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Supporting Information

RGD-peptides modifying dexamethasone: To enhance the efficacy of anti-inflammation and limit the risk of osteoporosis

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Experimental

1. General

The protected amino acids and the chemicals were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA) and were purified when necessary. Column chromatography was performed using silica gel (200-300 mesh, Qingdao Haiyang Chemical Co., Qingdao, P. R. China). Purity of intermediates (>95%) and products (>98%) were determined by TLC analysis (Qingdao silica gel plates of GF254) and HPLC analysis (CHIRALPAK AH-H column, 4.6 × 250 mm, Daicel Chemical IND., LTD.). ^{1}H NMR (300 MHz) and ^{13}C NMR (75 MHz) spectra were recorded on Varian INOVA-300 MHz spectrometer with DMSO-*d6* as the solvent and tetramethylsilane as internal standard. ^{1}H NMR (800 MHz), ^{13}C NMR (200 MHz) and the ROESY 2D NMR spectra were recorded on Bruker Avance II 800 MHz spectrometer with DMSO-*d6* as the solvent and tetramethylsilane as internal standard. ESI/MS was tested on ZQ 2000 (Waters, US) and solariX FT-ICR mass spectrometer (Bruker Daltonik) consisting of an ESI/MALDI dual ion source and 9.4 T superconductive magnet.

2. Synthesis

Boc-Arg(NO_2)-Gly-OBzl At 0 ºC to a solution of 800 mg (2.5 mmol) of Boc-Arg(NO_2) in 10 mL of anhydrous THF the solution of 338 mg (2.5 mmol) HOBt and 619 mg (3.0 mmol) of DCC in 5 mL of anhydrous THF was added and stirred for 20 min. To this solution a solution of 775 mg (2.3 mmol) of Tos·Gly-OBzl and 230 mg (2.3 mmol) of N-methylmorpholine in 5 mL of anhydrous THF was added, stirred at

room temperature for 24 h, and TLC (CH_2Cl_2/MeOH, 20/1) indicated the disappearance of Tos·Gly-OBzl. The reaction mixture was evaporated under vacuum, the residue was dissolved with 150 mL of ethyl acetate, the solution was successively washed with saturated aqueous $NaHCO_3$ (30 mL × 3), saturated aqueous NaCl (30 mL × 3), saturated aqueous $KHSO_4$ (30 mL × 3) and saturated aqueous NaCl (30 mL × 3). The ethyl acetate phase was dried with anhydrous Na_2SO_4. After filtration the filtrate was evaporated under vacuum to provide 1.28 g (94%) of the title compound as colourless powders. ESI-MS(m/z) 568 $[M + H]^+$.

Boc-Arg(NO_2)-Gly At 0 ºC to a solution of 1.167 g (2.5 mmol) of Boc-Arg(NO_2)-Gly-OBzl in 20 mL of CH_3OH, 2.5 mLof aqueous NaOH (2 M) was added, stirred for 30 min, and TLC (CH_2Cl_2/MeOH, 20/1) indicated the disappearance of Boc-Arg(NO_2)-Gly-OBzl. The reaction mixture was adjusted to pH 2 with hydrochloric acid (2 N), extracted with ethyl acetate (50 mL × 3), and the ethyl acetate phase was dried with anhydrous Na_2SO_4. After filtration the filtrate was evaporated under vacuum to provide 1.075 g (90%) of the title compound for next reaction.

Boc-Ser(Bzl)-OBzl At room temperature to a solution of 739 mg (2.5 mmol) of Boc-Ser(Bzl) in 10 mL of ethanol a solution of 405 mg (1.25 mmol) of Cs_2CO_3 in 2 mL of distilled water was added, the reaction mixture was stirred for 15 min, and TLC (ethyl acetate/petrolum, 1/1) indicated the disappearance of Boc-Ser(Bzl)-OCs, which was dissolved in 2 mL of anhydrous DMF, mixed with 0.3 mL (2.5 mmol) of $BrCH_2C_6H_5$, at 55-60 ℃ stirred for 4 h, and TLC ($CHCl_3$/MeOH, 30/1) indicated the disappearance of Boc-Ser(Bzl). The reaction mixture was evaporated under vacuum, the residue was

dissolved with 50 mL of ethyl acetate, filtrated, the filtrate was washed successively washed with saturated aqueous $NaHCO_3$ (30 mL × 3) and saturated aqueous NaCl (30 mL × 3). The ethyl acetate phase was dried with anhydrous Na_2SO_4. After filtration the filtrate was evaporated under vacuum to provide 873 mg (91%) of the title compound as colourless syrupy. ESI-MS(m/z) 386 $[M + H]^+$.

HCl·Ser(Bzl)-OBzl At 0 ºC to a solution of 962 mg (2.5 mmol) of Boc-Ser(Bzl)-OBzl in 6 mL of ethyl acetate 10 mL of the solution of hydrogen chloride in ethyl acetate (4 N) was added, stirred for 2 h, and TLC ($CHCl_3$/MeOH, 20/1) indicated the disappearance of Boc-Ser(Bzl)-OBzl. The reaction mixture was evaporated under vacuum, the residue was crystalized in a 1/1 mixture of methanol and ether to provide the title compound for the next reaction.

Boc-Asp(OBzl)-Ser(Bzl)-OBzl Using the procedure of preparing Boc-Arg(NO_2)-Gly-OBzl from 807 mg (2.5 mmol) of Boc-Asp(OBzl) and 742 mg (2.3 mmol) of HCl·Ser(Bzl)-OBzl 1.28 g (94％) of the title compound was obtained as colorless powders. ESI-MS(m/z) 592 $[M + H]^+$.

HCl·Asp(OBzl)-Ser(Bzl)-OBzl Using the procedure of preparing HCl·Ser(Bzl)-OBzl from 1.477 g (2.5 mmol) of Boc-Asp(OBzl)-Ser(Bzl)-OBzl 1.277 g (97%) of the title compound was obtained for the next reaction. ESI-MS (m/z) 492 $[M + H]^+$.

Boc-Arg(NO_2)-Gly-Asp(OBzl)-Ser(Bzl)-OBzl Using the procedure of preparing Boc-Arg(NO_2)-Gly-OBzl from 1195 mg (2.5 mmol) of Boc-Arg(NO_2)-Gly and 1317 mg (2.3 mmol) of HCl·Asp(OBzl)-Ser(Bzl)-OBzl 2.115 g (90%) of the title compound was obtained as colorless powders. ESI-MS (m/z) 941 $[M + H]^+$.

Arg-Gly-Asp-Ser At 0 ºC to a solution of 941 mg (1.0 mmol) of Boc-Arg(NO_2)-Gly-Asp(OBzl)-Ser(Bzl)-OBzl in 3 mL of CF_3CO_2H 1 mL of trifluomethane sulfonic acid was added, the reaction mixture was stirred for 20 min, and TLC (CH_2Cl_2/MeOH, 10/1) indicated the disappearance of Boc-Arg(NO_2)-Gly-Asp(OBzl)-Ser(Bzl)-OBzl. To the reaction mixture 80 mL of anhydrous ether was added to form colorless precipitates. The precipitates were collected by filtration, and dissolved in 1 mL of distilled water. The solution was adjusted to pH 8 with concentrated ammonia, filtrated and the filtrate was desalt on column of Sephadex G10. The fraction was evaporated under vacuum to provide 350 mg (81%) of the title compound as colorless powders. ESI-MS (m/z) 432 $[M - H]^-$.

Boc-Asp(OBzl)-Val-OBzl Using the procedure of preparing Boc-Arg(NO_2)-Gly-OBzl from 807 mg (2.5 mmol) of Boc-Asp(OBzl) and 947 mg (2.3 mmol) of Tos·Val-OBzl 1.28 g (94%) of the title compound was obtained as colorless powders. ESI-MS (m/z) 510 $[M + H]^+$.

HCl·Asp(OBzl)-Val-OBzl Using the procedure of preparing HCl·Ser(Bzl)-OBzl from 1.272 g (2.5 mmol) of Boc-Asp(OBzl)-Val-OBzl 1.00 g (92%) of the title compound was obtained for the next reaction. ESI-MS (m/z) 410 $[M + H]^+$.

Boc-Arg(NO_2)-Gly-Asp(OBzl)-Val-OBzl Using the procedure of preparing Boc-Arg(NO_2)-Gly-OBzl from 1195 mg (2.5 mmol) of Boc-Arg(NO_2)-Gly and 1087 mg (2.3 mmol) HCl·Asp(OBzl)-Val-OBzl 1.925 g (89%) of the title compound was obtained as colorless powders. ESI-MS (m/z) 863 $[M + H]^+$.

Arg-Gly-Asp-Val Using the procedure of preparing Arg-Gly-Asp-Ser from 862 mg

(1.0 mmol) of Boc-Arg(NO_2)-Gly-Asp(OBzl)-Val-OBzl 301 mg (68%) of the title compound was obtained as colorless powders. ESI-MS (m/z) 444 $[M - H]^-$.

Boc-Asp(OBzl)-Phe-OBzl Using the procedure of preparing Boc-Arg(NO_2)- Gly-OBzl from 807 mg (2.5 mmol) of Boc-Asp(OBzl) and 1067 mg (2.3 mmol) of Tos·Phe-OBzl 1.28 g (92%) of the title compound was obtained as colorless powders. ESI-MS (m/z) 558 $[M + H]^+$.

HCl·Asp(OBzl)-Phe-OBzl Using the procedure of preparing Arg-Gly-Asp-Ser from 1.395 g (2.5 mmol) of Boc-Asp(OBzl)-Phe-OBzl 1.171 g (95%) of the title compound was obtained as colorless powders. ESI-MS (m/z) 458 $[M + H]^+$.

Boc-Arg(NO_2)-Gly-Asp(OBzl)-Phe-OBzl Using the procedure of preparing Boc-Arg(NO_2)-Gly-OBzl from 1195 mg (2.5 mmol) of Boc-Arg(NO_2)-Gly and 1232 mg (2.3 mmol) of HCl·Asp(OBzl)-Phe-OBzl 2.004 g (88%) of the title compound was obtained as colorless powders. ESI-MS (m/z) 911 $[M + H]^+$.

Arg-Gly-Asp-Phe Using the procedure of preparing Arg-Gly-Asp-Ser from 910 mg (1.0 mmol) of Boc-Arg(NO_2)-Gly-Asp(OBzl)-Phe-OBzl 320 mg (65%) of the title compound was obtained as colorless powders. ESI-MS (m/z) 492 $[M - H]^-$.

(9R,10S,11S,13S,16R,17R)-9-Fluoro-7,8,11,12,13,15,16,17-octahydro-11,17-dihydroxy-17-(2-carboxyethylcarbonyloxy)-10,13,16-trimethyl-6H-cyclopenta[a]phenanthren-3(9H,10H,14H)one (1) In the presence of 1.46 g (12 mmol) of DMAP (dimethylaminopyridine) 3.93 g (10 mmol) of Dex in 150 mL of anhydrous THF was treated with 1.3 g (13 mmol) of butanedionic by preventing from light for 48 h, and TLC (CH_2Cl_2/CH_3OH, 20/1) indicated the disappearance of Dex.

After addition of 30 mL of water the reaction mixture was evaporated under vacuum to remove THF, the residue was adjusted to pH 2 with hydrochloric acid (2 M), the formed precipitates were collected with filtration to provide 4.64 g (94%) of the title compound as colorless powders. ESI/MS (m/z) 491 $[M - H]^-$. ^{1}H NMR (BHSC-300, DMSO-d_6): δ/ppm = 7.29 (d, J = 10.2 Hz, 1H), 6.23 (d, J = 9.3 Hz, 1H), 6.01 (s, 1H), 5.42 (s, 1H), 5.17 (s, 1H), 5.05 (d, J = 17.7 Hz, 1H), 4.80 (d, J = 17.7 Hz, 1H), 4.15 (m, 1H), 2.88 (m, 1H), 2.52 (m, 2H), 2.61 (m, 3H), 2.34 (m, 2H), 2.15 (m, 2H), 1.77 (m, 1H), 1.61 (m, 2H), 1.49 (s, 3H), 1.35 (m, 1H), 1.08 (m, 1H), 0.88 (s, 3H), 0.79 (d, J = 7.2 Hz, 3H). ^{13}C NMR (BHSC-75, DMSO-d_6): δ/ppm = 205.16, 185.78, 173.63, 172.10, 167.56, 153.23, 129.44, 124.55, 102.87, 100.55, 90.91, 71.12, 70.63, 68.50, 48.38, 43.74, 36.07, 34.17, 32.37, 31.13, 30.74, 29.05, 19.00, 16.90, 15.70. **(9R,10S,11S,13S,16R,17R)-9-Fluoro-7,8,11,12,13,15,16,17-octahydro-11,17-dihydroxy-17-(2-pyrrolidine-2,5-dione-1-yl-oxycarbonylthylcarbonyloxy)-10,13,16-tri- methyl-6H-cyclopenta[a]phenanthren-3(9H,10H,14H)one (2)** In the presence of 2.50 g (13 mmol) of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) 4.92 g (10 mmol) of (9R,10S,11S,13S,16R,17R)-9-fluoro-7,8,11,12,13,15,16,17-octahydro- 11,17-dihydroxy-17-(2-carboxyethylcarbonyloxy)-10,13,16-trimethyl-6H-cyclopenta-[a]phenanthren-3(9H,10H,14H)-one (**1**) in 100 mL of THF and 10 mL of DMF was treated with 1.50 g (13 mmol) of N-hydroxylsuccimmide by preventing from light for 24 h, and TLC (CH_2Cl_2/CH_3OH, 20/1) indicated the disappearance of **1**. The reaction mixture was evaporated under vacuum to remove THF, the residue was dissolved in 150 mL of ethyl acetate, and the

solution was successively washed with saturated aqueous $NaHCO_3$ (30 mL × 3), saturated aqueous NaCl (30 mL × 3), saturated aqueous $KHSO_4$ (30 mL × 3) and saturated aqueous NaCl (30 mL × 3). The ethyl acetate phase was dried with anhydrous Na_2SO_4. After filtration the filtrate was evaporated under vacuum, the residue was purified on silica gel column (petrolum/acetone, 1/1) to provide 5.37 g (91%) of the title compound as colorless powders. ESI/MS (m/z) 612 $[M + Na]^+$. ^1H NMR (BHSC-300, DMSO-d_6): δ/ppm = 7.29 (d, J = 10.2 Hz, 1H), 6.23 (d, J = 10.2 Hz, 1H), 6.01 (s, 1H), 5.41 (d, J = 3.9 Hz, 1H), 5.18 (s, 1H), 5.10 (d, J = 17.4 Hz, 1H), 4.82 (d, J = 17.7 Hz, 1H), 4.16 (m, 1H), 2.98 (t, J = 6.3 Hz, 1H), 2.81 (m, 6H), 2.63 (m, 1H), 2.34 (m, 2H), 2.16 (t, J = 11.7 Hz, 2H), 1.78 (m, 1H), 1.64 (d, J = 11.7 Hz, 1H), 1.56 (d, J = 13.5 Hz, 1H), 1.50 (s, 3H), 1.34 (m, 1H), 1.08 (m, 1H), 0.89 (s, 3H), 0.80 (d, J = 7.2 Hz, 3H). ^{13}C NMR (BHSC-75, DMSO-d_6): δ/ppm = 205.02, 185.74, 171.07, 170.53, 168.67, 167.49, 153.19, 129.45, 124.57, 102.87, 100.55, 90.92, 71.22, 70.73, 68.75, 48.56, 43.76, 36.14, 33.93, 32.38, 31.13, 30.74, 28.35, 27.75, 26.16, 25.90, 23.61, 17.01, 16.18.

(9R,10S,11S,13S,16R,17R)-9-Fluoro-7,8,11,12,13,15,16,17-octahydro-11,17-dihydroxy-17-(2-Arg-Gly-Asp-Val-oxycarbonylthylcarbonyloxy)-10,13,16-trimethyl- 6H-cyclopenta[a]phenanthren-3(9H,10H,14H)one (4a) To a solution of 782 mg (1.4 mmol) of (9R,10S,11S,13S,16R,17R)-9-fluoro-7,8,11,12,13,15,16,17-octahydro- 11,17-dihydroxy-17-(2-pyrrolidine-2,5-dione-1-yl-oxycarbonylthylcarbonyloxy)-10,13,16-trimethyl-6H-cyclopenta[a]phenanthren-3(9H,10H,14H)-one (**2**) in 20 mL of DMF, 10 mL of water and 623 mg (1.4 mmol) of

RGDV were successively added. The reaction mixture was adjusted to pH 8 with N-methylmorpholine, stirred by preventing from light for 48 h, and TLC (CH_3OH/ HOAC, 1000/5) indicated the disappearance of **2**. The reaction mixture was evaporated under vacuum to remove DMF, the residue was extracted with methanol (20 mL × 5), the extract was evaporated under vacuum, the formed colorless powders were washed with ether/acetone (5/1, 20 mL × 6), and purified on preparative TLC to provide 240 mg (26%) of the title compound as colorless powders. Mp 139 - 143 ºC. ESI/MS (m/z) 919 $[M - H]^-$. $[\alpha]_D^{20}$ = 34.8 (c = 0.13, CH_3OH). IR (KBr) 3380, 2959, 1664, 1580, 1409, 1178, 1043, 891, 652 cm^{-1}. ^1H NMR (BHSC-300, DMSO-d_6) δ/ppm = 10.14 (s, 1H), 9.16 (d, *J* = 8.1 Hz, 1H), 9.03 (m, 1H), 8.64 (d, *J* = 7.5 Hz, 1H), 7.83 (m, 2H), 7.34 (d, *J* = 9.9 Hz, 1H), 7.03 (d, *J* = 8.1 Hz, 1H), 6.22 (d, *J* = 10.2 Hz, 1H), 6.00 (s, 1H), 5.74 (s, 1H), 5.08 (d, *J* = 17.4 Hz, 1H), 4.80 (d, *J* = 17.7 Hz, 1H), 4.38 (d, *J* = 5.2 Hz, 1H), 4.25 (m, 1H), 4.16 (d, *J* = 10.2 Hz, 1H), 4.00 (d, *J* = 16.2 Hz, 1H), 3.80 (m, 1H), 3.54 (m, 2H), 3.17 (s, 3H), 2.88 (m, 2H), 2.60 (m, 3H), 2.32 (m, 2H), 2.16 (m, 3H), 1.66 (m, 3H), 1.56 (m, 3H), 1.49 (s, 3H), 1.34 (m, 2H), 1.07 (m, 1H), 0.89 (s, 3H), 0.79 (d, *J* = 6.9 Hz, 3H), 0.75 (d, *J* = 6.3 Hz, 6H). ^{13}C NMR (BHSC-75, DMSO-d_6) δ/ppm = 211.61, 206.95, 185.79, 178.19, 176.00, 172.31, 171.24, 169.21, 168.58, 167.60, 153.40, 129.37, 124.55, 102.88, 90.80, 72.69, 66.58, 60.53, 59.57, 53.20, 51.18, 48.95, 47.91, 43.74, 36.25, 34.27, 32.52, 31.54, 30.78, 29.31, 28.50, 27.74, 25.52, 23.48, 20.07, 18.60, 17.07, 15.73.

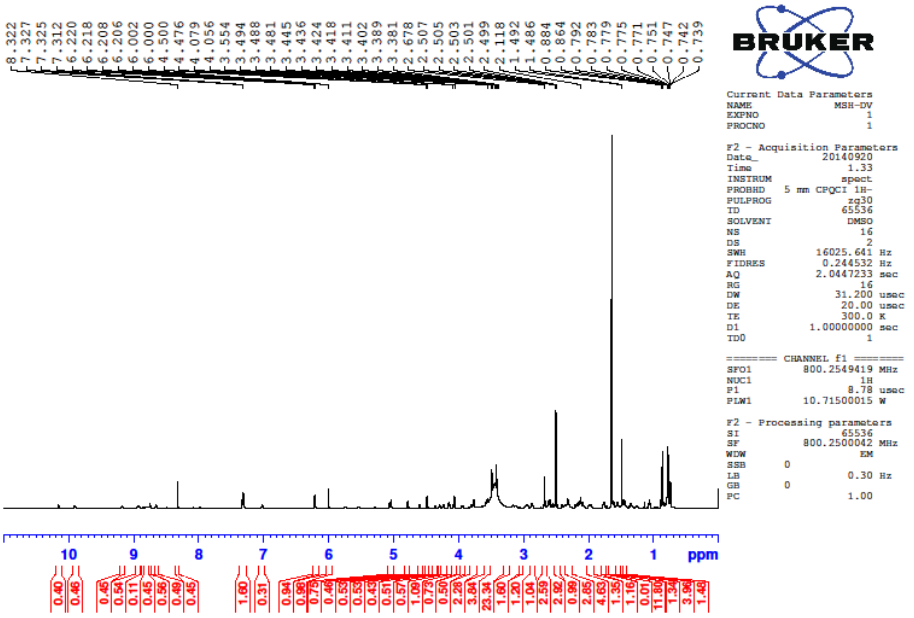

Figure S1 [1]HNMR of RGDV-Dex (**4a**).

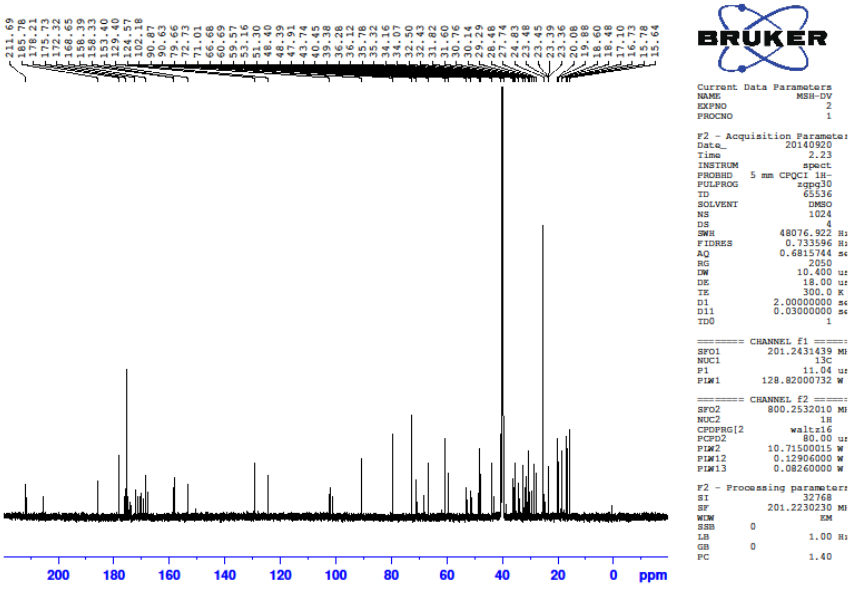

Figure S2 [13]CNMR of RGDV-Dex (**4a**).

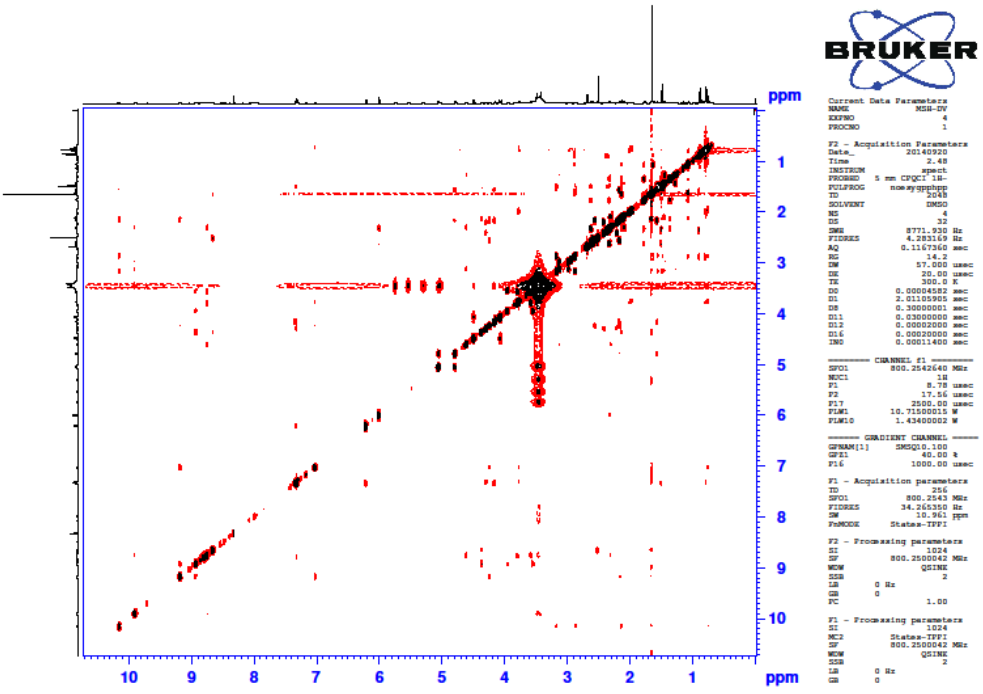

Figure S3 ROESY 2D NMR spectrum of RGDV-Dex (**4a**).

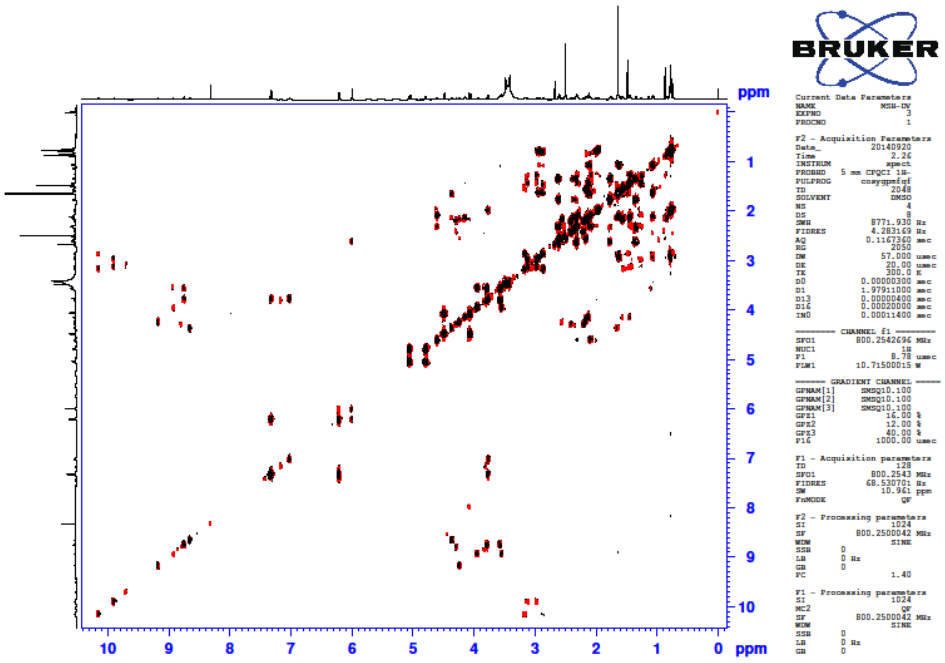

Figure S4 COSY 2D NMR spectrum of RGDV-Dex (**4a**).

(9R,10S,11S,13S,16R,17R)-9-Fluoro-7,8,11,12,13,15,16,17-octahydro-11,17-dihydroxy-17-(2-Arg-Gly-Asp-Ser-oxycarbonylthylcarbonyloxy)-10,13,16-

trimethyl- 6H-cyclopenta[a]phenanthren-3(9H,10H,14H)one (4b) Using the procedure of preparing **4a** from 782 mg (1.4 mmol) of **2** and 606 mg (1.4 mmol) of RGDS 318 mg (35%) of the title compound was obtained as colorless powders. Mp 177 - 179 ºC. ESI/MS (m/z) 907 $[M - H]^-$. $[\alpha]_D^{20}$ = 72.1 (c = 0.14, CH_3OH). IR (KBr) 3363, 2942, 1663, 1405, 1246, 1180, 1041, 891, 650 cm^{-1}; ^1H NMR (BHSC-300, DMSO-d_6) δ/ppm = 10.02 (s, 1H), 8.73 (m, 1H), 8.51 (d, *J* = 7.5 Hz, 1H), 8.39 (d, *J* = 7.5 Hz, 1H), 7.91 (m, 1H), 7.58 (m, 2H), 7.33 (d, *J* = 10.0 Hz, 1H), 7.23 (d, *J* = 5.0 Hz, 1H), 6.22 (d, *J* = 9.5 Hz, 1H), 6.01 (s, 1H), 5.57 (s, 1H), 5.06 (d, *J* = 17.5 Hz, 1H), 4.80 (d, *J* = 17.5 Hz, 1H), 4.30 (m, 2H), 4.13 (m, 1H), 3.92 (d, *J* = 17.0 Hz, 1H), 3.78 (m, 2H), 3.58 (d, *J* = 16.5 Hz, 1H), 3.50 (m, 2H), 3.18 (m, 1H), 2.93 (m, 3H), 2.68 (m, 1H), 2.65 (m, 4H), 2.36 (m, 3H), 2.02 (m, 1H), 1.78 (m, 1H), 1.70 (s, 3H), 1.64 (d, *J* = 11.5 Hz, 1H), 1.57 (d, *J* = 13.0 Hz, 1H), 1.50 (s, 3H), 1.45 (m, 2H), 1.36 (m, 2H), 0.90 (s, 3H), 0.80 (d, *J* = 7.0 Hz, 3H). ^{13}C NMR (BHSC-75, DMSO-d_6) δ/ppm = 211.65, 185.80, 178.17, 175.83, 172.37, 171.34, 169.21, 168.79, 167.59, 153.35, 129.41, 124.55, 102.40, 90.83, 71.22, 66.63, 62.92, 56.13, 52.76, 50.96, 48.54, 47.92, 43.74, 36.27, 35.35, 34.06, 32.50, 30.77, 29.25, 28.53, 27.73, 25.14, 24.50, 23.39, 17.09, 16.72, 15.74.

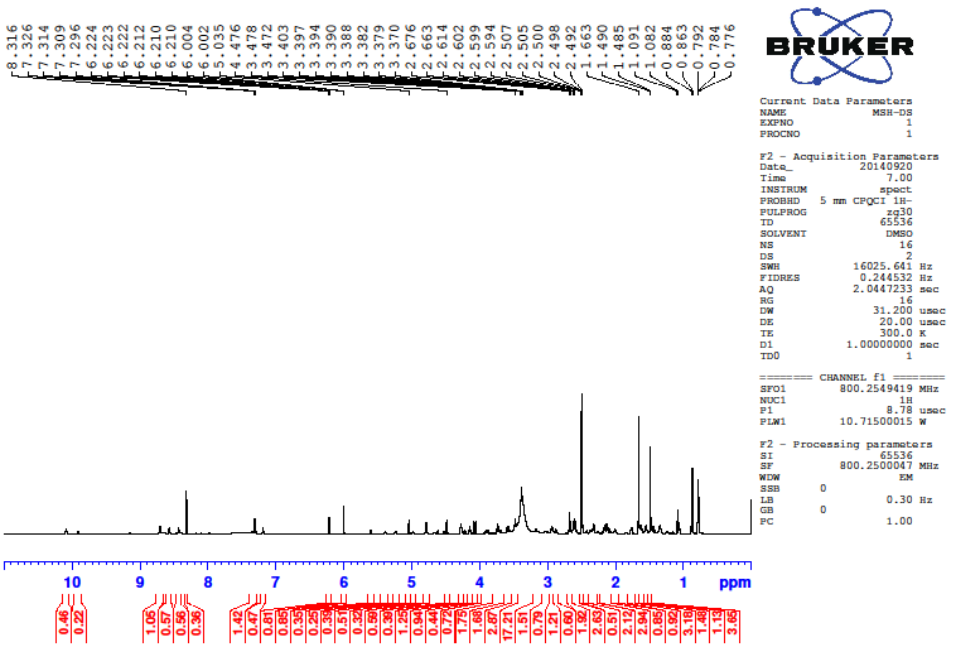

Figure S5 [1]HNMR of RGDS-Dex (**4b**).

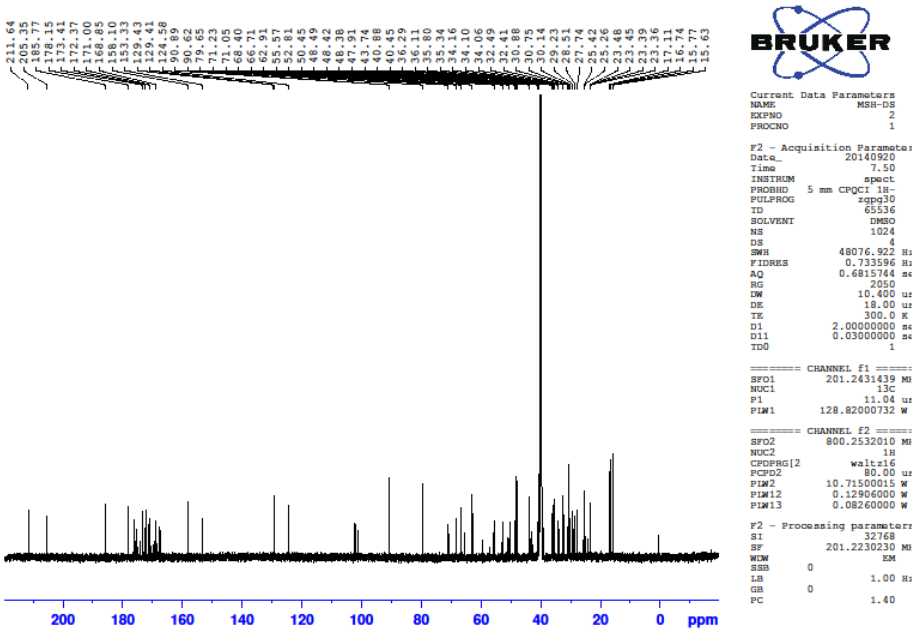

Figure S6 [13]CNMR of RGDS-Dex (**4b**).

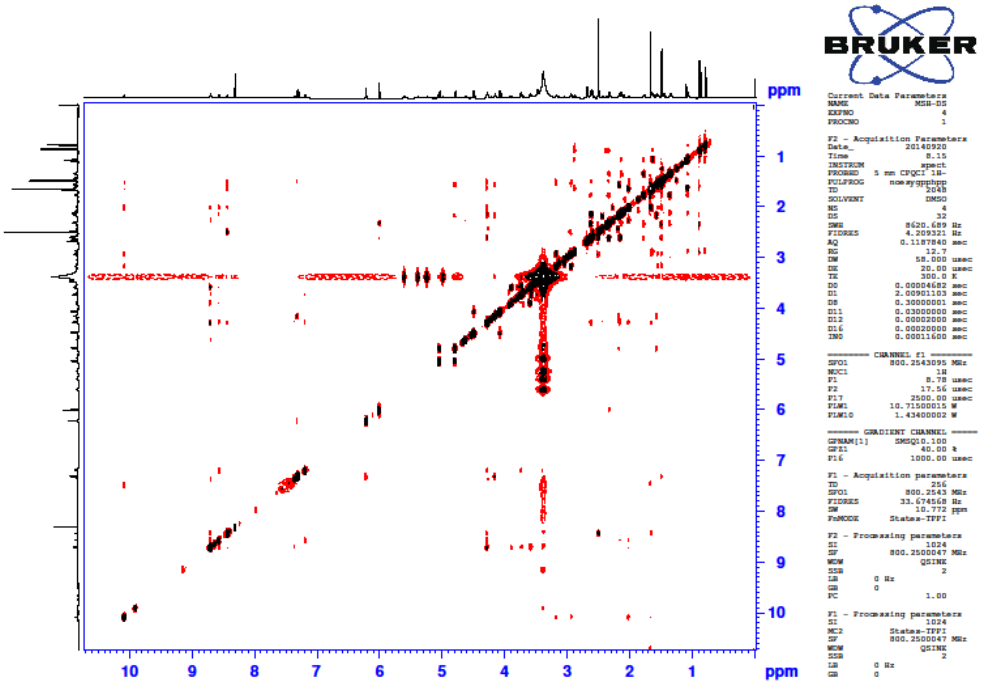

Figure S7 ROESY 2D NMR spectrum of RGDS-Dex (**4b**).

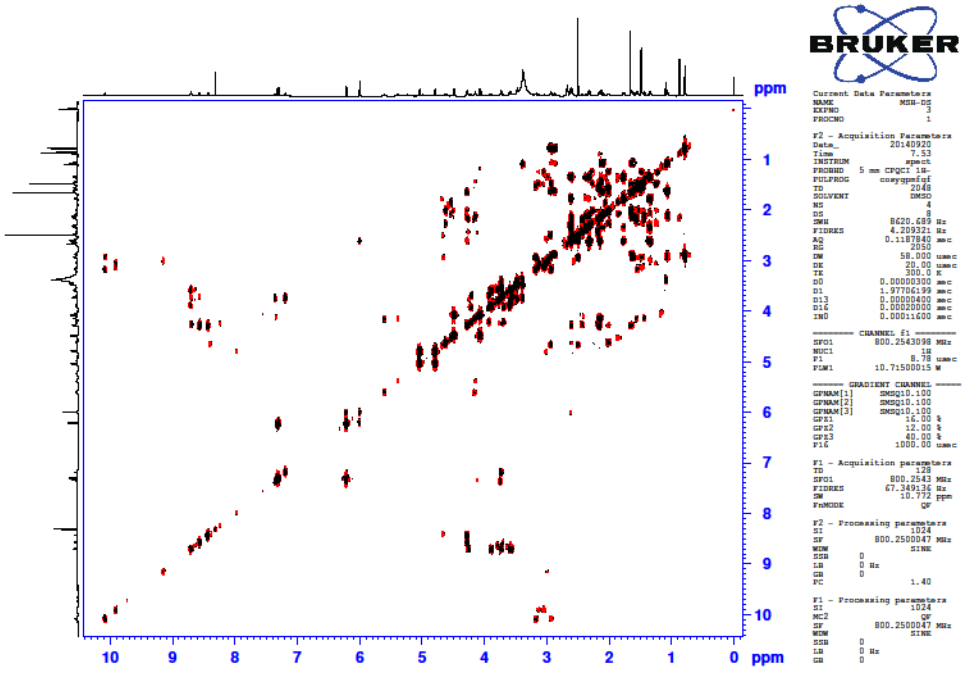

Figure S8 COSY 2D NMR spectrum of RGDS-Dex (**4b**).

(9R,10S,11S,13S,16R,17R)-9-Fluoro-7,8,11,12,13,15,16,17-octahydro-11,17-

dihydroxy-17-(2-Arg-Gly-Asp-Phe-oxycarbonylthylcarbonyloxy)-10,13,16-trimethyl- 6H-cyclopenta[a]phenanthren-3(9H,10H,14H)one (4c) Using the procedure of preparing **4a** from 782 mg (1.4 mmol) of **2** and 690 mg (1.4 mmol) of RGDF 231 mg (24%) of the title compound was obtained as colorless powders. Mp 181 - 184 ºC. ESI/MS (m/z) 967 $[M - H]^-$. $[\alpha]_D^{20}$ = 68.8 (c = 0.11, CH_3OH). IR (KBr) 3370, 2940, 1663, 1580, 1411, 1284, 1171, 1042, 891, 650 cm^{-1}. ^1H NMR (BHSC-300, DMSO-d_6) δ/ppm = 9.93 (s, 1H), 8.73 (m, 1H), 8.51 (d, J = 6.6 Hz, 1H), 8.17 (m, 1H), 7.79 (m, 2H), 7.44 (m, 1H), 7.35 (d, J = 9.6 Hz, 1H), 7.15 (m, 5H), 7.09 (m, 1H), 6.22 (d, J = 9.6 Hz, 1H), 6.01 (s, 1H), 5.78 (m, 1H), 5.07 (d, J = 17.4 Hz, 1H), 4.79 (d, J = 17.4 Hz, 1H), 4.30 (m, 2H), 4.17 (m, 2H), 4.05 (m, 3H), 3.87 (m, 4H), 3.58 (m, 3H), 2.97 (m, 2H), 2.89 (m, 3H), 2.63 (m, 2H), 2.31 (m, 3H), 2.17 (m, 3H), 1.93 (m, 1H), 1.59 (m, 2H), 1.50 (s, 3H), 1.35 (m, 1H), 0.89 (s, 3H), 0.79 (d, J = 6.6 Hz, 3H). ^{13}C NMR (BHSC-75, DMSO-d_6) δ/ppm = 211.66, 185.75, 178.10, 176.36, 172.34, 171.11, 168.92, 167.53, 158.35, 153.37, 129.97, 128.09, 125.97, 124.57, 102.89, 90.89, 71.36, 67.48, 65.35, 60.19, 56.02, 53.09, 51.06, 48.61, 43.75, 36.30, 35.34, 34.02, 32.53, 30.78, 28.54, 27.75, 25.59, 23.42, 21.21, 17.08, 16.72, 15.75, 14.55.

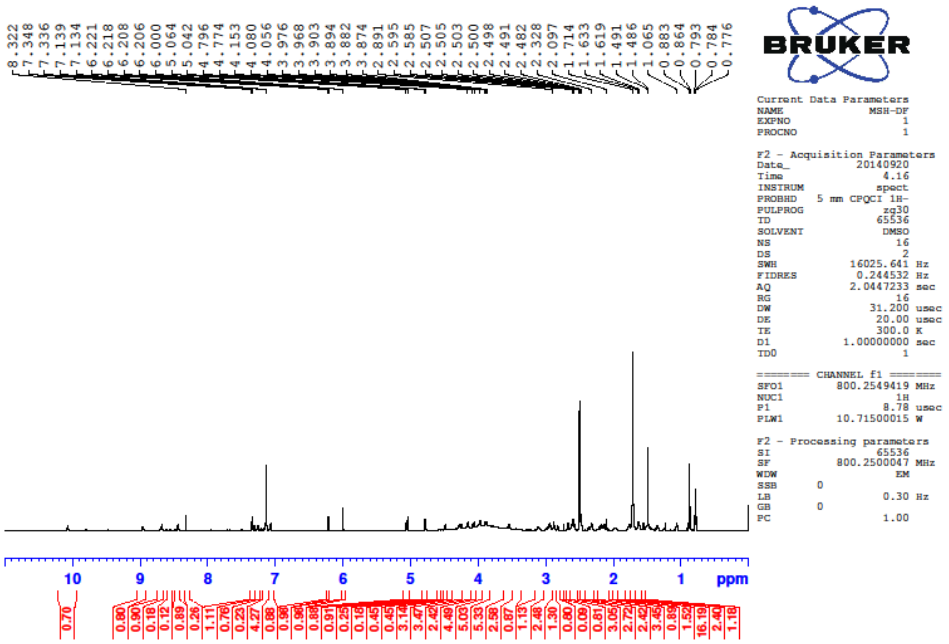

Figure S9 [1]HNMR of RGDF-Dex (**4c**).

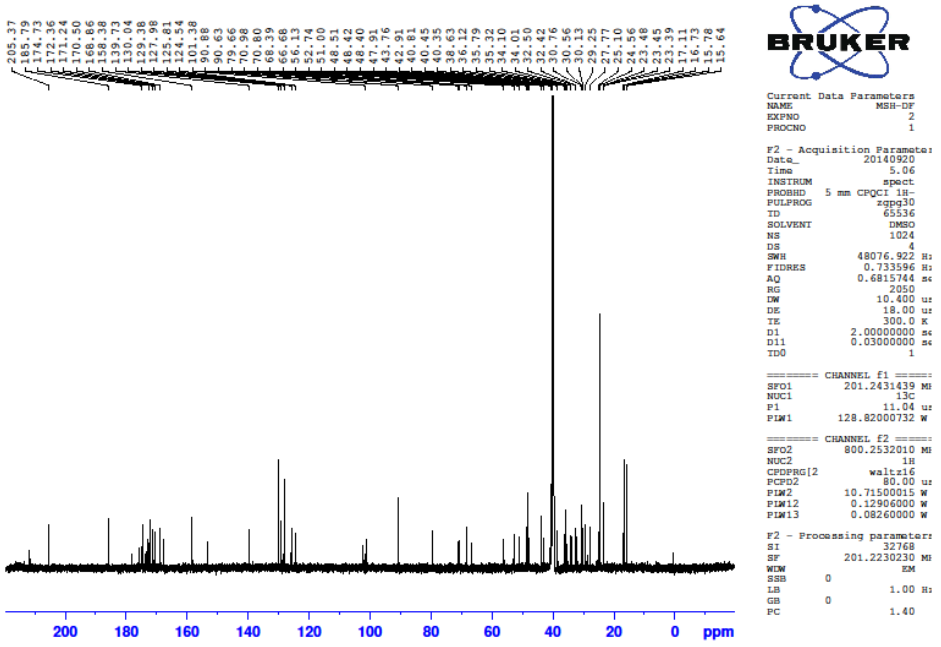

Figure S10 [13]CNMR of RGDF-Dex (**4c**).

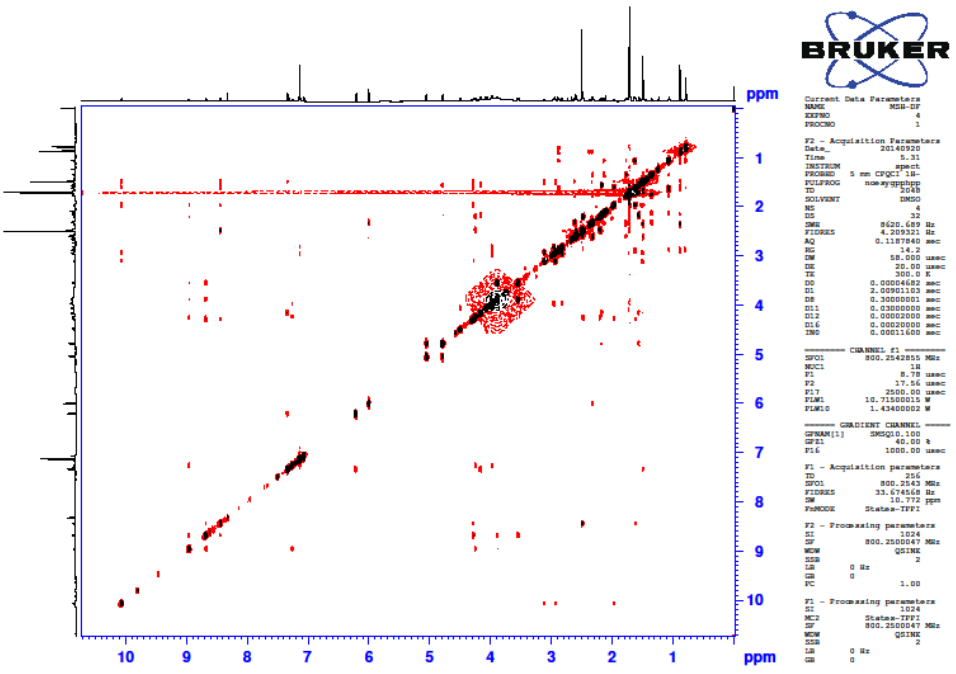

Figure S11 ROESY 2D NMR spectrum of RGDF-Dex (**4c**).

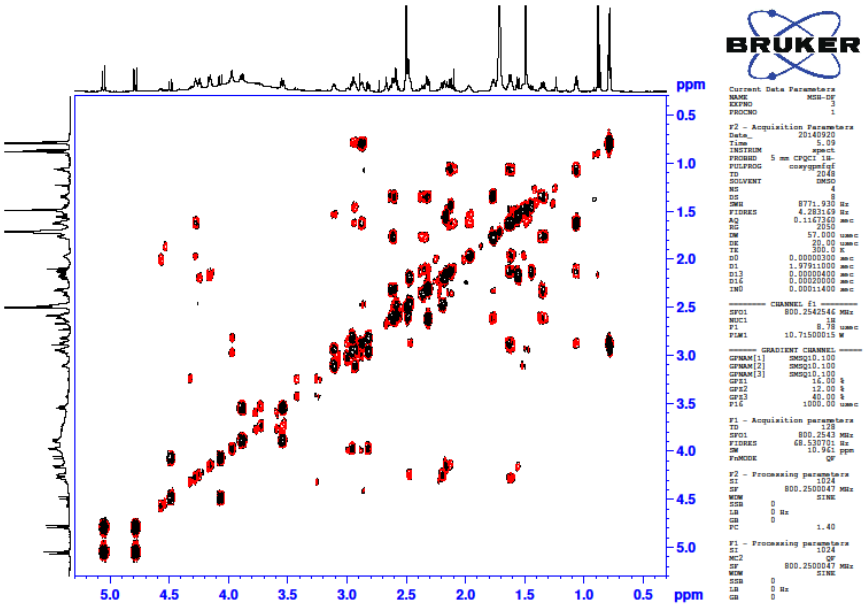

Figure S12 COSY 2D NMR spectrum of RGDF-Dex (**4c**).

3. Characterization

ESI-Mas test

The ESI mass spectrum was acquired using a SolariX FT-ICR mass spectrometer (Bruker Daltonics) consisting of an ESI ion source and 9.4 T superconductive magnet. The measurement was carried out in the positive ion mode. A smart-beam-II laser (wavelength, 355 nm; focus setting, medium; repetition rate, 1000 Hz) was used as the ion source. The qCID mass was set to proper m/z, and the isolation window was 5 m/z. Data were acquired using SolariXcontrol software. Spectral data were processed with DataAnalysis software (Bruker Daltonics).

3D structure generation

The 2D structures of RGDV-Dex, RGDS-Dex and RGDF-Dex were sketched with ChemDraw Ultra 10.0. The 3D structures were created and energy minimized until the minimum rms reached 0.001 in Chem3D Ultra 10.0. Then the 3D structures were energy minimized with Discovery Studio 2.1 in the MMFF force field. The energy-minimized conformation was utilized as starting conformation for the conformation generation. The energy-minimized conformations were sampled in the whole conformational space via systematic search method and BEST method in Discovery Studio 2.1. Both the methods were performed with the SMARTminimizer using the CHARMM force field. The 20 kcal/mol/300 K of the energy threshold was set. The maximum minimization step was set to 200, and the minimization of the rms gradient was set to 0.1 Å. The maximum generated conformations were set to 255 with a rmsd cutoff of 0.2 Å. According to the 3D structures and the intermolecular interactions identified with their ROESY 2D NMR spectra and their ESI-MS spectra, the present

polymerization models were proposed.

Calculation to predict the formation of the nanoparticles

The mesoscale simulation software was used to perform the calculation and to predict how much trimers of RGDV-Dex, RGDS-Dex and RGDF-Dex can form a nanoparticle of definite size. Discover module of the Materials Studio software was used for the simulation. The molecules of RGDV-Dex, RGDS-Dex and RGDF-Dex were built and optimized simply in the Visualizer window. “Beads” were constructed from atomistic simulation and placed at the center-of-mass of groups of atoms corresponding to the particular parts of the molecules of RGDV-Dex, RGDS-Dex and RGDF-Dex.

TEM Test

The examinations of the shape and size of the nanospecies of RGDV-Dex, RGDS-Dex and RGDF-Dex were performed on a transmission electron microscope (TEM, JSM- 6360 LV, JEOL, Tokyo, Japan). The 1 nM aqueous solutions of RGDV-Dex, RGDS- Dex and RGDF-Dex were dripped onto a Formvarcoated copper grid, and then a drop of anhydrous ethanol was added to promote water removal. The grid was first allowed to thoroughly dry in air and was then heated at 37 °C for 24 h. The samples were viewed under the TEM. The shape and size distribution of the nano-species were determined from counting over 100 species in randomly selected regions on the TEM copper grid. All the determinations were carried out in triplicate grids. The TEM was operated at 80 kV, electron beam accelerating voltage. Images were recorded on an imaging plate (Gatan Bioscan Camera model 1792) with 20 eV energy

windows at 6000-400 000 × and were digitally enlarged.

4. Bioassays

Male ICR mice and BALB/C mice were purchased from the Animal Center of Peking University. Work performed was 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 in accordance to the NIH Guide for Care and Use of Laboratory Animals. Statistical analyses of all the biological data were carried out by use of ANOVA. $P < 0.05$ were considered statistically significant. The mice were maintained at 21 °C with a natural day/night cycle in a conventional animal colony.

Total volumetric bone mineral density assay of femurs

Secondary osteoporosis is common in patients treated with glucocorticoids. Chronic exposure to effective dose of Dex causes side reaction, osteoporosis. To examine the risk induced by RGD-tetrapeptide covalent modification the BALB/C mice (male, 14 weeks in age) were orally administered with 0.5 % CMC-Na or 1.43 μmol/kg/day of Dex or RGDV-Dex or RGDS-Dex or RGDF-Dex for 15 consecutive days to measure the total volumetric bone mineral density (vBMD) and the peripheral quantitative CT (pQCT). Computed tomography (CT) allows testing 3D bone geometry and provides size- independent vBMD. The peripheral quantitative CT (pQCT) allows quantitative test of 3D bone geometry and quantitatively provides size-independent vBMD.

Femur weight assay

After vBMD and pQCT measurements the mice orally treated with 0.5 % CMC-Na or

1.43 μmol/kg/day of Dex or RGDV-Dex or RGDS-Dex or RGDF-Dex for 15 consecutive days received euthanasia, their left femurs were immediately collected, completely removed the muscle to measure their lengths, immersed in a 2/1 solution of chloroform and methanol (3 h × 2, each) to defate, heated at 120 ºC for 6 h, cooled and weighed to record their dry weight. The femurs were calcined in a furnace at 800 ºC for 8 h, cooled, weighed to record the ash weight, and the rate of the ash weight to dry femur weight (the mineral content of the femur) was calculated. The ashes of the left femurs were dissolved in 0.5 mL of hydrochloric acid (6 M) and diluted to 5.00 mL with ultrapure water, from which 0.05 mL of the solution was drawn and diluted to 1.00 mL with ultrapure water before use. The content of calcium in the solution was tested with the method of o-methylphenolphthalein complexing ketone, while the content of phosphorus in the solution was tested with the method of molybdenum blue.

Anti-inflammatory assay

The anti-inflammatory assay of Dex, RGDV-Dex, RGDS-Dex and RGDF-Dex were performed with xylene-induced ear edema, and the clinical dose of Dex in the anti-inflammatory therapy was converted for the mice. In brief, male ICR mice (18 ± 2 g) were randomly divided into five groups, each 12 mice. The mice in vehicle control group were orally given 0.5 % CMC-Na at a dose of 0.2 mL/mouse, and the mice in positive group were orally given the suspension of Dex in 0.5 % CMC-Na at a dose of 25.5 μmol/kg, and the mice in RGDV-Dex, RGDS-Dex and RGDF-Dex groups were orally given the suspension of RGDV-Dex, RGDS-Dex and RGDF-Dex in 0.5 % CMC-Na at a dose of 25.5 μmol/kg. Thirty minutes after the administration, 30 μL of

xylene was applied to the anterior and posterior surfaces of the right ear, with the left ear as control. Two hours after xylene application, the mice received euthanasia to remove both ears. By using a cork borer of 7 mm in diameter the circular slices were collected and weighed. The increased weight caused by the irritant was calculated by subtracting the weight of the untreated left ear slice from that of the treated right ear slice.

Effect of dose on the anti-inflammation activity

The 25.5, 2.55 and 0.255 μmol/kg of RGDS-Dex was used as the representatives to reveal the dose-activity relationship. In brief by using the same procedure as that of item **Anti-inflammatory assay** the xylene-induced ear edema of the mice treated with 25.5, 2.55 and 0.255 μmol/kg of RGDS-Dex were tested.

DNA expression assay

DNA expression assay was carried out with A549 cells. In brief, aliquots of 3×10^5 cells were seeded in six well plates, incubated at 37 ºC for 24 h, treated with fresh medium alone (negative control), or with fresh medium containing Dex, RGDF-Dex, RGDS-Dex or RGDV-Dex (final concentration, 1 μM) and incubated for another 48 h. The cells were harvested, centrifuged at 2500 g for 5 min, resuspended in 200 μL PBS, to which 20 μL Proteinase K and 20 μL RNaseA were added according to Genomic DNA Kits (Catalog Numbers K182001, Invitrogen, 3175 Staley Road, Grand Island, New York, USA). The wells were mixed by brief vortexing, and incubate at room temperature for 2 min. To the cells 200 μL lysis and binding buffer were added and mixed well by vortexing to obtain a homogenous solution, and then incubateed at 55

°C for 10 min to promote the digestion of the protein. To each well 200 μL ethanol (96%) was added and the lysate was mixed by vortexing for 5 sec to yield a homogenous solution. At room temperature the lysate (~640 μL) was centrifuged at 10000 g for 1 min, and 500 μL wash buffer was add and centrifuged at room temperature at 16000 g for 3 min. To the residues 30 μL of elution buffer was added, incubated at room temperature for 1 min, and then centrifuged at 16000 g for 1 min at room temperature. The supernatant solution was transferred to a sterile 1.5 mL microcentrifuge tube for analysis. The DNA concentration was measured by Nanodrop 1000 (NanoDrop products, 3411 Silverside Rd, Bancroft Building, Wilmington, DE 19810, USA).

Plasma TNF-α assay

To understand the possible mechanism of RGD-tetrapeptide covalent modification enhancing anti-inflammatory activity the TNF-α of the plasma of xylene-induced ear edema of mice orally treated with 0.5% CMC-Na, or the suspensions of Dex, RGDS-Dex, RGDF-Dex and RGDV-Dex in 0.5% CMC-Na were measured. In brief, to prepare the plasma sample 9 mL of blood collected from health mice or xylene-induced ear edema mice receiving 0.5% CMC-Na or 25 μmol/kg Dex, or 25 μmol/kg conjugates into a syringe containing 1 mL 3.8% sodium citrate. Sample was centrifuged at 4 °C and 3000 × g for 30 min to prepare plasma sample. To each of blank wells nothing was added. To each of standard wells 50 μL standard solutions and 100 μL HRP-conjugate were added. To each of testing wells 10μL plasma of inflammation mice 40μL sample diluents and 100 μL HRP-conjugate were

successively added. The wells were aspirated, washed by filling with 400 μL wash solution via a squirt bottle and the liquid was completely moved, which was repeated for 5 times. The plate was inverted and blotted with clean paper towels, closed with closure plate membrane and incubated at 37 °C for 60 min. On uncovering plate membrane the liquid were discarded, the wells were dried by swing, to the residue sufficient washing buffer was added, stilled for 30s and then drained. This procedure was repea- ted for 5 times, the wells were dried by pat. To the residue 50 μL chromogen solutions A and B were added, gently mixed and at 37 °C and in dark incubated for 10 min. To each well 50 μL stop solution was added, and the color in the well changed from blue to yellow. The plate was read at 450 nm using a microtiter plate reader within 15 min to record O.D. value. According to the standard curve the concentration of plasma TNF-α was calculated.

Plasma IL-8 assay

To understand the possible mechanism of RGD-tetrapeptide covalent modification enhancing anti-inflammatory activity the IL-8 of the plasma of xylene-induced ear edema of mice orally treated with 0.5% CMC-Na, or the suspensions of Dex, RGDS-Dex, RGDF-Dex and RGDV-Dex in 0.5% CMC-Na were measured. In brief, in the preparation of the plasma sample 9 mL of blood collected from health mice or xylene-induced ear edema mice receiving 0.5% CMC-Na or 25 μmol/kg Dex, or 25 μmol/kg conjugates into a syringe containing 1 mL 3.8% sodium citrate. Sample was centrifuged at 4 °C and 3000 × g for 30 min to prepare plasma sample. To each of blank wells 100 μL sample diluent was added. To each of standard wells 50 μL

standard solution and 50 μL Streptavidin-HRP were added. To each of the wells 40 μL plasma sample of the mice receiving 0.5% CMC-Na, 25 μmol/kg Dex, RGDS-Dex, RGDF-Dex and RGDV-Dex, 10 μL of anti-IL-8-antibody and 50 μL of Streptavidin-HRP were added. The plate was closed with plate membrane and at 37 °C incubated for 60 min. On uncovering plate membrane the liquid was discarded and the wells were dried by swing, to the residue sufficient washing buffer was added, stilled for 30s and then drained. The procedure was repeated for 5 times and the wells were dried by pat. To the residue 50 μL chromogen solutions A and B were added, gently mixed and at 37 °C in dark incubated for 10 min. To each well 50 μL stop solution was added, and the plate was read at 450 nm using a microtiter plate reader within 15 min to record O.D. value. According to the standard curve the concentrations of IL-8 in plasma were calculated.

ROESY 2D NMR spectra showing the hydroxyl of Dex modified by RGD-peptides

To show which hydroxyl of Dex was modified by RGD-peptides the ROESY 2D NMR spectra were analyzed, the cross-peaks were marked with blue rings and are shown in Figures S13-S15. As seen, the spectra consistently give a cross peak from -CO-CH_2-O- (4.20 ppm) and -O-CO-CH_2-CH_2-CO- (2.16 ppm). Thus among 11-hydroxy, 17-hydroxy and 17-(2-hydroxyacetyl) of Dex only the latter was modified.

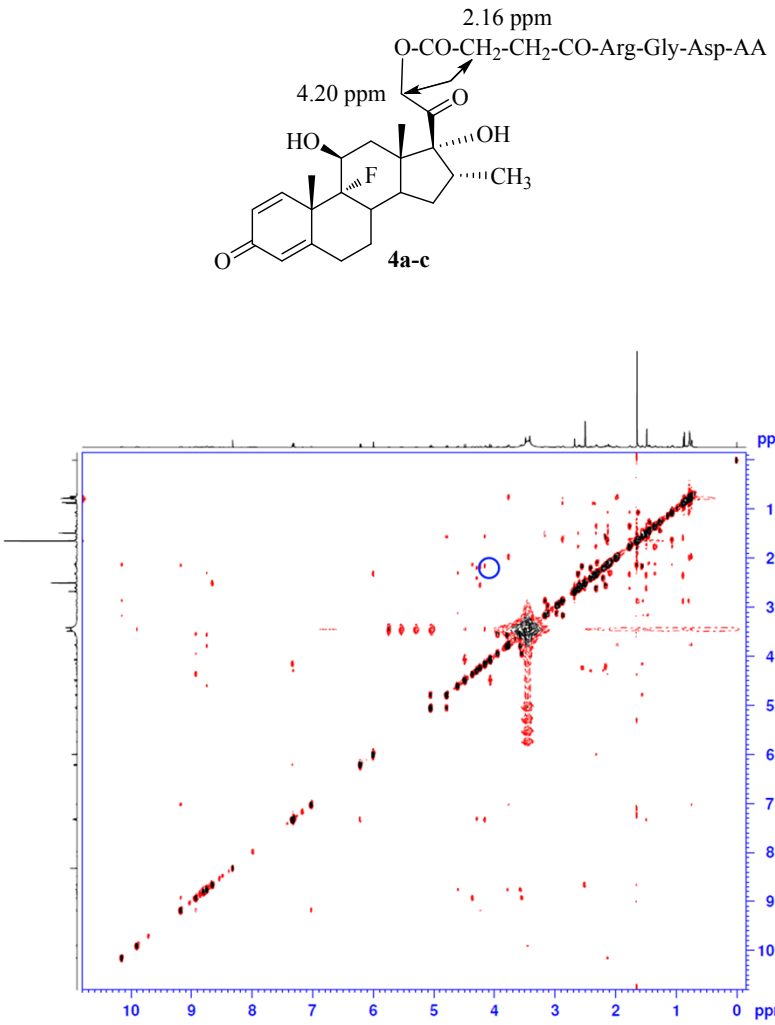

Figure S13 ROESY 2D NMR spectrum of RGDV-Dex (**4a**)

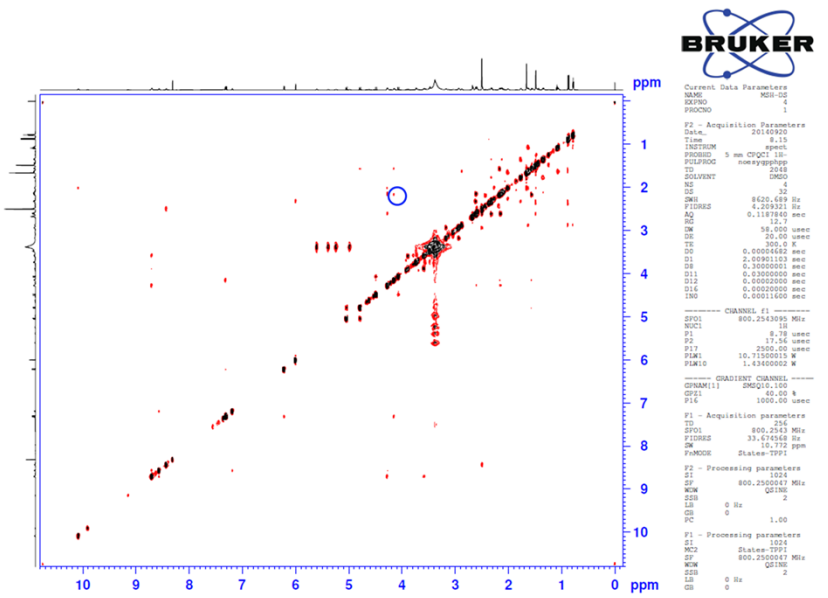

Figure S14 ROESY 2D NMR spectrum of RGDS-Dex (**4b**)

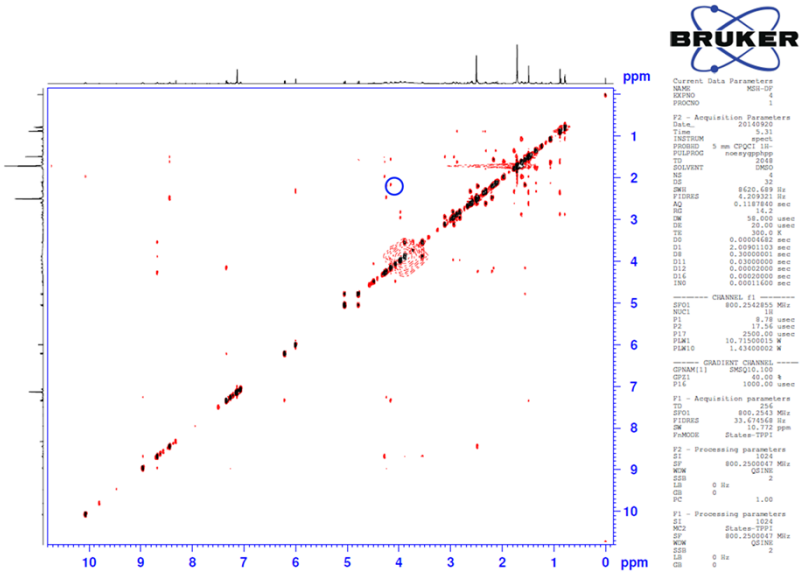

Figure S15 ROESY 2D NMR spectrum of RGDV-Dex (**4c**)

TEM images of 1 µM 4a-c

To show the

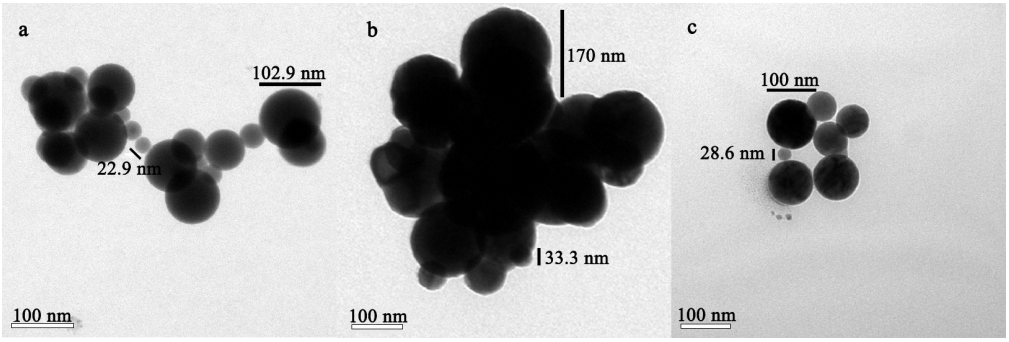

Figure S16 TEM images of 1 µM solution of the conjugates in ultrapure water: (a) TEM images of RGDV-Dex; (b) TEM images of RGDS-Dex; (c) TEM images of RGDF-Dex.