Synthesis of L-2-Amino-8-oxodecanoic Acid: An Amino Acid Component of Apicidins

M. Lourdes Linares,1 F. Javier Agejas,2 Ramón Alajarín,8 J. José Vaquero, Julio Alvarez-Builla*
Departamento de Química Orgánica, Universidad de Alcalá, Alcalá de Henares, 28871 Madrid, Spain
Fax +34(91)8854606; E-mail: julio.alvarez@uah.es
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Abstract: The synthesis of L-2-amino-8-oxodecanoic acid (Aoda) is described. This is a rare amino acid component of apicidins, a family of new cyclic tetrapeptides, inhibitors of histone deacetylase. Aoda was synthesised in seven steps from L-glutamic acid, along with some derivatives.

Key words: amino acid, Aoda, apicidins, histone deacetylase, inhibitors

Apicidins 1a–d are a new family of natural cyclic tetrapeptides that have been recently identified as antiprotozoal agents inhibiting parasite histone deacetylase (HDAC) in vitro. HDACs are nuclear isozymes that regulate gene transcription via the dynamic acetylation/deacetylation at specific residues in histones.1 Three analogues, apicidin (1a), apicidin A (1b), apicidin B (1c) and apicidin C (1d) have been recently isolated from the fermentation broth of Fusarium palidoderum (ATCC 74289) (Figure 1).2,3 They are structurally related to other naturally occurring cyclic tetrapeptides described in the literature as, for instance, HC-toxin, trapoxin A, WFS161, Cly-2, and chlamydacin, which display potent anti-neoplastic or antiprotozoal activity.4–10 Also, 1a has shown in vivo efficacy against Plasmodium berghei malaria. The potential use of 1a for the treatment of cancer has been suggested.11 Thus, four patents have been reported claiming apicidins and derivatives as therapeutic agents against protozoal infections or as components of anti-tumour compositions.12 Additional potent inhibitors of HDAC are largely restricted to hydroxamic acid containing molecules such as the natural product trichostatin A and derivatives.13–15

The four apicidins are structurally related by the presence of (2S)-amino-8-oxodecanoic acid (Aoda; 2) and Trp moieties (Figure 1). The ethyl ketone side-chain in 2 is unique to apicidins and is not present in related HDAC cyclic tetrapeptide inhibitors. The C-8 keto group of the 2 moiety mimics the C-8 keto group of the acetylated lysine residue of histones 3 (Figure 1). Although chemical modification of 216–18 and Trp20–21 residues in 1a produced some transition-state analogues, it only led to a limited SAR. The presence of a C-8 oxo group in the side-chain in 2 has been shown of significance for the activity of analogues of 1a.

Recently, three syntheses of apicidins have been reported. First, 1a was synthesised using a precursor of 2 bearing a silyl ether group in the C-8 position. In this synthesis 2 was not synthesised but its 8-tert-butyldiphenylsilyloxy derivative (23.8% from L-Ser) that was transformed to the 8-keto group once the cyclic tetrapeptide core was built. After cyclisation, silyl ether was deprotected and the resulting secondary alcohol oxidised to give 1a.22 The first synthesis of 2 as its 2-benzoylcarbonyl methyl ester derivative was performed from L-glutamic acid in six steps (23% yield) and was used to prepare 1b.23 The key step was a Michael addition on ethyl vinyl ketone under photolytic conditions in the presence of tri-n-butyltin hydride. Finally, 2 was also synthesised as its N-Boc-derivative from Garner’s aldehyde in six steps (33.6% yield) to prepare a tetrapeptoid analogue of 1c and apicidin A (1a) through solution- and solid-phase synthesis, respectively.24

In this article we report a synthesis of 2 and derivatives which can be suitable for developing apicidine analogues by modification at any amino acid residue in 1a–d (Scheme 1). Starting from commercially available 4, the
reaction with triphenylphosphine in ethyl acetate, in the presence of sodium iodide gave quantitatively the insoluble phosphonium salt 5 (99%). The oxo group was then protected as 1,3-dioxolane or 1,3-dithiolane to give 6a (85%) and 6b (83%) respectively. Wittig reaction between 6a,b and 7 led to a satisfactory yield of 9a (72%; cis/trans = 93:7) but only a low yield of 9b (31%; cis-isomer). Aldehyde 7 was obtained from L-glutamic acid (8) in two steps in an improved yield to that reported (91%).25 This approach to long-chain amino acids has been previously described for the synthesis of an inhibitor of pancreatic lipase.26 Both 6a and 7 were synthesised on 200 g scale in our pilot plant facilities.27 Compounds 9a,b were subsequently hydrogenated on Pd/C to quantitatively yield 10a (98%) and 10b (93%). Compound 10a was sequentially hydrolysed under basic and acidic conditions to give Aoda (2; 79%; 47% overall yield from 4) as its hydrochloride 11.28 Further treatment of 11 with FmocCl in the presence of 10% potassium carbonate led to 12 (85%). Finally, allyl ester formation was carried out using allyl bromide and N,N-dissopropylethylamine to give 13 (95%; 39% overall yield from 4). Sequential hydrolysis of 10b under basic and acidic conditions gave 14 (98%). Further treatment with di-tert-butyl dicarbonate and triethylamine yielded 15 (78%; 18.1% overall yield from 4). Further treatment with clayfen gave Boc-Aoda (16; 88%). In the order to improve the low yield for 9b, 10a was hydrolysed by acid and then treated with ethanedithiol before final basic hydrolysis to give 14 (76%). After protection of amine group 16 was obtained in an improved yield (78%; 35.2% overall yield from 4).

In summary, a synthesis of 2 has been developed with an improved overall yield, in comparison to previously reported syntheses, from readily accessible precursors, and can be easily scaled up in initial steps.

Solvents and reagents were purchased from Aldrich Co. and were not further purified. Clayfen was prepared as previously reported.29 Solvents were dried by standard methods. Reactions under anhydrous conditions were performed under argon using deoxygenated and anhydrous solvents.30 1H NMR spectra were recorded on a Varian Unity 300 or Varian Unity 500 (300 MHz and 500 MHz, respectively) spectrometers.31 13C NMR spectra were recorded on a Varian Unity 300 (75 MHz) spectrometer. Mass spectra were obtained on a Hewlett-Packard 5988A instrument (EI or CI, 70 eV) by direct inlet or electrospray. Microanalyses were performed on a Heraeus CHN Rapid instrument. Optical rotations were measured with a Perkin-Elmer 341 polarimeter. Melting points were measured with an Electrothermal IA 6304 instrument.

3-Oxopentyltriphenyphosphonium Iodide (5)
To a solution of 4 (25 g, 0.207 mol) in EtOAc (210 mL), Ph,P (54.38 g, 0.207 mol) and NaI (31.03 g, 0.207 mol) were added. The reaction mixture was stirred at reflux temperature for 22 h. The precipitated NaCl was filtered off and the remaining mixture was washed with EtOAc. The solvent was evaporated under reduced pressure and the residue was treated with EtO. The precipitated solid was filtered and dried, yielding 5 (97.14 g, 99%) as a white solid; mp 214–215 °C (hexane–EtOH).

3H NMR (300 MHz, CDCl3): δ = 6.60–7.80 (m, 15 H), 3.86 (p, J = 6.8 Hz, 2 H), 3.10–3.20 (m, 2 H), 2.43 (q, J = 7.2 Hz, 2 H), 0.87 (t, J = 7.2 Hz, 2 H).

3C NMR (75 MHz, CDCl3): δ = 229.7, 134.9, 134.9, 133.4, 133.3, 130.4, 130.3, 130.2, 118.3, 117.1, 36.1, 35.2, 17.6, 16.9, 7.7.

MS (EI): m/z (%) = 262 (82), 183 (100), 128 (92), 108 (68).

[2-(2-Ethyl[1,3]dioxolane-2-yl)ethyl]triphenylphosphonium Iodide (6a)

To a slurry of 25.5 (46 g, 96.84 mmol) in toluene (300 mL), PPTS (2.47 g, 9.08 mmol) and ethylene glycol (13.53 mL, 242.1 mmol) were added. H₂O was removed using a Dean–Stark trap, heating at reflux temperature for 14 h. The solvent was evaporated under reduced pressure. The residue was dissolved in CH₂Cl₂ and washed with H₂O and sat. NaCl solution. The organic layer was dried over MgSO₄, filtered and evaporated. The residual oil was treated with THF, yielding 6a (41.13 g, 82%) as a white solid; mp 137–137 °C (hexane–EtOH).

H NMR (300 MHz, CDCl₃): δ = 7.70–7.80 (m, 15 H), 4.04 (t, J = 6.8 Hz, 2 H), 3.91 (t, J = 6.8 Hz, 2 H), 3.40–3.50 (m, 2 H), 1.80–2.10 (m, 1 H), 1.63 (q, J = 7.6 Hz, 2 H), 0.78 (t, J = 7.2 Hz, 2 H).

13C NMR (75 MHz, CDCl₃): δ = 135.0, 133.3, 133.1, 130.5, 130.3, 117.9, 116.8, 72.3, 73.2, 41.7, 39.4, 36.5, 35.2, 28.8, 21.8, 11.2.

MS (EI): m/z (%) = 252 (77), 183 (100), 128 (18), 108 (69).


(2-(2-Ethyl[1,3]dithiolan-2-yl)ethyl]triphenylphosphonium Iodide (6b)

To a solution of 25.5 (40.8 g, 86.9 mmol) in CH₂Cl₂ (330 mL), 1,2-ethanediol (21.6 mL, 258 mmol) and BF₃·Et₂O (4.3 mL, 34 mmol) were added. The reaction mixture was stirred at r.t. for 20 h under an argon atmosphere. The solvent was removed under reduced pressure. Residual oil was triturated with hexane, yielding 6b (41.20 g, 87%) as a white solid; mp 124–125 °C (hexane–EtOH).

H NMR (300 MHz, CDCl₃): δ = 7.70–7.80 (m, 15 H), 3.60–3.70 (m, 2 H), 3.20–3.30 (m, 4 H), 2.00–2.10 (m, 2 H), 2.00 (q, J = 7.0 Hz, 2 H), 1.00 (t, J = 7.0 Hz, 3 H).

13C NMR (75 MHz, CDCl₃): δ = 135.1, 133.3, 133.2, 130.6, 130.4, 117.9, 116.8, 72.1, 73.2, 41.9, 40.9, 36.5, 37.2, 35.3, 28.8, 21.8, 11.2.

MS (EI): m/z (%) = 262 (83), 183 (100), 128 (33), 77 (70).


(S)-2-[(2,3,4,5-Tetrahydro-2H-pyran-2-yl)methoxy]carbonylaminomethyl-2-ethyl[1,3]dioxol-2-yl)hept-5-ene Acid Methyl Ester (9a)

To a solution of 9a (16.6 g, 36.32 mmol) in EtOAc (15 mL), 10% Pd/C (3.86 g, 3.63 mmol) was added. The mixture was stirred at r.t. under a H₂ atmosphere (5 atm) for 15 h. The reaction mixture was filtered through Celite and washed with EtOH. The solvent was evaporated under reduced pressure, yielding 9a (16.33 g, 98%) as a colourless oil; [α]D²⁵ = +26.0 (c = 0.33, CHCl₃).

H NMR (300 MHz, CDCl₃): δ = 3.89 (dd, J = 5.1, 9.5 Hz, 1 H), 3.88 (s, 4 H), 3.87 (s, 3 H), 3.20–3.40 (m, 1 H), 2.92–2.10 (m, 2 H), 1.80–1.90 (m, 2 H), 1.50–1.70 (m, 3 H), 1.46 (s, 18 H), 1.20–1.40 (m, 4 H), 0.86 (t, J = 7.5 Hz, 3 H).

13C NMR (75 MHz, CDCl₃): δ = 121.0, 119.7, 113.6, 111.7, 82.8, 64.9, 55.1, 52.6, 36.7, 29.9, 29.7, 28.2, 26.3, 23.8, 8.3.

MS (EI): m/z (%) = 460 (42) [M⁺ + 1], 430 (15), 101 (87), 57 (100).

Anal. Calcd for C₃₂H₄₃NO₄: C, 60.11; H, 8.99; N, 3.05. Found: C, 60.11; H, 9.10; N, 3.10.

(S)-2-[(2,3,4,5-Tetrahydro-2H-pyran-2-yl)methoxy]carbonylaminomethyl-2-ethyl[1,3]dioxol-2-yl)hept-5-ene Acid Methyl Ester (10a)

To a solution of 9a (14.7 g, 2.86 mmol) in EtOAc (10 mL), 10% Pd/C (0.30 g, 0.28 mmol) was added. The reaction mixture was stirred at 35 °C under H₂ (5 atm) for 18 h. After filtering through Celite and washing with EtOAc, the filtrate was evaporated under reduced pressure yielding 10a (1.3 g, 95%) as a colourless oil; [α]D²⁵ = +26.3 (c = 1.03, CHCl₃).

H NMR (300 MHz, CDCl₃): δ = 3.84 (dd, J = 5.1, 9.5 Hz, 1 H), 3.70 (s, 3 H), 3.24 (s, 4 H), 2.00–2.10 (m, 1 H), 1.90–2.00 (m, 4 H), 1.48 (s, 18 H), 1.30–1.40 (m, 7 H), 1.02 (t, J = 7.2 Hz, 3 H).

13C NMR (75 MHz, CDCl₃): δ = 137.1, 135.7, 82.9, 72.3, 58.1, 52.2, 42.9, 39.6, 36.3, 30.0, 29.8, 29.6, 28.1, 26.9, 26.3, 11.5.

MS (EI): m/z (%) = 492 (100) [M⁺ + 1].

Anal. Calcd for C₃₂H₄₃NO₄: C, 56.18; H, 8.40; N, 2.85. Found: C, 56.78; H, 8.90; N, 2.83.
(S)-2-Amino-8-oxodecanoic Acid Hydrochloride (11)
To a solution of 10a (11 g, 23.96 mmol) in MeOH (156 mL), 1 M NaOH (72 mL) was added at 0 °C and the mixture was stirred at r.t. for 12 h. The solvent was removed under reduced pressure and the aqueous basic solution was extracted with EtO (2 x 20 mL). The aqueous layer was treated with 3.5% HCl until pH = 4-5 and then extracted with EtO (3 x 50 mL). The organic layer was dried over MgSO₄, filtered and evaporated under reduced pressure. The residue was dissolved in THF (35 mL), cooled at 0 °C and treated with 1 N HCl (34.5 mL). The mixture was stirred at r.t. for 8 h. The solvent was removed under reduced pressure. The residual oil was dissolved in EtOAc and treated with EtOH, yielding 11 (9.99 g, 92%) as a white solid; mp 207-208 °C (EtOH-EtOH); [α]D 25 4.4 (c = 1.06, MeOH).

1H NMR (300 MHz, CDCl₃): δ = 2.34 (d, J = 6.0 Hz, 2H); 2.45 (q, J = 7.2 Hz, 2H); 1.8-2.0 (m, 2H), 1.5-1.6 (m, 6H), 1.00 (t, J = 7.2 Hz, 3H), 3.12 (H, CH₂CH₃)

13C NMR (75 MHz, CDCl₃): δ = 211.5, 172.2, 155.8, 143.8, 143.7, 141.3, 131.5, 127.6, 127.0, 125.0, 119.9, 118.9, 66.9, 65.9, 53.8, 47.1, 42.1, 35.8, 32.5, 28.7, 24.9, 23.4, 7.8.


(S)-2-Amino-7-(2-ethyl-1,3)dithiolan-2-yl)heptanoic Acid (14)
To a solution of 10a (2.47 g, 5.39 mmol) in EtOAc (7.6 mL), 4 M HCl (7.6 mL, 30.4 mmol) was added at 0 °C and the mixture was stirred at r.t. for 8 h. The solvents were removed under reduced pressure and the residue was treated with EtOAc, filtered and washed (EtOAc and EtOH). The solid was suspended in anhyd CHCl₃ (50 mL) and 1,2-ethanediol (0.66 mL, 8.08 mmol) and BF₃·Et₂O (0.54 mmol) were added under an argon atmosphere. The mixture was stirred at r.t. for 20 h. The solvent was removed to dryness and the residue was dissolved in H₂O (50 mL) and extracted with EtO (2 x 25 mL). The aqueous layer was evaporated to dryness, dissolved in MeOH (30 mL) and treated with 1 N NaOH (27 mL, 27 mmol) at r.t. for 12 h. MeOH was evaporated under reduced pressure and the aqueous residue was neutralised with 3.5% HCl yielding 14 (1.46 g, 98%) as a white solid; mp 222-223 °C; [α]D 19 2.0 (c = 1.0, MeOH).

1H NMR (300 MHz, CDCl₃): δ = 2.49 (t, J = 5.3 Hz, 1H), 2.34 (s, 4H), 1.80-1.90 (m, 4H), 1.70-1.80 (m, 2H), 1.40-1.50 (m, 7H), 1.03 (t, J = 7.1 Hz, 3H).

13C NMR (75 MHz, CDCl₃): δ = 174.0, 70.3, 56.1, 44.1, 40.4, 37.4, 32.4, 30.7, 27.8, 26.3, 11.6.

MS (ESI): m/z (%) = 276 (100) [M+H]+. Anal. Calcd for C₁₃H₂₃NO₃S₂: C, 51.96; H, 8.36; N, 5.05; S, 23.07. Found: C, 56.68; H, 8.20; N, 4.83; S, 22.96.

(S)-2-Amino-7-(2-ethyl-1,3)dithiolan-2-yl)heptanoic Acid (15)
To a solution of 14 (1.46 g, 5.39 mmol) in MeOH (36 mL), di-tert-butyl dicarbonate (1.8 g, 8.6 mmol) and Et₂N (4.13 mL, 29.67 mmol) were added. The reaction mixture was heated at 60 °C for 1 h, and then left at r.t. for 2 h. The solvent was concentrated to dryness and treated with 3.5% HCl to pH 4-5. The mixture was extracted with EtOAc (3 x 25 mL) and the organic layer was washed with sat. NaCl, dried over MgSO₄, filtered and evaporated under reduced pressure to yield 15 (1.58 g, 78%) as a brownish oil; [α]D 25 7.4 (c = 1.06, CHCl₃).

1H NMR (300 MHz, CDCl₃): δ = 2.62 (br s, 1H), 5.01 (d, J = 8.2 Hz, 1H), 4.11 (d, J = 7.1 Hz, 1H), 3.62 (s, 4H), 1.80-2.00 (m, 4H), 1.00-1.70 (m, 1H), 1.44 (s, 9H), 1.30-1.40 (m, 7H), 1.02 (t, J = 7.2 Hz, 3H).

13C NMR (75 MHz, CDCl₃): δ = 172.3, 155.5, 80.1, 72.3, 53.4, 42.8, 39.5, 36.2, 32.4, 29.3, 28.3, 26.6, 25.2, 11.2.


(S)-2-Amino-7-(2-ethyl-1,3)dithiolan-2-yl)heptanoic Acid (16)
To a solution of 15 (0.1 g, 0.26 mmol) in CH₂Cl₂ (10 mL), clayfen (0.27 g, 0.29 mmol) was added and the mixture was stirred at r.t. for 2 h. After filtering through Celite, the filtrate was evaporated to dryness to yield 16 (70.2 mg, 88%) as a colourless oil; [α]D 25 -2.5 (c = 1.05, MeOH).

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1H NMR (400 MHz, CDCl3): δ = 5.10 (d, J = 7.9 Hz, 1 H), 4.20–4.30 (m, 1 H), 2.49–2.50 (m, 4 H), 1.70–1.80 (m, 1 H), 1.50–1.70 (m, 4 H), 1.45 (s, 9 H), 1.30–1.40 (m, 4 H), 1.05 (t, J = 7.3 Hz, 3 H).

12C NMR (100 MHz, CDCl3): δ = 212.0, 176.8, 155.6, 80.1, 53.2, 42.1, 35.8, 32.2, 28.6, 28.2, 24.9, 23.5, 7.7.

HRMS (ES+): m/z calc'd for C11H16N2O2: 300.1802; found: 300.1811.

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(27) Planta Piloto de Química Fina, Universidad de Alcalá de Henares, 28871 Alcalá de Henares, Madrid, Spain. Head: Prof. J. Alvarez-Builla, E-mail: julio.alvarez@uah.es.