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Synthesis and Biological Activities of NB-506 Analogues Modified at the Glucose Group

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Abstract—A new indolocarbazole compound, NB-506 (**1**), modified at the glucose group yielded a β-D-glucopyranoside, J-107,088 (**2**), which showed potent anticancer activity. A β-D-ribofuranoside, J-109,534 (**3**), was found to be 6 times more potent than J-107,088 at inhibiting topoisomerase I.

It is well known that DNA-topoisomerase I is an attractive target for cancer chemotherapy.[1,2] NB-506 (**1**),[3–5] a new indolocarbazole anticancer agent developed via modification of a natural compound, BE-13793C (**4**),[6] was found to be a potent topoisomerase I inhibitor. Previous studies showed that synthetic NB-506 analogues differed in the 6-*N*-amino formyl group,[7] and that the presence of two hydroxyl groups at the benzene ring[8] yielded the most potent anticancer drug, J-107,088 (**2**), against human stomach cancer cells, MKN-45, implanted in mice. This compound had an extremely broad safety margin.[9,10] The potent anticancer activity and wide safety margin of J-107,088 may be due to its high concentration in the target cancer cells. In order to enhance intracellular penetration, an extensive study was conducted of the modification of J-107,088 at the glucose group. In this paper, we report the synthesis and biological activities of a series of sugar analogues of J-107,088, and discuss the in vivo anticancer effects of several potent analogues (Fig. 1).

Chemistry

A series of sugar analogues of J-107, 088 (**2**, **3**, **9**–**29**) was synthesized by applying two types of previously reported[11,12] glycosylation reactions starting from indolocarbazole compound **5**[13] or bisindolylmaleimide compound **6**[13] as shown in Scheme 1. Most of the key intermediate glycosides (**7**) were prepared by method A, in which a glycosylation reaction with a 1-chloro-*O*-benzyl-D-sugar[14,15] was carried out using potassium hydroxide (KOH) as a base in the presence of sodium sulfate. In method B, the Mitsunobu reaction was very useful when a halosugar such as D-erythrose or D-mannopyranose was unstable under the basic conditions used in method A.

As shown in Table 1, the stereoselectivity of the glycosylation reaction in method A was almost entirely β-selective, but in the case of D-allopyranose, poor selectivity (Run 9, $\alpha/\beta = 2/3$) was found because the β-chloride was easily inverted to α-chloride by steric hindrance due to the 1,3-dipolar alignment in the six-membered ring system. Interestingly, in the case of 2-deoxy-D-glucopyranose (Run 15, 16), stereoselectivity was completely controlled by the glycosylation method. The reason for this is not clear, but it may be that the β-conformer of the 1-chloro-2-deoxy-3,4,6-tri-*O*-benzyl-D-glucopyranose is more stable than the α-conformer in method A. The benzyl groups of the 6-*N*-methyl compounds (**7**) were removed by hydrogenolysis with palladium hydroxide followed by treatment with 2.0 M aqueous potassium hydroxide to yield anhydride compound **8**. In the case of D-erythrose compound, 2,3-*O*-isopropylidene-D-erythrose was used as a sugar source, and deprotection of isopropylidene of compound **7** occurred at a hydrogenolysis step. Final compounds **2**, **3** and **10**–**29** were obtained by a coupling reaction of **8** with hydrazine **9** in dimethylformamide (DMF) at

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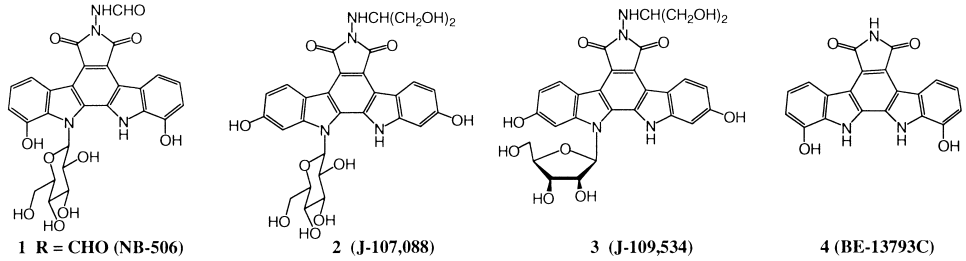
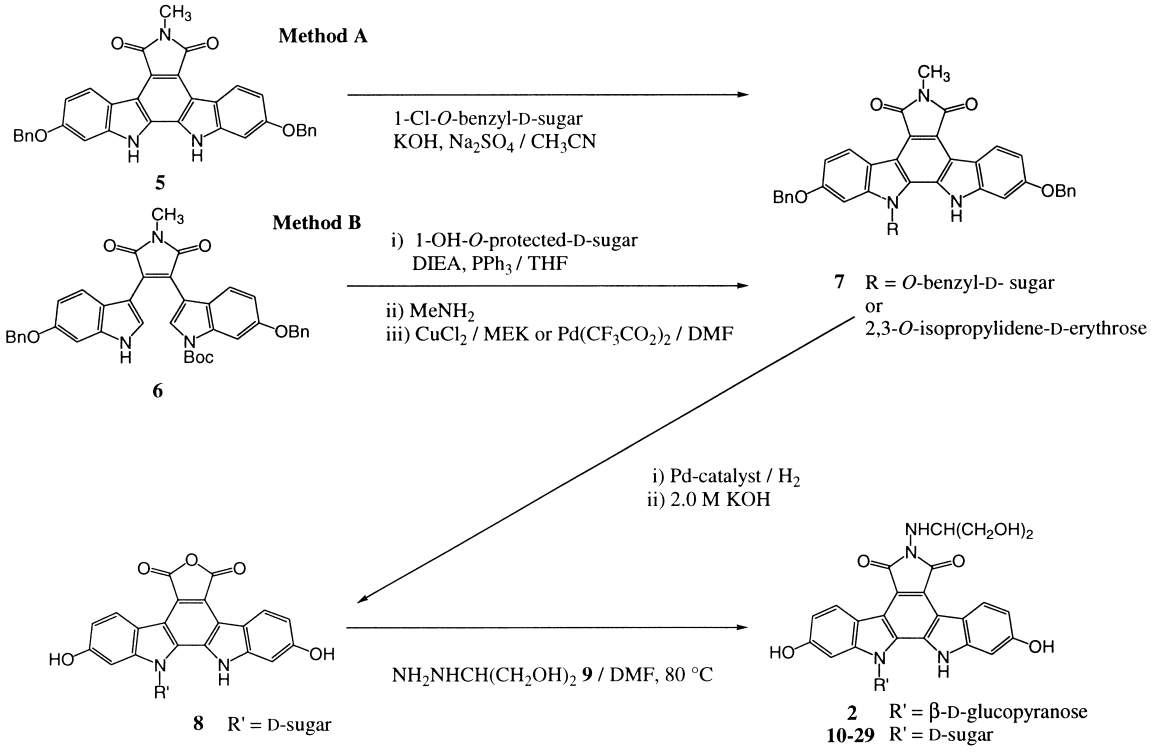


Figure 1.



Scheme 1.

80 °C. The final structures of the analogues were determined by both ^{1}H NMR and MS.[16]

Results and Discussion

As shown in Table 2, the β-D-ribofuranose derivative, J-109,534 (**3**) was the most potent inhibitor of topoisomerase I in both the enzyme assay (Topo-I cleavage EC_{50}) and cellular assay (K^+/SDS EC_{200}). However, in these sugar analogues, correlation between the enzyme and cellular topoisomerase I inhibition assay was not observed, probably due to the difference in penetration into cells. Interestingly, the cytotoxicities of the compounds did not correlate with the cellular assay (K^+/SDS EC_{200}) results, probably because of the presence of other mechanisms of cytotoxicity. Most of the compounds showed complete selectivity for topoisomerase II and protein kinase C (PKC); however, the 2-deoxy-D-ribofuranose derivative **17** showed moderate inhibitory activity against PKC, perhaps due to structural similarity to adenosine triphosphate (ATP). To determine the

Table 1. Results of the glycosylation reaction

	Sugar[a]	Method	Yield (%)	α/β[b]
1	Galactopyranose	A	99	1:99
2	Glucopyranose	A	89	1:49
3	Xylofuranose	A	60	1:3.3
4	Xylopyranose	A	84	β only[c]
5	Allopyranose	A	52	2:3
6	6-Deoxyglucopyranose	A	99	β only
7	Mannopyranose	B	22	1:2
8	Arabinofuranose	A	99	β only
9	Ribofuranose	A	96	1:6
10	Allofuranose	A	81	1:7
11	Glucofuranose	A	62	2:7
12	2-Deoxyribofuranose	A	36	2:5
13	Maltose	A	44	β only
14	Erythrose	B	35	2:5
15	2-Deoxyglucopyranose	A	72	α only
16	2-Deoxyglucopyranose	B	50	β only

[a]All sugars were D-enantiomers and protected with benzyl ether except erythrose.
[b]Determined by HPLC or ^{1}H NMR.
[c]The isomer was not detectable by ^{1}H NMR measurement.

Table 2. In vitro activities of various compounds

	R	Topo-I[a] Cleavage EC_{50} (nM)	Topo-II[a] Cleavage EC_{50} (μM)	K^+/SDS[b] (P388/S) EC_{200} (nM)	PKC[c] IC_{50} (μM)	CTX[d] P388/S IC_{50} (nM)	CTX[e] MKN-45 IC_{50} (nM)
10	β-Glucofuranose	20	>50	1700	>200	14	29
11	β-Allofuranose	42	>50	2000	>200	17	70
12	β-Arabinofuranose	32	>50	430	120	5.3	93
13	α-Arabinofuranose	100	>50	2600	>200	8.5	43
3	β-Ribofuranose	8	>50	32	20	1.8	50
14	α-Ribofuranose	30	>50	450	21	19	130
15	β-Xylofuranose	17	>50	60	>200	0.96	6.0
16	α-Xylofuranose	17	>50	400	>200	12	68
17	β-2-Deoxyribofuranose	25	>50	170	3	4.4	45
18	β-Erythrose	40	NT[f]	NT	12	2.4	37
2	β-Glucopyranose	51	>50	100	200	1.5	4.8
19	α-Glucopyranose	300	>50	450	23	19	8.5
20	β-Allopyranose	26	>50	160	17	13	33
21	α-Allopyranose	26	NT	1600	72	18	20
22	β-Mannopyranose	230	>50	>10000	20	36	63
23	α-Mannopyranose	80	>50	930	50	15	23
24	β-Galactopyranose	140	NT	>10000	>200	7.1	9.3
25	β-2-Deoxyglucopyranose	240	>50	3000	130	5.6	42
26	α-2-Deoxyglucopyranose	130	>50	1200	150	16	33
27	β-6-Deoxyglucopyranose	140	>50	6500	60	3.0	38
28	β-Xylopyranose	100	>50	300	>200	3.8	50
29	β-Maltose	>3000	>50	>10000	>200	3.5	73

[a]Topoisomerase-mediated DNA cleavage assay was carried out using supercoiled pBR322 plasmid DNA.[4]
[b]Effects on the formation of protein-DNA complex in P388 cells were investigated by the K^+/SDS method.[4]
[c]Histone II-As was used as a substrate for protein kinase C.[4]
[d]Cytotoxicity (CTX) against murine leukemia cells (P388) was measured by the colorimetric tetrazolium–formazan method.[4]
[e]CTX against human stomach cancer cells (MKN-45) was measured by the colorimetric tetrazolium–formazan method and the sulforhodamine B dye-staining method.[4]
[f]NT: not tested.

effect of stereochemistry at the anomeric carbon, α-isomers were also synthesized and tested. As shown in Table 2, α-isomers were less potent than the corresponding β-isomers. Only one disaccharide analogue was synthesized, but compound **29** did not inhibit either topoisomerase I and II or PKC. In general, the furanose analogues showed greater inhibitory activities against topoisomerase I than did the pyranose analogues at the enzyme level, possibly indicating that the sugar moiety has some interaction with the 2-deoxy-D-ribofuranose parts of DNA. Some analogues that showed potent inhibition of topoisomerase I were tested for anticancer effects in mice and compared with J-107,088 (**2**).

As shown in Table 3, β-D-ribofuranose derivative **3** and β-D-xylofuranose derivative **16**, though more potent than J-107,088 (**2**) at inhibiting topoisomerase I, were less potent with respect to anticancer effects against MKN-45 xenografts in mice. Moreover, their safety margins were not as wide as that of J-107,088 (**2**). The greater anticancer effects of the β-isomers relative to those of the corresponding α-isomers were reflected in the potencies of topoisomerase I inhibition. These studies revealed that a β-D-glucopyranose compound, J-107,088 (**2**), showed anticancer activity superior to that of other glycosides, probably due to good distribution to the target cancer cells, and had a very wide safety margin.

Table 3. Anticancer effects

No[a]	R	Topo-I cleavage EC_{50} (nM)	K^+/SDS (P388/S) EC_{200} (nM)	CTX MKN-45 IC_{50} (nM)	MKN-45 GID_{75}[b] mg/m^2	LD_{10}[c] mg/m^2	Safety margin LD_{10} / GID_{75}[d]
3	β-Ribofuranose	8	40	50	290	570	2.0
14	α-Ribofuranose	30	450	130	540	1000	1.9
15	β-Xylofuranose	17	60	6.0	300	420	1.4
11	β-Allofuranose	42	2000	70	260	370	1.4
2	β-Glucopyranose	51	100	4.8	45	>1600	>36.0
19	α-Glucopyranose	300	450	8.5	110	170	1.5
20	β-Allopyranose	26	160	33	440	990	2.3

[a]Compounds were injected intravenously five times/week for 2 weeks, and treatment was initiated when tumors grew to 0.2 cm^3 or larger.
[b]GID_{75}: approximate 75% growth inhibition dose reflects the anticancer effect on MKN-45 human stomach cancer cells implanted subcutaneously into a side flank of nude mice.
[c]LD_{10}: approximate 10% lethal dose at the treatment schedule.
[d]Safety margin: the ratio LD_{10}/GID_{75}.

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16. Physical data for a representative compound, J-109,534 [6-*N*-(1-hydroxymetyl-2-hydroxy)ethylamino-12,13-dihydro-2,10-dihydroxy-13-(β-D-ribofuranosyl)-5*H*-indolo[2,3-*a*]pyrrolo[3,4-*c*]-carbazole-5,7(6*H*)-dione] (**3**): mp >250 °C; [α] +72.2° (*c* 1.00 DMSO); IR (KBr) ν_{max} 3350, 1749, 1697, 1398, 1338, 1197, 1064 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6), d 3.27 (1H, m), 3.49 (4H, m), 3.92–4.12 (4H, m), 4.34 (1H, m), 4.50 (2H, m), 5.22 (2H, m), 5.53 (2H, d, $J=2.7$ Hz), 6.13 (1H, d, $J=7.5$ Hz),6.38 (1H, m), 6.81 (1H, d, $J=1.8$, 8.4 Hz), 6.87 (1H, d, $J=1.8$, 8.4 Hz), 7.03 (1H, d, $J=1.8$ Hz), 7.15 (1H, d, $J=1.8$ Hz), 8.81 (1H, d, $J=8.4$ Hz), 8.93 (1H, d, $J=8.4$ Hz), 9.75 (1H, brs), 9.87 (1H, brs), 11.22 (1H, brs); HRMS (FAB) calcd for $C_{28}H_{26}N_4O_{10}$ 578.1649, found 578.1655.