Syntheses and Antiproliferative Activities of New Rebeccamycin Derivatives with the Sugar Unit Linked to Both Indole Nitrogens

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The synthesis of new rebeccamycin derivatives, in which the carbohydrate moiety is attached to both indole nitrogens, is described. The newly synthesized compounds were tested for their abilities to block the cell cycle of murine leukemia L1210 cells and their in vitro antiproliferative activities against four tumor cell lines (murine L1210 leukemia and human HT29 colon carcinoma, A549 non-small-cell lung carcinoma, K-562 leukemia). Their biological activities are compared with those of the parent compound rebeccamycin. Some of the new compounds exhibit potent antiproliferative activities, either against the four cell lines or mostly the two leukemias (L1210 and K-562 cell lines). The 3,9-diformyl analogue **9** was selective toward L1210 cells, whereas the 3,9-dibromo **16** was strongly cytotoxic toward the four cell lines tested. Nonselective compound **16** and 3,9-dinitro **13**, which exhibited selectivity toward leukemia tumor cell lines, were selected for in-depth evaluation, including in vivo experiments.

Introduction

Indolopyrrolocarbazoles are a class of compounds capable of inducing topoisomerase I mediated DNA breaks. The most significant of these are NB-506 (L-753,000), ED-749 (J-107088), and NCS 655649 (Chart 1), which are presently undergoing clinical trials.[1−3] Although topoisomerase I mediated DNA cleavage by these compounds has been clearly demonstrated in vitro, yet unknown mechanisms of action may interplay to kill treated cells.[3] Other indolocarbazoles including staurosporine and UCN-01 are well-known as kinase inhibitors. Staurosporine has no effect against topoisomerase I. KT-6528, KT-6006, and KT-6124, derivatives of the microbial metabolite K-252a, unlike their parent compound K-252a, inhibit the catalytic activity of topoisomerase I by stabilizing the cleavable complex (Chart 1). They are also nonselective protein kinase inhibitors.[4−7] Examination of the structures shown in Chart 1 does not allow us to determine the parameters responsible for the discrimination topoisomerase I/kinases. The main differences in the structures are the functionality in the upper heterocycle (imide or amide), the sugar moiety linked to one or both indole nitrogens, and the carbohydrate heterocycle (furanose or pyranose). Rebeccamycin **1** is a microbial metabolite isolated from cultures of *Saccharothrix aerocolonigenes*. It is well-known for its antiproliferative properties and its inhibitory potency toward topoisomerase I.[8,9] To improve its pharmacological profile, various series of analogues have been prepared either by total synthesis or by semisynthesis.[10−15]

Recently, we have prepared from rebeccamycin a novel series of derivatives in which the sugar moiety is linked to both indole nitrogens (Chart 1).[16] This series presents a structural analogy to the kinases inhibitor staurosporine. But contrary to staurosporine, compounds **2** and **3** are not protein kinase C (PKC) inhibitors and their antiproliferative properties cannot be solely due to their relatively weak topoisomerase I inhibitory potencies. Their weak solubility was suspected to limit their biological properties. In this study, our purpose was to investigate the influence on the antiproliferative activities of modifications of compound **3** by substitutions on the imide nitrogen or on the aromatic moieties and by functional modifications on the carbohydrate part. The in vitro antiproliferative activities against two solid tumors (human HT29 colon carcinoma, A549 non-small-cell lung carcinoma) and two leukemia (murine L1210 and human K-562) were tested. The examination of the effect on the L1210 cell cycle revealed that L1210 cells were mostly accumulated in the G2+M phases.

Results and Discussion

Chemistry. Compound **3** (Scheme 1) was prepared from rebeccamycin as already reported.[16] The stereochemistry of the new bond with the second indole nitrogen was assigned from crystallographic data (unpublished results). Anhydride **4** was obtained by treatment of **3** in a basic medium.

Compounds **5**, **6**, and **7** were formed by reaction of anhydride **4** with methylamine, hydroxylamine hydrochloride, and diethylethylenediamine, respectively.

To improve the solubility of analogues in this new series, various functional groups were introduced on the indole rings (Scheme 2). Before substitution on the indole parts with formyl groups, it has been necessary to protect the hydroxyl functions of the sugar moiety

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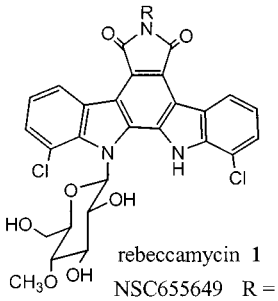
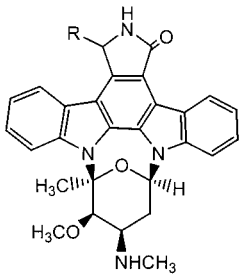
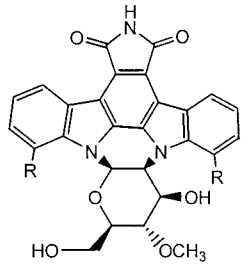
Chart 1



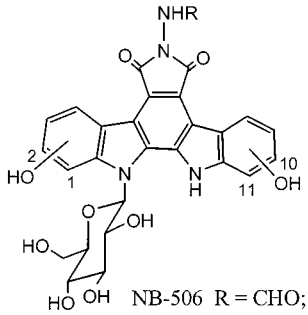
rebeccamycin **1** R = H
NSC655649 R = $(CH_2)_2NEt_2$



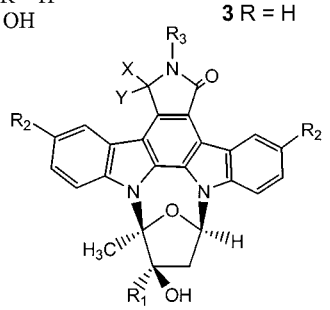
staurosporine R = H
UCN-01 R = OH



2 R = Cl
3 R = H

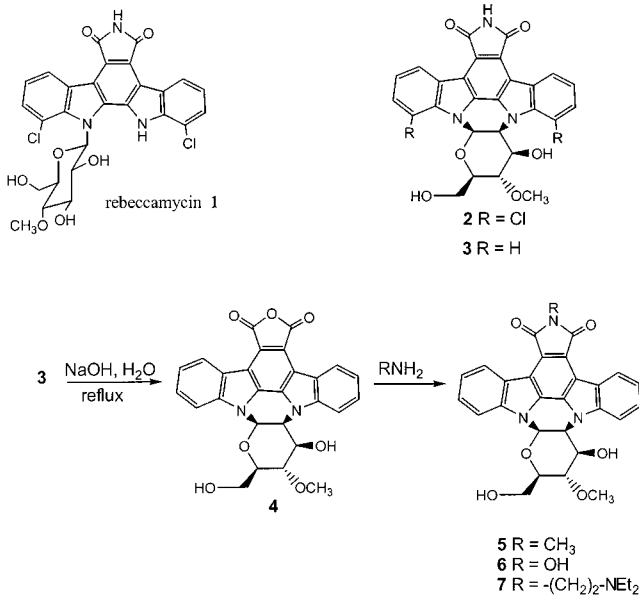


NB-506 R = CHO; OH at 1,11
ED-749 R = $CH(CH_2OH)_2$; OH at 2,10

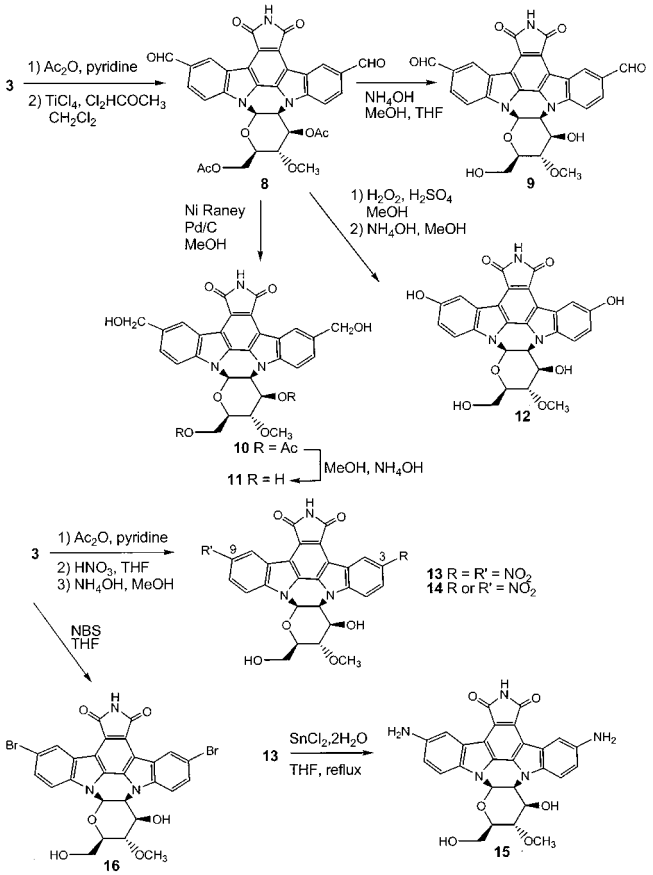


K-252a $R_1 = COOCH_3$; R_2 = H; R_3 = H; X,Y = H
KT-6528 $R_1 = CH_2OH$; R_2 = H; R_3 = OH; X,Y = O
KT-6006 $R_1 = COOCH_3$; R_2 = OH; R_3 = H; X,Y = H
KT-6124 $R_1 = CH_2OH$; R_2 = H; $R_3 = NH_2$; X,Y = O

Scheme 1

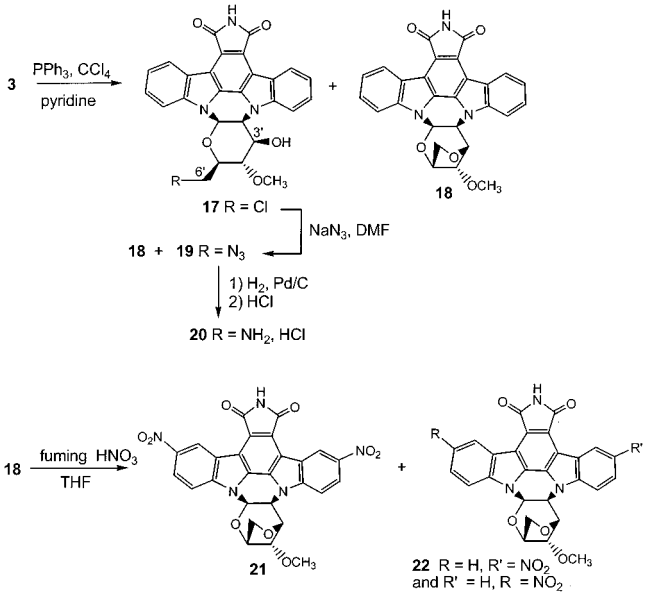


Scheme 2

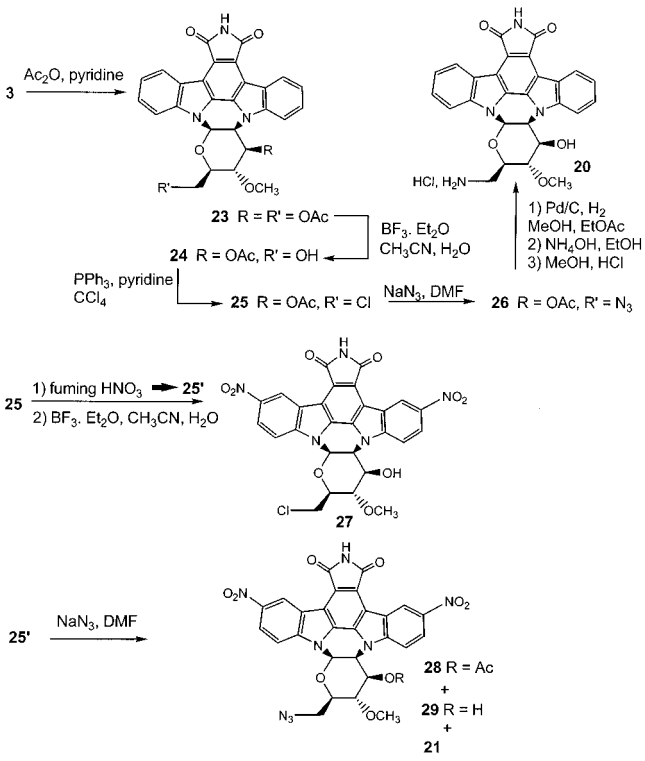


as acetates to avoid side reactions. First, formylation was performed using dichloromethyl methyl ether in the presence of titanium chloride, leading to **8**.[17] Removal of the hydroxyl protective groups allowed the obtention of compound **9**. On the other hand, reduction of the formyl groups, in the presence of Raney nickel as the catalyst, was at first attempted on deprotected compound **9**, but a better yield was obtained afterward on the diacetylated intermediate **8** followed by hydrolysis of the acetates to give diol **11**. Baeyer−Villiger oxidation of the diacetylated intermediate **8** with H_2O_2/H_2SO_4 in methanol afforded, after hydrolysis of the acetates, diphenol **12**.[18] Nitration of compound **3** was performed using an identical sequence of reactions as shown in Scheme 2. According to the strength of the nitric acid used, fuming or concentrated, dinitrated or mononitrated products were obtained, respectively. The mononitrated compound **14** was obtained as a mixture of 3-NO_2 and 9-NO_2 regioisomers in a 1.5/1 ratio determined from ^1H NMR data and $^1H-^1H$ COSY correla-

Scheme 3



Scheme 4



tions. The aromatic nitro functions of **13** were reduced to amino groups (compound **15**) using $SnCl_2 \cdot 2H_2O$ in THF.[19] Bromination yielding **16** was directly performed on compound **3** with *N*-bromosuccinimide.

Since an amphiphilic amino function on a sugar moiety is present in many biologically active compounds and is expected to improve the penetration of the drugs into the cells, our aim was to prepare an analogue bearing this function at the 6′ position. For this purpose, a chlorine atom was introduced selectively at the 6′ position using triphenylphosphine and CCl_4 (Scheme 3).[20] Compound **17** was obtained in poor yield (23%), the major product of the reaction being the 3′,6′-anhydro derivative **18** due to the attack of the deprotonated 3′-hydroxyl group on the 6′-oxotriphenylphosphonium intermediate.[21,22] Nucleophilic substitution of **17** with sodium azide led to azido compound **19** as the minor product of the reaction. The major product was once more compound **18**, the nitration of which, using fuming HNO_3, gave 3,9-dinitrated and a mixture of 3- or 9-mononitrated derivatives (compounds **21** and **22**, respectively). To improve the yields in the sequence of reactions affording the primary amine at the 6′ position from the chloride and via the azide, the monoacetylated compound at the 3′ position (**24**, Scheme 4) was prepared to avoid the formation of byproduct **18**. The diacetylated intermediate **23** was specifically hydrolyzed at the 6′ position using $BF_3 \cdot Et_2O$ in wet CH_3CN,[23] yielding **24**, which was further transformed into the chloro and then the azido derivatives **25** and **26**, respectively. Reduction of the azide followed by hydrolysis of the 3′-acetate gave the corresponding amine which was transformed to hydrochloride **20**. Nitration of compound **25** using fuming HNO_3 gave the 3′-acetylated compound **25′**, which was deprotected with $BF_3 \cdot Et_2O$ in wet CH_3CN to yield **27**. Treatment of compound **25′** with sodium azide led to a mixture of acetylated azide **28**, deacetylated azide **29**, together with small amounts (4%) of compound **21**.

Cytotoxicity. The antiproliferative activities were tested in vitro against four tumor cell lines, two solid tumors (human HT29 colon carcinoma and A549 non-small-cell lung carcinoma), and two leukemias (murine L1210 and human K-562). Results, expressed as IC_{50}, are reported in Table 1. The most efficient compound against L1210 cells is the mixture of regioisomers **14**, which could not be separated either by chromatography or by HPLC. In this mixture the ratio 3-nitro/9-nitro was 1.5/1. Another sample in which the ratio 3-nitro/9-nitro was 1.1/1 was found to exhibit weaker antiproliferative activities (IC_{50} on L1210: 0.14 μM), suggesting that the 3-nitro derivative is more efficient. The

Table 1. Antiproliferative Activities against Four Tumor Cell Lines, Murine Leukemia L1210 and Human HT-29, A549, and K-562 (IC_{50}, μM), and Effect on the Cell Cycle of L1210 Cells

compd	L1210	HT29	A549	K-562	% of L1210 cells in the G2+M phases at a given drug concentration[a]
1	0.1	0.3	0.3	0.2	69 (1 μM)
2	1.3	3.5	3.3	0.8	75 (5 μM)
3	0.9	2.5	2.0	0.5	72 (10 μM)
4	70.3	>10	>10	>10	ne
5	3.1	ne	ne	ne	ne
6	0.6	3.0	3.1	0.4	81 (2.5 μM)
7	0.4	0.2	0.3	<0.1	61 (2 μM)
9	0.2	>10	>10	>10	72 (1 μM)
11	18.1	>10	>10	0.8	69 (5 μM)
12	0.7	>10	>10	0.1	55 (2.5 μM)
13	0.1	>10	>10	<0.1	70 (0.5 μM)
14	0.08	0.3	0.3	<0.1	76 (0.25 μM)
15	19.3	>10	>10	>10	ne
16	0.2	0.1	0.2	<0.1	76 (0.5 μM)
18	2.9	>10	>10	>10	*b*
20	0.2	0.2	0.2	0.2	61 (1 μM)
21	0.2	<0.1	0.1	0.1	61 (1μM)
22	0.7	0.3	0.3	0.2	69 (2.5 μM)
27	0.5	0.3	0.5	0.4	74 (5 μM)
29	6.5	4	16.6	1.1	42 (10−50 μM)

[a] 24% of untreated control cells were in the G2+M phases of the cell cycle, 44% were in the G1 phase, and 28% were in the S phase; ne = not evaluated. [b] G1, 72% (10 μM).

regioisomers **14** are globally as potent as rebeccamycin, the four cell lines being sensitive to both compounds. Interestingly, the efficiency against L1210 of the dinitro analogue **13** is in the same range but compound **13** exhibits a marked selectivity; HT29 and A549 were at least 100-fold more resistant than L1210 and K-562. Diformyl **9** was even more selective, only L1210 being sensitive to this derivative. Compounds that have strong antiproliferative activities without selectivity are **14**, rebeccamycin **1**, dibromo **16**, *N*-ethyldiethylamino **7**, 6′-amino **20**, dinitroanhydro **21**, and mononitroanhydro **22**. Replacement of the imide function at the upper heterocycle by an anhydride function (compound **4**) abolishes the antiproliferative activity. The introduction of nitro functions at the 3 and 9 positions, which increase the solubility, enhances the cytotoxicity (compare **3** with **13** and **14**, and compare **18** with **21** and **22**). That is also the case for the introduction of bromine atoms at these positions (compare **3** with **16**), but amino functions (compound **15**) led to poor cytotoxic activities. 3,9-Dihydroxymethyl **11** and 3,9-dihydroxy **12** show the same profile of selectivity, but **12** is more cytotoxic toward the nonsolid tumor cell lines tested.

Effect on L1210 Cell Cycle. The effect on the L1210 cell cycle of compounds that exhibited the strongest antiproliferative activities against this tumor cell line was studied (Table 1). It was observed that, with most of them, the cells were accumulated in the G2+M phases except for anhydro **18** with which 72% of the cells were accumulated in the G1 phase at 10 μM. 3,9-Dinitro **13** and 3,9-dibromo **16** are specially interesting with 70% and 76% of cells in the G2+M phases, respectively, at a drug concentration of 0.5 μM.

In conclusion, the development of a new series of rebeccamycin analogues with the sugar moiety linked to both indole nitrogens led to the obtention of efficient antiproliferative compounds. The IC_{50} values for some of them are in the nanomolar range. Except for 6′-amino **20** and nitroanhydro **21, 22, 27**, which are nonselective against the four tumor cell lines tested, the other efficient derivatives exhibit stronger cytotoxicities toward leukemia L1210 and K-562 than toward solid tumor cell lines HT29 and A549. Nonselective dibromo **16** and selective dinitro **13** have been selected for in vivo evaluation. Like most rebeccamycin analogues previously studied, the compounds of this series (except anhydro **18**) induced arrest of the cell cycle of L1210 leukemia cells in the G2+M phases. From the structure−activity relationships studies of rebeccamycin analogues performed in our laboratory, we could conclude that if topoisomerase I represents a target for these compounds, their antiproliferative activities could be due to the interaction with other proteins or kinases essential for cell multiplication. For example, during the G2+M phases, the complex cyclinB/CDK1 contribute to the progression of the cell cycle toward mitosis. A possible inhibition of this kinase could led to the blockage in G2+M. Anhydro **18** induced an arrest of the cell cycle in the G1 phase. Its mechanism of action could be due to other or additional protein interactions such as cyclin D/CDK4 or cyclin D/CDK6. The inhibitory potencies of the compounds in this series against various kinases that regulate the cell cycle have to be examined.

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 are in ppm, and the following abbreviations are used: singlet (s), doublet (d), doubled doublet (dd), pseudotriplet (pt), multiplet (m), tertiary carbons (C tert), quaternary carbons (C quat), broad signal (br s)). The signals were assigned from $^1H-^1H$ COSY, $^{13}C-^1H$ correlations, exhange with D_2O, and inverse gate decoupling. Mass spectra (FAB+) were determined on a high-resolution Fisons Autospec-Q spectrometer at CESAMO (Talence, France). Chromatographic purifications were performed by Kieselgel 60 (Merck) 0.063−0.200 mm column chromatography. For purity tests, TLC were performed on fluorescent silica gel plates (60 F_{254} from Merck).

12,13-[1,2-(4-*O*-Methyl-D-mannopyranosyl)]-6,7,12,13-tetrahydro-5,7-dioxo(5*H*)-indolo[2,3-*a*]furo[3,4-*c*]carbazole (4). A mixture of compound **3**[16] (200 mg, 0.414 mmol), water (70 mL), and NaOH (420 mg) was refluxed for 3 h. After it was poured into water, acidified with 1 N HCl, and then extracted with EtOAc, the organic phase was washed with saturated aqueous $NaHCO_3$ and brine and then was dried over $MgSO_4$. After evaporation of the solvent, the residue was purified by flash chromatography (eluent, EtOAc/cyclohexane, 70:30) to give **4** (182 mg, 0.376 mmol, 91% yield) as a yellow solid. Mp = 300 °C. IR (KBr): $\nu_{C=O}$ = 1750, 1820 cm^{-1}, ν_{OH} = 3200−3600 cm^{-1}. HRMS (FAB+) $(M + H)^+$: calcd for $C_{27}H_{21}N_2O_7$, 485.1349; found, 485.1333. ^1H NMR (400 MHz, DMSO-d_6): δ 3.44 (1H, m), 3.51 (2H, m), 3.62 (3H, s, OCH_3), 3.86 (1H, m), 4.63 (2H, m, H + OH), 5.28 (1H, t, J = 2.5 Hz), 6.78 (1H, d, J = 5.0 Hz, OH), 6.89 (1H, d, J = 2.0 Hz, $H_{1'}$), 7.50 (1H, t, J = 8.0 Hz), 7.53 (1H, t, J = 8.0 Hz), 7.70 (1H, dt, J_1 = 7.5 Hz, J_2 = 1.0 Hz), 7.73 (1H, dt, J_1 = 7.5 Hz, J_2 = 1.0 Hz), 8.04 (1H, d, J = 7.9 Hz), 8.55 (1H, d, J = 7.9 Hz), 8.66 (1H, d, J = 7.4 Hz), 8.92 (1H, d, J = 8.9 Hz). ^{13}C NMR (100 MHz, DMSO-d_6): δ 59.9 (OCH_3), 60.0 ($C_{6'}$), 63.1, 71.4, 76.1, 78.7, 80.2 ($C_{1'}$, $C_{2'}$, $C_{3'}$, $C_{4'}$, $C_{5'}$), 112.6, 112.9, 118.4, 118.8, 123.1, 123.2, 131.0, 131.8, 140.6, 142.5 (C quat arom), 112.3, 115.5, 121.5, 122.3, 123.6, 123.7, 127.6 (2C) (C tert arom), 164.6, 164.8 (C=O).

6-Methyl-12,13-(4-*O*-methyl-D-mannopyranosyl)-6,7,-12,13-tetrahydroindolo[2,3-*a*]pyrrolo[3,4-*c*]carbazole-5,7-dione (5). A mixture of anhydride **4** (57 mg, 0.118 mmol) and a 2 M solution of methylamine in THF (14 mL) was stirred in a sealed tube at 70 °C for 16 h. After cooling, the mixture was poured into water. The yellow precipitate was filtered off and washed with water, then purified by flash chromatography (eluent, EtOAc/cyclohexane, 8:2) to give **5** (30 mg, 0.06 mmol, 51% yield) as a yellow solid. Mp > 300 °C. IR (KBr): $\nu_{C=O}$ 1690 cm^{-1}, $\nu_{NH,OH}$ = 3200−3600 cm^{-1}. HRMS (FAB+) M^+: calcd for $C_{28}H_{23}N_3O_6$, 497.1587; found, 497.1591. ^1H NMR (400 MHz, DMSO-d_6): δ 3.12 (3H, s, NCH_3), 3.35−3.45 (2H, m), 3.50 (1H, m), 3.62 (3H, s, OCH_3), 3.81 (1H, m), 4.58 (1H, t, J = 5.5 Hz, OH), 4.62 (1H, m), 5.18 (1H, s), 6.76 (1H, d, J = 4.7 Hz, OH), 6.81 (1H, s, $H_{1'}$), 7.44 (1H, t, J = 7.9 Hz), 7.47 (1H, t, J = 7.9 Hz), 7.63 (1H, d, J = 8.7 Hz), 7.65 (1H, t, J = 7.1 Hz), 7.93 (1H, d, J = 8.7 Hz), 8.65 (1H, d, J = 7.9 Hz), 8.78 (1H, d, J = 7.1 Hz), 8.88 (1H, d, J = 8.7 Hz). ^{13}C NMR (100 MHz, DMSO-d_6): δ 23.5 (NCH_3), 59.9 (OCH_3), 60.0 ($C_{6'}$), 63.2, 71.8, 76.1, 78.8, 80.2 ($C_{1'}$, $C_{2'}$, $C_{3'}$, $C_{4'}$, $C_{5'}$), 111.8, 115.3, 120.9, 121.7, 124.2, 124.3, 127.0 (2C) (C tert arom), 112.3, 112.7, 119.4, 119.7, 123.5, 123.6, 129.9, 130.8, 140.6, 142.5 (C quat arom), 169.5, 169.7 (C=O).

6-Hydroxy-12,13-(4-*O*-methyl-D-mannopyranosyl)-6,7,-12,13-tetrahydroindolo[2,3-*a*]pyrrolo[3,4-*c*]carbazole-5,7-dione (6). To a solution of anhydride **4** (100 mg, 0.207 mmol) in DMF (5 mL) was added hydroxylamine hydrochloride (1 g, 14.4 mmol) and then triethylamine (2 mL, 14.4 mmol). The mixture was stirred at 70 °C for 23 h, and then 1 N HCl, EtOAc, and THF were added. The organic phase was washed with water, saturated aqueous $NaHCO_3$, and brine, then dried over $MgSO_4$. The solvent was removed, and the residue was purified by flash chromatography (eluent, THF/MeOH, 95:5) to give **6** (72 mg, 0.143 mmol, 69% yield) as an orange solid.

Mp > 260 °C (dec). IR (KBr): $\nu_{C=O}$ = 1700, 1760 cm^{-1}, $\nu_{NH,OH}$ = 3100−3600 cm^{-1}. HRMS (FAB+) (M + H)$^+$: calcd for $C_{27}H_{22}N_3O_7$, 500.1457; found, 500.1445. ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.41 (1H, m), 3.51 (2H, m), 3.62 (3H, s, OCH_3), 3.82 (1H, m), 4.58 (1H, t, J = 5.5 Hz, $OH_{6'}$), 4.62 (1H, m), 5.24 (1H, br s), 6.78 (1H, d, J = 5.0 Hz, OH), 6.87 (1H, d, J = 1.4 Hz, $H_{1'}$), 7.48 (1H, t, J = 7.5 Hz), 7.52 (1H, t, J = 7.5 Hz), 7.66 (1H, dt, J_1 = 8.4 Hz, J_2 = 1.5 Hz), 7.70 (1H, dt, J_1 = 7.3 Hz, J_2 = 1.5 Hz), 7.99 (1H, d, J = 8.2 Hz), 8.70 (1H, d, J = 7.9 Hz), 8.82 (1H, d, J = 7.6 Hz), 8.94 (1H, d, J = 8.6 Hz), 10.66 (1H, s, NOH). ^{13}C NMR (100 MHz, DMSO-d_6): δ 59.9 (OCH_3), 60.0 ($C_{6'}$), 63.3, 71.8, 76.1, 78.8, 80.2 ($C_{1'}$, $C_{2'}$, $C_{3'}$, $C_{4'}$, $C_{5'}$), 111.9, 115.3, 121.0, 121.9, 124.3, 124.4, 127.2 (2C) (C tert arom), 112.5, 112.9, 116.0, 116.4, 123.4, 123.5, 130.2, 131.1, 140.7, 142.6 (C quat arom), 166.2, 166.5 (C=O).

6-Diethylaminoethyl-12,13-[1,2-(4-*O*-methyl-D-mannopyranosyl)]-6,7,12,13-tetrahydro(5*H*)-indolo[2,3-*a*]pyrrolo-[3,4-*c*]carbazole-5,7-dione Hydrochloride (7). To a solution of anhydride **4** (58.5 mg, 0.121 mmol) in THF (7 mL) was added dropwise commercial *N*,*N*-diethylethylenediamine (26 μL, 0.181 mmol). The light-protected mixture was stirred at 65 °C for 4 days, then cooled, and 1 N HCl (40 mL) and EtOAc were poured into the mixture. The aqueous phase was adjusted to pH 12 with saturated aqueous $NaHCO_3$. After extraction with EtOAc, the organic phases were dried over $MgSO_4$ and the solvent was removed to give the crude amine. To a solution of the amine at 0 °C in methanol (200 μL) was added dropwise 1.14 N HCl (108 μL). Cyclohexane was added to the stirred mixture. The precipitate was filtered off to give hydrochloride **7** (63.4 mg, 0.103 mmol, 85% yield) as a red solid. Mp = 250 °C. IR (KBr): $\nu_{C=O}$ = 1700, 1750 cm^{-1}, $\nu_{NH,OH}$ = 3200−3600 cm^{-1}. HRMS (FAB+) (M + H)$^+$: calcd for $C_{33}H_{35}N_4O_6$, 583.2556; found, 583.2557. ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.32 (6H, t, J = 7.0 Hz), 3.30 (4H, m), 3.40 (1H, m), 3.48 (4H, m), 3.62 (3H, s, OCH_3), 3.83 (1H, m), 3.90−4.15 (4H, m), 4.63 (1H, dd, J_1 = 9.0 Hz, J_2 = 2.3 Hz), 5.22 (1H, s), 6.86 (1H, s, $H_{1'}$), 7.47 (1H, t, J = 7.6 Hz), 7.49 (1H, t, J = 7.6 Hz), 7.65 (1H, t, J = 7.4 Hz), 7.67 (1H, t, J = 7.1 Hz), 7.98 (1H, d, J = 8.2 Hz), 8.67 (1H, d, J = 7.8 Hz), 8.80 (1H, d, J = 7.8 Hz), 8.90 (1H, d, J = 8.5 Hz), 10.50 (1H, s, NH). ^{13}C NMR (100 MHz, DMSO-d_6): δ 8.2 (2C) (CH_3), 32.0, 46.1 (2C), 48.1 (CH_2), 59.8 (OCH_3), 60.0 ($C_{6'}$), 63.1, 71.6, 76.1, 78.6, 80.2 ($C_{1'}$, $C_{2'}$, $C_{3'}$, $C_{4'}$, $C_{5'}$), 112.0, 115.3, 121.0, 121.8, 124.1 (2C), 127.1 (2C) (C tert arom), 112.5, 112.8, 119.3, 119.7, 123.4, 123.5, 130.1, 130.9, 140.7, 142.5 (C quat arom), 169.2, 169.4 (C=O).

3,9-Diformyl-12,13-(3,6-di-*O*-acetyl-4-*O*-methyl-D-mannopyranosyl)-6,7,12,13-tetrahydroindolo[2,3-*a*]pyrrolo-[3,4-*c*]carbazole-5,7-dione (8). A mixture of compound **3** (100 mg, 0.207 mmol) and pyridine (2 mL) was cooled to 0 °C before addition of acetic anhydride (200 μL). The mixture was stirred at room temperature for 18 h. Water was added (5 mL), and the mixture was stirred for 40 min and then extracted with EtOAc. The organic phase was washed successively with 1 N HCl, water, saturated aqueous $NaHCO_3$, and water and was dried over $MgSO_4$. The solvent was removed, and the residue was dissolved in CH_2Cl_2 (4 mL). Dichloromethyl methyl ether (380 μL, 4.2 mmol) was added, and the mixture was cooled to 0 °C before addition of 1 M solution of $TiCl_4$ in CH_2Cl_2 (4.2 mL, 4.2 mmol). The mixture was stirred at room temperature for 24 h and then was poured into water (50 mL). The mixture was stirred for 1 h before extraction with EtOAc. The organic phase was dried over $MgSO_4$, and the solvent was removed. The residue was purified by flash chromatography (eluent, EtOAc/cyclohexane, 50:50) to give a pure fraction of **8** and another one in mixture with an intermediate. The fraction containing the mixture was dissolved in water (5 mL) and DMSO (15 mL) and warmed at 60 °C for 2.5 h to allow the hydrolyzation of the intermediate. After extraction with EtOAc and removal of the solvent, the residue was purified by flash chromatography (eluent, EtOAc/cyclohexane, 50:50) to give a total amount of 97 mg of **8** (0.156 mmol, 75% yield) as a yellow solid. Mp > 200 °C. IR (KBr): ν_{CO} = 1690, 1720, 1760 cm^{-1}, $\nu_{NH,OH}$ = 3100−3600 cm^{-1}. ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.65 (3H, s), 1.95 (3H, s), 3.59 (3H, s, OCH_3), 3.74 (1H, pt, J = 6.9 Hz), 4.18−4.30 (2H, m), 4.43 (1H, dt, J_1 = 7.1 Hz, J_2 = 3.2 Hz), 5.75 (1H, t, J = 3.1 Hz), 5.86 (1H, dd, J_1 = 5.8 Hz, J_2 = 3.1 Hz), 6.96 (1H, d, J = 3.6 Hz, $H_{1'}$), 8.08 (2H, m), 8.19 (1H, dd, J_1 = 8.6 Hz, J_2 = 1.5 Hz), 8.25 (1H, d, J = 8.7 Hz), 8.74 (1H, s), 8.87 (1H, s), 9.91 (1H, s, CHO), 10.08 (1H, s, CHO), 11.09 (1H, s, NH). ^{13}C NMR (100 MHz, DMSO-d_6): δ 20.2, 20.5 (CH_3), 63.0 ($C_{6'}$), 56.5, 58.9, 69.2, 74.1, 75.1, 80.0 (OCH_3, $C_{1'}$, $C_{2'}$, $C_{3'}$, $C_{4'}$, $C_{5'}$), 112.2, 112.4, 121.1, 121.2, 123.2, 123.6, 129.0, 129.8, 130.0, 130.7, 144.1, 144.2 (C quat arom), 112.5, 112.8, 127.0, 127.1, 128.0, 128.2 (C tert arom), 168.6, 170.0 (CHO), 191.4, 191.9 (C=O).

3,9-Diformyl-12,13-(4-*O*-methyl-D-mannopyranosyl)-6,7,12,13-tetrahydro(5*H*)-indolo[2,3-*a*]pyrrolo[3,4-*c*]carbazole-5,7-dione (9). To a solution of compound **8** (46 mg, 0.074 mmol) in THF (10 mL) were added methanol (5 mL) and then 28% aqueous NH_4OH (5 mL). The mixture was stirred at room temperature for 30 h. The solvents were removed, and the yellow solid obtained was dissolved in CH_2Cl_2 and washed with water. The organic phase was evaporated, and the residue was washed with acetone to give **9** (29 mg, 0.054 mmol, 73% yield) as a yellow solid. Mp > 300 °C. IR (KBr): $\nu_{C=O}$ = 1680, 1710, 1750 cm^{-1}, $\nu_{NH,OH}$ = 3100−3650 cm^{-1}. HRMS (FAB+) (M + H)$^+$: calcd for $C_{29}H_{22}N_3O_8$, 540.1407; found, 540.1402. ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.39−3.57 (3H, m), 3.63 (3H, s, OCH_3), 3.90 (1H, m), 4.63 (2H, m, H+OH), 5.40 (1H, m), 6.82 (1H, d, J = 4.9 Hz, OH), 6.96 (1H, d, J = 1.8 Hz, $H_{1'}$), 8.17 (1H, d, J = 9.9 Hz), 8.18 (1H, d, J = 9.9 Hz), 8.23 (1H, dd, J_1 = 8.5 Hz, J_2 = 1.4 Hz), 8.98 (1H, d, J = 8.8 Hz), 9.11 (1H, s), 9.24 (1H, d, J = 1.5 Hz), 10.16 (1H, s), 10.17 (1H, s), 11.15 (1H, br s, NH). ^{13}C NMR (100 MHz, DMSO-d_6): δ 59.8 (OCH_3), 60.0 ($C_{6'}$), 62.9, 71.0, 76.1, 78.7, 80.3 ($C_{1'}$, $C_{2'}$, $C_{3'}$, $C_{4'}$, $C_{5'}$), 112.6, 115.3, 126.9 (2C), 128.1, 128.4 (C tert arom), 112.7, 113.2, 121.5, 121.7, 123.7, 123.8, 129.9, 130.7 (2C), 131.6, 144.0, 145.7 (C quat arom), 170.5, 170.7 (C=O), 192.2 (2C, CHO).

3,9-Dihydroxymethyl-12,13-(3,6-di-*O*-acetyl-4-*O*-methyl-D-mannopyranosyl)-6,7,12,13-tetrahydroindolo[2,3-*a*]-pyrrolo[3,4-*c*]carbazole-5,7-dione (10). A mixture of **8** (72 mg, 0.116 mmol), methanol (60 mL), and Raney nickel (50% w/w in water, 101 mg) was hydrogenated at room temperature for 48 h. Raney nickel (591 mg) was added, and the mixture was hydrogenated for 5 days. The mixture was filtered over Celite, and the residue was washed with THF and methanol. The filtrate was evaporated, and the residue was purified by flash chromatography (eluent, EtOAc/cyclohexane, 90:10) to give **10** (30 mg, 0.045 mmol, 41% yield) as a yellow solid. Mp > 180 °C. IR (KBr): $\nu_{C=O}$ = 1720 cm^{-1}, $\nu_{OH,NH}$ = 3150−3650 cm^{-1}. ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.75 (3H, s), 1.90 (3H, s), 3.59 (3H, s, OCH_3), 3.71 (1H, t, J = 7.0 Hz), 4.12−4.38 (3H, m), 4.71 (2H, s, CH_2OH), 4.73 (2H, s, CH_2OH), 5.58 (1H, t, J = 3.1 Hz), 5.89 (1H, dd, J_1 = 6.3 Hz, J_2 = 3.1 Hz), 6.84 (1H, d, J = 3.4 Hz, $H_{1'}$), 7.61 (1H, dd, J_1 = 8.6 Hz, J_2 = 1.6 Hz), 7.64 (1H, dd, J_1 = 8.8 Hz, J_2 = 1.6 Hz), 7.87 (1H, d, J = 8.4 Hz), 8.13 (1H, d, J = 8.6 Hz), 8.61 (1H, s), 8.68 (1H, s), 11.07 (1H, s, NH). ^{13}C NMR (100 MHz, DMSO-d_6): δ 20.4, 20.5 (3 CH_3), 56.7, 59.0, 69.9, 74.0, 75.1, 80.1 (OCH_3, $C_{1'}$, $C_{2'}$, $C_{3'}$, $C_{4'}$, $C_{5'}$), 63.0, 63.2, 63.3 (2CH_2, $C_{6'}$), 111.8 (2C), 122.6, 122.9, 126.2, 126.6, 122.9, 126.2, 126.6 (C tert arom), 112.0, 112.3, 120.6, 120.8, 123.5, 123.7, 129.3, 130.1, 135.5, 136.3, 140.1, 140.3 (C quat arom), 168.8, 170.0, 170.8, 170.9 (C=O).

3,9-Dihydroxymethyl-12,13-(4-*O*-methyl-D-mannopyranosyl)-6,7,12,13-tetrahydro(5*H*)-indolo[2,3-*a*]pyrrolo[3,4-*c*]carbazole-5,7-dione (11). A mixture of compound **10** (30 mg, 0.048 mmol), methanol (20 mL), THF (5 mL), and 28% aqueous NH_4OH (5 mL) was stirred at room temperature for 6.5 h. After removal of the solvents, water was added to the residue. After filtration and washing with water, **11** (23 mg, 0.042 mmol, 89% yield) was obtained as a yellow solid. Mp > 300 °C. IR (KBr): ν_{CO} = 1710, 1750 cm^{-1}, $\nu_{NH,OH}$ = 3150−3600 cm^{-1}. HRMS (FAB+) (M + H)$^+$: calcd for $C_{29}H_{25}N_3O_8$, 543.1641; found, 543.1654. ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.42 (1H, m), 3.49 (2H, m), 3.62 (3H, s, OCH_3), 3.80 (1H, m), 4.56 (1H, t, J = 5.5 Hz, OH), 4.62 (1H, m), 4.75 (4H, d, J = 4.7 Hz), 5.21 (1H, br s), 5.32 (1H, t, J = 5.5 Hz, OH), 5.37 (1H, t, J = 5.7

Hz, OH), 6.76 (1H, d, J = 4.8 Hz, OH), 6.82 (1H, s, $H_{1'}$), 7.58 (1H, d, J = 8.9 Hz), 7.62 (1H, d, J = 8.6 Hz), 7.91 (1H, d, J = 8.3 Hz), 8.67 (1H, s), 8.80 (1H, s), 8.86 (1H, d, J = 8.7 Hz), 11.07 (1H, s, NH). ^{13}C NMR (100 MHz, DMSO-d_6): δ 60.0 ($C_{6'}$), 63.3 (2C, CH_2OH), 59.9, 63.1, 71.8, 76.1, 78.7, 80.2 (OCH_3, $C_{1'}$, $C_{2'}$, $C_{3'}$, $C_{4'}$, $C_{5'}$), 111.3, 114.7, 122.5 (2C), 126.1, 126.2 (C tert arom), 112.4, 112.7, 120.4, 120.8, 123.5, 123.8, 130.6, 131.3, 135.1, 136.1, 139.8, 141.6 (C quat arom), 170.9, 171.1 (C═O).

3,9-Dihydroxy-12,13-(4-*O*-methyl-D-mannopyranosyl)-6,7,12,13-tetrahydro(5*H*)-indolo[2,3-*a*]pyrrolo[3,4-*c*]carbazole-5,7-dione (12). To a solution of compound **8** (132 mg, 0.211 mmol) in methanol (6 mL) was added 50% aqueous H_2O_2 (37 μL, 0.64 mmol) and then 95% H_2SO_4 (11 μL). The mixture was stirred for 72 h at room temperature, and then water (20 mL) was added. After the mixture was stirred for 30 min and then extracted with EtOAc, the organic phase was washed with brine and dried over $MgSO_4$. After removal of the solvent, the residue was purified by flash chromatography (eluent, toluene/THF, 65:35) to give a mixture of deprotected and monoacetylated compounds (125 mg) as an orange solid, which was further dissolved in methanol (125 mL). To this solution was added 30% aqueous NH_4OH (24 mL), and the mixture was stirred at room temperature for 14 h. The solvent was removed, the residue was dissolved in EtOAc and washed with brine, and the organic phase was dried over $MgSO_4$. The solvent was removed, and the residue was purified by flash chromatography (eluent, acetone/cyclohexane, 50:50) to give **12** as an orange solid (76.3 mg, 0.148 mmol, 51% yield). Mp > 258 °C (decomposition). IR (KBr): ν_{CO} = 1700, 1750 cm^{-1}, $\nu_{NH,OH}$ = 3100−3600 cm^{-1}. HRMS (FAB+) (M + H)$^+$: calcd for $C_{27}H_{21}N_3O_8$, 515.1328; found, 515.1326. ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.35−351 (3H, m), 3.61 (3H, s, OCH_3), 3.75 (1H, m), 4.50 (1H, m), 4.55 (1H, t, J = 5.4 Hz, $OH_{6'}$), 5.05 (1H,br s), 6.71 (1H, s, $OH_{3'}$), 6.72 (1H, d, J = 4.9 Hz, $H_{1'}$), 7.08 (1H, dd, J_1 = 9.3 Hz, J_2 = 2.9 Hz), 7.10 (1H, dd, J_1 = 8.9 Hz, J_2 = 2.4 Hz), 7.73 (1H, d, J = 8.2 Hz), 8.12 (1H, d, J = 2.5 Hz), 8.26 (1H, d, J = 2.5 Hz), 8.72 (1H, d, J = 8.9 Hz), 9.40 (1H, s, OH), 9.48 (1H, s, OH), 10.90 (1H, s, NH). ^{13}C NMR (100 MHz, DMSO-d_6): δ 59.9 ($C_{6'}$), 60.0 (OCH_3), 63.3, 72.1, 76.0, 78.7, 80.2 ($C_{1'}$, $C_{2'}$, $C_{3'}$, $C_{4'}$, $C_{5'}$), 109.0, 109.3, 112.1, 115.8 (3C) (C tert arom), 111.9, 112.3, 120.0, 120.5, 124.6, 124.8, 130.9, 131.7, 134.4, 136.4, 152.0, 152.6 (C quat arom), 171.1, 171.3 (C═O).

3,9-Dinitro-12,13-(4-*O*-methyl-D-mannopyranosyl)-6,7,-12,13-tetrahydro(5*H*)-indolo[2,3-*a*]pyrrolo[3,4-*c*]carbazole-5,7-dione (13). To a solution of compound **3** (237 mg, 0.492 mmol) in pyridine (4.8 mL) at 0 °C was added acetic anhydride (488 μL, 4.92 mmol). The mixture was stirred at room temperature for 24 h. Water was added. The organic phase was successively washed with 1 N HCl, saturated aqueous $NaHCO_3$, and then brine and was dried over $MgSO_4$. The solvent was removed, and the diacetylated compound was obtained as a yellow powder. Fuming HNO_3 (5.6 mL) in THF (10 mL) was added, and the mixture was stirred at 40 °C for 5 days. More fuming HNO_3 (2.8 mL) was added, and the mixture was stirred at 40 °C for 30 h. Water and EtOAc were added. The organic phase was washed with brine and dried over $MgSO_4$. Removal of the solvent led to an orange residue of crude nitrated compound. To the residue in methanol (120 mL) was added dropwise a 40% aqueous NH_4OH solution (100 mL). The mixture was stirred at room temperature for 24 h. After evaporation, water and EtOAc were added to the residue. After extractions with EtOAc, the organic phase was washed with brine, then dried over $MgSO_4$. The solvent was removed, and the residue was purified by flash chromatography (eluent, THF/toluene, 3:2) to give **13** (135 mg, 0.235 mmol, 47% yield) as a yellow solid. Mp > 300 °C. IR (KBr): $\nu_{C=O}$ 1690, 1740 cm^{-1}, $\nu_{NH,OH}$ = 3170−3640 cm^{-1}. ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.40−3.54 (3H, m), 3.62 (3H, s, OCH_3), 3.90 (1H, m), 4.63 (2H, m), 5.43 (1H, t, J = 2.2 Hz, OH), 6.91 (1H, d, J = 5.1 Hz, OH), 7.02 (1H, d, J = 1.8 Hz, $H_{1'}$), 8.21 (1H, d, J = 9.1 Hz), 8.54 (1H, dd, J_1 = 9.2 Hz, J_2 = 2.5 Hz), 8.59 (1H, dd, J_1 = 9.0 Hz, J_2 = 2.4 Hz), 9.02 (1H, d, J = 9.4 Hz), 9.33 (1H, dd, J = 2.4 Hz), 9.48 (1H, d, J = 2.5 Hz), 11.35 (1H, s, N_{imide}−H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 59.8 (OCH_3), 59.9 ($C_{6'}$), 63.2, 70.9, 75.9, 78.7, 80.3 ($C_{1'}$, $C_{2'}$, $C_{3'}$, $C_{4'}$, $C_{5'}$), 112.6, 115.4, 120.2, 120.4, 122.4, 122.8 (C tert arom), 113.2 (2C), 121.8, 121.9, 123.1 (2C), 131.0, 132.0, 141.4, 142.2, 143.7, 145.4 (C quat arom), 170.2, 170.4 (C═O).

3- Or 9-Nitro-12,13-(4-*O*-methyl-D-mannopyranosyl)-6,7,12,13-tetrahydro(5*H*)-indolo[2,3-*a*]pyrrolo[3,4-*c*]carbazole-5,7-dione (Regioisomers 14). The procedure was identical to that described for the preparation of **13** except the use of concentrated HNO_3 (instead of fuming HNO_3) and the temperature of the reaction (room temperature instead of 40 °C). From **3** (50 mg, 0.103 mmol), compound **14** (21 mg, 0.04 mmol, 39% yield) was obtained after purification by flash chromatography (eluent, THF/toluene, 1:1) as a yellow solid mixture of regioisomers. ^{1}H−^{1}H COSY experiments allowed the assignments of the signals, and the identification of the 3-nitro as the major isomer in the mixture (1.5/1 **A**/**B**). Mp = 293 °C. IR (KBr): $\nu_{C=O}$ 1690, 1750 cm^{-1}, $\nu_{NH,OH}$ = 3100−3590 cm^{-1}. HRMS (FAB+) (M + H)$^+$: calcd for $C_{27}H_{20}N_4O_8$, 529.1359; found, 529.1356. ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.40−3.56 (6H, m, $4H_{6'}$ + $2H_{4'}$), 3.61 (6H, s, $2OCH_3$), 3.80 (1H m, $H_{5'}$), 3.90 (1H, m, $H_{5'}$), 4.55 (1H, t, J = 5.5 Hz, $OH_{6'}$), 4.60 (1H, m, $H_{3'}$), 4.64 (1H, m, $H_{3'}$), 4.65 (1H, t, J = 5.6 Hz, $OH_{6'}$), 5.28 (1H, s, $H_{2'}$), 5.35 (1H, s, $H_{2'}$), 6.65 (1H, d, J = 4.9 Hz, OH), 6.91 (1H, s, $H_{1'}$), 6.94 (1H, s, $H_{1'}$), 7.01 (1H, d, J = 5.2 Hz, OH), 7.48 (1H, t, J = 7.6 Hz, H_{2B}), 7.52 (1H, t, J = 7.6 Hz, H_{9A}), 7.65 (1H, t, J = 7.6 Hz, H_{3B}), 7.72 (1H, t, J = 7.4 Hz, H_{10A}), 7.99 (1H, d, J = 8.3 Hz, H_{11A}), 8.18 (1H, d, J = 9.1 Hz, H_{11B}), 8.52 (1H, dd, J_1 = 9.4 Hz, J_2 = 2.6 Hz, H_{2A}), 8.55 (1H, dd, J_1 = 9.0 Hz, J_2 = 2.3 Hz, H_{10B}), 8.67, 8.77, 8.80 (3H, 3d, J = 7.9, 7.1, and 8.0 Hz, H_{8A}, H_{8B}, H_{1B}), 9.11 (1H, d, J = 9.5 Hz, H_{1A}), 9.44 (1H, d, J = 2.3 Hz, H_{8B}), 9.58 (1H, d, J = 2.4 Hz, H_{4A}), 11.22 (1H, s, NH), 11.25 (1H, s, NH). ^{13}C NMR (100 MHz, DMSO-d_6): δ 59.6, 60.0 (OCH_3), 59.8, 60.1 ($C_{6'}$), 62.1, 64.2, 71.0, 71.6, 75.8, 76.2, 78.6, 78.8, 80.1, 80.4 ($C_{1'}$, $C_{2'}$, $C_{3'}$, $C_{4'}$, $C_{5'}$), 111.6, 112.4, 113.4, 120.4, 120.9, 122.1, 123.3, 123.4, 123.5, 130.4, 130.9, 132.2, 140.7, 141.1, 141.9, 142.4, 143.6, 145.6 (C quat arom), 112.0, 112.4, 114.9, 115.6, 120.0, 120.2, 121.2, 121.9, 122.3, 124.5, 124.6, 127.4, 127.5, 128.6, 131.6 (C tert arom), 170.6, 170.7, 170.8, 170.9 (C═O).

3,9-Diamino-12,13-(4-*O*-methyl-D-mannopyranosyl)-6,7,-12,13-tetrahydro(5*H*)-indolo[2,3-*a*]pyrrolo[3,4-*c*]carbazole-5,7-dione (15). A mixture of compound **13** (54 mg, 0.094 mmol), THF (13 mL), and $SnCl_2{\cdot}2H_2O$ (356 mg, 1.88 mmol) was refluxed for 63 h. After cooling, the mixture was poured into water and then filtered off, and the solid was washed with AcOEt. The filtrate was adjusted to pH 10 using $NaHCO_3$. After extraction with EtOAc, the organic phase was dried over $MgSO_4$. Removal of the solvent led to **15** (26.7 mg, 0.052 mmol, 55% yield) as a red-orange powder. Mp > 155 °C (dec). IR (KBr): ν_{CO} = 1700, 1710, 1750 cm^{-1}, $\nu_{NH,OH,NH2}$ = 3000−3600 cm^{-1}. HRMS (ESI) (M$^+$): calcd for $C_{27}H_{23}N_5O_6$, 514.1726; found, 514.1755. ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.45−3.52 (2H, m), 3.62 (3H, s, OCH_3), 3.72 (2H, m), 4.54 (1H, m), 4.58 (1H, t, J = 5.0 Hz, OH), 4.96 (1H, br s), 5.15 (4H, br s, NH_2), 6.61 (1H, s, $H_{1'}$), 6.64 (1H, d, J = 4.5 Hz, OH), 6.92 (1H, dd, J_1 = 9.1 Hz, J_2 = 2.4 Hz), 6.94 (1H, dd, J_1 = 8,8 Hz, J_2 = 2.1 Hz), 7.59 (1H, d, J = 8.6 Hz), 7.90 (1H, d, J = 2.1 Hz), 8.04 (1H, d, J = 2.2 Hz), 8.58 (1H, d, J = 9.0 Hz), 10.87 (1H, s, NH). ^{13}C NMR (100 MHz, DMSO-d_6): δ 59.9 (OCH_3), 60.0 ($C_{6'}$), 63.1, 72.3, 76.1, 78.7, 80.1 ($C_{1'}$, $C_{2'}$, $C_{3'}$, $C_{4'}$, $C_{5'}$), 107.7, 107.9, 111.7, 115.3, 115.4, 115.5 (C tert arom), 111.9, 112.2, 119.7, 120.3, 124.7, 124.9, 130.6, 131.4, 133.1, 135.2, 143.1, 143.9 (C quat arom), 171.1, 171.3 (C═O).

3,9-Dibromo-12,13-(4-*O*-methyl-D-mannopyranosyl)-6,7,12,13-tetrahydro(5*H*)-indolo[2,3-*a*]pyrrolo[3,4-*c*]carbazole-5,7-dione (16). To a solution of compound **3** (60 mg, 0.124 mmol) in THF (3 mL) at 0 °C was added dropwise a solution of *N*-bromosuccinimide (221 mg, 1.24 mmol) in THF (6 mL). The mixture was stirred for 4 days at room temperature and then poured into water (50 mL). After hydrolysis (H_2O, 50 mL, 10 min) and then extraction with EtOAc, the organic phase was dried over $MgSO_4$, the solvent was removed, and the residue was purified by flash chromatography (eluent, EtOAc/cyclohexane, 7:3) to give **16** as a yellow solid (67 mg,

0.104 mmol, 84% yield). Mp > 295 °C. IR (KBr): $\nu_{C=O}$ = 1710, 1760 cm^{-1}, $\nu_{NH,OH}$ = 2700−3300 cm^{-1}. HRMS (FAB+) (M^+): calcd for $C_{27}H_{19}N_3O_6Br_2$, 640.9621; found, 640.9593. ^1H NMR (400 MHz, DMSO-d_6): δ 3.45 (4H, m), 3.60 (3H, s, OCH_3), 3.80 (1H, m), 4.58 (1H, m), 5.24 (1H, br s), 6.85 (2H, br s, OH + $H_{1'}$), 7.77 (1H, d, J = 9.5 Hz), 7.82 (1H, d, J = 9.0 Hz), 7.96 (1H, d, J = 8.7 Hz), 8.73 (1H, s), 8.86 (1H, s), 8.90 (1H, d, J = 7.4 Hz), 11.18 (1H, s, NH). ^{13}C NMR (100 MHz, DMSO-d_6): δ 59.9 ($C_{6'}$), 60.0 (OCH_3), 63.4, 71.6, 75.9, 78.7, 80.2 ($C_{1'}$, $C_{2'}$, $C_{3'}$, $C_{4'}$, $C_{5'}$), 111.3, 111.7, 113.2, 113.8, 121.0, 121.3, 125.2, 125.3, 130.5, 131.3, 139.3, 141.2 (C quat arom), 113.9, 117.3, 126.2, 126.4, 129.3, 129.5 (C tert arom), 170.7, 170.9 (C═O).

12,13-[1,2-(6-Chloro-6-deoxy-4-*O*-methyl-D-mannopyranosyl)]-6,7,12,13-tetrahydro(5*H*)-indolo[2,3-*a*]pyrrolo-[3,4-*c*]carbazole-5,7-dione (17) and 12,13-[1,2-(3,6-Anhydro-4-*O*-methyl-D-mannopyranosyl)]-6,7,12,13-tetrahydro(5*H*)-indolo[2,3-*a*]pyrrolo[3,4-*c*]carbazole-5,7-dione (18). To a mixture of compound **3** (216 mg, 0.047 mmol) and pyridine (2 mL) was added PPh_3 (468 mg, 1.78 mmol) and then CCl_4 (86 μL, 0.89 mmol). The mixture was stirred at room temperature for 3 h, then poured into aqueous 1 N HCl, extracted with EtOAc, and washed with brine. The organic phase was dried over $MgSO_4$, and the solvent was removed. The residue was purified by column chromatography and then PLC (eluent, EtOAc/CH_2Cl_2, 10:90) to give **17** (51 mg, 0.102 mmol, 23% yield) and **18** (110 mg, 0.236 mmol, 53% yield) as yellow solids.

17. Mp > 280 °C (dec). IR (KBr): ν_{CO} = 1710, 1750 cm^{-1}, ν_{NH} = 3150−3600 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6): δ 3.56 (1H, t, J = 8.6 Hz), 3.67 (3H, s, OCH_3), 3.70 (1H, dd, J_1 = 12.4 Hz, J_2 = 5.7 Hz), 3.78 (1H, dd, J_1 = 12.2 Hz, J_2 = 2.4 Hz), 4.17 (1H, m), 4.66 (1H, m), 5.26 (1H, br s), 6.90 (1H, d, J = 5.1 Hz, OH), 6.93 (1H, br s, $H_{1'}$), 7.46 (1H, t, J = 7.5 Hz), 7.50 (1H, t, J = 7.5 Hz), 7.64 (1H, t, J = 8.1 Hz), 7.68 (1H, t, J = 7.8 Hz), 7.96 (1H, d, J = 8.2 Hz), 8.71 (1H, d, J = 7.9 Hz), 8.83 (1H, d, J = 7.8 Hz), 8.88 (1H, d, J = 8.6 Hz), 11.10 (1H, s, NH). ^{13}C NMR (100 MHz, DMSO-d_6): δ 44.6 (CH_2Cl), 60.2, 62.9, 71.5, 76.6, 77.1, 80.1 (OCH_3, $C_{1'}$, $C_{2'}$, $C_{3'}$, $C_{4'}$, $C_{5'}$), 111.7, 115.1, 121.0, 121.9, 124.4, 124.5, 127.0, 127.1 (C tert arom), 112.4, 112.8, 120.6, 121.0, 123.6, 123.8, 130.0, 131.0, 140.6, 142.4 (C quat arom), 170.9, 171.2 (C═O).

18. Mp > 300 °C. IR (KBr): ν_{CO} = 1720, 1750 cm^{-1}, ν_{NH} = 3100−3600 cm^{-1}. HRMS (FAB+) (M + H)$^+$: calcd for $C_{27}H_{20}N_3O_5$, 466.1403; found, 466.1388. ^1H NMR (400 MHz, DMSO-d_6): δ 3.55 (1H, d, J = 10.6 Hz), 3.67 (3H, s, OCH_3), 3.73 (1H, m), 4.17 (1H, m), 4.69 (1H, d, J = 6.2 Hz), 4.90 (1H, br s), 5.55 (1H, d, J = 5.5 Hz), 6.66 (1H, d, J = 5.5 Hz, $H_{1'}$), 7.41 (2H, t, J = 7.4 Hz), 7.61 (3H, m), 8.00 (1H, d, J = 8.2 Hz), 8.60 (1H, d, J = 6.8 Hz), 8.62 (1H, d, J = 6.9 Hz), 10.93 (1H, s, NH). ^{13}C NMR (100 MHz, DMSO-d_6): δ 53.2, 57.3, 71.9, 73.6, 78.1, 81.1 (OCH_3, $C_{1'}$, $C_{2'}$, $C_{3'}$, $C_{4'}$, $C_{5'}$), 69.4 (CH_2O), 110.5, 113.0, 121.0, 121.5, 124.3, 124.6, 126.7, 127.0 (C tert arom), 110.5, 111.7, 120.0, 120.6, 123.3, 123.9, 128.1, 128.9, 139.2, 142.2 (C quat arom), 171.0 (2C═O).

12,13-[1,2-(6-Azido-6-deoxy-4-*O*-methyl-D-mannopyranosyl)]-6,7,12,13-tetrahydro(5*H*)-indolo[2,3-*a*]pyrrolo-[3,4-*c*]carbazole-5,7-dione (19). A mixture of compound **17** (51 mg, 0.102 mmol) in DMF (1 mL) and sodium azide (66 mg, 1.02 mmol) was stirred at 80 °C for 48 h. The mixture was dissolved in EtOAc and then washed with water. The organic phase was dried over $MgSO_4$, and the solvent was removed. The residue was purified by chromatography (eluent, EtOAc/CH_2Cl_2, 5:95) to give **19** (19 mg, 0.037 mmol, 38% yield) and **18** (24 mg, 0.052 mmol, 51% yield) as yellow solids.

19. Mp > 250 °C (dec). IR (KBr): $\nu_{C=O}$ = 1700, 1750 cm^{-1}, $\nu_{N=N}$ = 2100 cm^{-1}, $\nu_{NH,OH}$ = 3150−3600 cm^{-1}. HRMS (FAB+) (M^+): calcd for $C_{27}H_{20}N_6O_5$, 508.1495; found, 508.1475. ^1H NMR (400 MHz, DMSO-d_6): δ 3.27 (1H, dd, J_1 = 13,7 Hz, J_2 = 6,0 Hz), 3.48 (2H, m), 3.63 (3H, s, OCH_3), 4.10 (1H, m), 4.65 (1H, m), 5.27 (2H, br s), 6.96 (2H, m, OH + $H_{1'}$), 7.47 (1H, t, J = 7.9 Hz), 7.49 (1H, t, J = 8.3 Hz), 7.65 (1H, dt, J_1 = 8.4 Hz, J_2 = 1.1 Hz), 7.68 (1H, dt, J_1 = 7.3 Hz, J_2 = 1.1 Hz), 7.97 (1H, d, J = 8.2 Hz), 8.70 (1H, d, J = 7.8 Hz), 8.85 (1H, d, J = 7.5 Hz), 9.95 (1H, d, J = 8.6 Hz), 11.10 (1H, s, NH). ^{13}C NMR (100 MHz, DMSO-d_6): δ 50.5 ($C_{6'}$), 60.2, 63.3, 71.8, 76.7, 77.0, 80.1 (OCH_3, $C_{1'}$, $C_{2'}$, $C_{3'}$, $C_{4'}$, $C_{5'}$), 111.7, 115.2, 121.0, 121.8, 124.4, 124.5, 127.0 (2C) (C tert arom), 112.5, 112.9, 120.5, 120.9, 123.7, 123.8, 130.1, 131.0, 140.5, 142.5 (C quat arom), 170.9, 171.2 (C═O).

3,9-Dinitro-12,13-[1,2-(3,6-anhydro-4-*O*-methyl-D-mannopyranosyl)]-6,7,12,13-tetrahydro(5*H*)-indolo[2,3-*a*]pyrrolo[3,4-*c*]carbazole-5,7-dione (21) and 3- or 9-Nitro-12,13-[1,2-(3,6-anhydro-4-*O*-methyl-D-mannopyranosyl)]-6,7,12,13-tetrahydro(5*H*)-indolo[2,3-*a*]pyrrolo[3,4-*c*]carbazole-5,7-dione (22). To a solution of fuming nitric acid (0.3 mL) in THF (2.15 mL) cooled to 0 °C was added compound **18** (147 mg, 0.32 mmol). The reaction mixture was stirred at room temperature for 19 h before another addition of fuming nitric acid (0.3 mL). After 24 h (5 h after the last addition of fuming nitric acid), water was added to the mixture. After extraction with EtOAc, the organic phase was washed with H_2O until pH 6 was attained and was dried over $MgSO_4$. The solvent was removed, and the residue was purified by flash chromatography (eluent, EtOAc/cyclohexane, 60:40) to give **22** (88 mg, 0.17 mmol, 54% yield) and (eluent, EtOAc/cyclohexane, 1:4) to give **21** (36 mg, 0.07 mmol, 20% yield) as yellow solids.

21. Mp > 300 °C. IR (KBr): ν_{CO} = 1710, 1760 cm^{-1}, ν_{NH} = 3200 cm^{-1}. HRMS (FAB+) (M + H)$^+$: calcd for $C_{27}H_{18}N_5O_9$, 556.1104; found, 556.1106. ^1H NMR (400 MHz, DMSO-d_6): δ 3.34 (1H, d, J = 10.8 Hz), 3.69 (1H, m), 3.70 (3H, s, OCH_3), 4.21 (1H, dd, J_1 = 6.4 Hz, J_2 = 2.5 Hz), 4.68 (1H, d, J = 6.9 Hz), 4.97 (1H, t, J = 2.4 Hz), 5.90 (1H, d, J = 5.4 Hz), 6.80 (1H, d, J = 5.4 Hz), 7.90 (1H, d, J = 9.3 Hz), 8.13 (1H, d, J = 9.4 Hz), 8.42 (1H, dd, J_1 = 9.3 Hz, J_2 = 2.5 Hz), 8.48 (1H, dd, J_1 = 9.4 Hz, J_2 = 2.5 Hz), 8.91 (1H, s), 8.92 (1H, s), 11.10 (1H, s, NH). ^{13}C NMR (100 MHz, DMSO-d_6): δ 54.1, 57.5, 72.2, 73.8, 78.0, 81.3 (OCH_3, $C_{1'}$, $C_{2'}$, $C_{3'}$, $C_{4'}$, $C_{5'}$), 69.5 ($C_{6'}$), 111.0, 111.8, 120.7, 121.2, 122.5, 122.9, 128.8, 129.7, 141.5, 141.8, 142.3, 144.9 (C quat arom), 111.5, 113.4, 120.0, 120.3, 122.5, 122.8 (C tert arom), 169.5, 169.8 (C═O).

22 Regioisomers. Mp > 300 °C. IR (KBr): ν_{CO} = 1710, 1750 cm^{-1}, ν_{NH} = 3200 cm^{-1}. HRMS (FAB+) (M + H)$^+$: calcd for $C_{27}H_{19}N_4O_7$, 511.1253; found, 511.1244. ^1H NMR (400 MHz, DMSO-d_6): δ 3.34 and 3.47 (1H, 2d, J = 10.8 and 10.3 Hz), 3.64−3.76 (4H, s + m), 4.15 (1H, m), 4.62 and 4.67 (1H, 2d, J = 5.4 and 5.9 Hz), 4.91 (1H, m), 5.63 and 5.67 (1H, 2d, J = 5.9 and 5.4 Hz), 6.60 and 6.71 (1H, 2d, J = 5.4 Hz), 7.35 (1H, d, J = 7.4 and 7.9 Hz), 7.60 and 7.74 (1H, m and d, J = 9.4 Hz), 7.96 and 8.05 (1H, 2d, J = 8.5 and 8.9 Hz), 8.30 and 8.40 (2H, dd, J_1 = 9.4 Hz, J_2 = 2.5 Hz, m), 9.05 and 9.08 (1H, 2d, J = 2.5 Hz), 10.91 and 10.94 (1H, 2s, NH). ^{13}C NMR (100 MHz, DMSO-d_6): δ 53.4, 53.9, 57.4 (2C), 71.9, 72.2, 73.5, 74.0, 78.0, 78.1, 81.1, 81.3 (OCH_3, $C_{1'}$, $C_{2'}$, $C_{3'}$, $C_{4'}$, $C_{5'}$), 69.4, 69.6 ($C_{6'}$), 110.2, 111.0, 111.3, 112.6, 119.6, 120.3, 121.3, 121.4, 122.9, 123.0, 123.4, 128.1, 128.8, 129.0, 129.8, 139.3, 141.2, 141.6, 142.2, 142.3, 144.9 (C quat arom), 110.8, 110.9, 113.1, 119.5, 120.3, 121.7, 121.8, 122.0, 122.3, 124.4, 124.7, 127.3, 127.6 (C tert arom), 170.3, 170.4, 170.5, 170.6 (C═O).

12,13-(3,6-Di-*O*-acetyl-4-*O*-methyl-D-mannopyranosyl)-6,7,12,13-tetrahydro(5*H*)-indolo[2,3-*a*]pyrrolo[3,4-*c*]carbazole-5,7-dione (23). To a solution of compound **3** (351 mg, 0.73 mmol) in pyridine (7 mL) was added dropwise acetic anhydride (0.68 mL, 7.27 mmol). The mixture was stirred for 19 h at room temperature. After hydrolysis (H_2O, 30 mL) and then extraction with EtOAc, the organic phase was washed successively with 1 N HCl, water, saturated aqueous $NaHCO_3$, and water and was dried over $MgSO_4$ and the solvent was removed. The residue was purified by flash chromatography (eluent, EtOAc/cyclohexane, 4:6) to give **23** (309 mg, 0.55 mmol, 75% yield) and a mixture of unseparable di- and triacetylated compounds (72.6 mg) as yellow solids.

23: Mp = 106 °C. IR (KBr): $\nu_{C=O}$ = 1720, 1750 cm^{-1}, $\nu_{NH,OH}$ = 3100−3600 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6): δ 1.78 (3H, s), 1.90 (3H, s), 3.59 (3H, s, OCH_3), 3.67 (1H, pt, J = 7.0 Hz), 4.12−4.30 (2H, m), 4.34 (1H, dt, J_1 = 7.1 Hz, J_2 = 2.7 Hz), 5.65 (1H, pt, J = 2.9 Hz), 5.90 (1H, dd, J_1 = 6.1 Hz, J_2 = 2.7 Hz), 6.90 (1H, d, J = 3.3 Hz, $H_{1'}$), 7.48 (1H, t, J = 7.1 Hz), 7.50 (1H, t, J = 7.0 Hz), 7.67 (1H, t, J = 8.2 Hz), 7.72 (1H, t, J = 7.9 Hz), 7.95 (1H, d, J = 8.3 Hz), 8.22 (1H, d, J = 8.4 Hz),

8.68 (1H, d, J = 7.9 Hz), 8.77 (1H, d, J = 7.8 Hz), 11.10 (1H, s, NH). ^{13}C NMR (100 MHz, DMSO-d_6): δ 20.4 (2C) (CH_3), 56.8, 58.9, 69.9, 74.0, 74.1, 80.1 ($C_{1'}$, $C_{2'}$, $C_{3'}$, $C_{4'}$, $C_{5'}$, OCH_3), 63.0 ($C_{6'}$), 112.1 (2C), 120.8, 120.9, 123.6, 123.8, 129.1, 129.9, 141.0, 141.3 (C quat arom), 112.3 (2C), 121.3, 121.9, 124.4, 124.7, 127.1, 127.3 (C tert arom), 168.7, 169.9, 170.9, 171.0 (C=O).

12,13-(3-*O*-Acetyl-4-*O*-methyl-D-mannopyranosyl)-6,7,-12,13-tetrahydro(5*H*)-indolo[2,3-*a*]pyrrolo[3,4-*c*]carbazole-5,7-dione (24). To a solution compound **23** (337 mg, 0.594 mmol) in a mixture of acetonitrile (30 mL) and water (3 mL) at 0 °C was added dropwise BF_3/Et_2O (0.92 mL, 7.26 mmol). After the mixture was stirred for 24 h at room temperature, water (3 mL) and BF_3/Et_2O (0.92 mL, 7.26 mmol) were added. After this mixture was stirred at room temperature for 24 h, saturated aqueous $NaHCO_3$ was added. After extraction with EtOAc, the organic phase was washed with brine and was dried over $MgSO_4$. The solvent was removed, and the residue was purified by flash chromatography (eluent, EtOAc/cyclohexane 1:1) to give **24** (110.8 mg, 0.211 mmol, 35% yield), the unreacted 3,6-diacetylated compound (212 mg, 0.374 mmol), and compound **3** (8.1 mg, 0.016 mmol) as yellow solids.

24. Mp = 294 °C. IR (KBr): $\nu_{C=O}$ = 1720, 1750 cm^{-1}, $\nu_{NH,OH}$ = 3100−3600 cm^{-1}. ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.95 (3H, s, CH_3CO), 3.60 (3H, s, OCH_3), 3.61 (1H, m), 3.77 (1H, t, J = 7.7 Hz), 3.84 (1H, m), 4.04 (1H, m), 4.79 (H, t, J = 5.4 Hz, $OH_{6'}$), 5.53 (1H, br s), 5.92 (1H, dd, J_1 = 7.3 Hz, J_2 = 2.9 Hz), 6.94 (1H, d, J = 2.6 Hz), 7.50 (2H, t, J = 7.5 Hz), 7.67 (1H, t, J = 7.5 Hz), 7.73 (1H, t, J = 7.9 Hz), 8.00 (1H, d, J = 8.3 Hz), 8.35 (1H, d, J = 8.5 Hz), 8.69 (1H, d, J = 7.9 Hz), 8.81 (1H, d, J = 7.8 Hz), 11.07 (1H, s, NH). ^{13}C NMR (100 MHz, DMSO-d_6): δ 20.7 (CH_3), 60.1 ($C_{6'}$), 58.6, 59.3, 71.1, 74.3, 77.7, 80.2 ($C_{1'}$, $C_{2'}$, $C_{3'}$, $C_{4'}$, $C_{5'}$, OCH_3), 112.2, 112.8, 121.2, 121.8, 124.4, 124.7, 127.1, 127.4 (C tert arom), 112.3, 112.4, 120.8, 120.9, 123.7, 123.8, 129.4, 130.3, 140.8, 141.7 (C quat arom), 169.0, 170.9, 171.1 (C=O).

12,13-(3-*O*-Acetyl-6-chloro-6-deoxy-4-*O*-methyl-D-mannopyranosyl)-6,7,12,13-tetrahydro(5*H*)-indolo[2,3-*a*]pyrrolo[3,4-c]carbazole-5,7-dione (25). To a solution of compound **24** (77 mg, 0.147 mmol) in pyridine (1.7 mL) were successively added triphenylphosphine (154 mg, 0.587 mmol) and dropwise CCl_4 (43 μL, 0.441 mmol). The mixture was stirred at 40 °C for 65 h, cooled, and then poured into water (30 mL). After extraction with EtOAc, the organic phase was successively washed with 1 N HCl, water, saturated aqueous $NaHCO_3$, and water and was dried over $MgSO_4$. The solvent was removed, and the residue was purified by flash chromatography (eluent, EtOAc/cyclohexane 35:55) to give **25** (49.5 mg, 0.048 mmol, 62% yield) as a yellow solid and unreacted starting product (10.4 mg, 0.020 mmol).

25. Mp > 300 °C. IR (KBr): $\nu_{C=O}$ = 1700, 1730, 1770 cm^{-1}, $\nu_{NH,OH}$ = 3100−3600 cm^{-1}. HRMS (FAB+) (M^+): calcd for $C_{29}H_{22}N_3O_6Cl$, 543.1197; found, 543.1196. ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.97 (3H, s), 3.61 (3H, s, OCH_3), 3.73−3.88 (3H, m), 4.33 (1H, m), 5.46 (1H, m), 5.94 (1H, dd, J_1 = 7.3 Hz, J_2 = 2.3 Hz), 6.93 (1H, br s, $H_{1'}$), 7.44 (1H, t, J = 7.6 Hz), 7.48 (1H, t, J = 7.6 Hz), 7.66 (1H, t, J = 7.9 Hz), 7.71 (1H, t, J = 7.8 Hz), 7.91 (1H, d, J = 8.2 Hz), 8.29 (1H, d, J = 8.4 Hz), 8.63 (1H, d, J = 7.8 Hz), 8.74 (1H, d, J = 7.7 Hz), 11.00 (1H, s, NH). ^{13}C NMR (100 MHz, DMSO-d_6): δ 20.7 (CH_3), 44.4 ($C_{6'}$), 58.3, 59.5, 70.8, 75.7, 75.9, 80.0 ($C_{1'}$, $C_{2'}$, $C_{3'}$, $C_{4'}$, $C_{5'}$, OCH_3), 111.9, 112.6, 121.2, 121.9, 124.5, 124.7, 127.1, 127.4 (C tert arom), 112.3, 112.4, 120.7, 120.9, 123.7 (2C), 129.0, 129.9, 140.6, 141.5 (C quat arom), 169.0, 170.7, 170.9 (C=O).

12,13-(3-*O*-Acetyl-6-azido-6-deoxy-4-*O*-methyl-D-mannopyranosyl)-6,7,12,13-tetrahydro(5*H*)-indolo[2,3-*a*]pyrrolo[3,4-*c*]carbazole-5,7-dione (26). To a solution of compound **25** (57 mg, 0.105 mmol) in DMF was added NaN_3 (68.2 mg, 1.05 mmol). The mixture was stirred at 90 °C for 65 h. After it was cooled and extracted with EtOAc, the organic phase was washed with water and dried over $MgSO_4$ and the solvent was removed. The residue was purified by flash chromatography (eluent, cyclohexanes/EtOAc, 7:3) to give **26** (46 mg, 0.084 mmol, 80% yield) as a yellow solid. Mp = 258 °C. IR (KBr): $\nu_{C=O}$ = 1700, 1720−1750 cm^{-1}, ν_{N3} = 2100 cm^{-1}, ν_{NH} = 3100−3500 cm^{-1}. HRMS (FAB+) (M^+): calcd for $C_{29}H_{22}N_6O_6$, 550.1601; found, 550.1611. ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.04 (3H, s, CH_3CO), 3.40 (1H, m), 3.56 (1H, m), 3.58 (3H, s, OCH_3), 3.71 (1H, pt, J = 8.2 Hz), 4.28 (1H, dt, J_1 = 8.4 Hz, J_2 = 2.2 Hz), 5.51 (1H, br s), 5.94 (1H, dd, J_1 = 7.8 Hz, J_2 = 2.6 Hz), 7.03 (1H, d, J = 1.5 Hz, $H_{1'}$), 7.49 (2H, t, J = 7.5 Hz), 7.67 (1H, t, J = 7.8 Hz), 7.73 (1H, t, J = 7.8 Hz), 7.95 (1H, d, J = 8.3 Hz), 8.37 (1H, d, J = 8.5 Hz), 8.67 (1H, d, J = 7.8 Hz), 8.80 (1H, d, J = 7.8 Hz), 11.07 (1H, s, NH). ^{13}C NMR (100 MHz, DMSO-d_6): δ 20.7 (CH_3CO), 50.6 ($C_{6'}$), 58.8, 59.6, 71.1, 75.5, 75.9, 80.0 ($C_{1'}$, $C_{2'}$, $C_{3'}$, $C_{4'}$, $C_{5'}$, OCH_3), 111.9, 112.8, 121.3, 121.9, 124.5, 124.7, 127.1, 127.4 (C tert arom), 112.4, 112.6, 120.8, 120.9, 123.7, 123.8, 129.3, 130.2, 140.6, 141.7 (C quat arom), 169.0, 170.8, 171.0 (C=O).

12,13-(6-Deoxy-6-amino-4-*O*-methyl-D-mannopyranosyl)-6,7,12,13-tetrahydro(5*H*)-indolo[2,3-*a*]pyrrolo[3,4-*c*]carbazole-5,7-dione hydrochloride (20). To a solution of compound **26** (69.6 mg, 0.126 mmol) in methanol (15 mL) and EtOAc (14 mL) was added 10% Pd/C (7 mg). The light-protected mixture was hydrogenated (1 bar) for 40 h at room temperature. After filtration over Celite, the solid was washed with methanol, THF, and EtOAc. After removal of the solvents, the residue was dissolved in a mixture of 1 N HCl (20 mL)/ EtOAc (30 mL). The organic phase was washed with 1 N HCl. After extraction with EtOAc, the organic phase was washed with saturated aqueous Na HCO_3, and then the acetylated amine was extracted with EtOAc. The organic phase was dried over $MgSO_4$, and the solvent was removed. The residue was dissolved in methanol (18 mL), and 28% NH_4OH (6 mL) was added dropwise. The light-protected mixture was stirred at room temperature for 5 h. After evaporation of the solvents, a mixture of water and EtOAc was poured into the residue and the amine was extacted with EtOAc. The organic phase was dried over $MgSO_4$; the solvent was removed to give the amine as a yellow solid. To a solution of the amine at 0 °C in methanol (130 mL) was added dropwise 1.14 N HCl (88 μL). Cyclohexane was added, and the precipitate was filtered off to give hydrochloride **20** (31.8 mg, 0.061 mmol, 49% yield) as an orange solid. Mp > 300 °C. IR (KBr): ν_{CO} = 1710, 1760 cm^{-1}, $\nu_{NH,OH}$ = 3270−3600 cm^{-1}. HRMS (FAB+) (M + H)$^+$: calcd for $C_{27}H_{23}N_4O_5$, 483.1668; found, 483.1672. ^{1}H NMR (400 MHz, CD_3OD): δ 3.05 (1H, dd, J_1 = 9.5 Hz, J_2 = 13.5 Hz), 3.44 (1H, dd, J_1 = 3.2 Hz, J_2 = 13.5 Hz), 3.68 (1H, pt, J = 8.7 Hz), 3.93 (3H, s, OCH_3), 4.27 (1H, pdt, J_1 = 3.2 Hz, J_2 = 8.7 Hz), 4.91 (1H, dd, J_1 = 3.2 Hz, J_2 = 8.7 Hz), 5.21 (1H, m), 6.82 (1H, br s, $H_{1'}$), 7.51 (1H, t, J = 7.1 Hz), 7.52 (1H, t, J = 7.1 Hz), 7.73 (1H, t, J = 9.5 Hz), 7.75 (1H, t, J = 7.9 Hz), 7.98 (1H, d, J = 8.7 Hz), 8.77 (1H, d, J = 7.9 Hz), 8.92 (1H, d, J = 7.1 Hz), 8.97 (1H, d, J = 8.7 Hz). ^{13}C NMR (100 MHz, CD_3OD): δ 42.1 ($C_{6'}$), 61.5, 64.7, 74.1, 76.5, 79.6, 82.4 ($C_{1'}$, $C_{2'}$, $C_{3'}$, $C_{4'}$, $C_{5'}$, OCH_3), 112.5, 116.5, 122.6, 123.4, 126.3, 126.5, 128.5 (2C) (C tert arom), 114.8, 115.4, 122.2, 122.7, 125.8, 126.1, 131.5, 132.5, 142.4, 144.4 (C quat arom), 172.7, 172.9 (C=O).

3,9-Dinitro-12,13-(6-chloro-6-deoxy-4-*O*-methyl-D-mannopyranosyl)-6,7,12,13-tetrahydro(5*H*)-indolo[2,3-*a*]pyrrolo[3,4-*c*]carbazole-5,7-dione (27). To a solution of compound **25** (50.6 mg, 0.093 mmol) in THF (5 mL) at 0 °C was added dropwise fuming HNO_3 (3.5 mL). After being stirred at 0 °C for 2 h, the mixture was allowed to reach room temperature then stirred at room temperature for 21 h. Water (40 mL) was added. After extraction wth 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, toluene/THF, 7:3) to give a dinitrated product **25′** (44.9 mg, 0.071 mmol, 76% yield). This compound was very hygroscopic, and the NMR spectra in DMSO were not interpretable. To a solution of this compound (22.3 mg, 0.038 mmol) in CH_3CN (3 mL), water (0.3 mL), and THF (2 mL) was added dropwise $BF_3{\cdot}Et_2O$ (48 μL, 0.38 mmol). After the mixture was stirred for 48 h at 40 °C, water (0.3 mL) and $BF_3.Et_2O$ (1.0 mL, 7.89 mmol) were added. After the mixture was stirred for 24 h at room temperature, saturated aqueous $NaHCO_3$ was added. After extraction with EtOAc, the organic phase was

washed with brine and then dried over $MgSO_4$. The solvent was removed, and the residue was purified by flash chromatography (eluent, toluene/THF, 1:1) to give **27** (2.7 mg, 4.56×10^{-3} mmol, 12% yield) as a yellow solid. Mp > 300 °C. IR (KBr): ν_{CO} = 1704 cm^{-1}, $\nu_{NH,OH}$ = 3300−3600 cm^{-1}. Mass (electrospray) (M − HCl): 569. ^1H NMR (400 MHz, DMSO-d_6): δ 3.48 (1H, m), 3.65 (3H, s, OCH_3), 3.64−3.78 (2H, m), 4.23 (1H, m), 4.67 (1H, m), 5.46 (1H, br s), 7.06 (2H, m, $H_{1'}$ + $OH_{3'}$), 8.17 (1H, d, J = 9.1 Hz), 8.43 (1H, d, J = 9.2 Hz), 8.60 (1H, d, J = 8.9 Hz), 8.99 (1H, d, J = 9.5 Hz), 9.29 (1H, br s), 9.42 (1H, br s), 11.34 (1H, s, NH). ^{13}C NMR (100 MHz, DMSO-d_6): δ 43.7 ($C_{6'}$), 60.0, 62.8, 70.1, 76.5, 76.8, 79.9 ($C_{1'}$, $C_{2'}$, $C_{3'}$, $C_{4'}$, $C_{5'}$, OCH_3), 113.0, 116.0, 119.9, 120.3, 123.2, 123.7 (C tert arom), 113.1, 113.6, 122.0, 122.6, 123.7, 124.0, 131.8, 132.4, 142.2, 143.2, 144.5, 146.0 (C quat arom), 170.4, 170.6 (C═O).

3,9-Dinitro-12,13-(3-*O*-acetyl-6-azido-6-deoxy-4-*O*-methyl-D-mannopyranosyl)-6,7,12,13-tetrahydro(5*H*)-indolo[2,3-*a*]pyrrolo[3,4-*c*]carbazole-5,7-dione (28) and 3,9-Dinitro-12,13-(6-azido-6-deoxy-4-*O*-methyl-D-mannopyranosyl)-6,7,12,13-tetrahydro(5*H*)-indolo[2,3-*a*]pyrrolo[3,4-*c*]carbazole-5,7-dione (29). To a solution of **25′** dried over P_2O_5 (50 mg, 0.079 mmol) in DMF (4 mL) was added NaN_3 (318 mg, 4.89 mmol). The mixture was stirred at 90 °C for 36 h. After the mixture was cooled, water (20 mL) was added and the azide was extracted with EtOAc. The organic phase was dried over $MgSO_4$, and the solvent was removed. The residue was purified by flash chromatography (eluent, cyclohexanes/EtOAc, 2:3) to give **28** (17.1 mg, 0.027 mmol, 34% yield) and **29** (7.8 mg, 0.013 mmol, 16% yield) as yellow solids. Compound **21** (1.7 mg, 3.06×10^{-3} mmol, 4% yield) was also formed in this reaction.

28. Mp = 253 °C. IR (KBr): ν_{CO} = 1720, 1760 cm^{-1}, ν_{N3} = 2100 cm^{-1}, ν_{NH} = 3200−3600 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6): δ 2.03 (3H, s, CH_3CO), 3.43 (1H, m), 3.59 (3H, s, OCH_3), 3.62 (1H, m), 3.71 (1H, pt, J = 7.9 Hz), 4.35 (1H, m), 5.79 (1H, br s), 5.95 (1H, dd, J_1 = 7.5 Hz, J_2 = 2.4 Hz), 7.25 (1H, d, J = 2.1 Hz, $H_{1'}$), 8.20 (1H, d, J = 9.2 Hz), 8.53 (1H, d, J = 9.3 Hz), 8.57 (1H, pt, J = 2.5 Hz), 8.59 (1H, pt, J = 2.5 Hz), 9.34 (1H, br s), 9.44 (1H, d, J = 3.0 Hz), 11.40 (1H, s, NH). ^{13}C NMR (100 MHz, DMSO-d_6): δ 20.6 (CH_3CO), 50.4 ($C_{6'}$), 59.3, 59.7, 70.4, 75.2, 76.1, 80.1 ($C_{1'}$, $C_{2'}$, $C_{3'}$, $C_{4'}$, $C_{5'}$, OCH_3), 112.6, 113.6, 120.2 (2C), 122.9 (2C) (C tert arom), 112.5, 112.8, 121.6, 121.7, 123.0, 123.1, 130.0, 130.8, 141.6, 142.3, 143.6, 144.7 (C quat arom), 169.0, 169.8, 169.9 (C═O).

29. Mp > 300 °C. IR (KBr): ν_{CO} = 1720, 1760 cm^{-1}, ν_{N3} = 2100 cm^{-1}, $\nu_{NH,OH}$ = 3200−3600 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6): δ 3.28 (1H, dd, J_1 = 13.8 Hz, J_2 = 6.1 Hz), 3.42−3.47 (2H, m), 3.62 (3H, s, OCH_3), 4.16 (1H, m), 4.65 (1H, m), 5.46 (1H, br s), 7.09 (1H, br s, $H_{1'}$), 7.12 (1H, d, J = 4.5 Hz, $OH_{3'}$), 8.18 (1H, d, J = 9.1 Hz), 8.53 (1H, dd, J_1 = 9.4 Hz, J_2 = 2.5 Hz), 8.58 (1H, dd, J_1 = 9.4 Hz, J_2 = 2.5 Hz), 9.03 (1H, d, J = 9.4 Hz), 9.22 (1H, br s), 9.37 (1H, br s), 11.30 (1H, s, NH). ^{13}C NMR (100 MHz, DMSO-d_6): δ 50.4 ($C_{6'}$), 60.1, 63.2, 70.9, 76.8 (2C), 80.0 ($C_{1'}$, $C_{2'}$, $C_{3'}$, $C_{4'}$, $C_{5'}$, OCH_3), 112.4, 115.4, 120.1, 120.2, 122.4, 122.8 (C tert arom), 112.6, 113.2, 121.5, 121.7, 123.0, 123.1, 130.7, 131.7, 141.4, 142.2, 143.6, 145.4 (C quat arom), 169.9, 170.1 (C═O).

Growth Inhibition Assays. Tumor cells were provided by American Type Culture Collection (Frederik, MD). 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, then a total of 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 was solubilized by 100 μL of DMSO. Results are expressed as IC_{50}, the concentration at which the optical density of treated cells with respect to untreated controls is reduced by 50%.

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, then fixed by 70% ethanol (v/v), and 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 low-pass filter.

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