Tetrahedron 71 (2015) 9626–9629

Contents lists available at ScienceDirect

Tetrahedron

journal homepage: www.elsevier.com/locate/tet

Total synthesis of splenocin B, a potent inhibitor of the pro-inflammatory cytokine from marine-derived *Streptomyces* sp.

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ARTICLE INFO

Article history:
Received 29 September 2015
Received in revised form 26 October 2015
Accepted 27 October 2015
Available online 3 November 2015

Keywords:
Splenocin B
9-Membered dilactone
Anti-inflammatory
Kita–Trost lactonization
Antimycin-class antibiotics

ABSTRACT

The first total synthesis of splenocin B (**1**), a new potent anti-inflammatory antimycin-class antibiotic, has been described. The synthesis of **1** has been accomplished in 8 linear steps, starting from commercially available *N*-Boc-L-threonine benzyl ester **4** and 3,4-dihydroxypentanoic acid derivative **2**. Kita–Trost lactonization via an ethoxyvinyl ester intermediate was utilized for the construction of the 9-membered dilactone core.

1. Introduction

Splenocins were isolated from an organic extract of marine-derived *Streptomyces* strain CNQ431 as potent anti-inflammatory antibiotics in 2009, which displayed low nanomolar activity in the suppression of cytokine production by OVA-stimulated splenocytes.[1a] Splenocins exhibit inhibitory activities toward not only the production of TH2 cytokines IL-5 and IL-13 but also the production of the dendritic cell-associated cytokins IL-1 and TNF-α, which provide great benefits in the treatment of asthma. The structures of splenocins are similar to those of antimycin A_3 (AA)[2,3] and UK-2A, another antibiotic in the antimycin class, which was first isolated in 1996 from a soil sample collected at our campus.[4] These consist of 9-membered dilactone rings linked via an amide bond to an aromatic acid moiety (Fig. 1); splenocins and AAs have 3-formamidosalicylic moieties, while UK-2A possesses a 3-hydroxy-4-methoxy-picolinic moiety.

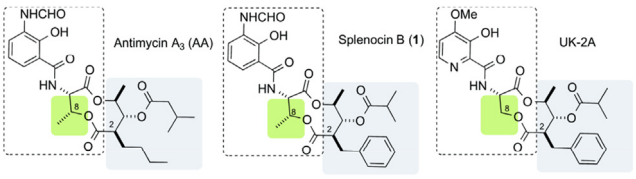

Fig. 1. Structures of antimycin A_3, splenocin B (**1**) and UK-2A.

Splenocin B (**1**) is reported to be as effective as dexamethasone in inhibiting TH2 cytokine production and is a hybrid molecule combining some structural features of both UK-2A and AA; the benzyl group at the C2 position in **1** had not been reported in AAs. In our continuing studies on UK-2A and AAs,[5] we have been very interested in the structure and biological activities of splenocin B. Herein we report the first total synthesis of splenocin B (**1**).

2. Results and discussion

Our synthesis of **1** commences with the formation of the 3,4-dihydroxypentanoic acid derivative **2**, which was achieved through the Evans aldol reaction between aldehyde **3** and *N*-hydrocinnamoyloxazolidine, as previously reported (Scheme 1).[6] Condensation of **2** with commercially available *N*-Boc-L-threonine benzyl ester **4** was conducted in the presence of EDCI·HCl (3.0 equiv)–DMAP (0.30 equiv) to afford **5** in 93% yield. Removal of the two benzyl groups by hydrogenolysis with $Pd(OH)_2$ in EtOH afforded the cyclization precursor seco acid **6** in 98% yield.

Next, we focused on the construction of a nine-membered dilactone moiety, which appear to be the more difficult due to both enthalpic and entropic factors. The use of 2-methyl-6-nitrobenzoic anhydride (MNBA) with a DMAP or DMAPO catalyst,[7] which was found to be optimal in the previous reports on the synthesis of AAs,[3e,3j] provided only dimeric 18-membered tetralactone. After several attempts, including the use of a 2-pyridinethiol ester-

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http://dx.doi.org/10.1016/j.tet.2015.10.075
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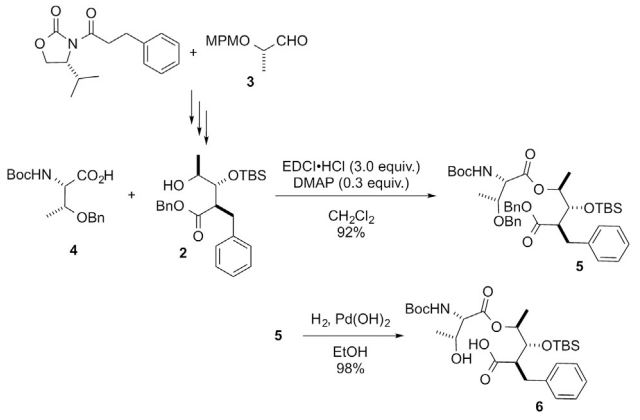
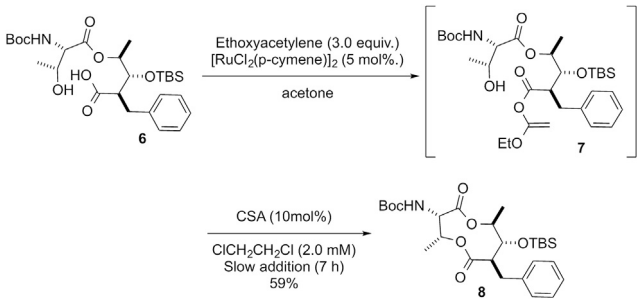


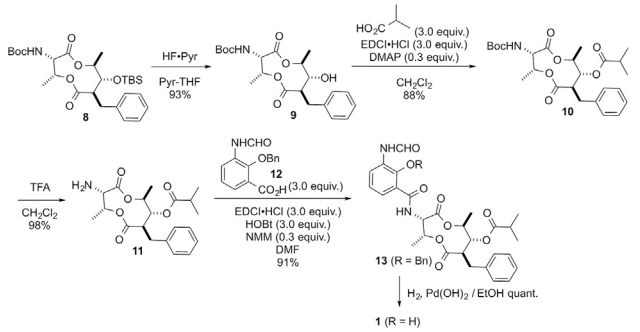
Scheme 1. Synthesis of seco acid **6**.

silver salt,[8] we executed the Kita–Trost method.[9] Treatment of **6** with ethoxyacetylene (3.0 equiv) in the presence of $[RuCl_2(p\text{-cymene})]_2$ (5 mol%) afforded the corresponding ethoxyvinyl ester intermediate **7**. To a solution of CSA (10 mol%) in 1,2-dichloroethane, a dilute solution of **7** in 1,2-dichloroethane was slowly added over 7 h at 80 °C, followed by stirring for 24 h. The desired reaction proceeded to afford dilactone **8** in 59% yield (Scheme 2).



Scheme 2. Construction of the nine-membered dilactone core.

To complete the synthesis of **1**, the TBS group was removed by treatment of **8** with an HF·pyridine complex in a mixture of pyridine and THF to afford **9**, which was condensed with isobutyric acid in the presence of EDCI·HCl (3.0 equiv)–DMAP (0.30 equiv) to afford **10** (Scheme 3). The *N*-Boc moiety was removed with TFA in dichloromethane to afford **11**. Amide formation of **11** with acid **12** was achieved in DMF with EDCI·HCl, HOBt, and NMM in 91% yield. Hydrogenolysis of **13** with $Pd(OH)_2$ in EtOH afforded splenocin B (**1**) in 98% yield. The spectral data of synthetic **1** were identical to those reported for the natural sample. The optical rotation of synthetic **1** ($[\alpha]_D$ +24.8, *c* 0.11, $CHCl_3$) was in agreement with that of the natural product ($[\alpha]_D$ +21.2, *c* 0.01, $CHCl_3$).[1a]



Scheme 3. Final steps in the synthesis of **1**.

3. Conclusions

The total synthesis of splenocin B (**1**) has been achieved via macrolactonization using ethoxyvinyl ester as a key reagent for furnishing the 9-membered dilactone ring skeleton. Investigations into the application of the developed protocol to the structure–activity relationship are currently underway in our laboratory.

4. Experimental section

4.1. General remarks

Unless mentioned otherwise, ^1H and ^{13}C NMR spectra were recorded on a JEOL JNM-LA 300 (300 and 75 MHz), a JEOL JNM-LA 400 (400 and 100 MHz), a Bruker AVANCE 300 (300 and 75 MHz), or a Bruker AVANCE III 600 (600 and 150 MHz). Data are reported as follows: chemical shift, multiplicity (s=singlet, d=doublet, t=triplet, q=quartet, m=multiplet, and br=broad), coupling constant in Hz, integration. Coupling constants were determined directly from ^1H and ^{13}C NMR spectra. The chemical shifts are reported in δ (ppm) values relative to $CHCl_3$ (δ 7.26 ppm for ^1H NMR and δ 77.0 ppm for ^{13}C NMR) and Me_4Si (δ 0.00 ppm for ^1H NMR). Mass spectra were obtained on a JEOL JMS-700T (EI, CI, FAB) or a Bruker solariX (ESI) spectrometer. IR spectra were recorded on a JASCO FT/IR-4600 spectrometer. Optical rotations were measured on a PERKIN-ELMER 241 polarimeter with a path length of 1 dm or on a JASCO P-1030 with a path length of 0.1 dm at ambient temperature; the concentrations are reported in g dL^{-1}. Melting points were determined on a Yanaco MP-I3 micro melting point apparatus and the thermometer was used without correction. All air- and moisture-sensitive reactions were conducted in a flame-dried, argon-flushed, two-necked flask sealed with a rubber septa, and dry solvents and reagents were introduced using a syringe. Tetrahydrofuran (THF) was freshly distilled under an argon atmosphere from sodium benzophenone ketyl. Dichloromethane (CH_2Cl_2) was freshly distilled from phosphoric pentoxide (P_2O_5). Flash column chromatography was conducted on a Kanto Chemical silica gel 60N (spherical, neutral, 40–50 μm), and pre-coated Merck silica gel plates (Art5715 Kieselgel $60F_{254}$, 0.25 mm) were used for thin-layer chromatography (TLC). TLC visualization was accompanied by the use of a UV lamp (254 nm) or a charring solution (ethanolic *p*-anisaldehyde, ethanolic phosphomolybdic acid).

4.2. (2*R*,3*R*,4*S*)-2-Benzyl-4-[(2*S*,3*R*)-3-benzyloxy-2-*tert*-butoxycarbonylamino-propionyloxy]-3-(*tert*-butyl-dimethyl-silanyloxy)-pentanoic acid benzyl ester (5)

To a stirred, cooled (0 °C) solution of freshly prepared **2**[6b] (454 mg, 1.06 mmol) and *N*-Boc-L-Thr (OBn) **4** (989 mg, 3.20 mmol) in CH_2Cl_2 (8 mL), DMAP (39 mg, 0.32 mmol) and EDCI HCl (613 mg, 3.20 mmol) were added successively. After stirring overnight at 0 °C to rt, the resulting mixture was diluted with Et_2O–hexane (1:2, 30 mL) and filtered through a short-pass silica gel column. The filtrate was concentrated and purified by silica gel column chromatography (EtOAc–hexane) to provide **5** (614 mg, 0.975 mmol, 92%) as a clear oil. $[\alpha]_D$ +6.7 (*c* 1.3, MeOH); ^1H NMR (600 MHz, $CDCl_3$) δ 0.05 (s, 3H), 0.070 (s, 3H), 0.92 (s, 9H), 1.23 (d, *J*=6.7 Hz, 6H), 1.43 (s, 9H), 2.78–2.84 (m, 2H), 3.09–3.16 (m, 1H), 4.01–4.04 (m, 1H), 4.09–4.14 (m, 1H), 4.31 (dd, *J*=9.8, 2.0 Hz, 1H), 4.36 (d, *J*=11.7 Hz, 1H), 4.50 (d, *J*=11.7 Hz, 1H), 4.89–4.95 (m, 1H), 4.92 (s, 2H), 5.30 (d, *J*=9.8 Hz, 1H), 7.03 (d, *J*=7.3 Hz, 1H), 7.04 (d, *J*=5.3 Hz, 1H), 7.10 (d, *J*=6.7 Hz, 2H), 7.18 (t, *J*=6.8 Hz, 2H), 7.18 (t, *J*=7.3 Hz, 1H), 7.18–7.20 (m, 1H), 7.20–7.26 (m, 4H), 7.26–7.29 (m, 1H), 7.33 (t, *J*=7.1 Hz, 2H); ^{13}C NMR (150 MHz, $CDCl_3$) δ −4.52, −4.05, 14.28, 16.27, 18.31, 25.94, 28.27, 35.23, 52.14, 58.35, 66.44,

70.87, 74.09, 74.89, 75.15, 79.73, 126.31, 127.63, 127.72, 128.07, 128.27, 128.38, 128.41, 128.49, 128.87, 135.33, 137.85, 139.03, 155.79, 170.37, 173.00; IR(KBr) ν_{max} 2931, 1781, 1722, 1497, 1171, 778, 748, 699 cm^{-1}; HRFABMS calcd for $C_{41}H_{58}NO_8Si$ $[M+H]^+$ 720.3931: found 720.3929.

4.3. (2*R*,3*R*,4*S*)-2-Benzyl-3-(*tert*-butyl-dimethyl-silanyloxy)-4-hydroxy-pentanoic acid (1′*R*,2′*S*)-2-*tert*-butoxycarbonylamino-2-carboxy-1-methyl-ethyl ester (6)

To a stirred solution of **5** (328 mg, 0.52 mmol) in EtOH (15 mL), 10% $Pd(OH)_2$ (51 mg) was added. The resulting suspension was placed under H_2 gas (1 atm) and stirred vigorously at rt for several hours. Then, the mixture was filtered through a pad of Celite. The filtrate was concentrated to provide crude seco acid **6** (276 mg, 0.511 mmol, 98%) as a pale yellow oil. $[\alpha]_D$=−7.8 (*c* 0.66, MeOH). ^1H NMR (400 MHz, $CDCl_3$) δ 0.10 (s, 3H), 0.12 (s, 3H), 0.94 (s, 9H), 1.21 (d, *J*=5.6 Hz, 3H), 1.29 (d, *J*=6.3 Hz, 3H), 1.44 (s, 9H), 2.74−2.89 (m, 2H), 3.02−3.08 (m, 1H), 4.02 (dd, *J*=6.1, 4.4 Hz, 1H), 4.22−4.34 (m, 2H), 4.99 (br s, 1H), 5.43 (br d, *J*=9.3 Hz, 1H), 7.16−7.28 (m, 5H). ^{13}C NMR (100 MHz, $CDCl_3$) δ −4.51, −4.14, 15.63, 18.18, 19.55, 25.86, 28.21, 34.17, 51.84, 58.80, 74.13, 75.00, 77.38, 126.45, 128.48, 128.90, 139.03, 156.14, 170.71, 177.17; IR(KBr) ν_{max} 3423, 2932, 1717, 1509, 1164, 778, 700 cm^{-1}; HRFABMS calcd for $C_{27}H_{44}NO_8Si$ $[M-H]^-$ 538.2837: found 538.2838.

4.4. (3*S*, 4*R*, 7*R*, 8*R*, 9*S*)[7-Benzyl-8-(*tert*-butyl-dimethyl-silanyloxy)-4,9-dimethyl-2,6-dioxo-[1,5]dioxonan-3-yl]-carbamic acid *tert*-butyl ester (8)

Under Ar atmosphere, ethoxyacetylene (40 wt. % solution in hexanes, 221 μL, 0.924 mmol) was slowly added to a solution of $[RuCl_2(p\text{-cymene})]_2$ (9.3 mg, 15 μmol) in acetone (1.5 mL) at 0 °C. After being stirred at 0 °C for 5 min, **6** (166 mg, 0.308 mmol) in acetone (1.5 mL) was slowly added to the solution at 0 °C. The resulting mixture was stirred at rt for 1 h and then filtered through a short neutral SiO_2 pad column elucidated with EtOAc. The filtrate was concentrated to afford the corresponding ethoxyvinyl ester (EVE) **7**, which was used without further purification. A solution of crude EVE **7** in 1,2-dichloroethane (31 mL) was slowly added to a highly diluted (±)-10-camphorsulfonic acid (0.05 M in 1,2-dichloroethane−CH_3CN 1:1, 620 μL, 31 μmol) solution in 1,2-dichloroethane (94 mL) over 7 h at 80 °C. Stirring was continued at 80 °C for an additional 17 h. The resulting mixture was cooled to rt before the addition of Et_3N (42 μL, 30 μmol). Concentration and purification by silica gel column chromatography (EtOAc−hexane) provided **8** as a pale yellow solid (87.0 mg, 0.167 mmol, 59%). Mp 96 °C; $[\alpha]_D$=+49 (*c* 0.13, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ 0.16 (s, 3H), 0.21 (s, 3H), 0.96 (s, 9H), 1.09 (d, *J*=6.6 Hz, 3H), 1.38 (d, *J*=6.8 Hz, 3H), 1.42 (s, 9H), 2.63 (ddd, *J*=10.4, 9.2, 2.8 Hz, 1H), 2.79 (dd, *J*=12.8, 10.4 Hz, 1H), 3.08 (dd, *J*=12.8, 2.8 Hz, 1H), 3.77 (t, *J*=9.2 Hz, 1H), 4.70−4.90 (m, 2H), 5.20 (d, *J*=7.6 Hz, 1H), 5.32−5.45 (m, 1H), 7.12 (d, *J*=7.3 Hz, 2H), 7.11−7.31 (m, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ −3.17, −3.14, 14.60, 18.68, 18.99, 25.93, 28.15, 35.48, 54.67, 55.30, 71.32, 76.94, 77.54, 126.42, 128.40, 128.85, 138.84, 154.93, 170.68, 173.77; IR(KBr) ν_{max} 3367, 1739, 1709, 1521, 1454, 1361, 1249, 1169, 1095, 780 cm^{-1}; HRFABMS calcd for $C_{27}H_{44}NO_7Si$ $[M+H]^+$ 522.2887: found 522.2880.

4.5. (3*S*, 4*R*, 7*R*, 8*R*, 9*S*)-(7-Benzyl-8-hydroxy-4,9-dimethyl-2,6-dioxo-[1,5]dioxonan-3-yl)-carbamic acid *tert*-butyl ester (9)

Dilactone **8** (104 mg, 0.200 mmol) was treated with (HF·pyridine complex)-pyridine-THF (5:6:8, 3.2 mL) at rt until the starting material disappeared. The mixture was diluted with EtOAc, poured into stirred saturated aq $NaHCO_3$, and extracted with EtOAc (2×). The combined extracts were washed with brine, dried over Na_2SO_4, concentrated, and purified by silica gel column chromatography (EtOAc−hexane) to provide **9** (75.7 mg, 0.186 mmol, 93%) as a clear oil. $[\alpha]_D$=+55 (*c* 0.29, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ 1.15 (d, *J*=6.6 Hz, 3H), 1.43 (s, 9H), 1.45 (d, *J*=6.5 Hz, 3H), 2.66 (ddd, *J*=10.8, 9.6, 3.6 Hz 1H), 2.98 (dd, *J*=13.4, 10.8 Hz, 1H), 3.18 (dd, *J*=13.4, 3.6 Hz, 1H), 3.69 (t, *J*=9.6 Hz, 1H), 4.75−4.87 (m, 2H), 5.17−5.27 (m, 1H), 5.41 (m, 1H), 7.14−7.29 (m, 5H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 14.38, 18.38, 28.24, 31.58, 35.06, 53.90, 54.32, 71.06, 75.95, 80.36, 128.48, 128.53, 128.84, 138.67, 154.85, 170.65, 172.96; IR(KBr) ν_{max} 3396, 1687, 1544, 1509, 1135, 1044, 698 cm^{-1}; HRFABMS calcd for $C_{21}H_{28}NO_7$ $[M-H]^-$ 406.1866: found 406.1861.

4.6. Isobutyric acid (3*S*, 4*R*, 7*R*, 8*R*, 9*S*)-[7-benzyl-3-*tert*-butoxycarbonylamino-4,9-dimethyl-2,6-dioxo-[1,5]dioxonan-8-yl] ester (10)

To a stirred, cooled (0 °C) solution of **9** (17.8 mg, 44 μmol) and isobutyric acid (18 μL) in CH_2Cl_2 (0.5 mL) was added DMAP (2 mg, 16 μmol) and EDCI HCl (36 mg, 18 μmol) successively. After stirring overnight at rt, the resulting mixture was poured into H_2O and extracted with EtOAc (3×). The combined extracts were washed with H_2O (2×) and brine, dried over Na_2SO_4, concentrated, and purified by silica gel column chromatography (20% EtOAc−hexane) to give **10** as a white solid (18.4 mg, 39 μmol, 88%). Mp 122 °C; $[\alpha]_D$=+57 (*c* 0.11, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ 1.15 (d, *J*=6.6 Hz, 3H), 1.217 (d, *J*=6.8 Hz, 3H), 1.224 (d, *J*=6.8 Hz, 3H), 1.30 (d, *J*=6.3 Hz, 3H), 1.43 (s, 9H), 2.59 (sept, *J*=6.8 Hz, 1H), 2.66 (dd, *J*=3.2, 13.4 Hz, 1H), 2.85 (ddd, *J*=11.6, 10.0, 3.2 Hz, 1H), 2.97 (dd, *J*=11.6, 13.4 Hz, 1H), 4.83−4.97 (m, 2H), 5.17 (t, *J*=10.0 Hz, 1H), 5.16−5.19 (m, 1H), 5.42−5.45 (m, 1H), 7.07−7.30 (m, 5H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.45, 17.77, 18.86, 28.15, 34.03, 34.45, 51.75, 54.27, 71.28, 74.08, 75.25, 126.59, 128.54, 128.76, 138.15, 154.62, 170.67, 171.99, 175.73; IR(KBr) ν_{max} 3368, 1741, 1699, 1510, 1369, 1149, 743, 704 cm^{-1}; HRFABMS calcd for $C_{25}H_{36}NO_8$ $[M+H]^+$ 478.2441: found 478.2438.

4.7. Isobutyric acid (3*S*, 4*R*, 7*R*, 8*R*, 9*S*)-[3-amino-7-benzyl-4,9-dimethyl-2,6-dioxo-[1,5]dioxonan-8-yl] ester (11)

To a solution of **10** (9.2 mg, 19 μmol) in CH_2Cl_2 (1 mL), trifluoroacetic acid (0.5 mL, 0.759 mmol) was added dropwise. After stirring at rt for 2 h, the resulting mixture was concentrated, diluted with saturated aq $NaHCO_3$, and extracted with EtOAc (2×). The combined extracts were washed with brine, dried over Na_2SO_4, and concentrated to produce crude **11** (8.5 mg, 23 μmol, 98%) as a white solid. This was used for the next reaction without further purification. Mp 124 °C; $[\alpha]_D$=+0.54 (*c* 0.56, MeOH); ^1H NMR (400 MHz, $CDCl_3$) δ 1.20 (d, *J*=6.3 Hz, 3H), 1.218 (d, *J*=6.8 Hz, 3H), 1.224 (d, *J*=6.8 Hz, 3H), 1.29 (d, *J*=6.3 Hz, 3H), 2.58 (sept, *J*=6.8 Hz, 1H), 2.65 (dd, *J*=2.8, 13.6 Hz, 1H), 2.88 (ddd, *J*=11.2, 10.0, 2.8 Hz, 1H), 2.99 (dd, *J*=13.6, 11.2 Hz, 1H), 4.13 (d, *J*=8.1 Hz, 1H), 4.82 (dq, *J*=10.0, 6.3 Hz, 1H), 5.13 (t, *J*=10.0 Hz, 1H), 5.17−5.20 (m, 1H), 7.11−7.30 (m, 5H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 13.00, 18.93, 18.96, 29.69, 34.11, 34.56, 51.64, 53.82, 72.08, 73.86, 75.26, 126.50, 128.49, 128.73, 138.28, 171.51, 174.14, 175.75; IR(KBr) ν_{max} 3397, 2983, 1741, 1454, 1177, 702 cm^{-1}; HRFABMS calcd for $C_{20}H_{28}NO_6$ $[M+H]^+$ 378.1916: found 378.1921.

4.8. Isobutyric acid (3*S*, 4*R*, 7*R*, 8*R*, 9*S*)-7-benzyl-3-(3-formylamino-2-benzyloxy-benzoylamino)-4,6-dimethyl-2,6-dioxo-[1,5]dioxonan-8-yl ester (13)

To a stirred solution of **11** (8.5 mg, 23 μmol) and **12** (18.7 mg, 69 μmol) in DMF (0.5 mL), HOBt (9.3 mg, 69 μmol), EDCI HCl (13.2 mg, 69 μmol) and NMM (5.5 μL, 6.9 μmol) were added successively. After stirring for 18 h at rt, the mixture was poured into H_2O and extracted with EtOAc (3×). The combined extracts were washed with H_2O (2×) and brine, dried over Na_2SO_4, concentrated,

and purified by silica gel column chromatography (40% EtOAc–hexane) to produce **13** (13.2 mg, 21 μmol, 91%) as a pale yellow oil.[10] $[\alpha]_D$=+24 (*c* 0.78, MeOH); ^{1}H NMR (600 MHz, $CDCl_3$) δ 1.14 (d, *J*=6.7 Hz, 3H), 1.23 (d, *J*=7.0 Hz, 3H), 1.24 (d, *J*=7.0 Hz, 3H), 1.32 (d, *J*=6.3 Hz, 3H), 2.61 (sept, *J*=7.0 Hz, 1H), 2.69 (dd, *J*=13.5, 3.0 Hz, 1H), 2.89 (ddd, *J*=11.4, 10.3, 3.0 Hz, 1H), 3.00 (dd, *J*=13.5, 11.4 Hz, 1H), 4.82 (A of ABq, *J*=11.5 Hz, 1H), 4.99–5.09 (m, 1H), 5.15 (B of ABq, *J*=11.5 Hz, 1H, Δν=198.5 Hz) 5.21 (t, *J*=10.3 Hz, 1H), 5.33 (dd, *J*=7.8, 7.2 Hz, 1H), 5.54–5.61 (m, 1H), 7.13 (d, *J*=7.8 Hz, 2H), 7.18–7.39 (m, 5H), 7.71 (d, *J*=7.8 Hz, 1H), 8.02 (d, *J*=7.8 Hz, 1H), 8.13 (s, 1H), 8.42 (d, *J*=7.8 Hz, 1H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 14.87, 17.91, 18.95, 22.63, 34.54, 51.88, 53.91, 71.35, 74.35, 78.76, 121.44, 124.78, 125.50, 126.42, 126.59, 128.52, 128.72, 128.74, 129.10, 129.40, 131.31, 135.10, 138.03, 145.94, 158.44, 160.93, 164.68, 170.47, 171.93, 175.59; IR(KBr) ν_{max} 3368, 2934, 1736, 1626, 1545, 1059, 739, 696 cm^{-1}; HRFABMS calcd for $C_{35}H_{37}N_2O_9$ $[M-H]^-$ 629.2499: found 629.2488.

4.9. Splenocin B (1)

To a stirred solution of **13** (2.0 mg, 3.2 μmol) in ethanol (0.5 mL), 10% $Pd(OH)_2$ (3.0 mg) was added. The resulting suspension was placed under H_2 gas at 1 atm and stirred vigorously at rt for 15 h. Next, the mixture was filtered through a pad of Celite and concentrated to produce splenocin B (1.6 mg, 3.0 μmol, 98%) as white amorphous powder.[10] Mp 137 °C;[11] $[\alpha]_D$=+63 (*c* 0.10, MeOH), natural:[1a]: $[\alpha]_D$=+68 (*c* 0.1, MeOH); ^{1}H NMR (600 MHz, $CDCl_3$) δ 1.16 (d, *J*=7.0 Hz, 3H), 1.215 (d, *J*=7.1 Hz, 3H), 1.221 (d, *J*=6.9 Hz, 3H), 1.33 (d, *J*=6.2 Hz, 3H), 2.61 (sept, *J*=7.0 Hz, 1H), 2.70 (dd, *J*=2.0, 12.4 Hz, 1H), 2.91 (ddd, *J*=2.5, 9.2, 11.3 Hz, 1H), 3.00 (dd, *J*=11.5, 12.5 Hz, 1H), 5.02–5.05 (m, 1H), 5.22 (t, *J*=9.5 Hz, 1H), 5.27 (dd, *J*=7.6, 7.6 Hz, 1H), 5.62 (quint, *J*=6.9 Hz, 1H), 6.91 (t, *J*=8.1 Hz, 1H), 6.99 (d, *J*=7.7 Hz, 1H), 7.13 (d, *J*=7.2 Hz, 2H), 7.19 (d, *J*=7.4 Hz, 1H), 7.20 (d, *J*=8.4 Hz, 1H), 7.24 (t, *J*=7.5 Hz, 2H), 7.89 (s, 1H), 8.50 (s, 1H), 8.54 (d, *J*=7.2 Hz, 1H), 12.60 (s, 1H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 14.71, 17.81, 18.95, 34.11, 34.53, 51.91, 53.46, 70.92, 74.78, 75.06, 112.54, 118.94, 120.08, 124.80, 126.65, 127.44, 128.55, 128.72, 137.90, 150.62, 159.06, 169.33, 170.07, 171.94, 175.65; IR(KBr) ν_{max} 3352, 2932, 1737, 1524, 1376, 1151, 749, 703 cm^{-1}; HRESIMS calcd for $C_{28}H_{31}N_2O_9$ $[M-H]^-$ 539.2035: found 539.2035.

Acknowledgements

This paper is dedicated to the memory of late Professor Kozo Shibata, Osaka City University. We thank Dr. Matsumi Doe, Analytical Division, Osaka City University for NMR measurements.

Supplementary data

Supplementary data (^{1}H and ^{13}C NMR spectra) associated with this article can be found in the online version, at http://dx.doi.org/10.1016/j.tet.2015.10.075.

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10. ^{1}H and ^{13}C NMR spectra indicated that it existed as a mixture of two rotamers.
11. Mp was not reported in ref 1a.