Total synthesis of proposed cephalosporolides H and I

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ABSTRACT

An efficient total synthesis toward spiroketal diastereomers of cephalosporolides H and I was achieved, respectively, taking advantage of intramolecular Wacker-type spiroketalization on the common olefin-containing dihydroxy- γ -lactone substrate. By comparing the physical data of natural products with synthetic samples, we suggest that the reported stereochemical assignments for cephalosporolides H and I are incorrect. Our work suggests that the stereochemistry on C-6 of the core spiroketal skeleton should be revised to S from its original R assignment, and the real structures of these natural compounds should be reconsidered.

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1. Introduction

Most of naturally occurring spiroketals are polyketide-derived polyether antibiotics produced by bacteria as metabolites.¹ The increasing pharmacological importance of compounds with spiroketal blocks has triggered intense interest in both preparation and chemical reactivity. A rigid spiroketal framework may provide pinpoint orientation of pendant functional groups, which facilitates interactions in complex biochemical systems. Recently, a class of natural products containing 5,5-spiroketal-cis-fused- γ -lactone moiety have been isolated, and their structures and biological activities have been investigated. Cephalosporolides E, F,^{2–4} H, I,⁵ penisporolides A, B,⁶ and ascospiroketal B⁷ are structural representatives of this family, having the central tricyclic core of spiral lactone skeleton with stereochemistry variations at C-3,4 of γ -lactone and C-9 of the side chain.

Since the first report on chemical preparation of (–)-cephalosporolide E and (+)-cephalosporolide F by Ramana and co-workers,⁸ many efforts have been made in the total synthesis of related natural structural analogues.^{9–14} Nevertheless, metal-mediated alkynediol spiroketalization and acid-catalyzed spiroketalization were major approaches in the synthesis of these molecules, to afford spiroketal diastereomers.¹⁵ A recent plausible chelation-controlled epimerization approach to spiroketals

involves Zn salts, which raised the selectivity to as high as a 20:1 diastereomeric mixture.⁹

We were interested in the total synthesis of cephalosporolides H and I due to the challenging structures of spiroketal-fused lactone (Fig. 1), as well as our previous experience in exploration of PdCl₂-catalyzed Wacker oxidative cyclization.¹⁶ Both cephalosporolides H and I possess a *gem*-dimethyl group α to the γ -lactone and differ only in the substituent at C-9. Cephalosporolide H has a saturated heptyl side chain, whereas cephalosporolide I has a four carbon chain bearing a terminal carboxylic acid. Preliminary biological testing on these natural products demonstrated inhibition of the enzymes 3 α -hydroxysteroid dehydrogenase and xanthine oxidase, which implicates these natural products as potential *anti-inflammatory* agents.^{17,18} In particular, to the best of our knowledge, the total synthesis of cephalosporolide I has not been reported so far. Herein, we disclose our synthesis and structural revision of cephalosporolides H and I via an intra-molecular Wacker-type spiroketalization of dihydroxy- γ -lactone precursor.

Cephalosporolide H, R = (CH₂)₆CH₃
Cephalosporolide I, R = (CH₂)₃CO₂H

Fig. 1. Structures of cephalosporolides H and I.

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2. Results and discussion

Retrosynthetic analysis of cephalosporolides H (**1**) and I (**2**) is summarized in Scheme 1. The skeleton of compounds **1** and **2** could be accomplished with Wacker oxidation via intramolecular spiroketalization of precursor **3**, which could be assembled from segments epoxide **4**, allylmagnesium bromide **5**, and γ -lactone **6**, sequentially. We envisaged that the stereogenic centers at C-3,4 of **6** could be introduced from commercially available D-glucono- δ -lactone.

Scheme 1. Retrosynthetic analysis of cephalosporolides H and I.

We started from the synthesis of building block **6** as shown in Scheme 2. Treatment of commercially available D-glucono- δ -lactone with HBr in AcOH, followed by zinc reduction, afforded the desired γ -lactone **7** (Scheme 1) according to a modified procedure.¹⁹ Dimethylation of **7** with two successive additions of lithium diisopropylamide (LDA) (2.2 equiv for each addition) and methyl iodide (3 equiv for each addition) gave the core intermediate **6** in good yield. Ring opening of the S-epoxide **8** at the terminal position by the addition of an allyl cuprate in THF, which was generated in situ from Li₂CuCl₄ and allylmagnesium bromide **5**,²⁰ afforded secondary alcohol **9** in 93% yield. A further copper-catalyzed coupling reaction of **9** and n-hexylmagnesium bromide **10** furnished alkyl side chain **11** of cephalosporolide H in the presence of catalytic amount of CuCl.²¹ The cross metathesis reaction of **11** and **6** catalyzed by Grubbs-II led to key dihydroxy lactone **12** in 77% yield with *E*-olefin isomer as the unique product,

Scheme 2. Total synthesis of cephalosporolide H. Reagents and conditions: (a) 33% HBr in AcOH, 50 °C, 1 h; (b) Zn, AcOH/H₂O (1:1), –10–60 °C, 4 h; (c) LDA, CH₃I, THF, –78 °C to rt, overnight; (d) (5 mol %) Li₂CuCl₄, THF, –40 °C, 1 h; (e) (3 mol %) CuCl, THF, rt, 1.5 h; (f) (2 mol %) Grubbs-II catalyst, DCM, reflux, 12 h; (g) PdCl₂, CuCl, O₂, DMF/H₂O (4:1), rt, 1 h.

confirmed by a large coupling constant of $J_{1,2}$ =15.6 Hz in **12**. The intramolecular Wacker oxidative spiroketalization of **12** with PdCl₂ and CuCl in aqueous DMF accomplished spiroketal diastereomers of cephalosporolide H (**1a**:**1b**=45:39, 84% total yield). The physical data (¹H NMR, ¹³C NMR, and [α]_D) for our synthetic cephalosporolides (**1a** and **1b**) were in satisfied agreement with those of reported synthetic counterparts.^{9–13} However, some significant differences were observed between our synthetic *R* epimer **1a** and the corresponding natural product having *R* assignment at C-6. In contrast, the characteristic of our *S*-product **1b** matched well with the data of natural product,⁵ except for C-12 on alkyl chain shown chemical shift at 33.8 ppm for natural product and 35.5 ppm for synthetic sample in ¹³C NMR spectra. In terms of the specific optical rotation, synthetic **1b** and natural product gave [α]_D²⁵ +52 (c 0.66, MeOH) and [α]_D²⁵ +57.6 (c 0.70, MeOH), respectively.

To prepare both cephalosporolides H and I efficiently, a more reliable and stereo-controllable strategy is required. We thus focused on the investigation of Wacker oxidation²² for the common core structure **3**. Replacement of X group with chloride, Ms, and Ts in **3**, respectively, followed by Wacker oxidation under standard reaction conditions afforded some encouraging results. In the case of Cl and Ms, C-6 stereo isomers were obtained in varied ratios, while Ts substitution generated a surprised result with *S* epimer at C-6 as the solo product. The *S* configuration was clearly indicated by the peak of H-4 shown as a doublet of doublet of doublets at δ 4.98 ppm with coupling constants of J 6.6, 3.7, 1.6 Hz on ¹H NMR spectrum.¹³ Thus, the cross metathesis reaction of **6** and **9** (Scheme 3) was proceeded as described for the preparation of **12**, gave tosylated compound **13** in an approximately 20:1 ratio (*E*/*Z* was determined by ¹H NMR spectroscopy), which was subjected to Pd-catalyzed spiroketalization without further purification affording **14** as a single spiroketal isomer. Iodination of **14** with NaI in acetone gave the core building block **15** in excellent yield. CuCl-catalyzed cross-coupling of **15** and **10** furnished **1b** in 86% yield, further affirming the *S* configuration at C-6 of **14**. Conveniently, a NaBH₃CN/AIBN promoted radical Michael addition²³ between **15** and benzyl acrylate furnished the corresponding benzyl ester **16**, which was converted into (*S*)-cephalosporolide I (**2b**) with H₂ in the presence of 10% Pd/C in MeOH.

Scheme 3. Stereocontrol synthesis of (*S*)-cephalosporolides H and I. Reagents and conditions: (a) (2 mol %) Grubbs-II catalyst, DCM, reflux, 12 h; (b) PdCl₂, CuCl, O₂, DMF/H₂O (4:1), rt, 1 h; (c) NaI, acetone, reflux, 13 h; (d) **10**, (3 mol %) CuCl, THF, rt, 1 h; (e) Benzyl acrylate, NaBH₃CN, AIBN, CH₃CN, reflux, 0.5 h; (f) Pd/C, H₂, MeOH, 15 min.

Interestingly, treatment of the key spiroketal iodide **15** with *p*-toluenesulfonic acid (PTSA) in MeOH could drive the transformation²⁴ of **15** into its C-6 epimer **17** in a ratio of 1:5 with 83% yield. DFT computational result indicated that the enthalpy of **17** is 1.43 kcal/mol lower than that of **15**, suggesting a thermodynamic

preference of **17** in acid promoted transformation. Following the same procedures as described in the synthesis of compounds **1b** and **2b**, the spiroketal diastereomers **1a** and **2a** were accomplished in excellent yields (Scheme 4).

Scheme 4. Stereo-controlled synthesis of (*R*)-cephalosporolides H and I. Reagents and conditions: (a) (1 mol %) PTSA, MeOH, rt, 3 h; (b) **10**, (3 mol %) CuCl, THF, rt, 1 h; (c) Benzyl acrylate, NaBH₃CN, AIBN, CH₃CN, reflux, 0.5 h; (d) Pd/C, H₂, MeOH, 15 min.

By comparison of the physical data of natural cephalosporolide I and our synthetic samples **2a** and **2b**, there are some significant differences between reported natural product and the assigned *R* diastereomer **2a**, as well as the opposite sign of specific optical rotation (+70 for natural product, +72.9 for **2b**, but −7.1 for **2a**). The characteristic data of our **2b** matched well with the core structure of natural cephalosporolide I. The *ddd* splitting peak of H-4, criteria for *S* assignment, also matched with reported *dt* peak in natural product, while *R* stereomer gave clear triplet for H-4. However, C-12 and C-13 on the side alkyl chain shown chemical shifts at 33.4 and 24.3 ppm for natural product, whereas the corresponding peaks for synthetic sample appeared respectively at 34.7 and 21.4 ppm in ¹³C NMR spectra, indicating that the real structure of natural cephalosporolide I needs to be reconsidered.

3. Conclusion

We have successfully developed an efficient route toward the divergent total synthesis of cephalosporolides H and I taking advantage of intramolecular Wacker-type spiroketalization on olefin-containing dihydroxy-γ-lactone substrate. The spiroketal diastereomers on C-6 for both cephalosporolides H and I have been prepared, and their physical data are compared with the corresponding natural counterparts. Our results suggest that the stereochemistry on C-6 of the core spiroketal skeleton should be in *S* configuration for the natural cephalosporolides H and I, instead of the original *R* assignments. Our strategy provides a general method toward the total synthesis of other family members, and should be applicable to the total synthesis of cephalosporolides E/F, penisporolides A/B, and ascospiroketal B.

4. Experimental

4.1. General

The commercially available reagents were used without further purification. Reactions were monitored by thin-layer chromatography (TLC) on gel F₂₅₄ plates. Flash chromatography (FC) was performed using silica gel (200–300 mesh) according to the standard protocol. Optical rotations were recorded in either a 5-cm microcell or a 2.5-cm microcell. High-resolution mass spectrometry data (HRMS) were acquired with a Q-TOF analyzer. ¹H and ¹³C NMR spectra were recorded on a Bruker 400 MHz (100 MHz for ¹³C) NMR spectrometer. IR spectra were recorded on KBr with a NICO-LET 8700 infrared spectrometer.

4.2. (4*R*,5*R*)-4-Hydroxy-3,3-dimethyl-5-vinyldihydrofuran-2(3*H*)-one (**6**)

n-BuLi (8.3 mL, 20 mmol, 2.4 M solution in hexane, 2.0 equiv) was added to a solution of diisopropylamine (3.1 mL, 22 mmol, 2.2 equiv) in THF (30 mL) at −78 °C to generate freshly prepared LDA. After stirring for 0.5 h, a solution of β-hydroxy lactone **7** (1.281 g, 10 mmol) in THF (10 mL) was added. The solution was stirred for 2 h at 0 °C and then cooled again to −78 °C before the addition of methyl iodide (1.6 mL, 26 mmol, 2.6 equiv). After stirring for 6 h at room temperature, the reaction mixture was cooled to −78 °C, and a second addition of LDA (generated from *n*-BuLi in situ as described above) and methyl iodide was carried out repeatedly. The reaction mixture was stirred overnight, then aqueous NH₄Cl (2 mL) was added to quench the reaction, and the crude product was extracted with EtOAc (2×20 mL). The organic phase was washed with brine, dried over Na₂SO₄ and evaporated under vacuum to give a residue, which was purified by flash column chromatography (hexanes/EtOAc 2:1) to give compound **6** as a yellowish oil (1.39 g, 89%); [α]_D²⁵ +56.4 (c 0.66, CHCl₃); IR ν_{max} 3380, 2924, 1769, 1454, 1296, 1218, 1158, 1075, 985, 772 cm^{−1}; ¹H NMR (400 MHz, CDCl₃): δ 5.98–5.90 (m, 1H), 5.61–5.50 (m, 2H), 5.04–5.01 (m, 1H), 4.03–4.02 (d, *J*=4.0 Hz, 1H), 1.78 (br s, 1H, OH), 1.29 (s, 3H), 1.28 (s, 3H); ¹³C NMR (100 MHz, CDCl₃): δ 180.4, 130.7, 120.8, 80.7, 77.2, 45.6, 22.7, 18.0; ESI-HRMS calcd for C₈H₁₂O₃ ([M+Na]⁺) 179.0679, found 179.0671.

4.3. (S)-2-Hydroxyhex-5-en-1-yl 4-methylbenzenesulfonate (**9**)

To a solution of lithium tetrachlorocuprate (0.1 M solution in THF, 5.0 mL, 0.5 mmol), allylmagnesium bromide **5** (1.0 M solution in THF, 13 mL, 13 mmol) was added at −40 °C. After stirred for 1 h, a pre-cooled solution of tosylated glycidol **8** (2.28 g, 10 mmol) in THF (35 mL) was added dropwise through dropping funnel in about 1 h at −40 °C and stirred further for 1 h. The reaction mixture was quenched with aqueous NH₄Cl and warmed to room temperature, then extracted with EtOAc (2×20 mL). The aqueous layers were extracted with EtOAc and the combined organic layers were washed with brine, dried over Na₂SO₄ and concentrated in vacuo. The residue was purified by flash column chromatography (hexanes/EtOAc 3:1) to afford **9** as a colorless oil (2.51 g, 93%); [α]_D²⁵ +3.2 (c 0.13, CHCl₃); IR ν_{max} 3441, 2921, 1653, 1357, 1178, 1067, 985, 932, 828, 725 cm^{−1}; ¹H NMR (400 MHz, CDCl₃): δ 7.79 (d, 2H, *J*=8.4 Hz), 7.35 (d, 2H, *J*=8 Hz), 5.81–5.70 (m, 1H), 5.00–4.97 (m, 2H), 4.02–3.98 (m, 1H), 4.03 (dd, 1H, *J*=2.4 Hz), 3.92–3.83 (m, 2H), 2.45 (s, 3H), 2.22–2.05 (m, 2H), 1.57–1.49 (m, 2H); ¹³C NMR (100 MHz, CDCl₃): δ 145.3, 137.7, 132.8, 130.1, 128.1, 115.6, 74.0, 69.0, 31.9, 29.5, 21.8; ESI-HRMS calcd for C₁₃H₁₈O₃S ([M+Na]⁺) 293.0818, found 293.0813.

4.4. (R)-Dodec-1-en-5-ol (**11**)

To a solution of CuCl (3 mg, 0.03 mmol) and **9** (137 mg, 0.51 mmol) in THF (5 mL) was added hexylmagnesium bromide **10** (0.8 M, 1.4 mL, 2.2 equiv) dropwise. The reaction was stirred for 1.5 h at rt. Then it was quenched by addition of 1 mL of saturated NH₄Cl aqueous solution. The organic phase in the resulting solution mixture was extracted with ether (3×20 mL), dried over Na₂SO₄, filtered and concentrated in vacuo. The residue was purified by flash column chromatography on silica gel (hexanes/EtOAc 4:1) to give **11** (92 mg, 98%); [α]_D²⁵ +7.9 (c 0.66, CHCl₃); IR ν_{max} 3400, 2906, 1658, 1406, 965, 913, 675 cm^{−1}; ¹H NMR (400 MHz, CDCl₃): δ 5.88–5.79 (m, 1H), 5.07–4.95 (m, 2H), 3.62–3.60 (m, 1H), 2.22–2.10 (m, 2H), 1.60–1.53 (m, 2H), 1.51–1.28 (m, 12H), 0.88 (t, *J*=6.8 Hz, 3H); ¹³C NMR (100 MHz, CDCl₃): δ 138.9, 114.9, 71.7, 37.7,

36.7, 32.0, 30.3, 29.8, 29.5, 25.8, 22.8, 14.3; ESI-HRMS calcd for $C_{12}H_{24}O$ ($[M+Na]^+$) 207.1719, found 207.1726.

4.5. (4*R*,5*R*)-4-Hydroxy-5-((*R*,*E*)-5-hydroxydodec-1-en-1-yl)-3,3-dimethyldihydro furan-2(3*H*)-one (**12**)

Grubbs second catalyst (4 mg, 0.004 mmol) was added to a mixture of **6** (31 mg, 0.2 mmol) and **11** (47 mg, 0.26 mmol) in dry DCM (3 mL). The mixture was heated under reflux for 12 h. The solution was concentrated under reduced pressure and purified by flash chromatography on silica gel (hexanes/EtOAc 2:1) to give **12** (46 mg, 77%); $[\alpha]_D^{25} +86.3$ (c 0.8, $CHCl_3$); IR ν_{max} 3429, 2976, 2910, 1731, 1468, 1365, 847, 659 cm^{-1} ; 1H NMR (400 MHz, $CDCl_3$): δ 6.01–5.94 (m, 1H), 5.63–5.57 (dd, $J=15.6$, 6.4 Hz, 1H), 4.98–4.96 (m, 1H), 3.96–3.95 (d, $J=4.0$ Hz, 1H), 3.65–3.60 (m, 1H), 2.30–2.25 (m, 2H), 1.70–1.58 (m, 1H), 1.56–1.45 (m, 4H), 1.44–1.26 (m, 15H), 0.88 (t, $J=6.8$ Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 180.9, 137.6, 122.9, 81.0, 77.7, 71.9, 45.6, 37.9, 36.3, 32.0, 29.8, 29.4, 29.3, 25.8, 22.9, 22.8, 18.2, 14.3; ESI-HRMS calcd for $C_{18}H_{32}O_4$ ($[M+Na]^+$) 335.2193, found 333.2188.

4.6. Spiroketal diastereomers of cephalosporolide **H** (**1a**) and (**1b**)

To a suspension of palladium (II) chloride (9 mg, 0.052 mmol) and copper (I) chloride (52 mg, 0.182 mmol) in DMF/ H_2O (3 mL, v/v=4:1) at room temperature under oxygen atmosphere was added a solution of **12** (40 mg, 0.13 mmol) in DMF/ H_2O (2 mL, v/v=4:1). The reaction mixture was stirred for 1 h at room temperature, then filtered through a short pad of silica gel column to give a mixture of **1a** and **1b**. The filtrate was concentrated and purified by column chromatography (hexane/EtOAc 3:1) to afford **1a** (18 mg, 45%) and **1b** (15 mg, 39%), respectively.

For **1a**: $[\alpha]_D^{25} -7.8$ (c 0.66, MeOH); IR ν_{max} 2978, 2932, 2860, 1772, 1458, 1325, 857, 689 cm^{-1} ; 1H NMR (400 MHz, $CDCl_3$): δ 5.07 (t, $J=4.8$ Hz, 1H), 4.29 (d, $J=4.4$ Hz, 1H), 4.01–3.94 (m, 1H), 2.44 (d, $J=14.4$ Hz, 1H), 2.14–1.98 (m, 4H), 1.68–1.61 (m, 2H), 1.29 (s, 3H), 1.25 (m, 11H), 1.21 (s, 3H), 0.87 (t, $J=6.8$ Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 180.7, 115.2, 87.2, 82.0, 79.9, 44.6, 41.7, 37.5, 36.4, 31.9, 30.9, 29.7, 29.3, 26.1, 24.9, 22.7, 18.4, 14.2; ESI-HRMS calcd for $C_{18}H_{30}O_4$ ($[M+Na]^+$) 333.2036, found 333.2028.

For **1b**: $[\alpha]_D^{25} +52$ (c 0.66, MeOH); IR ν_{max} 2936, 2858, 1782, 1468, 1395, 893, 657 cm^{-1} ; 1H NMR (400 MHz, $CDCl_3$): δ 5.03 (ddd, $J=6.4$, 3.6, 1.6 Hz, 1H), 4.29 (d, $J=4.0$ Hz, 1H), 4.07–4.00 (m, 1H), 2.51 (dd, $J=14.8$, 6.4 Hz, 1H), 2.36 (dd, $J=15.2$, 1.2 Hz, 1H), 2.13–1.99 (m, 3H), 1.55–1.48 (m, 1H), 1.27 (s, 12H), 1.22 (s, 3H), 0.88 (t, $J=6.8$ Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 181.0, 115.5, 85.2, 80.6, 79.0, 44.5, 42.4, 35.7, 35.5, 31.8, 30.0, 29.6, 29.2, 25.8, 23.1, 22.6, 18.1, 14.1; ESI-HRMS calcd for $C_{18}H_{30}O_4$ ($[M+Na]^+$) 333.2036, found 333.2028.

4.7. ((2*S*,3*a'*,*R*,5*S*,6*a'*,*R*)-6',6'-Dimethyl-5'-oxohexahydro-3*H*,3'*H*-spiro[furan-2,2'-furo[3,2-*b*]furan]-5-yl)methyl 4-methylbenzenesulfonate (**14**)

Grubbs second catalyst (85 mg, 0.1 mmol) was added to a mixture of **6** (0.78 g, 5.0 mmol) and **9** (1.62 g, 6.0 mmol) in dry DCM (25 mL). The mixture was heated under reflux for 12 h, and concentrated under reduced pressure to give a crude olefin **13**, which was used for the next step without further purification.

To a suspension of palladium (II) chloride (267.4 mg, 1.5 mmol) and copper (I) chloride (1.49 mg, 15.0 mmol) in DMF/ H_2O (15 mL, v/v=4:1) at room temperature under oxygen atmosphere was added a solution of **13** in DMF/ H_2O (5 mL, v/v=4:1). The reaction mixture was stirred for 1 h at room temperature, filtrated through a short pad of silica gel column, and the filtrate was concentrated and purified by column chromatography (hexanes/EtOAc 3:1) to afford

14 as a pale yellow oil (1.46 g, 74% for two steps); $[\alpha]_D^{25} +48.8$ (c 0.25, MeOH); IR ν_{max} 2950, 2923, 2853, 1776, 1729, 1660, 1600, 1463, 1367, 1286, 1132, 772 cm^{-1} ; 1H NMR (400 MHz, $CDCl_3$): δ 7.78 (d, 2H, $J=8.4$ Hz), 7.35 (d, 2H, $J=8$ Hz), 4.98 (ddd, 1H, $J=5.6$, 3.6, 1.2 Hz), 4.26 (m, 2H), 4.03 (dd, 1H, $J=10.8$, 4.0 Hz), 3.95 (m, 1H), 2.45 (m, 4H), 2.32 (d, $J=14.4$ Hz, 1H), 2.18–2.13 (m, 1H), 2.04–1.95 (m, 2H), 1.74–1.70 (m, 1H), 1.24 (s, 3H), 1.21 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 180.7, 145.0, 133.1, 129.9, 128.0, 116.3, 85.6, 80.3, 75.7, 71.3, 44.5, 41.7, 35.0, 26.5, 23.1, 21.7, 18.1; ESI-HRMS calcd for $C_{19}H_{24}O_7S$ ($[M+Na]^+$) 419.1135, found 419.1128.

4.8. (2*R*,3*a'*,*R*,5*S*,6*a'*,*R*)-5-(Iodomethyl)-6',6'-dimethyltetrahydro-3*H*,3'*H*-spiro[furan-2,2'-furo[3,2-*b*]furan]-5'(3*a'*,*H*)-one (**15**)

A mixture of **14** (794 mg, 2.0 mmol) and NaI (1.49 g, 10.0 mmol) in acetone (20 mL) was heated under reflux for 13 h, and concentrated to dryness. The residue was suspended in H_2O (5 mL), brine (20 mL), and $Na_2S_2O_3$ (200 mg) and then was extracted with Et_2O (4×25 mL). The combined extracts were dried over Na_2SO_4 , concentrated and purified by column chromatography (hexanes/EtOAc 4:1) to give **15** (673.3 mg, 96%) as a pale yellow oil; $[\alpha]_D^{25} +66.0$ (c 0.3, MeOH); IR ν_{max} 2970, 2932, 2871, 1774, 1605, 1459, 1349, 1122, 1004, 824, 623 cm^{-1} ; 1H NMR (400 MHz, $CDCl_3$): δ 5.03 (ddd, 1H, $J=5.6$, 4.0, 1.6 Hz), 4.29 (d, 1H, $J=3.6$ Hz), 4.07–4.05 (m, 1H), 3.24–3.22 (m, 2H), 2.60 (dd, $J=15.2$, 6.8 Hz, 1H), 2.38 (dd, $J=15.2$, 1.2 Hz, 1H), 2.26–2.21 (m, 1H), 2.12–2.08 (m, 2H), 1.74–1.69 (m, 1H), 1.26 (s, 3H), 1.22 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 180.7, 116.5, 85.6, 80.4, 77.5, 44.5, 42.1, 35.4, 30.7, 23.2, 18.2, 10.4; ESI-HRMS calcd for $C_{12}H_{17}O_4I$ ($[M+Na]^+$) 375.0064, found 375.0052.

4.9. (*S*)-Cephalosporolide **H** (**1b**)

A 0.8 M solution of hexylmagnesium bromide (commercially available, 0.09 mL, 0.072 mmol) was added to a 2.0 mL of THF solution containing CuCl (0.2 mg, 0.002 mmol) and **15** (21 mg, 0.06 mmol). The reaction mixture was stirred at room temperature for 1 h. It was quenched by the addition of 1 mL of saturated NH_4Cl aqueous solution. The organic phase in the resulting solution mixture was extracted with ether (3 times, 10 mL each), dried over Na_2SO_4 , filtered and concentrated in vacuo. The residue was purified by flash chromatography on silica gel (hexanes/EtOAc 5:1) to give **1b** (16 mg, 86%).

4.10. (*S*)-Cephalosporolide **I** (**2b**)

The mixture of **15** (50 mg, 0.15 mmol), benzyl acrylate (36 mg, 0.23 mmol), $NaBH_3CN$ (28 mg, 0.45 mmol), AIBN (5 mg, 0.03 mmol) in CH_3CN (1.5 mL) was stirred under reflux for 0.5 h. At the end of which time, a saturated ammonium chloride aqueous solution (1.0 mL) was added, then the mixture was poured into water (3 mL) and extracted with Et_2O (10 mL×3). The organic layer was washed with brine and dried over Na_2SO_4 and then filtered and concentrated in vacuo to dryness, and the residue was purified by flash chromatography on silica gel (hexanes/ Et_3N 70:1) to give syrupy **16** (56 mg, 97%); $[\alpha]_D^{25} +8.7$ (c 0.3, MeOH); IR ν_{max} 2920, 2850, 1776, 1733, 1051, 772 cm^{-1} ; 1H NMR (400 MHz, $CDCl_3$): δ 7.37–7.33 (m, 5H), 5.11 (s, 2H), 5.00 (ddd, 1H, $J=6.0$, 4.0, 1.8 Hz), 4.26 (d, 1H, $J=4.0$ Hz), 4.04 (m, 1H), 2.49 (dd, $J=14.8$, 6.4 Hz, 1H), 2.41–2.32 (m, 3H), 2.09–2.01 (m, 3H), 1.70–1.48 (m, 5H), 1.26 (s, 3H), 1.22 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 181.0, 173.4, 136.1, 128.6, 128.3, 128.2, 115.6, 85.3, 80.6, 78.4, 66.3, 44.5, 42.4, 35.6, 34.9, 34.2, 30.1, 23.2, 21.4, 18.2; ESI-HRMS calcd for $C_{22}H_{28}O_6$ ($[M+Na]^+$) 411.1778, found 411.1786.

A solution of **16** (56 mg, 0.145 mmol) in MeOH (2 mL) was added Pd/C (2 mg, 20%) and the suspension was hydrogenated at 3 bar of

H₂ pressure for 15 min. The reaction mixture was filtered through a Celite, and the filtrate was washed with MeOH (2×2 mL). The combined organic phase was evaporated to dryness to yield a yellow oil **2b** (43 mg, 100%); [α]_D²⁵ +72.9 (c 0.27, MeOH); IR $\nu_{\max}$ 2932, 2871, 1774, 1736, 1709, 1458, 1349, 1210, 1123, 1087, 833 cm⁻¹; ¹H NMR (400 MHz, CDCl₃): δ 5.03 (m, 1H), 4.27 (d, 1H, J =3.2 Hz), 4.05 (m, 1H), 2.51 (dd, J =15.2, 6.0 Hz, 1H), 2.38–2.34 (m, 3H), 2.15–2.00 (m, 3H), 1.66–1.51 (m, 5H), 1.27 (s, 3H), 1.22 (s, 3H); ¹³C NMR (100 MHz, CDCl₃): δ 180.9, 178.5, 115.5, 85.3, 80.5, 78.3, 44.6, 42.4, 35.5, 34.7, 33.7, 30.0, 23.1, 21.2, 18.1; ESI-HRMS calcd for C₁₅H₂₂O₆ ([M+Na]⁺) 321.1309, found 321.1326. The NMR spectra data was also obtained in CD₃OD as follows: ¹H NMR (400 MHz, CD₃OD): δ 5.15 (ddd, 1H, J =5.2, 3.6, 1.2 Hz), 4.30 (d, 1H, J =3.6 Hz), 4.07–4.04 (m, 1H), 2.53 (dd, J =15.2, 6.4 Hz, 1H), 2.37–2.27 (m, 3H), 2.11–2.03 (m, 3H), 1.62–1.51 (m, 3H), 1.38–1.20 (m, 2H), 1.21 (s, 6H); ¹³C NMR (100 MHz, CD₃OD): δ 183.2, 178.4, 117.1, 86.7, 82.6, 79.9, 46.0, 43.2, 36.6, 36.2, 35.6, 31.1, 23.3, 22.9, 18.5.

4.11. (2S,3a'R,5S,6a'R)-5-(Iodomethyl)-6',6'-dimethyltetrahydro-3H,3'H-spiro[furan-2,2'-furo[3,2-b]furan]-5'-(3a'H)-one (17)

Compound **15** (70 mg, 0.2 mmol) and PTSA (0.38 mg, 0.002 mmol) were mixed with MeOH (2 mL). After 3 h, the reaction mixture was added with drops of Et₃N and then extracted with CH₂Cl₂ (10 mL), washed with brine (3 mL), and dried over MgSO₄. The reaction mixture was concentrated under reduced pressure and purified over silica gel (deactivated with Et₃N) to afford **17** (58 mg, 83%) with 12 mg of **15** recovered; [α]_D²⁵ –15.7 (c 0.3, MeOH); IR $\nu_{\max}$ 2980, 2926, 2861, 1768, 1609, 1463, 1359, 1132, 1012, 832, 603 cm⁻¹; ¹H NMR (400 MHz, CDCl₃): δ 5.07 (t, 1H, J =4.8 Hz), 4.30 (d, 1H, J =4.4 Hz), 4.28–4.25 (m, 1H), 3.33 (dd, J =9.2, 4.4 Hz, 1H), 3.03 (t, 1H, J =9.6 Hz), 2.50 (d, J =14.4 Hz, 1H), 2.33–2.26 (m, 1H), 2.17–2.03 (m, 3H), 1.82–1.76 (m, 1H), 1.28 (s, 3H), 1.22 (s, 3H); ¹³C NMR (100 MHz, CDCl₃): δ 180.4, 116.5, 87.6, 81.2, 79.7, 44.7, 41.9, 36.5, 31.3, 24.5, 18.5, 9.6; ESI-HRMS calcd for C₁₂H₁₇O₄I ([M+Na]⁺) 375.0064, found 375.0052.

4.12. (R)-Cephalosporolide H (1a)

A 0.8 M solution of hexylmagnesium bromide (commercially available, 0.09 mL, 0.072 mmol) was added to a premixed solution of CuCl (0.2 mg, 0.002 mmol) in THF (2.0 mL) and **17** (21 mg, 0.06 mmol). The reaction mixture was stirred at room temperature for 1 h, quenched by addition of saturated NH₄Cl aqueous solution (1 mL), and the resulting solution mixture was extracted with ether (3×10 mL), dried over Na₂SO₄, filtered and concentrated in vacuo. The residue was purified by flash chromatography on silica gel (hexanes/EtOAc 5:1) to give cephalosporolide H (**1a**, 15 mg, 81%).

4.13. (R)-Cephalosporolide I (2a)

The mixture of **17** (50 mg, 0.15 mmol), benzyl acrylate (36 mg, 0.23 mmol), NaBH₃CN (28 mg, 0.45 mmol), AIBN (5 mg, 0.03 mmol) in CH₃CN (1.5 mL) were stirred under reflux for 0.5 h. At the end of which time, a saturated ammonium chloride aqueous solution (1.0 mL) was added, then the mixture was poured into water (3 mL) and extracted with Et₂O (10 mL×3). The combined organic layer was washed with brine and dried over Na₂SO₄, then filtered and concentrated. The residue was purified by flash chromatography on silica gel (hexanes/Et₃N 60:1) to give syrupy **18** (55 mg, 96%); [α]_D²⁵ –7.1 (c 0.3, MeOH); IR $\nu_{\max}$ 2926, 2853, 1778, 1730, 1058, 770 cm⁻¹;

¹H NMR (400 MHz, CDCl₃): δ 7.36–7.31 (m, 5H), 5.10 (s, 2H), 5.07 (t, 1H, J =5.2 Hz), 4.28 (d, 1H, J =4.8 Hz), 3.98–3.96 (m, 1H), 2.44 (d, J =14.0 Hz, 1H), 2.39–2.32 (m, 2H), 2.15–1.98 (m, 4H), 1.71–1.51 (m, 5H), 1.27 (s, 3H), 1.21 (s, 3H); ¹³C NMR (100 MHz, CDCl₃): δ 180.7, 173.5, 136.2, 128.6, 128.3, 128.2, 115.2, 87.2, 81.2, 79.8, 66.2, 44.6, 41.7, 36.6, 36.3, 34.1, 30.8, 24.8, 21.5, 18.3; ESI-HRMS calcd for C₂₂H₂₈O₆ ([M+Na]⁺) 411.1778, found 411.1783.

To a solution of **18** (55 mg, 0.14 mmol) in MeOH (2 mL) was added Pd/C (2 mg, 20%) and the suspension was hydrogenated at 3 bar of H₂ pressure for 15 min. The reaction mixture was filtered through a plug of Celite, washed with additional MeOH and evaporated to dryness to yield a yellow oil **2a** (41 mg, 100%); [α]_D²⁵ –7.4 (c 0.3, MeOH); IR $\nu_{\max}$ 2931, 2868, 1772, 1706, 1463, 1346, 1214, 1133, 1082, 818 cm⁻¹; ¹H NMR (400 MHz, CDCl₃): δ 5.08 (t, 1H, J =4.8 Hz), 4.28 (d, 1H, J =4.4 Hz), 3.97 (m, 1H), 2.43 (d, J =14.4 Hz, 1H), 2.37–2.24 (m, 2H), 2.14–1.97 (m, 4H), 1.69–1.51 (m, 5H), 1.27 (s, 3H), 1.21 (s, 3H); ¹³C NMR (100 MHz, CDCl₃): δ 180.9, 179.1, 115.1, 87.0, 81.2, 79.9, 44.6, 41.5, 36.4, 36.1, 34.1, 30.7, 24.6, 21.4, 18.2; ESI-HRMS calcd for C₁₅H₂₂O₆ ([M+Na]⁺) 321.1309, found 321.1317.

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Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.tet.2015.04.025>.

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