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Original article

Synthesis, evaluation and 3D QSAR analysis of novel estradiol–RGD octapeptide conjugates with oral anti-osteoporosis activity

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ABSTRACT

To enhance the potency, reduce the side effects and improve oral property of estradiol in estrogen replacement therapy (ERT), 6 novel estradiol–RGD octapeptide conjugates have been prepared. In an ovariectomized mouse osteoporotic model, at an oral dosage of 110.3 nmol/kg per day, their anti-osteoporosis activity was significantly higher than that of estradiol and estradiol–RGD tetrapeptide conjugates, and their risks of thrombogenesis and endometrial hyperplasia were significantly lower than that of estradiol and estradiol–RGD tetrapeptide conjugates. Using QSAR module of Cerius2, the 3D QSAR was performed for both femur weights and femur ash weights of estradiol–RGD peptide conjugates receiving mice. The r^2 of the 3D QSAR equations up to 0.995 and 0.988 indicates that they are capable of predicting a comparatively exact anti-osteoporosis activity for a conjugate.

1. Introduction

The ovarium atrophy leads almost all postmenopausal women confronting with osteoporosis [1]. More than 50% of these osteoporosis women suffered from fracture and some of them died of secondary affection. Therefore osteoporosis is being a crushing pressure for our society [2,3]. It is well known that the lack of estrogen resulted from ovarium atrophy is responsible for postmenopausal women developing osteoporosis. Consequently estrogen replacement therapy (ERT) or estrogen–progestogen replacement therapy (HRT) is clinically used for preventing osteoporosis [4]. In fact, ERT or HRT not only inhibits bone loss but also decreases the risk of postmenopausal women developing coronary heart disease [5,6]. It was found that a five-year HRT could decrease the fracture risk of postmenopausal women by 50% [7]. In spite of the efficacy of ERT or HRT, a long-term therapy may induce a series of dose-related side effects such as breast cancer, endometrial hyperplasia and the thromboembolic events [8–12]. To improve the clinical use of ERT or HRT structure modifications of estrogen, such as conjugating estrogen with peptides, have attracted a lot of interests [13–15].

It is commonly accepted that osteoporosis is related to both the decrease of bone formation modulated by osteoblast and the increase of bone resorption modulated by osteoclast. In ERT or HRT the role of estrogen is up-modulating the activity and proliferation of osteoblast, and regulating the gene expression in osteoblast and osteoclast [16–18]. Bone resorption is regulated by osteoclast binding to bone surface, and depends on osteoclast adhering to bone surface, which was mediated by integrin recognizing RGD containing protein of osteoclasts [19]. This knowledge lead to a hypothesis that the activity and proliferation of osteoblast and the adhesion of osteoclast could be simultaneously upregulated and downregulated by estrogen–RGD peptide conjugates. As a practice a series of estrogen–RGD tetrapeptide conjugates were prepared and bioassayed. On osteoporotic female mouse model, the conjugates possessed significantly higher potency and lower side effects than both estrogen and RGD tetrapeptides. On the other hand however, the comparisons of the administration routes explored that even though both oral and intraperitoneal administrations of the conjugates gave similarly low side effects, the oral potency was significantly lower than that of intraperitoneal one, for instance the femur ash weights, one of the important bone parameters, of orally and intraperitoneally treated mice ranged from 28.7 mg to 33.7 mg and from 34.4 mg to 40.0 mg, respectively [20]. These data suggest that the conjugates' oral potency needs to be increased and a corresponding strategy could be extending the peptide chain.

As a perspective experiment of this study the probability increasing the oral stability via extending RGD peptide chain was evaluated by pepsin promotion in vitro hydrolysis, for which RGDF and RGDFRGDF

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were selected as the substrates. Briefly, the solution of 3.2 mg pepsin, 2.0 mg NaCl, 7 μl concentrated hydrochloric acid and 1 ml water was centrifuged at 0 °C and 3500*g* for 5 min and the upper layer was stored at 4 °C as pepsin stock solution. To 100 μl of pepsin stock solution 49 μg (0.1 μmol) of RGDF or 97 μg (0.1 μmol) of RGDFRGDF was added, the formed mixture was incubated at 37 °C for 4 h and the incubator was monitored by HPLC–ESI–MS at 30 min intervals for 6 h. Our unpublished MS data explored that under pepsin promotion RGDF was completely degraded within 90 min, while in the 4 h incubation system of RGDFRGDF/pepsin the molecule ion of RGDFRGDF was still testable. Besides, in the literature it was pointed out that using artificial extracellular matrix proteins containing a repetitive Arg-Gly-Asp the cell adhesion ability was enhanced without increasing side effects [21]. These observations imply that the modification of estrogen with repetitive Arg-Gly-Asp peptide chain is worthwhile. Thus, in the present paper 6 novel estradiol–RGD octapeptide conjugates were designed and synthesized. Their oral anti-osteoporosis activity, endometrial hyperplasia risk and thromboembolic side effect were evaluated on osteoporotic female mouse model using 6 estradiol–RGD tetrapeptide conjugates as the reference compounds. Besides, dependence of their oral anti-osteoporosis activity on their structure was rationalized with 3D QSAR.

2. Results and discussion

2.1. Preparing estradiol–RGD peptide conjugates

To develop orally active estrogen–RGD peptide conjugates estradiol–RGD octapeptide conjugates were prepared via introducing the building blocks RGD octapeptides on to the 3- and 17-β positions of estradiol. On the other hand RGD octapeptides and estradiol–RGD tetrapeptide conjugates, which were prepared via introducing the building blocks RGD tetrapeptides on to the 3- and 17-β positions of estradiol, were used as the reference compounds of estradiol–RGD octapeptide conjugates.

2.1.1. Preparation of RGD octapeptide conjugates

RGD tetrapeptides and octapeptides were prepared via liquid phase method according to the route depicted in Scheme 1. The coupling of Boc-Asp(OBzl) with L-Ser(Bzl)-OBzl, L-Val-OBzl, L-Phe-OBzl and removal of Boc group provided three C-terminal fragments HCl·H-Asp(OBzl)-Ser(Bzl)-OBzl, HCl·H-Asp(OBzl)-Val-OBzl and HCl·H-Asp(OBzl)-Phe-OBzl in 94%, 92% and 87% yield, respectively. The coupling of Boc-Asp(OBzl) with L-Ser(Bzl), L-Val and L-Phe provided another three tetrapeptides Boc-Asp(OBzl)-Ser(Bzl), Boc-Asp(OBzl)-Val and Boc-Asp(OBzl)-Phe in 74%, 71% and 80% yield, respectively. The coupling of Boc-Arg(NO_2) with Gly provided Boc-Arg(NO_2)-Gly in 84% yield. The condensation of these dipeptide fragments and removal of Boc group provided HCl·H-Arg(NO_2)-Gly-Asp(OBzl)-Ser(Bzl)-OBzl (**1**), HCl·H-Arg(NO_2)-Gly-Asp(OBzl)-Val-OBzl (**2**), HCl·H-Arg(NO_2)-Gly-Asp(OBzl)-Phe-OBzl (**3**), HCl·H-Arg(NO_2)-Gly-Asp(OBzl)-Ser(Bzl)-Arg(NO_2)-Gly-Asp(OBzl)-Ser(Bzl)-OBzl (**4**), HCl·H-Arg(NO_2)-Gly-Asp(OBzl)-Val-Arg(NO_2)-Gly-Asp(OBzl)-Val-OBzl (**5**), HCl·H-Arg(NO_2)-Gly-Asp(OBzl)-Phe-Arg(NO_2)-Gly-Asp(OBzl)-Phe-OBzl (**6**) in 89%, 76%, 81%, 82%, 66% and 69% yield, respectively. After hydrogenation **4**–**6** were converted into the corresponding peptides RGDSRGDS (**7**), RGDVRGDV (**8**) and RGDFRGDF (**9**) in 86%, 73% and 76% yield respectively.

2.1.2. Preparing estradiol-3-RGD peptide conjugates

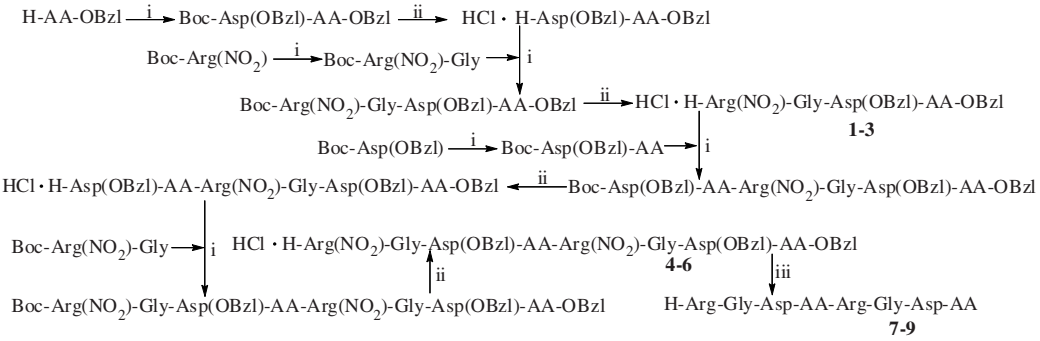
In the preparations of estradiol-3-RGD tetrapeptide conjugates and estradiol-3-RGD octapeptide conjugates the 3-hydroxyl group of estradiol was firstly alkylated by ethyl bromoacetate to provide ethyl estradiol-3-oxylacetate (**10**) in 83% yield. By saponification **10** was converted into the acid **11** (96% yield), to which HCl·H-Arg(NO_2)-Gly-Asp(OBzl)-AA-OBzl (**1**–**3**, AA = Ser(Bzl), Val and Phe) or HCl·H-Arg(NO_2)-Gly-Asp(OBzl)-AA-Arg(NO_2)-Gly-Asp(OBzl)-AA-OBzl (**4**–**6**) was introduced and the corresponding estradiol-3-Arg(NO_2)-Gly-Asp(OBzl)-AA-OBzl (**12**–**14**, AA = Ser(Bzl), Val and Phe) and estradiol-3-Arg(NO_2)-Gly-Asp-(OBzl)-AA-Arg(NO_2)-Gly-Asp(OBzl)-AA-OBzl [**15**–**17**, AA = Ser(Bzl), Val and Phe] were obtained in 53%, 37%, 61%, 71%, 62% and 71% yield, respectively. Removing all protective groups by catalytic hydrogenation estradiol-3-Arg-Gly-Asp-AA (**18**–**20**, AA = Ser, Val and Phe) and estradiol-3-Arg-Gly-Asp-AA-Arg-Gly-Asp-AA (**21**–**23**, AA = Ser, Val and Phe) were obtained in 63%, 91%, 48%, 41%, 52% and 71% yield, respectively (Scheme 2).

2.1.3. Preparing estradiol-17β-RGD peptide conjugates

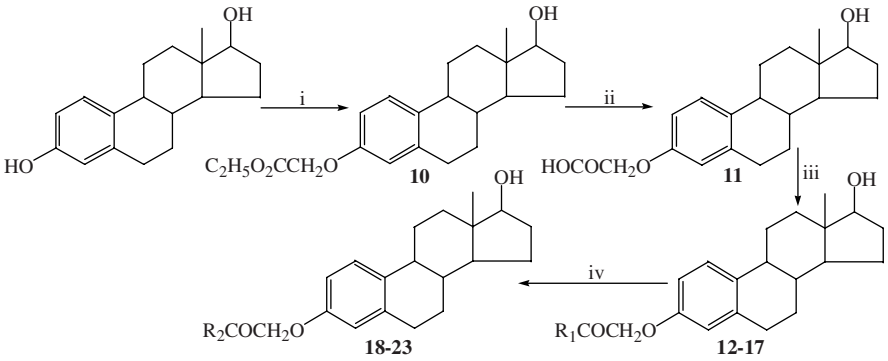
Treating estradiol with succinic anhydride the estradiol-17-monoester of succinic acid (**24**) was obtained in 95% yield. Coupling **24** and **1**–**3** estradiol-17β-Arg(NO_2)-Gly-Asp(OBzl)-AA-OBzl [**25**–**27**, AA = Ser(Bzl), Val and Phe] were obtained in 48%, 45% and 42% yield, respectively. Coupling **24** and **4**–**7** estradiol-17β-Arg(NO_2)-Gly-Asp(OBzl)-AA-Arg(NO_2)-Gly-Asp(OBzl)-AA-OBzl [**28**–**30**, AA = Ser(Bzl), Val and Phe] were obtained in 48%, 45%, 42%, 71%, 62% and 71% yield, respectively. Removing all protective groups by catalytic hydrogenation estradiol-17-Arg-Gly-Asp-AA–OH (**31**–**33**, AA = Ser, Val and Phe) and estradiol-17-Arg-Gly-Asp-AA-Arg-Gly-Asp-AA (**34**–**36**, AA = Ser, Val and Phe) were obtained in 79%, 84%, 86%, 41%, 52% and 71% yield, respectively (Scheme 3).

2.2. Evaluating oral anti-osteoporosis activity of estradiol–RGD conjugates

To estimate the oral anti-osteoporosis activity of estradiol–RGD octapeptide conjugates the ovariectomized mouse osteoporotic model was used. After oral administration of estradiol, RGD



Scheme 1. Preparation of RGD tetrapeptides and octapeptides, in **1** and **4** AA = Ser(Bzl), **2** and **5** AA = Val, **3** and **6** AA = Phe, **7** AA = Ser, **8** AA = Val, **9** AA = Phe; (i) DCC, HOBt and NMM; (ii) hydrogen chloride in ethyl acetate; (iii) Pt/H_2.



Scheme 2. Preparation of estradiol-3-RGD tetrapeptide and octapeptide conjugates, in **12** R_1 = -Arg(NO_2)-Gly-Asp(OBzl)-Ser(Bzl)-OBzl, in **13** R_1 = -Arg(NO_2)-Gly-Asp(OBzl)-Val-OBzl, in **14** R_1 = -Arg(NO_2)-Gly-Asp(OBzl)-Phe-OBzl, in **15** R_1 = -Arg(NO_2)-Gly-Asp(OBzl)-Ser(Bzl)-Arg(NO_2)-Gly-Asp(OBzl)-Ser(Bzl)-OBzl, in **16** R_1 = -Arg(NO_2)-Gly-Asp(OBzl)-Val-Arg(NO_2)-Gly-Asp(OBzl)-Val-OBzl, in **17** R_1 = -Arg(NO_2)-Gly-Asp(OBzl)-Phe-Arg(NO_2)-Gly-Asp(OBzl)-Phe-OBzl, in **18** R_2 = -Arg-Gly-Asp-Ser, in **19** R_2 = -Arg-Gly-Asp-Val, in **20** R_2 = -Arg-Gly-Asp-Phe, in **21** R_2 = -Arg-Gly-Asp-Ser-Arg-Gly-Asp-Ser, in **22** R_2 = -Arg-Gly-Asp-Val-Arg-Gly-Asp-Val, in **23** R_2 = -Arg-Gly-Asp-Phe-Arg-Gly-Asp-Phe; (i) $BrCH_2CO_2C_2H_5$ and $NaOC_2H_5$; (ii) NaOH in methanol; (iii) DCC, HOBt, NMM and **1**–**6**; (iv) Pt/H_2.

octapeptides (**7**–**9**), estradiol-3-RGD tetrapeptide conjugates (**18**–**20**), estradiol-17β-RGD tetrapeptide conjugates (**31**–**33**), estradiol-3-RGD octapeptide conjugates (**21**–**23**), estradiol-17β-RGD octapeptide conjugates (**34**–**36**), the serum calcium, phosphorous and ALP, the femur calcium, phosphorous and mineral, the weights of dry femurs and femur ash, and the lengths of the femurs were measured to know their anti-osteoporosis potencies.

To assign a proper oral dose for estradiol–RGD octapeptide conjugates RGDFRGDF and estradiol–RGDFRGDF were selected as model compounds and as the pre-treatment a series doses (0.01, 0.11, 0.15, 0.30, 0.50, 1.0, 2.0 and 4.0 μmol/kg) of them were orally administered to osteoporotic female mice to determine their ex vivo anti-aggregation activities and anti-osteoporosis activities. The ex vivo anti-platelet aggregation assay explored that for ADP and PAF induced mouse platelet aggregation RGDFRGDF had 0.31 μmol/kg and 0.26 μmol/kg IC_{50} values, respectively, while estradiol–RGDFRGDF had 0.15 μmol/kg and 0.11 μmol/kg IC_{50} values, respectively. The anti-osteoporosis assay explored that 4.0 μmol/kg RGDFRGDF exhibited no anti-osteoporosis activity, whereas 0.11 μmol/kg estradiol–RGDFRGDF exhibited significant anti-osteoporosis activity. All assays gave no evidence for side effects. Thus 0.11 μmol/kg was selected as general dose in all experiments.

2.2.1. Estradiol–RGD conjugates decreasing the content of calcium and ALP in mouse serum

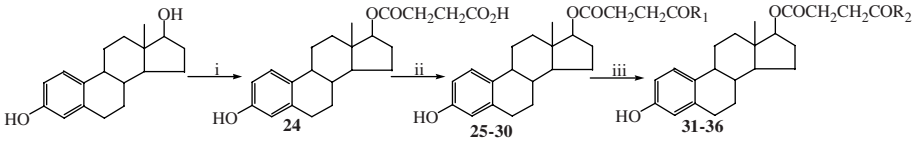
In order to evaluate the influence of estradiol–RGD octapeptides on the bone loss the serum of estradiol, RGD octapeptides, estradiol–RGD tetrapeptide conjugates and estradiol–RGD octapeptide conjugates receiving mice were collected, treated and measured following the procedure of Section 4.7 to determine the calcium and alkaline phosphatase (ALP) contents of the serum. The data are listed in Table 1, which indicated that the contents of serum calcium was decreased by estradiol–RGD conjugates **18**–**23** and **31**–**36**, and neither RGD octapeptides **7**–**9** nor the mixture of estradiol and **7**–**9** decreased the content of serum calcium. However the decreased extent of serum calcium by conjugates **21**–**23** and **34**–**36** was significantly higher than that decreased by conjugates **18**–**20** and **31**–**33**. Though estradiol, octapeptides **7**–**9**, the mixture of estradiol and **7**–**9**, conjugates **18**–**23** and **31**–**36** decreased the content of ALP the decreasing extent of conjugates **21**–**23** and **34**–**36** was significantly higher than that of the other conjugates. On the other hand all of them did not affect the content of serum phosphorous of the mice.

2.2.2. Estradiol–RGD conjugates increasing the weights of dry femurs and femur ashes

In order to evaluate the influence of estradiol–RGD conjugates on the bone loss, the femurs of estradiol, octapeptides **7**–**9**, the mixture of estradiol and **7**–**9**, conjugates **18**–**23** and **31**–**36** receiving mice were collected, treated and measured following the procedure of Section 4.7 to record the weights of dry femurs and their ashes and femur lengths, the data are listed in Table 2. The measurements indicated that conjugates **18**–**23** and **31**–**36** increased the weights of dry femurs and their ashes, and the increased extents by conjugates **21**–**23** and **34**–**36** were significantly higher than those by conjugates **18**–**20** and **31**–**33**. On the other hand neither octapeptides (**7**–**9**) nor the mixture of estradiol and **7**–**9** increased the weights of dry femurs and femur ashes. However estradiol, octapeptides **7**–**9**, the mixture of estradiol and **7**–**9**, conjugates **18**–**23** and **31**–**36** did not affect the length of mouse femur.

2.2.3. Estradiol–RGD conjugates increasing the femur calcium, phosphorous and mineral

In order to evaluate the influence of estradiol–RGD octapeptides on the bone loss the femurs of estradiol, octapeptides **7**–**9**, the mixture of estradiol and **7**–**9**, conjugates **18**–**23** and **31**–**36** receiving mice were collected, treated and measured following the procedure of Section 4.7 to record the calcium, phosphorous and



Scheme 3. Preparation of estradiol-17-RGD tetrapeptide and octapeptide conjugates, in **25** R_1 = -Arg(NO_2)-Gly-Asp(OBzl)-Ser(Bzl)-OBzl, in **26** R_1 = -Arg(NO_2)-Gly-Asp(OBzl)-Val-OBzl, in **27** R_1 = -Arg(NO_2)-Gly-Asp(OBzl)-Phe-OBzl, in **28** R_1 = -Arg(NO_2)-Gly-Asp(OBzl)-Ser(Bzl)-Arg(NO_2)-Gly-Asp(OBzl)-Ser(Bzl)-OBzl, in **29** R_1 = -Arg(NO_2)-Gly-Asp(OBzl)-Val-Arg(NO2)-Gly-Asp(OBzl)-Val-OBzl, in **30** R_1 = -Arg(NO_2)-Gly-Asp(OBzl)-Phe-Arg(NO_2)-Gly-Asp(OBzl)-Phe-OBzl, in **31** R_2 = -Arg-Gly-Asp-Ser, in **32** R_2 = -Arg-Gly-Asp-Val, in **33** R_2 = -Arg-Gly-Asp-Phe, in **34** R_2 = -Arg-Gly-Asp-Ser-Arg-Gly-Asp-Ser, in **35** R_2 = -Arg-Gly-Asp-Val-Arg-Gly-Asp-Val, in **36** R_2 = -Arg-Gly-Asp-Phe-Arg-Gly-Asp-Phe; (i) succinic anhydride; (ii) DCC, HOBt, NMM and **1**–**6**; (iii) Pt/H_2.

Table 1
Effect of estradiol–RGD conjugates on calcium, phosphorous and ALP of mouse serum.

Group[a]	Calcium (mM)	Phosphorous (mM)	ALP
OVX	3.39 ± 0.15	3.18 ± 0.28	28.78 ± 2.74
Sham	3.30 ± 0.11	2.82 ± 0.29[h]	22.97 ± 1.71[e]
Estradiol	3.24 ± 0.24	3.01 ± 0.27	26.22 ± 2.92[f]
7	3.27 ± 0.25	3.13 ± 0.20	25.45 ± 2.34[f]
Estradiol + **7**	3.29 ± 0.11	3.29 ± 0.14	26.29 ± 2.58[f]
18	3.23 ± 0.20[c]	2.98 ± 0.20	26.62 ± 2.12[f]
31	3.18 ± 0.17[b]	3.01 ± 0.27	23.93 ± 2.90[e]
21	3.01 ± 0.18[d]	2.91 ± 0.49	20.27 ± 2.88[g]
34	3.00 ± 0.20[d]	2.60 ± 0.29[h]	20.18 ± 2.55[g]
8	3.28 ± 0.19	2.94 ± 0.35	25.12 ± 2.44[f]
Estradiol + **8**	3.21 ± 0.32	3.12 ± 0.41	26.19 ± 2.48[f]
19	3.22 ± 0.19[c]	2.99 ± 0.23	26.01 ± 2.71[f]
32	3.26 ± 0.19[c]	3.04 ± 0.12	22.96 ± 2.96[e]
22	3.04 ± 0.14[d]	2.70 ± 0.34[h]	20.64 ± 2.08[g]
35	3.02 ± 0.09[d]	3.04 ± 0.12	19.56 ± 1.96[g]
9	3.27 ± 0.18	3.24 ± 0.52	26.09 ± 2.09[f]
Estradiol + **9**	3.27 ± 0.22[c]	3.33 ± 0.60	25.99 ± 1.67[f]
20	3.24 ± 0.10[c]	3.19 ± 0.61	25.42 ± 1.35[f]
33	3.21 ± 0.15[c]	3.01 ± 0.28	23.18 ± 2.79[e]
23	3.05 ± 0.09[d]	3.01 ± 0.40	20.06 ± 2.15[g]
36	3.01 ± 0.15[d]	3.01 ± 0.28	20.18 ± 2.19[g]

[a] Dosage = 110.3 nmol/kg, $n = 12$.
[b] Compare to ovariotomy $P < 0.01$.
[c] Compare to ovariotomy $P < 0.05$.
[d] Compare to ovariotomy $P < 0.01$, to **18**–**20** and **31**–**33** $P < 0.05$.
[e] Compare to ovariotomy $P < 0.01$.
[f] Compare to ovariotomy $P < 0.05$.
[g] Compare to ovariotomy $P < 0.01$, to **18**–**20** and **31**–**33** $P < 0.01$.
[h] Compare to ovariotomy $P < 0.01$.

mineral contents in the femurs and the data are listed in Table 3, which indicated that the content of femur calcium was increased by octapeptides **7**–**9**, the mixture of estradiol and **7**–**9**, conjugates **18**–**23** and **31**–**36**, and the increased extent of femur calcium by conjugates **21**–**23** and **34**–**36** was significantly higher than that increased by **7**–**9**, the mixture of estradiol and **7**–**9**, the conjugates **18**–**20** and **31**–**33**. The contents of femur phosphorous were increased only by conjugates **18**–**23** and **31**–**36**, and neither octapeptides **7**–**9** nor the mixture of estradiol and **7**–**9** increased the contents of femur phosphorous. On the other hand the increased

Table 2
Effect of estradiol–RGD conjugates on the length and the weight of mouse femur.

Group[a]	Weight of dry femur (mg)	Weight of femur ash (mg)	Length of femur (cm)
OVX	51.77 ± 2.50	31.57 ± 2.11	1.641 ± 0.235
Sham	53.77 ± 2.04[b]	34.11 ± 2.66[d]	1.644 ± 0.209
Estradiol	52.35 ± 2.22	32.22 ± 2.02	1.642 ± 0.284
7	50.98 ± 2.11	29.10 ± 2.69	1.6.21 ± 0.228
Estradiol + **7**	52.12 ± 2.36	30.10 ± 2.04	1.622 ± 0.241
18	53.89 ± 2.20[b]	34.08 ± 2.94[d]	1.646 ± 0.202
31	53.78 ± 2.08[b]	34.17 ± 2.23[d]	1.659 ± 0.283
21	56.60 ± 2.70[c]	36.42 ± 2.73[e]	1.657 ± 0.254
34	56.41 ± 2.82[c]	36.48 ± 2.05[e]	1.665 ± 0.223
8	50.44 ± 2.69	29.92 ± 2.19	1.622 ± 0.231
Estradiol + **8**	49.78 ± 2.47	32.12 ± 2.40	1.640 ± 0.264
19	53.97 ± 2.31[b]	33.99 ± 2.92[d]	1.670 ± 0.268
32	54.01 ± 2.67[b]	33.70 ± 2.14[d]	1.672 ± 0.243
22	57.98 ± 2.48[c]	36.19 ± 2.35[e]	1.671 ± 0.298
35	57.31 ± 2.67[c]	36.70 ± 2.94[e]	1.672 ± 0.243
9	53.17 ± 2.89	30.90 ± 2.70	1.661 ± 0.204
Estradiol + **9**	52.98 ± 2.97	31.80 ± 2.11	1.636 ± 0.241
20	53.87 ± 2.15[b]	33.56 ± 2.42[d]	1.669 ± 0.201
33	54.00 ± 2.55[b]	33.87 ± 2.04[d]	16.40 ± 0.256
23	56.96 ± 2.22[c]	36.74 ± 2.32[e]	1.641 ± 0.224
36	56.60 ± 2.55[c]	36.87 ± 2.04[e]	16.40 ± 0.256

[a] Dosage = 110.3 nmol/kg, $n = 12$.
[b] Compare to ovariotomy $P < 0.05$.
[c] Compare to ovariotomy $P < 0.01$, to **18**–**20** and **31**–**33** $P < 0.05$.
[d] Compare to ovariotomy $P < 0.05$.
[e] Compare to ovariotomy $P < 0.01$, to **18**–**20** and **31**–**33** $P < 0.05$.

Table 3
Effect of estradiol–RGD octapeptides on calcium, phosphorous and mineral in mouse femur.

Group[a]	Calcium (%)	Phosphorous (%)	Mineral (%)
OVX	54.45 ± 1.35	19.31 ± 1.16	0.603 ± 0.011
Sham	62.08 ± 2.40[b]	20.16 ± 0.19[d]	0.633 ± 0.017[f]
Estradiol	57.28 ± 0.87[b]	19.81 ± 0.21	0.608 ± 0.018
7	55.61 ± 1.22[b]	18.58 ± 0.65	0.610 ± 0.017
Estradiol + **7**	57.58 ± 1.28[b]	18.91 ± 0.95	0.607 ± 0.032
18	58.76 ± 2.13[b]	20.24 ± 0.04[d]	0.605 ± 0.011
31	59.34 ± 1.22[b]	20.37 ± 0.12[d]	0.604 ± 0.010
21	63.12 ± 3.15[c]	21.56 ± 0.44[e]	0.626 ± 0.014[g]
34	65.16 ± 2.42[c]	21.40 ± 0.23[e]	0.625 ± 0.010[g]
8	56.45 ± 2.01[b]	19.85 ± 0.26	0.572 ± 0.039
Estradiol + **8**	56.91 ± 1.24[b]	19.78 ± 0.04	0.591 ± 0.021
19	57.84 ± 1.76[b]	20.24 ± 0.42[d]	0.611 ± 0.022
32	57.49 ± 0.98[b]	20.14 ± 0.28[d]	0.612 ± 0.007
22	64.22 ± 0.99[c]	21.12 ± 0.18[e]	0.624 ± 0.009[g]
35	67.49 ± 0.98[c]	21.94 ± 0.28[e]	0.624 ± 0.007[g]
9	57.57 ± 3.47[b]	19.43 ± 0.84	0.583 ± 0.044
Estradiol + **9**	57.67 ± 3.29[b]	19.39 ± 1.18	0.592 ± 0.022
20	57.42 ± 1.04[b]	20.09 ± 0.19[d]	0.596 ± 0.027
33	59.36 ± 1.17[b]	20.56 ± 0.26[d]	0.605 ± 0.013
23	63.92 ± 2.14[c]	21.33 ± 0.68[e]	0.623 ± 0.011[g]
36	64.36 ± 1.17[c]	21.56 ± 0.26[e]	0.625 ± 0.013[g]

[a] Dosage = 110.3 nmol/kg, $n = 12$.
[b] Compare to ovariotomy $P < 0.01$.
[c] Compare to ovariotomy $P < 0.01$, to **18**–**20** and **31**–**33** $P < 0.01$.
[d] Compare to ovariotomy $P < 0.01$.
[e] Compare to ovariotomy $P < 0.01$, to **18**–**20** and **31**–**33** $P < 0.01$.
[f] Compare to ovariotomy $P < 0.01$.
[g] Compare to ovariotomy $P < 0.01$, to **18**–**20** and **31**–**33** $P < 0.05$.

extents of femur phosphorous by conjugates **21**–**23** and **34**–**36** were significantly higher than those increased by conjugates **18**–**23** and **31**–**33**. The contents of femur mineral were increased by conjugates **31**–**36** only.

2.3. Side effects of estradiol–RGD octapeptide conjugates receiving mice

To estimate the side effects of orally administration of estradiol–RGD conjugates the osteoporosis mouse model was also used. After oral administration of estradiol, octapeptides **7**–**9**, conjugates **18**–**23** and **31**–**36** the weights of the organs and the coagulation were tested.

2.3.1. Estradiol–RGD conjugates affecting no kidneys, lungs, livers, and spleens of the mice

In order to evaluate the side effects of estradiol–RGD conjugates, the kidneys, lungs, livers, and spleens of estradiol, octapeptides **7**–**9**, the mixture of estradiol and **7**–**9**, conjugates **18**–**23** and **31**–**36** receiving mice were collected and measured following the procedure of Section 4.7 to record their morphology and weights which are listed in Table 4. It was found that oral administration of octapeptides **7**–**9**, the mixture of estradiol and **7**–**9**, the conjugates **18**–**23** and **31**–**36** changed neither the morphology nor the weights of the kidneys, lungs, livers, and spleens of the mice.

2.3.2. Estradiol–RGD conjugates increasing no risks of coagulation and uterine weight getting of mice

In order to further evaluate the side effects of estradiol–RGD conjugates the tail bleeding time and uterine weight of estradiol, octapeptides **7**–**9**, conjugates **18**–**23** and **31**–**36** receiving mice were measured following the procedure of Section 4.8, which are listed in Table 5. The data indicate that excepting estradiol and the mixture of estradiol and **7**–**9**, which shortened the tail bleeding time, oral administration of estradiol–RGD conjugates **18**–**23** and **31**–**36** did not change the tail bleeding time. On the other hand the uterine weights of the osteoporosis mice receiving estradiol and the

Table 4
Effect of estradiol–RGD conjugates on mouse kidney, lung, liver, and spleen.

Group[a]	Spleen weight (g)	Lung weight (g)	Kidney weight (g)	Liver weight (g)
OVX	0.145 ± 0.018	0.121 ± 0.015	0.168 ± 0.022	1.490 ± 0.177
Sham	0.106 ± 0.013[b]	0.107 ± 0.005[c]	0.168 ± 0.015	1.342 ± 0.117[f]
Estradiol	0.144 ± 0.017	0.120 ± 0.016	0.173 ± 0.020	1.552 ± 0.101
7	0.120 ± 0.044	0.114 ± 0.009	0.169 ± 0.027	1.425 ± 0.142
Estradiol + **7**	0.125 ± 0.023	0.118 ± 0.009	0.169 ± 0.014	1.506 ± 0.119
18	0.142 ± 0.029	0.128 ± 0.013	0.193 ± 0.010[e]	1.492 ± 0.145
31	0.150 ± 0.033	0.120 ± 0.010	0.182 ± 0.015	1.537 ± 0.227
21	0.133 ± 0.029	0.110 ± 0.009[d]	0.170 ± 0.009	1.448 ± 0.162
34	0.149 ± 0.026	0.116 ± 0.013	0.174 ± 0.014	1.479 ± 0.130
8	0.122 ± 0.014[b]	0.105 ± 0.004[c]	0.156 ± 0.007	1.398 ± 0.208
Estradiol + **8**	0.114 ± 0.016[b]	0.109 ± 0.007[d]	0.160 ± 0.016	1.360 ± 0.198
19	0.133 ± 0.017	0.109 ± 0.014	0.167 ± 0.014	1.400 ± 0.053
32	0.149 ± 0.027	0.125 ± 0.011	0.178 ± 0.013	1.550 ± 0.155
22	0.145 ± 0.034	0.118 ± 0.028	0.164 ± 0.023	1.469 ± 0.121
35	0.149 ± 0.027	0.125 ± 0.011	0.178 ± 0.013	1.550 ± 0.155
9	0.122 ± 0.016[b]	0.113 ± 0.024	0.167 ± 0.018	1.319 ± 0.136[f]
Estradiol + **9**	0.117 ± 0.020[b]	0.109 ± 0.010[d]	0.157 ± 0.005	1.235 ± 0.133[g]
20	0.177 ± 0.008[b]	0.112 ± 0.009	0.159 ± 0.017	1.336 ± 0.182[f]
33	0.135 ± 0.032	0.109 ± 0.011[d]	0.160 ± 0.009	1.266 ± 0.201[f]
23	0.131 ± 0.020	0.119 ± 0.007	0.169 ± 0.017	1.439 ± 0.099
36	0.135 ± 0.032	0.109 ± 0.011[d]	0.160 ± 0.009	1.266 ± 0.201[f]

[a] Dosage = 110.3 nmol/kg, $n = 12$.
[b] Compare to ovariotomy $P < 0.01$.
[c] Compare to ovariotomy $P < 0.01$.
[d] Compare to ovariotomy $P < 0.05$.
[e] Compare to ovariotomy $P < 0.01$.
[f] Compare to ovariotomy $P < 0.05$.
[g] Compare to ovariotomy $P < 0.01$.

mixture of estradiol and **7**–**9** were significantly higher than those of OVX, sham, **7**–**9**, **18**–**23** and **31**–**36**.

2.4. 3D QSAR analysis of the in vivo activity of estradiol–RGD conjugates

To gain insight into the correlation between the anti-osteoporosis activity with the structure of estradiol–RGD conjugate, the femur weights and femur ash weights of the mice receiving estradiol–RGD conjugates **18**–**23** and **31**–**36** were selected as the activity parameter for 3D QSAR analysis. By using QSAR module of Cerius^2 and following a standard procedure two equations were established to reflect the relationships of the femur weights and the femur ash weights and the structures. The resulted equations with 0.995 and 0.988 of r^2 demonstrated that both the femur weights and the femur ash weights of the mice receiving **18**–**23** and **31**–**36** quantitatively correlated with their structures, suggesting these equations are capable of exactly predicting the anti-osteoporosis activity for unknown estradiol–RGD peptide conjugate.

Table 5
Effect of estradiol–RGD conjugates on tail bleeding time and uterine weights.

Group[a]	Bleeding time (s) after last administration		Uterine weight (g)
	30 min	90 min	
OVX	542 ± 50	548 ± 49	0.081 ± 0.022
Sham	562 ± 53	568 ± 59	0.086 ± 0.026
Estradiol	417 ± 57[b]	441 ± 43[b]	0.108 ± 0.021[c]
7	551 ± 55	548 ± 59	0.084 ± 0.012
Estradiol + **7**	422 ± 53[b]	426 ± 49[b]	0.110 ± 0.015[c]
18	582 ± 50	577 ± 52	0.064 ± 0.015[d]
31	575 ± 51	561 ± 54	0.063 ± 0.015[d]
21	564 ± 53	559 ± 45	0.062 ± 0.014[d]
34	562 ± 54	556 ± 43	0.065 ± 0.011[d]
8	541 ± 53	549 ± 50	0.079 ± 0.024
Estradiol + **8**	412 ± 43[b]	416 ± 40[b]	0.116 ± 0.028[c]
19	569 ± 54	577 ± 52	0.064 ± 0.011[d]
32	551 ± 48	560 ± 51	0.064 ± 0.013[d]
22	566 ± 54	558 ± 81	0.061 ± 0.018[d]
35	581 ± 61	580 ± 64	0.064 ± 0.013[d]
9	555 ± 49	551 ± 48	0.076 ± 0.012
Estradiol + **9**	412 ± 43[b]	416 ± 40[b]	0.118 ± 0.023[c]
20	557 ± 45	560 ± 51	0.067 ± 0.021
33	576 ± 59	568 ± 61	0.060 ± 0.028
23	592 ± 49	585 ± 55	0.061 ± 0.018[d]
36	584 ± 45	571 ± 51	0.060 ± 0.018[d]

[a] Dosage = 110.3 μmol/kg, $n = 12$.
[b] Compare to ovariotomy $P < 0.01$.
[c] Compare to ovariotomy $P < 0.01$.
[d] Compare to ovariotomy $P < 0.05$.

2.4.1. Alignment of estradiol–RGD conjugates *18*–*23* and *31*–*36*

To establish the valid 3D QSAR models, a proper alignment procedure of estradiol–RGD conjugates **18**–**23** and **31**–**36** was practiced using the target model align strategy in the align module within Cerius^2. Based on the assumption that each structure of estradiol–RGD conjugates **18**–**23** and **31**–**36** exhibits activity at the same binding site of the receptor, they were aligned in a pharmacological active orientation. To obtain a consistent alignment, the estradiol ring, the common pharmacophore, was selected as the template for superposing estradiol–RGD conjugates **18**–**23** and **31**–**36**. The method used for performing the alignment was the maximum common subgraph (MCS) [22]. MCS looks at molecules as points and lines, and uses the techniques out of graph theory to identify the patterns. Then MCS finds the largest subset of atoms in estradiol ring that is shared by estradiol–RGD conjugates **18**–**23** and **31**–**36**. This subset was used for the alignment. A rigid fit of atom pairings was performed to superimpose each structure onto the target model estradiol ring. Stereoview of aligned estradiol–RGD conjugates **18**–**23** and **31**–**36** used for molecular field generation is shown in Fig. 1. The alignment stereoview explores that to superimpose onto the estradiol ring, the RGD peptide chain of each structure has to take individual conformation. This individual RGD peptide chain conformation will affect the anti-osteoporosis activity.

2.4.2. QSAR module of Cerius^2 based MFA of estradiol–RGD conjugates *18*–*23* and *31*–*36*

After energy minimization using the MMFF94 (Merck Molecular Force Field), the Molecular Field Analysis (MFA) was performed for estradiol–RGD conjugates **18**–**23** and **31**–**36** using the QSAR module of Cerius^2 [23]. A five-step-procedure consisted of generating

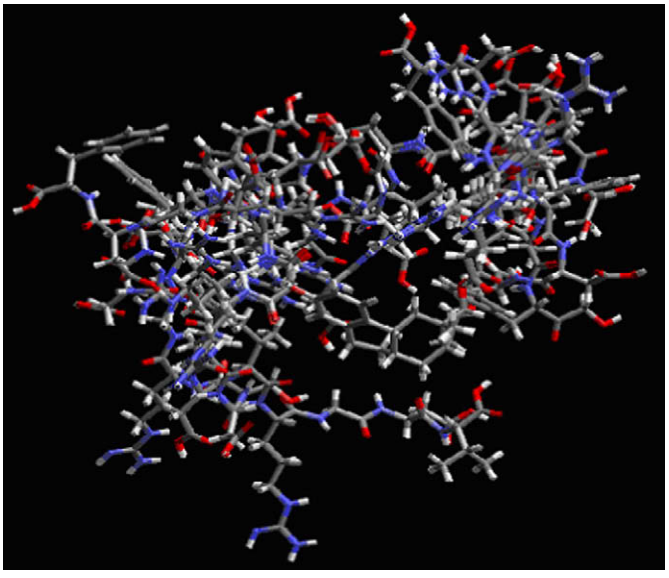


Fig. 1. Stereoview of the alignment of estradiol–RGD peptide conjugates **18**–**23** and **31**–**36** for generating molecular field.

conformers, energy minimization, matching atoms and aligning molecules, setting preferences, and regression analysis was automatically practiced in MFA. Molecular electrostatic and steric fields were created by use of proton and methyl groups as probes, respectively. These fields were sampled at each point of a regularly spaced grid of 1 Å. An energy cutoff of ±30.0 kcal/mol was set for both electrostatic and steric fields. The total grid points generated were 672. Though the spatial and structural descriptors such as dipole moment, polarizability, radius of gyration, number of rotatable bonds, molecular volume, principal moment of inertia, AlogP98, number of hydrogen bond donors and acceptors, and molar refractivity were also considered, only the highest variance holder proton, methyl and hydroxyl descriptors were used. Regression analysis was carried out using the genetic partial least squares (G/PLS) method consisting of 50 000 generations with a population size of 100. The number of components was set to 5. Cross-validation was performed with the leave-one-out procedure. PLS analysis was scaled, with all variables normalized to a variance of 1.0.

The regions where variations in the steric or electrostatic features of estradiol–RGD peptide conjugates (**18**–**23** and **31**–**36**) in the training set lead to increase or decrease femur weights were specified. Proton descriptor with positive coefficient indicates a region favorable for electropositive group, while negative coefficient indicates electronegative group required at this position. Methyl descriptor with positive coefficient indicates a region favorable for large group, while negative coefficient indicates small group required at this position. Hydroxyl descriptor with positive coefficient indicates a region favorable for hydrogen bond forming group, while negative coefficient indicates hydrogen bond forming group not required at this position.

*2.4.2.1. MFA model for femur weights of the mice receiving estradiol–RGD conjugates **18**–**23** and **31**–**36**.* The MFA model for femur weights of the mice receiving estradiol–RGD conjugates **18**–**23** and **31**–**36** in terms of the descriptors proton, methyl and hydroxyl groups is aligned as Fig. 2 and expressed by Eq. (1). The correlation of the femur weight tested on the in vivo osteoporosis mouse model and the femur weight calculated using Eq. (1) is explained by Fig. 3.

$$\begin{aligned}\text{Activity} = {} & 53.3225 + 0.01235(\text{H}^+/2949) - 0.018056(\text{HO}^-/2766) \\ & + 0.024866(\text{HO}^-/3218) - 0.036975(\text{H}^+/3220) \\ & + 0.072378(\text{CH}_3/3760) + 0.0121532(\text{HO}^-/2742) \\ & - 0.035892(\text{H}^+/2659) - 0.067739(\text{HO}^-/2633) \\ & - 0.04947(\text{H}^+/1649) - 0.050404(\text{HO}^-/1850) \end{aligned} \quad (1)$$

In Eq. (1) the data points (n), correlation coefficient (r), square correlation coefficient (r^2), cross-validated correlation coefficient (r^2cv), bootstrap correlation coefficient (r^2_{BS}) and least square error (LSE) were 12, 0.995, 0.991, 0.104, 0.737 and 0.0383, respectively. This equation contains term of 0.01235(H^+/2949) with positive coefficient, which means that at this position an electron-releasing group will affect on the femur weight positively, and terms of 0.036975(H^+/3220), 0.035892(H^+/2659) and 0.04947(H^+/1649) with negative coefficients, which means that at these positions electron-withdrawing groups will affect on the femur weight positively, from proton descriptor. Eq. (1) contains term of 0.072378(CH_3/3760) with positive coefficient, which means that at this position a large group will affect on the femur weight positively, from methyl descriptor. Eq. (1) contains also terms of 0.0121532(HO^-/2742) and 0.024866(HO^-/3218) with positive coefficients, which means that at these positions electron-releasing group will affect the femur weight positively, and terms of 0.018056(HO^-/2766), 0.067739(HO^-/2633) and 0.050404(HO^-1850) with negative coefficients, which means that at these positions electron-withdrawing groups will affect the femur weight positively, from hydroxyl descriptor.

As examples Fig. 4 gives two representatives **34** (a), which has electron-withdrawing groups near H^+/1649, H^+/2949, H^+/3220, HO^-/1850 and HO^-/2742 and totally increasing its femur weight, as well as **22** (b), which has electron-withdrawing groups near H^+/2949, H^+/3220, HO^-/3218 and a larger group near CH_3/3760 and also totally increasing its femur weight.

*2.4.2.2. MFA model for femur ash weights of the mice receiving estradiol–RGD conjugates **18**–**23** and **31**–**36**.* The MFA model for femur weights of the mice receiving estradiol–RGD peptide conjugates **18**–**23** and **31**–**36** in terms of the descriptors proton, methyl and hydroxyl groups is aligned as Fig. 5 and expressed by Eq. (2). The correlation of the femur ash weight tested on the in vivo

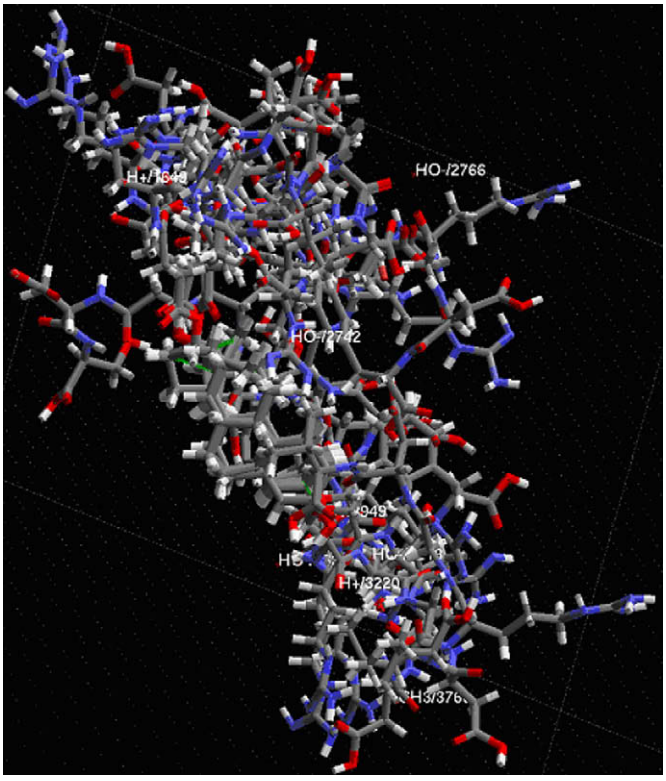


Fig. 2. Stereoview of the alignment of estradiol–RGD peptide conjugates **18**–**23** and **31**–**36** with the femur weight as the activity parameter.

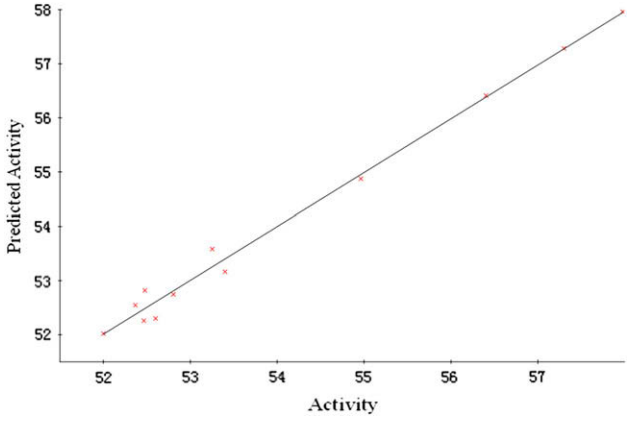


Fig. 3. Graph of femur weights of the mice receiving estradiol–RGD peptide conjugates **18**–**23** and **31**–**36** against the predicted femur weights by 3D QSAR.

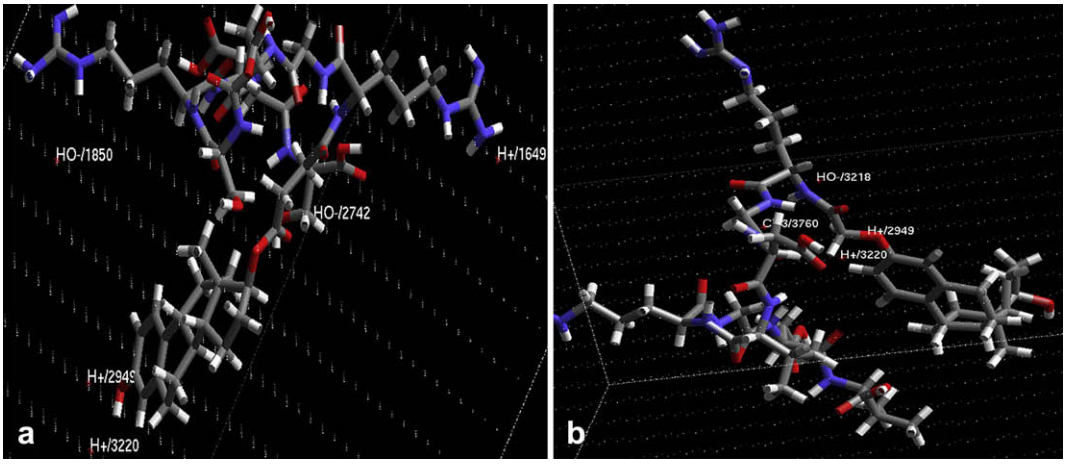


Fig. 4. Electrostatic and environments of conjugates **34** (a) and **22** (b) within 3D grid of Eq. (1).

osteoporosis mouse model and the femur ash weight calculated using equation Eq. (2) is explained by Fig. 6.

$$\begin{aligned}\text{Activity} = {} & 33.3666 - 0.070947(\mathrm{H^+}/4346) - 0.076576(\mathrm{CH_3}/2721) \\ & + 0.059459(\mathrm{CH_3}/4059) + 0.002937(\mathrm{HO^-}/2661) \\ & - 0.021407(\mathrm{CH_3}/1679) - 0.03634(\mathrm{HO^-}/2648) \\ & + 0.058069(\mathrm{CH_3}/2741) - 0.00753(\mathrm{HO^-}/2770) \\ & - 0.05163(\mathrm{HO^-}/3188) + 0.000807(\mathrm{H^+}/2418) \\ & - 0.050821(\mathrm{H^+}/3230) \end{aligned} \quad (2)$$

In Eq. (2) the data points (n), correlation coefficient (r), square correlation coefficient (r^2), cross-validated correlation coefficient (r^2cv), bootstrap correlation coefficient (r^2_{BS}) and least square error (LSE) were 12, 0.988, 0.976, 0.085, 0.863 and 0.007, respectively.

This equation contains term of 0.000807(H^+/2418) with positive coefficient, which means that at this position an electron-releasing group will affect the femur ash weight positively, and terms of 0.070947(H^+/4346) and 0.050821(H^+/3230) with negative coefficients, which means that at these positions electron-withdrawing groups will affect the femur ash weight positively, from proton descriptor. It contains terms of 0.059459(CH_3/4059) and 0.058069(CH_3/2741) with positive coefficient, which means that at these positions large groups will affect the femur ash weight positively, and terms of 0.076576(CH_3/2721) and 0.021407(CH_3/1679) with negative coefficient, which means that at these positions small groups will affect the femur ash weight positively, from methyl descriptor. Eq. (2) contains also term of 0.002937(HO^-/2661) with positive coefficient, which means that at this position an electron-releasing group will be favorable for increasing femur ash weight, and terms of 0.03634(HO^-/2648), 0.00753(HO^-/2770) and 0.05163(HO^-/3188) with negative coefficients, which means that at these positions electron-withdrawing groups will be favorable for increasing femur ash weight, from hydroxyl descriptor.

As examples Fig. 7 gives two representatives **34** (a), which has electron-withdrawing group near HO^-/2770 and large group near CH_3/2741 and totally increasing its femur ash weight, as well as **22** (b), which has electron-withdrawing groups near H^+/4346 and HO^-/2648 and a larger group near CH_3/2741 and CH_3/4059 and also totally increasing its femur ash weight.

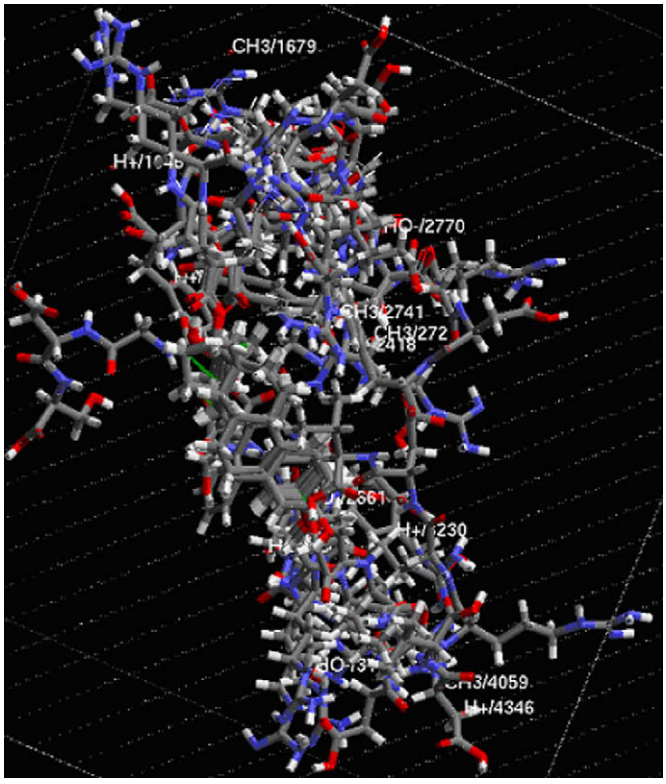


Fig. 5. Stereoview of the alignment of estradiol–RGD peptide conjugates **18**–**23** and **31**–**36** with the femur ash weight as the activity parameter.

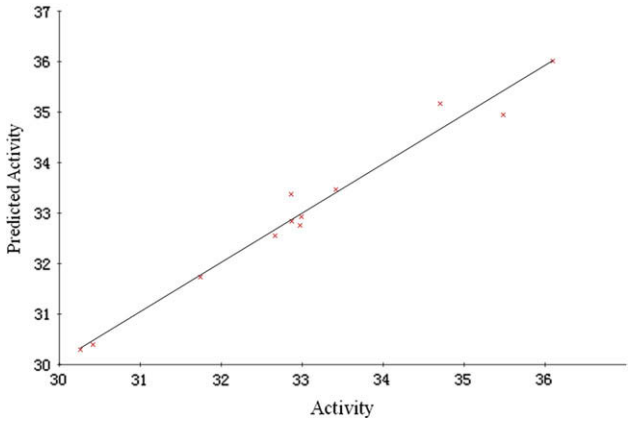


Fig. 6. Graph of femur ash weights of the mice receiving estradiol–RGD peptide conjugates **18**–**23** and **31**–**36** against the predicted femur ash weights by 3D QSAR.

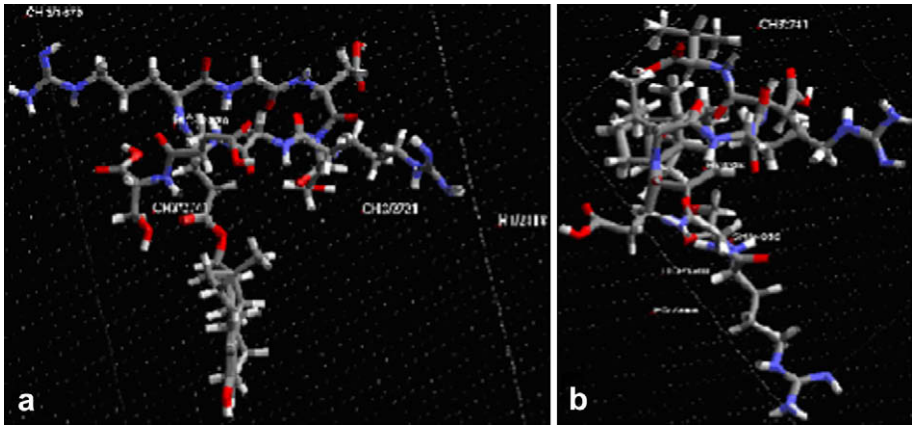


Fig. 7. Electrostatic and environments of conjugates **34** (a) and **22** (b) within 3D grid of Eq. (2).

3. Conclusion

Osteoporosis is being a crushing pressure for our society, and the modification of estradiol to improve the ERT or HRT is clinically important. The observations provided here explored that the risks of thrombogenesis and endometrial hyperplasia of the mice receiving 110.3 nmol/kg of estradiol–RGD peptide conjugates were significantly lower than that of mice receiving 110.3 nmol/kg of estradiol, which suggested that the use of estradiol–RGD peptide conjugates can simultaneously up-regulate the activity and proliferation of osteoblasts and down-regulate the adhesiveness of osteoclasts. In addition, the development levels of thrombogenesis of the mice receiving 110.3 nmol/kg of estradiol–RGD octapeptide conjugates were significantly lower than those of mice receiving 110.3 nmol/kg of RGD tetrapeptide conjugates, which suggested that introducing repeated RGD sequence to estradiol is a useful strategy to further increase the activity of these conjugates. The 0.995 and 0.988 of the r^2 of 3D QSAR analysis resulted equations of estradiol–RGD tetrapeptide and octapeptide conjugates explained that the anti-osteoporosis activity of unknown conjugate can be exactly predicted.

4. Experimental section

4.1. General

All the reactions were carried out under nitrogen (1 bar). ^{1}H (300 and 500 MHz) and ^{13}C (75 and 125 MHz) NMR spectra were recorded on Bruker AMX-300 and AMX-500 spectrometers for solution DMSO-d_6 or $CDCl_3$ with tetramethylsilane as internal standard. IR spectra were recorded with a Perkin–Elmer 983 instrument. FAB/MS was determined on VG-ZAB-MS and TOF-MS was recorded on MDS SCIEX QSTAR. Melting points were measured on a XT5 hot stage microscope (Beijing key electro-optic factory). All L-amino acids were purchased from China Biochemical Corp. TLC was made with Qingdao silica gel GF_{254}. Chromatography was performed with Qingdao silica gel H_{60} or Sephadex-LH_{20}. All solvents were distilled and dried before use by reference to literature procedures. Optical rotations were determined with a Jasco P-1020 Polarimeter at 20 °C. The statistical analysis of all the biological data was carried out by use of ANOVA test, $P < 0.05$ is considered significant.

4.2. Preparing RGD peptides

4.2.1. Boc-Arg(NO_2)-Gly-OCH_3

At 0 °C to the solution of 638 mg (2.0 mmol) of Boc-Arg(NO_2) and 297 mg (2.2 mmol) of 1-hydroxybenzotriazole (HOBt) in 20 ml of anhydrous tetrahydrofuran (THF) 453 mg (2.2 mmol) of dicyclohexylcarbodiimide (DCC) was added. The solution was stirred at 0 °C for 30 min, to which a solution of 250 mg (2.0 mmol) of HCl·H-Gly-OCH_3 in 10 ml of anhydrous THF was added. At 0 °C the reaction mixture was adjusted to pH 8.5 with *N*-methylmorpholine (NMM), then stirred at room temperature for 8 h, and TLC (chloroform/methanol, 5:1) indicated the disappearance of Boc-Arg(NO_2). The precipitates of dicyclohexylurea (DCU) were removed by filtration. The filtrate was evaporated under reduced pressure and the residue was dissolved in 50 ml of ethyl acetate. The solution was washed successively with a 5% aqueous solution of sodium bicarbonate, a 5% aqueous solution of citric acid and a saturated aqueous solution of sodium chloride. The organic phase was dried over anhydrous sodium sulfate. After filtration and evaporation under reduced pressure, 0.688 g (88%) of the title compound was provided as colorless powder. Mp. 94–95 °C, $[\alpha]_D^{20} = 0.4$ ($c = 1.07$, CH_3OH), ESI-MS (m/z) 391 $[M+H]^+$.

4.2.2. Boc-Arg(NO_2)-Gly

At 0 °C to the solution of 5.72 g (14.7 mmol) of Boc-Arg(NO_2)-Gly-OCH_3 in 70 ml of methanol 30 ml of aqueous NaOH (2 N) was added. The reaction mixture was stirred at 0 °C for 30 min, adjusted to pH 5.5 with hydrochloric acid (2 N). After filtration the filtrate was evaporated under reduced pressure. The residue was dissolved in 30 ml of methanol and the solution was filtered. The filtrate was evaporated under reduced pressure and the residue was solidified in 10 ml of anhydrous ether to provide 5.28 g (96%) of the title compound as colorless powder. Mp. 130–132 °C, $[\alpha]_D^{20} = -4.8$ ($c = 1.13$, CH_3OH), ESI-MS (m/z) 377 $[M+H]^+$.

4.2.3. Boc-Asp(OBzl)-Ser(Bzl)-OBzl

Using the same procedure as described for Boc-Arg(NO_2)-Gly-OCH_3 from 5.04 g (15.5 mmol) of Boc-Asp(OBzl), 2.31 g (16.5 mmol) of HOBt, 3.52 g (17.1 mmol) of DCC and 5.00 g (15.6 mmol) of HCl·H-Ser(Bzl)-OBzl 9.55 g (98%) of the title compound was obtained as yellow syrup. $[\alpha]_D^{20} = -12.1$ ($c = 1.13$, MeOH), ESI-MS (m/z) 592 $[M+H]^+$.

4.2.4. HCl·H-Asp(OBzl)-Ser(Bzl)-OBzl

To 9.0 g (1.3 mmol) of Boc-Asp(OBzl)-Ser(Bzl)-OBzl 80 ml of 4 N solution of hydrochloride in ethyl acetate was added. The reaction mixture was stirred at room temperature for 60 min and TLC (chloroform/methanol, 5:1) indicated the disappearance of Boc-Asp(OBzl)-Ser(Bzl)-OBzl. The reaction mixture was evaporated under reduced pressure, the residue was dissolved in 40 ml of ethyl acetate, the solution was again evaporated under reduced pressure and the residue was washed with anhydrous ether to provide 6.02 g

(94%) of the title compound as colorless powder. Mp. 105–106 °C, $[\alpha]_D^{20} = -13.2$ ($c = 1.27$, MeOH), ESI-MS (m/z) 492 $[M + H]^+$.

4.2.5. Boc-Asp(OBzl)-Val-OBzl

Using the same procedure as described for Boc-Arg(NO_2)-Gly-OCH_3, from 3.59 g (11.1 mmol) of Boc-Asp(OBzl), 1.57 g (11.2 mmol) of HOBt, 2.39 g (11.7 mmol) of DCC and 4 g (10.5 mmol) of TosH·H-Val-OBzl 5.15 g (90%) of the title compound was obtained as yellow syrup. $[\alpha]_D^{20} = -33.3$ ($c = 1.07$, CH_3OH), ESI-MS (m/z) 514 $[M + H]^+$.

4.2.6. HCl·H-Asp(OBzl)-Val-OBzl

Using the same procedure as described for HCl·H-Asp(OBzl)-Ser(Bzl)-OBzl, from 6.15 g of Boc-Asp(OBzl)-Val-OBzl and 80 ml of 4 N solution of hydrochloride in ethyl acetate 4.01 g (total yield 92%) title compound was obtained, Mp 105–107 °C, $[\alpha]_D^{20} = -18.6$ ($c = 1.20$, CH_3OH), ESI-MS (m/z) 414 $[M + H]^+$.

4.2.7. Boc-Asp(OBzl)-Phe-OBzl

Using the same procedure as described for Boc-Arg(NO_2)-Gly-OCH_3, from 2.28 g (7.0 mmol) of Boc-Asp(OBzl), 1.04 g (7.4 mmol) of HOBt, 1.59 g (7.8 mmol) of DCC and 3.00 g (7.0 mmol) of TosH·H-Phe-OBzl 4.15 g of the title compound was obtained as yellow powder. Mp 81.9–82.8 °C, $[\alpha]_D^{20} = 0.4$ ($c = 1.27$, MeOH), ESI-MS (m/z) 562 $[M + H]^+$.

4.2.8. HCl·H-Asp(OBzl)-Phe-OBzl

Using the same procedure as described for HCl·H-Asp(OBzl)-Ser(Bzl)-OBzl, from 4.15 g of Boc-Asp(OBzl)-Phe-OBzl and 60 ml of 4 N solution of hydrochloride in ethyl acetate 3.03 g (total yield 86.5%) of the title compound was obtained as yellow powder. Mp 123.6–125.0 °C, $[\alpha]_D^{20} = -17.1$ ($c = 1.00$, CH_3OH), ESI-MS (m/z) 462 $[M + H]^+$.

4.2.9. Boc-Asp(OBzl)-Val

Using the same procedure as described for Boc-Arg(NO_2)-Gly-OCH_3, from 4.80 g (14.8 mmol) of Boc-Asp(OBzl), 1.04 g (16.3 mmol) of *N*-hydroxyl-succinamide (HOSu), 3.36 g (16.4 mmol) of DCC and 1.90 g (16.3 mmol) of L-Val 4.419 g (71%) of the title compound was obtained as colorless syrup. $[\alpha]_D^{20} = -8.8$ ($c = 0.73$, CH_3OH), ESI-MS (m/z) 424 $[M + H]^+$.

4.2.10. Boc-Asp(OBzl)-Phe

Using the same procedure as described for Boc-Arg(NO_2)-Gly-OCH_3, from 4.80 g (14.8 mmol) of Boc-Asp(OBzl), 1.04 g (16.3 mmol) of HOSu, 3.36 g (16.4 mmol) of DCC and 2.69 g (16.3 mmol) of L-Phe 5.5 g (80%) of the title compound was obtained as colorless powder. Mp 80–81 °C, $[\alpha]_D^{20} = 6.3$ ($c = 0.90$, CH_3OH), ESI-MS (m/z) 472 $[M + H]^+$.

4.2.11. Boc-Arg(NO_2)-Gly-Asp(OBzl)-Ser(Bzl)-OBzl

Using the same procedure as described for Boc-Arg(NO_2)-Gly-OCH_3, from 2.62 g (7.0 mmol) of Boc-Arg(NO_2)-Gly-OH, 3.67 g (7.0 mmol) of HCl·H-Asp(OBzl)-Ser(Bzl)-OBzl, 1.03 g (7.4 mmol) of HOBt and 1.58 g (7.7 mmol) of DCC to provide 5.65 g (95%) of the title compound as yellow powder. Mp 82–83 °C, $[\alpha]_D^{20} = -18.9$ ($c = 1.00$, CH_3OH), ESI-MS (m/z) 850 $[M + H]^+$.

*4.2.12. HCl·H-Arg(NO_2)-Gly-Asp(OBzl)-Ser(Bzl)-OBzl (**1**)*

Using the same procedure as described for HCl·H-Asp(OBzl)-Ser(Bzl)-OBzl, from 6.23 g of Boc-Arg(NO_2)-Gly-Asp(OBzl)-Ser(Bzl)-OBzl and 60 ml of 4 N solution of hydrochloride in ethyl acetate 4.02 g (total yield 89%) of the title compound was obtained as colorless powder. Mp 106–108 °C, $[\alpha]_D^{20} = -4.3$ ($c = 0.70$, CH_3OH), ESI-MS (m/z) 750 $[M + H]^+$.

4.2.13. Boc-Arg(NO_2)-Gly-Asp(OBzl)-Val-OBzl

Using the same procedure as described for Boc-Arg(NO_2)-Gly-OCH_3, from 4.93 g (13.09 mmol) of Boc-Arg(NO_2)-Gly, 5.89 g (13.1 mmol) of HCl·H-Asp(OBzl)-Val-OBzl, 1.95 g (13.9 mmol) of HOBt and 2.97 g (14.5 mmol) of DCC 9.31 g (92%) of the title compound was obtained as colorless powder. Mp 59–62 °C, $[\alpha]_D^{20} = -22.7$ ($c = 1.00$, CH_3OH), ESI-MS (m/z) 772 $[M + H]^+$.

*4.2.14. HCl·H-Arg(NO_2)-Gly-Asp(OBzl)-Val-OBzl (**2**)*

Using the same procedure as described for HCl·H-Asp(OBzl)-Ser(Bzl)-OBzl, from 6.23 g of Boc-Arg(NO_2)-Gly-Asp(OBzl)-Val-OBzl and 60 ml of 4 N solution of hydrochloride in ethyl acetate 6.69 g (total yield 76%) of the title compound was obtained as colorless powder. Mp 98–100 °C, $[\alpha]_D^{20} = -12.4$ ($c = 1.00$, CH_3OH), ESI-MS (m/z) 672 $[M + H]^+$.

4.2.15. Boc-Arg(NO_2)-Gly-Asp(OBzl)-Phe-OBzl

Using the same procedure as described for Boc-Arg(NO_2)-Gly-OCH_3, from 5.08 g (13.5 mmol) of Boc-Arg(NO_2)-Gly, 6.35 g (12.8 mmol) of HCl·H-Asp(OBzl)-Phe-OBzl, 2.00 g (14.3 mmol) of HOBt and 3.05 g (14.9 mmol) of DCC 10.95 g (99%) of the title compound was obtained as colorless powder. Mp 102–104 °C, $[\alpha]_D^{20} = -20.6$ ($c = 1.20$, CH_3OH), ESI-MS (m/z) 820 $[M + H]$.

*4.2.16. HCl·H-Arg(NO_2)-Gly-Asp(OBzl)-Phe-OBzl (**3**)*

Using the same procedure as described for HCl·H-Asp(OBzl)-Ser(Bzl)-OBzl, from 10.95 g of Boc-Arg(NO_2)-Gly-Asp(OBzl)-Phe-OBzl and 100 ml of 4 N solution of hydrochloride in ethyl acetate 7.84 g (total yield 81%) was obtained as colorless powder. Mp 102–104 °C, $[\alpha]_D^{20} = -9.3$ ($c = 1.47$, CH_3OH), ESI-MS (m/z) 720 $[M + H]^+$.

4.2.17. Boc-Ser(Bzl)-Arg(NO_2)-Gly-Asp(OBzl)-Ser(Bzl)-OBzl

Using the same procedure as described for Boc-Arg(NO_2)-Gly-OCH_3, from 1.13 g (3.8 mmol) of Boc-Ser(Bzl), 3.0 g (3.8 mmol) of HCl·H-Arg(NO_2)-Gly-Asp(OBzl)-Ser(Bzl)-OBzl, 0.57 g (4.1 mmol) of HOBt and 0.87 g (4.2 mmol) of DCC 3.87 g (99%) of the title compound was obtained as colorless powder. Mp 103–104 °C, $[\alpha]_D^{20} = +0.4$ ($c = 0.77$, CH_3OH), ESI-MS (m/z) 1027 $[M + H]^+$.

4.2.18. HCl·H-Ser(Bzl)-Arg(NO_2)-Gly-Asp(OBzl)-Ser(Bzl)-OBzl

Using the same procedure as described for HCl·H-Asp(OBzl)-Ser(Bzl)-OBzl, from 3.87 g of Boc-Ser(Bzl)-Arg(NO_2)-Gly-Asp(OBzl)-Ser(Bzl)-OBzl and 60 ml of 4 N solution of hydrochloride in ethyl acetate 3.31 g (95%) of the title compound was obtained as colorless powder. Mp 159–163 °C, $[\alpha]_D^{20} = -0.3$ ($c = 0.40$, CH_3OH), ESI-MS (m/z) 927 $[M + H]^+$.

4.2.19. Boc-Asp(OBzl)-Ser(Bzl)-Arg(NO_2)-Gly-Asp(OBzl)-Ser(Bzl)-OBzl

Using the same procedure as described for Boc-Arg(NO_2)-Gly-OCH_3, from 1.12 g (3.4 mmol) of Boc-Asp(OBzl), 3.31 g (3.4 mmol) of HCl·H-Ser(Bzl)-Arg(NO_2)-Gly-Asp(OBzl)-Ser(Bzl)-OBzl, 0.51 g (3.6 mmol) of HOBt and 0.78 g (3.8 mmol) of DCC 4.16 g (98%) of the title compound was obtained as colorless powder. Mp 123–124 °C, $[\alpha]_D^{20} = -8.0$ ($c = 0.67$, CH_3OH), ESI-MS (m/z) 1233 $[M + H]^+$.

4.2.20. HCl·H-Asp(OBzl)-Ser(Bzl)-Arg(NO_2)-Gly-Asp(OBzl)-Ser(Bzl)-OBzl

Using the same procedure as described for HCl·H-Asp(OBzl)-Ser(Bzl)-OBzl, from 4.16 g of Boc-Asp(OBzl)-Ser(Bzl)-Arg(NO_2)-Gly-Asp(OBzl)-Ser(Bzl)-OBzl and 60 ml of 4 N solution of hydrochloride in ethyl acetate 3.78 g (96%) of the title compound was obtained as colorless powder. Mp 129–131 °C, $[\alpha]_D^{20} = -6.9$ ($c = 1.00$, CH_3OH), ESI-MS (m/z) 1133 $[M + H]^+$.

4.2.21. Boc-Asp(OBzl)-Val-Arg(NO_2)-Gly-Asp(OBzl)-Val-OBzl

Using the same procedure as described for Boc-Arg(NO_2)-Gly-OCH_3, from 2.11 g (5.0 mmol) of Boc-Asp(OBzl)-Val, 3.52 g (5.0 mmol) of HCl·H-Arg(NO_2)-Gly-Asp(OBzl)-Val-OBzl, 0.74 g

(5.3 mmol) of HOBt and 1.13 g (5.5 mmol) of DCC 5.14 g (96%) of the title compound was obtained as colorless powder. Mp 157–158 °C, $[\alpha]_D^{20} = -23.6$ ($c = 0.87$, CH_3OH), ESI-MS (m/z) 1077 $[M + H]^+$.

4.2.22. HCl·H-Asp(OBzl)-Val-Arg(NO_2)-Gly-Asp(OBzl)-Val-OBzl

Using the same procedure as described for HCl·H-Asp(OBzl)-Ser(Bzl)-OBzl, from 5.14 g of Boc-Asp(OBzl)-Val-Arg(NO_2)-Gly-Asp(OBzl)-Val-OBzl and 80 ml of 4 N solution of hydrochloride in ethyl acetate 4.33 g (90%) of the title compound was obtained as colorless powder. Mp 116–118 °C, $[\alpha]_D^{20} = -11.9$ ($c = 1.00$, CH_3OH), ESI-MS (m/z) 977 $[M + H]^+$.

4.2.23. Boc-Asp(OBzl)-Phe-Arg(NO_2)-Gly-Asp(OBzl)-Phe-OBzl

Using the same procedure as described for Boc-Arg(NO_2)-Gly-OCH_3, from 0.47 g (1.0 mmol) of Boc-Asp(OBzl)-Phe, 0.75 g (1.0 mmol) of HCl·H-Arg(NO_2)-Gly-Asp(OBzl)-Phe-OBzl, 0.15 g (1.1 mmol) of HOBt and 0.23 g (1.1 mmol) of DCC 1.18 g (99%) of the title compound was obtained as colorless powder. Mp 92–94 °C, $[\alpha]_D^{20} = -12.7$ ($c = 1.00$, CH_3OH), ESI-MS (m/z) 1173 $[M + H]^+$.

4.2.24. HCl·H-Asp(OBzl)-Phe-Arg(NO_2)-Gly-Asp(OBzl)-Phe-OBzl

Using the same procedure as described for HCl·H-Asp(OBzl)-Ser(Bzl)-OBzl, from 1.18 g of Boc-Asp(OBzl)-Phe-Arg(NO_2)-Gly-Asp(OBzl)-Phe-OBzl and 20 ml of 4 N solution of hydrochloride in ethyl acetate 1.01 g (total yield 92%) of the title compound was obtained as colorless powder. Mp 145–146 °C, $[\alpha]_D^{20} = -5.4$ ($c = 0.63$, CH_3OH), ESI-MS (m/z) 1073 $[M + H]^+$.

4.2.25. Boc-Arg(NO_2)-Gly-Asp(OBzl)-Ser(Bzl)-Arg(NO_2)-Gly-Asp(OBzl)-Ser(Bzl)-OBzl

Using the same procedure as described for Boc-Arg(NO_2)-Gly-OCH_3, from 1.42 g (3.8 mmol) of Boc-Arg(NO_2)-Gly, 4.42 g (3.8 mmol) of HCl·H-Asp(OBzl)-Ser(Bzl)-Arg(NO_2)-Gly-Asp(OBzl)-Ser(Bzl)-OBzl, 0.56 g (4.0 mmol) of HOBt and 0.86 g (4.2 mmol) of DCC 5.14 g (91%) of the title compound was obtained as colorless powder. Mp 134–136 °C, $[\alpha]_D^{20} = -6.3$ ($c = 1.07$, CH_3OH), ESI-MS (m/z) 1491$[M + H]^+$.

*4.2.26. HCl·H-Arg(NO_2)-Gly-Asp(OBzl)-Ser(Bzl)-Arg(NO_2)-Gly-Asp(OBzl)-Ser(Bzl)-OBzl (**4**)*

Using the same procedure as described for HCl·H-Asp(OBzl)-Ser(Bzl)-OBzl, from 2.50 g of Boc-Arg(NO_2)-Gly-Asp(OBzl)-Ser(Bzl)-Arg(NO_2)-Gly-Asp(OBzl)-Ser(Bzl)-OBzl and 60 ml of 4 N solution of hydrochloride in ethyl acetate 2.15 g (90%) of the title compound was obtained as colorless powder. Mp. 157–159 °C, $[\alpha]_D^{20} = -4.3$ ($c = 0.60$, CH_3OH), ESI-MS (m/z) 1391 $[M + H]^+$.

4.2.27. Boc-Arg(NO_2)-Gly-Asp(OBzl)-Val-Arg(NO_2)-Gly-Asp(OBzl)-Val-OBzl

Using the same procedure as described for Boc-Arg(NO_2)-Gly-OCH_3, from 1.17 g (3.1 mmol) of Boc-Arg(NO_2)-Gly, 3.0 g (3.0 mmol) of HCl·H-Asp(OBzl)-Val-Arg(NO_2)-Gly-Asp(OBzl)-Val-OBzl, 0.46 g (3.3 mmol) of HOBt and 0.68 g (3.3 mmol) of DCC 2.73 g (69%) of the title compound was obtained as colorless powder. Mp 129–130 °C, $[\alpha]_D^{20} = -17.7$ ($c = 1.00$, CH_3OH), ESI-MS (m/z) 1435 $[M + H]^+$.

*4.2.28. HCl·H-Arg(NO_2)-Gly-Asp(OBzl)-Val-Arg(NO_2)-Gly-Asp(OBzl)-Val-OBzl (**5**)*

Using the same procedure as described for HCl·H-Asp(OBzl)-Ser(Bzl)-OBzl, from 2.50 g of Boc-Arg(NO_2)-Gly-Asp(OBzl)-Val-Arg(NO_2)-Gly-Asp(OBzl)-Val-OBzl and 50 ml of 4 N solution of hydrochloride in ethyl acetate 2.25 g (95%) of the title compound was obtained as colorless powder. Mp 135–137 °C, $[\alpha]_D^{20} = -33.2$ ($c = 1.20$, CH_3OH), ESI-MS (m/z) 1335 $[M + H]^+$.

4.2.29. Boc-Arg(NO_2)-Gly-Asp(OBzl)-Phe-Arg(NO_2)-Gly-Asp(OBzl)-Phe-OBzl

Using the same procedure as described for Boc-Arg(NO_2)-Gly-OCH_3, from 0.89 g (2.3 mmol) of Boc-Arg(NO_2)-Gly, 2.5 g (3.4 mmol) of HCl·H-Asp(OBzl)-Phe-Arg(NO_2)-Gly-Asp(OBzl)-Phe-OBzl, 0.34 g (2.4 mmol) of HOBt and 0.51 g (2.5 mmol) of DCC 2.50 g (78%) of the title compound was obtained as colorless powder. Mp 124–126 °C, $[\alpha]_D^{20} = -12.0$ ($c = 0.60$, CH_3OH), ESI-MS (m/z) 1431 $[M + H]^+$.

*4.2.30. HCl·H-Arg(NO_2)-Gly-Asp(OBzl)-Phe-Arg(NO_2)-Gly-Asp(OBzl)-Phe-OBzl (**6**)*

Using the same procedure as described for HCl·H-Asp(OBzl)-Ser(Bzl)-OBzl, from 2.50 g of Boc-Arg(NO_2)-Gly-Asp(OBzl)-Phe-Arg(NO_2)-Gly-Asp(OBzl)-Phe-OBzl and 50 ml of 4 N solution of hydrochloride in ethyl acetate 2.1 g (88%) of the title compound was obtained as colorless powder. Mp 127–129 °C, $[\alpha]_D^{20} = -6.5$ ($c = 1.07$, CH_3OH), ESI-MS (m/z) 1331 $[M + H]^+$.

*4.2.31. HCl·H-Arg-Gly-Asp-Ser-Arg-Gly-Asp-Ser (**7**)*

To the suspension of 100 mg (0.07 mmol) of **4**, 8 ml of methanol, 2 ml of water, 10 mg of Pd/C (10%) hydrogen gas was bubbled for 20 h and TLC indicates complete disappearance of **4**. After filtration the filtrate was evaporated under vacuum and the residue was triturated with ether repeatedly to provide the title compound in 51 mg (86% yield) as colorless powder. Mp 117–119 °C, $[\alpha]_D^{20} = -37.0$ ($c = 0.95$, CH_3OH), ESI-MS 849 $[M + H]^+$. ^1H NMR (BHSC-300, $CDCl_3$) $\delta = 11.0$ (s, 3H), 7.89 (d, $J = 5.2$ Hz, 7H), 5.7 (s, 2H), 4.62 (q, $J = 6.3$ Hz, 1H), 4.86 (q, $J = 6.2$ Hz, 2H), 4.55 (q, $J = 6.3$ Hz, 1H), 4.53 (t, $J = 6.3$ Hz, 1H), 4.09 (d, $J = 6.2$ Hz, 4H), 4.03 (d, $J = 7.0$ Hz, 4H), 3.56 (q, $J = 6.5$ Hz, 1H), 2.73 (t, $J = 6.6$ Hz, 4H), 2.65 (q, $J = 6.8$ Hz, 4H), 2.0 (s, 10H), 1.79 (q, $J = 7.4$ Hz, 4H), 1.55 (m, 4H). Anal. Calcd for $C_{30}H_{52}N_{14}O_{15}$: C 42.45, H 6.17, N 23.10; Found C 42.64, H 6.30, N 23.28.

*4.2.32. HCl·H-Arg-Gly-Asp-Val-Arg-Gly-Asp-Val (**8**)*

Using the same procedure as described for **7**, from 100 mg (0.07 mmol) of **5** 48 mg (73%) of the title compound was obtained as colorless powder. Mp 121–23 °C, $[\alpha]_D^{20} = -69.0$ ($c = 1.01$, CH_3OH), ESI-MS 873 $[M + H]^+$. ^1H NMR (BHSC-300, $CDCl_3$) $\delta = 11.0$ (s, 3H), 7.89 (d, $J = 5.2$ Hz, 7H), 5.70 (s, 2H), 4.86 (q, $J = 6.2$ Hz, 2H), 4.53 (t, $J = 6.3$ Hz, 2H), 4.45 (q, $J = 6.0$ Hz, 1H), 4.09 (d, $J = 6.0$ Hz, 4H), 3.56 (q, $J = 6.5$ Hz, 1H), 2.78 (m, 1H), 2.73 (t, $J = 6.6$ Hz, 4H), 2.68 (m, 1H), 2.65 (q, $J = 6.0$ Hz, 4H), 2.00 (s, 8H), 1.79 (q, $J = 7.4$ Hz, 4H), 1.55 (m, 4H), 1.01 (d, $J = 8.1$ Hz, 12H). Anal. Calcd for $C_{34}H_{60}N_{14}O_{13}$: C 46.78, H 6.93, N 22.46; Found C 46.60, H 6.88, N 22.65.

*4.2.33. HCl·H-Arg-Gly-Asp-Phe-Arg-Gly-Asp-Phe (**9**)*

Using the same procedure as described for **7**, from 100 mg (0.07 mmol) of **6** 55 mg (77%) of the title compound was obtained as colorless powder. Mp 149–151 °C, $[\alpha]_D^{20} = -17.0$ ($c = 1.06$, CH_3OH), ESI-MS 969 $[M + H]^+$. ^1H NMR (BHSC-300, $CDCl_3$) $\delta = 11.0$ (s, 3H), 7.89 (d, $J = 5.2$ Hz, 7H), 7.21 (d, $J = 7.3$ Hz, 4H), 7.12 (m, 4H), 7.08 (t, $J = 7.2$ Hz, 2H), 5.70 (s, 2H), 4.92 (q, $J = 6.5$ Hz, 1H), 4.86 (q, $J = 6.2$ Hz, 3H), 4.53 (t, $J = 6.3$ Hz, 1H), 4.09 (d, $J = 6.2$ Hz, 4H), 3.56 (q, $J = 6.5$ Hz, 1H), 3.05 (d, $J = 6.3$ Hz, 4H), 2.73 (t, $J = 6.6$ Hz, 4H), 2.65 (q, $J = 6.8$ Hz, 4H), 2.00 (s, 8H), 1.79 (q, $J = 7.4$ Hz, 4H), 1.55 (m, 4H). Anal. Calcd for $C_{42}H_{60}N_{14}O_{13}$: C 52.06, H 6.24, N 20.24; Found C 51.87, H 6.38, N 20.07.

4.3. Preparing N-(estradiol-3-oxyacetyl)-RGD octapeptide conjugates

*4.3.1. Ethyl estradiol-3-oxyacetate (**10**)*

The solution of 1.00 g (3.7 mmol) of estradiol in 15 ml of anhydrous THF and the solution of 7.40 ml of sodium ethoxide in

anhydrous ethanol (2 M) were mixed and stirred at room temperature for 30 min, and then 1.64 ml ($d = 1.514$ g/ml, 12.2 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 for 4 times to completely remove the residual ethyl bromoacetate. The residue was purified on silica gel column (chloroform/ether, 10:0.3) to provide 1.22 g (92%) of the title compound as 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) δ/ppm = 7.12 (d, $J = 7.5$ Hz, 1H), 6.71 (s, 1H), 6.52 (d, $J = 7.5$ Hz, 1H), 5.14 (s, 2H), 4.61 (q, $J = 4.8$ Hz, 1H), 4.20 (q, $J = 4.4$ Hz, 2H), 2.97 (t, $J = 4.6$ Hz, 2H), 2.91 (m, $J = 4.2$ Hz, 1H), 2.55 (s, 1H), 2.03 (m, $J = 4.8$ Hz, 2H), 2.01 (m, $J = 4.3$ Hz, 2H), 1.87 (m, $J = 4.2$ Hz, 1H), 1.84 (m, $J = 4.4$ Hz, 2H), 1.76 (m, $J = 4.6$ Hz, 2H), 1.70 (m, $J = 4.3$ Hz, 1H), 1.65 (m, $J = 4.7$ Hz, 2H), 1.41 (t, $J = 4.4$ Hz, 3H), 0.78 (s, 3H). ESI-MS (m/z) 359 $[M + H]^+$, $[\alpha]_D^{20} = +60.0$ ($c = 1.00$, THF).

4.3.2. Estradiol-3-oxyacetic acid (**11**)

At 0 °C to the solution of 1.30 g (3.6 mmol) of **10** in 10 ml of methanol 10 ml of NaOH aqueous solution (2 N) was added. The reaction mixture was stirred at room temperature for 1.5 h and TLC (ethyl acetate/petroleum, 6:1) indicated complete disappearance of **10**. 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 1.11 g (93%) of the title compound as 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) δ/ppm = 10.55 (s, 1H), 7.00 (d, $J = 7.5$ Hz, 1H), 6.63 (s, 1H), 6.51 (d, $J = 7.5$ Hz, 1H), 5.00 (s, 2H), 4.42 (q, $J = 4.8$ Hz, 1H), 2.97 (t, $J = 4.6$ Hz, 2H), 2.89 (m, $J = 4.3$ Hz, 1H), 2.56 (s, 1H), 2.02 (m, $J = 4.8$ Hz, 2H), 2.01 (m, $J = 4.3$ Hz, 2H), 1.86 (m, $J = 4.2$ Hz, 1H), 1.84 (m, $J = 4.4$ Hz, 2H), 1.75 (m, $J = 4.6$ Hz, 2H), 1.70 (m, $J = 4.3$ Hz, 1H), 1.65 (m, $J = 4.7$ Hz, 2H), 0.783 (s, 3H). ESI-MS (m/z) 331 $[M + H]^+$. $[\alpha]_D^{20} = 50.0$ ($c = 0.60$, THF).

4.3.3. N-(Estradiol-3-oxyacetyl)-Arg(NO_2)-Gly-Asp(OBzl)-Ser(Bzl)-Arg(NO_2)-Gly-Asp(OBzl)-Ser(Bzl)-OBzl (**15**)

Using the same procedure as described for Boc-Arg(NO_2)-Gly-OCH_3, from 0.17 g (0.52 mmol) of **11**, 0.70 g (0.49 mmol) of **4**, 0.08 g (0.57 mmol) of HOBt and 0.12 g (0.57 mmol) of DCC 0.60 g (71.4%) of the title compound was obtained as colorless powder. Mp 176–178 °C, ESI-MS (m/z) 1703 $[M + H]^+$, $[\alpha]_D^{20} = 3.0$ ($c = 1$, DMSO). ^1H NMR (DMSO-d_6) δ/ppm = 8.46 (d, $J = 5.1$ Hz, 2H), 8.36 (t, $J = 7.2$ Hz, 2H), 8.28 (d, $J = 4.8$ Hz, 2H), 8.29 (d, $J = 5.1$ Hz, 2H), 8.25 (d, $J = 4.5$ Hz, 2H), 7.35 (s, 2H), 7.34 (s, 2H), 7.26–7.32 (m, 25H), 7.15 (d, $J = 8.7$ Hz, 1H), 6.69 (d, $J = 8.7$ Hz, 1H), 6.63 (s, 1H), 5.15 (s, 2H), 5.14 (s, 2H), 5.14 (s, 2H), 5.08 (d, $J = 5.1$ Hz, 1H), 4.82 (s, 2H), 4.79 (s, 2H), 4.70 (s, 2H), 4.56 (m, 2H), 4.54 (m, 2H), 4.55 (m, 2H), 4.48 (m, 2H), 3.76 (d, $J = 2.1$ Hz, 2H), 3.75 (d, $J = 2.1$ Hz, 2H), 3.53 (d, $J = 3.3$ Hz, 1H), 2.97 (d, $J = 2.4$ Hz, 2H), 2.92 (d, $J = 2.7$ Hz, 2H), 2.91 (d, $J = 1.8$ Hz, 2H), 2.82 (m, 1H), 2.74 (m, 2H), 2.73 (m, 2H), 1.95 (m, 2H), 1.94 (m, 2H), 1.82 (m, 2H), 1.82 (m, 2H), 1.81 (m, 2H), 1.80 (m, 2H), 1.73 (m, 2H), 1.72 (m, 2H), 1.69 (m, 2H), 1.67 (m, 1H), 1.65 (m, 1H), 1.43 (m, 2H), 1.41 (m, 2H), 0.87 (s, 3H). ^{13}C NMR (DMSO-d_6) δ/ppm = 171.01, 170.79, 170.49, 170.20, 170.16, 169.21, 168.97, 159.80, 157.15, 138.51, 138.29, 136.47, 136.24, 128.83, 128.67, 128.41, 128.32, 128.15, 127.96, 127.86, 126.63, 112.63, 115.00, 80.54, 72.79, 72.69, 69.52, 67.34, 66.62, 66.24, 66.18, 53.55, 53.16, 52.82, 52.47, 50.06, 49.76, 49.51, 48.02, 43.99, 39.02, 37.08, 36.90, 36.71, 33.81, 30.42, 29.66, 26.48, 25.82, 24.91, 23.25, 11.70.

4.3.4. N-(Estradiol-3-oxyacetyl)-Arg(NO_2)-Gly-Asp(OBzl)-Val-Arg(NO_2)-Gly-Asp(OBzl)-Val-OBzl (**16**)

Using the same procedure as described for Boc-Arg(NO_2)-Gly-OCH_3, from 0.19 g (0.60 mmol) of **11**, 0.70 g (0.60 mmol) of **5**, 0.09 g (0.60 mmol) of HOBt and 0.13 g (0.60 mmol) of DCC 0.53 g (61.8%) of the title compound was obtained as colorless powder. Mp 168–172 °C, ESI-MS (m/z) 1547 $[M + H]^+$, $[\alpha]_D^{20} = -4.4$ ($c = 1$, DMSO). ^1H NMR (DMSO-d_6) δ/ppm = 8.48 (d, $J = 5.1$ Hz, 2H), 8.46 (t, $J = 7.2$ Hz, 2H), 8.34 (d, $J = 4.8$ Hz, 2H), 8.28 (d, $J = 5.1$ Hz, 2H), 8.16 (d, $J = 4.5$ Hz, 2H), 7.35 (s, 2H), 7.34 (s, 2H), 7.27–7.40 (m, 15H), 7.14 (d, $J = 8.7$ Hz, 1H), 6.68 (d, $J = 8.7$ Hz, 1H), 6.62 (s, 1H), 5.11 (s, 2H), 5.09 (s, 2H), 5.07 (s, 2H), 5.05 (d, $J = 6.6$ Hz, 1H), 5.03 (m, 1H), 5.03 (m, 1H), 4.52 (m, 2H), 4.51 (m, 2H), 4.11 (m, 2H), 4.10 (m, 2H), 3.61 (d, $J = 2.1$ Hz, 2H), 3.57 (d, $J = 2.1$ Hz, 2H), 3.28 (d, $J = 3.3$ Hz, 1H), 2.96 (d, $J = 2.4$ Hz, 2H), 2.91 (d, $J = 2.7$ Hz, 2H), 2.90 (d, $J = 1.8$ Hz, 2H), 2.86 (d, $J = 2.7$ Hz, 2H), 2.81 (m, 1H), 2.34 (m, 1H), 2.20 (m, 1H), 1.98 (m, 2H), 1.98 (m, 2H), 1.93 (m, 2H), 1.90 (m, 2H), 1.87 (m, 2H), 1.86 (m, 2H), 1.74 (m, 2H), 1.72 (m, 2H), 1.68 (m, 2H), 1.64 (m, 2H), 1.63 (m, 1H), 1.61 (m, 1H), 1.10 (s, 3H), 0.97 (d, $J = 1.8$ Hz, 3H), 0.97 (d, $J = 1.8$ Hz, 3H), 0.96 (d, $J = 1.8$ Hz, 3H), 0.96 (d, $J = 1.8$ Hz, 3H). ^{13}C NMR (DMSO-d_6) δ/ppm = 171.96, 171.49, 171.19, 171.04, 170.57, 170.49, 170.22, 169.27, 169.09, 168.52, 159.75, 157.09, 155.94, 137.99, 136.45, 136.27, 133.51, 128.84, 128.56, 128.49, 128.42, 128.33, 126.64, 112.59, 114.92, 80.51, 67.48, 66.41, 58.25, 58.12, 50.01, 49.77, 49.54, 47.98, 43.98, 43.26, 37.04, 36.70, 33.81, 31.13, 30.86, 30.38, 30.24, 27.26, 25.79, 24.92, 23.23, 19.67, 19.34, 18.57, 18.37, 11.70.

4.3.5. N-(Estradiol-3-oxyacetyl)-Arg(NO_2)-Gly-Asp(OBzl)-Phe-Arg(NO_2)-Gly-Asp(OBzl)-Phe-OBzl (**17**)

Using the same procedure as described for Boc-Arg(NO_2)-Gly-OCH_3, from 0.18 g (0.54 mmol) of **11**, 0.70 g (0.51 mmol) of **6**, 0.08 g (0.57 mmol) of HOBt and 0.12 g (0.59 mmol) of DCC 0.59 g (70.6%) of the title compound was obtained as colorless powder. Mp 146–148 °C, ESI-MS (m/z) 1643 $[M + H]^+$, $[\alpha]_D^{20} = +4.5°$ ($c = 1$, DMSO). ^1H NMR (DMSO-d_6) δ/ppm = 8.40 (d, $J = 5.1$ Hz, 2H), 8.34 (t, $J = 7.2$ Hz, 2H), 8.23 (d, $J = 4.8$ Hz, 2H), 8.13 (d, $J = 5.1$ Hz, 2H), 8.05 (d, $J = 4.5$ Hz, 2H), 7.34 (s, 2H), 7.30 (s, 2H), 7.13–7.28 (m, 25H), 7.14 (d, $J = 8.7$ Hz, 1H), 6.67 (d, $J = 8.7$ Hz, 1H), 6.65 (s, 1H), 5.10 (d, $J = 5.5$ Hz, 1H), 5.08 (s, 2H), 5.07 (s, 2H), 5.05 (s, 2H), 5.04 (m, 1H), 5.03 (m, 1H), 4.79 (s, 2H), 4.60 (m, 2H), 4.54 (m, 2H), 3.75 (d, $J = 2.1$ Hz, 2H), 3.74 (d, $J = 2.1$ Hz, 2H), 3.20 (d, $J = 3.3$ Hz, 1H), 3.10 (d, $J = 2.4$ Hz, 2H), 2.79 (m, 1H), 2.79 (m, 2H), 2.58 (m, 2H), 2.58 (m, 2H), 1.95 (m, 2H), 1.92 (m, 2H), 1.92 (m, 2H), 1.84 (m, 2H), 1.82 (m, 2H), 1.81 (m, 2H), 1.81 (m, 2H), 1.71 (m, 2H), 1.71 (m, 2H), 1.69 (m, 2H), 1.68 (m, 1H), 1.64 (m, 1H), 1.48 (m, 2H), 1.42 (m, 2H), 0.91 (s, 3H). ^{13}C NMR (DMSO-d_6) δ/ppm = 171.98, 171.46, 171.24, 170.87, 170.57, 170.45, 170.21, 169.09, 168.52, 159.76, 157.09, 155.9, 137.98, 137.30, 136.43, 136.08, 133.44, 129.70, 129.57, 128.85, 128.49, 128.35, 127.06, 126.70, 114.86, 112.56, 80.51, 67.48, 66.50, 54.38, 52.72, 52.42, 49.96, 49.62, 49.41, 47.96, 43.97, 43.25, 42.21, 37.57, 36.93, 33.81, 30.35, 29.67, 27.26, 26.44, 25.78, 24.93, 23.22, 16.40.

4.3.6. N-(Estradiol-3-oxyacetyl)-Arg-Gly-Asp-Ser-Arg-Gly-Asp-Ser (**21**)

Using the same procedure as described for **7**, from 300 mg (0.176 mmol) of **15** 180 mg (88%) of the title compound was obtained as colorless powder. Mp 250 °C (decomp.), $[\alpha]_D^{20} = -3.6$ ($c = 1.48$, DMSO), ESI-MS (m/z): 1161 $[M + H]^+$, 1057 $[M - (Ser) + H]^+$, 942 $[M - (Asp\text{-}Ser) + H]^+$, 885 $[M - (Gly\text{-}Asp\text{-}Ser) + H]^+$, 729 $[M - (Arg\text{-}Gly\text{-}Asp\text{-}Ser) + H]^+$, 642 $[M - (Ser\text{-}Arg\text{-}Gly\text{-}Asp\text{-}Ser) + H]^+$, 527 $[M - (Asp\text{-}Ser\text{-}Arg\text{-}Gly\text{-}Asp\text{-}Ser) + H]^+$, 470 $[M - (Gly\text{-}Asp\text{-}Ser\text{-}Arg\text{-}Gly\text{-}Asp\text{-}Ser) + H]^+$. ^1H NMR (DMSO-d_6) δ/ppm = 9.88 (s, 1H), 9.665 (s, 2H), 8.42 (d, $J = 5.4$ Hz, 2H), 8.37 (t, $J = 6.8$ Hz, 2H), 8.26 (d, $J = 4.8$ Hz, 2H), 8.12 (d, $J = 5.4$ Hz, 2H), 8.11 (d, $J = 4.5$ Hz, 2H), 8.01 (s, 1H), 7.98 (s, 1H), 7.34 (s, 2H), 7.32 (s, 2H), 7.13 (d, $J = 8.7$ Hz, 1H), 6.67 (d, $J = 8.7$ Hz, 1H), 6.63 (s, 1H),

4.60 (t, $J = 3.6$ Hz, 1H), 4.58 (t, $J = 3.6$ Hz, 1H), 4.64 (s, 2H), 4.63 (d, $J = 4.5$ Hz, 1H), 4.62 (m, 1H), 4.52 (m, 1H), 4.51 (m, 1H), 4.50 (m, 1H), 4.41 (m, 2H), 3.88 (d, $J = 3.6$ Hz, 2H), 3.67 (d, $J = 3.3$ Hz, 2H), 3.62 (d, $J = 2.1$ Hz, 2H), 3.60 (d, $J = 2.1$ Hz, 2H), 3.58 (m, 1H), 3.54 (d, $J = 3.3$ Hz, 2H), 3.42 (d, $J = 3.6$ Hz, 2H), 2.86 (t, $J = 2.1$ Hz, 2H), 2.74 (m, 1H), 2.47 (m, 2H), 2.10 (m, 2H), 1.98 (m, 2H), 1.87 (m, 2H), 1.75 (m, 2H), 1.73 (m, 2H), 1.62 (m, 2H), 1.60 (m, 2H), 1.52 (m, 2H), 1.46 (m, 2H), 1.36 (m, 2H), 1.28 (m, 1H), 1.26 (m, 1H), 1.24 (m, 2H), 0.67 (s, 3H). ^{13}C NMR (DMSO-d_6) δ/ppm = 176.03, 175.29, 174.86, 173.54, 173.08, 172.61, 171.45, 170.83, 168.80, 168.39, 157.72, 157.10, 137.98, 133.37, 126.66, 114.87, 112.59, 80.52, 67.05, 62.77, 50.08, 56.50, 47.98, 44.00, 42.75, 42.59, 37.04, 35.61, 34.75, 33.82, 30.37, 29.70, 27.30, 26.48, 25.79, 24.93, 23.25, 15.38. Anal. Calcd for $C_{50}H_{76}N_{14}O_{18}$: C 51.72, H 6.60, N 16.89; Found C 51.52, H 6.46, N 16.70.

4.3.7. N-(Estradiol-3-oxyacetyl)-Arg-Gly-Asp-Val-Arg-Gly-Asp-Val (**22**)

Using the same procedure as described for **7**, from 300 mg (0.194 mmol) of **16** 140 mg (60.8%) of the title compound was obtained as colorless powder. Mp 228–230 °C, $[\alpha]_D^{20} = -5.1$ ($c = 1.80$, DMSO), ESI-MS (m/z): 1186 $[M+H]^+$, 1069 $[M-(Val)+H]^+$, 954 $[M-(Asp\text{-}Val)+H]^+$, 897 $[M-(Gly\text{-}Asp\text{-}Val)+H]^+$, 741 $[M-(Arg\text{-}Gly\text{-}Asp\text{-}Val)+H]^+$, 642 $[M-(Val\text{-}Arg\text{-}Gly\text{-}Asp\text{-}Val)+H]^+$, 527 $[M-(Asp\text{-}Val\text{-}Arg\text{-}Gly\text{-}Asp\text{-}Val)+H]^+$, 470 $[M-(Gly\text{-}Asp\text{-}Val\text{-}Arg\text{-}Gly\text{-}Asp\text{-}Val)+H]^+$. ^{1}H NMR (DMSO-$d_6$) δ/ppm = 10.14 (s, 1H), 9.90 (s, 2H), 8.64 (d, $J = 5.4$ Hz, 2H), 8.51 (t, $J = 7.2$ Hz, 2H), 8.34 (d, $J = 5.1$ Hz, 2H), 8.16 (d, $J = 4.5$ Hz, 2H), 8.09 (d, $J = 4.5$ Hz, 2H), 8.07 (s, 1H), 8.04 (s, 1H), 7.35 (s, 2H), 7.18 (d, $J = 8.1$ Hz, 1H), 7.13 (s, 2H), 6.78 (d, $J = 8.7$ Hz, 1H), 6.61 (s, 1H), 4.61 (d, $J = 3.3$ Hz, 1H), 4.56 (s, 2H), 4.52 (m, 1H), 4.42 (m, 1H), 4.41 (m, 1H), 4.26 (m, 1H), 4.25 (m, 1H), 4.23 (m, 1H), 4.11 (m, 2H), 3.68 (d, $J = 3.6$ Hz, 2H), 3.59 (d, $J = 3.3$ Hz, 2H), 3.27 (m, 1H), 3.21 (d, $J = 2.1$ Hz, 2H), 3.20 (d, $J = 2.1$ Hz, 2H), 2.87 (t, $J = 2.1$ Hz, 2H), 2.84 (m, 1H), 2.60 (m, 2H), 2.58 (m, 2H), 2.57 (m, 1H), 2.20 (m, 2H), 2.099 (m, 2H), 1.96 (m, 2H), 1.87 (m, 1H), 1.78 (m, 2H), 1.78 (m, 2H), 1.66 (m, 2H), 1.58 (m, 2H), 1.50 (m, 2H), 1.41 (m, 2H), 1.36 (m, 1H), 1.32 (m, 1H), 1.04 (s, 3H), 0.836 (d, $J = 1.8$ Hz, 3H), 0.82 (d, $J = 1.8$ Hz, 3H), 0.81 (d, $J = 1.8$ Hz, 3H), 0.80 (d, $J = 1.8$ Hz, 3H). ^{13}C NMR (DMSO-d_6) δ/ppm = 176.05, 174.01, 172.71, 172.53, 171.33, 170.76, 169.04, 168.89, 168.27, 168.27, 158.04, 157.90, 138.03, 133.56, 126.63, 112.65, 114.93, 80.54, 67.24, 58.58, 57.68, 52.17, 50.08, 48.02, 47.32, 44.01, 38.04, 37.08, 33.80, 31.11, 30.68, 30.41, 29.86, 27.30, 26.50, 25.81, 25.22, 24.91, 19.66, 19.47, 18.40, 17.97, 15.35. Anal. Calcd for $C_{54}H_{84}N_{14}O_{16}$: C 54.72, H 7.14, N 16.54; Found C 54.53, H 7.30, N 16.35.

4.3.8. N-(Estradiol-3-oxyacetyl)-Arg-Gly-Asp-Phe-Arg-Gly-Asp-Phe (**23**)

Using the same procedure as described for **7**, from 300 mg (0.194 mmol) of **17** 110 mg (47.0%) of the title compound was obtained as colorless powder. Mp 210–212 °C, $[\alpha]_D^{20} = -10.1$ ($c = 1.64$, DMSO), ESI-MS (m/z) 1281 $[M+H]^+$, 1117 $[M-(Phe)+H]^+$, 1002 $[M-(Asp\text{-}Phe)+H]^+$, 945 $[M-(Gly\text{-}Asp\text{-}Phe)+H]^+$, 789 $[M-(Arg\text{-}Gly\text{-}Asp\text{-}Phe)+H]^+$, 642 $[M-(Phe\text{-}Arg\text{-}Gly\text{-}Asp\text{-}Phe)+H]^+$, 527 $[M-(Asp\text{-}Phe\text{-}Arg\text{-}Gly\text{-}Asp\text{-}Phe)+H]^+$, 470 $[M-(Gly\text{-}Asp\text{-}Phe\text{-}Arg\text{-}Gly\text{-}Asp\text{-}Phe)+H]^+$. ^{1}H NMR (DMSO-$d_6$) δ/ppm = 10.01 (s, 1H), 9.79 (s, 2H), 8.43 (d, $J = 5.4$ Hz, 2H), 8.35 (t, $J = 6.8$ Hz, 2H), 8.24 (d, $J = 4.8$ Hz, 2H), 8.12 (d, $J = 5.4$ Hz, 2H), 8.15 (d, $J = 4.5$ Hz, 2H), 8.01 (s, 1H), 8.00 (s, 1H), 7.35 (s, 2H), 7.33 (s, 2H), 7.10–7.21 (m, 10H), 7.13 (d, $J = 8.7$ Hz, 1H), 6.67 (d, $J = 8.7$ Hz, 1H), 6.63 (s, 1H), 4.65 (s, 2H), 4.64 (d, $J = 4.5$ Hz, 1H), 4.64 (m, 1H), 4.55 (m, 1H), 4.54 (m, 1H), 4.53 (m, 1H), 4.42 (m, 2H), 3.74 (d, $J = 3.6$ Hz, 2H), 3.73 (d, $J = 3.3$ Hz, 2H), 3.69 (d, $J = 2.1$ Hz, 2H), 3.69 (d, $J = 2.1$ Hz, 2H), 3.62 (m, 1H), 3.50 (d, $J = 3.3$ Hz, 2H), 3.48 (d, $J = 3.6$ Hz, 2H), 2.84 (t, $J = 2.1$ Hz, 2H), 2.83 (m, 1H), 2.58 (m, 2H), 2.04 (m, 2H), 1.98 (m, 2H), 1.97 (m, 2H), 1.74 (m, 2H), 1.72 (m, 2H), 1.61 (m, 2H), 1.60 (m, 2H), 1.50 (m, 2H), 1.47 (m, 2H), 1.36 (m, 2H), 1.35 (m, 1H), 1.34 (m, 1H), 1.27 (m, 2H), 0.78 (s, 3H). ^{13}C NMR (DMSO-d_6) δ/ppm = 175.89, 174.35, 172.64, 172.55, 171.42, 170.0, 169.03, 168.74, 168.33, 158.16, 156.03, 139.23, 138.81, 138.00, 133.46, 129.87, 128.49, 128.05, 126.00, 114.91, 112.60, 80.54, 67.15, 56.51, 55.75, 52.34, 50.31, 50.04, 48.01, 44.00, 43.28, 38.50, 37.06, 33.81, 30.40, 29.89, 29.41, 27.30, 25.81, 23.26, 11.72. Anal. Calcd for $C_{62}H_{84}N_{14}O_{16}$: C 58.11, H 6.61, N 15.30; Found C 58.30, H 6.78, N 15.49.

4.4. Preparing estradiol-17β-O-carbonylpropinyl-RGD octapeptide conjugates

4.4.1. Estradiol-17-β-O-carbonylpropinic acid (**24**)

To the warm solution of 100 mg (1.0 mmol) of succinic anhydrate 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, the ethyl acetate phase was separated and dried with anhydrous Na_2SO_4. After filtration the filtrate was evaporated at reduced pressure to give a yellow syrup. The syrup 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 strolled at room temperature for 18 h. The solution was adjusted to pH 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 for 3 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 refrigerator 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}. ^{1}H NMR (DMSO-d_6) δ/ppm = 12.34 (s, 2H), 7.14 (d, $J = 8.7$ Hz, 1H), 6.57 (d, $J = 2.4$ Hz, 1H), 6.63 (s, 1H), 4.62 (t, $J = 9.0$ Hz, 1H), 3.60 (s, 2H), 3.49 (t, $J = 8.7$ Hz, 2H), 3.36 (dt, $J = 8.1$ Hz, $J = 6.9$ Hz, 1H), 2.11 (t, $J = 4.8$ Hz, 2H), 2.06 (t, $J = 9.0$ Hz, 2H), 1.78 (dt, $J = 4.8$ Hz, $J = 3.3$ Hz, 2H), 1.56 (dt, $J = 7.1$ Hz, $J = 3.9$ Hz, 2H), 1.51 (dt, $J = 3.6$ Hz, $J = 2.7$ Hz, 2H), 1.48 (t, $J = 6.6$ Hz, 2H), 1.47 (dt, $J = 7.8$ Hz, $J = 3.0$ Hz, 1H), 1.38 (dt, $J = 6.3$ Hz, $J = 4.5$ Hz, 2H), 1.34 (dt, $J = 6.6$ Hz, $J = 4.8$ Hz, 1H), 1.17 (s, 3H). ^{13}C NMR (DMSO-d_6) δ/ppm = 173.93, 172.33, 170.85, 155.95, 137.89, 133.19, 114.54, 112.30, 82.37, 64.79, 49.97, 49.48, 43.99, 43.69, 37.02, 36.84, 30.35, 29.65, 27.52, 27.19, 26.47, 23.25, 12.31. ESI-MS (m/z) 373 $[M+H]^+$. $[\alpha]_D^{20} = 35.0$ ($c = 1.00$, THF).

4.4.2. Estradiol-17-O-β-carbonylpropionyl-Arg(NO$_2$)-Gly-Asp(OBzl)-Ser(Bzl)-Arg(NO$_2$)-Gly-Asp(OBzl)-Ser(Bzl)-OBzl (**28**)

Using the same procedure as described for Boc-Arg(NO$_2$)-Gly-OCH$_3$ from 0.19 g (0.52 mmol) of **24**, 0.70 g (0.49 mmol) of **5**, 0.08 g (0.57 mmol) of HOBt and 0.12 g (0.57 mmol) of DCC 0.55 g (65.3%) of the title compound was obtained as colorless powder. Mp 162–164 °C, ESI-MS (m/z): 1745 $[M+H]^+$, $[\alpha]_D^{20} = 1.5$ ($c = 1$, DMSO). ^{1}H NMR (DMSO-d_6) δ/ppm = 8.98 (s, 1H), 8.49 (d, $J = 5.1$ Hz, 2H), 8.47 (t, $J = 7.2$ Hz, 2H), 8.23 (d, $J = 5.4$ Hz, 2H), 8.22 (d, $J = 5.1$ Hz, 2H), 8.21 (d, $J = 4.5$ Hz, 2H), 7.35 (s, 2H), 7.31 (s, 2H), 7.26–7.30 (m, 25H), 7.08 (d, $J = 8.7$ Hz, 1H), 6.60 (d, $J = 8.7$ Hz, 1H), 6.49 (s, 1H), 5.12 (s, 2H), 5.11 (s, 2H), 5.10 (s, 2H), 5.07 (t, $J = 5.1$ Hz, 1H), 5.06 (t, $J = 5.1$ Hz, 1H), 4.71 (s, 2H), 4.70 (s, 2H), 4.60 (m, 2H), 4.53 (m, 2H), 4.22 (m, 2H), 3.96 (t, $J = 2.1$ Hz, 1H), 3.76 (d, $J = 2.1$ Hz, 2H), 3.74 (d, $J = 3.3$ Hz, 1H), 3.46 (d, $J = 2.4$ Hz, 2H), 3.41 (d, $J = 2.7$ Hz, 2H), 2.91 (d, $J = 1.$ 8 Hz, 2H), 2.82 (m, 1H), 2.80 (t, $J = 2.4$ Hz, 2H), 2.79 (t, $J = 2.4$ Hz, 2H), 2.64 (m, 2H), 2.62 (m, 2H), 1.89 (m, 2H), 1.89 (m, 2H), 1.80 (m, 2H), 1.80 (m, 2H), 1.79 (m, 2H), 1.78 (m, 2H), 1.78 (m, 2H), 1.75 (m, 2H), 1.72 (m, 2H), 1.71 (m, 2H), 1.70 (m, 1H), 1.68

(m, 1H), 1.39 (m, 2H), 1.36 (m, 2H), 1.04 (s, 3H). ^{13}C NMR (DMSO-d_6) δ/ppm = 172.41, 171.82, 171.02, 170.80, 170.49, 170.21, 170.17, 169.79, 169.33, 168.97, 159.79, 157.15, 138.52, 138.29, 136.47, 136.24, 128.84, 128.67, 128.41, 128.32, 128.15, 127.97, 127.86, 72.78, 70.02, 66.62, 66.23, 66.18, 53.54, 53.15, 53.02, 52.82, 51.74, 49.75, 49.50, 48.02, 42.44, 42.24, 38.80, 36.89, 36.66, 33.81, 30.25, 29.63, 29.25, 26.34, 24.91, 23.27, 14.51.

4.4.3. Estradiol-17-O-β-carbonylpropionyl-Arg(NO$_2$)-Gly-Asp(OBzl)-Val-Arg(NO$_2$)-Gly-Asp(OBzl)-Val-OBzl (**29**)

Using the same procedure as described for Boc-Arg(NO$_2$)-Gly-OCH$_3$, from 0.22 g (0.58 mmol) of **24**, 0.70 g (0.55 mmol) of **6**, 0.09 g (0.64 mmol) of HOBt and 0.13 g (0.64 mmol) of DCC 0.66 g (76.4%) of the title compound was obtained as colorless powder. Mp 170–173 °C, ESI-MS (*m*/*z*) 1589 $[M+H]^+$, $[\alpha]_D^{20} = +2.4$ (*c* = 1, DMSO). ^{1}H NMR (DMSO-d_6) δ/ppm = 9.01 (s, 1H), 8.28 (d, *J* = 5.1 Hz, 2H), 8.26 (t, *J* = 7.2 Hz, 2H), 8.20 (d, *J* = 5.4 Hz, 2H), 8.19 (d, *J* = 5.1 Hz, 2H), 8.10 (d, *J* = 4.5 Hz, 2H), 7.36 (s, 2H), 7.32 (s, 2H), 7.29–7.32 (m, 15H), 7.07 (d, *J* = 8.7 Hz, 1H), 6.67 (d, *J* = 8.7 Hz, 1H), 6.44 (s, 1H), 5.10 (s, 2H), 5.09 (s, 2H), 5.07 (d, *J* = 6.6 Hz, 1H), 5.04 (m, 1H), 5.03 (m, 1H), 4.53 (m, 2H), 4.53 (m, 2H), 4.12 (m, 2H), 4.11 (m, 2H), 3.61 (d, *J* = 2.1 Hz, 2H), 3.56 (d, *J* = 2.1 Hz, 2H), 3.29 (d, *J* = 3.3 Hz, 1H), 2.97 (d, *J* = 2.4 Hz, 2H), 2.92 (d, *J* = 2.7 Hz, 2H), 2.91 (d, *J* = 1.8 Hz, 2H), 2.87 (d, *J* = 2.7 Hz, 2H), 2.85 (t, *J* = 2.1 Hz, 2H), 2.84 (t, *J* = 2.1 Hz, 2H), 2.81 (m, 1H), 2.40 (m, 1H), 2.36 (m, 1H), 1.99 (m, 2H), 1.97 (m, 2H), 1.94 (m, 2H), 1.93 (m, 2H), 1.88 (m, 2H), 1.86 (m, 2H), 1.75 (m, 2H), 1.73 (m, 2H), 1.69 (m, 2H), 1.66 (m, 2H), 1.63 (m, 1H), 1.62 (m, 1H), 1.11 (s, 3H), 0.90 (d, *J* = 1.8 Hz, 3H), 0.87 (d, *J* = 1.8 Hz, 3H), 0.86 (d, *J* = 1.8 Hz, 3H), 0.85 (d, *J* = 1.8 Hz, 3H). ^{13}C NMR (DMSO-d_6) δ/ppm = 172.62, 172.44, 171.98, 171.48, 171.22, 171.03, 170.58, 170.50, 170.24, 169.44, 169.12, 159.76, 157.09, 155.43, 137.56, 136.48, 136.29, 128.84, 128.55, 128.49, 128.42, 128.39, 128.33, 128.29, 126.50, 113.24, 115.43, 82.42, 67.50, 66.42, 58.31, 58.14, 49.58, 47.96, 43.72, 43.08, 38.79, 36.94, 36.70, 33.82, 30.85, 30.39, 30.27, 29.70, 25.57, 27.32, 25.60, 24.92, 23.26, 19.68, 19.34, 18.57, 18.40, 12.37.

4.4.4. Estradiol-17-O-β-carbonylpropionyl-Arg(NO$_2$)-Gly-Asp(OBzl)-Phe-Arg(NO$_2$)-Gly-Asp(OBzl)-Phe-OBzl (**30**)

Using the same procedure as described for Boc-Arg(NO$_2$)-Gly-OCH$_3$, from 0.20 g (0.54 mmol) of **24**, 0.70 g (0.51 mmol) of **7**, 0.07 g (0.59 mmol) of HOBt and 0.12 g (0.59 mmol) of DCC 0.66 g (76.5%) of the title compound was obtained as colorless powder. Mp 161–163 °C, ESI-MS (*m*/*z*) 1685 $[M+H]^+$, $[\alpha]_D^{20} = 3.5$ (*c* = 1, DMSO). ^{1}H NMR (DMSO-d_6) δ/ppm = 8.99 (s, 1H), 8.63 (d, *J* = 5.1 Hz, 2H), 8.53 (t, *J* = 7.2 Hz, 2H), 8.43 (d, *J* = 4.8 Hz, 2H), 8.21 (d, *J* = 5.1 Hz, 2H), 8.13 (d, *J* = 4.5 Hz, 2H), 7.40 (s, 2H), 7.38 (s, 2H), 7.19–7.35 (m, 25H), 7.07 (d, *J* = 8.7 Hz, 1H), 6.67 (d, *J* = 8.7 Hz, 1H), 6.65 (s, 1H), 5.08 (s, 2H), 5.07 (s, 2H), 5.04 (m, 1H), 5.03 (m, 1H), 4.78 (s, 2H), 4.51 (m, 2H), 4.50 (m, 2H), 3.74 (d, *J* = 2.1 Hz, 2H), 3.73 (d, *J* = 2.1 Hz, 2H), 3.21 (d, *J* = 3.3 Hz, 1H), 3.13 (d, *J* = 2.4 Hz, 2H), 2.86 (t, *J* = 2.1 Hz, 2H), 2.85 (t, *J* = 2.1 Hz, 2H), 2.78 (m, 1H), 2.78 (m, 2H), 2.57 (m, 2H), 2.56 (m, 2H), 1.98 (m, 2H), 1.97 (m, 2H), 1.94 (m, 2H), 1.89 (m, 2H), 1.81 (m, 2H), 1.85 (m, 2H), 1.87 (m, 2H), 1.78 (m, 2H), 1.73 (m, 2H), 1.69 (m, 2H), 1.63 (m, 1H), 1.61 (m, 1H), 1.49 (m, 2H), 1.43 (m, 2H), 0.90 (s, 3H). ^{13}C NMR (DMSO-d_6) δ/ppm = 171.96, 171.41, 171.21, 170.81, 170.52, 170.44, 170.21, 169.93, 169.54, 168.95, 159.76, 157.12, 155.42 138.00, 137.56, 137.32, 136.47, 136.11, 130.66, 129.71, 129.56, 128.84, 128.81, 128.74, 128.48, 128.42, 128.33, 127.05, 126.51, 113.24, 115.42, 82.41, 67.50, 66.53, 54.47, 54.36, 49.57, 49.49, 48.00, 43.71, 43.08, 38.78, 37.01, 36.94, 36.80, 33.82, 29.63, 29.56, 27.60, 27.31, 25.60, 24.92, 23.26, 12.37.

4.4.5. Estradiol-17β-O-carbonylpropionyl-Arg-Gly-Asp-Ser-Arg-Gly-Asp-Ser (**34**)

Using the same procedure as described for **7**, from 300 mg (0.172 mmol) of **28** 110 mg (53.5%) of the title compound was obtained as colorless powder. Mp 250 °C (decomp.), $[\alpha]_D^{20} = -3.3$ (*c* = 1.50, DMSO), ESI-MS (*m*/*z*): 1203 $[M+H]^+$, 1099 $[M-(Ser)+H]^+$, 984 $[M-(Asp\text{-}Ser)+H]^+$, 927 $[M-(Gly\text{-}Asp\text{-}Ser)+H]^+$, 771 $[M-(Arg\text{-}Gly\text{-}Asp\text{-}Ser)+H]^+$, 684 $[M-(Ser\text{-}Arg\text{-}Gly\text{-}Asp\text{-}Ser)+H]^+$, 569 $[M-(Asp\text{-}Ser\text{-}Arg\text{-}Gly\text{-}Asp\text{-}Ser)+H]^+$, 512 $[M-(Gly\text{-}Asp\text{-}Ser\text{-}Arg\text{-}Gly\text{-}Asp\text{-}Ser)+H]^+$. ^{1}H NMR (DMSO-$d_6$) δ/ppm = 10.11 (s, 1H), 9.84 (s, 2H), 8.86 (s, 1H), 8.63 (d, *J* = 5.4 Hz, 2H), 8.53 (t, *J* = 6.8 Hz, 2H), 8.24 (d, *J* = 4.8 Hz, 2H), 8.12 (d, *J* = 5.4 Hz, 2H), 8.12 (d, *J* = 4.5 Hz, 2H), 8.00 (s, 1H), 7.85 (s, 1H), 7.32 (s, 2H), 7.23 (s, 2H), 7.13 (d, *J* = 8.7 Hz, 1H), 6.76 (d, *J* = 8.7 Hz, 1H), 6.61 (s, 1H), 4.65 (t, *J* = 3.6 Hz, 1H), 4.64 (t, *J* = 3.6 Hz, 1H), 4.57 (m, 1H), 4.50 (m, 1H), 4.48 (m, 1H), 4.47 (m, 1H), 4.25 (m, 2H), 3.91 (d, *J* = 3.6 Hz, 2H), 3.76 (d, *J* = 3.3 Hz, 2H), 3.52 (d, *J* = 2.1 Hz, 2H), 3.53 (d, *J* = 2.1 Hz, 2H), 3.53 (t, *J* = 4.5 Hz, 1H), 3.46 (d, *J* = 3.3 Hz, 2H), 3.34 (d, *J* = 3.6 Hz, 2H), 2.94 (t, *J* = 2.1 Hz, 2H), 2.72 (m, 1H), 2.24 (t, *J* = 2.1 Hz, 2H), 2.16 (t, *J* = 2.1 Hz, 2H), 2.14 (m, 2H), 2.10 (m, 2H), 1.98 (m, 2H), 1.78 (m, 2H), 1.71 (m, 2H), 1.67 (m, 2H), 1.64 (m, 2H), 1.59 (m, 2H), 1.46 (m, 2H), 1.32 (m, 2H), 1.31 (m, 2H), 1.27 (m, 1H), 1.20 (m, 1H), 1.18 (m, 2H), 0.77 (s, 3H). ^{13}C NMR (DMSO-d_6) δ/ppm = 176.38, 175.74, 174.65, 173.33, 172.56, 172.09, 171.17, 170.43, 169.12, 157.93, 155.43, 138.97, 137.55, 130.66, 126.49, 113.25, 115.46, 82.35, 55.47, 53.15, 51.69, 49.60, 48.00, 43.72, 43.08, 38.82, 38.18, 36.71, 33.34, 31.41, 30.35, 29.88, 29.25, 27.12, 26.45, 23.19, 12.39. Anal. Calcd for $C_{52}H_{78}N_{14}O_{19}$: C 51.91, H 6.53, N 16.30; Found C 51.74, H 6.38, N 15.77.

4.4.6. Estradiol-17β-O-carbonylpropionyl-Arg-Gly-Asp-Val-Arg-Gly-Asp-Val (**35**)

Using the same procedure as described for **7**, from 300 mg (0.189 mmol) of **29** 150 mg (64.6%) of the title compound was obtained as colorless powder. Mp 213–216 °C, $[\alpha]_D^{20} = -24.1$ (*c* = 1.55, DMSO), ESI-MS (*m*/*z*): 1227 $[M+H]^+$, 1111 $[M-(Val)+H]^+$, 996 $[M-(Asp\text{-}Val)+H]^+$, 939 $[M-(Gly\text{-}Asp\text{-}Val)+H]^+$, 783 $[M-(Arg\text{-}Gly\text{-}Asp\text{-}Val)+H]^+$, 684 $[M-(Val\text{-}Arg\text{-}Gly\text{-}Asp\text{-}Val)+H]^+$, 512 $[M-(Gly\text{-}Asp\text{-}Val\text{-}Arg\text{-}Gly\text{-}Asp\text{-}Val)+H]^+$. ^{1}H NMR (DMSO-$d_6$) δ/ppm = 10.17 (s, 1H), 9.90 (s, 2H), 8.99 (s, 1H), 8.62 (d, *J* = 5.4 Hz, 2H), 8.47 (t, *J* = 7.2 Hz, 2H), 8.33 (d, *J* = 5.1 Hz, 2H), 8.14 (d, *J* = 4.5 Hz, 2H), 8.04 (d, *J* = 4.5 Hz, 2H), 8.02 (s, 1H), 7.98 (s, 1H), 7.34 (s, 2H), 7.17 (d, *J* = 8.1 Hz, 1H), 7.13 (s, 2H), 6.79 (d, *J* = 8.7 Hz, 1H), 6.65 (s, 1H), 4.62 (m, 2H), 4.59 (m, 1H), 4.39 (m, 1H), 4.30 (m, 1H), 4.23 (m, 1H), 4.22 (m, 1H), 4.19 (m, 1H), 4.10 (m, 2H), 3.88 (d, *J* = 3.6 Hz, 2H), 3.56 (d, *J* = 3.3 Hz, 2H), 3.39 (t, *J* = 3.6 Hz, 1H), 3.14 (d, *J* = 2.1 Hz, 2H), 2.98 (d, *J* = 2.1 Hz, 2H), 2.86 (t, *J* = 2.1 Hz, 2H), 2.70 (m, 1H), 2.61 (t, *J* = 2.1 Hz, 2H), 2.57 (m, *J* = 2.1 Hz, 2H), 2.53 (m, 1H), 2.23 (m, 2H), 2.10 (m, 2H), 2.08 (m, 2H), 1.95 (m, 2H), 1.84 (m, 1H), 1.76 (m, 2H), 1.73 (m, 2H), 1.65 (m, 2H), 1.49 (m, 2H), 1.49 (m, 2H), 1.39 (m, 2H), 1.31 (m, 1H), 1.29 (m, 1H), 1.09 (s, 3H), 0.84 (d, *J* = 1.8 Hz, 3H), 0.83 (d, *J* = 1.8 Hz, 3H), 0.82 (d, *J* = 1.8 Hz, 3H), 0.78 (d, *J* = 1.8 Hz, 3H). ^{13}C NMR (DMSO-d_6) δ/ppm = 176.12, 175.67, 174.27, 173.00, 172.78, 172.57, 171.39, 170.69, 169.02, 158.01, 155.42, 137.55, 130.64, 126.49, 113.25, 115.43, 82.34, 58.69, 57.61, 50.38, 49.60, 43.72, 43.08, 38.79, 38.19, 36.93, 31.12, 30.51, 30.25, 29.74, 29.55, 27.32, 26.35, 23.28, 19.71, 18.52, 15.35. Anal. Calcd for $C_{56}H_{86}N_{14}O_{17}$: C 54.80, H 7.06, N 15.98; Found C 54.61, H 7.19, N 15.79.

4.4.7. Estradiol-17β-O-carbonylpropionyl-Arg-Gly-Asp-Phe-Arg-Gly-Asp-Phe (**36**)

Using the same procedure as described for **7**, from 300 mg (0.178 mmol) of **30** 120 mg (51.1%) of the title compound was obtained as colorless powder. Mp 222–224 °C, $[\alpha]_D^{20} = -25.8$ (*c* = 1.85, DMSO), ESI-MS (*m*/*z*) 1323 $[M+H]^+$, 1159 $[M-(Phe)+H]^+$, 1044 $[M-(Asp\text{-}Phe)+H]^+$, 987 $[M-(Gly\text{-}Asp\text{-}Phe)+H]^+$, 831 $[M-(Arg\text{-}Gly\text{-}Asp\text{-}Phe)+H]^+$, 684 $[M-(Phe\text{-}Arg\text{-}Gly\text{-}Asp\text{-}Phe)+H]^+$, 569 $[M-(Asp\text{-}Phe\text{-}Arg\text{-}Gly\text{-}Asp\text{-}Phe)+H]^+$, 512 $[M-(Gly\text{-}Asp\text{-}Phe\text{-}Arg\text{-}Gly\text{-}Asp\text{-}Phe)+H]^+$. ^{1}H NMR (DMSO-$d_6$) δ/ppm = 9.80 (s, 1H), 9.40 (s, 2H), 8.73 (s, 1H), 8.51 (d, *J* = 5.4 Hz, 2H), 8.32 (t, *J* = 6.8 Hz, 2H), 8.21 (d,

J = 4.8 Hz, 2H), 8.14 (d, J = 5.4 Hz, 2H), 8.11 (d, J = 4.5 Hz, 2H), 7.94 (s, 1H), 7.46 (s, 1H), 7.34 (s, 2H), 7.34 (s, 2H), 7.04–7.19 (m, 10H), 7.13 (d, J = 8.7 Hz, 1H), 6.49 (d, J = 8.7 Hz, 1H), 6.44 (s, 1H), 4.61 (m, 1H), 4.59 (m, 1H), 4.56 (m, 1H), 4.37 (m, 1H), 4.36 (m, 2H), 4.10 (d, J = 3.6 Hz, 2H), 3.87 (d, J = 3.3 Hz, 2H), 3.83 (d, J = 2.1 Hz, 2H), 3.56 (d, J = 2.1 Hz, 2H), 3.52 (t, J = 4.5 Hz, 1H), 3.22 (d, J = 3.3 Hz, 2H), 3.16 (d, J = 3.6 Hz, 2H), 3.07 (t, J = 2.1 Hz, 2H), 2.92 (m, 1H), 2.89 (m, 2H), 2.46 (t, J = 2.1 Hz, 2H), 2.34 (t, J = 2.1 Hz, 2H), 2.08 (m, 2H), 1.90 (m, 2H), 1.88 (m, 2H), 1.80 (m, 2H), 1.73 (m, 2H), 1.65 (m, 2H), 1.62 (m, 2H), 1.51 (m, 2H), 1.49 (m, 2H), 1.36 (m, 2H), 1.35 (m, 1H), 1.31 (m, 1H), 1.28 (m, 2H), 0.76 (s, 3H). ^{13}C NMR (DMSO-d_6) δ/ppm = 175.81, 174.97, 174.19, 173.68, 173.01, 172.58, 171.42, 170.89, 170.61, 169.87, 169.04, 157.93, 155.43, 138.97, 138.66, 137.55, 130.66, 129.84, 129.52, 128.48, 128.17, 126.49, 113.25, 115.46, 82.35, 55.47, 53.65, 52.70, 49.60, 48.00, 43.72, 43.08, 38.80, 38.21, 37.80, 36.93, 34.78, 32.15, 30.27, 29.74, 27.61, 26.34, 25.16, 23.28, 12.37. Anal. Calcd for $C_{64}H_{86}N_{14}O_{17}$: C 58.08, H 6.55, N 14.82; Found C 57.88, H 6.41, N 15.01.

4.5. Preparing N-(estradiol-3-oxyacetyl)-RGD tetrapeptide conjugates

4.5.1. N-(Estradiol-3-oxyacetyl)-Arg(NO_2)-Gly-Asp(OBzl)-Ser(Bzl)-OBzl (**12**)

Using the same procedure as described for Boc-Arg(NO_2)-Gly-OCH_3, from 0.17 g (0.52 mmol) of **11**, 0.40 g (0.50 mmol) of **1**, 84 mg (0.60 mmol) of HOBt and 123 mg (0.60 mmol) of DCC 0.47 g (89%) of the title compound was obtained as colorless powder. ESI-MS (m/z) 1061 $[M+H]^+$. $[\alpha]_D^{20}$ = 55.0 (c = 0.5, CH_3OH). ^{1}H NMR (DMSO-d_6) δ/ppm = 8.38 (s, 1H), 8.20 (s, 1H), 8.08 (s, 1H), 8.00 (s, 1H), 7.34 (t, J = 7.8 Hz, 1H), 7.342 (t, J = 7.8 Hz, 1H), 7.33 (t, J = 7.8 Hz, 1H), 7.30 (t, J = 7.8 Hz, 2H), 7.29 (t, J = 7.8 Hz, 2H), 7.25 (t, J = 7.8 Hz, 2H), 7.24 (d, J = 7.8 Hz, 2H), 7.18 (d, J = 7.8 Hz, 2H), 7.17 (d, J = 7.8 Hz, 2H), 7.12 (d, J = 7.5 Hz, 1H), 7.03 (s, 1H), 6.70 (s, 1H), 6.52 (d, J = 7.5 Hz, 1H), 6.41 (s, 1H), 6.374 (s, 1H), 5.16 (s, 2H), 5.14 (s, 2H), 5.13 (s, 2H), 5.00 (s, 2H), 4.90 (dt, J = 6.3 Hz, J = 6.6 Hz, 1H), 4.71 (t, J = 6.5 Hz, 1H), 4.70 (t, J = 6.4 Hz, 1H), 4.61 (t, J = 4.7 Hz, 1H), 3.96 (d, J = 6.0 Hz, 2H), 3.76 (d, J = 4.1 Hz, 2H), 2.97 (t, J = 4.5 Hz, 2H), 2.91 (m, J = 4.2 Hz, 1H), 2.78 (t, J = 5.1 Hz, 2H), 2.62 (t, J = 6.5 Hz, 2H), 2.55 (s, 1H), 2.02 (m, J = 4.7 Hz, 2H), 2.01 (m, J = 4.3 Hz, 2H), 1.86 (m, J = 4.2 Hz, 1H), 1.84 (m, J = 4.4 Hz, 2H), 1.75 (m, J = 4.5 Hz, 2H), 1.72 (m, J = 6.3 Hz, 2H), 1.70 (m, J = 4.2 Hz, 1H), 1.65 (m, J = 4.7 Hz, 2H), 1.62 (m, J = 6.3 Hz, 2H), 0.78 (s, 3H).

4.5.2. N-(Estradiol-3-oxyacetyl)-Arg(NO_2)-Gly-Asp(OBzl)-Val-OBzl (**13**)

Using the same procedure as described for Boc-Arg(NO_2)-Gly-OCH_3, from 0.20 g (0.60 mmol) of **11**, 0.40 g (0.60 mmol) of **2**, 0.10 g (0.70 mmol) of HOBt and 0.14 g (0.68 mmol) of DCC 0.55 g (88.7%) of the title compound was obtained as colorless powder. ESI-MS (m/z) 983 $[M+H]^+$. $[\alpha]_D^{20}$ = 60.0 (c = 1.00, MeOH). ^{1}H NMR (DMSO-d_6) δ/ppm = 8.38 (s, 1H), 8.22 (s, 1H), 8.08 (s, 1H), 8.00 (s, 1H), 7.34 (t, J = 7.8 Hz, 1H), 7.33 (t, J = 7.8 Hz, 1H), 7.30 (t, J = 7.8 Hz, 2H), 7.29 (t, J = 7.8 Hz, 2H), 7.24 (d, J = 7.8 Hz, 2H), 7.18 (d, J = 7.8 Hz, 2H), 7.12 (d, J = 7.5 Hz, 1H), 7.03 (s, 1H), 6.71 (s, 1H), 6.520 (d, J = 7.5 Hz, 1H), 6.41 (s, 1H), 6.37 (s, 1H), 5.16 (s, 2H), 5.14 (s, 2H), 5.00 (s, 2H), 4.90 (dt, J = 6.3 Hz, J = 6.6 Hz, 1H), 4.71 (t, J = 6.5 Hz, 1H), 4.70 (t, J = 6.4 Hz, 1H), 4.61 (t, J = 4.7 Hz, 1H), 3.96 (d, J = 6.0 Hz, 2H), 3.26 (m, J = 4.1 Hz, 1H), 2.96 (t, J = 4.5 Hz, 2H), 2.91 (m, J = 4.2 Hz, 1H), 2.79 (t, J = 5.1 Hz, 2H), 2.63 (t, J = 6.5 Hz, 2H), 2.55 (s, 1H), 2.02 (m, J = 4.7 Hz, 2H), 2.01 (m, J = 4.3 Hz, 2H), 1.86 (m, J = 4.2 Hz, 1H), 1.83 (m, J = 4.4 Hz, 2H), 1.75 (m, J = 4.5 Hz, 2H), 1.72 (m, J = 6.3 Hz, 2H), 1.70 (m, J = 4.2 Hz, 1H), 1.65 (m, J = 4.7 Hz, 2H), 1.62 (m, J = 6.3 Hz, 2H), 0.81 (s, 3H), 0.78 (d, J = 4.1 Hz, 6H).

4.5.3. N-(Estradiol-3-oxyacetyl)-Arg(NO_2)-Gly-Asp(OBzl)-Phe-OBzl (**14**)

Using the same procedure as described for Boc-Arg(NO_2)-Gly-OCH_3, from 0.18 g (0.56 mmol) of **11**, 0.40 g (0.53 mmol) of **3**, 0.09 g (0.61 mmol) of HOBt and 0.13 g (0.63 mmol) of DCC 0.52 g (88.3%) of the title compound was obtained as colorless powder. ESI-MS (m/z) 1031 $[M+H]^+$. $[\alpha]_D^{20}$ = 51.0 (c = 1.0, CH_3OH). ^{1}H NMR (DMSO-d_6) δ/ppm = 8.38 (s, 1H), 8.22 (s, 1H), 8.08 (s, 1H), 8.00 (s, 1H), 7.34 (t, J = 7.8 Hz, 1H), 7.39 (t, J = 7.8 Hz, 1H), 7.39 (t, J = 7.8 Hz, 1H), 7.33 (t, J = 7.8 Hz, 1H), 7.30 (t, J = 7.8 Hz, 2H), 7.29 (t, J = 7.8 Hz, 2H), 7.25 (t, J = 7.8 Hz, 2H), 7.20 (d, J = 7.8 Hz, 2H), 7.18 (d, J = 7.8 Hz, 2H), 7.17 (d, J = 7.8 Hz, 2H), 7.12 (d, J = 7.5 Hz, 1H), 7.03 (s, 1H), 6.70 (s, 1H), 6.52 (d, J = 7.5 Hz, 1H), 6.41 (s, 1H), 6.37 (s, 1H), 5.16 (s, 2H), 5.14 (s, 2H), 5.01 (s, 2H), 4.90 (dt, J = 6.3 Hz, J = 6.6 Hz, 1H), 4.70 (t, J = 6.5 Hz, 1H), 4.69 (t, J = 6.4 Hz, 1H), 4.60 (t, J = 4.7 Hz, 1H), 4.00 (s, 2H), 3.98 (d, J = 6.0 Hz, 2H), 2.96 (t, J = 4.5 Hz, 2H), 2.91 (m, J = 4.2 Hz, 1H), 2.78 (t, J = 5.1 Hz, 2H), 2.63 (t, J = 6.5 Hz, 2H), 2.56 (s, 1H), 2.02 (m, J = 4.7 Hz, 2H), 2.01 (m, J = 4.3 Hz, 1H), 1.86 (m, J = 4.2 Hz, 1H), 1.83 (m, J = 4.4 Hz, 2H), 1.75 (m, J = 4.5 Hz, 2H), 1.72 (m, J = 6.3 Hz, 2H), 1.70 (m, J = 4.2 Hz, 1H), 1.65 (m, J = 4.7 Hz, 2H), 1.62 (m, J = 6.3 Hz, 2H), 0.78 (s, 3H).

4.5.4. N-(Estradiol-3-oxyacetyl)-Arg-Gly-Asp-Ser (**18**)

Using the same procedure as described for **7**, from 0.29 g (0.27 mmol) of **12** 0.13 g (63.0%) of the title compound was obtained as colorless powder. ESI-MS (m/z): 745 $[M+H]^+$. $[\alpha]_D^{20}$ = 39.0 (c = 0.50, CH_3OH). ^{1}H NMR (DMSO-d_6) δ/ppm = 9.07 (s, 1H), 9.07 (s, 1H), 8.02 (s, 1H), 8.019 (s, 1H), 8.01 (s, 1H), 8.00 (s, 2H), 7.24 (s, 2H), 7.04 (d, J = 7.5 Hz, 1H), 7.03 (s, 1H), 6.75 (s, 1H), 6.51 (d, J = 7.5 Hz, 1H), 6.40 (s, 1H), 5.00 (s, 2H), 4.85 (dt, J = 6.3 Hz, J = 6.6 Hz, 1H), 4.67 (t, J = 6.5 Hz, 1H), 4.60 (t, J = 4.7 Hz, 1H), 4.57 (t, J = 6.4 Hz, 1H), 4.01 (d, J = 6.4 Hz, 2H), 3.97 (d, J = 6.0 Hz, 2H), 2.96 (t, J = 4.5 Hz, 2H), 2.90 (m, J = 4.2 Hz, 1H), 2.87 (t, J = 5.1 Hz, 2H), 2.80 (d, J = 6.6 Hz, 2H), 1.83 (m, J = 4.4 Hz, 2H), 1.76 (m, J = 4.5 Hz, 2H), 1.72 (m, J = 6.3 Hz, 2H), 1.70 (m, J = 4.2 Hz, 1H), 1.65 (m, J = 4.7 Hz, 2H), 1.62 (m, J = 6.3 Hz, 2H), 1.60 (m, J = 4.5 Hz, 2H), 1.57 (m, J = 4.5 Hz, 2H), 1.54 (m, J = 4.5 Hz, 2H), 0.73 (s, 3H).

4.5.5. N-(Estradiol-3-oxyacetyl)-Arg-Gly-Asp-Val (**19**)

Using the same procedure as described for **7**, from 0.22 g (0.22 mmol) of **13** 0.15 g (91%) of the title compound was obtained as colorless powder. ESI-MS (m/z) 758 $[M+H]^+$. $[\alpha]_D^{20}$ = 45.0 (c = 0.50, CH_3OH). ^{1}H NMR (DMSO-d_6) δ/ppm = 9.10 (s, 1H), 9.08 (s, 1H), 8.02 (s, 1H), 8.02 (s, 1H), 8.01 (s, 1H), 8.00 (s, 2H), 7.24 (s, 2H), 7.04 (d, J = 7.5 Hz, 1H), 7.03 (s, 1H), 6.75 (s, 1H), 6.51 (d, J = 7.5 Hz, 1H), 6.40 (s, 1H), 5.00 (s, 2H), 4.85 (dt, J = 6.3 Hz, J = 6.6 Hz, 1H), 4.67 (t, J = 6.5 Hz, 1H), 4.57 (t, J = 6.4 Hz, 1H), 4.60 (t, J = 4.7 Hz, 1H), 3.97 (d, J = 6.0 Hz, 2H), 2.96 (t, J = 4.5 Hz, 2H), 2.90 (m, J = 4.2 Hz, 1H), 2.87 (t, J = 5.1 Hz, 2H), 2.80 (d, J = 6.6 Hz, 2H), 1.83 (m, J = 4.4 Hz, 2H), 1.76 (m, J = 4.5 Hz, 2H), 1.72 (m, J = 6.3 Hz, 2H), 1.70 (m, J = 4.2 Hz, 1H), 1.65 (m, J = 4.7 Hz, 2H), 1.62 (m, J = 6.3 Hz, 2H), 1.60 (m, J = 4.5 Hz, 2H), 1.57 (m, J = 4.5 Hz, 2H), 1.54 (m, J = 4.5 Hz, 2H), 0.93 (d, J = 4.2 Hz, 6H), 0.74 (s, 3H).

4.5.6. N-(Estradiol-3-oxyacetyl)-Arg-Gly-Asp-Phe (**20**)

Using the same procedure as described for **7**, from 0.35 g (0.22 mmol) of **14** 0.13 g (47.6%) of the title compound was obtained as colorless powder. ESI-MS (m/z): 805 $[M+H]^+$. $[\alpha]_D^{20}$ = 48.0 (c = 0.50, CH_3OH). ^{1}H NMR (DMSO-d_6) δ/ppm = 9.07 (s, 1H), 9.06 (s, 1H), 8.03 (s, 1H), 8.01 (s, 1H), 8.00 (s, 1H), 7.98 (s, 2H), 7.27 (t, J = 7.8 Hz, 2H), 7.21 (s, 1H), 7.12 (d, J = 7.8 Hz, 2H), 7.07 (d, J = 7.8 Hz, 1H), 7.06 (d, J = 7.5 Hz, 1H), 7.02 (s, 1H), 6.74 (s, 1H), 6.66 (d, J = 7.5 Hz, 1H), 6.64 (s, 1H), 4.99 (s, 2H), 4.84 (dt, J = 6.3 Hz, J = 6.6 Hz, 1H), 4.67 (t, J = 6.5 Hz, 1H), 4.59 (t, J = 4.7 Hz, 1H), 4.57 (t, J = 6.4 Hz, 1H), 3.96 (d, J = 6.0 Hz, 2H), 3.22 (t, J = 4.7 Hz, 2H), 2.96 (t, J = 4.5 Hz, 2H), 2.90 (m, J = 4.2 Hz, 1H), 2.87 (t, J = 5.1 Hz, 2H), 2.80 (d, J = 6.5 Hz, 2H), 1.84 (m, J = 4.4 Hz, 2H), 1.76 (m, J = 4.5 Hz, 2H), 1.72 (m, J = 6.3 Hz, 2H), 1.67 (m, J = 4.7 Hz, 2H), 1.62 (m, J = 6.3 Hz, 2H), 1.60 (m, J = 4.5 Hz, 2H), 1.58 (m, J = 4.5 Hz, 1H), 1.57

(m, J = 4.5 Hz, 2H), 1.54 (m, J = 4.5 Hz, 2H), 1.46 (m, J = 4.5 Hz, 1H), 0.75 (s, 3H).

4.6. Preparing estradiol-17β-O-carbonylpropinyl-RGD tetrapeptide conjugates

4.6.1. Estradiol-17β-O-carbonylpropionyl-Arg(NO_2)-Gly-Asp(OBzl)-Ser(Bzl)-OBzl (**25**)

Using the same procedure as described for Boc-Arg(NO_2)-Gly-OCH_3 from 0.20 g (0.53 mmol) of **24**, 0.40 g (0.51 mmol) of **1**, 0.08 g (0.58 mmol) of HOBt and 0.12 g (0.58 mmol) of DCC 0.47 g (80.3%) of the title compound was obtained as colorless powder. ESI-MS (m/z) 1103 $[M+H]^+$. $[\alpha]_D^{20} = 20.0$ (c = 1.00, $CHCl_3$:CH_3OH = 10:1). ^{1}H NMR (DMSO-d_6) δ/ppm = 8.94 (s, 1H), 8.38 (s, 1H), 8.22 (s, 1H), 8.08 (s, 1H), 8.00 (s, 1H), 7.34 (t, J = 7.8 Hz, 1H), 7.34 (t, J = 7.9 Hz, 1H), 7.33 (t, J = 7.8 Hz, 1H), 7.30 (t, J = 7.9 Hz, 2H), 7.29 (t, J = 7.9 Hz, 2H), 7.25 (t, J = 7.8 Hz, 2H), 7.24 (d, J = 7.8 Hz, 2H), 7.18 (d, J = 7.8 Hz, 2H), 7.17 (d, J = 7.8 Hz, 2H), 7.04 (d, J = 7.5 Hz, 1H), 7.03 (s, 1H), 6.74 (s, 1H), 6.51 (d, J = 7.5 Hz, 1H), 6.40 (s, 1H), 6.37 (s, 1H), 5.16 (s, 2H), 5.14 (s, 2H), 5.13 (s, 2H), 4.90 (dt, J = 6.3 Hz, J = 6.6 Hz, 1H), 4.71 (t, J = 6.5 Hz, 1H), 4.70 (t, J = 6.4 Hz, 1H), 4.61 (t, J = 4.7 Hz, 1H), 3.96 (d, J = 6.0 Hz, 2H), 3.76 (d, J = 4.1 Hz, 2H), 2.97 (t, J = 4.5 Hz, 2H), 2.91 (m, J = 4.2 Hz, 1H), 2.88 (t, J = 5.1 Hz, 2H), 2.82 (d, J = 6.6 Hz, 2H), 2.78 (t, J = 5.1 Hz, 2H), 2.62 (t, J = 6.5 Hz, 2H), 2.02 (m, J = 4.7 Hz, 2H), 2.01 (m, J = 4.3 Hz, 2H), 1.87 (m, J = 4.2 Hz, 1H), 1.84 (m, J = 4.4 Hz, 2H), 1.76 (m, J = 4.5 Hz, 2H), 1.72 (m, J = 6.3 Hz, 2H), 1.70 (m, J = 4.2 Hz, 1H), 1.65 (m, J = 4.7 Hz, 2H), 1.62 (m, J = 6.3 Hz, 2H), 0.77 (s, 3H).

4.6.2. Estradiol-17β-O-carbonylpropionyl-Arg(NO_2)-Gly-Asp(OBzl)-Val-OBzl (**26**)

Using the same procedure as described for Boc-Arg(NO_2)-Gly-OCH_3, from 0.22 g (0.59 mmol) of **24**, 0.40 g (0.57 mmol) of **2**, 0.09 g (0.65 mmol) of HOBt and 0.14 g (0.68 mmol) of DCC 0.49 g (79.3%) of the title compound was obtained as colorless powder. ESI-MS (m/z) 1025 $[M+H]^+$. $[\alpha]_D^{20} = 30.0$ (c = 1.00, $CHCl_3$:CH_3OH = 10:1). ^{1}H NMR (DMSO-d_6) δ/ppm = 8.94 (s, 1H), 8.38 (s, 1H), 8.22 (s, 1H), 8.08 (s, 1H), 8.00 (s, 1H), 7.33 (t, J = 7.8 Hz, 1H), 7.33 (t, J = 7.8 Hz, 1H), 7.30 (t, J = 7.8 Hz, 2H), 7.29 (t, J = 7.8 Hz, 2H), 7.24 (d, J = 7.8 Hz, 2H), 7.18 (d, J = 7.8 Hz, 2H), 7.04 (d, J = 7.5 Hz, 1H), 7.037 (s, 1H), 6.74 (s, 1H), 6.51 (d, J = 7.5 Hz, 1H), 6.40 (s, 1H), 6.37 (s, 1H), 5.12 (s, 2H), 5.13 (s, 2H), 5.00 (dt, J = 6.3 Hz, J = 6.6 Hz, 1H), 4.89 (d, J = 6.5 Hz, 1H), 4.53 (d, J = 6.5 Hz, 1H), 4.42 (d, J = 6.4 Hz, 1H), 4.10 (d, J = 4.7 Hz, 2H), 3.07 (m, J = 4.1 Hz, 1H), 2.97 (t, J = 4.5 Hz, 2H), 2.91 (m, J = 4.2 Hz, 1H), 2.88 (t, J = 5.1 Hz, 2H), 2.82 (d, J = 6.6 Hz, 2H), 2.78 (t, J = 5.1 Hz, 2H), 2.62 (t, J = 6.5 Hz, 2H), 2.02 (m, J = 4.7 Hz, 2H), 2.01 (m, J = 4.3 Hz, 2H), 1.87 (m, J = 4.2 Hz, 1H), 1.84 (m, J = 4.4 Hz, 2H), 1.76 (m, J = 4.5 Hz, 2H), 1.72 (m, J = 6.3 Hz, 2H), 1.70 (m, J = 4.2 Hz, 1H), 1.65 (m, J = 4.7 Hz, 2H), 1.62 (m, J = 6.3 Hz, 2H), 1.01 (d, J = 4.1 Hz, 6H), 0.77 (s, 3H).

4.6.3. Estradiol-17β-O-carbonylpropionyl-Arg(NO_2)-Gly-Asp(OBzl)-Phe-OBzl (**27**)

Using the same procedure as described for Boc-Arg(NO_2)-Gly-OCH_3, from 0.21 g (0.56 mmol) of **24**, 0.40 g (0.53 mmol) of **3**, 85 mg (0.61 mmol) of HOBt and 0.125 mg (0.61 mmol) of DCC 0.51 g (84.9%) of the title compound was obtained as colorless powder. ESI-MS (m/z) 1073 $[M+H]^+$. $[\alpha]_D^{20} = 23.0$ (c = 1.00, $CHCl_3$:CH_3OH = 10:1). ^{1}H NMR (DMSO-d_6) δ/ppm = 8.93 (s, 1H), 8.37 (s, 1H), 8.22 (s, 1H), 8.08 (s, 1H), 8.00 (s, 1H), 7.34 (t, J = 7.8 Hz, 1H), 7.33 (t, J = 7.8 Hz, 1H), 7.33 (t, J = 7.8 Hz, 1H), 7.30 (t, J = 7.8 Hz, 2H), 7.29 (t, J = 7.8 Hz, 2H), 7.27 (t, J = 7.8 Hz, 2H), 7.24 (d, J = 7.8 Hz, 2H), 7.18 (d, J = 7.8 Hz, 2H), 7.17 (d, J = 7.8 Hz, 2H), 7.04 (d, J = 7.5 Hz, 1H), 7.03 (s, 1H), 6.74 (s, 1H), 6.50 (d, J = 7.5 Hz, 1H), 6.40 (s, 1H), 6.36 (s, 1H), 5.12 (s, 2H), 5.13 (s, 2H), 5.01 (dt, J = 6.3 Hz, J = 6.5 Hz, 1H), 4.88 (t, J = 6.5 Hz, 1H), 4.53 (t, J = 6.5 Hz, 1H), 4.41 (t, J = 6.4 Hz, 1H), 4.10 (d, J = 4.7 Hz, 2H), 3.16 (d, J = 6.4 Hz, 2H), 2.96 (t, J = 4.5 Hz, 2H), 2.90 (m, J = 4.2 Hz, 1H), 2.87 (t, J = 5.1 Hz, 2H), 2.82 (d, J = 6.5 Hz, 2H), 2.78 (t, J = 5.1 Hz, 2H), 2.61 (t, J = 6.5 Hz, 2H), 2.02 (m, J = 4.7 Hz, 2H), 2.01 (m, J = 4.3 Hz, 2H), 1.86 (m, J = 4.2 Hz, 1H), 1.84 (m, J = 4.4 Hz, 2H), 1.76 (m, J = 4.5 Hz, 2H), 1.72 (m, J = 6.3 Hz, 2H), 1.70 (m, J = 4.2 Hz, 1H), 1.65 (m, J = 4.7 Hz, 2H), 1.61 (m, J = 6.3 Hz, 2H), 0.82 (s, 3H).

4.6.4. Estradiol-17β-O-carbonylpropionyl-Arg-Gly-Asp-Ser (**31**)

Using the same procedure as described for **7**, from 240 mg (0.172 mmol) of **25** 150 mg (79.2%) of the title compound was obtained as colorless powder. ESI-MS (m/z) 788 $[M+H]^+$. $[\alpha]_D^{20} = 50.0$ (c = 1.00, CH_3OH). ^{1}H NMR (DMSO-d_6) δ/ppm = 9.07 (s, 1H), 9.06 (s, 1H), 9.06 (s, 1H), 8.04 (s, 1H), 8.03 (s, 1H), 8.01 (s, 1H), 8.00 (s, 2H), 7.24 (s, 2H), 7.04 (d, J = 7.5 Hz, 1H), 7.03 (s, 1H), 6.75 (s, 1H), 6.51 (d, J = 7.5 Hz, 1H), 6.40 (s, 1H), 4.85 (dt, J = 6.3 Hz, J = 6.6 Hz, 1H), 4.68 (t, J = 6.5 Hz, 1H), 4.60 (t, J = 4.7 Hz, 1H), 4.59 (t, J = 6.4 Hz, 1H), 4.02 (d, J = 6.4 Hz, 2H), 3.96 (d, J = 6.0 Hz, 2H), 2.97 (t, J = 4.5 Hz, 2H), 2.91 (m, J = 4.2 Hz, 1H), 2.87 (t, J = 5.1 Hz, 2H), 2.82 (d, J = 6.6 Hz, 2H), 2.77 (t, J = 5.1 Hz, 2H), 2.60 (t, J = 6.5 Hz, 2H), 2.02 (m, J = 4.7 Hz, 2H), 2.01 (m, J = 4.3 Hz, 2H), 1.87 (m, J = 4.2 Hz, 1H), 1.84 (m, J = 4.4 Hz, 2H), 1.76 (m, J = 4.5 Hz, 2H), 1.72 (m, J = 6.3 Hz, 2H), 1.70 (m, J = 4.2 Hz, 1H), 1.65 (m, J = 4.7 Hz, 2H), 1.62 (m, J = 6.3 Hz, 2H), 0.77 (s, 3H).

4.6.5. Estradiol-17β-O-carbonylpropionyl-Arg-Gly-Asp-Val (**32**)

Using the same procedure as described for **7**, from 260 mg (0.26 mmol) of **26** 170 mg (84.0%) of the title compound was obtained as colorless powder. ESI-MS (m/z) 800 $[M+H]^+$. $[\alpha]_D^{20} = 56.0$ (c = 0.5, CH_3OH). ^{1}H NMR (DMSO-d_6) δ/ppm = 9.15 (s, 1H), 9.12 (s, 1H), 9.03 (s, 1H), 8.22 (s, 1H), 8.20 (s, 1H), 8.07 (s, 1H), 8.00 (s, 1H), 7.22 (s, 2H), 7.04 (d, J = 7.5 Hz, 1H), 7.03 (s, 1H), 6.72 (s, 1H), 6.51 (s, 1H), 6.44 (d, J = 7.5 Hz, 1H), 4.86 (d, J = 6.5 Hz, 1H), 4.61 (t, J = 6.3 Hz, 1H), 4.53 (d, J = 6.5 Hz, 1H), 4.42 (d, J = 6.4 Hz, 1H), 4.10 (d, J = 4.7 Hz, 2H), 2.96 (t, J = 4.5 Hz, 2H), 2.92 (m, J = 4.1 Hz, 1H), 2.90 (m, J = 4.2 Hz, 1H), 2.88 (t, J = 5.1 Hz, 2H), 2.82 (d, J = 6.5 Hz, 2H), 2.78 (t, J = 5.1 Hz, 2H), 2.61 (t, J = 6.5 Hz, 2H), 2.02 (m, J = 4.7 Hz, 2H), 2.01 (m, J = 4.3 Hz, 2H), 1.86 (m, J = 4.2 Hz, 1H), 1.84 (m, J = 4.4 Hz, 2H), 1.76 (m, J = 4.5 Hz, 2H), 1.72 (m, J = 6.3 Hz, 2H), 1.70 (m, J = 4.2 Hz, 1H), 1.65 (m, J = 4.7 Hz, 2H), 1.61 (m, J = 6.3 Hz, 2H), 0.86 (d, J = 4.1 Hz, 6H), 0.75 (s, 3H).

4.6.6. Estradiol-17β-O-carbonylpropionyl-Arg-Gly-Asp-Phe (**33**)

Using the same procedure as described for **7**, from 240 mg (0.22 mmol) of **27** 160 mg (86.4%) of the title compound was obtained as colorless powder. ESI-MS (m/z) 848 $[M+H]^+$. $[\alpha]_D^{20} = 45.0$ (c = 0.5, CH_3OH). ^{1}H NMR (DMSO-d_6) δ/ppm = 9.06 (s, 1H), 9.05 (s, 1H), 9.04 (s, 1H), 8.06 (s, 1H), 8.03 (s, 1H), 8.02 (s, 1H), 8.00 (s, 2H), 7.23 (s, 2H), 7.11 (t, J = 7.8 Hz, 2H), 7.07 (d, J = 7.8 Hz, 2H), 7.01 (t, J = 7.7 Hz, 1H), 7.00 (s, 1H), 6.95 (d, J = 7.5 Hz, 1H), 6.90 (s, 1H), 6.43 (d, J = 7.5 Hz, 1H), 4.91 (dt, J = 6.3 Hz, J = 6.5 Hz, 1H), 4.87 (t, J = 6.5 Hz, 1H), 4.84 (t, J = 6.5 Hz, 1H), 4.50 (t, J = 6.4 Hz, 1H), 4.12 (d, J = 4.7 Hz, 2H), 3.04 (d, J = 6.4 Hz, 2H), 2.96 (t, J = 4.5 Hz, 2H), 2.91 (m, J = 4.2 Hz, 1H), 2.87 (t, J = 5.1 Hz, 2H), 2.82 (d, J = 6.5 Hz, 2H), 2.77 (t, J = 5.1 Hz, 2H), 2.59 (t, J = 6.5 Hz, 2H), 2.02 (m, J = 4.7 Hz, 2H), 2.01 (m, J = 4.3 Hz, 2H), 1.86 (m, J = 4.2 Hz, 1H), 1.84 (m, J = 4.4 Hz, 2H), 1.75 (m, J = 4.5 Hz, 2H), 1.72 (m, J = 6.3 Hz, 2H), 1.70 (m, J = 4.2 Hz, 1H), 1.656 (m, J = 4.7 Hz, 2H), 1.61 (m, J = 6.3 Hz, 2H), 0.82 (s, 3H).

4.7. Anti-osteoporosis assay in vivo

The assessments described herein were performed based on a protocol reviewed and approved by the ethics committee of Capital Medical 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% carboxymethylcellulose just before use and kept in an ice bath. ICR 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, ip). The mice of OVX groups

were given abdominal ovariotomy by standard procedure and the mice of sham group were given abdominorotomy only. On the 5th day of surgical operation the mice of ovariotomy and sham groups were orally administrated 0.2 ml of aqueous solution of 0.5% carboxymethylcellulose, the mice of treatment groups were orally administrated the solution of 110.3 nmol/kg estradiol, the mixture of 110.3 nmol/kg estradiol and RGD peptide, 110.3 nmol/kg of the estradiol–RGD tetrapeptides or estradiol–RGD octapeptides in 0.2 ml aqueous solution of 0.5% carboxymethylcellulose 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, drawn blood via eye orbit, anesthetized with pentobarbital sodium (40.0 mg/kg, ip) and executed to remove the lungs, livers, spleens, uteri and left femurs.

After 30 min standing the blood was centrifuged 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 phosphorous content of the serum was measured by the method of molybdenum blue. The alkaline phosphatase content of the serum was measured by using disodium phenylphosphate as the substrate.

The lungs, livers, spleens and uteri were weighed directly. After completely removing the muscle the lengths of left femurs were measured and then immersed in the solution of chloroform–methanol (2:1) for two times (one time 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 muffle furnace at 800 °C for 8 h, cooled, weighed and recorded 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 phosphorous content of the aqueous solution was measured by the method of molybdenum blue.

4.8. In vivo tail bleeding time assay [24,25]

Thirty and ninety minutes later after the last administration, the mouse was placed in a tube holder with its tail protruding, and a 2 mm cut was made on the tail. Flowing blood was gently wiped away with a tissue every 30 s until bleeding ceased and the time recorded. The observed bleeding time at 30 min and 90 min after the ip injection or orally administered mice was recorded to yield the bleeding time.

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