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Orally Active Docetaxel Analogue: Synthesis of 10-Deoxy-10-*C*-morpholinoethyl Docetaxel Analogues

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Abstract—To improve cytotoxicity of 10-deoxy-10-*C*-morpholinoethyl docetaxel analogues against various tumor cell lines including resistant cells expressing P-glycoprotein (P-gp), we modified the 7-hydroxyl group to hydrophobic groups (methoxy, deoxy, 6,7-olefin, α-F, 7-β-8-β-methano, fluoromethoxy). Among these analogues, the 7-methoxy analogue showed the strongest cytotoxicity. This analogue showed potent activity against B16 melanoma BL6 in vivo by oral administration.

Paclitaxel (**1**, Taxol®)[1] and docetaxel (**2**, Taxotere®)[2] are currently considered to be some of the most important drugs in cancer chemotherapy. However, their low water-solubility requires co-injection of a detergent, Cremophor® EL or Tween® 80. These detergents frequently cause untoward hypersensitivity reactions, and patients receiving these drugs require premedication.[3] To resolve these problems, we previously described the synthesis of non-prodrug water-soluble docetaxel analogues, such as 10-*O*-*sec*-aminoethyl docetaxel analogues[4] and 10-deoxy-10-*C*-*sec*-aminoalkyl docetaxel analogues.[5] Among these analogues, 10-deoxy-10-*C*-morpholinoethyl docetaxel analogue (**3**) exhibited cytotoxicity equal to that of docetaxel (**2**) (Table 1).

However, the cytotoxicity of **3** against drug-resistant cell lines expressing P-gp was not sufficiently potent.

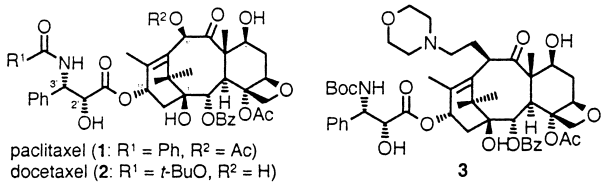

Figure 1. Structure of paclitaxel analogues.

Many patients whose initial response is good go into relapse because of the development of drug resistance after treatment.[6] To overcome this problem, we modified the 7-hydroxyl group of the 10-*C*-morpholinoethyl docetaxel analogue (**3**) to the groups that have been reported to be able to improve the cytotoxicity against cancer cell lines such as methoxy, deoxy, 6,7-olefin, etc. We found that the 7-methoxy analogue showed the most potent activity, regardless of P-gp expression. It has been reported that the poor oral bioavailability of docetaxel is caused by P-gp, which is present in the gastrointestinal tract.[7] Consequently, the oral bioavailability of paclitaxel could be increased markedly with P-gp inhibitors.[8] Therefore, a taxane that is a poor substrate of P-gp showed oral bioavailability.[9] On the basis of these observations, the 7-methoxy analogue, a poor substrate of P-gp, appeared to have good bioavailability and showed antitumor activity in vivo by oral administration. Here we report modification of the 7-hydroxyl group of 10-deoxy-10-*C*-morpholinoethyl docetaxel analogues, and the results of in vivo studies.

Chemistry

10-Deoxy-7-*O*-methyl-10-*C*-morpholinoethyl docetaxel (**7**) was synthesized from 10-deacetoxy-10-*C*-morpholinoethyl-7,13-*O*-bis-TES baccatin III (**4**)[5] (Scheme 1). Selective desilylation and substitution with MTM and

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following desulfurylation[10] gave the key intermediate (**6**). Introduction of the phenylisoserine side chain using protected β-lactam (**8**)[11] as a side-chain precursor and deprotection using the reported method[11] gave **7**.[12]

Synthesis of 7,10-di-deoxy-10-*C*-morpholinoethyl docetaxel (**12**) is described in Scheme 2. The key intermediate (**11**) was synthesized from **9**[13] in the reported manner.[5] Introduction of the phenylisoserine side chain, oxidation, reductive amination, and deprotection gave **12**.[14]

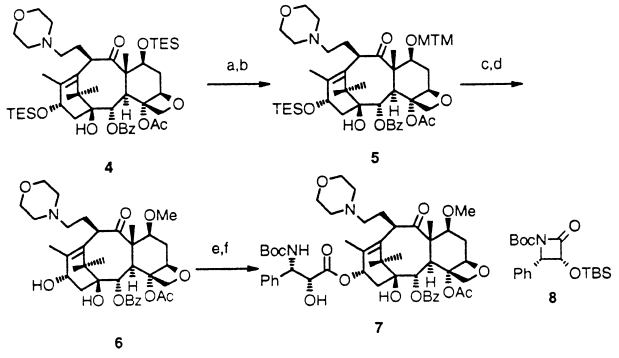
Synthesis of 6,7-dehydro-7,10-di-deoxy-10-*C*-morpholinoethyl docetaxel (**15**) is described in Scheme 3. The key intermediate (**14**) was synthesized from **13**[5] in high yield.[15] **14** was converted to **15**[16] by reactions similar to those used for preparation of **12**.

Synthesis of 7,10-di-deoxy-7-α-fluoro-10-*C*-morpholinoethyl docetaxel (**18**) is described in Scheme 4. The key intermediate (**17**) was synthesized from **16** in moderately high yield.[17] Compound **17** was converted to **18**.[18]

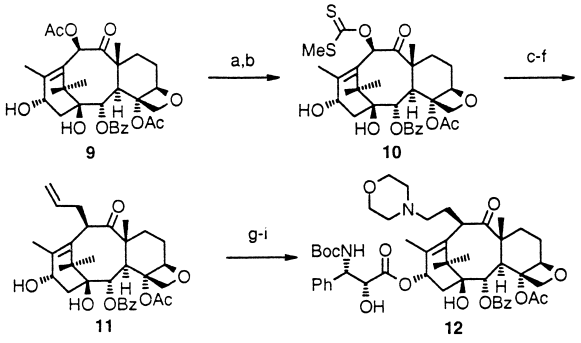
Table 1. Cytotoxicity of 10-*C*-morpholinoethyl docetaxel analogues modified 7-hydroxyl group[a]

	7-group	Cytotoxic activity GI50 (ng/mL)[b]		
		PC-6	PC-12	PC-6/VCR29-9
2		0.408–2.55	11.7–72.7	39.6–230
3	OH	1.72	21.5	76.3
7	OMe	0.266	0.756	9.01
12	Deoxy	0.761	1.47	20.4
15	olefin	0.500	0.824	18.0
18	α-F	0.379	2.55	16.2
21	Methano	0.873	3.56	38.6
27	OCH_2F	0.246	1.50	40.4

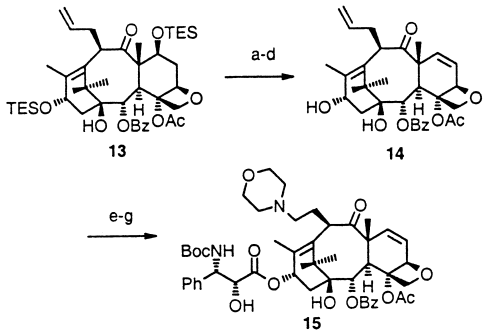
[a]The in vitro experiments were performed with three different cell lines: PC-6, a human small cell lung cancer,[23] and its variant, PC-6/VCR29-9, vincristine-resistant cell line expressing P-gp,[24] PC-12, a human non-small cell lung cancer cell line.[23] Determination of GI50 was performed using the MTT assay.[25] The cells were exposed continuously to the test compounds for 72 h.
[b]Growth inhibition of 50%: the concentration required to obtain half of the maximal inhibition for cell growth.



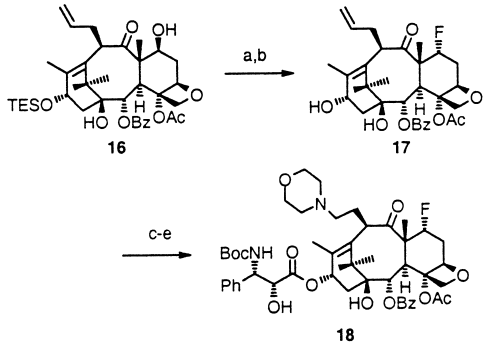
Scheme 1. Reagents and conditions: (a) TsOH, MeOH (71%); (b) Ac_2O, DMSO (42%); (c) Raney-Ni, EtOH, reflux (67%); (d) HF–Py (82%); (e) **8**, NaHMDS, THF, −78 °C (72%); (f) HF–Py (82%).



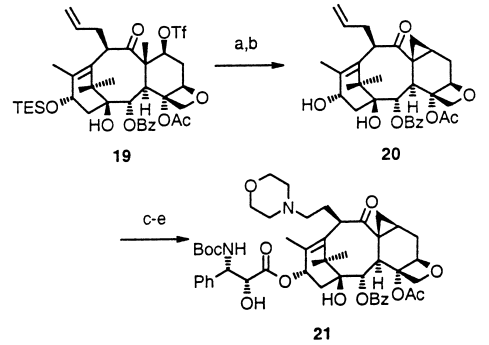
Scheme 2. Reagents and conditions: (a) hydrazine (53%); (b) CS_2, MeI, *n*-BuLi (55%); (c) *n*-Bu_3SnH, AIBN, acrolein (30%); (d) $NaBH_4$ (97%); (e) *o*-nitrophenylseleno cyanide, *n*-Bu_3P (75%); (f) mCPBA (89%); (g) **8**, NaH, THF (94%); (h) 1) OsO_4, *N*-methylmorpholine *N*-oxide, 2) $NaIO_4$ (63%); (i) 1) morpholine, H_2, Pd–C, 2) HF–Py (47%).



Scheme 3. Reagents and conditions: (a) cat. *p*-TsOH, MeOH (75%); (b) Tf_2O, Py (92%); (c) DBU (98%); (d) HF–Py (96%); (e) **8**, LiHMDS, THF (100%); (f) HF–Py (94%); (g) 1) OsO_4, *N*-methylmorpholine *N*-oxide, 2) $NaIO_4$, 3) morpholine, AcOH, $NaBH_3CN$ (95%).



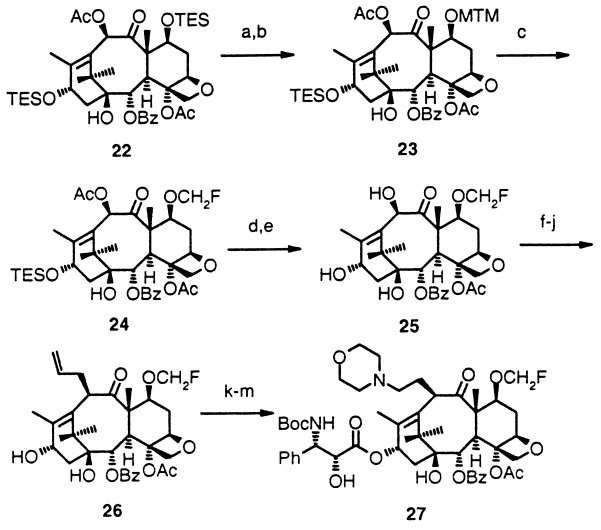
Scheme 4. Reagents and conditions: (a) DAST (50%); (b) HF–Py (50%); (c) **8**, LiHMDS, THF (62%); (d) 1) OsO_4, *N*-methylmorpholine *N*-oxide, 2) $NaIO_4$, 3) morpholine, AcOH, $NaBH_3CN$ (51%); (e) HF–Py (84%).



Scheme 5. Reagents and conditions: (a) silica gel, CH_3CN–THF, 55 °C (72%); (b) HF–Py (q.y.); (c) **8**, LiHMDS, THF (89%); (d) 1) OsO_4, *N*-methylmorpholine *N*-oxide, 2) $NaIO_4$, 3) morpholine, AcOH, $NaBH_3CN$ (76%); (e) HF–Py (91%).

Synthesis of 7,10-di-deoxy-7-β-8-β-methano-10-*C*-morpholinoethyl docetaxel (**21**) is described in Scheme 5. The key intermediate (**20**) was synthesized from **19** in high yield.[19] Compound **20** was converted to **21**.[20]

Synthesis of 10-deoxy-7-*O*-fluoromethyl-10-*C*-morpholinoethyl docetaxel (**27**) is described in Scheme 6. The key intermediate (**26**) was synthesized from **22**. We found that oxidative fluorination of 7-*O*-methylthiomethyl ether (**23**) using NIS and DAST afforded fluoromethyl ether (**24**) in good yield.[21] The 10-*C*-alkyl group was introduced in the reported manner.[5] Compound **26** was converted to **27**.[22]



Scheme 6. Reagents and conditions: (a) cat. TsOH, MeOH (80%); (b) Ac_2O, DMSO (49%); (c) NIS, DAST, CH_2Cl_2, 0 °C (64%); (d) HF–Py (80%); (e) hydrazine, EtOH (97%); (f) *n*-BuLi, carbon disulfide, methyl iodide, THF (91%); (g) acrolein, $(TMS)_3SiH$, AIBN, toluene (50%); (h) $NaBH_4$, THF (77%); (i) *o*-nitrophenylseleno cyanide, *n*-Bu_3P (79%); (j) mCPBA (71%); (k) **8**, LiHMDS, THF (92%); (l) HF–Py (84%); (m) 1) OsO_4, *N*-methylmorpholine *N*-oxide, 2) $NaIO_4$, 3) morpholine, AcOH, $NaBH_3CN$ (68%).

Table 2. Antitumor activity against B16 melanoma BL-6[a]

	Route	Dose (mg/kg)	IR (%)[b]	BWL max. (%)[c]	Mortality
2	iv	100.0	95.1	<0	0/6
	po	600.0	6.2	<0	0/6
7	po	112.5	99	20.0	4/6
		75.0	98.3	5.0	0/6
		50.0	96.2	1.7	0/6
		33.3	82.8	0.9	0/6
		22.2	81.7	<0	0/6
	iv	75.0	99.4	19.2	5/6
		50.0	98.6	18.0	0/6
		33.3	94.4	9.4	0/6
		22.2	93.7	5.7	0/6

[a]B16 melanoma BL6 was kindly provided by Dr. Tsuruo (Institute of Molecular and Cellular Biosciences, University of Tokyo) by courtesy of Dr. Fidler (The University of Texas M. D. Anderson Cancer Center).[26] B16 melanoma BL6 cells were subcutaneously inoculated into C57BL/6 mice on day 0. Compounds were administered intravenously or orally on day 4 (single administration). Tumor masses were weighed on day 15.
[b]IR (%) = (1−TWt/TWc)×100 TWt: the mean tumor weight of the treated group. TWc: the mean tumor weight of the control group.
[c]BWL max. (%): Maximum rate of body weight loss (<0 indicates no body weight loss).

These analogues (**7**, **12**, **15**, **18**, **21**, **27**) having a morpholinoethyl group are more soluble than paclitaxel and docetaxel in acidic solution.

Results and Discussion

Antitumor activities of the 10-deoxy-10-*C*-morpholinoethyl docetaxel analogues modified at the 7-hydroxyl group were determined in vitro and in vivo. The cytotoxicities of 7-modified docetaxel analogues (**7**, **12**, **15**, **18**, **21**, **27**) against two human lung cancers and resistant cell lines expressing P-gp were improved in comparison with those of 7-hydroxy analogue (**3**) (Table 1).

Among these analogues, 7-methoxy analogue (**7**) showed the most potent cytotoxic activity. We selected compound **7** as a candidate for further in vivo investigation. To evaluate the antitumor effects of compound **7** in vivo, we used B16 melanoma BL6 subcutaneously implanted into mice, and the activity of **7** was compared with that of docetaxel (**2**). Furthermore, we investigated whether compound **7** exhibits antitumor effects by oral administration or not. Compound **7** showed potent antitumor effects over a wide dose range by both iv and po administration. However, five of the six mice that received compound **7** intravenously at 75 mg/kg died from toxicity. The same dosage by oral administration exhibited potent antitumor activity with an IR value of 98.3% with body weight loss of only 5.0%. The near dosage (112.5 mg/kg) by oral administration gave death. Thus, we considered compound **7** to have excellent oral bioavailability in mice. In contrast, docetaxel at 600.0 mg/kg given po exhibited no antitumor activity, and showed no body weight loss. This result clearly showed that docetaxel has very poor bioavailability (Table 2).

In conclusion, we synthesized several 10-*C*-morpholinoethyl docetaxel analogues modified at the 7-hydroxyl group. The 7-methoxy analogue was the most potent of the analogues prepared and was highly active against B16 melanoma BL6 by both iv and po administration. Further investigations of orally active docetaxel analogues will be reported in the near future.

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12. ^{1}H NMR of **7** (400 MHz, $CDCl_3$) δ 1.14 (s, 3H), 1.19 (s, 3H), 1.35 (s, 9H), 1.67 (s, 3H), 1.85 (d, J = 1 Hz, 3H), 2.37 (s, 3H), 1.73–2.75 (m, 12H), 3.25 (s, 3H), 3.68 (m, 4H), 3.93–4.01 (m, 3H), 4.18 (d, J = 8 Hz, 1H), 4.30 (d, J = 8 Hz, 1H), 4.61 (s, 1H), 4.99 (d, J = 8 Hz, 1H), 5.27 (d, J = 10 Hz, 1H), 5.42 (d, J = 10 Hz, 1H), 5.63 (d, J = 7 Hz, 1H), 6.16 (t, J = 8 Hz, 1H), 7.29–7.42 (m, 5H), 7.49 (t, J = 8 Hz, 2H), 7.60 (t, J = 8 Hz, 1H), 8.10 (d, J = 8 Hz, 2H).
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14. ^{1}H NMR of **12** (400 MHz, $CDCl_3$) δ 1.09 (s, 3H), 1.20 (s, 3H), 1.31 (s, 9H), 1.3–1.6 (m, 3H), 1.71 (s, 3H), 1.80 (s, 3H), 1.85–2.05 (m, 2H), 2.1–2.6 (m, 11H), 2.40 (s, 3H), 3.68 (m, 4H), 3.92 (d, J = 7 Hz, 1H), 4.08 (t, J = 5 Hz, 1H), 4.24 (d, J = 8 Hz, 1H), 4.32 (d, J = 8 Hz, 1H), 4.60 (brs, 1H), 4.95 (dd, J = 3 Hz, 10 Hz, 1H), 5.28 (d, J = 9 Hz, 1H), 5.37 (d, J = 9 Hz, 1H), 5.65 (d, J = 7 Hz, 1H), 6.20 (t, J = 9 Hz, 1H), 7.31 (t, J = 7 Hz, 1H), 7.37 (d, J = 7 Hz, 2H), 7.40 (t, J = 7 Hz, 2H), 7.51 (t, J = 7 Hz, 2H), 7.61 (t, J = 7 Hz, 1H), 8.14 (d, J = 7 Hz, 2H).
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16. ^{1}H NMR of **15** (400 MHz, $CDCl_3$) δ 1.10 (s, 3H), 1.22 (s, 3H), 1.33 (s, 9H), 1.45–1.55 (m, 2H), 1.72 (s, 3H), 1.83 (s, 3H), 2.23 (dd, J = 8 Hz, 15 Hz, 1H), 2.39 (s, 3H), 2.30–2.60 (m, 8H), 3.68 (t, J = 4.7 Hz, 4H), 3.78 (d, J = 5 Hz, 7 Hz, 1H), 4.19 (d, J = 7 Hz, 1H), 4.31 (d, J = 8 Hz, 1H), 4.42 (d, J = 8 Hz, 1H), 4.60 (s, 1H), 5.11 (d, J = 6 Hz, 1H), 5.27 (d, J = 9 Hz, 1H), 5.41 (d, J = 9 Hz, 1H), 5.72 (d, J = 10 Hz, 1H), 5.83 (d, J = 7 Hz, 1H), 6.02 (dd, J = 6 Hz, 10 Hz, 1H), 6.18 (t, J = 9 Hz, 1H), 7.51 (t, J = 7 Hz, 2H), 7.62 (t, J = 7 Hz, 1H), 8.16 (d, J = 7 Hz, 2H).
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18. ^{1}H NMR of **18** (400 MHz, $CDCl_3$) δ 1.09 (s, 3H), 1.21 (s, 3H), 1.33 (s, 9H), 1.68 (s, 3H), 1.77 (s, 3H), 2.40 (s, 3H), 2.14–2.65 (m, 13H), 3.68 (m, 4H), 4.20–4.24 (m, 2H), 4.33, 4.36 (each d, J = 9 Hz, total 2H), 4.52 (dd, J = 3 Hz, 47 Hz, 1H), 4.61 (s, 1H), 5.02 (dd, J = 2 Hz, 9 Hz, 1H), 5.30 (d, J = 10 Hz, 1H), 5.41 (d, J = 10 Hz, 1H), 5.74 (d, J = 7 Hz, 1H), 6.19 (t, J = 8 Hz, 1H), 7.40–7.42 (m, 5H), 7.51 (t, J = 8 Hz, 2H), 7.61 (t, J = 8 Hz, 1H), 8.14 (d, J = 8 Hz, 2H).
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20. ^{1}H NMR of **21** (400 MHz, $CDCl_3$) δ 1.20 (s, 3H), 1.22 (s, 3H), 1.27 (s, 9H), 1.56 (t, J = 7 Hz, 1H), 1.79 (s, 3H), 1.88 (brs, 1H), 2.12 (m, 1H), 2.20 (m, 1H), 2.39 (m, 4H), 2.43 (m, 6H), 2.55 (m, 1H), 3.71 (m, 4H), 3.75 (m, 1H), 4.04 (d, J = 9 Hz, 1H), 4.27 (d, J = 7 Hz, 1H), 4.29 (d, J = 9 Hz, 1H), 4.60 (brs, 1H), 4.75 (d, J = 3 Hz, 1H), 5.29 (m, 1H), 5.35 (d, J = 10 Hz, 1H), 5.65 (d, J = 7 Hz, 1H), 6.22 (m, 1H), 7.30–7.40 (m, 5H), 7.50 (t, J = 8 Hz, 2H), 7.60 (t, J = 8 Hz, 1H), 8.16 (d, J = 8 Hz, 2H).
21. Experimental procedure of fluorination: To a cooled (ice-water) solution of 7-*O*-methylthiomethyl-13-*O*-TES baccatin III (100 mg, 0.13 mmol) in CH_2Cl_2, was added *N*-iodosuccinimide (45 mg, 0.2 mmol) and DAST (35 μL, 0.20 mmol). Stirring was continued for 1 h at 0 °C. The reaction mixture was poured into satd. $NaHCO_3$ and the mixture was stirred vigorously for 5 min. The organic phase was separated and dried over anhydrous $MgSO_4$ and evaporated. The residue was purified by TLC to give **24** as colorless solids (62 mg, 64%).
22. ^{1}H NMR of **27** (400 MHz, $CDCl_3$) δ 1.15 (s, 3H), 1.20 (s, 3H), 1.34 (s, 9H), 1.72 (s, 3H), 1.85 (s, 3H), 2.38 (s, 3H), 1.60–2.70 (m, 12H), 3.68 (m, 4H), 4.00–4.06 (m, 2H), 4.19 (d, J = 8 Hz, 1H), 4.31 (d, J = 8 Hz, 1H), 4.41 (dd, J = 7 Hz, 10 Hz, 1H), 4.60 (s, 1H), 4.96 (d, J = 8 Hz, 1H), 5.11 (dd, J = 8 Hz, 28 Hz, 1H), 5.22–5.28 (m, 2H), 5.41 (d, J = 10 Hz, 1H), 5.65 (d, J = 7 Hz, 1H), 6.17 (t, J = 8 Hz, 1H), 7.30–7.42 (m, 5H), 7.49 (t, J = 8 Hz, 2H), 7.61 (t, J = 8 Hz, 1H), 8.10 (d, J = 8 Hz, 2H).
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