Synthesis and Antitumor Cytotoxicity Evaluation of Novel Thiazole-Containing Glycosylated Polyamides

Rohtash Kumar[a] and J. William Lown*[a]

Keywords: Amides / Antitumor agents / DNA / Synthetic methods

Certain thiazole-containing water-soluble glycosylated polyamides were synthesized and preliminary anti-cancer evaluation carried out by NCI against three types of cancer-cells. Glycosyl moieties were introduced onto the polyamide backbone in order to increase the water-solubility of the longer polymers. This necessitated the development of novel synthetic methods.

Introduction

In recent years, progress has been made towards understanding molecular recognition processes between small molecules and peptides, proteins or nucleic acids.[1−3] In particular, considerable interest has been seen in the development of sequence-specific agents to target genomic DNA.[4,5] The guiding principle behind efforts to develop DNA sequence-specific agents is that a greater biological response may be achieved for a given drug, compared with the sequence-neutral agent, thereby reducing toxic side effects. A related goal is the selective suppression of transcription from particular gene-sequences.[6−10] There are a number of approaches to the problem of developing DNA sequence-specific agents, for example, the use of oligonucleotides,[6−8] or their backbone-modified counterparts,[10] which take advantage of the inherent Watson−Crick base-pairing to target single-strand sequences, or with hybrid probes incorporating an intercalator. Another approach is to use the property of certain oligonucleotides to form triplex structures, and thereby target double-stranded nucleic acid sequences, in what is referred to[11] as the antigene-strategy.

Polyamides containing pyrrole (Py) and imidazole (Im) units, based on the naturally-occurring compounds netropsin and distamycin, constitute a class of agents with DNA sequence-specificity.[12] Polyamides show specific and high-affinity binding for nucleotide-sequences in the minor groove of DNA, and in favorable cases, the binding strength is similar to sequence-specific DNA-binding proteins.[2] NMR spectroscopic studies show that under certain conditions, two distamycin molecules can bind simultaneously at the same site in the minor groove.[13] This observation has led to the development of three different types of polyamide compounds which have a 2:1 binding ratio with the minor groove of DNA; hairpin polyamide complexes,[14] cross-linked polyamide complexes,[15,16] and extended bis-polyamide complexes.[17,18] The biological characterization of polyamides reveals that they function by interfering with critical processes, including, but not limited to, the inhibition of DNA and RNA polymerases, topoisomerases, HIV integrases, gyrases, human tumor helicases and reverse transcriptases.[19−22]

Increasing the number of pyrrole (Py) and imidazole (Im) units in a polyamide results in a direct increase in the length of the sequence of base pairs recognized by the polyamide in the minor groove of the DNA. The disadvantage of increasing the number of such groups is that the aqueous solubility of the drug is progressively decreased, often resulting in substantially reduced cellular uptake or bioavailability. It used to be the case that a large number of polyamides that were synthesized and evaluated biologically against various targets could not be developed further because of their low aqueous solubility. In this context, our group recently submitted a patent for the synthesis of glycosylated polyamides with increased water-solubility.[23] Recently, we also compared the cytotoxic potency between PBD (pyrrolo[2,1-*c*][1,4]benzodiazepine)-water-insoluble and PBD-water-soluble polyamide conjugates,[24] and we also reported the cytotoxic activity of tryptophan-glycosylated water-soluble polyamide conjugates.[25] These studies showed that the water-soluble PBD-polyamide conjugates are generally more potent than the PBD-water-insoluble polyamide conjugates.

Encouraged by the results from the PBD-water-soluble glycosylated polyamide conjugates, we have begun a systematic exploration of several structural factors in the ligands that might be expected to contribute to the processes

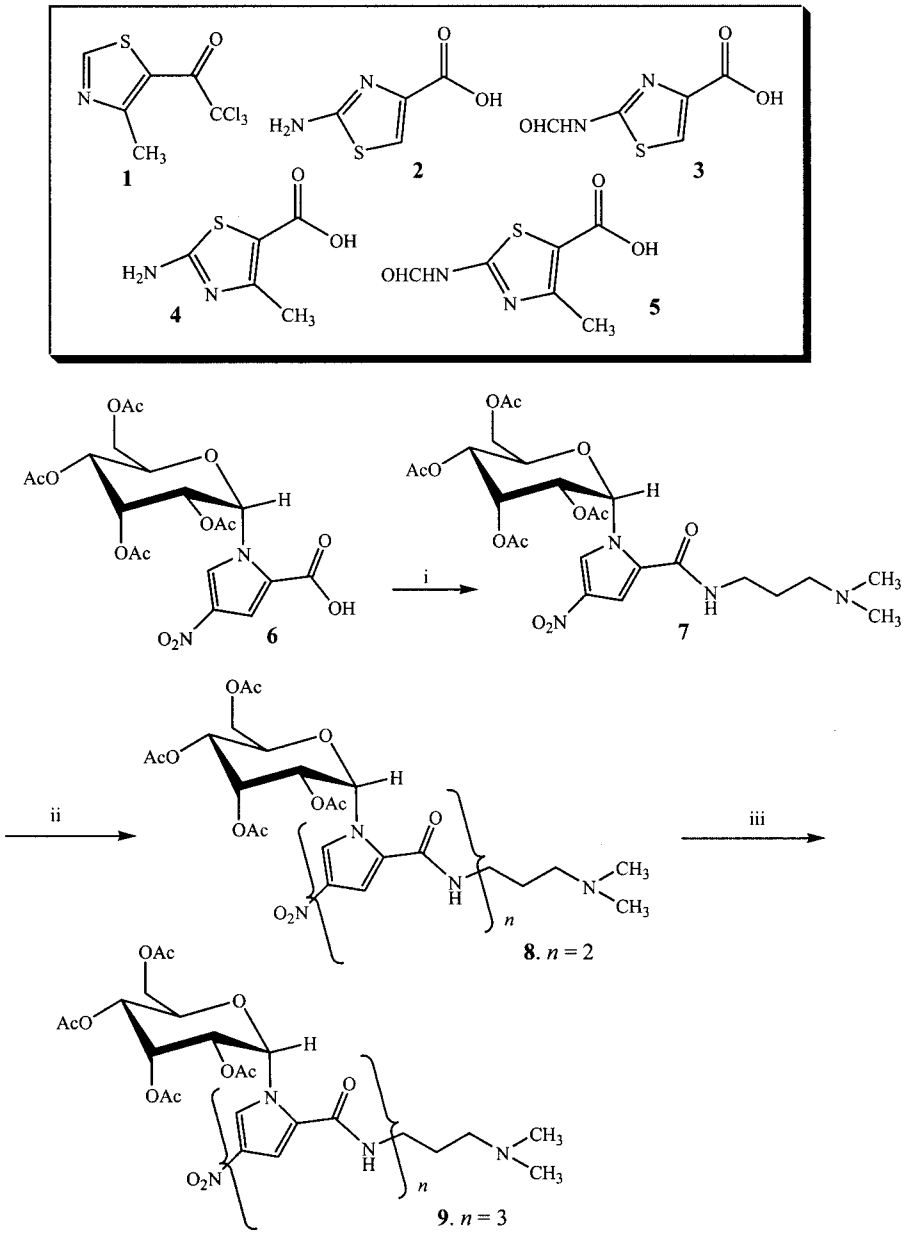
[a] Department of Chemistry, University of Alberta, Edmonton, AB, T6G 2G2, Canada
Fax: (internat.) + 1-780-492-8231
E-mail: Lynne.lechelt@ualberta.ca

  DOI: 10.1002/ejoc.200300532

of molecular recognition. One important aspect of molecular design in the context of polyamides is the introduction of heterocyclic moieties capable of specific DNA-recognition by hydrogen-bond acceptance and donation.[4]

The natural product distamycin was first modified for a change in sequence-recognition from an AT site to a GC site by Lown[26] by the introduction of an imidazole ring in place of the pyrrole ring in the natural product. Since then, the groups of Lown and Dervan have carried out extensive work on the introduction of new heterocycles in the polyamide system to alter sequence-specificity and recognition, compared with the natural product. One such modification performed by our group is the introduction of the thiazole ring in three ring systems and also to the cross-linked system. Recently, Dervan's group has incorporated this ring-modification into a hairpin system.[27]

We have studied the binding-affinities of a series of thiazole−imidazole−pyrrole (TIP) monomers and cross-linked dimers, and evaluated the effect on selectivity and binding affinity of the introduction of different N-terminal head groups attached to the leading thiazole ring, and of changing the length of the crosslinking methylene chain.[28,29] In a continuation of our ongoing research, we also reported the synthesis of thiazole-containing pyrrole-cross-linked polyamides, and evaluated these compounds for their gyrase-inhibition activity.[30]



(i) $(CH_3)_2NCH_2CH_2CH_2NH_2$, EDCI, HOBT DMF, r.t., 80% (ii) 10% Pd-C/ H_2, MeOH, **6**, EDCI, HOBT DMF, r.t., 75% (iii) 10% Pd-C/ H_2, MeOH, **6**, EDCI, HOBT DMF, r.t., 70%

Scheme 1

The structural consideration we examine in this paper is the complementary one (compared with the above-described substitution of imidazole for pyrrole) of site avoidance. In addition, we address the question of whether this property can be incorporated into the design of agents capable of recognizing and binding to unique sequences. Accordingly, we report the synthesis of novel thiazole-containing water-soluble polyamides in such a way that the sulfur of the thiazole is orientated towards the floor of the minor groove, thereby sterically preventing binding at GC sites.[4] We also report preliminary cytotoxicity data for these thiazole-containing water-soluble polyamide conjugates. The base-recognizing properties of the thiazole moiety are employed in the side chain of the glycopeptide antitumor antibiotic, bleomycin,[31] which is thought to determine its sequence-recognizing properties.[32] Thiazole-containing polyamides have shown a high preference for AT base-pairs. Therefore, it was considered to be of interest to design and synthesize thiazole-containing water-soluble polyamides, and to investigate the effects of these structural changes on their biological properties.[33,34] In a continuation of these efforts, we herein report the design, synthesis and preliminary in vitro antitumor cytotoxicity activities of these novel thiazole-containing water-soluble pyrrole polyamides.

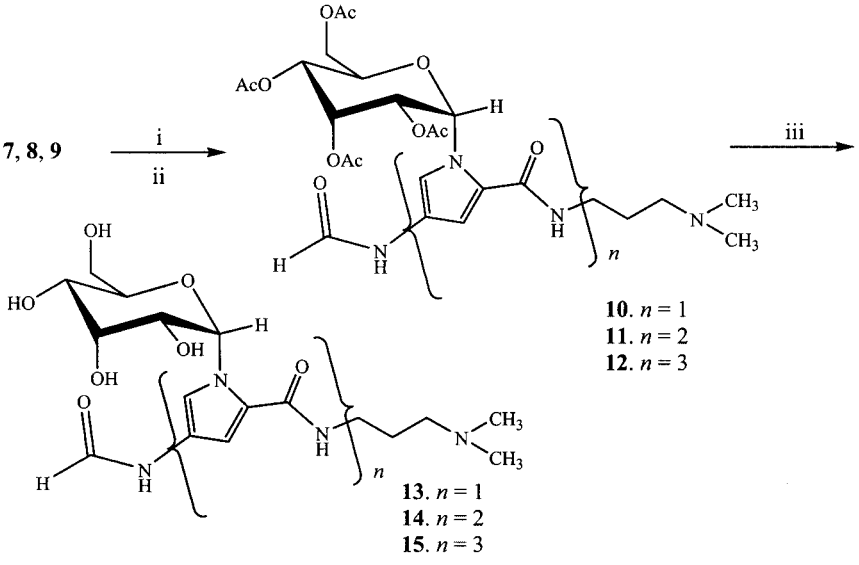
Results and Discussion

In our previous report, the thiazole [35] compounds **1**−**5** and the acetyl glycosylated pyrrole acid[24] **6** were synthesized using convenient routes and in good yield. Acetyl glycosylated pyrrole acid **6** was coupled with *N*,*N*-dimethylpropane-1,3-diamine using EDCI and HOBt as coupling reagents, to give compound **7** in 80 % yield. The nitro group of compound **7** was reduced with hydrogen in the presence of a Pd/C catalyst, to give the corresponding amino compound, which was then again coupled with the glycosylated pyrrole acid **6** using standard EDCI/HOBt coupling conditions, to afford compound **8** in 75 % yield. The nitro group of compound **8** was again reduced to the corresponding amino group, and the product again coupled with the acid **6** in presence of EDCI/HOBt (for chain-elongation of the glucopyrrole carboxamide peptide) to give compound **9** in 70 % yield (Scheme 1).

The nitro groups of the acetyl glycosylated pyrrole polyamide compounds **7**−**9** were reduced with hydrogen in the presence of Pd/C to give the corresponding amines. This was immediately followed by *N*-formylation using a mixture of formic acid and acetic anhydride, to give the desired acetyl glycosylated pyrrole polyamides **10**−**12** in 40−50 % yield. Hydrolysis of compounds **10**−**12** with 0.1 N NaOH at room temperature for 2−3 h gave water-soluble glycosylated pyrrole polyamides **13**−**15** in good yields. The final compounds were isolated as solids in 40−50 % yield from compounds **10**−**12** after passing through an ion-exchange column with methanol/water as eluent (Scheme 2).

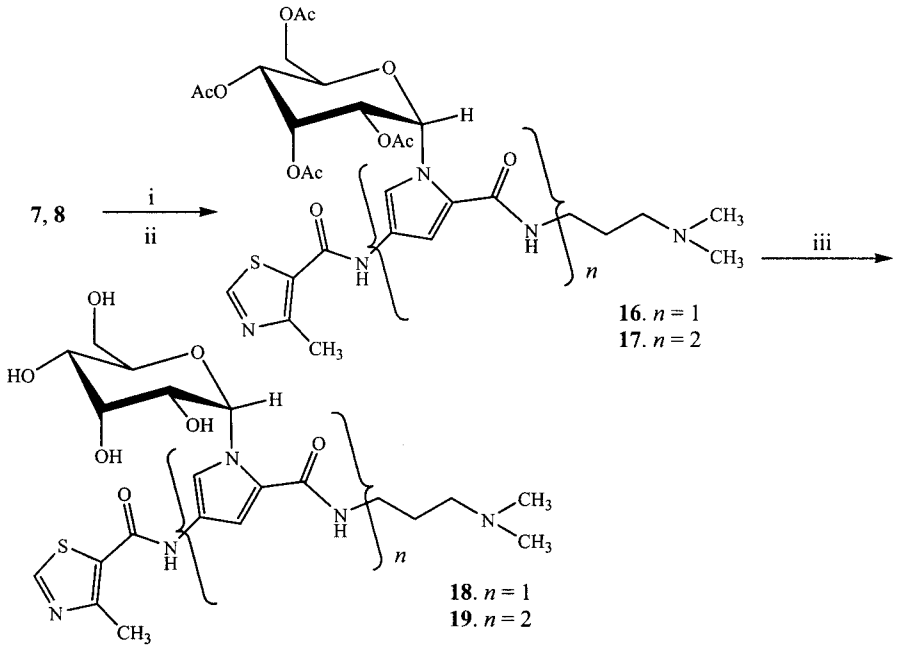
Catalytic reduction of the nitro groups of the acetyl glycosylated pyrrole polyamides **7** and **8** converted them into the corresponding amines, which were then treated with compound **1**[29] in the presence of triethylamine in dry THF, to afford the thiazole-containing acetyl glycosylated pyrrole polyamides **16** and **17**, respectively, in good yield. Hydrolysis of compounds **16** and **17** with 0.1 N NaOH solutions gave the target thiazole-containing glycosylated pyrrole polyamides **18** and **19** (Scheme 3).

The nitro groups of the acetyl glycosylated pyrrole polyamides **7** and **8** were reduced with hydrogen in the presence of Pd/C to give the corresponding amines. These were then coupled to the thiazole acids **2**, **3**, **4** and **5** using standard EDCI/HOBt coupling reagents, to give the amino- and for-



(i) 10% Pd–C/ H_2, MeOH (ii) HCOOH, Ac_2O, r.t., 40–50% (iii) 0.1N NaOH, THF:MeOH (1:1) 40–50%.

Scheme 2

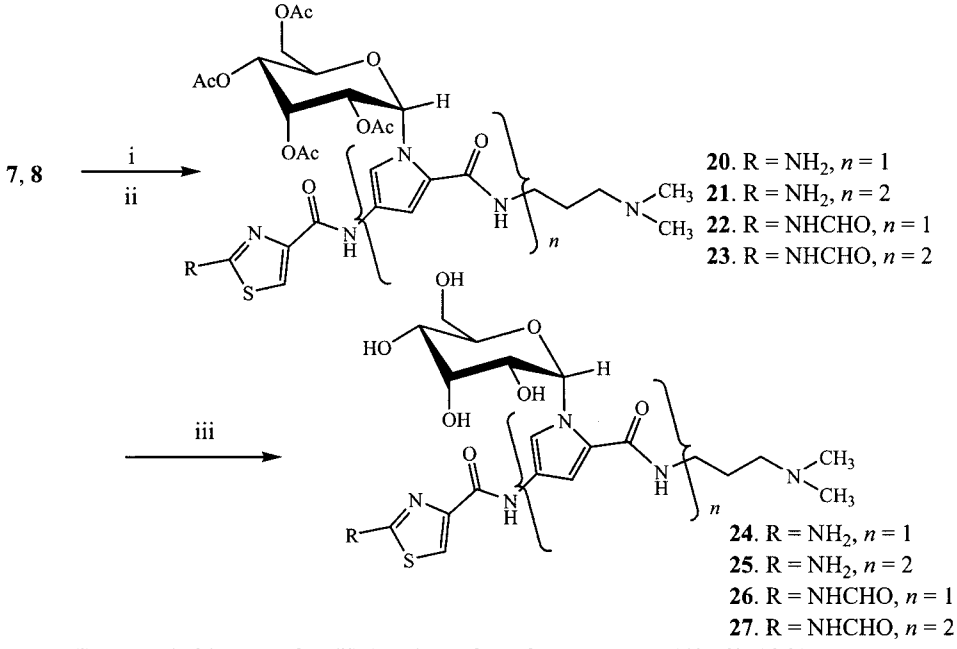


(i) 10% Pd–C/ H_2, MeOH (ii) **1**, Et_3N, THF, r.t., 4h, 78 % (iii) 0.1N NaOH, THF:MeOH (1:1) 40–50 %.

Scheme 3

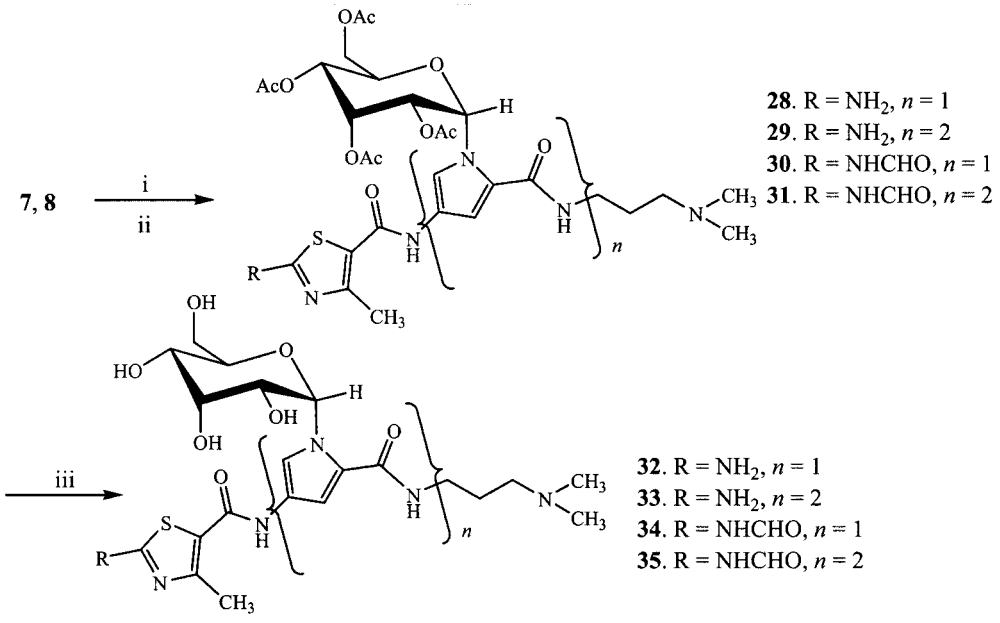
mylaminothiazole-containing acetyl glycosylated pyrrole polyamide conjugates **20**−**23** and **28**−**31** in 60−70 % yield. Hydrolysis of compounds **20**−**23** and **28**−**31** using 0.1 N NaOH solution at room temperature for 2−3 h gave amino and formylamino thiazole-containing glycosylated water-soluble pyrrole polyamides **24**−**27** and **32**−**35** in good yields. The final thiazole-containing glycosylated pyrrole polyamides were isolated as solids in 40−50 % yield from their acetyl derivatives after passing through an ion-exchange column with methanol/water as eluent (Scheme 4 and 5).

The glycosylated pyrrole and thiazole-containing glycosylated pyrrole polyamides **13**−**15**, **18**−**19**, **24**−**27** and **32**−**35** containing one or more glycosylated pyrrole units



(i) 10% Pd–C/ H_2, MeOH (ii) **2** or **3**, EDCI, HOBt, DMF, r.t.,12h, 60–70 %
(iii) 0.1N NaOH, THF:MeOH (1:1) 40–50 %.

Scheme 4



(i) 10% Pd-C/ H_2, MeOH (ii) **4** or **5**, EDCI, HOBt, DMF, r.t.,12h, 60–70 %
(iii) 0.1N NaOH, THF:MeOH (1:1) 40–50 %.

Scheme 5

were selected by the US National Cancer Institute for evaluation in an in vitro preclinical antitumor screening program for primary anticancer assays against three human tumor cell-lines consisting of MCF7 (Breast), NCI-H460 (Lung), and SF-268 (CNS) cells. In the current protocol, each cell-line is inoculated and pre-incubated on a microtiter plate. Test agents are then added at a single concentration, and the culture is incubated for 48 h. End-point determinations are made with alamar blue. Results of each test agent are reported as the percent of growth of the treated cells when compared to the untreated control cells. The compounds listed in Table 1 have been evaluated in the three-cell-line, one dose primary anticancer assay. It can be observed from the initial cytotoxic data that the compounds have varying cytotoxic potencies against these three cancer-cell-lines, with, in certain cases, significant inhibitory effects against MCF-7 and NCI-H460 cell-lines. From the initial biological data, it can be tentatively concluded that if the compound is too long, it might be out of phase in the minor groove of DNA, which lowers the DNA-binding-affinity, or, due to the large size of the molecule, might lead to low binding-affinity. Evidently, incorporation of thiazole moieties into the polyamide conjugate structure does not adversely affect the overall physical and biological properties. We are now in the course of performing cellular-uptake and subcellular localization studies of modifications of these compounds, which bear fluorescent labels. Cellular-uptake results from confocal microscopy, DNA-binding characteristics and more extensive cytotoxicity data will be published in due course.

Table 1. In vitro preclinical cytotoxic data of thiazole-containing glycosylated polyamides

Compound	Concentration	Units	(Breast) MCF-7	Growth Percentage (Non-small cell lung) NCI-H460	(CNS) SF-268
13	$1.000 \cdot 10^{-4}$	mol/L	90	141	173
14	$1.000 \cdot 10^{-4}$	mol/L	96	103	126
15	$1.000 \cdot 10^{-4}$	mol/L	99	109	124
18	$1.000 \cdot 10^{-4}$	mol/L	79	86	107
19	$1.000 \cdot 10^{-4}$	mol/L	106	102	112
24	$1.000 \cdot 10^{-4}$	mol/L	97	115	118
25	$1.000 \cdot 10^{-4}$	mol/L	104	102	123
26	$1.000 \cdot 10^{-4}$	mol/L	107	101	125
27	$1.000 \cdot 10^{-4}$	mol/L	108	99	114
32	$1.000 \cdot 10^{-4}$	mol/L	107	101	119
33	$1.000 \cdot 10^{-4}$	mol/L	68	57	104
34	$1.000 \cdot 10^{-4}$	mol/L	106	100	125
35	$1.000 \cdot 10^{-4}$	mol/L	105	99	105

In summary, we have described the first synthesis of water-soluble thiazole-containing glycosylated pyrrole polyamides, and also their preliminary anti-cancer evaluation.

Experimental Section

TLC plates were visualized with UV light. All compounds obtained commercially were used without further purification unless otherwise stated. Kieselgel 60 (230−400 mesh) of E. Merck was used for flash column chromatography, and precoated silica gel 60F-254 sheets of E. Merck were used for TLC, with the solvent system indicated in the procedure. Methanol and ethanol was freshly distilled from magnesium turnings; tetrahydrofuran was distilled from sodium benzophenone ketyl under an atmosphere of dry argon, diethyl ether was dried with sodium; dichloromethane was freshly distilled from calcium hydride, triethylamine was treated with potassium hydroxide then distilled from barium oxide and stored over 3Å molecular sieves. Dry dimethylformamide and all commercially available chemicals were purchased from Aldrich Chemical Co. The ^{1}H NMR spectra were recorded with a Bruker WH-300 spectrometer. Proton chemical shifts are reported in parts per million (δ) downfield from tetramethylsilane ($SiMe_4$) as an internal standard. Coupling constants (*J* values) are given in Hertz and spin multiplicates are described as follows: s (singlet), d (doublet), dd (doublet of doublets), t (triplet), q (quadruplet), p (pentuplet) or m (multiplet). FAB (fast atom bombardment) mass spectra with glycerol as the matrix were determined with Associate Electrical Ind. (AEI) MS − 9 and MS − 50 focusing high-resolution mass spectrometers.

***N*-[3-(Dimethylamino)propyl]-4-nitro-1-(2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl)-1*H*-pyrrole-2-carboxamide (7):** The *N*,*N*-dimethylpropane-1,3-diamine (0.46 g, 4.50 mmol) was dissolved in dry DMF and added to a mixture of the 1-(2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl)-4-nitro-1*H*-pyrrole-2-carboxylic acid (**6**) (2.0 g, 4.11 mmol), hydroxybenzotriazole (0.55 g, 4.07 mmol), and EDCI (1.97 g, 10.27 mmol), in dry DMF. This mixture was stirred at room temperature for 12 h, and after completion of the reaction, the solvent was removed under reduced pressure to afford a dark oil. This was purified by flash column chromatography on silica gel by using methanol/dichloromethane (5:95) as eluent, to afford **7** as a white solid in 80 % yield (1.89 g). ^{1}H NMR: (300 MHz, [D$_6$]DMSO): δ = 1.55 (q, *J* = 6.8 Hz, 2 H, −CH_2−), 1.95 (s, 3 H, $COCH_3$), 2.08 (s, 3 H, $COCH_3$), 2.10 (s, 3 H, $COCH_3$), 2.12 (s, 3 H, $COCH_3$), 2.18 [s, 6 H, −$N(CH_3)_2$], 2.25 (t, *J* = 7.0 Hz, 2 H, −C*H*$_2$N−), 3.15 (dt, *J* = 6.0, 7.0 Hz, 2 H, −NHC*H*$_2$−), 4.15 (ddd, *J* = 10.1, 4.4, 12.1 Hz, 1 H), 4.18 (m, 2 H), 4.32 (dd, *J* = 10.1, 9.5 Hz, 1 H), 5.20 (t, *J* = 9.5 Hz, 1 H), 5.50 (t, *J* = 9.5 Hz, 1 H), 6.61 (d, *J* = 3.85 Hz, 1 H), 7.62 (d, *J* = 1.8 Hz, 1 H, Py-H), 8.08 (d, *J* = 1.8 Hz, 1 H, Py-H), 8.45 (t, *J* = 6.5 Hz, 1 H, −N*H*CH_2−) ppm. HRMS calculated for $C_{24}H_{34}N_4O_{12}$ 570.21, found 593.20 [M + Na].

***N*-[3-(Dimethylamino)propyl]-4-[4-nitro-1-(2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl)-1*H*-pyrrol-2-ylcarbonylamino]-1-(2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl)-1*H*-pyrrole-2-carboxamide (8):** A solution of the acetyl glycosylated nitro polyamide **7** (1.5 g, 2.63 mmol) in MeOH or DMF was hydrogenated over 10 % Pd/C (0.300 g) at 50 psi pressure for 2 h, after which, the catalyst was removed by filtration through a Celite pad. The filtrate was concentrated to dryness under reduced pressure (at room temp.) to afford the corresponding amine. Owing to the sensitivity of the amine to oxidation, it was used for the next reaction immediately. It was dissolved in dry DMF, and a mixture of the acid **6** (1.27 g, 2.61 mmol), hydroxybenzotriazole (0.355 g, 2.62 mmol), and EDCI (1.26 g, 6.57 mmol) in dry DMF was added. This mixture was stirred at room temperature for 12 h, and after completion of the reaction, the solvent was removed under reduced pressure to afford a dark oil. This was purified by flash column chromatography on silica gel, by using methanol/dichloromethane as eluent, to afford the acetyl glycosylated polyamide **8** as a light yellow solid in 75 % yield (2.0 g). ^{1}H NMR: (300 MHz, [D$_6$]DMSO): δ = 1.56 (q, *J* = 6.8 Hz, 2 H, −CH_2−), 1.80 (s, 3 H, $COCH_3$), 1.86 (s, 3 H, $COCH_3$), 1.96 (s, 3 H, $COCH_3$), 1.99 (s, 3 H, $COCH_3$), 2.03 (s, 3 H, $COCH_3$), 2.05 (s, 3 H, $COCH_3$), 2.07 (s, 3 H, $COCH_3$), 2.08 (s, 3 H, $COCH_3$), 2.26 [s, 6 H, −$N(CH_3)_2$], 2.30 (t, *J* = 7.0 Hz, 2 H, −CH_2N−), 3.18 (dt, *J* = 6.0, 7.0 Hz, 2 H, −NHCH_2−), 3.90−4.32 (m, 8 H, sugar proton), 5.20−5.50 (m, 4 H, sugar proton), 6.71 (d, *J* = 3.85 Hz, 2 H), 6.96 (d, *J* = 1.8 Hz, 1 H, Py-H), 7.20 (d, *J* = 1.8 Hz, 1 H, Py-H), 7.56 (d, *J* = 1.8 Hz, 1 H, Py-H), 7.95 (d, *J* = 1.8 Hz, 1 H, Py-H), 8.15 (t, *J* = 6.5 Hz, 1 H, −HCH_2−, 9.62 (s, 1 H, −NH− ppm. HR-MS: *m*/*z* calculated for $C_{43}H_{56}N_6O_{22}$ 1008.34 found 1031.40 [M + Na].

Compound 9: 10 % Pd−C (0.300 g) was added to a solution of compound **8** (1.5 g, 1.48 mmol) in methanol (25.0 mL). The reaction mixture was hydrogenated in a Parr shaker at 50 psi for 2 h. The catalyst was removed by filtration, and the solvent was evaporated in vacuo. The residue was dissolved in dry DMF (20.0 mL), and a mixture of the acid **6** (0.723 g, 1.48 mmol), HOBt (0.201 g, 1.48 mmol), EDCI (0.713 g, 3.71 mmol) was added with stirring at room temperature under a nitrogen atmosphere. The reaction mixture was stirred at room temperature and for 12 h. After completion of the reaction, the residue was concentrated to dryness under reduced pressure, and the residue was purified by column chromatography eluting with NH_4OH/MeOH/DCM (0.2:1:9) to give compound **9** in 70 % yield (1.52 g) as a yellow solid. ^{1}H NMR: (300 MHz, [D$_6$]DMSO): δ = 1.54 (q, *J* = 6.8 Hz, 2 H, −CH_2−), 1.79−2.15 (12s, 36 H, 12x-$COCH_3$), 2.28 [s, 6 H, −$N(CH_3)_2$], 2.32 (t, *J* = 7.0 Hz, 2 H, −CH_2N−), 3.20 (dt, *J* = 6.0, 7.0 Hz, 2 H, −NHC*H*$_2$−), 3.91−4.35 (m, 12 H, sugar proton), 5.22−5.56 (m, 6 H, sugar proton), 6.73 (m, 3 H, sugar proton), 6.83 (d, *J* = 1.8 Hz, 1 H, Py-H), 6.97 (d, *J* = 1.8 Hz, 1 H, Py-H), 7.20 (d, *J* = 1.8 Hz, 1 H, Py-H), 7.29 (d, *J* = 1.8 Hz, 1 H, Py-H), 7.45 (d, *J* = 1.8 Hz, 1 H, Py-H), 7.76 (d, *J* = 1.8 Hz, 1 H, Py-H), 8.25 (t, *J* = 6.5 Hz, 1 H, −N*H*CH_2−), 9.61 (s, 1 H, −NH−), 9.95 (s, 1 H, −NH−) ppm. HR-MS: *m*/*z* calculated for $C_{62}H_{78}N_8O_{32}$ 1446.50 found 1469.47 [M + Na].

General Procedure A: A suspension of the acetyl glycosylated nitro polyamides **7**, **8** or **9** in methanol was hydrogenated over 10 % Pd on charcoal at room temperature. The catalyst was removed by filtration, and the filtrate was concentrated to give the respective amine. Owing to the instability of each amine, they were used immediately in the next reactions. The amine was dissolved in 98 % formic acid, acetic anhydride was added, and the reaction mixture was stirred at room temperature for 12 h. After completion of the reaction, the solvent was evaporated to dryness in vacuo, and the resulting residue was purified by column chromatography using NH_4OH/MeOH/DCM as eluting solvent to give compounds **10**−**13** in 40−50 % yield.

***N*-[3-(Dimethylamino)propyl]-4-formylamino-1-(2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl)-1*H*-pyrrole-2-carboxamide (10):** This compound was prepared according to the general procedure **A** starting from compound **7** (1.0 g, 1.75 mmol) and 98 % formic acid (5.0 mL), acetic anhydride (1.0 mL) as a light yellow solid in 45 % yield (0.45 g). ^{1}H NMR: (300 MHz, [D$_6$]DMSO): δ = 1.56 (q, *J* =

6.8 Hz, 2 H, $-CH_2-$), 1.94 (s, 3 H, $COCH_3$), 2.05 (s, 3 H, $COCH_3$), 2.09 (s, 3 H, $COCH_3$), 2.11 (s, 3 H, $COCH_3$), 2.18 [s, 6 H, $-N(CH_3)_2$], 2.25 (t, J = 7.0 Hz, 2 H, $-CH_2N-$), 3.18 (dt, J = 6.0, 7.0 Hz, 2 H, $-NHCH_2-$), 4.14 (ddd, J = 10.1, 4.4, 12.1 Hz, 1 H), 4.17 (m, 2 H), 4.34 (dd, J = 10.1, 9.5 Hz, 1 H), 5.25 (t, J = 9.5 Hz, 1 H), 5.58 (t, J = 9.5 Hz, 1 H), 6.62 (d, J = 3.85 Hz, 1 H), 7.55 (d, J = 1.8 Hz, 1 H, Py-H), 7.95 (d, J = 1.8 Hz, 1 H, Py-H), 8.10 (s, 1 H, $-CHO-$), 8.35 (t, J = 6.5 Hz, 1 H, $-NHCH_2-$), 10.00 (s, 1 H, $-NH-$) ppm. HR-MS calculated for $C_{25}H_{36}N_4O_{11}$ 568.25 found 591.21 [M + Na].

Compound 11: This compound was prepared starting from compound **8** (1.0 g, 0.992 mmol.) and 98 % formic acid (5.0 mL), acetic anhydride (1.0 mL) according to the general procedure **A** (0.42 g, 42 % yield) as a light yellow solid. ^{1}H NMR: (300 MHz, [D$_6$]DMSO): δ = 1.55 (q, J = 6.8 Hz, 2 H, $-CH_2-$), 1.81−2.12 (8s, 24 H, 8 × $-COCH_3$), 2.27 [s, 6 H, $-N(CH_3)_2$], 2.29 (t, J = 7.0 Hz, 2 H, $-CH_2N-$), 3.21 (dt, J = 6.0, 7.0 Hz, 2 H, $-NHCH_2-$), 3.91−4.35 (m, 8 H, sugar proton), 5.22−5.59 (m, 4 H, sugar proton), 6.69 (m, 2 H, sugar proton), 6.95 (d, J = 1.8 Hz, 1 H, Py-H), 7.25 (d, J = 1.8 Hz, 1 H, Py-H), 7.45 (d, J = 1.8 Hz, 1 H, Py-H), 7.75 (d, J = 1.8 Hz, 1 H, Py-H), 8.09 (s, 1 H, $-CHO-$), 8.35 (t, J = 6.5 Hz, 1 H, $-NHCH_2-$), 9.65 (s, 1 H, $-NH-$), 10.02 (s, 1 H, $-NH-$) ppm. HR-MS: m/z calculated for $C_{44}H_{58}N_6O_{21}$ 1006.41 found 1029.55 [M + Na].

Compound 12: The title compound was prepared according to the general procedure **A** using compound **9** (1.0 g, 0.691 mmol.) and 98 % formic acid (5.0 mL), acetic anhydride (1.0 mL) in 47 % yield (0.47 g) as a light yellow solid. ^{1}H NMR: (300 MHz, [D$_6$]DMSO): δ = 1.55 (q, J = 6.8 Hz, 2 H, $-CH_2-$), 1.80 −2.12 (12s, 36 H, 12 × $-COCH_3$), 2.25 [s, 6 H, $-N(CH_3)_2$], 2.30 (t, J = 7.0 Hz, 2 H, $-CH_2N-$), 3.18 (dt, J = 6.0, 7.0 Hz, 2 H, $-NHCH_2-$), 3.89−4.35 (m, 12 H, sugar proton), 5.20−5.57 (m, 6 H, sugar proton), 6.72 (m, 3 H, sugar proton), 6.86 (d, J = 1.8 Hz, 1 H, Py-H), 6.96 (d, J = 1.8 Hz, 1 H, Py-H), 7.21 (d, J = 1.8 Hz, 1 H, Py-H), 7.30 (d, J = 1.8 Hz, 1 H, Py-H), 7.46 (d, J = 1.8 Hz, 1 H, Py-H), 7.79 (d, J = 1.8 Hz, 1 H, Py-H), 8.10 (s, 1 H, $-CHO-$), 8.25 (t, J = 6.5 Hz, 1 H, $-NHCH_2-$), 9.61 (s, 1 H, $-NH-$), 9.95 (s, 1 H, $-NH-$), 10.01 (s, 1 H, $-NH-$) ppm. HR-MS: m/z calculated for $C_{63}H_{80}N_8O_{31}$ 1444.51 found 1467.40 [M + Na].

General Procedure B: A mixture of the respective compounds **10**−**12**, **16**−**17**, **20**−**23** and **28**−**31** in methanol/THF (1:1) and 0.1 N NaOH was placed in a flask, then the reaction mixture was stirred at room temperature until the starting materials completely disappeared as shown by TLC. The crude reaction mixture was passed through a small ion-exchange resin (Amberlite-15) column using MeOH/H$_2$O (80:20) as eluent. The solvent was removed under reduced pressure and lyophilization to give the final water-soluble glycosylated polyamides **13**−**15**, **18**−**19**, **24**−**27** and **32**−**35** in 40−50 % yield.

***N*-[3-(Dimethylamino)propyl]-4-formylamino-1-(α-D-glucopyranosyl)-1*H*-pyrrole-2-carboxamide (13):** This compound was prepared according to the general procedure **B** by using compound **10** (0.38 g, 0.669 mmol.) and 0.1 N NaOH solution as a white solid in 45 % yield (0.125 g). ^{1}H NMR: (300 MHz, D$_2$O): δ = 1.57 (q, J = 6.8 Hz, 2 H, $-CH_2-$), 2.20 [s, 6 H, $-N(CH_3)_2$], 2.26 (t, J = 7.0 Hz, 2 H, $-CH_2N-$), 3.20 (dt, J = 6.0, 7.0 Hz, 2 H, $-NHCH_2-$), 4.10−4.36 (m, 4 H, sugar proton), 5.21−5.56 (m, J = 9.5 Hz, 2 H), 6.62 (d, J = 3.85 Hz, 1 H), 7.56 (d, J = 1.8 Hz, 1 H, Py-H), 7.85 (d, J = 1.8 Hz, 1 H, Py-H), 8.10 (s, 1 H, $-CHO-$) ppm. HRMS calculated for $C_{17}H_{28}N_4O_7$ 400.21 found 423.43 [M + Na].

***N*-[3-(Dimethylamino)propyl]-4-{[4-formylamino-1-(α-D-glucopyranosyl)-1*H*-pyrrol-2-yl]carbonylamino}-1-(α-D-glucopyranosyl)-1*H*-pyrrole-2-carboxamide (14):** This compound was prepared starting from compound **11** (0.4 g, 0.397 mmol) and 0.1 N NaOH solution according to the general procedure **B** (0.126 g, 47 % yield) as a solid. ^{1}H NMR: (300 MHz, D$_2$O): δ = 1.56 (q, J = 6.8 Hz, 2 H, $-CH_2-$), 2.25 [s, 6 H, $-N(CH_3)_2$], 2.30 (t, J = 7.0 Hz, 2 H, $-CH_2N-$), 3.21 (dt, J = 6.0, 7.0 Hz, 2 H, $-NHCH_2-$), 3.91−4.39 (m, 8 H, sugar proton), 5.20−5.56 (m, 4 H, sugar proton), 6.69 (m, 2 H, sugar proton), 6.96 (d, J = 1.8 Hz, 1 H, Py-H), 7.22 (d, J = 1.8 Hz, 1 H, Py-H), 7.46 (d, J = 1.8 Hz, 1 H, Py-H), 7.76 (d, J = 1.8 Hz, 1 H, Py-H), 8.10 (s, 1 H, $-CHO-$) ppm. HR-MS: m/z calculated for $C_{28}H_{42}N_6O_{13}$ 670.28 found 693.40 [M + Na].

***N*-[3-(Dimethylamino)propyl]-1-(α-D-glucopyranosyl)-4-{4-[4-formylamino-1-(α-D-glucopyranosyl)-1*H*-pyrrol-2-ylcarbonylamino]-1-(α-D-glucopyranosyl)]-1*H*-pyrrol-2-ylcarbonylamino}-1*H*-pyrrole-2-carboxamide (15):** This compound was prepared according to the general procedure **B** by using compound **12** (0.4 g, 0.277 mmol.) and 0.1 N NaOH solution in 46 % yield (0.12 g) as a light yellow solid. ^{1}H NMR: (300 MHz, D$_2$O): δ = 1.57 (q, J = 6.8 Hz, 2 H, $-CH_2-$), 2.23 [s, 6 H, $-N(CH_3)_2$], 2.28 (t, J = 7.0 Hz, 2 H, $-CH_2N-$), 3.20 (dt, J = 6.0, 7.0 Hz, 2 H, $-NHCH_2-$), 3.95−4.38 (m, 12 H, sugar proton), 5.22−5.59 (m, 6 H, sugar proton), 6.65 (m, 3 H, sugar proton), 6.89 (d, J = 1.8 Hz, 1 H, Py-H), 6.95 (d, J = 1.8 Hz, 1 H, Py-H), 7.20 (d, J = 1.8 Hz, 1 H, Py-H), 7.31 (d, J = 1.8 Hz, 1 H, Py-H), 7.45 (d, J = 1.8 Hz, 1 H, Py-H), 7.75 (d, J = 1.8 Hz, 1 H, Py-H), 8.12 (s, 1 H, $-CHO-$) ppm. HR-MS: m/z calculated for $C_{39}H_{56}N_8O_{19}$ 940.37 found 963.50 [M + Na].

***N*-{5-[[3-(Dimethylamino)propyl]aminocarbonyl]-1*H*-pyrrol-3-yl}-4-methyl-1,3-thiazole-5-carboxamide (16):** 10 % Pd-C (0.200 g) was added to a solution of compound **7** (0.6 g, 1.05 mmol) in methanol (25.0 mL). The reaction mixture was hydrogenated in a Parr shaker at 50 psi for 2 h. The catalyst was removed by filtration, and the solvent was evaporated in vacuo. The residue was dissolved in dry THF (20.0 mL) and triethylamine (1.0 mL), and a solution of compound **1** (0.281 g, 1.15 mmol) in THF (10 mL) was added slowly with stirring at 0 °C under a nitrogen atmosphere. The reaction mixture was brought to room temperature and stirred for 2 h. After completion of the reaction, the residue was concentrated to dryness under reduced pressure, and the residue was purified by column chromatography, eluting with NH$_4$OH/MeOH/DCM (0.2:1:9) to give compound **16** in 78 % yield (0.55 g). ^{1}H NMR: (300 MHz, [D$_6$]DMSO): δ = 1.56 (q, J = 6.8 Hz, 2 H, $-CH_2-$), 1.90 (s, 3 H, $COCH_3$), 2.05 (s, 3 H, $COCH_3$), 2.10 (s, 3 H, $COCH_3$), 2.15 (s, 3 H, $COCH_3$), 2.25 [s, 6 H, $-N(CH_3)_2$], 2.30 (t, J = 7.0 Hz, 2 H, $-CH_2N-$), 2.45 (s, 3 H, Thio-CH_3), 3.18 (dt, J = 6.0, 7.0 Hz, 2 H, $-NHCH_2-$), 3.95−4.32 (m, 4 H, sugar proton), 5.21−5.58 (m, 2 H, sugar proton), 6.65 (d, J = 3.85 Hz, 1 H), 7.58 (s, 1 H, Thio-H), 7.67 (d, J = 1.8 Hz, 1 H, Py-H), 7.89 (d, J = 1.8 Hz, 1 H, Py-H), 8.35 (t, J = 6.5 Hz, 1 H, $-NHCH_2-$), 9.97 (s, 1 H, $-NH-$) ppm. HRMS calculated for $C_{29}H_{39}N_5O_{11}S$ 665.24 found 688.41 [M + Na].

Compound 17: This compound was prepared according to the method described for the compound **16**, using compound **8** (0.7 g, 0.694 mmol) and compound **1** (0.185 g, 0.761 mmol), in 78 % yield (0.60 g). ^{1}H NMR: (300 MHz, [D$_6$]DMSO): δ = 1.54 (q, J = 6.8 Hz, 2 H, $-CH_2-$), 1.79−2.15 (8s, 24 H, 8 × $-COCH_3$), 2.25 [s, 6 H, $-N(CH_3)_2$], 2.30 (t, J = 7.0 Hz, 2 H, $-CH_2N-$), 2.50 (s, 3 H, Thio-CH_3), 3.19 (dt, J = 6.0, 7.0 Hz, 2 H, $-NHCH_2-$), 3.95−4.36 (m, 8 H, sugar proton), 5.21−5.58 (m, 4 H, sugar proton), 6.65 (m, 2 H, sugar proton), 6.96 (d, J = 1.8 Hz, 1 H, Py-H), 7.25 (d, J = 1.8 Hz, 1 H, Py-H), 7.45 (d, J = 1.8 Hz, 1 H, Py-H),

7.62 (s, 1 H, Thio-H), 7.95 (d, J = 1.8 Hz, 1 H, Py-H), 8.35 (t, J = 6.5 Hz, 1 H, −NHCH$_2$−), 9.60 (s, 1 H, −NH−), 9.80 (s, 1 H, −NH−) ppm. HR-MS: *m*/*z* calculated for $C_{48}H_{61}N_7O_{21}S$ 1103.36 found 1126.51 [M + Na].

Compound 18: This compound was prepared according to the method described for compound **14**, using compound **16** (0.4 g, 0.601 mmol.) and 0.1 N NaOH solution, in 44 % yield (0.131 g) as a light yellow solid. ^{1}H NMR: (300 MHz, D_2O): δ = 1.54 (q, J = 6.8 Hz, 2 H, −CH$_2$−), 2.26 [s, 6 H, −N(CH$_3$)$_2$], 2.29 (t, J = 7.0 Hz, 2 H, −CH$_2$N−), 2.50 (s, 3 H, −Thio-CH$_3$), 3.20 (dt, J = 6.0, 7.0 Hz, 2 H, −NHC*H*$_2$−), 3.95−4.32 (m, 4 H, sugar proton), 5.21−5.58 (m, 2 H, sugar proton), 6.65 (d, J = 3.85 Hz, 1 H), 7.60 (s, 1 H, Thio-H), 7.69 (d, J = 1.8 Hz, 1 H, Py-H), 7.92 (d, J = 1.8 Hz, 1 H, Py-H) ppm. HRMS calculated for $C_{21}H_{31}N_5O_7S$ 497.19 found 520.35 [M + Na].

Compound 19: This compound was prepared according to the general procedure **B** using compound **17** (0.4 g, 0.362 mmol) and 0.1 N NaOH solution, in 43 % yield (0.12 g) as a solid. ^{1}H NMR: (300 MHz, D_2O): δ = 1.56 (q, J = 6.8 Hz, 2 H, −CH$_2$−), 2.26 [s, 6 H, −N(CH$_3$)$_2$], 2.30 (t, J = 7.0 Hz, 2 H, −C*H*$_2$N−), 2.49 (s, 3 H, Thio-CH$_3$), 3.20 (dt, J = 6.0, 7.0 Hz, 2 H, −NHC*H*$_2$−), 3.91−4.38 (m, 8 H, sugar proton), 5.20−5.57 (m, 4 H, sugar proton), 6.67 (m, 2 H, sugar proton), 6.96 (d, J = 1.8 Hz, 1 H, Py-H), 7.25 (d, J = 1.8 Hz, 1 H, Py-H), 7.48 (d, J = 1.8 Hz, 1 H, Py-H), 7.60 (s, 1 H, Thio-H), 7.93 (d, J = 1.8 Hz, 1 H, Py-H) ppm. HR-MS: *m*/*z* calculated for $C_{32}H_{45}N_7O_{13}S$ 767.28 found 790.50 [M + Na].

General Procedure C: A solution of the acetyl glycosylated nitropolyamides **7** or **8** in MeOH was hydrogenated over 10 % Pd/C at 50 psi pressure for 2 h, after which the catalyst was removed by filtration through a Celite pad. The filtrate was concentrated to dryness under reduced pressure (at room temp.) to afford the corresponding amine. Owing to the sensitivity of the amines to oxidation, they were used for the next reaction immediately. The amine was dissolved in dry DMF, and a mixture of the thio acids **2** or **3** and **4** or **5** (1.0 equivalent), hydroxybenzotriazole (1.0 equivalent), and EDCI (2.5 equivalent), in dry DMF was added. This mixture was stirred at room temperature for 12 h, and after completion of the reaction, the solvent was removed under reduced pressure to afford a dark oil. This was purified by flash column chromatography on silica gel using methanol/dichloromethane as eluent, to afford the acetyl glycosylated polyamides **20**−**23** and **28**−**31** respectively as light yellow solids in 60−70 % yield.

Compound 20: This compound was prepared according to the general procedure **C**, using compound **7** (0.7 g, 1.22 mmol.) and the thio acid **2** (0.176 g, 2.05 mmol), in 67 % yield (0.55 g) as a solid. ^{1}H NMR: (300 MHz, [D$_6$]DMSO): δ = 1.57 (q, J = 6.8 Hz, 2 H, −CH$_2$−), 1.85 (s, 3 H, COCH$_3$), 2.06 (s, 3 H, COCH$_3$), 2.08 (s, 3 H, COCH$_3$), 2.12 (s, 3 H, COCH$_3$), 2.26 [s, 6 H, −N(CH$_3$)$_2$], 2.31 (t, J = 7.0 Hz, 2 H, −CH$_2$N−), 3.20 (dt, J = 6.0, 7.0 Hz, 2 H, −NHC*H*$_2$−), 3.92−4.35 (m, 4 H, sugar proton), 5.21−5.58 (m, 2 H, sugar proton), 6.62 (d, J = 3.85 Hz, 1 H), 7.00 (br. s, 2 H, NH$_2$), 7.15 (s, 1 H, Thio-H), 7.67 (d, J = 1.8 Hz, 1 H, Py-H), 7.89 (d, J = 1.8 Hz, 1 H, Py-H), 8.32 (t, J = 6.5 Hz, 1 H, −N*H*CH$_2$−), 9.87 (s, 1 H, −NH−) ppm. HRMS calculated for $C_{28}H_{38}N_6O_{11}S$ 666.25 found 689.50 [M + Na].

Compound 21: This compound was prepared according to the method described for the compound **20**, using compound **8** (0.7 g, 0.694 mmol.) and the thio acid **2** (0.1 g, 0.694 mmol.) in 65 % yield (0.5 g). ^{1}H NMR: (300 MHz, [D$_6$]DMSO): δ = 1.55 (q, J = 6.8 Hz, 2 H, −CH$_2$−), 1.80−2.12 (8s, 24 H, 8x−COCH$_3$), 2.26 [s, 6 H, −N(CH$_3$)$_2$], 2.31 (t, J = 7.0 Hz, 2 H, −C*H*$_2$N−), 3.20 (dt, J = 6.0, 7.0 Hz, 2 H, −NHC*H*$_2$−), 3.92−4.38 (m, 8 H, sugar proton), 5.20−5.65 (m, 4 H, sugar proton), 6.62 (m, 2 H, sugar proton), 6.98 (d, J = 1.8 Hz, 1 H, Py-H), 6.95 (br. s, 1 H, NH$_2$), 7.18 (s, 1 H, Thio-H), 7.22 (d, J = 1.8 Hz, 1 H, Py-H), 7.42 (d, J = 1.8 Hz, 1 H, Py-H), 7.95 (d, J = 1.8 Hz, 1 H, Py-H), 8.32 (t, J = 6.5 Hz, 1 H, −NHCH$_2$−), 9.80 (s, 1 H, −NH−), 9.98 (s, 1 H, −NH−) ppm. HR-MS: *m*/*z* calculated for $C_{47}H_{60}N_8O_{21}S$ 1104.38 found 1127.40 [M + Na].

Compound 22: This compound was prepared according to the general procedure **C**, using compound **7** (0.7 g, 1.22 mmol.) and the thio acid **3** (0.21 g, 1.22 mmol.), in 65 % yield (0.56 g) as a solid. ^{1}H NMR: (300 MHz, [D$_6$]DMSO): δ = 1.55 (q, J = 6.8 Hz, 2 H, −CH$_2$−), 1.89 (s, 3 H, COCH$_3$), 2.06 (s, 3 H, COCH$_3$), 2.09 (s, 3 H, COCH$_3$), 2.12 (s, 3 H, COCH$_3$), 2.25 [s, 6 H, −N(CH$_3$)$_2$], 2.29 (t, J = 7.0 Hz, 2 H, −CH$_2$N−), 3.19 (dt, J = 6.0, 7.0 Hz, 2 H, −NHC*H*$_2$−), 3.91−4.32 (m, 4 H, sugar proton), 5.20−5.60 (m, 2 H, sugar proton), 6.62 (d, J = 3.85 Hz, 1 H), 7.68 (d, J = 1.8 Hz, 1 H, Py-H), 7.85 (d, J = 1.8 Hz, 1 H, Py-H), 7.90 (s, 1 H, Thio-H), 8.35 (t, J = 6.5 Hz, 1 H, −N*H*CH$_2$−), 8.50 (s, 1 H, −CHO−), 9.50 (s, 1 H, −NH−), 10.20 (s, 1 H, −NH−) ppm. HRMS calculated for $C_{29}H_{38}N_6O_{12}S$ 694.23 found 717.41 [M + Na].

Compound 23: This compound was prepared according to the general method **C**, using compound **8** (0.7 g, 0.694 mmol) and the thio acid **3** (0.118 g, 0.69 mmol) in 63 % yield (0.5 g) as a solid. ^{1}H NMR: (300 MHz, [D$_6$]DMSO): δ = 1.55 (q, J = 6.8 Hz, 2 H, −CH$_2$−), 1.80−2.18 (8s, 24 H, 8 × −COCH$_3$), 2.26 [s, 6 H, −N(CH$_3$)$_2$], 2.31 (t, J = 7.0 Hz, 2 H, −C*H*$_2$N−), 3.20 (dt, J = 6.0, 7.0 Hz, 2 H, −NHC*H*$_2$−), 3.92−4.36 (m, 8 H, sugar proton), 5.20−5.60 (m, 4 H, sugar proton), 6.67 (m, 2 H, sugar proton), 6.96 (d, J = 1.8 Hz, 1 H, Py-H), 7.22 (d, J = 1.8 Hz, 1 H, Py-H), 7.45 (d, J = 1.8 Hz, 1 H, Py-H), 7.92 (s, 1 H, Thio-H), 7.95 (d, J = 1.8 Hz, 1 H, Py-H), 8.35 (t, J = 6.5 Hz, 1 H, −N*H*CH$_2$−), 8.51 (s, 1 H, −CHO−), 9.55 (s, 1 H, −NH−), 9.80 (s, 1 H, −NH−), 10.24 (s, 1 H, −NH−) ppm. HR-MS: *m*/*z* calculated for $C_{48}H_{60}N_8O_{22}S$ 1132.35 found 1155.41 [M + Na].

Compound 24: This compound was prepared according to the general procedure **B**, using compound **20** (0.4 g, 0.60 mmol.) and 0.1 N NaOH solution in 43 % yield (0.13 g) as a light yellow solid. ^{1}H NMR: (300 MHz, D_2O): δ = 1.56 (q, J = 6.8 Hz, 2 H, −CH$_2$−), 2.27 [s, 6 H, −N(CH$_3$)$_2$], 2.30 (t, J = 7.0 Hz, 2 H, −CH$_2$N−), 3.21 (dt, J = 6.0, 7.0 Hz, 2 H, −NHC*H*$_2$−), 3.91−4.32 (m, 4 H, sugar proton), 5.21−5.60 (m, 2 H, sugar proton), 6.64 (d, J = 3.85 Hz, 1 H), 7.18 (s, 1 H, Thio-H), 7.65 (d, J = 1.8 Hz, 1 H, Py-H), 7.90 (d, J = 1.8 Hz, 1 H, Py-H) ppm. HRMS calculated for $C_{20}H_{30}N_6O_7S$ 498.19 found 521.40 [M + Na].

Compound 25: This compound was prepared according to the method described for compound **24**, using compound **21** (0.4 g, 0.362 mmol) and 0.1 N NaOH solution, in 43 % yield (0.12 g) as a light yellow solid. ^{1}H NMR: (300 MHz, D_2O): δ = 1.54 (q, J = 6.8 Hz, 2 H, −CH$_2$−), 2.26 [s, 6 H, −N(CH$_3$)$_2$], 2.30 (t, J = 7.0 Hz, 2 H, −C*H*$_2$N−), 3.19 (dt, J = 6.0, 7.0 Hz, 2 H, −NHC*H*$_2$−), 3.91−4.38 (m, 8 H, sugar proton), 5.21−5.62 (m, 4 H, sugar proton), 6.63 (m, 2 H, sugar proton), 6.96 (d, J = 1.8 Hz, 1 H, Py-H), 7.20 (s, 1 H, Thio-H), 7.26 (d, J = 1.8 Hz, 1 H, Py-H), 7.45 (d, J = 1.8 Hz, 1 H, Py-H), 7.90 (d, J = 1.8 Hz, 1 H, Py-H) ppm. HR-MS: *m*/*z* calculated for $C_{31}H_{44}N_8O_{13}S$ 768.27 found 791.40 [M + Na].

Compound 26: Prepared according to general method **B**, using compound **22** (0.4 g, 0.576 mmol.) and 0.1 N NaOH solution, as a solid in 44 % yield (0.135 g). ^{1}H NMR: (300 MHz, D_2O): δ = 1.56 (q,

J = 6.8 Hz, 2 H, $-CH_2-$), 2.26 [s, 6 H, $-N(CH_3)_2$], 2.30 (t, J = 7.0 Hz, 2 H, $-CH_2N-$), 3.20 (dt, J = 6.0, 7.0 Hz, 2 H, $-NHCH_2-$), 3.93−4.35 (m, 4 H, sugar proton), 5.21−5.58 (m, 2 H, sugar proton), 6.60 (d, J = 3.85 Hz, 1 H), 7.65 (d, J = 1.8 Hz, 1 H, Py-H), 7.84 (d, J = 1.8 Hz, 1 H, Py-H), 7.91 (s, 1 H, Thio-H), 8.52 (s, 1 H, −CHO−) ppm. HRMS calculated for $C_{21}H_{30}N_6O_8S$ 526.18 found 549.40 [M + Na].

Compound 27: This compound was prepared according to the method described for the compound **25**, using compound **23** (0.4 g, 0.353 mmol) and 0.1 N NaOH solution, in 46 % yield (0.13 g) as a solid. ^{1}H NMR: (300 MHz, D_2O): δ = 1.56 (q, J = 6.8 Hz, 2 H, $-CH_2-$), 2.25 [s, 6 H, $-N(CH_3)_2$], 2.30 (t, J = 7.0 Hz, 2 H, $-CH_2N-$), 3.19 (dt, J = 6.0, 7.0 Hz, 2 H, $-NHCH_2-$), 3.95−4.38 (m, 8 H, sugar proton), 5.20−5.65 (m, 4 H, sugar proton), 6.65 (m, 2 H, sugar proton), 6.95 (d, J = 1.8 Hz, 1 H, Py-H), 7.20 (d, J = 1.8 Hz, 1 H, Py-H), 7.42 (d, J = 1.8 Hz, 1 H, Py-H), 7.90 (s, 1 H, Thio-H), 7.98 (d, J = 1.8 Hz, 1 H, Py-H), 8.52 (s, 1 H, −CHO−) ppm. HR-MS: *m*/*z* calculated for $C_{32}H_{44}N_8O_{14}S$ 796.27 found 819.40 [M + Na].

Compound 28: This compound was prepared according to the general procedure **C**, using compound **7** (0.7 g, 1.22 mmol.) and the thio acid **4** (0.194 g, 1.22 mmol.), in 67 % yield (0.56 g) as a solid. ^{1}H NMR: (300 MHz, [D_6]DMSO): δ = 1.55 (q, J = 6.8 Hz, 2 H, $-CH_2-$), 1.80 (s, 3 H, $COCH_3$), 2.05 (s, 3 H, $COCH_3$), 2.08 (s, 3 H, $COCH_3$), 2.10 (s, 3 H, $COCH_3$), 2.26 [s, 6 H, $-N(CH_3)_2$], 2.31 (t, J = 7.0 Hz, 2 H, $-CH_2N-$), 2.48 (s, 3 H, Thio-CH_3), 3.20 (dt, J = 6.0, 7.0 Hz, 2 H, $-NHCH_2-$), 3.95−4.37 (m, 4 H, sugar proton), 5.20−5.60 (m, 2 H, sugar proton), 6.60 (d, J = 3.85 Hz, 1 H), 7.05 (br. s, 2 H, NH_2), 7.65 (d, J = 1.8 Hz, 1 H, Py-H), 7.90 (d, J = 1.8 Hz, 1 H, Py-H), 8.35 (t, J = 6.5 Hz, 1 H, $-NHCH_2-$), 9.92 (s, 1 H, −NH−) ppm. HRMS calculated for $C_{29}H_{40}N_6O_{11}S$ 680.25 found 703.20 [M + Na].

Compound 29: This compound was prepared according to the method described for the compound **28**, using compound **8** (0.7 g, 0.694 mmol) and the thio acid **4** (0.11 g, 0.696 mmol), in 67 % yield (0.52 g) as a light yellow solid. ^{1}H NMR: (300 MHz, [D_6]DMSO): δ = 1.56 (q, J = 6.8 Hz, 2 H, $-CH_2-$), 1.79−2.15 (8s, 24 H, 8x$-COCH_3$), 2.27 [s, 6 H, $-N(CH_3)_2$], 2.30 (t, J = 7.0 Hz, 2 H, $-CH_2N-$), 2.50 (s, 3 H, Thio-CH_3), 3.18 (dt, J = 6.0, 7.0 Hz, 2 H, $-NHCH_2-$), 3.95−4.38 (m, 8 H, sugar proton), 5.20−5.60 (m, 4 H, sugar proton), 6.65 (m, 2 H, sugar proton), 6.96 (d, J = 1.8 Hz, 1 H, Py-H), 7.00 (br. s, 1 H, NH_2), 7.25 (d, J = 1.8 Hz, 1 H, Py-H), 7.45 (d, J = 1.8 Hz, 1 H, Py-H), 7.90 (d, J = 1.8 Hz, 1 H, Py-H), 8.35 (t, J = 6.5 Hz, 1 H, $-NHCH_2-$), 9.85 (s, 1 H, −NH−), 9.98 (s, 1 H, −NH−) ppm. HR-MS: *m*/*z* calculated for $C_{48}H_{62}N_8O_{21}S$ 1118.38 found 1141.40 [M + Na].

Compound 30: This compound was prepared by employing compound **7** (0.7 g, 1.22 mmol) and the thio acid **5** (0.228 g, 1.22 mmol) according to general procedure **C**, in 63 % yield (0.55 g) as a light yellow solid. ^{1}H NMR: (300 MHz, [D_6]DMSO): δ = 1.54 (q, J = 6.8 Hz, 2 H, $-CH_2-$), 1.80 (s, 3 H, $COCH_3$), 2.08 (s, 3 H, $COCH_3$), 2.10 (s, 3 H, $COCH_3$), 2.15 (s, 3 H, $COCH_3$), 2.26 [s, 6 H, $-N(CH_3)_2$], 2.30 (t, J = 7.0 Hz, 2 H, $-CH_2N-$), 2.50 (s, 3 H, Thio-CH_3), 3.20 (dt, J = 6.0, 7.0 Hz, 2 H, $-NHCH_2-$), 3.95−4.38 (m, 4 H, sugar proton), 5.21−5.62 (m, 2 H, sugar proton), 6.60 (d, J = 3.85 Hz, 1 H), 7.70 (d, J = 1.8 Hz, 1 H, Py-H), 7.85 (d, J = 1.8 Hz, 1 H, Py-H), 8.31 (t, J = 6.5 Hz, 1 H, $-NHCH_2-$), 8.51 (s, 1 H, −CHO−), 9.50 (s, 1 H, −NH−), 10.22 (s, 1 H, −NH−) ppm. HRMS calculated for $C_{30}H_{40}N_6O_{12}S$ 708.24 found 731.31 [M + Na].

Compound 31: Prepared according to general procedure **C**, using compound **8** (0.7 g, 0.694 mmol) and the thio acid **5** (0.129 g, 0.693 mmol), in 64 % yield (0.51 g) as a light yellow solid. ^{1}H NMR: (300 MHz, [D_6]DMSO): δ = 1.56 (q, J = 6.8 Hz, 2 H, $-CH_2-$), 1.79−2.18 (8s, 24 H, 8 × $-COCH_3$), 2.25 [s, 6 H, $-N(CH_3)_2$], 2.31 (t, J = 7.0 Hz, 2 H, $-CH_2N-$), 2.51 (s, 3 H, Thio-CH_3), 3.19 (dt, J = 6.0, 7.0 Hz, 2 H, $-NHCH_2-$), 3.95−4.38 (m, 8 H, sugar proton), 5.21−5.59 (m, 4 H, sugar proton), 6.65 (m, 2 H, sugar proton), 6.95 (d, J = 1.8 Hz, 1 H, Py-H), 7.20 (d, J = 1.8 Hz, 1 H, Py-H), 7.43 (d, J = 1.8 Hz, 1 H, Py-H), 7.92 (d, J = 1.8 Hz, 1 H, Py-H), 8.32 (t, J = 6.5 Hz, 1 H, $-NHCH_2-$), 8.50 (s, 1 H, −CHO−), 9.58 (s, 1 H, −NH−), 9.80 (s, 1 H, −NH−), 10.24 (s, 1 H, −NH−) ppm. HR-MS: *m*/*z* calculated for $C_{49}H_{62}N_8O_{22}S$ 1146.37 found 1169.50 [M + Na].

Compound 32: This compound was prepared by using compound **28** (0.4 g, 0.588 mmol.) and 0.1 N NaOH solution according to general procedure **B** in 43 % yield (0.13 g) as a light yellow solid. ^{1}H NMR: (300 MHz, D_2O): δ = 1.56 (q, J = 6.8 Hz, 2 H, $-CH_2-$), 2.25 [s, 6 H, $-N(CH_3)_2$], 2.30 (t, J = 7.0 Hz, 2 H, $-CH_2N-$), 2.50 (s, 3 H, Thio-CH_3), 3.21 (dt, J = 6.0, 7.0 Hz, 2 H, $-NHCH_2-$), 3.95−4.36 (m, 4 H, sugar proton), 5.21−5.59 (m, 2 H, sugar proton), 6.62 (d, J = 3.85 Hz, 1 H), 7.65 (d, J = 1.8 Hz, 1 H, Py-H), 7.90 (d, J = 1.8 Hz, 1 H, Py-H) ppm. HRMS calculated for $C_{21}H_{32}N_6O_7S$ 512.21 found 535.30 [M + Na].

Compound 33: This compound was prepared according to the method described for the compound **32**, using compound **29** (0.4 g, 0.357 mmol) and 0.1 N NaOH solution, in 46 % yield (0.13 g) as a light yellow solid. ^{1}H NMR: (300 MHz, D_2O): δ = 1.55 (q, J = 6.8 Hz, 2 H, $-CH_2-$), 2.25 [s, 6 H, $-N(CH_3)_2$], 2.31 (t, J = 7.0 Hz, 2 H, $-CH_2N-$), 2.51 (s, 3 H, Thio-CH_3), 3.20 (dt, J = 6.0, 7.0 Hz, 2 H, $-NHCH_2-$), 3.92−4.38 (m, 8 H, sugar proton), 5.21−5.62 (m, 4 H, sugar proton), 6.65 (m, 2 H, sugar proton), 6.95 (d, J = 1.8 Hz, 1 H, Py-H), 7.26 (d, J = 1.8 Hz, 1 H, Py-H), 7.42 (d, J = 1.8 Hz, 1 H, Py-H), 7.91 (d, J = 1.8 Hz, 1 H, Py-H) ppm. HR-MS: *m*/*z* calculated for $C_{32}H_{46}N_8O_{13}S$ 782.29 found 805.40 [M + Na].

Compound 34: This compound was prepared according to general procedure **B**, using compound **30** (0.4 g, 0.564 mmol) and 0.1 N NaOH solution, as a light yellow solid in 44 % yield (0.135 g). ^{1}H NMR: (300 MHz, D_2O): δ = 1.55 (q, J = 6.8 Hz, 2 H, $-CH_2-$), 2.27 [s, 6 H, $-N(CH_3)_2$], 2.31 (t, J = 7.0 Hz, 2 H, $-CH_2N-$), 2.50 (s, 3 H, Thio-CH_3), 3.19 (dt, J = 6.0, 7.0 Hz, 2 H, $-NHCH_2-$), 3.94−4.37 (m, 4 H, sugar proton), 5.20−5.60 (m, 2 H, sugar proton), 6.62 (d, J = 3.85 Hz, 1 H), 7.69 (d, J = 1.8 Hz, 1 H, Py-H), 7.80 (d, J = 1.8 Hz, 1 H, Py-H), 8.50 (s, 1 H, −CHO−) ppm. HRMS calculated for $C_{22}H_{32}N_6O_8S$ 540.20 found 563.25 [M + Na].

Compound 35: Prepared according to the method described for the compound **33**, using compound **31** (0.4 g, 0.349 mmol) and 0.1 N NaOH solution, in 42 % yield (0.12 g) as a light yellow solid. ^{1}H NMR: (300 MHz, D_2O): δ = 1.55 (q, J = 6.8 Hz, 2 H, $-CH_2-$), 2.26 [s, 6 H, $-N(CH_3)_2$], 2.30 (t, J = 7.0 Hz, 2 H, $-CH_2N-$), 2.50 (s, 3 H, Thio-CH_3), 3.20 (dt, J = 6.0, 7.0 Hz, 2 H, $-NHCH_2-$), 3.95−4.37 (m, 8 H, sugar proton), 5.20−5.60 (m, 4 H, sugar proton), 6.62 (m, 2 H, sugar proton), 6.95 (d, J = 1.8 Hz, 1 H, Py-H), 7.22 (d, J = 1.8 Hz, 1 H, Py-H), 7.45 (d, J = 1.8 Hz, 1 H, Py-H), 7.75 (d, J = 1.8 Hz, 1 H, Py-H), 8.52 (s, 1 H, −CHO−) ppm. HR-MS: *m*/*z* calculated for $C_{33}H_{46}N_8O_{14}S$ 810.29, found 833.41 [M + Na].

Acknowledgments

We gratefully acknowledge the financial support of the Natural Sciences and Engineering Research Council of Canada (to J. W. L.).

[1] R. Schleif, *Science* **1988**, *241*, 1182−1187.
[2] P. B. Dervan, *Science* **1986**, *232*, 464−471.
[3] "*Molecular Basis of Specificity in Nucleic Acid-Drug Interaction*" (Eds.: B. Pullman, J. Jortner), Kluwer Academic Publishers, London **1990**.
[4] J. W. Lown, *Anti-Cancer Drug Design* **1988**, *3*, 25−40.
[5] P. E. Nielsen, *Bioconjugate Chem.* **1990**, *2*, 1−12.
[6] M. L. Stephenson, D. C. Zamecnik, *Proc. Natl, Acad, Sci., USA* **1978**, *75*, 285−288.
[7] J. G. Izant, H. Weintraub, *Cell* **1984**, *36*, 1007−1015.
[8] C. Helene, T. Monteray-Garestier, T. Saison, M. Takasugi, J. J. Toulme, V. Asseline, G. Lancelot, J. C. Maurizot, F. Toulme, N. T. Thuong, *Biochimie* **1985**, *67*, 777−783.
[9] J. T. Holt, G. T. Venkat, A. D. Moulton, A. W. Nienhuis, *Proc. Natl, Acad, Sci., USA* **1986**, *83*, 4794−4798.
[10] P. S. Miller, C. H. Agris, L. Aureliani, K. R. Blake, A. Murakami, M. P. Reddy, S. A. Spitz, P. O. P. Ts'O, *Biochimie* **1986**, *67*, 769−776.
[11] L. J. Maher, B. Wold, P. B. Dervan, *Science* **1989**, *245*, 725−730.
[12] J. M. Gottesfeld, L. Neely, J. W. Trauger, E. E. Baird, P. B. Dervan, *Nature* **1997**, *387*, 202−205.
[13] J. G. Pelton, D. E. Wemmer, *Proc. Natl, Acad, Sci., USA* **1989**, *86*, 5723−5727.
[14] M. Mrksich, M. E. Parks, P. B. Dervan, *J. Am. Chem. Soc.* **1994**, *116*, 7983−7988.
[15] Y. H. Chen, J. W. Lown, *J. Am. Chem. Soc.* **1994**, *116*, 6995−7005.
[16] M. Mrksich, P. B. Dervan, *J. Am. Chem. Soc.* **1994**, *116*, 3663−3664.
[17] R. S. Youngquist, P. B. Dervan, *J. Am. Chem. Soc.* **1985**, *107*, 5528−5529.
[18] W. Wang, J. W. Lown, *J. Med. Chem.* **1992**, *35*, 2890−2897.
[19] L. A. Dickinson, R. J. Gulizia, J. W. Trauger, E. E. Baird, D. E. Mosier, J. M. Gottesfeld, P. B. Dervan, *Proc. Natl. Acad. Sci. USA* **1998**, *95*, 12890−12895.
[20] J. W. Lown, R. G. Micetich, *US Patent*, Patent no. 5,616,606. Dated April, 01, **1997**.
[21] J. W. Lown, K. Krowicki, *US Patent*, Patent no. 4,912,199. Dated May, 10, **1995**.
[22] N. Neamati, A. Mazumder, S. Sunder, J. M. Owen, M. Tandon, J. W. Lown, Y. Pommier, *Mol. Pharmacology* **1998**, *54*, 280−290.
[23] J. W. Lown, S. K. Sharma, A general procedure for aqueous solubilization of DNA binding polyamides for increased cellular uptake., patent pending **2003**.
[24] R. Kumar, J. W. Lown, *Organic Biomolecular Chemistry* **2003**, *1*, 3327−3342.
[25] R. Kumar, J. W. Lown, *Oncology Research* **2003** (manuscript submitted).
[26] M. L. Kopka, C. Yoon, D. Goodsell, P. Pjura, E. Dickerson, *Proc. Natl Acad. Sci. USA* **1985**, *82*, 1376−1380; J. W. Lown, K. Krowicki, U. G. Bhat, A. Skorobogaty, J. C. Dabrowiak, *Biochemistry* **1986**, *25*, 7408−7416.
[27] M. L. Kopka, D. S. Goodsell, G. W. Han, T. K. Chiu, J. W. Lown, R. E. Dickerson, *Structure* **1997**, *5*, 1033−1046.; D. H. Nguyen, J. W. Szewczyk, E. E. Baird, P. B. Dervan, *Bioorg. Med. Chem.* **2001**, *9*, 7−17.
[28] C. C. O'Hare, D. Mack, M. Tandon, S. K. Sharma, J. W. Lown, M. L. Kopka, R. E. Dickerson, J. A. Hartley, *Proc. Natl Acad. Sci. USA* **2002**, *99*, 72−77.
[29] S. K. Sharma, M. Tandon, J. W. Lown, *J. Org. Chem.* **2000**, *65*, 1102−1107.
[30] S. K. Sharma, M. Tandon, J. W. Lown, *Tetrahedron* **2002**, *58*, 3417−3421.
[31] J. P. Henichart, J. L. Beuvien, N. Helbecque, R. Houssin, *Nucleic Acids Res.* **1985**, *13*, 6703−6717.
[32] H. Kasai, H. Naganawa, H. Takita, H. Umezawa, *J. Antibiot. (Tokyo)* **1978**, *32*, 1316−1320.
[33] K. E. Rao, R. G. Shea, B. Yadagri, J. W. Lown, *Anti-Cancer Drug Des.* **1990**, *5*, 3−20.
[34] M. L. Kopka, G. W. Han, D. S. Goodsell, T. K. Chiu, W. L. Walker, J. W. Lown, R. E. Dickerson, in *Structure, Motion, Interaction and Expression of Biological Macromolecules* (Eds.: R. H. Sarma, M. H. Sarma), Proceedings of the Tenth Conversation, State University New York, Albany, NY; Adenine Press: Guilderland, NY, **1998**; p. 177.
[35] K. E. Rao, B. Yadagiri, J. W. Lown, *J. Org. Chem.* **1990**, *55*, 728−737.

Received August 22, 2003

