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A novel simplified synthesis of acivicin

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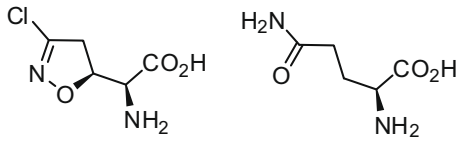
ABSTRACT

This report describes an efficient synthesis of the natural isomer of acivicin, which is the only one provided with a noteworthy biological activity. The present procedure allowed the synthesis of (+)-**1** in just five steps with a 34% overall yield. Due to the easy separation of the two diastereomers and to the availability of the starting material at low cost, the present procedure can be scaled-up to gram quantities.

1. Introduction

Acivicin [(α*S*,5*S*)-α-amino-3-chloro-4,5-dihydroisoxazol-5-yl acetic acid] (+)-**1**, a heterocyclic analogue of L-glutamine **2** (Fig. 1), was originally isolated from the fermentation broths of *Streptomyces sviceus*.[1,2] It is provided with potent anticancer activity, and a number of experimental evidences suggested its potential use in the treatment of tumors such as leukemia, melanoma, and a variety of carcinomas.[3,4] Unfortunately, during clinical trials the administration of acivicin was associated with the appearance of many severe gastrointestinal and neurological side effects.[5–8] The neurotoxic side effects of acivicin were dose related and spanned from lethargy, headaches and confusion to hallucinations, amnesia, and transient unconsciousness. Since these neurological side effects did not correlate with peak levels of acivicin, it was suggested that the drug is metabolized into a neurotransmitter antagonist.[3]



(+)-1 2

Figure 1. Model compounds.

Despite its failure as a drug candidate, acivicin has undergone extensive biochemical and pharmacological investigations to find out its mode of action as an antitumor agent.[3] It has been shown that acivicin behaves as a glutamine antimetabolite[9] that irreversibly inactivates several L-glutamine amidotransferases, including cytosine triphosphate synthetase (CTP-synthetase),[10] formylglycinamidine ribonucleotide synthetase,[11] γ-glutamyltranspeptidase (γ-GT),[12] asparagine and glutamate synthase (GltS).[13]

Since rapidly proliferating tumor cells have elevated activities of the glutamine amidotransferases, due to their involvement in the de novo biosynthesis of purine and pyrimidine nucleotides, the antitumor activity of acivicin is believed to be a consequence of the inhibition of the above-mentioned enzymes.

All the glutamine amidotransferases possess, in the pocket of the catalytic site, an essential cysteine residue that attacks the amide bond of glutamine to form a covalent γ-glutamyl thioester intermediate.[14,15] Acivicin contains a 4,5-dihydroisoxazole ring bearing a chloro substituent at position 3 which could be displaced by a nucleophilic attack of the thiolate anion of such a cysteine residue. The result is the formation of a covalent thioether bond with the dihydroisoxazole ring of acivicin and the irreversible inactivation of the enzyme. This mechanism of inhibition has been elucidated through X-ray diffraction analysis of the complex of acivicin with the carbamoyl phosphate synthetase from *Escherichia coli*.[16] A recent report on the crystal structure of the complex between γ-glutamyltranspeptidase and acivicin shows that the adduct was not derived simply by the nucleophilic displacement of the 3-chloro substituent of acivicin, but involved the opening of its 4,5-dihydroisoxazole nucleus followed by a closure with a shift of the double bond to positions 4 and 5.[17]

In the past, we had prepared a series of conformationally constrained analogues of acivicin in order to study its structure–activity relationship.[18] We evaluated the anticancer activity of new compounds in vitro against a panel of human tumor cell lines; we also measured their ability to inhibit glutamate synthase (GltS) from *Azospirillum brasilense*.[18]

With the aim of extending our investigation to different targets, for example, the capacity to inhibit trypanosome CTP-synthetase, a target for the treatment of African sleeping sickness,[19] we needed substantial amounts of acivicin to be used as the reference compound. Although acivicin is available through fermentation, large-scale production has been hampered to some extent by the occurrence of contaminants from which it is not easily separable.

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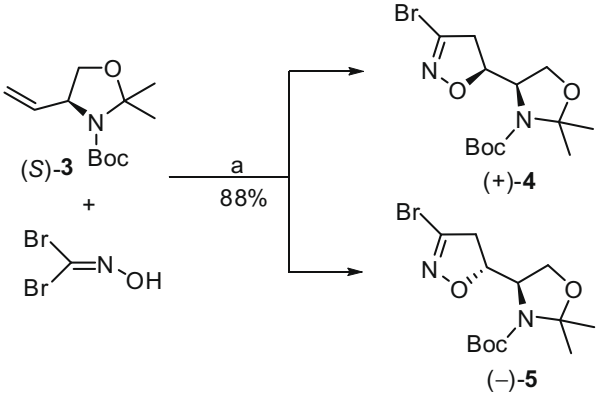
For such a reason, there has been interest in the total synthesis of acivicin with results ranging from stereorandom to stereoselective formation of the (αS,5S) natural diastereomer.[20–25]

Herein we report the total synthesis of (+)-**1** through the use of the bromonitrile N-oxide cycloaddition to dipolarophile (*S*)-**3**. We accomplished the exchange of bromide with chloride at the 3 position of the Δ^2-isoxazoline nucleus just by treating the appropriate intermediate with a THF solution of hydrochloric acid. The exchange is quantitative and takes place at room temperature in a few minutes.

2. Results and discussion

As already described in a previous paper,[26] the 1,3-dipolar cycloaddition of bromonitrile oxide to (*S*)-3-(*tert*-butoxycarbonyl)-2,2-dimethyl-4-vinyloxazolidine[27] [(*S*)-**3**; Scheme 1] yielded the two pairs of diastereomeric cycloadducts *erythro*-(+)-**4**/*threo*-(−)-**5** in a 65:35 ratio and in a 88% overall yield. The two isomers were easily separated by silica gel column chromatography.



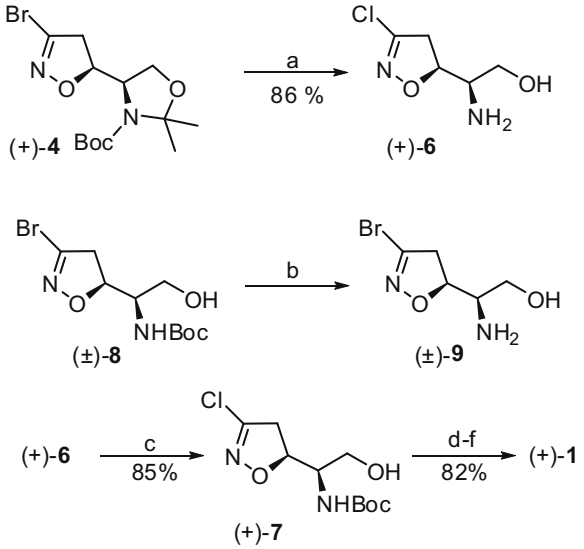
Scheme 1. Reagents: (a) $NaHCO_3$/AcOEt.

The *erythro* cycloadduct (+)-**4** was dissolved in a 10 M THF solution of HCl and was left stirring for 1 h at room temperature. After the usual work-up, amino alcohol (+)-**6** was recovered in 86% yield (Scheme 2). In order to exclude the presence of trace amounts of the corresponding 3-bromo-amino alcohol, we carried out an accurate analytical investigation based on a HRGC–MS analysis. For such a purpose, we prepared a pure sample of compound (±)-**9** by treating intermediate (±)-**8**[28] with a dichloromethane solution of trifluoroacetic acid. As detailed in Section 4, derivatives (+)-**6** and (±)-**9** possess different retention times, and consequently, we excluded the presence of the bromo derivative (±)-**9** in the reaction mixture of (+)-**6**. Furthermore, we found that the exchange is rapid and complete in 5 min.

Finally, the primary amino group of (+)-**6** was temporarily protected as a *tert*-butyl carbamate to give intermediate (+)-**7,** which was fully characterized. The primary alcohol was then oxidized to the corresponding carboxylic acid by treatment with pyridinium dichromate followed by removal of the amino protective group by treatment with a 4 N dioxane solution of HCl. After purification by cation exchange chromatography, acivicin (+)-**1** was obtained in its zwitterionic form. Crystallization from aqueous methanol afforded white crystals of (+)-**1**, whose chiroptical properties matched those of the material obtained from different synthetic procedures, for example, $[\alpha]_D^{20} = +157.6$ (*c* 0.13, H_2O) versus $[\alpha]_D^{20} = +139$ (*c* 0.14, H_2O)[25] or $[\alpha]_D^{20} = +148$ (*c* 0.845, H_2O).[21]

3. Conclusion

In summary, this report describes an efficient synthesis of the natural isomer of acivicin, which is the only one provided with a noteworthy biological activity. The present procedure allowed the synthesis of (+)-**1** in just five steps with a 34% overall yield. The limiting step is the 1,3-dipolar cycloaddition since the desired isomer (+)-**4** is obtained in high yield, but as a 65:35 mixture with its diastereomer (−)-**5**. Nevertheless, due to the easy separation of the two diastereomers and to the availability of the starting material at low cost, the present procedure can be scaled-up to gram quantities, and represents a significant improvement over the previously reported synthetic strategies.



Scheme 2. Reagents and conditions: (a) 10 M HCl/THF; (b) CF_3COOH/CH_2Cl_2; (c) Boc_2O, TEA/CH_2Cl_2; (d) PDC/DMF; (e) 4 N HCl/dioxane; (f) Amberlite IR-120 plus, 0.5 N NH_4OH (aq).

4. Experimental

4.1. Material and methods

All reagents were purchased from Sigma. Dibromoformaldoxime was prepared according to a literature procedure.[23] Dipolarophile (*S*)-3-(*tert*-butoxycarbonyl)-2,2-dimethyl-4-vinyloxazolidine[27] (*S*)-**3** was prepared from (*R*)-Garner's aldehyde following the procedure reported in the literature.

^1H NMR and ^{13}C NMR spectra were recorded with a Varian Mercury 300 (300 MHz) spectrometer. Chemical shifts (δ) are expressed in ppm, and coupling constants (*J*) are expressed in Hertz. Rotary power determinations were carried out with a Jasco J-810 spectropolarimeter coupled with a Haake N3-B thermostat. TLC analyses were performed on commercial Silica Gel 60 F_{254} aluminum sheets; spots were further evidenced by spraying with a dilute alkaline potassium permanganate solution or with ninhydrin. Melting points were determined on a model B 540 Büchi apparatus, and are uncorrected. The HRGC-FID analyses were performed using an Agilent Technologies (Palo Alto, CA) model HP 6890 series equipped with a split–splitless injector, electronic pressure control, HP 6890 autosampler, and flame ionization detector (FID); the column used was a J&W Scientific DB-5 (15 m × 0.32 mm i.d., 0.25 μm); nitrogen was used as the carrier at a flow rate of 1.3 mL/min; the injector and detector temperatures were 280 and 300 °C, respectively; the column temperature was programmed from an initial 100 °C to 300 °C at 10 °C/min; the total analysis time was 23 min. Direct infusion mass analyses were performed using a Varian 320-MS Triple Quadrupole. Microanalyses (C, H, N) of new compounds agreed with the theoretical value within ±0.4%.

4.1.1. Synthesis of (2*R*,5*S*)-2-amino-2-(3-chloro-4,5-dihydro-isoxazol-5-yl)-ethanol (+)-6

4-(3-Bromo-4,5-dihydro-isoxazol-5-yl)-2,2-dimethyl-oxazolidine-3-carboxylic acid *tert*-butyl ester (+)-**4** (7.00 g, 20.0 mmol) was dissolved in a 10 N THF solution of HCl (50 mL). The reaction mixture was stirred for 1 h. The reaction was monitored by HRGC-FID analysis to exclude the presence of the corresponding bromo-amino alcohol **9**. The solvent was evaporated, water (50 mL) was added, and the aqueous phase was extracted with AcOEt (2 × 100 mL). The aqueous layer was made basic with 0.5 N NaOH and was newly extracted with AcOEt (4 × 100 mL). The organic extracts were pooled and dried over anhydrous sodium sulfate. The solvent was removed under vacuum, and the crude material was purified by column chromatography (eluent: dichloromethane/methanol 8:2), obtaining 2.81 g of compound (+)-**6** (17.1 mmol, 86% yield).

(+)-**6**: pale yellow oil; R_f (dichloromethane/methanol 8:2) 0.5; $[\alpha]_D^{20} = +95.7$ (*c* 1.00, $CHCl_3$); ^1H NMR ($CDCl_3$): 2.07 (br s, 3H); 3.13 (dd, *J* = 10.7, 17.6, 1H); 3.15 (ddd, 4.4, 5.5, 5.8, 1H); 3.25 (dd, *J* = 9.0, 17.6, 1H); 3.54 (dd, *J* = 5.8, 11.0, 1H); 3.66 (dd, *J* = 4.4, 11.0, 1H); 4.73 (ddd, *J* = 5.5, 9.0, 10.7, 1H); ^{13}C NMR ($CDCl_3$): 39.9; 54.4; 63.4; 84.1; 150.0; HRGC retention time: 3.7 min; $[M+H]^+$ = 165.1. Anal. Calcd for $C_5H_9ClN_2O_2$ (164.59): C, 36.49; H, 5.51; N, 17.02. Found: C, 36.70; H, 5.69; N, 16.69.

4.1.2. Synthesis of (2*R**,5*S**)-2-amino-2-(3-bromo-4,5-dihydro-isoxazol-5-yl)-ethanol (±)-9

Intermediate (±)-**8** (100 mg, 0.32 mmol) was treated with a 30% dichloromethane solution of trifluoroacetic acid (250 μL) at 0 °C. The reaction mixture was stirred at room temperature until disappearance of the starting material (4 h). The volatiles were removed under vacuum. Water (5 mL) was added, and the aqueous phase was extracted with AcOEt (2 × 5 mL). The aqueous layer was made basic with 0.5 N NaOH and was newly extracted with AcOEt (4 × 10 mL). The organic extracts were pooled and dried over anhydrous sodium sulfate. The solvent was removed under vacuum and purified by column chromatography (eluent: dichloromethane/methanol 8:2), obtaining 58 mg of compound (±)-**9** (0.28 mmol, 85% yield).

(±)-**9**: pale yellow oil; R_f (dichloromethane/methanol 8:2) 0.5; ^1H NMR ($CDCl_3$): 1.65 (br s, 3H); 3.12–3.24 (m, 2H); 3.30 (dd, 8.8, 17.3, 1H); 3.56 (dd, *J* = 5.8, 11.0, 1H); 3.68 (dd, *J* = 4.4, 11.0, 1H); 4.67 (ddd, *J* = 5.2, 8.8, 10.4, 1H); ^{13}C NMR ($CDCl_3$): 42.9; 54.3; 63.8; 83.6; 138.3; HRGC retention time: 4.6 min; $[M+H]^+$ = 208.9. Anal. Calcd for $C_5H_9BrN_2O_2$ (209.04): C, 28.73; H, 4.34; N, 13.40. Found: C, 29.01; H, 5.81; N, 16.40.

4.1.3. Synthesis of (1*R*,5*S*)-[1-(3-chloro-4,5-dihydro-isoxazol-5-yl)-2-hydroxy-ethyl]-carbamic acid *tert*-butyl ester (+)-7

To a stirred solution of compound (+)-**6** (2.81 g, 17.1 mmol) in dichloromethane (50 mL) at 0 °C were added TEA (3.60 mL, 25.7 mmol) and Boc_2O (4.10 g, 18.8 mmol). The mixture was stirred overnight. The organic layer was washed with 0.5 N HCl (2 × 50 mL). The organic layer was dried over anhydrous Na_2SO_4, and the solvent was evaporated under vacuum. The crude material was purified by flash chromatography (eluent: cyclohexane/AcOEt 7:3) to give 3.84 g of compound (+)-**7** (14.5 mmol, yield 85%).

(+)-**7**: colorless needles from iPr_2O; mp 104–106 °C; R_f: (cyclohexane/AcOEt 1:1) 0.5; $[\alpha]_D^{20} = +98.2$ (*c* 1.00, $CHCl_3$); ^1H NMR (DMSO-d_6, *T* = 50 °C): 1.37 (s, 9H); 3.15 (dd, *J* = 8.2, 17.6, 1H); 3.28 (dd, *J* = 11.0, 17.6, 1H); 3.41 (dd, *J* = 5.8, 5.8, 2H); 3.65 (dddd, *J* = 5.8, 5.8, 6.6, 9.4, 1H); 4.65 (dd, *J* = 5.8, 5.8, 1H); 4.76 (ddd, *J* = 6.6, 8.2, 11.0, 1H); 6.62 (br s, 1H); ^{13}C NMR ($CDCl_3$): 28.5; 41.6; 54.2; 61.3; 80.5; 81.6; 150.2; 156.3; $[M+Na]^+$ = 287.0. Anal. Calcd for $C_{10}H_{17}ClN_2O_4$ (264.71): C, 45.37; H, 6.47; N, 10.58. Found: C, 45.64; H, 6.60; N, 10.39.

4.1.4. Synthesis of (α*S*,5*S*)-α-amino-3-chloro-4,5-dihydro isoxazol-5-yl acetic acid (+)-1

(a) To a solution of compound (+)-**7** (3.84 g, 14.5 mmol) in DMF (80 mL) pyridinium dichromate (85 g, 225 mmol) was added, and the mixture was stirred at room temperature for 6 h. The progress of the reaction was monitored by TLC (CH_2Cl_2/MeOH 95:5 + 1% acetic acid). Water was added (150 mL), and the mixture was extracted with AcOEt (3 × 150 mL). The organic layer was extracted with 1 M NaOH (3 × 50 mL), and the aqueous phase was made acidic with 2 M HCl and was extracted with AcOEt (3 × 50 mL). The organic extracts were washed with brine, dried over anhydrous Na_2SO_4, and the solvent was evaporated to give 3.62 g of the crude carboxylic acid.

(b) The crude material obtained from the previous step (3.62 g, 13.0 mmol) was treated with a 4 M HCl/dioxane solution. The reaction mixture was stirred at room temperature until disappearance of the starting material (1 h). The volatiles were removed under vacuum, and the residue was dissolved in water and submitted to cation exchange chromatography using Amberlite IR-120 plus. The acidic solution was slowly eluted onto the resin, and then the column was washed with water until the pH was neutral. The compound was eluted off the resin with 0.5 N aqueous ammonia, and the product-containing fractions (detected with ninhydrin stain on a TLC plate) were combined and the solvent was freeze-dried to give 2.13 g (11.9 mmol, 82% yield after two steps) of (+)-**1** as a white solid.

Recrystallization from aqueous methanol gave colorless prisms of (+)-**1**, mp dec >180 °C, R_f: 0.55 (MeOH–H_2O–pyridine: 20:5:1); $[\alpha]_D^{20} = +157.6$ (*c* 0.13, H_2O) {lit. $[\alpha]_D^{20} = +139$ (*c* 0.14, H_2O)[25] or $[\alpha]_D^{20} = +148$ (*c* 0.845, H_2O)[21]}; ^1H NMR (D_2O): 3.34 (dd, *J* = 8.3, 18.0, 1H); 3.43 (dd, *J* = 11.0, 18.0, 1H); 3.94 (d, *J* = 3.3, 1H), 5.18 (ddd, *J* = 3.3, 8.3, 11.0, 1H); ^{13}C NMR (D_2O): 39.8; 56.1; 80.5; 152.4; 170.1; $[M-H]^-$ = 176.5. Anal. Calcd for $C_5H_7ClN_2O_3$ (178.57): C, 33.63; H, 3.95; N, 15.69. Found: C, 33.42; H, 4.00; N, 15.59.

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