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SYNTHESIS AND BIOLOGICAL ACTIVITIES ON BATZELLADINE DERIVATIVES†

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Abstract – Structure-activity relationship studies on batzelladines A and D were examined. Seven batzelladine derivatives, including 24-*epi*-batzelladine A (**3**) and 7-*epi*-batzelladine D (**4**), were synthesized. The inhibitory activity of these derivatives on binding of gp120 with CD4 was evaluated by the use of ELISA-based assay method. For the potent biological activities of batzelladines, tricyclic guanidine moiety, a common structure of batzelladine A and D, and side chain bearing guanidine functional group were found to be mandatory.

Batzelladines A-I are members of a unique polycyclic guanidine alkaloids which were isolated from Bahamian and Jamaican sponges by a SmithKline Beecham group in 1995 and 1997.[1,2] These alkaloids were reported to control protein-protein interactions, i.e., batzelladines A and B inhibit the interaction between HIV gp120 and human CD4,[1] while batzelladines F and G induce dissociation of the complex between a tyrosine kinase $p56^{lck}$ and CD4.[2] Since protein-protein interactions play important roles in all aspects of molecular cell biology, elucidation of the inhibition/dissociation mechanisms controlled by those guanidine alkaloids is of great interest. Over the past decade, considerable efforts have been devoted to the synthesis of batzelladines.[3,4] We recently accomplished the synthesis of (+)-batzelladine

A (**1**)[5a] and (-)-batzelladine D (**2**)[5b] based upon a successive nitrone 1,3-dipolar cycloaddition strategy. We also elucidated their target protein to be CD4.[6] For further elucidation of the mechanism of their inhibitory activity of the protein-protein interaction by these alkaloids, molecular probes related to batzelladines are helpful, and therefore, structure-activity relationships (SAR) studies of batzelladines[7] are required for the design of those probes. In this communication, we describe the SAR studies based on batzelladine A (**1**) and D (**2**).

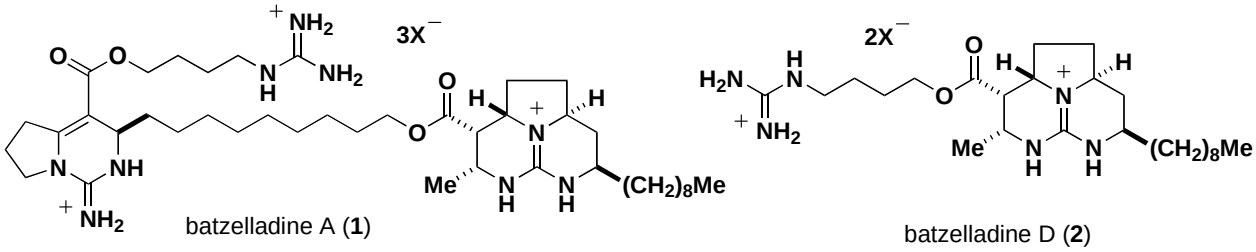
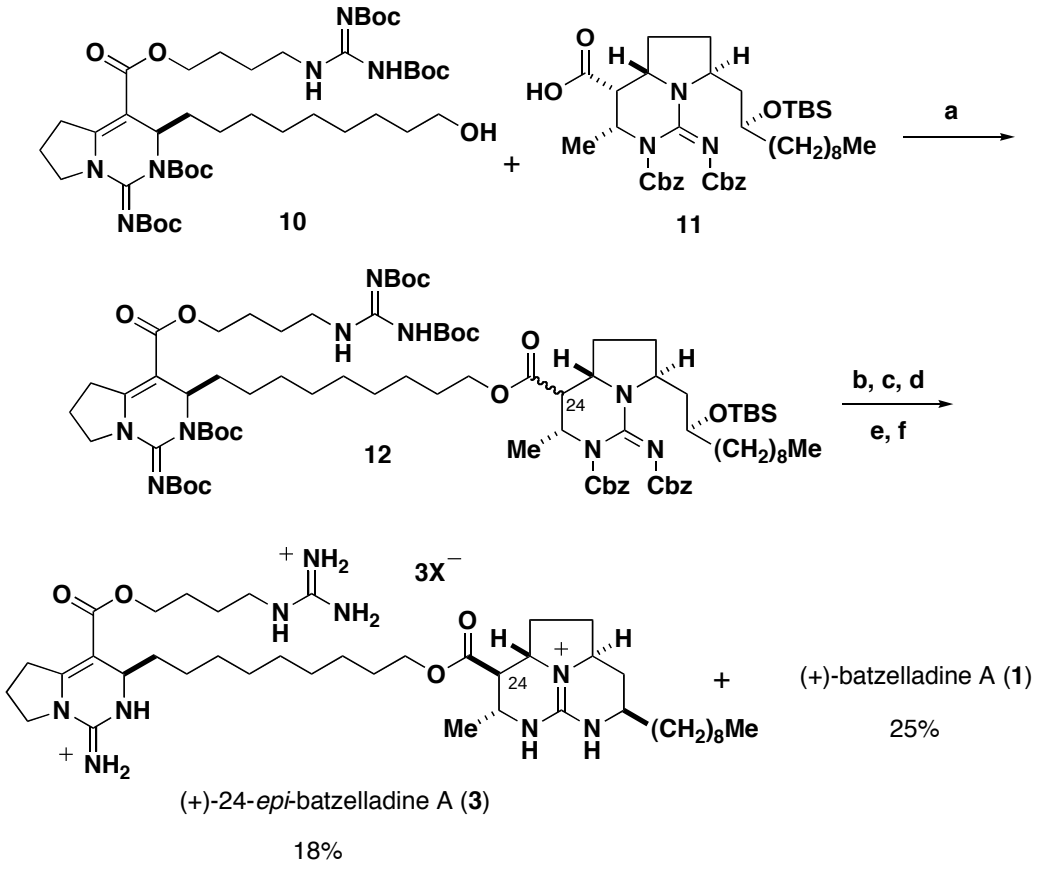
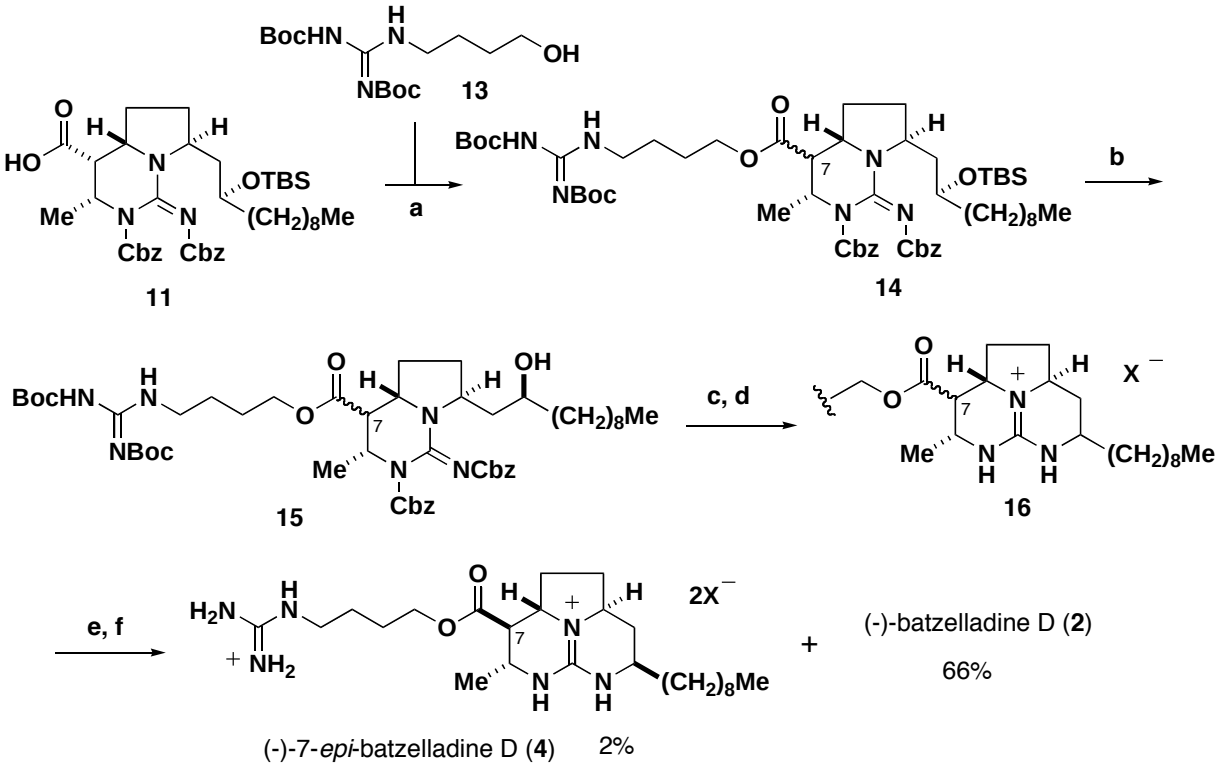


Figure 1. Structures of batzelladine A (**1**) and batzelladine D (**2**)

For the SAR studies, we planned the synthesis of seven batzelladine derivatives **3**~**9**. First, (+)-24-*epi*-batzelladine A (**3**) and (-)-7-*epi*-batzelladine D (**4**) were obtained through esterification of guanidine alcohols **10** and **13** with carboxylic acid **11** as the side product during the natural product synthesis (Scheme 1 and 2).



Scheme 1. Synthesis of (+)-24-*epi*-batzelladine A (**3**). Reagents and conditions; (a) EDCI, DMAP, rt, 39%; (b) HF-Py, THF, 0 °C, 71%; (c) Pd-C, H_2, AcOEt, rt; (d) DEAD, PPh_3, toluene, rt, 62% (2 steps); (e) TFA, CH_2Cl_2, rt; (f) HPLC separation.

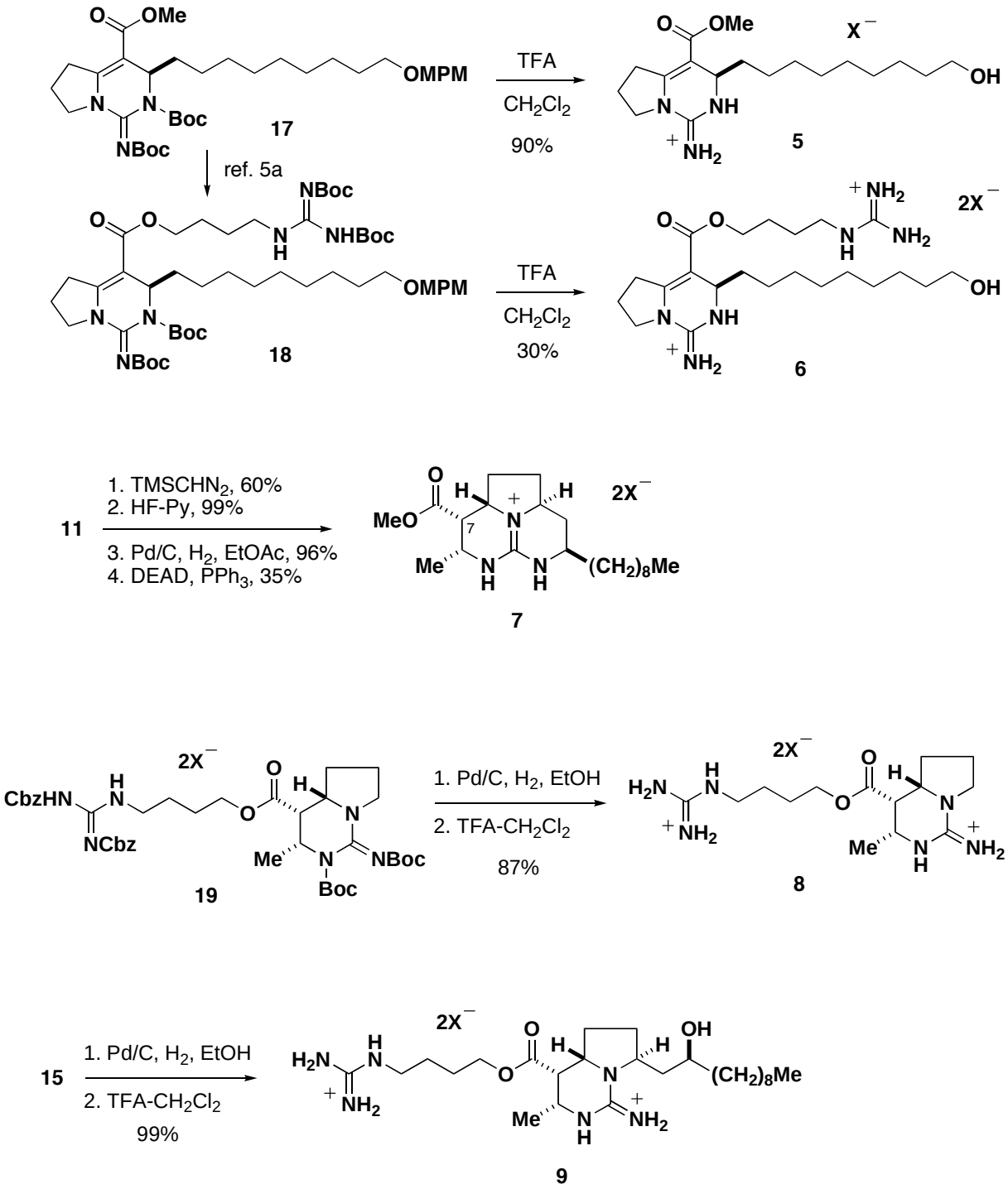


Scheme 2. Synthesis of (-)-7-*epi*-batzelladine D (**4**). Reagents and conditions; (a) EDCI, DMAP, rt, 68%; (b) HF-Py, THF, 0 °C, 80%; (c) Pd-C, H_2, AcOEt, rt; (d) DEAD, PPh_3, toluene, rt; (e) TFA, CH_2Cl_2, rt; (f) HPLC separation.

Thus, esterification of carboxylic acid **11**[5a] with the side chain bicyclic guanidine alcohol **10**[5a] was conducted in the presence of EDCI and DMAP at room temperature, and the ester **12** was obtained in 39% yield as a 1:1 stereoisomer mixture at C-24.[5a] After deprotection of the TBS and Cbz groups of **12** with HF/pyridine and hydrogen in the presence of 10% Pd-C, respectively, the tricyclic guanidine was formed under the Mitsunobu reaction conditions. Finally, deprotection of Boc groups was perforemed with trifluoroacetic acid to give (+)-24-*epi*-batzelladine A (**3**) together with (+)-batzelladine A (**1**). These mixtures were separated by HPLC to give **1** and **3** in 25% and 18% yield, respectively. (-)-7-*epi*-Batzelladine D (**4**) was also synthesized in a similar manner to that for **3** (Scheme 2).

The synthesis of batzelladine derivatives **5**~**9** was shown in Scheme 3. Alcohols **5** and **6**, which correspond to the bicyclic guanidine moiety of batzelladine A (**1**), were obtained from **17**[5a] and **18**[5a] by deprotection of Boc groups and MPM ether with TFA. A tricyclic guanidine ester **7**, a common

structure of batzelladine A (**1**) and batzelladine D (**2**), was synthesized from carboxylic acid **11** by a reaction with trimethylsilyldiazomethane followed by tricyclic guanidine construction under the Mitsunobu reaction conditions. Bicyclic guanidines **8** and **9** bearing a linear guanidine side chain were prepared from **19**[5b] and **15**, which are synthetic intermediates of batzelladine D (**2**), by sequential deprotection of Cbz and Boc groups with hydrogen in the presence of 10% Pd-C and TFA, respectively.



Scheme 3. Synthesis of batzelladine derivatives **5**~**9**.

With the compounds in hand, we examined the inhibitory activity of these derivatives against the binding of gp120 with CD4 by ELISA (Table 1).[8] CD4 and batzelladine A (**1**), D (**2**), and their derivatives **3**~**9** were added to gp120 immobilized on ELISA plates, and the bound CD4 was quantified with the anti-CD4 antibody. Batzelladine A (**1**), its stereoisomer **3**, batzelladine D (**2**), and its stereoisomer **4** showed inhibitory activities with IC_{50} values of 8, 7, 24, and 29 μM, respectively. On the other hand, bicyclic guanidine compounds, **5**, **6**, **8**, and **9**, and the tricyclic guanidine ester **7** were inactive at the concentration of 100 μM in this assay. These results suggest that both the tricyclic guanidine structure and the bicyclic guanidine or the linear guanidine moiety should be presented in the same molecule for the inhibitory activity of gp120-CD4 interaction.

Table 1 Inhibition of gp120 binding to CD4 using ELISA-based assay

Compound	IC_{50} value (μM)
(+)-baztelladine A (**1**)	8
(-)-batzelladine D (**2**)	24
(+)-24-*epi*-batzelladine A (**3**)	7
(-)-7-*epi*-batzelladine D (**4**)	29
5	>100
6	>100
7	>100
8	>100
9	>100

In summary, SAR studies on batzelladine derivatives were examined. Several cyclic guanidines **3**~**9**, i.e., bicyclic guanidines in batzelladine A, a tricyclic guanidine ester, and bicyclic guanidines in batzelladine D, were synthesized. The inhibitory activity of these compounds against the gp120-CD4 interaction was evaluated by ELISA, and it was found that the tricyclic guanidine moiety and the side chain having a guanidine functional group are mandatory for the inhibitory activity. Design and synthesis of probe molecules derived from batzelladines based on these SAR studies for elucidation of the mechanism of their activity against the protein-protein interaction is in progress.

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