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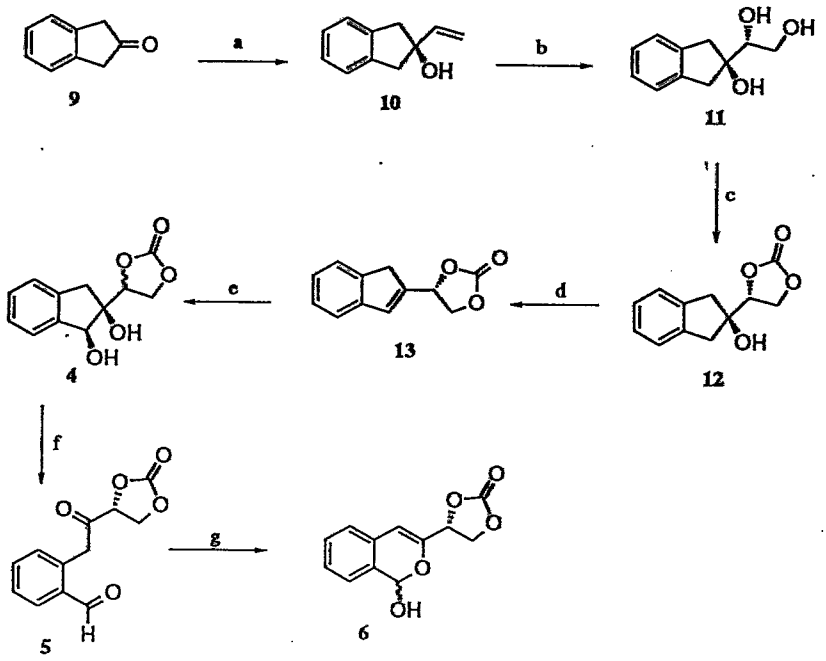
JL726-1

Preparation of Hydroxyisochromene **6**



[a](a) C_2H_3MgBr, THF (60%). (b) OsO_4, NMO, THF/H_2O (68%). (c) Cl_2CO, Et_3N, CH_2Cl_2, 0 °C (85%). (d) Martin Sulfurane, CH_2Cl_2, 0 °C (86%). (e) OsO_4, NMO, THF/H_2O (67%). (f) $NaIO_4$, THF/H_2O, 0 °C (89%). (g) HOAc, DMSO-d_6 (92%).

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Experimental Section:

General Procedures. All reactions were performed in flame dried flasks under argon unless otherwise noted. All commercial reagents were used as received with the following exceptions. Tetrahydrofuran and ethyl ether were distilled from sodium benzophenone ketyl. Dichloromethane and triethylamine were distilled from calcium hydride. Neocarzinostatin powder was obtained from Kayaku Ltd. (Japan), and was used as received. Infrared spectra were obtained using a Perkin-Elmer 1600 FT-IR spectrophotometer referenced to a polystyrene standard. Samples were analyzed as thin films on sodium chloride plates. Absorptions are reported in wavenumbers (cm^{-1}) and intensities are designated as broad (b), strong (s), medium (m), or weak (w). Proton magnetic resonance spectra (^1H NMR), carbon magnetic resonance spectra (^{13}C NMR), and the DEPT-90 spectrum were recorded on a JEOL JX-400 (400 MHz) spectrometer. The gradient-enhanced inverse detected HETCOR spectrum was recorded on a Bruker AMX-500 (500 MHz) spectrometer. Chemical shifts are measured in parts per million (ppm) relative to tetramethylsilane (TMS) and are referenced to residual protium in the NMR solvent. Coupling constants (*J* values) are in hertz (Hz). Multiplicities are designated as singlet (s), doublet (d), triplet (t), quartet (q), or multiplet (m). Peaks due to an additional diastereomer in a mixture are designated by a *. Melting points were determined on a Buchi SMP-20, are reported in °C, and are uncorrected. Analytical thin layer chromatography (TLC) was performed on Merck silica gel (60F-254) plates (0.25 mm) impregnated with a fluorescent indicator (254 nm). Visualization was effected with ultraviolet light and immersion in an acidic staining solution of *p*-anisaldehyde followed by heating on a hot plate. Flash column chromatography was performed as described by Still et al., employing E. Merck silica gel 60 (240-400 mesh). Reverse phase high performance liquid chromatography (HPLC) was performed using a Beckman SystemGold and Beckman Ultrasphere ODS C18 columns.

Isolation of NCS cyclization product (2,3). 2-mercaptoethanol (50 μL, 0.71 mmol, 68.6 equiv) was added to a solution of Neocarzinostatin (111.2 mg, 10.4 μmol, 1 equiv) and 2,5-dimethoxybenzyl alcohol (10.0 μmol, 1.0 equiv; internal standard for HPLC) in aqueous TRIS buffer (25 mM, pH 7.5, 10 mL). The resulting solution was vortexed briefly and was allowed to stand in a cold room (4 °C) for 14 hr. The reaction was purified in 10 1.0-mL portions by reverse phase HPLC (Beckman Ultrasphere ODS C18) using a gradient of 20% acetonitrile in aqueous ammonium acetate (pH 4.0) to 50% acetonitrile in aqueous ammonium acetate (pH 4.0) over 40 min. The fractions containing **3** were combined, the organic phase was removed on a

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rotary evaporator, and the aqueous phase was removed by lyophylization to give **3** (2.0 mg, 29%) as an off white powder:

Alcohol 10. A solution of 2-indanol (**9**, 758.2 mg, 5.74 mmol, 1 equiv) in tetrahydrofuran (6.0 mL) was added dropwise via pressure equalizing dropping addition funnel to a solution of vinlymagnesium bromide (1.0 M in THF, 8.0 mL, 8.0 mmol, 1.39 equiv) over 15 min. The addition was quantitated with additional tetrahydrofuran (2.0 mL), and was stirred at 23 °C for 15 min. The reaction was cooled to 0 °C and a saturated aqueous solution of ammonium chloride (3.0 mL) was added dropwise. The resulting biphasic, heterogeneous mixture was partitioned between water (40 mL) and ethyl ether (3 x 35 mL). The combined organic layers were dried over sodium sulfate, were filtered, and were concentrated. The residue was purified by flash column chromatography (17% ethyl acetate in hexanes) to give alcohol **10** (552.6 mg, 60%) as a white solid: mp 49.5-50.5 °C; R_f 0.24 (17% ethyl acetate in hexanes); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.16-7.25 (m, 4H), 6.17 (dd, 1H, *J*=17.2, 10.6), 5.41 (dd, 1H, *J*=17.2, 1.1), 5.15 (dd, 1H, *J*=10.6, 1.1), 3.19 (d, 1H, *J*=16.5), 2.98 (d, 1H, *J*=16.1), 1.87 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 142.8, 141.0, 126.7, 124.9, 111.5, 82.2, 47.4; IR 3530 (b), 3380 (b), 3071 (m), 3022 (m), 2944 (m), 2901 (m), 2836 (m), 1749 (w), 1642 (w), 1586 (w), 1483 (s), 1460 (s), 1412 (s), 1302 (w), 1278 (m), 1247 (s), 1212 (s), 1104 (s), 1042 (s), 1023 (m), 1002 (s), 922 (s), 896 (m), 740 (s), 676 (w) cm^{-1}.

Triol 11. 4-Methylmorpholine *N*-oxide (816.2 mg, 6.97 mmol, 2.1 equiv) and osmium tetroxide (4% w/w in H_2O, 1.0 mL, 0.16 mmol, 4.8 mol %) were added to a solution of allylic alcohol **10** (529.8 mg, 3.31 mmol, 1 equiv) in a mixture of tetrahydrofuran and water (5:1, respectively, 40 mL), and the resulting brown solution was stirred at 23 °C for 21 hr. The volatiles were removed on a rotary evaporator, an aqueous solution of sodium bisulfite (1.0 M, 50 mL) was added, and the resulting brown suspension was stirred at 23 °C for 20 min. The suspension was added to water (25 mL), and the mixture was saturated with sodium chloride. The aqueous phase was extracted with ethyl acetate (8 x 75 mL). The combined organic layers were dried over sodium sulfate, were filtered, and were concentrated. The solid residue was triturated with dichloromethane to give triol **11** (437.9 mg, 68%) as a beige solid: mp 161.0-162.0 °C; R_f 0.07 (67% ethyl acetate in hexanes), ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.13-7.17 (m, 2H), 7.06-7.10 (m, 2H), 4.73 (d, 1H, *J*=5.2), 4.51 (s, 1H), 4.42 (t, 1H, *J*=5.5), 3.68 (ddd, 1H, *J*=9.5, 5.9, 3.3), 3.51 (ddd, 1H, *J*=7.7, 5.2, 3.3), 3.38 (ddd, 1H, *J*=11.4, 7.4, 5.2), 3.20 (d, 1H, *J*=16.1), 3.10 (d, 1H, *J*=16.1), 2.65 (d, 1H, *J*=16.1); ^{13}C NMR (100 MHz, DMSO-d_6) d 142.0, 141.9, 125.8, 125.8, 124.6, 124.5, 82.8, 75.7, 62.8, 43.8, 42.1; IR

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3325 (b), 2932 (m), 2896 (m), 1487 (m), 1455 (m), 1434 (m), 1414 (m), 1226 (w), 1117 (m), 1092 (m), 1067 (s), 1038 (s), 996 (m), 876 (m), 818 (m), 742 (s) cm^{-1}.

Carbonate 12. Phosgene (20% w/w in H_2O, 1.7 mL, 3.28 mmol, 1.5 equiv) was added dropwise over 7 min to a solution of triol **11** (431.5 mg, 2.22 mmol, 1 equiv) and triethylamine (3.1 mL, 22.2 mmol, 10.0 equiv) in dichloromethane (30 mL) at 0 °C. The resulting solution was stirred at 0 °C for 30 min. The reaction was partitioned between a saturated aqueous solution of sodium bicarbonate (35 mL) and ethyl acetate (4 x 40 mL). The combined organic layers were dried over sodium sulfate, were filtered, and were concentrated. The residue was purified by flash column chromatography (40% ethyl acetate in hexanes) to give carbonate **12** (413.5 mg, 85%) as a white solid, along with small amounts of olefin **13** (29.8 mg, 7%). Carbonate **12** was recrystallized from a mixture of hexane and ethyl acetate (2:1, respectively): mp 162-163 °C; R_f 0.55 (25% hexanes in ethyl acetate); ^1H NMR (400 MHz, DMSO-d_6) δ 7.13-7.23 (m, 4H), 5.45 (s, 1H), 4.83 (dd, 1H, *J*=8.4, 5.8), 4.57 (dd, 1H, *J*=8.4, 8.4), 4.43 (dd, 1H, *J*=8.4, 5.8), 3.11 (d, 1H, *J*=16.5), 2.96 (d, 1H, *J*=16.4), 2.92 (d, 1H, *J*=14.7), 2.81 (d, 1H, *J*=16.4); ^{13}C NMR (100 MHz, DMSO-d_6) δ 155.0, 140.7, 140.3, 126.4, 126.4, 124.7, 124.7, 80.4, 80.0, 65.7, 43.1, 42.0; IR 3439 (b), 3025 (w), 2908 (w), 1781 (s), 1482 (m), 1399 (m), 1328 (m), 1255 (m), 1127 (s), 1088 (s), 1073 (s), 1020 (m), 979 (m), 773 (m), 745 (s) cm^{-1}.

Vinyl carbonate 13. A solution of the Martin sulfurane dehydrating agent (1.12 g, 1.67 mmol, 1.3 equiv) in dichloromethane (10 mL) was added via canula to a solution of carbonate alcohol **12** (289.9 mg, 1.32 mmol, 1 equiv) in dichloromethane (10 mL) at 0 °C. The resulting yellow solution was stirred at 0 °C for 45 min. The reaction mixture was concentrated in vacuo, and the residue was purified by flash column chromatography (dichloromethane) to give indene **13** (229.9 mg, 86%) as a white solid: mp 98-99 °C; R_f 0.44 (50% ethyl acetate in hexanes); ^1H NMR (400 MHz, $CDCl_3$) δ 7.48 (d, 1H, *J*=7.3), 7.41 (d, 1H, *J*=7.3), 7.31 (ddd, 1H, *J*=7.3, 7.3, 1.5), 7.26 (ddd, 1H, *J*=7.3, 7.3, 1.5), 6.98 (s, 1H), 5.68 (dd, 1H, *J*=8.1, 7.7), 4.71 (dd, 1H, *J*=8.8, 8.1), 4.40 (dd, 1H, *J*=8.8, 7.4), 3.50 (s, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 154.7, 143.0, 142.8, 141.1, 132.3, 126.9, 126.1, 124.1, 121.8, 75.3, 69.0, 37.0; IR 3072 (w), 3025 (w), 2978 (w), 2923 (w), 1790 (s), 1556 (w), 1480 (w), 1462 (m), 1391 (s), 1360 (m), 1334 (m), 1308 (w), 1218 (w), 1169 (s), 1073 (s), 1053 (s), 961 (w), 916 (s), 864 (s), 778 (s), 754 (s), 717 (s), 552 (m) cm^{-1}.

Diol carbonate 4. 4-Methylmorpholine *N*-oxide (227.6 mg, 1.94 mmol, 2.1 equiv) and osmium tetroxide (4% w/w in H_2O, 0.3 mL, 0.048 mmol, 5.1 mol %) were added to a solution of indene **13** (188.9

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mg, 0.93 mmol, 1 equiv) in a mixture of tetrahydrofuran and water (5:1, respectively, 12 mL), and the resulting pale yellow solution was stirred at 23 °C for 15 hr. An aqueous solution of sodium sulfite buffer (pH 7, 0.5 M in sodium bisulfite and 1.0 M in sodium sulfite, 10 mL) was added, and the resulting brown biphasic mixture was stirred vigorously at 23 °C for 35 min.. The mixture was added to a saturated aqueous solution of sodium chloride (20 mL), and was extracted with ethyl acetate (4 x 40 mL). The combined organic layers were dried over sodium sulfate, were filtered, and were concentrated. The residue was purified by flash column chromatography (45% hexanes in ethyl acetate) to give diol carbonate **4** (147.5 mg, 67%, 1.2:1 mixture of diastereomers) as a white solid: mp 138-144 °C; R_f 0.20 (50% ethyl acetate in hexanes); ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.18-7.35 (m, 8H), 5.74 (d, 1H, *J*=6.9), 5.57* (d, 1H, *J*=8.1 Hz), 5.07 (s, 1H), 4.96* (s, 1H), 4.88-4.94 (m, 2H), 4.77-4.81 (m, 2H), 4.64 (dd, 1H, *J*=8.0, 5.9), 4.50-4.59 (m, 5H), 2.98 (d, 1H, *J*=16.4), 2.93 (d, 1H, *J*=16.5), 2.85* (d, 1H, *J*=16.1), 2.81* (d, 1H, *J*=16.4); ^{13}C NMR (100 MHz, DMSO-d_6) δ 155.6, 143.9, 139.2, 128.6, 128.3*, 127.3, 127.1*, 125.5, 125.4*, 125.4, 125.3*, 125.0, 81.1, 80.7*, 80.3, 80.0*, 76.1, 66.2, 65.9*; IR 3448 (b), 3049 (w), 2931 (w), 1785 (s), 1479 (m), 1461 (w), 1399 (m), 1328 (m), 1302 (w), 1182 (s), 1078 (s), 971 (m), 748 (s), 618 (w) cm^{-1}.

Keto aldehyde 5 and hydroxyisochromene 6. Sodium meta-periodate (187.3 mg, 0.88 mmol, 1.3 equiv) was added to a solution of indandiol **4** (162.6 mg, 0.69 mmol, 1 equiv) in a mixture of tetrahydrofuran and water (1:1, 10 mL) at 0 °C. The resulting solution was stirred at 0 °C for 10 min. The reaction was partitioned between a saturated aqueous solution of sodium bicarbonate (40 mL) and ethyl acetate (4 x 40 mL). The combined organic layers were dried over sodium sulfate, were filtered, and were concentrated. The resulting white solid (143.5 mg, 89%) consisted almost entirely of keto aldehyde **5**: R_f 0.31 (50% ethyl acetate in hexanes); ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.97 (s, 1H), 7.95 (dd, 1H, *J*=7.7, 1.4), 7.65 (ddd, 1H, *J*=7.7, 7.3, 1.4), 7.56 (ddd, 1H, *J*=7.7, 7.3, 1.1), 7.36 (d, 1H, *J*=7.3), 5.52 (dd, 1H, *J*=9.5, 5.5), 4.79 (dd, 1H, *J*=9.5, 8.4), 4.65 (dd, 1H, *J*=8.4, 5.5), 4.41 (d, 1H, *J*=18.0), 4.29 (d, 1H, *J*=17.9); ^{13}C NMR (100 MHz, DMSO-d_6) δ 202.6, 194.3, 154.3, 134.6, 134.2, 133.9, 132.8, 127.9, 77.8, 66.4, 43.0. The crude keto aldehyde was purified by flash column chromatography (50% ethyl acetate in hexanes) to give an equimolar mixture of **5** and isochromene **6** (95.3 mg, 59%) as a white solid: IR 3433 (b), 2924 (w), 2849 (w), 2755 (w), 1790 (s), 1732 (s), 1694 (s), 1603 (m), 1577 (m), 1486 (m), 1392 (m), 1303 (m), 1173 (s), 1072 (s), 914 (m), 700 (s), 666 (w) cm^{-1}. Acetic acid (10 μL, 0.17 mmol, 0.4 equiv) was added to a solution of keto aldehyde **5** and isochromene **6** (95.3 mg, 0.41 mmol, 1 equiv) in perdeuterodimethyl sulfoxide (0.6

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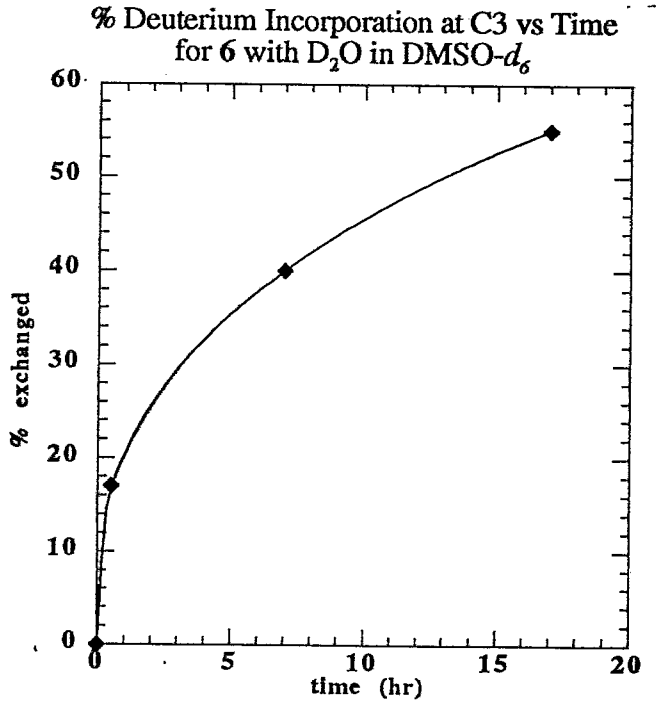
mL) in an NMR tube. After 75 min, ^{1}H NMR showed the mixture to be 12:1 **6**:**5**, where **6** exists as an equimolar mixture of two diastereomers: R_f 0.31 (50% ethyl acetate in hexanes); ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.48 (d, 1H, *J*=5.8), 7.47* (d, 1H, *J*=6.2), 7.19-7.35 (m, 8H), 6.38 (d, 1H, *J*=6.6), 6.36* (d, 1H, *J*=6.2), 6.34 (s, 1H), 6.31* (s, 1H), 5.46 (dd, 1H, *J*=8.4, 5.5), 5.46* (dd, 1H, *J*=8.4, 5.5), 4.72 (dd, 1H, *J*=8.4, 8.0), 4.69* (dd, 1H, *J*=8.4, 8.0), 4.45 (dd, 1H, *J*=8.0, 5.8), 4.44* (dd, 1H, *J*=8.0, 5.8); ^{13}C NMR (100 MHz, DMSO-d_6) δ 154.8, 146.2, 145.6*, 129.6, 128.9, 128.2, 128.1*, 127.6, 127.4*, 125.7, 124.4, 124.3*, 105.2, 104.0*, 92.8, 92.5*, 75.3, 75.1*, 66.6, 66.5*.

Deuterium exchange on hydroxyisochromene 6. Deuterium oxide (20 μL, 1.11 mmol, 10 equiv) was added to a solution of isochromene **6** (approx. 0.11 mmol, 1 equiv) in perdeuterodimethyl sulfoxide (0.6 mL) that had been prepared in a drybox in an NMR tube. The resulting solution was analyzed by ^{1}H NMR periodically to monitor the exchange of the C3 and C5 protons (NCS numbering). The C5 proton was observed to exchange completely within 5 min, whereas the C3 proton exchanged more slowly (Graph 1).

Graph 1



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Comparison of ^{13}C (100 MHz) NMR Data for 3 in DMSO-d_6, Saito et. al. and this work

Assignment	Saito (Tet. Lett. 1992)	Saito (Tetrahedron 1994)	This Work
1	135.5	128.4, 129.5*	127.7, 127.8*
2	136.4	134.5, 135.3*	140.2
3	139.7	139.2, 139.9*	119.7, 122.6*
4	132.8	132.9	133.3
5	88.2, 89.4*	88.1, 89.0*	88.6, 89.5*
6	155.0	156.2, 156.3*	156.6
7	118.8	117.7, 117.8*	118.1, 118.2*
8	122.2, 127.5*	127.2, 127.3*	127.5, 127.6*
9	139.0	138.2, 138.9*	139.6
10	82.3	82.1	82.5
11	84.5	84.2, 84.4*	84.7, 84.8*
12	50.6	50.3, 50.4*	50.7, 50.8*
13	76.1, 76.7*	76.1, 76.6*	76.5, 77.0*
14	68.7	68.6	69.0, 69.3*
15	154.3, 154.5*	154.6, 154.7*	155.0
16	34.9, 35.1*	34.6, 34.8*	34.6, 34.7*
17	61.0	61.0	61.5
1'	96.3	96.2, 96.3*	96.7, 96.8*
2'	58.2	58.1	58.6
3'	69.0	68.9	69.5
4'	71.0	70.8	71.3
5'	66.7	66.8	67.2
6'	16.8	16.8	17.2
2' NCH_3	34.0	33.9	34.2
1"	107.5	110.9, 111.1*	111.8, 111.9*
2"	161.0	168.2	168.5
3"	115.3	115.3	115.7
4"	129.3	129.3	129.6, 129.6*
4a"	121.5	120.1	122.5
5"	138.6	136.8	137.2
6"	116.5	116.6	117.0, 117.0*
7"	160.1	158.4	158.7
8"	100.7	100.5	100.9, 100.9*
8a"	136.0	133.4	133.8
5" CH_3	19.4	19.3	19.6
7" OCH_3	54.8	54.6, 54.8*	55.0, 55.2*
1" CO_2	172.9	172.6	172.5

* Peaks due to diastereomers at C5

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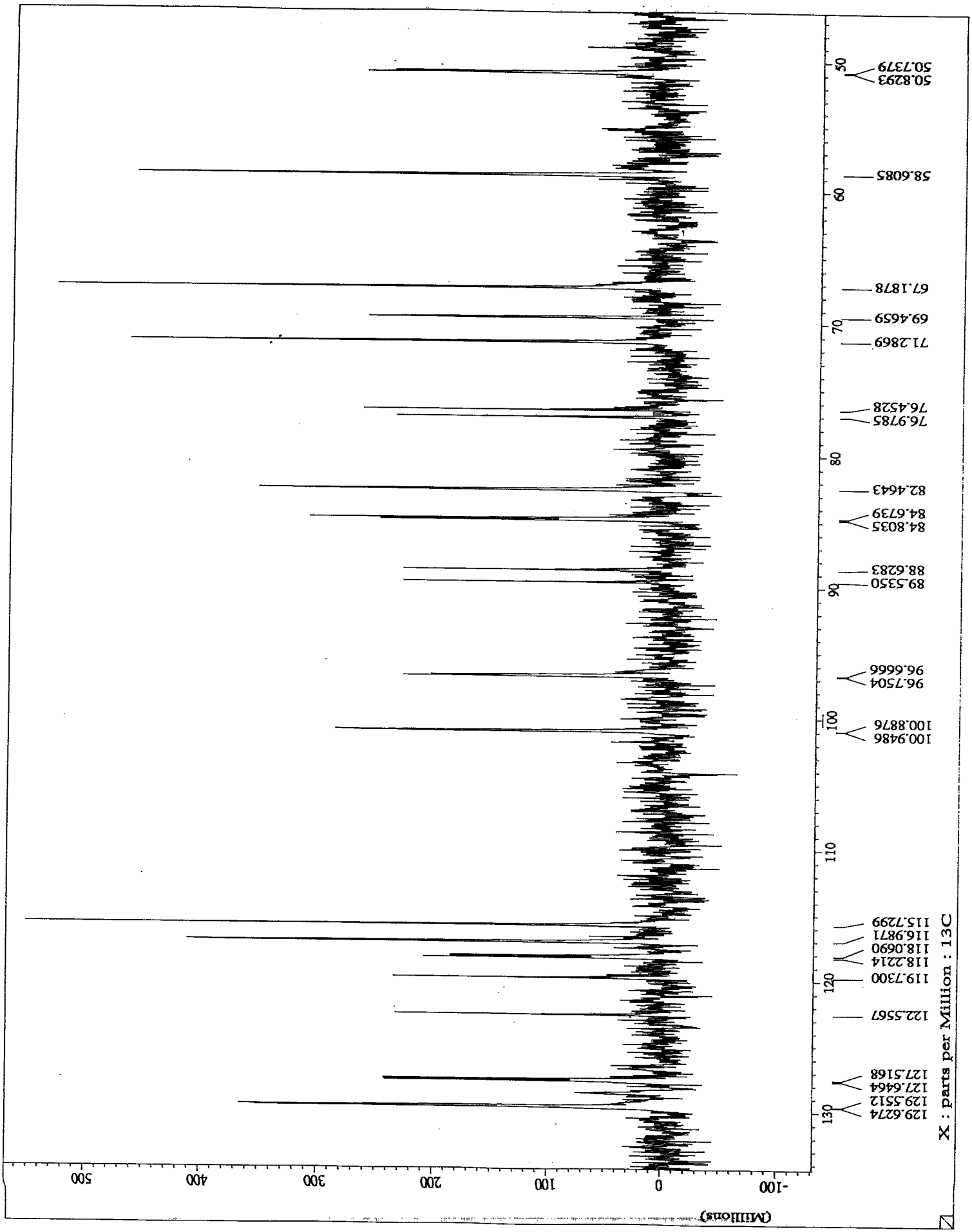
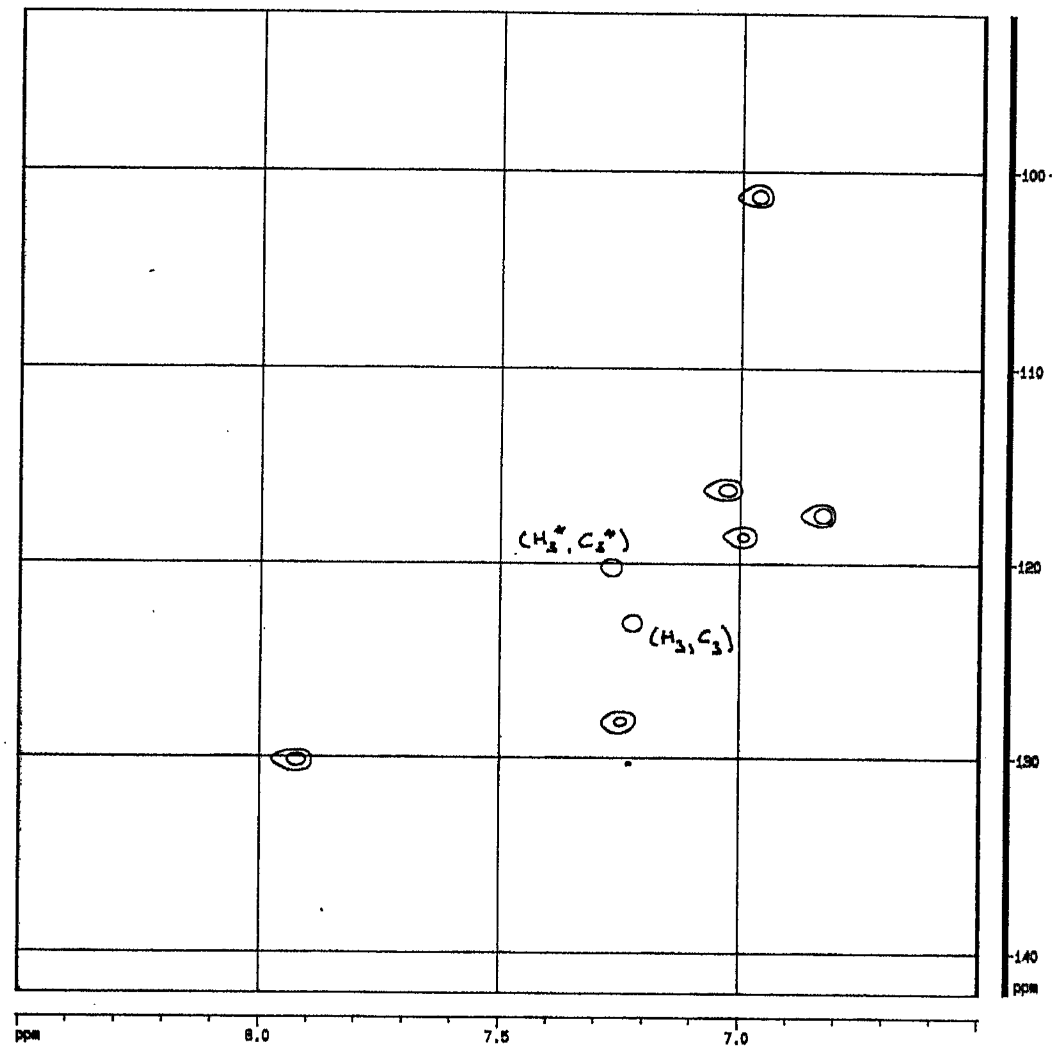


DEPT-90 on 3 (400MHz, DMSO-d_6)

^{1}H-^{13}C HETCOR on **3**, aromatic region (500 MHz, DMSO-d_6)



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D11	0.0300000	sec
DL0	10.00	dB
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D30	0.0002000	sec
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GB	0.001	
2D NMR plot parameters		
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CX1	20.00	cm
F2PLO	8.502	ppm
F2LO	4252.30	Hz
F2PHI	6.486	ppm
F2HI	3243.70	Hz
F1PLO	142.040	ppm
F1LO	17862.50	Hz
F1PHI	91.964	ppm
F1HI	11565.34	Hz
F2PPMCM	0.10084	ppm/cm
F2HZCM	50.43440	Hz/cm
F1PPMCM	2.50381	ppm/cm
F1HZCM	314.87896	Hz/cm

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