Angewandte Chemie
Eine Zeitschrift der Gesellschaft Deutscher Chemiker

Supporting Information

69451 Weinheim, Germany

Application of Stereocontrolled Stepwise [3+2] Cycloadditions to the Preparation of Inhibitors of $\alpha_4\beta_1$ Integrin-Mediated Hepatic Melanoma Metastasis

Aizpea Zubia, Lorea Mendoza, Silvia Vivanco, Eneko Aldaba, Teresa Carrascal, Begoña Lecea, Ana Arrieta, Tahl Zimmerman, Fernando Vidal-Vanaclocha, and Fernando P. Cossío*

A) Synthetic methods and analytical data of compounds 4, 5, 6, 10 and 11 (Schemes 1 and 2).

General procedure for the synthesis of α-benzyloxy esters **4.**

To a solution of L-amino acid **1** (25.0 mmol) in H_2SO_4 2N (37.5 ml, aqueous solution), cooled to 0ºC, was added a solution of $NaNO_2$ 2N (2.57 g, 37.0 mmol) in water (9 ml). The temperature was maintained below 5ºC during the addition, and the mixture was stirred at such a temperature for 24 h. The solution was saturated with $(NH_4)_2SO_4$, extracted with ether (3x25 ml), dried over Na_2SO_4 and evaporated under reduced pressure giving 2.72 g (23.0 mmol) of the corresponding α-hydroxy acid as an oil. A solution of this oily material, *p*-toluenesulfonic acid monohydrate (0.03 g, 0.16 mmol) and dimethoxypropane (2.8 ml, 23.0 mmol) in methanol (1.15 ml) was heated at 45ºC for 24 h. The reaction mixture was evaporated under reduced pressure providing the corresponding α-hydroxy methyl ester **2** as an oil. This material was dissolved in anhydrous tetrahydrofuran (40 ml) and anhydrous *N*,*N*-dimethylformamide (12 ml), under an inert atmosphere, was cooled to 0ºC. NaH (0.48 g, 20.0 mmol) was added and the mixture was stirred for 10 min. The corresponding bromide **3** (24.0 mmol) was added dropwise, and the mixture was allowed to reach room temperature. The reaction was monitored by TLC. When the reaction was complete, the reaction mixture was diluted in ether (130 ml) and washed with water (3x50 ml). The remaining organic layer was dried over Na_2SO_4 and evaporated under reduced pressure, providing the crude product **4**, which was purified by flash column chromatography.

(S)-Methyl 2-(benzyloxy)-3-methylbutanoate. This compound was previously described by Jouillé et al. See: W.-R. Li, W. R. Ewing, B. D. Harris, M. M. Jouillé *J. Am. Chem. Soc*. **1990**, *112*, 7659.

(S)-Methyl 2-(2-fluorobenzyloxy)-3-methylbutanoate. This compound was prepared from L-valine and 2-fluorobenzyl bromide in 40 % yield: bp 70-72 ºC (0.5 mm Hg); IR 1751, 1229 cm^{-1}; ^{1}H-NMR (δ ppm, $CDCl_3$) 7.49-6.97 (m, 4H), 4.71 (d, 1H, J= 12.1 Hz), 4.49 (d, 1H, J= 12.1 Hz), 3.74 (s, 3H), 3.71 (d, 1H, J=5.7 Hz), 2.18-2.00 (m, 1H), 0.96 (d, 3H, J=4.0 Hz), 0.92 (d, 3H, J=4.0 Hz); ^{13}C-NMR (δ ppm, $CDCl_3$) 172.8, 163.1, 158.2, 130.3, 130.2, 129.5, 129.4, 124.0, 123.9, 115.3, 114.9, 83.6, 66.1, 66.0, 51.7, 31.6, 18.7, 17.7; $[\alpha]_D^{25}$= -64.9 (c= 1.27, CH_2Cl_2).

(S)-Methyl 2-(2,6-difluorobenzyloxy)-3-methylbutanoate. This compound was prepared from L-valine and 2,6-difluorobenzyl bromide in 51 % yield: bp 78-80 ºC (0.3 mm Hg); IR 1751, 1275 cm^{-1}; ^{1}H-NMR (δ ppm, $CDCl_3$) 7.31-7.20 (m, 1H), 6.92-6.85 (m, 2H), 4.78 (d_b, 1H, J=11.3 Hz), 4.51 (d_b, 1H, J=11.3 Hz), 3.74 (s, 3H), 3.66 (d, 1H, J=5.7 Hz), 2.01-1.98 (m, 1H), 0.97 (d, 3H, J=4.0 Hz), 0.95 (d, 3H, J=4.0 Hz); ^{13}C-NMR (δ ppm, $CDCl_3$) 172.5, 164.4, 164.3, 159.4, 159.3, 130.5, 130.2, 130.0, 113.8, 113.2, 112.6, 111.3, 111.1, 110.9, 110.8, 83.2, 59.5, 51.4, 31.3, 18.3, 17.3; $[\alpha]_D^{25}$= -70.2 (c= 1.42, CH_2Cl_2).

(2S,3S)-Methyl 2-(benzyloxy)-3-methylpentanoate. This compound was prepared from L-isoleucine and benzyl bromide in 58 % yield: bp 80-82 ºC (0.8 mm Hg); IR 1750, 742, 700 cm^{-1}; ^{1}H-NMR (δ ppm, $CDCl_3$) 7.35-7.25 (m, 5H), 4.67 (d, 1H, J= 11.7 Hz), 4.36 (d, 1H, J= 11.7 Hz), 3.75 (d, 1H, J= 3.7 Hz), 3.73 (s, 3H), 1.92-1.76 (m, 1H), 1.68-1.47 (m, 1H), 1.35-1.13 (m, 1H), 0.90 (d, 3H, J=6.8 Hz), 0.85 (t, 3H, J=7.4 Hz); ^{13}C-NMR (δ ppm, $CDCl_3$) 172.4, 137.3, 127.9, 127.5, 127.3, 82.1, 72.0, 51.0, 37.6, 24.3, 14.8, 10.9; $[\alpha]_D^{25}$= -66.7 (c= 1.00, CH_2Cl_2).

(2S,3S)-Methyl 2-(3,5-difluorobenzyloxy)-3-methylpentanoate. This compound was prepared from L-isoleucine and 3,5-difluorobenzyl bromide in 63 % yield: bp 78-80 ºC (0.7 mm Hg); IR 1746, 1114, 850 cm^{-1}; ^{1}H-NMR (δ ppm, $CDCl_3$) 6.90-6.66 (m, 3H), 4.64 (d, 1H, J= 12.4 Hz), 4.33 (d, 1H, J= 12.4 Hz), 3.78 (d, 1H, J= 5.7 Hz), 3.76 (s, 3H), 2.01-1.79 (m, 1H), 1.65-1.44 (m, 1H), 1.40-1.13 (m, 1H), 0.96-0.85 (m, 6H); ^{13}C-NMR

(□ ppm, $CDCl_3$) 172.5, 165.5, 165.3, 160.6, 160.4, 142.0, 141.9, 141.7, 110.3, 110.1, 109.9, 109.8, 103.4, 102.9, 102.4, 83.1, 71.2, 51.7, 38.0, 24.6, 15.3, 11.3; $[\alpha]_D^{25}$= -55.3 (c= 1.10, CH_2Cl_2).

(2S,3S)-Methyl 2-(2,3-difluorobenzyloxy)-3-methylpentanoate. This compound was prepared from L-isoleucine and 2,3-difluorobenzyl bromide in 48 % yield: bp 77-78 ºC (0.3 mm Hg); IR 1744, 1287 cm^{-1}; ^{1}H-NMR (□ ppm, $CDCl_3$) 7.25-7.03 (m, 3H), 4.70 (d, 1H, J= 12.2 Hz), 4.48 (d, 1H, J= 12.8 Hz), 3.78 (d, 1H, J= 5.7 Hz), 3.75 (s, 3H), 1.93-1.77 (m, 1H), 1.59-1.42 (m, 1H), 1.36-1.13 (m, 1H), 0.92-0.82 (m, 6H); ^{13}C-NMR (□ ppm, $CDCl_3$) 172.6, 152.9, 152.6, 151.1, 150.9, 148.0, 147.7, 146.2, 145.9, 127.4, 127.2, 124.9, 124.8, 124.7, 124.1, 124.0, 123.9, 116.8, 116.5, 83.2, 65.7, 65.6, 65.5, 51.7, 37.9, 24.6, 15.2, 11.3; $[\alpha]_D^{25}$= -56.7 (c= 0.40, CH_2Cl_2).

General procedure for the synthesis of □-benzyloxy aldehydes **5**.

A solution of lithium aluminum hydride (0.76 g, 20.0 mmol) in anhydrous ether (30 ml), under an inert atmosphere, was cooled to 0ºC. The corresponding □-benzyloxy ester **4** (20.0 mmol) in anhydrous ether (40 ml) was added dropwise, and the mixture was stirred at room temperature for 3 h. Then, the reaction mixture was cooled in ice and treated with water (130 ml). The crude was filtrated through Celite, and the filtrate was washed with water (3x80 ml). The remaining organic layer was dried over Na_2SO_4 and evaporated under reduced pressure, providing the expected alcohol as an oil, which was purified by distillation under reduced pressure using a Kugelrohr. A solution of oxalyl chloride (1.82 ml, 21.0 mmol) in anhydrous methylene chloride (42 ml), under inert atmosphere, was cooled to –70ºC. Anhydrous dimethyl sulfoxide (1.96 ml, 28.0 mmol) in anhydrous methylene chloride (42 ml) was added dropwise during 10 min. The temperature was maintained below –60ºC during the addition. To this mixture, the previously prepared and purified alcohol (14.0 mmol) dissolved in anhydrous methylene chloride (45 ml) was added dropwise during 10 min. The temperature was maintained below –60ºC during the addition. Then the mixture was stirred at –70ºC for 20 min. Triethylamine (7.84 ml, 56.0 mmol) was added dropwise during 5 min. The reaction was monitored by TLC. When the reaction was complete the mixture was allowed to reach room temperature. HCl 1N (56 ml, aqueous solution) and hexanes (140 ml) were added. The aqueous layer was separated and extracted twice with 90 ml aliquots of

ether. The residual organic layer and ether extracts were combined and washed with $NaHCO_3$ saturated water solution (2x70 ml), water (2x70 ml) and brine (2x70 ml), dried over Na_2SO_4 and evaporated under reduced pressure providing an oily residue which was purified by distillation under reduced pressure using a Kugelrohr, yielding the corresponding aldehyde as a colorless oil.

(S)-2-(Benzyloxy)-3-methylbutanal. This compound was previously described by Jouillé et al. See: W.-R. Li, W. R. Ewing, B. D. Harris, M. M. Jouillé *J. Am. Chem. Soc*. **1990**, *112*, 7659.

(S)-2-(2-Fluorobenzyloxy)-3-methylbutanal. This compound was prepared from (*S*)-methyl 2-(2-fluorobenzyloxy)-3-methylbutanoate in 58 % yield: bp 76-78 ºC (0.3 mm Hg); IR 1732 cm^{-1}; ^{1}H-NMR (δ ppm, $CDCl_3$) 9.66 (d, 1H, J= 2.6 Hz), 7.48-7.09 (m, 4H), 4.72 (d, 1H, J= 12.0 Hz), 4.56 (d, 1H, J= 12.0 Hz), 3.59 (dd, 1H, J= 5.7 Hz, J'=2.6 Hz), 2.18-2.01 (m, 1H), 0.99 (d, 3H, J=1.7 Hz), 0.96 (d, 3H, J=1.7 Hz); ^{13}C-NMR (δ ppm, $CDCl_3$) 203.7, 162.9, 158.1, 130.1, 130.0, 129.6, 129.4, 123.9, 123.8, 115.2, 114.8, 88.2, 66.2, 29.7, 18.1, 17.2; $[\alpha]_D^{25}$ = -77.0 (c=1.51, CH_2Cl_2).

(S)-2-(2,6-Difluorobenzyloxy)-3-methylbutanal. This compound was prepared from (*S*)-methyl 2-(2,6-difluorobenzyloxy)-3-methylbutanoate in 73 % yield: bp 75-76 ºC (0.3 mm Hg); IR 1729, 1275 cm^{-1}; ^{1}H-NMR (δ ppm, $CDCl_3$) 9.65 (d, 1H, J=2.7 Hz), 7.37-7.22 (m, 1H), 6.96-6.86 (m, 2H), 4.73 (d, 1H, J=11.0 Hz), 4.60 (d, 1H, J=11.0 Hz), 3.45 (dd, 1H, J=5.9 Hz, J'=2.7 Hz), 2.12-1.96 (m, 1H), 0.95 (d, 3H, J=2.0 Hz), 0.91 (d, 3H, J=2.0 Hz); ^{13}C-NMR (δ ppm, $CDCl_3$) 204.1, 164.4, 164.2, 159.4, 159.3, 130.7, 130.6, 130.5, 113.6, 113.3, 111.5, 111.3, 111.2, 111.0, 88.5, 60.2, 60.1, 60.0, 29.8, 18.2, 17.3; $[\alpha]_D^{25}$ = -73.1 (c=1.14, CH_2Cl_2).

(2S,3S)-2-(Benzyloxy)-3-methylpentanal. This compound was prepared from (*2S,3S*)-methyl 2-(benzyloxy)-3-methylpentanoate in 88 % yield: bp 67-68 ºC (0.8 mm Hg); IR 1732 cm^{-1}; ^{1}H-NMR (δ ppm, $CDCl_3$) 9.67 (d, 1H, J= 2.8 Hz), 7.35-7.25 (m, 5H), 4.66 (d, 1H, J= 11.7 Hz), 4.48 (d, 1H, J= 11.7 Hz), 3.53 (t, 1H, J= 2.9 Hz), 1.92-1.79 (m, 1H), 1.69-1.47 (m, 1H), 1.41-1.18 (m, 1H), 0.96-0.83 (m, 6H); ^{13}C-NMR (δ ppm, $CDCl_3$) 204.0, 137.3, 128.2, 127.7, 87.0, 72.5, 36.2, 24.3, 14.8, 11.2; $[\alpha]_D^{25}$ = -84.5 (c=1.00, CH_2Cl_2).

(2S,3S)-2-(3,5-Difluorobenzyloxy)-3-methylpentanal. This compound was prepared from (*2S,3S*)-methyl 2-(3,5-difluorobenzyloxy)-3-methylpentanoate in 73 % yield: bp 92-93 ºC (0.5 mm Hg); IR 1734, 1113, 850 cm^{-1}; ^{1}H-NMR (δ ppm, $CDCl_3$) 9.69 (d, 1H, J= 2.5 Hz), 6.92-6.83 (m, 2H), 6.78-6.68 (m, 1H), 4.66 (d, 1H, J= 12.5 Hz), 4.42 (d, 1H, J= 12.5 Hz), 3.57 (dd, 1H, J=5.7, J'= 2.5 Hz), 2.08-1.81 (m, 1H), 1.72-1.46 (m, 1H), 1.43-1.19 (m, 1H), 0.99 (d, 3H, J=6.9 Hz), 0.90 (d, 3H, J=7.4 Hz); ^{13}C-NMR (δ ppm, $CDCl_3$) 203.3, 165.5, 165.3, 160.6, 160.3, 141.9, 141.7, 141.5, 110.1, 109.9, 109.8, 109.6, 103.4, 102.9, 102.4, 87.7, 71.2, 36.4, 24.5, 15.0, 11.3; $[\alpha]_D^{25}$ = -65.9 (c=1.00, CH_2Cl_2).

(2S,3S)-2-(2,3-Difluorobenzyloxy)-3-methylpentanal. This compound was prepared from (*2S,3S*)-methyl 2-(2,3-difluorobenzyloxy)-3-methylpentanoate in 85 % yield: bp 88-89 ºC (0.5 mm Hg); IR 1725, 1290 cm^{-1}; ^{1}H-NMR (δ ppm, $CDCl_3$) 9.69 (d, 1H, J= 2.5 Hz), 7.26-7.02 (m, 3H), 4.73 (dd, 1H, J= 12.0 Hz, J'=1.0 Hz), 4.56 (dd, 1H, J= 12.0 Hz, J'=1.0 Hz), 3.58 (dd, 1H, J=5.8, J'= 2.5 Hz), 1.97-1.84 (m, 1H), 1.62-1.46 (m, 1H), 1.40-1.17 (m, 1H), 0.96 (d, 3H, J=6.9 Hz), 0.88 (d, 3H, J=7.4 Hz); ^{13}C-NMR (δ ppm, $CDCl_3$) 203.3, 152.7, 152.4, 150.9, 150.7, 147.8, 147.5, 146.0, 145.7, 127.2, 126.9, 124.6, 124.5, 124.0, 123.9, 123.8, 116.7, 116.4, 87.7, 65.7, 36.2, 24.3, 14.7, 11.1; $[\alpha]_D^{25}$ = -64.7 (c=1.00, CH_2Cl_2).

General procedure for the synthesis of (E)-nitroalkenes ***6***

A mixture of the corresponding aldehyde **5** (16.0 mmol), nitromethane (4.30 ml, 80.0 mmol) and triethylamine (0.32 ml, 2.28 mmol) was stirred at room temperature for 16 h. The excess of nitromethane was removed by evaporation under reduced pressure. The nitroaldol thus obtained as a mixture of diastereomers was dissolved in anhydrous methylene chloride (32 ml) and cooled to -70ºC. Methanesulfonyl chloride (1.48 ml, 19.11 mmol) was added dropwise followed by a solution of *N,N*-diisopropylethylamine (6.85 ml, 39.81 mmol) in dry methylene chloride (8 ml), keeping the reaction mixture below –60ºC. The mixture was stirred at –70ºC for 2 h and then allowed to reach room temperature. The solution was washed with water (8 ml), HCl 1N (4x8 ml, aqueous solution) and brine (8 ml), dried over Na_2SO_4 and evaporated. The product corresponding nitroalkene was purified by flash column chromatography as a pale yellow oil.

[[(S,E)-4Methyl-1-nitropent-1-en-3yloxy]methyl]benzene. This compound was obtained from (S)-2-(benzyloxy)-3-methylbutanal in 68 % yield: b.p. 124-126 ºC (0.2 mm Hg); IR 1527, 1348 cm^{-1}; ^{1}H-NMR (δ ppm, $CDCl_3$) 7.47-7.28 (m, 6H), 7.21-7.06 (m, 2H), 4.58 (d, 1H, J= 11.7 Hz), 4.43 (d, 1H, J= 11.7 Hz), 3.86 (t, 1H, J=5.2 Hz), 2.03-1.90 (m, 1H), 0.97 (d, 3H, J=6.8 Hz), 0.97 (d, 3H, J=6.8 Hz); ^{13}C-NMR (δ ppm, $CDCl_3$) 140.7, 140.3, 138.4, 128.3, 127.7, 127.5, 80.0, 71.2, 17.9, 17.7; $[\alpha]_D^{25}$ = -33.7 (c=2.30, CH_2Cl_2).

1-[[(S,E)-4Methyl-1-nitropent-1-en-3yloxy]methyl]-2-fluorobenzene. This compound was obtained from (*S*)-2-(2-fluorobenzyloxy)-3-methylbutanal in 81 % yield: b.p. 110-111 ºC (0.3 mm Hg); IR 1527, 1349, 1230 cm^{-1}; ^{1}H-NMR (δ ppm, $CDCl_3$) 7.43-7.03 (m, 6H), 4.60 (d, 1H, J= 11.7 Hz), 4.49 (d, 1H, J= 11.7 Hz), 3.88 (t, 1H, J=5.3 Hz), 2.02-1.92 (m, 1H), 0.94 (t_b, 3H, J=7.1 Hz); ^{13}C-NMR (δ ppm, $CDCl_3$) 163.1, 158.2, 140.5, 140.4, 130.1, 130.0, 129.8, 129.7, 124.1, 124.0, 115.4, 115.0, 80.6, 65.6, 65.5, 32.5, 17.8; $[\alpha]_D^{25}$ = -27.2 (c=1.03, CH_2Cl_2).

1-[[(S,E)-4Methyl-1-nitropent-1-en-3yloxy]methyl]-2,6-difluorobenzene. This compound was obtained from (*S*)-2-(2,6-difluorobenzyloxy)-3-methylbutanal in 95 % yield: b.p. 110-111 ºC (0.3 mm Hg); IR 1527, 1353, 1276 cm^{-1}; ^{1}H-NMR (δ ppm, $CDCl_3$) 7.38-6.85 (m, 5H), 4.64 (dt_b, 1H, J= 11.6 Hz, J'=1.4 Hz), 4.56 (dt_b, 1H, J= 11.6 Hz, J'=1.4 Hz), 3.83 (dd, 1H, J=5.7 Hz, J'=3.7 Hz), 2.00-1.84 (m, 1H), 0.92 (d, 3H, J=4.7 Hz), 0.89 (d, 3H, J=4.7 Hz); ^{13}C-NMR (δ ppm, $CDCl_3$) 164.2, 164.0. 159.2, 159.1, 140.4, 140.2, 130.6, 130.4, 130.2, 113.4, 113.1, 111.1, 111.0, 110.8, 80.5, 59.1, 59.0, 58.9, 32.4, 17.6, 17.4; $[\alpha]_D^{25}$ = -40.5 (c=1.23, CH_2Cl_2).

[[(E,3S,4S)-4Methyl-1-nitrohex-1-en-3yloxy]methyl]benzene. This compound was obtained from (*S*)-2-(benzyloxy)-3-methylpentanal in 91 % yield: b.p. 97-98 ºC (0.9 mm Hg); IR 1526, 1344, 748, 697 cm^{-1}; ^{1}H-NMR (δ ppm, $CDCl_3$) 7.35-7.07 (m, 7H), 4.56 (d, 1H, J= 11.7 Hz), 4.44 (d, 1H, J= 11.7 Hz), 3.96 (t, 1H, J=5.1 Hz), 1.91-1.72 (m, 1H), 1.63-1.41 (m, 1H), 1.32-1.10 (m, 1H), 0.92 (t, 3H, J=4.5 Hz), 0.88 (d, 3H, J=3.3 Hz); ^{13}C-NMR (δ ppm, $CDCl_3$) 140.7, 140.3, 138.4, 128.3, 127.7, 127.5, 80.0, 71.2, 17.9, 17.7; $[\alpha]_D^{25}$ = -20.1 (c=1.00, CH_2Cl_2).

1-[[(E,3S,4S)-4Methyl-1-nitrohex-1-en-3yloxy]methyl]-3,5-difluorobenzene. This compound was obtained from (*S*)-2-(3,5-difluorobenzyloxy)-3-methylpentanal in 82 %

yield: b.p. 115-116 ºC (0.3 mm Hg); IR 1522, 1344, 1113, 850 cm^{-1}; ^{1}H-NMR (δ ppm, $CDCl_3$) 7.25-7.05 (m, 2H), 6.85-6.69 (m, 3H), 4.54 (d, 1H, J= 12.6 Hz), 4.42 (d, 1H, J= 12.6 Hz), 3.98 (t, 1H, J=5.1 Hz), 1.94-1.72 (m, 1H), 1.64-1.39 (m, 1H), 1.36-1.09 (m, 1H), 0.96-0.89 (m, 6H); ^{13}C-NMR (δ ppm, $CDCl_3$) 165.5, 165.3, 160.6, 160.3, 141.8, 141.7, 141.5, 140.6, 140.0, 109.9, 109.7, 109.6, 109.4, 103.4, 102.9, 102.4, 79.6, 70.2, 38.9, 25.1, 14.2, 11.4; $[\alpha]_D^{25}$ = -11.5 (c=1.00, CH_2Cl_2).

1-[[(E,3S,4S)-4Methyl-1-nitrohex-1-en-3yloxy]methyl]-2,3-difluorobenzene. This compound was obtained from (*S*)-2-(2,3-difluorobenzyloxy)-3-methylpentanal in 93 % yield: b.p. 119-120 ºC (0.6 mm Hg); IR 1530, 1352, 1104 cm^{-1}; ^{1}H-NMR (δ ppm, $CDCl_3$) 7.26-7.05 (m, 5H), 4.61 (dd, 1H, J= 12.0 Hz, J'=1.4 Hz), 4.52 (dd, 1H, J= 12.0 Hz, J'=1.4 Hz), 3.99 (dd, 1H, J=5.5 Hz, J'=4.2 Hz), 1.91-1.68 (m, 1H), 1.63-1.37 (m, 1H), 1.33-1.08 (m, 1H), 0.94-0.85 (m, 6H); ^{13}C-NMR (δ ppm, $CDCl_3$) 152.9, 152.7, 151.2, 150.9, 148.0, 147.8, 146.2, 146.0, 140.6, 140.2, 127.2, 126.9, 124.6, 124.5, 124.4, 124.3, 124.2, 124.1, 124.0, 117.1, 116.8, 79.8, 65.1, 65.0, 64.9, 38.9, 25.2, 14.4, 11.4; $[\alpha]_D^{25}$ = -11.0 (c=1.10, CH_2Cl_2).

General procedure for the synthesis of imines **9.**

These compounds were prepared following the procedure reported in Ref. 6 and were used as such in the next step since their instability precluded further chromatographic purification.

General procedure for the synthesis of pyrrolidines **10**

The imine **9** (5 mmol) was solved in CH_3CN (50 ml), and then TEA (1.4 ml, 10 mmol), the nitroalkene **6** (5 mmol) and AgOAc (0.13 g, 0.75 mmol) were added. The progress of the reaction was monitored by TLC. After completion of the reaction, the mixture was filtered through a Celite pad and washed with NH_4Cl saturated water solution (2 x 10 ml) and water (2 x 10 ml). After drying (Na_2SO_4), the solution was evaporated, and the crude mixture purified by flash chromatography (Ethyl acetate/hexanes). The resulting oily product was solved in DME (25 ml) and cooled down to 0°C. LiOH 1N aqueous solution (15 ml) was added dropwise, and the progress of the reaction was monitored by TLC. After completion of the reaction, citric acid 10% aqueous solution

(15 ml, pH 6) was added. The resulting solution was extracted with CH_2Cl_2 (3 x 20 ml), and the combined organic fractions were dried and evaporated. The crude product was triturated in Et_2O yielding the corresponding pyrrolidine **10** as a white solid.

(2S,3R,4S,5S)-3-[(S)-1-(Benzyloxy)-2-methypropyl]-4-nitro-5-phenyl-pyrrolidine-2-carboxylic acid **(10a):** 68 % yield; mp 154-155 ºC (dec.); IR 1734, 1551, 1364 cm^{-1}; ^1H NMR (δ ppm, $CDCl_3$) 9.30 (s_b, 2H), 7.40-7.21 (m, 10H), 5.43 (d, 1H, J=5.8 Hz), 4.80 (d, 1H, J=5.8 Hz), 4.68 (s_b, 2H), 4.16 (d, 1H, J=7.4 Hz), 3.63 (d, 1H, J=6.5 Hz), 3.09 (d, 1H, J=7.4 Hz), 2.11-1.92 (m, 1H), 0.97 (d, 3H, J=6.7 Hz), 0.88 (d, 3H, J=6.7 Hz); ^{13}C NMR (δ ppm, $CDCl_3$) 173.2, 138.5, 135.8, 128.4, 128.2, 128.0, 127.6, 126.7, 91.8, 83.1, 73.5, 67.0, 62.7, 51.5, 30.9, 18.4. Anal. Calcd. for $C_{22}H_{26}N_2O_5$: C, 66.30; H, 6.59; N, 7.03. Found: C, 65.89; H, 6.64; N, 7.02 %; $[\alpha]_D^{25}$ = + 33.0 (c = 1.04, CH_2Cl_2).

(2S,3R,4S,5S)-5-tert-Butyl-3-[(S)-1-(benzyloxy)-2-methypropyl]-4-nitropyrrolidine-2-carboxylic acid **(10b):** 55 % yield; mp 155-157 ºC (dec.); IR 1734, 1551, 1364 cm^{-1}; ^1H NMR (δ ppm, $CDCl_3$) 10.35 (s_b, 2H), 7.42-7.25 (m, 5H), 5.09 (d, 1H, J=4.6 Hz), 4.81 (d, 1H, J=10.2 Hz), 4.60 (d_b, 2H, J=4.9 Hz), 4.56 (d, 1H, J=10.2 Hz), 3.72 (d, 1H, J=4.6 Hz), 3.62 (tb, 1H, J=4.0 Hz), 3.05-2.95 (m, 1H), 2.10-1.90 (m, 1H), 1.10-0.85 (m, 15H); ^{13}C NMR (δ ppm, $CDCl_3$) 172.3, 138.2, 128.4, 127.8, 86.5, 85.1, 74.5, 70.1, 63.5, 50.8, 32.2, 31.5, 26.5, 19.0, 17.7. Anal. Calcd. for $C_{20}H_{30}N_2O_5$: C, 63.45; H, 8.00; N, 7.40. Found: C, 62.91; H, 7.94; N, 7.44 %; $[\alpha]_D^{25}$ = + 33.0 (c = 1.04, CH_2Cl_2).

(2S,3R,4S,5S)-3-[(S)-1-(2-Fluorobenzyloxy)-2-methypropyl]-4-nitro-5-phenyl-pyrrolidine-2-carboxylic acid **(10c):** 66 % yield; mp 158-159 ºC (dec.); IR 1734, 1551, 1364 cm^{-1}; ^1H NMR (δ ppm, $CDCl_3$) 7.47-6.70 (m, 11H), 5.39 (dd, 1H, J=6.1 Hz, J'=1.7 Hz), 4.80-4.66 (m, 3H), 4.06 (d, 1H, J=7.2 Hz), 3.62 (d_b, 1H, J=6.8 Hz), 3.13 (d_b, 1H, J=7.4 Hz), 2.15-1.95 (m, 1H), 1.01 (d, 3H, J=6.7 Hz), 0.89 (d, 3H, J=6.7 Hz); ^{13}C NMR (δ ppm, $CDCl_3$) 173.3, 163.4, 158.5, 132.8, 130.6, 130.5, 130.2, 130.0, 128.8, 126.2, 124.9, 124.6, 124.4, 124.3, 115.7, 115.3, 90.5, 84.5, 68.4, 6.3, 66.7, 63.0, 51.6, 31.5, 19.0, 18.4. Anal. Calcd. for $C_{22}H_{25}N_2O_5F$: C, 63.44; H, 6.06; N, 6.73. Found: C, 62.96; H, 6.07; N, 6.72 %; $[\alpha]_D^{25}$ = + 22.4 (c = 1.13, CH_2Cl_2).

(2S,3R,4S,5S)-3-[(1S,2S)-1-(Benzyloxy)-2-methylbutyl]-4-nitro-5-phenyl-pyrrolidine-2-carboxylic acid **(10d):** 71% yield; mp 162-164 ºC (dec.); IR 3435, 1627, 1555, 1377 cm^{-1}; ^1H NMR (δ ppm, $CDCl_3$) 7.37-7.22 (m, 10H), 5.69 (s_b, 2H), 5.43 (dd, 1H, J = 6.2

Hz, J' = 1.8 Hz), 4.76 (d, 1H, J = 11.3 Hz), 4.71 (d, 1H, J = 6.7 Hz), 4.54 (d, 1H, J = 11.3 Hz), 3.94 (d, 1H, J = 7.0 Hz), 3.74 (d, 1H, J = 5.2 Hz), 3.09 (d_b, 1H, J = 7.2 Hz), 1.94-1.78 (m, 1H), 1.57-1.39 (m, 1H), 1.28-1.05 (m, 1H), 0.96-0.85 (m, 6H); ^{13}C NMR (□ ppm, $CDCl_3$) 173.3, 137.9, 133.2, 128.8, 128.6, 128.1, 127.8, 126.2, 90.8, 82.9, 73.4, 67.1, 63.4, 51.1, 37.4, 26.0, 14.5, 11.7. Anal. Calcd. for $C_{23}H_{28}N_2O_5$: C, 66.96; H, 6.85; N, 6.79. Found: C, 66.78; H, 6.82; N, 6.72 %; $[\square]_D^{25}$ = + 52.2 (c = 0.60, CH_2Cl_2).

(2S,3R,4S,5S)-3-[(S)-1-(2,6-Difluorobenzyloxy)-2-methypropyl]-4-nitro-5-phenyl-pyrrolidine-2-carboxylic acid **(10e):** 60 % yield; mp 169-170 ºC; IR 1734, 1551, 1364 cm^{-1}; ^{1}H NMR (□ ppm, $CDCl_3$) 7.40-7.20 (m, 6H), 7.00-6.86 (m, 2H), 5.39 (s_b, 2H), 5.28 (dd, 1H, J=6.2 Hz, J'=1.8 Hz), 4.89 (d, 1H, J=10.5 Hz), 4.66 (d_b, J=6.9 Hz), 3.88 (d, 1H, J=6.8 Hz), 3.57 (dd, 1H, J=7.1 Hz, J'=1.3 Hz), 3.15 (d_b, 1H, J=7.4 Hz), 2.18-1.98 (m, 1H), 1.09 (d, 3H, J=6.7 Hz), 0.93 (d, 3H, J=6.7 Hz); ^{13}C NMR (□ ppm, $CDCl_3$) 173.7, 164.3, 164.1, 159.3, 159.2, 133.1, 130.9, 130.6, 130.4, 128.7, 126.2, 113.4, 112.3, 111.8, 111.6, 111.4, 111.3, 90.7, 84.4, 66.9, 63.1, 61.5, 61.4, 51.9, 31.6, 18.8, 18.5. Anal. Calcd. for $C_{22}H_{24}N_2O_5F_2$: C, 60.81; H, 5.58; N, 6.45. Found: C, 60.15; H, 5.49; N, 6.54 %; $[\square]_D^{25}$ = + 25.9 (c = 1.00, CH_2Cl_2).

(2S,3R,4S,5S)-3-[(1S,2S)-1-(Benzyloxy)-2-methylbutyl]-5-cyclohexyl-4-nitropyrrolidine-2-carboxylic acid **(10f):** 55% yield; mp 194-196ºC; IR 3437, 1635, 1553, 1380 cm^{-1}; ^{1}H NMR (□ ppm, $CDCl_3$) 7.40-7.29 (m, 5H), 5.23 (d, 1H, J = 4.9 Hz), 4.73 (d, 1H, J = 10.4 Hz), 4.57 (d, 1H, J = 10.4 Hz), 4.33 (d, 1H, J= 7.1 Hz), 3.72 (d, 1H, J = 5.5 Hz), 3.55 (dd, 1H, J = 4.9 Hz, J' = 3.9 Hz), 2.99 (d, 1H, J = 6.0 Hz), 2.04-2.01 (m, 1H), 1.83-1.41 (m, 8H), 1.32-1.13 (m, 5H), 0.99-0.85 (m, 7H); ^{13}C NMR (□ ppm, $CDCl_3$) 172.1, 138.3, 128.6, 128.5, 128.0, 87.8, 84.1, 74.2, 67.3, 64.0, 50.7, 38.0, 36.8, 30.3, 30.2, 25.8, 25.7, 24.8, 14.8, 11.9. Anal. Calcd. For $C_{23}H_{34}N_2O_5$: C, 65.99; H, 8.20; N, 6.69. Found: C, 65.79; H, 8.10, N, 6.83 %; $[\square]_D^{25}$ = + 51.0 (c = 1.0, CH_2Cl_2).

(2S,3R,4S,5S)-3-[(1S,2S)-1-(3,5-Difluorobenzyloxy)-2-methylbutyl]-4-nitro-5-phenyl-pyrrolidine-2-carboxylic acid **(10g):** 69% yield; mp 146-147 ºC (dec.); IR 3428, 1626, 1555, 1378, 1119 cm^{-1}; ^{1}H NMR (□ ppm, $CDCl_3$) 7.43-7.26 (m, 5H), 7.09-6.68 (m, 5H), 5.46 (dd, 1H, J = 5.9 Hz, J' = 2.0 Hz), 4.75 (d, 1H, J = 5.9 Hz), 4.67 (d, 1H, J = 11.7 Hz), 4.60 (d, 1H, J = 11.7 Hz), 4.13 (d, 1H, J = 8.2 Hz), 3.75 (d, 1H, J = 6.1 Hz), 3.06 (d_b, 1H, J = 7.9 Hz), 1.94-1.70 (m, 1H), 1.55-1.30 (m, 1H), 1.28-0.97 (m, 1H), 0.92-0.78

(m, 6H); ^{13}C NMR (□ ppm, $CDCl_3$) 172.6, 165.6, 165.4, 160.7, 160.4, 141.9, 141.8, 141.6, 131.5, 129.0, 125.9, 110.7, 110.5, 110.4, 110.2, 103.7, 103.2, 102.7, 90.1, 83.0, 72.3, 65.9, 63.1, 51.1, 37.5, 25.7, 14.3, 11.6. Anal. Calcd. for $C_{23}H_{26}N_2O_5F_2$: C, 61.59; H, 5.86; N, 6.25. Found: C, 61.60; H, 5.89; N, 6.19 %; $[\square]_D^{25}$ = + 21.4 (c = 0.80, CH_2Cl_2).

(2S,3R,4S,5S)-3-[(1S,2S)-1-(Benzyloxy)-2-methylbutyl]-5-cyclopropyl-4-nitro-pyrrolidine-2-carboxylic acid **(10h):** 39% yield; mp 159-161ºC; IR 3248, 1557, 1389 cm^{-1}; ^{1}H NMR (□ ppm, $CDCl_3$) 7.32-7.27 (m, 5H), 5.19 (d, 1H, J = 4.9 Hz), 4.65 (d, 1H, J = 10.4 Hz), 4.50 (d, 1H, J = 10.2 Hz), 4.21 (d, 1H, J= 4.3 Hz), 3.70 (d, 1H, J = 5.4 Hz), 3.10 (m, 2H), 1.79-1.68 (m, 1H), 1.52-1.42 (m, 1H), 1.14-1.03 (m, 1H), 0.90-0.78 (m, 6H), 0.79-0.66 (m, 2H), 0.66-0.54 (m, 2H), 0.29-0.19 (m, 1H); ^{13}C NMR (□ ppm, $CDCl_3$) 172.3, 138.4, 128.8, 128.6, 128.2, 89.2, 83.8, 74.2, 67.9, 63.7, 50.8, 38.1, 30.0, 25.9, 14.9, 12.1, 7.8, 4.4, 1.4. Anal. Calcd. For $C_{20}H_{28}N_2O_5$: C, 63.81; H, 7.51; N, 7.44. Found: C, 63.99; H, 7.60, N, 7.50 %; $[\square]_D^{25}$ = + 37.8 (c = 0.86, CH_3OH).

(2S,3R,4S,5S)-3-[(1S,2S)-1-(2,3-Difluorobenzyloxy)-2-methylbutyl]-4-nitro-5-phenyl-pyrrolidine-2-carboxylic acid **(10i):** 58% yield; mp 148-149 ºC (dec.); IR 3428, 1626, 1555, 1386, 1287 cm^{-1}; ^{1}H NMR (□ ppm, $CDCl_3$) 8.23 (s_b, 2H) 7.30-7.05 (m, 8H), 5.43 (dd, 1H, J = 6.0 Hz, J' = 1.8 Hz), 4.80-4.65 (m, 3H), 4.06 (d, 1H, J = 7.6 Hz), 3.76 (d, 1H, J = 6.0 Hz), 3.05 (d_b, 1H, J = 7.8 Hz), 1.92-1.77 (m, 1H), 1.53-1.33 (m, 1H), 1.25-1.06 (m, 1H), 0.92-0.82 (m, 6H); ^{13}C NMR (□ ppm, $CDCl_3$) 172.8, 152.9. 152.7, 151.3, 151.1, 148.0, 147.8, 146.4, 146.1, 131.8, 128.9, 127.4, 127.1, 125.9, 125.2, 125.1, 125.0, 124.4, 124.3, 124.2, 117.2, 116.9, 90.3, 83.1, 66.6, 66.3, 63.4, 51.1, 37.4, 25.6, 14.3, 11.6. Anal. Calcd. for $C_{23}H_{26}N_2O_5F_2$: C, 61.59; H, 5.86; N, 6.25. Found: C, 61.57; H, 5.87; N, 6.25 %; $[\square]_D^{25}$ = + 26.4 (c = 0.77, CH_2Cl_2).

(2S,3S,4S,5S)-3-[(1S,2S)-1-(Benzyloxy)-2-methylbutyl]-2-methyl-4-nitro-5-phenyl-pyrrolidine-2-carboxylic acid **(10j):** 83% yield; mp 98-99 ºC (dec.); IR 3438, 1725, 1640, 1551, 1362 cm^{-1}; ^{1}H NMR (□ ppm, $CDCl_3$) 7.39-7.25 (m, 10H), 5.51 (dd, 1H, J=6.4 Hz, J'=2.5 Hz), 5.36 (s_b, 2H), 4.78 (d, 1H, J=6.9 Hz), 4.72 (d, 1H, J=11.2 Hz), 4.41 (d, 1H, J=11.2 Hz), 3.82 (d, 1H, J=3.8 Hz), 3.17 (d, 1H, J=2.2 Hz), 2.08-1.93 (m, 1H), 1.65 (s, 3H), 1.32-1.14 (m, 1H), 1.13-0.98 (m, 1H), 0.91 (t, 3H, J=7.1 Hz), 0.81 (d, 3H, J=6.9 Hz); ^{13}C NMR (□ ppm, $CDCl_3$) 176.0, 134.0, 131.7, 128.7, 128.4, 127.7,

127.4, 126.3, 90.7, 79.5, 70.5, 69.6, 64.3, 51.2, 36.2, 25.8, 18.7, 13.4, 12.0. Anal. Calcd. for $C_{24}H_{30}N_2O_5$: C, 67.57; H, 7.10; N, 6.57. Found: C, 66.94; H, 7.19; N, 6.56 %; $[\alpha]_D^{25}$ = + 9.9 (c = 0.53, CH_2Cl_2).

(2S,3R,4S,5S)-3-[(1S,2S)-1-(benzyloxy)-2-methylbutyl]-5-[(S)-1-(benzyloxy)-2-methylpropyl]-4-nitropyrrolidine-2-carboxylic acid **(10k)**: 20% yield; mp 172-174ºC; IR 3428, 1635, 1555, 1386 cm^{-1}; ^1H NMR (δ ppm, $CDCl_3$) 7.88 (s_b, 2H), 7.35-7.23 (m, 10H), 5.24 (d, 1H, J = 4.8 Hz), 4.65 (d, 1H, J = 10.3 Hz), 4.55 (d, 1H, J = 10.4 Hz), 4.51 (d, 1H, J = 11.1 Hz), 4.37 (d, 1H, J= 11.1 Hz), 4.22 (d, 1H, J = 6.1 Hz), 3.94 (t, 1H, J = 5.7 Hz, J' = 5.6 Hz), 3.65 (d, 1H, J = 5.9 Hz), 3.52 (t, 1H, J = 5.1 Hz, J' = 5.7 Hz), 2.94 (d, 1H, J = 6.1 Hz), 2.12-2.09 (m, 1H), 1.84-1.75 (m, 1H), 1.54-1.48 (m, 1H), 1.16-1.07 (m, 1H), 0.89-0.85 (m, 12H); ^{13}C NMR (δ ppm, $CDCl_3$) 172.9, 138.2, 137.9, 128.7, 128.2, 128.0, 87.0, 84.5, 82.2, 75.3, 74.4, 65.0, 64.3, 51.6, 38.1, 31.0, 26.0, 19.8, 17.3, 15.0, 11.0, 2.3. Anal. Calcd. For $C_{28}H_{38}N_2O_6$: C, 67.45; H, 7.70; N, 5.62. Found: C, 67.23; H, 7.51, N, 5.57 %; $[\alpha]_D^{25}$ = + 14.6 (c = 1.1, CH_2Cl_2).

General procedure for the synthesis of compounds **11**

To a round bottom flask under argon atmosphere, the cycloadduct **10** (1 mmol) and glycine hydrochloride methyl ester (1 mmol) in 2.5 ml of anhydrous DMF were introduced, and the mixture was cooled with an ice/water bath. DECP (0.18 ml, 1.2 mmol) in 0.5 ml of DMF and TEA (0.29 ml, 2.05 mmol) were added dropwise, and the resulting mixture was stirred at room temperature for 16 hours. Then AcOEt (100 ml) and toluene (100 ml) were added, and the organic solution was washed with 50 ml fractions of H_2O, $Na_2S_2O_3$ 1N aqueous solution, H_2O, $NaHCO_3$ saturated aqueous solution and NaCl saturated aqueous solution, dried (Na_2SO_4) and evaporated. The crude mixture was purified by flash chromatography (Ethyl acetate/hexanes). The resulting oily product was solved in DME (5 ml) and cooled down to 0°C. LiOH 1N aqueous solution (3 ml) was added dropwise, and the progress of the reaction was monitored by TLC. After completion of the reaction, citric acid 10% aqueous solution (3 ml, pH 6) was added. The resulting solution was extracted with CH_2Cl_2 (3 x 4 ml), and the combined organic fractions were dried and evaporated. The crude product was triturated in Et_2O yielding the corresponding product **11** as a white solid.

2-[(2S,3R,4S,5S)-3-[(S)-1-(Benzyloxy)-2-methylpropyl]-4-nitro-5-phenylpyrrolidine-2-carboxamido]acetic acid **(11a):** 68% yield; mp 71-72 ºC; IR 1736, 1641, 1545, 1368 cm^{-1}; ^1H NMR (□ ppm, $CDCl_3$) 7.71 (t_b, 1H, J = 5.1 Hz), 7.43-7.22 (m, 10H), 5.51 (s_b, 2H), 5.33 (dd, 1H, J = 6.6 Hz, J' = 2.3 Hz), 4.80 (d, 1H, J = 11.6 Hz), 4.59 (d, 1H, J = 6.6 Hz), 4.58 (d, 1H, J = 11.6 Hz), 4.14 (m, 2H), 3.77 (d, 1H, J = 7.4 Hz), 3.59 (d, 1H, J = 6.5 Hz), 3.18 (d_b, 1H, J = 7.4 Hz), 2.10-1.92 (m, 1H), 1.01 (d, 3H, J=6.8 Hz), 0.87 (d, 3H, J=6.8 Hz); ^{13}C NMR (□ ppm, $CDCl_3$) 173.1, 173.0, 138.2, 134.7, 128.8, 128.6, 127.9, 127.6, 126.6, 90.7, 84.1, 74.1, 66.8, 63.2, 50.8, 41.1, 31.4, 19.2, 18.3. Anal. Calcd. for $C_{24}H_{29}N_3O_6$: C, 63.27; H, 6.43; N, 9.23. Found: C, 62.87; H, 6.39; N, 9.23 %; $[\square]_D^{25}$ = + 30.7 (c = 0.88, CH_2Cl_2).

2-[(2S,3R,4S,5S)-5-tert-Butyl-3-[(S)-1-(benzyloxy)-2-methylpropyl]-4-nitropyrrolidine-2-carboxamido]acetic acid **(11b):** 76 % yield; mp 119-120 ºC; IR 3367, 3283, 1707, 1693, 1555, 1377 cm^{-1}; ^1H NMR (□ ppm, $CDCl_3$) 7.56 (t_b, 1H, J = 4.9 Hz), 7.40-7.25 (m, 5H), 5.08 (d, 1H, J=5.3 Hz), 4.74 (d, 1H, J=11.2 Hz), 4.49 (d, 1H, J=11.2 Hz), 4.12 (dd, 1H, J=19.5 Hz, J'=4.9 Hz), 4.00 (dd, 1H, J=19.5 Hz, J'=4.9 Hz), 3.85 (d, 1H, J=6.3 Hz), 3.49 (dd, 1H, J=6.0 Hz, J'=1.5 Hz), 3.14 (d, 1H, J=5.3 Hz), 2.93 (d, 1H, J=6.3 Hz), 2.06-1.95 (m, 1H), 1.01-0.88 (m, 6H). 0.90 (s, 9H); ^{13}C NMR (□ ppm, $CDCl_3$) 173.2, 171.8, 138.0, 128.5, 127.9, 127.6, 86.5, 85.0, 74.3, 73.1, 63.2, 51.6, 41.8, 32.4, 31.4, 26.5, 19.0, 18.4. Anal. Calcd. for $C_{22}H_{33}N_3O_6$: C, 60.66; H, 7.65; N, 9.65. Found: C, 60.61; H, 7.70; N, 9.78 %; $[\square]_D^{25}$ = + 10.0 (c = 1.00, CH_2Cl_2).

2-[(2S,3R,4S,5S)-3-[(S)-1-(2-Fluorobenzyloxy)-2-methylpropyl]-4-nitro-5-phenyl-pyrrolidine-2-carboxamido]acetic acid **(11c):** 65 % yield; mp 72-73 ºC; IR 3378, 3330, 1741, 1665, 1550, 1363, 1225 cm^{-1}; ^1H NMR (□ ppm, $CDCl_3$) 7.82 (t_b, 1H, J = 5.4 Hz), 7.79-7.02 (m, 9H), 6.00 (s_b, 2H), 5.29 (dd, 1H, J = 6.6 Hz, J' = 2.3 Hz), 4.77 (d, 1H, J = 11.1 Hz), 4.70 (d, 1H, J = 11.2 Hz), 4.61 (d, 1H, J = 6.6 Hz), 4.20 (dd, 1H, J=18.3 Hz, J'=5.4 Hz), 4.08 (dd, 1H, J=18.3 Hz, J'=5.4 Hz), 3.85 (d, 1H, J=7.3 Hz), 3.59 (d_b, 1H, J = 4.8 Hz), 3.19 (d_b, 1H, J=7.3 Hz), 2.10-1.90 (m, 1H), 1.02 (d, 3H, J=6.9 Hz), 0.86 (d, 3H, J=6.9 Hz); ^{13}C NMR (□ ppm, $CDCl_3$) 173.2, 173.0, 163.4, 158.5, 134.8, 130.4, 130.3, 130.1, 129.9, 128.8, 128.6, 126.6, 125.2, 124.9, 124.3, 124.2, 115.7, 115.3, 90.7, 84.4, 68.4, 68.3, 66.8, 63.2, 50.8, 41.1, 31.5, 19.1, 18.3. Anal. Calcd. for $C_{24}H_{28}N_3O_6F$: C, 60.86; H, 5.97; N, 8.87. Found: C, 60.14; H, 6.03; N, 8.75 %; $[\square]_D^{25}$ = + 25.3 (c = 0.66, CH_2Cl_2).

2-[(2S,3R,4S,5S)-3-[(1S,2S)-1-(Benzyloxy)-2-methylbutyl]-4-nitro-5-phenyl-pyrrolidine-2-carboxamido]acetic acid **(11d):** 78% yield; mp 156-157ºC; IR 3368, 3321, 1737, 1669, 1545, 1363 cm^{-1}; ^1H NMR (□ ppm, $CDCl_3$) 7.70 (t_b, 1H, J = 5.2 Hz), 7.38-7.25 (m, 10H), 6.39 (s_b, 2H), 5.37 (dd, 1H, J = 6.8 Hz, J' = 2.4 Hz), 4.78 (d, 1H, J = 11.5 Hz), 4.61 (d, 1H, J = 6.4 Hz), 4.54 (d, 1H, J = 11.4 Hz), 4.19 (dd, 1H, J = 18.4 Hz, J' = 3.1 Hz), 4.09 (dd, 1H, J = 18.2 Hz, J' = 2.9 Hz), 3.78 (d, 1H, J = 7.6 Hz), 3.73 (d, 1H, J = 5.9 Hz), 3.16 (d_b, 1H, J = 8.4 Hz), 1.97-1.73 (m, 1H), 1.59-1.36 (m, 1H), 1.27-1.01 (m, 1H), 0.95-0.83 (m, 6H); ^{13}C NMR (□ ppm, $CDCl_3$) 173.1, 172.9, 138.1, 134.6, 128.9, 128.6, 127.9, 127.6, 126.6, 90.8, 82.4, 73.1, 66.9, 63.5, 50.2, 41.1, 37.2, 25.9, 14.4, 11.7. Anal. Calcd. for $C_{25}H_{31}N_3O_6$: C, 63.99; H, 6.67; N, 8.96. Found: C, 63.94; H, 6.56; N, 9.05 %; $[\square]_D^{25}$ = + 39.2 (c = 0.60, CH_2Cl_2).

2-[(2S,3R,4S,5S)-3-[(S)-1-(2,6-Difluorobenzyloxy)-2-methylpropyl]-4-nitro-5-phenyl-pyrrolidine-2-carboxamido]acetic acid **(11e):** 65 % yield; mp 80-81 ºC; IR 3372, 3334, 1734, 1635, 1555, 1358, 1275 cm^{-1}; ^1H NMR (□ ppm, $CDCl_3$) 7.82 (t_b, 1H, J = 4.0 Hz), 7.36-7.22 (m, 6H), 6.97-6.87 (m, 2H), 5.22 (dd, 1H, J = 6.6 Hz, J' = 2.4 Hz), 4.86 (d, 1H, J = 10.4 Hz), 4.68 (d, 1H, J = 10.4 Hz), 4.59 (d, 1H, J = 6.6 Hz), 4.24 (dd, 1H, J=18.2 Hz, J'=4.0 Hz), 4.15 (s_b, 2H), 4.14 (dd, 1H, J=18.2 Hz, J'=4.0 Hz), 3.84 (d, 1H, J=7.5 Hz), 3.58 (d, 1H, J = 6.3 Hz), 3.18 (d_b, 1H, J=8.0 Hz), 2.10-1.88 (m, 1H), 1.02 (d, 3H, J=6.7 Hz), 0.86 (d, 3H, J=6.8 Hz); ^{13}C NMR (□ ppm, $CDCl_3$) 173.2, 173.0, 164.3, 164.2, 159.4, 159.2, 135.0, 130.7, 130.5, 130.3, 128.7, 128.5, 126.7, 114.0, 113.6, 113.2, 111.7, 111.5, 111.4, 111.2, 90.6, 84.5, 66.7, 63.2, 61.4, 50.9, 41.1, 31.6, 19.0, 18.4. Anal. Calcd. for $C_{24}H_{27}N_3O_6F_2$: C, 58.64; H, 5.55; N, 8.55. Found: C, 58.47; H, 5.52; N, 8.53 %; $[\square]_D^{25}$ = + 23.2 (c = 1.02, CH_2Cl_2).

2-[(2S,3R,4S,5S)-3-[(1S,2S)-1-(benzyloxy)-2-methylbutyl]-5-cyclohexyl-4-nitropyrrol-idine-2-carboxamido]acetic acid **(11f)**: 55% yield; mp 177-179ºC; IR 3377, 3286, 1678, 1552, 1387 cm^{-1}; ^1H NMR (□ ppm, DMSO-d_6) 12.63 (s_b, 1H), 8.33 (t, 1H, J = 5.8 Hz), 7.43-7.32 (m, 5H), 5.21 (s, 1H), 4.71 (d, 1H, J = 11.5 Hz), 4.63 (d, 1H, J = 11.5 Hz), 3.92-3.82 (m, 2H), 3.65 (d, 1H, J = 5.3 Hz), 3.49 (d, 1H, J = 7.5 Hz), 2.93 (s, 1H), 2.82 (d, 1H, J = 7.6 Hz), 1.88-1.75 (m, 3H), 1.68-1.60 (m, 3H), 1.46-1.38 (m, 1H), 1.22-0.94 (m, 7H), 0.90 (t, 3H, J = 7.3 Hz), 0.82 (d, 3H, J = 6.9 Hz); ^{13}C NMR (□ ppm, DMSO-d_6) 172.5, 138.5, 128.9, 128.3, 128.0, 88.9, 83.1, 73.6, 69.8, 64.2, 51.6, 41.8, 38.4, 37.8, 31.0, 30.8, 30.1, 26.3, 25.7, 25.5, 14.9, 12.1, 2.3. Anal. Calcd. For

$C_{25}H_{37}N_3O_6$: C, 63.12; H, 7.86; N, 8.84. Found: C, 62.90; H, 7.68, N, 8.59 %; $[\alpha]_D^{25}$ = + 16.6 (c = 0.80, CH_3OH).

2-[(2S,3R,4S,5S)-3-[(1S,2S)-1-(3,5-Difluorobenzyloxy)-2-methylbutyl]-4-nitro-5-phenylpyrrolidine-2-carboxamido]acetic acid **(11g):** 77 % yield; mp 140-141 ºC; IR 3466, 3381, 1734, 1687, 1555, 1386, 1122 cm^{-1}; ^1H NMR (δ ppm, $CDCl_3$) 7.81 (t_b, 1H, J = 5.3 Hz), 7.33-7.25 (m, 5H), 6.90-6.70 (m, 3H), 6.18 (s_b, 2H), 5.36 (dd, 1H, J = 5.7 Hz, J' = 1.9 Hz), 4.74 (d, 1H, J = 12.0 Hz), 4.60 (d, 1H, J = 7.3 Hz), 4.55 (d, 1H, J = 12.0 Hz), 4.17 (d, 2H, J = 5.3 Hz), 3.82 (d, 1H, J = 7.5 Hz), 3.76 (d, 1H, J = 6.1 Hz), 3.21 (d_b, 1H, J = 7.2 Hz), 1.95-1.70 (m, 1H), 1.63-1.41 (m, 1H), 1.33-1.06 (m, 1H), 0.91 (t, 3H, J=7.0 Hz), 0.83 (d, 3H, J=6.9 Hz); ^{13}C NMR (δ ppm, $CDCl_3$) 173.1, 173.0, 165.6, 165.4, 160.7, 160.4, 142.3, 142.1, 142.0, 134.5, 128.9, 128.6, 126.6, 110.0, 109.8, 109.7, 109.5, 103.5, 103.0, 102.5, 90.6, 83.0, 71.8, 67.0, 63.5, 50.0, 41.1, 37.2, 25.9, 14.3, 11.7. Anal. Calcd. for $C_{25}H_{29}N_3O_6F_2$: C, 59.39; H, 5.79; N, 8.31. Found: C, 58.87; H, 5.81; N, 8.27 %; $[\alpha]_D^{25}$ = + 29.7 (c = 0.69, CH_2Cl_2).

2-[(2S,3R,4S,5S)-3-[(1S,2S)-1-(benzyloxy)-2-methylbutyl]-5-cyclopropyl-4-nitropyrrol-idine-2-carboxamido]acetic acid **(11h)**: 46% yield; mp 161-163ºC; IR 3356, 1673, 1562, 1399 cm^{-1}; ^1H NMR (δ ppm, DMSO-d_6) 12.64 (s_b, 1H), 8.25 (t, 1H, J = 5.8 Hz), 7.41-7.31 (m, 5H), 5.17 (dd, 1H, J = 4.2 Hz, J' = 4.2 Hz), 4.68 (d, 1H, J = 11.6 Hz), 4.62 (d, 1H, J = 11.6 Hz), 3.91-3.82 (m, 2H), 3.63 (dd, 1H, J = 1.8 Hz, J' = 1.9 Hz), 3.46 (d, 1H, J = 8.3 Hz), 3.04-3.03 (m, 1H), 2.69 (t, 1H, J = 7.7 Hz), 1.79-1.69 (m, 1H), 1.47-1.39 (m, 1H), 1.13-1.04 (m, 1H), 0.90 (t, 3H, J = 7.3 Hz), 0.80 (d, 3H, J = 6.9 Hz), 0.64-0.58 (m, 1H), 0.51-0.39 (m, 2H), 0.34-0.26 (m, 2H); ^{13}C NMR (δ ppm, DMSO-d_6) 172.8, 171.9, 139.5, 129.2, 128.1, 91.3, 82.3, 73.5, 68.6, 64.5, 52.1, 41.5, 37.7, 26.0, 15.5, 12.4, 11.0, 3.7, 3.1. Anal. Calcd. For $C_{22}H_{31}N_3O_6$: C, 60.95; H, 7.22; N, 9.70. Found: C, 61.23; H, 7.35, N, 9.76 %; $[\alpha]_D^{25}$ = + 24.6 (c = 0.56, CH_2Cl_2).

2-[(2S,3R,4S,5S)-3-[(1S,2S)-1-(2,3-Difluorobenzyloxy)-2-methylbutyl]-4-nitro-5-phenylpyrrolidine-2-carboxamido]acetic acid **(11i):** 67 % yield; mp 137-138 ºC; IR 3371, 1734, 1682, 1565, 1376, 1295 cm^{-1}; ^1H NMR (δ ppm, $CDCl_3$) 7.79 (t_b, 1H, J = 5.8 Hz), 7.36-7.09 (m, 8H), 5.32 (dd, 1H, J=5.8 Hz, J'=2.5 Hz), 4.78 (d, 1H, J=11.2 Hz), 4.67 (d, 1H, J=11.2 Hz), 4.60 (d, 1H, J=6.8 Hz), 4.19 (d, 2H, J=5.8 Hz), 3.81 (d, 1H, J=7.9 Hz), 3.78 (d, 1H, J=6.4 Hz), 3.54 (s_b, 2H), 3.20-3.16 (m, 1H), 1.92-1.76 (m, 1H),

1.57-1.38 (m, 1H), 1.28-1.06 (m, 1H), 0.93 (t, 3H, J=7.2 Hz), 0.84 (d, 3H, J=6.9 Hz); ^{13}C NMR (δ ppm, $CDCl_3$) 173.2, 173.1, 153.1, 152.8, 151.4, 151.2, 148.1, 147.9, 146.5, 146.2, 134.7, 128.9, 128.6, 127.6, 127.3, 126.7, 124.9, 124.8, 124.7, 124.4, 124.2, 124.1, 117.3, 117.0, 90.7, 82.9, 66.9, 63.5, 50.1, 41.0, 37.2, 25.9, 14.4, 11.7. Anal. Calcd. for $C_{25}H_{29}N_3O_6F_2$: C, 59.39; H, 5.79; N, 8.31. Found: C, 58.83; H, 5.81; N, 8.39 %; $[\alpha]_D^{25}$ = + 40.4 (c = 0.50, CH_2Cl_2).

2-[(2S,3R,4S,5S)-3-[(1S,2S)-1-(Benzyloxy)-2-methylbutyl]-2-methyl-4-nitro-5-phenyl-pyrrolidine-2-carboxamido]acetic acid **(11j):** 72% yield; mp 89-90 ºC; IR 3381, 3306, 1748, 1668, 1551, 1367 cm^{-1}; ^{1}H NMR (δ ppm, $CDCl_3$) 8.27 (t_b, 1H, J = 3.6 Hz), 7.44-7.23 (m, 10H), 5.46 (dd, 1H, J=6.8 Hz, J'=2.9 Hz), 5.28-5.60 (s_b, 2H), 4.75 (d, 1H, J = 6.8 Hz), 4.70 (d, 1H, J = 11.2 Hz), 4.41 (d, 1H, J = 11.2 Hz), 4.15 (d, 1H, J = 3.6 Hz), 3.82 (d, 1H, J = 4.0 Hz), 3.22 (d, 1H, J = 2.9 Hz), 2.09-1.83 (m, 1H), 1.58 (s, 3H), 1.49-1.23 (m, 1H), 1.21-0.98 (m, 1H), 0.91 (t, 3H, J=7.2 Hz), 0.76 (d, 3H, J=7.0 Hz); ^{13}C NMR (δ ppm, $CDCl_3$) 176.7, 138.3, 135.7, 128.7, 128.4, 128.1, 127.5, 127.1, 126.9, 104.1, 91.4, 79.7, 70.3, 66.1, 64.9, 50.3, 35.9, 29.6, 26.1, 18.9, 13.3, 11.9. Anal. Calcd. for $C_{26}H_{33}N_3O_6$: C, 64.57; H, 6.89; N, 8.69. Found: C, 64.25; H, 6.95; N, 8.65 %; $[\alpha]_D^{25}$ = + 2.1 (c = 0.96, CH_2Cl_2).

2-[(2S,3R,4S,5S)-3-[(1S,2S)-1-(benzyloxy)-2-methylbutyl]-5-[(S)-1-(benzyloxy)-2-methylpropyl]-4-nitropyrrolidine-2-carboxamido]acetic acid **(11k)**: 44% yield; mp 153-155ºC; IR 3417, 1678, 1547, 1387 cm^{-1}; ^{1}H NMR (δ ppm, $CDCl_3$) 7.50-7.01 (m, 11H), 5.68 (s_b, 2H), 5.27 (s, 1H), 4.71 (d, 1H, J = 10.6 Hz), 4.50 (d, 1H, J = 10.8 Hz), 4.40 (d, 1H, J = 10.9 Hz), 4.33 (d, 1H, J = 10.6 Hz), 4.13-3.90 (m, 2H), 3.67 (d, 1H, J = 5.5 Hz), 3.59-3.41 (m, 2H), 3.33 (d, 1H, J = 3.6 Hz), 2.92 (d, 1H, J = 2.1 Hz), 2.03-1.94 (m, 1H), 1.87-1.77 (m, 1H), 1.56-1.50 (m, 1H), 1.23-1.12 (m, 2H), 1.06-0.74 (m, 11H); ^{13}C NMR (δ ppm, $CDCl_3$) 172.7, 172.6, 138.5, 138.4, 129.0, 128.7, 128.3, 128.2, 128.0, 88.1, 84.1, 83.1, 74.8, 74.0, 66.8, 65.0, 52.0, 41.7, 38.0, 30.9, 26.3, 19.9, 17.4, 15.0, 12.0. Anal. Calcd. For $C_{30}H4_1N_3O7$: C, 64.85; H, 7.45; N, 7.56. Found: C, 64.97; H, 7.66, N, 7.64 %; $[\alpha]_D^{25}$ = + 8.7 (c = 0.30, CH_2Cl_2).

B) Evaluation of the binding ability of compounds 11 to the I-domain of the α-subunit of integrin $\alpha_2\beta_1$.

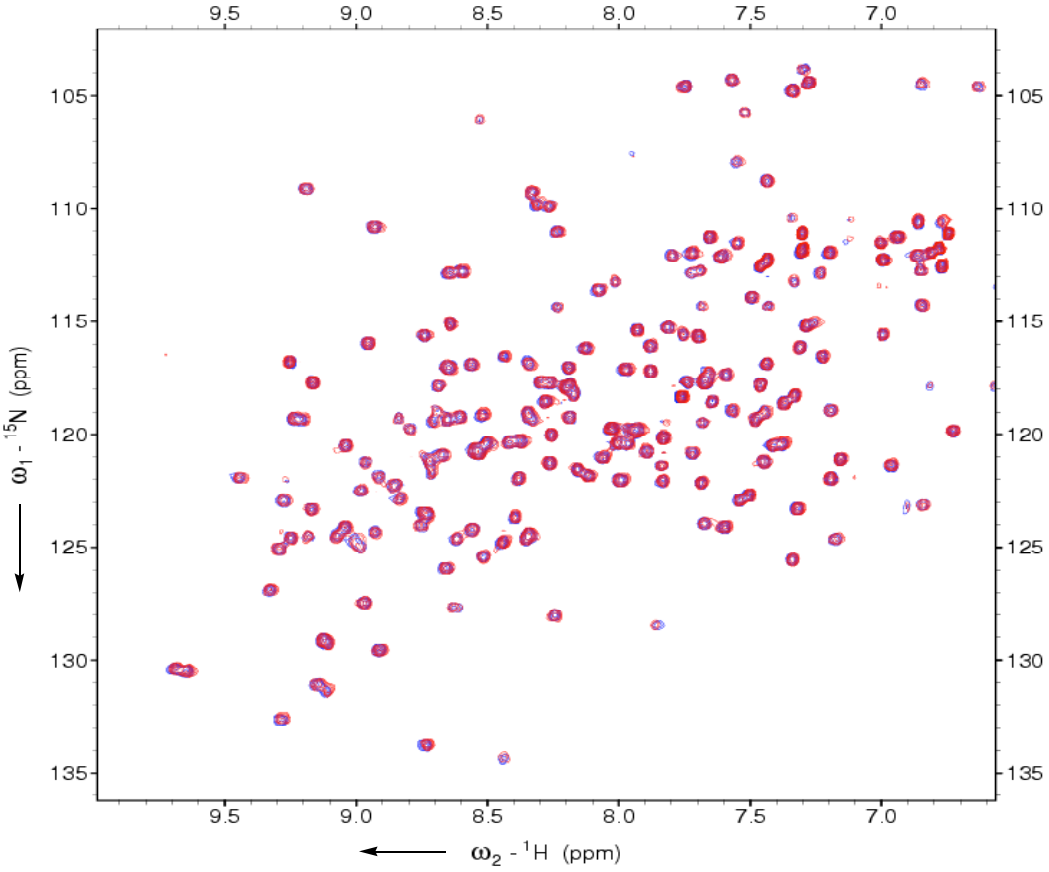

Figure S1. Overlay of spectra of HSQCs of sample containing compounds **11c,d,h,k** and reference sample of the I-domain of α_2 subunit. Signals in blue and red correspond to the I-domain alone and its mixture with the inhibitors, respectively. The spectra were obtained at 298 K and pH 7.4.

Compounds **11a-k** were grouped in several sets. Each set was dissolved in 80% DMSO-d_6 and mixed in a 1:2 ratio with 50 μM ^{15}N-labelled I-domain of $\alpha_2\beta_1$. HSQC experiments were conducted at pH 6.5 and 7.4 and at 283 K and 298 K. Heteronuclear correlation bidimensiaonal plots did not show any significant shift of the cross signals with respect to the reference experiments carried out in the absence of inhibitor (Figure S1). In particular, evaluation of the chemical shift deviation of the N-H signals (*CSD*, *ppm*) according to Eq. (1) did not show any significant contact between the I-domain of the α_2 subunit and compounds **11a-k** (Figure S2).

$$CSD = \sqrt{\frac{1}{2}\left[\Delta_{HN}^2 + \left(\frac{\Delta_{5N}}{5}\right)^2\right]} \quad (1)$$

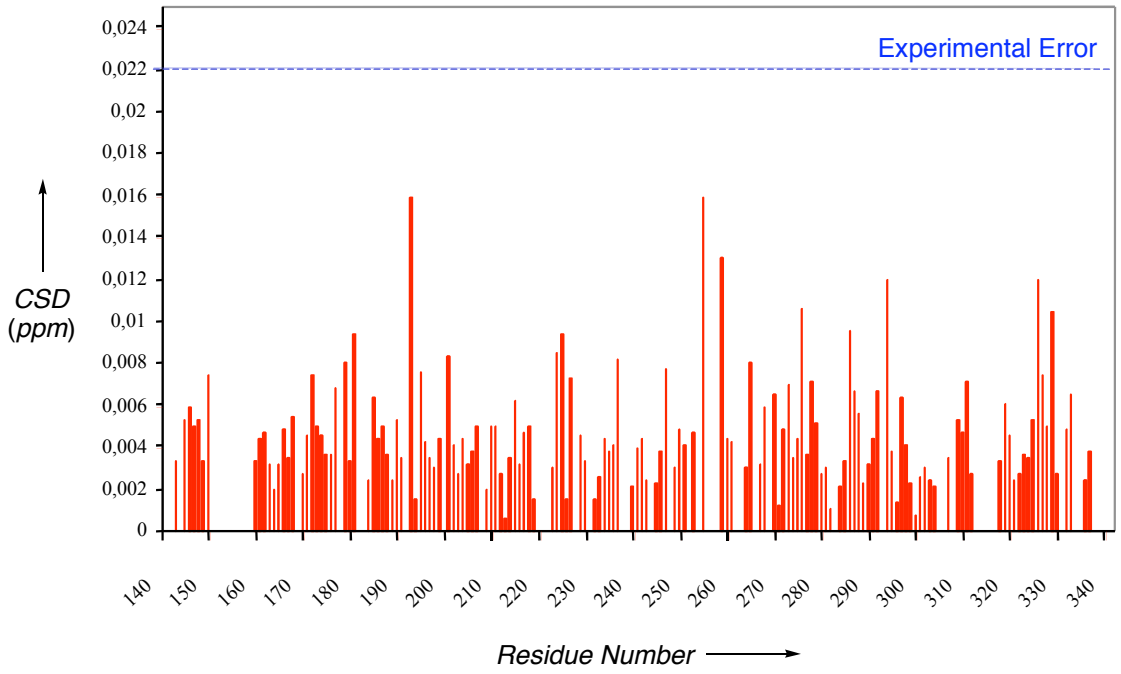

Figure S2. Chemical Shift Deviations (*CSD*, ppm, calculated according to Eq. (1)) obtained from the HSQCs spectra reported in Figure S1. The dashed line corresponds to the experimental error.

C) Effect of 11d on B16M cell adhesion to immobilized VCAM-1, on VEGF production and on B16M cell proliferation *in vitro*.

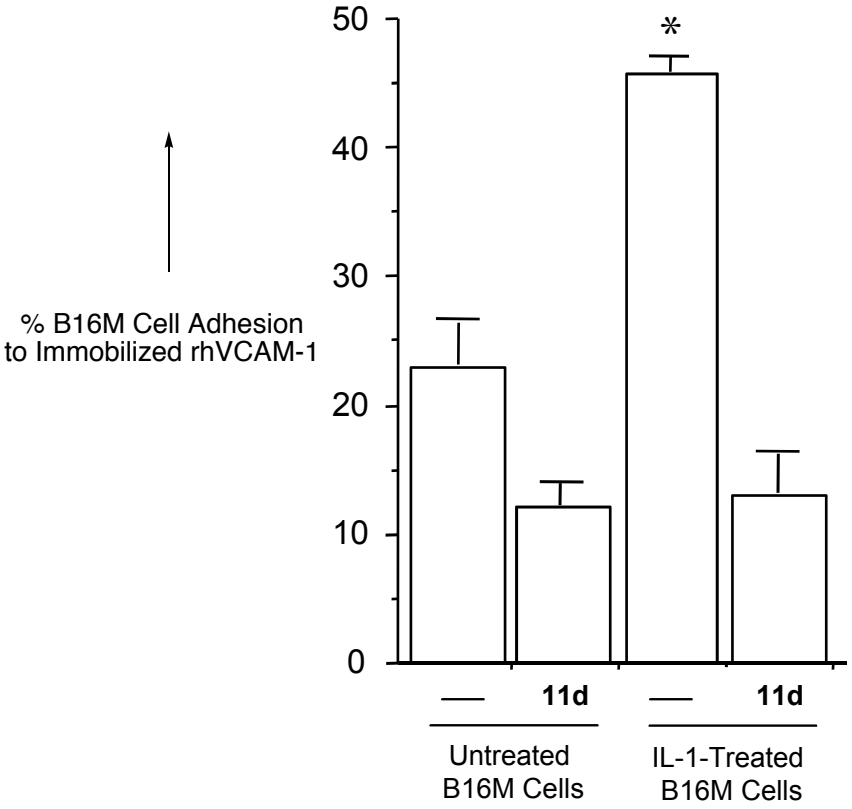

Figure S3. Effect of compound **11d** on the adhesion of untreated and interleukin-18 (IL-18)-treated B16M cells to immobilized VCAM-1 in vitro. B16M cells were treated with 1 ng/ml recombinant murine IL-18 for 6 hours. Untreated B16M cells received basal medium. Then, B16M cells were labeled with BCECF-AM and incubated either with basal medium or with 50 μg/ml of compound 11 at 37ºC for 15 minutes. Then, cells were washed and added to 96-well plate, which was precoated with 2 μg/ml recombinant VCAM-1 and blocked with 0.5% BSA. The results are the mean ±SD of three independent experiments, each in sextuplicate (n=18). Differences in the percent of adhering cells with respect to untreated B16M cells (*) were statistically significant (P<.001), by ANOVA and Bonferroni´s post-hoc test.

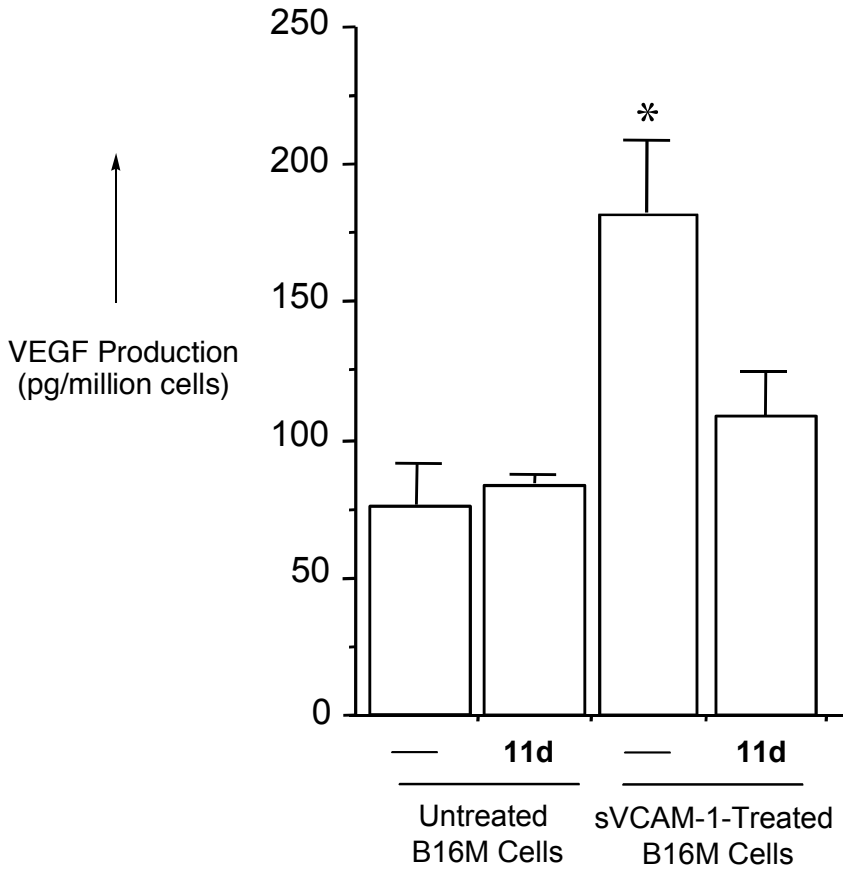

Figure S4. Effect of compound **11d** on vascular endothelial growth factor (VEGF) production by untreated and soluble VCAM-1-treated B16M cells in vitro. B16M cells were incubated either with basal medium or with 50 µg/ml of compound **11d** for 1 hour. Then, B16M cells were treated with soluble VCAM-1 for 11 hours. Untreated B16M cells received basal medium. Then, supernatant was collected and VEGF concentration evaluated by ELISA. The results are the mean ±SD of three independent experiments, each in sextuplicate (n=18). Differences in the VEGF concentration with respect to untreated B16M cells (*) were statistically significant (P<.001), by ANOVA and Bonferroni´s post-hoc test.

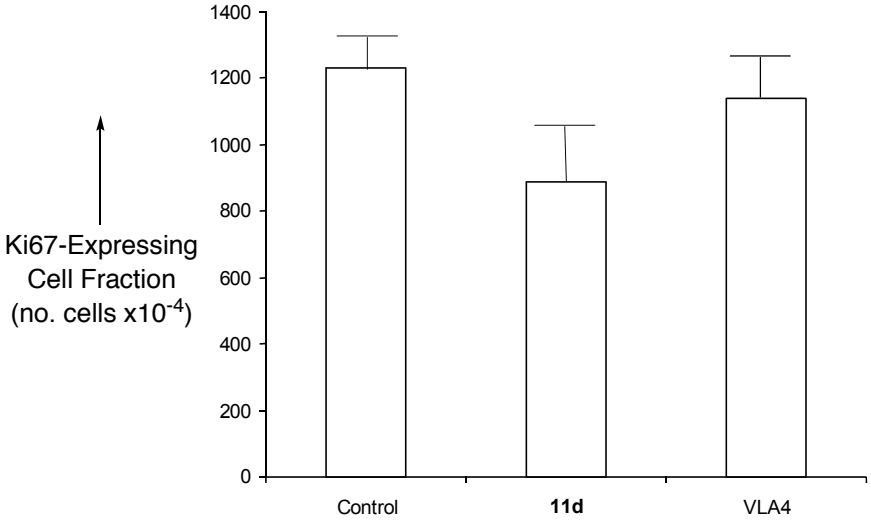

Figure S5. Effect of compound **11d** and anti-murine VLA-4 antibody on B16M cell proliferation *in vitro*. B16M cells were cultured for 24 hours in the presence 50 µg/ml of compound **11d**, or 5 µg/ml anti-murine VLA-4 antibody. Cultured cells were fixed and processed for the immunohistochemical detection of Ki-67 antigen expression. Next, a computed-aid microscopic image analysis system was used to discriminate stained cells (brown nuclei) and calculate the average number of Ki-67 antigen-expressing cells per $1x10^4$ cells. The results are the mean ±SD of 6 independent fields, from 3 different experiments (n=18).