Organic & Biomolecular Chemistry

PAPER

View Article Online
View Journal | View Issue

Cite this: *Org. Biomol. Chem.*, 2014, **12**, 5192

An efficient synthesis of methyl 2-cyano-3,12-dioxoursol-1,9-dien-28-oate (CDDU-methyl ester): analogues, biological activities, and comparison with oleanolic acid derivatives†

Liangfeng Fu,[a] Qi-Xian Lin,[a] Karen T. Liby,[b] Michael B. Sporn*[b] and Gordon W. Gribble*[a]

Received 31st March 2014,
Accepted 30th May 2014

DOI: 10.1039/c4ob00679h

www.rsc.org/obc

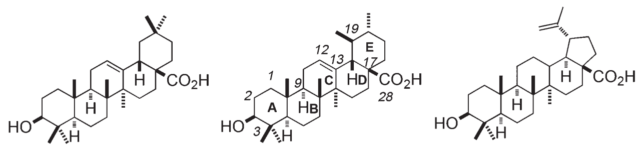
An efficient synthesis of methyl 2-cyano-3,12-dioxoursol-1,9-dien-28-oate (CDDU-methyl ester) from commercially available ursolic acid, which features an oxidative ozonolysis-mediated C-ring enone formation, and provides the first access to ursolic acid-derived cyano enone analogues with C-ring activation. These new ursolic acid analogues show potent biological activities, with potency of approximately five-fold less than the corresponding oleanolic acid derivatives.

Introduction

The biological importance of naturally occurring pentacyclic triterpenoids has long been recognized. The annual review of triterpenoids in *Natural Product Reports* is ample testimony to the ubiquitous worldwide distribution of these compounds, which include squalenes, lanostanes, fusidanes, dammaranes, euphanes, and others. Within each class is a stunning array of structural diversity and a range of biological activity.[1] For example, many oleanane and ursane triterpenoids, which are derived biosynthetically by cyclization of squalene,[2] have interesting biological, pharmacological, and medicinal activities not unlike those attributed to retinoids and steroids. These properties include anti-inflammatory actions, suppression of tumor promotion, suppression of immunoglobulin synthesis, protection of the liver against toxic injury, induction of collagen synthesis, and induction of differentiation in leukemia and teratocarcinoma cells.[3]

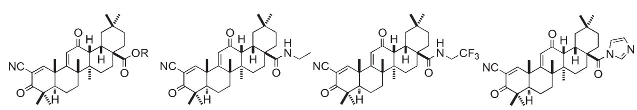
Oleanolic acid (**1**), ursolic acid (**2**), and betulinic acid (**3**) (Fig. 1), probably the most commonly studied triterpenoids, exhibit modest biological activity, although **2** has been marketed in China as an oral drug for the treatment of liver disorders in humans.[4]

Extensive studies report the use of oleanolic acid as a skeleton motif. Indeed, the highly potent oleanolic acid derivative



Oleanolic Acid (**1**) Ursolic Acid (**2**) Betulinic Acid (**3**)

Fig. 1 Oleanolic acid, ursolic acid, and betulinic acid.



CDDO-Me (**4**); R = Me
CDDO (**8**); R = H
CDDO-Ethyl Amide (**5**) CDDO-Trifluoroethyl Amide (**6**) CDDO-Imidazolide (**7**)

Fig. 2 CDDO methyl ester and analogues.

CDDO-methyl ester (**4**) (bardoxolone methyl) (Fig. 2), which was developed in our laboratory years ago,[5] has successfully completed a Phase I clinical trial for the treatment of cancer[6] and a Phase II clinical trial for the treatment of chronic kidney disease in type 2 diabetes patients.[7] During this research we synthesized a series of CDDO-methyl ester analogues (Fig. 2), such as CDDO-ethyl amide (**5**), CDDO-trifluoroethyl amide (**6**), and CDDO-imidazolide (**7**).[8] Details of their biological properties are described in recent review articles.[9–11]

Due to the limited structure–activity studies involving ursolic acid, we have undertaken and now describe an efficient synthesis of the ursolic acid analogue of CDDO-methyl ester, CDDU-methyl ester (methyl 2-cyano-3,12-dioxoursol-1,9-dien-28-oate), along with related derivatives, and describe their biological activities.

[a]*Department of Chemistry, Dartmouth College, Hanover, NH 03755, USA. E-mail: ggribble@dartmouth.edu*
[b]*Department of Pharmacology and Toxicology, Geisel School of Medicine, Hanover, NH 03755, USA*

†Electronic supplementary information (ESI) available. See DOI: 10.1039/c4ob00679h

To investigate the anti-inflammatory and cancer chemopreventive properties of these derivatives, we have adopted a preliminary screening assay system that measures inhibition of nitric oxide (NO) production as induced by interferon-γ (IFN-γ) in mouse macrophages, and CDDO (**8**) is 200 000–400 000 times more active than oleanolic acid in this inducible nitric oxide synthase (iNOS) assay. Also, CDDO at nanomolar concentrations suppresses the *de novo* synthesis of the inflammatory enzymes iNOS and cyclooxygenase-2 (COX-2) in activated macrophages.[12]

Results and discussion

Chemical synthesis

Our rationale for this project is that because ursolic acid is often more potent than oleanolic acid,[13] we deemed it important to synthesize and study the ursolic acid derivatives CDDU-methyl ester (**9**), CDDU-ethyl amide (**10**), CDDU-trifluoroethyl amide (**11**), and CDDU-imidazolide (**12**) (Fig. 3).

Our first goal was to prepare the C-ring enone of ursolic acid. A number of reagents and reaction conditions are known to oxidize the C11–C12 alkene of oleanolic acid, including *m*-CPBA,[14] MMPP,[15] ozone-mediated[16] hydroxy lactonization, DMDO-mediated chlorolactonization,[17] bismuth(III) triflate-mediated[18] lactonization, and bromolactonization.[19] Oxidation conditions, such as H_2O_2–AcOH[20] or *m*-CPBA-mediated epoxidation and isomerization,[21] ozonolysis[22] have also been developed for oleanolic acid esters (Scheme 1).

However, the corresponding oxidations of ursolic acid are either rare or unknown. For example, Bag and coworkers reported a selective Br_2/AcOH-induced bromolactonization of oleanolic acid in the presence of ursolic acid.[19] Recently, Csuk and Siewert reported a *m*-CPBA-mediated oxidative separation of oleanolic acid from ursolic acid.[14b] Under these conditions, oleanolic acid was completely converted to the oxidation product, whereas ursolic acid was recovered nearly quantitatively. The different C-ring reactivity between oleanolic acid and ursolic acid is presumed to be due to the steric hindrance imparted by the C19 methyl group in ursolic acid. However, Salvador, Jing and co-workers describe the introduction of a C-12 fluorine in an ursolic acid derivative[23] (Scheme 2).

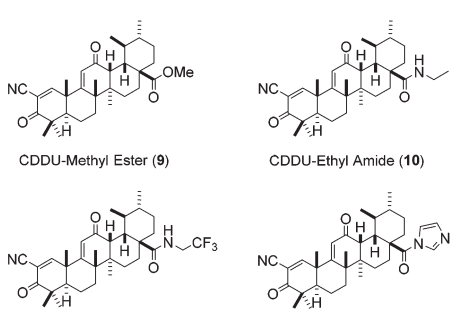
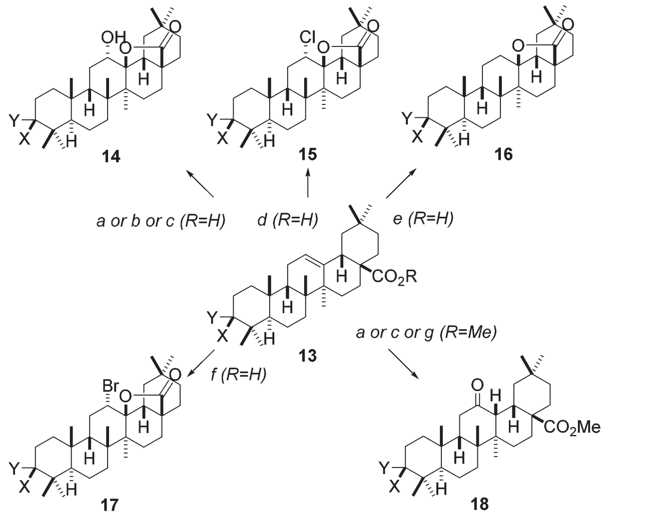
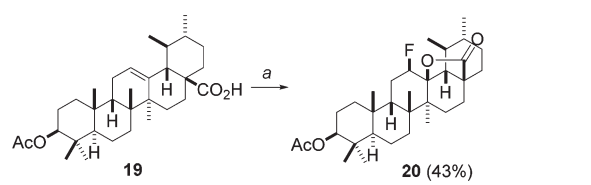


Fig. 3 CDDU-methyl ester and analogues.



Scheme 1 Reported C11–C12 oxidation of oleanolic acid. Reagents: (a) *m*-CPBA, CH_2Cl_2; (b) MMPP, acetone; (c) O_3, CH_2Cl_2; (d) DMDO, $CHCl_3$; (e) $Bi(OTf)_3$, CH_2Cl_2; (f) Br_2, AcOH; (g) H_2O_2, AcOH.

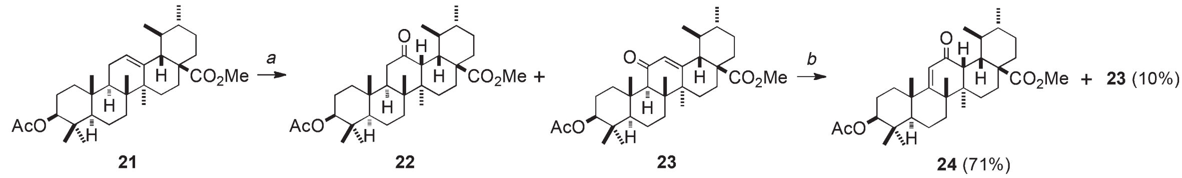


Scheme 2 Reported fluorolactonization on ursolic acid. Reagent: (a) Selectfluor, dioxane, nitromethane, 80 °C.

Not surprisingly, we encountered difficulty in the oxidation of the ursolic acid C-ring. Thus, an initial reaction of ursane ester **21** with *m*-CPBA as the oxidant resulted in almost quantitative recovery of **21**[24] (Scheme 3), and classic conditions employing hydrogen peroxide in acetic acid gave complex mixtures. Moreover, neither *t*-butyl hydroperoxide nor MMPP provided satisfactory results. Reductive conditions on **21** using excess borane also failed, yielding partial reduction of both the C-3 acetate and C-17 methyl ester, but leaving the C11–C12 alkene intact.

To our delight, treatment of **21** with ozone at −40 °C afforded a mixture of **22** and **23** (4 : 1) (Table 1, entry 3) as an inseparable mixture, which was evidenced by our proton and carbon NMR spectra. A temperature study revealed that −78 °C is optimal (Table 1, entry 4), and these conditions provided **22** and **23** in a ratio of 8 : 1. Without further purification, direct treatment with pyridinium perbromide in acetonitrile afforded ring-C enones **24** and **23** in 81% and 10% yield, respectively (Scheme 3).

Mild base-catalyzed hydrolysis of **24** afforded an alcohol **25** (not shown), which was oxidized to the corresponding ketone **26** with refluxing iodoxybenzoic acid. A subsequent two-step selenation/oxidation/elimination protocol gave bis-enone **27** in good yield. Regioselective iodination using iodine in carbon tetrachloride and pyridine in the presence of catalytic amount



Scheme 3 Ozonolysis-mediated C-ring enone formation. Reagent: (a) O_3, CH_2Cl_2, *T* °C; (b) $pyHBr_3$, CH_3CN, 50 °C.

Table 1 Optimization for ozonolysis-mediated C-ring enone formation of **22** plus **23**

Entry	*T* (°C)	Ratio (22 : 23)	Yield (%)	Yield of 22 (%)	Yield of 23 (%)
1	25	2 : 1	84	56	28
2	0	2 : 1	84	56	28
3	−40	4 : 1	87	70	17
4	**−78**	**8 : 1**	**91**	**81**	**10**

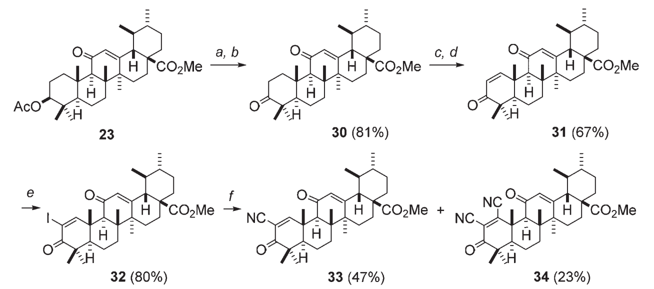
of dimethylaminopyridine gave iodoenone **28**. Final cyanation using copper(I) cyanide in dimethyl formamide unexpectedly provided a mixture of the desired cyanoenone **9** and bis-cyanoenone **29**, the latter of which was not observed in our recently developed CDDO-Me synthesis.[25] The formation of bis-cyanoenone **29** may indicate that CDDU-methyl ester (**9**) is a more reactive Michael acceptor and will prove more potent than CDDO-methyl ester (**4**) (Scheme 4).

In similar fashion, enone **23** was converted in five steps to cyanoenone **33** and bis-cyanoenone **34** (Scheme 5).

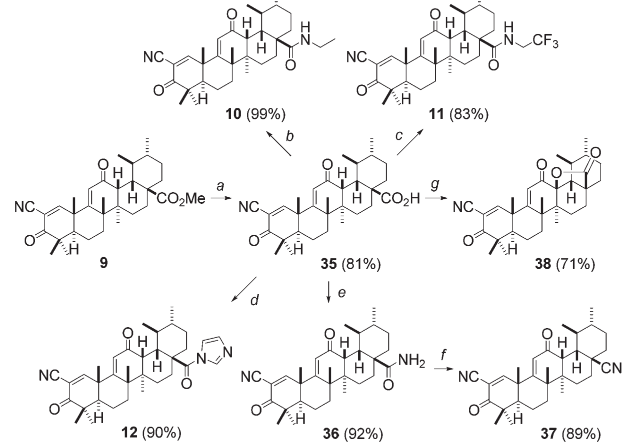
With CDDU-methyl ester (**9**) in hand, lithium iodide-mediated demethylation in refluxing pyridine afforded CDDU (**35**) in 81% yield (Scheme 6). Amides **10**, **11**, **36**, and imidazolide **12** were obtained in a two-step sequence from CDDU **35**, *via* the acid chloride, respectively. Treatment of **36** with trifluoroacetic anhydride and triethylamine in methylene chloride afforded bis-nitrile **37** in an 89% yield. Finally, direct treatment of **35** with DDQ in refluxing benzene provided lactone **38** in 71% yield (Scheme 6).[26]

Biological evaluation

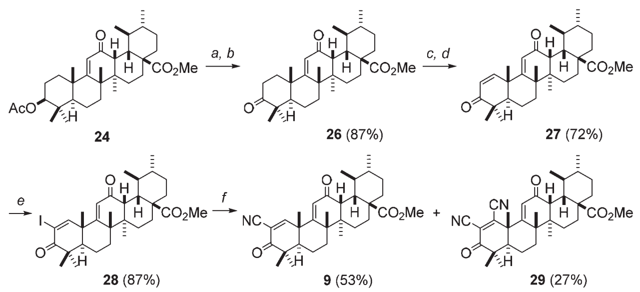
The anti-inflammatory activity of our ursolic acid cyanoenones as measured by the inhibition of nitric oxide (NO) production in RAW 264.7 macrophages is summarized in Table 2. The orientation of the ring-C enone is important, as the activity significantly decreases when this moiety is moved from the 9(11)-en-12-one position in CDDU-methyl ester (**9**) to the 12(13)-en-11-one position in **33**. Similarly, **29** is somewhat more potent than **34**. Notably, the addition of a cyano group at C1 (**29** and **34** compared to **9** and **33**) significantly decreases biological activity, presumably because the C1-cyano group retards Michael addition with a reactive cysteine or other nucleophiles on a target protein.



Scheme 5 Synthesis of cyano enones **33** and **34**. Reagent: (a) K_2CO_3, MeOH; (b) IBX, EtOAc, reflux; (c) LiHMDS, THF, −78 °C to 0 °C; PhSeCl, THF, −78 °C; (d) H_2O_2, THF, CH_2Cl_2, 0 °C to rt; (e) I_2, DMAP, pyridine, CCl_4, 90 °C; (f) CuCN, DMF, 120 °C.



Scheme 6 Synthesis of CDDU-methyl ester analogues. Reagent: (a) LiI, pyridine, reflux; (b) $(COCl)_2$, DMF, CH_2Cl_2; $EtNH_2{\cdot}HCl$, Et_3N, CH_2Cl_2; (c) $(COCl)_2$, DMF, CH_2Cl_2; $CF_3CH_2NH_2{\cdot}HCl$, Et_3N, CH_2Cl_2; (d) $(COCl)_2$, DMF, CH_2Cl_2; imidazole, PhH; (e) $(COCl)_2$, DMF, CH_2Cl_2; NH_3, MeOH; (f) TFAA, Et_3N, CH_2Cl_2; (g) DDQ, PhH, reflux.



Scheme 4 Synthesis of CDDU-methyl ester (**9**). Reagent: (a) K_2CO_3, MeOH; (b) IBX, EtOAc, reflux; (c) LiHMDS, THF, −78 °C to 0 °C; PhSeCl, THF, −78 °C; (d) H_2O_2, THF, CH_2Cl_2, 0 °C to rt; (e) I_2, DMAP, pyridine, CCl_4, 90 °C; (f) CuCN, DMF, 120 °C.

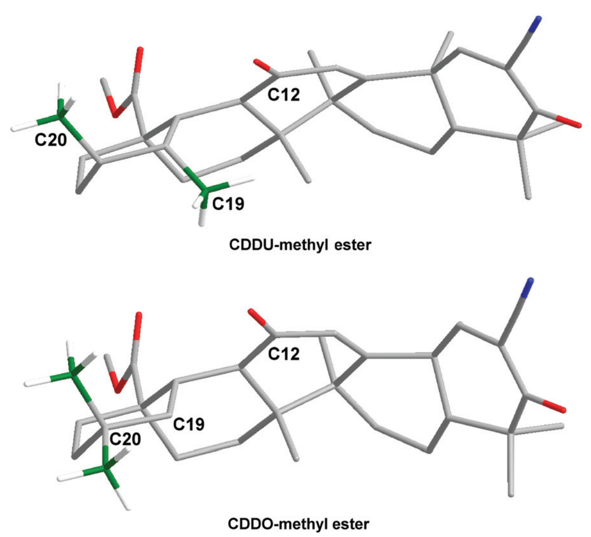
Table 2 Biological potency of triterpenoids with a comparison between ursolic and oleanolic analogues[a]

UA cmpds	IC_{50} (nM)	OA cmpds	IC_{50} (nM)
9	17 ± 1	CDDO-Me	4 ± 1.6
29	380 ± 38		
33	61 ± 7		
34	304 ± 38		
10	54 ± 2	CDDO-EA	5 ± 3
11	26 ± 4	CDDO-TFEA	8 ± 4
12	5 ± 0.2	CDDO-Im	1 ± 0.4
35	251 ± 41	CDDO	95 ± 21
36	48 ± 5	CDDO-$CONH_2$	7 ± 3
37	7 ± 0.4	CDDO-CN	1 ± 0.05
38	8 ± 2		

[a] IC_{50} values were determined by serial dilutions of the compounds (range 1 nM–1 μM). All experiments were repeated at least 3 times.

Compounds **9**, **10**, **11**, **12**, **35**, **36**, **37**, and **38** are all active at nanomolar concentrations in this assay (Table 2). Imidazolide **12**, nitrile **37**, and lactone **38** are the most potent. CDDU-Me (**9**) is slightly less active than the former three compounds, but more active than CDDU (**35**) itself.

As shown in Table 2, the oleanolic acid analogues are uniformly more potent than the corresponding ursolic acid derivatives, but with a similar trend. Thus, imidazolides (**12** and CDDO-Im), nitriles (**37** and CDDO-CN), and a lactone (**38**) are the most potent. As the only difference between the two scaffolds is the C19 axial methyl substituent for CDDU-methyl ester (**9**) *versus* C20 equatorial methyl for CDDO-methyl ester (Scheme 7), this simple transposition affects the biological activity by 5–10 fold. This result may be useful for future studies of structure–activity relationships related to the methyl substituents on oleanolic acid and ursolic acid. Especially important would seem to be the modification of the gem dimethyl group at C4, since the origin of the biological activity is primarily due to the reversible Michael addition mechanism at C-1 on ring-A.



Scheme 7 3D models for CDDU-methyl ester and CDDO-methyl ester.

Conclusions

An efficient synthesis of methyl 2-cyano-3,12-dioxoursol-1,9-dien-28-oate (CDDU-methyl ester) from commercially available ursolic acid is disclosed, which provides access to ursolic acid-derived cyanoenone analogues with C-ring enone activation. The conversion of the C ring C11–C12 alkene to a 9(11)-en-12-one features an ozonolysis-induced oxidation followed by enone formation mediated by pyridinium tribromide. Biological studies of these ursolic acid analogues display a similar structure–activity relationship to the corresponding oleanolic acid analogues, but are less potent by approximately half a log. Interestingly, introduction of a cyano substituent at C1 greatly decreases the biological activity, probably due to steric blocking of C1 to a Michael addition.

Experimental section

Chemistry

All reactions were performed in a single-neck, round-bottomed flask fitted with rubber septa under a positive pressure of nitrogen, unless otherwise noted. Organic solutions were concentrated by rotary evaporation below 30 °C. Flash-column chromatography was performed using silica gel (0.04–0.063 mm, 230–400 mesh ASTM) purchased from DAWN RUSSUP Macherey-Nagel Inc. (Bethlehem, PA). Analytical thin-layer chromatography (TLC) was performed using glass backed TLC extra hard layer pre-coated with silica gel (0.25 mm, 60 Å pore size) impregnated with a fluorescent indicator. TLC plates were visualized by exposure to ultraviolet light (UV) or/and submersion in PAA (*p*-anisaldehyde) or CAM (ceric ammonium molybdate) stains followed by brief heating on a hot plate (120 °C, 10–15 s). Commercial solvents and reagents were used as received. Proton nuclear magnetic spectra (^1H NMR) were recorded at 500 MHz at 24 °C, unless otherwise noted. Chemical shifts are expressed in parts per million (ppm, δ scale) downfield from tetramethylsilane and are referenced to residual protium in the NMR solvent ($CHCl_3$, δ 7.26). Data are represented as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet and/or multiple resonances, br = broad, app = apparent), integration, coupling constant in Hertz, and assignment. Proton-decoupled carbon nuclear magnetic resonance spectra (^{13}C NMR) were recorded at 500 MHz at 24 °C unless otherwise noted. Chemical shifts are expressed in parts per million (ppm, δ 77.0). IR spectra were recorded on a Jasco FT-IR 4100 Series spectrophotometer, γ_{max} (cm^{-1}) are partially reported. High resolution mass spectra were acquired from the Mass Spectrometry Laboratory of the University of Illinois (Urbana-Champaign, IL).

Biological evaluation

NO assay. RAW 264.7 cells (5×10^5 cells per well) were plated in 96-well plates. The next day, cells were incubated with synthetic triterpenoids and 10 ng mL^{-1} IFNγ (R & D systems) for 24 h. NO was measured as nitrite by the Griess reaction.

Methyl 3-acetoxyl-12-oxoursol-9(11)-en-28-oate (24). To a stirred solution of ester **21** (500 mg, 0.98 mmol, 1.0 equiv.) in methylene chloride (10 mL) was subjected to ozonolysis at −78 °C. Upon completion of the reaction, it was allowed to slowly warm to room temperature and kept at room temperature for 3 h. The solvent was then removed to give crude inseparable reaction mixtures with the desired ketone **22** and **23** (~8 : 1). The crude mixture was dissolved in acetonitrile (10 mL) and pyridinium perbromide (416 mg, 1.30 mmol, 1.3 equiv.) was added. The resulting mixture was then heated to 50 °C for 18 h. After the completion of the reaction, it was allowed to cool to room temperature and quenched with 20% aqueous sodium thiosulfate (20 mL). It was then extracted with methylene chloride (3 × 20 mL), the combined organic extracts were washed with saturated aqueous $NaHCO_3$ (20 mL), brine (20 mL), and dried over Na_2SO_4. Removal of solvent and flash column chromatography over silica gel using hexanes–EtOAc (4 : 1 & 2 : 1) gave **24** (52 mg, 10%) and **23** (365 mg, 71%) as white solids, respectively. For **24**: ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.87 (s, 1H), 4.47 (dd, 1H, J_1 = 11.7 Hz, J_2 = 4.6 Hz), 3.63 (s, 3H), 2.91 (dd, 1H, J_1 = 11.5 Hz, J_2 = 3.2 Hz), 2.39 (d, 1H, J = 3.7 Hz), 2.04 (s, 3H), 1.87–2.00 (m, 2H), 1.72–1.80 (m, 4H), 1.36–1.70 (m, 10H), 1.14–1.29 (m, 2H), 1.21 (s, 3H), 1.12 (s, 3H), 1.08 (s, 3H), 0.90 (s, 3H), 0.88 (s, 3H), 0.83–0.90 (m, 1H), 0.87 (d, 3H, J = 5.6 Hz), 0.73 (d, 3H, J = 6.6 Hz); ^{13}C NMR (500 MHz, $CDCl_3$) δ 201.0, 179.3, 178.5, 171.1, 123.3, 80.0, 52.1, 51.2, 50.3, 50.2, 45.4, 42.4, 40.9, 40.1, 39.6, 38.9, 38.3, 37.0, 36.2, 33.0, 31.3, 28.4, 28.3, 24.6, 24.4, 24.1, 24.0, 21.5, 20.9, 19.9, 19.7, 17.7, 16.9; IR (solution, $CHCl_3$, cm^{-1}): 3019, 2366, 2340, 1717, 1652, 1558, 1540, 1520, 1507, 1456, 1217, 772, 669, 463.

Methyl 3-hydroxyl-12-oxoursol-9(11)-en-28-oate (25). To a stirred solution of enone **24** (7.0 g, 13.3 mmol, 1.0 equiv.) in methanol (100 mL) was added potassium carbonate (7.0 g, 40.0 mmol, 3.0 equiv.). The reaction mixture was allowed stir at room temperature for 24 h. After completion of the reaction, it was quenched with water (200 mL) and extracted with methylene chloride (4 × 100 mL). The combined organic extracts were washed with brine (80 mL) and dried over Na_2SO_4. Removal of solvent and flash column chromatography over silica gel using hexanes–EtOAc (2 : 1) gave alcohol **25** (5.6 g, 87%) as a white solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.80 (s, 1H), 3.56 (s, 3H), 3.13 (dd, 1H, J_1 = 11.6 Hz, J_2 = 4.5 Hz), 2.83 (dd, 1H, J_1 = 11.4 Hz, J_2 = 3.0 Hz), 2.32 (d, 1H, J = 3.8 Hz), 2.25 (brs, 1H), 1.78–1.91 (m, 2H), 1.37–1.72 (m, 12H), 1.24–1.35 (m, 2H), 1.03–1.20 (m, 2H), 1.09 (s, 3H), 1.04 (s, 3H), 1.01 (s, 3H), 0.95 (s, 3H), 0.79 (d, 3H, J = 6.1 Hz), 0.73 (s, 3H), 0.65 (d, 3H, J = 6.6 Hz); ^{13}C NMR (500 MHz, $CDCl_3$) δ 201.0, 179.9, 178.4, 123.0, 77.9, 52.0, 51.1, 50.1, 45.4, 42.3, 40.8, 40.2, 39.5, 39.4, 38.8, 36.9, 36.4, 33.0, 31.3, 28.4, 27.6, 24.5, 24.4, 23.9, 20.9, 19.9, 19.7, 18.0, 15.9; IR (solution, $CHCl_3$, cm^{-1}): 3019, 2410, 2360, 2340, 1716, 1652, 1558, 1539, 1520, 1507, 1217, 772, 669, 624, 432.

Methyl 3,12-dioxoursol-9(11)-en-28-oate (26). To a stirred solution of alcohol **25** (4.9 g, 10.1 mmol, 1.0 equiv.) in ethyl acetate (80 mL) was added iodoxybenzoic acid (3.7 mg, 13.1 mmol, 1.3 equiv.) in one portion. The resulting suspension was heated to reflux for 24 h. After completion of the reaction, it was cooled in ice bath and was then filtered through Celite. The resulting filtrate was concentrated and flash column chromatography over silica gel using hexanes–EtOAc (2 : 1) gave ketone **26** (4.8 g, 99%) as a yellowish solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.87 (s, 1H), 3.60 (s, 3H), 2.86 (dd, 1H, J_1 = 11.2 Hz, J_2 = 2.7 Hz), 2.57 (m, 1H), 2.45 (m, 2H), 2.18 (m, 1H), 1.35–1.95 (m, 14H), 1.00–1.30 (m, 2H), 1.24 (s, 3H), 1.12 (s, 3H), 1.08 (s, 3H), 1.07 (s, 3H), 1.03 (s, 3H), 0.83 (d, 3H, J = 6.1 Hz), 0.67 (d, 3H, J = 6.3 Hz); ^{13}C NMR (500 MHz, $CDCl_3$) δ215.4, 199.9, 177.9, 177.6, 124.2, 51.8, 50.9, 50.5, 49.9, 47.3, 45.3, 42.2, 40.7, 39.4, 39.3, 38.7, 36.8, 36.7, 34.1, 32.0, 31.1, 28.3, 26.5, 24.3, 24.2, 23.8, 21.4, 20.8, 19.7, 19.5, 19.0; IR (solution, $CHCl_3$, cm^{-1}): 3019, 2360, 2340, 1698, 1684, 1652, 1558, 1540, 1507, 1217, 772, 668; HRMS-ESI (calcd for $C_{31}H_{47}O_4$ $[M + H]^+$) 483.3474, found 483.3471.

Methyl 3,12-dioxoursol-1(2),9(11)-dien-28-oate (27). To a stirred solution of ketone **26** (4.8 g, 10.0 mmol, 1.0 equiv.) in anhydrous THF (150 mL) at −78 °C was slowly added LHMDS (20 mL, 1.0 M solution, 20.0 mmol, 2.0 equiv.); it was kept stirring at −78 °C for 30 min and then allowed to warm to 0 °C and kept at 0 °C for 30 min. It was then cooled back to −78 °C and phenylselenium chloride (3.8 g, 20.0 mmol, 2.0 equiv.) in anhydrous THF (10 mL) was added slowly. After the completion of the reaction, it was quenched with saturated aqueous ammonium chloride, and extracted with methylene chloride (3 × 100 mL). The combined organic extracts were washed with brine and dried over Na_2SO_4. The solvent was removed to give crude product, which was used directly for next step without further purifications. The selenite obtained above was dissolved in a 1 : 1 mixture of THF and methylene chloride (100 mL), and 30% aqueous hydrogen peroxide (5 mL) was added at 0 °C. The reaction mixture was allowed to warm to room temperature and stirred until disappearance of starting selenite. The reaction mixture was then diluted with ethyl acetate (100 mL) and washed with 20% aqueous sodium thiosulfate (10 mL), brine (10 mL), and dried over Na_2SO_4. Removal of solvent and flash column chromatography over silica gel using hexanes–EtOAc (4 : 1 & 2 : 1) gave bis-enone **27** (3.44 g, 72%) as a yellowish solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.32 (d, 1H, J = 10.7 Hz), 6.14 (s, 1H), 5.93 (d, 1H, J = 10.3 Hz), 3.66 (s, 3H), 2.95 (dd, 1H, J_1 = 11.2 Hz, J_2 = 3.2 Hz), 2.48 (d, 1H, J = 3.7 Hz), 1.89–1.98 (m, 1H), 1.70–1.84 (m, 7H), 1.44–1.65 (m, 4H), 1.40 (s, 3H), 1.10–1.29 (m, 3H), 1.20 (s, 3H), 1.18 (s, 3H), 1.12 (s, 3H), 1.11 (s, 3H), 0.88 (d, 3H, J = 6.1 Hz), 0.73 (d, 3H, J = 6.3 Hz); ^{13}C NMR (500 MHz, $CDCl_3$) δ 203.8, 200.0, 178.4, 173.0, 155.4, 126.5, 124.4, 52.1, 51.4, 50.2, 48.4, 45.8, 44.9, 42.5, 42.1, 41.1, 39.6, 38.9, 36.9, 32.2, 31.3, 28.5, 28.0, 27.5, 25.3, 24.0, 21.8, 20.9, 20.0, 19.6, 18.5; IR (solution, $CHCl_3$, cm^{-1}): 3019, 2360, 2340, 1716, 1699, 1652, 1558, 1540, 1520, 1507, 1216, 770, 668, 451; HRMS-ESI (calcd for $C_{31}H_{45}O_4$ $[M + H]^+$) 481.3318, found 481.3306.

Methyl 2-iodo-3,12-dioxoursol-1(2),9(11)-dien-28-oate (28). To a stirred solution of bisenone **27** (1.07 g, 2.3 mmol, 1.0 equiv.) in a 1 : 1 mixture of pyridine and carbon tetrachloride (10 mL) was added dimethylaminopyridine (56 mg, 0.46 mmol, 0.2 equiv.) and iodine (1.75 g, 6.9 mmol, 3.0 equiv.), and the resulting mixture was heated to 90 °C for 24 h without light. After the completion of the reaction, the solvent was removed under vacuum, and the residue was diluted with ethyl acetate (100 mL). The resulting solution was successively washed with 20% aqueous sodium thiosulfate (3 × 10 mL), 1 N aqueous HCl (3 × 10 mL), saturated $NaHCO_3$ (10 mL), brine (10 mL), and dried over Na_2SO_4. Removal of solvent and flash column chromatography over silica gel using hexanes–EtOAc (4 : 1 & 2 : 1) gave iodo-enone **28** (1.18 g, 87%) as a yellowish solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.11 (s, 1H), 6.11 (s, 1H), 3.66 (s, 3H), 2.95 (dd, 1H, J_1 = 11.3 Hz, J_2 = 3.0 Hz), 2.48 (d, 1H, J = 3.7 Hz), 1.90–1.99 (m, 1H), 1.70–1.87 (m, 7H), 1.59–1.65 (m, 1H), 1.44–1.57 (m, 3H), 1.41 (s, 3H), 1.15–1.26 (m, 3H), 1.18 (s, 3H), 1.22 (s, 3H), 1.17 (s, 3H), 1.13 (s, 3H), 0.89 (d, 3H, J = 6.1 Hz), 0.75 (d, 3H, J = 6.6 Hz); ^{13}C NMR (500 MHz, $CDCl_3$) δ 199.8, 197.0, 178.3, 171.5, 163.8, 124.6, 102.4, 52.2, 51.3, 50.2, 38.3, 36.5, 45.9, 45.4, 42.5, 41.2, 39.6, 38.9, 36.9, 31.9, 31.3, 28.6, 28.5, 28.0, 25.3, 24.0, 22.4, 20.9, 20.0, 19.7, 18.7; IR (solution, $CHCl_3$, cm^{-1}): 3019, 2360, 2340, 1717, 1652, 1540, 1520, 1507, 1217, 770, 669; HRMS-ESI (calcd for $C_{31}H_{44}IO_4$ $[M + H]^+$) 607.2284, found 607.2280.

Methyl 2-cyano-3,12-dioxoursol-1(2),9(11)-dien-28-oate (9) and methyl 1,2-dicyano-3,12-dioxoursol-1(2),9(11)-dien-28-oate (29). To a stirred solution of iodoenone **28** (322 mg, 0.5 mmol, 1.0 equiv.) in anhydrous dimethylformamide (5 mL) was added copper(I) cyanide (54 mg, 0.6 mmol, 1.2 equiv.), and the resulting mixture was allowed to heat to 120 °C for 12 h. After the completion of the reaction, it was cooled to room temperature, diluted with ethyl acetate (100 mL). The resulting solution was washed with water (3 × 10 mL), brine (3 × 10 mL), and dried over Na_2SO_4. Removal of solvent and flash column chromatography over silica gel using hexanes–EtOAc (4 : 1 & 2 : 1) gave cyanoenone **9** (142 mg, 53%) and biscyanoenone **29** (76 mg, 27%) as yellowish solids, respectively. For **9**: ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.04 (s, 1H), 6.11 (s, 1H), 3.67 (s, 3H), 2.96 (dd, 1H, J_1 = 11.5 Hz, J_2 = 2.7 Hz), 2.51 (d, 1H, J = 3.7 Hz), 1.91–1.99 (m, 1H), 1.72–1.85 (m, 7H), 1.60–1.68 (m, 1H), 1.47–1.58 (m, 3H), 1.48 (s, 3H), 1.16–1.30 (m, 2H), 1.26 (s, 3H), 1.21 (s, 3H), 1.18 (s, 3H), 1.12 (s, 3H), 0.90 (d, 3H, J = 6.1 Hz), 0.83–0.90 (m, 1H), 0.74 (d, 3H, J = 6.6 Hz); ^{13}C NMR (500 MHz, $CDCl_3$) δ 199.3, 196.9, 178.3, 169.8, 166.4, 124.9, 115.1, 114.7, 52.2, 51.4, 50.2, 47.9, 46.0, 45.3, 42.9, 42.6, 41.2, 39.6, 38.9, 36.9, 31.9, 31.3, 28.5, 27.6, 27.5, 25.4, 23.9, 21.8, 20.9, 20.0, 19.6, 18.4; IR (solution, $CHCl_3$, cm^{-1}): 3019, 2360, 2340, 1716, 1652, 1558, 1540, 1520, 1507, 1217, 772, 669, 464; HRMS-ESI (calcd for $C_{32}H_{44}NO_4$ $[M + H]^+$) 506.3270, found 506.3267. For **29**: ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.22 (s, 1H), 3.68 (s, 3H), 3.02 (dd, 1H, J_1 = 11.6 Hz, J_2 = 2.0 Hz), 2.51 (d, 1H, J = 3.4 Hz), 1.74–2.02 (m, 7H), 1.40–1.67 (m, 4H), 1.62 (s, 3H), 1.18–1.34 (m, 2H), 1.26 (s, 3H), 1.22 (s, 3H), 1.21 (s, 3H), 1.17 (s, 3H), 0.83–0.97 (m, 1H), 0.90 (d, 3H, J = 5.9 Hz), 0.74 (d, 3H, J = 6.6 Hz); ^{13}C NMR (500 MHz, $CDCl_3$) δ 198.0, 195.2, 178.3, 166.3, 146.8, 126.9, 124.6, 113.9, 112.1, 52.3, 51.6, 50.3, 47.3, 46.4, 46.2, 46.1, 42.7, 42.1, 39.8, 38.8, 36.8, 31.4, 29.1, 28.8, 26.6, 26.2, 26.1, 24.1, 21.0, 20.9, 20.0, 19.3, 19.2; IR (solution, $CHCl_3$, cm^{-1}): 3019, 2929, 2360, 2340, 1732, 1716, 1683, 1668, 1653, 1558, 1540, 1520, 1507, 1472, 1456, 1217, 772, 669, 473; HRMS-ESI (calcd for $C_{33}H_{43}N_2O_4$ $[M + H]^+$) 531.3474, found 531.3468.

2-Cyano-3,12-dioxoursol-1(2),9(11)-dien-28-carboxylic acid (35). To a stirred solution of CDDU-methyl ester (**9**) (6.0 g, 11.9 mmol, 1.0 equiv.) in pyridine (50 mL) was added LiI (16.0 g, 0.12 mol, 10.0 equiv.) and the resulting suspension was heated to reflux for 16 h. Additional LiI (3.2 g, 23.8 mmol, 2.0 equiv.) was added and the heating was continued for another 8 h. After the completion of the reaction, it was cooled to room temperature, and the solvent was removed by vacuum. The reaction was quenched with 2 N HCl (200 mL), and extracted with ethyl acetate (4 × 100 mL). The combined organic extracts were washed with saturated aqueous $NaHCO_3$ (50 mL), brine (50 mL), and dried over Na_2SO_4. Removal of solvent and flash column chromatography over silica gel using hexanes–EtOAc (2 : 1 & 1 : 1) gave acid **35** (4.7 g, 81%) as a yellowish solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.03 (s, 1H), 6.12 (s, 1H), 2.95 (dd, 1H, J_1 = 11.0 Hz, J_2 = 2.5 Hz), 2.59 (d, 1H, J = 3.4 Hz), 1.66–2.00 (m, 9H), 1.43–1.62 (m, 2H), 1.47 (s, 3H), 1.18–1.34 (m, 3H), 1.26 (s, 3H), 1.20 (s, 3H), 1.19 (s, 3H), 1.13 (s, 3H), 0.82–0.94 (m, 1H), 0.90 (d, 3H, J = 5.8 Hz), 0.75 (d, 3H, J = 6.4 Hz); ^{13}C NMR (500 MHz, $CDCl_3$) δ 199.3, 196.8, 170.0, 166.3, 124.8, 115.2, 114.6, 51.4, 47.9, 46.0, 45.3, 42.8, 42.6, 41.2, 39.5, 38.8, 37.0, 31.8, 31.2, 28.5, 27.6, 25.5, 23.8, 21.8, 20.9, 20.0, 19.6, 18.4; IR (solution, $CHCl_3$, cm^{-1}): 3019, 2360, 2340, 1716, 1698, 1684, 1652, 1558, 1540, 1520, 1507, 1456, 1217, 772, 669; HRMS-ESI (calcd for $C_{31}H_{42}NO_4$ $[M + H]^+$) 492.3114, found 492.3108.

2-Cyano-3,12-dioxoursol-1(2),9(11)-dien-28-carboxylic acid ethyl amide (10). To a stirred solution of acid **35** (400 mg, 0.81 mmol, 1.0 equiv.) in methylene chloride (15 mL) was added oxalyl chloride (0.35 mL, 4.05 mmol, 5.0 equiv.) and anhydrous dimethylformamide (0.11 μL, 2.0 μmol, catalytic) slowly at 0 °C, and it was allowed to warm to room temperature for 2 h. The solvent was removed, and toluene (10 mL) was added and removed by vacuum, which was repeated for three times to provide the corresponding acyl chloride. To a stirred solution of the resulting acyl chloride obtained above in methylene chloride (10 mL) was added triethylamine (0.58 mL, 4.05 mmol, 5.0 equiv.) followed by slow addition of ethylamine hydrochloride (340 mg, 4.05 mmol, 5.0 equiv.) at 0 °C. After the addition, the resulting mixture was allowed to warm to room temperature and kept stirring until the disappearance of acyl chloride. After the completion of the reaction, the solvent was removed and it was diluted with methylene chloride (80 mL), washed with 1 N HCl (10 mL), saturated aqueous $NaHCO_3$ (10 mL), brine (10 mL), and dried over Na_2SO_4. Removal of solvent and flash column chromatography over silica gel using hexanes–EtOAc (2 : 1 & 1 : 1) gave ethyl amide **10** (418 mg, 99%) as a yellowish solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.49 (s, 1H), 6.36 (s, 1H), 6.16 (t, 1H, J = 5.5 Hz), 3.33 (m, 1H), 3.21 (m, 1H), 2.68 (dd, 1H, J_1 = 10.7 Hz, J_2 = 2.2 Hz), 2.62 (d, 1H, J = 3.4 Hz), 1.60–1.99 (m, 9H), 1.46–1.60 (m, 2H), 1.44 (s, 3H), 1.05–1.30 (m, 3H), 1.28 (s, 3H), 1.24 (s, 3H), 1.19 (s, 3H), 1.10 (s, 3H), 0.85–0.95 (m, 1H), 0.90 (d, 3H, J = 6.1 Hz), 0.68 (d, 3H, J = 6.6 Hz); ^{13}C NMR (500 MHz, $CDCl_3$) δ 200.5, 197.1, 177.4, 171.1, 168.1, 124.9, 115.1, 114.8, 51.0, 49.3, 47.8, 46.2, 45.3, 43.8, 43.2, 41.2, 39.9, 39.0, 37.9, 34.6, 31.9, 31.4, 29.6, 28.2, 27.4, 27.3, 25.8, 23.7, 21.8, 21.0, 19.5, 19.4, 15.2; IR (solution, $CHCl_3$, cm^{-1}): 3019, 2979, 2360, 2340, 1698, 1684, 1652, 1636, 1558, 1540, 1520, 1507, 1456, 1217, 771, 669, 444; HRMS-ESI (calcd for $C_{33}H_{47}N_2O_3$ [M + H]$^+$) 519.3587, found 519.3585.

2-Cyano-3,12-dioxoursol-1(2),9(11)-dien-28-carboxylic acid trifluoroethyl amide (11). To a stirred solution of acid **35** (400 mg, 0.81 mmol, 1.0 equiv.) in methylene chloride (15 mL) was added oxalyl chloride (0.35 mL, 4.05 mmol, 5.0 equiv.) and anhydrous dimethylformamide (0.11 μL, 2.0 μmol, catalytic) slowly at 0 °C, and it was allowed to warm to room temperature for 2 h. The solvent was removed, and toluene (10 mL) was added and removed by vacuum, which was repeated for three times to provide the corresponding acyl chloride. To a stirred solution of the resulting acyl chloride obtained above in methylene chloride (10 mL) was added triethylamine (0.58 mL, 4.05 mmol, 5.0 equiv.) followed by slow addition of trifluoroethylamine hydrochloride (340 mg, 4.05 mmol, 5.0 equiv.) at 0 °C. After the addition, the resulting mixture was allowed to warm to room temperature and kept stirring until the disappearance of acyl chloride. After the completion of the reaction, the solvent was removed and it was diluted with methylene chloride (80 mL), washed with 1 N HCl (10 mL), saturated aqueous $NaHCO_3$ (10 mL), brine (10 mL), and dried over Na_2SO_4. Removal of solvent and flash column chromatography over silica gel using hexanes–EtOAc (2 : 1 & 1 : 1) gave trifluoroethyl amide **11** (387 mg, 83%) as a yellowish solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.14 (s, 1H), 6.92 (t, 1H, J = 6.5 Hz), 6.74 (t, 1H, J = 5.5 Hz), 4.20 (m, 1H), 3.55 (m, 1H), 2.89 (dd, 1H, J_1 = 9.9 Hz, J_2 = 2.8 Hz), 2.48 (d, 1H, J = 3.2 Hz), 1.93–2.15 (m, 1H), 1.69–1.92 (m, 6H), 1.47–1.65 (m, 4H), 1.55 (s, 3H), 1.05–1.30 (m, 3H), 1.29 (s, 3H), 1.23 (s, 3H), 1.18 (s, 3H), 1.05 (s, 3H), 0.85–0.95 (m, 1H), 0.87 (d, 3H, J = 6.6 Hz), 0.57 (d, 3H, J = 6.6 Hz); ^{13}C NMR (500 MHz, $CDCl_3$) δ 200.3, 197.2, 178.2, 171.2, 169.4, 125.0, 124.5 (q), 115.6, 114.4, 51.1, 49.8, 47.7, 46.1, 45.3, 43.4, 43.3, 41.1, 40.5 (s), 39.7, 38.7, 37.4, 31.9, 31.3, 29.6, 27.9, 27.1, 27.0, 25.9, 23.7, 21.8, 20.9, 19.4, 18.3; IR (solution, $CHCl_3$, cm^{-1}): 3019, 2957, 2869, 2360, 2340, 1652, 1558, 1539, 1520, 1507, 1465, 1436, 1376, 1364, 1217, 772, 669; HRMS-ESI (calcd for $C_{33}H_{44}N_2F_3O_3$ [M + H]$^+$) 573.3304, found 573.3307.

2-Cyano-3,12-dioxoursol-1(2),9(11)-dien-28-carboxyl-13-oxyl-actone (38). To a stirred solution of acid **35** (250 mg, 0.51 mmol, 1.0 equiv.) in benzene (15 mL) was added DDQ (136 mg, 0.61 mmol, 1.2 equiv.) and the resulting mixture was heated to reflux for 24 h. After the completion of the reaction, it was cooled to room temperature and filtered through Celite. Removal of solvent and flash column chromatography over silica gel using hexanes–EtOAc (4 : 1 & 2 : 1) gave the desired product **38** (175.5 mg, 71%) as a reddish solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.05 (s, 1H), 6.28 (s, 1H), 2.56 (d, 1H, J = 11.7 Hz), 2.12 (m, 1H), 1.71–1.92 (m, 4H), 1.60–1.70 (m, 3H), 1.57 (s, 3H), 1.50 (s, 3H), 1.39–1.50 (m, 2H), 1.22–1.44 (m, 1H), 1.28 (s, 3H), 1.21 (s, 3H), 1.18 (s, 3H), 1.00–1.10 (m, 1H), 0.95 (d, 3H, J = 6.3 Hz), 0.82–0.90 (m, 1H), 0.82 (d, 3H, J = 6.4 Hz); ^{13}C NMR (500 MHz, $CDCl_3$) δ 196.6, 192.2, 178.3, 174.3, 165.7, 123.4, 115.4, 114.5, 87.8, 55.1, 48.1, 46.4, 45.3, 45.1, 43.2, 42.9, 39.8, 37.5, 32.8, 31.3, 31.2, 30.8, 28.0, 27.6, 26.3, 21.9, 21.8, 20.3, 19.5, 18.6, 17.8; IR (solution, $CHCl_3$, cm^{-1}): 3019, 2969, 2936, 2360, 2340, 1772, 1715, 1697, 1675, 1652, 1558, 1540, 1520, 1507, 1456, 1215, 909, 749, 669; HRMS-ESI (calcd. for $C_{31}H_{40}NO_4$ [M + H]$^+$) 490.2957, found 490.2961.

2-Cyano-3,12-dioxoursol-1(2),9(11)-dien-28-carboxamide (36). To a stirred solution of acid **35** (860 mg, 1.75 mmol, 1.0 equiv.) in methylene chloride (15 mL) was added oxalyl chloride (0.50 mL, 5.72 mmol, 3.3 equiv.) and anhydrous dimethylformamide (0.15 μL, 2.7 μmol, catalytic) slowly at 0 °C, and it was allowed to warm to room temperature for 2 h. The solvent was removed, and toluene (10 mL) was added and removed by vacuum, which was repeated for three times to provide the corresponding acyl chloride. To a stirred solution of the resulting acyl chloride obtained above in methylene chloride (20 mL) was added ammonia (8.75 mL, 1.0 M in MeOH, 8.75 mmol, 5.0 equiv.), and the resulting mixture was stirred at room temperature until the disappearance of acyl chloride. After the completion of the reaction, the solvent was removed and flash column chromatography over silica gel using hexanes–EtOAc (1 : 1 & 1 : 2) gave amide **36** (791 mg, 92%) as a yellowish solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.06 (s, 1H), 6.28 (s, 1H), 2.51 (d, 1H, J = 11.7 Hz), 2.10 (m, 1H), 1.69–1.90 (m, 6H), 1.51–1.67 (m, 4H), 1.54 (s, 3H), 1.34–1.50 (m, 2H), 1.46 (s, 3H), 1.10–1.30 (m, 2H), 1.23 (s, 3H), 1.17 (s, 3H), 1.15 (s, 3H), 0.82–0.92 (m, 1H), 0.91 (d, 3H, J = 6.3 Hz), 0.78 (d, 3H, J = 6.1 Hz); ^{13}C NMR (500 MHz, $CDCl_3$) δ 200.3, 197.0, 180.4, 171.1, 167.7, 124.9, 115.0, 114.9, 51.2, 49.8, 47.8, 46.2, 45.3, 43.9, 43.1, 41.2, 39.9, 39.0, 37.9, 31.9, 31.4, 28.2, 27.5, 25.9, 23.7, 21.8, 20.9, 20.1, 19.5, 18.4; IR (solution, $CHCl_3$, cm^{-1}): 3019, 2360, 2340, 1652, 1558, 1540, 1507, 1217, 771, 669, 429; HRMS-ESI (calcd for $C_{31}H_{43}N_2O_3$ [M + H]$^+$) 491.3114, found 491.3105.

2-Cyano-3,12-dioxoursol-1(2),9(11)-dien-28-nitrile (37). To a stirred solution of amide **36** (600 mg, 1.22 mmol, 1.0 equiv.) in methylene chloride (40 mL) at 0 °C was added triethylamine (0.44 mL, 3.13 mmol, 2.5 equiv.) and trifluoroacetic anhydride (0.26 mL), and the resulting mixture was stirred at 0 °C until the disappearance of starting material. After the completion of the reaction, it was quenched with saturated aqueous $NaHCO_3$ (20 mL), and extracted with methylene chloride (3 × 20 mL). The combined organic extracts were washed with brine (10 mL) and dried over Na_2SO_4. Removal of solvent and flash column chromatography over silica gel using hexanes–EtOAc (4 : 1 & 2 : 1) gave the desired product **37** (407 mg, 89%) as a white solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.06 (s, 1H), 6.16 (s, 1H), 3.17 (d, 1H, J = 3.7 Hz), 2.72 (dd, 1H, J_1 = 11.2 Hz, J_2 = 3.2 Hz), 1.95–2.18 (m, 4H), 1.74–1.86 (m, 3H), 1.55–1.66 (m, 3H), 1.53 (s, 3H), 1.34–1.50 (m, 2H), 1.45 (s, 3H), 1.27 (s, 3H), 1.16–1.26 (m, 2H), 1.20 (s, 3H), 1.12 (s, 3H), 0.92 (d, 3H, J = 5.6 Hz), 0.82–0.92 (m, 1H), 0.76 (d, 3H, J = 6.6 Hz); ^{13}C NMR (500 MHz, $CDCl_3$) δ 198.1, 196.8, 170.4, 166.1, 125.0, 124.8, 115.2, 114.6, 51.5, 48.0, 46.1, 45.3, 44.3, 43.0, 41.5, 41.2, 39.0, 38.7, 36.7, 32.0, 30.6, 28.8, 27.6, 27.5, 25.8, 25.6, 21.8, 20.6, 19.8, 19.6, 18.4; IR (solution, $CHCl_3$, cm^{-1}): 3019, 2360, 2340, 1697, 1684, 1662, 1653, 1558, 1540, 1520, 1507, 1473, 1456, 1217, 909, 772, 669, 450; HRMS-ESI (calcd for $C_{31}H_{41}N_2O_2$ $[M + H]^+$) 473.3168, found 473.3165.

2-Cyano-3,12-dioxoursol-1(2),9(11)-dien-28-carboxylic acid imidazolide (12). To a stirred solution of acid **35** (460 mg, 1.0 mmol, 1.0 equiv.) in methylene chloride (20 mL) was added oxalyl chloride (0.50 mL, 5.8 mmol, 5.8 equiv.) and anhydrous dimethylformamide (0.15 μL, 2.0 μmol, catalytic) slowly at 0 °C, and it was allowed to warm to room temperature for 2 h. The solvent was removed, and toluene (10 mL) was added and removed by vacuum, which was repeated for three times to provide the corresponding acyl chloride. To a stirred solution of the resulting acyl chloride obtained above in benzene (10 mL) was added imidazole (350 mg, 5.0 mmol, 5.0 equiv.) and the resulting mixture was stirred at room temperature until the disappearance of acyl chloride. After the completion of the reaction, removal of solvent and flash column chromatography over silica gel using hexanes–EtOAc (4 : 1 & 2 : 1) gave imidazolide **12** (458 mg, 90%) as a yellowish solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.34 (s, 1H), 8.05 (s, 1H), 7.66 (s, 1H), 7.06 (s, 1H), 6.10 (s, 1H), 3.27 (d, 1H, J = 9.8 Hz), 2.43 (s, 1H), 0.98–2.06 (m, 11H), 1.44 (s, 3H), 1.23 (s, 3H), 1.16 (s, 9H), 0.96 (d, 3H, J = 5.1 Hz), 0.78 (d, 3H, J = 5.9 Hz); ^{13}C NMR (500 MHz, $CDCl_3$) δ 197.9, 196.8, 175.5, 170.2, 166.3, 137.8, 130.6, 124.6, 117.9, 115.1, 114.6, 52.6, 51.1, 47.9, 46.0, 45.2, 43.1, 42.9, 41.3, 39.9, 38.8, 35.8, 31.7, 31.0, 28.1, 27.5, 27.5, 25.4, 21.8, 20.8, 20.0, 19.7, 18.3; IR (solution, $CHCl_3$, cm^{-1}): 3019, 2360, 2340, 1716, 1698, 1652, 1558, 1540, 1520, 1507, 1214, 909, 773, 669, 438; HRMS-ESI (calcd for $C_{34}H_{44}N_3O_3$ $[M + H]^+$) 542.3383, found 542.3377.

Acknowledgements

We acknowledge support from Reata Pharmaceuticals.

Notes and references

1 R. A. Hill and J. D. Connolly, Triterpenoids, *Nat. Prod. Rep.*, 2012, **29**, 780–818; and previous reviews in this series.

2 (*a*) J. D. Connolly and K. H. Overton, The Triterpenoids, in *Chemistry of Terpenes and Terpenoids*, ed. A. A. Newman, Academic Press, New York, 1972, pp. 207–287; (*b*) K. Nakanishi and S. Ito, Biosynthesis of oleanane and ursane triterpenes in tissue culture of Isodon japonicas, in *Natural Products Chemistry*, ed. K. Nakanishi, T. Goto, S. Ito, S. Natori and S. Nozoe, Kodansha, Tokyo, 1983, vol. 3, pp. 185–187.

3 T. Honda, H. J. Finlay, G. W. Gribble, N. Suh and M. B. Sporn, New enone derivatives of oleanolic acid and ursolic acid as inhibitors of nitric oxide production in mouse macrophages, *Bioorg. Med. Chem. Lett.*, 1997, **7**, 1623–1628.

4 M. B. Sporn, K. T. Liby, M. M. Yore, L. Fu, J. M. Lopchuk and G. W. Gribble, New synthetic triterpenoids: potent agents for prevention and treatment of tissue injury caused by inflammatory and oxidative stress, *J. Nat. Prod.*, 2011, **74**, 537–545; and references therein.

5 T. Honda, B. V. Rounds, L. Bore, H. J. Finlay, F. G. Favaloro Jr., N. Suh, Y. Wang, M. B. Sporn and G. W. Gribble, Synthetic oleanane and ursane triterpenoids with modified ring A and C: a series of highly active inhibitors of nitric oxide production in mouse macrophages, *J. Med. Chem.*, 2000, **43**, 4233–4246.

6 D. S. Hong, R. Kurzrock, J. G. Supko, X. He, A. Naing, J. Wheler, D. Lawrence, J. P. Eder, C. J. Meyer, D. A. Ferguson, J. Mier, M. Konopleva, S. Konoplev, M. Andreeff, D. Kufe, H. Lazarus, G. I. Shapiro and B. J. Dezube, A phase I first-in-human trial of bardoxolone methyl in patients with advanced solid tumors and lymphomas, *Clin. Cancer Res.*, 2012, **18**, 3396–3406.

7 P. E. Pergola, P. Raskin, R. D. Toto, C. J. Meyer, J. W. Huff, E. B. Grossman, M. Krauth, S. Ruiz, P. Audhya, H. Christ-Schmidt, J. Wittes and D. G. Warnock, Bardoxolone methyl and kidney function in CKD with type 2 diabetes, *N. Engl. J. Med.*, 2011, **365**, 327–336.

8 T. Honda, Y. Honda, F. G. Favaloro Jr., G. W. Gribble, N. Suh, A. E. Place, M. H. Rendi and M. B. Sporn, A novel dicyanotriterpenoid, 2-cyano-3,12-dioxooloana-1,9(11)-dien-28-onitrile, active at picomolar concentrations for inhibition of nitric oxide production, *Bioorg. Med. Chem.*, 2002, **12**, 1027–1030.

9 K. T. Liby and M. B. Sporn, Synthetic oleanane triterpenoids: multifunctional drugs with a broad range of applications for prevention and treatment of chronic disease, *Pharmacol. Rev.*, 2012, **64**, 972–1003.

10 A. Petronelli, G. Pannitteri and U. Testa, Triterpenoids as new promising anticancer drugs, *Anti-Cancer Drugs*, 2009, **20**, 880–892.

11 M. B. Sporn, K. T. Liby, M. M. Yore, N. Suh, A. Albini, T. Honda, C. Sundararajan and G. W. Gribble, Platforms and networks in triterpenoid pharmacology, *Drug Dev. Res.*, 2007, **68**, 174–182.

12 R. D. Couch, R. G. Browning, T. Honda, G. W. Gribble, D. L. Wright, M. B. Sporn and A. C. Anderson, Studies on the reactivity of CDDO, a promising new chemopreventive and chemotherapeutic agent: implications for a molecular mechanism of action, *Bioorg. Med. Chem. Lett.*, 2005, **15**, 2215–2219.

13 J. A. R. Salvador, V. M. Moreira, B. M. F. Gonçalves, A. S. Leal and Y. Jing, Ursane-type pentacyclic triterpenoids as useful platforms to discover anticancer drugs, *Nat. Prod. Rep.*, 2012, **29**, 1463–1479.

14 (*a*) J. A. R. Salvador, V. M. Moreira, R. M. A. Pinto, A. S. Leal and C. Le Roux, Bismuth(III) triflate-based catalytic direct opening of oleanolic hydroxyl-γ-lactones to afford 12-oxo-28-carboxylic acids, *Adv. Synth. Catal.*, 2011, **353**, 2637–2642; (*b*) R. Csuk and B. Siewert, A convenient separation of ursolic and oleanolic acid, *Tetrahedron Lett.*, 2011, **52**, 6616–6618.

15 J. A. R. Salvador, V. M. Moreira, R. M. A. Pinto, A. S. Leal and J. A. Paixão, Efficient oxidation of oleanolic acid derivatives using magnesium bis(monoperoxyphthalate) hexahydrate (MMPP): a convenient 2-step procedure towards 12-oxo-28-carboxylic acid derivatives, *Beilstein J. Org. Chem.*, 2012, **8**, 164–169.

16 (*a*) S. Qian, H. Li, Y. Chen, W. Zhang, S. Yang and Y. Wu, Synthesis and biological evaluation of oleanolic acid derivatives as inhibitors of protein tyrosine phosphatase 1B, *J. Nat. Prod.*, 2010, **73**, 1743–1750; (*b*) T. Hida, Y. Fukui, K. Kawata, M. Kabaki, T. Masui, M. Fumoto and H. Nogusa, Practical application of oxidation using a novel Na_2WO_4-H_2O_2 system under neutral conditions for scale-up manufacturing of 12α-hydroxy-3-oxooleanano-28,13-lactone: key intermediate of endothelin A receptor antagonist s-0139, *Org. Process Res. Dev.*, 2010, **14**, 289–294; (*c*) A. García-Granados, P. E. López, E. Melguizo, A. Parra and Y. Simeó, Partial synthesis of C-ring derivatives from oleanolic and maslinic acids. Formation of several triene systems by chemical and photochemical isomerization processes, *Tetrahedron*, 2004, **60**, 1491–1503; (*d*) M. S. Ali, M. Jahangir, S. S. Hussan and M. I. Choudhary, Inhibition of α-glucosidase by oleanolic acid and its synthetic derivatives, *Phytochemistry*, 2002, **60**, 295–299.

17 S. Ogawa, K. Hosoi, N. Ikeda, M. Makino, Y. Fujimoto and T. Ilda, Oxyfunctionalization products of terpenoids with dimethyldioxirane and their biological activity, *Chem. Pharm. Bull.*, 2007, **55**, 247–250.

18 J. A. R. Salvador, R. M. A. Pinto, R. C. Santos, C. Le Roux, A. M. Beja and J. A. Paixão, Bismuth triflate-catalyzed Wagner-Meerwein rearrangement in terpenes. Application to the synthesis of the 18α-oleanane core and A-*neo*,-18α-oleanene compounds from lupanes, *Org. Biomol. Chem.*, 2009, **7**, 508–517.

19 B. G. Bag, P. P. Dey, S. K. Dinda, W. S. Sheldrick and I. M. Oppel, A simple route for renewable nano-sized arjunolic and Asiatic acids and self-assembly of arjuna-bromolactone, *Beilstein J. Org. Chem.*, 2008, **4**, 24–28.

20 (*a*) I. Okamoto, T. Takeya, Y. Kagawa and E. Kotani, Iron (III) picolinate-induced oxygenation and subsequent rearrangement of triterpenoid derivatives with hydrogen peroxide, *Chem. Pharm. Bull.*, 2000, **48**, 120–125; (*b*) S. Siddiqui, S. Begum, F. Hafeez and B. S. Siddiqui, Two triterpenes from the leaves of *Nerium Oleander*, *Phytochemistry*, 1989, **28**, 1187–1191.

21 (*a*) B. Bednarczyk-Cwynar, L. Zaprutko, P. Ruszkowski and B. Hladoń, Anticancer effect of A-ring or/and C-ring modified oleanolic acid derivatives on KB, MCF-7, and HeLa cell lines, *Org. Biomol. Chem.*, 2012, **10**, 2201–2205; (*b*) H. J. Finlay, T. Honda and G. W. Gribble, Synthesis of novel [3,2-*b*]indole fused oleanolic acids as potential inhibitors of cell proliferation, *ARKIVOC*, 2002, 38–46; (*c*) L. Zaprutko, D. Partyka and B. Bednarczyk-Cwynar, Triterpenoids. Part 21: Oleanolic acid azaderivatives as percutaneous transport promoters, *Bioorg. Med. Chem. Lett.*, 2004, **14**, 4723–4726.

22 (*a*) O. B. Kazakova, N. I. Medvedeva, O. Kukovinets, G. A. Tolstikov, E. F. Khusnutdinova, L. Zaprutko, B. Bednarczyk-Cwynar and Z. Paryzek, Chemoselective oxidation of oleanolic acid derivatives with ozone, *Chem. Nat. Compd.*, 2010, **46**, 397–399; (*b*) H.-J. Yang, B.-G. Li, X.-H. Cai, H.-Y. Qi, Y.-G. Luo and G.-L. Zhang, Chiral decalins: preparation from oleanolic acid and application in the synthesis of (−)-9-*epi*-Ambrox, *J. Nat. Prod.*, 2006, **69**, 1531–1538.

23 A. S. Leal, R. Wang, J. A. R. Salvador and Y. Jing, Semisynthetic ursolic acid fluorolactone derivatives inhibit growth with induction of $p21^{waf1}$ and induce apoptosis with upregulation of NOXA and downregulation of c-FLIP in cancer cells, *ChemMedChem*, 2012, **7**, 1635–1646.

24 A. V. Tkachev, A. Y. Denisov, Y. V. Gatilov, I. Y. Bagryanskaya, S. A. Shevtsov and T. V. Rybalova, Stereochemistry of hydrogen peroxide-acetic acid oxidation of ursolic acid and related compounds, *Tetrahedron*, 1994, **50**, 11459–11488.

25 L. Fu and G. W. Gribble, An efficient and scalable synthesis of bardoxolone methyl (CDDO-methyl ester), *Org. Lett.*, 2013, **15**, 1622–1625.

26 Y. Ding, Z. Huang, J. Yin, Y. Lai, S. Zhang, Z. Zhang, L. Fang, S. Peng and Y. Zhang, DDQ-promoted dehydrogenation from natural rigid polycyclic acids or flexible alkyl acids to generate lactones by a radical ion mechanism, *Chem. Commun.*, 2011, **47**, 9495–9497.