

# Synthesis of the Tripeptide Antibiotic Resormycin

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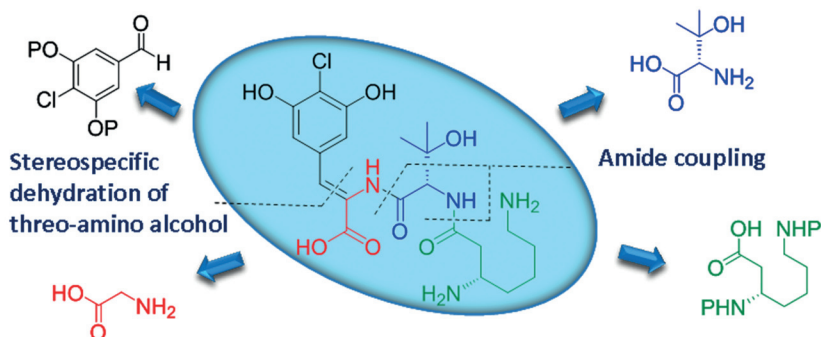
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**Abstract** A short and efficient synthesis of resormycin, a metabolite of *Streptomyces platensis* MJ953-SF5 with herbicidal and antifungal activity, is described. The key step in our synthetic approach is a late-stage stereospecific dehydration of a  $\beta$ -hydroxy amino acid to install the Z-olefin. Because of the modular nature of the synthesis, access to analogues for biological evaluation is readily available.

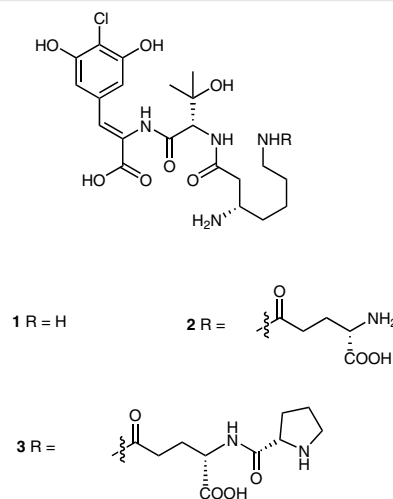
**Key words** *Streptomyces*, antifungal agents, tripeptides, natural products, amino acids, total synthesis

Natural products have been and still are a large reservoir of new biologically active substances, and to this very day, numerous drugs and agrochemicals have been developed from naturally occurring lead compounds.<sup>1</sup>

In 1997, Takeuchi et al. isolated the novel metabolite resormycin<sup>2</sup> (**1**) from *Streptomyces platensis* MJ953-SF5, a strain collected from the soil at Yokohama, Japan (Figure 1). The molecule showed remarkable growth inhibition of monocotyledonous and dicotyledonous weeds. Moreover, the compound inhibited the growth of phytopathogenic fungi, in particular *Cercospora beticola*, *Pyricularia oryzae*, *Botrytis cinerea*, and *Ustilago maydis* with a minimal inhibitory concentration (MIC) lower than 10  $\mu$ g/mL.<sup>2</sup>

Resormycin is composed of three rare unnatural amino acid residues,  $\beta$ -homolysine, 3-hydroxy-L-valine, and an unusual chlorinated resorcylic-2,3-dehydropropenoic amino acid at the C terminus. The resorcylic fragment is attached to the dipeptide unit via a Z-alkene.<sup>2</sup> In 2015, Momose et al. isolated two novel peptide metabolites from *Streptomyces* sp. MK932-CF8, androprostamine A (**2**) and B (**3**),<sup>3</sup> both sharing the tripeptide backbone core with resormycin (Fig-

ure 1). The compounds inhibit androgen-dependent growth of human prostate cancer cells and repress the androgen-induced expression of androgen-receptor-regulated genes.<sup>3</sup>



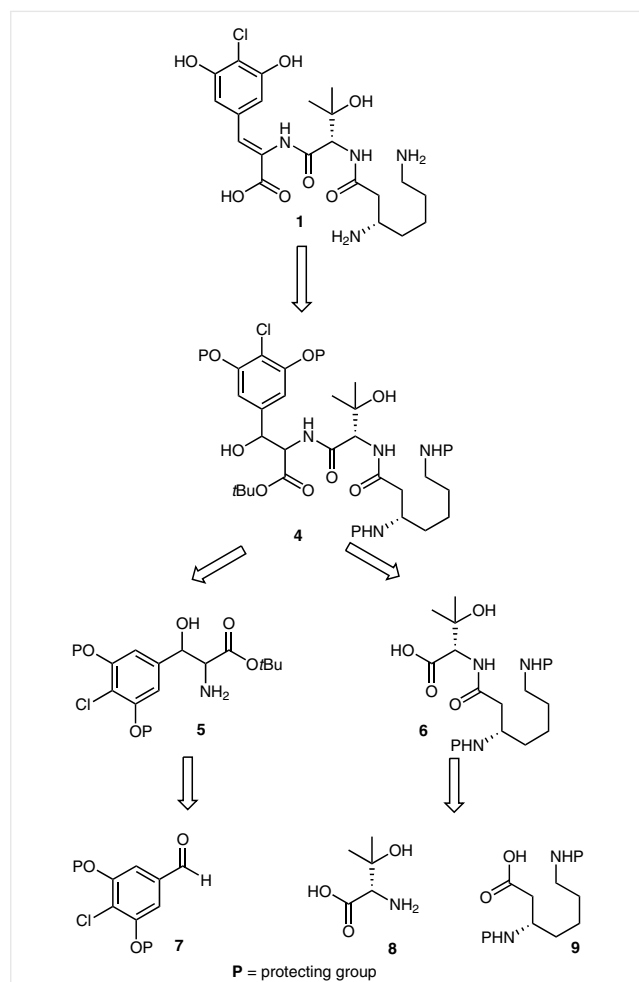
**Figure 1** Structures of resormycin (**1**), androprostamine A (**2**), and androprostamine B (**3**)

Our continuous interest in natural compounds endowed with biological activity, together with the distinctive structural architecture of resormycin, prompted us to develop a synthesis of **1**, which may, in principle, have value in the preparation of **2** and **3** as well as other analogues. The strong interest in these molecules is confirmed by the recent synthesis of resormycin (**1**) and androprostamine A (**2**) by Shibasaki et al.<sup>4</sup> reported in the literature during the last part of this work.

The Shibasaki strategy<sup>4</sup> is based on the use of Horner–Wadsworth–Emmons (HWE) olefination as a key reaction to install the 2,3-dehydroamino acid moiety. The olefination

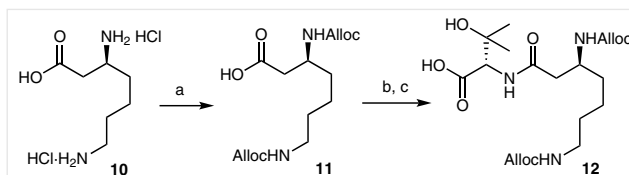
step proceeds with good *Z* selectivity (7% of *E* isomer), but in poor yield (26% crude), and requires a troublesome purification. The synthesis of resormycin is achieved in a 9% overall yield considering the longest linear sequence of six steps.

Our retrosynthetic plan is depicted in Scheme 1. We envisioned that the *Z*-olefin could be obtained by stereospecific dehydration of *N*-acyl- $\beta$ -hydroxy  $\alpha$ -amino acid **4**.<sup>5</sup> The aldol condensation of *N*-(diphenylmethylene)glycine *tert*-butyl ester with the appropriately protected aldehyde **7** was expected to furnish amino alcohol **5**, whereas its counterpart, the acid **6**, could be obtained by coupling 3-hydroxy-L-valine **8** and suitably protected  $\beta$ -homolysine **9**.



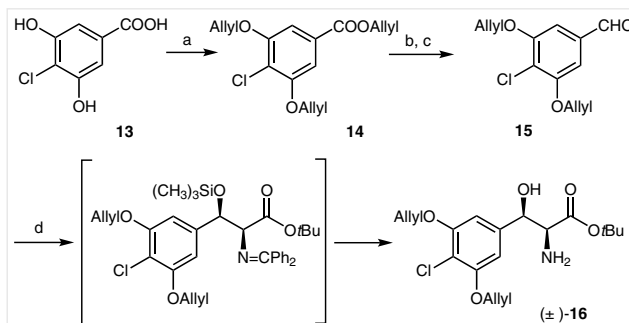
**Scheme 1** Retrosynthetic approach to resormycin (**1**)

Di-Alloc- $\beta$ -homolysine **11** (Alloc = allyloxycarbonyl) was obtained in 73% yield by treatment of  $\beta$ -homolysine **10** with allyl chloroformate and  $K_2CO_3$  in water<sup>6</sup> (Scheme 2); reaction of **11** with *N*-hydroxysuccinimide and EDC-HCl in DMF, followed by coupling of the NHS ester with 3-hydroxy-L-valine produced acid **12** in 86% yield over two steps.



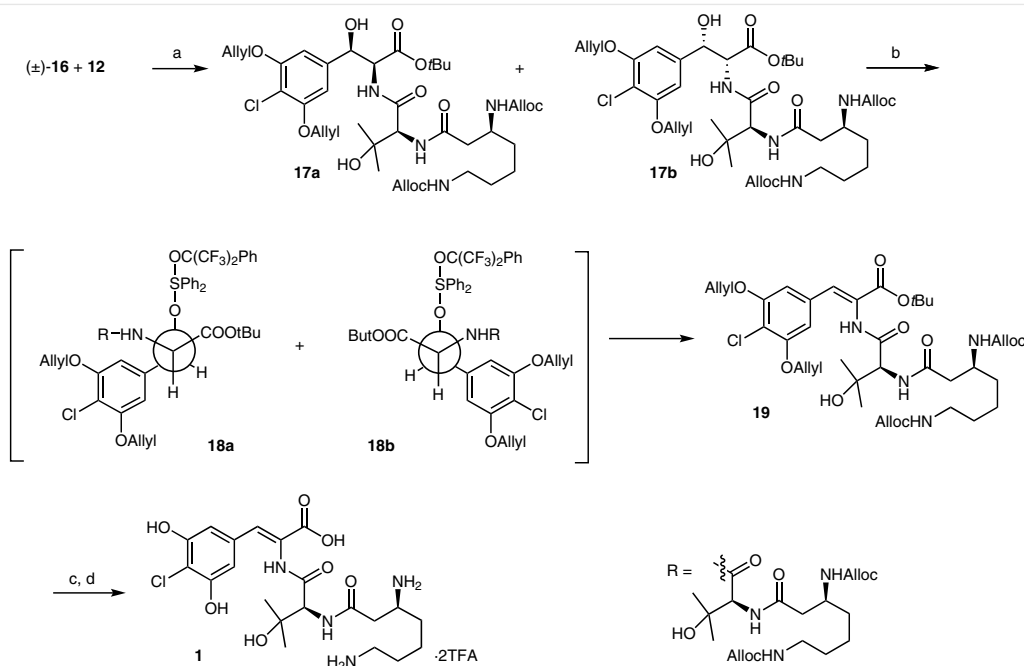
**Scheme 2** Synthesis of dipeptide **12**. Reagents and conditions: (a) allyl chloroformate,  $K_2CO_3$ ,  $H_2O$ , 0 °C to r.t., 16 h, 73%; (b) NHS, EDC-HCl, DMF, r.t., 16 h; (c) 3-hydroxy-L-valine,  $Et_3N$ , dioxane- $H_2O$  (2:1), r.t., 2 h, 86% (over 2 steps).

The synthesis of the aryl core is depicted in Scheme 3. We envisioned that the shortest and easiest approach for the synthesis of aldehyde **15** could be the chlorination of commercially available 3,5-dihydroxybenzaldehyde at the C-4 position. Initially, several attempts using NCS/PTSA/NaCl,<sup>7</sup> NaOCl/KOH,<sup>8</sup> NCS/AcOH,<sup>9</sup> and NCS/MeOH<sup>10</sup> were tried to obtain 4-chloro-3,5-dihydroxybenzaldehyde, but in all the cases either chlorination occurred exclusively at the C-2 position or there was no product formation at all. Thus, we followed a different strategy. Alkylation of commercially available 4-chloro-3,5-dihydroxybenzoic acid (**13**) by using  $K_2CO_3$ /DMF<sup>11</sup> at room temperature gave ester **14** in 92% yield (Scheme 3). Treatment of ester **14** with DIBAL-H in  $CH_2Cl_2$  at  $-78$  °C<sup>12</sup> cleanly furnished the benzyl alcohol, which was immediately oxidized by using PCC/NaOAc in  $CH_2Cl_2$  to give aldehyde **15** in 80% yield over two steps.



**Scheme 3** Synthesis of the aryl core. Reagents and conditions: (a) allyl bromide,  $K_2CO_3$ , DMF, 0 °C to r.t., 2 h, 92%; (b) DIBAL-H,  $CH_2Cl_2$ ,  $-78$  °C, 1 h; (c) PCC, NaOAc, 10 °C to r.t., 2 h, 80% (over 2 steps); (d) 1.  $i$ -Pr<sub>2</sub>NH,  $n$ -BuLi, THF, 0 °C, 30 min; 2. *N*-(diphenylmethylene)glycine *tert*-butyl ester in THF,  $-78$  °C, 30 min; 3. TMSCl,  $-78$  °C to r.t. over 1 h; 4.  $ZnCl_2$  (cat.), **15** in THF, r.t., 2 h; 5. 10% citric acid, r.t., 16 h, 81% (from **15**).

The aldehyde now had to be converted into the corresponding amino alcohol ( $\pm$ )-**16**, for the coupling with the dipeptide counterpart. Kirk et al.<sup>13</sup> reported that  $ZnCl_2$ -catalyzed aldol condensation of a glycine equivalent with aromatic aldehydes mainly gave the *threo* diastereomers. Thus, following Kirk's protocol, compound **15** was condensed with *N*-(diphenylmethylene)glycine *tert*-butyl ester at  $-78$  °C in the presence of 5 mol% of  $ZnCl_2$ . The imine end



**Scheme 4** Synthesis of resormycin. *Reagents and conditions:* (a) HBTU, DMAP, DMF, 0 °C, 2 h, then r.t., 1 h, 73%; (b) Martin's sulfurane, CH<sub>2</sub>Cl<sub>2</sub>, 0 °C to r.t., 16 h, 60%; (c) SnBu<sub>3</sub>H, AcOH, [PdCl<sub>2</sub>(PPh<sub>3</sub>)<sub>2</sub>], CH<sub>2</sub>Cl<sub>2</sub>, r.t., 12 h; (d) TFA, CH<sub>2</sub>Cl<sub>2</sub>, 0 °C to r.t., 2 h, 97% (over 2 steps).

the silyl ether functionalities were removed by mild hydrolysis with 10% citric acid to provide the key intermediate (±)-16 in 81% yield (*threo/erythro*, 95:5).

The amide coupling of amino alcohol (±)-16 and acid 12 by using HBTU/DMAP furnished amide 17 in 73% yield as a mixture of *threo* isomers (Scheme 4). Stereospecific dehydration of the *threo*-N-acyl-β-hydroxy-α-amino acid fragment was performed by using Martin's sulfurane [diphenylbis(1,1,1,3,3,3-hexafluoro-2-phenyl-2-propyl)sulfurane] under neutral conditions.<sup>5</sup> The stereospecific formation of the Z-dehydroamino acid is compatible with an E<sub>2</sub> elimination process. Since Martin's sulfurane is known to react with alcohols to give ROSPh<sub>2</sub>OC(F<sub>3</sub>)<sub>2</sub>Ph intermediates, the reaction is likely to proceed through the formation of intermediate 18, followed by *trans* E<sub>2</sub> elimination from the anti-periplanar conformation.<sup>14,15</sup> The dehydration smoothly occurred at room temperature without base to produce Z-alkene 19 in 60% yield. One-pot deprotection of the allyl and Alloc groups by using Pd(PPh<sub>3</sub>)<sub>4</sub> as catalyst in combination with N-methylaniline and dimesedone<sup>16</sup> as an allyl scavenger gave only a partially deprotected compound. Conversely, the palladium-catalyzed hydrostannolytic cleavage of Alloc and allyl groups using SnBu<sub>3</sub>H and AcOH in CH<sub>2</sub>Cl<sub>2</sub>, followed by careful treatment with TFA in CH<sub>2</sub>Cl<sub>2</sub> at 0 °C<sup>17</sup> resulted in clean deprotection to give the final product resormycin (1), whose spectroscopic data matched those reported in the literature.<sup>4</sup>

In conclusion, we have designed and accomplished a convergent total synthesis of resormycin with the longest linear sequence of eight steps in 25% overall yield. The key steps in our synthetic approach include the late-stage stereospecific dehydration of a β-hydroxy amino acid to install the Z-olefin and palladium-catalyzed one-pot deprotection of allyl and Alloc groups. This straightforward synthetic approach constitutes also a formal synthesis of androprostamines<sup>3,4</sup> and is amenable to generate diverse analogues for biological investigation.

All reagents and solvents were reagent grade or were purified by standard methods before use. Melting points were determined in open capillaries on an SMP3 apparatus and are uncorrected. <sup>1</sup>H NMR spectra were recorded on Varian Mercury 300 MHz and Bruker AV600 spectrometers; TMS was used as an internal standard. <sup>13</sup>C NMR spectra were recorded on Varian 300 MHz and Bruker AV600 spectrometers. Optical rotations were measured on a Perkin Elmer 241 polarimeter. IR spectra were recorded on a Perkin Elmer 1310 spectrophotometer. The elemental analyses were recorded on a CARLO ERBA EA 1108 instrument. HPLC analyses were performed by using a Jasco PU-980 pump equipped with a Jasco UV-975 (λ = 220 nm) UV-vis detector and a Phenomenex Lux Amylose-2 column (4.6 mm i.d. × 150 mm, 5 μm) at a flow rate of 1 mL·min<sup>-1</sup>, using *n*-hexane-*i*-PrOH (7:3) as eluent. Preparative HPLC was performed using a 1525 Extended Flow Binary HPLC pump, equipped with a Waters 2489 UV-vis detector and a Phenomenex Lux Amylose-2 column (21.2 mm i.d. × 250 mm) at a flow rate of 15 mL·min<sup>-1</sup> using *n*-hexane-*i*-PrOH (1:1) as eluent. Solvents were routinely distilled prior to use; anhydrous THF and Et<sub>2</sub>O were obtained by distillation from sodium benzophenone ketyl;

anhydrous  $\text{CH}_2\text{Cl}_2$  was obtained by distillation from phosphorus pentoxide. All reactions requiring anhydrous conditions were performed under a positive nitrogen flow and all glassware were oven-dried and/or flame-dried. Isolation and purification of the compounds were performed by flash column chromatography on silica gel 60 (230–400 mesh). Analytical TLC was conducted on TLC plates (silica gel 60 F254, aluminum foil). Compounds on TLC plates were detected under UV light at 254 and 365 nm or were revealed by spraying with 10% phosphomolybdic acid (PMA) in EtOH. 3-Hydroxy-L-valine was purchased from Acros Organic. L- $\beta$ -Homolysine dihydrochloride (**10**) was purchased from abcr, Germany (<http://www.abcr.de>).

#### (S)-3,7-Bis(allyloxycarbonylamino)heptanoic Acid (**11**)

A cold solution of  $\text{K}_2\text{CO}_3$  (948 mg, 6.86 mmol) in  $\text{H}_2\text{O}$  (2 mL), followed by allyl chloroformate (0.36 mL, 3.43 mmol), was added dropwise to a solution of  $\beta$ -homolysine dihydrochloride (**10**; 320 mg, 1.37 mmol) in  $\text{H}_2\text{O}$  (2 mL) at 0 °C. After complete addition, the reaction mixture was stirred at r.t. for 16 h; then it was cooled to 0 °C and acidified by using 2 N HCl. The aqueous layer was extracted with EtOAc (2  $\times$  10 mL). The combined organic extracts were washed with brine (7 mL), dried over anhydrous  $\text{Na}_2\text{SO}_4$ , and concentrated in vacuo. Purification by flash column chromatography (silica gel, MeOH– $\text{CH}_2\text{Cl}_2$ , 7–10%) yielded compound (–)-**11**.

Yield: 330 mg (73%); colorless solid; mp 77–78 °C;  $[\alpha]_{\text{D}}^{23}$  –12.8 (c 1.15,  $\text{CHCl}_3$ );  $R_f$  = 0.4 (MeOH– $\text{CH}_2\text{Cl}_2$ , 10:90).

IR (film): 3480, 3400, 3100, 3000, 1750, 1720, 1680, 1550, 1440, 1300, 800, 785, 730  $\text{cm}^{-1}$ .

$^1\text{H}$  NMR (300 MHz,  $\text{CD}_3\text{OD}$ ):  $\delta$  = 5.99–5.85 (m, 2 H), 5.31 (ddt,  $J$  = 17.1, 1.6, 1.5 Hz, 2 H), 5.16 (ddt,  $J$  = 10.6, 1.6, 1.5 Hz, 2 H), 4.50 (dt,  $J$  = 5.4, 1.5 Hz, 4 H), 4.02–3.84 (m, 1 H), 3.09 (t,  $J$  = 6.7 Hz, 2 H), 2.44 (dd,  $J$  = 6.9, 2.0 Hz, 2 H), 1.66–1.27 (m, 6 H).

$^{13}\text{C}$  NMR (75 MHz,  $\text{CD}_3\text{OD}$ ):  $\delta$  = 175.2, 158.8, 158.2, 134.6, 134.5, 117.3 (2 C), 66.2 (2 C), 49.4, 41.5, 40.9, 35.3, 30.6, 24.2.

Anal. Calcd for  $\text{C}_{15}\text{H}_{24}\text{N}_2\text{O}_6$ : C, 54.87; H, 7.37; N, 8.53. Found: C, 54.96; H, 7.36; N, 8.55.

#### (S)-2-[(S)-3,7-Bis(allyloxycarbonylamino)heptanamido]-3-hydroxy-3-methylbutanoic Acid (**12**)

To a stirred solution of compound (–)-**11** (508 mg, 1.55 mmol) in anhydrous DMF (5.1 mL, 0.3 M), NHS (409 mg, 3.56 mmol) and EDC·HCl (682 mg, 3.56 mmol) were sequentially added under a  $\text{N}_2$  atmosphere. The clear solution obtained was stirred overnight at r.t. The reaction mixture was poured into ice water (30 mL) and the aqueous layer was extracted with EtOAc (2  $\times$  10 mL). The combined organic extracts were washed with cold brine (5  $\times$  5 mL), dried over anhydrous  $\text{Na}_2\text{SO}_4$ , and concentrated in vacuo to give the NHS ester (785 mg). The crude ester was used without further purification. To a solution of 3-hydroxy-L-valine (269 mg, 2.02 mmol) in dioxane– $\text{H}_2\text{O}$  (1:1, 26.6 mL) was added  $\text{Et}_3\text{N}$  (0.64 mL, 4.60 mmol) followed by the dropwise addition of a solution of the above NHS ester (785 mg, 1.84 mmol) in dioxane (13.4 mL). The resultant solution was stirred at r.t. for 2 h. The solvent was removed in vacuo and diluted with aq  $\text{NaHCO}_3$  (5 mL), and the aqueous layer was washed with EtOAc (2  $\times$  10 mL). The aqueous layer was cooled to 0 °C and acidified by using 2 N HCl; then it was extracted with EtOAc (2  $\times$  20 mL), washed with brine (10 mL), and dried over anhydrous  $\text{Na}_2\text{SO}_4$ . The solvent was concentrated in vacuo to yield **12**.

Yield: 595 mg (86%); pale yellow solid; mp 63–65 °C;  $[\alpha]_{\text{D}}^{23}$  –18.4 (c 1.0,  $\text{CHCl}_3$ );  $R_f$  = 0.1 (MeOH– $\text{CH}_2\text{Cl}_2$ , 10:90).

IR (film): 3490, 3380, 3105, 3000, 1730, 1670, 1625, 1550, 1445, 1295, 770, 730  $\text{cm}^{-1}$ .

$^1\text{H}$  NMR (300 MHz,  $\text{CD}_3\text{OD}$ ):  $\delta$  = 5.99–5.83 (m, 2 H), 5.28 (ddt,  $J$  = 17.1, 1.6, 1.5 Hz, 2 H), 5.16 (ddt,  $J$  = 10.6, 1.6, 1.5 Hz, 2 H), 4.50 (dt,  $J$  = 5.4, 1.5 Hz, 4 H), 4.42 (s, 1 H), 3.95–3.91 (m, 1 H), 3.09 (t,  $J$  = 6.4 Hz, 2 H), 2.45 (dd,  $J$  = 6.9, 2.6 Hz, 2 H), 1.68–1.29 (m, 6 H), 1.29 (s, 3 H), 1.26 (s, 3 H).

$^{13}\text{C}$  NMR (75 MHz,  $\text{CD}_3\text{OD}$ ):  $\delta$  = 178.0, 173.3, 158.8, 158.3, 134.6, 134.5, 117.5, 117.4, 73.4, 66.4, 66.2, 62.6, 49.9, 42.8, 41.5, 35.5, 30.6, 27.8, 26.1, 24.1.

Anal. Calcd for  $\text{C}_{20}\text{H}_{33}\text{N}_3\text{O}_8$ : C, 54.16; H, 7.50; N, 9.47. Found: C, 54.22; H, 7.48; N, 9.49.

#### Allyl 3,5-Bis(allyloxy)-4-chlorobenzoate (**14**)

A suspension of 4-chloro-3,5-dihydroxybenzoic acid (**13**; 0.50 g, 2.65 mmol) and anhydrous  $\text{K}_2\text{CO}_3$  (2.50 g, 18.55 mmol) in anhydrous DMF (5 mL, 0.5 M) was stirred for 10 min and cooled to 0 °C. Allyl bromide (0.80 mL, 9.28 mmol) was added dropwise at 0 °C and the mixture was stirred for 1 h at r.t. Then the reaction mixture was poured into ice water (25 mL) and the aqueous layer was extracted with  $\text{Et}_2\text{O}$  (2  $\times$  10 mL); the combined organic extracts were washed with brine (5 mL) and dried over anhydrous  $\text{Na}_2\text{SO}_4$ . The solvent was removed in vacuo. Purification by flash column chromatography (silica gel, EtOAc–PE, 3:97) afforded compound **14**.

Yield: 750 mg (92%); colorless oil;  $R_f$  = 0.3 (EtOAc–PE, 5:95).

IR (film): 3040, 3100, 3000, 1750, 1625, 1450, 1350, 1295, 1270, 1150, 1130, 1000  $\text{cm}^{-1}$ .

$^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ ):  $\delta$  = 7.29 (s, 2 H), 6.18–5.94 (m, 3 H), 5.53–5.28 (m, 6 H), 4.82 (dt,  $J$  = 5.9, 1.3 Hz, 2 H), 4.67 (dt,  $J$  = 5.2, 1.6 Hz, 4 H).

$^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ ):  $\delta$  = 165.7, 155.2 (2 C), 132.4 (2 C), 132.2, 128.9, 118.7, 118.3 (3 C), 107.3 (2 C), 70.1 (2 C), 66.1.

Anal. Calcd for  $\text{C}_{16}\text{H}_{17}\text{ClO}_4$ : C, 62.24; H, 5.55. Found: C, 62.33; H, 5.56.

#### 3,5-Bis(allyloxy)-4-chlorobenzaldehyde (**15**)

A 1 M solution of DIBAL-H in  $\text{CH}_2\text{Cl}_2$  (4.30 mL, 4.30 mmol) was added dropwise to a stirred solution of **14** (532 mg, 1.72 mmol) in  $\text{CH}_2\text{Cl}_2$  (11.4 mL, 0.15 M) at –78 °C under a  $\text{N}_2$  atmosphere. After complete addition, the reaction mixture was stirred at –78 °C for 1 h. A solution of saturated aq Rochelle salt (1 mL) was added followed by 2 N HCl (5 mL). The reaction mixture was stirred vigorously until the two layers became clear. The organic layer was separated and the aqueous layer was extracted with  $\text{CH}_2\text{Cl}_2$  (10 mL); the combined organic extracts were washed with brine (6 mL), dried over anhydrous  $\text{Na}_2\text{SO}_4$ , and concentrated in vacuo to afford the benzyl alcohol (418 mg) as a colorless oil. The alcohol was used for the next step without further purification.

To a stirred suspension of PCC (709 mg, 3.29 mmol) and NaOAc (67 mg, 0.82 mmol) in anhydrous  $\text{CH}_2\text{Cl}_2$  (3 mL) cooled to 10 °C, the above benzyl alcohol (418 mg, 1.64 mmol) in  $\text{CH}_2\text{Cl}_2$  (5 mL) was added dropwise. The reaction mixture was stirred for 2 h at r.t. In vacuo concentration followed by flash column chromatography (silica gel) furnished **15**.

Yield: 346 mg (80%, over 2 steps); white solid; mp 88–89 °C;  $R_f$  = 0.2 (EtOAc–PE, 5:95).

IR (film): 3080, 2965, 2920, 1720, 1615, 1460, 1280, 1120, 760  $\text{cm}^{-1}$ .



$^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ ):  $\delta$  = 9.88 (s, 1 H), 7.09 (s, 2 H), 6.15–5.99 (m, 2 H), 5.49 (ddt,  $J$  = 17.0, 1.6, 1.5 Hz, 2 H), 5.33 (ddt,  $J$  = 10.1, 1.6, 1.5 Hz, 2 H), 4.68 (dt,  $J$  = 5.0, 1.5 Hz, 4 H).

$^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ ):  $\delta$  = 190.0, 155.9 (2 C), 135.0, 132.0 (2 C), 118.4 (3 C), 106.8 (2 C), 70.0 (2 C).

Anal. Calcd for  $\text{C}_{13}\text{H}_{13}\text{ClO}_3$ : C, 61.79; H, 5.19. Found: C, 61.70; H, 5.18.

***tert*-Butyl 2-Amino-3-[3,5-bis(allyloxy)-4-chlorophenyl]-3-hydroxypropanoate (16)**

A 1.6 M solution of *n*-BuLi in hexane (1.3 mL, 1.37 mmol) was added dropwise to a stirred solution of *i*-Pr<sub>2</sub>NH (0.20 mL, 1.43 mmol) in anhydrous THF (4 mL) at 0 °C under a N<sub>2</sub> atmosphere. The pale yellow solution obtained was stirred at 0 °C for 30 min. The thus generated LDA solution was added dropwise to a solution of *N*-(diphenylmethylene)glycine *tert*-butyl ester (0.37 g, 1.25 mmol) in THF (4 mL) cooled to –78 °C under a N<sub>2</sub> atmosphere. The wine red solution obtained was stirred at –78 °C for 30 minutes. TMSCl (0.47 mL, 3.75 mmol) was added dropwise (color changes from wine red to pale yellow); after complete addition, the reaction mixture was warmed to r.t. over 1 h. The yellow solution obtained was added dropwise to a stirred solution of **15** (0.316 g, 1.25 mmol) and  $\text{ZnCl}_2$  (0.16 mL, 0.16 mmol) in anhydrous THF (4 mL) at r.t., and stirred for 2 h. The reaction mixture was cooled to 0 °C and quenched by dropwise addition of 10% citric acid (6 mL). After complete addition, the reaction mixture was stirred overnight at r.t. THF was removed in vacuo and the aqueous layer was cooled to 0 °C and basified by using saturated aq NaHCO<sub>3</sub> and extracted with EtOAc (2 × 25 mL). The combined organic extracts were washed with brine (15 mL) and dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>; the solvent was then removed in vacuo. The crude was purified by flash column chromatography (silica gel, MeOH–CH<sub>2</sub>Cl<sub>2</sub>, 0.5:99.5) to give **16**.

Yield: 0.393 g (81%); colorless oil;  $R_f$  = 0.25 (MeOH–CH<sub>2</sub>Cl<sub>2</sub>, 2:98).

IR (film): 3440, 3360, 3100, 3020, 2985, 2930, 1750, 1615, 1480, 1445, 1400, 1290, 1180, 1155, 1120, 950, 880, 765 cm<sup>–1</sup>.

$^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ ):  $\delta$  (major stereoisomers in *threo/erythro* mixture, 95:5) = 6.61 (s, 2 H), 6.15–5.98 (m, 2 H), 5.46 (ddt,  $J$  = 17.5, 1.6, 1.5 Hz, 2 H), 5.30 (ddt,  $J$  = 10.4, 1.6, 1.5 Hz, 2 H), 4.72 (d,  $J$  = 4.9 Hz, 1 H), 4.61 (dt,  $J$  = 5.0, 1.5 Hz, 4 H), 3.49 (d,  $J$  = 4.9 Hz, 1 H), 1.40 (s, 9 H).

$^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ ):  $\delta$  (major stereoisomer) = 172.2, 155.4 (2 C), 140.8, 132.7 (2 C), 117.8 (3 C), 104.7 (2 C), 82.1, 74.2, 70.0 (2 C), 60.9, 28.0 (3 C).

Anal. Calcd for  $\text{C}_{19}\text{H}_{26}\text{ClNO}_5$ : C, 59.45; H, 6.83; N, 3.65. Found: C, 59.51; H, 6.84; N, 3.65.

***tert*-Butyl (5*S*,9*S*)-9-(Allyloxycarbonylamino)-2-[[3,5-bis(allyloxy)-4-chlorophenyl](hydroxy)methyl]-5-(2-hydroxypropan-2-yl)-4,7,15-trioxo-16-oxa-3,6,14-triazanonadec-18-enoate (17)**

A stirred solution of acid **12** (201 mg, 0.45 mmol) and amino alcohol **16** (190 mg, 0.50 mmol) in anhydrous DMF (4.5 mL, 0.1 M) was cooled to 0 °C. HBTU (188.8 mg, 1.58 mmol) and DMAP (60.8 mg, 0.498 mmol) were sequentially added at 0 °C under a N<sub>2</sub> atmosphere. The reaction mixture was stirred at 0 °C for 2 h and then for 1 h at r.t. The reaction mixture was poured into cold water (30 mL) and the aqueous layer was extracted with EtOAc (2 × 15 mL). The combined organic extracts were washed with brine (10 mL), dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>, and concentrated in vacuo. The residue was purified by column chromatography (silica gel, MeOH–CH<sub>2</sub>Cl<sub>2</sub>, 1.5 to 2.5%) to afford amide **17**.

Yield: 270 mg (73%, mixture of diastereomers);  $R_f$  = 0.25 (MeOH–CH<sub>2</sub>Cl<sub>2</sub>, 3:97).

$^1\text{H}$  NMR (300 MHz,  $\text{CD}_3\text{OD}$ ):  $\delta$  = 6.80–6.70 (m, 2 H), 6.17–6.00 (m, 2 H), 5.98–5.83 (m, 2 H), 5.55–5.47 (m, 2 H), 5.34–5.11 (m, 6 H), 4.74–4.37 (m, 10 H), 3.98–3.80 (m, 1 H), 3.15–3.05 (m, 2 H), 2.47–2.30 (m, 2 H), 1.60–1.39 (m, 15 H).

$^{13}\text{C}$  NMR (75 MHz,  $\text{CD}_3\text{OD}$ ): major  $\delta$  = 173.2, 172.5, 172.2, 170.6, 170.4, 156.3 (2 C), 142.4, 134.4 (4 C), 117.6 (4 C), 117.4, 105.3 (2 C), 83.2, 73.6, 72.9, 70.7 (3 C), 66.2, 61.2, 60.4, 50.0, 42.4, 41.5, 35.2, 30.5, 28.3 (5 C), 24.1.

Anal. Calcd for  $\text{C}_{39}\text{H}_{57}\text{ClN}_4\text{O}_{12}$ : C, 57.88; H, 7.10, N, 6.92. Found: C, 57.79; H, 7.08; N, 6.90.

***tert*-Butyl (5*S*,9*S*)-9-(Allyloxycarbonylamino)-2-[(*Z*)-3,5-bis(allyloxy)-4-chlorobenzylidene]-5-(2-hydroxypropan-2-yl)-4,7,15-trioxo-16-oxa-3,6,14-triazanonadec-18-enoate (19)**

A solution of Martin's sulfurane (49.8 mg, 0.07 mmol) in anhydrous CH<sub>2</sub>Cl<sub>2</sub> (0.4 mL) was added dropwise to a stirred solution of alcohol **17** (30 mg, 0.04 mmol) in anhydrous CH<sub>2</sub>Cl<sub>2</sub> (0.6 mL) at 0 °C under a N<sub>2</sub> atmosphere. The reaction mixture was stirred at r.t. for 3 h. More Martin's sulfurane (0.074 mmol) was added and the reaction mixture was stirred at r.t. for 12 h. The reaction mixture was concentrated in vacuo and the crude material was purified by flash column chromatography (silica gel, MeOH–CH<sub>2</sub>Cl<sub>2</sub>, 1.5%). At this stage, the product was purified by semi-preparative HPLC (Phenomenex Lux Amylose-2 column, 21.2 mm i.d. × 250 mm; flow rate 15 mL·min<sup>–1</sup>; *n*-hexane–*i*-PrOH, 1:1;  $t_r$  = 4.6 min;  $\lambda$  = 220 nm) to furnish pure compound **19**.

Yield: 17.5 mg (60%); white solid; mp 72–73 °C;  $[\alpha]_D^{23}$  –3.59 (c 0.004, CHCl<sub>3</sub>);  $R_f$  = 0.2 (MeOH–CH<sub>2</sub>Cl<sub>2</sub>, 2:98).

IR (film): 3360, 3045, 2990, 1770, 1755, 1720, 1665, 1610, 1540, 1400, 1300, 1280, 1055, 1010, 950, 870 cm<sup>–1</sup>.

$^1\text{H}$  NMR (600 MHz,  $\text{CDCl}_3$ ):  $\delta$  = 8.09 (s, 1 H), 7.23 (s, 1 H), 6.71 (s, 2 H), 6.65 (d,  $J$  = 8.3 Hz, 1 H), 6.10–6.02 (m, 2 H), 5.94–5.83 (m, 2 H), 5.56 (d,  $J$  = 7.5 Hz, 1 H), 5.48 (ddt,  $J$  = 17.2, 1.5, 1.6 Hz, 2 H), 5.32–5.24 (m, 4 H), 5.21–5.12 (m, 2 H), 4.87 (br s, 1 H), 4.65–4.46 (m, 8 H), 4.39 (d,  $J$  = 8.3 Hz, 1 H), 3.84–3.78 (m, 1 H), 3.76 (s, 1 H), 3.15–3.04 (m, 2 H), 2.50 (dd,  $J$  = 14.6, 5.0 Hz, 1 H), 2.46 (dd,  $J$  = 14.6, 5.9 Hz, 1 H), 1.53 (s, 9 H), 1.48–1.38 (m, 4 H), 1.33 (s, 3 H), 1.32–1.24 (m, 2 H), 1.21 (s, 3 H).

$^{13}\text{C}$  NMR (150 MHz,  $\text{CDCl}_3$ ):  $\delta$  = 171.8, 170.2, 164.2, 156.6, 156.2, 155.4 (2 C), 133.2, 133.0, 132.8 (2 C), 132.7, 132.2, 125.9, 118.0 (2 C), 117.9, 117.7, 113.6, 107.7 (2 C), 83.0, 72.0, 70.1 (3 C), 65.7, 60.2, 49.0, 40.8, 40.4, 33.9, 29.4, 28.2, 28.0, 27.8, 27.6, 25.8, 23.1.

Anal. Calcd for  $\text{C}_{39}\text{H}_{55}\text{ClN}_4\text{O}_{11}$ : C, 59.19; H, 7.01; N, 7.08. Found: C, 59.35; H, 7.03; N, 7.06.

**Resormycin (1) TFA Salt**

$\text{SnBu}_3\text{H}$  (0.04 mL, 0.17 mmol) was added in one portion to a stirred solution of compound **19** (23 mg, 0.03 mmol), AcOH (0.02 mL, 0.35 mmol), and  $[\text{PdCl}_2(\text{PPh}_3)_2]$  (2 mg, 0.003 mmol) in anhydrous CH<sub>2</sub>Cl<sub>2</sub> (0.8 mL, 0.03 M) under a N<sub>2</sub> atmosphere. The reaction mixture was stirred at r.t. for 2 h and again  $\text{SnBu}_3\text{H}$  (0.04 mL, 0.17 mmol) was added in one portion. The reaction mixture was stirred overnight at r.t., then it was concentrated in vacuo to dryness and the solid obtained was triturated with hexane (2 mL), followed by hexane–MeCN (9:1) to give crude *tert*-butyl (*Z*)-3-(4-chloro-3,5-dihydroxyphenyl)-2-[(*S*)-2-[(*S*)-3,7-diaminoheptanamido]-3-hydroxy-3-methylbutanamido]-acrylate;  $R_f$  = 0.2 (3% NH<sub>3</sub> in MeOH–H<sub>2</sub>O, 1:1).

A solution of the above ester (20 mg) in CH<sub>2</sub>Cl<sub>2</sub> (0.5 mL) was cooled to 0 °C. TFA (0.2 mL) was added dropwise and the reaction mixture was stirred at r.t. for 2 h. The mixture was concentrated to dryness and the residue was triturated with Et<sub>2</sub>O to afford title compound **1**.

Yield: 13 mg (97%); white solid; mp 123–124 °C;  $[\alpha]_D^{23} +123.2$  (c 0.004, MeOH) {Lit.<sup>4</sup> for TFA salt:  $[\alpha]_D^{23} +127.7$  (c 0.44, MeOH)};  $R_f = 0.8$  (5%  $\text{NH}_3$  in MeOH– $\text{H}_2\text{O}$ , 1:1).

$^1\text{H}$  NMR (600 MHz,  $\text{CD}_3\text{OD}$ ):  $\delta = 7.35$  (s, 1 H), 6.83 (s, 2 H), 4.54 (s, 1 H), 3.59–3.52 (m, 1 H); 2.90 (t,  $J = 7.7$  Hz, 2 H), 2.78–2.73 (m, 2 H), 1.75–1.45 (m, 6 H), 1.40 (s, 3 H), 1.38 (s, 3 H).

$^1\text{H}$  NMR (600 MHz,  $\text{DMSO}-d_6$ )  $\delta = 12.78$  (br s, 1 H), 9.80 (br s, 2 H), 9.42 (br s, 1 H), 8.35 (d,  $J = 8.4$  Hz, 1 H), 8.02–7.50 (m, 6 H), 7.00 (s, 1 H), 6.70 (s, 2 H), 5.00–4.90 (m, 1 H), 4.49 (d,  $J = 8.4$  Hz, 1 H), 3.40–3.35 (m, 1 H), 2.72 (t,  $J = 7.5$  Hz, 2 H), 2.70–2.50 (m, 2 H), 1.63–1.25 (m, 6 H), 1.20 (s, 6 H).

$^{13}\text{C}$  NMR (150 MHz,  $\text{DMSO}-d_6$ ):  $\delta = 169.9$ , 169.4, 166.1, 158.2 (TFA), 158.0 (TFA), 153.9 (2 C), 132.2, 130.9, 126.7, 121.3 (TFA), 121.2 (TFA), 108.6 (2 C), 108.5, 71.3, 60.4, 48.0, 38.5, 37.1, 31.5, 27.1, 26.5, 26.1, 21.3.

Anal. Calcd for  $\text{C}_{25}\text{H}_{33}\text{ClF}_6\text{N}_4\text{O}_{11}$ : C, 42.00; H, 4.65; N, 7.84. Found: C, 41.85; H, 4.66; N, 7.86.

## Supporting Information

Supporting information for this article is available online at <https://doi.org/10.1055/s-0036-1588553>.

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