Journal of Medicinal Chemistry

Article

pubs.acs.org/jmc

Oxaphosphinanes: New Therapeutic Perspectives for Glioblastoma

Ludovic Clarion,[†,‡,∥] Carine Jacquard,[†,∥] Odile Sainte-Catherine,[§] Séverine Loiseau,[‡] Damien Filippini,[‡] Marie-Hélène Hirlemann,[‡] Jean-Noël Volle,[‡] David Virieux,[‡] Marc Lecouvey,[§] Jean-Luc Pirat,*[,‡] and Norbert Bakalara*[,†]

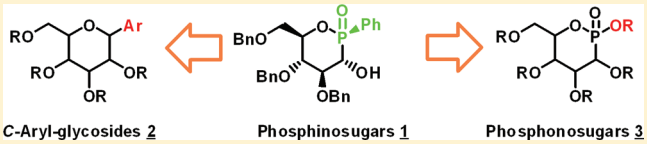
[†]INSERM U-1051, Institut des Neurosciences de Montpellier, 80 rue Augustin Fliche 34091 Montpellier, France

[‡]UMR 5253, ICG Montpellier, Equipe AM2N, ENSCM, 8, rue de l'Ecole Normale, 34296 Montpellier cedex 5, France

[§]UMR 7244 CSPBAT, Equipe CBS Université Paris 13, 74 rue Marcel Cachin, 93017 Bobigny cedex, France

Supporting Information

ABSTRACT: This paper reports the design and the synthesis of a new family of compounds, the phostines, belonging to the [1,2]oxaphosphinane family. Twenty-six compounds have been screened for their antiproliferative activity against a large panel of NCI cancer cell lines. Because of its easy synthesis and low EC_{50} value (500 nM against the C6 rat glioma cell line), compound **3.1a** was selected for further biological study. Moreover, the specific biological effect of **3.1a** on the glioblastoma phylogenetic cluster from the NCI is dependent on its stereochemistry. Within that cluster, **3.1a** has a higher antiproliferative activity than Temozolomide and is more potent than paclitaxel for the SF295 and SNB75 cell lines. In constrast with paclitaxel and vincristine, **3.1a** is devoid of astrocyte toxicity. The original activity spectrum of **3.1a** on the NCI cancer cell line panel allows the development of this family for use in association with existing drugs, opening new therapeutic perspectives.



C-Aryl-glycosides 2 Phosphinosugars 1 Phosphonosugars 3

1. INTRODUCTION

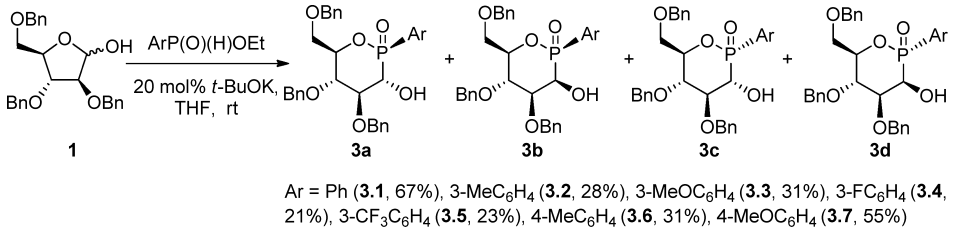
Malignant gliomas are the most frequent primary brain tumors. Glioblastomas (WHO grade IV) account for 60−70% of primary malignant gliomas with poor prognosis. Moreover, low-grade glioma will inescapably become malignant. The best treatment, which consists of surgical resection followed by chemotherapy with Temozolomide (TMZ) and radiotherapy, gives a median survival of 15 months as compared to 55 days with biopsy alone.[1] We therefore realize the need of developing new drugs with antiproliferative activity on glioblastoma cells. So far, molecular agents currently in clinical trial for high-grade glioma have emerged from existing molecules selected for other types of cancer. Mainly, these molecules target intracellular signaling pathways relative to either growth or angiogenesis and have been efficient in preclinical models of gliomas.[2] Nevertheless, systemic antiangiogenic treatment can increase glioblastoma invasion in patients and induce side effects like intracranial hemorrhage and ischemic stroke.[3,4] Moreover, inhibition of tyrosine kinase-coupled growth factor receptors by small chemical compounds (Gefitinib) or monoclonal antibodies (cetuximab) adds little in terms of overall survival or progression free survival.[5,6] Innovative aspects of glioblastoma treatment lie in combinations of molecules such as PCV (procarbazine, lomustine, and vincristine)[7] and in local chemotherapy with biodegradable carmustine wafers (Gliadel).[8] Improvements have been attained at the surgery level by the use of magnetic resonance imaging (MRI)[9] and fluorescent-guided surgery[10] to increase the extent of resection. To develop a new antiglioma therapy, we decided to design and synthesize a new family of compounds screened for their antiproliferative activity, in a first intention on commonly reported glioblastoma cell line such as the C6 one and in a second step against the human National Cancer Institute (NCI) cell line panel.

Phostines are a set of compounds that present an 1,2-oxaphosphinane heterocyclic core and are considered as mimes of glycosides. We thought that the phosphinolactone structure possesses unique and unexploited features: besides purely physical and geometrical considerations, the phosphinolactone group can be regarded as a surrogate of lactol and could present an opportunity for medicinal chemists to explore uncovered regions of chemical space.[11] In addition to its isosteric relationship with the lactol group, phosphinolactone is an outstanding hydrogen bond acceptor and metal-complexing agent, which makes it an attractive structural motif for drug discovery. Whatever the nature of the phosphoryl bond (P═O), such compounds exhibit high Lewis/Bronsted basicity.[12] As observed with glycopyranoside derivatives, phosphorus six-membered rings show also stereoelectronic-dependent interactions with phosphorus atoms that are closely related to the anomeric effect.[13] Another important feature of oxaphosphinanes is that such phosphorus heterocycles are considerably more resistant to the ring-opening/closure occurring between the open-chain and the cyclic pyranose forms.[14,15]

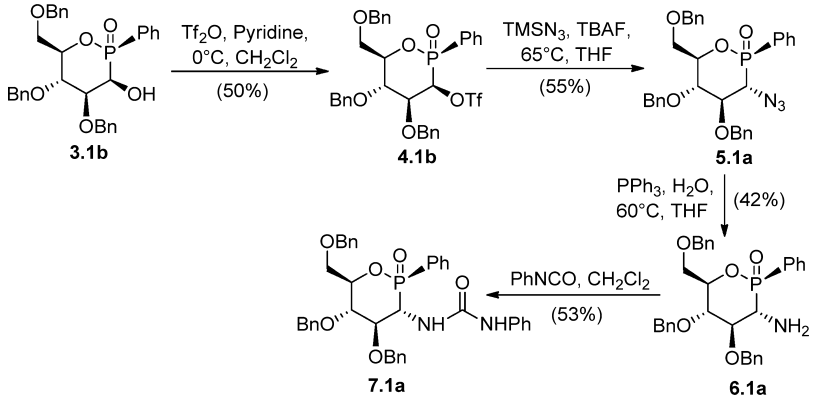
Similar modifications of lactol groups have already been exploited in *C*-glycosides and aza- or thio-sugars, leading to compounds of therapeutic interests.[16] However, these compounds are different from the phostone[17−19] family because of the exocyclic P−C bond, which confers to the molecules a higher stability. In this paper, we describe the synthesis[20] of a

Received: November 2, 2011
Published: January 23, 2012

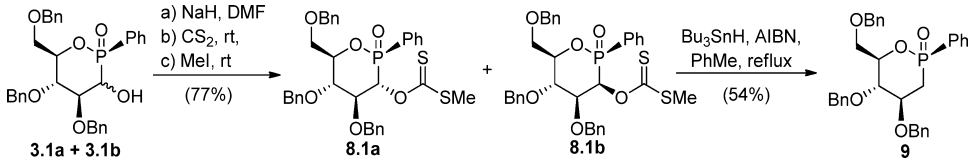
Scheme 1. Synthetic Route to hydroxy-oxaphosphinanes 3.1-3.7



Scheme 2. Synthetic Route to the α-D-Amino-glucose Analogues 6a and 7a



Scheme 3. Synthesis of the Deoxyphosphinosugar 9



new family of compounds, the phostines, obtained from the reaction of *H*-phosphinates with protected furanosyl glycosides in the presence of a base. The antiproliferative properties of the phostines were screened on C6 glial cells.

The toxicity of the best compound, **3.1a**, was compared with its diastereoisomer **3.1b** on cancer cell lines from different tissues and species, C6 glial cells, and nonproliferative brain cells. This study shows that **3.1a** is an antiglioma compound with low glial cell toxicity and leads us to think that it can be a therapeutic compound in preclinical glioma models.

2. RESULTS

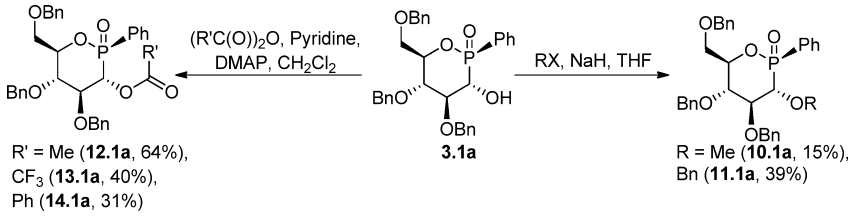
2.1. Oxaphosphinane Synthesis. Phostines were specifically designed as a combination of glycopyranosides and *C*-aryglycosides, and their synthesis was achieved by the reaction of tris(*O*-benzyl protected)-D-arabinofuranose **1** with various ethyl arylphosphinates **2**[21] in the presence of catalytic amount of potassium *t*-butoxide (Scheme 1).[20,22] This tandem sequence involved first the nucleophilic Pudovik addition of *H*-phosphinate anions **2** to the open-chain form of arabinofuranose. The second step consisted of a subsequent prototropy and a 6-*exotet* cyclization, affording the six-membered ring phosphinolactones (21−67% yield, Scheme 1). This procedure offered an easy tuning of the aryl substituent directly bound to the phosphorus atom. Four enantiopure diastereomers **3.a−d** were formed, arising from the creation of two chiral centers with moderate diastereoselectivities. The reaction appeared general, and oxaphosphinanes **3.1−3.6** were initially tested as a mixture of stereoisomers. When the IC_{50} values were lower than 15 μM, they were separated by preparative reverse phase high-performance liquid chromatography (HPLC) and tested separately.

When the Ar group was a phenyl, the diastereomers **3.1a−d** were obtained in 28/26/19/27 proportion. The four stereoisomers were fully characterized, allowing the attribution of all of the stereogenic centers. The first two diastereomers **3.1a** and **3.1b** are epimer at carbon 2, and they correspond, respectively, to phosphino-α-D-glucose and phosphino-α-D-mannose.[23] The two other, **3.1c** and **3.1d**, can be perceived as the β-anomers, but they were revealed to be less configurationally stable at the phosphorus center due to the presence of the phenyl ring in axial position. So, only the **3.1a** and **3.1b** isomers were finally tested.

In a second set of reactions, different chemical modifications were introduced on the carbon adjacent to the phosphorus atom. Then, amino and urea derivatives **6a** and **7a** were synthesized starting from the mannose-like stereoisomer, **3.1b**. The transformation of the alcohol function into a good leaving group led to the mesylate **4.1b** in 50% yield. Substitution by trimethylsilylazide gave the azido derivative **5.1a** with inversion of configuration. As expected, the Staudinger reaction furnished the glucoselike oxaphosphinane **6.1a**. Finally, when **6.1a** was reacted with phenylisocyanate, the corresponding urea **7.1a** was isolated in 53% yield. Starting from the glucoselike stereoisomer **3.1a**, a similar scheme allowed the formation of the

dx.doi.org/10.1021/jm201428a | J. Med. Chem. 2012, 55, 2196−2211

Scheme 4. Synthesis of the α-D-Glucose Analogues



Scheme 5. Synthetic Routes of the α-D-Glucose Analogues

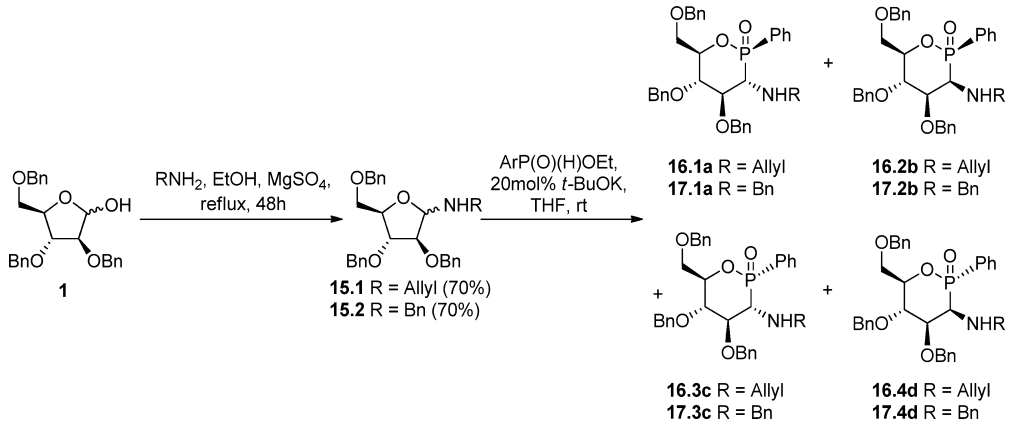
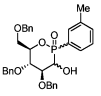
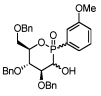
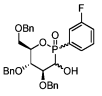
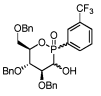
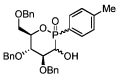
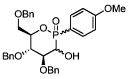
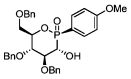
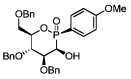
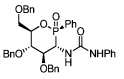
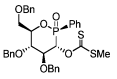
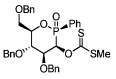
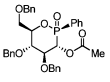
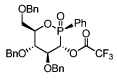
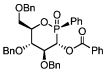
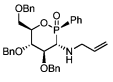


Table 1. Antiproliferative Values of the Phostines on C6[a]

Compounds	3.1a-d	3.1a	3.1b	3.2a-d	3.3a-d	3.4a-d	3.5a-d
							
EC50μM	12±1	0.52±0.03	23.81±0.49	>25	>25	>25	>25

Compounds	3.6a-d	3.7a-d	3.7a	3.7b	5.1a	5.1b	6.1a
							
EC50μM	>25	15±2	8.4±1.8	2.9±1.9	16.7±0.1	>25	16.3±0.1

Compounds	6.1b	7.1a	8.1a	8.1b	9	10.1a	11.1a
							
EC50μM	18.7±0.2	4.4±1	14±2	>25	8.3±0.1	16.6±0.5	5.3±0.2

Compounds	12.1a	13.1a	14.1a	16.1a	17.1a
					
EC50μM	11.9±0.7	>25	>25	0.49±0.01	2.68±0.07

[a]Values were calculated from at least three independent experiments, and for each set of experiments, each point was repeated six times. EC_{50} estimation was determined with Prism 4.0 software using the Hill equation.

dx.doi.org/10.1021/jm201428a | J. Med. Chem. 2012, 55, 2196–2211

Table 2. Antiproliferative Activities of 3.1a and 3.1b on Different Cell Lines[a]

tissues		brain	skin			liver	intestine	prostate	breast
cell types		glioma	melanoma		epidermoid carcinoma	hepato-carcinoma	colorectal adeno-carcinoma	prostatic carcinoma (brain metastasis)	adeno-carcinoma (pleural metastasis)
cell lines		C6	MDAMB-435	B16F10	A431	HuH7	CaCo2	DU145	MDAMB-231
species		rat	human	mouse	human	human	human	human	human
3.1a	LTC[b] (μM)	0.001	0.001	0.5	0.1	0.1	0.5	5	0.1
	10 μM toxicity (%)[c]	82 ± 1	42 ± 0	73 ± 0	32 ± 1	22 ± 1	43 ± 1	40 ± 0	30 ± 1
	1 μM toxicity (%)	63 ± 1	22 ± 1	25 ± 0	18 ± 1	16 ± 1	10 ± 1	Nt[d]	17 ± 1
	EC_{50} (μM)[e]	**0.5 ± 0.1**[f]	13.9 ± 0.5	**4.9 ± 0.3**	14.5 ± 0.5	30 ± 3	57 ± 8	65 ± 8	Nqe[g]
3.1b	LTC (μM)	0.1	0.1	25	5	0.1	5	1	5
	10 μM toxicity (%)	31 ± 1	43 ± 0	Nt	33 ± 1	55 ± 1	15 ± 1	14 ± 1	18 ± 1

[a]Values were calculated from at least three independent experiments, and for each set of experiments, each point was repeated six times. Data are results of MTT assay, 48 h after product addition. [b]LTC is calculated after mean comparison between vehicle and product and ANOVA posthoc Fisher test ($p < 0.0001$). [c]Toxicity (10 μM) was calculated as the percentage of MTT absorbance loss as compared to the vehicle. [d]Nt, no toxicity. [e]EC_{50} estimation was determined with Prism 4.0 software using Hill equation with four parameters and with the bottom fixed with Triton X-100 2% and the top fixed with vehicle. [f]EC_{50} values under 10 μM are in bold. [g]Nqe, no quantified estimation.

phosphino-α-D-mannose analogues **4.1a**, **5.1b**, and **6.1b** in, respectively, 46, 86, and 54% yields (Scheme 2).

To estimate whether or not the presence of the free hydroxy group affects the IC_{50}, the corresponding deoxyphosphinolactone **9** was also synthesized. This oxaphosphinane **9** was directly accessible from the mixture of diastereomers **3.1a** and **3.1b** by the Barton−McCombie deoxygenation. The dithiocarbonate intermediates **8.1a** and **8.1b** were separated to further test them (Scheme 3).

Other modifications on the hydroxy group were introduced by the formation of the corresponding ester and ether derivatives. Starting from the diastereoisomer **3.1a**, the desired targets were prepared by Williamson or by acylation reactions (Scheme 4).

Direct synthesis of amino-oxaphosphinanes can be performed starting from the alkylamino-arabinofuranose. Thus, **15** and **15′** were obtained quantitatively by treatment of 2,3,5-tri-*O*-benzyl-D-arabinofuranose with an excess of benzylamine or allylamine.[24,25]

Then, the phostines **16** and **17** were synthesized according to the conditions of reaction mentioned above. Only one enantiopure stereoisomer **16.1a** and **17.1a** (among the four diastereomers) was isolated after purification in 12 and 7% yield, respectively (Scheme 5).

2.2. Antiproliferative and Stereoselective Activity of 3.1a on Brain Cancer Cell Lines. Phostines were screened on the C6 rat glioblastoma cell line using a dimethylthiazolyl-2,5-diphenyltetrazolium bromide (MTT) test. Compounds **3.1a** and **16.1a** proved to be the most potent inhibitors of C6 cell growth (Table 1). However, the following studies were carried out with **3.1a**, which is readily synthesized in yields that are better than for **16.1a**. Moreover, a promising diastereoselective synthesis to increase the yield of **3.1a** even further is already under investigation.

In all cancer cell lines derived from other tissue than brain (i.e., skin, liver, intestine, prostate, and breast) **3.1a** at 1 and 10 μM is always less toxic and has a higher lower toxic concentration (LTC) and half maximal effective concentration (EC_{50}) values (Table 2), suggesting specificity for cancers that form from brain tissue. That hypothesis is reinforced by the low sensitivity of DU-145 (65 ± 8 μM) to **3.1a**. As a matter of fact, DU-145 is a metastatic brain tumor that started from a primary prostate cancer (Figure 1 and Table 2). Furthermore, because

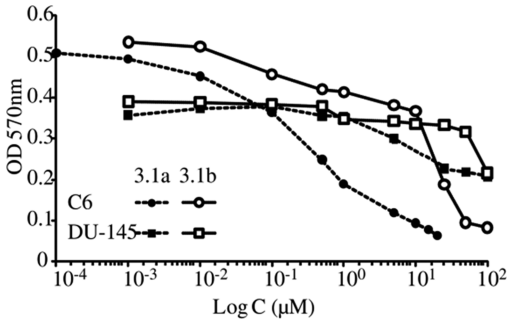


Figure 1. Antiproliferative activities of **3.1a** and **3.1b**. Antiproliferative activities were measured by MTT reduction, 48 h after drug addition (**3.1a** and **3.1b**) on different cell lines on rat C6 glioblastoma cells (C6) and brain metastasis of prostatic cells (DU-145). OD 570 nm, optical density measured at 570 nm; C, concentration.

3.1a always has a higher antiproliferative effect than **3.1b** at 10 μM and lower LTC and EC_{50} values (Figure 1 and Table 2) on skin cancer cell lines (MDA-MB-435, B16F10, and A431), colorectal cancer cells (CaCo2) and breast cancer cells (MDA-MB-231), a stereodependent mode of action of **3.1a** is suggested. Conversely, **3.1b** has greater antiproliferative activity on the hepatocarcinoma cell line (Huh7, Table 2).

In a next step, we investigated whether or not the brain tumor specificity and stereospecificity of **3.1a** hold also for other human primary cell lines (ie. Leukemia, lung, ovarian, etc.). For that purpose, a screen test on the NCI human cancer cell lines has been performed.

2.3. Higher Antiproliferative Activity of 3.1a Than 3.1b against a Large Panel of Human Cancer Cell Lines and Brain Specificity. The NCI quantified the effects of **3.1a** and **3.1b** at 10 μM on the growth of 57 cell lines (Figure 2A,B) using the sulforhodamine test. The results obtained by this test

dx.doi.org/10.1021/jm201428a | J. Med. Chem. 2012, 55, 2196−2211

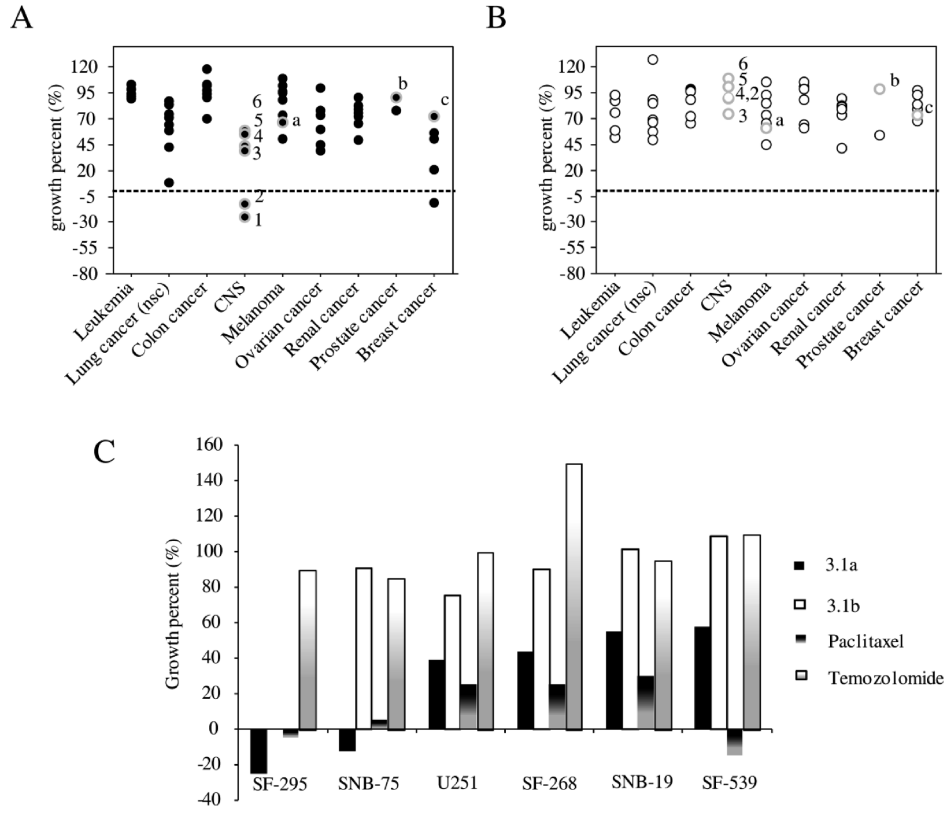


Figure 2. Oxaphosphinanes effect on NCI60 GP and effects comparison with paclitaxel on the CNS cell lines. (A and B) Growth percentage of NCI-60 cell lines after 48 h of incubation of 10 μM **3.1a** (A) or 10 μM **3.1b** (B) as quantified with sulforhodamine B assay. Gray circles represent NCI 60 CNS cell lines and cell lines used for the MTT test in the primary screening (Table 1). (C) Oxaphosphinanes, TMZ, and paclitaxel effects on brain cell lines growth percentage. Comparison of CNS cell lines growth percentage with 10 μM **3.1a**, **3.1b**, TMZ, and paclitaxel. Black, **3.1a**; white, **3.1b**; black and gray, paclitaxel; and white and gray, TMZ. Nsc, nonsmall cell; CNS, central nervous system; 1, SF-295; 2, SNB-75; 3, U-251; 4, SF-268; 5, SNB-19; 6, SF-539; a, MDA-MB-435; b, DU-145; and c, MDA-MB-231.

on the MDA-MB-435, MDA-MB-231, and DU-145 cell lines show similar levels of antiproliferative activity as those initially observed with the MTT test (Table 2). This confirms on the one hand that **3.1a** has a greater antiproliferative activity than **3.1b** (Figure 2A–C) and on the other hand its brain selectivity. As a matter of fact, the median growth percent (GP) value of **3.1a** is inferior to 50% only for the central nervous system (CNS) cell line cluster (Figure 2A). Negative GP values, indicating a cytotoxic effect, were found for two CNS cell lines, SF-295 and SNB75 (Figure 2A). Interestingly, Hs-578T is the only breast cancer cell line highly sensitive to **3.1a** (Figure 2A). Other cell lines are moderately sensitive to **3.1a** (i.e., $0 < GP < 50$). They include CNS cell lines (SF-268 and U251), nonsmall lung cancer cells (HOP-62 and NCI-H522), ovarian cell line (SK-OV-3), and one breast cancer cell line (MDA-MB-468). The GP mean of 81% $\pm$ 18 of **3.1b** indicates that it has a generally weak antiproliferative activity. Because the median GP values in different tissues are similar, we concluded that it is not tissue specific. Nevertheless, two cell lines are moderately sensitive to **3.1b** with GP values comprised between 45 and 50%: a melanoma cell line (LOX-IMVI) and a renal cell line (UO-31).

Next, the sensitivities of six CNS cell lines to paclitaxel, TMZ, and **3.1a** were compared. The antiproliferative activity of **3.1a** used at 10 μM is higher than 10 μM paclitaxel on the two cell lines, SF-295 and SNB75, and higher to 10 μM TMZ on the six cell lines (Figure 2C). These last data prompted us to compare the antiproliferative activities of **3.1a** and **3.1b** with reference drugs used in the treatment of malignant glioma.

2.4. Comparison of the Antiproliferative Activities of 3.1a and 3.1b and Reference Drugs Used in the Treatment of Malignant Glioma on the C6 Glioma Model. To evaluate the position of the most active compound of the new phostine family, the antiproliferative activity of **3.1a** was compared to **3.1b** (Figure 3A) and to some therapeutic agents currently prescribed or in clinical trial for glioblastoma[7,26,27] (Figure 3B). By measuring neutral red accumulation, we confirmed the data obtained with MTT, which indicates that **3.1a** is more active on the C6 cell line than its diastereoisomer **3.1b**. We can see that the antiproliferative activity of **3.1a** is lower than the antimitotic drugs paclitaxel and vincristine used at 0.1, 1, or 10 μM concentration. By adding 100 μM TMZ, a slight antiproliferative activity on C6 cells appeared, but it still remained lower than for 10 μM **3.1a** (Figure 3B). In conclusion, although **3.1a** is less active than the alkylating agent carmustine, it shows a higher activity than TMZ, the alkylat agent used in first line for primary and recurrent glioblastoma.

To evaluate if the best antiproliferative phostine, **3.1a**, is less toxic for normal than clinical drugs and if the antiproliferative activity occurs at lower concentrations than for toxicity, we next measured its effect on primary culture of nonproliferative brain cells.

2.5. Comparison of Toxicity between 3.1a and Clinical Drugs on Nonproliferative Astrocytes in Primary

dx.doi.org/10.1021/jm201428a | J. Med. Chem. 2012, 55, 2196–2211

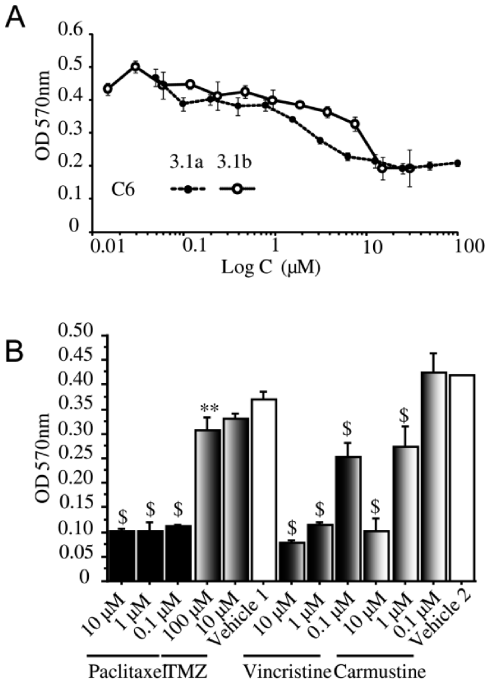


Figure 3. Comparison of the antiproliferative activities of phostines with clinical drugs on C6 cells. Neutral red incorporation in C6 cells was measured 48 h after product addition. The percentage of toxicity was calculated in comparison to vehicle. nt, no toxicity. Results in bold are above 50% of toxicity. OD, optical density.

Culture. Twenty-one day primary cell cultures of astrocytes were not proliferative because they were not sensitive to cytabarine (AraC) as measured by neutral red test (Figure 4A), Hoechst nuclei staining (Figure 4B), and propidium iodide incorporation (Figure 4C) and expressed markers of differentiated cells like glial fibrillary acidic protein (GFAP) (data not shown). At all concentrations, **3.1a** has no toxic effect on nonproliferative astrocytes, the major brain cell population. In contrast, vincristine used at 1 and 10 μM induced a significant decrease of viability (60% in Figure 4A), which was associated with its cytotoxicity as shown by the incorporation of propidium iodide (PI) in the majority of the remaining cells (Figure 4C). However, at 0.1 μM, vincristine induced cytotoxicity without a decrease of cell number (Figure 4A–C). Paclitaxel used at 10 μM induced a weak decrease of viability of 28% (Figure 4A) and a dose-dependent cytotoxicity (Figure 4C) without affecting cell number (Figure 4B). Like **3.1a**, TMZ and carmustine used at 0.1 and 100 μM neither induced cytotoxicity nor decreased viability (Figure 4A–C).

Taken together, these data indicate that **3.1a** has a better antiproliferative activity on glioma than TMZ and a lower toxicity than paclitaxel and a comparable one to carmustine on 21 day astrocytes. With no toxicity at 100 μM (Figure 4) and an antiproliferative activity starting at 1 nM (Table 1), **3.1a** shows a good in vitro "therapeutic index".

2.6. Cytotoxic Effect of 3.1a and 3.1b on C6 Cells. To evaluate if the antiproliferative effects of the two diastereoisomers could be due to their cytotoxicity, we measured the release of lactate dehydrogenase (LDH) in the cell medium. This test shows that **3.1a** and **3.1b** are toxic for the C6 glioblastoma cell line and that at 10 μM **3.1a** is more toxic than **3.1b** (Figure 5). It has to be noticed that the LTC values measured with the MTT test were 0.001 and 0.1 μM for **3.1a** and **3.1b**, respectively. These values are much lower than the 10 and 25 μM measured using the LDH release test. Although the LDH test is known as an insensitive assay, these differences indicate that the MTT test measured inhibition of proliferation rather than cell lysis.

2.7. Time–Course Effects of 3.1a on C6 Cells Viability. To estimate the effect of **3.1a** on cell density in time, we measured impedance (cell index value) (Figure 6). Compound **3.1a** cytotoxicity is confirmed at 25, 50, and 100 μM because the cell index value measured at these concentrations was lower than at t = 0. With 100 μM, the cell index value decreased as soon as 1 h after drug addition. At 50 and 25 μM, a decrease of the cell index value was only detected 2 and 3 h after **3.1a** addition. With 10 μM, the cell index value remained constant throughout the experiment and below vehicle. This result suggests that at 10 μM, **3.1a** is cytostatic. The cell index value still decreases with 5 μM **3.1a**, while no decrease is observable with 1 μM.

3. DISCUSSION AND CONCLUSION

From a chemical point of view, a two-step sequence starting from aryl-*H*-phosphinates and substituted arabinofuranoses afforded the synthesis of structurally diverse phostines. The purification of the active compounds was achieved either by a direct selective precipitation of phostines from the crude mixture or by reverse-phase preparative HPLC. This methodology allowed an easy scale-up and offered the opportunities to introduce stereochemical modifications leading to different phostines. Qualification and/or quantification of the biological effects perceived on the modified 2-oxo-2λ^5-[1,2]-oxaphosphinanes allowed the selection of five active phosphino-heterocycles (**3.1a**, **3.7b**, **11.1a**, **16.1a**, and **17.1a**) with IC_{50} values lower than 5 μM. Interestingly, the activity profile was affected both by the nature of the substituent and by their relative stereochemistry. The combination of these two features has motivated the synthesis of the small focused library presented in this paper. Refinement of our lead compounds into a drug will require the extension of the chemical space. Then, our work will be focused on the determination of a quantitative structure–activity relationship to choose the best drug candidate.

This study has evidenced that phostine drugs exhibit stereospecific antiproliferative properties with respect to CNS cell lines and, very importantly, that these drugs are devoid of toxicity on normal astrocytes. Such an unusual brain tumor specificity has been previously described for 2-methyl-9-methoxyellipticinium.[28] Also, when compared to paclitaxel, **3.1a** has a different action on the CNS cell lines (Figure 3), indicating different and potentially complementary modes of action of these compounds.

In addition to CNS cell lines, **3.1a** inhibits the growth of the breast cancer HS578T and the nonsmall cell cancer lung HOP-62 cell lines. Phylogenetic trees of the 57 NCI cell lines indicate that these two cell lines cluster with the CNS mesenchymal cell line cluster.[29,30] Because microRNA expression has been shown to be related to the chemosensitivity of tumor cells,[31] we looked into the microRNA expression of the NCI cancer cell lines. By this analysis, HS578T also clusters with the CNS cell lines cluster.[32] Moreover, the HOP-62 also clusters with the CNS cell lines (in dendrograms based on drug sensitivity).[29] Although HS578T did not group with the CNS cluster on a dendrogram-based cell line drug sensitivities,[29] it appeared to be sensitive to **3.1a**. Therefore, we conclude that the drug

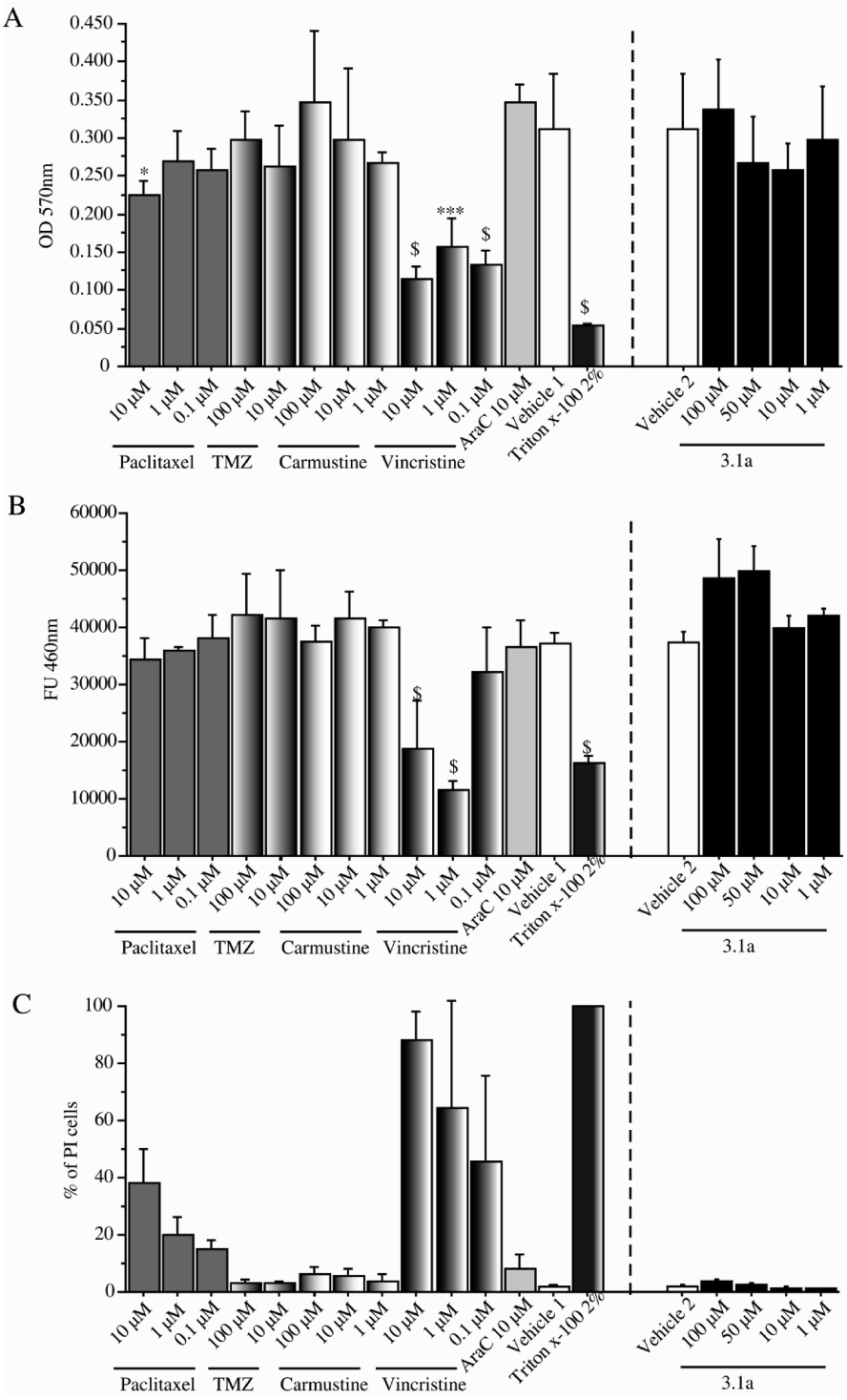


Figure 4. Comparison of the toxicity of OXP430.1 with clinical drugs on nonproliferative astrocytes. Astrocyte viability was quantified 48 h after drug addition on nonproliferative cells maintained in cell culture during 3 weeks after isolation from mouse pups cortical brain. (A and B) Graphics with means ± standard deviations (statview) show neutral red incorporation level (A) and Hoechst 33342 (5 μg/mL) cytofluor intensity measurement. The mean fluorescence was then calculated from three different wells per condition. (C) Dual labeling for PI and Hoechst was performed using the Cellomics array scan vti (Thermofisher). The object detection was fixed on the Hoechst 33342 channel, and the graph shows the percentage of Hoechst 33342 cells stained with PI. The control fluorescence intensities were measured on Triton X-100 (2%)-treated cells. The mean percentage of PI incorporation was calculated from three different wells per condition. OD, optical density; and FU, fluorescent unit. *, significant activity ($p < 0.05$); ***, significant activity ($p < 0.001$); and $, significant activity ($p < 0.0001$).

sensitivity of the CNS cell lines, HOP-62 and HS-578T, can be attributed to a common biochemical mechanism depending on a set of genes commonly expressed by these cell lines. Running the COMPARE program from the NCI evidenced no other clinically used standard agents with a similar profile as **3.1a**. Taken together, these data suggest that **3.1a** inhibits a biochemical process that has not been targeted by standard anticancer agents. Otherwise, the selective cytotoxicity to this

dx.doi.org/10.1021/jm201428a | J. Med. Chem. 2012, 55, 2196–2211

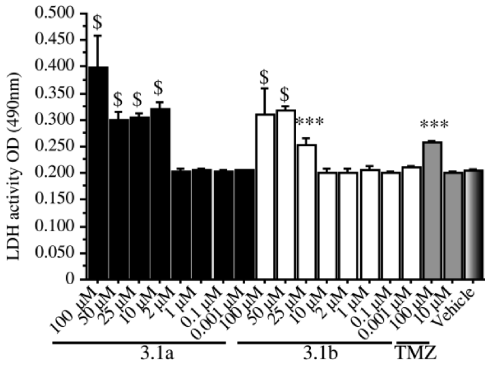


Figure 5. Cytotoxic activity of **3.1a** and **3.1b** on C6 cells. The graph shows the mean ± standard deviation (statview) optical density measured at 490 nm 48 h after product addition on C6 glial cells. $, significant activity ($p < 0.0001$); and ***, significant activity ($p < 0.001$).

cell line cluster might be the consequence of a preferential uptake in the sensitive lines. Thus, in such a way, stereoselectivity will play a central role in a metabolism where enzymes are involved.

Finally, it is important to note that **3.1a** is toxic for different cell types identified as drug-resistant. Compound **3.1a** kills one multidrug-resistant CNS cell line (SF-295) and reduces the cell growth of two other MDR cell types: the nonsmall cell lung tumor cell line (HOP-62) and to a lesser extent the renal tumor cell line (RXF-393).[33] Moreover, a greater sensitivity to carmustine was reported for SF-268 than for SF-295,[34] and we observe a greater toxicity of **3.1a** on SF-295 than on SF-268. This last result suggests that **3.1a** could be used on strains developing resistance against alkylating agents.

When taken into account, the results from the different viability tests on C6 glioblastoma cells, the effects of **3.1a** are dual. At concentrations higher than or equal to 10 μM, **3.1a** induces necrosis as revealed by LDH release. At concentrations below 5 μM, we observed stable cell index values, a decrease of MTT reduction, a decrease of neutral red accumulation, and no LDH release. These data suggest that at least two different effects are mediated by the drugs. At high concentrations, **3.1a** is toxic, whereas at medium concentrations (between 1 and 10 μM), a cytostatic effect is observed.

In conclusion, this work characterized a new family of compounds targeting CNS cancer without affecting normal astrocytes. The easy tuning of the substituent groups at the phosphorus and carbon 2 atoms of phostines will allow the development of this family for therapeutic use.

4. EXPERIMENTAL SECTION

4.1. Chemistry. Commercial reagents were purified by distillation or sublimation, and 2,3,5-tri-*O*-benzyl-D-arabinofuranose was purchased from Carbosynth and dried under vacuum before use. The analytical method used to determine the purity of all of the compounds described in this paper has been determined by phosphorus and proton NMR confirming a purity ≥95%. Analytical and preparative HPLC show a purity ≥99% for the compounds **3.1a** and **3.1b** for which the biological activities have been described.

All reactions were carried out under dry nitrogen in flame-dried glassware. Solvents were dried according to current method, distilled, and stored under nitrogen atmosphere.

Nuclear magnetic resonance (^{1}H, ^{31}P, ^{13}C, and ^{19}F NMR) spectra were recorded at 400.13 MHz for proton, 161.97 MHz for phosphorus, 100.6 MHz for carbon, and 376.50 for fluorine (Bruker DRX 400). Chemical shifts are reported in ppm relative to the solvent signals [^{1}H: chloroform-d_1 (7.26 ppm), acetone-d_6 (2.05 ppm), dimethylsulfoxide (DMSO)-d_6 (2.50 ppm), D_2O (4.79 ppm), or methanol-d_4 (3.31 ppm); ^{31}P: H_3PO_4 85%; ^{13}C: chloroform-d_1 (77.16 ppm), acetone-d_6 (29.84 ppm), DMSO-d_6 (39.52 ppm), or methanol-d_4 (49.00 ppm); ^{19}F: reported from $CFCl_3$]. NMR coupling constants are noted *J* and reported in Hertz (Hz). These abbreviations are used for express the multiplicity: s (singulet), d (doublet), t (triplet), q (quadruplet), qu (quintuplet), m (multiplet), and b (broad).

Low- and high-resolution mass spectra were acquired using a WATERS Micromass Q-Tof spectrometer with as internal reference H_3PO_4 (0.1% in water/acetonitrile, 1/1) in positive mode (ESI). Flash chromatography was performed using a CombiFlash Companion/TS with prepacked column (6−120 g scale) with particle size of 35−70 μm. Analytical HPLC (column, Waters SunFire C18, 5 μm, 4.6 mm × 250 mm; eluent, acetonitrile/water (63:34); flow, 1 mL min^{-1}). Preparative HPLC (Waters SunFire Prep C18 OBD; column, Waters SunFire C18, 50 mm × 250 mm; flow, 17 mL min^{-1}).

4.2. General Procedure for Oxaphosphinane Synthesis: Compounds 3. Ethyl arylphosphinate was prepared using the method described in the literature by our group.[21] Under dry nitrogen, 2,3,5-tri-*O*-benzyl-D-arabinofuranose (1 equiv) was added under nitrogen to a solution of ethyl arylphosphinate (1 equiv) in THF (1.4 mmol/mL), and then, freshly sublimated potassium *tert*-butoxide (0.2 equiv) was added to the solution. The reaction mixture was stirred at room temperature for 15 h. After evaporation of the solvent under vacuum, chloroform (3 mmol/mL) was added to the crude oil. The organic solution was washed with a saturated aqueous solution of ammonium chloride (3 × 1.5 mmol/mL). The organic

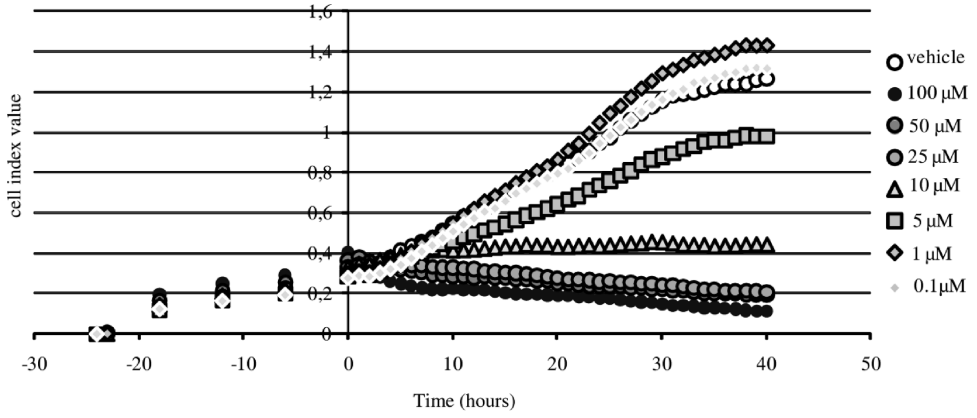


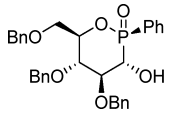
Figure 6. Time−course effect of **3.1a** on C6 cell growth. Compound **3.1a** was used at concentrations ranging between 100 nM and 100 μM, and impedance was measured 25 h after plating ($t = 0$) for 44 h. The electrode impedance is displayed as a cell index (CI) value.

dx.doi.org/10.1021/jm201428a | J. Med. Chem. 2012, 55, 2196−2211

layer was dried over sodium sulfate and filtered, and the solvent was evaporated under vacuum.

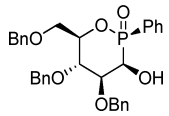
*4.2.1. 3-Hydroxy-4,5-bis-benzyloxy-6-benzyloxymethyl-2-phenyl-2-oxo-2λ⁵-[1,2]oxaphosphinane (**3.1**).* From ethyl phenylphosphinate (8.5 g, 50 mmol), a yellow oil residue containing a mixture of four diastereomers was obtained (25.7 g, 28/26/19/27). The solid was purified by normal phase chromatography on silica gel with a mixture of chloroform/ethyl acetate (60/40) as the eluent to give a mixture of four diastereomers **3.1a–d** (18.3 g, 67%). After chromatography on preparative reverse-phase HPLC with a Waters SunFire column C18, 50 mm × 250 mm, and with a mixture of acetonitrile/water (60:40, flow of 17 mL min^{-1}) as the eluent, **3.1a**, **3.1b**, **3.1c**, and **3.1d** were all separated and recovered purely.

*4.2.2. (S_PSSSR)-3-Hydroxy-4,5-bis-benzyloxy-6-benzyloxymethyl-2-phenyl-2-oxo-2λ⁵-[1,2]oxaphosphinane (**3.1a**).*



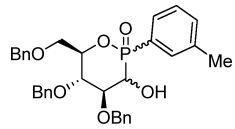
^{31}P NMR (161.97 MHz, $CDCl_3$): δ = 36.1 ppm. ^1H NMR (400.13 MHz, $CDCl_3$): δ = 3.69 (dd, 1H, J = 11.1, 2.3 Hz), 3.88 (ddd, 1H, J = 11.1, 2.7, 2.5 Hz), 3.90 (dd, 1H, J = 9.5, 9.3 Hz), 3.93 (dd, 1H, ^3J = 9.8, 2.0 Hz), 4.10 (ddd, 1H, J = 9.8, 9.5, 2.6 Hz), 4.46 (dddd, 1H, J = 9.3, 3.1, 2.7, 2.3 Hz), 4.46 (d, 1H, J = 12.1 Hz), 4.54 (d, 1H, J = 12.1 Hz), 4.59 (d, 1H, J = 10.8 Hz), 4.83 (d, 1H, J = 11.1 Hz), 4.84 (d, 1H, J = 10.8 Hz), 4.88 (d, 1H, J = 11.1 Hz), 7.38–7.43 (m, 15H), 7.50–7.55 (m, 3H), 7.75–7.80 (m, 2H) ppm. ^{13}C NMR (100.6 MHz, $CDCl_3$): δ = 68.64 (d, J = 9.2 Hz), 72.19 (d, J = 97.3 Hz), 73.45, 75.24 (d, J = 5.2 Hz), 75.50, 76.25, 77.65, 84.34 (d, J = 6.9 Hz), 127.65, 127.76, 127.82, 127.84, 128.15, 128.45, 127.49 (d, J = 135.8 Hz), 128.71 (d, J = 13.4 Hz), 131.91 (d, J = 10.4 Hz), 133.30 (d, J = 2.8 Hz), 137.92, 137.98, 138.37 ppm. HRMS (ESI) m/z calcd for $C_{32}H_{34}O_6P$ $(M + H)^+$, 545.2093; found, 545.2089. Analytical HPLC: column Waters SunFire C18, 5 μm, 4.6 mm × 250 mm, acetonitrile/water (63/37); flow, 1 mL min^{-1}; retention time = 18.53 min.

*4.2.3. (S_PRSSR)-3-Hydroxy-4,5-bis-benzyloxy-6-benzyloxymethyl-2-phenyl-2-oxo-2λ⁵-[1,2]oxaphosphinane (**3.1b**).*



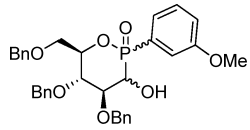
^{31}P NMR (161.97 MHz, $CDCl_3$): δ = 36.6 ppm. ^1H NMR (400.13 MHz, $CDCl_3$): δ = 2.67 (s, 1H), 3.80 (dd, 1H, J = 11.3, 2.0 Hz), 4.00 (ddd, 1H, J = 11.3, 3.3, 3.2 Hz), 4.30 (dd, 1H, J = 9.5, 2.8 Hz), 4.36 (dd, 1H, J = 9.5, 2.8 Hz), 4.43 (dd, 1H, J = 3.5, 2.8 Hz), 4.56 (dddd, 1H, J = 10.0, 3.3, 3.2, 2.0 Hz), 4.59 (d, 1H, J = 12.1 Hz), 4.62–4.80 (m, 4H), 4.93 (d, 1H, J = 10.8 Hz), 7.24–7.38 (m, 15H), 7.48–7.54 (m, 2H), 7.62–7.68 (m, 1H,), 7.96–8.03 (m, 2H) ppm. ^{13}C NMR (161.99 MHz, $CDCl_3$): δ = 66.81 (d, J = 105.1 Hz), 68.91 (d, J = 8.8 Hz), 72.48, 73.43, 73.74 (d, J = 2.2 Hz), 75.46 (d, J = 5.6 Hz), 75.64, 81.38 (d, J = 2.9 Hz), 126.53 (d, J = 138.8 Hz), 127.23, 127.68, 127.80, 127.84, 127.98, 128.20, 128.31 (d, J = 13.4 Hz), 128.42, 128.65, 132.99 (d, J = 9.9 Hz), 133.34 (d, J = 2.8 Hz), 137.32, 138.07, 138.09 ppm. HRMS (ESI) m/z calcd for $C_{32}H_{34}O_6P$ $(M + H)^+$, 545.2093; found, 545.2092. Analytical HPLC: column Waters SunFire C18, 5 μm, 4.6 mm × 250 mm, acetonitrile/water (63/37); flow, 1 mL min^{-1}; retention time = 15.97 min.

*4.2.4. 3-Hydroxy-4,5-bis-benzyloxy-6-benzyloxymethyl-2-[3-methylpheny]l-2-oxo-2λ⁵-[1,2]oxaphosphinane (**3.2a–d**).*



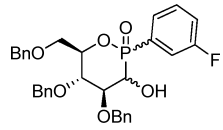
From ethyl 3-methylphenylphosphinate (3.00 g, 16.3 mmol), 2,3,5-tri-*O*-benzyl-D-arabinofuranose (6.85 g, 16.3 mmol), and freshly sublimated potassium *tert*-butoxide (0.37 g, 3.3 mmol), **3.2** was isolated as a yellow oil containing a mixture of four diastereomers (26/29/16/29, 2.54 g, 28%). ^{31}P NMR (161.97 MHz, DMSO-d_6): δ = 37.37, 35.68, 33.41, 32.35 ppm. HRMS (ESI) m/z calcd for $C_{33}H_{36}O_6P$ $(M + H)^+$, 559.2250; found, 559.2243.

*4.2.5. 3-Hydroxy-4,5-bis-benzyloxy-6-benzyloxymethyl-2-[3-methoxypheny]l-2-oxo-2λ⁵-[1,2]oxaphosphinane (**3.3a–d**).*



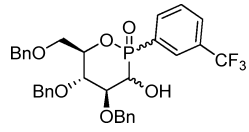
From ethyl 3-methoxyphenylphosphinate (4.0 g, 20.0 mmol), 2,3,5-tri-*O*-benzyl-D-arabinofuranose (8.4 g, 20.0 mmol) and freshly sublimated potassium *tert*-butoxide (0.45 g, 4.0 mmol), **3.3** was isolated as a yellow oil containing a mixture of four diastereomers (23/26/31/20, 3.56 g, 31%). ^{31}P NMR (161.97 MHz, DMSO-d_6): δ 37.8, 36.6, 34.4, 34.2 ppm. HRMS (ESI) m/z calcd for $C_{33}H_{36}O_7P$ $(M + H)^+$, 575.2199; found, 575.2228.

*4.2.6. 3-Hydroxy-4,5-bis-benzyloxy-6-benzyloxymethyl-2-[3-fluoropheny]l-2-oxo-2λ⁵-[1,2]oxaphosphinane (**3.4a–d**).*



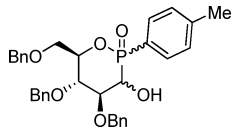
From ethyl 3-fluorophenylphosphinate (0.85 g, 4.5 mmol), 2,3,5-tri-*O*-benzyl-D-arabinofuranose (1.89 g, 4.5 mmol) and freshly sublimated potassium *tert*-butoxide (0.10 g, 0.9 mmol), **3.4** was isolated as a yellow oil containing a mixture of four diastereomers (27/29/18/26, 0.53 g, 21%). ^{31}P NMR (161.97 MHz, DMSO-d_6): δ 34.9 (d, J = 7.9 Hz), 34.1 (d, J = 7.9 Hz), 32.3 (d, J = 1.9 Hz), 29.8 (d, J = 5.9 Hz) ppm. HRMS (ESI) m/z calcd for $C_{32}H_{33}FO_6P$ $(M + H)^+$, 563.1967; found, 563.1964.

*4.2.7. 3-Hydroxy-4,5-bis-benzyloxy-6-benzyloxymethyl-2-[3-trifluoromethylpheny]l-2-oxo-2λ⁵-[1,2]oxaphosphinane (**3.5a–d**).*



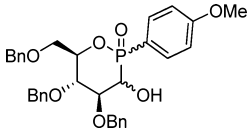
From ethyl 3-trifluoromethylphenylphosphinate (1.07 g, 4.5 mmol), 2,3,5-tri-*O*-benzyl-D-arabinofuranose (1.89 g, 4.5 mmol) and freshly sublimated potassium *tert*-butoxide (0.10 g, 0.9 mmol), **3.5** was isolated as a yellow oil containing a mixture of four diastereomers (26/32/19/23, 0.63 g, 23%). ^{31}P NMR (161.97 MHz, DMSO-d_6): δ 35.0, 34.3, 32.3, 30.1 ppm. HRMS (ESI) m/z calcd for $C_{33}H_{33}F_3O_6P$ $(M + H)^+$, 613.1886; found, 613.3000.

*4.2.8. 3-Hydroxy-4,5-bis-benzyloxy-6-benzyloxymethyl-2-[4-methylpheny]l-2-oxo-2λ⁵-[1,2]oxaphosphinane (**3.6a–d**).*



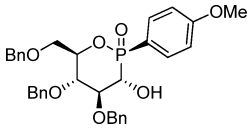
From ethyl 4-methylphenylphosphinate (3.68 g, 20.0 mmol), 2,3,5-tri-*O*-benzyl-D-arabinofuranose (8.40 g, 20.0 mmol) and freshly sublimated potassium *tert*-butoxide (0.45 g, 4.0 mmol), **3.6** was isolated as a yellow oil containing a mixture of four diastereomers (30/25/15/30, 3.83 g, 34%). ^{31}P NMR (161.97 MHz, DMSO-d_6): δ 37.1, 35.7, 33.3, 32.1 ppm. HRMS (ESI) m/z calcd for $C_{33}H_{36}O_6P$ $(M + H)^+$, 559.2250; found, 559.2245.

*4.2.9. 3-Hydroxy-4,5-bis-benzyloxy-6-benzyloxymethyl-2-[4-methoxypheny]l-2-oxo-2λ⁵-[1,2]oxaphosphinane (**3.7a–d**).*



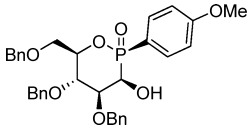
From ethyl 4-methoxylphenylphosphinate (4.42 g, 22.1 mmol), 2,3,5-tri-*O*-benzyl-D-arabinofuranose (9.27 g, 22.1 mmol) and freshly sublimated potassium *tert*-butoxide (0.49 g, 4.4 mmol), **3.7** was isolated as a yellow oil containing a mixture of four diastereomers (27/25/18/30, 6.32 g, 50%). ^{31}P NMR (161.97 MHz, DMSO-d_6): δ 37.3, 37.1, 35.2, 32.5 ppm. HRMS (ESI) m/z calcd for $C_{33}H_{36}O_7P$ $(M + H)^+$, 575.2199; found, 575.2206.

*4.2.10. (S_PSSR)-3-Hydroxy-4,5-bis-benzyloxy-6-benzyloxymethyl-2-[4-methoxypheny]l-2-oxo-2λ⁵-[1,2]oxaphosphinane (**3.7a**).*



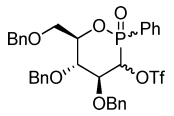
^{31}P NMR (161.97 MHz, $CDCl_3$): δ 37.6 ppm. ^{1}H NMR (400.13 MHz, $CDCl_3$): δ 3.68 (dd, 1H, J = 11.1, 2.1 Hz), 3.77 (s, 3H), 3.85–3.91 (m, 3H), 4.09 (dd, 1H, J = 25.5, 9.1 Hz), 4.44–4.60 (m, 4H), 4.81–4.89 (m, 3H), 6.88–7.72 (m, 19H) ppm. ^{13}C NMR (100.61 MHz, $CDCl_3$): δ 55.43, 68.58, 72.57, 73.47, 75.09, 75.44, 76.15, 77.69, 113.66, 114.27, 114.41, 127.64, 127.75, 127.82, 127.85, 127.89, 128.06, 128.44, 128.46 ppm. HRMS (ESI) m/z calcd for $C_{33}H_{36}O_7P$ $(M + H)^+$, 575.2199; found, 575.2193. Analytical HPLC: column Waters SunFire C18, 5 μm 4.6 mm × 250 mm, acetonitrile/water (60:40); flow, 1 mL min^{-1}; retention time = 23.51 min.

*4.2.11. (S_PRSSR)-3-Hydroxy-4,5-bis-benzyloxy-6-benzyloxymethyl-2-[4-methoxypheny]l-2-oxo-2λ⁵-[1,2]oxaphosphinane (**3.7b**).*



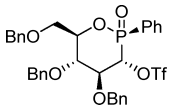
^{31}P NMR (161.97 MHz, $CDCl_3$): δ 36.9 ppm. ^{1}H NMR (400.13 MHz, $CDCl_3$): δ 3.60–3.79 (m, 2H), 3.87 (s, 3H), 3.9 (d, 1H, J = 9.6 Hz), 4.20–4.35 (m, 4H), 4.40–4.90 (m, 6H), 4.83 (d, 1H, J = 10.6 Hz), 6.90 (dd, 2H, J = 8.6, 2.9 Hz), 7.00–7.35 (m, 15H), 7.83 (dd, 2H, J = 11.5, 8.8 Hz) ppm. ^{13}C NMR (100.61 MHz, $CDCl_3$): δ 55.39, 68.92, 69.00, 72.42, 73.42, 73.76, 75.33, 75.61, 76.75, 77.07, 77.39, 81.46, 113.87, 114.01, 127.68, 127.78, 127.99, 128.17, 128.42, 128.64, 135.02, 135.12, 137.40, 138.12, 163.67 ppm. HRMS (ESI) m/z calcd for $C_{33}H_{36}O_7P$ $(M + H)^+$, 575.2199; found, 575.2191. Analytical HPLC: column Waters SunFire C18, 5 μm, 4.6 mm × 250 mm, acetonitrile/water (60:40); flow, 1 mL min^{-1}; retention time = 20.12 min.

*4.2.12. 3-O-Trifluoromethanesulfonyl-4,5-bis-benzyloxy-6-benzyloxymethyl-2-[4-methoxypheny]l-2-oxo-2λ⁵-[1,2]oxaphosphinane (**4a,b**).*



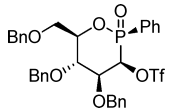
Under dry nitrogen, pyridine (0.63 g, 0.64 mL, 8.0 mmol) and trifluoromethanesulfonic anhydride (1.13 g, 0.66 mL, 4.0 mmol) were added to a solution of a mixture of **3.1a**/**3.1b** (1.09 g, 2.0 mmol, 60/40) in dichloromethane (CH_2Cl_2) (20 mL) at 0 °C. The reaction mixture was stirred for 2 h, and then, water (10 mL) was added. The aqueous layer was extracted with ethyl acetate (3 × 10 mL). The combined organic layers were dried over magnesium sulfate, filtered, and concentrated under vacuum to give yellow oil. The oily residue was purified by normal-phase chromatography on silica gel with a mixture of *n*-heptane/ethyl acetate (60/20) as the eluent, and a mixture of **4.1a**/**4.1b** (60/40) was obtained in 46% yield. ^{31}P NMR (161.97 MHz, DMSO-d_6): δ = 24.29, 27.92 ppm.

*4.2.13. (S_PSSR)-3-O-Trifluoromethanesulfonyl-4,5-bis-benzyloxy-6-benzyloxymethyl-2-[4-methoxypheny]l-2-oxo-2λ⁵-[1,2]-oxaphosphinane (**4.1a**).*



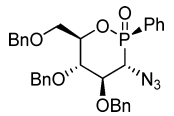
^{31}P NMR (161.97 MHz, $CDCl_3$): δ 28.1 ppm. ^{19}F NMR (376.50 MHz, $CDCl_3$): δ −75.00 ppm. ^{1}H NMR (400.13 MHz, $CDCl_3$): δ 3.68 (dd, 1H, J = 11.2, 2.0 Hz), 3.88 (dt, 1H, J = 11.1, 2.5 Hz), 4.09 (t, 1H, J = 9.7 Hz), 4.36 (m, 1H), 4.40–4.60 (m, 4H), 4.80 (d, 2H, J = 10.6 Hz), 4.900 (d, 1H, J = 10.4 Hz), 4.98 (dd, 1H, J = 10.4, 1.0 Hz), 7.00–7.30 (m, 15H), 7.49 (m, 2H), 7.62 (m, 1H), 7.80 (m, 2H) ppm. ^{13}C NMR (100.61 MHz, $CDCl_3$): δ 67.85, 67.95, 73.51, 75.52, 75.58, 75.64, 78.49, 80.31, 80.35, 80.94, 81.90, 116.38, 119.56, 124.10, 125.54, 127.58, 127.67, 127.71, 127.80, 127.92, 127.93, 128.01, 128.10, 128.37, 128.52, 128.60, 128.62, 128.96, 129.10, 132.37, 132.48, 134.48, 134.51, 137.03, 137.32, 137.55. HRMS (ESI) m/z calcd for $C_{32}H_{33}O_8PF_3S$ $(M + H)^+$, 677.1586; found, 677.1582.

*4.2.14. (S_PRSSR)-3-O-Trifluoromethanesulfonyl-4,5-bis-benzyloxy-6-benzyloxymethyl-2-[4-methoxypheny]l-2-oxo-2λ⁵-[1,2]-oxaphosphinane (**4.1b**).*



^{31}P NMR (161.97 MHz, DMSO-d_6): δ 33.4 ppm.

*4.2.15. (S_PSSR)-3-Azido-4,5-bis-benzyloxy-6-benzyloxymethyl-2-[4-methoxypheny]l-2-oxo-2λ⁵-[1,2]oxaphosphinane (**5.1a**).*

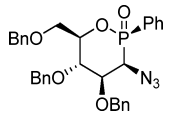


dx.doi.org/10.1021/jm201428a | J. Med. Chem. 2012, 55, 2196–2211

Under dry nitrogen, trimethylsilyl azide (0.173 g, 0.197 mL, 1.5 mmol) and a solution of tetrabutylammonium fluoride (1.5 mmol) in THF (15 mL) were added to a solution of **4.1a**/**4.1b** (0.406 g, 0.6 mmol, 40/60) in THF (20 mL). The reaction was stirred at 65 °C overnight, and then, the solvent was evaporated under vacuum to give a yellow oil. The residue was purified by chromatography on silica gel with as eluent a mixture of *n*-heptane/ethyl acetate (60/40) to give **5.1b** and **5.1a** in an overall yield of 62%.

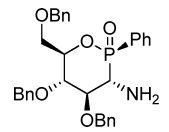
^{31}P NMR (161.97 MHz, $CDCl_3$): δ 31.9 ppm. ^{1}H NMR (400.13 MHz, $CDCl_3$): δ 3.61 (dd, 1H, J = 10.6, 5.8 Hz), 3.70 (dd, 1H, J = 11.1, 2.0 Hz), 3.91 (dt, 1H, J = 11.1, 2.5 Hz), 4.00 (t, 1H, J = 9.7 Hz), 4.21 (m, 1H, J = 10.6, 9.3, 2.5 Hz), 4.40−4.70 (m, 4H), 4.80−4.90 (m, 3H), 7.10−7.35 (m, 15H), 7.42−7.52 (m, 2H), 7.55−7.63 (m, 1H), 7.78−7.86 (m, 1H) ppm. ^{13}C NMR (100.61 MHz, $CDCl_3$): δ 61.65, 62.60, 68.32, 68.41, 73.50, 75.27, 75.33, 75.53, 76.25, 78.41, 81.74, 81.77, 126.26, 127.63, 127.66, 127.67, 127.85, 127.94, 128.05, 128.11, 128.49, 128.52, 128.90, 129.04, 131.92, 132.02, 133.77, 133.80, 137.43, 137.67, 137.78 ppm. HRMS (ESI) m/z calcd for $C_{32}H_{33}N_3O_5P$ $(M + H)^+$, 570.2158; found, 570.2151.

*4.2.16. (S_PRSSR)-3-Azido-4,5-bis-benzyloxy-6-benzyloxymethyl-2-[4-methoxypheny]l-2-oxo-2λ^5-[1,2]oxaphosphinane (**5.1b**).*



^{31}P NMR (161.97 MHz, $CDCl_3$): δ 33.4 ppm. ^{1}H NMR (400.13 MHz, $CDCl_3$): δ 3.77 (dd, 1H, J = 11.2, 1.9 Hz), 3.97 (dt, 1H, J = 11.2, 2.9, Hz), 4.26 (dd, 1H, J = 8.2, 3.2 Hz), 4.30 (d, 1H, J = 9.5 Hz), 4.50 (dd, 1H, J = 9.5, 3.2 Hz), 4.54 (dm, 1H, J = 9.9 Hz), 4.59 (d, 1H, J = 12.4 Hz), 4.64 (d, 1H, J = 10.6 Hz), 4.72 (d, 1H, J = 12.4 Hz), 4.79 (s, 2H), 4.94 (d, 1H, J = 10.9 Hz), 7.18−7.42 (m, 15 H), 7.52−7.60 (m, 2H), 7.68 (tq, 1H, J = 7.7, 1.3 Hz), 7.93−8.00 (m, 2H) ppm. ^{13}C NMR (100.6 MHz, $CDCl_3$): δ 59.04, 60.00, 68.67, 68.77, 68.72, 72.99, 73.41, 74.06, 74.08, 75.74, 76.01, 76.07, 80.81, 80.84, 127.69, 127.74, 127.84, 127.90, 127.93, 128.11, 128.42, 128.45, 128.54, 128.62, 128.68 ppm. HRMS (ESI) m/z calcd for $C_{32}H_{33}N_3O_5P$ $(M + H)^+$, 570.2167; found, 570.2158.

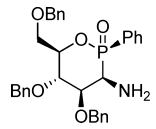
*4.2.17. (S_PSSSR)-3-Amino-4,5-bis-benzyloxy-6-benzyloxymethyl-2-phenyl-2-oxo-2λ^5-[1,2]oxaphosphinane (**6.1a**).*



Under dry nitrogen, polymer-bound triphenylphosphine (3 mmol.g^{-1}, 0.17 g, 0.510 mmol) and water (0.03 g, 0.03 mL, 1.67 mmol) were added to a solution of **5.1a** (0.095 g, 0.167 mmol) in THF (2.5 mL). The reaction mixture was stirred at 60 °C for 3 h, cooled at room temperature, and filtered through microporous filter. The solvent was evaporated under vacuum to give **6.1a** as colorless oil (0.074 g, 82%).

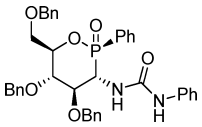
^{31}P NMR (161.97 MHz, $CDCl_3$): δ 38.7 ppm. ^{1}H NMR (400.13 MHz, $CDCl_3$) δ 1.46 (s, 2H), 2.97 (dd, 1H, J = 10.1, 9.8 Hz), 3.70 (dd, J = 11.8, 2.0 Hz), 3.82 (m, 1H, J = 10.1, 9.0, 2.5 Hz), 3.90 (dd, 1H, J = 9.6, 9.0 Hz), 3.92 (ddd, 1H, J = 11.8, 4.3, 2.5 Hz), 4.44−5.10 (m, 7H), 7.05−7.18 (m, 15H), 7.41−7.5 (m, 2H), 7.5−7.62 (m, 1H), 7.79−7.90 (m, 1H) ppm. ^{13}C NMR (100.6 MHz, $CDCl_3$): δ 53.82, 54.79, 68.61, 68.70, 73.49, 75.32, 75.36, 75.41, 76.35, 76.72, 78.82, 84.76, 84.82, 127.24, 127.66, 127.77, 127.82, 127.92, 127.99, 128.07, 128.45, 128.52, 128.59, 128.68, 128.73, 132.95, 133.05, 133.13, 133.11, 137.84, 137.93, 138.08 ppm. HRMS (ESI) m/z calcd for $C_{32}H_{35}NO_5P$ $(M + H)^+$, 544.2253; found, 544.2258.

*4.2.18. (S_PRSSR)-3-Amino-4,5-bis-benzyloxy-6-benzyloxymethyl-2-phenyl-2-oxo-2λ^5-[1,2]oxaphosphinane (**6.1b**). Starting from **5.1b**.*



^{31}P NMR (161.97 MHz, $CDCl_3$): δ 39.5 ppm. ^{1}H NMR (400.13 MHz, $CDCl_3$): δ 1.65 (s, 2H, $N\underline{H}_2$), 3.71 (dd, 1H, J = 11.1, 2.0 Hz), 3.77 (dd, 1H, J = 5.8, 3.3 Hz), 3.88 (ddd, 1H, J = 11.1, 3.0, 2.5 Hz), 4.25−4.31 (m, 2H, 4.41−4.65 (m, 6H), 4.84 (d, 1H, J = 10.84 Hz), 7.05−7.21 (m, 15H), 7.43−7.48 (m, 2H), 7.54−7.60 (m, 1H), 7.77−7.93 (m, 2H, J = 11.6, 7.4, 1.5 Hz) ppm. ^{13}C NMR (100.6 MHz, $CDCl_3$): δ 49.37, 50.34, 68.94, 69.04, 72.14, 73.29, 73.32, 73.40, 75.42, 75.60, 75.65, 80.99, 127.74, 127.87, 127.95, 128.39, 128.44, 128.54, 128.57, 132.69, 132.79, 133.16, 133.19, 137.75, 138.04, 138.14 ppm. HRMS (ESI) m/z calcd for $C_{32}H_{35}NO_5P$ $(M + H)^+$, 544.2253; found, 544.2245.

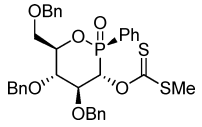
*4.2.19. (S_PRSSR)-3-Phenylureido-4,5-bis-benzyloxy-6-benzyloxymethyl-2-phenyl-2-oxo-2λ^5-[1,2]oxaphosphinane (**7.1a**).*



Under dry nitrogen, phenyl isocyanate (0.024 g, 0.20 mmol) was added to a solution of **6.1a** (0.100 g, 0.18 mmol) in CH_2Cl_2 (3 mL). The reaction mixture was stirred at room temperature overnight, and then, the solvent was evaporated under vacuum. The residue was purified by chromatography on silica gel with as eluent a mixture of *n*-heptane/ethyl acetate (100/0 to 0/100) to give **7.1a** (0.063 g, 53%).

^{31}P NMR (161.97 MHz, $CDCl_3$): δ 39.0 ppm. ^{1}H NMR (400.13 MHz, $CDCl_3$): δ 3.81 (dd, 1H, J = 11.1, 1.8 Hz), 3.94 (ddd, 1H, J = 10.9, 2.8, 2.5 Hz), 4.07 (ddd, 1H, J = 10.6, 9.4, 2.8 Hz), 4.18 (dd, 1H, J = 9.3, 9.3 Hz), 4.53−4.83 (m, 8H), 6.87−8.49 (m, 25H) ppm. ^{13}C NMR (100.6 MHz, $CDCl_3$): δ 50.73 (d, J = 97.3 Hz), 68.65 (d, J = 9.5 Hz), 73.36, 75.45, 76.27 (d, J = 5.9 Hz), 76.63, 78.30, 82.97 (d, J = 8.8 Hz), 118.25, 121.86, 125.47−128.73 (m, 15C), 127.10 (d, J = 23.6 Hz), 129.12 (d, J = 13.2 Hz), 131.74 (d, J = 11.0 Hz), 133.87, 137.59, 137.80, 137.87, 139.71, 155.22 ppm. HRMS (ESI) m/z calcd for $C_{39}H_{40}N_2O_6P$ $(M + H)^+$, 663.2631; found, 663.2624.

*4.2.20. (S_PSSSR)-3-Dithiocarbonyl-S-methylester-4,5-bis-benzyloxy-6-benzyloxymethyl-2-phenyl-2-oxo-2λ^5-[1,2]oxaphosphinane (**8.1a**).*

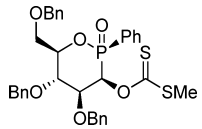


Under dry nitrogen, carbon disulfide (2.34 g, 1.90 mL, 30.7 mmol) and sodium hydride (60% in oil, 0.16 g, 4.0 mmol) were added to a solution of a mixture **3.1a**/**3.1b** (1.69 g, 3.1 mmol, 40/60) in DMF (15 mL). The reaction mixture was stirred at room temperature for 30 min, and then, methyl iodide (4.36 g, 1.90 mL, 30.7 mmol) was added. After 1 h of stirring, water (30 mL) was added, and the aqueous layer was extracted with ethyl acetate (3 × 30 mL). The combined organic layers were dried over magnesium sulfate, filtered, and concentrated under vacuum to give a white solid (1.70 g). The solid was purified by normal-phase chromatography on silica gel with a mixture of

n-heptane/ethyl acetate (70/30) as the eluent to give **8.1a** and **8.1b** (overall yield, 75%).

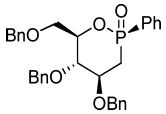
^{31}P NMR (161.97 MHz, $CDCl_3$): δ 30.1 ppm. ^{1}H NMR (400.13 MHz, $CDCl_3$): δ 2.39 (s, 3H), 3.69 (dd, 1H, J = 11.1, 1.5 Hz), 3.91 (d, 1H, J = 11.2 Hz), 4.08 (t, 1H, J = 9.7 Hz), 4.38−4.50 (m, 2H), 4.51−4.65 (m, 3H), 4.7 (s, 2H), 4.80 (d, 1H, J = 10.9 Hz), 6.52 (d, 1H, J = 10.3 Hz), 7.18−7.25 (m, 15H), 7.35−7.43 (m, 2H), 7.45−7.58 (m, 1H), 7.72−7.83 (m, 2H). ^{13}C NMR (100.6 MHz, $CDCl_3$): δ 19.50, 68.31, 68.40, 73.50, 75.12, 75.17, 75.68, 76.28, 78.05, 78.20, 79.19, 81.92, 81.98, 126.30, 127.68, 127.78, 127.82, 127.87, 127.90, 128.08, 128.39, 128.48, 128.70, 128.83, 129.22, 130.80, 130.87, 132.05, 132.16, 133.68, 133.71, 137.66, 137.79, 193.66, 193.94, 194.70, 194.76, 215.47 ppm. HRMS (ESI) m/z calcd for $C_{34}H_{36}O_6PS_2$ $(M + H)^+$, 635.1691; found, 635.1682.

*4.2.21. (S_PRSSR)-3-Dithiocarbonyl-S-methylester-4,5-bis-benzyloxy-6-benzyloxymethyl-2-phenyl-2-oxo-2λ^5-[1,2]oxaphosphinane (**8.1b**).*



^{31}P NMR (161.97 MHz, $CDCl_3$): δ 32.1 ppm. ^{1}H NMR (400.13 MHz, $CDCl_3$): δ 2.30 (s, 3H), 3.75 (dd, 1H, J = 11.1, 1.8 Hz), 3.95 (dt, 1H, J = 11.1, 2.8 Hz), 4.25 (t, 1H, J = 9.9 Hz), 4.45 (dd, 1H, J = 9.6, 2.5 Hz), 4.48 (d, 1H, J = 11.1 Hz), 4.50 (d, 1H, J = 11.9 Hz), 4.53 (d, 1H, J = 11.1 Hz), 4.57 (m, 1H), 4.77 (d, 1H, J = 11.1 Hz), 4.85 (d, 1H, J = 11.1 Hz), 6.88 (dd, 1H, J = 3.78, 2.78 Hz), 7.23−7.40 (m, 17H), 7.50 (m, 1H), 7.78 (m, 2H) ppm. ^{13}C NMR (100.6 MHz, $CDCl_3$): δ 18.02, 28.67, 67.85, 67.94, 71.11, 73.10, 73.25, 73.26, 74.06, 74.67, 75.05, 75.11, 78.67, 78.72, 123.82, 125.26, 126.53, 126.66, 126.76, 126.87, 126.92, 127.19, 127.35, 127.37, 127.39, 127.51, 131.72, 131.83, 132.59, 132.61, 136.18, 136.93, 137.10, 213.38 ppm. HRMS (ESI) m/z calcd for $C_{34}H_{36}O_6PS_2$ $(M + H)^+$, 635.1691; found, 635.1698.

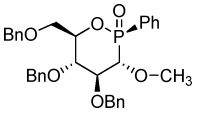
*4.2.22. (S_PSSR)-4,5-Bis-benzyloxy-6-benzyloxymethyl-2-phenyl-2-oxo-2λ^5-[1,2]oxaphosphinane (**9**).*



Tributyltin hydride (0.23 g, 0.21 mL, 0.79 mmol) and AIBN (0.002 g, 0.0122 mmol) were added to a solution of **8.1a**/**8.1b** (0.39 g, 0.61 mmol) in toluene (8 mL) at room temperature. The reaction mixture was stirred at 115 °C for 3 h. The solvent was evaporated affording a yellow oil. The residue was purified by chromatography on silica gel with a mixture of *n*-heptane/ethyl acetate (60:40) as the eluent to give **9** as a white solid (0.094 g, 29%).

^{31}P NMR (161.97 MHz, $CDCl_3$): δ 37.9 ppm. ^{1}H NMR (400.13 MHz, $CDCl_3$): δ 1.92 (m, 1H), 2.62 (dt, 1H, J = 13.6, 4.5 Hz), 3.68 (dd, 1H, J = 11.1, 2.0 Hz), 3.93 (m, 2H), 4.25 (m, 1H), 4.40−4.50 (m, 2H), 4.50−4.65 (m, 4H), 4.88 (d, 1H, J = 10.6 Hz), 7.25−7.30 (m, 15H), 7.30−7.47 (m, 2H), 7.49−7.58 (m, 1H), 7.70−7.78 (m, 2H) ppm. ^{13}C NMR (400.13 MHz, $CDCl_3$): δ 32.47, 33.29, 68.96, 69.05, 72.14, 73.42, 75.44, 75.50, 75.53, 127.64, 127.69, 127.75, 127.80, 127.85, 127.90, 129.13, 130.51, 131.42, 131.53, 132.95, 132.98, 137.87, 138.09, 138.13 ppm. HRMS (ESI) m/z calcd for $C_{32}H_{34}O_5P$ $(M + H)^+$, 529.2144; found, 529.2136.

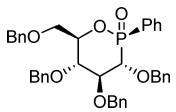
*4.2.23. (S_PSSSR)-3-Methoxy-4,5-bis-benzyloxy-6-benzyloxymethyl-2-phenyl-2-oxo-2λ^5-[1,2]oxaphosphinane (**10.1a**).*



Under dry nitrogen, sodium hydride (50% in oil, 0.01 g, 0.021 mmol) was added to a solution of **3.1a** (0.10 g, 0.18 mmol) in dry THF (3 mL). The reaction mixture was stirred at room temperature for 30 min, and then, methyl iodide (13 μL, 0.21 mmol) was added. The reaction mixture was stirred for one night. After solvent evaporation under vacuum, ethyl acetate (3 mL) was poured into the crude oil. The combined organic layers were dried over magnesium sulfate, filtered, and concentrated under vacuum. The residue was purified by chromatography on silica gel using a mixture of *n*-heptane/ethyl acetate (100/0 to 0/100) as the eluent to give **10.1a** as a white solid (0.015 g, 15%).

^{31}P NMR (161.97 MHz, $CDCl_3$): δ = 32.8 ppm. ^{1}H NMR (400.13 MHz, $CDCl_3$): δ 3,23 (s, 3H), 3.63 (d, 1H, J = 10.0 Hz), 3.67 (dd, 1H, J = 11.1, 1.8 Hz), 3.88 (ddd, 1H, J = 11.1, 2.6, 2.4 Hz), 3.94 (dd, 1H, J = 9.8, 9.6 Hz), 4.17 (ddd, 1H, J = 9.8, 9.6, 2.6 Hz), 4.43−4.62 (m, 3H), 4.78−4.89 (m, 2H), 7.12−7.24 (m, 15H), 7.43−7.57 (m, 3H), 7.81−7.85 (m, 2H) ppm. ^{13}C NMR (100.6 MHz, $CDCl_3$): δ 61.19, 68.59, 73.43, 74.85, 75.55, 76.43, 77.83, 81.30, 82.31, 83.46, 83.53, 127.59, 127.73, 127.77, 127.81, 127.99, 128.42, 128.44, 128.78, 128.91,131.78, 131.88, 133.25, 137.98, 138.32 ppm. HRMS (ESI) m/z calcd for $C_{33}H_{36}O_6P$ $(M + H)^+$, 559.2250; found, 559.2250.

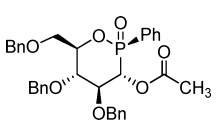
*4.2.24. (S_PSSSR)-3-Benzyloxy-4,5-bis-benzyloxy-6-benzyloxymethyl-2-phenyl-2-oxo-2λ^5-[1,2]oxaphosphinane (**11.1a**).*



Under dry nitrogen, sodium hydride (50% oil, 0.01 g, 0.21 mmol) was added to a solution of **3.1a** (0.10 g, 0.18 mmol) in dry THF (3 mL). The reaction mixture was stirred at room temperature for 30 min. Benzyl chloride (25 μL, 0.22 mmol) was added, and the reaction mixture was stirred for one night. After solvent evaporation under vacuum, ethyl acetate (3 mL) was added to the crude oil. The combined organic layers were dried over magnesium sulfate, filtered, and concentrated under vacuum. The residue was purified by chromatography on silica gel using as eluent a mixture of *n*-heptane/ethyl acetate (100/0 to 0/100) to give **11.1a** as a white solid (0.045 g, 39%).

^{31}P NMR (161.97 MHz, $CDCl_3$): δ = 33.4 ppm. ^{1}H NMR (400.13 MHz, $CDCl_3$): δ 3.65 (dd, 1H, J = 11.2, 2.0 Hz), 3.85 (d, 1H, J = 10.2 Hz), 3.88 (ddd, 1H, J = 11.2, 2.6, 2.3 Hz), 3.92 (dd, 1H, J = 9.8, 9.6 Hz), 4.28 (ddd, 1H, J = 10.0, 9.8, 2.7 Hz), 4.39−4.61 (m, 6H), 4.84 (d, 1H, J = 10.7), 4.91 (d, 1H, J = 10.7), 6.80−6.90 (m, 2H), 6.95−7.05 (m, 2H), 7.05−7.15 (m, 3H), 7.15−7.25 (m, 13H), 7.35−7.45 (m, 2H), 7.05−7.15 (m, 3H), 7.50−7.55 (m, 1H), 7.65−7.75 (m, 2H) ppm. ^{13}C NMR (100.6 MHz, $CDCl_3$): δ 68.52, 68.61, 73.44, 74.42, 74.46, 74.74, 75.54, 76.39, 77.43, 78.03, 78.43, 83.72, 83.79, 127.72, 127.86, 128.14, 128.17, 128.42, 128.44, 128.69, 128.82, 131.88, 131.98 ppm. HRMS (ESI) m/z calcd for $C_{39}H_{40}O_6P$ $(M + H)^+$, 635.2563; found, 635.2559.

*4.2.25. (S_PSSSR)-3-Acetoxy-4,5-bis-benzyloxy-6-benzyloxymethyl-2-phenyl-2-oxo-2λ^5-[1,2]oxaphosphinane (**12.1a**).*

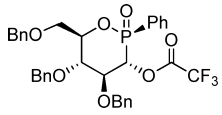


dx.doi.org/10.1021/jm201428a | J. Med. Chem. 2012, 55, 2196−2211

Under dry nitrogen, acetic anhydride (0.062 g, 57 μL, 0.61 mmol), pyridine (30 μL, 0.41 mmol), and DMAP (0.005 g, 0.041 mmol) were added to a solution of **3.1a** (0.225 g, 0.41 mmol) in dry CH_2Cl_2 (5 mL). The reaction mixture was stirred for one night. After solvent evaporation under vacuum, ethyl acetate (10 mL) was added to the crude oil. The combined organic layers were dried over magnesium sulfate, filtered, and concentrated under vacuum. The residue was purified by normal-phase chromatography on silica gel using as eluent a mixture of *n*-heptane/ethyl acetate (100/0 to 0/100) to give **12.1a** (0.154 g, 64%).

^{31}P NMR (161.97 MHz, $CDCl_3$): δ 32.1 ppm. ^{1}H NMR (400.13 MHz, $CDCl_3$): δ 1.82 (s, 3H), 3.67 (dd, 1H, *J* = 11.2, 2.0 Hz), 3.90 (ddd, 1H, *J* = 11.2, 2.7, 2.4 Hz), 4.04 (t, 1H, *J* = 9.7 Hz), 4.23 (ddd, 1H, *J* = 10.4, 9.4, 2.9 Hz), 4.44−4.83 (m, 7H), 5.42 (dd, 1H, *J* = 10.4, 1.0 Hz), 7.12−7.25 (m, 15H), 7.40−7.50 (m, 2H), 7.50−7.60 (m, 1H), 7.74−7.80 (m, 2H) ppm. ^{13}C NMR (100.6 MHz, $CDCl_3$): δ 20.46, 68.31, 68.40, 70.16, 71.17, 73.46, 75.20, 75.25, 75.59, 76.21, 78.21, 81.77, 81.82, 127.60, 127.63, 127.87, 127.93, 128.49, 128.69, 131.82, 133.61, 133.64, 137.70, 137.83, 137.97, 169.54, 169.58 ppm. HRMS (ESI) *m*/*z* calcd for $C_{34}H_{36}O_7P$ $(M + H)^+$, 587.2199; found, 587.2186.

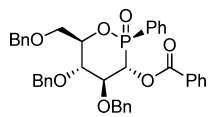
*4.2.26. (S_PSSR)-3-Trifluoroacetoxy-4,5-bis-benzyloxy-6-benzyloxymethyl-2-phenyl-2-oxo-2λ^5-[1,2]oxaphosphinane (**13.1a**).*



Under dry nitrogen, trifluoroacetic anhydride (0.084 g, 56 μL, 0.40 mmol), pyridine (0.021 g, 21 μL, 0.27 mmol), and DMAP (0.003 g, 0.027 mmol) were added to a solution of **3.1a** (0.150 g, 0.27 mmol) in dry CH_2Cl_2 (3 mL). The reaction mixture was stirred during night. Ethyl acetate (5 mL) was added to the crude mixture, and the mixture was washed with water saturated with NaCl. The organic layer was dried over magnesium sulfate, filtered, and concentrated under vacuum. The residue was purified by chromatography on silica gel with as eluent a mixture of *n*-heptane/ethyl acetate (100/0 to 0/100) to give **13.1a** (0.069 g, 40%).

^{19}F NMR (376.46 MHz, $CDCl_3$): δ −74.4 ppm. ^{31}P NMR (161.97 MHz, $CDCl_3$): δ 28.7 ppm. ^{1}H NMR (400.13 MHz, $CDCl_3$): 3.81 (d, 1H, *J* = 11.2 Hz), 4.00 (d, 1H, *J* = 11.0 Hz), 4.18 (dd, 1H, *J* = 9.8, 9.6 Hz), 4.46 (dd, 1H, *J* = 9.4, 8.4 Hz), 4.50−5.00 (m, 5H), 5.48 (d, 1H, *J* = 10.6 Hz), 7.15−7.45 (m, 15H), 7.50−7.63 (m, 2H), 7.65−7.75 (m, 1H), 7.80−7.95 (m, 2H) ppm. ^{13}C NMR (100.6 MHz, $CDCl_3$): δ 68.05, 68.13, 73.51, 74.11, 75.09, 75.42, 75.47, 75.67, 76.47, 78.17, 80.67, 127.66, 127.70, 127.74, 128.05, 128.53, 129.00, 129.14, 131.81, 131.91, 134.17, 137.16, 137.45, 137.64 ppm. MS (ESI) *m*/*z* for $C_{34}H_{33}F_3O_7P$ $(M + H)^+$, 641.2.

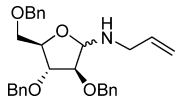
*4.2.27. (S_PSSR)-3-Benzoate-4,5-bis-benzyloxy-6-benzyloxymethyl-2-phenyl-2-oxo-2λ^5-[1,2]oxaphosphinane (**14.1a**).*



Under dry nitrogen, benzoic anhydride (0.090 g, 0.40 mmol), pyridine (0.021 g, 21 μL, 0.27 mmol), and DMAP (0.003 mg, 0.027 mmol) were added to a solution of **3.1a** (0.150 g, 0.27 mmol) in dry CH_2Cl_2 (3 mL). The reaction mixture was stirred during night. Ethyl acetate (5 mL) was added to the crude mixture and washed with water saturated with NaCl. The organic layer was dried over magnesium sulfate, filtered, and concentrated under vacuum. The residue was purified by chromatography on silica gel with a mixture of *n*-heptane/ethyl acetate (100/0 to 0/100) as the eluent to give **14.1a** (0.054 g, 31%).

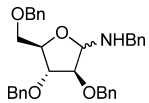
^{31}P NMR (161.97 MHz, $CDCl_3$): δ 31.8 ppm. ^{1}H NMR (400.13 MHz, $CDCl_3$): δ 3.72 (d, 1H, *J* = 11.2 Hz), 3.92 (d, 1H, *J* = 11.0 Hz), 4.11 (t, 1H, *J* = 9.6 Hz), 4.41 (m,1H), 4.46−4.65 (m, 5H), 4.74 (d, 1H, *J* = 11.0 Hz), 4.84 (d, 1H, *J* = 11.0 Hz), 5.70 (d, 1H, *J* = 10.4 Hz), 6.90−7.60 (m, 21H), 7.70−7.85 (m, 2H), 7.94 (d, 1H, *J* = 7.2 Hz) ppm. ^{13}C NMR (100.6 MHz, $CDCl_3$): δ 68.35, 68.44, 73.46, 75.29, 75.60, 76.33, 78.29, 81.60, 127.64, 127.83, 127.98, 128.27, 128.42, 128.48, 128.73, 128.87, 130.06, 131.90, 133.48, 137.43, 137.77, 137.85 ppm. MS (ESI) *m*/*z* for $C_{39}H_{38}O_7P$ $(M + H)^+$, 649.3.

*4.2.28. Compound **15.1**.*



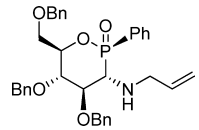
The synthesis was according to Cipolla et al.[24]

*4.2.29. Compound **15.2**.*



The synthesis was according to Cipolla et al.[24]

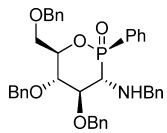
*4.2.30. (S_PSSR)-3-Allylamino-4,5-bis-benzyloxy-6-benzyloxymethyl-2-phenyl-2-oxo-2λ^5-[1,2]oxaphosphinane (**16.1a**).*



Under dry nitrogen, compound **15.1** (10.0 g, 21.7 mmol) was added to a solution of ethyl phenylphosphinate (3.7 g, 21.7 mmol) in THF (250 mL), and then, freshly sublimated potassium *tert*-butoxide (0.7 g, 6.24 mmol) was poured to the solution. The reaction mixture was stirred at room temperature for one night. The crude mixture was washed with water saturated with NaCl. The organic layer was dried over magnesium sulfate, filtered, and concentrated under vacuum. The residue was purified by chromatography on silica gel with as eluent a mixture of *n*-heptane/ethyl acetate (100/0 to 0/100) to give **16.1a** (1.60 g, 12%).

^{31}P NMR (161.97 MHz, $CD_3C(O)CD_3$): δ 35.5 ppm. ^{1}H NMR (400.13 MHz, $CDCl_3$): 3.08−3.23 (m, 3H), 3.77 (dd, 1H, *J* = 11.0, 2.2 Hz), 3.98−4.15 (m, 3H), 4.45−5.05 (m, 9H), 5.45−5.65 (m, 1H), 7.20−7.40 (m, 15H), 7.46−7.56 (m, 1H), 7.58−7.66 (m, 1H), 7.85−7.95 (m, 2H) ppm. Analytical HPLC [column, Waters SunFire C18, 5 μm, 4.6 mm × 250 mm; eluent, acetonitrile/water (80:20); flow, 1 mL min^{-1}; retention time = 13.66 min (99%)]. HRMS (ESI) *m*/*z* calcd for $C_{35}H_{39}NO_5P$ $(M + H)^+$, 584.2566; found, 584.2565.

*4.2.31. (S_PSSR)-3-Benzylamino-4,5-bis-benzyloxy-6-benzyloxymethyl-2-phenyl-2-oxo-2λ^5-[1,2]oxaphosphinane (**17.1a**).*



Under dry nitrogen, compound **15.2** (10.20 g, 19.6 mmol) was added to a solution of ethyl phenylphosphinate (3.34 g, 19.6 mmol) in THF (250 mL), and then, freshly sublimated potassium *tert*-butoxide (0.65 g, 5.8 mmol) was poured into the solution. The reaction mixture was stirred at room temperature for one night. The crude mixture was washed with a saturated aqueous solution of NaCl. The organic layer was dried over magnesium sulfate, filtered, and concentrated under vacuum.

The residue was purified by chromatography on silica gel with as eluent a mixture of *n*-heptane/ethyl acetate (100/0 to 0/100) to give **17.1a** (0.97 g, 7%).

^{31}P NMR (161.97 MHz, $CDCl_3$): δ = 36.8 ppm. ^{1}H NMR (400.13 MHz, $CD_3C(O)CD_3$): 3.08 (dd, 1H, J = 10.1, 5.5 Hz), 3.64−3.70 (m, 3H), 3.844.10 (m, 3H), 4.30−5.00 (m, 7H), 6.61−6.90 (m, 2H), 6.90−7.05 (m, 3H), 7.05−7.15 (m, 2H), 7.15−7.30 (m, 13H), 7.30−7.45 (m, 2H), 7.45−7.60 (m, 2H), 7.70−7.85 (m, 2H) ppm. ^{13}C NMR (100.6 MHz, $CDCl_3$): δ 52.58, 52.65, 53.62, 58.63, 60.09, 68.69, 68.84, 71.76, 74.94, 75.02, 75.35, 76.47, 76.62, 77.12, 78.76, 83.96, 84.08, 126.88, 127.63, 127.71, 127.76, 127.84, 128.08, 128.13, 128.42, 128.47, 128.48, 128.59, 128.64, 128.80, 130.75, 131.87, 132.04, 132.98, 133.02 ppm. HRMS (ESI) m/z calcd for $C_{39}H_{41}NO_5P$ $(M + H)^+$, 634.2722; found, 634.2724.

4.3.1. Cell Cultures. *Cell Lines.* A431, MDA-MB-231, DU145, MDA-MB-435, B16F10, CacO2, and HuH7 cells were obtained from the American Type Culture Collection. The C6 cells were a gift from M. Mersel (Institut des neurosciences de Montpellier, INM). These cell lines were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal calf serum (FCS), 2 mM L-glutamine, 1 mM sodium pyruvate, and 50 U mL^{-1} streptomycin.

4.3.2. Astrocytes. Astrocytes were isolated from newborn mice (day 2) cortex as previously described.[35]

4.4. Drug Addition. TMZ, carmustine, vincristine, paclitaxel, cytosine arabinoside (AraC), MTT, neutral red, phosphate-buffered saline (PBS), triton X 100, Hoechst 33342, and PI were from Sigma. Phostine solutions were prepared in ethanol/DMSO (80/20 v/v). For TMZ and paclitaxel, solutions at 100 mM were prepared in 100% DMSO. Carmustine was at 100 mM in ethanol. Vincristine (100 mM) was dissolved, and AraC (10 μM) was prepared in PBS.

4.5.1. Measurements of the Oxaphosphinane Effects on C6 and Astrocytes in Primary Culture. *MTT Assay.* The MTT assay was carried out as previously described[36] 48 h after drug addition.

4.5.2. Neutral Red Assay. The neutral red assay was carried out as previously described[36] 24 and 48 h after drug addition.[37]

4.5.3. LDH Assay. LDH release was measured using the LDH kit from Roche for 60 min and according to the manufacturer's instructions.

4.5.4. NCI Assay. The NCI assays were carried out at 10 μM performing a sulforhodamine test at 24 h.

4.5.5. Hoechst 33342 and Propidium Iodide Fluorescence Measurements and Impedance Measurement. After 48 h of OXP treatment, 5 μg/mL Hoechst 33342 and 10 μM PI were added in the culture medium for 30 min at 37 °C. Cells were then washed in PBS and fixed during 10 min with 4% paraformaldehyde. The mean fluorescence at four positions in each well at 460 nm was measured (Cytofluor multiwell plate reader 4000, Perseptive biosystems) with excitation at 360 nm and a gain of 90 dB. The mean fluorescence of three wells per condition was then calculated.

Dual labeling with PI and Hoechst 33342 was performed using the Cellomics array scan vti (Thermofisher). Fluorescence was detected with an ORCA camera (ORCA ER;0.63), a 20× objective, and a XF 93 filter, gain of 25 dB, and exposure times were 24 and of 280 ms for Hoechst 33342 and PI, respectively. Images were analyzed using the array scan vti 6.0 software. The object detection was fixed on the Hoechst 33342 channel with a threshold intensity of 400. The object area was fixed between 10 and 160 to separate nuclei. The object counting validation was fixed at 2500/well counting less than 15 fields containing a minimum of five objects. Control fluorescence intensities for red and blue staining were measured on Triton X-100 (2%)-treated cells. The mean percentage of PI incorporation was calculated from three different wells per condition.

4.5.6. Impedance Measurement. The Xcelligence system, kindly lent by Roche diagnostics (Meylan, France), measured the electrical impedance across interdigitated microelectrodes integrated on the bottom of tissue culture E-Plates (ACEA biosciences). Impedance was recorded for 69 h using RTCA software every 10 min. The effects of OXP (1 nM to 100 μM) were measured 25 h after plating (t = 0) for 44 h. The electrode impedance was displayed as a cell index (CI) value. The mean CI value calculated from triplicate measurements was normalized to vehicle-treated cells. The primary effects of OXP were determined by a statistical analysis of the 0−5 h interval after drug addition. Indeed, when plates were coated with an inhibitor of cell adhesion like polyhydroxymethacrylate (data not shown), the CI value remained constant over time. The primary effects of **3.1a** were determined by a statistical analysis on the 0−5 h interval after drug addition.

4.6. Statistical Analyses. EC_{50} values were calculated using the Hill equation of the dose−log response curves using Prism 5.0 software (Graphpad) using the vehicle as the top value and Triton X-100 as the bottom value. For group comparison, one-way ANOVA followed by Fisher's posthoc PLSD test was performed using the Statview 5.0 software.

■ ASSOCIATED CONTENT

Supporting Information

All spectra and analyses. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*Tel: +33 467 147 243. Fax: +33 467 144 319. E-mail: jean-luc.pirat@enscm.fr (J.-L.P.). Tel: +33 607 106 844. Fax: +33 499 636 020. E-mail: norbert.bakalara@enscm.fr (N.B.).

Author Contributions

‖L.C. and C.J. equally contributed to the paper.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We thank Didier Petite, Clovis Rakotoarivelo, Marcel Mersel, Omar Munoz, Marissa Teigell, and Alina Florentine Cristian for expert technical support, Romain Filhol and Julien Cau for Cellomic expertise, Nicolas Gauthier from Roche for Xcelligence system expertise, and Jan de Weille for carefully reading the manuscript and for English language. This research was supported by l'Agence Nationale de la Recherche (ANR-07-PCVI-0038-01), L'INCa (DM/FC/mc/25-03-09), l'Institut National de la Santé et de la Recherche Médicale (INSERM), and L'Ecole Nationale Supérieure de Chimie de Montpellier (ENSCM).

■ DEDICATION

This paper is dedicated in honor of Prof. Francisco Palacios on the occasion of his 60th birthday.

■ ABBREVIATIONS USED

NCI, National Cancer Institute; EC_{50}, half maximal effective concentration; PCV, procarbazine, lomustine, and vincristine; MRI, magnetic resonance imaging; MTT, dimethylthiazolyl-2,5-diphenyltetrazolium bromide; LTC, lower toxic concentration; GP, growth percent; CNS, central nervous system; AraC, cytabarine; GFAP, glial fibrillary acidic protein; PI, propidium iodide; TMZ, Temozolomide; LDH, lactate dehydrogenase; CH_2Cl_2, dichloromethane; HPLC, high-performance liquid chromatography; INM, Institut des neurosciences de Montpellier; DMEM, Dulbecco's modified Eagle's medium; FCS, fetal calf serum; DMSO, dimethylsulfoxide

REFERENCES

(1) Bauchet, L.; Mathieu-Daude, H.; Fabbro-Peray, P.; Rigau, V.; Fabbro, M.; Chinot, O.; Pallusseau, L.; Carnin, C.; Laine, K.; Schlama, A.; Thiebaut, A.; Patru, M. C.; Bauchet, F.; Lionnet, M.; Wager, M.; Faillot, T.; Taillandier, L.; Figarella-Branger, D.; Capelle, L.; Loiseau, H.; Frappaz, D.; Campello, C.; Kerr, C.; Duffau, H.; Reme-Saumon, M.; Tretarre, B.; Daures, J. P.; Henin, D.; Labrousse, F.; Menei, P.; Honnorat, J. Oncological patterns of care and outcome for 952 patients with newly diagnosed glioblastoma in 2004. *Neuro-Oncology* **2010**, *12*, 725−735.

(2) Van Meir, E. G.; Hadjipanayis, C. G.; Norden, A. D.; Shu, H. K.; Wen, P. Y.; Olson, J. J. Exciting new advances in neuro-oncology: The avenue to a cure for malignant glioma. *CA Cancer J. Clin.* **2010**, *60*, 166−193.

(3) Gerstner, E. R.; Chen, P. J.; Wen, P. Y.; Jain, R. K.; Batchelor, T. T.; Sorensen, G. Infiltrative patterns of glioblastoma spread detected via diffusion MRI after treatment with cediranib. *Neuro-Oncology* **2010**, *12*, 466−472.

(4) Fraum, T. J.; Kreisl, T. N.; Sul, J.; Fine, H. A.; Iwamoto, F. M. Ischemic stroke and intracranial hemorrhage in glioma patients on antiangiogenic therapy. *J. Neurooncol.* **2011**, *105*, 287−289.

(5) Uhm, J. H.; Ballman, K. V.; Wu, W.; Giannini, C.; Krauss, J. C.; Buckner, J. C.; James, C. D.; Scheithauer, B. W.; Behrens, R. J.; Flynn, P. J.; Schaefer, P. L.; Dakhill, S. R.; Jaeckle, K. A. Phase II Evaluation of Gefitinib in Patients With Newly Diagnosed Grade 4 Astrocytoma: Mayo/North Central Cancer Treatment Group Study N0074. *Int. J. Radiat. Oncol. Biol. Phys.* **2011**, *80*, 347−353.

(6) Neyns, B.; Sadones, J.; Joosens, E.; Bouttens, F.; Verbeke, L.; Baurain, J. F.; D'Hondt, L.; Strauven, T.; Chaskis, C.; In't Veld, P.; Michotte, A.; De Greve, J. Stratified phase II trial of cetuximab in patients with recurrent high-grade glioma. *Ann. Oncol.* **2009**, *20*, 1596−1603.

(7) Brada, M.; Stenning, S.; Gabe, R.; Thompson, L. C.; Levy, D.; Rampling, R.; Erridge, S.; Saran, F.; Gattamaneni, R.; Hopkins, K.; Beall, S.; Collins, V. P.; Lee, S. M. Temozolomide versus procarbazine, lomustine, and vincristine in recurrent high-grade glioma. *J. Clin. Oncol.* **2010**, *28*, 4601−4608.

(8) Westphal, M.; Hilt, D. C.; Bortey, E.; Delavault, P.; Olivares, R.; Warnke, P. C.; Whittle, I. R.; Jaaskelainen, J.; Ram, Z. A phase 3 trial of local chemotherapy with biodegradable carmustine (BCNU) wafers (Gliadel wafers) in patients with primary malignant glioma. *Neuro-Oncology* **2003**, *5*, 79−88.

(9) Sabbah, P.; Foehrenbach, H.; Dutertre, G.; Nioche, C.; DeDreuille, O.; Bellegou, N.; Mangin, J. F.; Leveque, C.; Faillot, T.; Gaillard, J. F.; Desgeorges, M.; Cordoliani, Y. S. Multimodal anatomic, functional, and metabolic brain imaging for tumor resection. *Clin. Imaging* **2002**, *26*, 6−12.

(10) Stummer, W.; Pichlmeier, U.; Meinel, T.; Wiestler, O. D.; Zanella, F.; Reulen, H. J. Fluorescence-guided surgery with 5-aminolevulinic acid for resection of malignant glioma: a randomised controlled multicentre phase III trial. *Lancet Oncol.* **2006**, *7*, 392−401.

(11) Volle, J.-N.; Filippini, D.; Krawczyk, B.; Kaloyanov, N.; Van der Lee, A.; Maurice, T.; Pirat, J.-L.; Virieux, D. Drug discovery: Phosphinolactone, in vivo bioisostere of the lactol group. *Org. Biomol. Chem.* **2010**, *8*, 1438−1444.

(12) Chemical properties and reactions of phosphine chalcogenides. In *The Chemistry of Organophosphorus Compounds*; Hartley, F. R., Ed.; John Wiley & Sons: Chichester, 1992; Vol. 2, pp 287−408.

(13) Hernández, J.; Ramos, R.; Sastre, N.; Meza, R.; Hommer, H.; Salas, M.; Gordillo, B. Conformational analysis of six-membered ring dioxaphosphinanes. Part 1: Anancomeric thiophosphates. *Tetrahedron* **2004**, *60*, 10927−10941.

(14) Wadsworth, S.; Emmons, W. D. Bicyclic phosphites. *J. Am. Chem. Soc.* **1962**, *84*, 610−617.

(15) Volle, J.-N.; Virieux, D.; Starck, M.; Monbrun, J.; Clarion, L.; Pirat, J.-L. Chiral phosphinyl analogues of 2-C-arylmorpholinols: 2-Aryl-3,5-diphenyl-[1,4,2]-oxazaphosphinanes. *Tetrahedron: Asymmetry* **2006**, *17*, 1402−1408.

(16) McDonald, F. E.; Zhu, H. Y. H.; Holmquist, C. R. Rhodium-catalysed alkaline cyclotrimerization strategies for 6-arylglycoside synthesis. *J. Am. Chem. Soc.* **1995**, *117*, 6605−6606.

(17) Harvey, T. C.; Simiand, C.; Weiler, L.; Withers, S. G. Synthesis of Cyclic Phosphonate Analogs of Ribose and arabinose. *J. Org. Chem.* **1997**, *62*, 6722−6725.

(18) Hanessian, S.; Rogel, O. Synthesis of a phostone glycomimetic of the endothelin converting enzyme inhibitor phosphoramidon. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 2441−2446.

(19) Stoianova, D. S.; Whitehead, A.; Hanson, P. R. P-Heterocyclic Building Blocks: Application to the Stereoselective Synthesis of P-Sugars. *J. Org. Chem.* **2005**, *70*, 5880−5889.

(20) Pirat, J.-L.; Virieux, D.; Volle, J.-N.; Clarion, L.; Bakalara, N.; Mersel, M.; Monbrun, J.; Cristau, H.-J. New phosphorus containing heterocyclic compounds, sugar analogs, and compositions having anticancer activity containing the same. PCT Int. Appl. WO 2009/004096 A1, 2009.

(21) Volle, J.-N.; Filippini, D.; Midrier, C.; Virieux, D.; Pirat, J.-L.; Sobecki, M.; Drag, M. Revisited Synthesis of Aryl-H-phosphinates. *Synthesis* **2011**, 2490−2494.

(22) Cristau, H.-J.; Monbrun, J.; Schleiss, J.; Virieux, D.; Pirat, J.-L. First synthesis of P-aryl-phosphinosugars, organophosphorus analogues of C-arylglycosides. *Tetrahedron Lett.* **2005**, *46*, 3741−3744.

(23) Filippini, D.; Loiseau, S.; Bakalara, N.; Dziuganowska, Z. A.; Van der Lee, A.; Volle, J.-N.; Virieux, D.; Pirat, J. L. Dramatic effect of modified boranes in diastereoselective reduction of chiral cyclic α-ketophosphinates. *RSC Adv.* **2012**, *2*, 816−818.

(24) Cipolla, L.; Lay, L.; Nicotra, F.; Pangrazio, C.; Panza, L. Synthesis of Azasugars by Grignard Reaction on Glycosylamines. *Tetrahedron* **1995**, *51*, 4679−4690.

(25) Behr, J. M.; Guillerm, G. Synthesis of 6-deoxy-homoDMDP and its C(5)-epimer: Absolute stereochemistry of natural products from Hyacinthus orientalis. *Tetrahedron: Asymmetry* **2002**, *13*, 111−113.

(26) Stupp, R.; Mason, W. P.; Van den Bent, M. J.; Weller, M.; Fisher, B.; Taphoorn, M. J.; Belanger, K.; Brandes, A. A.; Marosi, C.; Bogdahn, U.; Curschmann, J.; Janzer, R. C.; Ludwin, S. K.; Gorlia, T.; Allgeier, A.; Lacombe, D.; Cairncross, J. G.; Eisenhauer, E.; Mirimanoff, R. O. Radiotherapy plus concomitant and adjuvant Temozolomide for glioblastoma. *N. Engl. J. Med.* **2005**, *352*, 987−996.

(27) Koziara, J. M.; Lockman, P. R.; Allen, D. D.; Mumper, R. J. Paclitaxel nanoparticles for the potential treatment of brain tumors. *J. Controlled Release* **2004**, *99*, 259−269.

(28) Acton, E. M.; Narayanan, V. L.; Risbood, P. A.; Shoemaker, R. H.; Vistica, D. T.; Boyd, M. R. Anticancer specificity of some ellipticinium salts against human brain tumors in vitro. *J. Med. Chem.* **1994**, *37*, 2185−2189.

(29) Scherf, U.; Ross, D. T.; Waltham, M.; Smith, L. H.; Lee, J. K.; Tanabe, L.; Kohn, K. W.; Reinhold, W. C.; Myers, T. G.; Andrews, D. T.; Scudiero, D. A.; Eisen, M. B.; Sausville, E. A.; Pommier, Y.; Botstein, D.; Brown, P. O.; Weinstein, J. N. A gene expression database for the molecular pharmacology of cancer. *Nat. Genet.* **2000**, *24*, 236−244.

(30) Ross, D. T.; Scherf, U.; Eisen, M. B.; Perou, C. M.; Rees, C.; Spellman, P.; Iyer, V.; Jeffrey, S. S.; Van de Rijn, M.; Waltham, M.; Pergamenschikov, A.; Lee, J. C.; Lashkari, D.; Shalon, D.; Myers, T. G.; Weinstein, J. N.; Botstein, D.; Brown, P. O. Systematic variation in gene expression patterns in human cancer cell lines. *Nat. Genet.* **2000**, *24*, 227−235.

(31) Blower, P. E.; Chung, J. H.; Verducci, J. S.; Lin, S.; Park, J. K.; Dai, Z.; Liu, C. G.; Schmittgen, T. D.; Reinhold, W. C.; Croce, C. M.; Weinstein, J. N.; Sadee, W. MicroRNAs modulate the chemosensitivity of tumor cells. *Mol. Cancer Ther.* **2008**, *7*, 1−9.

(32) Blower, P. E.; Verducci, J. S.; Lin, S.; Zhou, J.; Chung, J. H.; Dai, Z.; Liu, C. G.; Reinhold, W.; Lorenzi, P. L.; Kaldjian, E. P.; Croce, C. M.; Weinstein, J. N.; Sadee, W. MicroRNA expression profiles for the NCI-60 cancer cell panel. *Mol. Cancer Ther.* **2007**, *6*, 1483−1491.

(33) Lee, J. S.; Paull, K.; Alvarez, M.; Hose, C.; Monks, A.; Grever, M.; Fojo, A. T.; Bates, S. E. Rhodamine efflux patterns predict P-

glycoprotein substrates in the National Cancer Institute drug screen. *Mol. Pharmacol.* **1994**, *46*, 627−638.
(34) Rutka, J. T.; Giblin, J. R.; Dougherty, D. Y.; Liu, H. C.; McCulloch, J. R.; Bell, C. W.; Stern, R. S.; Wilson, C. B.; Rosenblum, M. L. Establishment and characterization of five cell lines derived from human malignant gliomas. *Acta Neuropathol.* **1987**, *75*, 92−103.
(35) Audouy, S.; Mallet, J.; Privat, A.; Gimenez y Ribotta, M. Adenovirus-mediated suicide gene therapy in an in vitro model of reactive gliosis. *Glia* **1999**, *25*, 293−303.
(36) Mosmann, T. Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. *J. Immunol. Methods* **1983**, *65*, 55−63.
(37) Repetto, G.; del Peso, A.; Zurita, J. L. Neutral red uptake assay for the estimation of cell viability/cytotoxicity. *Nat. Protoc.* **2008**, *3*, 1125−1131.