Improved Anti-Osteoporosis Potency and Reduced Endometrial Membrane Hyperplasia During Hormone Replacement Therapy with Estrogen−RGD Peptide Conjugates

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To improve the specificity and potency of estrogen replacement therapy therapeutics while also minimizing the side effects such as bone resorption and thickening of the uterine wall, a series of novel estrogen-derived conjugates estradiol-3-RGD, estradiol-17-RGD, and estrone-3-RGD peptides have been prepared. In a mouse model, intraperitoneal (i.p.) administration of these estrogen−RGD peptide conjugates resulted in decreased serum concentrations of calcium and alkaline phosphatase, as well as increased levels of calcium, phosphorus, and minerals in the mouse femur. Furthermore, the anti-osteoporosis action of these conjugates followed a dose-dependent manner and was accompanied with no observable effects on endometrial cell hyperplasia. In addition to all of these compounds exhibiting biological activity when administered by the i.p. route, we were particularly pleased to note that the estradiol−3-RGD and estradiol−17-RGD conjugates were both orally active.

1. Introduction

Owing to ovarium atrophy, almost all postmenopausal women over 55 years of age confront osteoporosis to varying extents, with over 50% suffering from bone fractures and secondary complications.[1−3] Because the lack of estrogen resulting from ovarium atrophy is responsible for the onset and progression of osteoporosis, many postmenopausal women undergo estrogen replacement therapy (ERT[a]) or estrogen/progesterone replacement therapy (HRT).[4] For postmenopausal women, ERT or HRT not only inhibit bone loss,[5] but also decrease the risk of coronary heart disease.[6] Michealsson et al. have shown that if postmenopausal women receive HRT for at least 5 years their fracture risk will be decreased by about 50%;[7] however, long-term therapy has been linked to a series of dose-related side effects such as breast cancer and hyperplasia of the uterine membrane.[8−11]

In previous work, we have demonstrated that the preparation of conjugates comprised of steroids and peptides can result in synergism of both molecular "partners". For example, in the case of hydrocortisone−KTP and estrone−KTP conjugates, the analgesic activities of hydrocortisone, estrone, and kyotorphin (KTP) were all enhanced.[12] In hydrocortisone−urotoxin and prednisolone−urotoxin conjugates, the immunosuppressive activities of hydrocortisone, prednisolone, and the urotoxins were all enhanced.[13] In estrogen−GHRPs conjugates, the anti-osteoperotic effects of estradiol, estrone, and growth hormone releasing peptides (GHRPs) were enhanced.[14]

It is commonly accepted that osteoporosis relates not only to a decrease in bone formation modulated by osteoblasts, but also to an increase in bone resorption modulated by osteoclasts. In ERT and HRT, estrogen is used to treat the decrease in skeletal muscle and bone by direct modulation of osteoblastic activity and proliferation or by regulation of gene expression in osteoblasts and osteoclasts.[15−17] Bone resorption is regulated by the binding of osteoclasts to the bone surface and is, therefore, dependent upon osteoclast adhesiveness. This bone adhesion process is mediated by the Arg-Gly-Asp (RGD)-binding cell-surface integrin receptor.[18]

Based on this information, we surmised that a "two-pronged" approach, combining the effects of estrogen on upregulation of osteoblastic activity and proliferation with the adhesion properties of the RGD peptide to downregulate osteoclast adhesiveness, and the synergism for both estrogen and peptide resulted from their conjugation may prove useful in the design of more efficacious osteoperosis inhibitors. Herein, we report our preliminary work in this area, which includes the synthesis and in vivo characterization of three kinds of estrogen−peptide conjugates, wherein the RGD peptide is incorporated at the 3- or 17-position of estradiol and the 3-position of estrone.

2. Results and Discussion

2.1. Preparation of RGD Peptides. Boc-Arg(NO_2)-Gly-Asp-(OBzl)-Ser(Bzl)-OBzl, Boc-Arg(NO_2)-Gly-Asp(OBzl)-Val-OBzl, and Boc-Arg(NO_2)-Gly-Asp(OBzl)-Phe-OBzl were prepared via the solution-phase method according to the route depicted in Scheme 1. The stepwise synthesis was carried out from C-terminal to N-terminal with L-Ser(Bzl)-OBzl, L-Val-OBzl, and L-Phe-OBzl as the C-terminal residue, respectively. Total yields were in a range of 76−82%. Upon removal of the *N*-terminal Boc groups, the building blocks HCl·H-Arg(NO_2)-Gly-Asp(OBzl)-Ser(Bzl)-OBzl (**1**), HCl·H-Arg(NO_2)-Gly-Asp-(OBzl)-Val-OBzl (**2**), and HCl·H-Arg(NO_2)-Gly-Asp(OBzl)-Phe-OBzl (**3**) were obtained in 93, 94, and 93% yield. Treating

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[a] Abbreviations: ERT, estrogen replacement therapy; ALP, alkaline phosphatase; HRT, hormone replacement therapy; ca., circa; KTP, kyotorphin; GHRPs, growth hormone releasing peptides; AA, amino acid; DCC, *N*,*N*′-dicyclohexylcarbodiimide; HOBt, 1-hydroxybenzotriazole hydrate; i.p., intraperitoneal; μL, microlitre; wt, weight; ESI-MS, electrospray ionization mass spectrometery; OVX, ovariotomy; FAB-MS, fast atom bombardment mass spectrometery; Boc, butyloxycarbonyl group; Bzl, benzyl group; THF, tetrahydrofuran; ESI-MS, electrospray ionization mass spectrometery; Anal. Calcd, analytical calculated; DMSO-d_6, dimethyl-d_6 sulfoxide; HPLC, high performance liquid chromatography; CMC, carboxymethylcellulose.

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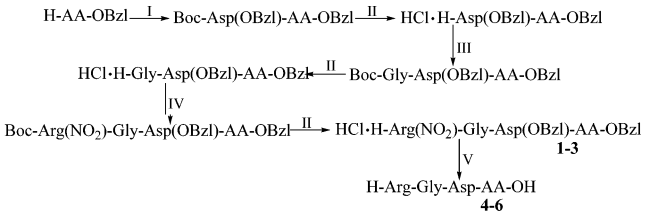
Scheme 1. Preparation of RGD Peptides[a]



[a] For H-AA-OBzl, AA = Ser(Bzl), Val, and Phe; for **1**, AA = Ser(Bzl), for **2** and **5**, AA = Val, for **3** and **6**, AA = Phe; and for **4**, AA = Ser. Reagents: (I) DCC, HOBt, and Boc-Gly-OH; (II) hydrogen chloride in ethyl acetate; (III) DCC, HOBt, and Boc-Asp(OBzl)-OH; (IV) DCC, HOBt, and Boc-Arg(NO_2)-OH; (V) Pt/H_2.

1−**3** with Pd/H_2 removed all protecting groups and the RGD peptides (**4**−**6**) were obtained in 89−92% yield.

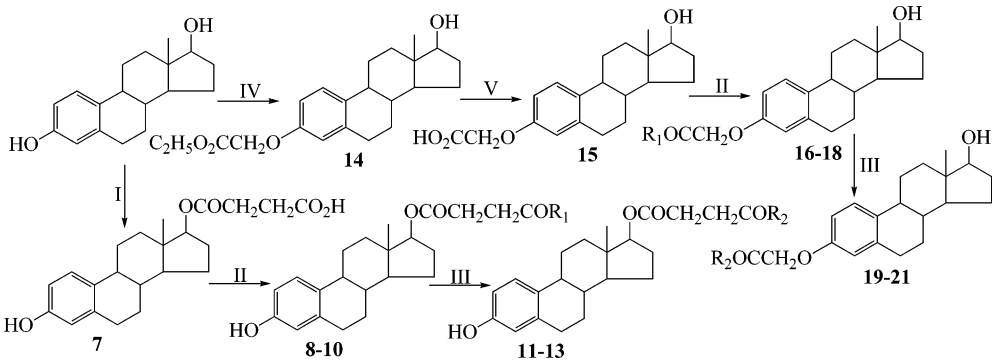
2.2. Preparation of Estradiol−3-RGD Peptide Conjugates. The estradiol−3-RGD peptide conjugates were prepared as outlined in Scheme 2. Thus, the C_3-hydroxyl group of estradiol was first alkylated with ethyl bromoacetate to provide ethyl estradiol-3-oxylacetate (**14**) in 83% yield. Saponification of **14** resulted in acid **15** in excellent yield (96%). Conjugation of **14** with HCl·H-Arg(NO_2)-Gly-Asp(OBzl)-AA-OBzl (**1**−**3**) then yielded the corresponding estradiol−3-Arg(NO_2)-Gly-Asp-(OBzl)-AA-OBzl derivatives **16**−**18** in 93, 74, and 61% yield, respectively. Following global deprotection by catalytic hydrogenation, estradiol−3-Arg-Gly-Asp-AA-OH (**19**−**21**) were obtained in 70, 73, and 77% yield, respectively. The yields indicate that with the methylcarbonyl group as the linker RGD peptides can be smoothly introduced into the 3-position of estradiol.

2.3. Preparation of Estradiol−17-RGD Peptide Conjugates. The estradiol-17-monoester of succinic acid (**7**) was first prepared by treating estradiol with succinic anhydride in 95% yield. Coupling of **7** and HCl·H-Arg(NO_2)-Gly-Asp(OBzl)-AA-OBzl (**1**−**3**) the corresponding estradiol−17-Arg(NO_2)-Gly-Asp(OBzl)-AA-OBzl (**8**−**10**) were obtained in 61, 53, and 53% yield, respectively. Following global deprotection by catalytic hydrogenation, estradiol−3-Arg-Gly-Asp-AA-OH (**11**−**13**) were obtained in 62, 68, and 58% yield, respectively. The yields indicate that with the carbonylethylcarbonyl group as the linker RGD peptides can be smoothly introduced into the 17-position of estradiol.

2.4. Preparation of Estrone−3-RGD Peptide Conjugates. As outlined in Scheme 3, the preparation of the estrone 3-RGD peptide conjugates commenced with the alkylation of the C_3-hydroxyl group of estrone using ethyl bromoacetate to give alkyl ether **22** in 90% yield. Saponification of **22** yielded acid **23** (96% yield) to which HCl·H-Arg(NO_2)-Gly-Asp(OBzl)-AA-OBzl (**1**−**3**) were coupled to give the corresponding estrone−3-Arg(NO_2)-Gly-Asp(OBzl)-AA-OBzl conjugates **24**−**26** in 62, 68, and 70% yield, respectively. Following global deprotection by catalytic hydrogenation, estrone−3-Arg-Gly-Asp-AA-OH conjugates **27**−**29** were obtained in 57, 76, and 56% yield, respectively. The yields demonstrate that through the same linker RGD peptides can also be smoothly introduced into the 3-position of estrone.

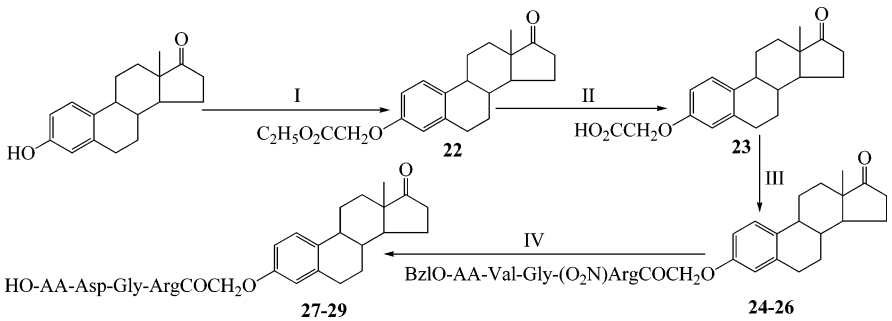
2.5. Estrogen−RGD Peptide Conjugates Inhibit Body Weight Increase of Mice. To develop osteoporosis, female Kuiming mice weighing 30.7 ± 3.1 g were subjected to abdominal ovariotomy (OVX) according to standard procedures.[19] To compensate for possible effects of the surgical procedure, the mice of a sham group were subjected to an abdominorotomy. On the fifth day after surgery, the mice then received an intraperitoneal (i.p.) injection of an aqueous solution of carboxymethylcellulose (CMC; 2 μL, 0.5 wt %), containing

Scheme 2. Preparation of 3- and 17-RGD Peptide Conjugates of Estradiol[a]



[a] Reagents: (I) succinic anhydride; (II) DCC, HOBt, **1**, **2**, or **3**; (III) Pt/H_2; (IV) ethyl bromoacetyl acetate; (V) NaOH. In **8** and **16**, R_1 = Arg(NO_2)-Gly-Asp(OBzl)-Ser-OBzl; in **9** and **17**, R_1 = Arg(NO_2)-Gly-Asp(OBzl)-Val-OBzl; in **10** and **18**, R_1 = Arg(NO_2)-Gly-Asp(OBzl)-Phe-OBzl; in **11** and **19**, R_2 = Arg-Gly-Asp-Ser-OH; in **12** and **20**, R_2 = Arg-Gly-Asp-Val-OH; and in **13** and **21**, R_2 = Arg-Gly-Asp-Phe-OH.

Scheme 3. Preparation of Estrone−3-RGD Peptide Conjugates[a]



[a] Reagents: (I) ethyl 2-bromoacetate; (II) NaOH; (III) DCC, HOBt, **1**, **2**, or **3**; (IV) H_2/Pd. In **24**, AA = Ser(Bzl); in **25** and **28**, AA = Val; in **26** and **29**, AA = Phe; and in **27**, AA = Ser.

Table 1. Effect of i.p. Injection of Estrogen-RGDAA on Body Weight of Mice[a]

group	before treatment (g)	after treatment (g)
OVX	31.1 ± 2.2	37.2 ± 2.6
sham	30.0 ± 1.5	34.5 ± 2.9[b]
estradiol	31.0 ± 2.6	34.1 ± 2.3[b]
RGDS	30.7 ± 2.5	34.0 ± 2.4[b]
estradiol + RGDS	30.6 ± 2.7	33.9 ± 2.7[c]
11	30.3 ± 1.8	35.4 ± 2.7[b]
19	30.9 ± 1.5	35.2 ± 1.6[b]
RGDV	30.3 ± 1.5	33.4 ± 2.5[c]
estradiol + RGDV	30.7 ± 2.0	33.6 ± 2.3[c]
12	30.4 ± 1.9	34.5 ± 2.7[b]
20	30.5 ± 2.6	35.2 ± 2.5[b]
RGDF	29.8 ± 1.7	33.3 ± 2.0[c]
estradiol + RGDF	30.5 ± 2.3	33.9 ± 1.9
13	29.8 ± 2.9	34.8 ± 2.7[b]
21	30.1 ± 2.0	33.1 ± 2.2[c]
estrone	30.4 ± 2.5	34.3 ± 2.1[c]
estrone + RGDS	30.6 ± 2.1	34.4 ± 2.2[c]
27	29.9 ± 1.9	32.9 ± 2.3[c]
estrone + RGDV	30.1 ± 2.4	33.5 ± 2.5[c]
28	30.3 ± 2.1	34.1 ± 2.6[b]
estrone + RGDF	31.0 ± 2.2	33.4 ± 2.3[c]
29	30.4 ± 2.2	34.5 ± 2.8[b]

[a] Dose = 110.3×10^{-3} mmol/kg, $n = 12$. OVX = ovariotomy. The statistical analysis of the data was carried out by the use of an ANOVA test, and $P < 0.05$ was considered significant. [b] Compared to OVX, $P < 0.05$. [c] Compared to OVX, $P < 0.01$.

estradiol, estrone, RGDS, RGDV, RGDF, a mixture of estradiol and RGDS, a mixture of estradiol and RGDV, a mixture of estradiol and RGDF, a mixture of estrone and RGDS, a mixture of estrone and RGDV, a mixture of estrone and RGDF, estradiol−17-RGD peptide conjugates (**11**−**13**), estradiol−3-RGD peptide conjugates (**19**−**21**), and estrone−3-RGD peptide conjugates (**27**−**29**; each at 110.3×10^{-3} mmol/kg). The mice were injected once daily for 4 weeks. On the day of the last administration, the mice were weighed and the body weights were recorded (Table 1). As shown, the data demonstrate that the surgical operation and the treatment with estrogen and estrogen−RGD peptide conjugates inhibits mouse body weight increase, which suggests that the inhibition of the increase of the body weight of mice is independent of the conjugations.

2.6. Estrogen−RGD Peptide Conjugates Decrease the Serum Levels of Calcium and ALP in Mice. To explore the effect of estrogen−RGD peptide conjugates on the content of calcium and ALP in the serum, blood was drawn by retrorbital puncture on the day after the last day of the 4 week dosing regime. The blood was allowed to stand at room temperature for 30 min and was then centrifuged (3000 *g* for 20 min). The serum was removed by pipet and stored at −20 °C and serum calcium content was measured using *o*-methylphenolphthalein, as previously described[20] (Table 2). The serum phosphorus content was measured using molybdenum blue, as previously described.[21] The serum alkaline phosphatase (ALP) level was measured using a functional assay with disodium phenylphosphate as the substrate. The data listed in Table 2 indicate that the content of calcium in the serum is significantly decreased after administration of **11**−**13**, **19**−**21**, and **27**−**29**, but is unchanged by treatment with estradiol, estrone, the mixtures of estradiol and RGD peptides, and the mixture of estrone and RGD peptides. Serum ALP levels were clearly reduced by the administration of estradiol, estrone, the mixtures of estradiol and RGD peptides, and the mixture of estrone and RGD peptides. However, the conjugates **11**−**13**, **19**−**21**, and **27**−**29** were far more effective at reducing ALP levels. The significantly higher inhibition of serum calcium and ALP levels in mice for estrogen−RGD peptide conjugates demonstrates that the conjugation of estrogen and RGD peptides is helpful for enhancing their corresponding inhibitions.

Table 2. Effect of i.p. Injection of Estrogen−RGD Peptide Conjugates on Serum ALP Level, Serum Calcium Concentration, and Serum Phosphorus Concentration of Mice[a]

group	calcium (mmol/L)	phosphorous (mmol/L)	ALP (U/L, king)
OVX	2.039 ± 0.0445	1.601 ± 0.00268	40.086 ± 2.884
sham	2.024 ± 0.0783	1.602 ± 0.00519	34.171 ± 3.341[c]
estradiol	2.013 ± 0.0382	1.601 ± 0.00521	35.286 ± 1.733[c]
estradiol + RGDS	2.013 ± 0.0433	1.602 ± 0.00408	36.328 ± 2.113[c]
RGDS	2.010 ± 0.0417	1.603 ± 0.00371	36.643 ± 1.787[c]
11	2.002 ± 0.0271[b]	1.602 ± 0.00385	32.771 ± 2.050[d]
19	2.004 ± 0.0347[b]	1.599 ± 0.00487	31.429 ± 1.837[d]
estradiol + RGDV	2.012 ± 0.0389	1.603 ± 0.00417	36.005 ± 1.772[c]
RGDV	2.011 ± 0.0181	1.605 ± 0.00669	35.614 ± 1.486[c]
12	2.005 ± 0.0258[b]	1.605 ± 0.00405	31.057 ± 0.996[d]
20	2.003 ± 0.0270[b]	1.600 ± 0.00492	30.514 ± 1.301[d]
estradiol + RGDF	2.011 ± 0.0367	1.601 ± 0.00520	36.084 ± 1.890[c]
RGDF	2.012 ± 0.0324	1.599 ± 0.00733	35.643 ± 2.756[c]
13	2.005 ± 0.0258[b]	1.605 ± 0.00498	31.486 ± 1.285[d]
21	2.005 ± 0.0160[b]	1.605 ± 0.00537	31.529 ± 1.676[d]
estrone	2.012 ± 0.0175	1.601 ± 0.00417	36.271 ± 3.228[c]
estrone + RGDS	2.013 ± 0.0441	1.603 ± 0.00439	36.204 ± 1.913[c]
27	2.000 ± 0.0284[b]	1.604 ± 0.00268	32.371 ± 1.910[e]
estrone + RGDV	2.010 ± 0.0395	1.603 ± 0.00550	35.970 ± 1.983[c]
28	2.008 ± 0.0179[b]	1.602 ± 0.00560	32.229 ± 1.467[e]
estrone + RGDF	2.013 ± 0.0382	1.601 ± 0.00488	36.433 ± 1.936[c]
29	2.008 ± 0.0344[b]	1.602 ± 0.00315	32.407 ± 2.137[e]

[a] Dosage = 110.3×10^{-3} mmol/kg, $n = 12$. OVX = ovariotomy. The statistical analysis of the data was carried out by the use of an ANOVA test, and $p < 0.05$ was considered significant. [b] Compared to OVX, $P < 0.05$. [c] Compared to OVX, $P < 0.01$. [d] Compared to OVX, estradiol, and RGD peptides, $P < 0.01$. [e] Compared to OVX, estrone, and RGD peptides, $P < 0.01$.

2.7. Estrogen−RGD Peptide Conjugates Inhibit Bone Loss of Mice. To evaluate the direct influence of the estrogen−RGD peptide conjugates on bone loss, the weights of dry femurs, the weights of femur ashes, and the lengths of femurs from the mice of all groups were measured. After body weighing and blood drawing, the mice were euthanized by anesthetization (pentobarbital sodium, 40.0 mg/kg, i.p.) followed by removal of the left femur. After complete removal of the muscle, the femur length was measured and the femurs were then defatted by immersion in a solution of chloroform−methanol (2:1) for 3 h (repeated 2 times). The femurs were then heated at 120 °C for 6 h, cooled, and weighed to record the dry weight. The femurs were then incinerated in a furnace at 800 °C for 8 h, cooled, and the residual ash was weighed. Dry femur weights listed in Table 3 indicate that estradiol, estrone, the mixtures of estradiol and RGD peptides, and the mixture of estrone and RGD peptides did not inhibit bone loss in the recipient mice. The weights of dry femurs from mice receiving estradiol, estrone, the mixtures of estradiol and RGD peptides, and the mixture of estrone and RGD peptides are significantly lower than that of the abdominorotomy-receiving mice (sham group). However, estrogen−RGD peptide conjugates did inhibit bone loss in the recipient mice, as the weights of dry femurs of the mice receiving **11**−**13**, **19**−**21**, and **27**−**29** are significantly higher than that of vehicle-receiving mice (OVX group) and no significant difference is found between the weights of dry femurs of the mice receiving estrogen−RGD peptide conjugates and the weight of

Table 3. Effect of i.p. Injection of Estrogen−RGD Peptide Conjugates on the Length of Femur and the Weight of Dry Femur and Femur Ash of Mice[a]

group	wt of dry femur (mg)	wt of femur ash (mg)	length of femur (cm)
OVX	58.5 ± 3.94	17.8 ± 2.17	1.597 ± 0.0390
sham	74.0 ± 8.32[c]	42.9 ± 4.83[c]	1.646 ± 0.0438[c]
estradiol	57.7 ± 6.96	28.5 ± 6.80[c]	1.630 ± 0.0646
estradiol + RGDS	58.2 ± 6.33	27.9 ± 5.10[c]	1.620 ± 0.0540
RGDS	59.8 ± 4.65	27.8 ± 4.06[c]	1.617 ± 0.0397
11	66.4 ± 5.38[d]	34.4 ± 5.65[g]	1.634 ± 0.0358[b]
19	63.4 ± 6.39[e]	37.0 ± 6.25[g]	1.642 ± 0.0500[b]
estradiol + RGDV	59.0 ± 6.37	28.1 ± 3.60[c]	1.618 ± 0.0523
RGDV	58.8 ± 4.65	27.8 ± 3.53[c]	1.614 ± 0.0455
12	62.6 ± 3.68[e]	35.8 ± 3.13[g]	1.655 ± 0.0490[c]
20	63.7 ± 6.27[e]	40.0 ± 4.81[g]	1.640 ± 0.0306[c]
estradiol + RGDF	59.5 ± 6.55	28.2 ± 3.59[c]	1.621 ± 0.0451
RGDF	60.0 ± 6.49	22.5 ± 7.43[b]	1.620 ± 0.0343
13	66.0 ± 5.48[d]	34.8 ± 4.67[g]	1.635 ± 0.0273[b]
21	64.2 ± 5.32[f]	35.8 ± 4.37[g]	1.635 ± 0.0474[b]
estrone	61.1 ± 5.87	20.8 ± 4.30[b]	1.606 ± 0.0317
estrone + RGDS	60.8 ± 6.19	23.4 ± 4.50[b]	1.600 ± 0.0522
27	66.9 ± 5.70[d]	22.6 ± 2.97[c]	1.634 ± 0.0459[b]
estrone + RGDV	58.6 ± 5.89	26.2 ± 4.48[b]	1.604 ± 0.0320
28	63.9 ± 7.10[e]	21.9 ± 6.32[b]	1.628 ± 0.0309[b]
estrone + RGDF	59.7 ± 6.24	25.5 ± 6.01[b]	1.606 ± 0.0340
29	65.9 ± 5.37[d]	24.7 ± 4.53[h]	1.639 ± 0.0456[b]

[a] Dosage = 110.3×10^{-3} mmol/kg, $n = 12$. OVX = ovariotomy. The statistical analysis of the data was carried out by the use of an ANOVA test, and $p < 0.05$ was considered significant. [b] Compared to OVX, $P < 0.05$. [c] Compared to OVX, $P < 0.01$. [d] Compared to OVX and estradiol, $P < 0.01$, to estrone and RGD peptides, $P < 0.05$. [e] Compared to OVX, estradiol, and RGD peptides, $P < 0.05$. [f] Compared to OVX, $P < 0.01$, to estradiol and RGD peptides, $P < 0.05$. [g] Compared to OVX and estradiol, $P < 0.01$, and to RGD peptides, $P < 0.05$. [h] Compared to OVX, $P < 0.01$, and to estrone, $P < 0.05$.

dry femurs of sham group mice. The data in Table 3 indicate that though the femur ash weights of mice receiving estradiol, estrone, the mixtures of estradiol and RGD peptides, and the mixture of estrone and RGD peptides are significantly higher than that of vehicle receiving mice (OVX group), they are significantly less than that of mice receiving **11**−**13** and **19**−**21**. Similarly, except the femur length of the mice receiving estrone, the mixtures of estrone and RGD peptides, estradiol, and the mixtures of estradiol and RGD peptides, the femur length of the mice receiving **11**−**13**, **19**−**21**, and **27**−**29** are significantly higher than that of the vehicle-receiving mice (OVX group). All of the data listed in Table 3 suggest that estrogen−RGD peptide conjugates **11**−**13**, **19**−**21**, and **27**−**29** are able to inhibit bone loss in OVX mice.

2.8. Effect of Estrogen−RGD Peptide Conjugates on Bone Calcium, Phosphorus Content, and Mineral Content. The femurs were then incinerated in a furnace at 800 °C for 8 h, cooled, and weighed to record the ash weight and calculate the ratio of the ash weight to dry femur weight (i.e., the mineral content of the femur). To investigate the influence of estrogen−RGD peptide conjugates on the content of calcium, phosphorus, and minerals in the mouse femurs, the weighed ashes of the left femurs were dissolved in hydrochloric acid (6 N, 0.5 mL) and diluted with ultrapure water (4.5 mL). An aliquot (0.05 mL) of the solution was drawn and diluted to 1 mL with ultrapure water. The calcium content of the aqueous solution was measured using *o*-methylphenolphthalein. The phosphorus content of the aqueous solution was measured by the method of molybdenum blue.[22] Based on the measured data, the ratios of calcium and phosphorus in the ash were calculated. The data are listed in Table 4. As shown, the calcium, phosphorus, and mineral content in the femurs of the mice in the sham and the drug treatment groups are significantly higher than that of the

Table 4. Effect of i.p. Injection of Estrogen−RGD Peptide Conjugates on the Calcium, Phosphorus, and Mineral Content of Mouse Femurs[a]

group	calcium (%)	phosphorous (%)	mineral (ratio)
OVX	39.545 ± 2.551	22.951 ± 1.960	0.304 ± 0.0272
sham	53.213 ± 2.365[c]	24.811 ± 1.049[c]	0.487 ± 0.0922[c]
estradiol	44.466 ± 3.041[c]	24.995 ± 2.307[b]	0.496 ± 0.0945[c]
estradiol + RGDS	43.903 ± 2.402[c]	24.891 ± 2.067[b]	0.413 ± 0.0557[c]
RGDS	43.480 ± 2.331[c]	24.888 ± 2.059[b]	0.396 ± 0.0460[c]
11	51.312 ± 2.602[d]	24.920 ± 1.623[b]	0.520 ± 0.0819[c]
19	54.885 ± 1.668[d]	24.949 ± 2.221[b]	0.582 ± 0.0512[f]
estradiol + RGDV	43.009 ± 2.055[c]	24.823 ± 2.217[b]	0.402 ± 0.0406[c]
RGDV	42.739 ± 1.816[c]	24.759 ± 2.267[b]	0.394 ± 0.0411[c]
12	53.078 ± 2.349[d]	24.867 ± 1.536[b]	0.625 ± 0.0399[d]
20	58.350 ± 0.926[d]	24.991 ± 1.338[b]	0.630 ± 0.0690[d]
estradiol + RGDF	44.103 ± 3.121[c]	24.765 ± 2.258[b]	0.407 ± 0.0411[c]
RGDF	43.815 ± 1.792[c]	24.585 ± 1.204[b]	0.423 ± 0.0685[c]
13	52.798 ± 1.376[d]	24.665 ± 1.069[b]	0.533 ± 0.0921[c]
21	56.690 ± 1.474[d]	25.460 ± 1.265[c]	0.585 ± 0.0555[f]
estrone	43.840 ± 2.659[c]	23.979 ± 1.087	0.430 ± 0.0522[c]
estrone + RGDS	43.466 ± 2.450[c]	24.756 ± 2.260[b]	0.426 ± 0.0548[c]
27	47.613 ± 1.891[e]	24.399 ± 1.110[b]	0.438 ± 0.0388[c]
estrone + RGDV	43.634 ± 2.569[c]	24.767 ± 1.309[b]	0.422 ± 0.0681[c]
28	47.173 ± 1.772[e]	24.750 ± 1.083[b]	0.429 ± 0.0387[c]
estrone + RGDF	43.722 ± 2.490[c]	24.766 ± 2.249[b]	0.420 ± 0.0556[c]
29	49.550 ± 2.436[e]	24.588 ± 1.080[b]	0.462 ± 0.0510[c]

[a] Dosage = 110.3×10^{-3} mmol/kg, $n = 12$. OVX = ovariotomy. The statistical analysis of the data was carried out by the use of an ANOVA test, and $p < 0.05$ was considered significant. [b] Compared to OVX, $P < 0.05$. [c] Compared to OVX, $P < 0.01$. [d] Compared to OVX, estradiol, and RGD peptides, $P < 0.01$. [e] Compared to OVX, estrone, and RGD peptides, $P < 0.01$. [f] Compared to OVX and RGD peptides, $P < 0.01$, and to estradiol, $P < 0.05$.

mice in OVX group. When compared to the mice receiving estradiol, estrone, the mixtures of estradiol and RGD peptides, and the mixtures of estrone and RGD peptides, the femurs of the mice receiving **11**−**13**, **19**−**21**, and **27**−**29** show significantly higher contents of calcium and the femurs of the mice receiving **12**, **19**, and **21** show significantly higher contents of mineral. Thus, the estrogen−RGD peptide conjugates substantially increase the content of calcium, phosphorus, and mineral in the femur. This is not only consistent with their inhibition of bone loss, but also with the advantage that resulted from the conjugation of estrogen and RGD peptides.

2.9. Effect of Estrogen−RGD Peptide Conjugates on Organ Weights. To examine the effect of estrogen−RGD peptide conjugates on organ weights, the removed lungs, livers, spleens, and uteri were weighed directly and the weights are listed in Table 5. The data indicate that though the liver weight of the mice receiving estradiol is occasionally higher than that of the mice in the OVX group, the weights of lungs, livers, and spleens of the mice in all groups show no significant difference. However, the weights of the uteri of mice receiving estradiol, estrone, the mixtures of estradiol and RGD peptides, and the mixtures of estrone and RGD peptides were significantly heigher than that of OVX, sham and estrogen−RGD peptide conjugates receiving mice. These results indicate that estrogen−RGD peptide conjugates exhibited no observable influence on endometrial cell hyperplasia, which reveals that the conjugation of estrogen and RGD peptides is helpful for eliminating the dose-related side effects of estrogen.

2.10. Dose-Related Inhibition of Mouse Bone Loss by Estrogen−RGD Peptide Conjugates 19−21. To evaluate the effect of the dose of estrogen−RGD peptide conjugates on bone

Table 5. Effect of i.p. Injection of Estrogen−RGD Peptide Conjugates on the Weight of Lung, Liver, Spleen, and Uterus of Mice[a]

group	lung (mg)	liver (g)	spleen (mg)	uterus (mg)
OVX	163.0 ± 23.1	1.329 ± 0.156	130.0 ± 31.9	89.0 ± 24.9[c]
sham	164.0 ± 24.7	1.367 ± 0.198	151.0 ± 41.0	91.5 ± 30.0[c]
estradiol	168.0 ± 30.8	1.565 ± 0.199[b]	151.0 ± 35.7	177.1 ± 43.6[b]
estradiol + RGDS	165.0 ± 26.1	1.372 ± 0.190	148.0 ± 32.3	178.0 ± 42.5[b]
RGDS	168.0 ± 22.7	1.295 ± 0.186	138.0 ± 37.6	71.0 ± 52.2[c]
11	151.0 ± 18.8	1.371 ± 0.178	130.0 ± 37.2	55.5 ± 34.4[d]
19	173.0 ± 20.1	1.377 ± 0.185	124.0 ± 36.4	42.5 ± 18.7[d]
estradiol + RGDV	167.0 ± 24.7	1.370 ± 0.187	129.0 ± 33.6	177.7 ± 42.7[b]
RGDV	171.0 ± 25.9	1.153 ± 0.110[b]	127.0 ± 33.0	61.1 ± 28.7[d]
12	167.0 ± 27.9	1.353 ± 0.221	143.0 ± 36.8	69.4 ± 33.6[d]
20	162.0 ± 19.0	1.363 ± 0.133	132.0 ± 45.0	50.3 ± 31.1[d]
estradiol + RGDF	165.0 ± 24.4	1.422 ± 0.183	130.0 ± 25.8	178.2 ± 44.1[b]
RGDF	163.0 ± 17.7	1.413 ± 0.122	134.0 ± 25.5	69.8 ± 41.9[d]
13	164.0 ± 24.5	1.391 ± 0.129	142.0 ± 32.0	71.2 ± 26.0[d]
21	169.0 ± 30.3	1.395 ± 0.160	131.0 ± 34.4	55.4 ± 28.8[d]
estrone	176.0 ± 25.9	1.318 ± 0.113	150.0 ± 31.0	141.9 ± 45.7[b]
estrone + RGDS	170.0 ± 24.9	1.331 ± 0.159	146.0 ± 32.3	143.2 ± 46.0[b]
27	173.0 ± 16.4	1.347 ± 0.146	129.0 ± 26.8	63.3 ± 43.4[d]
estrone + RGDV	172.0 ± 23.7	1.387 ± 0.155	144.0 ± 33.0	145.5 ± 47.0[b]
28	168.0 ± 10.7	1.389 ± 0.139	131.0 ± 37.2	51.9 ± 32.0[d]
estrone + RGDF	169.0 ± 22.3	1.410 ± 0.187	139.0 ± 40.7	143.7 ± 41.2[b]
29	179.0 ± 16.0	1.458 ± 0.199	140.0 ± 41.1	55.8 ± 32.6[d]

[a] Dosage = 110.3×10^{-3} mmol/kg, $n = 12$. OVX = ovariotomy. The statistical analysis of the data was carried out by the use of an ANOVA test, and $p < 0.05$ was considered significant. [b] Compared to OVX, $P < 0.01$. [c] Compared to estradiol and estrone, $P < 0.01$. [d] Compared to OVX, estradiol, and estrone, $P < 0.01$.

Table 6. Effect of **19**−**21** at Different Doses (i.p.) on the Femur Length, the Dry Femur Weight, and the Femur Ash Weight of Recipient Mice[a]

group	dosage (mmol/kg)	femur weight (mg)	femur ash weight (mg)	femur length (cm)
19	110.3×10^{-3}	63.4 ± 6.25	37.0 ± 6.25	1.642 ± 0.0500
	55.15×10^{-3}	56.3 ± 6.99[b]	31.6 ± 4.43[b]	1.622 ± 0.0430
	11.03×10^{-3}	48.8 ± 4.97[c]	18.1 ± 4.23[c]	1.642 ± 0.0421
20	110.3×10^{-3}	63.7 ± 6.27	40.0 ± 4.81	1.640 ± 0.0306
	55.15×10^{-3}	57.5 ± 4.87[b]	32.3 ± 4.70[b]	1.643 ± 0.0300
	11.03×10^{-3}	50.9 ± 6.75[c]	20.6 ± 7.33[c]	1.618 ± 0.0520
21	110.3×10^{-3}	61.2 ± 6.74	35.8 ± 4.37	1.623 ± 0.0475
	55.15×10^{-3}	53.9 ± 4.08[b]	31.6 ± 2.87[b]	1.624 ± 0.0580
	11.03×10^{-3}	46.9 ± 6.74[c]	20.8 ± 7.11[c]	1.647 ± 0.0414

[a] $n = 12$. The statistical analysis of the data was carried out by the use of an ANOVA test, and $p < 0.05$ was considered significant. [b] Compared to 110.3×10^{-3} mmol/kg, $P < 0.05$. [c] Compared to 55.15×10^{-3} mmol/kg, $P < 0.01$.

loss, the weights of dry femurs, weights of femur ashes, the lengths of femurs, the calcium content of femurs, the phosphorus content of femurs, the mineral content of femurs, the serum calcium content, the serum phosphorus content of and the serum ALP activity of the mice receiving **19**−**21**, the estradiol−RGD peptide conjugates with higher anti-osteoporosis potency at a dose of 110.3×10^{-3} mmol/kg (high dose), 55.15×10^{-3} mmol/kg (moderate dose) and, 11.03×10^{-3} mmol/kg (low dose), respectively, were measured. The data are listed in Tables 6−8. The data in Table 6 demonstrate that the femur length, the dry femur weight, and the femur ash weight of the mice receiving a high dose of **19**−**21** are significantly higher than that of the mice receiving a moderate dose of **19**−**21**. On the other hand, the femur length, the dry femur weight, and the femur ash weight of the mice receiving a moderate dose of **19**−**21** are also significantly higher than that of the mice receiving a low dose of **19**−**21**. Similarly, the data in Table 7 demonstrate that the femur calcium content, the femur phosphorus content, and the femur mineral content of the mice receiving a high dose of **19**−**21** are significantly higher than that of the mice receiving a moderate dose of **19**−**21**, and the femur calcium content the femur phosphorus content and the femur mineral content of the mice receiving a moderate dose of **19**−**21** are significantly higher than that of the mice receiving a low dose of **19**−**21**. The same dose-related effects of **19**−**21** on the serum calcium content, the serum phosphorus content, and the serum ALP activity are also revealed in the data of Table 8. All the data support that the effect of **19**−**21** on bone loss follows a dose-dependent manner.

2.11. Dose Effects of Estradiol−RGD Peptide Conjugates 19−21 on Uterine Weights of Mice. To examine the risk of of dose-related side effects, the weight of the liver, the lung, the spleen, and especially the uterine of the mice receiving **19**−**21**, the estradiol−RGD peptide conjugates with a higher anti-osteoporosis potency, at a dose of 110.3×10^{-3} mmol/kg (high dose), 55.15×10^{-3} mmol/kg (moderate dose), and 11.03×10^{-3} mmol/kg (low dose), respectively, were measured. The data are listed in Table 9. It is clear that the weight of the liver, the lung, the spleen, and the uterine of the mice receiving high, moderate, and low doses of **19**−**21** show no significant difference. The results indicate that the anti-osteoporosis action of estradiol−RGD peptide conjugates followed a dose-dependent manner and was accompanied with no observable effects on endometrial cell hyperplasia.

2.12. Oral Administration of Estrogen−RGD Peptide Conjugates Inhibited Bone Loss of Mice. To explore if estrogen−RGD peptide conjugates are orally active, **11**−**13** and **19**−**20** were administered orally. The female Kuiming mice weighing 30.7 ± 3.1 g were subjected to abdominal OVX, as mentioned above. To compensate for the possible effects of the surgical procedure, the mice of a sham group were subjected to an abdominorotomy. On the fifth day after surgery, the mice then received an oral administration of an aqueous solution of CMC (0.2 mL, 0.5 wt %), containing estradiol, a mixture of estradiol and RGDS, estradiol−17-RGD peptide conjugates (**11**−**13**) and estradiol−3-RGD peptide conjugates (**19**−**21**; each at 110.3×10^{-3} mmol/Kg). The mice were orally administered once daily for 4 weeks. On the day of the last administration, the blood of the mice was drawn by retrorbital puncture. The blood was allowed to stand at room temperature for 30 min and was then centrifuged (3000 g for 20 min). The serum was removed by pipet and stored at −20 °C and the serum calcium

Table 7. Effect of **19**−**21** at Different Doses (i.p.) on Femur Calcium Content, Femur Phosphorus Content, and Femur Mineral Content of Mice[a]

group	dosage (mmol/kg)	calcium (%)	phosphorous (%)	mineral (ratio)
19	110.3×10^{-3}	54.885 ± 1.668	24.949 ± 2.221	0.582 ± 0.0512
	55.15×10^{-3}	52.076 ± 2.399[c]	24.274 ± 2.394	0.479 ± 0.0686[c]
	11.03×10^{-3}	41.548 ± 3.792[d]	23.984 ± 2.711	0.310 ± 0.0776[d]
20	110.3×10^{-3}	58.353 ± 0.926	24.991 ± 2.338	0.630 ± 0.0690
	55.15×10^{-3}	57.130 ± 1.113[c]	24.315 ± 2.123	0.539 ± 0.0973[b]
	11.03×10^{-3}	42.110 ± 3.759[d]	23.978 ± 3.076	0.345 ± 0.0120[d]
21	110.3×10^{-3}	56.693 ± 1.474	25.460 ± 3.265	0.585 ± 0.0555
	55.15×10^{-3}	54.493 ± 1.381[c]	24.845 ± 2.214	0.504 ± 0.0401[c]
	11.03×10^{-3}	42.855 ± 3.447[d]	24.347 ± 1.869	0.338 ± 0.0121[d]

[a] $n = 12$. The statistical analysis of the data was carried out by the use of an ANOVA test, and $p < 0.05$ was considered significant. [b] Compared to 110.3×10^{-3} mmol/kg, $P < 0.05$. [c] Compared to 110.3×10^{-3} mmol/kg, $P < 0.01$. [d] Compared to 55.15×10^{-3} mmol/kg, $P < 0.01$.

Table 8. Effect of **19**−**21** at Different Doses (i.p.) on Serum ALP Level, the Serum Calcium Concentration, and Serum Phosphorus Concentration of Mice[a]

group	dosage (mmol/kg)	calcium (mmol/L)	phosphorous (mmol/L)	ALP (U/L, king)
19	110.3×10^{-3}	2.014 ± 0.0347	1.599 ± 0.00487	31.429 ± 1.837
	55.15×10^{-3}	2.047 ± 0.0198[b]	1.607 ± 0.00356	33.616 ± 2.643[b]
	11.03×10^{-3}	2.087 ± 0.0254[e]	1.611 ± 0.00265	40.327 ± 1.526[e]
20	110.3×10^{-3}	2.005 ± 0.0270	1.600 ± 0.00492	30.514 ± 1.301
	55.15×10^{-3}	2.084 ± 0. 0483[c]	1.586 ± 0.00274	32.786 ± 2.143[c]
	11.03×10^{-3}	2.117 ± 0.0115[d]	1.605 ± 0.00324	38.457 ± 1.546[e]
21	110.3×10^{-3}	2.0150 ± 0.016	1.605 ± 0.00537	31.529 ± 1.676
	55.15×10^{-3}	2.040 ± 0.0369[b]	1.602 ± 0.00314	33.746 ± 1.724[c]
	11.03×10^{-3}	2.093 ± 0.0176[e]	1.600 ± 0.00264	38.643 ± 1.204[e]

[a] $n = 12$. The statistical analysis of the data was carried out by use of an ANOVA test, and $p < 0.05$ was considered significant. [b] Compared to 110.3×10^{-3} mmol/kg, $P < 0.05$. [c] Compared to 110.3×10^{-3} mmol/kg, $P < 0.01$. [d] Compared to 55.15×10^{-3} mmol/kg, $P < 0.05$. [e] Compared to 55.15×10^{-3} mmol/kg, $P < 0.01$.

Table 9. Effect of **19**−**21** at Different Doses (i.p.) on the Liver Weight, Lung Weight, Spleen Weight, and Uterine Weight of the Mice[a]

group	dosage (mmol/kg)	liver weight (g)	lung weight (mg)	spleen weight (mg)	uterine weight (mg)
19	110.3×10^{-3}	1.377 ± 0.185	173.0 ± 20.1	124.0 ± 36.0	42.5 ± 18.7
	55.15×10^{-3}	1.397 ± 0.231	167.0 ± 33.3	136.0 ± 46.3	48.6 ± 28.2
	11.03×10^{-3}	1.424 ± 0.179	181.0 ± 24.9	125.0 ± 22.9	52.8 ± 38.8
20	110.3×10^{-3}	1.363 ± 0.133	182.0 ± 19.0	132.0 ± 45.0	50.3 ± 31.1
	55.15×10^{-3}	1.462 ± 0.165	179.0 ± 33.2	140.0 ± 40.6	57.6 ± 43.9
	11.03×10^{-3}	1.477 ± 0.227	188.0 ± 56.0	156.0 ± 53.0	55.7 ± 35.5
21	110.3×10^{-3}	1.395 ± 0.160	169.0 ± 30.3	131.0 ± 34.0	55.4 ± 28.8
	55.15×10^{-3}	1.407 ± 0.227	155.0 ± 45.7	123.0 ± 36.8	55.3 ± 19.1
	11.03×10^{-3}	1.575 ± 0.239	174.0 ± 33.3	145.0 ± 62.0	60.6 ± 47.3

[a] $n = 12$; the statistical analysis of the data was carried out by the use of an ANOVA test, and $p < 0.05$ was considered significant.

Table 10. Effect of Orally Administered Estradiol−RGD Peptide Conjugates on Femur Weight, Femur Ash Weight and Femur Length of Mice[a]

group	weight of dry femur (mg)	weight of femur ash (mg)	length of femur (cm)
OVX	58.8 ± 4.15	18.0 ± 2.20	1.601 ± 0.0394
sham	73.3 ± 7.18[c]	42.4 ± 4.31[c]	1.642 ± 0.0440[c]
estradiol	63.1 ± 6.54	22.2 ± 4.23	1.622 ± 0.0447
estradiol + RGDS	62.9 ± 5.20	22.3 ± 3.97	1.620 ± 0.0512
11	68.8 ± 6.42[d]	29.3 ± 4.54[e]	1.654 ± 0.0550[c]
estradiol + RGDV	63.0 ± 5.14	22.0 ± 4.30	1.621 ± 0.0509
12	68.2 ± 5.32[d]	33.0 ± 5.81[e]	1.645 ± 0.0470[c]
estradiol + RGDF	62.6 ± 5.24	21.7 ± 4.11	1.610 ± 0.0439
13	62.7 ± 4.77[b]	30.0 ± 4.67[e]	1.638 ± 0.0420[b]
19	68.6 ± 5.67[d]	30.4 ± 6.18[e]	1.635 ± 0.0362[b]
20	68.4 ± 4.68[d]	33.7 ± 7.46[e]	1.614 ± 0.0329
21	63.1 ± 5.15[b]	28.7 ± 3.89[e]	1.626 ± 0.0296

[a] Dosage = 110.3×10^{-3} mmol/kg, $n = 12$. OVX = ovariotomy. The statistical analysis of the data was carried out by the use of an ANOVA test, and $p < 0.05$ was considered significant. [b] Compared to OVX, $P < 0.05$. [c] Compared to OVX, $P < 0.01$. [d] Compared to OVX, $P < 0.01$, and to estradiol, $P < 0.05$. [e] Compared to OVX and estradiol, $P < 0.01$.

content, the serum phosphorus content, and the serum ALP level were measured as mentioned above. After blood drawing, the mice were euthanized by anesthetization (pentobarbital sodium, 40.0 mg/kg, i.p.), followed by removal of the left femur. After the treatment of the left femurs by use of the same procedure as mentioned above, dry femur weights, femur ash weights, femur length, femur calcium content, femur phosphorus content, and femur mineral content of the mice receiving vehicle (CMC), estradiol, a mixture of estradiol and RGD peptides, **11**−**13** and **19**−**21** were tested. The data are listed in Tables 10−12. The data of the tables indicate that though oral administration of estradiol is unable to inhibit the bone loss of the mice, the oral administration of **11**−**13** and **19**−**21** is able to inhibit the bone loss of the mice. The femur weight, femur ash weight, femur length, femur calcium content, femur phosphorus content, and femur mineral content of the mice orally administered with **11**−**13** and **19**−**21** are significantly higher than that of the mice orally administered with vehicle (OVX group), estradiol, and a mixture of estradiol and RGD peptides. The results suggest that the estradiol−3-RGD peptide conjugates and estradiol−17-RGD peptide conjugates are both orally active.

2.13. Oral Administration of Estrogen−RGD Peptide Conjugates Gave No Influence on the Uterus of the Mice. To examine the effect of oral administration of estradiol−RGD peptide conjugates on the weights of organs, the removed lungs, livers, spleens, and uteri of the mice orally receiving $110.3 \times$

Table 11. Effect of Orally Administered Estradiol−RGD Peptide Conjugates on Femur Calcium Content, Femur Phosphorus Content, and Femur Mineral Content of Mice[a]

group	calcium (%)	phosphorous (%)	mineral (ratio, %)
OVX	39.606 ± 2.563	22.972 ± 1.958	0.371 ± 0.0779
sham	53.400 ± 2.566[d]	25.101 ± 1.925[c]	0.520 ± 0.0811[d]
estradiol	41.729 ± 2.670	24.530 ± 2.173	0.411 ± 0.0822
estradiol + RGDS	41.699 ± 2.701	24.499 ± 2.007	0.413 ± 0.0746
11	51.201 ± 2.653[d]	24.885 ± 2.300[b]	0.515 ± 0.0807[c]
estradiol + RGDV	41.694 ± 2.658	24.532 ± 2.187	0.408 ± 0.0818
12	52.003 ± 2.776[d]	24.879 ± 1.963[b]	0.525 ± 0.0795[d]
estradiol + RGDF	41.705 ± 2.712	24.465 ± 2.185	0.409 ± 0.0791
13	51.903 ± 2.822[d]	24.869 ± 2.126[b]	0.516 ± 0.0812[d]
19	51.988 ± 2.689[d]	24.994 ± 2.211[b]	0.522 ± 0.0783[d]
20	52.197 ± 2.765[d]	24.997 ± 2.312[b]	0.519 ± 0.0794[d]
21	53.001 ± 2.699[d]	25.532 ± 2.209[c]	0.525 ± 0.0859[d]

[a] Dosage = 110.3×10^{-3} mmol/kg, $n = 12$. OVX = ovariotomy. The statistical analysis of the data was carried out by the use of an ANOVA test, and $p < 0.05$ was considered significant. [b] Compared to OVX, $P < 0.05$. [c] Compared to OVX, $P < 0.01$. [d] Compared to OVX and estradiol, $P < 0.01$.

Table 12. Effect of Oral Estradiol−RGD Peptide Conjugates on Serum Calcium Content, Serum Phosphorus Content, and ALP Level of Mice[a]

group	calcium (%)	phosphorous (%)	ALP (U/L, king)
OVX	2.026 ± 0.0450	1.604 ± 0.00471	40.223 ± 2.947
sham	2.033 ± 0.0482	1.606 ± 0.00519	34.691 ± 3.155[b]
estradiol	2.022 ± 0.0469	1.605 ± 0.00488	37.705 ± 3.221
estradiol + RGDS	2.030 ± 0.0671	1.602 ± 0.00530	38.114 ± 3.196
11	2.040 ± 0.0490	1.604 ± 0.00497	35.400 ± 3.134[b]
estradiol + RGDV	2.029 ± 0.0477	1.605 ± 0.00525	38.006 ± 3.200
12	2.033 ± 0.0474	1.606 ± 0.00499	33.411 ± 2.780[c]
estradiol + RGDF	2.029 ± 0.0465	1.603 ± 0.00519	37.995 ± 3.100
13	2.036 ± 0.0476	1.602 ± 0.00590	35.511 ± 2.939[b]
19	2.032 ± 0.0489	1.604 ± 0.00438	35.211 ± 2.896[b]
20	2.035 ± 0.0491	1.606 ± 0.00496	34.521 ± 2.488[d]
21	2.041 ± 0.0526	1.603 ± 0.00319	33.700 ± 2.682[c]

[a] Dosage = 110.3×10^{-3} mmol/kg, $n = 12$. OVX = ovariotomy. The statistical analysis of the data was carried out by the use of an ANOVA test, and $p < 0.05$ was considered significant. [b] Compared to OVX, $P < 0.01$. [c] Compared to OVX and estradiol, $P < 0.01$. [d] Compared to OVX, $P < 0.01$, and to estradiol, $P < 0.05$.

Table 13. Effect of Oral Estradiol−RGD Peptide Conjugates on the Mice Uterus Weight

group	lung (mg)	liver (g)	spleen (mg)	uterus (mg)
OVX	165.0 ± 23.9	1.332 ± 0.176	130.0 ± 31.9	89.0 ± 24.9
sham	164.0 ± 24.2	1.337 ± 0.191	131.0 ± 41.5	41.5 ± 30.1
estradiol	168.0 ± 25.1	1.335 ± 0.198	141.0 ± 35.9	40.1 ± 18.4
11	161.0 ± 25.5	1.331 ± 0.189	135.0 ± 35.5	36.7 ± 13.0
12	167.0 ± 24.9	1.340 ± 0.188	133.0 ± 36.7	50.7 ± 31.9
13	167.0 ± 24.5	1.338 ± 0.199	142.0 ± 38.0	38.2 ± 10.8
19	166.0 ± 24.8	1.329 ± 0.194	138.0 ± 33.5	43.2 ± 23.6
20	163.0 ± 24.4	1.337 ± 0.199	134.0 ± 37.6	40.7 ± 19.6
21	165.0 ± 24.0	1.339 ± 0.188	135.0 ± 38.7	47.5 ± 30.5

[a] Dosage = 110.3×10^{-3} mmol/kg, $n = 12$. OVX = ovariotomy.

10^{-3} mmol/kg of estradiol, **11**−**13**, and **19**−**21** were directly weighed and the data are listed in Table 13. As shown in Table 13, the weights of lungs, livers, spleens, and uteri of the mice of all groups exhibit no significant difference. These results suggest that the anti-osteoporosis action of oral administration of estradiol−RGD peptide conjugates had no observable effects on endometrial cell hyperplasia even though an obvious anti-osteoporosis action was observed.

2.14. Conclusion. In conclusion, through the conjugation of estrogen and RGD peptides, the present paper provides a modification method for estrogen to improve the efficacy of HRT. By the in vivo assay data of nine novel estrogen−RGD peptide conjugates, it is recognized that this kind of conjugation is a promising new approach by which anti-osteoperotic drugs with improved therapeutic efficacy might be developed for use in HRT. For instance, in a mouse model, i.p. administration of the estradiol−3-RGD, estradiol−17-RGD, and estrone−3-RGD peptide conjugates resulted in decreased serum concentrations of calcium and the level of ALP, as well as increased levels of calcium, phosphorus, and minerals in the femur. Furthermore, the anti-osteoporosis action of these compounds followed a dose-dependent manner and was accompanied with no observable effects on endometrial cell hyperplasia. In addition to these compounds all exhibiting biological activity when administered by the i.p. route, we were particularly pleased to note that the estradiol−3-RGD and estradiol−17-RGD peptide conjugates were both orally active.

3. Experimental Section

3.1. Chemical Synthesis. The protected amino acids (AAs) with L-configuration were purchased from Sigma Chemical Co. All coupling and deprotection reactions were carried out under anhydrous conditions. Chromatography was performed on Qingdao silica gel H. The purity of the intermediates and the products was confirmed by TLC (Merck silica gel plates of type 60 F_{254}, 0.25 mm layer thickness) and HPLC (Waters, C_{18} column 4.6 × 150 mm). The AA analysis was determined by Hitachi 835-50 instrument. FAB-MS were determined by VG-ZAB-MS high-resolution GC/MS/DS and HP ES-5989x. Optical rotations were determined on Schmidt+Haensch Polartronic D instrument.

3.1.1. General Procedure for Removal of the Boc of the C-Terminal Component. The solution of 0.20 mmol of Boc-protected compound in 2 mL of hydrogen chloride in ethyl acetate (4 mol/L) was stirred at room temperature for 3 h. The reaction mixture was evaporated to remove the solvent. The residue was dissolved in 10 mL of ethyl acetate, and the solution was evaporated to dryness. The resultant solid was used directly for a subsequent coupling reaction.

3.1.2. Boc-Arg(NO_2)-Gly-Asp(OBzl)-Ser(Bzl)-OBzl. (1) Boc-Asp(OBzl)-Ser(Bzl)-OBzl. To a solution of 420 mg (1.30 mmol) of Boc-Asp(OBzl)-OH in 2 mL of anhydrous THF, 176 mg (1.30 mmol) of HOBt and 268 mg (1.30 mmol) of DCC were added. The mixture was stirred at 0 °C for 0.5 h to provide solution A. At 0 °C, to a solution of 418 mg (1.30 mmol) of HCl·H-Ser(Bzl)-OBzl in 2 mL of anhydrous THF, 155 μL of *N*-methylmorpholine was added, and the mixture was stirred for 10 min to provide solution B. At 0 °C, solutions A and B were mixed. The solution was adjusted with *N*-methylmorpholine to pH 8.5. The reaction mixture was stirred at room temperature for 8 h, and TLC ($CHCl_3$/CH_3OH, 10:1) indicated the complete disappearance of Boc-Asp-(OBzl)-OH. On evaporation, the residue was dissolved in 50 mL of ethyl acetate. The solution was washed successively with 5% sodium bicarbonate, 5% citric acid, and saturated sodium chloride and the organic phase was dried over anhydrous sodium sulfate. Filtration, evaporation under reduced pressure, and purification by chromatography ($CHCl_3$/CH_3OH, 30:1) provided 722 mg (94%) of the title compound as a colorless powder. ESI-MS (*m*/*e*) 591 $[M + H]^+$.

(2) HCl·H-Asp(OBzl)-Ser(Bzl)-OBzl. A solution of 500 mg (1.30 mmol) of Boc-Asp(OBzl)-Ser(Bzl)-OBzl in 3 mL of hydrogen chloride in ethyl acetate (4 mol/L) was stirred at room temperature for 2 h, and TLC ($CHCl_3$/CH_3OH, 10:1) indicated the complete disappearance of Boc-Asp(OBzl)-Ser(Bzl)-OBzl. The reaction mixture was evaporated to remove the solvent. The residue was dissolved in 10 mL of ethyl acetate, and the solution was evaporated to dryness. The resulting solid was used for a subsequent coupling reaction directly. ESI-MS (*m*/*e*) 591 $[M + H]^+$.

(3) Boc-Gly-Asp(OBzl)-Ser(Bzl)-OBzl. When the same procedure for preparing Boc-Asp(OBzl)-Ser(Bzl)-OBzl was used, from 227 mg (1.30 mmol) of Boc-Gly-OH and 767 mg (1.30 mmol) of HCl·H-Asp(OBzl)-Ser(Bzl)-OBzl, 765 mg (91%) of the title compound was obtained as a colorless powder. ESI-MS (*m*/*e*) 648 $[M + H]^+$.

(4) HCl·H-Gly-Asp(OBzl)-Ser(Bzl)-OBzl. When the same procedure for preparing HCl·H-Asp(OBzl)-Ser(Bzl)-OBzl was used, from 841 mg (1.30 mmol) of Boc-Gly-Asp(OBzl)-Ser(Bzl)-OBzl, 663 mg (93%) of the title compound was obtained. The resulting solid was used for a subsequent coupling reaction directly. ESI-MS (*m*/*e*) 548 $[M + H]^+$.

(5) Boc-Arg(NO_2)-Gly-Asp(OBzl)-Ser(Bzl)-OBzl. When the same procedure for preparing Boc-Asp(OBzl)-Ser(Bzl)-OBzl was used, from 415 mg (1.30 mmol) of Boc-Arg(NO_2)-OH and 758 mg (1.30 mmol) of HCl·H-Gly-Asp(OBzl)-Ser(Bzl)-OBzl, 982 mg (89%) of the title compound was obtained as a colorless powder. IR (KBr) 3350, 3345, 3323, 3031, 1681, 1600, 1503, 1462, 1445, 1393, 1370, 740, 696 cm^{-1}. ^1H NMR (DMSO-d_6) 8.951 (s, 1H), 8.401 (s, 1H), 8.237 (s, 1H), 8.110 (s, 1H), 8.010 (s, 1H), 7.350 (t, J = 7.86 Hz, 1H), 7.343 (t, J = 7.88 Hz, 1H), 7.337 (t, J = 7.84 Hz, 1H), 7.304 (t, J = 7.88 Hz, 2H), 7.293 (t, J = 7.88 Hz, 2H), 7.253 (t, J = 7.84 Hz, 2H), 7.246 (d, J = 7.84 Hz, 2H), 7.186 (d, J = 7.82 Hz, 2H), 7.175 (d, J = 7.82 Hz, 2H), 7.038 (s, 1H), 6.746 (s, 1H), 5.166 (s, 2H), 5.149 (s, 2H), 5.135 (s, 2H), 4.902 (dt, J = 6.31 Hz, J = 6.60 Hz, 1H), 4.714 (t, J = 6.50 Hz, 1H), 4.703 (t, J = 6.44 Hz, 1H), 3.967 (s, 2H), 3.771 (d, J = 6.44 Hz, 2H), 2.830 (d, J = 6.60 Hz, 2H), 2.627 (t, J = 6.48 Hz, 2H), 1.840 (m, J = 4.42 Hz, 2H), 1.627 (m, J = 6.36 Hz, 2H), 1.386 (s, 9H). ESI-MS (*m*/*e*) 849 $[M + H]^+$. Mp 72−74 °C. $[\alpha]^{20}_D$ 5.0 (*c* 1.0, $CHCl_3$/MeOH 10:1). AA analysis: calcd, Arg/Gly/Asp/Ser = 1.00:1.00:1.00:1.00; found, Arg/Gly/Asp/Ser = 0.98:1.00:1.04:0.97.

3.1.3. Boc-Arg(NO_2)-Gly-Asp(OBzl)-Val-OBzl. (1) Boc-Asp-(OBzl)-Val-OBzl. When the same procedure for preparing Boc-Asp(OBzl)-Ser(Bzl)-OBzl was used, from 420 mg (1.30 mmol) of Boc-Asp(OBzl)-OH and 315 mg (1.30 mmol) of HCl·H-Val-OBzl, 632 mg (95%) of the title compound was obtained as a colorless powder. ESI-MS (*m*/*e*) 513 $[M + H]^+$.

(2) HCl·H-Asp(OBzl)-Val-OBzl. When the same procedure for preparing HCl·H-Asp(OBzl)-Ser(Bzl)-OBzl was used, from 666 mg (1.30 mmol) of Boc-Asp(OBzl)-Val-OBzl, 505 mg (94%) of the title compound was obtained as a colorless powder. The resulting solid was used directly for a subsequent coupling reaction. ESI-MS (*m*/*e*) 413 $[M + H]^+$.

(3) Boc-Gly-Asp(OBzl)-Phe-OBzl. When the same procedure for preparing Boc-Asp(OBzl)-Ser(Bzl)-OBzl was used, from 227 mg (1.30 mmol) of Boc-Gly-OH and 582 mg (1.30 mmol) of HCl·H-Asp(OBzl)-Val-OBzl, 711 mg (96%) of the title compound was obtained as a colorless powder. Mp 77−79 °C; ESI-MS (*m*/*e*) 571 $[M + H]^+$.

(4) HCl·H-Gly-Asp(OBzl)-Val-OBzl. When the same procedure for preparing HCl·H-Asp(OBzl)-Ser(Bzl)-OBzl was used, from 741 mg (1.30 mmol) of Boc-Gly-Asp(OBzl)-Val-OBzl, 581 mg (95%) of the title compound was obtained. The resulting solid was used directly for a subsequent coupling reaction. ESI-MS (*m*/*e*) 471 $[M + H]^+$.

(5) Boc-Arg(NO_2)-Gly-Asp(OBzl)-Val-OBzl. When the same procedure for preparing Boc-Asp(OBzl)-Ser(Bzl)-OBzl was used, from 415 mg (1.30 mmol) of Boc-Arg(NO_2)-OH and 658 mg (1.30 mmol) of HCl·H-Gly-Asp(OBzl)-Val-OBzl, 901 mg (90%) of the title compound was obtained as a colorless powder. Mp 95−97 °C. IR (KBr) 3347, 3340, 3323, 3030, 1680, 1603, 1504, 1460, 1446, 1395, 1372, 747, 696 cm^{-1}. ^1H NMR (DMSO-d_6) 8.942 (s, 1H), 8.386 (s, 1H), 8.223 (s, 1H), 8.085 (s, 1H), 8.011 (s, 1H), 7.336 (t, J = 7.85 Hz, 1H), 7.330 (t, J = 7.86 Hz, 1H), 7.302 (t, J = 7.85 Hz, 2H), 7.290 (t, J = 7.86 Hz, 2H), 7.240 (d, J = 7.85 Hz, 2H), 7.184 (d, J = 7.86 Hz, 2H), 7.035 (s, 1H), 6.743 (s, 1H), 5.130 (s, 2H), 5.129 (s, 2H), 5.001 (dt, J = 6.30 Hz, J = 6.61 Hz, 1H), 4.887 (d, J = 6.48 Hz, 1H), 4.423 (d, J = 6.42 Hz, 1H), 4.104 (d, J = 4.70 Hz, 2H), 3.078 (m, J = 4.14 Hz, 1H), 2.833 (d, J = 6.62 Hz, 2H), 2.631 (t, J = 6.47 Hz, 2H), 1.843 (m, J = 4.44 Hz, 2H), 1.629 (m, J = 6.37 Hz, 2H),1.015 (d, J = 4.14 Hz, 6H), 1.388 (s, 9H). ESI-MS (*m*/*e*) 771 $[M + H]^+$. $[\alpha]^{20}_D$ 9.0 (*c* 1.0, $CHCl_3$/MeOH 10:1). AA analysis: calcd, Arg/Gly/Asp/Val = 1.00:1.00:1.00:1.00; found, Arg/Gly/Asp/Val = 0.97:1.00:0.98:0.98.

3.1.4. Boc-Arg(NO_2)-Gly-Asp(OBzl)-Phe-OBzl. (1) Boc-Asp-(OBzl)-Phe-OBzl. When the same procedure for preparing Boc-Asp(OBzl)-Ser(Bzl)-OBzl was used, from 420 mg (1.30 mmol) of Boc-Asp(OBzl)-OH and 376 mg (1.30 mmol) of HCl·H-Phe-OBzl, 626 mg (96%) of the title compound was obtained as a colorless powder. Mp 88−90 °C, ESI-MS (*m*/*e*) 561 $[M + H]^+$.

(2) HCl·H-Asp(OBzl)-Phe-OBzl. When the same procedure for preparing HCl·H-Asp(OBzl)-Ser(Bzl)-OBzl was used, from 728 mg (1.30 mmol) of Boc-Asp(OBzl)-Phe-OBzl, 571 mg (95%) of the title compound was obtained as a colorless powder. The resulting solid was used directly for subsequent coupling reaction. ESI-MS (*m*/*e*) 461 $[M + H]^+$.

(3) Boc-Gly-Asp(OBzl)-Val-OBzl. When the same procedure for preparing Boc-Asp(OBzl)-Ser(Bzl)-OBzl was used, from 227 mg (1.30 mmol) of Boc-Gly-OH and 645 mg (1.30 mmol) of HCl·H-Asp(OBzl)-Val-OBzl, 754 mg (94%) of the title compound was obtained as a colorless powder. Mp 72−74 °C. ESI-MS (*m*/*e*) 618 $[M + H]^+$.

(4) HCl·H-Gly-Asp(OBzl)-Phe-OBzl. When the same procedure for preparing HCl·H-Asp(OBzl)-Ser(Bzl)-OBzl was used, from 802 mg (1.30 mmol) of Boc-Gly-Asp(OBzl)-Phe-OBzl, 633 mg (94%) of the title compound was obtained as a colorless powder. The resulting solid was used directly for a subsequent coupling reaction. ESI-MS (*m*/*e*) 518 $[M + H]^+$.

(5) Boc-Arg(NO_2)-Gly-Asp(OBzl)-Phe-OBzl. When the same procedure for preparing Boc-Asp(OBzl)-Ser(Bzl)-OBzl was used, from 415 mg (1.30 mmol) of Boc-Arg(NO_2)-OH and 719 mg (1.30 mmol) of HCl·H-Gly-Asp(OBzl)-Phe-OBzl, 926 mg (87%) of the title compound was obtained as a colorless powder. Mp 148−150 °C. IR (KBr) 3350, 3342, 3326, 3030, 1682, 1601, 1500, 1463, 1444, 1392, 1374, 748, 702 cm^{-1}. ^1H NMR (DMSO-d_6) 8.933 (s, 1H), 8.375 (s, 1H), 8.222 (s, 1H), 8.084 (s, 1H), 8.001 (s, 1H), 7.338 (t, J = 7.83 Hz, 1H), 7.333 (t, J = 7.84 Hz, 1H), 7.328 (t, J = 7.82 Hz, 1H), 7.305 (t, J = 7.83 Hz, 2H), 7.290 (t, J = 7.82 Hz, 2H), 7.274 (t, J = 7.84 Hz, 2H), 7.240 (d, J = 7.84 Hz, 2H), 7.180 (d, J = 7.83 Hz, 2H), 7.176 (d, J = 7.82 Hz, 2H), 7.035 (s, 1H), 6.743 (s, 1H), 5.125 (s, 2H), 5.133 (s, 2H), 5.007 (dt, J = 6.30 Hz, J = 6.56 Hz, 1H), 4.880 (t, J = 6.51 Hz, 1H), 4.416 (t, J = 6.40 Hz, 1H), 4.100 (d, J = 4.70 Hz, 2H), 3.168 (d, J = 6.40 Hz, 2H), 2.827 (d, J = 6.50 Hz, 2H), 2.618 (t, J = 6.48 Hz, 2H), 1.844 (m, J = 4.45 Hz, 2H), 1.622 (m, J = 6.30 Hz, 2H), 1.374 (s, 9H). ESI-MS (*m*/*e*) 819 $[M + H]^+$. $[\alpha]^{20}_D$ 7.0 (*c* 1.0, $CHCl_3$/MeOH = 10:1). AA analysis: calcd, Arg/Gly/Asp/Phe = 1.00:1.00:1.00:1.00; found, Arg/Gly/Asp/Phe = 0.98:1.00:1.04:0.97.

3.1.5. General Procedure for the Removal of NO_2 and Bzl of the Protective Peptides. A suspension of 1.00 mmol of NO_2 and Bzl protected peptides, 25 mg of Pd/C (5%), and 30 mL of formic acid in methanol (4.4%) was agitated with hydrogen (0.02 Mpa) at room temperature for 24 h. The reaction mixture was filtrated. The filtrate was evaporated, the residue was triturated with ether, and the resulting solid was purified on a Sephadex G-10 column with water as the mobil phase. The collected fractions were lyophilized to provide the corresponding peptide.

3.1.6. H-Arg-Gly-Asp-Ser-OH. (1) HCl·H-Arg(NO_2)-Gly-Asp-(OBzl)-Ser(Bzl)-OBzl. When the general Boc deprotection procedure was used, from 848 mg (1.00 mmol) of Boc-Arg(NO_2)-Gly-Asp(OBzl)-Ser(Bzl)-OBzl, 710 mg (93%) of the title compound was obtained. ESI-MS (*m*/*e*) 739 $[M + H]^+$.

(2) H-Arg-Gly-Asp-Ser-OH (4). When the general procedure for catalytic hydrogenation of the protected peptides was used, from 774 mg (1.00 mmol) of HCl·H-Arg(NO_2)-Gly-Asp(OBzl)-Ser(Bzl)-OBzl, 399 mg (92%) of the title compound was obtained as a colorless powder. Mp 183−187 °C. IR (KBr) 3451, 3348, 3340, 3325, 2455, 1680 cm^{-1}. ^1H NMR (DMSO-d_6) 10.89 (s, 1H), 10.80 (s, 1H), 8.760 (s, 2H), 8.270 (s, 1H), 8.213 (s, 1H), 8.006 (s, 1H), 7.238 (s, 2H), 6.668 (s, 1H), 6.543 (s, 1H), 4.670 (t, J = 6.62 Hz, 1H), 4.528 (t, J = 6.52 Hz, 1H), 4.512 (t, J = 6.40 Hz, 1H), 4.113

(d, $J = 6.40$ Hz, 2H), 3.816 (s, 2H), 2.793 (d, $J = 6.40$ Hz, 2H), 2.633 (t, $J = 4.40$ Hz, 2H), 2.049 (s, 1H), 1.768 (m, $J = 4.40$ Hz, 2H), 1.605 (m, $J = 6.01$ Hz, 2H). ESI-MS (*m*/*e*) 434 $[M + H]^+$. $[\alpha]^{20}_D$ 6.0 (*c* 1.0, H_2O). AA analysis: calcd, Arg/Gly/Asp/Ser = 1.00:1.00:1.00:1.00; found, Arg/Gly/Asp/Ser = 0.96:1.00:1.05:0.99.

3.1.7. H-Arg-Gly-Asp-Val-OH. (1) HCl·H-Arg(NO_2)-Gly-Asp-(OBzl)-Val-OBzl. When the general Boc deprotection procedure was used, from 770 mg (1.00 mmol) of Boc-Arg(NO_2)-Gly-Asp-(OBzl)-Val-OBzl, 664 mg (94%) of the title compound was obtained. ESI-MS (*m*/*e*) 671 $[M + H]^+$.

(2) H-Arg-Gly-Asp-Val-OH (5). When the general procedure for catalytic hydrogenation of the protected peptides was used, from 706 mg (1.00 mmol) of HCl·H-Arg(NO_2)-Gly-Asp(OBzl)-Val-OBzl, 396 mg (89%) of the title compound was obtained. Mp 180−182 °C. IR (KBr) 3349, 3337, 3330, 2430, 1682, 1390, 1372 cm^{-1}. ^1H NMR (DMSO-d_6) 10.899 (s, 1H), 10.880 (s, 1H), 8.661 (s, 2H), 8.299 (s, 1H), 8.217 (s, 1H), 8.053 (s, 1H), 7.234 (s, 2H), 6.700 (s, 1H), 6.537 (s, 1H), 5.003 (d, $J = 6.59$ Hz, 1H), 4.837 (d, $J = 6.46$ Hz, 1H), 4.395 (d, $J = 6.40$ Hz, 1H), 3.997 (d, $J = 4.73$ Hz, 2H), 2.891 (m, $J = 6.37$ Hz, 1H), 2.782 (d, $J = 6.40$ Hz, 2H), 2.628 (t, $J = 6.45$ Hz, 2H), 1.763 (m, $J = 6.44$ Hz, 2H), 1.604 (m, $J = 6.35$ Hz, 2H), 1.009 (d, $J = 6.37$ Hz, 6H). ESI-MS (*m*/*e*) 446 $[M + H]^+$. $[\alpha]^{20}_D$ 3.0 (*c* 1.0, H_2O). AA analysis: calcd, Arg/Gly/Asp/Val = 1.00:1.00:1.00:1.00; found, Arg/Gly/Asp/Val = 0.96:1.00:0.98:0.97.

3.1.8. H-Arg-Gly-Asp-Phe-OH. (1) HCl·H-Arg(NO_2)-Gly-Asp(OBzl)-Phe-OBzl. When the general Boc deprotection procedure was used, from 834 mg (1.00 mmol) of Boc-Arg(NO_2)-Gly-Asp(OBzl)-Phe-OBzl, 700 mg (93%) of the title compound was obtained. ESI-MS (*m*/*e*) 718 $[M + H]^+$.

(2) H-Arg-Gly-Asp-Phe-OH (6). When the general procedure for catalytic hydrogenation of the protected peptides was used, from 734 mg (1.00 mmol) of Boc-Arg(NO_2)-Gly-Asp(OBzl)-Phe-OBzl, 454 mg (92%) of the title compound was obtained. Mp 158−160 °C. IR (KBr) 3353, 3345, 3322, 2422, 1684, 1601, 1500, 1460, 1450, 700 cm^{-1}. ^1H NMR (DMSO-d_6) 10.975 (s, 1H), 10.901 (s, 1H), 8.620 (s, 2H), 8.251 (s, 1H), 8.174 (s, 1H), 8.032 (s, 1H), 7.312 (s, 2H), 7.227 (t, $J = 7.80$ Hz, 2H), 7.156 (d, $J = 7.80$ Hz, 2H), 7.098 (t, $J = 7.80$ Hz, 1H), 6.927 (s, 1H), 6.746 (s, 1H), 4.968 (t, $J = 6.47$ Hz, 1H), 4.815 (d, $J = 6.40$ Hz, 1H), 4.433 (t, $J = 6.42$ Hz, 1H), 4.102 (d, $J = 4.70$ Hz, 2H), 3.009 (d, $J = 6.42$ Hz, 2H), 2.801 (d, $J = 6.47$ Hz, 2H), 2.614 (t, $J = 6.45$ Hz, 2H), 1.825 (m, $J = 5.99$ Hz, 2H), 1.617 (m, $J = 6.46$ Hz, 2H). ESI-MS (*m*/*e*) 494 $[M + H]^+$. $[\alpha]^{20}_D$ 4.0 (*c* 2.0, H_2O). AA analysis: calcd, Arg/Gly/Asp/Phe = 1.00:1.00:1.00:1.00; found, Arg/Gly/Asp/Phe = 0.96:1.00:0.98:0.98.

3.1.9. Estradiol-17-β-O-carbonylpropionic Acid (7). To a warm solution of 100 mg (1.0 mmol) of succinic anhydride in 1 mL of pyridine, 100 mg (0.37 mmol) of estradiol was added. The reaction mixture was stirred at 90 °C for 10 h and TLC (chloroform/methanol/acetic acid, 20:1:0.4) indicated complete disappearance of estradiol. The reaction mixture was cooled to room temperature, mixed with 20 mL of ice water and 75 mg of sodium chloride, and stirred vigorously. The mixture was then extracted with ethyl acetate and the ethyl acetate phase was separated and dried with anhydrous Na_2SO_4. After filtration, the filtrate was evaporation at reduced pressure to give a yellow syrup, which was dissolved in 1 mL of methanol. The solution was adjusted to pH 8.5−9.0 with cold aqueous K_2CO_3 (10%) and stirred at room temperature for 18 h. The solution was adjusted to a pH of 6.0 with acetic acid (50%) and evaporated at reduced pressure. The residue was mixed with 2 mL of ice water and 75 mg of sodium chloride. The mixture was extracted with 50 mL of ethyl acetate, and the ethyl acetate phase was separated. After washing with ice water three times and drying with anhydrous Na_2SO_4, the ethyl acetate phase was evaporated to give a yellowish syrup. The syrup was kept in a refrigerater for 18 h to give 137 mg (95%) of the title compound as a colorless powder. Mp 148−150 °C. IR (KBr) 3253, 3035, 2867, 1742, 1602, 1504, 1463, 1372, 876, 831 cm^{-1}. ^1H NMR (DMSO-d_6) 12.192 (s, 1H), 8.993 (s, 1H), 7.043 (d, $J = 7.50$ Hz, 1H), 6.517 (d, $J = 7.50$ Hz, 1H), 6,400 (s,1H), 4.618 (t, $J = 4.78$ Hz, 1H), 2.972 (t, $J = 4.55$ Hz, 2H), 2.910 (m, $J = 4.22$ Hz, 1H), 2.883 (t, $J = 5.11$ Hz, 2H), 2.780 (t, $J = 5.11$ Hz, 2H), 2.028 (m, $J = 4.78$ Hz, 2H), 2.015 (m, $J = 4.28$ Hz, 2H), 1.871 (m, $J = 4.20$ Hz, 1H), 1.840 (m, $J = 4.42$ Hz, 2H), 1.761 (m, $J = 4.55$ Hz, 2H), 1.703 (m, $J = 4.282$ Hz, 1H), 1.654 (m, $J = 4.72$ Hz, 2H), 0.774 (s, 3H). ESI-MS(*m*/*e*) 373 $[M + H]^+$. $[\alpha]^{20}_D$ 35.0 (*c* 1.00, THF). Anal. Calcd for $C_{22}H_{28}O_5$: C, 70.94; H, 7.58. Found: C, 71.10; H, 7.69.

3.1.10. Estradiol-17-β-O-carbonylpropionyl-Arg(NO_2)-Gly-Asp(OBzl)-Ser(Bzl)-OBzl (8). At 0 °C, to the solution of 88 mg (0.24 mmol) of estradiol-17-β-O-carbonylpropionic acid and 32 mg (0.24 mmol) of HOBt in 5 mL of anhydrous THF, 49 mg (0.24 mmol) of DCC was added. The mixture was stirred at 0 °C for 30 min and then the solution of 185 mg (0.24 mmol) of HCl·H-Arg(NO_2)-Gly-Asp(OBzl)-Ser(Bzl)-OBzl was added. The reaction mixture was adjusted to pH 8.0 and stirred at room temperature for 8 h, and TLC (ethyl acetate/petroleum, 6:1) indicated complete disappearance of HCl·H-Arg(NO_2)-Gly-Asp(OBzl)-Ser(Bzl)-OBzl. The reaction mixture was filtered, and the filtrate was evaporated under vacuum. The residue was dissolved in 20 mL of chloroform and washed with citric acid aqueous solution (5%), sodium bicarbonate aqueous solution (5%), and saturated NaCl aqueous solution succesively, then dried with anhydrous Na_2SO_4. After filtration and evaporation, the residue was purified by flash chromatography (chloroform/methanol, 10:1) to give 158 mg (61%) of the title compound as a colorless powder. Mp 143−145 °C. IR (KBr) 3353, 3342, 3325, 3084, 3070, 3033, 1745, 1687, 1603, 1501, 1460, 1445, 1397, 1368, 742, 694 cm^{-1}. ^1H NMR (DMSO-d_6) 8.942 (s, 1H), 8.389 (s, 1H), 8.223 (s, 1H), 8.087 (s, 1H), 8.004 (s, 1H), 7.344 (t, $J = 7.88$ Hz, 1H), 7.341 (t, $J = 7.90$ Hz, 1H), 7.334 (t, $J = 7.86$ Hz, 1H), 7.301 (t, $J = 7.90$ Hz, 2H), 7.291 (t, $J = 7.90$ Hz, 2H), 7.250 (t, $J = 7.86$ Hz, 2H), 7.243 (d, $J = 7.86$ Hz, 2H), 7.185 (d, $J = 7.84$ Hz, 2H), 7.172 (d, $J = 7.84$ Hz, 2H), 7.046 (d, $J = 7.52$ Hz, 1H), 7.035 (s, 1H), 6.744 (s, 1H), 6.515 (d, $J = 7.51$ Hz, 1H), 6.403 (s, 1H), 6.372 (s, 1H), 5.164 (s, 2H), 5.147 (s, 2H), 5.132 (s, 2H), 4.900 (dt, $J = 6.30$ Hz, $J = 6.62$ Hz, 1H), 4.711 (t, $J = 6.54$ Hz, 1H), 4.700 (t, $J = 6.47$ Hz, 1H), 4.612 (t, $J = 4.76$ Hz, 1H), 3.964 (d, $J = 6.02$ Hz, 2H), 3.768 (d, $J = 4.12$ Hz, 2H), 2.974 (t, $J = 4.56$ Hz, 2H), 2.915 (m, $J = 4.25$ Hz, 1H), 2.880 (t, $J = 5.16$ Hz, 2H), 2.824 (d, $J = 6.62$ Hz, 2H), 2.783 (t, $J = 5.13$ Hz, 2H), 2.624 (t, $J = 6.50$ Hz, 2H), 2.024 (m, $J = 4.76$ Hz, 2H), 2.017 (m, $J = 4.31$ Hz, 2H), 1.874 (m, $J = 4.22$ Hz, 1H), 1.843 (m, $J = 4.40$ Hz, 2H), 1.765 (m, $J = 4.52$ Hz, 2H), 1.723 (m, $J = 6.32$ Hz, 2H), 1.700 (m, $J = 4.28$ Hz, 1H), 1.656 (m, $J = 4.70$ Hz, 2H), 1.625 (m, $J = 6.38$ Hz, 2H), 0.776 (s, 3H). $[\alpha]^{20}_D$ 20.0 (*c* 1.00, $CHCl_3$/MeOH, 10:1). ESI-MS 1103 $[M + H]^+$. Anal. Calcd for $C_{58}H_{70}N_8O_{14}$: C, 63.14; H, 6.40; N, 10.16. Found: C, 63.40; H, 6.58; N, 10.00. AA analysis: calcd, Arg/Gly/Asp/Ser = 1.00:1.00:1.00:1.00; found, Arg/Gly/Asp/Ser = 0.97:1.00:1.07:0.98.

3.1.11. Estradiol-17-β-O-carbonylpropionyl-Arg(NO_2)-Gly-Asp(OBzl)-Val-OBzl (9). When the same procedure as that used in the preparation of **8** was used, from 121 mg (0.33 mmol) of estradiol-17-β-O-carbonylpropinic acid and 230 mg (0.33 mmol) of HCl·H-Arg(NO_2)-Gly-Asp(OBzl)-Val-OBzl, 177 mg (53%) of the title compound was obtained as a colorless powder. Mp 151−153 °C. IR (KBr) 3350, 3344, 3326, 3087, 3071, 3032, 1746, 1684, 1601, 1500, 1462, 1448, 1386, 1370, 746, 698 cm^{-1}. ^1H NMR (DMSO-d_6) 8.940 (s, 1H), 8.385 (s, 1H), 8.220 (s, 1H), 8.083 (s, 1H), 8.007 (s, 1H), 7.338 (t, $J = 7.87$ Hz, 1H), 7.332 (t, $J = 7.88$ Hz, 1H), 7.304 (t, $J = 7.87$ Hz, 2H), 7.294 (t, $J = 7.86$ Hz, 2H), 7.241 (d, $J = 7.87$ Hz, 2H), 7.186 (d, $J = 7.85$ Hz, 2H), 7.044 (d, $J = 7.54$ Hz, 1H), 7.037 (s, 1H), 6.746 (s, 1H), 6.517 (d, $J = 7.53$ Hz, 1H), 6.401 (s, 1H), 6.370 (s, 1H), 5.127 (s, 2H), 5.135 (s, 2H), 5.007 (dt, $J = 6.32$ Hz, $J = 6.60$ Hz, 1H), 4.892 (d, $J = 6.51$ Hz, 1H), 4.534 (d, $J = 6.50$ Hz, 1H), 4.421 (d, $J = 6.45$ Hz, 1H), 4.106 (d, $J = 4.73$ Hz, 2H), 3.071 (m, $J = 4.12$ Hz, 1H), 2.970 (t, $J = 4.54$ Hz, 2H), 2.911 (m, $J = 4.26$ Hz, 1H), 2.882 (t, $J = 5.15$ Hz, 2H), 2.825 (d, $J = 6.60$ Hz, 2H), 2.784 (t, $J = 5.14$ Hz, 2H), 2.620 (t, $J = 6.51$ Hz, 2H), 2.025 (m, $J = 4.75$ Hz, 2H), 2.014 (m, $J = 4.30$ Hz, 2H), 1.870 (m, $J = 4.20$ Hz, 1H), 1.844 (m, $J = 4.42$ Hz, 2H), 1.763 (m, $J = 4.50$ Hz, 2H),

1.724 (m, J = 6.33 Hz, 2H), 1.702 (m, J = 4.29 Hz, 1H), 1.653 (m, J = 4.72 Hz, 2H), 1.621 (m, J = 6.35 Hz, 2H), 1.012 (d, J = 4.12 Hz, 6H), 0.779 (s, 3H). Anal. Calcd for $C_{53}H_{68}N_8O_{13}$: C, 62.09; H, 6.69; N, 10.93. Found: C, 61.92; H, 6.51; N, 11.12. AA analysis: calcd, Arg/Gly/Asp/Val = 1.00:1.00:1.00:1.00; found, Arg/Gly/Asp/Val = 0.98:1.00:0.97:0.98. $[\alpha]^{20}_D$ 30.0 (*c* 1.00, $CHCl_3$/MeOH, 10:1). ESI-MS 1025 $[M + H]^+$.

3.1.12. Estradiol-17-β-O-carbonylpropionyl-Arg(NO_2)-Gly-Asp(OBzl)-Phe-OBzl (10). When the same procedure as that used in the preparation of **8** was used, from 39 mg (0.11 mmol) of estradiol-17-β-O-carbonylpropionic acid and 78 mg (0.11 mmol) of HCl•H-Arg(NO_2)-Gly-Asp(OBzl)-Phe-OBzl, 65 mg (53%) of the title compound was obtained as a colorless powder. Mp 156−158 °C. IR (KBr) 3352, 3345, 3323, 3084, 3068, 3030, 1744, 1680, 1603, 1502, 1465, 1446, 1390, 1364, 745, 701 cm^{-1}. ^1H NMR (DMSO-d_6) 8.935 (s, 1H), 8.377 (s, 1H), 8.225 (s, 1H), 8.087 (s, 1H), 8.002 (s, 1H), 7.340 (t, J = 7.85 Hz, 1H), 7.336 (t, J = 7.86 Hz, 1H), 7.330 (t, J = 7.84 Hz, 1H), 7.307 (t, J = 7.85 Hz, 2H), 7.292 (t, J = 7.84 Hz, 2H), 7.272 (t, J = 7.80 Hz, 2H), 7.243 (d, J = 7.86 Hz, 2H), 7.184 (d, J = 7.83 Hz, 2H), 7.177 (d, J = 7.80 Hz, 2H), 7.040 (d, J = 7.55 Hz, 1H), 7.032 (s, 1H), 6.741 (s, 1H), 6.509 (d, J = 7.51 Hz, 1H), 6.403 (s, 1H), 6.368 (s, 1H), 5.124 (s, 2H), 5.131 (s, 2H), 5.011 (dt, J = 6.33 Hz, J = 6.58 Hz, 1H), 4.886 (t, J = 6.50 Hz, 1H), 4.530 (t, J = 6.51 Hz, 1H), 4.417 (t, J = 6.42 Hz, 1H), 4.101 (d, J = 4.72 Hz, 2H), 3.165 (d, J = 6.45 Hz, 2H), 2.967 (t, J = 4.55 Hz, 2H), 2.907 (m, J = 4.27 Hz, 1H), 2.879 (t, J = 5.14 Hz, 2H), 2.822 (d, J = 6.55 Hz, 2H), 2.780 (t, J = 5.15 Hz, 2H), 2.611 (t, J = 6.50 Hz, 2H), 2.027 (m, J = 4.73 Hz, 2H), 2.017 (m, J = 4.32 Hz, 2H), 1.869 (m, J = 4.22 Hz, 1H), 1.843 (m, J = 4.43 Hz, 2H), 1.760 (m, J = 4.52 Hz, 2H), 1.725 (m, J = 6.30 Hz, 2H), 1.700 (m, J = 4.27 Hz, 1H), 1.655 (m, J = 4.70 Hz, 2H), 1.617 (m, J = 6.32 Hz, 2H), 0.824 (s, 3H). $[\alpha]^{20}_D$ 23.0 (*c* 1.00, $CHCl_3$/MeOH, 10:1). ESI-MS 1073 $[M + H]^+$. Anal. Calcd for $C_{57}H_{68}N_8O_{13}$: C, 63.79; H, 6.39; N, 10.44. Found: C, 63.52; H, 6.23; N, 10.61. AA analysis: calcd, Arg/Gly/Asp/Phe = 1.00:1.00:1.00:1.00; found, Arg/Gly/Asp/Phe = 0.97:1.00:1.05:0.98.

3.1.13. Estradiol-17-β-O-carbonylpropionyl-Arg-Gly-Asp-Ser-OH (11). A suspension of 100 mg (0.091 mmol) of estradiol-17-β-O-carbonylpropionyl-Arg(NO_2)-Gly-Asp(OBzl)-Ser(Bzl)-OBzl (**8**), 15 mg of Pd/C (10%), 3 mL of methanol, and 1 mL of THF were stirred at room temperature. To this stirred suspension, hydrogen was bubbled for 24 h and TLC (ethyl acetate/petroleum, 6:1) indicated complete disappearance of **8**. The reaction mixture was filtered, and the filtrate was evaporated under vacuum. The residue was triturated with ether and the colorless powder was purified on a Sephadex LH 20 column (10−30% ethanol). The desirable fraction was evaporated under vacuum to give 44 mg (62%) of the title compound as a colorless powder. Mp 178−180 °C. IR (KBr) 3440, 3351, 3345, 3323, 3031, 1693, 1604, 1506, 1465, 1442, 746, 700 cm^{-1}. ^1H NMR (DMSO-d_6) 9.072 (s, 1H), 9.068 (s, 1H), 9.063 (s, 1H), 8.043 (s, 1H), 8.035 (s, 1H), 8.017 (s, 1H), 8.004 (s, 2H), 7.241 (s, 2H), 7.044 (d, J = 7.50 Hz, 1H), 7.039 (s, 1H), 6.751 (s, 1H), 6.518 (d, J = 7.50 Hz, 1H), 6.407 (s, 1H), 4.854 (dt, J = 6.32 Hz, J = 6.60 Hz, 1H), 4.683 (t, J = 6.53 Hz, 1H), 4.603 (t, J = 4.74 Hz, 1H), 4.590 (t, J = 6.45 Hz, 1H), 4.021 (d, J = 6.44 Hz, 2H), 3.960 (d, J = 6.00 Hz, 2H), 2.972 (t, J = 4.55 Hz, 2H), 2.913 (m, J = 4.26 Hz, 1H), 2.879 (t, J = 5.15 Hz, 2H), 2.820 (d, J = 6.60 Hz, 2H), 2.778 (t, J = 5.12 Hz, 2H), 2.602 (t, J = 6.52 Hz, 2H), 2.025 (m, J = 4.75 Hz, 2H), 2.016 (m, J = 4.32 Hz, 2H), 1.870 (m, J = 4.23 Hz, 1H), 1.841 (m, J = 4.42 Hz, 2H), 1.763 (m, J = 4.53 Hz, 2H), 1.721 (m, J = 6.30 Hz, 2H), 1.702 (m, J = 4.27 Hz, 1H), 1.653 (m, J = 4.72 Hz, 2H), 1.622 (m, J = 6.35 Hz, 2H), 0.773 (s, 3H). $[\alpha]^{20}_D$ 50.0 (*c* 1.00, MeOH). ESI-MS 788 $[M + H]^+$. Anal. Calcd for $C_{37}H_{53}N_7O_{12}$: C, 56.41; H, 6.78; N, 12.44. Found: C, 56.60; H, 6.92; N, 12.26. AA analysis: calcd, Arg/Gly/Asp/Ser = 1.00:1.00:1.00:1.00; found, Arg/Gly/Asp/Ser = 1.02:1.00:1.01:0.97.

3.1.14. Estradiol-17-β-O-carbonylpropinyl-Arg-Gly-Asp-Val-OH (12). When the same procedure as that used in the preparation of **11** was used, from 100 mg (0.098 mmol) of estradiol-17-β-O-carbonylpropinyl-Arg(NO_2)-Gly-Asp(OBzl)-Val-OBzl (**9**), 54 mg (68%) of the title compound was obtained as a colorless powder. Mp 193−195 °C. IR (KBr) 3360, 3357, 3348, 3030, 1689, 1603, 1501, 1460, 1445, 1382, 1373, 749, 703 cm^{-1}. ^1H NMR (DMSO-d_6) 9.155 (s, 1H), 9.127 (s, 1H), 9.032 (s, 1H), 8.226 (s, 1H), 8.203 (s, 1H), 8.075 (s, 1H), 8.002 (s, 1H), 7.225 (s, 2H), 7.040 (d, J = 7.54 Hz, 1H), 7.039 (s, 1H), 6.722 (s, 1H), 6.512 (s, 1H), 6.449 (d, J = 7.50 Hz, 1H), 4.867 (d, J = 6.52 Hz, 1H), 4.611 (t, J = 6.32 Hz, 1H), 4.532 (d, J = 6.51 Hz, 1H), 4.423 (d, J = 6.43 Hz, 1H), 4.101 (d, J = 4.74 Hz, 2H), 2.962 (t, J = 4.56 Hz, 2H), 2.920 (m, J = 4.13 Hz, 1H), 2.903 (m, J = 4.27 Hz, 1H), 2.880 (t, J = 5.14 Hz, 2H), 2.822 (d, J = 6.57 Hz, 2H), 2.780 (t, J = 5.15 Hz, 2H), 2.614 (t, J = 6.50 Hz, 2H), 2.027 (m, J = 4.76 Hz, 2H), 2.016 (m, J = 4.32 Hz, 2H), 1.866 (m, J = 4.21 Hz, 1H), 1.845 (m, J = 4.43 Hz, 2H), 1.760 (m, J = 4.51 Hz, 2H), 1.720 (m, J = 6.30 Hz, 2H), 1.700 (m, J = 4.29 Hz, 1H), 1.655 (m, J = 4.70 Hz, 2H), 1.617 (m, J = 6.34 Hz, 2H), 0.862 (d, J = 4.15 Hz, 6H), 0.754 (s, 3H). Anal. Calcd for $C_{39}H_{57}N_7O_{11}$: C, 58.56; H, 7.18; N, 12.26. Found: C, 58.75; H, 7.32; N, 12.45. AA analysis: calcd, Arg/Gly/Asp/Val = 1.00:1.00:1.00:1.00; found, Arg/Gly/Asp/Val = 0.99:1.00:1.03:0.97. $[\alpha]^{20}_D$ 56.0 (*c* 0.5, MeOH). ESI-MS (*m*/*e*) 800 $[M + H]^+$.

3.1.15. Estradiol-17-β-O-carbonylpropionyl-Arg-Gly-Asp-Phe-OH(13). When the same procedure as that used in the preparation of **11** was used, from 100 mg (0.093 mmol) of estradiol-17-β-O-carbonylpropionyl-Arg(NO_2)-Gly-Asp(OBzl)-Phe-OBzl (**10**), 46 mg (58%) of the title compound was obtained as a colorless powder. Mp 196−198 °C. IR (KBr) 3391, 3382, 3377, 3032, 1681, 1605, 1505, 1462, 743, 700 cm^{-1}. ^1H NMR (DMSO-d_6) 9.065 (s, 1H), 9.054 (s, 1H), 9.045 (s, 1H), 8.061 (s, 1H), 8.035 (s, 1H), 8.020 (s, 1H), 8.001 (s, 2H), 7.233 (s, 2H), 7.116 (t, J = 7.81 Hz, 2H), 7.075 (d, J = 7.80 Hz, 2H), 7.010 (t, J = 7.79 Hz, 1H), 7.006 (s, 1H), 6.955 (d, J = 7.56 Hz, 1H), 6.900 (s, 1H), 6.432 (d, J = 7.50 Hz, 1H), 4.910 (dt, J = 6.30 Hz, J = 6.55 Hz, 1H), 4.870 (t, J = 6.52 Hz, 1H), 4.846 (t, J = 6.50 Hz, 1H), 4.500 (t, J = 6.43 Hz, 1H), 4.122 (d, J = 4.70 Hz, 2H), 3.042 (d, J = 6.43 Hz, 2H), 2.963 (t, J = 4.53 Hz, 2H), 2.910 (m, J = 4.28 Hz, 1H), 2.872 (t, J = 5.16 Hz, 2H), 2.820 (d, J = 6.56 Hz, 2H), 2.776 (t, J = 5.16 Hz, 2H), 2.593 (t, J = 6.52 Hz, 2H), 2.024 (m, J = 4.70 Hz, 2H), 2.014 (m, J = 4.34 Hz, 2H), 1.865 (m, J = 4.24 Hz, 1H), 1.840 (m, J = 4.42 Hz, 2H), 1.755 (m, J = 4.53 Hz, 2H), 1.720 (m, J = 6.32 Hz, 2H), 1.702 (m, J = 4.26 Hz, 1H), 1.656 (m, J = 4.71 Hz, 2H), 1.613 (m, J = 6.30 Hz, 2H), 0.821 (s, 3H). $[\alpha]^{20}_D$ 45.0 (*c* 0.5, MeOH). ESI-MS (*m*/*e*) 848 $[M + H]^+$. Anal. Calcd for $C_{43}H_{57}N_7O_{11}$: C, 60.91; H, 6.78; N, 11.56. Found: C, 61.10; H, 6.96; N, 11.37. AA analysis: calcd, Arg/Gly/Asp/Phe = 1.00:1.00:1.00:1.00; found, Arg/Gly/Asp/Phe = 0.98:1.00:1.03:0.99.

3.1.16. Ethyl Estradiol-3-oxyacetate (14). A solution of 500 mg (1.85 mmol) of estradiol in 10 mL of anhydrous THF and 1.32 mL of sodium ethoxide in anhydrous ethanol (2 mmol/L) was mixed and stirred at room temperature for 30 min, and then 0.612 mL (5 mmol) of ethyl bromoacetate was added. The mixture was stirred at 55 °C for 16 h and TLC (ethyl acetate/petroleum, 6:1) indicated complete disappearance of estradiol. The reaction mixture was filtered, and the filtrate was evaporated under vacuum. The residue was dissolved in 15 mL of ethyl acetate and evaporated under vacuum, which was repeated four times to completely remove the residual ethyl bromoacetate. The residue was purified by flash chromatography (chloroform/ether, 10:0.3) to provide 550 mg (83%) of the title compound as a colorless powder. Mp 88−90 °C. IR (KBr) 3352, 3031, 2864, 1745, 1604, 1502, 1464, 1370, 874, 832 cm^{-1}. ^1H NMR (DMSO-d_6) 7.121 (d, J = 7.52 Hz, 1H), 6.710 (s, 1H), 6.523 (d, J = 7.52 Hz, 1H), 5.141 (s, 2H), 4.613 (q, J = 4.77 Hz, 1H), 4.203 (q, J = 4.44 Hz, 2H), 2.974 (t, J = 4.56 Hz, 2H), 2.907 (m, J = 4.24 Hz, 1H), 2.551 (s, 1H), 2.025 (m, J = 4.76 Hz, 2H), 2.013 (m, J = 4.26 Hz, 2H), 1.867 (m, J = 4.22 Hz, 1H), 1.842 (m, J = 4.43 Hz, 2H), 1.757 (m, J = 4.56 Hz, 2H), 1.704 (m, J = 4.28 Hz, 1H), 1.653 (m, J = 4.73 Hz, 2H), 1.411 (t, J = 4.44 Hz, 3H), 0.781 (s, 3H). ESI-MS(*m*/*e*) 359 $[M + H]^+$. $[\alpha]^{20}_D$ 60.0 (*c* 1.00, THF). Anal. Calcd for $C_{22}H_{30}O_4$: C, 73.71; H, 8.44. Found: C, 73.58; H, 8.61.

3.1.17. Estradiol-3-oxyacetic acid (15). At 0 °C to a solution of 450 mg (1.26 mmol) of 3-ethyloxycarbonylmethylenoxylestradiol (**14**) in 3 mL of anhydrous ethanol, 0.5 mL of NaOH aqueous solution (2 mol/L) was added. The reaction mixture was stirred at room temperature for 2.5 h and TLC (ethyl acetate/petroleum, 6:1) indicated complete disappearance of **14**. The pH of the reaction mixture was adjusted to 2 by adding $KHSO_4$ powder. The reaction mixture was extracted by ethyl acetate, and the ethyl acetate phase was dried over anhydrous Na_2SO_4. After filtration, the filtrate was evaporated under vacuum to provide 397 mg (96%) of the title compound as a colorless powder. Mp 214−215 °C. IR (KBr) 3350, 3033, 2866, 1726, 1602, 1501, 1463, 1372, 875, 831 cm^{-1}. ^1H NMR (DMSO-d_6) 10.551 (s, 1H), 7.001 (d, J = 7.50 Hz, 1H), 6.631 (s, 1H), 6.511 (d, J = 7.51 Hz, 1H), 5.002 (s, 2H), 4.415 (q, J = 4.76 Hz, 1H), 2.966 (t, J = 4.55 Hz, 2H), 2.889 (m, J = 4.25 Hz, 1H), 2.560 (s, 1H), 2.020 (m, J = 4.75 Hz, 2H), 2.010 (m, J = 4.25 Hz, 2H), 1.862 (m, J = 4.23 Hz, 1H), 1.837 (m, J = 4.44 Hz, 2H), 1.750 (m, J = 4.55 Hz, 2H), 1.700 (m, J = 4.25 Hz, 1H), 1.647 (m, J = 4.74 Hz, 2H), 0.783 (s, 3H). ESI-MS (*m*/*e*) 331 $[M + H]^+$. $[\alpha]^{20}_D$ 50.0 (*c* 0.60, THF). Anal. Calcd for $C_{20}H_{26}O_4$: C, 72.70; H, 7.93. Found: C, 72.54; H, 8.11.

3.1.18. *N*-(Estradiol-3-oxyacetyl)-Arg(NO_2)-Gly-Asp(OBzl)-Ser(Bzl)-OBzl (16). When the same procedure as that used in the preparation of **8** was used, from 50 mg (0.15 mmol) of estradiol-3-oxyacetic acid and 119 mg (0.15 mmol) of HCl·H-Arg(NO_2)-Gly- Asp(OBzl)-Ser(Bzl)-OBzl, 150 mg (93.2%) of the title compound was obtained as a colorless powder. Mp 114−116 °C. IR (KBr) 3355, 3340, 3322, 3086, 3074, 3031, 1748, 1683, 1601, 1500, 1462, 1443, 1395, 1366, 741, 692 cm^{-1}. ^1H NMR (DMSO-d_6) 8.382 (s, 1H), 8.220 (s, 1H), 8.083 (s, 1H), 8.005 (s, 1H), 7.346 (t, J = 7.85 Hz, 1H), 7.342 (t, J = 7.88 Hz, 1H), 7.336 (t, J = 7.84 Hz, 1H), 7.303 (t, J = 7.85 Hz, 2H), 7.292 (t, J = 7.88 Hz, 2H), 7.254 (t, J = 7.84 Hz, 2H), 7.245 (d, J = 7.85 Hz, 2H), 7.186 (d, J = 7.88 Hz, 2H), 7.175 (d, J = 7.86 Hz, 2H), 7.123 (d, J = 7.51 Hz, 1H), 7.037 (s, 1H), 6.708 (s, 1H), 6.522 (d, J = 7.51 Hz, 1H), 6.415 (s, 1H), 6.374 (s, 1H), 5.166 (s, 2H), 5.143 (s, 2H), 5.135 (s, 2H), 5.006 (s, 2H), 4.902 (dt, J = 6.32 Hz, J = 6.60 Hz, 1H), 4.713 (t, J = 6.53 Hz, 1H), 4.705 (t, J = 6.46 Hz, 1H), 4.615 (t, J = 4.78 Hz, 1H), 3.965 (d, J = 6.00 Hz, 2H), 3.769 (d, J = 4.15 Hz, 2H), 2.970 (t, J = 4.55 Hz, 2H), 2.912 (m, J = 4.26 Hz, 1H), 2.785 (t, J = 5.15 Hz, 2H), 2.627 (t, J = 6.52 Hz, 2H), 2.553 (s, 1H), 2.029 (m, J = 4.74 Hz, 2H), 2.014 (m, J = 4.33 Hz, 2H), 1.866 (m, J = 4.25 Hz, 1H), 1.841 (m, J = 4.42 Hz, 2H), 1.756 (m, J = 4.53 Hz, 2H), 1.727 (m, J = 6.34 Hz, 2H), 1.704 (m, J = 4.27 Hz, 1H), 1.652 (m, J = 4.72 Hz, 2H), 1.629 (m, J = 6.36 Hz, 2H), 0.782 (s, 3H). $[\alpha]^{20}_D$ 55.0 (*c* 0.5, MeOH). ESI-MS 1061 $[M + H]^+$. Anal. Calcd for $C_{56}H_{68}N_8O_{13}$: C, 63.38; H, 6.46; N, 10.56. Found: C, 63.22; H, 6.29; N, 10.71. AA analysis: calcd, Arg/Gly/Asp/Ser = 1.00:1.00: 1.00:1.00; found, Arg/Gly/Asp/Ser = 0.99:1.00:1.02:0.97.

3.1.19. *N*-(Estradiol-3-oxyacetyl)-Arg(NO_2)-Gly-Asp(OBzl)-Val-OBzl (17). When the same procedure as that used in the preparation of **8** was used, from 50 mg (0.15 mmol) of estradiol-3-oxyacetic acid and 107 mg (0.15 mmol) of HCl·H-Arg(NO_2)-Gly-Asp(OBzl)-Val-OBzl, 111 mg (74%) of the title compound was obtained as a colorless powder. Mp 130−132 °C. IR (KBr) 3355, 3342, 3328, 3089, 3073, 3034, 1745, 1682, 1600, 1505, 1463, 1446, 1388, 1375, 748, 699 cm^{-1}. ^1H NMR (DMSO-d_6) 8.380 (s, 1H), 8.223 (s, 1H), 8.081 (s, 1H), 8.002 (s, 1H), 7.343 (t, J = 7.82 Hz, 1H), 7.337 (t, J = 7.85 Hz, 1H), 7.301 (t, J = 7.84 Hz, 2H), 7.290 (t, J = 7.86 Hz, 2H), 7.241 (d, J = 7.82 Hz, 2H), 7.183 (d, J = 7.86 Hz, 2H), 7.121 (d, J = 7.52 Hz, 1H), 7.039 (s, 1H), 6.711 (s, 1H), 6.520 (d, J = 7.52 Hz, 1H), 6.418 (s, 1H), 6.376 (s, 1H), 5.164 (s, 2H), 5.140 (s, 2H), 5.003 (s, 2H), 4.900 (dt, J = 6.30 Hz, J = 6.61 Hz, 1H), 4.715 (t, J = 6.50 Hz, 1H), 4.707 (t, J = 6.43 Hz, 1H), 4.611 (t, J = 4.75 Hz, 1H), 3.968 (d, J = 6.02 Hz, 2H), 3.262 (m, J = 4.12 Hz, 1H), 2.966 (t, J = 4.58 Hz, 2H), 2.915 (m, J = 4.25 Hz, 1H), 2.793 (t, J = 5.16 Hz, 2H), 2.632 (t, J = 6.55 Hz, 2H), 2.556 (s, 1H), 2.024 (m, J = 4.76 Hz, 2H), 2.016 (m, J = 4.30 Hz, 2H), 1.862 (m, J = 4.26 Hz, 1H), 1.833 (m, J = 4.46 Hz, 2H), 1.759 (m, J = 4.54 Hz, 2H), 1.725 (m, J = 6.32 Hz, 2H), 1.702 (m, J = 4.26 Hz, 1H), 1.653 (m, J = 4.70 Hz, 2H), 1.627 (m, J = 6.34 Hz, 2H), 0.816 (s, 3H), 0.780 (d, J = 4.14 Hz, 6H). Anal. Calcd for $C_{51}H_{66}N_8O_{12}$: C, 62.31; H, 6.77; N, 11.40. Found: C, 62.14; H, 6.59; N, 11.57. AA analysis: calcd, Arg/Gly/Asp/Val = 1.00:1.00:1.00:1.00; found, Arg/Gly/Asp/Val = 0.99:1.00:0.98:0.97. $[\alpha]^{20}_D$ 60.0 (*c* 1.00, MeOH). ESI-MS 983 $[M + H]^+$.

3.1.20. *N*-(Estradiol-3-oxyacetyl)-Arg(NO_2)-Gly-Asp(OBzl)-Phe-OBzl (18). When the same procedure as that used in the preparation of **8** was used, from 50 mg (0.15 mmol) of estradiol-3-oxyacetic acid and 119 mg (0.15 mmol) of HCl·H-Arg(NO_2)-Gly-Asp(OBzl)-Phe-OBzl, 97 mg (61%) of the title compound was obtained as a colorless powder. Mp 136−138 °C. IR (KBr) 3354, 3343, 3326, 3081, 3065, 3031, 1742, 1683, 1601, 1500, 1466, 1445, 1392, 1362, 748, 700 cm^{-1}. ^1H NMR (DMSO-d_6) 8.381 (s, 1H), 8.223 (s, 1H), 8.081 (s, 1H), 8.002 (s, 1H), 7.340 (t, J = 7.82 Hz, 1H), 7.396 (t, J = 7.82 Hz, 1H), 7.392 (t, J = 7.81 Hz, 1H), 7.339 (t, J = 7.82 Hz, 1H), 7.301 (t, J = 7.83 Hz, 2H), 7.290 (t, J = 7.86 Hz, 2H), 7.251 (t, J = 7.82 Hz, 2H), 7.207 (d, J = 7.82 Hz, 2H), 7.184 (d, J = 7.85 Hz, 2H), 7.176 (d, J = 7.83 Hz, 2H), 7.121 (d, J = 7.50 Hz, 1H), 7.033 (s, 1H), 6.705 (s, 1H), 6.521 (d, J = 7.50 Hz, 1H), 6.411 (s, 1H), 6.377 (s, 1H), 5.160 (s, 2H), 5.144 (s, 2H), 5.011 (s, 2H), 4.900 (dt, J = 6.30 Hz, J = 6.62 Hz, 1H), 4.708 (t, J = 6.51 Hz, 1H), 4.691 (t, J = 6.45 Hz, 1H), 4.608 (t, J = 4.75 Hz, 1H), 4.006 (s, 2H), 3.981 (d, J = 6.03 Hz, 2H), 2.965 (t, J = 4.56 Hz, 2H), 2.910 (m, J = 4.28 Hz, 1H), 2.781 (t, J = 5.14 Hz, 2H), 2.631 (t, J = 6.50 Hz, 2H), 2.560 (s, 1H), 2.022 (m, J = 4.75 Hz, 2H), 2.012 (m, J = 4.35 Hz, 1H), 1.861 (m, J = 4.26 Hz, 1H), 1.832 (m, J = 4.43 Hz, 2H), 1.754 (m, J = 4.52 Hz, 2H), 1.720 (m, J = 6.30 Hz, 2H), 1.700 (m, J = 4.26 Hz, 1H), 1.650 (m, J = 4.73 Hz, 2H), 1.625 (m, J = 6.38 Hz, 2H), 0.786 (s, 3H). $[\alpha]^{20}_D$ 51.0 (*c* 1.0, MeOH). ESI-MS 1031 $[M + H]^+$. Anal. Calcd for $C_{55}H_{66}N_8O_{12}$: C, 64.06; H, 6.45; N, 10.87. Found: C, 64.22; H, 6.62; N, 10.70. AA analysis: calcd, Arg/Gly/Asp/Phe = 1.00:1.00:1.00:1.00; found, Arg/Gly/Asp/Phe = 0.98:1.00:0.97:0.99.

3.1.21. *N*-(Estradiol-3-oxyacetyl)-Arg-Gly-Asp-Ser-OH (19). When the same procedure as that used in the preparation of **11** was used, from 100 mg (0.093 mmol) of estradiol-17-β-O-carbonylpropinyl-Arg(NO_2)-Gly-Asp(OBzl)-Ser(Bzl)-OBzl, 49 mg (70%) of the title compound was obtained as a colorless powder. Mp 155−157 °C. IR (KBr) 3443, 3387, 3342, 3325, 3028, 1685, 1601, 1503, 1464, 1446, 745, 701 cm^{-1}. ^1H NMR (DMSO-d_6) 9.076 (s, 1H), 9.071 (s, 1H), 8.026 (s, 1H), 8.019 (s, 1H), 8.011 (s, 1H), 8.002 (s, 2H), 7.244 (s, 2H), 7.042 (d, J = 7.51 Hz, 1H), 7.032 (s, 1H), 6.755 (s, 1H), 6.515 (d, J = 7.51 Hz, 1H), 6.400 (s, 1H), 5.004 (s, 2H), 4.851 (dt, J = 6.31 Hz, J = 6.60 Hz, 1H), 4.677 (t, J = 6.54 Hz, 1H), 4.600 (t, J = 4.72 Hz, 1H), 4.579 (t, J = 6.42 Hz, 1H), 4.017 (d, J = 6.42 Hz, 2H), 3.971 (d, J = 6.03 Hz, 2H), 2.966 (t, J = 4.52 Hz, 2H), 2.905 (m, J = 4.27 Hz, 1H), 2.874 (t, J = 5.12 Hz, 2H), 2.807 (d, J = 6.61 Hz, 2H), 1.832 (m, J = 4.43 Hz, 2H), 1.762 (m, J = 4.55 Hz, 2H), 1.725 (m, J = 6.31 Hz, 2H), 1.700 (m, J = 4.26 Hz, 1H), 1.655 (m, J = 4.70 Hz, 2H), 1.629 (m, J = 6.34 Hz, 2H), 1.600 (m, J = 4.52 Hz, 2H), 1.579 (m, J = 4.54 Hz, 2H), 1.544 (m, J = 4.55 Hz, 2H), 0.733 (s, 3H). $[\alpha]^{20}_D$ 39.0 (*c* 0.50, MeOH). ESI-MS 745 $[M + H]^+$. Anal. Calcd for $C_{36}H_{52}N_6O_{11}$: C, 58.05; H, 7.04; N, 11.28. Found: C, 58.20; H, 7.11; N, 11.11. AA analysis: calcd, Arg/Gly/Asp/Ser = 1.00:1.00:1.00:1.00; found, Arg/Gly/Asp/Ser = 1.01:1.00:0.97:0.98.

3.1.22. *N*-(Estradiol-3-oxyacetyl)-Arg-Gly-Asp-Val-OH (20). When the same procedure as that used in the preparation of **11** was used, from 100 mg (0.093 mmol) of estradiol-17-β-O-carbonylpropionyl-Arg(NO_2)-Gly-Asp(OBzl)-Val-OBzl, 54 mg (73%) of the title compound was obtained as a colorless powder. Mp 174−176 °C. IR (KBr) 3402, 3355, 3343, 3032, 1687, 1601, 1500, 1462, 1446, 1380, 1375, 746, 700 cm^{-1}. ^1H NMR (DMSO-d_6) 9.104 (s, 1H), 9.087 (s, 1H), 8.029 (s, 1H), 8.021 (s, 1H), 8.013 (s, 1H), 8.004 (s, 2H), 7.241 (s, 2H), 7.044(d, J = 7.53 Hz, 1H), 7.030 (s, 1H), 6.754 (s, 1H), 6.512(d, J = 7.53 Hz, 1H), 6.403 (s, 1H), 5.002 (s, 2H), 4.855 (dt, J = 6.30 Hz, J = 6.61 Hz, 1H), 4.673 (t, J = 6.51 Hz, 1H), 4.574 (t, J = 6.40 Hz, 1H), 4.602 (t, J =

4.71 Hz, 1H), 3.979 (d, J = 6.00 Hz, 2H), 2.963 (t, J = 4.50 Hz, 2H), 2.902 (m, J = 4.25 Hz, 1H), 2.876 (t, J = 5.10 Hz, 2H), 2.801 (d, J = 6.60 Hz, 2H), 1.837 (m, J = 4.45 Hz, 2H), 1.768 (m, J = 4.56 Hz, 2H), 1.722 (m, J = 6.30 Hz, 2H), 1.704 (m, J = 4.28 Hz, 1H), 1.657 (m, J = 4.71 Hz, 2H), 1.625 (m, J = 6.35 Hz, 2H), 1.602 (m, J = 4.51 Hz, 2H), 1.576 (m, J = 4.52 Hz, 2H), 1.549 (m, J = 4.56 Hz, 2H), 0.935 (d, J = 4.25 Hz, 6H), 0.742 (s, 3H). $[\alpha]^{20}_D$ 45.0 (*c* 0.50, MeOH). ESI-MS 758 $[M + H]^+$. Anal. Calcd for $C_{38}H_{56}N_6O_{10}$: C, 63.30; H, 7.46; N, 11.10. Found: C, 63.47; H, 7.57; N, 11.28. AA analysis: calcd, Arg/Gly/Asp/Val = 1.00:1.00:1.00:1.00; found, Arg/Gly/Asp/Val = 0.98:1.00:0.98:0.97.

3.1.23. *N*-(Estradiol-3-oxyacetyl)-Arg-Gly-Asp-Phe-OH (21). When the same procedure as that used in the preparation of **11** was used, from 100 mg (0.095 mmol) of estradiol-17-β-O-carbonylpropionyl-Arg(NO_2)-Gly-Asp(OBzl)-Phe-OBzl, 59 mg (77%) of the title compound was obtained as a colorless powder. Mp 180−182 °C. IR (KBr) 3405, 3380, 3375, 3030, 1690, 1602, 1503, 1460, 745, 701 cm^{-1}. ^1H NMR (DMSO-d_6) 9.070 (s, 1H), 9.063 (s, 1H), 8.035 (s, 1H), 8.010 (s, 1H), 8.003 (s, 1H), 7.986 (s, 2H), 7.275 (t, J = 7.80 Hz, 2H), 7.217 (s, 1H), 7.127 (d, J = 7.80 Hz, 2H), 7.079 (d, J = 7.85 Hz, 1H), 7.063 (d, J = 7.50 Hz, 1H), 7.026 (s, 1H), 6.744 (s, 1H), 6.667 (d, J = 7.51 Hz, 1H), 6.645 (s, 1H), 4.991 (s, 2H), 4.840 (dt, J = 6.32 Hz, J = 6.60 Hz, 1H), 4.670 (t, J = 6.52 Hz, 1H), 4.597 (t, J = 4.70 Hz, 1H), 4.573 (t, J = 6.40 Hz, 1H), 3.966 (d, J = 6.00 Hz, 2H), 3.227 (t, J = 4.72 Hz, 2H), 2.960 (t, J = 4.54 Hz, 2H), 2.900 (m, J = 4.28 Hz, 1H), 2.871 (t, J = 5.14 Hz, 2H), 2.800 (d, J = 6.56 Hz, 2H), 1.840 (m, J = 4.45 Hz, 2H), 1.766 (m, J = 4.52 Hz, 2H), 1.728 (m, J = 6.30 Hz, 2H), 1.670 (m, J = 4.72 Hz, 2H), 1.626 (m, J = 6.30 Hz, 2H), 1.604 (m, J = 4.54 Hz, 2H), 1.584 (m, J = 4.58 Hz, 1H), 1.572 (m, J = 4.54 Hz, 2H), 1.543 (m, J = 4.52 Hz, 2H), 1.467 (m, J = 4.56 Hz, 1H), 0.752 (s, 3H). $[\alpha]^{20}_D$ 48.0 (*c* 0.50, MeOH). ESI-MS 805 $[M + H]^+$. Anal. Calcd for $C_{42}H_{56}N_6O_{10}$: C, 62.67; H, 7.01; N, 10.44. Found: C, 61.73; H, 7.07; N, 10.63. AA analysis: calcd, Arg/Gly/Asp/Phe = 1.00:1.00:1.00:1.00; found, Arg/Gly/Asp/Phe = 0.98:1.00:0.98:0.97.

3.1.24. Ethyl Estrone-3-oxyacetate (22). To the solution of 500 mg (1.85 mmol) of estrone in 10 mL of anhydrous THF, 1.4 mL of sodium ethoxide in anhydrous ethanol (2.0 mol/L) was added. The mixture was stirred for 0.5 h, to which 0.750 mL (5.5mmol) of ethyl bromoacetate was added. The reaction mixture was stirred at 56 °C for 16 h and TLC ($CHCl_3$/CH_3OH, 10:1) indicated the complete disappearance of estrone. The reaction mixture was evaporated to remove the solvent and excess of ethyl bromoacetate. After purification on chromatography column of silica gel 593 mg (90%) of the title compound was obtained as a colorless powder. Mp. 98−100 °C. ESI-MS(*m*/*e*) 357 $[M + H]^+$. $[\alpha]^{20}_D$ 138.0 (*c* 0.50, THF).

3.1.25. Estrone-3-oxyacetic Acid (23). At 0 °C to the solution of 500 mg (1.40 mmol) of ethyl estrone-3-oxyacetate in 3 mL of anhydrous ethanol, 2 mL of aqueous solution of NaOH (2 mol/L) was added dropwise. The reaction mixture was stirred at room temperature for 2.5 h and TLC ($CHCl_3$/CH_3OH, 10:1) indicated the complete disappearance of methyl estrone-3-oxyacetate. The reaction mixture was adjusted with $KHSO_4$ powder to pH 7.0 and evaporated to remove the organic solvent. To the residue, 3 mL of water was added and adjusted with $KHSO_4$ powder to pH 2.0. The mixture was extracted with ethyl acetate and dried over anhydrous Na_2SO_4. After filtration the filtrate was evaporated to give 442 mg (96%) of the title compound as a colorless powder. Mp 214−215 °C. ESI-MS (*m*/*e*) 329 $[M + H]^+$. $[\alpha]^{20}_D$ 159.0 (*c* 0.45, THF).

3.1.26. Estrone-3-oxyacetyl-Arg(NO_2)-Gly-Asp(OBzl)-Ser-(Bzl)-OBzl (24). When the same procedure as that used in the preparation of **8** was used, from 50 mg (0.15 mmol) of estrone-3-oxyacetic acid and 119 mg (0.15 mmol) of HCl·H-Arg(NO_2)-Gly-Asp(OBzl)-Ser(Bzl)-OBzl, 99 mg (62%) of the title compound was obtained as a colorless powder. Mp 133−135 °C. IR (KBr) 3352, 3343, 3326, 3084, 3072, 3028, 1749, 1722, 1680, 1603, 1505, 1460, 1445, 744, 693 cm^{-1}. ^1H NMR (DMSO-d_6) 8.380 (s, 1H), 8.217 (s, 1H), 8.081 (s, 1H), 8.002 (s, 1H), 7.342 (t, J = 7.82 Hz, 1H), 7.339 (t, J = 7.84 Hz, 1H), 7.334 (t, J = 7.82 Hz, 1H), 7.300 (t, J = 7.82 Hz, 2H), 7.290 (t, J = 7.84 Hz, 2H), 7.250 (t, J = 7.82 Hz, 2H), 7.242 (d, J = 7.82 Hz, 2H), 7.183 (d, J = 7.84 Hz, 2H), 7.172 (d, J = 7.82 Hz, 2H), 7.120 (d, J = 7.50 Hz, 1H), 7.034 (s, 1H), 6.710 (s, 1H), 6.520 (d, J = 7.50 Hz, 1H), 6.412 (s, 1H), 6.376 (s, 1H), 5.167 (s, 2H), 5.145 (s, 2H), 5.136 (s, 2H), 5.002 (s, 2H), 4.900 (dt, J = 6.30 Hz, J = 6.58 Hz, 1H), 4.711 (t, J = 6.50 Hz, 1H), 4.702 (t, J = 6.44 Hz, 1H), 4.613 (t, J = 4.76 Hz, 1H), 3.964 (d, J = 6.01 Hz, 2H), 3.767 (d, J = 4.17 Hz, 2H), 2.972 (t, J = 4.56 Hz, 2H), 2.919 (m, J = 4.24 Hz, 1H), 2.790 (d, J = 5.16 Hz, 2H), 2.633 (t, J = 6.50 Hz, 2H), 2.058 (t, J = 4.76 Hz, 2H), 2.017 (m, J = 4.36 Hz, 2H), 1.845 (m, J = 4.46 Hz, 2H), 1.750 (m, J = 4.55 Hz, 2H), 1.729 (m, J = 6.32 Hz, 2H), 1.707 (m, J = 4.29 Hz, 1H), 1.654 (m, J = 4.75 Hz, 2H), 1.626 (m, J = 6.34 Hz, 2H), 0.792 (s, 3H). ESI-MS (*m*/*e*) 1059 $[M + H]^+$. $[\alpha]^{20}_D$ 32.0 (*c* 0.5, $CHCl_3$.MeOH, 10:1). Anal. Calcd for $C_{56}H_{66}N_8O_{13}$: C, 63.50; H, 6.28; N, 10.58. Found: C, 63.65; H, 6.35; N, 10.41. AA analysis: calcd, Arg/Gly/Asp/Ser = 1.00:1.00:1.00:1.00; found, Arg/Gly/Asp/Ser = 0.97:1.00:1.01:0.98.

3.1.27. Estrone-3-oxyacetyl-Arg(NO_2)-Gly-Asp(OBzl)-Val-OBzl (25). When the same procedure as that used in the preparation of **8** was used, from 50 mg (0.15 mmol) of estrone-3-oxyacetic acid and 107 mg (0.15 mmol) of HCl·H-Arg(NO_2)-Gly-Asp(OBzl)-Val-OBzl, 101 mg (68%) of the title compound was obtained as a colorless powder. Mp 124−126 °C. IR (KBr) 3359, 3345, 3331, 3086, 3077, 3030, 1747, 1724, 1680, 1602, 1507, 1465, 1448, 1389, 1378, 744, 695 cm^{-1}. ^1H NMR (DMSO-d_6) 8.377 (s, 1H), 8.220 (s, 1H), 8.078 (s, 1H), 8.000 (s, 1H), 7.345 (t, J = 7.80 Hz, 1H), 7.339 (t, J = 7.83 Hz, 1H), 7.305 (t, J = 7.82 Hz, 2H), 7.294 (t, J = 7.84 Hz, 2H), 7.245 (d, J = 7.80 Hz, 2H), 7.185 (d, J = 7.84 Hz, 2H), 7.124 (d, J = 7.50 Hz, 1H), 7.042 (s, 1H), 6.714 (s, 1H), 6.523 (d, J = 7.50 Hz, 1H), 6.422 (s, 1H), 6.378 (s, 1H), 5.169 (s, 2H), 5.146 (s, 2H), 5.007 (s, 2H), 4.904 (dt, J = 6.32 Hz, J = 6.60 Hz, 1H), 4.718 (t, J = 6.52 Hz, 1H), 4.709 (t, J = 6.41 Hz, 1H), 4.613 (t, J = 4.76 Hz, 1H), 3.971 (d, J = 6.00 Hz, 2H), 2.967 (t, J = 4.55 Hz, 2H), 2.919 (m, J = 4.28 Hz, 1H), 2.795 (t, J = 5.14 Hz, 2H), 2.634 (t, J = 6.52 Hz, 2H), 2.027 (m, J = 4.74 Hz, 2H), 2.019 (m, J = 4.35 Hz, 2H), 1.866 (m, J = 4.29 Hz, 1H), 1.835 (m, J = 4.48 Hz, 2H), 1.762 (m, J = 4.56 Hz, 2H), 1.733 (m, J = 6.30 Hz, 2H), 1.706 (m, J = 4.27 Hz, 1H), 1.656 (m, J = 4.72 Hz, 2H), 1.629 (m, J = 6.35 Hz, 2H), 0.819 (s, 3H), 0.785 (d, J = 4.16 Hz, 6H). Anal. Calcd for $C_{51}H_{64}N_8O_{12}$: C, 62.43; H, 6.58; N, 11.42. Found: C, 62.57; H, 6.50; N, 11.61. AA analysis: calcd, Arg/Gly/Asp/Val = 1.00:1.00:1.00:1.00; found, Arg/Gly/Asp/Val = 0.98:1.00:0.97:0.98. ESI-MS(*m*/*e*) 981 $[M + H]^+$. $[\alpha]^{20}_D$ 26.0 (*c* 0.5, $CHCl_3$/MeOH, 10:1).

3.1.28. Estrone-3-oxyacetyl-Arg(NO_2)-Gly-Asp(OBzl)-Phe-OBzl (26). When the same procedure as that used in the preparation of **8** was used, from 50 mg (0.15 mmol) of estrone-3-oxyacetic acid and 119 mg (0.15 mmol) of HCl·H-Arg(NO_2)-Gly-Asp(OBzl)-Phe-OBzl, 110 mg (70%) of the title compound was obtained as a colorless powder. Mp 130−132 °C. IR (KBr) 3357, 3346, 3328, 3083, 3062, 3029, 1745, 1720, 1681, 1600, 1504, 1467, 1443, 747, 703 cm^{-1}. ^1H NMR (DMSO-d_6) 8.385 (s, 1H), 8.227 (s, 1H), 8.084 (s, 1H), 8.010 (s, 1H), 7.342 (t, J = 7.80 Hz, 1H), 7.399 (t, J = 7.80 Hz, 1H), 7.396 (t, J = 7.77 Hz, 1H), 7.342 (t, J = 7.80 Hz, 1H), 7.304 (t, J = 7.81 Hz, 2H), 7.293 (t, J = 7.84 Hz, 2H), 7.255 (t, J = 7.80 Hz, 2H), 7.211 (d, J = 7.80 Hz, 2H), 7.187 (d, J = 7.83 Hz, 2H), 7.177 (d, J = 7.81 Hz, 2H), 7.124 (d, J = 7.52 Hz, 1H), 7.036 (s, 1H), 6.707 (s, 1H), 6.525 (d, J = 7.51 Hz, 1H), 6.414 (s, 1H), 6.382 (s, 1H), 5.163 (s, 2H), 5.147 (s, 2H), 5.014 (s, 2H), 4.903 (dt, J = 6.32 Hz, J = 6.64 Hz, 1H), 4.712 (t, J = 6.50 Hz, 1H), 4.694 (t, J = 6.43 Hz, 1H), 4.002 (s, 2H), 3.985 (d, J = 6.00 Hz, 2H), 2.962 (t, J = 4.57 Hz, 2H), 2.913 (m, J = 4.27 Hz, 1H), 2.784 (t, J = 5.16 Hz, 2H), 2.634(t, J = 6.51 Hz, 2H), 2.025 (m, J = 4.74 Hz, 2H), 2.014 (m, J = 4.36 Hz, 1H), 1.865 (m, J = 4.27 Hz, 1H), 1.835 (m, J = 4.44 Hz, 2H), 1.756 (m, J = 4.54 Hz, 2H), 1.723 (m, J = 6.28 Hz, 2H), 1.706 (m, J = 4.27 Hz, 1H), 1.655 (m, J = 4.74 Hz, 2H), 1.628 (m, J = 6.36 Hz, 2H), 0.791 (s, 3H). ESI-MS (*m*/*e*) 1029 $[M + H]^+$. $[\alpha]^{20}_D$ 28.0 (*c* 0.5, $CHCl_3$/MeOH, 10:1). Anal. Calcd for $C_{55}H_{64}N_8O_{12}$: C, 64.19; H, 6.27; N, 10.89. Found: C, 64.34; H, 6.38; N, 10.71. AA

analysis: calcd, Arg/Gly/Asp/Phe = 1.00:1.00:1.00:1.00; found, Arg/Gly/Asp/Phe = 0.99:1.00:0.98:0.98.

3.1.29. Estrone-3-oxyacetyl-Arg-Gly-Asp-Ser-OH (27). When the same procedure as that used in the preparation of **11** was used, from 100 mg (0.094 mmol) of estrone-3-oxyacetyl-Arg(NO_2)-Gly-Asp(OBzl)-Ser(Bzl)-OBzl (**24**), 40 mg (57%) of the title compound were obtained as a colorless powder. Mp 170−172 °C. IR (KBr) 3449, 3392, 3347, 3329, 3031, 1726, 1682, 1604, 1505, 1462, 1445, 741, 700 cm^{-1}. ^1H NMR (DMSO-d_6) 9.073 (s, 1H), 9.067 (s, 1H), 8.023 (s, 1H), 8.015 (s, 1H), 8.006 (s, 1H), 8.000 (s, 2H), 7.241 (s, 2H), 7.038 (d, J = 7.50 Hz, 1H), 7.029 (s, 1H), 6.751 (s, 1H), 6.512 (d, J = 7.50 Hz, 1H), 6.404 (s, 1H), 5.008 (s, 2H), 4.847 (dt, J = 6.30 Hz, J = 6.58 Hz, 1H), 4.674 (t, J = 6.52 Hz, 1H), 4.604 (t, J = 4.70 Hz, 1H), 4.575 (t, J = 6.40 Hz, 1H), 4.013 (d, J = 6.40 Hz, 2H), 3.968 (d, J = 6.00 Hz, 1H), 2.962 (t, J = 4.54 Hz, 2H), 2.902 (m, J = 4.29 Hz, 1H), 2.872 (t, J = 5.13 Hz, 2H), 2.811 (d, J = 6.59 Hz, 2H), 1.830 (m, J = 4.45 Hz, 2H), 1.766 (m, J = 4.54 Hz, 2H), 1.728 (m, J = 6.31 Hz, 2H), 1.708 (m, J = 4.25 Hz, 1H), 1.659 (m, J = 4.72 Hz, 2H), 1.624 (m, J = 6.30 Hz, 2H), 1.607 (m, J = 4.50 Hz, 2H), 1.576 (m, J = 4.52 Hz, 2H), 1.548 (m, J = 4.56 Hz, 2H), 0.827 (s, 3H). ESI-MS (*m*/*e*) 744 [M + H]$^+$. $[\alpha]^{20}_D$ 55.0 (*c* 1.0, MeOH). Anal. Calcd for $C_{36}H_{50}N_6O_{11}$: C, 58.21; H, 6.78; N, 11.31. Found: C, 58.40; H, 6.73; N, 11.13. AA analysis: calcd, Arg/Gly/Asp/Ser = 1.00:1.00:1.00:1.00; found, Arg/Gly/Asp/Ser = 1.02:1.00:0.98:0.98.

3.1.30. Estrone-3-oxyacetyl-Arg-Gly-Asp-Val-OH (28). When the same procedure as that used in the preparation of **11** was used, from 100 mg (0.102 mmol) of estrone-3-oxyacetyl-Arg(NO_2)-Gly-Asp(OBzl)-Val-OBzl (**25**), 59 mg (76%) of the title compound was obtained as a colorless powder. Mp 164−166 °C. IR (KBr) 3411, 3353, 3347, 3030, 1725, 1684, 1605, 1503, 1467, 1442, 1385, 1378, 748, 703 cm^{-1}. ^1H NMR (DMSO-d_6) 9.114 (s, 1H), 9.093 (s, 1H), 8.034 (s, 1H), 8.025 (s, 1H), 8.017 (s, 1H), 8.001 (s, 2H), 7.245 (s, 2H), 7.042 (d, J = 7.50 Hz, 1H), 7.033 (s, 1H), 6.756 (s, 1H), 6.516 (d, J = 7.50 Hz, 1H), 6.412 (s, 1H), 5.011 (s, 2H), 4.853 (dt, J = 6.31 Hz, J = 6.60 Hz, 1H), 4.675 (t, J = 6.50 Hz, 1H), 4.572 (t, J = 6.41 Hz, 1H), 4.610 (t, J = 4.70 Hz, 1H), 3.974 (d, J = 6.01 Hz, 1H), 2.966 (t, J = 4.52 Hz, 2H), 2.907 (m, J = 4.28 Hz, 1H), 2.875 (t, J = 5.10 Hz, 2H), 2.808 (d, J = 6.62 Hz, 2H), 1.839 (m, J = 4.48 Hz, 2H), 1.764 (m, J = 4.53 Hz, 2H), 1.725 (m, J = 6.27 Hz, 2H), 1.713 (m, J = 4.26 Hz, 1H), 1.659 (m, J = 4.70 Hz, 2H), 1.628 (m, J = 6.33 Hz, 2H), 1.613 (m, J = 4.54 Hz, 2H), 1.578 (m, J = 4.50 Hz, 2H), 1.545 (m, J = 4.52 Hz, 2H), 0.938 (d, J = 4.27 Hz, 6H), 0.747 (s, 3H). ESI-MS (*m*/*e*) 755 [M + H]$^+$. $[\alpha]^{20}_D$ 44.0 (*c* 0.5, MeOH). Anal. Calcd for $C_{38}H_{54}N_6O_{10}$: C, 60.46; H, 7.21; N, 11.13. Found: C, 60.62; H, 7.30; N, 11.27. AA analysis: calcd, Arg/Gly/Asp/Val = 1.00:1.00:1.00:1.00; found, Arg/Gly/Asp/Val = 0.97:1.00:0.98:0.99.

3.1.31. Estrone-3-oxyacetyl-Arg-Gly-Asp-Phe-OH (29). When the same procedure as that used in the preparation of **11** was used, from 100 mg (0.097 mmol) of estrone-3-oxyacetyl-Arg(NO_2)-Gly-Asp(OBzl)-Phe-OBzl (**26**), 44 mg (56%) of the title compound was obtained as a colorless powder. Mp 164−166 °C; IR (KBr) 3408, 3384, 3376, 3034, 1722, 1693, 1600, 1505, 1467, 748, 704 cm^{-1}. ^1H NMR (DMSO-d_6) 9.075 (s, 1H), 9.068 (s, 1H), 8.037 (s, 1H), 8.014 (s, 1H), 8.006 (s, 1H), 7.982 (s, 2H), 7.271 (t, J = 7.81 Hz, 2H), 7.213 (s, 1H), 7.120 (d, J = 7.82 Hz, 2H), 7.072 (d, J = 7.83 Hz, 1H), 7.060 (d, J = 7.51 Hz, 1H), 7.022 (s, 1H), 6.741 (s, 1H), 6.663 (d, J = 7.50 Hz, 1H), 6.643 (s, 1H), 4.993 (s, 2H), 4.843 (dt, J = 6.30 Hz, J = 6.58 Hz, 1H), 4.671 (t, J = 6.50 Hz, 1H), 4.590 (t, J = 4.71 Hz, 1H), 4.576 (t, J = 6.42 Hz, 1H), 3.223 (t, J = 4.74 Hz, 2H), 2.962 (t, J = 4.55 Hz, 2H), 2.903 (m, J = 4.29 Hz, 1H), 2.873 (t, J = 5.16 Hz, 2H), 2.802 (d, J = 6.53 Hz, 2H), 1.843 (m, J = 4.47 Hz, 2H), 1.763 (m, J = 4.50 Hz, 2H), 1.721 (m, J = 6.27 Hz, 2H), 1.674 (m, J = 4.70 Hz, 2H), 1.623 (m, J = 6.27 Hz, 2H), 1.602 (m, J = 4.52 Hz, 2H), 1.582 (m, J = 4.55 Hz, 1H), 1.570 (m, J = 4.56 Hz, 2H), 1.540 (m, J = 4.50 Hz, 2H), 1.464 (m, J = 4.53 Hz, 1H), 0.810 (s, 3H). ESI-MS (*m*/*e*) 803 [M + H]$^+$. $[\alpha]^{20}_D$ 32.0 (*c* 0.5, MeOH). Anal. Calcd for $C_{42}H_{54}N_6O_{10}$: C, 62.83; H, 6.78; N, 10.47. Found: C, 62.65; H, 6.70; N, 10.66. AA analysis: calcd, Arg/Gly/Asp/Phe = 1.00:1.00:1.00:1.00; found, Arg/Gly/Asp/Phe = 0.99:1.00: 0.97: 0.99.

3.1.32. Bioassay In Vivo. The assessments described herein were performed based on a protocol reviewed and approved by the ethics committee of Peking University. The committee assures the welfare of the animals was maintained in accordance to the requirements of the animal welfare act and according to the guide for care and use of laboratory animals. The tested compound was dissolved in aqueous solution of 0.5% CMC just before use and kept in an ice bath. Female Kuiming mice weighing 30.7 ± 3.1 g (7 weeks old, purchased from Animal Center of Peking University) were anesthetized with pentobarbital sodium (40.0 mg/kg, i.p.). The mice of OVX groups were given an abdominal OVX by the standard procedure, and the mice of the sham group were given abdominorotomy only. On the fifth day after the surgical operation, the mice of the OVX and sham groups were intraperitoneally injected with 2 μL of an aqueous solution of 0.5% CMC, the mice of the treatment groups were intraperitoneally injected with 2 μL of the solution of estrogen, the mixture of estrogen and RGD peptide, or 0.1103 μmol/Kg of the estrogen-RGD peptide in an aqueous solution of 0.5% CMC once a day. All of the mice were treated according to the corresponding procedure for 4 weeks. On the next day of the last administration, the mice were weighed and blood was drawn from the eye orbit. The mice were then anesthetized with sodium pentobarbital (40.0 mg/kg, i.p.) and executed to remove the lungs, livers, spleens, uteri, and left femurs.

After 30 min of standing, the blood was centrifugated at 3000 *g* for 20 min and the serum was stored at −20 °C before use. The calcium content of the serum was measured by the method of o-methylphenolphthalein complexing ketone. The phosphorus content of the serum was measured by the method of molybdenum blue. The ALP content of the serum was measured using disodium phenylphosphate as the substrate.

The lungs, livers, spleens, and uteri were weighed directly. After completely removing the muscle, the lengths of the left femurs were measured and then immersed in a solution of chloroform−methanol (2:1) two times (one time for 3 h). After defatting, the left femurs were heated at 120 °C for 6 h, cooled, and weighed to record the dry weight. The femurs were incinerated in a furnace at 800 °C for 8 h, cooled, weighed to record the ash weight and calculate the rate of the ash weight to dry femur weight (namely, the mineral content of the femur).

The ashes of the left femurs were dissolved in 0.5 mL of hydrochloric acid (6 N) and diluted to 5 mL with ultrapure water, from which 0.05 mL of the solution was drawn and diluted to 1 mL with ultrapure water before use. The calcium content of the aqueous solution was measured by the method of o-methylphenolphthalein complexing ketone. The phosphorus content of the aqueous solution was measured by the method of molybdenum blue.

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Supporting Information Available: Physical, analytical, and spectrometric data of the target compounds (**11**−**13**, **19**−**21**, and **27**−**29**). This material is available free of charge via the Internet at http://pubs.acs.org.

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