Journal Name

RSCPublishing

ARTICLE

Design, Synthesis and Biological Evaluation of Novel Dimeric and Tetrameric cRGD-Paclitaxel Conjugates for Integrin-Assisted Drug Delivery

Cite this: DOI: 10.1039/x0xx00000x

Received 00th January 2012,
Accepted 00th January 2012

DOI: 10.1039/x0xx00000x

www.rsc.org/

A. Bianchi,†,[a] D. Arosio,†,[b] P. Perego,[c] M. De Cesare,[c] N. Carenini,[c] N. Zaffaroni,[c] M. De Matteo,[a] and L. Manzoni[b,*]

Integrins are implicated in tumour cell survival and progression, and their expression has been shown to be increased in tumours. Thus, four novel conjugates of the tripeptide integrin ligand Arg-Gly-Asp (RGD) and the cytotoxic agent paclitaxel (cRGD-PTX) were prepared to investigate the potential of the multivalent presentation of the RGD moiety in improving the antitumor efficacy of PTX by tumour targeting. PTX was conjugated to two or four integrin recognizing ligands. The influence of multivalent presentation on *in vitro* $\alpha_v\beta_3$-receptor affinity was confirmed. For all the conjugates compared to the previous synthesized monovalent counterparts, an enhancement of the binding strength was observed; this behaviour was more pronounced if considering the tetravalent presented RGD-conjugate. Cell growth inhibition assays on a panel of human tumour cell lines showed remarkable cytotoxic activity for all conjugates with IC_{50} values in nanomolar range. Among the four conjugates, the bivalent derivative **3b**, was selected for *in vivo* studies in an ovarian carcinoma cell model xenografted in immunodeficient mice. A marked antitumor activity was observed, similar to that of PTX, but with a much more favourable toxicity profile. Overall, the novel cRGD-PTX conjugates here disclosed represent promising candidates for further advancement in the domain of targeted anti-tumour therapy.

Introduction

Anti-angiogenic therapeutic approaches are regarded as promising in antitumor therapy because they could provide selectivity toward activated endothelial cells, possibly avoiding pharmacological resistance due to the low tendency of these cells to mutate. Among the crucial players of angiogenesis, integrins play a key role, being involved in cell adhesion and migration processes. Specifically, α_v and $\alpha_5\beta_1$ integrins are overexpressed on activated endothelial cells, and particularly on the surfaces of certain tumour cells, whereas they are barely detectable on quiescent vessels and in normal tissues.[1] These integrins recognize the RGD (Arg-Gly-Asp) sequence which was incorporated in a number of peptidic and peptidomimetic constructs[2,3] originating potent and selective ligands. However, phase III clinical trials in patients with glioblastoma multiforme with Cilengitide (cRGDf-N(Me)V) developed by Kessler and co-workers[4] failed. Moreover, Cilengitide resulted inactive in the treatment of other human cancers, suggesting that the real target of this molecule are integrins expressed by glioblastoma cells rather than by activated endothelial cells.[5] Thus, the available evidence suggests that normalization of tumour vasculature appear not to be sufficient to fulfil a pharmacological action able to eradicate advanced tumours. Recent studies have also underlined a paradoxical effect of RGD-based integrin ligands suggesting that, at nanomolar concentration, they are pro-angiogenic.[5] Nevertheless, anti-angiogenic therapies could be considered complements of current cytotoxic therapies, characterized by low selectivity for tumour cells, toxicity for normal tissues and often limited in their curative potential by the development of resistance. In spite of some concerns about the antitumor activity of RGD-based therapeutics, α_v integrins overexpressed on the surface of tumour cells can be still considered a useful target for tumour homing of cytotoxic and/or imaging cargos,[3,6-8] as supported by the numerous reports appeared in the literature concerning the synthesis and biological evaluation of targeted therapeutics where the RGD-homing function is coupled to the drugs under the form either of covalent conjugates[3,8-11] or nanosized particles.[3,10-15]

The microtubule-interfering agent paclitaxel (PTX) has often been selected for the preparation of RGD-conjugates. The reasons for this choice are linked to the drawbacks of the drug, such as low aqueous solubility, short half-life, poor bioavailability, and systemic toxicity.

RGD-conjugation could overcome all or a part of these disadvantages, possibly increasing the therapeutic index. However, among the broad spectrum of tumour-addressing integrin ligands only some examples of linear RGD, and no more than a couple of cyclic peptide [i.e. c(RGDfK) or c(RGDyK), and c[DKPRGD]] has been reported as PTX conjugates.[16-19]

Our contribution to this field started from the synthesis of cyclic RGD pentapeptide analogues incorporating 1-aza-2-oxobicycloalkane amino acids. These studies have produced some specific ligands with nanomolar affinity for $\alpha_v\beta_3$ and $\alpha_v\beta_5$ integrins, which were subsequently modified to introduce a functionalized site suitable for conjugation[20] to different functional units with applications in medical diagnosis[21-24] and therapy.[14,25] In particular, we have recently reported our preliminary studies on the employment of the integrin ligand DB58 for the synthesis of conjugates with PTX.[25]

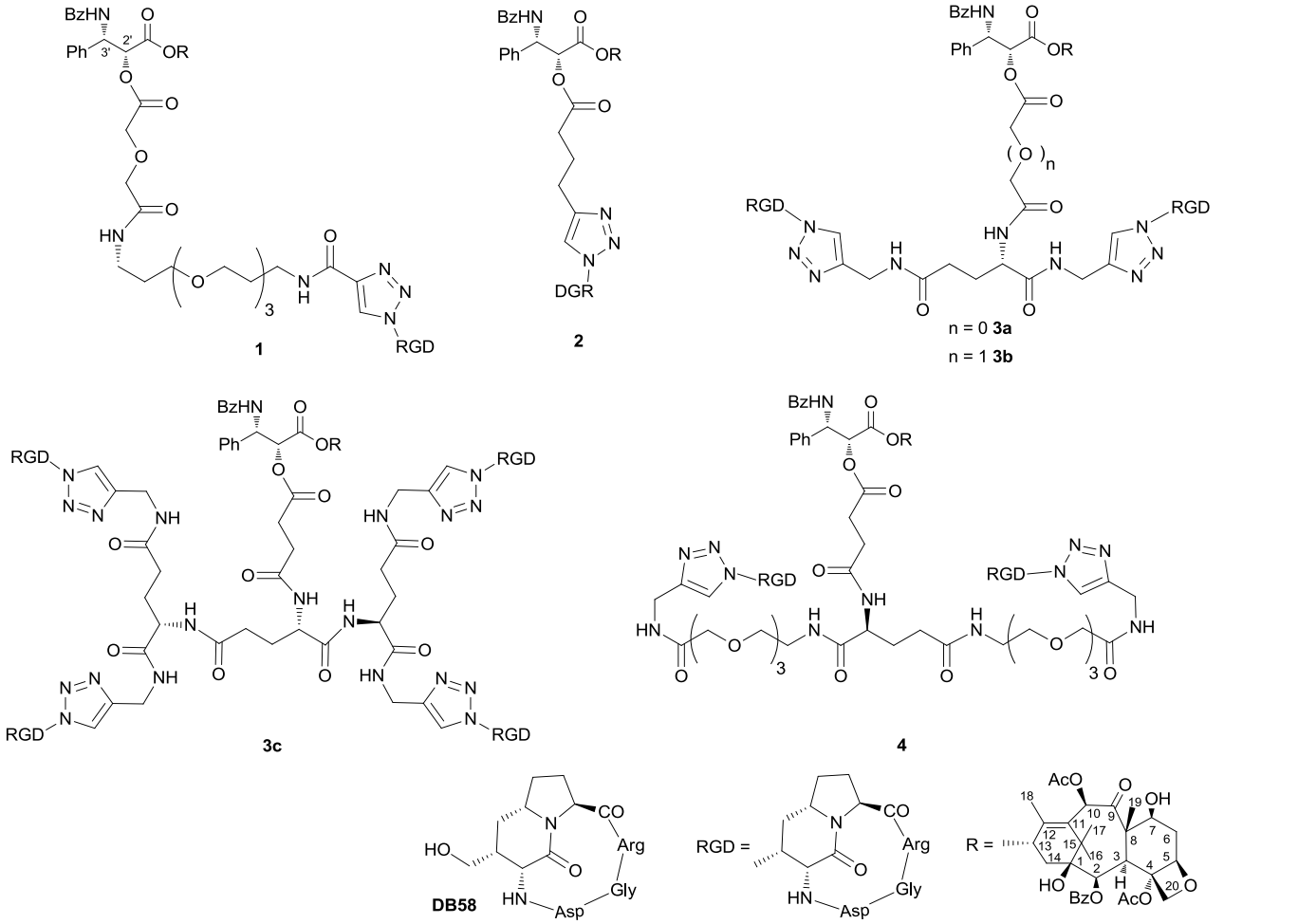


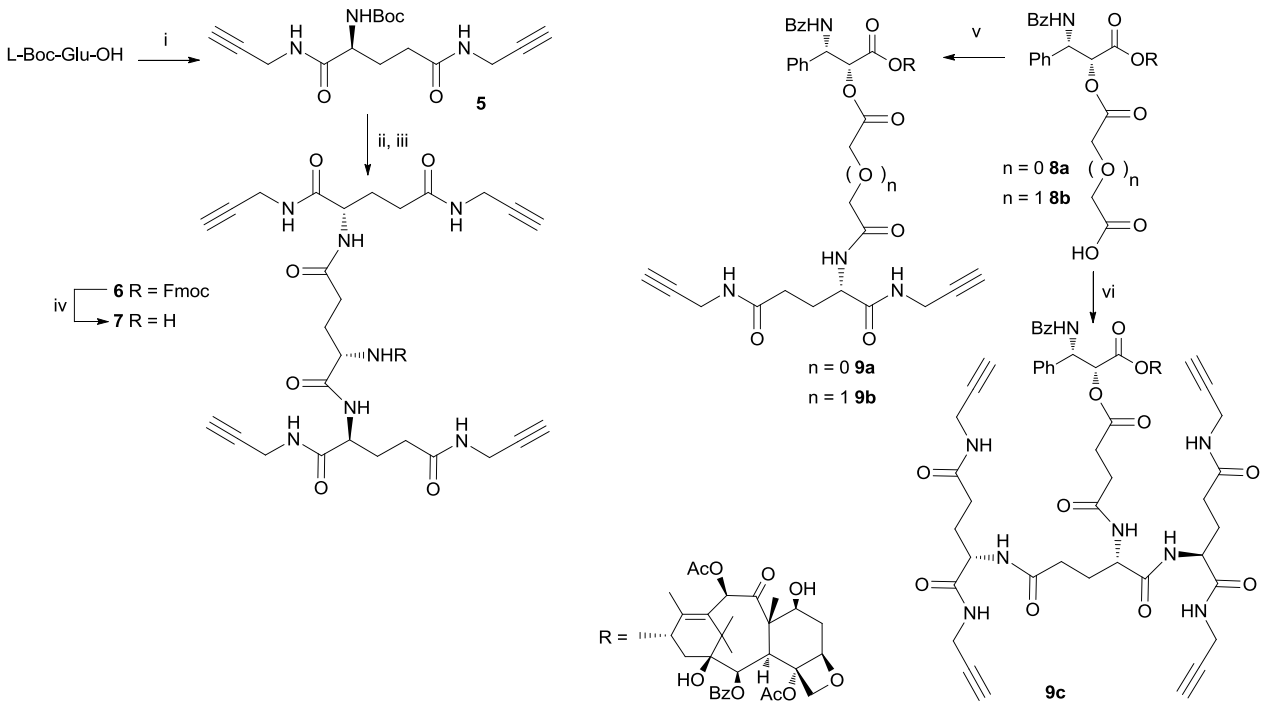
Figure 1. RGD-Paclitaxel conjugates

Among the nine synthesized conjugates, derivative **1** (Figure 1), bearing a robust triazole ring connected to ethylene glycol unit by an amide function and showing excellent cell growth inhibitory properties, was selected for *in vivo* studies in an ovarian carcinoma model xenografted in immunodeficient mice. Remarkable antitumor activity was obtained, superior to that of PTX itself, which was associated with a marked induction of aberrant mitoses, consistent with the mechanism of action of spindle poisons. This result prompted us to further investigate the potential of RGD-PTX conjugates for targeted drug delivery, specifically evaluating the employment of RGD multivalent presentations in an attempt to improve the tumour targeting and thus the PTX antitumor efficacy. To this end, we prepared three divalent RGD-PTX conjugates exploring different linker length (**3a-b**, **4** Figure 1) and a tetravalent RGD-PTX compound (**3c**, Figure 1) and evaluated their *in vitro* and *in vivo* ability to inhibit tumour cell and tumour growth.

Results and discussion

Our approach to the synthesis of multivalent systems is based on glutamic acid dendrons. These compounds can be easily prepared using glutamic acid as the branching unit and multiple copies of the ligand can be bound to this inert branching core

(Scheme 1). We decided to use copper catalyzed Huisgen 1,3-dipolar cycloaddition for the final step of conjugation; thus, the terminal carboxylic groups were functionalized with propargylamine. The synthesis proceed from commercially available L-Boc glutamic acid that was activated with *N*-hydroxy succinimide (NHS) in the presence of *N,N*-diisopropylcarbodiimide (DIC). The activated ester was coupled with propargylamine giving the protected compound **5** which was treated with TFA to remove Boc protection. Reaction with L-Fmoc glutamic acid gave the tetravalent dendron **6**. Deprotection of **6** with polymer bound piperazine gave the corresponding free amine **7** (Scheme 1).



Scheme 1. i.1) NHS, DIC, DMF; 2) propargylamine, 90% ii. TFA, anisole, DCM; iii. L-Fmoc-Glu, NHS, DIC, DMF, DIPEA, 50% over two steps; iv. polymer bound piperazine, DMF, M.W., quant.; v. **5**, TBTU, HOBt, DMF, DIPEA, 81% for **9a** and 93% for **9b**; vi. **7**, TBTU, HOBt, DMF, DIPEA, 70%.

Paclitaxel derivatives **8a-b** were obtained by derivatisation of the 2'-hydroxy function with succinic or diglycolic anhydride, according to reported procedures.[26] The paclitaxel hemisuccinate and hemidiglycolate esters **8a-b** were then activated with *O*-(benzotriazol-1-yl)-*N,N,N',N'*-tetramethyluronium tetrafluoroborate (TBTU) and 1-hydroxybenzotriazole (HOBt) and coupled with amines **5** or **7** to give terminal triple bonds functionalized dendrons **9a-c**.

The decoration of the dendron ends with cyclic RGD peptides is based on the 1,3-dipolar cycloaddition between terminal triple bonds and azides.[27,28] Alkyne dendrons **9a-c** were subjected to the cycloaddition reaction conditions in the presence of the deprotected azido cyclo-RGD peptide **10**[21] to give di- and tetravalent cyclo-RGD peptide dendrons **3a-c** (Scheme 2). The reaction proceeded smoothly giving the final multivalent compounds in acceptable yields (54-57%). The main by-products, consisting in partially reacted dendrons, were eliminated through HPLC purification.

To explore the effect of the spatial separation of the cyclo-RGD moieties, a spacer was introduced in the structure. Polyethylene glycol (PEG) derivatives are ideal for this purpose because they are inexpensive, water soluble and available in a variety of lengths. To this purpose, acid **11** was converted in the corresponding isopropyl ester **12** by activation with 1,1'-carbonyldiimidazole followed by treatment with an excess of 2-propanol. Hydrogenolysis of carbobenzyloxy group with 10% Pd-C in the presence of 1M HCl afforded the amine hydrochloride that was coupled with activated L-Boc-glutamic acid to give the divalent dendron **13**. Hydrolysis of isopropyl esters with 1M NaOH in dioxane followed by coupling of the activated carboxylic functions with propargylamine and subsequent Boc deprotection with TFA gave the desired amine **14**. Compound **14** was then coupled with activated acid **8a** to give alkyne **15** (Scheme 3) that was then subjected to the cycloaddition reaction in the presence of the deprotected azido cyclo-RGD peptide **10** to give divalent cyclo-RGD peptide dendron **4**.

Biology

Solid Phase Receptor Binding Assay.

The ability of the three divalent conjugates **3a-b** and **4**, and of tetravalent derivative **3c** to inhibit the binding of biotinylated vitronectin to human, isolated $\alpha_v\beta_3$ integrin receptors *in vitro* was determined by competitive binding assay (Table 1, and SuppInfo). To better evaluate the influence of multivalent presentation on the binding capability of the RGD conjugates **3a-b** and **4**, we compared the results obtained from these compounds with those obtained for the monovalent counterparts. On this purpose we selected, among the monovalent compounds previously synthesized compound **1** and compound **2** (figure 1) both conjugated trough a triazole and representative of short and long linkers.[25] It is interesting to note that both divalent compounds **3a** (IC_{50}=22 ± 8.4 nM) and **3b** (IC_{50}=35 ± 7.4 nM) bind integrin $\alpha_v\beta_3$ better than the monovalent compound **2** (IC_{50}=266 ± 75 nM) and present an IC_{50} similar to that of the unconjugated compound DB58 (IC_{50}=53.7 ± 17.3 nM). An even better improvement is observed comparing compound **4** (IC_{50}=1.95 ± 0.1 nM), where a short polyethylenic linker further distance the RGD moiety from the citotoxyc drug, with the monovalent analog **1** (IC_{50}=220.0 ± 13.2 nM) (Table 1).

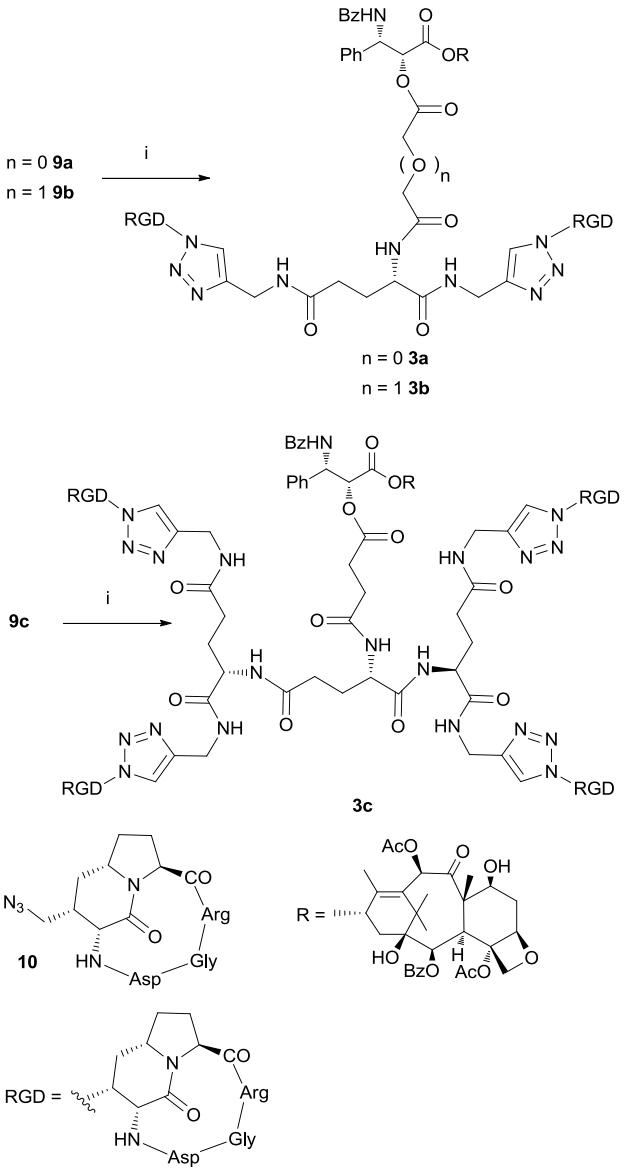
Table 1. Inhibition of biotinylated vitronectin binding to $\alpha_v\beta_3$ receptors.[a]

Entry	Compound	IC_{50} (nM) ± SD for $\alpha_v\beta_3$
1	c(RGDfV)[b]	3.2 ± 1.3
2	**DB58**[b]	53.7 ± 17.3
3	**3a**	22 ± 8
4	**3b**	35 ± 7
5	**3c**	2.5 ± 1.7
6	**4**	1.95 ± 0.1
7	**2**[b]	266 ± 75
8	**1**[b]	220.0 ± 13.2

[a] IC_{50} values were calculated as the concentration of compound required for 50% inhibition of biotinylated vitronectin binding as estimated by Prism GraphPad program. All values are the mean (± standard deviation) of triplicate determinations. $\alpha_v\beta_3$ and $\alpha_v\beta_5$ inhibition curves have been reported in SuppInfo.

[b] ref. 25

The role of multivalency in increasing the binding affinity of conjugates can further be appreciate if we consider the result obtained for tetravalent compound **3c** (IC_{50} = 2.5 ± 1.7 nM), that binds integrin $\alpha_v\beta_3$ 100-fold better than the monovalent counterpart **2** and 10-fold better in comparison to the divalent compound **3a**. These values suggest that multivalent interactions enhanced the binding strength in a way that is greater than the sum of the affinity of the involved ligands and somehow proportional to the number of conjugated ligands.



Scheme 2. i. **10**, copper(II) acetate, sodium L-ascorbate, 3:2 t-butanol/water, 54% for **3a**, 57% for **3b**, and 24% for **3c**.

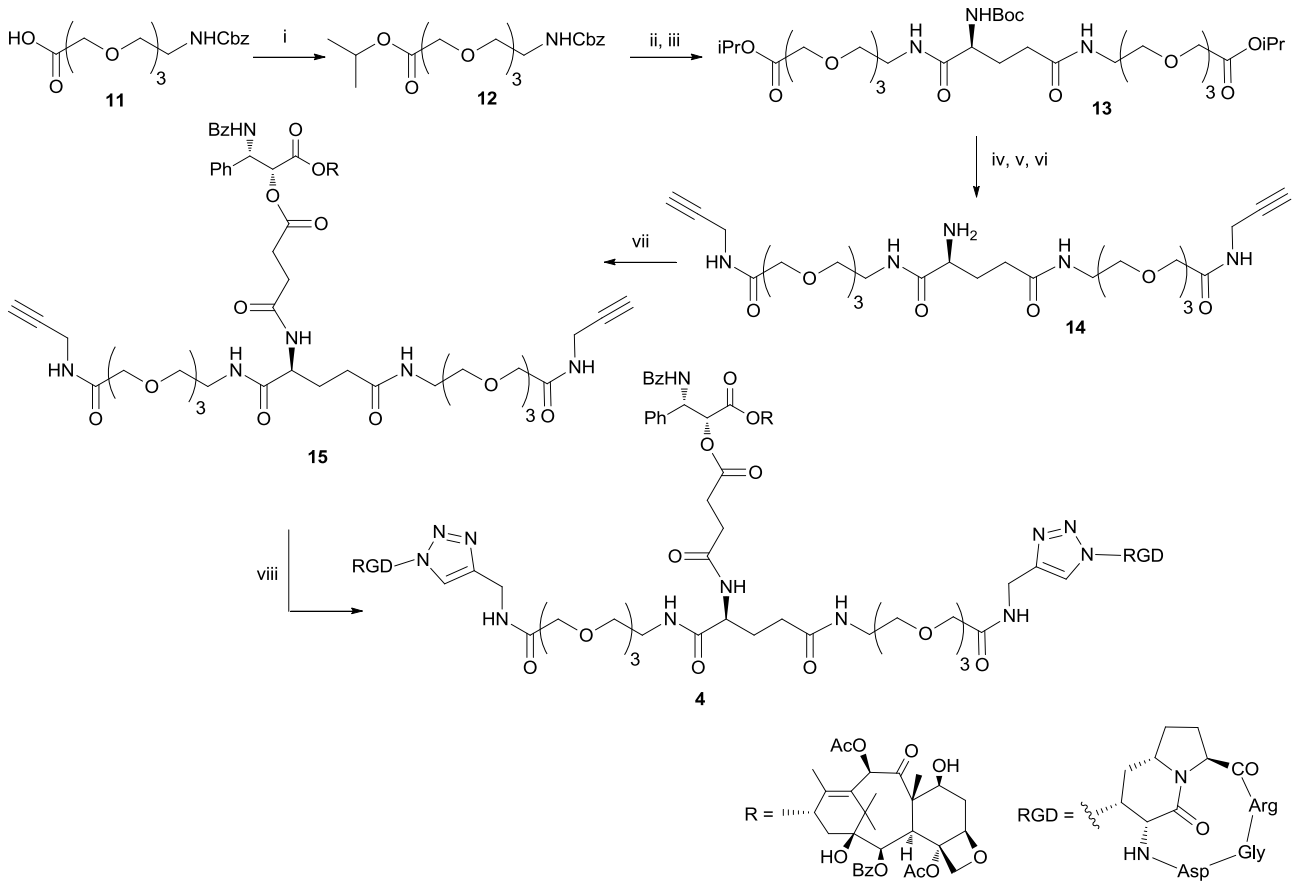
Cell Sensitivity Assays.

The antiproliferative activity of the compounds was studied in cell lines of different tumour types (Table 2, and SuppInfo). These cellular models were chosen due to their variable expression of integrins as previously shown.[25] In particular, the expression of $\alpha_v\beta_5$ was more marked in the osteosarcoma U2-OS cells than in the other cell systems, and the ovarian carcinoma mutant p53 cisplatin-resistant IGROV-1/Pt1 subline exhibited the highest level of expression of $\alpha_v\beta_3$. Using growth inhibition assays in which PTX was employed for comparison, all compounds exhibited a marked growth inhibitory effect in the IGROV-1/Pt1 variant as compared to the parental wild-type p53 cisplatin-sensitive IGROV-1 cell line. In such a subline, the effect of compound **3b** was similar to that of paclitaxel as shown by IC_{50} values. In H460 cells and U2-OS cells, the IC_{50} values were generally higher than in IGROV-1/Pt1 cells, being similar to those of PTX in H460 cells, whereas they were higher than that of the reference compound in U2-OS cells. Overall, all compounds were characterized by marked potency because the IC_{50} values were all in the nanomolar range.

Table 2. Cell sensitivity of different tumour cell lines to compounds **3a, 3b, 3c** and **4**[a]

Compound	IGROV-1 IC_{50} nM	IGROV-1/Pt1 IC_{50} nM	H460 IC_{50} nM	U2-OS IC_{50} nM
3a	16.46±4.38	5.87±3.50	13.44±3.06	18.00±8.76
3b	6.65±1.30	4.52±1.74	11.56±3.91	13.17±8.69
3c	12.18±0.99	8.81±2.14	13.62±2.67	10.76±5.34
4	32.67±7.5	6.65±3.76	12.62±3.76	19.00±11.27
Paclitaxel	58.55±11.71	4.3±1.17	11.43±7.03	4.40±0.0

[a]Cell sensitivity was evaluated by growth inhibition assays based on cell counting. Cells were seeded and 24 h later they were exposed to the compounds for 72 h. At the end of treatment, cells were counted using a cell counter. Dose response curves for inhibition of cell growth have been reported in SuppInfo.



Scheme 3. i. **10**, THF, 1,1'-carbonyldiimidazole, 2-propanol, quant.; ii. H_2, Pd/C, iPrOH, HCl, quant.; iii. *N*-Boc-glutamic acid, NHS, DIC, DIPEA, DMF, 76%; iv. NaOH, dioxane, quant.; v. DMF, TBTU, HOBt, propargylamine, 50%; vi. TFA, DCM, quant.; vii. TBTU, HOBt, DMF, **8a**, DIPEA, 76%; viii. **10**, copper(II) acetate, sodium L-ascorbate, 3:2 t-butanol/water, 55%.

***In Vivo* Antitumor Activity.**

Due to the good growth inhibition capability displayed *in vitro* by compound **3b**, and for its synthetic accessibility it was selected to be further examined for *in vivo* studies. The effects of the PTX-conjugates **3b** was examined and compared to those of PTX in athymic nude mice s.c. bearing the IGROV-1/Pt1 human ovarian carcinoma. The compounds were administered i.v. every fourth day

for four times (q4dx4) at a dose level of 30mg/kg. The results are reported in Table 3 and Figure 2. A significant antitumor activity was observed after administration of **3b**, which induced a TVI of 86% similarly to PTX. However, after treatment with the new drug, 2 out of 10 tumours disappeared without any sign of toxicity, whereas severe body weight loss was observed in PTX-treated animals, in which no CR were observed. No lethal toxicity occurred. Thus, **3b** showed an antitumor activity similar to that of PTX and compound **1**[25] against the growth of the IGROV-1/Pt1 carcinoma, but with a much more favourable toxicity profile, indicating that the maximum tolerated dose has still to be reached.

The observed improved antitumor activity suggests that compound **3b** do not undergo premature release of the cytotoxic cargo. This is also supported by previous study using fluorescent $\alpha_v\beta_3$ probes chemically similar to those employed in the present experiments.[21] In that study we observed that fluorescence was distributed at the cell surface and in putative cytosolic vesicles, suggesting that compounds are internalized upon binding to integrins by endocytosis.

Table 3. Antitumor activity of i.v. paclitaxel (PTX) and its RGD-conjugates **3b**, 30mg/kg, q4dx4 on the IGROV-1/Pt1 human ovarian carcinoma xenografted s.c. in female nude mice.[a]

Drug	TVI%[b]	CR[c]	BWL%[d]	Tox[e]
PTX	86**	0/ 10	16	0/5
3b	86**	2/ 10	2	0/5

[a] Tumour fragments were implanted on both flanks on day 0. Treatment started when tumours were just palpable (day 3).

[b] Tumour volume inhibition % in treated over control mice, assessed 6 days after the last treatment.

[c] Complete responses, i.e. disappearance of tumour lasting at least 10 days.

[d] Body weight loss % induced by drug treatment, the highest change is reported.

[e] Dead/treated mice.

**P<0.01 by Student' t test vs. tumour volumes in control mice.

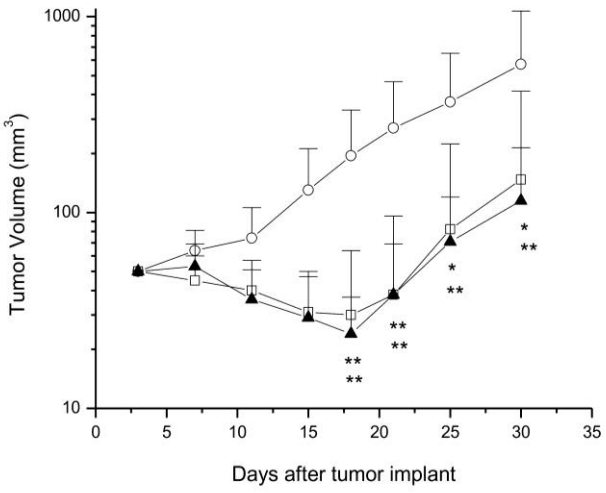


Figure 2. Antitumor activity studies on IGROV-1/Pt1 ovarian carcinoma. Efficacy of compound 3b (□) and paclitaxel (▲) administered intravenously every fourth day for four times on the ovarian carcinoma IGROV-1/Pt1 xenografted subcutaneously in athymic nude mice. The solvent was injected for the control group (○). Each point represents the mean tumour volume from 10 tumours. Bars represent S.D. *, $P < 0.05$; **, $P < 0.01$ by Student's two-tail t test on tumour volumes over control mice.

Conclusions

The study focused on the synthesis and biological evaluation of four PTX-conjugates compounds **3a-3c** and **4**, with the aim to investigate the multivalent presentation in an attempt to improve the tumour targeting and consequently the PTX antitumor efficacy. The main goals of the work have been accomplished. The synthesis provided the four conjugates in good yields, which were fully characterized after purification. The influence of multivalent presentation on in vitro $\alpha_v\beta_3$-receptor affinity was also confirmed. For all the bivalent conjugates compared to the previous synthesized monovalent counterparts, has been observed an enhancement of the binding strength greater than the sum of the affinity of the involved ligands and proportional to the number of conjugated ligands. This behaviour is even more pronounced if we consider the tetravalent presented RGD-conjugate **3c** that binds integrin $\alpha_v\beta_3$ 100-fold better than the monovalent counterpart **2** and 10-fold better in comparison to the divalent compound **3a**. In vitro growth inhibition assays on a panel of $\alpha_v\beta_3/\alpha_v\beta_5$-overexpressing human tumour cell lines showed remarkable cytotoxic activity in the nanomolar range. Finally, *in vivo* evaluation of conjugate **3b** confirmed the effectiveness of this compound to inhibit tumour growth of the IGROV-1/Pt1 carcinoma, displaying an antitumor activity similar to that of PTX and a much more favourable toxicity profile. This result is further strengthened by the fact that the effective quantity of PTX administered to the animals treated with compound **3b** was almost one third of the dose administered to PTX-treated animals, given the different molecular weight of compounds **3b** and PTX.

Experimental

General

Solvents were dried by standard procedures: dichloromethane, pyridine and *N*,*N*-diisopropylethylamine were dried over calcium hydride; tetrahydrofuran was dried over sodium; dry *N*,*N*-dimethylformamide was purchased from Sigma-Aldrich.

^{1}H, and ^{13}C-NMR spectra were recorded at 300 K on a Bruker AVANCE-600 or Bruker AVANCE-400 spectrometer. Chemical shifts δ for ^{1}H and ^{13}C are expressed in ppm relative to internal Me_4Si as standard. Signals were abbreviated as s, singlet; bs, broad singlet; d, doublet; t, triplet; q, quartet; m, multiplet. Mass spectra were obtained with Agilent 1100 analytical HPLC equipped with diode array detector and Bruker ion-trap Esquire 3000+ with ESI. HRMS spectra were obtained by Fourier transform ion cyclotron resonance (FT-ICR) Mass Spectrometer APEX II & Xmass software (Bruker Daltonics) 4.7 T Magnet (Magnex). Thin layer chromatography (TLC) was

carried out with pre-coated Merck F_{254} silica gel plates. Flash chromatography was carried out with Macherey-Nagel silica gel 60 (230-400 mesh) or using SP1 Biotage flash purification system (with silica or C18 cartridges). Preparative HPLC was performed using Waters MS-based preparative HPLC system (See Supplementary Information). ^{1}H-, ^{13}C-NMR and MS analysis confirmed the purity and identity of all synthesized compounds.

Synthesis of (S)-2-amino-N^{1},N^{5}-di(prop-2-yn-1-yl)pentanediamide, (5). At room temperature and under a nitrogen atmosphere, a solution of *N*-Boc-glutamic acid (1 g, 4.04 mmol) and *N*-hydroxy-succinimide (1.4 g, 12.13 mmol) in dry DMF (0.2 M) was prepared. *N*,*N*-diisopropylcarbodiimide (1.53 g, 12.13 mmol) was added and the solution was stirred at room temperature overnight (after a while, a white precipitate appeared). Propargylamine (832 μL, 12.13 mmol) was added and the cloudy solution was stirred for 3 h. After reaction completion, the solvent was evaporated under reduced pressure. The residue was taken up with acetonitrile and the white solid (*N*,*N*-diisopropylurea) was filtered on a celite pad. The solvent was evaporated under reduced pressure and the crude product was purified by flash chromatography using 9:1 EtOAc/ETP as the eluent to afford the Boc-protected compound (90%). ^{1}H-NMR ($CDCl_3$, 400 MHz): 1.46 (s, 9H, Boc), 2.03-1.94 (m, 1H, $CH_2\beta$), 2.15-2.06 (m, 1H, $CH_2\beta$), 2.26-2.22 (m, 2H, C≡CH), 2.45-2.30 (m, 2H, $CH_2\gamma$), 4.10-3.98 (m, 4H, H_2C-C≡), 5.78 (d, 1H, NHBoc, J = 6.8 Hz), 6.77 (bs, 1H, NHCO), 4.23-4.15 (m, 1H, CHα), 7.28 (bs, 1H, NHCO); ^{13}C-NMR ($CDCl_3$, 100.6 MHz): 172.4, 171.4, 80.3, 79.4, 79.3, 71.6, 53.5, 32.4, 29.3, 29.1, 28.3; MS (ESI): m/z = 343.9 $[M+Na]^+$, calcd for $C_{16}H_{23}N_3O_4$ m/z = 321.37 [M].

Synthesis of (9H-fluoren-9-yl)methyl ((8S,11S,16S)-5,10,14,19-tetraoxo-8,16-bis(prop-2-yn-1-ylcarbamoyl)-4,9,15,20-tetraazatricosa-1,22-diyn-11-yl)carbamate, (6). At room temperature, a solution of compound **5** (1.17 g, 3.64 mmol) in DCM (18.2 mL, 0.2 M) was prepared. Anisole (593 μL, 5.46 mmol) and trifluoroacetic acid (2.62 mL, 36.4 mmol) were added and the solution was stirred at room temperature. After 2 h, the solvent was evaporated under reduced pressure and the residue washed twice with toluene. The residue was then taken up in DCM and water. The trifluoroacetate salt can be recovered by evaporation of water and the crude product was used without any further purification. ^{1}H-NMR (D_2O, 400 MHz): 2.11-2.04 (m, 2H, CH_2 β), 2.36-2.30 (m, 2H, $CH_2\gamma$), 2.52-2.49 (m, 1H, C≡CH), 2.57-2.54 (m, 1H, C≡CH), 3.87-3.83 (m, 2H, H_2C-C≡), 3.95-3.89 (m, 3H, CHα, H_2C-C≡); MS (ESI): m/z = 243.6 $[M+Na]^+$, calcd for $C_{11}H_{15}N_3O_2$ m/z = 221.26 [M].
At room temperature and under a nitrogen atmosphere, a solution of *N*-Fmoc-glutamic acid (231.5 mg, 0.63 mmol) and *N*-hydroxy-succinimide (216.7 mg, 1.88 mmol) in dry DMF (3.15 mL, 0.2 M) was prepared. *N*,*N*-diisopropylcarbodiimide (237.6 mg, 1.88 mmol) was added and the solution was stirred at room temperature overnight (after a while, a white precipitate appeared). At room temperature and under a nitrogen atmosphere, a solution of above compound (trifluoroacetate salt, 2.51 mmol) and dry diisopropylethylamine (874 μL, 5.02 mmol) in dry DMF was prepared and then added to the solution containing the activated *N*-Fmoc-glutamic acid. The mixture was stirred at room temperature and the reaction was monitored by LC-MS. After 2h, the solution was acidified with 2M HCl to pH 2 and the solvent was evaporated under reduced pressure. The crude product was purified by reverse phase chromatography affording **6** (50% over two steps). ^{1}H-NMR (DMSO, 400 MHz): 2.23-2.02 (m, 6H, $CH_2\gamma$, 1.97-1.64 (m, 6H, $CH_2\beta$), 3.14-3.03 (m, 4H, C≡CH), 3.90-3.79 (m, 8H, H_2C-C≡), 4.00-3.93 (m, 1H, CHα), 4.32-4.18 (m, 5H, CHα, Fmoc-CH, -CH_2), 7.34 (t, 2H, aromatics, J = 7.6 Hz), 7.42 (t, 2H, aromatics, J = 7.6 Hz), 7.58 (d, 1H, NH, J = 7.6 Hz), 7.74 (t, 2H, aromatics, J = 6.0 Hz), 7.89 (d, 2H, aromatics, J = 7.6 Hz), 8.09 (d, 1H, NH, J = 7.6 Hz), 8.14 (d, 1H, NH, J = 8.0 Hz), 8.29-8.20 (m, 2H, NH), 8.48-8.33 (m, 2H, NH); ^{13}C-NMR (DMSO, 100.6 MHz): 171.9, 171.6, 144.3, 141.2, 128.2, 128.1, 127.6, 120.6, 73.6, 73.3, 66.2, 52.5, 47.1, 32.0, 28.5; MS (ESI): m/z = 776.3 $[M+H]^+$, 798.3 $[M+Na]^+$, calcd for $C_{42}H_{45}N_7O_8$ m/z = 775.85 [M].

Synthesis of (2S,2'S)-2,2'-(((S)-2-aminopentanedioyl)bis(azanediyl))bis(N^{1},N^{5}-di(prop-2-yn-1-yl)pentanediamide), (7). At room temperature, a solution of **6** (106 mg, 0.137 mmol) and polymer bound piperazine (366 mg, 0.55 mmol, resin loading: 1.5 mmol/g) in dry DMF (2.75 mL, 0.025 M) was prepared. The suspension was heated at 80°C in a microwave for 3 h. After reaction completion, the resin was filtered and washed with DMF. The solvent was then evaporated under reduced pressure and the crude product was used without any further purification. ^{1}H-NMR (DMSO, 400 MHz): 1.94-1.55 (m, 6H, $CH_2\beta$), 2.22-2.04 (m, 6H, $CH_2\gamma$), 3.15-3.03 (m, 4H, C≡CH), 3.92-3.76 (m, 8H, H_2C-C≡), 4.14-4.05 (m, 1H, CHα), 4.28-4.15 (m, 2H, CHα), 7.62 (d, 1H, J = 7.6 Hz, NH), 8.11 (d, 1H, J = 8.0 Hz, NH), 8.32-8.24 (m, 2H, NH), 8.45 (dd, 1H, J = 5.4 Hz, NH), 8.51 (dd, 1H, J = 5.4 Hz, NH); MS (ESI): m/z = 554.4 $[M+H]^+$, calcd for $C_{27}H_{35}N_7O_6$ m/z = 553.26 [M].

Synthesis of (2aR,4S,4aS,6R,9S,11S,12R,12aR,12bS)-9-(((2R)-3-benzamido-2-((4-(((S)-1,5-dioxo-1,5-bis(prop-2-yn-1-ylamino)pentan-2-yl)amino)-4-oxobutanoyl)oxy)-3-phenylpropanoyl)oxy)-12-(benzoyloxy)-4,11-dihydroxy-4a,8,13,13-tetramethyl-5-oxo-2a,3,4,4a,5,6,9,10,11,12,12a,12b-dodecahydro-1H-7,11-methanocyclodeca[3,4]benzo[1,2-b]oxete-6,12b-diyl diacetate, (9a). At room temperature and under a nitrogen atmosphere, a solution of acid **8a** (54.9 mg, 0.058 mmol) containing TBTU, (22.2 mg, 0.069 mmol) and HOBt, (9.3 mg, 0.069 mmol) in dry DMF (575 μL, 0.1 M) was prepared. After 10 minutes, a solution of **5** (38.6 mg, 0.115 mmol) and dry DIPEA (30 μL, 0.172 mmol) in dry DMF (575 μL, 0.2 M) was added. The resulting solution was stirred at room temperature. After reaction completion (2 h), the solvent was evaporated under reduced pressure. The residue was taken up with DCM and washed with water. The organic phase was dried over Na_2SO_4, filtered and evaporated under reduced pressure. The crude product was purified by reverse phase chromatography affording **9a** (81%). ^{1}H-NMR (CD_3CN, 400 MHz): 1.11 (s, 3H, -CH_3), 1.14 (s, 3H, -CH_3), 1.61 (s, 3H, -CH_3), 1.80-1.69

(m, 3H, Hβ, H6, H14), 1.90 (s, 3H, -CH_3), 2.02-1.95 (m, 1H, Hβ), 2.17 (s, 3H, -$COCH_3$), 2.22-2.12 (m, 3H, Hγ, H14), 2.38 (s, 3H, -$COCH_3$), 2.47-2.42 (m, 2H, HC≡), 2.54-2.49 (m, 3H, -NHCOC$\underline{H}_2$CH_2COO, H6), 2.77-2.72 (m, 2H, -NHCOCH_2C$\underline{H}_2$COO-), 2.85 (s, 1H, -OH), 2.96 (d, 1H, J = 6.0 Hz, -OH), 3.74 (d, 1H, J= 7.2 Hz, H3), 3.95-3.91 (m, 4H, H_2C-C≡), 4.16 (s, 2H, H20), 4.23-4.16 (m , 1H, Hα), 4.38-4.31 (m, 1H, H7), 4.97 (d, 1H, J = 8.0 Hz, H5), 5.41 (d, 1H, J= 6.4 Hz, H2'), 5.59 (d, 1H, J= 7.2 Hz, H2), 5.79 (dd, 1H, J = 6.4 Hz, J = 8.4 Hz, H3'), 6.02 (dd, 1H, J = 6.8 Hz, J = 8.0 Hz, H13), 6.34, (s, 1H, H10), 6.86-6.79 (m, 1H, -NHCH_2CH_2), 7.06 (d, 1H, J = 11.4 Hz, -NHCO), 7.11 (t, 1H, J = 8.4 Hz, -NHCH_2CH_2), 7.29 (t, 1H, J = 6.8 Hz, aromatic), 7.52-7.44 (m, 5H, aromatics), 7.68-7.54 (m, 4H, aromatics), 7.72 (t, 1H, J = 7.6 Hz, aromatic), 7.87-7.82 (m, 3H, aromatics, NH), 8.13 (d, 2H, J = 7.6 Hz, aromatics); ^{13}C-NMR (CD_3CN, 100.6 MHz): 203.4, 172.4, 172.2, 171.4, 171.1, 170.2, 170.2, 169.0, 167.3, 165.9, 141.2, 137.5, 134.3, 133.5, 133.3, 131.7, 130.1, 130.0, 128.9, 128.7, 128.6, 128.5, 128.4, 127.8, 127.5, 84.02, 80.7, 78.0, 75.9, 75.4, 74.8, 74.7, 71.5, 71.5, 58.0, 54.0, 53.0, 46.2, 43.1, 36.1, 35.4, 31.6, 30.0, 28.8, 28.3, 28.2, 27.3, 26.1, 22.4, 21.3, 20.1, 14.1, 9.3; MS (ESI): m/z = 1157.5 $[M+H]^+$, m/z = 1179.3 $[M+Na]^+$, calcd for $C_{62}H_{68}N_4O_{18}$ m/z = 1156,45 [M].

Synthesis of (2aR,4S,4aS,6R,9S,11S,12R,12aR,12bS)-9-(((2R,10S)-2-(benzamido(phenyl)methyl)-4,8,13-trioxo-10-(prop-2-yn-1-ylcarbamoyl)-3,6-dioxa-9,14-diazaheptadec-16-yn-1-oyl)oxy)-12-(benzoyloxy)-4,11-dihydroxy-4a,8,13,13-tetramethyl-5-oxo-2a,3,4,4a,5,6,9,10,11,12,12a,12b-dodecahydro-1H-7,11-methanocyclodeca[3,4]benzo[1,2-b]oxete-6,12b-diyl diacetate, (9b). At room temperature and under a nitrogen atmosphere, a solution of acid **8b** (46.8 mg, 0.048 mmol) containing TBTU, (18.6 mg, 0.058 mmol) and HOBt, (7.8 mg, 0.058 mmol) in dry DMF (480 μL, 0.1 M) was prepared. After 10 minutes, a solution of **5** (48.3 mg, 0.144 mmol) and dry DIPEA (33 μL, 0.192 mmol) in dry DMF (720 μL, 0.2 M) was added. The resulting solution was stirred at room temperature overnight. After reaction completion (2 h), the solvent was evaporated under reduced pressure. The residue was taken up with DCM and washed with water. The organic phase was dried over Na_2SO_4, filtered and evaporated under reduced pressure. The crude product was purified by reverse phase chromatography affording **9b** (93%). ^{1}H-NMR (CD_3CN, 400 MHz): 8.13 (d, 2H, J = 8.0 Hz, aromatics), 7.87 (d, 1H J = 7.6 Hz, -N$\underline{H}$COPh), 7.81 (d, 2H, J = 7.6 Hz, aromatics), 7.74-7.69 (m, 1H, aromatic), 7.66-7.60 (m, 2H, aromatics), 7.59-7.44 (m, 8H, aromatics), 7.30 (t, 1H, J = 7.2 Hz, aromatic), 7.17 (bs, 1H, -N$\underline{H}$CH_2), 6.87 (bs, 1H, -N$\underline{H}$CH_2), 6.34, (s, 1H, H10), 6.03 (dd, 1H, J = 6.8 Hz, J = 8.0 Hz, H13), 5.85 (dd, 1H, J = 6.8 Hz, J = 7.6 Hz, H3'), 5.59 (d, 1H, J= 6.4 Hz, H2), 5.54 (d, 1H, J= 6.4 Hz, H2'), 4.98 (d, 1H, J = 8.0 Hz, H5), 4.42-4.29 (m, 4H, H7, Hα, -NHCOCH_2O$\underline{C}H_2$COO-), 4.16 (s, 2H, H20), 4.03 (s, 2H, -NHCO$\underline{C}H_2$OCH_2COO-), 3.95-3.88 (m, 4H, H_2C-C≡), 3.75 (d, 1H, J= 6.8 Hz, H3), 2.92 (d, 1H, J = 6.0 Hz, -OH), 2.86 (s, 1H, -OH), 2.51-2.40 (m, 3H, H6, HC≡), 2.39 (s, 3H, -$COCH_3$), 2.26-2.12 (m, 3H, Hγ, H14), 2.16 (s, 3H, -$COCH_3$), 2.08-1.68 (m, 4H, Hβ, H6, H14), 1.91 (s, 3H, -CH_3), 1.61 (s, 3H, -CH_3), 1.14 (s, 3H, -CH_3), 1.11 (s, 3H, -CH_3); ^{13}C-NMR (CD_3CN, 100.6 MHz): 203.4, 172.0, 170.9, 170.2, 169.7, 168.9, 168.6, 167.4, 165.9, 141.1, 137.1, 134.3, 133.5, 133.4, 131.7, 130.1, 130.0, 128.9, 128.7, 128.5, 127.5, 127.4, 84.0, 80.7, 78.0, 75.9, 75.3, 74.8, 71.8, 71.5, 71.0, 70.9, 70.5, 68.1, 58.0, 53.9, 52.1, 46.2, 43.1, 36.1, 35.4, 31.5, 28.2, 27.8, 26.1, 22.4, 21.3, 20.1, 14.0, 9.3; MS (ESI): m/z = 1173.5 $[M+H]^+$, m/z = 1195.4 $[M+Na]^+$, calcd for $C_{62}H_{68}N_4O_{19}$ m/z = 1172.45 [M].

Synthesis of (2aR,4S,4aS,6R,9S,11S,12R,12aR,12bS)-9-(((2R,9S,14S)-2-(benzamido(phenyl)methyl)-9-(((S)-1,5-dioxo-1,5-bis(prop-2-yn-1-ylamino)pentan-2-yl)carbamoyl)-4,7,12,17-tetraoxo-14-(prop-2-yn-1-ylcarbamoyl)-3-oxa-8,13,18-triazahenicos-20-yn-1-oyl)oxy)-12-(benzoyloxy)-4,11-dihydroxy-4a,8,13,13-tetramethyl-5-oxo-2a,3,4,4a,5,6,9,10,11,12,12a,12b-dodecahydro-1H-7,11-methanocyclodeca[3,4]benzo[1,2-b]oxete-6,12b-diyl diacetate, (9c). At room temperature and under a nitrogen atmosphere, a solution of acid **8a** (33 mg, 0.035 mmol) containing TBTU, (13.3 mg, 0.042 mmol) and HOBt, (5.6 mg, 0.042 mmol) in dry DMF (1.38 mL, 0.025 M) was prepared. After 10 minutes, a solution of **7** (38.3 mg, 0.069 mmol) in dry DMF (345 μL, 0.2 M) was added. The resulting solution was stirred at room temperature overnight. After reaction completion (24 h), the solvent was evaporated under reduced pressure. The crude product was purified by reverse phase chromatography affording **9c** (70%). The presence of the product was confirmed by MS and **9c** was submitted to the next reaction without further characterization; MS (ESI): m/z = 1490.7 $[M+H]^+$, calcd for $C_{78}H_{88}N_8O_{22}$ m/z = 1489.57 [M].

General procedure for the click reaction.

At room temperature, a solution of the alkyne derivative (1 eq) in a 3:2 mixture of t-butanol/water was prepared. The aqueous solution of the azide (2.1 eq) was then added (concentration of the taxol derivative is 0.02 M). 0.3 M copper(II) acetate (0.8 eq) and 0.9 M sodium L-ascorbate (1.6 eq) were added and the yellow solution was stirred at room temperature. The reaction was monitored by LC-MS. After reaction completion, the solvent was removed by lyophilisation.

The crude product was purified by preparative HPLC (see Supporting Information).

Paclitaxel-RGD conjugate, (3a). (54%) ^{1}H-NMR (3:2 CD_3CN/D_2O, 600 MHz): 1.05 (bs, 6H, -CH_3, -CH_3), 1.32-1.18 (m, 2 H, H5a), 1.55 (s, 3H, -CH_3), 1.60-1.49 (m, 9H, H14, H8a, Hβ-Arg, Hγ-Arg), 1.79 (s, 3H, -CH_3), 1.85-1.69 (m, 2H, Hβ-Glu, H6), 2.13 (s, 3H, -$COCH_3$), 1.94-1.86 (m, 1H, H14), 2.08-1.94 (m, 5H, H8a, Hβ-Glu, Hβ-Arg), 2.27 (s, 3H, -$COCH_3$), 2.47-2.11 (m, 7H, H6, H5a, H7a Hγ-Glu), 2.60-2.47 (m, 4H, 2Hβ-Asp, -NHCOC$\underline{H}_2$CH_2COO-), 2.75-2.67 (m, 2H, -NHCOCH_2C$\underline{H}_2$COO-), 2.96-2.85 (m, 2H, Hβ-Asp), 3.22-3.05 (m, 6H, H4a, Hδ-Arg), 3.46-3.37 (m, 2H, CHGly), 3.69-3.62 (m, 1H, H3), 4.00-3.90 (m, 2H, H6a), 4.27-4.01 (m, 10H, Hα-Glu, H20, H7, H10a, H9a, CHGly), 4.45-4.28 (m, 10H, H3a, H10a, Hα-Asp, -NCH_2-triazole), 4.57-4.48 (m, 2H, Hα-Arg), 5.02-4.93 (m, 1H, H5), 5.42-5.33 (m, 1H, H2'), 5.57-5.48 (m, 1H, H2), 5.65-5.58 (m, 1H, H3'), 5.93-5.83 (m, 1H, H13), 6.30 (s, 1H, H10), 7.27-7.16 (m, 1H, aromatic), 7.52-7.40 (m, 6H, aromatics), 7.60-7.52 (m, 1H, aromatic), 7.68-7.60 (m, 2H, aromatics), 7.84-7.69 (m, 5H, HC=, aromatics), 8.09-8.01 (m, 2H, aromatics), 8.37-8.27 (m, 2H, aromatics); ^{13}C-NMR DEPT (3:2 CD_3CN/D_2O, 150.9 MHz): 134.1, 132.3, 130.1, 129.2, 129.1, 128.9, 128.8, 128.7, 127.7, 127.5, 84.4, 76.3, 75.6, 75.0, 74.9, 71.9, 71.0, 62.2, 55.7, 54.6, 53.3, 53.0, 52.1, 51.6, 51.4,

46.5, 42.5, 40.6, 36.4, 35.9, 34.4, 34.5, 34.4, 32.9, 32.4, 30.2, 29.7, 28.7, 27.4, 27.2, 25.8, 24.7, 22.5, 21.2, 20.4, 14.1, 9.6; MS (ESI): m/z = 1142.5 $[M+2H]^+/2$, calcd for $C_{106}H_{134}N_{26}O_{32}$ m/z = 2282.97 [M]; HRMS (ESI): m/z = 1153.48348 $[M+H+Na]^+/2$, calcd for $C_{106}H_{135}N_{26}O_{32}Na$ m/z = 1153.48112 $[M+H+Na]^+/2$.

Paclitaxel-RGD conjugate, (3b). (57%) ^{1}H-NMR (3:2 CD_3CN/D_2O, 600 MHz): 8.40-8.27 (m, 1H, aromatic), 8.08-8.01 (m, 2H, aromatics), 7.81-7.69 (m, 5H, HC=, aromatics), 7.66-7.59 (m, 2H, aromatics), 7.58-7.51 (m, 1H, aromatic), 7.49-7.40 (m, 6H, aromatics), 7.24-7.19 (m, 1H, aromatic), 6.32 (s, 1H, H10), 5.93-5.87 (m, 1H, H13), 5.70-5.66 (m, 1H, H3'), 5.54-5.50 (m, 1H, H2), 5.48-5.44 (m, 1H, H2'), 4.98 (d, 1H, J = 9.0 Hz, H5), 4.54-4.48 (m, 2H, Hα-Arg), 4.45-4.25 (m, 13H, H3a, H10a, Hα-Glu, Hα-Asp, -NHCOCH_2O, -NCH_2-triazole), 4.25-4.01 (m, 11H, H7, H20, H10a, H9a, -OCH_2CONH, CHGly), 3.99-3.90 (m, 2H, H6a), 3.69-3.65 (m, 1H, H3), 3.41 (d, 1H, J = 13.8 Hz, CHGly), 3.20-3.05 (m, 6H, H4a, Hδ-Arg), 2.93-2.83 (m, 2H, Hβ-Asp), 2.54-2.41 (m, 3H, Hβ-Asp, H6), 2.40-2.19 (m, 8H, H5a, H7a, H8a, Hγ-Glu), 2.31 (s, 3H, -COCH_3), 2.14 (s, 3H, -COCH_3), 2.08-1.93 (m, 5H, H14, Hβ-Glu, Hβ-Arg), 1.81-1.69 (m, 3H, H8a, H6), 1.84 (s, 3H, -CH_3), 1.63-1.39 (m, 9H, H14, H7a, Hβ-Arg, Hγ-Arg), 1.56 (s, 3H, -CH_3), 1.32-1.19 (m, 2 H, H5a), 1.06 (bs, 6H, -CH_3,-CH_3); ^{13}C-NMR (3:2 CD_3CN/D_2O, 150.9 MHz): 204.4, 172.4, 172.2, 171.2, 171.1, 170.7, 170.5, 169.5, 169.2, 166.5, 156.7, 140.7, 136.6, 134.0, 133.6, 133.4, 132.2, 130.0, 129.7, 129.2, 128.9, 128.7, 127.6, 127.5, 127.4, 84.4, 80.7, 77.8, 76.2, 75.6, 75.1, 74.8, 72.2, 70.9, 70.0, 67.9, 62.2, 57.9, 55.6, 54.4, 52.9, 52.7, 52.0, 51.6, 51.3, 46.5, 43.1, 42.4, 40.6, 36.6, 35.8, 34.9, 34.5, 34.4, 32.9, 32.4, 31.7, 30.1, 27.4, 27.2, 25.9, 24.6, 22.5, 21.2, 20.3, 14.0, 9.6; MS (ESI): m/z = 1150.5 $[M+2H]^+/2$, calcd for $C_{106}H_{134}N_{26}O_{33}$ m/z = 2298.96 [M]; HRMS (ESI): m/z = 1161.48006 $[M+H+Na]^+/2$, calcd for $C_{106}H_{135}N_{26}O_{33}Na$ m/z = 1161.47858 $[M+H+Na]^+/2$.

Paclitaxel-RGD conjugate, (3c). (24%) ^{1}H-NMR (3:2 CD_3CN/D_2O, 400 MHz): 1.04 (bs, 6H, -CH_3, -CH_3), 1.30-1.14 (m, 4H, H5a), 1.46 (s, 3H, -CH_3), 1.60-1.33 (m, 17H, H14, H7a, Hβ-Arg, Hγ-Arg), 1.78 (s, 3H, -CH_3), 2.09-1.67 (m, 18H, H6, H8a, Hβ-Glu, Hγ-Glu, Hβ-Arg), 2.13 (s, 3H, -COCH_3), 2.25 (s, 3H, -COCH_3), 2.47-2.10 (m, 17H, H6, H14, H5a, H7a, Hβ-Asp, Hγ-Glu), 2.55-2.47 (m, 2H, -NHCOC$\underline{H}_2$CH$_2$COO-), 2.66-2.55 (m, 2H, -NHCOCH$_2$C$\underline{H}_2$COO-), 2.84-2.67 (m, 4H, Hβ-Asp), 3.19-3.03 (m, 12H, H4a, Hδ-Arg), 3.45-3.34 (m, 4H, CHGly), 3.69-3.62 (d, 1H, J = 7.2 Hz, H3), 3.99-3.85 (m, 4H, H6a), 4.25-4.00 (m, 18H, Hα-Glu, H20, H7, H10a, H9a, CHGly), 4.43-4.20 (m, 20H, H3a, H10a, Hα-Asp, -NCH_2-triazole), 4.53-4.43 (m, 4H, Hα-Arg), 4.96 (d, 1H, J = 9.6 Hz, H5), 5.33 (d, 1H, J = 8.4 Hz, H2'), 5.48 (d, 1H, J = 7.2 Hz, H2), 5.60 (m, 1H, J = 8.4 Hz, H3'), 5.91-5.83 (m, 1H, H13), 6.29 (s, 1H, H10), 7.21-7.14 (m, 1H, aromatic), 7.49-7.38 (m, 5H, aromatics), 7.55-7.49 (m, 1H, aromatic), 7.66-7.58 (m, 2H, aromatics), 7.82-7.68 (m, 6H, HC=, aromatics), 8.01 (d, 2H, aromatics, J = 8.4 Hz), 8.43-8.28 (m, 2H, aromatics); ^{13}C-NMR HSQC (3:2 CD_3CN/D_2O, 100.6 MHz): 130.4, 129.4, 129.3, 128.1, 127.8, 124.7, 85.0, 76.7, 76.0, 75.4, 75.3, 72.2, 71.4, 62.6, 56.1, 55.9, 53.5, 53.5, 53.2, 52.0, 51.8, 46.8, 43.1, 41.0, 36.8, 36.6, 34.9, 33.3, 32.9, 30.5, 30.1, 29.1, 27.7, 27.7, 26.3, 25.1, 22.9, 21.7, 20.9, 14.6, 10.1; MS (ESI): m/z = 1873.2 $[M+2H]^+/2$, 1249.1 $[M+3H]^+/3$, 937.3 $[M+4H]^+/4$, calcd for $C_{166}H_{220}N_{52}O_{50}$ m/z = 3743.84 [M]; HRMS (ESI) m/z = 947.90724 $[M+2H+2Na]^+/4$, calcd for $C_{166}H_{222}N_{52}O_{50}Na_2$ m/z = 947.90639 $[M+2H+2Na]^+/4$.

Synthesis of isopropyl 3-oxo-1-phenyl-2,7,10,13-tetraoxa-4-azapentadecan-15-oate, (12). At room temperature and under a nitrogen atmosphere, a solution of **11** (500 mg, 1.46 mmol) in dry THF (14.6 mL, 0.1 M) containing CDI, (949 mg, 5.85 mmol) was prepared. The mixture was stirred for 2 h before adding 2-propanol (5.6 mL, 76.2 mmol). The reaction was monitored by TLC using 86:10:4 $CHCl_3$/MeOH/AcOH as the eluent. After 1 h, the solvent was removed under reduced pressure. The residue was taken up with DCM and washed with 1M HCl. The organic layer was dried over Na_2SO_4, filtered and the solvent was evaporated under reduced pressure. The crude product was used without any further purification. ^{1}H-NMR ($CDCl_3$, 400 MHz): 1.26 (d, 6H, J = 6.4 Hz, -CH(C$\underline{H}_3$)$_2$), 3.41 (t, 2H, J = 4.8 Hz, -CH_2NHCbz), 3.58 (dd, 2H, J = 5.6 Hz, J = 5.2 Hz, -OCH_2), 4.09 (s, 2H, -CH_2COO-), 3.74-3.63 (m, 8H, -OCH_2), 5.11 (s, 2H, -OCH_2Ph), 5.14-5.09 (m, 1H, -C$\underline{H}$(CH$_3$)$_2$), 7.41-7.32 (m, 5H, aromatics); MS (ESI): m/z = 384.2 $[M+H]^+$, 406.2 $[M+Na]^+$, calcd for $C_{19}H_{29}NO_7$ m/z = 383.19 [M].

Synthesis of (S)-diisopropyl 14-((tert-butoxycarbonyl)amino)-13,17-dioxo-3,6,9,21,24,27-hexaoxa-12,18-diazanonacosane-1,29-dioate, (13). At room temperature, a solution of **12** (559 mg, 1.46 mmol) in iPrOH (29.2 mL, 0.05 M) was prepared and 1M HCl (2.19 mL) was added. The protecting group was removed by hydrogenolysis over 10 % Pd-C catalyst (10 % w/w). The mixture was stirred under a hydrogen atmosphere for 18 h and the reaction was monitored by TLC using 86:10:4 $CHCl_3$/MeOH/AcOH as the eluent. After reaction completion, the catalyst was filtered and the solvent was evaporated under reduced pressure. The crude product was obtained as a white foam and it was used without any further purification. ^{1}H-NMR ($CDCl_3$, 400 MHz): 1.29 (d, 6H, J = 5.6 Hz, -CH(C$\underline{H}_3$)$_2$), 3.35-3.22 (m, 2H, -CH_2NH$_3$Cl), 3.84-3.69 (m, 6H, -OCH_2), 4.10-3.98 (m, 4H, -OCH_2), 4.18 (s, 2H, -CH_2COO-), 5.12 (sept, 1H, J= 5.6 Hz, -C$\underline{H}$(CH$_3$)$_2$,); MS (ESI): m/z = 250.1 $[M+H]^+$, calcd for $C_{11}H_{23}NO_5$ m/z = 249.16 [M].

At room temperature and under a nitrogen atmosphere, a solution of *N*-Boc-glutamic acid (180.5 mg, 0.73 mmol) and *N*-hydroxy-succinimide (252 mg, 2.19 mmol) in dry DMF (3.65 mL, 0.2 M) was prepared. DIC (339 μL, 2.19 mmol) was added and the solution was stirred at room temperature overnight (after a while, a white precipitate appeared). A solution of the above compound (1.46 mmol) in dry DMF (3.65 mL) and dry DIPEA (509 μL, 2.92 mmol) were then added and the cloudy solution was stirred for 2 h (the reaction was monitored by LC-MS). After reaction completion, the solvent was evaporated under reduced pressure. The residue was taken up with DCM and washed with water. The organic layer was dried over Na_2SO_4, filtered and the solvent was evaporated under reduced

pressure. The crude product was purified by flash chromatography using 9:1 DCM/MeOH as the eluent affording **13** (76%). ^{1}H-NMR ($CDCl_3$, 600 MHz): 1.26 (d, 6H, J = 6.0 Hz, -CH($C\underline{H}_3$)$_2$), 1.42 (s, 9H, Boc), 2.11-1.94 (m, 2H, $CH_2\beta$), 2.39-2.23 (m, 2H, $CH_2\gamma$), 3.53-3.36 (m, 4H, -CH_2NHCO), 3.74-3.55 (m, 20H, -OCH_2), 4.10 (s, 4H, -CH_2COO-), 4.15-4.09 (m, 1H, CHα), 5.08 (sept, 2H, J= 6.0 Hz, -C$\underline{H}$(CH_3)$_2$), 5.68 (bs, 1H, NHBoc), 6.77 (bs, 1H, NHCO), 7.11 (bs, 1H, NHCO); ^{13}C-NMR ($CDCl_3$, 100.6 MHz): 172.8, 171.8, 170.0, 79.6, 70.8, 70.5, 70.2, 69.7, 68.8, 68.7, 68.5, 53.9, 39.3, 32.5, 29.3, 28.3, 21.8; MS (ESI): m/z = 710.6 $[M+H]^+$, calcd for $C_{32}H_{59}N_3O_{14}$: 709.40 [M].

Synthesis of (S)-2-amino-N^1,N^5-bis(11-oxo-3,6,9-trioxa-12-azapentadec-14-yn-1-yl)pentanediamide, (14). At room temperature and under a nitrogen atmosphere, a solution of **13** (392 mg, 0.55 mmol) in dioxane (5.5 mL, 0.1 M) was prepared. 1M NaOH (1.1 mL, 1.1 mmol) was added and the solution was stirred at room temperature for 1 h. After reaction completion, the solvent was evaporated under reduced pressure. The crude product was used without any further purification. ^{1}H-NMR (D_2O, 400 MHz): 1.36 (s, 9H, Boc), 1.87-1.77 (m, 1H, CHβ), 2.05-1.88 (m, 1H, CHβ), 2.33-2.26 (m, 2H, $CH_2\gamma$), 3.39-3.31 (m, 4H, -CH_2NHCO), 3.59-3.53 (m, 4H, -OCH_2), 3.67-3.60 (m, 16H, -OCH_2), 3.88 (s, 4H, -CH_2COO-), 3.94-3.85 (m, 1H, CHα); ^{13}C-NMR (D_2O, 100.6 MHz): 177.9, 175.1, 174.5, 157.3, 81.5, 69.8, 69.7, 69.6, 69.6, 69.4, 69.4, 68.8, 66.6, 54.5, 39.0, 32.0, 27.6, 27.3; MS (ESI): m/z = 626.5 $[M+H]^+$, calcd for $C_{26}H_{47}N_3O_{14}$ m/z = 625.31 [M].

At room temperature and under a nitrogen atmosphere, a solution diacid (216 mg, 0.345 mmol) in dry DMF (3.45 mL, 0.1 M) was prepared. TBTU, (277.1 mg, 0.863 mmol) and HOBt, (116.6 mg, 0.863 mmol) were added and the solution was stirred at room temperature. After 20 minutes, propargylamine (95 μL, 1.38 mmol) was added and the solution was stirred overnight. After reaction completion, the solvent was evaporated under reduced pressure and the crude product was purified by reverse phase chromatography affording the Boc derivative (50%). ^{1}H-NMR ($CDCl_3$, 400 MHz): 1.45 (s, 9H, Boc), 2.11-1.91 (m, 2H, $CH_2\beta$), 2.39-2.20 (m, 4H, $CH_2\gamma$, HC≡C), 3.54-3.38 (m, 4H, -CH_2NHCO), 3.77-3.55 (m, 20H, -OCH_2), 4.18-4.06 (m, 9H, CHα, H_2C≡C, OCH_2CO), 5.73 (d, 1H, J= 7.2 Hz, NHBoc), 6.78 (bs, 1H, NHCO), 7.23 (bs, 1H, NHCO), 7.48-7.33 (bs, 2H, NHCO); MS (ESI): m/z = 700.6 $[M+H]^+$, 722.6 $[M+Na]^+$, calcd for $C_{32}H_{53}N_5O_{12}$ m/z = 699.37 [M].

At room temperature, a solution of the above substrate (37.6 mg, 0.054 mmol) in dry DCM (540 μL, 0.1 M) was prepared. Anisole (8.8 μL, 0.081 mmol) and trifluoroacetic acid (39.9 μL, 0.54 mmol) were added and the solution was stirred at room temperature. After 2 h, the solvent was evaporated under reduced pressure and the residue washed twice with toluene. The residue was then taken up in DCM and water. The trifluoroacetate salt can be recovered by evaporation of water and the crude product was used without any further purification. ^{1}H-NMR (D_2O, 400 MHz): 2.08-2.01 (m, 2H, $CH_2\beta$), 2.34-2.27 (m, 2H, $CH_2\gamma$), 2.51 (bs, 2H, C≡CH), 3.43-3.26 (m, 4H, -CH_2NHCO), 3.68-3.49 (m, 20H, -OCH_2), 3.95-3.88 (m, 5H, CHα, H_2C-C≡), 3.99 (bs, 4H, OCH_2CO); ^{13}C-NMR (D_2O, 100.6 MHz): 173.9, 172.5, 169.1, 79.5, 71.9, 70.4, 69.7, 69.5, 69.4, 68.8, 68.6, 52.7, 39.1, 39.0, 30.7, 28.3, 26.6; MS (ESI): m/z = 600.5 $[M+H]^+$, calcd for $C_{27}H_{45}N_5O_{10}$ m/z = 599.32 [M].

Synthesis of (2aR,4S,4aS,6R,9S,11S,12R,12aR,12bS)-9-(((2R,9S)-2-(benzamido(phenyl)methyl)-4,7,12,24-tetraoxo-9-((11-oxo-3,6,9-trioxa-12-azapentadec-14-yn-1-yl)carbamoyl)-3,16,19,22-tetraoxa-8,13,25-triazaoctacos-27-yn-1-oyl)oxy)-12-(benzoyloxy)-4,11-dihydroxy-4a,8,13,13-tetramethyl-5-oxo-2a,3,4,4a,5,6,9,10,11,12,12a,12b-dodecahydro-1H-7,11-methanocyclodeca[3,4]benzo[1,2-b]oxete-6,12b-diyl diacetate, (15). At room temperature and under a nitrogen atmosphere, a solution of acid **8a** (42.7 mg, 0.045 mmol) containing TBTU, (17.2 mg, 0.054 mmol) and HOBt, (7.3 mg, 0.054 mmol) in dry DMF (450 μL, 0.1 M) was prepared. After 10 minutes, a solution of **14** (38.3 mg, 0.054 mmol) and dry DIPEA (15.7 μL, 0.09 mmol) in dry DMF (540 μL, 0.1 M) was added. The resulting solution was stirred at room temperature. After reaction completion (24 h), the solvent was evaporated under reduced pressure. The crude product was purified by reverse phase chromatography affording **15** (76%). ^{1}H-NMR (CD_3CN, 400 MHz): 1.11 (s, 3H, -CH_3), 1.14 (s, 3H, -CH_3), 1.61 (s, 3H, -CH_3), 1.88-1.69 (m, 3H, Hβ, H6, H14), 1.89 (s, 3H, -CH_3), 1.99-1.89 (m, 1H, Hβ), 2.17 (s, 3H, -$COCH_3$), 2.25-2.11 (m, 3H, Hγ, H14), 2.37 (s, 3H, -$COCH_3$), 2.54-2.41 (m, 5H, -NHCOC$\underline{H}_2$$CH_2$COO-, H6, HC≡), 2.76-2.67 (m, 2H, -NHCOCH_2C$\underline{H}_2$COO-), 2.88 (s, 1H, -OH), 3.15 (d, 1H, J = 6.0 Hz, -OH), 3.40-3.26 (m, 4H, -$NHCH_2$), 3.49 (t, 4H, J = 5.2 Hz, -$NHCH_2$C$\underline{H}_2$O), 3.67-3.55 (m, 16H, –OCH_2), 3.74 (d, 1H, J= 7.2 Hz, H3), 3.96-3.94 (m, 4H, –OCH_2CONH), 4.04-3.97 (m, 4H, H_2C-C≡), 4.16 (s, 2H, H20), 4.23-4.16 (m ,1H, Hα), 4.39-4.31 (m, 1H, H7), 4.97 (d, 1H, J = 9.6 Hz, H5), 5.42 (d, 1H, J= 6.4 Hz, H2'), 5.59 (d, 1H, J= 6.8 Hz, H2), 5.77 (dd, 1H, J = 6.4 Hz, J = 8.4 Hz, H3'), 6.00 (dd, 1H, J = 6.8 Hz, J = 8.4 Hz, H13), 6.34 (s, 1H, H10), 6.80-6.73 (m, 1H, $NHCH_2CH_2$), 7.00-6.93 (m, 1H, $NHCH_2CH_2$), 7.12 (m, 1H, J = 7.6 Hz, NHCO), 7.29 (t, 1H, J = 7.6 Hz, aromatic), 7.59-7.43 (m, 8H, aromatics, NH), 7.63 (t, 2H, J = 7.6 Hz, aromatics), 7.72 (t, 1H, J = 7.6 Hz, aromatic), 7.85 (d, 2H, J = 7.6 Hz, aromatics), 7.93 (d, 1H, J = 8.4 Hz, NH), 8.13 (d, 2H, J = 7.6 Hz, aromatics); ^{13}C-NMR (CD_3CN, 100.6 MHz): 203.3, 172.6, 172.3, 171.4, 171.1, 170.2, 169.9, 169.0, 167.2, 165.9, 141.2, 137.6, 134.3, 133.5, 133.3, 131.6, 130.1, 130.0, 128.9, 128.7, 128.5, 128.4, 127.5, 127.4, 84.0, 80.7, 78.0, 75.9, 75.4, 74.8, 74.7, 71.5, 71.4, 70.8, 70.1, 69.9, 69.2, 69.2, 58.1, 54.1, 53.1, 46.3, 43.1, 39.0, 38.9, 36.2, 35.4, 31.9, 30.0, 28.8, 27.9, 27.6, 26.1, 22.3, 21.3, 20.1, 14.1, 9.35; MS (ESI): m/z = 1535.6 $[M+H]^+$, calcd for $C_{78}H_{98}N_6O_{26}$ m/z = 1534.65 [M].

Paclitaxel-RGD conjugate, (4). (55%) Compound has been prepared following the general procedure for the click reaction. ^{1}H-NMR (3:2 CD_3CN/D_2O, 400 MHz): 1.05 (bs, 6H, -CH_3), 1.29-1.19 (m, 2 H, H5a), 1.54 (s, 3H, -CH_3), 1.60-1.48 (m, 9H, H14, H7a, H8a, Hγ-Arg), 1.80 (s, 3H, -CH_3), 1.83-1.67 (m, 3H, Hβ-Glu, Hγ-Glu, H6), 2.13 (s, 3H, -$COCH_3$), 2.06-1.84 (m, 6H,

H14, H8a, Hβ-Glu, Hβ-Arg), 2.27 (s, 3H, -$COCH_3$), 2.41-2.12 (m, 6H, H5a, H7a, Hγ-Glu), 2.57-2.42 (m, 5H, H6, Hβ-Asp, -NHCOC$\underline{H}_2CH_2COO$-), 2.71-2.64 (m, 2H, -NHCOC$H_2C\underline{H}_2COO$-), 2.85 (dd, 2H, J = 16.4, 6.5 Hz, Hβ-Asp), 3.20-3.04 (m, 6H, H4a, Hδ-Arg), 3.31-3.22 (m, 4H, $CONHCH_2$), 3.50-3.42 (m, 6H, CHGly, –OCH_2), 3.67-3.50 (m, 17H, H3, –OCH_2), 4.01-3.90 (m, 6H, H6a, –OCH_2CO), 4.25-4.03 (m, 10H, Hα-Glu, H20, H7, H10a, H9a, CHGly), 4.46-4.30 (m, 10H, H3a, H10a, Hα-Asp, -NCH_2-triazole), 4.53-4.47 (m, 2H, Hα-Arg), 4.97 (d, 1H, J = 9.5 Hz, H5), 5.34 (d, 1H, J = 7.6 Hz, H2'), 5.49 (d, 1H, J = 7.6 Hz, H2), 5.59 (d, 1H, J = 7.6 Hz, H3'), 5.90-5.83 (m, 1H, H13), 6.30 (s, 1H, H10), 7.22-7.16 (m, 1H, aromatic), 7.49-7.39 (m, 6H, aromatics), 7.58-7.51 (m, 1H, aromatic), 7.66-7.59 (m, 2H, aromatics), 7.82-7.69 (m, 5H, aromatics), 8.06-8.01 (m, 2H, HC=), 8.35-8.30 (m, 2H, aromatics); ^{13}C-NMR DEPT (3:2 CD_3CN/D_2O, 100.6 MHz): 205.1, 176.7, 175.4, 174.7, 173.64, 173.6, 172.4, 173.4, 173.0, 172.4, 171.7, 171.7, 171.6, 170.4, 169.7, 169.4, 167.1, 157.2, 145.2, 141.3, 137.2, 134.6, 134.1, 133.8, 132.7, 130.5, 130.1, 129.6, 129.4, 129.3, 128.1, 127.9, 124.8, 84.9, 81.1, 78.3, 76.7, 76.1, 75.4, 75.3, 72.4, 71.3, 70.9, 70.3, 70.3, 70.2, 70.2, 70.0, 69.4, 69.4, 62.6, 57.4, 56.1, 55.0, 53.7, 53.4, 52.6, 52.1, 51.8, 47.0, 43.5, 42.9, 41.0, 39.5, 39.4, 36.8, 36.3, 35.6, 34.4, 33.3, 32.8, 32.4, 30.6, 30.2, 29.2, 28.1, 27.6, 26.3, 25.1, 22.9, 21.7, 20.8, 14.5, 10.1; MS (ESI): m/z = 1332.9 $[M+2H]^+/2$, 889.0 $[M+3H]^+/3$, calcd for $C_{122}H_{164}N_{28}O_{40}$ m/z = 2662.77 [M]; HRMS (ESI): 1342.58330 $[M+H+Na]^+/2$, calcd for $C_{122}H_{165}N_{28}O_{40}Na$ m/z = 1342.58123 $[M+H+Na]^+/2$.

Acknowledgements

We gratefully acknowledge "Ministero dell'Università e della Ricerca" for financial support (PRIN project 2010NRREPL: Synthesis and biomedical applications of tumour-targeting peptidomimetics).

Notes and references

[a] Centro Interdipartimentale Studi Biomolecolari e Applicazioni Industriali, Università degli Studi di Milano, Via Fantoli 16/15, I-20138 Milano, Italy; present address: CISI scrl, Via Fantoli 16/15, I-20138 Milano, Italy.

[b] Istituto di Scienze e Tecnologie Molecolari, Consiglio Nazionale delle Ricerche, Via Golgi 19, I-20133 Milano, Italy.

[c] Fondazione IRCCS Istituto Nazionale dei Tumori, Dipartimento di Oncologia Sperimentale e Medicina Molecolare, Via Amadeo 42, I-20133 Milan, Italy.

† These authors contributed equally to the project.

Electronic Supplementary Information (ESI) available: [^{1}H and ^{13}C NMR spectra of new synthesized compounds; HPLC purification conditions; solid-phase receptor binding assay procedure; in vitro cell sensitivity assay and in vivo anti-tumour activity protocols]. See DOI: 10.1039/b000000x/

1 J. S. Desgrosellier, D. A. Cheresh, *Nat. Rev. Cancer*, 2010, **10**, 9.

2 D. Cox, M. Brennan, N. Moran, *Nat. Rev. Drug Discov*., 2010, **9**, 804.

3 L. Auzzas, F. Zanardi, L. Battistini, P. Burreddu, P. Carta, G. Rassu, C. Curti, G. Casiraghi, *Curr. Med. Chem*., 2010, **17**, 1255.

4 M. A. Dechantsreiter,. E. Planker, B. Mathä, E. Lohof, G. Hölzemann, A. Jonczyk, S. L. Goodman, H. Kessler, *J. Med. Chem*., 1999, **42**, 3033.

5 A. R. Reynolds, I. R. Hart, A. R. Watson, J. C. Welti, R. G. Silva, S. D. Robinson, G. Da Violante, M. Gourlaouen, M. Salih, M. C. Jones, D. T. Jones, G. Saunders, V. Kostourou, F. Perron-Sierra, J. C. Norman, G. C. Tucker, K. M. Hodivala-Dilke, *Nat. Med.*, 2009, **15**, 392.

6 M. Schottelius, B. Laufer, H. Kessler, H.-J. Wester, *Acc. Chem. Res*., 2009, **42**, 969.

7 A. J. Beer, H. Kessler, H. J. Wester, M. Schwaiger, *Theranostics*, 2011, **17**, 48.

8 K. Temming, R. M. Schiffelers, G. Molema, R. J. Kok, *Drug Resistance Updates*, 2005, **8**, 381.

9 W. Arap, R. Pasqualini, E. Ruoslahti, *Science*, 1998, **279**, 377.

10 Z. Wang, W. K. Chui, P. C. Ho, *Expert Opin. Drug Deliv*., 2010, **7**, 159.

11 K. Chen, X. Chen, *Theranostics*, 2011, **17**, 189.

12 D. Arosio, C. Casagrande, L. Manzoni, *Curr. Med. Chem*., 2012, **19**, 3128.

13 U. K. Marelli, F. Rechenmacher, T. R. Sobahi, C. Mas-Moruno, H. Kessler, *Front. Oncol*., 2013, **3**, 222.

14 L. Battistini, P. Burreddu, A. Sartori, D. Arosio, L. Manzoni, L. Paduano, G. D'Errico, R. Sala, L. Reia, S. Bonomini, G. Rassu, F. Zanardi, *Mol Pharm*. 2014, **11**, 2280.

15 D. Peer, J. M. Karp, S. Hong, O. C. Farokhzad, R. Margalit, R. Langer, *Nat. Nanotechnol.*, 2007, **2**, 751.

16 X. Chen, C. Plasencia, Y. Hou, N. Neamati, *J. Med. Chem*., 2005, **48**, 1098. (corrigendum *J. Med. Chem*. 2005, **48**, 5874).

17 Q. Cao, Z.-B. Li, K. Chen, Z. Wu, L. He, N. Neamati, X. Chen, *Eur. J. Nucl. Med. Mol. Imaging*, 2008, **35**, 1489.

18 C. Ryppa, H. Mann-Steinberg, M. L. Biniossek, R. Satchi-Fainaro, F. Kratz, *Int. J. Pharm*., 2009, **368**, 89.

19 R. Colombo, M. Mingozzi, L. Belvisi, D. Arosio, U. Piarulli, N. Carenini, P. Perego, N. Zaffaroni, M. De Cesare, V. Castiglioni, E. Scanziani, C. Gennari, *J. Med. Chem*., 2012, **55**, 10460.

20 L. Manzoni, L. Belvisi, D. Arosio, M. Civera, M. Pilkington-Miksa, D. Potenza, A. Caprini, E. M.V. Araldi, E. Monferini, M. Mancino, F. Podestà, C. Scolastico, *ChemMedChem*, 2009, **4**, 615.

21 D. Arosio, L. Manzoni, E. M. V. Araldi, A. Caprini, E. Monferini, C. Scolastico, *Bioconj. Chemistry*, 2009, 1611.

22 S. Lanzardo, L. Conti, C. Brioschi, M. P. Bartolomeo, D. Arosio, L. Belvisi, L. Manzoni, A. Maiocchi, F. Maisano, G. Forni, *Contrast Media Mol. Imaging*, 2011, **6**, 449.

23 L. Manzoni, L. Belvisi, D. Arosio, M. P. Bartolomeo, A. Bianchi, C. Brioschi, F. Buonsanti, C. Cabella, C. Casagrande, M. Civera, M. De Matteo, L. Fugazza, L. Lattuada, F. Maisano, L. Miragoli, C. Neira, M. Pilkington-Miksa, C. Scolastico, *ChemMedChem*, 2012, **7**, 1084.

24 L. Menichetti, C. Kusmic, D. Panetta, D. Arosio, D. Petroni, M. Matteucci, P. A. Salvadori, C. Casagrande, A. L'Abbate, L. Manzoni, *Eur. J. Nucl. Med. Mol. Imaging*, 2013, **40**, 1265.

25 M. Pilkington-Miksa, D. Arosio, L. Battistini, L. Belvisi, M. De Matteo, F. Vasile, P. Burreddu, P. Carta, G. Rassu, P. Perego, N. Carenini, F. Zunino, M. De Cesare, V. Castiglioni, E. Scanziani, C.

Scolastico, G. Casiraghi, F. Zanardi, L. Manzoni, *Bioconj. Chemistry*, 2012, 1610.
26 H. M. Deutsch, J. A. Glinski, M. Hernandez, R. D. Haugwitz, V. L. Narayanan, M. Suffness, L. H. Zalkow, *J. Med. Chem.* 1989, **32**, 788.
27 V. V. Rostovtsev, L. G. Green, V. V. Fokin, K. B. Sharpless, *Angew. Chem. Int Ed.* 2002, **41**, 2596.
28 Y. L. Angell, K. Burgess, *Chem. Soc. Rev.*, 2007, **36**, 1674.

Graphical abstract

Novel RGD-PTX multivalent conjugates, presenting enhanced binding for $\alpha_v\beta_3$ integrin, have been reported. In vivo evaluation of **3b** showed tumor growth inhibition though administering one third of the PTX dose.