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The New Metabolite (*S*)-Cinnamoylphosphoramide from *Streptomyces* sp. and Its Total Synthesis

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The tunicate-associated strain *Streptomyces* sp. JP90 produces the unprecedented metabolite cinnamoylphosphoramide (**1**) among several other compounds. Structure elucidation was accomplished by NMR spectroscopic studies and efficient total synthesis. The absolute configuration at phosphorus was determined by synthesis of both enantiomers of **1** performing a resolution of the corresponding diastereomeric phosphoramides of L-phenylalanine ethyl ester. Unusual cinnamic acid derivative **1** represents the first bacterial organophosphoramide. As it matches the Schrader's formula for insecticidal organophosphates, its biological activity was investigated. A weak inhibition of acetylcholinesterase was observed in in vitro tests, and water-soluble analogues of **1** were prepared.

Introduction

Marine organisms are a rich source of secondary metabolites many of which show unique structures and possess a wide range of pharmacological properties.[1] Natural products isolated from marine – and also terrestrial – macroorganisms are frequently assumed to actually be of bacterial origin. Occurring structural similarities to known metabolites from microorganisms, bacteria or non-related eukaryotes, underline this hypothesis.[2] For example, several bacteria were cultivated from tunicates and yielded structurally diverse metabolites; however, systematic studies of such bacterial communities and their metabolite patterns, as well as compound localizations, are rare.[3,4]

One of us (J. P.) and his coworkers identified a large community of Actinomycetes bacteria associated with the marine ascidian *Aplidium lenticulum* found in the Great Barrier Reef (Australia), which is a prolific source of new natural products.[5] Cultivations of one of the isolated strains *Streptomyces* sp. JP90 yielded several metabolites of low molecular weight, such as the new organophosphate cinnamoylphosphoramide (**1**; Figure 1). Its structure comprises a methyl cinnamate moiety *para*-substituted with an unusual phosphoramide methyl ester, which is unique among natural products. However, phosphoramides are known as synthetic anti-HIV pharmaceuticals [e.g., L-alaninyl-d4T-MP (**2**)] and antihepatitis C virus (HCV) drug candidates (e.g., azidoadenosin phosphoramides from the current promising "ProTide technology" approach).[6] Also, they are structurally related to organophosphate poisons, for example, the famous synthetic insecticide methyl paraoxon (**3**), an inhibitor of acetylcholinesterase (AChE).[7] Moreover, tetracoordinate organophosphates show high configurational stability and, therefore, are of great importance for asymmetric synthesis in modern organic chemistry.[8] On the contrary,

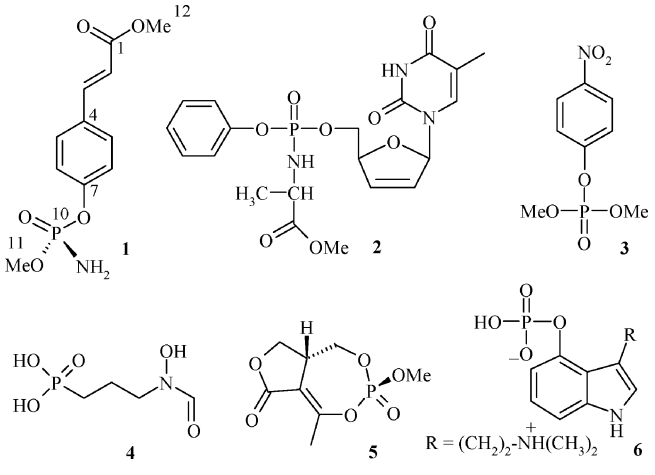

Figure 1. Structures of phosphorus-containing bioactive compounds.

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only very few such compounds originating from microbial secondary metabolism are described in the literature. The well-known herbicide bialaphos, the potent antimalarial fosmidomycin (**4**), and the strongly insecticidal cyclophostin (**5**) all originate from *Streptomyces* sp.[9] In addition, the indole alkaloid psilocybin (**6**) and its dephosphorylated analogue psilocin are ingredients of "magic mushrooms" and responsible for their hallucinogenic effect.[10]

Herein, we present a comprehensive study of new cinnamoylphosphoramide (**1**) comprising isolation, structure elucidation, total synthesis, and biological activity.

Results and Discussion

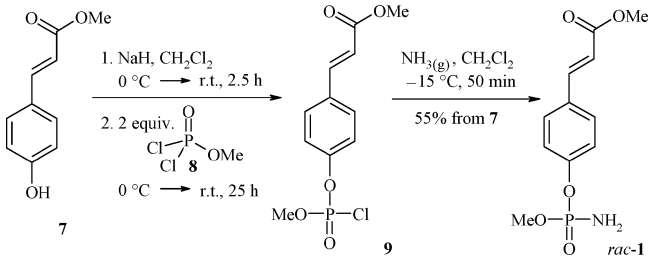
Isolation and Chemical Characterization

Fermentations of *Streptomyces* sp. (strain JP90) were initially performed in 300-mL shaking flasks for 96 h by using a complex medium (1% starch, 1% glucose, 1% glycerol, 0.25% cornsteep powder, 0.5% peptone). The culture filtrate was adsorbed on XAD-16 resin and elution with methanol yielded the crude extract. The metabolite pattern was analyzed,[11] and several substances were isolated by column chromatography on Sephadex LH-20/MeOH and on silica gel.[5] This led to pure compounds such as the immunosuppressive siderophore desferrioxamine E, the monoterpene rosiridol, the new compound 6-hydroxy-6-methylheptanoic acid, cyclo(tyrosylprolyl), *N*-acetyl tyramine, and 3-indolecarboxylic acid, which were identified by NMR spectroscopy, MS analysis, and database search.[12–16] However, an apparently unknown secondary metabolite aroused our interest. Pure colorless solid **1** was isolated from the crude extract in a yield of 1.2 mg L^{-1}.

The ESI mass spectrum of **1** showed an ion peak at *m*/*z* = 294 $[M + Na]^+$, and the molecular formula was determined by ESI HRMS to be $C_{11}H_{14}NO_5P$. Characteristic IR absorption peaks suggested an α,β-unsaturated ester (1716 cm^{-1}), an alkene (1602, 1508 cm^{-1}), and a P=O moiety (1250–1300 cm^{-1}). The UV spectra showed an absorption maximum at 283 nm with a significant bathochromic shift upon addition of HCl (316 nm) or NaOH (359 nm). The constitution of **1** was deduced from ^1H, ^{13}C, ^{31}P, and 2D NMR spectroscopic experiments (CD_2Cl_2). The ^1H NMR spectrum exhibited signals of 14 protons with two signals pointing at methoxy groups [δ_H = 3.76 (s), 3.79 (d, $^3J_{H,P}$ = 11.8 Hz) ppm]. The presence of a *para*-substituted methyl cinnamate was confirmed by two methine proton signals [δ_H = 6.37, 7.62 ppm, (*E*)-$^3J_{H,H}$ = 16.0 Hz] and four aromatic protons (δ_H = 7.23, 7.50 ppm). The ^{13}C NMR spectrum showed nine resonances, three of which appeared as doublets at δ = 53.9 ($^2J_{C,P}$ = 5.8 Hz), 121.0 ($^3J_{C,P}$ = 4.5 Hz), 152.7 ($^2J_{C,P}$ = 6.8 Hz) ppm and thus indicated $^{13}C,^{31}P$ coupling. In conclusion, compound **1** could be identified as methyl 4-(aminomethoxyphosphoryloxy)cinnamate, although an imido ester could not be finally excluded on the basis of all spectroscopic data.[17]

Total Synthesis

To unambiguously confirm the constitution of cinnamoylphosphoramide a total synthesis of *rac*-**1** was performed by starting from methyl 4-hydroxycinnamate (**7**), which was readily prepared by Heck reaction of 4-bromophenol with methyl acrylate (Scheme 1).[17] A one-pot procedure was envisioned for the attachment of the phosphoramide moiety involving consecutive phosphorylation, methoxylation, and amidation with aqueous ammonia, and phenol was used as a model substrate.[18] Different methods for its selective reaction with phosphoryl chloride were tested, yet all yielded complex mixtures of mono- and diphenoxylated species, which decomposed upon attempted separation by distillation.[19] Therefore, the reaction sequence was altered and methoxy phosphoryl dichloride (**8**) was used as starting material.[20] Slow addition of a prestirred mixture of sodium hydride and phenol **7** to this reagent yielded **9**,[21] which was immediately treated with gaseous ammonia to afford *rac*-cinnamoylphosphoramide (**1**) in 55% yield over the two steps from **7** (Scheme 1).[22]



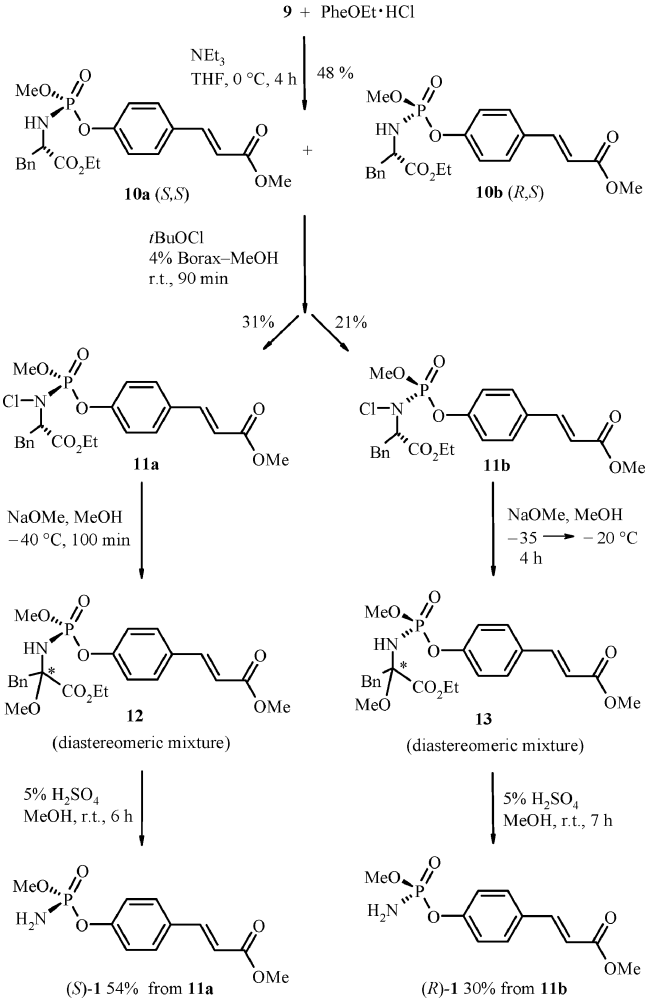
Scheme 1. Total synthesis of *rac*-**1**.

The aminomethoxyphosphoryloxy moiety proved to be highly sensible towards acidic and basic conditions, and decomposition to phenol **7** was observed during chromatography on silica gel. However, **1** could be successfully purified on reverse-phase (RP) silica gel. Comparison of the spectroscopic data confirmed the assigned structure of the first microbial organophosphoramide **1**.

Absolute Configuration

For the determination of the absolute configuration at phosphorus, the single enantiomers were both prepared following a protocol of Koizumi et al.[23] Treatment of racemic phosphoryl monochloride **9** with the hydrochloride of L-phenylalanine ethyl ester afforded a diastereomeric mixture of phosphoramides **10a**,**b** in a 13:7 *dr*, as judged from the integrals of the P–OMe signals in the ^1H NMR spectrum (Scheme 2).

Unfortunately, the diastereomers could not be separated by crystallization (e.g., from *n*-hexane) or various chromatographic methods including chromatography on silica gel ($CHCl_3$/MeOH; *n*-hexane/EtOAc/MeOH), reverse-phase silica gel (MeOH/water), chiral plates (MeOH/water; acetonitrile/water; MeOH/water/acetonitrile), or HPLC (Nucleosil C_8 column, acetonitrile/water). Therefore, the mixture of **10a**,**b** was subjected to *N*-chlorination with *tert*-butyl hypo-



Scheme 2. Resolution of racemic phosphoryl chloride **9** and preparation of (*S*)-**1** and (*R*)-**1**.

chlorite in 4% methanolic borax yielding chloro derivatives **11a**,**b**.[24] Both diastereomers were readily distinguishable by the ^{1}H NMR signals of the P–OMe group (δ = 3.03 and 3.93 ppm, *dr* 13:10) and by their R_f values on silica-gel plates (hexane/EtOAc, 2:1). A rapid flash column chromatography at 4 °C furnished pure **11a** and **11b** in yields of 31 and 21%, respectively.[25] This ratio does not reflect the original *dr*, as partial dechlorination of compounds **11a**,**b** occurred during chromatography leading to the formation of some starting material **10a**,**b**. Separated diastereomers **11a**,**b** were then treated with sodium methoxide to give N,O-acetals **12** from **11a**, and **13** from **11b** (Scheme 2).[26] These reactions destroyed the configurational integrity of the former amino acid, as they proceeded by initial elimination of HCl and thus formation of an *N*-phosphorylimine, which then undergoes addition of MeOH. Finally, cleavage of the C,N bonds in crude products **12** and **13** was effected by acid-catalyzed hydrolysis by employing 5% methanolic H_2SO_4, and purification by RP silica gel chromatography yielded (*S*)-**1** in 54% yield and (*R*)-**1** in 30% yield from **11a** and **11b**, respectively.

This configurational assignment to the synthetic enantiomers of cinnamoylphosphoramide (**1**) was performed by comparison of the physicochemical properties of compounds **11a**,**b** with those of the analogous diastereomeric phosphoramides **14a**,**b**, which have previously been prepared by Koizumi et al. (Figure 2).[23] A characteristic feature of both pairs of diastereomers is the significant difference between the chemical shifts of the P–OMe signals in the ^{1}H NMR spectra ($\Delta\delta$ = 0.8–0.9 ppm, Table 1).

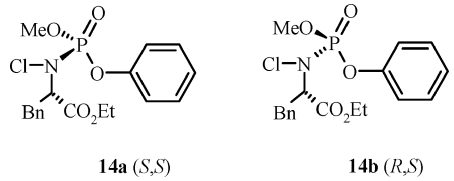


Figure 2. Analogous *N*-chlorophosphoramides **14a**,**b**.[23]

Table 1. Comparison of physicochemical properties of *N*-chlorophosphoramides **14a**,**b** and **11a**,**b**.

	14a[a]	**14b**[a]	**11a**	**11b**
Configuration	*S*,*S*	*R*,*S*	**X**,*S*	**Y**,*S*
^{1}H NMR, δ [ppm] P–OMe	2.98	3.80	3.03	3.93
R_f values on silica gel	0.67[b]	0.58[b]	0.54[c]	0.36[c]
Optical rotation values of respective hydrolysis product [°]	–13.6	+14.0	–6.6	+6.6
Deduced configurations **X** and **Y** at the phosphorus atom	*S*	*R*	*S*	*R*

[a] Data taken from refs.[23,27] [b] Benzene/EtOAc, 5:1. [c] Hexane/EtOAc, 2:1.

Moreover, in both pairs the diastereomers with the high-field shifted methoxy signal also have higher R_f values on silica gel. Another analogy arises from the optical rotation values of the cleaved phosphoramides, that is, of enantiomers **1** in the case of diastereomers **11a**,**b** and their respective phenoxy analogues from **14a**,**b**: the enantiomers obtained from the diastereomers with the high-field shifted methoxy signal exhibit negative rotation values. On the basis of X-ray crystal structure analysis of the dechlorinated derivative of **14b**, Koizumi et al. had assigned the (*R*,*S*) configuration to this diastereomer.[27] By analogy, the (*S*,*S*) and (*R*,*S*) configuration were assigned to diastereomers **11a** and **11b**, respectively. Thus, pure enantiomers **1** are (*S*)-(–)-cinnamoylphosphoramide and (*R*)-(+)-cinnamoylphosphoramide. Isolated metabolite **1** was compared with the synthetically prepared enantiomers by chiral HPLC analysis (Daicel Chiralpak IA column, *n*-hexane/2-propanol). Single chromatography runs with the three single compounds and with mixed samples represent experimental sets,[17] which unambiguously proved the identity of the natural product with the (*S*)-(–)–enantiomer **1**.

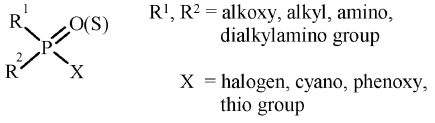
Biosynthesis

The carbon skeleton of cinnamoylphosphoramide (**1**) indicates biosynthesis by the shikimate pathway.[17] Within the scope of biosynthetic studies on other metabolites,

tracer experiments were carried out by adding the sodium salts of [1,2-$^{13}C_2$]acetate and [U-$^{13}C_3$]glycerol to the growing cultures of the strain *Streptomyces* sp. JP90 (12 h of continuous feeding starting 48 h after inoculation). The isolated metabolites were purified and analyzed by ^{13}C NMR spectroscopy. Whereas other compounds such as polyketides, amino acid derived metabolites, and terpenoids were intensively labeled,[5] no specific enrichments were detected for **1**. This might indicate that cinnamate **1** or precursors thereof were possibly produced within the first 48 h of fermentation.[17]

Biological Activity

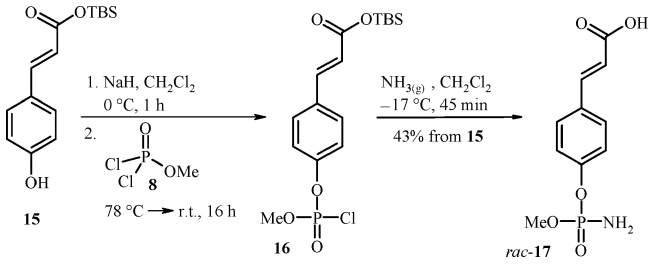
Cinnamoylphosphoramide (**1**) comprises an unusual natural phosphoramide methyl ester, which is very rare among natural products, and it entirely matches Schrader's formula describing the chemical structure of highly active organophosphate poisons and the resulting mode of action as strong electrophiles in acetylcholinesterase (AChE) inhibition (Figure 3).[28] The important structural motifs are the P,O double bound, basic substituents R^1 and R^2 such as alkoxy, alkyl, or amino groups, and a leaving group X such as a halide or phenoxy substituent, which provides the electrophilicity at the phosphorus atom as a prerequisite for the biological activity as an AChE inhibitor. On the basis of this analogy, we investigated the biological activity of **1**.



Phosphoramide **1**: R^1 = OMe, R^2 = NH_2, X = aryloxy

Figure 3. Schrader's formula.

In a cooperation with U. Holzgrabe et al., Ellmann tests were performed but revealed only very weak inhibition of AChE by methyl ester **1** (IC_{50} > 350 μM).[29] This was ascribed to its poor solubility in the used buffer solution, and thus, we decided to prepare free carboxylic acid **17** as a water-soluble analogue of **1**. This turned out to be quite challenging, as compound **1** undergoes fast dephosphorylations under acidic and basic conditions, and decomposition to phenol **7** was observed upon attempted ester hydrolysis with lithium hydroxide.[17] Enzymatic ester hydrolysis with different enzymes was investigated next, but the desired compound was not obtained.[17] A reduction–oxidation sequence was also examined for the transformation of **1** into the free acid, but treatment with $LiAlH_4$ just led to decomposition, whereas reaction of **1** with DIBAL-H at –78 °C merely furnished a mixture of the desired allylic alcohol of **1** and the dephosphorylated 4-[(*E*)-3-hydroxyprop-1-enyl] phenol.[17] Finally, synthesis of water-soluble analogue **17** was successfully accomplished by starting from TBS-ester **15** (TBS = *tert*-butyldimethylsilyl). In contrast to the synthesis of **1**, the addition order had to be reversed in that phosphoryl chloride **8** was added to the sodium salt of **15** at –78 °C (Scheme 3). After subsequent treatment with gaseous ammonia, desired acid **17** was obtained in 43% overall yield from **15**.



Scheme 3. Synthesis of water-soluble *rac*-**17**.

Ellmann tests performed with **17** again showed only very weak inhibition of AChE (IC_{50} > 350 μM), whereas control experiments with the aminoacridine tacrine gave the expected results (IC_{50} = 1.5 nM).[30] Moreover, the inhibition potential of **1** and **17** on butyryl cholinesterase (BChE) was investigated in collaboration with C. Meier et al. and revealed an IC_{50} = 250 μM for **1**, but no significant activity for **17**.

Cinnamoylphosphoramide (**1**) showed no antibacterial and antifungal activity in plate diffusions assays with *E. coli*, *B. subtilis*, *S. aureus*, and *C. albicans*. Phosphates and their derivatives are known as ubiquitous energy-rich intermediates in metabolic pathways and mediators for the activation of organic substances. Thus, it was assumed that metabolite **1** with its unusual phosphoramide moiety could be an activated biosynthetic precursor. To evaluate the biological function of **1** for the producer strain JP90, feeding experiments were performed by adding synthetic **1** and related cinnamic acid derivatives (e.g., methyl 4-hydroxycinnamate, cinnamic acid, methyl cinnamate) to the growing cultures (12 and 16 h after inoculation). The metabolite pattern was analyzed by methods of chemical screening (HPLC–MS–UV and TLC), yet revealed no detectable amounts of new metabolites, but production of already known compounds. Therefore, the biological function of cinnamoylphosphoramide (**1**) for the producer strain remains unclear, as for most other interesting Streptomycetes products, but might be of future interest in terms of investigations of chemical biology.

Conclusions

The new cinnamoylphosphoramide (**1**) is a structurally unusual microbial natural product from the tunicate-associated *Streptomyces* sp. strain JP90 due to its phosphoramide moiety. Although its constitution could be deduced from analysis of the isolated material, the elucidation of its configuration required a total synthesis of both enantiomers (*S*)-**1** and (*R*)-**1**. The evaluation of the biological activity of **1** was of special interest, as its organophosphate structure complies with the criteria of Schrader's formula for insecticides and nerve poisons of extraordinary potency. Whereas

strong AChE and BChE inhibition was not observed in vitro, preliminary studies indicate biological activity against arthropods. Similar apparently opposed results have been described before and additional inhibition of cell membrane functions are presently discussed for organophosphates,[28b,31] which invites us to more future investigations of **1**.

The physiological function of such unique microbial organophosphates like **1** for the tunicate-associated *Streptomyces* strain is yet fully unknown. We tackled this question with a first experimental series of feeding experiments of synthetic **1** and derivatives to growing cultures of the producer strain, and have not yet detected altered metabolite patterns. Our finding of the first microbial organophosphate amide emphasizes the value of natural product discoveries in raising questions and enriching research in both chemical synthesis and chemical biology.

Experimental Section

General: ^{1}H, ^{13}C, ^{31}P, and 2D NMR spectra were recorded with a Varian Inova-600, Varian Mercury-300, Varian Unity-300, or Bruker AM 250 spectrometer. Chemicals shifts are reported as δ values (ppm) with the residual protonated solvent as the internal reference; the multiplicity of the carbon signals was determined by the DEPT or APT technique and quoted as follows: (+) for CH_3 and CH, (–) for CH_2, and (C_{quat}) for quaternary carbon atoms. Electron impact (EI) mass spectra were recorded with a Finnigan MAT 95 spectrometer (70 eV), electrospray ionization (ESI) mass spectra with a Finnigan LC-Q spectrometer (70 eV), high-resolution (HR) mass spectra (ESI) with a Bruker APEX-Q 7T IV spectrometer; preselected ion-peak matching at $R >> 10000$ were within ±2 ppm of the exact masses. Elemental analysis was performed at the Mikroanalytisches Labor der Universität Göttingen (Germany). IR spectra were recorded with a Perkin-Elmer Model 1600 spectrometer. UV spectra were recorded with a Varian Model Cary 3E spectrophotometer. Optical rotations were recorded with a Perkin-Elmer 241 polarimeter. Melting points are uncorrected. The solvents used for extraction and chromatography were of technical grade and distilled prior to use. All moisture-sensitive reactions were carried out under nitrogen or argon in oven- and/or flame-dried glassware. THF was distilled from sodium benzophenone ketyl; dichloromethane and triethylamine were distilled from CaH_2; methanol was distilled from magnesium. Column chromatography was carried out on silica gel (Merck; grade 60, 70–230 mesh or Machery & Nagel; grade 60, 230–400 mesh), Sephadex LH-20 (Pharmacia), Li-Chroprep ®RP-18 (Merck) and Lobar RP-18 (Merck). TLC analysis was performed on silica plates (Merck 60 F_{254}, 0.25 mm, RP-18 F/UV_{254} and Machery Nagel chiral plates). HPLC analyses were performed by using a Grom Supher-sphere-100 RP-18, 4 µm (100 × 2 mm) column, a Knauer Nucleosil 100 C8, 5 µm (250 × 3 mm) column or a Daicel Chiralpak IA (0.46 × 25 cm) column, a Jasco pump PU-2080 Plus, a Kontron pump Model 322, a UV detector MD-2010 Plus and a Kontron diode Array Detector 440. Staining reagents were anisaldehyde/sulfuric acid [anisaldehyde (1.0 mL) in methanol (85 mL) with conc. sulfuric acid (5 mL) and acetic acid (10 mL)], 4-dimethylaminobenzaldehyde/hydrochloric acid [4-dimethylaminobenzaldehyde (1 g) in methanol (75 mL) with conc. hydrochloric acid (25 mL)], orcin/sulfuric acid; iron(III)chloride (1 g) in sulfuric acid (100 mL) with 6% alcoholic solution of orcin (ratio 1:1), molybdatophosphoric acid solution; 5% molybdatophosphoric acid in ethanol. Fermentation was carried out in a Braun Incubator BS4, Braun Certomat RM, 250-mL or 1-L Erlenmeyer flasks with three spoilers; Nutrient solutions: Medium S: starch (10 g L^{-1}), glycerol (4 g L^{-1}), casein peptone (4 g L^{-1}), yeast extract (0.5 g L^{-1}), meat extract (0.5 g L^{-1}), liver extract (0.5 g L^{-1}), NaCl (1 g L^{-1}) adjusted to pH 7.0 prior to sterilization; complex medium (SGG): glucose (10 g L^{-1}), glycerol (10 g L^{-1}), starch (10 g L^{-1}), cornsteep powder (2.5 g L^{-1}), casein peptone (5 g L^{-1}), yeast extract (2.0 g L^{-1}), NaCl (1 g L^{-1}), $CaCO_3$ (3 g L^{-1}) adjusted to pH 7.3 prior to sterilization; medium M^{2+}: malt extract (10 g L^{-1}), yeast extract (4 g L^{-1}), glucose (4 g L^{-1}), $CaCO_3$ (0.3 g L^{-1}) adjusted to pH 7.0 prior to sterilization. Labeled precursors: sodium [1,2-$^{13}C_2$]acetate (99% ^{13}C; Cambridge Isotope Lab.), [U-$^{13}C_3$]glycerol (99% ^{13}C; Chemotrade).

Fermentation: Strain JP90 (*Streptomyces* sp.) was incubated for 6 d at 28 °C and maintained on agar plates (medium M^{2+}). A 1-cm^2 piece of incubated agar plates was used to inoculate 100 mL of medium SGG in 250-mL flasks (180 rpm, 28 °C, 48 h). Production in medium SGG was carried out in 250-mL Erlenmeyer flasks (with three spoilers, 180 rpm, 28 °C, 96 h) by using 1% of the preculture as inoculum. Under optimized conditions, a 1-cm^2 piece of incubated agar plates was used to inoculate 100 mL of medium S in 300-mL flasks (180 rpm, 28 °C, 48 h). Production in medium S was carried out in 1-L Erlenmeyer flasks (180 rpm, 28 °C, 96 h).

Isolation and Purification: The culture broth was adjusted to pH 5.0 and separated by filtration from the mycelia, which was discarded. The culture filtrate was adsorbed on XAD-16 resin and elution with methanol yielded the crude extract. The solvent was removed by evaporation. Purification was achieved by flash column chromatography on silica gel ($CHCl_3$/MeOH, 9:1), gel permeation chromatography on Sephadex LH-20 (MeOH) and column chromatography on reverse-phase silica gel (MeOH/H_2O, 7:3), thus yielding 1.2 mg L^{-1} of **1**. Optimized conditions: The culture broth was adjusted to pH 5.6 and separated by filtration from the mycelia. The culture filtrate was extracted with ethyl acetate (2 × 500 mL), and the solvent was removed by evaporation. Purification was achieved by column chromatography on silica gel ($CHCl_3$/MeOH, 95:5), twofold chromatography on reverse-phase silica gel (acetone/H_2O, 3:1), thus yielding 2.0 mg L^{-1} of **1**.

Methyl (*S*)-(2*E*)-3-[4-(Aminomethoxyphosphoryloxy)phenyl]acrylate (1): The title compound was isolated as described above as a pure colorless solid. $C_{11}H_{14}NO_5P$: M_r = 272.21. R_f (silica gel) = 0.35 ($CHCl_3$/MeOH, 9:1), R_f = 0.52 (MeOH/H_2O, 7:3). M.p. 130 °C. $[\alpha]_D^{20}$ = –5.2 (c = 0.25, MeOH). IR (KBr): $\tilde{\nu}$ = 3417 (N–H), 2953 (C–H), 1716 (C=O), 1637 (N–H), 1602 (C=C), 1508 (C=C), 1437, 1326, 1226 (P=O), 1171, 1053, 1014, 982, 925, 836, 796 cm^{-1}. UV (MeOH): λ_{max} (log ε) = 283 (4.86) nm. ^{1}H NMR (600 MHz, CD_2Cl_2, 25 °C): δ = 3.76 (s, 3 H, 12-H_3), 3.79 (d, $^3J_{H,P}$ = 11.8 Hz, 3 H, 11-H_3), 6.37 (d, $^3J_{H,H}$ = 16.0 Hz, 1 H, 2-H), 7.23 [d, $^3J_{H,H}$ = 8.5 Hz, 2 H, 6(8)-H], 7.50 [d, $^3J_{H,H}$ = 8.4 Hz, 5(9)-H], 7.62 (d, $^3J_{H,H}$ = 16.0 Hz, 1 H, 3-H) ppm. ^{13}C NMR (75.5 MHz, CD_2Cl_2, 25 °C): δ = 51.8 (+, C-12), 53.9 (+, d, $^2J_{C,P}$ = 5.8 Hz, C-11), 117.9 (+, C-2), 121.0 [+, d, $^3J_{C,P}$ = 4.5 Hz, C-6(8)], 129.8 [+, C-5(9)], 131.4 (C_{quat}, C-4), 143.7 (+, C-3), 152.7 (d, $^2J_{C,P}$ = 6.8 Hz, C-7, C_{quat}), 167.4 (C_{quat}, C-1) ppm. ^{31}P NMR (121.5 MHz, CD_2Cl_2, 25 °C): δ = 6.1 (br.) ppm. MS (ESI): m/z (%) = 294 (32) [M + Na]$^+$, 565 (100) [2M + Na]$^+$. HRMS (ESI): calcd. for $C_{11}H_{15}NO_5P$ [M + H]$^+$ 272.0681; found 272.0684.

Methyl (2*E*)-3-[4-(Chloromethoxyphosphoryloxy)phenyl]acrylate (9): Methyl 4-hydroxycinnamate (**7**; 301 mg, 1.68 mmol) was dissolved in dichloromethane (22 mL) and cooled to 0 °C, and sodium hydride (60% in mineral oil, 72.9 mg, 1.82 mmol), was added. After

stirring for 0.5 h at 0 °C, the suspension was warmed to room temperature and stirred for another 2 h. The resulting suspension was added dropwise to a vigorously stirred solution of methoxy phosphoryl dichloride (**8**; 503 mg, 3.38 mmol)[20] in dichloromethane (6 mL) over a period of 9 h at 0 °C. The reaction mixture was warmed to room temperature and stirred for 16 h. The mixture was filtered under a nitrogen atmosphere through a 2-cm pad of Celite. The solids were washed with dichloromethane (3 × 50 mL), and the filtrate was concentrated in vacuo, thus yielding 586 mg of **9** as a yellow oil, which without purification was transformed in the following reaction. $C_{11}H_{12}ClO_5P$: M_r = 290.64. ^{1}H NMR (250 MHz, $CDCl_3$, 25 °C): δ = 3.80 (s, 3 H, 12-H_3), 4.03 (d, $^3J_{H,P}$ = 14.1 Hz, 3 H, 11-H_3), 6.39 (d, $^3J_{H,H}$ = 15.9 Hz, 1 H, 2-H), 7.29 [d, $^3J_{H,H}$ = 8.4 Hz, 2 H, 6(8)-H], 7.54 [d, $^3J_{H,H}$ = 8.4 Hz, 2 H, 5(9)-H], 7.66 (d, $^3J_{H,H}$ = 15.9 Hz, 1 H, 3-H) ppm. ^{31}P NMR (121.5 MHz, $CDCl_3$, 25 °C): δ = 1.81 (br.) ppm.

Methyl (2*E*)-3-[4-(Aminomethoxyphosphoryloxy)phenyl]acrylate (1): Crude product **9** from the reaction described above (586 mg) was dissolved in dichloromethane (18 mL) and cooled to –15 °C. A stream of gaseous ammonia was passed for 50 min through the vigorously stirred solution, brine (20 mL) was added, and the layers were separated. The aqueous phase was extracted with ethyl acetate (3 × 30 mL), and the combined organic layer was dried with $MgSO_4$ and concentrated in vacuo. The crude product was purified by column chromatography on reverse-phase silica gel (Li-Chroprep ®RP-18; MeOH/H_2O, 6:4) to yield 252 mg (55% from **7**) of the title compound **1**. All spectroscopic data were consistent with the data of cinnamoylphosphoramide (**1**) isolated from *Streptomyces* sp. JP90. $C_{11}H_{14}NO_5P$ (271.2): calcd. C 48.72, H 5.20, N 5.16; found C 48.40, H 5.21, N 5.00.

Methyl (*S*,*S*)- and (*R*,*S*)-(2*E*)-3-{4-[*N*-(1-Ethoxycarbonyl-2-phenylethyl)aminomethoxyphosphoryloxy]phenyl}acrylate (10a,b): To a solution of L-phenylalanine ethyl ester hydrochloride (2.59 g, 11.3 mmol) dissolved in THF (4 mL) was added NEt_3 (5.04 mL, 36.2 mmol) at 0 °C. After being stirred for 1 h the resulting suspension was added dropwise to a solution of **9** (3.28 g, 11.3 mmol) in THF (48 mL), and the reaction mixture was vigorously stirred for 3 h at 0 °C. The mixture was filtered through a 2-cm pad of Celite, the solids were washed with THF (2 × 40 mL), and the filtrate was concentrated in vacuo to a volume of approx. 20 mL. Then, the solution was diluted with diethyl ether (60 mL), washed successively with brine (40 mL), hydrochloric acid (1 N, 40 mL), saturated $NaHCO_3$ solution (40 mL), and brine (40 mL), and dried with $MgSO_4$. The solvent was removed in vacuo, and the residue was purified by column chromatography on silica gel (180 g; hexane/EtOAc, 1:1) to yield 2.42 g (48%) of diastereomeric mixture **10a**,**b** (*dr* 13:7) as a colorless oil, which slowly started to crystallize. $C_{22}H_{26}NO_7P$: M_r = 447.43. R_f = 0.35 (hexane/EtOAc, 1:1). IR (KBr): $\tilde{\nu}$ = 3446 (N–H), 2949 (C–H), 1734 (C=O), 1636 (N–H), 1559 (C=C), 1508 (C=C), 1457, 1386 (P=O), 1170, 1044, 986, 845, 759 cm^{-1}. ^{1}H NMR (250 MHz, $CDCl_3$, 25 °C): δ = 1.14/1.23 (2 × t, $^3J_{H,H}$ = 7.0 Hz, 3 H, CH_2CH_3), 2.89–3.05 (m_c, 2 H, 3′-H), 3.53/3.66 (2 × d, $^3J_{H,P}$ = 11.5 Hz, 3 H, 11-H_3), 3.76 (s, 3 H, 12-H_3), 4.03–4.23 (m, 3 H, 2′-H, CH_2CH_3), 6.33 (d, $^3J_{H,H}$ = 16.0 Hz, 1 H, 2-H), 7.07–7.23 [m, 7 H, 6(8)-H, Ar-H], 7.42 [d, $^3J_{H,H}$ = 8.5 Hz, 2 H, 5(9)-H], 7.61 (d, $^3J_{H,H}$ = 16.0 Hz, 1 H, 3-H) ppm. ^{13}C NMR (63.9 MHz, $CDCl_3$, 25 °C): δ = 14.1 (CH_2CH_3), 40.4 (C-3′), 51.7 (C-12), 53.4/53.6 (d, $^2J_{C,P}$ = 5.7 Hz, C-11), 55.5/55.8 (C-2′), 61.4 (CH_2CH_3), 117.4 (C-2), 120.5 [d, $^3J_{C,P}$ = 5.2 Hz, C-6(8)], 127.0 (C-7′), 128.4 [C-6′(8′)*], 129.4 [C-5′(9′)*], 129.5 [C-5(9)*], 130.9 (C-4), 135.6/135.8 (C-4′), 143.7 (C-3), 152.2 (d, $^2J_{C,P}$ = 6.5 Hz, C-7), 167.3 (C-1), 172.1/172.2 (C-1′) ppm. MS (ESI): *m*/*z* (%) = 917 (100) [2M + Na]$^+$, 470 (12) [M + Na]$^+$. HRMS (ESI): calcd. for $C_{22}H_{27}NO_7P$ [M + H]$^+$ 448.1520; found 448.1520.

Methyl (*S*,*S*)- and (*R*,*S*)-(2*E*)-3-{4-[*N*-Chloro-*N*-(1-ethoxycarbonyl-2-phenylethyl)aminomethoxyphosphoryloxy]phenyl}acrylate (11a,b): A solution of *tert*-butyl hypochlorite (1.28 mL, 11.3 mmol) in 4% methanolic borax (34 mL) was added to a vigorously stirred solution of diastereomers **10a**,**b** (*dr* 13:7, 2.96 g, 6.62 mmol) in 4% methanolic borax (20 mL). Due to incomplete conversion, an additional amount of *tert*-butyl hypochlorite (1.02 mL, 9.02 mmol) in 4% methanolic borax (31 mL) was added after 50 min, and the mixture was stirred for another 45 min. The resulting mixture was diluted with chloroform (40 mL), washed with water (40 mL), dried with $MgSO_4$, and concentrated in vacuo. The residue was purified by flash column chromatography on silica gel at 4 °C (260 g; hexane/EtOAc, 2:1) to yield 996 mg (31%) of (*S*,*S*) diastereomer **11a** (R_f = 0.54) as a yellow oil and 661 mg (21%) of (*R*,*S*) diastereomer **11b** (R_f = 0.36) as yellow solid. Data for **11a**: $C_{22}H_{25}ClNO_7P$: M_r = 481.87. ^{1}H NMR (250 MHz, $CDCl_3$, 25 °C): δ = 1.20 (t, $^3J_{H,H}$ = 7.2 Hz, 3 H, CH_2CH_3), 3.03 (d, $^3J_{H,P}$ = 12.6 Hz, 3 H, 11-H_3), 3.14 (dd, $^3J_{H,H}$ = 10.9 Hz, $^2J_{H,H}$ = 14.5 Hz, 1 H, 3′-H_A), 3.37 (dt, $^3J_{H,H}$ = 3.6 Hz, $^2J_{H,H}$ = 14.5 Hz, 1 H, 3′-H_B), 3.79 (s, 3 H, 12-H_3), 4.09 (q, $^3J_{H,H}$ = 7.2 Hz, 2 H, CH_2CH_3), 4.91–4.99 (m, 1 H, 2′-H), 6.34 (d, $^3J_{H,H}$ = 15.9 Hz, 1 H, 2-H), 7.18–7.28 [m, 7 H, 6(8)-H, Ar-H], 7.46 [d, $^3J_{H,H}$ = 7.2 Hz, 2 H, 5(9)-H], 7.63 (d, $^3J_{H,H}$ = 15.9 Hz, 1 H, 3-H) ppm. ^{13}C NMR (63.9 MHz, $CDCl_3$, 25 °C): δ = 14.0 (+, CH_2CH_3), 40.4 (–, d, $^3J_{C,P}$ = 5.0 Hz, C-3′), 51.7 (+, C-12), 53.5 (+, d, $^2J_{C,P}$ = 5.7 Hz, C-11), 61.7 (–, CH_2CH_3), 63.7 (+, d, $^2J_{C,P}$ = 4.3 Hz, C-2′), 117.6 (+, C-2), 120.9 [+, d, $^3J_{C,P}$ = 5.0 Hz, C-6(8)], 126.9 (+, C-7′), 128.5 [+, C-6′(8′)*], 129.3 [+, C-5′(9′)*], 129.4 [+, C-5(9)*], 131.3 (C_{quat}, C-4), 136.5 (C_{quat}, C-4′), 143.6 (+, C-3), 152.0 (d, $^2J_{C,P}$ = 7.4 Hz, C-7, C_{quat}), 167.3 (C_{quat}, C-1), 169.2 (C_{quat}, C-1′) ppm. MS (ESI): *m*/*z* (%) = 504 (18) [M + Na]$^+$, 985 (100) [2M + Na]$^+$. Data for **11b**: $C_{22}H_{25}ClNO_7P$: M_r = 481.87. ^{1}H NMR (250 MHz, $CDCl_3$, 25 °C): δ = 1.32 (t, $^3J_{H,H}$ = 7.2 Hz, 3 H, CH_2CH_3), 3.06 (dd, $^3J_{H,H}$ = 10.9 Hz, $^2J_{H,H}$ = 14.5 Hz, 1 H, 3′-H_A), 3.33 (dt, $^3J_{H,H}$ = 3.6 Hz, $^2J_{H,H}$ = 14.5 Hz, 1 H, 3′-H_B), 3.83 (s, 3 H, 12-H_3), 3.93 (d, $^3J_{H,P}$ = 12.6 Hz, 3 H, 11-H_3), 4.21–4.33 (m, 2 H, CH_2CH_3), 4.84–4.94 (m, 1 H, 2′-H), 6.34 (d, $^3J_{H,H}$ = 15.9 Hz, 1 H, 2-H), 6.86 [d, $^3J_{H,H}$ = 7.2 Hz, 2 H, 6(8)-H], 7.13 (m_c, 5 H, Ar-H), 7.29 [d, $^3J_{H,H}$ = 7.2 Hz, 2 H, 5(9)-H], 7.61 (d, $^3J_{H,H}$ = 15.9 Hz, 1 H, 3-H) ppm. ^{13}C NMR (63.9 MHz, $CDCl_3$, 25 °C): δ = 14.1 (+, CH_2CH_3), 40.4 (–, C-3′), 51.7 (+, C-12), 55.1 (+, d, $^2J_{C,P}$ = 6.0 Hz, C-11), 61.8 (–, CH_2CH_3), 64.2 (+, d, $^2J_{C,P}$ = 4.0 Hz, C-2′), 117.5 (+, C-2), 120.4 [+, d, $^3J_{C,P}$ = 5.1 Hz, C-6(8)], 126.6 (+, C-7′), 128.4 [+, C-6′(8′)*], 129.0 [+, C-5′(9′)*], 129.3 [+, C-5(9)*], 131.1 (C_{quat}, C-4), 136.1 (C_{quat}, C-4′), 143.7 (+, C-3), 151.4 (d, $^2J_{C,P}$ = 6.9 Hz, C-7, C_{quat}), 167.3 (C_{quat}, C-1), 169.6 (C_{quat}, C-1′) ppm. MS (DCI, 200 eV): *m*/*z* (%): 178 (9), 194 (100) [$C_{10}H_9O_3$ + NH_3], 211 (29).

Methyl (*S*,*S*)- and (*S*,*R*)-(2*E*)-3-{4-[*N*-(1-Ethoxycarbonyl-1-methoxy-2-phenylethyl)aminomethoxyphosphoryloxy]phenyl}acrylate (12): (*S*,*S*) Diastereomer **11a** (288 mg, 0.598 mmol) was dissolved in methanol (1.33 mL) and treated at –40 °C with sodium methoxide (1.0 M in methanol, 0.60 mL, 0.60 mmol), and the solution was stirred for 100 min at –40 °C. The reaction mixture was neutralized with acetic acid and concentrated in vacuo. The residue was extracted with ethyl acetate (3 × 10 mL), and the combined organic phases were washed successively with aqueous solutions of citric acid (10%, 15 mL), $NaHCO_3$ (1 M, 15 mL), and brine (15 mL) and dried with $MgSO_4$. Concentration in vacuo yielded 270 mg of **12** as a slightly impure mixture of both diastereomers (*dr* 1:1). $C_{23}H_{28}NO_8P$: M_r = 477.44. R_f = 0.41 (hexane/EtOAc, 1:1). ^{1}H NMR (250 MHz, $CDCl_3$, 25 °C): δ = 1.23/1.32 (2 × t, $^3J_{H,H}$ = 7.2 Hz, 3 H, CH_2CH_3), 3.26 (d, $^2J_{H,H}$ = 14.5 Hz, 1 H, 3′-H_A), 3.27/

3.28 (2 × s, 3 H, OCH_3), 3.36 (d, $^2J_{H,H}$ = 14.5 Hz, 1 H, 3′-H_B), 3.78 (2 × s, 3 H, 12-H_3), 3.74/3.86 (2 × d, $^3J_{H,P}$ = 12.6 Hz, 3 H, 11-H_3), 4.12/4.23 (2 × q, $^3J_{H,H}$ = 7.2 Hz, 2 H, CH_2CH_3), 4.69 (2 × d, $^2J_{H,P}$ = 8.0 Hz, 1 H, NH), 6.36/6.37 (2 × d, $^3J_{H,H}$ = 15.6 Hz, 1 H, 2-H), 7.05–7.19 [m, 7 H, 6(8)-H, Ar-H], 7.47 [2 × d, $^3J_{H,H}$ = 8.6 Hz, 2 H, 5(9)-H], 7.64/7.66 (2 × d, $^3J_{H,H}$ = 15.6 Hz, 1 H, 3-H) ppm. ^{13}C NMR (75.5 MHz, $CDCl_3$, 25 °C): δ = 14.0/14.0 (+, CH_2CH_3), 43.1 (–, C-3′), 51.5/51.5 (+, OCH_3), 51.7/51.7 (+, C-12), 54.2/54.3 (+, d, $^2J_{C,P}$ = 6.0 Hz, C-11), 62.7/62.8 (–, CH_2CH_3), 90.8/90.8 (d, $^2J_{C,P}$ = 5.3 Hz, C-2′, C_{quat}), 117.5/117.7 (+, C-2), 120.7/121.0 [+, d, $^3J_{C,P}$ = 4.5 Hz, C-6(8)], 126.9/127.9 (+, C-7′), 128.0/128.1 [+, C-6′(8′)*], 129.5/129.5 [+, C-5′(9′)*], 130.2/130.3 [+, C-5(9)*], 131.0/131.3 (C_{quat}, C-4), 134.5/134.8 (C_{quat}, C-4′), 143.7/143.7 (+, C-3), 152.2/152.6 (d, $^2J_{C,P}$ = 6.8 Hz, C-7, C_{quat}), 167.3 (C_{quat}, C-1), 172.0/172.1 (C_{quat}, C-1′) ppm.

Methyl (*R*,*S*)- and (*R*,*R*)-(2*E*)-3-{4-[*N*-(1-Ethoxycarbonyl-1-methoxy-2-phenylethyl)aminomethoxyphosphoryloxy]phenyl}acrylate (13): (*R*,*S*) Diastereomer **11b** (136 mg, 0.282 mmol) was dissolved in methanol (10 mL) and treated at –35 °C with sodium methoxide (1.0 M in methanol, 0.28 mL, 0.28 mmol), and the solution was stirred for 2.5 h at –35 °C. Due to incomplete conversion, additional sodium methoxide (1.0 M in methanol, 0.14 mL, 0.14 mmol) was added, and stirring was continued for 50 min. The reaction mixture was then warmed to –20 °C, treated with sodium methoxide (1.0 M in methanol, 0.12 mL, 0.12 mmol), and stirred for another 0.5 h. Workup as described above yielded 106 mg of **13** as a slightly impure mixture of both diastereomers (*dr* 1:1). $C_{23}H_{28}NO_8P$: M_r = 477.44. R_f = 0.39 (hexane/EtOAc, 1:1). ^{1}H NMR (250 MHz, $CDCl_3$, 25 °C): δ = 1.23/1.32 (2 × t, $^3J_{H,H}$ = 7.2 Hz, 3 H, CH_2CH_3), 3.26 (d, $^2J_{H,H}$ = 14.5 Hz, 1 H, 3′-H_A), 3.27/3.28 (2 × s, 3 H, OCH_3), 3.36 (d, $^2J_{H,H}$ = 14.5 Hz, 1 H, 3′-H_B), 3.78 (2 × s, 3 H, 12-H_3), 3.74/3.86 (2 × d, $^3J_{H,P}$ = 12.6 Hz, 3 H, 11-H_3), 4.12/4.23 (2 × q, $^3J_{H,H}$ = 7.2 Hz, 2 H, CH_2CH_3), 4.66 (2 × d, $^2J_{H,P}$ = 8.0 Hz, 1 H, NH), 6.36/6.37 (2 × d, $^3J_{H,H}$ = 15.6 Hz, 1 H, 2-H), 7.05–7.19 [m, 7 H, 6(8)-H, Ar-H], 7.47 [2 × d, $^3J_{H,H}$ = 8.6 Hz, 2 H, 5(9)-H], 7.64/7.66 (2 × d, $^3J_{H,H}$ = 15.6 Hz, 1 H, 3-H) ppm. ^{13}C NMR (75.5 MHz, $CDCl_3$, 25 °C): δ = 14.0/14.0 (+, CH_2CH_3), 43.1 (–, C-3′), 51.5/51.5 (+, OCH_3), 51.7/51.7 (+, C-12), 54.2/54.3 (+, d, $^2J_{C,P}$ = 6.0 Hz, C-11), 62.75/62.82 (–, CH_2CH_3), 90.8/90.8 (d, $^2J_{C,P}$ = 5.3 Hz, C-2′, C_{quat}), 117.5/117.6 (+, C-2), 120.6/120.9 [+, d, $^3J_{C,P}$ = 4.5 Hz, C-6(8)], 126.9/127.0 (+, C-7′), 128.0/128.1 [+, C-6′(8′)*], 129.5/129.5 [+, C-5′(9′)*], 130.2/130.3 [+, C-5(9)*], 131.1/131.3 (C_{quat}, C-4), 134.5/134.8 (C_{quat}, C-4′), 143.6/143.7 (+, C-3), 152.2/152.6 (d, $^2J_{C,P}$ = 5.3 Hz, C-7, C_{quat}), 167.3 (C_{quat}, C-1), 172.0/172.1 (C_{quat}, C-1′) ppm.

Methyl (*S*)-(2*E*)-3-[4-(Aminomethoxyphosphoryloxy)phenyl]acrylate [(*S*)-1]: An aqueous solution of H_2SO_4 (5%, 2.1 mL) was added to a solution of diastereomeric mixture **12** (1:1; 270 mg) in methanol (4.1 mL), and the reaction mixture was stirred for 6 h at room temperature. Water (15 mL) was added, and the solution was extracted with chloroform (3 × 15 mL). The combined organic layer was washed with brine (20 mL), dried with $MgSO_4$, and concentrated in vacuo. The residue was purified by column chromatography on reverse-phase silica gel (Li-Chroprep ®RP-18; MeOH/H_2O, 6:4) to yield 87.0 mg (54% from **11a**) of the title compound (*S*)-**1** as a colorless solid. The optical rotation {$[\alpha]_D^{20}$ = –6.6 (*c* = 0.14, MeOH)} and all spectroscopic data were consistent with the data of cinnamoylphosphoramide (**1**) isolated from *Streptomyces* sp. JP90.

Methyl (*R*)-(2*E*)-3-[4-(Aminomethoxyphosphoryloxy)phenyl]acrylate [(*R*)-1]: An aqueous solution of H_2SO_4 (5%, 0.82 mL) was added to a solution of diastereomeric mixture **13** (1:1; 106 mg) in methanol (1.7 mL), and the reaction mixture was stirred for 7 h at room temperature. Workup as described above and purification by column chromatography on reverse-phase silica gel (Li-Chroprep ®RP-18; MeOH/H_2O, 6:4) yielded 22.8 mg (30% from **11b**) of (*R*)-**1** as a colorless solid. All spectroscopic data were consistent with the data of cinnamoylphosphoramide (**1**) isolated from *Streptomyces* sp. JP90, and the optical rotation {$[\alpha]_D^{20}$ = +6.6 (*c* = 0.14, MeOH)} indicated (*R*)-**1** to be the nonnatural enantiomer.

***tert*-Butyldimethylsilyl (2*E*)-3-(4-Hydroxyphenyl)acrylate (15):** To a solution of 4-hydroxycinnamic acid (1.65 g, 10.1 mmol) in THF (70 mL) was added NEt_3 (1.4 mL, 10 mmol) and *tert*-butyldimethylsilyl chloride (2.5 M in toluene, 4.0 mL, 10 mmol). The solution was stirred for 30 min and then added to an aqueous phosphate buffer solution (pH 7, 75 mL) and extracted with diethyl ether (3 × 75 mL). The combined organic phase was dried with Na_2SO_4 and concentrated in vacuo to yield 2.71 g (97%) of the title compound as a colorless solid whose purity was >95%. An analytical sample was purified by column chromatography on silica gel (hexane/EtOAc, 5:1 plus 2.5% MeOH), which, however, occurred with partial desilylation. $C_{15}H_{22}O_3Si$: M_r = 278.42. R_f = 0.28. M.p. 95 °C. IR (KBr): $\tilde{\nu}$ = 3244 (O–H), 2956 (C–H), 1659 (C=O), 1472, 1462, 1411, 1296, 1275, 1253, 1173, 991, 873, 822, 793, 741 cm^{-1}. ^{1}H NMR (250 MHz, $CDCl_3$, 25 °C): δ = 0.34 (s, 6 H, $SiMe_2tBu$), 1.05 (s, 9 H, $SiMe_2tBu$), 6.28 (d, $^3J_{H,H}$ = 15.9 Hz, 1 H, 2-H), 6.88 [d, $^3J_{H,H}$ = 8.5 Hz, 2 H, 6(8)-H], 7.41 [d, $^3J_{H,H}$ = 8.5 Hz, 5(9)-H], 7.60 (d, $^3J_{H,H}$ = 15.9 Hz, 1 H, 3-H) ppm. ^{13}C NMR (63.9 MHz, $CDCl_3$, 25 °C): δ = –4.7 (+, $SiMe_2tBu$), 17.8 {C_{quat}, $SiMe_2[C(CH_3)_3]$}, 25.6 {+, $SiMe_2[C(CH_3)_3]$}, 115.9 [+, C-6(8)], 116.9 (+, C-2), 126.8 (C_{quat}, C-4), 130.1 [+, C-5(9)], 145.5 (+, C-3), 158.3 (C_{quat}, C-7), 168.1 (C_{quat}, C-1) ppm. MS (EI): *m*/*z* (%) = 278 (<1) $[M]^+$, 263 (2) $[M - CH_3]^+$, 221 (100) $[M - tBu]^+$, 203 (14), 177 (16), 147 (18), 119 (6), 75 (24). $C_{15}H_{22}O_3Si$ (278.4): calcd. C 64.71, H 7.96; found C 64.88, H 7.62.

***tert*-Butyldimethylsilyl (2*E*)-3-[4-(Chloromethoxyphosphoryloxy)-phenyl]acrylate (16):** Silyl ester **15** (555 mg, 1.99 mmol) was dissolved in dichloromethane (30 mL) and cooled to 0 °C, and sodium hydride (60% in mineral oil, 111 mg, 2.78 mmol) was added. After being stirred for 1 h at 0 °C, the suspension was cooled to –78 °C and methoxy phosphoryl dichloride (**8**; 0.30 g, 2.0 mmol)[20] was added dropwise under vigorous stirring. The reaction mixture was warmed to room temperature, stirred for 16 h, and then transformed in the next reaction without purification. An analytical sample was concentrated in vacuo leading to the isolation of the free carboxylic acid. ^{1}H NMR (250 MHz, $CDCl_3$, 25 °C): δ = 4.10 (d, $^3J_{H,P}$ = 12.4 Hz, 3 H, 11-H_3), 6.43 (d, $^3J_{H,H}$ = 15.8 Hz, 1 H, 2-H), 7.31 [d, $^3J_{H,H}$ = 7.9 Hz, 2 H, 6(8)-H], 7.58 [d, $^3J_{H,H}$ = 7.9 Hz, 2 H, 5(9)-H], 7.76 (d, $^3J_{H,H}$ = 15.8 Hz, 1 H, 3-H) ppm. ^{13}C NMR (63.9 MHz, $CDCl_3$, 25 °C): δ = 56.3 (+, d, $^2J_{C,P}$ = 7.3 Hz, C-11), 118.0 (+, C-2), 120.9 [+, d, $^3J_{C,P}$ = 4.7 Hz, C-6(8)], 130.0 [+, C-5(9)], 132.2 (C_{quat}, C-4), 145.2 (+, C-3), 151.5 (d, $^2J_{C,P}$ = 7.6 Hz, C-7, C_{quat}), 171.6 (C_{quat}, C-1) ppm. ^{31}P NMR (121.5 MHz, $CDCl_3$, 25 °C): δ = 1.87 ppm.

(2*E*)-3-[4-(Aminomethoxyphosphoryloxy)phenyl]acrylic Acid (17): Crude product **16** from the reaction described above was diluted with dichloromethane (20 mL) and cooled to –17 °C. A stream of gaseous ammonia was passed for 45 min through the vigorously stirred solution, which was then added to an aqueous phosphate buffer solution (pH 4, 25 mL). The aqueous phase was extracted with ethyl acetate (3 × 75 mL), and the combined organic layer was dried with Na_2SO_4 and concentrated in vacuo. The crude product was purified by column chromatography on silica gel (80 g; $CHCl_3$/MeOH, 6:1 gradually increased to 2:1) yielding 220 mg (43%) of

phosphoramide **17** as a colorless solid. $C_{10}H_{12}NO_5P$: M_r = 257.18. R_f = 0.33 ($CHCl_3$/MeOH, 4:1). R_f = 0.84 (RP; MeOH/H_2O, 7:3). M.p. 163–164 °C. IR (KBr): $\tilde{\nu}$ = 3363 (N–H), 2947 (C–H), 1713 (C=O), 1639 (N–H), 1601 (C=C), 1507 (C=C), 1438, 1317, 1218 (P=O), 1173, 1060, 1002, 986, 931, 839, 793 cm^{-1}. UV (MeOH): λ_{max} (log ε) = 272 (2.22) nm. ^{1}H NMR (600 MHz, CD_3OD, 25 °C): δ = 3.80 (d, $^3J_{H,P}$ = 11.5 Hz, 3 H, 11-H_3), 6.44 (d, $^3J_{H,H}$ = 16.0 Hz, 1 H, 2-H), 7.26 [d, $^3J_{H,H}$ = 8.5 Hz, 2 H, 6(8)-H], 7.61 [d, $^3J_{H,H}$ = 8.5 Hz, 2 H, 5(9)-H], 7.62 (d, $^3J_{H,H}$ = 16.0 Hz, 1 H, 3-H) ppm. ^{13}C NMR (125.7 MHz, CD_3OD, 25 °C): δ = 54.1 (+, d, $^2J_{C,P}$ = 5.7 Hz, C-11), 120.3 (+, C-2), 122.0 [+, d, $^3J_{C,P}$ = 4.9 Hz, C-6(8)], 130.6 [+, C-5(9)], 132.8 (C_{quat}, C-4), 144.4 (+, C-3), 153.9 (d, $^2J_{C,P}$ = 6.6 Hz, C-7, C_{quat}), 171.1 (C_{quat}, C-1) ppm. ^{31}P NMR (121.5 MHz, [D_6]DMSO, 25 °C): δ = 9.25 ppm. MS (ESI): *m*/*z* (%) = 537 (100) [2M + Na]$^+$. HRMS (ESI): calcd. for $C_{10}H_{13}NO_5P$ [M + H]$^+$ 258.05259; found 258.05262.

Supporting Information (see footnote on the first page of this article): Detailed experimental procedures; comparison of the spectroscopic data of the natural product and α,β-unsaturated carboxylic esters related to **1** and related imido esters from the literature; chiral HPLC traces; hypothesis for the biosynthetic pathway of cinnamoylphosphoramide.

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