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Chemical resolution of (±)-calanolide A, (±)-cordatolide A and their 11-demethyl analogues

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Abstract—The chemical resolution of (±)-calanolide A and (±)-cordatolide A into their corresponding optically active enantiomers is described. Their inhibitory activities against HIV-1 are tested in vitro.

Coumarin is one of the main natural product scaffolds displaying a broad range of biological activities. Its derivatives have been widely used as therapeutic agents, active media for tunable dye lasers, optical bleaching agents, luminescent probes, and triplet sensitizers.[1] Since 1992, medicinal scientists have been interested in some *Calophyllum* coumarins (**1**–**3**, Fig. 1) because of their potent inhibitory activity against HIV-1.[2] Especially, (+)-calanolide A (**1**) was found to inhibit not only the wild type of HIV-1, but also clinically isolated resistant strains such as A17 (Y181C mutant).[3] Recently, it was demonstrated that (+)-calanolide A could also inhibit *Mycobacterium tuberculosis* (TB) at MIC 3.13 μg/ml level.[4]

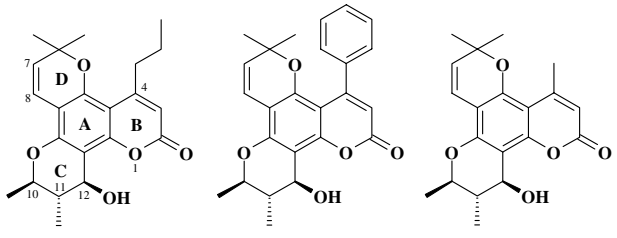

Figure 1. The chemical structures of the biologically active *Calophyllum* coumarins.

The total synthesis of racemic calanolide A has been reported by Chenera,[5] Kucherenko,[6] and us[7,8] with phloroglucinol as the starting material. Its 4-ring system was consecutively constructed through the three skeletal rings, coumarin (ring **A** and **B**), 2,3-dimethylchromanol (ring **C**), and 2,2-dimethyl chromene (ring **D**). In 1994, Palmer[9] reported general synthetic routes for racemic calanolide A, inophyllum B, and cordatolide A through a ten-step approach with lower total yields. Furthermore, optically active (+)-calanolide A has been successfully synthesized either by the chiral borane-participated allylation of 8-formyl coumarin derivative,[10] or by the Pd-catalyzed asymmetric *O*-allylation of 7-hydroxycoumarin,[11] or by the application of a (−)-quinine-catalyzed intramolecular oxo-Michael addition (IMA),[12,13] or by optically enzymatic resolution of 8-(3-hydroxy-2-methyl propionyl)coumarin,[14] or by resolution through chiral preparative HPLC.[15,16] However, their chemical resolution has not been previously reported.

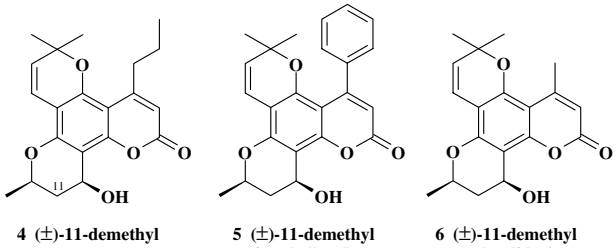

Figure 2. The racemic 11-demethyl analogues of *Calophyllum* coumarins.

Keywords: HIV-1; Chemical resolution; Calanolide A; Cordatolide A.
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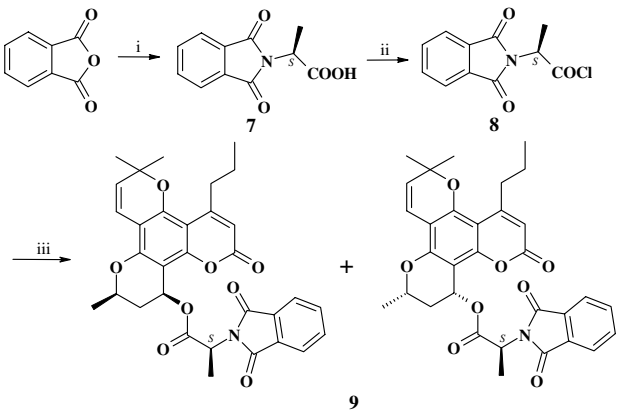


Scheme 1. Reagents and conditions: (i) L-alanine, 160 °C, 2 h (68%); (ii) $SOCl_2$, toluene, reflux, 5 h (88%); (iii) racemic 11-demethyl calanolide A, 4-dimethylaminopyridine (DMAP), dichloromethane (DCM), room temperature (rt) (41%).

In our laboratory, the concise total synthesis of the racemic compounds and their 11-demethyl analogues (**1**–**6**, Figs. 1 and 2) has been developed with fewer reaction steps and higher total yields.[17,18] Moreover, their corresponding demethyl analogues were also found to be anti-HIV active at a similar level to (+)-calanolide A in vitro. Therefore, the chemical resolution was then explored to obtain their corresponding optically active enantiomers.

Table 1. The chemical shifts (δ) of characteristic ^{1}H signals and their ratios of the two diastereoisomers

H	*S*-(−)-α-phthalyl-L-alanine-(+)-11-demethyl calanolide A	*S*-(−)-α-phthalyl-L-alanine-(−)-11-demethyl calanolide A	Δ
8-H	6.616 (52.5%)	6.532 (37.5%)	0.084
7-H	5.536 (62.6%)	5.486 (37.4%)	0.050
12-H	6.314 (65.1%)	6.387 (34.9%)	0.073
CO-CH-N (CH$_3$)	5.112 (53.5%)	5.022 (46.5%)	0.090
10-H	4.474 (59.3%)	4.357 (40.7%)	0.117

S-(−)-α-Phthalyl-L-alanine (**7**) was first selected as the chemical resolution reagent, because it could be conveniently prepared through condensation of commercially available phthalic anhydride with L-alanine. With the racemic 11-demethyl calanolide A as the template compound, *S*-(−)-α-phthalyl-L-alanine was converted readily into its corresponding acyl chloride (**8**) in the presence of $SOCl_2$ with toluene as the solvent (Scheme 1). A pair of diastereoisomers (**9**) was obtained subsequently. How-

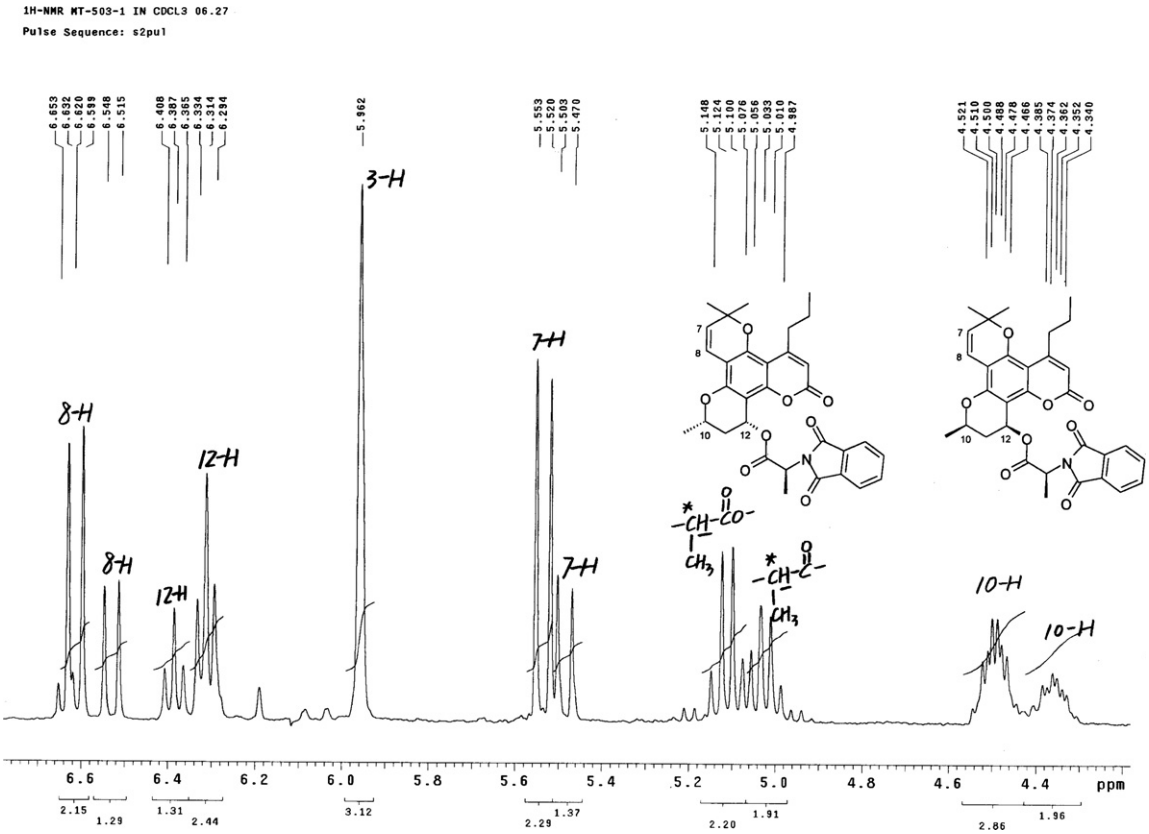
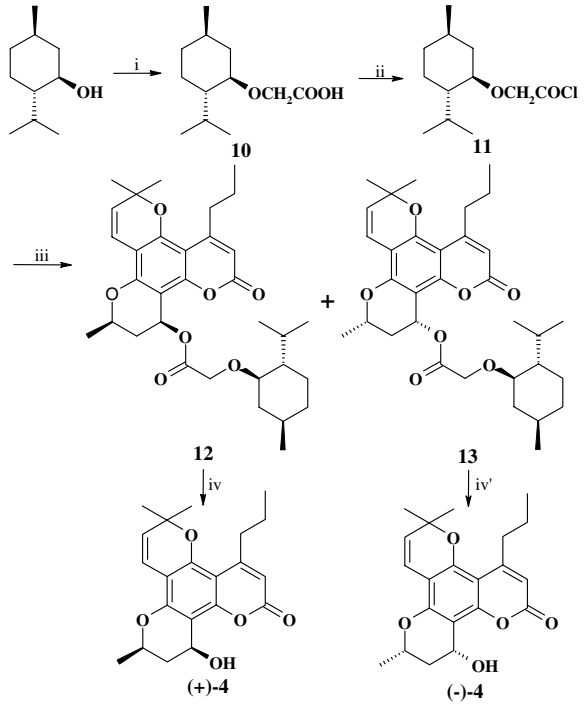


Figure 3. The magnified ^{1}H NMR spectrum (300 MHz, $CDCl_3$, ppm) of the mixed diastereoisomers (**9**, only downfield is shown). The two sets of ^{1}H signals were assigned to the mixed diastereoisomers, respectively. The chemical shifts of 12-H signals in compound **9** range from 6.3 to 6.4 ppm (t, J = 6.6 Hz), compared to 5.239 ppm (dd, J = 7.35 Hz, 8.7 Hz) in compound (±)-**4**. That difference apparently indicated that the 12-OH was acylated by the resolution reagent (**8**).



Scheme 2. Reagents and conditions: (i) $ClCH_2COOH$, NaH, 1,4-dioxane, reflux, 4 h (41%); (ii) $SOCl_2$, toluene, reflux, 4 h (86%); (iii) racemic 11-demethyl calanolide A, DMAP, DCM, room temperature (42% and 37%); (iv and iv′) 0.1 mol/L NaOH, THF, rt (86% and 77%).

ever, they could not be effectively separated from each other through classical purification methods such as silica gel column chromatography and preparative thin layer chromatography. The mixed diastereoisomers were identified by ^{1}H NMR analysis (Fig. 3). Their relative ratios could be consequently approximately calculated through their corresponding integration areas (Table 1).

An alternative approach was then developed. (−)-Menthol-acetic acid (**10**) was finally selected as the resolution reagent, which had three asymmetric carbon centers in its scaffold. (−)-Menthol-acetyl chloride (**11**) was readily reacted with racemic 11-demethyl calanolide A to produce the anticipative pair of diastereoisomers (**12**, **13**), which were effectively separated and purified using silica gel H column chromatography (Scheme 2, Fig. 4). The two separated diastereoisomers (**12**, **13**), which were characterized by ^{1}H NMR spectra (Table 2), were dissolved in tetrahydrofuran (THF), respectively, and were treated with 0.1 mol/L NaOH, respectively, to eventually gain their corresponding optical enantiomers [(+)-**4** and (−)-**4**] (Fig. 5).[19]

Table 2. The chemical shifts (δ) of characteristic ^{1}H signals between the purified diastereoisomers (**12** and **13**)

H	(−)-Menthol-acetyl-(+)-11-demethyl calanolide A (**12**)	(−)-Menthol-acetyl-(−)-11-demethyl calanolide A (**13**)	Δ
12-H	6.346	6.335	0.011
10-H	4.356	4.336	0.020
OCH_2COO	4.196	4.179	0.017
1′-H	3.151	3.296	0.145
a-CH_3	0.918	0.894	0.024
b-CH_3	0.886	0.888	0.002
c-CH_3	0.642	0.831	0.189

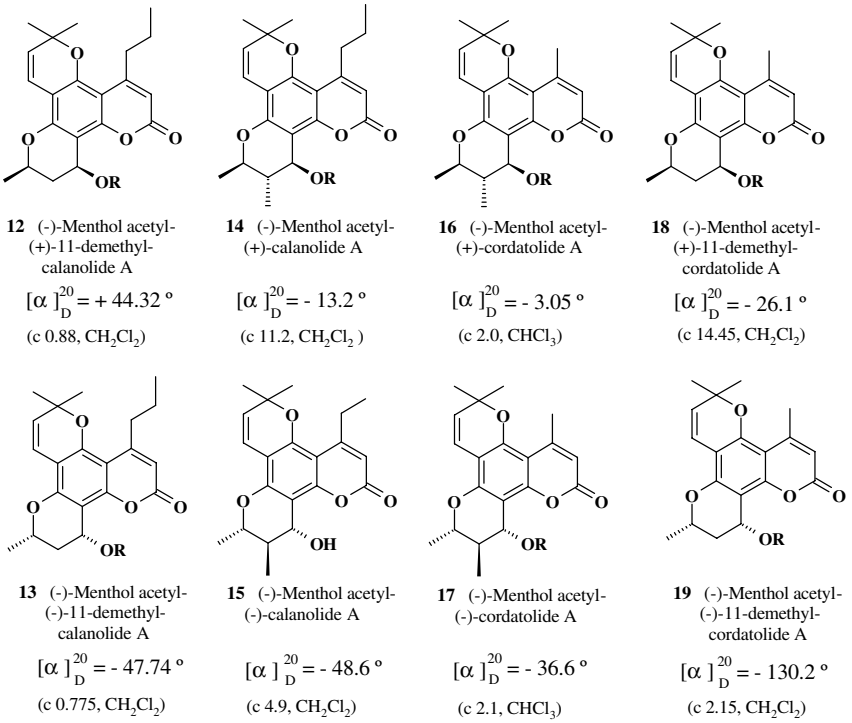


Figure 4. The four pairs of optically active 12-*O*-(−)-menthol acetyl derivatives. R represents (−)-menthol acetyl group.

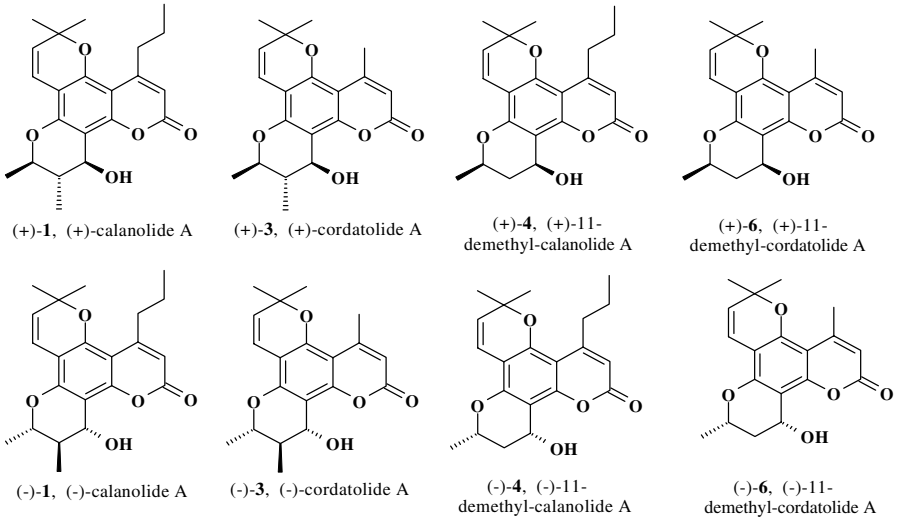


Figure 5. The four pairs of optically active enantiomers.

Table 3. The anti-HIV-1 activities and cytotoxicities of the optically active calanolide A, cordatolide A, and their corresponding 11-demethyl analogues

Entry	$[\alpha]_D^{20}$	Inhibition (%) 10 μM	Inhibition (%) 1.0 μM	Cytotoxicity (%) (10 μM)	Cytotoxicity (%) (1.0 μM)
(±)−**1**	0	97	81	0	0
(+)−**1**	+58.1° (*c* 0.93, CH_2Cl_2)[a]	98	86	6	1
(−)−**1**	−56.6° (*c* 0.60, CH_2Cl_2)[b]	97	67	0	0
(+)−**3**	+51.8° (*c* 1.3, $CHCl_3$)	85	44	0	0
(−)−**3**	−58.1° (*c* 1.1, $CHCl_3$)	0	0	0	0
(+)−**4**	+38.4° (*c* 3.2, CH_3OH)	98	75	54	0
(−)−**4**	−33.3° (*c* 0.9, CH_3OH)	0	0	0	0
(+)−**6**	+46.2° (*c* 1.3, CH_2Cl_2)	0	0	0	0
(−)−**6**	−44.1° (*c* 1.7, CH_2Cl_2)	0	0	0	0

[a] $[\alpha]_D^{25} = +60°$ (*c* 0.5, $CHCl_3$),[2] +68.8° (*c* 0.7, $CHCl_3$),[16] +66° (*c* 0.5, $CHCl_3$).[10]
[b] $[\alpha]_D^{25} = -75.6°$ (*c* 0.7, $CHCl_3$),[16] −66° (*c* 0.5, $CHCl_3$),[10] −68° (*c* 1.36, $CHCl_3$).[22]

Using the same strategy, the other three racemic *Calophyllum* coumarins (**1**, **3**, and **6**) were chemically resolved into their corresponding optical enantiomers with (−)-menthol-acetyl chloride as a resolution reagent. All the four pairs of optical diastereoisomers and enantiomers are summarized in Figures 4 and 5, respectively.

The inhibitory activities against HIV-1 of all the enantiomers were tested in vitro using a pseudotyped viral assay as previously described.[20] The assay results in cell culture and their cytotoxicity are outlined in Table 3.[21] Obviously, (−)-11-demethyl calanolide A [(−)-**4**], (+) and (−)-11-demethyl cordatolide A [(+)-**6** and (−)-**6**], and (−)-cordatolide A [(−)-**3**] enantiomers lost their activities against HIV-1. (+)-Cordatolide A [(+)-**3**] had lower inhibitory ability compared with (+)-, or (−)-calanolide [(+)-**1**, or [(−)-**1**]. Interestingly, (−)-calanolide A [(−)-**1**] also showed inhibitory activity against HIV-1 in the assay that was different from previous studies.[15,16] The racemic calanolide A [(±)-**1**] was also tested in the assay, and it had very close inhibitory activity to (+)-1. This difference will be further investigated to determine the impalpable structure–activity relationship of the stereo configurations of calanolide A. We also determined the cytotoxicity to the host cells induced by (+)-**4** or (+)-**1** at 10 μM. The findings indicate that the removal of a methyl group at the 11-position of calanolide A increases the cytotoxicity of this type of optical enantiomer.

In summary, the concise total synthesis of the racemic *Calophyllum* coumarins and their 11-demethyl analogues has been developed in our laboratory with fewer reaction steps and higher total yields. The chemical resolution of (±)-calanolide A and (±)-cordatolide A into their corresponding optically active enantiomers is described in this paper. Their inhibitory activities against HIV-1 were tested in vitro.

Acknowledgments

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2007.12.008.

References and notes

1. Wang, L.; Ma, T.; Liu, G. *Medicinal Chemistry of Bioactive Natural Products (Chapter 8)* **2006**, 325, and references therein.
2. Kashman, Y.; Gustafson, K. R.; Fuller, R. W.; Cardellina, J. H.; McMahon, J. B.; Currens, M. J.; Buckheit, R. W.; Hughes, S. H.; Cragg, G. M.; Boyd, M. R. *J. Med. Chem.* **1992**, *35*, 2735.
3. Ma T.; Wang L.; Liu G. *Oral presentation at the 40th IUPAC Congress*, Beijing, August 15–19, 2005, p 207.
4. Xu, Z. Q.; Barrow, W. W.; Suling, W. J.; Westbrook, L.; Barrow, E.; Lin, Y. M.; Flavin, M. T. *Bioorg. Med. Chem.* **2004**, *12*, 1199.
5. Chenera, B.; West, M. L.; Finkelstein, J. A.; Dreyer, G. B. *J. Org. Chem.* **1993**, *58*, 5605.
6. Kucherenko, A.; Flavin, M. T.; Boulanger, W. A.; Khilevich, A.; Shone, R. L.; Rizzo, J. D.; Sheikman, A. K.; Xu, Z. Q. *Tetrahedron Lett.* **1995**, *36*, 5475.
7. Zhou, C. M.; Wang, L.; Zhang, M. L.; Zhao, Z. Z.; Zhang, X. Q.; Chen, X. H.; Chen, H. S. *Chin. Chem. Lett.* **1998**, *9*, 433.
8. Zhou, C. M.; Wang, L.; Zhang, M. L.; Zhao, Z. Z.; Zhang, X. Q.; Chen, X. H.; Chen, H. S. *Acta Pharm. Sin.* **1999**, *34*, 673.
9. Palmer, C. J.; Josephs, J. L. *Tetrahedron Lett.* **1994**, *35*, 5363.
10. Deshpande, P. P.; Tagliaferri, F.; Victory, S. F.; Yan, S. J.; Baker, D. C. *J. Org. Chem.* **1995**, *60*, 2964.
11. Trost, B. M.; Toste, F. D. *J. Am. Chem. Soc.* **1998**, *120*, 9074.
12. Tanaka, T.; Kumamoto, T.; Ishikawa, T. *Tetrahedron Lett.* **2000**, *41*, 10229.
13. Sekino, E.; Kumamoto, T.; Tanaka, T.; Ikeda, T.; Ishikawa, T. *J. Org. Chem.* **2004**, *69*, 2760.
14. Khilevich, A.; Mar, A.; Flavin, M. T.; Rizzo, J. D.; Lin, L.; Dzekhtser, S.; Brankovic, D.; Zhang, H. P.; Chen, W.; Liao, S. Y.; Zembower, D. E.; Xu, Z. Q. *Tetrahedron: Asymmetry* **1996**, *7*, 3315.
15. Cardellina, J. H.; Bokesch, H. R.; McKee, T. C.; Boyd, M. R. *Bioorg. Med. Chem. Lett.* **1995**, *5*, 1011.
16. Flavin, M. T.; Rizzo, J. D.; Khilevich, A.; Kucherenko, A.; Sheinkman, A. K.; Vilaychack, V.; Lin, L.; Chen, W.; Greenwood, E. M.; Pengsuparp, T.; Pezzuto, J. M.; Hughes, S. H.; Flavin, T. M.; Cibulski, M.; Boulanger, W. A.; Shone, R. L.; Xu, Z. Q. *J. Med. Chem.* **1996**, *39*, 1303.
17. Wang L.; Liu G.; Gao Q.; Ma T.; Zhao Z. Z. Chinese patent application for invention No. 03123628.6.
18. Liu G.; Ma T.; Chen Z. W.; Wang L. Chinese patent application for invention No. 200610098615.8.
19. Analytical data for **12**: colorless oil; ^{1}H NMR (500 MHz, $CDCl_3$, ppm): 6.620 (d, 1H, J = 10.0 Hz, 8-H), 6.346 (t, 1H, J = 7.0 Hz, 12-H), 5.936 (s, 1H, 3-H), 5.540 (d, 1H, J = 10.0 Hz, 7-H), 4.356 (m, 1H, 10-H), 4.196 (q, 2H, OCH_2COO), 3.151 (dt, 1H, J = 4.0 Hz, 10.5 Hz, 1′-H), 2.864 (m, 2H, 4-CH_2CH$_2$CH$_3$), 2.220 (m, 2H, 11-CH$_2$), 2.016 (m, 2H, CH$_2$), 1.637 (m, 2H, CH$_2$), 1.508 (m, 2H, 4-CH$_2$CH_2CH$_3$), 1.471, 1.451 (2s, 6H, 6-CH$_3$), 1.313 (d, 3H, J = 6.0 Hz, 10-CH$_3$), 0.989 (t, 3H, J = 7.5 Hz, 4-CH$_2$CH$_2$CH_3), 0.918 (d, 3H, J = 6.5 Hz, a-CH$_3$), 0.886 (d, 3H, J = 6.5 Hz, b-CH$_3$), 0.642 (d, 3H, J = 6.5 Hz, c-CH$_3$).
    Analytical data for **13**: colorless oil; ^{1}H NMR (500 MHz, $CDCl_3$, ppm): 6.620 (d, 1H, J = 10.0 Hz, 8-H), 6.335 (t, 1H, J = 7.0 Hz, 12-H), 5.940 (s, 1H, 3-H), 5.540 (d, 1H, J = 10.0 Hz, 7-H), 4.336 (m, 1H, 10-H), 4.179 (q, 2H, OCH_2COO), 3.296 (dt, 1H, J = 4.0 Hz, 10.5 Hz, 1′-H), 2.880 (m, 2H, 4-CH_2CH$_2$CH$_3$), 2.222 (m, 2H, 11-CH$_2$), 2.014 (m, 2H, CH$_2$), 1.638 (m, 2H, CH$_2$), 1.508 (m, 2H, 4-CH$_2$CH_2CH$_3$), 1.472, 1.464 (2s, 6H, 6-CH$_3$), 1.310 (d, 3H, J = 6.0 Hz, 10-CH$_3$), 0.979 (t, 3H, J = 7.5 Hz, 4-CH$_2$CH$_2$CH_3), 0.894 (d, 3H, J = 7.0 Hz, a-CH$_3$), 0.888 (d, 3H, J = 7.5 Hz, b-CH$_3$), 0.831 (d, 3H, J = 7.0 Hz, c-CH$_3$). Analytical data for (+)-**4**: off-white powder; ^{1}H NMR (500 MHz, $CDCl_3$, ppm): 6.620 (d, 1H, J = 10.0 Hz, 8-H), 5.949 (s, 1H, 3-H), 5.543 (d, 1H, J = 10.0 Hz, 7-H), 5.247 (t, 1H, J = 8.0 Hz, 12-H), 4.270 (m, 1H, 10-H), 2.890 (m, 2H, 13-CH$_2$), 2.369 (m, 1H, 11-He), 1.934 (m, 1H, 11-Ha), 1.649 (m, 2H, 14-CH$_2$), 1.506, 1.464 (2s, 6H, 6-CH$_3$), 1.481 (d, 3H, J = 6.0 Hz, 10-CH$_3$), 1.034 (t, 3H, J = 7.5 Hz, 15-CH$_3$); Anal. Calcd for $C_{21}H_{24}O_5$: C, 70.77; H, 6.79. Found: C, 70.51; H, 6.74.
    Analytical data for (−)-**4**: off-white powder; ^{1}H NMR (500 MHz, $CDCl_3$, ppm): 6.620 (d, 1H, J = 10.5 Hz, 8-H), 5.947 (s, 1H, 3-H), 5.542 (d, 1H, J = 10.5 Hz, 7-H), 5.247 (t, 1H, J = 7.5 Hz, 12-H), 4.270 (m, 1H, 10-H), 2.895 (m, 2H, 13-CH$_2$), 2.364 (m, 1H, 11-He), 1.929 (m, 1H, 11-Ha), 1.650 (m, 2H, 14-CH$_2$), 1.506, 1.464 (2s, 6H, 6-CH$_3$), 1.480 (d, 3H, J = 6.5 Hz, 10-CH$_3$), 1.034 (t, 3H, J = 8.0 Hz, 15-CH$_3$); Anal. Calcd for $C_{21}H_{24}O_5$: C, 70.77; H, 6.79. Found: C, 70.71; H, 6.75.
20. Chen, Z. W.; Zhou, P.; Ho, D. D.; Landau, N. R.; Marx, P. A. *J. Virol.* **1997**, *71*, 2705.
21. HIV inhibition assay. A serial diluted drug (10 μM, 1 μM, 100 nM, 10 nM, 1 nM, and 0.1 nM), was tested against 100 TCID50 HIVADA infection in a pseudovirus-based assay. The assay quantifies the activity of a drug to inhibit HIV-induced reporter luciferase activity. The viral infection was determined on day 3 by measuring the reporter luciferase activity in TZM. CD4+. CCR5+ cells post-infection using commercially available kits. Antiviral data were reported as the quantity of drug required to inhibit production by percentage. Controls include (1) infected cells without adding any drugs as a baseline, (2) pseudovirus with non-specific envelope (e.g., HCV) which cannot infect TZM. CD4+. CCR5+ cells, and (3) an HIV-specific RT inhibitor as a positive control (e.g. AZT). The cell toxicity of the compounds against HIV was evaluated by similar microtiter assays with TZM. CD4+. CCR5+ and Vero cells. Serial diluted drugs (10 mM, 1 mM, 100 μM, 10 μM, 1 μM and 0.1 μM) were tested in cells using the cell viability assay which is commercially available (Promega). The toxicity data are reported as the quantity of a drug required to reduce cell viability by percentage.
22. Galinis, D. L.; Fuller, R. W.; Mckee, T. C.; Cardellina, J. H.; Gulakowski, R. J.; Mcmahon, J. B.; Boyd, M. R. *J. Med. Chem.* **1996**, *39*, 4507.