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# Syntheses and Antiproliferative Activities of Rebeccamycin **Analogues Bearing Two 7-Azaindole Moieties**

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Abstract—As a part of structure–activity relationship studies on rebeccamycin analogues, compounds containing two aza-indole moieties were synthesized bearing either a methyl group or a hydrogen atom on the imide nitrogen. The azaindole substructures were expected to enhance the cytotoxicity toward tumor cell lines through stronger hydrogen bonding with the target enzyme(s). The cytotoxicities of compounds 8, 10 and 19 against a panel of tumor cell lines were examined and compared with those of rebeccamycin, dechlorinated rebeccamycin 2 and N-methylated analogue A. Their effect on the L1210 cell cycle was also evaluated. Compound 19, having an imide NH function had the strongest cytotoxicity towards L1210 cells and induced the largest accumulation of cells in the G2+M phases of the cell cycle. In contrast to their non-aza analogues, which were cytotoxic for all the cell lines tested, diaza compounds 10 and 19 showed selectivity for some cell lines.

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# Introduction

Rebeccamycin, a microbial metabolite isolated from cultures of Saccharothrix aerocolonigenes, is an antitumor antibiotic that inhibits topoisomerase I by stabilizing the topoisomerase I-DNA cleavable complex.<sup>1,2</sup> However, its toxicity prohibits its use in cancer chemotherapy. Structure-activity relationship studies have been carried out with a view to improve the pharmacological profile of rebeccamycin,<sup>3-7</sup> and have led to the development of a schematic representation of a drug-topoisomerase I-DNA ternary complex. According to this model, the indolocarbazole chromophore intercalates with DNA and the carbohydrate side chain is oriented toward the minor groove, while the imide part occupies a recognition pocket of topoisomerase I.<sup>8</sup> It has been showed that although topoisomerase I is a target for most of rebeccamycin derivatives, the inhibition of other enzymes may also be a contributing factor to their cytotoxicity.

Azaindoles, as biosteres for indoles, present considerable biological importance. Azaindoles have been used as various pharmaceutical agents such as anti-inflammatory and anti-psychotic agents. 7-Aza-indoles are found in several natural products such as variolins, compounds isolated from Antarctic sponge Kirk-patrickia varialosa<sup>9</sup> which exhibit antiproliferative activity against murine leukemia P388 cell lines. Over the past few years, the chemistry and biological applications of 7-aza-indoles have been widely investigated.<sup>10–13</sup> New NADH models in the pyrrolo[2,3-b] pyridine series and 7-azaindole derivatives with high affinity for the dopamine D4 receptor have been synthesized. The syntheses of 7-azaindole analogues of natural products such as melatonin, vincamin, ellipticin, tryptophan have been described.<sup>14–16</sup> In 7-aza-rebeccamycin analogues, the replacement of carbon atoms by nitrogen atoms introduces a basic character into the molecule that can modify the pharmacological properties. These nitrogen atoms may also contribute to the formation of hydrogen bonds within the active site of target enzyme(s) and/ or DNA and thereby stabilize or modify the orientation of the drug in the drug-enzyme complexes.

In this paper, we present the syntheses of rebeccamycin analogues bearing two 7-azaindole moieties, in some cases substituted with a methyl group on the imide nitrogen (Fig. 1). Their antiproliferative activities in

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Rebeccamycin 1 R = ClDechlorinated rebeccamycin 2 R = H

#### Figure 1.

vitro against a panel of murine and human tumor cells lines are described and compared with those of rebeccamycin 1, dechlorinated rebeccamycin  $2,^4$  and the previously synthesized analogue  $A,^3$  which bears a methyl group on the imide nitrogen.

#### **Results and Discussion**

# Chemistry

The synthesis of compound **10** is outlined in Scheme 1. Compound **3** was obtained by coupling 7-azaindole magnesium bromide with *N*-methyl-dibromomaleimide in toluene solution. *N*-methyl-dibromomaleimide was obtained by methylation of dibromomaleimide using dimethylsulfate and potassium carbonate in acetone, according to a classical procedure.<sup>17</sup> Dibromomaleimide was obtained in 67% yield by treatment of maleimide with bromine in DMF at 55 °C for 3 days. This method is easier than the classical one from succinimide.<sup>18</sup> The indole nitrogen of **3** was protected with a benzenesulphonyl group<sup>9</sup> using benzenesulphonyl chloride in the presence of sodium hydride. A second nucleophilic attack of lithiated 7-azaindole on intermediate 4 in toluene led to bis-azaindole 5.<sup>19</sup> Several methods of Nglycosylation of either bis-indolyl maleimides or indolocarbazoles have been described in the literature.<sup>20</sup> Since the desired coupling product possesses a  $\beta$ -N-glycosidic bond, the sugar part was introduced via a Mitsunobu reaction using 2,3,4,6-tetra-O-acetyl- $\alpha$ -Dglucopyranose. This carbohydrate was prepared in three steps from commercial 1,2,3,4,6-penta-O-acetyl- $\alpha$ -Dglucopyranose (Scheme 2).<sup>21</sup> The intermediate alkene, with an  $\alpha$  anomeric bond, was obtained by reaction of tetraacetylglucopyranose with 3-buten-1-ol in the presence of SnCl<sub>4</sub> as a catalyst. Ozonolysis led to the corresponding aldehyde, which was treated with potassium carbonate to give the required sugar.

The Mitsunobu reaction with compound **5** gave  $\beta$ -*N*-glycosylated compound **6** as the major product of the reaction (65% yield). The  $\beta$ -configuration of **6** was indicated in the <sup>1</sup>H NMR spectrum by the H<sub>1</sub>'-H<sub>2</sub>'



Scheme 1.



## Scheme 2.

coupling constant value (9 Hz), consistent with axialaxial coupling.

The second azaindole nitrogen was deprotected using tetrabutylammonium fluoride (TBAF) to yield 7. Deacetylation of the hydroxyl groups of the sugar moiety was performed with NH<sub>4</sub>OH in methanol leading to compound 8 (Scheme 3), designed as an analogue in bisaza-indolylmaleimide series.  $\beta$ -*N*-Glycosylated compound 7 was cyclized to aza-carbazole 9 by oxidative irradiation in benzene in the presence of iodine. Hydrolysis of the acetate groups of the carbohydrate part led to the required rebeccamycin analogue 10.

An identical synthetic scheme was used to obtain *N*benzyloxymethyl-dibromomaleimide, the precursor of compounds having a free NH imide function. The differences were in the final deprotection steps (Scheme 4). *N*-BOM-dibromomaleimide was prepared from dibromomaleimide using benzyloxymethyl chloride in the presence of potassium carbonate in acetone. During the coupling with the second aza-indole moiety using LiHMDS, partial deprotection of the aza-indolic nitrogen was observed, leading to 14. The final deprotection of compound 17 was performed in two steps. First, hydrogenolysis with a catalytic amount of Pd/C in an ethyl acetate/methanol mixture lead to 18 in low yield, due to product adsorption on the catalyst and partial hydrolysis of the acetates. Aminolysis yielded the azarebeccamycin analogue **19**.

## In vitro antiproliferative activities

The in vitro antiproliferative activities were tested against three tumor cell lines, murine leukemia (L1210), and two human solid tumors: colon carcinoma (HT29), and non-small cell lung carcinoma A549. The results, expressed as IC<sub>50</sub> values, are reported in Table 1. Compared with A and 10, compound 8, lacking an indolocarbazole framework, is inactive. The planar structure of an indolocarbazole or diazaindolocarbazole seems necessary for the cytotoxicity. The aza-analogue 19 with the free imide NH exhibits the strongest cytotoxicity toward L1210 cells, being slightly more potent than rebeccamycin 1 and dechlorinated rebeccamycin 2 and about ten times more efficient than the N-methyl analogue 10. However, very different results were obtained on the two human tumor cell lines : 10 is inactive on both lines, while 19 is potent on HT29. These two compounds thus appear to have some selectivity against certain cell lines. To compare the cytotoxicity profile of 10 with those of 1, 2 and A, the antiproliferative activities against six other tumor cell lines were examined, human leukemia (K-562) and five human solid tumors: one ovarian carcinoma (IGROV1), one neuroblastoma (SK-N-MC), one small-cell lung carcinoma (H69) and





#### Scheme 4.

**Table 1.** In vitro antiproliferative activities ( $IC_{50} \mu M$ ) against three tumor cell lines: murine leukemia (L1210), human colon carcinoma (HT29), human non-small cell lung carcinoma (A549)

Compd	L1210	HT29	A549	
1	0.14	0.3	0.3	
2	0.11	2.5	2.0	
A	0.67	0.86	0.94	
8	66.1	>100	>100	
10	0.58	97	>100	
19	0.067	0.57	41.5	

two epidermoïd carcinomas (A431 and KB-3-1) (Table 2). While **A** is almost equally potent in all the cell lines tested (IC<sub>50</sub> range of 0.25–0.88  $\mu$ M), **10** appears highly cytotoxic for A431 cells, slightly less cytotoxic for SK-N-MC, NCI-H69 and inactive in IGROV1 cells. Like compound **A**, rebeccamycin **1** was non selective. This different cytotoxicity profile suggests that the aza-analogues do not share the same mechanism of action, or that additional targets are affected by the non aza-analogues. The basis for the selectivity of **10** is currently being investigated.

#### Effect on L1210 cell cycle

The effect on the L1210 cell cycle was investigated for compounds **10** and **19** (i.e., those compounds which exhibit strong antiproliferative activities against this tumor cell line). As already observed for most rebeccamycin analogues tested so far, these compounds induced

accumulation in the G2+M phases. The most efficient compound was aza-analogue **19**, for which 66% of cells were arrested in the G2+M phases at a drug concentration of 0.25  $\mu$ M (Table 3). Interestingly, the most selective compound **10** is devoid of a significant effect on L1210 cell cycle, suggesting a different mechanism of action at the molecular level.

In conclusion, as a contribution to structure-activity relationship studies in rebeccamycin analogues, the synthesis of diazaindole analogues, with or without a methyl group on the imide nitrogen, has been carried out. The new compounds having azaindolocarbazole frameworks have strong antiproliferative properties against the murine L1210 tumor cell line. Compared with rebeccamycin 1, dechlorinated rebeccamycin 2, and *N*-methyl derivative **A**, compound **19** is more cytotoxic. The cytotoxicity profiles of aza-analogues 10 and 19 are quite different from those of the parent non-aza compounds, which are non selective, and display similarly cytotoxicity for all the cell lines. These data suggest additional targets for the non-aza compounds. Consequently, the aza-analogues might be less toxic in vivo than rebeccamycin. Compared with 1 and 2, the effect of 19 on the cell cycle of L1210 is about four times greater for the accumulation of the cells in the G2 + Mphases. By introducing nitrogen atoms in the indole rings, our aim was to reinforce the interactions with the active site of the target enzyme(s) or with DNA. The first biological results seem to validate our hypothesis

**Table 2.** In vitro antiproliferative activities (IC<sub>50</sub>  $\mu$ M) against six human tumor cell lines: leukemia (K-562) and five solid tumors: ovarian carcinoma (IGROV1), neuroblastoma (SK-N-MC), small-cell lung carcinoma (H69) and two epidermoïd carcinomas (A431 and KB-3-1). Ne: not evaluated

Compd	IgROV1	SK-N-MC	A431	NCI-H69	K-562	KB-3-1
1	0.25	< 0.1	0.25	< 0.1	0.2	0.3
2	2.6	< 0.1	3.8	< 0.1	< 0.1	0.28
Α	0.88	0.25	0.84	0.33	ne	0.6

**Table 3.** Effect on the cell cycle. Results are expressed as percentage of cells recovered in the G2 + M phases (concentration of the drug)

% of L1210 cells in the $G2 + M$ phase		
24%		
69% (1 μM)		
$71\%$ (1 $\mu$ M)		
77% (2.5 μM)		
31% (5-20 µM)		
66% (0.25 μM)		

which has now to be confirmed by DNA binding experiments, topoisomerase I inhibition evaluation and the search for new targets.

### Experimental

# Chemistry

IR spectra were recorded on a Perkin–Elmer 881 spectrometer (v in cm<sup>-1</sup>). NMR spectra were performed on a Bruker AC 400 (<sup>1</sup>H: 400 MHz, <sup>13</sup>C: 100 MHz) (chemical shifts  $\delta$  in ppm, the following abbreviations are used: singlet (s), broad singlet (br s), doublet (d), doubled doublet (dd), triplet (t), pseudo-triplet (pt), multiplet (m), tertiary carbons (C *tert*), quaternary carbons (C quat). Mass spectra (FAB+) were determined at CESAMO (Talence, France) on a high resolution Fisons Autospec-Q spectrometer. Chromatographic purifications were performed by flash silicagel Geduran SI 60 (Merck) 0.040-0.063 mm or Kieselgel 60 (Merck) 0.063-0.200 mm column chromatography. For purity tests, TLC were performed on fluorescent silica gel plates (60 F<sub>254</sub> from Merck).

1-Methyl-3-[1-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-1*H*-pyrrolo[2,3-*b*]pyridin-3-yl]-4-[1-(phenylsulphonyl)-1*H*pyrrolo[2,3-*b*]pyridin-3-yl]-1*H*-pyrrole-2,5-dione (6). To a solution of 5 (250 mg, 0.518 mmol) in THF (20 mL), were added 2,3,4,6-*O*-α-D-acetylglucopyranose (486 mg, 1.40 mmol) and triphenylphosphine (367 mg, 1.40 mmol). The mixture was cooled to -78 °C, then DEAD (220 µL, 1.40 mmol) was added dropwise. The mixture was stirred at room temperature for 15 h. Water was added. After extraction with EtOAc, the organic phase was dried over MgSO<sub>4</sub>, the solvent was removed and the residue purified by flash chromatography (eluent toluene/EtOAc, 30:25 then cyclohexane/EtOAc, 3:2) to give β-glycosylated compound **6** (273 mg, 0.336 mmol, 65% yield) as a yellow solid.

Mp 116–118 °C; IR (KBr),  $\nu_{CO}$  1710, 1750 cm<sup>-1</sup>. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>): 1.65 (3H, s), 2.04 (3H, s), 2.09 (3H, s), 2.11 (3H, s), 3.20 (3H, s, NCH<sub>3</sub>), 4.03–4.08 (1H, m), 4.19 (1H, dd,  $J_1$ =1.7 Hz,  $J_2$ =12.6 Hz), 4.32 (1H, dd,  $J_1$ =4.6 Hz,  $J_2$ =12.6 Hz), 5.29 (1H, m), 5.51 (2H, m), 6.26 (1H, m), 6.61 (1H, dd,  $J_1$ =7.9 Hz,  $J_2$ =4.7 Hz), 6.87 (1H, dd,  $J_1$ =8.0 Hz,  $J_2$ =4.8 Hz), 6.94 (1H, dd,  $J_1$ =7.9 Hz,  $J_2$ =1.1 Hz), 7.34 (1H, dd,  $J_1$ =7.9 Hz,  $J_2$ =1.2 Hz), 7.50 (1H, t, J=7.7 Hz), 7.52 (1H, t, J=7.9 Hz), 7.63 (1H, t, J=7.6 Hz), 8.13 (1H, br s), 8.18–8.21 (4H, m), 8.32 (1H, d, J=4.6 Hz). <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>): 20.1, 20.6, 20.7, 20.9 (CH<sub>3</sub>CO), 22.0 (NCH<sub>3</sub>), 61.8 (C<sub>6</sub>'), 68.1, 71.0, 73.2, 75.0, 80.5 (C<sub>1</sub>', C<sub>2</sub>', C<sub>3</sub>', C<sub>4</sub>', C<sub>5</sub>'), 117.9, 119.1, 128.3 (2C), 129.1, 129.2 (2C), 129.6, 129.9, 130.7, 134.5, 144.2, 145.6 (C *tert* arom), 105.8, 109.0, 118.0, 120.9, 125.1, 130.4, 137.9, 146.9, 147.8 (C quat arom), 168.9, 169.5, 170.0, 170.7, 170.8° (2C) (C=O).

1-Methyl-3-[1-(2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl)-1H-pyrrolo[2,3-b]pyridin-3-yl]-4-(1H-pyrrolo[2,3-b]pyridin-3yl)-1*H*-pyrrole-2,5-dione (7). To a solution of  $\beta$ -glycosylated compound 6 (37 mg, 0.046 mmol) in THF (4 mL) was added a solution of tetrabutylammonium fluoride (1,1 M in THF) (0.125 mL, 0.137 mmol). The mixture was stirred at room temperature for 2.5 h. Water was added. After extraction with EtOAc, the organic phase was dried over MgSO<sub>4</sub>, the solvent was removed and the residue purified by flash chromatography (eluent cyclohexane/EtOAc, 3:7) to give 7 (24 mg, 0.036 mmol, 78% yield) as a yellow-orange solid. Mp 148-150 °C; IR (KBr),  $\nu_{\rm CO}$  1700, 1760 cm<sup>-1</sup>,  $\nu_{\rm NH}$  3300–3600 cm<sup>-1</sup>. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>): 1.70 (3H, s), 2.04 (3H, s), 2.09 (3H, s), 2.11 (3H, s), 3.21 (3H, s, NCH<sub>3</sub>), 4.07 (1H, m), 4.18 (1H, dd,  $J_1 = 12.5$  Hz,  $J_2 = 1.1$  Hz), 4.31 (1H, dd,  $J_1 = 12.6$  Hz,  $J_2 = 4.6$  Hz), 5.18 (1H, pt, J = 9.4 Hz), 5.51 (1H, pt, J=9.5 Hz), 5.57 (1H, pt, J=9.4 Hz), 6.29  $(1H, d, J=8.6 Hz, H_{1'}), 6.74 (1H, dd, J_1=7.9 Hz,$  $J_2 = 7.7$  Hz), 6.83 (1H, dd,  $J_1 = 7.9$  Hz,  $J_2 = 7.7$  Hz), 7.20 (1H, d, J=7.9 Hz), 7.42 (1H, d, J=7.9 Hz), 8.05 (1H, s), 8.06 (1H, s), 8.21 (2H, d, J=4.0 Hz), 11.08 (1H, s, NH). <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>): 20.2, 20.7, 20.8, 21.1 (CH<sub>3</sub>CO), 24.4 (NCH<sub>3</sub>), 61.8 (C<sub>6'</sub>), 68.1, 70.9, 73.2, 74.9, 80.4 ( $C_{1'}$ ,  $C_{2'}$ ,  $C_{3'}$ ,  $C_{4'}$ ,  $C_{5'}$ ), 116.7, 117.6, 128.7, 129.1, 130.4, 130.6, 143.5, 144.0 (C tert arom), 105.3, 106.6, 118.4, 118.9, 126.5, 128.3, 147.7, 148.6 (C quat arom), 168.9, 169.5, 170.0, 170.8, 171.6, 171.8 (C=O).

1-Methyl-3-[1-(β-D-glucopyranosyl)-1H-pyrrolo[2,3-b]pyridin-3-yl]-4-(1H-pyrrolo[2,3-b]pyridin-3-yl)-1H-pyrrole-2,5dione (8). To a solution of 7 (36 mg, 0.054 mmol) in methanol (14 mL) was added 28% aqueous NH<sub>4</sub>OH (10 mL). The mixture was stirred for 26 h at room temperature. After removal of the solvents, the residue was purified by flash chromatography (eluent EtOAc/ methanol, 9:1) to give 8 (18 mg, 0.036 mmol, 67% yield) as a red-orange solid. Mp 195-197°C; IR (KBr) <sub>CO</sub> 1700, 1710 cm<sup>-1</sup>,  $\nu_{\rm NH,OH}$  3200–3600 cm<sup>-1</sup>. HRMS  $(FAB^+)$   $(M+H)^+$  calcd for  $C_{25}H_{24}N_5O_7$  506.1676, found 506.1681. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>): 3.10 (3H, s, NCH<sub>3</sub>), 3.38 (1H, m), 3.49 (1H, d, *J*=7.7 Hz), 3.53 (2H, m), 3.77 (1H, m), 3.87 (1H, m), 4.95 (1H, t, J=4.0 Hz, OH), 5.19 (1H, d, J=4.3 Hz, OH), 5.28 (1H, d, J=3.4 Hz, OH), 5.33 (1H, d, J=4.6 Hz, OH), 5.89  $(1H, d, J=7.5 Hz, H_{1'}), 6.65 (1H, dd, J_1=6.5 Hz,$  $J_2 = 3.6$  Hz), 6.68 (1H, dd,  $J_1 = 6.6$  Hz,  $J_2 = 3.6$  Hz), 7,06 (1H, d, J=6, 4 Hz); 7,27 (1H, d, J=6, 4 Hz); 7,93 (1H, d, J=6, 4 Hz);s); 8,14 (1H, s); 8,16 (1H, d, J=3.6 Hz), 8.20 (1H, d, J=3.6 Hz), 12.34 (1H, s, NH). <sup>13</sup>C NMR (100 MHz, DMSO-*d*<sub>6</sub>): 24.1 (NCH<sub>3</sub>), 62.8 (C<sub>6'</sub>), 69.6, 72.0, 77.5,  $80.1, 82.4 (C_{1'}, C_{2'}, C_{3'}, C_{4'}, C_{5'}), 116.2, 116.7, 129.1,$ 129.3, 129.9, 130.1, 143.2, 143.4 (C tert arom), 104.0, 104.5, 117.6, 118.0, 125.9, 128.3, 147.7, 148.3 (C quat arom), 171.2 (2C) (C=O).

6-Methyl-12-(2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl)-12,13-dihydro-5*H*-pyrido[2,3-*b*]pyrido[3',2':4,5]pyrrolo[3,2g|pyrrolo[3,4-e|indole-5,7(6H)dione (9). A mixture of 7 (112 mg, 0.166 mmol) in benzene (150 mL) and iodine (537 mg, 2.11 mmol) was irradiated for 1.5 h with a medium pressure mercury lamp (400 W). The solvent was removed and the residue dissolved in EtOAc (50 mL), washed with aqueous sodium thiosulfite then with brine. The organic phase was dried over MgSO<sub>4</sub>, the solvent was removed and the residue purified by flash chromatography (eluent cyclohexane:EtOAc, 1:1) to give 9 (85 mg, 0.127 mmol, 76% yield) as a pale-yellow solid. Mp > 300 °C; IR (KBr)  $\nu_{CO}$  1703, 1757 cm<sup>-1</sup>,  $\nu_{NH}$ 3373 cm<sup>-1</sup>. HRMS (FAB<sup>+</sup>) (M+H)<sup>+</sup> calcd for C<sub>33</sub>H<sub>30</sub>N<sub>5</sub>O<sub>11</sub> 672.1942, found 672.1938. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>): 1.03 (3H, s), 1.90 (3H, s), 2.13 (3H, s), 2.65 (3H, s), 3.21 (3H, s, NCH<sub>3</sub>), 4.44 (1H, d, J=9.9 Hz), 4.48 (1H, d, J = 13.3 Hz), 4.87 (1H, d, J = 12.0 Hz), 5.31 (1H, pt, J = 9.3 Hz), 5.61 (1H, pt, J = 9.4 Hz), 5.70 (1H, pt, J=9.6 Hz), 6,87 (1H, d, J=9.5 Hz,  $H_{1'}$ ), 7,33 (1H, dd,  $J_1 = 7.9$  Hz,  $J_2 = 4.8$  Hz), 7.35 (1H, dd,  $J_1 = 7.8$ Hz, J<sub>2</sub>=4.9 Hz), 8.52 (1H, d, J=3.7 Hz), 8.56 (1H, d, J=3.6 Hz,), 9.25 (2H, d, J=7.7 Hz), 10.19 (1H, s, NH). <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>): 19.0, 20.5, 20.6, 21.1 (CH<sub>3</sub>CO), 23.8 (NCH<sub>3</sub>), 60.7 (C<sub>6'</sub>), 67.2, 70.5, 72.9, 76.5, 82.1 (C<sub>1'</sub>, C<sub>2'</sub>, C<sub>3'</sub>, C<sub>4'</sub>, C<sub>5'</sub>), 117.7, 118.3, 133.7, 134.0, 147.3, 148.1 (C tert arom), 114.8, 115.1, 116.7, 117.0, 120.0, 122.2, 127.0, 128.1, 152.1, 152.5 (C quat arom), 168.0, 169.2, 169.3, 169.4, 169,9, 171.8 (C=O).

6-Methyl-12-(β-D-glucopyranosyl)-12,13-dihydro-5H-pyrido[2,3-b]pyrido[3',2':4,5]pyrrolo[3,2-g]pyrrolo[3,4-e]indole-5,7(6H)dione (10). To a solution of 9 (44 mg, 0.066 mmol) in methanol (40 mL) was added 28% aqueous NH<sub>4</sub>OH (28 mL). The mixture was stirred for 26 h at 55 °C. After removal of the solvents, water and EtOAc were added to the residue. After filtration, the solid was washed with EtOAc then methanol. Compound 10 (5 mg, 9.54.10<sup>-3</sup> mmol, 14% yield) was obtained as a yellow solid: mp > 300 °C; IR (KBr)  $\nu_{CO}$  1650, 1700 cm<sup>-1</sup>.  $\nu_{\rm NH,OH}$  3200–3600 cm<sup>-1</sup>. HRMS (FAB<sup>+</sup>) (M+H)<sup>+</sup> calcd for C<sub>25</sub>H<sub>22</sub>N<sub>5</sub>O<sub>7</sub> 504.1519, found 504.1509. <sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>): 3.23 (3H, s, NCH<sub>3</sub>), 3.62 (2H, m), 3.83 (1H, d, J=10.3 Hz), 3.91 (1H, d, J=9.5 Hz), 4.04 (2H, d, J=11.9 Hz), 5.01 (1H, d, J=4.1 Hz, OH), 5.26 (1H, d, J=4.0 Hz, OH), 5.41 (1H, d, J=4.7 Hz, OH), 5;75 (1H, m, OH), 6.64 (1H, d, J=8.6 Hz,  $H_{1'}$ , 7.54 (2H, m), 8.69 (2H, br s), 9.32 (1H, d, J=8.2Hz), 9.35 (1H, d, J = 8.2 Hz), 11.66 (1H, br s, NH). <sup>13</sup>C NMR (100 MHz, DMSO-d<sub>6</sub>): 23.8 (NCH<sub>3</sub>), 58.2 (C<sub>6'</sub>),  $67.9, 72.8, 76.8, 79.1, 83.0 (C_{1'}, C_{2'}, C_{3'}, C_{4'}, C_{5'}), 117.3,$ 117.6, 132.5, 132.8, 147.5, 148.4 (C tert arom), 114.0 (2C), 115.6, 116.1, 119.9, 121.0, 127.2, 128.3, 152.3, 152.7 (C quat arom), 169.4 (2C) (C=O).

1-[(Benzyloxy)methyl]-3-bromo-4-(1*H*-pyrrolo[2,3-*b*]pyridin-3-yl]-1*H*-pyrrole-2,5-dione (11). A solution of ethylmagnesium bromide was prepared from Mg (146 mg, 6.00 mmol) and  $C_2H_3Br$  (452 µL, 6.00 mmol) in THF (2.5 mL). The mixture was stirred for 1 h at room temperature then 7-azaindole (708 mg, 6.00 mmol) in toluene (20 mL) was added dropwise. The mixture was stirred at room temperature for 1.5 h then a solution of N-benzyloxymethyl-2,3-dibromomaleimide (756 mg, 2.01 mmol) in toluene (20 mL) was added dropwise. After stirring for 20 min, dichloromethane (30 mL) was added then the mixture was stirred for 65 h at 40 °C. Saturated aqueous NH<sub>4</sub>Cl was added. After extraction with EtOAc, the organic phase was dried over MgSO<sub>4</sub>, the solvent was removed and the residue purified by flash chromatography (eluent cyclohexane/EtOAc, 3:2, then toluene/ EtOAc, 7:3) to give 11 (471 mg, 1.14 mmol, 57% yield) as a yellow solid. Mp 168–170 °C. IR (KBr) v<sub>CO</sub> 1714, 1774 cm<sup>-1</sup>, v<sub>NH</sub> 3340–3500 cm<sup>-1</sup>. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) 4.70 (2H, s, CH<sub>2</sub>), 5.20 (2H, s, CH<sub>2</sub>), 7.23-7.40 (6H, m), 8.37 (1H, s), 8.48 (1H, d, J=3.8 Hz), 8.56 (1H, dd, J<sub>1</sub>=7.9 Hz,  $J_2 = 0.9$  Hz), 13.26 (1H, s, NH). <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) 67.7, 71.9 (CH<sub>2</sub>), 117.4, 127.7 (2C), 127.9, 128.5 (2C), 131.5, 132.6, 143.5 (C tert arom), 103.8, 115.7, 118.3, 137.1, 137.4, 149.2 (C quat arom), 166.1, 168.9 (C=O).

1-[(Benzyloxy)methyl]-3-bromo-4-[1-(phenylsulphonyl)-1Hpyrrolo[2,3-b]pyridin-3-yl]-1H-pyrrole-2,5-dione (12). To a suspension of HNa (60% in mineral oil) (51 mg, 1.27 mmol) in THF (5 mmol) at 0 °C was added dropwise a solution of 11 (330 mg, 0.800 mmol) in THF (10 mL). The mixture was stirred at 0 °C for 1 h, then benzenesulphonyl chloride (125 µL, 0.98 mmol) was added dropwise. The mixture was stirred at room temperature for 4 h, then saturated aqueous NH<sub>4</sub>Cl was added. After extraction with EtOAc, the organic phase was washed with brine, dried over MgSO<sub>4</sub>, the solvent was removed and the residue purified by flash chromatography (eluent cyclohexane/EtOAc, 4:1) to give 12 (333 mg, 0.60 mmol, 75% yield) as a yellow solid. Mp 123–125 °C. IR (KBr)  $\nu_{\rm CO}$  1730, 1780 cm<sup>-1</sup>. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) 4.68 (2H, s, CH<sub>2</sub>), 5.18 (2H, s, CH<sub>2</sub>), 7.24-7.37 (6H, m), 7.55 (2H, t, J = 7.5 Hz), 7.65 (1H, t, J = 7.5 Hz), 8.24 (1H, dd, dd)J<sub>1</sub>=8.0 Hz, J<sub>2</sub>=1.3 Hz), 8.29 (1H, br s), 8.30 (1H, br s), 8.48 (1H, s), 8.51 (1H, dd,  $J_1 = 4.7$  Hz,  $J_2 = 1.3$  Hz). <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) 67.9, 72.1 (CH<sub>2</sub>), 119.6, 127.6 (2C), 128.0, 128.5 (3C), 128.6 (2C), 129.3 (2C), 132.0, 134.8, 146.0 (C tert arom), 107.0, 119.8, 121.5, 135.4, 137.3, 137.5, 146.9 (C quat arom), 165.2, 167.9 (C=O).

1-[(Benzyloxy)methyl-3]1-(phenylsulphonyl)-1H-pyrrolo]2,3b]pyridin-3-yl]-4(1H-pyrrolo[2,3-b]pyridin-3-yl)-1H-pyrrole-2,5-dione (13) and 1-[(benzyloxy)methyl-3(1H-pyrrolo[2,3-b]pyridin-3-yl)-4(1H-pyrrolo[2,3-b]pyridin-3-yl)-1Hpyrrole-2,5-dione (14). To a solution of 7-azaindole (155 mg, 1.36 mmol) in toluene (10 mL) at -15 °C was added dropwise a solution of LiHMDS (1M in hexane) (1.52 mL, 1.52 mmol). The solution was stirred at -15 °C for 1 h then a solution of 12 (300 mg, 0.543 mmol) in toluene (10 mL) was added dropwise at -20 °C. The mixture was stirred at room temperature for 24 h before addition of saturated aqueous NH<sub>4</sub>Cl, then the pH was adjusted to 7. After extraction with EtOAc, the organic phase was washed with brine, dried over MgSO<sub>4</sub>, the solvent was removed and the residue purified by flash chromatography (eluent cyclohexane/EtOAc, 3:2) to give 13 (99 mg, 0.168 mmol, 31% yield) and 14 (24 mg, 0.053 mmol, 10% yield) as orange solids.

**13**: mp 115–117 °C; IR (KBr)  $\nu_{CO}$  1707, 1730 cm<sup>-1</sup>,  $\nu_{NH}$  3300–3400 cm<sup>-1</sup>. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) 4.73

(2H, s, CH<sub>2</sub>), 5.24 (2H, s, CH<sub>2</sub>), 6.61 (1H, dd,  $J_1$ =8.1 Hz,  $J_2$ =4.8 Hz), 6.86 (1H, dd,  $J_1$ =8.0 Hz,  $J_2$ =4.7 Hz), 7.03 (1H, dd,  $J_1$ =8.0 Hz,  $J_2$ =1.2 Hz), 7.19–7.31 (4H, m), 7.39 (2H, dd,  $J_1$ =8.4 Hz,  $J_2$ =1.3 Hz), 7.53 (2H, t, J=7.6 Hz), 7.64 (1H, dt,  $J_1$ =7.5 Hz,  $J_2$ =1.2 Hz), 8.17 (2H, br s), 8.21–8.25 (3H, m), 8.36 (1H, dd,  $J_1$ =4.7 Hz,  $J_2$ =1.5 Hz), 12.93 (1H, s, NH). <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) 67.5, 71.9 (CH<sub>2</sub>), 116.8, 119.1, 127.6, 127.8, 128.3 (2C), 128.4 (2C), 128.6, 128.8, 129.2, 129.8, 130.3, 130.6, 131.2, 134.5, 143.3, 145.7 (C *tert* arom), 104.3, 108.9, 118.0, 120.7, 123.9, 131.4, 137.7, 137.8, 146.8, 148.9 (C quat arom), 170.4, 170.7 (C=O).

14: mp 239–241 °C. IR (KBr)  $\nu_{CO}$  1710, 1760 cm<sup>-1</sup>,  $\nu_{NH}$ 3300–3600 cm<sup>-1</sup>. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) 4.67 (2H, s, CH<sub>2</sub>), 5.12 (2H, s, CH<sub>2</sub>), 6.77 (2H, dd, *J*<sub>1</sub> = 7.6 Hz, *J*<sub>2</sub> = 4.7 Hz), 7.10 (2H, d, *J* = 7.8 Hz), 7.27–7.39 (5H, m), 7.97 (2H, s), 8.16 (2H, d, *J* = 3.8 Hz), 12.39 (2H, s, NH). <sup>13</sup>C NMR (100 MHz, DMSO-*d*<sub>6</sub>) 67.1, 70.5 (CH<sub>2</sub>), 115.9 (2C), 127.4 (2C), 127.5 (2C), 128.2 (2C), 128.9 (2C), 129.9, 143.4 (2C) (C *tert* arom), 103.8 (2C), 117.4 (2C), 127.1, 137.9 (2C), 148.4 (2C) (C quat arom), 170.9 (2C) (C=O).

1-[(Benzyloxy)methyl]-3-[1-(2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl)-1H-pyrrolo[2,3-b]pyridin-3-yl]-4-[1-(phenylsulphonyl)-1H-pyrrolo[2,3-b]pyridin-3-yl]-1H-pyrrole-2,5dione (15). To a solution of 13 (356 mg, 0.604 mmol) in THF (30 mL) were added 2,3,4,6-O-acetylglucopyranose (372 mg, 1.09 mmol) and triphenylphosphine (285 mg, 1.09 mmol). The mixture was cooled to -78 °C then DEAD (174 µL, 1.09 mmol) was added dropwise. The mixture was stirred at room temperature for 15 h. Water was added. After extraction with EtOAc, the organic phase was dried over MgSO<sub>4</sub>, the solvent was removed and the residue purified by flash chromatography (eluent cyclohexane/EtOAc, 1:1, then toluene/ EtOAc, 3:2) to give  $\beta$ -glycosylated compound 15 (377) mg, 0.410 mmol, 68% yield) as a yellow solid. Mp 108-110 °C. IR (KBr)  $\nu_{CO}$  1720, 1760 cm<sup>-1</sup>. HRMS (FAB<sup>+</sup>) calcd C<sub>46</sub>H<sub>42</sub>N<sub>5</sub>O<sub>14</sub>S 920.2449,  $(M + H)^{+}$ found 920.2443. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>): 1.62 (3H, s, CH<sub>3</sub>CO), 1.99 (3H, s, CH<sub>3</sub>CO), 2.03 (3H, s, CH<sub>3</sub>CO), 2.04 (3H, s, CH<sub>3</sub>CO), 4.05 (1H, m), 4.15 (1H, d, J=11,2 Hz), 4.28 (1H, dd,  $J_1 = 12.6$  Hz,  $J_2 = 4.6$  Hz), 4.65 (2H, s, CH<sub>2</sub>), 5.17 (2H, s, CH<sub>2</sub>), 5.27 (1H, m), 5.45-5.52 (2H, m), 6.24 (1H, m), 6.51 (1H, m), 6.78–6.84 (2H, m), 7.13 (1H, t, J=7.2 Hz), 7.19-7.25 (3H, m), 7.31 (2H, d, m)J = 7.4 Hz), 7.46 (2H, t, J = 7.9 Hz), 7.57 (1H, t, J = 7.4Hz), 8.06 (1H, br s), 8.11-8.14 (3H, m), 8.13 (1H, s), 8.26 (1H, d, J = 4.4 Hz). <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>): 19.9, 20.3 (2C), 20.4 (CH<sub>3</sub>CO), 61.6 (C<sub>6</sub>), 67.3, 71.6 (CH<sub>2</sub>), 67.9, 71.0, 72.7, 74.7, 80.3 (C<sub>1</sub>', C<sub>2</sub>', C<sub>3</sub>', C<sub>4</sub>', C<sub>5</sub>'), 117.6, 118.9, 128.0 (2C), 128.2 (2C), 128.6 (2C), 129.0 (2C), 129.6 (2C), 129.7, 130.2, 131.8, 134.3, 144.0, 145.4 (C tert arom), 105.3, 108.5, 117.7, 120.5, 125.0, 130.6, 137.5 (2C), 146.6, 147.5 (C quat arom), 168.6, 169.3, 169.7, 169.9, 170.0, 170.4 (C=O).

1-[(Benzyloxy)methyl]-3-[1-(2,3,4,6-tetra-O-acetyl- $\beta$ -D-glucopyranosyl)-1*H*-pyrrolo[2,3-*b*]pyridin-3-yl]-4-(1*H*pyrrolo[2,3-*b*]pyridin-3-yl)-1*H*-pyrrole-2,5-dione (16). To a solution of  $\beta$ -glycosylated compound 15 (154 mg, 0.168 mmol) in THF (6 mL) was added a solution of tetrabutylammonium fluoride (1.1 M in THF) (457  $\mu$ L, 0.503 mmol). The mixture was stirred for 3 h at room temperature. Water was added. After extraction with EtOAc, the organic phase was dried over MgSO<sub>4</sub>, the solvent was removed and the residue purified by flash chromatography (eluent cyclohexane/EtOAc, 2:3) to give 16 (100 mg, 0.128 mmol, 76% yield) as an orange solid. Mp 127–129 °C; IR (KBr) v<sub>CO</sub> 1710, 1750 cm<sup>-1</sup>,  $\nu_{\rm NH}$  3300–3500 cm<sup>-1</sup>. HRMS (FAB<sup>+</sup>) (M+H)<sup>+</sup> calcd  $C_{40}H_{38}N_5O_{12}\,$  780.2517, found 780.2518.  $^1H\,$  NMR (400 MHz, CDCl<sub>3</sub>): 1.71 (3H, s), 2.03 (3H, s), 2.07 (3H, s), 2.09 (3H, s), 4.09 (1H, m), 4.18 (1H, d, J=12.3 Hz), 4.30 (1H, dd,  $J_1 = 12,6$  Hz,  $J_2 = 4,6$  Hz); 4,71 (2H, s, CH<sub>2</sub>); 5,22 (2H, s, CH<sub>2</sub>); 5,31 (1H, t, J=9,6 Hz), 5.53 (1H, pt, J=9.1 Hz), 5.59 (1H, t, J=9.4 Hz), 6.31 (1H, d, J=8.8 Hz,  $H_{1'}$ ), 6.69 (1H, t, 4.3 Hz), 6.72 (1H, t, 4.3 Hz), 7.15 (2H, t, J=7.5 Hz), 7.21 (1H, d, J=7.1 Hz), 7.28 (2H, t, J=7.3 Hz), 7.38 (2H, d, J=7.4 Hz), 8.07 (1H, s), 8.13 (1H, s), 8.20 (2H, dd,  $J_1 = 9.4$  Hz,  $J_2 = 4.3$ Hz), 12.78 (1H, s, NH). <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>): 20.1, 20.6, 20.7, 21.0 (CH<sub>3</sub>CO), 61.8 (C<sub>6'</sub>), 67.3, 71.6  $(CH_2)$ , 68.1, 70.9, 73.1, 74.8, 80.3  $(C_{1'}, C_{2'}, C_{3'}, C_{4'}, C_{5'})$ , 116.4, 117.5, 127.6 (3C), 127.7 (2C), 128.3 (2C), 130.3, 130.5, 142.9, 143.8 (C tert arom), 104.6, 106.3, 118.5, 118.8, 126.0, 128.6, 137.6, 147.5, 148.7 (C quat arom), 168.8, 169.5, 170.0, 170.6, 170.9, 171.1 (C=O).

6-[(Benzyloxy)methyl]-12-(2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl)-12,13-dihydro-5H-pyrido[2,3-b]pyrido[3',2':4,5]pyrrolo[3,2-g]-pyrrolo[3,4-e]indole-5,7(6H)dione (17). A solution of 16 (96 mg, 0.123 mmol) and iodine (376 mg, 1,48 mmol) in benzene (150 mL) was irradiated for 1.5 h with a medium pressure mercury lamp (400 W). The solvent was removed and the residue dissolved in EtOAc (50 mL), washed with aqueous sodium thiosulfite then with brine. The organic phase was dried over MgSO<sub>4</sub>, the solvent was removed and the residue purified by flash chromatography (eluent cyclohexane/EtOAc, 2:3) to give 17 (73 mg, 0.094 mmol, 76% yield) as a pale-yellow solid. Mp 194–196 °C; IR (KBr)  $\nu_{\rm CO}$  1690, 1730 cm<sup>-1</sup>,  $\nu_{\rm NH}$  3300–  $cm^{-1}$ . HRMS (FAB<sup>+</sup>) (M+H)<sup>+</sup> 3400 calcd C40H36N5O12 778.2360, found 778.2388. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>): 1.08 (3H, s), 1.93 (3H, s), 2.14 (3H, s), 2.65 (3H, s), 4.39 (1H, dd, J<sub>1</sub> = 10.2 Hz, J<sub>2</sub> = 1.9 Hz), 4.44 (1H, dd,  $J_1 = 12.8$  Hz,  $J_2 = 1.7$  Hz), 4.79 (2H, AB system, J = 12.2 Hz,  $\Delta v = 12.1$  Hz), 4.85 (1H, dd,  $J_1 = 13.1 \text{ Hz}, J_2 = 2.0 \text{ Hz}$ , 5,30 (2H, AB system, J = 11.0Hz,  $\Delta v = 8.9$  Hz), 5.36 (1H, t, J = 9.4 Hz), 5.60 (1H, t, J=9.4 Hz), 5.71 (1H, t, J=9.6 Hz), 6.93 (1H, d, J=9.5 Hz, H<sub>1</sub>'), 7.25 (1H, m), 7.32–7.42 (4H, m), 7.48 (2H, dd,  $J_1 = 7.1$  Hz,  $J_2 = 1.3$  Hz), 8.54 (1H, dd,  $J_1 = 4.8$  Hz,  $J_2 = 1.6$  Hz), 8.62 (1H, dd,  $J_1 = 4.6$  Hz,  $J_2 = 1.5$  Hz), 9.32 (1H, dd,  $J_1 = 7.9$  Hz,  $J_2 = 1.8$  Hz), 9.34 (1H, dd,  $J_1 = 7.9$ Hz,  $J_2 = 1.5$  Hz), 10.30 (1H, s, NH). <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>): 19.1, 20.5, 20.6, 21.1 (CH<sub>3</sub>CO), 60.6 (C<sub>6'</sub>), 66.9, 71.7 (CH<sub>2</sub>), 67.2, 70.6, 72.9, 76.5, 82.1 (C<sub>1'</sub>,  $C_{2'}, C_{3'}, C_{4'}, C_{5'}$ , 117.8, 118.4, 127.9 (3C), 128.0 (2C), 133.8, 134.2, 147.4, 148.3 (C tert arom), 114.8, 115.1, 116.9, 117.2, 119.7, 121.9, 127.4, 128.4, 137.6, 152.0, 152.5 (C quat arom), 168.0, 168.9, 169.3, 169.9, 171.8 (2C) (C=O).

6-(Hydroxy)methyl-12-(2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl)-12,13-dihydro-5H-pyrido[2,3-b]pyrido[3',2':4,5] pyrrolo[3,2-g]-pyrrolo[3,4-e]indole-5,7(6H)-dione (18). A mixture of 17 (70 mg, 0.090 mmol) and 10% Pd/C (84 mg) in methanol (4.5 mL) and EtOAc (1.5 mL) was hydrogenated for 24 h (1 bar). After 24 h, 10% Pd/C (42 mg) was added and the mixture was hydrogenated for 24 h (1 bar). After filtration over Celite, the solid was washed with methanol and CHCl<sub>3</sub>. After removal of the solvents, purification of the residue by flash chromatography (cyclohexane/EtOAc, 3:2), gave the starting product 17 (19 mg, 0.024 mmol, 27%), compound 18 (cyclohexane/EtOAc, 1:1) (9 mg, 0.013 mmol, 15% yield) as a pale-yellow solid and a mixture of compounds partially deacetylated (41 mg) (EtOAc/methanol, 9:1).

**18**: mp > 300 °C; IR (KBr)  $\nu_{CO}$  1710, 1760 cm<sup>-1</sup>  $\nu_{\rm NH} = 3300 - 3600 \text{ cm}^{-1}$ . HRMS (FAB<sup>+</sup>) (M+H)<sup>+</sup> calcd C<sub>33</sub>H<sub>30</sub>N<sub>5</sub>O<sub>12</sub> 688.1891, found 688.1898. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>: 0.85 (3H, s), 1.83 (3H, s), 2.12 (3H, s), 2.69 (3H, s), 4.50 (1H, d, J=9.8 Hz), 4.81-4.95 (3H, m, 2H + OH), 5.04 (1H, d, J = 12.6 Hz), 5.23 (1H, d, J = 10.8 Hz), 5.32 (1H, dd,  $J_1 = 11.5$  Hz,  $J_2 = 4.5$  Hz), 5.49 (1H, t, J=9.3 Hz), 5.57 (1H, t, J=9.6 Hz), 6.72 (1H, d, J=9.5 Hz,  $H_{1'}$ ), 7.26 (1H, dd,  $J_1=7.6$  Hz,  $J_2 = 4.9$  Hz), 7.48 (1H, dd,  $J_1 = 7.8$  Hz,  $J_2 = 4.8$  Hz), 8.30 (1H, d, J=4.4 Hz), 8.71 (1H, d, J=4.7 Hz), 8.82 (1H, d, J=7.8 Hz), 9.18 (1H, d, J=7.8 Hz), 9.85 (1H, s, NH). <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>): 18.8, 20.5, 20.6, 21.4 (CH<sub>3</sub>CO), 60.7, 60.9 (CH<sub>2</sub>OH, C<sub>6</sub>), 67.0, 70.2, 72.9, 76.5, 82.6 (C<sub>1'</sub>, C<sub>2'</sub>, C<sub>3'</sub>, C<sub>4'</sub>, C<sub>5'</sub>), 117.8, 118.5, 132.9, 135.1, 147.9, 148.2 (C tert arom), 113.8, 115.4, 116.5, 118.1, 121.9, 126.3, 127.7, 128.6, 151.6, 151.7 (C quat arom), 167.6, 167.7, 168.0, 169.3, 169.9, 172.2 (C=O).

12-(β-<sub>D</sub>-Glucopyranosyl)-12,13-dihydro-5*H*-pyrido[2,3b]pyrido[3',2':4,5]pyrrolo[3,2-g]-pyrrolo[3,4-e]indole-5,7(6H)-dione (19). A solution of 18 (36 mg, 0.053 mmol) and 28% aqueous NH<sub>4</sub>OH (13 mL) in methanol (15 mL) was stirred for 21 h at 40 °C. The solvents were removed, water and EtOAc were added to the residue. After filtration, the solid was washed with EtOAc then methanol. Compound 19 was obtained (8 mg, 0.016 mmol, 30% yield) as a yellow-brown solid. Mp > 300 °C; IR (KBr)  $\nu_{CO}$  1710, 1760 cm<sup>-1</sup>,  $\nu_{NH,OH}$  3200–3600  $cm^{-1}$ . HRMS (FAB<sup>+</sup>) (M+H)<sup>+</sup> calcd C<sub>24</sub>H<sub>20</sub>N<sub>5</sub>O<sub>7</sub> 490.1363, found 490.1369. <sup>1</sup>H NMR (400 MHz, DMSO $d_6$ ): 3.54–3.68 (2H, m), 3.82 (1H, d, J = 10.3 Hz), 3.91 (1H, d, J=9.2 Hz), 4.03 (2H, d, J=9.2 Hz), 5.01 (1H, br s, OH), 5.26 (1H, br s, OH), 5.41 (1H, d, J=4.6 Hz, OH), 5.73 (1H, br s, OH), 6.65 (1H, d, J = 8.6 Hz,  $H_{1'}$ ), 7.57 (2H, m), 8.69 (2H, m), 9.34 (1H, d, J=8.1 Hz), 9.37 (1H, d, J=7.9 Hz), 11.26 (1H, s, NH), 11.66 (1H, s, s)NH). <sup>13</sup>C NMR (100 MHz, DMSO- $d_6$ ): 58.2 (C<sub>6</sub>), 67.9, 72.8, 76.8, 79.1, 83.0 ( $C_{1'}$ ,  $C_{2'}$ ,  $C_{3'}$ ,  $C_{4'}$ ,  $C_{5'}$ ), 117.2, 117.6, 132.6, 132.9, 147.5, 148.3 (C tert arom), 114.0 (2C), 115.5, 115.7, 120.9, 122.0, 128.1, 129.8, 153.1, 153.3 (C quat arom), 170.7 (2C) (C=O).

Growth inhibition assays. Tumor cells were provided by American Type Culture Collection (Frederik, MD, USA). Nine cell lines were used: one murine leukemia (L1210), one human leukemia (K-562) and seven human solid tumors: one ovarian carcinoma (IGROV1), one neuroblastoma (SK-N-MC), one colon carcinoma (HT29), one non-small cell lung carcinoma (A549), one small-cell lung carcinoma (H69) and two epidermoid carcinomas (A431 and KB-3-1). They were cultivated in RPMI 1640 medium (Life Science technologies, Cergy-Pontoise, France) supplemented with 10% fetal calf serum, 2 mM L-glutamine, 100 units/mL penicillin, 100 µg/mL streptomycin, and 10 mM HEPES buffer (pH = 7.4). Cytotoxicity was measured by the microculture tetrazolium assay as described.22 Cells were continuously exposed to graded concentrations of the compounds for four doubling times, then 15 µL of 5 mg/mL 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide were added to each well and the plates were incubated for 4 h at 37 °C. The medium was then aspirated and the formazan solubilized by 100  $\mu$ L of DMSO. Results are expressed as  $IC_{50}$ , concentration which reduced by 50% the optical density of treated cells with respect to untreated controls.

Cell cycle analysis. For the cell cycle analysis, L1210 cells  $(2.5 \times 10^5 \text{ cells/mL})$  were incubated for 21 h with various concentrations of the compounds, then fixed by 70% ethanol (v/v), washed and incubated in PBS containing 100 µg/mL RNAse and 25 µg/mL propidium iodide for 30 min at 20 °C. For each sample, 10<sup>4</sup> cells were analyzed on a XL/MCL flow cytometer (Beckman Coulter). The fluorescence of propidium iodide was collected through a 615 nm long-pass filter. Results are expressed as the highest% of cells accumulated in the G2+M phases of the cell cycle by a non-toxic (no cell lysis) concentration.

#### **References and Notes**

- 1. Nettleton, D. E.; Doyle, T. W.; Krishnan, B.; Matsumoto, G. K.; Clardy, J. *Tetrahedron Lett.* **1985**, *26*, 4011.
- 2. Bush, J. A.; Long, B. H.; Catino, J. J.; Bradner, W. T.; Tomita, K. J. Antibiot. **1987**, 40, 668.
- 3. Anizon, F.; Belin, L.; Moreau, P.; Sancelme, M.; Voldoire,
- A.; Prudhomme, M.; Ollier, M.; Sevère, D.; Riou, J-F.; Bailly,
- C.; Fabbro, D.; Meyer, T. J. Med. Chem. 1997, 40, 3456.
- 4. Moreau, P.; Anizon, F; Sancelme, M.; Prudhomme, M.; Bailly, C.; Carrasco, C.; Ollier, M.; Sevère, D.; Riou, J.-F.; Fabbro, D.; Meyer, T.; Aubertin, A.-M. *J. Med. Chem.* **1998**, *41*, 1631.
- 5. Kaneko, T.; Wong, H.; Utzig, J.; Schurig, J.; Doyle, T. W.
- J. Antibiot. 1990, 43, 125.
- 6. Prudhomme, M. Curr. Med. Chem. 2000, 7, 1189.
- 7. Voldoire, A.; Sancelme, M.; Prudhomme, M.; Colson, P.; Houssier, C.; Bailly, C.; Leonce, S.; Lambel, S. *Bioorg. Med. Chem.* **2001**, *9*, 357.
- 8. Bailly, C.; Qu, X.; Anizon, F.; Prudhomme, M.; Riou, J.-F.; Chaires, J. *Mol. Pharmacol.* **1999**, *55*, 377.
- 9. Alvarez, M.; Fernandez, D.; Joule, J. A. *Synthesis* **1999**, *4*, 615. 10. Chi, S. M.; Choi, J.-K.; Yum, E. K.; Chi, D. Y. Tetrahedron Lett. **2000**, *41*, 919.
- 11. Mérour, J.-Y.; Joseph, B. Curr. Org. Chem. 2001, 5, 471.
- 12. Levacher, V.; Benoît, R.; Duflos, J.; Dupas, G.; Bour-
- guignon, J.; Queguigner, G. Tetrahedron 1991, 47, 429.
- 13. Löber, S.; Hübner, H.; Gmeiner, P. Bioorg. Med. Chem. 1999, 9, 97.

- 14. Mazéas, D.; Guillaumet, G.; Viaud, M.-C. *Heterocycles* 1999, 50, 1065.
- 15. Robinson, M. M.; Robinson, B. L. J. Am. Chem. Soc. 1955, 77, 457.
- 16. Robinson, M. M.; Robinson, B. L. J. Am. Chem. Soc. 1956, 78, 1247.
- 17. Brenner, M.; Rexhausen, H.; Steffan, B.; Steiglich, W. Tetrahedron 1988, 44, 2887.
- 18. Ciamician, G. L.; Silber, P. Chem. Ber. 1884, 17, 1743.
- 19. Routier, S.; Coudert, G.; Merour, J. Y.; Caignard, D. H. *Tetrahedron Lett.* **2002**, *43*, 2561.
- 20. Prudhomme, M.; Anizon, F.; Moreau, P. Recent Res. Devel. Synth. Organic. Chem. 1999, 2, 79.
- 21. Bayle, C.; Defaye, J.; Horton, D.; Lehman, J.; Scheuring, M. Carbohydrate Res. **1992**, 232, 375.
- 22. Léonce, S.; Pérez, V.; Casabianca-Pignède, M. R.; Anstett, M.; Bisagni, E.; Atassi, G. *Invest. New Drugs* **1996**, *14*, 169.