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Design, synthesis and *in vitro* cytotoxicity studies of novel pyrrolo [2,1][1,4] benzodiazepine-glycosylated pyrrole and imidazole polyamide conjugates

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Received 16th June 2003, Accepted 5th August 2003
First published as an Advance Article on the web 8th September 2003

The design, synthesis and biological evaluation of novel pyrrolo [2,1][1,4] benzodiazepine-water insoluble **31**–**38** and water soluble **39**–**46** glycosylated pyrrole and imidazole polyamide conjugates are described that involved mercuric chloride mediated cyclization of the corresponding amino diethyl thioacetals. The compounds were prepared with varying numbers of pyrrole and imidazole containing polyamides and incorporating glucose moieties in order to improve the water solubility of PBD-polyamide conjugates and probe the structural requirements for optimal *in vitro* antitumor activity. These compounds were tested against a panel of 60 human cancer cells by the National Cancer Institute, and demonstrated that the water soluble PBD-polyamide compounds exhibited a higher level of cytotoxic activity than the existing natural and synthetic pyrrolo [2,1-*c*][1,4] benzodiazepines. The cytotoxic activities of these compounds dramatically increase after hydrolysis of their acetylated counterparts. The activity data summarized in Table 1 and Table 2 show that the solubility of the PBD-polyamides and also the type of heterocycle play important roles influencing the cytotoxic activity of the PBD-polyamide conjugates. The PBD-glycosylated polyamide (water soluble) conjugates **39**–**46** are highly cytotoxic against many human cancer cell lines in comparison with the PBD-polyamide (water insoluble version) conjugates.

Introduction

The biological activity of certain low molecular weight antitumour compounds appears to be related to their mode and specificity of interaction with particular DNA sequences. Such small molecules are of considerable interest in chemistry, biology and medicine. Many of the anticancer drugs employed clinically also exert their antitumour effect by inhibiting nucleic acid (DNA or RNA) or protein synthesis. Inhibition can occur for example through cross-linking of bases in DNA or binding to and inactivation of enzymes necessary for key processes. It is evident that DNA is an important cellular target for many anticancer agents. DNA is a well-characterized intracellular target but its large size and sequential nature makes it an elusive target for selective drug action. Binding of low molecular weight ligands to DNA causes a wide variety of potential biological responses. In this context PBDs (pyrrolo [2,1-*c*][1,4] benzodiazepines), a group of potent naturally occurring antitumor antibiotics produced by various *Streptomyces* species,[1,2] are one of the promising types of lead compounds. They differ in the number, type and position of substituent in both their aromatic A-ring and pyrrolidine C-rings, and in the degree of saturation of the C-rings which can be either fully saturated or unsaturated at either the C2–C3 (endocyclic) or C2 (exocyclic) positions. There is either an imine or carbinolamine methyl ether moiety at the N10–C11 position.[3–6] This latter is an electrophilic centre responsible for alkylating DNA.

To date, 13 structures, including such compounds as anthramycin, tomaymycin, chicamycin, neothramycin, and DC-81 (Fig. 1) have been isolated from various *Streptomyces* species. Their common interaction with DNA has been extensively investigated and it is considered unique since they bind within the minor groove of DNA forming a covalent aminal bond between the C11-position of the central B-ring and the N2 amino group of guanine base.[7] The cytotoxicity and antitumor activity of PBDs are thus attributed to their ability to form covalent DNA adducts. Molecular modeling, solution NMR, flourimetry experiment and DNA footprinting experiments have shown that these molecules have a preferred selectivity for Pu–G–Pu[8] sequences and are oriented with their A-rings pointed either towards the 3′- or –5′ end of the covalently bonded DNA strand (as in the case of anthramycin and tomaymycin). The PBDs have been shown to interfere with the action of endonuclease enzymes on DNA[9] and to block transcription by inhibiting DNA polymerase in a sequence specific manner,[10] processes, which may be relevant for their biological activity.

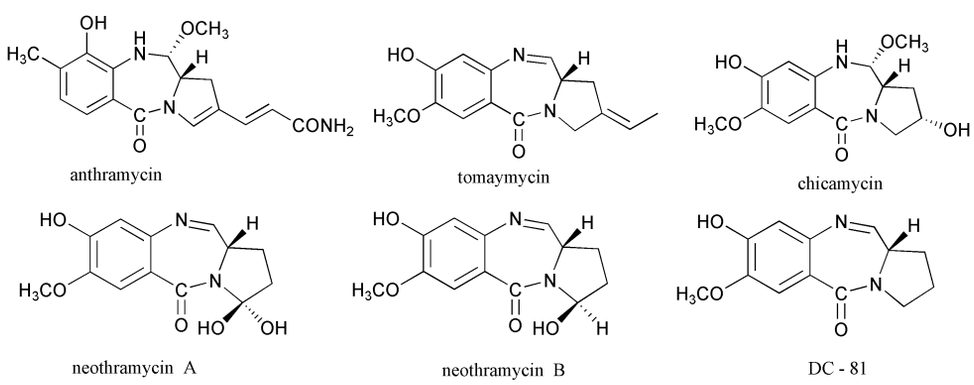


Fig. 1 Compounds isolated from various *Streptomyces* species.

DOI: 10.1039/b306685a

In the search for compounds with better antitumour selectivity and DNA sequence specificity many PBD analogues have been synthesized in an attempt to increase their potency against tumor cells. Confalone and coworkers[11] have synthesized PBD analogues with an epoxide group attached at the C-11a position with the objective of producing a PBD structure with DNA cross-linking activity. Recently, Thurston and coworkers, in order to increase cytotoxicity through the production of DNA cross-links, have designed and synthesized PBD dimers[12] comprising two PBD units joined through their A-rings. Similarly, Baraldi and coworkers[13] as well as Lown and coworkers[14–19] have prepared new PBD-pyrrole and imidazole polyamide conjugates to explore their biological properties. Kamal and coworkers[20] recently designed and synthesized non-cross-linking mixed imine–amide PBD dimers that have significant DNA-binding affinity and potent anti-tumour activity.

Recently a large number of structurally modified PBDs compounds have also been prepared and evaluated for their biological activity, particularly their antitumour potential.[21,22] A number of these compounds have been selected for preclinical studies but unfortunately most of them did not proceed beyond that stage, due to problems related to poor bioavailability and low water solubility. Therefore Kamal and coworkers[23,24] recently designed and synthesized PBD-naphthalimides, morpholine, *N*-methyl piperizine and *N*, *N*-diethylamine hybrids in attempts to improve the water solubility and cytotoxicity of the PBDs compounds.

In a complementary approach polyamides containing pyrrole (Py) and imidazole (Im) moieties, that are based on the naturally occurring compounds netropsin and distamycin, constitute a class of compounds having DNA sequence specificity.[25] Polyamides show specific and high affinity binding for nucleotide sequences in the minor groove of DNA and in favourable cases the binding strength is comparable to sequence-specific DNA binding proteins. 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.[26–29] Polyamides can block the activity of processive enzymes complexes or interfere with the binding of DNA-binding proteins. The binding of transcription factors (TFs) to DNA cis elements is central to cellular regulation, and the identification of novel agents which can be targeted at regulating this binding interaction would therefore be of great therapeutic value.

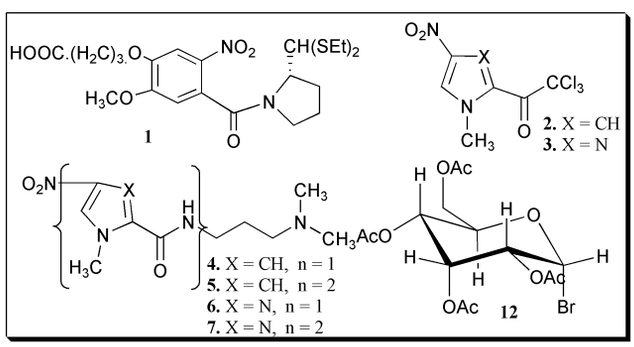
Increasing the number of pyrrole (Py) and imidazole (Im) groups in a polyamide compound results in a direct increase in the length of base pairs recognized by the polyamide in the minor groove of the DNA. The disadvantage to increasing the number of such groups is that the aqueous solubility of the drug is progressively decreased, often resulting in substantially reduced cellular uptake or bioavailability. Earlier 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. Thus there is an urgent need for water soluble polyamides which could overcome the solubility problem of conventional polyamides.[30]

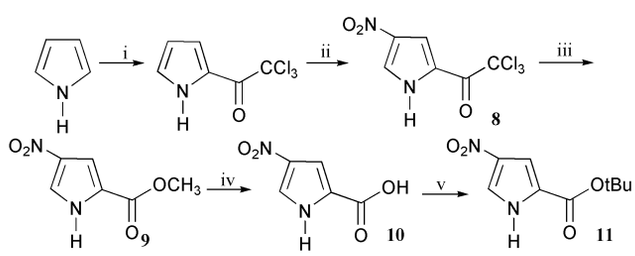
In view of the commonly observed activity of polyamides and PBDs, we have also been interested in structural modifications of the PBD ring system and also towards the development of new synthetic PBD hybrids with polyamides. Therefore it was considered of interest to design and synthesize C-8 linked PBD-water soluble polyamide conjugates and to investigate the effects of these structural changes on their biological properties. In continuation of these efforts, we herein report the design, synthesis and *in vitro* antitumour cytotoxicity activities of novel C-8 linked PBD-water soluble pyrrole and imidazole polyamide hybrids.

Results and discussion

Synthesis

In our previous report the (2*S*)-*N*-[5-methoxy-4-[3-(carboxy) propyloxy]-2-nitrobenzoyl] pyrrolidine-2-carboxaldehyde diethyl thioacetal (**1**) and compounds **2**–**7** were synthesized.[15,19] by using convenient routes in good yield. The synthesis of a pyrrole with a D-glucose moiety attached at the 1-position of the heterocycle was performed using the route outlined in Scheme 1. The functionalization of the pyrrole ring was performed at positions 2 and 4 by trichloroacetyl chloride and fuming nitric acid, successively, to yield compound **8**, which when treated with methanol gave the methyl ester **9** in good yield. Basic hydrolysis of compound **9** gave the corresponding acid **10** which was then converted into an acidic labile *tert*-butyl ester **11** by treating the acid with isobutylene under acidic conditions according to Scheme 1. Bromination of the anomeric acetate group of the 2,3,4,6-tetra-*O*-acetyl-α-D-glucose with hydrobromic acid in acetic acid gave compound 2,3,4,6-tetra-*O*-acetyl-α-D-bromoglucose (**12**) in 85% yield. The coupling of the *tert*-butyl ester pyrrole unit **11** with the 2,3,4,6-tetra-*O*-acetyl-α-D-bromoglucose **12** was achieved using KOH and a phase transfer catalyst (18-crown-6-ether) to give compound **13** in 80% yield (Scheme 2).

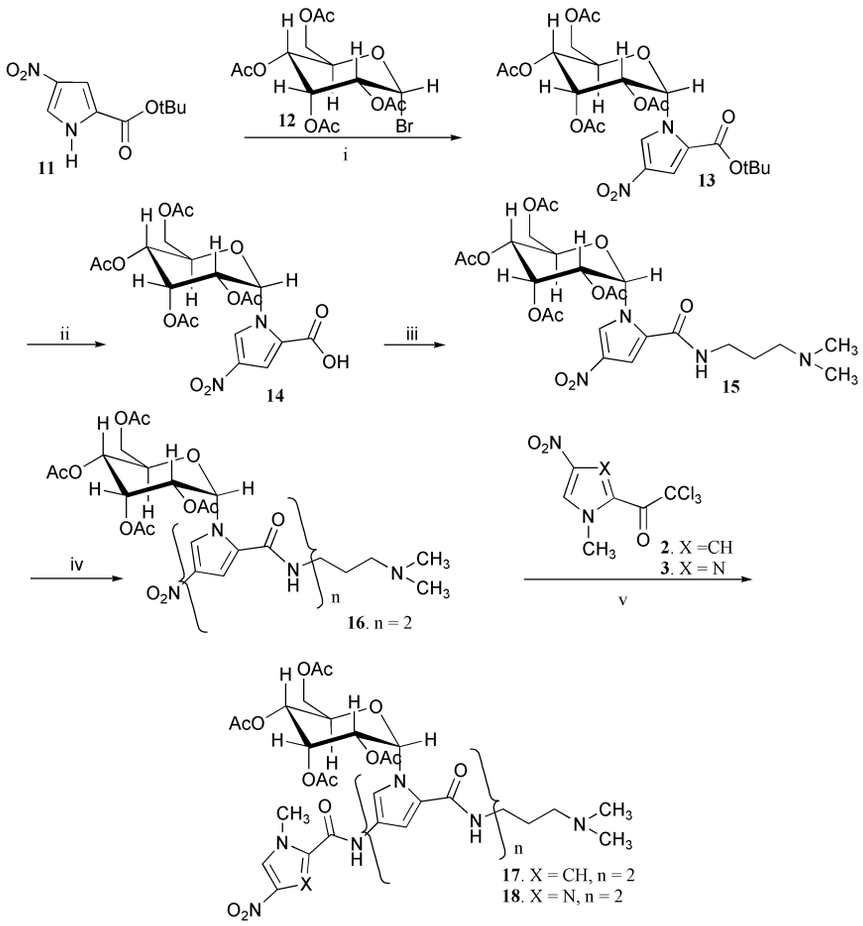




Scheme 1 (i) Trichloroacetyl chloride, TEA CH_2Cl_2 0 °C to RT, 6 h, 80%; (ii) fuming HNO_3, acetic anhydride, CH_2Cl_2, −40 °C to RT, 12 h, 70%; (iii) MeOH, DMAP, 60 °C, 10 h, 88%; (iv) 1 N NaOH, MeOH, 60 °C 20 h, 80%; (v) isobutylene, conc. H_2SO_4, diethylether, −40 °C, RT, 15 h, 83%.

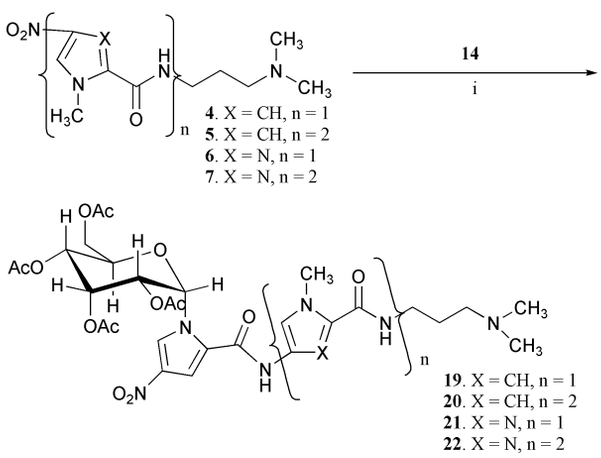
The *tert*-butyl ester group of compound **13** was hydrolyzed using 1 M $TiCl_4$ solution in dichloromethane to afford acid **14** in 78% yield. This acid **14** was then coupled with 3-dimethylaminopropylamine using EDCI/HOBt as coupling reagents to give compound **15** in 77% yield. The nitro group of compound **15** was reduced with hydrogen in the presence of Pd/C catalyst into the corresponding amino compound which was then coupled (for the chain elongation of the glucopyrrole carboxamide peptide) with acid **14** using standard EDCI/HOBt coupling conditions to afford compound **16** in 80% yield. The nitro group of compound **16** was converted into its amino counterpart with hydrogen in the presence of Pd/C catalyst and then treated with compounds **2 or 3** in the presence of triethylamine in dry THF give polyamides **17** and **18**, respectively in good yields (Scheme 2).

The nitro groups of compound **4**, **5**, **6**, and **7** were similarly



Scheme 2 (i) KOH, DMF, 18-crown-6-ether, RT, 4 h, 80%; (ii) 1.0 M $TiCl_4$ solution in dichloromethane, CH_2Cl_2, RT, 12 h 78%; (iii) $NH_2(CH_3)_3N(CH_3)_2$, EDCI, HOBt, DMF, RT, 12 h, 77%; (iv) (a) H_2 Pd/C, MeOH, RT, (b) EDCI, HOBt, DMF, RT, 12 h, 80% (v) (a) H_2 Pd/C, MeOH, RT, (b) **2 or 3**, TEA, THF, RT, 6 h.

reduced with hydrogen in the presence of Pd/C catalyst into their corresponding respective amino compounds which were then coupled with acid **14** using standard EDCI/HOBt as coupling reagents to give compounds **19**–**22**, respectively in good yields (Scheme 3).



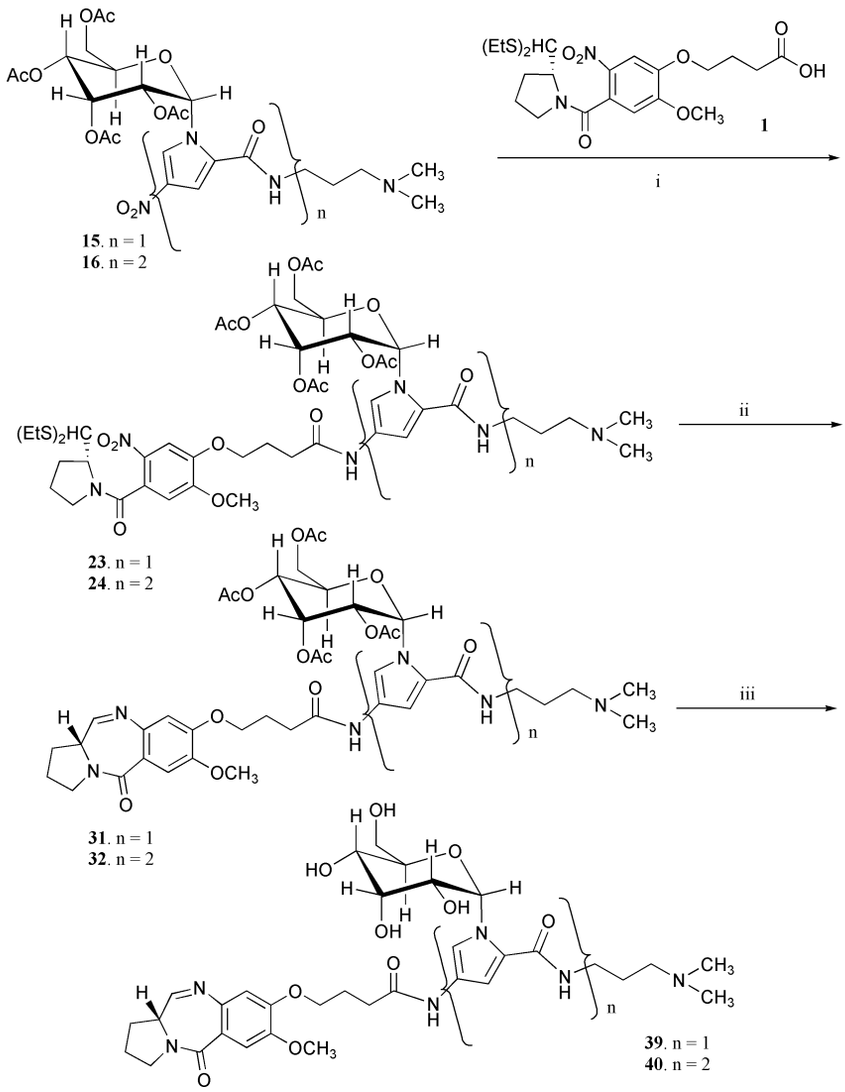
Scheme 3 (i) (a) H_2 Pd/C, MeOH, RT, (b) EDCI, HOBt, DMF, RT, 12 h, 75–81%.

Coupling of the (2*S*)-*N*-[5-methoxy-4-[3-(carboxy) propyloxy]-2-nitrobenzoyl] pyrrolidine-2-carboxaldehyde diethyl thioacetal (**1**) with the amine moiety of the polyamides **15**–**22**, using EDCI and HOBt as coupling agents in dry DMF afforded the corresponding coupled compounds **23**–**30**, respectively in good yields.

The nitro groups of compounds **23**–**50** were reduced with hydrogen in the presence of Pd/C catalyst into their corresponding respective amino compounds which were then subjected to deprotective cyclization with $HgCl_2$/HgO in aqueous acetonitrile at room temperature affording the corresponding imines **31**–**38** in 40–45% yield. Hydrolysis of compounds **31**–**38** with 0.1 N NaOH at room temperature for 2–3 h gave water soluble PBD-glycosylated pyrrole and imidazole polyamide conjugates **39**–**46** in good yield. Since the conjugation of the polyamides with PBD resulted in highly polar imines, this necessitated the use of methanol in combination with chloroform as eluent during the purification of the imines by column chromatography and therefore the product was obtained as a mixture of imine and its methyl ether in approximately a 1 : 1 ratio. The presence of both the forms was confirmed by NMR and mass spectra. Since the methyl ether form is fully equivalent to the imine form in terms of their reaction with DNA, the compounds were isolated as the mixture of imines and methyl ethers. This did not present any problems in evaluating their biological activities. The final compounds were isolated as pale yellow crystalline compounds in 50–60% overall yields after passing through an ion exchange column with methanol/water (Schemes 4, 5, and 6).

Anticancer cytotoxicity

The pyrrolo [2,1][1,4] benzodiazepine-acetyl glycosylated (water insoluble) **31**–**38** and glycosylated (water soluble) **39**–**46** pyrrole and imidazole polyamide conjugates, which contain one or more pyrrole and imidazole units, were selected by the US National Cancer Institute for evaluation in the *in vitro* preclinical antitumor screening program against sixty human tumour cell lines derived from nine cancer types, leukemia, non-small cell lung cancer, colon cancer, CNS cancer, melan-



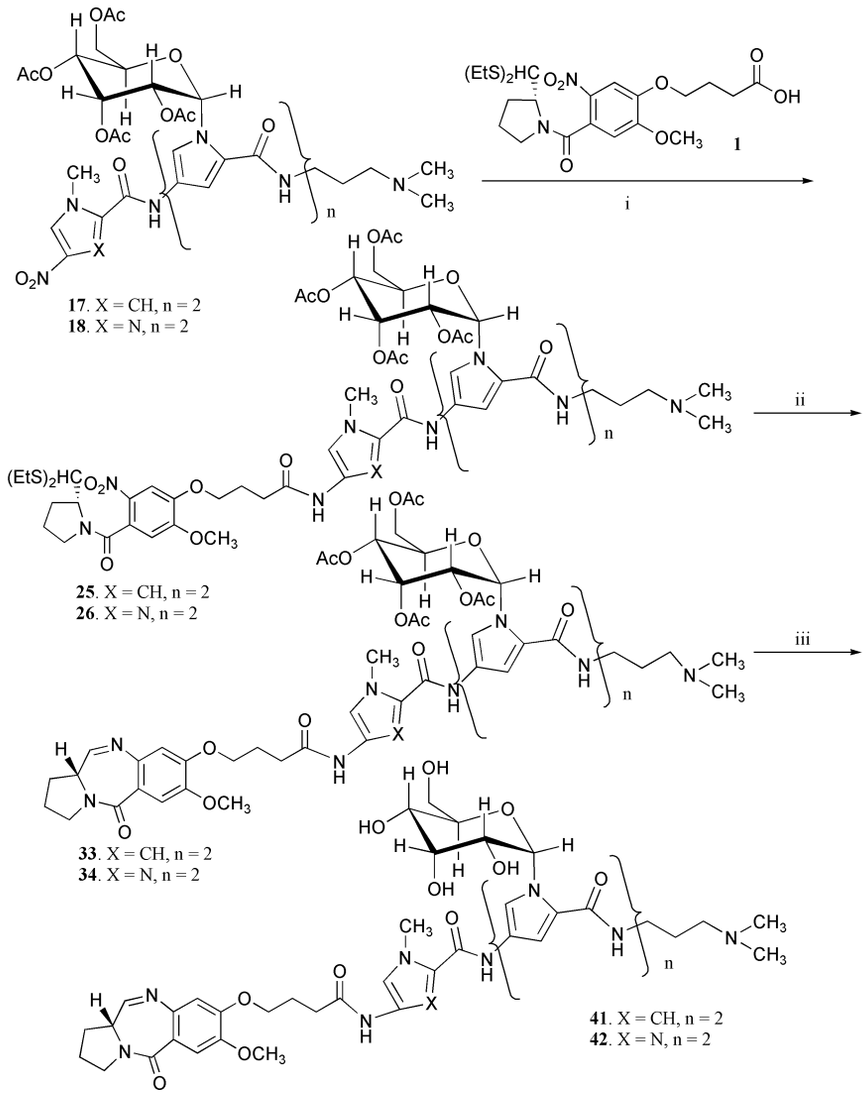
Scheme 4 (i) (a) H_2 Pd/C, MeOH, RT, (b) EDCI, HOBt, DMF, RT, 12 h, 73–74%; (ii) (a) H_2 Pd/C, MeOH, RT, (b) $HgCl_2$, HgO, 75% aq CH_3CN, 41–43%; (iii) 0.1 N NaOH, MeOH/THF (1 : 1) RT, 53–60%.

oma, ovarian cancer, renal cancer, prostate cancer and breast cancer. For each compound, dose response curves for each cell line were measured at a minimum of five concentrations at 10 fold dilutions in a protocol of 48 h continuous drug exposure, and a sulfurhodamine B (SRB) protein assay was used to estimate cell viability or growth. The concentration causing 50% cell growth inhibition (GI_{50}), total cell growth inhibition (TGI, 0% growth), and 50% cell death (LC_{50}, −50% growth) compared with the control was calculated. The LC_{50} values (concentration for killing 50% of the cells) for compounds **31**–**38** and **39**–**46** against the 60 different tumor cells are given in Table 1 (water insoluble) and Table 2 (water soluble).

In general all the compounds are active against most of the cell lines with the MG_MID (mean graph mid point) values ranging from −4.00 to −6.23. Compound **31** which has one acetyl glycosylated pyrrole unit shows almost the same cytotoxicity with the $\log_{10}LC_{50}$ values ranging from −5.05 to −5.27 against non-small cell lung, colon, melanoma, renal and breast cancer cells, while its water soluble counterpart **39** shows somewhat lower cytotoxicity against the same cell lines. Compound **32**, bearing two acetyl glycosylated pyrrole units displayed lower cytotoxicity compared with the compound **31** against the same cell lines, while its water soluble version compound **40** displays much higher cytotoxicity against the same cell lines with $\log_{10}LC_{50}$ values ranging from −5.07 to −5.23.

Compound **33**, having two acetyl glycosylated pyrrole units and one *N*-methyl pyrrole unit, displayed similar cytotoxic potencies against the non-small cell lung, CNS, melanoma, renal and breast cancer cell lines with $\log_{10}LC_{50}$ values ranging from −5.04 to −5.41. Compound **34**, bearing two acetyl glycosylated pyrrole units and one *N*-methyl imidazole unit, shows notably higher cytotoxicity against the melanoma cancer cells M14 and SK-MEL-5 with $\log_{10}LC_{50}$ value −6.04 and −6.25. This compound **34** has also shown promising activity against colon cancer cells HCT-116 and KM-12 with $\log_{10}LC_{50}$ value −5.71 and −6.08. On the other hand its water soluble version, compound **42**, shows somewhat lower cytotoxic potency against the same cell lines but, in contrast the water soluble version of compound **33** *i.e.* compound **41** the higher cytotoxicity against the renal cancer cell 786-O ($\log_{10}LC_{50}$ value −5.92). From the biological data from Table 1 and Table 2 we can conclude that the compounds which bear two glycosylated pyrrole units and one *N*-methyl pyrrole unit **33** and **41** are much more potent compared with their counterparts the *N*-methyl imidazole compounds **34** and **42**.

Compound **35**, with one acetyl glycosylated pyrrole unit and one *N*-methyl pyrrole unit, displayed a higher potency against colon cancer cells Colo-205 and HCT-116 with $\log_{10}LC_{50}$ values −6.21 and −6.01. This compound **35** also shows higher cytotoxicity against renal cancer cell 786-O ($\log_{10}LC_{50}$ value −6.11) and breast cancer cell T-47D ($\log_{10}LC_{50}$ value −6.01), while its water soluble version compound **43** displayed somewhat lower cytotoxicity against the same cell lines. Compound **37**, which has one acetyl glycosylated pyrrole unit and one *N*-methyl

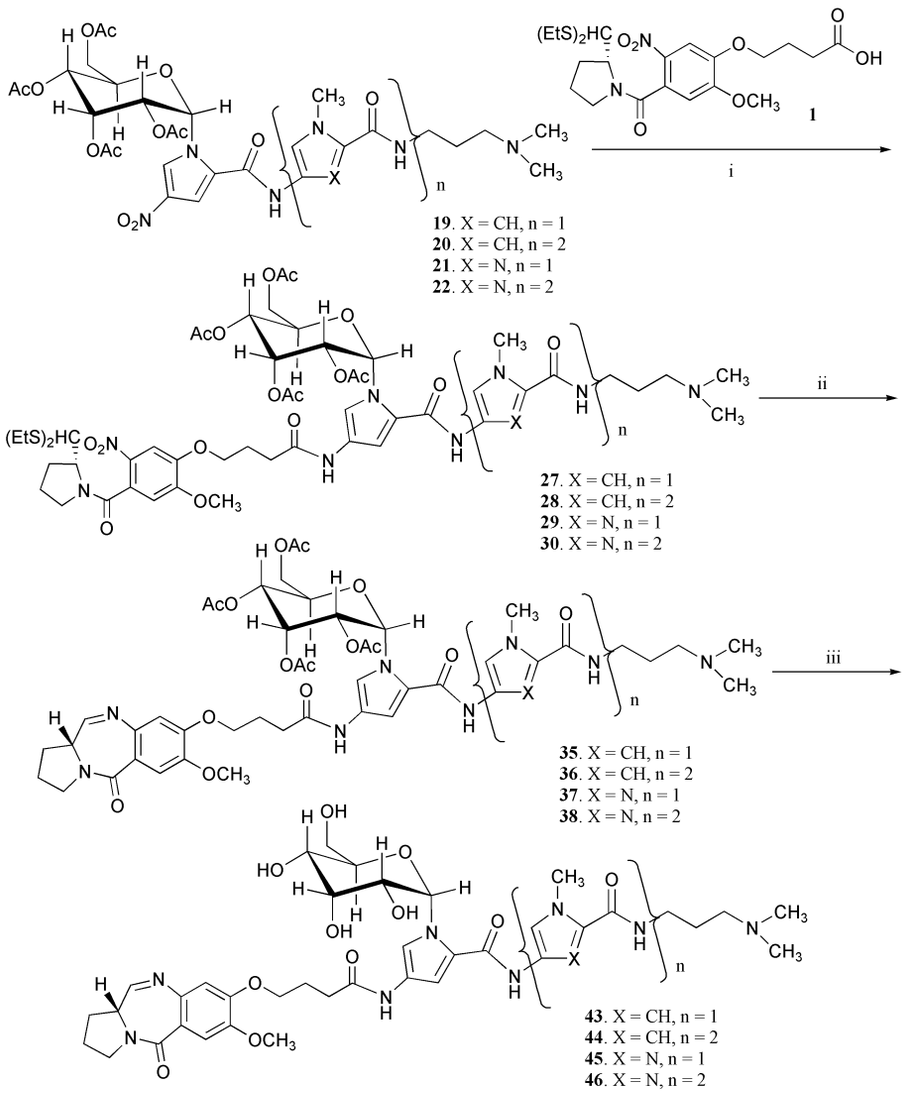


Scheme 5 (i) (a) H_2 Pd/C, MeOH, RT, (b) EDCI, HOBt, DMF, RT, 12 h, 75–77%; (ii) (a) H_2 Pd/C, MeOH, RT, (b) $HgCl_2$, HgO, 75% aq CH_3CN, 40–42%; (iii) 0.1 N NaOH, MeOH/THF (1 : 1) RT, 50–57%.

imidazole unit, displayed higher potency against the colon cancer cell Colo-205 ($log_{10}LC_{50}$ value −6.10) and renal cancer cell 786-O ($log_{10}LC_{50}$ value −6.18), while its water soluble version compound **45** displayed somewhat lower cytotoxicity against the same cell lines. However compound **45** displays higher cytotoxic potency against the CNS cancer cell line SNB-75 ($log_{10}LC_{50}$ value −5.13) and renal cancer cell ACHN ($log_{10}LC_{50}$ value −4.87).

Compound **36**, bearing one acetyl glycosylated pyrrole unit and two *N*-methyl pyrrole units displayed very good potencies against the colon cancer cells Colo-205 and HCT-116 with the $log_{10}LC_{50}$ values −6.18 and −5.19, and also showed higher cytotoxicity against the renal cancer cell line 786-O ($log_{10}LC_{50}$ value −6.24) and breast cancer cell T-47D ($log_{10}LC_{50}$ value −6.16). It is noteworthy that its water soluble counterpart compound **44** is also more potent against non-small cell lung, colon, melanoma, renal and breast cancer cell lines with the $log_{10}LC_{50}$ values ranging from −5.03 to −5.30. Compound **38**, having one acetyl glycosylated pyrrole unit and two *N*-methyl imidazole units displayed almost the same cytotoxic potencies against all the human cancer cell lines used by the NIH, and this compound displayed $log_{10}LC_{50}$ value −4.30 against the colon cancer cell HCT-116. The water soluble version of this compound *i.e.* **46** shows markedly higher cytotoxicities against non-small cell lung cancer cell EKVX ($log_{10}LC_{50}$ value −7.16), colon cancer cells Colo-205 and HCT-116 ($log_{10}LC_{50}$ values < −8.00 and 7.13), melanoma cancer cells Malme-3M, M14, SK-MEL-28, SK-MEL-5 with $log_{10}LC_{50}$ values < −8.00, −7.95, −7.15, −7.07. Compound **46** is also highly potent against all the renal cancer cell lines with $log_{10}LC_{50}$ values ranging from −7.19 to < −8.00. In addition Compound **46** displayed higher cytotoxic potencies against the breast cancer cell lines MDA-MB-231/ATCC, MDA-MB-435 and BT-549 with $log_{10}LC_{50}$ value −7.30, 7.11 and 7.37. From these results we can conclude that the water soluble compound **46** with one glycosylated pyrrole unit and two *N*-methyl imidazole units, is much more potent in comparision with the compounds **43**, **44** and **45**.

It can be seen from the comparison of the cytotoxic data presented in Table 1 (water insoluble) and in Table 2 (water soluble) that in individual cases certain PBD-acetyl glycosylated polyamide conjugates are active in comparison to their water soluble version. However, in general, most of the PBD-glycosylated polyamide (water soluble) conjugates are more highly potent compared with their water insoluble counterparts. For example in the case of compounds **39**, **40**, **41**, **44**, and **46** these water soluble versions are significantly more highly potent then their water insoluble counterparts. It is evident that the cytotoxic activity of these compounds increases dramatically after hydrolysis of their acetyl derivatives (Fig. 2). There are individual exceptions, *e.g.* in the case of compounds **34**, **35**, and **37**, where the water insoluble versions are more potent than the water soluble version. Comparing the cytotoxic data among the PBD-polyamide



Scheme 6 (i) (a) H_2 Pd/C, MeOH, RT, (b) EDCI, HOBt, DMF, RT, 12 h, 70–77%; (ii) (a) H_2 Pd/C, MeOH, RT, (b) $HgCl_2$, HgO, 75% aq CH_3CN, 40–45%; (iii) 0.1 N NaOH, MeOH/THF (1 : 1) RT, 51–56%.

Table 1 *In vitro*, cytotoxic potencies ($log_{10}LC_{50}$) of novel pyrrolo [2,1][1,4] benzodiazepine-acetyl glycosylated pyrrole and imidazole polyamide conjugates **31**–**38** (water insoluble) against nine panels of human cell lines

	$log_{10}LC_{50}/\mu M$ [a]							
Panels/cancer cell lines	**31**	**32**	**33**	**34**	**35**	**36**	**37**	**38**
Leukemia								
CCRF-CEM	> −4.00	> −4.00	−4.28	> −4.00	> −4.00	> −4.00	> −4.00	> −4.00
HL-60 (TB)	—	—	—	> −4.00	> −4.00	> −4.00	> −4.00	—
K-562	> −4.00	—	−4.48	—	—	—	—	> −4.00
MOLT-4	> −4.00	> −4.00	> −4.00	> −4.00	> −4.00	> −4.00	> −4.00	> −4.00
RPMI-8226	> −4.00	> −4.00	> −4.00	> −4.00	> −4.00	> −4.00	> −4.00	> −4.00
Non-small cell lung cancer								
A549/ATCC	> −4.00	−4.05	−4.24	> −4.00	> −4.00	> −4.00	—	> −4.00
EKVX	−5.12	> −4.00	−5.08	> −4.00	−5.06	−5.09	−4.30	> −4.00
HOP-62	−5.14	−4.13	−5.07	> −4.00	−5.22	−5.26	−5.19	−4.12
HOP-92	−5.10	−4.19	−5.20	> −4.00	−5.22	−5.28	−5.17	−4.11
NCI-H226	—	—	—	> −4.00	—	—	—	—
NCI–H23	−5.19	−4.04	−5.12	—	−5.26	−5.28	−5.28	> −4.00
NCI–H322M	> −4.00	−4.17	−4.41	−4.88	−4.46	−4.30	−4.23	−4.04
NCI–H460	> −4.00	> −4.00	−4.33	> −4.00	> −4.00	> −4.00	> −4.00	> −4.00
NCI–H522	> −4.00	> −4.00	> −4.00	> −4.00	> −4.00	> −4.00	> −4.00	> −4.00
Colon cancer								
COLO 205	−5.17	−4.19	−4.98	−4.74	−6.21	−6.18	−6.10	> −4.00
HCC-2998	−5.01	−4.12	—	—	—	—	—	> −4.00
HCT-116	> −4.00	> −4.00	—	−5.71	−6.05	−5.89	−5.21	−4.30
HCT-15	−5.01	> −4.00	−5.23	> −4.00	−5.23	−5.24	−5.19	> −4.00
HT29	> −4.00	> −4.00	−4.59	> −4.00	−5.26	−5.27	−4.20	> −4.00

Table 1 (*Contd.*)

	$\log_{10}LC_{50}/\mu M$[a]							
Panels/cancer cell lines	**31**	**32**	**33**	**34**	**35**	**36**	**37**	**38**
KM12	> −4.00	−4.16	−4.46	−6.08	−5.29	−5.21	−4.02	> −4.00
SW-620	> −4.00	> −4.00	−4.92	> −4.00	−5.04	—	−5.05	> −4.00
CNS cancer								
SF-268	−4.41	−4.05	−4.46	> −4.00	−5.09	> −4.00	−5.16	> −4.00
SF-295	> −4.00	−4.11	−4.05	−4.32	−5.20	−5.24	−5.22	> −4.00
SF-539	−5.22	−4.51	−5.31	−4.44	—	—	—	> −4.00
SNB-19	−4.23	−4.20	−4.38	−5.21	−5.26	−5.31	−5.27	> −4.00
SNB-75	−4.38	−4.07	−4.33	—	−5.24	−5.28	−4.27	−4.09
U251	−5.18	> −4.00	−5.28	—	−5.23	−5.25	−5.24	> −4.00
Melanoma								
LOX IMVI	−5.27	> −4.00	−5.41	−5.21	−5.21	−5.23	−5.19	> −4.00
MALME-3M	> −4.00	−4.19	−5.04	−5.16	−5.21	−5.27	−4.21	> −4.00
M14	−5.10	−4.12	−5.27	−6.04	−5.24	−5.30	−5.26	> −4.00
SK-MEL-2	> −4.00	> −4.00	−4.00	> −4.00	−5.18	−5.17	−5.14	> −4.00
SK-MEL-28	−5.14	−4.04	−5.15	—	−5.25	−5.23	−4.41	−4.04
SK-MEL-5	−5.14	−4.13	−5.16	−6.25	−5.22	−5.24	−5.20	−4.02
UACC-257	> −4.00	> −4.00	−4.54	> −4.00	−5.14	−5.15	−5.21	> −4.00
UACC-62	−5.16	−4.26	−5.10	−5.33	−5.23	−5.27	−5.25	−4.17
Ovarian cancer								
IGROV 1	> −4.00	> −4.00	−4.43	> −4.00	> −4.00	> −4.00	> −4.00	> −4.00
OVCAR-3	> −4.00	−4.10	—	> −4.00	−5.27	−5.31	—	> −4.00
OVCAR-4	> −4.00	> −4.00	−4.55	> −4.00	−5.25	−5.25	−4.22	> −4.00
OVCAR-5	−5.07	−4.13	−4.78	> −4.00	−5.20	−5.28	−5.24	> −4.00
OVCAR-8	−5.07	−4.20	−5.19	> −4.00	> −4.00	> −4.00	—	> −4.00
SK-OV-3	−4.04	−4.12	−4.31	−5.18	−5.26	−5.26	−5.26	−4.17
Renal cancer								
786-0	−5.25	−4.22	−5.32	—	−6.11	−6.24	−6.18	−4.14
A498	−4.21	−4.20	−4.89	—	−4.35	—	—	> −4.00
ACHN	−5.18	−4.03	−5.26	—	−5.24	−5.27	−5.23	−4.08
CAKI-1	−5.10	−4.23	—	> −4.00	−5.31	−5.32	−5.27	> −4.00
RXF-393	−5.05	−4.16	−4.69	> −4.00	−5.11	> −4.00	−5.11	> −4.00
SN12C	−5.14	−4.22	−5.24	—	—	−5.23	—	−4.12
TK-10	> −4.00	−4.09	—	> −4.00	−5.24	−5.27	−4.56	> −4.00
UO-31	−4.22	> −4.00	−5.13	> −4.00	−5.41	−5.32	−5.35	−4.06
Prostate cancer								
PC-3	> −4.00	> −4.00	−5.12	> −4.00	−5.16	−5.20	−5.04	> −4.00
DU-145	−4.54	−4.12	−5.26	—	−5.18	−5.26	—	−4.08
Breast cancer								
MCF 7	> −4.00	> −4.00	> −4.00	> −4.00	> −4.00	> −4.00	> −4.00	> −4.00
NCI/ADR-RES	−5.19	−4.01	−5.14	—	−5.14	−5.24	—	> −4.00
MDA-MB231/ATCC	−5.17	−4.22	−5.19	> −4.00	−5.22	−5.30	−5.17	> −4.00
HS578T	> −4.00	> −4.00	> −4.00	> −4.00	> −4.00	> −4.00	> −4.00	> −4.00
MDA-MB-435	−5.05	−4.14	−4.13	—	−5.23	−5.26	−5.22	> −4.00
BT-549	−5.19	−4.20	−5.22	> −4.00	−5.19	−5.25	−5.14	−4.06
T-24D	—	> −4.00	−4.72	−4.62	−6.01	−6.16	−5.38	> −4.00
MGM[b]	−4.53	−4.09	−4.75	−4.38	−5.00	−4.99	−4.84	−4.03

[a] The cytotoxicity $\log_{10}LC_{50}$ values are the concentrations corresponding to 50% growth inhibition. [b] Mean graph midpoint (μM) for growth inhibition against all human cancer cell lines tested.

Table 2 *In vitro*, cytotoxic potencies ($\log_{10}LC_{50}$) of novel pyrrolo [2,1][1,4] benzodiazepine-glycosylated (water soluble) pyrrole and imidazole polyamide conjugates **39**–**46** (water soluble) against nine panels of human cell lines

	$\log_{10}LC_{50}/\mu M$[a]							
Panels/cancer cell lines	**39**	**40**	**41**	**42**	**43**	**44**	**45**	**46**
Leukemia								
CCRF-CEM	—	> −4.00	> −4.00	> −4.00	> −4.00	> −4.00	> −4.00	> −4.00
HL-60 (TB)	—	> −4.00	> −4.00	> −4.00	> −4.00	—	−4.10	—
K-562	—	> −4.00	> −4.00	—	—	> −4.00	—	—
MOLT-4	> −4.00	> −4.00	> −4.00	> −4.00	> −4.00	> −4.00	−4.10	> −4.00
RPMI-8226	> −4.00	> −4.00	> −4.00	> −4.00	> −4.00	> −4.00	> −4.00	> −4.00
Non-small cell lung cancer								
A549/ATCC	−4.11	> −4.00	> −4.00	> −4.00	> −4.00	> −4.00	> −4.00	—
EKVX	−4.19	—	−4.16	> −4.00	> −4.00	−5.15	> −4.00	−7.16

Table 2 (*Contd.*)

Panels/cancer cell lines	$\log_{10}LC_{50}/\mu M$ [a]							
	39	**40**	**41**	**42**	**43**	**44**	**45**	**46**
HOP-62	−4.28	−5.14	> −4.00	> −4.00	> −4.00	−5.06	−4.47	−6.34
HOP-92	−4.09	−5.06	−5.18	> −4.00	> −4.00	−5.22	> −4.00	—
NCI–H226	—	—	> −4.00	> −4.00	> −4.00	—	−4.36	—
NCI–H23	−4.21	—	> −4.00	> −4.00	> −4.00	—	−5.25	—
NCI–H322M	> −4.00	−4.27	> −4.00	> −4.00	> −4.00	−4.24	> −4.00	−6.02
NCI–H460	> −4.00	> −4.00	> −4.00	> −4.00	> −4.00	> −4.00	> −4.00	> −4.00
NCI–H522	−4.14	> −4.00	—	> −4.00	> −4.00	—	> −4.00	—
Colon cancer								
COLO 205	−4.30	−4.69	> −4.00	> −4.00	> −4.00	−5.16	−4.33	< −8.00
HCC-2998	—	−5.36	−4.33	—	—	> −4.00	—	—
HCT-116	—	> −4.00	—	> −4.00	> −4.00	−5.32	−4.61	—
HCT-15	−4.26	−5.15	−4.25	> −4.00	> −4.00	−5.24	> −4.00	−7.13
HT29	−4.14	> −4.00	> −4.00	> −4.00	> −4.00	> −4.00	−4.52	> −4.00
KM12	−4.12	> −4.00	−4.16	> −4.00	−4.21	−4.04	−4.74	—
SW-620	−4.15	> −4.00	—	> −4.00	> −4.00	—	> −4.00	—
CNS cancer								
SF-268	−4.19	—	−4.64	> −4.00	> −4.00	> −4.00	> −4.00	—
SF-295	> −4.00	—	−4.16	> −4.00	> −4.00	> −4.00	−4.30	−6.20
SF-539	—	—	−5.23	—	> −4.00	—	−4.02	< −8.00
SNB-19	> −4.00	−5.12	> −4.00	> −4.00	> −4.00	−4.11	−4.47	−6.22
SNB-75	−4.19	−5.11	−4.11	> −4.00	> −4.00	−4.55	−5.13	−6.18
U251	−5.29	−5.18	−4.29	> −4.00	> −4.00	—	−4.32	—
Melanoma								
LOX IMVI	—	—	−4.59	> −4.00	> −4.00	−4.16	> −4.00	—
MALME-3M	−4.17	−5.10	−5.16	> −4.00	> −4.00	—	−4.24	< −8.00
M14	—	—	−5.21	> −4.00	> −4.00	−5.29	> −4.00	−7.95
SK-MEL-2	−4.20	> −4.00	−4.15	> −4.00	> −4.00	−4.41	> −4.00	—
SK-MEL-28	−4.21	−5.07	> −4.00	> −4.00	> −4.00	−5.19	−4.36	−7.15
SK-MEL-5	−4.29	−5.11	−5.16	> −4.00	> −4.00	−5.23	−5.24	−7.07
UACC-257	> −4.00	> −4.00	> −4.00	> −4.00	> −4.00	> −4.00	> −4.00	> −4.00
UACC-62	−4.29	−5.19	−4.30	> −4.00	> −4.00	−5.26	−4.57	−6.59
Ovarian cancer								
IGROV 1	−4.07	> −4.00	−4.00	> −4.00	> −4.00	> −4.00	> −4.00	> −4.00
OVCAR-3	−4.12	—	−5.07	> −4.00	> −4.00	> −4.00	−4.46	—
OVCAR-4	−4.09	> −4.00	−4.09	> −4.00	> −4.00	> −4.00	−4.19	—
OVCAR-5	−4.26	−5.15	−5.24	> −4.00	> −4.00	—	−4.18	—
OVCAR-8	−4.20	> −4.00	−4.03	> −4.00	> −4.00	—	> −4.00	—
SK-OV-3	−4.11	> −4.00	> −4.00	> −4.00	> −4.00	−4.06	−4.57	−6.10
Renal cancer								
786-0	−4.26	−5.23	−5.92	> −4.00	> −4.00	−5.36	−4.61	< −8.00
A498	−4.24	−4.41	−4.24	—	> −4.00	−4.26	—	−5.86
ACHN	−4.25	−5.17	−4.63	> −4.00	> −4.00	−5.26	−4.87	−7.34
CAKI-1	−4.20	−5.15	−5.07	> −4.00	> −4.00	−5.03	> −4.00	−7.51
RXF-393	−4.20	−5.07	−5.14	> −4.00	> −4.00	—	−4.03	−7.19
SN12C	−4.27	—	−4.28	> −4.00	> −4.00	—	—	−7.30
TK-10	−4.19	> −4.00	−4.39	> −4.00	> −4.00	—	−4.59	< −8.00
UO-31	−4.24	−5.11	−5.17	> −4.00	> −4.00	−5.21	> −4.00	—
Prostate cancer								
PC-3	> −4.00	> −4.00	−4.30	> −4.00	> −4.00	—	> −4.00	—
DU-145	−4.27	−5.36	−5.21	—	> −4.00	−5.25	—	−7.25
Breast cancer								
MCF 7	> −4.00	> −4.00	> −4.00	> −4.00	> −4.00	> −4.00	> −4.00	> −4.00
NCI/ADR-RES	−4.13	—	—	> −4.00	> −4.00	> −4.00	−4.25	—
MDA-MB231/ATCC	−4.29	—	−4.35	> −4.00	> −4.00	−5.22	> −4.00	−7.30
HS578T	> −4.00	> −4.00	—	> −4.00	> −4.00	> −4.00	> −4.00	> −4.00
MDA-MB-435	−4.18	−5.02	−4.34	> −4.00	> −4.00	−5.26	> −4.00	−7.11
BT-549	−4.27	−5.20	−5.22	> −4.00	> −4.00	−5.30	> −4.00	−7.37
T-24D	> −4.00	> −4.00	> −4.00	> −4.00	> −4.00	> −4.00	−4.44	> −4.00
MG_MID [b]	−4.16	−4.51	−4.42	−4.00	−4.00	−4.53	−4.21	−6.23

[a] The cytotoxicity $\log_{10}LC_{50}$ values are the concentrations corresponding to 50% growth inhibition. [b] Mean graph midpoint (μM) for growth inhibition against all human cancer cell lines tested.

conjugates in our group[15,19] the activity data presented in Table 1 and Table 2 show that the solubility of the PBD-polyamides and also the nature of the heterocycles play important roles in influencing the cytotoxic activities of the PBD-polyamide conjugates. This study found that PBD-glycosylated polyamide (water soluble) conjugates **39**–**46** in general are

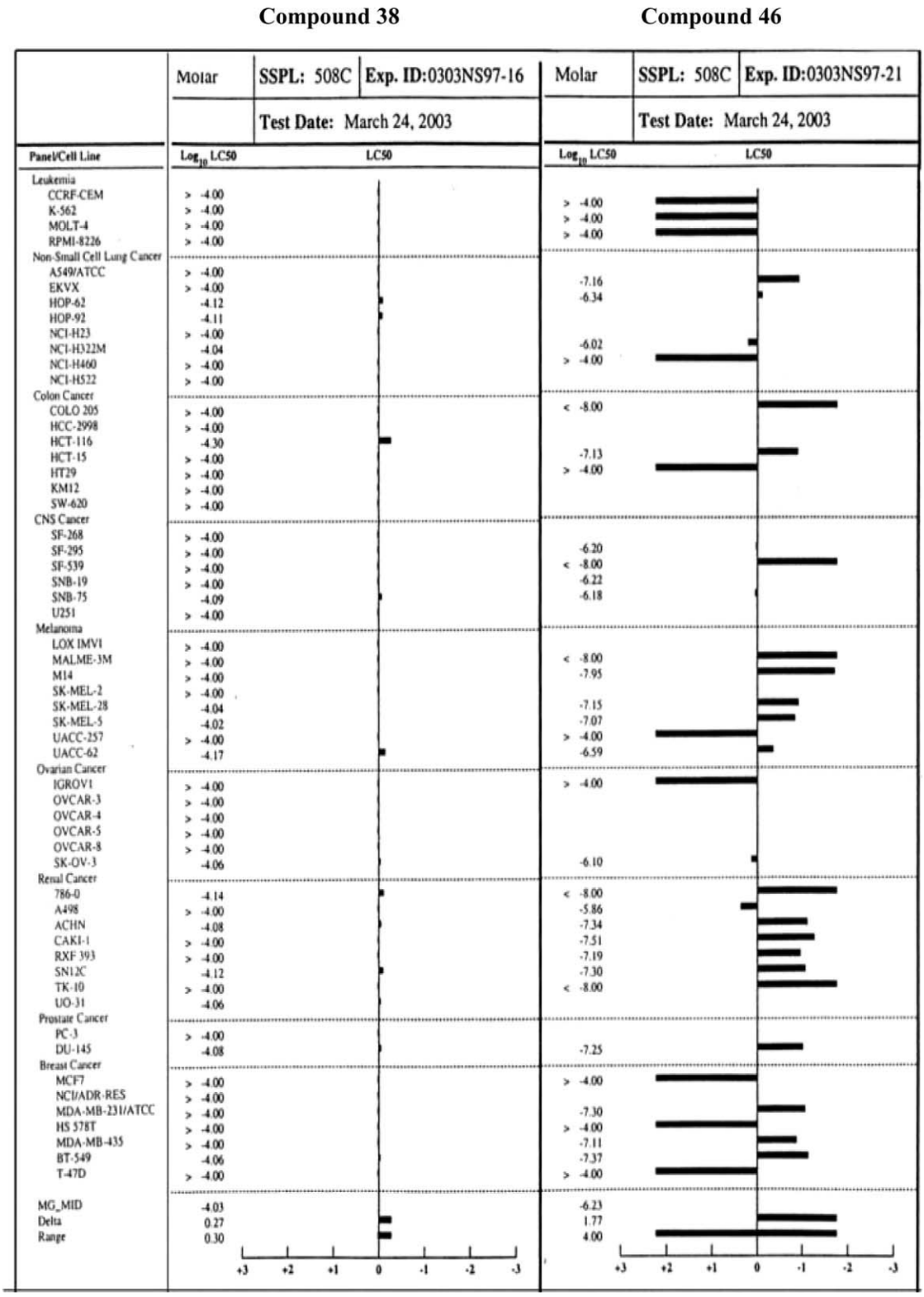


Fig. 2 Comparison between PBD-water insoluble acetylglycosylated polyamide compound **38** and PBD-water soluble glycosylated polyamide compound **46**.

highly potent against many human cancer cell lines and in comparison with the PBD-polyamide (water insoluble version) conjugates.

In summary, we have described the first synthesis of the PBD-water soluble pyrrole and imidazole polyamide conjugates and also their anticancer evaluation against 60 human tumor cell lines in 9 cancer panels. More details of the biophysical, intracellular uptake and localization and additional biological evaluation will be reported in due course.

Experimental

Kieselgel 60 (230–400 mesh) from E. Merck was used for flash column chromatography, and precoated silica gel 60F-254 sheets from E. Merck were used for TLC, with the solvent system indicated in the procedure. TLC plates were visualized by using UV light. All compounds obtained commercially were used without further purification unless otherwise stated. Methanol and ethanol was freshly distilled over magnesium

turnings; tetrahydrofuran was distilled over sodium benzophenone ketyl under an atmosphere of dry argon, ether was dried over sodium; methylene chloride was freshly distilled from calcium hydride, triethylamine was treated with potassium hydroxide then distilled from barium oxide and stored over 3 Å molecular sieves, dry dimethylformamide and all commercially available chemicals were purchased from Aldrich Chemical Co. The ^{1}H NMR spectra were recorded on 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 (quartet), p (pentet) or m (multiplet). FAB (fast atom bombardment) mass spectra with glycerol as the matrix were determined on Associate Electrical Ind. (AEI) MS-9 and MS-50 focusing high-resolution mass spectrometers.

2,2,2-Trichloro-1-(4-nitro-1*H*-pyrrol-2-yl)-ethanone (8)

To a solution of 2,2,2-trichloro-1-(1*H*-pyrrol-2-yl)-ethanone (5.3 g, 24.94 mmol) in acetic anhydride (40.0 ml) at −40 °C was added fuming nitric acid 8.0 ml over a period of 30 min while a temperature of −40 °C was maintained. The reaction mixture was carefully allowed to warm to room temperature and stirred for an additional 6 h. After being stirred for 6 h the solvent and acetic anhydride were removed under reduced pressure and the residue was purified by column chromatography eluting with EtOAc/DCM (2 : 98) to give compound **8** 4.5 g in 70% yield as a solid. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.26 (d, 1H, J = 1.8 Hz, Py–H), 7.76 (d, 1H, J = 1.8 Hz, Py–H), 10.10 (brs, 1H, –NH–). MS: *m*/*z* calculated for $C_6H_3Cl_3N_2O_3$ 257.50, found 280.15 (M + Na).

4-Nitro-1*H*-pyrrole-2-carboxylic acid methyl ester (9)

A solution of the 2,2,2-trichloro-1-(4-nitro-1*H*-pyrrol-2-yl)-ethanone (**8**) (4.5 g, 17.47 mmol) in MeOH and DMAP (catalyst) was stirred at 60 °C temperature until all the starting material disappeared. After completion the reaction mixture was cooled and concentrated to dryness under reduced pressure (at RT) and crystallized to afford the ester **9** in 88% yield (2.41 g). ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.98 (s, 1H, –$COOCH_3$), 7.20 (d, 1H, J = 1.8 Hz, Py–H), 7.72 (d, 1H, J = 1.8 Hz, Py–H), 10.15 (brs, 1H, –NH–). MS: *m*/*z* calculated for $C_6H_6N_2O_4$ 170.00, found 171.03 (M + 1).

4-Nitro-1*H*-pyrrole-2-carboxylic acid (10)

A mixture of 4-nitro-1*H*-pyrrole-2-carboxylic acid methyl ester (**9**) (2.0 g, 28.84 mmol) methanol (100 ml) and 10 ml of 1 N NaOH was placed in a flask, then the reaction mixture was stirred at 60 °C temperature until the ester completely disappeared as shown by TLC. The reaction mixture was cooled in ice with stirring and neutralized with 0.5 N HCl to pH 2 when a solid separated out from the solution. The solid was collected and washed with water and dried under reduced pressure to give acid **10** in 80% yield (1.45 g). ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.25 (d, 1H, J = 1.8 Hz, Py–H), 7.78 (d, 1H, J = 1.8 Hz, Py–H), 10.15 (brs, 1H, –NH–), 12.98 (brs, 1H, –COOH). MS: *m*/*z* calculated for $C_5H_4N_2O_4$ 156.00, found 156.10.

4-Nitro-1*H*-pyrrole-2-carboxylic acid *tert*-butyl ester (11)

4-Nitro-1*H*-pyrrole-2-carboxylic acid (**10**) (4.5 g, 28.84 mmol) was added to 200 ml of diethyl ether and 6 ml of concentrated sulfuric acid in a round bottom pressure bottle. The colloidal solution was cooled to −60 °C and a slow stream of isobutylene was bubbled through this solution for several minutes. The solution was capped tightly with a teflon cork and allowed to warm to room temperature and stirred for 36 h. The crude reaction mixture was washed with saturated $NaHCO_3$ repeatedly. The crude product was further purified by column chromatography using DCM as eluent to give 4-nitro-1*H*-pyrrole-2-carboxylic acid *tert*-butyl ester (**11**) as a white solid in 83% yield (5.1 g). ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.59 (s, 9H, –$C(CH_3)_3$), 7.26 (d, 1H, J = 1.8 Hz, Py–H), 7.76 (d, 1H, J = 1.8 Hz, Py–H), 10.10 (brs, 1H, –NH–). HR-MS: *m*/*z* calculated for $C_9H_{12}N_2O_4$ 212.07, found 212.15.

4-Nitro-1-(3,4,5-triacetoxy-6-acetoxymethyl-tetrahydro-pyran-2-yl)-1*H*-pyrrole-2-carboxylic acid *tert*-butyl ester (13)

4-Nitro-1*H*-pyrrole-2-carboxylic acid *tert*-butyl ester (**11**) (2.5 g, 11.79 mmol) was taken in dry DMF and to it crushed potassium hydroxide (0.86 g, 15.35 mmol) was added and the reaction mixture was stirred at room temperature for 30 min and the yellow colored potassium salt was obtained. The reaction mixture was then treated with the acetyl bromo-sugar compound **12** (7.25 g, 17.68 mmol) followed by the addition of a catalytic amount of 18-crown-6-ether (10 mg). The reaction mixture was stirred for 3 h at room temperature. TLC examination showed the consumption of all the starting material. The reaction mixture was concentrated under reduced pressure and the residue was dissolved in ethyl acetate and washed with water. The organic was removed and dried over sodium sulfate, filtered and concentrated *in vacuo*, to give crude compound **13** which was purified by column chromatography using ethyl acetate/dichloromethane (2 : 98) as a white colored foam in 80% yield (5.15 g). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.70 (s, 9H, $C(CH_3)_3$), 1.85 (s, 3H, $COCH_3$), 2.00 (s, 3H, $COCH_3$), 2.12 (s, 3H, $COCH_3$), 2.16 (s, 3H, $COCH_3$), 4.00 (ddd, 1H, J = 10.1, 4.4, 12.1 Hz), 4.20 (m, 2H), 4.56 (dd, 1H, J = 10.1, 9.5 Hz), 5.19 (t, 1H, J = 9.5 Hz), 5.62 (t, 1H, J = 9.5 Hz), 6.64 (d, 1H, J = 3.85 Hz), 7.30 (d, 1H, J = 1.8 Hz, Py–H), 7.90 (d, 1H, J = 1.8 Hz, Py–H). HR-MS: *m*/*z* calculated for $C_{23}H_{30}N_2O_{13}$ 542.174, found 565.40 (M + Na).

4-Nitro-1-(3,4,5-triacetoxy-6-acetoxymethyl-tetrahydro-pyran-2-yl)-1*H*-pyrrole-2-carboxylic acid (14)

A solution of the compound **13** (4.0 g, 7.38 mmol) was prepared in dry dichloromethane (50 ml) and to it 1.0 M $TiCl_4$ solution in dichloromethane (10 ml) was added dropwise slowly with constant stirring at room temperature. After complete addition the stirring was continued for 24 h. TLC observation at this time indicated completion of the reaction. The reaction mixture was concentrated *in vacuo* and purified by column chromatography. Elution with EtOAC/DCM (3 : 7) gave pure 4-nitro-1-(3,4,5-triacetoxy-6-acetoxymethyl-tetrahydro-pyran-2-yl)-1*H*-pyrrole-2-carboxylic acid (**14**) as a white solid in 78% yield (2.8 g). ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.92 (s, 3H, $COCH_3$), 2.05 (s, 3H, $COCH_3$), 2.08 (s, 3H, $COCH_3$), 2.12 (s, 3H, $COCH_3$), 4.06 (ddd, 1H, J = 10.1, 4.4, 12.1 Hz), 4.15 (m, 2H), 4.36 (dd, 1H, J = 10.1, 9.5 Hz), 5.21 (t, 1H, J = 9.5 Hz), 5.56 (t, 1H, J = 9.5 Hz), 6.62 (d, 1H, J = 3.85 Hz), 7.58 (d, 1H, J = 1.8 Hz, Py–H), 7.98 (d, 1H, J = 1.8 Hz, Py–H), 12.95 (brs, 1H, –COOH). HR-MS: *m*/*z* calculated for $C_{19}H_{22}N_2O_{13}$ 486.12, found 509.12 (M + Na).

Acetic acid 4,5-diacetoxy-6-acetoxymethyl-2-[2-(3-dimethyl-amino-propylcarbamoyl)-4-nitro-pyrrol-1-yl]-tetrahydro-pyran-3-yl ester (15)

3-Dimethylaminopropylamine (0.46 g, 4.50 mmol) was dissolved in dry DMF and added to a mixture of the 4-nitro-1-(3,4,5-triacetoxy-6-acetoxymethyl-tetrahydro-pyran-2-yl)-1*H*-pyrrole-2-carboxylic acid (**14**) (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 RT for 12 h and after completion of the reaction the solvent was removed

under reduced pressure to afford a dark oil which was purified by flash column chromatography on silica gel by using methanol/dichloromethane (5 : 95) as eluent to afford the acetic acid 4,5-diacetoxy-6-acetoxymethyl-2-[2-(3-dimethylamino-propylcarbamoyl)-4-nitro-pyrrol-1-yl] tetrahydro-pyran-3-yl ester (**15**) as a white solid in 77% yield (1.89 g). ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.55 (q, 2H, J = 6.8 Hz, –CH_2–), 1.95 (s, 3H, $COCH_3$), 2.08 (s, 3H, $COCH_3$), 2.10 (s, 3H, $COCH_3$), 2.12 (s, 3H, $COCH_3$), 2.18 (s, 6H, –$N(CH_3)_2$), 2.25 (t, 2H, J = 7.0 Hz, –C**H**$_2$N–), 3.15 (dt, 2H, J = 6.0, 7.0 Hz, –NHC**H**$_2$–), 4.15 (ddd, 1H, J = 10.1, 4.4, 12.1 Hz), 4.18 (m, 2H), 4.32 (dd, 1H, J = 10.1, 9.5 Hz), 5.20 (t, 1H, J = 9.5 Hz), 5.50 (t, 1H, J = 9.5 Hz), 6.61 (d, 1H, J = 3.85 Hz), 7.62 (d, 1H, J = 1.8 Hz, Py–H), 8.08 (d, 1H, J = 1.8 Hz, Py–H), 8.45 (t, 1H, J = 6.5 Hz, –**NH**CH_2–). HR-MS: m/z calculated for $C_{24}H_{34}N_4O_{12}$ 570.21, found 593.20 (M + Na).

General procedure A

A solution of the nitropolyamides **15** and **4**–**7** in MeOH or DMF was hydrogenated over 10% Pd/C at 50 psi pressure for 2 h and the catalyst was removed by filtration through a Celite pad. The filtrate was concentrated to dryness under reduced pressure (at RT) 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 **14** (1.0 equivalent), hydroxybenzotriazole (1.0 equivalent), and EDCI (2.5 equivalent), in dry DMF was added. This mixture was stirred at RT for 12 h and after completion of the reaction the solvent was removed under reduced pressure to afford a dark oil which was purified by flash column chromatography on silica gel by using methanol–dichloromethane as eluent to afford the polyamides **16**, and **19**–**22** respectively in good yields.

Acetic acid 4,5-diacetoxy-6-acetoxymethyl-2-{2-[5-(3-dimethylamino-propylcarbamoyl)-1-(3,4,5-triacetoxy-6-acetoxymethyl-tetrahydro-pyran-2-yl)-1*H*-pyrrol-3-ylcarbamoyl]-4-nitro-pyrrol-1-yl}-tetrahydro-pyran-3-yl ester (16)

This compound was prepared starting from acetic acid 4,5-diacetoxy-6-acetoxymethyl-2-[2-(3-dimethylamino-propylcarbamoyl)-4-nitro-pyrrol-1-yl] tetrahydro-pyran-3-yl ester (**15**) (1.5 g, 2.63 mmol) and the acid **14** (1.27 g, 2.61 mmol) according to the general procedure A (2.1 g, 80% yield) as a light yellow solid. ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.55 (q, 2H, J = 6.8 Hz, –CH_2–), 1.79 (s, 3H, $COCH_3$), 1.86 (s, 3H, $COCH_3$), 1.96 (s, 3H, $COCH_3$), 1.99 (s, 3H, $COCH_3$), 2.03 (s, 3H, $COCH_3$), 2.05 (s, 3H, $COCH_3$), 2.07 (s, 3H, $COCH_3$), 2.08 (s, 3H, $COCH_3$), 2.26 (s, 6H, –$N(CH_3)_2$), 2.30 (t, 2H, J = 7.0 Hz, –C**H**$_2$N–), 3.18 (dt, 2H, J = 6.0, 7.0 Hz, –NHC**H**$_2$–), 3.90–4.32 (m, 8H, sugar proton), 5.20–5.50 (m, 4H, sugar proton), 6.71 (d, 2H, J = 3.85 Hz), 6.96 (d, 1H, J = 1.8 Hz, Py–H), 7.20 (d, 1H, J = 1.8 Hz, Py–H), 7.56 (d, 1H, J = 1.8 Hz, Py–H), 7.95 (d, 1H, J = 1.8 Hz, Py–H), 8.15 (t, 1H, J = 6.5 Hz, –**NH**CH_2–), 9.62 (s, 1H, –NH–). HR-MS: m/z calculated for $C_{43}H_{56}N_6O_{22}$ 1008.34, found 1031.40 (M + Na).

Acetic acid 4,5-diacetoxy-6-acetoxymethyl-2-[2-(3-dimethylamino-propylcarbamoyl)-4-({1-(3,4,5-triacetoxy-6-acetoxymethyl-tetrahydro-pyran-2-yl)-4-[(1-methyl-4-nitro-1*H*-pyrrole-2-carbonyl)-amino]-1*H*-pyrrole-2-carbonyl}-amino)-pyrrol-1-yl]-tetrahydro-pyran-3-yl ester (17)

To a solution of compound **16** (1.2 g, 1.19 mmol) in 25.0 ml of methanol was added 0.200 g of 10% Pd–C. 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 (10.0 ml), triethylamine (2.0 ml) and a solution of 4-nitro-2-(trichloroacetyl)-1-methylpyrrole (**2**) (0.354 g, 1.30 mmol) in THF (10 ml), was added slowly with stirring at 0 °C under 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_4OH/MeOH/DCM 0.2 : 1 : 9; to give compound **17**, 1.10 g in 82% yield. ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.55 (q, 2H, J = 6.8 Hz, –CH_2–), 1.78 (s, 3H, $COCH_3$), 1.85 (s, 3H, $COCH_3$), 1.95 (s, 3H, $COCH_3$), 1.99 (s, 3H, $COCH_3$), 2.04 (s, 3H, $COCH_3$), 2.06 (s, 3H, $COCH_3$), 2.08 (s, 3H, $COCH_3$), 2.09 (s, 3H, $COCH_3$), 2.26 (s, 6H, –$N(CH_3)_2$), 2.30 (t, 2H, J = 7.0 Hz, –CH_2N–), 3.18 (dt, 2H, J = 6.0, 7.0 Hz, –NHCH_2–), 3.85 (s, 3H, –NCH_3), 3.90–4.33 (m, 8H, sugar proton), 5.22–5.56 (m, 4H, sugar proton), 6.71 (d, 2H, J = 3.85 Hz), 6.81 (d, 1H, J = 1.8 Hz, Py–H), 6.96 (d, 1H, J = 1.8 Hz, Py–H), 7.18 (d, 1H, J = 1.8 Hz, Py–H), 7.25 (d, 1H, J = 1.8 Hz, Py–H), 7.45 (d, 1H, J = 1.8 Hz, Py–H), 7.86 (d, 1H, J = 1.8 Hz, Py–H), 8.18 (t, 1H, J = 6.5 Hz, –NHCH_2–), 9.61 (s, 1H, –NH–), 9.95 (s, 1H, –NH–). HR-MS: m/z calculated for $C_{49}H_{62}N_8O_{23}$ 1130.39, found 1153.40 (M + Na).

Acetic acid 4,5-diacetoxy-6-acetoxymethyl-2-[2-(3-dimethylamino-propylcarbamoyl)-4-({1-(3,4,5-triacetoxy-6-acetoxymethyl-tetrahydro-pyran-2-yl)-4-[(1-methyl-4-nitro-1*H*-imidazole-2-carbonyl)-amino]-1*H*-pyrrole-2-carbonyl}-amino)-pyrrol-1-yl]-tetrahydro-pyran-3-yl ester (18)

This compound was prepared according to the method described for the compound **17**, employing compound **16** (1.2 g, 1.19 mmol) and 4-nitro-2-(trichloroacetyl)-1-methylimidazole (**3**) (0.356 g, 1.30 mmol) in 78% yield (1.05 g). ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.56 (q, 2H, J = 6.8 Hz, –CH_2–), 1.76 (s, 3H, $COCH_3$), 1.86 (s, 3H, $COCH_3$), 1.97 (s, 3H, $COCH_3$), 1.99 (s, 3H, $COCH_3$), 2.02 (s, 3H, $COCH_3$), 2.04 (s, 3H, $COCH_3$), 2.06 (s, 3H, $COCH_3$), 2.09 (s, 3H, $COCH_3$), 2.28 (s, 6H, –$N(CH_3)_2$), 2.29 (t, 2H, J = 7.0 Hz, –C**H**$_2$N–), 3.18 (dt, 2H, J = 6.0, 7.0 Hz, –NHC**H**$_2$–), 3.86 (s, 3H, –NCH_3), 3.90–4.33 (m, 8H, sugar proton), 5.22–5.56 (m, 4H, sugar proton), 6.70 (d, 2H, J = 3.85 Hz), 6.96 (d, 1H, J = 1.8 Hz, Py–H), 7.20 (d, 1H, J = 1.8 Hz, Py–H), 7.40 (d, 1H, J = 1.8 Hz, Py–H), 7.68 (s, 1H, Im–H), 7.79 (d, 1H, J = 1.8 Hz, Py–H), 8.15 (t, 1H, J = 6.5 Hz, –NHCH_2–), 9.51 (s, 1H, –NH–), 9.93 (s, 1H, –NH–). HR-MS: m/z calculated for $C_{48}H_{61}N_9O_{23}$ 1131.39, found 1154.45 (M + Na).

Acetic acid 4,5-diacetoxy-6-acetoxymethyl-2-{2-[5-(3-dimethylamino-propylcarbamoyl)-1-methyl-1*H*-pyrrol-3-ylcarbamoyl]-4-nitro-pyrrol-1-yl}-tetrahydro-pyran-3-yl ester (19)

This compound was prepared starting from compound **4** (0.574 g, 2.25 mmol) and the acid **14** (1.0 g, 2.05 mmol) according to the general procedure A (1.15 g, 81% yield) as a light yellow solid. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.56 (q, 2H, J = 6.8 Hz, –CH_2–), 1.85 (s, 3H, $COCH_3$), 2.00 (s, 3H, $COCH_3$), 2.04 (s, 3H, $COCH_3$), 2.09 (s, 3H, $COCH_3$), 2.20 (s, 6H, –$N(CH_3)_2$), 2.27 (t, 2H, J = 7.0 Hz, –C**H**$_2$N–), 3.18 (dt, 2H, J = 6.0, 7.0 Hz, –NHC**H**$_2$–), 3.85 (s, 3H, –NCH_3), 4.15 (ddd, 1H, J = 10.1, 4.4, 12.1 Hz), 4.18 (m, 2H), 4.34 (dd, 1H, J = 10.1, 9.5 Hz), 5.22 (t, 1H, J = 9.5 Hz), 5.56 (t, 1H, J = 9.5 Hz), 6.63 (d, 1H, J = 3.85 Hz), 6.89 (d, 1H, J = 1.8 Hz, Py–H), 7.25 (d, 1H, J = 1.8 Hz, Py–H), 7.42 (d, 1H, J = 1.8 Hz, Py–H), 7.82 (d, 1H, J = 1.8 Hz, Py–H), 8.25 (t, 1H, J = 6.5 Hz, –**NH**CH_2–), 9.59 (s, 1H, –NH–). HR-MS: m/z calculated for $C_{30}H_{40}N_6O_{13}$ 692.27, found 693.30 (M + 1).

Acetic acid 4,5-diacetoxy-6-acetoxymethyl-2-(2-{5-[5-(3-dimethylamino-propylcarbamoyl)-1-methyl-1*H*-pyrrol-3-ylcarbamoyl]-1-methyl-1*H*-pyrrol-3-ylcarbamoyl}-4-nitro-pyrrol-1-yl)-tetrahydro-pyran-3-yl ester (20)

The title compound was prepared according to the general procedure A by using compound **5** (0.851 g, 2.26 mmol) and the

acid **14** (1.0 g, 2.05 mmol) in 80% yield (1.35 g) as a yellow solid. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.55 (q, 2H, J = 6.8 Hz, –CH_2–), 1.87 (s, 3H, $COCH_3$), 2.01 (s, 3H, $COCH_3$), 2.03 (s, 3H, $COCH_3$), 2.06 (s, 3H, $COCH_3$), 2.19 (s, 6H, –$N(CH_3)_2$), 2.28 (t, 2H, J = 7.0 Hz, –C**H**$_2$N–), 3.15 (dt, 2H, J = 6.0, 7.0 Hz, –NHC**H**$_2$–), 3.85 (s, 3H, –NCH_3), 3.88 (s, 3H, –NCH_3), 4.12 (ddd, 1H, J = 10.1, 4.4, 12.1 Hz), 4.17 (m, 2H), 4.32 (dd, 1H, J = 10.1, 9.5 Hz), 5.21 (t, 1H, J = 9.5 Hz), 5.53 (t, 1H, J = 9.5 Hz), 6.61 (d, 1H, J = 3.85 Hz), 6.83 (d, 1H, J = 1.8 Hz, Py–H), 6.96 (d, 1H, J = 1.8 Hz, Py–H), 7.20 (d, 1H, J = 1.8 Hz, Py–H), 7.35 (d, 1H, J = 1.8 Hz, Py–H), 7.45 (d, 1H, J = 1.8 Hz, Py–H), 7.80 (d, 1H, J = 1.8 Hz, Py–H), 8.20 (t, 1H, J = 6.5 Hz, –**NH**CH_2–), 9.59 (s, 1H, –NH–), 9.97 (s, 1H, –NH–). HR-MS: *m*/*z* calculated for $C_{36}H_{46}N_8O_{14}$ 814.31, found 837.40 (M + Na).

Acetic acid 4,5-diacetoxy-6-acetoxymethyl-2-{2-[2-(3-dimethyl-amino-propylcarbamoyl)-1-methyl-1*H*-imidazol-4-ylcarbamoyl]-4-nitro-pyrrol-1-yl}-tetrahydro-pyran-3-yl ester (21)

This compound was prepared according to the method described for compound **20**, employing compound **6** (0.577 g, 2.26 mmol) and the acid **14** (1.0 g, 2.05 mmol), in 77% yield (1.10 g) as a light yellow solid. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.55 (q, 2H, J = 6.8 Hz, –CH_2–), 1.90 (s, 3H, $COCH_3$), 2.00 (s, 3H, $COCH_3$), 2.05 (s, 3H, $COCH_3$), 2.10 (s, 3H, $COCH_3$), 2.20 (s, 6H, –$N(CH_3)_2$), 2.29 (t, 2H, J = 7.0 Hz, –C**H**$_2$N–), 3.15 (dt, 2H, J = 6.0, 7.0 Hz, –NHC**H**$_2$–), 3.87 (s, 3H, –NCH_3), 4.14 (ddd, 1H, J = 10.1, 4.4, 12.1 Hz), 4.19 (m, 2H), 4.32 (dd, 1H, J = 10.1, 9.5 Hz), 5.21 (t, 1H, J = 9.5 Hz), 5.55 (t, 1H, J = 9.5 Hz), 6.60 (d, 1H, J = 3.85 Hz), 7.15 (d, 1H, J = 1.8 Hz, Py–H), 7.35 (s, 1H, Im–H), 7.75 (d, 1H, J = 1.8 Hz, Py–H), 8.18 (t, 1H, J = 6.5 Hz, –**NH**CH_2–), 9.65 (s, 1H, –NH–). HR-MS: *m*/*z* calculated for $C_{29}H_{39}N_7O_{13}$ 693.26, found 716.20 (M + Na).

Acetic acid 4,5-diacetoxy-6-acetoxymethyl-2-(2-{2-[2-(3-di-methylamino-propylcarbamoyl)-1-methyl-1*H*-imidazol-4-ylcar-bamoyl]-1-methyl-1*H*-imidazol-4-ylcarbamoyl}-4-nitro-pyrrol-1-yl)-tetrahydro-pyran-3-yl ester (22)

The title compound was prepared according to the general procedure A, using compound **7** (0.855 g, 2.26 mmol), and the acid **14** (1.0 g, 2.05 mmol) in 75% yield (1.25 g) as a yellow solid. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.56 (q, 2H, J = 6.8 Hz, –CH_2–), 1.89 (s, 3H, $COCH_3$), 2.03 (s, 3H, $COCH_3$), 2.07 (s, 3H, $COCH_3$), 2.10 (s, 3H, $COCH_3$), 2.15 (s, 6H, –$N(CH_3)_2$), 2.26 (t, 2H, J = 7.0 Hz, –C**H**$_2$N–), 3.18 (dt, 2H, J = 6.0, 7.0 Hz, –NHC**H**$_2$–), 3.86 (s, 3H, –NCH_3), 3.89 (s, 3H, –NCH_3), 4.15 (ddd, 1H, J = 10.1, 4.4, 12.1 Hz), 4.19 (m, 2H), 4.32 (dd, 1H, J = 10.1, 9.5 Hz), 5.21 (t, 1H, J = 9.5 Hz), 5.55 (t, 1H, J = 9.5 Hz), 6.62 (d, 1H, J = 3.85 Hz), 6.89 (d, 1H, J = 1.8 Hz, Py–H), 7.25 (s, 1H, Im–H), 7.45 (d, 1H, J = 1.8 Hz, Py–H), 7.95 (s, 1H, Im–H), 8.18 (t, 1H, J = 6.5 Hz, –**NH**CH_2–), 9.56 (s, 1H, –NH–), 9.95 (s, 1H, –NH–). HR-MS: *m*/*z* calculated for $C_{34}H_{44}N_{10}O_{14}$ 816.30, found 839.37 (M + Na).

General procedure B

Solution of the respective nitropolyamides **15**–**22** in MeOH or DMF were hydrogenated over 10% Pd/C at 50 psi pressure for 2 h and the catalyst was removed by filtration through a Celite pad. The filtrate was concentrated to dryness under reduced pressure (at RT) 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 (2*S*)-*N*-[5-methoxy-4-[3-(carboxy) propyloxy]-2-nitrobenzoyl] pyrrolidine-2-carboxaldehyde diethyl thioacetal (**1**) (1.0 equivalent), hydroxybenzotriazole (1.0 equivalent), and EDCI (2.5 equivalent), in dry DMF was added. This mixture was stirred at RT for 12 h and after completion of the reaction the solvent was removed under reduced pressure to afford a dark oil which was purified by flash column chromatography on silica gel by using methanol–dichloromethane–aq ammonia as eluent to afford the PBD-nitro dithioacetal glycosylated polyamides **23**–**30** in good yield.

Compound 23

This compound was prepared according to the general procedure **B**, using compound **15** (1.28 g, 2.24 mmol) and the (2*S*)-*N*-[5-methoxy-4-[3-(carboxy) propyloxy]-2-nitrobenzoyl] pyrrolidine-2-carboxaldehyde diethyl thioacetal (**1**) (1.0 g, 2.05 mmol), as a yellow solid in 73% yield (1.5 g). IR: (Nujol) ν_{max} 3289, 2855, 2420, 2061, 1721, 1703, 1640, 1156, 889, 810, 756 cm^{-1}. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 1.24 (s, 6H, –$(CH_3)_2$), 1.56–1.61(m, 4H), 1.69 (m, 2H), 1.95 (m, 2H), 2.01 (s, 3H, –$COCH_3$), 2.03 (s, 3H, –$COCH_3$), 2.05 (s, 3H, –$COCH_3$), 2.09 (s, 3H, –$COCH_3$), 2.18 (m, 2H), 2.28 (s, 6H, –$N(CH3)_2$), 2.36 (m, 2H), 2.48 (m, 4H), 2.95 (m, 2H), 3.30 (m, 2H), 3.75 (s, 3H, –OCH_3), 3.89 (m, 1H), 3.95 (m, 2H), 4.12 (m, 1H), 4.16–4.35 (m, 4H, sugar proton), 5.21–5.60 (m, 2H, sugar proton), 6.56 (m, 1H, sugar proton), 6.91 (d, 1H, J = 1.7 Hz, Py–H), 7.12 (d, 1H, J = 1.7 Hz, Py–H), 7.61 (s, 1H, Ar–H), 7.75 (s, 1H, Ar–H), 8.20 (t, J = 6.5 Hz, 1H), 9.98 (s, 1H, –NH–). HR-MS: *m*/*z* calculated for $C_{45}H_{64}N_6O_{16}S_2$, 1008.35 found 1009.10 (M + 1).

Compound 24

This compound was prepared according to the method described for the compound **23**, employing glycosylated polyamide **16** (2.06 g, 2.04 mmol) and the acid **1** (1.0 g, 2.05 mmol) in 74% yield (2.21 g) as a yellow solid. IR: (Nujol) ν_{max} 3285, 2850, 2415, 2161, 1720, 1640, 1155, 890, 810, 756 cm^{-1}. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 1.25 (s, 6H, –$(CH_3)_2$), 1.56–1.60 (m, 4H), 1.65 (m, 2H), 1.98 (m, 2H), 2.00–2.12 (8 singlets, 24H, $COCH_3$), 2.16 (m, 2H), 2.30 (s, 6H, –$N(CH3)_2$), 2.35 (m, 2H), 2.50 (m, 4H), 2.97 (m, 2H), 3.30 (m, 2H), 3.78 (s, 3H, –OCH_3), 3.89 (m, 1H), 3.97 (m, 2H), 4.15 (m, 1H), 4.16–4.41 (m, 8H, sugar proton), 5.20–5.65 (m, 4H, sugar proton), 6.61 (m, 2H, sugar proton), 6.83 (d, 1H, J = 1.7 Hz, Py–H), 6.96 (d, 1H, J = 1.7 Hz, Py–H), 7.12 (d, 1H, J = 1.7 Hz, Py–H), 7.35 (d, 1H, J = 1.7 Hz, Py–H), 7.60 (s, 1H, Ar–H), 7.76 (s, 1H, Ar–H), 8.25 (t, J = 6.5 Hz, 1H), 9.98 (s, 1H, –NH–), 10.05 (s, 1H, –NH–). MS: *m*/*z* calculated $C_{64}H_{86}N_8O_{26}S_2$ 1446.51, found 1447.50 (M + 1).

Compound 25

This compound was prepared starting from the polyamide **17** (1.27 g, 1.12 mmol) and the acid **1** (0.50 g, 1.02 mmol), according to the general procedure described for compound **24**, as a yellow solid (1.21 g, 75% yield). IR: (Nujol) ν_{max} 3288, 2960, 2690, 1720, 1640, 1578, 1523, 1405, 1220, 1156, 1098, 886, 810, 756 cm^{-1}. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 1.24 (s, 6H, –$(CH_3)_2$), 1.56–1.61 (m, 4H), 1.65 (m, 2H), 1.95 (m, 2H), 2.01–2.12 (8 singlets, 24H, $COCH_3$), 2.16 (m, 2H), 2.32 (s, 6H, –$N(CH3)_2$), 2.35 (m, 2H), 2.48 (m, 4H), 2.95 (m, 2H), 3.31 (m, 2H), 3.76 (s, 3H, –OCH_3), 3.85 (s, 3H, –NCH_3), 3.89 (m, 1H), 3.95 (m, 2H), 4.10 (m, 1H), 4.16–4.39 (m, 8H, sugar proton), 5.21–5.59 (m, 4H, sugar proton), 6.60 (m, 2H, sugar proton), 6.84 (d, 1H, J = 1.7 Hz, Py–H), 6.96 (d, 1H, J = 1.7 Hz, Py–H), 7.12 (d, 1H, J = 1.7 Hz, Py–H), 7.20 (d, 1H, J = 1.7 Hz, Py–H), 7.45 (d, 1H, J = 1.7 Hz, Py–H), 7.56 (d, 1H, J = 1.7 Hz, Py–H), 7.60 (s, 1H, Ar–H), 7.76 (s, 1H, Ar–H), 8.28 (t, J = 6.5 Hz, 1H), 9.95 (s, 1H, –NH–), 10.05 (s, 1H, –NH–), 10.08 (s, 1H, –NH–). MS: *m*/*z* calculated for $C_{70}H_{92}N_{10}O_{27}S_2$ 1568.52, found 1569.60 (M + 1).

Compound 26

This compound was prepared according to the method described for the compound **23**, employing the polyamide **18**

(1.27 g, 1.12 mmol) and the thio acid **1** (0.50 g, 1.02 mmol), in 77% yield (1.25 g) as a yellow solid. IR: (Nujol) ν_{max} 3288, 2960, 1730, 1640, 1578, 1523, 1405, 1156, 886, 810, 756 cm^{-1}. ^1H NMR (DMSO-d_6, 300 MHz): δ 1.25 (s, 6H, $-(CH_3)_2$), 1.56–1.61 (m, 4H), 1.65 (m, 2H), 1.95 (m, 2H), 2.01–2.12 (8 singlets, 24H, $COCH_3$), 2.16 (m, 2H), 2.30 (s, 6H, $-N(CH3)_2$), 2.34 (m, 2H), 2.48 (m, 4H), 2.95 (m, 2H), 3.31 (m, 2H), 3.75 (s, 3H, $-OCH_3$), 3.87 (s, 3H, $-NCH_3$), 3.90 (m, 1H), 3.95 (m, 2H), 4.12 (m, 1H), 4.16–4.39 (m, 8H, sugar proton), 5.20–5.59 (m, 4H, sugar proton), 6.62 (m, 2H, sugar proton), 6.96 (d, 1H, J = 1.7 Hz, Py–H), 7.12 (d, 1H, J = 1.7 Hz, Py–H), 7.20 (d, 1H, J = 1.7 Hz, Py–H), 7.56 (d, 1H, J = 1.7 Hz, Py–H), 7.60 (s, 1H, Ar–H), 7.76 (s, 1H, Ar–H), 7.82 (s, 1H, Im–H), 8.25 (t, J = 6.5 Hz, 1H), 9.97 (s, 1H, –NH–), 10.06 (s, 1H, –NH–), 10.08 (s, 1H, –NH–). MS: m/z calculated for $C_{69}H_{91}N_{11}O_{27}S_2$ 1569.52, found 1570.68 (M + 1).

Compound 27

This compound was prepared according to the general procedure C, using compound **19** (1.17 g, 1.69 mmol) and the acid **1** (0.75 g, 1.54 mmol) in 77% yield (1.35 g), as a yellow solid. IR: (Nujol) ν_{max} 3288, 2960, 1640, 1578, 1410, 1205, 1156, 1098, 890, 810, 756 cm^{-1}. ^1H NMR (DMSO-d_6, 300 MHz): δ 1.23 (s, 6H, $-(CH_3)_2$), 1.56–1.61 (m, 4H), 1.65 (m, 2H), 1.95 (m, 2H), 2.00 (s, 3H, $-COCH_3$), 2.04 (s, 3H, $-COCH_3$), 2.05 (s, 3H, $-COCH_3$), 2.10 (s, 3H, $-COCH_3$), 2.17 (m, 2H), 2.25 (s, 6H, $-N(CH3)_2$), 2.37 (m, 2H), 2.50 (m, 4H), 2.97 (m, 2H), 3.30 (m, 2H), 3.76 (s, 3H, $-OCH_3$), 3.85 (s, 3H, $-NCH_3$), 3.89 (m, 1H), 3.95 (m, 2H), 4.12 (m, 1H), 4.15–4.38 (m, 4H, sugar proton), 5.21–5.55 (m, 2H, sugar proton), 6.60 (m, 1H, sugar proton), 6.86 (d, 1H, J = 1.7 Hz, Py–H), 6.91 (d, 1H, J = 1.7 Hz, Py–H), 7.12 (d, 1H, J = 1.7 Hz, Py–H), 7.26 (d, 1H, J = 1.7 Hz, Py–H), 7.61 (s, 1H, Ar–H), 7.75 (s, 1H, Ar–H), 8.22 (t, J = 6.5 Hz, 1H), 9.95 (s, 1H, –NH–), 10.05 (s, 1H, –NH–). MS: m/z calculated for $C_{51}H_{70}N_8O_{17}S_2$ 1130.40, found 1131.30 (M + 1).

Compound 28

This compound was prepared according to the general procedure B, using compound **20** (1.37 g, 1.68 mmol) and the acid **1** (0.75 g, 1.54 mmol), in 77% yield (1.49 g) as a yellow solid. IR: (Nujol) ν_{max} 3288, 2960, 2650, 1710, 1640, 1578, 1523, 1405, 1210, 1156, 1090, 886, 810, 756 cm^{-1}. ^1H NMR (DMSO-d_6, 300 MHz): δ 1.24 (s, 6H, $-(CH_3)_2$), 1.56–1.61(m, 4H), 1.66 (m, 2H), 1.95 (m, 2H), 2.01 (s, 3H, $-COCH_3$), 2.03 (s, 3H, $-COCH_3$), 2.06 (s, 3H, $-COCH_3$), 2.08 (s, 3H, $-COCH_3$), 2.15 (m, 2H), 2.27 (s, 6H, $-N(CH_3)_2$), 2.35 (m, 2H), 2.48 (m, 4H), 2.95 (m, 2H), 3.30 (m, 2H), 3.75 (s, 3H, $-OCH_3$), 3.84 (s, 3H, $-NCH_3$), 3.86 (s, 3H, $-NCH_3$), 3.90 (m, 1H), 3.95 (m, 2H), 4.12 (m, 1H), 4.15–4.38 (m, 4H, sugar proton), 5.20–5.76 (m, 2H, sugar proton), 6.61 (m, 1H, sugar proton), 6.84 (d, 1H, J = 1.7 Hz, Py–H), 6.96 (d, 1H, J = 1.7 Hz, Py–H), 7.15 (d, 1H, J = 1.7 Hz, Py–H), 7.20 (d, 1H, J = 1.7 Hz, Py–H), 7.35 (d, 1H, J = 1.7 Hz, Py–H), 7.65 (d, 1H, J = 1.7 Hz, Py–H), 7.71 (s, 1H, Ar–H), 7.77 (s, 1H, Ar–H), 8.20 (t, J = 6.5 Hz, 1H), 9.95 (s, 1H, –NH–), 9.98 (s, 1H, –NH–), 10.05 (s, 1H, –NH–). MS: m/z calculated for $C_{57}H_{76}N_{10}O_{18}S_2$ 1252.48, found 1253.35 (M + 1).

Compound 29

This compound was prepared starting from compound **21** (1.17 g, 1.68 mmol) and the acid **1** (0.75 g, 1.54 mmol) according to the general procedure B (1.22 g, 70% yield) as a yellow solid. IR: (Nujol) ν_{max} 3289, 2980, 2640, 1640, 1578, 1415, 1156, 1098, 810, 756 cm^{-1}. ^1H NMR (DMSO-d_6, 300 MHz): δ 1.24 (s, 6H, $-(CH_3)_2$), 1.56–1.61 (m, 4H), 1.65 (m, 2H), 1.95 (m, 2H), 2.02 (s, 3H, $-COCH_3$), 2.05 (s, 3H, $-COCH_3$), 2.08 (s, 3H, $-COCH_3$), 2.10 (s, 3H, $-COCH_3$), 2.16 (m, 2H), 2.27 (s, 6H, $-N(CH3)_2$), 2.36 (m, 2H), 2.48 (m, 4H), 2.95 (m, 2H), 3.30 (m, 2H), 3.75 (s, 3H, $-OCH_3$), 3.86 (s, 3H, $-NCH_3$), 3.89 (m, 1H), 3.94 (m, 2H), 4.12 (m, 1H), 4.16–4.35 (m, 4H, sugar proton), 5.21–5.60 (m, 2H, sugar proton), 6.61 (m, 1H, sugar proton), 6.94 (d, 1H, J = 1.7 Hz, Py–H), 7.20 (d, 1H, J = 1.7 Hz, Py–H), 7.61 (s, 1H, Ar–H), 7.75 (s, 1H, Ar–H), 7.80 (s, 1H, Im–H), 8.20 (t, J = 6.5 Hz, 1H), 9.98 (s, 1H, –NH–), 10.06 (s, 1H, –NH–). MS: m/z calculated for $C_{50}H_{69}N_9O_{17}S_2$ 1131.43 found 1132.25 (M + 1).

Compound 30

Prepared according to the general procedure B using compound **22** (1.38 g, 1.69 mmol) and the acid **1** (0.75 g, 1.54 mmol) in 72% yield (1.40 g) as a yellow solid. IR: (Nujol) ν_{max} 3290, 2980, 2650, 1720, 1640, 1578, 1415, 1156, 1110, 886, 810, 756 cm^{-1}. ^1H NMR (DMSO-d_6, 300 MHz): δ 1.24 (s, 6H, $-(CH_3)_2$), 1.56–1.61 (m, 4H), 1.66 (m, 2H), 1.95 (m, 2H), 2.01 (s, 3H, $-COCH_3$), 2.03 (s, 3H, $-COCH_3$), 2.06 (s, 3H, $-COCH_3$), 2.08 (s, 3H, $-COCH_3$), 2.15 (m, 2H), 2.27 (s, 6H, $-N(CH3)_2$), 2.35 (m, 2H), 2.48 (m, 4H), 2.95 (m, 2H), 3.30 (m, 2H), 3.75 (s, 3H, $-OCH_3$), 3.86 (s, 3H, $-NCH_3$), 3.88 (s, 3H, $-NCH_3$), 3.90 (m, 1H), 3.95 (m, 2H), 4.12 (m, 1H), 4.15–4.38 (m, 4H, sugar proton), 5.20–5.55 (m, 2H, sugar proton), 6.60 (m, 1H, sugar proton), 6.84 (d, 1H, J = 1.7 Hz, Py–H), 6.96 (d, 1H, J = 1.7 Hz, Py–H), 7.20 (d, 1H, J = 1.7 Hz, Py–H), 7.65 (s, 1H, Im–H), 7.70 (s, 1H, Ar–H), 7.75 (s, 1H, Ar–H), 7.84 (s, 1H, Im–H), 8.21 (t, J = 6.5 Hz, 1H), 9.95 (s, 1H, –NH–), 9.99 (s, 1H, –NH–), 10.04 (s, 1H, –NH–). MS: m/z calculated for $C_{55}H_{74}N_{12}O_{18}S_2$ 1254.47, found 1255.35 (M + 1).

General procedure C

Nitrodithioacetal glycosylated polyamides **23**–**30** were dissolved in methanol (50.0 ml) respectively and hydrogenated over 10% Pd–C at 50 psi pressure for 2 h and then the catalyst was removed by filtration through a Celite pad. The filtrate was concentrated to dryness under reduced pressure. The resultant amino compounds were dissolved in CH_3CN/H_2O (4 : 1) and $HgCl_2$ (1.5 equivalent) and HgO (1.5 equivalent) were added and the mixture was stirred slowly at RT for 12 h. When TLC ($CHCl_3$/MeOH/ammonia) indicated the complete disappearance of starting materials, the reaction mixtures were charged directly on to a short silica column and first eluted with ethyl acetate to remove $HgCl_2$. After complete removal of $HgCl_2$, the column was eluted with $CHCl_3$/MeOH system by which all other impurities were removed. Then the column was further eluted with $CHCl_3$/MeOH/ammonia and the ammonia concentration were slowly increased from 1% through 2%. The imine compounds were collected at different percentage of ammonia from 1–3%. The imine and corresponding methyl ether move together as they have close Rf values. Evaporation of the solvents under high vacuum, at RT, afforded an inseparable mixture of imines and methyl ethers **31**–**38** in almost 1 : 1 ratio in 40–45% yield.

Compound 31

This was prepared according to the general procedure C by using compound **23** (1.1 g, 1.09 mmol) in 43% yield (0.40 g) as a yellow solid after purification. ^1H NMR (DMSO-d_6, 300 MHz): δ 1.59–1.62 (m, 4H), 1.65 (m, 2H), 1.98 (m, 2H), 2.01–2.10 (4 singlets, 12H, 4× $-COCH_3$), 2.16 (m, 2H), 2.27 (s, 6H, $-N(CH_3)_2$), 2.36 (m, 2H), 2.96 (m, 2H), 3.30 (m, 1H), 3.35 (m, 2H), 3.73 (s, 3H, $-ArOCH_3$), 3.89 (m, 3H, $-OCH_3$ for its methyl ether), 3.95 (m, 2H), 4.16–4.35 (m, 4H, sugar proton), 5.20–5.62 (m, 2H, sugar proton), 6.50 (m, 1H, sugar proton), 6.80 (s, 1H, Ar–H), 6.92 (d, 1H, J = 1.7 Hz, Py–H), 7.10 (d, 1H, J = 1.7 Hz, Py–H), 7.35 (s, 1H, Ar–H), 7.50 (d, 1H, J = 4.2 Hz, imine proton), 8.21 (t, J = 6.5 Hz, 1H), 9.95 (s, 1H, –NH–). MS: m/z calculated for $C_{41}H_{54}N_6O_{14}$ 854.37, found 855.30 (M + 1) for its imine compound and 887.10 (M + 1) for its methyl ether compound.

Compound 32

This compound was prepared starting from compound **24** (1.5 g, 1.03 mmol) according to the general procedure C (0.55 g, 41% yield) as a yellow solid. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 1.56–1.62 (m, 4H), 1.65 (m, 2H), 1.95 (m, 2H), 1.98–2.12 (8 singlets, 24H, $COCH_3$), 2.15 (m, 2H), 2.28 (s, 6H, $-N(CH_3)_2$), 2.35 (m, 2H), 2.95 (m, 2H), 3.30 (m, 1H), 3.34 (m, 2H), 3.75 (s, 3H, $-ArOCH_3$), 3.89 (m, 3H, $-OCH_3$ for its methyl ether), 3.95 (m, 2H), 4.15–4.45 (m, 8H, sugar proton), 5.21–5.65 (m, 4H, sugar proton), 6.52 (m, 2H, sugar proton), 6.80 (s, 1H, Ar–H), 6.86 (d, 1H, J = 1.7 Hz, Py–H), 6.96 (d, 1H, J = 1.7 Hz, Py–H), 7.18 (d, 1H, J = 1.7 Hz, Py–H), 7.35 (s, 1H, Ar–H), 7.45 (d, 1H, J = 1.7 Hz, Py–H), 7.50 (d, 1H, J = 4.2 Hz, imine proton), 8.22 (t, J = 6.5 Hz, 1H), 9.95 (s, 1H, –NH–), 10.05 (s, 1H, –NH–). MS: *m*/*z* calculated for $C_{60}H_{76}N_8O_{24}$ 1292.48, found 1293.25 (M + 1) for its imine compound and 1325.33 (M + 1) for its methyl ether compound.

Compound 33

This compound was prepared according to the general procedure C by using compound **25** (1.5 g, 0.956 mmol) in 42% yield (0.57 g) as a yellow solid. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 1.55–1.60 (m, 4H), 1.65 (m, 2H), 1.95 (m, 2H), 1.98–2.12 (8 singlets, 24H, $COCH_3$), 2.15 (m, 2H), 2.28 (s, 6H, $-N(CH_3)_2$), 2.35 (m, 2H), 2.96 (m, 2H), 3.30 (m, 1H), 3.34 (m, 2H), 3.75 (s, 3H, $-ArOCH_3$), 3.84 (s, 3H, $-NCH_3$), 3.89 (m, 3H, $-OCH_3$ for its methyl ether), 3.95 (m, 2H), 4.15–4.45 (m, 8H, sugar proton), 5.21–5.65 (m, 4H, sugar proton), 6.52 (m, 2H, sugar proton), 6.80 (s, 1H, Ar–H), 6.84 (d, 1H, J = 1.7 Hz, Py–H), 6.96 (d, 1H, J = 1.7 Hz, Py–H), 7.15 (d, 1H, J = 1.7 Hz, Py–H), 7.20 (d, 1H, J = 1.7 Hz, Py–H), 7.35 (s, 1H, Ar–H), 7.43 (d, 1H, J = 1.7 Hz, Py–H), 7.52 (d, 1H, J = 4.2 Hz, imine proton), 7.72 (d, 1H, J = 1.7 Hz, Py–H), 8.20 (t, J = 6.5 Hz, 1H), 9.95 (s, 1H, –NH–), 10.00 (s, 1H, –NH–), 10.05 (s, 1H, –NH–). MS: *m*/*z* calculated for $C_{66}H_{82}N_{10}O_{25}$ 1414.50, found 1415.41 (M + 1) for its imine compound and 1447.42 (M + 1) for its methyl ether compound.

Compound 34

This compound was prepared according to the method described for the compound **33**, by employing compound **26** (1.5 g, 0.956 mmol) in 40% yield (0.54 g) as a yellow solid. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 1.56–1.62 (m, 4H), 1.65 (m, 2H), 1.95 (m, 2H), 1.98–2.10 (8 singlets, 24H, $-COCH_3$), 2.12 (m, 2H), 2.27 (s, 6H, $-N(CH_3)_2$), 2.35 (m, 2H), 2.96 (m, 2H), 3.31 (m, 1H), 3.36 (m, 2H), 3.77 (s, 3H, $-ArOCH_3$), 3.85 (s, 3H, $-NCH_3$), 3.90 (m, 3H, $-OCH_3$ for its methyl ether), 3.96 (m, 2H), 4.15–4.49 (m, 8H, sugar proton), 5.21–5.60 (m, 4H, sugar proton), 6.55 (m, 2H, sugar proton), 6.81 (s, 1H, Ar–H), 6.83 (d, 1H, J = 1.7 Hz, Py–H), 6.96 (d, 1H, J = 1.7 Hz, Py–H), 7.17 (d, 1H, J = 1.7 Hz, Py–H), 7.32 (s, 1H, Ar–H), 7.47 (d, 1H, J = 1.7 Hz, Py–H), 7.50 (d, 1H, J = 4.2 Hz, imine proton), 7.80 (s, 1H, Im–H), 8.21 (t, J = 6.5 Hz, 1H), 9.96 (s, 1H, –NH–), 10.01 (s, 1H, –NH–), 10.05 (s, 1H, –NH–). MS: *m*/*z* calculated for $C_{65}H_{81}N_{11}O_{25}$ 1415.52, found 1416.40 (M + 1) for its imine compound and 1448.44 (M + 1) for its methyl ether compound.

Compound 35

This compound was prepared according to the general procedure C, using compound **27** (1.2 g, 1.06 mmol) in 43% yield (0.45 g) as a yellow solid. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 1.59–1.62 (m, 4H), 1.65 (m, 2H), 1.98 (m, 2H), 2.01–2.10 (4 singlets, 12H, 4× $-COCH_3$), 2.16 (m, 2H), 2.27 (s, 6H, $-N(CH_3)_2$), 2.36 (m, 2H), 2.96 (m, 2H), 3.30 (m, 1H), 3.35 (m, 2H), 3.73 (s, 3H, $-ArOCH_3$), 3.84 (s, 3H, $-NCH_3$), 3.89 (m, 3H, $-OCH_3$ for its methyl ether), 3.95 (m, 2H), 4.16–4.35 (m, 4H, sugar proton), 5.20–5.62 (m, 2H, sugar proton), 6.51 (m, 1H, sugar proton), 6.80 (s, 1H, Ar–H), 6.86 (d, 1H, J = 1.7 Hz, Py–H), 6.92 (d, 1H, J = 1.7 Hz, Py–H), 7.22 (d, 1H, J = 1.7 Hz, Py–H), 7.38 (s, 1H, Ar–H), 7.42 (d, 1H, J = 1.7 Hz, Py–H), 7.50 (d, 1H, J = 4.2 Hz, imine proton), 8.20 (t, J = 6.5 Hz, 1H), 9.97 (s, 1H, –NH–), 10.05 (s, 1H, –NH–). MS: *m*/*z* calculated for $C_{47}H_{60}N_8O_{15}$ 976.40, found 977.01 (M + 1) for its imine compound and 1009.07 (M + 1) for its methyl ether compound.

Compound 36

This compound was prepared according to the method described for the compound **34**, employing compound **28** (1.2 g, 0.958 mmol) in 44% yield (0.47 g) as a yellow solid. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 1.56–1.60 (m, 4H), 1.66 (m, 2H), 1.95 (m, 2H), 2.00–2.10 (4 singlets, 12H, 4× $-COCH_3$), 2.14 (m, 2H), 2.27 (s, 6H, $-N(CH_3)_2$), 2.34 (m, 2H), 2.95 (m, 2H), 3.30 (m, 1H), 3.34 (m, 2H), 3.75 (s, 3H, $-ArOCH_3$), 3.84 (s, 3H, $-NCH_3$), 3.86 (s, 3H, $-NCH_3$), 3.89 (m, 3H, $-OCH_3$ for its methyl ether), 3.96 (m, 2H), 4.15–4.40 (m, 4H, sugar proton), 5.21–5.55 (m, 2H, sugar proton), 6.50 (m, 1H, sugar proton), 6.81 (s, 1H, Ar–H), 6.84 (d, 1H, J = 1.7 Hz, Py–H), 6.95 (d, 1H, J = 1.7 Hz, Py–H), 7.20 (d, 1H, J = 1.7 Hz, Py–H), 7.35 (s, 1H, Ar–H), 7.45 (d, 1H, J = 1.7 Hz, Py–H), 7.50 (d, 1H, J = 4.2 Hz, imine proton), 7.75 (d, 1H, J = 1.7 Hz, Py–H), 8.21 (t, J = 6.5 Hz, 1H), 9.94 (s, 1H, –NH–), 10.00 (s, 1H, –NH–), 10.05 (s, 1H, –NH–). MS: *m*/*z* calculated for $C_{53}H_{66}N_{10}O_{16}$ 1098.45, found 1099.12 (M + 1) for its imine compound and 1131.15 (M + 1) for its methyl ether compound.

Compound 37

This compound was prepared starting from compound **29** (1.2 g, 1.06 mmol) according to the general procedure C (0.42 g, 40% yield) as a yellow solid. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 1.58–1.62 (m, 4H), 1.66 (m, 2H), 1.95 (m, 2H), 2.03–2.12 (4 singlets, 12H, 4× $-COCH_3$), 2.15 (m, 2H), 2.27 (s, 6H, $-N(CH_3)_2$), 2.36 (m, 2H), 2.96 (m, 2H), 3.30 (m, 1H), 3.35 (m, 2H), 3.73 (s, 3H, $-ArOCH_3$), 3.85 (s, 3H, $-NCH_3$), 3.89 (m, 3H, $-OCH_3$ for its methyl ether), 3.95 (m, 2H), 4.15–4.35 (m, 4H, sugar proton), 5.20–5.62 (m, 2H, sugar proton), 6.50 (m, 1H, sugar proton), 6.80 (s, 1H, Ar–H), 6.92 (d, 1H, J = 1.7 Hz, Py–H), 7.22 (d, 1H, J = 1.7 Hz, Py–H), 7.38 (s, 1H, Ar–H), 7.50 (d, 1H, J = 4.2 Hz, imine proton), 7.80 (s, 1H, Im–H), 8.25 (t, J = 6.5 Hz, 1H), 9.95 (s, 1H, –NH–), 10.04 (s, 1H, –NH–). MS: *m*/*z* calculated for $C_{46}H_{59}N_9O_{15}$ 977.41, found 978.02 (M + 1) for its imine compound and 1010.04 (M + 1) for its methyl ether compound.

Compound 38

This compound was prepared according to the general procedure C by using compound **30** (1.2 g, 0.956 mmol) in 44% yield (0.44 g) as a yellow solid. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 1.56–1.65 (m, 4H), 1.70 (m, 2H), 1.95 (m, 2H), 2.00–2.10 (4 singlets, 12H, 4× $-COCH_3$), 2.14 (m, 2H), 2.26 (s, 6H, $-N(CH_3)_2$), 2.35 (m, 2H), 2.95 (m, 2H), 3.30 (m, 1H), 3.34 (m, 2H), 3.74 (s, 3H, $-ArOCH_3$), 3.84 (s, 3H, $-NCH_3$), 3.86 (s, 3H, $-NCH_3$), 3.88 (m, 3H, $-OCH_3$ for its methyl ether), 3.95 (m, 2H), 4.15–4.38 (m, 4H, sugar proton), 5.21–5.62 (m, 2H, sugar proton), 6.51 (m, 1H, sugar proton), 6.82 (s, 1H, Ar–H), 6.92 (d, 1H, J = 1.7 Hz, Py–H), 7.20 (d, 1H, J = 1.7 Hz, Py–H), 7.35 (s, 1H, Ar–H), 7.47 (s, 1H, Im–H), 7.52 (d, 1H, J = 4.2 Hz, imine proton), 7.80 (s, 1H, Im–H), 8.25 (t, J = 6.5 Hz, 1H), 9.95 (s, 1H, –NH–), 10.00 (s, 1H, –NH–), 10.04 (s, 1H, –NH–). MS: *m*/*z* calculated for $C_{51}H_{64}N_{12}O_{16}$ 1100.42, found 1101.13 (M + 1) for its imine compound and 1133.15 (M + 1) for its methyl ether compound.

General procedure D

A mixture of the respective compounds **31**–**38** 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 mixtures were passed through small ion exchange resin (Amberlite-15) columns using MeOH/H_2O (80 : 20). The solvent was removed under reduced pressure and the final water soluble PBD-glycosylated polyamides **39**–**46** dried by lyophilization in 50–60% yield.

Compound 39

This compound was prepared according to the general procedure D employing compound **31** (0.35 g, 1.48 mmol) and 0.1 N NaOH in 53% yield (0.15 g) as a yellow solid. ^{1}H NMR (D_2O, 300 MHz): δ 1.59–1.60 (m, 4H), 1.65 (m, 2H), 1.98 (m, 2H), 2.18 (m, 2H), 2.26 (s, 6H, –$N(CH_3)_2$), 2.35 (m, 2H), 2.95 (m, 2H), 3.30 (m, 1H), 3.35 (m, 2H), 3.40–3.49 (m, 3H, sugar proton), 3.65 (m, 2H, –C**H**$_2$OH), 3.73 (s, 3H, –$ArOCH_3$), 3.76 (m, 1H, sugar proton), 3.89 (m, 3H, –OCH_3 for its methyl ether), 3.95 (m, 2H), 5.89 (m, 1H, sugar proton), 6.81 (s, 1H, Ar–H), 6.92 (d, 1H, J = 1.7 Hz, Py–H), 7.12 (d, 1H, J = 1.7 Hz, Py–H), 7.32 (s, 1H, Ar–H), 7.50 (d, 1H, J = 4.2 Hz, imine proton). HR-MS: m/z calculated for $C_{33}H_{46}N_6O_{10}$ 686.30, found 687.20 (M + 1) for its imine compound and 719.65 (M + 1) for its methyl ether compound.

Compound 40

This compound was prepared starting from compound **32** (0.45 g, 0.348 mmol) and 0.1 N NaOH according to the general procedure D (0.20 g, 60% yield) as a yellow solid. ^{1}H NMR (D_2O, 300 MHz): δ 1.56–1.60 (m, 4H), 1.65 (m, 2H), 1.95 (m, 2H), 2.15 (m, 2H), 2.28 (s, 6H, –$N(CH_3)_2$), 2.35 (m, 2H), 2.95 (m, 2H), 3.30 (m, 1H), 3.34 (m, 2H), 3.40–3.50 (m, 6H, sugar proton), 3.66 (m, 4H, 2× –C**H**$_2$OH), 3.74 (s, 3H, –$ArOCH_3$), 3.78 (m, 2H, sugar proton), 3.89 (m, 3H, –OCH_3 for its methyl ether), 3.95 (m, 2H), 5.90 (m, 2H, sugar proton), 6.82 (s, 1H, Ar–H), 6.86 (d, 1H, J = 1.7 Hz, Py–H), 6.95 (d, 1H, J = 1.7 Hz, Py–H), 7.15 (d, 1H, J = 1.7 Hz, Py–H), 7.35 (s, 1H, Ar–H), 7.42 (d, 1H, J = 1.7 Hz, Py–H), 7.50 (d, 1H, J = 4.2 Hz, imine proton). HR-MS: m/z calculated for $C_{44}H_{60}N_8O_{16}$ 956.40, found 957.00 (M + 1) for its imine compound and 989.03 (M + 1) for its methyl ether compound.

Compound 41

This compound was prepared according to the general procedure D using compound **33** (0.45 g, 0.318 mmol) and 0.1 N NaOH in 55% yield (0.19 g) as a yellow solid. ^{1}H NMR (D_2O, 300 MHz): δ 1.56–1.60 (m, 4H), 1.65 (m, 2H), 1.95 (m, 2H), 2.17 (m, 2H), 2.27 (s, 6H, –$N(CH_3)_2$), 2.36 (m, 2H), 2.95 (m, 2H), 3.30 (m, 1H), 3.35 (m, 2H), 3.41–3.51 (m, 6H, sugar proton), 3.66 (m, 4H, 2× –C**H**$_2$OH), 3.74 (s, 3H, –$ArOCH_3$), 3.77 (m, 2H, sugar proton), 3.84 (s, 3H, –NCH_3), 3.89 (m, 3H, –OCH_3 for its methyl ether), 3.95 (m, 2H), 5.89 (m, 2H, sugar proton), 6.81 (s, 1H, Ar–H), 6.83 (d, 1H, J = 1.7 Hz, Py–H), 6.96 (d, 1H, J = 1.7 Hz, Py–H), 7.16 (d, 1H, J = 1.7 Hz, Py–H), 7.21 (d, 1H, J = 1.7 Hz, Py–H), 7.36 (s, 1H, Ar–H), 7.45 (d, 1H, J = 1.7 Hz, Py–H), 7.51 (d, 1H, J = 4.2 Hz, imine proton), 7.72 (d, 1H, J = 1.7 Hz, Py–H). HR-MS: m/z calculated for $C_{50}H_{66}N_{10}O_{17}$ 1078.46, found 1079.10 (M + 1) for its imine compound and 1111.15 (M + 1) for its methyl ether compound.

Compound 42

This compound was prepared according to the method described for the compound **40** employing compound **34** (0.48 g, 0.339 mmol) and 0.1 N NaOH in 57% yield (0.21 g) as a yellow solid. ^{1}H NMR (D_2O, 300 MHz): δ 1.56–1.62 (m, 4H), 1.65 (m, 2H), 1.95 (m, 2H), 2.12 (m, 2H), 2.27 (s, 6H, –$N(CH_3)_2$), 2.35 (m, 2H), 2.96 (m, 2H), 3.31 (m, 1H), 3.36 (m, 2H), 3.41–3.51 (m, 6H, sugar proton), 3.66 (m, 4H, 2× –C**H**$_2$OH), 3.74 (s, 3H, –$ArOCH_3$), 3.78 (m, 2H, sugar proton), 3.85 (s, 3H, –NCH_3), 3.90 (m, 3H, –OCH_3 for its methyl ether), 3.96 (m, 2H), 5.90 (m, 2H, sugar proton), 6.81 (s, 1H, Ar–H), 6.83 (d, 1H, J = 1.7 Hz, Py–H), 6.96 (d, 1H, J = 1.7 Hz, Py–H), 7.17 (d, 1H, J = 1.7 Hz, Py–H), 7.32 (s, 1H, Ar–H), 7.47 (d, 1H, J = 1.7 Hz, Py–H), 7.50 (d, 1H, J = 4.2 Hz, imine proton), 7.80 (s, 1H, Im–H). HR-MS: m/z calculated for $C_{49}H_{65}N_{11}O_{17}$ 1079.45, found 1080.10 (M + 1) for its imine compound and 1112.12 (M + 1) for its methyl ether compound.

Compound 43

This compound was prepared according to general procedure D using compound **35** (0.4 g, 0.409 mmol) 0.1 N NaOH in 56% yield (0.185 g) as a yellow solid. ^{1}H NMR (D_2O, 300 MHz): δ 1.59–1.62 (m, 4H), 1.65 (m, 2H), 1.98 (m, 2H), 2.15 (m, 2H), 2.27 (s, 6H, –$N(CH_3)_2$), 2.36 (m, 2H), 2.95 (m, 2H), 3.30 (m, 1H), 3.35 (m, 2H), 3.40–3.51 (m, 3H, sugar proton), 3.65 (m, 2H, –C**H**$_2$OH), 3.73 (s, 3H, –$ArOCH_3$), 3.76 (m, 1H, sugar proton), 3.84 (s, 3H, –NCH_3), 3.89 (m, 3H, –OCH_3 for its methyl ether), 3.95 (m, 2H), 5.89 (m, 1H, sugar proton), 6.80 (s, 1H, Ar–H), 6.86 (d, 1H, J = 1.7 Hz, Py–H), 6.92 (d, 1H, J = 1.7 Hz, Py–H), 7.22 (d, 1H, J = 1.7 Hz, Py–H), 7.38 (s, 1H, Ar–H), 7.42 (d, 1H, J = 1.7 Hz, Py–H), 7.50 (d, 1H, J = 4.2 Hz, imine proton). HR-MS: m/z calculated for $C_{39}H_{52}N_8O_{11}$ 808.36, found 809.30 (M + 1) for its imine compound and 841.46 (M + 1) for its methyl ether compound.

Compound 44

This compound was prepared according to the method described for the compound **42**, employing compound **36** (0.41 g, 0.373 mmol) 0.1 N NaOH in 54% yield (0.19 g) as a yellow solid. ^{1}H NMR (D_2O, 300 MHz): δ 1.56–1.60 (m, 4H), 1.66 (m, 2H), 1.95 (m, 2H), 2.14 (m, 2H), 2.27 (s, 6H, –$N(CH_3)_2$), 2.34 (m, 2H), 2.95 (m, 2H), 3.30 (m, 1H), 3.34 (m, 2H), 3.40–3.49 (m, 3H, sugar proton), 3.65 (m, 2H, –C**H**$_2$OH), 3.73 (s, 3H, –$ArOCH_3$), 3.75 (m, 1H, sugar proton), 3.84 (s, 3H, –NCH_3), 3.87 (s, 3H, –NCH_3), 3.89 (m, 3H, –OCH_3 for its methyl ether), 3.96 (m, 2H), 5.90 (m, 1H, sugar proton), 6.81 (s, 1H, Ar–H), 6.84 (d, 1H, J = 1.7 Hz, Py–H), 6.95 (d, 1H, J = 1.7 Hz, Py–H), 7.20 (d, 1H, J = 1.7 Hz, Py–H), 7.35 (s, 1H, Ar–H), 7.45 (d, 1H, J = 1.7 Hz, Py–H), 7.50 (d, 1H, J = 4.2 Hz, imine proton), 7.75 (d, 1H, J = 1.7 Hz, Py–H). HR-MS: m/z calculated for $C_{45}H_{58}N_{10}O_{12}$ 930.40, found 931.21 (M + 1) for its imine compound and 963.04 (M + 1) for its methyl ether compound.

Compound 45

This compound was prepared starting from compound **37** (0.40 g, 0.409 mmol) 0.1 N NaOH according to the general procedure D (0.17 g, 51% yield) as a yellow solid. ^{1}H NMR (D_2O, 300 MHz): δ 1.58–1.62 (m, 4H), 1.66 (m, 2H), 1.95 (m, 2H), 2.15 (m, 2H), 2.27 (s, 6H, –$N(CH_3)_2$), 2.36 (m, 2H), 2.96 (m, 2H), 3.30 (m, 1H), 3.35 (m, 2H), 3.40–3.49 (m, 3H, sugar proton), 3.65 (m, 2H, –C**H**$_2$OH), 3.74 (s, 3H, –$ArOCH_3$), 3.77 (m, 1H, sugar proton), 3.85 (s, 3H, –NCH_3), 3.89 (m, 3H, –OCH_3 for its methyl ether), 3.95 (m, 2H), 5.89 (m, 1H, sugar proton), 6.80 (s, 1H, Ar–H), 6.92 (d, 1H, J = 1.7 Hz, Py–H), 7.22 (d, 1H, J = 1.7 Hz, Py–H), 7.38 (s, 1H, Ar–H), 7.50 (d, 1H, J = 4.2 Hz, imine proton), 7.80 (s, 1H, Im–H). HR-MS: m/z calculated for $C_{38}H_{51}N_9O_{11}$ 809.37, found 810.57 (M + 1) for its imine compound and 842.40 (M + 1) for its methyl ether compound.

Compound 46

This compound was prepared according to the general procedure D using compound **38** (0.4 g, 0.363 mmol) 0.1 N NaOH in 53% yield (0.18 g) as a yellow solid. ^{1}H NMR (D_2O, 300 MHz): δ 1.56–1.65 (m, 4H), 1.70 (m, 2H), 1.95 (m, 2H), 2.15 (m, 2H), 2.26 (s, 6H, –$N(CH_3)_2$), 2.35 (m, 2H), 2.95 (m, 2H), 3.30 (m, 1H), 3.34 (m, 2H), 3.41–3.50 (m, 3H, sugar proton), 3.65

(m, 2H, –C**H**$_2$OH), 3.73 (s, 3H, –ArOC**H**$_3$), 3.77 (m, 1H, sugar proton), 3.84 (s, 3H, –NC**H**$_3$), 3.86 (s, 3H, –NC**H**$_3$), 3.88 (m, 3H, –OC**H**$_3$ for its methyl ether), 3.95 (m, 2H), 5.89 (m, 1H, sugar proton), 6.82 (s, 1H, Ar–H), 6.92 (d, 1H, J = 1.7 Hz, Py–H), 7.20 (d, 1H, J = 1.7 Hz, Py–H), 7.35 (s, 1H, Ar–H), 7.47 (s, 1H, Im–H), 7.52 (d, 1H, J = 4.2 Hz, imine proton), 7.80 (s, 1H, Im–H). HR-MS: *m*/*z* calculated for $C_{43}H_{56}N_{12}O_{12}$ 932.41, found 933.15 (M + 1) for its imine compound and 965.02 (M + 1) for its methyl ether compound.

Acknowledgements

We thank the National Cancer Institute, Maryland for the anticancer assay in human cell lines. This research was supported by a grant (to J.W.L.) from the Natural Sciences and Engineering Research Council of Canada.

References

1 L. H. Hurley, *J. Antibiot.*, 1977, **30**, 349.
2 L. H. Hurley, J. S. Rokem and R. L. Petrusek, *Biochem. Pharmacol.*, 1980, **29**, 1307.
3 D. E. Thurston and D. S. Bose, *Chem. Rev.*, 1994, **94**, 433.
4 M. L. Kopka, D. S. Goodsell, I. Baikalov, K. Grzeskowaik, D. Cascio and R. E. Dickerson, *Biochemistry*, 1994, **33**, 13593.
5 A. Kamal, Y. Damayanthi, B. S. N. Reddy, B. Lakminarayana and B. S. P. Reddy, *Chem. Commun.*, 1997, 1015.
6 A. Kamal, B. S. N. Reddy and B. S. P. Reddy, *Biorg. Med. Chem. Lett.*, 1997, **7**, 1825.
7 (*a*) L. H. Hurley and D. E. Thurston, *Pharm. Res.*, 1984, **2**, 52; (*b*) B. S. P. Reddy, S. M. Sondhi and J. W. Lown, *Pharmacol. Ther.*, 1999, **84**, 1.
8 (*a*) L. H. Hurley, T. Reck, D. E. Thurston, D. R. Langley, K. G. Holden, R. P. Hertzberg, J. R. E. Hoover, G. Gallagher, Jr., L. F. Faucette, S. M. Mong and R. K. Johnson, *Chem. Res. Toxicol.*, 1988, **1**, 258–268; (*b*) F. L. Boyd, D. Stewart, W. A. Remers, M. D. Barkley and L. H. Hurley, *Biochemistry*, 1990, **29**, 2387–2403.
9 M. S. Puvada, J. A. Hartley, T. C. Jenkins and D. E. Thurston, *Nucl. Acids Res.*, 1993, **21**, 3671–3675.
10 M. S. Puvada, S. A. Forrow, J. A. Hartley, P. Stephenson, I. Gibson, T. C. Jenkins and D. E. Thurston, *Biochemistry*, 1997, **36**, 2478–2484.
11 P. N. Confalone, E. M. Huie, S. S. Ko and G. M. Cole, *J. Org. Chem.*, 1988, **53**, 482.
12 (*a*) D. S. Bose, A. S. Thompson, J. A. Ching, M. D. Berardini, T. C. Jenkins, S. Neidle, L. H. Hurley and D. E. Thurston, *J. Am. Chem. Soc.*, 1992, **114**, 4939; (*b*) D. E. Thurston, D. S. Bose, A. S. Thompson, P. W. Howard, A. Leoni, S. J. Croker, T. C. Jenkins, S. Neidle, J. A. Hartley and L. H. Hurley, *J. Org. Chem.*, 1996, **61**, 8141.
13 P. G. Baraldi, G. Balboni, B. Cacciari, A. Guiotto, S. Manfridini, R. Romagnoli, G. Spalluto, D. E. Thurston, P. W. Howard, N. Bianchi, C. Rutigliano, C. Mischiati and R. Gambari, *J. Med. Chem.*, 1999, **42**, 5131.
14 Y. Damayanthi, B. S. P. Reddy and J. W. Lown, *J. Org. Chem.*, 1999, **64**, 290.
15 B. S. P. Reddy, Y. Damayanthi, B. S. N. Reddy and J. W. Lown, *Anti-Cancer Drug Des.*, 2000, **15**, 225.
16 B. S. P. Reddy, Y. Damayanthi and J. W. Lown, *Synth. Lett.*, 1999, 1112.
17 R. Kumar, B. S. N. Reddy and J. W. Lown, *Heterocycl. Commun.*, 2002, **8**, 19–26.
18 R. Kumar and J. W. Lown, *Heterocycl. Commun.*, 2002, **8**, 115–122.
19 R. Kumar and J. W. Lown, *Oncol. Res.*, 2002, **13**(4), 221–233.
20 A. Kamal, G. Ramesh, N. Laxman, P. Ramulu, O. Srinivas, K. Neelima, A. K. Kondapi, V. B. Sreenu and H. A. Nagarajaram, *J. Med. Chem.*, 2002, **45**(21), 4679.
21 S. J. Gregson, P. W. Howard, K. E. Corcoran, T. C. Jenkins, L. R. Kelland and D. E. Thurston, *Bioorg. Med. Chem. Lett.*, 2001, **11**, 2859.
22 N. Langlois, A. R. Rousseau, C. Gaspard, G. H. Werner, F. Darro and R. Kiss, *J. Med. Chem.*, 2001, **44**, 3754.
23 A. Kamal, N. Laxman, G. Ramesh, O. Srinivas and P. Ramulu, *Bioorg. Med. Chem. Lett.*, 2002, **12**, 1917.
24 A. Kamal, B. S. N. Reddy, G. S. K. Reddy and G. Ramesh, *Bioorg. Med. Chem. Lett.*, 2002, **12**, 1933.
25 J. M. Gottesfeld, L. Neely, J. W. Trauger, E. E. Baird and P. B. Dervan, *Nature*, 1997, **387**, 202.
26 L. A. Dickinson, R. J. Gulizia, J. W. Trauger, E. E. Baird, D. E. Mosier, J. M. Gottesfeld and P. B. Dervan, *Proc. Natl. Acad. Sci. U.S.A*, 1998, **95**, 12890.
27 J. W. Lown and R. G. Micetich, *US Patent*, Patent no. 5,616,606, dated April 1, 1997.
28 J. W. Lown and K. Krowicki, *US Patent*, Patent no. 4,912,199, dated May 10, 1995.
29 N. Neamati, A. Mazumder, S. Sunder, J. M. Owen, M. Tandon, J. W. Lown and Y. Pommier, *Mol. Pharmacol.*, 1998, **54**, 280.
30 J. W. Lown and S. K. Sharma, 2003, patent pending.