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Synthesis of Hsp90 inhibitor dimers as potential antitumor agents

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ABSTRACT

Structure-based drug design was used to systematically synthesize PU3-dimers. The cytotoxicity of PU3 dimers **6** against breast cancer cell lines was evaluated, and their potency increased as the length of the bridging linker increased. Among the compounds tested, **6e** with a C-20 linker was the most potent and exhibited a 20- to 30-fold increase in activity compared with that of the parent compound **5**. Western blot analyses of the cell lysates treated with **6c** revealed that **6c** resulted in the concentration-dependent degradation of the Hsp90 client protein Her2, which is consistent with other Hsp90 inhibitors.

1. Introduction

The heat shock protein 90 (Hsp90) family is a group of molecular chaperons that play a key role in the folding of polypeptide chains in the crowded environment of the cell and that operate under normal conditions as well as under various kinds of stress.[1–4] Polypeptides folded by Hsp90 are called as client proteins, and include a growing list of over 100 members.[5] Since Hsp90 and other molecular chaperons are overexpressed in many human cancers, it has been suggested that they could be of prognostic value in breast, renal and endometrial malignancies.[6] Increased expression of Hsp90 helps the cancer cell manage not only the stress caused by mutation and overexpression of oncogenes but also the stress due to hypoxia, nutrient deprivation and acidosis.[7,8] Cancer cells acquire sets of functional capacities known as the six hallmarks of cancer.[9] Moreover, many of the Hsp90 client proteins, for example, Bcr-abl, Akt, C-Raf, CDK4, Her2, and the estrogen receptor (ER), are oncogenic and are also considered hallmarks of the disease.[10,11] Inhibition of Hsp90 leads to degradation of these various client proteins by a proteasome. The simultaneous combinatorial depletion of many cancer-causing client proteins and the modulation of all of the hallmarks of cancer are the major advantages of Hsp90 inhibitors. Hsp90 exists predominantly as a dimer in the cell, with each subunit being made up of three functional domains: an N-terminal ATP-binding domain; a middle domain; and a C-terminal dimerization domain that contains tetratricopeptide repeat-binding motifs. Geldanamycin[12] (GDM, **1**) and radicicol[13] (RDC, **2**) are natural products and are known to be strong inhibitors of the Hsp90 N-terminal domain (775 nM for GDM, 2.7 nM for RDC).[14] These natural products are competitive with an ATP, which is a natural substrate of the Hsp90 N-terminal domain.[15–17] They exhibit growth inhibitory activity against a range of cancer cell lines, including human breast cancer cells such as MCF-7 (49 nM for GDM, 23 nM for RDC). Although GDM and RDC have certain drawbacks as potential drugs, the 17-allylamino derivative (17-AAG, **3**) and the 17-dimethylaminoethyl derivative (17-DMAG, **4**) of GDM are currently in phase II studies as single agents, and combination studies with cytotoxic agents are also underway.[18] The 8,9-disubstituted purine class of molecules, for example, PU3 (**5**),[19] is the first fully synthetic Hsp90 inhibitor with the ability to bind at the ATP-binding site of the N-terminal domain. This class of synthetic inhibitors is also active in cells causing degradation of Erb B2 kinase, ER and *C*-Raf kinase; however, these activities are minimal compared to those of GDM and RDC[20] (Fig. 1).

When a target molecule is a homodimer, one of the strategies used in developing a high affinity ligand to this target is to make a divalent ligand by combining two ligands with an appropriate linker.[21] With this approach, a multiplier effect would be expected since the conformational restriction by cross-linking monomers reduces the free energy associated with the restriction of the translational and rotational freedom of the ligand and the target. A recently reported X-ray crystal structure of the full length Hsp90 dimer[23] revealed that the distance between each ATP-binding site of the N-terminal domains is at least 25 Å, which corresponds to a linear 20 carbon chain in length. Knowing that Hsp90 exists

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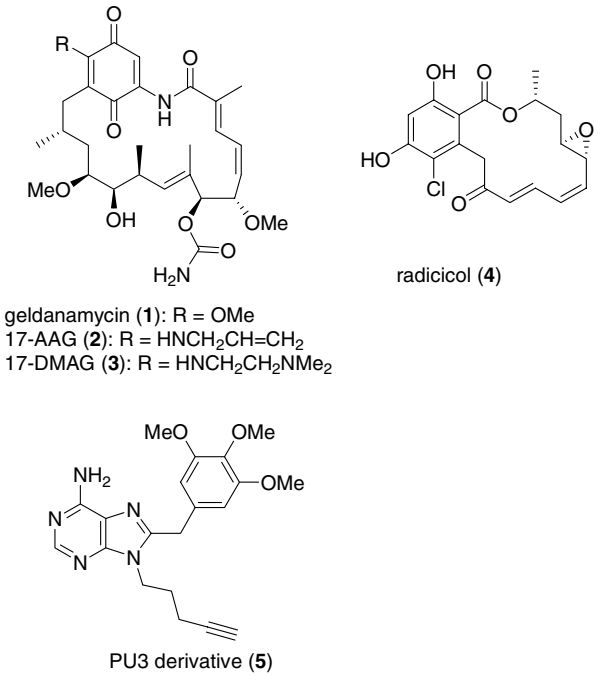


Figure 1. Structures of Hsp90 N-terminal domain inhibitors.

predominantly as dimers, researchers have prepared several dimers of GDM and evaluated their biological activity.[22] For example, the four-carbon tethered GDM dimer **7a** exhibited the greatest inhibitory activity for the series of compounds and demonstrated high selectivity toward the Her2 family of receptors. Given the distance between the ATP-binding sites of the N-terminal domains, it is unlikely that the four-carbon tethered GDM dimer **7a** would act as a divalent ligand against the Hsp90 dimer. The linker connecting the two ligands would have to be lengthened in the divalent ligands in order to develop divalent ligands for the Hsp90 N-terminal domains. Herein we describe the design, synthesis and the biological evaluation of a series of dimeric compounds of PU3 **6a**–**e** (Fig. 2).

2. Results and discussion

2.1. Molecular design

In order to observe more easily the impact of the dimerization of ligands in developing divalent inhibitors, we selected PU3, a low affinity N-terminal inhibitor. We planned to systematically prepare the dimers of PU3 **6a**–**e** by varying the length of the linker enough to cover the distance of the two ATP-binding sites of the N-terminal domains. The X-ray crystal structures of the PU3 and Hsp90 complex[24] indicated that the terminal alkyl moiety at the N^9-position of PU3 was exposed to the solvent-accessible region and did not participate in the binding to Hsp90. Therefore, it was decided that the terminus of the alkyne could be the position where the linker was attached. After considering water-solubility and the increase in molecular weight caused by dimerization, we chose an amine as the pivotal functional group connecting PU3 and the linker. We also prepared the GDM dimers **7a**–**c**, including several known compounds, in order to compare their biological activity with that of the PU3 dimers and to reinvestigate the activity of the GDM dimers.

2.2. Chemistry

The synthesis of the PU3 dimers **6a**–**e** is outlined in Scheme 1. The hydroxyl group of 4-pentyn-1-ol (**8**) was temporarily protected with a TBDPS group to afford **9** in quantitative yield. Deprotonation of the terminal alkyne moiety of **9** with *n*-BuLi followed by substitution of the resulting lithium acetylide with methyl chloroformate afforded the corresponding methyl ester. Deblocking the TBDPS group of this compound with TBAF in the presence of acetic acid gave **10** in good yield. The absence of acetic acid in the deprotection step caused the further cyclization of **10** and furnished a stable 2-methoxycarbonylmethylidenetetrahydrofurane.[25] The Mitsunobu reaction of **10** and 8-(3,4,5-trimethoxybenzyl)adenine[26] **11** in toluene/CH_2Cl_2 gave the desired N^9-substituted adenine derivative **12** as the major product along with the corresponding N^3- and N^7-isomers. The methoxycarbonyl group of **12** was reduced to the hydroxymethyl group with DIBAL-H providing the alcohol **13** in 75% yield. Installation of two PU3 moieties

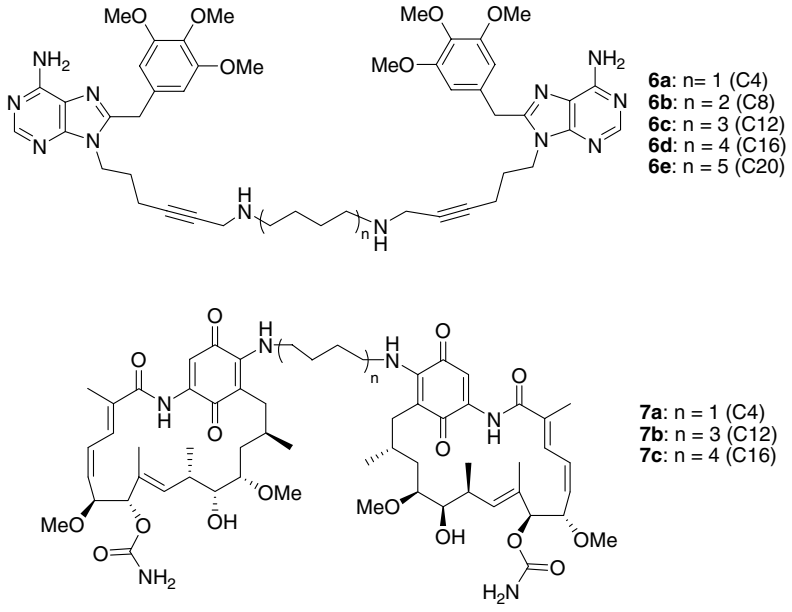
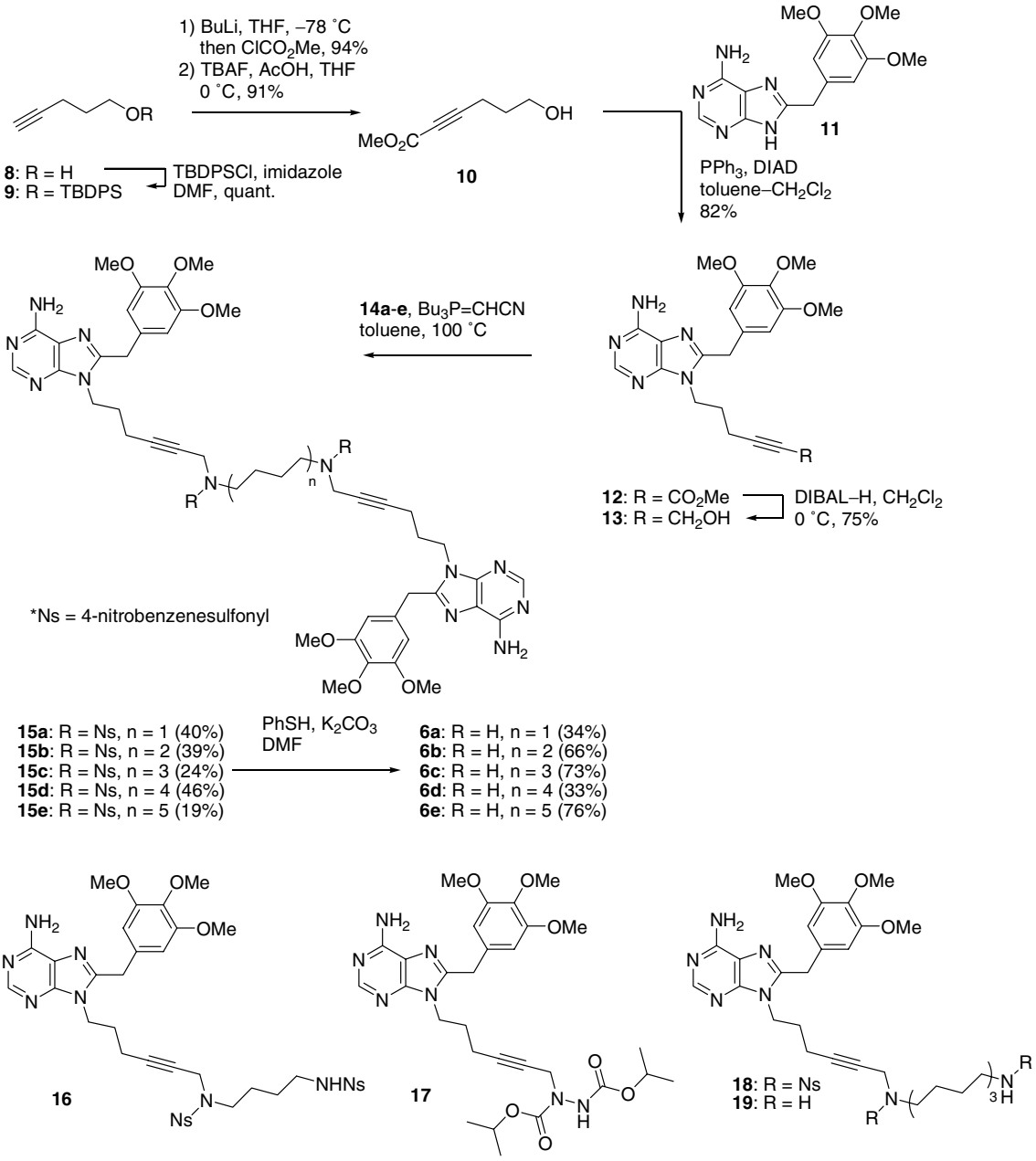
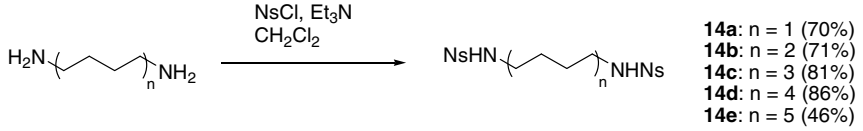


Figure 2. Structures of dimers of Hsp90 N-terminal domain inhibitors.

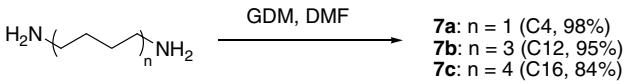


Scheme 1. Synthesis of dimers of PU3.

on the alkyldiamines was conducted by a Mitsunobu reaction using the corresponding *N*,*N*′-bis-4-nitrobenzenesulfonyl (Ns)[27] derivatives **14a**–**e**, which were prepared by simple sulfonylation of the alkyldiamines (Scheme 2). Optimization of the reaction conditions was first examined with **14a**. When Ph_3P and DIAD were used, only a trace amount of the desired **15a** was obtained. Careful analysis of the reaction revealed the formation of the *N*-alkylhydrazine derivative **17**, which was formed by the nucleophilic substitution of the triphenylphosphonium intermediate by hydrazine-*N*,*N*′-diisopropylcarbonate, a side-product of the reaction. The formation of **17** consumed the electrophile to give the *N*,*N*′-bis-Ns amide. In order to increase the nucleophilicity of the sulfonamide, the 2,4-dinitrobenzenesulfonamide derivative was used instead of **14a**; however no improvement was observed. The Mitsunobu-type reaction with cyanomethylene tributylphosphorane (CMBP), which was developed by Tsunoda et al., allows the use of nucleophiles having a pK_a higher than 13.[28] In addition, there are no nucleophiles such as hydrazine-*N*,*N*′-diisopropylcarbonate present in



Scheme 2. Preparation of alkyldiamines protected with 4-nitrobenezenesulfonyl group.



Scheme 3. Synthesis of GDM dimers.

the reaction. When the *N*,*N*′-bis-Ns amides **14a** were treated with CMBP at 100 °C, the desired **15a** was produced in 40% yields together with the monomeric compound **16**. Similarly, compounds **15b–e** were also obtained in acceptable yields (19–46%). Finally the Ns groups were removed by thiophenol and K_2CO_3 in DMF to afford the desired dimers **6a–e** (33–76%).

As described above, the GDM dimers **7a–c** with various lengths of linker were also synthesized in order to compare their biological activity with that of the PU3 dimers **6a–e**. In a manner similar to that reported previously,[22] simple treatment of GDM with 1,4-butanediamine, 1,12-dodecanediamine, and 1,16-hexadecanediamine gave the corresponding GDM dimers, respectively, in high yields (84–98%, Scheme 3).

2.3. Biological activity

The cytotoxic activity of the series of synthesized dimers was evaluated against the MCF-7 and SKBr3 human breast cancer cell lines, as shown in Table 1. Under conditions where the IC_{50} for the parent PU3 **5** exhibited the previously reported activity (51.6 μM for MCF-7, 28.9 μM for SKBr3), the monomer **13** showed no cytotoxicity at 100 μM. As for the activity of the PU3 dimers, the results revealed that the longer the length of the linker, the more potent the cytotoxicity. The IC_{50}s for **6e** possessing a C-20 linker were 1.38 μM (MCF-7) and 1.29 μM (SKBr3), respectively, and a 20- to 30-fold increase in cytotoxicity was observed when compared with that of **5**. To determine whether the observed cytotoxicity was related to Hsp90 inhibition, **5** and **6c** were incubated with MCF-7 breast cancer cells for 24 h. As can be seen from Western blot analyses of the protein lysates, **6c** resulted in the concentration-dependent degradation of the Hsp90 client protein Her2 (Fig. 3). The dimer **6c** exhibited higher activity than that of **5**, and the amount of the Her2 degradation activity is in good accordance with the cytotoxic data. Although moderate, Hsp70 levels increased in a concentration-dependent manner, which is consistent with other Hsp90 inhibitors. Since actin is not dependent on the Hsp90 protein folding machinery, actin levels remained unchanged. Of importance is the effect of dimerization. The mono-PU3 derivative **19**, which was obtained by deprotection of the Ns group of **18**, exhibited weak cytotoxic activity (IC_{50} = 55.1 μM for MCF-7, 13.1 μM for SKBr3). This result indicated that the dimer **6** could act as a divalent ligand for Hsp90, as we expected, although more detailed mechanistic study is necessary. These molecules contain two nitrogen atoms within the linker, which are protonated under physiological conditions to form ammonium ions that increase water-solubility. The fact that the dimers **6** exhibited client degradation activity indicates that the newly synthesized dimers reach the target Hsp90 inside the cells even though they are relatively large molecules.

Table 1
Cytotoxic activity of synthesized analogs

Compound	IC_{50} (μM)	
	MCF-7	SKBr3
2	0.0892	0.00364
5	45.9	31.8
6a	>100	>100
6b	30.6	85.7
6c	4.68	5.73
6d	1.57	3.18
6e	1.46	1.31
7a	1.11	0.258
7b	0.628	0.296
7c	0.431	0.150
13	>100	>100
19	55.1	13.1

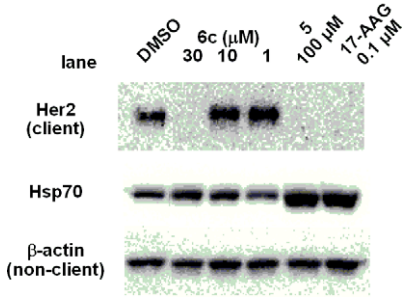


Figure 3. Her2 Degradation assay. MCF-7 cells were plated 2×10^5 cells/well and seeded for 24 h. After 24 h incubation in the presence of drugs or DMSO, lysates were prepared using M-PER® reagent. Clarified protein lysates were analyzed by Western blotting with anti-Her2, anti-Hsp70 and anti-β-actin.

The cytotoxicity of the GDM dimers **7a–c** was also evaluated. The dimers **7a–c** exhibited cytotoxicity against MCF-7 cells within the same range (IC_{50} = 0.31 μM for **7a**, 0.27 μM for **7b**, 0.15 μM for **7c**). However, it is unknown whether they act as divalent ligands, since the cytotoxicity of **7a–c** was less potent than that of the parent GDM.

2.4. Conclusion

The PU3-dimers were systematically synthesized and their biological properties were evaluated according to a structure-based drug design. It was found that by increasing the length of the bridging linker, the PU3 dimers became more cytotoxic against human breast cancer cell lines. The IC_{50}s for **6e** with a C-20 linker exhibited a 20- to 30-fold increase in cytotoxicity compared with that of the parent compound. In this study, PU3, a relatively weak inhibitor of the N-terminal domain, was used as a model. If more potent inhibitors were used, it conceivably would develop more potent dimeric inhibitors. If the N-terminal domain dimers **6** synthesized in this study actually indeed acts as divalent inhibitors, it would be theoretically possible to capture the different conformations of the Hsp90 dimer. Namely, it is expected that these dimers could modulate the functions of Hsp90 leading to an investigation of its dynamic-functional relationship.

3. Experimental

3.1. General experimental methods

NMR spectra were obtained on a JEOL EX270, JEOL GX270, JEOL AL400 or JEOL ECA500, and were reported in parts per million (δ) relative to tetramethylsilane (0.00 ppm) as internal standard otherwise noted. Coupling constant (J) was reported in hertz (Hz). Abbreviations of multiplicity were as follows; s: singlet, d: doublet, t: triplet, q: quartet, m: multiplet, br: broad. Data were presented as follows; chemical shift (multiplicity, integration, coupling constant). Assignment was based on ^1H–^1H COSY, HMBC and HMQC NMR spectra. FAB-MS was obtained on a JEOL JMS-HX101 or JEOL JMS-700TZ. Analytical thin layer chromatography (TLC) was performed on Merck silica gel 60F254 plates. Normal-phase column chromatography was performed on Merck silica gel 5715 or Kanto Chemical silica gel 60N (neutral). Flash column chromatography was performed on Merck silica gel 60.

3.1.1. 1-*O*-*tert*-Butyldiphenylsilyl-4-pentyn-1-ol (9)

A solution of 4-pentyn-1-ol (**8**, 930 μL, 10 mmol) and imidazole (1.5 g, 22 mmol) in DMF (50 mL) was treated with TBDPSCl (2.8 mL, 11 mmol) at room temperature for 1 h. The reaction was quenched with H_2O, and the mixture was partitioned between AcOEt (200 mL) and H_2O (2× 100 mL). The organic layer was washed with brine (100 mL), dried (Na_2SO_4), filtered and concentrated. The residue was purified by silica gel column chromatography (8 × 10 cm, 10% hexane/AcOEt) to give **9** (3.2 g, 99%) as a colorless syrup: ^{1}H NMR (270 MHz, $CDCl_3$) δ 7.67 (m, 4H, phenyl), 7.40 (m, 6H, phenyl), 3.75 (t, 2H, OCH_2, J = 5.9 Hz), 2.35 (dt, 2H, CH_2, J = 7.3, 2.6 Hz), 1.91 (t, 1H, CH, J = 2.6 Hz), 1.80 (tt, 2H, CH_2, J = 7.3, 5.9 Hz), 1.05 (s, 9H, *tert*-butyl); ^{13}C NMR (125 MHz, $CDCl_3$) δ 135.5, 133.8, 129.6, 127.6, 84.2, 68.3, 62.2, 31.4, 26.8, 19.2, 14.9; FABMS-LR m/z 323.5 (MH^+); FABMS-HR calcd for $C_{21}H_{27}OSi$ 323.1831, found 323.1820 (MH^+).

3.1.2. Methyl 6-hydroxy-6-*O*-*tert*-butyldiphenylsiloxy-hex-2-ynoate

Butyl lithium (1.58 M solution in THF, 2.1 mL, 3.3 mmol) was added dropwise to a solution of **9** (870 mg, 2.7 mmol) in THF (10 mL) at −78 °C, and the mixture was stirred at the same temperature for 20 min. Methyl chloroformate (260 μL, 3.3 mmol) was added dropwise to the mixture, which was stirred at 0 °C for 30 min. The reaction was quenched with saturated aqueous NH_4Cl, and the mixture was partitioned between AcOEt (200 mL) and H_2O (2× 100 mL). The organic layer was washed with brine (100 mL), dried (Na_2SO_4), filtered and concentrated. The residue was purified by silica gel column chromatography (2 × 10 cm, 17% hexane/AcOEt) to give the title compound (960 mg, 94%) as a colorless syrup: ^{1}H NMR (270 MHz, $CDCl_3$) δ 7.66 (m, 4H, phenyl), 7.40 (m, 6H, phenyl), 3.76 (s, 3H, OMe), 3.73 (t, 2H, OCH_2, J = 5.9 Hz), 2.50 (t, 2H, CH_2, J = 7.3 Hz), 1.81 (tt, 2H, CH_2, J = 5.9, 7.3 Hz), 1.04 (s, 9H, *tert*-butyl); ^{13}C NMR (125 MHz, $CDCl_3$) δ 154.3, 135.7, 133.7, 129.8, 127.8, 89.5, 73.1, 62.1, 52.6, 30.6, 26.9, 19.3, 15.4: FABMS-LR m/z 403.2 (MNa^+); FABMS-HR calcd for $C_{23}H_{28}O_3SiNa$ 403.1705, found 403.1715 (MNa^+).

3.1.3. Methyl 6-hydroxyhex-2-ynoate (10)

A mixture of methyl 6-hydroxy-6-*O*-*tert*-butyldiphenylsiloxy-hexy-2-ynoate (960 mg, 2.5 mmol), TBAF (1.0 M solution in THF, 3.0 mL, 3.0 mmol) and AcOH (350 μL, 6.1 mmol) in THF (25 mL) was stirred at 0 °C for 3 h. After AcOH (0.5 mL) was added, the mixture was concentrated in vacuo. The residue was purified by silica gel column chromatography (2 × 10 cm, 20% hexane/AcOEt) to give **10** (330 mg, 91%) as a pale yellow syrup: ^{1}H NMR (270 MHz, $CDCl_3$) δ 3.75 (s, 3H, OMe), 3.75 (t, 2H, OCH_2, J = 6.3Hz), 2.48 (t, 2H, CH_2, J = 6.9 Hz), 1.83 (tt, 2H, CH_2, J = 6.9, 6.3 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ 154.2, 89.1, 72.9, 60.8, 52.5, 30.1, 15.0; FABMS-LR m/z 143.1 (MH^+); FABMS-HR calcd for $C_7H_{10}O_3$ 143.0708, found 143.0710 (MH^+).

3.1.4. 6-Amino-9-(5-methoxycarbonylpent-4-ynyl)-8-(3,4,5-trimethoxybenzyl)-9*H*-purine (12)

A suspension of **11** (200 mg, 0.64 mmol), **10** (120 mg, 0.83 mmol), and PPh_3 (370 mg, 1.4 mmol) in toluene/CH_2Cl_2 (6.5:1) was treated with DIAD (610 μL, 3.2 mmol) at room temperature for 2 h. The solvent was removed in vacuo, and the residue was purified by flash silica gel column chromatography (1.5 × 10 cm, 2% MeOH/$CHCl_3$) to give **12** (240 mg, 82%) as a yellow foam: ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.31 (s, 1H, H-2), 6.48 (s, 2H, phenyl), 5.58 (br s, 2H, 6-NH_2), 4.24 (s, 2H, PhCH_2), 4.18 (t, 2H, NCH_2, J = 7.3 Hz), 3.82, 3.80 (each s, 9H, 3× OMe), 3.77 (s, 3H, OMe), 2.33 (t, 2H, CH_2, J = 6.8 Hz), 2.00 (tt, 2H, CH_2, J = 7.3, 6.8 Hz); ^{13}C NMR (67.5 MHz, $CDCl_3$) δ 154.1, 153.2, 152.1, 151.0, 150.4, 150.3, 149.7, 144.7, 130.6, 124.4, 105.2, 105.1, 60.4, 55.8, 52.2, 41.4, 34.2, 26.5, 15.5, 14.5; FABMS-LR m/z 440.2 (MH^+); FABMS-HR calcd for $C_{22}H_{26}N_5O_5$ 440.1934, found 440.1934 (MH^+).

3.1.5. 6-Amino-9-(6-hydroxyhex-4-ynyl)-8-(3,4,5-trimethoxy benzyl)-9*H*-purine (13)

Diisobutylaluminiumhydride (1.5 M solution in toluene, 280 μL, 0.42 mmol) was added dropwise to a solution of **12** (60 mg, 0.14 mmol) in CH_2Cl_2 (2 mL) at 0 °C, and the mixture was stirred at the same temperature for 1 h. The reaction was quenched with saturated aqueous Rochelle salt (50 mL). The mixture was stirred at room temperature overnight, and was extracted with $CHCl_3$ (2× 20 mL). The separated organic layers were washed with brine (10 mL), dried (Na_2SO_4), filtered and concentrated. The residue was purified by silica gel column chromatography (1.5 × 10 cm, 4% MeOH/$CHCl_3$) to give **13** (43 mg, 75%) as a pale yellow foam: ^{1}H NMR (270 MHz, $CDCl_3$) δ 8.32 (s, 1H, H-2), 6.47 (s, 2H, phenyl), 5.69 (br s, 2H, 6-NH_2), 4.23 (s, 2H, PhCH_2), 4.17 (m, 4H, NCH_2, OCH_2), 3.82, 3.80 (each s, 9H, 3× OMe), 2.19 (t, 2H, CH_2, J = 6.6 Hz), 1.87 (m, 2H, CH_2); ^{13}C NMR (67.5 MHz, $CDCl_3$) δ 153.7, 153.6, 153.5, 153.2, 152.5, 151.5, 151.1, 117.2, 105.9, 60.9, 56.4, 50.9, 42.0, 34.8, 27.9, 17.8, 16.1; FABMS-LR m/z 412.2 (MH^+); FABMS-HR calcd for $C_{21}H_{26}N_5O_4$ 412.1985, found 412.2000 (MH^+).

3.1.6. *N*,*N*′-Di-(4-nitrobenzenesulfonyl)-1,4-diaminobutane (14a)

4-Nitrobenzenesulfonyl chloride (490 mg, 2.2 mmol) was added to a mixture of 1,4-diaminobutane (1.0 mmol, 100 μL) and triethylamine (310 μL, 2.2 mmol) in CH_2Cl_2 (10 mL) at 0 °C, and the mixture was stirred at the same temperature for 1 h. The reaction mixture was allowed to room temperature, and stirred for further 3 h. The reaction was quenched with MeOH, and the solvent was concentrated in vacuo. The residue was triturated from AcOEt to give **14a** (320 mg, 70%) as a white solid: ^{1}H NMR (270 MHz, DMSO-d_6) δ 8.40 (d, 4H, Ns, J = 8.6 Hz), 7.98 (d, 4H, Ns, J = 8.6 Hz), 7.94 (t, 2H, 2× NH, J = 5.3 Hz), 2.71 (m, 4H, 2× NHCH_2), 1.33 (m, 4H, 2× CH_2); ^{13}C NMR (125 MHz, DMSO-d_6) δ 149.5, 146.1, 128.0, 127.9, 124.7, 124.5, 42.0, 26.1; FABMS-LR m/z 459.1 (MH^+); FABMS-HR calcd for $C_{16}H_{19}N_4O_8S_2$ 459.0644, found 459.0641 (MH^+).

3.1.7. *N*,*N*′-Di-(4-nitrobenzenesulfonyl)-1,8-diaminooctane (14b)

Compound **14b** (720 mg, 71%) was obtained from 1,8-diaminoctane (290 mg, 2.0 mmol) as described for the synthesis of **14a**: ^{1}H NMR (270 MHz, DMSO-d_6) δ 8.41 (d, 4H, Ns, J = 8.8 Hz), 8.02 (d, 4H, Ns, J = 8.6 Hz), 7.94 (t, 2H, 2× NH, J = 5.9 Hz), 2.77 (m, 4H, 2× NHCH_2), 1.33 (m, 4H, 2× CH_2), 1.12 (m, 8H, 4× CH_2); ^{13}C NMR (125 MHz, DMSO-d_6) δ 149.5, 146.2, 128.1, 127.9, 124.7, 124.5, 42.5, 28.9, 28.3, 25.8; FABMS-LR m/z 515.2 (MH^+); FABMS-HR calcd for $C_{20}H_{27}N_4O_8S_2$ 515.1270, found 515.1277 (MH^+).

3.1.8. *N*,*N*′-Di-(4-nitrobenzenesulfonyl)-1,12-diaminododecane (14c)

Compound **14c** (1.4 g, 81%) was obtained from 1,12-diaminododecane (3.0 mmol, 600 mg) as described for the synthesis of **14a**: ^{1}H NMR (270 MHz, DMSO-d_6) δ 8.40 (d, 4H, Ns, J = 9.2 Hz), 8.02 (d, 4H, Ns, J = 9.2 Hz), 7.93 (t, 2H, 2× NH, J = 5.6 Hz), 2.75 (m, 4H, 2× NHCH_2), 1.30 (m, 4H, 2× CH_2), 1.09 (m, 16H, 8× CH_2); ^{13}C NMR (125 MHz, DMSO-d_6) δ 149.5, 146.3, 128.1, 128.0, 124.7, 124.5, 42.5, 29.0, 28.9, 28.8, 28.4, 25.9; FABMS-LR m/z 571.2 (MH^+); FABMS-HR calcd for $C_{24}H_{35}N_4O_8S_2$ 571.1896, found 571.1888 (MH^+).

3.1.9. *N,N′*-Di-(4-nitrobenzenesulfonyl)-1,16-diaminohexadecane (14d)

A solution of *p*-toluenesulfonyl chloride (2.2 g, 11.6 mmol) in CH_2Cl_2 (10 mL) was added dropwise to a mixture of 1,16-hexadecanediol (1.0 g, 3.8 mmol), Et_3N (2.1 mL, 15.2 mmol) and $Me_3N{\cdot}HCl$ (73 mg, 0.76 mmol) in CH_2Cl_2 (30 mL) at 0 °C, and the mixture was stirred at the same temperature for 30 min. The reaction was quenched with MeOH, and whole the mixture was concentrated in vacuo. The residue was partitioned between $CHCl_3$ and H_2O, the organic layer was washed with brine, dried (Na_2SO_4), filtered and concentrated. The residue was triturated from AcOEt/hexane to give *O,O′*-ditosyl-1,16-hexadecanediol (2.0 g, 92%) as a white solid: ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.78 (d, 4H, 2× Ts, J = 8.6 Hz), 7.33 (d, 4H, 2× Ts, J = 8.6 Hz), 4.01 (t, 4H, 2× OCH_2, J = 6.5 Hz), 2.44 (s, 6H, 2× Me), 1.61 (m, 4H, 2× CH_2), 1.27 (m, 4H, 2× CH_2), 1.21 (m, 20H, 10× CH_2); ^{13}C NMR (125 MHz, $CDCl_3$) δ 144.6, 133.2, 129.8, 127.8, 70.7, 29.6, 29.5, 29.4, 29.3, 28.9, 28.8, 25.3, 21.6. A solution of *O,O′*-ditosyl-1,16-hexadecanediol (1.1 g, 2.0 mmol) in DMF (10 mL) was treated with NaN_3 (390 mg, 6.0 mmol) at 60 °C for 9 h. The mixture was partitioned between AcOEt (100 mL) and H_2O (3× 100 mL), and the organic layer was washed with brine, dried (Na_2SO_4), filtered and concentrated. A solution of the residue in THF/H_2O (9:1; 20 mL) was treated with PPh_3 (1.1 g, 4.4 mmol) at room temperature for 12 h. The mixture was concentrated in vacuo, and the residue was triturated from AcOEt to give 1,16-diaminohexadecane (280 mg, 55% in two steps) as a white solid. A mixture of **7** (200 mg, 0.78 mmol) and triethylamine (260 μL, 1.9 mmol) in CH_2Cl_2 (10 mL) was treated with 4-nitrobenzenesulfonyl chloride (380 mg, 1.7 mmol) at 0 °C, and the mixture was stirred at room temperature for 3 h. The reaction was quenched with MeOH (5 mL), and the mixture was concentrated in vacuo. The residue was triturated from AcOEt to give **14d** (420 mg, 86%) as a white solid: ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.40 (d, 4H, 2× Ns, J = 8.6 Hz), 8.02 (d, 4H, 2× Ns, J = 8.6 Hz), 7.94 (t, 2H, 2× NH, J = 5.8 Hz), 2.77 (dt, 4H, 2× NCH_2, J = 5.8, 6.9 Hz), 1.33 (m, 4H, 2× CH_2, J = 6.9 Hz), 1.15 (m, 24H, 12× CH_2); ^{13}C NMR (125 MHz, DMSO-d_6) δ 149.5, 146.4, 128.2, 128.0, 124.8, 124.5, 42.5, 29.1, 28.9, 28.5, 25.9; FABMS-LR m/z 627.3 (MH^+); FABMS-HR calcd for $C_{28}H_{43}N_4O_8S_2$ 627.2522, found 627.2516 (MH^+).

3.1.10. *N,N′*-Di-(4-nitrobenzenesulfonyl)-1,20-diaminoeicosane (14e)

A mixture of 1,20-eicosanediol (500 mg, 1.6 mmol), Et_3N (0.90 mL, 6.4 mmol) and Me_3NHCl (30 mg, 0.32 mmol) in CH_2Cl_2 (30 mL) was treated with MsCl (0.37 mL, 4.8 mmol) at 0 °C for 10 min. The reaction was quenched with H_2O (30 mL), and the mixture was extracted with $CHCl_3$ (2× 50 mL). The organic layers were washed with brine (30 mL), dried (Na_2SO_4), filtered and concentrated. The residue was triturated from hexane to give 1,20-*O,O′*-dimethanesulfonyl eicosanediol (340 mg, 0.72 mmol, 45%) as a white solid: ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.21 (t, 4H, 2× OCH_2, J = 6.8 Hz), 3.00 (s, 6H, Ms2), 1.73 (tt, 4H, 2× CH_2, J = 6.8, 7.3 Hz), 1.40–1.25 (br m, 32H, 16× CH_2). A solution of 1,20-*O,O′*-dimethanesulfonyl eicosanediol (240 mg, 0.50 mmol) in DMF (5.0 mL) was treated with NaN_3 (98 mg, 1.5 mmol) at 60 °C for 12 h. The mixture was partitioned between AcOEt (100 mL) and H_2O (2× 50 mL), The organic layer was washed with brine, dried (Na_2SO_4), filtered and concentrated. A solution of the residue (140 mg) in THF/H_2O (9:1, 5 mL) was treated with PPh_3 (220 mg, 0.84 mmol) at room temperature for 6 h. The mixture was concentrated in vacuo, and the residue was triturated from AcOEt to give 1,20-diaminoeicosane (60 mg, 50% over two steps) as a white solid. A suspension of 1,20-diaminoeicosane (150 mg, 0.48 mmol), Et_3N (0.28 mL, 2.0 mmol) and 4-nitrobenzenesulfonyl chloride was stirred at 0 °C for 10 min. The reaction mixture was allowed to room temperature and stirred for further 8 h. The reaction was quenched with MeOH (5 mL), and the solvent was removed in vacuo. The residue was triturated from AcOEt and $CHCl_3$ to give **14** (150 mg, 46%) as a white solid: ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.40 (d, 4H, 2× Ns, J = 8.1 Hz), 8.02 (d, 4H, 2× Ns, J = 8.1 Hz), 7.94 (t, 2H, 2× NH, J = 5.7 Hz), 2.77 (m, 4H, 2× NCH_2, J = 5.7, 6.3 Hz), 1.31 (m, 4H, 2× CH_2, J = 6.3 Hz), 1.20–1.12 (br m, 32H, 16× CH_2); ^{13}C NMR (100 MHz, DMSO-d_6) δ 149.5, 146.4, 128.1, 124.7, 42.6, 29.1, 29.0, 28.5, 26.0; FABMS-LR m/z 683.4 (MH^+); FABMS-HR calcd for $C_{32}H_{51}N_4O_8S_2$ 683.3141, found 683.3134 (MH^+).

3.1.11. *N,N′*-Di-(4-nitrobenzenesulfonyl)-PU3-C4-dimer (15a)

A mixture of **13** (85 mg, 0.20 mmol), **14a** (45 mg, 0.098 mmol) and cyanomethylene tributylphosphorane (CMBP, 71 mg, 0.30 mmol) in toluene (0.5 mL) was stirred at 100 °C for 2 h. After cooling, the solvent was removed in vacuo. The residue was purified by preparative TLC (5% 3× MeOH/$CHCl_3$) to give **15a** (48 mg, 40%) as a brownish foam and **16** (26 mg, 31%) as a brownish foam. Data for **15a**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.29 (s, 2H, 2× H-2), 8.26 (d, 4H, 2× Ns, J = 8.8 Hz), 7.97 (d, 4H, 2× Ns, J = 8.8 Hz), 6.42 (s, 4H, 2× phenyl), 5.89 (br s, 4H, 2× 6-NH_2), 4.17 (s, 4H, 2× PhCH_2), 4.0 (s, 4H, 2× H-d), 3.97 (t, 4H, J = 7.3 Hz), 3.81, 3.80 (each s, 18H, 6× OMe), 3.23 (m, 4H), 1.88 (t, 4H, J = 6.9 Hz), 1.63 (tt, 4H, J = 7.3, 6.9 Hz), 1.59 (m, 4H); ^{13}C NMR (67.5 MHz, $CDCl_3$) δ 180.6, 154.3, 153.1, 151.9, 151.1, 150.1, 149.5, 144.1, 136.9, 130.6, 128.5, 123.5, 118.0, 116.7, 105.2, 84.2, 60.4, 55.8, 44.7, 41.7, 36.0, 34.3, 27.3, 23.4, 22.9, 15.6; FABMS-LR m/z 1245.2 (MH^+); FABMS-HR calcd for $C_{58}H_{64}N_{14}O_{14}S_2$ 1245.4246, found 1245.4246 (MH^+). Data for **16**: ^{1}H NMR (270 MHz, $CDCl_3$) δ 8.34 (d, 2H, Ns, J = 8.6 Hz), 8.31 (s, 1H, H-2), 8.20 (d, 2H, Ns, J = 8.9 Hz), 8.05 (d, 2H, Ns, J = 8.9 Hz), 7.92 (d, 2H, Ns, J = 8.6 Hz), 6.71 (br s, 1H, NsNH), 6.41 (s, 2H, Ph), 5.94 (br s, 2H, 6-NH_2), 4.17 (s, 2H, PhCH_2), 4.03 (t, 2H, J = 6.3 Hz), 3.84 (s, 2H), 3.82 (s, 9H, 3× OMe), 3.05 (m, 4H), 1.89 (m, 4H), 1.70 (m, 2H), 1.53 (m, 2H); ^{13}C NMR (67.5 MHz, $CDCl_3$) δ 184.5, 155.1, 154.1, 152.9, 152.5, 152.4, 152.2, 151.3, 150.5, 147.3, 146.5, 145.1, 131.4, 129.3, 128.6, 124.9, 124.4, 109.3, 84.9, 73.1, 61.4, 56.8, 56.5, 46.3, 46.1, 43.0, 37.8, 35.3, 27.9, 26.6, 24.9, 16.6; FABMS-LR m/z 852.2 (MH^+); FABMS-HR calcd for $C_{37}H_{42}N_9O_{11}S_2$ 852.2445, found 852.2447 (MH^+).

3.1.12. *N,N′*-Di-(4-nitrobenzenesulfonyl)-PU3-Ns-C8-dimer (15b)

Compound **15b** (44 mg, 39%) was obtained from **13** (75 mg, 0.18 mmol), **14b** (45 mg, 0.087 mmol) and CMBP (84 mg, 0.35 mmol) as described for the synthesis of **15a**: ^{1}H NMR (270 MHz, $CDCl_3$) δ 8.29 (s, 2H, 2× H-2), 8.26 (d, 4H, 2× Ns, J = 8.8 Hz), 7.97 (d, 4H, 2× Ns, J = 8.8 Hz), 6.42 (s, 4H, 2× phenyl), 5.75 (br s, 4H, 2× 6-NH_2), 4.17 (s, 4H, 2× PhCH_2), 4.07 (s, 4H), 3.97 (t, 4H, J = 7.3 Hz), 3.80 (s, 18H, 6× OMe), 3.17 (t, 4H, J = 7.1 Hz), 1.85 (t, 4H, J = 6.9), 1.61 (tt, 4H, J = 6.9, 7.3 Hz), 1.52 (m, 4H), 1.26 (m, 8H); ^{13}C NMR (67.5 MHz, $CDCl_3$) δ 154.2, 153.2, 152.0, 151.0, 150.0, 149.5, 144.5, 136.9, 130.6, 128.4, 123.4, 118.1, 105.2, 83.9, 72.8, 60.4, 56.9, 55.8, 45.8, 41.6, 36.0, 34.3, 28.4, 27.5, 26.8, 25.7, 15.5; FABMS-LR m/z 1301.1 (MH^+); FABMS-HR calcd for $C_{62}H_{73}N_{14}O_{14}S_2$ 1301.4872, found 1301.4892 (MH^+).

3.1.13. *N,N′*-Di-(4-nitrobenzenesulfonyl)-PU3-C12-dimer (15c)

Compound **15c** (17 mg, 29%) and **18** (18 mg, 41%) were obtained from **13** (42 mg, 0.10 mmol), **14c** (26 mg, 0.046 mmol) and CMBP (36 mg, 0.15 mmol) as described for the synthesis of **15a**. Data for **15c**: ^{1}H NMR (270 MHz, $CDCl_3$) δ 8.28 (s, 2H, 2× H-2), 8.26 (d, 4H, 2× Ns, J = 8.6 Hz), 7.98 (d, 4H, 2× Ns, J = 8.6 Hz), 6.41 (s, 4H, 2× phenyl), 5.92 (br s, 4H, 2× 6-NH_2), 4.16 (s, 4H, 2× PhCH_2), 4.08 (s, 4H), 3.96 (t, 4H, J = 7.3 Hz), 3.81, 3.80 (each s, 18H, 6× OMe), 3.16 (t, 4H, J = 7.2 Hz), 1.87 (t, 4H, J = 6.8 Hz), 1.61 (tt, 4H, J = 7.3, 6.8 Hz), 1.51 (m, 4H), 1.24–1.19 (m, 16H); ^{13}C NMR

(125 MHz, $CDCl_3$) δ 154.9, 153.8, 152.6, 151.6, 150.7, 150.1, 145.2, 137.5, 131.2, 129.1, 124.0, 118.7, 105.7, 84.5, 73.5, 61.0, 56.4, 46.6, 42.2, 36.6, 34.9, 29.6, 29.3, 28.1, 27.7, 26.6, 16.2; ESI-LR m/*z* 1379.8 (MNa^+); ESI-HR calcd for $C_{66}H_{80}N_{14}O_{14}S_2Na$ 1379.5318, found 1379.5334 (MNa^+). Data for **18**: ^1H NMR (270 MHz, $CDCl_3$) δ 8.34–8.29 (m, 4H, Ns), 8.26 (s, 1H, H-2), 8.05–7.98 (m, 4H, Ns), 6.63 (t, 1H, NsNH, *J* = 6.3 Hz), 6.42 (s, 4H, phenyl), 5.89 (br s, 4H, 6-NH_2), 4.15 (s, 2H, PhCH_2), 4.08 (s, 2H), 3.93 (t, 2H, *J* = 7.3 Hz), 3.81 (s, 9H, 3× OMe), 3.19 (t, 2H, *J* = 6.9 Hz), 3.02 (m, 2H, *J* = 6.3 Hz), 1.88 (m, 2H), 1.81 (m, 2H), 1.62 (m, 2H), 1.40 (m, 2H), 1.21 (m, 8H); ^{13}C NMR (125 MHz, $CDCl_3$) δ154.9, 153.8, 152.2, 151.5, 150.8, 150.1, 150.0, 146.5, 145.1, 137.5, 131.1, 129.0, 128.4, 124.5, 124.1, 118.7, 105.7, 84.5, 73.6, 61.0, 56.4, 46.7, 43.3, 42.1, 36.9, 34.9, 29.7, 28.9, 28.8, 28.2, 27.7, 26.4, 26.3, 16.1; FAB-LR *m*/*z* 908.3 (MH^+); FAB-HR calcd for $C_{41}H_{50}N_9O_{11}S_2$ 908.3071, found 908.3068 (MH^+).

3.1.14. *N,N′*-Di-(4-nitrobenzenesulfonyl)-PU3-C16-dimer (15d)

Compound **15d** (72 mg, 46%) was obtained from **13** (100 mg, 0.24 mmol), **4d** (69 mg, 0.11 mmol) and CMBP (106 mg, 0.44 mmol) as described for the synthesis of **15a**: ^1H NMR (400 MHz, $CDCl_3$) δ 8.26 (s, 2H, 2× H-2), 8.24 (d, 4H, 2× Ns, *J* = 9.1 Hz), 7.96 (d, 4H, 2× Ns, *J* = 9.1 Hz), 6.39 (s, 4H, 2× phenyl), 6.17 (br s, 4H, 2× 6-NH_2), 4.14 (s, 4H, 2× PhCH_2), 4.07 (s, 4H), 3.95 (t, 4H, *J* = 7.3 Hz), 3.79 (s, 6H, 2× OMe), 3.77 (s, 12H, 4× OMe), 3.15 (t, 4H, *J* = 7.2 Hz), 1.86 (t, 4H , *J* = 6.8 Hz), 1.58 (tt, 4H, *J* = 7.3, 6.8 Hz), 1.49 (m, 4H, *J* = 7.2 Hz), 1.23 (m, 4H), 1.20 (m, 20H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 155.0, 153.7, 152.5, 151.4, 150.5, 150.0, 145.1, 137.4, 131.2, 129.0, 124.0, 118.6, 105.6, 84.4, 77.4, 73.4, 61.0, 56.4, 46.5, 42.1, 36.5, 34.8, 29.7, 29.6, 29.5, 29.2, 28.1, 27.5, 26.5, 16.1; ESIMS-LR *m*/*z* 1413.41 (MH^+); ESIMS-HR calcd for $C_{70}H_{89}N_{14}O_{14}S_2$ 1413.6124, found 1413.60925 (MH^+).

3.1.15. *N,N′*-Di-(4-nitrobenzenesulfonyl)-PU3-C20-dimer (15e)

Compound **15e** (30 mg, 19%) was obtained from **13** (100 mg, 0.24 mmol), **14e** (75 mg, 0.11 mmol) and CMBP (84 mg, 0.35 mmol) as described for the synthesis of **15e**: ^1H NMR (500 MHz, $CDCl_3$) δ 8.28 (s, 2H, 2× H-2), 8.25 (d, 4H, 2×Ns, *J* = 8.6 Hz), 7.97 (d, 4H, 2× Ns, *J* = 8.6 Hz), 6.41 (s, 4H, 2× phenyl), 5.97 (br s, 4H, 2× 6-NH_2), 4.16 (s, 4H, 2× PhCH_2), 4.08 (s, 4H), 3.96 (t, 4H, *J* = 7.4 Hz), 3.80 (s, 6H, 2× OMe), 3.79 (s, 12H, 4× OMe), 3.16 (t, 4H, *J* = 6.9 Hz), 1.87 (t, 4H, *J* = 6.9 Hz), 1.60 (tt, 4H, *J* = 7.4, 6.9 Hz), 1.52 (m, 4H, *J* = 6.9 Hz), 1.25–1.22 (m, 32H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 154.9, 153.7, 152.6, 151.5, 150.6, 150.0, 145.2, 137.4, 131.2, 129.0, 124.0, 118.6, 105.7, 84.4, 73.5, 61.0, 56.4, 46.6, 42.1, 36.6, 34.8, 29.8, 29.7, 29.6, 29.3, 28.1, 27.6, 26.6, 16.1; ESIMS-LR *m*/*z* 1491.65 (MNa^+); ESIMS-HR calcd for $C_{74}H_{96}N_{14}O_{14}S_2Na$ 1491.6570, found 1491.6570 (MNa^+).

3.1.16. PU3-C4-dimer (6a)

Thiophenol (17 μL, 0.17 mmol) was added to a mixture of **15a** (35 mg, 0.17 mmol) and K_2CO_3 (20 mg, 0.15 mmol) in DMF (0.30 mL), and the mixture was stirred at room temperature for 6 h. The mixture was directly applied to a silica gel column (NH silica, 1 × 7 cm, 2% MeOH/$CHCl_3$). Appropriate fractions were evaporated, and the residue was further purified by C18 reverse phase column chromatography (1 × 5 cm, 1 N aqueous HCl/MeOH = 1:1). Appropriate fractions were evaporated, and the residue was lyophilized to give **6a** (9 mg, 34%) as dihydrochloride salt: ^1H NMR (500 MHz, D_2O) δ 8.22 (s, 2H, 2× H-2), 6.53 (s, 4H, 2× phenyl), 4.16 (s, 4H, 2× PhCH_2), 4.13 (t, 4H, *J* = 7.4 Hz), 3.69 (s, 4H), 3.66 (s, 12H, 4× OMe), 3.59 (s, 6H, 2× OMe), 2.95 (m, 4H), 2.08 (t, 4H, *J* = 6.9 Hz), 1.60 (tt, 4H, *J* = 6.9, 7.4 Hz), 1.57 (m, 4H): ^{13}C NMR (125 MHz, D_2O) δ 155.2, 153.6, 151.1, 150.5, 146.9, 136.8, 132.5, 117.8, 107.1, 90.6, 76.2, 61.8, 56.7, 46.1, 43.2, 37.2, 34.2, 28.0, 23.2, 16.0: FABMS-LR *m*/*z* 875.3 [(M−2HCl)H^+]: FABMS-HR calcd for $C_{46}H_{59}N_{12}O_6$ 875.4681, found 875.4683 [(M−2HCl)H^+].

3.1.17. PU3-C8-dimer (6b)

Compound **6d** (20 mg, 66%) was obtained from **15b** (40 mg, 0.031 mmol) as described for the synthesis of **6a**: ^1H NMR (500 MHz, D_2O) δ 8.24 (s, 2H, 2× H-2), 6.53 (s, 4H, 2× phenyl), 4.22 (s, 4H, 2× PhCH_2), 4.19 (t, 4H, *J* = 7.4 Hz), 3.67 (s, 4H), 3.66 (s, 12H, 4× OMe×), 3.60 (s, 6H, 2× OMe), 2.89 (t, 4H, *J* = 7.5 Hz), 2.13 (m, 4H), 1.68 (t, 4H, *J* = 7.4, 6.9 Hz), 1.40 (tt, 4H, *J* = 7.5, 8.0 Hz), 1.03 (m, 4H), 0.97 (m, 4H): ^{13}C NMR (125 MHz, D_2O) δ 156.0, 153.5, 150.4, 149.8, 145.0, 136.8, 132.2, 117.7, 107.3, 88.7, 70.8, 61.5, 56.7, 46.9, 43.3, 37.1, 34.2, 28.7, 28.0, 26.3, 25.9, 16.0: ESIMS-LR *m*/*z* 931.48 [(M−2HCl)H^+]: ESIMS-HR calcd for $C_{50}H_{67}N_{12}O_6$ 931.5307, found 931.5300 [(M−2HCl)H^+].

3.1.18. PU3-C12-dimer (6c)

Compound **6c** (17 mg, 73%) was obtained from **15c** (30 mg, 0.022 mmol) as described for the synthesis of **6a**: ^1H NMR (500 MHz, D_2O) δ 8.25 (s, 2H, 2× H-2), 6.55 (s, 4H, 2× phenyl), 4.20 (s, 4H, 2× PhCH_2), 4.18 (t, 4H, *J* = 7.5), 3.69 (s, 4H), 3.66 (s, 12H, 4× OMe), 3.60 (s, 6H, 2× OMe), 2.91 (t, 4H, *J* = 7.5 Hz), 2.17 (t, 4H, *J* = 7.4 Hz), 1.66 (tt, 4H, *J* = 7.4, 7.5 Hz), 1.42 (tt, 4H, *J* = 7.5, 6.9 Hz), 1.05 (tt, 4H, *J* = 6.9, 8.0 Hz), 0.89 (tt, 4H, *J* = 8.0, 7.5 Hz), 0.77 (m, 4H, *J* = 7.5 Hz), 0.67 (m, 4H): ^{13}C NMR (125 MHz, D_2O) δ 155.9, 153.7, 150.5, 150.3, 145.6, 137.0, 132.3, 117.9, 107.3, 88.9, 61.6, 56.8, 46.7, 43.4, 37.0, 34.3, 29.6, 29.4, 29.1, 28.2, 26.5, 25.8, 16.1: ESIMS-LR *m*/*z* 987.58 [(M−2HCl)H^+]: ESIMS-HR calcd for $C_{54}H_{75}N_{12}O_6$ 987.5933, found 987.5935 [(M−2HCl)H^+].

3.1.19. PU3-C16-dimer (6d)

Compound **6d** (14 mg, 33%) was obtained from **15d** (57 mg, 0.040 mmol) as described for the synthesis of **6a**: ^1H NMR (500 MHz, D_2O) δ 8.36 (s, 2H, 2× H-2), 6.65 (s, 4H, 2× phenyl), 4.30 (s, 4H, 2× PhCH_2), 4.29 (t, 4H, *J* = 8.0 Hz), 3.82 (s, 4H), 3.76 (s, 12H, 4× OMe), 3.69 (s, 6H, 2× OMe), 3.05 (t, 4H, *J* = 8.0 Hz), 2.28 (t, 4H, *J* = 6.9 Hz), 1.79 (tt, 4H, *J* = 8.0, 6.9 Hz), 1.57 (tt, 4H, *J* = 8.0, 7.4 Hz), 1.20 (tt, 4H, *J* = 7.4, 6.9 Hz), 1.05 (tt, 4H, *J* = 8.0, 6.9 Hz), 0.95 (m, 4H, *J* = 8.0 Hz), 0.83–0.80 (m, 12H): ^{13}C NMR (125 MHz, D_2O) δ 156.0, 153.6, 150.3, 149.6, 144.7, 136.8, 132.1, 117.7, 107.2, 88.8, 70.9, 61.65 56.7, 46.4, 43.3, 36.8, 34.2, 29.6, 29.5, 29.4, 29.2, 28.7, 28.1, 26.3, 25.5, 16.0: ESIMS-LR *m*/*z* 1043.6 [(M−2HCl)H^+]: ESIMS-HR calcd for $C_{58}H_{83}N_{12}O_6$ 1043.6559, found 1043.6584 [(M−2HCl)H^+].

3.1.20. PU3-C20-dimer (6e)

Compound **6e** (16 mg, 76%) was obtained from **15e** (27 mg, 0.018 mmol) as described for the synthesis of **6a**: ^1H NMR (500 MHz, D_2O) δ 8.34 (s, 2H, 2× H-2), 6.62 (s, 4H, 2× phenyl), 4.26 (m, 8H), 3.81 (s, 4H), 3.72 (s, 12H, 4× OMe), 3.61 (s, 6H, 2× OMe), 3.04 (t, 4H, *J* = 7.5 Hz), 2.22 (m, 4H), 1.71 (m, 4H, *J* = 6.9 Hz), 1.61 (m, 4H, *J* = 7.5 Hz), 1.22 (m, 4H), 1.14 (m, 4H), 1.06 (m, 4H), 0.98 (m, 24H); ^{13}C NMR (125 MHz, D_2O) δ 155.8, 153.6, 150.3, 150.0, 144.7, 136.8, 132.0, 117.6, 107.1, 88.8, 70.8, 61.2, 56.6, 46.3, 43.3, 36.8, 34.3, 30.0, 29.8, 29.6, 29.2, 28.1, 26.6, 25.7, 16.1; ESIMS-LR *m*/*z* 1099.7 [(M−2HCl)H^+]; ESIMS-HR calcd for $C_{62}H_{91}N_{12}O_6$ 1099.7185, found 1099.7148 [(M−2HCl)H^+].

3.1.21. PU3-C12-monomer (19)

Compound **19** was (22 mg, 43%) was obtained from **18** (75 mg, 0.077 mmol) as described for the synthesis of **6a**: ^1H NMR (500 MHz, D_2O) δ 8.39 (s, 1H, H-2), 6.71 (s, 2H, phenyl), 4.35 (m, 2H, PhCH_2), 4.32 (t, 2H, *J* = 7.4 Hz), 3.80 (s, 6H, 2× OMe), 3.80 (s, 2H), 3.74 (s, 3H, OMe), 3.04 (t, 2H, *J* = 7.4 Hz), 2.95 (t, 2H, *J* = 7.4 Hz), 2.30 (t, 2H, *J* = 6.8 Hz), 1.71 (tt, 2H, *J* = 7.4, 6.8 Hz), 1.57 (m, 4H), 1.25 (m, 2H), 1.19 (m, 4H), 1.06–0.94 (m, 10H); ^{13}C

NMR (125 MHz, D_2O) δ 156.0, 153.6, 150.4, 149.7, 136.9, 132.2, 117.7, 107.2, 88.7, 61.5, 56.7, 46.6, 43.3, 40.1, 36.9, 34.2, 29.4, 29.3, 29.2, 29.0, 28.9, 28.1, 27.4, 26.4, 25.7, 16.0; ESIMS-LR *m/z* 594.4 [(M−2HCl)H^+]; ESIMS-HR calcd for $C_{33}H_{52}N_7O_3$ 594.4132, found 594.4130 [(M−2HCl)H^+].

3.1.22. GMD-C4-dimer (7a)

A mixture of geldanamycin (6.0 mg, 0.011 mmol) and 1,4-diaminobutane (0.1 M solution in DMF; 50 μL, 5.0 μmol) in DMF (0.3 mL) was stirred at room temperature for 14 h. The solvent was removed in vacuo. The residue was purified by silica gel column chromatography (0.5 × 6.0 cm, 33% acetone/CH_2Cl_2) to give **7a** (5.7 mg, 98%) as a purple solid: ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.15 (br s, 2H), 7.29 (s, 2H), 6.95 (d, 2H, *J* = 12.5 Hz), 6.58 (dd, 2H, *J* = 12.5, 11.5 Hz), 6.24 (t, 2H, *J* = 5.7 Hz), 5.90 (m, 2H, *J* = 8.6 Hz), 5.86 (m, 2H, *J* = 11.5, 10.3 Hz), 5.19 (s, 2H), 5.00–4.50 (br s, 4H), 4.31 (d, 2H, *J* = 10.3 Hz), 4.20 (br s, 2H), 3.79–3.54 (m, 6H, *J* = 7.4 Hz), 3.45 (d, 2H, *J* = 8.6 Hz), 3.36 (s, 6H), 3.27 (s, 6H), 2.79–2.69 (m, 4H, *J* = 13.7 Hz), 2.37 (dd, 2H, *J* = 13.7, 10.9 Hz), 2.02 (s, 6H), 1.79 (br s, 14H), 1.72 (m, 2H), 0.99 (d, 6H, *J* = 7.4 Hz), 0.96 (d, 6H, *J* = 6.9 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ 184.0, 181.1, 168.5, 156.2, 144.7, 141.4, 136.1, 135.1, 133.8, 132.9, 127.1, 126.7, 109.0, 109.0, 81.8, 81.6, 81.3, 72.8, 69.7, 57.3, 56.9, 53.9, 45.4, 35.2, 34.6, 32.5, 31.9, 29.4, 28.8, 27.3, 23.1, 13.0, 12.8, 12.6, 0.1; ESIMS-LR *m/z* 1167.6 (MNa^+); ESIMS-HR calcd for $C_{60}H_{84}N_6O_{16}Na$ 1167.5842, found 1167.5817 (MNa^+).

3.1.23. GMD-C12-dimer (7b)

Compound **7b** (6.0 mg, 95%) was obtained after purification by silica gel column chromatography (0.5 × 6.0 cm, 25% acetone/CH_2Cl_2) as a purple solid as described for the synthesis of **7a**: ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.19 (br s, 2H), 7.27 (s, 2H), 6.95 (d, 2H, *J* = 11.5 Hz), 6.58 (dd, 2H, *J* = 11.5, 10.9 Hz), 6.30 (t, 2H, *J* = 5.2 Hz), 5.92 (d, 2H, *J* = 9.8 Hz), 5.86 (dd, 2H, *J* = 10.9, 10.3 Hz), 5.19 (s, 2H), 5.00–4.60 (br s, 4H), 4.39 (m, 2H), 4.29 (d, 2H, *J* = 10.3 Hz), 3.50 (m, 4H), 3.44 (m, 4H), 3.36 (s, 6H), 3.27 (s, 6H), 2.73 (m, 2H, *J* = 9.8, 7.4 Hz), 2.65 (d, 2H, *J* = 13.2 Hz), 2.43 (dd, 2H, *J* = 13.2, 10.9 Hz), 2.02 (s, 6H), 1.80 (m, 10H), 1.78–1.65 (m, 6H), 1.40 (m, 6H), 1.30–1.25 (m, 10H), 0.99 (d, 6H, *J* = 7.4 Hz), 0.96 (d, 6H, *J* = 6.9 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 184.1, 180.7, 168.6, 156.2, 145.0, 141.6, 135.9, 135.1, 133.9, 132.8, 127.1, 126.7, 108.8, 108.4, 81.8, 81.6, 81.3, 72.8, 69.7, 57.3, 56.9, 53.9, 46.0, 35.2, 34.6, 32.5, 31.9, 29.9, 29.8, 29.6, 29.5, 29.4, 29.3, 28.6, 26.9, 23.1, 12.9, 12.8, 12.5, 0.1; ESIMS-LR *m/z* 1279.7 (MNa^+); ESIMS-HR calcd for $C_{68}H_{100}N_6O_{16}Na$ 1279.7094, found 1279.7069 (MNa^+).

3.1.24. GMD-C16-dimer (7c)

Compound **7c** (5.5 mg, 84%) was obtained after purification by silica gel column chromatography (0.5 × 10 cm, 25% acetone/CH_2Cl_2) as a purple solid as described for the synthesis of **7a**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.19 (br s, 2H), 7.27 (s, 2H), 6.95 (d, 2H, *J* = 11.3 Hz), 6.58 (dd, 2H, *J* = 11.3, 10.9 Hz), 6.30 (t, 2H, *J* = 5.5 Hz), 5.90 (d, 2H, *J* = 9.5 Hz), 5.86 (dd, 2H, *J* = 10.9, 9.5 Hz), 5.19 (s, 2H), 5.00–4.60 (br s, 4H), 4.40 (br s, 2H), 4.29 (d, 2H, *J* = 9.5 Hz), 3.55 (m, 4H), 3.44 (m, 4H), 3.36 (s, 6H), 3.26 (s, 6H), 2.73 (m, 2H, *J* = 9.5, 7.3 Hz), 2.66 (d, 2H, *J* = 13.2 Hz), 2.43 (dd, 2H, *J* = 13.2, 10.9 Hz), 2.02 (s, 6H), 1.79 (m, 10H), 1.72 (m, 6H), 1.40–1.26 (m, 24H), 0.99 (d, 6H, *J* = 7.3 Hz), 0.96 (d, 6H, *J* = 6.4 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 184.1, 180.7, 168.6, 156.2, 145.0, 141.6, 135.9, 135.1, 134.0, 132.9, 127.0, 126.7, 108.8, 108.4, 81.8, 81.6, 81.4, 72.7, 69.7, 57.3, 56.9, 53.9, 46.0, 41.1, 35.2, 34.6, 32.4, 31.9, 29.9, 29.8, 29.8, 29.7, 29.6, 29.4, 29.3, 28.6, 26.9, 23.1, 12.9, 12.8, 12.5, 0.1; ESIMS-LR *m/z* 1335.8 (MNa^+); ESIMS-HR calcd for $C_{72}H_{108}N_6O_{16}Na$ 1335.7720, found 1335.7705 (MNa^+).

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2008.04.070.

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