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

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Abstract - The first total synthesis of lamellarin $\alpha$ 20-sulfate (1), a selective inhibitor of HIV-1 integrase, has been completed. The lamellarin $\alpha$ 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 debenzylation 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. Unfortunately, however, no clinically useful integrase inhibitors have been developed so far.

Lamellarins are polycyclic marine alkaloids having a unique
14-phenyl-6H-[1]benzopyran[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 \(\alpha\) 20-sulfate (1) displayed the most favorable therapeutic index. The sulfate 1 inhibited the integrase terminal cleavage activity with an IC\(_{50}\) of 16 \(\mu M\), the strand transfer activity with an IC\(_{50}\) of 22 \(\mu M\), and growth of the HIV-1 virus in cell culture with an IC\(_{50}\) of 8 \(\mu M\). The MTT assay of 1 toward Hela cells displayed the least toxicity with an LD\(_{50}\) of 274 \(\mu M\). Protection of the phenolic hydroxyl group as the sulfate could reduce the cytotoxicity of the parental lamellarins in general.

A synthetic approach to lamellarin \(\alpha\) 20-sulfate (1) was reported by Faulkner and coworkers in 2002.\(^5\) They prepared lamellarin \(\alpha\) (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-SO\(_3\) complex failed and afforded only lamellarin \(\alpha\) 13,20-disulfate (3) in low yield. Recently, Taylor developed a reliable method to produce aryl sulfates \textit{via} mixed aryl 2,2,2-trichloroethyl sulfate intermediates.\(^7\) In this Letter, we report the first total synthesis of lamellarin \(\alpha\) 20-sulfate (1) using Taylor’s protocol for the final steps of sulfate formation.\(^8\)

The pivotal lamellarin \(\alpha\) 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. This includes Hinsberg-type pyrrole synthesis and palladium-catalyzed Suzuki-Miyaura coupling 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 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. 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 in the presence of 2 mol% of Pd(PPh$_3$)$_4$ and aqueous Na$_2$CO$_3$ 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 using 8 mol% of Pd(PPh$_3$)$_4$ 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 Cu$_2$O provided 15 in 96 % yield. Intramolecular oxidative biaryl coupling of 15 under Kita’s conditions [phenyliodine bis(trifluoroacetate) (PIFA)/BF$_3$Et$_2$O] 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-isopropyllamellarin 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. Selective removal of the isopropyl protecting group of 18 with boron trichloride proceeded cleanly without affecting the trichloroethylsulfate moiety to give 19 in 96% yield. Final reductive deprotection of the trichloroethyl ester with Zn/HCO$_2$NH$_4$ 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) BrCH₂CO₂Me (2.2 equiv.), NaHCO₃, CH₃CN, reflux, 2.5 h (91%); (b) (CO₂Me)₂ (2.0 equiv.), NaH (4.0 equiv.), THF, reflux, 4.5 h (85%); (c) (CF₃SO₂)₂O (2.2 equiv.), pyridine, 0 ºC, 2 h (92%); (d) 9 (1.0 equiv.), Pd(PPh₃)₄ (2 mol %), aq. Na₂CO₃, THF, reflux, 5 h (80%); (e) 11 (2.0 equiv.), Pd(PPh₃)₄ (8 mol %), aq. Na₂CO₃, 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, CH₂Cl₂, reflux, 1 h (77%); (h) Cu₃O, quinoline, 220 ºC, 10 min (96%); (i) Phi(OOCF₃)₂ (1.2 equiv.), BF₃·OEt₂ (2.4 equiv.), CH₂Cl₂, -40 ºC, 1.5 h (95%); (j) DDQ (1.0 equiv.), CH₂Cl₂, reflux, 30 h (99%); (k) H₂, 10% Pd-C (20 wt %), AcOEt, r.t., 2 h (99%); (l) CISO₂CH₂CCl₃ (2.0 equiv.), DMAP (1.0 equiv.), Et₃N (2.0 equiv.), THF, r.t., 4 h (96%); (m) BCl₃ (3.0 equiv.), CH₂Cl₂, -78 ºC, 0.5 h, then 0 ºC, 4 h (96%); (n) (1) Zn powder (2 equiv.), HCO₂NH₂ (6 equiv.), THF-MeOH (1:1), 4 h, (2) Amberlite IRC-50 (Na⁺ form), MeOH, (3) Sephadex LH-20, MeOH-CH₂Cl₂ (1:1) (80%).
The spectroscopic data of the synthetic 117 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-d6) 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.

References

8. During the course of our synthesis of lamellarin α 20-sulfate (1), Fürstner completed the total synthesis of the marine alkaloid dictyodendrin B, in which Taylor’s protocol was successfully used for the final sulfate formation, see: Fürstner, A.; Domostoj, M. M.; Scheiper, B. *J. Am. Chem. Soc.* 2005, 127, 11620-11621.
13. The boronic acid 11 was prepared from O-benzylisovanillin in a similar manner as described for the synthesis of 4-isopropoxy-5-methoxy-2-methoxymethoxyphenylboronic acid. See reference 10.
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-d₆): 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-d₆): 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); $^1$H NMR (100 MHz, 17 mg of 1 in 0.7 mL of DMSO-$d_6$): $\delta$ 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 C$_{29}$H$_{22}$NNa$_2$O$_{11}$S [(M+Na)$^+$]: 638.0709. Found: 638.0710. HRFABMS (negative ion mode) m/z. Calcd for C$_{29}$H$_{22}$NO$_{11}$S [(M–Na)$^-$]: 592.0914. Found: 592.0913.
Graphical abstract

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14 steps
24% overall yield

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