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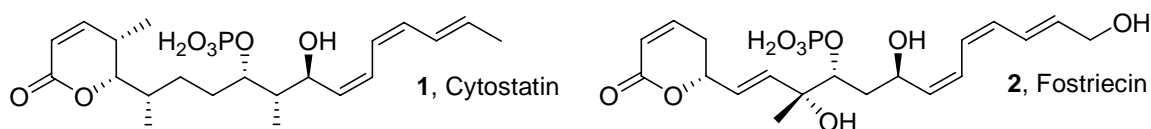
## TOTAL SYNTHESIS OF CYTOSTATIN<sup>†</sup>

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**Abstract** – The total synthesis of cytostatin, an antitumor agent belonging to the fostriecin family of natural products, is disclosed. The convergent route features a key epoxide opening reaction to join the two stereotriad units and a single-step late stage, stereoselective installation of the sensitive triene through a  $\beta$ -chelation controlled nucleophilic addition. The synthesis provides rapid access to the C4–C6 and C10–C11 stereoisomers of cytostatin and additional analogues to define the substituent role in PP2A binding.

Cytostatin (**1**),<sup>1</sup> an antitumor agent isolated from *Streptomyces sp.* MJ654-Nf4 belonging to the fostriecin family of natural products,<sup>2</sup> displays potent cytotoxic activity, induces apoptosis,<sup>3</sup> and inhibits lung tumor metastasis.<sup>4</sup> Like the parent of its class, fostriecin (**2**), **1** was disclosed without a definition of its relative or absolute stereochemistry and is a potent and selective inhibitor of protein phosphatases 2A and 4 (PP2A IC<sub>50</sub> = 210 nM, PP2A/PP1 >1000).<sup>5</sup>

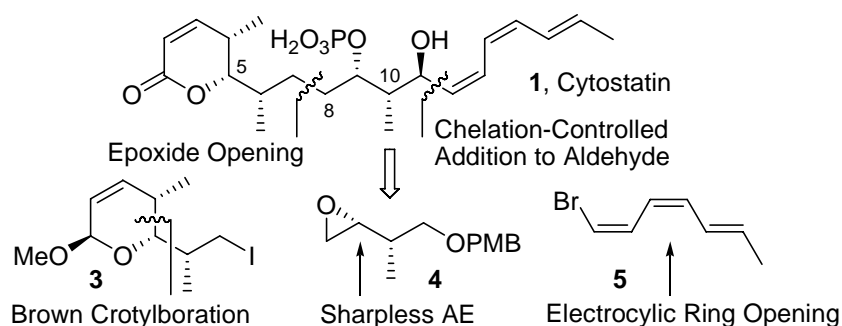


In preceding efforts, we determined the stereochemical configuration of fostriecin (**2**), reported its first total synthesis, and prepared analogues that were used to demonstrate the importance of the phosphate monoester and define the role of the unsaturated lactone for selective PP2A inhibition.<sup>6</sup> In these studies, we provided evidence that the unsaturated lactone serves as a critical electrophile that reacts with C269 of PP2A, and this has now been confirmed in biochemical studies of PP2A inhibition by the related natural product phoslactomycin.<sup>7</sup> In 2002, Waldmann reported the total synthesis of cytostatin (**1**) and several

<sup>†</sup> We would like to dedicate this work to Professor S. M. Weinreb, a superb scientist and wonderful friend, on the occasion of his 65<sup>th</sup> birthday.

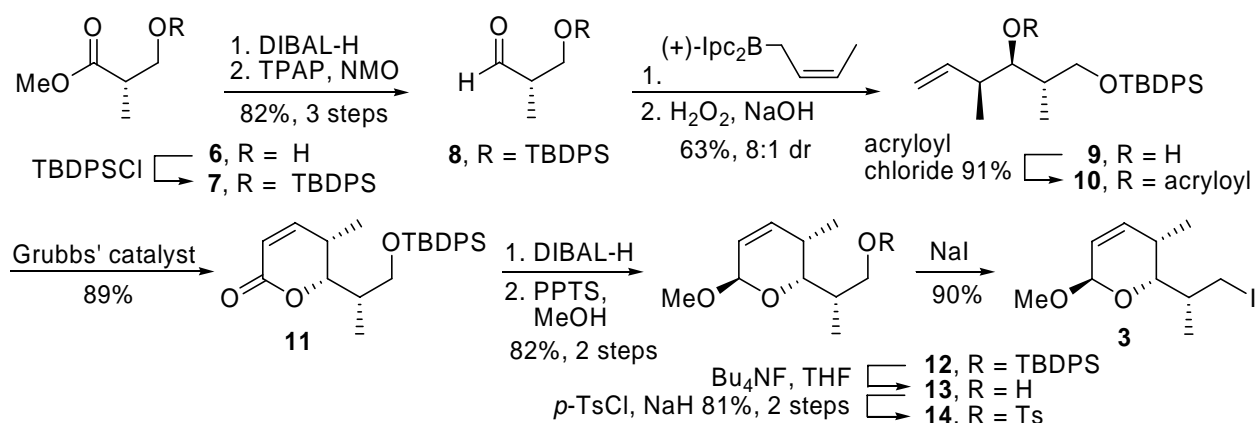
analogues,<sup>8</sup> and Marshall has since disclosed an alternative route to an advanced intermediate of the Waldmann synthesis.<sup>9</sup> Herein, we disclose a complementary total synthesis of **1** that further confirms its stereochemical assignment and will facilitate further delineation of the cytostatin–PP2A interaction.

The route was designed to provide rapid access to the C10–C11 stereoisomers of **1** to define the role of the C11-hydroxy group in PP2A binding, and to employ intermediates that could be used to decipher the C4–C6 stereochemistry of **1** en route to the natural product. Key features of the convergent approach include installation of the triene unit in a single step by addition of **5** to a C11 aldehyde, enlisting substrate control to set the C11 stereochemistry, and assembly of the C7–C8 bond by coupling a cuprate derived from iodide (**3**) with epoxide (**4**). This bond construction was chosen to isolate the two stereotriads allowing for independent adjustment of their stereochemistry. Brown crotylboration was used to install the C4 and C5 stereochemistry while a Sharpless epoxidation served to set the C9 and C10 stereochemistry. The triene was synthesized in a short, stereospecific approach relying on an electrocyclic ring opening to set the geometry of the three olefins (Figure 1).



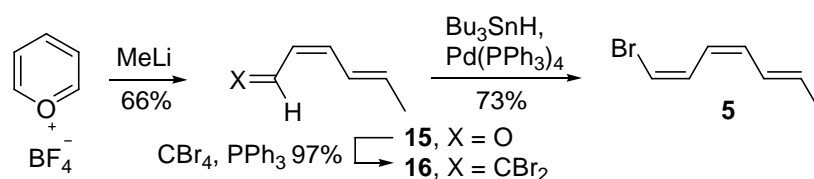
**Figure 1** Synthetic Plan for Cytostatin.

Synthesis of iodide (**3**) (Scheme 1) began with silylation (TBDPSCl, imidazole, DMF, 25 °C, 2 h, 99%) of methyl (*S*)-3-hydroxy-2-methylpropionate followed by conversion of the ester to the corresponding aldehyde (**8**) (DIBAL-H, toluene, –78 °C, 1 h; TPAP, NMO, CH<sub>2</sub>Cl<sub>2</sub>, 0 °C, 30 min, 82%, 2 steps).<sup>10</sup> Crotylation of **8** (*cis*-2-butenyldiisopinocampheylborane, THF, ether, –78 °C, 12 h; NaOH, H<sub>2</sub>O<sub>2</sub>, 70 °C, 5 h, 63%, 8:1 dr)<sup>11</sup> gave alcohol (**9**) which was converted to the  $\alpha,\beta$ -unsaturated lactone (**11**) via acylation (acryloyl chloride, *i*-Pr<sub>2</sub>NEt, CH<sub>2</sub>Cl<sub>2</sub>, 0 °C, 2 h, 91%) and subsequent ring closing metathesis (Grubbs' I catalyst, CH<sub>2</sub>Cl<sub>2</sub>, 40 °C, 12 h, 89%).<sup>12</sup> Notably, this sequence allows access to all possible stereoisomers of **11** by treating (*R*)- or (*S*)-**8** with the appropriate crotylboration reagent.<sup>13</sup> At this stage, the lactone was masked by reduction (DIBAL-H, CH<sub>2</sub>Cl<sub>2</sub>, –78 °C, 30 min) and conversion of the intermediate lactol into methyl acetal (**12**) (PPTS, MeOH, 25 °C, 10 min, 82%, 2 steps).<sup>14</sup> The silyl ether was converted to iodide (**3**) through desilylation (Bu<sub>4</sub>NF, THF, 25 °C, 12 h), tosylation of the resulting alcohol (*p*-TsCl, NaH, benzene, 25 °C, 4 h, 81%, 2 steps), and iodine displacement (NaI, acetone, 56 °C, 12 h, 90%).



**Scheme 1** Synthesis of **3**.

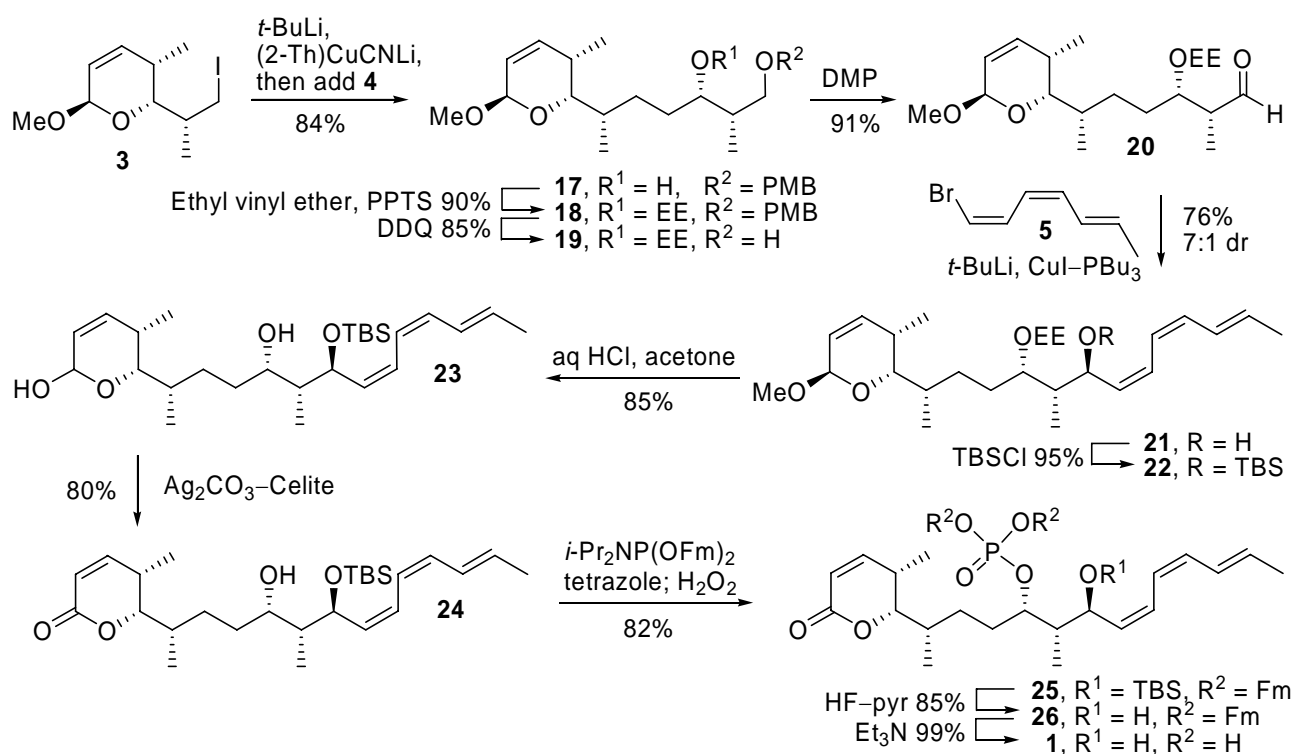
In a modification of Taylor's synthesis of *Z,E*-dienals,<sup>15</sup> addition of MeLi (THF,  $-78\text{ }^{\circ}\text{C}$ , 2 h) to pyrylium tetrafluoroborate followed by electrocyclic ring opening provided **15** in 66% as a single isomer (Scheme 2). Transformation of **15** to dibromoolefin (**16**) ( $\text{CBr}_4$ ,  $\text{PPh}_3$ ,  $\text{Et}_3\text{N}$ ,  $\text{CH}_2\text{Cl}_2$ ,  $0\text{ }^{\circ}\text{C}$ , 10 min, 97%)<sup>16</sup> and selective (*E*)-bromide reduction ( $\text{Bu}_3\text{SnH}$ ,  $\text{Pd}(\text{PPh}_3)_4$ , ether,  $0\text{ }^{\circ}\text{C}$ , 15 min, 73%)<sup>17</sup> gave **5** stereoselectively.



**Scheme 2** Synthesis of **5**.

Assembly of **1** (Scheme 3) was initiated by converting iodide (**3**) into the corresponding cuprate (*t*-BuLi, ether,  $-78\text{ }^{\circ}\text{C}$ , 5 min; then (2-Th)CuCNLi, THF,  $-78$  to  $0\text{ }^{\circ}\text{C}$ , 5 min)<sup>18</sup> and adding epoxide (**4**)<sup>19</sup> at  $0\text{ }^{\circ}\text{C}$  to give **17** in 84%. The coupling of **3** and **4** was slow when alternative metalated forms of **3** were used (i.e.  $\text{R}_2\text{CuLi}$ ,  $\text{RMgI}/\text{cat.}$   $\text{CuI}$ ,  $\text{R}_2\text{CuCNLi}_2$ ,  $\text{RLi}/\text{BF}_3$ ), and the success of the transformation was dependent on the use of the higher order cuprate at  $0\text{ }^{\circ}\text{C}$  and at concentrations of 50 mM or greater. Through a sequence of acetal formation (ethyl vinyl ether, PPTS,  $\text{CH}_2\text{Cl}_2$ ,  $25\text{ }^{\circ}\text{C}$ , 30 min, 90%), PMB removal (DDQ,  $\text{CH}_2\text{Cl}_2$ ,  $\text{H}_2\text{O}$ ,  $25\text{ }^{\circ}\text{C}$ , 30 min, 85%), and oxidation (DMP,  $\text{CH}_2\text{Cl}_2$ ,  $25\text{ }^{\circ}\text{C}$ , 15 min, 91%),<sup>20</sup> **17** was converted to aldehyde (**20**), setting the stage for installation of the triene. Following Still's precedent,<sup>21</sup> conversion of **5** to its  $\text{Bu}_3\text{P}$ -stabilized cuprate (*t*-BuLi, ether,  $-78\text{ }^{\circ}\text{C}$ , 1.5 h;  $\text{CuI}-\text{PBu}_3$ , 10 min) prior to addition of **20** ( $-78\text{ }^{\circ}\text{C}$ , 30 min, 76%) produced **21** as a pair of chromatographically-separable diastereomers in a ratio of 7:1 favoring the desired 10,11-*anti* product. Following silylation (TBSCl, imidazole, DMF,  $25\text{ }^{\circ}\text{C}$ , 2 h, 95%) of the resulting secondary alcohol, treatment of **22** with dilute HCl for short reaction times (0.02 N HCl, acetone, water,  $25\text{ }^{\circ}\text{C}$ , 10 min, 85%) induced simultaneous C1 and C9 acetal hydrolysis without affecting the silyl ether. In this context, the use of the C9 EE protecting group

proved valuable as it served to direct the formation of the C11 stereocenter (through chelation) and could be effectively removed in the presence of the labile silyl ether and conjugated triene. Selective oxidation ( $\text{Ag}_2\text{CO}_3$ -Celite, benzene, 80 °C, 2 h, 80%) of lactol (**23**) produced **24**, which was phosphorylated ( $i\text{-Pr}_2\text{NP}(\text{OFm})_2$ , tetrazole,  $\text{CH}_2\text{Cl}_2$ ,  $\text{CH}_3\text{CN}$ , 25 °C, 15 min;  $\text{H}_2\text{O}_2$ , 10 min, 82%) using the protocol introduced by Waldmann<sup>8</sup> to give **25**. Desilylation ( $\text{HF}$ -pyr, THF, pyridine, 25 °C, 4 h, 85%) followed by fluorenylmethyl removal ( $\text{Et}_3\text{N}$ ,  $\text{CH}_3\text{CN}$ , 25 °C, 17 h, 99%) provided **1**, identical to a sample of natural cytostatin ( $^1\text{H}$  NMR, TLC, HPLC, HRMS).<sup>22</sup>



**Scheme 3** Synthesis of Cytostatin.

A convergent route to **1** that features a key epoxide opening reaction to join the two stereotriad units and a single-step late stage, stereoselective installation of the sensitive triene through a  $\beta$ -chelation controlled nucleophilic addition is disclosed. The synthesis provides rapid access (11 steps from convergence point) to the C10–C11 diastereomers which have been prepared to probe the PP2A interaction and used to unequivocally establish the relative and absolute stereochemistry of cytostatin. These and related studies will be disclosed in due time.

## ACKNOWLEDGEMENTS

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