Eur. J. Org. Chem. **2008** · © WILEY-VCH Verlag GmbH & Co. KGaA, 69451 Weinheim, 2008 · ISSN 1434–193X

SUPPORTING INFORMATION

Title: The New Metabolite (*S*)-Cinnamoylphosphoramide from *Streptomyces* sp. and Its Total Synthesis
Author(s): Melanie Quitschau, Tim Schuhmann, Jörn Piel, Paultheo von Zezschwitz,* Stephanie Grond*
Ref. No.: O200800654

Table of Content:

---

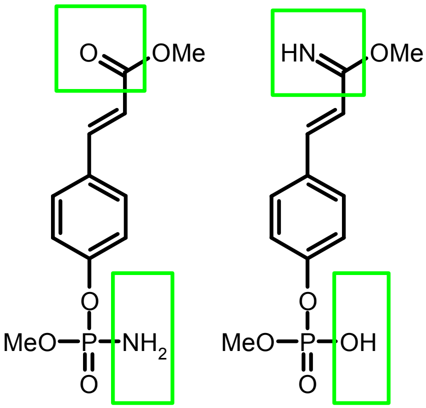
1. General Experimental Methods

^{1}H, ^{13}C, ^{31}P and 2D NMR spectra were recorded on 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 proponated 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, CH, (–) CH_2, and (C_{quat}) for quaternary carbons. Electron impact (EI) mass spectra were recorded on a Finnigan MAT 95 spectrometer (70 eV); electrospray ionisation (ESI) mass spectra on a Finnigan LC-Q spectrometer (70 eV); high-resolution (HR) mass spectra (ESI) on 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 on a Perkin-Elmer Model 1600 spectrometer. UV spectra were recorded on a Varian Model Cary 3E spectrophotometer. Optical rotations were recorded on 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. **Chromatography:** 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 using a Grom Suphersphere-100 RP-18, 4 µm (100 × 2 mm) column, a Knauer Nucleosil 100 C8, 5 µm (250 x 3 mm) column or a Daicel Chiralpak IA (0.46 × 25 cm) column, a Jasco pump PU-2080 Plus, a Kontron pump Modell 322, a UV detector MD-2010 Plus and a Kontron diode Array Detector 440. Staining reagents were anisaldehyde/sulfuric acid (anisaldeyhde (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 gL^{-1}), glycerol (4 gL^{-1}), casein peptone (4 gL^{-1}), yeast extract (0.5 gL^{-1}), meat extract (0.5 gL^{-1}), liver extract (0.5 gL^{-1}), NaCl (1 gL^{-1}) adjusted to pH 7.0 prior to sterilization; complex medium (SGG): glucose (10 gL^{-1}), glycerol (10 gL^{-1}), starch (10 gL^{-1}), cornsteep powder (2.5 gL^{-1}), casein peptone (5 gL^{-1}), yeast extract (2.0 gL^{-1}), NaCl (1 gL^{-1}), $CaCO_3$ (3 gL^{-1}) adjusted to pH 7.3 prior to sterilization; medium M^{2+}: malt extract (10 gL^{-1}), yeast extract (4 gL^{-1}), glucose (4 gL^{-1}), $CaCO_3$ (0.3 gL^{-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).

2. Feeding Experiments: Feeding experiments with the ^{13}C-labeled compounds were carried out under the fermentation conditions described. In general, the precursors were administered to the fermentation as sterile aqueous solutions, and continuous feeding was carried out within 48–60 h of fermentation using a low-rate pump. The following amounts were added: sodium [1,2-$^{13}C_2$]acetate: 550 mgL^{-1} (6.70 $mmolL^{-1}$); [U-$^{13}C_3$]glycerol: 500 mgL^{-1} (9.05 $mmolL^{-1}$). The feeding experiments with synthetic **1** and further cinnamic acid derivatives such as methyl 4-hydroxycinnamate, cinnamic acid and methyl cinnamate were conducted under the fermentation conditions described. Sterile solutions of the substances in DMSO were added in two portions (12 h and 16 h after inoculation) to the fermentations. The following amounts were added: synthetic **1**: 72 mgL^{-1} (0.59 $mmolL^{-1}$); methyl 4-hydroxycinnamate: 100 mgL^{-1} (0.94 $mmolL^{-1}$); cinnamic acid: 100 mgL^{-1} (1.12 $mmolL^{-1}$); methyl cinnamate: 100 mgL^{-1} (1.03 $mmolL^{-1}$). Cinnamoylphosphoramide **1** was isolated in a mixture with rosiridol which was intensively labelled, **1** not.

3. Spectroscopic Data of Cinnamoylphosphoramide

The spectroscopic data of the natural product isolated from *Streptomyces* sp. JP90 (Table 1) is consistent with both, structures **1** or **18**. Both structures contain unusual structural elements for microbial secondary metabolites. **1** comprises a cinnamoyl moiety and a phosphate amide methyl ester, structure **18** an imido ester and a phosphoric acid diester moiety. Imido esters are very unstable under aqueous conditions, thus, structure **1** was favoured due to its stability and it was selected for a total synthesis. Indeed, comparison of the spectroscopic data of the metabolite and the synthetic material confirmed the natural compound to be the cinnamoylphosphoramide **1**.[S1,S2]

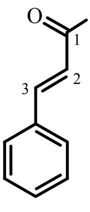
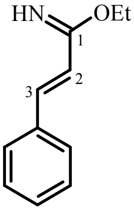
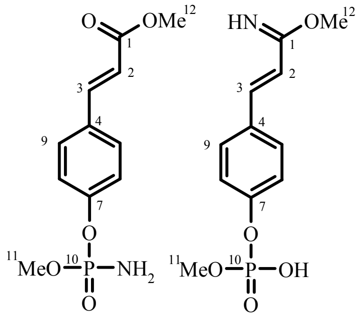
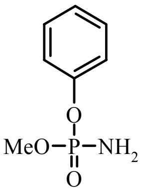


1 **18**

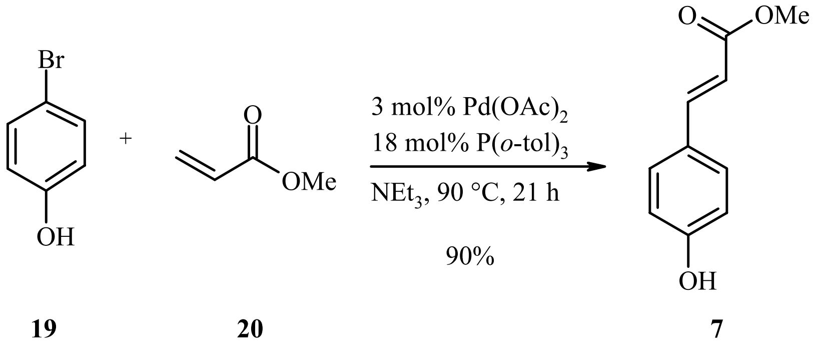
Figure S1. Possible structures of the natural product.

Spectroscopic data of (*E*)-3-[4-(aminomethoxyphosphoryloxy)phenyl]acrylic acid methyl ester from total synthesis (1): $R_f = 0.34$ ($CHCl_3$/MeOH 9:1), $R_f = 0.56$ (MeOH/H_2O 7:3); m.p. = 130–131 °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} (lg ε) =283 (4.30) nm; ^{1}H NMR (300 MHz, $CDCl_3$, 25 °C): $\delta = 3.32$ (br. s, 2 H, 13-H_2), 3.77 (s, 3 H, 12-H_3), 3.80 (d, $^3J_{H,P} = 11.4$ Hz, 3 H, 11-H_3), 6.33 (d, $^3J_{H,H} = 16.0$ Hz, 1 H, 2-H), 7.22 (d, $^3J_{H,H} = 8.4$ Hz, 2 H, 6(8)-H), 7.46 (d, $^3J_{H,H} = 8.4$ Hz, 5(9)-H), 7.61 (d, $^3J_{H,H} = 16.0$ Hz, 1 H, 3-H) ppm; ^{13}C NMR (75.5 MHz, $CDCl_3$, 25 °C): $\delta = 51.7$ (+, C-12), 53.8 (+, d, $^2J_{C,P} = 5.3$ Hz, C-11), 117.5 (+, C-2), 120.7 (+, d, $^3J_{C,P} = 5.3$ Hz, C-6(8)), 129.5 (+, C-5(9)), 131.1 (C_{quat}, C-4), 143.7 (+, C-3), 152.3 (C_{quat}, d, $^2J_{C,P} = 6.8$ Hz, C-7), 167.3 (C_{quat}, C-1) ppm; ^{31}P NMR (121.5 MHz, $CDCl_3$, 25 °C): $\delta = 7.1$ (s) ppm; EI-MS (70 eV), *m/z* (%): 271 (92) $[M]^+$, 240 (76), 178 (40) $[C_{10}H_{10}O_3]^+$, 147 (89), 119 (40), 94 (100) $[CH_6NO_2P]^+$, 91 (39); ESI-MS, *m/z* (%): 565 (100) $[2M + Na]^+$.

Table S1: Comparison of spectroscopic data of the natural product and data of α,β-unsaturated carboxylic esters related to **1** and imido esters related to **18** from the literature.

	Data from Literature[S3,S4]		**Spectroscopical Data of the Natural Product**	**Data from Literature**[S5,S6]	
			 **1** **18**		General spectroscopic data
^{1}H [ppm]	7.70 (3-H)	7.24 (3-H)	7.62 (3-H)	–	6–8
	6.44 (2-H)	6.39 (2-H)	6.37 (2-H)	–	5–7
^{13}C [ppm]	167.8 (C-1)	161.1 (C-1)	167.4 (C-1)	–	150–190 140–168
	145.3 (C-3)	136.4 (C-3)	143.7 (C-3)	–	90–155
	118.2 (C-2)	120.6 (C-2)	117.9 (C-2)	–	
IR $[cm^{-1}]$	–	3321	3417	3260	3500–3300 (N–H)
	1720	–	1716	–	1750–1735 (C=O)
	–	1644	–	–	1690–1520 (C=NH)
	1638	1627	1637, 1602	–	1640–1590 (C=C)
	–	–	–	1580	–
IR $[cm^{-1}]$ Finger print	–	–	1437	–	–
			1326		
	–	–	1226	1220	–
	–	1170	1171	1171	–
	–	–	1053	1050	–
	981	–	982	–	–
	–	–	925	920	–
	769	–	836, 796	–	–

4. Synthesis of Methyl 4-Hydroxycinnamate 7:[S7]



Scheme S1. Optimized synthesis of methyl 4-hydroxycinnamate.

A mixture of $Pd(OAc)_2$ (195 mg, 0.869 mmol), tri-*o*-tolylphosphane (1.58 g, 5.19 mmol), *p*-bromophenol (**19**) (5.00 g, 28.9 mmol), NEt_3 (29.0 mL) and methyl acrylate (**20**) (4.98 g, 57.8 mmol) was stirred in a capped, nitrogen-filled Pyrex-bottle at 90 °C for 21 h. After cooling to room temperature HCl (1 M, 289 mL) was added. The aqueous phase was separated and extracted with diethyl ether (3 × 150 mL). The combined organic layers were dried over $MgSO_4$ and concentrated in vacuo. The residue was purified by column chromatography on silica gel (270 g, hexane/EtOAc 4:1, $R_f = 0.28$) to yield 4.65 g (90%) of **7** as a yellow solid: m.p. 135–137 °C (literature: m.p. 135.5–137 °C).

5. Expansions of Chiral HPLC Analysis

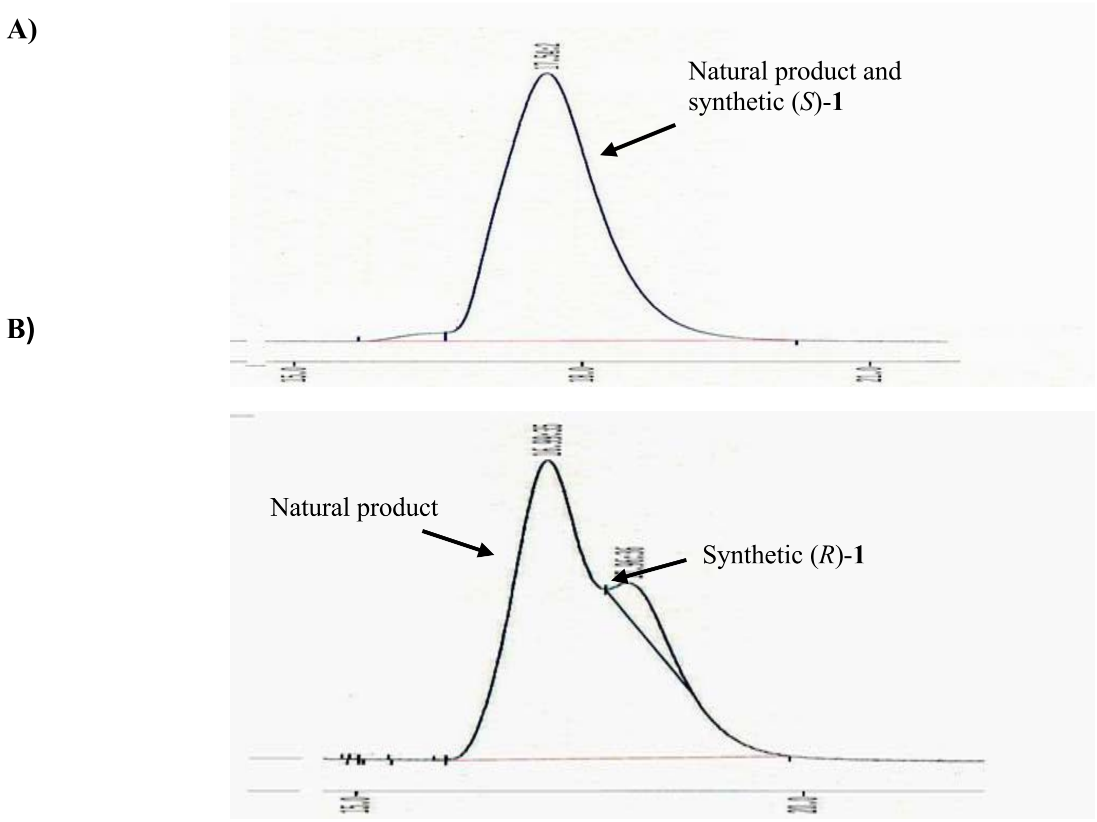
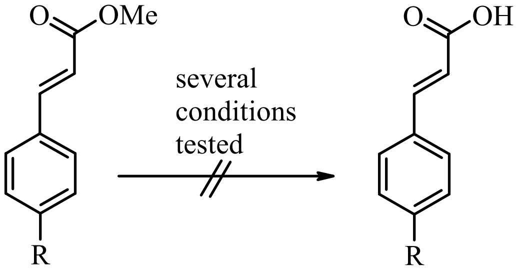


Figure S2. A): Natural product and synthetic (*S*)-**1**; B): Natural product and synthetic (*R*)-**1**.

6. Experiments towards the Total Synthesis of the Water-Soluble Analogue 17

6.1. Attempted hydrolysis of methyl ester 1 under mild basic or enzymatic conditions

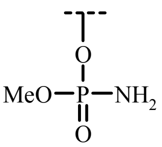
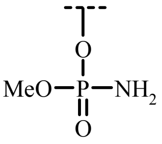


Scheme S2. Attempted hydrolysis of methyl ester **1**.

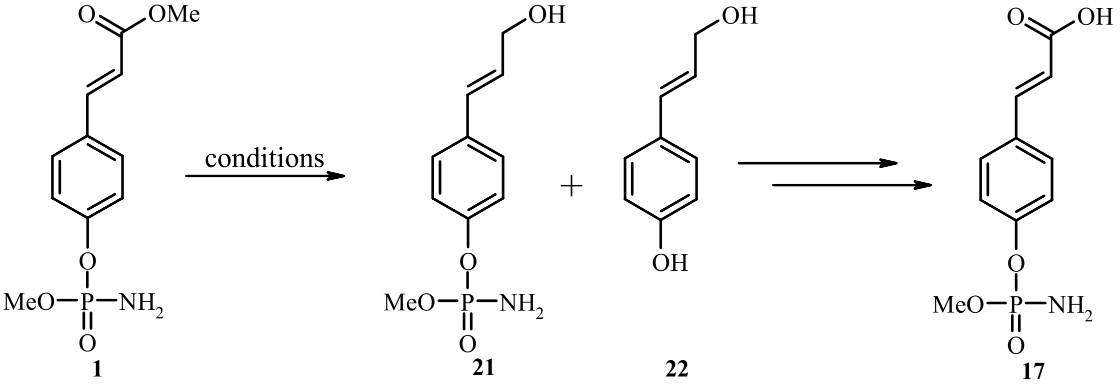
Condition A: Cinnamoylphosphoramide **1** (10 mg, 37 µmol) was dissolved in THF (88.5 µL), and LiOH × H_2O (3.6 mg, 86 µmol) was added. The resulting mixture was stirred at room temperature and monitored by TLC on reversed phase silica gel (MeOH/H_2O 7:3). After 30 min of stirring decomposition to the phenol **7** was observed.

Condition B: Cinnamoylphosphoramide **1** (20.4 mg, 75.2 µmol) was suspended in water (1.13 mL, pH 7.7) and treated with porcine liver esterase (25.8 µL, 75.2 µmol, 75.2 units). The pH was continuously adjusted to 7.7 by the pH-controlled addition of 0.5 M NaOH. The resulting mixture was stirred at room temperature and monitored by TLC on reversed phase silica gel (MeOH/H_2O 7:3).[S8]

Table S2. Hydrolysis under mild basic or enzymatic conditions.

R	Conditions	Note
 $\mathrm{MeO{-}\underset{\underset{O}{\|}}{\overset{\overset{|}{O}}{P}}{-}NH_2}$	A) 2.4 equiv. LiOH × H_2O, THF, r.t., 2 h, TLC monitored B) 1.0 equiv. LiOH × H_2O, THF, r.t., 30 min, TLC monitored	decomposition to phenol **7**
H	1.0 equiv. porcine liver esterase, H_2O (pH = 7.7), r.t., 25 min, TLC monitored	clean and complete conversion
 $\mathrm{MeO{-}\underset{\underset{O}{\|}}{\overset{\overset{|}{O}}{P}}{-}NH_2}$	A) 1.0-10 equiv. porcine liver esterase, H_2O (pH = 7.7), r.t., 14 d, TLC monitored B) 1.0-3.0 equiv. cholesterine esterase, H_2O (pH = 7.0), 37 °C, 14 d, TLC monitored	TLC analysis: traces of a more polar compound, no preparative isolation
OH	1.0-3.0 equiv. porcine pancrease lipase, H_2O (pH = 7.4), 37 °C, 14 d, TLC monitored	TLC analysis: traces of a more polar compound, no preparative isolation

6.2. Attempted reduction of methyl ester 1



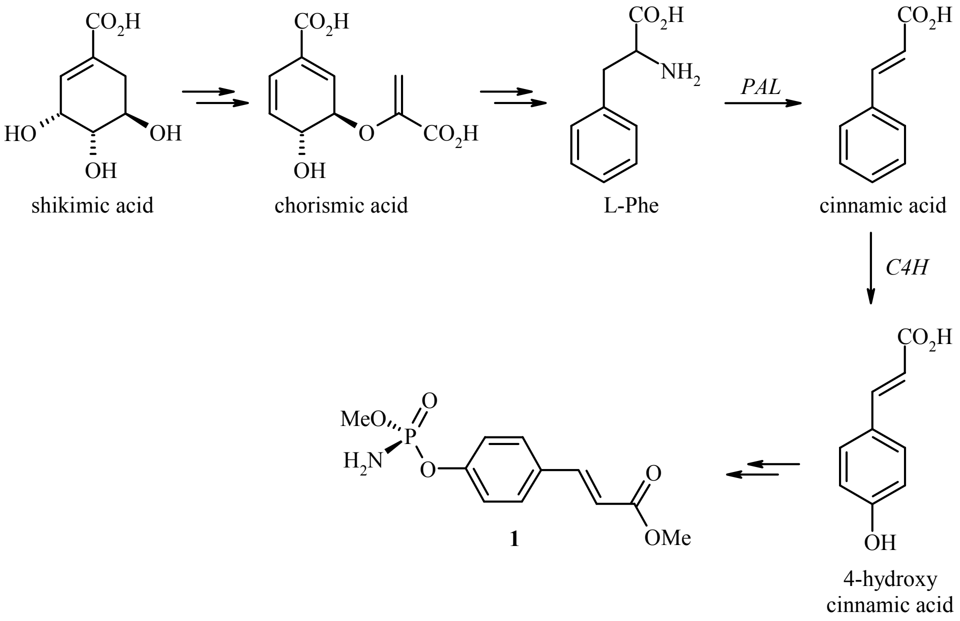
Scheme S3. Envisioned reduction-oxidation sequence for the synthesis of **17**.

Condition A: A solution of cinnamoylphosphoramide **1** (12.2 mg, 45.0 µmol) in THF (1.5 mL) was treated at –78 °C with DIBAL-H (1.0 M in hexane, 90 µL, 90 µmol) and stirred at –78 ° for 3.5 h. The reaction was quenched by addition of MeOH (27 µL) and of a saturated solution of Rochelle salt (103 µL). The resulting mixture was warmed to room temperature and the aqueous phase was diluted with water and extracted with ethyl acetate (3 × 3 mL). The combined organic layers were dried over Na_2SO_4, filtered and concentrated in vacuo to give a yellow oil.[S9]

Table S3. Attempted reductions of methyl ester **1**.

Reagent	Conditions	Note
DIBAL-H (1.0 M)	A) 2.0 equiv., THF, –78 °C, 3.5 h	**21** + **22**
DIBAL-H (1.0 M)	B) 1.0 equiv., CH_2Cl_2, –78 °C, 3.5 h	decomposition to phenol **7**
DIBAL-H (1.0 M)	C) 2.0 equiv., THF, –78 °C, 40 min	decomposition to phenol **7**
$LiAlH_4$	1.3 equiv., THF, –78 °C, 30 min	decomposition to phenol **7**

7. Hypothesis for the Biosynthetic Pathway of Cinnamoylphosphoramide



Scheme S4. Hypothesis for the biosynthetic pathway of cinnamoylphosphoramide (*PAL*: phenylalanine ammonia lyase; *C4H*: Cinnamate 4-hydroxylase).

The microbial metabolite **1** from *Streptomyces* sp. comprises a 4-hydroxycinnamate moiety which is known as a biosynthetic product from the shikimic acid pathway. In our case, the biosynthesis of the key intermediate 4-hydroxycinnamic acid probably also proceeds from shikimic acid via chorismic acid to L-phenylalanine (L-Phe) which is converted by phenylalanine ammonia lyase (PAL) to cinnamic acid followed by enzymatic conversion by cinnamate-4-hydroxylase.[S10] Subsequent methylation, phosphorylation and the introduction of the methoxy and amino group to the phosphate moiety – these steps in still unknown order - should complete the formation of the cinnamoylphosphoramide **1**.

8. References for Supporting Information:

[S1] T. Schuhmann, *Dissertation*, University Göttingen **2004**.
[S2] M. Quitschau, *Diploma thesis*, University Göttingen **2006**.
[S3] M. Hatsuda, T. Kuroda, M. Seki, *Synth. Commun.* **2003**, *33*, 427–434.
[S4] A. J. Gutierrez, K. J. Shea, J. J. Svoboda, *J. Org. Chem.* **1989**, *54*, 4335–4344.
[S5] Y. Kobayashi, T. Koizumi, E. Yoshi, *Chem. Pharm. Bull.* **1979**, *27*, 1641–1650.
[S6] M. Hesse, H. Meier, B. Zeeh, *Spektroskopische Methoden in der Organischen Chemie*, 7. Auflage, Georg Thieme Verlag Stuttgart, **2005**.
[S7] C. B. Ziegler, R. F. Heck, *J. Org. Chem.* **1978**, *43*, 2941–2946.
[S8] a) Sigma-Aldrich: notes. – b) P. G. Hultin, F. J. Mueseler, J. B. Jones, *J. Org. Chem.* **1991**, *56*, 5375–5380.
[S9] W. R. Roush, D. S. Coffey, D. J. Madar, *J. Am. Chem. Soc.* **1997**, *119*, 11331–11332.
[S10] a) K. Yazaki, L. Heide, M. Tabata, *Phytochemistry* **1991**, *30*, 2233–2236. b) C. Hertweck, B.S. Moore, *Tetrahedron* **2000**, *56*, 9115–9120.