

The first total synthesis of lamellarin α 20-sulfate, a selective inhibitor of HIV-1 integrase

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Abstract- The first total synthesis of lamellarin α 20-sulfate (**1**), a selective inhibitor of HIV-1 integrase, has been completed. The lamellarin α core in which 13-OH and 20-OH were differentially protected by isopropyl and benzyl groups, respectively, was constructed by using Hinsberg-type pyrrole synthesis and Suzuki-Miyaura coupling as the key reactions. The 20-sulfate was prepared by a sequence including debenylation of 20-OBn, 2,2,2-trichloroethylsulfation of the resulting 20-OH, deprotection of 13-Oi-Pr, and final reductive cleavage of the 2,2,2-trichloroethyl ester.

Keywords: HIV-1 integrase inhibitor; lamellarin; sulfate; Hinsberg reaction; Suzuki-Miyaura coupling

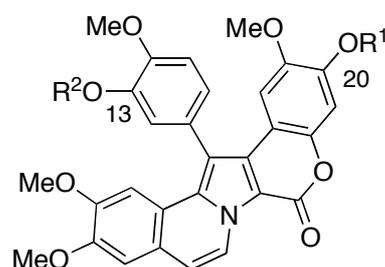
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Human immunodeficiency virus (HIV) encodes three enzymes, namely, reverse transcriptase, protease, and integrase. Anti-HIV drugs targeting the first two enzymes have been successfully employed for treatment of acquired immune deficiency syndrome (AIDS). Integrase is another attractive and safe target against HIV because it is essential for HIV replication and, unlike reverse transcriptase and protease, there is no similar enzyme in the host cell.¹ Unfortunately, however, no clinically useful integrase inhibitors have been developed so far.

Lamellarins are polycyclic marine alkaloids having a unique

14-phenyl-6*H*-[1]benzopyrano[4',3':4,5]pyrano[2,1-*a*]isoquinolin-6-one ring-system.² So far, over 30 lamellarins have been isolated from mollusks, tunicates, and sponges. These alkaloids have received considerable attention as new leads for anticancer agents.³ In 1999, Faulkner and coworkers discovered a series of lamellarin alkaloids exhibit selective inhibition of HIV-1 integrase.⁴ Within the alkaloids tested, lamellarin α 20-sulfate (**1**) displayed the most favorable therapeutic index. The sulfate **1** inhibited the integrase terminal cleavage activity with an IC₅₀ of 16 μ M, the strand transfer activity with an IC₅₀ of 22 μ M, and growth of the HIV-1 virus in cell culture with an IC₅₀ of 8 μ M. The MTT assay of **1** toward Hela cells displayed the least toxicity with an LD₅₀ of 274 μ M. Protection of the phenolic hydroxyl group as the sulfate could reduce the cytotoxicity of the parental lamellarins in general.

A synthetic approach to lamellarin α 20-sulfate (**1**) was reported by Faulkner and coworkers in 2002.⁵ They prepared lamellarin α (**2**) using an intramolecular 1,3-dipolar cycloaddition strategy developed by Banwell.⁶ An attempt to synthesize **1** by titration of **2** with a conventional DMF-SO₃ complex failed and afforded only lamellarin α 13,20-disulfate (**3**) in low yield. Recently, Taylor developed a reliable method to produce aryl sulfates *via* mixed aryl 2,2,2-trichloroethyl sulfate intermediates.⁷ In this Letter, we report the first total synthesis of lamellarin α 20-sulfate (**1**) using Taylor's protocol for the final steps of sulfate formation.⁸

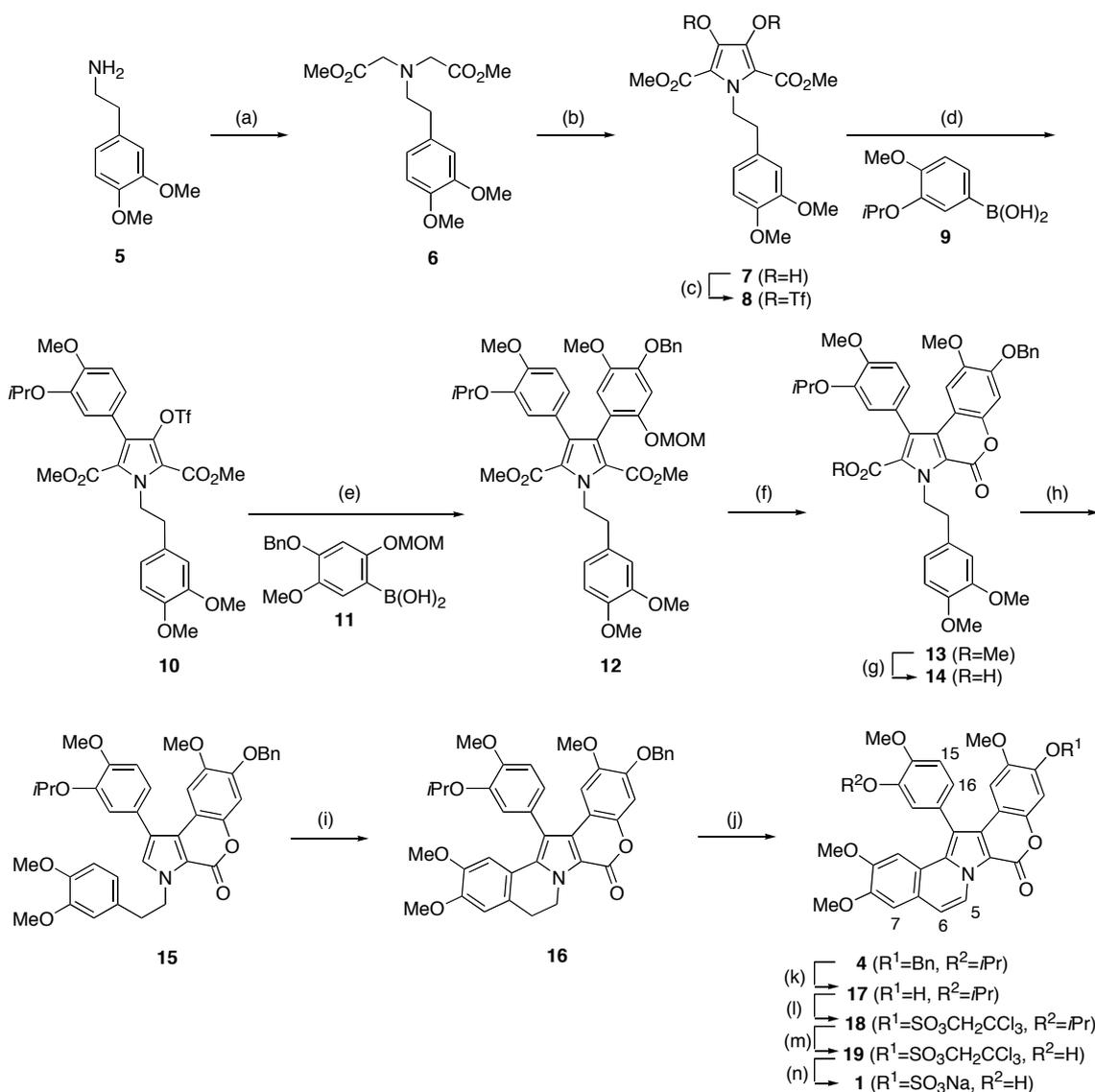


lamellarin α 20-sulfate (**1**) (R¹=SO₃Na, R²=H)
 lamellarin α (**2**) (R¹=R²=H)
 lamellarin α 13,20-disulfate (**3**) (R¹=R²=SO₃Na)
 lamellarin α core (**4**) (R¹=Bn, R²=*i*-Pr)

The pivotal lamellarin α core **4** in which 13-OH and 20-OH are differentially protected for the selective introduction of a sulfate group was constructed by a strategy developed

in our laboratories.^{9, 10} This includes Hinsberg-type pyrrole synthesis¹¹ and palladium-catalyzed Suzuki-Miyaura coupling¹² of the 3,4-dihydroxypyrrole bistriflate as the key reactions. The total synthesis of lamellarin α 20-sulfate (**1**) based upon this strategy is shown in the Scheme.

Alkylation of the commercially available 2-(3,4-dimethoxyphenyl)ethylamine (**5**) with 2.2 equiv. of methyl bromoacetate gave the iminodiacetate **6** in 91% yield. Hinsberg reaction of **6** with dimethyl oxalate under the conventional NaOMe/MeOH conditions^{9, 11} provided 3,4-dihydroxypyrrole **7** in only 49% yield. However, the yield was greatly improved to 85% by carrying out the reaction in dry THF using sodium hydride as a base.¹⁰ Reaction of **7** with 2.0 equiv. of trifluoromethanesulfonic anhydride in pyridine gave the corresponding bistriflate derivative **8** in good yield. The bistriflate **8** was coupled with 1.0 equiv. of the boronic acid **9**¹⁰ in the presence of 2 mol% of Pd(PPh₃)₄ and aqueous Na₂CO₃ in refluxing THF to give the mono-arylated pyrrole **10** in 80% yield. The second cross-coupling of this product with 2.0 equiv. of **11**¹³ using 8 mol% of Pd(PPh₃)₄ produced 3,4-disubstituted pyrrole **12** in 90% yield. Deprotection of the MOM group of **12** with HCl in methanol caused concomitant lactonization to give **13** in 93% yield. Alkaline hydrolysis of **13** followed by treatment with *p*-TsOH in refluxing dichloromethane gave the acid **14** in 77% yield. Decarboxylation of this compound in hot quinoline in the presence of Cu₂O provided **15** in 96 % yield.¹⁴ Intramolecular oxidative biaryl coupling of **15** under Kita's conditions¹⁵ [phenyliodine bis(trifluoroacetate) (PIFA)/BF₃·Et₂O] proceeded cleanly to produce the cyclized compound **16** in 95% yield. Dehydrogenation of this compound with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) gave 20-benzyl-13-isopropylamellarin α (**4**) in 99% yield. Deprotection of the benzyl group by hydrogenolysis over palladium on charcoal afforded **17**, which was reacted with trichloroethyl chlorosulfate in pyridine to give the mixed sulfate **18** in 96% yield.⁶ Selective removal of the isopropyl protecting group of **18** with boron trichloride¹⁶ proceeded cleanly without affecting the trichloroethylsulfate moiety to give **19** in 96% yield. Final reductive deprotection of the trichloroethyl ester with Zn/HCO₂NH₄ followed by ion exchange over Amberlite IRC-50 (Na⁺ form) and Sephadex purification produced lamellarin α 20-sulfate (**1**) in 80% yield.



Scheme. Total synthesis of lamellarin α 20-sulfate (**1**). *Reagents and conditions:* (a) $\text{BrCH}_2\text{CO}_2\text{Me}$ (2.2 equiv.), NaHCO_3 , CH_3CN , reflux, 2.5 h (91%); (b) $(\text{CO}_2\text{Me})_2$ (2.0 equiv.), NaH (4.0 equiv.), THF, reflux, 4.5 h (85%); (c) $(\text{CF}_3\text{SO}_2)_2\text{O}$ (2.2 equiv.), pyridine, 0 °C, 2 h (92%); (d) **9** (1.0 equiv.), $\text{Pd}(\text{PPh}_3)_4$ (2 mol %), aq. Na_2CO_3 , THF, reflux, 5 h (80%); (e) **11** (2.0 equiv.), $\text{Pd}(\text{PPh}_3)_4$ (8 mol %), aq. Na_2CO_3 , THF, reflux, 20 h (90%); (f) conc. HCl, MeOH, reflux, 2 h (93%); (g) (1) 40% aq. KOH-EtOH (1:1), reflux, 3 h, (2) cat. *p*-TsOH, CH_2Cl_2 , reflux, 1 h (77%); (h) Cu_2O , quinoline, 220 °C, 10 min (96%); (i) $\text{PhI}(\text{OCOCF}_3)_2$ (1.2 equiv.), $\text{BF}_3\cdot\text{OEt}_2$ (2.4 equiv.), CH_2Cl_2 , -40 °C, 1.5 h (95%); (j) DDQ (1.0 equiv.), CH_2Cl_2 , reflux, 30 h (99%); (k) H_2 , 10% Pd-C (20 wt %), AcOEt, r.t., 2 h (99%); (l) $\text{ClSO}_3\text{CH}_2\text{CCl}_3$ (2.0 equiv.), DMAP (1.0 equiv.), Et_3N (2.0 equiv.), THF, r.t., 4 h (96%); (m) BCl_3 (3.0 equiv.), CH_2Cl_2 , -78 °C, 0.5 h, then 0 °C, 4 h (96%); (n) (1) Zn powder (2 equiv.), HCO_2NH_4 (6 equiv.), THF-MeOH (1:1), 4 h, (2) Amberlite IRC-50 (Na^+ form), MeOH, (3) Sephadex LH-20, MeOH- CH_2Cl_2 (1:1) (80%).

The spectroscopic data of the synthetic **1**¹⁷ were shown to be identical with those reported for the natural product.⁴ It is noteworthy that the ¹H NMR absorptions of aromatic (H-5, 6, 7, 15, 16) and hydroxylic protons of **1** shift considerably depending on the concentration of the samples.¹⁷ The ¹H NMR data of the synthetic **1** obtained at the low concentration (1.0 mg of **1** in 0.7 mL of DMSO-*d*₆) were found to be identical with those reported for the natural product.

In conclusion, we have achieved the first total synthesis of lamellarin α 20-sulfate (**1**) in 14 steps from the commercially available 2-(3,4-dimethoxyphenyl)ethylamine (**4**) in excellent overall yield (24%). This synthesis opens the way to produce diverse sulfated lamellarins, which enable us to undertake the structure-activity relationship studies on integrase-inhibiting and anti-HIV activities. Studies along this line are in progress in our laboratories.

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 17. Lamellarin α 20-sulfate (**1**). Mp 263-269 °C (dec.) (sealed capillary) (lit.⁴ mp 145-148 °C); IR (KBr): 3448, 1695, 1487, 1419, 1272, 1223, 1167, 1048, 839 cm⁻¹; ¹H NMR (400 MHz, 17 mg of **1** in 0.7 mL of DMSO-*d*₆): δ 3.37 (s, 3H), 3.37 (s, 3H), 3.86 (s, 3H), 3.87 (s, 3H), 6.80 (s, 1H), 6.94 (dd, *J*= 2.0 and 8.0 Hz, 1H), 7.05 (d, *J*= 2.0 Hz, 1H), 7.18 (s, 1H), 7.19 (d, *J*= 8.0 Hz, 1H), 7.29 (d, *J*= 7.4 Hz, 1H), 7.38 (s, 1H), 7.57 (s, 1H), 8.48 (br s, 1H), 9.03 (d, *J*= 7.4 Hz, 1H); ¹H NMR (400 MHz, 1.0 mg of **1** in 0.7 mL of DMSO-*d*₆): δ 3.38 (s, 3H), 3.38 (s, 3H), 3.87 (s, 3H), 3.89 (s, 3H), 6.81 (s, 1H), 7.03 (d, *J*= 2.0 Hz, 1H), 7.04 (dd, *J*= 2.0 and 8.0 Hz, 1H),

7.21 (s, 1H), 7.26 (d, $J= 8.0$ Hz, 1H), 7.35 (d, $J= 7.4$ Hz, 1H), 7.44 (s, 1H), 7.56 (s, 1H), 9.09 (d, $J= 7.4$ Hz, 1H), 9.45 (br s, 1H); ^{13}C NMR (100 MHz, 17 mg of **1** in 0.7 mL of $\text{DMSO-}d_6$): δ 54.36, 54.96, 55.48, 55.97, 104.63, 105.64, 106.80, 108.00, 108.66, 111.13, 111.40, 112.70, 113.42, 118.00, 118.05, 121.36, 121.88, 124.09, 126.83, 127.80, 133.26, 143.00, 144.93, 146.48, 147.87, 147.99, 148.71, 149.71, 153.96. HRFABMS (positive ion mode) m/z . Calcd for $\text{C}_{29}\text{H}_{22}\text{NNa}_2\text{O}_{11}\text{S}$ $[(\text{M}+\text{Na})^+]$: 638.0709. Found: 638.0710. HRFABMS (negative ion mode) m/z . Calcd for $\text{C}_{29}\text{H}_{22}\text{NO}_{11}\text{S}$ $[(\text{M}-\text{Na})^-]$: 592.0914. Found: 592.0913.

Graphical abstract

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