Syntheses and Antiproliferative Activities of 7-Azarebeccamycin Analogues Bearing One 7-Azaindole Moiety

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Rebeccamycin analogues containing one azaindole unit, with and without a methyl group on the imide nitrogen and with the sugar moiety coupled either to the indole nitrogen or to the azaindole nitrogen were synthesized. To increase the solubility and induce stronger interactions with the target macromolecules, a bromo or nitro substitutent was introduced on the indole unit. The DNA binding and topoisomerase I inhibition properties were investigated together with the antiproliferative activities toward nine tumor cell lines. In addition, the effect of the compounds on the cell cycle of L1210 leukemia cells was examined. The nonaza analogues were found to be cytotoxic against all cell lines of the panel whereas the aza-analogues showed a selective action toward certain cell lines. They strongly inhibited the proliferation of SK-N-MC neuroblastoma, A431 epidermoid carcinoma and NCI-H69 small cell lung carcinoma cells, but showed little or no cytotoxic effect against IGROV ovary carcinoma, HT29 colon carcinoma, and A549 non small cell lung carcinoma cells. Whatever their cytotoxicity profile, all compounds induce similar cell cycle effects, with a marked G2+M block observed with L1210 leukemia cells. The data suggest that the molecular mechanism of action of the aza-analogue derivatives is different from that of rebeccamycin.

Introduction

Indolocarbazole compounds bearing a carbohydrate moiety attached to one of the indole nitrogens have been largely studied for their antiproliferative activities and their ability to inhibit topoisomerase I.[1] These compounds are related to the antibiotics BE-13793C and rebeccamycin. Extensive structure−activity relationships studies have led to the development of analogues which are currently undergoing clinical trials, in particular NB-506, a glycosylated derivative of BE-13793C, and the rebeccamycin derivative NCS655649.[2−11] Our previous studies on rebeccamycin analogues have revealed that topoisomerase is a privileged but not unique target for these drugs. The structural analogy with staurosporine, a nonselective kinases inhibitor which interacts with the ATP binding site of the enzymes,[12] raises the possibility that some of the rebeccamycin analogues could also function as protein kinase inhibitors.

In this paper, we report the syntheses of rebeccamycin analogues bearing a azaindole unit and a carbohydrate residue attached either to the azaindole nitrogen (compounds **10**, **11**, **22**−**24**, Figure 1) or the indole nitrogen (compounds **32** and **14**, Figure 1). The compounds were synthesized from 7-azaindole which is commercially available. The bromo compounds **11** and **23** and nitro compound **24** have been prepared with the aim of enhancing the water solubility. Rebeccamycin is known as a nonselective cytotoxic agent, and it is too toxic to be used in the clinic.[11] To investigate whether the compounds could present some degree of selectivity against certain cell lines, the newly designed compounds were tested on a panel of nine tumor cell lines including seven human solid tumors. In an attempt to gain information on their mechanism of action, the effect of the compounds on the cell cycle was studied. The activities of the new compounds were compared with their parent compounds, rebeccamycin **A**, dechlorinated rebeccamycin **B**, and a synthetic compound **C**,[16] methylated at the imide nitrogen.

Results and Discussion

Chemistry. The synthesis of compound **10** bearing a methyl group at the imide nitrogen is outlined in Scheme 1. Aglycone **5** was prepared according to the classical method[13] modified by Routier et al.[14] to introduce a 7-azazindole. Indolylmagnesium bromide was coupled to *N*-methyldibromomaleimide, and then the indole nitrogen was protected with a *tert*-butyloxycarbonyl group (Boc) before a second nucleophilic attack by lithiated azaindole. Oxidative cyclization of compound **3** in the presence of iodine led to carbazole **4** which was further deprotected using tetrabutylammonium fluoride (TBAF) to yield aglycone **5**. Various methods of *N*-glycosylations of indole or indolocarbazole moieties are described in the literature:[15−22] (i) coupling of indolocarbazoles to an α-D-glucopyranosyl bromide via

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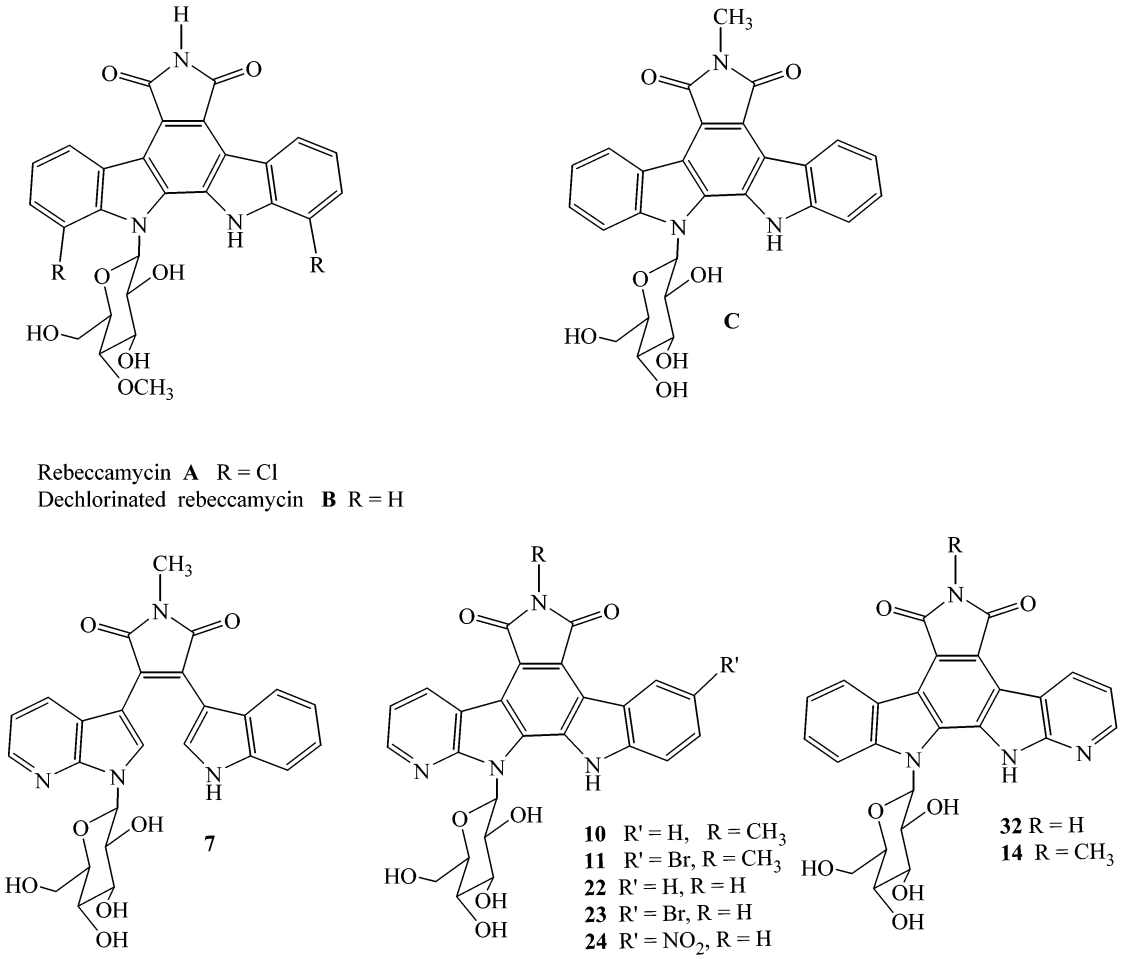


Figure 1.

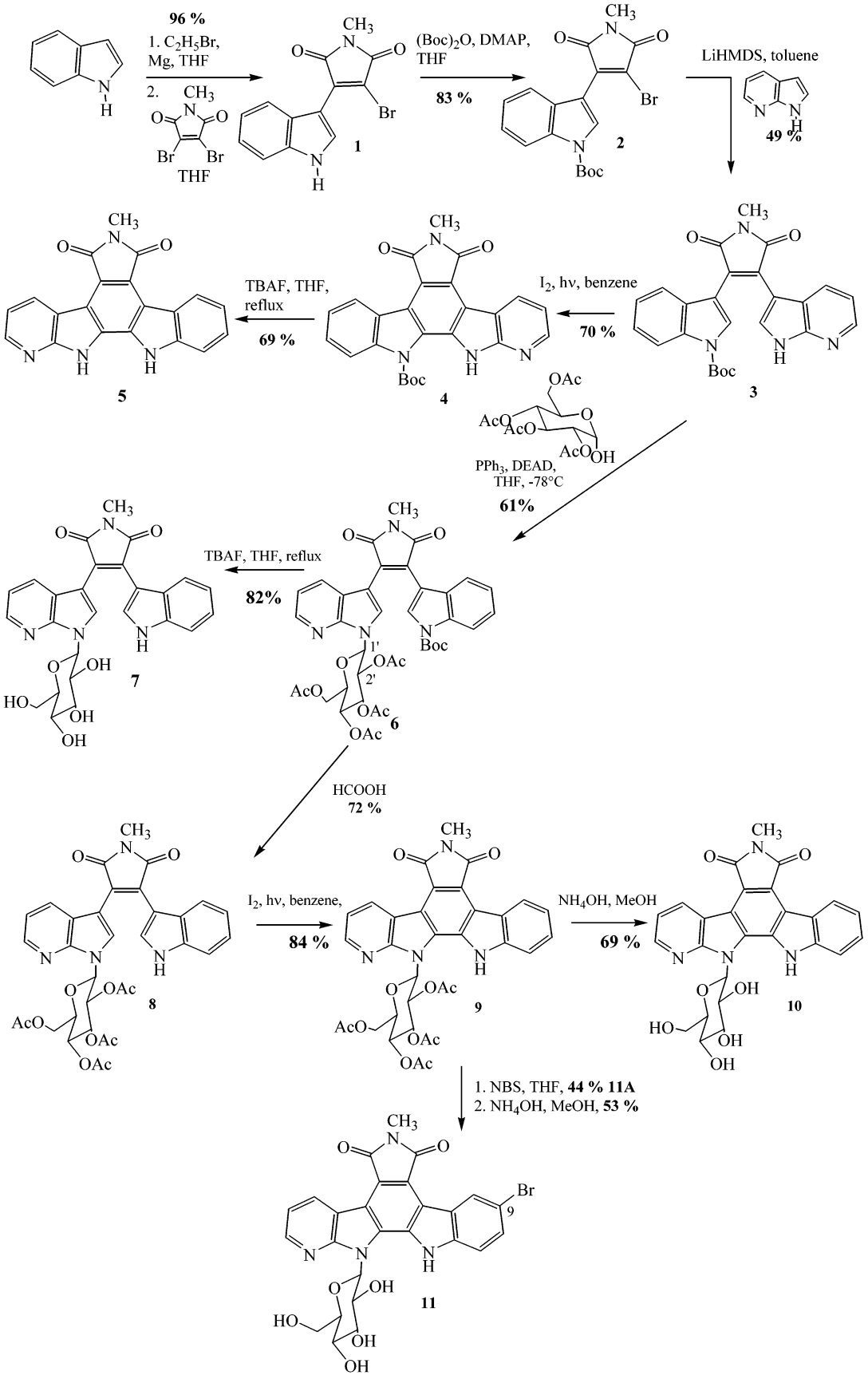
the Koenigs−Knorr method but it provides essentially α-*N*-glycosylated compounds; (ii) coupling of an anhydrosugar to an indole or bis-indole moiety and in this case, the *β*-*N*-glycosylated compound is favored; (iii) reaction in heterogeneous basic medium with an indolocarbazole and α-tetra-*O*-benzyl-glucopyranosyl chloride. This reaction is highly stereoselective, with the *β*-*N*-glycosylated compound as the major product; (iv) a Mitsunobu reaction for the coupling of a bis-indole or an indole with α-2,3,4,6-tetra-*O*-benzyl-glucose. In this case, the *β*-*N*-glycosylated compound is favored but an electron-withdrawing group is required on the substrate.

Compound **5** been particularly insoluble, a Mitsunobu reaction was first performed with compound **3** using α-2,3,4,6-tetra-*O*-acetyl glucose prepared according to the method described by Bayle et al.[23] in the presence of diethylazodicarboxylate (DEAD) and triphenylphosphine. *β*-*N*-Glycosylated **6** was obtained as the major product of the reaction in 61% yield. Its stereochemistry was assigned from the coupling constant $J_{1'-2'}$. The value (9 Hz) is in agreement with an axial−axial coupling. Treatment of **6** with TBAF led to compound **7** with simultaneous removal of the Boc group and the acetyl groups of the sugar moiety. Due to the insolubility of **7**, the presence of the acetyl groups on the carbohydrate part were required for further steps. Consequently, an alternative method of deprotection of the indole nitrogen was investigated. Removal of the Boc protecting group only was achieved using formic acid leading to **8**. Oxidative cyclization of **8** by irradiation in the presence of iodine yielded **9**, and the aminolysis of this compound gave the required rebeccamycin analogue **10**. To improve the solubility and/or to increase the stability of the complex drug-target macromolecule, bromination of **9** was performed with *N*-bromosuccinimide followed by regeneration of the hydroxyl functions of the sugar. In rebeccamycin analogues of previous series,[7] bromination was found to enhance both topoisomerase I inhibitory potency and in vitro antiproliferative activity. Bromination occurred at position 9 as already observed in the previous series and only on the indole moiety yielding **11**.

N-Glycosylation was performed from aglycone **5** in heterogeneous medium according to the method described by Ohkubo et al.[19,20] with tetra-*O*-benzyl-glucopyrasosyl chloride. N-Glycosylations occurred both on the indole (*β*-*N*-glycosylated compound **13**, 42% yield) and the azaindole (*β*-*N*-glycosylated compound **12**, 10%) nitrogens. Removal of the benzyl groups of the sugar moiety of the major product by hydrogenolysis in a EtOAc/MeOH mixture in the presence of catalytic amounts of Pd/C provided compound **14** in 85% yield (Scheme 2).

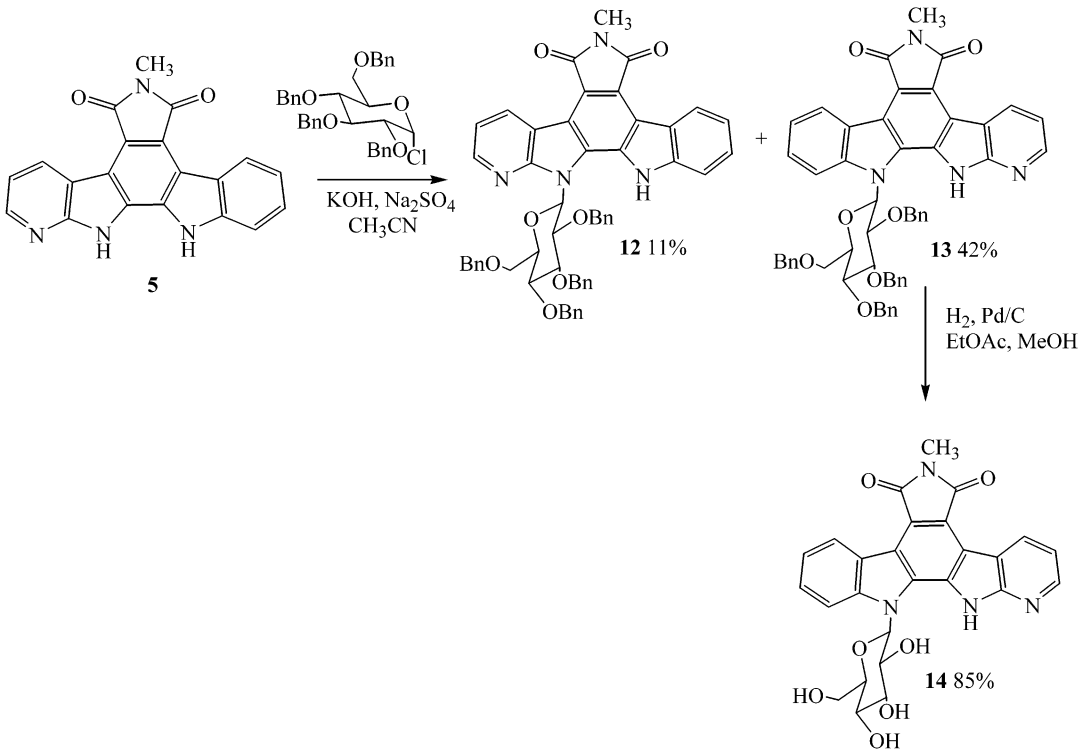
For the synthesis of **22**, the analogue of **10** with a free imide nitrogen, a synthetic scheme similar to that presented in Scheme 1 was investigated. The removable imide protecting group, benzyloxymethyl (BOM), was used. The synthetic procedure is outlined in Scheme 3.

Scheme 1



The phenylsulfonyl protecting group on the indole nitrogen was introduced using sodium hydride as a base. The phenylsulfonyl group was removed after the Mitsunobu reaction. Final deprotection of the imide nitrogen was performed in two steps: first, hydrogenolysis gave compound **21** bearing a hydroxymethyl group at

Scheme 2



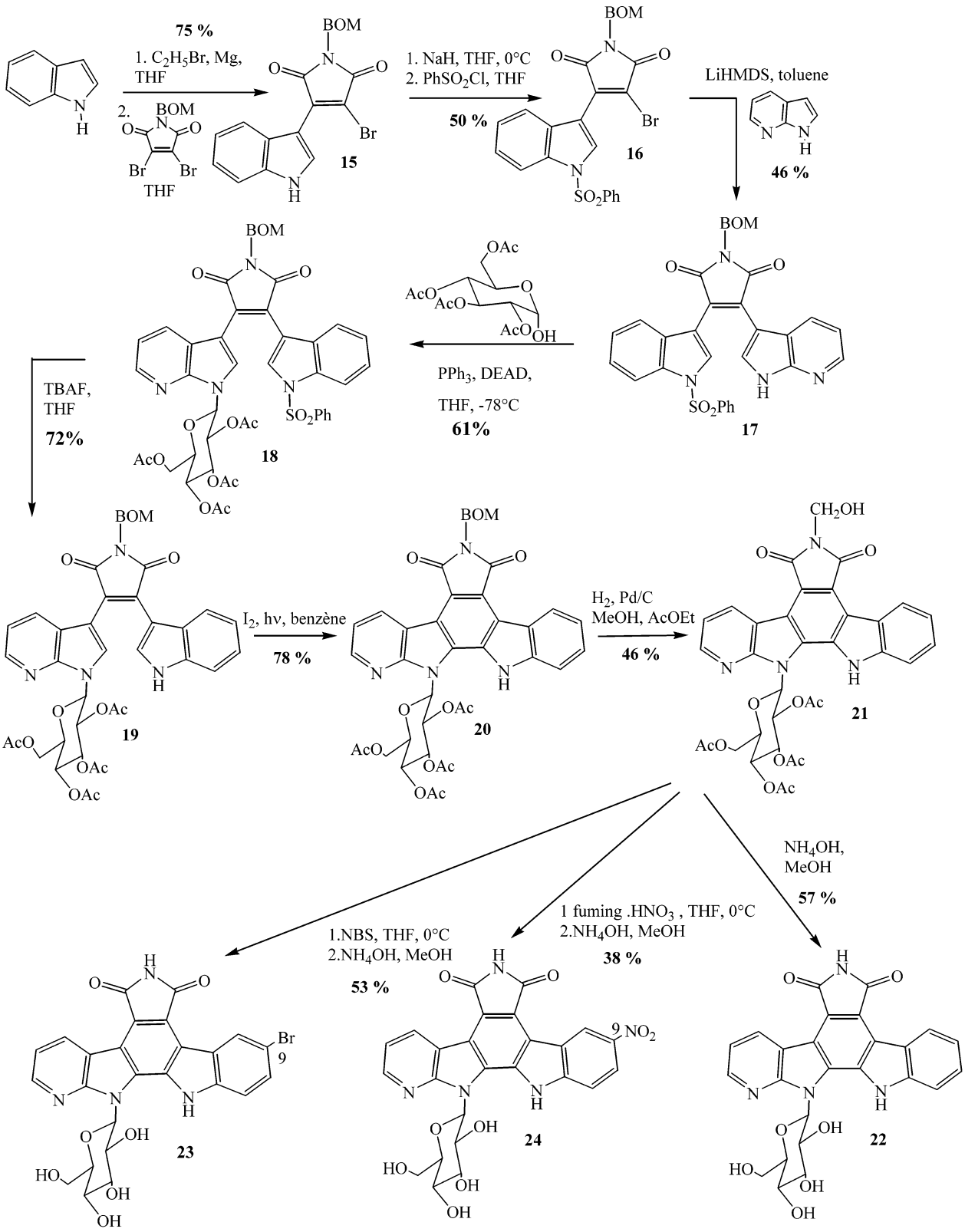
the imide nitrogen and second, aminolysis served to remove both the imide and sugar protecting groups, thus providing compound **22**. Bromination and nitration were achieved from the more soluble acetylated compound **21** yielding compounds **23** and **24**, respectively. Aromatic substitution was observed exclusively on the indole moiety. Nitration of the indole part of these rebeccamycin analogues has been found to promote biological activity.[11]

To complete this study, the analogue **32**, in which the imide nitrogen is free and the sugar moiety attached to the indole nitrogen, was synthesized (Scheme 4). The nitrogen of the azaindole was protected with a phenylsulfonyl group to allow *N*-glycosylation at the indole part. Compound **26** was prepared by substitution of **25**, the aza-analogue of **13**, with indolylmagesium bromide in toluene. *N*-glycosylation via a Mitsunobu reaction allowed the formation of *β*-*N*-glycosylated compound **27** with a 37% yield. A Koenigs−Knorr reaction with 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide, followed by deprotection of the azaindole nitrogen using TBAF to separate the required coupling compound from sugar residues, provided **28** in only 6% yield. The α-*N*-glycosylated compound **29**, the major product of the reaction was isolated in 51% yield. Compound **27** was treated with TBAF, then photocyclized in the presence of iodine to yield **30**. The final deprotections were performed as already shown in Scheme 3, hydrogenolysis and then aminolysis.

DNA Binding and Topoisomerase I Inhibition. DNA and topoisomerase I represent potential (but not unique) targets for the aza-rebeccamycins. We evaluated the DNA binding properties of the new compounds and their capacity to interfere with the DNA breakage−reunion reaction catalyzed by human topoisomerase I. To compare the relative affinities of the drugs for DNA, melting temperature measurements were performed with calf thymus DNA and the polynucleotide poly-$(dAT)_2$. The results of these T_m measurements, carried out at a drug/DNA-phosphate (D/P) ratio of 0.5, are presented in Figure 2. The position of the newly incorporated nitrogen atom in the indolocarbazole framework has a major effect on the DNA binding capacity of the drug. Added to the unsubstituted indole, it promotes DNA binding. Indeed, both compounds **14** and **32** give high ΔT_m values, even superior to those of dechlorinated rebeccamycin **B**. In contrast, when added to the sugar-substituted indole, it abolishes the DNA binding capacity. Compounds **10** and **22** as well as their bromo (**11**, **23**) and nitro (**24**) derivatives fail to elevate the melting temperature of DNA, suggesting that they have little, if any, interaction with DNA.

The effect of the drug on human topoisomerase I was investigated using a DNA sequencing assay to identify the site of DNA cleavage in a ^{32}P-labeled 117-bp DNA restriction fragment. The DNA substrate was incubated with each drug at 10 or 50 μM prior to initiating DNA cleavage by topoisomerase I. The cleavage patterns observed with the indolocarbazole drugs were comparable, but however slightly distinct, to those seen in the presence of the reference topoisomerase I poison camptothecin. The gel in Figure 3b compares the effects of the azaindole **22** with those of its bromo (**23**) and nitro (**24**) derivatives. The cleavage intensities at the prominent TG26 site vary in the order **24**>**23**>**22**. The substitution of the indole ring with a nitro group markedly reinforces the anti-topoisomerase I activity. The azaindole **32** is significantly more potent than the azaindole **22** at stimulating DNA cleavage by topoisomerase I (Figure 3a). At first sight, this may be correlated with the higher DNA affinity of **32** vs **22**, but we know from previous studies that in general there is

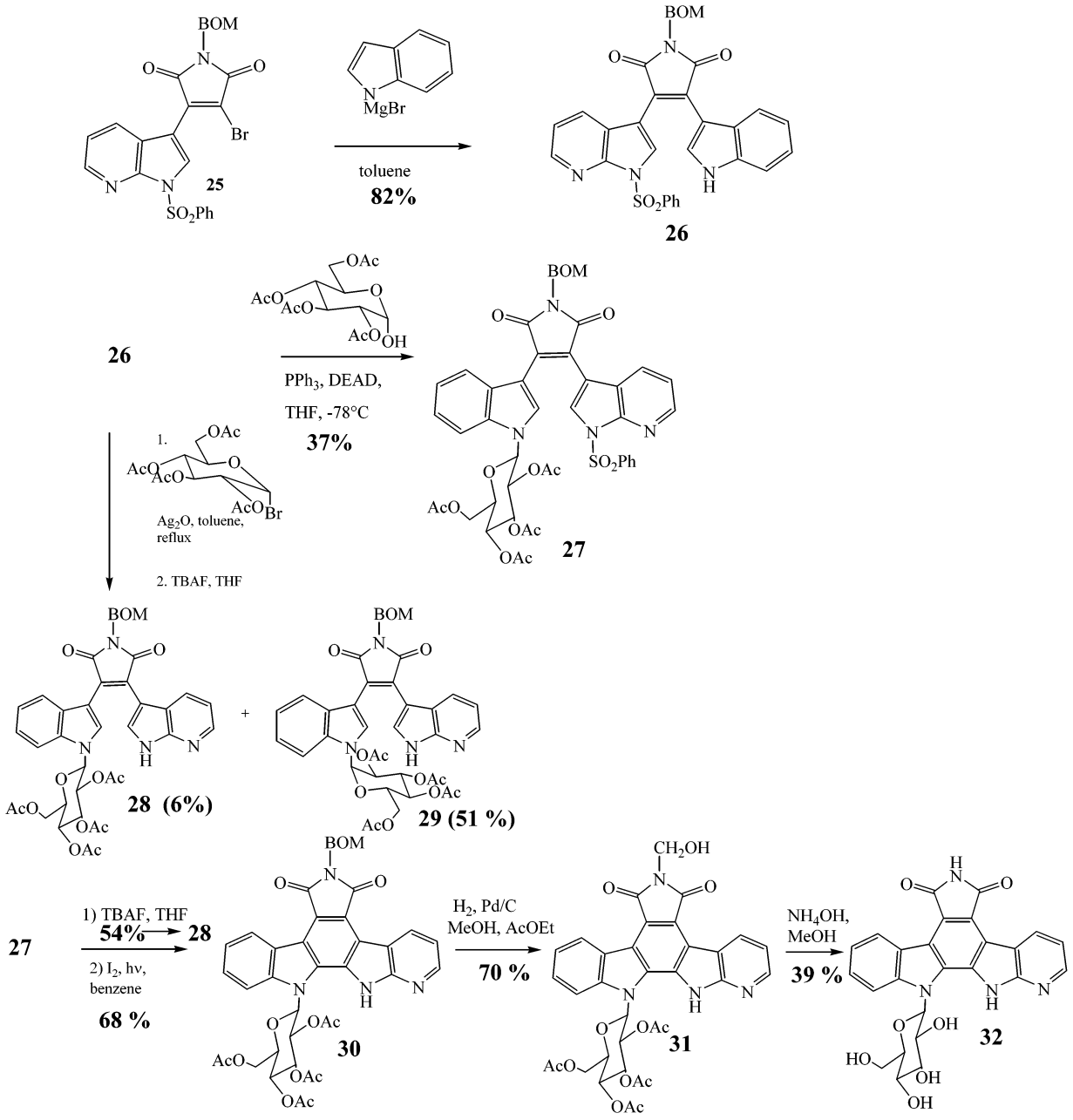
Scheme 3



no direct relationships between DNA binding affinities and topoisomerase I inhibition.

In Vitro Antiproliferative Activities. The in vitro antiproliferative activities were tested against nine tumor cell lines: murine leukemia (L1210), human leukemia (K-562), and seven human solid tumors: ovarian carcinoma (IGROV1), neuroblastoma (SK-N-MC), colon carcinoma (HT29), non small cell lung carcinoma (A549), small-cell lung carcinoma (H69), and two epidermoïd carcinomas (A431 and KB-3-1). The IC_{50} values are collated in Table 1. Non cyclized compound **7** is inactive. Comparison of **10** and **22**, both bearing the sugar moiety attached to the azaindole nitrogen, showed that the NH compound is more active against

Scheme 4



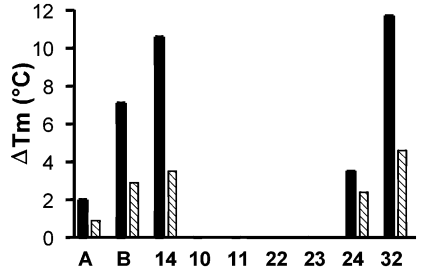


Figure 2. Variation of the ΔT_m ($T_m^{\text{drug–DNA complex}} - T_m^{\text{DNA alone}}$) of the complexes between DNA and the test compounds. Melting temperature measurements were performed in BPE (6 mM Na_2HPO_4, 2 mM NaH_2PO_4, 1 mM EDTA) buffer at pH 7.0. with a drug/DNA ratio of 0.5, using calf thymus DNA (hatched bars) or poly(dAT)$_2$ (black bars).

L1210, SK-N-MC and NCI-H69 cells than the *N*-methylated analogue. However, compound **10** is more cytotoxic toward KB-3-1 and A431 than **22**. Both compounds must be considered as selective cytotoxic agent because some cell lines are highly sensitive to these two compounds (A431, SK-N-MC, NCI-H69) and other are resistant (IGROV1, HT29, A549). In contrast, the three compounds possessing two indole units, **A**, **B**, and **C** are highly cytotoxic against all cell lines. Brominated derivatives **11** and **23** are about 2 times more cytotoxic against L1210, SK-N-MC, and NCI-H69 cells than the parent compounds **10** and **22**. The high cytotoxicity of these bromo derivatives may be attributed to their increased solubility. Interestingly, almost identical profiles of cytotoxicity are observed with all the aza derivatives, suggesting a common mechanism of action.

The comparison between **22** and **32** with the free imide nitrogen and **10** and **14** with a methyl group at the imide nitrogen suggests that compounds for which

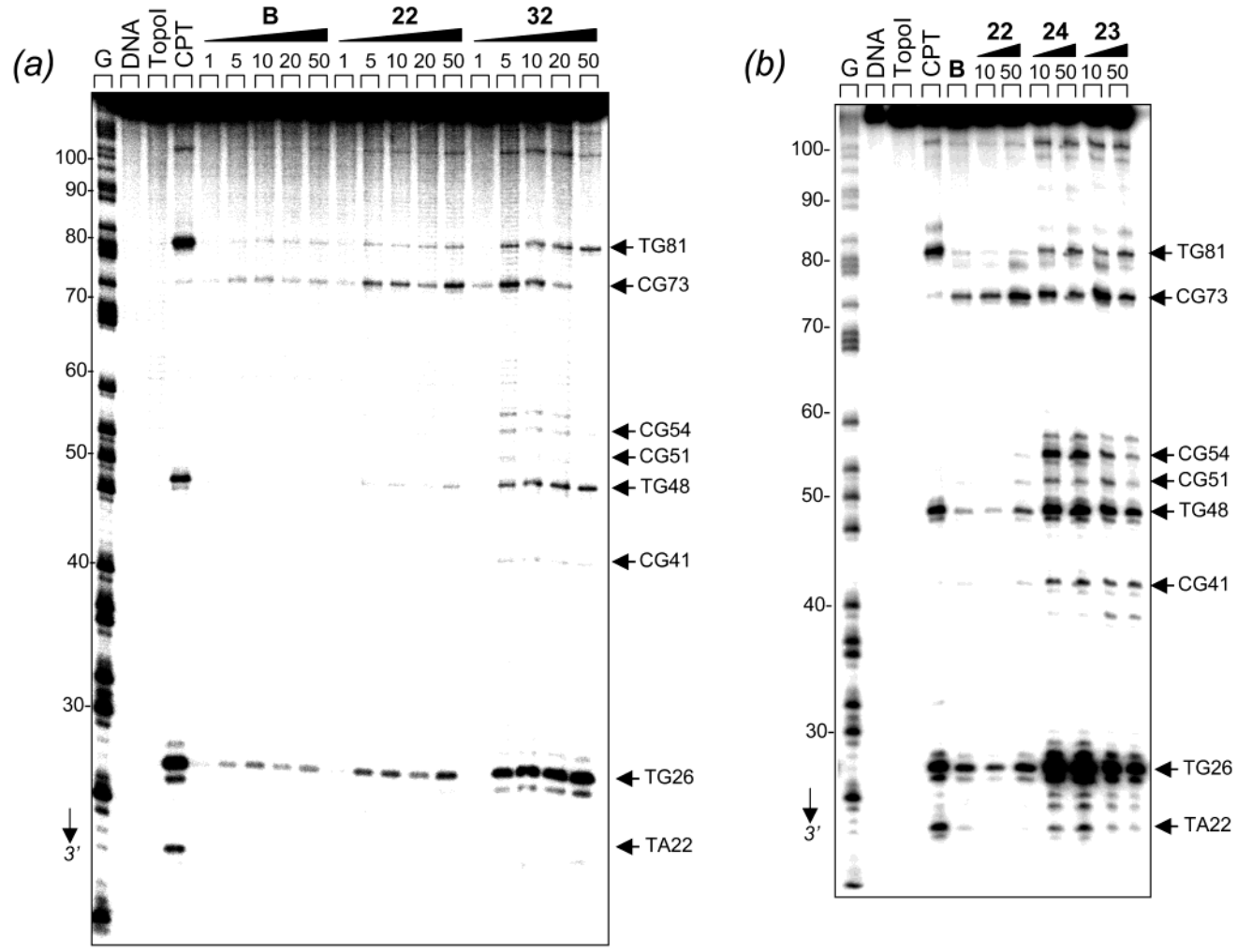


Figure 3. DNA cleavage by human topoisomerase I in the presence of the aza-indolocarbazoles. Gel (a) compares the two azaindoles **22** and **32**; gel (b) refers to the bromo and nitro derivatives. The 117-bp 3′-end labeled fragment (DNA) was incubated in the absence (lane TopoI) or presence of the test drug at the indicated μM concentration. Camptothecin (CPT) was used at 40 μM. Topoisomerase I cleavage reactions were analyzed on 8% denaturing polyacrylamide gels. Numbers at the left side of the gels show the nucleotide positions, determined with reference to the guanine tracks labeled G. The nucleotide positions and sequences to the cleavage sites are indicated.

Table 1. In Vitro Antiproliferative Activities against Nine Tumor Cell Lines: Murine Leukemia (L1210), Human Leukemia (K-562), and Seven Human Solid Tumors: Ovarian Carcinoma (IGROV1), Neuroblastoma (SK-N-MC), Colon Carcinoma (HT29), Non Small Cell Lung Carcinoma (A549), Small Cell Lung Carcinoma (H69), and Two Epidermoïd Carcinomas (A431 and KB-3-1) (IC_{50} μM)

compd	L1210	IgROV	SK-N-MC	HT29	A549	A431	NCI-H69	K-562	KB-3-1
A	0.14	0.25	<0.1	0.3	0.3	0.25	<0.1	0.2	0.3
B	0.11	2.6	<0.1	2.5	2	3.8	<0.1	<0.1	0.3
C	0.67	0.88	0.25	0.86	0.94	0.84	0.33	ne	0.6
14	1.3	ne[a]	ne	17.8	47.2	ne	ne	ne	ne
7	51.3	78.8	28	58.3	98.7	29.1	11.8	70.5	32.6
10	0.45	8.5	0.18	67.2	59.9	0.012	0.11	0.7	0.11
11	0.27	19.5	0.041	27.5	32	0.053	0.043	ne	1.2
22	0.13	68.8	0.059	>100	>100	0.238	0.01	ne	2.56
23	0.061	24.7	0.018	32.3	85.2	0.229	0.007	ne	0.93
24	0.07	33.4	ne	>100	>100	10	0.017	ne	0.552
32	0.066	ne	ne	4.8	5.3	ne	ne	ne	ne

[a] ne: not evaluated.

the sugar part is linked to the indole nitrogen are less selective than those having the carbohydrate attached to the azaindole nitrogen. Either different targets or different expression of these targets in the various tumor cell lines may account for the difference of selectivity observed between compounds in which the sugar moiety is linked to the indole nitrogen and compounds in which the sugar moiety is linked to the aza-indole nitrogen.

Effect on L1210 Cell Cycle. The compounds highly toxic toward the L1210 leukemia cell line were used to investigate the cell cycle effects. Bromo **23** and nitro **24** derivatives as well as compound **32** induce a marked accumulation of the cells in the G2+M phase of the cell

Table 2. L1210 Cytotoxicity and Cell Cycle Effects

compd	antiproliferative activities IC_{50}, μM	cells in the G2+M phase (%) (drug concentration)
control	–	24
A	0.14	69 (1 μM)
B	0.11	71 (1 μM)
C	0.67	77 (2.5 μM)
14	1.3	ne[a]
10	0.45	26 (5−20 μM)
11	0.27	78 (5 μM)
22	0.13	79 (0.5 μM)
23	0.061	85 (0.25 μM)
24	0.07	87 (0.25 μM)
32	0.066	78 (0.25 μM)

[a] ne: not evaluated

cycle (about 80% at 0.25 μM). Although four times less potent, compounds **A**, **B**, and **C** similarly modify the cell cycle. Globally, the effect on the cell cycle appears to be linked to the antiproliferative properties, with the exception of compound **10** which is relatively potent but does not significantly perturb the cell cycle.

In conclusion, in contrast to the compounds possessing two indole units, some of the new compounds described here exhibit a very interesting profile of cytotoxicity, with a high selectivity for some tumor lines. A wide IC_{50} range was observed, from 10 to 20 nM to >100 μM. Such important differences are not commonly observed with the majority of cytotoxic agents. What are the molecular events underlying these effects? Although the tumor cell lines used in this panel could present different permeability properties and/or cellular metabolism, it seems unlikely that they can account for the dramatic selectivity observed for some cell lines versus other ones. Rather, it is possible that the molecular targets of the compounds (for example kinases) were differently expressed in the cell lines, and differently affected by analogues, even though they are structurally related.

Interestingly, the replacement of indole unit by its bioisostere azaindole led to quite different profiles of cytotoxicity. The aza-analogues are much more selective. From our previous works, it could be concluded that topoisomerase I is not the only target for rebeccamycin analogues. The differences in the selectivity seem to indicate more specific targets for the aza-analogues. Compounds **10** and **14** unsubstituted on the indole ring and possessing a methyl group at the imide nitrogen are less selective than their parent compounds with a free imide nitrogen **22** and **32**. However, **10** was strongly cytotoxic against epidermoid carcinoma A431. At this stage, it is necessary to investigate the biological targets of these molecules. Unfortunately, the cell cycle effect of these compounds gave no clues to answer these questions, the G2+M arrest being observed for all the cytotoxic compounds, whatever the degree of selectivity they present. It should be kept in mind that a G2+M arrest could be induced by many compounds having unrelated mechanisms of action. Experiments are in progress to study the cell cycle effect on the sensitive human tumor cell lines identified in this work.

Surprisingly, DNA binding experiments showed major differences between the compounds bearing the sugar part either on the azaindole moiety or on the indole moiety. Compounds with the sugar linked to the indole (**14** and **32**) exhibited higher affinity for DNA than the parent dechlorinated rebeccamycin **B**. In contrast, when the sugar was attached to the azaindole (**10**, **11**, **22**, **23**, **24**), DNA binding properties were abolished. Concerning topoisomerase I inhibition, **32** was more efficient than **22**. This effect seems to be correlated with its higher DNA affinity and its stronger cytotoxicity against the tumor cell lines tested. Bromo **23** and nitro **24** analogues were more potent topoisomerase I inhibitors than the unsubstituted analogue **22**; however, there is no clear correlation between the anti-topoisomerase I activities and the cytotoxicities toward the various tumor cell lines tested.

Do these compounds inhibit kinases involved in the transformed phenotype and/or the proliferative capability? If they do, is this inhibition specific toward some kinases which could explain at least partially their specificity against the different tumor cell lines tested. To answer these questions, the inhibitory activities of these compounds against various cyclin-dependent kinases are being investigated.

Experimental Section

Chemistry. IR spectra were recorded on a Perkin-Elmer 881 spectrometer (ν in cm^{-1}). NMR spectra were performed on a Bruker AC 400 (^1H: 400 MHz, ^{13}C: 100 MHz) (chemical shifts δ in ppm, the following abbreviations are used: singlet (s), broad singlet (br s), doublet (d), doubled doublet (dd), triplet (t), doubled triplet (dt), 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 silica gel Geduran SI 60 (Merck) 0.040−0.063 mm or Kieselgel 60 (Merck) 0.063−0.200 mm column chromatography. TLC was performed on fluorescent silica gel plates (60 F_{254} from Merck). HPLC analyses were carried out on Perkin-Elmer (series 200) equipment using a Nucleosil 5 μm C18 column (250 × 4.6 mm) and a flow rate of 1.00 mL/min at 25 °C (UV detection at 280 nm with a diode array detector) eluting with (1) MeCN/H_2O+H_3PO_4 at pH 2 (60:40), (2) MeCN/H_2O+H_3PO_4 at pH 2 (80:20). HPLC results are presented as retention times (min), purity (%).

***tert*-Butyl 3-{4-[1-(2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl)-1*H*-pyrrolo[2,3-*b*]pyridin-3-yl]-1-methyl-2,5-dioxo-2,5-dihydro-1*H*-pyrrol-3-yl}-1*H*-indole-1-carboxylate (6).** To a solution of **3** (217 mg, 0.491 mmol) in THF (15 mL) were added 2,3,4,6-*O*-acetylglucopyranose (380 mg, 1.09 mmol) and triphenylphosphine (286 mg, 1.09 mmol). The mixture was cooled to −78 °C, and then DEAD (172 μL, 1.09 mmol) was added dropwise. The mixture was stirred at room temperature for 15 h, and then water was added. After extraction with EtOAc, the organic phase was dried over $MgSO_4$, the solvent was removed, and the residue was purified by flash chromatography (eluent cyclohexane:EtOAc:triethylamine, 4:1:1%) to give β-glycosylated compound **6** (231 mg, 0.299 mmol, 61% yield) as a yellow solid.

6: mp 89−91 °C. IR (KBr) ν_{CO} 1700, 1750 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) 1.66 (3H, s), 1.70 (9H, s, *t*-Bu), 2.03 (3H, s), 2.06 (3H, s), 2.09 (3H, s), 3.20 (3H, s, NCH_3), 4.04 (1H, m), 4.12 (1H, m), 4.24 (1H, dd, ^1J = 12.6 Hz, ^2J = 7.9 Hz), 5.25 (1H, pt, J = 9.7 Hz), 5.48 (1H, pt, J = 9.9 Hz), 5.53 (1H, pt, J = 9.7 Hz), 6.26 (1H, d, J = 8.8 Hz, $H_{1'}$), 6.67 (4H, m), 7.13 (1H, m), 7.27 (1H, m), 8.06 (1H, s), 8.14 (1H, s), 8.18 (1H, dd, ^1J = 4.7 Hz, ^2J = 1.3 Hz). ^{13}C NMR (100 MHz, $CDCl_3$) 20.1, 20.6, 20.8, 21.1 (CH_3CO), 24.4 (NCH_3), 28.2 (3C) (CH_3 of *t*-Bu), 62.2 ($C_{6'}$), 68.1, 70.7, 73.2, 74.8, 80.2 ($C_{1'}$, $C_{2'}$, $C_{3'}$, $C_{4'}$, $C_{5'}$), 84.8 (C-CH_3 of *t*-Bu), 115.2, 117.7, 121.4, 122.8, 124.7, 128.9, 129.5, 130.4, 143.9 (C tert arom), 106.6, 110.3, 119.0, 126.5, 127.7, 128.8, 135.1, 147.6 (C quat arom), 149.2, 168.9 (2C), 169.5, 170.0, 170.8, 171.3 (C=O).

3-(1*H*-Indol-3-yl)-4-[1-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-1*H*-pyrrolo[2,3-*b*]pyridin-3-yl]-1-methyl-1*H*-pyr

role-2,5-dione (8). A mixture of β-glycosylated compound **6** (88 mg, 0.114 mmol) in HCOOH (10 mL) was stirred at room temperature for 24 h. Triethylamine was added dropwise until neutralization. Saturated aqueous HCO_3Na (20 mL) was added. After extraction with EtOAc, the organic phase was dried over $MgSO_4$, the solvent was removed, and the residue was purified by flash chromatography (eluent cyclohexane: EtOAc, 1:1) to give **8** (55 mg, 0.082 mmol, 72% yield) as an orange solid: mp 119−121 °C; IR (KBr) ν_{CO} 1700, 1752 cm^{-1}, ν_{NH} 3300−3500 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) 1.69 (3H, s), 2.02 (3H, s), 2.06 (3H, s), 2.08 (3H, s), 3.17 (3H, s, NCH_3), 4.03 (1H, m), 4.11 (1H, m), 4.25 (1H, dd, ^1J = 4.6 Hz, ^2J = 12.5 Hz), 5.26 (1H, pt, J = 9.7 Hz), 5.48 (1H, pt, J = 9.4 Hz), 5.58 (1H, pt, J = 9.4 Hz), 6.27 (1H, d, J = 9.3 Hz, $H_{1'}$), 6.68 (1H, t, J = 7.1 Hz), 6.70 (1H, m), 6.83 (1H, d, J = 8.1 Hz), 7.01 (1H, t, J = 7.5 Hz), 7.19 (1H, d, J = 7.9 Hz), 7.29 (1H, d, J = 8.1 Hz), 7.79 (1H, d, J = 1.0 Hz), 8.00 (1H, s), 8.16 (1H, d, J = 4.3 Hz), 9.24 (1H, s, NH). ^{13}C NMR (100 MHz, $CDCl_3$) 20.2, 20.6, 20.8, 21.1 (CH_3CO), 24.3 (NCH_3), 61.9 ($C_{6'}$), 68.2, 70.6, 73.3, 74.7, 80.2 ($C_{1'}$, $C_{2'}$, $C_{3'}$, $C_{4'}$, $C_{5'}$), 111.4, 117.3, 120.5, 121.6, 122.7, 127.9, 128.9, 130.6, 143.6 (C tert arom), 106.6, 107.0, 119.2, 125.3, 125.6, 129.3, 136.0, 147.6 (C quat arom), 168.9, 169.5, 170.0, 170.8, 171.8, 172.1 (C═O).

13-(2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl)-6-methyl-12,13-dihydro-5*H*-pyrido[3′,2′:4,5]pyrrolo[2,3-*a*]pyrrolo[3,4-*c*]carbazole-5,7(6*H*)-dione (9). A mixture of **8** (55 mg, 0.083 mmol) and iodine (252 mg, 0.99 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) and washed with aqueous sodium thiosulfate and then with brine. The organic phase was dried over $MgSO_4$, the solvent was removed, and the residue was purified by flash chromatography (eluent cyclohexane:EtOAc, 1:1) to give **9** (47 mg, 0.070 mmol, 84% yield) as a yellow solid: mp 302−304 °C; IR (KBr) ν_{CO} 1700, 1750 cm^{-1}, ν_{NH} 3200−3600 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) 1.11 (3H, s), 1.93 (3H, s), 2.15 (3H, s), 2.30 (3H, s), 3.23 (3H, s, NCH_3), 4.44 (2H, d, J = 10.9 Hz), 4.82 (1H, dd, ^1J = 13.4 Hz, ^2J = 3.6 Hz), 5.48 (1H, pt, J = 9.2 Hz), 5.61 (1H, pt, J = 9.4 Hz), 5.68 (1H, pt, J = 9.5 Hz), 6.90 (1H, d, J = 9.4 Hz, $H_{1'}$), 7.37 (1H, dd, ^1J = 7.8 Hz, ^2J = 4.8 Hz), 7.44 (1H, t, J = 7.5 Hz), 7.59 (1H, t, J = 7.8 Hz), 7.64 (1H, d, J = 8.0 Hz), 8.56 (1H, d, J = 4.3 Hz), 9.19 (1H, d, J = 8.0 Hz), 9.34 (1H, dd, ^1J = 7.0 Hz, ^2J = 0.9 Hz), 9.88 (1H, s, NH). ^{13}C NMR (100 MHz, $CDCl_3$) 19.2, 20.5, 21.0, 21.4 (CH_3CO), 23.8 (NCH_3), 61.3 ($C_{6'}$), 67.4, 70.6, 72.8, 75.9, 82.1 ($C_{1'}$, $C_{2'}$, $C_{3'}$, $C_{4'}$, $C_{5'}$), 111.6, 118.3, 121.7, 125.9, 127.8, 134.1, 146.9 (C tert arom), 115.4, 116.2, 119.8, 119.9, 122.0, 122.6, 127.2, 129.8, 140.9, 152.0 (C quat arom), 168.0, 169.5, 169.6, 169.7, 169.9, 170.6 (C═O).

13-(β-D-Glucopyranosyl)-6-methyl-12,13-dihydro-5*H*-pyrido[3′,2′:4,5]pyrrolo[2,3-*a*]pyrrolo[3,4-*c*]carbazole-5,7-(6*H*)-dione (10). A mixture of **9** (45 mg, 0.067 mmol) and 28% aqueous NH_4OH (31 mL) in methanol (20 mL) was stirred for 22 h at 65 °C. After removal of the solvents, water and EtOAc were added to the residue. After filtration, the solid was washed with EtOAc. Compound **10** was obtained (23 mg, 0.046 mmol, 69% yield) as a yellow solid: mp > 300 °C. IR (KBr) ν_{CO} 1690, 1750 cm^{-1}, $\nu_{NH,OH}$ 3300−3600 cm^{-1}. HRMS $(M + H)^+$ (FAB$^+$) calcd for $C_{26}H_{23}N_4O_7$ 503.1566, found 503.1560. ^1H NMR (400 MHz, DMSO-d_6) 3.25 (3H, s, NCH_3), 3.58 (2H, m), 3.87 (1H, d, J = 10.3 Hz), 3.95 (1H, d, J = 10.8 Hz), 4.02 (1H, m), 4.12 (1H, d, J = 9.4 Hz), 4.99 (1H, br s, OH), 5.22 (1H, br s, OH), 5.42 (1H, d, J = 5.4 Hz, OH), 6.12 (1H, br s, OH), 6.64 (1H, d, J = 8.4 Hz, $H_{1'}$), 7.44 (1H, t, J = 7.4 Hz), 7.53 (1H, dd, ^1J = 7.9 Hz, ^2J = 4.9 Hz), 7.64 (1H, t, J = 7.9 Hz), 7.75 (1H, d, J = 8.4 Hz), 8.67 (1H, d, J = 4.0 Hz), 9.12 (1H, d, J = 7.9 Hz), 9.36 (1H, d, J = 7.4 Hz), 11.71 (1H, s, NH). ^{13}C NMR (100 MHz, DMSO-d_6) 23.8 (NCH_3), 58.4 ($C_{6'}$), 67.6, 72.7, 76.6, 78.7, 83.1 ($C_{1'}$, $C_{2'}$, $C_{3'}$, $C_{4'}$, $C_{5'}$), 112.3, 117.4, 120.7, 124.4, 127.5, 132.7, 147.1 (C tert arom), 114.2, 115.4, 117.8, 119.0, 121.2 (2C), 127.2, 129.5, 141.1, 152.5 (C quat arom), 169.6 (2C) (C═O). HPLC >97% pure, 1) t_R = 5.41 min, 2) t_R = 3.61 min.

3-(1*H*-Indol-3-yl)-4-[1-(β-D-glucopyranosyl)-1*H*-pyrrolo[2,3-*b*]pyridin-3-yl]-1-methyl-1*H*-pyrrole-2,5-dione (7). To a solution of **6** (40 mg, 0.051 mmol) in THF (6 mL) was added a solution of tetrabutylammonium fluoride (1.1 M in THF) (2.1 mL, 2.3 mmol). The mixture was refluxed for 15 h. After removal of the solvent, the residue was purified by flash chromatography (eluent EtOAc:methanol, 93:7) to give **7** (21 mg, 0.042 mmol, 82% yield) as a red solid: mp 176−178 °C; IR (KBr) ν_{CO} 1690, 1700 cm^{-1}, $\nu_{NH,OH}$ 3200−3600 cm^{-1}. HRMS (FAB$^+$) $(M + H)^+$ calcd for $C_{26}H_{25}N_4O_7$ 505.1723, found 505.1725. ^1H NMR (400 MHz, DMSO-d_6) 3.10 (3H, s, NCH_3), 3.29−3.54 (3H, m), 3.71 (1H, t, J = 3.3 Hz), 3.75 (1H, dd, ^1J = 10.4 Hz, ^2J = 4.5 Hz), 3.86 (1H, m), 4.64 (1H, t, J = 5.5 Hz, OH), 5.18 (1H, d, J = 5.5 Hz, OH), 5.27 (1H, d, J = 5.0 Hz, OH), 5.30 (1H, d, J = 5.9 Hz, OH), 5.87 (1H, d, J = 9.4 Hz, $H_{1'}$), 6.70−6.77 (2H, m), 6.95 (1H, d, J = 8.0 Hz), 6.99 (1H, dd, ^1J = 8.0 Hz, ^2J = 1.5 Hz), 7.00 (1H, t, J = 7.3 Hz), 7.43 (1H, d, J = 8.2 Hz), 7.82 (1H, d, J = 2.8 Hz), 8.15 (1H, s), 8.16 (1H, dd, ^1J = 4.6 Hz, ^2J = 1.5 Hz), 11.88 (1H, s, NH). ^{13}C NMR (100 MHz, DMSO-d_6) 24,0 (NCH_3), 52.0 ($C_{6'}$), 70.0, 72.0, 77.5, 80.0, 82.3 ($C_{1'}$, $C_{2'}$, $C_{3'}$, $C_{4'}$, $C_{5'}$), 111.8, 116.5, 119.8, 121.0, 121.8, 128.7, 129.2, 131.5, 143.0 (C tert arom), 104.9, 105.3, 118.3, 125.2, 125.3, 128.8, 135.9, 147.7 (C quat arom), 171.4 (2C) (C═O). HPLC >96% pure, 1) t_R = 3.48 min, 2) t_R = 2.76 min.

13-(2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl)-9-bromo-6-methyl-12,13-dihydro-5*H*-pyrido[3′,2′:4,5]pyrrolo[2,3-*a*]-pyrrolo[3,4-*c*]carbazole-5,7(6*H*)-dione (11A). To a solution of **9** (41 mg, 0.061 mmol) in THF (2 mL) at 0 °C was added dropwise a solution of *N*-bromosuccinimide (109 mg, 0.61 mmol) in THF (2 mL). The mixture was stirred at room temperature for 4 days with light protection. Then a solution of *N*-bromosuccinimide (109 mg, 0.61 mmol) in THF (2 mL) was added dropwise. Water was added. After hydrolysis for 15 min, saturated aqueous sodium thiosulfate (20 mL) was poured into the mixture. After extraction with EtOAc, the organic phase was dried over $MgSO_4$, the solvent was removed, and the residue was purified by flash chromatography (eluent cyclohexane:EtOAc, 3:2) to give **11A** (20 mg, 0.027 mmol, 44% yield) as a yellow solid and a mixture of brominated compounds partially deacetylated.

11A: mp 280−282 °C; IR (KBr) ν_{CO} 1700, 1760 cm^{-1}, ν_{NH} 3360−3400 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) 1.11 (3H, s), 1.93 (3H, s), 2.15 (3H, s), 2.28 (3H, s), 3.25 (3H, s, NCH_3), 4.41−4.47 (2H, m), 4.85 (1H, dd, ^1J = 13.0 Hz, ^2J = 3.5 Hz), 5.44 (1H, pt, J = 9.3 Hz), 5.58−5.66 (2H, m), 6.92 (1H, d, J = 9.4 Hz, $H_{1'}$), 7.40 (1H, dd, ^1J = 7.9 Hz, ^2J = 4.9 Hz), 7.54 (1H, t, J = 8.5 Hz), 7.67 (1H, dd, ^1J = 8.7 Hz, ^2J = 2.0 Hz), 8.60 (1H, dd, ^1J = 4.6 Hz, ^2J = 1.6 Hz), 9.32 (1H, s), 9.33 (1H, dd, ^1J = 7.9 Hz, ^2J = 1.6 Hz), 9.95 (1H, s, NH). ^{13}C NMR (100 MHz, $CDCl_3$) 19.2, 20.5, 20.7, 20.8 (CH_3CO), 23.9 (NCH_3), 61.4 ($C_{6'}$), 67.4, 70.6, 73.0, 76.3, 82.2 ($C_{1'}$, $C_{2'}$, $C_{3'}$, $C_{4'}$, $C_{5'}$), 113.1, 118.5, 128.2, 130.7, 134.2, 147.2 (C tert arom), 114.5, 115.3, 116.6, 118.6, 120.2, 122.0, 124.1, 127.2, 130.0, 139.5, 152.1 (C quat arom), 168.1, 169.4, 169.5, 169.6, 169.9, 170.5 (C═O).

13-(β-D-Glucopyranosyl)-9-bromo-6-methyl-12,13-dihydro-5*H*-pyrido[3′,2′:4,5]pyrrolo[2,3-*a*]pyrrolo[3,4-*c*]carbazole-5,7(6*H*)-dione (11). A solution of **11A** (20 mg, 0.026 mmol) and 28% aqueous NH_4OH (8 mL) in methanol (8 mL) was stirred for 22 h at room temperature with light protection. The solvents were removed, and then water and EtOAc were added to the residue. After fitration, the solid was washed with EtOAc to give **11** (8 mg, 0.014 mmol, 53% yield) as a yellow solid: mp > 300 °C, IR (KBr) ν_{CO} 1680, 1760 cm^{-1}, $\nu_{NH,OH}$ 3300−3600 cm^{-1}. HRMS (FAB$^+$) $(M + H)^+$ calcd for $C_{26}H_{22}N_4O_7$-Br 581.0672, found 581.0668. ^1H NMR (400 MHz, DMSO-d_6) 3.25 (3H, s, NCH_3), 3.52−3.62 (2H, m), 3.88 (1H, d, J = 10.5 Hz), 3.95−4.04 (2H, m), 4.12 (1H, d, J = 10.3 Hz), 4.99 (1H, d, J = 4.9 Hz, OH), 5.21 (1H, d, J = 5.0 Hz, OH), 5.42 (1H, d, J = 5.0 Hz, OH), 6.18 (1H, m, OH), 6.65 (1H, d, J = 8.6 Hz, $H_{1'}$), 7.55 (1H, dd, ^1J = 7.7 Hz, ^2J = 4.8 Hz), 7.71 (1H, d, J = 8.7 Hz), 7.81 (1H, dd, ^1J = 8.7 Hz, ^2J = 1.6 Hz), 8.69 (1H, dd, ^1J = 4.6 Hz, ^2J = 1.3 Hz), 9.28 (1H, d, J = 1.3 Hz), 9.36 (1H, dd, ^1J = 6.6 Hz, ^2J = 1.3 Hz), 11.83 (1H, s, NH). ^{13}C NMR (100 MHz, DMSO-d_6) 23.8 (NCH_3), 58.3 ($C_{6'}$), 67.6, 72.7, 76.5, 78.7, 83.1 ($C_{1'}$, $C_{2'}$, $C_{3'}$, $C_{4'}$, $C_{5'}$), 114.3, 117.6, 126.4, 129.9, 132.7, 147.4 (C tert arom), 112.5, 114.1, 115.8, 116.5, 119.5, 120.8,

123.0, 127.2, 129.8, 139.7, 152.4 (C quat arom), 169.4, 169.5 (C=O). HPLC >95% pure, 1) t_R = 13.55 min, 2) t_R = 9.28 min.

12-(2,3,4,6-Tetra-*O*-benzyl-*β*-D-glucopyranosyl)-6-methyl-12,13-dihydro-5*H*-pyrido[3′,2′:4,5]pyrrolo[2,3-*a*]pyrrolo-[3,4-*c*]carbazole-5,7(6*H*)-dione (13). To a suspension of KOH (55 mg) and Na_2SO_4 (338 mg) in acetonitrile (10 mL) was added aglycone **5** (45 mg, 0.132 mmol). The mixture was stirred for 30 min at room temperature, before addition of 2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl chloride (221 mg, 0.396 mmol) in acetonitrile (3 mL). After stirring for 15 h at room temperature, water (20 mL) was added and the mixture was acidified with 1 N HCl. After extraction with EtOAc, the organic phase was washed with brine and then dried over $MgSO_4$, and the solvent was removed. The residue was purified by flash chromatography (eluent cyclohexane:EtOAc, 1:9) to give compound **13** (48 mg, 0.056 mmol, 42% yield) and **12** (12 mg, 0.014 mmol, 11% yield) as yellow solids.

13: mp > 80−82 °C, IR (KBr) ν_{CO} 1700, 1750 cm^{-1}, ν_{NH} 3200−3600 cm^{-1}. ^{1}H NMR (400 MHz,$CDCl_3$) 3.33 (3H, s), 3.88−3.91 (2H, m), 3.95−4.08 (4H, m), 4.38 (1H, d, J = 10.4 Hz), 4.49 (1H, d, J = 9.2 Hz), 4.73 (1H, d, J = 12.6 Hz), 4.86−4.92 (3H, m), 5.40 (1H, d, J = 12.4 Hz), 6.05 (1H, d, J = 8.2 Hz, $H_{1'}$), 6.10 (1H, d, J = 7.6 Hz), 6.80 (2H, t, J = 7.6 Hz), 7.00 (1H, t, J = 7.4 Hz), 7.09−7.11 (2H, m), 7.27−7.40 (15H, m), 7.51 (1H, t, J = 7.7 Hz), 7.60−7.68 (3H, m), 8.68 (1H, dd, ^1J = 4.7 Hz, ^2J = 1.5 Hz), 9.40 (1H, d, J = 7.9 Hz), 9.48 (1H, d, J = 7.8 Hz), 11.47 (1H, s, NH). ^{13}C NMR (100 MHz, $CDCl_3$) 23.9 (CH_3), 65.5, 73.3, 75.0, 75.4, 76.0 (CH_2), 76.3, 77.8, 81.0, 84.6, 85.7 ($C_{1'}$, $C_{2'}$, $C_{3'}$, $C_{4'}$, $C_{5'}$), 110.6, 117.2, 121.7, 125.9, 127.7 (2C), 127.75 (2C), 127.8 (2C), 127.9 (2C), 128.0 (2C), 128.2 (2C), 128.3 (2C), 128.5 (2C), 128.6 (2C), 129.4 (3C), 133.5, 148.2 (C tert arom), 115.2, 116.6, 119.7, 120.2, 121.6, 122.2, 128.9, 135.9, 137.7 (2C), 137.9, 138.1, 141.8, 153.5 (C quat arom), 170.0 (2C) (C=O).

12-(*β*-D-Glucopyranosyl)-6-methyl-12,13-dihydro-5*H*-pyrido[3′,2′:4,5]pyrrolo[2,3-*a*]pyrrolo[3,4-*c*]carbazole-5,7-(6*H*)-dione (14). A mixture of compound **13** (47 mg, 0.054 mmol), catalytic amounts of 10% Pd/C in EtOAc (2 mL), and methanol (5 mL) was hydrogenated (1 bar) for 3 days. The solvents were removed, and the residue was purified by flash chromatography (eluent EtOAc:MeOH, 98:2 then 9:1) to give compound **14** (23 mg, 0.046 mmol, 85% yield) as a yellow solid. mp > 275−277 °C, IR (KBr) ν_{CO} 1690, 1750 cm^{-1}, $\nu_{NH,OH}$ 3100−3600 cm^{-1}. HRMS (FAB$^+$) (M + H)$^+$ calcd for $C_{26}H_{23}N_4O_7$ 503.1567, found 503.1568. ^{1}H NMR (400 MHz, DMSO-d_6) 3.06 (3H, s, CH_3), 3.38 (1H, m), 3.51 (1H, m), 3.61−3.71 (3H, m), 4.40 (1H, d, J = 3.2 Hz), 4.58 (2H, dt, ^1J = 17.3 Hz, ^2J = 3.7 Hz), 4.73 (1H, d, J = 3.8 Hz), 4.91 (1H, t, J = 2.9 Hz), 5.40 (1H, d, J = 6.2 Hz), 6.21−6.29 (2H, m), 6.41 (1H, t, J = 5.6 Hz), 6.70 (1H, d, J = 6.4 Hz), 7.15 (1H, dd, ^1J = 3.5 Hz, ^2J = 1.0 Hz), 7.57 (1H, d, J = 6.0 Hz), 7.69 (1H, dd, ^1J = 5.9 Hz, ^2J = 0.8 Hz), 9.25 (1H, d, J = 5.2 Hz), 9.30 (1H, d, J = 5.3 Hz), 11.60 (1H, s, NH). ^{13}C NMR (100 MHz, DMSO-d_6) 23.8 (CH_3), 58.2 ($C_{6'}$), 67.9, 73.2, 76.7, 79.0, 84.4 ($C_{1'}$, $C_{2'}$, $C_{3'}$, $C_{4'}$, $C_{5'}$), 111.8, 117.0, 120.8, 124.3, 127.2, 132.3, 147.9 (C tert arom), 114.1, 114.9, 117.3, 119.1, 120.4, 120.6, 128.0, 128.3, 142.2, 152.5 (C quat arom), 169.5 (2C) (C=O). HPLC >98% pure, 1) t_R = 4.70 min, 2) t_R = 3.51 min.

1-[(Benzyloxy)methyl]-3-bromo-4-(1*H*-indol-3-yl)-1*H*-pyrrole-2,5-dione (15). A solution of ethylmagnesium bromide was prepared from magnesium (164 mg, 6.75 mmol) and bromoethane (509 μL, 6.75 mmol) in THF (5 mL). The mixture was stirred for 15 min at room temperature and then at 40 °C for 20 min. A solution of indole (790 mg, 6.75 mmol) in THF (40 mL) was then added dropwise. The mixture was stirred at 40 °C for 1 h and then cooled to room temperature before dropwise addition of a solution of *N*-benzyloxymethyl-2,3-dibromomaleimide (1.27 g, 3.38 mmol) in THF (40 mL). The mixture was stirred for 15 h at room temperature, before addition of saturated aqueous NH_4Cl. After extraction with EtOAc, the organic phase was dried over $MgSO_4$, the solvent was removed, and the residue was purified by flash chromatography (eluant cyclohexane:EtOAc, 4:1) to give **15** (1.043 g, 2.54 mmol, 75% yield) as a yellow solid. Mp 115−117 °C. IR (KBr), $\nu_{C=O}$ 1700, 1770 cm^{-1}, ν_{NH} 3200−3500 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$): 4.70 (2H, s, CH_2), 5.19 (2H, s, CH_2), 7.27−7.44 (8H, m), 7.94 (1H, d, J = 2.5 Hz), 8.04 (1H, dd, ^1J = 6.3 Hz, ^2J = 2.0 Hz), 9.25 (1H, s, NH). ^{13}C NMR (100 MHz, $CDCl_3$) 67.6, 71.9 (CH_2), 112.0, 121.5, 122.9, 123.5, 127.8 (2C), 128.0, 128.5 (2C), 130.5 (C tert arom), 105.1, 114.9, 124.6, 136.3, 137.2, 137.9 (C quat arom), 166.5, 169.1 (C=O).

1-[(Benzyloxy)methyl]-3-bromo-4-[1-(phenylsulfonyl)-1*H*-indol-3-yl]-1*H*-pyrrole-2,5-dione (16). A suspension of NaH (60% in mineral oil) (218 mg, 5.45 mmol) in THF (10 mL) was cooled to 0 °C, and then a solution of **15** (1.038 g, 2.53 mmol) in THF (20 mL) was added dropwise. The mixture was stirred at 0 °C for 1 h, and then benzenesulfonyl chloride (516 mL, 4.04 mmol) was added dropwise. The mixture was stirred at room temperature for 4 h before addition of saturated aqueous NH_4Cl. After extraction with EtOAc, the organic phase was dried over $MgSO_4$, the solvent was removed, and the residue was purified by flash chromatography (eluant cyclohexane:EtOAc, 85:15) to give **16** (702 mg, 1.27 mmol, 50% yield) as a yellow solid. Mp 49−51 °C. IR (KBr), $\nu_{C=O}$ 1740, 1790 cm^{-1}. HRMS (FAB$^+$) (M + H)$^+$ calcd $C_{26}H_{20}N_2O_5BrS$ 550,-0198, found 550.0203. ^{1}H NMR (400 MHz, $CDCl_3$): 4.68 (2H, s, CH_2), 5.18 (2H, s, CH_2), 7.24−7.38 (6H, m), 7.42 (1H, t, J = 7.5 Hz), 7.52 (2H, t, J = 7.7 Hz), 7.61 (1H, t, J = 7.5 Hz), 7.80 (1H, d, J = 8.0 Hz), 7.99 (2H, d, J = 7.8 Hz), 8.05 (1H, d, J = 8.3 Hz), 8.22 (1H, s). ^{13}C NMR (100 MHz, $CDCl_3$): 67.6, 71.6 (CH_2), 113.2, 122.4, 122.8, 123.7, 125.4, 126.7, 126.8, 127.2, 127.5, 127.7, 127.9, 128.1, 129.0, 131.6, 134.4 (C tert arom), 109.7, 121.7, 125.5, 135.6, 137.0, 137.2, 146.4 (C quat arom), 165.0, 167.6 (C=O).

1-[(Benzyloxy)methyl]-3-[1-(phenylsulfonyl)-1*H*-indol-3-yl]-4-(1*H*-pyrrolo[2,3-*b*]pyridin-3-yl)-1*H*-pyrrole-2,5-dione (17). To a solution of 7-azaindole (182 mg, 1.542 mmol) in toluene (10 mL) at −15 °C was added dropwise a solution of LiHMDS (1 M in hexane) (1.78 mL). The mixture was stirred at −15 °C for 1 h, and then a solution of **16** (351 mg, 0.637 mmol) in toluene (10 mL) was added dropwise at −20 °C. The mixture was stirred for 24 h at room temperature before addition of saturated aqueous NH_4Cl (20 mL), and then the pH was adjusted to pH 7. After extraction with EtOAc, the organic phase was dried over $MgSO_4$, the solvent was removed, and the residue was purified by flash chromatography (eluent cyclohexane:EtOAc, 3:2) to give **17** (173 mg, 0.294 mmol, 46% yield) as an orange solid. Mp 94−96 °C. IR (KBr), $\nu_{C=O}$ 1710, 1770 cm^{-1}, ν_{NH} 3300−3600 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$): 4.73 (2H, s, CH_2), 5.24 (2H, s, CH_2), 6.48 (1H, dd, ^1J = 7.9 Hz, ^2J = 4.4 Hz), 6.81 (1H, d, J = 1.0 Hz), 6.82 (1H, d, J = 3.0 Hz), 6.95 (1H, dd, ^1J = 8.4 Hz, ^2J = 1.5 Hz), 7.17−7.25 (2H, m), 7.29 (2H, t, J = 6.9 Hz), 7.39 (2H, d, J = 6.9 Hz), 7.53 (2H, t, J = 6.9 Hz), 7.64 (1H, dt, ^1J = 6.9 Hz, ^2J = 1.5 Hz), 7.97−8.01 (3H, m), 8.10 (1H, s), 8.14 (1H, s), 8.18 (1H, dd, ^1J = 4.4 Hz, ^2J = 1.5 Hz), 12.05 (1H, s, NH). ^{13}C NMR (100 MHz, $CDCl_3$): 67.5, 71.8 (CH_2), 113.6, 116.6, 121.9, 123.5, 125.3, 127.1 (2C), 127.7 (3C), 127.8, 128.5 (3C), 129.5 (2C), 130.2, 134.3, 143.2 (C tert arom), 104.8, 112.4, 118.5, 124.2, 127.8, 131.5, 134.6, 137.7, 137.8, 148.7 (C quat arom), 170.5, 170.9 (C=O).

1-[(Benzyloxy)methyl]-3-[1-(phenylsulfonyl)-1*H*-indol-3-yl]-4-[1-(2,3,4,6-tetra-*O*-acetyl-*β*-D-glucopyranosyl)-1*H*-pyrrolo[2,3-*b*]pyridin-3-yl]-1*H*-pyrrole-2,5-dione (18). To a solution of **17** (545 mg, 0.927 mmol) in THF (40 mL) were added 2,3,4,6-*O*-acetylglucopyranose (677 mg, 1.95 mmol) and triphenylphosphine (510 mg, 1.95 mmol). The mixture was cooled to −78 °C, and then DEAD (306 μL, 1.95 mmol) was added dropwise. The mixture was allowed to reach room temperature and then stirred for 15 h. Water (100 mL) was added. After extraction with EtOAc, the organic phase was dried over $MgSO_4$, the solvent was removed, and the residue was purified by flash chromatography (eluent cyclohexane: EtOAc, 65:35, then toluene:EtOAc, 3:2) to give *β*-glycosylated compound **18** (519 mg, 0.565 mmol, 61% yield) as a yellow solid. Mp 105−107 °C. IR (KBr), $\nu_{C=O}$ 1720, 1760 cm^{-1}. HRMS (FAB$^+$) (M + H)$^+$ calcd $C_{47}H_{43}N_4O_{14}S$ 919.4296, found 919.2501. ^{1}H NMR (400 MHz, $CDCl_3$): 1.66 (3H, s, CH_3CO), 2.05 (3H, s, CH_3CO), 2.10 (3H, s, CH_3CO), 2.13 (3H, s, CH_3CO), 4.05 (1H,

m), 4.18 (1H, dd, ^1J = 12.5 Hz, ^2J = 1.5 Hz), 4.30 (1H, dd, ^1J = 12.6 Hz, ^2J = 4.7 Hz), 4.72 (2H, s, CH_2), 5.23 (2H, s, CH_2), 5.30 (1H, m), 5.51 (2H, m), 6.25 (1H, m), 6.48 (1H, dd, ^1J = 8.0 Hz, ^2J = 4.7 Hz), 6.83 (1H, s), 6.84 (1H, s), 6.85 (1H, dd, ^1J = 8.1 Hz, ^2J = 1.0 Hz), 7.16−7.32 (4H, m), 7.39 (2H, d, J = 7.4 Hz), 7.52 (2H, t, J = 7.9 Hz), 7.63 (1H, t, J = 7.6 Hz), 7.95−7.99 (3H, m), 8.06 (1H, br s), 8.15 (2H, m), 12.05 (1H, s, NH). ^{13}C NMR (100 MHz, $CDCl_3$): 20.0, 20.5 (2C), 20.7 (CH_3CO), 61.7 ($C_{6'}$), 67.4, 71.7 (CH_2), 68.0, 70.9, 72.9, 74.8, 80.3 ($C_{1'}$, $C_{2'}$, $C_{3'}$, $C_{4'}$, $C_{5'}$), 113.4, 117.5, 121.9, 123.4, 125.1, 126.8 (2C), 127.5 (3C), 127.6, 128.3 (3C), 129.4 (2C), 129.8, 134.2, 143.9 (C tert arom), 105.8, 112.1, 118.2, 125.3, 128.2, 130.4, 134.4, 137.6 (2C), 147.4 (C quat arom), 168.7, 169.4, 169.8, 170.1, 170.3, 170.6 (C═O).

1-[(Benzyloxy)methyl]-3-(1*H*-indol-3-yl)-4-[1-(2,3,4,6-tetra-*O*-acetyl-*β*-D-glucopyranosyl)-1*H*-pyrrolo[2,3-*b*]pyridin-3-yl]-1*H*-pyrrole-2,5-dione (19). To a solution of compound **18** (519 mg, 0.565 mmol) in THF (20 mL) was added a 1.1 M solution of nBu$_4$NF in THF (1.70 mL, 1.86 mmol). The mixture was stirred for 2.5 h at room temperature. Water (45 mL) was added. After extraction with EtOAc, the organic phase was dried over $MgSO_4$, the solvent was removed, and the residue was purified by flash chromatography (eluent cyclohexane:EtOAc, 2:3) to give **19** (318 mg, 0.409 mmol, 72% yield) as an orange solid. Mp 117−119 °C. IR (KBr), $\nu_{C=O}$ 1710, 1760 cm^{-1}, ν_{NH} 3300−3500 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$): 1.71 (3H, s, CH_3CO), 2.03 (3H, s, CH_3CO), 2.07 (3H, s, CH_3CO), 2.09 (3H, s, CH_3CO), 4.05 (1H, m), 4.15 (1H, J = 11.7 Hz), 4.27 (1H, dd, ^1J = 12.6 Hz, ^2J = 4.7 Hz), 4.72 (2H, s, CH_2), 5.22 (2H, d, J = 2.0 Hz, CH_2), 5.28 (1H, t, J = 9.9 Hz), 5.50 (1H, t, J = 9.4 Hz), 5.59 (1H, t, J = 9.4 Hz), 6.29 (1H, d, J = 9.3 Hz, $H_{1'}$), 6.69 (1H, t, J = 7.8 Hz), 6.70 (1H, t, J = 7.7 Hz), 6.80 (1H, d, J = 8.1 Hz), 7.02 (1H, t, J = 7.4 Hz), 7.16−7.33 (5H, m), 7.39 (2H, d, J = 7.4 Hz), 7.82 (1H, d, J = 2.1 Hz), 8.00 (1H, s), 8.17 (1H, dd, ^1J = 3.7 Hz, ^2J = 1.0 Hz), 9.35 (1H, s, NH). ^{13}C NMR (100 MHz, $CDCl_3$): 20.1, 20.5, 20.6, 20.8 (CH_3CO), 61.9 ($C_{6'}$), 67.3, 71.7 (CH_2), 68.1, 70.6, 73.3, 74.7, 80.3 ($C_{1'}$, $C_{2'}$, $C_{3'}$, $C_{4'}$, $C_{5'}$), 111.5, 117.3, 120.6, 121.6, 122.7, 127.7 (2C), 127.8, 128.1, 128.4 (2C), 129.2, 130.6, 143.7 (C tert arom), 106.3, 106.8, 119.1, 125.2, 125.6, 129.4, 136.0, 137.7, 147.6 (C quat arom), 168.9, 169.5, 170.0, 170.8, 171.2, 171.5 (C═O).

6-[(Benzyloxy)methyl]-13-(2,3,4,6-tetra-*O*-acetyl-*β*-D-glucopyranosyl)-12,13-dihydro-5*H*-pyrido[3′,2′:4,5]pyrrolo-[2,3-*a*]pyrrolo[3,4-*c*]carbazole-5,7(6*H*)-dione (20). A mixture of **19** (318 mg, 0.409 mmol), benzene (500 mL), and iodine (1.245 g, 4.90 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 (150 mL) was washed with saturated aqueous sodium thiosulfate and then brine. The organic phase was dried over $MgSO_4$. After removal of the solvent, the residue was purified by flash chromatography (eluent cyclohexane:EtOAc, 3:2) to give **20** (247 mg, 0.318 mmol, 78% yield) as a pale yellow solid. Mp 109−111 °C. IR (KBr), $\nu_{C=O}$ 1710, 1760 cm^{-1}, ν_{NH} 3300−3500 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$): 1,87 (3H, s, CH_3CO), 2.01 (3H, s, CH_3CO), 2.11 (3H, s, CH_3CO), 2.32 (3H, s, CH_3CO), 4.34 (1H, dd, ^1J = 9.9 Hz, ^2J = 2.0 Hz), 4.55 (1H, d, J = 11.6 Hz), 4.75 (2H, AB system, J = 11.9 Hz, $\Delta\nu$ = 20.8 Hz), 4.88 (1H, dd, ^1J = 13.1 Hz, ^2J = 3.1 Hz), 5.14 (2H, AB system, J = 11.0 Hz, $\Delta\nu$ = 9.2 Hz), 5.30 (1H, pt, J = 9.6 Hz), 5.50 (1H, pt, J = 9.2 Hz), 5.57 (1H, pt, J = 9.7 Hz), 6.84 (1H, d, J = 9.5 Hz, $H_{1'}$), 7.23 (1H, t, J = 7.3 Hz), 7.29−7.37 (4H, m), 7.43−7.49 (4H, m), 8.54 (1H, dd, ^1J = 4.6 Hz, ^2J = 1.5 Hz), 9.11 (1H, d, J = 8.0 Hz), 9.15 (1H, dd, ^1J = 7.8 Hz, ^2J = 1.6 Hz), 9.80 (1H, s, NH). ^{13}C NMR (100 MHz, $CDCl_3$): 18.9, 20.3, 20.4, 21.2 (CH_3CO), 61.1 ($C_{6'}$), 66.6, 71.4 (CH_2), 67.1, 70.4, 72.8, 76.1, 81.9 ($C_{1'}$, $C_{2'}$, $C_{3'}$, $C_{4'}$, $C_{5'}$), 111.4, 118.1, 121.4, 125.5, 127.7, 127.9, 128.3 (2C), 128.7 (2C), 133.9, 146.9 (C tert arom), 115.0, 116.0, 118.9, 119.3, 121.2, 122.1, 127.1, 129.5, 137.6, 140.5, 151.8 (C quat arom), 167.8, 168.6, 168.8, 169.3, 169.6, 170.4 (C═O).

6-(Hydroxymethyl)-13-(2,3,4,6-tetra-*O*-acetyl-*β*-D-glucopyranosyl)-12,13-dihydro-5*H*-pyrido[3′,2′:4,5]pyrrolo-[2,3-*a*]pyrrolo[3,4-*c*]carbazole-5,7(6*H*)-dione (21). A mixture of **20** (52 mg, 0.067 mmol), methanol (3 mL), EtOAc (1 mL), and 10% Pd/C (18 mg) was hydrogenated (1 bar) at room temperature for 24 h. 10% Pd/C (21 mg) was added and the mixture hydrogenated (1 bar) for 48 h. After filtration over Celite, the solid was washed with methanol and EtOAc. After removal of the solvent, the residue was purified by flash chromatography (eluent cyclohexane:EtOAc, 65:35) to give unreacted compound **20** (10.5 mg, 0.0135 mmol), **21** (21 mg, 0.031 mmol, 46% yield) (eluent cyclohexane:EtOAc, 3:2) as a pale yellow solid, and a mixture of partially deacetylated compounds.

21: Mp 154−156 °C. IR (KBr), $\nu_{C=O}$ 1705, 1760 cm^{-1}, ν_{NH} 3200−3600 cm^{-1}. HRMS (FAB$^+$) (M + H)$^+$ calcd $C_{34}H_{31}N_4O_{12}$ 687.1938, found 687.1939. ^{1}H NMR (400 MHz, $CDCl_3$): 0.81 (3H, s, CH_3CO), 1.80 (3H, s, CH_3CO), 2.11 (3H, s, CH_3CO), 2.43 (3H, s, CH_3CO), 4.53 (1H, m), 4.88 (1H, m), 5.08 (1H, dd, ^1J = 13.1 Hz, ^2J = 2.4 Hz), 5.17 (1H, d, J = 12.3 Hz), 5.26−5.32 (3H, m, 2H + OH), 5.42−5.48 (2H, m), 6.65 (1H, d, J = 9.6 Hz, $H_{1'}$), 6.93 (1H, m), 7.26−7.31 (2H, m), 7.47 (1H, dd, ^1J = 7.8 Hz, ^2J = 4.9 Hz), 8.70 (1H, dd, ^1J = 4.8 Hz, ^2J = 1.5 Hz), 8.75 (1H, dd, ^1J = 7.8 Hz, ^2J = 1.6 Hz), 9.03 (1H, m), 9.36 (1H, s, NH). ^{13}C NMR (100 MHz, $CDCl_3$): 18.8, 20.4, 20.5, 21.7 (CH_3CO), 60.7, 61.1 (CH_2OH, $C_{6'}$), 66.9, 70.2, 73.1, 76.8, 83.0 ($C_{1'}$, $C_{2'}$, $C_{3'}$, $C_{4'}$, $C_{5'}$), 111.2, 118.4, 121.0, 124.8, 127.9, 135.1, 147.5 (C tert arom), 115.7, 115.9, 117.7, 118.8, 121.4, 121.6, 126.2, 129.0, 140.1, 151.7 (C quat arom), 167.5 (2C), 168.3, 169.5, 169.8, 170.6 (C═O).

13-(*β*-D-Glucopyranosyl)-12,13-dihydro-5*H*-pyrido[3′,2′f:4,5]pyrrolo[2,3-*a*]pyrrolo[3,4-*c*]carbazole-5,7(6*H*)-dione (22). A mixture of **21** (21 mg, 0.030 mmol), methanol (9.4 mL), and 28% aqueous NH_4OH (8 mL) was stirred for 19 h at room temperature. After removal of the solvents, a mixture of water/EtOAc was added to the residue. After filtration, the solid was washed successively with EtOAc and then methanol. Compound **22** (8,4 mg, 0.017 mmol, 57% yield) was obtained as a yellow solid. Mp > 300 °C. IR (KBr), $\nu_{C=O}$ 1710, 1740 cm^{-1}, $\nu_{NH,OH}$ 3100−3600 cm^{-1}. HRMS (FAB$^+$) (M + H)$^+$ calcd $C_{25}H_{21}N_4O_7$ 489.1410, found 489.1404. ^{1}H NMR (400 MHz, DMSO-d_6): 3.58 (2H, m), 3.87 (1H, d, J = 10.1 Hz), 3.95 (1H, d, J = 9.5 Hz), 4.04 (1H, m), 4.12 (1H, d, J = 10.3 Hz), 4.97 (1H, br s, OH), 5.22 (1H, br s, OH), 5.44 (1H, d, J = 4.2 Hz, OH), 6.14 (1H, br s, OH), 6.64 (1H, d, J = 6.6 Hz, $H_{1'}$), 7.43 (1H, t, J = 7.4 Hz), 7.53 (1H, t, J = 6,5 Hz), 7.64 (1H, t, J = 7.4 Hz), 7.76 (1H, d, J = 8.2 Hz), 8.67 (1H, d, J = 3.6 Hz), 9.11 (1H, d, J = 7.9 Hz), 9.36 (1H, d, J = 7.6 Hz), 11.25 (1H, s, NH), 11.72 (1H, s, NH). ^{13}C NMR (100 MHz, DMSO-d_6): 58.3 ($C_{6'}$), 67.6, 72.7, 76.6, 78.7, 83.1 ($C_{1'}$, $C_{2'}$, $C_{3'}$, $C_{4'}$, $C_{5'}$), 112.2, 117.4, 120.6, 124.5, 127.5, 132.8, 147.0 (C tert arom), 114.3, 115.3, 117.7, 120.0, 121.3, 121.6, 127.4, 129.6, 141.0, 152.4 (C quat arom), 170.9 (2C) (C═O). HPLC >97% pure, 1) t_R = 3.77 min, 2) t_R = 3.05 min.

9-Bromo-13-(*β*-D-glucopyranosyl)-12,13-dihydro-5*H*-pyrido[3′,2′:4,5]pyrrolo[2,3-*a*]pyrrolo[3,4-*c*]carbazole-5,7-(6*H*)-dione (23). To a solution of **21** (30 mg, 0.043 mmol) in THF (2 mL) cooled to 0 °C was added dropwise a solution of *N*-bromosuccinimide (154 mg, 0.866 mmol) in THF (1.5 mL). The light-protected mixture was stirred for 5 days at room temperature. Water (30 mL) was added, and the mixture was stirred for 15 min before addition of saturated aqueous sodium thiosulfate (20 mL). After extraction with EtOAc, the organic phase was dried over $MgSO_4$. After removal of the solvent, brominated derivative was obtained as a yellow solid which was further dissolved in methanol (8 mL), and then 28% aqueous NH_4OH (9 mL) was added. The light-protected mixture was stirred for 22 h at room temperature. After removal of the solvents, a mixture of water/EtOAc was added to the residue. After filtration, the solid was washed with EtOAc. Compound **23** (13 mg, 0.023 mmol, 53% yield) was obtained as a yellow solid. Mp > 300 °C. IR (KBr), $\nu_{C=O}$ 1710, 1750 cm^{-1}, $\nu_{NH,OH}$ 3200−3600 cm^{-1}. HRMS (FAB$^+$) (M + H)$^+$ calcd $C_{25}H_{20}N_4O_7Br$ 567.0515, found 567.0519. ^{1}H NMR (400 MHz, DMSO-d_6): 3.48−3.61 (2H, m), 3.87 (1H, d, J = 10.4 Hz), 3.93−4.01 (2H, m), 4.11 (1H, d, J = 12.2 Hz), 4.98 (1H, d, J = 4.8 Hz, OH), 5.21 (1H, d, J = 5.1 Hz, OH), 5.42 (1H, d, J = 5.0 Hz, OH), 6.17 (1H, br s, OH), 6.65 (1H, d, J = 8.5 Hz,

$H_{1'}$), 7.54 (1H, dd, ^1J = 7.3 Hz, ^2J = 4.7 Hz), 7.71 (1H, d, J = 8.7 Hz), 7.80 (1H, dd, ^1J = 8.7 Hz, ^2J = 1.3 Hz), 8.68 (1H, d, J = 4.7 Hz), 9.26 (1H, d, J = 1.5 Hz), 9.35 (1H, dd, ^1J = 7.7 Hz, ^2J = 1.4 Hz), 11.84 (1H, s, NH). ^{13}C NMR (100 MHz, DMSO-d_6): 58.3 ($C_{6'}$), 67.6, 72.7, 76.5, 78.7, 83.1 ($C_{1'}$, $C_{2'}$, $C_{3'}$, $C_{4'}$, $C_{5'}$), 114.2, 117.6, 126.5, 129.9, 132.9, 147;3 (C tert arom), 112.5, 114.1, 115.7, 116.4, 120.5, 121.7, 123.1, 127.4, 129.8, 139.6, 152.4 (C quat arom), 170.8, 170.9 (C═O). HPLC >95% pure, 1) t_R = 4.64 min, 2) t_R = 3.28 min.

9-Nitro-13-(*β*-D-glucopyranosyl)-12,13-dihydro-5*H*-pyrido[3′,2′:4,5]pyrrolo[2,3-*a*]pyrrolo[3,4-*c*]carbazole-5,7-(6*H*)-dione (24). To Mallinckrodt ether (a solution of fuming nitric acid (2,1 mL) in THF (13 mL)) at 0 °C was added dropwise **21** (42 mg, 0.061 mmol) cooled to 0 °C. After stirring for 10 min, the mixture was allowed to reach room temperature and then stirred for 21 h. Water (40 mL) was added. After extraction with EtOAc, the organic phase was dried over $MgSO_4$, the solvent was removed to give the nitrated compound which was further dissolved in methanol (10 mL), and then 28% aqueous NH_4OH (17 mL) was added. The light-protected mixture was stirred for 16 h at room temperature. After removal of the solvents, a mixture of water/EtOAc was added to the residue. After filtration, the solid was washed with EtOAc. Compound **24** (12.5 mg, 0.023 mmol, 38% yield) was obtained as a brown solid. Mp > 300 °C. IR (KBr), $\nu_{C=O}$ 1710, 1760 cm^{-1}, $\nu_{NH,OH}$ 3300−3600 cm^{-1}. HRMS (FAB$^+$) (M + H)$^+$ calcd $C_{25}H_{20}N_5O_9$ 534.1261, found 534.1268. ^{1}H NMR (400 MHz, DMSO-d_6): 3.56−3.59 (2H, m), 3.91 (1H, d, J = 7.9 Hz), 4.00 (2H, m), 4.13 (1H, m), 4.99 (1H, br s, OH), 5.20 (1H, br s, OH), 5.44 (1H, br s, OH), 6.29 (1H, br s, OH), 6.66 (1H, d, J = 7.9 Hz, $H_{1'}$), 7,55 (1H, m), 7.87 (1H, d, J = 8.7 Hz), 8.54 (1H, m), 8.69 (1H, m), 9.35 (1H, d, J = 7.1 Hz), 10.00 (1H, m), 11.47 (1H, s, NH), 12.19 (1H, s, NH). ^{13}C NMR (100 MHz, DMSO-d_6): 58.3 ($C_{6'}$), 67.6, 72.8, 76.4, 78.7, 83.1 ($C_{1'}$, $C_{2'}$, $C_{3'}$, $C_{4'}$, $C_{5'}$), 112.6, 117.7, 121.1, 122.6, 133.0, 147.6 (C tert arom), 114.0, 116.5, 117.6, 120.9, 121.5, 121.7, 127.2, 130.7, 141.2, 144.0, 152.4 (C quat arom), 170.5 (2C) (C═O). HPLC >96% pure, (1) t_R = 3.75 min, (2) t_R = 3.47 min.

1-[(Benzyloxy)methyl]-3-bromo-4-[1-(phenylsulfonyl)-1*H*-pyrrolo[2,3-*b*]pyridin-3-yl]-1*H*-pyrrole-2,5-dione (25). A solution of ethylmagnesium bromide was prepared from Mg (146 mg, 6.00 mmol) and C_2H_5Br (452 μL, 6.00 mmol) in THF (2.5 mL). The mixture was stirred for 1 h at room temperature, and then commercially available 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, and 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_4Cl was added. After extraction with EtOAc, the organic phase was dried over $MgSO_4$, the solvent was removed, and the residue was purified by flash (eluent cyclohexane:EtOAc, 3:2, then toluene:EtOAc, 7:3) to give 1-[(benzyloxy)methyl]-3-bromo-4-[1*H*-pyrrolo[2,3-*b*]pyridin-3-yl]-1*H*-pyrrole-2,5-dione (471 mg, 1.14 mmol, 57% yield). 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 the previous isolated compound (330 mg, 0.800 mmol) in THF (10 mL). The mixture was stirred at 0 °C for 1 h, and then benzenesulfonyl chloride (125 mL, 0.98 mmol) was added dropwise. The mixture was stirred at room temperature for 4 h, and then saturated aqueous NH_4Cl was added. After extraction with EtOAc, the organic phase was washed with brine and dried over $MgSO_4$, the solvent was removed, and the residue was purified by flash chromatography (eluent cyclohexane:EtOAc, 4:1) to give **25** (333 mg, 0.60 mmol, 75% yield) as a yellow solid: mp123−125 °C. IR (KBr) ν_{CO} 1730, 1780 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) 4.68 (2H, s, CH_2), 5.18 (2H, s, CH_2), 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, ^1J = 8.0 Hz, ^2J = 1.3 Hz), 8.29 (1H, br s), 8.30 (1H, br s), 8.48 (1H, s), 8.51 (1H, dd, ^1J = 4.7 Hz, ^2J = 1.3 Hz). ^{13}C NMR (100 MHz, $CDCl_3$) 67.9, 72.1 (CH_2), 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*H*-indol-3-yl)-4-[1-(phenylsulfonyl)-1*H*-pyrrolo[2,3-*b*]pyridin-3-yl]-1*H*-pyrrole-2,5-dione (26). A solution of ethylmagnesium bromide was prepared from Mg (15 mg, 0.62 mmol) and bromoethane (47 μL, 0.62 mmol) in THF (0.4 mL). The solution was stirred at room temperature for 15 min and then at 40 °C for 20 min. A solution of indole (76 mg, 0.65 mmol) in toluene (3 mL) was then added dropwise. After stirring for 1 h at 40 °C, the mixture was cooled, then a solution of **X** (140 mg, 0.254 mmol) in toluene (5 mL) was added dropwise. The mixture was stirred for 15 h and then hydrolyzed with saturated aqueous NaCl (15 mL). After extraction with EtOAc, the organic phase was dried over $MgSO_4$, the solvent was removed, and the residue was purified by flash chromatography (eluent cyclohexane: EtOAc, 3:2) to give **26** (122 mg, 0.208 mmol, 82% yield) as a red solid: mp 86−88 °C; IR (KBr) ν_{CO} 1710, 1730 cm^{-1}, ν_{NH} 3300−3440 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) 4.75 (2H, s, CH_2), 5.24 (2H, s, CH_2), 6.53 (1H, dt, ^1J = 7.1 Hz, ^2J = 0.6 Hz), 6.60 (1H, d, J = 7.9 Hz), 6.89 (1H, dd, ^1J = 8.0 Hz, ^2J = 4.7 Hz), 7.07 (1H, td, ^1J = 7.2 Hz, ^2J = 0.9 Hz), 7.21−7.40 (7H, m), 7.51 (2H, t, J = 8.0 Hz), 7.63 (1H, td, ^1J = 7.4 Hz, ^2J = 0.9 Hz), 7.91 (1H, d, J = 2.9 Hz), 8.04 (1H, s), 8.12 (1H, d, J = 1.3 Hz), 8.16 (1H, br s), 8.34 (1H, dd, ^1J = 4.8 Hz, ^2J = 1.5 Hz), 9.11 (1H, s, NH). ^{13}C NMR (100 MHz, $CDCl_3$) 67.3, 71.7 (CH_2), 112.0, 118.9, 120.9, 121.2, 122.8, 127.6 (2C), 127.7, 127.8, 128.1, 128.2, 128.3 (2C), 129.1, 129.2, 130.4, 131.0, 134.3, 145.3 (C tert arom), 105.6, 109.4, 121.1, 122.8, 124.2, 132.2, 136.2, 137.5, 137.7, 146.6 (C quat arom), 170.7, 170.9 (C═O).

1-[(Benzyloxy)methyl]-3-[1-(2,3,4,6-tetra-*O*-acetyl-*β*-D-glucopyranosyl)-1*H*-indol-3-yl]-4-[1-(phenylsulfonyl)-1*H*-pyrrolo[2,3-*b*]pyridin-3-yl]-1*H*-pyrrole-2,5-dione (27). To a solution of **26** (650 mg, 1.105 mmol) in THF (10 mL) were added 2,3,4,6-*O*-acetylglucopyranose (616 mg, 1.769 mmol) and PPh_3 (464 mg, 1.769 mmol). The mixture was cooled to −78 °C, and then DEAD (282 μL, 1.769 mmol) was added dropwise. The mixture was allowed to reach room temperature and then stirred at room temperature for 15 h. Water (80 mL) was added. After extraction with EtOAc, the organic phase was dried over $MgSO_4$, the solvent was removed, and the residue was purified by flash chromatography (cyclohexane:EtOAc, 1:1, then toluene:EtOAc, 3:2) to give *β*-glycosylated compound **27** (373 mg, 0.40 mmol, 37% yield), a red solid as the major product of the reaction. Mp 80−82 °C. IR (KBr), $\nu_{C=O}$ 1710, 1760 cm^{-1}. HRMS (FAB$^+$) (M + H)$^+$ calcd $C_{47}H_{43}N_4O_{14}S$ 919.2496, found 919.2501. ^{1}H NMR (400 MHz, $CDCl_3$): 1.72 (3H, s, CH_3CO), 2.00 (3H, s, CH_3CO), 2.09 (3H, s, CH_3CO), 2.12 (3H, s, CH_3CO), 4.11−4.27 (2H, m), 4.35 (1H, dd, ^1J = 12.6 Hz, ^2J = 4.7 Hz), 4.69 (2H, s, CH_2), 5.20 (2H, s, CH_2), 5.48−5.50 (2H, m), 5.66 (1H, m), 6.43−6.51 (2H, m), 6.88 (1H, dd, ^1J = 8.0 Hz, ^2J = 4.8 Hz), 7.09 (1H, t, J = 7.4 Hz), 7.21 (1H, d, J = 7.4 Hz), 7.25 (3H, t, J = 7.2 Hz), 7.36 (3H, d, J = 7.5 Hz), 7.44−7.54 (3H, m), 7.61 (1H, t, J = 4.6 Hz), 7.98 (1H, s), 8.01 (1H, s), 8.10 (2H, d, J = 7.6 Hz), 8.30 (1H, d, J = 4.6 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): 20.0, 20.4, 20.6, 20.7 (CH_3-CO), 61.7 ($C_{6'}$), 67.0, 71.8 (CH_2), 68.0, 70.9, 72.8, 75.0, 84.3 ($C_{1'}$, $C_{2'}$, $C_{3'}$, $C_{4'}$, $C_{5'}$), 110.9, 119.0, 121.6, 121.7, 123.3, 127.6 (2C), 127.8, 128.2, 128.3 (2C), 128.4 (2C), 129.1 (2C), 130.0, 130.9, 134.3, 145.4 (C tert arom), 106.7, 109.2, 121.1, 124.5, 125.5, 130.8, 135.7, 137.6, 137.8, 146.7 (C quat arom), 168.5, 169.4, 170.1, 170.4, 170.6, 171.2 (C═O).

1-[(Benzyloxy)methyl]-3-[1-(2,3,4,6-tetra-*O*-acetyl-*β*-D-glucopyranosyl)-1*H*-indol-3-yl]-4-(1*H*-pyrrolo[2,3-*b*]pyridin-3-yl)-1*H*-pyrrole-2,5-dione (28) and 1-[(Benzyloxy)-methyl]-3-[1-(2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl)-1*H*-indol-3-yl]-4-(1*H*-pyrrolo[2,3-*b*]pyridin-3-yl)-1*H*-pyrrole-2,5-dione (29). To a solution of **26** (50 mg, 0.085 mmol) in toluene (7 mL) were added Ag_2O (197 mg, 0.85 mmol) and 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide (175 mg, 0.425 mmol). The mixture was refluxed for 3 h and then cooled and filtered over Celite. After removal of the solvent, the residue was purified by flash chromatography (eluent cyclohexane:EtOAc, 65:35) to give a mixture of α-glycosylated

compound **29A** and unreacted sugar (73 mg), and then (eluent cyclohexane:EtOAc, 1:1) a mixture of β-glycosylated compound **28A** and unreacted sugar (6 mg).

To a solution of a mixture of α-glycosylated compound **29A** and α-D-acetobromoglucose (73 mg) in THF (5 mL) was added a 1.1 M solution of nBu$_4$NF in THF (217 mL, 0.238 mmol). The mixture was stirred at room temperature for 3 h. Water (20 mL) was added. After extraction with EtOAc, the organic phase was dried over $MgSO_4$, the solvent was removed, and the residue was purified by flash chromatography (eluent cyclohexane:EtOAc, 1:1) to give **29** (34 mg, 0.043 mmol, 51% yield over 2 steps) as an orange solid.

To a solution of a mixture of β-glycosylated compound **28A** and α-D-acetobromoglucose (6 mg) in THF (2 mL) was added a 1.1 M solution of nBu$_4$NF in THF (18 mL, 0.016 mmol). The mixture was stirred at room temperature for 3 h. Water (5 mL) was added, and after identical workup as described above, a purification by flash chromatography (eluent cyclohexane: EtOAc, 1:1) gave **28** (4 mg, 0.005 mmol, 6% yield for two steps) as a red solid.

28 was also obtained from **27** in 51% yield by treatment with nBu$_4$NF in THF according to identical procedure as above-described.

28: Mp 115−117 °C. IR (KBr), $\nu_{C=O}$ 1700, 1760 cm^{-1}, ν_{NH} 3100−3600 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$): 1.79 (3H, s, CH_3-CO), 2.05 (3H, s, CH_3CO), 2.11 (3H, s, CH_3CO), 2.14 (3H, s, CH_3CO), 4.06 (1H, m), 4.26 (1H, dd, ^1J = 12.5 Hz, ^2J = 2.0 Hz), 4.36 (1H, dd, ^1J = 12.6 Hz, ^2J = 4.7 Hz), 4.73 (2H, s, CH_2), 5.23 (2H, s, CH_2), 5.35 (1H, t, J = 9.8 Hz), 5.50 (1H, t, J = 9.4 Hz), 5.57 (1H, t, J = 9.3 Hz), 5.67 (1H, d, J = 8.8 Hz), 6.69−6.78 (3H, m), 7.11 (1H, t, J = 7.1 Hz), 7.24 (2H, d, J = 7.3 Hz), 7.32 (2H, t, J = 7.3 Hz), 7.40 (2H, d, J = 7.3 Hz), 7.46 (1H, d, J = 8.4 Hz), 7.90 (1H, s), 8.05 (1H, s), 8.21 (1H, d, J = 4.5 Hz), 11.97 (1H, s, NH). ^{13}C NMR (100 MHz, $CDCl_3$): 20.0, 20.5 (2C), 20.7 (CH_3CO), 61.7 ($C_{6'}$), 67.1, 71.5 (CH_2), 67.9, 70.6, 72.9, 74.9, 84.3 ($C_{1'}$, $C_{2'}$, $C_{3'}$, $C_{4'}$, $C_{5'}$), 110.5, 116.3, 121.3, 122.0, 123.0, 127.5 (2C), 127.6 (2C), 128.3, 128.7, 129.6, 130.6, 143.0 (C tert arom), 105.2, 107.4, 118.8, 126.5, 126.6, 128.0, 135.4, 137.6, 148.4 (C quat arom), 168.5, 169.3, 170.0, 170.5, 171.2, 171.4 (C═O).

29: Mp 107−109 °C. IR (KBr), $\nu_{C=O}$ 1710, 1750 cm^{-1}, ν_{NH} 3300−3600 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$): 1.98 (3H, s, CH_3-CO), 2.05 (3H, s, CH_3CO), 2.13 (3H, s, CH_3CO), 2.16 (3H, s, CH_3CO), 4.02 (1H, dd, ^1J = 5.1 Hz, ^2J = 2.4 Hz), 4.08 (1H, m), 4.22 (1H, dd, ^1J = 12.2 Hz, ^2J = 5.7 Hz), 4.28 (1H, dd, ^1J = 12.2 Hz, ^2J = 2.7 Hz), 4.73 (2H, s, CH_2), 4.94 (1H, d, J = 9.4 Hz), 5.24 (2H, s, CH_2), 5.27 (1H, pt, J = 1.8 Hz), 5.52 (1H, d, J = 5.1 Hz, $H_{1'}$), 6.73 (1H, dd, ^1J = 8.1 Hz, ^2J = 4.8 Hz), 7.00 (1H, t, J = 7.7 Hz), 7.04 (1H, dd, ^1J = 7.8 Hz, ^2J = 1.0 Hz), 7.20−7.34 (5H, m), 7.41 (2H, d, J = 7.4 Hz), 7.58 (1H, s), 7.76 (1H, d, J = 8.3 Hz), 8.09 (1H, s), 8.25 (1H, dd, ^1J = 4.7 Hz, ^2J = 1.0 Hz), 11.69 (1H, s, NH). ^{13}C NMR (100 MHz, $CDCl_3$): 20.7, 20.8, 20.9, 24.1 (CH_3CO), 63;2 ($C_{6'}$), 67.3, 71.7 (CH_2), 67.2, 68.3, 69.4, 73.0, 97.3 ($C_{1'}$, $C_{2'}$, $C_{3'}$, $C_{4'}$, $C_{5'}$), 112.7, 116.0, 121.5, 122.1, 123.5, 127.7 (2C), 127.8, 128.2, 128.4 (2C), 130.2, 130.8, 143.2 (C tert arom), 105.1, 107.0, 113.1, 117.8, 127.4, 128.6, 134.4, 137.8, 148.9 (C quat arom), 169.0, 169.6, 170.8, 171.2, 171.3, 171.4 (C═O).

6-[(Benzyloxymethyl)]-12-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-12,13-dihydro-5*H*-pyrido[3′,2′:4,5]pyrrolo-[2,3-*a*]pyrrolo[3,4-*c*]carbazole-5,7(6*H*)-dione (30). To a solution of **28** (194 mg, 0.249 mmol) in benzene (150 mL) was added iodine (758 mg, 2.99 mmol). The mixture was irradiated for 1.5 h with a medium-pressure mercury lamp (400 W). The solvent was removed, and the residue dissolved in EtOAc (80 mL) was washed with aqueous sodium thiosulfate then with brine. The organic phase was dried over $MgSO_4$, the solvent was removed, and the residue was purified by flash chromatography (eluent cyclohexane:EtOAc, 65:35) to give **30** (131 mg, 0.168 mmol, 68% yield) as a pale yellow solid. Mp 166−168 °C. IR (KBr), $\nu_{C=O}$ 1710, 1760 cm^{-1}, ν_{NH} 3360−3420 cm^{-1}. HRMS (FAB$^+$) (M + H)$^+$ calcd $C_{41}H_{37}N_4O_{12}$ 777.2408, found 777.2409. ^{1}H NMR (400 MHz, $CDCl_3$): 0.99 (3H, s, CH_3CO), 1.91 (3H, s, CH_3CO), 2.14 (3H, s, CH_3CO), 2.67 (3H, s, CH_3-CO), 4.31 (1H, d, J = 10.1 Hz), 4.51 (1H, d, J = 12.4 Hz), 4.80 (2H, AB system, J = 12.0 Hz, $\Delta\nu$ = 7.8 Hz), 4.88 (1H, d, J = 11.8 Hz), 5.23−5.31 (3H, m), 5.49 (1H, t, J = 9.5 Hz), 5.66 (1H, t, J = 9.7 Hz), 6.03 (1H, d, J = 8.9 Hz, $H_{1'}$), 7.27−7.42 (5H, m), 7.48−7.51 (3H, m), 7.61 (1H, t, J = 7.7 Hz), 8.48 (1H, d, J = 3.7 Hz), 9.12 (1H, d, J = 7.9 Hz), 9.33 (1H, d, J = 7.6 Hz), 10.23 (1H, s, NH). ^{13}C NMR (100 MHz, $CDCl_3$): 18.5, 20.5, 20.6, 21.1 (CH_3CO), 60.5 ($C_{6'}$), 66.9, 71.7 (CH_2), 67.0, 70.8, 72.8, 76.7, 84.3 ($C_{1'}$, $C_{2'}$, $C_{3'}$, $C_{4'}$, $C_{5'}$), 110.1, 117.6, 122.3, 126.1, 127.7, 128.0, 128.1 (2C), 128.5 (2C), 133.6, 147.8 (C tert arom), 114.8 (2C), 116.5, 119.1, 120.6 (2C), 121.6, 128.4, 137.7, 141.2, 152.3 (C quat arom), 167.8, 168.8, 169.0, 169.1, 170.0, 171.9 (C═O).

6-(Hydroxymethyl)-12-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-12,13-dihydro-5*H*-pyrido[3′,2′:4,5]pyrrolo-[2,3-*a*]pyrrolo[3,4-*c*]carbazole-5,7(6*H*)-dione (31). A mixture of **30** (70 mg, 0.090 mmol), methanol (40 mL), EtOAc (20 mL), and 10% Pd/C (60 mg) was hydrogenated (1 bar) at room temperature for 17 h. Pd/C (10%) (31 mg) was added and the mixture hydrogenated for 21 h. After filtration over Celite, the residue was washed with methanol and chloroform. The solvents were removed, and the residue was purified by flash chromatography (eluent cyclohexane:EtOAc, 1:1) to give **31** (44 mg, 0.063 mmol, 70% yield) as a pale yellow solid. Mp 264−266 °C. IR (KBr), $\nu_{C=O}$ 1710, 1760 cm^{-1}, ν_{NH} 3300−3600 cm^{-1}. HRMS (FAB$^+$) (M + H)$^+$ calcd $C_{34}H_{31}N_4O_{12}$ 687.1938, found 687.1946. ^{1}H NMR (400 MHz, $CDCl_3$): 0.89 (3H, s, CH_3CO), 1.87 (3H, s, CH_3CO), 2.12 (3H, s, CH_3CO), 2.62 (3H, s, CH_3-CO), 4.44 (1H, d, J = 9.9 Hz), 4.64 (1H, d, J = 12.5 Hz), 4.99 (1H, d, J = 11.4 Hz), 5.12 (1H, t, J = 9.3 Hz), 5.30 (2H, AB system, J = 11.2 Hz, $\Delta\nu$ = 7.0 Hz), 5.51 (1H, t, J = 9.5 Hz), 5.62 (1H, t, J = 9.7 Hz), 5.97 (1H, d, J = 9.3 Hz, $H_{1'}$), 7.25 (1H, dd, ^1J = 7.7 Hz, ^2J = 4.8 Hz), 7.32−7.35 (2H, m), 7.49 (1H, t, J = 7.5 Hz), 8.49 (1H, d, J = 4.1 Hz), 9.06 (1H, d, J = 6.6 Hz), 9.07 (1H, d, J = 7.3 Hz), 10.23 (1H, s, NH). ^{13}C NMR (100 MHz, $CDCl_3$): 18.8, 20.3, 20.5, 21.2 (CH_3CO), 60.5, 61.3 (CH_2OH, $C_{6'}$), 67.2, 70.5, 72.8, 77.0, 84.4 ($C_{1'}$, $C_{2'}$, $C_{3'}$, $C_{4'}$, $C_{5'}$), 110.0, 117.5, 122.0, 125.8, 127.7, 133.8, 147.8 (C tert arom), 114.9, 116.1, 119.0, 120.6, 121.3, 122.2, 128.2, 128.3, 141.2, 152.0 (C quat arom), 167.8, 168.3, 168.8, 169.2, 170.1, 171.8 (C═O).

12-(β-D-Glucopyranosyl)-12,13-dihydro-5*H*-pyrido[3′,2′f:4,5]pyrrolo[2,3-*a*]pyrrolo[3,4-*c*]carbazole-5,7(6*H*)-dione (32). A mixture of **31** (28 mg, 0.040 mmol), methanol (13 mL), and 28% aqueous NH_4OH (9 mL) was stirred for 15 h at room temperature. After removal of the solvents, a mixture EtOAc/water was added to the residue. After fitration, the solid was washed successively with EtOAc and methanol to give **32** (8 mg, 0.016 mmol, 39% yield) as a yellow solid. Mp > 250 °C (degradation). IR (KBr), $\nu_{C=O}$ 1720, 1760 cm^{-1}, $\nu_{NH,OH}$ 3100−3600 cm^{-1}. HRMS (FAB$^+$) (M + H)$^+$ calcd $C_{25}H_{21}N_4O_7$ 489.1410, found 489.1416. ^{1}H NMR (400 MHz, DMSO-d_6): 3.65 (1H, d, J = 5.5 Hz), 3.81 (1H, d, J = 10.2 Hz), 3.87−4.60 (8H, m), 6.32 (1H, d, J = 6.0 Hz, $H_{1'}$), 7.41−7.55 (2H, m), 7.66 (1H, t, J = 7.0 Hz), 8.05 (1H, d, J = 8.3 Hz), 8.69 (1H, d, J = 3.8 Hz), 9.21 (1H, d, J = 7.8 Hz), 9.36 (1H, d, J = 7.3 Hz), 11.28 (1H, s, NH), 11.66 (1H, s, NH). ^{13}C NMR (100 MHz, DMSO-d_6): 58.2 ($C_{6'}$), 67.8, 73.2, 76.7, 78.9, 84.4 ($C_{1'}$, $C_{2'}$, $C_{3'}$, $C_{4'}$, $C_{5'}$), 111.8, 117.0, 120.8, 124.5, 127.2, 132.7, 147.6 (C tert arom), 114.3, 114.7, 119.1, 120.3 (2C), 121.3, 128.2, 128.4, 142.1, 152.2 (C quat arom), 170.9 (2C) (C═O). HPLC >96% pure, 1) t_R = 3.28 min, 2) t_R = 3.31 min.

Melting Temperature Studies. Melting curves were measured using an Uvikon 943 spectrophotometer coupled to a Neslab RTE111 cryostat. For each series of measurements, 12 samples were placed in a thermostatically controlled cell-holder, and the quartz cuvettes (10 mm path length) were heated by circulating water. Measurements were performed in BPE buffer pH 7.1 (6 mM Na_2HPO_4, 2 mM NaH_2PO_4, 1 mM EDTA). The temperature inside the cuvette was measured with a platinum probe; it was increased over the range 20−100 °C with a heating rate of 1 °C/min. The "melting" temperature T_m was taken as the midpoint of the hyperchromic transition.

Sequencing of topoisomerase I-mediated DNA cleavage sites. The 117 base pairs DNA fragment was prepared by 3′-[32P]-end labeling of the *Eco*RI-*Pvu*II double digest of the plasmid pBS using α-[32P]-dATP (3000 Ci/mMol) and AMV reverse transcriptase. For the topoisomerase I experiments, each reaction mixture contained 2 μL of 3′-end [32P] labeled DNA (~1 μM), 5 μL of water, 2 μL of 10X topoisomerase I buffer, and 10 μL of drug solution at the desired concentration (10−50 μM). After 10 min incubation to ensure equilibration, the reaction was initiated by addition of 2 μL (20 units) calf thymus topoisomerase I (Life Technologies). Samples were incubated for 45 min at 37 °C prior to adding SDS to 0.25% and proteinase K to 250 μg/mL to dissociate the drug−DNA-topoisomerase I cleavable complexes. The DNA was precipitated with ethanol and then resuspended in 5 μL of formamide-TBE loading buffer, denatured at 90°C for 4 min, and then chilled in ice for 4 min prior to loading on to the sequencing gel. DNA cleavage products were resolved by polyacrylamide gel electrophoresis under denaturing conditions.

Growth Inhibition Assays. Tumor cells were provided by American Type Culture Collection (Frederik, MD). 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.[24] Cells were continuously exposed to graded concentrations of the compounds for four doubling times, 15 μL of 5 mg/mL 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide was 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 μL of DMSO. Results are expressed as IC_{50}, the 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 (2.5×10^5 cells/mL) were incubated for 21 h with various concentrations of the compounds and 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, 10^4 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.

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