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Design and Synthesis of Non-peptide RGD Mimics for Evaluation of Their Utility as Anti-platelet Agents

Kazuhiro Higuchi,* Hideki Hikita, Asumi Murayama, Daichi Yuri, Natsu Kobayakawa, Takashi Takahashi, Shigeru Kojima, Hiroko Ueno, Tomomi Hatakeyama, Airi Kato, Masanori Tayu, Etsuko Oyama, Shigeo Sugiyama, Kazuyuki Ishii, Hidenobu Takahashi, and Tomomi Kawasaki*

Meiji Pharmaceutical University; 2-522-1 Noshio, Kiyose, Tokyo 204-8588, Japan.

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Arg-Gly-Asp (RGD) mimics were synthesized and their anti-platelet activity was evaluated. A concise method was developed for the synthesis of the target compounds from dehydroepiandrosterone and Wieland–Miescher and Hajos–Parrish ketones, which are suitable for readily available platform. Among the synthesized compounds, the perhydronaphthalene framework with a 3-(4-piperidinyl)propoxyl structure **3e possessed the highest anti-aggregative activity. The IC_{50} values of **3e** were 0.91 mM (ADP initiation) and 0.54 mM (collagen initiation).**

Key words non-peptide Arg-Gly-Asp (RGD) mimic; antiplatelet agent; dehydroepiandrosterone; Wieland–Miescher ketone; Hajos–Parrish ketone

Platelets play an important role in hemostasis; however, its aggregation triggers several serious diseases, including cardiovascular, cerebrovascular, and peripheral vascular diseases. Platelet aggregation process is occurred by the stimulation of various physiological activators such as ADP, collagen and thrombin.^{1,2)} Finally the stimulation derives the structural transformation of glycoprotein IIb/IIIa (integrin $\alpha IIb\beta 3$) on the platelet surface. The activated GPIIb/IIIa receptor interacts with fibrinogen to induce platelet aggregation.^{3–5)} The interaction between GPIIb/IIIa and fibrinogen is mediated by the Arg-Gly-Asp (RGD) sequence in fibrinogen.^{6–8)} Additionally, the β -turn structure formed by the sequence enhances the interaction of each molecules.^{9,10)} Therefore, RGD mimics are valuable platelet aggregation antagonists, and many compounds have been developed.^{11–14)} Among them, tirofiban (**1**)¹⁵⁾ and eptifibatide (**2**)¹⁶⁾ were launched for clinical use (Fig. 1).

To develop a more efficient approach to RGD mimics, we assumed that the shape and volume of the β -turn structure is equal to that of perhydro polycyclic hydrocarbon structures such as perhydro-phenanthrene,^{17,18)} -naphthalene, and -indene (Fig. 2). Therefore, these hydrocarbons, with acidic and basic moieties of the appropriate position and size, were hypothesized to be good candidate for antiplatelet agents. Moreover, such compounds should be biologically stable and show better oral bioavailability than the peptide ligands. Additionally, a

method for stereoselective transformation of such compounds is available, based on established concepts in steroid chemistry, enabling detailed conformational analysis of the ligand docking site. We planned to develop a method for synthesis of the orally active antiplatelet agents **3a–e** from the readily available dehydroepiandrosterone (**4**) and Wieland–Miescher (**5**) and Hajos–Parrish ketones (**6**). Herein, we report the synthesis of the target peptide mimic compounds and an evaluation of those antiplatelet activities.

Chemistry

First, for preparation of the perhydrophenanthrene compounds **3a** and **b**, the common intermediate **11** was synthesized from dehydroepiandrosterone **4** (Chart 1). Acetalization of the ketone **4**¹⁹⁾ and Oppenauer oxidation of the secondary alcohol accompanying isomerization of the olefin yielded an enone.²⁰⁾ The stereoselective 1,4-reduction of the enone under Birch conditions²¹⁾ quenching with solid NH_4Cl afforded **7**,²²⁾ and then Horner–Wadsworth–Emmons olefination provided the α,β -unsaturated ester **8a**. One-electron reduction of the α,β -unsaturated ester in **8a** with magnesium metal²³⁾ gave a mixture of stereoisomers ($\beta:\alpha=1:1$); however, Birch reduction of α,β -unsaturated carboxylic acid **8b**²⁴⁾ gave the β -rich isomer **9** with excellent selectivity ($\beta:\alpha>10:1$). Methylation of carboxylic acid **9b** and deacetalization of **9a** afforded compound

Fig. 1. RGD Mimics for Acute Platelet Diseases

* To whom correspondence should be addressed. e-mail: khiguchi@my-pharm.ac.jp; kawasaki@my-pharm.ac.jp

Fig. 2. Synthetic Strategy for RGD Mimics **3a–e**

10, which was converted to the intermediate **11** by ozonolysis of the enol acetate of **10**, followed by methylation of the carboxylic acid provided.^{25,26)}

Side-chain elongation of intermediate **11** was carried out by Horner–Wadsworth–Emmons (HWE) olefination with diethyl cyanomethyl phosphonate to yield **12** (Chart 2). Hydrogenation of the α,β -unsaturated nitrile in **12** with Adams' catalyst^{27,28)} followed by *tert*-butoxycarbonyl (Boc) protection afforded the carbamate **13**. Hydrolysis of the primary ester and subsequent deprotection afforded a primary amine. Finally, the guanidine formation from the amine with *N,N'*-bis(Boc)-1*H*-pyrazole-1-carboxamide and subsequent deprotection²⁹⁾ gave the target compound **3a**.

An alternative target compound, **3b**, which is one carbon shorter at the side chain than compound **3a**, was synthesized from intermediate **11**, by the following procedure (Chart 3).

Wittig chain elongation of intermediate **11**, using methoxymethyl triphenylphosphonium chloride followed by hydrolysis of the enolate, produced aldehyde **14**. Condensation between aldehyde **14** and hydroxylamine,³⁰⁾ followed by hydrogenation³¹⁾ and protection of the amine produced compound **15**, at 78% yield. The following transformation to the target compound **3b** was achieved by the same procedure as the synthesis of compound **3a**.

For regulation of the hydrophilicity of the test compounds, we synthesized bicyclic compounds such as perhydro-naphthalene and -indene from Wieland–Miescher (**5**) and Hajos–Par-

Reagents and conditions: (a) $(CH_2OH)_2$, CSA, benzene, reflux, 80%; (b) $Al(OPr^i)_3$, cyclohexanone, toluene, reflux, 97%; (c) Li, liq. NH_3 , THF, $-78^\circ C$, then NH_4Cl , 88%; (d) $(EtO)_2P(O)CH_2CO_2Me$, $KOBu^t$, DMF, $0^\circ C$ –r.t., 97%; (e) KOH, EtOH/ H_2O , $90^\circ C$; (f) Li, liq. NH_3 , t -BuOH, THF/dioxane, $-78^\circ C$, $\beta:a \geq 10:1$; (g) MeI, NaH, DMF, r.t.; (h) 1.5M HCl, THF, r.t., 91% in 4 steps; (i) isopropenyl acetate, conc. H_2SO_4 , reflux, 75%; (j) O_3 , CH_2Cl_2 /AcOH, then Me_2S , AcOH/ H_2O , $-78^\circ C$ to r.t.; (k) CH_2N_2 , Et_2O , $0^\circ C$, 83% in 2 steps.

Chart 1

Reagents and conditions: (a) $(EtO)_2P(O)CH_2CN$, $KOBu^t$, DMF, $0^\circ C$ to r.t., 96%; (b) PtO_2 , H_2 , HCl/MeOH/ $CHCl_3$, r.t.; (c) Boc_2O , Et_3N , CH_2Cl_2 , $0^\circ C$ to r.t., 95% in 2 steps; (d) 10% LiOH aq., Bu_4NHSO_4 , MeOH/THF, $0^\circ C$ to r.t., quant.; (e) TFA, CH_2Cl_2 , $0^\circ C$ to r.t.; (f) *N,N'*-bis(*tert*-butoxycarbonyl)-1*H*-pyrazole-1-carboxamide, pyridine, $0^\circ C$ to r.t., quant. in 2 steps; (g) TFA, CH_2Cl_2 , $0^\circ C$ to r.t., 87%.

Chart 2

rich ketones (**6**) (Charts 4, 5).

At first, a keto-acetal **17** was produced from **5**, via a previously reported procedure^{32–34} (Chart 4). To introduce the carboxyl unit, a HWE reaction of **17** yielded the α,β -unsaturated ester **18a** at 93% yield, as a mixture of geo-

metric isomers ($E:Z=1:1$). Hydrogenation or one-electron reduction of **18a** with magnesium metal afforded a mixture of epimers ($\beta:\alpha=1:1$ to 8:1). In contrast, the Birch reduction of carboxylic acid **18b** followed by deacetalization and esterification gave the desired β compound **19** in good yield.

Reagents and conditions: (a) $\text{MeOCH}_2\text{PPh}_3\cdot\text{Cl}$, LHMDS, THF, -78°C , 4 h, 73%; (b) $p\text{-TsOH}\cdot\text{H}_2\text{O}$, acetone/ H_2O , 0°C to r.t., 73%; (c) $\text{NH}_2\text{OH}\cdot\text{HCl}$, Et_3N , EtOH, r.t.; (d) PtO_2 , H_2 , AcOH, r.t.; (e) Boc_2O , Et_3N , CH_2Cl_2 , 0°C to r.t., 78% in 3 steps; (f) 10% LiOH aq., Bu_4NHSO_4 , MeOH/THF, 0°C to r.t., quant.; (g) TFA, CH_2Cl_2 , 0°C to r.t.; (h) N,N' -bis(*tert*-butoxycarbonyl)-1*H*-pyrazole-1-carboxamide, pyridine, 0°C to r.t., 71% in 2 steps; (i) TFA, CH_2Cl_2 , 0°C to r.t., quant.

Chart 3

Reagents and conditions: (a) $(\text{EtO})_2\text{P}(\text{O})\text{CH}_2\text{CO}_2\text{Me}$, KOBu^t , DMF, r.t., 98%; (b) KOH , EtOH/ H_2O , 90°C , 93%; (c) Li, liq. NH_3 , THF, -78°C ; (d) 1.5 M HCl, THF, r.t.; (e) MeI, K_2CO_3 , acetone, r.t., 91% in 3 steps; (f) $(\text{EtO})_2\text{P}(\text{O})\text{CH}_2\text{CN}$, KOBu^t , DMF, 70°C , 95%; (g) 10% Pd/C, H_2 , EtOH, 1.5 h, quant. ($\beta:\alpha=14:1$); (h) PtO_2 , H_2 , 10% HCl-MeOH, r.t.; (i) N,N' -bis(*tert*-butoxycarbonyl)-1*H*-pyrazole-1-carboxamide, Et_3N , DMF, r.t., 83% in 2 steps; (j) 10% LiOH aq., MeOH-THF, 0°C to r.t., 75%; (k) recrystallization 13%; (l) 4 M HCl-AcOEt, 0°C to r.t., quant.

Chart 4

Reagents and conditions: (a) Li, liq. NH_3 , THF, -78°C , then NH_4Cl , 70%; (b) $(\text{EtO})_2\text{P}(\text{O})\text{CH}_2\text{CO}_2\text{Me}$, KOBu^t , DMF, r.t., 97%; (c) KOH , EtOH/ H_2O , r.t., 71%; (d) Li, liq. NH_3 , THF, -78°C , then NH_4Cl ; (e) 1.5 M HCl, THF, r.t.; (f) MeI, K_2CO_3 , acetone, r.t., 98% in 3 steps; (g) $(\text{EtO})_2\text{P}(\text{O})\text{CH}_2\text{CN}$, NaH, DMF, r.t., 93%; (h) 10% Pd/C, H_2 , EtOH, r.t., 80%; (i) PtO_2 , H_2 , 10% HCl-MeOH; (j) N,N' -bis(*tert*-butoxycarbonyl)-1*H*-pyrazole-1-carboxamide, Et_3N , DMF, r.t., 13% in 2 steps; (k) 10% LiOH aq., MeOH-THF, r.t., 82%; (l) 4 M HCl-AcOEt, r.t., 56%.

Chart 5

Reagents and conditions: (a) NaBH_4 , MeOH, 0°C , 83%; (b) *tert*-butyl 4-(3-bromopropyl)piperidine-1-carboxylate, NaH, DMF, r.t., 29%; (c) 4M HCl–AcOEt, r.t., 75%.

Chart 6

Side-chain elongation with diethyl cyanomethylphosphonate and potassium *tert*-butoxide gave **20** at 95% yield. The direct transformation of the cyanomethylidene moiety in **20** to an aminoethyl group, using Adams' catalyst, resulted in a mixture of diastereomers ($\beta:\alpha=1:1$). Stepwise transformation by hydrogenation of the olefin in **20** with 10% palladium on carbon ($\beta:\alpha=14:1$, determined by $^1\text{H-NMR}$ spectrum) and hydrogenation of the cyano group in **21** with Adams' catalyst followed by amidylation of the amine gave the guanidino compound **22** at good yield and diastereoselectivity. After hydrolysis of the ester **22** with 10% LiOH aq., the carboxylic acid was recrystallized to give the diastereomerically pure β -compound **24**. Finally, deprotection of the *N*-Boc guanidino compound **24** using 4M HCl yielded the target compound **3c**.

The indene core compound **3d** was also synthesized from a Hajos–Parrish ketone (**6**),^{35,36} following the same procedure as described for compound **3c** (Chart 5).

We also synthesized a pyrrolidino compound, **3e**, from the intermediate **19** (Chart 6). The reduction of ketone **19**, which was synthesized in Chart 4, with NaBH_4 afforded the alcohol **29** ($\beta:\alpha=8.5:1$, determined by the $^1\text{H-NMR}$ spectrum). Etherification of the secondary alcohol in **29** with *N*-Boc bromopropyl piperidine³⁷ gave compound **30**. Finally, deprotection of the *N*-Boc guanidino compound **30**, using 4M HCl, afforded the target compound **3e**.

Thus, accessibility to test compounds such as **3e** will facilitate evolution of the structural diversity of RGD mimic analogs.

Biological Activity

In a preliminary study, the tricyclic compounds **3a** and **b** were only slightly soluble in a buffer solution and dimethyl sulfoxide (DMSO), preventing testing of their anti-platelet activity. Therefore, we investigated the anti-platelet activity of the soluble bicyclic compounds **3c–e**, which have low-lipophilicity cores relative to **3a** and **b**. Initially, the dependence of the anti-platelet activity of compound **3c** on concentration was measured by initiation with both ADP (Fig. 3, left) and collagen (Fig. 3, right) in platelet-rich plasma (PRP). The extent of platelet aggregation was measured at 5 min after addition of ADP. Maximum aggregation of the control was set as 100%. A strong correlation was observed between the concentration and the antiplatelet activity of **3c**, using each inducer.

Next, the anti-aggregative activity of compounds **3c–e** was investigated in ADP-induced platelet aggregation (Fig. 4). The aggregation ratio of the perhydronaphthalene **3c** was

much higher than that of the perhydroindane **3d** (Fig. 4, left). Comparison of the activities of the perhydronaphthalenes **3c** and **e** indicated that the 3-(4-piperidinyl)propoxyl moiety in **3e** is more effective than the guanidino group in **3c**. In addition, Arg-Gly-Asp-Ser (RGDS) showed extremely smooth and strong anti-platelet activity compared to **3e** (Fig. 4, right). Antiplatelet activity of RGD mimics were evaluated from maximum aggregation value of each compound against that of control (Fig. 5). The maximum anti-aggregation ratio of **3e** (60%) was much higher than those of compounds **3c** (41%) and **d** (17%).

Conclusion

We have studied the synthesis of RGD-mimic compounds and evaluated the anti-platelet activity of each compound. We have developed a concise method for synthesis of RGD mimics from readily available steroid and bicyclic ketones. Among the compounds synthesized, the perhydronaphthalene framework with the 3-(4-piperidinyl)propoxyl structure **3e** possessed the highest anti-aggregative activity. Further analogue syntheses and those biological evaluation are under investigation.

Experimental

General Methods All melting points were measured on a Yanagimoto micro melting point apparatus and are uncorrected. Optical rotations were obtained using a JASCO P-2200 polarimeter. Optical purities were determined on a JASCO HPLC (PU-2089 plus, MD-2015 plus) instrument equipped with Daicel Chemical chiral columns. IR spectra were recorded on a Shimadzu IRPrestige-21 spectrophotometer. ^1H - and ^{13}C -NMR spectra were measured on a JEOL JNM-AL300 (300 MHz), JEOL JMN-AL400 (400 MHz), JEOL JNM-LA500 (500 MHz) or JNM-ECA500 (500 MHz) spectrometers with tetramethylsilane as an internal standard. *J*-Values are given in Hertz. Mass spectra were recorded on a JEOL JMS-DX302 or JEOL JMS 700 instruments with a direct inlet system. Elemental analysis was performed using a Yanaco MT-6 elemental analyzer. Column chromatography was carried out on silica gel [Kanto Chemical Co., Inc., Japan (Silica Gel 60N, Spherical, neutral 40–50 μm) and Merck Ltd. (Silica Gel 60F₂₅₄, 230–400 mesh)]. TLC was carried out on silica gel plates [Merck Ltd., Germany (TLC Silica gel 60F₂₅₄)] and visualized with UV254 light and/or staining with phosphomolybdic acid in EtOH. The purity of compounds **3a–e** were measured by the internal standard method using 1,4-bis(trimethylsilyl)benzene.³⁸

Fig. 3. Concentration Dependence of Platelet Aggregation by Compound **3c**, Initiated by ADP (Left) and Collagen (Right)

Fig. 4. Anti-platelet Activity of Compounds **3c–e** and RGDS (1.0 mM) on ADP-Induced Platelet Aggregation

Anti-platelet activities were monitored during 5 min after addition of an inducer.

Fig. 5. Anti-platelet Activity of Compounds **3c–e** and RGDS (1.0 mM) on ADP-Induced Platelet Aggregation

Data are expressed as the mean $\pm$ standard error (S.E.) ($n=6$ for **3c–e**, $n=5$ for RGDS). ** $p<0.01$ compared with RGDS.

(3*S*,8*R*,9*S*,10*R*,13*S*,14*S*)-10,13-Dimethyl-1,2,3,4,7,8,9,10,11,12,13,14,15,16-tetradecahydrospiro[cyclopenta[*a*]phenanthrene-17,2'-[1,3]dioxolan]-3-ol To a solution of **4** (25.0 g, 86.7 mmol) in benzene (420 mL) were added ethylene glycol (14.5 mL, d 1.11, 259 mmol) and camphorsulfonic acid (4.0 g, 17.2 mmol). The reaction mixture was heated under reflux using Dean-Stark apparatus for 3 h. The mixture was cooled to room temperature (r.t.) and neutralized using satd aqueous NaHCO_3 on an ice cooling bath. The mixture was extracted with EtOAc and the combined organic layer was washed with brine. The extract was dried over MgSO_4 and concentrated under reduced pressure. The residue was purified using column chromatography (AcOEt-*n*-hexane=1:8) and acetal (23 g, 80%) was obtained as a white powder.

mp 166–168°C (AcOEt-*n*-hexane); $[\alpha]_D^{27}$ -92.3 ($c=0.48$, CHCl_3); *Anal.* Calcd for $\text{C}_{21}\text{H}_{32}\text{O}_3$: C, 75.86; H, 9.70. Found: C, 75.82; H, 9.60.

(8*R*,9*S*,10*R*,13*S*,14*S*)-10,13-Dimethyl-1,6,7,8,9,10,11,12,13,14,15,16-dodecahydrospiro[cyclopenta[*a*]phenanthrene-17,2'-[1,3]dioxolan]-3(2*H*)-one Under nitrogen atmosphere, $\text{Al}(\text{O}i\text{-Pr})_3$ (2.6 g, 12.7 mmol) and cyclohexanone (39.7 mL, d 0.95, 383 mmol) were added to a toluene (255 mL) solution of acetal (8.5 g, 25.5 mmol) at r.t. After the 2 h reflux, the reaction mixture was cooled to r.t. and added satd aqueous potas-

sium sodium tartrate. The aqueous layer was extracted with AcOEt and the combined extract was dried over MgSO_4 . The concentrated residue was purified by column chromatography (AcOEt–*n*-hexane=1:3) to give enone (8.2 g, 97%) as colorless crystals.

mp 148–149°C (AcOEt–*n*-hexane); $[\alpha]_{\text{D}}^{27} +62.2$ ($c=0.49$, CHCl_3); *Anal.* Calcd for $\text{C}_{21}\text{H}_{30}\text{O}_3$: C, 76.33; H, 9.15. Found: C, 76.17; H, 8.98.

(5*S*,8*R*,9*S*,10*S*,13*S*,14*S*)-10,13-Dimethyltetradecahydro-spiro[cyclopenta[*a*]phenanthrene-17,2'-[1,3]dioxolan]-3(2*H*)-one (7) Under a nitrogen atmosphere, a lithium wire (1.0 g, 144 mmol) was added to liquid ammonia (300 mL) at –78°C. To the reaction mixture was added the solution of enone (4.0 g, 12.1 mmol) in tetrahydrofuran (THF) (150 mL) and stirred for 30 min. After the substrate disappearance was monitored by TLC, solid NH_4Cl was added until the mixture turned white suspension. The mixture was allowed to r.t. and the liquid ammonia was evaporated through the night. The residue was suspended with CH_2Cl_2 and filtrated. The concentrated residue was purified by column chromatography (AcOEt–*n*-hexane=1:3) to give compound **7** (3.5 g, 88%) as white crystals.

mp 203–204°C (MeOH); $[\alpha]_{\text{D}}^{28} +2.1$ ($c=0.47$, CHCl_3); *Anal.* Calcd for $\text{C}_{21}\text{H}_{32}\text{O}_3$: C, 75.86; H, 9.70. Found: C, 75.96; H, 9.91.

Methyl 2-((5*S*,8*R*,9*S*,10*S*,13*S*,14*S*)-10,13-Dimethyltetradecahydrospiro[cyclopenta[*a*]phenanthrene-17,2'-[1,3]dioxolan]-3(2*H*)-ylidene)acetate (8*a*) To a suspension of *t*-BuOK (5.3 g, 47.5 mmol) in anhydrous DMF (95 mL) was added methyl diethylphosphonoacetate (10.5 mL, d 1.15, 57.0 mmol) at r.t. and stirred for 1 h under a nitrogen atmosphere. The mixture was cooled to 0°C and added the solution of **7** (6.3 g, 19.0 mmol) in anhydrous DMF (95 mL) and stirred for 12.5 h. The reaction mixture was poured into water and extracted with Et_2O . The combined extracts were washed with brine, dried over MgSO_4 and concentrated under the reduced pressure. The residue was purified by column chromatography (AcOEt–*n*-hexane=1:6) to give compound **8a** (7.1 g, 97%) as a white powder.

mp 199–201°C (MeOH); $[\alpha]_{\text{D}}^{28} +68.8$ ($c=0.16$, CHCl_3); IR (CHCl_3) cm^{-1} : 2945, 1705, 1643, 1204, 1165; $^1\text{H-NMR}$ (CDCl_3 , 400 MHz) δ : 5.59 (t, 1H, $J=1.6$ Hz), 4.04–3.80 (m, 4H), 3.67 (s, 3H), 2.44–2.26 (m, 1H), 2.22–2.08 (m, 1H), 2.06–0.45 (m, 20H), 0.94 (s, 3H), 0.85 (s, 3H); $^{13}\text{C-NMR}$ (CDCl_3 , 100 MHz) δ : 167.0, 163.5, 119.3, 112.1, 65.2, 64.6, 54.1, 50.8, 50.4, 47.6, 46.0, 40.2, 36.2, 35.8, 34.3, 33.7, 32.3, 31.4, 30.8, 29.0, 22.8, 20.7, 14.6, 12.1; HR-MS (EI) Calcd for $\text{C}_{24}\text{H}_{36}\text{O}_4$ m/z [M^+]: 388.2614. Found 388.2612; *Anal.* Calcd for $\text{C}_{24}\text{H}_{36}\text{O}_4$: C, 74.19; H, 9.34. Found: C, 74.04; H, 9.59.

Methyl 2-((3*S*,5*S*,8*R*,9*S*,10*S*,13*S*,14*S*)-10,13-Dimethyl-17-oxohexadecahydro-1*H*-cyclopenta[*a*]phenanthren-3-yl)-acetate (10) To a suspension of **8a** (7.1 g, 18.3 mmol) in 1:1 EtOH/ H_2O (184 mL) was added KOH (10.3 g, 183 mmol) and the mixture was heated at 90°C for 1.5 h. The reaction mixture was concentrated and neutralized with dilute HCl on the ice bath. The mixture was extracted with CH_2Cl_2 and the combined organic layer was dried over MgSO_4 . The filtrate was concentrated to obtain the crude α,β -unsaturated carboxylic acid **8b**.

Under the nitrogen atmosphere, a lithium wire (1.3 g, 187 mmol) was added to liquid ammonia (30 mL) at –78°C. To the mixture was added the solution of the above crude

α,β -unsaturated carboxylic acid **8b** in 1,4-dioxane (95 mL), THF (95 mL) and *t*-BuOH (7.1 mL, d 0.78, 74.2 mmol). After 3 h stirring, MeOH was added until the mixture turned white suspension. The mixture was allowed to r.t. and the liquid ammonia was evaporated through the night. The residue was suspended with CH_2Cl_2 and filtrated. The solution was acidified using dilute HCl and the water layer was extracted by CH_2Cl_2 . The combined organic layer was dried over MgSO_4 and the concentrated residue was evaporated to give the crude carboxylic acid **9b**. The ratio of isomers was determined the benzyl esters of **9b** by $^1\text{H-NMR}$.

Under the nitrogen atmosphere, sodium hydride (NaH) (2.2 g, 60% oil suspension, 54.9 mmol) was added to the above crude carboxylic acid **9b** in anhydrous DMF (183 mL) at r.t. After the reaction mixture was stirred for 1 h, methyl iodide (MeI) (8.0 mL, 54.9 mmol) was added and the reaction mixture was stirred overnight. The reaction was concentrated, diluted by Et_2O and washed with H_2O . The organic layer was dried over MgSO_4 and concentrated to give crude methyl ester.

To the above crude methyl ester in THF (183 mL) was added 1.5*N* HCl (245 mL) using the dropping funnel at 0°C and the mixture was stirred for 4 h at r.t. The reaction mixture was neutralized with 10% NaOH and concentrated under reduced pressure. The residue was extracted with AcOEt and the organic layer was dried over MgSO_4 . The concentrated residue was purified by column chromatography (AcOEt–*n*-hexane=1:7) to give compound **10** (5.8 g, 91%, 4 steps) as a white powder.

mp 110–115°C (AcOEt–*n*-hexane); $[\alpha]_{\text{D}}^{28} +74.3$ ($c=0.50$, CHCl_3); IR (CHCl_3) cm^{-1} : 3012, 2920, 2855, 1728, 1439; $^1\text{H-NMR}$ (CDCl_3 , 400 MHz) δ : 3.64 (s, 3H), 2.41 (dd, 1H, $J=19.0$, 8.7 Hz), 2.18 (d, 2H, $J=7.1$ Hz), 1.91 (dt, 1H, $J=19.0$, 9.0 Hz), 1.97–1.86 (m, 1H), 1.86–0.60 (m, 20H), 0.84 (s, 3H), 0.77 (s, 3H); $^{13}\text{C-NMR}$ (CDCl_3 , 100 MHz) δ : 220.9, 173.2, 54.6, 51.5, 51.4, 47.8, 46.4, 41.8, 38.3, 36.0, 35.9, 35.24, 35.21, 35.1, 31.7, 31.0, 28.59, 28.57, 21.9, 20.4, 13.9, 12.4; HR-MS (EI) Calcd for $\text{C}_{22}\text{H}_{34}\text{O}_3$ m/z [M^+]: 346.2508. Found 346.2511.

Methyl 2-((3*S*,5*S*,8*R*,9*S*,10*S*,13*S*,14*S*)-17-Acetoxy-10,13-dimethyl-2,3,4,5,6,7,8,9,10,11,12,13,14,15-tetradecahydro-1*H*-cyclopenta[*a*]phenanthren-3-yl)acetate To a solution of **10** (6.1 g, 17.6 mmol) in isopropenyl acetate (38.4 mL) was added the solution of conc. H_2SO_4 (0.1 mL) in isopropenyl acetate (5.0 mL) under a nitrogen atmosphere. After stirring for 5.5 h at 90°C, above H_2SO_4 in isopropenyl acetate (1.9 mL) was added and then the reaction mixture was stirred for 4.5 h at same temperature. The reaction mixture was diluted with Et_2O and neutralized with satd aqueous NaHCO_3 at 0°C. The mixture was extracted with Et_2O and the combined organic layer was dried over MgSO_4 . The concentrated residue was purified by column chromatography (AcOEt–*n*-hexane=1:15) to give acetate (5.2 g, 75%) as a white solid.

IR (CHCl_3) cm^{-1} : 2920, 2855, 1732, 1229, 1202; $^1\text{H-NMR}$ (CDCl_3 , 400 MHz) δ : 5.44 (dd, 1H, $J=2.9$, 1.5 Hz), 3.65 (s, 3H), 2.29–2.07 (m, 1H), 2.19 (d, 2H, $J=7.1$ Hz), 2.13 (s, 3H), 1.98–0.67 (m, 20H), 0.87 (s, 3H), 0.77 (s, 3H); $^{13}\text{C-NMR}$ (CDCl_3 , 100 MHz) δ : 173.2, 168.6, 159.5, 111.1, 55.1, 54.2, 51.4, 46.7, 44.9, 41.9, 38.1, 36.1, 35.3 (CH), 35.3 (CH₂), 33.7, 33.5, 31.2, 29.0, 28.7, 28.6, 21.3, 20.6, 15.7, 12.4; HR-MS (EI) Calcd for $\text{C}_{24}\text{H}_{36}\text{O}_4$ m/z [M^+]: 388.2614. Found 388.2617.

Methyl (1*S*,2*S*,4*aS*,4*bS*,7*S*,8*aS*,10*aR*)-7-(2-Methoxy-2-oxoethyl)-2,4*b*-dimethyl-1-(2-oxoethyl)tetradecahydrophen-

anthrene-2-carboxylate (11) To a solution of acetate (1.0 g, 2.6 mmol) and AcOH (2.1 mL) in CH_2Cl_2 (26 mL) was bubbled with ozone at -78°C for 3 h. The dissolved ozone gas was removed by Ar bubbling through the solution. Then Me_2S (3.0 mL, d 0.85, 41.0 mmol) was added to the mixture, and the resulting mixture was stirred at 0°C for 6.5 h. After addition of AcOH (24 mL) and H_2O (8 mL), the mixture was stirred at r.t. for 1 d. The reaction mixture was added H_2O and extracted with CH_2Cl_2 . The organic layer was dried over MgSO_4 and concentrated *in vacuo*. The residue was diluted with Et_2O , and a solution of CH_2N_2 in Et_2O was added until the resulting solution turned to yellow. The mixture was warmed to r.t. and stirred overnight. The concentrated residue was purified by column chromatography (Et_2O -*n*-hexane=1:5) to give compound **11** (845 mg, 83% in 2 steps) as a white solid.

mp $68\text{--}70^\circ\text{C}$ (AcOEt -*n*-hexane); IR (CHCl_3) cm^{-1} : 3026, 2949, 2855, 1721, 1435; $[\alpha]_{\text{D}}^{27} -26.6$ ($c=0.36$, CHCl_3); $^1\text{H-NMR}$ (CDCl_3 , 400 MHz) δ : 9.68 (d, 1H, $J=3.2$ Hz), 3.654 (s, 3H), 3.646 (s, 3H), 2.42–2.31 (m, 1H), 2.27 (ddd, 1H, $J=17.2$, 7.2, 3.2 Hz), 2.19 (d, 2H, $J=6.8$ Hz), 2.10 (dd, 1H, $J=17.2$, 2.2 Hz), 1.88–0.67 (m, 18H), 1.11 (s, 3H), 0.72 (s, 3H). $^{13}\text{C-NMR}$ (CDCl_3 , 100 MHz) δ : 201.9, 178.1, 173.2, 53.3, 52.0, 51.4, 47.4, 46.7, 45.8, 41.8, 41.7, 38.1, 37.4, 36.7, 35.9, 35.2, 35.0, 31.9, 28.7, 28.5, 19.8, 15.6, 12.3; HR-MS (FAB) Calcd for $\text{C}_{23}\text{H}_{37}\text{O}_5$ m/z $[\text{MH}^+]$: 393.2641. Found 393.2641.

Methyl (1S,2S,4aS,4bS,7S,8aS,10aR)-1-(3-Cyanoallyl)-7-(2-methoxy-2-oxoethyl)-2,4b-dimethyltetradecahydrophenanthrene-2-carboxylate (12) To a suspension of KO^tBu (44.0 mg, 0.38 mmol) in DMF (640 μL) was added diethyl cyanomethylphosphonate (59.6 μL , d 1.13, 0.38 mmol) and the mixture was stirred at 0°C for 1 h under a nitrogen atmosphere. To the solution was added compound **11** (50.0 mg, 0.13 mmol) in anhydrous DMF (640 μL) and stirred at r.t. for 10 h. The reaction mixture was poured into H_2O and extracted with Et_2O . The combined extract was dried over MgSO_4 and concentrated. The residue was purified by column chromatography (Et_2O -*n*-hexane=1:3) to give compound **12** (50.5 mg, 96%) as white amorphous.

IR (CHCl_3) cm^{-1} : 2949, 2922, 2224, 1721, 1630, 1435, 1236; $[\alpha]_{\text{D}}^{27} -28.2$ ($c=0.24$, CHCl_3); $^1\text{H-NMR}$ (CDCl_3 , 300 MHz, $E:Z=1:1.8$) δ : 6.70 (dt, 0.36H, $J=16.5$, 7.2 Hz), 6.49 (ddd, 0.64H, $J=10.9$, 6.8, 6.6 Hz), 5.28 (dt, 0.36H, $J=16.5$, 1.7 Hz), 5.21 (dt, 0.64H, $J=10.9$, 1.7 Hz), 3.670 (s, 1.1H), 3.665 (s, 1.9H), 3.663 (s, 1.9H), 3.661 (s, 1.1H), 2.51–2.30 (m, 0.64H), 2.26–2.09 (m, 1H), 2.20 (d, 2H, $J=7.0$ Hz), 2.08–1.95 (m, 0.36H), 1.91–0.73 (m, 19H), 1.15 (s, 1.9H), 1.10 (s, 1.1H), 0.74 (s, 1.9H), 0.73 (s, 1.1H); $^{13}\text{C-NMR}$ (CDCl_3 , 100 MHz, $E:Z=1:5.5$) δ : 178.2, 172.9, 155.7, 115.7, 98.2, 53.3, 51.7, 51.2, 47.4, 46.9, 45.6, 41.6, 38.0, 37.3, 37.2, 35.8, 35.0, 34.9, 34.3, 31.5, 28.6, 28.3, 19.6, 15.2, 12.2; HR-MS (FAB) Calcd for $\text{C}_{25}\text{H}_{38}\text{NO}_4$ m/z $[\text{MH}^+]$: 416.2801. Found 416.2806.

Methyl (1S,2S,4aS,4bS,7S,8aS,10aR)-1-(4-((*tert*-Butoxycarbonyl)amino)butyl)-7-(2-methoxy-2-oxoethyl)-2,4b-dimethyltetradecahydrophenanthrene-2-carboxylate (13) Under a hydrogen atmosphere (10 atm), the suspension of compound **12** (144 mg, 0.35 mmol), Pt_2O (27 mg) and 10% HCl -MeOH (9.0 mL) in CHCl_3 (6.0 mL) was stirred at for 11.5 h in an autoclave at r.t. The reaction mixture was filtered through a Celite pad and the filtrate was concentrated. The residue was dissolved in CH_2Cl_2 (4.0 mL) and added Et_3N (146 μL , d 0.73, 1.05 mmol) and Boc_2O (161 μL , d 0.95,

0.70 mmol). After 7 h stirring, the reaction mixture was washed with H_2O and dried over MgSO_4 . The concentrated residue was purified by column chromatography (AcOEt -*n*-hexane=1:5) to give compound **13** (174 mg, 95%, 2 steps) as a pale yellow oil.

$[\alpha]_{\text{D}}^{28} -16.7$ ($c=0.46$, CHCl_3); IR (CHCl_3) cm^{-1} : 3453, 3024, 2928, 2857, 2359, 1715, 1506; $^1\text{H-NMR}$ (CDCl_3 , 300 MHz) δ : 4.49 (br, 1H), 3.64 (s, 3H), 3.63 (s, 3H), 3.03 (dd, 2H, $J=11.8$, 6.3 Hz), 2.18 (d, 2H, $J=6.9$ Hz), 2.00–0.50 (m, 25H), 1.42 (s, 9H), 1.05 (s, 3H), 0.70 (s, 3H); $^{13}\text{C-NMR}$ (CDCl_3 , 100 MHz) δ : 179.2, 173.5, 155.9, 78.9, 53.3, 51.5, 51.3, 47.7, 47.5, 45.8, 41.7, 40.3, 38.0, 37.7, 36.9, 35.8, 35.1, 35.0, 31.8, 31.6, 30.5, 28.8, 28.4, 28.4, 27.6, 19.6, 15.1, 12.2; HR-MS (EI) Calcd for $\text{C}_{30}\text{H}_{51}\text{NO}_6$ m/z $[\text{M}^+]$: 521.3716. Found 522.3797.

2-((2S,4aS,4bS,7S,8S,8aR,10aS)-8-(4-((*tert*-Butoxycarbonyl)amino)butyl)-7-(methoxycarbonyl)-4a,7-dimethyltetradecahydrophenanthren-2-yl)acetic Acid To a solution of **13** (400 mg, 0.77 mmol) and Bu_4NHSO_4 (40 mg) in 1:1 MeOH/THF (15.4 mL) was added 10% LiOH (3.6 mL, 15.2 mmol) and stirred at r.t. for 3.5 h. The mixture was concentrated *in vacuo* and dilute with CH_2Cl_2 . The mixture was neutralized with 10% KHSO_4 and the aqueous layer was extracted with CH_2Cl_2 . The concentrated residue was purified by column chromatography (CH_2Cl_2 -MeOH=10:1) to give a desired carboxylic acid (389 mg, quant.) as a white amorphous.

$^1\text{H-NMR}$ (CDCl_3 , 300 MHz) δ : 4.51 (br, 1H), 3.64 (s, 3H), 3.04 (dd, 2H, $J=11.6$, 6.2 Hz), 2.22 (d, 2H, $J=6.9$ Hz), 1.95–0.65 (m, 25H), 1.43 (s, 9H), 1.11 (s, 3H), 0.72 (s, 3H); $^{13}\text{C-NMR}$ (CDCl_3 , 100 MHz) δ : 179.0, 178.2, 155.7, 79.0, 53.4, 51.6, 47.9, 47.6, 46.0, 41.8, 40.4, 38.2, 37.9, 37.0, 36.0, 35.1, 35.0, 32.0, 31.8, 30.7, 29.0, 28.5, 28.5, 27.8, 19.8, 15.3, 12.4.

2-((2S,4aS,4bS,7S,8S,8aR,10aS)-8-(4-Guanidinobutyl)-7-(methoxycarbonyl)-4a,7-dimethyltetradecahydrophenanthren-2-yl)acetic Acid (3a) To a solution of the above carboxylic acid (350 mg, 0.71 mmol) in CH_2Cl_2 (7.1 mL) was added trifluoroacetic acid (TFA) (5.5 mL, d 1.48, 71 mmol) at 0°C , and the mixture was stirred at r.t. for 4.5 h. The reaction mixture was concentrated, and then pyridine (7.1 mL) and *N,N'*-bis(*tert*-butoxycarbonyl)-1*H*-pyrazole-1-carboxamidine (1.1 g, 3.55 mmol) was added to the residue. The mixture was stirred for 11 d and the reaction mixture was diluted with Et_2O . The mixture was neutralized with 10% KHSO_4 and the water layer was extracted by Et_2O . The organic layer was dried over MgSO_4 and concentrated *in vacuo*. The residue was purified by column chromatography (CH_2Cl_2 -MeOH=20:1) to give a *N*-Boc guanidine derivative (462 mg, 2 steps quant.) as white amorphous. To the solution of above compound (50 mg, 0.08 mmol) in CH_2Cl_2 (769 μL) was added TFA (592 μL , d 1.48, 7.69 mmol) at 0°C and stirred at r.t. for 5 h. The reaction mixture was concentrated to give crude guanidine **3a** (30 mg, 87%, 68% purity) as a colorless oil.

$[\alpha]_{\text{D}}^{28} -5.75$ ($c=0.72$, EtOH); IR (KBr) cm^{-1} : 3354, 3171, 2937, 2857, 1705, 1662, 1451, 1383, 1243, 1148, 1120; $^1\text{H-NMR}$ (CD_3OD , 50°C , 500 MHz) δ : 3.64 (s, 3H), 3.11 (t, 2H, $J=7.0$ Hz), 2.15 (d, 2H, $J=7.0$ Hz), 1.92 (ddd, 1H, $J=12.5$, 6.7, 3.4 Hz), 1.85–0.60 (m, 24 H), 1.10 (s, 3H), 0.77 (s, 3H); $^{13}\text{C-NMR}$ (CD_3OD , 50°C , 100 MHz) δ : 180.5, 176.5, 158.5, 55.1, 52.1, 49.0, 47.5, 42.8, 42.4, 39.5, 39.2, 39.1, 38.3, 37.1, 36.5, 36.3, 33.4, 32.8, 30.5, 30.2, 29.6, 28.7, 20.9, 15.7, 12.7; HR-MS (FAB) Calcd for $\text{C}_{25}\text{H}_{44}\text{N}_3\text{O}_4$ m/z $[\text{MH}^+]$: 450.3332. Found 450.3339.

Methyl (1S,2S,4aS,4bS,7S,8aS,10aR)-7-(2-Methoxy-2-oxoethyl)-1-(3-methoxyallyl)-2,4b-dimethyltetradecahydrophenanthrene-2-carboxylate To a suspension of (methoxymethyl)triphenylphosphonium chloride (263 mg, 0.78 mmol) in THF (2.6 mL) was added LHMDs (765 μ L, 0.765 mmol) and stirred at -78°C for 15 min under a nitrogen atmosphere. After the solution of **11** (100 mg, 0.255 mmol) in THF (2.6 mL) was added to the reaction mixture and stirred for 4.5 h. The reaction was quenched with satd aqueous NH_4Cl and extracted with Et_2O . The organic layer was washed with brine and dried over MgSO_4 . The concentrated residue was purified by column chromatography (AcOEt - n -hexane=1:10) to give a desired enol ether (78.4 mg, 73%) as a colorless oil.

$^1\text{H-NMR}$ (CDCl_3 , 300 MHz, $E:Z=1:1.2$) δ : 6.16 (d, 0.46H, $J=12.6$ Hz), 5.74 (dt, 0.54H, $J=6.2, 1.6$ Hz), 4.64 (dt, 0.46H, $J=12.6, 7.6$ Hz), 4.28 (dd, 0.54H, $J=13.6, 7.1$ Hz), 3.66 (s, 3H), 3.631 (s, 1.4H), 3.625 (s, 1.6H), 3.55 (s, 1.6H), 3.47 (s, 1.4H), 2.20 (d, 2H, $J=6.9$ Hz), 2.14–0.52 (m, 21H), 1.11 (s, 1.6H), 1.10 (s, 1.4H), 0.73 (s, 3H); $^{13}\text{C-NMR}$ (CDCl_3 , 100 MHz, $E:Z=1:1.2$) δ : 179.1 (Z), 179.0 (E), 173.23 (Z), 173.21 (E), 147.0 (E), 145.0 (Z), 106.9 (Z), 102.8 (E), 59.4, 55.9, 53.5, 51.5 (Z), 51.4 (E), 48.0 (E), 47.5 (Z), 47.1, 45.9 (0.54), 45.8 (E), 41.8, 38.2, 37.9 (E), 37.7 (Z), 37.4 (0.54), 37.3 (E), 36.0, 35.21 (Z), 35.20 (E), 35.16 (Z), 35.12 (E), 31.8 (E), 31.5 (0.54), 29.2 (E), 28.93 (Z), 28.91 (E), 28.6 (Z), 25.8, 19.93 (E), 19.90 (Z), 15.4 (E), 15.3 (Z), 12.3; HR-MS (FAB) Calcd for $\text{C}_{25}\text{H}_{41}\text{O}_5$ m/z $[\text{MH}^+]$: 421.2954. Found 421.2963.

Methyl (1S,2S,4aS,4bS,7S,8aS,10aR)-7-(2-Methoxy-2-oxoethyl)-2,4b-dimethyl-1-(3-oxopropyl)tetradecahydrophenanthrene-2-carboxylate (14) To a solution of the enol ether (114 mg, 0.271 mmol) in acetone (9 mL)– H_2O (300 μ L) was added $\text{TsOH}\cdot\text{H}_2\text{O}$ (262 mg, 1.35 mmol) at 0°C and stirred at r.t. for overnight. The reaction mixture was neutralized with satd aqueous NaHCO_3 and extracted with Et_2O . The combined organic layer was washed with brine and dried over MgSO_4 . The concentrated residue was purified by column chromatography (AcOEt - n -hexane=1:5) to give compound **14** (80.0 mg, 73%) as a colorless oil.

IR (CHCl_3) cm^{-1} : 3516, 2949, 2922, 2727, 1732, 1711, 1385, 1238; $^1\text{H-NMR}$ (CDCl_3 , 300 MHz) δ : 9.62 (t, 1H, $J=1.4$ Hz), 3.62 (s, 3H), 3.62 (s, 3H), 2.43 (td, 2H, $J=7.8, 1.4$ Hz), 2.16 (d, 2H, $J=7.2$ Hz), 1.82–0.73 (m, 21H), 1.08 (s, 3H), 0.70 (s, 3H); $^{13}\text{C-NMR}$ (CDCl_3 , 100 MHz) δ : 201.8, 178.6, 173.1, 53.3, 51.7, 51.3, 47.8, 46.7, 45.8, 45.0, 41.7, 38.1, 37.9, 37.2, 35.9, 35.1, 35.0, 31.9, 28.8, 28.5, 24.0, 19.7, 15.2, 12.3; HR-MS (EI) Calcd for $\text{C}_{24}\text{H}_{38}\text{O}_5$ m/z $[\text{M}^+]$: 406.2719. Found 406.2717.

Methyl (1S,2S,4aS,4bS,7S,8aS,10aR)-1-(3-((tert-Butoxycarbonyl)amino)propyl)-7-(2-methoxy-2-oxoethyl)-2,4b-dimethyltetradecahydrophenanthrene-2-carboxylate (15) To a solution of **14** (355 mg, 0.87 mmol) in EtOH (9 mL) was added Et_3N (487 μ L, d 0.73, 3.49 mmol) and hydroxylamine hydrochloride (152 mg, 2.18 mmol) and stirred vigorously at r.t. for 2.5 h. The reaction mixture was diluted with Et_2O and washed with water and brine. The organic layer was dried over MgSO_4 and concentrated *in vacuo* to give crude oxime. Under a hydrogen atmosphere (30 atm), a suspension of the crude oxime (165 mg, 0.39 mmol), PtO_2 (16.5 mg) in AcOH (15 mL) was vigorously stirred at r.t. for 10 h in an autoclave. The reaction mixture was filtered through a Celite pad and concentrated. The residue was dissolved in CH_2Cl_2 (4.0 mL) and added Et_3N (163 μ L, d 0.73, 1.17 mmol) and Boc_2O

(179 μ L, d 0.95, 0.78 mmol). After 2 h stirring, the reaction mixture was washed with H_2O and dried over MgSO_4 . The concentrated residue was purified by column chromatography (AcOEt - n -hexane=1:5) to give compound **15** (155 mg, 78%) as a white amorphous.

$[\alpha]_{\text{D}}^{27} +18.2$ ($c=0.51$, CHCl_3); IR (CHCl_3) cm^{-1} : 3455, 2947, 2924, 1715, 1506; $^1\text{H-NMR}$ (CDCl_3 , 500 MHz) δ : 4.66 (br, 1H), 3.66 (s, 3H), 3.66 (s, 3H), 2.99 (quintet, 2H, $J=6.2$ Hz), 2.20 (d, 2H, $J=7.4$ Hz), 1.88–0.73 (m, 23H), 1.43 (s, 9H), 1.08 (s, 3H), 0.73 (s, 3H); $^{13}\text{C-NMR}$ (CDCl_3 , 125 MHz) δ : 179.0, 173.3, 155.8, 78.6, 53.2, 51.4, 51.1, 47.7, 47.0, 45.7, 41.6, 40.7, 37.9, 37.7, 36.8, 35.7, 34.94, 34.89, 31.7, 30.6, 29.0, 28.7, 28.3, 19.5, 15.0, 12.1; HR-MS (FAB) Calcd for $\text{C}_{29}\text{H}_{50}\text{NO}_6$ m/z $[\text{MH}^+]$: 508.3638. Found 508.3637.

2-((2S,4aS,4bS,7S,8S,8aR,10aS)-8-(3-((tert-Butoxycarbonyl)amino)propyl)-7-(methoxycarbonyl)-4a,7-dimethyltetradecahydrophenanthren-2-yl)acetic Acid To a solution of **15** (126 mg, 0.25 mmol) and Bu_4NHSO_4 (13 mg) in 1:1 MeOH/THF (5.0 mL) was added 10% aqueous LiOH (1.2 mL, 5.0 mmol) and stirred at r.t. overnight. The reaction mixture was concentrated *in vacuo* and diluted with CH_2Cl_2 . The solution was neutralized with 10% aqueous KHSO_4 at 0°C , and the aqueous layer was extracted with CH_2Cl_2 . The concentrated residue was purified by column chromatography (CH_2Cl_2 - $\text{MeOH}=20:1$) to give a desired carboxylic acid (122 mg, quant) as a white amorphous.

mp 176 – 180°C (AcOEt - n -hexane); $[\alpha]_{\text{D}}^{27} +83.1$ ($c=0.045$, CHCl_3); IR (CHCl_3) cm^{-1} : 3455, 2945, 2924, 2857, 1709, 1506; $^1\text{H-NMR}$ (CDCl_3 , 400 MHz) δ : 4.56 (br, 1H), 3.66 (s, 3H), 3.00 (br, 2H), 2.22 (d, 2H, $J=5.1$ Hz), 1.87–0.68 (m, 23H), 1.43 (s, 9H), 1.08 (s, 3H), 0.73 (s, 3H); $^{13}\text{C-NMR}$ (CDCl_3 , 100 MHz) δ : 179.0, 178.1, 155.7, 79.0, 53.4, 51.7, 47.9, 47.2, 45.9, 41.8, 41.0, 38.2, 37.9, 37.1, 36.0, 35.1, 35.0, 32.0, 31.5, 30.9, 29.3, 28.9, 28.5, 19.8, 15.3, 12.4; HR-MS (FAB) Calcd for $\text{C}_{28}\text{H}_{48}\text{NO}_6$ m/z $[\text{MH}^+]$: 494.3482. Found: 494.3477.

2-((2S,4aS,4bS,7S,8S,8aR,10aS)-8-(3-((Z)-2,3-Bis(tert-butoxycarbonyl)guanidino)propyl)-7-(methoxycarbonyl)-4a,7-dimethyltetradecahydrophenanthren-2-yl)acetic Acid (16) To a solution of the carboxylic acid (110 mg, 0.22 mmol) in CH_2Cl_2 (2.2 mL) was added TFA (1.7 mL, 22 mmol) at 0°C and the mixture was stirred at r.t. for 4.5 h. The reaction mixture was concentrated, and then pyridine (2.2 mL) and N,N' -bis(tert-butoxycarbonyl)-1H-pyrazole-1-carboximidine (140 mg, 0.44 mmol) was added to the residue. The solution was stirred for 6 d and the reaction mixture was diluted with Et_2O . The mixture was neutralized with 10% aqueous KHSO_4 , and the aqueous layer was extracted by Et_2O . The organic layer was dried over MgSO_4 and concentrated *in vacuo*. The residue was purified by column chromatography (CH_2Cl_2 - $\text{MeOH}=20:1$) to give compound **16** (101 mg, 71%) as white amorphous.

IR (CHCl_3) cm^{-1} : 3688, 3327, 2928, 1719, 1634, 1616, 1578; $[\alpha]_{\text{D}}^{20} -13.8$ ($c=0.87$, CHCl_3); $^1\text{H-NMR}$ (CDCl_3 , 400 MHz) δ : 8.24 (t, 1H, $J=4.4$ Hz), 3.66 (s, 3H), 3.27 (m, 2H), 2.22 (d, 2H, $J=7.1$ Hz), 1.83–0.72 (m, 23H), 1.48 (s, 18H), 1.02 (s, 3H), 0.72 (s, 3H); $^{13}\text{C-NMR}$ (CDCl_3 , 100 MHz) δ : 178.8, 178.2, 163.3, 155.8, 153.0, 82.9, 79.1, 53.4, 51.7, 47.9, 47.2, 46.0, 41.8, 41.4, 38.1, 37.8, 37.1, 36.0, 35.1, 35.0, 32.0, 30.0, 29.4, 28.9, 28.5, 28.4, 28.1, 19.8, 15.3, 12.4; HR-MS (FAB) Calcd for $\text{C}_{34}\text{H}_{58}\text{N}_3\text{O}_8$ m/z $[\text{MH}^+]$: 635.4146. Found 636.4224;

2-((2S,4aS,4bS,7S,8S,8aR,10aS)-8-(3-Guanidinopropyl)-

7-(methoxycarbonyl)-4a,7-dimethyltetradecahydrophenanthren-2-yl)acetic Acid (3b) To a solution of **16** (50 mg, 0.08 mmol) in CH_2Cl_2 (786 μL) was added TFA (606 μL , d 1.48, 7.86 mmol) at 0°C , and the mixture was stirred at r.t. for 5 h. The reaction mixture was concentrated to give a crude guanidine **3b** (34 mg, quant, 69% purity) as a colorless oil.

IR (CHCl_3) cm^{-1} : 2952, 2924, 1710, 1678, 1199; $[\alpha]_{\text{D}}^{27}$ -56.0 ($c=0.03$, EtOH); $^1\text{H-NMR}$ (CDCl_3 , 400 MHz) δ : 3.65 (s, 3H), 3.07 (t, 2H, $J=6.7$ Hz), 2.17 (d, 2H, $J=7.1$ Hz), 1.94 (dd, 1H, $J=12.5$, 2.8 Hz), 1.84–0.60 (m, 22 H), 1.11 (s, 3H), 0.78 (s, 3H); $^{13}\text{C-NMR}$ (CD_3OD , 100 MHz) δ : 180.4, 176.6, 158.3, 55.0, 52.3, 49.1, 48.6, 47.4, 42.75, 42.69, 39.4, 39.2, 38.4, 37.1, 36.5, 36.2, 33.3, 31.0, 30.3, 30.1, 29.5, 20.8, 16.0, 12.8; HR-MS (FAB) Calcd for $\text{C}_{24}\text{H}_{42}\text{N}_3\text{O}_4$ m/z $[\text{M}+\text{H}^+]$: 436.3186. Found 436.3181.

Methyl 2-((4a*S*,8a*S*)-8a-Methylhexahydro-2*H*-spiro[naphthalene-1,2'-[1,3]dioxolan]-6(5*H*)-ylidene)acetate (18a) Under a nitrogen atmosphere, to a suspension of *t*-BuOK (3.0 g, 27 mmol) in anhydrous DMF (22 mL) was added methyl diethylphosphonoacetate (4.9 mL, d 1.15, 27 mmol) at r.t. and stirred for 1.5 h. The mixture was cooled to 0°C and the solution of **17** (2.0 g, 8.9 mmol) in anhydrous DMF (23 mL) was added, and the resulting mixture was stirred for 12.5 h. The reaction mixture was poured into water and extracted with Et_2O . The combined extracts were washed with brine, dried over MgSO_4 and concentrated under the reduced pressure. The residue was purified by column chromatography ($\text{AcOEt-n-hexane}=1:4$) to give compound **18a** (2.6 g, 98%) as a colorless oil.

IR (CHCl_3) cm^{-1} : 2949, 1707, 1165; $[\alpha]_{\text{D}}^{26}$ $+0.88$ ($c=0.90$, EtOH); $^1\text{H-NMR}$ (CDCl_3 , 400 MHz, $E:Z=1:1$) δ : 5.59 (1/2H, s), 5.58 (1/2H, s), 3.95–3.81 (4H, m), 3.77 (1/2H, d, $J=14.4$ Hz), 3.67 (3/2H, s), 3.66 (3/2H, s), 3.56 (1/2H, d, $J=13.6$ Hz), 2.43–1.21 (12H, m), 1.10 (3H, s); $^{13}\text{C-NMR}$ (CDCl_3 , 100 MHz, $E:Z=1:1$) δ : 167.2, 167.1, 163.0, 162.9, 112.7, 112.54, 112.50, 112.4, 65.1, 65.0, 64.93, 64.87, 50.67, 50.66, 42.8, 42.3, 42.2, 42.0, 39.6, 33.1, 31.6, 31.4, 31.0, 30.5, 30.4, 27.9, 27.7, 24.8, 22.80, 22.77, 13.5, 13.3; HR-MS (EI) Calcd for $\text{C}_{16}\text{H}_{24}\text{O}_4$ m/z $[\text{M}^+]$: 280.1675. Found 280.1672.

2-((4a*S*,8a*S*)-8a-Methylhexahydro-2*H*-spiro[naphthalene-1,2'-[1,3]dioxolan]-6(5*H*)-ylidene)acetic Acid (18b) To a suspension of **18a** (2.5 g, 8.6 mmol) in 1:1 EtOH/ H_2O (86 mL) was added KOH (4.8 g, 86 mmol), and the mixture was heated at 90°C for 0.5 h. The reaction mixture was concentrated and neutralized with 3M HCl on an ice bath. The mixture was extracted with CH_2Cl_2 and the combined organic layer was dried over MgSO_4 . The concentrated residue was purified by column chromatography ($\text{CH}_2\text{Cl}_2\text{-MeOH}=20:1$) to give α,β -unsaturated carboxylic acid **18b** (2.1 g, 93%) as a white amorphous.

IR (CHCl_3) cm^{-1} : 2933, 1685, 1631; $[\alpha]_{\text{D}}^{26}$ -12.7 ($c=0.06$, EtOH); $^1\text{H-NMR}$ (CDCl_3 , 400 MHz, $E:Z=1:1$) δ : 11.8 (1H, brs), 5.61 (1/2H, s), 5.60 (1/2H, s), 3.98–3.82 (4H, m), 3.77 (1/2H, d, $J=14.8$ Hz), 3.55 (1/2H, d, $J=13.2$ Hz), 2.35 (1/2H, dt, $J=4.8$, 13.6 Hz), 2.35–2.10 (m, 1H), 2.05–1.90 (m, 1H), 1.87–1.45 (m, 15/2H), 1.41–1.17 (m, 2H), 1.10 (s, 3/2H), 1.09 (s, 3/2H); $^{13}\text{C-NMR}$ (CDCl_3 , 100 MHz, $E:Z=1:1$) δ : 172.3, 172.2, 165.8, 165.6, 112.8, 112.6 (2C), 112.5, 65.14, 65.08, 65.0, 64.9, 42.9, 42.3, 42.2, 42.1, 39.9, 33.3, 31.6 (2C), 31.1, 30.5, 30.4, 27.9, 27.7, 25.1, 22.80, 22.77, 13.5, 13.4; HR-MS (EI) Calcd for $\text{C}_{15}\text{H}_{22}\text{O}_4$ m/z $[\text{M}^+]$: 266.1518. Found 266.1517.

Methyl 2-((2*S*,4a*S*,8a*S*)-4a-Methyl-5-oxodecahydronaph-

thalen-2-yl)acetate (19) Under a nitrogen atmosphere, a lithium wire (0.5 g, 68 mmol) was added to liquid ammonia (96 mL) at -78°C . To this mixture was added a solution of **18b** (1.8 g, 6.8 mmol) in THF (45 mL), and the resulting mixture was stirred for 3 h. After the substrate disappearance was monitored by TLC, solid NH_4Cl was added until the mixture turned white suspension. The mixture was allowed to r.t. and the liquid ammonia was evaporated through the night. The residue was suspended with CH_2Cl_2 and filtrated. To the concentrated residue in THF (68 mL) was added 1.5M HCl (0.11 L) using a dropping funnel at 0°C , and the mixture and stirred for 1 h at r.t. The reaction mixture was extracted with CH_2Cl_2 and dried over MgSO_4 . To the concentrated residue was added MeI (6.9 mL, 68 mmol) and K_2CO_3 (1.9 g, 14 mmol), and then the mixture was stirred at r.t. overnight. The reaction was concentrated, diluted with Et_2O and washed with H_2O . The organic layer was dried over MgSO_4 and concentrated. The crude material was purified by column chromatography ($\text{AcOEt-n-hexane}=1:2$) to give compound **19** (1.5 g, 91%, in 3 steps) as a colorless oil.

IR (CHCl_3) cm^{-1} : 3010, 2933, 2862, 1730, 1699; $[\alpha]_{\text{D}}^{20}$ -7.9 ($c=0.15$, EtOH); $^1\text{H-NMR}$ (CDCl_3 , 300 MHz) δ : 3.67 (3H, s), 2.66–2.63 (1H, m), 2.28–2.17 (3H, m), 2.08–1.96 (1H, m), 1.84–1.41 (9H, m), 1.26–1.12 (2H, m), 1.09 (3H, s); $^{13}\text{C-NMR}$ (CDCl_3 , 100 MHz) δ : 215.9, 173.1, 51.3, 47.8, 45.2, 41.3, 37.3, 34.6, 33.8, 32.1, 27.7, 27.3, 26.0, 15.5; HR-MS (EI) Calcd for $\text{C}_{14}\text{H}_{22}\text{O}_3$ m/z $[\text{M}^+]$: 238.1569. Found 238.1562.

Methyl 2-((2*S*,4a*S*,8a*S*)-5-(Cyanomethylene)-4a-methyl-decahydronaphthalen-2-yl)acetate (20) To a suspension of KO^tBu (3.6 g, 32 mmol) in DMF (30 mL) was added diethyl cyanomethylphosphonate (5.0 mL, d 1.13, 32 mmol) under a nitrogen atmosphere, and the mixture was stirred at 0°C for 1 h. To the solution was added **19** (2.5 g, 11 mmol) in anhydrous DMF (25 mL) and the resulting mixture was stirred at 90°C for 1 h. The reaction mixture was added H_2O and extracted with Et_2O . The combined extracts were dried over MgSO_4 and concentrated. The residue was purified by column chromatography ($\text{AcOEt-n-hexane}=1:5$) to give compound **20** (2.6 g, 95%) as a colorless oil.

IR (CHCl_3) cm^{-1} : 2983, 2929, 2216, 1730; $[\alpha]_{\text{D}}^{25}$ -71.9 ($c=0.46$, EtOH); $^1\text{H-NMR}$ (CDCl_3 , 400 MHz) δ : 5.03 (1H, d, $J=1.6$ Hz), 3.67 (3H, s), 2.94–2.78 (1H, m), 2.42–2.30 (1H, m), 2.29–2.10 (2H, m), 1.98–1.67 (3H, m), 1.66–1.57 (1H, m), 1.51–1.03 (8H, m), 1.01 (3H, s); $^{13}\text{C-NMR}$ (CDCl_3 , 100 MHz) δ : 175.9, 173.0, 117.7, 90.2, 51.3, 45.2, 41.2, 40.6, 35.5, 34.58, 34.55, 30.1, 28.2, 28.0, 27.0, 16.9; HR-MS (EI) Calcd for $\text{C}_{16}\text{H}_{23}\text{NO}_2$ m/z $[\text{M}^+]$: 261.1729. Found 261.1725.

Methyl 2-((2*S*,4a*S*,5*R*,8a*S*)-5-(Cyanomethyl)-4a-methyl-decahydronaphthalen-2-yl)acetate (21) Under a hydrogen balloon atmosphere, a suspension of compound **20** (12 mg, 0.046 mmol), 10% Pd/C (2.0 mg) in EtOH (1.4 mL) was stirred at r.t. for 1.5 h. The reaction mixture was filtered through a Celite pad and concentrated. The residue was purified by column chromatography ($\text{AcOEt-n-hexane}=1:5$) to give compound **21** (12 mg, quant, $\beta:\alpha=14:1$) as a colorless oil.

IR (CHCl_3) cm^{-1} : 2927, 2858, 2247, 1730; $[\alpha]_{\text{D}}^{23}$ $+100$ ($c=0.004$, EtOH); $^1\text{H-NMR}$ (CDCl_3 , 400 MHz) δ : 3.66 (3H, s), 2.63–2.35 (1H, m), 2.21 (2H, d, $J=7.2$ Hz), 2.00 (1H, dd, $J=16.4$, 10.4 Hz), 1.90–1.71 (3H, m), 1.67–1.56 (2H, m), 1.53–0.96 (10H, m), 0.72 (3H, s); $^{13}\text{C-NMR}$ (CDCl_3 , 100 MHz) δ : 173.3, 120.1, 51.4, 45.7, 45.6, 41.5, 38.1, 36.3, 35.0, 34.8,

28.5, 28.2, 27.8, 25.9, 18.3, 10.9; HR-MS (EI) Calcd for $C_{16}H_{25}NO_2$ m/z [M^+]: 263.1885. Found 263.1881.

Methyl 2-((2*S*,4*aS*,5*R*,8*aS*)-5-(2-((*Z*)-2,3-Bis(*tert*-butoxycarbonyl)guanidinoethyl)-4*a*-methyldecahydronaphthalen-2-yl)acetate (22**)** Under a hydrogen atmosphere (balloon), a suspension of compound **21** (0.11 g, 0.45 mmol) and PtO₂ (20 mg) in 10% HCl–MeOH (12 mL) was stirred at r.t. for 140 min. The reaction mixture was filtered through a Celite pad and concentrated. The residue was dissolved in DMF (3.9 mL), and Et₃N (0.16 mL, d 0.73, 1.2 mmol) and *N,N'*-bis(*tert*-butoxycarbonyl)-1*H*-pyrazole-1-carboxamide (0.15 g, 0.47 mmol) was added. After 1 h stirring, the reaction mixture was added H₂O, extracted with Et₂O, and dried over MgSO₄. The concentrated residue was purified by column chromatography (AcOEt–*n*-hexane=1:5) to give compound **22** (114 mg, 83%, 2 steps) as a colorless amorphous.

IR (CHCl₃) cm^{-1} : 2981, 2926, 2856, 1720, 1633; [α]_D²⁶ +2.2 ($c=0.09$, EtOH); ¹H-NMR (CDCl₃, 400 MHz) δ : 11.5 (1H, brs), 8.25 (1H, brs), 3.66 (3H, s), 3.62–3.22 (2H, m), 2.20 (2H, d, $J=6.8$ Hz), 1.99–1.38 (10H, m), 1.26 (9H, s), 1.22–0.70 (13H, m), 0.53 (3H, s); ¹³C-NMR (CDCl₃, 100 MHz) δ : 173.4, 163.6, 156.0, 153.3, 82.9, 79.1, 51.3, 46.3, 45.9, 41.7, 40.2, 37.9, 36.3, 35.2, 29.6, 28.9, 28.8, 28.4, 28.3, 28.0, 27.7, 26.3, 11.1; HR-MS (FAB) Calcd for $C_{27}H_{47}N_3O_6$ m/z [M^+]: 510.3543. Found 510.3543.

2-((2*S*,4*aS*,5*R*,8*aS*)-5-(2-((*Z*)-2,3-Bis(*tert*-butoxycarbonyl)guanidinoethyl)-4*a*-methyldecahydronaphthalen-2-yl)acetic Acid (24**)** To a solution of **22** (1.5 g, 3.1 mmol, $\beta:\alpha=14:1$) in MeOH–THF (62 mL, 1:1) was added 10% aqueous LiOH (15 mL, 0.2 M) and stirred at r.t. for 4 h. The mixture was concentrated *in vacuo* and diluted with CH₂Cl₂. The solution was neutralized with dil HCl and aqueous layer was extracted with CH₂Cl₂ and dried over MgSO₄. The concentrated residue was purified by column chromatography (CH₂Cl₂–MeOH=10:1) to give compound **23** (1.2 g, 75%, $\beta:\alpha=14:1$) as a white solid. This product was recrystallized from Et₂O to give β -isomer **24** as a white powder (0.20 g, 13%).

mp 108–112°C (AcOEt–*n*-hexane); IR (CHCl₃) cm^{-1} : 3332, 2926, 1720, 1641, 1394; [α]_D²¹ –6.6 ($c=0.09$, EtOH); ¹H-NMR (CDCl₃, 400 MHz) δ : 8.24 (1H, brs), 3.49–3.38 (1H, m), 3.33–3.24 (1H, m), 2.24 (2H, d, $J=6.8$ Hz), 1.89–1.58 (8H, m), 1.50 (18H, s), 1.43–0.92 (11H, m), 0.69 (3H, s); ¹³C-NMR (CDCl₃, 100 MHz) δ : 178.7, 163.5, 156.0, 153.7, 83.0, 79.2, 46.3, 41.7, 40.3, 37.9, 36.3, 36.1, 35.1, 28.89, 28.85, 28.4, 28.3, 28.0, 27.6, 26.3, 11.1; HR-MS (FAB) Calcd for $C_{26}H_{46}N_3O_6$ m/z [M^+]: 496.3387. Found 496.3384.

2-((2*S*,4*aS*,5*R*,8*aS*)-5-(2-Guanidinoethyl)-4*a*-methyldecahydronaphthalen-2-yl)acetic Acid (3c**)** To a solution of **24** (39 mg, 0.080 mmol) was added HCl–AcOEt (0.80 mL, 0.4 M) at 0°C and stirred at r.t. for 31 h. The mixture was concentrated *in vacuo* to give compound **3c** (24 mg, quant, 70% purity) as a white amorphous.

IR (CHCl₃) cm^{-1} : 3435, 3417, 1633, 1261, 1095, 1022, 802; [α]_D²³ +22.7 ($c=0.22$, EtOH); ¹H-NMR (CDCl₃, 300 MHz) δ : 7.48–7.35 (1H, m), 3.24–2.82 (2H, m), 2.10 (2H, d, $J=7.2$ Hz), 1.90–0.83 (20H, m), 0.66 (3H, s); ¹³C-NMR (CDCl₃, 125 MHz) δ : 173.6, 156.6, 53.5, 52.3, 49.4, 37.5, 35.9, 34.7, 28.5, 28.3, 27.8, 27.6, 26.9, 25.9, 11.0, 0.05; HR-MS (FAB) Calcd for $C_{16}H_{30}N_3O_2$ m/z [M^+]: 296.2338. Found 296.2335.

(3*aS*,7*aS*)-7*a*-Methylhexahydrospiro[indene-1,2'-[1,3]-

dioxolan]-5(4*H*)-one (25**)** Under a nitrogen atmosphere, a lithium wire (0.16 g, 23.0 mmol) was added to liquid ammonia (30 mL) at –78°C. To the reaction mixture was added the solution of acetal (1.5 g, 7.2 mmol) in THF (18 mL), and the resulting mixture was stirred for 100 min. After the substrate disappearance was monitored by TLC, solid NH₄Cl was added until the mixture turned white suspension. The mixture was allowed to r.t. and the liquid ammonia was evaporated through the night. The residue was suspended with CH₂Cl₂ and filtrated. The concentrated residue was purified by column chromatography (AcOEt–*n*-hexane=1:4) to give compound **25** (1.0 g, 70%) as a colorless oil.

IR (CHCl₃) cm^{-1} : 2956, 1705; [α]_D²² +58.8 ($c=0.17$, EtOH); ¹H-NMR (CDCl₃, 400 MHz) δ : 3.97–3.87 (4H, m), 2.52–2.19 (5H, m), 2.02–1.78 (4H, m), 1.72–1.66 (1H, m), 1.42–1.30 (1H, m), 1.16 (3H, s); ¹³C-NMR (CDCl₃, 100 MHz) δ : 213.0, 119.4, 65.2, 64.4, 44.3, 44.1, 42.2, 37.1, 32.9, 29.4, 25.7, 18.4; HR-MS (EI) Calcd for $C_{12}H_{18}O_3$ m/z [M^+]: 210.1256. Found 210.1257.

Methyl 2-((3*aS*,7*aS*)-7*a*-Methylhexahydrospiro[indene-1,2'-[1,3]dioxolan]-5(4*H*)-ylidene)acetate To a suspension of KO^tBu (0.11 g, 1.1 mmol) in DMF (0.75 mL) was added methyl 2-(diethoxyphosphoryl)acetate (0.19 mL, d 1.15, 1.1 mmol), and the mixture was stirred at 0°C for 10 min under a nitrogen atmosphere. To the mixture was added compound **25** (74 mg, 0.35 mmol) in anhydrous DMF (1.0 mL) and the resulting mixture was stirred at r.t. for 30 h. The reaction mixture was added H₂O and extracted with Et₂O. The combined extract was dried over MgSO₄ and concentrated. The residue was purified by column chromatography (AcOEt–*n*-hexane=1:4) to give α,β -unsaturated ester (90 mg, 97%) as a colorless oil.

IR (CHCl₃) cm^{-1} : 3012, 2949, 2879, 1708, 1649; [α]_D²¹ +65.5 ($c=0.07$, EtOH); ¹H-NMR (CDCl₃, 400 MHz, $E:Z=1:1$) δ : 5.76 (1/2H, s), 5.64 (1/2H, s), 3.95–3.81 (4H, m), 3.69 (3/2H, s), 3.68 (3/2H, s), 3.62–3.50 (1H, m), 2.47–1.19 (10H, m), 1.06 (3/2H, s), 1.05 (3/2H, s); ¹³C-NMR (CDCl₃, 100 MHz, $E:Z=1:1$) δ : 167.0, 166.7, 161.7, 161.6, 120.0 (2C), 115.0, 114.6, 65.2 (2C), 64.2, 64.1, 50.7 (2C), 44.7, 44.6, 43.9, 43.8, 37.2, 33.1, 32.7, 32.6, 31.3, 29.8, 28.7, 25.1, 23.9, 23.5, 17.6, 16.9; HR-MS (EI) Calcd for $C_{15}H_{22}O_4$ m/z [M^+]: 266.1518. Found 266.1516.

2-((3*aS*,7*aS*)-7*a*-Methylhexahydrospiro[indene-1,2'-[1,3]dioxolan]-5(4*H*)-ylidene)acetic Acid (26**)** To a solution of α,β -unsaturated ester (57 mg, 0.21 mmol) in 1:1 EtOH/H₂O (2.0 mL) was added powdered KOH (0.12 g, 2.1 mmol) and the mixture was heated at 90°C for 15 min. The reaction mixture was concentrated and neutralized with 10% HCl on an ice bath. The mixture was extracted with CH₂Cl₂ and the combined organic layer was dried over MgSO₄. The concentrated residue was purified by column chromatography (AcOEt–*n*-hexane=1:3) to give compound **26** (38 mg, 71%) as a white amorphous.

IR (CHCl₃) cm^{-1} : 3016, 2949, 2879, 1687; [α]_D²⁵ +100 ($c=0.02$, EtOH); ¹H-NMR (CDCl₃, 400 MHz, $E:Z=1:1$) δ : 5.78 (1/2H, s), 5.67 (1/2H, s), 3.95–3.79 (4H, m), 3.55 (1H, d, $J=16.0$ Hz), 2.49–1.10 (11H, m), 1.06 (3/2H, s), 1.05 (3/2H, s); ¹³C-NMR (CDCl₃, 100 MHz, $E:Z=1:1$) δ : 165.6, 165.5, 164.8, 164.6, 120.1, 120.1, 114.8, 114.3, 65.3, 64.3, 64.2, 44.7, 44.7, 44.1, 44.0, 37.6, 33.5, 32.8, 32.7, 31.3, 29.8, 29.1, 25.4, 24.1, 23.7, 17.9, 17.0; HR-MS (EI) Calcd for $C_{14}H_{20}O_4$ m/z [M^+]: 252.1362. Found 252.1356.

Methyl 2-((3a*S*,5*S*,7a*S*)-7a-Methyl-1-oxooctahydro-1*H*-inden-5-yl)acetate (27) Under a nitrogen atmosphere, a lithium wire (0.74 g, 0.10 mol) was added to liquid ammonia (150 mL) at -78°C . To the reaction mixture was added the solution of **26** (2.7 g, 11 mmol) in THF (100 mL), and the mixture was stirred for 25 min at r.t. After the substrate disappearance was monitored by TLC, solid NH_4Cl was added until the mixture turned white suspension. The mixture was allowed to r.t. and the liquid ammonia was evaporated through the night. The residue was suspended with CH_2Cl_2 and filtrated. To the concentrated residue in THF (100 mL) was added 1.5 M HCl (40 mL) using a dropping funnel at 0°C , and the mixture and stirred for 1.5 h at r.t. The reaction mixture was extracted with CH_2Cl_2 and dried over MgSO_4 . To the concentrated residue was dissolved in acetone (100 mL), and MeI (11 mL, 0.10 mol) and K_2CO_3 (6.0 g, 43 mmol) were added, and then the mixture was stirred for overnight. The reaction was concentrated, diluted with Et_2O and washed with H_2O . The organic layer was dried over MgSO_4 and concentrated. The crude material was purified by column chromatography (AcOEt-*n*-hexane=1:2) to give compound **27** (2.4 g, 98%, 3 steps) as a colorless oil.

IR (CHCl_3) cm^{-1} : 2953, 2929, 2358, 2339, 1730; $[\alpha]_{\text{D}}^{20} +35.6$ ($c=0.03$, EtOH); $^1\text{H-NMR}$ (CDCl_3 , 400 MHz) δ : 3.68 (3H, s), 2.54–2.38 (1H, m), 2.32–1.80 (7H, m), 1.75–1.56 (2H, m), 1.50–1.35 (2H, m), 1.29–1.10 (2H, m), 1.07 (3H, s); $^{13}\text{C-NMR}$ (CDCl_3 , 100 MHz) δ : 222.2, 172.9, 51.2, 46.6, 42.3, 41.0, 35.9, 30.9, 29.0, 27.7, 27.3, 22.9, 18.8; HR-MS (EI) Calcd for $\text{C}_{13}\text{H}_{20}\text{O}_3$ m/z $[\text{M}^+]$: 224.1412. Found 224.1412.

Methyl 2-((3a*S*,5*S*,7a*S*)-1-(Cyanomethylene)-7a-methyloctahydro-1*H*-inden-5-yl)acetate To a suspension of NaH (26 mg, 60% oil suspension, 0.66 mmol) in DMF (0.50 mL) was added diethyl cyanomethylphosphonate (0.10 mL, d 1.13, 0.66 mmol) and stirred at 0°C for 1 h under a nitrogen atmosphere. To the solution was added compound **11** (550 mg, 0.22 mmol) in anhydrous DMF (0.51 mL) and stirred at r.t. for 11 h. The reaction mixture was added H_2O and extracted with Et_2O . The combined extract was dried over MgSO_4 and concentrated. The residue was purified by column chromatography (Et_2O -*n*-hexane=1:4) to give the desired α,β -unsaturated nitrile (50 mg, 93%) as a colorless oil.

IR (CHCl_3) cm^{-1} : 3018, 2954, 2927, 2216, 1730; $[\alpha]_{\text{D}}^{26} +11.7$ ($c=0.29$, EtOH); $^1\text{H-NMR}$ (CDCl_3 , 400 MHz) δ : 5.10 (1H, t, $J=2.4$ Hz), 3.65 (3H, s), 2.90–2.69 (1H, m), 2.57–2.46 (1H, m), 2.21 (2H, d, $J=6.8$ Hz), 2.07–1.15 (10H, m), 1.07 (3H, s); $^{13}\text{C-NMR}$ (CDCl_3 , 100 MHz) δ : 182.1, 172.9, 117.3, 89.2, 51.3, 44.9, 44.8, 41.0, 33.0, 30.9, 30.2, 28.8, 28.1, 26.0, 21.8; HR-MS (EI) Calcd for $\text{C}_{15}\text{H}_{21}\text{NO}_2$ m/z $[\text{M}^+]$: 247.1572. Found 247.1572.

Methyl 2-((1*R*,3a*S*,5*S*,7a*S*)-1-(Cyanomethyl)-7a-methyloctahydro-1*H*-inden-5-yl)acetate (28) Under a hydrogen balloon atmosphere, a suspension of the α,β -unsaturated nitrile (23 mg, 0.090 mmol), 10% Pd/C (2.3 mg) in EtOH (3.0 mL) was stirred at r.t. for 2 h. The reaction mixture was filtered through a Celite pad and concentrated. The residue was purified by column chromatography (AcOEt-*n*-hexane=1:5) to give nitrile **28** (18.6 mg, 80%, β : $\alpha=10:1$) as a colorless oil.

IR (CHCl_3) cm^{-1} : 2953, 2926, 1730; $[\alpha]_{\text{D}}^{26} -29.0$ ($c=0.06$, EtOH); $^1\text{H-NMR}$ (CDCl_3 , 400 MHz) δ : 3.67 (3H, s), 2.40–2.25 (1H, m), 2.20 (2H, d, $J=5.6$ Hz), 2.17–1.03 (14H, m), 1.02 (30/11H, s), 0.95 (3/11, s); $^{13}\text{C-NMR}$ (CDCl_3 , 100 MHz) δ : 173.3, 119.8, 51.4, 47.9, 45.6, 41.6, 41.0, 31.0, 29.0, 27.9, 27.6, 26.8, 25.5, 22.6, 17.1; HR-MS (EI) Calcd for $\text{C}_{15}\text{H}_{23}\text{NO}_2$ m/z

$[\text{M}^+]$: 249.1729. Found 249.1727.

Methyl 2-((1*R*,3a*S*,5*S*,7a*S*)-1-(2-((*E*)-2,3-Bis(*tert*-butoxycarbonyl)guanidino)ethyl)-7a-methyloctahydro-1*H*-inden-5-yl)acetate Under a hydrogen atmosphere (balloon), a suspension of nitrile **28** (29 mg, 0.11 mmol) and PtO_2 (3 mg) in 10% HCl-MeOH (2.7 mL) was stirred at r.t. for 2 h. The reaction mixture was filtered through a Celite pad and concentrated. The residue was dissolved in DMF (1.1 mL), and Et_3N (45 μL , d 0.73, 0.33 mmol) and *N,N'*-bis(*tert*-butoxycarbonyl)-1*H*-pyrazole-1-carboxamide (40 mg, 0.13 mmol) were added. After 1 h stirring, the reaction mixture was added H_2O , extracted with Et_2O , and dried over MgSO_4 . The concentrated residue was purified by column chromatography (AcOEt-*n*-hexane=1:5) to give *N,N'*-bisBoc guanidine (11 mg, 13%) as a colorless amorphous.

IR (CHCl_3) cm^{-1} : 2927, 1716, 1635; $[\alpha]_{\text{D}}^{21} +15.0$ ($c=0.02$, EtOH); $^1\text{H-NMR}$ (CDCl_3 , 400 MHz, 10:1 diastereo mixture) δ : 11.5 (1H, s), 8.27 (1H, s), 3.66 (3H, s), 3.43–3.33 (2H, m), 2.25 (2/11H, d, $J=7.6$ Hz), 2.18 (20/11H, d, $J=7.2$ Hz), 2.14–1.78 (2H, m), 1.77–1.54 (5H, m), 1.51 (9H, s), 1.49 (9H, s), 1.47–0.98 (8H, m), 0.94 (30/11H, s), 0.86 (3/11H, s); $^{13}\text{C-NMR}$ (CDCl_3 , 100 MHz) δ : 173.6, 163.6, 156.0, 153.3, 83.0, 79.2, 51.4, 49.6, 45.7, 41.8, 41.1, 40.7, 31.3, 29.3, 29.2, 28.30, 28.26, 28.1, 27.7, 27.1, 26.1, 22.7; HR-MS (FAB) Calcd for $\text{C}_{26}\text{H}_{46}\text{N}_3\text{O}_6$ m/z $[\text{MH}^+]$: 496.3386. Found 496.3390.

2-((1*R*,3a*S*,5*S*,7a*S*)-1-(2-((*E*)-2,3-Bis(*tert*-butoxycarbonyl)guanidino)ethyl)-7a-methyloctahydro-1*H*-inden-5-yl)-acetic Acid To a solution of the *N,N'*-bisBoc guanidine (36 mg, 0.070 mmol) in MeOH (0.70 mL) and THF (0.70 mL) was added 10% aqueous LiOH (0.70 mL, 0.1 M), and the mixture was heated at r.t. for 4 h. The reaction mixture was concentrated and neutralized with dil HCl on an ice bath. The mixture was extracted with CH_2Cl_2 , and the combined organic layer was dried over MgSO_4 . The concentrated residue was purified by column chromatography (AcOEt-*n*-hexane=1:1) to give the desired carboxylic acid (28 mg, 82%) as a white amorphous.

IR (CHCl_3) cm^{-1} : 2981, 2927, 2872, 1714, 1633; $^1\text{H-NMR}$ (CDCl_3 , 400 MHz) δ : 11.5 (1H, s), 8.27 (1H, s), 3.53–3.33 (2H, m), 2.18 (2H, d, $J=7.6$ Hz), 2.14–1.54 (7H, m), 1.53 (9H, s), 1.49 (9H, s), 1.47–0.98 (9H, m), 0.94 (3H, s); $^{13}\text{C-NMR}$ (CDCl_3 , 100 MHz) δ : 156.0, 153.3, 83.0, 79.3, 53.4, 49.6, 45.6, 41.1, 40.7, 31.2, 31.0, 29.1, 28.3, 28.2, 28.1, 27.7, 27.1, 26.1, 22.7; HR-MS (FAB) Calcd for $\text{C}_{25}\text{H}_{44}\text{N}_3\text{O}_6$ m/z $[\text{MH}^+]$: 481.3230. Found 482.3233.

2-((1*R*,3a*S*,5*S*,7a*S*)-1-(2-Guanidinoethyl)-7a-methyloctahydro-1*H*-inden-5-yl)acetic Acid (3d) To a solution of the carboxylic acid (24 mg, 0.050 mmol) was added 4 M HCl-AcOEt (0.5 mL) at 0°C , and the mixture was stirred at r.t. for 6.5 h. The mixture was concentrated *in vacuo* to give compound **3d** (7.8 mg, 56, 75% purity) as a pale yellow oil.

IR (CHCl_3) cm^{-1} : 3152, 2956, 2928, 2868, 1725, 1678, 1642, 1615, 1252, 1148; $[\alpha]_{\text{D}}^{20} -5.0$ ($c=0.12$, EtOH); $^1\text{H-NMR}$ ($\text{DMSO}-d_6$, 300 MHz) δ : 11.95 (5H, brs), 8.07 (1H, brs), 7.15 (3H, brs), 3.24–2.97 (2H, m), 2.08 (2H, d, $J=9.2$ Hz), 1.08–0.97 (15H, m), 0.92 (3H, s); $^{13}\text{C-NMR}$ ($\text{DMSO}-d_6$, 100 MHz) δ : 173.7, 171.9, 156.7, 48.5, 45.4, 41.4, 30.7, 28.6, 28.4, 27.7, 26.9, 26.6, 25.6, 22.6, 21.0; HR-MS (FAB) Calcd for $\text{C}_{15}\text{H}_{28}\text{N}_3\text{O}_2$ m/z $[\text{MH}^+]$: 282.2181. Found 282.2184.

Methyl 2-((2*S*,4a*S*,5*S*,8a*S*)-5-Hydroxy-4a-methyldecahydronaphthalen-2-yl)acetate (29) To a solution of **19** (10 mg,

0.040 mmol) in MeOH (0.2 mL) was added NaBH₄ at 0°C, and the mixture was stirred at r.t. for 5 min. The reaction mixture was concentrated and then purified by column chromatography (AcOEt-*n*-hexane=1:4) to give compound **29** (8.0 mg, 83%, β : α =8.5:1) as a colorless oil.

IR (CHCl₃) cm⁻¹: 2927, 2858, 1730; [α]_D²⁵ -13.0 (*c*=0.26, EtOH); ¹H-NMR (CDCl₃, 400 MHz) δ : 3.67 (3H, s), 3.23 (1H, dd, *J*=12.0, 4.0 Hz), 2.22 (2H, d, *J*=4.0 Hz), 2.60–0.90 (15H, m), 0.79 (3H, s); ¹³C-NMR (CDCl₃, 100 MHz) δ : 173.5, 79.5, 51.4, 43.5, 41.7, 38.7, 36.9, 35.2, 34.2, 30.4, 28.1, 27.8, 24.3, 9.8; HR-MS (EI) Calcd for C₁₄H₂₄O₃ *m/z* [M⁺]: 240.1725. Found 240.1724.

2-((2*S*,4*aS*,5*S*,8*aS*)-5-(3-(1-(*tert*-Butoxycarbonyl)piperidin-4-yl)propoxy)-4*a*-methyldecahydronaphthalen-2-yl)acetic Acid (30**)** To a solution of **29** (22 mg, 0.090 mmol, β : α =8.5:1) in anhydrous DMF (1.0 mL) was added NaH (5.5 mg, 0.23 mmol, 60% oil suspension), and the mixture was stirred at r.t. for 1 h under a nitrogen atmosphere. The mixture was added *tert*-butyl 4-(3-bromopropyl)piperidine-1-carboxylate (50 mg, 0.090 mmol) and stirred for 30 h. Then the solution was neutralized with satd aqueous NH₄Cl and extracted with Et₂O. The extract was washed with brine and dried over Na₂SO₄. The concentrated residue was purified by column chromatography (AcOEt-*n*-hexane=1:3) to give compound **30** (12 mg, 29%) as a colorless oil and **29** (12 mg, 53%) was recovered.

IR (CHCl₃) cm⁻¹: 3444, 2929, 2358, 1722, 1678; [α]_D²⁸ +3.0 (*c*=0.82, CHCl₃); ¹H-NMR (CDCl₃, 500 MHz) δ : 4.05 (2H, t, *J*=6.5 Hz), 3.23 (1H, dd, *J*=4.0 Hz), 2.68 (2H, t, *J*=12.5 Hz), 2.20 (2H, d, *J*=6.0 Hz), 1.93–1.47 (11H, m), 1.45 (9H, s), 1.43–0.84 (15H, m), 0.80 (3H, s); ¹³C-NMR (CDCl₃, 100 MHz) δ : 173.1, 154.9, 79.5, 79.2, 64.3, 43.7, 43.5, 42.0, 38.7, 36.9, 35.6, 35.3, 34.2, 32.7, 32.1, 30.4, 28.4, 28.0, 27.8, 25.8, 24.3, 24.0, 10.9, 9.8; HR-MS (EI) Calcd for C₂₆H₄₅NO₅ *m/z* [M⁺]: 451.3298. Found 451.3295.

2-((2*S*,4*aS*,5*S*,8*aS*)-4*a*-Methyl-5-(3-(piperidin-4-yl)propoxy)decahydronaphthalen-2-yl)acetic Acid (3e**)** To a solution of **30** (6.2 mg, 0.010 mmol) was added 4 M HCl-AcOEt (0.1 mL) at 0°C, and the mixture was stirred at r.t. for 5 h. The reaction mixture was concentrated *in vacuo* to give compound **3e** (3.6 mg, 75, 72% purity) as a white amorphous.

IR (CHCl₃) cm⁻¹: 3685, 2928, 2857, 1722, 1601, 1453; [α]_D²⁸ +2.4 (*c*=0.45, EtOH); ¹H-NMR (CD₃OD, 400 MHz) δ : 4.21–4.02 (2H, m), 3.37 (2H, d, *J*=12.4 Hz), 3.15 (1H, dd, *J*=4.8, 11.2 Hz), 2.96 (2H, t, *J*=12.4 Hz), 2.21 (2H, d, *J*=7.2 Hz), 2.12–0.90 (25H, m), 0.90 (1H, s), 0.79 (2H, s); ¹³C-NMR (CD₃OD, 100 MHz) δ : 174.8, 80.2, 65.3, 45.3 (2C), 45.2, 42.8, 40.0, 38.4, 36.8, 35.5, 34.5, 33.4, 31.3, 30.0 (2C), 29.19, 29.16, 26.7, 25.6, 10.3; HR-MS (FAB) Calcd for C₂₁H₃₇NO₃ *m/z* [M⁺]: 352.2852. Found 352.2857.

Platelet Aggregation Assay Rabbit platelet rich-plasma (PRP) was obtained from fresh rabbit blood collected into a one-tenth volume of 3.8% sodium citrate by centrifugation at 1000 rpm (50×*g*) for 20 min at r.t. Platelet aggregometry followed the methods of Born³⁹⁾ and O'Brien.⁴⁰⁾ Rabbit PRP (175 μ L) was added to 10 mM CaCl₂ (25 μ L) and the mixture was preincubated at 37°C for 1 min. The mixture was added to each sample in 0.02 M Tris-HCl buffer (pH 7.4) containing 0.15 M NaCl (25 μ L) and incubated at 37°C for 5 min. A total of 25 μ L of ADP (final concentration: 1–2 μ M) or collagen (final concentration: 1 μ g/mL) was then added and incubated

at 37°C for 5–7 min. Platelet aggregation was measured by the turbidimetric method using HEMA TRACER 704 from MC MEDICAL Inc. (Tokyo, Japan).

Statistical Analysis Statistical analyses were performed by using Microsoft Excel 2007 software (Microsoft Co., Redmond, WA, U.S.A.). The statistical significance of differences was determined according to the student's *t*-test. *p*<0.05 was considered to be statistically significant.

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Conflict of Interest The authors declare no conflict of interest.

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