Inhibition of Mitosis by Glycopeptide Dendrimer Conjugates of Colchicine

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Abstract: Glycopeptide dendrimers have been prepared bearing four or eight identical glycoside moieties at their surface (β-glucose, α-galactose, α-*N*-acetyl-galactose, or lactose), natural amino acids within the branches (Ser, Thr, His, Asp, Glu, Leu, Val, Phe), 2,3-diaminopropionic acid as the branching unit, and a cysteine residue at the core. These dendrimers have been used as drug-delivery devices for colchicine. Colchicine was attached to the dendrimers at the cysteine thiol group through a disulfide or thioether linkage. The biological activities of the glycopeptide dendrimer conjugates were evaluated in HeLa tumor cells and non-transformed mouse embryonic fibroblasts (MEFs). The concentrations of glycopeptide dendrimer drug conjugates required to achieve inhibition of cell proliferation by interference with the tubulin system were found to be higher ($IC_{50} > 1$ μM) compared to the required colchicine concentration. On the other hand, the glycopeptide dendrimer conjugates inhibited the proliferation of HeLa cells 20–100 times more effectively than the proliferation of MEFs. In comparison, non-glycosylated dendrimers and colchicine itself showed a selectivity of 10-fold or less for HeLa cells.

Keywords: amino acids · antitumor agents · carbohydrates · dendrimers · drug delivery · glycopeptides

Introduction

Dendrimers are tree-like macromolecules composed of a core, a branched structure, and end groups that form the dendrimer surface.[1] One particularly attractive potential application of dendrimers is in drug delivery, whereby an inactive dendrimer–drug conjugate is delivered to a diseased cell and is degraded within the cell to release the active principle, for example by a cascade release mechanism.[2] Dendrimers are already in use as gene transfection agents.[3] In principle, a dendritic drug-delivery system should include a tissue- or cell-targeting molecule and an inactive prodrug that can be released by a selective triggering mechanism. Folic acid has been placed at the surface of dendrimers as a targeting device for the release of methotrexate to cancer cells.[4] Arrays of multiple carbohydrate moieties at a dendrimer surface, which may be assembled by a variety of dendrimer-assembly chemistries,[5] should provide optimal targeting devices. Various glycoprotein receptors occur at cell surfaces, where they mediate cell-cell recognition and the uptake of macromolecules.[6] For example, galactose and *N*-acetyl-galactosamine groups are known to bind to asialoglycoprotein receptors and have been used to target anticancer compounds to hepatic cancer cells.[7]

A key aspect of dendrimer-mediated drug delivery is that the dendrimer should undergo biodegradation within the target cell after delivery. Dendrimers consisting of peptide building blocks would be optimally suited for this purpose since they would be sensitive to proteolytic cleavage. We have recently prepared peptide dendrimers consisting of alternating sequences of proteinogenic α-amino acid and diamino acid building blocks.[8] These dendrimers can be prepared by Fmoc-based solid-phase peptide synthesis and provide functional artificial protein models exhibiting enzyme-like catalytic properties and binding affinities.[9] We have also discovered that these dendrimers are readily degraded by proteases, with cleavage occurring both at the surface and within the branches of the dendrimers.[10] This suggests that peptide dendrimers might be suitable for targeting drugs to cancer cells. One interesting drug is the well-known antimitotic agent colchicine, which is able to bind tubulin and thus prevents its assembly during mitosis.[11] This process precludes cell division and can induce apoptosis. The antiproliferative function of colchicine makes it an interesting agent for tumor therapy, but its use is restricted as it lacks specificity and inhibits proliferation of both tumor cells and

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Supporting information for this article is available on the WWW under http://www.chemeurj.org/ or from the author.

 DOI: 10.1002/chem.200401294

healthy cells. Therefore, it is of potential interest to produce colchicine derivatives that show a bias towards the inhibition of tumor cells.

Herein, we report the preparation of glycopeptide dendrimer–drug conjugates of colchicine equipped with surface glycosides as targeting devices. The dendrimers display multiple glycoside moieties at the surface and carry a covalently bound colchicine unit at the core. The dendrimers are shown to inhibit mitosis much more potently in cancer cells as compared to non-transformed fibroblasts (as a model for healthy cells), presumably by a mechanism involving cellular uptake followed by intracellular degradation and release of active colchicine, which can then bind to tubulin (Figure 1).

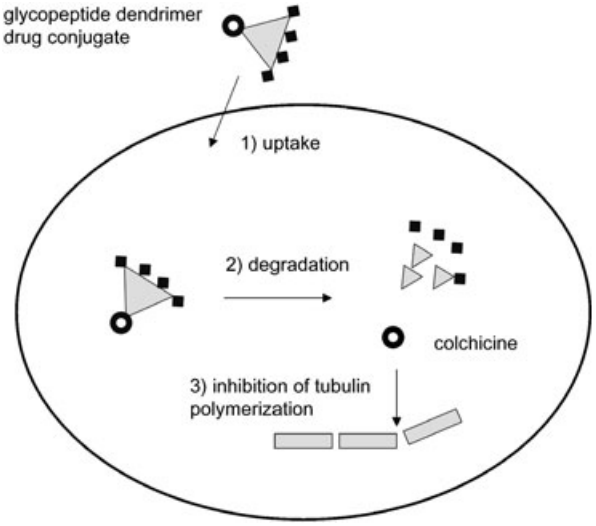


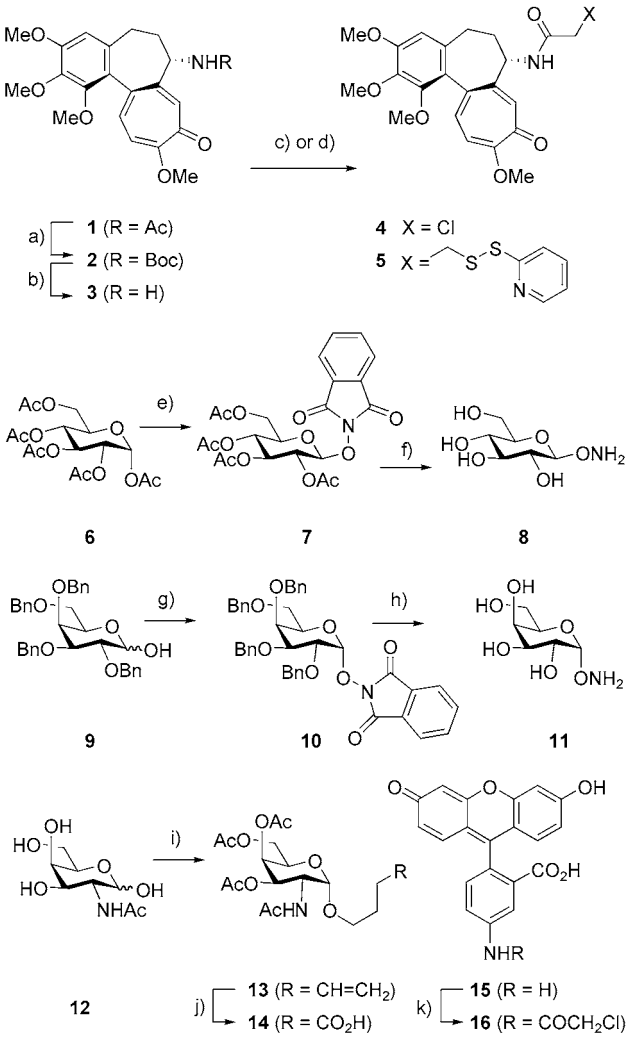
Figure 1. Hypothetical mechanism for mitosis inhibition by glycopeptide conjugates of colchicine. The glycopeptide dendrimer drug conjugate is taken into the cell (1), and is then degraded by intracellular proteases to release colchicine (2). Colchicine binds to tubulin and inhibits its polymerization (3), thereby blocking mitosis.

Although less potent than colchicine itself, these glycopeptide dendrimer–drug conjugates show an enhanced selectivity towards cancer cells versus immortalized but non-transformed cells.

Results and Discussion

Design: Colchicine (**1**) is a tricyclic plant alkaloid that has a cytostatic effect triggered by a binding interaction to tubulin, which prevents cell division.[12] Colchicine is abundant and inexpensive, yet is not useful therapeutically due to its toxicity, presumably as a result of a lack of selectivity. Its usefulness as a drug might therefore be improved if it could be deployed within a selective drug-delivery device. Colchicine can be readily modified at various positions.[13] In particular, it is possible to append various groups at the C(7) amide position and preserve bioactivity, as long as these groups are relatively small in size. We envisaged that conjugating colchicine to the core of a peptide dendrimer would form a biologically inactive derivative, but that degradation of the conjugate might release a smaller fragment carrying colchicine, which should be biologically active. The peptide dendrimer would be functionalized with glycosidic groups to facilitate cellular uptake and provide optimal water solubility (Figure 1).

Synthesis of colchicine and glycoside components: Colchicine **1** was first modified at C(7) to allow conjugation to peptide dendrimers (Scheme 1). Deacetylation at C(7) was performed by using a known procedure involving the inter-

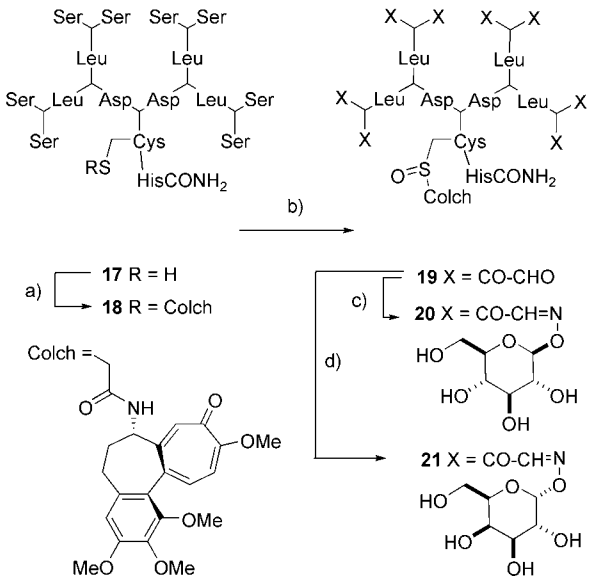


Scheme 1. Synthesis of colchicine and carbohydrate building blocks. Conditions : a) 1) Boc_2O, Et_3N, DMAP, CH_2Cl_2, 3 h, 25 °C (30 %), 2) NaOMe, MeOH, 0 °C, 30 min (80 %); b) CF_3CO_2H/CH_2Cl_2, 1:1, 25 °C, 3 h (100 %); c) $ClCH_2COCl$, Na_2CO_3, acetone/H_2O, 0 °C, 3 h (26 %); d) $PySSCH_2CH_2$-CONHS, DMAP, CH_2Cl_2, 2 h, 25 °C (54 %); e) 1) HBr/AcOH, CH_2Cl_2, 25 °C, 3 h, 2) Bu_4N^+ HSO_4^-, *N*-hydroxyphthalimide, CH_2Cl_2/1 M aq. Na_2CO_3, 25 °C, 15 h (22 %); f) $MeNHNH_2$, 25 °C, 15 h (76 %); g) *N*-hydroxyphthalimide, Et_3N, $Et_2O{\cdot}BF_3$, CH_2Cl_2, 25 °C, 3 h (64 %); h) 1) H_2, Pd/C, MeOH/CH_2Cl_2, 25 °C, 2 h (52 %); 2) $MeNHNH_2$, 25 °C, 15 h (80 %); i) 4-pentenol, $Et_2O{\cdot}BF_3$, 95 °C, 4 h, then Ac_2O/pyridine, 25 °C, 24 h (60 %); j) $NaIO_4$, cat. $RuCl_3$, $CCl_4/CH_3CN/H_2O$, 25 °C, 3 h (73 %); k) $ClCH_2COCl$, H_2O/acetone, Na_2CO_3, 0 °C, 1 h (67 %).

mediacy of the Boc derivative **2** to give deacetylcolchicine **3**.[14] The amino group was then acylated to give either the 7-chloroacetamido derivative **4**,[15] to be used for thioether bond formation with the core cysteine of the dendrimers, or the dithiopyridyl-propionyl derivative **5**,[16] to be used for disulfide coupling to cysteine. Penta-*O*-acetyl-α-D-glucopyranoside (**6**) was converted to the corresponding bromide by reaction with hydrogen bromide,[17] and then glycosylated with *N*-hydroxyphthalimide under phase-transfer conditions, as described for the reaction with *N*-hydroxysuccinimide,[18] to give intermediate **7**. Deacetylation and removal of the phthalimide protection was finally accomplished by treatment with methylhydrazine to give the sensitive β-D-glucopyranosyloxyamine (**8**). Oxyamine **8** was purified by precipitation from ethanol/dichloromethane, and would be used in oxime ligation for glycodendrimer synthesis. The related α-D-galactopyranosyloxyamine (**11**) was prepared by using a known procedure[19] involving α-selective glycosylation of tetra-*O*-benzyl-α-D-galactose (**9**)[20] with *N*-hydroxyphthalimide to give intermediate **10**, followed by a short hydrogenation for debenzylation, removal of the phthalimide protecting group by treatment with methylhydrazine, and purification by precipitation as above. Glycosylation of *N*-acetylgalactosamine **12** with 4-penten-1-ol using $Et_2O{\cdot}BF_3$ as catalyst followed by acetylation gave the corresponding α-D-galactopyranoside **13**.[21] Oxidation of the vinyl group with $NaIO_4$ and a catalytic amount of $RuCl_3$[22] gave the corresponding acid **14**, ready for amide bond formation with *N*-terminal amino acids on a solid support. Finally, chloroacetylation of amino-fluorescein **15** gave *N*-chloroacetamido-fluorescein **16** for glycopeptide dendrimer labeling studies.

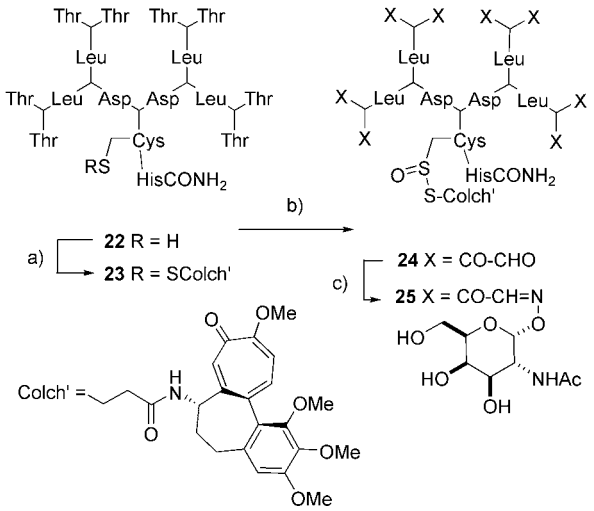
Synthesis of glycopeptide dendrimer–colchicine conjugates: The peptide dendrimers were prepared by solid-phase peptide synthesis (SPPS). All of the dendrimers possessed a cysteine residue at the core for the conjugation of colchicine. Various combinations of hydrophilic (serine, threonine, histidine, aspartate, glutamate) and hydrophobic (leucine, valine, phenylalanine) amino acid residues were used within the branches. Three different synthetic strategies were explored for the attachment of carbohydrates at the dendrimer surface.

The first approach relied on chemoselective ligation[23] of oxyamine glycosides to surface carbonyl groups generated by periodate cleavage of N-terminal serine or threonine residues (Scheme 2 and Scheme 3). This strategy has been used previously for the glycosylation of peptides[24] and peptide dendrimers.[25] The peptide dendrimer **17** was first prepared by SPPS, then cleaved and purified by preparative HPLC. Colchicine was then coupled to the cysteine thiol group as the chloroacetamide derivative **4** to give conjugate **18** (Scheme 2). Oxidation of the N-terminal serine residues gave the corresponding glyoxamides. MS analysis showed a molecular mass with +16 relative to the value expected for the peptide dendrimer. This was attributed to oxidation of the thioether linkage to a sulfoxide.[26] The glyoxamide dendrimer **19** thus obtained was combined with either β-D-glucopyranosyloxyamine (**8**) or α-D-galactopyranosyloxyamine



Scheme 2. Synthesis of oxime-linked glycopeptide colchicine conjugates **20** and **21**. Conditions: a) **4**, aq. phosphate buffer pH 8, 25 °C, 15 h (58%); b) $NaIO_4$, H_2O, 25 °C, 2 h (62%); c) **8**, H_2O/AcOH, 25 °C, 15 h (64%); d) **11**, H_2O/AcOH, 25 °C, 15 h (36%). Branching points in the dendrimers consist of Dap ((*S*)-2,3-diaminopropionic acid).

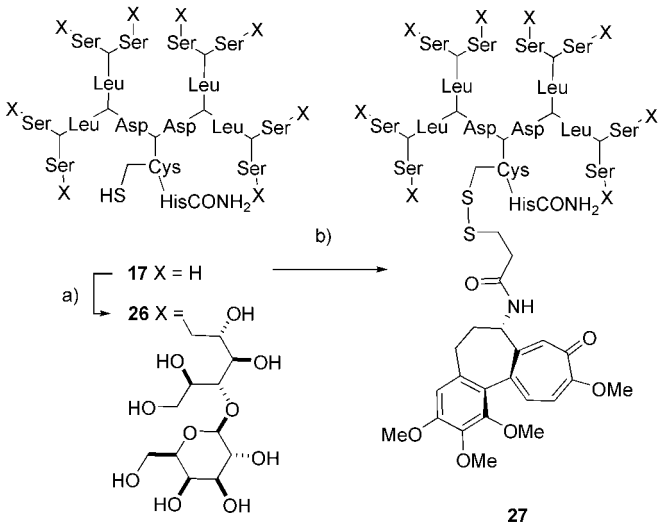
(**11**) under catalysis by acetic acid to give the oxime-linked glycopeptide dendrimer–colchicine conjugates **20** and **21**, respectively. In a similar sequence, the threonine-terminated dendrimer **22** was prepared and coupled to dithiopyridylpropionyl-colchicine **5** by disulfide exchange to yield the dendrimer–colchicine conjugate **23** (Scheme 3). Subsequent oxidation with sodium periodate produced the corresponding glyoxamide-terminated dendrimer **24**. As for dendrimer **18** above, MS analysis revealed a mass difference of +16,



Scheme 3. Synthesis of glycopeptide dendrimer colchicine conjugate **25**. Conditions: a) **5**, MeOH, CH_3CN, 25 °C, 2 h (60%); b) $NaIO_4$, H_2O, 25 °C, 2 h (56%); c) D-GalNAc-α-ONH_2, H_2O, AcOH, 25 °C, 15 h (23%). Branching points in the dendrimers consist of Dap ((*S*)-2,3-diaminopropionic acid).

which was attributed to a single oxidation of the disulfide bridge. Subsequent coupling with *N*-acetyl-α-D-galactopyranosyl-oxyamine[19] in the presence of acetic acid finally gave peptide dendrimer conjugate **25**. It should be noted that attempts to functionalize the dendrimers on a solid support using glycosyloxyamines and levulinate end groups were unsuccessful.

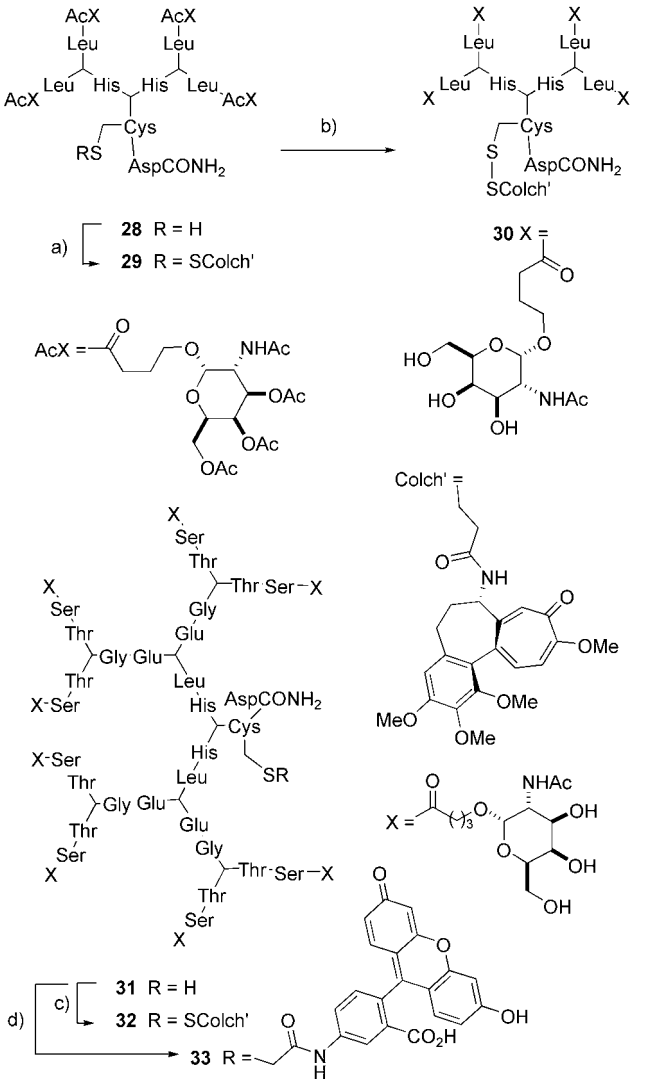
In the second approach to glycopeptide dendrimer synthesis, the carbohydrate was attached to the N-termini by reductive amination (Scheme 4).[27] This approach is known to



Scheme 4. Synthesis of lactose-capped peptide dendrimer colchicine conjugate **27**. Conditions: a) lactose, $NaBH_3CN$, AcOH, MeOH, 70 °C, 15 h (44 %); b) **5**, CH_3CN, MeOH, 25 °C, 2 h (58 %). Branching points in the dendrimers consist of Dap ((*S*)-2,3-diaminopropionic acid).

modify whole proteins,[28] and has recently been applied by Stoddart et al. for the preparation of glycodendrimers.[29] Treatment of dendrimer **17** with lactose in the presence of sodium cyanoborohydride in methanol resulted in clean conversion to glycodendrimer **26** bearing eight galactose end groups. The product was purified by semipreparative HPLC and coupled with the thiopyridyl-activated colchicine **5** to yield conjugate **27**.

In the third approach, the dendrimers were directly functionalized with a carbohydrate during SPPS by amide bond formation to the N-terminal residues (Scheme 5). *O*-Acetyl-protected 4-(α-*N*-acetyl-galactosyloxy)butyric acid **14** was used for amide bond formation to the N-terminal residues at the dendrimer surface under standard peptide-coupling conditions. Deprotection and cleavage from the resin gave the *O*-acetyl-protected glycodendrimer **28**, which was then coupled with the thiopyridyl derivative of colchicine **5** to give **29**. The *O*-acetyl protecting groups were then removed by treatment with methanolic ammonia to provide the glycopeptide dendrimer colchicine conjugate **30** bearing four *N*-acetyl-galactosamine moieties at the surface. In a similar approach, glycopeptide dendrimer **31** bearing eight *N*-acetyl-galactosamine moieties was obtained after N-terminal

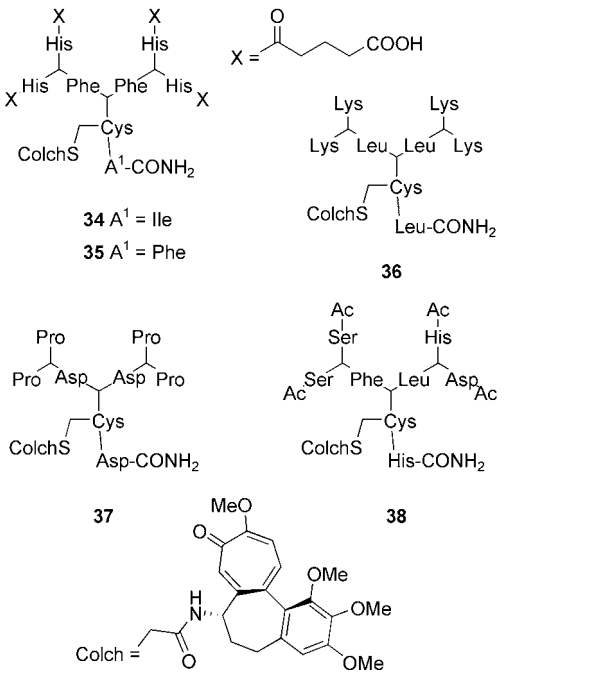


Scheme 5. Synthesis of $(GalNAc)_4$ and $(GalNAc)_8$ glycopeptide dendrimer colchicine conjugates **30** and **32**. Conditions: a) **5**, CH_3CN/MeOH, 25 °C, 2 h (30 %); b) NH_4OH, MeOH/H_2O, 25 °C, 12 h (79 %); c) **5**, CH_3CN/MeOH, 25 °C, 2 h (13 %); d) **16**, aq. phosphate buffer, pH 8, MeOH, 25 °C, 15 h (20 %). Branching points in the dendrimers consist of Dap ((*S*)-2,3-diaminopropionic acid).

coupling with **14**, followed by cleavage from the resin, deacetylation of the crude cleavage product with methanolic ammonia, and purification. Dendrimer **31** was then coupled with either thiopyridylcolchicine **5** or the chloroacetyl-fluorescein derivative **16** to produce the glycopeptide dendrimer conjugates **32** and **33**, respectively.

Finally, chloroacetylcolchicine **4** was conjugated to peptide dendrimers available from other projects,[9] bearing a variety of non-carbohydrate surface groups (carboxylate, ammonium, and acetyl groups), to provide dendrimer conjugates **34**–**38** (Scheme 6).

Biological evaluation: The biological activities of the various colchicine derivatives were compared by using immortalized mouse embryonic fibroblasts (MEFs) and HeLa cervix carcinoma cells, which are oncogenically transformed



Scheme 6. Peptide dendrimer conjugates of colchicine. Branching points are formed by 1,3-diamino-2-propyloxy-acetic acid in dendrimers **34**, **35**, **37**, **38** and by (*S*)-1,2-diaminopropionic acid in dendrimer **36**.

by the papillomavirus proteins E6 and E7. As colchicine binding to tubulin inhibits mitosis and thus cell division, the determination of cell numbers allows a direct comparison of the biological activities of colchicine and its derivatives. Equal numbers of cells were plated and either left untreated or incubated with various concentrations of some of the compounds, including the lactose-terminated dendrimer **27** and the GalNAc-terminated dendrimers **30** and **32**. After three days, the cell number was determined. The dendrimers were found to be generally much less active than colchicine itself or its chloroacetyl analogue **4** (Figure 2). Optimal inhibition of cell division was observed at a concentration of 5 μM.[30] A survey of the cytostatic activities of the colchicine glycopeptide dendrimer conjugates against MEFs and HeLa tumor cells was therefore carried out at this concentration (Table 1). While glycopeptide dendrimer **31** not bearing a colchicine substituent was not cytostatic, all glycopeptide dendrimer conjugates of colchicine showed cytostatic activity. The glycopeptide conjugates generally showed a higher selectivity against the HeLa tumor cells over the non-transformed MEF cells when compared to colchicine itself. The effect was most pronounced with dendrimer **20** bearing eight oxime-linked β-glucose units at its surface. In future studies, it will be interesting to determine whether dendrimer **20** also shows this bias for other tumor cells.

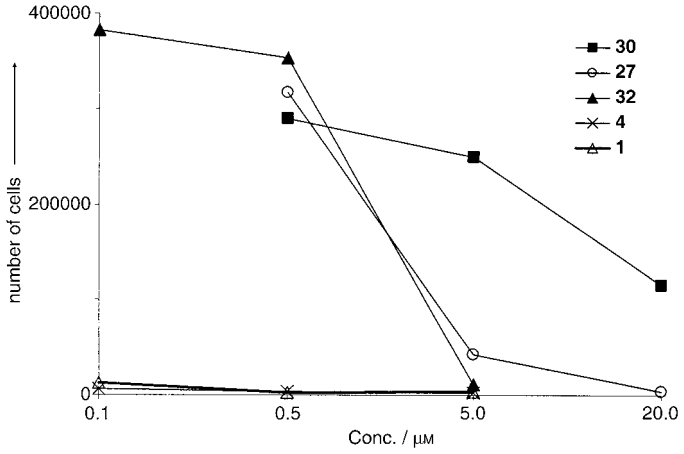


Figure 2. Concentration dependence of the cytostatic effect of colchicines **1** and **4** and of the glycopeptide dendrimer conjugates **27**, **30**, and **32**. The indicated concentrations of colchicine and its derivatives were added to a constant number of HeLa cells. After three days, the cell number was determined, which is given on the *y* axis.

Table 1. Inhibition of mitosis by colchicine glycopeptide and peptide dendrimer conjugates.

Compd	Dend. surf.[a]	Link[b]	Colch.[c]	HeLa [%][d]	MEF [%][e]	Selec.[f]
blank				100.0	100.0	1
1				0.2	2.4	12
4				0.2	0.6	3
20	8×Glc	oxime	S(O)	0.7	113.8	163
21	8×Gal	oxime	S(O)	2.1	92.2	44
25	8×GalNAc	oxime	SS(O)	0.9	93.4	104
27	8×Lac	amine	SS	0.4	34.1	85
30	4×GalNAc	amide	SS	0.5	47.9	96
32	8×GalNAc	amide	SS	0.3	6.6	22
31	8×GalNAc	amide	–	95.6	81.8	1
34	4×glutarate	–	S	3	2	1
35	4×glutarate	–	S	1	10	10
36	4×lysine	–	S	16	106	7
37	4×proline	–	S	48	86	2
38	4×acetyl	–	S	17	84	5

[a] Glycoside type at dendrimer surface. [b] Type of glycopeptide link. [c] Link between colchicine and cysteine thiol. [d] Percent HeLa tumor cells after 3 days in culture with 5 μM of indicated compounds. [e] Percent surviving MEF cells after three days in culture with 5 μM of indicated compounds. [f] Ratio of percent surviving MEFs to HeLa cells.

The non-glycosylated dendrimers **34**–**38** were generally found to be less active than the glycopeptide dendrimers. Only dendrimer conjugates **34** and **35** showed bioactivity comparable to that of the glycopeptide dendrimer conjugates, albeit without noticeable selectivity towards HeLa cells as observed for the glycopeptide dendrimers such as **20**. The activity of **34** and **35** in comparison to the less active dendrimers **36**–**38** might stem from the presence of the negatively charged carboxylate groups at the surface, although there are also other differences in amino acid composition. The bioactivity of **34** and **35** further indicates that the nature of the colchicine–dendrimer link (thioether, sulfoxide, disulfide, thiosulfoxide) is not the primary determinant of bioactivity of the conjugates.

The effect of the tumor cell selective conjugate **20** was investigated in more detail. To test whether it affects the spindle apparatus or acts by additional pathways, human lung cancer H1299 cells were transiently transfected to express green fluorescent protein (GFP)-tagged tubulin and re-

mained either untreated or were incubated with colchicine or conjugate **20**. Immune fluorescence microscopy revealed a normal cell morphology for control cells, while treated cells often displayed double or inadequately separated nuclei, which is indicative of a disturbed tubulin system (Figure 3). These results suggest that the colchicine den-

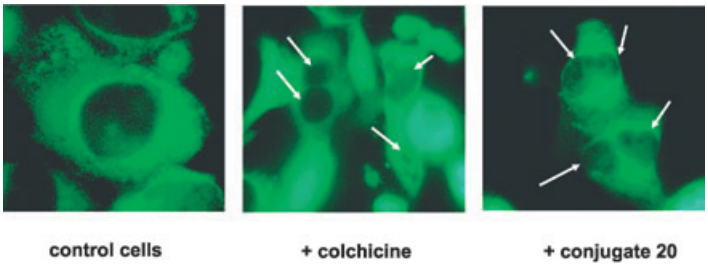


Figure 3. H1299 cells expressing GFP-tubulin were treated for 30 h as indicated and analyzed by immune fluorescence microscopy. The malformed double nuclei are shown by arrows. Typical examples are shown.

drimer conjugate **20** acts in the same manner as colchicine itself, that is, by disrupting tubulin function.

Glycopeptide dendrimer fluorescein conjugate **33** was incubated with cultures of HeLa or MEF cells under the conditions of the bioassay. HPLC analysis of the supernatant did not reveal any noticeable degradation of the conjugate, showing only unchanged dendrimer. When the cells were washed and examined under a fluorescence microscope, both the HeLa and MEF cells showed a homogeneous weak fluorescein fluorescence, indicating that the dendrimers were able to enter inside the cells during the course of the experiment.

A possible mechanism of action of our dendrimer–colchicine conjugates involves either passive or active uptake of the dendrimer-bound colchicine into the cells, followed by intracellular proteolytic degradation of the peptide structure to release an active colchicine compound, which then interferes with tubulin and blocks mitosis. The proposed prodrug-type mechanism of action involving dendrimer degradation would also explain the fact that structurally diverse glycopeptide dendrimer conjugates show similar activities.

Conclusion

A series of glycopeptide dendrimer colchicine conjugates has been prepared using different strategies for glycoside attachment. The glycoside attachment methods used have included oxime bond formation to N-terminal glyoxyamide generated by periodate cleavage of N-terminal serine or threonine residues, reductive alkylation of peptide amino termini with lactose/$NaBH_3CN$, and amide bond formation with an acetylated carboxypropyl glycoside directly on a solid support as the last step of the peptide dendrimer synthesis followed by cleavage and deacetylation. The latter strategy proved to be the most practical because only the colchicine conjugation step was necessary after the solid-supported peptide dendrimer synthesis. Non-glycosylated peptide dendrimer colchicine conjugates have also been prepared.

Cytostatic properties have been investigated against HeLa cells, as a model for cancer cells, and MEF cells, as a model for immortalized but non-transformed cells. Although the dendrimer conjugates were found to be less active than colchicine itself, the glycopeptide dendrimer colchicine conjugates showed enhanced selectivity towards HeLa cells over MEF cells as compared to colchicine. Non-glycosylated peptide dendrimer colchicine conjugates were generally found to be less active and less selective than the glycosylated conjugates. Dendrimers without colchicine were inactive. The bioactivity of glycopeptide dendrimer conjugate **20** has been traced to the inhibition of mitosis and nuclear separation during cell division, as observed for colchicine itself. There was no degradation of the dendrimers in the cell culture medium. The bioactivity of the glycopeptide dendrimer colchicine conjugates can best be explained in terms of internalization followed by degradation with the release of an active colchicine derivative.

These experiments show that glycopeptide dendrimers can be readily prepared and provide a suitable selective vehicle for the delivery of cytotoxic compounds to cancer cells. Dendrimer glycosylation induces a selective cytotoxicity towards Hela cells over MEF cells. This effect might become even more pronounced when applied to cell types expressing specific receptors for carbohydrates, such as hepatic cancer cells. Optimization of the dendritic glycopeptide array for optimal targeting to such cells and for the delivery of drugs other than colchicine is currently under investigation.

Experimental Section

General: Preparative HPLC purifications were performed on a Waters Prepak cartridge packed with 500 g of RP-C18, particle size 20 μm, pore size 300 Å, flow rate 100 mL min^{-1}, eluent A: 0.1% TFA in water; eluent B: acetonitrile/water (3:2) + 0.1% TFA; gradient +1% B min^{-1}, detection by UV at $\lambda = 214$ nm.

Semipreparative RP-HPLC purifications were performed on a Vydac 218TP510, C18, 1.0 cm × 25 cm, flow rate 4 mL min^{-1}, eluent A: 0.1% TFA in water; eluent B: acetonitrile/water (3:2) + 0.1% TFA, gradient +1% B min^{-1}, detection by UV at $\lambda = 220$ nm.

***N*-[(*tert*-Butoxy)carbonyl]colchicine**: Di(*tert*-butyl) pyrocarbonate (1.55 g, 7.10 mmol, 6.0 equiv) was added portionwise to a mixture of colchicine **1** (0.40 mg, 1.25 mmol), Et_3N (0.2 mL, 1.43 mmol, 1.1 equiv), and 4-(dimethylamino)pyridine (0.18 mg, 1.46 mmol, 1.2 equiv) in CH_2Cl_2 (3 mL), and the mixture was stirred for 3 h at 25 °C. The solvent was then removed in vacuo and the residue was purified by flash chromatography on silica gel (AcOEt/acetone, 4:1, $R_f = 0.28$) to give *N*-Boc-colchicine (0.187 mg, 0.38 mmol, 30%) as a yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 7.55$ (s, 1H), 7.18 (d, $J = 10.88$ Hz, 1H), 6.74 (d, $J = 10.88$ Hz, 1H), 6.51 (s, 1H), 5.13 (q, $J = 6.11$ Hz, 1H), 3.95 (s, 3H), 3.92 (s, 3H), 3.88 (s, 3H), 3.64 (s, 3H), 2.70–2.43 (m, 3H), 2.26 (s, 3H), 1.95 (m, 1H), 1.54 ppm (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 179.9$, 164.7, 154.2, 154.1, 151.8, 151.2, 142.3, 137.5, 136.3, 134.7, 133.3, 126.7, 113.7, 107.8, 85.5, 62.2, 62.1, 58.5, 57.0, 56.7, 33.3, 30.8, 30.3, 28.5,

26.2 ppm; EI-MS: *m/z*: 500 [M^+]; HRMS: calcd for $C_{27}H_{33}NO_8$ 500.2284; found 500.2295.

***N*-[(*tert*-Butoxy)carbonyl]deacetylcolchicine (2)**: A mixture of *N*-Boc-colchicine (89.3 mg, 0.18 mmol) and NaOMe (36.2 mg, 0.67 mmol, 3.7 equiv) in MeOH (2 mL) was stirred for 30 min at 0 °C. It was then neutralized by the addition of NH_4Cl and the solvent was evaporated. The residue was purified by flash chromatography on silica gel (AcOEt/acetone, 4:1, R_f = 0.39) to give **2** (65.8 mg, 0.144 mmol, 80 %) as a pale-yellow solid. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.48 (s, 1H), 7.26 (d, J = 10.74 Hz, 1H), 6.79 (d, J = 10.74 Hz, 1H), 6.51 (s, 1H), 4.98 (d, J = 7.72 Hz, 1H), 4.38 (m, 1H), 3.97 (s, 3H), 3.91 (s, 3H), 3.88 (s, 3H), 3.63 (s, 3H), 2.52–2.21 (m, 3H), 1.66 (m, 1H), 1.35 ppm (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 180.2, 164.6, 154.9, 154.1, 151.8, 142.2, 136.6, 135.6, 134.9, 131.8, 126.3, 112.7, 107.8, 80.5, 62.1, 61.2, 61.0, 56.9, 56.7, 53.7, 38.3, 30.6, 28.9 ppm; MS (ES+): *m/z*: 458.14 [M^+]; HRMS: calcd for $C_{25}H_{31}NO_7$ 458.2178; found 458.2175.

Deacetylcolchicine (3): A solution of compound **2** (158.1 mg, 0.346 mmol) in CH_2Cl_2 (4 mL) containing TFA (0.4 mL) was stirred for 3 h. Toluene was then added, the mixture was concentrated in vacuo, and the residue was purified by flash chromatography on silica gel (CH_2Cl_2/MeOH, 9:1, R_f = 0.34) to give compound **4** (123.1 mg, 0.345 mmol, quantitative) as a yellow solid. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.66 (s, 1H), 7.33 (d, J = 11.11 Hz, 1H), 6.88 (d, J = 11.11 Hz, 1H), 6.57 (s, 1H), 4.12 (m, 1H), 3.92 (s, 3H), 3.91 (s, 6H), 3.57 (s, 3H), 2.71–2.59 (m, 2H), 2.48–2.28 ppm (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 179.6, 164.7, 154.7, 151.6, 147.4, 142.2, 137.3, 137.2, 134.4, 132.0, 125.1, 114.4, 108.3, 61.9, 61.9, 57.2, 56.7, 54.5, 36.4, 30.2 ppm; EI-MS: *m/z*: 357 [M^+]; HRMS: calcd for $C_{20}H_{23}NO_5$ 357.157623; found 357.157530.

***N*-[2-(Chloromethyl)carbonyl]deacetylcolchicine (4)**: A solution of chloroacetyl chloride (30 μL, 0.376 mmol, 1.1 equiv) in acetone (0.1 mL) was added dropwise to a solution of **3** (135.0 mg, 0.378 mmol) and Na_2CO_3 (20 mg) in water/acetone (1 mL, 1:1) at 0 °C. The resulting mixture was stirred for 1 h and then the solvent was removed in vacuo. The residue was extracted with AcOEt, the solvent was removed from the extract, and the crude product was purified by flash chromatography on silica gel (CH_2Cl_2/MeOH, 19:1, R_f = 0.37) to give **4** (42.8 mg, 0.097 mmol, 26 %) as a yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.40 (s, 1H), 7.32 (d, J = 10.84 Hz, 1H), 7.12 (d, J = 7.16 Hz, NH), 6.83 (d, J = 10.84 Hz, 1H), 6.54 (s, 1H), 4.63 (m, 1H), 4.03 (m, 2H), 4.00 (s, 3H), 3.94 (s, 3H), 3.91 (s, 3H), 3.64 (s, 3H), 2.56 (m, 1H), 2.44 (m, 1H), 2.85 (m, 1H), 1.91 ppm (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 180.1, 166.4, 164.8, 154.3, 151.9, 150.8, 142.5, 136.8, 136.1, 134.6, 131.3, 126.2, 113.2, 108.1, 62.1, 62.1, 57.1, 56.8, 53.3, 43.1, 37.5, 30.4 ppm; EI-MS: *m/z*: 434 [M^+]; HRMS: calcd for $C_{22}H_{24}ClNO_6$ 434.1370; found 434.1382.

***N*-[3-(2-Pyridyldithio)propionyl]deacetylcolchicine (5)**: A solution of **3** (46.7 mg, 0.131 mmol) in CH_2Cl_2 (0.5 mL) was treated with *N*-succinimidyl 3-(2-pyridyldithio)propionate (80 %, 51.1 mg, 0.131 mmol, 1.0 equiv) and DMAP (16.6 mg, 0.131 mmol, 1.0 equiv). The resulting mixture was stirred for 2 h at 25 °C. Thereafter, the solvent was removed in vacuo and the residue was purified by flash chromatography on silica gel ($CHCl_3$/MeOH, 94:6, R_f = 0.54) and subsequently precipitated from CH_2Cl_2/petroleum ether to give the thiopyridyl-activated colchicine **5** (39.5 g, 0.071 mmol, 54 %) as an orange solid. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.48 (m, J = 4.89 Hz, 1H), 7.65–7.56 (m, 3H), 7.47 (s, 1H), 7.28 (d, J = 10.82 Hz, 1H), 7.14–7.10 (m, NH), 6.81 (d, J = 10.82 Hz, 1H), 6.53 (s, 1H), 4.67 (m, 1H), 3.98 (s, 3H), 3.94 (s, 3H), 3.90 (s, 3H), 3.65 (s, 3H), 3.01 (m, 2H), 2.69 (m, 2H), 2.68–2.25 (m, 3H), 1.93–1.83 ppm (m, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 180.1, 171.1, 164.7, 160.1, 154.1, 151.9, 151.6, 150.2, 142.4, 137.8, 136.8, 135.8, 134.8, 131.6, 126.4, 121.9, 121.1, 112.9, 108.0, 62.2, 62.1, 57.0, 56.8, 53.0, 37.6, 36.4, 35.8, 30.6 ppm; LSI-MS: *m/z*: 555 [M^+]; HRMS: calcd for $C_{28}H_{30}N_2O_6S_2$ 555.1623; found 555.1632.

***O*-(2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl)-*N*-oxyphthalimide (7)**: A 30 % solution of HBr in AcOH (8.2 mL, 0.142 mol, 11 equiv) was added dropwise to a solution of α-D-glucose pentaacetate **6** (5.06 g, 0.013 mol) in CH_2Cl_2 (30 mL) at 0 °C. The resulting mixture was stirred at 0 °C for 30 min and then at room temperature for 3 h. After the addition of further CH_2Cl_2, the solution was washed with cold water and with a cold solution of $NaHCO_3$. The organic phase was then dried with Na_2SO_4 and concentrated in vacuo to give tetra-*O*-acetyl-α-D-glucopyranosyl bromide. This product was dissolved in CH_2Cl_2 (100 mL) and treated with tetrabutylammonium hydrogen sulfate (4.41 g, 0.013 mol, 1.0 equiv) and *N*-hydroxyphthalimide (10.6 g, 0.065 mol, 5 equiv) dissolved in 1 M aqueous Na_2CO_3 solution (100 mL) and the resulting mixture was vigorously stirred overnight at 25 °C. Thereafter, CH_2Cl_2 was added and the mixture was washed with water, aqueous $NaHCO_3$ solution, and saturated aqueous NaCl solution. The organic phase was dried with Na_2SO_4 and concentrated. The residue was purified by flash chromatography on silica gel (CH_2Cl_2/AcOEt, 9:1, R_f = 0.40) to furnish **7** (1.44 g, 2.9 mmol, 22 %) as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.88–7.77 (m, 4H), 5.32–5.24 (m, 3H), 5.11 (dd, J = 6.30 and 1.59 Hz, 1H), 4.34 (dd, J = 12.42 and 4.89 Hz, 1H), 4.14 (dd, J = 12.42 and 2.64 Hz, 1H), 3.80–3.74 (m, 1H), 2.20 (s, 3H), 2.06 (s, 3H), 2.04 (s, 3H), 2.03 ppm (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 171.3, 170.8, 170.2, 170.0, 163.3, 135.5, 129.5, 124.6, 105.8, 73.1, 73.1, 70.3, 68.8, 62.5, 21.4, 21.3, 21.3 ppm.

***O*-β-D-Glucopyranosyl-oxyamine (8)**: Compound **7** (170 mg, 0.34 mmol) was dissolved in methylhydrazine (2.0 mL, 37.59 mmol) and the solution was stirred overnight at 25 °C. The solvent was then evaporated in vacuo and the residue was purified by precipitation from the minimum volume of MeOH and CH_2Cl_2. After filtration, compound **8** (51 mg, 0.26 mmol, 76 %) was obtained as a white solid. TLC: CH_2Cl_2/EtOH, 1:1, R_f = 0.38; ^{1}H NMR (300 MHz, D_2O): δ = 4.49 (d, J = 8.46 Hz, 1H), 3.86 (dd, J = 12.51 and 2.19 Hz, 1H), 3.65 (dd, J = 12.51 and 5.90 Hz, 1H), 3.46–3.19 ppm (m, 4H); ^{13}C NMR (75 MHz, D_2O): δ = 107.5, 78.3, 78.3, 74.1, 72.0, 63.2 ppm; HRMS: calcd for $C_6H_{14}NO_6^+$ 196.0821; found 196.0824.

***O*-(2,3,4,6-Tetra-*O*-benzyl-α-D-galactopyranosyl)-*N*-oxyphthalimide (10)**: $Et_2O{\cdot}BF_3$ (9.0 mL, 71.66 mmol, 4 equiv) was added to a solution of tetra-*O*-benzyl-D-galactose **9** (9.74 g, 18.00 mmol), *N*-hydroxyphthalimide (2.92 g, 18.00 mmol, 1.0 equiv), and Et_3N (2.5 mL, 17.93 mmol, 1 equiv) in CH_2Cl_2 (70 mL) at 0 °C. The mixture was stirred for 3 h at 25 °C and then washed with water and a saturated solution of $NaHCO_3$. The organic phase was concentrated and the residue was purified by flash chromatography on silica gel (hexane/AcOEt, 7:3, R_f = 0.46) to yield **10** (7.89 g, 11.50 mmol, 64 %) as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.86–7.73 (m, 4H), 7.61–7.29 (m, 20H), 5.71 (d, J = 3.78 Hz, 1H), 5.13 (d, J = 11.49 Hz, 1H), 5.04–4.79 (m, 5H), 4.66–4.51 (m, 3H), 4.33 (dd, J = 10.56 and 3.87 Hz, 1H), 4.22–4.17 (m, 2H), 3.70–3.57 ppm (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 164.0, 139.4, 139.2, 138.9, 138.6, 135.1, 129.6, 129.0, 128.9, 128.9, 128.9, 128.8, 128.4, 128.3, 128.2, 128.2, 128.1, 124.2, 103.3, 78.8, 76.0, 75.6, 75.6, 74.1, 73.8, 73.5, 71.6, 68.8 ppm.

***O*-α-D-Galactopyranosyl-oxyamine (11)**: A mixture of compound **10** (1.70 g, 2.48 mmol) and 10 % Pd/C (214 mg, 1.98 mmol, 0.8 equiv) in CH_2Cl_2/MeOH (1:1, 5 mL) was stirred under a hydrogen atmosphere for 2 h at 25 °C. The catalyst was then removed by filtration through Celite, and the filtrate was concentrated. The solid residue was purified by flash chromatography on silica gel (CH_2Cl_2/MeOH, 8.5:1.5; TLC: CH_2Cl_2/MeOH, 9:1, R_f = 0.23) to yield *O*-α-D-galactopyranosyl-*N*-oxyphthalimide (0.42 g, 1.28 mmol, 52 %) as a white solid. This product (0.809 g, 2.49 mmol) was dissolved in methylhydrazine (22.2 mL, 0.42 mol) and the solution obtained was stirred overnight at 25 °C. It was then concentrated in vacuo and the residue was purified by precipitation from the minimum volume of MeOH and CH_2Cl_2. After filtration, compound **11** (0.387 mg, 1.98 mmol, 80 %) was obtained as a white solid; TLC: CH_2Cl_2/EtOH, 1:1, R_f = 0.38; ^{1}H NMR (300 MHz, D_2O): δ = 4.96 (d, J = 3.93 Hz, 1H), 3.92–3.88 (m, 2H), 3.82–3.68 ppm (m, 4H); ^{13}C NMR (75 MHz, D_2O): δ = 104.3, 73.4, 71.8, 71.7, 70.3, 63.6 ppm; LSI-MS (DTT/DTE): *m/z*: 196 [*M*]; HRMS: calcd for $C_6H_{13}NO_6^+$ 196.0821; found 196.0815.

4-Pentenyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-galactopyranoside (13): Dry 4-pentenol (8 mL, 78 mmol, 34 equiv) and freshly distilled $Et_2O{\cdot}BF_3$ (0.05 mL, 0.42 mmol, 0.19 equiv) were added to *N*-acetyl-galactosamine **12** (501 mg, 2.26 mmol) and the mixture was stirred for 4 h at 95 °C. The excess 4-pentenol was then removed in vacuo and the residue was dissolved in Ac_2O (5 mL) and pyridine (10 mL). After allowing the reaction to proceed for 24 h, toluene was added and the solution was concentrated. The residue was purified by flash chromatography on silica gel (AcOEt/hexane, 4:1, R_f = 0.33) to yield **13** (552 mg, 1.33 mmol,

60%) as an orange oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 5.85–5.75 (m, 1H), 5.58 (d, J = 9.68 Hz, 1H), 5.36 (d, J = 2.44 Hz, 1H), 5.15 (dd, J = 11.4 and 3.30 Hz, 1H), 5.05–4.98 (m, 2H), 4.85 (d, J = 3.68 Hz, 1H), 4.59–4.30 (m, 1H), 4.16–4.03 (m, 3H), 3.72–3.66 (m, 1H), 3.47–3.41 (m, 1H), 2.15 (s, 3H), 2.14–2.04 (m, 2H), 2.03 (s, 3H), 1.99 (s, 3H), 1.95 (s, 3H), 1.74–1.67 ppm (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 171.6, 171.0, 171.0, 170.5, 138.3, 115.9, 98.3, 69.1, 68.4, 68.0, 67.3, 62.6, 48.4, 30.9, 29.1, 23.9, 21.4, 21.4, 21.3 ppm; EI-MS: m/z: 416 [M^+]; HRMS: calcd for $C_6H_{13}NO_6$ 416.1920; found 416.1906.

3-Carboxypropyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-galactopyranoside (14): $NaIO_4$ (1.66 g, 7.74 mmol, 4.1 equiv) and $RuCl_3$ (20 mg, 0.076 mmol, 0.04 equiv) were added to a solution of **13** (783 mg, 1.89 mmol) in CCl_4 (3.5 mL)/CH_3CN (3.5 mL)/H_2O (5 mL) and the mixture was stirred for 3 h at room temperature. It was then extracted with CH_2Cl_2 and the extracts were washed with H_2O. The combined organic layers were concentrated and the residue was purified by flash chromatography on silica gel (Et_2O/CH_2Cl_2/MeOH, 7:2.5:0.5 + 1% AcOH, R_f = 0.48) to yield **14** (599 mg, 1.38 mmol, 73%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 6.14 (d, J = 9.68 Hz, 1H), 5.33 (d, J = 2.48 Hz, 1H), 5.10 (dd, J = 11.32 and 3.24 Hz, 1H), 4.83 (d, J = 3.52 Hz, 1H), 4.57–4.50 (m, 1H), 4.14–4.05 (m, 3H), 3.80–3.75 (m, 1H), 3.47–3.41 (m, 1H), 2.50–2.32 (m, 2H), 2.13 (s, 3H), 2.11–2.05 (m, 2H), 2.02 (s, 3H), 1.96 (s, 3H), 1.96 ppm (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 177.7, 171.6, 171.6, 171.1, 170.9, 98.4, 69.0, 68.1, 67.9, 67.3, 62.5, 48.3, 31.9, 25.4, 23.4, 21.3, 21.2, 21.2 ppm; EI-MS: m/z: 434 [M^+]; HRMS: calcd for $C_6H_{13}NO_6Na$ 456.1481; found 456.1495.

Chloroacetamido-fluorescein (16): A solution of chloroacetyl chloride (23 μL, 0.29 mmol, 1.1 equiv) in acetone (0.1 mL) was added dropwise to a solution of fluoresceinamine **15** (90.1 mg, 0.26 mmol) and Na_2CO_3 (14.4 mg) in water/acetone (1 mL, 1:1) at 0°C. The resulting mixture was stirred for 1 h and then the solvent was removed in vacuo. After extraction with AcOEt and removal of the solvent, the crude product was purified by flash chromatography on silica gel (acetone/AcOEt/hexane, 3:2:5) to give **16** (74 mg, 0.17 mmol, 67%) as a yellow solid. ^{1}H NMR (400 MHz, [D$_6$]acetone): δ = 8.42 (dd, J = 2.02 and 0.48 Hz, 1H), 7.95 (dd, J = 8.31 and 2.02 Hz, 1H), 7.24 (dd, J = 8.31 and 0.48 Hz, 1H), 6.74 (d, J = 2.32 Hz, 2H), 6.72 (s, 1H), 6.70 (s, 1H), 6.64 (d, J = 2.44 Hz, 1H), 6.62 (d, J = 2.44 Hz, 1H), 4.32 ppm (s, 2H); ^{13}C NMR (75 MHz, [D$_6$]acetone): δ = 206.9, 206.9, 206.9, 166.7, 160.9, 154.0, 153.9, 146.3, 141.7, 130.9, 130.8, 128.1, 127.0, 126.0, 122.4, 115.9, 115.0, 114.0, 113.9, 112.3, 104.0, 44.7 ppm; EI-MS: m/z: 423 [M^+]; HRMS: calcd for $C_{22}H_{14}ClNO_6$ 424.0587; found 424.0572.

[{(NH$_2$-Ser)$_2$DapLeu}$_2$DapAsp]$_2$DapCysHisNH$_2$ (17): The dendrimer synthesis was carried out by means of standard Fmoc SPPS. In each step, the resin was acylated with 3 equivalents of the suitably protected Fmoc-amino acid or Fmoc-protected (*S*)-2,3-diaminopropanoic acid in the presence of 3 equiv of BOP (benzotriazol-1-yl-oxy-tris(dimethylamino)phosphonium hexafluorophosphate) and 5 equiv of DIEA (*N*,*N*-diisopropylethylamine). After reaction for 30 min, the resin was successively washed with DMF, MeOH, and CH_2Cl_2 (2× with each solvent) and then checked for free amino groups by carrying out a TNBS (2,4,6-trinitrobenzenesulfonic acid) test. The Fmoc protecting group was removed by treating the resin with a 20% solution of piperidine in DMF for two periods of 10 min, and then the resin was separated from the solution by filtration. The resin was again washed with DMF, MeOH, and CH_2Cl_2 (2× with each). At the end of the synthesis, the resin was dried and the product was cleaved using trifluoroacetic acid/1,2-ethanedithiol/water/triisopropylsilane (94:2.5:2.5:1) for 4 h. The peptide was precipitated with methyl *tert*-butyl ether and then dissolved in a water/acetonitrile mixture. Starting with 50 mg of NovaSyn TGR resin (0.25 mmol g^{-1}), dendrimer **17** was obtained as a colorless foamy solid after purification by preparative HPLC (16.2 mg, 13%). MS (ES+): calcd for $C_{86}H_{151}N_{33}O_{35}S$: 2238.08, found: 2238.50.

[{(NH$_2$-Ser)$_2$DapLeu}$_2$DapAsp]$_2$DapCys(S-Colch)HisNH$_2$ (18): Dendrimer **17** (7.8 mg, 3.32 μmol) was dissolved in an oxygen-free phosphate buffer solution (0.3 mL, pH 8, 0.1 mM) and a solution of chloroacetylcolchicine **4** (1.4 mg, 3.23 μmol, 1 equiv) in degassed MeOH (50 μL) was added. The mixture was stirred overnight under nitrogen. Subsequent purification by semipreparative RP-HPLC gave **18** (5.1 mg, 1.93 μmol, 58%). MS (ES+): calcd for $C_{108}H_{174}N_{34}O_{41}S$: 2635.23, found: 2636.39.

[{(CHO-CO)$_2$DapLeu}$_2$DapAsp]$_2$DapCys(S(O)-Colch)HisNH$_2$ (19): Dendrimer **18** (1.7 mg, 0.45 μmol) was dissolved in H_2O (100 μL) and $NaIO_4$ (4.9 mg, 22.9 μmol, 51 equiv) was added. The solution was stirred for 2 h under nitrogen and then worked-up by semipreparative RP-HPLC. The fraction corresponding to the main peak (t_R = 14.4 min; A/B = 70:30 to 40:60 in 30 min) was collected and lyophilized to give **19** (0.7 mg, 0.28 μmol, 62%). MS (ES+): calcd for $C_{100}H_{133}N_{25}O_{43}S$: 2403.87, found: 2403.36.

[{(D-Glc-β-ONCH-CO)$_2$DapLeu}$_2$DapAsp]$_2$DapCys(S(O)-Colch)HisNH$_2$ (20): Dendrimer **19** (1.0 mg, 0.40 μmol) was dissolved in H_2O (0.1 mL) containing 2 drops of AcOH, and oxyamine **8** (7.7 mg, 39.5 μmol, 12 equiv/aldehyde function) was added. The solution was kept overnight under nitrogen at 25°C and then worked-up by semipreparative HPLC. Collection of the fraction corresponding to the main peak (t_R = 9.6 min; A/B = 70:30 to 40:60 in 30 min) and lyophilization gave **20** (1.0 mg, 0.25 μmol, 64%). MS (ES+): calcd for $C_{148}H_{222}N_{34}O_{82}S$: 3819.4, found: 3820.25.

[{(D-Gal-α-ONCH-CO)$_2$DapLeu}$_2$DapAsp]$_2$DapCys(S(O)-Colch)HisNH$_2$ (21): Treating dendrimer **19** (0.7 mg, 0.28 μmol) as above, but using oxyamine **11** (3.9 mg, 20.00 μmol, 9 equiv/aldehyde function), followed by purification by semipreparative HPLC and isolation of the fraction corresponding to the main peak (t_R = 18.4 min; A/B = 80:20 to 50:50 in 30 min) gave dendrimer **21** (0.4 mg, 0.10 μmol, 36%). MS (ES+): calcd for $C_{148}H_{222}N_{34}O_{82}S$: 3819.4, found: 3820.88.

[{(NH$_2$-Thr)$_2$DapLeu}$_2$DapAsp]$_2$DapCysHisNH$_2$ (22): Starting with 50 mg of NovaSyn TGR resin (0.25 mmol g^{-1}), the dendrimer was prepared by standard Fmoc-SPPS (see the preparation of **17**). Cleavage, precipitation, and purification by preparative HPLC gave, after lyophilization of the fraction corresponding to the main peak (t_R = 20.0 min; A/B = 90:10 to 60:40 in 30 min), dendrimer **22** (20.3 mg, 16%) as a colorless foamy solid. MS (ES+): calcd for $C_{94}H_{167}N_{33}O_{35}S$: 2350.20, found: 2351.25.

[{(NH$_2$-Thr)$_2$DapLeu}$_2$DapAsp]$_2$DapCys(SS(CH$_2$)$_2$Colch)HisNH$_2$ (23): Dendrimer **22** (4.7 mg, 1.39 μmol) was dissolved in degassed MeOH/CH_3CN (1:1, 0.2 mL) and dithiopyridylpropionylcolchicine **5** (8.0 mg, 14.4 μmol, 10 equiv) was added. The solution was kept at 25°C for 2 h under nitrogen. Purification by semipreparative RP-HPLC, isolation of the fraction corresponding to the main peak (t_R = 10.8 min; A/B = 80:20 to 50:50 in 30 min), and lyophilization gave dendrimer **23** (3.2 mg, 0.84 μmol, 60%). MS (ES+): calcd for $C_{117}H_{192}N_{34}O_{41}S_2$: 2793.34, found: 2793.75.

[{(CHO-CO)$_2$DapLeu}$_2$DapAsp]$_2$DapCys(S(O)S(CH$_2$)$_2$Colch)HisNH$_2$ (24): Dendrimer **23** (3.2 mg, 0.84 μmol) was dissolved in H_2O (0.2 mL) and $NaIO_4$ (9.2 mg, 43 μmol, 51 equiv) was added. The solution was kept at 25°C for 2 h under nitrogen. Purification by semipreparative RP-HPLC and isolation of the fraction corresponding to the main peak (t_R = 23.6 min; A/B = 80:20 to 50:50 in 30 min) gave dendrimer **24** (1.2 mg, 0.47 μmol, 56%), which was used directly for the next step.

[{(D-GalNAc-α-ONCH-CO)$_2$DapLeu}$_2$DapAsp]$_2$DapCys(S(O)S-(CH$_2$)$_2$Colch)HisNH$_2$ (25): Dendrimer **24** (1.2 mg, 0.40 μmol) was dissolved in H_2O (0.15 mL) containing 2 drops of AcOH, and α-D-*N*-acetylgalactosyloxyamine (3.5 mg, 14.8 μmol, 4.6 equiv/aldehyde function) was added. The solution was kept at 25°C overnight under nitrogen. Purification by semipreparative RP-HPLC, isolation of the fraction corresponding to the main peak (t_R = 20.8 min; gradient: A/B = 80:20 to 50:50 in 30 min), and lyophilization gave dendrimer **25** (0.4 mg, 0.09 μmol, 23%). MS (ES+): calcd for $C_{165}H_{248}N_{42}O_{82}S_2$: 4193.60, found: 4196.00.

[{(Lactyl-NH-Ser)$_2$DapLeu}$_2$DapAsp]$_2$DapCysHisNH$_2$ (26): Dendrimer **17** (4.5 mg, 1.38 μmol) was dissolved in MeOH (1 mL) containing 3 drops of AcOH. Lactose (12.2 mg, 33.9 μmol, 25 equiv) and $NaBH_3CN$ (1.9 mg, 30.2 μmol, 22 equiv) were added and the mixture was stirred overnight at 70°C. The methanol was then evaporated and the residue was purified by semipreparative RP-HPLC. Collection of the fraction corresponding to the main peak (t_R = 26.0 min; gradient: A/B = 100:0 to 70:30 in 30 min)

and lyophilization gave **26** (3.0 mg, 0.60 μmol, 44%) as a white solid. MS (ES+): calcd for $C_{182}H_{327}N_{33}O_{115}S$: 4847.05, found: 4848.13.

[{(Lactyl-NH-Ser)$_2$DapLeu}$_2$DapAsp]$_2$DapCys(SS(CH$_2$)$_2$Colch)HisNH$_2$ (27): Dendrimer **26** (2.4 mg, 0.48 μmol) was dissolved in degassed MeOH/CH_3CN (1:1, 0.2 mL) and dithiopyridylpropionylcolchicine **5** (2.6 mg, 4.69 μmol, 10 equiv) was added. The solution was kept at 25°C for 2 h under nitrogen. Purification by semipreparative RP-HPLC, collection of the fraction corresponding to the main peak (t_R = 16.0 min; gradient: A/B = 80:20 to 50:50 in 30 min), and lyophilization gave dendrimer **27** (1.5 mg, 0.28 μmol, 58%). MS (ES+): calcd for $C_{205}H_{352}N_{34}O_{121}S_2$: 5290.19, found: 5291.63.

[(D-Ac$_3$GalNAc-α-O(CH$_2$)$_3$CO-Leu)$_2$DapHis]$_2$DapCysAspNH$_2$ (28): Starting with 100 mg of NovaSyn TGR resin (0.25 mmol g^{-1}), the dendrimer was prepared by standard Fmoc-SPPS (see the preparation of **17**). For the final coupling step, the resin was treated with 3 equiv of **14** for 5 h. Cleavage from the resin, precipitation, and purification by preparative HPLC gave, after lyophilization of the fraction corresponding to the main peak (t_R = 6.8 min; gradient: A/B = 40:60 to 10:90 in 30 min), glycodendrimer **28** (13.4 mg, 19%) as a colorless foamy solid. MS (ES+): calcd for $C_{124}H_{189}N_{23}O_{53}S$: 2880.25, found: 2881.25.

[(D-Ac$_3$GalNAc-α-O(CH$_2$)$_3$CO-Leu)$_2$-DapHis]$_2$DapCys(SS(CH$_2$)$_2$Colch)-AspNH$_2$ (29): Dendrimer **28** (5.0 mg, 1.67 μmol) was dissolved in degassed MeOH/CH_3CN (1:1; 0.2 mL) and dithiopyridylpropionylcolchicine **5** (9.2 mg, 16.6 μmol, 10 equiv) was added. The solution was kept at 25°C for 2 h under nitrogen and purified by semipreparative RP-HPLC. Collection of the fraction corresponding to the main peak (t_R = 22.8 min; gradient: A/B = 50:50 to 20:80 in 30 min) and lyophilization gave dendrimer **29** (1.7 mg, 0.49 μmol, 30%). MS (ES+): calcd for $C_{147}H_{214}N_{24}O_{59}S_2$: 3323.39, found: 3324.25.

[(D-GalNAc-α-O(CH$_2$)$_3$CO-Leu)$_2$-DapHis]$_2$DapCys(SS(CH$_2$)$_2$Colch)AspNH$_2$ (30): A mixture of MeOH/H_2O/NH_4OH (100 μL, 8:1:1) was added to dendrimer **29** (0.9 mg, 0.25 μmol). The solution was stirred overnight at 25°C. Subsequent evaporation of the solvent and semipreparative HPLC purification gave, after lyophilization of the fraction corresponding to the main peak (t_R = 8.2 min; gradient: A/B = 60:40 to 30:70 in 30 min), dendrimer **30** (0.6 mg, 0.20 μmol, 79%) as a yellow solid. MS (ES+): calcd for $C_{123}H_{190}N_{24}O_{47}S_2$: 2819.27, found: 2819.63.

[{(D-GalNAc-α-O(CH$_2$)$_3$COSerThr)$_2$DapGlyGlu}$_2$DapLeuHis]$_2$Dap-CysAspNH$_2$ (31): Starting with 150 mg of NovaSyn TGR resin (0.25 mmol g^{-1}), the dendrimer was prepared by standard Fmoc-SPPS (see the preparation of **17**). For the final coupling step, the resin was treated with 3 equiv of **14** for 5 h. The resin was dried and the cleavage was carried out using CF_3CO_2H/1,2-ethanedithiol/H_2O/triisopropylsilane (94:2.5:2.5:1) for 4 h. The peptide was precipitated with methyl *tert*-butyl ether and then dissolved in a water/acetonitrile mixture. The dendrimer was directly deacetylated with MeOH/H_2O/NH_4OH (2 mL, 8:1:1). The solution was stirred overnight at 25°C and then the methanol was evaporated in vacuo. Purification by preparative HPLC gave, after lyophilization of the fraction corresponding to the main peak (t_R = 23.4 min; gradient: A/B = 100:0 to 70:30 in 30 min), glycodendrimer **31** (14.6 mg, 7%) as a colorless foamy solid. MS (ES+): calcd for $C_{232}H_{379}N_{57}O_{119}S$: 5899.51, found: 5901.63.

[{(D-GalNAc-α-O(CH$_2$)$_3$COSerThr)$_2$DapGlyGlu}$_2$DapLeuHis]$_2$DapCys-(SS(CH$_2$)$_2$Colch)-AspNH$_2$ (32): Dendrimer **31** (4.2 mg, 0.69 μmol) was dissolved in degassed MeOH/CH_3CN (1:1, 0.2 mL) and dithiopyridylpropionylcolchicine **5** (3.6 mg, 6.5 μmol, 10 equiv) was added. The solution was stirred at 25°C for 2 h under nitrogen and purified by semipreparative RP-HPLC. Collection of the fraction corresponding to the main peak (t_R = 24.0 min; gradient: A/B = 100:0 to 30:70 in 30 min) and lyophilization gave dendrimer **32** (0.6 mg, 0.09 μmol, 13%). MS (ES+): calcd for $C_{255}H_{404}N_{58}O_{125}S_2$: 6342.65, found: 6345.00.

[{(D-GalNAc-α-O(CH$_2$)$_3$COSerThr)$_2$DapGlyGlu}$_2$DapLeuHis]$_2$Dap-Cys(SCH$_2$CONH-Fluorescein)AspNH$_2$ (33): Dendrimer **31** (3.4 mg, 0.57 μmol) was dissolved in an oxygen-free phosphate buffer solution (0.1 mL, pH 8, 0.1 mM) and a solution of **16** (0.7 mg, 1.65 μmol, 2.9 equiv) in degassed MeOH (40 μL) was added. The mixture was stirred overnight under nitrogen. Subsequent purification by semipreparative RP-HPLC, collection of the fraction corresponding to the main peak (t_R = 12.0 min; gradient: A/B = 80:20 to 60:40 in 20 min) gave **33** (0.7 mg, 0.11 μmol, 20%). MS (ES+): calcd for $C_{254}H_{392}N_{58}O_{125}S$: 6286.58, found: 6290.75.

[(HO$_2$C(CH$_2$)$_3$CO-His)$_2$DipPhe]$_2$DipCys(S-Colch)IleNH$_2$ (34): A sample of the purified peptide dendrimer (3.6 mg, 1.54 μmol) was dissolved in an oxygen-free phosphate buffer solution (0.3 mL, pH 8, 0.1 mM) and a solution of chloroacetylcolchicine **4** (1.0 mg, 2.3 μmol, 1.5 equiv) in degassed MeOH (50 μL) was added. The mixture was stirred overnight under nitrogen. Subsequent purification by semipreparative RP-HPLC (t_R = 18.0 min; A/B = 70:30 to 50:50 in 20 min) gave **34** (2.5 mg, 0.90 μmol, 58%). MS (ES+): calcd for $C_{108}H_{142}N_{24}O_{32}S$: 2318.99, found: 2319.50.

[(HO$_2$C(CH$_2$)$_3$CO-His)$_2$DipPhe]$_2$DipCys(S-Colch)PheNH$_2$ (35): A sample of the purified peptide dendrimer (6.1 mg, 2.53 μmol) was dissolved in an oxygen-free phosphate buffer solution (0.6 mL, pH 8, 0.1 mM) and a solution of chloroacetylcolchicine **4** (1.0 mg, 2.3 μmol, 0.9 equiv) in degassed MeOH (50 μL) was added. The mixture was stirred overnight under nitrogen. Subsequent purification by semipreparative RP-HPLC (t_R = 37.6 min; A/B = 90:10 to 40:60 in 50 min) gave **35** (2.1 mg, 0.75 μmol, 30%). MS (ES+): calcd for $C_{111}H_{140}N_{24}O_{32}S$: 2352.98, found: 2354.00.

[(Lys)$_2$DapLeu]$_2$DapCys(S-Colch)LeuNH$_2$ (36): A sample of the purified peptide dendrimer (4.3 mg, 2.53 μmol) was dissolved in an oxygen-free phosphate buffer solution (0.3 mL, pH 8, 0.1 mM) and a solution of chloroacetylcolchicine **4** (1.0 mg, 2.3 μmol, 0.9 equiv) in degassed MeOH (50 μL) was added. The mixture was stirred overnight under nitrogen. Subsequent purification by semipreparative RP-HPLC (t_R = 30.0 min; A/B = 100:0 to 50:50 in 50 min) gave **36** (2.9 mg, 1.39 μmol, 55%). MS (ES+): calcd for $C_{76}H_{130}N_{20}O_{17}S$: 1626.96, found: 1628.00.

[(Pro)$_2$DipAsp]$_2$DipCys(S-Colch)AspNH$_2$ (37): A sample of the purified peptide dendrimer (4.3 mg, 2.53 μmol) was dissolved in an oxygen-free phosphate buffer solution (0.3 mL, pH 8, 0.1 mM) and a solution of chloroacetylcolchicine **4** (1.0 mg, 2.3 μmol, 0.9 equiv) in degassed MeOH (50 μL) was added. The mixture was stirred overnight under nitrogen. Subsequent purification by semipreparative RP-HPLC (t_R = 15.4 min; A/B = 80:20 to 40:60 in 40 min) gave **37** (2.5 mg, 1.19 μmol, 47%). MS (ES+): calcd for $C_{72}H_{104}N_{16}O_{26}S$: 1640.70, found: 1641.50.

[(AcSer)$_2$DipPhe][(AcHis)(AcAsp)DipLeu]DipCys(S-Colch)HisNH$_2$ (38): A sample of the purified peptide dendrimer[9c] (4.1 mg, 2.53 μmol) was dissolved in an oxygen-free phosphate buffer solution (0.3 mL, pH 8, 0.1 mM) and a solution of chloroacetylcolchicine **4** (1.0 mg, 2.3 μmol, 0.9 equiv) in degassed MeOH (50 μL) was added. The mixture was stirred overnight under nitrogen. Subsequent purification by semipreparative RP-HPLC (t_R = 32.6 min; A/B = 90:10 to 40:60 in 50 min) gave **38** (1.8 mg, 0.73 μmol, 30%). MS (ES+): calcd for $C_{85}H_{118}N_{20}O_{28}S$: 1898.81, found: 1899.25.

Cell culture experiments: HeLa cervix carcinoma cells, mouse embryonic fibroblasts (MEFs), and human lung cancer H1299 cells were grown at 37°C in DMEM (Dulbecco's modified Eagle medium), 10% heat-inactivated foetal calf serum, and 1% (v/v) penicillin/streptomycin (all from Life Technologies, Grand Island, NY). All cells were grown in a humidified incubator in an atmosphere containing 5% CO_2. One day prior to the addition of colchicine or glycopeptide dendrimer conjugates, equal numbers of cells were seeded on 6-well plates. After three days of incubation, cells were trypsinized and the cell number was determined in duplicate by counting in a Neubauer chamber. H1299 cells were transiently transfected with 50 ng of GFP-tubulin[31] using Rotifect (Roth) according to the instructions of the manufacturer, and were analyzed by fluorescence microscopy.

Acknowledgements

This work was supported by the University of Berne and the Swiss National Science Foundation.

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Received: December 16, 2004
Published online: April 29, 2005