Enzymatic Dehydration and Skeleton Rearrangement of Paclitaxel Precursors

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Supplimentary Material

Synthesis of 4a from 2a. To a stirred solution of **2a** (63 mg, 0.12 mmol) and RDL (*Rhizopus delema* lipase, Fluka, 4.5 mg) in dried THF (1.5 mL) was added TCAA (133 μL, 0.72 mmol). The reaction mixture was allowed to be stirred for 1.5 h while the starting material disappeared. The reaction was stopped by adding dropwise sat'd aq $NaHCO_3$ until no gas was produced. The solution was diluted and extracted twice with ethyl acetate. The combined organic layers were dried with anhyrous Na_2SO_4, evaporated, and subjected to column chromatography to give **4a** (46.2 mg, 0.088 mmol, 73%) as a white powder: UV (CH_2Cl_2, nm) 237 (ε = 1.45), 275 (ε = 0.16); IR (film, cm^{-1}) 3437, 2968, 1736, 1723, 1450, 1372, 1314, 1265, 1241, 1176, 1093, 1070, 1048, 1026, 991, 968, 850; ^1H NMR ($CDCl_3$, ppm) 7.26-7.94 (*m*, 5 H, Bz), 5.80 (*s*, 1 H, 1-H), 5.56 (*s*, 1 H, 2-H), 5.44 (*dd*, *J* = 3.96 and 9.81 Hz, 1 H, 13-H), 5.38 (*d*, *J* = 8.34 Hz, 1 H, 20-H1), 5.19 (*br t*, 1 H, 5-H), 4.81 (*d*, *J* = 8.25 Hz, 1 H, 20-H2), 4.44 (*dd*, *J* = 4.04 and 7.58 Hz, 1 H, 7-H), 3.66 (*s*, 3-H), 3.05 (*dd*, *J* = 11.3 and 14.3 Hz, 1 H, 14-H1), 2.73 (*dd*, *J* = 4.68 and 15.1 Hz, 1 H, 14-H2), 2.60 (*m*, 1H, 6-H1), 2.30 (*m*, 6-H2), 2.14 (*s*, 3 H, Ac), 2.02 (*s*, 3 H, 16-H), 1.97 (*s*, 3 H, 17-H), 1.62 (*s*, 3 H, 18-H), 1.32 (*s*, 3 H, 19-H). ^{13}C-NMR ($CDCl_3$, ppm) 213.02, 201.99, 171.30, 165.60, 142.00, 138.80, 133.95, 130.32,

129.96, 129.37, 129.04, 120.56, 85.11, 81.99, 79.18, 75.83, 72.50, 69.72, 57.85, 40.85, 38.89, 35.76, 26.14, 22.72, 21.80, 20.47, 11.92; HRMS calc. for $C_{29}H_{35}O_9$ (M^+H^+) 527.2281 found 527.2245.

Synthesis of 5b from 2b. To a stirred solution of **2b** (0.1078 g, 0.16 mmol) and RDL (5 mg) in dried THF (3 mL) was added TCAA (154 μL, 0.96 mmol). The solution was allowed to be stirred for 4 h and stopped by filtration of the enzymes. The resulting solution was evaporated under reduced pressure, diluted with ethyl acetate and washed with sat'd aq $NaHCO_3$ solution. The organic layer was dried with anhydrous Na_2SO_4, evaporated, and subjected to column chromatography to give **5a** (0.0888 g, 0.14 mmol, 88%) as a sticky oil: Mass (FAB) calc. for $C_{35}H_{47}O_8Si$ (M^+H^+) 623.30 found 623.12.

The intermediate **5a** (17 mg, 0.033 mmol) was dissolved in a mixture of THF (0.5 mL) and MeOH (0.5 mL). To the reaction mixture was added 1 N HCl (1 mL). The resulting solution was stirred for 0.5 h and then evaporated under reduced pressure. The residue was diluted with ethyl acetate and washed with sat'd aq $NaHCO_3$ and brine in a sequence. The organic layer was dried with anhydrous Na_2SO_4, evaporated, and subjected to flash chromatogaphy to give **5b** (17 mg, 0.033 mmol, 94%) as a white powder: UV (CH_2Cl_2, nm) 245 (ε = 1.70), 261 (ε =1.18); IR (film, cm^{-1}) 3491, 2970, 2922, 1732, 1691, 1583, 1452, 1371, 1315, 1271, 1243, 1175, 1090, 1069, 1025, 992, 903, 850; ^1H NMR ($CDCl_3$, ppm) 7.45-7.96 (*m*, 5 H, Bz), 6.67 (*s*, 1 H, 10-H), 5.48 (*s*, 1 H, 2-H), 5.51 (*m, overlapped*, 1 H, 13-H), 5.27 (*d*, J = 8.34 Hz, 1 H, 20-H1), 5.33 (*br s*, 16-H1), 5.25 (*br s*, 16-H2), 5.14 (*br t*, 1 H, 5-H), 4.48 (*d*, J = 8.58 Hz, 1H, 20-H2), 4.62 (*m*, 1 H, 7-H), 3.75 (*s*, 3-H), 3.08 (*dd*, J = 14.7 and 11.4 Hz, 1 H, 14-H1), 2.74 (*ddd*, J =

1.1 Hz, *J* = 4.5 and 6.0 Hz, 1 H, 14-H2), 2.62 (*m*, 1 H, 6-H1), 2.22 (*m*, 6-H2), 2.09 (*s*, 3 H, Ac), 2.04 (*s*, 3 H, 17-H), 2.00 (*s*, 3 H, 18-H), 1.37 (*s*, 3 H, 19-H); ^{13}C-NMR ($CDCl_3$, ppm) 203.00, 205.99, 171.60, 165.70, 142.05, 139.90, 133.90, 153.15, 130.02, 129.50, 129.02, 123,70, 121.24, 119.27, 85.30, 82.11, 80.00, 69.35, 69.34, 58.42, 41.56, 40.17, 32.00, 25.60, 21.82, 20.21, 9.93; HRMS calc. for $C_{29}H_{33}O_8$ 509.2175 (M^+H^+) found 509.2179.

Synthesis of 6 from 5b. The compound **5b** (23.1 mg, 0.046 mmol) was dissolved in a mixture of MeOH (0.5 mL) and THF (0.5 mL). To this solution was added glacial acetic acid (ca. 0.5 mL). The resulting mixture was refluxed for 2 h. A white solid precipitated. The solvent was removed using a disposable spoid and the solid was washed once with methanol and three times with ethyl ether to give **6** (21mg, 0.043 mmol, 94%) as a white powder: UV (CH_2Cl_2, nm) 232 (ε = 0.83), 272 (ε = 1.62); IR (cm^{-1}, film) 3495, 2970, 2927, 2360,2342, 1726, 1698, 1583, 1451, 1370, 1275, 1244, 1175, 1151, 1120, 1027, 980, 906; ^{1}H NMR ($CDCl_3$, ppm) 8.35 (*s*, 10-H), 7.88 (*s*, 13-H), 7.45-8.08 (*m*, 5 H, Bz), 6.00 (*d*, *J* = 11.24 Hz, 1 H, 2-H), 5.24 (*s*, 1 H, 16-H1), 5.00 (*d*, *J* = 7.47 Hz, 1 H, 5-H), 4.88 (*s*, 1 H, 16-H1), 4.67 (*d*, *J* = 8.11 Hz, 1 H, 20-H1), 4.56 (*d*, *J* = 8.11 Hz, 1 H, 20-H2), 4.54 (*overlapped*, 1 H, 7-H), 3.24 (*d*, *J* = 12.91 Hz, 1 H, 3-H), 2.76 (*m*, 1 H, 6-H1), 2.31 (*s*, 3 H, 18-H), 2.00 (*m*, 6-H2), 2.04 (*s*, 3 H, Ac), 1.85 (*s*, 3 H, 17-H), 1.78 (*s*, 3 H, 19-H); ^{13}C NMR ($CDCl_3$, ppm) 192.44, 170.13, 166.03, 151.00, 149.37, 145.59, 134.49, 133.83, 130.36, 130.14, 128.82, 128.64, 127.69, 121.96, 115.90, 84.08, 78.30, 74.21, 72.43, 72.43, 45.92, 44.11, 39.01, 24.39, 21.60, 19.69, 16.85; Mass (CI) calc. for $C_{29}H_{31}O_7$ 491.2047 (M^+H^+) found 491.20.

The single crystal for X-ray crystallography was prepared from ethyl acetate.

The crystal structure and data are given in the attached sheets.

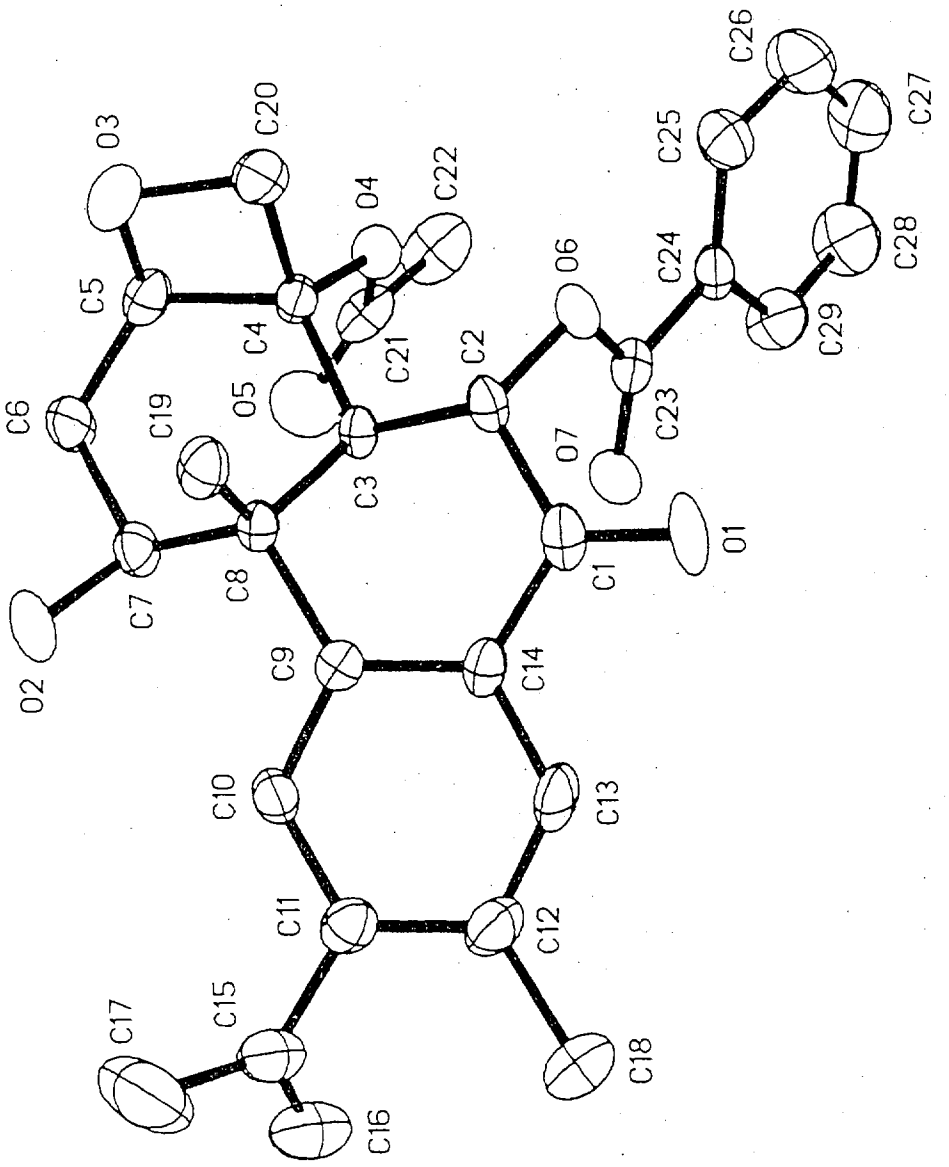

Figure 1. ORTEP drawing of the structure of compound 6.

Table 1. Crystal data and structure refinement for compound 6.

Identification code	compound 6
Empirical formula	$C_{29}H_{30}O_7$
Formula weight	490.53
Temperature	188(2) K
Wavelength	0.71073 Å
Crystal system	orthorhombic
Space group	P2(1)2(1)2(1)
Unit cell dimensions	a = 9.0905(19) Å alpha = 90° b = 12.189(3) Å beta = 90° c = 22.597(5) Å gamma = 90°
Volume, Z	2503.8(9) $Å^3$, 4
Density (calculated)	1.301 Mg/m^3
Absorption coefficient	0.093 mm^{-1}
F(000)	1040
Crystal size	0.35 x 0.15 x 0.15 mm
θ range for data collection	2.80 to 28.29°
Limiting indices	$-11 \le h \le 12$, $-11 \le k \le 16$, $-27 \le l \le 30$
Reflections collected	15298
Independent reflections	6043 (R_{int} = 0.1111)
Completeness to θ = 28.29°	98.9 %
Max. and min. transmission	0.9862 and 0.9683
Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	6043 / 1 / 328
Goodness-of-fit on F^2	1.294
Final R indices [I>2σ(I)]	R1 = 0.0891, wR2 = 0.1321
R indices (all data)	R1 = 0.1487, wR2 = 0.1542
Largest diff. peak and hole	0.300 and -0.297 $eÅ^{-3}$

Table 2. Atomic coordinates [x 10^4] and equivalent isotropic displacement parameters [$Å^2$ x 10^3] for compound 6. U(eq) is defined a one third of the trace of the orthogonalized U_{ij} tensor.

	x	y	z	U(eq)
O(1)	-8326(3)	-498(3)	-824(2)	54(1)
O(2)	-1268(3)	-25(3)	-667(2)	47(1)
O(3)	-2443(3)	-3374(3)	-503(1)	42(1)
O(4)	-4638(3)	-3385(2)	-1614(1)	28(1)
O(5)	-3299(4)	-2436(3)	-2274(1)	48(1)
O(6)	-7153(3)	-2470(2)	-1131(1)	27(1)
O(7)	-7151(3)	-1448(2)	-1960(1)	38(1)
C(1)	-6985(4)	-541(3)	-852(2)	31(1)
C(2)	-6231(4)	-1643(3)	-851(2)	24(1)
C(3)	-4651(4)	-1614(3)	-1102(2)	21(1)
C(4)	-4003(4)	-2768(3)	-1127(2)	22(1)
C(5)	-2315(4)	-2867(3)	-1092(2)	30(1)
C(6)	-1398(4)	-1843(3)	-1085(2)	42(1)
C(7)	-2168(4)	-730(3)	-1021(2)	28(1)
C(8)	-3740(4)	-754(3)	-747(2)	22(1)
C(9)	-4499(4)	367(3)	-838(2)	21(1)
C(10)	-3735(4)	1355(3)	-878(2)	27(1)
C(11)	-4414(4)	2368(3)	-960(2)	30(1)
C(12)	-5968(5)	2423(4)	-970(2)	37(1)
C(13)	-6726(4)	1465(3)	-923(2)	34(1)
C(14)	-6052(4)	440(3)	-868(2)	26(1)
C(15)	-3468(5)	3344(3)	-1052(2)	40(1)
C(16)	-3513(6)	3914(4)	-1561(2)	52(1)
C(17)	-2464(8)	3648(5)	-570(3)	88(2)
C(18)	-6781(6)	3505(4)	-1024(3)	68(2)
C(19)	-3662(4)	-953(3)	-70(2)	29(1)
C(20)	-4022(4)	-3480(4)	-573(2)	33(1)
C(21)	-4149(5)	-3155(4)	-2168(2)	35(1)
C(22)	-4820(6)	-3936(4)	-2609(2)	53(1)
C(23)	-7515(4)	-2267(3)	-1697(2)	28(1)
C(24)	-8429(4)	-3165(3)	-1960(2)	28(1)
C(25)	-8726(5)	-4128(4)	-1660(2)	40(1)
C(26)	-9565(6)	-4930(4)	-1940(2)	54(1)
C(27)	-10095(6)	-4767(4)	-2503(3)	57(1)
C(28)	-9787(6)	-3818(4)	-2798(2)	57(2)
C(29)	-8962(5)	-3007(4)	-2533(2)	45(1)

Table 3. Bond lengths [Å] and angles [°] for compound 6.

O(1)-C(1)	1.222(4)	O(2)-C(7)	1.431(5)
O(3)-C(20)	1.449(5)	O(3)-C(5)	1.470(5)
O(4)-C(21)	1.359(5)	O(4)-C(4)	1.452(4)
O(5)-C(21)	1.193(5)	O(6)-C(23)	1.344(4)
O(6)-C(2)	1.455(4)	O(7)-C(23)	1.208(5)
C(1)-C(14)	1.466(6)	C(1)-C(2)	1.508(5)
C(2)-C(3)	1.545(5)	C(3)-C(4)	1.526(5)
C(3)-C(8)	1.558(5)	C(4)-C(20)	1.523(5)
C(4)-C(5)	1.541(5)	C(5)-C(6)	1.501(6)
C(6)-C(7)	1.534(5)	C(7)-C(8)	1.557(5)
C(8)-C(9)	1.544(5)	C(8)-C(19)	1.551(5)
C(9)-C(10)	1.394(5)	C(9)-C(14)	1.416(5)
C(10)-C(11)	1.393(5)	C(11)-C(12)	1.414(5)
C(11)-C(15)	1.484(6)	C(12)-C(13)	1.361(6)
C(12)-C(18)	1.516(6)	C(13)-C(14)	1.397(5)
C(15)-C(16)	1.344(6)	C(15)-C(17)	1.470(7)
C(21)-C(22)	1.507(6)	C(23)-C(24)	1.496(5)
C(24)-C(25)	1.383(6)	C(24)-C(29)	1.397(6)
C(25)-C(26)	1.391(6)	C(26)-C(27)	1.376(7)
C(27)-C(28)	1.365(7)	C(28)-C(29)	1.378(6)

C(20)-O(3)-C(5)	91.0(3)	C(21)-O(4)-C(4)	117.5(3)
C(23)-O(6)-C(2)	115.4(3)	O(1)-C(1)-C(14)	122.9(4)
O(1)-C(1)-C(2)	119.4(4)	C(14)-C(1)-C(2)	117.6(3)
O(6)-C(2)-C(1)	110.8(3)	O(6)-C(2)-C(3)	113.0(3)
C(1)-C(2)-C(3)	113.7(3)	C(4)-C(3)-C(2)	110.6(3)
C(4)-C(3)-C(8)	115.7(3)	C(2)-C(3)-C(8)	108.7(3)
O(4)-C(4)-C(20)	108.8(3)	O(4)-C(4)-C(3)	110.6(3)
C(20)-C(4)-C(3)	119.3(3)	O(4)-C(4)-C(5)	113.2(3)
C(20)-C(4)-C(5)	85.6(3)	C(3)-C(4)-C(5)	117.0(3)
O(3)-C(5)-C(6)	112.7(3)	O(3)-C(5)-C(4)	90.1(3)
C(6)-C(5)-C(4)	119.2(3)	C(5)-C(6)-C(7)	118.8(3)
O(2)-C(7)-C(6)	108.9(3)	O(2)-C(7)-C(8)	108.3(3)
C(6)-C(7)-C(8)	116.2(3)	C(9)-C(8)-C(19)	106.8(3)
C(9)-C(8)-C(7)	110.0(3)	C(19)-C(8)-C(7)	110.6(3)
C(9)-C(8)-C(3)	106.9(3)	C(19)-C(8)-C(3)	115.2(3)
C(7)-C(8)-C(3)	107.2(3)	C(10)-C(9)-C(14)	116.1(4)
C(10)-C(9)-C(8)	123.4(3)	C(14)-C(9)-C(8)	120.5(3)
C(11)-C(10)-C(9)	123.6(3)	C(10)-C(11)-C(12)	119.2(4)
C(10)-C(11)-C(15)	118.2(3)	C(12)-C(11)-C(15)	122.6(4)
C(13)-C(12)-C(11)	117.6(4)	C(13)-C(12)-C(18)	120.4(4)
C(11)-C(12)-C(18)	122.0(4)	C(12)-C(13)-C(14)	123.5(4)
C(13)-C(14)-C(9)	119.9(4)	C(13)-C(14)-C(1)	118.5(3)
C(9)-C(14)-C(1)	121.6(4)	C(16)-C(15)-C(17)	121.6(5)
C(16)-C(15)-C(11)	121.2(4)	C(17)-C(15)-C(11)	117.2(4)
O(3)-C(20)-C(4)	91.6(3)	O(5)-C(21)-O(4)	123.3(4)
O(5)-C(21)-C(22)	126.4(4)	O(4)-C(21)-C(22)	110.3(4)
O(7)-C(23)-O(6)	123.5(4)	O(7)-C(23)-C(24)	124.1(3)
O(6)-C(23)-C(24)	112.3(3)	C(25)-C(24)-C(29)	120.2(4)
C(25)-C(24)-C(23)	122.4(4)	C(29)-C(24)-C(23)	117.4(4)
C(24)-C(25)-C(26)	118.8(4)	C(27)-C(26)-C(25)	120.7(5)
C(28)-C(27)-C(26)	120.2(5)	C(27)-C(28)-C(29)	120.5(5)
C(28)-C(29)-C(24)	119.5(5)		

Table 4. Anisotropic displacement parameters [$Å^2 \times 10^3$] for compound 6.

The anisotropic displacement factor exponent takes the form:

$-2\pi^2 [(ha^*)^2 U_{11} + \ldots + 2hka^*b^* U_{12}]$

	U11	U22	U33	U23	U13	U12
O(1)	10(1)	53(2)	99(3)	-22(2)	4(2)	0(1)
O(2)	15(1)	42(2)	86(3)	-24(2)	-4(2)	1(1)
O(3)	36(2)	46(2)	43(2)	7(2)	-15(1)	7(2)
O(4)	30(1)	30(2)	23(1)	-6(1)	-4(1)	1(1)
O(5)	53(2)	55(2)	36(2)	-1(2)	14(2)	-2(2)
O(6)	21(1)	31(2)	29(1)	1(1)	-3(1)	-7(1)
O(7)	35(2)	37(2)	41(2)	9(2)	-13(1)	-10(2)
C(1)	19(2)	39(2)	33(2)	-9(2)	-1(2)	1(2)
C(2)	18(2)	29(2)	26(2)	-6(2)	-2(2)	-3(2)
C(3)	16(2)	26(2)	20(2)	-4(2)	0(1)	2(2)
C(4)	20(2)	23(2)	24(2)	-1(2)	-3(2)	2(2)
C(5)	27(2)	30(2)	33(2)	-3(2)	-2(2)	10(2)
C(6)	19(2)	28(2)	79(3)	-3(2)	2(2)	2(2)
C(7)	19(2)	33(2)	30(2)	2(2)	3(2)	-2(2)
C(8)	17(2)	24(2)	23(2)	-2(2)	-1(2)	2(2)
C(9)	23(2)	23(2)	17(2)	-3(2)	-1(2)	3(2)
C(10)	21(2)	29(2)	32(2)	-4(2)	-2(2)	-2(2)
C(11)	32(2)	27(2)	29(2)	-5(2)	-2(2)	6(2)
C(12)	35(2)	31(2)	45(3)	-1(2)	-3(2)	11(2)
C(13)	19(2)	41(2)	42(3)	-8(2)	1(2)	11(2)
C(14)	19(2)	32(2)	28(2)	-4(2)	-1(2)	6(2)
C(15)	46(3)	26(2)	47(3)	-1(2)	-3(2)	3(2)
C(16)	64(3)	34(3)	58(3)	-1(2)	9(3)	5(3)
C(17)	111(5)	53(3)	99(5)	16(4)	-52(4)	-35(4)
C(18)	50(3)	37(3)	116(5)	-3(3)	-5(3)	17(3)
C(19)	28(2)	33(2)	25(2)	-4(2)	-7(2)	3(2)
C(20)	34(2)	33(2)	31(2)	5(2)	-7(2)	-2(2)
C(21)	44(3)	36(2)	26(2)	-2(2)	-3(2)	16(2)
C(22)	70(4)	57(3)	32(3)	-10(2)	-10(2)	18(3)
C(23)	21(2)	34(2)	30(2)	1(2)	-3(2)	3(2)
C(24)	18(2)	30(2)	36(2)	-9(2)	-3(2)	2(2)
C(25)	34(2)	36(2)	49(3)	-4(2)	-7(2)	-5(2)
C(26)	56(3)	43(3)	63(3)	-5(3)	-18(3)	-14(3)
C(27)	50(3)	49(3)	72(4)	-23(3)	-15(3)	-3(3)
C(28)	60(3)	61(4)	50(3)	-12(3)	-23(3)	-6(3)
C(29)	52(3)	42(3)	41(3)	-5(2)	-18(2)	1(2)