Supporting Information

Fluorinated Betulinic Acid Derivatives and Evaluation of Their Anti-HIV Activities

Jizhen Li [a], Masuo Goto [b], Xiaoming Yang [b], Susan L. Morris-Natschke [b], Li Huang [c,]*, Chin-Ho Chen [c,] *[*], Kuo-Hsiung Lee[b,d,]*

[a] *Department of Organic Chemistry, College of Chemistry, Jilin University, 2519 Jiefang Road, Changchun, 130023, China*

[b] *Natural Products Research Laboratories, UNC Eshelman School of Pharmacy, University of North Carolina, Chapel Hill, North Carolina, 27599, United States*

[c] *Surgical Science, Department of Surgery, Duke University Medical Center, Durham, North Carolina 27710, United States*

[d] *Chinese Medicine Research and Development Center, China Medical University and Hospital, Taichung, Taiwan*

Corresponding author E-mail:khlee@unc.edu

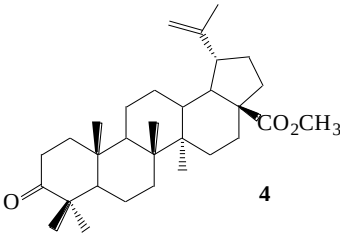
Contents

General methods

All reagents were purchased from commercial sources and used without treatment, unless otherwise indicated. All solvents were dried and newly distilled. The ^{1}H NMR spectra were obtained on a Bruker AVANCE-400 NMR spectrometer recorded at 400 MHz in $CDCl_3$ with TMS as internal standard. All shifts were given in ppm. All coupling constants (*J* values) were reported in Hertz (Hz). Column chromatography was performed on silica gel 100-200 mesh or 200-300 mesh. ESIMS data were measured on an Agilent 1200 series LCMS/MS system.

Synthetic procedures

Synthesis of 4

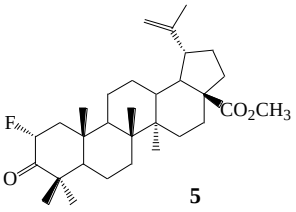


4

To a suspension of betulonic acid (**3**, 1.2 g, 2.64 mmol) and K_2CO_3 (0.73 g, 5.28 mmol) in DMF (10 mL) was added dropwise CH_3I (2.64 mL, 42.24 mmol) at rt. The reaction mixture was heated to 40 °C and stirred for 5 h. Then the reaction mixture was poured into water and extracted with EtOAc. The organic layer was washed with brine, dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. The residue was purified by silica gel column (hexane/EtOAc as eluent) to give product **4** (1.11 g).

White solid, yield 90%. ^{1}H NMR ($CDCl_3$, 400 MHz, ppm) δ: 4.74 (d, J=1.6 Hz, 1H, C=C<u>H</u>, in C29), 4.61 (d, J=1.6 Hz, 1H, C=C<u>H</u>, in C29), 3.67 (s, 3H, $CO_2C\underline{H_3}$), 2.99 (m, 1H, in C19), 2.49-2.39 (2m, 2H, in C2), 1.68, 1.07, 1.02, 0.97, 0.95, 0.92 (6s, 6╳3H, 6╳CH3, in C30, C23, C24, C25, C26, C27). MS (ESI-) m/z: 469.35 $(M+H)^+$ for $C_{31}H_{48}O_3$.

Synthesis of 5

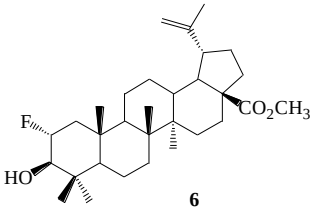


5

[1]Compound **4** (1.01 g, 2.15 mmol) was dissolved in anhydrous THF (10 mL) under nitrogen protection and cooled to -78 °C with a dry ice-acetone solution. LDA [$LiN(i\text{-}Pr)_2$, 1 M/L) (2.15 mL, 2.15 mmol) was added slowly to the stirred reaction solution. After 1 h, NFSI (*N*-fluorobenzene sulfonamide) (0.68 g, 2.15 mmol) dissolved in 2-3 mL THF was added dropwise to the reaction mixture. After 1 h, the reaction was allowed to continue for another 3 h at rt. The solvent was then removed under reduced pressure, and the residue was separated directly by silica gel column chromatography (hexane/EtOAc as eluent) to give product **5** (0.52 g).

White solid, yield 43%. ^1H NMR ($CDCl_3$, 400 MHz, ppm) δ: 5.36, 5.21 (2dd, 1H, J_1=48Hz, J_2=12Hz, J_3=8Hz, C<u>H</u>-F, in C2), 4.74 (s, 1H, C=C<u>H</u>, in C29), 4.61 (s, 1H, C=C<u>H</u>, in C29), 3.67 (s, 3H, $CO_2C\underline{H_3}$), 2.97-3.01 (m, 1H, in C19), 1.68, 1.14, 1.07, 0.97, 0.95, 0.92 (6s, 6╳3H, 6╳CH3, in C30, C23, C24, C25, C26, C27). MS (ESI-) m/z: 487.30 $(M+H)^+$ for $C_{31}H_{47}FO_3$.

Synthesis of 6

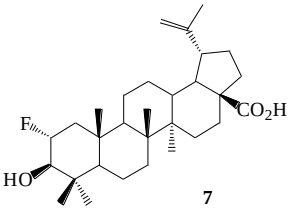


6

Compound **5** (188 mg, 0.39 mmol) was dissolved in EtOH/THF (25 mL, 10mL/15mL) at rt. $NaBH_4$ (150 mg, 3.9 mmol) was added to the solution, which was stirred for 24 h. Then 3 N HCl was added dropwise to a solution pH of 5~7. The organic solvent was removed under reduced pressure. The mixture was extracted with EtOAc, washed with brine, dried over anhydrous Na_2SO_4. After concentration *in vacuo*, the residue was purified by silica gel column chromatography (hexane/EtOAc as eluent) to give product **6** (144 mg).

White solid, yield 81%. ^{1}H NMR ($CDCl_3$, 400 MHz, ppm) δ: 4.73 (s, 1H, C=C<u>H</u>, in C29), 4.61 (s, 1H, C=C<u>H</u>, in C29), 4.62, 4.45 (2m, 1H, J_1=48Hz, C<u>H</u>-F, in C2), 3.67 (s, 3H, $CO_2C\underline{H_3}$), 3.26-3.20 (m, 1H, C<u>H</u>-OH, in C3), 2.96 (m, 1H, in C19), 1.67, 1.04, 0.95, 0.90, 0.88, 0.81 (6s, 6╳3H, 6╳CH3, in C30, C23, C24, C25, C26, C27). MS (ESI-) m/z: 489.40 $(M+H)^+$ for $C_{31}H_{49}FO_3$.

Synthesis of 7

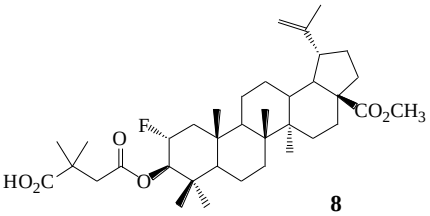


7

Compound **6** (0.12 g, 0.24 mmol) was dissolved in anhydrous DMF (8 mL), and LiI (0.33 g, 2.4 mmol) was slowly added to the solution. The glass bottle containing the reaction mixture was shaken by hand for 2~5 min. Then the reaction mixture was stirred at 140°C for 48 h under argon protection. The reaction mixture was poured into water, extracted with EtOAc, washed with brine, dried over anhydrous Na_2SO_4, and concentrated *in vacuo*. The target product **7** (66 mg) was separated by silica gel column chromatography (hexane/EtOAc as eluent).

White solid, yield 58%. ^{1}H NMR ($CDCl_3$, 400 MHz, ppm) δ: 4.73 (s, 1H, C=C<u>H</u>, in C29), 4.61 (s, 1H, C=C<u>H</u>, in C29), 4.62, 4.49 (2m, 1H, C<u>H</u>-F, in C2), 3.26-3.20 (q, 1H, J_1=12 Hz, J_2=4.4 Hz, C<u>H</u>-OH, in C3), 2.99 (m, 1H, in C19), 1.68, 1.04, 0.97, 0.92, 0.88, 0.80 (6s, 6╳3H, 6╳CH3, in C30, C23, C24, C25, C26, C27). MS (ESI-) m/z: 473.30 $(M-H)^-$ for $C_{30}H_{47}FO_3$.

Synthesis of 8



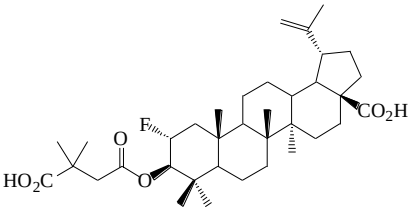
8

Compound **6** (16 mg, 0.032 mmol), 2,2-dimethyl succinic anhydride (41 mg, 0.32 mmol), DMAP (8 mg, 0.064 mmol) and pyridine (1.5 mL) were put into a 10 mL glass tube and sealed. The mixture was stirred at 120 °C for about 12 h. The reaction mixture was then transferred into a 50 mL flask. Pyridine was removed under reduced pressure. 3N HCl (10 mL) was added, the

mixture was extracted three times with EtOAc, and the organic layer washed with 3 N HCl for a second time. Then the organic layer was washed with brine, dried over anhydrous Na_2SO_4, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (hexane/EtOAc as eluent) to provide target product **8** (11 mg).

White solid, yield 54%. ^{1}H NMR ($CDCl_3$, 400 MHz, ppm) δ: 4.76 (d, 1H, J=12Hz, C<u>H</u>-OCO), in C3), 4.73 (s, 1H, C=C<u>H</u>, in C29), 4.60 (s, 1H, C=C<u>H</u>, in C29), 4.67, 4.51 (2m, 1H, C<u>H</u>-F, in C2), 3.66 (s, 3H, $CO_2C\underline{H_3}$), 2.98 (m, 1H, in C19), 2.76-2.62 (2d, J=16 Hz, CO-C<u>H_2</u>-C$(CH_3)_2$), 1.67 (s, CH_3, in C30), 1.32 (s, 6H, $2CH_3$, CO_2H-C(C<u>H_3</u>)<u>$_2$</u>-CH_2-), 0.90, 0.88, 0.86, 0.83, 0.81 (5s, 5╳3H, 5 ╳CH3, in C23, C24, C25, C26, C27). MS (ESI-) m/z: 639.45 $(M+Na)^+$, 655.40 $(M+K)^+$ for $C_{37}H_{57}FO_6$.

Synthesis of 9

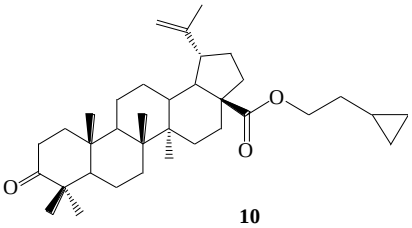


9

Compound **7** (20 mg, 0.0421 mmol) was converted to product **9** (12.5 mg) using the same procedure as for the synthesis of **8** described above.

White solid, yield 50%. ^{1}H NMR ($CDCl_3$, 400 MHz, ppm) δ: 5.30-5.17 (d, 1H, J=8Hz, C<u>H</u>-OCO, in C3), 4.95, 4.81 (2dm, 1H, J=48Hz, C<u>H</u>-F, in C2), 4.74 (s, 1H, C=C<u>H</u>, in C29), 4.62 (s, 1H, C=C<u>H</u>, in C29), 2.98 (m, 1H, in C19), 2.77-2.67 (m, CO-C<u>H_2</u>-C$(CH_3)_2$), 1.70 (s, CH_3, in C30), 1.35, 1.35 (2s, 6H, $2CH_3$, CO_2H-C(C<u>H_3</u>)<u>$_2$</u>-CH_2-), 1.09, 0.93, 0.91, 0.89, 0.88 (5s, 5╳3H, 5╳CH3, in C23, C24, C25, C26, C27). MS (ESI-) m/z: 625.40 $(M+Na)^+$, 641.40 $(M+K)^+$ for $C_{36}H_{55}FO_6$.

Synthesis of 10

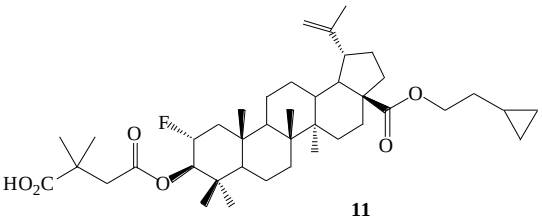


10

To a stirred solution of betulonic acid (**3**, 0.883g, 1.94 mmol) in anhydrous CH_2Cl_2 (10 mL) was added oxylchoride (1.70 mL, 19.45 mmol) at rt and kept for 2 h. The solvent was removed under vaccum, and another 3 mL CH_2Cl_2 was added to the residue and then removed again. To the residue was added anhydrous CH_2Cl_2 (8 mL), 2-cyclopropylethanol (0.5025 g, 5.83 mmol) and triethyl amine (2.71mL, 19.44 mmol), and the mixture was stirred for 24 h at rt. After removal of solvent under vaccumn, the residue was purified by silica gel column chromatography (hexane/EtOAc as eluent) to provide desired compound **10** (760 mg).

White solid, yield 75%. ^{1}H NMR ($CDCl_3$, 400 MHz, ppm) δ: 4.71 (s, 1H, C=C<u>H</u>, in C29), 4.58 (s, 1H, C=C<u>H</u>, in C29), 4.13 (m, 2H, CO_2C**<u>H</u>**$_2$-CH_2), 2.99 (m, 1H, in C19), 2.40-2.34 (2m, 2H, in C2), 1.67 (s, CH_3, in C30), 1.05, 1.00, 0.94, 0.92, 0.90 (5s, 5╳3H, 5╳CH3, in C23, C24, C25, C26, C27), 0.71 (m, 1H, $CO_2CH_2CH_2$-C**<u>H</u>**-), 0.45 (dd, J1=4Hz, J2=8Hz, 2H, $CO_2CH_2CH_2$-CH-C**<u>H</u>**$_2$), 0.08 (dd, J1=4Hz, J2=8Hz, 2H, $CO_2CH_2CH_2$-CH-C**<u>H</u>**$_2$). MS (ESI-) m/z: 523.40 $(M+H)^+$ for $C_{35}H_{54}O_3$.

Synthesis of 11



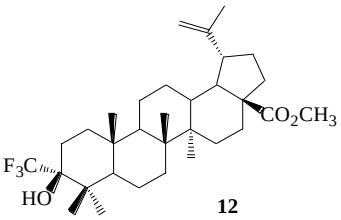
11

The fluorination of **10** (277mg, 0.5302 mmol) was accomplished according to the synthesis of **5** described above, to provide a fluorinated intermediate (158 mg, 55% yield, white solid). The carbonyl group of this fluorinated intermediate (150 mg, 0.278 mmol) was reduced to a hydoxyl group (113 mg, yield 75%, white solid) in same manner as for the synthesis of **6.** The reduced product (20 mg, 0.037 mmol) was esterified as described in the synthesis of **8**, to provide target product **11** (14.3 mg).

White solid, yield 58%. ^{1}H NMR ($CDCl_3$, 400 MHz, ppm) δ: 4.93-4.91, 4.79-4.72 (2dd, 1H, J_1=48Hz, J_2=12Hz, J_3=8Hz, CH-F, in C2), 4.72 (s, 1H, C=C<u>H</u>, in C29), 4.59 (s, 1H, C=C<u>H</u>, in C29), 4.65, 4.52 (2m, 1H, in C3), 4.13 (m, 2H, CO_2C**<u>H</u>**$_2$-CH_2), 3.01 (m, 1H, in C19), 2.76-2.61

(m, 2H, CO-C<u>H</u>$_2$-C(CH$_3$)$_2$), 1.68 (s, CH$_3$, in C30), 1.33, 1.30 (2s, 6H, 2CH$_3$, CO$_2$H-C(C**<u>H</u>$_3$**)$_2$-CH$_2$-), 1.07, 0.98, 0.94, 0.90, 0.84 (5s, 5╳3H, 5╳CH3, in C23, C24, C25, C26, C27), 0.72 (m, 1H, CO$_2$CH$_2$CH$_2$-C**<u>H</u>**-), 0.47 (d, J=8Hz, 2H, CO$_2$CH$_2$CH$_2$-CH-C**<u>H</u>$_2$**), 0.08 (d, J=4Hz, 2H, CO$_2$CH$_2$CH$_2$-CH-C**<u>H</u>$_2$**). MS (ESI-) m/z: 669.90 $(M-H)^-$ for $C_{41}H_{63}FO_6$.

Synthesis of 12

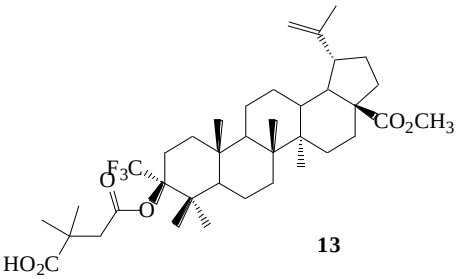


12

$TMSSiCF_3$ (200 mg, 0.43 mmol) was added to the ester 4 (200 mg, 1.40 mmol) in anhydrous THF (8 mL), and the mixture was cooled to 0 ℃ in an ice bath. $Bu_4N^+F^-$ (1M/THF, 0.56 mL, 0.56 mmol) was added slowly to the stirring solution over 20 min, The reaction mixture continued to stir for 24 h at rt. After removing the organic solvent under vaccumn, 1.0 N HCl 30 ml was added to the residue, and 2.0 mL THF was added into the flask again. The mixture was kept for an additional 15 h at rt. After extraction with EtOAc, the solution was washed with brine and dried over anhydrous Na_2SO_4. The desired product (192 mg)was purified by silica gel column chromatography (hexane/EtOAc as eluent).

White solid, yield 83%. ^{1}H NMR ($CDCl_3$, 400 MHz, ppm) δ: 4.72 (s, 1H, C=C<u>H</u>, in C29), 4.59 (s, 1H, C=C<u>H</u>, in C29), 3.66 (s, 3H, CO$_2$C<u>H</u>$_3$), 2.97 (m, 1H, in C19), 2.24-2.19 (m, 2H, in C2), 1.67, 1.02, 0.95, 0.92, 0.90, 0.88 (6s, 6╳3H, 6╳CH3, in C30, C23, C24, C25, C26, C27). MS (ESI-) m/z: 539.35 $(M+H)^+$ for $C_{32}H_{49}F_3O_3$.

Synthesis of 13



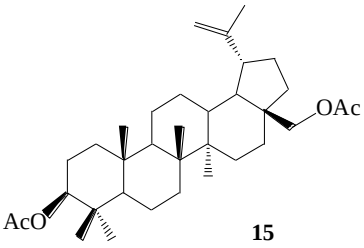
13

The procedure described for the synthesis of **8** was applied to **12** (25 mg, 0.046 mmol) to provide

the desired product **13** (11.7 mg).

White solid, yield 38%. ^{1}H NMR ($CDCl_3$, 400 MHz, ppm) δ: 4.71 (s, 1H, C=C<u>H</u>, in C29), 4.59 (s, 1H, C=C<u>H</u>, in C29), 3.67 (s, 3H, $CO_2C\underline{H_3}$), 2.98 (m, 1H, in C19), 2.76-2.61 (m, 2H, CO-C<u>H$_2$</u>-C(CH_3)$_2$), 2.24-2.20 (m, 2H, in C2), 1.68 (s, 3H, CH_3, in C30), 1.34, 1.31 (2s, 6H, 2CH_3, CO_2H-C(C<u>**H$_3$**)$_2$</u>-CH_2-), 1.04, 0.95, 0.92, 0.90, 0.88 (6s, 5╳3H, 5╳CH3, in C23, C24, C25, C26, C27). MS (ESI-) m/z: 706.35 $(M+K)^+$ for $C_{38}H_{57}F_3O_6$.

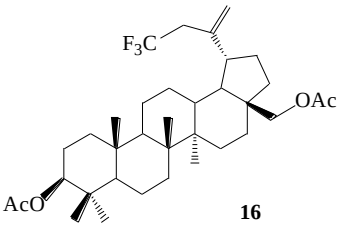
Synthesis of 15



15

Compound **15** was synthesized from **14** according to methods provided in *Journal of Medicinal Chemistry*, **2006**, *49(18)*, 5462-5469.

Synthesis of 16



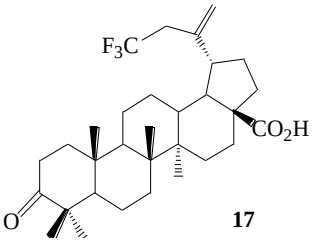
16

[2, 3]Compound **15** (100 mg, 0.19 mmol), CuTc (8 mg, 3.8 mmol) and 2,4,6-collidine (50 mg, 0.38 mmol) were dissolved in 1 mL anhydrous DMAC. The solution was stirred for 10 min. Then (S-trifluoromethyl)dibenzothiophenium triflate (92 mg, 0.23 mmol) was added to the reaction solution. The mixture was stirred at 40 ℃ overnight under argon protection. The reaction mixture was poured into water, extracted with EtOAc, washed with brine, and dried over anhydrous Na_2SO_4. After concentration *in vacuo*, the residue was purified by silica gel column chromatography (hexane/EtOAc as eluent) to provide product **16** (28 mg).

White solid, yield 25%. ^{1}H NMR ($CDCl_3$, 400 MHz, ppm) δ: 5.74, 5.43 (2m, 1H, in C3), 5.07 (s, 1H, C=C<u>H</u>, in C29), 4.99 (s, 1H, C=C<u>H</u>, in C29), 4.45 (m, 2H, C<u>H$_2$</u>OCOCH$_3$), 2.99 (m, 1H, in

C19), 3.21, 3.10 (2m, 1H, in C19), 2.74 (m, 2H, C**H**$_2$-CF_3), 2.48-2.33 (m, 2H, in C2), 1.04, 1.02, 0.95, 0.94 (s, s, ws, s, 3H, 3H, 6H, 3H, 5╳CH3, in C23, C24, C25, C26, C27). MS (ESI-) m/z: 617.35 $(M+Na)^+$ for $C_{35}H_{53}F_3O_4$.

Synthesis of 17



17

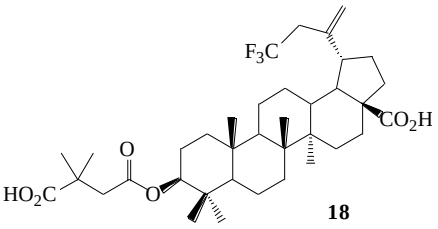
2N NaOH (5 mL) was added to a mixture of compound **16** (0.63 g, 1.1 mmol) dissolved in THF/MeOH (volume 1:2, 10 mL), and the reaction was stirred for 2 days at rt. The organic solvent was removed under reduced pressure, and 2N HCl solution was added to the residue to achieve a pH of 6~7. The solution was extracted with EtOAc. The organic layer was washed with brine, dried over anhydrous Na_2SO_4, and concentrated under vacuum. Te residue was purified by silica gel column chromatograpny (hexane/EtOAc as eluent) to provide a deprotected intermediate (0.49 g, white solid, yield 90%).

Then, to a solution of this intermediate (0.46 g, 0.9 mmol) in acetone (60 mL) was added freshly prepared Jones' reagent (CrO_3 3.34 g, H_2SO_4 2.8 mL, in H_2O until total volume 13 mL) dropwise at 0 ℃. The reaction solution was stirred for another 1.5 h at 0 ℃. The reaction was quenched with MeOH (20 mL) and the mixture stirred mixture for 10 min. H_2O (40 mL) was added to the solution, and the organic solvent was removed under reduced pressure. The aqueous solution was extracted with EtOAc. The organic layer was washed with brine and dried over anhydrous Na_2SO_4. After the solution was filtered and concentrated, the residue was purified by silica gel column chromatography (hexane/EtOAc as eluent) to give the desired compound **17** (0.28 g).

White solid, yield 60%. ^{1}H NMR ($CDCl_3$, 400 MHz, ppm) δ: 5.12 (s, 1H, C=C<u>H</u>, in C29), 5.03 (d, J=1.6 Hz, 1H, C=C<u>H</u>, in C29), 3.05 (m, 1H, in C19), 2.96-2.93, 2.81-2.78 (m, 2H, C**H**$_2$-CF_3), 2.49-2.39 (2m, 2H, in C2), 1.07, 1.02, 0.99, 0.97, 0.92 (5s, 5╳3H, 5╳CH3, in C23, C24, C25,

C26, C27). MS (ESI-) m/z: 521.35 $(M-H)^-$ for $C_{31}H_{45}F_3O_3$.

Synthesis of 18

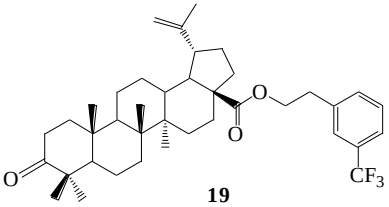


18

The carbonyl group in **17** (35 mg, 0.067 mmol) was reduced to a hydroxyl group with $NaBH_4$ according to the same method described above for the synthesis of **6**. A hydroxy-substituted intermediate was obtained in 82% yield (28 mg, white solid). Esterification with dimethylsuccinic anhydride was performed according to the synthesis of **8**. The desired compound **18** (8.4 mg) from 14 mg (0.027 mmol) of the starting material.

White solid, yield 48%. ^{1}H NMR ($CDCl_3$, 400 MHz, ppm) δ: ^{1}H NMR ($CDCl_3$, 400 MHz, ppm) δ: 5.35-5.25 (m, 1H, C<u>H</u>-OCO), in C3), 4.72 (s, 1H, C=C<u>H</u>, in C29), 4.59 (s, 1H, C=C<u>H</u>, in C29), 2.92 (m, 1H, in C19), 2.81 (m, 2H, CO-C<u>H$_2$</u>-C$(CH_3)_2$), 2.76-2.60 (m, 2H, C<u>**H$_2$**</u>-CF_3), 2.44 (m, 2H, in C2), 1.41, 1.36 (2s, 6H, $2CH_3$, CO_2H-C(C<u>H$_3$</u>)<u>$_2$</u>-CH_2-), 1.02, 0.96, 0.87, 0.83, 0.79 (5s, 5╳3H, 5 ╳CH3, in C23, C24, C25, C26, C27). MS (ESI-) m/z: 675.40 $(M+Na)^+$, 651.45 $(M-H)^-$ for $C_{37}H_{55}F_3O_6$.

Synthesis of 19



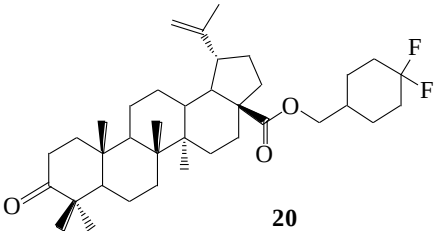
19

The synthetic method for compound **19** was identical to that for the synthesis of **10**. From starting material **3** (175 mg, 0.38 mmol) and 3-(trifluoromethyl) phenethyl alcohol (75 mg, 0.40 mmol), 132 mg of product was obtained.

White solid, yield 55%. ^{1}H NMR ($CDCl_3$, 400 MHz, ppm) δ: 7.50, 7.43 (2s, 4H, Ar-H), 4.69 (s,

1H, C=C<u>H</u>, in C29), 4.58 (s, 1H, C=C<u>H</u>, in C29), 4.32 (m, 2H, $CO_2C\underline{\mathbf{H_2}}CH_2$), 3.02 (m, 2H, $CO_2CH_2C\underline{\mathbf{H_2}}$-Ar), 2.93 (m, 1H, in C19), 2.40 (m, 2H, in C2), 1.65 (s, 3H, CH_3, in C30), 1.05, 1.01, 0.92, 0.89, 0.81 (5s, 5╳3H, 5╳CH3, C23, C24, C25, C26, C27). MS (ESI-) m/z: 628.45 $(M+H)^+$, 627.20 $(M-H)^-$ for $C_{39}H_{53}F_3O_3$.

Synthesis of 20

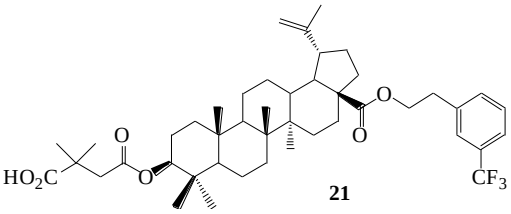


20

Compound **20** was synthesized in the same manner as **10**. From starting material **3** (175 mg, 0.38 mmol) and 4,4-difluorocyclohexane methanol (70 mg, 0.47 mmol), 104 mg of product was obtained.

White solid, yield 47%. ^{1}H NMR ($CDCl_3$, 400 MHz, ppm) δ: 4.73 (s, 1H, C=C<u>H</u>, in C29), 4.61 (s, 1H, C=C<u>H</u>, in C29), 3.97 (m, 2H, $CO_2C\underline{\mathbf{H_2}}$-CH), 3.01 (m, 1H, in C19), 2.36-2.47 (2m, 2H, in C2), 1.69 (s, 3H, CH_3, in C30), 1.07, 1.02, 0.98, 0.95, 0.92 (5s, 5╳3H, 5╳CH_3, C23, C24, C25, C26, C27). MS (ESI-) m/z: 587.45 $(M+H)^+$ for $C_{37}H_{56}F_2O_3$.

Synthesis of 21



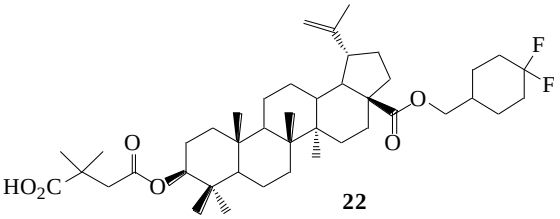
21

Target compound **21** (13.9 mg) was obtained from intermediate **19** (112 mg, 0.17 mmol) in two steps: carbonyl reduction according to the synthesis of **6** to give a hydroxy compound (84 mg, yield 75%), followed by esterification of the hydroxy intermediate (25 mg, 0.04 mmol) according to the synthesis of **8**.

White solid, yield 46%. ^{1}H NMR ($CDCl_3$, 400 MHz, ppm) δ: 7.49, 7.41 (2s, 4H, Ar-H), 4.68 (s, 1H, C=C<u>H</u>, in C29), 4.57 (s, 1H, C=C<u>H</u>, in C29), 4.45 (m, 1H, in C3), 4.33-4.28 (m, 2H, $CO_2C\underline{\mathbf{H_2}}CH_2$-Ar), 3.01 (t, J=8Hz, 2H, $CO_2CH_2C\underline{\mathbf{H_2}}$-Ar), 2.89 (m, 1H, in C19), 2.68-2.57 (dd,

J_1=15Hz, J_2=20Hz, 2H, CO-C<u>H$_2$</u>-C(CH$_3$)$_2$), 1.65 (s, 3H, CH$_3$, in C30), 1.28, 1.25 (2s, 6H, 2×CH$_3$, CO-C<u>H$_2$</u>-C(C**<u>H$_3$)$_2$**), 0.90, 0.82, 0.79, 0.79, 0.76 (5s, 5×3H, 5×CH3, C23, C24, C25, C26, C27). MS (ESI-) m/z: 755.35 $(M-H)^-$ for $C_{45}H_{63}F_3O_6$.

Synthesis of 22

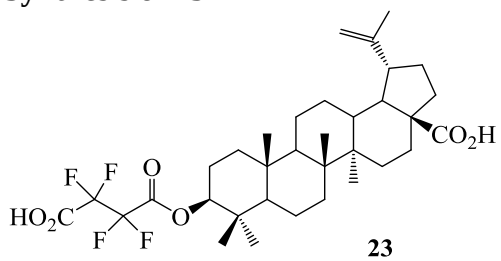


22

Compound **20** (100 mg, 0.17 mmol) was converted to a hydoxy intermediate (76 mg, yield 75%) according to the synthesis of **6**. Then, the obtained white solid (25 mg, 0.04 mmol) was esterified according to the synthesis of **8**, to provide target product **22** (14.1 mg).

White solid, yield 49%. ^{1}H NMR ($CDCl_3$, 400 MHz, ppm) δ: 4.72 (s, 1H, C=C<u>H</u>, in C29), 4.59 (s, 1H, C=C<u>H</u>, in C29), 4.48 (m, 1H, in C3), 3.95 (m, 2H, CO$_2$C**<u>H$_2$</u>**-CH), 3.00 (m, 1H, in C19), 2.68-2.57 (dd, J_1=16Hz, J_2=20Hz, 2H, CO-C**<u>H$_2$</u>**-C(CH$_3$)$_2$), 1.67 (s, 3H, CH$_3$, in C30), 1.31, 1.25 (2s, 6H, 2×CH$_3$, CO-C<u>H$_2$</u>-C(C**<u>H$_3$)$_2$</u>**), 0.97, 0.89, 0.82, 0.82, 0.79 (5s, 5×3H, 5×CH$_3$, C23, C24, C25, C26, C27). MS (ESI-) m/z: 739.45 $(M+Na)^+$, for $C_{43}H_{66}F_2O_6$.

Synthesis of 23

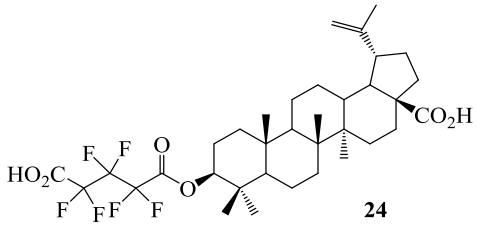


23

A mixture of betullinic acid (**2**, 50 mg, 0.11 mmol) and DMAP (26.7 mg, 0.22 mmol) in anhydrous CH_2Cl_2 (3.0 mL) was cooled to 0~5℃ in an ice-water bath. Then tetrafluorosuccinic anhydride (76 mg, 0.44 mmol) was added to the solution and the mixture was stirred for 1 h, then warmed to rt and stirred for another 12 h. The solution was filtered and the obtained white solid was recrystallized from THF to provide 21 mg of the desired product.

White powder, yield 30%. ^{1}H NMR ($CDCl_3$+CCl_4), 400 MHz, ppm) δ: 4.75 (s, 1H, C=C<u>H</u>, in C29), 4.59 (s, 1H, C=C<u>H</u>, in C29), 4.53 (m, 1H, in C3), 3.00 (m, 1H, in C19), 1.68 (s, 3H, CH_3, in C30), 1.04, 0.97, 0.89, 0.82, 0.82 (5s, 5╳3H, 5╳CH_3, C23, C24, C25, C26, C27). MS (ESI-) m/z: 650.20 $(M+Na)^+$, 626.25 $(M-H)^-$ for $C_{34}H_{48}F_4O_6$.

Synthesis of 24



24

The synthetic method was identical to that described above for **23**. The product (24 mg) was obtained in 32% yield from betulinic acid (**2**, 50 mg, 0.11 mmol) and hexafluroglutaric anhydride (97.7 mg, 0.44 mmol).

White powder, yield 32%. ^{1}H NMR ($CDCl_3$+CCl_4), 400 MHz, ppm) δ: 4.77 (s, 1H, C=C<u>H</u>, in C29), 4.60 (s, 1H, C=C<u>H</u>, in C29), 4.52 (m, 1H, in C3), 3.00 (m, 1H, in C19), 1.67 (s, 3H, CH_3, in C30), 1.02, 0.98, 0.89, 0.82, 0.82 (5s, 5╳3H, 5╳CH_3, C23, C24, C25, C26, C27).MS (ESI-) m/z: 678.25 $(M+H)^+$, 676.20 $(M-H)^-$ for $C_{35}H_{48}F_6O_6$.

Biological Methods

Anti-HIV Assay. MT4 cells were infected with HIV-1 NL4-3 (multiplicity of infection = 0.001) in the presence of various concentrations of compounds. Fresh medium, which contained appropriate concentrations of the compounds, was added to the culture 48 h after infection to maintain normal cell growth. Virus replication was analyzed on day 4 postinfection using p24 ELISA kits from Perkin-Elmer. The compound effective concentration that inhibited HIV-1 replication by 50% (EC_{50}) was calculated by using the biostatistics software Calcusun (Biosoft).

Cytotoxicity Determination. Cytotoxicity of the purified compounds toward MT4 cells was determined by using a cell viability kit provided by Promega. The CellTiter-Glo luminescent cell

viability assay is a simple method of determining the viability of the cells in culture based on quantitation of ATP in metabolically active cells. The CellTiter-Glo reagent was added to MT4 cells that were cultured parallel to the antiviral assays. The compound cytotoxic concentration that decreased the cell viability by 50% (CC_{50}) was calculated by using Calcusun.

References

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2. J. Xu, Y. Fu, and L. Liu. "*Copper-Catalyzed Trifluoromethylation of Terminal Alkenes through Allylic C-H Bond Activation*", *J. Am. Chem. Soc*. **2011**, *133*, 15300-15303.
3. L.l. Chu and F.L.Qing. "*Copper-Catalyzed OxidativeTrifluoromethylation of Terminal Alkenes Using Nucleophilic CF3SiMe3: Efficient C(sp3)-CF3 Bond Formation*", *Org. Lett*. **2012**, *14(8)*, 2106-2109.