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Divergent Strategy for the Synthesis of α-Aryl-Substituted Fosmidomycin Analogues

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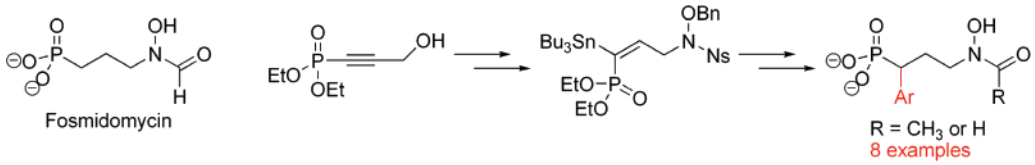

Fosmidomycin is the first representative of a new class of antimalarial drugs acting through inhibition of 1-deoxy-D-xylulose 5-phosphate (DOXP) reductoisomerase (DXR), an essential enzyme in the non-mevalonate pathway for the synthesis of isoprenoids. This work describes a divergent strategy for the synthesis of a series of α-aryl-substituted fosmidomycin analogues, featuring a palladium-catalyzed Stille coupling as the key step. An α-(4-cyanophenyl)fosmidomycin analogue emerged as the most potent analogue in the present series. Its antimalarial activity clearly surpasses that of the reference compound fosmidomycin.

Introduction

According to the WHO, malaria still causes 1.5 to 2 million casualties in Africa alone, among which are mainly children. One of the major reasons for the spread of the disease is the growing resistance of *Plasmodium falciparum*, the parasite species responsible for most of the diagnosed cases, toward almost every antimalarial drug currently available. Therefore, the discovery of novel inhibitors targeting new biochemical pathways is of high priority.

In 1980, Okuhura and colleagues reported the first isolation of fosmidomycin (**1**), a structurally simple antibiotic from *Streptomyces lavendulae*.[1,2]

In 1998, 1-deoxy-D-xylulose 5-phosphate (DOXP) reductoisomerase, an enzyme involved in the 2*C*-methyl-D-erythritol 4-phosphate (MEP) pathway for the biosynthesis of isoprenoids and absent in humans, was identified as the molecular target for fosmidomycin.[3,4]

Fosmidomycin was found to be a potent inhibitor of DOXP reductoisomerase (DXR) of *P. falciparum*.[4] FR900098 (**2**), the acetyl congener of fosmidomycin, was shown to be approximately twice as active against *P. falciparum* in vitro, as well as in a *P. vinckei* mouse model.[4]

Because of this promising antimalarial activity, fosmidomycin received considerable attention, and recent clinical trials conducted in Gabon and Thailand confirmed the potential of fosmidomycin as an antimalarial drug.[5,6]These important discoveries stimulated research on the chemical synthesis of

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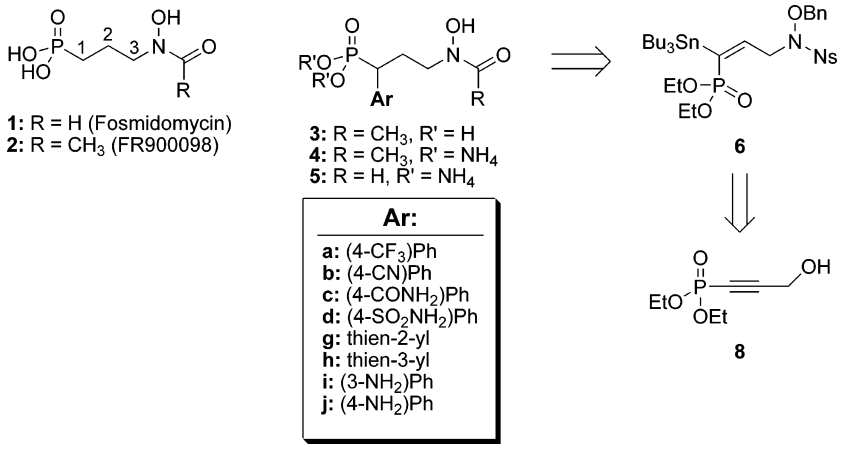
CHART 1. Target Fosmidomycin and FR900098 Analogues and Retrosynthetic Plan Involving Key Intermediate 6



fosmidomycin analogues. Structural changes of fosmidomycin and FR900098 could provide essential information on the structure−activity relationship and produce improved leads for the treatment of malaria. Mostly, attention was focused on the modification of the phosphonate and hydroxamate moieties, yielding, on one hand, various phosphonate prodrugs aimed to enhance the oral bioavailability, and on the other hand, modifications of the retrohydroxamate moiety into a hydroxamic acid, among others.[7−11] Structural variations of the three-carbon spacer, however, are scarce. Our group recently reported the synthesis of conformationally restricted analogues in which the carbon spacer was part of a cyclopropyl or cyclopentyl ring.[12,13]

To date, the most promising modifications of the carbon spacer consist of the introduction of an aryl substituent in α-position of fosmidomycin or FR900098.[14] Recently, we identified the α-3,4-dichlorophenyl-substituted fosmidomycin analogue which exhibits a 12 times stronger *in vitro* antimalarial activity (Dd2 and 3D7 strains of *P. falciparum*) compared to fosmidomycin. Lately, Kurz and co-workers evaluated the effect of α-substituents on pivaloyloxymethyl ester prodrugs of fosmidomycin and FR900098.[15,16] The α-methyl and phenyl analogues proved almost equipotent and the 3,4-difluorophenyl analogue slightly better than the FR900098 prodrug in inhibiting growth of a 3D7 *P. falciparum* strain.[15] Incorporation of an arylmethyl moiety in α-position generally resulted in a marked decrease in activity.[16] Unfortunately, the strategy used to synthesize the α-aryl-substituted analogues involved a *de novo* synthetic approach, which only permitted synthesis of a limited number of examples. To further explore the structure−activity relationship of this class, a strategy involving the incorporation of the aryl moiety at a later stage in the synthesis would be desirable.

Results and Discussion

Here we describe a highly efficient divergent synthesis of a series of α-aryl-substituted fosmidomycin analogues **3**−**5**, involving the α-tributylstannyl derivative **6** as a key intermediate (Chart 1). The vinylic tributyltin functionality of this intermediate was subjected to a palladium(0)-catalyzed Stille coupling with a variety of commercially available or readily synthesized aryl iodides.

The synthesis of said key intermediate **6** started with the conversion of the THP-protected propargyl alcohol **7** to diethyl (3-hydroxypropynyl)phosphonate (**8**) in a yield of 64%.[17] Subsequently, the hydroxyl group of compound **8** was displaced by a protected hydroxylamino moiety under Mitsunobu conditions. Nucleophilic substitution of the corresponding tosylate proved unsuccessful because of 1,4-addition of the nucleophile to the alkynyl phosphonate. Although extensive examples of successful Mitsunobu couplings are found in literature using carbamoyl-protected hydroxylamines,[18,19] introduction of a protected hydroxylamine moiety only succeeded using *N*-benzyloxy-2-nitrobenzenesulfonamide.[20] Indeed, the 2-nitrobenzenesulfonamide (or nosyl/Ns) moiety effectively enhances the acidity of the proton on the nitrogen, whereas in the case of the Boc-protected hydroxylamine, its acidity is not sufficient for reaction to occur.

Subsequently, a palladium(0)-catalyzed addition of Bu_3SnH to the triple bond of compound **9** afforded 1-tributylstannylpropenylphosphonate **6** as a single isomer in an excellent yield of 90%. The *E*-geometry was assigned based on the $^3J_{PH}$ coupling constant in the ^1H NMR spectrum (62.7 Hz), which is in accordance with the large coupling constant typically found

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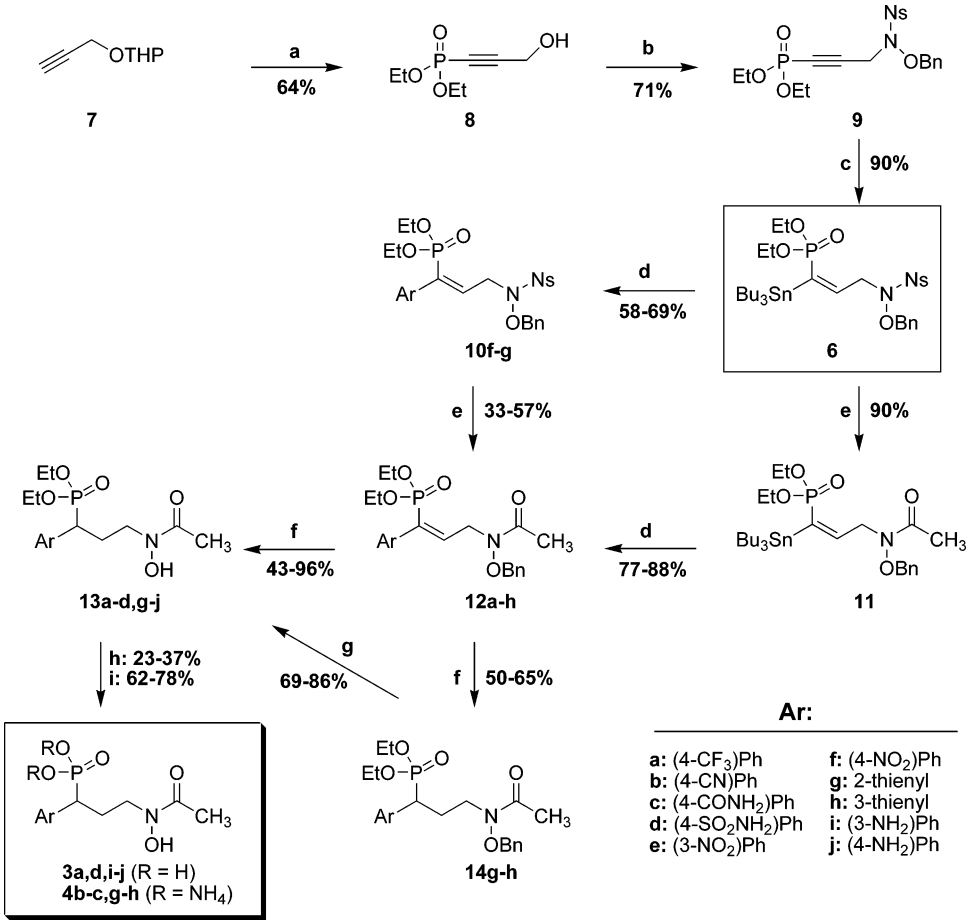
SCHEME 1. Synthesis of α-Aryl-Substituted *N*-Acetylfosmidomycin Analogues[a]



[a] Reagents and conditions: (a) (i) *n*-BuLi, THF, −78 °C; (ii) $(EtO)_2P(O)Cl$; HOAc; (iii) *p*-TsOH, MeOH, rt; (b) NsNHOBn, DEAD, PPh_3, THF, rt; (c) Bu_3SnH, $Pd(PPh_3)_4$, THF, 0 °C; (d) Ar−I, Pd_2dba_3•$CHCl_3$, $(2\text{-furyl})_3P$, CuI, NMP, rt; (e) (i) PhSH, K_2CO_3, MeCN, DMSO, 70 °C; (ii) Ac_2O; (f) H_2, Pd/C, Na_2CO_3, THF, rt; (g) BCl_3, CH_2Cl_2, −50 °C; (h) TMSBr, MeCN, rt; C-18 RP-HPLC; (i) TMSBr, MeCN, rt; $NH_4OH_{(aq)}$; CF11-cellulose chromatography.

for a vinylic proton trans to a phosphonate.[21] Optimization of the Stille coupling on the organotin derivative **6** afforded compounds **10f**,**g** in good yields using a combination of Pd_2-dba_3•$CHCl_3$, tri(2-furyl)phosphine, and anhydrous CuI in NMP.[22] Under these conditions, apparently no homo-coupling of **6** was detected and the geometry of the double bond was retained. Removal of the nosyl group of **10f**,**g** with thiophenol and K_2-CO_3 followed by *in situ* acylation, however, provided compounds **12f**,**g** in rather disappointing yields.[23] Apparently, the specific conditions used for the deprotection of the nosyl group are not fully compatible with the (substituents on the) aryl moiety. Therefore, we investigated if steps d and e could be interchanged. An additional advantage of moving the nosyl deprotection step forward in the synthesis is that it enhances the divergent character of the sequence. Thus, compound **6** was first deprotected and *in situ* acylated in excellent yield. Subsequent Stille coupling on the organotin compound **11** using the above-mentioned conditions led to compounds **12a**−**h** in good to excellent yields. The method proves highly compatible with a wide range of diverse functionalities, including heteroaromatic rings (**12g**,**h**). The current methodology requires the use of aryl iodides, since aryl bromides only induced homo-coupling of **6**. 4-Iodobenzamide, required for preparing **12c**, is not commercially available, but was readily synthesized from 4-iodobenzonitrile.

Reduction of the α,*β*-double bond, combined with removal of the benzyl protecting group on compounds **12a**−**f**, was accomplished using palladium on carbon (10% Pd/C) and anhydrous Na_2CO_3 in dry THF under slightly positive atmospheric H_2-pressure. Reaction times varied from 3 to 14 h to afford compounds **13a**−**d**,**i**,**j** in moderate to excellent yields. During this hydrogenation step little or no deoxygenation of the hydroxamic acid was observed, in contrast to previously reported N−O bond cleavage during catalytic hydrogenation.[13] In case of the conversion of compound **12b**, short reaction times were necessary to prevent reduction of the nitrile substituent on the phenyl ring. Reduction of the nitro group in compounds **12e**,**f** was unavoidable, resulting in amino-compounds **13i**,**j**. Furthermore, reduction of the 2-thienyl (**12g**) and 3-thienyl (**12h**) compounds was successfully accomplished only when using more than 1 equiv of Pd/C. Presumably the sulfur atom in the thiophene ring is poisoning the catalyst. Remarkably, simultaneous removal of the benzyl protecting group was not observed for these thienyl compounds, yielding the O-protected saturated compounds **14g**,**h**. Subsequent deprotection of the benzyl protecting group with BCl_3 afforded compounds **13g**,**h** in good to excellent yields. Finally, the phosphonate ester groups of the

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TABLE 1. Inhibition of Recombinant *E. coli* DXR by Analogues 3−5

compound	Ar	R	R	IC_{50} (μM)
3a	(4-CF_3)Ph	CH_3	H	0.360
4b	(4-CN)Ph	CH_3	NH_4	0.119
4c	(4-$CONH_2$)Ph	CH_3	NH_4	2.84
3d	(4-SO_2NH_2)Ph	CH_3	H	0.318
4g	2-thienyl	CH_3	NH_4	0.603
4h	3-thienyl	CH_3	NH_4	0.483
3i	(3-NH_2)Ph	CH_3	H	1.45
3j	(4-NH_2)Ph	CH_3	H	3.60
5	(4-CN)Ph	H	NH_4	0.054
fosmidomycin (**1**)	H	H	Na	0.056

α-aryl FR900098 analogues **13a−d**,**g**,**j** were cleaved with TMSBr. Two different methods for purification of the final phosphonic acid compounds were used. The first, more traditional method involves removal of the solvent, addition of water, lyophilization of the solution, and purification of the solids via reversed phase chromatography. This method was used for the purification of compounds **3a**,**d**,**i**,**j** but suffers from some serious drawbacks. First, the resulting phosphonic acids are only sparingly soluble in water or MeOH. Second, the C-18 reversed phase column used for purification strongly absorbs the phosphonic acids **3a**,**d**,**i**,**j**, resulting in a low recovery. This pronounced retention was not observed for more polar fosmidomycin analogues,[12] hence it could be correlated to the affinity of the aryl moiety for the stationary phase of the HPLC column. Third, the C-18 HPLC column used only allows for a maximum loading of 30 mg of crude material each time, which is very time-consuming for the purification of larger amounts of crude analogues. Therefore, a second methodology was investigated, comprising removal of the solvent and addition of water followed by dropwise addition of a 5% aqueous NH_4OH solution until pH 8−9 was reached. Subsequently, the solution was lyophilized and the product was purified via column chromatography using Whatman CF11-cellulose.[24] LC-MS analysis of the crude solids before chromatographic purification only showed NH_4Br (resulting from a reaction of TMSBr with NH_4OH) as a contaminant. The CF11-cellulose proved very effective in separating this contaminant from compounds **4b**,**c**,**g**,**h**, which were isolated in high yields due to the low affinity for the stationary phase. Additionally the resulting bis-ammonium salts of the α-aryl phosphonates showed excellent solubility in water.

Because of the difficulties associated with the handling of *P. falciparum* DXR, we investigated the ability of the synthesized α-aryl-substituted FR900098 analogues **3a**,**d**,**i**,**j** and **4b**,**c**,**g**,**h** to inhibit the highly homologous recombinant *E. coli* DXR. The conversion of DOXP to MEP by the enzyme was determined in an assay based on the NADPH dependency of the reaction.[25] With respect to the slow, tight-binding properties of fosmidomycin and probably most of its derivatives the inhibitors were preincubated with the enzyme and NADPH before the reaction was started by the addition of DOXP.[7,11,26] The results are summarized in Table 1. Compared to fosmidomycin, all synthesized analogues were found to be weaker inhibitors of *E. coli* DXR. Analysis of the order of DXR inhibitory potency (**4b** > **3d** > **3a** > **3j**) suggests that activity is Hammett σ^+-controlled (4-CN > 4-SO_2NH_2 > 4-CF_3 > 4-NH_2). A similar trend was observed for the previously reported α-phenyl-substituted analogues.[14] The weak inhibitory activity of the *p*-carbamoylphenyl analogue **4c** of *p*-carbamoylphenyl analogue **4c** illustrates that the activity pattern is not exclusively Hammett σ^+-controlled. Also, incorporation of a 2- or 3-thienyl moiety in analogues (**4g**,**h**) negatively affects the inhibitory potency.

In previous studies, the α-aryl-substituted *N*-formyl-fosmidomycin analogues were generally superior DXR inhibitors as compared to their *N*-acetyl counterparts. Hence, we decided to synthesize the *N*-formyl congener of **4b**, the most potent *N*-acetyl analogue in the present study. Toward the synthesis of **5** the above-mentioned strategy proved equally useful upon substituting *in situ* N-acetylation for N-formylation, using a mixture of HCOOH and 1,1′-carbonyldiimidazole.[13] (Scheme 2).

In the enzyme assay (Table 1), compound **5** was approximately twofold more active than its acetyl congener **4b** and equally active to fosmidomycin **(1)**. More importantly, an in vitro antimalarial assay using intraerythrocytic stages of the *P. falciparum* D2d strain, proved that the activities of **4b** (IC_{50} = 0.27 μM) and **5** (IC_{50} = 0.27 μM) surpass the activity of fosmidomycin (IC_{50} = 1.1 μM) and even FR900098 (IC_{50} = 0.49 μM) to inhibit parasite growth.

In conclusion, a highly accessible, divergent procedure for the preparation of α-aryl-substituted fosmidomycin analogues **3−5** with varying substituents was developed using a palladium(0)-catalyzed Stille coupling as a key step. The required organotin precursor **6** was prepared regio- and stereoselectively in four steps from protected propargyl alcohol **7**, using a Pd(0)-catalyzed addition of tributyltin hydride as a key step. Two compounds (**4b** and **5**), both possessing an α-4-cyanophenyl group, exhibited a promising *in vitro* inhibitory activity for recombinant *E. coli* DXR comparable to that of fosmidomycin and were even found to show a higher activity than fosmidomycin in an *in vitro* test against *P. falciparum* D2d strain.

Experimental Section

Diethyl 3-Hydroxy-propynylphosphonate (8). A 1.6 M *n*-BuLi in hexanes (47.6 mL, 71.3 mmol) was added dropwise to a stirred solution of tetrahydro-2-(2-propynyloxy)-2*H*-pyran (**7**, 10 g, 71.3 mmol) in dry THF (100 mL) at −78 °C. After stirring for 30 min at the same temperature, a solution of diethyl chlorophosphate (11.4 mL, 78.5 mmol) in dry THF (100 mL) was added dropwise, after which the mixture was stirred for another 30 min at −78 °C. The reaction was quenched with saturated aqueous NH_4Cl (100 mL), and the aqueous layer was extracted three times with Et_2O (100 mL). The combined organic layers were dried on anhydrous $MgSO_4$ and filtered, and the solvents were removed under reduced pressure. The residual oil was dissolved in MeOH (200 mL), *p*-TsOH (1.36 g, 7.13 mmol) was added, and the reaction mixture was stirred overnight at rt. The solvent was removed under reduced pressure, and the residual oil was purified via column chromatography (pentane/acetone: 3/2) yielding 8.75 g of a colorless oil (64%). R_f 0.30 (pentane/acetone: 3/2); ^{1}H NMR (300.13 MHz, $CDCl_3$) δ 1.36 (6H, td, J = 7.1 and 0.6 Hz), 2.89 (1H, br s), 4.16 (4H, dq, J = 8.3 and 7.0 Hz), 4.35 (2H, d, J = 3.7 Hz); ^{13}C NMR (75.47 MHz, $CDCl_3$) δ 16.0 (CH_3, $^3J_{PC}$ = 7.1 Hz), 50.6 (OCH_2, $^3J_{PC}$ = 4.4 Hz), 63.5 (OCH_2, $^2J_{PC}$ = 5.5 Hz), 76.3 (≡C−P, $^1J_{PC}$ = 298.6 Hz), 100.0 (≡C, $^2J_{PC}$ = 50.5 Hz); ^{31}P NMR (121.50 MHz, $CDCl_3$) δ −6.7; ESMS *m*/*z* 193 $[M + H]^+$.

Diethyl 3-[*N*-Benzyloxy,*N*-(2-nitrobenzenesulfonyl)amino]-propynylphosphonate (9). To a stirred solution of alcohol **8** (6.58

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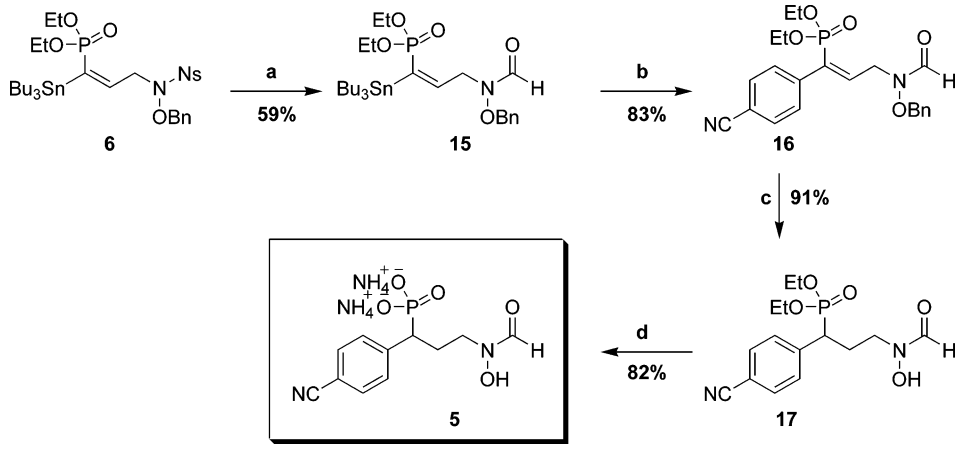
SCHEME 2. Synthesis of α-Aryl-Substituted Fosmidomycin Analogue 5[a]



[a] Reagents and conditions: (a) (i) PhSH, K_2CO_3, MeCN, DMSO, 70 °C; (ii) 1,1′-carbonyldiimidazole, HCOOH, 0 °C; (b) 4-iodobenzonitrile, Pd_2dba_3•$CHCl_3$, $(2\text{-furyl})_3P$, CuI, NMP, rt; (c) H_2, Pd/C, Na_2CO_3, THF, rt; (d) TMSBr, MeCN, rt; $NH_4OH_{(aq)}$; CF11-cellulose chromatography.

g, 34.2 mmol), *N*-benzyloxy,*N*-(2-nitrobenzene)sulfonamide (11.5 g, 37.3 mmol), and PPh_3 (9.8 g, 37.3 mmol) in dry THF (180 mL) was added a solution of DEAD (6.91 mL 85%, 37.3 mmol) in dry THF (90 mL) over 1 h at rt. The mixture was stirred for 20 h at room temperature. The solvent was removed under reduced pressure, and the residual oil was purified via column chromatography (column 1: pentane/CH_2Cl_2/acetone: 2/7/1, column 2: Et_2O → CH_2Cl_2/acetone: 1/1), yielding 11.7 g of a thick amber oil (71%). R_f 0.40 (pentane/CH_2Cl_2/acetone: 2/7/1); ^{1}H NMR (300.13 MHz, $CDCl_3$) δ 1.29 (6H, dt, J = 7.1 and 0.6 Hz), 3.99−4.06 (4H, m), 4.18 (2H, d, J = 3.9 Hz), 5.15 (2H, s), 7.36−7.45 (5H, m), 7.56 (1H, dd, J = 7.8 and 1.2 Hz), 7.67 (1H, app dt, J = 7.7 and 1.3 Hz), 7.78 (1H, app dt, J = 7.7 and 1.5 Hz), 8.04 (1H, dd, J = 7.9 and 1.3 Hz); ^{13}C NMR (75.47 MHz, $CDCl_3$) δ 16.0 (CH_3, $^4J_{PC}$ = 7.2 Hz), 43.1 (NCH_2, $^3J_{PC}$ = 5.5 Hz), 63.3 (OCH_2, $^3J_{PC}$ = 5.5 Hz), 76.1 (≡C−P, $^1J_{PC}$ = 293.7 Hz), 80.6 (OCH_2), 91.9 (≡C, $^2J_{PC}$ = 51.0 Hz), 123.9 (=CH), 126.O (=C), 128.7 (=CH), 129.2 (=CH), 129.9 (=CH), 131.4 (=CH), 133.2 (=CH), 134.2 (=C), 135.6 (=CH), 149.5 (=C); ^{31}P NMR (121.50 MHz, $CDCl_3$) δ 8.2; Exact mass (ESI-MS): calculated for $C_{20}H_{23}N_2O_8PS$ $[M + H]^+$ 483.0991; found 483.0965.

Diethyl (*E*)-3-[*N*-(Benzyloxy),*N*-(2-nitrobenzenesulfonyl)-amino]-1-(tributylstannanyl)-propenylphosphonate (6). To a cooled (0 °C) solution of **9** (10.4 g, 21.6 mmol) and $Pd(PPh_3)_4$ (474 mg, 0.430 mmol) in dry THF (100 mL) was added Bu_3SnH (6.28 g, 21.6 mmol) over a period of 40 min. The reaction mixture was stirred at 0 °C for 1 h and then allowed to warm to room temperature in 2 h. The solvent was removed and the crude oil was purified by column chromatography (pentane/acetone: 3/1), yielding 15 g of an amber oil (90%). R_f 0.31 (pentane/acetone: 3/1); ^{1}H NMR (300.13 MHz, $CDCl_3$) δ 0.87 (9H, t, J = 7.2 Hz), 0.93−0.98 (6H, m), 1.19 (6H, t, J = 7.0 Hz), 1.26−1.35 (6H, m), 1.42−1.52 (6H, m), 3.88−3.95 (4H, m), 4.39−4.41 (2H, m), 5.06 (2H, s), 6.53 (1H, dt, J = 62.7 and 5.5 Hz), 7.33−7.35 (5H, m), 7.54 (1H, d, J = 7.7 Hz), 7.62 (1H, app t, J = 7.6 Hz), 7.71 (1H, app t, J = 7.5 Hz), 8.04 (1H, d, J = 8.0 Hz); ^{13}C NMR (75.47 MHz, $CDCl_3$) δ 10.8 (CH_2), 13.6 (CH_3), 27.3 (CH_2), 28.8 (CH_2), 55.2 (NCH_2, $^3J_{PC}$ = 13.8 Hz), 61.1 (OCH_2, $^3J_{PC}$ = 5.5 Hz), 80.2 (OCH_2), 123.8 (=CH), 126.6 (=C), 128.5 (=CH), 128.9 (=CH), 129.6 (=CH), 131.1 (=CH), 132.6 (=CH), 136.2 (=C), 136.4 (=C−P, $^1J_{PC}$ = 247.6 Hz), 149.8 (=C), 152.1 (=CH, $^2J_{PC}$ = 1.7 Hz); ^{31}P NMR (121.50 MHz, $CDCl_3$) δ 21.4; Exact mass (ESI-MS): calculated for $C_{32}H_{51}N_2O_8PSSn$ $[M(^{120}Sn) + H]^+$ 797.2024; found 797.1999.

Diethyl 3-[*N*-Benzyloxy,*N*-(2-nitrobenzenesulfonyl)amino]-1-(4-nitrophenyl)-propenylphosphonate (10f). $(2\text{-Furyl})_3P$ (12 mg, 0.051 mmol) was added to a solution of Pd_2dba_3•$CHCl_3$ (6 mg, 0.0065 mmol) in anhydrous NMP (2 mL), and the mixture was stirred for 1 h at rt. A solution of **6** (216 mg, 0.280 mmol) and 1-iodo-4-nitrobenzene (62 mg, 0.249 mmol) in anhydrous NMP (5 mL) was added dropwise over a 15 min period. After the addition of anhydrous CuI (46 mg, 0.280 mmol), the reaction mixture was stirred overnight protected from light. EtOAc (100 mL) was added, and the organic layer was washed three times with 5% NH_4OH (10 mL) and three times with saturated aqueous NaCl (10 mL). The organic layer was dried over anhydrous $MgSO_4$ and filtered, and the solvents were removed under reduced pressure. The residual oil was purified by column chromatography (pentane/CH_2Cl_2/acetone: 3/1/1) yielding 98 mg of a pale yellow oil (58%). R_f 0.48 (pentane/acetone: 1/1); ^{1}H NMR (300.13 MHz, $CDCl_3$) δ 1.16 (6H, t, J = 7.0 Hz), 3.92 (2H, ddq, J = 10.4, 8.4 and 7.1 Hz), 4.01 (2H, ddq, J = 10.3, 8.1 and 7.0 Hz), 4.47 (2H, dd, J = 5.7 and 3.3 Hz), 5.01 (2H, s), 6.05 (1H, dt, J = 46.0 and 6.0 Hz), 7.16−7.33 (7H, m), 7.61 (1H, dd, J = 7.7 and 1.4 Hz), 7.72 (1H, app dt, J = 7.7 and 1.4 Hz), 7.79 (1H, app dt, J = 7.7 and 1.5 Hz), 8.1 (1H, dd, J = 8.0 and 1.4 Hz), 8.14 (2H, d, J = 8.4 Hz); ^{13}C NMR (75.47 MHz, $CDCl_3$) δ 16.1 (CH_3, $^3J_{PC}$ = 6.6 Hz), 53.1 (NCH_2, $^3J_{PC}$ = 5.4 Hz), 62.3 (OCH_2, $^3J_{PC}$ = 6.0 Hz), 79.6 (OCH_2), 123.4 (=CH), 123.9 (=CH), 126.0 (=C), 128.5 (=CH), 128.8 (=CH, $^3J_{PC}$ = 1.1 Hz), 129.0 (=CH), 130.1 (=CH), 131.3 (=CH), 132.6 (=C−P, $^1J_{PC}$ = 176.2 Hz), 132.7 (=CH), 134.8 (=C) 135.2 (=CH), 145.3 (=C, $^2J_{PC}$ = 10.2 Hz), 145.8 (=CH, $^2J_{PC}$ = 8.2 Hz), 147.3 (=C, $^5J_{PC}$ = 1.1 Hz), 149.9 (=C); ^{31}P NMR (121.50 MHz, $CDCl_3$) δ 13.2; Exact mass (ESI-MS): calculated for $C_{26}H_{28}N_3O_{10}PS$ $[M + H]^+$ 606.1311; found 606.1301.

Diethyl (*E*)-3-[*N*-Acetyl,*N*-(benzyloxy)amino)-1-(tributylstannyl)-propenylphosphonate (11). To a stirred solution of **6** (15 g, 19.4 mmol) and anhydrous K_2CO_3 (10.8 g, 77.8 mmol) in MeCN (500 mL) containing dry DMSO (10 mL) was added PhSH (6 mL, 58.3 mmol) over a 30 min-period at 70 °C. After stirring for 1 h at the same temperature, Ac_2O (18.3 mL, 195 mmol) was added and the reaction was stirred for another 30 min. After cooling to room temperature, the reaction was quenched with saturated $NaHCO_3$. The aqueous layer was extracted with CH_2Cl_2; the combined organic fractions were dried over anhydrous $MgSO_4$, filtered, and concentrated under vacuum. The residual oil was purified via column chromatography (pentane/CH_2Cl_2/acetone: 2/1/1) yielding 11 g of a yellow oil (90%). R_f 0.35 (pentane/CH_2Cl_2/acetone: 3/1/1); ^{1}H NMR (300.13 MHz, $CDCl_3$) δ 0.85 (9H, t, J = 7.3 Hz), 0.94−0.99 (6H, m), 1.22−1.34 (12H, m), 1.41−1.51 (6H, m), 2.07 (3H, s), 3.94−4.11 (4H, m), 4.86 (2H, s), 4.89−4.99 (2H, m), 6.48 (1H, dt, J = 63.5 and 5.9 Hz); ^{13}C NMR (75.47 MHz, $CDCl_3$) δ 10.6 (CH_2), 13.6 (CH_3), 16.4 (CH_3, $^3J_{PC}$ = 6.6 Hz), 20.5 (CH_3), 27.3 (CH_2), 28.7 (CH_2), 57.1 (NCH_2), 61.1 (OCH_2, $^2J_{PC}$ = 5.5 Hz), 76.0 (OCH_2), 128.6 (=CH), 128.9 (=CH), 129.5 (=CH), 134.4 (=C),

135.1 (=C−P, $^1J_{PC}$ = 133.4 Hz), 155.0 (=CH), 172.1 (N−C=O); Exact mass (ESI-MS): calculated for $C_{28}H_{50}NO_5PSn$ [M(^{120}Sn) + H]$^+$ 632.2527; found 632.2528.

Diethyl (*Z*)-3-[*N*-Acetyl,*N*-(benzyloxy)amino]-1-(4-trifluoromethylphenyl)-propenylphosphonate (12a). (2-Furyl)$_3$P (27.2 mg, 0.116 mmol) was added to a solution of Pd_2dba_3·$CHCl_3$ (13.6 mg, 0.0149 mmol) in anhydrous NMP (3 mL), and the mixture was stirred at rt for 1 h. A solution of **11** (400 mg, 0.634 mmol) and 1-iodo-4-trifluoromethylbenzene (154 mg, 0.566 mmol) in anhydrous NMP (5 mL) was added over a period of 15 min. After the addition of anhydrous CuI (104 mg, 0.634 mmol), the reaction mixture was stirred overnight protected from light. EtOAc (200 mL) was added and the organic layer was washed three times with 5% NH_4OH (24 mL) and three times with saturated aqueous NaCl (24 mL). The organic layer was dried over anhydrous $MgSO_4$, filtered, and concentrated under vacuum. The residual oil was purified by column chromatography (pentane/acetone: 55/45) to yield 228 mg of a pale yellow oil (88%). R_f 0.43 (pentane/acetone: 3/2); ^{1}H NMR (300.13 MHz, $CDCl_3$) δ 1.24 (6H, t, J = 7.1 Hz), 2.09 (3H, s), 3.98−4.18 (4H, m), 4.91 (2H, s), 5.06 (2H, dd, J = 5.8 and 2.9 Hz), 6.46 (1H, dt, J = 47.1 and 6.4 Hz), 7.35−7.41 (5H, m), 7.43 (2H, d, J = 8.1 Hz), 7.56 (2H, d, J = 8.4 Hz); ^{13}C NMR (75.47 MHz, $CDCl_3$) δ 16.2 (CH_3, $^3J_{PC}$ = 6.6 Hz), 20.5 (CH_3), 45.3 (NCH_2), 62.2 (OCH_2, $^2J_{PC}$ = 5.5 Hz), 76.4 (OCH_2), 123.9 (CF_3, q, $^1J_{FC}$ = 286.5 Hz), 128.5 (=CH, q, $^3J_{FC}$ = 3.4 Hz), 128.7 (=CH), 129.0 (=CH), 129.4 (=CH), 129.8 (=C, dq, $^2J_{FC}$ = 32.0 Hz, $^5J_{PC}$ = 1.1 Hz), 132.9 (=C−P, $^1J_{PC}$ = 196.5 Hz), 134.0 (=C), 142.5 (=C, $^2J_{PC}$ = 11.5 Hz), 147.4 (=CH, $^2J_{PC}$ = 10.5 Hz), 172.6 (N−C=O); ^{31}P NMR (121.50 MHz, $CDCl_3$) δ 14.3; ESMS m/z 486 [M + H]$^+$.

Diethyl 3-[*N*-Acetyl,*N*-(hydroxy)amino]-1-(4-trifluoromethylphenyl)-propylphosphonate (13a). To a solution of **12a** (266 mg, 0.547 mmol) in dry THF (8.5 mL) were added anhydrous Na_2CO_3 (174 mg, 1.64 mmol) and Pd/C (133 mg, 10%). The suspension was hydrogenated at room temperature and at a slightly positive atmospheric pressure for 4 h. The catalyst was removed by filtration through celite, which was washed with portions of CH_2Cl_2. The filtrate was evaporated under reduced pressure and the residual oil was purified by column chromatography (CH_2Cl_2/MeOH: 94/6) yielding a colorless oil (85%). R_f 0.29 (CH_2Cl_2/acetone: 1/1); ^{1}H NMR (300.13 MHz, $CDCl_3$) δ 1.19 (3H, t, J = 7.1 Hz), 1.23 (3H, t, J = 7.1 Hz), 2.12 (3H, s), 2.14−2.61 (2H, m), 3.18 (1H, dt, J = 23.1 and 6.1 Hz), 3.36 (1H, dt, J = 14.2 and 4.8 Hz), 3.44−4.02 (5H, m), 7.40 (2H, d, J = 7.7 Hz), 7.57 (2H, d, J = 7.9 Hz), 9.44 (1H, br s); ^{13}C NMR (75.47 MHz, $CDCl_3$) δ 16.2 (CH_3, $^3J_{PC}$ = 6.0 Hz), 20.5 (CH_3), 27.9 (CH_2, $^2J_{PC}$ = 2.2 Hz), 41.0 (P−CH, minor, $^1J_{PC}$ = 139.9 Hz), 41.8 (P−CH, major, $^1J_{PC}$ = 137.2 Hz), 46.2 (NCH_2, major, $^3J_{PC}$ = 10.4 Hz), 46.5 (NCH_2, minor, $^3J_{PC}$ = 15.9 Hz), 62.8 (OCH_2, $^2J_{PC}$ = 7.2 Hz), 63.2 (OCH_2, $^2J_{PC}$ = 7.7 Hz), 121.3 (CF_3, q, $^1J_{FC}$ = 273.2 Hz), 125.5 (=CH), 129.5 (=CH, $^3J_{PC}$ = 6.6 Hz), 129.6 (=C, dq, $^2J_{FC}$ = 33.2 Hz, $^5J_{PC}$ = 2.2 Hz), 140.8 (=C, $^2J_{PC}$ = 7.7 Hz), 172.5 (N−C=O); ^{31}P NMR (121.50 MHz, $CDCl_3$) δ 26.6, 28.7 (minor, major); Exact mass (ESI-MS): calculated for $C_{16}H_{23}F_3NO_5P$ [M + H]$^+$ 398.1344; found 398.1331.

Diethyl 3-[*N*-Acetyl,*N*-(benzyloxy)amino]-1-(thien-2-yl)-propylphosphonate (14g). The title compound was prepared according to the procedure described for **13a**, but using 1.2 eq of 10% Pd/C, resulting in a thick gray oil (50%). R_f 0.44 (pentane/acetone: 1/1); ^{1}H NMR (300.13 MHz, $CDCl_3$) 1.15 (3H, t, J = 7.0 Hz), 1.26 (3H, t, J = 7.2 Hz), 2.03 (3H, s), 2.12−2.26 (1H, m), 2.40−2.54 (1H, m), 3.39 (1H, ddd, J = 22.9, 11.3 and 3.7 Hz), 3.52 (1H, ddd, J = 14.3, 8.5 and 4.9 Hz), 3.66−3.73 (1H, m), 3.77−4.12 (4H, m), 4.71 (1H, br s), 4.81 (1H, br s), 6.95−7.00 (2H, m), 7.20−7.23 (1H, m), 7.29−7.27 (5H, m); ^{13}C NMR (75.47 MHz, $CDCl_3$) δ 16.3 (CH_3, $^3J_{PC}$ = 6.0 Hz), 16.4 (CH_3, $^3J_{PC}$ = 6.0 Hz), 20.5 (CH_3), 28.5 (CH_2, $^2J_{PC}$ = 1.7 Hz), 33.6 (P−CH, minor, $^1J_{PC}$ = 139.8 Hz), 37.5 (P−CH, major, $^1J_{PC}$ = 143.8 Hz), 43.7 (NCH_2), 61.5 (OCH_2, $^2J_{PC}$ = 6.6 Hz, major), 61.6 (OCH_2, $^2J_{PC}$ = 6.6 Hz, minor), 62.3 (OCH_2, $^2J_{PC}$ = 7.1 Hz, major), 62.9 (OCH_2, $^2J_{PC}$ = 7.1 Hz, minor), 76.2 (OCH_2, major), 76.4 (OCH_2, minor), 124.8 (=CH, $^5J_{PC}$ = 2.8 Hz), 126.9 (=CH,$^3J_{PC}$ = 3.3 Hz), 127.0 (=CH, $^4J_{PC}$ = 8.2 Hz), 128.7 (=CH), 128.9 (=CH), 129.2 (=CH), 134.4 (=CH), 137.4 (=C, $^2J_{PC}$ = 8.8 Hz), 171.2 (N−C=O); ^{31}P NMR (121.50 MHz, $CDCl_3$) δ 25.9; Exact mass (ESI-MS): calculated for $C_{20}H_{28}NO_5PS$ [M + H]$^+$ 426.1504; found 426.1488.

Diethyl 3-[*N*-Acetyl,*N*-(hydroxy)amino]-1-(thien-2-yl)-propylphosphonate (13g). A 1 M solution of BCl_3 in hexanes (1.6 mL, 1.60 mmol) was added dropwise to a stirred solution of **14g** (169 mg, 0.397 mmol) in dry CH_2Cl_2 (7 mL) at −50 °C. After stirring for 30 min at −50 °C, the reaction was quenched with saturated $NaHCO_3$ (10 mL) and the reaction mixture was allowed to warm to rt. The aqueous layer was extracted with CH_2Cl_2, dried over anhydrous $MgSO_4$, and filtered, and the solvents were removed under reduced pressure. The residual oil was purified by column chromatography (CH_2Cl_2/MeOH: 94/6) to give the title compound as a thick gray oil (69%). R_f 0.19 (CH_2Cl_2/Acetone: 1/1); ^{1}H NMR (300.13 MHz, $CDCl_3$) δ 1.15−1.34 (6H, m), 2.14 (3H, s) 2.17−2.60 (2H, m), 3.46 (1H, dt, J = 22.8 and 5.3 Hz), 3.61−4.12 (6H, m), 6.95−7.02 (2H, m), 7.20−7.22 (1H, m), 9.42 (1H, br s); ^{13}C NMR (75.47 MHz, $CDCl_3$) δ 16.3 (CH_3, $^3J_{PC}$ = 5.4 Hz), 20.6 (CH_3), 29.6 (CH_2, major, $^2J_{PC}$ = 3.3 Hz), 29.7 (CH_2, minor, $^2J_{PC}$ = 3.3 Hz), 37.1 (P−CH, $^1J_{PC}$ =142.2 Hz), 46.3 (NCH_2), 62.9 (OCH_2, $^2J_{PC}$ = 6.0 Hz), 63.4 (OCH_2, $^2J_{PC}$ = 7.7 Hz), 124.9 (=CH), 127.0 (=CH, $^4J_{PC}$ = 7.5 Hz), 127.2 (=CH), 138.1 (=C), 172.5 (N−C=O); ^{31}P NMR (121.50 MHz, $CDCl_3$) δ 25.6, 28.0 (minor,-major); Exact mass (ESI-MS): calculated for $C_{13}H_{22}NO_5PS$ [M + Na]$^+$ 358.0854; found 358.0842.

3-[*N*-Acetyl,*N*-(hydroxy)amino]-1-(4-trifluoromethylphenyl)-propylphosphonic Acid (3a). To a solution of **13a** (150 mg, 0.377 mmol) in dry MeCN (3.8 mL) was added dropwise TMSBr (497 μL, 3.77 mmol) at rt, and the mixture was stirred for 2 days at rt. The solvents were removed under reduced pressure, and the remaining traces of TMSBr were removed under high vacuum (0.05 mbar). The residual oil was dissolved in 2 mL of distilled water and lyophilized. The resulting solid was purified via reversed phase HPLC using a 5 mM NH_4OAc solution for 5 min followed by a gradient elution of 5 mM NH_4OAc solution to MeCN in 15 min. The appropriate fractions were lyophilized to give 23 mg of the phosphonic acid as a colorless hygroscopic solid (23%). ^{1}H NMR (300.13 MHz, D_2O) δ 1.75 (3H, minor, s), 2.01 (3H, major, s), 2.25−2.61 (2H, m), 3.00−3.18 (1H, m), 3.43−3.67 (2H, m), 7.46−7.64 (2H, m), 7.66−7.84 (2H, m); 19.3 (CH_3), 26.7 (CH_3, major), 26.9 (CH_3, minor), 43.5 (P−CH, minor, $^1J_{PC}$ = 131.1 Hz), 44.2-(P−CH, major, $^1J_{PC}$ = 130.0 Hz), 46.3 (NCH_2, $^3J_{PC}$ = 17.6 Hz), 121.9 (CF_3, q, $^1J_{FC}$ = 271.1 Hz) 125.3 (=CH, major), 125.6 (=CH, minor), 128.1 (=C, dq, $^2J_{FC}$ = 31.8 Hz, $^5J_{PC}$ = 3.3 Hz), 129.6 (=CH, $^3J_{PC}$ = 5.5 Hz), 142.8 (=C, minor), 143.0 (=C, major, $^2J_{PC}$ = 7.2 Hz), 173.7 (N−C=O, major)? 173.8 (N−C=O, minor); ^{31}P NMR (121.50 MHz, D_2O) δ 21.5; Exact mass (ESI-MS): calculated for $C_{12}H_{15}F_3NO_5P$ [M − H]$^-$ 340.0562; found 340.0564.

Bisammonium 3-[*N*-Acetyl,*N*-(hydroxy)amino]-1-(4-cyanophenyl)-propylphosphonate (4b). To a solution of **13b** (130 mg, 0.367 mmol) in dry MeCN (3.7 mL) was added dropwise TMSBr (484 μL, 3.67 mmol) at rt, and the mixture was stirred for 24 h at rt. The solvents were removed under reduced pressure, and the traces of TMSBr were removed under high vacuum (0.05 mbar). The residual oil was dissolved in 2 mL of Type I water, and the pH of the mixture was adjusted to 8−9 with a 5% NH_4OH solution. The solution was lyophilized, and the residual solid was purified by Whatman CF11 cellulose column chromatography (MeCN/$NH_4OH_{(aq,\ 1\ M)}$: 3/1). The fractions were assayed using cellulose TLC, and the spots were visualized under UV-light (365 nm) after dipping in a pinacryptol yellow solution (0.1% in H_2O) and drying the plate

under a stream of hot air.[27] The appropriate fractions were lyophilized, yielding 79 mg of a pale amber hygroscopic solid (66%). R_f 0.33 (cellulose TLC, MeCN/$NH_4OH_{(aq,1M)}$: 3/1); ^{1}H NMR (300.13 MHz, D_2O) δ 1.64 (3H, minor, s), 1.90 (3H, major, s), 2.12−2.49 (2H, m), 2.94 (1H, ddd, J = 22.0, 12.1 and 2.8 Hz), 3.35 (1H, app dt, J = 14.4 and 5.7 Hz), 3.50 (1H, ddd, J = 14.4, 8.4 and 6.1 Hz), 7.39 (2H, dd, J = 8.3 and 1.7 Hz), 7.65 (2H, d, J = 8.2 Hz); ^{13}C NMR (75.47 MHz, D_2O) δ 19.1 (CH_3, major), 19.3 (CH_3, minor), 26.5 (CH_2, major), 26.7 (CH_2, minor), 44.0 (P−CH, minor, $^1J_{PC}$ = 126.8 Hz), 44.5 (P−CH, major, $^1J_{PC}$ = 127.4 Hz), 46.1 (NCH_2, major, $^3J_{PC}$ = 17.1 Hz), 49.6 (NCH_2, minor, $^3J_{PC}$ = 17.0 Hz), 108.8 (=C, $^5J_{PC}$ = 3.3 Hz), 120.0 (C≡N), 129.8 (=CH, $^3J_{PC}$ = 6.1 Hz), 132.3 (=CH, major, $^4J_{PC}$ = 2.2 Hz), 132.6 (=CH, minor, $^4J_{PC}$ = 1.6 Hz), 145.1 (=C, $^2J_{PC}$ = 7.2 Hz), 173.6 (N−C=O); ^{31}P NMR (121.50 MHz, D_2O) δ 20.0, 20.5 (minor, major) Exact mass (ESI-MS): calculated for $C_{12}H_{21}N_4O_5P$ [M − 2(NH_4) + H]$^-$ 297.0640; found 297.0645.

Diethyl (*E*)-3-[*N*-(Benzyloxy),*N*-formylamino)-1-(tributylstannyl)-propenylphosphonate (15). To a stirred solution of **6** (3.04 g, 3.93 mmol) and anhydrous K_2CO_3 (728 mg, 5.23 mmol) in MeCN (100 mL), containing dry DMSO (2 mL), was added PhSH (404 μL, 3.93 mmol) over a 30 min period at 70 °C, and the reaction mixture was stirred overnight at the same temperature. The reaction mixture was then cooled to 0 °C, and the formylating solution [prepared in a separate flask by adding HCOOH (99%, 163 μL, 4.32 mmol) dropwise to a solution of 1,1′-carbonyldiimidazole (702 mg, 4.32 mmol) in dry CH_2Cl_2 (6.4 mL) at 0 °C for and stirring for 1 h] was added dropwise at 0 °C. The reaction mixture was stirred for 1 h at 0 °C. The reaction was quenched with saturated $NaHCO_3$, and the aqueous layer was extracted with CH_2Cl_2. The combined organic fractions were dried over anhydrous $MgSO_4$ and filtered, and the solvents were removed under reduced pressure. The residual oil was purified by column chromatography (pentane/CH_2Cl_2/acetone: 4/1/1) to give 1.42 g of the title compound as a yellow oil (59%). R_f 0.43 (hexane/acetone: 7/3); ^{1}H NMR (300.13 MHz, $CDCl_3$) δ 0.86 (6H, minor, t, J = 7.3 Hz), 0.87 (6H, major, t, J = 7.2 Hz), 0.95−1.00 (6H, m), 1.22−1.34 (12H, m), 1.41−1.57 (6H, m), 3.95−4.06 (6H, m), 4.71 (2H, minor, s), 4.87 (4H, major, m), 6.34−6.55 (1H, minor, m), 6.71 (1H, major, dt, J = 64.2 and 5.8 Hz), 7.27−7.35 (5H, m), 8.16 (1H, br s); ^{13}C NMR (75.47 MHz, $CDCl_3$) δ 10.6 (CH_2, minor), 10.7 (CH_2, major), 13.6 (CH_3, minor), 13.6 (CH_3, major), 16.3 (CH_3, minor, $^3J_{PC}$ = 6.6 Hz); 16.4 (CH_3, major, $^3J_{PC}$ =7.2 Hz), 27.2 (CH_2, minor) 27.3 (CH_2, major), 28.7 (CH_2 minor), 28.8 (CH_2, major), 45.7 (NCH_2, major, $^3J_{PC}$ = 15.4 Hz), 54.0 (NCH_2, minor, $^3J_{PC}$ = 13.2 Hz), 61.0 (OCH_2, minor, $^2J_{PC}$ = 5.5 Hz), 61.1 (OCH_2, major, $^2J_{PC}$ = 5.5 Hz), 76.1 (OCH_2, minor), 77.0 (OCH_2, major), 127.8 (=CH, minor), 128.2 (=CH, minor), 128.3 (=CH, minor), 128.6 (=CH, major), 129.0 (=CH, major), 129.5 (=CH, major), 133.9 (=C−P, $^1J_{PC}$ = 133.4 Hz), 137.7 (=C), 153.6 (=CH, minor), 157.8 (=CH, major), 162.6 (N−C=O); ^{31}P NMR (121.50 MHz, $CDCl_3$) δ 21.7, 22.4 (minor, major); Exact mass (ESI-MS): calculated for $C_{27}H_{48}NO_5PSn$ [M(^{120}Sn) + H]$^+$ 618.2370; found 618.2373.

DOXP Reductoisomerase Inhibition Assay. The assay was performed in a reaction mixture containing 100 mM Tris HCl (pH 7.5), 0.2% bovine serum albumin, 1 mM $MnCl_2$, 1 mM DOXP, 0.3 mM NADPH, and 1 μg/ml recombinant DOXP reductoisomerase of *E. coli*. The mixture without substrate was preincubated with a serial dilution of the test compounds on a 96-well plate at 30 °C for 5 min. The compounds had been dissolved in 100 mM Tris HCl (pH 7.5) and prediluted to the 10-fold final concentrations on a separate plate. The reaction was started by the addition of DOXP. The decrease of absorption was monitored at 340 nm.

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Supporting Information Available: Experimental details (including ^{1}H, ^{13}C, ^{31}P NMR, MS data) for intermediates and final products (**3d**,**i**,**j**, **4c**,**g**,**h**, **5**, **10g**, **12b**−**h**, **13b**−**d**,**h**−**j**, **14h**, **16**, and **17**), as well as full APT spectra of all new compounds. This material is available free of charge via Internet at http://pubs.acs.org.

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