Design, Synthesis, and Biological Evaluation of Biotin Conjugates of 2-Cyano-3,12-dioxooleana-1,9(11)-dien-28-oic Acid for the Isolation of the Protein Targets

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2-Cyano-3,12-dioxooleana-1,9(11)-dien-28-oic acid (CDDO, **1**) and related compounds [for example, CDDO-Me (**2**) and CDDO-Im (**3**)] are potential anti-inflammatory, cancer chemopreventive, and chemotherapeutic agents. However, the mechanisms responsible for the multiple effects of CDDO are still unclear. Clarification of these mechanisms and particularly isolation of the protein targets are essential for the development of CDDO and its analogues as clinically useful drugs. Such knowledge would provide superior opportunities for designing new compounds with improved potency and selectivity. Therefore, to isolate protein targets using affinity chromatography with immobilized streptavidin as a carrier, we have designed and synthesized C-17 and C-23 biotin conjugates of CDDO (**4**, **5**, and **6**) on the basis of our established structure−activity relationships. For the synthesis of **6**, a new important precursor, 23-hydroxy-CDDO-Me (**29**) was synthesized from **20** by a C-23 oxidation protocol, which involves cyclopalladation of the C-4 methyl group from a 3-one oxime. The inhibitory activity of C-23 conjugate **6** is only about 3 times less potent than the mother compound, CDDO, against the proliferation of MCF-7 breast cancer cells. Consequently, **6** may be a very promising tool for the isolation of the protein targets of CDDO.

Introduction

Triterpenoids are natural products with 30 carbon atoms, biosynthetically derived from the cyclization of squalene.[1] Many triterpenoids are reported to have various interesting biological, pharmacological, and medicinal activities including anti-inflammatory and anticarcinogenic activities.[2] However, in many cases, the potency of these triterpenoids is relatively weak. To discover new anti-inflammatory, cancer chemopreventive, and chemotherapeutic drugs from triterpenoids, we have previously synthesized and biologically evaluated over 270 derivatives of oleanolic and ursolic acids, commercially available naturally occurring triterpenoids.[3]

In these investigations, we have found that 2-cyano-3,12-dioxooleana-1,9(11)-dien-28-oic acid (CDDO, **1**) and its related compounds [for example, CDDO-Me (**2**) and CDDO-Im (**3**)] show high inhibitory activity against production of nitric oxide (NO) induced by interferon-γ (IFN-γ) in mouse macrophages.[3b,c,e,f] We and others have also found the following biological activities of CDDO. CDDO induces differentiation of human myeloid leukemia cells and mouse 3T3-L1 fibroblasts, enhances nerve-growth-factor-induced neuronal differentiation of rat PC12 cells,[4,5] inhibits the proliferation of human myeloid leukemia and carcinoma cell lines,[4] blocks de novo synthesis of inducible nitric oxide synthase (iNOS) and inducible cyclooxygenase (COX-2) in mouse macrophages, microglia, and fibloblasts,[4] induces apoptosis of human myeloid leukemia,[6−8] osteosarcoma,[9] lung cancer,[10] and CLL cells,[11] and shows inhibitory activity against mouse peritoneal inflammation induced by thioglycollate and IFN-γ and L1210 leukemia and B16 melanoma models of murine cancer.[12]

However, the mechanisms responsible for the multiple effects of CDDO are still unclear. Clarification of these mechanisms and particularly isolation of the protein targets are essential for the development of CDDO and its analogues as clinically useful drugs. Also, such knowledge would provide superior opportunities for designing totally new compounds with improved potency and selectivity.

Affinity chromatography, based on the principle of specific protein−ligand recognition, is an efficient method for the refinement of proteins and has been successfully used for the purification of proteins, antibodies, and receptor−ligand complexes.[13] Biotinylated compounds were chosen as an effective means of selectively identifying their protein targets within the proteome of a cell because streptavidin, a 66 kDa homotetrameric protein isolated from *Streptomyces avidinni*, has a very high affinity for biotin ($K_a \approx 10^{15}$ M^{-1}),[14] and immobilized streptavidin[15] is commercially available, which permits the affinity purification of complexes of biotinylated compounds and the protein targets. Appropriately designed biotinylated compounds[16] would serve as

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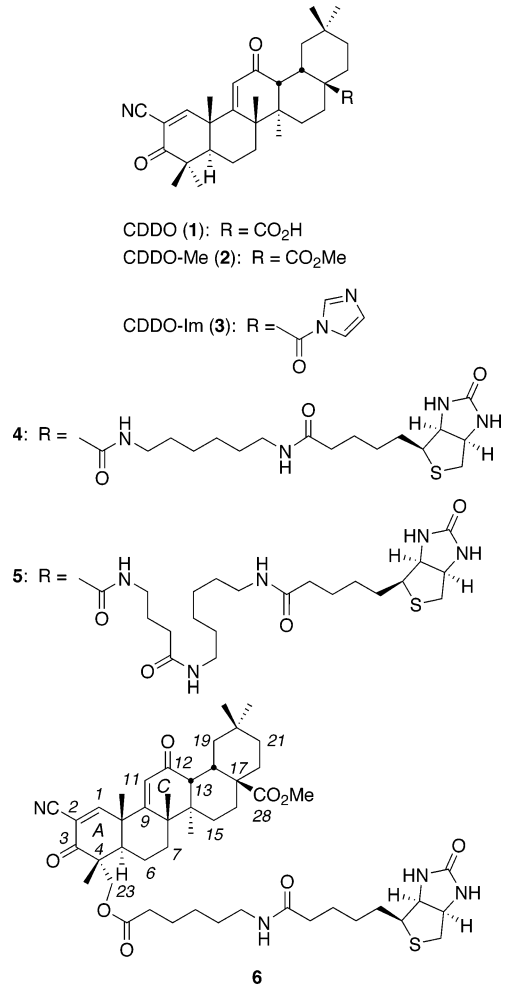


a molecular bridge between their protein target and the immobilized streptavidin and would ultimately lead to an understanding of the mode of action of the mother compound. In this methodology, it is essential to design and synthesize appropriate biotinylated compounds that retain potency in bioassays.

Therefore, we have designed two C-17 biotin conjugates of CDDO, **4** and **5**, and a C-23 biotin conjugate of CDDO-Me, **6**, for the following reasons. (1) Our preliminary structure−activity relationships show that a 2-cyano-1-en-3-one in ring A and a 9(11)-en-12-one in ring C are essential for the extremely high potency of CDDO and its biologically active analogues.[3b,c,e] (2) Although the substituents at C-17 are also important for potency, we have found that C-17 modifications do not drastically decrease the potency except for a few derivatives.[3f] (3) CDDO has only one functionality at C-17 that can be modified. (4) Two spacer lengths (**4** vs **5**) are designed in order to avoid steric interference between the protein and the bead and because it was not certain, a priori, which spacer would accomplish the task. (5) Our C-23 oxidation protocol, which involves cyclopalladation of the methyl group at C-4 from a 3-one oxime functionality,[17,18] could be applied to a known compound **20** to afford a new important precursor, 23-hydroxy-CDDO-Me (**29**) for the synthesis of **6**. (6) It has been reported that a biotinylated radicicol derivative with an ester linkage is a good affinitic probe for identification of cellular radicicol-binding proteins.[16d] (7) These syntheses, particularly that of **6**, are very interesting from the perspective of synthetic organic chemistry.

We herein describe a full account of our syntheses of biotin conjugates **4**−**6** and the interesting biological results.

Results and Discussion

Chemistry. The C-17 biotin conjugate **4** was synthesized in four steps from CDDO[3b,e] (Scheme 1). Treatment of CDDO with oxalyl chloride in CH_2Cl_2 gave acyl chloride **7** quantitatively. Condensation of **7** with commercially available 6-[(*tert*-butoxycarbonyl)amino]hexylamine hydrochloride in aqueous $NaHCO_3$ solution and benzene afforded **8** in 81% yield.

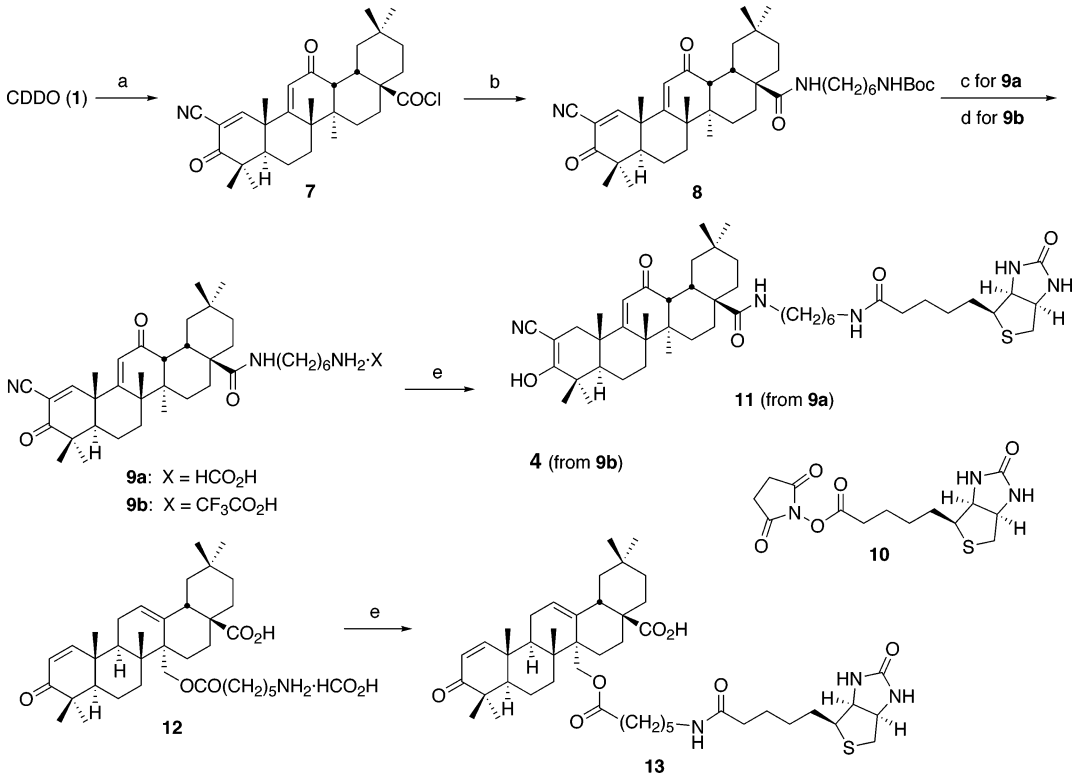
Initially, we removed the Boc group of **8** with formic acid to give the formic acid salt **9a** (quantitative yield). Unexpectedly, condensation of **9a** and 1.2 equiv of commercially available *N*-hydroxysuccinimidobiotin (**10**) in the presence of DMAP in pyridine gave **11** with a reduced ring A as a major product (76% yield) and the desired conjugate **4** as a minor product (6% yield). Other conditions (e.g., pyridine without DMAP, DMAP in DMF) for the condensation of **9a** and **10** also gave the same reduced conjugate **11**. The carbonyl group at C-3 of **11** is completely enolized in CD_3OD because in the ^1H NMR spectrum two protons at C-1 were observed as an AB quartet (J = 15.0 Hz) at 2.43 and 2.27 ppm.

Because formic acid was thought to be a hydride source for the reduction, we used TFA for the removal of the Boc group of **8** instead of formic acid. Treatment of **8** with TFA gave TFA salt **9b**[19] quantitatively. As expected, condensation of **9b** and **10** under the same conditions as for **11** gave the desired conjugate **4** in 79% yield without coformation of **11**.

This unexpected reduction seems to be unique for the combination of the cyanoenone structure in ring A, formic acid, and *N*-hydroxysuccinimidobiotin (**10**) for the following reasons. We were able to synthesize the biotin conjugate **13** (65% yield) using the same conditions for the condensation of formic acid salt **12** without a nitrile group at C-2 and **10** (unpublished data). Also, the cyanoenone of **9a** was not reduced although excess formic acid was used for the removal of the Boc group of **8**. Finally, because three different conditions (DMAP in pyridine, pyridine, and DMAP in DMF) gave **11**, we can exclude the possibility that they participate in the reduction. The role of *N*-hydroxysuccinimidobiotin (**10**) in this reduction is unclear.

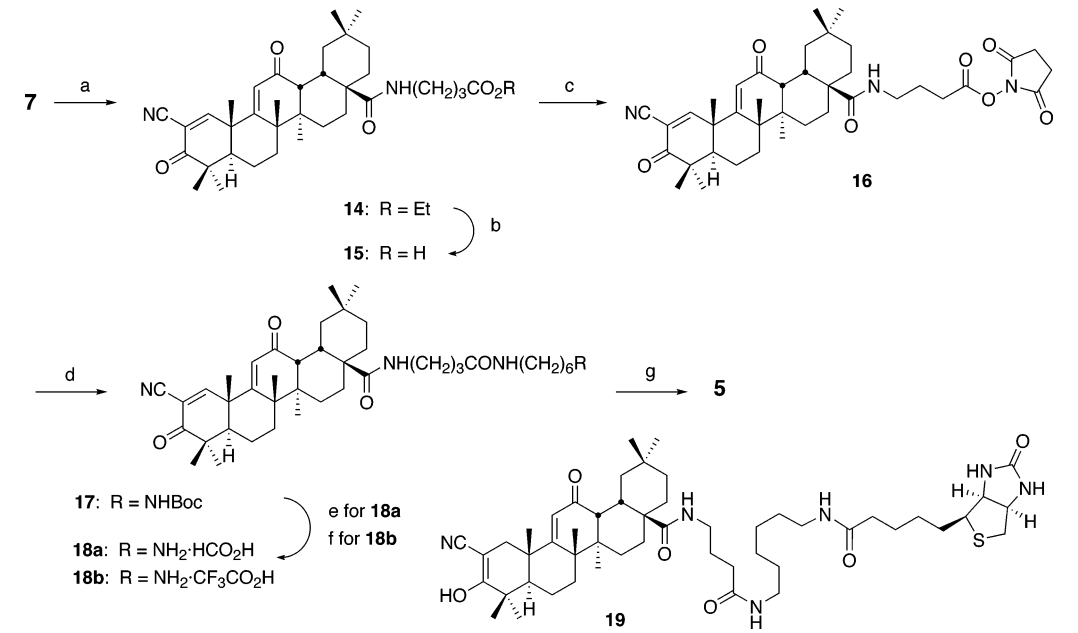
The C-17 biotin conjugate **5** with a longer spacer than **4** was synthesized in five steps from **7** (Scheme 2). Condensation of **7** and ethyl 4-aminobutanoate hydrochloride under the same conditions as for **8** gave ethyl ester **14** in 84% yield. Alkaline hydrolysis of **14** gave acid **15** in 93% yield. *N*-Succinimide ester **16** was prepared in 89% yield from **15** with *N*-hydroxysuccinimide in the presence of EDCI in DMF. Condensation of **16** and 6-[(*tert*-butoxycarbonyl)amino]hexylamine hydrochloride in aqueous $NaHCO_3$ solution and CH_2Cl_2 afforded **17** in 89% yield. Removal of the Boc group of **17** with TFA gave TFA salt **18b**[19] in quantitative yield. Condensation of **18b** and *N*-hydroxysuccinimidobiotin (**10**) under the same conditions as for **4** gave the desired conjugate **5** in 63% yield. Once again, when we used the formic acid salt **18a**, prepared from **17** with formic acid,

Scheme 1[a]



[a] Reagents: (a) $(COCl)_2$, CH_2Cl_2; (b) $HCl{\cdot}H_2N(CH_2)_6NHBoc$, $NaHCO_3$, PhH, water; (c) HCO_2H; (d) TFA; (e) **10**, DMAP, pyridine.

Scheme 2[a]

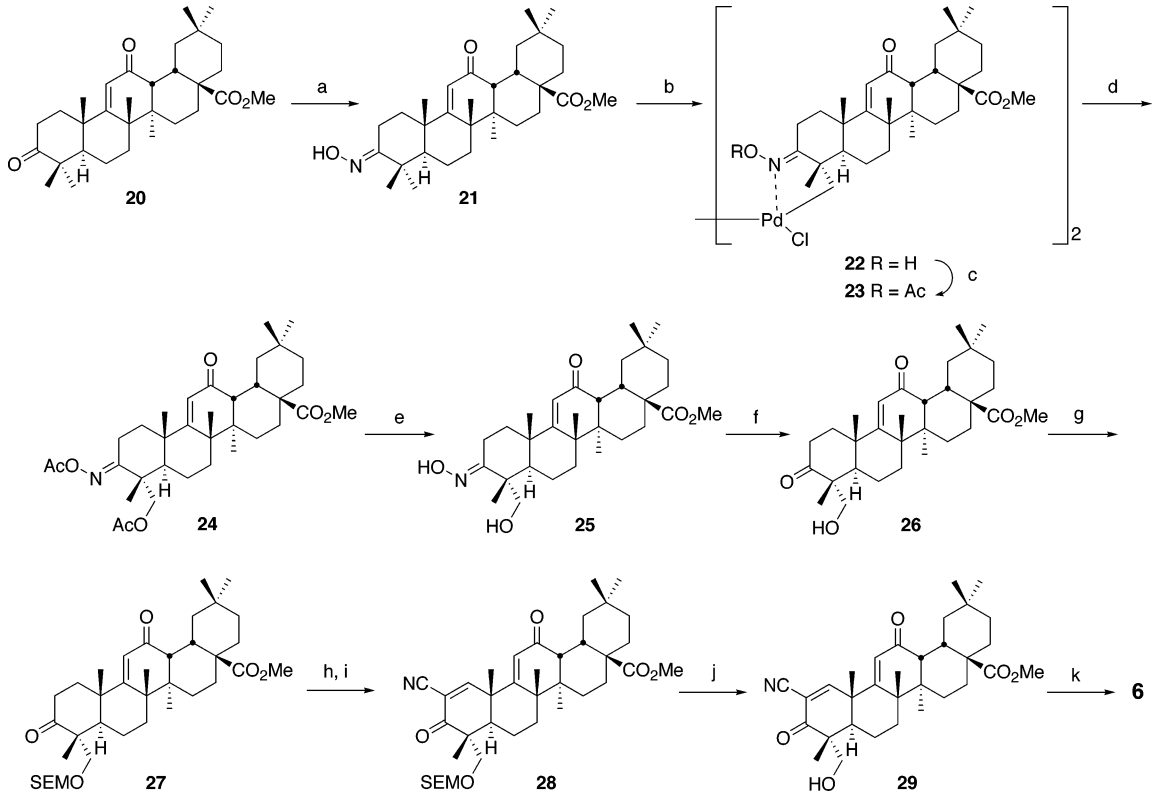


[a] Reagents: (a) $HCl{\cdot}H_2N(CH_2)_3CO_2Et$, $NaHCO_3$, PhH, water; (b) aqueous KOH, THF; (c) *N*-hydroxysuccinimide, EDCI, DMF; (d) $HCl{\cdot}H_2N(CH_2)_6NHBoc$, $NaHCO_3$, CH_2Cl_2, water; (e) HCO_2H; (f) TFA; (g) **10**, DMAP, pyridine.

for the condensation with **10**, conjugate **19** with a reduced ring A was obtained in 54% yield. This result suggests that the length of the spacer does not affect this reduction.

The C-23 biotin conjugate **6** was successfully synthesized in 11 steps via 23-hydroxy-CDDO-Me (**29**) from the known compound **20**,[3e] which was prepared in six steps in 75% yield from oleanolic acid by the sequence shown in Scheme 3. This approach involves functionalization of the hindered C-4 equatorial methyl group of **20** through a cyclopalladation reaction.[18] Condensation of **20** with hydroxylamine hydrochloride in the presence of NaOAc in a mixture of CH_2Cl_2 and MeOH selectively gave C-3 oxime **21** in 97% yield. The dimeric organopalladium complex **22** was prepared from **21** with Na_2PdCl_4 and NaOAc in AcOH. Acetylation of **22** with Ac_2O in the presence of Et_3N and DMAP in CH_2Cl_2 gave an unstable acetate **23** that was immediately oxidized with $Pb(OAc)_4$ and pyridinium acetate in THF, followed by reductive workup with $NaBH_4$ to afford diacetate **24**

Scheme 3[a]

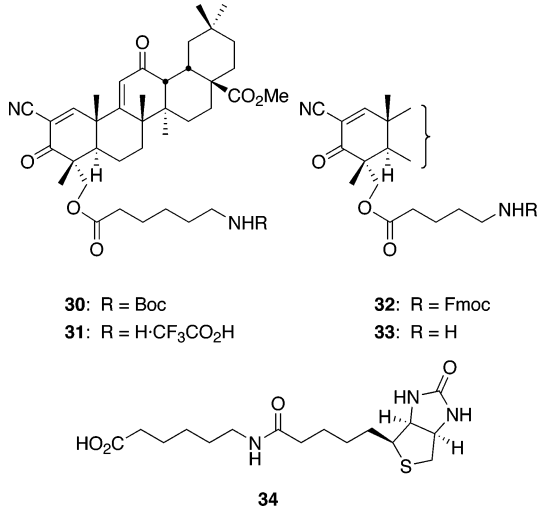


[a] Reagents: (a) $NH_2OH{\cdot}HCl$, NaOAc, MeOH, CH_2Cl_2; (b) Na_2PdCl_4, NaOAc, AcOH; (c) Ac_2O, Et_3N, DMAP, CH_2Cl_2; (d) $Pb(OAc)_4$, pyridine, AcOH, THF; $NaBH_4$, aqueous NaOH; (e) Na_2CO_3, MeOH; (f) $TiCl_3$, NH_4OAc, aqueous THF; (g) SEMCl, $(i\text{-}Pr)_2EtN$, CH_2Cl_2; (h) LDA, *p*-TsCN, THF; (i) DDQ, PhH; (j) aqueous HF, CH_3CN; (k) **34**, DCC, DMAP, DMF.

(56% yield from **21**). Deacetylation of **24** with Na_2CO_3 in MeOH gave oxime **25** (76% yield), which was hydrolyzed with $TiCl_3$ in aqueous THF to give ketol **26** in 88% yield.

Because **26** has an α-hydroxymethylketone functionality in ring A, the hydroxymethyl group is readily cleaved via a retro-aldol reaction under basic conditions. Therefore, we attempted to protect the hydroxyl group at C-23 of **26** with TBSCl in the presence of imidazole in DMF.[20] However, these conditions only led to recovered **26** presumably because of steric hindrance. Even under other conditions using a stronger base, Et_3N and DMAP in DMF,[21] only **26** was recovered. Therefore, we employed the trimethylsilylethoxymethyl (SEM) group, which is less bulky than the TBS group, for the hydroxyl protection, and this reaction (SEMCl and $(i\text{-}Pr)_2NEt$ in CH_2Cl_2)[22] gave **27** in 96% yield. Cyanation of the enolate of **27** with *p*-toluenesulfonyl cyanide (*p*-TsCN)[23] in THF, followed by DDQ oxidation, gave cyanoenone **28** in 63% yield. The SEM group of **28** was removed with a mixture of 48% aqueous HF solution and CH_3CN (1:10)[24] to afford 23-hydroxy-CDDO-Me (**29**) in quantitative yield.

Our initial strategy for the synthesis of the C-23 biotin conjugate **6** involved a "step by step" approach similar to that used for the C-17 conjugates. However, removal of the Boc group of **30** with TFA did not give the desired unprotected TFA salt **31**. Also, removal of Fmoc group of **32** with $(n\text{-}Bu)_4NF$ in THF[25] gave **29** instead of amine **33**. Therefore, we decided to prepare the C-23 biotin conjugate **6** by direct esterification of **29** with 6-(biotinylamido)hexanoic acid (**34**). The requisite acid **34** was prepared from *N*-succinimidobiotin (**10**) and methyl 6-aminohexanoate hydrochloride, followed by alkaline hydrolysis of the methyl ester according to a known method.[26] Because of the low solubility of **34** in various organic solvents, the methods for the esterification that we could use were severely limited. For example, we could not prepare the acyl chloride of **34**. Finally, the synthesis of conjugate **6** was successfully achieved by the condensation of **29** and **34** in the presence of DCC and DMAP in DMF at 60 °C for 3 days (32% yield, 78% yield based on consumed **29**). The low isolated yield is thought to be due to the low solubility of **34**.



Biological Results. It is obviously important that these biotinylated compounds retain potency in bioassays. We now report the inhibitory activity of biotin conjugates **4**−**6** against the proliferation of MCF-7

Table 1. Inhibitory Activity of Biotin Conjugates of CDDO against the Proliferation of MCF-7 (ER Positive) Breast Cancer Cells

compd	activity[a] IC_{50} (μM)	compd	activity[a] IC_{50} (μM)
4	3.31	**29**	0.085
5	5.26	CDDO (**1**)	0.16
6	0.55	CDDO-Me (**2**)	0.056

[a] IC_{50} values of compounds were determined in the range of 1 nM to 10 μM (3-fold dilutions). Values are an average of two separate experiments. None of the compounds were toxic to MCF-7 cells at 10 μM.

[estrogen receptor (ER) positive] breast cancer cells (Table 1). Interestingly, the C-23 conjugate **6** is only 3−10 times less potent than the mother compounds 23-hydroxy-CDDO-Me (**29**), CDDO-Me (**2**), and CDDO (**1**).[27] Also, the C-17 conjugates **4** and **5** are 20−30 times less potent than the mother compound CDDO.

It is very difficult to assign a value to the necessary affinity of the conjugated inhibitor to the protein target for successful affinity chromatography. However, a good example is methotrexate agarose, commonly and successfully used to purify dihydrofolate reductase (DHFR) from cell extract. Methotrexate agarose exhibits 100-fold lower affinity toward the target than methotrexate itself. The affinity of methotrexate for DHFR is approximately 10 nM, and the conjugated compound has an affinity of approximately 1 μM.[28] The affinity of the conjugated methotrexate is very similar to the measured affinity for the conjugates **4**−**6**. Therefore, these conjugates, particularly **6**, may be very promising tools for the isolation of the protein targets.

Further syntheses of new C-23 and C-17 biotin conjugates (e.g., conjugates with various spacers) are in progress. Preliminary studies for the isolation of the protein targets using **4**−**6** are also in progress, and we will report the results elsewhere.

Experimental Section

General Experimental Procedures. Melting points were determined on a Laboratory Devices Mel Temp capillary melting point apparatus and are uncorrected. Optical rotations were measured with a Jasco DIP-370 digital polarimeter. UV and IR spectra were recorded on a Hewlett-Packard 8451A diode array UV spectrophotometer and Thermo Nicolet AVATAR 330 FT-IR, respectively. ^{1}H (300 MHz) and ^{13}C (75 MHz) NMR spectra were recorded on a Varian XL-300 Fourier transform spectrometer. The chemical shifts are reported in δ (ppm) using the δ 7.27 signal of C*H*Cl$_3$ and δ 3.31 signal of C*H*D$_2$OD (^{1}H NMR) and the δ 77.23 signal of *C*DCl$_3$ and δ 49.15 of *C*D$_3$OD (^{13}C NMR) as internal standards. Low-resolution mass spectra and high-resolution MS data were obtained on a Micromass 70-VSE. Elemental analyses were performed by Atlantic Microlab Inc., Norcross, GA. TLC and preparative TLC were performed with Merck precoated TLC plates silica gel 60 F_{254}. Flash column chromatography was done with Select Scientific silica gel (230−400 mesh). Anhydrous CH_2Cl_2 and anhydrous THF were distilled from calcium hydride and sodium benzophenone ketyl−sodium metal under a nitrogen atmosphere, respectively. All experiments were performed under a nitrogen atmosphere. The standard workup method was as follows: an organic extract was washed with water (three times) and then saturated aqueous NaCl solution (three times), dried over anhydrous $MgSO_4$, and filtered. The solvent was removed in vacuo from the filtrate.

2-Cyano-*N*-[6-({5-[(3a*S*,4*S*,6a*R*)-2-oxohexahydro-1*H*-thieno[3,4-*d*]imidazol-4-yl]pentanoyl}amino)hexyl]-3,12-dioxooleana-1,9(11)-dien-28-amide (4). A solution of **9b** (61 mg, 0.087 mmol), *N*-hydroxysuccinimidobiotin (**10**) (36 mg, 0.11 mmol), and DMAP (13 mg, 0.11 mmol) in dry pyridine (2.4 mL) was stirred at room temperature overnight. Removal of pyridine in vacuo gave a residue (147 mg). The residue was purified by preparative TLC [CH_2Cl_2−MeOH (9:1)] to give **4** (56 mg, 79%) as an amorphous solid: $[\alpha]^{27}_D$ +34° (*c* 0.70, MeOH). UV (EtOH) λ_{max} (log ϵ): 240 (4.08) nm. IR (neat): 3315, 2931, 2859, 2234, 1705, 1654 cm^{-1}. ^{1}H NMR (CD$_3$OD): δ 8.42 (1H, s), 7.94 (1H, t, J = 5.5 Hz), 7.78 (1H, t, J = 5.9 Hz), 6.13 (1H, s), 4.50 (1H, dd, J = 4.4, 7.7 Hz), 4.30 (1H, dd, J = 4.4, 7.7 Hz), 3.28−3.11 (5H, m), 3.04 (1H, m), 2.93 (1H, dd, J = 4.9, 12.8 Hz), 2.71 (1H, d, J = 12.8 Hz), 2.20 (2H, t, J = 7.3 Hz), 2.07 (1H, m), 1.53, 1.36, 1.25, 1.17, 1.06, 0.99, 0.90 (each 3H, s). ^{13}C NMR (CD$_3$OD): δ 201.9, 198.9, 179.9, 175.9, 171.7, 168.7, 166.1, 125.0, 116.0, 115.1, 63.5, 61.7, 57.2, 48.6, 47.7, 47.3, 46.2, 44.2, 43.3, 41.2, 40.7, 40.6, 40.3, 37.2, 37.0, 35.8, 35.2, 34.0, 32.9, 32.6, 31.7, 30.8, 30.5, 29.9, 29.6, 29.0, 27.9, 27.7, 27.3, 27.1, 26.4, 25.6, 23.7, 23.5, 22.22, 22.16, 19.3. MS (ESI+) *m*/*z*: 816 [M + H]$^+$. HRMS (ESI+) Calcd for $C_{47}H_{69}N_5O_5S$ + H: 816.5098. Found: 816.5099. Anal. ($C_{47}H_{69}N_5O_5S\cdot{}^5/_4H_2O$) C, H, N, S.

2-Cyano-*N*-{3-[6-({5-[(3a*S*,4*S*,6a*R*)-2-oxohexahydro-1*H*-thieno[3,4-*d*]imidazol-4-yl]pentanoyl}amino)hexyl]aminocarbonyl}propyl-3,12-dioxooleana-1,9(11)-dien-28-amide (5). A solution of **18b** (25 mg, 0.032 mmol), *N*-hydroxysuccinimidobiotin (**10**) (13 mg, 0.038 mmol), and DMAP (4.7 mg, 0.038 mmol) in dry pyridine (0.9 mL) was stirred at room temperature overnight. Removal of pyridine in vacuo gave a residue (58 mg). The residue was purified by preparative TLC [CH_2Cl_2−MeOH (9:1)] to give **5** (18 mg, 63%) as an amorphous solid: $[\alpha]^{27}_D$ +37° (*c* 0.60, MeOH). UV (EtOH) λ_{max} (log ϵ): 240 (4.21) nm. IR (neat): 3306, 2930, 2860, 2235, 1690, 1642 cm^{-1}. ^{1}H NMR (CD$_3$OD): δ 8.41 (1H, s), 7.89 (1H, t, J = 5.6 Hz), 6.12 (1H, s), 4.50 (1H, dd, J = 4.4, 7.7 Hz), 4.30 (1H, dd, J = 4.4, 7.7 Hz), 3.28−3.11 (7H, m), 3.04 (1H, m), 2.93 (1H, dd, J = 4.9, 12.9 Hz), 2.71 (1H, d, J = 12.9 Hz), 2.20 (4H, m), 2.08 (1H, m), 1.52, 1.36, 1.24, 1.17, 1.06, 0.99, 0.90 (each 3H, s). ^{13}C NMR (CD$_3$OD): δ 202.0, 198.9, 180.2, 176.0, 175.4, 171.7, 168.7, 166.2, 125.1, 115.9, 115.1, 63.5, 61.7, 57.2, 48.7, 47.8, 47.3, 46.2, 44.2, 43.3, 41.2, 40.5, 40.44, 40.36, 37.2, 37.0, 35.8, 35.2, 34.7, 34.0, 32.9, 32.6, 31.7, 30.5, 29.9, 29.7, 29.1, 27.7, 27.3, 27.2, 27.11, 27.06, 25.5, 23.7, 23.6, 22.2, 22.1, 19.3. MS (ESI+) *m*/*z*: 901 [M + H]$^+$. HRMS (ESI+) Calcd for $C_{51}H_{76}N_6O_6S$ + H: 901.5625. Found: 901.5648. Anal. ($C_{51}H_{76}N_6O_6S\cdot{}^5/_6CH_2Cl_2$)[29] C, H, N, S.

Methyl 2-Cyano-23-[6-({5-[(3a*S*,4*S*,6a*R*)-2-oxohexahydro-1*H*-thieno[3,4-*d*]imidazol-4-yl]pentanoyl}amino)hexanoyl]oxy-3,12-dioxooleana-1,9(11)-dien-28-oate (6). A mixture of **29** (32 mg, 0.062 mmol), 6-(biotinylamido)hexanoic acid (**34**)[26] (35 mg, 0.097 mmol), DCC (25 mg, 0.12 mmol), and DMAP (15 mg, 0.12 mmol) in anhydrous DMF (0.5 mL) was stirred at 60 °C for 3 days. After the mixture was cooled, colorless needles precipitated. After removal of insoluble matter by filtration, DMF was removed in vacuo to give an amorphous solid (93 mg). The solid was purified by preparative TLC [CH_2Cl_2−MeOH (10:1)] to afford starting material **29** (19 mg) and **6** (17 mg, 32%, 78% based on consumed **29**) as an amorphous solid: $[\alpha]^{27}_D$ +48° (*c* 1.7, $CHCl_3$). UV (EtOH) λ_{max} (log ϵ): 241 (4.19) nm. IR (neat): 3285, 2933, 2862, 2236, 1697, 1661 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 8.19 (1H, s), 6.36 (1H, brs), 6.07 (1H, s), 4.54 (1H, dd, J = 4.4, 7.9 Hz), 4.36 (1H, dd, J = 4.0, 7.9 Hz), 4.22 (1H, d, J = 11.1 Hz), 4.17 (1H, d, J = 11.1 Hz), 3.70 (3H, s), 3.22 (2H, t, J = 6.8 Hz), 3.04 (1H, m), 2.96 (1H, d, J = 4.8 Hz), 2.92 (1H, brs), 2.76 (1H, d, J = 12.8 Hz), 2.24 (4H, t, J = 7.1 Hz), 2.12 (1H, m), 1.52, 1.33, 1.15, 1.02, 1.00, 0.90 (each 3H, s). ^{13}C NMR ($CDCl_3$): δ 199.1, 194.9, 178.3, 173.4, 173.1, 168.2, 166.7, 124.5, 115.3, 114.3, 67.5, 62.0, 60.4, 55.8, 52.1, 49.9, 48.6, 47.3, 45.9, 42.4, 42.3, 41.2, 40.8, 39.3, 36.2, 36.0, 34.6, 34.0, 33.4, 32.9, 31.7, 31.5, 30.8, 29.3, 28.3, 28.2, 28.1, 27.6, 26.4, 25.8, 24.9, 24.8, 23.2, 22.8, 21.8, 18.2, 17.5. MS (ESI+) *m*/*z*: 861 [M + H]$^+$. HRMS (ESI+) Calcd for $C_{48}H_{68}N_4O_8S$ + H: 861.4836. Found: 861.4808. Anal. ($C_{48}H_{68}N_4O_8S\cdot CH_2Cl_2$)[29] C, H, N, S.

2-Cyano-3,12-dioxooleana-1,9(11)-dien-28-oyl Chloride (7). A mixture of CDDO (**1**)[3b,e] (3.0 g, 6.1 mmol) and oxalyl chloride (5 mL) in anhydrous CH_2Cl_2 (50 mL) was stirred at room temperature overnight. The solvent was removed in vacuo, and the residue was coevaporated with benzene three times to give **7** as a crystalline solid (3.1 g, 100%): mp >196 °C (dec). $[\alpha]^{26}_D$ +26° (*c* 0.52, $CHCl_3$). UV (EtOH) λ_{max} (log ϵ): 241 (4.05) nm. IR (neat): 2946, 2866, 2236, 1781, 1694, 1663 cm^{-1}. ^1H NMR ($CDCl_3$): δ 8.04 (1H, s), 5.99 (1H, s), 3.09 (1H, m), 2.96 (1H, d, J = 4.8 Hz), 1.51, 1.38, 1.26, 1.18, 1.04, 1.02, 0.93 (each 3H, s). ^{13}C NMR ($CDCl_3$): δ 198.0, 196.7, 181.0, 168.8, 165.7, 128.5, 124.1, 114.8, 114.5, 57.9, 49.4, 47.9, 46.0, 45.2, 42.8, 42.3, 36.2, 34.5, 33.1, 31.9, 31.8, 30.7, 27.9, 27.2, 26.8, 24.8, 23.9, 23.1, 21.8, 21.7, 18.4. HRMS (EI) Calcd for $C_{31}H_{40}NO_3Cl$: 509.2697. Found: 509.2695.

1,1-Dimethylethyl 6-[(2-Cyano-3,12-dioxooleana-1,9(11)-dien-28-oyl)amino]hexylcarbamate (8). To a solution of **7** (180 mg, 0.35 mmol) in benzene (5.4 mL) was added a solution of 6-[(*tert*-butoxycarbonyl)amino]hexylamine hydrochloride (380 mg, 1.50 mmol) and $NaHCO_3$ (126 mg, 1.5 mmol) in water (3.6 mL). The mixture was stirred at room temperature overnight. The organic layer was separated and then diluted with EtOAc (15 mL). It was worked up by the standard method to give an amorphous solid (270 mg). The solid was purified by flash column chromatography [hexanes−EtOAc (1:1.5)] to give **8** (198 mg, 81%) as an amorphous solid: $[\alpha]^{27}_D$ +32° (*c* 0.48, $CHCl_3$). UV (EtOH) λ_{max} (log ϵ): 240 (4.25) nm. IR (neat): 3357, 2931, 2861, 2235, 1690, 1660 cm^{-1}. ^1H NMR ($CDCl_3$): δ 8.10 (1H, s), 6.10 (1H, brs), 5.99 (1H, s), 4.59 (1H, brs) 3.22 (2H, m), 3.05 (3H, m), 2.88 (1H, m), 1.47 (3H, s), 1.40 (9H, s), 1.31, 1.22, 1.14, 0.99, 0.95, 0.86 (each 3H, s). ^{13}C NMR ($CDCl_3$): δ 199.3, 196.8, 177.1, 168.8, 166.2, 156.2, 124.1, 114.58, 114.57, 79.2, 49.6, 47.8, 46.5, 46.0, 45.1, 42.7, 42.2, 40.3, 39.4, 36.2, 34.7, 34.2, 33.4, 32.0, 31.8, 30.7, 30.2, 29.8, 28.5, 27.9, 27.1, 26.7, 26.4, 26.2, 25.0, 23.2, 23.0, 21.9, 21.7, 18.3. MS (ESI+) *m*/*z*: 712 $[M + Na]^+$, 690 $[M + H]^+$. HRMS (ESI+) Calcd for $C_{42}H_{63}N_3O_5$ + H: 690.4846. Found: 690.4851.

***N*-(6-Aminohexyl)-2-cyano-3,12-dioxooleana-1,9(11)-dien-28-amide Formate (9a).** A solution of **8** (58 mg, 0.084 mmol) in 95% formic acid (1.7 mL) was stirred at room temperature for 1 h. The formic acid was removed in vacuo, and the residue was coevaporated with benzene (three times) to yield **9a** (53 mg, 100%) as a colorless glass. ^1H NMR (CD_3OD):[19] δ 8.41 (1H, s), 8.29 (2H, brs), 7.81 (1H, t, J = 5.7 Hz), 6.13 (1H, s), 3.22 (2H, m), 3.14 (1H, d, J = 4.4 Hz), 3.04 (1H, m), 2.92 (2H, t, J = 7.5 Hz), 1.52, 1.36, 1.25, 1.17, 1.06, 0.98, 0.90 (each 3H, s). ^{13}C NMR (CD_3OD): δ 201.8, 198.7, 180.1, 171.8, 168.6, 125.0, 115.9, 115.2, 48.6, 47.8, 47.3, 46.2, 44.2, 43.4, 40.7, 40.6, 37.2, 35.8, 35.2, 34.0, 32.9, 32.7, 31.7, 30.6, 29.0, 28.6, 27.6, 27.3, 27.2, 27.0, 25.5, 23.7, 23.5, 22.2, 19.3.

***N*-(6-Aminohexyl)-2-cyano-3,12-dioxooleana-1,9(11)-dien-28-amide Trifluoroacetate (9b).** A solution of **8** (20 mg, 0.029 mmol) in TFA (0.6 mL) was stirred at room temperature for 30 min. The TFA was removed in vacuo, and the residue was coevaporated with benzene (three times) to give **9b** (21 mg, 100%) as a colorless glass. UV (EtOH) λ_{max} (log ϵ): 240 (4.08) nm. IR (neat): 3390, 2942, 2864, 2237, 1779, 1682, 1660, 1617 cm^{-1}. ^1H NMR (CD_3OD):[19] δ 8.41 (1H, s), 6.12 (1H, s), 3.21 (2H, t, J = 7.0 Hz), 3.04 (1H, m), 2.92 (2H, t, J = 7.5 Hz), 1.52, 1.35, 1.24, 1.16, 1.05, 0.98, 0.90 (each 3H, s). ^{13}C NMR (CD_3OD): δ 202.0, 198.9, 180.1, 171.9, 168.6, 125.1, 115.9, 115.2, 48.7, 47.8, 47.3, 46.2, 44.2, 43.3, 40.8, 40.5, 37.2, 35.8, 35.2, 33.9, 32.9, 32.6, 31.7, 30.6, 29.0, 28.6, 27.6, 27.3, 27.1, 27.0, 25.5, 23.7, 23.5, 22.2, 22.1, 19.3. MS (ESI+) *m*/*z*: 612 $[M + Na]^+$, 590 $[M + H]^+$. HRMS (ESI+) Calcd for $C_{37}H_{55}N_3O_3$ + H: 590.4322. Found: 590.4325.

2-Cyano-3-hydroxy-*N*-[6-({5-[(3a*S*,4*S*,6a*R*)-2-oxohexahydro-1*H*-thieno[3,4-*d*]imidazol-4-yl]pentanoyl}amino)hexyl]-12-oxooleana-2,9(11)-dien-28-amide (11). A solution of **9a** (53 mg, 0.084 mmol), *N*-hydroxysuccinimidobiotin (**10**) (34 mg, 0.10 mmol), and DMAP (12 mg, 0.1 mmol) in dry pyridine (2.8 mL) was stirred at room temperature overnight. Removal of pyridine in vacuo gave a residue (138 mg). The residue was purified by preparative TLC [CH_2Cl_2−MeOH (10:1)] to give **4** (4 mg, 6%) and **11** (52 mg, 76%) as amorphous solids, respectively. **11**: $[\alpha]^{26}_D$ +46° (*c* 1.56, MeOH). UV (EtOH) λ_{max} (log ϵ): 238 (4.22) nm. IR (neat): 3305, 2931, 2859, 2204, 1706, 1644 cm^{-1}. ^1H NMR (CD_3OD): δ 7.94 (1H, t, J = 5.5 Hz), 7.76 (1H, t, J = 5.5 Hz), 5.80 (1H, s), 4.49 (1H, dd, J = 4.9, 7.9 Hz), 4.30 (1H, dd, J = 4.6, 7.9 Hz), 3.28−3.11 (5H, m), 3.03 (1H, m), 2.93 (1H, dd, J = 5.1, 12.8 Hz), 2.70 (1H, d, J = 12.8 Hz), 2.43 (1H, d, J = 15.0 Hz), 2.27 (1H, d, J = 15.0 Hz), 2.19 (2H, t, J = 7.3 Hz), 2.06 (1H, m), 1.30, 1.26, 1.20, 1.13, 1.05, 0.98, 0.90 (each 3H, s). ^{13}C NMR (CD_3OD): δ 202.8, 180.1, 178.9, 176.0, 175.1, 173.4, 166.2, 125.2, 120.6, 79.5, 63.5, 61.8, 57.2, 50.8, 49.9, 47.8, 47.1, 43.1, 41.2, 40.8, 40.4, 40.3, 40.2, 40.0, 37.4, 37.0, 35.9, 35.3, 34.0, 32.7, 32.6, 31.7, 30.5, 30.0, 29.7, 29.3, 28.1, 27.9, 27.8, 27.1, 24.7, 24.1, 23.7, 23.6, 22.2, 20.2, 20.1. MS (ESI+) *m*/*z*: 818 $[M + H]^+$. HRMS (ESI+) Calcd for $C_{47}H_{71}N_5O_5S$ + H: 818.5254. Found: 818.5252.

Ethyl 4-[(2-Cyano-3,12-dioxooleana-1,9(11)-dien-28-oyl)amino]butanoate (14). A mixture of **7** (1.12 g, 2.2 mmol) in benzene (20 mL), and ethyl 4-aminobutanoate hydrochloride (820 mg, 4.89 mmol) and $NaHCO_3$ (1.0 g, 11.9 mmol) in water (20 mL) was stirred at room temperature overnight. The layers were separated, and the aqueous layer was extracted with benzene (20 mL). The combined organic layers were worked up by the standard method to give **14** as an amorphous solid (1.12 g, 84%): $[\alpha]^{27}_D$ +31° (*c* 0.50, $CHCl_3$). UV (EtOH) λ_{max} (log ϵ): 240 (4.21) nm. IR (neat): 3399, 2941, 2235, 1730, 1688, 1659 cm^{-1}. ^1H NMR ($CDCl_3$): δ 8.07 (1H, s), 6.12 (1H, t, J = 5.7 Hz), 5.98 (1H, s), 4.13 (2H, q, J = 7.1 Hz), 3.31 (2H, q, J = 6.9 Hz), 3.06 (1H, d, J = 4.5 Hz), 2.90 (1H, m), 2.35 (2H, t, J = 6.9 Hz), 1.84 (2H, t, J = 6.9 Hz), 1.48, 1.33 (each 3H, s), 1.26 (3H, t, J = 7.1 Hz), 1.25, 1.17, 1.02, 1.00, 0.90 (each 3H, s). ^{13}C NMR ($CDCl_3$): δ 199.2, 196.8, 177.4, 173.7, 168.7, 166.0, 124.2, 114.7, 114.6, 60.8, 49.7, 47.9, 46.6, 46.1, 45.2, 42.7, 42.3, 39.5, 36.2, 34.8, 34.3, 33.5, 32.1, 32.1, 31.8, 30.8, 28.0, 27.2, 26.8, 25.0, 24.8, 23.3, 23.2, 21.9, 21.8, 18.4, 14.4. MS (EI) *m*/*z*: 604 $[M]^+$ (75), 445 (100). HRMS (EI) Calcd for $C_{37}H_{52}N_2O_5$: 604.3876. Found: 604.3877.

4-[(2-Cyano-3,12-dioxooleana-1,9(11)-dien-28-oyl)amino]-butanoic Acid (15). To a solution of **14** (1.12 g, 1.85 mmol) in freshly distilled THF (40 mL) was added a solution of KOH (0.72 g, 12.8 mmol) in water (40 mL). The resultant mixture was stirred at room temperature overnight. It was thereafter concentrated at reduced pressure, acidified to pH ∼5 by addition of AcOH, diluted with water (50 mL), and extracted with CH_2Cl_2 (3 × 50 mL). The combined organic extracts were worked up by the standard method to give **15** as an amorphous solid (990 mg, 93%): $[\alpha]^{27}_D$ +30° (*c* 0.30, $CHCl_3$). UV (EtOH) λ_{max} (log ϵ): 240 (4.13) nm. IR (neat): 3392, 2942, 2235, 1724, 1689, 1659 cm^{-1}. ^1H NMR ($CDCl_3$): δ 8.11 (1H, s), 6.66 (1H, t, J = 5.4 Hz), 6.06 (1H, s), 3.34 (2H, q, J = 6.4 Hz), 3.01 (1H, d, J = 4.2 Hz), 2.88 (1H, m), 2.38 (2H, t, J = 6.6 Hz), 1.86 (2H, t, J = 6.6 Hz), 1.48, 1.33, 1.25, 1.16, 1.01, 0.95, 0.89 (each 3H, s). ^{13}C NMR ($CDCl_3$): δ 200.6, 196.7, 177.9, 177.0, 170.3, 165.9, 124.1, 114.8, 114.6, 49.9, 47.8, 46.9, 46.2, 45.2, 42.8, 42.3, 39.4, 36.4, 34.7, 34.2, 33.5, 32.1, 31.8, 31.6, 30.8, 27.9, 27.2, 26.7, 24.9, 24.8, 23.2, 22.7, 22.1, 21.8, 18.4. MS (EI) *m*/*z*: 576 $[M]^+$ (32), 491 (43), 445 (100). HRMS (EI) Calcd for $C_{35}H_{48}N_2O_5$: 576.3563. Found: 576.3564.

***N*-Hydroxysuccinimido 4-[(2-Cyano-3,12-dioxooleana-1,9(11)-dien-28-oyl)amino]butanoate (16).** A mixture of **15** (980 mg, 1.70 mmol), *N*-hydroxysuccinimide (217 mg, 1.89 mmol), and EDCI (328 mg, 1.71 mmol) in anhydrous DMF (16 mL) was stirred for 23 h. Upon addition of water (75 mL), compound **16** precipitated out of the solution. It was collected by filtration, washed with several portions of water, and dried in vacuo to give **16** (1.02 g, 89%) as an amorphous solid: $[\alpha]^{27}_D$ +29° (*c* 0.52, $CHCl_3$). UV (EtOH) λ_{max} (log ϵ): 240 (4.26) nm. IR (neat): 3410, 2944, 2235, 1814, 1784, 1737, 1688, 1658 cm^{-1}. ^1H NMR ($CDCl_3$): δ 8.06 (1H, s), 6.43 (1H, t, J = 3.3 Hz), 5.95 (1H, s), 3.55−3.45 (1H, m), 3.31−3.21 (1H, m), 3.01 (1H, d, J = 4.5 Hz), 2.90−2.78 (5H, m), 2.64 (2H, t, J = 7.2 Hz), 2.00 (2H, quintet, J = 6.9 Hz), 1.49, 1.36, 1.25, 1.17 (each 3H, s), 1.00 (6H, s), 0.90 (3H, s). ^{13}C NMR ($CDCl_3$): δ 199.3, 196.8,

177.9, 169.7, 169.0, 168.6, 166.1, 124.1, 114.7, 114.6, 49.8, 47.9, 46.8, 46.1, 45.2, 42.7, 42.3, 38.7, 36.3, 34.7, 34.3, 33.5, 31.9, 31.8, 30.8, 29.0, 28.0, 27.1, 26.8, 25.9, 25.0, 24.9, 23.3, 22.9, 22.0, 21.7, 18.4. MS (FAB+) *m/z*: 674 $[M + H]^+$. HRMS (FAB+) Calcd for $C_{39}H_{51}N_3O_7$ + H: 674.3805. Found: 674.3807.

1,1-Dimethylethyl 6-({3-[(2-Cyano-3,12-dioxooleana-1,9(11)-dien-28-oyl)amino]propanoyl}amino)hexylcarbamate (17). To a solution of **16** (477 mg, 0.708 mmol) in CH_2Cl_2 (10 mL) was added 6-[(*tert*-butoxycarbonyl)amino]-hexylamine hydrochloride (230 mg, 0.91 mmol), followed by $NaHCO_3$ (370 mg, 4.4 mmol) in water (4.5 mL). The resulting mixture was stirred at room temperature overnight. It was diluted with CH_2Cl_2 (25 mL) and water (25 mL). The layers were separated, and the aqueous layer was extracted with CH_2Cl_2 (10 mL). The combined organic layers were worked up by the standard method to give **17** (490 mg, 89%) as an amorphous solid: $[\alpha]^{27}_D$ +20° (*c* 0.50, $CHCl_3$). UV (EtOH) λ_{max} (log ϵ): 240 (4.25) nm. IR (neat): 3330, 2931, 2862, 2235, 1689, 1654 cm^{-1}. ^1H NMR ($CDCl_3$): δ 8.11 (1H, s), 6.80 (1H, t, J = 5.1 Hz), 6.59 (1H, t, J = 5.1 Hz), 5.99 (1H, s), 4.69 (1H, br), 3.30−3.16 (4H, m), 3.09−3.03 (3H, m), 2.95 (1H, m), 2.21 (2H, m), 1.46 (3H, s), 1.40 (9H, s), 1.30, 1.22, 1.13, 0.98, 0.94, 0.85 (each 3H, s). ^{13}C NMR ($CDCl_3$): δ 199.4, 196.8, 177.9, 172.9, 168.6, 166.3, 156.3, 124.2, 114.6, 114.5, 79.1, 49.5, 47.7, 46.4, 45.9, 45.1, 42.7, 42.2, 40.3, 39.5, 39.4, 36.1, 34.8, 34.1, 33.4, 31.7, 31.7, 30.7, 30.1, 29.4, 28.5, 27.9, 27.0, 26.7, 26.4, 26.2, 25.5, 24.9, 23.3, 23.0, 21.8, 21.7, 18.3. MS (ESI+) *m/z*: 797 $[M + Na]^+$, 775 $[M + H]^+$. HRMS (ESI+) Calcd for $C_{46}H_{70}N_4O_6$ + H: 775.5374. Found: 775.5403.

***N*-[3-(6-Aminohexyl)aminocarbonyl]propyl-2-cyano-3,12-dioxooleana-1,9(11)-dien-28-amide Formate (18a).** A solution of **17** (490 mg, 0.63 mmol) in 95% formic acid (7 mL) was stirred at room temperature for 1 h. The formic acid was removed in vacuo, and the residue was coevaporated with CH_2Cl_2 (three times) to yield **18a** (454 mg, 100%) as a colorless glass. ^1H NMR (CD_3OD):[19] δ 8.35 (1H, s), 7.82 (1H, m), 6.06 (1H, s), 3.20−3.00 (5H, m), 2.97 (1H, m), 2.86 (2H, t, J = 7.7 Hz), 2.15 (2H, t, J = 7.5 Hz) 1.49, 1.29, 1.18, 1.10, 0.99, 0.92, 0.84 (each 3H, s). ^{13}C NMR (CD_3OD): δ 201.9, 198.9, 180.3, 175.5, 171.8, 168.6, 125.0, 115.9, 115.2, 48.7, 47.8, 47.3, 46.2, 44.2, 43.4, 40.7, 40.5, 40.3, 37.2, 35.8, 35.2, 34.7, 34.0, 32.9, 32.7, 31.7, 30.3, 29.0, 28.6, 27.5, 27.3, 27.1, 27.0, 25.5, 23.7, 23.5, 22.2, 22.1, 19.3.

***N*-[3-(6-Aminohexyl)aminocarbonyl]propyl-2-cyano-3,12-dioxooleana-1,9(11)-dien-28-amide Trifluoroacetate (18b).** A solution of **17** (100 mg, 0.13 mmol) in TFA (2.4 mL) was stirred at room temperature for 30 min. The TFA was removed in vacuo, and the residue was coevaporated with benzene (three times) to give **18b** (102 mg, 100%) as a colorless glass. UV (EtOH) λ_{max} (log ϵ): 239 (4.04) nm. IR (neat): 2943, 2866, 2236, 1776, 1654 cm^{-1}. ^1H NMR (CD_3OD):[19] δ 8.40 (1H, s), 6.12 (1H, s), 3.29−3.12 (4H, m), 3.03 (1H, m), 2.92 (2H, t, J = 7.4 Hz), 2.22 (2H, t, J = 7.3 Hz), 1.52, 1.35, 1.23, 1.16, 1.04, 0.97, 0.89 (each 3H, s). ^{13}C NMR (CD_3OD): δ 202.1, 198.9, 180.2, 175.6, 171.9, 168.6, 125.0, 115.9, 115.2, 48.7, 47.7, 47.3, 46.2, 44.2, 43.3, 40.8, 40.4, 40.3, 37.2, 35.8, 35.2, 34.6, 34.0, 32.8, 32.6, 31.6, 30.2, 29.0, 28.5, 27.4, 27.3, 27.1, 27.0, 25.5, 23.7, 23.5, 22.2, 22.1, 19.2. MS (ESI+) *m/z*: 697 $[M + Na]^+$, 675 $[M + H]^+$. HRMS (ESI+) Calcd for $C_{41}H_{62}N_4O_4$ + H: 675.4849. Found: 675.4842.

2-Cyano-3-hydroxy-*N*-{3-[6-({5-[(3a*S*,4*S*,6a*R*)-2-oxohexahydro-1*H*-thieno[3,4-*d*]imidazol-4-yl]pentanoyl}amino)hexyl]aminocarbonyl}propyl-12-oxooleana-2,9(11)-dien-28-amide (19). A solution of **18a** (222 mg, 0.31 mmol), *N*-hydroxysuccinimidobiotin (**10**) (113 mg, 0.33 mmol), and DMAP (41 mg, 0.33 mmol) in dry pyridine (6.0 mL) was stirred at room temperature overnight. Removal of pyridine in vacuo gave a residue. To a solution of the residue in MeOH (50 mL) was added water to give a precipitate. The precipitate was collected by filtration and washed with several portions of water. It was air-dried to give **19** as an amorphous solid (150 mg, 54%): $[\alpha]^{26}_D$ +47° (*c* 0.52, MeOH). UV (EtOH) λ_{max} (log ϵ): 239 (4.26) nm. IR (neat): 3306, 2931, 2860, 2205, 1697, 1643 cm^{-1}. ^1H NMR (CD_3OD): δ 7.96 (1H, m), 7.86 (1H, m), 5.78 (1H, s), 4.60 (1H, brs), 4.49 (1H, dd, J = 4.8, 7.8 Hz), 4.30 (1H, dd, J = 4.5, 7.8 Hz), 3.28−3.11 (7H, m), 3.02 (1H, m), 2.92 (1H, dd, J = 5.0, 12.9 Hz), 2.70 (1H, d, J = 12.9 Hz), 2.41 (1H, d, J = 14.7 Hz), 2.26 (1H, d, J = 14.7 Hz), 2.19 (4H, m), 2.07 (1H, m), 1.28, 1.24, 1.19, 1.11, 1.04, 0.97, 0.89 (each 3H, s). ^{13}C NMR (CD_3OD): δ 202.8, 180.4, 178.9, 176.1, 175.5, 173.4, 166.2, 125.2, 120.6, 79.5, 63.5, 61.7, 57.2, 50.7, 50.0, 49.5, 47.9, 47.1, 43.1, 41.2, 40.5, 40.44, 40.37, 40.3, 40.2, 40.0, 37.4, 37.0, 35.8, 35.3, 34.7, 34.0, 32.7, 32.5, 31.7, 30.5, 29.9, 29.7, 29.3, 28.1, 27.7, 27.2, 27.1, 24.7, 24.0, 23.7, 23.6, 22.2, 20.2, 20.1. MS (ESI+) *m/z*: 925 $[M + Na]^+$, 903 $[M + H]^+$. HRMS (ESI+) Calcd for $C_{51}H_{78}N_6O_6S$ + H: 903.5782. Found: 903.5780.

Methyl 3-Hydroxyimino-12-oxoolean-9(11)-en-28-oate (21). To a solution of **20**[3e] (4.83 g, 10.0 mmol) in anhydrous CH_2Cl_2 (50 mL) and anhydrous MeOH (50 mL) were added hydroxylamine hydrochloride (1.39 g, 20.0 mmol) and NaOAc (1.64 g, 20.0 mmol). The solution was stirred at 40 °C for 42 h. After the mixture was cooled, water (150 mL) was added, and the mixture was extracted with CH_2Cl_2 (4 × 50 mL). The combined organic layers were worked up by the standard method to give a colorless solid, which was crystallized from hexanes−Et_2O to give two crops of **21** (3.23 and 1.09 g, respectively). The mother liquors were subjected to flash column chromatography [hexanes−EtOAc (3:2)] to give more **21** (0.53 g). Overall, 4.85 g (97%) of **21** was obtained as a crystalline solid: mp 140−144 °C. $[\alpha]^{27}_D$ +6.9° (*c* 0.55, $CHCl_3$). UV (EtOH) λ_{max} (log ϵ): 248 (4.03) nm. IR (neat): 3286, 2948, 2867, 1721, 1661 cm^{-1}. ^1H NMR ($CDCl_3$): δ 7.7 (1H, brs), 5.76 (1H, s), 3.69 (3H, s), 3.25−3.17 (1H, m), 3.02 (1H, m), 2.86 (1H, d, J = 4.5 Hz), 2.28 (1H, ddd, J = 15.9, 12.9, 6.0 Hz), 2.13−2.05 (1H, m), 1.30, 1.27, 1.21, 1.12, 1.00, 0.97, 0.89 (each 3H, s). ^{13}C NMR ($CDCl_3$): δ 200.6, 178.5, 177.6, 165.7, 123.5, 52.0, 51.2, 49.6, 47.4, 45.5, 41.9, 40.8, 40.1, 36.6, 35.9, 34.6, 33.4, 33.0, 32.5, 31.6, 30.8, 28.2, 26.9, 23.9, 23.7, 23.30, 23.26, 22.8, 21.8, 18.7, 17.4. MS (FAB+) *m/z*: 498 $[M + H]^+$. Anal. ($C_{31}H_{47}NO_4 \cdot {}^1/_4H_2O$) C, H, N.

Methyl 23-Acetoxy-3-acetoxyimino-12-oxoolean-9(11)-en-28-oate (24). A mixture of **21** (2.45 g, 4.92 mmol), NaOAc (0.45 g, 5.49 mmol), and Na_2PdCl_2 (1.61 g, 5.47 mmol) in AcOH (200 mL) was stirred at room temperature for 72 h. It was then poured onto ice (∼500 g). After a few hours, the precipitate was collected by filtration, air-dried, and then dried further at reduced pressure at 60 °C to give the complex **22** (2.67 g) as a pale-beige solid, which was used in the next step without further purification. A mixture of **22** (2.67 g, 2.09 mmol), DMAP (12 mg), Et_3N (0.9 mL), and Ac_2O (0.7 mL) was stirred in anhydrous CH_2Cl_2 (175 mL) at room temperature for 45 min. It was washed with water and dried over $MgSO_4$. Evaporation of the solvent gave **23** as a tan glass, which was dissolved in anhydrous THF (170 mL). Pyridine (0.35 mL) was added, and the solution was stirred at room temperature for 15 min. It was thereafter cooled to −78 °C, and $Pb(OAc)_4$ (95%, 2.14 g, 4.59 mmol) dissolved in AcOH (70 mL) was added slowly. After complete addition, the mixture was allowed to warm to room temperature and was then stirred at room temperature for 15.5 h. A solution of $NaBH_4$ (180 mg) in 1 N aqueous NaOH solution (62 mL) was added, and stirring was continued for 10 min. The mixture was filtered through Celite, diluted with CH_2Cl_2 (200 mL), and washed with saturated aqueous $NaHCO_3$ solution until all AcOH was removed. Drying of the organic layer over $MgSO_4$ and evaporation of the solvents gave a yellow foam, which was subjected to flash column chromatography [hexanes−EtOAc, (3:2)] to give **24** (1.64 g, 56% from **21**) as a crystalline solid: mp 197−199 °C. $[\alpha]^{27}_D$ +52° (*c* 0.58, $CHCl_3$). UV (EtOH) λ_{max} (log ϵ): 246 (4.02) nm. IR (neat): 2952, 2928, 2886, 1769, 1744, 1717, 1658 cm^{-1}. ^1H NMR ($CDCl_3$): δ 5.72 (1H, s), 4.21 (1H, d, J = 11.4 Hz), 4.16 (1H, d, J = 11.4 Hz), 3.63 (3H, s), 3.00−2.86 (2H, m), 2.83 (1H, d, J = 4.8 Hz), 2.53 (1H, ddd, J = 7.2, 11.1, 17.7 Hz), 2.13, 1.97 (each 3H, s), 1.21 (6H, s), 1.15 (3H, s), 0.94 (6H, s), 0.83 (3H, s). ^{13}C NMR ($CDCl_3$): δ 200.2, 178.4, 176.4, 170.9, 169.4, 169.1, 124.3, 67.2, 52.0, 49.7, 47.4, 46.0, 44.5, 44.1, 41.9, 39.3, 36.0, 35.2, 34.6, 33.4, 33.0, 31.8, 31.7, 30.8, 28.2,

24.0, 23.9, 23.3, 22.8, 21.7, 21.0, 20.4, 20.1, 19.5, 18.8. MS (FAB+) *m/z*: 598 $[M + H]^+$. Anal. ($C_{35}H_{51}NO_7$) C, H, N.

Methyl 23-Hydroxy-3-hydroxyimino-12-oxoolean-9(11)-en-28-oate (25). A mixture of **24** (1.64 g, 2.74 mmol) and Na_2CO_3 (1.31 g, 12.4 mmol) in anhydrous MeOH (150 mL) was stirred at room temperature for 16 h. After removal of the solvent in vacuo, the residue was partitioned between CH_2Cl_2 (50 mL) and 5% aqueous AcOH solution (100 mL). The aqueous layer was extracted with additional CH_2Cl_2 (2 × 50 mL). The combined organic extracts were washed three times with saturated aqueous $NaHCO_3$ solution and dried over $MgSO_4$. The solvent was removed in vacuo to give a residue, which was purified by flash column chromatography [hexanes−EtOAc, (1:1)] to give **25** (1.07 g, 76%) as a crystalline solid: mp 218−220 °C; $[\alpha]^{27}_D$ +2.2° (*c* 0.50, $CHCl_3$). UV (EtOH) λ_{max} (log ϵ): 248 (4.05) nm. IR (neat): 3286, 2945, 2863, 1724, 1717, 1661 cm^{-1}. ^1H NMR ($CDCl_3$): δ 5.77 (1H, s), 5.44 (2H, brs), 3.73 (1H, d, J = 11.7 Hz), 3.69 (3H, s), 3.53 (1H, d, J = 11.7 Hz), 3.34−3.26 (1H, m), 3.02 (1H, m), 2.87 (1H, d, J = 4.8 Hz), 2.21−2.07 (2H, m), 1.93−1.79 (2H, m), 1.32, 1.26, 1.05, 0.99, 0.98, 0.88 (each 3H, s). ^{13}C NMR ($CDCl_3$): δ 200.8, 178.6, 177.2, 165.2, 123.3, 66.9, 52.0, 49.7, 47.5, 45.52, 45.46, 45.0, 42.0, 39.8, 36.2, 36.0, 34.6, 33.4, 33.0, 32.3, 31.7, 30.8, 28.2, 24.0, 23.8, 23.3, 22.8, 21.9, 18.7, 18.5, 17.8. MS (FAB+) *m/z*: 514 $[M + H]^+$. HRMS (EI) Calcd for $C_{31}H_{47}NO_5$: 513.3454. Found: 513.3447. Anal. ($C_{31}H_{47}NO_5$) C, H, N.

Methyl 23-Hydroxy-3,12-dioxoolean-9(11)-en-28-oate (26). To a solution of NH_4OAc (3.41 g, 44.2 mmol) in water (125 mL) at room temperature was added $TiCl_3$ (30% w/w solution in 2 N aqueous HCl solution, 3.82 mL), followed by dropwise addition at room temperature of a solution of **25** (842 mg, 1.64 mmol) in freshly distilled THF (120 mL) over 15 min. The resulting mixture was stirred at room temperature for 4 h. It was thereafter extracted with Et_2O (3 × 100 mL) and CH_2Cl_2 (50 mL). The combined organic extracts were washed with saturated aqueous $NaHCO_3$ solution and dried over $MgSO_4$. Evaporation of the solvents gave **26** (723 mg, 88%) as a crystalline solid: mp 228−231 °C. $[\alpha]^{27}_D$ +27° (*c* 0.52, $CHCl_3$). UV (EtOH) λ_{max} (log ϵ): 248 (4.00) nm. IR (neat): 3404, 2928, 2867, 1724, 1691, 1660 cm^{-1}. ^1H NMR ($CDCl_3$): δ 5.78 (1H, s), 3.69 (1H, d, J = 11.4 Hz), 3.66 (3H, s), 3.38 (1H, d, J = 11.4 Hz), 3.00 (1H, m), 2.87 (1H, d, J = 4.5 Hz), 2.70 (1H, ddd, J = 6.3, 12.9, 16.2 Hz), 2.49 (1H, brs), 2.39 (1H, ddd, J = 2.4, 5.1, 16.2 Hz), 2.58−2.18 (1H, m), 1.37, 1.27, 1.01, 0.98, 0.97, 0.86 (each 3H, s). ^{13}C NMR ($CDCl_3$): δ 217.1, 200.4, 178.4, 176.3, 123.7, 66.4, 52.8, 52.0, 49.7, 47.4, 45.5, 44.4, 42.0, 39.3, 36.8, 35.9, 35.5, 34.6, 33.4, 33.0, 32.1, 31.6, 30.8, 28.2, 24.0, 23.8, 23.2, 22.8, 21.9, 18.8, 17.1. HRMS (FAB+) Calcd for $C_{31}H_{46}O_5$ + H: 499.3424. Found: 499.3425. Anal. ($C_{31}H_{46}O_5$) C, H.

Methyl 23-[2-(Trimethylsilyl)ethyloxy]methoxy-3,12-dioxoolean-9(11)-en-28-oate (27). To a mixture of **26** (285 mg, 0.571 mmol) and SEMCl (0.3 mL, 1.7 mmol) in dry CH_2Cl_2 (10 mL) was added (*i*-Pr)$_2$NEt (0.5 mL, 2.9 mmol) at room temperature. The solution was stirred at room temperature for 18 h and was thereafter diluted with CH_2Cl_2 (25 mL). The reaction mixture was worked up by the standard method to give a yellow oily residue. It was subjected to flash column chromatography [hexanes−EtOAc (3:1)] to give **27** (345 mg, 96%) as an amorphous solid: $[\alpha]^{27}_D$ +25° (*c* 0.74, $CHCl_3$). UV (EtOH) λ_{max} (log ϵ): 247 (4.11) nm. IR (neat): 2948, 2872, 1722, 1708, 1660 cm^{-1}. ^1H NMR ($CDCl_3$): δ 5.84 (1H, s), 4.59 (1H, d, J = 6.9 Hz), 4.56 (1H, d, J = 6.9 Hz), 3.68 (3H, s), 3.59 (1H, d, J = 9.3 Hz), 3.53 (1H, t, J = 8.4 Hz), 3.36 (1H, d, J = 9.3 Hz), 3.03 (1H, m), 2.89 (1H, d, J = 4.5 Hz), 2.64−2.46 (2H, m), 2.21−2.13 (2H, m), 1.27, 1.26, 1.02, 0.99, 0.98, 0.88 (each 3H, s), 0.01 (9H, s). ^{13}C NMR ($CDCl_3$): δ 214.4, 200.4, 178.5, 176.5, 124.4, 95.0, 72.0, 65.3, 52.0, 51.3, 49.8, 47.4, 45.6, 43.3, 42.0, 39.2, 36.0, 35.7, 35.5, 34.6, 33.4, 33.0, 31.8, 31.7, 30.8, 28.3, 24.0, 23.8, 23.3, 22.9, 21.9, 19.1, 18.2, 18.0, −1.2. HRMS (FAB+) Calcd for $C_{37}H_{60}O_6Si$ + H: 629.4237. Found: 629.4226.

Methyl 2-Cyano-23-[2-(trimethylsilyl)ethyloxy]methoxy-3,12-dioxooleana-1,9(11)-dien-28-oate (28). To a solution of **27** (188 mg, 0.30 mmol) in anhydrous THF (12 mL) was added LDA (2 M in THF/*n*-heptane, 0.22 mL, 0.44 mmol) dropwise at −78 °C. After complete addition, the mixture was allowed to reach room temperature over 20 min and was then cooled to −78 °C. A solution of *p*-TsCN (106 mg, 0.58 mmol) in dry THF (3 mL) was added dropwise, and the resulting solution was stirred at −78 °C for 20 min, whereupon it was quenched by addition of saturated aqueous NH_4Cl solution (5 mL). After warming to room temperature, the mixture was diluted with water (15 mL) and extracted with EtOAc (3 × 15 mL). The combined organic extracts were worked up by the standard method to give a tan glass, which was dissolved in anhydrous benzene (12 mL). DDQ (68 mg, 0.30 mmol) was added in one portion, and the mixture was heated at reflux for 20 min. After the mixture was cooled to room temperature, the solids were removed by filtration through Celite. The pad was washed with small portions of benzene. The combined filtrate and washings were concentrated in vacuo to give a reddish-brown residue, which was subjected to flash column chromatography [hexanes−EtOAc (3:2)] to give **28** (123 mg, 63%) as a pale-tan glass: $[\alpha]^{27}_D$ +38° (*c* 0.50, $CHCl_3$). UV (EtOH) λ_{max} (log ϵ): 241 (4.27) nm. IR (neat): 2948, 2235, 1721, 1692, 1664 cm^{-1}. ^1H NMR ($CDCl_3$): δ 8.03 (1H, s), 6.00 (1H, s), 4.55 (2H, s), 3.73 (1H, d, J = 9.3 Hz), 3.70 (3H, s), 3.50 (2H, ddd, J = 1.5, 6.9, 8.7 Hz), 3.46 (1H, d, J = 9.3 Hz), 3.05 (1H, m), 2.94 (1H, d, J = 4.5 Hz), 2.51−2.44 (1H, m), 1.49, 1.32, 1.06, 1.03, 1.00, 0.90 (each 3H, s), 0.03 (9H, s). ^{13}C NMR ($CDCl_3$): δ 199.1, 195.7, 178.4, 168.9, 165.7, 124.5, 115.9, 114.4, 95.0, 72.0, 65.6, 52.1, 50.0, 49.3, 47.4, 46.0, 42.4, 42.3, 40.7, 35.9, 34.6, 33.4, 32.9, 31.7, 31.6, 30.8, 28.2, 28.0, 24.7, 23.2, 22.8, 21.9, 18.2, 18.2, 17.6, −1.2. HRMS (FAB+) Calcd for $C_{38}H_{57}NO_6Si$ + H: 652.4033. Found: 652.4009.

Methyl 2-Cyano-23-hydroxy-3,12-dioxooleana-1,9(11)-dien-28-oate (29). To a solution of **28** (123 mg, 0.189 mmol) in CH_3CN (6 mL) was added 48% aqueous HF solution (0.6 mL). The resulting mixture was stirred at room temperature overnight. It was thereafter diluted with a mixture of Et_2O and CH_2Cl_2 (2:1, 20 mL), washed with water and saturated aqueous $NaHCO_3$ solution, and dried over $MgSO_4$. Evaporation of the solvents in vacuo gave **29** (98 mg, 100%) as an amorphous solid. This material was used for the next reaction without further purification. An analytically pure sample was obtained by flash column chromatography [hexanes−EtOAc (1:1)] as an amorphous solid: $[\alpha]^{27}_D$ +51° (*c* 1.1, $CHCl_3$). UV (EtOH) λ_{max} (log ϵ): 241 (4.16) nm. IR (neat): 3501, 2946, 2236, 1720, 1689, 1662 cm^{-1}. ^1H NMR ($CDCl_3$): δ 8.10 (1H, s), 5.98 (1H, s), 3.92 (1H, d, J = 10.8 Hz), 3.69 (3H, s), 3.51 (1H, d, J = 10.8 Hz), 3.04 (1H, m), 2.94 (1H, d, J = 4.5 Hz), 2.42−2.38 (1H, m), 1.51, 1.32, 1.05, 1.02, 0.98, 0.89 (each 3H, s). ^{13}C NMR ($CDCl_3$): δ 199.2, 197.2, 178.4, 168.6, 166.5, 124.2, 115.4, 114.3, 66.9, 52.1, 50.6, 49.9, 47.4, 45.9, 42.5, 42.3, 40.6, 35.9, 34.6, 33.4, 32.9, 31.7, 31.5, 30.8, 28.2, 27.7, 24.8, 23.2, 22.8, 21.9, 18.1, 17.2. HRMS (FAB+) Calcd for $C_{32}H_{43}NO_5$ + H: 522.3219. Found: 522.3229. Anal. ($C_{32}H_{43}NO_5 \cdot {}^3/_4CH_2Cl_2$)[29] C, H, N.

Evaluation Methods.[4,12] 1. Reagents. All chemicals were purchased from Sigma Chemicals Co. (St. Louis, MO). Inhibitory test compounds were dissolved in DMSO and kept at −80 °C before addition to MCF-7 (ER positive) cell culture; final concentrations of DMSO were 0.1% or less. A control with DMSO alone was run in this assay.

2. Cell Culture. The MCF-7 breast cancer cell line was purchased from American Type Culture Collection (Manassas, VA) and maintained in DMEM/F12 supplemented with 10% FBS and penicillin/streptomycin (50 units/mL penicillin and 50 μg/mL streptomycin). MCF-7 cells were incubated in 10% CO_2.

3. Measurement of Cellular Proliferation of MCF-7. MCF-7 cells were treated with the test compounds at 37 °C for 3 days, and cellular proliferation was measured by incorporation of radioactive thymidine. IC_{50} values (μM) of the test compounds were determined in the range of 1 nM to 10 μM (3-fold dilutions). Values are an average of two separate experiments.

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Supporting Information Available: Elemental analysis results of **4**, **5**, **6**, **21**, **24**, **25**, **26**, and **29**. This material is available free of charge via the Internet at http://pubs.acs.org.

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