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Construction of polyamine-modified uridine and adenosine derivatives—evaluation of DNA binding capacity and cytotoxicity in vitro

Johannes Ghatnekar,[a] Margareta Hägerlöf,[b] Stina Oredsson,[c] Kersti Alm,[c] Sofi K. C. Elmroth[b,*] and Tina Persson[a,†]

[a]*Organic Chemistry, Chemical Center, Lund Univesity, PO Box 124, SE-221 00 Lund, Sweden*
[b]*Biochemistry, Chemical Center, Lund University, PO Box 124, SE-221 00 Lund, Sweden*
[c]*Cell and Organism Biology, Lund University, Helgonav. 3B, SE-223 62 Lund, Sweden*

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Abstract—We here report the synthesis of the two polyamine-based nucleoside derivatives 5-{[bis-(3-aminopropyl)amino]acetamido-1-propynyl}uridine and 2-{[bis-(3-aminopropyl)amino]-acetamido-1-propynyl}adenosine. The various polyamine derivatives have been used in thermal melting analysis using DNA from herring testes, and in cellular studies using four different cell lines. The compounds were all found to be non-toxic, thus holding good promise for future use as siRNA building blocks.

1. Introduction

Polyamines are small aliphatic organic molecules with positive character that are present in prokaryotic and eukaryotic cells.[1] The naturally occurring polyamines putrescine, spermidine and spermine are involved in multiple cellular functions.[2,3] Many studies support that polyamines are involved in cell growth and differentiation,[4,5] but the mechanisms of action are still poorly understood. Polyamines are also involved in chromatin condensation, maintenance of DNA and RNA structure, RNA processing, translation, and protein activation.[6,7] Recently, functionalized polyamines have been shown to bind specifically to structured RNA indicating that polyamines might play a regulatory role on RNA level in the cellular machinery.[8] Thus, development of polyamine derivatives is of interest for future design of polyamine-based drugs acting on RNA level. Because of their simple structure and multiple roles, further studies of analogues of the natural polyamines are important, both from a medicinal chemistry perspective and to obtain a more detailed fundamental understanding of their mode of action in vivo. In the latter context, modified polyamine derivatives are of particular interest for potential use as inhibitors of the endogenous polyamine biosynthetic pathway. In this study, the synthesis and activity of a series of novel polyamine analogues is reported. The polyamine structure has been altered by chemical synthesis, and the analogues have been studied with respect to their affinity to DNA, their influence on DNA structure, and evaluated with respect to their growth inhibitory activity in four different breast cancer cell lines.[9]

2. Results and discussion

2.1. Chemical synthesis

The polyamine group was connected to the nucleoside via linker to the C-5 position in the heterocyclic moiety of uridine and to the C-2 position in the heterocyclic

Abbreviations: *t*-Boc, *tert*-butylcarbamate; DCC, *N,N*-Dicyclohexylcarbodiimide; DIEA, diisopropylethylamine; TFA, trifluoroacetic acid; HCl, hydrochloric acid; DCM, dichloromethane; Et_3N, triethylamine; DMF, dimethylformamide; PFP, pentafluorophenol; MeOH, methanol; RP C-18, reversed-phase C-18.

Keywords: Modified nucleoside; Adenosine; Uridine; Polyamine derivatives.

* Corresponding author. Tel.: +46 46 222 3692; fax: +46 46 222 4116; e-mail addresses: sofi.elmroth@biochemistry.lu.se; tina.persson@organic.lu.se
† Tel.: +46 46 222 8210; fax: +46 46 222 8209.

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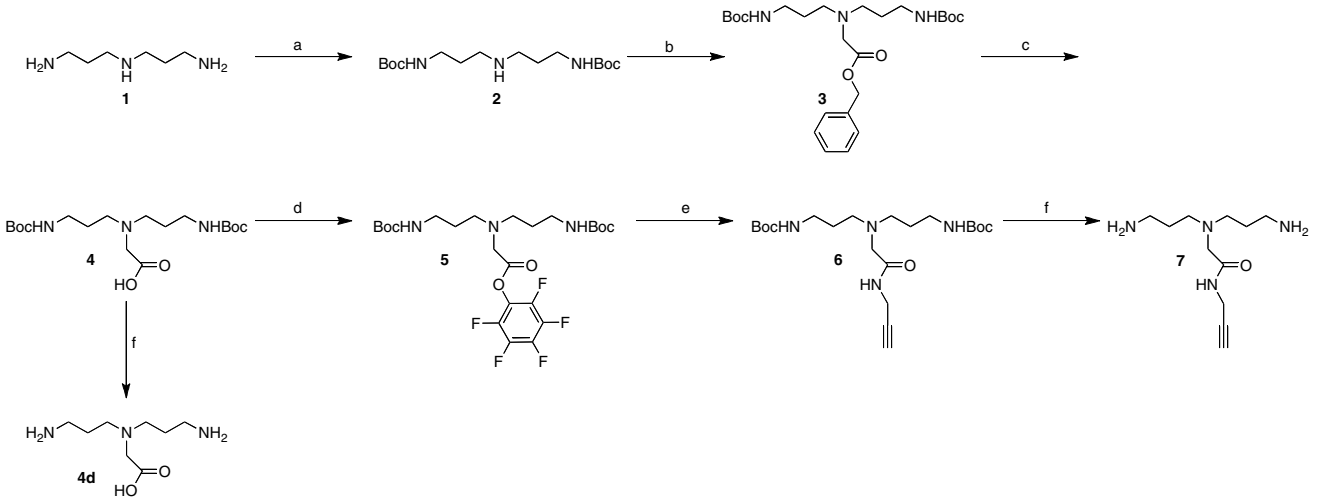
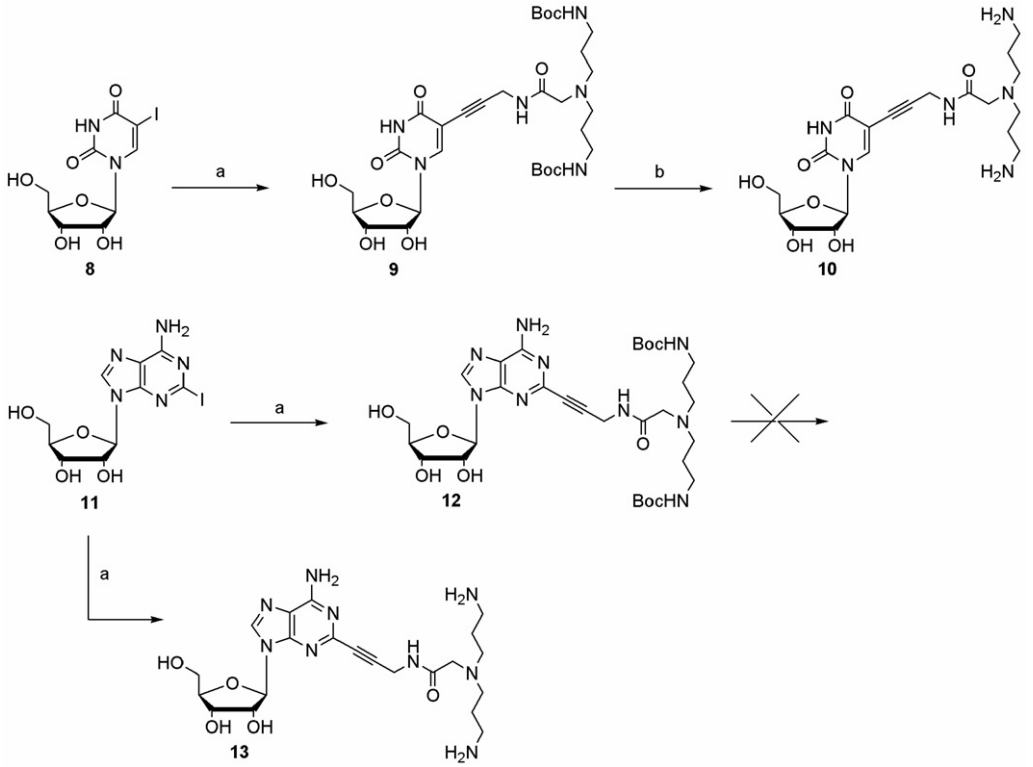
moiety of adenosine, respectively.[10] In the first step, the polyamine linker was functionalized with a triple bond enabling Pd-catalysed coupling reactions to 5-iodouridine and 2-iodoadenosine, respectively, using the Sonogashira reaction.[11] The starting material 5-iodouridine was commercially available, but the corresponding 2-iodoadenosine was synthesized in four steps according to the literature.[12–14] In the first step, toward polyamine derivative **7** (Scheme 1), the primary amine functions in the commercially available polyamine 3,3′-diaminodipropylamine **1** were protected using *t*-Boc groups[15,16] producing compound **2** in 84% yield. The *t*-Boc groups were used as they are stable toward hydrogenation and basic reaction conditions. In the next step, benzyl bromo acetate was used as alkylation reagent in the presence of DIEA using DMF as solvent. Purification of **3** by silica gel column chromatography using EtOAc/heptane (70:30) as eluent produced white crystals in 97% yield. The benzyl protecting group was removed by hydrogenation at 60 psi using MeOH as solvent.[17,18] After evaporation, a white foam was formed and polyamine derivative **4** was obtained in 90% yield. Formation of the carboxylic acid is essential in order to generate the desired amide bond between the *N*-propargylic amine and the polyamine derivative. For our purpose, the amide bond was chosen since it is more stable toward hydrolysis in aqueous media. Prior to connecting *N*-propargylamine, the carboxylic acid was activated by reacting with pentafluorphenol in the presence of DCC.[19] The reaction mixture was stirred overnight, whereupon it was filtered through a Celite pad. The resulting mother liquid was evaporated to dryness and the crude product **5** was used in the next step without further purification. The activated polyamine derivative **5** was reacted with *N*-propargylamine in the presence of DIEA, and stirred under an inert atmosphere overnight.[11] The product was purified by silica gel column chromatography using EtOAc/Heptane (80:20) as eluent producing **6** in 61% yield. Deprotection of polyamine derivatives **4** and **6** was accomplished by using TFA in DCM giving the deprotected polyamine derivatives **4d** and **7** in 95% yields. The polyamine derivative **6** was successfully connected to 5-iodouridine **8** and 2-iodoadenosine **11**, respectively, by using Sonogashira reaction conditions (Scheme 2).

Nucleoside derivatives **8** and **11** were dissolved in dry DMF, treated with CuI, $Pd(PPh_3)_4$ and reacted with **6** overnight at room temperature. The corresponding polyamine-based nucleoside analogues **9** and **12** were purified by silica gel column chromatography using a mixture of DCM/MeOH (9:1) and DCM/MeOH (98:2–9:1), respectively, as eluent. The Pd-cross coupling reaction between the polyamine derivative **6** and 5-iodouridine **8** or 2-iodoadenosine **11** resulted in 95 and 51% yields of 9 and 12, respectively.

Removal of the *t*-Boc groups was first performed with the uridine derivative **9** using TFA and anisole as reagents. The resulting oil was dissolved in water and washed with DCM, whereupon the aqueous phase was evaporated to dryness under reduced pressure. The product **10** was obtained as a brown oil in a yield of about 25%. Exposure of the corresponding adenosine derivative **12** to TFA and anisole resulted in a brown syrup-like mixture which was difficult to dissolve in water. Various attempts to separate the produced product mixture failed. Therefore, an alternative approach was considered with the aim to couple the deprotected polyamine derivative **7** to 2-iodoadenosine **11**. By using the above described Sonogashira reaction conditions, the polyamine-based adenosine analogue **13** was obtained in 19% yield. To simplify the purification procedure, a slight excess of **11** over the deprotected polyamine derivative **7** was used and after RP C-18 column chromatography using MeOH as eluent. Analysis of ^{1}H NMR indicated correct product formation and removal of impurities. Further purification was accom-



Scheme 1. Reagents and conditions: (a) **1**, *tert*-butyl phenyl carbonate, DMF, rt, 12 h, 84%; (b) **2**, benzyl bromoacetate, DMF, DIEA, rt, 4 h, 97%; (c) **3**, Pd/C 10%, MeOH, 60 psi, rt, 22 h, 90%; (d) **4**, solution; i—pentafluorophenol, EtOAc, solution; ii—DCC, EtOAc; combine (i) and (ii) and react at rt; used in the next step without purification; (e) **5**, solution; i—DIEA, DCM solution; ii—*N*-propargylamine, DCM, combine (i) and (ii) and react at rt, 61%; (f) **6**, TFA, DCM, rt, 95%.



Scheme 2. Reagents and conditions: (a) **6** or **7**, $Pd(PPh_3)_4$, CuI, DMF, Et_3N, rt; (b) **9**, TFA, anisole, MeOH, rt, 10%.

plished by using RP C-18 column chromatography and H_2O as eluent producing the polyamine-based analogue **13** as a yellow oil in 19% yield.

2.2. Thermal melting analysis

The thermal melting point (T_m) of DNA from herring testes (Sigma) was determined to be 65 °C in 10 mM NaCl, which is comparable to results obtained by others.[20] Thermal melting studies were further performed with DNA from herring testes in the presence of polyamines **1**, **4d**, **7**, **10** and **13**, see Schemes 1 and 2 for illustrations. In the presence of **1**, the T_m was increased by 12 °C to 77 °C as expected from theory and previous reports.[21] In the presence of **4d** and **7**, the T_m was however decreased (Fig. 1). The effect can be ascribed to the presence of the tertiary substituted central N-atom of **4** and **7** which introduces more steric constrains on the interaction with DNA. The electron density at the central N is also changed. In contrast, the modified nucleosides **10** and **13** did not have any effect on the melting temperature (Table 1). However, a careful inspection of the melting curves obtained for these two latter compounds shows a slightly altered profile above T_m, indicating a slower transition from duplex to single-stranded coil for these compounds, see Figure 1. Only minor pH changes of the solution were observed after addition of the polyamines, for example, an increase of 0.06 pH units after addition of **1** and decrease of 0.08 and 0.14 units for **4d** and **7**, respectively.

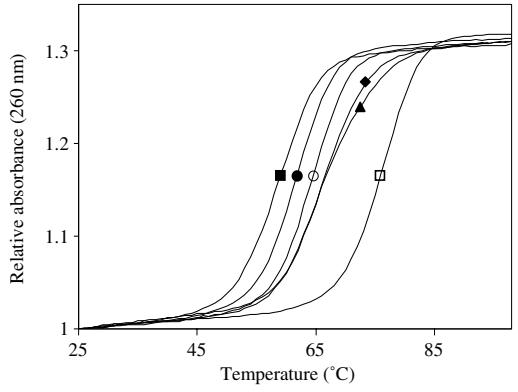


Figure 1. Absorbance versus temperature profiles of herring testes DNA alone (○) and in the presence of **1** (□), **4d** (●), **7** (■), **10** (♦) and **13** (▲). The DNA concentration was kept to 100 μM, and the concentration of the polyamines was 10 μM. The measurements were performed in 10 mM NaCl.

Table 1. Thermal melting points for herring testes DNA in the presence of the polyamines **1**, **4d**, **7**, **10** and **13**

Polyamine	Conc (μM)	T_m (°C)	ΔT_m (°C)
—	—	65	—
1	10	77	12
4d	1	62	−3
	10	61	−4
	100	50	−15
7	10	58	−7
	50	52	−13
	100	51	−14
10	10	66.5	1.5
13	10	66	1

The concentration of DNA was typically 100 μM and the measurements were performed in 10 mM NaCl.

2.3. Circular dichroism

The influence of compounds **1**, **4d**, **7**, **10**, and **13** on the circular dichroism (CD) spectra of B-DNA[22] is shown in Figure S1. After addition of the polyamines, the spectra show the characteristics of B-DNA, that is, a positive peak at 275 nm, a cross-over at 258 nm, and a negative peak at 246 nm. The polyamine with the largest effect on DNA is **1**, which induces both a higher peak at 275 nm and an elevated minimum at 246 nm. Polyamines **4d** and **13** induce similar spectral changes as does **1**, while the effect of **7** and **10** are slightly less pronounced.

2.4. Cellular studies

In the MTT assay, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) is added to the medium of the cells in the microwell. After removal of the MTT containing medium, the formazan crystals formed were dissolved by addition of dimethyl sulfoxide (DMSO) and the absorbance was monitored. The amount of formazan generated is assumed to be directly proportional to the cell number when using homogenous cell populations.[23–25]

The toxicity of **4d**, **7**, **10** and **13** on four human breast cancer cell lines is shown in Figure 2.

As can be seen here, none of the compounds exhibited strong cytotoxicity in any of the cell lines studied. The figure also shows that there are no clear patterns in the response to the compounds. However, the general conclusion may be drawn that HCC1937 and L56Br-C1 show a more similar response than the other two cell lines. SK-BR-3 cells appear to be the least responsive, while even stimulation of MTT reduction was found in MCF-7 cells.

3. Conclusions

In summary, we synthesized two new polyamine-based nucleoside derivatives. Both nucleoside derivatives and their corresponding polyamine precursors were shown to have a limited influence on DNA structure and thermal stability. In comparison to the unmodified 3,3′-diaminodipropylamine **1**, the polyamine derivatives **4d** and **7** were found to have a slight destabilizing effect on the DNA structure, whereas the nucleoside derivatives **10** and **13** had little effect. None of the polyamine derivatives showed any toxic effect in the breast cancer cell lines. The compounds thus seem to be well suited for further work aiming at synthetic incorporation of these bases into, e.g., modified siRNAs, since the toxic side effects arising from tentative degradation products are likely to be minimal.

4. Experimental

4.1. General information

^{1}H NMR was recorded on a Bruker DRX400 spectrometer operating at 400 MHz for ^{1}H and 100.6 MHz for ^{13}C. A Sanyo Gallenkamp melting point apparatus was used for determination of the melting points of the polyamine derivatives. All melting points are uncorrected. IR spectra were recorded on a Nicolet Avastar 360 FT-IR ESP. TLC was carried out on alumina plates covered with silica (Merck 60 F^{254}). Ninhydrin 1% was used for visualization of polyamine derivatives. Column

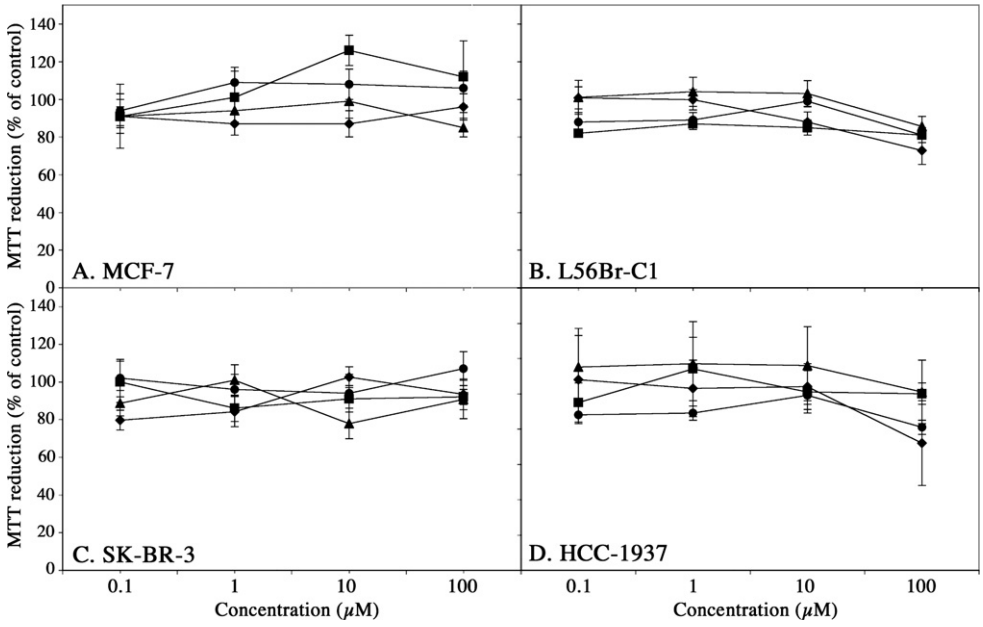


Figure 2. Effect of compounds **4d** (●), **7** (■), **10** (♦) and **13** (▲) on four human breast cancer cell lines evaluated with a MTT assay. Cells were seeded in 96-well plates and incubated for 24 h before addition of the compounds to the final concentrations (shown in the figure). After 48 h of treatment, the effects were evaluated using MTT assay. Each data point is the mean value of 12–18 independent wells from 2 to 3 independent experiments.

chromatography was performed on silica 60 Å (0.35–0.70) and silica C18—reversed phase, 17% C (40–63 μm). Celite 545 was used as media in various filtration steps. All solvents were of PA quality and prior to use dried over molecular sieves 4 Å. Chemicals and solvents were purchased from Aldrich and Acros. DNA from herring testes was purchased from Sigma, and the concentration was determined spectroscopically using the average molecular extinction coefficient per DNA base, $\varepsilon_{260} = 6521\ M^{-1}\,cm^{-1}$.[26] 3,3′-Diaminodipropylamine **1** was purchased from Aldrich. Concentrations of **4d** and **7** were determined by use of molecular weights of 186.12 g mol^{-1} for **4d** and 262.32 g mol^{-1} for **7**, respectively.

4.2. Synthesis of Bis-(3-*tert*-butoxycarbonyl-aminopropyl)amine (2)

Compound **2** was synthesized according to the literature procedure. The isolated product was obtained as white crystals in a yield of 84% (8.0 g). Analysis of the product was made according to the literature.[15]

4.3. Synthesis of [Bis-(3-*tert*-butoxycarbonyl-aminopropyl)amino]acetic acid benzylester (3)

Compound **2** (4.0 g, 12 mmol) and 9 ml DMF were dissolved in a three-necked flask under stirring. The mixture was cooled by using an ice bath followed by addition of benzylbromo acetate (2.4 ml, 15 mmol) and DIEA (2.1 ml, 12 mmol) over a time period of 30 min. The resulting reaction mixture was stirred at 0 °C for additional 30 min, whereupon it was allowed to reach rt. The reaction mixture was reacted for additional 4 h at rt. Ether (50 ml) was added and the formed white precipitate was collected by filtration and the mother liquid was evaporated to dryness. The produced oil was dissolved in 50 ml DCM and carefully washed sequentially with 3 × 40 ml water, 2 × 40 ml 1 M HCl, and 3 × 40 ml water. The organic phase was dried over Na_2SO_4 followed by evaporation to dryness. The crude product was purified by silica gel column chromatography using EtOAc/Heptane (70:30) as eluent and **3** was obtained in a yield of 5.6 g (97%); IR (KBr, cm^{-1}): 1733 (C=O); ^{1}H NMR (400 MHz, $CDCl_3$, ppm): δ 1.43 (s, 18H), 1.60 (m, J = 6.4, 6.3 Hz, 4H), 2.57 (t, J = 6.5 Hz, 4H), 3.17 (m, J = 5.9 Hz, 4H), 3.32 (s, 2H), 5.15 (s, 2H), 5.28 (bs, 2H), 7.34 (m, 5H); ^{13}C NMR (100.6 MHz, $CDCl_3$, ppm): δ 27.5, 28.7, 40.0, 52.3, 55.1, 66.6, 79.1, 128.6, 128.8, 135.8, 156.3, 171.4; M.p. 69.9-71.5°C; HRMS (FAB$^+$) m/z calcd for $C_{25}H_{41}O_6N_3$: 479.2995. Found: 480.3071 (M+H)$^+$.

4.4. Synthesis of [Bis-(3-*tert*-butoxycarbonyl-aminopropyl)amino]acetic acid (4)

Compound **3** (1.7 g, 3,5 mmol), Pd/C 10% (262 mg, 15 wt%), and 80 ml MeOH were mixed in a sealed tube, whereupon hydrogen pressure of 60 psi was applied. The mixture was reacted for 22 h at rt. The resulting reaction mixture was filtered through a Celite pad and carefully washed with 100 ml MeOH. The mother liquid was evaporated to dryness and a white product was produced. Compound **4** was obtained in a yield of 1.2 g (90%); IR (KBr, cm^{-1}): 1696 (C=O); ^{1}H NMR (400 MHz, CD_3OD, ppm): δ 1.44 (s, 18H), 1.87 (m, J = 7.6, 7.1, 6.3 Hz, 4H), 3.17 (m, J = 6.6 Hz, 8H), 3.62 (s, 2H); ^{13}C NMR (100.6 MHz, CD_3OD, ppm): δ 26.2, 28.9, 38.3, 54.7, 57.0, 80.5, 158.9, 170.2; HRMS (FAB$^+$) m/z calcd for $C_{18}H_{35}O_6N_3$: 389.2526. Found: 390.2605 (M+H)$^+$. Compound **4d**, used for cellular, thermal, melting, and CD studies, was obtained after addition of TFA to **4** as described below (Section 4.7).

4.5. Synthesis of [Bis-(3-*tert*-butoxycarbonyl-aminopropyl)amino]acetic acid 2,3,4,5,6-pentafluorophenyl ester (5)

Compound **4** (1.2 g, 3.2 mmol) and pentafluorophenol (0.77 g, 4.2 mmol) were dissolved in 11 ml dry EtOAc. In a second flask, DCC (890 mg, 4.3 mmol) was dissolved in 2 ml EtOAc. Each flask was cooled on ice bath while stirring under an argon atmosphere. The two solutions were combined, the reaction mixture was stirred overnight and the reaction temperature was allowed to slowly reach room temperature. The resulting reaction mixture was cooled on an ice bath and filtered through a Celite pad and carefully washed with EtOAc. The mother liquid was evaporated resulting in a clear viscous oil. The product was used in the next step without further purification and analysis.

4.6. Synthesis of [Bis-(3-*tert*-butoxycarbonyl-aminopropyl)amino]acetamido-1-propynyl (6)

Compound **5** (2.3 g, 4.1 mmol) and DIEA (1.4 ml, 8.2 mmol) were dissolved in 13 ml DCM. In parallel, a solution of *N*-propargylamine (0.53 ml, 8.2 mmol) was dissolved in 3.5 ml DCM. The two solutions were mixed and reacted for 12 h at rt under argon atmosphere. The resulting reaction mixture was concentrated under reduced pressure, and the crude product was purified by silica gel column chromatography using EtOAc/Heptane (80:20) as eluent. The product was obtained as a red oil in a yield of 61% (906 mg); IR (NaCl, cm^{-1}): 2114; ^{1}H NMR (400 MHz, $CDCl_3$, ppm): δ 1.41 (s, 18H), 1.59 (m, J = 6.9 Hz, 4H), 2.20 (t, J= 2.4 Hz, 1H), 2.47 (t, J = 7 Hz, 4H), 3.00 (s, 2H), 3.14 (m, J = 6.2 Hz, 4H), 4.04 (dd, J = 3.2, 2.5 Hz, 2H), 4.87 (br s, 2H), 7.59 (br s, 1H); ^{13}C NMR (100.6 MHz, $CDCl_3$, ppm): δ 27.8, 28.6, 28.7, 38.5, 50.7, 58.7, 71.3, 79.4, 79.9, 156.3, 171.6; HRMS (FAB$^+$) m/z calcd for $C_{21}H_{38}O_5N_4$: 426.2842. Found: 427.2933 (M+H)$^+$.

4.7. Synthesis of [Bis-(3-aminopropyl)amino]acetamido-1-propynyl (7)

TFA (2.2 ml, 28.6 mmol) was slowly added to a flask containing **6** (497 mg, 1.16 mmol) dissolved in 10 ml dry DCM, whereupon the mixture was stirred overnight at rt. The reaction mixture was evaporated to dryness under reduced pressure resulting in a brown oil in a yield of 95% (249 mg); IR (KBr, cm^{-1}): 2131; ^{1}H NMR (400 MHz, CD_3OD, ppm): δ 1.85 (m, J = 6.9 Hz, 4H), 2.63 (t, J = 2.5 Hz, 1H), 2.67 (t, J = 6.8 Hz, 4H), 3.02 (t, J = 7.0, 4H), 3.28 (s, 2H), 4.01 (d, J = 2.5 Hz, 2H); ^{13}C NMR (100.6 MHz, CD_3OD, ppm): δ 27.8, 29.9,

37.9, 53.7, 55.5, 73.1, 79.8, 165.8; HRMS (FAB^+) *m/z* calcd for $C_{11}H_{22}ON_4$: 226.1794. Found: 227.1881 $(M+H)^+$.

4.8. Synthesis of 5-{[Bis-(3-*tert*-butoxycarbonyl-aminopropyl)amino]acetamido-1-propynyl}uridine (9)

Compound **8** (159 mg, 0.43 mmol), CuI (28.6 mg, 0.15 mmol), $Pd(PPh_3)_4$ (48.5 mg, 42 μmol), and compound **6** (526 mg, 1.23 mmol) were dissolved in 1 ml DMF and Et_3N (114 μl, 0.82 mmol). After dissolution, 4 ml DMF was added and the reaction was stirred under argon atmosphere for 22 h at rt. The reaction mixture was concentrated under reduced pressure, and the crude product was purified by silica gel column chromatography using DCM/MeOH (9:1) as eluent. The product was isolated as yellow crystals in a yield of 95% (273 mg); IR (KBr, cm^{-1}): 3062; ^1H NMR (400 MHz, CD_3OD, ppm): δ 1.43 (s, 18H), 1.64 (dd, J = 6.9, 6.6 Hz, 4H), 2.53 (t, J = 7.1, 6.8 Hz, 4H), 3.10 (t, J = 7.1, 6.6 Hz, 6H), 3.75 (dd, J = 9.5, 2.7 Hz, 1H), 3.88 (dd, J = 9.6, 2.6 Hz, 1H), 4.00 (m, 1H), 4.16 (m, 2H), 4.20 (s, 2H), 5.88 (d, J = 3.6 Hz, 1H), 8.37 (s, 1H); ^{13}C NMR (100.6 MHz, CD_3OD, ppm): δ 28.6, 29.1, 30.2, 39.5, 53.9, 59.3, 62.1, 71.2, 75.1, 76.2, 80.1, 86.6, 90.4, 91.1, 100.2, 145.7, 151.6, 158.7, 164.6, 174.3; mp 82.1–88.1 °C; HRMS (FAB^+) *m/z* calcd for $C_{30}H_{48}O_{11}N_6$: 668.3381. Found: 669.3483 $(M+H)^+$.

4.9. Synthesis of 2-{[Bis-(3-*tert*-butoxycarbonyl-aminopropyl)amino]acetamido-1-propynyl}adenosine (12)

Compound **11** (152 mg, 0.39 mmol), CuI (25 mg, 0.13 mmol), $Pd(PPh_3)_4$ (45 mg, 0.039 mmol), and compound **6** (496 mg, 1.16 mmol) were dissolved in 1 ml DMF and Et_3N (107 μl, 0.77 mmol). After dissolution, 4 ml DMF was added and the reaction mixture was stirred at rt for 17 h under argon atmosphere. The produced mixture was evaporated to dryness and the crude product was purified by silica gel column chromatography using a gradient of DCM/MeOH (98:2–9:1) as eluent. The isolated product was obtained in a yield of 51% (136 mg); IR (KBr, cm^{-1}): 2246; ^1H NMR (400 MHz, CD_3OD, ppm): δ 1.39 (s, 18H), 1.67 (dd, J = 7.1, 6.7 Hz, 4H), 2.56 (t, J = 7.0, Hz, 4H), 3.11 (t, J = 6.7 Hz, 4H), 3.14 (s, 2H), 3.75 (dd, J = 9.9, 2.7 Hz, 1H), 3.88 (dd, J = 10.1, 2.5 Hz, 1H), 4.17 (q, J = 2.6, 2.5 Hz, 1H), 4.28 (s, 2H), 4.33 (q, J = 2.7, 2.3 Hz, 1H), 4.70 (t, J = 6.0, 5.3 Hz, 1H), 5.93 (d, J = 6.2, Hz, 1H), 8.34 (s, 1H); ^{13}C NMR (100.6 MHz, CD_3OD, ppm): δ 28.5, 28.9, 29.8, 39.5, 54.0, 59.3, 63.6, 72.6, 75.6, 80.1, 82.2, 83.5, 88.2, 91.3, 120.6, 142.9, 147.0, 150.2, 157.4, 158.7, 174.2; mp 92.2–102.6 °C; HRMS (FAB^+) *m/z* calcd for $C_{31}H_{49}O_9N_9$: 691.3653. Found: 714.3559 $(M+Na)^+$.

4.10. Synthesis of 5-{[Bis-(3-aminopropyl)amino]acetamido-1-propynyl}uridine (10)

Compound **9** (54 mg, 80 mmol), TFA (300 μl, 4.0 mmol) and anisole (150 μl, 1.4 mmol) were dissolved in 4 ml dry MeOH. The reaction mixture was stirred overnight at rt and evaporated to dryness under reduced pressure. The obtained oil was dissolved in water and extracted with 3 × 5 ml DCM. The aqueous phase was evaporated to dryness and the produced brown oil was dissolved in MeOH, 1 ml DCM, whereupon about 110 mg Dowex 1 × 8 − 200 was added to the solution. The mixture was stirred for additional 30 min followed by filtration and evaporation to dryness. The resulting product was obtained in 25% yield; ^1H NMR (400 MHz, CD_3OD, ppm): δ 1.70 (m, J = 6.8, 6.7 Hz, 4H), 2.55 (t, J = 6.9 Hz, 4H), 2.77 (t, J = 6.7, Hz, 4H), 3.13 (s, 2H), 3.74 (dd, J = 9.4, 2.9, Hz, 1H), 3.86 (dd, J = 9.9, 2.5 Hz, 1H), 4.00 (m, 1H), 4.14 (m, J = 3.2 Hz, 2H), 4.20 (s, 2H), 5.89 (bs, 1H), 8.23 (s, 1H); ^{13}C NMR (100.6 MHz, CD_3OD, ppm): δ 24.5, 30.6, 38.8, 53.7, 56.7, 62.1, 71.2, 75.5, 76.2, 86.7, 89.8, 91.1, 100.0, 145.7, 151.5, 164.6, 174.1; HRMS (FAB^+) *m/z* calcd for $C_{20}H_{32}O_7N_6$: 468.2332. Found: 469.2405 $(M+H)^+$.

4.11. Synthesis of 2-{[Bis-(3-aminopropyl)amino]acetamido-1-propynyl}adenosine (13)

Compound **11** (150 mg, 0.38 mmol), CuI (24 mg, 0.13 mmol), $Pd(PPh_3)_4$ (44 mg, 38 μmol) were dissolved in 1 ml DMF and Et_3N (105 μl, 0.75 μmol). Compound **7** dissolved in 4 ml DMF was added to the mixture and the resulting solution was stirred overnight under an argon atmosphere. The solvent was removed by reduced pressure and the resulting brown oil was dissolved in water and purified by reversed-phase column chromatography, C-18, using MeOH as eluent. A second purification was accomplished made on C-18 column using H_2O as eluent. The product was isolated as yellow oil in a yield of 19% (35.0 mg); ^1H NMR (400 MHz, CD_3OD, ppm): δ 1.88 (m, J = 6.7, 6.5 Hz, 4H), 2.66 (t, J = 6.5 Hz, 4H), 3.06 (t, J = 6.8 Hz, 4H), 3.28 (s, 2H), 3.75 (dd, J = 10.0, 2.5 Hz, 1H), 3.87 (dd, J = 10.2, 2.3, 2.0, Hz, 1H), 4.17 (m, J = 2.3 Hz, 1H), 4.27 (m, J = 2.5, 2.4 Hz, 2H), 4.33 (t, J = 2.4, 2.5 Hz, 1H), 4.68 (t, J = 5.8, 5.5 Hz, 1H), 6.96 (d, J = 6.3 Hz, 1H), 8.33 (s, 1H); ^{13}C NMR (100.6 MHz, CD_3OD, ppm): δ 25.5, 30.0, 39.8, 53.6, 58.0, 63.6, 72.7, 75.8, 82.2, 83.3, 88.3, 91.0, 116.9, 142.8, 146.9, 150.2, 157.4, 173.8; HRMS (FAB^+) *m/z* calcd for $C_{21}H_{33}O_5N_9$: 491.2605. Found: 492.2693 $(M+H)^+$.

4.12. Melting experiments

Thermal melting profiles were obtained in 10 mM NaCl to be directly comparable with previous investigations.[20] Melting temperatures were determined for DNA from herring testes alone, and herring testes DNA in the presence of compounds **1**, **4d**, **7**, **10** and **13**. The concentration of DNA was typically 100 μM and the concentration of polyamine was 10 μM. Measurements were performed using a Cary 300 Bio spectrophotometer at 260 nm. The temperature was increased at a rate of 0.4 °C/min over a range of 25–98 °C, allowing the system to stabilize for 15 min before taking the first reading. Readings were taken at every degree using a signal average time of 2 s. The thermal melting point, T_m, was determined from the peak of the first derivative of the absorbance versus temperature curves (Figure

S2). The pH was measured before and after addition of the polyamines.

4.13. Circular dichroism

Circular dichroism measurements (CD) of DNA were performed in the presence of the polyamines **1**, **4d**, **7**, **10**, and **13**. The concentration of DNA was 100 μM and the concentration of the polyamines was 10 μM. The measurements were performed in 10 mM NaCl at ambient temperature. All spectra were collected four times consecutively on a Jasco J600 Spectropolarimeter and an average was taken. Scans were obtained from 320 to 220 nm at a rate of 20 nm/min in a 3-ml cuvette with a path length of 1 cm. The ellipticity given, θ (mdeg), was converted to the decadic molar extinction coefficient, $\Delta\varepsilon$, by first subtracting the base line and then applying the following equation,

$$\Delta\varepsilon = \frac{\theta}{32.98 * cl} \quad (1)$$

where c is the concentration in $\mathrm{mol} \times \mathrm{dm}^{-3}$ and l is the path length in cm.

4.14. Cellular experiments

4.14.1. Materials. Growth medium components were purchased from Biochrom, Berlin, Germany. Tissue culture plastics were purchased from Nunc, Roskilde, Denmark. 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) was purchased from ICN Biomedicals Inc., Aurora, OH, USA. Phosphate-buffered saline (PBS: 8 g/l NaCl, 0.2 g/l KCl, 1.15 g/l Na_2HPO_4, 0.2 g/l KH_2PO_4, pH 7.3) was purchased from Oxoid Ltd., Basingstoke, Hampshire, United Kingdom. DMSO was purchased from Merck KGaA, Darmstadt, Germany. The MCF-7 (HTB-22), HCC1937 (CRL-2336), and SK-BR-3 (HTB-30) cell lines were purchased from American Type Culture Collection, Manassas, VA, USA. The L56Br-C1 cell line was established in Lund.[9]

4.14.2. Cell culture experiments. All cell lines were cultured at 37 °C, in a humidified incubator with 5% CO_2 in air. The human epithelial adenocarcionoma breast cancer cell line MCF-7 was cultured in RPMI 1640 medium supplemented with 10% fetal calf serum (FCS), non-essential amino acids, insulin (10 μg/ml), penicillin (50 U/ml), and streptomycin (50 mg/ml). The human adenocarcinoma breast cancer cell line SK-BR-3 was cultured in the same medium as MCF-7 cells, but without the addition of insulin. The human carcinoma breast cancer cell line HCC1937[27] was cultured in MEM α-medium supplemented with 10% heat-inactivated FCS, non-essential amino acids, gentamycin (0.1 mg/ml), epidermal growth factor (EGF) (20 ng/ml) and insulin (10 μg/ml). The human breast cancer cell line L56Br-C1 was cultured in RPMI 1640 medium supplemented with 10% heat-inactivated FCS, non-essential amino acids, insulin (10 μg/ml), penicillin (50 U/ml), and streptomycin (50 rmug/ml).

4.14.3. MTT assay. Cells in late plateau phase were trypsinized, counted, and resuspended to a final concentration of 0.055×10^6 cells per millilitre. A 180-μl aliquot of the cell suspension was seeded per well in 96-well plates. After 24 h of incubation, the compounds to be tested were added in 20 μl aliquots to the medium to give a final concentration of 0.1, 1, 10, or 100 μM. The compounds were dissolved in H_2O or PBS as 4 or 20 mM stock solutions. The stock solutions were sterile-filtered and stored at −20 °C. Further dilutions before addition to the 96-well plates were done in PBS. Controls received 20 μl PBS. At 24, 48, and 72 h after addition of compounds, 20 μl of a MTT solution was added to each well and the 96-well plates were returned to the incubator for 1 h. The sterile-filtered MTT solution (5 mg/ml in PBS) was stored protected from light at −20 °C until usage. Then, the MTT containing medium was removed and each well was gently washed with PBS. The blue formazan product was dissolved by the addition of 100 μl of 100% DMSO per well. The plates were swirled gently for 10 min to dissolve the precipitate. Absorbance was monitored at 540 nm using a Labsystems iEMS Reader MF (Labsystems Oy, Helsinki, Finland) and the software DeltaSoft II v. 4.14 (Biometallics Inc., Princeton, NJ, USA). The outermost wells were not used for the calculations. The values were expressed as percentage of control ± one standard deviation.

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Supplementary data

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References and notes

1. Tabor, C. W.; Tabor, H. *Annu. Rev. Biochem.* **1984**, *53*, 749–790.
2. Oredsson, S. M. *Biochem. Soc. Trans.* **2003**, *31*, 366–370.
3. Williams, L. J.; Barnett, G. R.; Ristlow, J. L.; Pitkin, J.; Perriere, M.; Davis, R. H. *Mol. Cell. Biol.* **1992**, *12*, 347–359.
4. Thomas, T.; Thomas, T. J. *Cell. Mol. Life Sci.* **2001**, *58*, 244–258.
5. Wallace, H. M.; Fraser, A. V.; Hughes, A. *Biochem. J.* **2003**, *376*, 1–14.
6. Childs, A. C.; Mehta, D. J.; Gerner, E. W. *Cell. Mol. Life Sci.* **2003**, *60*, 1394–1406.
7. Yoshida, M.; Kashiwagi, K.; Shigemasa, A.; Taniguchi, S.; Yamamoto, K.; Makinoshima, H.; Ishihama, A.; Igarashi, K. *J. Biol. Chem.* **2004**, *279*, 46008–46013.
8. Lawton, G. R.; Appella, D. H. *J. Am. Chem. Soc.* **2004**, *126*, 12762–12763.

9. Holst, C. M.; Frydman, B.; Marton, L. M.; Oredsson, S. M. *Toxicology* **2006**, *223*, 71–81.
10. Putnam, W. C.; Daniher, A. T.; Trawick, B. N.; Bashkin, J. K. *Nucleic Acid Res.* **2001**, *29*, 2199–2204.
11. Hobbs, F. W. *J. Org. Chem.* **1989**, *54*, 3420–3422.
12. Matsuda, A.; Shinozaki, M.; Yamaguchi, T.; Homma, H.; Nomoto, R.; Miyasaka, T.; Watanabe, Y.; Abiru, T. *J. Med. Chem.* **1992**, *35*, 241–252.
13. Nair, V.; Turner, G. A.; Buenger, G. S.; Chamberlain, S. D. D. *J. Org. Chem.* **1988**, *53*, 3051–3057.
14. Robins, M. J.; Uznański, B. *Can. J. Chem* **1981**, *59*, 2601–2607.
15. Pittelkow, M.; Lewinsky, R.; Christensen, J. B. *Synthesis* **2002**, *15*, 2195–2202.
16. Hansen, J. B.; Nielsen, M. C.; Buchardt, O. *Synthesis* **1982**, *5*, 404–405.
17. Fouace, S.; Gaudin, C.; Picard, S.; Corvaisier, S.; Renault, J.; Carboni, B.; Felden, B. *Nucleic Acids Res.* **2004**, *32*, 151–157.
18. Muller, D.; Zeltser, I.; Bitan, G.; Gilon, C. *J. Org. Chem.* **1997**, *62*, 411–416.
19. Kim, Y.; Zeng, F.; Zimmerman, S. C. *Chem. Eur. J.* **1999**, *5*, 2133–2138.
20. Rodger, A.; Taylor, S.; Adlam, G.; Blagbrough, I. S.; Haworth, I. S. *Bioorg. Med. Chem.* **1995**, *3*, 861.
21. Marquet, R.; Houssier, C. J. *Biomol. Struct. Dyn.* **1988**, *6*, 235–246.
22. Johnson, C. W., Jr. In *Circular Dichroism and the Conformational Analysis of Biomolecules*; Fasman, G. D., Ed.; Plenum Press: London, 1996; pp 433–468.
23. Mosmann, T. *J. Immunol. Methods* **1983**, *65*, 55–63.
24. Berridge, M. V.; Tan, A. S. *Arch. Biochem. Biophys.* **1993**, *303*, 474–482.
25. Denizot, F.; Lang, R. *J. Immunol. Methods* **1986**, *89*, 271–277.
26. Bloomfield, V. A.; Crothers, D. M.; Tinoco, I., Jr.. In *Nucleic Acids, Structures, Properties, and Functions*; University Science Books: Sausalito, California, 1999, p 176.
27. Tomlinson, G. E.; Chen, T. T-L.; Stastny, V. A.; Virmani, A. K.; Spillman, M. A.; Tonk, V.; Blum, J. L.; Schneider, N. R.; Wistuba, I. I.; Shay, J. W.; Minna, J. D.; Gazdar, A. F. *Cancer Res.* **1998**, *58*, 3237–3242.