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Letter

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Total Synthesis of the GRP78-Downregulatory Macrolide (+)-Prunustatin A, the Immunosuppressant (+)-SW-163A, and a JBIR-04 Diastereoisomer That Confirms JBIR-04 Has Nonidentical Stereochemistry to (+)-Prunustatin A

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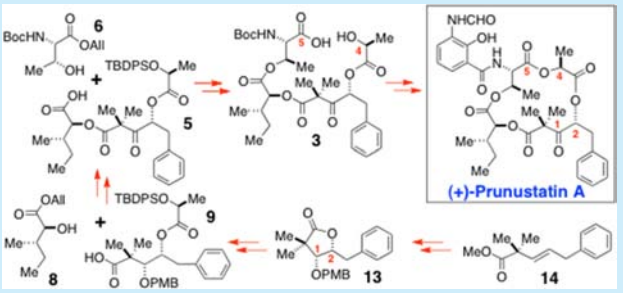
Supporting Information

ABSTRACT: A unified total synthesis of the GRP78-downregulator (+)-prunustatin A and the immunosuppressant (+)-SW-163A based upon [1 + 1 + 1 + 1]-fragment condensation and macrolactonization between O(4) and C(5) is herein described. Sharpless asymmetric dihydroxylation was used to set the C(2) stereocenter present in both targets. In like fashion, coupling of the (+)-prunustatin A macrolide amine with benzoic acid furnished a JBIR-04 diastereoisomer whose NMR spectra did not match those of JBIR-04, thus confirming that it has different stereochemistry than (+)-prunustatin A.



(+)-Prunustatin A is a chemically alluring β-keto ester macrolide first discovered by Shin-ya and co-workers[1] in fermentation broths of *Streptomyces violaceoniger* 4521-SVS3, an actinomycete found in soil of the Okinawan island of Kumejima. While initially a full stereostructure for (+)-prunustatin A could not be proposed, later chemical degradation and fragment correlation studies by Shin-ya did eventually reveal the full absolute stereostructure to be as that shown in Scheme 1.[2]

From a therapeutic perspective, (+)-prunustatin A is of considerable pharmaceutical interest because of its pronounced downregulatory effects on GRP78/BIP (78 kDa glucose-regulated protein) expression in *glucose-deprived* HT1080 human fibrosarcoma cells at very low drug concentrations (IC_{50} = 11.5 nM), with total inhibition of GRP78 expression occurring at the 80 nM level and full cancer cell apoptosis occurring at the slightly higher drug concentration of 100 nM.[1] Importantly, (+)-prunustatin A is non-cytotoxic toward HT1080 cells *under normal conditions*, where it functions as a cytostatic agent even at concentrations as high as 500 nM. This remarkable property of (+)-prunustatin A to *selectively* induce apoptosis within highly stressed, glucose-deprived, cancer cells suggests that it might potentially be useful to combat hypoxic human tumors while leaving normal healthy tissue undamaged.

Upregulated GRP78 expression within hypoxic solid tumors is now thought to contribute significantly toward them becoming refractory toward treatment with drugs and radiotherapy. There is thus a very good medical case for clinically establishing whether (+)-prunustatin A will be of value for treating such cancers. However, preliminary screening of (+)-prunustatin A against xenografted tumors in mice has not been possible to date because of the dearth of material that is presently available for testing. This has prompted a number of groups to devise elegant total syntheses of (+)-prunustatin A to increase the supply, with the teams of Kawanishi[3] and Usuki[4] scoring particularly notable successes in this regard.

Apart from the potential value of GRP78 inhibitors for treating drug-resistant cancers, such molecules could possibly sensitize drug-resistant bacteria to the effects of existing antibiotics (e.g., Gram-negative *Neisseria gonorrheae* and *Neisseria meningitides* strains),[5] and they might also prove useful for counteracting many lethal viral infections. In the latter regard, many viruses rely on GRP78-regulated machinery to create functionally active virions (e.g., the Ebola, Lassa, and Marburg hemorrhagic RNA viruses).[5] Because of this, we became interested in developing a new synthetic route to (+)-prunustatin A to expedite its future clinical development and that of its powerful reduced immunosuppressant congener, (+)-SW-163A.[6] In this Letter we report our success in these endeavors.

Following several abortive attempts to synthesize (+)-prunustatin A by strategies involving macrocyclization between O(7) and C(8), *which each furnished prunustatin A diastereomers*, we eventually decided to pursue a new synthetic plan wherein the C(1) keto group would be installed before ring closure between (O)4 and C(5). In our newly proposed strategem (Scheme 1), a late-stage macrolactonization would now be effected on *seco*-acid **3**.[3] The resulting macrolide would then be converted into amine−lactone **2**, which would be coupled to acid **1**. The resulting product would then be O-debenzylated. Compound **3** would itself be acquired from **4** by O-deallylation and O-

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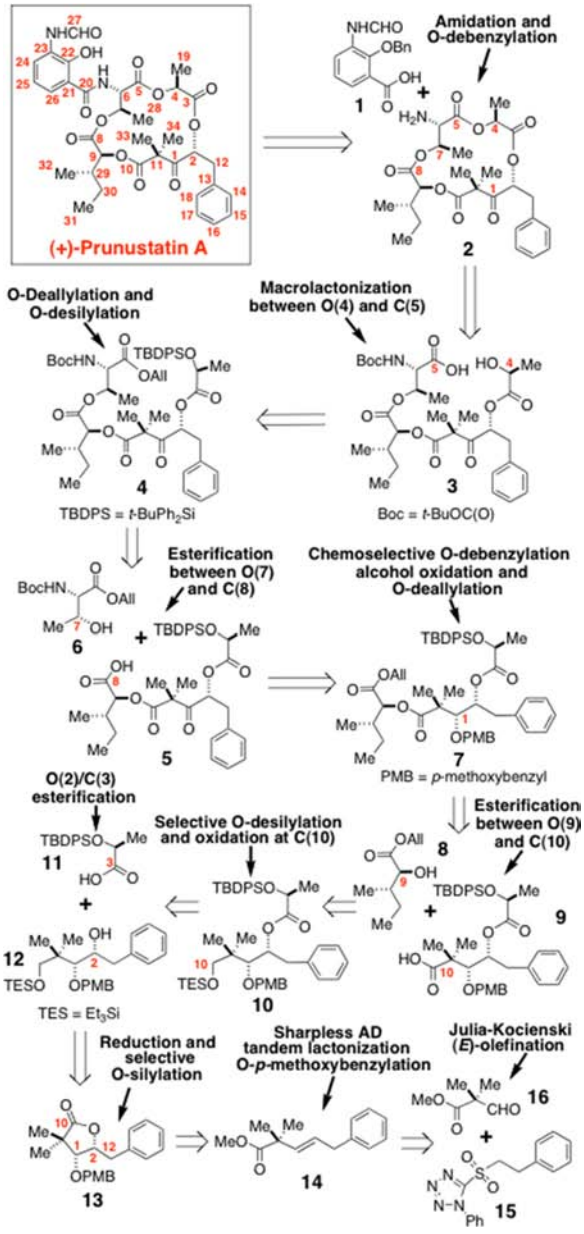
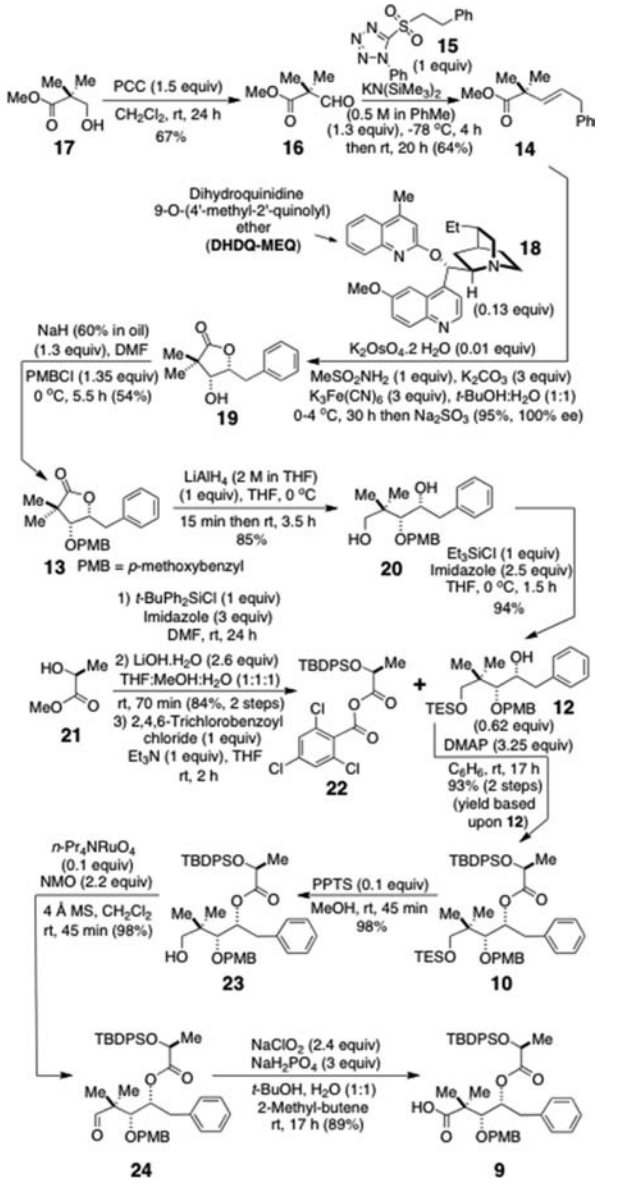
Scheme 1. Our Retrosynthetic Planning for Prunustatin A



desilylation, with tetraester **4** being assembled from the *sterically hindered* acid **9** by two successive esterifications, the first involving **8** and **9** and the second involving **5** and **6**. Keto acid **5** would be derived from triester **7** by O-debenzylation at C(1), oxidation of the alcohol to the ketone, and O-deallylation. Acid **9** was envisioned to emanate from alcohol **12** by esterification with protected L-lactic acid derivative **11** allied with selective O-desilylation and oxidation of the primary alcohol to the acid. The main issue associated with constructing alcohol **12** would be correct positioning of the various protecting groups to enable the alcohol at C(2) to be selectively presented to **11**. For this, we hoped to take advantage of a Sharpless asymmetric dihydroxylation (AD) reaction[7] on (*E*)-alkene **14** accompanied by in situ lactonization to give the corresponding *β*-hydroxybutyrolactone with high ee. If successful, such an approach would nicely allow protection of the secondary hydroxyl at C(1) as an *O-p*-methoxybenzyl (OPMB) ether, as in compound **13**, to allow the remaining features to be elaborated by reduction and selective O-silylation. Of course, a good option for constructing the (*E*)-olefin in **14** would be a Julia–Kocienski olefination[8] between tetrazolylsulfone **15** and known *β*-aldehydo ester **16**.[9]

Our new (+)-prunustatin A campaign began with a repetition of the synthesis of *β*-aldehydo ester **16**,[9] which was typically prepared in 67% yield from **17** by pyridinium chlorochromate (PCC) oxidation in CH_2Cl_2 (Scheme 2). Aldehyde **16** was then

Scheme 2. Our Synthesis of Acid 9



condensed with the anion derived from **15** to give (*E*)-alkene **14** in 64% yield with total stereocontrol. While initially we accessed **19** in near optically pure condition via Sharpless AD with AD-mix-*β*,[7a] we found this process to be inconveniently slow, needing 7 days to reach completion. We therefore evaluated less hindered Sharpless ligands for this purpose. A considerable improvement in the reaction rate was found when the AD was conducted with catalytic potassium osmate (1 mol %) and the DHQD-MEQ ligand[7b] (13 mol %), which afforded the lactonized product **19** in 95% yield with 100% ee after only 30 h of stirring at 0 °C, which represented a considerable

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operational improvement and was also much cheaper to carry out.

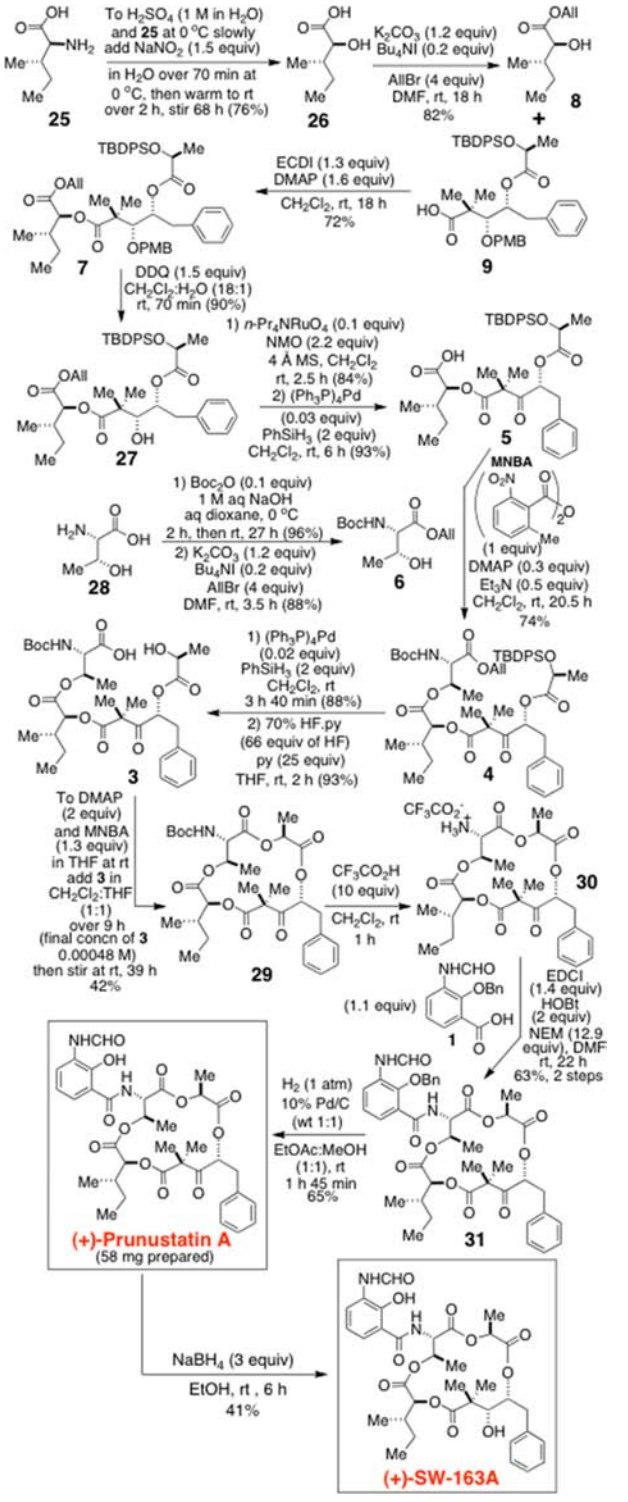
The alcohol in **19** was then protected as an OPMB ether with NaH and PMBCl in DMF, and the product, lactone **13**, was reduced with $LiAlH_4$ to obtain diol **20** in 85% yield. The less hindered primary hydroxyl of **20** was next regioselectively protected as an *O*-triethylsilyl (OTES) ether to allow the all-important ester bond to be grafted onto O(2). For this, (*S*)-lactic acid derivative **21** was first converted into the Yamaguchi[10] 2,4,6-trichlorobenzoic acid mixed anhydride **22**, and this was reacted with **12** in C_6H_6 at rt for 17 h in the presence of 4-(dimethylamino)pyridine (DMAP) (3.25 equiv). This proved to be the optimal method for esterifying this system, furnishing **10** in 93% yield. Having reliably fulfilled its alcohol-differentiating role, the primary OTES ether was selectively cleaved from **10** by catalytic pyridinium *p*-toluenesulfonate (PPTS) (0.1 equiv) in MeOH over 45 min at rt. A two-stage oxidation thereafter converted alcohol **23** into carboxylic acid **9**. In this sequence, a Ley−Griffith catalytic *n*-Pr_4NRuO_4/*N*-methylmorpholine *N*-oxide (NMO) oxidation[11] first furnished aldehyde **24** in near quantitative yield, and a Pinnick oxidation subsequently provided **9** in 89% yield.[12]

We next focused our attention on converting L-isoleucine (**25**) into L-isoleucic acid (**26**) and the latter into *O*-allyl ester **8** (Scheme 3).[13] To access the former, we followed the diazotization procedure of Plenkiewicz and Poterala,[13a] which worked very well in our hands, and generated the HNO_2 in situ from 1 M aqueous H_2SO_4 and $NaNO_2$. It delivered the crystalline acid **26** in 76% yield on a large scale from **25** (53 g). Chemoselective O-allylation[13b] was next achieved with K_2CO_3/allyl bromide/Bu_4NI in DMF at rt; the product ester **8** was isolated in 82% yield. It was then coupled to acid **9** using excess *N*-(3-(dimethylamino)propyl)-*N*-ethyl carbodiimide hydrochloride (EDCI) and DMAP as the acid activators; triester **7** was formed in 72% yield after 18 h of stirring at rt in CH_2Cl_2.

DDQ was now used to chemoselectively remove the PMB group from O(1) without disturbing the potentially sensitive *O*-allyl ester. The resulting alcohol **27** was oxidized to the ketone with *n*-Pr_4NRuO_4/NMO in MeCN, and the *O*-allyl ester was detached with $PhSiH_3$ and Pd(0). The acid **5** so produced was then coupled to partially protected L-threonine derivative **6** using 2-methyl-6-nitrobenzoic anhydride (MNBA)[14] and DMAP in CH_2Cl_2, affording the desired product **4** in 74% yield. Subsequently, **4** was O-deallylated with $PhSiH_3$ and catalytic $Pd(PPh_3)_4$ in CH_2Cl_2,[15] and the product acid was O-desilylated with HF·pyridine complex in a mixture of pyridine/THF. Both deprotections proceeded cleanly to provide the required *seco*-acid **3** in 82% yield over the two steps. The latter was then macrolactonized on a 0.3 g scale under high-dilution conditions by addition of a solution of **3** in CH_2Cl_2/THF (1:1) over 9 h to a solution of DMAP (2 equiv) and MNBA[14] (1.3 equiv) in dry THF at rt, attaining a final reaction concentration of ca. 0.00048 M with respect to **3**. The reactants were then allowed to stir at rt for 39 h to bring about the desired ring closure. Macrolide **29** was isolated pure in 42% yield after SiO_2 flash chromatography. The structure of **29** was unambiguously confirmed by single-crystal X-ray analysis (see the Supporting Information). Importantly, the 400 MHz ^1H NMR spectrum of **29** in $CDCl_3$ matched that of Kawanishi.[3]

Although we did attempt to repeat the 50 °C macrolactonization protocol of Yamakoshi and Kawanishi[3,16] on **3** at the reaction concentration of 0.0012 M that they reported, we found it extremely difficult to control the rate of the addition of the *seco*-acid solution to the MNBA/DMAP solution, when either a syringe pump or slow cannulation was used to deliver the THF/CH_2Cl_2 solution of **3**. Not only did the hot vapor from the reaction mixture consistently oppose a carefully controlled slow addition of the solution of **3** into the reaction flask, but also, the heating process caused much more variable reaction outcomes, with the attendant formation of more complex mixtures. Our very best yield of **29** from adhering to the 50 °C cyclization

Scheme 3. Completion of Our Total Synthesis of (+)-Prunustatin A and the Immunosuppressant SW-163A



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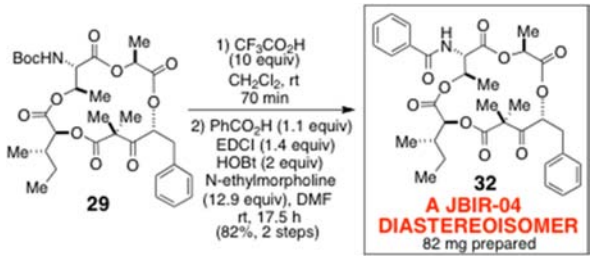
protocol in ref 3 was 49%, *but this was not the norm*. Because of the significant technical difficulties and reaction variations that attend this method,[3] we recommend that other workers use *the much more consistent ultrahigh-dilution rt cyclization procedure* that we have described in the Supporting Information. However, even under our rt conditions, intermolecular dimerization of **3** still continues to be significant, but generally less so than under the 50 °C reaction conditions.[3,16]

In order to complete our synthesis of (+)-prunustatin A, the Boc group of **29** was detached with neat CF_3CO_2H in CH_2Cl_2, and the crude TFA salt **30** was coupled with **1**[3,4,18] using EDCI, *N*-ethylmorpholine (NEM), and 1-hydroxybenzotriazole (HOBt). The desired product **31** was isolated in 63% yield after SiO_2 flash chromatography; it was identical to the same compound prepared by Usuki.[4] Compound **31** was then deprotected by catalytic hydrogenation with 10% Pd/C in EtOAc/MeOH (1:1) at 1 atm; synthetic (+)-prunustatin A was isolated in 65% yield after SiO_2 chromatography (0.74% overall). Its spectroscopic values closely matched those reported by Shin-ya,[1,2] Kawanishi,[3] and Usuki,[4] thus confirming that the natural product had indeed been synthesized. $NaBH_4$ reduction of (+)-prunustatin A in EtOH also furnished the immunosuppressant (+)-SW-163A,[6] in accord with Shin-ya's 2007 limited report.[2]

Given that we had unambiguously proven the stereochemistry of **29**, we next deprotected its Boc group and coupled **30** to $PhCO_2H$ in order to secure what we hoped was going to be the structurally related natural product JBIR-04[17] (Scheme 4),

Scheme 4. Our Attempted Synthesis of JBIR-04



whose absolute stereostructure has not been assigned to date. Unfortunately, our spectroscopic comparisons of **32** with JBIR-04 soon confirmed that JBIR-04 has different absolute stereochemistry than (+)-prunustatin A, which perhaps explains why its GRP78-downregulatory effects are 200 times lower.

In conclusion, we have devised unified, highly stereoselective total syntheses of (+)-prunustatin A, SW-163A, and JBIR-04 diastereoisomer **32**.[18] The latter synthesis also revealed that the absolute stereochemistry of JBIR-04 differs from that found in (+)-prunustatin A. We expect that our new synthetic pathway to these molecules will prove useful for fashioning analogues, including biotinylated ones, which would have potential value for new drug target retrieval by affinity chromatography.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.6b01235.

Full experimental procedures for all steps, copies of the IR, HMRS, and $^1H/^{13}C$ NMR spectra of every intermediate, and X-ray plots and crystallographic data for **19**, **13**, and **29** (including CCDC accession numbers) (PDF)

SQUEEZE-processed crystallographic data for **29** (CIF)

Original crystallographic data for **29** (CIF)

Crystallographic data for **13** (CIF)

Crystallographic data for **19** (CIF)

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Notes

The authors declare no competing financial interest.

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