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Synthesis of alafosfalin and its phosphinic analogue and their fungicidal activity

Radii M. Khomutov,*[a] Tatyana I. Osipova,[a] Elena N. Khurs[a] and Vitalii G. Dzhavakhiya[b]

[a] *V. A. Engelhardt Institute of Molecular Biology, Russian Academy of Sciences, 119991 Moscow, Russian Federation. Fax: +7 499 135 1405; e-mail: khomutov@genome.eimb.relarn.ru*
[b] *All-Russian Research Institute of Phytopathology, 143050 Golitsyno, Moscow Region, Russian Federation. Fax: +7 496 233 409 02; e-mail: vitaly@vniif.rosmail.com*

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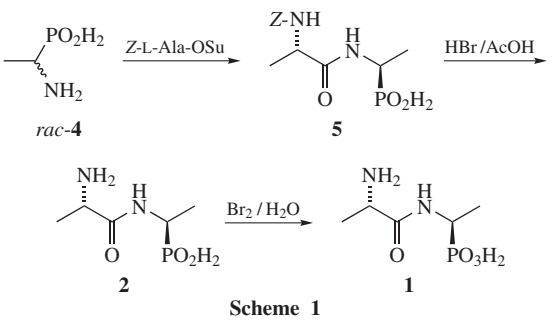
A new convenient method for the synthesis of the phosphonic and phosphinic analogues of L-Ala-L-Ala has been developed and their fungicidal activity has been demonstrated.

Alafosfalin, *i.e.*, (*R*)-[1-*N*-(*S*-alanyl)aminoethylphosphonic acid] **1**, has high antibacterial activity (ID_{50} = 0.1–4 μg cm^{-3} for gram-negative bacteria†) and provides a good example of a successful solution to the problem of transportation of compounds that are active *in vitro* into a bacterial cell.[1] Its phosphine analogue, (*R*)-[1-*N*-(*S*-alanyl)aminoethylphosphinic acid] **2**, is also efficient against pathogenic bacteria.[2] In the known synthesis of compound **1**, original racemic 1-aminoethylphosphonic acid **3** was obtained from an ester of α-bromopropionic acid (four stages).[3] After that, the diastereomeric salts of *N*-benzyloxycarbonyl derivative **3** with (*S*)-methylbenzylamine were separated by crystallisation, protection was removed, the (*R*)-isomer of acid **3** was acylated with an activated ester of *N*-benzyloxycarbonyl-L-alanine, and compound **1** was obtained after protection removal (four stages).[4] Phosphinic analogue **2** was synthesised by a similar procedure including the stage of isomer separation.[2,5]

Aiming at practical aspects of the utilisation of phosphorus analogues of dipeptides, we developed a new synthesis of analogues **1** and **2** starting from racemic 1-aminoethylphosphinic acid **4** obtained in one stage from acetaldoxime and H_3PO_2.[6] The key stage of the synthesis involves the separation of diastereomeric *N*-benzyloxycarbonyl derivatives of dipeptide **5** by usual crystallisation from water.‡ Protection removal results in target analogue **2**,§ which was oxidised to give alafosfalin **1**¶ (Scheme 1). The suggested synthetic method is based on accessible phosphinate **4**, excludes laborious crystallisation of salts with chiral amines, considerably reduces the number of stages, and allows the synthesis of analogues **1** and **2** to be performed using a common procedure.

It has been shown previously that the fungicidal activity of acid **4** is due to the inhibition of the polyketide pathways of biosynthesis of the melanin pigment, which is a component of the cytoderm in fungi.[8] In *in vitro* experiments with the *Magnaporthe grisea* (Hebert) Barr fungi, the originator of rice blast, we compared the effect of (*R*)-phosphinate **4** and (*R*)-acid **3**, as well as analogues **1** and **2**, on the pigmentation and growth of mycelium under various growing conditions on solid media in Petri dishes.†† On an agarized medium containing no amino acids, the minimum suppressing concentrations (MSC)



Scheme 1

† ID_{50} represents the concentration that inhibited growth to 50% with respect to the reference.

‡ A mixture of compound **4** (818 mg, 7.5 mmol), $NaHCO_3$ (1.26 g, 15 mmol) and 25% aqueous dioxane (20 ml) was stirred at 40 °C until dissolution and cooled to 20 °C, then a solution of *Z*-(L)-Ala-OSu (2.4 g, 7.5 mmol) in dioxane (40 ml) was added over 30 min with stirring. The mixture was stirred for 20 h at 20 °C, concentrated to 25 ml *in vacuo*, and extracted with ethyl acetate (2×10 ml); the aqueous portion was acidified with conc. HCl to pH 1 and kept for 12 h at +4 °C. The residue was separated, washed with ice water (2×2.5 ml), and dried *in vacuo* over KOH to give 1.62–1.75 g of a raw mixture of diastereomers **5**, mp 158–166 °C (decomp.). 155 mg of this mixture was dissolved in water (6 ml) with stirring at 90 °C and the solution was kept for 12 h at 20 °C; the precipitate was separated, washed with hot water (90–95 °C, 2×1 ml) on a warm filter, and dried *in vacuo* over KOH to give 44 mg of compound **5** [39% with respect to the (*R*)-isomer **4**], mp 183–186 °C (decomp.) [lit.,[2] 180–182 °C (decomp.)]. $[\alpha]_D^{22}$ –61 (*c* 2, AcOH) {lit.,[2] $[\alpha]_D^{22}$ –61 (*c* 2, AcOH)}. R_f 0.66 (A; Pr^iOH–25% NH_4OH–H_2O, 7:1:2).

§ Compound **5** (220 mg, 0.7 mmol) was added at +4 °C with stirring to 2 ml AcOH saturated with HBr. After keeping for 30 min at 20 °C, the mixture was evaporated to dryness *in vacuo*. The residue was triturated with diethyl ether, dissolved in 5 ml of anhydrous EtOH, and a solution of propylene oxide in acetone was added until formation of a precipitate stopped. After keeping for 1 h at +4 °C, the precipitate was separated, washed with acetone and diethyl ether, and dried *in vacuo* over KOH to give 106 mg (84%) of compound **2**, mp 273–277 °C (decomp.) [lit.,[2] 276 °C (decomp.)]. $[\alpha]_D^{22}$ –79 (*c* 2, H_2O) {lit.,[2] $[\alpha]_D^{22}$ –80.1 (*c* 2, H_2O)}. R_f 0.52 (A).

¶ Compound **5** (220 mg, 0.7 mmol) was treated with AcOH saturated with HBr similarly to the procedure described above. The residue was triturated with diethyl ether and dissolved in water (3 ml), Br_2 (0.05 ml) was added, the mixture was washed for 30 min at 20 °C and evaporated to dryness *in vacuo*. The residue was dissolved in water (5 ml) and evaporated to dryness *in vacuo*; after repeating this operation, the residue was dissolved in 5 ml of anhydrous EtOH. Alafosfalin **1** (124 mg, 90%) was isolated similarly to the procedure described above; mp 293–297 °C (decomp.) [lit.,[4] 295–297 °C (decomp.)]. $[\alpha]_D^{22}$ –44.7 (*c* 2, H_2O) {lit.,[4] $[\alpha]_D^{20}$ –45.4 (*c* 1, H_2O)}. R_f 0.17 (A).

†† The (*R*) isomers of acids **3** and **4** were obtained using the procedures from refs. 5 and 4, respectively. The effect of analogues on the growth and pigmentation of the mycelium of phytopathogenic fungi *Magnaporthe grisea* was studied according to ref. 7.

of (*R*)-phosphinate **4** and dipeptide **2** for mycelium pigmentation and growth were 1 and 5 μg cm^{-3}, respectively. Under these conditions, alafosfalin **1** was effective at higher concentrations (by an order), while (*R*)-acid **3** was inactive at 1000 μg cm^{-3}. On full broth containing casein hydrolysate, dipeptide **2** retained both types of activity at the same level, whereas the efficiency of (*R*)-phosphinate **4** was retained only for melanin genesis inhibition but was totally suppressed for mycelium growth. Under the same conditions, the effect of alafosfalin **1** decreased several times, while (*R*)-acid **3** was inactive. Thus, aminoacylation provided the transportation of the pharmacophore to the pathogen cell and made it possible to overcome competition with amino acids existing in the environment, which was significant for ensuring *in vivo* activity.

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