E was carried out within the 18.5−192.4 MBq range, and the average isolated yield was 93%. The largest conducted radiolabeling proceeded with stannane **24a** (∼120 μg) and [^{125}I]NaI/NaOH (60 μL, 192.4 MBq). The HPLC purification of the crude product was best achieved on ACE C18, 100 Å (5 μm, 4.6 mm × 250 mm) column; eluent, solvent A 50% MeCN in water, solvent B MeCN. The column was eluted at 1.0 mL/min flow rate, with a linear gradient of B from 0% to 95% over 60 min, followed by 95% B for 30 min. The product **24b** (177.5 MBq, 92%) collected within 26−28 min after the injection of 500 μL (∼188.7 MBq) of the reaction mixture was fully separated from an excess of the tin precursor **24a**, which eluted ∼9 min later (37.0−37.6 min). Fractions containing the product were combined. The solvent was evaporated with a stream of nitrogen and the residue further dried in a high vacuum. A mixture of purified **24b** (∼445 kBq, 10 μL) and the nonradioactive analogue **24** (∼15 μg, 20 μL) was prepared in acetonitrile and analyzed on the HPLC, using the same settings as applied during the separation of the product. The analysis showed diastereomer **24b-*fast***, t_R = 26.1 min, and **24b-*slow***, t_R = 26.7 min, coeluting with the [^{127}I]iodo analogs **24-*fast***, t_R = 25.9 min, and **24-*slow***, t_R = 26.5 min. Diastereomers of **24b** were separated on a ACE C18, 100 Å (5 μm, 4.6 mm × 250 mm) column; eluent, solvent A, 50% MeCN in water, solvent B, MeCN. The column was eluted with a linear gradient of B from 0% to 10% over 60 min at 0.8 mL/min flow rate. Each diastereomer, **24b-*fast*** (t_R = 38.1 min) and **24b-*slow*** (t_R = 40.7 min), was ≥98% pure (Bioscan NaI(T) detector). In the single HPLC run a full separation of both diastereomers was possible if the total amount of **24b** loaded onto the column was ≤20.6 MBq and the column was eluted with 50% MeCN (isocratic) at 0.8 mL/min flow rate. Larger lots of single diastereomers were obtained by repetitive HPLC injections or using a larger column, Columbus C18, 100 Å (5 μm, 10 mm × 250 mm); elution at the 2.2 mL/min flow rate with 57% MeCN in water.

Method II: Direct Coupling of Phosphoramidite (18) with Non-Carrier-Added 5-[^{125}I]Iodo-3′-fluoro-2′,3′-dideoxyuridine (21b). This preparation was repeated five times, starting with 39.6, 50.7, 81.1, 254.2, and 353.4 MBq purified **21b**. An average isolated yield of the purified product was 43%. Reaction tubes were flame-dried and equipped with rubber septa and magnetic microstirrers. All preparation steps were conducted under argon atmosphere, and evaporation of solvents was accomplished with a stream of dry nitrogen. Typically, 1 day before the planned experiment, 2,4-bis(*tert*-butyl)-5-fluorosalicyl alcohol (0.05 g, 0.2 mmol) was placed in the reaction tube and dissolved in dry diethyl ether (2 mL). After the mixture was cooled to −17 °C, phosphorus trichloride (19 μL, 0.22 mmol) was injected into the reaction mixture, using a syringe and argon pressure. This was followed by the slow, dropwise addition of the triethylamine solution (60 μL, 0.43 mmol) in dry diethyl ether (1 mL). The resulting mixture was left overnight at −20 °C to facilitate complete precipitation of triethylamine hydrochloride. The following day the reaction tube was centrifuged briefly and ∼2 mL of the clear reaction mixture was withdrawn and immediately transferred into a second reaction tube. The solvent was evaporated and MeCN (1 mL) added to the residue. After the mixture was cooled to −20 °C, a solution of DIPA (46 μL, 0.38 mmol) in 0.5 mL of dry MeCN was added. During this addition the temperature of the reaction mixture was kept below 0 °C. The resulting solution of the crude saligenyl

N,*N*-diisopropylaminophosphoramidite was transferred via a needle with help of argon pressure to a third reaction tube, which was immersed in the −40 °C cooling bath and contained 3′-fluoro-2′,3′-dideoxyuridine **21b** (50.7 MBq, ∼6.2×10^{-4} μmol) and a solution (∼0.45 M) of 1*H*-tetrazole (50 μL, 2.5×10^{-2} mmol) in dry MeCN. The resulting mixture was stirred for 5 min, sonicated briefly, and allowed to reach ambient temperature. The stirring continued for 10 min, and after the mixture was cooled to −40 °C for a second time, a solution of *tert*-butyl hydroperoxide (5 μL, 3.25×10^{-2} mmol) in *n*-decane was added. A the cooling bath was removed and when the reaction mixture reached ambient temperature (∼20 min), the solvent was evaporated and 1 mL of ethyl acetate and 5% solution of $NaHSO_3$ (1 mL) were added. The mixture was vortexed, centrifuged, and the aqueous phase was washed with EtOAc (3 × 100 μL). The organic phase, combined with washes, was partially evaporated to a total of ∼500 μL and injected into the HPLC instrument, equipped with a semipreparative Columbus C18, 100 Å (5 μm, 10 mm × 250 mm) column; eluent, solvent A water, solvent B MeCN. Elution was at 2.5 mL/min flow rate, with a linear gradient of B from 0% to 95% over 30 min and with 95% B for 60 min. The main radioactivity peak (20.39 MBq, 40%) was collected between 34.1 and 35.2 min after the injection. The solvent was evaporated. A receiver tube was further dried in a high vacuum and the residue reconstituted in dry MeCN (∼37 kBq/μL). The 10 μL aliquot was injected on an analytical ACE C18, 100 Å (5 μm, 4.6 mm × 250 mm) column; eluent, solvent A 50% MeCN in water, solvent B MeCN; elution at 1.0 mL/min with a linear gradient of B from 0% to 95% over 90 min. The analysis showed diastereomer **24b-*fast*** at t_R = 31.0 min and **24b-*slow*** at t_R = 31.9 min (≥98% pure, Bioscan NaI(T)). The HPLC co-injections of this product with the **24b**, previously prepared by the destannylation of **24a** according to method I and also co-injections with the [^{127}I]iodo analogue **24**, further confirmed the product identity.

Lipophilicity Measurements. The partition ratio (*P*) of compounds **6b**−**8b** and **21b** was determined by the shake-tube method. To a glass tube containing a dried residue of [^{125}I]iodinated compound (740−1295 kBq), *n*-octanol (3.5 mL) was added. The solution was vortexed for 1 min. The three aliquots (1.0 mL each) were transferred into clean tubes, and to each tube 1 mL of aqueous phosphate buffered saline (10 mM, pH 6.8) was added. The mixtures, in tightly closed tubes, were vortexed vigorously for 5 min until a homogeneous suspension has formed. This was repeated twice. Organic and water layers were separated by centrifugation at 4000 rpm for 10 min at 20 °C. Aliquots of 10, 25, and 40 μL, from each phase were carefully removed and counted in a γ counter in triplicate. Each *P* was calculated as an average of $n \geq 18$.

Chemical Hydrolysis of the cycloSal phosphate triesters. Experiments to determine and compare the chemical stability and hydrolysis of the synthesized triesters were conducted in phosphate buffer containing ∼15% of acetonitrile. The presence of acetonitrile was necessary because of lower water solubility of compounds **8**, **14**, and **24**. Phosphotriesters **6**−**8**, **12**−**14**, and **24** were each dissolved in acetonitrile (1.1−2.4 mg/mL) just before the experiment, and aliquots (100 μL) were withdrawn and transferred into vials containing phosphate buffer (550 μL, 50 mM, pH 7.3). Solutions were briefly vortexed, filtered through the membrane filter (ABF, 0.22 μm), and the initial concentration was determined from the UV concentration standard curve for every compound. Vials were tightly covered and the mixtures equilibrated at 37 °C. Samples of the identical volume (40−60 μL, depending on the initial concentration of the triester) were removed periodically from the hydrolyzed mixture. The reaction was terminated by the addition of acetic acid (5 μL), and each sample was analyzed by analytical HPLC. Formation of all hydrolysis products was monitored (peak area %), and the degradation profiles were established. In the second set of experiments, the course of hydrolysis was monitored as often as possible and the chromatograms were analyzed by the integration of the decreasing areas under peaks of the phosphotriesters. Subsequently, the rate constants (*k*) were determined from the slopes of the degradation curves and the half-lives ($t_{1/2}$) calculated from the rate constants. The corresponding ^{125}I-iodolabeled triesters **6b**−**8b**, **12b**−**14b**, and **24b** were evaluated in the same way, within the initial concentration range of 25.9−40.7 kBq/μL.

Proteins, Sera, Buffers, and Cell Culture Reagents. Human recombinant BChE (20 mg/mL, 994 U/mg protein), insulin, human serum, and mouse serum were purchased from Sigma-Aldrich (St. Louis, MO). BChE from human serum (3.5 U/mL) was a generous gift from Dr. Oksana Lockridge (UNMC, Omaha, NE). ATCC-formulated Eagle's minimum essential medium was from the American Type Culture Collection (ATCC; Manassas, VA). Fetal bovine serum (FBS), RPMI-1640 medium, L-glutamine, penicillin−streptomycin, and sodium pyruvate were from GIBCO Invitrogen Cell Culture (Carlsbad, CA). NE-PER nuclear and cytoplasmic extraction reagents were from Thermo Fisher Scientific (Rockford, IL). The Genomic-tips 100/G were from QIAGEN (QIAGEN Inc., Valencia, CA). CellTiter96 AQueous One Solution Cell Proliferation Assay was from Promega Corporation (Madison, WI).

Gel Electrophoresis. All analyses were conducted on precast 4−20% or 10% Mini-PROTEAN TGX polyacrylamide gels (10-well, 30 μL) using the Mini-PROTEAN electrophoresis cell (Bio-Rad, Hercules, CA). Gels were run at a constant voltage (150 or 190 V) for 1 h. When analyses included radioactive compounds, gels were placed on the MidSci classic autoradiography film BX (MidSci, St. Louis, MO) at −80 °C for 1−48 h, depending on the amount of radioactivity applied. Native gels were stained for BChE activity with 1 mM butyrylthiocholine iodide as a substrate using the Karnovsky and Roots[73] staining procedure as described by Lockridge.[74] Analyses of hBChE interactions with the radioactive compounds were also conducted on SDS−PAGE nonreducing gels.

Determination of IC_{50}. Multiwell assays employed to determine IC_{50} were developed in this laboratory and utilized either UV or fluorescence detection. For UV-based assays, 0.05 mL/well of human BChE in 0.1 M potassium phosphate, pH 7.0 (3.5 U/mL), was placed in the desired numbers of wells of a 96-well plate. The investigated drug was diluted with DMSO to produce concentrations from 0 to 1.5 mM, and aliquots of 2 μL/well of these dilutions were added to BChE-containing wells to produce the range of final drug concentrations from 0.0005 to 10.5 μM. Mixtures were incubated at room temperature for 30 min. A reagent consisting of BChE substrate, 1 mM (2-mercaptoethyl)trimethylammonium iodide butyrate, and 0.5 mM 5,5′-dithiobis(2-nitrobenzoic acid) in 0.1 M potassium phosphate, pH 7.0 (prepared fresh for each assay), was added, 0.25 mL per well. Absorption at λ = 405 nm was read at 1 min after the addition of this reagent using the Opsys MRplate reader (Dynex Technologies, Chantilly, VA). The time of incubation was increased for poor BChE inhibitors. IC_{50} values reported in Table 2 were calculated using absorption values read after 1 min. Data were analyzed and IC_{50} values calculated using the four-parameter logistic equation (variable Hill slope) provided with the GraphPad Prism software (GraphPad Software, La Jolla, CA).

Cells. NIH:OVCAR-3 human ovarian epithelial adenocarcinoma cells, LS 174T human colorectal adenocarcinoma cells, and U-87 MG glioblastoma cells were purchased from ATCC. OVCAR-3 cells were grown in RPMI-1640 medium supplemented with 0.01 mg/mL bovine insulin, 2 mM L-glutamine, and FBS to a final concentration of 20%. When sufficient numbers of OVCAR-3 cells were produced, athymic NCr-*nu/nu* female mice were injected intraperitoneally (ip) with OVCAR-3 cells suspended in 0.4 mL of serum-free RPMI-1640 medium, and further propagation of these cells was from the ip xenografts. When the abdominal distention became apparent, mice were killed and the peritoneal cavity was lavaged with 2 mL of sterile PBS to recover nonadherent OVCAR-3 cells. Cells were centrifuged. The supernatant was discarded, and the cell pellet was resuspended in complete growth medium (95%) containing DMSO (5%). Cells were stored in liquid N_2 until ready for use. LS 174T cells were grown in the ATCC-formulated Eagle's minimum essential medium containing 10% FBS. Cells used in these experiments have been maintained by using 0.25% trypsin in the subculture protocol. The subcultivation ratio of 1:3 was routinely applied. The same formulation of growth medium was used for U-87 MG cells with 0.5% trypsin plus 0.53 mM EDTA in

the subculture protocol. All cell culture media were supplemented with penicillin (100 U/mL) and streptomycin (100 μg/mL).

Uptake Kinetics. For each drug tested, cells were plated in four six-well plates at 2×10^5 cells/well in 3 mL of growth medium. After 24 h in culture, radioactive drugs were added to wells at predetermined times. The cells were incubated with drugs up to 360 min. Each point in time was tested in triplicate. Aliquots of medium (0.5 mL) were removed from each well for γ counting to determine the radioactive concentration in each well. At the end of incubation, the radioactive medium was aspirated and disposed. Cells were washed twice with 3 mL of ice-cold PBS. Aliquots of wash PBS (0.5 mL) were also taken for γ counting. Cells were trypsinized with 1.5 mL of trypsin/EDTA, and 1 mL aliquot of the cell suspension was counted in a γ counter to determine the cell-associated radioactivity.

Clonogenic Assays. *Colorectal Adenocarcinoma.* LS174T cells, 2×10^6 cells/flask, were plated in T75 flasks. After ∼18 h in culture, the growth medium was removed from all flasks and replaced with either 15 mL of fresh medium containing **6b-*fast***, 114.5 ± 0.2 kBq/mL, or 15 mL fresh medium containing **6b-*slow***, 114.4 ± 0.4 kBq/mL. Control cells were given 15 mL of fresh nonradioactive medium containing PBS in amounts identical to those added with the radioactive compounds. Triplicate 0.1 mL aliquots of medium were withdrawn from each flask and counted in a γ counter to determine the final concentration of radiolabeled compounds. After 4 h in the incubator at 37 °C, the growth medium was removed from all flasks. Monolayers were washed once with full medium, and 15 mL of fresh medium was added to each flask. Cells were allowed to grow undisturbed for 24 h, at which time cells were trypsinized, counted, and their viability was determined. Cells were replated in T25 flasks at plating densities of 500 and 200 cells/flask. Each cell density was tested in quadruplicate. After 17−21 days, colonies were washed with 5 mL of ice-cold PBS, followed by 5 mL of PBS/methanol (1:1; v/v), and fixed in 5 mL of methanol for 10 min. Methanol was removed, and flasks were left open to dry for a few hours. Crystal violet (5 mL, 0.25% in 1:1 PBS/methanol) was added to each flask to stain cells. After approximately 10 min, the dye was removed and flasks were rinsed first with tap water followed by distilled water and were left to dry. Colonies were counted manually by two observers using the Wheaton colony counter (Wheaton, Millville, NJ, U.S.).

To determine if the reduced exposure time with higher compound concentrations produces similar radiotoxic effects, an alternative procedure with a higher concentration of the radioactive compounds and a shorter exposure time to the radioactive compounds was also employed. LS174T cells (2×10^6) were plated in T75 flasks and allowed to recover and attach for ∼18 h, after which time the medium was removed and replaced with medium containing radioactive compounds **7b-*fast*** and **7b-*slow*** at 185 kBq. Cells in control flasks received nonradioactive medium. Triplicate 0.1 mL aliquots were taken from each flask for γ counting. Cells were returned to the incubator for 4 h. The medium was removed from all flasks, including controls, and the cell monolayer was washed once with fresh nonradioactive medium without FBS. Then 5 mL of 2.5% trypsin−EDTA was added to each flask to dissociate the monolayer. Fresh FBS-containing medium was added to stop the action of trypsin and to form a single cell suspension. Cell numbers and cell viability were determined. All cell suspensions were centrifuged at 800 rpm for 10 min at 4 °C. Fresh FBS-containing medium was added to cell pellets to produce 1×10^6 cells/mL suspension. The 1 mL of each suspension was counted in a γ counter to determine cell-associated radioactivity. Cells were plated in duplicate T25 flasks at densities of 1000 , 500, and 100 cells/flask. The medium was changed approximately once a week. After 3 weeks, colonies were processed as described above.

Glioblastoma. U-87 MG cells were plated in the ATCC-formulated Eagle's minimum essential medium with 10% FBS 24 h before treatment and then treated for 24 h with various concentrations of **6b-*slow*** and **6b-*fast*** ranging from 18.5 to 185 kBq/mL dissolved in PBS and added to growth medium. Cells were harvested, washed and their numbers counted in Cellometer disposable cell counting chambers (Nexcelom Bioscience, Lawrence, MA). Cells were diluted in fresh medium to concentrations suitable for the clonogenic assay. Cells from each concentration of **6b** were plated in three T25 flasks at two cell densities, 100 and 500 cells/flask. Control cells were given medium containing the identical amount of PBS and processed in a manner identical to cells treated with **6b**. Cells were periodically examined, and their growth medium was replaced every 5−7 days. Numerous colonies formed 3 weeks after plating control cells. These colonies were treated and stained as described above. The **6b**-treated cells were monitored for an additional 4 weeks, during which time colonies did not form, not even in flasks plated at 500 cells/flask.

In a separate experiment, U-87 MG cells were treated with **6b-*fast*** and **6b-*slow*** at several concentrations ranging from 18.5 to 129.5 kBq/mL in triplicate. After 24 h, the radioactive medium was removed, cells were washed twice with fresh medium warmed to 37 °C, and fresh medium with 10% FBS was added. Cells were allowed to grow for an additional 144 h. Cells from one set of plates were harvested, counted in a Cellometer, and their radioactive content was determined in a γ counter. Monolayers in the second set of cells were stained with crystal violet, washed as described above, dried, and solubilized in 35 mM sodium dodecyl sulfate/ethanol mixture (1:1, v/v). Triplicate samples were collected from each flask, and absorbance was measured at λ = 560 nm.

The radiotoxicity of **24b** in U-87 MG cells was also measured. To evaluate the radiotoxicity of **24b**, U-87 cells were plated in T75 flasks at ∼1×10^6 cells/flask in the ATCC-formulated Eagle's minimum essential medium with 10% FBS. Cells were maintained in the FBS-containing media for 24 h, at which time the used medium was aspirated. The used medium was replaced with fresh medium containing either **24b-*fast*** or **24b-*slow*** at 3.7 kBq/mL. Cells were allowed to grow in the presence of radioactive drugs for 1 h. Two treatment flasks and one control flask were processed after 1 h of exposure to **24b**. The cells in the remaining flasks were given fresh growth medium and were processed 24 h later. Cells were processed as follows: the radioactive medium was aspirated and saved for radioactivity determinations. Cells washed with PBS were harvested using 0.5% trypsin−EDTA. Harvested cell suspensions were centrifuged at 1000 rpm for 15 min at 4 °C. Supernatants were collected and aliquots counted in a γ counter. Cell pellets were washed with full medium and centrifuged again. The supernatant was collected for γ counting, and cell pellets were resuspended. Two 1-mL aliquots of the cell suspension were counted in a γ counter to determine the total radioactive content of cells. Cell numbers and their viability were determined. Cells from each treatment concentration were replated in triplicate T25 flasks at 500 and 100 cells/flask for the clonogenic assay. Control flasks received medium containing vehicle and were processed alongside the treated cells for clonogenic assay. The remaining cell suspensions were processed for the determination of the subcellular distribution of radioactivity. The cells were collected as a pellet after centrifugation at 1000 rpm (4 °C) for 15 min, resuspended in 1 mL of PBS, and transferred into microcentrifuge tubes. The subcellular fractionation was conducted using NE-PER nuclear and cytoplasmic extraction reagents. The clonogenic assay cells were allowed to grow undisturbed for 4 weeks. The medium was changed once a week. After 28 days, colonies were processed as described above. Colonies were stained with crystal violet as described above and were counted with the aid of ImageJ software (http://rsb.info.nih.gov/ij/). Two independent readers were employed to count the colonies. The stained colonies were also solubilized in a 35 mM sodium dodecyl sulfate/ethanol mixture (1:1, v/v). Triplicate samples were collected from each flask, and absorbance was measured at λ = 560 nM.

Subcellular Fractionation. Cancer cells were plated into six T150 flasks and allowed to attach overnight. The growth medium was removed and replaced with 10 mL of fresh medium containing the tested radioactive drug. Cells were exposed to the drug for 1 h, after which time the radioactive medium was removed and replaced with 12 mL of fresh medium. Aliquots of all radioactive growth media were counted in the γ counter before and after the cell culture. Cells in three flasks were processed immediately. Cells in the remaining three flasks

were cultured in fresh nonradioactive medium for 24 h and then processed. Cell monolayers were rinsed with 5 mL of PBS and trypsinized. Cells were counted, and their viability was determined. Using NE-PER nuclear and cytoplasmic extraction reagents,[75] the cell content was fractionated and counted in a γ counter to determine the drug distribution in various compartments of the cancer cell. The DNA associated radioactivity was also measured using the QIAGEN Genomic-tip 100/G procedures[76] (QIAGEN Inc., Valencia, CA). To determine the effects of the duration of the exposure on the subcellular distribution, cells were grown with radioactive compounds for 40, 96, 120, and 144 h. Cells were plated in T75 flasks and were allowed to attach for 24 h. Radioactive compounds were added to cells with fresh medium. Aliquots of media were taken for γ counting to ensure that concentrations of ***fast*** and ***slow*** diastereomers were comparable. After the prescribed time, cells were washed with nonradioactive medium and either harvested and fractionated as described above or allowed to continue to grow for up to 120 h and then harvested and processed.

■ ASSOCIATED CONTENT

Supporting Information

Preparation and analytical details for **9**, **9a**, **9b**, **10**, **10a**, **10b**, **11**, **11a**, **11b**, **20**, and **21**; HPLC analyses and conditions, stability, and hydrolysis studies; cancer cell interactions with human BChE. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS USED

IUdR, 5-iodo-2′-deoxyuridine; BChE, butyrylcholinesterase; LET, linear energy transfer; %ID/g, percent injected dose per gram; ESI-HR, high resolution electrospray ionization

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