# Syntheses and Biological Activities of Rebeccamycin Analogues with Uncommon Sugars

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Rebeccamycin analogues containing uncommon sugars and substitutions on the imide nitrogen have been synthesized. Their cytotoxicities were tested in colon cancer and leukemia cells. Their ability to target topoisomerase I was examined using the in vivo complex of the topoisomerase bioassay in Hela cells. Compared with aglycon **1**, the modified compounds with various sugar moieties showed more potent cytotoxicities and topo I targeting ability. In addition, the rebeccamycin analogues with various uncommon sugars showed distinct cytotoxicities and topo I targeting activities. The activity of compounds with 2-deoxyglucose (**8** and **9**) > compounds with 2,6-deoxyglucose (**5** and **6**) > compounds with 2,3,6-deoxyglucose (**10**). Furthermore, the anticancer activity of compounds correlated with their ability to target endogenous topo I. These results suggest that the sugar moiety, especially the 2-OH and 6-OH group of the sugar, rather than the modifications in imide structure on the indolocarbazole ring, is a key element for its activity.

## Introduction

Rebeccamycin and BE-13793C (Figure 1) were isolated from fermentation cultures of *Saccharotrix aerocolonigenes* (ATCC 39243)[1] and *Streptoverticillium mobaraense* (FERM P-10489).[2] Their structures, which were elucidated in 1985[3,4] and 1991,[2] share a common indolocarbazole pharmacophore. Since the potent anticancer activities were discovered for these compounds, the indolocarbazole compounds have been extensively studied. Structure−activity relationships studies have led to the development of several analogues. For instance, NB-506,[5−7] a glycosylated derivative of BE-13793C, the rebeccamycin derivative NCS655649,[8−10] and J-107088[11−13] are currently in clinical trials.

Generally, there are three modification approaches to synthesize indolocarbazole derivatives for new antitumor drug candidates with improved pharmaceutical properties: (i) replacement of the hydrogen on the imide functionality with other groups, especially a hydrophilic group; (ii) substitution on the fused aromatic ring or a change of the benzene ring to another hetero-aromatic ring, and (iii) replacement of the $\beta$-glycoside with other sugar moieties. More than 300 rebeccamycin derivatives were synthesized and reported to date. Most of the current modifications through the first two approaches are to improve the water solubility of the compounds. A few papers in recent years reported the synthesis and activity of indolocabazoles with a modified sugar moiety.[14−16] These studies suggest that altering glycosylation patterns on indolocarbazole has a high potential for generating novel chemotherapeutics. For instance, alterations from glucose to other sugars such as galactose, mannose, and fucose do not change the biological profile significantly.[15] In sharp contrast, however, the introduction of an amino group on the glucose sugar moiety greatly enhance the binding affinity to DNA, leading to enhanced cytotoxicity. While the 2-amino group does not prevent indolocarbazoles from inhibiting topo I,[16] the 6-amino group almost abolishes topoisomerase inhibition activity of the compound.[14] To date, there are no systematic studies to depict the biological effect of the functionalities on the sugar moiety.

In our present study, we intend to investigate a series of rebeccamycin analogues for which various uncommon sugars are attached to one of the indole nitrogens via an $\alpha$ or $\beta$-*N*-glycosidic linkage for their cytotoxicities and inhibition of topoisomerase I. Uncommon sugars have received special attention, due to the increased recognition of their vital roles in the biological function of many natural products. They add important features to the shape and the stereoelectronic properties of a molecule and often play an essential role in the biological activity of many natural product drugs. Areas in which their significance has been well-established include cellular adhesion and cell−cell recognition, fertilization, protein folding, neurobiology, xenotransplantation, and target recognition in the immune response.[17−20] In addition, a methyl group is introduced on the imide nitrogen of the indolocarbazole chromophore. Methyl-containing indolocarbazoles may provide a different mechanism for the

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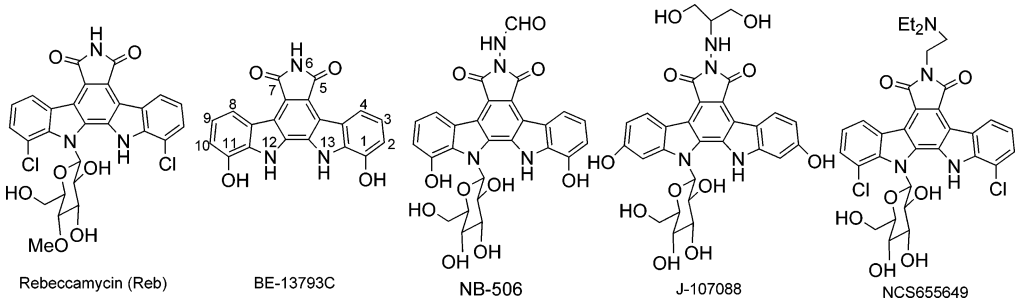



**Figure 1.** Structures of rebeccamycin and its analogues.

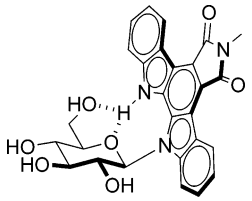


**Figure 2.** Favorable conformation of compound **2** with bifurcated intramolecular hydrogen bond.

development of antitumor drugs since two rebeccamycin analogues (AT2433-A1 and AT2433-B1) with a methyl group on the imide nitrogen exhibited significant antitumor properties via an inhibition of topoisomerase I.[9,21]

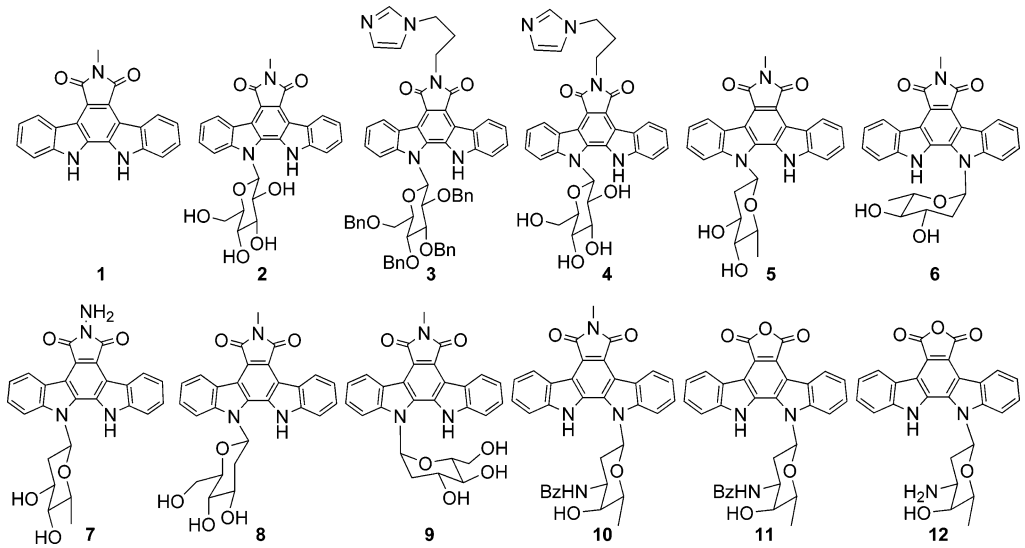
Furthermore, it has been reported that the closed conformation involving a hydrogen bond between the pyranose oxygen and the indole NH of rebeccamycin is essential for the activity to access DNA binding sites and inhibit topoisomerase I[10,22,23] (Figure 2). Since both 6-OH and 1-oxygen can form a hydrogen bond with indole NH thus keeping the closed conformation, the importance of all OH groups in the sugar moiety needs to be further investigated.

To explore the importance of OH group at the 2, 3, 6 positions in the sugar and the impact of an uncommon aminosugar, three uncommon sugars **25** (2-deoxy sugar), **17** (2,6-dideoxy sugar), and **34** (2,3,6-trideoxy aminosugar) were selected as glycosyl donors. Currently, we have prepared a series of rebeccamycin analogues (Scheme 1) with various uncommon sugars and substitution on the imide nitrogen with a methyl group (**5**, **6**, **8**, **9**, **10**) or amino group (**3**, **4**, **7**), and the corresponding anhydride analogues (**11**, **12**). The cytotoxicities and topoisomerase I inhibition of these compounds were tested in cancer cells in comparison with their parent compounds, synthetic compounds **1** and **2**, methylated at the imide nitrogen.

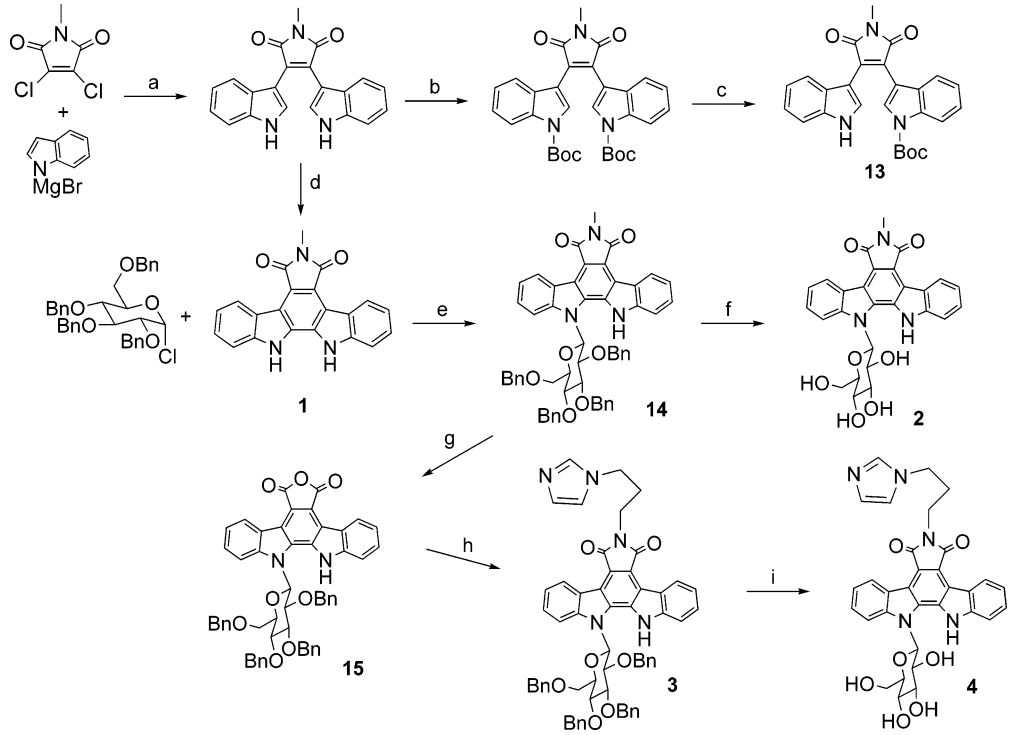
## Results and Discussion

**Chemistry.** The synthesis of compounds **1**, **2** and **13** bearing a methyl group at the imide nitrogen is outlined in Scheme 2. The coupling reaction of indolylmagnesium bromide with *N*-methyldichloromaleimide provided the bisindolylmaleimide intermediate.[24] Treatment of the intermediate with 2 equiv of *tert*-butyl dicarbonate followed by the selective mono-deprotection gave compound **13**,[25] which was employed as the glycosylation aglycon in the following synthetic routes. Compound **1** was prepared via oxidative photocyclization of the bisindolylmaleimide intermediate.[26] *N*-Glycosylation of compound **1** with tetra-*O*-benzyl glucopyranosyl chloride was performed in the presence of potassium hydroxide according to the methods described by Ohkubo et al.,[27−29] and the hydrogenation debenzylation led to compound **2**. Various methods of *N*-glycosylations of indole or indolocarbazole moieties were described in the literature:[4,9,15,27−31] (i) coupling of indolocarbazoles to an α-D-glucopyranosyl bromide via the Koenigs-Knorr method provided essentially α-*N*-glycosylated compounds; (ii) coupling of an anhydrosugar to an indole or bis-indole moiety favored the *β*-*N*-glycosylated product; (iii) reaction in heterogeneous basic medium with an indolocarbazole and α-tetra-*O*-benzyl-glucopyranosyl chloride gave the major *β*-*N*-glycosylated compounds.

**Scheme 1.** Synthesized Compounds



**Scheme 2**[a]



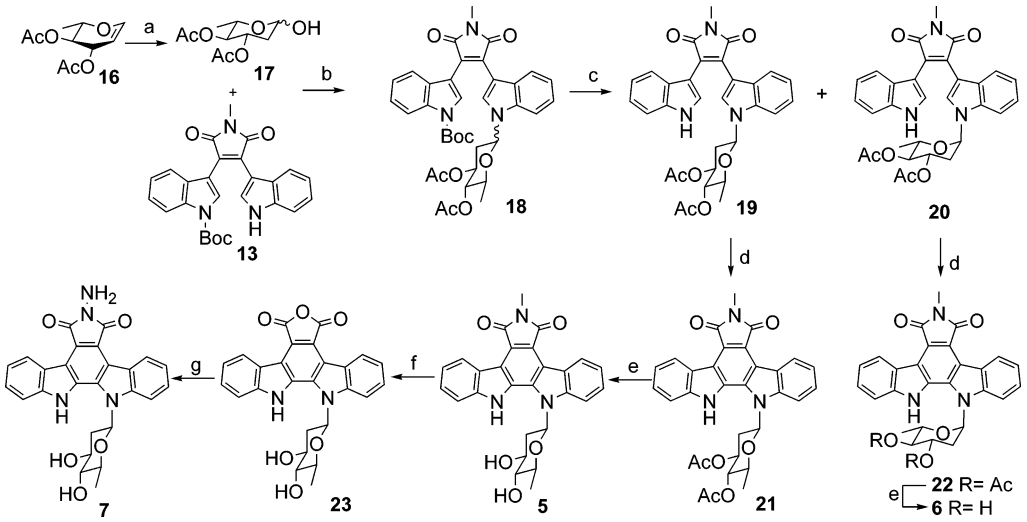

[a] Reagents and conditions: (a) $Et_2O$/toluene, 88 °C, 24 h (78%); (b) $Boc_2O$, DMAP, THF, rt, 1 h; (c) TBAF, THF, reflux, 8 h (75%, two steps); (d) *hv*, air, $I_2$, THF/MeCN, 15 h (80%); (e) NaOH, $Na_2SO_4$, MeCN, r.t, 24 h (92%); (f) Pd/C, $H_2$ 45 psi, 10 h (87%); (g) EtOH/toluene (1:3), 48% KOH, rt, overnight; then 10% citric acid (80%); (h) 1-(3-aminopropyl)-imidazole, 60 °C, 24 h (80%); (i) Pd/C, $H_2$, 45 psi, 1 M HCl, THF/EtOH, 24 h (80%).

This reaction is highly stereoselective; (iv) a Mitsunobu reaction for the coupling of a bis-indole or an indole with α-2,3,4,6-tetra-*O*-benzyl-glucose afforded the *β*-*N*-glycosylated compound with an electron-withdrawing group on the substrate.

To synthesize the *β*-glycoside of rebeccamycin, methods for stereospecifically forming *β*-glycoside of rebeccamycin derivatives are mostly desired. In a convergent design, it is favorable to form the indoloacarbazole core before the glycosylation. However, the indolocarbazole acceptor is a weaker nucleophile than the bis(indoly1)-maleimide or indole acceptor, which limits the direct application of many established glycosylation methodologies to the indolocarbazole aglycones. This challenge was overcome by Ohkubo[28] in 1997 when armed chloride donor was used in heterogeneous basic media for the synthesis of NB-506. This attractive methodology afforded both excellent yield and high stereoselectivity.[15,28,32−34] In the case of the *β*-*N*-glycosylation of 2-deoxysugar, however, this method is unsuitable. The glycosylation reaction of 3,4,6-tri-*O*-benzyl-2-deoxy glucopypyranosyl chloride with an indolocarbazole only produced α-isomer.[15] Currently, there is no general methodology to directly couple 2-deoxy uncommon sugar with an indolocarbazole core with *β*-selectivity. As an alternative approach, glycosylation can be executed before the indolocarbazole core formation. For instance, the indole or bis(indolyl)maleimide derivative is used as the glycosylation acceptor.[15] Mitsunobu reaction and 1,2-anhydrosugar donors[30,31] are the two most frequently employed methods, both of which stereoselectively generate the *β*-isomers. A particular superiority of the Mitsunobu approach to the chloride donor methodology lies in the glycosylation reaction of mannose and erythrose whose halosugars are not stable.[15]

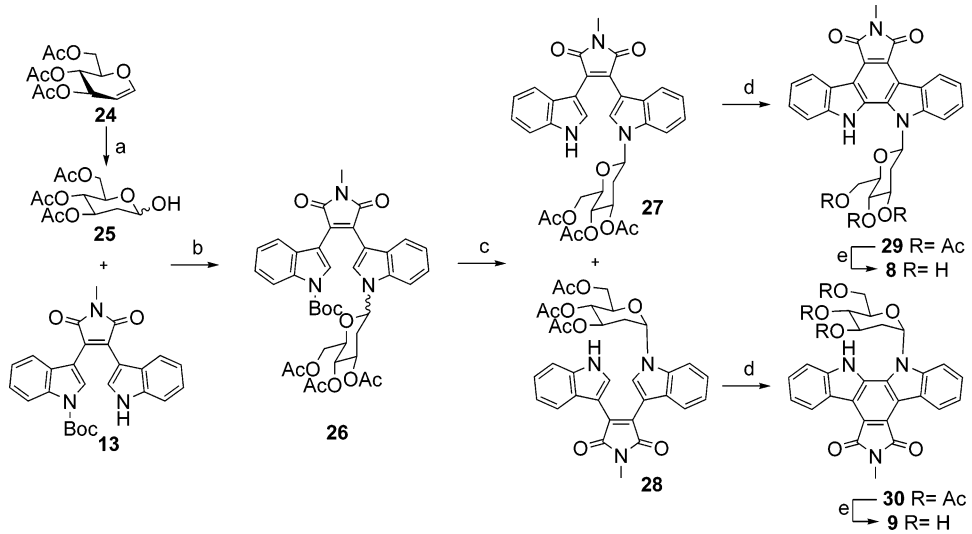
The Mitsunobu reaction was first performed with compound **1** using 2,6-dideoxy-3,4-di-*O*-acetyl-L-rhamnopyranose (**17**) according to the method described by Sabesan et al.[35] in the presence of diethyl azodicarboxylate (DEAD) and triphenylphosphine. However, no reaction was observed due to the particularly insolubility of **1**. Using bis(indolyl)maleimide derivative **13** instead of **1** as the glycosylated acceptor, an inseparable anomeric mixture **18** was produced with 32% yield. The $^1$H NMR spectrum of the mixture indicates the presence of α,*β*-anomers in the ratio 1:2.5. Treatment of the mixture **18** with formic acid at room temperature afforded *β*-*N*-glycoside **19** and α-*N*-glycoside **20** with yield of 91%. Their stereochemistry was assigned from the coupling constant $J_{1',2'}$. The values (10.3 Hz of **19**, and 4.4 Hz of **20**) are in agreement with axial−axial, axial−equatorial coupling, respectively. Oxidative cyclization of **19** and **20** by irradiation in the presence of iodine yielded the corresponding **21** and **22**, and the deacetylation of these compounds gave the required rebeccamycin analogues **5** and **6** with high yields (Scheme 3). The conversion of **5** to anhydride **23** was accomplished by treatment with aqueous KOH in toluene/EtOH overnight at room temperature. The reaction initially generates a mixture of regioisomeric amic acid intermediates, which upon adjustment to pH 6−7 with aqueous citric acid undergoes dehydration to form the anhydride **23** over the course of 3 h at room temperature. The coupling of hydrazine with anhydride **23** was run in THF at 50 °C produced analogue **7** with 80% yield from **5**.

**Scheme 3**[a]



[a] Reagents and conditions (a) LiBr·$H_2O$, AG 50W-X2 resin, $CH_3CN$, rt, 15 min (72%); (b) $Ph_3P$, DEAD, THF, −78 °C, 15 h (32%, α:β = 1:2.5); (c) 88% $HCO_2H$, rt, 6 h (91%); (d) *hν*, air, $I_2$, benzene, 8 h (**21**: 82%, **22**: 57%); (e) TBAF, THF, reflux, 15 h (**5**: 90%, **6**: 70%); (f) EtOH/toluene (1:3), 48% KOH, rt, overnight; then 10% citric acid; (g) $N_2H_4$·$H_2O$ (80% from **5**).

**Scheme 4**[a]



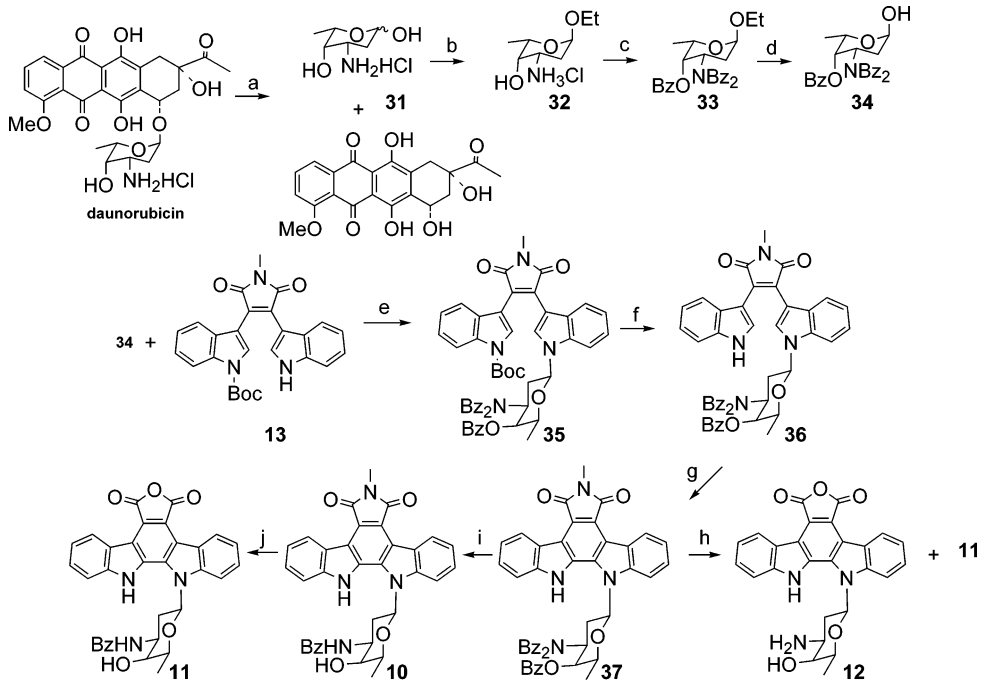
[a] Reagents and conditions: (a) LiBr·$H_2O$, AG 50W-X2 resin, $CH_3CN$, rt, 1 h (71%); (b) $Ph_3P$, DEAD, THF, −78 °C, 15 h (35%, α:β = 1:2.2); (c) 88% $HCO_2H$, rt, 6 h (88%); (d) *hν*, air, $I_2$, benzene, 8h (**29**: 82%, **30**: 68%); (e) TBAF, THF, reflux, 15 h (**8**: 91%, **9**: 92%).

For the synthesis of **8** and **9** from **13** and 2-deoxy-3,4,6-tri-*O*-acetyl-D-glucopyranose (**25**),[35] a synthetic route similar to that presented in Scheme 3 was investigated. The desired rebeccamycin analogues were obtained and the synthetic procedure is outlined in Scheme 4.

Aqueous acidic hydrolysis of hydrochloride of daunorubicin with 0.2 M hydrochloric acid at 90 °C gave daunorubicinone and hydrochloride of daunosamine.[36] To get a pure hydochloride of daunosamine, we tried to purify the crude product by recrystallization with ethanol. The ethyl glycoside **32** was obtained in 83% yield instead of hydrochloride of daunosamine. A glycosylation occurred in the recrystallization process. Benzoxylation of **32** with benzoxy anhydride followed by acidic hydrolysis with 3 M hydrogen chloride produced the glycosylated donor **34**. *N*-Glycosylation of **13** with **34** in THF and subsequent removal of the Boc protecting group using formic acid led to **36** as a single β-isomer ($^1$H NMR: δ 5.81 ppm 1H, dd, $J_{1',2eq'}$ = 2.1 Hz, $J_{1',2ax'}$ = 10.8 Hz, H-1′). Oxidative cyclization of compound **36** in the presence of iodine led to compound **37** (85% yield), which was treated with tetrabutylammonium fluoride (TBAF) or sodium methoxide in methanol, to yield analogues **10** in yields of 89% and 91%, respectively (Scheme 5). Treatment of **10** according to the similar procedure as described for **23** yielded the anhydride analogues **11** (80% yield). The conversion of **37** to **11** and **12** was performed in two steps: treatment of **37** with powdered NaOH in 2-methoxyethanol under reflux followed by acidification with 10% aqueous citric acid to produce anhydride analogues **11** and **12**, in yields of 40% and 42%, respectively.

To study the effect of base group at imide nitrogen on the bioactivity of rebeccamicin analogues, the derivatives **4,** 3-imidazol-1-yl-propyl connected to imide nitrogen, was prepared from **14**. The conversion of **14** to **15** was performed according to the method described for **23** yielded **15** in 80% yield. Treatment of **15** with 3-(amino propyl) imidazole and the hydrogenation de-

**Scheme 5**[a]




[a] Reagents and conditions: (a) 0.2 M HCl, 90 °C, 1 h; (b) EtOH, reflux (83% from dunorubicin); (c) $Bz_2O$, Pyridine, rt, overnight (74%); (d) 3 M HCl, THF, 60 °C, 15 h (80%); (e) $Ph_3P$, DEAD, THF, −78 °C, 18 h (34%); (f) 88% $HCO_2H$, rt, 22 h (81%); (g) *hν*, air, $I_2$, benzene, 15 h (85%); (h) NaOH, $MeOCH_2CH_2OH$, reflux, overnight, then 10% citric acid (**11**:40%, **12**: 42%). (i) TBAF, THF, reflux, 14 h (89%); or NaOMe/MeOH, rt, overnight (91%); (j) EtOH/toluene (1:3), 48% KOH, reflux, overnight; then 10% citric acid (80%).

**Table 1.** Anticancer Activity ($IC_{50}$) of Synthesized Compounds in Two Cancer Cell Lines ($\mu$M)

| | **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** | **9** | **10** | **11** | **12** | Reb |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| SW620 | >100 | 5 | >50 | 2 | 24 | 34 | >100 | 13 | 32 | >100 | 76 | >100 | 4 |
| K562 | 40 | 3 | >50 | 4 | 26 | 19 | >100 | 12 | 12 | 34 | 53 | 55 | 6 |

benzylation led to compound **4** with 64% overal yield (Scheme 2).

## Biology

**Cytotoxicity.** The cytotoxicities of these conjugates were tested with MTS assay in two cancer cell lines, including colorectal carcinoma cells (SW620) and leukemia cells (K562) (Table 1).

The rebeccamycin (Reb) and its analogues **2**, **5**, **6**, **8**, **9** with different sugar moieties are much more active ($IC_{50}$ 3−34 $\mu$M) than the corresponding aglycon **1** ($IC_{50}$ > 100 $\mu$M), which lacking the sugar moiety. Compared with rebeccamycin (Reb, $IC_{50}$ 4−6 $\mu$M), compound **2** ($IC_{50}$ 3−5 $\mu$M) displayed similar activity, despite the substitution of chlorine atoms at the C1, C11 positions and methyl substitution at the N6 position. These results suggest that the sugar moiety on the indolocarbazole ring is a key element for its cytotoxicity. Comparing compounds with a $\beta$-linkage (**8**, **5**) ($IC_{50}$ 12−26 $\mu$M) to compounds with a α-linkage (**9**, **6**) ($IC_{50}$ 12−34 $\mu$M), no activity difference was observed between $\beta$-configuration and α-configuration**.** In addition, it seems that compounds with 2-deoxyglucose (**8** and **9)** are slightly more active than compounds with 2,6-dideoxyglucose (**5** and **6**). Interestingly, when the sugar moiety was changed to 2,3,6-trideoxy-3-benzylaminoglucose in rebeccamycin anlogues (compound **10**), it showed the least cytotoxicity ($IC_{50}$ > 100 $\mu$M), which is similar to that of the aglycon **1** without sugar moieties. Thus, all the above results indicate that the 2-OH, 3-OH, and 6-OH groups in sugar moieties played a very important role in maintaining rebeccamycin anticancer activity. The 6-OH group may have a more significant role than other OH groups in the sugar moiety.

The cytotoxicity of rebeccamycin analogues with substitution at the N6 position of the imide was also compared in SW620 and K562 cells. Despite the imidazole substitution at N6 of compound **4** ($IC_{50}$ 2−4 $\mu$M), it showed identical activity to that of Reb and **2**, which indicates that the sugar moiety, rather than the N6 substitution, is the activity determinant. This mechanism was confirmed by the activity of compound **3**, the protected sugar moiety derivative of **4**. When the sugar moiety was protected with a benzyl group (compound **3**), it lost the anticancer activity ($IC_{50}$ > 50 $\mu$M). The observation is further verified by the activity of compound **7**, where $NH_2$ attached at N6 of the imide did not rescue the loss of activity by sugar modification with 2,6-deoxyglucose.

Cytotoxicity of compound **10**, **11**, and **12**, differing in substitution at the N6 or O6 position, was also determined in SW620 and K562 cells. All three compounds showed low activity similar to that of aglycon **1** without sugar moieties ($IC_{50}$ > 50−100 $\mu$M). Comparing compound **10** and **11**, the anhydride function does not confer higher activities compared to the amide function. In comparison of compound **11** and **12**, deprotected $NH_2$ at the C3′ of the sugar moiety did not rescue its anticancer activity, which indicates either 3-OH in the sugar moiety is critical or the polarity of this position

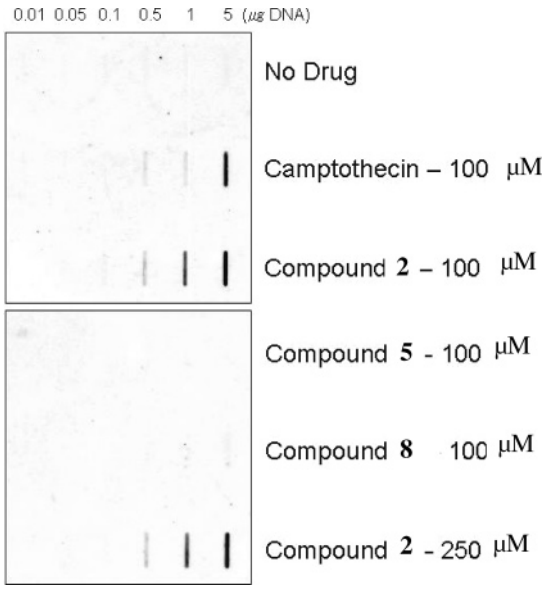



**Figure 3.** Standard ICT assay in HeLa cells comparing camptothecin (CPT) with Rebeccamycin−sugar derivatives **2**, **5**, and **8**. Exponentially growing HeLa cells were incubated with the compounds indicated to the right of the blot for 30 min at 37 °C, followed by sarkosyl lysis and a standard ICT assay (CsCl gradient separation of DNA, recovery of DNA and spotting from 0.01 to 5 $\mu$g of DNA on the blot). The blot was probed with anti-topo I antibody. Signals were developed using ECL and a short (1 min) exposure.

is not a key element for its activity. Further validation and analysis with different compounds need to be performed for further clarification.

**Inhibition of Topoisomerase I.** The compounds were also tested for their inhibition of topoisomerase I with in vivo complex of topoisomerase (ICT) bioassay in HeLa cells. Formation of the covalent complex (topoisomerase/DNA) is essential for the cytotoxic action of the topoisomerase poisons. The unique nature of topoisomerase/DNA interaction (viz., formation of the covalent intermediate) can be exploited to quantify topoisomerase-mediated DNA damage in the intact cell. In the presence of topo I poisons, the DNA breaks are stabilized and a covalent topo−DNA complex can be trapped upon addition of protein denaturants. The Standard ICT assay was performed on HeLa cells comparing camptothecin (CPT) with three rebeccamycin analogues (**5**, **8**, and **2**) that differ only in their sugar side chains where compound **2** was linked with glucose, compound **8** with 2-deoxyglucose, and compound **5** with 2,6-deoxyglucose. To ensure uniformity between six different treatments, a range of DNA concentrations was probed from 0.01 to 5 $\mu$g; thus, the signals can be directly compared between each culture. Very surprisingly, their topoisomerase inhibition is in agreement with the anticancer activity (Figure 3). Compound **2** with glucose moiety showed similar strong topoisomerase inhibition as that of CPT (positive control) at the same molar concentration, which was indicated by the formation of topo−DNA complex in the immunoblot. However, compound **8** with 2-deoxy-D-glucose moiety gave a very weak (but detectable on longer exposure) signal while compound **5** with 2,6-dideoxy glucose moiety was inactive. Clearly, the 2-OH and 6-OH of the sugar moiety in rebeccamycin analogues strongly modulate drug activity in topoisomerase I inhibition in vivo. This is surprising because the N6 region of rebeccamycin was thought to be involved in topoisomerase inhibition while the sugar moiety is involved in DNA binding. Our results clearly demonstrated that the sugar moiety also modulates the topoisomerase I activity, which is worthy of further investigation with various modifications in sugar moiety.

## Conclusion

In summary, several rebeccamycin analogues were synthesized, which contain uncommon sugars, and substitutions on the imide nitrogen such as a methyl group or amino group, as well as two corresponding anhydride analogues. The results suggest that the sugar moiety on the indolocarbazole ring rather than N6 substitution is a key element for its anticancer activity and topoisomerase inhibition. In addition, among the compounds tested in this study, the rebeccamycin analogues with various uncommon sugars showed distinct cytotoxicities and topoisomerase inhibition. These compounds with glucose (**2**, **4**, and Reb) are more active than compounds with 2-deoxyglucose (**8** and **9**) or with 2,6-deoxyglucose (**5** and **6**). Compounds with 2,3,6-deoxyglucose (compound **10**) showed the worst activity. These data indicate that the 2-OH, 3-OH, and 6-OH groups in sugar moieties, rather than the modifications in the imide structure, play a very important role in maintaining their anticancer activity. The better activities of compound **2**, **8**, and **4** imply that the 6-OH group may have a more significant role than other OH groups in the sugar moiety. The 6-OH group on the carbohydrate residue may form a hydrogen bond with the indole NH, which may maintain the carbohydrate in a fixed conformation for optimal activity and interaction with the topoisomerase I−DNA complex. In addition, the cytotoxicities of these compounds correlated with the inhibition of topoisomerase I, where compounds with various sugar moieties show more potent topoisomerase inhibition compared to the aglycon **1** without a sugar moiety. Compounds with 2-deoxyglucose showed better topoisomerase I inhibition than the compounds with 2,6-deoxyglucose. This indicates that the 2-OH and 6-OH of the sugar moiety also modulate the topoisomerase I activity in cancer cells, which is worthy of further investigation with various modifications in the sugar moiety. Therefore, the modifications of rebeccamycin with uncommon sugars may provide a new class of anticancer compounds.

## Experimental Section

**Chemistry.** All solvent was dried with a solvent-purification system from Innovative Technology, Inc. All reagents were used as obtained from commercial sources. Analytical TLC was carried out on E. Merck silica gel 60 F254 aluminum-backed plates. Preparative TLC was carried out on EMD Chemicals, Inc. silica gel 60 F254 plates (20 × 20 cm, 1 mm). The 230−400 mesh size of the same absorbent was utilized for all chromatographic purifications. $^1$H and $^{13}$C NMR spectra are at the indicated field strengths. The high-resolution mass spectra were recorded at The Ohio State University Campus Chemical Instrumentation Center.

**2-Deoxy-3,4-di-*O*-acetyl-L-rhamnopyranose (17).**[35] AG 50W−X2 resin (0.3 g) and water (0.6 mL) were added to a solution of 3,4-di-*O*-acetyl-L-rhamnal (0.4 g) and lithium bromide hydrate (0.5 g) in acetonitrile (15 mL). The mixture was stirred at room temperature for 15 min. The reaction

mixture was filtered, neutralized with triethylamine, and evaporated to dryness. The residue was dissolved in dichloromethane and washed with water, ice-cold 1 M hydrochloric acid, and saturated sodium bicarbonate solution. The colorless solid ($\alpha$:$\beta$ = 2:1) of compound **17** (310 mg, 72% yield) was obtained after purification on a column of silica gel using ethyl acetate−hexane (1:3) as eluant: $^1$H NMR (250 MHz, $CDCl_3$) 5.24 (d, $J$ = 3.4 Hz, H-1$\alpha$), 5.20 (m, H-3$\alpha$), 4.88 (m, H-3$\beta$), 4.80 (dd, $J$ = 1.9 Hz, $J$ = 9.4 Hz, H-1$\beta$), 4.63 (t, $J$ = 9.6 Hz, H-4), 4.00 (m, H-5$\alpha$), 3.43 (m, H-5$\beta$), 2.26 (m, H-2$\beta_{eq}$), 2.13 (m, H-2$\alpha_{eq}$), 1.70 (m, H-2$\beta_{ax}$), 1.60 (m, H-2$\alpha_{ax}$), 1.11 (d, $J$ = 6.2 Hz, H-6$\beta$), 1.05 (d, $J$ = 6.2 Hz, H-6$\alpha$).

**2,5-Dihydro-3-[1-(*tert*-butyloxycarbonyl)-1*H*-indol-3-yl]-4-[1-(2-deoxy-3,4-di-*O*-acetyl-L-rhamnopyranosyl)-1*H*-indol-3-yl]-1*H*-pyrrole-2,5-dione (18).** To a solution of **13** (1.50 g, 3.40 mmol) in THF (100 mL) were added **17** (1.95 g, 8.41 mmol) and triphenylphosphine (2.20 g, 8.41 mmol). The mixture was cooled to −78 °C, and then DEAD (1.33 mL, 8.41 mmol) was added dropwise. The mixture was allowed to reach room temperature and stirred at room temperature for 15 h, and then water was added. After extraction with ethyl acetate, the combined organic phase was dried over $Na_2SO_4$. The solvent was removed, and the residue was purified through column chromatography on silica gel using benzene-ethyl acetate (9:1) to give unreacted substrate **13** (1.0 g) and the anomeric mixture **18** (0.7 g, 32%, $\alpha$:$\beta$ = 1:2.5) as an orange solid: $^1$H NMR (250 MHz, $CDCl_3$) 5.24 (dd, $J$ = 1.75 Hz, $J$ = 11 Hz, H-1$\beta$), 5.96 (d, $J$ = 4.7 Hz, H-1$\alpha$).

**2,5-Dihydro-3-(1*H*-indol-3-yl)-4-[1-(2-deoxy-3,4-di-*O*-acetyl-$\beta$-L-rhamnopyranosyl)-1*H*-indol-3-yl]-1*H*-pyrrole-2,5-dione(19) and 2,5-Dihydro-3-(1*H*-indol-3-yl)-4-[1-(2-deoxy-3,4-di-*O*-acetyl-$\alpha$-L-rhamnopyranosyl)-1*H*-indol-3-yl]-1*H*-pyrrole-2,5-dione (20).** A solution of **18** (310 mg, 0.47 mmol) in 88% formic acid (40 mL) was stirred at room temperature and monitored by TLC hourly. After 6 h, the reaction was completed. Trimethylamine was added dropwise until neutralization. Saturated aqueous NaOAc was added. After extraction with ethyl acetate, the combined organic phase was dried over $Na_2SO_4$. The solvent was removed, and the residue was purified by prep-TLC (benzene−ethyl acetate, 8:1) to afford **19** (red solid, 170 mg) and **20** (red solid, 68 mg) with 91% yield.

**19**: HRMS (M + Na)$^+$ (ESI$^+$) calcd for $C_{31}H_{29}N_3O_7Na^+$ 578.1898, found 578.1896. $^1$H NMR (400 MHz, $CDCl_3$) 8.52 (1H, s, NH), 7.72 (1H, s), 7.67 (1H, s), 7.34 (1H, d, $J$ = 8.2 Hz), 7.30 (1H, d, $J$ = 8.2 Hz), 7.11 (1H, t, $J$ = 7.4 Hz), 7.06 (1H, t, $J$ = 7.4 Hz), 6.97 (2H, d, $J$ = 8.8 Hz), 6.77 (2H, dd, $J$ = 7.3 Hz, $J$ = 12.6 Hz), 5.65 (1H, d, $J$ = 10.3 Hz, H-1′), 5.14 (1H, m, H-3′), 4.87 (1H, t, $J$ = 9.6 Hz, H-4′), 3.75 (1H, m, H-5′), 3.17 (3H, s, $NCH_3$), 2.47 (1H, m, H-2$_{eq}$), 2.23 (1H, m, H-2$_{ax}$), 2.08 (3H, s, $COCH_3$), 2.04 (3H, s, $COCH_3$), 1.24 (3H, d, $J$ = 6.3 Hz, H-6′). $^{13}$C NMR (400 MHz, $CDCl_3$) 18.2 (C$_{6'}$), 21.2, 21.3 ($CH_3CO$), 24.6 ($NCH_3$), 36.1 (C$_{2'}$), 71.4, 73.3, 74.1, 81.4 (C$_{1'}$, C$_{3'}$, C$_{4'}$, C$_{5'}$), 110.5, 111.6, 120.9, 121.4, 122.5, 122.9, 123.1, 123.2, 128.4, 128.8 (C tert arom), 107.6, 107.9, 125.7, 126.9, 127.5, 128.7, 136.1, 136.3 (C quat arom), 170.4, 170.7, 172.6, 172.7 (C═O). **20**: HRMS (M + Na)$^+$ (ESI$^+$) calcd for $C_{31}H_{29}N_3O_7Na^+$ 578.1897, found 578.1895. $^1$H NMR (400 MHz, $CDCl_3$) 8.59 (1H, s, NH), 7.88 (1H, s), 7.80 (1H, d, $J$ = 2.7 Hz), 7.58 (1H, d, $J$ = 8.3 Hz), 7.29 (1H, d, $J$ = 8.2 Hz), 7.20 (1H, d, $J$ = 8.0 Hz), 7.11 (1H, t, $J$ = 8.2 Hz), 7.03 (1H, t, $J$ = 8.1 Hz), 6.88 (2H, t, $J$ = 8.1 Hz), 6.72 (1H, t, $J$ = 8.0 Hz), 5.99 (1H, d, $J$ = 4.4 Hz, H-1′), 4.87 (2H, m, H-4′, H-3′), 3.31 (1H, m, H-5′), 3.18 (3H, s, $NCH_3$), 2.75 (1H, m, H-2$_{eq}$), 2.17 (1H, m, H-2$_{ax}$), 2.03 (3H, s, $COCH_3$), 2.02 (3H, s, $COCH_3$), 1.12 (3H, d, $J$ = 6.3 Hz, H-6′). $^{13}$C NMR (400 MHz, $CDCl_3$) 17.6 (C$_{6'}$), 21.2, 21.3 ($CH_3CO$), 24.6 ($NCH_3$), 32.7 (C$_{2'}$), 67.9, 69.9, 73.6, 80.5 (C$_{1'}$, C$_{3'}$, C$_{4'}$, C$_{5'}$), 111.5, 112.4, 120.6, 121.6, 122.4, 122.6, 123.1, 123.4, 129.1, 129.6 (C tert arom), 107.5, 108.0, 125.4, 127.4, 127.6, 128.9 136.5, 137.0 (C quat arom), 170.4, 170.8, 172.6, 172.7 (C═O).

**6-Methyl-12-(2-deoxy-3,4-di-*O*-acetyl-$\beta$-L-rhamnopyranosyl)-6,7,12,13-tetrahydroindolo[2,3-*a*]pyrrolo[3,4-*c*]carbazole-5,7-dione (21).** A red solution of **19** (50 mg, 0.09 mmol) in benzene (150 mL) was treated with iodine (1 mg). Air was continuously bubbled through the reaction while was irradiated with a medium-pressure mercury lamp equipped with a Vycor filter for 8 h. Benzene was added to the reaction every 2 h to keep the solvent volume constant. The solvent was removed, and the residue was dissolved in EtOAc and washed with aqueous $Na_2S_2O_3$ and brine. The organic phase was dried over $Na_2SO_4$. After removal of the solvent, the residue was purified by Prep-TLC using EtOAc−hexane (1:9) as developing solvent to give the product **21** (41 mg, 82% yield) as a yellow solid: HRMS (M + Na)$^+$ (ESI$^+$) calcd for $C_{31}H_{27}N_3O_7Na^+$ 576.1741, found 576.1751. $^1$H NMR (400 MHz, $CDCl_3$) 10.15 (1H, s, NH), 9.25 (1H, d, $J$ = 7.8 Hz), 9.15 (1H, d, $J$ = 7.9 Hz), 7.59−7.40 (6H, m), 6.16 (1H, dd, $J$ = 2.9 Hz, $J$ = 10.7 Hz, H-1′), 5.29 (2H, m, H-3′, H-4′), 4.15 (1H, m, H-5′), 3.21 (3H, s, $NCH_3$), 2.25 (2H, m, H-2), 2.17 (3H, s, $COCH_3$), 1.90 (3H, s, $COCH_3$), 1.67 (3H, d, $J$ = 6.2 Hz, H-6′). $^{13}$C NMR (400 MHz, $CDCl_3$) 19.1 (C$_{6'}$), 21.1, 21.2 ($CH_3CO$), 24.1 ($NCH_3$), 36.1 (C$_{2'}$), 70.7, 73.9, 75.2, 82.7 (C$_{1'}$, C$_{3'}$, C$_{4'}$, C$_{5'}$), 109.7, 111.5, 121.7, 122.5, 126.1, 126.5, 127.8, 127.9, (C tert arom), 118.9, 119.7, 120.1, 121.8, 122.7, 123.3, 128.5, 130.0, 140.6, 140.7 (C quat arom), 170.2, 170.3, 170.3, 170.4 (C═O).

**6-Methyl-12-(2-deoxy-3,4-di-*O*-acetyl-$\alpha$-L-rhamnopyranosyl)-6,7,12,13-tetrahydroindolo[2,3-*a*]pyrrolo[3,4-*c*]carbazole-5,7-dione (22).** Following the same procedure described for **21**, compound **22** was obtained from **20** as a yellow solid (57% yield): HRMS (M + Na)$^+$ (ESI$^+$) calcd for $C_{31}H_{27}N_3O_7Na^+$ 576.1741, found 576.1756. $^1$H NMR (500 MHz, $CDCl_3$) 10.46 (1H, s, NH), 9.36 (1H, d, $J$ = 7.6 Hz), 9.26 (1H, d, $J$ = 8.2 Hz), 7.61−7.39 (6H, m), 6.16 (1H, d, $J$ = 9.2 Hz, H-1′), 5.25 (1H, br, H-4′), 5.02 (1H, br, H-3′), 4.87 (1H, m, H-5′), 3.32 (3H, s, $NCH_3$), 2.60, 1.83 (2H, 2m, H-2), 2.32 (3H, s, $COCH_3$), 2.24 (3H, s, $COCH_3$), 1.86 (3H, d, $J$ = 7.3 Hz, H-6′). $^{13}$C NMR (500 MHz, $CDCl_3$) 16.0 (C$_{6'}$), 21.5, 21.8 ($CH_3CO$), 24.1 ($NCH_3$), 32.1 (C$_{2'}$), 68.9, 68.9, 74.8, 75.8, (C$_{1'}$, C$_{3'}$, C$_{4'}$, C$_{5'}$), 109.7, 110.9, 121.2, 122.2, 126.3, 126.6, 127.5, 127.6, (C tert arom), 118.5, 120.0, 120.1, 121.3, 122.7, 123.2, 128.4, 129.7, 140.4, 140.5 (C quat arom), 169.1, 169.6, 170.2 (C═O).

**6-Methyl-12-(2-deoxy-$\beta$-L-rhamnopyranosyl)-6,7,12,13-tetrahydroindolo[2,3-*a*]pyrrolo[3,4-*c*]carbazole-5,7-dione (5).** A solution of tetrabutylammonium fluoride (4 mL, 1.0 M in THF) was added to a solution of **21** (70 mg, 0.07 mmol) in THF (10 mL). The mixture was refluxed for 15 h, diluted with EtOAc (80 mL), washed with water, and dried over $Na_2SO_4$. The solvent was removed, and the residue was purified on a column of silica gel using MeOH−$CH_2Cl_2$ (1:50) as eluent to give the product **5** (63 mg, 90% yield) as a yellow solid: HRMS (M + Na)$^+$ (ESI$^+$) calcd for $C_{27}H_{23}N_3O_5Na^+$ 492.1530, found 492.1529. $^1$H NMR (500 MHz, DMSO) 11.35 (1H, s, NH), 9.09 (1H, d, $J$ = 7.9 Hz), 8.97 (1H, d, $J$ = 8.0 Hz), 8.02 (1H, d, $J$ = 8.4 Hz), 7.73 (1H, d, $J$ = 8.2 Hz), 7.55 (2H, m), 7.33 (2H, m), 6.61 (1H, dd, $J$ = 2.4 Hz, $J$ = 11.1 Hz, H-1′), 5.41 (1H, br, H-4′), 5.09 (1H, br, HO-3′), 3.94 (1H, m, H-5′), 3.34 (2H, m, H-3′, HO-4′), 3.03(3H, s, $NCH_3$), 3.11, 2.25 (2H, m, H-2), 1.49 (3H, d, $J$ = 6.1 Hz, H-6′). $^{13}$C NMR (500 MHz, DMSO) 19.3 (C$_{6'}$), 24.4 ($NCH_3$), 39.1 (C$_{2'}$), 71.1, 76.1, 77.5, 83.0 (C$_{1'}$, C$_{3'}$, C$_{4'}$, C$_{5'}$), 112.6, 113.7, 121.4, 121.9, 125.2, 125.5, 127.8, 128.1, (C tert arom), 118.1, 118.2, 119.5, 120.7, 121.4, 122.9, 128.8, 129.1, 140.5, 141.3 (C quat arom), 170.1, 170.2 (C═O).

**6-Methyl-12-(2-deoxy-$\alpha$-L-rhamnopyranosyl)-6,7,12,13-tetrahydroindolo[2,3-*a*]pyrrolo[3,4-*c*]carbazole-5,7-dione (6).** Followed the same procedure described for **5**, **6** was obtained from **22** as a yellow solid (70% yield): HRMS (M + Na)$^+$ (ESI$^+$) calcd for $C_{27}H_{23}N_3O_5Na^+$ 492.1530, found 492.1523. $^1$H NMR (400 MHz, DMSO) 12.06 (1H, s, NH), 9.20 (1H, d, $J$ = 8.1 Hz), 9.09 (1H, d, $J$ = 8.0 Hz), 7.79 (1H, d, $J$ = 8.4 Hz), 7.60 (3H, m), 7.38 (2H, m), 6.69 (2H, m, H-1′, 4′), 5.56 (1H, d, $J$ = 3.0 Hz, HO-3′), 4.53 (1H, m, H-3′), 4.19 (1H, br, H-5′), 3.80 (1H, br, HO-4′), 3.(3H, s, $NCH_3$), 2.73, 1.83 (2H,m, H-2), 1.47 (3H, d, $J$ = 6.1 Hz, H-6′). $^{13}$C NMR (500 MHz, DMSO) 16.0 (C$_{6'}$), 24.5 ($NCH_3$), 34.5 (C$_{2'}$), 68.6, 69.2, 73.9, 77.9 (C$_{1'}$, C$_{3'}$, C$_{4'}$, C$_{5'}$), 110.4, 112.2, 121.1, 121.7, 125.3, 125.6, 127.8, 128.1, (C tert arom), 117.4, 118.1, 119.1, 120.9, 121.5, 121.8, 128.5, 129.4, 140.3, 141.2 (C quat arom), 170.43, 170.45 (C═O).

**6-Amino-12-(2-deoxy-β-L-rhamnopyranosyl)-6,7,12,13-tetrahydroindolo[2,3-*a*]pyrrolo[3,4-*c*]carbazole-5,7-dione (7).** A mixture of **5** (30 mg, 0.064 mmol), 48% KOH aqueous (1 g), EtOH (3.0 mL) and toluene (1.0 mL) was stirred at room temperature for overnight. The color of the reaction solution was changed from deep red to pale yellow. The solution was acidified with 10% citric acid to pH 6, and stirred for additional 3 h at room temperature, then extracted with EtOAc. After removal of the solvent, the crude compound **23** was obtained (HRMS (M + Na)$^+$ (ESI$^+$) calcd for $C_{26}H_{20}N_2O_6Na^+$ 479.1213, found 479.1207). THF (2.0 mL) and hydrazine hydrate (1.0 mL) was added to the crude compound **23**. The mixture was stirred at 50 °C for 3 h, and then poured into brine (20 mL). The resulted mixture was stirred at room-temperature overnight. The solid was collected with EtOAc and dried over $Na_2SO_4$. The solvent was removed, and the residue was purified on a prepTLC using MeOH−$CH_2Cl_2$ (1:25) as developing solvent to give the product **7** (24 mg, 80% yield from **5**) as a yellow solid: HRMS (M + Na)$^+$ (ESI$^+$) calcd for $C_{26}H_{22}N_4O_5Na^+$ 493.1482, found 493.1472. $^1$H NMR (500 MHz, DMSO+$D_2O$) 9.10 (1H, d, $J$ = 8.0 Hz), 9.01 (1H, d, $J$ = 7.9 Hz), 7.95 (1H, d, $J$ = 8.4 Hz), 7.76 (1H, d, $J$ = 8.2 Hz), 7.57 (2H, m), 7.37 (2H, m), 6.54 (1H, dd, $J$ = 2.1 Hz, $J$ = 11.0 Hz, H-1′), 3.94 (2H, m, H-5′, H-3′), 3.38 (1H, m, H-4′), 2.18 (2H, m, H-2), 1.47 (3H, d, $J$ = 6.1 Hz, H-6′). $^{13}$C NMR (500 MHz, DMSO+$D_2O$) 18.7 ($C_{6'}$), 38.5 ($C_{2'}$), 60.5, 70.3, 76.7, 82.3 ($C_{1'}$, $C_{3'}$, $C_{4'}$, $C_{5'}$), 112.3, 112.4, 121.8, 122.2, 124.8, 125.1, 127.9, 128.1 (C tert arom), 117.1, 117.7, 117.9, 118.5, 121.3, 121.4, 128.4, 128.7, 140.2, 140.6 (C quat arom), 168.9, 169.0, (C=O).

**2-Deoxy-3,4,6-trii-*O*-acetyl-D-glucopyranose (25).** AG 50W−X2 resin (0.4 g) and water (0.6 mL) were added to a solution 3,4,6-tri-*O*-acetyl-D-glucal (0.45 g) and lithium bromide hydrate (0.5 g) in acetonitrile (15 mL). The mixture was stirred at room temperature for 1 h. The product was isolated as described for **17** and purified on a column of silica gel using ethyl acetate−hexane (1:3) as eluant to give the compound **25** (340 mg, 71% yield) as a colorless solid.[35]

**2,5-Dihydro-3-[1-(*tert*-butyloxycarbonyl)-1*H*-indol-3-yl]-4-[1-(2-deoxy-3,4,6-tri-*O*-acetyl-D-glucopyranosyl)-1*H*-indol-3-yl]-1*H*-pyrrole-2,5-dione (26).** Triphenylphosphine (894 mg, 3.40 mmol) and DEAD (1 g, 3.40 mmol) were added to a solution of **13** (0.68 g, 1.80 mmol) in THF (50 mL). The mixture was cooled to −78 °C, and then DEAD (0.54 mL., 3.40 mmol) was added dropwise. The mixture was allowed to reach room temperature and stirred for 15 h, and then water was added. After extraction with ethyl acetate, the combined organic phase was dried over $Na_2SO_4$. The solvent was removed, and the residue was purified on a column of silica gel using ethyl acetate−hexane-trimethylamine (1:3:0.01) as eluant to give the anomeric mixture **26** (0.38 g, 35% yield, α:β = 1:2.2) as an orange solid, which could not be separated by chromatography.

**2,5-Dihydro-3-(1*H*-indol-3-yl)-4-[1-(2-deoxy-3,4,6-tri-*O*-acetyl-β-D-glucopyranosyl)-1*H*-indol-3-yl]-1*H*-pyrrole-2,5-dione(27) and 2,5-Dihydro-3-(1*H*-indol-3-yl)-4-[1-(2-deoxy-3,4,6-tri-*O*-acetyl-α-D-glucopyranosyl)-1*H*-indol-3-yl]-1*H*-pyrrole-2,5-dione (28).** A solution of **26** (40 mg, 0.06 mmol) in formic acid (88%, 4.0 mL) was stirred at room temperature and monitored by TLC every hour. After being stirred 6 h, the reaction was completed. Trimethylamine was added dropwise until neutralization. Saturated aqueous NaOAc was added. After extraction with ethyl acetate, the combined organic phase was dried over $Na_2SO_4$. The solvent was removed, and the residue was purified on prepTLC (ethyl acetate−hexane (2:3) to afford **27** (red solid, 20 mg) and **28** (red solid, 10 mg), the yield was 88%.

**27**: HRMS (M + Na)$^+$ (ESI$^+$) calcd for $C_{33}H_{31}N_3O_9Na^+$ 636.1952, found 636.1944. $^1$H NMR (500 MHz, $CDCl_3$) 8.64 (1H, s, NH), 7.69 (1H, s), 7.62 (1H, d, $J$ = 2.6 Hz), 7.34 (1H, d, $J$ = 8.3 Hz), 7.26 (1H, d, $J$ = 8.1 Hz), 7.11 (1H, t, $J$ = 7.4 Hz), 7.05 (1H, t, $J$ = 7.4 Hz), 6.97 (2H, dd, $J$ = 2.9 Hz, $J$ = 8.1 Hz), 6.77 (2H, dd, $J$ = 8.1 Hz, $J$ = 15.6 Hz), 5.66 (1H, dd, $J$ = 1.3 Hz, $J$ = 11.0 Hz, H-1′), 5.20 (1H, m, H-3′), 5.10 (1H, t, $J$ = 9.7 Hz, H-4′), 4.27 (1H, dd, $J$ = 5.2 Hz, $J$ = 12.4 Hz, H-6′), 4.12 (1H, dd, $J$ = 2.9 Hz, $J$ = 12.4 Hz, H-6′), 3.85 (1H, m, H-5′), 3.20 (3H, s, $NCH_3$), 2.47 (1H, m, H-$2_{eq}$), 2.26 (1H, m, H-$2_{ax}$), 2.08 (3H, s, $COCH_3$), 2.05 (3H, s, $COCH_3$), 2.04 (3H, s, $COCH_3$). $^{13}$C NMR (500 MHz, $CDCl_3$) 21.1, 21.2, 21.3 ($CH_3CO$), 24.6 ($NCH_3$), 35.7 ($C_{2'}$), 62.7, 69.0, 71.4, 74.9, 81.7 ($C_{1'}$, $C_{3'}$, $C_{4'}$, $C_{5'}$, $C_{6'}$), 110.5, 111.6, 120.9, 121.5, 122.5, 122.9, 123.1, 123.3, 128.3, 129.0 (C tert arom), 107.5, 108.1, 125.6, 125.9, 126.9, 127.3, 136.2, 136.3 (C quat arom), 170.2, 170.6, 171.1, 172.6, 172.7 (C=O). **28**: HRMS (M + Na)$^+$ (ESI$^+$) calcd for $C_{33}H_{31}N_3O_9Na^+$ 636.1952, found 636.1948. $^1$H NMR (500 MHz, $CDCl_3$) 8.63 (1H, s, NH), 7.86 (1H, s), 7.82 (1H, d, $J$ = 2.7 Hz), 7.59 (1H, d, $J$ = 8.3 Hz), 7.29 (1H, d, $J$ = 8.3 Hz), 7.22 (1H, t, $J$ = 8.1 Hz), 7.12 (1H, t, $J$ = 7.4 Hz), 7.04 (1H, t, $J$ = 7.3 Hz), 6.89 (1H, t, $J$ = 7.1 Hz), 6.83 (1H, d, $J$ = 8.2 Hz), 6.69 (1H, t, $J$ = 8.1 Hz), 6.07 (1H, d, $J$ = 4.9 Hz, H-1′), 5.10 (1H, t, $J$ = 9.6 Hz, H-4′), 4.99 (1H, m, H-3′), 4.26 (1H, dd, $J$ = 5.2 Hz, $J$ = 12.5 Hz, H-6′), 4.12 (1H, dd, $J$ = 2.8 Hz, $J$ = 12.5 Hz, H-6′), 3.40 (1H, m, H-5′), 3.19 (3H, s, $NCH_3$), 2.75 (1H,m, H-$2_{eq}$), 2.21 (1H,m, H-$2_{ax}$), 2.03 (3H, s, $COCH_3$), 2.02 (3H, s, $COCH_3$), 1.98 (3H, s, $COCH_3$). $^{13}$C NMR (500 MHz, $CDCl_3$) 21.1, 21.2, 21.6 ($CH_3CO$), 24.7 ($NCH_3$), 34.6 ($C_{2'}$), 62.1, 68.5, 70.0, 80.7 ($C_{1'}$, $C_{3'}$, $C_{4'}$, $C_{5'}$, $C_{6'}$), 111.6, 112.5, 120.6, 121.8, 122.3, 122.6, 123.1, 123.5, 129.3, 129.4 (C tert arom), 107.4, 108.4, 125.3, 125.9, 127.3, 127.5, 136.5, 136.9 (C quat arom), 170.1, 170.7, 171.0, 172.6, 172.7 (C=O).

**6-Methyl-12-(2-deoxy-3,4,6-tri-*O*-acetyl-β-D-glucopyranosyl)-6,7,12,13-tetrahydroindolo[2,3-*a*]pyrrolo[3,4-*c*]carbazole-5,7-dione (29).** A red solution of **27** (120 mg, 0.20 mmol) in benzene (200 mL) was treated with of iodine (35 mg). Air was continuously bubbled through the reaction which was irradiates with a medium-pressure mercury lamp equipped with a Vycor filter for 8 h. Benzene was added to the reaction each 2 h to keep the solvent volume constant. The solvent was removed, and the residue was dissolved in EtOAc and washed with aqueous $Na_2S_2O_3$ and brine. The organic phase was dried over $Na_2SO_4$, and the residue was purified on a Prep-TLC using EtOAc−benzene (1:4) as developing solvent to give the product **29** (98 mg, 82% yield) as a yellow solid: HRMS (M + Na)$^+$ (ESI$^+$) calcd for $C_{33}H_{29}N_3O_9Na^+$ 634.1796, found 634.1792. $^1$H NMR (500 MHz, $CDCl_3$) 10.16 (1H, s, NH), 9.32 (1H, d, $J$ = 7.9), 9.24 (1H, d, $J$ = 7.9 Hz), 7.72 (1H, d, $J$ = 8.0 Hz), 7.62 (2H, m), 7.55 (1H, d, $J$ = 8.3 Hz), 7.46 (2H, m), 6.25 (1H, dd, $J$ = 2.4 Hz, $J$ = 11.4 Hz, H-1′), 5.65 (1H, t, $J$ = 9.8 Hz, H-4′), 5.39 (1H, m, H-3′), 4.80 (1H, dd, $J$ = 3.3 Hz, $J$ = 12.9 Hz, H-6′), 4.39 (1H, dd, $J$ = 2.1 Hz, $J$ = 12.9 Hz, H-5′), 4.29 (1H, dd, $J$ = 2.7 Hz, $J$ = 12.9 Hz, H-6′), 3.27 (3H, s, $NCH_3$), 2.47 (1H, m, H-$2_{eq}$), 2.30 (1H, m, H-$2_{ax}$), 2.29 (3H, s, $COCH_3$), 2.18 (3H, s, $COCH_3$), 1.98 (3H, s, $COCH_3$). $^{13}$C NMR (500 MHz, $CDCl_3$) 20.68, 20.69, 21.2 ($CH_3CO$), 25.6 ($NCH_3$), 35.5 ($C_{2'}$), 61.6, 67.6, 70.7, 76.2, 82.7 ($C_{1'}$, $C_{3'}$, $C_{4'}$, $C_{5'}$, $C_{6'}$), 109.5, 111.5, 121.5, 122.5, 125.8, 126.2, 127.4, 127.5 (C tert arom), 118.9, 119.5, 119.7, 122.2, 122.8, 128.1, 128.3, 129.6, 140.2, 140.6 (C quat arom), 169.6, 169.8, 169.9, 170.0, 170.5 (C=O).

**6-Methyl-12-(2-deoxy-3,4,6-tri-*O*-acetyl-α-D-glucopyranosyl)-6,7,12,13-tetrahydroindolo[2,3-*a*]pyrrolo[3,4-*c*]carbazole-5,7-dione (30).** Followed the same procedure described for **29**, Compound **30** was obtained from **28** as a yellow solid (68% yield): HRMS (M + Na)$^+$ (ESI$^+$) calcd for $C_{33}H_{29}N_3O_9Na^+$ 634.1796, found 634.1790. $^1$H NMR (500 MHz, $CDCl_3$) 10.16 (1H, s, NH), 9.28 (1H, d, $J$ = 7.9), 9.19 (1H, d, $J$ = 7.9 Hz), 7.67 (1H, d, $J$ = 8.4 Hz), 7.64 (1H, m), 7.51 (1H, m), 7.43 (3H, m), 6.64 (1H, dd, $J$ = 2.5 Hz, $J$ = 9.7 Hz, H-1′), 5.23 (1H, br, H-3′), 5.05 (1H, br, H-4′), 4.78 (1H, m, H-5′), 5.38 (1H, dd, $J$ = 9.8 Hz, $J$ = 12.3 Hz, H-5′), 4.25 (1H, dd, $J$ = 4.3 Hz, $J$ = 12.3 Hz, H-6′), 3.19 (3H, s, $NCH_3$), 2.59 (1H, m, H-$2_{eq}$), 1.78 (1H, m, H-$2_{ax}$), 2.27 (3H, s, $COCH_3$), 2.25 (3H, s, $COCH_3$), 2.24 (3H, s, $COCH_3$). $^{13}$C NMR (500 MHz, $CDCl_3$) 20.7, 221.0, 21.3 ($CH_3CO$), 23.7 ($NCH_3$), 34.2 ($C_{2'}$), 59.2, 65.6, 67.8, 76.0, 798.1 ($C_{1'}$, $C_{3'}$, $C_{4'}$, $C_{5'}$, $C_{6'}$), 109.8, 110.7, 121.0, 121.9, 125.8, 126.1, 127.3, 127.5 (C tert arom), 118.2, 119.2, 119.6, 121.3, 122.3, 122.9, 128.4, 129.6, 140.3, 140.5 (C quat arom), 168.7, 169.1, 169.8, 169.9, 170.9 (C=O).

**6-Methyl-12-(2-deoxy-β-D-glucopyranosyl)-6,7,12,13-tetrahydroindolo[2,3-*a*]pyrrolo[3,4-*c*]carbazole-5,7-dione (8).** A solution of tetrabutylammonium fluoride (1.0 M in THF, 7 mL) was added to a solution of **29** (98 mg, 0.016 mmol) in THF (20 mL). The mixture was refluxed for 15 h, diluted with EtOAc (100 mL), washed with water, and dried over $Na_2SO_4$. The solvent was removed, and the residue was purified on a column of silica gel using MeOH−$CH_2Cl_2$ (1:25) as eluent to give the product **8** (70 mg, 91% yield) as a yellow solid: HRMS $(M + Na)^+$ $(ESI^+)$ calcd for $C_{27}H_{23}N_3O_6Na^+$ 508.1479, found 508.1484. $^1H$ NMR (500 MHz, DMSO) 12.09 (1H, s, NH), 9.18 (1H, d, $J = 7.5$), 9.09 (1H, d, $J = 8.0$ Hz), 8.03 (1H, d, $J = 8.4$ Hz), 7.73 (1H, d, $J = 8.2$ Hz), 7.62 (1H, dd, $J = 7.4$ Hz, $J = 8.1$ Hz), 7.56 (1H, dd, $J = 7.2$ Hz, $J = 8.0$ Hz), 7.41 (1H, t, $J = 10.8$), 7.35 (1H, t, $J = 7.8$), 6.63 (1H, dd, $J = 6.9$ Hz, $J = 13.5$ Hz, H-1′), 6.01 (1H, t, $J = 3.9$ Hz, H-4′), 5.35 (1H, d, $J = 3.7$, OH-6′), 5.00 (1H, d, $J = 4.4$, OH-3′), 4.11 (1H, m, H-5′), 3.86 (3H, m, OH-4′, H-3′, H-6′), 3.17 (3H, s, $NCH_3$), 1.83 (2H, m, H-2). $^{13}C$ NMR (500 MHz, DMSO) 25.6 ($NCH_3$), 34.5 ($C_{2'}$), 58.9, 69.4, 70.7, 79.4, 81.6 ($C_{1'}$, $C_{3'}$, $C_{4'}$, $C_{5'}$, $C_{6'}$), 111.4, 112.7, 120.9, 122.0, 124.8, 125.1, 127.5, 127.7 (C tert arom), 117.5, 118.6, 118.9, 121.7, 121.8, 125.4, 128.6, 129.7, 140.5, 141.3 (C quat arom), 170.0, 170.1 (C=O).

**6-Methyl-12-(2-deoxy-α-L-rhamnopyranosyl)-6,7,12,13-tetrahydroindolo[2,3-*a*]pyrrolo[3,4-*c*]carbazole-5,7-dione (9).** Following the same procedure described for **8**, Compound **9** was obtained from **30** as a yellow solid (92% yield): HRMS $(M + Na)^+$ $(ESI^+)$ calcd for $C_{27}H_{23}N_3O_6Na^+$ 508.1479, found 508.1472. $^1H$ NMR (500 MHz, DMSO) 12.06 (1H, s, NH), 9.19 (1H, d, $J = 7.8$), 9.08 (1H, d, $J = 7.9$ Hz), 7.77 (1H, d, $J = 8.5$ Hz), 7.64 (2H, m), 7.58 (1H, m), 7.40 (1H, m), 7.35 (1H, m), 6.79, (1H, br, OH-4′), 6.70 (1H, d, $J = 8.7$ Hz, H-1′), 5.60 (1H, s, OH-3′), 4.85 (1H, s, HO-6′), 4.38 (2H, m, H-5′, H-6′), 4.17 (1H, br, H-3′), 3.90 (1H, s, H-4′), 3.77 (1H, m, H-6′), 3.14 (3H, s, $NCH_3$), 2.71, 1.87 (2H,m, H-2). $^{13}C$ NMR (500 MHz, DMSO) 24.1 ($NCH_3$), 34.8 ($C_{2'}$), 58.5, 66.6, 67.4, 74.2, 83.9 ($C_{1'}$, $C_{3'}$, $C_{4'}$, $C_{5'}$, $C_{6'}$), 110.3, 111.8, 120.5, 121.4, 124.9, 125.1, 127.5, 127.6 (C tert arom), 117.1, 117.7, 118.7, 120.7, 121.2, 125.4, 128.1, 129.1, 140.0, 140.8 (C quat arom), 170.0, 170.1 (C=O).

**Hydrochloride of Ethyl 3-Amino-2,3,6-trideoxy-α-L-lyxo-hexoside (32).** The hydrochloride of daunorubicin (10.0 g) was heated in 0.2 M hydrochloric acid (800 mL) at 90 °C for 1 h. On cooling, the orange precipitate was filtered off and dried to give daunorubicinone (6.4 g, 91% yield). The filtrate was evaporated to dryness, which was dissolved in refluxing ethanol. The solution was cooled to room temperature and left for overnight at room temperature. The white precipitate was filtered off to give **32** (3.08 g, 83% yield): $^1H$ NMR (500 MHz, DMSO) 8.01 (3H, s, $NH_3$), 5.40 (1H, d, $J = 6.1$, HO-4), 4.82 (1H, d, $J = 2.2$ Hz, H-1), 3.76 (1H, m, H-5), 3.56 (2H, m, H-4, $CH_2$), 3.38 (2H, m, H-3, $CH_2$), 1.82 (1H, tt, $J = 3.4$ Hz, $J = 12.7$ Hz, H-$2_{eq}$), 1.68 (1H, dd, $J = 4.24$ Hz, $J = 12.6$ Hz, H-$2_{ax}$), 1.12 (3H, t, $J = 7.1$ Hz, $CH_3$), 1.08, (1H, d, $J = 6.5$ Hz, H-6). $^{13}C$ NMR (500 MHz, DMSO) 15.8, 17.6 ($CH_3$, $C_6$), 28.9 ($C_2$), 62.7, ($CH_2$), 47.4, 66.3, 66.7 ($C_1$, $C_4$, $C_5$), 96.1 ($C_3$).

**Ethyl 4-*O*-Benzoxyl-3-dibenzoxy amido-2,3,6-trideoxy-α-L-lyxo-hexoside (33).** Benzoxy chloride (10 mL) was added dropwise to a cold solution of **32** (1.5 g, 7.143 mmol) in anhydrous pyridine (30 mL). The mixture was stirred at room temperature for overnight, then quenched with methanol (5 mL). The solvent was removed, and the residue was dissolved in ethyl acetate, washed with 0.05 M $H_2SO_4$ solution, saturated $Na_2CO_3$ solution, and brine, and dried over $Na_2SO_4$. Compound **33** (2.55 g, 73% yield) as a white solid was obtained by removal of the solvent and purification on a column of silica gel using ethyl acetate−hexane (1:9) as eluant: HRMS $(M + Na)^+$ $(ESI^+)$ calcd for $C_{29}H_{29}NO_6Na^+$ 510.1893, found 510.1893. $^1H$ NMR (500 MHz, DMSO) 8.01 (3H, s, $NH_3$), 5.40 (1H, d, $J = 6.1$, HO-4), 4.82 (1H, d, $J = 2.2$ Hz, H-1), 3.76 (1H, m, H-5), 3.56 (2H, m, H-4, $CH_2$), 3.38 (2H, m, H-3, $CH_2$), 1.82 (1H, tt, $J = 3.4$ Hz, $J = 12.7$ Hz, H-$2_{eq}$), 1.68 (1H, dd, $J = 4.24$ Hz, $J = 12.6$ Hz, H-$2_{ax}$), 1.12 (3H, t, $J = 7.1$ Hz, $CH_3$), 1.08, (1H, d, $J = 6.5$ Hz, H-6); $^{13}C$ NMR (500 MHz, DMSO) 14.8, 16.6 ($CH_3$, $C_6$), 28.9 ($C_2$), 61.9 ($CH_2$), 51.9, 65.2, 70.9 ($C_1$, $C_4$, $C_5$), 95.9 ($C_3$).

**4-*O*-Benzoxyl-3-dibenzoxy amido-2,3,6-trideoxy-α-L-lyxo-hexose (34) and 2,5-Dihydro-3-[1-(*tert*-butyloxycarbonyl)-1*H*-indol-3-yl]-4-[1-(4-*O*-benzoxyl-3-dibenzoxy amido-2,3,6-trideoxy-β-L-lyxo-hexosyl)-1*H*-indol-3-yl]-1*H*-pyrrole-2,5-dione (35).** A mixture of **33** (100 mg), 3 M hydrochloric acid aqueous (2.2 mL), and THF (1.4 mL) was stirred at 60 °C for 15 h. TLC showed the reaction was completed. Solid sodium bicarbonate was added to the reaction mixture until pH 7. After extraction with ethyl acetate, the combined organic phase was dried over $Na_2SO_4$. The solvent was removed, and the residue was purified on a column of silica gel using ethyl acetate−hexane (1:4) as eluant to give **34** (75 mg, 80% yield) as a colorless solid: HRMS $(M + Na)^+$ $(ESI^+)$ calcd for $C_{27}H_{25}NO_6Na^+$ 482.1580, found 482.1843.

To a solution of **13** (0.94 g, 2.14 mmol) in THF (20 mL) were added **34** (1.19 g, 2.59 mmol) and triphenylphosphine (678 mg, 2.59 mmol). The mixture was cooled to −78 °C, and then DEAD (0.41 mL, 2.59 mmol) was added dropwise. The mixture was allowed to reach room temperature and stirred at room temperature for 18 h, and then water was added. After extraction with ethyl acetate, the combined organic phase was dried over $Na_2SO_4$. The solvent was removed, and the residue was purified on a column of silica gel using ethyl acetate-benzene (1:15) as eluant to give the product **35** (0.64 g, 34% yield) as an orange solid: HRMS $(M + Na)^+$ $(ESI^+)$ calcd for $C_{53}H_{46}N_4O_9Na^+$ 905.3156, found 905.3121.

**2,5-Dihydro-3-[1*H*-indol-3-yl]-4-[1-(4-*O*-benzoxyl-3-dibenzoxy amido-2,3,6-trideoxy-β-L-lyxo-hexosyl)-1*H*-indol-3-yl]-1*H*-pyrrole-2,5-dione (36).** A solution of **35** (630 mg, 0.72 mmol) in 88% formic acid (70 mL) and THF (3 mL) was stirred at room temperature and monitored by TLC every hour. After being stirred 22 h, the reaction was completed. Trimethylamine was added dropwise until neutralization, and then saturated aqueous NaOAc was added. After extraction with ethyl acetate, the combined organic phase was dried over $Na_2SO_4$. The solvent was removed, and the residue was purified on a column of silica gel (ethyl acetate−hexane (3:7) to afford **36** (454 mg, 81% yield) as a orange solid: HRMS $(M + Na)^+$ $(ESI^+)$ calcd for $C_{48}H_{38}N_4O_7Na^+$ 805.2632, found 805.2647. $^1H$ NMR (500 MHz, $CDCl_3$) 8.57 (1H, s, NH), 7.72 (1H, s), 8.07 (1H, d, $J = 7.2$ Hz), 7.84 (1H, s), 7.65 (1H, d), 7.54 (2H, m), 7.42 (2H, m), 7.27 (5H, m), 7.12 (11H, m), 6.78 (2H, m), 5.81 (1H, dd, $J = 2.1$ Hz, $J = 10.8$ Hz, H-1′), 5.68 (1H, br, H-4′), 5.41 (1H, m, H-3′), 4.26 (1H, m, H-5′), 3.18 (3H, s, $NCH_3$), 3.57 (1H,m, H-$2_{eq}$), 2.14 (1H,m, H-$2_{ax}$), 1.30 (3H, d, $J = 6.4$ Hz, H-6′). $^{13}C$ NMR (500 MHz, $CDCl_3$) 17.7 ($C_{6'}$), 24.6 ($NCH_3$), 34.6 ($C_{2'}$), 68.4, 70.9, 74.2 ($C_{1'}$, $C_{4'}$, $C_{5'}$), 84.8 ($C_{3'}$), 107.4, 107.5, 111.6, 120.9, 121.3, 122.6, 122.9, 123.0, 123.1, 125.7,125.9, 127.0, 128.6, 128.8, 128.9, 129.3, 129.4, 130.2, 130.3, 132.5, 133.5, 136.0, 136.2, 136.3, 137.2 (arom), 167.0, 172.6, 172.8, 173.5 (C=O).

**6-Methyl-12-(4-*O*-benzoxyl-3-dibenzoxyamido-2,3,6-trideoxy-β-L-lyxo-hexosyl)-6,7,12,13-tetrahydroindolo[2,3-*a*]pyrrolo[3,4-*c*]carbazole-5,7-dione (37).** A red solution of **36** (454 mg, 0.58 mmol) in benzene (400 mL) was treated with iodine (120 mg). Air was continuously bubbled through the reaction which was irradiates with a medium-pressure mercury lamp equipped with a Vycor filter for 15 h. Benzene was added to the reaction each 2 h to keep the solvent volume constant. The solvent was removed, and the residue was dissolved in EtOAc and washed with aqueous $Na_2S_2O_3$ and brine. The organic phase was dried over $Na_2SO_4$, the solvent was removed, and the residue was purified on a column of silica gel using EtOAc−hexane (1:4) as eluant to give the product **37** (387 mg, 85% yield) as a yellow solid: HRMS $(M + Na)^+$ $(ESI^+)$ calcd for $C_{48}H_{36}N_4O_7Na^+$ 803.2476, found 803.2457. $^1H$ NMR (500 MHz, $CDCl_3$) 10.73 (1H, s, NH), 9.38 (1H, d, $J = 7.3$ Hz), 9.24 (1H, d, $J = 7.9$ Hz), 7.79 (2H, d, $J = 7.7$ Hz), 7.63 (2H, br), 7.44 (2H, m), 7.30 (1H, m), 7.20 (4H, m), 7.09 (5H, m), 6.94 (4H, m), 6.64 (1H, d, $J = 8.1$ Hz), 6.41 (1H, d, $J = 10.8$ Hz, H-1′), 6.04 (1H, br, H-4′), 5.59 (1H, d, $J = 13.1$ Hz, H-3′), 4.77 (1H, m, H-5′), 3.31 (3H, s, $NCH_3$), 3.44

(1H,m, H-$2_{eq}$), 2.14 (1H,m, H-$2_{ax}$), 1.74 (3H, d, $J$ = 6.4 Hz, H-6′). $^{13}$C NMR (500 MHz, $CDCl_3$) 17.7 ($C_{6'}$), 23.8 ($NCH_3$), 32.2 ($C_{2'}$), 60.4, 70.8, 76.2 ($C_{1'}$, $C_{4'}$, $C_{5'}$), 85.1 ($C_{3'}$), 109.2, 111.6, 118.4, 119.0, 119.4, 120.7, 121.3, 121.8, 122.1, 123.0, 125.3, 126.2, 126.6, 127.3, 128.1, 128.2, 128.5, 128.6, 128.9, 129.7, 130.1, 132.2, 133.3, 136.3, 139.9, 140.0 (arom), 166.8, 170.1, 170.2, 173.0 (C=O).

**6-Methyl-12-(3-benzoxy amido-2,3,6-trideoxy-β-L-lyxo-hexosyl)-6,7,12,13-tetrahydroindolo[2,3-*a*]pyrrolo[3,4-*c*]-carbazole-5,7-dione(10). Method 1**: A solution of tetrabutylammonium fluoride (1.0 M in THF, 3 mL) was added to a solution of **37** (50 mg, 0.064 mmol) in THF (8 mL). The mixture was refluxed for 15 h, diluted with EtOAc (40 mL), washed with water, and dried over $Na_2SO_4$. The solvent was removed, and the residue was purified on a column of silica gel using MeOH−$CH_2Cl_2$ (1:50) as eluent to give the product **10** (32 mg, 89% yield) as a yellow solid.

**Method 2**: A solution of **37** (50 mg, 0.064 mmol) and sodium methoxide (cat.) in anhydrous methanol (10 mL) was stirred at room-temperature overnight. The solvent was evaporated under reduced pressure. The residue was dissolved in dichloromethane, washed with water, and dried over $Na_2SO_4$. The solvent was removed, and the residue was purified on a column of silica gel using MeOH−$CH_2Cl_2$ (1:50) as eluent to give the product **10** (33 mg, 91% yield) as a yellow solid.

**10**: HRMS (M + Na)$^+$ (ESI$^+$) calcd for $C_{34}H_{28}N_4O_5Na^+$ 595.1952, found 595.1974. $^1$H NMR (500 MHz, $CD_3CN$) 11.76 (1H, s, NH), 9.16 (1H, d, $J$ = 7.8 Hz), 9.03 (1H, d, $J$ = 7.9 Hz), 8.00 (1H, d, $J$ = 7.1 Hz), 7.81 (2H, d, $J$ = 7.3 Hz), 7.72 (1H, d, $J$ = 8.4 Hz), 7.64 (1H, d, $J$ = 8.1 Hz), 7.48 (3H, m), 7.45−7.26 (4H, m), 6.42 (1H, dd, $J$ = 5.3 Hz, $J$ = 11.4 Hz, H-1′), 4.72 (1H, m, H-3′), 4.25 (1H, m, H-5′), 4.10 (1H, d, $J$ = 1.6 Hz, HO-4′), 3.09 (3H, s, $NCH_3$), 2.71 (1H, q, $J$ = 12.2 Hz, H-$2_{eq}$), 2.04 (1H,m, H-$2_{ax}$), 1.47 (3H, d, $J$ = 6.4 Hz, H-6′). $^{13}$C NMR (500 MHz, $CD_3CN$) 17.8 ($C_{6'}$), 24.1 ($NCH_3$), 31.9 ($C_{2'}$), 50.9, 69.5, 76.4 ($C_{1'}$, $C_{4'}$, $C_{5'}$), 82.6 ($C_{3'}$), 110.5, 112.4, 119.0, 121.2, 121.8, 122.0, 122.3, 122.7, 125.8, 126.1, 126.4, 128.0, 128.1, 128.3, 128.7, 129.4, 129.5, 130.0, 130.6, 132.4, 134.1, 135.5, 141.0, 141.9 (arom), 167.6, 170.9, 171.1 (C=O).

**11,12-Dihydro-11-(3-benzoxy amido-2,3,6-trideoxy-β-L-lyxo-hexosyl) indolo[2,3-*a*]carbazole-5,6-dicarboxylic Anhydride (11).** A mixture of **10** (10 mg, 0.017 mmol), KOH aqueous (48%, 12 mL), and EtOH (4 mL) was stirred at reflux for overnight. The color of the reaction solution was changed from deep red to pale yellow. The solution was acidified with 10% citric acid to pH 6, stirred for additional 24 h at room temperature, then extracted with EtOAc. After removal of the solvent, the crude compound **11** was purified on a column of silica gel using MeOH−$CH_2Cl_2$ (1:25) to give **11** (8 mg, 80% yield): HRMS (M + Na)$^+$ (ESI$^+$) calcd for $C_{33}H_{25}N_3O_6Na^+$ 582.1635, found 582.1650. $^1$H NMR (500 MHz, pyridine-$d_5$) 12.66 (1H, s, NH), 9.45 (2H, m), 9.28 (1H, m), 8.22 (2H, d, $J$ = 7.6 Hz), 7.95 (2H, m), 7.69 (1H, m), 7.59 (1H, m), 7.52−7.34 (5H, m), 6.81 (1H, d, $J$ = 8.4, H-1′), 5.23 (1H, m, H-3′), 4.55 (1H, br, H-4′), 4.40 (1H, q, $J$ = 6.4 Hz, H-5′), 2.94 (1H, q, $J$ = 12.6 Hz, H-$2_{eq}$), 2.24 (1H,m, H-$2_{ax}$), 1.61 (3H, d, $J$ = 6.4 Hz, H-6′). $^{13}$C NMR (500 MHz, pyridine-$d_5$) 17.8 ($C_{6'}$), 31.7 ($C_{2'}$), 50.9, 68.8, 76.5 ($C_{1'}$, $C_{4'}$, $C_{5'}$), 82.4 ($C_{3'}$), 110.4, 112.1, 117.9, 118.1, 118.9, 119.9, 121.3, 121.8, 121.9, 122.1, 123.1, 123.8, 123.9, 125.0, 125.3, 127.9, 128.0, 128.1, 128.6, 129.4, 130.5, 135.8, 140.5, 141.5 (arom), 165.5, 165.6, 167.8 (C=O).

**11,12-Dihydro-11-(3-amono-2,3,6-trideoxy-β-L-lyxo-hexosyl) indolo[2,3-*a*]carbazole-5,6-dicarboxylic Anhydride (12).** A mixture of **37** (240 mg, 0.31 mmol), solid NaOH (1.50 g), methoxyethanol (23 mL), and water (7 mL) was stirred at refluxing for overnight. The solution was acidified with 10% citric acid to pH 7, stirred for additional 24 h at room temperature, then extracted with EtOAc. After removal of the solvent, the crude compound was purified on a column of silica gel to give **11** (70 mg, 40% yield) using MeOH−$CH_2Cl_2$ (1:25) as eluant, and **12** (58 mg, 42% yield) using MeOH−$CH_2Cl_2$ (1:5) as eluant. **12**: HRMS (M + Na)$^+$ (ESI$^+$) calcd for $C_{26}H_{21}N_3O_5Na^+$ 478.1373, found 478.1376. $^1$H NMR (500 MHz, DMSO−$D_2O$) 8.61 (1H, d, $J$ = 7.9 Hz), 8.47 (1H, d, $J$ = 7.9 Hz), 7.90 (1H, d, $J$ = 8.4 Hz), 7.64 (1H, d, $J$ = 8.2 Hz), 7.48 (2H, m), 7.25 (1H, t, $J$ = 7.5 Hz), 7.16 (1H, t, $J$ = 7.5 Hz), 6.49 (1H, dd, $J$ = 2.9, $J$ = 10.7, H-1′), 3.94 (2H, m, H-3′, H-5′), 3.32 (1H, m, H-4′), 2.08 (2H, m, H-2′), 1.48 (3H, d, $J$ = 6.1 Hz, H-6′). $^{13}$C NMR (500 MHz, DMSO-$D_2O$) 18.8 ($C_{6'}$), 38.7 ($C_{2'}$), 70.4, 75.9, 76.8, 82.7 ($C_{1'}$, $C_{3'}$, $C_{4'}$, $C_{5'}$), 112.5, 113.3, 121.6, 122.1, 123.9, 124.2, 128.1, 128.4 (C tert arom), 117.5, 117.7, 117.9, 118.7, 120.9, 121.8, 129.1, 129.4, 140.1, 140.5 (C quat arom), 164.58, 164.64 (C=O).

**11,12-Dihydro-11-(3-amono-2,3,6-trideoxy-β-L-lyxo-hexosyl)indolo[2,3-*a*]carbazole-5,6-dicarboxylic Anhydride (15) and 6-(3-Imidazol-1-yl-propyl)-12-(2,3,4,6-tetra-*O*-benzyl-β-D-glucopyranosyl)-6,7,12,13-tetrahydroindolo-[2,3-*a*]pyrrolo[3,4-*c*]carbazole-5,7-dione (3).** Followed the same procedure described for **23**, compound **15** was obtained from 6-(3-imidazol-1-yl-propyl)-12-(2,3,4,6-tetra-*O*-benzyl-β-D-glucopyranosyl)-6,7,12,13-tetrahydroindolo[2,3-*a*]pyrrolo[3,4-*c*]carbazole-5,7-dione (**14**) as a yellow solid (80% yield): HRMS (M + Na)$^+$ (ESI$^+$) calcd for $C_{54}H_{44}N_2O_8Na^+$ 871.2989, found 871.3018. THF (2 mL) and 3-(amino propanyl) imidazole (1.0 mL) was added to the crude compound **15** (50 mg, 0.06 mmol). The mixture was stirred at 60−70 °C for 24 h, and then diluted with EtOAc (80 mL), washed with brine, and dried over $Na_2SO_4$. The solvent was removed, and the residue was purified on a column of silic gel using MeOH−$CH_2Cl_2$ (1:30) as eluant to give the product **3** (45 mg, 80%) as a yellow solid: HRMS (M + Na)$^+$ (ESI$^+$) calcd for $C_{60}H_{53}N_5O_7Na^+$ 978.3837, found 978.3859. $^1$H NMR (500 MHz, $CDCl_3$) 10.84 (1H, s, NH), 9.41 (1H, d, $J$ = 7.9 Hz, Ar−H), 9.28 (1H, d, $J$ = 7.7 Hz, Ar−H), 7.69 (3H, m, Ar−H), 7.43−7.29 (21H, m, Ar−H), 7.11 (2H, m), 7.02 (1H, t, $J$ = 7.5 Hz, Ar−H), 6.89 (1H, t, $J$ = 7.7 Hz, Ar−H), 6.10 (1H, d, $J$ = 7.7 Hz, Ar−H), 6.05 (1H, d, $J$ = 9.0 Hz, H-1′), 5.04 (1H, d, $J$ = 10.6 Hz), 4.88 (2H, br), 4.76 (2H, m), 4.66(1H, d, $J$ = 12.5 Hz), 4.41(1H, t, $J$ = 9.6 Hz), 4.15(2H, d, $J$ = 7.1 Hz), 4.07−3.84 (6H, m), 2.93 (1H, d, $J$ = 9.6 Hz), 2.24 (2H, m). $^{13}$C NMR (DEPT135) (500 MHz, pyridine-$d_{5'}$) 30.8 ($CH_2$), 35.0, 44.7 (N−$CH_2$), 66.9 ($C_{6'}$), 74.2, 75.1, 75.5, 76.1 ($PhCH_2$), 75.9, 77.2, 77.7, 81.1, 84.8, 85.7 ($C_{1'}$,$C_{2'}$, $C_{3'}$, $C_{4'}$, $C_{5'}$).

**6-(3-Imidazol-1-yl-propyl)-12-β-D-glucopyranosyl-6,7,-12,13-tetrahydroindolo[2,3-*a*]pyrrolo[3,4-*c*]carbazole-5,7-dione (4).** A mixture of **3** (40 mg, 0.067 mmol), Pd/C (40 mg, palladium, 10 wt % on activativated carbon), and HCl (1 M, 2 mL) in MeOH−THF (60 mL) was hydrogenated at room temperature/45 psi for 24 h, then filtered and washed with ethanol. The filtrate was concentrated to remove the solvent. The residue was dissolved in 80 mL EtOAc, washed with saturated aqueous $NaHCO_3$ and brine, and dried over $Na_2SO_4$. The solvent was removed, and the residue was purified on a prepTLC using MeOH−$CH_2Cl_2$ (1:10) as developing solvent to give the product **4** (21 mg, 80% yield) as a orange solid: HRMS (M + Na)$^+$ (ESI$^+$) calcd for $C_{26}H_{22}N_4O_5Na^+$ 493.1482, found 493.1472. $^1$H NMR (500 MHz, DMSO+$D_2O$) 9.13 (1H, d, $J$ = 7.8 Hz, Ar−H), 9.05 (1H, d, $J$ = 8.0 Hz, Ar−H), 7.92 (1H, d, $J$ = 8.7 Hz, Ar−H), 7.71 (1H, s, imidazole-H), 7.67 (1H, m, Ar−H), 7.56 (2H, m, Ar−H), 7.38 (2H, m, Ar−H), 7.02 (1H, t, $J$ = 7.5 Hz, Ar−H), 6.89 (1H, t, $J$ = 7.7 Hz, Ar−H), 6.10 (1H, d, $J$ = 7.7 Hz, Ar−H), 7.26 (1H, s, imidazole-H), 6.89 (1H, s, imidazole-H), 6.24 (1H, d, $J$ = 9.0 Hz, H-1′), 4.09 (3H, m), 3.95 (2H, m), 3.76 (1H, m), 3.72−3.48(4H, m), 2.15 (2H, m). $^{13}$C (500 MHz, DMSO+$D_2O$) 30.5 ($CH_2$), 35.3, 44.4 (N−$CH_2$), 58.6, 67.9, 73.4, 76.8, 78.9, 84.8 ($C_{1'}$,$C_{2'}$, $C_{3'}$, $C_{4'}$, $C_{5'}$ $C_{6'}$), 112.2, 112.6, 117.6, 118.7, 119.1, 119.9, 120.4, 121.0, 121.3, 121.4, 121.7, 124.8, 127.6, 127.8, 128.6129.9, 137.8, 141.2, 142.6 (arom), 170.1, 170.2 (C=O).

**Cell Culture.** Cell lines SW620 and K562 were cultured in RPMI 1640 supplemented with 10% fetal bovine serum (FBS), 1% nonessential amino acid, and penicillin (100 units/mL)−streptomycin (100 μg/mL) in a humidified atmosphere of 5% $CO_2$ and 95% air at 37 °C. The culture mediums were changed every 2−3 days.

**Cytotoxicity of Synthesized Compounds (Reb, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 and 12) (MTS assay).** Cells (2000−10 000) were seeded in 96-well plates in RPMI-1640 and incubated for 24 h. The exponentially growing cancer cells were

incubated with various concentrations of compounds for 72 h at 37 °C (5% $CO_2$, 95% humidity). After 72 h incubation, tetrazolium [3-(4,5-dimethythiazol-2-yl)]-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2*H*-tetrazolium, inner salt (MTS, 2 mg/mL) and phenazine methosulfate (PMS, 25 $\mu$M) were mixed and added directly to the cells. After incubated for 3 h at 37 °C, the absorbance of formazan (the metabolite of MTS by viable cells) was measured at 490 nm. The $IC_{50}$ values of the carbohydrate−drug conjugates for cytotoxicity were calculated by the dose−response curves of percentage of cell growth vs control (no compound added).

**Inhibition of Topoisomerase I with ICT Bioassay.** ICT bioassay was performed according to previously described,[37−39] Briefly, HeLa cells were incubated with or without 10 $\mu$M camptothecin or tested compounds for 30 min during exponential growth at 37 °C. Following detergent lysis with sarkosyl, DNA, and denatured proteins were fractionated by CsCl gradient centrifugation. The fraction was collected from the bottom. The DNA plus covalently bonded proteins are associated with fractions while free proteins and debris are at the top of the gradient. Each fraction (50 $\mu$L) was slotted onto a HybondECL membrane (Amersham Biosciences), and the blot was probed with anti-topo I antibody. The immunoblotsignals were visualized by chemiluminescence (Amersham Biosciences).

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