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Stereoselective Synthesis and Osteogenic Activity of Subglutinols A and B

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Immunosuppressive drugs are used to prevent rejection of transplanted organs and treat autoimmune diseases, including rheumatoid arthritis, multiple sclerosis, and insulin-dependent type-1 diabetes.[1] While clinically approved immunosuppressive drugs (e.g., cyclosporin A, FK506) are anabolic on bone at low concentrations, as they increase osteoblast differentiation and bone mass,[2] they elicit an opposite catabolic response at clinically relevant higher concentrations, causing undesirable side effects on bone structure (dose-dependent biphasic effects), including osteopenia, osteoporosis, and increased incidence of bone fractures.[3] As a result, considerable effort has been devoted to the identification of immunosuppressive drugs that lack the undesirable biphasic effects and promote bone formation in a dose-dependent manner. Such drugs with dose-dependent osteogenic activity might help reduce bone-associated side effects and be clinically useful for bone tissue transplantation. Herein, we report the stereoselective synthesis of subglutinols A (**1a**) and B (**1b**) and present initial biological data showing the significant potential of **1a** as an immunosuppressive drug with dose-dependent osteogenic activity. We also show that activating protein 1 (AP-1) family transcription factors could be one of the key regulators of the anabolic activity of **1a**.

Compounds **1a** and **1b** (Figure 1) are diterpene pyrones isolated from the endophytic fungus *Fusarium subglutinans*.[4] The structures and relative stereochemistry of natural **1a** and **1b** were determined by extensive NMR spectroscopic and X-ray diffraction analysis, but the absolute stereochemistries were not established. There have been reports on structurally related and biologically interesting diterpene pyrones.[5−7] Compounds **1a** and **1b** are equipotent in the mixed lymphocyte reaction (MLR) and thymocyte proliferation (TP) assays (IC_{50} 0.1 μM).[4] Because of the lack of toxicity, **1a** and **1b** have been hypothesized to be promising new immunosuppressive drugs.

Figure 1 summarizes our approach for the stereoselective synthesis of **1a** and **1b** from the common intermediate **7**. The strategy underlying our synthetic plan for **1a** was to apply $BF_3 \cdot OEt_2$-promoted deoxygenation of the cyclic hemiketal **5** followed by stereoselective reduction of the oxocarbenium ion intermediate to afford **4a**. For the synthesis of **1b**, a novel tandem procedure of cross-metathesis (CM) of **7** with allyl chloride followed by intramolecular S_N2' cyclization of the hydroxyalkene **6** would stereoselectively provide the key intermediate **4b** in the synthesis of **1b**. Completion of the synthesis of **1a** and **1b** would be accomplished by Cu(I)-catalyzed intermolecular S_N2' reaction of

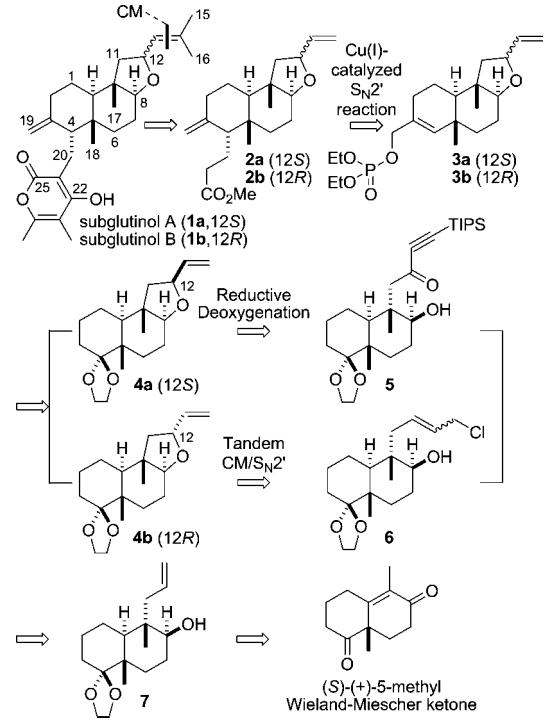

Figure 1. Retrosynthesis of subglutinols A (**1a**) and B (**1b**).

the phosphates **3a** and **3b** with a propionate moiety and subsequent aldol reaction.

As Scheme 1 shows, the synthesis of the key intermediate **4b** leading to **1b** began with CM[8] of **7**,[9] the common intermediate readily prepared from the enantiomerically pure (*S*)-(+)-5-methyl-Wieland−Miescher ketone.[10] Treatment of **7** with allyl chloride in the presence of Grubbs' second-generation catalyst and subsequent intramolecular S_N2' reaction[11] of the corresponding hydroxyalkene **6** (tandem CM/S_N2' reaction) provided **4b** as a single diastereomer. The stereochemical outcome observed in the tandem reaction can be rationalized on the basis that the unfavorable 1,3-diaxial interaction of the C12 allyl substituent and the C17 methyl group in conformation **6A** is larger than that of the hydrogen and the methyl group in conformation **6B**, thus preferentially affording the 2,3-*trans*-2,5-*trans*-tetrahydrofuran **4b**.[12] The configuration of the newly formed C12 stereocenter of **4b** was confirmed by single-crystal X-ray diffraction analysis. To the best of our knowledge, the tandem CM/S_N2' reaction has never been reported for stereoselective synthesis of tetrahydrofurans and other heterocycles.[13] In fact, few approaches for the stereoselective synthesis of tetrahydrofurans and tetrahydropyrans involve intramolecular S_N2' reac-

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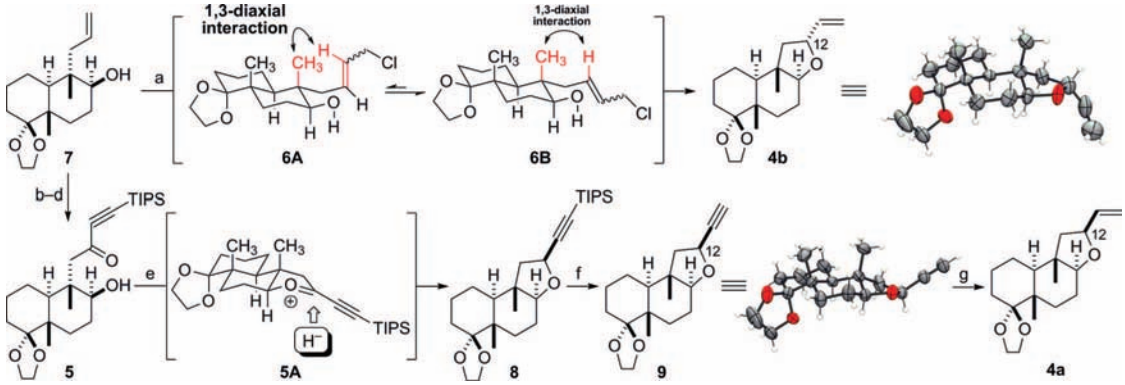
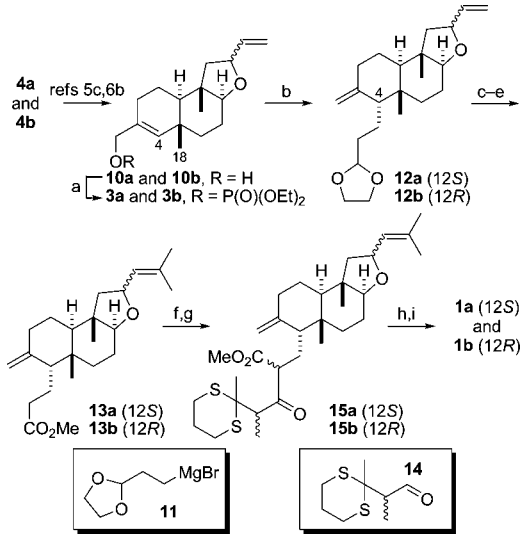
Scheme 1. Stereoselective Synthesis of Tetrahydrofurans[a]



[a] Reagents and conditions: (a) allyl chloride, Grubbs' second-generation catalyst (20 mol %), CH_2Cl_2, reflux, 30 h, 53% (76% BRSM); (b) O_3, EtOAc, −78 °C, 5 min, then Ph_3P, 25 °C, 6 h, 100%; (c) (*i*-Pr_3Si)−C≡C−Li, THF, −78 to 0 °C, 5 h, 89%; (d) MnO_2, CH_2Cl_2, 25 °C, 1 h, 95%; (e) BF_3·OEt_2, Et_3SiH, CH_2Cl_2, −78 to −20 °C, 2 h, 91%; (f) TBAF, THF, 25 °C, 1 h, 97%; (g) H_2, Lindlar's catalyst (10 wt %), 30:1 EtOAc/pyridine, 25 °C, 3 h, 99%.

Scheme 2. Completion of the Synthesis of Subglutinols A and B[a]



[a] Reagents and conditions: (a) $ClP(O)(OEt)_2$, Et_3N, DMAP, CH_2Cl_2, 0 to 25 °C, 30 min, **3a**: 92%, **3b**: 97%; (b) **11**, CuI·2LiCl, Et_2O/THF, 25 °C, 10 min, then phosphate **3**, 25 °C, 30 min, **3a**: 64%, **3b**: 80%; (c) Jones' reagent, acetone, 25 °C, 30 min; (d) MeI, K_2CO_3, DMF, 25 °C, 2 h, then NaOMe, MeOH, 25 °C, 1 h, **3a**: 34% (44% BRSM) for two steps, **3b**: 45% (57% BRSM) for two steps; (e) 2-methylpropene, Grubbs' second-generation catalyst, CH_2Cl_2, 50 °C, 72 h, **3a**: 91%, **3b**: 85%; (f) LDA, THF, −78 °C, 30 min, then **14**, −78 °C, 30 min, **3a**: 74%, **3b**: 91%; (g) Dess−Martin periodinane, $NaHCO_3$, CH_2Cl_2, 25 °C, 30 min, **3a**: 72%, **3b**: 92%; (h) MeI, $CaCO_3$, 3:1 CH_3CN/H_2O, 25 °C, 24 h; (i) DBU, benzene, reflux, 1 h, **3a**: 53% for two steps, **3b**: 54% for two steps.

tions, perhaps because of the low nucleophilicity of oxygen and a less well-defined transition state.[11c]

After synthesizing **4b** leading to **1b**, we turned our attention to the stereoselective synthesis of the 2,3-*trans*-2,5-*cis*-tetrahydrofuran **4a**, the key intermediate leading to **1a**. Our preliminary study showed that addition of a variety of nucleophiles to oxocarbenium ion intermediates derived from γ-lactol derivatives could be employed in the stereoselective synthesis of tetrahydrofurans.[14] After an extensive search for a surrogate for the vinyl group,[15] ozonolysis of **7**, addition of (*i*-Pr_3Si)−C≡C−Li, and MnO_2 oxidation proceeded to give γ-hydroxyketone **5** (Scheme 1). We anticipated that BF_3·OEt_2-promoted deoxygenation of **5** followed by reduction of the corresponding oxocarbenium ion intermediate **5A** with a reducing agent would stereoselectively provide the 2,3-*trans*-2,5-*cis*-tetrahydrofuran **8** via addition of the hydride from the direction opposite to the C17 methyl group.[12] As expected, the reaction conditions for reductive deoxygenation afforded **8** as a single diastereomer. Deprotection of the TIPS group of **8** by treatment with TBAF followed by partial reduction of the alkyne **9** with Lindlar's catalyst gave the key intermediate **4a**.

Compounds **4a** and **4b** were converted to the appropriately functionalized alcohols **10a** and **10b** following the procedures established by Danishefsky[5c] and Katoh[6b] (see the Supporting Information). With the appropriately functionalized alcohols **10a** and **10b** in hand, we turned our attention to the installation of the α-pyrone moiety (Scheme 2). Unfortunately, there are few successful examples of direct functionalization at the sterically congested neopentyl C4 position of decalins. Danishefsky[5c] and Katoh[6b] utilized sigmatropic rearrangement reactions to install precursors to α- or γ-pyrone. To establish a more straightforward and efficient method for the installation of α-pyrone, we extensively investigated the scope of regio- and stereoselective intermolecular S_N2' reaction of a propionate moiety. With respect to the regioselectivity, we expected that the propionate group should be added from the α-face opposite to the axially oriented C18 methyl group, which is analogous to the intramolecular sigmatropic rearrangement reactions reported by Danishefsky and Katoh. However, intermolecular S_N2' alkylation at the sterically hindered neopentyl C4 position was expected to be more challenging. After an extensive search of reaction conditions, we were delighted to find that conversion of **10a** and **10b** to the phosphates **3a** and **3b** followed by Cu(I)-catalyzed intermolecular S_N2' addition of **11** to **3a** and **3b** in the presence of CuI·2LiCl provided **12a** and **12b**, respectively, as single diastereomers with good regioselectivity (S_N2'/S_N2 = 5:1).[16]

Oxidation of **12a** and **12b**, methyl ester formation, and CM with 2-methylpropene proceeded smoothly to provide **13a** and **13b** (Scheme 2). Aldol reaction of **13a** and **13b** with **14**[17] followed by Dess−Martin oxidation set the stage for the final cyclization to the α-pyrone. Deprotection of the 1,3-dithiane and subsequent DBU-mediated cyclization to the α-pyrone completed the syntheses of **1a** and **1b**, which proved identical in all respects to the authentic natural products.[4] The optical rotations of our synthetic **1a** and **1b** were nearly identical to those of natural **1a** and **1b**, indicating that natural **1a** and **1b** possess 12*S* and 12*R* absolute stereochemistries, respectively.

Upon completion of the syntheses of **1a** and **1b**, we evaluated their immunosuppressive and osteogenic activities. The MLR assay revealed that **1a** exhibited a potent level of immunosuppressive activity (IC_{50} = 25 nM), as described in the original report.[18]

The effect of **1a** on the bone morphogenetic protein 2 (BMP-2)-induced commitment of murine pluripotent mesenchymal precur-

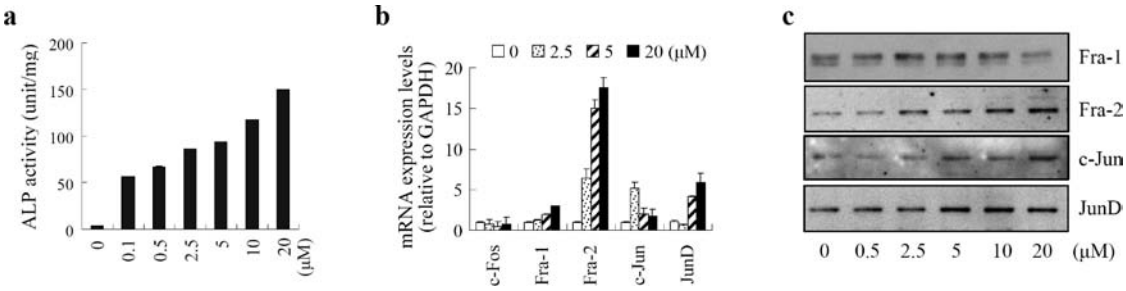


Figure 2. Effect of **1a** on (a) ALP protein level/activity in C2C12 cells and (b) mRNA and (c) protein expression level of AP-1 family transcription factors.

sor C2C12 cells into osteoblasts was determined by the expression level of alkaline phosphatase (ALP), an early phase marker of osteoblast differentiation.[19] Up to the highest concentration examined (20 μM), **1a** increased the expression/activity of ALP in a dose-dependent manner (Figure 2a). The dose-dependent induction of ALP expression/activity by **1a** shows the great clinical potential of **1a** as an immunosuppressive drug without undesirable side effects on bone structure.

Since the expression of Fra-2, an AP-1 family transcription factor, has been shown to be critical in osteoblast differentiation induced by cyclosporin A,[20] we examined the effect of **1a** on mRNA expression of Fra-2 and other AP-1 family transcription factors. As Figure 2b shows, **1a** dramatically induced the expression of Fra-1, Fra-2, c-Jun, and Jun D at a transcript level. In addition, Western blot analysis showed that the protein expression level of Fra-1, Fra-2, c-Jun, and Jun D in the nucleus was increased by **1a** (Figure 2c). These data imply that AP-1 family transcription factors could be one of the key regulators of the anabolic activity of **1a**.

In summary, we completed the stereoselective synthesis of **1a** and **1b** from the readily available common intermediate **7**. We applied an unprecedented tandem CM/intramolecular S_N2' reaction of **7** to the synthesis of **4b**. $BF_3 \cdot OEt_2$-promoted deoxygenation of the cyclic hemiketal **5** followed by stereoselective reduction of the oxocarbenium ion intermediate was explored for the synthesis of **4a**. In addition, we demonstrated that Cu(I)-catalyzed intermolecular S_N2' addition of **11** is a straightforward and efficient method for the synthesis of the α-pyrone moiety. The absolute stereochemistries of natural **1a** and **1b** were assigned as 12*S* and 12*R*, respectively. These synthetic strategies provide access to more potent analogues and tools for the study of their molecular mechanism of action. We also demonstrated the synergistic effect of **1a** with BMP-2 on the commitment of C2C12 cells into osteoblasts, suggesting that **1a** increases signal transduction of BMP-2 in mesenchymal stem cells. Further study would be required to evaluate the *in vivo* bone-forming efficacy of **1a** in order to show the possibility that the combination of **1a** with BMPs can be used in autogenous bone graft materials.

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Supporting Information Available: General experimental procedures, including spectroscopic and analytical data for **1**−**5**, **8**−**10**, **12**, **13**, and **15** along with copies of ^1H and ^{13}C NMR spectra; detailed assay procedures; and CIF files for **4b** and **9**. This material is available free of charge via the Internet at http://pubs.acs.org.

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(15) Addition of CH_2═$CHMgBr$, MnO_2 oxidation, and reductive deoxygenation ($BF_3 \cdot OEt_2$, $NaBH_3CN$, −78 °C, 10 min) of the corresponding γ-hydroxyketone gave the 2-ethyltetrahydrofuran as a single diastereomer instead of the desired 2-ethenyltetrahydrofuran through 1,4-addition of a hydride to the oxocarbenium intermediate followed by 1,2-addition of a hydride.
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